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Physostigmine emulsion: a new injectable controlled release delivery system

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Summary

Physostigmine salicylate was incorporated in a soybean injectable emulsion in an attempt to control the drug release from the emulsion. In vitro examination of the physicochemical properties of the emulsions revealed that a mixture of an anionic (phospholipid) and non-ionic surfactant (Pluronic) was required to prepare stable physostigmine emulsions.

Variation in concentration of either the anionic or non-ionic emulsifier decreased the mean droplet size while the zeta potential did not show any clear correlation with increasing phospholipid concentration but decreased, reached a plateau and fell again with increasing Pluronic concentration. In the presence of physostigmine salicylate, instability was introduced which changed the profile of zeta potential dependency on phospholipids and Pluronic concentrations. Zeta potential increased with physostigmine concentration as a result of increasing ionization of various polar groups at the oil–water droplet interface. Moreover, physostigmine incorporated in the emulsion was protected from its aqueous decomposition and remained intact. Stable physostigmine emulsions preserved over long periods of time were obtained using appropriate experimental conditions probably as a result of optimization of effects due to surface potential increase, droplet size reduction and formation of a resistant close-packed interfacial mixed film, which confers steric stabilization to the dispersed droplets.

Introduction

In the last decade, there has been renewed interest in the emulsion as a vehicle for delivering drugs to the body (Kakemi et al., 1972; Noguchi et al., 1975; Attwood and Florence, 1983; Nakamoto et al., 1975). Incorporation of various active ingredients in emulsions was shown to improve their biological availability following oral administration (Daeschner et al., 1957; Carrigan and Bates, 1973). However, problems associated with the oral administration of emulsions such as the low pH and high ionic strength of stomach environment and their deleterious effects on emulsion stability, limited the therapeutic application of these interesting dosage forms. The parenteral route of administration appeared much less harmful to emulsion stability and has been explored for prolonging the effect of drugs with short biological half-lives, poor oral bioavailability, or having very low aqueous solubility.

The pharmacological activity of barbiturates was shown to be prolonged by incorporating these drugs in a soybean oil emulsion administrered intravenously (Ljungberg and Jeppsson, 1970; Jeppsson and Ljungberg, 1973).

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Physostigmine, an anticholinesterase which readily enters the brain, has been shown to reverse the effects of various classes of drugs which act in different ways to reduce cholinergic transmission. These include tricyclic anti-depressants (Aquilonius and Hedstrand, 1978), ketamine (Balmer and Wyte, 1977), anticholinergics (Holzgraff et al., 1973), and narcotic analgesic drugs (Weinstock et al., 1980).

The objective of our investigation was to design a controlled injectable emulsion delivery system of physostigmine which would prolong and improve the therapeutic efficacy while reducing significantly the side-effects which are due to marked fluctuations of blood levels after repeated parenteral administration.

Preliminary encouraging in vivo results using this delivery system in rabbits have been presented recently (Friedman et al., 1985).

The present study is an in vitro examination of the physicochemical properties of the physostigmine emulsions.

Materials and Methods

Materials

Physostigmine salicylate, cottonseed and soybean oils, crude phospholipids, mannitol were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Polyoxyethylenepolyoxypropylene emulsifier (Pluronic F68) was obtained from BASF (Ludwigshafen, F.R.G.). The phospholipids were purified according to the method reported by Schuberth and Wrethind (1961) prior to use. This complex emulsifier mixture is composed mainly of phosphatidylcholine and of some anionic phospholipids as determined using standard TLC procedures (Christie, 1973).

Methods

Emulsion preparation

The purified phospholipids and physostigmine were dissolved in the oil phase. The non-ionic emulsifier (Pluronic F68) and mannitol were dissolved in the aqueous phase. Both phases were heated separately to 70°C and dispersed by a magnetic stirrer. Further heating was then applied on the coarse emulsion which was emulsified using a high-speed mixer (Ultraturrax) for 1 min at a given temperature. The resulting fine emulsion was cooled rapidly below 20°C. A typical formulation (% w/w) consisted of drug q.s. vegetable oils 20.0, purified phospholipids 1.0, Pluronic F68 2.0, mannitol 6.0 and double-distilled water to 100 g.

The effect of the emulsification temperature, the concentration of physostigmine, the surfactants and the nature of the vegetable oil on the physicochemical properties of the emulsions formed were examined. Each emulsion batch was prepared in triplicate.

Emulsion evaluations

Particle size analysis. The mean droplet size of most of the emulsions prepared was found to lie between 300 to 1000 nm and was determined by means of a laser light scattering apparatus (Coulter Nanosizer). This was similar to that used by Burnham et al. (1983) to determine the mean droplet size of fat emulsions and is widely used to characterize the mean particles of various liposome formulations (Benita et al., 1984; Henry-Michelland et al., 1983). Each emulsion sample was analyzed twice and for each diluted system three size determinations were made. The data were expressed as a mean diameter and polydispersity factor that defines the deviation of the system from a monodispersed distribution. According to the manufacturer's instructions, a polydispersity factor value of 0–1 indicates a monodispersed distribution while values ranging from 3 to 5 indicate a range distribution by diameter of 2 : 1 in the emulsion samples.

Electrophoretic mobility. The charge on emulsion droplets was measured using the moving boundary electrophoresis technique which has been shown to yield accurate electrophoretic mobility data (Shaw, 1969). The electrophoresis cell was developed with modifications, from an earlier technique (Ottewil and Shaw, 1967). Appropriate experimental conditions were found which yielded initially sharp boundaries without further disturbances. The electrolyte consisted of an aqueous solution containing 3% mannitol and 0.5% Pluronic F68 which helped to stabilize the boundary without

altering the electrophoretic mobility. Emulsions were diluted with distilled water (1 : 10) prior to examination. The electrophoretic mobility was measured at 20°C and converted to zeta potential using the Helmholtz-Smoluchowski equation:

$$Z_p = \frac{u \times 4n}{E}$$

where Z_p is the zeta potential, u is the electrophoretic mobility, n is the viscosity and E is the dielectric constant. Viscosity measurement of the diluent were made with an Ostwald Capillary Viscosometer according to the B.P. method. Similar treatments were reported by various authors (Kawilarang et al., 1980; Dawes and Groves, 1978). The zeta potential values presented in this study are the mean of triplicate determinations for each emulsion batch.

Zeta potentials of various marketed intralipid fat emulsions measured and calculated by this technique have been found to be identical, within the limits of experimental error, to corresponding zeta potential values published in the literature, confirming the reliability of the method used.

Stability studies. Long-term stability studies were conducted at various temperatures, 4, 20 and 37°C. The chemical and physical changes that might occur in the emulsion during the storage were followed up by visual observations (phase separation, creaming, etc.).

The emulsion samples were stored in 10 ml, stoppered, graduated measuring cylinders and the degrees of creaming and of separation of the oily phase were assessed at given time intervals.

Results and Discussion

Influence of increasing emulsification temperature

There is no doubt as reported by various authors (for example, Boyd et al., 1972) that emulsification temperature is an important factor with regard to the particle size distribution of the emulsified droplet and consequently the emulsion stability. Temperature would influence the interaction between the aqueous phase and the hydrophilic groups of the emulsifiers and between the oil phase and the lipophilic groups. On the other hand, it would be wrong to consider the oil phase as an inert carrier or even simply as a reservoir. Recent work has shown that the oil phase exerts some biological effects on the availability of the drugs (Yamahira et al., 1979; Palin et al., 1980). The nature of the oil phase would also alter the properties of the emulsions formed as can be seen from Fig. 1 where the effect of the temperature on the mean droplet size for two oils are reported. The minimum droplet size was reached with soybean oil at 85°C and with cottonseed oil at 90°C emulsification temperature, whereas particle size decreased to 442 nm and 799 nm, respectively. Mean droplet size, first decreased and then increased with increasing temperature (Fig. 1) probably as a result of hydration and dehydration, respectively, of the non-ionic surfactant molecules which alter their adsorption at the oil–water interface and the interaction between the non-ionic

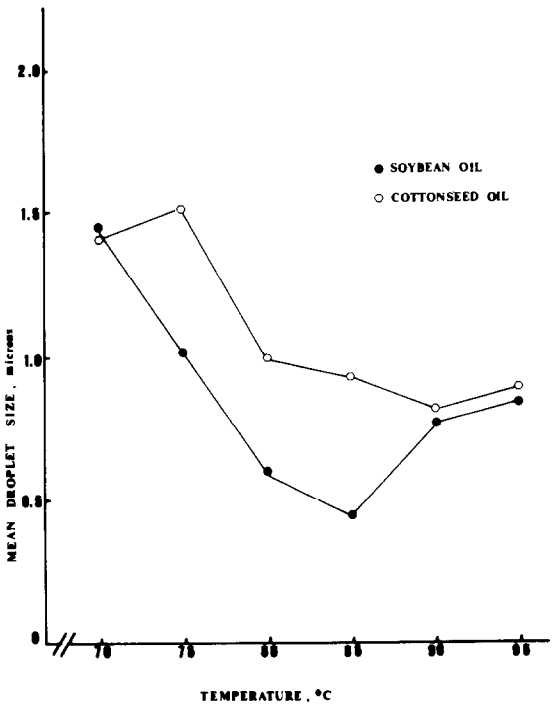

Fig. 1. Effect of increasing emulsification temperature on mean droplet size of the emulsion (polydispersity index values ranging from 3 to 5; formulation: oily phase 20.0 g, purified phospholipids 1.0, Pluronic F68 2.0, mannitol 6.0 and water to 100 g).

and anionic emulsifier molecules. It is evident that the optimum emulsion formed when the surfactant ratios were such that the droplet sizes were at a minimum (Depraetere et al., 1980). Therefore, two optimum emulsification temperatures were yielded as a function of the nature of the oil phase where the best close-packed mixed interfacial film of both emulsifiers was formed at the oil–water interface. No difference in the composition of the interfacial film between the phospholipid and Pluronic was detected since the zeta potential of the emulsions at the various temperatures remained practically constant. In any case, the phase inversion (Shinoda and Sarto, 1969) was not observed even at the highest temperature since the specific conductivity of the emulsions increased with increasing temperature. The lack of reduction in the specific conductivity of the emulsion monitored according to the method of Enever (1976) confirmed that no phase inversion had occurred.

Effect of purified phospholipids concentration

Mean droplet size of the emulsion vehicle decreased with increasing phospholipids concentration, but zeta potential did not show any clear tendency, rather an initial decrease at early low concentration of phospholipids occurred (Fig. 2). The initial fall in the surface potential on the emulsion droplets with increasing concentration of phospholipids should be attributed to a decrease in the ionization of the ionic surfactant molecular groups at the oil–water interface of the dispersed droplets. However, the surface of the emulsion droplets remained sufficiently charged to repel other similarly charged emulsion droplets when they come close together.

It should be noted that the resulting zeta potential value was high enough (around 60 mV) to prevent coalescence of the droplets and preserved the stability of the emulsion formed. Mean droplet size declined progressively with increasing phospholipid concentration reflecting the formation of better close-packed mixed film of both emulsifying agents (phospholipids and Pluronic F68) at the oil–water interface of the emulsified droplets. This interfacial film acted as a stabilizer at the earlier stage of the emulsification process by forming a high energy barrier which caused repulsion of

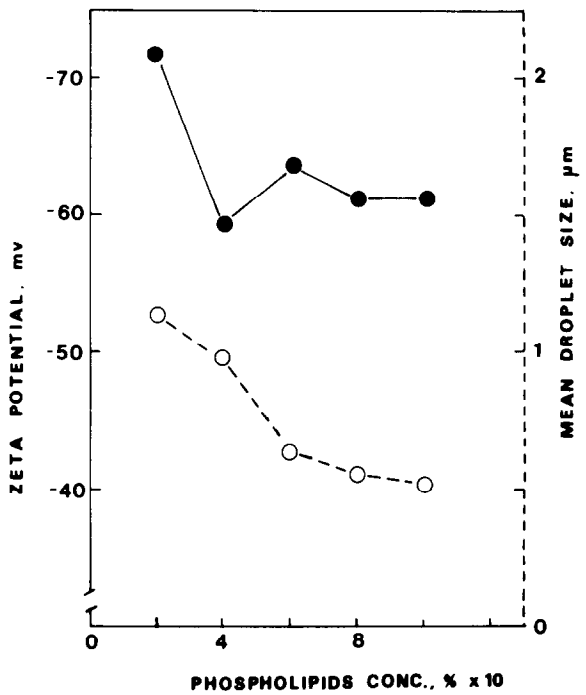

Fig. 2. Effect of phospholipid concentration on zeta potential (●) and mean droplet size (○) of the emulsion vehicle (formulation: soybean oil phase 20.0 g, Pluronic F68 2.0, mannitol 6.0 and water 100 g).

adjacent droplets and led to the formation of stabilized emulsified droplets of progressively decreasing size.

However, in the presence of physostigmine salicylate, some instability was introduced and changed the profile of zeta potential dependency on phospholipids concentration (Fig. 3).

At least 0.8% of phospholipids were required to maintain adequate values of zeta potential and stability.

These results indicated that physostigmine salicylate interfered at the oil–water interface and influenced zeta potential.

Physostigmine salicylate presumably interacted with the complex mixed emulsifier changing the degree of ionization of the polar groups at the oil–water interface without altering significantly the nature of the interfacial films since mean droplet size remained practically constant.

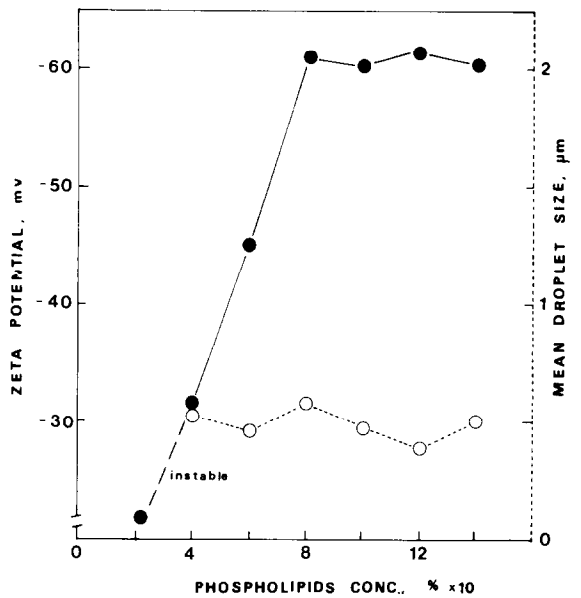

Fig. 3. Effect of phospholipid concentration on zeta potential (●) and mean droplet size (○) of physostigmine emulsion (soybean oil phase 20.0 g, Pluronic F68 2.0, mannitol 6.0, physostigmine salicylate 0.1 and water to 100 g).

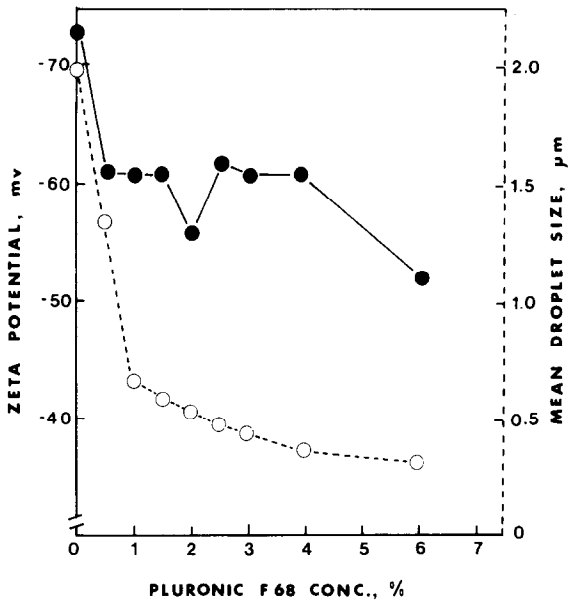

Fig. 4. Effect of Pluronic F68 concentration on zeta potential (●) and mean droplet size (○) of the emulsion vehicle (soybean oil phase 20.0 g, phospholipids 1.0, mannitol 6.0 and water to 100 g).

Effect of non-ionic emulsifier concentration

It is interesting to note that the variation of the non-ionic Pluronic F68 emulsifier concentration affected the zeta potential of the emulsion vehicle as shown in Fig. 4. Zeta potential values decreased, reached a plateau and fell again with increasing Pluronic F68 concentration.

The initial fall could be explained by a change in the interfacial layer composition which contained fewer ionic polar heads and reached an equilibrium at 0.5% regardless of the bulk concentration of the non-ionic surfactant.

The final reduction in zeta potential was probably due to a multilayer formation of surfactants around the droplets which could be attributed to an increase in the hydrophilicity of the surfactant mixtures (Tadros, 1976).

It could be also observed from Fig. 4 that mean droplet size decreased moderately but significantly at the beginning with increasing Pluronic F68 concentration.

Again the incorporation of physostigmine salicylate changed the influence of Pluronic F68 concentration on zeta potential. As shown in Fig. 5 the plateau disappeared reflecting the prevention of the equilibrium as a result of the molecular interactions between the drug and the surfactant mixtures at the oil–water interface of the emulsified droplets.

Effect of physostigmine concentration

Physostigmine salicylate increased the zeta potential (Fig. 6). Before going into an explanation of this parameter, it should be emphasized that values of the zeta potential presented in Fig. 6 differed from values reported earlier, due to utilization of different batches of purified phospholipids. In the present case, the ratio of ionic-to-neutral phospholipids was higher than in the previous experiments producing higher zeta potential values under identical experimental conditions. Nevertheless, similar behaviour was observed but the plateau was reached at a different zeta potential value, always above 60 mV.

The difference in the batch composition of purified phospholipids arises from the difference

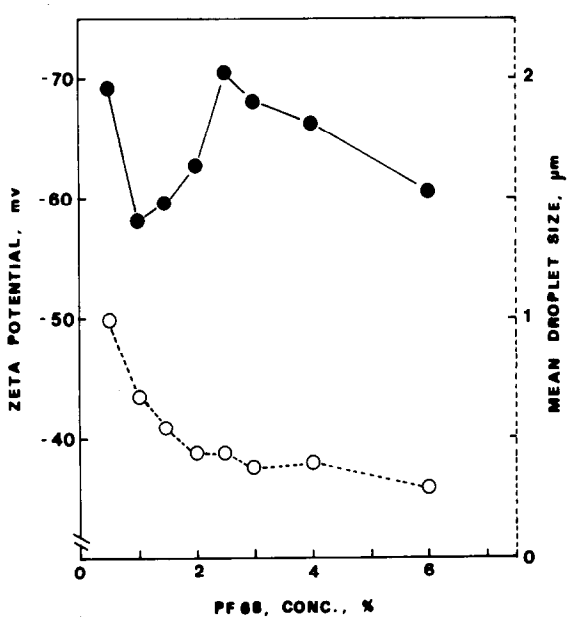

Fig. 5. Effect of Pluronic F68 concentration on zeta potential (●) and mean droplet size (○) of physostigmine emulsion (soybean oil phase 20.0 g, phospholipids 1.0, mannitol 6.0, physostigmine salicylate 0.1 and water to 100 g).

between egg yolk sources. Such batch variation experiments were carried out to ascertain the reproducibility of the process and end product as a function of crude egg yolk of various sources.

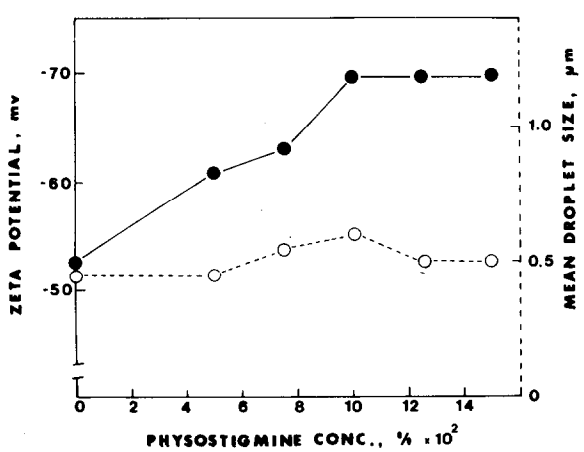

Fig. 6. Effect of physostigmine salicylate concentration on zeta potential (●) and mean droplet size (○) of the emulsion (formulation: soybean oil phase 20.0 g, purified phospholipid fraction 1.0, mannitol 6.0 and water to 100 g).

The increase in zeta potential values with increasing physostigmine salicylate concentration can be attributed to an increase in the ionization of various polar groups including the active ingredient at the interface.

The reasons for the increase in zeta potential are not completely clear, but may be due to any or all of the following factors. By incorporating physostigmine at the interface, molecules of nonionic emulsifier are repulsed thereby increasing electrical charge on the droplets. Molecular interactions between physostigmine salicylate with one or more of the ionic emulsifiers which increased total ionization on the interface is also a possibility. A change in the microenvironment induced by physostigmine which affects the ionic strength and thereby the ionization of the polar groups should also be taken into consideration.

In any case, most of the physostigmine appears to be localized at the interface in spite of its high aqueous solubility. This was confirmed by the influence of physostigmine on the interfacial tension between oily and aqueous phases identical to those of the emulsion but without the emulsifiers.

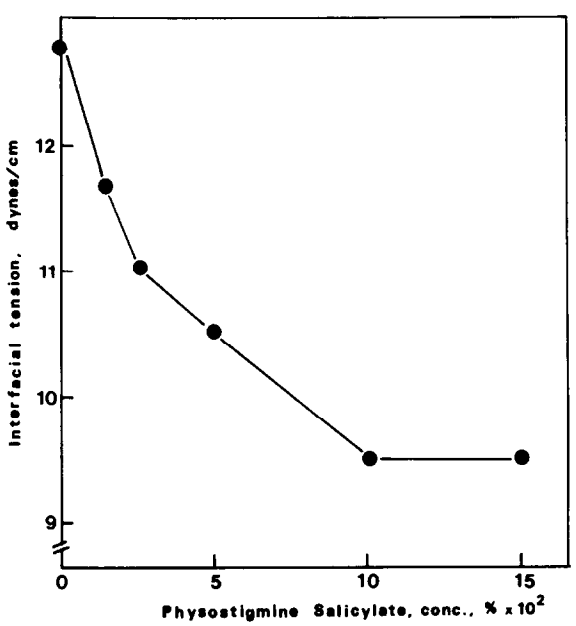

Fig. 7. Effect of physostigmine salicylate concentration on interfacial tension between the oily and aqueous phases of the emulsion without the emusifiers.

Physostigmine salicylate reduced the interfacial tension as shown in Fig. 7 proving that the drug tends to migrate to the interface.

Physostigmine decomposes rapidly by hydrolysis and oxidation. Hydrolysis is catalyzed in the presence of proton and hydroxide. Physostigmine is most stable at pH 3.3 while at pH 5.5 (pH of the emulsions) and above it decomposes rapidly resulting in a red solution which indicates the presence of rubreserine which is pharmacologically inactive (Rogers and Smith, 1973).

It should be emphasized that physostigmine salicylate incorporated in the emulsion was protected from the aqueous decomposition and remained remarkably stable. The sensitive methylcarbamate side-chain was probably localized in the external layer of the oily phase preventing direct contact with the aqueous phase. These findings explain the ability of physostigmine salicylate to change the zeta potential.

Stability studies

The shelf-life of the emulsions as a function of the Pluronic F68 concentration at room temperature is shown in Fig. 8. Shelf-life was defined as the minimal period of time for appearance of tiny oily droplets in the emulsion indicating phase separation. For comparison purposes, the emulsions which are still stable, even after 6 months storage, are also presented in Fig. 8. It was observed that for identical unstable formulations shelf-lives of the emulsion vehicles were always longer than the corresponding shelf-lives of the drug emulsions. These findings reflected the influence of physostigmine on the zeta potential and mean droplet size of the various emulsions. At least 2% Pluronic was needed to maintain stability over a period longer than 6 months.

The results shown in Figs. 4, 5 and 8 indicate that emulsion stability was not dependent on the magnitude of the zeta potential or mean droplet size only but rather on a combined effect of the latter factors associated with the formation of thick well solvated adsorbed surfactant layers. The latter can be attributed to the presence of Pluronic molecules at the oil–water interface.

The adsorbed layers on the droplets, evidently act by forming a high-energy barrier with the

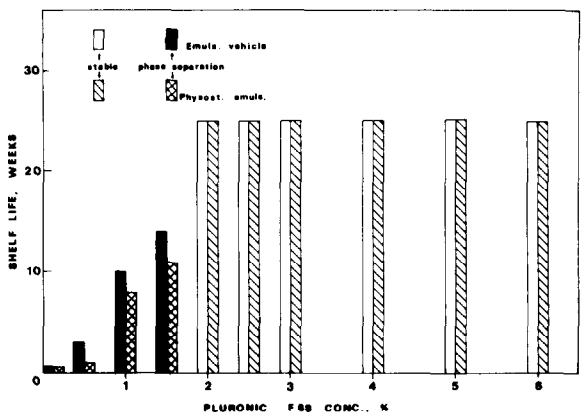

Fig. 8. Effect of Pluronic F68 concentration on the stability of the vehicle and physostigmine emulsions.

macromolecule chains oriented toward the continuous phase, an arrangement leading to steric stabilization by repulsion of surfactant chains adsorbed on adjacent dispersed droplets as they approach each other.

In the present case it appeared, therefore, that the following coincident conditions should be maintained to guarantee prolonged stability. The zeta potential values should range above 60 mV, mean droplet size below 500 nm and a coherent interfacial complex film which prevents coalescence by steric hindrance should be formed on the dispersed droplets.

We did not find any correlation between creaming rate and phase separation which could be detected by the visual appearance of oily droplets.

Storage temperature did not affect significantly the emulsion stability. Identical results were obtained at 4°C while storage at 37°C yielded reduced shelf-lives in the unstable formulations but did not alter the prolonged stability of the optimal emulsions.

Similar stability behaviour of the emulsion was also observed as a function of the phospholipid concentration.

Conclusion

Stable emulsions containing physostigmine salicylate were obtained. It appeared therefore,

that at optimal experimental conditions the purified phospholipids formed a close-packed interfacial film with the Pluronic F68 molecules at the oil–water interface of the dispersed droplets and this acted as a mechanical barrier which stabilized the emulsion. Droplet coalescence was also prevented by mutual electrostatic repulsion due to a net negative charge that resided on the droplets. No changes were observed in these emulsions with regard to particle size or zeta potential after 6 months storage at different temperatures. Since the oily phase did not exceed 20% of the total volume these emulsions can be injected using different parenteral routes without being limited by their viscosity. They were well tolerated by the rabbits used in preliminary pharmacological experiments.

Acknowledgements

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