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CARNAUBA WAX MICROSPHERES LOADED WITH VALPROIC ACID: PREPARATION AND EVALUATION OF DRUG RELEASE

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ABSTRACT

To minimize unwanted toxic effects of valproic acid (**1**) by the kinetic control of drug release, gastroresistant carnauba wax microspheres loaded with the antiepileptic agent were prepared. The preparation was based on a technique involving melting and dispersion of drug-containing wax in an aqueous medium. The resulting emulsion after cooling under rapid stirring produced solid, discrete, reproducible free flowing microspheres which converted the liquid drug droplets into solid material. About 94% of the isolated microspheres were of particle size range 200-425 μm. The microspheres were analyzed to determine the drug content in various particle size range and to characterize the *in vitro* release profile. The average drug content was 26% w/w. The intestinal drug discharge of **1** from the carnauba wax microspheres was studied and compared with the release patterns observed for white beeswax and hexadecanol microspheres previously described. The drug release performance was greatly affected by the material used in the microencapsulation process. In the intestinal environment carnauba wax microspheres exhibited more rapid initial rate of release and about 80% of the entrapped drug was discharged in 120 min while complete release occurred in about 8 h.

INTRODUCTION

Valproic acid (2-propylpentanoic acid) (**1**) has been widely used in the treatment of several types of epilepsy (1). However, the long therapeutic regimen with **1**

has often been marred by high incidence of adverse drug reactions on the gastrointestinal tract as well on hepatic, pancreatic, renal, endocrine, immunologic, cutaneous, central nervous and reproductive systems (2), likely arising from zinc and selenium deficiency due to binding by the drug (3,4). Severe hepatotoxicity (5), hyperammonemia (6), embryotoxicity (7,8), teratogenic effects (9), skeletal defects (10), neurotoxicity and spina bifida in fetuses of women exposed to the drug in early pregnancy are reported (11,12). The drug should be dosed at least three, and preferably four, times a day because of its short half-life and the frequency of adverse effects may be dose-related (13,14). Depression of side effects of **1** could be obtained controlling the drug discharge from administration form (15-17). As demonstrated by pharmacokinetic studies on **1** (18), the ingestion of a single controlled release enteric coated tablet is effective even when administered once a day. These findings suggested that kinetic control is an effective method for evident lack of toxicity.

Microspheres for oral use, modifying the dissolution of drugs, allow the administration of much smaller doses than are normally required and provide a method of achieving controlled release rates in the desired site. This reduces local irritation when compared to single-unit dosage forms.

Previous experimental results from these laboratories demonstrated the aptitude of beeswax and hexadecanol in the preparation of microspheres loaded with lipophilic drugs (19-22). The microspheres prevent drug discharge in the stomach, thus ensuring dissolution in the desired part of the digestive tract employing known rates of release.

These considerations led to the objective of this study to investigate and evaluate the behaviour of carnauba wax in the preparation of microspheres containing **1** and the related drug release kinetic. The system was chosen because the easiness of manufacture and the wax physical properties and *in vivo* behaviour (23,24).

MATERIALS AND METHODS

Valproic acid was purchased from Janssen Pharmaceutica, Geel (Belgium), carnauba wax from A.C.E.F., Fiorenzuola D'Arda (Italy) and the surfactants Tweens® 21, 60, 80, 85 and Spans® 20 and 40 from Fluka, Buchs (Switzerland). All the solvents and reagents were of analytical grade (Carlo Erba, Milan, Italy) and were used without further purification. Preparation of microspheres of hexadecanol and white beeswax containing **1** was accomplished as described before (21,22).

Microsphere preparation.

The drug was incorporated as liquid particles in the melted material in aqueous dispersion. Carnauba wax (10 g) was melted on an oil bath preheated at 110°C.

On wax becoming molten, **1** (3 g) was stirred into it to obtain a homogeneous melt. To the mixture were then added 100 ml of a pH 4.5 solution (to minimize the solubility of **1**) previously combined with 100 ml of glycerine (to increase the boiling point) and heated to a temperature higher than the melting point of the carnauba wax (104°C). To the mixture was then added the surfactant (0.26 g). The whole mixture was mechanically stirred at a constant predetermined speed of either 600, 800, 1000 or 1200 rpm using a Polymix stirrer (mod. RW 20) equipped with a KCH-TRON digital spin counter (Kinematica, Switzerland) and fitted with a four-bladed impeller of approx. 45 mm diameter. The molten mass upon dispersion in the aqueous medium formed spherical particles. The temperature was maintained with stirring at 104°C for 3 min after which time iced water was added until room temperature was reached. The carnauba wax solidified enveloping the drug. The resultant solid spherical particles were recovered by flotation, collected by filtration and extensively washed with water to remove any drug and surfactant residues. Air drying at room temperature for 48 h gave solid free-flowing microspheres. The recovery yield was about 94% of the starting material.

All batches of microspheres were monitored under optical microscope with transmitted light (magnification 500 x) to confirm morphological characteristics and evaluate quality, shape size and homogeneity.

Size distribution of microspheres.

The separation of the microspheres into various size fractions was carried out using an Endecotts Octagon 200 sieve shaker (Endecotts Ltd, England) and standard mesh wire sieves (Endecotts). A series of six standard stainless steel sieves in the range 100-710 μm were arranged in the order of decreasing aperture size. Drug loaded microspheres (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 (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. Batches of spheres, prepared at the same drug loading and stirring speed, were reproducible in terms of mean size.

Determination of the microspheres content.

The total amount of active ingredient incorporated into the microspheres was determined by a modified method of gas-liquid chromatography developed by Johno and others (25). Amounts of microspheres of each batch and size were randomly selected, microscopically observed, accurately weighed, quantitatively transferred into 100 ml measuring flask and completed to volume by carbon tetrachloride (GC grade). After sonification the waxy material was dissolved

releasing all of the incorporated drug. The amount of **1** into the final solution was measured by a Perkin-Elmer 8500 gas-chromatograph equipped with a DB-1 J & W, 15 m, 0.53 mm I.D., fused silica megabore column. Chromatography was accomplished at 90°C in isothermal condition using N_2 as carrier gas (P = 110 kPa), injector and detector (FID) at 250°C. As internal standard a carbon tetrachloride (GC grade) solution containing 1 mg/ml of methyl pelargonate was used. The samples were resolved in about 4 min. The ratio between the peak area of **1** and the standard was 0.80. In these conditions the R_t of **1** was 1.83 min and the R_t of the standard 0.98 min. The average drug content resulted 26% w/w.

Release from microspheres.

Release of the active ingredient from microspheres was measured at 37.0 ± 0.2°C using perspex screw capped tubes and a constant-rate adjustable, thermostatated stirrer (Polymix, EH2 Reco® S5, Kinematica, Switzerland). Accurately weighed amounts of loaded microspheres 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). To improve the wettability of microspheres 0.2% of Tween® 20 was added to the buffer solution. At fixed time intervals after the start of experiments, samples (3 ml) of the solutions were withdrawn from the tube and the volume was readjusted with fresh buffer solution to keep the volume of dissolution medium constant. The samples were extracted with the same volume of carbon tetrachloride (GC grade)(3 x 1 ml) and dried with anhydrous sodium sulphate. The quantitative determination of **1** in the collected fractions was carried out using the GC method, by measuring the peak area and comparing with a calibration curve of the standard. The residual drug content in the microspheres after release studies was determined for selected samples by extraction with carbon tetrachloride. The amount of drug released and the residual drug content matched the original drug content within 2 and 8%. No significant differences were observed in the release profile varying the microspheres size. Experiments were carried out six times, and mean results were reported. Reproducibility was within 5% of the mean. Release patterns were constructed from the drug concentrations determined via GC analyses.

RESULTS AND DISCUSSION

Evidence has been accumulated in recent years that waxy materials own physical properties suitable to prepare gastroresistant, biocompatible microspheres allowing release in the intestinal lumen for those drugs characterized by adequate physicochemical properties for incorporation into lipid carriers (19-22). High

TABLE

Size fraction (μm)	Microspheres in each fraction (% ± SE)	Drug content (%)
100 - 200	6.2 ± 0.24	25.3
200 - 250	21.4 ± 0.68	25.7
250 - 300	57.5 ± 1.78	26.2
300 - 425	7.2 ± 0.29	26.2
425 - 710	7.7 ± 0.28	26.5
> 710	-	-

Size distribution and drug content of microspheres expressed as percent. Results are reported as the mean of six batches.

partition coefficient exhibited by valproic acid suggested good percentage of drug entrapment into lipophilic microspheres made of carnauba wax.

In the present study carnauba wax microspheres containing valproic acid in a core to coat ratio of 2 : 5 were prepared using the technique involving cooling-induced solidification of the oily phase of a two-phase system. The drug and the wax were mixed in a homogeneous melt and emulsified into the aqueous external phase; solid spherical, free-flowing microspheres were formed after rapid cooling. Being the partition coefficient dependent by the pH value of the aqueous medium the microspheres preparation was carried out at pH 4,5. The gradual increase of the pH value gradually diminished the microsphere content. The amount of drug entrapped modulated from 26% to 10% when pH value changed from 4,5 to 7.

Incorporation of **1** into the microspheres needed the addition of a surfactant at a minimal concentration to decrease the interfacial tension between the hydrophobic material and the aqueous external phase and make wettable the waxy material. The optimum surfactant concentration to produce discrete microspheres which exhibit good flow properties was 2% (w/w). It was found that surfactants having a hydrophilic-lipophilic balance (HLB) value of 8.6 was more appropriate to increase substantially dispersion of lipophilic material and promote drug incorporation in the microspheres.

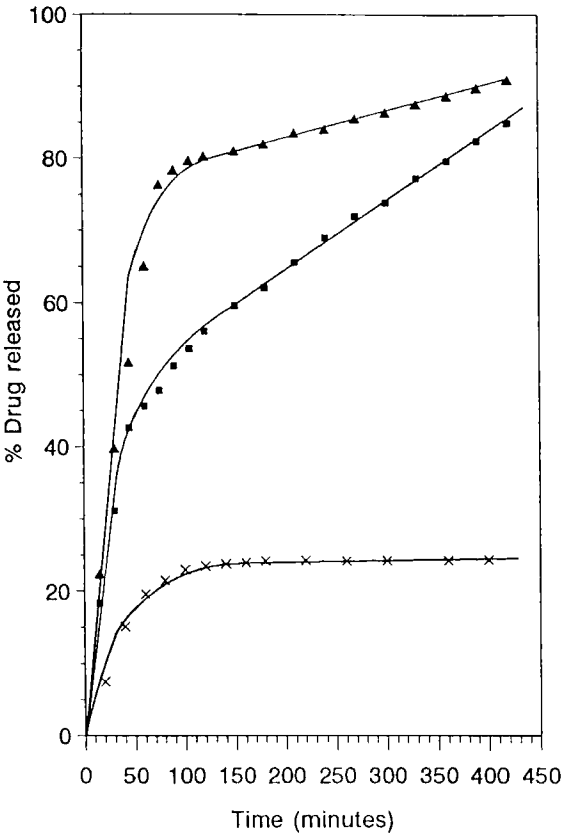

FIGURE 1

Percent amount of released valproic acid from (▪) beeswax microspheres, (▲) carnauba microspheres and (X) hexadecanol microspheres in the intestinal environment against time

Sieve analysis showed that most of the isolated microspheres were of particle size range 200-425 μm, and about 80% were of size fraction 200-250 μm. Drug content determinations in various particle size ranges were performed; no marked variation in the drug amount to particle size ratio was observed, indicating that the ratio between drug and waxy material remained practically constant. Size distribution and drug content are reported in Table. The average drug content for all size fraction combined resulted 25.98% w/w. The incorporation efficiency within the microspheres was 86,7%. No significant differences were observed in drug content with the use of the various surfactants in the range 6.7-11 HLB value.

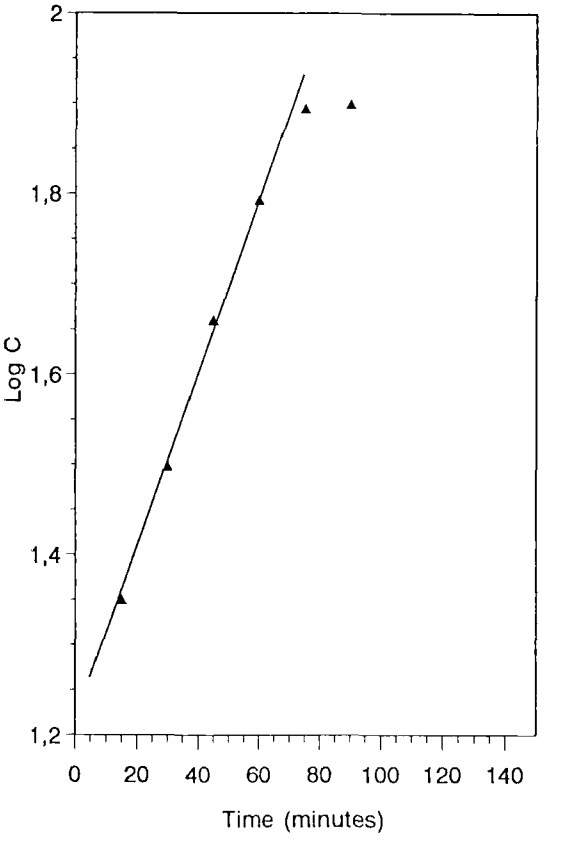

FIGURE 2

Logarithm of valproic acid percent amount released from carnauba microspheres in the intestinal environment against time before the range of 50-60% of drug release.

The optimum stirring speed to obtain reproducible particle size and yields was 800 rpm. Higher percentages of smaller particles were observed when stirring rate was increased over 800 rpm and amounts of microspheres were lost during successive washings; on the other hand the decrease of the speed lower 800 rpm leaded to the loss of melted material which adhered to the beaker walls during the cooling process.

The valproic acid *in vitro* release from the microspheres was accomplished in conditions approaching those in the gastro-intestinal tract. Drug dissolution was followed by periodically measuring the released amount in the simulated fluid. In

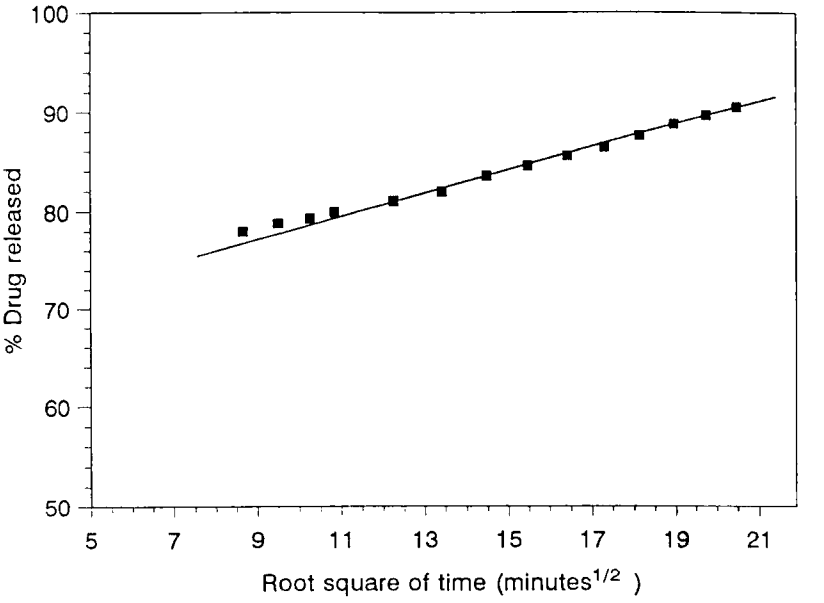

FIGURE 3

Percent amount of released valproic acid from carnauba microspheres in the intestinal environment against the root square of time over the range of 50-60% of drug release.

the gastric juice the active ingredient released was undetectable indicating that carnauba wax is a suitable material to produce gastroresistant multipartuculate delivery systems. The results of dissolution experiments in the intestinal environment are reported in Fig. 1 which shows the percent amount of **1** released from microspheres *versus* time.

The drug was released in a biphasic manner consisting of an initial fast-release stage followed by a slow-release stage. The initial fast stage suggested that the first order equation was the most appropriate one for describing the release behaviour as shown in Fig 2 where the logarithm of discharged drug amounts are reported *versus* time.

Moreover the first order equation gave consistently higher values for the correlation coefficient (0.996-0.998) than did the diffusion equation (0.978-0.990). Over 70% of drug release (Fig 3), treatment of the experimental data on the basis of the diffusion-controlled model indicated that in the slow release phase the drug discharged increased linearly with the square root of time as

indicated by the higher values of the correlation coefficients (0.995-0.999 for the diffusion equation and 0.987- 0.991 for the first order equation).
In previous publications (21,22) have been discussed the behaviour and the main release models used to describe the drug discharged from white beeswax and hexadecanol microspheres loaded with **1**. From each composition studied the drug was released in the intestinal environment at a rate that decreased with time. While hexadecanol microspheres released **1** only by a first order kinetic pattern more rapid initial rate was observed for white beeswax and carnauba wax microspheres. Carnauba wax microspheres exhibited faster initial rate of release and about 80% of the entrapped drug was discharged in 120 min while complete release occurred in about 8 h. The drug release performance was greatly affected by the material used in the microencapsulation process.

CONCLUSIONS

Reproducible microspheres could be prepared for intestinal release of valproic acid using a technique quite simple, rapid, economical which does not imply the use of organic solvents. The method described achieves good incorporation efficiency (86.7%; average drug content 26%) and should be very useful for the development of controlled release dosage forms. Carnauba wax was a suitable material for the entrapment of **1** in biocompatible microspheres converting the liquid drug droplets into solid material. The drug discharge was rapid in the initial stage and slower in the second stage of release. The kinetic performance for all size of microspheres fits initially the first order equation followed by a diffusional behaviour. The cumulative amount was sufficient for oral use. The drug release profiles were significantly affected by the properties of the waxy materials used in the microsphere preparation.
The waxes represent a class of biocompatible materials which are able to prepare multiparticulate delivery systems and release lipophilic drugs in the intestinal tract.

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