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Polymer-coated microparticles for the sustained release of nitrofurantoin

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Abstract

Suspensions of nitrofurantoin (NTF) microparticles for controlled release were investigated in this study. The microparticles were enteric coated with various combinations of the two polymers, cellulose acetate phthalate/cellulose acetate butyrate (CAP/CAB) by a modified solvent evaporation method. Ratios of NTF to the two polymers (NTF/CAP/CAB) ranged from 1.0:1.6:0.4, 1.0:1.0:1.0, 1.0:0.4:1.6 to 1.0:0.0:2.0. The encapsulation efficiency, percentage yield, determined by comparing the final mass of the microparticles with the initial mass of the ingredients used, distribution of particle size and the in-vitro dissolution profiles of the microparticles were determined. Based on light photographs for the evaluation of the microparticle morphology, the drug crystals appeared to be encapsulated sufficiently by the enteric polymers. In our study, the microparticles enteric coated with CAP/CAB in the ratio of 0.4:1.6 displayed the most satisfactory in-vitro release profile (reduced release in the simulated gastric fluid and sustained release in the simulated intestinal fluid). Thus, microparticles with NTF/CAP/CAB in the ratio of 1.0:0.4:1.6 were formulated into a suspension for further bioavailability and ulcerogenicity studies in Sprague–Dawley rats, with the suspension of NTF crystals as a control. The bioavailability study was carried out in eight rats fed with either the free NTF or the corresponding microparticles in a cross-over design. The ulcerogenicity study was carried out in three groups of six rats each: one group received no drug treatment; the control group was treated with free NTF; and the third group was treated with enteric-coated NTF microparticles. The bioavailability of NTF from the microparticles was comparable with the control. More importantly, there was notably less ulceration of the gastric mucosa observed after dosing with the microparticle suspension compared with that after the administration of the control suspension.

Introduction

Nitrofurantoin (NTF) is a urinary tract antiseptic. Administration of NTF in conventional dosage forms often produces intolerably high concentrations of the drug in the gastrointestinal tract of patients with urinary tract infection, and this in turn causes many undesirable side-effects (Brumfitt et al 1985; Brumfitt & Hamilton-Miller 1998). Its clinical use is often limited by side-effects such as nausea and vomiting, probably as a result of its rapid absorption and gastric irritation (Reynolds 1989). In addition, NTF has a short biological half-life of less than 1 h (Kunin 1967; Bennet et al 1970). Conventional dosage forms are required to be administered frequently, causing patient non-compliance (Carlsen et al 1985).

In order to minimize the side-effects of NTF after oral administration, some investigators (Brumfitt et al 1985; Brumfitt & Hamilton-Miller 1998) formulated the solid dosage form with the macrocrystalline NTF, which has slower dissolution and absorption rates. These macrocrystals would therefore produce less gastrointestinal side-effects. However, they were also found to produce lower serum concentrations than the microcrystalline form, and take longer to achieve peak concentration in the urine, causing bioavailability problems (Paul et al 1967; Meyer et al 1974). The recommendation is for this macrocrystalline solid dosage form to be taken together with food, since the presence of food in the gastrointestinal tract could minimize drug irritation on the stomach, increase the bioavailability and prolong the duration of the therapeutic urinary concentration (Cadwallader & Jun 1976).

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However, the gastric side-effects could not be completely overcome by using macrocrystals in the formulation. Therefore, many attempts have been made to reduce the gastric side-effects by controlling the drug release in the stomach, for example by using sustained release tablets, hard gelatin capsules and microcapsules (Baichwal & Shetty 1982; Eldem & Capan 1983; Ertan et al 1994). However, when these dosage forms are used to treat childhood urinary tract infections, which occur in 2% of boys and 8% of girls by 10 years of age (Roy 1999), the difficulty of swallowing tablets or capsules could result in non-compliance. To date, there is no suitable dosage form of NTF available for paediatric patients.

Cellulose derivatives are pharmaceutical excipients commonly used for enteric coating of tablets and capsules with the purpose of preventing gastric irritation (Edgar et al 2001). We postulated that a dosage form of NTF as enteric-coated microparticles of less than 200 μm in diameter would be able to overcome the clinical problems associated with the administration, bioavailability and gastric side-effects of NTF in paediatric patients. Particles of less than 200 μm in diameter could be free of the gritty sensation when administered as a suspension to paediatric patients (Lewis et al 1998). Cellulose derivatives, such as cellulose acetate butyrate (CAB) and cellulose acetate phthalate (CAP), that are insoluble in water but soluble in organic solvents, have been used as enteric coatings for microparticulate drug delivery systems (Conti et al 1997). CAP is further characterized by its pH-dependent solubility. The CAP-coated dosage forms resist drug release in acidic medium, but disintegrate in-vitro at a pH of approximately 6.5 (Chambliss 1992). CAB and CAP would be useful to minimize the gastric effects of NTF. The nature of polymers (Bhardwaj et al 1995) and their combination ratios (Babay et al 1988) can affect the patterns of drug release and the particle size distribution of microparticles. In this study, microparticles of NTF encapsulated with CAB and CAP were prepared by a simple emulsion–solvent evaporation method (Maharaj et al 1984). The effects of polymers on drug release and particle size distribution were then evaluated. Finally, the selected batch of the microparticles was further assessed in-vivo to compare its ulcerogenicity effect against the control formulation of free NTF.

Materials and Methods

Materials

NTF, CAB and methylcellulose were purchased from Sigma Chemical Co. St. Louis, MO. CAP was obtained from Fluka Chemie AG CH-9471 Buchs, and Span 85 was obtained from Tokyo Kasei Co. Japan. Sodium chloride and potassium dihydrogen orthophosphate were supplied by AnalaR, UK. Phosphoric acid was provided by Mallinckrodt Baker, USA. All other chemicals, such as acetanilide, dimethylformamide (DMF), acetone, hydrochloric acid 37%, sodium dihydrogen phosphate monohydrate, sodium hydroxide and citric acid were purchased from Merck Darmstadt, Germany, and were of analytical grade or pharmaceutical quality.

Preparation of microparticles

Microparticles were prepared by a modified solvent evaporation method (Maharaj et al 1984). NTF (1 g) was dissolved in 200 mL acetone/alcohol (9:1). The solution was then added with stirring (2500 rev min^{-1}) into 200 mL liquid paraffin containing 2% Span 85. The temperature was maintained at 27°C throughout the process. In the liquid paraffin, the dissolved NTF re-precipitated as microparticles when the organic solvent was evaporated off. After stirring for 2 h, 100 mL acetone/alcohol (9:1) solution containing 2 g CAP/CAB in various combination ratios (2.0:0.0, 1.0:1.0, 1.6:0.4, 0.4:1.6 or 0.0:2.0) was poured into the liquid paraffin containing the dispersed NTF particles. Stirring was continued for another 2 h to allow the microparticles to be encapsulated with the polymers and for the volatile solvent to be completely evaporated.

Solid microparticles were separated by passing the suspension through filter paper (Whatman, 0.70 μm) under vacuum, followed by washing with 2.5 L of diluted hydrochloric acid (0.058 M) to remove any residual NTF attached to the surface of the microparticles. The microparticles were air-dried at ambient temperature in the fume cupboard overnight. The dried microparticles were then sieved through meshes of between 250 and 180 μm. Only the microparticles that could pass through the 180-μm sieve were formulated into suspensions for further studies.

Determination of the microparticle yield

The yield was determined by comparing the final amount of microparticles with the initial amounts of ingredients used (NTF, CAP and CAB).

Microparticle size distribution

Microscopic analysis was used to determine the size distribution of the sieved microparticles. A small spatula of the respective batch of microparticles was placed in oil on a slide and viewed under a light microscope (Model BH-2; Olympus, Japan) at a magnification of 10×. The particle sizes were measured through a digital monitor with a calliper. More than 375 microparticles for each batch were counted and the percentage size distribution was presented.

Microscopic evaluation

The microparticles were examined and photographs were taken for morphological analysis under a light microscope (Model BH-2) at a magnification of 40×.

HPLC assay of NTF

NTF and its internal standard (acetanilide) were detected and quantitated by a HPLC system (1050-Series; Hewlett-Packard, GmbH, Waldbronn, Germany). The system consisted of a quaternary pump, an online degasser, an auto-

sampler and a diode array detector. The separation of compounds was made on a reversed-phase C18 column (Hypersil 5 μm, 200 × 4.6 mm; Hewlett-Packard, GmbH), which was protected by a guard column (C_{18}, 4 × 3.0 mm; Phenomenex, USA).

For in-vitro studies, 10 mg NTF was dissolved in 10 mL DMF and 10 mg acetanilide, the internal standard, in 20 mL methanol, and stored at −20°C as stock solutions. Six calibration samples over the concentration range of 0.2 to 0.0125 mg mL^{-1} were prepared from a series of dilutions of the stock solutions. Each volume of sample was diluted with an equal volume of acetanilide (0.05 mg mL^{-1}) in methanol. A 15-μL sample of the mixture was auto-injected and the concentration was determined by HPLC. The mobile phase consisted of 0.043 M phosphate buffer (pH 7)/acetonitrile (85:15, v/v). The wavelength was set at 254 nm and the flow rate was 1.0 mL min^{-1}. Under these chromatographic conditions, NTF eluted at 5.3 min and acetanilide at 9.4 min. All detections were made at ambient temperature.

For in-vivo studies, in order to avoid interference from endogenous substances in the urine samples, different chromatographic conditions were used. Briefly, the respective urine samples were diluted with an appropriate volume of acetonitrile/water (50:50, v/v). A 20-μL sample of the mixture was injected onto the HPLC system. The concentrations of NTF in the urine samples were estimated with reference to the external standard. The mobile phase consisted of 0.005 M phosphate buffer (pH 3)/acetonitrile (72:28, v/v). The wavelength was set at 370 nm and the flow rate was 1.2 mL min^{-1}. Under these chromatographic conditions, NTF eluted at 3.3 min. Seven calibration samples over the concentration range of 40 to 0.25 μg mL^{-1} were prepared from a series of dilutions of the stock solution.

The HPLC assays for both in-vitro and in-vivo assays were established and validated. The calibration plots were linear within their respective ranges ($r^2 \geqslant 0.999$); the % coefficients of variation for intra- and inter-day assay precision in triplicate were less than 5% for all calibration concentrations.

Estimation of drug content in the microparticles

To determine the encapsulation efficiency, 50 mg of NTF microparticles from each batch were dissolved in 100 mL DMF, and the concentration of NTF was assayed using the same chromatographic conditions as for the in-vitro studies.

In-vitro dissolution studies

In-vitro dissolution studies were carried out on batches of microparticles, each containing an equivalent of 50 mg NTF, using the standard US Pharmacopeia (USP) XXII paddle apparatus. The dissolution fluid was either the USP simulated gastric medium (SGM, pH 1.2) or the simulated intestinal medium (SIM, pH 7.5), but without enzymes. The microparticles were suspended in 1 L of the respective dissolution medium and stirred at 100 rev min^{-1}. The temperature was constantly maintained at 37 ± 0.5°C. During the experiment, a series of 1.5-mL samples were withdrawn from the dissolution medium at selected times with the aid of a syringe fitted with a Millipore 0.45-μm filter. An equal volume of medium was returned to the system after each withdrawal. The release of NTF from the microparticles was continuously analysed spectrophotometrically with HPLC. Each batch of microparticles was tested in 5 replicates. T50 values, that is the time required to release 50% of the drug from microparticles, were compared with that of the control free NTF crystals.

Preparation of dispersing medium and suspensions of NTF for in-vivo studies

Methylcellulose (1.5%, w/v, 1500 cps) was used as the suspending agent. The suspending medium was buffered at pH 3.5 with 0.2 M citric acid for the remaining studies to ensure minimum drug release from the microparticles in the suspension, while avoiding extreme acidity. NTF/CAP/CAB (1.0:0.4:1.6) microparticles were selected for incorporation into suspensions for further studies in-vivo, as they exhibited the most desirable dissolution profile when compared with other formulations. The crystalline NTF powder and the NTF/CAP/CAB (1.0:0.4:1.6) microparticles were, respectively, made into suspension. The two suspensions, containing an equivalent of 0.1%, w/v, NTF in 20 mL of suspending medium, were prepared immediately before experiments for bioavailability studies.

In-vivo bioavailability studies

The protocol for the in-vivo studies was approved by the local Animal Care and Ethics Committee. Eight Sprague–Dawley rats (180–230 g) were used. They were separated into two groups of four rats each. The rats were kept in metal metabolic cages, with free access to water, but no food for 12 h before and during the course of the experiment. Each formulation was administered to one group of rats at a dose of 2 mg kg^{-1} NTF, using a gavage needle. Urine samples were collected immediately before administration and at 1.5, 3, 4.5, 6 and 7.5 h after administration. The volume of urine was recorded at each collection time and the samples were stored at −20°C until assay. Each rat was also injected with 3 mL normal saline intraperitoneally 1 h before treatment and after each urine collection point in order to maintain adequate urine output. The experiments were repeated in a cross-over arrangement with a washout period of 3 days.

Calculation of the cumulative amount of NTF excreted in urine and statistical analysis for in-vivo studies

The cumulative percentage of NTF excreted in urine (X_n) at each time interval was calculated according to the following equation:

$$X_n = \sum_{n=1}^{n} V_n C_n / D \times 100\%$$

where V_n is the volume of urine collected at each time point, C_n is the NTF concentration of the urine sample collected at each time point, and D is dose of drug administered. The cumulative percentages of NTF against time were then plotted for each suspension.

In-vivo ulcerogenicity studies

Eighteen Sprague–Dawley rats (180–230 g) were divided into three groups of six rats each. They were fasted 24 h before and during treatment, but water was freely available. By using an oral gavage needle, the first group, serving as a control, was fed with the dispersion medium; the second group received the free NTF crystal suspension at 25 mg kg^{-1}; and the third group received the NTF/CAP/CAB (1.0:0.4:1.6) microparticle suspension, containing an equivalent of 25 mg kg^{-1} NTF.

The rats were dosed twice daily for 3 days. At 12 h after the last dose, the animals were killed; their stomachs were excised and filled with saline. The contents of the stomach were emptied. The stomach was then opened up along the greater curvature, gently wiped clean with a swab dipped in saline, and examined for the presence of any gastric bleeding under a magnifier. The severity of mucosal damage was assessed by a slightly modified scheme reported previously (Cioli et al 1980; Dalal & Narurkar 1991): no lesions = 0.0; punctiform lesions (lesions < 1 mm) = 0.5; five or more punctiform lesions = 1.0; one to five small ulcers (1–2 mm) = 2.0; more than five small ulcers or one large ulcer (> 2 mm) = 3.0; more than one large ulcer = 4.0; more than one large ulcer and swollen heavily on stomach = 5.0.

Based on the severity of the mucosal damage, the specimen was assigned an ordinal score according to the scoring scheme. The control specimens did not exhibit the formation of lesions or ulcers and accordingly the controls had a score of 0. The median and range of scores of each group were computed as the severity indices of stomach irritation.

Statistical analysis

Statistical differences in microparticle yields, sizes and T50 values among various microparticle formulations were compared using a one-way analysis of variance. The cumulative percentages of NTF recovered in urine after administration of suspensions of the tested microparticles and free NTF were compared with respect to time and formulation using a two-way analysis of variance. Post-hoc multiple comparisons were done by Tukey's test for significance at $\alpha = 0.05$. Statistical comparisons of the damage severity index on rat gastric mucosa were conducted using a non-parametric Kruskal–Wallis test.

Results and Discussion

It is generally more difficult to formulate a drug into a suspension with sustained release enteric-coated microparticles than sustained release tablets or capsules. Microparticles of the suspension have enormous surface areas, leading to leaching of the drug into the surrounding media. Therefore, it is critical to adjust the formulation factors to optimize the release profiles. In this study, the ratio of polymers was found to have significant effects on the pharmaceutical properties of the microparticles.

Encapsulation efficiency, percentage yield and particle size evaluation

Microparticles prepared with NTF/CAP/CAB (1.0:2.0:0.0) were so large that virtually no microparticles could pass through the 180-μm sieve and so this formulation was discarded and not subjected to further study.

It was found that the percentage yield increased, whereas the drug content encapsulated within the microparticles decreased with a decrease in the CAP/CAB ratio (Table 1). The size distribution for different batches of microparticles is shown in Figure 1. The sizes of these microparticles were mostly over the range of 60 to 120 μm. Our results also indicated that the drug to polymer ratios had no influence on the size distribution of the microparticles.

The yield of microparticles depends on good emulsification of two phases, and so the action of the surfactant is very important. In the preparation of microparticles for the

Table 1 Effect of the different nitrofurantoin/cellulose acetate phthalate/cellulose acetate butyrate (NTF/CAP/CAB) ratios on the characteristics of NTF microparticles.

	NTF/CAP/CAB microparticles			
	1:1.6:0.4	1.0:1.0:1.0	1.0:0.4:1.6	1.0:0.0:2.0
Microparticle yield (%)[a]	50.8±4.3	68.7±5.6[c]	78.5±4.8[c]	90.0±7.2[c,d]
Drug content (%) w/w	17.8	13.8	10.4	10.4
Mean particle size (μm)[b]	67.1±33.1	105.4±44.2[c]	70.8±33.6[d]	83.1±33.8[c,d]
Encapsulation efficiency (%)[e]	53.5%	41.4%	31.2%	31.2%

[a]Each value represents the mean±s.d., n = 4; [b]Each value represents the mean±s.d., (n ⩾ 375); [c]Significantly different from the first column ($P < 0.05$); [d]Significantly different from the second column ($P < 0.05$); [e]Encapsulation efficiency is defined as the percentage of the measured drug content over the theoretical drug content of 33.3%, w/w.

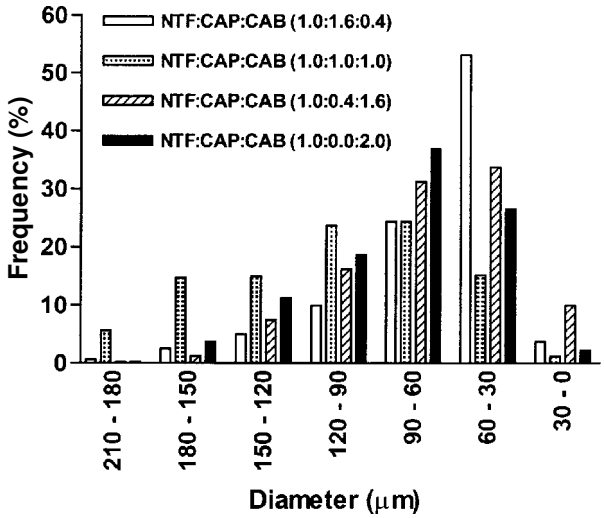

Figure 1 Influence of the nitrofurantoin/cellulose acetate phthalate/cellulose acetate butyrate (NTF/CAP/CAB) ratios on the size distribution of NTF microparticles.

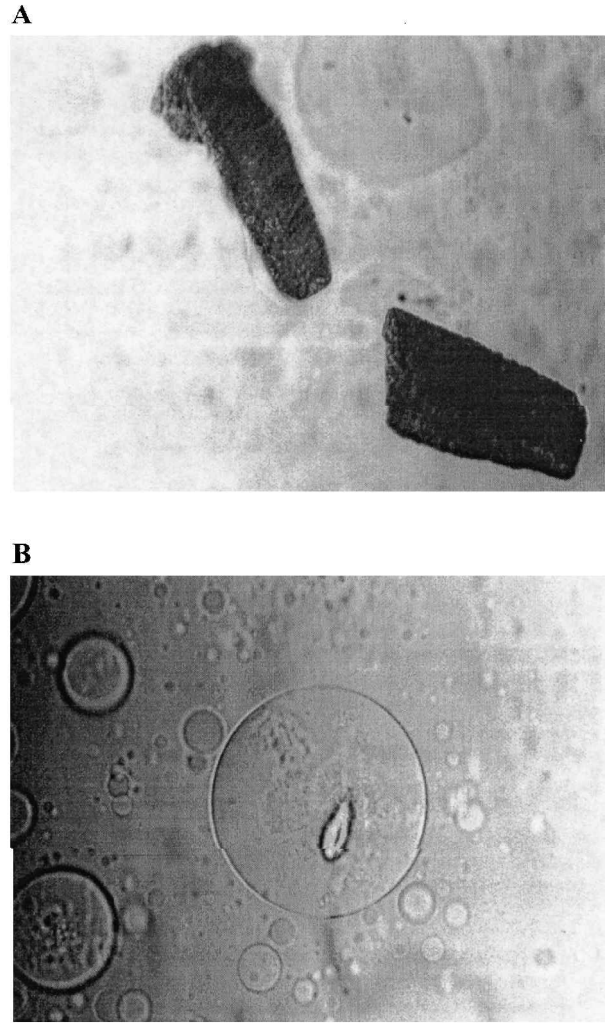

Figure 2 Light micrographs at a magnification ×400 of pure nitrofurantoin (NTF) crystals (A) and microparticles with NTF/cellulose acetate phthalate/cellulose acetate butyrate in the ratio of 1.0:0.4:1.6 (B).

controlled release of carbamazepine, Arnaud et al (1996) observed that the yield of the small sized microparticles was a function of the internal phase viscosity when the same conditions of surfactant were present in the two phases during the process of encapsulation. CAB has a lower glass transition temperature than CAP (Ceccorulli et al 1993; Roxin et al 1998) and is considered to be a "softer" polymer. The decrease in the ratio of CAP to CAB might increase the yield by decreasing the viscosity of the internal phase. As shown in Table 1, the yield of NTF/CAP/CAB in the ratio of 1.0:0.0:2:0 was 90%, but the drug content of the microparticles was only 10.4%, w/w, which was equivalent to about one-third of the theoretical drug content. Therefore, the increase in the yield could be partly owing to incorporation of certain amounts of liquid paraffin and surfactant into the microparticles. At the same time, it would also reduce the drug content by reducing the encapsulation rate.

Microscopic evaluation

Microscopic evaluation showed that the original NTF crystals were generally greater than 200 μm, whereas the size of most microparticles prepared with different combinations of polymers was found to be less than 100 μm (Figures 1 and 2). This can be explained by the recrystallization of NTF in a rapid evaporation of the solvent mixture (acetone/alcohol) to obtain fine particles ready for encapsulation. It was apparent under the light microscope that these microparticles were efficiently encapsulated (Figure 2).

In-vitro dissolution studies

The release behaviour of the pure drug and the encapsulated microparticles in SGM and SIM were evaluated. In SGM, the release of drug from the various batches of microparticles was very much retarded compared with that from the control of pure NTF (Figure 3A). Microparticles prepared with the combination of NTF/CAP/CAB in the ratio of 1.0:0.4:1.6 showed the greatest delay in drug release in SGM. Earlier work by Ertan et al (1994) showed that a sustained-release dosage form of NTF as microcapsules could be prepared by a carboxymethylcellulose–aluminium sulfate coacervation technique. With their polymer mixture, approximately 60% of the drug was released in SGM in 5 h compared with the 20% released by our formulation of NTF/CAP/CAB (1.0:0.4:1.6). This indicates that the polymer mixture of CAP/CAB could provide better protection in SGM.

The percentage release of NTF into SIM is shown in Figure 3B. Almost all of the free NTF crystals were dissolved in 10 min, whereas 80% of the drug entrapped in all batches of microparticles was slowly released into SIM

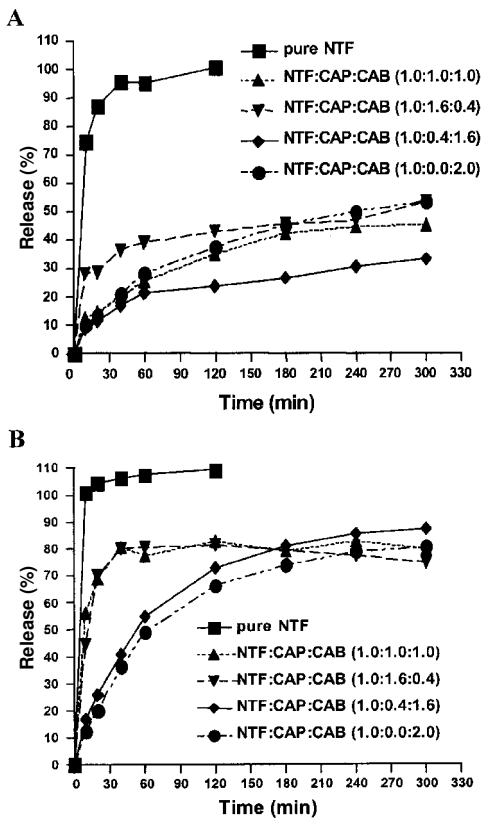

Figure 3 Percentage release of nitrofurantoin (NTF) from crystals and various batches of microparticles in simulated gastric fluid at pH 1.2 (A) and in simulated intestinal fluid at pH 7.5 (B) (n = 5).

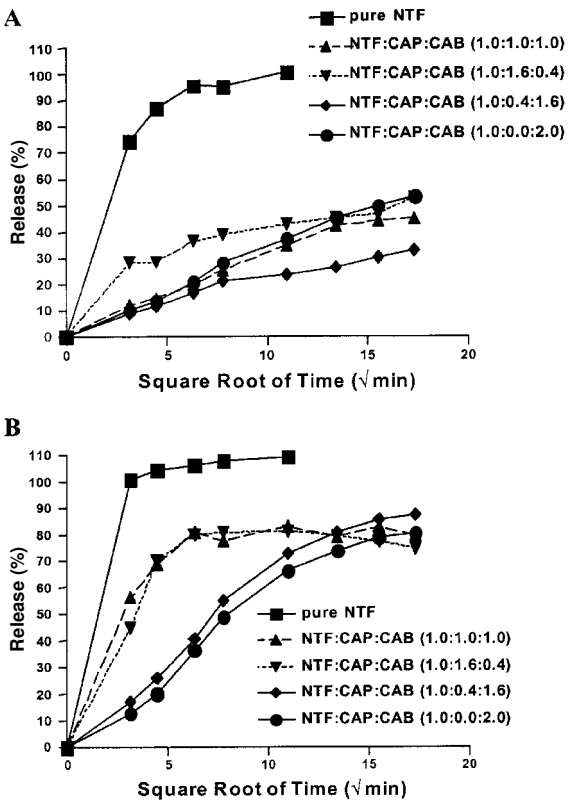

Figure 4 Percentage release of nitrofurantoin (NTF) from crystals and microparticles according to the Higuchi square root of time equation ($Q = Kt^{1/2}$) in simulated gastric fluid at pH 1.2 (A) and in simulated intestinal fluid at pH 7.5 (B) (n = 5).

over 5 h. It was also observed that the matrix of the particles eroded into the medium during dissolution. Su et al (1994) suggested that for small particles with a mean radius of 106 μm, both the first-order and the cube-root models could adequately describe the dissolution profiles of these particles. Since the sizes of our particles were less than 200 μm, their dissolution profiles were subsequently fitted to the first-order model:

$$M_t/M_\infty = 1 - \exp(-kt)$$

where M_t and M_∞ are the amounts of drug released at time t and infinity, respectively, and k is the drug release constant. The T50 values were calculated from the equation 0.693/k. According to this calculation, the T50 values of the batches of NTF/CAP/CAB in the ratios of 1.0:1.0:1.0, 1.0:1.6:0.4, 1.0:0.4:1.6 and 1.0:0.0:2.0 were found to be 12.2, 13.9, 61.0 and 81.5 min, respectively. The T50 of the latter two batches of microparticles was significantly longer than that of the first two batches ($P < 0.05$), and therefore confers greater sustained release of the drug in SIM. As indicated earlier, in our study, the formulation of NTF/CAP/CAB (1.0:0.4:1.6) also released the least amount of drug (approx. 20%) into SGM. Although the chitosan/calcium alginate microcapsules developed by Hari et al (1996) for intestinal delivery of NTF achieved similar dissolution profiles in SGM and SIM, their beads of 900–1000 μm would not be suitable for formulation into a suspension. In contrast, the relatively small size of the microparticles developed in this study would enable them to be formulated into a pharmaceutically acceptable suspension.

When the amounts of NTF released in SGM were plotted against the square root of time, a near linear relationship was obtained for the respective batches of NTF/CAP/CAB in the ratios of 1.0:1.0:1.0, 1.0:0.4:1.6 and 1.0:0.0:2.0 (Figure 4A). This indicated that the release of NTF from these microparticles in the first 5 h displayed Higuchi-type kinetics (Higuchi 1963). Thus, the dissolution of NTF microparticles was postulated to occur predominantly by the diffusion mechanism as suggested by Higuchi. For the dissolution of these microparticles in SIM, no linear relationship of the amounts released versus square root of time was found in any of the plots (Figure 4B). Thus, the release of NTF from these microparticles might be owing to a complex mechanism of leaching and disintegration; a gradual disintegration of the enteric coating material accelerated the release of NTF together with an increment of the NTF surface area.

In-vivo bioavailability studies

As the formulation containing NTF/CAP/CAB (1.0:0.4:1.6) displayed favourable in-vitro dissolution characteris-

Table 2 The cumulative percentages of nitrofurantoin (NTF) excreted in urine over time after oral administration of suspensions of the free NTF and the polymer-coated NTF microparticles with NTF/cellulose acetate phthalate/cellulose acetate butyrate in the ratio of 1.0:0.4:1.6.

Time (h)	Free NTF suspension	Polymer-coated NTF microparticle suspension
1.5	23.6±3.9[a,b]	15.4±2.6[a,b]
3.0	38.8±4.3	34.7±4.7[b]
4.5	44.9±4.8	42.0±5.2
6.0	47.2±5.9	44.7±5.5
7.5	47.5±6.1	45.0±5.6

Data are mean±s.d., n = 8. [a]Significant formulation effect ($P < 0.05$); [b]Significant time effect ($P < 0.05$).

tics, suspension of these microparticles was further investigated by comparing it with a suspension of free crystalline NTF in the in-vivo study. The cumulative percentage of NTF excreted in urine after oral administration of both the microparticle and free crystalline suspensions of NTF to the respective groups of rats (n = 8) in a cross-over manner is shown in Table 2. The results were analysed by using a two-way analysis of variance and a statistically significant difference ($P < 0.01$) between the two formulations was only found at 1.5 h after administration. With respect to time, a significant difference was found at 1.5 and 3 h in the microparticle formulation, and at 1.5 h in the free NTF suspension. It was noted that from 3 h onwards, the total cumulative urinary recovery of NTF from the suspension of microparticles was not significantly different from the suspension of pure drug crystals. It was confirmed that NTF was as equally well absorbed from the suspensions of microparticles as from the pure drug.

In-vivo ulcerogenicity studies

In the control group of rats fed with the dispersion medium, gastric ulcer was completely absent. In contrast, large ulcers were observed in the gastric mucosa of rats dosed with the suspension of pure drug. In this group, two out of the six stomach specimens were swollen and badly bruised after treatment. Ulceration was notably absent after the administration of the suspension of microparticles at the same dose. The lack of mucosal damage after administration of the suspension of microparticles was probably linked to the encapsulation of NTF in the polymer matrix and its subsequent reduced release in the stomach. The damage severity indices (median, range) of the control group and the groups fed with the suspensions of the polymer-coated NTF microparticles and the free NTF were 0 (0–0), 1.0 (0.5–2) and 4.0 (4–5), respectively. The latter two values were found to be significantly different from the control ($P < 0.001$). There was a very small increase in the gastric severity index in the group receiving the suspension of microparticles when compared with the control group receiving the suspending medium. This may have been caused by the presence of free NTF crystals adhering to the surface of the microparticles and/or partial release of the drug from the microparticles, thereby contributing to the dose-dependent effect on the gastric mucosa. The reduced ulcerogenic effects of the microparticles were probably owing to the reduced release and, therefore, absorption of drug in the stomach, which corresponded to the reduced urinary recovery at 1.5 h after administration of the microparticles.

Conclusion

Cellulose esters, including CAB and CAP, have been used either alone or in combination with other polymers as enteric coatings for extended release tablets (Edgar et al 2001). Some of these products have been marketed and used clinically. A similar approach can also be used to prepare enteric-coated microparticles with the purpose of formulating them into a suspension. It has been described in a patent that CAB was used to enteric coat theophylline microparticles (Edgar et al 2001). CAB has also been used to enteric coat carbamazepine for controlled release (Arnaud et al 1996). In this study, the modified solvent evaporation method was used to prepare enteric-coated NTF microparticles in the desirable size range for formulation into a suspension. With an appropriate ratio of NTF/CAP/CAB, the release of NTF from these microparticles in SGM and SIM could be controlled. The microparticles coated with CAP/CAB in the ratio of 0.4:1.6 displayed the most satisfactory in-vitro release profile: the impeded release of NTF in SGM and the sustained release of NTF in SIM. In the in-vivo experiment, the suspensions of these microparticles (NTF/CAP/CAB, 1.0:0.4:1.6) showed similar bioavailability to free NTF, but with much less ulcerogenicity. These findings indicate that a suspension of polymer-coated microparticles could be developed as a suitable formulation for oral administration of NTF to paediatric patients to minimize the gastric side-effects associated with NTF. However, this formulation is not without limitations. First, the suspension of these microparticles had to be prepared in-situ just before administration in this study. Prolonged suspension of these microparticles in a medium would cause leaching of the drug into the medium. The polymer enteric coating is supposed to be resistant to the acidic medium. However, as illustrated by the dissolution profile in SGM, the enteric-coated microparticles still released as much as 20% of their contents in 5 h. Second, although the microparticles gave a retarded release of drug in SGM and a sustained release of drug in SIM, this in-vitro dissolution profile did not translate into a much extended sustained release of drug in-vivo. According to the cumulative percentages of NTF excreted in urine over time after oral administration of suspensions of free NTF and polymer-coated NTF microparticles, there was a delayed absorption of the drug in the first 1.5 h. Thereafter, the extent of drug absorption from these two formulations was similar. Further studies to develop similar suspensions of sustained release microparticles, for admin-

istration to paediatric patients with the purpose of prolonging the duration of the therapeutic urinary concentration and reducing the dosing frequency, are warranted.

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