*review articles*

# *Plasma or serum in therapeutic drug monitoring and clinical toxicology*

DONALD R.A. UGES

## Introduction

In therapeutic drug monitoring and clinical toxicology most determinations of the concentration of a substance are carried out in blood or blood components. Whole blood can only be analysed if the blood has been sampled in collecting tubes containing an anticoagulant. When such samples are centrifuged, plasma is obtained. Therefore, plasma always contains one or more anticoagulants. Heparin sodium (3-100 U/ml) is most widely used for this purpose, but citrate, edetate, oxalate and fluoride are also suitable additives. Serum is the clear liquid that separates from blood when blood is allowed to clot completely and then centrifuged. Therefore serum contains no fibrogen and no anticoagulants.

The choice between whole blood on the one hand and either plasma or serum on the other is clear. Whole blood concentrations are only measured if the compound is concentrated in the erythrocytes (*e.g.* lead, cyanide, mercury, carbon monoxide, chlorthalidone),[1-3] if there is a fluctuating erythrocyte–plasma ratio (ciclosporin A),[4] or because of the risk of loss during storage or centrifugation.[5]

The clinical effect of several compounds correlates better with the concentration in a tissue compartment than with the serum or plasma concentration. Considering erythrocytes as a readily available tissue, whole blood should be the matrix of choice in therapeutic drug monitoring and toxicology.[6,7] Sometimes lymphocyte concentration can predict the therapeutic effect better (epirubicin, mitoxantrone).[8,9] In nearly all other cases, plasma or serum concentrations are measured.

## Is there any difference between serum and plasma concentration?

The expression 'plasma concentration' is a part of the title of many articles in the literature. Reading these articles, however, it appears that serum samples have often been taken. The authors very seldom present their arguments for the choice between plasma or serum.

Some of the advantages of plasma over serum are:
- large volume;
- no delayed clotting;
- less risk of haemolysis;
- the sample is often suitable for both whole blood and plasma monitoring.

Some of the disadvantages of plasma over serum are:
- the (unknown) influence of the anticoagulant on the assay, the protein binding and the stability of the sample;
- the (unknown) influence of additives or impurities in the anticoagulant on the assay and the concentration;
- the risk of the formation of small clots (incomplete mixing, instability);
- dilution of the sample;
- sampling is more expensive;
- choice of anticoagulant can be confusing.

### ADVANTAGES OF PLASMA OVER SERUM

*Larger available volume*

If blood is allowed to clot and is then centrifuged,

Uges DRA. Plasma or serum in therapeutic drug monitoring and clinical toxicology. Pharm Weekbl [Sci] 1988;10:185-8.

**Keywords**
Anticoagulants
Blood coagulation
Blood preservation
Plasma
Protein binding
Serum
Therapeutic drug monitoring
Toxicology

*Laboratory for Clinical and Forensic Toxicology and Drug Monitoring, Department of Pharmacy, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen.

**Abstract**

The relative merits of plasma and serum in blood analysis are reviewed. The expression 'plasma concentration' is often used in the literature, although serum samples have been taken. In most cases serum and plasma concentrations of analytes are the same. The choice depends mostly on the policy of the hospital or the availability of the test tubes in the ward. Some of the advantages of plasma over serum are large volume, no delayed clotting, less risk of haemolysis. In addition, the sample is often suitable for both whole blood and plasma monitoring. Some of the disadvantages of plasma over serum are the (unknown) influence of the anticoagulant on the assay, on the protein binding and on the stability of the sample, the (unknown) influence of additives or impurities in the anticoagulants on the assay and on the concentration, the risk of the formation of small clots and dilution of the sample.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

about 30 to 50% of the original volume is collected as serum (upper layer). A slightly larger sample (in general 50%), known as plasma, can be obtained by centrifugation of a blood sample collected in a tube containing an anticoagulant. Serum and plasma are not significantly different with respect to drug analysis.

Despite the fact that serum is devoid of the proteins associated with the clotting process, the most important proteins (quantitatively speaking), such as the albumins and globulins, are present in similar amounts (approximately 2% wt/vol) in both serum and plasma. Thus plasma is in general preferred because of its greater yield from blood samples. The greater yield the greater the amount of drug and the fewer the problems with sensitivity, or with sampling neonates.[10 11]

*Delay through time needed for clotting*

Clotting can sometimes be of long duration. This is especially true when samples are taken in polypropylene test tubes, in which case clotting can continue even after centrifugation.

*Decreased risk of haemolysis*

The chlorthalidone concentration in erythrocytes is about 40 times as high as that in plasma.[8] One percent of haemolysis (which cannot easily be seen) will increase the apparent plasma concentration by 25%. This problem is even more acute when serum samples are produced from whole blood.

The separation by centrifugation should be carried out as quickly as possible in order to prevent any effects due to lysis of the blood clot.[11] This phenomenon is well known in the analysis of the endogenous compounds potassium and iron in serum.

*Suitability of the sample for both whole blood and plasma analysis*

If several analyses have to be carried out on the blood of a single patient, determinations can be carried out in whole blood as well as plasma from one blood sample containing a suitable anticoagulant. In bedside chemistry whole blood is preferred to plasma or serum, because this avoids a centrifugation step.[12]

## DISADVANTAGES OF PLASMA IN RELATION TO SERUM

*The influence of the anticoagulant on the assay*

Walters and Roberts showed that heparin at a concentration of 100 U per ml interfered with the gentamicin EMIT® assay, but not with the $TD_X$® FPIA assay.[13] This effect is thought to be due to the inhibition of enzyme activity rather than disturbance of the antigen–antibody reaction. Blood collected into heparinized tubes usually contains approximately 30 U of heparin per ml and at this level no interference with the EMIT® assay occurs. However, plasma derived from specimens collected from arterial and venous blood is likely to yield erroneous results.

The formation of a gentamicin–heparin complex has been demonstrated by Myers *et al.*[14] Cipolle *et al.* concluded that heparinized tubes should not be used when collecting blood samples for assays where inactivation of gentamicin would give false values.[15] Edetate was for gentamicin a suitable alternative. On the other hand, the Dutch foundation 'Quality control clinical drug analysis and toxicology' (KKGT) found with its quality control programme that edetate interfered strongly with the FPIA assay and not with the EMIT® assay of carbamazepine and with the EMIT® assay and not with FPIA assay of valproic acid. The reason of this interference is unknown (Dijkhuis IC, personal communication, 1988).

*The influence of the anticoagulant on protein binding*

A discrepancy in the measurement of lidocaine in heparinized blood and in serum could be expected, as heparin increases the free fraction of lidocaine.[16] Lidocaine is subsequently redistributed from the plasma into red blood cells.

The percentage of free ibuprofen in heparinized plasma is significantly higher than in non-heparinized plasma.[17] Although small doses of heparin can affect drug binding, the extent and variability of the effect depends on the biological activity of the heparin, and varies with the manufacturer and batch, time of sampling, and food intake.[18] Although the intravenous administration of heparin results in a high lipolytic activity *in vivo* and consequently increases the concentrations of non-esterified fatty acids *in vitro*, it is unlikely that this will happen after adding heparin to the tube.

Non-esterified fatty acid can displace numerous drugs from their binding sites in plasma. When the non-esterified fatty acids concentration increases *in vitro*, phenytoin will be displaced from the binding sites. The free phenytoin then shifts from the plasma into the erythrocytes, resulting in a lower phenytoin plasma level (up to 20% below its original value!).[19] The same phenomenon was found with amitriptyline, imipramine and maprotiline.[6] The recovery of chlorpromazine added to heparinized plasma *in vitro* was reduced compared with that obtained with water or oxalated plasma.[20]

*The influence of the anticoagulant on the stability of the sample*

When heparin deteriorates or adsorbs onto the wall of the collecting tube, clotting will still occur in a clear plasma sample. The pH of the sample can be changed by using sodium citrate, or oxalate as anticoagulant. This change of pH may influence the stability of the drug in the sample (*e.g.* atracurium). Sodium fluoride can be used as anticoagulant (cal-

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

cium binder), and as a preservative (antimicrobial) of ethanol. Blood samples that are to be tested for ethanol must contain 10 mg sodium fluoride per ml during storage.[21 22]

*The influence of additives or impurities in the anticoagulant on the assay*

Benzyl alcohol, which may be added to heparin as a preservative, can disturb the fluorimetric assay of corticosteroids.[23] Serum zinc concentrations are 16% higher than plasma concentrations. This phenomenon was explained by a release of zinc from platelets during coagulation of the blood samples.[24] Keyzer *et al.*, however, found no difference between the serum and plasma zinc levels of fifty volunteers.[25]

*The influence of additives or impurities in the anticoagulant on the concentration*

Anticoagulants can contain impurities which are precisely the compounds to be determined, *e.g.* lead, aluminium, copper, fluoride.

*The risk of clot formation because of poor mixing or poor stability*

Unfortunately, blood samples in heparinized tubes are sometimes not very well mixed. In such cases a partially clotted sample will arrive in the laboratory.

*Improper dilution of the sample*

We have had a number of incidents in which blood samples of about 5 ml were diluted with about 1 ml of a solution of anticoagulant (20% dilution!). Differences in serum and plasma transcobalamin II levels in the literature have been explained by dilution by EDTA or sodium fluoride solutions.[26]

*Cost*

Sample tubes with heparin are approximately 10% more expensive than non-heparinized tubes.

*The choice of anticoagulant*

There are several anticoagulants, each of which must be used in a different concentration. Examples are sodium heparin, lithium heparin, potassium edetate, sodium edetate, sodium citrate, and sodium fluoride. The differences in the influence of these anticoagulants on the various assays are largerly unknown.

Differences were demonstrated between serum and plasma levels of endogenous compounds, which may be of sufficient magnitude to alter clinical interpretation of some results when using different radioassay procedures and different anticoagulants.[27] These differences are in general larger with immunoassays than with chromatographic methods. Cholinesterase activity can be determined in serum or heparinized plasma. Sodium fluoride and citrate should not be used as anticoagulants because they depress cholinesterase activity as measured by several methods.[28]

Anticoagulants used in sampling plasma can confound diffusion assays by causing alterations in the agar gel matrix. Therefore, in the microbiological measurement of, for example, erythromycin, cytarabine or 5-fluorouracil the use of serum is recommended.[29]

**Conclusion**

In conclusion, we can say that in most cases serum and plasma analyte concentrations are the same. The choice depends mostly on the established practice in the hospital or the kind of available test tubes in the ward. In special cases such as the determination of free drug levels, however, there are important differences. It is advisable, therefore, that during kinetic studies of drugs, the similarity of serum and plasma levels for the compound being studied be proven. Furthermore, to avoid confusion, authors should be careful and precise in their presentations so that readers are aware that blood, plasma or serum is the sample under discussion.

**References**


1 Casarett LJ, Doull J. Toxicology. The basic science of poisons. New York: Macmillan Publishing Company, 1975.

2 Moffat AC, ed. Clarke's isolation and identification of drugs. 2nd ed. London: The Pharmaceutical Press, 1986.

3 Beermann B, Groschinsky-Grind M. Clinical pharmacokinetics of diuretics. Clin Pharmacokinet 1980;5:221-45.

4 Keown PA, Stiller CR, Stawecki M, McMichael J, Howson W. Pharmacokinetics and interactions of ciclosporin. In: Schindler R, ed. Ciclosporin in autoimmune diseases. Berlin: Springer-Verlag, 1985:39-42.

5 Uges DRA, Van der Paauw HJW. Alcohol, de bloedproef en de contra-expertise [Alcohol, blood test and counterexpertise]. Ned Forens Tijdschr 1982;2:7-9.

6 Maguire KP, Burrows GD, Norman JR, Scoggings BA. Blood plasma distribution ratios of psychotropic drugs. Clin Chem 1980;26:1624-5.

7 Linnoila M, Dorrity F, Jobson K. Plasma and erythrocyte levels of tricyclic antidepressants in depressed patients. Am J Psychiatry 1978;135:557-61.

8 De Vries EGE, Greidanus J, Mulder NH, et al. A phase I pharmacokinetic study with 21-day continuous infusion of epirubicin. J Clin Oncol 1987;5:1445-51.

9 De Vries EGE, Greidanus J, Mulder NH, et al. A phase I and pharmacokinetic study with 21-day continuous infusion of mitoxantrone (MX). Am Soc Clin Oncol 1988;7:58.

10 Smits RV, Stewart JT. Textbook of biopharmaceutic analysis. Philadelphia: Lea & Febiger, 1981:3-16.

11 Toseland PA. Samples and sampling. In: Moffat AC, ed. Clarke's isolation and identification of drugs. 2nd ed. London: The Pharmaceutical Press, 1986:111-7.

12 Hichs JA, Iosefsohn M. Another physician's office analyser: the Abbott vision evaluated. Clin Chem 1987;33:817-9.

13 Walters MI, Roberts WH. Gentamicin/heparin interactions: effects on two immunoassays and on protein


DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

binding. Ther Drug Monitor 1984;6:199-202.
[14] Meyers DR, DeFehr J, Bennett WM, Porter GA, Olsen GD. Gentamicin binding to serum and plasma proteins. Clin Pharmacol Ther 1978;23:356-60.
[15] Cipolle RJ, Zaske DE, Crossley K. Gentamicin/tobramycin. Therapeutic use and serum concentration monitoring. In: Taylor WJ, Finn LA, eds. Individualizing drug therapy. 1. New York: Frank Cross Townsend Inc., 1981:138.
[16] Dube LM, Ho-Ngoc A, Davies RF, Beaulands DS, Mousseau N, McGilveray IJ. Influence of protamine on heparine induced increases of lidocaine free fraction. Res Commun Chem Pathol Pharmacol 1983;42:401-15.
[17] Pearce GA, Brown KF. Heat inhibition of *in vitro* lipolysis and $^{14}$C ibuprofen protein binding in plasma from heparinized uraemic subjects. Life Sci 1983; 33:1457-66.
[18] Naranjo CA, Sellers EM, Khouw V, Alexander P, Fan T, Shaw J. Variability in heparin effect on serum drug binding. Clin Pharmacol Ther 1980;28:545-50.
[19] Schulz P, Abang A, Giacomini JC, Blaschke TF, Giacomini KM. Effect of heparin on the red blood cell-to-plasma concentration ratio of diphenylhydantoin and prazosin. Ther Drug Monitor 1983;5:497-9.
[20] Whelpton R. Tricyclic antidepressants and neuroleptics. In: Curry AS, ed. Antalytical methods in human toxicology. Part 1. Weinheim: Verlag Chemie, 1985: 139-58.
[21] Flanagan RJ, Widdop B. Clinical toxicology. In: Curry AS, ed. Analytical methods in human toxicology. Part 1. Weinheim: Verlag Chemie, 1985:37-66.
[22] Froentjes W, Schute JB, Strengers Th, Verwey AMA. Analytisch-chemische aspecten van de wijziging van de Wegenverkeerswet (II) [Analytical and clinical aspects of the change in the Traffic Act (II)]. Pharm Weekbl 1976;111:349-62.
[23] Werk EE jr. Interference of heparin containing benzyl alcohol in the fluorometric determination of plasma corticosteroids. J Clin Endocrinol 1967;27:1350.
[24] Foley B, Johnson SA, Hackley B, Smith JC jr, Halsted JA. Zinc content of human platelets. Proc Soc Exp Biol Med 1968;128:265-9.
[25] Keyzer JJ, Oosting E, Wolthers BG, Muskiet FAJ, Hindriks FR, Van der Slik W. Zinc in plasma and serum: influence of contamination due to the collection tubes. Pharm Weekbl [Sci] 1983;5:248-51.
[26] Carmel R. Vitamin B12-binding proteins in serum and plasma in various disorders. Effects of anticoagulants. Am J Pathol 1978;69:319-25.
[27] Kubasik NP, Sine HE. Results for serum and plasma compared in 15 selected radioassays. Clin Chem 1978;24:137-9.
[28] Huizinga JR, Gips CH. Evaluation of the UV-340 spectrophotometric determination for pseudocholinesterase activity (EC 3.1.1.8) in human serum. J Clin Chem Clin Biochem 1987;25:161-5.
[29] Smits RV, Stewart JT. Microbiologic assay methods. In: Textbook of biopharmaceutic analysis. Philadelphia: Lea & Febiger, 1981:249-59.

Received December 1987.
Revised April 1988.
Accepted May 1988.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.