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COMPARATIVE IN VITRO EVALUATION OF CUMULATIVE RELEASE OF THE URINARY ANTISEPTICS NALIDIXIC ACID, PIPEMIDIC ACID, CINOXACIN, AND NORFLOXACIN FROM WHITE BEESWAX MICROSPHERES

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ABSTRACT

The *in vitro* diffusion of nalidixic acid (**1**), pipemidic acid (**2**), cinoxacin (**3**), and norfloxacin (**4**) was studied. The transfer rate constants (k_d) from simulated gastro-intestinal juices to simulated plasma, throughout artificial wall lipid membranes, were defined. The k_d values suggested that the four drugs are absorbed both in gastric and intestinal environments in similar amounts. To obtain lack of gastric unwanted effects white beeswax microspheres containing **1**, **2**, **3**, and **4** were investigated as a vehicle for the drug intestinal release; they were prepared by the meltable dispersion process using wetting agents. Discrete, reproducible free flowing microspheres were obtained. The drug content increased when the particle size growed; it ranged from 4% to 18%. More than 95% of the isolated microspheres were of particle size range 100-500 μm.

The drug release was evaluated *in vitro*. Dissolution of entrapped active ingredients was greatly retarded allowing absorption only in the intestinal tract as result of microsphere formation.

INTRODUCTION

The widely used synthetic antibacterial urinary tract agents, nalidixic acid (**1**), pipemidic acid (**2**), cinoxacin (**3**), and norfloxacin (**4**) are generally considered almost safe drugs also after prolonged therapy. Following peroral administration they are rapidly absorbed from the gastro-intestinal tract, partially metabolised in the liver, and rapidly excreted through the kidneys and the gall.(1-5) However the quinolone agents administration is often associated with severe adverse reactions.(6-8) CNS effects including drowsiness, weakness, headache, dizziness, vertigo, brief convulsions, increased intracranial pressure, and toxic psychosis have been reported.(9-10) Within the class visual disturbances including overbrightness of lights, change in colour perception, difficulty in focusing, decrease in visual acuity, and double vision have been also reported. (11) Main side effects imply gastro-intestinal disorders, abdominal pain, anorexia, nausea, vomiting and diarrhoea (12-13). Toxic epidermal dermatitis, photosensitivity, and other skin disorders were also reported.(14-15) It was claimed that these effects are blood concentration-dependent (16).

In this study, we have determined the *in vitro* transfer rate constant (k_d) of **1**, **2**, **3** and **4** from simulated gastro-intestinal juices to simulated plasma, through artificial wall lipid membranes. The k_d values showed that the four drugs were absorbed both from stomach and intestinal tract in nearly equivalent amounts. Recent years have seen increasing attention directed towards suitable formulations designed to release the drug in some specific region of the gastro-intestinal tract. Enteric coating which resists dissolution in the

stomach may provide release of therapeutic agents in designated sites such as the colon from the administered form and reduce or eliminate the unwanted effects. It has been reported that the gastric irritation induced by quinolone antibiotics administration could be lowered by coating drug particles with a special enteric cellulose polymer (17), or entrapping the active agent in lipid vesicles,(18) or through pro-drug synthesis. (19-20)

In preceding papers from these laboratories we studied the ability of white beeswax to produce spherical free-flowing, gastro-resistant microparticles using the meltable dispersion in aqueous medium process.(21-22) The purpose of the present study was to asses the feasibility of formulating microspheres of **1**, **2**, **3** and **4** and obtain a suitable release pattern in the intestinal tract. Microspheres were prepared using white beeswax as a biocompatible, biodegradable gastro-resistant material which undergoes enzymatic cleavage in the intestinal environment.

MATERIALS AND METHODS

Nalidixic acid was purchased from Fluka, Buchs (Switzerland), pipemidic acid, cinoxacin and norfloxacin were purchased from Sigma Chemie, Deisenhofen (Germany). White beeswax was purchased from A.C.E.F., Fiorenzuola D'Arda (Italy). The surfactants used were Span 40, Tweens 20, 40, 60, and 85, (Fluka, Buchs, Switzerland). All chemicals were of analytical grade. UV spectra were recorder as aqueous solutions with a Kontron Uvikon model 860 spectrophotometer. In *vitro* diffusion experiments were carried out on a Sartorius SM 16750 apparatus.

In *vitro* diffusion of the pure drugs

The diffusion of the drugs was evaluated by using the Sartorius absorption simulator (23). As simulated gastric juice, a buffer pH 1.1

solution of HCl, NaCl and glycine was used (phase I) and as simulated intestinal juice buffer pH 6 or 7 solutions of Na_2HPO_4 and KH_2PO_4 were used (phase II). The initial volumes of the aqueous phases were 100 ml in every experiment. As an artificial stomach wall lipid membrane, the M1 barrier supported by an RS type Sartorius membrane filter (kit SM 15701) was used. As an artificial intestinal membrane, the D1 barrier supported by an RS type Sartorius membrane filter (kit SM 15702) was used. The temperature of the aqueous solutions, phase I and phase II, was maintained at 39 $\pm$ 1°C during experiments in order to obtain 37 $\pm$ 1 °C in the diffusion chamber. Samples of fluids (1.5 ml) were taken from each phase every 30 min and the total volume sampled from containers did not exceed 25% of the initial volume. The effective barrier area was 40 cm^2 and the experimental time 5 h. The output of the peristaltic pump was 15 ml/min during the experiments. The concentrations were measured by UV spectrophotometry to determine the percent of diffused material in phase II and the cumulative percent of undiffused material in phase I. The amounts of drugs used were 10 mg. Every experiment was repeated four times; the average deviation was $\pm$ 5%. Because the substance concentrations are affected by volume variations, the amount of diffused drug in phase II was corrected according to Stricker's equation (23). The diffusion patterns in gastric and intestinal juices are shown in figs. 1 and 2 respectively.

Microspheres preparation.

Microspheres were prepared incorporating the drugs in the melted wax. Compounds **1**, **2**, **3**, and **4**, (0.5 g) were finely powdered in a mortar and dispersed in the molten wax (2 g) with the surfactant (0.05 g). The dispersion medium (50 g) was heated to a temperature higher than the m.p. of the beeswax (60°C), added to the mixture and mechanically stirred at a rate of 600 rpm by a stirrer (Polymix

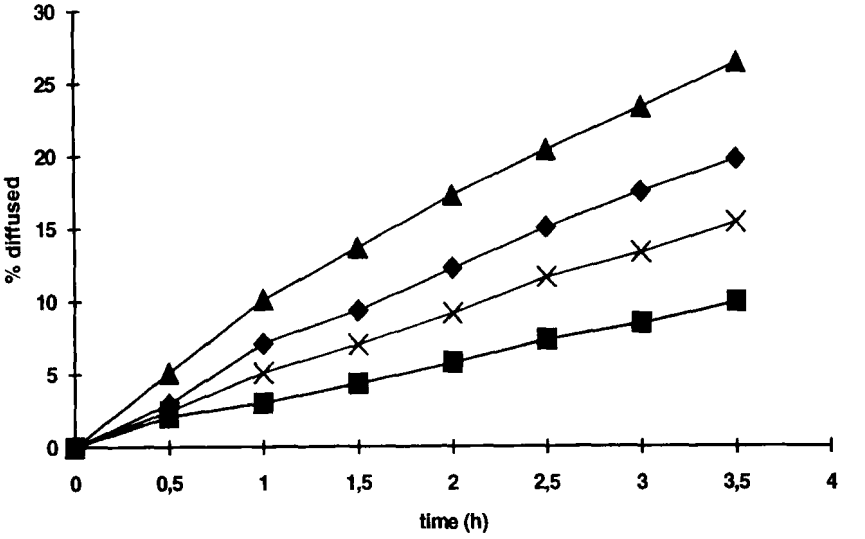

FIGURE 1

Percent amount of diffused drug through an artificial stomac wall lipid membrane against time: (◆) nalidixic acid, (■) pipemid acid, (▲) cinoxacin, (X) norfloxacin

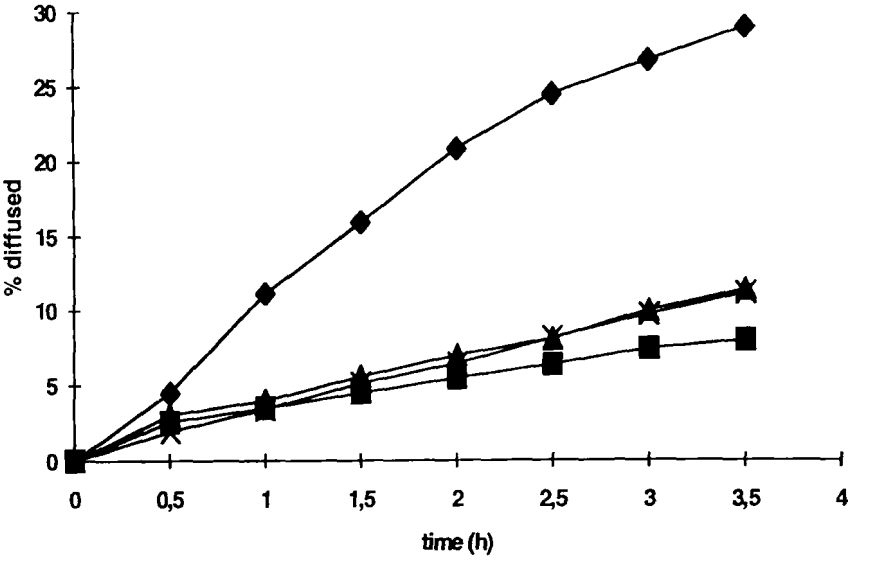

FIGURE 2

Percent amount of diffused drug through an artificial intestinal wall lipid membrane against time: (◆) nalidixic acid, (■) pipemidic acid, (▲) cinoxacin, (X) norfloxacin

stirrer, mod. RW 20 equipped with a KCH-TRON digital spin counter, Kinematica, Switzerland). The temperature was maintained at 60°C for 3 min with stirring (600 rpm) followed by rapid lowering at room temperature. The beeswax solidified enveloping the drug. The microspheres obtained were recovered by decantation, collected and washed twelve times with H_2O (12x40 ml) to remove any surfactant or drug residue. Air drying at room temperature for 24 h gave essentially solid free-flowing microspheres. The recovery yield was about 85% of the starting material. As dispersion medium distilled water (50 ml) was used to prepare microspheres containing cinoxacin or norfloxacin and glycerine (50 g) to prepare microspheres containing nalidixic acid or pipemidic acid.

The prepared microspheres were examined under transmission microscope for morphological evaluation.

Size distribution of microspheres.

Drug incorporated microspheres (5 g) were placed on the top sieve of a series of six standard stainless steel sieves ranged from 100 μm to 710 μm. The sieves were mechanically shaken for 15 min on an Endecotts, Octagon 200 shaker apparatus. The sieving analysis results are summarised in Table 2 as mean value of four batches.

Determination of the microspheres content.

Microspheres (100 mg) of each batch and size were randomly selected, microscopically observed and finely powdered in a mortar. The suitable solvent (50 ml) (dichloromethane for microspheres containing **1**, **3**, and **4**; water:acetic acid = 98:2 for microspheres containing **2**) was added in portions to the mortar content, quantitatively transferred into 100 ml measuring flask and completed to volume by the solvent. The amounts of **1**, **2**, **3**, and **4** into the final solutions were measured spectrophotometrically using the appropriate blank and a calibration curve at 258 nm for **1**, at 274 nm

for **2**, at 353 nm for **3**, and 286 nm for **4**. Drug content increased when the particle size grown; it ranged from 4% to 18% as shown in fig. 3.

In *vitro* drug release from microspheres

The release of the active ingredient from the microspheres were performed in conditions approaching the gastro-intestinal environments. Loaded microspheres (10 mg) were suspended in 100 ml of the appropriate simulated gastric (buffer pH 1.1 solution) or intestinal (buffer pH 6.0 or 7.0 solutions) juices in a perspex screw capped tube and kept in a water bath at 37.0 ± 0.2°C with constant stirring (100 rpm). Every 30 min samples of 1.5 ml were withdrawn from the tube and equivalent amounts of the corresponding fresh gastro-intestinal juice were added to the suspension to maintain the original volume. Drug concentrations were determined spectrophotometrically. In the gastric juice the amount of **1**, **2**, **3**, and **4**, released was practically undetectable. The intestinal release patterns are shown in fig. 4. Release experiments were triplicated to confirm reproducibility.

RESULTS AND DISCUSSION

The diffusion behaviour of **1, 2, 3** and **4**, under conditions approaching those in the gastro-intestinal tract, and the transport into the biophase were evaluated using the Sartorius absorption simulator. By this method, it was possible to assess the diffusion rate constant K_d, the absorbability and the effectiveness, and compare both gastric and intestinal administration of the four molecules. The diffusion rate constant from simulated gastro-intestinal juice to simulated plasma through lipid membranes was determined by plotting the % diffused *versus* time according to Stricker's method.

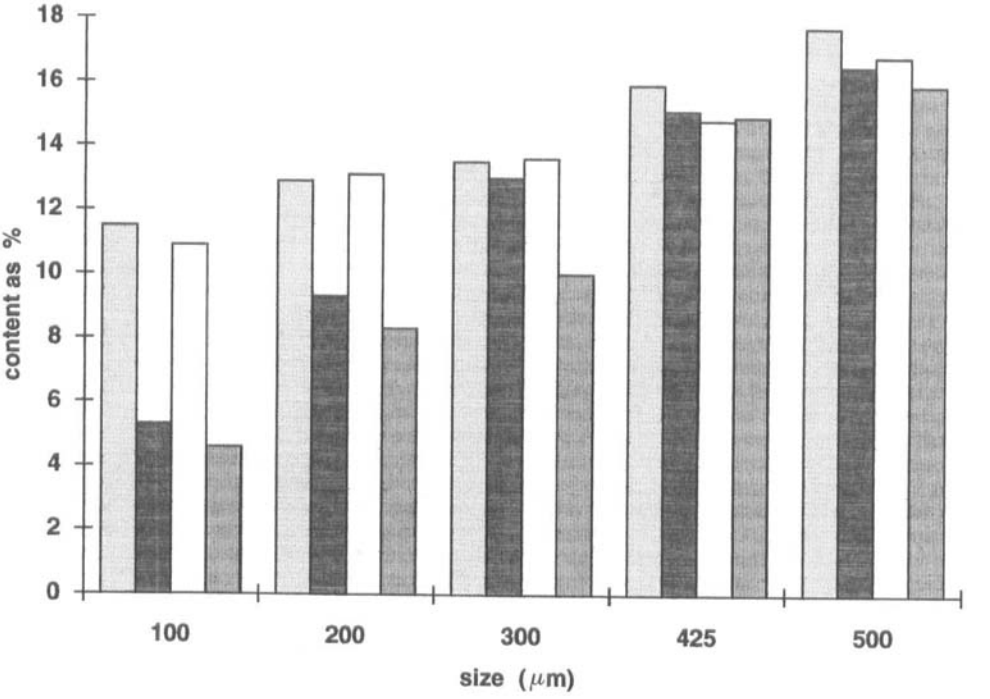

FIGURE 3

Percent amount of ▨ nalidixic acid, ▤ pipemidic acid, □ cinoxacin and ▥ norfloxacin entrapped within white beeswax microspheres of various size fractions

Figs. 1 and 2 illustrate the concentration-time curves of diffusion of **1**, **2**, **3** and **4**, from simulated gastric juice and simulated intestinal juice to plasma, respectively. The K_d values are reported in table 1.

The microspheres were prepared by the meltable dispersion process dispersing the molten wax in aqueous external phase. The molten mass formed spherical particles upon omogeneization. Solid, free-flowing microspheres without drug crystals were obtained after cooling. Incorporation of **1**, **2**, **3**, and **4** within the microspheres needed the addition of a surfactant to make materials wettable. It

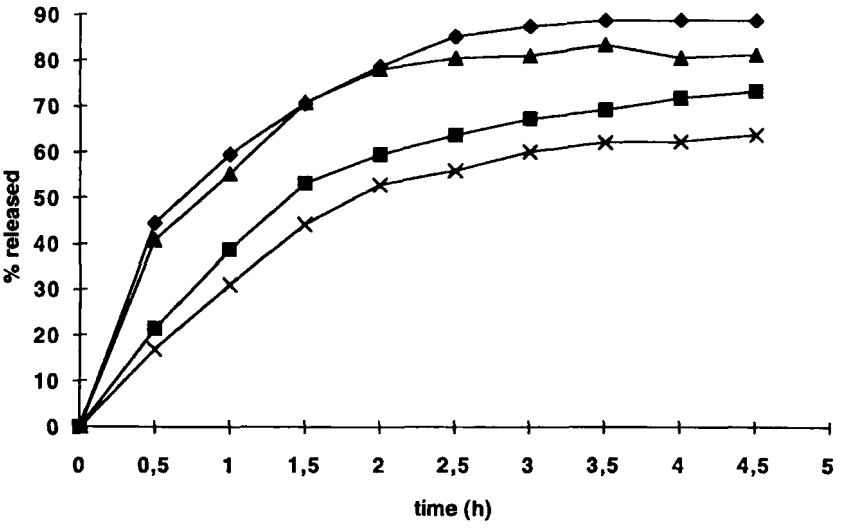

FIGURE 4

Percent drug released from white beeswax microspheres against time: (◆) nalidixic acid, (■) pipemidic acid, (▲) cinoxacin, (X) norfloxacin

TABLE 1

Diffusion rate constants

Compound	K_d [cm·min^{-1}] · 10^{-3}	
	(gastric pH 1.1)	(intestinal pH 6 or 7)
Nalidixic acid	2.29	3.76
Pipemidic acid	1.15	0.82
Cinoxacin	2.51	1.16
Norfloxacin	1.69	1.54

TABLE 2

Size distribution of microspheres obtained expressed as percent

Compound	Size range [μm]					
	100	200	300	425	500	710
Nalidixic acid	28.1	30.6	23.3	8.5	9.5	-
Pipemidic acid	34.4	45	16.8	2.7	1.7	-
Cinoxacin	0.6	9.4	56.9	24.0	9.1	-
Norfloxacin	6.8	22.4	39.5	16.3	15	-

Mean of four batches

was found that the best surfactant hydrophilic-lipophilic balance (HLB) value was of 6.7-14. The optimum concentration to produce a stable dispersion and discrete microspheres was about 2%. Sieve analysis (table 2) showed that most of the isolated microspheres were of particle size range 100-500 μm and about 70% were of size fraction from 200 to 300 μm. Repeat batches treated in this way proved to have reproducible particle sizes, indicating that stirring conditions, cooling rate and separation process were well controlled. Drug content determinations showed that the entrapped drug amount increased when the microsphere size grown. The average content ranged between 4 and 18%. The drug content was lower when the particle size was smaller and increased with the particle diameter. Fig 3 illustrates the content variations against the

microspheres size. The recovered yielded microspheres were about 85% of the starting material.

In *vitro* drug release profiles were obtained in conditions simulating the gastro-intestinal tract at 37°C and followed periodically measuring the UV absorptions. Small random samples were selected and observed microscopically before measurements of release. Optical microscopy showed that during all the drug release experiments the microspheres remained intact, even after complete exhaustion of the drug. Fig. 4 shows the release profiles from beeswax microspheres containing **1**, **2**, **3** and **4** in the intestinal environment. In the gastric juice the amount of drug released was undetectable. The release curves and the encapsulation efficiency were highest for nalidixic acid followed by cinoxacin, pipemidic acid and norfloxacin. This order was correlated to the aqueous solubilities of the four drugs.

CONCLUSIONS

White beeswax microspheres containing the urinary antiseptics nalidixic acid, pipemidic acid, cinoxacin and norfloxacin were successfully prepared by the easy, rapid and inexpensive melt-dispersion method. The microspheres wich had release rates suitable for oral use could be filled into capsules or formulated as oral suspension. The release in the intestinal lumen induce to think a better biodisponibility of the entrapped drugs. Moreover the preparation technique does not imply the use of organic solvents and the potential problem of toxic residues to the human body could be avoided. The method is highly reproducible and represents a very useful technique for microencapsulation of a large number of slight water soluble drug materials.

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