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PREPARATION OF WHITE BEESWAX MICROSPHERES LOADED WITH VALPROIC ACID AND KINETIC STUDY OF DRUG RELEASE

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ABSTRACT

The well known antiepileptic valproic acid (**1**) due to the long treatment of epilepsy may induce many adverse side effects on various systems. To minimize unwanted toxic effects by kinetic control of drug release, **1** was physically entrapped into white beeswax microspheres using the meltable dispersion process utilizing wetting agents. Solid, discrete, reproducible free flowing microspheres were obtained converting the liquid drug droplets into solid material. The average drug content was 17% w/w. More than 95% of the isolated microspheres were of particle size range 200-425 μm. The microspheres were analyzed to quantify the amount of incorporated drug and to characterize the *in vitro* release profile. The matematical approach to drug release using standard

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equations indicated that the first order equationis was the most appropriate one for describing the initial drug release profile; after about the 50-60% of drug was discharged numerical data fit well with the root of time equation. The drug removal from microspheres was compared with that obtained from a commercially available enteric coated oral formulation of **1** (Depakin®). The drug release performance was greatly affected by the microsphere formation which allows absorption only in the intestinal tract.

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INTRODUCTION

The 2-propylpentanoic acid also named Valproic acid (**1**) is well known as an antiepileptic agent. (1) After oral administration it is rapidly dissociated to the valproate ion, partially absorbed from stomach although at a slower rate than from the whole intestinal tract. (2) Recently it has been proposed an intestinal pH-dependent absorption mechanism that primarily involves a proton gradient-dependent carrier system for the transport through the brush-border membrane. (3) Considering the long regimen of antiepileptic therapy the administration of **1** may induce adverse side effects on the gastrointestinal tract as well on hepatic, pancreatic, renal, endocrine, immunologic, cutaneous, central nervous and reproductive systems. (4) Side effects could be lowered controlling the drug release from an implantable and refillable drug reservoir device, (5) or adjusting the absorption rate by formulation of peroral dosage forms, (6) or delivering the drug in the intestinal environment from particles coated with a special enteric cellulose polymer (Depakin®) or entrapping the drug into liposomes, (7) or administering polymer containing controlled release dosage forms of **1** or its derivatives. (8) These findings suggested that kinetic control is an effective method for evident lack of toxicity.

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The formation of microspheres is one of the methods used by the formulation development to obtain optimum control of kinetics of drug release from the administered form. (9) Previous experimental results demonstrated the release behaviour of drugs entrapped into microspheres (10-12) of beeswax which represents a biocompatible material, non-immunogenic and able to release drugs in the intestinal tract.

In the present study would describe the method of preparation, characterization and the in vitro drug release profile of microspheres of white beeswax loaded with **1**; the drug discharge from the microspheres is modelled using standard drug release equation and compared with that of the commercially available enteric coated oral formulation Depakin ®.

MATERIALS AND METHODS

Valproic acid was purchased from Janssen Pharmaceutica, Geel (Belgium), white beeswax 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). Depakin® was obtained from Sigmatau, Pomezia (Italy). All the solvents and reagents were of analytical grade and were used without further purification.

Microsphere preparation.

White beeswax (10 g) was melted in a water bath at 100°C; on becoming molten, **1** (3 g) was stirred into it to obtain a homogeneous melt; this mixture was then poured into 200 ml of acidified (HCl until pH 4.5), deionized water previously heated to a temperature higher than the melting point of the wax (80° C). To the mixture was then added the surfactant (0.26 g). The whole mixture was mechanically stirred at a constant predetermined speed of 800 rpm using a

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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 formed spherical particles upon dispersion in the aqueous medium. The temperature was maintained with stirring (800 rpm) at 80°C for 3 min after which time iced water was added until room temperature was reached. 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 92% of the starting material.

All batches of microspheres were monitored under optical microscope with transmitted light (Fig. 1) to confirm morphological characteristics and evaluate quality, shape, size and homogeneity of the powder.

Size distribution of microspheres.

Drug incorporated microspheres (10 g) were placed on the top of a series of six standard stainless steel sieves in the range 100-710 μm (Endecotts Ltd, England), staked from bottom to top in ascending order of aperture size. The sieves were mounted on the mechanical sieve shaker (Endecotts, Octagon 200) and shaken for 15 min. The weight of material on each sieve 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.

Loaded microspheres (100 mg) of each batch and size were randomly selected, microscopically observed and finely powdered in a mortar. Methylene chloride (50 ml)(GC grade) was added in

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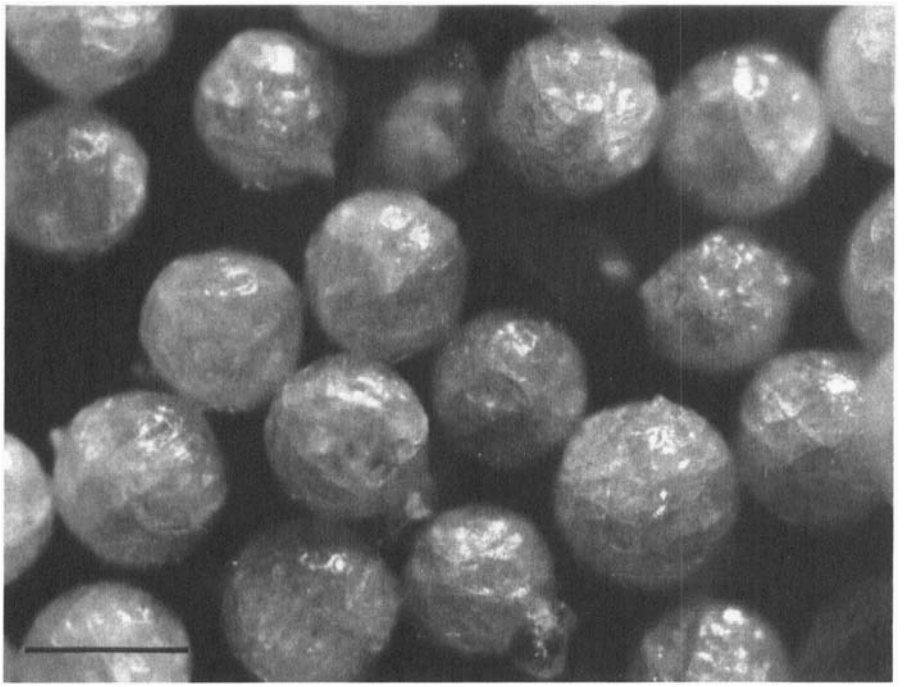

FIGURE 1

Transmission electron microphotograph showing the morphological appearance of a batch of beeswax microspheres containing valproic acid; magnification, x 45000; bar, 250 μm

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portions to the mortar content, quantitatively transferred into 100 ml measuring flask and completed to volume by methylene chloride (GC grade). Under these conditions the wax was completely dissolved releasing all of the incorporated drug. The total amount of **1** into the final solution was measured by GC (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 carried

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out 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 methylene chloride (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 17% w/w .

In vitro drug release from microspheres.

Accurately weighed quantities 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) in a perspex screw capped tube and kept in a water bath at 37.0 ± 0.2°C with constant stirring (100 rpm). A small amount of surfactant (0.2% Tween 20) was added to the buffer to improve the wettability of microspheres. At appropriate intervals of time samples of 3 ml 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 methylene chloride (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. The residual drug content in the microspheres after release studies was determined for selected samples by extraction in methylene chloride. 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 four 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.

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In vitro release from Depakin®. Amounts of Depakin® enteric coated tablet equivalent to a total 100 mg of **1** were suspended in 100 ml of buffered pH 6, pH 6,5 and pH 7 simulated intestinal juices. Samples of 3 ml were withdrawn from the tube every 1 min and treated as described before. Release patterns were constructed from the drug concentrations determined via GC analyses.

RESULTS AND DISCUSSION

The white beeswax due to its physical properties and behaviour in the intestinal lumen proved to be suitable to prepare gastro-resistant microspheres. (11) The method used in this study, involving cooling-induced solidification of the oily phase of a two-phase system, is advantageous for the entrapment of the water-insoluble drugs. In this method the drug and wax were mixed to obtain a homogeneous melt. The mixture was then emulsified into the aqueous phase and spherical, free-flowing microspheres were formed after cooling. **1** could be entrapped as liquid particles because its poor aqueous solubility and hence low partitioning into the external aqueous phase during microsphere formation. However the entrapment amount of **1** was pH dependent. As the pH value of the external phase diminished, the solubility of **1** lowered and the encapsulated amount of the drug increased. The maximum load was obtained at pH 4.5. The increase of pH value to 7 dropped the microsphere content below 10%.

Addition of a surfactant at a minimal concentration was required to make the hydrophobic material wettable and to form individual microspheres. An attempt to incorporate liquid **1** inside of the microspheres without the addition of a surfactant failed and resulted an aggregated cake during the solidification of the wax likely due to repulsion resulting from high interfacial tension between the

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TABLE 1

Size range [μm]					
100	200	250	300	425	710
4.7	12.8	64.8	9.9	7.8	-

Size distribution of microspheres expressed as percent
Mean of six batches

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hydrophobic material and the aqueous external phase. For an optimal surfactant concentration, various concentrations ranging from 0.2 to 10% (w/w) of the total formulation were examined. Concentrations of surfactant ranging from 0.2 to 1.8% (w/w) gave not reproducible microspheres and the resulted products were composed of irregular masses in which was impossible to distinguish individual particles. 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. Solid, free-flowing microspheres were obtained after cooling converting the liquid drug droplets into solid material.

To obtain discrete microspheres suitable for further manipulations the optimum drug to wax ratio was nearly 3 : 10 w/w. It was found that higher amounts of **1** led to the formation of droplets which aggregated during the cooling process as a consequence of the

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lowered melting of the waxy material; the produced masses were unsuitable for pharmaceutical uses. Fig 1 shows the typical morphological appearance of a batch of beeswax microspheres in the transmission electron microscopy; the particles were spherical and possessed a continuous surface.

Sieve analysis showed that most of the isolated microspheres were of particle size range 200-425 μm, and about 65% were of size fraction 250 μm. No marked differences were observed in the particle size distribution as a function of surfactant HLB value between 6.7 and 11. The sieving analysis results obtained from the mean of six batches are listed in Table 1.

The main factor influencing the size distribution was the stirring rate used during the preparation of spheres. There was little difference in the size of the spheres made at a stirring speed of 800 rpm and the yield was about 92-94% w/w of the starting material. Repeat batches treated in this way proved to have reproducible particle sizes, indicating that stirring condition, cooling rate and separation process were well controlled. We observed that increasing the stirring speed in the range from 800 to 1200 rpm the decrease of the average size of spheres was sharp as well the recovery yield of the microspheres. It seemed that recovery was lower when the particle size was smaller because amounts of smaller microspheres were lost during successive washings. When the stirring speed was slower than 800 rpm larger pellets were formed and amounts of melted material adhered to the beaker walls during the cooling process lowering the recovery yield.

Drug content determinations in various particle size ranges were performed. The average content of **1** resulted 17% w/w. No significant variation in the drug amount to particle size ratio was observed.

The releases of the active ingredient from the microspheres and from Depakin®, which is a commercially available enteric coated

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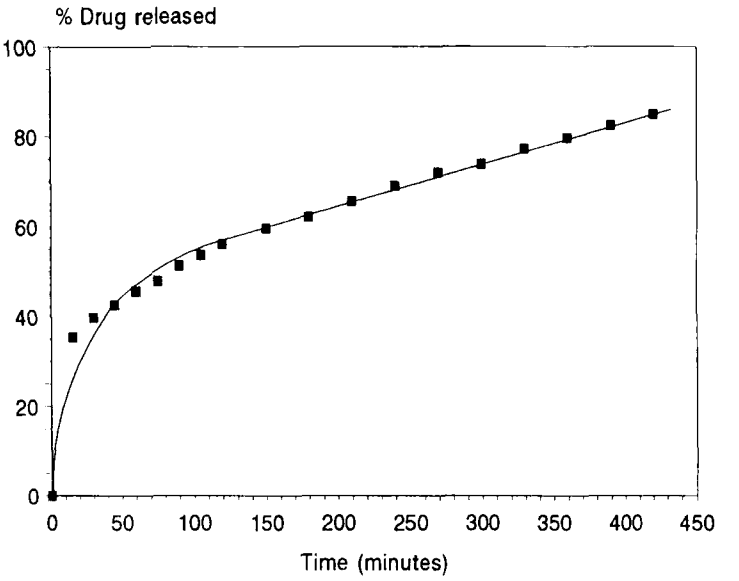

FIGURE 2

Percent amount of released valproic acid from beeswax microspheres in the intestinal environment against time

solid dosage formulation of **1**, were performed at 37°C in conditions approaching those in the gastro-intestinal tract and followed by periodically measuring the released drug amount. Random selected samples of microspheres were observed microscopically before measurements of release. Fig 2 shows the % amount of **1** released from wax microspheres *versus* time in the intestinal environment. In the gastric juice the released amount was undetectable. The observed profiles of released **1** were compared with those obtained from an equivalent amount of Depakin® (Fig 3). The microspheres considerably retarded the drug release when compared to non-encapsulated commercial formulation.

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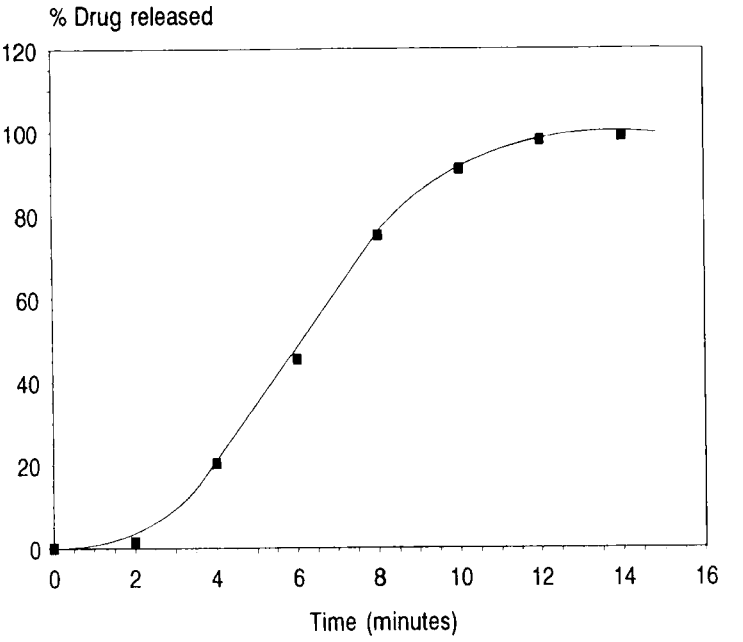

FIGURE 3

Percent amount of released valproic acid from Depakin® the intestinal environment against time

The mathematical approach to kinetic behaviour of microparticles offers three equations that are frequently used to describe the drug release (13-15): the first order equation (**a**), the Higuchi diffusion-controlled model (square root of time) (**b**) and the cube root equation (**c**).

$$\log Q_t = \log Q_o - \frac{k\,t}{2{,}303} \qquad (\mathbf{a})$$

$$Q_t = k\,t^{1/2} \qquad (\mathbf{b})$$

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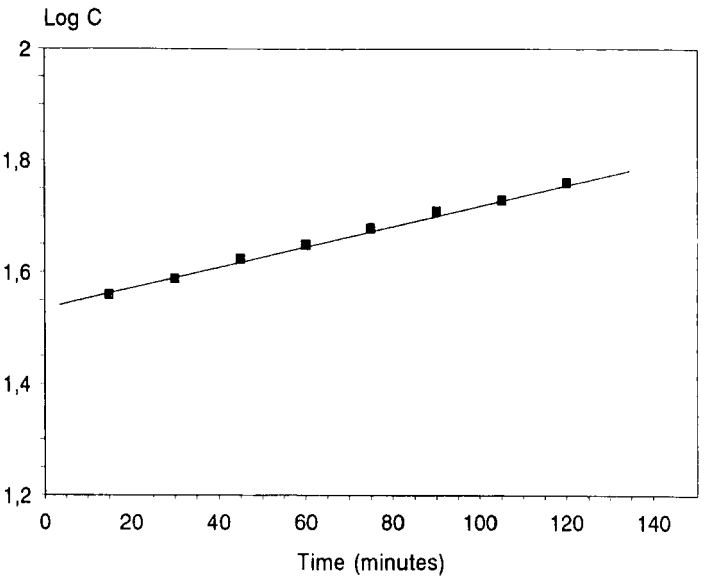

FIGURE 4

Log of percent amount of released valproic acid from beeswax microspheres in the intestinal environment against time before the range of 45-60% of drug release

$$\left(1 - \frac{Q_t}{Q_\infty}\right)^{1/3} = -kt \qquad (\mathbf{c})$$

were Q_t is the amount of drug discharged from microspheres after time t, Q_0 is the initial drug content and Q_∞ is the amount of drug released after $t = \infty$; k are constants depending on model.

The equation (**c**) is used for pellets of cubic shape which do not change in shape during drug release. The analysis of the release mechanism by means of the cube root equation did not show either linear plots or good correlation coefficients. Moreover optical

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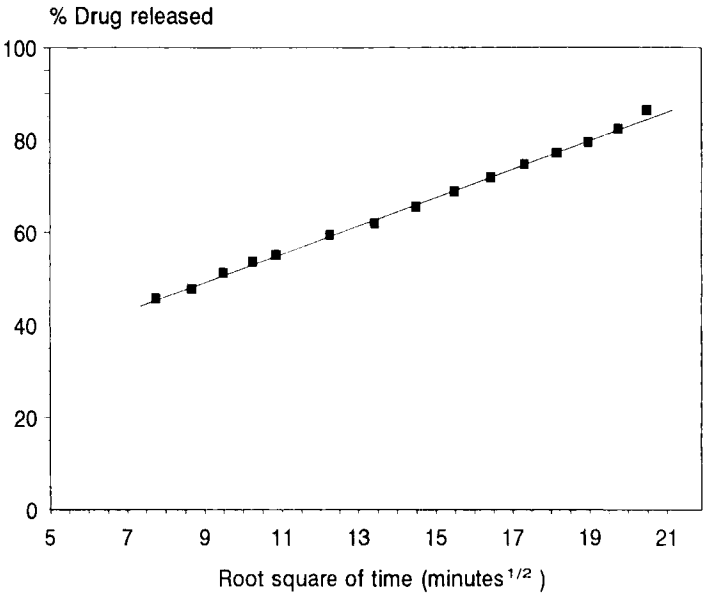

FIGURE 5

Percent amount of released valproic acid from beeswax microspheres in the intestinal environment against the root square of time over the range of 45-60% of drug release.

microscopy showed that the particles before the release tests were of spherical shape and during all the experiments became like an amorphous powder until complete exhaustion of the drug. The cube root equation was therefore ruled out.

Numerical fits indicated that the first order equation was the most appropriate one for describing initial release behaviour as shown in Fig 4 where Log Q_t is reported *versus* time. Treatment of the experimental data on the basis of the diffusion-controlled model indicated that the drug concentration increases linearly with the square root of time over the range of 50-60% of drug release (Fig 5).

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Exhaustion of microspheres occurred in about 11-12 h as obtained by extrapolation of the kinetic results.

These findings are in line with the results obtained in a preceding study on release of **1** together with vitamin E from hexadecanol microspheres. (12)

CONCLUSIONS

The results reported show that reproducible microspheres could be prepared for intestinal release of valproic acid using the meltable dispersion method. The technique is easy, rapid, inexpensive and does not imply the use of organic solvents which could be poisonous to the human body. Beeswax was a suitable material for the entrapment of **1** in biocompatible microspheres converting the liquid drug droplets into solid material. The drug release was sufficient for oral use. These results demonstrate the potential of waxes for the production of delivery devices for many lipophilic or water insoluble drugs.

ACKNOWLEDGMENTS

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REFERENCES

1. E.J. Hammond, B.J. Wilder, J. Bruni; *Life Sci..*, 29, 256, (1981).
2. N.D. Yeomans, F.J.E. Vajda, J. Baldas; *Clin. Exp. Pharmacol. Physiol..* 9, 173, (1982).

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3. A. Tsuji, M.T. Simanjuntak, I. Tamai, T. Terasaki; *J. Pharm. Sci.*. 79, 1123, (1990).
4. T. Yamauchi; *Shinkei Seishin Yakuri.* 11, 111, (1989).
5. H. Nau, P. Finley, J. Williams, K. Brendel; *Biopharm. Drug Dispos.*. 4, 173, (1983).
6. M. Bialer, M. Friedman, J. Dubrovsky; *Biopharm. Drug Dispos.*. 5, 1, (1984).
7. S. Ohta; *Fukushima Igaku Zasshi.*. 39, 597, (1990).
8. M. Friedman, M. Bialer, A. Rubinstein, U. Dufrovsky; **U.S. US 4,913,906** (Cl. 424-499; A61K9/50), 03 Apr. 1990, *Chem. Abstracts* 113, 84884d, (1990).
9. J.P. Benoit, S. Benita, P. Puisieux, C. Thies, in "Microspheres and Drug Therapy", S.S. Davis, L. Illum, J. C. Mc Vie, E. Tomlinson, eds., Elsevier, Amsterdam,1984, pp. 91-102.
10. L.I. Giannola, V. Di Stefano, V. De Caro; *Pharmazie.* **48**, 123, (1993).
11. L.I. Giannola, V. De Caro, V. Di Stefano; *Drug Dev. Ind. Pharm.*., in press.
12. L.I. Giannola, V. De Caro, V. Di Stefano, M.C. Rizzo; *Pharmazie.* **48**, 917, (1993).
13. L.P. Wong, C.A. Gilligan, A. Li Wan Po; *Int. J. Pharm.*. 83, 95, (1992).
14. Y. Samuelov, M. Donbrow, M. Friedman; *J. Pharm. Sci.*, 68, 325, (1979).
15. G. Muhuri, T.K. Pal; *Boll. Chim. Farm.*. 11, 169, (1991).

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