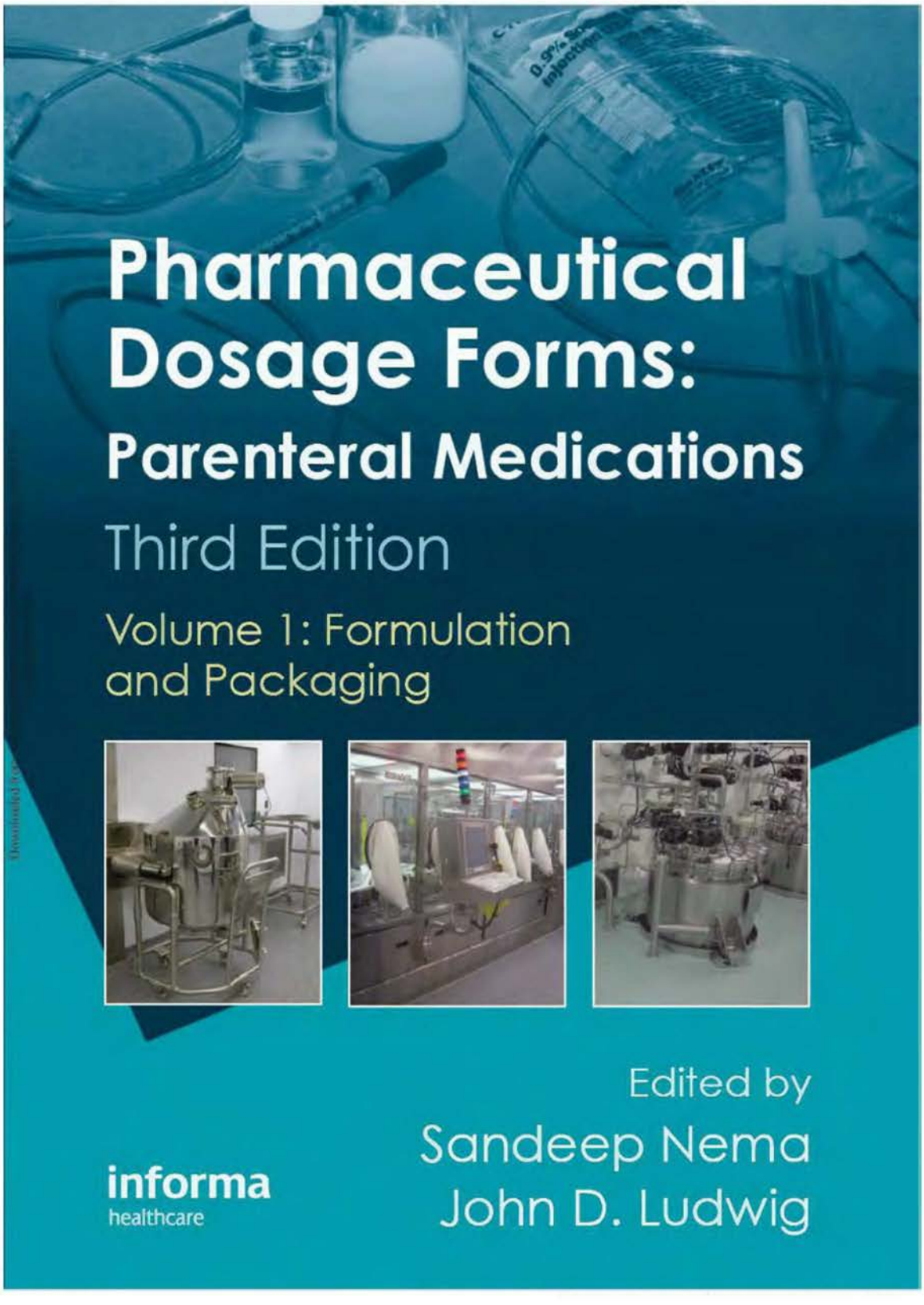


Pharmaceutical Dosage Forms

This page intentionally left blank

Pharmaceutical Dosage Forms

Parenteral Medications
Third Edition

Volume 1
Formulation and Packaging

Edited by

Sandeep Nema
Pfizer, Inc.
Chesterfield, Missouri, U.S.A.

John D. Ludwig
Pfizer, Inc.
Chesterfield, Missouri, U.S.A.

informa
healthcare
New York London

First published in 1984 by Marcel Dekker, Inc., New York, New York.
This edition published in 2010 by Informa Healthcare, Telephone House, 69 77 Paul Street, London EC2A 4LQ, UK.

Simultaneously published in the USA by Informa Healthcare, 52 Vanderbilt Avenue, 7th Floor, New York, NY 10017, USA.

Informa Healthcare is a trading division of Informa UK Ltd. Registered Office: 37 41 Mortimer Street, London W1T 3JH, UK. Registered in England and Wales number 1072954.

A CIP record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data available on application

ISBN 13: 9781420086430
ISBN 13: 9781420086539 (three volume set)

Orders may be sent to: Informa Healthcare, Sheepen Place, Colchester, Essex CO3 3LP, UK
Telephone: +44 (0)20 7017 5540
Email: CSDhealthcarebooks@informa.com
Website: http://informahealthcarebooks.com/

For corporate sales please contact: CorporateBooksIHC@informa.com
For foreign rights please contact: RightsIHC@informa.com
For reprint permissions please contact: PermissionsIHC@informa.com

Typeset by MPS Limited, A Macmillan Company
Printed and bound in India

We dedicate this work to those who have inspired us.
To my parents Walter and Ruth Ludwig and my wife Sue Ludwig
To my parents Hari and Pratibha Nema and my wife Tina Busch Nema

This page intentionally left blank

Foreword

I was a faculty member at the University of Tennessee and a colleague of Dr. Kenneth Avis when he conceived, organized, and edited (along with H.A. Lieberman and L. Lachman) the first edition of this book series that was published in 1984. It was so well received by the pharmaceutical science community that an expanded three-volume second edition was published in 1992. Dr. Avis did not survive long enough to oversee a third edition, and it was questionable whether a third edition would ever be published until two of his graduate students, Drs. Nema and Ludwig, took it upon themselves to carry on Dr. Avis' tradition.

Their oversight of this third edition is work that their mentor would be highly pleased and proud of. From 29 chapters in the second edition to 43 chapters in this new edition, this three-volume series comprehensively covers both the traditional subjects in parenteral science and technology as well as new and expanded subjects. For example, separate chapter topics in this edition not found in previous editions include solubility and solubilization, depot delivery systems, biophysical and biochemical characterization of peptides and proteins, container-closure integrity testing, water systems, endotoxin testing, focused chapters on different sterilization methods, risk assessment in aseptic processing, visual inspection, advances in injection devices, RNAi delivery, regulatory considerations for excipients, techniques to evaluate pain on injection, product specifications, extractables and leachables, process analytical technology, and quality by design.

The editors have done an outstanding job of convincing so many top experts in their fields to author these 43 chapters. The excellent reputations of the authors and editors of this book will guarantee superb content of each chapter. There is no other book in the world that covers the breadth and depth of parenteral science and technology better than this one. In my opinion, the editors have achieved their primary objectives publishing a book that contains current and emerging sterile product development and manufacturing information, and maintaining the high standard of quality that readers would expect.

Michael J. Akers
Baxter BioPharma Solutions
Bloomington, Indiana, U.S.A.

This page intentionally left blank

Preface

Pharmaceutical Dosage Forms: Parenteral Medications was originally published in 1984 and immediately accepted as a definitive reference in academic institutions and the pharmaceutical industry. The second edition was published in 1993. The ensuing years have produced incredible technological advancement. Classic small-molecule drugs are now complemented by complex molecules such as monoclonal antibodies, antibody fragments, aptamers, antisense, RNAi therapeutics, and DNA vaccines. There have been significant innovations in delivery devices, analytical techniques, in-silico modeling, and manufacturing and control technologies. In addition, the global regulatory environment has shifted toward greater emphasis on science-based risk assessment as evidenced by the evolving cGMPs, quality by design (QbD), process analytical technology (PAT), continuous processing, real time release, and other initiatives. The rapidly changing landscape in the parenteral field was the primary reason we undertook the challenging task of updating the three volumes. Our objectives were to (*i*) revise the text with current and emerging sterile product development and manufacturing science and (*ii*) maintain the high standard of quality the readers expect.

The third edition not only reflects enhanced content in all the chapters, but also more than half of the chapters are new underscoring the rapidly advancing technology. We have divided the volumes into logical subunits volume 1 addresses formulation and packaging aspects; volume 2, facility design, sterilization and processing; and volume 3, regulations, validation and future directions. The authors invited to contribute chapters are established leaders with proven track records in their specialty areas. Hence, the textbook is authoritative and contains much of the collective experience gained in the (bio)pharmaceutical industry over the last two decades. *We are deeply grateful to all the authors who made this work possible.*

Volume 1 begins with a historical perspective of injectable drug therapy and common routes of administration. Formulation of small molecules and large molecules is presented in depth, including ophthalmic dosage forms. Parenteral packaging options are discussed relative to glass and plastic containers, as well as elastomeric closures. A definitive chapter is provided on container closure integrity.

Volume 2 presents chapters on facility design, cleanroom operations, and control of the environment. A chapter discussing pharmaceutical water systems is included. Key quality attributes of sterile dosage forms are discussed, including particulate matter, endotoxin, and sterility testing. The most widely used sterilization techniques as well as processing technologies are presented. Volume 2 concludes with an in-depth chapter on lyophilization.

Volume 3 focuses on regulatory requirements, risk-based process design, specifications, QbD, and extractables/leachables. In addition, we have included chapters on parenteral administration devices, siRNA delivery systems, injection site pain assessment, and control, PAT, and rapid microbiology test methods. Volume 3 concludes with a forward-looking chapter discussing the future of parenteral product manufacturing.

These three volumes differ from other textbooks in that they provide a learned review on developing parenteral dosage forms for *both* small molecules and biologics. Practical guidance is provided, in addition to theoretical aspects, for how to bring a drug candidate forward from discovery, through preclinical and clinical development, manufacturing, validation, and eventual registration.

The editors wish to thank Judy Clarkston and Lynn O'Toole-Bird (Pfizer, Inc.) for their invaluable assistance and organizational support during this project, and Sherri Niziolek and Bianca Turnbull (Informa Healthcare) for patiently leading us through the publishing process.

We also acknowledge the assistance of Pfizer, Inc. colleagues Lin Chen and Min Huang for reviewing several of the chapters.

We would like to express special gratitude to the late Kenneth E. Avis (University of Tennessee College of Pharmacy) for his dedication to teaching and sharing practical knowledge in the area of parenteral medications to so many students over the years, including us. Finally, we acknowledge the contributions of Dr Avis, Leon Lachman, and Herbert A. Lieberman who edited the earlier editions of this book series.

Sandeep Nema
John D. Ludwig

Contents

Foreword Michael J. Akers *vii*
Preface *ix*
Contributors *xiii*

1. **Parenteral dosage forms: introduction and historical perspective** 1
   John D. Ludwig

2. **Parenteral drug administration: routes of administration and devices** 7
   Himanshu Bhattacharjee and Laura A. Thoma

3. **Biopharmaceutics of NCEs and NBEs** 30
   Balaji Agoram, Kazuko Sagawa, Ravi M. Shanker, and Satish K. Singh

4. **Preformulation** 57
   N. Murti Vemuri

5. **Formulation development of small and large volume injections** 76
   Madhav Kamat and Patrick P. DeLuca

6. **Drug solubility and solubilization** 134
   Ching Chiang Su, Lan Xiao, and Michael Hageman

7. **Formulation of depot delivery systems** 158
   James J. Cunningham, Marc J. Kirchmeier, and Sachin Mittal

8. **Biophysical and biochemical characterization of peptide and protein drug product** 194
   Tapan K. Das and James A. Carroll

9. **Formulation of protein- and peptide-based parenteral products** 222
   Gaozhong Zhu and Y. John Wang

10. **Development of ophthalmic formulations** 254
    Paramita Bandyopadhyay, Martin J. Coffey, and Mohannad Shawer

11. **Glass containers for parenteral products** 287
    Robert Swift

12. **Plastic packaging for parenteral drug delivery** 305
    Vinod D. Vilivalam and Frances L. DeGrazio

13. **Elastomeric closures for parenterals** 324
Renaud Janssen

14. **Parenteral product container closure integrity testing** 358
Dana Morton Guazzo

Index 389

Contributors

Balaji Agoram Pfizer, Inc., Sandwich, U.K.

Paramita Bandyopadhyay Bausch & Lomb, Rochester, New York, U.S.A.

Himanshu Bhattacharjee Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A.

James A. Carroll BioTherapeutics Pharmaceutical Sciences, Pfizer, Inc., Chesterfield, Missouri, U.S.A.

Martin J. Coffey Bausch & Lomb, Rochester, New York, U.S.A.

James J. Cunningham Pharmaceutical Research and Development Sciences, Merck Research Laboratories, West Point, Pennsylvania, U.S.A.

Tapan K. Das BioTherapeutics Pharmaceutical Sciences, Pfizer, Inc., Chesterfield, Missouri, U.S.A.

Frances L. DeGrazio West Pharmaceutical Services, Inc., Lionville, Pennsylvania, U.S.A.

Patrick P. DeLuca Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, Kentucky, U.S.A.

Dana Morton Guazzo RxPax, LLC, Bridgewater, New Jersey, U.S.A.

Michael Hageman Bristol Myers Squibb Research, Princeton, New Jersey, U.S.A.

Renaud Janssen Helvoet Pharma, Alken, Belgium

Madhav Kamat Biopharmaceutics R&D, Bristol Myers Squibb Company, New Brunswick, New Jersey, U.S.A.

Marc J. Kirchmeier Vaccine Formulation Development, Variation Biotechnologies, Inc., Cambridge, Massachusetts, U.S.A.

John D. Ludwig BioTherapeutics Pharmaceutical Sciences, Pfizer, Inc., Chesterfield, Missouri, U.S.A.

Sachin Mittal Pharmaceutical Research and Development Sciences, Merck Research Laboratories, West Point, Pennsylvania, U.S.A.

Kazuko Sagawa Pfizer Global R&D, Groton, Connecticut, U.S.A.

Ravi M. Shanker Pfizer Global R&D, Groton, Connecticut, U.S.A.

Mohannad Shawer Bausch & Lomb, Rochester, New York, U.S.A.

Satish K. Singh BioTherapeutics Pharmaceutical Sciences, Pfizer, Inc., Chesterfield, Missouri, U.S.A.

Ching-Chiang Su Bristol Myers Squibb Research, Princeton, New Jersey, U.S.A.

Robert Swift Amgen, Inc., Thousand Oaks, California, U.S.A.

Laura A. Thoma Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A.

N. Murti Vemuri Sanofi Aventis, Bridgewater, New Jersey, U.S.A.

Vinod D. Vilivalam West Pharmaceutical Services, Inc., Lionville, Pennsylvania, U.S.A.

Y. John Wang Genentech, South San Francisco, California, U.S.A.

Lan Xiao Bristol Myers Squibb Research, Princeton, New Jersey, U.S.A.

Gaozhong Zhu Shire Human Genetic Therapies, Inc., Cambridge, Massachusetts, U.S.A.

1 Parenteral dosage forms: introduction and historical perspective

John D. Ludwig

INTRODUCTION

Parenteral dosage forms are those administered directly into body tissues rather than via the alimentary canal. "Parenteral" is derived from the Greek words *para* (beside) and *enteron* (the intestine) and most often refers to subcutaneous (SC), intramuscular (IM), or intravenous (IV) administration of drugs. Parenteral drug delivery can pose significant risk to the patient since the natural barriers of the body (gut, skin, and mucous membranes) are bypassed. The highest standards for quality and purity must be maintained throughout dosage form manufacture to protect the patient from physical, chemical, and microbial contaminants. A single contaminated vial out of a batch of thousands can seriously injure a patient (or worse). Further, if improper or poor aseptic technique is used while administering an injection the patient could be similarly harmed. The minimum quality standards for pharmaceutical manufacturers are expressed in the current good manufacturing practices (cGMPs), which are constantly evolving as technology advances. An equal burden of responsibility is placed on physicians, pharmacists, nurses, and other health professionals to follow strict good aseptic practices (GAPs) as they administer parenteral dosage forms to patients. Nosocomial infections associated with parenteral drug therapy remain a significant issue (1 4).

ADVANTAGES AND DISADVANTAGES OF PARENTERAL DRUG DELIVERY

Parenteral drug delivery provides a number of advantages for the patient. The parenteral route provides an effective way to dose patients who are unconscious or those who cannot or would not take oral medications. A drug administered parenterally generally produces an immediate therapeutic effect and is therefore desirable in emergency situations. Parenteral administration also provides a mechanism for dosing drugs that are not bioavailable via noninjectable routes such as many protein and peptide therapeutics. Total parenteral nutrition can be provided for seriously ill patients where tube feeding is not an alternative. In addition, large amounts of fluid and electrolytes can be given relatively quickly via the IV route to patients with serious fluid loss from dehydration or gastrointestinal infections.

A significant disadvantage of injectable drug administration is that once a drug has been dosed it is difficult to reverse its effect. For example, in the event of a dosing error (overdose) with an oral tablet, gastric lavage, induced emesis, or activated charcoal can be employed. The options for reversing an IV overdose are usually very limited. Secondly, the risk of infection is always present with parenteral dosing both in the hospital/clinic setting as well as home administration. Finally, the cost per dose of parenteral drugs is typically higher than for oral medications.

PARENTERAL DRUG DELIVERY ROUTES

Routes of parenteral drug delivery are summarized in Table 1. SC, IM, and IV are the most common modes of administration. The fastest onset of action is achieved via the IV route since the injection is directly into a vein. Relatively large amounts of fluid can be delivered quickly and efficiently using the IV route. Slower and more variable onset of action typically occurs following SC and IM administration since the drug must be absorbed into the bloodstream from the site of injection. The absorption step can be exploited for drugs requiring chronic administration. Formulations can be designed to provide sustained-release profiles therefore reducing the number of injections required and the associated risk. Examples of "depot" formulations include DEPO-PROVERA® Contraceptive Injection, which is administered deep IM every 13 weeks and depo-subQ provera 104™ which is administered SC in the anterior thigh or abdomen every 12 to 14 weeks. Intravitreal dosing has increased significantly in recent

Table 1 Parenteral Drug Delivery Routes

Route	Administration volume
Subcutaneous (SC)	Low, generally <2 mL
Intramuscular (IM)	Medium, 2 mL 5 mL
Intravenous (IV)	High
Intravitreal	Low, generally <0.1 mL
Intradermal (ID)	Low, 0.1 mL
Intra articular	Medium
Intrathecal	Low
Intraepidural	Low
Intracisternal	Medium
Intra arterial	High
Intracardiac	Medium
Intrapleural	Medium
Intraperitoneal	High
Intraosseous	Medium

years because of new treatments for neovascular wet age-related macular degeneration (AMD) such as Lucentis® (ranibizumb injection) and Macugen® (pegaptanid sodium injection). The intradermal (ID) route is commonly used for very small volume injections (0.1 mL) such as the tuberculosis skin test [or tuberculin purified protein derivative (PPD) test]. Intra-articular injections directly into joint synovial fluid are routinely used to administer corticosteroids or hyaluronic acid derivatives to relieve the symptoms of osteoarthritis. Intrathecal (intraspinal) and intraepidural injections are used to deliver anesthesia, analgesics, anti-infectives, and some cancer therapies. Intracisternal administration is used to deliver critical therapeutics directly to the caudal region of the brain. Less common parenteral routes include intra-arterial, intracardiac (e.g., epinephrine for cardiac resuscitation), intrapleural, intraperitoneal, and intraosseous (bone) (5,6).

QUALITY ATTRIBUTES OF PARENTERAL DOSAGE FORMS

Quality attributes specific to parenteral dosage forms are shown in Table 2. Injectable products must be manufactured using the highest quality active drug substance and excipients. The regulatory review process requires that each ingredient in the formulation must be justified as

Table 2 Quality Aspects of Parenteral Dosage Forms

Attribute	Comment
Highest level of purity for the active drug substance and excipients	Highly purified "parenteral grade" excipients are available.
Formulation containing the fewest number and the simplest excipients possible	The presence and amount of each excipient must be justified in regulatory filings.
Physical and chemical stability	Minimal degradation during shelf life.
Container closure system with low extractable/ leachable profile	Minimize the impact of the container on product purity and stability.
Sterile	Sterility assurance is critical for patient safety.
Pyrogen free	Pyrogens cause febrile response. The most potent pyrogens are bacterial endotoxins.
Free from visible particulate matter	Subvisible particulate matter must be excluded as much as possible as defined by compendial requirements.
Container closure integrity	Product container maintains microbiological integrity during shelf life.
Injection site tolerability	Formulation does not cause significant injection site irritation or tissue damage. Products are frequently formulated as isotonic solutions.
Detailed dosing and administration instructions including evaluation of compatibility with coadministered drugs	In clinical practice, multiple drugs are frequently administered through the same IV line to avoid the risk of an additional venipuncture.

to why it was included and the relative amount. As a general rule, formulations with the fewest excipients and simplest composition are highly desired. The quality and robustness of the container-closure system must also be described and justified relative to extractables/leachables, container integrity (microbiological, oxygen transmission, moisture transmission), and intended clinical use. Parenteral products must be sterile, pyrogen-free, and free from visible particulate matter and remain so throughout shelf-life. Adverse injection site events are widely reported and can cause significant tissue damage. Often, the formulation can be modified to increase injection site tolerability, for example, by changing buffers and/or decreasing buffer concentration as well as rendering the dosing solution isotonic. The compatibility of the formulation should be assessed with the most likely drugs that will be coadministered with the new product. Compatibility results are generally included in the approved dosing instructions to assist pharmacists, nurses, and other health care providers.

MILESTONES IN PARENTERAL DRUG THERAPY

Various scholars have summarized the development of parenteral drug therapy (7 13). A compiled historical timeline is presented in Table 3. The reader should be aware there is disagreement in the literature about exact dates as well as who was "first," particularly for

Table 3 Historical Milestones in Parenteral Drug Delivery

Year	Milestone
1616	William Harvey described the circulation of blood. His findings were published in 1628.
1656	Christopher Wren infused dogs with opiates and alcoholic beverages using a sharpened quill and animal bladder.
1665	Johannes Escholtz described techniques for IV infusion of drugs into humans.
1796	Edward Jenner vaccinated children against smallpox using intradermal administration with cowpox virus.
1818	James Blundell performed a successful blood transfusion following postpartum hemorrhage.
1831	William O'Shaughnessy studied the blood of cholera patients and developed the concepts for IV water and electrolyte replacement therapy.
1832	Thomas Latta established the first clinical practice of IV infusions of water and salts to treat cholera patients, based on O'Shaughnessy's work.
1855	Alexander Wood developed the first modern hypodermic syringe with a steel barrel and hollow steel needle.
1867	Joseph Lister developed the concepts of antisepsis using carbolic acid (phenol) solutions to sanitize hands, instruments, and wounds to reduce postsurgery infections.
1860s 1880s	Louis Pasteur confirmed the germ theory of disease, discovered techniques for pasteurization of milk, and developed vaccinations against chicken cholera, bovine anthrax, and rabies.
1879	Charles Chamberland invented the autoclave.
1884	Charles Chamberland invented the "Chamberland filter" (porcelain) that removed bacteria from solutions prior to dosing.
1891	R.M. Matas demonstrated the effective use of IV saline solutions to treat shock.
1912	Using a rabbit model, E.C. Hort and W.J. Penfold determined the pyrogenic response following many IV injections was caused by a substance produced by gram negative bacterial contamination of the solution (14 16).
1918	Richard Zsigmondy and W. Bachman developed technology to manufacture microporous membrane filters from cellulose esters (nitrocellulose, acetyl cellulose, cellulose acetate).
1923	Florence Siebert and L.B. Mendel developed a definitive rabbit pyrogen test model and showed that endotoxin from gram negative bacteria was the substance responsible for the pyrogenic response following injection with sterile solutions (17 19,20).
1923	Frederick Banting and J.J.R. Macleod share the Nobel Prize in Physiology or Medicine for the extraction of insulin and demonstration of clinical efficacy.
1923	Purified insulin product marketed (Iletin®).
1924	R.M. Matas demonstrates continuous IV "drip" (21).
1933	L. Rademaker reported that after installation of a distilled water system for pharmaceutical production, pyrogenic reactions by surgery patients to parenteral injections dropped from 30% to 4% (22).
1938	Lloyd A. Hall and Carroll L. Griffith patented the use of ethylene oxide to sterilize and preserve spices. This technology was applied to sterile pharmaceutical product manufacturing during the 1940s.

(*Continued*)

Table 3 Historical Milestones in Parenteral Drug Delivery (*Continued*)

Year	Milestone
1942	Rabbit pyrogen test (Seibert and Mendel) published in the U.S. Pharmacopeia.
1940s	High Efficiency Particulate Air (HEPA) filters designed and installed for clean air supply in rudimentary cleanrooms at Manhattan project sites and biological weapons research laboratories at Fort Detrick, Maryland (10,23,24).
1946	Parenteral Drug Association founded.
1950s	Cleanrooms with HEPA filtered air supply widely used for pharmaceutical fill/finish (10,23,24).
1961	Willis J. Whitfield pioneered the concept of laminar air flow and constructed the first modern cleanroom at Sandia Corporation in Albuquerque, New Mexico (10,23,24).
1961	Arvid Wretlind and O. Schuberth formulated the first lipid emulsion, Intralipid®, suitable for IV infusion (7,25).
1964	Arvid Wretlind developed a total parenteral nutrition (TPN) program providing half of the calories from lipid and half from glucose. Recognized as the father of TPN (7,25).
1967	Stanley J. Dudrick reported comprehensive technique to provide long term total parenteral nutrition (TPN) (7,25).
1969	DW Wilmore and Stanley J Dudrick used an in line filter to reduce the risk of IV infusions (7, 25).
1971	James F. Cooper, Jack Levin, and H.N. Wagner Jr. pioneered use of the limulus amebocyte lysate test for screening parenteral drug products for endotoxin contamination (26).
1973	Infusion Nurses Society founded.
1976	Food and Drug Administration publishes *Current Good Manufacturing Practice in the Manufacture, Processing, Packing, or Holding of Large Volume Parenterals* (never formally adopted).
1978 1979	Human insulin cloned. Human growth hormone cloned.
1980s	First steps toward barrier isolator technology for aseptic fill/finish operations gray side maintenance (24).
1980s	Sterilizable isolators introduced for compendial sterility testing (27).
1982	Humulin® (human insulin recombinant) marketed.
1985	Protropin® (somatrem for injection) and Somatonorm® (somatrem) marketed. (methionyl human somatropin).
1986	Orthoclone® OTK3 marketed to treat the rejection of transplanted organs.
1987	FDA publishes *Industry Guideline on Sterile Drug Products Produced by Aseptic Processing and Guideline on General Principles of Process Validation.*
1987	Humatrope® (somatropin recombinant) and Genotropin® [somatropin (rDNA) for injection] marketed.
1987	First dual chamber pen injector launched (KabiPen®).
1990s	Barrier isolator technology for fill/finish operations Restricted Access Barrier Systems (RABS) and Isolators (24).
1992	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is established.
1994	FDA publishes *Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products.*
1996	*Note for Guidance on Manufacture of the Finished Dosage Form* issued by the Committee For Proprietary Medicinal Products (CPMP), CPMP/QWP/486/95.
1997	First monoclonal antibody to treat cancer approved Rituxan® (rituximab).
1999	*Decision Trees for the Selection of Sterilization Methods* finalized by the CPMP, CPMP/QWP/ 054/98.
2003	Pharmaceutical Compounding Sterile Preparations <797> became official in the U.S. Pharmacopeia.
2003	European Commission: Ad Hoc GMP Inspections Services Group, EC Guide to Good Manufacturing Practice Revision to Annex 1, Title: *Manufacture of Sterile Medicinal Products.*
2004	FDA publishes *Guidance for Industry Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice* (replaces 1987 version).
2006	Infusion Nurses Society publishes updated *Infusion Nursing Standards of Practice* (28).
2008	Heparin recalls due to intentional contamination during production of active pharmaceutical ingredient.
2009	European Commission: EudraLex The Rules Governing Medicinal Products in the European Union, Volume 4, EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, Annex 1, *Manufacture of Sterile Medicinal Products* (replaces 2003 version).

Abbreviation: IV, intravenous.

discoveries prior to the 20th century. Therefore, the author attempted to arrive at reasonable dates after consulting multiple sources. It is clear early scientific findings were not disseminated quickly because of lack of modern communication tools, and scientists were often working without knowledge of similar research occurring in other laboratories. In addition, advancements were occasionally "forgotten" only to be rediscovered independently a century later, all adding to the fascinating history of medicines and health care. Specific references have been included in Table 3 for recent advances and milestones.

CONCLUSION

The advent of safe, effective parenteral therapy has resulted in tremendous improvement in the quality of medical care around the world. Those of us fortunate enough to work in this exciting area whether in research, dosage form development, manufacturing, or clinical practice share a common goal of providing the highest standard of care. To do so requires diligence at each step in the process, be it synthesis of the active ingredient and excipients, production of the container and closure, compounding of the formulation, or aseptic fill/finish of the final product. The minimum quality standards are provided in the cGMPs, but regulatory and ethical expectations go well beyond the written requirements. Providing the highest standard of care also requires strict adherence to GAPs as the health care professional or family member is preparing and administering the dose to the patient. The risk of introducing infection and causing harm is ever present. Maxine B. Perdue of the Infusion Nurses Society summarized these sentiments as follows (29):

> "My word for competency is *excellence*. Excellence is not perfection; it is stellar performance. It is keeping current and complying with evidence-based practice standards. It is not accepting the status quo, rather, being visionary and innovative and a catalyst for research. It is sharing information with others by writing articles. . .and speaking at meetings. Each day is an opportunity to step outside the box and look at how we practice infusion therapy and to focus on each aspect of what we do as a chance to improve infusion care."

The constant pursuit of *excellence* is what drives us to the highest standard of care. Our patients deserve nothing less.

ACKNOWLEDGMENT

The author wishes to dedicate this chapter to Kenneth E. Avis, major professor and friend, who passed away in January 1999, and to Michael J. Akers for many years of mentoring and collaboration.

REFERENCES

1. Nichols RL, Smith JW. Bacterial contamination of an anesthetic agent. N Engl J Med 1995; 333(3): 184 185.
2. Mattner F, Gastmeier P. Bacterial contamination of multiple dose vials: a prevalence study. AJIC 2004; 32(1):12 16.
3. Nogler Semenitz E, Lass Flörl C, Nogler M, et al. Bacterial contamination of solutions for parenteral administration for single and multiple dose vials after multiple use in the hospital. Wein Med Wochenschr 2007; 157(15 16):398 401.
4. Kotwal A. Innovation, diffusion and safety of a medical technology: a review of the literature on injection practices. Soc Sci Med 2005; 1133 1147.
5. Wheeler CA. Pediatric intraosseous infusion: an old technique in modern health care technology. J Intravenous Nursing 1989; 12(6):371 376.
6. Foëx BA. Discovery of the intraossesous route for fluid administration. J Accid Emerg Med 2000; 17:136 137.
7. Dudrick SJ. History of vascular access. J Parenter Enteral Nutr 2006; 30(1):S47 S56.
8. Bartecchi CE. Intravenous therapy: from humble beginnings through 150 years. Southern Medical J 1982; 75(1):61 64.
9. Millam D. The history of intravenous therapy. J Intravenous Nursing 1996; 19(1):5 14.

10. Avis KE, Morris BG. The dosage form and its historical development. In: Avis KE, Lachman L, Lieberman HA, eds. Pharmaceutical Dosage Forms: Parenteral Medications. 1st ed. Vol. 1. New York: Marcel Dekker, 1984:1 12.
11. Helmstädter A. 350 years of intravenous injection. Pharmaceutical Historian 2006; 36(1):6 8.
12. Helmstädter A, Van Zundert A. 1656 2006: 350 years of intravenous injection. Acta Anaesth Belg 2006; 57:27.
13. Zimmerman JJ, Strauss RH. History and current application of intravenous therapy in children. Pediatr Emerg Care 1989; 5(2):120 127.
14. Hort EC, Penfold WF. Microorganisms and their relation to fever. J Hyg 1912; 12:361 390.
15. Hort EC, Penfold WF. A critical study of experimental fever. Proc R Soc Lond (Biol) 1912; 85:174 186.
16. Hort EC, Penfold WF. The relation of salvarsan fever to other forms of injection fever. Proc R Soc Med (Pt. III) 1912; 5:131 139.
17. Seibert FB, Mendel LB. Temperature variations in rabbits. Am J Physiol 1923; 67:83 89.
18. Seibert FB. Fever producing substances found in some distilled waters. Am J Physiol 1923; 67:90 104.
19. Seibert FB, Mendel LB. Protein fevers. Am. J. Physiol 1923; 67:105 123.
20. Seibert FB. The cause of many febrile reactions following intravenous injections. Am J Physiol 1925; 71:621 651.
21. Matas RM. The continuous intravenous "drip." Ann Surg 1924; 79:643 661.
22. Rademaker L. Reactions after intravenous infusions. Surg Gynec and Obstet 1933; 56:956 958.
23. Whitfield WJ. Microbiological studies of laminar flow rooms. Bull Parenter Drug Assoc 1967; 21:37 45.
24. Lysfjord JP, Haas PJ, Melgaard HL, et al. Barrier isolation technology: a systems approach. In: Wagner CM, Akers JE, eds. Isolator Technology Applications in the Pharmaceutical and Biotechnology Industries. Buffalo Grove: Interpharm Press, 1995:231 280.
25. Vinnars E, Wilmore D. History of parenteral nutrition. J Parenter Enteral Nutr 2003; 27(3):225 231.
26. Cooper JF, Levin J, Wagner HN Jr. Quantitative comparison of in vitro and in vivo methods for the detection of endotoxin. J Lab Clin Med 1971; 78:138 148.
27. Meyer D. Developing a barrier/isolator implementation plan. In: Wagner CM, Akers JE, eds. Isolator Technology Applications in the Pharmaceutical and Biotechnology Industries. Buffalo Grove: Interpharm Press, 1995:97 122.
28. Infusion Nursing Standards of Practice. J Infusion Nursing 2006; 29:1S.
29. Perdue, MB. 30 years and the hallmarks of infusion therapy remain unchanged. J Infusion Nursing 2007; 30(5):253 254.

2 Parenteral drug administration: routes of administration and devices

Himanshu Bhattacharjee and Laura A. Thoma

INTRODUCTION

The word Parenteral is coined from the greek words "*para enteron*" meaning "to avoid the intestines." Drugs administered via any route other than oral or rectal routes, are considered to be parenteral. However, common usage more closely associates the term as being synonymous with "injectable." These include drugs that are topically administered to the eye, ear, and skin or even inhaled may be considered broadly as parenterals. It is estimated that 40% of all drugs administered in hospitals are in the form of an injection. In some institutional settings, the percentage of injectables is greater than 40%. However, medical and pharmacy practioners of today generally limit the classification of parenterals to include only those drugs administered directly into tissues, tissue spaces, or compartments by injection or infusion.

Injectable products are sterile products and may require special handling and administration. Estimates indicate that over one billion disposable plastic syringes are used annually in American hospitals. With increasing complexity of the drugs being administered by the parenteral route, significant development with respect to techniques for parenteral administration have evolved in recent years and continue to do so. Moreover, development of site specific, efficacious, safe, and reproducible administration techniques have led to the development of highly advanced stand alone drug delivery devices. Some of these developments have addressed significant safety and efficacy concerns but the area of drug delivery device research is an active field of study. This chapter is an attempt to review and to update the current usage of parenteral drugs and their routes of administration. Additionally, this chapter will address currently available parenteral drug delivery devices and the trends of existing technology in the field.

PARENTERAL ADMINISTRATIONS CONCEPTS

Although oral administration is more prevalent in the current market place, parenteral administration of drugs has a number of distinct advantages over the former. Increasing complexity of new drug entities (e.g., biomolecules) and treatment regimens to treat life threatening diseases have led many formulation groups utilize parenteral routes. In some instances, parenteral administration is essential for the drug to be absorbed in active form. For example, almost all protein drugs are administered by injection, rather than administration by the oral route, because protein drugs are broken down by stomach acid and digestive enzymes. Absorption through the parenteral route is usually more rapid and predictable than when a drug is administered orally. Because of its predictable rate of absorption and bio-availability, parenteral drugs are routinely used in emergency therapy. If a patient is unconscious, uncooperative, or unable to retain anything administered orally, parenteral therapy may become a necessity.

Parenteral dosage forms ensure delivery of therapeutic concentrations of drug/s to its desired site/s of action (diseased tissues or target areas of the body). This factor becomes more significant especially when inadequate or marginal transport of drug/s into the tissues or target areas occurs or is anticipated. One such example is a direct intra-articular injection of drugs, (e.g. anti-inflammatory drugs such as the steroids) which exhibit poor transport characteristics into the synovial spaces between joints, may be used to reduce inflammation. Additionally, injectable drugs allow researcher to exert direct control over pharmacological parameters, such as the time of drug onset, serum peak and trough levels, tissue distribution, clearance and rates of elimination of the drug from the body; for example, sustained or prolonged action of intramuscular (IM) insulin administration. Parenteral administration of drugs, in some cases may aid in decreased side effects of the drug by avoiding the traditional

oral route. Methotrexate, an antimetabolite, used for blood malignancies, exhibits varied physiological side effects when administered via the intravenous (IV) route and shows poor blood-brain barrier (BBB) penetration. However in patients suffering from acute lymphoblastic leukemina (ALL) (1), methotraxate can be administered intrathecally to avoid systemic side effects. In a clinical setting, parenterally administered drugs are commonly employed for immediate correction of electrolyte or fluid imbalance, for example, dehydration or excessive blood loss due to trauma. Patients who require hyper alimentation can also be administered total parenteral nutrition consisting of minerals, amino acids, vitamins, and carbohydrates via the IV route.

Although parenterally administered drugs have a number of advantages they do suffer from certain shortcomings. One of the major disadvantages is the possiblity for infections resulting from inadequate aseptic technique during product administration. Asepsis must be maintained to avoid infection, particularly for an intravascular or intraventricular injection. Apart from infections, other life threatening conditions like AIDS (2) and hepatitis C (3) can be attributed to improper use of parenteral devices. Disinfection of the patient's skin with an antibacterial solution or rubbing alcohol before injection and using a new syringes and needles for each administration is considered a best practice. Since injecting a needle into vascular compartments or body cavities can be considered as invasive processes, pain may be an additional factor. This is especially a significant factor for patients who perform self-administration (e.g., insulin, human growth hormone). Many of the products in the current market are highly specialized drug products and expense is still a major consideration.

Although in many instances precaution are unique to the route to be utilized, several factors need to be emphasized. Needless to say good aseptic technique and sterile practices is an absolute necessity. The health practitioner should always examine the product carefully before administration to identify potential or real contamination by microorganisms or particulate matter unless the product is supplied as a suspension or emulsion. Adequate attention should be given to details with respect to dosage, mixing, potential drug interaction, and storage. Informed actions and precautions should be taken during handling of accessory or delivery devices necessary to accomplish the task of injection or infusion or to monitor the patient's conditions. Selection of correct equipment for administration of the drug product, careful assessment of the patient history, evaluation of risk factors (e.g., bleeding diathesis, previous drug interactions, predisposition to infection, etc.) and a careful observation of the patient during and after parenteral administration are recommended.

The need for good practices in storage and handling of parenteral drugs or infusions is also an important factor and should be appropriately emphasized. From the moment a parenteral drug product is manufactured, its purity and sterility are constantly threatened by handling or storage errors. Such problems are not unique to manufacturers but extend throughout the life of the product in all areas of delivery, receiving, and distribution. Difficulties encountered may range from inadequate temperature control of storage temperatures, to outdated shelf lives, to defective containers and closures (4). On the other hand errors encountered during handling or compounding usually occur at the hospital pharmacy or at bedside. Past attempts by hospital pharmacies emphasizing a "central additive programs" as a method of reducing such errors have led to reduced admixing errors (4). In such a setting sterile parenteral product received from the manufacturer is mixed in a central location (usually in the pharmacy) with specific agents or fluid formulas that physicians may have prescribed. The central location is isolated and compounding is performed aspectically under a laminar flow hood. Complex formulas are often generated in these specialized units to satisfy the therapeutic needs of an extremely difficult medical or surgical problem (e.g. hyperalimentation). Upon compounding, the product/s is shipped to the hospital ward for administration to the patient. Newer infusion devices like the "smart pumps" or "intelligent pumps" are now available that have shown to significantly reduce compounding errors related to dose accuracy (5). Central additive programs reduce the high risk of compounding and contaminating errors which may occur because of personnel variability.

In addition to these problems, difficulties exist in securing properly trained, highly intelligent, motivated health care personnel to employ correctly and responsibly the complicated methods often utilized in the modern hospital or clinic setting. Such personnel,

in addition to being expensive and scarce, must be constantly educated on new techniques and problems (continual education). Similarly, some of the devices employed in administration are not only expensive but also highly advanced, and in some instances possess inherent or generated problems too difficult to identify with 100% assurance with even the best quality control techniques. The actively engaged personnel or administrator must able to identify real and potential dangers associated with such delivery systems.

General hazards or complications are at risk of occurring regardless of the agent or class of drugs being administered, whereas specific hazards or complications are unique or peculiar to certain agents and methods of administration. An important fact to remember about all parenteral injections is that if a reaction or adverse side effect of any sort occurs, it is usually impossible to retrieve or locally neutralize the offending agent, whereas with oral agents, recovery or expulsion of the medication is possible.

ROUTES OF ADMINISTRATION

The major routes of parenteral administration are IV, subcutaneous (SC), and IM. These three routes satisfy to a large extent the four principal reasons for administering parenterals: (1) for therapy (definitive or palliative), (2) for prevention, (3) for diagnosis, and (4) for temporarily altering tissue function(s) to facilitate other forms of therapy. Besides these three primary routes, additional ones are utilized under special circumstances: for example, intrathecal, subconjunctival, intraocular, intrathecal, intra-articular, and so on. A comprehensive description of the most commonly used routes of administration is discussed in the following section.

Intravenous Route

Injections or infusions directly into a vein are termed as IV administration (Fig. 1). Such administrations of true solution drug products is considered to be 100%. Drug absorption and factors concerning absorption are circumvented by IV injection of drugs in aqueous solution. At the desired concentration of a drug in the blood an accurate and immediate action is obtained that is not always possible by other procedures. It is of the most common parenteral routes employed in hospitals for drugs, fluids, and/or electrolytes. It offers a convenient route for rapidly infusing large volumes of fluid. If the dose is administered over a few minutes, it is

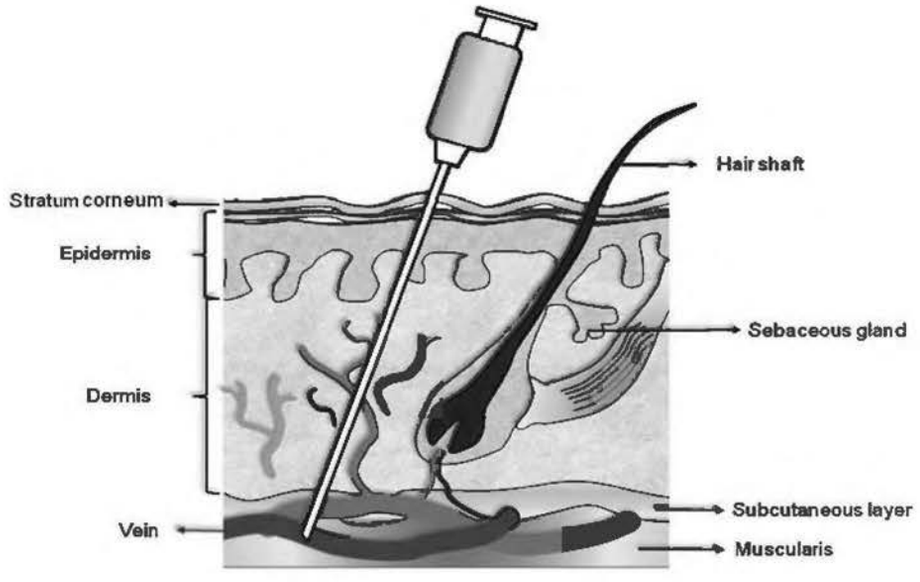


Figure 1 Schematic representation of an intravenous administration.

called a bolus dose and is primarily administered by a syringe directly into the vein. If the drug product is administered over hours from an infusion bag, it is termed an IV drip or infusion. Unfavorable reactions are prone to occur, since high concentrations of drug may be attained rapidly in both plasma and tissues. Repeated IV injections are dependent on the ability to maintain a patent vein. For prolonged IV use, flexible plastic catheters are better than sharp metal needles that may puncture through the other side of the vein.

Examples of drugs that are commonly administered by the IV route are analgesics, general anesthetics, antiviral agents, antibiotics, immunosuppressive agents, antifungal agents, antibacterial agents, antihypertensive agents, vasodilators, antiarrhythmic drugs, and chemotherapeutic agents. The preferred route for strong analgesics is a continuous IV infusion, because it produces less fluctuation in serum concentrations of the drug than do intermittent IM injections. Today, many IV drips are made in the pharmacy or by a special team rather than bedside preparations to insure accuracy of the drug product being administered.

The most common indication for use of this route are: (1) to guarantee delivery and distribution when hypotension or shock exists; (2) to restore rapidly electrolyte and fluid balance; (3) to achieve an immediate pharmacological effect, especially in emergencies, such as the treatment of certain arrhythmias or of seizures; (4) to treat serious, life threatening infections or conditions; (5) to provide continuous nutrition (hyperalimentation) when patients are unable to be fed by mouth; and (6) to avoid complications which might result if other administration routes are employed (e.g., hematomas at the site of IM injections in a patient with a bleeding diathesis). In addition, the IV route may be used for a variety of other purposes, such as plasmapheresis, blood transfusion, and hemodynamic monitoring, among others. Patient-controlled analgesia (PCA) is another unique mode of IV administration and is designed to deliver IV bolus doses in addition to a slow, continuous IV by this route for narcotic analgesics such as fentanyl, methadone, and morphine (6). Programmable infusion pumps with limited patient controls are often used for this type of administration and only allow the patient to receive an additional dose within limited time periods (7).

The IV route is not without adverse effects. Generally IV injections are administered directly into the venous circulation, and hence highly vascular and perfused organs, such as the heart, lungs, liver, and kidney, rapidly acquire the drug. However, a sudden increase in serum drug concentration may lead to toxicity and adversely affect the vital organs. This can be prevented by giving a slow IV bolus injection or controlling an IV drip. Some drugs with poor aqueous solubility may precipitate from solution and produce an embolism, for example, phenytoin IV injection. Hence, in such instances, it is important that proper selection of the diluent and slow IV administration be carried out; the latter allows for proper mixing of the drug into the circulation. Some vehicles may cause adverse effects in pediatric patients. For example, phenobarbital sodium when dissolved in propylene glycol may cause hyperosmolality in infants. In addition, because the alcohol and aldehyde dehydrogenase pathway that metabolizes propylene glycol is not well developed in infants and children younger than four years, repeated use of IV injections containing propylene glycol can lead to toxicity (8). Some lipid-soluble drugs, like diazepam, can cross the BBB and are effective when given by the IV route. Thus, lipid-soluble drugs, especially central nervous system (CNS) active drug, for example, sedatives, depressants, etc., often need to be administered by specialized routes of delivery that bypass the BBB. Other complications that may occur using the IV route are as follows: (1) thrombosis with or without complicating infection at the site of injection or infusion; (2) injection of microorganisms, toxins, particulate matter, or air; (3) the occurrence of physical or chemical incompatibilities between agents prior to or at the time of injection; (4) uncontrolled or excessive administration of drugs or fluids; and (5) extravasation of injections or infusions at the site of administration. When indwelling catheters are utilized, rarely the catheter tip may break off and lodge in a major vessel, in the heart, or in the lung and can cause fatalities.

To administer drugs through the IV route the upper extremities are chosen whenever possible for the site of injection or infusion. The most peripheral veins (e.g., over the hand) are selected for initial use. When arm sites are no longer available, the leg veins (femoral and saphenous) or dorsal foot veins may be utilized; and in small children the scalp veins. A recent improved in locating veins in pediatric and geriatric population is being used in clinical trials and is based on noninvasive infrared technology (Fig. 2). This unique device captures a near

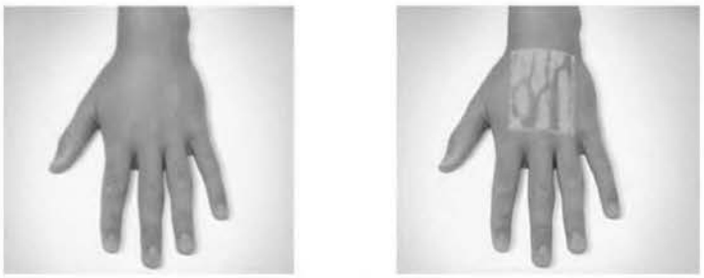


Figure 2 Visualization of veins using the proprietary VeinViewer® instrument form LuminetX, LLC.

infrared vein image, processes it, and projects it onto the skin using green light thus aiding phlebotomy (9). Selection of a vein depends on the size of the needle intended for use, type of fluids to be infused, flow rate anticipated, volume to be received, concomitant medications to be given, degree of patient mobility desired, and of course the skill of the person performing the venipuncture or catheterization. The veins in the antecubital fossa are among the most commonly chosen, because they are large and readily punctured. Other veins utilized commonly are basilic, cephalic, radial at the wrist, and the metacarpal and dorsal venous plexuses. Needles are generally preferred to indwelling IV catheters, as the risk of infection is believed to be less. Even after apparent exhaustion of all available venous sites, surgical cut downs of deep veins with insertion of catheters may be performed. When long-term, repeated usage is expected or when prolonged infusion is anticipated, the subclavian or internal jugular in the upper chest may be utilized. For peripheral veins and single or short-term usage, a 1 to 2 inch long, beveled, 18- to 22-gauge, stainless steel needle is commonly used.

For long-term and/or repeated IV administration, a sterile plastic catheter may be inserted into the vein percutaneously through or over the needle that was used for the initial puncture. The needle is then removed and the catheter is left in place. The indwelling needle or catheter, whichever is utilized, is anchored to the extremity or body by means of appropriate, sterile occlusive or nonocclusive dressings, often impregnated with an antibiotic ointment. Indwelling catheters may contain a heparin lock to ensure against clotting and loss of patency through venous thrombosis.

Intramuscular Route

An IM injection is defined as an injection directly into the body of a relaxed muscle (Fig. 3). The IM route is one of the most popular and convenient routes available, both for the administrator and for the patient, and a route of choice especially for pediatric subjects. Therefore, whenever it is possible and practicable, the IM route is used. The IM route provides a means for prolonged release of drugs formulated as aqueous or oily solutions or suspensions.

The IM route is preferred over the SC route when a rapid rate of absorption is desired for certain life threatening conditions. For example, administration of epinephrine via the IM route causes a higher peak plasma concentration compared with the SC route (10). However the rate of absorption is slow when compared with the IV route. One reason for using the IM route is because of the inability to administer the drug directly into the vascular compartment. Drugs commonly injected by IM administration include lidocaine, cephalosporins, aminoglycosides, diazepam, phenytoin, insoluble salts of penicillin G (procaine penicillin G), corticosteroids, narcotics, narcotic antagonists, and contraceptive steroids.

Although IM injections are much easier to administer than other injections, the main precaution is to avoid entering a blood vessel (especially an artery), which might lead to infusion of a toxic agent or a toxic vehicle directly to an organ or tissue. This can be prevented usually by pulling back on the plunger of the syringe; if blood does not appear, the needle is probably not in a vessel. Also, the accidental striking of or injection into a peripheral nerve may result in a peripheral nerve palsy with or without sensory damage. Occasionally, when a large bolus of drug is injected into the muscle, local damage or muscle infarction may result,

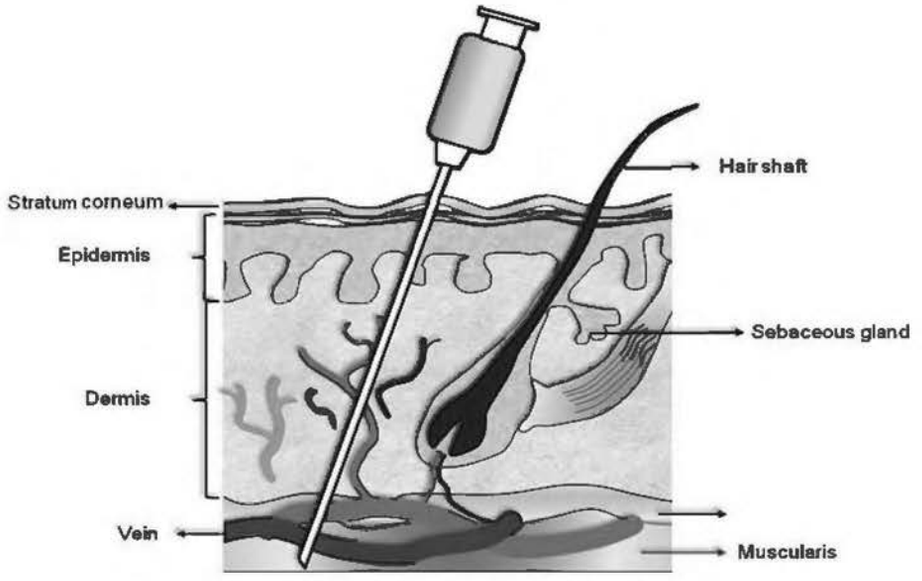


Figure 3 Schematic representation of an intramuscular administration.

leading to a sterile abscess or elevation of serum levels of muscle enzymes. The latter complication may present confusing diagnostic problems, especially in patients under suspicion of having a myocardial infarction or hepatitis.

If materials contaminated with microorganisms are injected, a septic abscess may result. Therefore, appropriate precautions must be taken to ensure sterility prior to injection. In patients with poor hygiene or skin care, microorganisms from the skin flora may be punched in by the needle at the time of injection, resulting in staphylococcal or streptococcal abscesses and rarely gas gangrene (11) or tetanus (12). An important note of caution: the IM route should never be employed in patients with significant heart failure or shock, where uptake into the vascular compartment may be expectantly poor. This caution should be followed especially if immediately high serum or plasma concentrations of the drug are desired or if rapid distribution to a distal organ is mandatory.

Various muscle sites are available for delivery, including the gluteal, deltoid, triceps, pectoral, and vastus lateralis muscles. In adults the site of choice often is the gluteal muscle, because large volumes of drug may be injected and tolerated. However, the vastus lateralis of the thigh may also be used because it not only tolerates large volumes of medication, but it is also away from any major vessels or nerves. For rapid absorption and small volumes (<2 mL), the deltoid muscle is preferred, as some studies suggest that blood flow in the deltoid muscle is 7% greater than that of the vastus lateralis and 17% greater than that of the gluteus maximus (4). In infants and small children, the vastus lateralis of the thigh is often preferred because it is better developed than other muscle groups.

With IM injections a beveled, 19- to 22-gauge, 1 to 2 inch long, stainless steel needle is used and no more than 5 mL of fluid is injected, depending on the site selected. The skin is first cleaned with alcohol or a suitable disinfectant, and the plunger on the syringe is always retracted prior to injection to be sure that the needle is not in a vessel. For deep IM injections, as might be used for irritating medications such as iron preparations, a "z-track" injection method is employed (4).

Subcutaneous Route

A SC injection (abbreviated as SC, SQ, sub-cu, sub-Q or subcut) is administered as a bolus into the subcutis, the layer of skin directly below the dermis and epidermis, collectively referred to

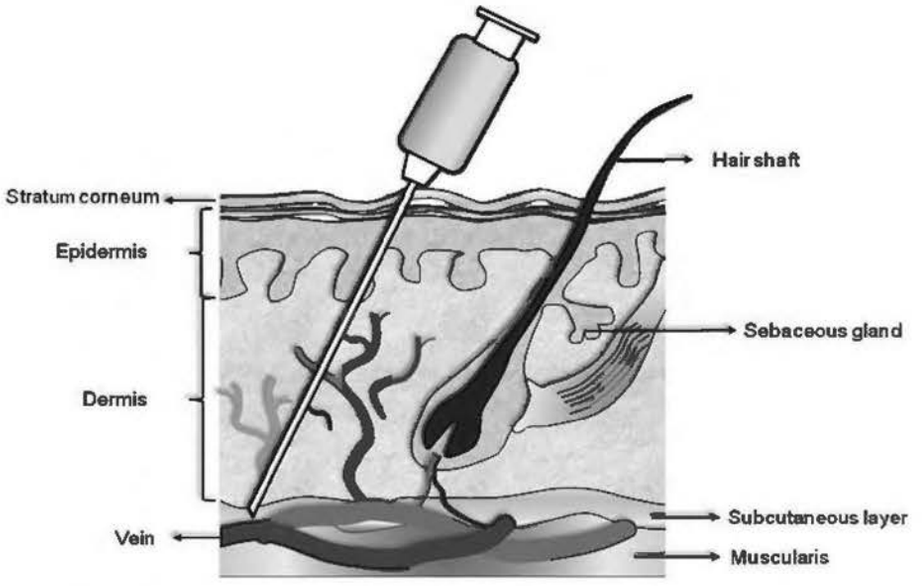


Figure 4 Schematic representation of a subcutaneous administration.

as the cutis (Fig. 4). SC injections are highly effective in administering vaccines and such medications as insulin, morphine, diacetylmorphine or goserelin. This route may be utilized if drugs cannot be administered orally because of lack of absorption from or inactivation by the contents of the gastrointestinal tract, if the patient is unable to ingest medications by mouth or if self-medication of parenterals (e.g., insulin) is desired. Drugs are more rapidly and more predictably absorbed by this route than by the oral route. However absorption of drugs via this route is slower and less predictable compared to the IM route and this effect can be attributed to the difference in vascularity of the muscle and dermis. Medications commonly administered subcutaneously include insulin, vaccines, narcotics, epinephrine, and vitamin B12. As with the IM route, if heart failure, shock, or vascular collapse exists, this route should not be depended on. Hypodermoclysis is a special form of SC administration, namely, the infusion of large amounts of fluid into the SC tissues when IV sites are not available. This form of administration is rarely (if ever) used today but in the recent past was a common mode of replenishment of fluid and electrolytes in infants and elderly patients.

Medications that are highly acidic, alkaline, or irritating, causing the production of pain, inflammation, and/or necrosis of tissues, should not be administered by this route. Infection, as with all parenteral injections, may occur, particularly in a patient with poor skin hygiene and particularly in situations where self-administration is practiced. Generally, a beveled, 24- to 25-gauge, 0.25 to 0.625 inch long, stainless steel needle is utilized. The volume injected generally does not exceed 0.5 to 1. 5 mL. Injection sites include the abdomen at the level of the umbilicus, the upper back, the upper arms, and the upper hip. The skin over the site of administration should be disinfected prior to injection with a sterile alcohol sponge. Prior to injection, aspiration should be attempted to be certain that the needle has not inadvertently entered a vessel. If blood does not appear in the syringe when the plunger is retracted, then the product is not injected.

It is advisable that the area of injection must be rotated for long-term therapies like administration of insulin or human growth hormone. Changing the injection site keeps lumps or small dents called lipodystrophies from forming in the skin. However, patients should try to use the same body area for injections that are given at the same time each day. Using the same body area for these routine injections lessens the possibility of changes in the timing and action of drugs like insulin.

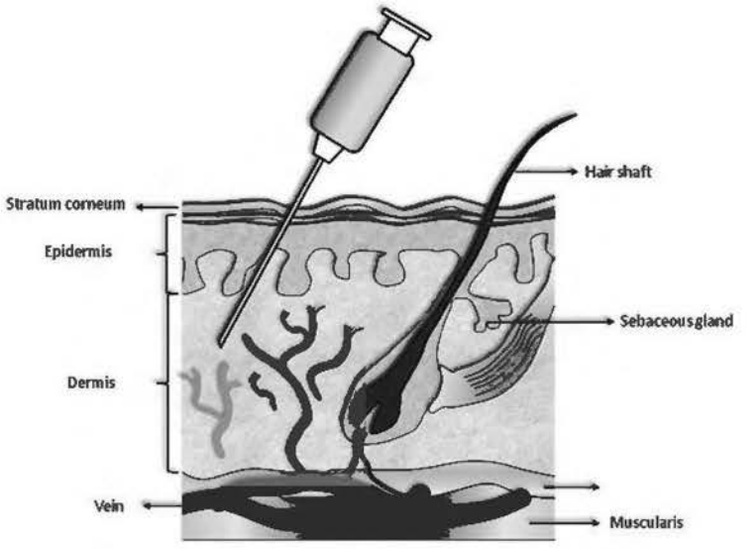


Figure 5 Schematic representation of an intradermal administration.

Intradermal Route

Injection into the dermis, located just beneath and adjacent to the epidermis is called an intradermal injection (Fig. 5). A number of diagnostic agents, antigens (e.g., tuberculin) and vaccines (e.g., smallpox) are administered by this route. The volume of fluid injected generally does not exceed 0.1 mL. Absorption by the intradermal route is very fast compared with the SC or IM route.

Generally a beveled, 26- or 30-gauge, 0.375 inch long, stainless steel needle is utilized. The skin at the site of administration should be cleaned prior to injection with 70% alcohol. Certainty of intradermal injection is evident by the appearance of a localized swelling of the skin, giving the appearance of an orange peel. The most common mistakes in intradermal injections are injecting beneath the skin rather than into it or permitting materials to leak out of the needle tip if it is not inserted completely into the skin.

Intra-arterial Route

The intra-arterial route is infrequently used route. Injection of a drug into an artery terminates in a target area, which may be an organ. Almost every artery is approachable by arterial catheterization and none are inaccessible to the skilled surgeon or radiologist.

The nature of the drug and the physiology of the circulatory system require IV injection to be diluted in the blood rather than going directly to an organ or tissue where the effects will be localized. The intra-arterial route is employed generally for diagnostic purposes, such as injecting radiopaque substances for roentgenographic studies of the vascular supply of various organs or tissues (e.g., coronary, cerebral, pulmonary, renal, enteric, or peripheral arteries). The usual reason for using the intra-arterial route is to introduce radiopaque materials for diagnostic purposes, such as for arteriograms. This route can be extremely hazardous, because products administered intra-arterially are not adequately diluted nor are they filtered by the lungs, liver, or kidneys before contact with peripheral tissue/s or vital organs nourished by the artery. Products contaminated with microorganisms, endotoxin, and/or particulate matter may result in serious complications or reactions, such as infection (either intra-arterial or extra-arterial) or arterial thromboembolism or vasospasm. This may result in ischemia, infarction, or gangrene of the tissues or organs supplied. In addition, if the technique of entry is faulty, damage to the arterial intima and vessel wall may occur resulting in serious hemorrhagic

extravagation or a dissecting aneurysm. If air is infused accidentally, air embolism with consequent ischemia and/or infarction of the tissue may occur; an event which usually does not occur when small amounts of air are infused into the venous system.

Usage of the intra-arterial route for treatment purposes is infrequent and limited generally to organ-specific chemotherapy, such as treating certain localized cancers (e.g., malignant melanomas of the lower extremities), where regional perfusion with high concentrations of toxic drugs (which when given intravenously may be associated with serious systemic reactions) can be achieved. Arterial spasm and subsequent gangrene present problems that make the intra-arterial route hazardous.

Either a suitably sized, smooth-bore, stainless steel needle or a short, flexible, plastic catheter is surgically inserted into the desired artery or a lengthy catheter is guided over a stylet or needle through a percutaneous entry site (sometimes under fluoroscopy) until the desired artery, organ, or tissue is reached; or the skin over the artery may be punctured directly, and the needle then inserted into the artery. Also, an open operative incision through the skin may be made (a "cut-down"), by which the artery is surgically exposed and under direct visualization is entered; a catheter is then inserted into the artery and sewn in place. Regardless of the method used, strict aseptic technique is practiced and appropriate occlusive or nonocclusive dressings are employed.

Intracisternal Route

Administration of drug products directly into the cisternal space surrounding the base of the brain is called as intracisternal injection. This route is employed mainly for diagnostic purposes. Additionally this route is used to decrease elevated intracranial pressures and reduce the risk of herniation of the brain if fluid is removed from the lumbar sac. Diseases involving the cisterns generally extend to nearby, contiguous structures are treated by utilizing the intraventricular route. Rarely, in order to locate and define a particular disease process; especially a spinal tumor or abscess, various contrast materials are injected into the cisterns. Intrathecal or intracisteral injections do not result in distribution of the drug into the ventricular space; thus disease within the ventricles would not be treated by these routes.

Many of the precautions concerning the use of the intraventricular route are applicable to the use of the intracisternal route, particularly as regards to aseptic practices and the threat of physicochemical irritation of the substances injected. One very serious drawback to the use of this route is the danger of producing permanent, serious, neurological injury or death due to possible damage to the midbrain. The space entered is relatively small, and insertion of a needle into it should be attempted only when other routes may not be used and only by the most experienced personnel. For intracisternal puncture the patient is placed in a head-down position and the entry approach is posterior between the occiput and the first cervical vertebrae. The cisterna magnum is punctured and extreme care is exercised to continue aspirating with a syringe while inserting the needle.

Intraventricular Route

Here the drug product is injected or infused directly into the lateral ventricles of the brain. This route is employed mainly in the treatment of infections (such as bacterial or fungal meningitis and/or ventriculitis) or of malignancies (such as leukemic infiltrates of the meninges or carcinomatoses) involving the membranes and cerebrospinal fluid surrounding the CNS. It is used especially in situations where the drugs involved are known to diffuse or pass poorly from the vascular compartment into the ventricles and subarachnoid space and/or where reduction of systemic side effects from a particular agent are desired. One such example is the treatment of fungal meningitis with amphotericin B (13) or in the therapy of leukemic infiltrates with methotrexate (14). Often, therapy via this route is complemented by the IV administration of the same agent which has been injected into the ventricles.

In the treatment of diseases of these areas, the intraventricular route often is preferred over the intracisternal or intrathecal. This is because the flow of cerebrospinal fluid is unidirectional and originates principally in the choroid plexus of the lateral ventricles and pursues a path through the third and fourth ventricles out the foramina of Luschka and

Magendie into the posterior fossa at the level of the pons, down over the spinal cord, and then finally reversing itself to flow up over the cerebral hemispheres. In addition, the ventricle provides a large fluid space in which to inject drugs, thereby diluting such drugs in a large volume of cerebrospinal fluid, thus minimizing potential, localized physicochemical irritation to the cells lining the ventricle and subsequent damage from a host reaction. In addition, if intracranial pressures are excessive, the risk of brain stem herniation may be avoided, a known risk factor for intracisternal route. Radiopaque tracers, radiolabeled, or dyes may be injected into the intraventricular space for studies of either the anatomy or patency of the system or for studies of the flow of cerebrospinal fluid.

Since cerebrospinal fluid bathes such critical organs as the brain and spinal cord and since one of its functions is believed to be a protective or cushioning fluid for these organs, any disturbance of this fluid or the membranes containing it may be deleterious and possibly lethal. Any foreign material, chemical or biological, when injected into the system may precipitate an inflammatory response anywhere or everywhere within the system. Strict aseptic techniques should be adhered to when entering the ventricles to prevent iatrogenic infections, and care should be exercised to be certain that the substances injected or infused are not irritating to the cells lining the ventricular or subarachnoid spaces. If irritating drugs are injected, ventriculitis or myelitis may result (sometimes progressive), producing obstruction of the system (hydrocephalus) or permanent neurological injury.

The vehicles employed for intraventricular injection should have physical characteristics as close to the cerebrospinal fluid as possible. If the ventricles are small or almost closed because of intracerebral edema, these spaces may be difficult to locate, and undesirable intracerebral injection of the drug with subsequent neurological injury may result. In addition, hemorrhages in the subdural, epidural, intraventricular, or intracerebral regions may occur. If the ventricular needle is inserted too far, passing through the ventricles, damage to the basal ganglia, thalamus, or other vital structures may occur. The procedure should be carried out only by experienced personnel.

To administer drug products via this route a 3.5 inch long, smooth-bore, 18-gauge, stainless steel, blunt-ended ventricular needle is used. The patient's skin is prepared as in any surgical procedure, taking extreme care to maintain strict aseptic technique. A twist drill puncture of the cranium is first performed, generally over the coronal suture about 2 cm from the midline and in line with the ipsilateral pupil. The needle, which is a special blunt, open-ended needle, is passed through the frontal lobe into the lateral ventricle. When repeated injections or infusions are required, use of an Ommaya (15) or Rickam (16) reservoir or similar silicone, elastomer, SC reservoir is recommended. Surgical placement of the reservoir may be accomplished in a variety of ways. Often with these devices no local anesthetic is required for reinjection, and the system may be sampled and injected repeatedly with minimum disturbance to the patient and with reduced risk of infection.

Intrathecal Route

Intrathecal (Latin *intra* "inside," Greek *theka* "capsule," "hull") is an adjective that refers to events that happen inside the spinal canal. An intrathecal injection (often simply called "intrathecal") is an injection into the spinal canal (intrathecal space surrounding the spinal cord), as in a spinal anesthesia or in chemotherapy or pain management applications (Fig. 6). This route is also used for some infections, particularly postneurosurgical. Drugs given intrathecally often have to be made up specially by a pharmacist or technician because they cannot contain any preservative or other potentially harmful inactive ingredients that are sometimes found in standard injectable drug preparations.

This route is a very popular for a single 24-hour dose of analgesia (opioid with local anesthetic). However extreme control had to be employed during dosing as most narcotic pain medications can cause a late onset respiratory depression when administered through this route. Often reserved for spastic cerebral palsy, intrathecally-administered baclofen is done through a intrathecal pump implanted just below the skin of the stomach with a tube connected directly to the base of the spine, where it bathes the appropriate nerves using low dose baclofen (17). Intrathecal baclofen also carries none of the side effects, such as sedation, that typically occur with oral baclofen. It is the preferred route for long-term management of

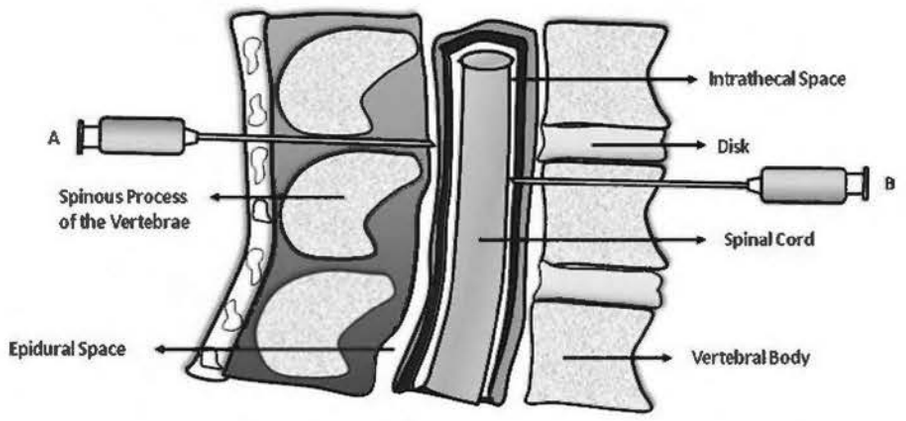


Figure 6 Schematic representation of an intrathecal administration (A) epidural route; (B) intrathecal route.

spasticity in people with cerebral palsy for whom other procedures, such as rhizotomy or orthopedic surgery, are inappropriate.

The same precautions required for intraventricular administration apply to use of the intrathecal route. In addition, a real threat of tonsilar or brain stem herniation (and possibly death) exists if this procedure is performed while intracranial pressure is elevated. Great care must be exercised to avoid this complication, which usually occurs one to two hours or sooner after removal of fluid. To administer via this route a 3.5 inch long, smooth-bore, beveled, 20- to 22-gauge stainless steel spinal needle is used for adults. The patient's skin is prepared as in any surgical procedure, taking the greatest caution to use aseptic technique. The needle is inserted posteriorly at the midline into any space below the third lumbar spinal process. The patient is in the lateral decubitus position with head, back, and thighs flexed. If intracranial pressure is diffusely elevated, the special precautions outlined above should be taken, but if intracranial masses are suspected, this procedure should not be done.

Epidural Route

The epidural space (or extradural space or peridural space) is a part of the human spine. It is the space inside the bony spinal canal but outside the membrane called the dura mater (Fig. 5). In contact with the inner surface of the dura is another membrane called the arachnoid matter. The arachnoid encompasses the cerebrospinal fluid that surrounds the spinal cord. The term epidural is often synonymous with epidural anesthesia, is a form of regional anesthesia involving injection of drugs through a catheter placed into the epidural space. The injection can cause both a loss of sensation and analgesia, by blocking the transmission of signals through nerves in or near the spinal cord.

Injecting medication into the epidural space is primarily performed for analgesia (18). This may be performed using a number of different techniques and for a variety of reasons. A patient receiving an epidural for pain relief typically receives a combination of local anesthetics and opioids (19). This combination works better than either type of drug used alone. Common local anesthetics include lidocaine, bupivacaine, ropivacaine, and chloroprocaine. Common opioids include morphine, fentanyl, sufentanil, and meperidine in the United States. These are injected in relatively small doses. Occasionally, other agents may be used, such as clonidine or ketamine.

When a catheter is placed into the epidural space, a continuous infusion can be maintained for several days, if needed. Epidural analgesia may be used for the following: (*i*) Analgesia alone especially where surgery is not contemplated. An epidural for pain relief (e.g., in childbirth) is unlikely to cause loss of muscle power, but is not usually sufficient for

surgery. (*ii*) An adjunct to general anesthesia. The anesthetist may use epidural analgesia in addition to general anesthesia. This may reduce the patient's requirement for opioid analgesics. This is suitable for a wide variety of surgery, for example, gynecological surgery (e.g., hysterectomy), orthopedic surgery (e.g., hip replacement), general surgery (e.g., laparotomy) and vascular surgery (e.g., open aortic aneurysm repair). (*iii*) As a sole technique for surgical anesthesia. Some operations, most frequently cesarean section, may be performed using an epidural anesthetic as the sole technique. Typically the patient would remain awake during the operation. The dose required for anesthesia is much higher than that required for analgesia. (*iv*) For postoperative analgesia, in either of the two situations above. Analgesics are given into the epidural space for a few days after surgery, provided a catheter has been inserted. Through the use of a patient-controlled epidural analgesia (PCEA) infusion pump (20), a patient may be given the ability to control postsurgical pain medications administered through the epidural. (*v*) For the treatment of back pain. Injection of analgesics and steroids into the epidural space may improve some forms of back pain. (*vi*) For the treatment of chronic pain or palliation of symptoms in terminal care, usually in the short or medium term. The epidural space is more difficult and risky to access as one ascends the spine, so epidural techniques are most suitable for analgesia for the chest, abdomen, pelvis or legs. They are much less suitable for analgesia for the neck, or arms and are not possible for the head.

There are certain instances where the risks of an epidural are higher than normal. Anatomical abnormalities, such as spina bifida, meningomyelocele or scoliosis could be a major limiting factor for using this route. If the patient has previous history of spinal surgery, which can lead to scar tissue, can potentially cause disruption in the distribution of the medication. Use of this route is not recommended for patient suffering from certain CNS disorders like multiple sclerosis. Certain heart-valve problems such as aortic stenosis, where the vasodilation induced by the anesthetic may impair blood supply to the thickened heart muscle, may be fatal.

A particular type of needle known as a *Tuohy* needle is used. This needle is specially designed for locating the epidural space safely, and has several specific features. The needle is inserted to the ligamentum flavum and a loss of resistance to injection technique is used to identify the epidural space. This technique works because the ligamentum flavum is extremely dense, and injection into it is almost impossible. The anesthesiologist attaches a syringe to the Tuohy needle and advances it slowly. The syringe may contain air or saline. The principles are the same, but the specifics of the technique are different because of the greater compressibility of air with respect to saline. When the tip of the needle enters a space of negative or neutral pressure (such as the epidural space), there occurs a "loss of resistance" and is possible to inject through the syringe (21).

Traditionally anesthesiologists have used either air or saline for identifying the epidural space, depending on their personal preference. However, evidence is accumulating that saline may result in more rapid and satisfactory quality of analgesia (22,23). In addition to the loss of resistance technique, real-time observation of the advancing needle is becoming more common. This may be done using a portable ultrasound scanner, fluoroscopy or real-time X-ray (1).

Intra-articular Route

Injection or infusion into the synovial sacs of accessible joints is termed as an intra-articular injection (Fig. 7). Antibiotics, lidocaine, and antiinlammatory drugs, like corticosteroid, may be administered into joints for the treatment of infections, pain, inflammation, or other problems resulting from inflammatory diseases (e.g., rheumatoid arthritis or trauma). Some agents are administered in single injections and some (e.g., antibiotics) via continuous infusion and "bathing" of the joint.

Intra-articular injections are easily accomplished in the knee, ankle, wrist, elbow, shoulder, phalangeal, sternoclavicular, and acromioclavicular joints. Joints deformed by any disease process (e.g., rheumatoid arthritis or trauma) may be more difficult to enter and inject. Usually, the intra-articular approach is utilized when no more than one or two joints are involved. Often it supplements systemic therapy since; when the synovium is inflamed it is

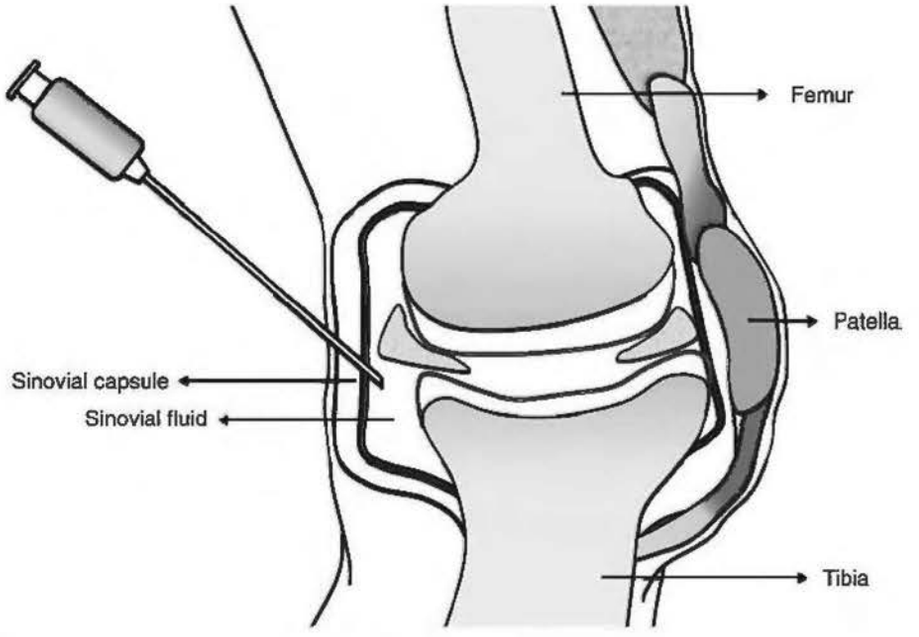


Figure 7 Schematic representation of an intra articular administration.

often highly vascularized, permitting a multitude of agents to enter with ease from the intravascular compartment.

Iatrogenic infection is always a threat following intra-articular injection. The consequences of such infection may result in destruction of the joint. Administration of corticosteroids is particularly troublesome because if serious infection does occur, recognition may be delayed because of suppression of the local inflammatory response; thus destruction of the joint and the cartilage may occur before the identification of a complicating infection. Severe, recurrent, intra-articular hemorrhage may be produced if a bleeding diathesis, such as hemophilia or severe hypoprothrombinemia, is present. Ordinarily, such blood is resorbed, but with recurrent hemorrhage eventual destruction of weight-bearing joints may occur. If the therapist is inexperienced, tendons may be ruptured if appropriate administration technique is not employed.

The anatomy of the joint to be treated should be studied by X-ray or imaging techniques prior to injection. Entry should be at the point where the synovial cavity is most superficial and free of large vessels and nerves. The site of skin entry is cleaned and prepared as with any surgical procedure; strict aseptic technique is mandatory. A sterile, 19- to 22-gauge, stainless steel needle attached to a syringe is inserted into the synovial cavity. The synovial fluid should be first aspirated to ensure that the needle is within the joint space. The syringe is changed, and one containing the drugs to be injected is attached and administered.

Intra-abdominal Route

This route is also known as the intraperitoneal route. An injection or infusion directly into the peritoneal cavity via a needle or indwelling catheter or directly into an abdominal organ, such as the liver, kidney, or bladder is defined as a intra-abdominal injection. The intra-abdominal route may be employed to treat local or widespread intra-abdominal disease due to microbial infection or tumor. The route is also employed to dialyze (peritoneal dialysis) various toxic substances from the abdomen when severe renal failure prohibits excretion. Another

application of this route is to determine the patency, as well as the structure, of various vascular or lymphatic systems employing radio opaque agents.

The intra-abdominal route of administration can cause serious abdominal infection (peritonitis) and hemorrhage. The source of infection may be extrinsic (e.g., from skin or contaminated drugs or infusates) or intrinsic (e.g., from puncture of the bowel). The risk of infection is enhanced if an indwelling catheter, rather than a single injection using a sterile needle, is utilized. Such infections are particularly difficult to treat, especially in the presence of ascites; thus every precaution should be taken to prevent them. In addition, an aseptic peritonitis may be induced if the agent or fluid injected is highly irritable or contains endotoxin. The chance of inducing hemorrhage is related generally to the size of the needle employed, the anatomical site selected for injection, the skill of the technician, and any tendencies of the patient to bleed (i. e., coagulation problems). If hemorrhage is induced, it may be difficult to control and may require surgical intervention and repair.

Drugs injected into the intraperitoneal space are usually absorbed into the vascular compartment, and under certain pathological conditions this can be unpredictable. This can result in an uncontrolled risk of toxicity or therapeutic failure. To administer a drug intraperitonially, suitable aseptic preparation of the skin should be carried out. A 16- or 18-gauge, stainless steel needle is then inserted through the anterior abdominal wall just lateral to the rectus muscles. If ascites is present, there is little risk of bowel puncture; however, if the peritoneal cavity is "dry," puncture of the bowel may occur (indicated by aspiration of fecal contents). Bowel puncture may be avoided by shallow punctures and withdrawing on the plunger while advancing the needle.

Intracardiac Route

An injection directly into chambers of the heart or the cardiac muscle is called as an intracardiac injection. The use of this route is not common for delivery of drugs. Nevertheless, under unusual circumstances and in certain emergency situations, such as cardiac arrest, in which drugs may have to reach the myocardium immediately, intracardiac injections may be employed.

One of the major risk factors is the damage inflicted on the heart muscle, coronary arteries, or the conducting system due to trauma of an injecting needle or by the drug injected. Occasionally, hemorrhage into the myocardium or pericardium may result, leading to infarction or pericardial tamponade. If extracardiac structures such as the lung are inadvertently punctured, a pneumothorax may result and breathing may be impaired.

Selection of the route may be influenced by the presence of left or right ventricular hypertrophy, the former being better suited for the anterolateral approach and the latter being better suited for the medial approach, or any anatomical derangements of the chest which may exist. Generally, a beveled, 18- to 21-gauge, 4 to 6 inch long, stainless steel needle is used.

Intraocular Route

Injection of drug products directly into the various chambers of the eye is collectively termed as intraocular injection (Fig. 8). Four types of intraocular injections are utilized. These include (*i*) anterior chamber: injection or irrigation directly into the anterior chamber of the eye; (*ii*) intravitreal: injection directly into the vitreous cavity of the eye; (*iii*) retrobulbar: injection around the posterior segment of the globe; and (*iv*) subconjunctival (4). Although included under this heading, subconjunctival (and retrobulbar) injections are not intraocular (Fig. 9). Instead, such injections are adminstered beneath the conjunctiva, so that medication diffuses through the limbus and sclera into the eye. This route is generally used in the treatment of infections and inflammatory diseases of the eye which are not treated effectively by topical or systemic drug administration for anesthesia of the globe (retrobulbar) and occasionally for pupillary dilation with cycloplegics and mydriatics. Absorption of drugs into the eye is challenging, as intraocular transport and diffusion are poor. Intraocular injections are complemented frequently by IV infusions of the therapeutic drugs employed. Selection of the type of intraocular injection depends on the disease present and the precise location of that disease within the eye.

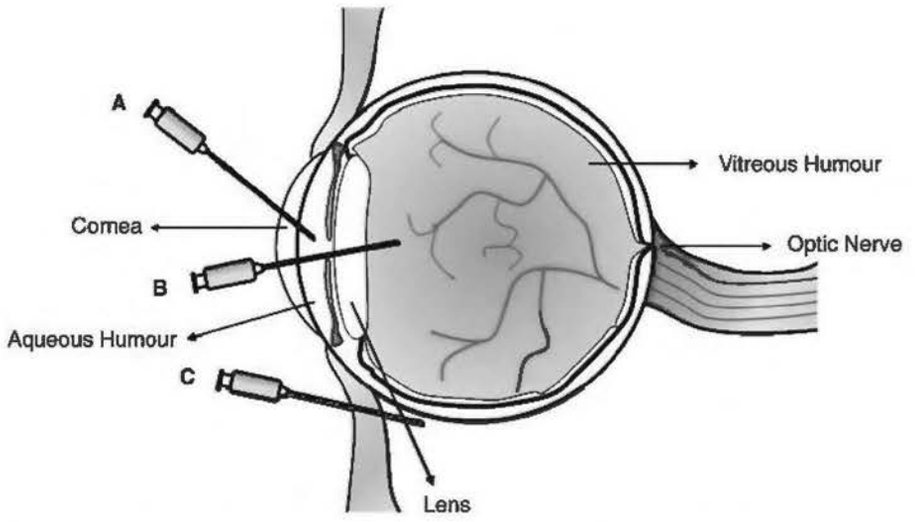


Figure 8 Schematic representation of an intraocular administration: (**A**) anterior chamber injection, (**B**) intravitreal injection, and (**C**) retrobulbar injection.

Extreme care and precise technique are required to minimize or prevent damage to the eye, especially to the corneal endothelium. Complications that can occur, depending on the route selected, are optic nerve damage, hemorrhage, retinal detachment, retinal necrosis, cataracts, and injection of the drug directly into the circulation with consequent systemic effects. Infection is always a threat and must be avoided as such infections may result in rapid destruction of the eye and/or blindness. The volume of solution that may be injected into the eye is severely restricted, generally to not more than 0.1 to 0.2 mL. Since an excellent knowledge of the anatomy and function of the eye is required, only an ophthalmologist should attempt these procedures.

The anterior chamber (containing the aqueous humor) is entered at a point located on the edge of the cornea (the limbus) with a 25-gauge or smaller, stainless steel needle, withdrawing a volume of fluid prior to injection equal to that to be instilled. For intraocular injections excluding the anterior chamber, a drop of 1:100,000 dilution epinephrine may be placed on the iris to dilate the pupil. Great care must be taken not to inject or damage the lens, as this may result in cataract formation.

Entry into the vitreous humor is accomplished by injection through the pars plana (junction of retina and ciliary body) with a 25-gauge stainless steel needle. The vitreous appears to be an inert fluid which is not replaced once removed. During injection, great care must be taken not to detach the retina. Again, a volume of fluid equal to that to be injected must be removed before instillation. Generally, not more than 0.1 mL may be injected. Injection of steroids into this chamber can be dangerous, resulting in destruction of the retina (retinal necrosis).

Entering the retrobulbar space involves insertion of the needle at the junction of the lateral and medial third of the orbital rim and then advancing the needle toward the apex of the orbit. Care must be taken not to inject the optic nerve directly. A 1 to 0.5 inch long, 25-gauge stainless steel needle is generally employed. Subconjunctival injections generally do not exceed volumes of 0.5 mL. This route is especially used in treating corneal abscesses. Injection of the sub-Tenon fascia is utilized for the treatment of uveitis (e.g., secondary to localized sarcoidosis) or chronic cyclitis. Again, care must be taken not to inject or nick the orbit.

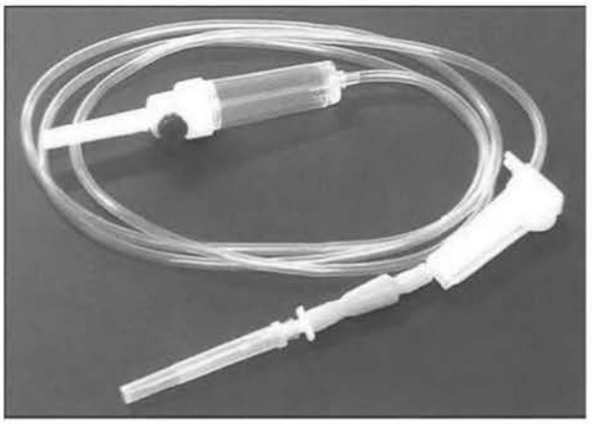


Figure 9 Picture of a standard infusion set indicating its components: a piercing spike; a vent; a drop chamber; a connec tion tubing; a roller clamp; a luer fitting; and a protective cap on the spike.

PARENTERAL DRUG ADMINISTRATION: METHODS AND DEVICES

This section describes the factors which determine the necessity of exact dosage as well as those which affect the flow of the infusion. Various infusion techniques such as gravity infusion, positive pressure infusion as well as other highly specialized types of infusion equipment will be discussed. Related information about their function and areas of application will be provided.

GENERAL CONCEPTS

Venous or arterial administration of a liquid into the circulatory system requires an accurate dosage and the infusion technique employed determines the accuracy of the dosage. The required dosage accuracy is generally dependent on the patient's status as well as on the type and amount of fluid to be infused, and the infusion equipment used. The flow of the infusion is affected by a range of factors including resistance in the channel of the piercing spike; resistance in the tubing and in the connector pieces; speed of drop formation; variability of the delivery pressure; and physicochemical characteristics of the solution.

GRAVITY INFUSION

The technique is the most frequently used one comprising of more than 80% of all infusions performed. The accuracy of the dosage and the infusion rate requirements are low for this type of infusion (±50%). The volume administered is based on the hydrostatic pressure differential between the patient and the infusion container. The rate of fluid administration can only be accelerated through compression of the container or by increasing the internal pressure of the container. Over the years, a standardised infusion set (Fig. 9) has been developed. Components used for this type of infusion are; a piercing spike; a vent; a drop chamber; a connection tubing; a roller clamp; a luer fitting; and a protective cap on the spike.

Depending on the type of container to be used with, the piercing spike is sharp for rubber stoppers or rounded and blunt for bag insertion sites. The infusion bag contains one channel for fluid and optionally a second channel for venting with a cap or stopper. Upon opening of a cap or stopper air flows into the container. The vent usually is equipped with a bacterial filter. A drop generator is located at the top of the drop chamber, which produces drops of a certain size. The chamber is partially filled with liquid to prevent air bubbles from entering the tubing. A particle filter is often located at the bottom outlet of the chamber. The connecting tube is usually 150 cm long and made of PVC. These are also available in other lengths and materials for special applications. The roller clamp supplied within the connecting tube is used to

regulate the flow rate of infusion by controlled compression of the tubing. The Luer fittings at the end of the line, guarantees a secure connection to all other products by means of the standardized Luer cone. In the lock version the lock connection is further secured against jerks and pressure by means of a screw thread. This prevents damage to the packaging and thus loss of sterility. The standardised infusion set is connected to a infusion container (bottle, bag) using the spike.

The rate of the infusion is a critical factor for gravity infusion and is mainly regulated by means of the roller clamp in most of the hospital settings. The roller clamp is positioned on the infusion tubing of the infusion set in such a way that the lumen of the infusion tubing is compressed from outside. With respect to gravity infusion the rate of infusion is calculated on the basis of number of drops/min. Most standard infusion sets are designed to deliver approximately 20 drops/min (equivalent to 1 mL/min). Specialized roller clamps are available that allow for drop rates of 60 drops/min. However, even with higher drop rates, the microdroppers (e.g., Dosifix® from B. Braun) still delivers only 1 mL/min; that is, 60 drops = 1 mL/min.

Another type of flow regulator is the tubing independent flow regulators that can replace the traditional roller clamp for improved control of dosage accuracy. The flow rate is controlled by varying the size of an accurately designed flow channel and flow rates can range from 3 200 mL/hr. These units are used for infusion solutions which are carrier solutions for drugs that need to be administered at a specific concentration for longer duration. It is important to note that an ideal flow regulator is the one that can maintain the desired flow rate irrespective of changes in the infusion height and patient activities.

PRESSURE INFUSION

In certain instances during IV administrations using infusion or transfusion bags, a pressure infusion may be performed. For this purpose a pressure cuff is used which is pumped up with an inflation bulb in a similar manner as with a blood pressure measurement instrument, thus exerting pressure on the container. A pressure of up to a maximum of 300 mmHg can be exerted on a regular infusion bag. Other types of positive pressure infusion equipments are available and employed for such infusions. They are especially used when the dosage accuracy is required or increased rate of infusion is needed or when a constant rate of delivery during long-term infusions is desired. The infusion equipment used should meet certain and the important criteria: (*i*) requirement-based infusion rate, (*ii*) exact dosage, (*iii*) robustness of equipment, (*iv*) quick functional readiness, (*v*) simple and safe operation, (*vi*) alarms for interruption of infusion or in the event of danger, (*vii*) mains-independent operation, and (*viii*) easy cleaning.

Depending of different applications and administrations to be performed, the required infusion rates extend over a wide range. Pressured infusion rates may vary from 1 mL/hr and > 1000 mL/hr (e.g., shock therapy) for adult patients. Such a type of infusion is generally used in an intensive care medicine scenario. Cost of equipment for pressured infusion can also be a limiting factor for many settings. The degree of accuracy of dosage depends on the status of the patient, the solution to be infused and other factors. Also, the degree of accuracy a dosage can have is determined by the kind of infusion technique that is employed.

With regard to these techniques, distinctions are made between gravity infusion, pressure infusion and the use of infusion equipment. Additional infusion equipment is required when the dosage accuracy should be increased, the rate of infusion should be raised or when a constant rate of delivery during long-term infusions should be achieved. In equipment-supported infusion techniques, distinctions are made between infusion regulators, that is, electronic medical devices without a delivery drive, infusion pumps and syringe pumps. In contrast to the infusion regulators, infusion pumps have their own delivery drives. Depending on the type of drive, there is a distinction between roller pumps, peristaltic pumps and plunger or syringe pumps. The accuracy of the dosage mainly depends on how the pumps are regulated. Syringe pumps are pressure infusion devices which administer the content of one or more syringes simultaneously using a precision linear drive. This form of infusion is particularly suited for an exact administration of drugs.

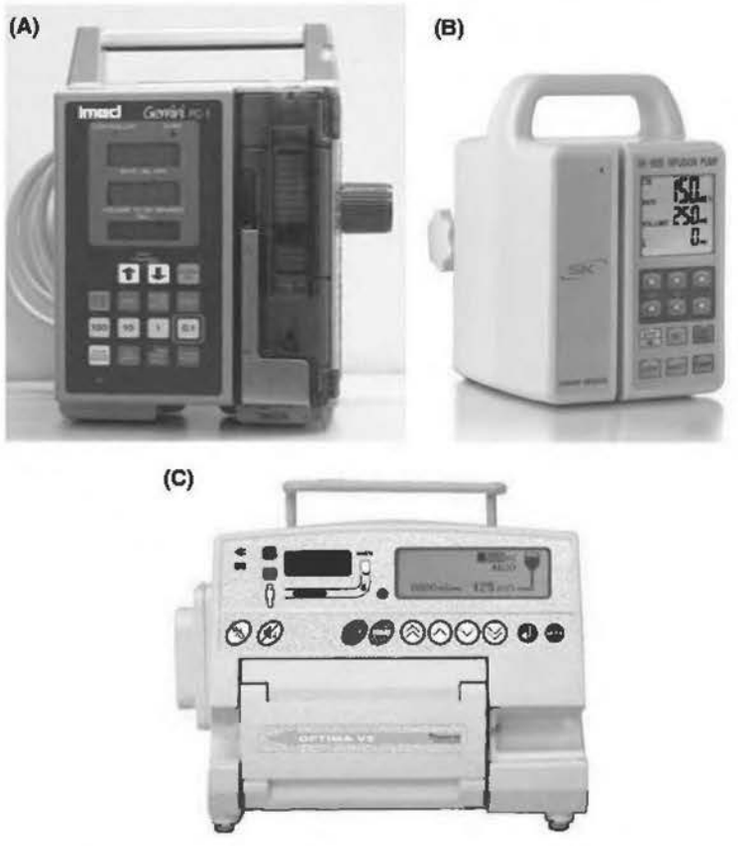


Figure 10 Examples of different types of infusion pumps: (**A**) a roller infusion pump, (**B**) a syringe driven pump, and (**C**) a peristaltic infusion pump.

Types of Equipment

Over the recent years significant advances have occurred in the area of pressure infusion or positive pressure infusion. Most of the infusion systems available in today's market are highly sophisticated, precise, and electronically advanced requiring specialized training. They can be broadly classified into three distinct classes: (*i*) infusion regulators, (*ii*) infusion pumps, and (*iii*) syringe pumps. Other infusion devices like the disposable infusion pumps, smart pumps, and associated accessories are regularly employed in different medical settings (Figure 10).

a. *Infusion regulators*: Infusion regulators are electronic medical devices which do not have their own delivery drive. They regulate and monitor the supply of fluid in the flow process. Simply stated, they are mechanized roller clamps. The dosage accuracy is often sufficient for everyday clinical purposes and ranges between ±10% and 20%.
b. *Infusion pumps*: In contrast to the regulators, infusion pumps are equipped with their own delivery drive. Depending on the type of drive, it can be classified as roller pumps, peristaltic pumps and piston pumps (Fig. 10). The main purpose of an infusion pump is to deliver medication(s) at a regulated rate and thereby in a regulated dose. Control of infusion pumps can either be drop based or volume based. The basic design of infusion pumps comprise of a delivery drive, a control or regulating system, and an infusion set. The dosage mainly depends on how the

pump is regulated. Roller pumps and peristaltic pumps are examples of volume-based pumps. The delivery principle of a roller pump is based on the rollers bringing a set amount of fluid into the tubing which is then transported by help of rotation in the flow direction. On the other hand, delivery principle of a peristaltic pump depend on the successive compression of the tubing by the individual fingers, makes the fluid be advanced forward.

In the case of the drop regulated infusion pumps, the dosage accuracy of these pumps relates to the number of drops (per minute) and depends on the volume of the drops. The drop accuracy is subject to several important conditions such as the viscosity of a solution, the solution's surface tension and the flow behavior resulting from these factors. Dosage accuracy is ±1 0%.

Accurate fluid infusion and drug administration is crucial for the optimum management of a critically ill patient. Continuous and controlled IV delivery of common medications, such as inotropic agents, vasodilators, aminophylline, insulin, heparin, etc., via infusion pump is the preferred mode of therapy in acute care. This is especially true for drugs with short half lives, so as to maintain a desirable constant serum concentration and in situations when constant infusion of glucose is needed. Patients with compromised renal, cardiac or pulmonary function have limited fluid tolerance and hence it is essential to use infusion pumps so as to prevent inadvertent volume overload. For intensive care, more than one infusion pump is often used when drug dosage, concentration, interaction and fluid volume require separate infusion rates. The use of infusion pumps has been advocated over manual flow control system on the basis of assuring precise and accurate delivery of prescribed fluid volumes over a specified time and to help in better nursing management.

The performance of infusion pumps is generally acceptable for clinical use, but the volume that may be infused is limited by the syringe capacity and infusion must be stopped whenever it is necessary to replace or refill the syringe. The largest syringe accepted by these pumps accommodates 100 mL of drug product. The small weight and no interference of gravity and positioning makes these syringe pumps suitable for transport. These pumps can be mounted on an IV pole or on the operating table. In addition these are small and light weight and have an occlusion alarm pressure of 570 mmHg.

Recently introduced modern infusion pumps incorporate a soft key interface by which a range of body weight and drug concentrations can be entered. Bolus doses can be easily and rapidly administered at any time during the infusion. These systems are also modifiable to accept all syringe sizes from 10-100 mL and have two independent microprocessors to monitor and control infusion processes for consistent delivery.

c. *Syringe pumps*: The syringe pump has been defined as a power driven device for pushing the plunger of a syringe forward at an accurately controlled rate. These are pressure infusion devices which supply the content of one or more syringes simultaneously by means of a precision linear drive. The dosage accuracy with these pumps is ±2% since a precise syringe volume is delivered through these pumps and all the error sources involved in drop regulation do not apply. This form of infusion is particularly suited for an exact administration of drugs with a dosage rate of 0.1 to 200 mL/hr. Special syringes of 10, 20, and 50/60 mL are commercially available. Because infusion pumps work with a maximum pressure of 1 bar, all tubings connected with such pumps need to be pressure resistant for safety reasons.

Previous research has demonstrated that variation occurs when different types of syringes are used with electronic syringe drivers (Medical Device Amendment (MDA), 2003). For example, it has been reported that there is a difference in the amount of drug delivered and the occlusion to alarm time in two different types of syringe (24). Similar findings are associated with spring devices (25). Luer-lock syringes are commonly recommended to avoid separation of the syringe and infusion set. This is particularly important for subjects who may be restless or lack of

understanding about the importance of protecting the device. Clearly, the type of syringe should be standardized to avoid variation in infusion rate and ensuing symptom control. The MDA (2003) recommends using specific types of syringes as indicated by the manufacturer of the pump used.

Nonelectronic spring driven devices work on the principle that the syringe compresses the spring and the flow of liquid from the syringe is controlled by tubing with a restrictive narrow bore (Springfusor®). Such devices are reported to be advantageous in comparison with electronic devices in terms of cost and simplicity of use. A number of researchers have compared the two in terms of accuracy and reliability. One disadvantage of the Springfusor is that it is calibrated at 25°C and is affected by temperature variation. When the temperature rises, for example, if the device is close to the skin or under the bed clothes, the flow rate increases. Although this is not expected to cause clinical effects in adults, it may well have implications for children in terms of over-infusion (26).

SMART PUMPS

Studies indicate that although 38% of errors occur at the time of drug administration, only 2% are actually caught (27). Roughly 35% to 60% of all harmful IV medication errors can be directly associated with the use of an infusion pump device (28). Because many of these harmful errors occur with drugs that are classified as high-alert medications it is not a surprise that safety-minded organizations are choosing to convert their infusion pumps to the newest form of "smart infusion devices." The term "smart" or "intelligent" is used to describe this pump technology because these infusion devices contain error reduction software with the ability to store organization-specific dosing guidelines, and they produce real-time alerts for practitioners when attempts are made to program doses outside of the established safe range. Smart pumps are computerized infusion devices with dose-error reduction software designed to help avert IV programming errors, as well as other errors associated with infusions (29). Smart pumps differ from older pumps because they can be programmed to include facility customized drug libraries lists of IV medications and their concentrations. Software provides point-of-care decision support for high or low infusion rates. The device prompts the user to choose a medication from the library, confirm the selection, input a volume to be infused, and input an infusion rate or dose. For all medications selected from the library, the keypad entry of an infusion rate in milliliters will automatically calculate the equivalent dose in units, milligrams or micrograms (5).

PATIENT-CONTROLLED ANALGESIA

One of the most common methods for providing postoperative analgesia is via patient-controlled analgesia (PCA). Although the typical approach is to administer opioids via a programmable infusion pump, other drugs and other modes of administration are available. There are several advantages of using a PCA (30). It reduces the time between when the patient feels pain and/or the need to receive analgesia and when it is administered (activation automatically pumps the dose into a preexisting IV line into the patient). It also reduces the workload of the nursing staff (an amount of the prescribed analgesic is preloaded into the PCA, enough for multiple doses) and the chances for medication errors. The PCA is programmed per the physician's order for amount and interval between doses and "locks out" the patient if he or she attempts excessive self-administeration. Patients can receive medicine when they need it, instead of having to wait for nurse practitioner or caretaker. Patients who use PCAs report better analgesia and lower pain scores than those patients who have to request analgesia from the nursing staff when they are in pain. Additionally careful examination of the syringes in a PCA provides a measurement of how much pain an individual patient is experiencing from one day to the next. It involves patients in their own care, giving them control and ultimately rendering better patient outcomes.

PCAs do suffer from certain disadvantages. Patients may be unwilling to use the PCA or be physically or mentally unable to. However, PCA pumps are rated among the world's most accessible pieces of equipment since all manufacturers must have alternative switch access

built into their PCA pumps. Most companies employ a TASH (The Association for Persons with Severe Handicaps) approved switch interface connection as TASH is one of the industry standards in accessibility switches (31). The pumps are often expensive and may malfunction.

DISPOSABLE INFUSION PUMPS

All nonelectric disposable pumps exploit the same physical principle: mechanical restriction within the flow path determines the speed of pressurized fluid. The pressure on the fluid is generated by a variety of mechanisms using nonelectric power, including a stretched elastomer or compressed spring, pressure generated during a chemical reaction (32), and pressure supplied from a cartridge of pressurized gas. The restriction of flow in all disposable pumps is caused by narrow-bore tubing. Tubing diameter has a determining influence on the device's flow rate. Therefore, flow restrictors are usually made of materials whose dimensions change little with temperature to maintain accuracy. Glass capillary-flow restrictors are typically used for devices infusing at a rate of 0.5 10 mL/hr; plastic is typically used for flow restrictors of pumps infusing at rates of 50 250 mL/hr. The flow restrictor is always integral to the administration set. The administration set can be integrated within or can be detachable from the pump reservoir.

Elastomeric infusion pumps are disposable devices, in which the pressure on the fluid is generated by the force of a stretched elastomer. Elastomeric disposable pumps consist of an elastomeric membrane, which contains the drug that is contained within an outer protective shell. The outer protective shell can either be a conformable elastomer (e.g., Homepump Eclipse®, BBraun) or a more rigid plastic (e.g., Infusor®). A soft elastomeric outer shell offers less protection against sharps puncture but requires less storage and disposal space. The membranes of elastomeric pumps are made of various elastomers, both natural and synthetic (e.g., isoprene rubber, latex, and silicon), and can be made of a single or multiple layers. The type of elastomer and the geometry of the elastomeric balloon determine the pressure generated on the fluid when the balloon is stretched (33). Multiple-layer elastomeric membranes can generate higher pressures than the single-layer membranes. Elastomeric pumps operate with a driving pressure of 260 520 mmHg and infuse at rates of 0.5 500 mL/hr.

Another type of disposable pump used is negative-pressure pumps. With negative-pressure pumps, a driving force is generated from the pressure difference across two sides of the pump's low-pressure chamber wall, with one side being at very low pressure (inside a vacuum chamber) and another side being at atmospheric pressure. The very low pressure in the vacuum chamber is created by the user while filling the device. Expansion of the drug reservoir, caused by the addition of fluid to the drug-containing reservoir, causes simultaneous expansion of the reduced pressure chamber, thus creating a significant vacuum. During infusion delivery, pressure on the movable wall plunger is generated by the large pressure difference between its two sides, causing it to move and compress the fluid in the drug-containing chamber.

SUMMARY

Although over the years the different routes of administration used for parenteral medications has remained the same, the science behind the design, development, and delivery of parenteral dosage forms have become complex. With continued and ever increasing need for superior dosage administration control, accuracy, and efficacy the development of newer dosage forms as well as parenteral drug delivery devices have become highly sophisticated. Additionally, new as well as older highly potent and difficult to formulate drug molecules are being rescrutinized and drugs once thought to be not viable because of poor oral bio-availability are seeing a comeback as parenteral dosage forms. These potent drug entities require accurate control of dose and higher safety margins. The advent of smarter and sleeker electronics and computers have helped to achieve this and also helped in the development of "error proof" infusion systems that have increased patient compliance and have lead to improved therapeutic outcomes. Some of these systems have considerably reduced the risks involved with parenteral administration of drugs and others show promise for safe and efficacious administration of drugs via this route.

REFERENCES

1. Schrappe M, Reiter A, Zimmermann M, et al. Long term results of four consecutive trials in childhood ALL performed by the ALL BFM study group from 1981 to 1995. Berlin Frankfurt Munster. Leukemia 2000; 14(12):2205 2222.
2. Masurier C, Salomon B, Guettari N, et al. Dendritic cells route human immunodeficiency virus to lymph nodes after vaginal or intravenous administration to mice. J Virol 1998; 72(10):7822 7829.
3. Wong T, Lee SS. Hepatitis C: a review for primary care physicians. CMAJ 2006; 174(5):649 659.
4. Duma RA, Akers MJ. Parenteral Drug Administration, Routes, Precaution, Problems, and Complications. Vol. 1. New York and Bassel: Marcel Dekker, 1984.
5. Christopher DA, Campbell A, Dateshidze J. Working smarter with intelligent pumps. Nurs Manage 2008; (Suppl):9 10, 12.
6. Argoff CE, Silvershein DI. A comparison of long and short acting opioids for the treatment of chronic noncancer pain: tailoring therapy to meet patient needs. Mayo Clin Proc 2009; 84(7):602 612.
7. Viscusi ER. Patient controlled drug delivery for acute postoperative pain management: a review of current and emerging technologies. Reg Anesth Pain Med 2008; 33(2):146 158.
8. Wheless JW. Pediatric use of intravenous and intramuscular phenytoin: lessons learned. J Child Neurol 1998; 13(Suppl 1), S11 S114; discussion S30 S32.
9. Miyake RK, Zeman HD, Duarte FH, et al. Vein imaging: a new method of near infrared imaging, where a processed image is projected onto the skin for the enhancement of vein treatment. Dermatol Surg 2006; 32(8):1031 1038.
10. Simons FE, Gu X, Simons KJ. Epinephrine absorption in adults: intramuscular versus subcutaneous injection. J Allergy Clin Immunol 2001; 108(5):871 873.
11. Van Hook R, Vandevelde AG. Letter: gas gangrene after intramuscular injection of epinephrine: report of fatal case. Ann Intern Med 1975; 83(5):669 670.
12. LaForce FM, Young LS, Bennett JV. Tetanus in the United States (1965 1966): epidemiologic and clinical features. N Engl J Med 1969; 280(11):569 574.
13. Williams PL. Coccidioidal meningitis. Ann N Y Acad Sci 2007; 1111:377 384.
14. Nowacki P, Zyluk B. Lymphomatous infiltrates within leptomeninges and spinal roots in adult patients with non Hodgkin's lymphomas after intrathecal methotrexate administration. Folia Neuropathol 1996; 34(2):114 118.
15. Ratcheson RA, Ommaya AK. Experience with the subcutaneous cerebrospinal fluid reservoir. Preliminary report of 60 cases. N Engl J Med 1968; 279(19):1025 1031.
16. Gnanalingham KK, Lafuente J, Harkness W. Intraventricular migration of a Rickham reservoir: endoscopic retrieval. Childs Nerv Syst 2003; 19(12):831 833.
17. Vogtle LK. Pain in adults with cerebral palsy: impact and solutions. Dev Med Child Neurol 2009; 51(Suppl 4):113 121.
18. Rigg JR, Jamrozik K, Myles PS, et al. Epidural anaesthesia and analgesia and outcome of major surgery: a randomised trial. Lancet 2002; 359(9314):1276 1282.
19. Jacobson L, Chabal C, Brody MC. A dose response study of intrathecal morphine: efficacy, duration, optimal dose, and side effects. Anesth Analg 1988; 67(11):1082 1088.
20. Lim Y, Sia AT, Ocampo C. Automated regular boluses for epidural analgesia: a comparison with continuous infusion. Int J Obstet Anesth 2005; 14(4):305 309.
21. Leighton BL. A greengrocer's model of the epidural space. Anesthesiology 1989; 70(2):368 369.
22. Norman D. Epidural analgesia using loss of resistance with air versus saline: does it make a difference? Should we reevaluate our practice? Aana J 2003; 71(6):449 453.
23. Beilin Y, Bernstein HH, Zucker Pinchoff B. The optimal distance that a multiorifice epidural catheter should be threaded into the epidural space. Anesth Analg 1995; 81(2):301 304.
24. Clinton CW, Morrell DF. Effect of two different syringes on syringe driver function. S Afr J Surg 1992; 30(1):7 9.
25. Bruera E, Macmillan K, Kuehn N, et al. Evaluation of a spring loaded syringe driver for the subcutaneous administration of narcotics. J Pain Symptom Manage 1991; 6(3):115 118.
26. McNeilly P, Price J, McCloskey S. The use of syringe drivers: a paediatric perspective. Int J Palliat Nurs 2004; 10(8):399 404.
27. Bates DW, Cullen DJ, Laird N, et al. Incidence of adverse drug events and potential adverse drug events. Implications for prevention. ADE Prevention Study Group. JAMA 1995; 274(1):29 34.
28. Husch M, Sullivan C, Rooney D, et al. Insights from the sharp end of intravenous medication errors: implications for infusion pump technology. Qual Saf Health Care 2005; 14(2):80 86.
29. Wilson K, Sullivan M. Preventing medication errors with smart infusion technology. Am J Health Syst Pharm 2004; 61(2):177 183.

30. Capdevila X, Macaire P, Aknin P, et al. Patient controlled perineural analgesia after ambulatory orthopedic surgery: a comparison of electronic versus elastomeric pumps. Anesth Analg 2003; 96(2): 414 417, table of contents.
31. Robinson SL, Rowbotham DJ, Mushambi M. Electronic and disposable patient controlled analgesia systems. A comparison of the Graseby and Baxter systems after major gynaecological surgery. Anaesthesia 1992; 47(2):161 163.
32. Bayne CG. Removing the confusion about infusion. Nurs Manage 1997; 28(2):15 18.
33. Chung IS, Cho HS, Kim JA, et al. The flow rate of the elastomeric balloon infusor is influenced by the internal pressure of the infusor. J Korean Med Sci 2001; 16(6):702 706.

3 | Biopharmaceutics of NCEs and NBEs

Balaji Agoram, Kazuko Sagawa, Ravi M. Shanker, and Satish K. Singh

INTRODUCTION

The term Biopharmaceutics is the study of the influence of formulation on the biological activity of a drug product, including its in vitro properties such as its physicochemical characteristics, formulation, and delivery technology (1). Pharmacokinetics (PK) is used to define the science of in vivo performance of a drug such as its bioavailability/absorption and systemic disposition, and is an important marker of the likely intensity and duration of the biological activity of the drug. Therefore, an understanding of the underlying processes governing drug absorption and disposition within the human body, methods of analyzing the characterizing the concentration-time profile, and the temporal relation between the measured concentration-time profile and the efficacy and safety time profiles are all critical elements in the design of appropriate dosage forms. This chapter has been designed to provide an overview of these topics.

The first part of this chapter focuses on the physicochemical properties of small-molecule drugs that influence their absorption by the parenteral route. An increasingly important category of injectable drugs now also includes biotherapeutics. Biotherapeutics (also called biologicals, biologics, or biopharmaceuticals) are compounds that are biologically produced as opposed to chemically synthesized. Some common examples of biotherapeutics are peptides, proteins and monoclonal antibodies. Most biotherapeutics are large hydrophilic molecules with complex tertiary structures. While the biopharmaceutical properties of small-molecule therapeutics have been extensively studied, the number of corresponding publications on injected biologics molecules is relatively rare (2). Therefore, biotherpeutics have also been considered in this chapter. However, many of the discussions on the basics of exposure (PK) and exposure-response (pharmacodynamics) analysis in this chapter are applicable to both biotherapeutics and small molecules. The impact of key physiological and physicochemical parameters on PK is also discussed in this chapter. A key biopharmaceutical aspect unique to biotherapeutics is their potential to cause immunological reactions, which can affect both PK and safety/efficacy profile. Immunogenicity, and the impact of formulation changes on immunogenicity is therefore covered in this chapter. Finally, the concept of comparability for biotherapeutics is discussed from the bioequivalence and PK perspective.

PHYSICOCHEMICAL PROPERTIES OF SMALL-MOLECULE DRUGS AFFECTING ABSORPTION BY THE PARENTERAL ROUTE

Takeru Higuchi, known as the "father of physical pharmacy" is credited with the introduction of many of the basic principles of physical chemistry that are known to influence the absorption, distribution, metabolism and excretion of drugs from the body. Although much of the literature on factors influencing absorption of drugs has focused on gaining detailed understanding after oral administration (3), the same physicochemical properties of molecules are important for absorption after administration via subcutaneous (SC), intramuscular (IM), intraperitoneal, and other extravascular routes delivered via injection. On the other hand, when a drug is injected directly into the vascular system, that is, via intravenous (IV) route then there are no physicochemical factors that affect absorption. Figure 1 provides a simplified, schematic overview of the relationships of administered dose of an injectable drug to the elicitation of the pharmacological effect, which includes therapeutic benefits as well as undesirable side effects. In the case of direct vascular injection via a bolus dose or as an infusion, the drug must be dissolved prior to administration to avoid the risks of causing

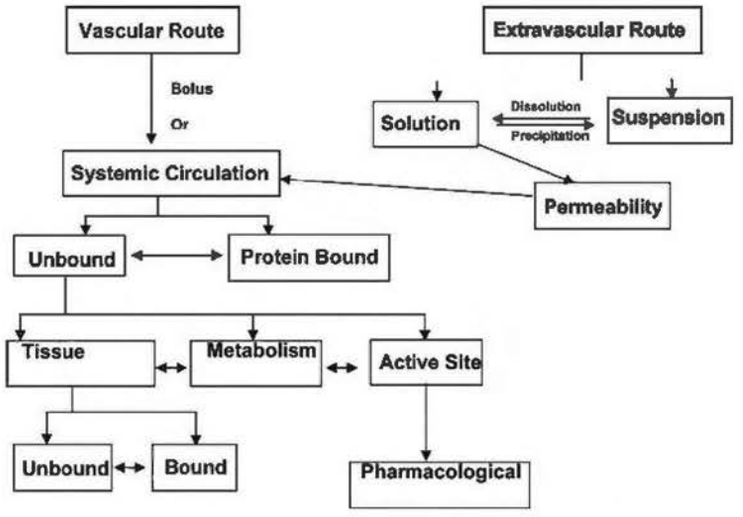


Figure 1 A schematic overview of fundamental relationships between routes of administration of injectable drugs to their ability to elicit pharmacological response.

blockage of capillaries that can affect the safety of the patient. However, injections through the extravascular route may be administered as either solutions or as suspensions of particles in aqueous or oil-based vehicles. Ultimately, for the drug to reach its intended target of the diseased tissue to elicit a pharmacological response, it is imperative that the drug must dissolve in the aqueous environment of the interstitial fluid and in the blood. Similarly, for the drug to reach the site of action from extravascular sites, it must have the ability to diffuse through cell membranes. These two essential properties of drug molecules: dissolution in aqueous and biological environment, and diffusing to reach the site of action, are governed by a multitude of physicochemical properties. The aim of this section is to provide the formulator of injectable drugs, a basic understanding of physicochemical properties of drugs that influence their PK as well as pharmacodynamics to assist with the design of drug products that can utilize these properties; to help identify formulation approaches to overcome limitations presented by any of these properties and also to assist in troubleshooting suboptimal performance of either novel or purportedly equivalent injectable drug products.

To consider the physicochemical properties of drugs that influence their absorption, distribution, metabolism and excretion it is essential to consider the anatomical and physiological characteristics of the vascular and extravascular injection sites. Detailed discussion of these factors and their impact on the design consideration of injectable dosage forms have been provided in preceding chapters. Similarly detailed discussions of preformulation and formulation approaches to quantitiatively understand the solubility and stability of a variety of injectable dosage forms are covered in various chapters. Factors such as the pH of blood, intracellular and extracellular fluid; the nature of ions and ionic strength of these physiological fluids; blood flow as well as number of capillaries at extravascular sites; the presence of lymphatic network; muscle movement; body temperature; nature of disease state; and, age of the patient are important considerations in understanding the PK and pharmacodynamics of drugs. The physicochemical properties of drugs may be broadly classified into two categories: (*i*) intrinsic properties and (*ii*) adjustable or changeable properties. Examples of intrinsic properties are molecular structure, functional groups, the ionization constant (pK_a the negative logarithm of the ionization constant) of the functional groups, partition coefficient (log*P*), melting point, and intrinsic aqueous solubility (of the unionized form of the drug). Examples of properties that can either adjusted or selected by the

formulator include salt forms of ionizable drugs, particle size, degree of crystallinity, amorphous form and solubilization via selection of excipients that can alter the solubility of the drug.

Ionization (pK_a)

A molecule or an atom group in a molecule may lose or gain a proton when the molecule is placed in an aqueous solution. The symbol K_a is used to describe the tendency of compounds to accept protons and is called the ionization constant. Expressed in mathematical terms, the negative logarithm ($\log_{10}$) of the ionization constant (K_a) is defined as pK_a. Since pH is the negative logarithm of the hydrogen ion concentration ($\log_{10}$ [H^+]), the relationship between pH and pK_a for an acidic drug can be expressed as follows:

$$\mathrm{pH} = \mathrm{p}K_a \quad \log \frac{[\text{Unionized}]}{[\text{Ionized}]}$$

This mathematical relationship provides an ability to calculate the fraction or percentage of ionized and unionized species of a drug in the pH of physiological interest by knowing the pK_a of the drug. This understanding of distribution of species is extremely important in predicting and quantifying the solubility, distribution coefficient (log*D*) and thus the drug's PK (ADME) and pharmcodynamics. The unionized form is the only species that diffuses through cell membranes; however, it is also the form that has the lowest aqueous solubility. Therefore, an injectable drug product when formulated at a pH to take advantage of its increased solubility in the ionized state stands the risk of precipitation of drug upon encountering physiological pH at the site of injection. This phenomenon of precipitation of drug can result in phlebitis as well as significant pain at the site of injection (4,5).

Partition Coefficient (log*P* and log*D*)

Partition coefficient (*P*) of a drug is the ratio of its concentration in the two phases of a mixture of two immiscible solvents at equilibrium. Conventionally, one of the solvents chosen is water while the second is octanol (6). Logarithm of the partition coefficient is referred to as log*P* as is defined as the ratio of the concentration of the unionized species in octanol divided by the concentration of unionized species in water.

$$P = \frac{[\text{Unionized Species}]_{\text{Octanol}}}{[\text{Unonized Species}]_{\text{Water}}}$$

Similarly, log*D*, refers to the logarithm of the distribution coefficient (*D*), which is defined as the ratio of the concentration of all the species, that is, unionized and ionized in octanol divided by the concentration of all species in water.

$$D = \frac{[\text{Unionized Species} + \text{Ionized Species}]_{\text{Octanol}}}{[\text{Unonized Species} + \text{Ionized Species}]_{\text{Water}}}$$

Since the fraction of unionized and ionized species in aqueous solution is governed by the pH of the solution and the pK_a of the molecule, therefore, the log*D* or distribution of the drug is dependent on pH and pK_a. Since only the unionized molecule diffuses through biological membrane, therefore, the permeability of the drug is dependent on log*D*. The interrelationships between ionization, pH, and partitioning of the drug through biological membrane are often referred to as the "pH-partition hypothesis" (Fig. 2). These interrelations are summarized in Figure 3. The pH-partition hypothesis was first proposed to explain the influence of pH of the gastrointestinal tract on the oral absorption of drugs (7). The concept is extensively used for not only understanding oral absorption but also the toxicity of drug molecules as well as the accumulation of drugs in specific tissues. Therefore, the interrelationships between the degree of ionization, the pH of biological fluid and the distribution coefficient is important for understanding the biopharmaceutical aspects of drugs.

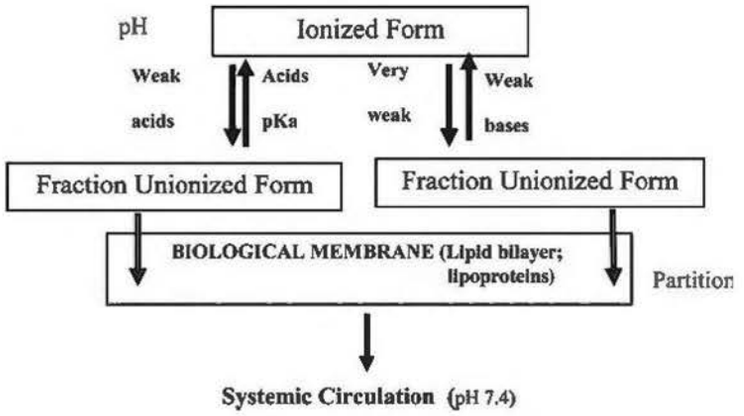


Figure 2 The pH partition theory for the absorption of drugs across biological membrane from extravascular sites of administration of injections.

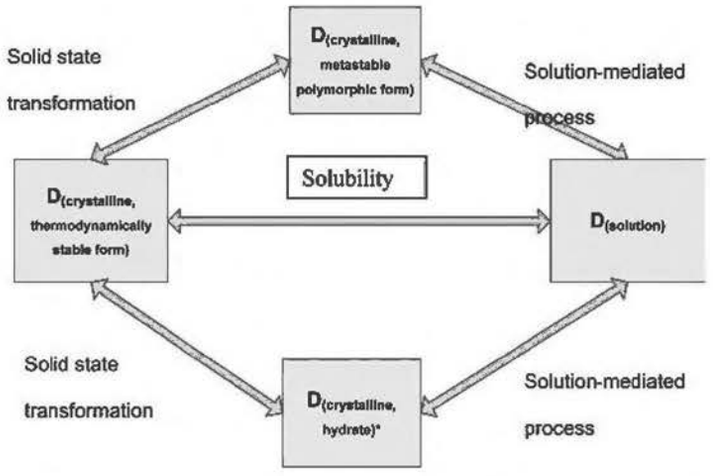


Figure 3 Potential pathways for transformation of solid form of drug (basic description for unionized drug since ionized form can undergo additional transformations to unionized form) during determination of equilibrium solubility or during transit through the body upon injection. Each arrow depicts a forward rate and a backward rate to maintain equilibrium.

Diffusion and Permeability (P_{app})

Molecular diffusion, often referred to as just diffusion is the physical phenomenon of transport of molecules via random molecular motion from a region of high concentration to one of low concentration. The phenomenon is typically described by Fick's laws of diffusion; the first law relates the diffusive flux to the concentration gradient and the second law predicts how the diffusion of molecules causes the concentration field to change with time. Mathematical expressions based on Fick's first law have been used to model transport processes in many systems including drugs across biological membranes. Fick's first law is expressed as follows:

$$J = PA(C_2 \quad C_1)$$

where J is the diffusion flux in units of [(amount of substance) length^{-2} time^{-1}]; P is the permeability of the membrane (e.g., biological cell membrane) for a given molecule at a given

temperature; A is the surface area over which diffusion is taking place; and $C_2 \quad C_1$ is the difference in concentration or concentration gradient of the molecule across the membrane in the direction of flow of molecules ($C_1 \quad C_2$). Biological membranes consisting of lipid bilayer are semi-permeable in nature and are also known as selectively permeable membranes, that is, they allow certain molecules or ions to diffuse through. There are several factors that influence the permeability of organic molecules through biological, semipermeable membranes such as molecular size (molecular weight), charge on the molecule, lipohilicity (log$P$ or log$D$) of the molecule, polar surface area, number of rotatable bonds, etc. Although it is possible to utilize formulation factors to change the concentration gradient to influence the flux across the biological membrane, it is not possible to alter the intrinsic permeability of a compound using formulation approaches. Because of the challenges of experimentally determining the permeability of drugs across biological membranes, several in vitro approaches, based on cell cultures, have been utilized extensively to ascertain the apparent permeability (P_{app}). Understanding and predicting molecular descriptors that can influence permeability of drugs across biological membranes continues to be a matter of extensive fundamental and applied research (8,9).

Solubility, Dissolution, and Solubilization

The pharmaceutical literature in the past few decades has used multiple terms to describe solubility and dissolution of drugs which has often resulted in confusion and misunderstanding (10). Terms such as thermodynamic solubility, equilibrium solubility, intrinsic solubility, kinetic solubility, apparent solubility, intrinsic equilibrium solubility, dissolution rate, intrinsic dissolution rate, etc., have been used by researchers to describe different aspects of experimental observations. Aqueous solubility of solutes is a relatively straightforward thermodynamic concept, especially for crystalline drug molecules since solubility represents the concentration of drug in solution which is in an equilibrium two phase system consisting of the drug in the solid state and the solution state. This concept is often schematically illustrated by

$$\text{Solute}_{(\text{solid, crystalline, excess})} + \text{Solvent} \overset{K}{\Leftrightarrow} \text{Solute}_{(\text{solution})}$$

where K, the equilibrium constant, is the ratio of activity of solute in solution to that in the solid. Typically, when the solute concentrations are low then the solute activity coefficients are essentially unity. Since solubility is equilibrium constant, it is dependent on temperature and pressure. However, in the context of drug delivery and biopharmaceutics, pressure is not considered to be a variable. The above definition of solubility highlights the importance of characterizing the solid at equilibrium in addition to measuring the concentration of drug in solution. If the solid form undergoes a change in its solid state relative to the initial form that was used for experimentally assessing solubility then the equilibrium solubility is reflective of the new solid form (polymorph, hydrate, solvate, etc.) rather than the original form. Solid state transformations can either be mediated through solution or directly through the solid state. Figure 3 provides a schematic overview of potential transformations that can occur during experimental determination of solubility determination or during the time the dosage form is present in the body.

Since a change in drug's solid state (polymorph, hydrate, solvate, amorphous form, etc.) can result in significant change in its solubility as well as dissolution rate, such transformations as depicted in Figure 3 can have a direct impact on the biopharmaceutic performance of an injectable drug product. An additional aspect of understanding the equilibrium constant between the drug in solid state and drug in solution is the rates of the forward and backward processes.

If both the forward rate, that is, dissolution and backward rate, that is, crystallization as shown in Figure 3 were completely controlled by diffusion process, then these rates would be identical at equilibrium, which however is rarely the case. Crystallization is not merely based on diffusion but is known to be a stochastic (probability-driven) process that requires random collisions to form a critical size of nuclei before crystal growth can occur. Therefore, it is imperative to understand the impact of dilution and mixing of solubilizing excipients with biological fluids at the site of injection as the resulting decrease in solubility of the drug can

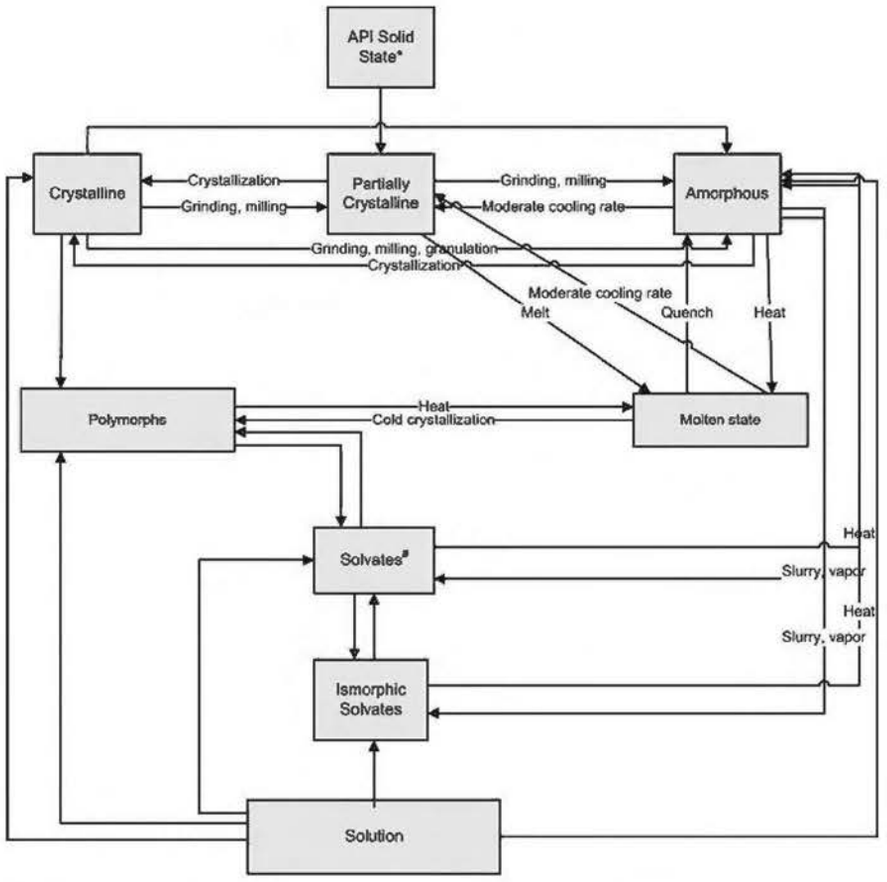


Figure 4 Processing options available for solid state transformation of drug to facilitate isolation of preferred API form for development of injectable formulations.

lead to precipitation of drug and consequently lead to decrease in available concentration of drug at the site, pain and phlebitis. Understanding of the thermodynamics and kinetics of interconversion of the solid state transformations can facilitate the development of processes that isolate the preferred stable form (thermodynamically or kinetically stable) for manufacture of dosage form. Figure 4 provides a schematic overview of the processing options available to the formulator.

In addition to equilibrium solubility, the time required to reach solubility, that is, dissolution is an extremely important phenomenon for the biopharmaceutics characteristics of an injectable suspension. There are several theories that model dissolution of solids to form solutions and the most frequently used relationship known as Nernst-Brunner equation, which was a modification of the original Noyes-Whitney equation (11). The Nernst-Brunner equation, shown below, is derived from Fick's law of diffusion and takes into account the presence of an aqueous diffusion boundary layer on the surface of the dissolving solid.

$$\text{Dissolution Rate} = \frac{A_{\text{solid}(t)} D_{\text{Drug}}}{h_{(t)}} \left(S_{\text{Bulk}} \quad \frac{X_{\text{Solution}(t)}}{V_{\text{Bulk}}} \right)$$

where $A_{solid(t)}$ is the total surface area of the solid at time t; D_{Drug} is the diffusion coefficient of the drug; $h_{(t)}$ is the thickness of the diffusion layer at time t; S_{Bulk} is the solubility of the drug in the bulk liquid; $X_{solution(t)}$ is the amount of drug dissolved in bulk solution at time t; and V_{bulk} is the volume of the bulk solution. Although the Nernst-Brunner equation is useful, it is not always applicable for biopharmaceutical applications. Modeling of dissolution kinetics especially of powders is of significant practical importance especially for injectable drug products. To model dissolution of particles, knowledge of particle size distribution as well as an estimate of the thickness of the aqueous diffusion layer as a function of particle size are necessary. In terms of particle size, it is important to take into account the poydispersity of the particle size (12). Several mathematical relationships have been developed to model the dissolution of powders. However, it is important to note that the dissolution of particles from an extravascular site of injection does not follow these models adequately because of poor mixing and agitation at the site. Therefore, biopharmaceutical considerations of particle size-dependent dissolution for injectables requires the development of more complex mathematical models (13).

The influence of degree of ionization of an ionizable drug on its partitioning into biological membranes was discussed previously. Similarly, the degree of ionization greatly affects the solubility of the drug. The ionized form (either acid or base) has higher solubility than the unionized form. Theoretical pH-solubility profiles of ionizable drugs is given by the Henderson-Hasselbalch equation, which relates the solubility of the unionized form of the drug (S_0) to the dissociation constant (pK_a) to obtain the total solubility (S_T) of the drug.

The underlying assumption in these predictions is that the drug molecule does not self-associate in solution either in the unionized or ionized states. However, these relationships cannot predict the pH independent, limiting solubility of the salt forms of ionizable drugs. There are no theoretical methods available to predict the solubility product (K_{sp}) of a drug with a specific counterion. Therefore, it is essential to determine the K_{sp} experimentally. Although salt forms of ionizable drugs can provide wide ranges of solubility enhancement, it is not possible to a priori predict a preferred salt form for any drug on the basis of any basic principles. Furthermore, the rate of conversion of a salt form to its unionized state upon being subjected to a change in pH is also not predicted by any known theory or good empirical model. Knowledge of the expected solid form (as predicted by the phase rule) at equilibrium at any given pH is extremely useful in ascertaining whether the formulation as drug product or after administration at the injection site has reached equilibrium or is in the metastable state. Generally, according to the phase rule, the solid form at equilibrium is the unionized form of the drug at all pH values in the K_{sp} controlled region ($pH < pH_{max}$ for bases and $pH > pH_{max}$ for acids).

For monobasic compounds, the relationships are as follows:

$$S_T = S_0\left(1 + \frac{K_a}{[H^+]}\right) \quad \text{when } pH < pH_{max}$$

$$S_T = S_0\left(1 + \frac{[H^+]}{K_a}\right) \quad \text{when } pH > pH_{max}$$

For monoacidic compounds,

$$S_T = S_0\left(1 + \frac{K_a}{[H^+]}\right) \quad \text{when } pH < pH_{max}$$

$$S_T = S_0\left(1 + \frac{[H^+]}{K_a}\right) \quad \text{when } pH > pH_{max}$$

The importance of aqueous solubility of drug has been discussed specifically in the context of biopharmaceutical properties injectable drugs. It is equally important to discuss the fundamental factors that contribute to make drugs insoluble. Considering the general solubility equation (14) provides insights into the physicochemical reasons that make drugs insoluble.

$$\log S_0 = 0.5 \quad 0.01[T_m(^\circ C) \quad 25] \quad \log P$$

According to the above equation, aqueous solubility (S_0) of a drug would be reduced if the melting point (T_m) is high, that is, the solid in the crystalline state has high lattice energy. Alternatively, insolubility may also arise because of high log$P$ or lipophilicity of the drug. If both factors, that is, lattice energy and lipophilicity are high then the drug would also be highly insoluble. There are many approaches available for the formulator to enhance the solubility of insoluble compounds. Knowledge of the key factors contributing to insolubility along with its molecular structure can assist in the selection of appropriate solubilization technologies. A few of the many solubility enhancing options available to the formulation scientist developing injectable drug products include pH adjustment, cosolvent solubilization, solubilization by lipids, micellar solubilization, complexation (e.g., with cyclodextrins), amorphous forms of drugs, emulsification, liposomes, etc. Similarly, enhancement of dissolution rates can be achieved by particle size reduction including generation of attrition milled nanoparticles. This discussion has briefly outlined why it is important to consider the various thermodynamic but also the kinetic aspects of equilibrium for all of the physicochemical properties in designing, optimizing and troubleshooting the biopharmaceutical properties of injectable drug products.

DRUG EXPOSURE/PHARMACOKINETICS

Pharmacokinetics (PK) refers to the in vivo time course of blood concentration of an exogenously administered drug. In most cases, this is described by observing the plasma or serum concentration of the drug over time. The various processes that govern the observed concentration-time profile are *a*bsorption, *d*istribution, *m*etabolism, and *e*xcretion. Together, these four processes are described by the acronym ADME. Any changes in the ADME processes, for example, due to disease in the target population, could have changes in the PK profiles and hence the observed safety/efficacy profile of the compound.

Figure 5 illustrates the events that occur after administration of a drug. If the drug is administered in a "depot" [e.g., orally, subcutaneously (SC), etc.], a delay may occur before the drug appears in the blood circulation, possibly because of the time taken for the transport from the site of administration to the circulation. The initial increase in the drug concentration corresponds to the accumulation of the drug in the blood because of absorption from the site of administration. When the absorption rate becomes slower the elimination rate of the drug, the observed plasma concentration profile enters the declining phase. The inflection point at the top of the curve represents the change in this balance of the absorption and elimination rates. A distribution phase occurs where the absorbed drug is deemed to have distributed from the central circulation to other tissues or components of the blood. A rapid decline in the observed concentrations in blood represents distribution from the blood to other tissues such as the liver, kidneys, fat, etc., rather than elimination from the body.

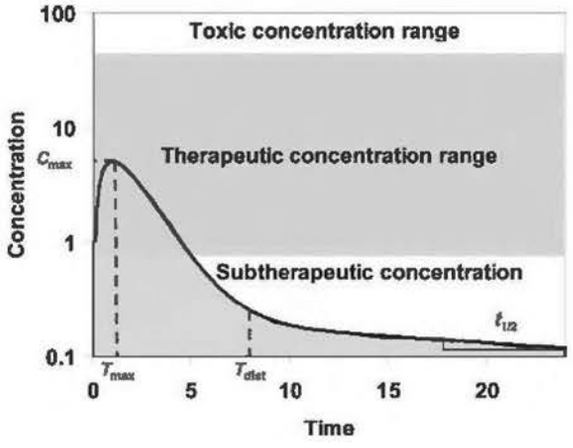


Figure 5 Absorption, distribution, metab olism, and excretion processes and phar macokinetic summary parameters.

Elimination of the drug occurs as soon as a drug is absorbed and enters the systemic circulation. The last phase of the decline in blood concentrations typically corresponds to the elimination of the drug from the body because absorption is completed and distribution equilibrium is established. The elimination phase is also sometimes referred to as the terminal phase.

The concentration-time profile is often summarized using a set of PK parameters: the maximum concentration (C_{max}), the time to attain maximum concentration (T_{max}), the time taken for the concentration to decline by half (half-life; $t_{1/2}$), and the area under the plasma concentration versus time curve (AUC) (Fig. 5). C_{max} and T_{max}, can be read from the concentration time profiles. The half-life can be read as the slope of the concentration-time curve, where the concentrations are plotted on a log scale. The AUC is typically calculated from the observed concentration-time profile through standard area calculation algorithms.

Taken together, the PK parameters C_{max}, T_{max}, and AUC can be used to characterize the rate and extent of absorption of a drug. Thus, they can be used to compare the relative extents to which a particular compound is bioavailable that is, reaches systemic circulation and is therefore available for therapeutic action after administration through different routes or from different formulations. Typically, the bioavailability (denoted by the symbol *F*) after an intravenous (IV) administration is assumed to be 100%, and that after other routes of administrations are expressed as fractions of the IV bioavailability.

Most therapeutic compounds exert their pharmacological effect by reversibly interacting with their targets for example, receptors, enzymes, ion channels, etc. When the systemic drug concentration declines, the extent of modulation of the target also reduces. Thus, the desired therapeutic effect for most compounds, which is the primary objective of the therapeutic dosing regimen, is obtained by maintaining a drug concentration above effective level (therapeutic concentration; Fig. 5). If the concentration is too low, loss of efficacy occurs because of lack of adequate modulation of the receptor. If the concentration is too high, toxicity might occur because of excessive modulation and potential exaggerated pharmacology or because of increasing expression of secondary pharmacological effects such as modulation of other subclasses of receptors. The difference (or the ratio) between the required therapeutic concentration and the toxic concentration is called the therapeutic index of a drug. Drugs with small differences between the therapeutic and toxic concentrations are referred to as narrow therapeutic index drugs and pose challenges in their clinical usage. A successful biopharmaceutical strategy would be effective in maintaining the concentration of the drug within the therapeutic concentration range.

Many biotherapeutics, especially macromolecules, because of their structure and physicochemical properties, possess distinct ADME properties from typical synthetic small molecules. As opposed to small molecules, a detailed understanding of these ADME mechanisms is not yet available for biotherapeutics. However, understanding the ADME processes for biotherapeutics is essential to appropriately design dosing regimens that maximize the therapeutic potential of these compounds.

Absorption

Before a drug can exert a pharmacological effect by modulating its target, it has to be absorbed from the site of administration into the bloodstream. For many synthetic small molecules, the oral route of administration is the preferred route of delivery because of the ease of administration and the related high level of patient compliance. However, biotherapeutics such as peptides, proteins and other macromolecules are, in general, not highly bioavailable after oral administration because of mainly two factors: (*i*) degradation in the gastrointestinal tract and (*ii*) lack of permeability across the GI mucosal barrier. Therefore, biotherapeutics such as monoclonal antibodies are typically administered through injections: IV, SC, and intramuscular (IM) routes being the preferred options. For example, of the 22 approved monoclonal antibodies (15), 4 are administered SC, 17 IV, and one each IM and intravitreally. Each of these sites of administration presents an absorption barrier with a unique set of properties.

For IV administration, there is no absorption barrier since the drug is directly delivered into the bloodstream. For extravascular routes of administration, the rate of absorption can vary widely depending on the site of administration.

SC doses are typically administered in the intradermal SC space in the shoulder, abdomen, thigh, or lower back. Similarly, IM doses are administered in the shoulder and gluteal muscles. After SC and IM injection, it is hypothesized that the drug is absorbed directly into systemic circulation via blood capillaries and through the lymphatic circulation. It has been shown through experiments in sheep that the lymphatic convective transport contributes substantially to the absorption of biotherapeutics after SC and IM administration and that the fraction of the drug absorbed through this process increases as the molecular weight increases (16). Consequently, it is hypothesized that for high molecular weight biotherapeutics such as monoclonal antibodies (approximate molecular weight of 150 kDa) are almost fully absorbed through the lymphatic system. Recent data in rats, where a low <3% contribution of the lymphatic route to the overall absorption was observed for erythropoietin, appear to contradict the findings in sheep. Suffice to say that a thorough quantitative understanding of the absorption processes after SC and IM administration of biotherapeutics is not yet available. Typically, absorption is slower for biotherapeutics than for small molecules with T_{max} values in the range of 24 to 72 hours post SC or IM dose.

The rate and extent of absorption from the extravascular site of administration depends on multiple factors and there is loss of drug prior to reaching systemic circulation (bioavailability is less than 100% compared with IV administration). A fraction of the drug administered after extravascular administered dose is subject to presystemic degradation, either at the site of administration, or during lymphatic transport hence, these routes are clinically relevant only when a limited amount of drug is required to be administered for efficacy.

Other routes of administration such as intravitreal and inhaled routes have also been explored for biotherapeutics. The intravitreal route has been pursued for ranibizumab (Lucentis®), a vascular endothelial growth factor antibody fragment and pegaptinib sodium (Macugen®), a polyethylene glycol conjugated aptamer to promote a local effect. Administration of the drug directly into the site of action typically overcomes systemic PK limitations such as short half-life and minimizes side effects due to interaction with therapeutically inactive targets or targets at organs other than the site of action, thus improving the therapeutic index of the compound. Recently, the inhaled route is being widely explored as an option for biotherapeutics. Exubera® is an inhaled form of insulin for diabetic control. The large surface area of the lungs and the rapid transport of many molecules across the lung epithelial barrier provide attractive options for delivery, especially when the target is present in the airways (17,18). The rate and extent of systemic absorption for biotherapeutics administered at the site of action can vary widely depending on the physiology of the site of action the density and porosity of the capillary bed, the lymphatic drainage of the site, any existing clearance mechanisms, and the effect of disease (see section "Absorption" under "Physiological Factors That Influence Pharmacokinetics of Injectable Drugs" for more details).

Distribution

Once the drug is absorbed from the site of administration into the blood circulation, it distributes to tissues, including the site of action, to exert its pharmacological effect. Unless the drug is designed to reach only a particular organ or tissue, this distribution of the drug occurs to various extents to all parts of the body. Within the PK field, the term distribution refers to the reversible partitioning of a drug to tissues within the body (19). The rate and extent of overall distribution of a drug from blood circulation to other tissues typically depends on many factors including the ability of the compound to cross tissue membranes, the perfusion rate of the tissues, partitioning into fat, and the tissue composition (20,21). Readers should note that the volume of distribution (V_d) commonly expressed as a PK parameter is a theoretical fluid volume that relates the administered dose and the observed blood concentrations and is not a strictly physiological quantity. For example, drugs that bind extensively to tissue targets have low blood concentrations after dosing, resulting in high estimated V_d, sometimes even higher than body volume (e.g., some basic drugs such as amphetamines)!

Except in the case of active transport, the distribution process for most small-molecule drugs is generally driven by concentration gradients. Therefore, at steady state, the free drug

concentrations in the blood and different tissues are at equilibrium. However, biotherapeutics are typically larger hydrophilic compounds with poor permeability across the tissue membranes. Entry into tissues is thought to be primarily through extracellular pathways (22,23), especially for tissues such as cerebrospinal fluid. Furthermore, return to blood from the tissue is in many cases through the lymphatic drainage (24), which is primarily a convective transport process not dependent on the concentration gradient and the biochemical properties of the compound such as permeability and tissue affinity. Therefore, the concentrations of the drug in blood and other tissues do not reach equilibrium, which is generally the case for small molecules. For example, the serum to cerebrospinal fluid concentration ratio of albumin is approximately 200:1 (22,23). Other investigations have shown that the blood: tissue ratio may also be dependent on the size of the biologic (24). Distribution of a drug to targets is another important factor to consider in the case of biotherapeutics. Many biotherapeutics, because of the very high affinity to their targets, are dosed at stoichiometrically equal molar concentrations to the target. Therefore, binding to the target constitutes a significant distribution pathway. Because the fraction of a drug bound to targets decreases with dose, target binding can lead to nonlinear distribution characteristics that is, dose-dependant volume of distribution for some biotherapeutics.

Metabolism

Most drugs begin to be metabolized after they enter the body. The majority of small-molecule drug metabolism is carried out in the liver by *redox* enzymes, termed cytochrome P (CYP)450 enzymes (ubiquitously expressed in the body). As metabolism occurs, a (parent) drug is chemically converted to metabolites. Metabolism eliminates the administered dose of a parent drug. When metabolites are pharmacologically inert, metabolism reduces pharmacological effects in the body as a parent drug is eliminated. Metabolites may also be pharmacologically active, sometimes more so than a parent drug (active metabolites).

The term catabolism is more relevant to describe the process by which biotherapeutics are broken down into smaller molecules such as amino acids. Proteolytic processes through enzymes such as proteases perform this function for biotherapeutics rather than CYP450 types of enzymes. The rate of proteolysis depends on many factors such as the size, carbohydrate content (glycosylation), potential for preproteolytic modification such as desialylation, the primary and tertiary structures (25). The sites of catabolism is also varied with liver, kidneys, and other extravascular sites such as sites of injection, for example, SC space have been implicated in protein catabolism. Many therapeutic proteins such as monoclonal antibodies are glycosylated proteins and are thought to interact with the asialoglycoprotein receptor (ASGPR) expressed on the sinusoidal surface of the parenchymal cells of the liver. ASGPR is believed to mediate the rapid removal and degradation of desialylated circulating proteins containing terminal galactose residues (26). It should be mentioned that characterizing the products of catabolism is substantially more difficult for biotherapeutics because of the wide range of catabolism products arising from an abundance of proteolysis sites and proteolytic enzymes.

An important site of catabolism of biotherapeutics is through the target. Binding of the biologic to the target has been shown to result in target-mediated endocytosis followed by lysosomal degradation for antibodies (27,28) and recombinant proteins (29).

Similar to V_d, clearance (CL) is a theoretical term that is the flow rate at a given concentration that is completely cleared of the drug in unit time and is calculated as the dose divided by the AUC under the assumption of constant clearance during drug elimination. Oxidative metabolism, catabolism, and other elimination processes all combine in achieving clearance of a xenobiotic.

Excretion/Elimination

Drugs and their metabolites are removed from the body via excretion, usually in the urine, in the feces or exhaled in the air. There are three major sites where drug excretion occurs. The kidneys, bile, and lungs. Many hydrophillic small molecules are cleared from the systemic circulation through the kidneys either intact or in the form of their metabolites (glomerular filtration) and excreted in the urine (renal elimination). Macromolecule biotherapeutics, because of their size are typically not cleared intact by filtration through the kidneys. However, small biotherapeutics such as some cytokines, insulin, granulocyte-colony stimulating factor,

interferon α, and erythropoietin have varying degrees of renal elimination, somewhat related to their size. In general, the renal elimination of intact biotherapeutics of molecular weight >30 kDa is expected to be negligible.

Larger molecular weight molecules are excreted into the bile and excrete in feces (biliary excretion). There are species differences in molecular weight cuttoffs for biliary excretion versus renal excretion. In human, the molecular weight cutoff required for biliary excretion is much greater than that for renal excretion. If the molecular weight is lower (e.g., <325 475 Da), the compound may be preferentially excreted in urine. Molecular weight from 325 to 850 Da may be eliminated via both renal and biliary routes. Excretion of molecules larger than 850 Da occurs mainly via biliary excretion. Physicochemical properties of the drug (polarity, lipophilicity, structure) are also critical to the extent of biliary excretion of a drug/metabolite. Biliary excretion has also been reported for biotherapeutics such as insulin (30) and epidermal growth factor.

PHYSIOLOGICAL FACTORS THAT INFLUENCE PHARMACOKINETICS OF INJECTABLE DRUGS

Physiological factors such as age, gender and disease states are known to alter PK of drugs. These factors can affect each component of PK absorption, distribution, metabolism and elimination described above.

Absorption

There is no absorption for IV administered drugs, as the drugs will directly circulate into the bloodstream. Therefore, the physiological factors, which influence absorption are minimal for IV dose. SC and IM administered drugs are taken up by the capillaries at the injection site and the permeability of the capillary wall membrane is affected by number of physiological factors.

Proteins larger than 16 to 20 kDa are generally taken up primarily by the lymphatic system and there is a linear correlation between molecular weight (MW 2,500 19,000) and the extent of recovery in the lymph (16). SC administered proteins generally exhibit a slower absorption and elimination compared with IV administration. Absolute bioavailability is generally low possibly because of protein degradation at the site of injection.

The factors affecting lymphatic transport of proteins after SC administration are summarized in the review by Porter and Charman (16). Lymph flow rate increases with exercise or mechanical injury (31). Massage is also known to increase lymph flow (32). Literatures show systemically administered insulin or gonadotropin increases capillary diameter and blood flow rate in rat cremaster muscle (33), although insulin-like growth factor-1 does not increase blood flow in human (34). The site of injection (injected to the abdomen vs. peripheral such as thigh or arm) influences the absorption (35) possibly because of differences in local blood flow and lymph flow.

SC blood flow increases in response to alterations in injection site, skin fold thickness, exercise, orthostatic changes, and ambient temperature (36).

Lymph flow is known to decrease with age (36,37). Membrane fluidity also decreases with age (38). Membrane permeability is also known to be altered with various disease states and with pharmacological agents (39).

Metabolism

Administered small-molecule drugs either orally or injection are mainly metabolized in the liver where the major metabolizing enzymes are located. Numerous literature reports suggest age and gender differences in CYP450 enzymes mediated metabolisms (40), however it is difficult to interpret those reports to general terms as those reports use probe drugs and majority of the studies is done in preclinical species.

The liver volume, liver blood flow and biliary function correlate well with body surface area (BSA). The liver size and blood flow decrease with aging, and therefore drug metabolism is reduced with advancing age (41). Renal clearance decreases with age and lower in women than in men at all ages.

Pelletier et al, demonstrated that the gut proteolytic activity is spread over a wide range of pH in younger animals than older ones with a shift from higher pH toward lower pH values with increasing age (42). A review article by Bota and Davies summarizes the regulation of

proteolytic enzymes in human diseases and ageing (43). Several disease states such as muscular dystrophy, cancer, Alzheimer's disease, neurological injury, ischemic injury, atherosclerosis, diabetes and cataract formation are known to alter the regulation of protease activities (44). Similarly, disease severity may also be related to increasing expression of the target and result in increased clearance of some biologics such as herceptin (cleared through the HER-2 receptor pathway) and omalizumab [cleared through immunoglobulin E (IgE)].

Distribution

Intravascular volumes, organ volumes and muscle volumes are generally smaller in elderly than younger people. The impact of reduced volumes is evident when the drug is distributed to those particular organs including muscles.

Drug distribution is also known to change with age because of relative changes in body fat. Lipophilic drugs such as midazolam and diazepam tend to get distributed to fatty tissue resulting in an increased volume of distribution(V_d) in elderly subjects (45,46). Divoll et al. studied PK of diazepam in young and elderly men and women (47). The authors found that the V_d was larger in women than in men but increased with age regardless of gender. Elimination half-life was longer in elderly than in young men partly because of the increased V_d as well as to a reduction in total metabolic clearance. It is noteworthy that the neither age nor gender influenced oral absorption and diazepam was nearly completely absorbed after IM administration (47). The level of α acid glycoprotein increase with age and as a consequence (48) the V_d can decrease for those drugs which bind to this particular protein.

As described above, biotherapeutics are distributed to tissues by blood or lymph, any disease states or aging which alter the blood flow and/or lymph flow can alter the tissue distribution of those large molecules. As mentioned earlier, the expression levels of target tissues (e.g., receptors) can be largely altered by the disease states as well. For example, the level of IgE correlates with the severity of asthma and the distribution of omalizumab, an anti-IgE monoclonal antibody, is related to the level of IgE present in the patient.

EXPOSURE-RESPONSE ANALYSIS

Pharmacokinetic Analysis

The primary aim of PK analysis is to summarize available plasma concentration versus time profiles (PK profiles) for interpretation, comparison, and predictions through the use of a set of parameters. These parameters can be obtained directly from an observation of the PK profile without the assumption of an underlying model quantitatively describing the different ADME processes. This is commonly referred to as a nonparametric, noncompartmental or model-independent analysis. These parameters include maximum concentration, time to reach C_{max}, area under the curve, the clearance [CL derived from the dose and AUC (CL = dose/AUC)]. While this analysis is simple and can represent simple PK characteristics of a compound, it has limited extrapolation ability beyond the studied regimen.

The PK profile can also be described by a set of PK parameters, assuming an underlying mathematical model typically, a mammillary model with first-order kinetic processes describing the ADME process. This analysis is commonly referred to as compartmental modeling. A simple model is a one-compartment model, which represents central compartment (blood/plasma compartment) (Fig. 6). The rate of drug in (k_a, first-order absorption rate constant) and out (k_{el}, first-order elimination rate constant) of the central compartment is described by first-order kinetics.

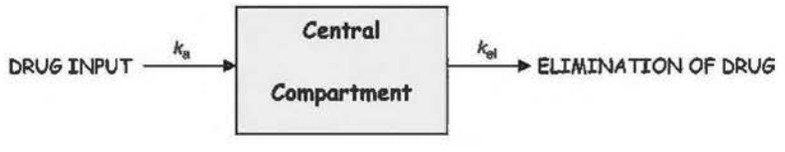


Figure 6 Schematic of a simple compartmental pharmacokinetic model.

The versatility of the parametric analysis is founded in the ability of simple mathematical constructs to describe complex ADME phenomenon. By fitting the data to the right model, the model parameters can be estimated and these model parameters can be used to simulate time versus concentration curve with different dose or different routes of administration. Further complexity can be added to the simple one-compartment model to describe more complex PK; standard additions include second (and third) distribution compartments to describe distribution at different rates to different sets of tissues and multiple absorption routes and windows. It should be noted that in this approach, the parameters of the model Ka, CL, V_d, etc., do not have a direct physiological meaning even though they are related to physiological phenomenon.

Physiology-based pharmacokinetic modeling (PBPK) could be considered a special case of compartmental modeling, where the compartments and transfer rates correspond to physiological quantities such as tissues and organ volumes and blood flow rates. PBPK modeling is particularly useful when one wishes to predict the disposition in a particular organ.

Pharmacokinetic-Pharmacodynamic Analysis

The original concept of pharmacokinetic-pharmacodynamic (PK/PD) was described by Gerhard Levy in 1966 (49). PK is a study of a time and drug concentration relationship. Pharmacodynamics is a study of pharmacological responses. PK/PD analysis is a study of the relationship between PK and pharmacodynamics (PD). Understanding the PK/PD relationship is critical to determine the clinical dose and dosing regimen.

There are many different types of pharmacological responses. Mainly they can be categorized as either direct or indirect responses. A direct response is when the observed time course of response is temporally similar to the PK. A simple example of direct response is a receptor binding type response where the relationship between blood drug concentrations and the effect can be described with Hill function (50).

$$E = \frac{E_{max} \times C^{\gamma}}{EC_{50} + C^{\gamma}}$$

where E_{max} is the maximum efficacy (capacity), EC_{50} is the concentration to produce 50% of effect (sensitivity), and γ is Hill factor. Direct PK/PD responses are observed when the drug target is present in blood or when equilibrium is established rapidly between plasma concentration and biophase (Fig. 7). Examples of direct responses are neuromuscular blocking agents, etc., where the response is directly related to the drug concentration and pharmacological effect can be seen immediately.

Those target tissues are often not in the blood and therefore, it is necessary to establish the relationship between plasma concentrations (PK) and the concentrations at the target tissue to understand the PK/PD relationship. The concept of "biophase" (target tissue) was first introduced by Segre in 1968 (51). Indirect PK/PD response is used to describe the case where the time course of PD is time-shifted from that of the PK that is, the maximum PD response does not occur at the maximum blood concentration (Fig. 8). Such responses occur when the pharmacological effects are results of a cascade of events such as induction, synthesis, secretion or cell trafficking. The very first work in this area was done with anticoagulants by Levy et al. (52,53). The diagram below shows the effect compartment model where the rate of onset and offset of effect is governed by the drug distribution and elimination from the biophase (effect compartment or target tissue).

Basic PK/PD Models

The relationship between PK and PD time courses is usually derived using a PK/PD model. Either observed or model-predicted blood concentrations are used as the forcing function for the PD response and the appropriate PD response parameters for example, E_{max}, EC_{50}, and γ in the Hill equation are estimated. PK/PD modeling enables us to quantify pharmacological effects as a function of time in relation to drug concentrations. The direct effect and indirect

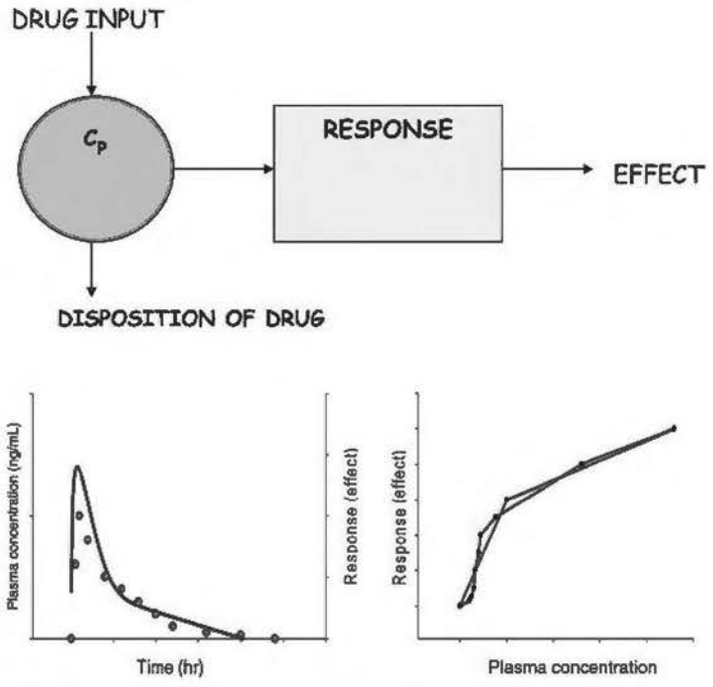


Figure 7 Illustration of a direct pharmacokinetic pharmacodynamic model. The solid line represents PK profile and the dots represent the PD measures.

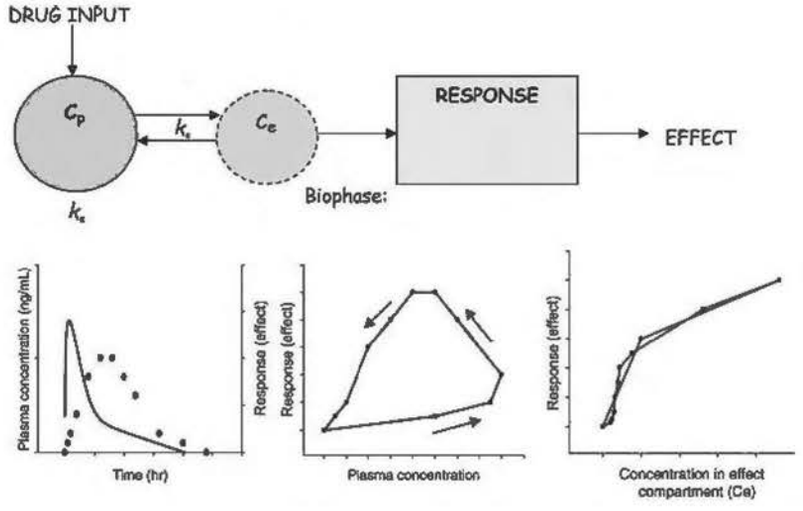


Figure 8 Illustration of an "indirect" or delayed pharmacokinetic pharmacodynamic effect. In the left panel, the solid lines represent the PK profile and the dots represent PD measures.

effect compartment models shown above are two of the simplest models to describe PK/PD relationships. As stated above, there are many other types of pharmacological responses which cause delayed responses. Because of the diversity of in vivo pharmacological responses, the variety of PK/PD models is quite large and cannot be dealt with in detail here.

BIOTHERAPEUTICS FORMULATION AND IMMUNOGENICITY

All biotherapeutics are potentially immunogenic, and this immunological reaction has the potential to impact the biopharmaceutics of the product. Thus, understanding and mitigating the causes of immunogenicity are critical to the successful application of the biotherapeutic (54).

The causes of immunogencity of biotherapeutics vary widely, and is not necessarily related simply to the amino acid sequence being of foreign origin. General immunological or safety concerns with protein therapeutics include acute infusion or injection site reactions (anaphylactic or anaphylactoid), serum sickness, effects related to the generation of antibodies against the therapeutic, as well as antibodies to therapeutic that may cross-react with endogenous proteins. The latter type of immunological reaction carries the greatest risk because of its potential to impact both safety and efficacy (55,56).

Therapeutic proteins can lead to antibody induction via two pathways: a T cell independent and a T cell dependent pathway (57 60). Analysis of antibodies from clinical studies suggests that IgG antibodies make up the majority of the antidrug antibody (ADA) responses, implicating the T cell dependent pathway as the primary mechanism.

The T cell dependent pathway requires a cognate T cell B cell interaction. To initiate the response, the protein must interact with antigen-presenting cells (APCs) such as dendritic cells (DCs), B cells, or macrophages. APCs internalize the antigen (i.e., therapeutic protein), digest it in the endosome, generate peptides that can be loaded into an appropriate MHC class II molecule and present them in a linear conformation on the surface as a complex. These peptides are called T-cell epitopes and may be recognized by T-cell receptors on naïve T (helper) cells in lymph nodes. In parallel naïve B cells also take up the antigen via their specific membrane-bound antigen (B-cell) receptors, process and subsequently present epitopes in MHC class II molecules on their surface. Helper T cells that have been already activated by recognizing the epitope on the APCs, must then proliferate, migrate and encounter B cells with the same epitope on the same MHC class II molecule at the lymphoid follicles. Binding of the T-cell receptor to the peptide:MHC class II complex on the B-cell surface then leads to the expression of costimulatory molecules and secretion of cytokines from T-cell surface that trigger the B cell to differentiate and mature into antibody-secreting cells. A mature but naïve B cell will initially produce an IgM response. Further helper T-cell interactions induce isotype switching to IgG (and other isotype) responses. This T cell dependent immune response is usually long lasting and of high titer. Once the switch has occurred, some of the activated B cells become long-lived memory cells which react rapidly to rechallenge with the characteristic IgG production. This mechanism requires that B cells (via B-cell receptors) and T cells respond to the same antigen although not necessarily the same epitope. Another important requirement is a costimulatory signal to activate the T cells. These costimulatory molecules can be induced by infection or inflammation a distress or danger signal in the form of cytokines such as tumor necrosis factor (TNF). In the absence of these distress signals, the peptide:MHC class II complex alone on the APC cannot activate the T cells, thus promoting anergy or tolerance in naïve T cells. On the other hand, the presence of additional molecules that are associated with the therapeutic protein that act like adjuvants (e.g., HCPs or endotoxins), can activate toll-like receptors on the APCs, and may result in reversing tolerance or abrogating T- and B-cell anergy, thus inducing the generation of an immune response.

B cells can also be activated without cognate T-cell help by the so-called T cell independent pathway. For this purpose, the antigen has to be engulfed by specialized blood-borne peripheral DCs, and presented to B cells. B-cell stimulatory signals are generated when a number of B-cell receptors simultaneously bind to the antigen resulting in their crosslinking and subsequent cell proliferation. A costimulatory signal (e.g., a cytokine) is however required for the activation step. Antibodies produced in this situation are of the IgM type, transient, of low titer and poor specificity. Because of lack of affinity maturation, there is no class switching or generation of memory. This pathway is typically evoked by particulate antigens displaying repetitive epitopes termed pathogen-associated molecular patterns, usually found on bacteria. Again, delivery of a second signal by helper T cells or via pathways mediated by Toll-like receptors would allow for affinity maturation and class switching, creating a more efficient IgG response.

Table 1 Factors That May Impact Immunogenicity of Biotherapeutics

Product related factors	Patient related factors	Treatment related factors
Protein structure (human/nonhuman, posttranslational or chemical modifications)	Disease state being treated	Dose
Product quality parameters (isoforms, chemical and physical degradants)	General immune status of patient	Route
Contaminants and impurities	Genetic background (MHC genotype, HLA phenotypes)	Frequency of dosing
	Concurrent illnesses and concomitant therapy	Length of treatment

Immune response to foreign (exogeneous) proteins also called the "classical" immune response, arises via the T cell dependent pathway. On the other hand, the human immune system is usually tolerant or anergic to proteins of human origin. In the absence of a neoantigen, an immune response against a human protein though not impossible, is highly unlikely unless the protein is presented to the immune system in a fashion that can reverse tolerance or T- and B-cell anergy by the above T cell dependent pathway. The likelihood of breakage of tolerance to proteins of human origin or recombinant autologous proteins, is considered a function of the abundance of the endogenous soluble protein. For proteins of low abundance, the immunological tolerance is not complete. T and B cells specific for low-abundance proteins (autoantigens) may not be completely eliminated during early development. Under sufficient provocation (e.g., presence of molecules with adjuvant-like characteristics), these might generate an immune response.

ADAs are broadly classified as binding (BAbs) or neutralizing (NAbs). For biologics of human origin, BAbs and NAbs are of concern because of the possibility of impacting efficacy and PK. BAbs bind to the protein but do not neutralize it. They may mediate infusion reactions or alter the PK/PD profile of the therapeutic. BAbs can enhance clearance or prolong systemic exposure. BAbs can be precursor or triggers for the generation of NAbs through epitope spreading. NAbs bind to the therapeutic molecule and disrupt its ability to bind to the target, that is, neutralize its function. When present at low titers, the impact on efficacy may be minimal but efficacy and biological activity may be impacted at high titers. The most serious type of NAbs response are those that cross-react and neutralize the function of the endogenous analog, especially one that serves a biologically unique function and has no redundancies (61).

There are many factors that can be involved in breaking of tolerance to a protein biotherapeutic and can be broadly classified into three categories as given in Table 1.

Although the factors are categorized above, in practice it is very difficult to deconvolute the impact of specific product attributes from the number of patient and dosing regimen related factors (62 64).

When considered from the perspective of a product development scientist, the causes of immunogencity can be divided into two broad categories.

1. Intrinsic to the molecule and treatment regimen
2. Extrinsic factors related to CMC aspects of the product

Immunogenicity as a Consequence of Molecule and Treatment Aspects of the Biotherapeutic

This category is concerned with the selection and design of the molecule itself and is often the result of a discovery effort intended to realize a certain therapeutic effect. A detailed consideration of this category is therefore outside the scope here but some relevant concepts are covered to provide a background for the subsequent discussion. A nonhuman protein (e.g., streptokinase, botulinum toxin) will induce antibodies by the classical immune response. A similar response can be generated in people who do not have tolerance to a certain protein. For

example, patients with severe hemophilia A involving large deletions or nonsense mutations of the factor VIII gene are more likely to have an antibody response to exogeneous factor VIII than patients with mild or moderate disease since patients with the severe form of the disease do not express functional factor VIII antigen and hence have no immune tolerance (65). In these instances, the generation of ADAs may be considered as a vaccine-like reaction to a foreign protein. As in vaccines, the response is related to a number of factors such as (number, frequency and amount) of dose administered, length of treatment, delivery route, and presence of "adjuvants" (66). More surprising is the observation that "self" proteins can induce an immunological response even in individuals who are not deficient in the protein, but simply produce an insufficient amount for the desired biological effect (67).

Foreign proteins can induce antibodies after a single injection while human proteins may require longer exposure of up to six months (68). Yet, as exemplified by insulin and growth hormone, chronic therapy need not compromise the therapeutic efficacy of the protein. The fact that both types of proteins can induce antibodies implies that the molecular characteristic evoking antibody response is at least more complex than simply being self or nonself to the human system. Nature of the therapeutic (immunostimulatory vs. immunosuppressive) proteins and host immune status also play a role in the observed effect. Cell surface binding therapeutic antibodies generally will have more potential to be immunogenic than those that interact with soluble targets.

The probability of an antibody immune response is considered highest after SC injection, followed by IM, intranasal, and intraveneous routes. SC administration localizes the protein to a small area with a short path to drain into the lymph nodes where B and T cells are present (69). Clinical experience with pulmonary administration of insulin suggests that this route also carries a high risk for generation of immunological reaction (70).

The type of disease plays a role, likely related to the immune status of the patient. Patients with weak or compromised immune systems or those on immune-suppression therapy are less likely to develop ADAs than those with intact immune systems. Acute therapy is less likely to be immunogenic than chronic therapy, although intermittent treatment is more likely to elicit a response than continuous therapy. Also, lower doses are generally more immunogenic than higher, probably related to the fact that the immune system is generally less tolerant of low-abundance proteins.

Porter (71) has prepared a comprehensive review of the literature on immune response to recombinant proteins used in therapy. Among the significant conclusions drawn are that the presence of antibodies has not necessarily been detrimental to the clinical efficacy and that no particular property of a protein has been identified as an obvious predictor of immunogenicity in humans.

Immunogenicity as a Consequence of Chemistry Manufacturing and Control (CMC) Aspects of the Biotherapeutic

The characteristics of a parenteral products are determined by three major factors: process, formulation and package. From the perspective of a product development scientist, the CMC aspects that can play a role in the immunogenicity profile of the product begin with the gene design and cell line selection. Gene sequences are mutated to avoid degradation and aggregation hotspots as well as antigenic epitopes, while maintaining potency (72 74). The choice of host cell line determines the presence (or absence) of glycosylation and the glycosylation pattern. The upstream (bioreactor/fermentation, harvest) process impacts the distribution of glycoforms and other product variants, for example, deamidated variants, disulfide scrambling, and also determines the type of host cell impurities that may ultimately remain in the product. The protein then further undergoes a complex series of processing steps for purification including viral removal. While the overall objective of the post harvest steps is to purify the protein by removing impurities (e.g., host cell proteins, DNA, endotoxins), and product related species (e.g., truncated, hydrolyzed, aggregated, deamidated, oxidized, and improperly glycosylated forms), it is nevertheless impossible to completely eliminate these. The current state of purification processes is such that impurities are routinely reduced to levels well below what is considered a risk in the particular case. Product variants are not as

easily eliminated and a certain minor fraction of some or all of these variants make their way into the final bulk solution. The bulk solution after the purification steps may be stored for a period of time either as a liquid or frozen, before it is finally filtered and pumped into vials or syringes. In some cases, it is subject to the final processing step of lyophilization, before being shipped over an appropriately designed (cold) transport chain to the clinic or pharmacy. Thus, an important objective of the process and formulation development is to stabilize the native state of the molecule and minimize physical and chemical degradation over the shelf-life of the product.

Impact of Process and Formulation

The process that a biotherapeutic undergoes in its product has a significant impact on the product characterisitc. The formulation is intended to stabilize the product during the process and during storage and use. Some aspects of the process and formulation that have the potential to impact immunogenicity are considered below.

Glycosylation. Glycosylation refers to the enzymatic addition of saccharides to the protein as a post-traslational modification. Glycosylation is present in approximately 50% of human proteins and an almost similar proportion of approved biopharmaceuticals. The presence and nature of the glycoform may impact primary functional activity, folding, stability, trafficking and immunogenicity. Although glycosylation is in a way intrinsic to the molecule, it can also be impacted by the production process. For this reason, the choice of the expression system is a critical activity in the development of a biotherapeutic. As mammalian expression systems produce mainly human glycans, these have become the dominant platform for production of therapeutic glycoproteins. However, these platforms require good process control since they display an inherent glycan heterogeneity that is sensitive to culture conditions. Glycosylation can have direct impact on immunogenicity through patterns that are not present in humans. CHO cells produce gycosylation patterns that are close to human, although these cells also express *N*-glycolylneuranimic acid (NGNA), a form of sialic acid not found in humans and reported as immunogenic. Mouse cell lines (e.g., NS0, SP2/0) also produce NGNA in addition to or instead of the *N*-acetylneuraminic acid (NANA) present in human IgGs (75,76). Galactose α(1-3) galactose linkages or terminal α(1-3) galactose can also be added by murine cells (e.g., C127, NS0, SP2/0). This residue has been shown to be recognized by up to 1% of circulating IgG in humans (77). Glycoslation can have an indirect effect on immunogenicty through its impact on folding solubility and (structural) stability. Glycosylation can affect local secondary structure and thereby direct the generation of tertiary structure. Altered or absent glycosylation can therefore alter or eliminate epitopes or expose/generate new ones. Glycosylation can increase solubility by shielding hydrophobic patches and reducing tendency to aggregate, and enhance stability by participating in intrachain stabilizing interactions (78 80).

Purity. Host cell proteins and DNA are contaminants that carry the risk of functioning as adjuvants and thus triggering an immunogenic reaction to the therapeutic given the appropriate antigenic determinants. Lundin et al. (81) summarized that the early pituitary preparations of hGH resulted in about 45% patients developing antibodies. Improvements in processing and purification led to a marked decrease in antibody formation to less than 10% (pituitary source), while it was <2% for the purest commercial pituitary preparation. Early recombinant preparations, on the other hand, also led to unexpectedly high antibody levels, but were related to *E coli* proteins remaining as impurities in the preparations (82). Bacterial DNA contains unmethylated CpG motifs that are known to activate Toll-Like receptors and are themselves being studied as adjuvants for vaccines. Process improvements resulting in greater purity by reduction of product-related and unrelated species have led to a clear reduction in ADA response. Current purification processes reduce host cell and process contaminants to very low levels.

Product-related impurities and degradation products. Product-related impurities and degradation products for biotherapeutics often overlap and are not readily distinguishable.

For example, charge variants encoded as a consequence of the cell line (e.g., sialylation) and/or generated in the upstream/downstream processes will often overlap with deamidation/isomerization products. Oxidation of susceptible residues can occur at any stage in the production process or subsequent storage and use, as can fragmentation/hydrolysis. Finally, size variants such as truncated, misfolded and aggregated species can also arise at all stages. However, among all the possible chemical and structural changes, the one that causes the most concern is aggregation involving association of multiple protein molecules in partially/wholly unfolded forms, and even in their native state.

Aggregates can form as a result of a variety of interactions between the protein molecules including hydrophobic interactions as well as because of covalent changes caused by chemical modifications such as oxidation. The protein molecules making up the aggregates can be in their native, partially or fully unfolded states. Aggregation is governed by the conformational, that is, thermodynamic stability self-association tendency, that is, colloidal stability of the molecule. A full discussion of the mechanisms of aggregation is outside the scope of this chapter but a number of good reviews are available [Chi et al. (83), Wang (84), Mahler et al. (85)]. Other factors that can impact the level of aggregation in a protein solution includes conditions such as pH, temperature, concentration, ions and ionic strength and stresses such as freeze/thaw, air/liquid and liquid/solid interfacial stress. Chemical modifications such as oxidation can also lead to loss of structural stability and aggregation. Since a protein can undergo aggregation by multiple pathways, all of these factors have to be addressed as part of the formulation development program for the biotherapeutic.

Aggregates are hypothesized to cause immunogenicity through their "repetitive" display of epitopes that are seen by the immune system as resembling the external surfaces of invasive species. As reviewed by Rosenberg (86), it is not the low MW aggregates such as dimers or trimers but the large multimers with molecular weights exceeding 100 kDa that are efficient inducers of immune responses. Native aggregates in which the protein retains a large part of its structure are of greater concern since antibodies could be generated against epitopes that are present on the native monomeric version. Antibodies generated against nonnative aggregates (generated by misfolded species or chemical modifications) could still result in increased clearance as well as raise potential safety concerns. Experiments on animal models have shown that aggregated proteins can lead to an immunological response, but the relevance to human experience is debated (61,87,88).

Aggregation is considered a strong risk factor for generation of immunological reaction and therefore has to be minimized by proper design of process and product. It is a fundamental attribute to assess the quality of a biotherapeutic and control of this parameter is an important aspect of biotherapeutic product development.

Container/Closure System

Container/closure are an integral part of a biological product, be it a vial/stopper, a prefilled syringe or a dual-chamber cartridge. Some component materials which come into contact with the product include the container (glass or plastic, vial or syringe), closure (stopper), administration and infusion components (syringes, bags, infusion lines). The concern for packaging component-dosage form interaction for biologics again arises because of the potential for alteration of the structure of the protein through aggregation or chemical degradation pathways such as oxidation. The impact on the protein can occur directly through the container interface, but also indirectly through any chemical compound that may leach out of the container. Some common leachables from the common container/closures used for biologics include metals, antioxidants, plasticizers, lubricants as well as degradation products of the various components. For example, tungsten residues left behind when preparing staked-needle syringes have been shown to cause oxidation of protein solutions. The FDA also considers the compatibility of container/closure with product as a key requirement in the development of parenteral products. The FDA guidance document on container closure considers inhalation aerosols and solutions, injections and injectable suspensions as products with the greatest level of concern when accounting for route of administration and risk for packaging component-dosage form interaction (89).

Silicone oil coating is commonly used on stoppers and on the inside of syringes or cartridges as a lubricant to enable movement of the plunger. Silicone oil contamination by the syringes used for injecting insulin has been well documented [Chantelau et al. (90), Bernstein (90,91)]. Current processes for siliconization of prefilled syringes or cartridges apply well controlled amounts and involves baking of the silicone emulsion. This tends to reduce the levels of silicone oil extracted into the formulation but the possibility exists. Fibrous aggregates have been shown to form in a a number of model proteins when incubated with silicone oil (92).

Selection of the container/closure system for any product is a critical task. The container/closure must provide adequate protection to the product from the environment and prevent contamination. It must also be compatible with the product and not leach any compunds that could harm the product or pose a safety risk. The experience with vials and stoppers is extensive, but use of devices such as inhalers and injectors increases the complexity of this task.

Safety/Tolerability of Excipients

As stated before, formulation development for a biologic is carried out to identify the optimal composition that will keep the biologic stable for an economically viable length of time. The product format can be a liquid or a lyophilized powder. Excipients are added to accomplish this. A review of the formulation composition for biologics shows that the vast majority comprise a buffer, a tonicity modifier, cryo- or lyoprotectant, and a surfactant. Other additives such as a chelator, antioxidant, and a preservative are occasionally found (93). Most common excipients are generally safe and have long precedence of use, although precautions may be in order in certain cases. Among the common ingredients is the surfactant polysorbate 20 or 80, comprising partial fatty acid esters of sorbital and its anhydrides copolymerized with ethylene oxide. These is known to cause anaphylactic reactions in dogs, and may have an allergenic potential in susceptible individuals. Intravenous immunoglobulin (IVIG) therapy has been connected with numerous episodes of acute renal toxicity and osmotic nephropathy because of a very high sucrose load. The sucrose is added to the product to reduce formation of aggregates as a consequence of the pathogen-removal steps in the process. Sucrose and sorbitol as well as maltitol and fructose can also be contraindicated in patients with hereditary fructose intolerance, the glucose-galactose malabsorption syndrome or sucrose-isomaltase deficiency.

Evaluation and Prediction of Immunogenicity

Animal models have traditionally been used to evaluate the safety of (bio)pharmaceuticals, but their utility in evaluation or prediction of clinical immunogenicity is controversial. Data generated from the animal models must be placed in context of the type of molecule. Bugelski and Treacy (94) group recombinant proteins into classes on the basis of preclinical immunogenicity. For some classes, for example, bacterial proteins, immunogenicity in animals is often predictive for humans. For others, such as fully human proteins, even data from nonhuman primates may have little predictive value. Nonhuman primates with a high level of sequence homology with humans are often seen as most relevant. However, the evidence for success is limited, and mainly governed by the degree of conservation across species. Limited homology means that the animal models are generally over-predictive of human immunogenicity. Transgenic mice that express the appropriate human transgene allow the protein to be tested without generating a xenogenic response. There are many caveats and limitations of this approach (62,94,95), the least of them being that the wild-type strain must be capable of making antibodies to the protein in questions. Limitation in the use of animal models is magnified when trying to decipher the relative impact on immunogenicity of a few percent of product degradants. To be able to detect such changes, the animal models must have a low baseline immune response or a slow development trajectory for immunogenicity, while the studies have to be carefully controlled. In summary, the utility of animal models would primarily lie in assessing the relative immunogenicity risk of CMC related factors.

Computational tools are also being developed for assessment of intrinsic immunogenicity of protein therapeutics including identification and modification or removal of T-cell epitopes (72). Further research is required to develop models with the ability to assess the impact of CMC factors in general and aggregation in particular on immunogenicity.

BIOTHERAPEUTICS BIOEQUIVALENCE/COMPARABILITY

Manufacturers of biotechnological/biological products frequently make changes to manufacturing processes of their products both during development and post-approval. These changes, however minor, could cause undetectable changes in the physicochemical composition of the primary active ingredient of the drug substance or in the profile coproduced compounds such as host cell proteins and other potential impurities. Also, as discussed in previous sections, the dose, frequency, and route of administration all have the potential to change the PK/PD and immunogenicity characteristics. Thus, even minor changes in the drug manufacturing and/or administration process have the potential to affect the overall safety/efficacy profile of the drug product. Demonstration of comparability of the pre- and postchange product is a sequential process, beginning with quality studies (limited or comprehensive) and supported, as necessary, by nonclinical, clinical and/or pharmacovigilance studies. For most changes to the manufacturing process, physicochemical and (quality-related) biological testing can demonstrate that there is no difference in quality of the product that could adversely impact the safety and efficacy of a product. Thus the comparability exercise may be limited to strict process validation of the change or be extended to various quality criteria such as in-process controls, thorough analytical and biological characterization of the product and stability data. However, sometimes an effect on efficacy and/or safety can be expected on the basis of observed difference(s) or cannot be ruled out in spite of the state of the art physicochemical and biological tests. In such cases, additional nonclinical and/or clinical studies will be necessary.

PK studies are a key component of the in vivo comparability testing and are typically performed when analytical characterization is not sufficient to detect differences, or the clinical implication of analytically detected differences is unknown. The study could be performed in animals, if a relevant animal model exists, or in humans. PK studies may not be appropriate for comparability testing when the PK variability of the reference product is in general very high or when the PK variability is of no clinical relevance, when PK is insensitive to clinically relevant changes to the active substance (e.g., in the case of misfolded proteins), drug is active at the site of administration and blood exposure is not a relevant biomarker for safety/efficacy. Despite these limitations, PK testing remains a valuable comparative tool.

Some of the key considerations of a PK for biologics study are: the study population patients or subjects, dosing regimen single or multiple doses, parallel or crossover study, the duration of sampling, route and method of administration, doses for evaluation in the study, PK parameters of interest, and the criteria for claiming equivalence. While many of these considerations are also relevant for a small-molecule drug, the PK characteristics of biologics pose a unique challenge in the design and conduct of these studies. The choice of study population depends on the PK and safety profile of the compound of interest and its mode of action. For compounds that are generally well-tolerated and where the PK in healthy subjects is known to be predictive of that in the target patient population, healthy subjects might be appropriate for comparative testing. In other cases, a patient PK study might be considered, especially where relevant PD information can also be gathered. Similarly, many biologics have a long half-life, from days to weeks. Therefore, standard crossover studies can pose limitations due to the duration of treatment and follow-up. Parallel studies could be considered if the duration of the study could become unfeasible. Furthermore, the potential for immunogenic reactions, typically observable after three to four weeks after a single dose, should also be considered in crossover designs. The route of administration should be in accordance with the intended clinical use. If the product is planned to be administered by more than one route (e.g., SC and IV), it may become necessary to test all routes. The selected dose should be in the steep portion of the dose-response curve to detect relevant differences, especially if PD markers are being monitored in the study. Apart from standard PK parameters describing absorption and

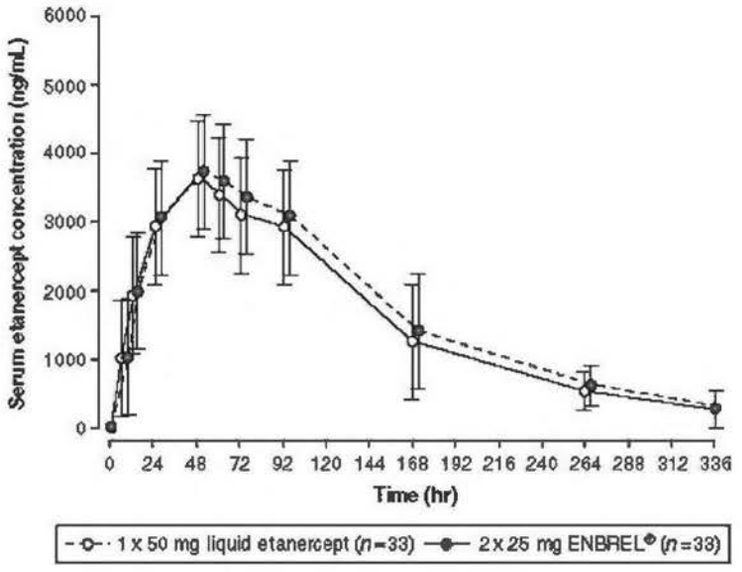


Figure 9 Illustration of pharmacokinetic equivalence for two formulations of etanercept. *Source*: From Ref. 97.

bioavailability (such as C_{max} and AUC), other PK parameters such as elimination half-life and clearance should also be considered for comparability, because of potential changes in the heterogeneity of active substance due to process changes.

The following example by Sullivan et al. (96) illustrate the concept of PK-based comparability assessment for a new formulation of Enbrel® (etanercept). Etanercept is a soluble, fully human, TNF receptor that competitively inhibits the interaction of TNF with cell surface receptors. Etanercept is currently approved for reducing signs and symptoms, inhibiting the progression of structural damage, and improving physical function in patients with rheumatoid arthritis. It is also approved for reducing the signs and symptoms and inhibiting the progression of structural damage in patients with psoriatic arthritis and for reducing the signs and symptoms of active ankylosing spondylitis, juvenile rheumatoid arthritis, and psoriasis. Etanercept was originally introduced commercially in vials containing 25 mg lyophilized powder requiring reconstitution, and to date most patients have received the reconstituted formulation. A 50-mg/mL liquid formulation supplied in a prefilled syringe was approved recently for commercial use. Sullivan et al. (96) present the results of a study in healthy volunteers comparing the PK of the liquid etanercept formulation with that of the reconstituted formulation (Fig. 9). The study was conducted in healthy male and female subjects, where each subject received both formulations (50 mg of etanercept per dose) in a crossover fashion with a minimum of 28 days washout period in between doses. The following PK parameters, obtained from the observed PK profile using noncompartmental analysis, were reported: AUC (to till the final sample collection timepoint and extrapolated to infinity), C_{max}, T_{max}, and terminal $t_{1/2}$. The point estimate of the ratio of geometric means of the PK parameters (AUC and C_{max}) were generated along with their 90% confidence intervals. Equivalence of the two formulations was concluded since the 90% confidence interval of the ratio of PK parameter means lay between 80% and 125%, which is the standard bioequivalence criterion.

Similarly, Paulson et al. (97) performed a PK comparability assessment for adalimumab (Humira) in healthy subjects between two administration routes as an autoinjector pen and a prefilled syringe. Adalimumab is a murine monoclonal antibody prescribed for the treatment of rheumatoid arthritis, and has a half-life of two to three weeks (PI). Therefore, a parallel group study in 290 subjects was performed in this case to assess the PK equivalence in this

case. The duration of PK assessment was appropriately adjusted to account for the long half-life. The PK and statistical data analysis was similar to that described by Sullivan et al. Comparability was concluded in this case also.

REFERENCES

1. Wagner D. History of pharmacokinetics. Pharm Ther 1981; 12:537 562.
2. Meibohm B. Pharmacokinetics and Pharmacodynamics of Biotech Drugs. 1st ed. Weinheim: Germany, Wiley VCH, 2006.
3. Martinez MN, Amidon GL. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. J Clin Pharmacol 2002; 42:620 643.
4. Yalkowsky SH, Krzyzaniank JF, Ward GH. Formulation related problems associated with intrave nous drug delivery. J Pharm Sci 1998; 87:787 796.
5. Narazaki R, Sanghvi R, Yalkowsky SH. Estimation of drug precipitation upon dilution of pH controlled formulations. Mol Pharm 2007; 4:550 555.
6. Leo A, Hansch C, Elkins D. Partition coefficient and their uses. Chem Rev 1971; 71:525 616.
7. Shore PA, Brodie BB, Hogben AM. The gastric secretion of drugs: a pH partition hypothesis. J Pharmacol Exp Therap 1957; 119:361 369.
8. Hou T, Wang J, Zhang W, et al. Recent advances in computation prediction of drug absorption and permeability in drug discovery. Curr Med Chem 2006; 13:2653 2667.
9. Jung SS, Choi SO, Um SY, et al. Prediction of the permeability of drugs through study of quantitative structure permeability relationship. J Pharm Biomed Anal 2006; 41:469 475.
10. Sugano K, Okazaki A, Sugimoto S, et al. Solubility and dissolution profile assessment in drug discovery. Drug Metab Pharmacokinet 2007; 22:225 254.
11. Wurster DE, Taylor PE. Dissolution rates. J Pharm Sci 1965; 54:169 175.
12. Lu ATK, Frisella ME, Johnson KC. Dissolution modeling. Factors affecting the dissolution rates of polydisperse powders. Pharm Res 1993; 10:1308 1314.
13. Hirano K, Yamada H. Studies on the absorption of practically water insoluble drugs following injection VIII. Comparison of the sub cutaneous absorption rates from aqueous suspensions in mouse, rat and rabbit. J Pharm Sci 1983; 72:608 612.
14. Ran Y, He Y, Yang G, et al. Estimation of aqueous solubility of organic compounds by using the general solubility equation. Chemosphere 2002; 48:487 509.
15. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 2008; 84(5):548 558.
16. Porter CJH, Charman SA. Lymphatic transport of proteins after subcutaneous administration. J Pharm Sci 2000; 89(3):297 310.
17. Patton J. Breathing life into protein drugs. Nat Biotechnol 1998; 16(2):141 143.
18. Patton JS, Fishburn CS, Weers JG. The lungs as a portal of entry for systemic drug delivery. Proc Am Thorac Soc 2004; 1(4):338 344.
19. Rowland M, Tozer TN. Clinical Pharmacokinetics. Concepts and Applications. Malvern: Lea and Febiger, 1989.
20. Poulin P, Theil FP. Prediction of pharmacokinetics prior to in vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition. J Pharm Sci 2002; 91(5):1358 1370.
21. Poulin P, Theil FP. Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism based prediction of volume of distribution. J Pharm Sci 2002; 91(1):129 156.
22. Banks WA. Are the extracellular [correction of extracelluar] pathways a conduit for the delivery of therapeutics to the brain? Curr Pharm Des 2004; 10(12):1365 1370.
23. Banks WA, Jumbe NL, Farrell CL, et al. Passage of erythropoietic agents across the blood brain barrier: a comparison of human and murine erythropoietin and the analog darbepoetin alfa. Eur J Pharmacol 2004; 505(1 3):93 101.
24. Wallis WJ, Simkin PA, Nelp WB. Protein traffic in human synovial effusions. Arthritis Rheum 1987; 30 (1):57 63.
25. Ferraiolo BL, Mohler MA, Gloff CA. Protein Pharmacokinetics and Metabolism. New York: Plenum, 1992.
26. Stockert RJ. The asialoglycoprotein receptor: relationships between structure, function, and expres sion. Physiol Rev 1995; 7:591 609.
27. Ng CM, Stefanich E, Anand BS, et al. Pharmacokinetics/pharmacodynamics of nondepleting anti CD4 monoclonal antibody (TRX1) in healthy human volunteers. Pharm Res 2006; 23(1):95 103.
28. Agoram B, Aoki K, Doshi S, et al. Investigation of the effects of altered receptor binding activity on the clearance of erythropoiesis stimulating proteins: nonerythropoietin receptor mediated pathways may play a major role. J Pharm Sci 2009; 98(6):2198 2211.

29. Gross AW, Lodish HF. Cellular traficking and degradation of erythropoeitin and novel erythropoeisis stimulating protein (NESP). J Biol Chem 2006; 281:2024 2032.
30. LaRusso NF. Proteins in bile: how they get there and what they do. Am J Physiol 1984; 247(3 pt 1): G199 G205.
31. Szczesny G, Veihelmann A, Nolte D, et al. Changes in the local blood and lymph microcirculation in response to direct mechanical trauma applied to leg: in vivo study in an animal model. J Trauma 2001; 51:508 517.
32. Ikomi F, Hunt J, Hanna G, et al. Interstitial fluid, plasma protein, colloid, and leukocyte uptake into initial lymphatics. J Appl Physiol 1996; 81:2060 2067.
33. Iwashita S, Yanagi K, Ohshima N, et al. Insulin increases blood flow rate in the microvasculature of cremaster muscke of the anesthetized rats. In Vivo 2001; 15:11 16.
34. Franzeck UK, Doerffler Melly J, Hussain MA, et al. Effects of subcutaneous insulin like growth factor 1 infusion on skin microcirculation. Int J Microcirc Clin Exp 1995; 15(1):10 13.
35. ter Braak EW, Woodworth JR, Bianchi R, et al. Injection site effects on the pharmacokineitcs and glucodynamics of insulin lispro and regular insulin. Diabetes Care 1996; 19:1437 1440.
36. Chevalier S, Ferland G, Tuchweber B. Lymphatic absorption of retinol in young, mature, and old rats: influence of dietary restriction. FASEB J 1996; 10:1085 1090.
37. Carlson GW. Age and the incidnece of sentinel lymph node metastases in melanoma. Ann Surg Oncol 2004; 11(3):236 237.
38. Alvarez E, Ruiz Gutierrez V, Sobrino F, et al. Age related changes in membrance lipid composition, fluidity and respiratory burst in rat peritoneal neutrophils. Clin Exp Immunol 2001; 124:95 102.
39. Travis S, Menzies L. Intestinal permeability: functional assessment and significance. Clin Sci 1992; 82:471 488.
40. McLean AJ, Couteur DGL. Aging biology and geriatric clinical pharmacology. Pharmacol Rev 2004; 56:163 184.
41. Cusack BJ. Pharmacokinetics in older persons. Am J Geriatr Pharmacother 2004; 2(4):274 302.
42. Pelletier G, Lanoe J, Dunnigan J. Changes in rat gastric proteases with age: effect of hydrocortisone. Rev Can Biol 1979; 38(4):317 319.
43. Bota DA, Davies KJA. Protein degradation in mitrochondria: implications for oxidative stress, aging and disease: a novel etiological classification of mitochondrial proteolytic disorders. Mitochondrion 2001; 1:33 49.
44. Carragher NO. Calpain inhibition: a therapeutic strategy trageting multiple disease states. Curr Pharm Des 2006; 12:615 638.
45. Durnas C, Loi CM, Cusack BJ. Hepatic drug metabolism and aging. Clin Pharmacokinet 1990; 19: 359 389.
46. Tabuchi Y. The effect of age and gender on the effect of midazolam as intramuscular premedicant. Masui 1992; 41(6):938 944.
47. Divoll M, Greenblatt D, Ochs HR, et al. Absolute bioavailability of oral and intramuscular Diazepam: effects of age and sex. Anesth Analg 1983; 62:1 8.
48. Woo J, Chan HS, Or KH, et al. Effect of age and disease in two drug binding proteins: albumin ad alpha 1 acid glycoprotein. Clin Biochem 1994; 27:289 292.
49. Levy G. Kinetics of pharmacologic effects. Clin Pharm Ther 1966; 7(3):362 372.
50. Hill AV. The possible effect of the aggregation of molecules of haemoglobin on its dissociation curve. J Physiol 1910; 40:4 7.
51. Segre G. Kinetics of interaction between drugs and biological systems. Farmaco Sci 1968; 23(10): 907 918.
52. Yacobi A, Lai CM, Levy G. Pharmacokinetic and pharmacodynamic studies of acute interaction between warfarin enantiomers and chloramphenicol in rats. J Pharm Exp Ther 1984; 231(1):80 84.
53. Yacobi A, Lai C M, Levy G. Comparative pharmacokinetics of coumarin anticoagulants. XLV: pharmacokinetic and pharmacodynamic studies of acute interaction between warfarin and phenyl butazone in rats. J Pharm Sci 1980; 69(1):14 20.
54. Sauerborn M, Brinks V, Jiskoot W, et al. Immunological mechanism underlying the immune response to recombinant human protein therapeutics. Trends Pharmacol Sci 2010; 31(2):53 59.
55. Schellekens H. Immunogenicity of therapeutic proteins: clinical implications and future prospects. Clin Ther 2002; 24(11):1720 1740.
56. Rosenberg AS, Worobec A. A risk based approach to immunogenicity concerns of therapeutic protein products. part I: considering consequences of the immune response to a protein. BioPharm Int 2004; 17(11):22 26.
57. Delves PJ, Roitt IM. The immune system. Part I. N Engl J Med 2000; 343(1):37 49.
58. Delves PJ, Roitt IM. The immune system. Part II. N Engl J Med 2000; 343(2):108 117.

59. Mukovozov I, Sabljic T, Hortelano G, et al. Factors that contribute to the immunogenicity of therapeutic recombinant human proteins. Thromb Haemost 2007; 99:874 882.
60. Parkin J, Cohen B. An overview of the immune system. Lancet 2001; 357:1777 1789.
61. Ponce R, Abad L, Amarvadi L, et al. Immunogenicity of biologically derived therapeutics: assessment and interpretation of nonclinical safety studies. Regul Toxicol Pharmacol 2009; 54:164 182.
62. Herzyk D. The immunogenicity of therapeutic cytokines. Curr Opin Mol Ther 2003; 5(2):167 171.
63. Pendley C, Schantz A, Wagner C. Immunogenicity of therapeutic monoclonal antibodies. Curr Opin Mol Ther 2003; 5(2):172 179.
64. Tabrizi MA, Roskos LK. Preclinical and clinical safety of monoclonal antibodies. Drug Discov Today 2007; 12(13/14):540 547.
65. Fakharzadeh SS, Kazazian HH. Correlation between factor VIII genotype and inhibitor development in hemophilia A. Semin Thromb Hemost 2000; 26(2):167 171.
66. De Groot AS, Scott DW. Immunogenicity of protein therapeutics. Trends Immunol 2007; 28(11): 482 490.
67. Diamond B. Speculations on the immunogenicity of self proteins. Dev Biol 2003; 112:29 34.
68. Schellekens H. How to predict and prevent the immunogenicity of therapeutic proteins. Biotechnol Annu Rev 2008; 14:191 202.
69. van Regenmortel MHV, Boven K, Bader F. Immunogenicity of biopharmaceuticals: an example from erythropoietin. BioPharm Int 2005; 18(8):36 52.
70. Fineberg SE, Kawabata T, Finco Kent D, et al. Antibody response to inhaled insulin in patients with type 1 or type 2 diabetes. An analysis of initial phase II and III inhaled insulin (exubera) trials and a two year extension trial. J Clin Endocrinol Metab 2005; 90:3287 3294.
71. Porter S. Human immune response to recombinant human proteins. J Pharm Sci 2001; 90(1):1 11.
72. De Groot AS, McMurry J, Moise L. Prediction of immunogenicity: in silico paradigms, ex vivo and in vivo correlates. Curr Opin Pharmacol 2008; 8:620 626.
73. Hwang I, Park S. Computational design of protein therapeutics. Drug Discov Today Technol 2008; 5(2 3):e43 e48.
74. Wang X, Das TK, Singh SK, et al. Potential aggregation prone regions in biotherapeutics: a survey of commercial monoclonal antibodies. MAbs 2009; 1(3):254 267.
75. Sethuraman N, Stadheim TA. Challenges in therapeutic glycoprotein production. Curr Opin Biotechnol 2006; 17:341 346.
76. Shantha Raju T. Glycosylation variations with expression systems and their impact on biological activity of therapeutic immunoglobulins. BioProcess Int 2003; 1(4):44 53.
77. Galili U, Macher BA, Buehler J, et al. Human natural anti α galactosyl IgG. II. The specific recognition of α(1 3) linked galactose residues. J Exp Med 1985; 162:573 582.
78. Jefferis R. Glycosylation of recombinant antibody therapeutics. Biotechnol Prog 2005; 21:11 16.
79. Sola RJ, Griebenow K. Effects of glycosylation on the stability of protein pharmaceuticals. J Pharm Sci 2009; 98(4):1223 1245.
80. Walsh G, Jefferis R. Post translational modifications in the context of therapeutic proteins. Nat Biotechnol 2006; 24(10):1241 1252.
81. Lundin K, Berger L, Blomberg F, et al. Development of anti hGH antibodies during therapy with authentic human growth hormone. Acta Paediatr Scand 1991; 372(Suppl):167 168.
82. Massa G, Vanderschueren Lodeweyckx M, Bouillion R. Five year follow up of growth hormone antibodies in growth hormone defieient children treated with recombinant human growth hormone. Clin Endocrinol 1993; 38:137 142.
83. Chi EY, Krishnan S, Randolph TW, et al. Physical stability of proteins in aqueous solutions: mechanisms and driving forces in non native protein aggregation. Pharm Res 2003; 20(9):1325 1336.
84. Wang W. Protein aggregation and its inhibition in biopharmaceuticals. Int J Pharm 2005; 289:1 30.
85. Mahler H C, Freiss W, Grauschopf U, et al. Protein aggregation: pathways, induction factors and analysis. J Pharm Sci 2009; 98(9):2909 2934.
86. Rosenberg AS. Effect of protein aggregates: an immunologic perspective. AAPS J 2006; 8(3):E501 E507.
87. Jahn E M, Schneider C. How to systematically evaluate immunogenicity of therapeutic proteins regulatory considerations. N Biotechnol 2009; 25(5):280 286; [Epub April 5, 2009].
88. Rosenberg AS, Worobec A. A risk based approach to immunogenicity concerns of therapeutic protein products. Part III: effects of manufacturing changes on immunogenicity and the utility of animal immunogenicity studies. BioPharm Int 2005; 18(1):32 36.
89. FDA. Guidance for industry: container closure systems for packaging human drug and biologics, 1999.
90. Chantelau EA, Berger M, Bohlken B. Silicone oil released from disposable insulin syringes. Diabetes Care 1986; 9(6):672 673.

91. Bernstein RK. Clouding and deactivation of clear (regular) human insulin: association with silicone oil from disposable syringes. Diabetes Care 1987; 10(6):786 787.
92. Jones LS, Kaufman A, Middaugh CR. Silicone oil induced aggregation of proteins. J Pharm Sci 2005; 94(4):918 927.
93. Carpenter JF, Pikal MJ, Chang BS, et al. Rational design of stable lyophilized protein formulations: some practical advice. Pharm Res 1997; 14(8):969 975.
94. Bugelski PJ, Treacy G. Predictive power of preclinical studies in animals for the immunogenicity of recombinant therapeutic proteins in humans. Curr Opin Mol Therap 2004; 6(1):10 16.
95. Chapman K, Pullen N, Graham M, et al. Preclinical safety testing of monoclonal antibodies: the significance of species relevance. Nat Rev Drug Discov 2007; 6:120 126.
96. Sullivan JT, Ni L, Sheelo C, et al. Bioequivalence of liquid and reconstituted lyophilized etanercept subcutaneous injections. J Clin Pharmacol 2006; 46(6):654 661.
97. Paulson S, Noertersheuser P, Garimella T, Doan T, Assessment of relative bioavailability, safety, and tolerability of single doses of adalimumab administered via an autoinjector pen and a prefilled syringe. J Am Acad Dermatol 2007; 56(2) Suppl 2, AB9.

4 | Preformulation

N. Murti Vemuri

INTRODUCTION

Parenteral medication refers to drugs administered by routes other than the oral, typically implying injectable medications. Injectable medications could be presented in various volumes (small volume and large volume), primary packaging (ampoules, vials, cartridges, bags) and specified routes (e.g., intravenous, intramuscular). Many of the preformulation and formulation principles applicable to injectable medications can often be extended to ophthalmic and nasal spray dosage forms as well.

Rational formulation development of parenteral medication should be based on the desired product profile, the physicochemical properties of the drug substance and its interaction with other formulation ingredients, primary packaging components under storage conditions defined by the product profile, as well as the pharmacokinetic properties of the drug substance. Preformulation research comprises pharmaceutical and analytical investigations in acquiring such knowledge base, and these investigations both precede and support formulation development.

On a drug development timescale, preformulation research enables data-driven decisions related to the drug substance and drug product such as salt form selection, polymorph selection, excipient selection, identification of suitable toxicology formulations, and, finally, selection of compositions for clinical and commercial formulations. Additionally, understanding the physical and chemical attributes of the drug substance can often help in troubleshooting formulation, stability, and processing issues that may arise.

Many good reviews and book chapters (1) have been written on the subject of preformulation and physicochemical characterization of drug substances. Although most articles focus on oral formulations, many of the principles carry over to development of parenteral medications. This chapter will attempt to focus more on aspects relevant to development of parenteral dosage forms. Much of the discussion will focus on small molecules and solutions dosage forms, but later sections will touch on specificities related to macromolecules and specialized dosage forms.

CHARACTERIZATION OF THE DRUG SUBSTANCE

Understanding the physicochemical properties of the drug substance is the first step (2) toward building quality into a product using rational formulation design. Drug substances are investigated at various levels of scrutiny to fully understand their behavior at the molecular/material level, at the particulate level and also at a bulk property level. Table 1 shows a representation of this hierarchy of physicochemical properties. The intended dosage form often dictates where to place the greatest emphasis. For a solid dosage form, it is important to also fully understand the bulk properties, but for parenteral dosage forms, greater emphasis is on understanding the molecular and material properties of the active pharmaceutical ingredient (API).

Molecular Properties

Prior to initiation of preformulation studies, the molecular structure of the drug substance is identified and confirmed by appropriate spectroscopic (NMR, MS) evidence. The material is further identified by its characteristic IR and UV spectrum.

Physicochemical Constants

Two key physicochemical constants of importance are the partition coefficient and the ionization constant. The partition coefficient is an indication of the lipophilicity of a compound and is measured as a ratio of the equilibrium concentrations of the drug in an oily (e.g., octanol) and an aqueous (e.g., water) phase in contact with each other and held at a constant temperature. The

Table 1 Physicochemical Properties of Drug Compounds

Molecular properties *Properties defined by the molecular structure*	**Material properties** *Properties intrinsic to the material or particle*	**Bulk properties** *Properties related to bulk powders*
Molecular weight	Salt form	Powder flow
log *P*/log *D*, pK_a	Crystal form (XRPD)	Bulk density
Chemical stability	Crystal habit	Wettability
Melting point		Powder electrostatics
Solid state stability		
Solubility		
Spectral characterization (UV, IR, NMR)	Particle size	
	Specific surface area	
	Hygroscopicity	

Abbreviation: XRPD, X ray powder diffractometry.

logarithmic value of the ratio of these concentrations is often used and referred as log P, or partition coefficient. When an aqueous buffer solution (often pH 7.4) is used instead of water, the value is referred to as log D, or distribution coefficient. These coefficients, which are descriptions of the lipophilicity of a compound, are often correlated to the ability of a compound to cross biological membranes as well as their ability to dissolve in formulation vehicles.

The ionization constant (K_a), an intrinsic property of the molecule, describes the ionization behavior of a compound as a function of pH. The negative logarithm of K_a is often used and referred to as pK_a. The pK_a is equal to the pH value when the ratio of the ionized and unionized species is one. The pK_a is thus an important determinant in the pH dependence of ionization and hence solubility as well as salt formation ability of a molecule. These concepts will be further expanded elsewhere in this chapter. If a compound has multiple ionizable groups, each group has a corresponding pK_a value.

The molecular structure of the compound can be utilized for obtaining additional first estimate of properties such as dissociation constants and partition coefficients utilizing prediction software (e.g., from ACD/Labs, Simulations Plus, etc.). Such software packages can also provide a first estimate of the solubility and pH-solubility profiles. These data are especially useful during early development when compound supply is very short and there is a need to provide formulations for discovery pharmacology and early toxicology studies.

Solubility

Solubility is the concentration of drug in solution at equilibrium with excess solid. Typically, when the solid drug is brought in contact with a solvent, it dissolves into the solvent over a period of time and achieves equilibrium asymptotically. Aqueous solubility is of particular relevance to biological activity, bioavailability, and formulation strategy (3).

Solubility is experimentally measured by placing an excess solid in a test tube in contact with a particular solvent with mild agitation and determining the concentration of the drug in a supernatant solution over a period of time using appropriate analytical techniques such as UV spectrophotometry or high-performance liquid chromatography (HPLC). In determining equilibrium solubility, it is important to ascertain that (*i*) an asymptotic value has been achieved (constant over multiple time-points) and (*ii*) the identity of the solid in contact with the solvent is unchanged. The identity of the residual phase can be confirmed by analyzing the residue using techniques such as differential scanning calorimetry (DSC) or X-ray powder diffractometry (XRPD).

During preformulation studies, it is common to determine solubility of the drug compound in aqueous and nonaqueous vehicles used in pharmaceutical formulations. Aqueous systems include buffers, surfactant solutions, and complexant solutions. Nonaqueous

systems include cosolvents (e.g., ethanol, glycerol, polyethylene glycols) and oils (soyabean oil, glycofurol). A more detailed list of excipients is discussed later in this chapter.

pH-solubility profile. Many pharmaceutical compounds contain acidic or basic functional groups and hence show pH dependence in their aqueous solubility. Solubilities can vary significantly in accordance with the pK_a across acceptable pH range. Hence, adjusting pH to achieve requisite solubility can be an important tool in formulating injectable solutions.

The pH dependence of solubility of acids and bases is derived from the ionic equilibria occurring across the pK_a of a compound and is described by the Henderson-Hasselbalch equation (4).

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad \text{(for an acid)} \tag{1}$$

$$pH = pK_a + \log \frac{[B]}{[BH^+]} \quad \text{(for a base)} \tag{2}$$

Taking the example of a free base, the total solubility of at any given pH is the sum of the solubility of the unionized species (S_0) and the ionized species.

$$S = S_0 + [BH^+] \tag{3}$$

Figure 1 shows a hypothetical pH-solubility profile for a weak base. At a high pH (pH >> pK_a), the solubility is practically independent of pH and is essentially S_0. As the pH approaches the pK_a, the fraction of ionized species and hence the total solubility increase and are described by

$$S = S_0\left(1 + \frac{[H^+]}{K_a}\right) \tag{4}$$

The ionized species can associate with a charged counterion to form a salt. This linear increase in solubility ends abruptly when the solubility of the salt form is reached, and at this point the solubility is governed by the solubility product (K_{sp}) of the salt form. For example,

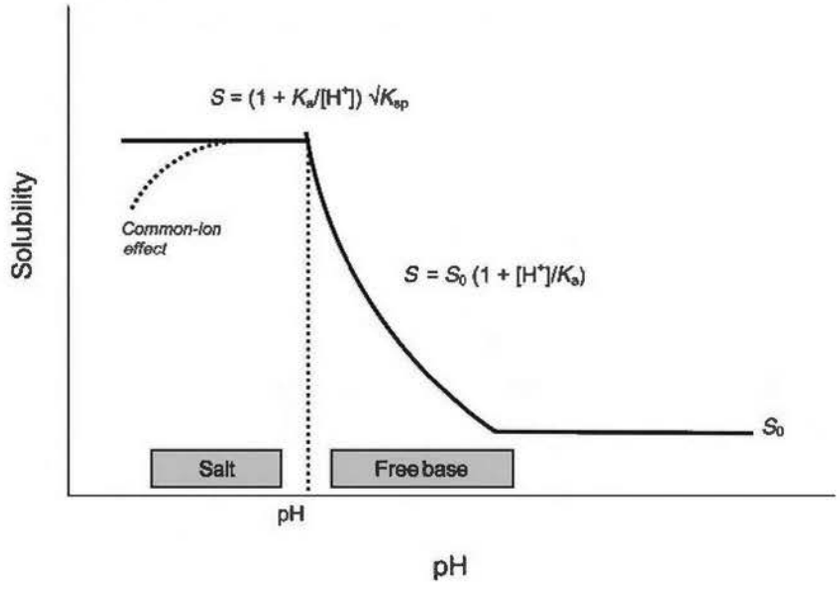


Figure 1 pH solubility profile of a hypothetical weak base.

Table 2 Properties of Some Commonly Used Solvents

Solvent	Dielectric constant (ε)	log P	Surface tension (γ) (dynes/cm)
Water	81.0	4.00	72.0
Glycerin	42.5	2.60	64.9
Propylene glycol	36.7	1.93	48.8
Ethanol	24.3	0.31	22.2

assuming that the pH was being changed by titrating with hydrochloric acid, the solubility product is

$$K_{sp} = [BH^+][Cl^-] \tag{5}$$

and the total solubility at this pH_{max} would be

$$S = \left(1 + \frac{K_a}{[H^+]}\right)\sqrt{K_{sp}} \tag{6}$$

Rearranging the equation, the pH_{max} can be determined if the solubility product is known.

$$pH_{max} = pK_a + \log\frac{S_0}{\sqrt{K_{sp}}} \tag{7}$$

Common-ion effect or salting-out effect is also depicted in Figure 1, representing the pH-solubility profile of a weakly basic drug. From the pH of maximum solubility, as one moves toward lower pH values, there is an increase in the concentration of the counterion (e.g., $[Cl^-]$). Depending on the value of the solubility product (a function of the nature of the drug and the counterion), this increase may be compensated by a decrease in the concentration of the ionized drug molecule. This decrease occurs through a precipitation of the drug in its corresponding salt form. This phenomenon is known as "salting-out" or common-ion effect and can be an important consideration in selecting salt forms or buffer systems for formulations.

Solubility in cosolvent systems. Cosolvents such as ethanol, propylene glycol, polyethylene glycols, and glycerol are routinely used in formulating to a higher solubility when aqueous solubility alone is not sufficient to achieve required levels. In case of some drug compounds, the use of appropriate cosolvents can increase the solubility quite significantly. The mechanism behind the increased solubility is frequently related to modifying the polarity or dielectric constant of the solvent system. The principle of "like dissolves like" works less polar molecules would be better dissolved in a less polar solvent system. Adding a cosolvent with a smaller dielectric constant to water will bring down the overall dielectric constant of the resultant solvent system and make it a better medium for dissolving a less polar or nonpolar molecule. Table 2 shows some physical parameters of common cosolvents (5).

Although cosolvents can be quite effective in achieving solubilization, it should be noted that as excipients these can have toxicological effects (e.g., hemolysis) and potential for local irritation depending on the concentrations used. Additionally, it is very important to consider the potential for the drug to precipitate upon dilution (6). This risk can be assessed both by calculating the degree of precipitation that could occur and by experimentally simulating the dilution that could occur and testing for precipitation potential (7).

Solubility in surfactant systems. Surfactants, a common class of excipients, are amphiphilic molecules (hydrophilic head group and hydrophobic tails), which strongly orient themselves at interfaces. In an aqueous system surfactant molecules would mainly be present at the water-air interface with a small but finite concentration in the bulk of the solution. Surfactants oriented at the water-air interface cause a reduction in the surface tension of water and thereby

improve wettability of drugs being exposed to such a system. With increasing concentration of surfactant in the system, the interface becomes crowded, and at a specific concentration, the surfactant molecules in the bulk orient themselves in micellar structures. Micelles consist of spherical structures with the hydrophobic (lipophilic) tails toward the core and hydrophilic heads forming the external surface. The concentration at which this occurs is called the critical micelle concentration (CMC). Above the CMC, aqueous surfactant systems would contain micellar structures in the bulk.

Lipophilic drugs can be incorporated into the core of micelles, thereby increasing the total solubility of a drug into aqueous systems. The lipophilic cores of micelles present a different environment to the drug molecule providing, in some instances, a stabilizing effect against chemical degradation. Surfactants will preferentially orient toward the surface of nuclei during a precipitation phenomenon and can prevent precipitation occurring due to dilution effects. Thus, surfactants can be a very useful tool in formulating aqueous injectable solutions and suspensions.

Examples of surfactants commonly used in injectable formulations include polyoxyethylene sorbitan monoesters (Tweens), polyoxyethylene-polyoxypropylene copolymers (Pluronics), sodium lauryl sulfate, and lecithins.

Solubility in complexant systems. A complex is an entity formed when two molecules, such as a drug and a solubilizing ligand, are held together by weak, noncovalent forces (dipole-dipole, hydrophobic, or hydrogen bond interactions). Cyclodextrins are a class of such solubilizing ligands that have found a significant application to pharmaceutical compounds. α-, β-, and γ- Cyclodextrins are cyclic oligomers of glucose containing six, seven, or eight glucose residues. Cyclodextrins have gained popularity from a pharmaceutical standpoint because of the ability of these materials to interact with poorly water-soluble drugs and drug candidates resulting in an increase in their apparent water solubility. The mechanism for this solubilization is rooted in the ability of cyclodextrin to form noncovalent dynamic inclusion complexes in solution. As a result of their structure, cyclodextrins present a hydrophilic exterior but a core that is more lipophilic and hence provides a microenvironment for lipophilic drug molecules to engage via hydrophobic interactions. In certain cases, the modified microenvironment of the cyclodextrin core results in improved chemical stability similar to micellar systems. The ability of the cyclodextrin to solubilize a drug compound depends on steric factors (size of the cavity) and thermodynamic factors (decrease in free energy of the system). Additionally, the solubility of the cyclodextrin in water is another key determinant. β-Cyclodextrin has relatively low water solubility (~18.5 mg/mL), but chemical modifications of the basic β-cyclodextrin have imparted improved solubility and lower toxicity. Two of the modified β-cyclodextrins that have gained greater acceptance are hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD). These have water solubilities of about 600 mg/mL and 500 mg/mL, respectively. Both of these modified cyclodextrins have been used in developing injectable formulations that are now FDA-approved products.

During preformulation studies, it is common to assess the solubility of a poorly soluble drug candidate in such cyclodextrins. If solubilization via cyclodextrin complexation is identified as a potential formulation approach, then it is also important to fully characterize the interactions in terms of stoichiometry of the complex as well as the equilibrium constant for the complexation. A number of excellent reviews cover the theoretical and experimental considerations for such determinations in detail (8 10).

Stability and Drug Degradation

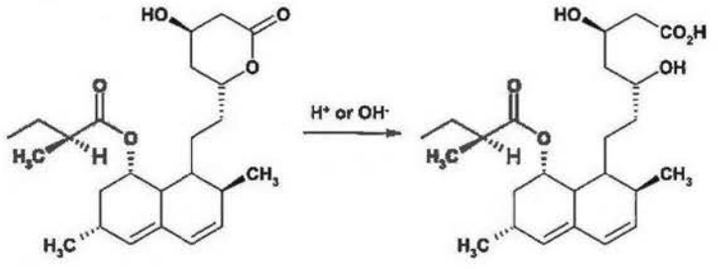
In addition to solubility, stability of the active drug compound is a key determinant in the viability of parenteral drug product. First, it is essential for a drug product to maintain potency relative to label claim over the shelf life to deliver an accurate dose. Second, degradation in the drug product can result in changes in appearance (color, precipitation) or bioavailability. Finally, degradation of the active compound can result in degradation products that may have toxicity that is more significant than that of the active drug substance. Depending on the daily

dose of the active and levels of such degradation products anticipated in a drug product, they may be subject to additional toxicological qualifications as described in ICH Guidance Q3B (R2) (11). When impurities or degradation products are identified as potentially genotoxic, they have to be controlled to very low levels if not completely avoided. This process is detailed in the EMEA Guidance on Limits for Genotoxic Impurities (12). Thus, it is essential to understand the stability and degradation of the active ingredient as a bulk drug substance and in formulation. Understanding the degradation pathways, kinetics, and mechanisms leads to development of a stable drug product (13,14).

During preformulation studies, the goal is to understand the modes of instability of a drug compound, kinetics of degradation, and factors (including formulation factors) influencing the kinetics of such degradation (15). One of the first steps is to develop a stability-indicating method that is capable of resolving and quantifying impurities and degradation products resulting from the drug compound. Typically, HPLC with UV detection is used in preformulation studies, but techniques such as LC-MS and NMR spectroscopy could often aid in the identification of degradation products. HPLC methods are developed to effectively resolve degradation products resulting from forced degradation studies (highly stressed condition of temperature, humidity, or pH).

Modes of degradation. Chemical degradation of small-molecule drugs can occur because of various chemical processes. However, a majority of these fall into three types of reactions.

Hydrolysis This is a very common pathway for drug degradation (16) and is essentially the cleavage of a molecule under the effect of water. Since water, either as a solvent or in the form of moisture in the air, is ubiquitous, the potential for this degradation pathway exists for most drugs. This is of particular relevance to parenteral products, which are mostly formulated in aqueous systems. Chemical bonds that commonly undergo hydrolytic degradation include lactam, ester, amide, and imide bonds. Aspirin is the most common example of a drug undergoing hydrolytic degradation. Lovastatin is a prodrug that undergoes activation through hydrolysis by carboxyesterases in vivo. In vitro it undergoes hydrolysis under acidic and basic conditions by cleavage of the lactone.



lovastatin
(hypolipidemic agent)

Hydrolytic reactions can be significantly influenced by the composition of the medium pH, buffer concentration, ionic strength, etc. The relationship of the rate of reaction (expressed as rate constant k_{obs}) with pH is quite informative both for understanding the mechanisms involved as well as a guide for formulation. When the reaction is catalyzed by the hydronium (H^+) ion, it results in a slope of negative one on a log k_{obs} versus pH profile, and similarly, when the reaction is catalyzed by the hydroxide ion (OH^-), a slope of one is observed. If no other catalyses are involved, then these two lines meet, forming a V-shaped profile. The pH at which they meet represents pH of maximum stability and is important to know during selection of formulation pH. The shape of curves can be more complicated (U shaped, additional inflections, etc.) depending on the number of ionic species involved (15,17).

In addition to the pH of the medium, concentration of the buffer itself can play a catalytic role in hydrolysis. This can be studied by studying the reaction rate as a function of buffer type and concentration while holding the pH constant. Ester hydrolysis of an experimental compound GW280430A was shown to be catalyzed by citrate, malate, and tartrate buffers but not by a glycine buffer (18). This phenomenon is termed as general acid/base catalysis or buffer catalysis and can often be the cause of deviation from a slope of −1 or +1 described in specific acid/base catalysis in the previous paragraph. Additionally, reactions can also be affected by ionic strength, which can be studied by holding the pH and buffer concentration constant and studying the reaction rate as a function of concentration of added ions (e.g., NaCl). Typically, this is not a big effect in pharmaceutical systems.

In summary, hydrolysis is a key degradation pathway for many drug compounds. pH-stability profiles can vary from a simple V shape to more complex profiles depending on the number of ionization states and the different reactivities they present. While some of the pathways can be predicted on the basis of the structure, evaluation of the pH-stability profile and effect of buffer catalysis can be very important in designing the formulation strategy.

Oxidation Oxidation is another common mode of drug degradation. Oxidation can be broadly defined as a loss of electrons in a system; alternately, it could be considered as an increase in oxygen or a decrease in hydrogen atoms. The reaction occurs in concert with reduction of the other reactant, thus forming a redox reaction. If molecular oxygen is involved in the reaction, this is termed as "auto-oxidation." Trace metals and light can catalyze oxidation reactions by initiating free radical chain reactions. Once formed, the radical can be propagated until a termination reaction or a suitable chemical inhibitor intervenes. These reactions can happen in aqueous and nonaqueous media.

Excipients used in formulation can be a source of trace metals and also peroxides, which can have significant effect on oxidative drug degradation. Table 3 shows levels of hydroperoxides measured in some commonly used pharmaceutical excipients (19).

To control oxidation reactions, antioxidants are often included in a formulation. Antioxidants used in a formulation could affect different stages of an oxidation reaction. True antioxidants (e.g., butylated hydroxy toluene, α-tocopherol) react with free radicals, resulting in termination of the chain reaction. Reducing agents (e.g., ascorbic acid) get preferentially oxidized and hence reduce the level of oxygen or the oxidant in the formulation. Chelating agents such as EDTA sequester trace metals which can catalyze oxidation and thereby function as antioxidant synergistic agents. Depending on the reaction involved, a combination of such agents may help control the oxidative degradation (20). Also, during manufacturing and in the primary package, an inert atmosphere generated by nitrogen blanketing can help control oxidative degradation.

Photolysis Photolysis, also referred to as photodegradation, occurs as a result of absorption of light (or radiation energy) (21). When the absorbed energy dissipates through a chemical change in the molecule, photolysis occurs. The changes may result in a color change, precipitate formation or may not be visually detectable. However, there is always loss of potency that is accompanied. Toxicity of the decomposition products is also of concern,

Table 3 Levels of HPO in Some Commonly Used Excipients

Excipient	Number of batches tested	Average HPO (nmol/gm)	Range of HPO (nmol/gm)
Polyvinylpyrrolidone	5	7,300	3,600 11,000
Polyethylene glycol 400	4	2,200	1,000 3,300
Polysorbate 80	8	1,500	180 4,600
Poloxamer[a]	7	30	10 50
Mannitol	5	<10	<10
Sucrose	5	<10	<10 20

[a]Different grades (188, 338, and 407) and batches tested.
Abbreviation: HPO, hydroperoxides.

especially when such products can form by the action of sunlight on the skin or eyes after administration (phototoxicity) (22).

Photodegradation depends on wavelength of the incident light as well as intensity. Primary photochemical reactions usually occur at wavelengths where the drug absorbs light, that is, in regions where the UV/VIS absorption spectrum of the drug overlaps with the spectrum of incident radiation. In some instances it is possible that the energy absorbed by a nondrug molecule (photosensitizer) in the formulation is transferred to the drug molecule, which eventually degrades. Examples of some common drugs that undergo photolytic degradation include methotrexate, furosemide, and tetracyclines. For many drug substances, the kinetics of photodegradation varies significantly with the ionization state of the molecule. Examples would include ciprofloxacin, midazolam, mefloquine, and ameloride (23).

Once a photoinstability is identified, it can be addressed during formulation development through different means. A protective market pack is one of the simplest solutions. Control of pH, ionic strength, trace metals, or even use of complexants (24) can be formulation approaches to also address such instability.

In addition to these major modes of degradation, many other routes are involved in drug degradation such as decarboxylation, racemization/epimerization, acylation, etc. Understanding the causes of drug instability allows for a rational design of a formulation.

Preformulation stability studies. Typically, the drug substance is studied in solid as well as solution states. Stability studies might involve storing the samples under stressed conditions of temperature and humidity such as 40°C/75% RH and 50°C. If the drug is fairly stable, conditions such as 80°C/75% RH and 80°C may be employed to get a first view of drug instability in a reasonable amount of time. These studies are conducted over a short duration such as four to six weeks.

Additionally, the solid drug and an aqueous solution of the drug are exposed to a representative duration and intensity of light in appropriate photostability chambers [as per ICH Q1B (25)]. These studies may be able to indicate not only potential need for protecting the drug product from light but also the need for conducting other stability studies under light-protected conditions. Failure to know this early can produce confounding results.

pH-stability profiles are determined by preparing aqueous solutions of the drug at various pH values ranging from 2 to 12 and studying the kinetics of degradation (loss of active/growth of degradation products) at an appropriate elevated temperature. The solutions are sampled at regular intervals and analyzed using a stability-indicating method. The time course of degradation at a particular pH can typically be expressed as the first-order rate constant k_{obs} (k observed). A log k_{obs} versus pH plot is referred to as the pH-rate profile and can be quite revealing of the mechanisms involved in drug degradation. The pH of maximum stability would be targeted as the pH for the formulation as long as it agrees with the required solubility and local tolerability at that pH.

Form Selection

The solid form of the drug compound can have a significant effect on parenteral drug product processing. During late discovery or early development stages, the solid form of the drug compound needs to be defined and fixed to develop formulations and processes consistent with the expected physical and chemical properties of the API. The solid form is typically described by the salt form used and the crystal polymorph of the chosen salt.

Salt Form

Many drugs are either weak acids or weak bases and can consequently form a range of salts by reacting with various bases and acids, respectively. Salt formation may be employed to alter the physicochemical, biopharmaceutical, and processing properties of a drug substance without modifying the pharmacologically relevant moiety (26).

To form stable salts, the pK_a of the basic center should be greater ($\Delta pK_a \geq 2$) than the pK_a of the conjugate acid to be utilized. Thus, for a basic drug, pK_a of the basic center will determine what salts are feasible.

In the case of parenteral medications, increased solubility is often desired from chosen salts. In general, utilizing counterions with greater acidity, utilizing more hydrophilic counterions (hydroxy acids), and lowering the melting point of the resultant salt (decreased crystal lattice energy) can result in increased solubility. Agharkar et al. (27) demonstrated an increased solubility of an experimental antimalarial drug as a result of decreased crystal lattice energy due to salt formation.

In the case of solution formulations, it is not essential that salt formation is only employed for obtaining a suitable solid form. Salts can be formed in situ in solutions by using the appropriate acid or base to adjust pH of the formulation (28). Sometimes the high aqueous solubility achieved prevents a salt from being easily isolated but can still be utilized as an effective solubilization approach, as previously discussed in the context of pH-solubility profiles.

Polymorph Selection

Polymorphism is defined as the ability of a substance (of constant chemical composition) to exist in two or more crystalline phases that differ in crystal packing arrangement and/or conformation of the molecules in the crystal lattice. The different crystalline forms are then termed as polymorphs.

Crystals are made up of repeating blocks called unit cells. Different polymorphs have distinct unit cells. Polymorphs can differ in various physical, physicochemical, and physicomechanical properties. Differences such as melting point, enthalpy of melt, true density, and powder X-ray diffraction patterns help characterize and differentiate between polymorphs. One can screen for polymorphs by crystallizing a drug from different systems of solvents, evaporation and cooling profiles, and then examining crystals obtained. However, it is not easy to search exhaustively for all possible crystal forms, and often new forms are discovered during development. To reduce the risk, many automated crystallization systems have been developed, which help examine a larger experimental space.

Polymorphism is commonly of concern in the context of solid dosage form bioavailability and processing (29). However, polymorphs also differ in properties that impact a parenteral drug product formulation of which solubility, dissolution rate, and hygroscopicity are of most relevance. Polymorphs differ in their free energy as a result of their packing, and this manifests itself as differences in solubility. The most stable polymorphic form has the lowest solubility. If a metastable polymorph is used in a solution or suspension formulation, there will be a risk of growing crystals of the stable form over a period of time. Solvent maturation studies and temperature cycling of prototype formulations can help identify such problems early.

When a solvent molecule incorporates itself into a crystal lattice associated with a drug compound, it is said to form a solvate. When this solvent is water, it is termed as a hydrate. A hydrate form of the drug is more stable than an anhydrous form and will exhibit lower solubility in an aqueous system. Thus, it is also important to understand and characterize solvate and hydrate forms of the drug compound.

Characterization of Material Properties

Appearance and Microscopy

The solid form of a drug substance is characterized by its appearance in terms of color and subjective description. Additionally, examination under a microscope reveals further details such as crystal morphology and habit.

Crystallinity

Crystalline material can be identified by polarized light optical microscopy where the sample displays birefringence. Crystallinity is also commonly examined by XRPD. An X-ray diffraction pattern is generated because of constructive and destructive interference of X rays reflected off the crystal planes of a powder sample as the angle of incidence is varied. This is described by the Bragg equation.

$$n\lambda = 2d \sin \theta \tag{8}$$

where θ is the incident angle, λ is the wavelength of the X radiation, d is the distance between the crystal planes, and n is an integer representing the order of reflection.

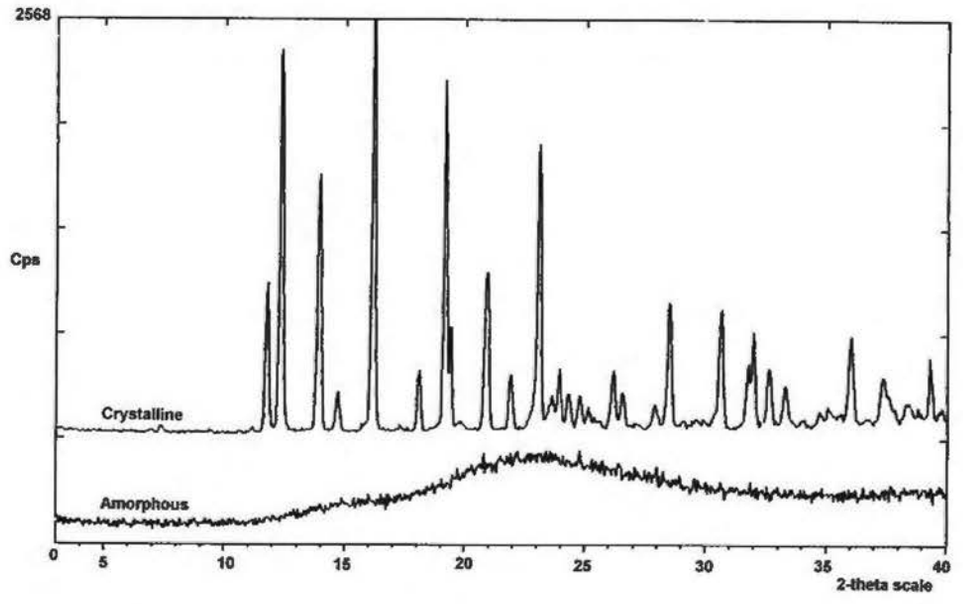


Figure 2 X ray powder diffraction patterns showing amorphous and crystalline states of an experimental drug compound.

Crystalline forms are characterized by sharp characteristic peaks, while an amorphous material displays a broad halo (Fig. 2) (30). XRPD can be used to distinguish between different polymorphs, solvates, and hydrates. Further, this technique can also be used to quantify mixtures of polymorphs and degree of crystallinity of a crystal form.

Thermal Properties

DSC measures the difference in the amount of heat required to raise the temperature of a sample and a reference as a function of a change in temperature. A typical output shows heat flow into (endothermic event) or out of (exothermic event) the sample as a function of temperature. Melting of a crystalline material is observed as an endothermic event characterized by an onset temperature (melting point) and heat of fusion measured as the area under the endothermic curve. At the glass transition temperature, amorphous materials undergo a transition from a glassy rigid state to a rubbery state of greater mobility (a higher heat capacity), and this is observed on the DSC as a baseline shift characterized by temperature (T_g) and change in heat capacity (ΔC_p). The glass transition is sometimes followed by a small endotherm of enthalpic relaxation related to time-dependent relaxation of this phase. Figure 3 shows the DSC thermogram of an experimental drug compound displaying these transitions along with an overlay of corresponding changes to the X-ray diffraction patterns as observed by variable-temperature XRPD (31).

Modulated DSC (mDSC) is a related technique where an oscillation of temperature is introduced on top of a linear heating rate. This allows deconvolution of the output into reversing (thermodynamic) and nonreversing (kinetic) components, allowing a further understanding of the transitions measured. This can be of particular utility in studying amorphous materials (29).

Thermogravimetric analysis (TGA) measures the weight of the sample as a function of increasing temperature. Loss of water, solvents, or volatile decomposition products can be observed as a weight loss at characteristic temperatures. This analysis is a key technique in characterizing solvates and hydrates. The technique is sometimes further coupled with an IR spectrometer or a mass spectrometer to characterize the evolved volatile components that come off during heating of the sample.

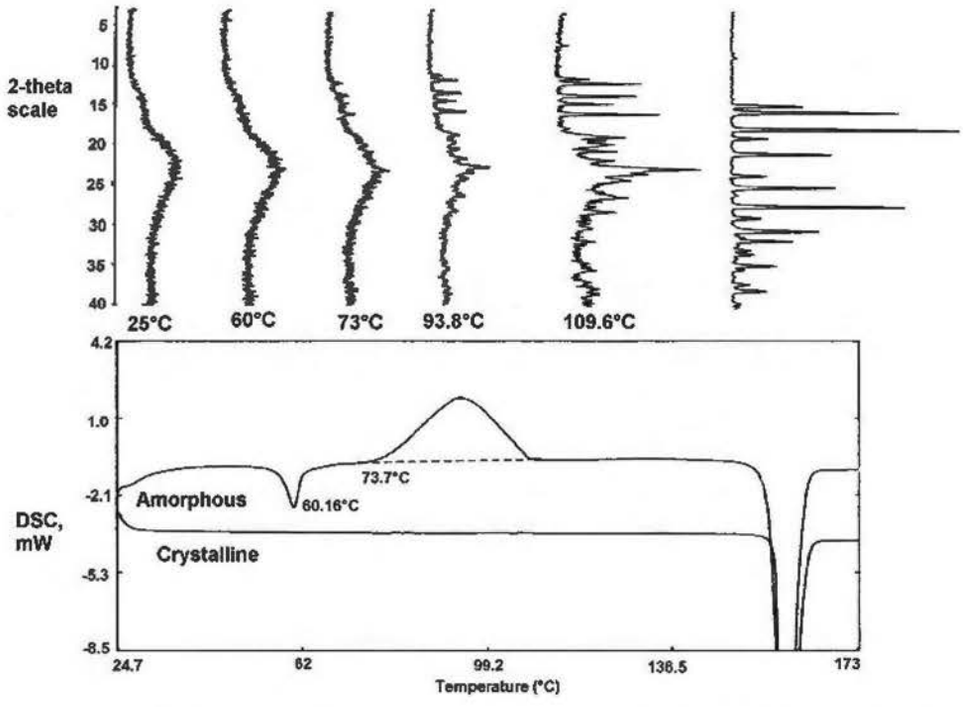


Figure 3 DSC curves of crystalline and amorphous phases of an experimental drug compound overlaid with XRD patterns of the amorphous phase obtained at temperatures corresponding to thermal events in the DSC curve. *Abbreviations*: DSC, differential scanning calorimetry; XRD, X ray diffraction.

Vapor (moisture) Sorption Analysis

The weight of the sample is monitored as it is exposed to different relative humidities for a period of time approaching equilibrium. The output is a moisture sorption profile, which depicts the sample weight as a function of relative humidity. When a material picks up enough water that causes a change in its physical properties, the material is considered hygroscopic. Crystalline materials typically *adsorb* small amounts of water on the surface unless they pick up water molecules into the crystal lattice to form hydrates. Hydrates are characterized by picking up stoichiometric amounts of water and are physically stable over a range of %RH. Deliquescence occurs when the material adsorbs enough water to dissolve into it thereby turning liquid. This can sometimes happen with salts of hydrophilic molecules and is characterized by a sharp increase in moisture uptake at humidity values greater than a threshold %RH.

Amorphous materials *absorb* water and other solvents into the bulk. The absorbed solvent acts as a plasticizer and reduces the apparent glass transition temperature. When the apparent glass transition temperature drops below storage temperature, the material goes into a mobile rubbery state from which collapse of the structure (liquefaction) with possible recrystallization can occur. This relationship of glass transition temperature as a function of absorbed water is critical to understand when developing a lyophilization process.

INTERACTION BETWEEN THE DRUG SUBSTANCE AND FORMULATION COMPONENTS

Formulation Components

In formulating a parenteral drug product, a number of excipients are employed, and these often form the bulk of a drug product. These excipients are included to dissolve the drug substance, increase the chemical or physical stability of the drug product, give the product

Table 4 Excipients Used in Parenteral Formulations

Solvents and cosolvents
- Glycerin
- Propylene glycol
- Ethanol
- Polyethylene glycol (300, 400)
- *N,N* dimethylacetamide
- Soyabean oil
- Corn oil
- Ethyl oleate
- Glycofurol

Surfactants (solubilizers, emulsifiers, and suspending agents)
- Polysorbate 80 (Tween 80)
- Polysorbate 20 (Tween 20)
- Polyoxyethylene polyoxypropylene copolymers (poloxamers)
- Cremophor EL
- Lecithin

Complexants
- Hydroxypropyl β cyclodextrin
- Sulfobutylether β cyclodextrin (Captisol®)

Buffers
- Citrate
- Phosphate
- Tartrate
- Tromethamine (TRIS)

Chelating agents
- Disodium ethylenediaminetetraacetic acid

Antioxidants
- Ascorbic acid
- Butylated hydroxy anisole
- Butylated hydroxyl toluene
- Sodium bisulfite
- Propyl gallate
- α Tocopherol

Preservatives
- Benzalkonium chloride
- Benzethonium chloride
- Benzyl alcohol
- Chlorbutanol
- Paraben (methyl, propyl)
- Thimerosal

Tonicity adjusters, bulking agents, lyoprotectants
- Sodium chloride
- Mannitol
- Glycine
- Sucrose
- Trehalose
- Dextran
- Povidone

microbiological protection, or control other product attributes. Since inclusion of new additives could require extensive pharmacological and toxicological evaluation, it is common for formulators to depend on materials already used in marketed parenteral products. Table 4 shows a representation of the classes of excipients that might be used in parenteral formulations and some examples of each of these categories. There is more discussion within this book on the functions and levels of these excipients. Additionally, the reader can refer to some excellent reviews that have been published on this topic (31,32). The FDA also maintains a listing of inactive ingredients used in approved products (33).

Designing Excipient Compatibility Studies

Excipients are often referred to as inactive or inert ingredients to distinguish them from the APIs. However, the lack of pharmacological activity does not necessarily result in a lack of chemical reactivity. Excipients can have significant expected and unexpected effects on the physical and chemical stabilities of a drug product. This is first assessed through well-designed excipient compatibility studies conducted at the preformulation stage (34).

Traditionally, thermal methods such as DSC have been employed as a first screen in determining incompatibilities (35). In these studies, the drug, excipient, and drug-excipient mixture are subjected to a temperature program. If the thermogram of the mixture is not representative (temperature and enthalpy) of the combination of the two single components, then an incompatibility could be suspected. Modifications such as a stepwise isothermal high-sensitivity DSC study have also been tried (36). However, DSC techniques have proved to be of limited predictability.

Isothermal heat conduction calorimetry is a technique that measures heat evolved or absorbed by a sample (relative to a suitable reference) with great sensitivity. Hence, even slow reactions occurring under isothermal (25°C, 45°C/75% RH) can be detected because of the

Table 5 A Plackett Burman Design

	Variable											Response
Trial	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	Y
1	+	+		+	+	+				+		
2	+		+	+	+				+		+	
3		+	+	+				+		+	+	
4	+	+	+				+		+	+		
5	+	+				+		+	+		+	
6	+				+		+	+		+	+	
7				+		+	+		+	+	+	
8			+		+	+		+	+	+		
9		+		+	+		+	+	+			
10	+		+	+		+	+	+				
11		+	+		+	+	+				+	
12												

sensitivity of the technique (37). This technique has been used to compare the heat signal from a drug-excipient mixture with the sum of the curves generated by the individual components under the same conditions. The magnitude of this interaction curve (difference curve) is an indicator of the extent of the incompatibility (38). However, this technique generally suffers from the fact that it is nonspecific and it is important to carefully design appropriate control experiments to make sure that the recorded heat pertains to a specific chemical incompatibility.

Given some of the challenges described above, the conventional method of chemical analysis of mixtures stored under accelerated storage conditions is still the most commonly employed method. The prerequisite for this methodology is having a stability-indicating method, and most commonly, this is an HPLC method. Since in a parenteral product, the drug and excipients are in very close contact (at a molecular level in the case of solution products) with each other, the stabilizing or destabilizing effect of an excipient is best studied in the presence of all formulation components (prototype formulations) including the targeted primary packaging when possible. High and low levels of each excipient or formulation factor are identified for testing on the basis of conventional levels used in experience or levels approved for use by regulatory authorities.

Different experimental designs can be used for obtaining the required information from a limited number of experimental runs. In such studies excipients constitute factors (at two levels high and low) in a factorial design of experiments. For such studies screening design is employed at first. A commonly used screening design is a fractional factorial design called a Plackett Burman design. Table 5 represents a possible design for studying 11 factors by performing 12 trials. This design was employed for a parenteral preformulation study for Naproxen as described by Peswani and Lalla (39). In this study they looked at effects of five excipients, pH, buffer type, autoclaving, and nitrogen blanketing by conducting 12 trials. Although these designs are quite efficient in terms of number of trials, it should be noted that these designs are not capable of identifying interaction terms (e.g., if two factors interact to produce an effect). If such confounding is suspected and needs to be resolved, a full factorial design study could be conducted on a smaller number of identified factors. The reader can get details of the advantages and disadvantages of different experimental designs from other reviews of this specific topic (40).

INTERACTION OF THE DRUG WITH PACKAGING COMPONENTS AND MANUFACTURING SURFACES

Parenteral drug products are in close contact with the primary package of the drug product; so it is useful to carefully consider primary package in the same way other formulation ingredients are evaluated. These packaging materials would include glass vials (or ampoules),

rubber stoppers, infusion bags, etc. Glass vials are most commonly type I (borosilicate glass), but that too can undergo different surface treatments at the manufacturer. Rubber stoppers (commonly butyl or other synthetic rubber and rarely natural rubber because of its sensitizing potential) and bag materials can be quite complex in composition. The formulation scientist works closely with the rubber manufacturer as with the glass manufacturer to choose the appropriate rubber formulation having consistent specifications and characteristics to maintain product stability. It is important during preformulation studies to include an evaluation of likely primary packaging materials to assess potential issues such as adsorption and incompatibilities. Also important to consider are other likely surfaces to be encountered during manufacturing steps, for example, stainless steel, glass, tubing, and filters.

Adsorption

Adsorption occurs when a molecule is attached to another solid surface, most commonly because of Van der Waals forces, hydrogen bonding, or electrostatic interactions. This can often occur with low-solubility hydrophobic compounds as they may prefer another surface as opposed to being in water. When a covalent bond is involved, the adsorption is chemisorption, but this is not commonly observed in the systems being discussed here.

To evaluate adsorption, the formulation (at the most dilute concentration likely) is exposed to the surface and then assayed for loss of drug concentration. For filters and tubing, this might involve passing through the tubing and filters for a fixed duration of time that will exceed the likely duration of a manufacturing run. For stoppers, it might be done by adding a fixed number of stoppers to flasks containing the formulation and storing for a fixed period of time before assaying the concentration. During development of an injectable formulation of Abbott-72517, Gupta et al. observed a 6% of loss of drug (250 mL recirculated for four hours) using a Pall Nylon 66® filter but no loss with a Millipore Durapore disk membrane (41). If adsorption to potential surfaces is identified early on, then it can be used to select appropriate materials for packaging and manufacturing processes.

Compatibility

In addition to adsorption, the degradation of the drug molecule can also be effected by packaging material or manufacturing surfaces. Thus, when feasible, it is useful to conduct excipient compatibility studies using preferred container closure systems. An early readout on any potential incompatibility can lead to an early assessment of alternatives and prevent the loss of time during development. For instance, rubber stoppers can leach out trace quantities of zinc into the formulation and effect oxidation of the drug. If a drug is particularly prone to oxidation, a steel surface may aggravate the issue and a glass-lined tank may be an appropriate measure. Protein drugs could be especially sensitive to silicone that is used on rubber stoppers. A nonsiliconized rubber with a bonded coating may be the answer to the issue.

SPECIALIZED FORMULATIONS

Suspensions and Nanosuspensions

Sterile injectable suspensions comprise of the active compound dispersed in a liquid vehicle either as a ready-to-use formulation or as a dry powder for reconstitution. Such formulations may be engaged either when the drug has solubility that is too low for a solution formulation or for prolonging the release of the drug through depot formulations. Aristocort® is a suspension of triamcinolone diacetate and may be administered by the intramuscular, intra-articular, or intrasynovial routes depending on the situation (42). NPH insulin is a suspension of crystalline zinc insulin combined with the positively charged polypeptide protamine. When injected subcutaneously, it has an intermediate duration of action. Depo-Medrol® is an anti-inflammatory glucocorticoid for intramuscular, intra-articular, soft-tissue, or intralesional injection. One of the challenges of formulating such products involves an evaluation of suspension physical stability with regard to resuspendability and caking.

Another area of specific concern for suspensions is syringeability (drawing a uniform dose) and injectability (pressure applied to expel product through a needle of specified gauge) of the product. The flow properties of the suspension can be characterized using techniques such as rheometry. This technique characterizes the flow of a fluid in response to a range of

applied stresses, resultant strains, and temperatures. Many suspensions and emulsions do not show a linear relationship between applied stress and strain (non-Newtonian behavior) and hence cannot be characterized by a single value for viscosity. A full discussion of this topic is out of the scope of this chapter and is well captured in many reviews on this topic.

For suspension formulations, the solid-state properties are quite relevant. Particle size of the dispersed phase can have a significant impact on the physical stability and syringeability of a suspension. Particle size distributions in suspensions can change over time because of Ostwald ripening a solution-mediated phenomenon during which larger particles grow at the expense of smaller particles dissolving. An appropriately selected medium and surfactant can minimize the impact of this phenomenon. During screening, subjecting prototype samples to temperature cycling can accelerate the event and help select systems that are the most stabilizing. Crystal growth can also occur because of a more stable polymorph precipitating or a salt being formed. A change in crystal habit can result in significant effects on syringeability and injectability. Hence, there is a greater emphasis to fully understand the solid properties of the drug being formulated as a suspension as opposed to a solution product.

Lately, there has been a growing interest in formulating poorly soluble drugs as nanoparticulate suspensions (43). For compounds that exhibit poor solubility in aqueous and oily vehicles, nanosuspensions could be a preferred formulation option resulting in improved bioavailability. Nanoparticles also form an interesting platform for attaching targeting moieties. Nanoparticles are produced by "top-down" (media milling) techniques (44) or by "bottoms-up" (controlled crystallization) approaches (45). More recently, there have been reports of generating engineered nanoparticles by printing techniques (46).

Well-formulated nanosuspensions are typically nonsettling and hence circumvent some of the concerns mentioned previously with conventional suspension formulations. In such formulations the natural tendency of these small particles to aggregate is overcome by a careful selection of stabilizers, which could include a mix of surfactants and polymers. Compatibility of the drug with a range of possible surfactants and polymers needs to be assessed in parallel to selecting the best options for stabilization. As in conventional suspensions, Ostwald ripening and crystal growth is a concern, and gaining a good understanding of the solid-state properties of the drug is very relevant. Prototype nanosuspensions can be stressed by temperature cycling and freeze-thaw studies to establish their physical stability. It is also useful to assess the physical and chemical stability of the formulated drug to autoclaving conditions to define the strategy for sterilization.

Emulsions

Injectable emulsions have been most commonly used for long-term parenteral nutrition (Intralipid®, Lipofundin®). However, emulsions can also be good carriers of drug substances with good lipid solubility (high log *P*) and poor aqueous solubility (47). Propofol (Diprivan®) and diazepam (Diazemul®) are examples of drugs formulated as emulsions (33), and there are reports on studies conducted with Taxol emulsions (48). With the increased interest in injectable lipid emulsions, there is also a greater awareness of safety issues surrounding such delivery (49).

Typical emulsion formulations consist of oils (long- and medium-chain triglycerides or high-quality food grade oils), emulsifiers (e.g., lecithins, poloxamers, Tweens, and Spans) and an aqueous phase containing appropriate additives to control pH, tonicity, etc. Antioxidants such as α-tocopherol could be included in the oil phase to prevent oxidation of the oils. The emulsions are typically prepared by dissolving the appropriate ingredients in the oil phase and water phase and then homogenizing (e.g., Microfluidizer®, Silverson® homogenizer) the two to obtain the emulsion.

Some attributes to be studied in the specific context of emulsion formulations include assessment of particle (droplet) size and surface charge. Droplet surface charge is measured in terms of the zeta potential. Essentially, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The zeta potential is determined using instruments that measure the elecrophoretic mobility of the particles. The surface change on droplets stabilizes emulsions because of electrostatic repulsion, which prevents coalescence of droplets. A zeta potential of ±30 mV or higher can

help stabilize a colloidal system. Measurement of zeta potential is equally useful while formulating suspensions and nanosuspensions.

SPECIFICITIES RELATED TO BIOLOGICS

Biotherapeutic molecules could range from small oligonucleotides or peptides synthesized using techniques such as solid-phase synthesis to proteins (including interferons, soluble receptors, antibodies, etc.) with tertiary and quaternary structures, which are often produced via genetic engineering technologies. Small oligonucleotides and peptides can often be formulated and analyzed by techniques similar to small molecules. More specialized analytical techniques and formulation considerations are needed for larger proteins. From a preformulation perspective, the goals are the same to characterize the drug compound and understand the solubility and stability of the drug as well as the interactions with potential excipients that would be used to formulate the drug compound. Early results may determine the formulation strategy of either a ready-to-use solution or a lyophilized product for reconstitution. On the basis of this strategy, additional preformulation studies may be needed to support the formulation choice.

Characterization

In addition to the conventional characterization described earlier in the chapter, additional parameters relevant to protein drugs need to be assessed (50). These include determination of molecular weight, amino acid sequence, and disulfide bonds. Because of a large number of charged groups, proteins are generally soluble in water but can be physically unstable at high concentrations because of their complex interaction with surrounding water. Proteins are zwitterionic in nature as a consequence of the amino and carboxylic groups of individual amino acids. At low pH values, proteins would have a net positive charge, and at higher pH values, due to ionization of the carboxyl groups, they carry a net negative charge. The isoelectric point, pI, is the pH of an aqueous solution of a peptide (or protein) at which the molecules on average have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged groups. This is an important parameter, which is most commonly determined using an electrophoresis technique called isoelectric focusing.

From a solid-state point of view, protein drugs are frequently amorphous and quite hygroscopic. For large proteins made by genetic engineering technologies, it is also quite common not to routinely isolate the protein as a solid but to hold it in a solution or frozen buffered and stabilized solution.

Stability

The pharmacological activity of proteins and peptides is largely dependent on their intact primary, secondary, tertiary, and quaternary structures. Proteins and peptides are quite fragile and can undergo physical and chemical degradation under a variety of conditions.

Chemical Stability

Chemical degradation can be triggered by changes in temperature, pH, oxygen levels, and trace metals and under the influence of light. Methionine, cysteine, tryptophane, and histidine residues can undergo oxidation under the influence of trace metals and light and higher levels of oxygen. Hydrolysis of the side chains of asparagine and glutamine residues can result in deamidation reaction. Hydrolysis of the amide bond in the protein backbone is another degradation route, which is mainly influenced by the solution pH. β-elimination of cysteine, serine, threonine, and lysine residues is also affected by the solution pH, temperature, and ionic composition.

To characterize the degradation pathways, a multitude of analytical techniques are employed. These include different sequencing (*N*-terminal sequencing), spectroscopic (UV spectral analysis), separation (e.g., ion exchange, reverse phase, gel electrophoresis with protein staining, isoelectric focusing) of the intact proteins or enzymatically digested proteins (peptide map), and mass spectroscopic analysis of proteins to define the chemical modifications occurring. Circular dichroism is used to assess secondary and tertiary structures.

Physical Stability

Native protein structures are not very thermodynamically stable. Proteins easily unfold (denaturation) under the influence of increased temperature and concentration, pH change, buffer species, or chemical and physical stress. Completely or partially unfolded proteins can associate to form irreversible aggregates. Aggregation is not necessarily visible to the eye, but with increasing aggregation, aggregate size increases, and eventually, precipitation can occur, which is clearly visible.

Fluorescence measurements, light scattering techniques (sometimes in combination with reverse-phase or size exclusion chromatographic separation) and field flow fractionation can be used to assess aggregation. Conformational changes leading to aggregation can also be measured by DSC.

Protein unfolding, adsorption to surfaces, and aggregation can be modulated by pH, buffer species, choice of preservatives, and use of appropriate surfactants and stabilizers (sugars) in the formulation. The formulation factors have to be tailored to individual proteins through well-executed studies evaluating formulation, processing, and storage conditions. Other chapters in this book cover protein characterization and formulation aspects in detail.

SUMMARY

The aim of preformulation studies is to gain a thorough understanding of the drug molecule, its physical and chemical properties, as well as its interaction with other formulation ingredients and packaging materials to drive a rational formulation design. This chapter has provided an overview of preformulation studies related to development of parenteral medications.

REFERENCES

1. Halbert GH. Preformulation. In: Florence AT and Shipman J, ed. Modern Pharmaceutics, Basic Principles and Systems. Vol. 1. New York: Informa Healthcare, 2009:327 355.
2. Gardner CR, Walsh CT and Almarsson O. Drugs as materials: valuing physical form in drug discovery. Nat Rev Drug Discov 2004; 3:926 934.
3. Bhattachar SN, Deschenes LA and Wesley JA. Solubility: it's not just for physical chemists. Drug Discov Today 2006; 11(21/22):1012 1018.
4. Anderson BD and Flora KP. Preparation of water soluble compounds through salt formation. In: Wermuth CG, ed. The Practice of Medicinal Chemistry. London: Academic Press, 1996:739 754.
5. Yalkowsky SH and Roseman TJ. Solubilization of drugs by cosolvents. In: Yalkowsky SH, ed. Techniques of Solubilization of Drugs. New York: Marcel Dekker, 1981:91 134.
6. Yalkowsky SH, Kryzyaniak JF and Ward GH. Formulation related problems associated with intravenous drug delivery. J Pharm Sci 1998; 87(7):787 796.
7. Yalkowsky SH, Valvani SC and Johnson BW. In vitro method for detecting precipitation of parenteral formulations after injection. J Pharm Sci 1983; 72(9):1014 1017.
8. Davis ME and Brewster ME. Cyclodextrin based pharmaceutics: past, present and future. Nat Rev Drug Discov 2004; 3:1023 1035.
9. Brewster ME and Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Del Rev 2006; 59:645 666.
10. Stella VJ and Rajewski RA. Cyclodextrins: their future in drug formulation and delivery. Pharm Res 1997; 14(5):556 567.
11. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research. Guidance for industry Q3B(R2) impurities in new drug products. Available at: http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm128033.pdf. Accessed December 2009.
12. European Medicines Agency. Evaluation of medicines for human use. Available at: http://www.emea.europa.eu/pdfs/human/swp/519902en.pdf. Accessed December 2009.
13. Alsante KM, Ando A, Brown R, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Del Rev 2006; 59:29 37.
14. Baertschi SW. Analytical Methodologies for discovering and profiling degradation related impurities. Trends Anal Chem 2006; 25(8):758 766.
15. Stella VJ. Chemical and physical bases determining the instability and incompatibilities of formulated injectable drugs. J Paren Sci Technol 1986; 40(4):142 163.

16. Waterman KC, Adami RC, Alsante KM et al. Hydrolysis in pharmaceutical formulations. Pharm Dev Technol 2002; 7(2):113 146.
17. Won CM, Molnar TE, Windisch VL et al., Kinetics and mechanism of degradation of klerval, a psuedo tetra peptide. Int J Pharm 1999; 190:1 11.
18. Zhu H, Meserve K and Floyd A. Preformulation studies for an ultrashort acting neuromuscular blocking agent GW280430A. I. Buffer and cosolvent effects on the solution stability, Drug Dev Ind Pharm 2002; 28(2):135 142.
19. Wasylaschuk WR, Harmon PA, Wagner G, et al. Evaluation of hydroperoxides in common pharmaceutical excipients. J Pharm Sci 2007; 96(1):106 116.
20. Won CM, Tang SY, Strohbeck CL. Photolytic and oxidative degradation of an antiemetic agent, RG 12915. Int J Pharm 1995; 121:95 105.
21. Tonnesen HH. Photodecomposition of drugs, In: Swarbrick J, ed. Encyclopedia of Pharmaceutical Technology. 3rd ed. New York: Informa Healthcare, 2006:2859 2865.
22. Cosa G. Photodegradation and photosensitization in pharmaceutical products: assessing drug phototoxicity. Pure Appl Chem 2004; 76(2):263 275.
23. Li YNB, Moore DE, Tattam BN. Photodegradation of amiloride in aqueous solution. Int J Pharm 1999; 183:109 116.
24. Lutka A. Effect of cyclodextrin complexation on aqueous solubility and photostability of phenothiazine. Pharmazie 1997; 55:120 123.
25. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research. Guidance for industry Q1B photostability testing of new drug substances and products. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073373.pdf. Accessed December 2009.
26. Serajuddin ATM. Salt formation to improve drug solubility. Adv. Drug Del Rev 2007; 59:603 616.
27. Agharkar S, Lindenbaum S, Higuchi T. Enhancement of solubility of drug salts by hydrophilic counterions: properties of organic salts of an antimalarial drug. J Pharm Sci 1976; 65:747 749.
28. Tong W Q and Whitesell G. In situ salt screening a useful technique for discovery support and preformulation studies. Pharm Dev Technol 1998; 3(2):215 223.
29. Cavatur RK, Vemuri NM and Suryanarayanan R. Preformulation studies for tablet formulation development. In: Hoag SW and Augsberger LL, ed. Pharmaceutical Dosage Forms: Tablets Vol. 1. Unit Operations and Mechanical Properties. New York: Informa Healthcare, 2007:465 483.
30. Vemuri NM, Chrzan Z and Cavatur R. Use of isothermal microcalorimetry in pharmaceutical preformulation studies part II: amorphous phase quantification in a predominantly crystalline phase. J Thermal Anal and Calorimetry 2004; 78:55 62.
31. Nema S, Washkuhn RJ and Brendel RJ. Excipients and their use in injectable products. PDA J Pharm Sci Technol 1997; 51(4):166 171.
32. Nema S., Brendel RJ and Washkuhn RJ. Excipients: parenteral dosage forms and their role. In: Swarbrick J, ed. Encyclopedia of Pharmaceutical Technology. 3rd ed. New York: Informa Healthcare, 2006:1622 1645.
33. U.S. Food and Drug Administration. Inactive ingredient search for approved drug products. Available at: http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm. Accessed December 2009.
34. Akers MA. Excipient drug interactions in parenteral formulations. J Pharm Sci 2002; 91(11): 2283 2300.
35. Durig T and Fassihi R. Identification of stabilizing and destabilizing effects excipient drug interactions in solid dosage form design, Int J Pharm 1993; 97:161 170.
36. Wissing S, Graig DQM, Barker SA et al. An investigation into the use of stepwise isothermal high sensitivity DSC as a means of detecting drug excipient incompatibility. Int J Pharm 2000; 199:141 150.
37. Phipps MA and Mackin LA. Application of isothermal microcalorimetry in solid state drug development. Pharm Sci Technol Today 2000; 3(1):9 17.
38. Cavatur R, Vemuri NM and Chrzan Z. Use of isothermal microcalorimetry in pharmaceutical preformulation studies, III: evaluation of excipient compatibility of a new chemical entity. J Thermal Anal and Calorimetry 2004; 78:63 72.
39. Peswani KS and Lalla JK. Naproxen parenteral formulation studies. J Parent Sci Technol 1990; 44(6):336 342.
40. Lewis GA, Mathieu D and Luu RPT. Screening. In: Pharmaceutical Experimental Design. New York: Marcel Dekker, 1999:23 78.
41. Gupta SL, Patel JP, Jones DL et al. Parenteral formulation development of renin inhibitor Abbott 72517. J Paren Sci Technol 1994; 48(2):86 91.
42. Wong J, Brugger A, Khare A et al. Suspensions for intravenous injection: a review of development, preclinical and clinical aspects. Adv. Drug Del Rev 2008; 60:939 954.

43. Boyd BJ. Past and future evolution in colloidal drug delivery systems. Expert Opin Drug Del 2008; 5(1):69 85.
44. Merisko Liversidge E, Liversidge GG and Cooper ER. Nanonizing: a formulation approach for poorly water soluble compounds. Eur J Pharm Sci 2003; 18:113 120.
45. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Disc 2004; 3:785 796.
46. Euliss LE, DuPont JA, Gratton S. et al. Imparting size, shape, and composition control of materials for nanomedicine. Chem Soc Rev 2006; 35:1095 1104.
47. Prankerd RJ and Stella VJ. The use of oil in water emulsions as a vehicle for parenteral drug administration. J Paren Sci Technol 1990; 44(3):139 149.
48. Constantinides PP, Lambert KJ, Tustian AK et al. Formulation development and antitumor activity of a filter sterelizable emulsion formulation of paclitaxel. Pharm Res 2000; 17(2):175 181.
49. Driscoll DF. Lipid injectable emulsions: pharmacopeial and safety issues. Pharm Res 2006; 23(9): 1959 1969.
50. Nguyen LT, Wiencek JM and Kirsch LE. Characterization methods for the physical stability of biopharmaceuticals. PDA J Paren Sci Technol 2003; 57 (6):429 445.

5 Formulation development of small and large volume injections

Madhav Kamat and Patrick P. DeLuca

INTRODUCTION

As described in the U.S. Pharmacopeia, USP-32/NF-27 (1), an injection is a preparation intended for parenteral administration and/or for constituting or diluting a parenteral article prior to administration. It is administered through the skin or other external boundary tissue, rather than through the alimentary canal, so that therapeutic substances, using gravity or force, can gain direct entry to a blood vessel, organ, tissue, or lesion. Parenteral products are required to meet pharmacopeial requirements for sterility, pyrogens, particulate matter, and other contaminants, and, where appropriate, contain inhibitors of the growth of microorganisms.

The USP (1) categorizes sterile preparations for parenteral use according to the physical state of the product as follows:

1. Liquid preparations that are drug substances or solutions thereof, for example, [drug] injection.
2. Dry solids that, upon the addition of suitable vehicles, yield solutions conforming in all respects to the requirements for injections, for example, [drug] for injection.
3. Liquid preparations of drug substances dissolved or dispersed in a suitable emulsion medium, for example, [drug] injectable emulsion.
4. Liquid preparations of solids suspended in a suitable liquid medium, for example, [drug] injectable suspension.
5. Dry solids that, upon the addition of suitable vehicles, yield preparations conforming in all respects to the requirements for injectable suspensions, for example, [drug] for injectable suspension.

Depending on the volume of injection in a package, the USP further designates injection, as either (*i*) small-volume injections or (*ii*) large-volume intravenous (IV) solutions. The term small-volume injection applies to an injection that is packaged in containers labeled as containing 100 mL or less. The large-volume IV solution applies to a single-dose injection that is intended for IV use and is packaged in containers labeled as containing more than 100 mL. Although the term sterile pharmaceutical is applicable to all injections (radiopharmaceuticals included), ophthalmic preparations, and irrigating solutions, this chapter emphasizes the formulation of injectable dosage forms.

FORMULATION OF SMALL-VOLUME INJECTIONS

In terms of number, the small-volume injections constitute the vast majority of all the injectable products in the market - small and large-volume injections combined. Whereas, large volume injections are administered exclusively as IV infusion, the small-volume injectables can be given by IV as well as other routes, although dictated by the volume of injection, as described later.

The goal of formulation development is to have a product that addresses all four requisites of an ideal product from a patient point of view: It should be safe, efficacious, stable, and acceptable/tolerable. From the point of marketing and commercial economics, the product should be easy to manufacture, relatively easy to use or present, and should have optimum shelf life at convenient storage conditions, such as room temperature. Although the preferred goal of the formulation scientist is to develop an injectable formulation that is ready to use (such as an aqueous solution), a number of codependent factors must be carefully evaluated in determining the most appropriate type of formulation. These factors are a) Biopharmaceutical considerations, b) Solubility, and c) Stability.

Biopharmaceutical considerations are aimed at achieving the required drug concentration for pharmacological response and include the intended mode of administration, desired onset of action, and the dose required. The formulation - the drug itself and the excipients used - must be compatible with body tissues, particularly taking care of properties such as hemolysis potential, pain on injection, precipitation of the drug upon administration, etc. Sterility, lack of pyrogenicity, and absence of particulate matter are other important considerations from general safety point of view.

Solubility issues become important when the drug does not have sufficient water solubility to achieve the target concentration in the formulation at physiologically acceptable pH range of 3 10. Various solubilization strategies must be employed to increase the solubility to achieve the required deliverable dose in a minimum possible volume. These techniques include use of buffers, salt formation, use of cosolvent, use of surfactants, etc.

Stability considerations are aimed at developing a formulation that provides sufficient shelf life, which is generally considered to be the time for 10% degradation. The product is optimized in such a way that its intrinsic degradation pathways, for example, the commonly encountered hydrolysis or oxidation, are minimized by appropriate modification of formulation composition, many times by using added substances, such as buffers, chelating agents, etc.

The successful formulation of an injectable small-volume preparation requires knowledge and expertise to effect rational decisions regarding the selection of

1. a suitable vehicle (aqueous, nonaqueous, or cosolvent),
2. added substances (buffers, antioxidants, antimicrobial agents, buffers, chelating agents, tonicity contributors, etc.), and
3. the appropriate container and closure components.

During the course of product development, formulation optimization is an iterative process and evolves as the product moves from the discovery to clinical to commercial stages. Inherent in the above decisions is the obligatory concern for product safety, effectiveness, stability, and reliability. As the injection formulation is finalized, a number of additional supportive studies must be undertaken to establish ruggedness of the formulation.

The majority of parenteral products are aqueous solutions, preferred because of their physiological compatibility and versatility with regard to route of administration. Survey of USP (1) shows that out of >300 pharmacopeial injection entries, nearly 70% are aqueous formulations (a similar trend is expected for nonpharmacopeial products as well). However, cosolvents or nonaqueous substances are often required to affect solution and/or stability of many compounds. Furthermore, for some other compounds, the desired properties must be attained through the use of an alternate dosage form such as suspension, emulsion, or even newer approaches such as liposomes and nanosuspensions.

Although each of these dosage forms have distinctive characteristics and formulation requirements, certain physical-chemical principles are common. Those common principles will be discussed in a general manner and the differences distinctive of each system will be emphasized. It is important to recognize that the pharmaceutical products derived from biotechnology are on the increase and the formulation of these products requires some unique skills and novel approaches. Formulation development aspects of these products are described elsewhere (see chap. 9).

Formulation Principles

Influence of the Route of Administration

Since parenteral preparations are introduced directly into the intra- or extracellular fluid compartments, the lymphatic system, or the blood, the nature of the product and the desired pharmacological action are factors determining the particular route of administration to be employed. The desired route of administration, in turn, places certain requirements and limitations on the formulations as well as the devices used for administering the dosage forms. Consequently, a variety of routes of administration (see chap. 2) are used.

One of the most important considerations in formulating a parenteral product is the appropriate volume into which the drug should be incorporated. The IV route is the only route

in which there are no strict limits of the volumes and as much as fifty milliliters can be administered by the IV route, via hypodermic injection, and several liters can be administered over the course of several hours through an IV administration system. Volumes up to 10 mL can be administered intraspinally, while the intramuscular route is normally limited to 3 mL, subcutaneous to 2 mL and intradermal to 0.2 mL.

The choice of the solvent system or vehicle is directly related to the intended route of administration of the product. IV and intraspinal injections are generally restricted to dilute aqueous solutions, whereas oily solutions, cosolvent solutions, suspensions, and emulsions can be injected intramuscularly and/or subcutaneously.

Isotonicity is another factor that must be taken into consideration. Although isotonic solutions are less irritating, cause less toxicity, and eliminate the possibility of hemolysis, it is not essential that all injections be isotonic. In fact, for subcutaneous and intramuscular injections hypertonic solutions are often used to facilitate absorption of drug because of local effusion of tissue fluids. With IV solutions, isotonicity becomes less important as long as administration is slow enough to permit dilution or adjustment in the blood. However, intraspinal injections must be isotonic because of slow circulation of the cerebrospinal fluid in which abrupt changes of osmotic pressure can give rise to severe side effects.

New routes of administration include intraarticular, directly into the synovial fluid for rheumatoidal diseases and even intradigital, between the fingers, in order to better target the lymphatics. The parenteral routes of administration will influence the design of novel dosage forms and drug delivery systems especially as more potent agents from biotechnology are developed.

This chapter focuses on the physicochemical aspects of formulating a stable product in a suitable container recognizing that safety must be established through evaluation of toxicity, tissue tolerance, pyrogenicity, sterility, and tonicity, and efficacy must be demonstrated through controlled clinical investigations.

Selection of Vehicle

Most parenteral products are aqueous solutions. Chemically, the high dielectric constant (DC) of water makes it possible to dissolve ionizable electrolytes and its hydrogen-bonding potential facilitates the solution of alcohols, aldehydes, ketones, and amines. Water for injection (WFI) is the solvent of choice for making parenterals. When it is not possible to use 100% aqueous solution for physical or chemical reasons, other means of solubilization including the addition of solubilizing agents or cosolvents may be necessary. For instance, nonpolar substances (i.e., alkaloidal bases) possess limited solubility in water and it is necessary to add a cosolvent such as glycerin, ethanol, propylene glycol, or polyethylene glycol. In other cases, to prevent chemical degradation (i.e., hydrolysis, oxidation, decarboxylation, or racemization) water may have to be eliminated partially or totally. Most proteins and peptides require an aqueous environment, and the addition of salt, buffer, or other additives for solubility purposes often leads to conformational changes. Consequently, parenteral product formulators should be aware of not only the nature of the solvent and solute in parenterals but also the solvent-solute interactions and the route of administration. Typically, aqueous solution formulations are prepared by simple solution of the drug and the excipients, by in situ salt formation of the drug in the solution (titrating against an acid or base), or by complexation of the drug with a complexing agent.

Solubility and solubilization. The solubility of a substance at a given temperature is defined quantitatively as the concentration of the dissolved solute in a saturated solution (i.e., the dissolved solute phase). Generally, drugs are present in solution at unsaturated or subsaturated concentrations; otherwise, crystallization of the drug may occur as a result of changes in pH, temperature, by seeding from other ingredients, or particulates in the solution.

The solubilization techniques for injectable formulations include pH adjustment, mixed aqueous/organic cosolvents, oily vehicles, surface-active agents, complexation, as well as formulating the drug in emulsion, suspension, liposomes, nanosuspensions, and combinations of techniques. An excellent review of the solubilizing excipients that could be used in the injectable formulations has been provided by Strickly (2).

Table 1 Expressions for Approximate Solubility

Term	Relative amount of solvent to dissolve
Very soluble	<1
Freely soluble	1 10
Soluble	10 30
Sparingly soluble	30 100
Slightly soluble	100 1,000
Very slightly soluble	1,000 10,000
Practically insoluble or insoluble	>10,000

Table 2 Typical Examples of Drugs Representing the Solubility Terms

Term	Drug	Solubility of drug
Very soluble	Chloral hydrate	>8 g/mL
Freely soluble	Isoniazid	0.330 g/mL
Soluble	Guaifensin	0.050 g/mL
Sparingly soluble	Pyrazinamide	0.015 g/mL
Slightly soluble	Salicylic acid	0.002 g/mL
Very slightly soluble	Griseofulvin	0.000,02 g/mL
Practically insoluble or insoluble	Diclofenec	0.000,002 g/mL

Source: Adapted from Ref. 3.

Solubility expressions. Solubility of a substance can be expressed in a number of ways. Generally, the concentration is expressed as percent (w/v), that is, grams per 100 mL of solution, but molarity and molality have been used. Molarity is defined as the number of moles per 1000 mL of solution. Molality is the number of moles of solute per 1000 g of solvent and, therefore, being a weight relationship, is not influenced by temperature. The USP lists solubility in terms of the number of milliliters of solvent required to dissolve 1 g of substance. If exact solubilities are not known, the USP provides general terms to describe a given range. These descriptive terms are listed in Table 1. Typical examples of drugs representing the solubility terms are listed in Table 2 (3).

Bonding forces. For a substance to dissolve, the forces of attraction that hold the molecules together must be overcome by the solvent. The solubility will be determined by the relative binding forces within the substance (solute-solute interactions) and between the substance and the vehicle (solute-solvent interactions). If an environment similar to that of the crystal structure can be provided by the solvent, then the greater the solubility (i.e., "like dissolves like"). Ionic compounds dissolve more readily in water by virtue of ion-dipole interactions, whereas hydrophobic substances dissolve more easily in organic solvents as a result of dipole or induced dipole interactions.

Often, the solubility of the drug substance is due in large part to the polarity of the solvent, generally expressed in terms of dipole moment, which is related to the DC. Solvents with high DCs dissolve ionic compounds and are water soluble, whereas solvents with low DCs are not water soluble and do not dissolve ionic compounds. The former are classified as polar solvents (e.g., water, glycerin, and ethanol), while the latter are nonpolar (e.g., chloroform, benzene, and the oils). Solvents with intermediate DCs (e.g., acetone and butanol) are classified as semipolar. The DCs of most pharmaceutical solvents are known (4) and values for a number of binary and tertiary blends have been reported (5) and, if not reported, can be readily estimated (6,7). Table 3 is a listing of the DCs of some solvents at 25°C.

The solubility profiles of a number of pharmaceuticals as a function of DC have been reported by Paruta and coworkers (8 10). By determining the solubility of a substance in a system at various DCs, a graph such as that shown in Figure 1 can be constructed to determine the DC that will provide the required solubility for a particular drug substance. As can be seen

Table 3 DCs of Some Solvents at 25°C

Solvent	DC
Water[a]	78.5
Glycerin[a]	40.1
N,N dimethyl acetamide[a]	37.8
Propylene glycol[a]	32.0 (30°C)
Methanol	31.5
Cremophor EL (R) (polyoxyl castor oil 35)[a]	27.0
Ethanol[a]	24.3
N propanol	20.1
Acetone	19.1
Benzyl alcohol[a]	13.1
Polyethylene glycol 400[a]	12.5
Cottonseed oil[a]	3.0
Benzene	2.3
Dioxane	2.2

[a]Solvents used in parenterals
Abbreviation: DC, dielectric constant.

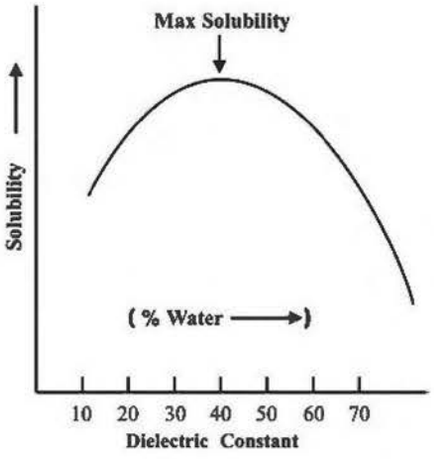


Figure 1 Hypothetical plot of solubility of a substance versus dielectric constant in various mixtures of dioxane and water.

from the plot, to obtain the maximum concentration, a DC of around 40 is required. Not all mixtures will show a maximum, but such a plot illustrates the required DC to obtain the desired concentration. For example, if a DC (DC) of 60 was selected, a mixture of water (DC = 78.5), polyethylene glycol (PEG) 400 (DC = 12.5) and ethanol (DC = 24.3) could be used. Selecting an amount of ethanol necessary to dissolve the drug (e.g., 10%), the percentages of PEG 400 and water can be calculated as follows:

$$(10)(24.3) + (X)(78.5) + (90 \quad X)(12.5) = (100)(60)$$

where X is the percentage of water required and is calculated to be 73.5%.

Therefore, the vehicle to provide a DC of 60 will have the following composition: Ethanol 10%, PEG 400 16.5%, and Water 73.5%

Since DC is a measure of the polarizability and dipole moment of a compound, several researchers have explored other parameters and polarity indices (11) which include molecular volume, solvent and solute interactions and specific interactions such as hydrogen bonding. In 1952, Hildebrand and Scott (12) introduced solubility parameters to predict solubility of regular solutions. Since pharmaceutical systems deviate from regular or ideal solutions, Martin and coworkers (13) modified the Hildebrand approach to include hydrogen-bonding and

dipolar interactions. The molecular surface area of the solute and interfacial tension between solute and solvent were further used by Amidon (14) and Yalkowsky (15) to predict solubility. Among the many theoretical models available to predict solubility in water, recent reports review the available models and discuss the potential and limitations of these computational approaches (16,17).

Hydrogen bonding, a type of dipole-dipole interaction, is an important determinant of solubility. Because of its small size, the hydrogen atom (proton donor) with its positive center, can approach the negative center (electron donor) of a neighboring dipole more closely than any other atom. As a result of this spatial maneuverability, both intramolecular bonding (i.e., between groups within a single molecule) and the intermolecular type (i.e., among molecules) can occur. The latter is responsible for association in most solvents and dissolution of most drugs. Alcohols dissolve in water by hydrogen bonding, up to an alkyl chain length of five carbon atoms. Phenols dissolve in water and alcohol and, as the number of hydroxyl groups increase, the water solubility is enhanced because of the increased opportunity for hydrogen bonding. Most aromatic carboxylic acids, steroids, and cardiac glycosides are not water soluble but dissolve in alcohol, glycerin, or glycols by hydrogen bonding.

Dipole-ion interaction is another important molecular property that is responsible for the dissolution of ionic crystalline substances in polar solvents (i.e., water or alcohol). Ions in aqueous solution are generally hydrated (surrounded by water molecules) by as many water molecules as can spatially fit around the ion. The attributes of a good solvent for electrolytes include: (*i*) a high-dipole moment; (*ii*) a small molecular size; and (*iii*) a high DC to reduce the force of attraction between the oppositely charged ions in the crystal. Water possesses all of these characteristics and is, therefore, a good solvent for electrolytes. The cation of the electrolyte is attracted to the negative oxygen atom, while the anion attracts the hydrogen atoms of the dipolar water molecules.

Symmetrical molecules, such as benzene and carbon tetrachloride, possess zero dipole moment and are nonpolar. Solubility of such molecules or their existence in a liquid state is due to van der Waals forces. Other intermolecular interactions, such as London forces or Debye interactions are also responsible for solubility of such nonpolar substances.

Effect of temperature. Substances generally dissolve faster if heat is applied to the system and the solubility of most solids is increased by an increase in temperature. This is true if the substance absorbs heat during the course of dissolution. The degree to which temperature can influence solubility is determined by the heat of solution, more specifically the differential heat of solution, ΔH, which represents the rate of change of the heat of solution per mole of solute in a solution of specified concentration. The higher the heat of solution, the greater is the influence of temperature on solubility.

The following equation shows the influence of temperature on solubility:

$$\frac{d\,Ln\,S}{dT} = \frac{\Delta H}{RT^2} \tag{1}$$

where S is the solubility or concentration of a saturated solution, often expressed in terms of molality, molarity, or mole fraction; R is the gas constant; and T is the absolute temperature. Equation (1) can be written as

$$\log S = \frac{\Delta H}{2.303R} \times \frac{1}{T} + \text{constant} \tag{2}$$

By plotting the logarithm of the solubility in moles per liter versus the reciprocal of the absolute temperature as shown in Figure 2, the differential heat of solution can be calculated from the slope of the line, which is equal to

$$\frac{\Delta H}{(2.303)(1.987)}$$

A positive heat of solution indicates that the process is endothermic (i.e., the solute absorbs heat when dissolving). Therefore, an increase in temperature will increase solubility. A

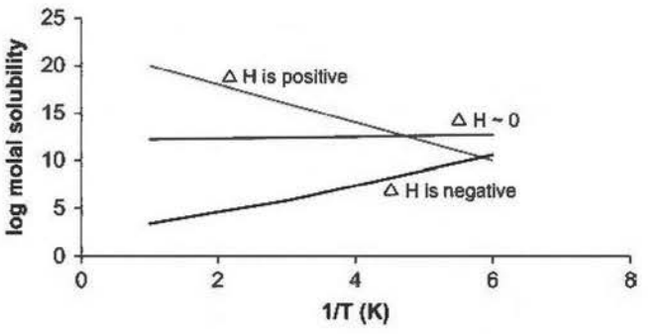


Figure 2 Effect of temperature on solubility of a substance. ΔH represents the differential heat of solution and is calculated from the slope of the line, $\frac{\Delta H}{(2.303)(1.987)}$.

negative value indicates that the process is exothermic (i.e., the solute evolves heat when dissolving). In this case, increase in temperature results in a decrease in solubility. A differential heat of solution around zero indicates that the solubility is not significantly influenced by temperature.

Measuring solubility. Methods for determining the solubility of drug substances in aqueous solvents have been described (18,19). The standard way to determine the solubility of a compound is to use the "shake-flask" solubility method. This method is inherently low-throughput, labor intensive, and necessitates the addition of drug in a powder form. It involves adding an excess quantity of solid material to a volume of buffer at a fixed pH and the saturated solution is agitated (shake-flask) until equilibrium is reached, generally 12 hours to seven days. Following separation by filtration or centrifugation, the compound in solution is analyzed and quantified by a suitable analytical technique such as UV/Vis spectroscopy or high-performance liquid chromatography (HPLC). The other classical experimental methods used to determine solubility are turbidimetric ranking assays, HPLC-based assays, and potentiometric methods. The newer high-throughput methods which determine both kinetic and thermodynamic (equilibrium) solubilities are based on screening multiple solutes and solvents, in array of compositions, using 96-well format that allows for solubility analysis in a single plate with very low drug amount (19,20).

Solubilization techniques. A variety of approaches to increase the aqueous solubility of an otherwise less soluble or insoluble drug substance to a desired level for optimum injectable product have been reported and reviewed (2,21,22). These include: 1) pH adjustment, 2) salt formation, 3) use of cosolvents, 4) surfactants as solubilizers, 5) use of complexing agents, and others. Metabolizable oils as vehicles have has also been used for certain class of compounds. Beyond these solubilization approaches, it may become necessary in some cases to change the formulation from solution to dispersed system such as emulsion, suspension, and more recently liposomes and nanosuspensions.

pH adjustment Most organic drug substances are weak electrolytes and, therefore, exist in solution in dissociated and undissociated forms. The ratio of these forms is determined by the pH of the solution as per the Henderson-Hasselbach relationship. As a result, properties such as solubility, partition coefficient, and chemical stability, which are markedly different for the undissociated and dissociated forms are influenced by pH.

Many of the organic electrolytes used in parenteral systems contain a basic nitrogen atom in the molecules. These include antihistamines, alkaloids, local anesthetics, and so on, which are practically insoluble in water but dissolve readily in dilute solutions of acids because of salt formation. The addition of alkali to these solutions increases the pH and causes free base to precipitate. Examples are atropine sulfate, ephedrine sulfate, lidocaine hydrochloride, and pyribenzamine hydrochloride.

In compounds that contain an electron withdrawing group, such as oxygen, a positive center is created, which in turn attracts electrons from adjacent nitrogen, and if a hydrogen

atom is attached, the N-H bond is weakened. As a result, in alkaline solution a more soluble anion is formed. The examples are phenobarbital and sulfanilamide.

The addition of acid to the solutions of these compounds will cause the free acid form to precipitate. Even the addition of a salt of a strong acid such as morphine sulfate will result in precipitation.

Most marketed injection products are in the pH range of 4 to 8 for biocompatibility reason, however, some are outside of this range. The pH solubility and pH stability-rate profiles of a drug usually determines the pH at which a product is formulated (23). Additional formulation variables to be considered are the necessity of a buffer, buffer capacity, and drug concentration. These variables are described in details in a further section (see "Added Substances").

Salt formation Salts of acidic and basic drugs usually exhibit higher solubility than their corresponding acid or base forms. Therefore, salt formation is the most preferred and effective method of increasing solubility and dissolution rates of acidic and basic drugs (24,25).

Solubility-pH profiles of weakly acidic or basic organic drugs may be visualized on the basis of classical Henderson-Hasselbach relationship. In the case of monoprotic acid, a saturated solution can be defined by the following equations and corresponding constants (26).

$$HA \Leftrightarrow H^+ + A^- K_a = \frac{[H^+][A^-]}{[HA]} \tag{3}$$

$$HA(\text{Solid}) \Leftrightarrow HA(\text{Solution}) S_0 = [HA] \tag{4}$$

where [HA] is the concentration of undissociated acid form, $[A^-]$ is the concentration of corresponding salt form, $[H^+]$ is the concentration of proton or dissociated hydrogen, and S_0 is the intrinsic solubility of the monoprotic acid. Solubility, S, at a particular pH is defined then as mass balance sum of the concentrations of all of the species dissolved in the aqueous phase.

$$S = [A]^- + [HA] \tag{5}$$

Rearranging equations (3), (4), and (5),

$$\begin{aligned} S &= K_a[HA]/[H^+] + [HA] \\ &= S_0(K_a/[H^+] + 1) \\ &= S_0\left(10^{-pK_a+pH} + 1\right), \text{ or} \\ \log S &= \log S_0 + \log\left(10^{-pK_a+pH} + 1\right) \end{aligned} \tag{6}$$

For a weakly acidic drug, depending on the pH of the solution, the term, $\log\left(10^{-pK_a+pH} + 1\right)$, changes solubility function according to the conditions below.

1. $pH >> pK_a$

   The exponent ($pK_a + pH$) remains positive and very large number compared with 1, and hence, 1 is ignored, and

$$\begin{aligned} &\log\left(10^{-pK_a+pH} + 1\right) \text{ becomes } \log\left(10^{-pK_a+pH}\right) \text{ or} \\ &(\ pK_a + pH) \log(10) \text{ or } (\ pK_a + pH) \\ &\text{Therefore,} \\ &\log S = \log S_0 \quad pK_a + pH \\ &\text{Since } pK_a \text{ is a constant.} \\ &\log S = (\log S_0 \quad pK_a) + pH \end{aligned} \tag{7}$$

   Equation (7) is of the form, $Y = c + mX$ or an equation of a straight line with associated intercept and slope, c and m, respectively.

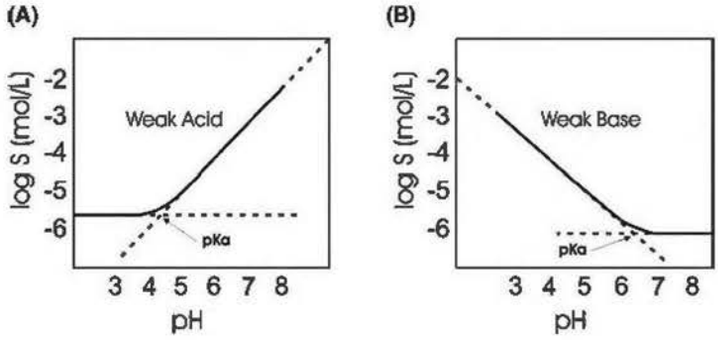


Figure 3 Solubility/pH profile for weak acid ($pK_a = 4.4$) and weak base ($pK_a = 6.1$). *Source*: Adapted from Ref. 26.

Therefore, a plot of log$S$ versus pH, will yield a straight line the slope of which will be equal to +1 and the intercept will be (log S_0 pK_a). A similar relationship can be made for a weakly basic drug, in which case, the slope will be equal to 1.

Figure 3 (26) shows the solubility-pH profile for a (*i*) weak acid (pK_a 4.4, logS_0 5.6) and (*ii*) weak base (pK_a 6.1, log S_0 5.9).

2. pH = pK_a, or at the inflection point in the curve.
   The exponent (pK_a + pH) becomes zero and the term

$$\log\left(10^{-pK_a+pH}+1\right) \text{ becomes } \log\left(10^0+1\right) \text{ or } \log(1+1).$$

   Therefore,

$$\log S = \log S_0 + 0.3$$

3. pH << pK_a, or at the flat line of the curve.
   The exponent (pK_a + pH) remains negative.

$$\log\left(10^{-pK_a+pH}+1\right) \text{ becomes } \log\left(0.000\ldots+1\right) \text{ or close to } 0.$$

   Therefore,

$$\log S \cong \log S_0$$

Whether certain acidic or basic drugs would form salts and, if salts are formed, dissociation back to the free acid or base forms would depend on several factors, such as pH, pK_a, S_0 (intrinsic solubility), k_{sp} (solubility product) and pH_{max} (pH of maximum solubility). The aqueous solubility of an acidic or basic drug as a function of pH determines if the compound will form suitable salts within the physiologically acceptable pH range. Moreover, the common-ion effect of the salt-forming agents is also important in determining the final solubility. It has been reported that dissolution rates of a hydrochloride salt decrease as the pH of an aqueous medium is lowered when HCl is added or if NaCl is added to the medium. Similarly, the dissolution rate of a sodium salt decreases in the presence of added NaCl in the medium. There are numerous reports in the literature indicating such common-ion effects on salts having relatively low aqueous solubilities (27).

A review by Serajuddin about the principles of salt formation and its utility in formulation has recently been published (28). It surveyed about 120 salts approved by the FDA during the 12-year period from 1995 to 2006 and showed that the hydrochloride salt was the predominant salt form among the basic drugs and the sodium salt was the predominant form for acidic drugs. About 77% of the salts of basic drugs were prepared with relatively stronger counterions (hydrochloride, hydrobromide/bromide, sulfate/bisulfate and nitrate). Similarly, 14 out of 19 salts of acidic drugs were prepared with strong alkalies such as NaOH and KOH.

Use of cosolvents If the pH adjustment or salt formation approach still results in aqueous solubility of a drug well below its therapeutic dose, a mixture of solvents may be used to achieve sufficiently high solubility. A cosolvent is a water-miscible organic solvent that is used to increase the solubility of a poorly water-soluble compound. The addition of cosolvent results in reduction of polarity of water which in effect reduces the surface tension, DC, and solubility parameter of water. The increase in solubility by cosolvents is much more dramatic for nonpolar solutes (can be several orders of magnitude), than for solutes of intermediate polarity. Another advantage of using cosolvents is that a change in solvent property may help considerably in stability for drugs which may exhibit hydrolytic degradation by reducing the concentration of water in the formulation. Cosolvent may also enhance the stability of a drug by providing a less suitable environment for the transition state of the reactants, provided the transition state is more polar than the reactants. It is reported that cosolvents are employed in approximately 10% of the FDA approved injectable products (22).

Cosolvents and solubility J. H. Hildebrand, in a series of papers published begining in 1916, deescribed the basic principles of solutions and solubility and introduced the cosolvency approach (29) and experimental tests of a general equation for solubility (30). Since then, numerous theoretical cosolvency models have been proposed that correlate and/or predict the solubility of drugs in water cosolvent mixtures (31 34) and have been reviewed extensively by Jouban (35). The simplest experimental cosolvency model, that is, the log-linear model of Yalkowsky (36 38), provides an estimate of drug solubility in water-cosolvent mixtures using aqueous solubility of the drug. It is expressed as:

$$\log S_m = f \log S_c + (1 \quad f) \log S_w \tag{8}$$

Where S_m is the solute's solubility in water-cosolvent mixture, f is the volume fraction of cosolvent, S_c is the solubility of drug in pure cosolvent, and S_w is the solubility of drug in water. S_x values can be expressed in g/L, mole fraction, etc. Equation (8) can be further simplified as

$$\log S_m = \log S_w + f\sigma \tag{9}$$

where

$$\sigma = \log ac_w \quad \log ac_c \tag{10}$$

And ac_w and ac_c are the activity coefficients for the drug in water and cosolvent, respectively. In a given cosolvent system, σ will be constant. Therefore, if one plots log S_m versus f, the slope will be σ. Comparing slopes of different cosolvent-water systems can easily be done by using σ as a measure of the solubilization potential of the cosolvent. In practice, experimental methods of characterizing the solubility of cosolvent systems can be utilized with the aid of statistical experimental design. Advantage of the experimental approach is that one can use additional excipients, for example, surfactants, buffers, etc., in screening experimental designs.

Cosolvents and stability Cosolvents cannot only increase the solubility of drugs but may also increase the stability of some drugs (31). The addition of cosolvent reduces the collision probability between a water molecule and a drug molecule which is necessary for hydrolysis. As mentioned earlier, the degradation rate of a drug may change with the DC of the medium. Decreasing the polarity of the reaction medium by the addition of cosolvent unfavors the formation of the charged species. It stabilizes a solute against any reaction that produces charged products or proceeds through a charged transition state (39,40). As a general rule, for reactions leading to products that are less polar than the starting material, a less polar medium may accelerate the reaction. On the other hand, reactions leading to products that are more polar than the starting material may proceed rapidly in polar media.

Improvement of stability of a drug in the presence of cosolvent was reported by Ni, et al (41). The authors studied the stability of an anticancer compound, SarCNU (a nitrosourea derivative), in several pharmaceutically acceptable solvents such as water, EtOH, propylene