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Micellar solutions of triblock copolymer surfactants with pilocarpine

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Abstract

Solutions of surface active triblock copolymer Pluronic F127 in the vicinity of the critical micellar concentration (cmc) were prepared with or without pilocarpine (either as the hydrochloride salt or the free base) in water and phosphate buffer. The characteristics parameters of the surface activity (cmc, Γ and a) were determined for F127 solutions. Additionally, it was found that the pilocarpine solutions without F127 in water exhibits a certain surface activity. The solutions containing F127 (2 wt.%) well above the cmc and pilocarpine (2 wt.% for the salt, or equimolar 1.7 wt.% for the base) were further tested in vivo (miotic response) on rabbit eye. Though the entrapment efficiency of the drug in the micelles was rather low (maximal 1.9%) the pharmacokinetic parameters (duration of miotic response and the area under miotic curve) were improved when compared to the standard pilocarpine solutions. The best results were obtained for the micellar pilocarpine base solution which exhibits significant prolongation of miotic activity and an increase of AUC for 64%.

Keywords: Pluronic F127; Pilocarpine; Surface tension; Critical micellar concentration; Miosis

1. Introduction

Several various attempts to improve the bioavailability and/or to prolong the activity of drugs used in ophthalmology have been described in recent years. Apart of more classical formulations novel drug delivery systems have been proposed and investigated. Among them are: controlled release systems (Chien, 1982), pilocarpine prodrugs (Saarinen-Savolainen et al., 1996), submicron emulsions (Muchtar and Benita, 1994; Naveh et al., 1994; Sznitowska et al., 2001), thermoreversible polymer gels (Desai and Blanchard, 1998; Miyazaki et al., 2001), non-ionic surfactant vesicles (Saettone et al., 1996) and liposomes (Law and Hung, 1998; Nagarsenker et al., 1999).

Since liposomes and non-ionic surfactant vesicles have been suggested as suitable ophthalmic drug carriers we have explored the potential of an alternative but similar system. For that purpose aqueous solutions of high molecular weight poly(oxyethylene)/poly(oxypropylene)/poly(oxyethylene) triblock copolymer surfactant Pluronic F127 was used. The copolymer forms micelles when present above the critical micellar concentration (cmc). It is generally accepted that the core of the micelles consists of dehydrated poly(oxypropylene) groups. The hydrophobic core is surrounded by an outer shell of hydrated poly(oxyethylene) groups (Wanka et al., 1994; Allen et al., 1999). The micelles of Pluronic copolymers

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have been proposed as microcontainers for various drugs (Kabanov et al., 2002; Torchilin, 2001).

The aim of this communication was a two-fold: (i) to prepare as simply as possible an ocular delivery system based on Pluronic solutions containing micelles with a model drug pilocarpine, and (ii) to investigate a possible difference in the prepared systems when two chemical forms of pilocarpine are used. Namely, pilocarpine hydrochloride and its base should be differently positioned in the micellar system because of their respective apparent partition coefficient values. Therefore, solutions of Pluronic F127 above the cmc with the hydrochloride salt and the free base were prepared, and some of their physico-chemical characteristics, as well as the miotic response in vivo were studied.

2. Materials and methods

2.1. Materials

Pilocarpine hydrochloride (Fluka) and Pluronic F127 ($EO_{100}PO_{65}EO_{100}$; M_w 12,600) (BASF) were used as received. Pilocarpine base was prepared from the salt by alkalifying and extraction with dichloromethane. Pyrogen-free and double distilled water (conductivity $<2\,\mu S\,cm^{-1}$) was used in all experiments. A standard phosphate buffer solution (pH 7.4) (0.1369 mol l^{-1} NaCl, 0.0014 mol l^{-1} KH_2PO_4 and 0.0168 mol l^{-1} Na_2HPO_4) was used. All chemicals and solvents were reagent grade or pharmacopoeial purity.

2.2. Solutions

Stock solutions of the drug (4 wt.%) and the copolymer (10 wt.%) were prepared and kept in a refrigerator for further dilutions for a maximum duration of seven days. The solutions of Pluronic F127 were prepared by dilution of the stock solution in the concentration range 6.2×10^{-4} to 2 wt.% (i.e. 5×10^{-8} to 1.59×10^{-3} mol l^{-1}). One set of solutions was prepared in water only (pH 6–7) and the other was prepared with phosphate buffer (pH 7.4, ionic strength 0.19). Diluted F127 solutions were kept overnight at a room temperature and used for surface tension measurements alone or with an addition of pilocarpine (hydrochloride salt 2 wt.% or free base 1.7 wt.%). In a separate experiment, the surface tension of pilocarpine solutions was measured in the concentration range 0.4–24.5 $\times 10^{-3}$ mol l^{-1}.

The final Pluronic solutions with pilocarpine (salt or base) intended for the in vivo studies (miosis test) were prepared as simply as possible containing 2 wt.% pilocarpine hydrochloride or an equimolar amount of the base (1.7 wt.%). The required amount of pilocarpine was weighed into a flask and the Pluronic solution was added slowly with periodical stirring. The solution was stored in the refrigerator until usage. These proportions were chosen since the drug amount is equal to the usual dosage in eye drops, while the polymer is well above the cmc. In that way a probable dilution of the applied solution with the tear fluid in the rabbit eye will not decrease the polymer concentration below the cmc value.

2.3. Surface tension measurement

The experiments were performed with the ring method (Fisher Model 215 Autotensiomat®, Surface Tension Analyser). All glass was cleaned in the usual manner (with a chromic acid and thoroughly washed with double distilled water). The measurements were performed at 25 °C in an exactly same manner, i.e. conditioning time of 30 min was allowed between the placement of the solution in the measurement cell and the actual reading, since Polat and Chander (1999) have shown that a certain time is needed for the systems to reach an equilibrium value. The readings were taken in triplicate.

2.4. Micellar size measurement

Size distribution of micelles was measured by dynamic light scattering with a vertically polarized He:Ne laser (Zetasizer 3000HS, Malvern, UK). The scattering angle was fixed at 90 °C and temperature was maintained at 25 °C. The samples were filtered through a Millipore 0.45 μm membrane before measurements.

2.5. Drug entrapment

The entrapment of the drug, defined as the percent of the drug in micelles versus the drug used

in preparation, was determined in order to compare amounts of the two forms of the drug either present in micelles or in solution. Separation was performed by dialysis as follows. Sample of micellar solution (1 ml) was placed in a dialysing tube (Dialysis Tubing-Visking; 12–14,000 Da) and extensively dialysed against water for several hours. The volume of medium was adjusted so that the concentration of drug in water was kept below the drug solubility. Drug concentration was determined spectrophotometrically at 215 nm.

2.6. In vivo evaluation

In order to assess the in vivo performance of the micellar solutions in the eye, the miotic response in rabbits was determined. Female albino rabbits ($n = 6$) weighing 2.5 ± 0.5 kg were used. The unanaesthetized animals were kept in cages placed in a room with standard lighting. Miosis-time data were obtained by administering the micellar solutions (25 μl) with a micropipette into the lower portion of the conjunctivas sac of the right eye of an animal, while an equal volume of solution without the drug was applied into the left eye as a control. The measurement of pupillary diameter was made at 25 min intervals with a micrometer by the same operator. In order to compare the effect of the micellar solutions with a standard eyedrops solution containing 2 wt.% of the drug but without F127 was tested in the same manner.

3. Results and discussion

F127 has been chosen for this investigation because of its aggregation behaviour. At low concentrations F127 exists in solution as individual coils (unimers) and thermodynamically stable micelles are formed with increasing copolymer concentration. The transition from unimers to micelles is not sharp and it takes place over a certain concentration range (Alexandridis and Hatton, 1995). Wanka et al. (1994) have studied phase diagrams of Pluronic copolymer and found that F127 has a relatively simple behaviour. In the temperature region, which is potentially interesting for medical application, i.e. up to about 37 °C, the copolymer solution is present as an isotropic phase up to approximately 20% of the copolymer and beyond that concentration the cubic phase is present. The solution with copolymer micelles are expected at more than 10-fold lower concentration of F127, i.e. around the cmc. The cmc is very important if one considers to use micelles or micellar solutions as a drug carrier and/or vehicle. The surface tension studies were performed in order to determine the cmc value of the systems prepared, since the cmc determines thermodynamic stability of the micelles against possible dilution of the drug delivery system on the spot of its application. The appropriate cmc value should be at such a copolymer concentration that the very existence of micelles will not be compromised, and their stability will be sustained. For instance, when a volume of 25 μl of the micellar solution is instilled to the eye, it will encounter lacrimal fluids (about 7 μl) and the dilution by the tear turnover of 0.66 μl min^{-1} (Chien, 1982). It should be added that the strong decrease in the cmc with increasing temperature, which can be more than four orders of magnitude with a temperature increase from 15 to 40 °C (Wanka et al., 1994), can help to sustain the existence of micelles even at high dilutions by the tear fluid, because the temperature in the conjunctivas sac (≈34 °C) is obviously higher than the room temperature. The size of micelles was determined by dynamic light scattering method and the values of the hydrodynamic diameter obtained for F127 in water, F127 in buffer solution, F127 with pilocarpine hydrochloride in water, F127 with pilocarpine hydrochloride in buffer and F127 with pilocarpine base in water are 16.5 ± 0.5 nm, 22.6 ± 0.2 nm, 18.7 ± 0.2 nm, 23.3 ± 0.5 nm, and 30.3 ± 0.3 nm, respectively. These values are in good correspondence with others authors results, e.g. Nagarajan (1999) has calculated the F127 diameter in water to be 21.5 nm, and Rill et al. (1998) have calculated the diameter of 18 nm in water. The micelle sizes depend on the addition of electrolytes into solutions. The micelles in the buffer solution are larger than in water, and also the drug present in micelles increases their size. One might speculate that the observed size increase of the micelles with pilocarpine hydrochloride is a consequence of the incorporation of the drug, at least partially, in the outer shell of hydrated poly(oxyethylene) groups. On the other hand, the size of the micelles with pilocarpine base is enlarged because their core is swollen by the solubilisation/incorporation of the nonionised drug.

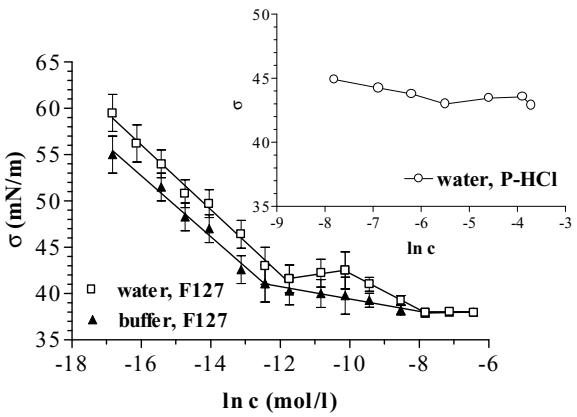

Fig. 1. The isotherm of surface tension in aqueous solution of Pluronic F127 copolymer at 25 °C; F127 solutions in water (pH 6–7) (squares); F127 solutions in buffer (pH 7.4) (triangles). Insert: pilocarpine HCl solution (pH 3.5–4.4) without copolymer at 25 °C (circles). Bars indicate the standard error of the mean.

The surface tension studies of Pluronic F127 in water and in phosphate buffer solutions are given in Fig. 1. Also in the insert, the data for pilocarpine alone is given in the same figure. The cmc was obtained from the intersection of two straight lines and for F127 in water the value was 0.5 wt.% (3.97×10^{-4} mol l^{-1}). The result is in a good correlation with previous results of other investigators, e.g. Alexandridis et al. (1994) have found the value of 0.7 wt.% at 25 °C, but it is quite different from the result by Polat and Chander (1999) who have cited the results of other investigators to be 4.2 or 7.0×10^{-6} mol l^{-1} at 25 °C. It should be noted that the cmc values of Pluronic copolymer show differences when determined by various investigators. Discrepancies were explained by the diversity of methods used for the determination and a rather broad distribution of copolymer species present in a particular sample. The cmc values obtained in water and phosphate buffer (Table 1) are the same. One might expect that the cmc in buffer solutions would yield a lower value than in water. However, it is well known that in similar systems such as non-ionic surfactants the micellar properties are little influenced by electrolyte addition in comparison to ionic surfactants (Florence and Attwood, 1994). The cmc is, as expected, rather low in comparison with the known values for other surface active agents (Florence and Attwood, 1994). Therefore, before mentioned dilution after application should not be a problem since the micelles are present at very low copolymer concentrations.

The general shape of the adsorption isotherm (Fig. 1) is characterised by a slope with two breaks. Three distinct separate regions were observed by other investigators also (Alexandridis and Hatton, 1995; Polat and Chander, 1999; Desai et al., 2001). These findings can be summarised briefly as follows. There is a general agreement that the second break in the curve is due to the formation of micelles, but for the first break at lower copolymer concentrations there is no general consent. Polat and Chander (1999) have suggested that the first break is a consequence of unimer aggregation into dimers and other larger aggregates. Also the formation of monomolecular micelles (consisting of a single triblock copolymer molecule) at lower concentrations of copolymers were suggested (Florence and Attwood, 1994). Yet another explanation is by Alexandridis and Hatton (1995) who have proposed that the first break is due to a change in configuration of the copolymer molecules at the air/water interface.

The results for the copolymer solution in the phosphate buffer are similar with water solution although the surface values are generally lower signifying an increased surface activity of F127 in the presence of an electrolyte and/or at higher pH. However, the

Table 1
Characteristic parameters of micellar solution of Pluronic F127 in water and phosphate buffer at 25 °C

Solution	cmc (wt.%)	cmc $\times 10^4$ (mol l^{-1})	$\Gamma \times 10^7$ (mol m^{-2})	a (nm^2 molecule^{-1})
F127 in water (pH 6–7)	0.50 ± 0.2	4.0	8.5	2.1
F127 in buffer (pH 7.4)	0.51 ± 0.3	4.0	3.5	4.7
F127 in water and pilocarpine HCl (2%) (pH 4.4)	0.25 ± 0.2	2.0	4.9	3.3
F127 in buffer and pilocarpine HCl (2%) (pH 7.4)	0.25 ± 0.2	2.0	2.8	5.9

constant surface tension values above the cmc were observed. Having in mind the ultimate purpose of pilocarpine formulations, i.e. the application to the eye slightly alkaline solutions might be considered beneficial because their bioavailability should be higher than that of slightly acetic or neutral solutions. Additionally, the surface tension data for pilocarpine solutions alone, i.e. without any Pluronic polymer present were measured and the results are shown in Fig. 1 (insert). The obtained surface tension values indicate a certain surface activity of the drug itself, and perhaps formation of small aggregates. In future this interesting phenomenon should be investigated further.

Apart of the cmc determination surface tension experiments provide a second important characteristic parameter, i.e. the slope of the surface tension against logarithm concentration curve (the adsorption isotherm). The slope before cmc, $d\sigma/d\ln c$, can be used in connection with the Gibbs adsorption isotherm for the calculation of the area per molecule, a which a copolymer occupies at the interface:

$$\Gamma = -\frac{1}{RT} \times \left(\frac{d\sigma}{d\ln c}\right), \quad \text{and} \quad a = \frac{1}{\Gamma N_{av}}$$

where Γ is the surface excess concentration of copolymer molecules which are adsorbed at monolayer coverage at the interface and c is the bulk concentration of the molecule. The area calculated gives information on the degree of packing and the orientation of the adsorbed molecule. The calculated values for Γ and a are given in Table 1 together with the characteristic values obtained for solutions with increasing concentration of F127 and a constant concentration of pilocarpine. The area of a single PO group is 0.11 nm^2 (Polat and Chander, 1999). As F127 consists of 65 PO groups it follows that the total packing area is 7.15 nm^2 for the extended molecule. The results in Table 1 show lower values for a indicating that the molecules are not adsorbed with their extended configuration, and only a fraction of PO groups resides at the surface. The calculated areas are higher for the solutions with pilocarpine and in phosphate buffer. It would appear that an addition of electrolytes and/or pilocarpine hydrochloride decreases Γ values and therefore increases the a values.

The surface tension of solutions in the range of the copolymer concentration between 6.2×10^{-4} and 2 wt.% was measured while the drug concentration

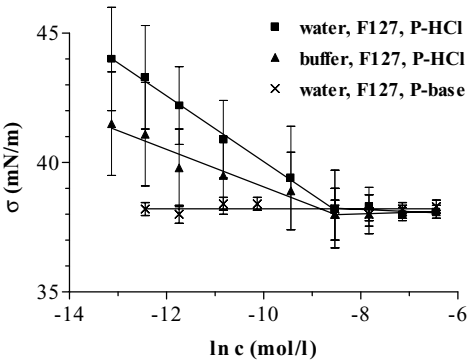

Fig. 2. The isotherm of surface tension in aqueous solutions of Pluronic F127 copolymer and constant pilocarpine amount at 25 °C; F127 in water and pilocarpine HCl (2 wt.%) (pH 4.4) (squares); F127 in buffer and pilocarpine HCl (2 wt.%) (pH 7.4) (triangles); F127 in water and pilocarpine base (1.7 wt.%) (pH 8.5) (crosses).

was kept constant at 2 wt.% for pilocarpine hydrochloride and 1.7 wt.% (equimolar) for pilocarpine base. The results are given in Fig. 2 as well as the calculated values in Table 1. The constant pilocarpine concentration was chosen because it corresponds to the concentration of the usually used in the eye drops formulations. Once again the intersection of two straight lines gives the cmc for a given solution and the surface tension values for buffer solutions are lower than those for the water solution. Saarinen-Savolainen et al. (1996) have studied amphiphilic properties of pilocarpine prodrugs and pilocarpine, and the apparent partition coefficient of pilocarpine between octanol and aqueous phase is 0.0182 and 1.023 for solutions at pH 5 and pH 7.4, respectively. They have found that the surface activity is increased when the lipophilicity of the prodrug is increased because the more lipophilic molecules have higher escaping tendency from the bulk solution to the air–water interface. It appears that a similar reasoning can be accepted for the systems studied herein (Figs. 1 and 2), and therefore the copolymer solutions either with or without the drug are more surface active at a higher pH.

Especially interesting are the data for the pilocarpine base solutions. Although the concentration of F127 was decreased as low as 8×10^{-8} mol l^{-1} a constant value of $\sigma = 38 \pm 2$ mN m^{-1} was obtained with all the concentrations investigated. This value is practically the same as the value achieved for the

cmc. There are two possible explanations either: (i) the pilocarpine base was completely solubilised in the micelles even with the smallest amounts of Pluronic used, or (ii) the surface activity of copolymer in combination with the drug action decreases the measured surface tension without a complete entrapment of the drug in micelles.

The drug entrapment was evaluated by dialysis experiments. The values obtained are rather low and in all cases were $1 \pm 0.9\%$. The sample containing pilocarpine base had the highest value of 1.9%. The entrapment efficiency is low because pilocarpine hydrochloride is ionised and very soluble in water, and even the free base is soluble, therefore only a small proportion of it can be entrapped either in the core or in the hydrophilic shell.

The low entrapment efficiency of a drug was found in similar systems. Muchtar and Benita (1994) have found that pilocarpine hydrochloride is located mainly in the water phase of an oil-in-water submicron emulsion intended for ocular administration. Also, Saettone et al. (1996) have shown that the entrapment efficiency of cyclopentolate hydrochloride in non-ionic surfactant vesicles used as ophthalmic carriers is negligible. However, in both the cases, the systems studied showed some evident advantages when compared to simple drug solutions.

Perhaps a remark might be in order: with an eye on the possible application of micellar solution studied we have tried to maintain the systems as simple as possible, and without any usual but not necessarily needed ingredients. On the other hand, the systems were still rather complicated from a physico-chemical standpoint. In the future work, it would be interesting to study surface tension activity of mixed surface active components such as pilocarpine and F127 further.

The results of the in vivo evaluation of micellar solutions of Pluronic F127 with pilocarpine are shown in Fig. 3 and Table 2. The mean change in pupillary diameters with time for two micellar solutions both with 2 wt.% of the copolymer but consisting of either the hydrochloride salt (2 wt.%) or the pilocarpine base (1.7 wt.%) were compared with the pilocarpine hydrochloride (2 wt.%) solution in water only. The general pattern of the curves obtained is similar in the following parameters: the time to reach the peak miotic response, t_{max}, which is ~25 min, and the maximal miotic response, $\Delta_{max} \approx 4$ mm. Two characteristics parameters are different for the three solutions: i.e. t_{mr} is the duration of miotic response (the time interval needed for the pupil diameter to return to its normal pre-treatment value), and AUC, the area under the temporal miotic response curve. A certain improvement is noticed with the micellar solution of pilocarpine hydrochloride in comparison with the solution without the copolymer. The t_{mr} value is prolonged (to

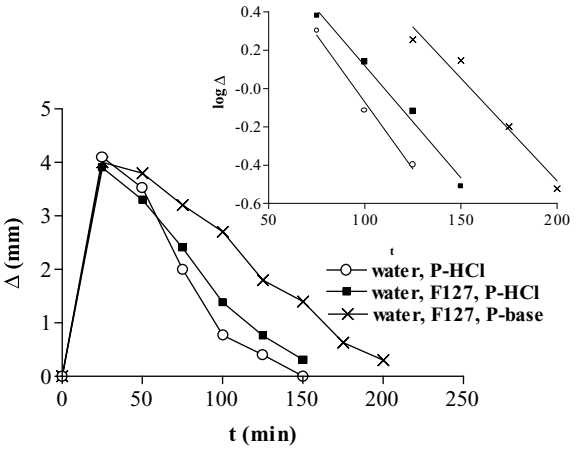

Fig. 3. The miotic response in rabbits of solutions with Pluronic F127 and pilocarpine; pilocarpine HCl (2 wt.%) solution (circles); F127 (2 wt.%) and pilocarpine HCl (2 wt.%) solution (squares); F127 (2 wt.%) and pilocarpine base (1.7 wt.%) solution (crosses).

Table 2
Pharmacokinetic parameters for the miotic response of pilocarpine solutions with Pluronic F127

Solution	t_{max} (min)	t_{mr} (min)	AUC (mm × min)	$k_{el,app}$[a] (min^{-1})
Pilocarpine HCl (2%)	~25	150	270	0.031
Pluronic F127 (2%) + pilocarpine HCl (2%)	~25	180	297 (10.2%)[b]	0.027
Pluronic F127 (2%) + pilocarpine base (1.7%)	~25	225	442 (64%)[b]	0.025

[a] Approximate values calculated from the slope $\ln \Delta/\Delta t$ for the terminal points of Δ vs. t curves (Fig. 3, insert).
[b] Percent increase in comparison with standard pilocarpine hydrochloride solution.

about 180 min), but the AUC is only slightly increased (from 270 to 297 mm × min). From a pharmacokinetic standpoint, it can be concluded that an increase of only 10.2% in the AUC is hardly important.

However, the more significant aspect of the results is evident for the micellar solution with the pilocarpine base: t_{mr} value was prolonged up to about 250 min and the AUC was 442 mm × min, i.e. an increase of 64%. As the AUC is proportional to the concentration of drug reaching the target receptor site (Desai and Blanchard, 1998) it can be concluded that the results presented here show a better bioavailability of the drug incorporated in micellar solutions. Also, it has been stated that the absorbed pilocarpine level in the aqueous humour of the eye following topical dosing in rabbit eye declines in a mono-exponential pattern with an elimination rate constant equal to 0.017 min^{-1} (Chien, 1982). With a robust approximation that the terminal segments of the miotic response curves shown here represent the elimination phase of the drug from the site of action the logarithm versus time plots for the terminal points is shown in Fig. 3 (insert). Three parallel straight lines were obtained ($r \geq 0.982$) and apparent elimination rate constants were calculated from the slopes (Table 2). Although the values are different from aforementioned it would appear that the rank order correlation was obtained.

For the sake of argument it should be mentioned that the reason for the higher t_{mr} and AUC values obtained with the micellar solutions is not clear. The entrapment efficiency is rather low (maximum 1.9%) and the drug is partitioned in water mainly due to its physico-chemical properties. Analogous results were described by other authors for different but similar systems, e.g. only 5.9% of pilocarpine was found in the oil phase of submicron emulsions (Muchtar and Benita, 1994) or negligible entrapment of cyclopentolate hydrochloride in non-ionic surfactant vesicle (Saettone et al., 1996), but still their formulations showed noticeable improvements in drug bioavailability when compared to the ordinary solutions. Saettone et al. (1996) have cautiously suggested a hypothesis that the permeability of the absorbing ocular membranes might be influenced by delivery system itself. Without any further evidence for the triblock copolymers investigated herein we may speculate along these lines, though at present there is no other information in support.

As the final conclusion it can be stated that micellar solutions prepared by the simplest method (dissolution of F127 and the drug) can offer some advantages over a standard solutions of pilocarpine and that further and additional studies of them were warranted.

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