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Penciclovir solubility in Eudragit films: a comparison of X-ray, thermal, microscopic and release rate techniques

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Abstract

The solubility of penciclovir ($C_{10}N_5O_3H_{17}$) in a novel film formulation designed for the treatment of cold sores was determined using X-ray, thermal, microscopic and release rate techniques. Solubilities of 0.15–0.23, 0.44, 0.53 and 0.42% (w/w) resulted for each procedure. Linear calibration lines were achieved for experimentally and theoretically determined differential scanning calorimetry (DSC) and X-ray powder diffractometry (XRPD) data. Intra- and inter-batch data precision values were determined; intra values were more precise. Microscopy was additionally useful for examining crystal shape, size distribution and homogeneity of drug distribution within the film. Whereas DSC also determined melting point, XRPD identified polymorphs and release data provided relevant kinetics.

Keywords: Eudragit films; Drug solubility; X-ray powder diffraction; Differential scanning calorimetry; Light microscopy; Release kinetics; Penciclovir

1. Introduction

Penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine], a synthetic nucleoside analogue, is a potent inhibitor of *Herpes simplex virus* (HSV1 and 2). It has been marketed in topical preparations (Vectavir/Denavir) for the treatment of cold sores. A semi-solid polymer formulation of penciclovir ($C_{10}N_5O_3H_{17}$) has been developed, which upon application to the affected area, rapidly dries leaving a thin protective film. This layer is clear, dry to touch, substantive and aesthetically acceptable.

It is important to characterise drug solubility within such a transdermal drug delivery system to understand and predict in vivo performance of the product [1]. The thermodynamic activity of the drug in the vehicle describes the potential of the active ingredient to become available for its therapeutic purpose, i.e. the leaving potential. Higuchi [2] postulated that to achieve the maximum rate of drug penetration, the highest thermodynamic potential should be utilised; this is usually a saturated system. The level of saturation depends on the amount and solubility of the drug in the vehicle and other factors such as the addition of solubility enhancers (e.g. propylene glycol), which may result in

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a sub-saturated system and hence reduce the rate of drug delivery. Many formulations overcome problems caused through using solubility enhancers by adding excessive amounts of drug to the formulation, leading to wastage of the active ingredient and poor efficiency of the product. Increasing the drug loading within a preparation does decrease potential problems caused by depletion of the active ingredient. Contrary wise, a high solubility may reduce drug partitioning into the skin. Therefore, bases selected should balance optimum solubility and release properties [1]. Also, knowledge of the physical state of the drug (dissolved or suspended) in the vehicle is required to model appropriately its release kinetics [3].

Hence, the solubility of a drug in its medium is an important determinant in formulation efficacy. However, it is difficult to measure such solubilities in semi-solids and films. Conventional methods such as filtration of a saturated drug solution and analysis [4] are inappropriate as it is difficult to remove excess crystals.

Several techniques have been used in attempts to measure solubility in semi-solids and films. For oxybenzone, Kobayashi and Saitoh [5] collected the residual liquid separated from an ointment on storage and measured concentration. They confirmed the absence of crystals by microscopy, and the solubility determined by the residual liquid approach was in a range consistent with microscopic examination. Optical methods for solubility measurements have also provided accurate data. Gopferich and Lee [6] measured clenbuterol solubility in polymer films; visible microscopy was the most sensitive of their techniques, with detection limit of 10% (w/w) compared to differential scanning calorimetry (DSC) and release studies (limits were 12 and 13.5% (w/w), respectively).

DSC has also been utilised to determine solubility of cholesterol in a silicone matrix [7] and for measuring propranolol [8], salicylic acid and chlorpheniramine [9] dispersed in polymer films. Plots of drug concentration versus enthalpy of fusion and extrapolation to the intersect provided data for the drugs, although clearly these determinations provided solubilities at the melting point, not at room (or skin) temperature.

Infra red attenuated total reflectance (IR-ATR) spectroscopy can determine solubility in acrylate adhesives [10,11]. The colorimetric determination of betamethasone in a topical vehicle by oxidation and then condensation of the 17 α-ketol group with phenylhydrazine has also been successfully demonstrated [12]. Chowhan and Pritchard [13] used partition data between the vehicle and an aqueous phase, together with release data, to determine concentrations of corticoids in ointment bases.

Interestingly, salicylic acid solubility in a hydrogel has been determined by X-ray powder diffractometry (XRPD). The intensities of salicylic acid peaks from its XRPD trace were linearly related to its weight percent in the formulation. The solubility of the acid in the hydrogel was taken as the intercept, determined to be 20% (w/w), but there was a large variance associated with this measure [14].

The objective of our work was to investigate the suitability of microscopy, DSC, XRPD and release experiments for determining penciclovir solubility in Eudragit NE30D films. Linear calibration lines were constructed for the DSC and XRPD data, intra- and inter-batch reproducibility of data was also determined. The solubility values from each of the methods were compared and advantages and disadvantages of the techniques were considered.

2. Experimental

2.1. Materials

Penciclovir (>99%, GSK, Weybridge, UK) was used as obtained, Eudragit NE30D (poly(ethyl acrylate methyl methacrylate)) was sourced from Rhom Pharma (Darmstad, Germany) and thickener Plasadone K90 (poly(vinylpyrolidine) (PVP)) was from ISP (Wayne, USA). HPLC grade methanol, buffer salt potassium dihydrogen orthophosphate and lithium fluoride standard (>99%) were supplied by Sigma (Dorset, UK).

2.2. Formulation preparation

The thickener PVP (0.5 g) was well stirred into the Eudragit NE30 dispersion (9.5 g). Penciclovir, 0.025–10% (w/w) were mixed into the vehicle and equilibrated overnight; three batches for each drug loading were prepared. A film forming aid or plasticizer was not required since soft flexible films resulted after drying at 32 °C.

2.3. Film casting

Films for microscopy, DSC and XRPD analysis were cast within a PVC template on a Teflon coated glass to obtain uniform sheets. The deposits were dried at 32 °C for 24 h; thickness of dry films was 0.5 ± 0.1 mm ($n = 30$). Films were stored in a humidity cabinet at 32 °C at 38% r.h.

2.4. Penciclovir loading in cast films

Penciclovir content in the films were assessed by dissolving 100 mg samples in 10 ml ethanol before HPLC determination. Three samples at each drug concentration from all three batches ($n = 9$), assessed drug homogeneity.

2.5. HPLC analysis

Penciclovir was analysed using a Hewlett-Packard 1100 HPLC instrument, with a flow rate of 1 ml min^{-1}, column temperature 30 °C, UV detection at λ_{max} of 254 nm and an injection volume of 100 μl. The mobile phase was composed of methanol–potassium phosphate (pH 7.0; 23 mM; 10:90 (v/v)), filtered and degassed. A guard column (Hypersil ODS C_{18} RP, 5 μm, 2.1 mm × 20 mm) cleaned the injected sample prior to separation on the main column (Hypersil ODS C_{18} 5 μm, 150 mm). The method gave a linear response with concentration over the range 0–100 μg ml^{-1} with $r^2 = 0.9999$; limit of detection was 0.016 μg ml^{-1} and limit of quantification 0.055 μg ml^{-1}.

2.6. Microscopy

Films were examined under a visible microscope (Nixon labophot 2A) at 20× magnification for the presence of penciclovir crystals and photographs taken with a Nikon C35 camera (Nikon, Japan).

2.7. Differential scanning calorimetry (DSC)

Temperature and enthalpy were calibrated with an indium standard and the thermal behaviour of the films was examined using DSC (Perkin-Elmer Series 7) using 10 °C min^{-1} over 25–300 °C. Samples in triplicate (8–10 mg), sealed in aluminium pans, were scanned against an empty reference pan. Since penciclovir content ranged from 10 to 0.025% (w/w), sample weights were so as to maintain constant drug amounts.

The enthalpy of fusion of penciclovir was calculated from the melting endotherm using Perkin-Elmer Pyris Software. The solubility at its melting point was determined from the intercept of a plot of enthalpy of fusion (J g^{-1}) versus drug loading (% (w/w)).

2.8. X-ray powder diffractometry (XRPD)

A Siemens D5000 powder diffractometer (Siemens, Karlsruhe, Germany) equipped with a scintillation counter detector produced film diffractograms. After calibration with lithium fluoride, samples were exposed to Cu Kα radiation, wavelength 1.5418 Å, through 2 nm slits from 2 to 60° 2θ with a step size of 0.05° 2θ and a count time of 1 s per step; the generator was set to 40 kV and 30 mA.

Samples (area = 3 cm^2) were weighed and placed in holders with triplicate determination of Batch 1 and one analysis for Batches 2 and 3, allowing calculation of intra- and inter-batch variation.

Integrated peak intensities (peak areas) were calculated from the diffractograms using GRAMS 32 version 5 software (Galactic Industries Corporation, USA). Integrated data were produced for five peaks in each diffractogram ($2\theta = 8, 11, 17, 18, 26°$), summed and adjusted for sample weight. A plot of I/I_0 (I: sum of five peaks at particular weight fraction, I_0: sum of five peaks for pure penciclovir powder) versus the weight fraction of drug yielded an intercept that provided the solubility.

2.9. Penciclovir release studies

Films were cast into holders (area = 1 cm^2) and placed in a oven for 24 h at 32 °C. A modified USP XXI rotating paddle method [8] determined the release. The receptor was 250 ml of a 10 mM pH 7.4 phosphate buffer maintained at 32 ± 1 °C (representing surface skin temperature) agitated by paddles at 50 rpm ensured sink conditions. Aliquots were removed at intervals, analysed using HPLC and replaced by fresh media. Formulations were tested in triplicate and release data were plotted according to Eq. (10). Solubility was determined from the differences in the rate of increase in the release rate constant as a function of drug loading.

2.10. Precision of data

Precision was assessed using percentage relative standard deviation (%R.S.D.) calculated as:

$$\%\text{R.S.D.} = \frac{\text{S.D.}}{\text{mean}} \times 100 \quad (1)$$

The precision for each point on the calibration plots was calculated for intra- and inter-batch data ($n = 3$).

3. Results and discussion

3.1. Penciclovir concentration in films

Casting the semi-solid formulations and solvent loss upon drying concentrated the drug in the resulting films. The concentration of penciclovir was determined using an HPLC assay (see Table 1). Data precision was within 4% R.S.D. indicating that the drug was homogeneously distributed for the 100 mg sample size tested.

3.2. Microscopy

Microscopic examination provided direct visual evidence for the presence or absence of solid penciclovir in the films with needle shaped crystals evident at high concentrations (Fig. 1A). As the penciclovir loading decreased, the number of crystals declined until at 0.23% (w/w) only a few fragments were visible; at 0.15% (w/w) none were apparent (Fig. 1B). Based on these observations, penciclovir solubility was estimated to be between 0.23 and 0.15% (w/w). The absence of any fine powder suggested that amorphous material or solid dispersions of penciclovir within film components were not formed.

Table 1
Penciclovir concentration in polymer films pre- and post-casting

Cast film	Polymer film	
Penciclovir concentration (% (w/w))	Penciclovir concentration (% (w/w) ± S.D.; $n = 6$)	Relative standard deviation (%)
10.0	14.66 (0.32)	2.2
7.5	10.94 (0.26)	2.4
5.0	7.39 (0.17)	2.3
2.5	3.81 (0.12)	3.1
1.0	1.54 (0.05)	3.2
0.75	1.14 (0.03)	2.6
0.5	0.77 (0.03)	3.9
0.25	0.39 (0.015)	4.0
0.15	0.23 (0.003)	3.8
0.1	0.15 (0.004)	2.7
0.05	0.076 (0.002)	2.6
0.025	0.039 (0.001)	2.6

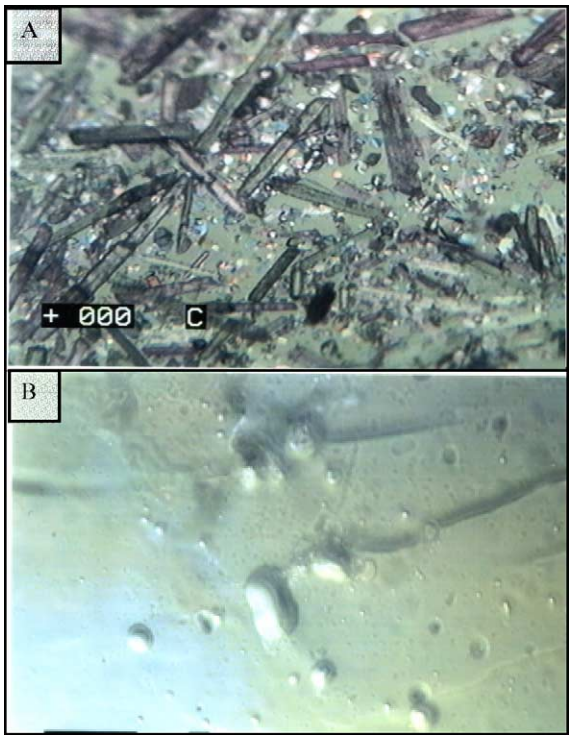

Fig. 1. Photomicrographs of penciclovir polymer film at (A) 14.66% (w/w) and (B) 1.5% (w/w) drug loading.

3.3. Differential scanning calorimetry

The penciclovir powder gave a single sharp endothermic peak with a melting point and enthalpy of fusion of 278 °C and 140 ± 5 J g^{-1} ($n = 3$) in agreement with product data sheet. Broad melting endotherms resulted at 276 °C for drug films (Fig. 2). As drug loading fell, the enthalpy of fusion correspondingly decreased up to 0.39% (w/w), beyond which no penciclovir melting events were recorded, implying that drug solubility was below 0.39% (w/w). The amorphous nature of the drug free films was shown by the absence of melting events and by a raised baseline;

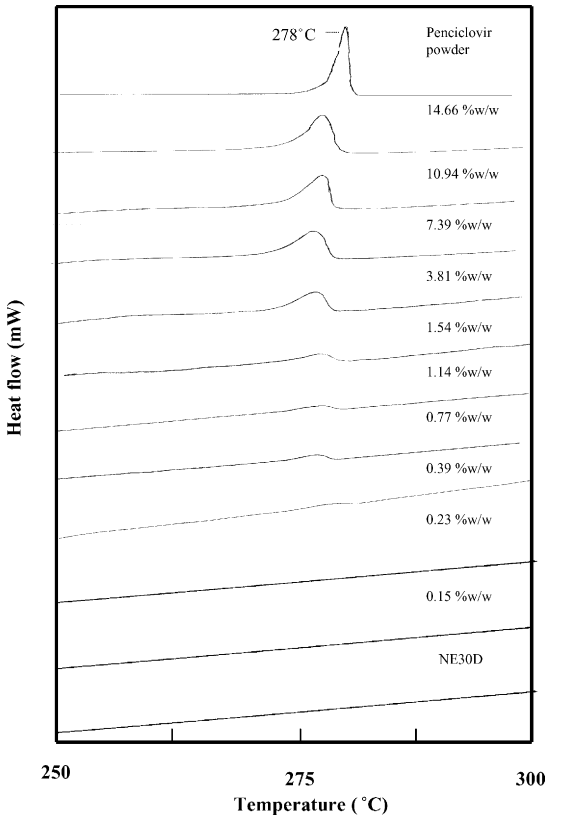

Fig. 2. Differential scanning calorimetry profiles (250–300 °C) of penciclovir powder, penciclovir-loaded polymer film at decreasing drug loading, and drug-free polymer film.

there were no interfering peaks at the drug melting point.

The theoretical enthalpy of fusion as a function of drug loading was calculated from that of pure drug:

$$\Delta H_t = x_p \, \Delta H_p \quad (2)$$

where ΔH_t and ΔH_p are the theoretical and pure penciclovir enthalpies of fusion, and x_p is the weight fraction of penciclovir [7]. Theoretical and experimental enthalpies were plotted versus drug loading (Fig. 3) resulting in linear calibration lines ($r^2 = 0.9989$ for experimental line); error bars on the theoretical lines were due to calculation from three values of ΔH_p. Experimental and theoretical lines agreed well. Since the theoretical line does not take into account the solubility of penciclovir in the film, whereas the experimental plot does, the difference between the two graphs

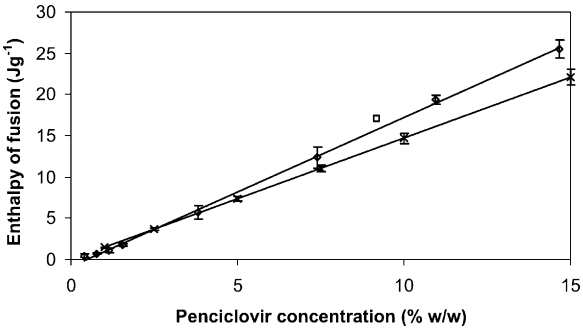

Fig. 3. (◇) Experimentally determined and (×) theoretically calculated enthalpy of fusion as a function of penciclovir loading in polymer films ($n = 3$). Error bars represent standard deviation.

could in principle be used to estimate drug solubility. However, in practice this approach was not possible since the experimental line overlapped that of the theoretical determination at low penciclovir levels and was raised above it at high drug levels. Higher than expected experimental enthalpies of fusion may have resulted because of drug interaction with the polymer; the broad endothermic event presented difficulties for an accurate determination of the integrated area. Additionally, the drug solubility was relatively low and, hence, the difference between theoretical and experimental lines was marginal; this approach may be more appropriate for systems with higher solubilities. Further difficulties arose due to the high water content in films (up to 25% (w/w)), the loss of which may further concentrate samples during analysis.

From the intercept of the experimental line a solubility of 0.44 ± 0.12% (w/w) ($n = 3$) was determined. This was close to <0.39% (w/w), which was the minimum drug concentration at which endotherms were observed on the thermograms. Thermal analysis results were higher than those estimated from visible microscopy (0.15–0.23% (w/w)) but used room temperature, whereas the DSC approach estimated solubility at the drug melting point. Thus, a higher value was expected for the thermal method.

For enthalpy of fusion values of penciclovir loaded films, precision of data expressed as %R.S.D. was good intra-batch (<5% R.S.D.) except for the 7.39 and 3.81% (w/w) samples where one outlying replicate caused a large %R.S.D. value for both intra- and inter-batch (Table 2). As expected, large R.S.D. values for inter-batch data resulted in comparison to

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