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RESEARCH PAPER

Design and Biopharmaceutical Evaluation of Nitrofurantoin-Loaded Eudragit RS100 Micropellets

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ABSTRACT

Nitrofurantoin, a synthetic bactericidal drug, was encapsulated with Eudragit RS 100 polymer by a coacervation phase separation technique using variable proportions of polyisobutylene (0% to 3%) as a protective colloid. The micropellets were evaluated by scanning electron microscopy (SEM), particle size distribution, wall thickness, and loss of wall polymer were determined. The in vitro release experiments were carried out over the entire pH range of the gastrointestinal tract, the data obtained from the dissolution profiles were compared in the light of different kinetic models, and the regression coefficients were compared. The in vivo studies were performed on female human volunteers. A linear correlation was obtained from in vitro–in vivo studies.

INTRODUCTION

Urinary tract infection is a common disorder at all ages and in both sexes. Some of the bacterial infections of the urinary tract that occur frequently are also notoriously resistant to the treatment and thus are likely to recur.

Nitrofurantoin is an orally active urinary tract antiseptic (1). It has a bacteriostatic effect against the common urinary pathogens. It is useful for chronic suppressive therapy and for patients who have mixed infections or infections accompanied by obtrusive uropathy (2). Its use is limited by the side effects (nausea, emesis, and skin rashes) that appear in some patients after oral administration of the conventional dosage form. Moreover, due to the short biological half-life (30 min or less) (3), the conventional dosage of 50 mg or 100 mg requires frequent administration (4), producing patient noncompliance and difficulty maintaining a constant serum drug level. Therefore, there is a clear need for a sustained-release dosage form of nitrofurantoin that would maintain the plasma drug level at a steady state within the therapeutic range over a prolonged period of time. This will provide better

management of urinary tract infection, reduced frequency of administration, and thus increased patient compliance and minimization of toxic manifestations.

MATERIALS AND METHODS

Materials

The materials were nitrofurantoin I.P. (generously supplied by SmithKline Beecham Pharmaceuticals Ltd., India, and Eskayef Ltd.) Eudragit RS100 (Rohm Pharma, GmbH, Weiterstadt, West Germany), and polyisobutylene (PIB) (MW 380,000) (National Chemicals, Baroda, India). All other reagents used were obtained commercially and used as such without further purification.

Preparation of Micropellets

The micropellets were prepared by a coacervation phase separation process using nitrofurantoin and Eudragit RS100 in the proportions 1:1 and 1:2. Coacervation was induced by nonsolvent addition in the presence of variable amounts of PIB (0% to 3%), which acted as a protective colloid. All the ingredients were used on a weight basis, and the total weight was always adjusted to 100 gm with trichloroethylene. The required quantity of PIB was weighed accurately and dissolved in some amount of trichloroethylene in a 100-ml clean, dry beaker. The solution was transferred to a 500-ml beaker. With the remaining amount of trichloroethylene, the first beaker was rinsed. Then, Eudragit RS100 was added gradually to the solvent system with stirring at 300–400 rpm until complete dissolution of the Eudragit RS100. It formed a uniform coating mixture at continuous stirring at the same speed, and nitrofurantoin was then added slowly into the coating solution until a homogeneous dispersion was obtained. Then, petroleum ether was added dropwise at a rate of 1.5–2.0 ml/min. At a particular time, phase separation occurred, and the coacervated particles could be seen through the magnifying glass. At this point, the system was cooled slowly about 2°C per min with ice. Addition of chilled petroleum ether with stirring continued. After some time, rigidized micropellets could be seen moving in the solution. They were filtered through a 100-mesh nylon cloth and washed with 100-ml portions of chilled petroleum ether with stirring up to 1 hr. Each time, any PIB adsorbed at the micropellet surface was removed. The micropellets were then air dried for 4 hr at ambient temperature, dried at 50°C for 3 hr, and stored in a desiccator over calcium chloride.

Evaluation of Micropellets

Size Analysis

Particle size distribution of the nitrofurantoin micropellets was done by sieving for 30 min using a nest of standard sieves (12–200 mesh) in a sieve shaker.

Scanning Electron Microscopy

The gold-coated samples were observed under a scanning electron microscope (SEM) (Hitachi, Model S-415A, Japan) to see their surface morphology.

Drug Content

About 100 mg accurately weighed micropellets were dissolved in 30 ml dimethylformamide. The volume was brought to 1000 ml with water, and the solution was mixed well. Of this solution, 5 ml was diluted to 100 ml with a solution containing 1.8% w/v of sodium acetate and 0.14% v/v of glacial acetic acid. The absorbance was measured at λ_{max} = 367 nm, and the content was calculated taking 765 as the value of A (1%, 1 cm) at λ_{max} = 367 nm.

Wall Thickness

The wall thickness was determined by a simplified indirect method (Eq. 1) in which micropellets were considered to be composed of two concentric spheres. Therefore, it can be called the mean spherical wall thickness (5,6).

$$R - r = \{[d_c/d_{eg}(1/F - 1) + 1]^{1/3} - 1\}r \qquad (1)$$

where R and r are radii of the micropellet and core particle, respectively, and d_c and d_{eg} are the densities of core material and Eudragit RS100, respectively. F is the fractional drug content. The densities of nitrofurantoin and Eudragit RS100 were determined in cyclohexane using a pycnometer. Dried material was used, and volume adjustment was completed in a few seconds to avoid inhibition and swelling. The densities of nitrofurantoin and Eudragit RS100 were found to be 1.428 g/cc and 1.176 g/cc, respectively.

Loss of Wall Polymer

Percentage loss of wall polymer was determined by the following equation (7):

$$Loss_p = 100[1 + W_0/W_{E0}\,(1 - 1/F)]$$

where W_0 is the initial amount of drug used, W_{E0} is the initial amount of wall polymer available for micropelletization, and F is the fractional drug content of isolated product by weight. For the case of a drug:coat ratio of 1:1, in which $W_0 = W_{E0}$, the above equation simplifies to $Loss_p = 100(2 - 1/F)$; in case of a drug:coat = 1:2, the above equation becomes $Loss_p = 50(3 - 1/F)$.

In Vitro Dissolution Studies

The release characteristics of nitrofurantoin from Eudragit RS 100 micropellets were determined over the whole pH range of the gastrointestinal tract fluid, from pH 1.2 to 7.6, with slight modification (8).

Accurately weighed dosage forms containing approximately 100 mg of nitrofurantoin were placed in the USP 20 dissolution basket maintained at 37°C ± 1°C at 100 rpm. The absorbance of the samples was measured at 367 nm against respective blanks.

Kinetic Assessment of Release Data

The data obtained from the in vitro dissolution studies with core-to-coat ratios of 1:1 and 1:2, which showed superior release characteristics, were analyzed in terms of different kinetic models (zero order, first order, Higuchi model, cube root equation, binomial equation, Weibull equation), and regression coefficients were compared.

In Vivo Study

Furadantin tablets (100 mg) (SmithKline Beecham India Ltd.) and the capsules containing Eudragit RS100 micropellets were administered orally to female human volunteers (27 years of age, average weight 50 kg, average height 160 cm). Since urinary excretion studies are the method of choice (9,10) and clearance is independent of urinary pH, urine samples were collected up to 12 hr at 2-hr intervals. A volume of 100 ml of water was provided to the volunteers after each collection to ensure adequate urine volume. The samples were frozen to provide stability and convenience in analysis and were allowed to thaw immediately before use. To 1 ml of the urine collected, 4 ml of 1% urea solution was added. The mixture was heated on a boiling water bath for 15 min. The absorbance was measured on a double beam spectrophotometer at 400 nm using 1 ml of water and 4 ml of 1% urea solution as a blank. The amount of drug present was read directly from the standard curve. Between the studies, a 2-week washout period was allowed.

RESULTS AND DISCUSSION

At low concentration or without PIB, fragile, brittle, poorly coated micropellets and sometimes small particles adhered with little polymer were obtained; uniformly coated microparticles were obtained at a higher PIB concentration. The product was more rigid and resistant to disintegration at higher concentrations of PIB. It was also observed that, above 3% PIB, lumps or an agglomerated mass was formed due to the higher viscosity of the medium, and at low or 0% PIB, loss of polymer by adherence to the wall of the container was increased.

At 0% PIB, the volume of petroleum ether required to induce phase separation was about 100 ml, whereas at 3% PIB, the volume was reduced to 40–45 ml. Therefore, PIB played a vital role in the micropelletization process, and at optimum concentration, the best micropellets could be produced.

The time and rate of cooling were also considered important for rigidization of the micropellets. After complete phase separation and formation of embryonic micropellets, the cooling should be initiated; otherwise, empty coacervated particles will be formed. Moreover, due to the tacky nature of Eudragit polymer, the embryonic micropellets became aggregated, resulting in the formation of lumps if the coatings were not rigidized by cooling immediately. Therefore, the rate of cooling should be sufficiently slow (i.e., 2°C/min) so that coacervation and rigidization can occur simultaneously and completely.

The size distributions of the micropellets are shown in Fig. 1 and Fig. 2. An increase in polymer-drug ratio increased the relative viscosity of the dispersed phase, and subdivision of the dispersed phase into smaller sizes was prevented by higher interfacial viscosity, thereby shifting the size distribution curves toward bigger particles and thus leading to an increase in average diameter. Similarly, an increase in the PIB concentration also increased the relative viscosity of the dispersed phase, leading to the formation of bigger micropellets while maintaining the same trend of increasing average diameter as the drug-polymer ratio was increased. More than 75% of the micropellets were found to be in the size range 250 μm–840 μm. Micropellets having an average diameter of 335 μm, 505 μm, and 715 μm were taken for evaluation.

The SEM of nitrofurantoin containing micropellets is shown in Fig. 3. At lower percentages of PIB, the coating appeared to be rough, granular, and aggregated by polymer bridging. Pores and channels were found in higher magnification of acrylic resin films, whereas a smooth

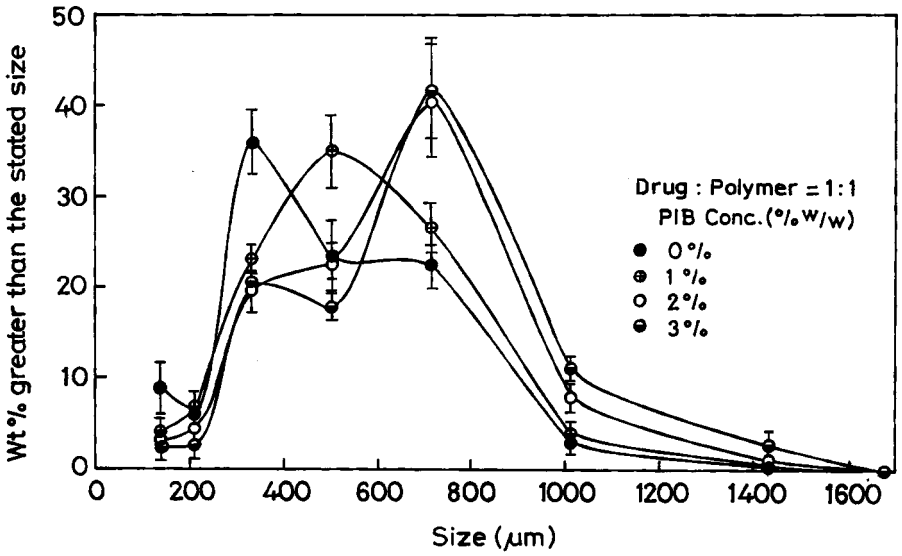

Figure 1. Particle size distribution of micropellets (drug:polymer = 1:1).

and uniform coating was obtained in 3% PIB, and the particle shape tended toward that of a sphere.

Drug content of different size micropellets of each of the formulations was found to remain apparently the same (Table 1). However, with both drug-polymer ratios of 1:1 and 1:2, the drug content was slightly lower with 3% PIB than at lower concentrations of PIB. These observations were in contrast to the findings by Benita et al.

The average drug content is an important factor that determines the wall thickness of micropellets, loss of wall polymer, and hence encapsulation efficiency. The wall thicknesses of different size micropellets of each formulation are shown in Table 1. The results indicate that the wall thickness decreased with decreasing particle size with core-to-coat ratios of 1:1 and 1:2, as also observed by Deasy. As the drug content in different size micropel-

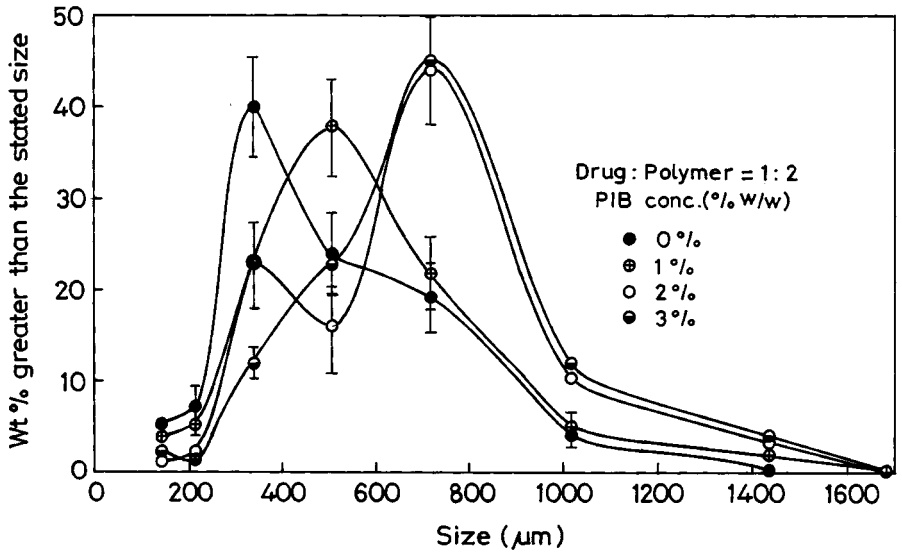

Figure 2. Particle size distribution of micropellets (drug:polymer = 1:2).

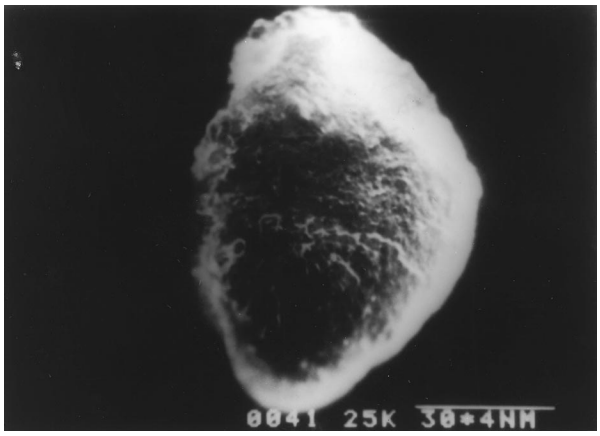

Figure 3. Scanning electron micrograph of micropellet (×75).

lets of each formulation remains apparently the same, the change in wall thickness is directly proportional to the particle size. Keeping the particle size constant and a fixed amount of core material, the wall thickness increased with the increase in the coating polymer. The coating thickness was higher in the core-to-coat ratio of 1:2 than in 1:1. Evidently, as there are fewer particles to be coated and a larger amount of polymer in the former ratio compared to the latter, the coating efficiency is better in the former. Another interesting observation was that, at these ratios, the wall thickness increased with the concentration of PIB, and the drug content decreased with the increase in PIB concentration.

The percentage Eudragit loss of different microparticles sizes for all the formulations is given in Table 1. It was found that the loss of Eudragit RS100 varies inversely with the wall thickness, whereas it varies directly with the drug content. As the PIB concentration increased, the percentage loss of Eudragit decreased as the uptake of the polymer by the core particles increased. Hence, we could say that encapsulation efficiency is bet-

Table 1

Coating Parameters of Nitrofurantion Micropellets

Formulation	PIB (%w/w)	Average Diameter (μm)	Drug Content (%)	Wall Thickness (μm)	Eudragit Loss (%)
1:1		715	50.05	69.56	24.71
	0	505	49.59	50.24	22.10
		335	50.12	32.76	24.13
		715	49.85	72.10	20.47
	1	505	49.23	52.39	16.88
		335	49.58	34.21	18.91
		715	49.12	74.60	16.41
	2	505	49.66	53.60	13.85
		335	49.83	35.80	12.91
		715	48.52	76.12	13.43
	3	505	48.98	54.96	10.35
		335	48.63	36.71	9.52
1:2		715	33.00	113.88	11.03
	0	505	32.15	80.40	11.11
		335	32.55	54.63	6.57
		715	32.54	118.86	2.72
	1	505	33.24	83.60	3.59
		335	32.56	56.10	1.19
		715	33.02	122.64	—
	2	505	32.58	85.26	—
		335	32.98	57.51	—
		715	31.94	125.10	—
	3	505	31.15	86.86	—
		335	31.08	58.32	—

ter at higher PIB concentrations. In a drug-coat ratio of 1:2, the percentage Eudragit loss was less than at the ratio of 1:1. Moreover, with 2% and 3% PIB, there was no loss of the wall polymer, indicating an even higher efficiency of nitrofurantoin encapsulation in the drug-coat ratio of 1:2 with higher PIB concentrations.

The in vitro release profiles of nitrofurantoin from Eudragit RS 100 micropellets with drug-polymer ratios of 1:1 and 1:2 in presence of varying concentrations of PIB are depicted in Fig. 4 and Fig. 5. It was noticed that the drug release was sustained for a longer period for the core-to-coat ratio of 1:2 than with the 1:1 ratio. Therefore, wall thickness plays a significant role. The concentrations of PIB were found to influence the release of nitrofurantoin from the micropellets to a great extent. As the concentration of PIB increased, the wall thickness increased, although in small increments, which could have led to the prolonged sustained release of the drug. It was possible to calculate a mean surface diameter of the microcapsules because of their essential spherical nature, and a plot of this against the time for 50% release of nitrofurantoin from the two batches with 3% PIB depicted a straight line (Fig. 6). This implied that a uniform diffusion gradient was set up through the wall, and it

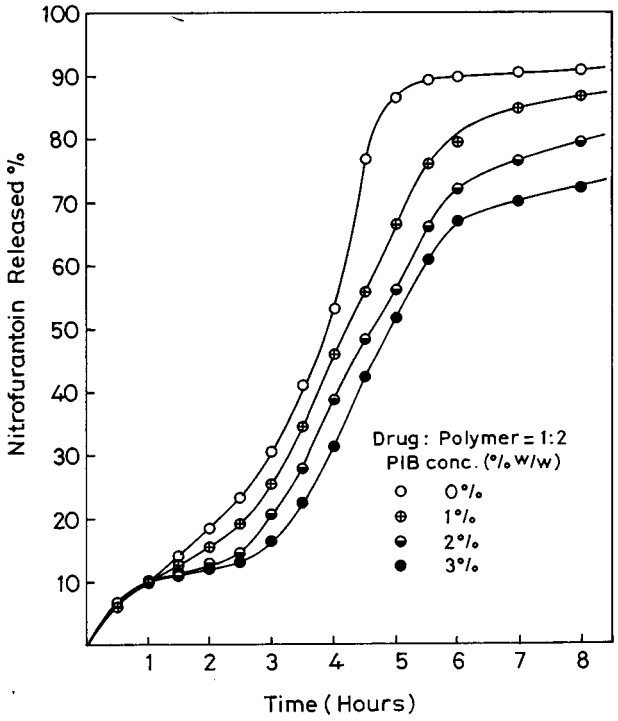

Figure 5. In vitro dissolution profiles of nitrofurantoin from Eudragit RS 100 micropellets (drug:polymer = 1:2).

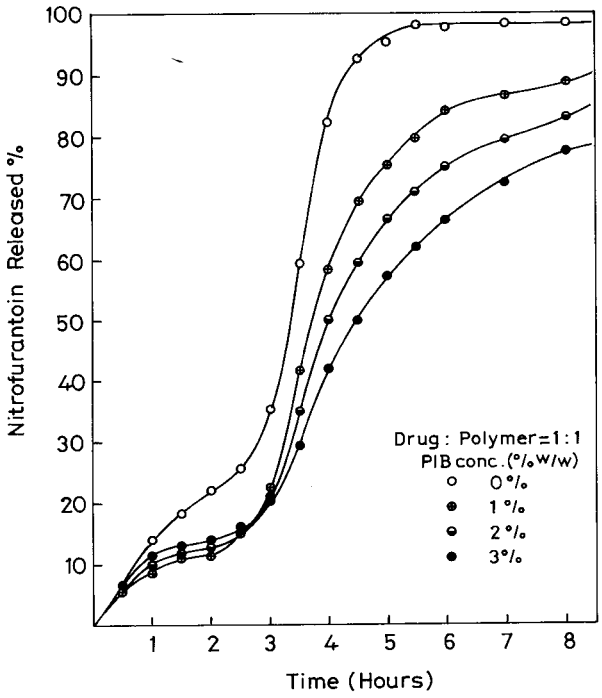

Figure 4. In vitro dissolution profiles of nitrofurantoin from Eudragit RS 100 micropellets (drug:polymer = 1:1).

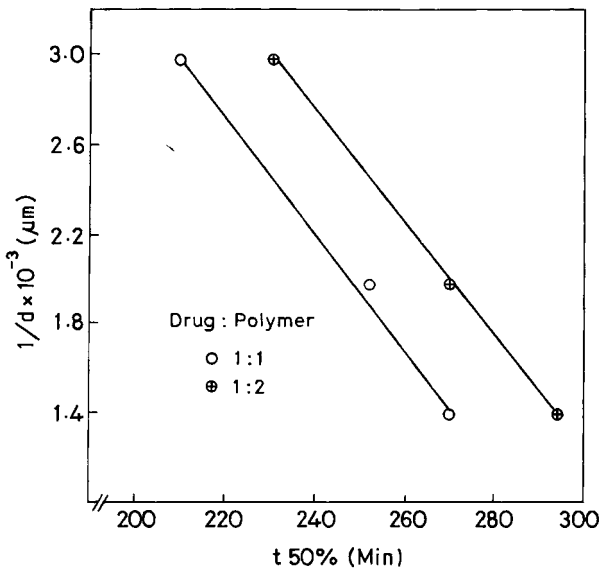

Figure 6. Effect of mean micropellet diameter on the time for 50% release of nitrofurantoin from Eudragit RS 100 micropellets.

Table 2

Kinetic Assessment of Release Data

Formulation Drug:Polymer	Regression Coefficient					
	Zero Order	First Order	Higuchi Model	Cube Root Model	Binomial Equation	Weibull Equation
1:1	0.9999430	0.9999081	0.999799	0.9698716	0.9999430	0.9995383
1:2	0.9999027	0.9998189	0.9996075	0.9716868	0.9999056	0.9993754

could be possible to predict the 50% release time from micropellets of known size. This in turn would allow the preparation of micropelleted dosage forms with a predictable release pattern.

It was evident from data presented in Table 2 that higher degree of good fit was obtained for the zero order, first order, Higuchi model, Weibull equation, and binomial model equation for nitrofurantoin release from micropellets. In the Weibull equation with a drug:polymer ratio of 1:1, the shape parameter was slightly more than 1, which expressed that the curve was a rising sigmoid with a smooth turning point. With a drug:polymer ratio of 1:2, the shape parameter, which was close to but less than unity, suggested a purely exponential form.

The batch with a drug-polymer ratio of 1:2 containing 3% PIB and an average micropellet diameter of 715 μm was found to give the best in vitro release pattern compared to the other batches. It released 75% of the drug in 9 hr, and the release was sustained for more than 12 hr, which was more than any other formulation. Hence, this formulation was subjected to in vivo studies in human volunteers. Nitrofurantoin concentrations in urine obtained after oral administration of the conventional dosage form (Furadantin tablet) and micropelleted dosage form to female human subjects are presented in Fig. 7. Nitrofurantoin is bacteriostatic at a concentration of 32 μg/ml or less (4). With conventional tablets, there was a sharp rise in its urine concentration, leading to the formation of a peak at about the fourth hour following oral administration. After attaining the peak level, there was a sharp decline in the nitrofurantoin concentration in urine that was much below the therapeutically effective drug concentration of 32 μg/ml. This effective concentration was maintained for just over 5.5 hr by the conventional dosage form. On the other hand, the nitrofurantoin concentration-time profile obtained after oral administration of the micropelleted dosage form was characterized by the absence of a sharp peak and a slight delay in the attainment of a therapeutic drug level at about 2 hr, but an effective nitrofurantoin concentration was maintained at pseudo–steady state over a prolonged period of more than 12 hr. After 12 hr, the total amount of nitrofurantoin excreted from the conventional tablet was 64.36%, but with the capsule containing Eudragit RS100 micropellets, it was 38.20% of the administered dose. This means that

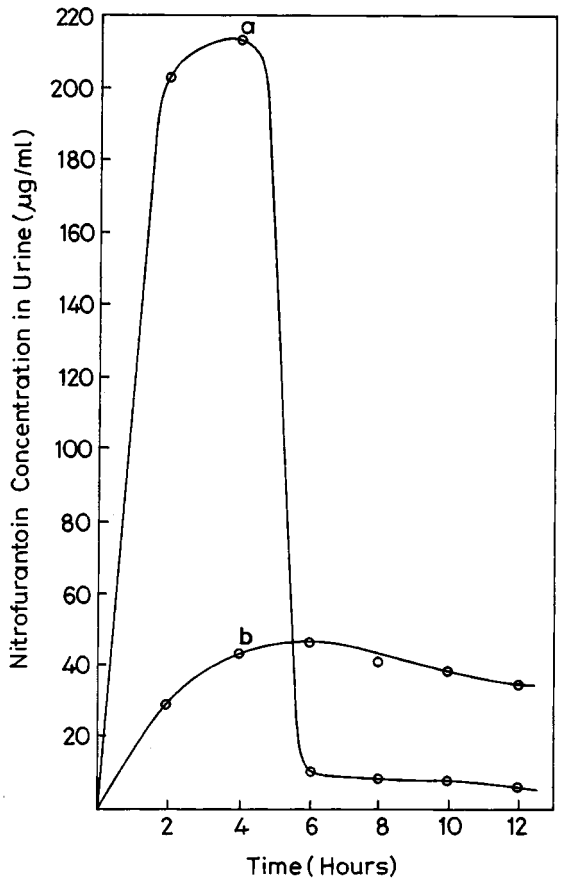

Figure 7. Urinary excretion profiles of nitrofurantoin in human volunteers following oral administration of (a) furadantin tablet (100 mg); (b) plain capsule containing Eudragit RS 100 micropellets of nitrofurantoin.

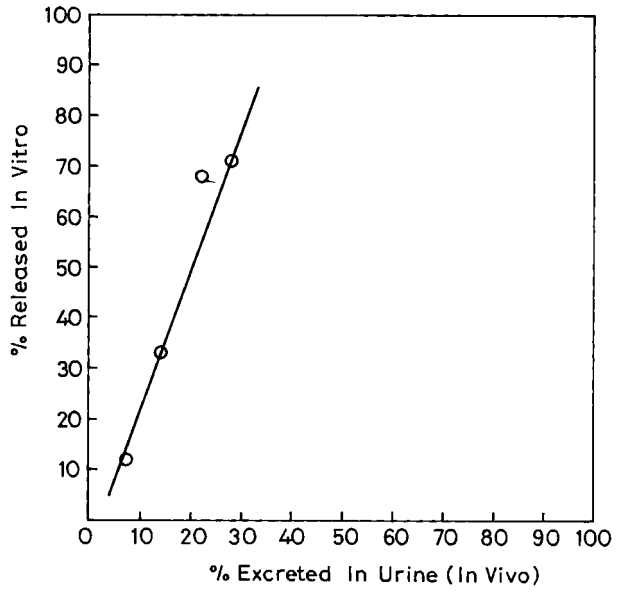

Figure 8. In vitro–in vivo correlation for nitrofurantoin-loaded Eudragit RS 100 micropellets.

the drug was still being excreted even after 12 hr of administration in the second case.

Again, the oral administration of the conventional tablet resulted in certain side effects, such as nausea, headache, and gastric irritation. One of the subjects also complained of skin eruptions, a common allergic reaction of nitrofurantoin. None of the volunteers who were administered the sustained-release capsules experienced any adverse reactions characteristic of the pure drug.

The amount excreted from the sustained-release preparations was lower than the amount excreted by the powdered drug; therefore, the absorption from the micropellets would be slower than from the conventional tablets. Since the first step in the absorption of a drug is the release from the dosage form and the release of drug from the micropellets was slow, it could be predicted that the absorption rate would be low as well, which explains the absence of side effects that were markedly noticed with the conventional tablets.

Percentage released in vitro versus percentage excreted in urine in vivo (Fig. 8) showed a linear correlation, indicating that the results were statistically validated. In conclusion, nitrofurantoin-loaded discrete, smooth, and uniformly coated Eudragit RS 100 micropellets having a high degree of incorporated drug can be prepared successfully by a coacervation phase separation technique. The designed controlled-release formulations exhibited prolonged and sustained release of nitrofurantoin as determined by the serum level. Thus, the therapeutically effective plasma drug concentration could be maintained at pseudo-steady-state level for more than 12 hr without untoward side effects.

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