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The stability and in vitro release kinetics of a clofibride emulsion

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Summary

Soybean and MCT submicron emulsions of clofibride were prepared using an optimal combination of phosphatidylcholine to poloxamer (molar ratio of 1 : 1). Both emulsions exhibited a mean droplet size in the range of 100–150 nm, and displayed identical Newtonian rheological behavior. The results of the accelerated stability tests indicated that the soybean emulsion was more resistant to various mechanical and thermal stresses than the MCT emulsion. The soybean emulsion remained stable even after an 18 month examination while the MCT emulsion showed signs of phase separation after 13 months storage at 25°C, confirming the findings of the accelerated tests. It was noted that the mean droplet size was not markedly altered by pH variation from 1.0 to 7.4 following 60 min of contact with the emulsion. Furthermore, the droplets of both emulsions remained practically unchanged as a function of contact time in artificial gastric medium. Whereas in artificial intestinal medium an increase in droplet size was observed only with the MCT emulsion. The in vitro release of clofibride from the emulsions was examined using various kinetic approaches. The dialysis sac technique was shown to be inadequate for drug release mechanism identification. However, two recent methods, the bulk-equilibrium reverse dialysis sac technique and the centrifugal ultrafiltration procedure, yielded rapid in vitro release profiles of clofibride from the emulsion. 70–90% of the clofibride content was released from the emulsion within 15–30 min, revealing that the kinetic process was probably controlled by the oil-water partition rate of the emulsion under perfect sink conditions. It was deduced from the overall results that the soybean emulsion of clofibride was definitely suitable for oral administration, since the presence of surface active agents may alter the drug absorption profile resulting in bioavailability enhancement.

Introduction

The potential use of o/w emulsions to enhance gastrointestinal absorption of poorly absorbed or instable drugs has been already outlined (Engel and Riggi, 1969; Carrigan and Bates, 1973). It was recently shown that incorporation of drugs administered orally in o/w emulsion significantly increased the absorption compared with the equivalent aqueous solution of the drugs (Palin et al., 1986; Benita et al., 1989; Kimura et al., 1989).

It can be inferred from the description of the

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stable drug emulsion formulations in the literature that most frequently at least two emulsifiers are required to establish a complex interfacial film emulgator with appropriate mechanical properties at the oil-water interface of the emulsified droplets. One of the most popular emulsifier combinations which have gained successes in the design of medicated emulsions is the mixture of phospholipids with poloxamer (Foster et al., 1988; Levy and Benita, 1989; Magdassi and Siman-Tov, 1990). Poloxamers are nonionic surface-active polyoxyethylene (POE) polyoxypropylene (POP) block copolymers which have received increasing interest for use in the formulation of dosage forms owing to their low toxicity (Leaf, 1967; Bentley et al., 1989). They were also reported to stabilize colloidal particles by a steric enthalpic-entropic mechanism where the POP group acts as an anchoring moiety and the POE segments provide stability in the surrounding medium by a repulsion effect (Naper and Netschey, 1971). Furthermore, it has been shown recently that poloxamer can penetrate soya phosphatidylcholine monolayers spread at the air-water interface, even in their condensed state, reflecting molecular interactions occurring between these emulsifiers (Santos Magalhaes et al., 1991).

Clofibride, a practically aqueous insoluble drug used in the treatment of hypercholesterolaemia and hypertriglyceridaemia, was reported to reach maximum effect 6 h after oral administration in a soft gelatin capsule. The half-life of the active metabolite was found to be 13 h (Harvengt and Desager, 1976; Dictionnaire Vidal, 1990).

The objective of the present study was to design a stable o/w emulsion formulation of clofibride for oral administration in an attempt to improve the pharmacokinetic parameters previously cited. The influence of the oily phase nature, soybean oil (SO) or medium chain triglycerides (MCT) which are absorbed differently from the gastrointestinal tract (Palin et al., 1986), on the emulsion properties was studied. In addition, the release profiles of clofibride from the emulsions, oily solutions and Lipenan® (a lipophilic marketed clofibride product) were compared using different in vitro kinetic approaches.

Materials and Methods

Materials

Clofibride (Scherer, Beinheim, France) and the corresponding marketed product, Lipenan® soft gelatin capsule, were offered by Laboratoires Bouchara (Paris, France). Medium chain triglycerides (MCT) were supplied by Societe Industrielle des Oleagineux (Saint-Laurent-Blangy, France). Soya phospholipids (Epikuron 200, Lucas Meyer Ltd, Hamburg, Germany), poloxamer (Synperonic F68, ICI Ltd, Clamart, France), purified soybean oil (Bertin Ltd, Courbevoie, France) and benzyl alcohol (a preservative from Carlo Erba, Milano, Italy) were used without further purification.

Methods

Emulsion preparations

Clofibride was first dissolved in the soybean or MCT oil. Poloxamer was dissolved in part of the aqueous phase where the phospholipids were dispersed. The remaining part of the water was used to dissolve benzyl alcohol. The oily and combined aqueous phases were heated to 70 and 75°C, respectively. Both phases were then mixed and emulsified applying further heating using a high shear mixer (Ultraturrax, Ika Werk, Staufen, Germany). The emulsion was then rapidly cooled and homogenized using a two-stage valve high pressure homogenizer (Gaulin, APV, Hilversum, The Netherlands).

Evaluation of physicochemical properties of emulsions

Emulsion characteristics Visual observations were made in order to determine macroscopic aspect, possible creaming, coalescence and phase separation.

Mean droplet diameter and size distribution were determined by a laser light scattering system (Super Nanosizer, Coulter model N4MD, Coultronics, Margency, France). Furthermore, the mean droplet diameter was also determined by an optical microscope (Zeitz, Wetzlar, Germany)

and scanning electron microscope, SEM (Camebax, Courbevoie, France), using a modified technique reported by Hamilton-Attwell and coworkers (1987). The emulsions were filtered through a Millipore membrane filter (0.5 μm, type VM). The lipid fixation on the filter was carried out using a 2% solution of osmium tetroxide (Fluka, Buchs, Switzerland).

Electrical conductivity measurements were performed using a conductivity meter (CDM 3, Radiometer, Copenhagen, Denmark).

The rheological properties of the emulsions were measured with a cone and plate viscosimeter (Carri-Med, Rheo, Palaiseau, France).

The pH of emulsions was measured with a micro pH 2001 (Barcelona, Spain).

The elecrophoretic mobility (Micrometrics 1202, Coultronics, Margency, France) and consequently the calculated zeta potential were determined using a technique previously described (Depraetere, 1980).

Accelerated stability tests The accelerated stability studies were conducted by ultracentrifugation of the emulsion samples (Ultracentrifuge L7-55, Beckman, Palo Alto, U.S.A.) at $25\,500 \times g$ over 1 h. The emulsions were also subjected to repeated freeze-thaw cycles (16 h of freezing at -18°C and 8 h of thawing at 25°C) and to excessive shaking at 150 strokes/min at 25°C over 48 h. The macroscopic aspect of the emulsions was observed and the mean droplet diameter and size distribution were measured before and after these accelerated tests.

Long-term stability assessment

Stability studies were conducted at various temperatures, 4, 25 and 40°C. The chemical and physical changes that might occur during storage were followed up by evaluation of the previously mentioned properties of the different emulsion samples.

Clofibride content Clofibride content of the emulsion was analyzed by using a Waters chain equipped with a UV detector at 225 nm, a model 501 pump, Wisp 712 auto injector and a model 745 computing integrator. The column was a 150 mm $\times$ 3.9 mm μBondapak C_{18} and the mobile phase consisted of methanol and water (70:30%) run at a flow rate of 1 ml/min. The calibration curve was prepared by injecting 10 μl of standard clofibride methanolic solutions ranging in concentration from 2 to 300 μg/ml. The various emulsion samples (1 ml) were diluted with methanol (1:50). Some of the samples yielded clear solutions while others remained cloudy, even after 1 h agitation. The samples were frozen at -18°C over 3 h to promote precipitation and separation of all the insoluble lipid materials in methanolic solutions. Finally, the supernatants were filtered and diluted to the appropriate concentration prior to HPLC injection (10 μl). Each experimental point was triplicated.

Apparent partition coefficient determinations 10 ml of the appropriate oily phase of clofibride at the same concentration of 5% (w/v) were kept in contact without agitation at 37°C with 40 ml of the buffer solution at pH 1 and 7.4 or with an aqueous phase having the same concentration of poloxamer (POL) or the same combination of phospholipid/poloxamer (PC/POL) as in the emulsion formulation. At different time intervals over a period of 48 h, samples were taken from the aqueous phases and clofibride concentrations were measured using the previously described HPLC technique, until an equilibrium was established between the oily and aqueous phases.

The apparent partition coefficient of clofibride in the intact SO and MCT emulsion was determined as follows. The pH of the various emulsions were first adjusted to pH 1 or 7.4 with HCl or NaOH (2%), respectively. After 48 h, total phase separation of the emulsions was induced by four successive freeze-thaw cycles. The clofibride was assayed by HPLC in the oily phase. The apparent partition coefficient was then calculated according to the equation $P = C_o/C_w$ where C_o and C_w are the equilibrium concentrations of clofibride in the oily and aqueous phases, respectively.

Resistance to pH environments The pH of the emulsion was adjusted from 1 to 7.4 with either HCl (2 N) or NaOH (0.1 or 2 N). The emulsions were examined while kept at 37°C under magnetic stirring.

The mean droplet diameter and size distribu-

tion and zeta potential of these emulsions were determined at given time intervals over 2 h.

Resistance to artificial gastrointestinal fluids The emulsions were diluted first with artificial gastric fluid (USP XXI) (1 : 10) and agitated moderately over 1 h at 37°C. Then artificial intestinal fluid was added in a volume equal to the previous volume resulting in a final dilution of 1 : 1. The pH was then adjusted to 7.4 with NaOH (2 N). The entire system was stirred moderately for a further 2 h period and the same emulsion properties as previously mentioned were examined.

In vitro release kinetic examination It was decided in this investigation to study the in vitro release profile from the various emulsions using the dialysis sac diffusion technique and the bulk-equilibrium reverse dialysis sac technique (Levy and Benita, 1990). For both techniques the same dialysis membrane was used (Spectra/por 4, diameter 25 mm, molecular weight cut-off 50000, Spectrum, LA, U.S.A.). In preliminary studies, it was found that the dialysis membrane (regenerated cellulose) did not adsorb or retain free clofibride, which had permeated freely into the sink solution within 60 min, when dissolved in methanol. The methanolic solution (5% w/v) was used as a reference for the calculation of the permeation constant through the dialysis membrane using a similar approach to that previously reported (Friedman and Benita, 1987). Furthermore, a new and interesting in vitro kinetic technique without any physical barrier recently applied to nanocapsules was also used (Ammoury, 1990). Since these various techniques differ in the sampling process of the drug released from the o/w emulsion, it will be interesting to compare the results yielded by the different methods.

(1) Dialysis sac diffusion technique
0.5 ml of the clofibride emulsion or oily solution was placed in the dialysis sac, hermetically sealed and dropped into 1 l of buffer sink solution at selected pH of 1 or 7.4. The entire system was kept at 37°C with continuous magnetic stirring. Samples (1 ml) were withdrawn from the sink solutions at predetermined time intervals and assayed for clofibride content by the HPLC technique.

(2) Bulk-equilibrium reverse dialysis sac technique
1.0 ml of the clofibride emulsion or 25 μl of the peanut oil solution incorporated in the marketed soft gelatin capsule were directly placed into 500 ml of a stirred buffer sink solution where 10 dialysis sacs containing 1 ml of the same buffer sink solution were previously immersed. It should be emphasized that the dialysis sacs were equilibrated with the buffer sink solutions for a few hours prior to experiments. At predetermined time intervals, a dialysis sac and 1 ml of the buffer sink solution are withdrawn from the stirred release solution, and the clofibride content of the dialysis sac and sink solution is assayed by HPLC. The kinetic experiments were performed at 37°C under constant magnetic stirring.

(3) Centrifugal ultrafiltration technique
The method was recently developed by Millipore Corp. (Bedford, MA, U.S.A.). The device, mainly based on an Eppendorf centrifuge tube separated from an enclosed tube by an ultrafiltration membrane, allows for separation of nanoparticles from microliter volumes of aqueous dispersion medium in a centrifuge (Ultra-free MC unit). This technique has been successfully applied by Ammoury (1990) to evaluate the in vitro release profile of indomethacin from PLA nanocapsules. 1.0 ml of the clofibride emulsion was directly placed in 250 ml of a stirred buffer sink solution at 37°C. At given time intervals, 400 μl of the release solution where the emulsion was dispersed are deposited in the Ultra-free-MC unit (10000 NMWL, low protein binding membrane, PLGC type) which is subjected to centrifugation at $5000 \times g$ for 5 min. 50 μl of the ultrafiltrate are then withdrawn and assayed for clofibride content by the HPLC technique. The percent release of clofibride is calculated from the ratio of drug concentration in the ultrafiltrate versus the total concentration of clofibride in the release solution.

Results and Discussion

The components in this formulation and especially the emulsifier combination of phospholipids-poloxamer were selected on the basis of preformulation studies. The phospholipids, used in this study, comprised mainly phosphatidylcholine according to manufacturer specifications. Various phosphatidylcholine/poloxamer ratios were studied, keeping constant the total concentration of both emulsifiers. It was found that the emulsions prepared using a ratio of phosphatidylcholine to poloxamer of 1:10 w/w, equivalent to a molar ratio of 1:1, maintained their initial properties over longer periods of time than the other emulsions.

A typical formulation (% w/w) consisted of soybean or MCT oil 20.0, clofibride 5.0, benzyl alcohol 1.0, phospholipids-poloxamer combination, 5.0 (at constant ratio of 1:10 w/w) and distilled water to 100.0 g.

Evaluation of physicochemical properties of emulsions

Emulsion characteristics

The various physicochemical properties of the SO and MCT emulsions are reported in Table 1.

TABLE 1

Physicochemical properties of the soybean (SO) and MCT emulsions

Properties	Emulsion	
	SO	MCT
Macroscopic aspect	stable; white; fluid	stable; white; fluid
Mean droplet diameter (± SD, nm)	166.0 ± 120.0	120.0 ± 100.0
Electrical conductivity (μS cm^{-1})	59.0	64.0
Viscosity (mPa s) × 10^3	7.9	5.8
pH	6.4	6.6
Zeta potential (mV)	−9.0	
Clofibride content (%)	4.91	4.89

Both emulsions were fluid, white and had the same morphological aspect.

No marked difference in mean particle size could be observed although wider distribution ranges were noted in both emulsions just after preparation as reflected by the high standard deviation (SD) values. This could be attributed to the presence of a large population of very tiny droplets which disappeared with time as a result of droplet coalescence. It should be pointed out that both emulsions were examined using an optical microscope and no droplets larger than 1 μm were detected.

The emulsions present close conductivity values of 59 and 64 μS cm^{-1} for the SO and MCT emulsions, respectively.

Both emulsions displayed identical rheological behavior and were Newtonian, indicating that the emulsified droplets did not aggregate or flocculate and remained individualized even when subjected to increasing rate shear. Since identical oil phase volume ratio was used and close particle size distribution profiles were obtained in both emulsions, the significant difference observed between the viscosity values could be explained by the different fluidity nature of the oil phase used, MCT being less viscous than SO.

The zeta potential of the emulsions was low (−9 mV) as expected, since the main constituent of the phospholipid mixture was phosphatidylcholine, a neutral phosphatide over a wide pH range.

Accelerated stability tests

The results of the accelerated stability tests are presented in Table 2. It could be observed that the SO emulsion is more resistant to various mechanical and thermal stresses than the MCT emulsion. This probably indicates that the properties of the mixed film formed at the oil-water interface of the SO emulsion are more effective in preventing phase separation than in the case of the MCT emulsion. It is well-known that the stress conditions normally employed for evaluating stability of emulsion cannot predict the normal shelf life of an emulsion. Although it appears that MCT emulsion exhibits instability problems related probably to unfavorable partitioning of

TABLE 2

Effect of accelerated ageing tests on emulsion stability

Emulsion type	Ultracentrifugation (25 500 × *g*, 60 min)		Freeze-thaw cycle		Oscillatory movement (150 strokes/min, 48 h)	
	Macroscopic aspect	Droplet size (nm) (± SD)	Macroscopic aspect	Droplet size (nm) (± SD)	Macroscopic aspect	Droplet size (nm) (± SD)
SO	stable	149 ± 27	1st cycle: creaming	1 : 247 ± 140	stable	418 ± 113
			2nd cycle: phase separation	2 : 4890		
MCT	light	105 ± 31	1st cycle: phase separation		phase separation	

the emulsifiers at the o/w interface, it was decided to carry out long term stability studies on both emulsions.

Long-term stability assessment

The SO emulsion remained stable even after 18 months examination while the MCT emulsion showed signs of phase separation after 13 months storage at 25°C, confirming the findings of the accelerated stability tests.

It should be added that increasing the storage temperature from 25 to 40°C reduced markedly the stability of both emulsions. Creaming and signs of phase separation (tiny oil droplets) were detected after storage at 40°C over 6 and 12 months for MCT and SO emulsion, respectively.

Furthermore, after 8 days storage at 4°C, phase separation is noted for both emulsions. This rapid instability at 4°C should be attributed to a marked decrease in solubility of clofibride in oil with decreasing temperature. A moderate precipitation was noted in an oily solution at 4°C. The crystals were identified as clofibride following isolation and dissolution in methanol by HPLC.

It can be seen first that the particle size increased moderately but progressively with time up to 18 months for the SO emulsion and up to 12 months for the MCT emulsion (Table 3). It should be noted that after a few months storage a small population of droplets with a mean diameter of 1–2 μm was detected using optical and scanning electron microscopes, reflecting a slow coalescence process of the small droplets which were not optimally stabilized. This is not in contradiction with the results presented in Table 1, since the super nanosizer is unable to detect the

TABLE 3

Physicochemical properties of the SO and MCT emulsions as a function of storage time at 25 °C

Time (months)	Soybean emulsion					MCT emulsion				
	Mean diameter (nm) (± SD)	σ (μS cm^{-1})	n (10^3 mPa s)	pH	ζ (mV)	Mean diameter (nm) (± SD)	σ (μS cm^{-1})	n (10^3 mPa s)	pH	ζ (mV)
0	166 ± 120	59	8.3	6.4	−9	120 ± 100	64	5.6	6.6	−9
1	131 ± 29	65	7.7			134 ± 15	74	5.6		
3	133 ± 47	87	7.9			141 ± 32	96	6.4		
6	148 ± 84	114	10.5	5.7		158 ± 23	110	6.5	5.9	
12	219 ± 150	116	10.4	5.4	−6	219 ± 150	–	7.1	5.6	−6
18	240 ± 87	295		5.3		phase separation				

σ, electrical conductivity; n, Newtonian viscosity; ζ, zeta potential.

population of droplets having a mean diameter of 1–2 μm especially if the percentage of this population in the emulsion is small.

The conductivity of both emulsions increased progressively with time indicating that some degradation of at least one of the emulsion components which becomes water soluble occurred with time.

The pH of both emulsions decreased from 6.6 and 6.4 to 5.4, confirming the conductivity results and indicating that an hydrolytic degradation process occurred.

The conductivity increase and pH decrease are probably due to the hydrolysis of the triglycerides and phospholipid moieties following the release of free fatty acids, a well-known phenomenon already reported by numerous authors.

It should be pointed out that clofibride retained its entire chemical integrity over the 18 months storage at 25°C as was confirmed by routine repeated HPLC analysis.

Resistance to pH environments

The effect of external pH variation on the mean droplet size as a function of time is depicted in Fig. 1. Two out of four pH values tested (ranging from 1.0 to 7.4) are presented. It can be clearly observed that the mean droplet size was not markedly altered by the pH following 60 min of contact when measured with the nanosizer. The SO emulsion showed a slight increase in the droplet size at pH 7.4 as a function of time, while the particle size of the MCT emulsion remains constant. However, a second albeit small droplet population was discerned by optical microscopy and SEM examinations as shown in Fig. 2 in both emulsions, indicating that some coalescence occurred which was not detected by the nanosizer apparatus.

The zeta potential declined with the variation of adjusted pH in the range tested, reaching a value close to zero. Similar behavior was also noted by other authors (Levy and Benita, 1989). However, no meaningful deduction could be drawn from these observations since the initial charge was low.

It is then assumed that the emulsifier system leads to the formation of almost an unionized

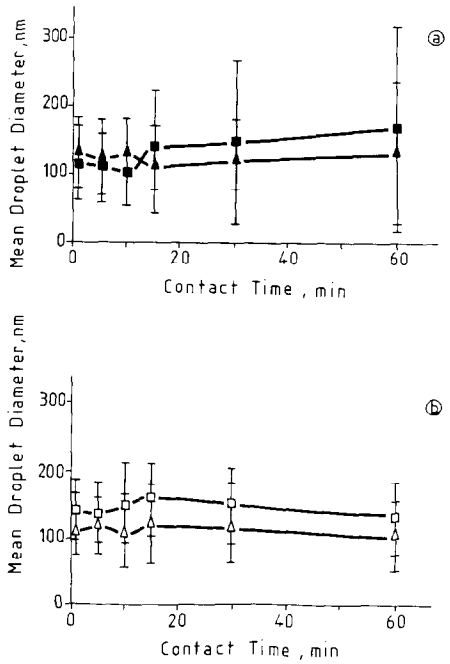

Fig. 1. Effect of pH variation on the mean droplet diameter size of: (a) SO emulsion [(▲) pH 1.0; (■) pH 7.4]; (b) MCT emulsion [(△) pH 1.0; (□) pH 7.4] as a function of time.

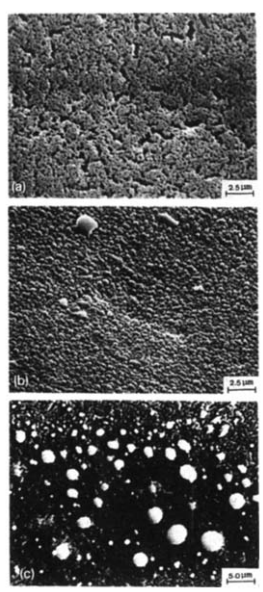

Fig. 2. Scanning electron micrograph of emulsion droplets subjected to Hamilton's technique: (a) Millipore membrane; (b) soybean emulsion; (c) soybean emulsion adjusted at pH 7.4.

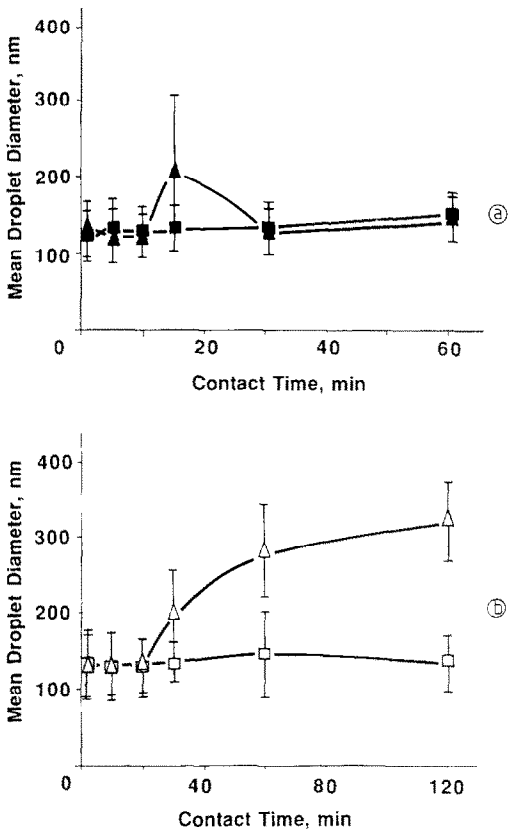

Fig. 3. Influence of artificial gastrointestinal juices on the mean droplet size of emulsions: (a) gastric juice [(■) SO; (▲) MCT]; (b) intestinal juice [(□) SO; (△) MCT].

interfacial mixed film. The emulsion stabilization should be attributed to the steric hindrance effect due mainly to the adsorbed solvated poloxamer molecules, the polar polymeric moeities of which extend towards the external aqueous phase. Such a steric effect resulted in a high energy barrier which led to repulsion of adjacent and approaching droplets.

Resistance to artificial gastrointestinal fluids

The droplets of both emulsions remained practically unchanged as a function of contact time in artificial gastric medium (Fig. 3a). In contrast, in intestinal medium an increase in droplet size was observed only with the MCT emulsion as depicted in Fig. 3b. This pronounced effect should be rather attributed to the presence of electrolytes which are known to affect the interfacial mixed emulsifier film integrity resulting in a reduced protection against substantial coalescence upon droplet collisions. These findings confirmed the previous deductions that MCT emulsion is less stable than SO emulsion even when subjected to short-term stress.

It is interesting to note that despite the emulsion droplet size increase, no phase separation was noted.

It can therefore be deduced from these results that the clofibride SO and MCT emulsions will maintain their dosage form integrity during the gastrointestinal transit. Thus, the emulsions appeared to be suitable for oral use.

In vitro release kinetic examination

An accurate analysis of in vitro drug release from emulsion requires first a knowledge of the distribution of the drug in the various phases of the emulsion. In preliminary studies, it was shown that clofibride partition is in favor of both oily phases comprising the emulsion use in contact with water at pH 1 and 7.4 (Table 4). However,

TABLE 4

Effect of oil nature, pH and emulsifiers on clofibride apparent partition coefficient

Oil phase	Aqueous phase composition	pH	Partition coefficient
Soybean	water	1.0	279.4
Soybean	water	7.4	227.9
MCT	water	1.0	545.0
MCT	water	7.4	273.0
Lipenan® Sol.	water	1.0	875.8
Lipenan® Sol.	water	7.4	644.4
Soybean	water/POL	6.5	70.9
Soybean	water/PC/POL	6.5	47.6
MCT	water/POL	6.5	108.5
MCT	water/PC/POL	6.5	71.1
SO emulsion	PC/POL	1.0 [a]	10.4
SO emulsion	PC/POL	7.4 [a]	4.5
MCT emulsion	PC/POL	1.0 [a]	12.4
MCT emulsion	PC/POL	7.4 [a]	8.8

[a] pH emulsion adjustment. For details see Materials and Methods.

clofibride is less localized in the oily phase when poloxamer is present in the aqueous phase. This effect is more pronounced in the presence of both emulsifiers in the water phase at the same concentration as in the emulsion but still into two separated phases (Table 4). Such a trend was expected since the presence of emulsifiers in the aqueous phase should induce the formation of a 'micellar' phase which should increase significantly the aqueous solubility of clofibride through a solubilization process. However, the apparent partition coefficient values decreased significantly in the intact emulsions by a 10-fold factor (Table 4) indicating that the results obtained in separated phases having similar composition to that of the intact emulsion could lead to misleading interpretations. The discrepancy could be explained by the fact that in the separated phases, substantial emulsifier molecules concentrate at the oil/water interface where the major part of the drug is localized. Therefore, any drug concentration measurement in the aqueous phase may not reflect the true partition profile of the drug in the various phases unless the concentration of the drug has been measured in the oily, aqueous phase and at the o/w interface. It is clearly deduced from the results exhibited in Table 4 that clofibride is mainly localized in the oily phase of the emulsion at pH 1 but raising the pH to 7.4 will significantly decrease the localization of the drug in the oily phase of the emulsion. In addition, it can be seen that MCT is capable of dissolving more clofibride than soybean oil irrespective of the emulsion pH.

It is difficult to characterize drug release profile from a colloidal carrier owing to the physical obstacles attributed to the very tiny size of the dispersed particles. Various techniques were used to evaluate drug release from colloidal carriers particularly from o/w submicron emulsions (Washington, 1989, 1990). Attempts were made to elucidate the release mechanism by using the dialysis sac diffusion technique recently criticized by Washington (1989). Levy and Benita (1990) recently proposed the bulk-equilibrium reverse dialysis sac technique to avoid the enclosure of the submicron emulsion dosage form in a dialysis sac. However, the kinetic system proposed is able

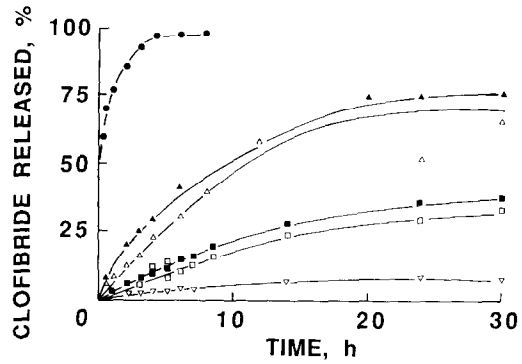

Fig. 4. Clofibride release profiles from different pharmaceutical forms: (●) methanolic solution; (▲) SO emulsion; (△) MCT emulsion; (■) SO solution; (□) MCT solution; (▽) Lipenan® at buffer sink solution pH 1.0 using the dialysis sac technique.

to differentiate between colloidal drug delivery carriers releasing their content over a period greater than 1 h. Finally, a more rapid stringent test based on a centrifugal ultrafiltration technique in the absence of any physical membrane was also used (Ammoury, 1990). All these tests were used to avoid any controversial in vitro release kinetic deductions.

Dialysis sac diffusion technique

It can be seen from Fig. 4 that clofibride at an initial concentration of 50 mg/ml in methanol diffused out rapidly (90% within 2 h) into the sink solution which consisted of methanol. These results clearly indicate that the membrane is not a rate-limiting step under the experimental conditions used where a given large clofibride gradient was established between the internal and external compartments separated by the membrane. Similar results were obtained with different membrane cut-offs. The clofibride release rate was slower from emulsions than from the methanolic solution. Furthermore, a marked decrease in clofibride release from the oil solutions and marketed product was observed (Fig. 4). Nevertheless, the kinetic behavior of both emulsions was similar despite the constraints and irrespective of the pH since sink conditions prevailed.

Numerous investigators have pointed out various reasons explaining the drastic decrease in the release rate of a drug from the emulsions using

the dialysis technique (Lostrito et al., 1978; Washington, 1989; Ammoury et al., 1990; Levy and Benita, 1990). It was assumed that slow drug release through the dialysis membrane under very low drug gradient was the main factor which should have drastically reduced the drug release from the colloidal carriers. For the purposes of confirming indirectly that the dialysis membrane was probably the rate-determining step in the overall kinetic process, a previous kinetic model, which was proposed for the description of drug release from emulsions, was applied (Friedman and Benita, 1987).

The drug kinetic model appears to be applicable in the present study since quite identical experimental conditions prevail in both cases. Such an approach was already used in a previous study dealing with physostigmine emulsion (Benita et al., 1989). This model concerns biphasic systems in which a drug is dissolved or partitioned between the lipophilic and hydrophilic phases of a dispersed system but separated from the sink solution by a membrane or a dialysis sac which should not be the rate-limiting step in the overall process. Drug diffusion through the membrane sac was assumed to obey Fick's first law; this was also confirmed experimentally. The drug is exchanged between three model compartments (a) those involving the oily phase of the emulsion, (b) the internal aqueous phase and (c) the sink solution where sampling is performed.

The expected curves were calculated by a curfit computer programme which consisted of a least-squares fit to a non-linear function with linearization of the fitting function (Benita et al., 1989).

A poor fit was achieved when the release curves predicted from the kinetic model equation, reported by Benita et al. (1989), were compared with the observed kinetic data curves (Fig. 5a,b). Therefore, it can be deduced that release of clofibride from SO and MCT emulsion at two pH values did not conform with the proposed kinetic model. This kinetic behavior could be explained by the fact that the release of clofibride from the emulsion was incomplete due to the low aqueous drug gradient achieved in the external aqueous phase of the emulsion inside the dialysis sac rendering the membrane the rate limiting step in the overall kinetic process. It can be definitely deduced that the dialysis sac technique could not be considered an appropriate method to evaluate the true release rate of a drug from a colloidal carrier, confirming findings previously reported (Washington, 1989; Ammoury et al., 1990; Levy and Benita, 1990).

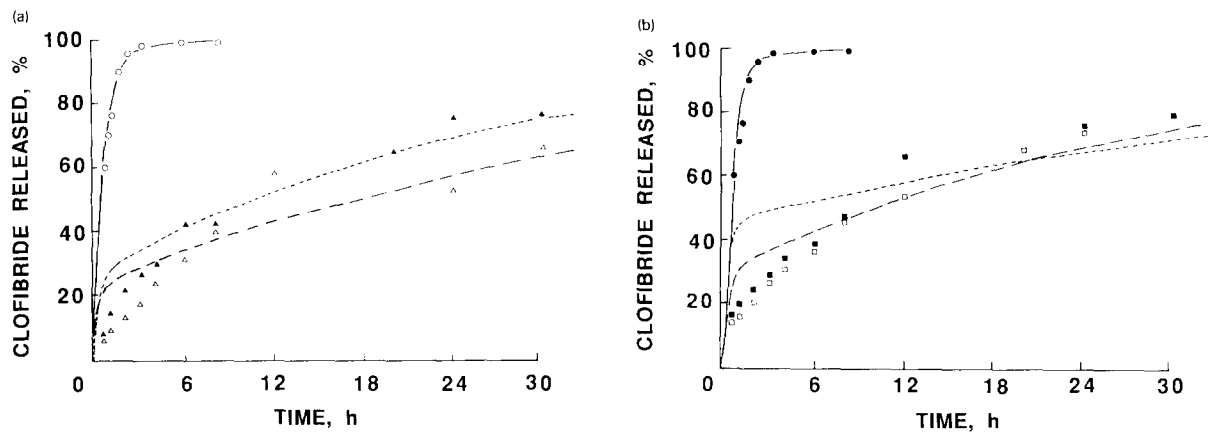

Fig. 5. Computer fitting and comparison of predicted release curves according to Eqn. 1 from Benita et al. (1989) with observed kinetic data: (a) (○) methanolic solution; (▲) SO emulsion and (△) MCT emulsion at buffer sink solution pH 1.0; (b) (●) methanolic solution; (■) SO emulsion and (□) MCT emulsion at buffer sink solution pH 7.4.

Bulk-equilibrium reverse dialysis sac technique

Upon clofibride emulsion immersion in the sink solution, an infinite dilution was achieved and a new equilibrium was re-established where drug partitioned between the oily nanodroplets and the sink solution which became the external phase of the emulsion without being separated from the oily droplets by any artificial membrane. The diffusion of the drug from the oily droplets to the sink solution will be governed by a true and real gradient existing between the oily and the new external aqueous phase. Drugs dissolved in the aqueous phase will then permeate into the dialysis sacs.

As previously reported, the percent drug released was calculated from the ratio of drug concentration measured at predetermined time intervals in the dialysis bags versus the total concentration of the drug in the sink solution where oily nanodroplets are also present.

It can be noted from Fig. 6 that the release of clofibride from the emulsions and marketed product into the sink solution is rapid. Practically all the clofibride is released in the sink solution within less than 1 h. No difference in the release profiles of clofibride from the various dosage forms was observed at a given pH value.

It is not yet possible to distinguish whether the drug release from the oily droplets is faster than the permeation rate of the dissolved drug through the dialysis membrane in the enclosed sink solution. This deduction was based on the similarity in the kinetic behavior yielded by the various pharmaceutical dosage forms. These observations were expected since a large dilution ($\times 500$), with an aqueous phase, was performed. This leads to drug partition largely in favor of the aqueous phase, as was confirmed by the apparent partition coefficient data presented in Table 4. Furthermore, a more rapid release rate was noted in buffer sink solution pH 7.4 (Fig. 6b) compared with drug release from buffer sink solution pH 1.0 (Fig. 6a). From the results presented in Table 4 on the drug distribution in the various phases, it can be deduced that less drug is retained in the dispersed oily droplets at pH 7.4 compared with pH 1.0, therefore, suggesting that the overall kinetic process is governed rather by the oil-water partition rate of the emulsion than by the diffusion of the drug through interfacial mixed emulgator film.

In cases where the permeation rate through the dialysis membrane should be the slowest step and consequently the rate-determining step in the overall kinetic process, a marked decrease in the initial drug concentration gradient should significantly alter and decrease the drug release profile.

The effect of initial drug concentration in the sink solution should therefore be addressed. The permeation rate of two different initial concentrations of clofibride in Lipenan® soft gelatin capsule (2.75×10^{-3} and 1.37×10^{-4} M, respectively) from the sink solution at pH 1.0 and 7.4 into the dialysis sacs was evaluated (Fig. 7). At both pH values, the low initial drug concentration resulting in a concentration gradient decrease by

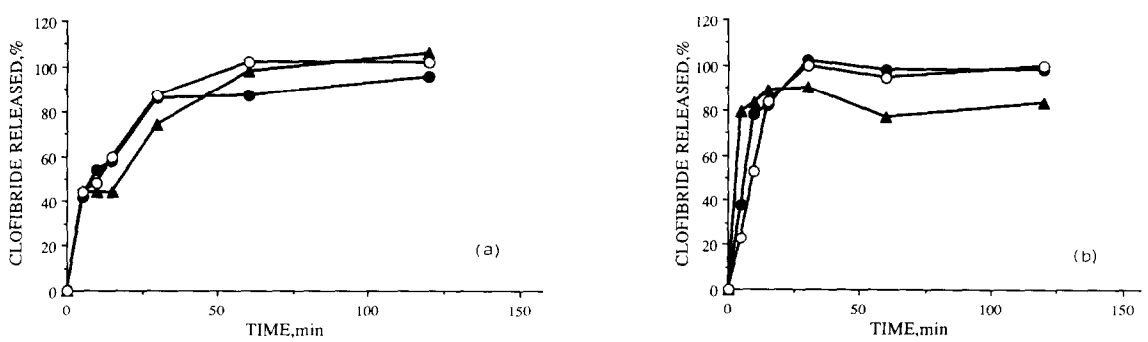

Fig. 6. Clofibride release profiles from: (a) (●) SO emulsion; (▲) MCT emulsion; (○) Lipenan® at buffer sink solution pH 1.0; (b) (●) SO emulsion; (▲) MCT emulsion; (○) Lipenan® at buffer sink solution pH 7.4 using reverse dialysis sac technique.

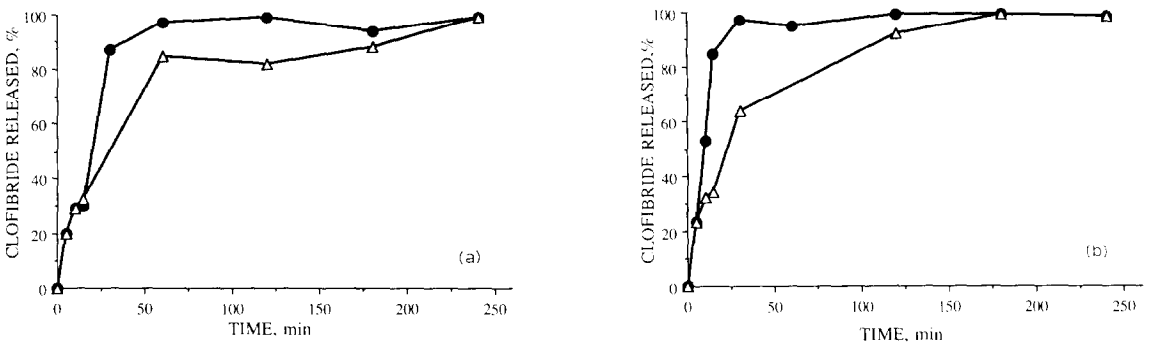

Fig. 7. Effect of initial concentration (mol l^{-1}) on the clofibride release from soft capsule gelatin Lipenan® by reverse dialysis at different buffer solutions: (a) (●) 1.37×10^{-4}, (△) 2.75×10^{-3} at pH 1.0; (b) (●) 1.37×10^{-4}, (△) 2.75×10^{-3} at pH 7.4.

20-fold yielded a faster release rate (Fig. 7), indicating that in the present case, the dialysis membrane was not the rate-determining step in the overall kinetic process. Again, at pH 7.4 (Fig. 7b) the drug was released more rapidly than at pH 1.0 (Fig. 7a), confirming the previous findings and supporting the hypothesis that the release of clofibride from the emulsion is probably controlled by the partition rate of the drug between the oily and aqueous phase of the emulsions.

For the purposes of confirming the previous deductions a new and rapid in vitro kinetic technique based on a centrifugal ultrafiltration was applied.

Centrifugal ultrafiltration

This technique yielded in vitro release profiles of clofibride from the emulsion similar to that already observed with the bulk-equilibrium reverse dialysis bag technique, indicating that the dialysis membrane was not really the rate-determining step as already mentioned. 75–90% of the clofibride content is released from the emulsion within 15 min (Fig. 8), confirming that the kinetic process is probably controlled by the oil-water partition rate of the emulsion under perfect sink conditions.

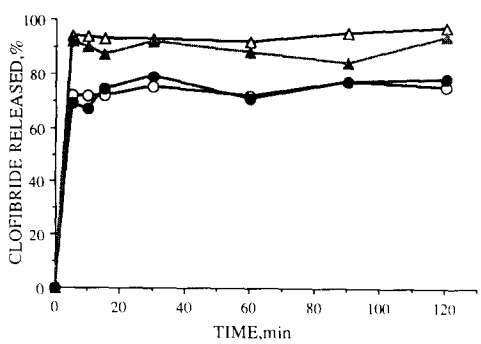

Fig. 8. Clofibride release profiles from emulsions: (●) SO; (▲) MCT at buffer sink solution pH 1.0; (○) SO; (△) MCT at buffer sink solution pH 7.4 using the centrifugal ultrafiltration technique.

It can be demonstrated from Table 4 that clofibride tends to accumulate preferably in the inner oil phase of the MCT emulsion as compared to that of the SO emulsion, irrespective of the pH.

In contrast, it can be observed from Fig. 8 that SO emulsion is able to retain clofibride over longer periods of time than the MCT emulsion, irrespective of pH. This does not correlate well with the apparent partition coefficient data exhibited in Table 4. No meaningful deductions can be made on these observations since the release in all the cases was very rapid and the difference in partition coefficient value is relatively small.

Conclusion

It could be concluded that the SO emulsion of clofibride is stable at room temperature over more than 18 months of storage, while the MCT emulsion showed clear signs of instability indicating that soybean oil exhibits more appropriate solubility properties to favor optimal partition of

the surfactant combination between the oily and aqueous phase. It can be deduced from the kinetic results that under perfect sink conditions clofibride release cannot be sustained by incorporating the drug in emulsions. However, it is expected, owing to the huge increase in surface area and to the presence of the surface active agents that such a dosage form may alter the drug absorption profile resulting in bioavailability enhancement. The SO emulsion of clofibride is definitely suitable for oral administration.

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