International Journal of Pharmaceutics, 65 (1990) 169–175
Elsevier

IJP 02181

Enhanced stability of physostigmine salicylate in submicron o/w emulsion

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(Received 1 March 1990)
(Accepted 20 April 1990)

Key words: Physostigmine salicylate; Stability; Submicronized emulsion; Phospholipid; Poloxamer

Summary

Submicronized emulsions of physostigmine salicylate (PS) with high batch to batch uniformity were prepared using phospholipids and poloxamer as a complex emulgator. The mean droplet size of the various emulsions was distributed around the value of 150 nm and no single droplet was larger than 400 nm. The chemical stability of PS was strongly affected by the adjusted pH. In accelerated tests, it was found that PS was most stable in the emulsion at pH 5.0 while rapid degradation was observed in aqueous solutions at the same pH regardless of the storage temperatures. In view of the overall results, it was deduced that despite its high aqueous solubility, PS appeared to be localized at the oil-water interface of the dispersed oil droplets of the emulsion. The observed protective effect displayed by the emulsion was partly due to the interaction of physostigmine with phospholipids at the oil-water interface and partly due to physostigmine dissolution in the internal phase of the emulsion.

Introduction

Improvement of memory functions in Alzheimer's disease patients by oral physostigmine treatment has been reported (Thal and Fuld, 1983; Beller et al., 1985). However, apart from its potential toxicity, adverse side effects, short half-life, and narrow effective dose range, oral therapeutic application of physostigmine is markedly limited by the low bioavailability due to an extended first-pass effect (Brufani et al., 1987).

Incorporation of drugs in emulsions was previously shown to accelerate the rate of absorption which was more complete following oral administration (Carrigan and Bates, 1973). Stable medicated emulsions were more recently shown to improve biological drug availability (Palin et al., 1986; Kimura et al., 1989). It was recently found that a fine micronized physostigmine emulsion, when given orally, although reducing the peak inhibition level of cholinesterase activity, and extending the therapeutic occupancy time in rabbits, did not markedly improve the bioavailability of physostigmine as compared with tablets (Benita et al., 1989). It was postulated that in order to improve significantly the bioavailability of the emulsion relative to the tablet, reduction in the droplet size is needed to increase the surface area of the oil-water interface at which pancreatic lipase hydrolyses the triglycerides resulting probably in better lymphatic uptake of physostigmine.

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Physostigmine is known to undergo rapid degradation in aqueous medium through hydrolysis and oxidation leading to decomposition products identified as eseroline and rubeserine (Rogers and Smith, 1973). The stability of physostigmine in solution has been studied by various authors (Christenson, 1969; Yang and Wilken, 1988a). They found adequate conditions to ensure long-term stability of PS in aqueous solutions but at pH 3.5. They reported that PS did not maintain its chemical integrity at pH 5.0, which is considered the optimal pH of the emulsion to ensure prolonged physical stability of the emulsion formulation, as will be seen later. Therefore, the chemical stability of PS in the emulsion should be examined.

The objective of the present work was the development of a PS submicronized emulsion, its physico-chemical characterization, and evaluation of the stability of physostigmine.

Materials and Methods

Materials

Physostigmine salicylate (PS) and crude phospholipids were purchased from Sigma (St. Louis, MO, U.S.A.). Purified soybean oil was obtained from Bertin (Courbevoie, France). Polyoxyethylene-polyoxypropylene emulsifier, poloxamer (Pluronic® F-68) was purchased from BASF (Ludwigshafen, F.R.G.). Methyl and butyl parabens, glycine, glycerol and the remaining ingredients were of pharmaceutical grade. The crude phospholipids were purified according to procedures described in previous works (Benita et al., 1986b; Friedman et al., 1989).

Methods

Preparation of the submicronized emulsion

The oil phase consisted of winterized soybean oil containing α-tocopherol (as an antioxidant), a stabilizing additive (oleic acid), purified phospholipids and physostigmine salicylate. The aqueous phase comprised the non-ionic poloxamer emulsifier, glycine, glycerol, methyl and butyl parabens, ascorbic acid (as an antioxidant), all dissolved in distilled water. Both phases were heated separately to 50 °C, then mixed using a magnetic stirrer, and further heated to 60 °C. Micronization of the emulsion was achieved using a high-speed Polytron homogenizer (Kinematica®, Switzerland). The resultant emulsion was then rapidly cooled in an ice bath. A fine monodispersed submicronized emulsion was achieved by passing the cooled emulsion through a two-stage homogenizing valve assembly (Gaulin® Homogenizer, APV, Hilversum, The Netherlands). CO_2 was bubbled through the emulsion during preparation in order to expel the dissolved oxygen and prevent PS degradation. The emulsion was filtered through a Millipore® filter (1.00 μm) to remove coarse globules and debris formed during the emulsification and homogenization processes. Finally, the pH of the emulsion was adjusted to the desired value (3.0, 5.0 and 7.0). The influence of the pH on either the physicochemical stability of the drug or the emulsion was further investigated. The various formulations were filled into 15 ml vials under a CO_2 atmosphere and sealed hermetically.

A typical formulation consisted of 0.1 g PS, 20.0 g oily phase (70% soybean oil and 30% oleic acid), 1.2 g purified phospholipids, 2.0 g poloxamer, 4.2 g glycine, 2.25 g glycerol, methyl and butyl parabens (0.2 and 0.075 g, respectively), 0.1 g ascorbic acid, 0.02 g α-tocopherol and double distilled water to 100.0 g.

The ingredients of this formulation were selected on the basis of preformulation studies and evaluation of the stability of PS in various oil and aqueous phase compositions that were kept at 50 °C.

Emulsion characterization

Particle size analysis The mean and droplet size distribution was measured with a computerized laser light scattering apparatus (Malvern® System 4700). Each emulsion sample was diluted appropriately before the measurement. The analysis of each sample was repeated twice and 10 measurements were performed for each dilution.

Electrophoretic mobility The charge on the emulsion droplets was measured using the moving

boundary electrophoresis technique which has been shown to yield accurate electrophoretic mobility data in a previous investigation of emulsions (Benita et al., 1986a).

Visual observations Possible creaming and phase separation were assessed visually at predetermined time intervals.

PS content The PS content of the emulsion was analysed using an HPLC system equipped with a UV detector (Tracor 97 A UV) at 254 nm according to the method reported by Whelpton (1983). The HPLC system comprised a 250 × 4.6 mm Econosil® 5 μm column (Alltech, U.S.A.), a Du Pont pump, a Waters WISP 710 B auto-injector and a Spectraphysics 4100 computing integrator. The mobile phase consisted of 9 parts methanol (HPLC grade) and 1 part 1 M ammonium nitrate solution in double-distilled water at pH 8.6. The flow rate was 1 ml/min. The calibration curves were constructed using freshly prepared samples by injecting standard solutions containing 10–300 ng of PS in 10 μl methanol. For calculation purposes, the linear fraction of the curve from 20 to 140 ng per 10 μl was used. The analysis procedure included dissolution of the emulsion samples in absolute methanol to suitable dilution followed by injection into a 20 μl loop.

Owing to the complexity of the submicronized emulsion formulation, it was not possible to adapt an alternative HPLC method (Yang and Wilken, 1988b) able to estimate quantitatively physostigmine, the decomposition products and other components of the preparation. The present HPLC method was able to detect accurately, with sufficient sensitivity, the levels of intact physostigmine at the various pH values and temperatures. No overlapping or interference with peaks yielded by the other decomposition products or excipients of the emulsion was noted.

Stability studies

Selected on the basis of stability in adjusted pH, the emulsions were stored at 4, 25, 37 and 50 °C in hermetically sealed 15 ml vials. The influence of temperature on the stability of emulsions and the chemical integrity of physostigmine was evaluated at predetermined time intervals.

Results and Discussion

The high reproducibility achieved in the physico-chemical properties of three different batches of the PS submicronized emulsion indicated that the manufacturing process was controlled. The mean droplet size of the various emulsions was distributed around the value of 150 nm and no single droplet was larger than 400 nm (Fig 1).

Since the emulsion is intended to be administered orally, the question of its stability at gastrointestinal physiological pH values was addressed. The effect of the adjusted pH on the zeta potential of the emulsion is presented in Fig. 2. A significant increase in the zeta potential was observed when the pH was raised from 3.0 to 7.0. This behavior was also observed in previous studies (Benita et al., 1986a; Friedman et al., 1989). The elevation in the zeta potential is assumed to be a result of the increased ionization of the negatively charged phosphatidylserine and phosphatidylethanolamine which were present in the phospholipid mixture of the present emulsion formulation. The variations in pH altered neither the mean droplet size nor distribution profiles of the emulsions (Fig. 2). However, the chemical stability of PS was markedly affected by the adjusted pH. Using accelerated tests, it was found that PS was most stable at pH 5.0, but rapidly degraded at pH

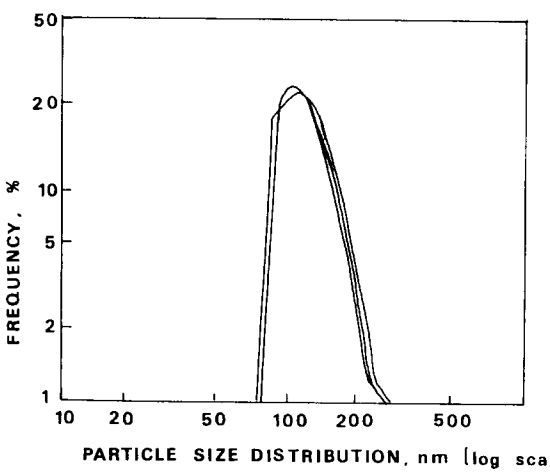

Fig. 1. Particle size-frequency plot of three emulsion batches prepared using identical manufacturing conditions.

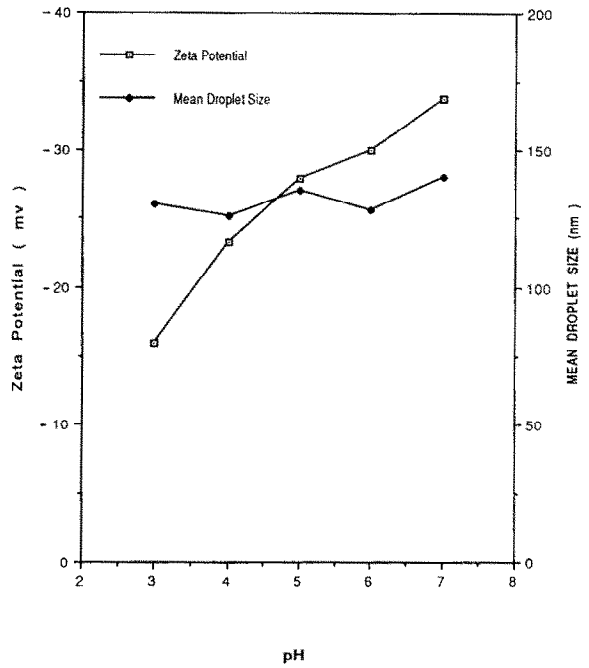

Fig. 2. Effect of initial adjusted pH on zeta potential and mean droplet size of the PS emulsion.

3.0 and 7.0, regardless of the storage temperatures (Fig. 3). As expected, the degradation of PS increased with rise in the storage temperature. At

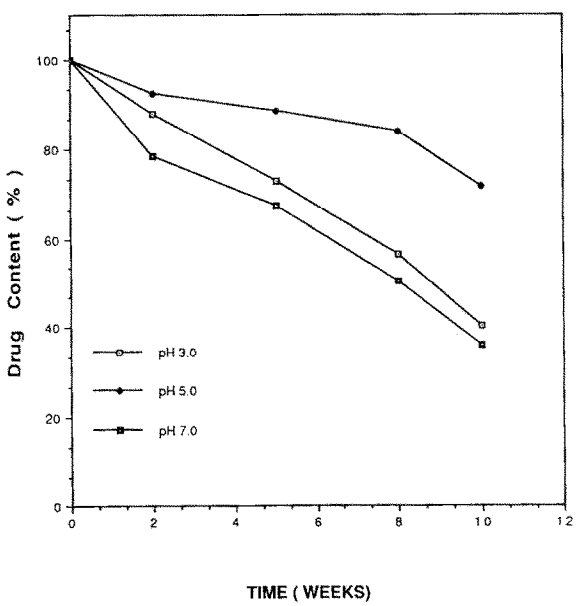

Fig. 3. Chemical stability of PS as a function of pH in an emulsion stored at 50 °C.

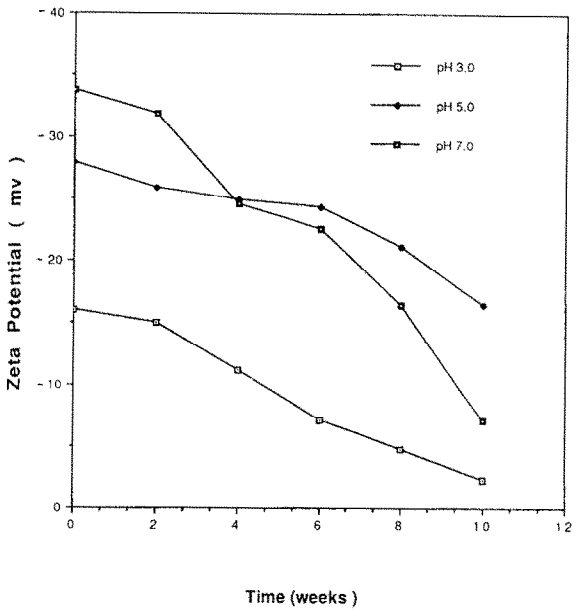

Fig. 4. Effect of pH on zeta potential of a physostigmine emulsion stored at 50 °C.

50 °C more than 10 weeks were required to degrade less than 30% of the initial PS concentration in the emulsion, whereas at pH 5.0 the PS aqueous solution totally decomposed within 1 week. Furthermore, no degradation of PS was observed in the PS emulsions stored at 4 °C over 18 months. These results indicated that the incorporation of PS into the internal oily phase of emulsion dosage form increased the stability and prolonged the shelf-life of this sensitive drug. The physical stability of the emulsion was also found to be temperature dependent: the zeta potential decreased with increasing temperature (Fig. 4). This can be attributed to the degradation of the oily components which formed free fatty acids that reduced the pH of emulsions following storage at elevated temperatures, in agreement with data reported previously by other authors on the investigation of fat emulsions (Kawilarang et al., 1980). The phospholipids stabilized the emulsion by either forming a mechanical barrier together with the poloxamer or producing an electrostatic charge on the droplet surface, thereby preventing the coalescence of droplets. Lowering the emulsion pH results in reduction of the surface potential which leads to

the instability of the emulsion. However, despite the decrease in the zeta potential values, no creaming was observed over 2 months at 50 °C. This may indicate that the physical barrier formed by the close-packed mixed film of poloxamer and phospholipids at the interface of the emulsified droplets was mechanically resistant and stable enough to prevent droplets coalescing.

Hydrolysis and oxidation are responsible for the deterioration of PS in aqueous preparations (Roger and Smith, 1973). Obviously, the rate of these reactions is pH and temperature dependent. Hydrolysis of PS is known to be subject to acid-base-catalysis. At pH 3.0, acid catalysis predominates while at pH 4.0 and above base catalysis dominates. In the pH and temperature ranges tested in this study, irrespective of the dosage form (solution or emulsion), pseudo first-order kinetics were observed for the degradation reaction, thus confirming the experimental results obtained by others in a study of the kinetics of physostigmine degradation in eye drop solutions as a function of pH (Yang and Wilken, 1988a).

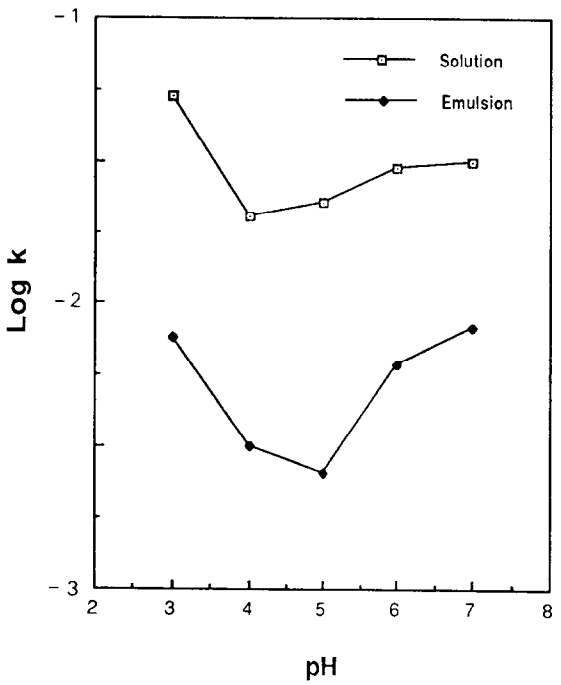

Fig. 5. pH profile of first-order degradation rate constants of PS submicronized emulsion and solution at 37 °C.

From the semi-logarithmic linear relationship of the percent drug remaining as a function of time, the values of K, the reaction constant at various pH values, were extracted for both the PS solution and emulsion and plotted vs pH (Fig. 5). An increase or decrease in the pH values around 5, although not affecting the physical properties of the submicronized emulsion, reduced the stability of PS in the emulsion (Fig. 5). When examined in aqueous solution, the degradation rate of PS was found to be considerably reduced at pH 4.0, corroborating previous results from the literature (Yang and Wilken, 1988a).

Monitoring of the stability of PS at different temperatures was carried out while the pH was kept constant. Obviously, the higher the temperature, the faster was the degradation rate observed. At optimal pH 5.0 the pseudo first-order degradation rate constants followed a pattern of exponential dependence on temperature, as expressed by the Arrhenius equation:

$$K = Ae^{(-E_a/RT)}$$

or in its logarithmic form:

$$\log K = \log A - \frac{E_a}{2.303RT}$$

Plots of log K vs $1/T$ at the optimal pH (pH 5.0) produced straight lines with slopes equal to $-E_a/R \times 2.303$ as observed in Fig. 6. In the above equation, K denotes the specific degradation rate constant for PS, R is the gas constant (1.987 cal degree^{-1} mol^{-1}), A represents a frequency factor, T is the absolute temperature, and E_a denotes the activation energy of the decomposition reaction (Fig. 6).

The degradation rates at 4 °C were calculated by extrapolation and found to be 5.01×10^{-4} and 7.08×10^{-3} day^{-1} for the emulsion and solution, respectively, leading to a predicted shelf life (90% drug remaining) of 210 days for the PS emulsion and 14 days for the PS solution.

In order to assess the validity of this extrapolation for the emulsion, the experimental result and the predicted value at 4 °C, calculated from the equation, $C = C_0e^{-kt}$, were compared. The PS

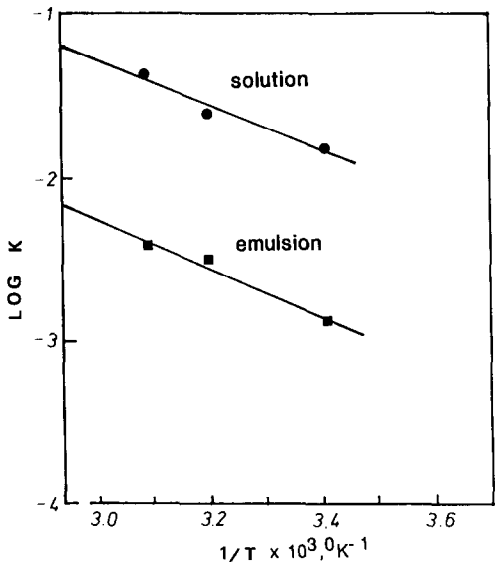

Fig. 6. Arrhenius plots of PS submicronized emulsion and solution at pH 5.0.

content in the emulsion was measured after 18 months storage at 4°C and found to be 95%. Long-term stability studies are now in progress.

The large discrepancy observed between the predicted value (210 days) and that obtained experimentally (540 days) could be explained by the instability of the emulsion and the phospholipid decomposition at elevated temperatures. Emulsions subjected to temperature variations undergo dramatic physico-chemical changes. Thus, long-term stability of emulsions and the subsequently induced protective effect vs sensitivity to drugs cannot be predicted from experiments carried out at high temperatures.

During 18 months storage at 4°C, the particle size distribution profile and the zeta potential values of the PS emulsion remained unchanged, and neither phase separation nor creaming could be detected. Therefore, it can be concluded that this emulsion conferred maximum protective effect to PS, as experimentally observed.

In previous studies on micronized emulsions, it was found that the addition of phospholipids to the emulsion oil phase markedly increased the solubility of PS (Friedman et al., 1989), and that the zeta potential of the emulsion increased with physostigmine concentration (Benita et al., 1989). It was suggested that these observations may be explained by molecular interactions that lead to a complex forming between PS and the phospholipids.

In view of the overall results, it can be deduced that, despite its high aqueous solubility, PS appears to be localized at the oil-water interface of dispersed oil droplets of the emulsion. The observed protective effect displayed by the emulsion is partly due to the interaction of physostigmine with phospholipids at the oil-water interface and partly due to the dissolution of physostigmine in the internal phase of the emulsion. Thus, a submicronized PS emulsion is much more stable than an aqueous solution of the drug. Based on kinetic calculations, the recommended storage temperature for PS emulsions is 4°C.

The PS emulsion formulation exhibits an interesting potential for enhancing PS bioavailability as compared to the solid dosage forms of this drug through possible lymphatic uptake. Direct uptake of drugs into the lymph capillaries followed by transport via the mesenteric lymph vessels and the thoracic duct has the advantage of circumventing the liver where physostigmine is prematurely metabolized. A recent study on dogs has indicated that the jejunal absorption of an emulsion of lipiodol with a mean droplet size of 258 nm appears to follow the pattern of lipid metabolism thereby promoting lymphatic uptake and accelerating lipiodol absorption (Damge et al., 1987). Animal studies are currently being performed in order to assess the potential of PS submicronized emulsions.

Acknowledgment

The authors would like to acknowledge the support of this study by a Grant from the Alzheimer's Disease and Related Disorders Association, Inc. (Chicago, U.S.A.).

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