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The in Vitro Release of Some Antimuscarinic Drugs from Monoolein/Water Lyotropic Liquid Crystalline Gels

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Purpose. To investigate the potential use of a monoolein/water lyotropic liquid crystalline gel for the vaginal delivery of the antimuscarinic drugs, propantheline bromide and oxybutynin hydrochloride to treat urinary incontinence, using Myverol 18-99 as a commercially available grade of monoolein.

Methods. The influence of propantheline bromide and oxybutynin hydrochloride on the phase structure of Myverol 18-99/water gels was investigated using polarising microscopy. The in-vitro release of the antimuscarinic drugs from Myverol 18-99/water gels was determined and the release pattern interpreted with the aid of results from swelling studies and partition coefficient determinations.

Results. Myverol 18-99 forms gels with lyotropic liquid crystalline structures in the presence of water. The addition of propantheline bromide and oxybutynin hydrochloride promoted the formation of gels with a lamellar phase structure. The gels absorbed water at a rate inversely proportional to their initial water content until they reached an equilibrium water content of ~40% w/w whilst maintaining their physical integrity. The release of the antimuscarinic drugs was sustained over a period of ~18 hours and followed square root of time kinetics indicating that the rate of release was diffusion controlled.

Conclusions. The in-vitro release behaviour of Myverol 18-99/water gels suggested that they are suitable carriers to deliver propantheline bromide or oxybutynin hydrochloride. The results of swelling studies indicated that a confined area, such as the vaginal cavity, would be a suitable site of administration.

KEY WORDS: monoolein; lyotropic liquid crystals; propantheline bromide; oxybutynin hydrochloride; drug release.

INTRODUCTION

Monoolein, glyceryl monooleate, is a polar lipid that forms gels with a lyotropic liquid crystalline structure in the presence of water (1). These gels have long range order due to the alignment and periodicity of the polar head groups of the lipid molecules and short range disorder due to the fluid hydrocarbon tails. The nature of the lyotropic liquid crystalline phase that forms depends on the ratio of monoolein to water, the temperature of the system and the presence of any additional substances (2).

Monoolein is of interest pharmaceutically because the amphiphilic nature of the gels formed in aqueous systems facilitates the incorporation of both hydrophilic and hydrophobic molecules. Monoolein is non toxic, biodegradable, biocompatible and generally recognised as safe (3–5). The gels can be manipulated for use as parenteral or enteral dosage forms but are suited particularly to transmucosal drug delivery systems because of their bioadhesive nature (6). However, there is little evidence of their pharmaceutical application in the literature.

The work contained in this report forms part of a study designed to characterise and investigate the potential use of a monoolein/water lyotropic liquid crystalline gel to deliver drugs via the vaginal cavity. The drugs of interest were propantheline bromide and oxybutynin hydrochloride. These antimuscarinic drugs are currently given orally to treat urinary incontinence and act by diminishing unstable detrusor contractions resulting in an increase in bladder capacity. The current usage of propantheline bromide and oxybutynin hydrochloride is limited because of a high incidence of side-effects, particularly dry mouth and constipation, attributed to a first-pass metabolite (7). As the majority of urinary incontinence sufferers are women, the delivery of these drugs by the vaginal route may be an advantageous alternative to the oral route. The vaginal delivery of these drugs may decrease the incidence of side-effects because drugs absorbed from the vaginal canal are subjected to a reduced first-pass effect, decreasing the production of metabolites. Furthermore, the vaginal wall is adjacent to the bladder and so the vaginal delivery of these antimuscarinic drugs offers the potential of administering a reduced dose as the drugs are delivered closer to their site of action.

MATERIALS & METHODS

Materials

A commercially available grade of monoolein, Myverol 18-99 distilled monoglycerides, was kindly donated by the Eastman Chemical Company and was used as received. Propantheline bromide, oxybutynin hydrochloride, sodium iodide, hydrogen peroxide 30 vol., p-aminophenol and octan-1-ol were obtained from Sigma Chemical Company. Potassium dihydrogen orthophosphate was obtained from BDH. Water was singly distilled.

Phase Determination

Myverol 18-99/water gels containing known amounts of water in the range 0–40% w/w were examined using a Nikon Optiphot 2 polarising microscope fitted with a Mettler FP82HT hot stage, after equilibration at room temperature for seven days. Each gel sample was heated at approximately 1°C per minute over the temperature range of 20–75°C whilst being constantly monitored between crossed polarisers for a change in the observed texture indicative of a phase transition. The cover slips of the samples were sheared frequently to prevent a homeotropically aligned anisotropic phase from being identified mistakenly as an isotropic phase and also to give an indication of the phase viscosity which was used as an identification aid. Photomicrographs were taken with Nikon FX-35 mm camera.

The influence of propantheline bromide and oxybutynin hydrochloride loading on the phase properties was investigated at defined ratios of Myverol 18-99 to water for a range of drug concentrations.

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Water Uptake/Swelling Studies

Myverol 18-99/water gel samples containing 0, 20 and 40% w/w water were allowed to equilibrate at 4°C for ~1 hour in a Teflon mould (10 mm × 6 mm), extruded, weighed and placed in 300 ml de-aerated 0.1M phosphate buffer (pH 4.5 at 37°C). At defined time intervals the gels were removed, blotted dry and re-weighed. The diameter and height of the gels were determined using a travelling microscope (Vickers Ltd.).

Gels prepared from Myverol 18-99/water with weight ratios of 100:0 and 80:20 loaded with 5% w/w drug, were placed into a 40 mesh basket and rotated at 100 rpm in 500 ml de-aerated 0.1M phosphate buffer (pH 4.5 at 37°C). After defined time intervals, the gel samples were removed and mounted between the crossed polarisers of a polarising light box (Sharples Photomechanics Ltd.) which revealed a birefringent core allowing gel thickness to be determined. Photographs were taken in both the horizontal and vertical planes using a Pentax ASAHI H100 camera.

In Vitro Drug Release

Dissolution studies were performed in triplicate using a perspex release apparatus based on the USP rotating-basket method (apparatus 1) (8). The release cell (10 cm diameter by 8.5 cm depth) was suspended in a water bath at 37°C. The gels were enclosed in a 40 mesh basket, due to their swellable nature, and rotated at 100 rpm in 500 ml de-aerated 0.1M phosphate buffer (pH 4.5). The amount of propantheline bromide or oxybutynin hydrochloride released into the dissolution medium was assayed spectrophotometrically at wavelengths of 282 nm and 230 nm respectively. The influence of initial gel composition on drug release was investigated using gels with known water contents between 10–40% w/w and a specified drug loading concentration between 2.5–7.5% w/w. The gels were prepared by mixing the required amount of propantheline bromide or oxybutynin hydrochloride with molten Myverol 18-99 and adding the appropriate amount of water. Drug particles could not be observed when the resulting gels were examined with a polarising microscope indicating that the drugs dissolved fully in the gels. Water-free Myverol 18-99 matrices loaded with 2.5, 5.0 and 7.5% w/w propantheline bromide or oxybutynin hydrochloride were also prepared and the release characteristics investigated. An indication of the solubility of each drug (<250 sieve aperture size fraction) in molten Myverol 18-99 (37°C) was determined at the specified drug loadings using a polarising light box to detect suspended drug particles.

The release profiles of both drugs from Myverol 18-99/water gels were compared with that of sodium iodide, chosen as a model ionic solute with negligible partitioning into the lipid bilayer. The amount of sodium iodide (5% w/w initial loading) released from Myverol 18-99/water systems containing 0 or 20% w/w initial water content was determined using a colorimetric assay (9). 2 ml aliquots of the release medium were removed at pre-set time intervals and reacted with 2 ml 0.05% w/w p-aminophenol solution in the presence of 2 drops of hydrogen peroxide. 2 ml 0.1M phosphate buffer was returned to the release vessel to maintain the dissolution volume at 500 ml. The colour development reaction was allowed to proceed for 100 minutes after which the solutions were assayed spectrophotometrically at 540 nm using a Cecil 1020 spectrophotometer. The limit of detection of the assay was 2.5 ppm.

RESULTS AND DISCUSSION

Phase Determination by Polarising Microscopy

Lamellar, cubic, L_2, and reversed hexagonal phases were identified in the Myverol 18-99/water phase diagram (Figure 1) according to the classification established by Rosevear (10).

Gels formed at ambient temperature with initial water contents of less than 15% w/w, had a lamellar (L_α) phase structure consisting of planar lipid bilayers with alternating water layers. The soft opaque gels were optically anisotropic and could be identified from the oily streak texture and the spherical, positive maltese cross units visible between crossed polarisers (Figure 2a). An increase in water content and/or temperature produced gels with a cubic (C) phase structure which were transparent, optically isotropic and viscous. These gels consist of a 3D network of curved bilayers forming a bicontinuous structure which coexists in equilibrium with excess water. At temperatures greater than 57°C, gels formed with a reversed hexagonal (H_{II}) phase structure consisting of water cylinders arranged in a 2D lattice separated by lipid bilayers. The gel samples were optically anisotropic and could be identified by the angular or fan-like textures when viewed between crossed polarisers (Figure 2b).

The phase diagram of Myverol 18-99 and water was similar to that of pure monoolein and water (11) in that the L_2, lamellar and cubic phases appeared in the same relative positions, although the locations of the phase boundaries were different. The most noticeable difference was the appearance of the reversed hexagonal phase at temperatures above 57°C instead of ~85°C as reported with pure monoolein.

Figure 3 represents the partial ternary phase diagram for Myverol 18-99/water showing the changes induced by the addition of propantheline bromide. The data points represent actual samples examined and the dotted lines represent probable phase boundaries based on these data. It can be seen that at ambient temperature, the samples with a water content of less than 15%w/w retained their lamellar structure when an increasing quantity of propantheline bromide was added. However, samples that formed a cubic phase gel in the absence of drug (~15–40% w/w water content), could only maintain their structure up to propantheline bromide loadings of less than 5% w/w. Above this drug loading, gels were formed that had a characteristic lamellar phase appearance when observed

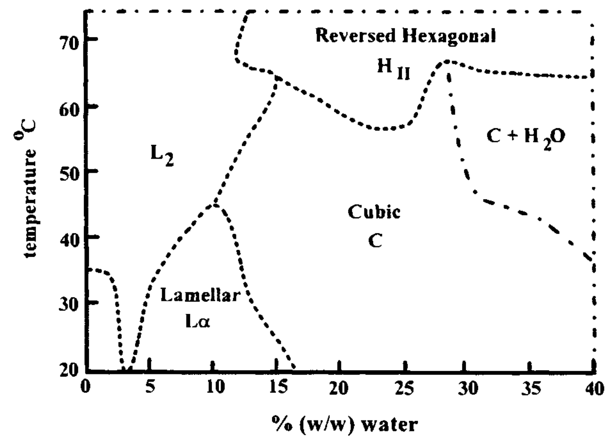

Fig. 1. Binary phase diagram of Myverol 18–99 and water.

Fig. 2. Photomicrographs showing **a)** the lamellar phase of a 90/10 wt ratio Myverol 18-99/water gel at ambient temperature and **b)** the reversed hexagonal phase of a 80/20 wt ratio Myverol 18-99/water gel at 65°C, as observed between crossed polarisers at ×100 magnification.

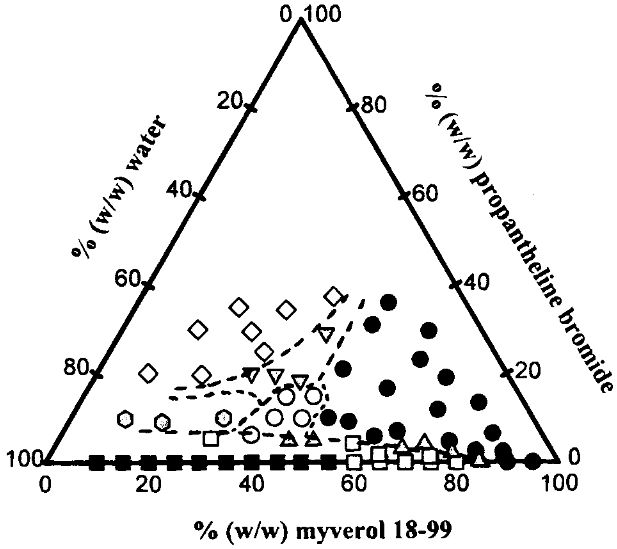

Fig. 3. Partial ternary phase diagram showing the lyotropic liquid crystalline phases formed by Myverol 18-99/water/propantheline bromide mixtures at ambient temperature. ○, Lamellar Phase (Clear Gel); ●, Lamellar Phase (Opaque Gel); □, Cubic Phase; ■, Cubic Phase (in Excess Water); ▲, Lamellar Phase/Cubic Phase; ◇, Isotropic Golden Liquid; ▼, Birefringent Droplets in Isotropic Liquid; ○, Isotropic White Dispersion.

observed for Myverol 18-99/water systems containing added oxybutynin hydrochloride. Both drugs are amphiphilic and therefore most likely to partition at the lipid-water interface. Both are in the fully ionised form at pH 4.5 (vaginal pH); oxybutynin hydrochloride has a pK_a of 8.04 (12) and propantheline bromide is a quaternary ammonium compound. The bulky, positively charged amino head groups present in these drug molecules may have prevented the close packing of the lipid molecules that is required to maintain the stability of the cubic phase (13). Thus, a transition to the lamellar phase occurred to relieve the induced packing stresses. The formation of lamellar phase gels was also observed by other workers when the cationic amphiphilic molecules, lidocaine (13) and CTAB (1), were added to monoolein and water mixtures.

Water Uptake/Swelling Studies

Figure 4 shows the percentage increase in weight of Myverol 18-99/water gels, with initial water contents of 0, 20 and 40% w/w, expressed as a function of time. The gels with 0 and 20% w/w initial water content were observed to hydrate rapidly up to a total water content of 40% w/w. The samples formulated with 40% w/w water did not hydrate or swell significantly as they already contained their equilibrium water content. When the data shown in Figure 4 were re-plotted as the percentage increase in weight of the dosage forms expressed as a fraction of their percentage increase in weight at equilibrium, the plots for the samples containing 0% and 20% w/w water were superimposible indicating that the dosage forms approached their equilibrium water content at the same rate. Thus, the rate of water uptake by the dosage forms was inversely proportional to their initial water content. A dramatic increase in the size of the dosage forms containing 0 and 20% w/w initial water content was observed within the first 15 minutes of hydration, after which the dimensions of the dosage forms remained relatively constant.

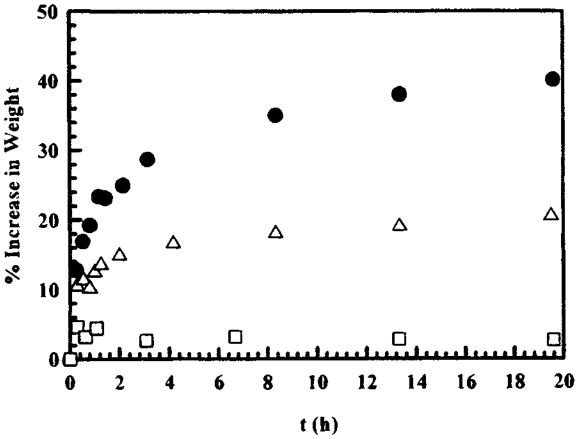

Fig. 4. Plots of the percentage increase in the weight of Myverol 18-99/water gels with initial water contents of ●, 0; △, 20 and □, 40% w/w, as a function of time when placed into excess phosphate buffer (pH 4.5, n = 2, sd = ±0.9).

When a Myverol 18-99 matrix containing 5% w/w oxybutynin hydrochloride was placed between the crossed polarisers of a polarising light box one hour after immersion in 0.1M phosphate buffer, a birefringent core was revealed, surrounded by an isotropic outer layer. Sectioning of the dosage form and examination of a sample of the interior by polarising microscopy identified the presence of a lamellar phase gel at the core. As the time of exposure to water increased, there was a decrease in the size of the core and a concomitant increase in the isotropic outer gel thickness. These observations suggested that as hydration continued, a cubic phase gel layer was forming around a core of lamellar phase gel. The core of the dosage form consisted of a lamellar phase gel because it had a lower water content and a higher oxybutynin hydrochloride concentration than the outer gel layer. The entire dosage form became an isotropic gel with a cubic phase structure within 5 hours of hydrating due to the increase in the amount of water permeating to the core and the counter current of drug diffusing away from the core as it was released. Samples containing propantheline bromide and sodium iodide were observed to behave in the same manner as those containing oxybutynin hydrochloride, in that they showed a progressive increase in isotropic outer gel layer thickness over the same time period.

In Vitro Drug Release

The Influence of Gel Composition

Figure 5 shows the fraction of the initial 5% w/w propantheline bromide loading released as a function of time for known initial gel compositions. Over a period of ~18 hours, the initial composition of the sample, i.e. the Myverol 18-99 to water ratio, did not affect the rate of propantheline bromide release. Similarly, release profiles obtained for the initial propantheline bromide loadings of 2.5% and 7.5% w/w and oxybutynin hydrochloride loadings of 2.5%, 5.0% and 7.5% w/w were also observed to be independent of the initial Myv-

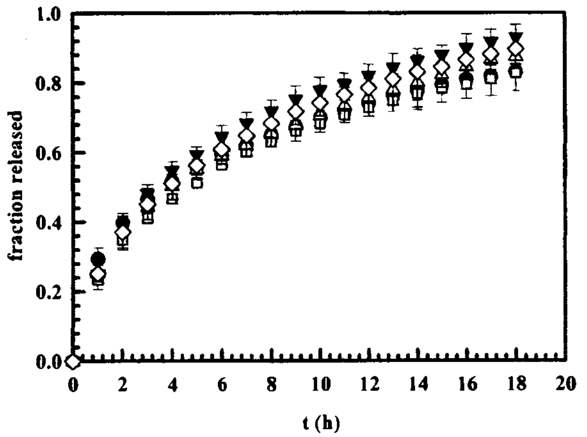

Fig. 5. Plots of the fraction of propantheline bromide (5% w/w initial loading) released as a function of time from Myverol 18-99/water gels with Myverol 18–99/water wt ratios of •, 100:0; □, 90:10; △, 80:20; ▼, 70:30 and ◇, 60:40, (n = 3, ±sd).

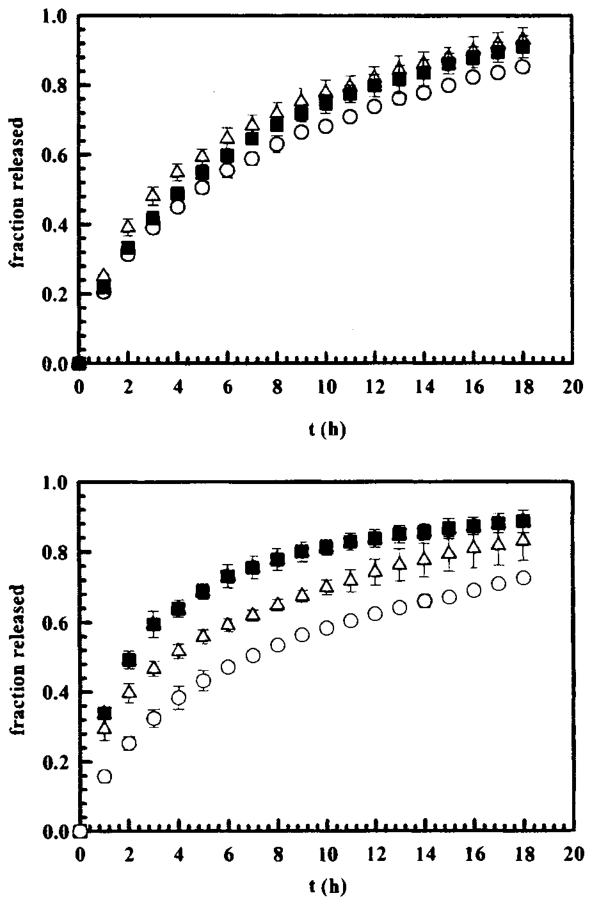

Fig. 6. Plots of the fraction of propantheline bromide released as a function of time from **a)** a 70/30 wt ratio Myverol 18-99/water gel and **b)** a water-free Myverol 18-99 matrix at initial propantheline bromide loadings of ○, 2.5; △, 5.0, and ■, 7.5% w/w (n = 3, ±sd).

erol 18-99/water gel composition. The gradient and correlation coefficient values obtained for plots of the fraction of propantheline bromide released from Myverol 18-99/water gels (70/30 wt ratio) containing initial drug loadings of 2.5, 5.0 and 7.5% w/w as a function as the square root of time were 0.23 h^{-1} (0.999), 0.28 h^{-1} (0.995) and 0.26 h^{-1} (0.999) respectively. The linear fit infers that the release kinetics could be satisfactorily described by the Higuchi square root of time law (14). It is proposed that the rate of drug release is controlled by the diffusion of molecules through the water channels of the cubic phase gel layer. The diffusional path length of the drug molecules increases with time, retarding the release rate. Swelling studies indicated that after a period of ~5 hours all the samples, regardless of initial composition, had equilibrated to form cubic phase gels with a water content of ~40% w/w. Thus, the rate of drug release was found to be independent of the initial water content of the gels as their equilibrium composition was the same.

The Influence of Drug Loading

Figure 6a shows the fraction of propantheline bromide released from a Myverol 18-99/water gel containing 30% w/w water, with initial drug loadings of 2.5%, 5.0%, and 7.5% w/w. These samples contained a sufficiently high initial water content to dissolve the propantheline bromide and the fraction of drug released with time was seen to be independent of initial drug loading. The fraction of propantheline bromide and of oxybutynin hydrochloride released from Myverol 18-99/water gels containing 10%, 20% and 40% w/w water was also shown to be independent of initial drug loading. These results were in agreement with the model proposed by Higuchi (14) for diffusion controlled release of dissolved drug from a matrix. However, plots of the log amount of drug remaining in the sample versus time for Myverol 18-99/water gels loaded with propantheline bromide and oxybutynin hydrochloride were also linear, possibly indicative of a first order drug release process. The mechanism of release of both drugs was determined using the analysis devised by Schwartz *et al* (15) which differentiates between first order and diffusion controlled kinetics. Figure 7a shows the non-linear fit obtained when the rate of release was plotted against the amount of drug released for a 70/30 wt ratio Myverol 18-99/water gel containing 5% w/w propantheline bromide indicating that first order release kinetics were not operative. However, the corresponding plot of release rate versus the reciprocal of the amount released (Figure 7b) resulted in a linear fit. A similar finding was obtained for oxybutynin hydrochloride, confirming that the release of propantheline bromide and oxybutynin hydrochloride from Myverol 18-99/water gels was diffusion controlled.

It was noted that the release of propantheline bromide and oxybutynin hydrochloride at drug loadings of 7.5% w/w from Myverol 18-99/water gels containing 40% w/w water was high when compared to gels with lower water contents. Visually these gels appeared more transparent than the opaque lamellar gels normally observed. The phase diagram in Figure 3 shows that further increases in the propantheline bromide loading whilst keeping the weight ratio of Myverol 18-99 to water constant at 60/40 resulted in the formation of a liquid crystalline dispersion and eventually an isotropic liquid, suggesting that there was possible limitation on the integrity of the gels formed with low Myverol 18-99 contents and high drug loadings.

Figure 6b shows that for matrices prepared from Myverol 18-99 in the absence of water and loaded with 2.5%, 5.0% and 7.5% w/w propantheline bromide, the fraction of drug released

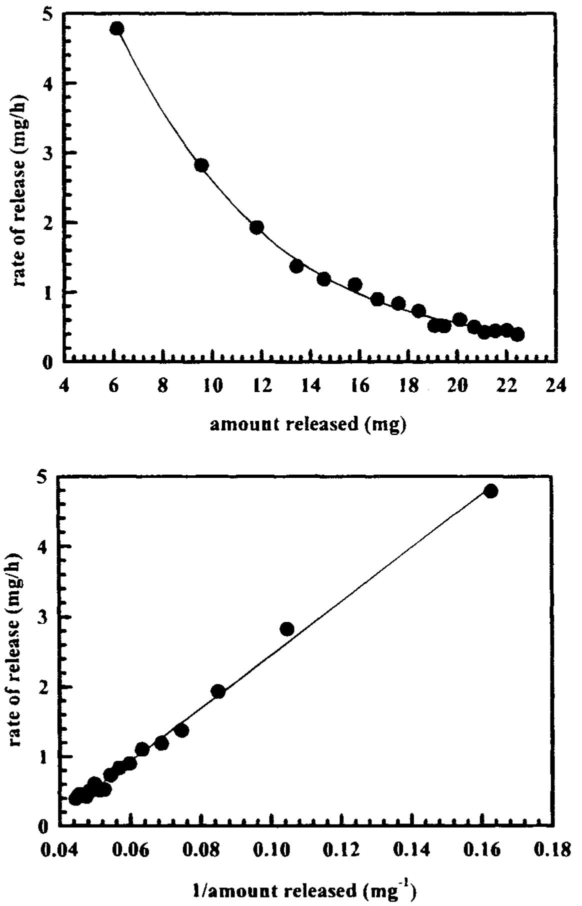

Fig. 7. Plot of the rate of propantheline bromide (5% w/w initial loading) release from a 70/30 Myverol 18-99/water gel as a function of **a)** amount released and **b)** 1/(amount released).

with time increased with an increase in initial drug loading. At propantheline bromide loadings of 5.0 and 7.5% w/w, undissolved drug particles were observed in molten Myverol 18-99 samples. In comparison, the fraction of oxybutynin hydrochloride released from water-free Myverol 18-99 matrices was found to be independent of the initial drug loading at 2.5 and 5.0% w/w, where the drug was seen to be dissolved, but increased at the 7.5% w/w loading where undissolved drug was observed in the samples. The release of both drugs from water-free Myverol 18-99 matrices could be fitted to models for the diffusion controlled release of dissolved or dispersed drug (14,16), as appropriate.

The Influence of Solute Type

The times for 50% release (T_{50}) of initial 5% w/w loadings of propantheline bromide, oxybutynin hydrochloride and sodium iodide from an 80/20 wt ratio Myverol 18-99/water gel were 3.94, 5.39 and 0.41 h respectively. Propantheline bromide and oxybutynin hydrochloride have similar structures and molecular weights and affected the phases formed by Myverol 18-99/water gels in a similar manner; the similarity of their release profiles was therefore expected. These surface active drugs are likely to partition at the lipid/water interface of the gels. The octanol-water partition coefficients of the drugs (P_{app}), determined by the shake flask method (17), were 25.49 (±3.15) and 0.18 (±0.02) at pH 4.5 for oxybutynin hydrochloride and propantheline bromide respectively, suggesting that oxybutynin hydrochloride would be more soluble in Myverol 18-99 and hence have a slower release rate than propantheline bromide. The release rate of sodium iodide from an equivalent Myverol 18-99/water gel was appreciably higher than that of the two drugs, as would be expected for a small ionic solute with negligible tendency to partition into lipid. The release kinetics of sodium iodide obeyed the square root of time dependence indicating that the mechanism of release was diffusion of the ions through the water-filled channels.

CONCLUSIONS

The commercial grade of monoolein, Myverol 18-99 forms lamellar, L_2, cubic and reversed hexagonal lyotropic liquid crystalline phases in the presence of water. The addition of the antimuscarinic agents propantheline bromide and oxybutynin hydrochloride was shown to promote the formation of the lamellar phase. The lyotropic liquid crystalline phases of Myverol 18-99/water systems sustained the release of propantheline bromide and oxybutynin hydrochloride over a period of 18 and 20 h respectively. When placed in excess aqueous media, the samples absorbed water at a rate inversely proportional to their initial water content and swelled to an equilibrium composition containing 40% w/w water whilst maintaining their physical integrity. On hydration, an outer cubic phase gel layer formed, across which the drug molecules diffused, most probably through the water-filled channels. The release kinetics followed a square root of time dependence indicating that the rate of release of propantheline bromide and oxybutynin hydrochloride from Myverol 18-99/water gels is diffusion controlled. The release profiles have also been found to be dependent upon the solubility of the drug in the lipid base and the extent of partitioning into the lipid bilayer.

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