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International Journal of Pharmaceutics 193 (2000) 231–237

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate

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Received 2 July 1999; received in revised form 22 August 1999; accepted 6 October 1999

Abstract

A systematic investigation on the influence of two cellulose polymers, methyl cellulose (MC) and hydroxypropyl cellulose (HPMC) on supersaturation and permeation of hydrocortisone acetate (HA) is reported. Diffusion of HA from a 0.5% Carbopol gel across a model silicone membrane was investigated using the Franz-cell technique. At constant polymer concentration, the flux increases proportionally with the degree of saturation up to 4.8 × but decreases thereafter. For a particular degree of supersaturation (4.8 ×), the flux increases with the concentration of polymer up to 1% and decreases at higher concentrations. The behaviour is found to be consistent with crystallisation experiments. The results suggest that optimisation of supersaturation and polymer content is necessary to achieve both high permeation rates and inherent stability.

Keywords: Supersaturation; Cosolvents; In vitro diffusion; Hydrocortisone acetate; Hydroxypropyl methyl cellulose; Methyl cellulose

1. Introduction

The stratum corneum acts as an excellent barrier to drug permeation. There have been several attempts to increase permeation using both physical and chemical methods. While physical methods such as iontophoresis use complex delivery devices, chemical methods such as penetration enhancers alter the barrier properties of the stratum corneum. The latter have an additional problem that they may induce irritancy or toxicity. Recently supersaturated systems have been used to increase drug permeation which may minimise the above problems. Moreover it is a relatively inexpensive technique.

In general, the flux of a drug from a saturated system across a membrane is constant provided that the solvent or any other component in the formulation does not alter the properties of the membrane. Hence the flux of a given drug is limited by its solubility. Any concentration above its solubility limit will increase its chemical potential leading to an increase in the flux across the membrane and hence increased bioavailability. Higuchi (1960) recognised the importance of supersaturation as a means of enhancing flux be-

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0378-5173/00/$ - see front matter
PII: S0378-5173(99)00345-2

yond the limiting value achieved with saturated systems. However the main problem encountered with supersaturated systems is their physical instability. Being thermodynamically unstable, the drug crystallises out and in the process the flux decreases.

Some polymers have been shown to inhibit nucleation and sustain supersaturation for prolonged periods of time. This has been examined in recent studies (Davis and Hadgraft, 1991; Pellett et al., 1994; Megrab et al., 1995; Pellett et al., 1997; Schwarb et al., 1999). The studies show a great potential however there has been a lack of any systematic investigation on the mechanism and suitable choice of polymer.

In this paper we investigate, systematically, the influence of two cellulose based polymers, namely hydroxypropyl methyl cellulose (HPMC) and methyl cellulose (MC) on the supersaturation and permeation of hydrocortisone acetate (HA) from Carbopol gels through a model silicone membrane.

2. Materials and methods

2.1. Materials

Hydrocortisone acetate was purchased from Sigma (Germany). Propylene glycol and HPLC grade methanol were obtained from Fisher Scientific International Company (UK). Hydroxypropylmethylcellulose grade 65SH-(viscosity 50cP) and methylcellulose grade SM (viscosity 100cP) both with the brand name of Metolose were obtained from Shin-Etsu Chemical (Japan). Carbopol 940 was provided by BF Goodrich Company (Cleveland, USA).

Triethanolamine was purchased from BDH Chemicals (UK) and silicone membranes with a thickness of 300 μm from Samco (UK).

2.2. Methods

2.2.1. Preparation of the gels

Firstly Carbopol was dissolved in an aqueous solution of HPMC or MC. An excess of HA was added to the solvent (PG) and the mixtures were agitated with a teflon coated magnetic bar and left in a water bath at 32°C for 48 h. The resultant saturated solution of HA in PG was centrifuged for 10 min and the supernatant added to the Carbopol-polymer solution. This solution was left to hydrate for 30 min. The final step was to stir the solution and add a drop of triethanolamine to form the gel.

Mixing different ratios of HA saturated solution in PG and the polymer solution in water allows the formation of supersaturated solutions. The simple saturated gel is obtained by dissolving HA in a mixture of PG and water (or polymer solution) and centrifuging before adding Carbopol at the end.

2.2.2. Solubility studies

HA was added, in excess, to a series of PG-water mixtures varying from 100% water to 100% PG and stirred in a water bath maintained at 32°C for 48 h. After ensuring that equilibrium had been reached, the solution was centrifuged and the supernatant solution diluted and assayed using HPLC.

2.2.3. Creation of supersaturation

Supersaturation was produced using the cosolvent method described previously by Davis and Hadgraft (1991). The cosolvents used were PG and water. Supersaturated systems were formed by mixing a saturated solution of HA in PG with either water or polymer solution. Supersaturation was calculated from the cosolvent solubility plot (Fig. 1) by dividing the concentration of the drug in the solution by its saturated solubility in the cosolvent mixture.

2.2.4. HPLC analysis

HPLC analysis of HA was performed using a Milton Roy Constametric III pump, set at a flow rate of 1.5 ml/min, with a Perkin-Elmer ISS-100 auto-sampler, a Varian 2550 variable wavelength UV detector set at 240 nm and a Milton Roy CI-4100 computing integrator.

The stationary phase was an Apex reverse phase ODS 5 μm packed column (250mm × 4.6 mm). The mobile phase was methanol: water (65:35% v/v). Calibration curves were constructed

on the basis of peak area measurements using standard solutions of known concentrations. The retention time was ≈ 4.5 min.

2.2.5. Microscopy

The gels and solutions were analysed for the presence of crystals by observing them on microscopes slides using a WILD Heerbrugg microscope (Switzerland) at a magnification of $160\times$.

2.2.6. Diffusion studies

Diffusion experiments were conducted using Franz-type diffusion cells that have a receptor volume of 3.9 ml and a diffusional surface area of about 0.95 cm^2. The receptor chambers had side arms through which samples could be taken. A sonicated solution of water: PG (75:25) was used as the receptor phase. The silicone membrane was cut to the appropriate size and allowed to soak overnight in isopropyl myristate. Silicone grease was used to produce a leakproof seal between the flanges of the two halves of the cell held together with a screw clamp. Samples of gels were introduced in the donor compartments and occluded using microscopic cover slips. The receptor compartment of the cells was maintained at 37°C in a water bath. Teflon coated magnets were used to agitate the receptor compartments to provide uniform mixing. The side arms were closed with caps to prevent evaporation. At predetermined intervals, every 2 h during 10 h, 0.4 ml of the receptor phase was removed and replaced with an equal volume of pre-thermostated receptor phase. Final samples were taken after 24 h and all samples assayed by HPLC.

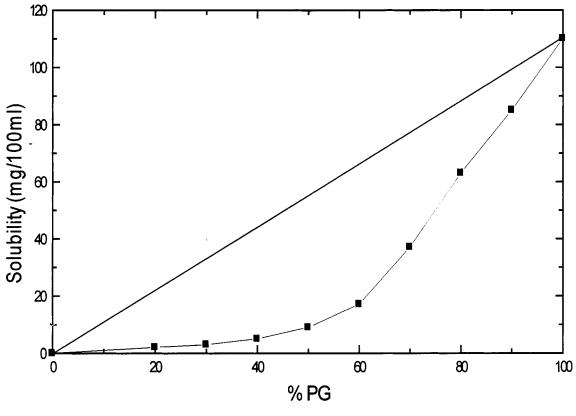

Fig. 1. Cosolvent solubility data of hydrocortisone acetate in PG-water mixtures.

3. Results and discussion

3.1. Solubility studies

Fig. 1 shows the saturated solubility plot of HA in the cosolvent system of propylene glycol and water at 32°C. The exponential increase in solubility values with increasing percent of propylene glycol is consistent with the data previously reported (Yalkowsky and Roseman, 1981; Davis and Hadgraft, 1991). Even though temperature can significantly affect the solubility, there does not appear to be any major influence in the present system. The solubility values obtained at 32°C from the present study compare well with those obtained at 21°C (Davis and Hadgraft, 1991).

The maximum supersaturation that can be obtained is limited by the solubility of HA in the cosolvent mixtures. In the present studies this was 8.33 times saturation for the PG: H_2O ratio of 20:80.

3.2. Transport of HA from supersaturated gels

Transport measurements were performed on supersaturated Carbopol gels without and with the addition of HPMC and MC. Two types of experiments were conducted;

1. constant polymer concentration but varying supersaturation,
2. constant supersaturation but varying polymer concentration.

The flux of HA from the gel was assessed from graphs of HA transported across the membrane with time and taking the mean of the gradients obtained from four different cells.

3.2.1. Constant polymer concentration but different supersaturations

Three different experiments were performed:

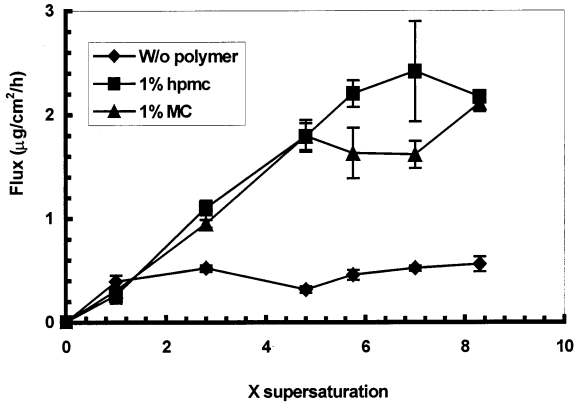

Fig. 2. Flux of HA as a function of degree of saturation at 1% polymer concentration ($n = 4$).

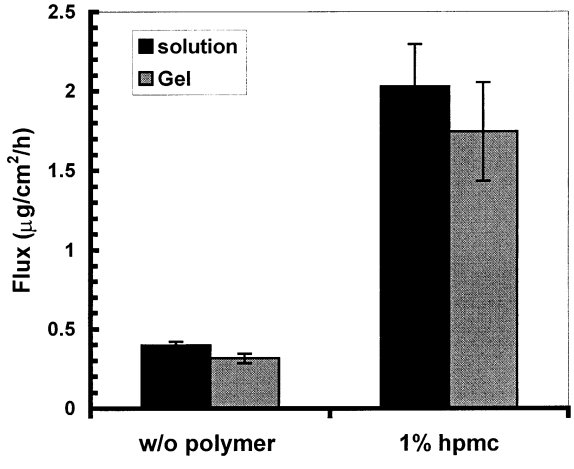

Fig. 3. Comparison of the fluxes of HA from a solution and a gel (4.8 × saturation).

1. gels saturated with HA without and with the polymer additives,
2. gels supersaturated with HA without the polymer additive,
3. gels supersaturated with HA with the polymer additive.

A 1% polymer concentration was used in all these studies. The flux data are combined and shown in Fig. 2.

For gels saturated with the drug, steady state flux was observed over the 24 h of the experiment. In the preparation of the saturated gels, the supernatant solution was used for producing the gel. Hence no excess drug was present to maintain the thermodynamic activity leading to a depletion of the drug in the donor compartment. However, over the 24 h period, no significant depletion effects were observed.

Irrespective of the presence (or absence) of the polymer, the flux from the saturated gels is similar in all the cases. This is expected since gels saturated with the HA have equal chemical potentials and will therefore give the same flux. The polymers had no effect suggesting no specific interaction between HA and the polymers nor polymer interaction with the silicone membrane.

Without the polymer additive, the supersaturated gels give fluxes similar to that of the saturated gel. In the absence of the polymer additive the drug precipitates in the gel as soon as the cosolvents are mixed. Crystallisation is facilitated by the thermodynamic instability created by supersaturation. Hence, supersaturation is not sustained and the gel returns to its saturation equilibrium giving a low flux. The transport measurements also show that steady state is maintained over the 24 h period. No significant depletion was observed as only $\approx 2\%$ of the drug had permeated over 24 h. The excess drug present in the donor phase replenishes the drug lost as a result of diffusion.

The similar values of the fluxes from the gels also indicate that the presence of Carbopol does not significantly influence the flux. This was also verified by performing transport measurements on supersaturated solutions of HA without Carbopol. The data are shown in Fig. 3. No significant variation was found for the flux values between the solution and Carbopol gels (ANOVA, XLSTAT).

The gels were observed under the microscope as soon as they were prepared. In the gels without the polymer additive, crystallisation of hydrocortisone acetate was observed when the cosolvents were mixed. There was no lag time for crystallisation in any of the gels showing that they were in the labile region.

Fig. 2 also shows the flux for gels with the polymers present. The profiles for the flux were quite similar for both HPMC and MC. For supersaturation at and below 4.8 × the degree of satu-

ration, the flux values were calculated over a period of at least 10 h. For supersaturation above 4.8 ×, the flux showed a non-linear behaviour as a function of time. The thermodynamic instability caused crystallisation of the drug leading to a decrease in the driving force for diffusion. The flux values presented are those determined over the initial 6 h.

The flux of HA is higher for the supersaturated gels containing the polymers compared to the values for the gels without the polymer additive. The flux is linear with degree of saturation up to 4.8 × supersaturation. It is well known that flux is proportional to the concentration for sub-saturated systems. The linear behaviour in supersaturated systems however is dependent on the stability of systems themselves. In other words, as long as the chemical potential (degree of supersaturation) remains the same one would expect a linear behaviour. Once crystallisation occurs, the chemical potential and hence the flux is reduced. This is clearly exhibited in Fig. 2 where, above 4.8 × supersaturation, the flux is not proportional to the degree of saturation.

In transport studies across membranes, Fick's 1st law of diffusion, which states that the amount of drug transported is a linear function of time, describes the steady state flux. In sub-saturated and saturated systems, any deviation from this linear behaviour implies a depletion of the donor phase. In the case of supersaturated systems a decrease in flux indicates the onset of instability of the gels. Once nucleation occurs, the chemical potential is reduced. The higher the degree of supersaturation the lower the nucleation time. This is clearly exhibited in Fig. 4 where the transport of HA is plotted as a function of time for two different degrees of supersaturation. In the case of saturated gel and 2.8 × supersaturation, the flux is linear over 24 h. Nucleation does not occur during the period of the study. For 6.9 × supersaturation a non-linear behaviour is observed. This tailing off is not due to depletion as only 6.5% of the drug had permeated over 24 h.

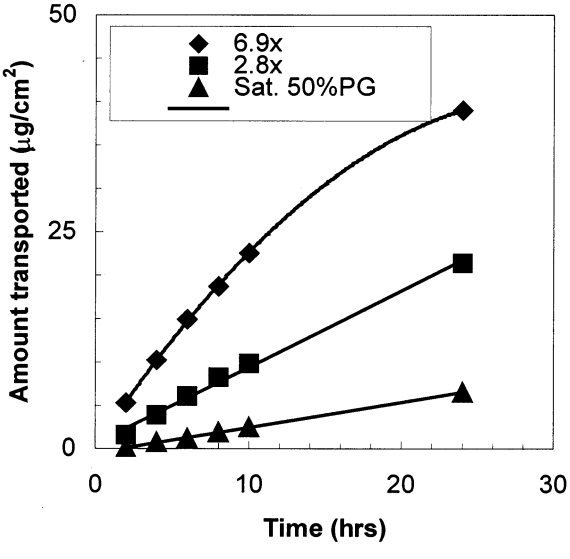

Fig. 4. Flux of HA showing a linear transport over 24 h at low degrees of saturation but a non-linear transport at 6.9 × saturation. A 1% hpmc concentration was used.

The crystallisation times of the supersaturated gels obtained from the crystallisation studies are much longer compared to the instability observed in the diffusion studies (Raghavan et al., submitted for publication). The difference may be explained by the fact that the particles observed under the microscope are crystallites of the order of few microns size. Nucleation takes places by formation of nuclei, which are of the order of tens of nanometers and cannot be observed using optical microscopy. In the presence of the polymer the growth rate of the HA crystals is reduced and it takes significantly longer to reach sizes observable under the microscope.

3.2.2. Different polymer concentrations: constant degree of supersaturation

Fig. 5 shows the transport of HA from Carbopol gels containing varying amounts of cellulose polymer. 4.8 × supersaturation was maintained in all the cases as this degree of supersaturation was found to provide stable gels for a period of more than 24 h. The polymer concentration was varied between 0–5%. The behaviour of the flux is similar for both MC and HPMC. The addition of hydroxypropyl group does not appear to affect the inhibition of nucleation. This suggests that this group does not take part in the inhibition process.

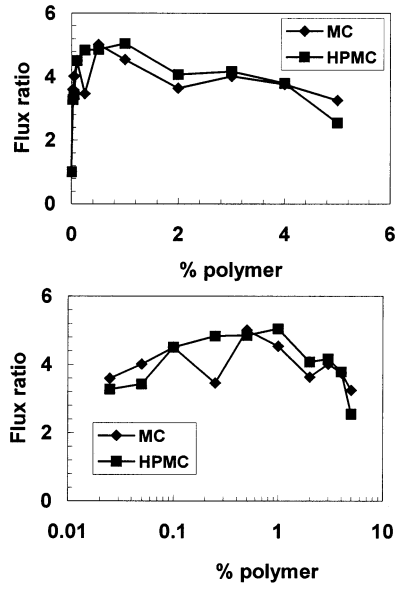

Fig. 5. (a) Ratio of flux of HA at different poymer concentrations to the flux from the gel without the polymer showing a maximum permeation at around 1% polymer concentration. 4.8 × saturation was used for all the samples. (b) Same graph plotted with polymer concentration using a logarithmic scale to show the flux at low polymer concentrations.

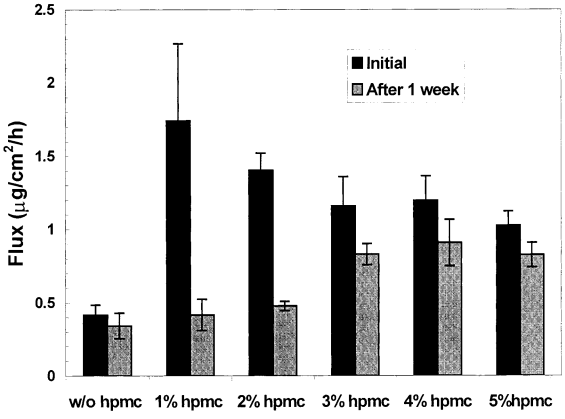

Fig. 6. Flux of HA before and after storage for one week at different hpmc concentrations (4.8 × saturation).

Flux enhancement was observed even for concentrations as small as 0.025%. The increase is less than that expected for 4.8 × supersaturation. With increase in the polymer concentration the flux increases and reaches a maximum at 1%. At further increases in the polymer concentration, the flux decreases slightly. The increase in flux at low polymer concentrations is understandable and can be attributed to the increased inhibition of crystallisation. However, as a result of increased stability, the flux may be expected to increase with further increases in polymer concentration. No depletion of the drug in the donor compartment was evident from the diffusion studies, as the transport was linear up to 24 h. Absence of nucleation was also confirmed from the crystallisation studies, which showed that the nucleation time increased with increase in polymer concentration. The decrease in flux at high polymer concentrations must be due to some other process. During the preparation of the gels it was seen that the gels at high polymer concentrations were very viscous and more difficult to handle than the gels containing low concentrations of the polymer. It is possible that the viscosity of the gel slows the diffusion of HA to the membrane interface. This would decrease the permeation rate.

3.3. Stability of the gels

As mentioned earlier, the main problem with supersaturated systems is their inherent instability. Polymer presence inhibits nucleation and stabilises supersaturation as confirmed by the diffusion and crystallisation studies. However, in pharmaceutical usage, these systems have to be stored for periods of time before application. Stability during storage is a problem that has to be addressed during formulation of a supersaturated topical system. This is also a major problem of the production of transdermal patches where drug crystallises during storage dependent on drug loading (Ma et al., 1996; Lipp, 1998).

The HA gels were stored for a week and diffusion studied again in order to determine if the fluxes remained the same. The results are shown in Fig. 6. The flux decreased to the saturation value after one week storage for polymer concentrations of $\leq 2\%$. At higher concentrations, the average flux is slightly lowered but the ANOVA calculations showed that there was no significant change (95% confidence limit). These studies show that even though the enhancement of the flux is

lower for high polymer concentrations, these concentrations stabilise the supersaturated systems when stored for short periods of time.

4. Conclusions

The present studies have shown that the flux from supersaturated gels of hydrocortisone acetate is proportional to the degree of saturation up to 4.8 × supersaturation. For a particular supersaturation (4.8 ×), the flux shows maximum enhancement for 1% polymer concentration and decreases at higher concentrations. The stability is better for gels with high polymer content. The results suggest that there is an optimum degree of supersaturation and polymer content to achieve high permeation rates. These results can be interpreted in terms of crystallisation kinetics.

Acknowledgements

The authors would like to acknowledge Engineering and Physical Science Research Council (EPSRC) and SmithKline Beecham Consumer HealthCare, Weybridge, UK for financial support. The authors would like to thank Monica Dias for help with the solubility studies.

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