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RESEARCH PAPER

Entrapment of Phenytoin into Microspheres of Oleaginous Materials: Process Development and In Vitro Evaluation of Drug Release

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ABSTRACT

A novel multiparticulate preparation of the antiepileptic agent phenytoin (1) was developed and evaluated in vitro. The preparation consists of gastroresistant microparticulate drug delivery system formulated with oleaginous material (lipospheres) to minimize unwanted effects of 1 on gastric apparatus. The drug was dispersed in a spherical micromatrix consisting of a mixture of stearyl alcohol and glycerol esters of various fatty acids. The best mixture to obtain discrete, reproducible, free-flowing lipospheres consisted of glyceryl monostearate dilaurate and stearyl alcohol (ratio 3:17). The lipospheres were obtained by a technique involving melting and dispersion of drug-containing oleaginous material in aqueous medium. The oily droplets of the resulting emulsion after cooling under rapid stirring were transformed into solid. About 99% of the lipospheres were of particle size range 100–800 μm. The lipospheres were analyzed to determine the drug content in various particle sizes and to characterize the in vitro release profile. The average drug content was 23.8% w/w. Drug encapsulation efficiency was about 93.6% and the yield of production ranged from 94 to 98%. The drug discharge pattern from the microparticulate system in the intestinal environment was evaluated. Kinetic results were analyzed to distinguish between various release models. The matrix diffusion-controlled equation was the most appropriate one in describing drug release.

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INTRODUCTION

The well-known phenytoin (5,5-diphenyl-2,4-imidazolidinedione) (**1**) has been widely used as an antiepileptic agent to control tonic-clonic (grand mal) and partial (focal) seizures (1). Phenytoin is absorbed almost completely after oral administration, most being absorbed from the upper intestine (2). The rate of absorption is variable and affected by the presence of food (3). Different formulations from the same manufacturer, as well as different brands of the drug, can vary considerably in their dissolution rates and in their bioavailability (4,5). Phenytoin exhibits nonlinear disposition, and plasma half-life varies and is greatly dose-dependent; slow-release dosage forms that produce acceptable steady-state plasma concentrations are desirable (6–8).

The treatment of epilepsy with **1** requires administration during very long periods of time and may often induce high incidence of adverse drug reactions on central nervous (9), integumentary (10), hemopoietic (11), immunologic (12), and cardiovascular systems (13). The long therapeutic regimen may also produce subtle effects on mental function and cognition, especially in children (14); moreover, when **1** is given by peroral route for long-term treatments, it may produce severe gastric irritation and should be taken with or after food with consequent fluctuation in the plasma concentrations (15).

Bioavailability studies on phenytoin demonstrated that the administration of drug emulsions in oil does not affect the amount adsorbed and is responsible for a longer absorption time (16).

Previous experimental results from these laboratories demonstrated the aptitude of oleaginous materials in the preparation of microspheres loaded with appropriate drugs (17–19).

Multiple-unit doses for oral use, modifying the dissolution of drugs, allowed the administration of much smaller drug amounts than single-unit doses and provided a method of releasing the active ingredient at desired rates (20). It has been reported that following oral administration of microcapsules, prolonged plasma concentrations of phenytoin were obtained (21,22). Drug discharge in the stomach may be hindered and local unwanted effects reduced or eliminated when a gastroresistant material is used for the microspheres preparation (23,24). Moreover, a multiple unit system offers the advantage that it spreads in a large area of the absorbing mucosa and prevents exposure to high drug concentration when compared to single-unit dosage forms on chronic dosing (25).

The objective of this study was to design and test a multiple-unit dosage form containing **1**. The following stages were involved: (i) the design of the microparticulate system, (ii) the choice of an oleaginous material suitable for the manufacture of the system, (iii) the evaluation of the physical characteristics of the system, and (iv) the evaluation of kinetics of drug release.

MATERIALS AND METHODS

Phenytoin (**1**) and octadecanoic (stearyl) alcohol (**2**) were purchased from Sigma Chemie (Deisenhofen, Germany); decanoic (capric) (**3**), dodecanoic (lauric) (**4**), tetradecanoic (myristic) (**5**), hexadecanoic (palmitic) (**6**), and octadecanoic (stearic) (**7**) acid chlorides were from Janssen Pharmaceutica, (Geel, Belgium); glyceryl monostearate (**8**) was purchased from Esperis (Milan, Italy); and the surfactants Tweens® 21, 60, 80, 85 and Spans® 20 and 40 were from Fluka (Buchs, Switzerland). All other chemicals used were of reagent grade (Carlo Erba, Milan, Italy) and were used without further purification.

Preparation of Glyceryl Triesters

A dry solution of the amount corresponding to 0.02 mol of the appropriate acyl chloride was gently added to a stirred warm solution (60 ± 1°C) of 3.58 g (0.01 mol) of glyceryl monostearate (**8**) in dry methylene chloride (50 ml). The reaction mixture was heated under reflux at 60°C with stirring for 48 hr, during which time HCl was evolved and product separated. The mixture was then evaporated off under reduced pressure, repeatedly washed with aqueous $NaHCO_3$, and water was added until pH 7. The resulting crude wax-like solid mass was collected by filtration, dried *in vacuo* and repeatedly crystallized from ethanol until gas-chromatographic purity. Four recrystallizations were sufficient to produce analytically pure samples of triesters. Analytical and spectral data were in line with the expected structures. Melting points and yields are reported in Table 1.

Lipospheres Preparation

The drug was incorporated in the melted material in aqueous dispersion. Fifteen grams of various composition mixtures of **2** and **9–13** were melted on a water bath preheated at 80°C until a homogeneous melt was attained. Compound **1** (5 g) was gradually added to the

Table 1

Melting Points and Yields of Glyceryl Triesters

Compound	Melting Point °C	Yield %
Glyceryl monostearate dicaprinate (**9**)	32–34	90
Glyceryl monostearate dilaurate (**10**)	38–40	97
Glyceryl monostearate dimyristate (**11**)	40–43	96
Glyceryl monostearate dipalmitate (**12**)	49–51	98
Glyceryl tristearate (**13**)	55–57	98

obtained mass under continuous stirring until homogeneous blend. To the molten mass were then added 200 ml of pre-heated (80°C) deionized water and the surfactant (0.5 g). The whole mixture was mechanically stirred at a constant predetermined speed of either 500, 700, 900, or 1200 rpm using a Polymix stirrer (model RW 20) equipped with a KCH-TRON digital spin counter (Kinematica, Switzerland) and fitted with a stainless steel four-blade impeller of approx. 45 mm diameter, pre-heated at about 70–80°C. The molten mass upon dispersion in the aqueous medium formed spherical oily particles. The temperature was maintained under stirring at 80°C for 3 min, after which time the mixture was rapidly cooled to room temperature. The oily material solidified, enveloping the drug. The resultant solid spherical particles were recovered by flotation, collected by filtration, extensively washed with water to remove any surfactant residues, and air-dried at room temperature for 48 hr. All batches of lipospheres were monitored under optical microscope with transmitted light (magnification 500×) to observe morphological characteristics and evaluate quality, shape, size, and homogeneity (Fig. 1).

Various compositions of mixed oleaginous materials were used. Most of them were not useful to obtain separated solid free-flowing lipospheres. The best mixture to obtain solid, discrete, reproducible, lipospheres consisted of glyceryl monostearate dilaurate (**10**) and stearyl alcohol (**2**) (ratio 3:17). The recovery yield was about 97% of the starting material.

Size Distribution of Lipospheres

The separation of the lipospheres into various size fractions was carried out using an Endecotts Octagon 200 test sieve shaker (Endecotts Ltd., England) and standard mesh wire sieves (Endecotts). A series of eight standard stainless steel sieves in the range 100–800 μm were arranged in the order of decreasing aperture size. Drug-loaded lipospheres (10 g) were placed on the upper sieve of the series. The sieves were mounted on the mechanical shaker operating for a period of time adequate for complete separation (about 15 min). The average sphere size of each fraction was determined as the arithmetic mean of the aperture size of the screen they were retained upon and the aperture size of the screen that they passed. The weight of separated material was measured and the size distribution determined (Table 2). Batches of spheres, prepared at the same drug

Figure 1. Morphological appearance of a batch of stearyl alcohol–glyceryl monostearate dilaurate (composition ratio 17:3) lipospheres sieved between 355 and 500 μm loaded with **1**. Transmission electron microphotograph; magnification 45,000×; 300 μmbar.

Table 2

Size Distribution and Drug Content of Stearyl Alcohol–Glyceryl Monostearate Dilaurate (Composition Ratio 17:3) Lipospheres Expressed as Percent. Results Are Reported as the Mean of Six Batches

Size Fraction (μm)	Lipospheres in Each Fraction (% ± SE)	Drug Content (%)
100–180	3.47 ± 0.24	23.24
180–250	9.56 ± 0.68	23.91
250–355	23.73 ± 1.38	24.19
355–500	27.03 ± 1.76	24.13
500–630	25.47 ± 1.49	23.65
630–800	9.66 ± 0.71	23.83
>800	1.08 ± 0.15	23.57

loading and stirring speed, were reproducible in terms of mean size.

Determination of Liposphere Content

The total amount of active ingredient incorporated into the lipospheres was obtained by first-derivative spectrophotometric determination. Amounts of lipospheres of each batch and size were randomly selected, microscopically observed, and accurately weighed, transferred into 100 ml measuring flask, and completed to volume by ethyl alcohol (UV grade). After sonification, the oleaginous material was dissolved, releasing all of the incorporated drug. The amount of **1** into the final solution was measured by first-derivative technique on a Shimadzu UV-1601 spectrophotometer, at λ = 247.4 nm, using an appropriate blank and a calibration curve. The first derivative curve of the absorption spectrum of phenytoin in ethyl alcohol showed negative peaks at 247.4, 260.8, and 268.0 nm. At 247.4 nm the peak was highly reproducible and linearly related to concentration over a range 0.2–10 mg/100 ml. The average drug content into lipospheres was 23.8% w/w (Table 2). Drug encapsulation efficiency was about 93.6%.

In Vitro Release from Lipospheres

Release of the active ingredient from lipospheres was measured using a system consisting of perspex screw-capped tubes thermostated at 37.0 ± 0.2°C (Polimix EH 2 bath equipped with a constant-rate adjustable stirrer RECO® S5 [Kinematica, Switzerland]).

Accurately weighed amounts of loaded lipospheres of each batch and size, equivalent to a total 100 mg of **1**, were suspended in 100 ml of simulated gastric (buffer pH 1.1 solution) or intestinal juices (saline phosphate pH 6, pH 6.5, and pH 7 buffer solutions) and kept at 37.0 ± 0.2°C with constant stirring (100 rpm). At fixed time intervals (20 min) after the start of experiments, samples (1 ml) of the solutions were withdrawn from the tube. In order to maintain the sink conditions, to the dissolution medium was added the same volume of fresh buffer solution at each time interval. The quantitative determination of **1** in the collected fractions was carried out using the second-derivative spectrophotometric technique and the appropriate calibration curve and blank. The curve in pH 6, pH 6.5, and pH 7 buffer solutions showed negative peaks at 241.4 and 264.4 nm. At 241.4 nm the peak was highly reproducible and linearly related to concentration over a range of 0.2–10 mg/100 ml.

The residual drug content in the lipospheres after release studies was determined for selected samples. The amount of drug released and the residual drug content matched the original drug content within 2–8%. No significant differences were observed in the release profile when liposphere size was varied. Experiments were carried out six times and mean results were reported (Fig. 2). Reproducibility was within 2.5% of the mean. Release patterns were constructed from the determined drug concentrations.

RESULTS AND DISCUSSION

The aim of this study was to evaluate the ability of fatty acid derivatives mixed with fatty alcohols as biocompatible materials in manufacturing microparticulate drug delivery systems. Such materials have adequate physicochemical properties for drug entrapment and allow delayed release in the intestinal lumen. Phenytoin (**1**), which exhibits high partition coefficient, allowed good percentage of drug to be entrapped into lipophilic mixtures.

In the present study oleaginous microspheres (lipospheres) containing **1** in a core:coat ratio of 1:3 were prepared. The technique involving melting and dispersion of drug-containing oleaginous material in aqueous medium and cooling-induced solidification of the system's oily phase was the method of choice for the entrapment of water-insoluble drugs. In this way, solid spherical, free-flowing lipospheres were formed after rapid cooling.

We selected stearyl alcohol (**2**), glyceryl monostearate dicaprinate (**9**), glyceryl monostearate

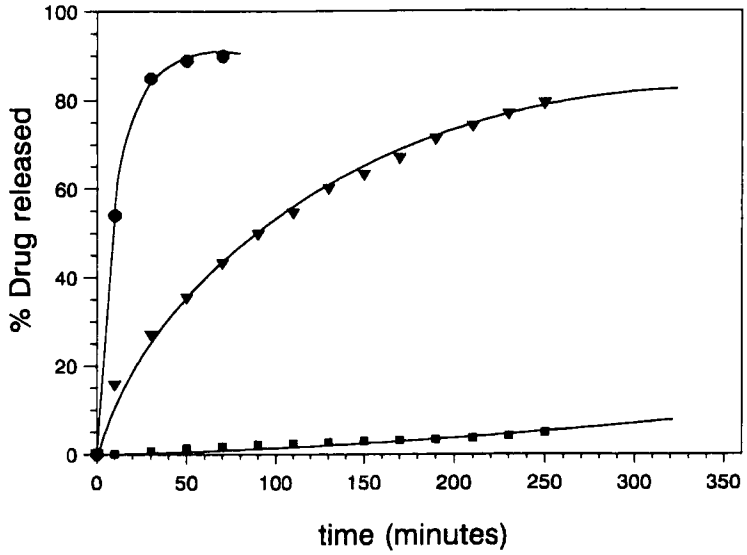

Figure 2. Percent of phenytoin released from (●) stearyl alcohol microspheres, (■) glyceryl tristearate lipospheres, (▼) stearyl alcohol–glyceryl monostearate dilaurate (composition ratio 17:3) lipospheres in the intestinal environment against time.

dilaurate (**10**), glyceryl monostearate dimyristate (**11**), glyceryl monostearate dipalmitate (**12**), and glyceryl tristearate (**13**) as oleaginous materials. The substances were chosen by the following criteria: melting interval should occur between 30 and 70°C; lower melting points could cause storage problems, higher melting points may cause difficulties from a manufacturing point of view. Materials should be characterized by water-insolubility to prevent rapid drug release and ability to dissolve the active ingredient for efficient drug entrapment. Moreover, a suitable chemical inertia toward the drug and the external phase was needed. Finally, materials should be biocompatible, nontoxic, inexpensive, and easily available.

For lipospheres preparation it was necessary to use the selected materials in combination. When stearyl alcohol alone was used as the matrix base, very low drug loading was observed as a consequence of the poor solubility of **1** in the matrix; moreover, the drug was leached out very quickly from stearyl alcohol lipospheres. When the tested triglycerides were used alone as the matrix base, the mass became low-melting and during the cooling–solidifying process, the major part resulted as aggregated cakes that adhered to the wall of the container or to the stainless impeller; liposphere formation did not occur owing to electrostatic phenomena. The drug release was also too slow when triglycerides were used alone as the matrix base. The release was correlated inversely with the melting points of the triglycerides used; fastest release was observed from lipomatrices of glyceryl monostearate dicaprinate (**9**), with the lowest melting point (32–34°C) when compared to those prepared with higher melting point triglycerides.

Drug loading and release seemed to be related to the hydrophobicity of the oleaginous material. The release was expected to be regulated by selecting an appropriate mixture of two or more materials.

Combinations of two components at various ratios were examined to obtain solid, free-flowing loaded lipospheres. Few of the mixtures investigated possessed adequate ability to form separated lipospheres containing drug amounts sufficient for therapeutic use. The best mixture was made of stearyl alcohol (**2**) and glyceryl monostearate dilaurate (**10**). The optimum composition ratio was 17:3. Higher levels of **10** in the mixture produced low-melting lipospheres while larger amount of **2**, diminishing drug solubility in the matrix, lowered the drug entrapment; solid drug crystals mixed with lipospheres were observed by optical microscopy.

Because the partition coefficient was dependent on the pH value of the aqueous medium liposphere preparation was carried out at pH 7. The gradual increase of the pH value gradually diminished the liposphere content, promoting drug dissolution in the external phase.

Incorporation of **1** into the lipospheres required the addition of a surfactant at a minimal concentration to

decrease the interfacial tension between the hydrophobic material and the aqueous external phase producing wettable oleaginous material. Various surfactants were used to determine the optimum hydrophilic–lipophilic balance (HLB) value. It was found that surfactant having HLB 8.6 was more appropriate to increase substantially dispersion of lipophilic material and promote drug incorporation into the lipospheres. The optimum surfactant concentration to produce discrete lipospheres that exhibit good flow properties was 5% (w/w).

The optimum stirring speed to obtain reproducible particle size and yields was 900 rpm. Higher percentages of smaller particles were observed when the stirring rate was increased over 900 rpm and amounts of liposheres were lost during successive washings; on the other hand, a stirring speed slower than 900 rpm led to losses of melted material that adhered to the beaker walls during the cooling process.

Sieve analysis showed that most of the isolated lipospheres were of particle size range 100–800 μm. Drug content determinations in various particle size ranges were performed; no marked variation in the drug amount:particle size ratio was observed, indicating that the ratio between drug and oleaginous materials remained practically constant. Size distribution and drug content are reported in Table 2. The average drug content for all size fraction combined was 23.8% w/w. The incorporation efficiency within the lipospheres was 95.2%. Using the mixture of optimum composition ratio, the recovery yield was about 97% of the starting material. When the composition ratio was altered the recovery yield of lipospheres dropped to 20–35%.

Transmission electron microscopy on all batches of lipospheres, prepared with the process conditions listed before, revealed completely formed microparticulate with spherical shape and smooth surface. No drug crystals were observed. Fig. 1 shows the photomicrograph of a batch of stearyl alcohol–glyceryl monostearate dilaurate (composition ratio 17:3) lipospheres.

Previous publications (19,23,24) have discussed the behavior and the main release models used to describe the drug discharged from loaded lipospheres of various hydrophobic materials like white beeswax, hexadecanol, and carnauba wax.

From each composition studied, the drug was released in the intestinal environment at a rate that decreased with time, and the kinetic performance was greatly affected by the material used in the microencapsulation process.

The phenytoin release kinetics from the lipospheres were evaluated using in vitro testing in conditions approaching those in the gastrointestinal tract. Drug dissolution was followed by periodically measuring the released amount in the simulated fluid. The active ingredient leached from the lipospheres was measured by the second-derivative spectrophotometric technique and the appropriate calibration curve and blank. The curve in buffer solutions at pH 6.0, 6.5, and 7.0 showed negative peaks at 241.4 and 264.4 nm. At 241.4 nm, the peak was highly reproducible and linearly related to concentration over a range of 0.2–10 mg/100 ml. During the release experiments the lipospheres were observed by optical microscopy to evaluate modifications in shape and size.

Although in the gastric juice the lipospheres changed shape, the amount of the discharged drug was undetectable. Very low amounts of **1** in the gastric juice were probably due to poor intrinsic drug solubility in the environment rather than the gastro-resistant characteristics of the material used for the drug entrapment. Fig. 2 shows the drug released in the intestinal environment from equivalent amounts of lipospheres containing 10 mg of phenytoin in a matrix, respectively made of stearyl alcohol, glyceryl tristearate and their mixture (ratio 17:3), with 355–500 μm diameter versus time. It was observed that decreasing the triglyceride content significantly increased drug release. Although stearyl alcohol has excellent ability to form microspheres, rapid drug release from this material would not be unexpected due to the leaching characteristics. On the other hand, glyceryl esters of fatty acids are hydrophobic in nature and retard the rate of dissolution fluid into the microparticles, consequently retarding drug release.

Optical microscopy showed that drug dissolution from the microparticulate system resulted in particles similar to spheres from which the drug had been leached, suggesting that the drug release was diffusion-controlled. We attempted to describe the release profile by a model function. On application of differential rate treatments and linear regression analysis, evidence supported the Higuchi matrix model for diffusion-controlled transport in a matrix. Fig. 3 shows the discharged drug amounts versus the square root of time. The Higuchi diffusion equation gave consistently higher values for the correlation coefficient (0.996–0.998) than did the main release models usually used to describe the drug discharge from microparticulate systems. Experimentally, the linear diffusion model held up to 80–90% drug release, after which the rate decreased progressively. The deviation would be explained by the exhaustion of drug phase from lipospheres, which as a consequence led to dependence on drug content.

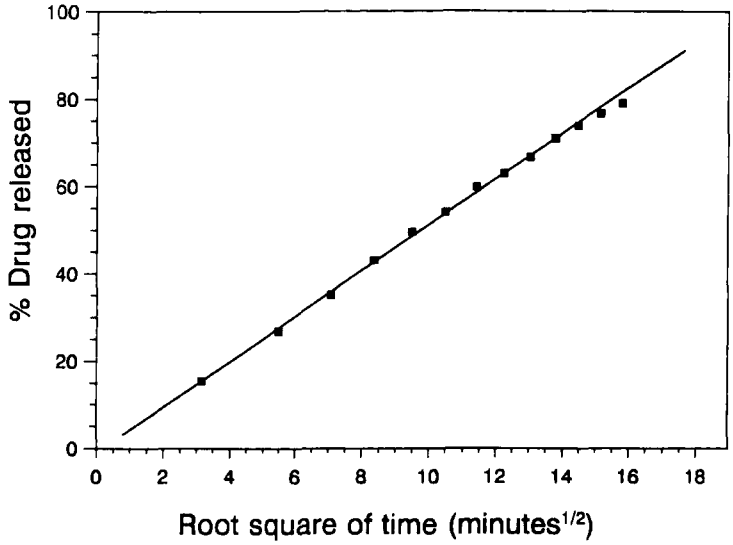

Figure 3. Percent of released phenytoin from stearyl alcohol–glyceryl monostearate dilaurate (composition ratio 17:3) lipospheres in the intestinal environment against the root square of time.

About 60% of the entrapped drug was discharged in 150 min of stay in the intestinal environment; complete release occurred in about 6 hr. The drug release from stearyl alcohol–glyceryl monostearate dilaurate (composition ratio 17:3) lipospheres was sufficient for oral use.

CONCLUSIONS

Drug-containing lipospheres were prepared by the meltable dispersion process using stearyl alcohol and glyceryl esters of fatty acids that are biocompatible, biodegradable, nontoxic materials. Combinations of two components at various ratios were examined to obtain solid, free-flowing loaded lipospheres. Few of the mixtures investigated possessed adequate ability to form separated lipospheres containing drug amounts sufficient for therapeutic use. The best mixture was made of stearyl alcohol and glyceryl monostearate dilaurate. The optimum composition ratio was 17:3. Reproducible lipospheres could be prepared for intestinal release of phenytoin using a quite simple, rapid, economical technique, that does not imply the use of organic solvents. The drug release profiles were significantly affected by the properties of the waxy materials used in the microsphere preparation. The kinetic performance fits well with a diffusional behavior. The cumulative amount was sufficient for oral use. The method described achieves good incorporation efficiency (93.6%; average drug content 23.8%) and should be very useful for the development of controlled-release dosage forms.

The used substances represent a class of biocompatible materials that can be used to prepare multiparticulate delivery systems and release lipophilic drugs in the intestinal tract.

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