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International Journal of Pharmaceutics 300 (2005) 113–124
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Novel gels and their dispersions—oral drug delivery systems for ciclosporin

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Available online 11 July 2005

Abstract

Amphiphilogels (gels that consist solely of surfactants) and gel-based emulsion (GEM) formulations (solutions that gel upon incorporation of small amounts of water) were investigated as oral delivery vehicles for ciclosporin A, in in vivo experiments in Beagle dogs. Both systems represent essentially self-dispersing non-lipidic drug delivery systems based on amphiphilic surfactants. Three different amphiphilogels (hydrophobic, hydrophilic and hydrophilic gel containing ethanol), the aqueous dispersions of the latter two amphiphilogels and of two GEM formulations were tested to determine the influence of (i) gel hydrophilicity/hydrophobicity, (ii) presence of ethanol, (iii) pre-dispersion of gels into aqueous medium prior to oral administration and (iv) size of dispersions, on drug absorption. It was found that all the formulations tested, except for the hydrophilic amphiphilogel and its aqueous dispersion, were bioequivalent to Neoral®, the commercially available preparation. High drug absorption from the bioequivalent formulations was thought to be due to the fact that following oral administration, ciclosporin remained in a soluble form, hence was available for absorption, despite relatively large droplet sizes of the formulations. The hydrophilic gel and its dispersion allowed less drug absorption; this was assigned to the fact that, when the hydrophilic amphiphilogel contacted an aqueous medium, there were no lipophilic domains in which the drug could remain soluble. It is possible that some drug precipitated out and was unavailable for absorption.

Keywords: Ciclosporin; Oral; Absorption; Drug delivery; Gels; Aqueous dispersions

1. Introduction

Ciclosporin A (CyA, ciclosporin), a powerful immunosuppressive agent which selectively inhibits T helper cells, has revolutionised organ transplantation. It is a neutral, lipophilic ($\log P \approx 3$), cyclic undecapeptide, with molecular weight 1202 Da and a very low aqueous solubility (0.04 mg/ml at 25 °C). Such a poor water solubility and the absence of adequate formulations in which ciclosporin could be administered almost led to the drug being abandoned for clinical development (Borel and Kis, 1991). Fortunately, this did not happen, lipid formulations were developed and ciclosporin remains the first line immunosuppressant in

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doi:10.1016/j.ijpharm.2005.06.002

organ and tissue transplantation. Recently, ciclosporin was approved for the treatment of psoriasis (Lebwohl et al., 1998) and is also being investigated for many other disorders of the immune system, such as, asthma (Rohatagi et al., 2000), moderate-to-severe eye diseases (Sall et al., 2000; Robert et al., 2001), inflammatory bowel disease (Sandborn, 1996), rheumatoid arthritis (Lee et al., 2001; Marra et al., 2000; Sarzi-Puttini et al., 2000). Different routes of drug administration, such as topical, inhalation, and ocular are also being investigated.

The commercially available proprietary formulations include Sandimmune® (the first preparation) and Neoral® which, when diluted with an aqueous medium (e.g. gastric contents upon ingestion or apple juice prior to oral administration) form a crude emulsion (droplet size 1 μm) and a finer homogeneous dispersion (droplet size < 100 nm) respectively (Vonderscher and Meinzer, 1994). The latter homogeneous dispersion is said to mimic the mixed micellar phase (which leads to rapid absorption) while the crude emulsion obtained from Sandimmune needs to be further emulsified to mixed micelles by bile salts before the oily droplets can be digested and drug can be released (Klyashchitsky and Owen, 1998). Thus, Neoral® has much improved pharmacokinetic and bioavailability profiles and a large proportion of transplant recipients in Europe and the US previously on Sandimmune® have been switched on to Neoral®. The latter, whose patent runs out in June 2112, has thus become very important economically and numerous alternative formulations of ciclosporin, such as liposomes, particulate systems, lipid-based formulations, are being investigated. Ciclosporin has a narrow therapeutic index and bioequivalence to Neoral® is almost always sought. To date, there have been four generic formulations of ciclosporin A, namely, SangCyA (Sangstat, recalled), Gengraf (Abbott), Cyclosporine Capsules USP Modified (EON) and Cyclosporine Capsules USP Modified (Sidmak) in the US market. First et al. (2000) estimated that the use of bioequivalent generic ciclosporin formulations can save almost $ 2000 per patient per year. Thus, new generic formulations are mainly created for economic reasons. However, they also provide a chance to evaluate different approaches for improving the bioavailability of poorly water-soluble drugs.

Lipid-based self-emulsifying systems which form a fine dispersion (droplet size in the 100 nm range) upon ingestion and dilution with gastric contents (or upon dilution with an aqueous medium prior to ingestion) form a large proportion of the alternative delivery systems designed for ciclosporin. They generally consist of the drug dissolved in a blend of excipients, which may be triglyceride oils, partial glycerides, hydrophilic and lipophilic surfactants and cosurfactants. Formation of a fine colloidal dispersion upon dilution with an aqueous medium is a prerequisite and great importance is given to the droplet size, though other factors such as the type of lipid phase and surfactants, droplet susceptibility to digestion and/or solubilisation by mixed micelles of bile salts and phospholipids, may be as, if not more, important than droplet size for drug absorption (Pouton, 2000; Andrýsek, 2001; Humberstone and Charman, 1997). Vrana and Andrýsek, (2001) showed bioequivalence of 10 different ciclosporin formulations despite a large variation in their droplet sizes (50–1000 nm). Pouton (2000) stressed the greater importance of keeping the drug solubilised following the dispersion of the lipid-based system in an aqueous medium, above that of drug solubility in the formulation. Pouton (2000) also suggested the classification of lipid-based systems into types I-IIIB. The latter differ in their proportion of hydrophilic component which dictates the type of dispersion obtained when the system interacts with an aqueous medium (for example in the stomach) and thus has a major role in drug bioavailability.

In this paper, we report our findings on the potential of two gel systems for oral ciclosporin delivery: polyglycerol ester solutions (which gel in contact with water) and novel amphiphilogels (gels which consist solely of surfactants, hence, the terminology). Amphiphilogels are different from other lipid-based systems for ciclosporin in that they contain no triglycerides or partial glycerides. The major components are the non-ionic surfactants, sorbitan monoesters and Polysorbates. Amphiphilogels are formed by dissolving/dispersing the gelator (in this case, sorbitan monostearate) in the fluid phase (e.g. sorbitan monooleate, polysorbate 80) at high temperatures, followed by cooling the sol phase to an opaque, semi-solid gel (Murdan et al., 1999; Jibry et al., 2004). Hydrophilic or hydrophobic amphiphilogels can be produced by choosing a hydrophilic or hydrophobic fluid phase component. Hydrophilic cosolvents such as ethanol can also be added to increase drug solubility (Jibry and Murdan, 2002). When placed

in an aqueous medium and stirred, the gels break up into dispersion. Thus, the amphiphilogels are a type of self-dispersing drug delivery system (SDDDS). The second formulation is a solution of ciclosporin in a mixture of polyglycerol esters, ethanol and cremophor. The mixture is a clear liquid at room temperature, but forms a gel upon the addition of water. Addition of excess water results in the gel breaking up into small gel particles of different shapes such as elipsoid, sigmoid and rod-like. Based on this behaviour, the system was denominated gel-based emulsion (GEM) (Andrýsek, 2001). A more precise description of this system would be a concentrate for dispersion into gel particles. However, we have decided to keep the abbreviation GEM to enable continuity in denominations.

We determined the oral bioavailability of ciclosporin from the two gel-based formulations and explored the factors which influence drug absorption. The nature of the dispersions obtained with the amphiphilogel and the polyglycerol solution depends on the nature of the original formulations. The type of dispersion can, in turn, affect the absorption profiles of drugs dissolved in these formulations. In the current study, fasted beagle dogs were used as the experimental animals in order to determine: (i) the effect of the nature of the amphiphilogel (hydrophobic, hydrophilic, inclusion of ethanol in gel), (ii) the effect of pre-dispersing the gel in an aqueous medium prior to oral administration, and (iii) to compare the two different gel-based formulations.

2. Materials and methods

2.1. Materials

Sorbitan monostearate, sorbitan monooleate, polysorbate 20, polysorbate 80 were purchased from Sigma, UK. Absolute ethanol was from Hayman, UK. Oleyl alcohol was from Merck, Germany. Polyglycerol esters were from Abitec, USA and POE-40-hydrogenated castor oil was from BASF, Germany. Ciclosporin was obtained from Ivax, Czech Republic. Distilled water was used throughout.

2.2. Preparation of formulations containing dissolved ciclosporin

Three amphiphilogels (A, hydrophobic; B, hydrophilic; C, hydrophilic gel containing ethanol) were prepared to investigate the effect of hydrophilicity/hydrophobicity of the gel and the effect of inclusion of ethanol (latter influences the drug solubility as well as gel structure). Two amphiphilogel dispersions (formulations D and E) were prepared to investigate the effect of pre-dispersing the gel prior to oral administration. Two GEM formulations (F and G) were prepared. The different formulations A–G were prepared as follows.

Hydrophobic gel (formulation A): The gelator, sorbitan monostearate (18%, w/w), the hydrophobic fluid phase, sorbitan mono-oleate (55%, w/w), polysorbate 20 (18%, w/w) and ciclosporin (9%, w/w) were weighed into a glass vial. The mixture was incubated in a water bath at 70 °C for 4 h. A clear solution was produced as the ciclosporin and the gelator dissolved in the fluid phase. On cooling at room temperature, the sol phase set to an opaque, semi-solid gel containing dissolved cyclosporin. The gel was filled into hard gelatin capsules and stored in closed glass vessels. Concentration of ciclosporin in the hydrophobic gel was 100 mg/1.1 g gel.

Hydrophilic gel (formulation B): Sorbitan monostearate (18.7%, w/w), the hydrophilic fluid phase, polysorbate 80 (74.7%, w/w) and ciclosporin (6.6%, w/w) were weighed into a vial and the amphiphilogel was prepared as described above. Concentration of ciclosporin in the hydrophilic gel was 100 mg/1.5 g gel.

Hydrophilic gel containing 10% ethanol (formulation C): Sorbitan monostearate (16.7%, w/w), polysorbate 80 (67.3%, w/w), ethanol (9.3%, w/w) and ciclosporin (6.7%, w/w) were weighed into a vial and the gel was produced as described above. Concentration of ciclosporin in the hydrophilic gel was 100 mg/1.5 g gel.

Aqueous dispersion of hydrophilic gel (formulation D): The dispersion was prepared immediately prior to administration to dogs. A 10 ml of distilled water was added to a glass vial containing 1.5 g of hydrophilic gel (gel B). The vial was incubated in a water bath at 40 °C for a few minutes and hand-shaken to disperse the gel in the water.

Aqueous dispersion of hydrophilic gel containing ethanol (formulation E): The dispersion was prepared, as detailed for formulation D, except for the fact that 10 ml of distilled water was added to 1.5 g of gel C.

GEM 304 (formulation F): Ethanol (12.0%, w/w), oleyl alcohol (10.0%, w/w), ciclosporin (10.0%, w/w), polyglyceryl-3-oleate (15.0%, w/w), polyglyceryl-10-oleate (25.0%, w/w) and POE-40-hydrogenated castor oil (28.0%, w/w) were weighed into a glass vial, heated at 60 °C and mixed with a magnetic stirrer until a clear, yellowish solution was formed. Immediately prior to oral administration to dogs, the yellowish solution was dispersed in distilled water-90 ml of water was added to a glass vial containing 10 g of solution and hand-shaken for 30 s.

GEM 101 (formulation G): Ethanol (12.0%, w/w), ciclosporin (9.5%, w/w), polyglyceryl-3-oleate (31.5%, w/w), polyglyceryl-10-oleate (19.0%, w/w) and POE-40-hydrogenated castor oil (28.0%, w/w) were weighed together in a glass vial, heated at 60 °C and mixed with a magnetic stirrer until a clear, yellowish solution was formed. The latter was dispersed in distilled water immediately prior to oral administration to dogs, as detailed for GEM 304.

The main difference between GEM 304 and GEM 101 is the presence of oleyl alcohol in GEM 304. Oleyl alcohol, being a solvent for ciclosporin (solubility of CyA in oleyl alcohol is 226 mg/ml) was included in GEM 304 to improve the solubilisation capacity of this formulation.

2.3. Light microscopy

The ciclosporin formulations were examined using a light microscope (Nikon Microphot-FXA, Japan) with attached camera (Nikon FX-35DX, Japan) and a hot-stage (Linkam TC93, UK).

2.4. Particle size analysis

To compare the dispersibility of the two different gel systems, the dispersions produced when the amphiphilogel and GEM solutions were mixed with excess water, were analysed by laser diffraction. Low angle laser scattering (LALS) technique was used for particle size evaluation of coarse particles. Working range of this method is 0.2–900 μm.

Samples were evaluated as follows: MasterSizer S, Malvern Instruments Ltd., UK equipped with small dispersion unit (SDU), 300 RF lens with 2.40 mm beam length was used. The equipment was located in an air-conditioned laboratory with temperature maintained on 24 ± 2 °C. The system was filled with deionized water, the pump was switched on at 1500 rpm and blank measurement was performed. Then, a sufficient amount of sample to obtain obscuration within 15–30% was added into the SDU. After 5 min equilibrium time, the particle size distribution was read (2000 sweeps). Evaluation of results is based on Frauhofers presentation expressed as volume distribution. In between measurements, the equipment was carefully rinsed out with water, ethanol and again, with water. Two samples were used for each formulation, each sample being measured 10 times.

2.5. Viscosity measurement

To evaluate rheological changes which occur when formulations F and G interact with increasing amounts of water, a viscometer (Brookfield DV-III, Brookfield, USA) with standard chamber SC4 and ultra thermostat (Brookfield TC 500, Brookfield, USA) were used. The rotation was varied from 1 to 49 rpm at 25 ± 1 °C. Measurements were made 3 times, using a fresh sample each time, i.e. $n = 3$.

2.6. In vivo studies in dogs

The amphiphilogels (in hard gelatin capsules) and gel dispersions were orally administered to fasted male Beagle dogs, in groups of 5–10. Neoral® was used as the control formulation. Each dog received 100 mg of ciclosporin. The animals were bled at times 1, 2, 3, 5, 8, 12 and 24 h post-administration, and the blood was analysed for ciclosporin concentration by specific radioimmunoassay as described previously (Jegorov et al., 2000; Šafarčík. et al., 2001).

2.7. Data analysis

The software WinNonlin was used to model the blood ciclosporin concentration profiles and C_{max}, T_{max} and area under the blood concentration-time profile (AUC) were obtained from the modelled curves. One way ANOVA was used to determine statistical differences between the AUC_{24} of the different formulations. Student's *t*-tests were conducted when two formulations were being compared.

3. Results and discussion

3.1. Amphiphilogels containing ciclosporin and their interactions with water

Amphiphilogels are a subset of organogels (gels where the fluid phase is organic in nature, rather than aqueous, in which case the gel is called a hydrogel). Many organogels are formed due to the differential solubility of the gelator in the fluid phase at different temperatures – high solubility at high temperature and low solubility at room temperature. Thus, organogels can be prepared simply by dissolving the gelator in the fluid phase at high temperature and cooling the resulting organosol. Upon cooling, the gelator solubility in the fluid phase decreases and gelator-solvent affinities are reduced. This results in gelator molecules coming out of solution and self-assembling into structures such as tubules and fibres which form a three-dimensional network and immobilise the fluid phase, i.e. a gel is formed.

In this study, amphiphilogels containing dissolved ciclosporin were produced by such a simple method. A hot clear solution containing the gelator (sorbitan monostearate), the fluid phase and ciclosporin was prepared, which cooled to an opaque gel. Light microscopy revealed that the gel was composed of clusters of tubules in the fluid phase (Fig. 1a–c). The tubules are assemblies of sorbitan monostearate and this microstructure is typical of amphiphilogels (Jibry et al., 2004). Ciclosporin is expected to be dissolved in the fluid phase. Light microscopy also revealed that no ciclosporin crystals were seen in the gel and indicated that the drug was present in a molecularly dispersed manner.

When ethanol was included in the amphiphilogels, the dissolution rate as well as the solubility of ciclosporin was increased. Ethanol is a good solvent for ciclosporin and is often added in ciclosporin formulations to enhance the drug's solubility and enable its formulation within suitable vehicles. Ethanol also had an effect on the gel itself. The latter was less rigid and the tubular clusters were larger (compare Fig. 1b and c). A softer gel in the presence of ethanol is easily explained. The gelator, sorbitan monostearate, is soluble in ethanol at room temperature, thus, the fluid phase – polysorbate 80 and ethanol in gel C – is a better solvent for the gelator compared to polysorbate 80 on its own (gel B). Consequently, when an organosol containing ethanol is cooled to form a gel, there may be a smaller amount of sorbitan monostearate which comes out of solution as self-assemblies to gel the fluid phase. A reduced gelator network thus leads to a softer gel. The effect of ethanol on the size of the tubular clusters is more difficult to explain. Theoretically, the fact that the addition of ethanol produces a better solvent for the gelator would have led us to expect smaller clusters of sorbitan monostearate as a smaller amount of the gelator comes out of solution on cooling.

In order to understand how the nature of the gels could affect ciclosporin absorption in vivo, the interactions between the gels and water were studied. It is expected that, following oral administration of the gels in hard gelatin capsules, the latter will disintegrate in the stomach, allowing the gels to be dispersed in the aqueous gastric contents. To determine the fate of the gel upon contact with an aqueous phase, 20 ml of distilled water at 40 °C was added to 0.2 g of gel in a glass vial. The vial was hand-shaken to disperse the gel and the resulting dispersion was examined using light microscopy. As expected, the hydrophobic gel dispersed very slowly in water and vigorous shaking of the container was needed in order to fully disperse the gel. The resulting aqueous dispersion was a mixture consisting of droplets of varying sizes and tubular clusters that were originally responsible for gelation (Fig. 2a). The droplets are expected to consist of sorbitan monooleate (which is immiscible with water) containing dissolved ciclosporin. In contrast, the hydrophilic gel dispersed faster as the water-soluble polysorbate 80 dispersed and dissolved in the aqueous medium. Light microscopy of the dispersion showed tubular clusters suspended in the aqueous phase (Fig. 2b).

The hydrophilic gel containing 10% (w/w) ethanol dispersed even more easily in water compared to the hydrophilic gel without ethanol. Easier dispersion reflects the 'softer' nature of the gel and faster ingress of water into the gel. Light microscopy revealed tubular clusters of the gelator as well as small droplets of uniform size (Fig. 2c). These droplets were totally unexpected. It was thought that the water-miscible polysorbate 80 and ethanol would disperse and dissolve in the water, leaving a suspension consisting of tubular clusters, as seen when a hydrophilic gel (without ethanol) was dispersed in water (Fig. 2b). Interestingly, when a hydrophilic gel containing 10% (v/v)

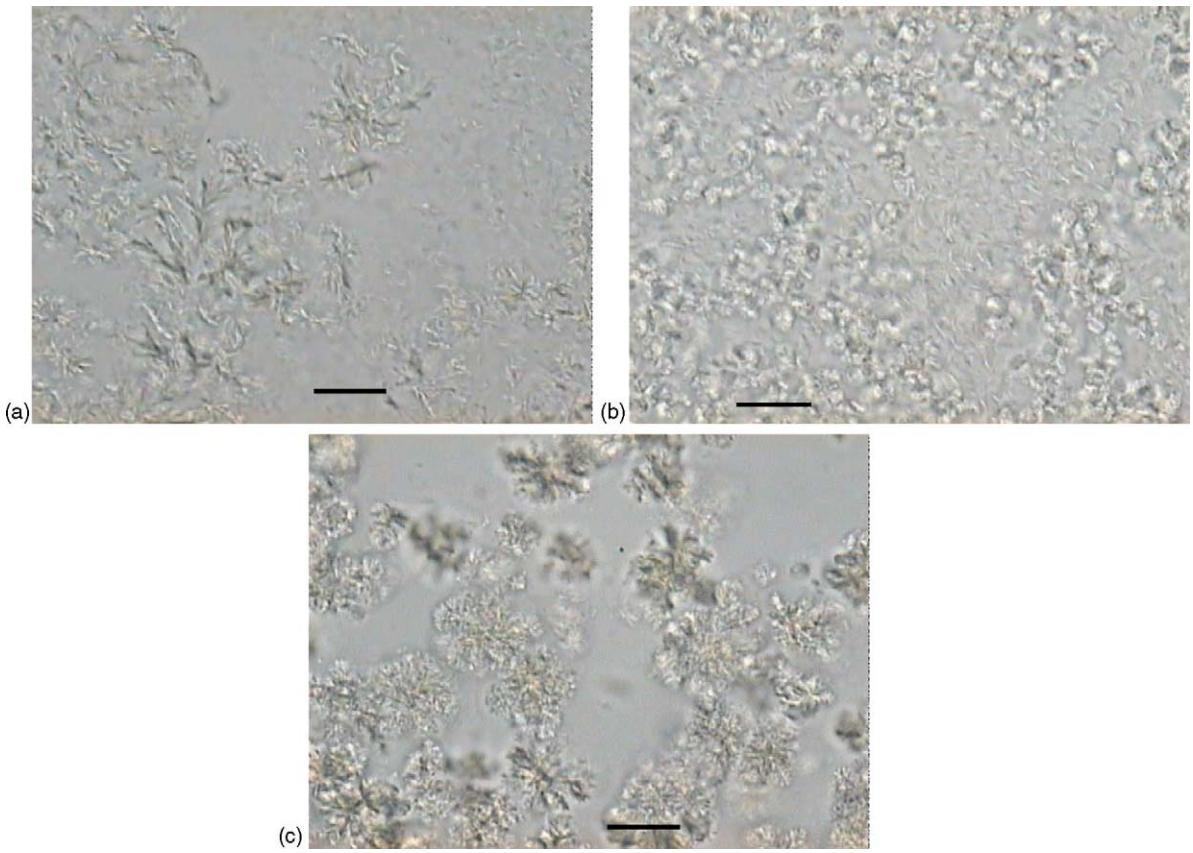

Fig. 1. (a) Light microscopy of ciclosporin-loaded hydrophobic gel. Clusters of tubular aggregates are seen dispersed in the fluid medium. Scale bar represents 20 μ. (b) Light microscopy of ciclosporin-loaded hydrophilic gel. Clusters of tubular aggregates are seen dispersed in the fluid medium. Scale bar represents 20 μ. (c) Light microscopy of hydrophilic gel containing 10% (w/w) ethanol. Clusters of tubular aggregates are larger compared to clusters in Fig. 1b. Scale bar represents 20 μ.

ethanol, but no ciclosporin, was dispersed in water, a suspension of the tubular clusters was obtained and no droplets were found (Fig. 2d). This led to the hypothesis that the droplets seen in Fig. 2c (which only form in the presence of both ethanol and ciclosporin) may be droplets of ethanol containing dissolved ciclosporin. To test this hypothesis, a solution of ciclosporin in ethanol was added to water. An oil-in-water (o/w) emulsion, where the oil phase is likely to consist of ethanol and ciclosporin was obtained (Fig. 2e). Dissolution of ciclosporin in ethanol seems to reduce the miscibility of ethanol with water, such that upon mixing the two liquids, an emulsion is formed. This behaviour is characteristic only for admixtures of ethanol and ciclosporin and was not observed with other commonly used hydrophilic solvents, such as propylene glycol, polyethylene glycols. In our study, it is expected that dispersion of gel C into an aqueous medium result in the ciclosporin being dissolved in the ethanol droplets as well as within micelles in the aqueous medium.

Particle size analysis of the three amphiphilogel dispersions is shown in Fig. 3. Firstly, it must be remembered that many different species are represented under each curve and it is difficult to assign an average diameter to specific species. For example, the profile of formulation C includes sizes of droplets and of tubular clusters, that of formulation B includes clusters of different sizes and possibly, aggregates of clusters, while that of formulation A includes droplets (of varying sizes), clusters and their aggregates. Fig. 3, therefore, only gives an indication of the average sizes. The smallest average size was obtained for the hydrophilic gel

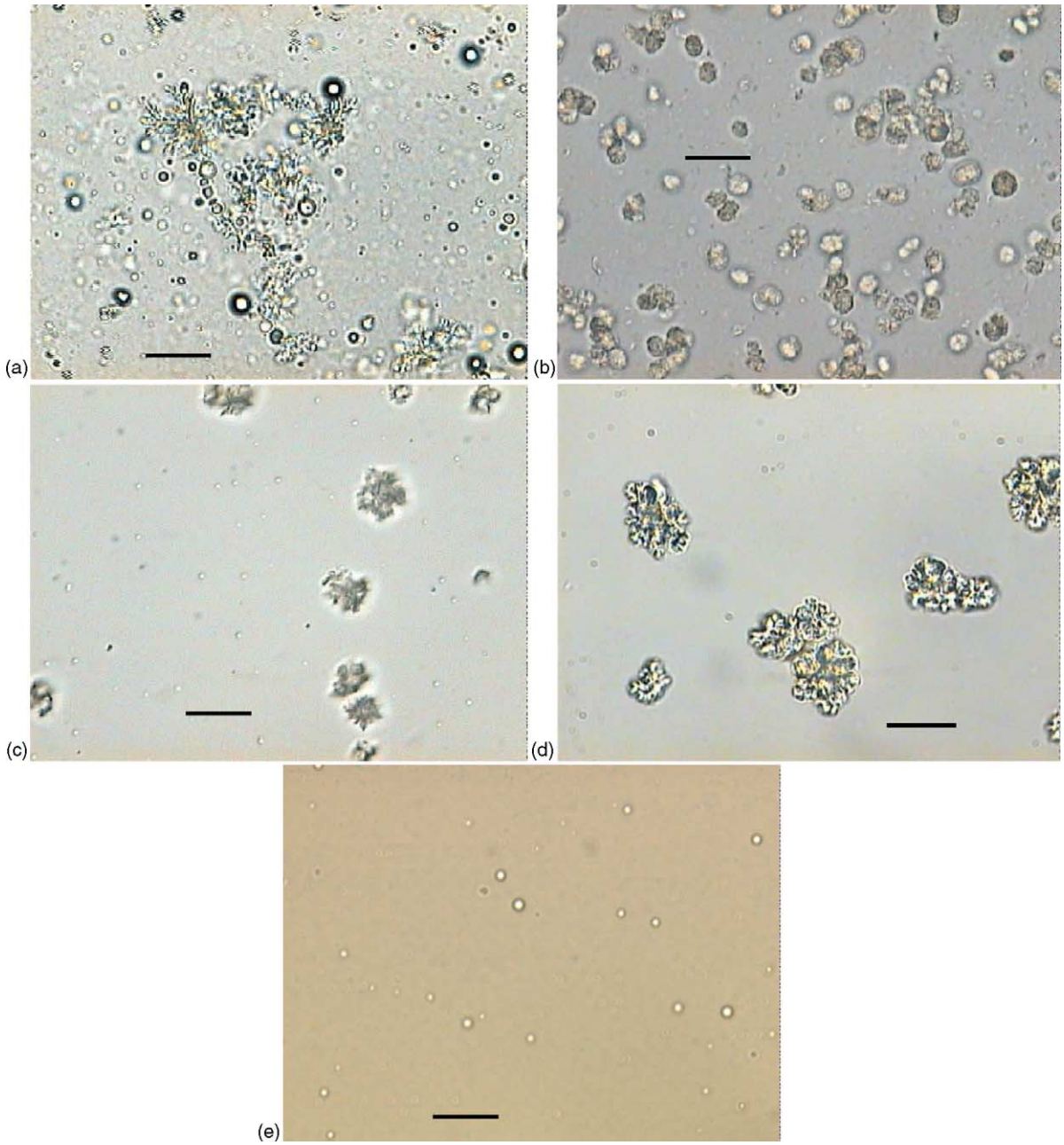

Fig. 2. (a) Light microscopy of an aqueous dispersion of ciclosporin-loaded hydrophobic gel. Clusters of tubular aggregates and droplet of varying sizes are seen. Scale bar represents 20 μ. (b) Light microscopy of an aqueous dispersion of ciclosporin-loaded hydrophilic gel. Dispersion consists mainly of clusters of tubular aggregates in the aqueous medium. Scale bar represents 20 μ. (c) Light microscopy of an aqueous dispersion of ciclosporin-loaded hydrophilic gel containing 10% ethanol. Clusters of tubular aggregates and droplets of relatively uniform size are seen. Scale bar represents 20 μ. (d) Light microscopy of an aqueous dispersion of hydrophilic gel containing 10% ethanol (gel does not contain any dissolved ciclosporin). Clusters of tubular aggregates are seen. Scale bar represents 20 μ. (e) Light microscopy of an emulsion formed when an ethanol solution of ciclosporin was mixed with water. Droplets of relatively uniform size (containing ethanol and ciclosporin) are seen. Scale bar represents 20 μ.

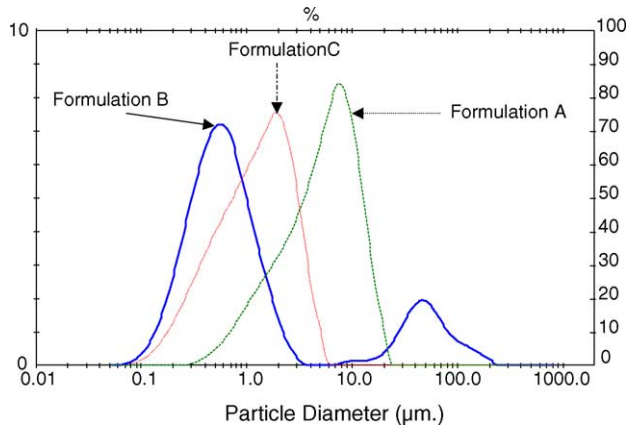

Fig. 3. Particle size analysis of aqueous dispersions of a hydrophobic gel (A), a hydrophilic gel (B) and a hydrophilic gel containing 10% ethanol (C). All the gels contained dissolved ciclosporin prior to dispersion into water. Span of size measurement of formulation A, 3.54; B, 1.26; C, 1.10.

B, followed by gel C, then gel A. This is reflected in the increasing size of the tubular clusters from gel B to C to A, as can be seen from the light micrographs in Figs. 1a–c and 2a–c. This shows that the particle size of the tubular clusters in the aqueous dispersions are the biggest contributors to the average particle size measured. However, there is no straightforward correlation between the measured particle sizes shown in Fig. 3 and the light micrographs shown in Fig. 2a–c. The same lack of correlation is observed for GEM formulations (Figs. 5 and 6). This shows the difficulties of measuring particle size of gel dispersions containing different species and the futility of using only the magnitude of the average particle size, when different formulations are compared.

3.2. GEM formulations and their interactions with water

The GEM formulations were clear organic solutions containing up to 10% ciclosporin. Like the amphiphilogels, water was easily incorporated within the GEM liquid solutions, which could also be easily dispersed in water, due to their amphiphilic nature. Small amounts of water (<10%) could be solubilised within the GEM solutions, which remained transparent

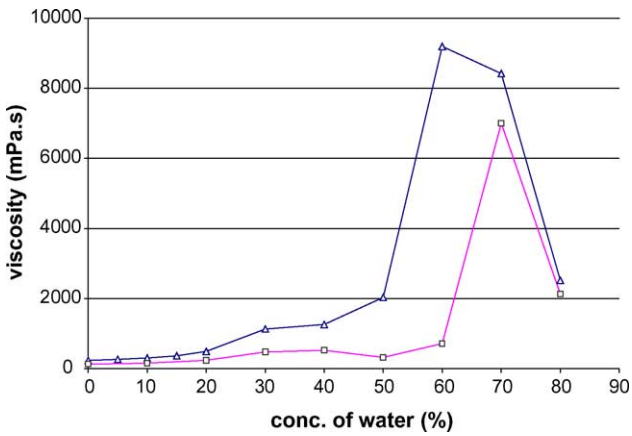

Fig. 4. Changes in viscosity of GEM formulations upon incorporation of increasing amounts of water, at a constant shear rate of 8.1 s^{-1}, at 25 °C (△) and at 37 °C (□).

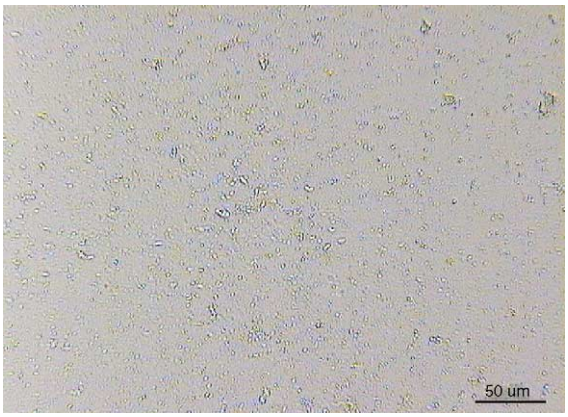

Fig. 5. Light microscopy of an aqueous dispersion of GEM, containing 90% water.

or became slightly opalescent. Increasing the amount of water in the GEM solutions resulted in increasing turbidity and viscosity of the mixture (Fig. 4). The highest viscosity was obtained when water was gently mixed into the formulation using a spatula at a concentration of 60%. The very large increase in viscosity (up to 45 times) has been assigned to the presence of lamellar liquid crystalline phase, as revealed by SAXS (Andrýsek et al., 2003; Uhrikova et al., 2004). It is likely that lyotropic liquid crystals are formed by the association and orientation of the different components present in the mixture. Further addition of water results in gel break-up into 'gel particles', their dispersion within the aqueous medium (Fig. 5) and a rapid reduction in viscosity as shown in Fig. 4. Changes in viscosity of the formulations followed the same trend at 25 °C and at 37 °C, except for the fact that viscosity was lower at 37 °C, and maximal viscosity was achieved at a greater water content. Particle size analysis of the dispersion in excess aqueous medium showed that the particles were fairly large (Fig. 6) and were much larger than particles in amphiphilogel dispersions. The larger particles formed when GEM 304 was dispersed in water could be due to the presence of oleyl alcohol which renders GEM 304 more lipophilic.

3.3. *In vivo* absorption of ciclosporin from gels and their dispersions

Following the oral administration of ciclosporin formulated in amphiphilogels, their aqueous dispersions and those of GEM formulations, drug absorption occurred rapidly and, in most cases, the maximum blood concentration was achieved within 2 h (Fig. 7). Table 1 shows the AUC_{24}, C_{max} and T_{max} obtained with the different formulations, including Neoral®, which was used as a control. Statistical analysis on the AUC_{24} indicates that the hydrophobic amphiphilogel, the hydrophilic amphiphilogel containing 10% (w/w) ethanol, the latter's dispersion in water, the aqueous dispersions of GEM101 and GEM304 and Neoral® were not significantly different from one another (one way ANOVA, $p > 0.05$). Compared to these formulations, drug absorption from the hydrophilic amphiphilogel was significantly lower. Absorption from an aqueous dispersion of the hydrophilic gel was even poorer as shown in Fig. 7.

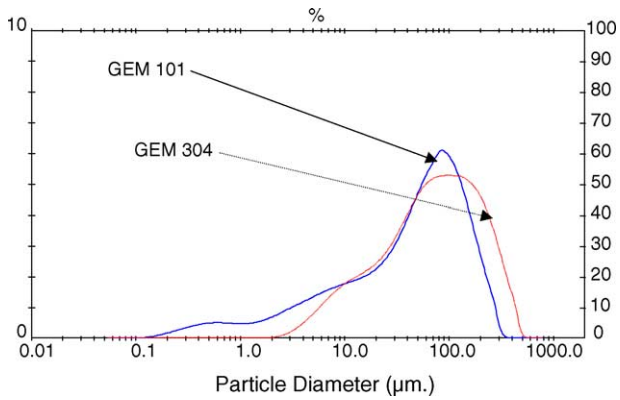

Fig. 6. Particle size analysis of GEM dispersions. Span of size measurement of GEM 101: 2.98; GEM 304: 1.43.

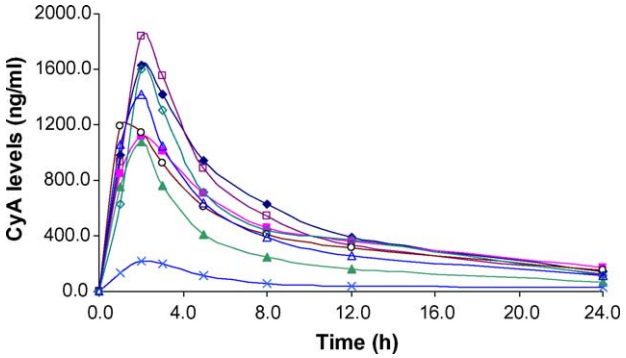

Fig. 7. Ciclosporin levels in blood, following oral administration of hydrophilic gel with 10% (w/w) ethanol (□), Neoral® (♦), GEM 304 (◊), GEM 101 (△), dispersion of a hydrophilic gel containing ethanol (○), hydrophobic gel (■), hydrophilic gel (▲), and hydrophilic gel dispersion (×).

Comparing the amphiphilogel formulations, the hydrophobic gel was a better vehicle for ciclosporin compared to the hydrophilic gel, allowing greater absorption of the drug (Student's *t*-test, $p < 0.05$, Fig. 8). Addition of 10% ethanol to a hydrophilic gel improved ciclosporin absorption (Student's *t*-test, $p < 0.01$, Fig. 9). Pre-dispersing a hydrophilic gel into water prior to oral administration severely reduced drug absorption (Student's *t*-test, $p < 0.001$, Fig. 7), whereas pre-dispersion of a gel containing ethanol did not cause any significant differences in AUC, T_{max} and C_{max} (Student's *t*-test, $p = 0.1$, Fig. 10).

The lower bioavailability of ciclosporin from the hydrophilic gel could be explained by the gel's interactions with water. As described in Section 3.1, when the hydrophilic gel is in contact with an aqueous phase, polysorbate 80 (the solvent in which ciclosporin is dissolved) disperses rapidly and dissolves in the aqueous medium. Following oral administration of the hydrophilic gel in vivo, it is possible that during gel dispersion in the stomach contents, some of the ciclosporin, which was originally dissolved

Table 1
AUC24, C_{max} and T_{max} (mean ± S.D.) of the different ciclosporin formulations

Formulation	AUC_{24} (h mg/ml)	C_{max} (mg/ml)	T_{max} (h)
Neoral ($n = 20$)	13.31 ± 3.01	1.84 ± 0.50	1.91 ± 0.93
Hydrophobic gel ($n = 5$)	10.77 ± 3.02	1.21 ± 0.24	2.25 ± 1.65
Hydrophilic gel ($n = 5$)	6.56 ± 1.18	1.25 ± 0.54	1.38 ± 0.67
Gel with ethanol ($n = 5$)	12.65 ± 2.13	1.81 ± 0.61	1.94 ± 0.37
Aqueous dispersion of hydrophilic gel ($n = 9$)	1.42 ± 1.05	0.24 ± 0.11	1.39 ± 0.92
Aqueous dispersion of gel containing ethanol ($n = 5$)	10.09 ± 2.43	1.54 ± 0.756	1.56 ± 1.06
GEM 304 ($n = 5$)	11.14 ± 7.07	1.62 ± 0.92	1.8 ± 0.40
GEM 101 ($n = 5$)	9.79 ± 2.13	1.57 ± 0.25	1.8 ± 0.75

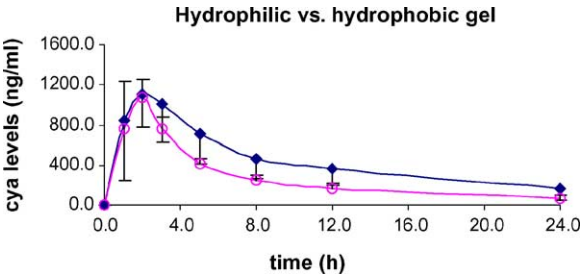

Fig. 8. Different ciclosporin levels in blood, following oral administration of a hydrophobic gel (♦) and a hydrophilic gel (○).

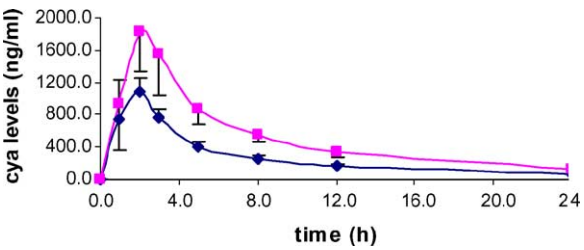

Fig. 9. Comparison between ciclosporin absorption from a hydrophilic gel (♦) and from a hydrophilic gel containing 10% (w/w) ethanol (■).

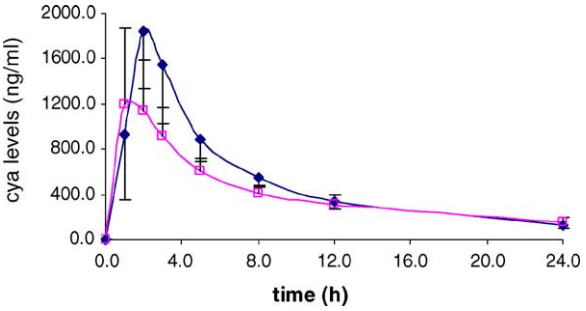

Fig. 10. Comparison between ciclosporin absorption from a hydrophilic gel containing 10% (w/w) ethanol (♦) and its dispersion in water (□).

in the gel, precipitates out. This would reduce the amount of soluble ciclosporin available for absorption from the gastro-intestinal tract. Partial loss of solvent capacity and consequent drug precipitation as the hydrophilic amphiphilogel is diluted with an aqueous phase is similar to the possibility of drug precipitation when lipid formulations which contain significant amounts of hydrophilic components are diluted with water (Pouton, 2000). Predictably, pre-dispersing a hydrophilic amphiphilogel in water prior to oral administration to dogs had an adverse effect on drug absorption (Fig. 7). A significant proportion of the drug must have precipitated out into the aqueous medium before administration. The poorer absorption profile of the aqueous dispersion compared to the hydrophilic gel may be due to the fact that when the hydrophilic gel disperses in vivo, following contact with the aqueous contents of the stomach, drug precipitation may be slow due to the 'solubilising and colloidal, stabilising environment' of the gut (Pouton, 2000). In contrast, when the gel was dispersed in water prior to oral administration, solvent loss occurred in a less favourable environment and more drug could have precipitated out. These observations concur with Humberstone's and Charman's point about the importance of keeping the drug in a solubilised state to enable absorption of poorly water-soluble drugs (Humberstone and Charman, 1997).

In contrast to the hydrophilic gel, aqueous dispersions of the hydrophobic amphiphilogel and of the hydrophilic gel containing 10% ethanol comprised 'oily' droplets (seen in Fig. 2a and c) where the ciclosporin could remain in the dissolved form and be available for absorption. The 'oily' droplets were very large (in the micron range); this shows that the size of the droplet does not seem to be an important factor in the absorption of ciclosporin from these formulations, as previously suggested by Andrýsek (2001). Similarly, the GEM formulations were found to have equivalent bioavailabilities to Neoral® microemulsion despite the fact that the particle size of their aqueous dispersions was much greater than those of Neoral® microemulsion which is less than 100 nm (Vonderscher and Meinzer, 1994). Although GEM 101 and GEM 304 have different ability to disperse (as shown in Fig. 6), both formulations achieved comparable bioavailability to Neoral®. It is obvious then, that particle size is not the most important factor in ensuring bioavailability of ciclosporin from GEM formulations or indeed from all the gel systems described in this paper. The most important contribution of the gel formulations is that they present the drug in a soluble form at the intestinal surface. Other beneficial properties of the gel formulations might include the ability of surfactants to enhance intestinal permeability, the action of polysorbate 80 as an inhibitor of the P-glycoprotein efflux transport pump which is known to exsorb ciclosporin from the blood into the intestinal lumen (Augustijns et al., 1993; Nerurkar et al., 1996) and the possibility of prolonged contact between gel particles and the intestinal wall which ensures a high concentration gradient of the drug at the site of absorption, unlike emulsion and microemulsion droplets.

4. Conclusions

In this paper, we have reported the development of gel formulations as oral vehicles for ciclosporin A. The gel formulations are easy to prepare and are stable. A number of these formulations show similar absorption profiles to the commercially available Neoral® microemulsion when orally administered to dogs. High drug absorption is thought to be linked to the ability of the gels to keep the drug in a solubilised form when the gel interacts with the aqueous gastric contents.

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