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In vitro and in vivo studies on biodegradable polyester microparticles containing sulphamethizole

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Summary

A matrix containing suspended drug and PHB or its copolymers PHB/PHV was prepared by a solvent evaporation process. The matrix was subsequently ground to give irregular particles of a desired size range and assayed in vitro and in vivo for its release characteristics. An increased drug loading from 16% to 50% increased the $t_{50\%}$ release in vitro significantly. In addition, decreasing the mean particle size of the product from 1590 μm to 101.5 μm showed a substantial increase in the release rate. Increasing the molecular weight of the PHB had a limited effect on the release rate. However, the use of copolymers of PHB and PHV, 17 and 30 mole percent enhanced the sustained release in vitro compared with the homopolymer (PHB). Overcoating of one of the products with L(-)PLA reduced the burst effect in addition to decreasing the overall in vitro release rate. Scanning electron microscopy revealed a porous product that increased in porosity after dissolution but appeared to remain intact. The mean in vivo sulphamethizole plasma profile of 6 dogs from one of the products chosen gave a more uniform and extended plasma level than a conventional product at a similar dose. These studies collectively indicate that PHB microparticles should be useful for the preparation of biodegradable sustained release dosage forms.

Introduction

Biodegradable polymers such as polylactic acid (PLA), polyglycolic acid, polyhydroxybutyric acid (PHB), polyalkylcyanoacrylates and more recently polycarbonates and polyanhydrides have been examined as materials for sustained release formulations. Reported applications of these polymer systems include fertility control (Jackanicz et al., 1973), narcotic antagonism (Woodland et al., 1973), anticancer chemotherapy (Yolles et al., 1975; Bissery et al., 1982; Couvreur et al., 1979; Lenaerts et al., 1984; Kawaguchi et al., 1983), local anaesthetics (Wakiyama et al., 1982; Kojima et al., 1984), and antimalarial chemotherapy (Wise et al., 1978). The formulations based on these polymers studied to date are mainly implants or injectable microparticles, microspheres or nanoparticles. In the present work, PHB and some copolymers have been used in an attempt to sustain the release of orally administered sulphamethizole.

A previous study using a natural biodegradable polymer, egg albumin, failed to achieve adequate

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sustained effect (Brophy and Deasy, 1984). Sulphamethizole was chosen because of its short plasma half-life and small extent of metabolism.

Materials and Methods

Materials

Sulphamethizole B.P. (Clonmel Chemicals), polyhydroxybutyric acid (PHB), number average molecular weights of 1.4×10^5 and 1×10^6, copolymers of polyhydroxybutyrate and polyhydroxyvalerate (PHB/PHV) containing 17 and 30 mole percent of hydroxyvalerate, number average molecular weights of 1.7×10^5 and 5×10^4, respectively (generously supplied by Marlborough Biopolymers Ltd., Stockton-on-Tees, Cleveland, U.K.), PHB, number average molecular weight of 5×10^5 and polyL(-)-lactic acid, (L(-)PLA), number average molecular weight of 7×10^4 (Polysciences, Northampton, U.K.), sulphamic acid, *n*-l-naphthylethylenediamine dihydrochloride, re-distilled chloroform and methylene chloride (BDH lab. reagents), sodium nitrite and hydrochloric acid (BDH Analar reagents) and distilled water were used.

Methods

Preparation of microparticles

A matrix containing sulphamethizole 16, 30 or 50% ω/ω was prepared using PHB of different molecular weights and copolymers of PHB/PHV (see Table 1). The appropriate weights of sulphamethizole, particle size < 53 μm were suspended in prefiltered solutions of the different polymers in chloroform. The concentration of the polymer solutions used were either 2% ω/ω (PHB 1.4×10^4, PHB 5×10^5 and PHB 1×10^6) or 10% ω/ω (PHB 1.4×10^4, PHB/PHV 17 mole % and PHB/PHV 30 mole %). The suspension was sonicated and evaporated on a rotary evaporator, temperature approximately 40°C, for about 30 min. The temperature was then increased to approximately 60°C to remove any residual solvent. The dried matrix was subsequently ground in a Moulinex grinder to give selected particle size ranges on sieving. Overcoating of product A (11) was done using a 10% ω/v solution of L(-)PLA in methylene chloride. The particles were suspended in the solution and allowed to air dry while stirred until approximately 8% ω/ω coating was achieved.

TABLE 1

PRODUCT MODIFICATIONS

	Polymer	Number average molecular weight	Drug loading	Particle size range (μm)
Product A				
(1)	PHB	1.4×10^5	50%	1180–2000
(11)	PHB	1.4×10^5	50%	425–600
(111)	PHB	1.4×10^5	50%	53–150
Product B	PHB	1.4×10^5	30%	1180–2000
Product C	PHB	1.4×10^5	16%	1180–2000
Product D	PHB	5×10^5	53%	1180–2000
Product E	PHB	1×10^6	47%	1180–2000
Product F	PHB/PHV 17 mole %	1.7×10^5	46%	1180–2000
Product G	PHB/PHV 30 mole %	5×10^4	52%	1180–2000
Product H	L(-)PLA--coated PHB	PLA : 7×10^4 PHB : 1.4×10^5	before coating 50 percent	before coating 425–600

Total drug content

40 mg samples were dissolved in 10 ml of chloroform and extracted with 5×20 ml vols. of 10% v/v HCl solution and assayed at 545 nm using the Bratton and Marshall procedure (1939).

In vitro dissolution studies

40 mg samples of microparticles were accurately weighed into a fine mesh bag, placed in a USP dissolution basket assembly and agitated at 100 rpm in 360 ml of 0.1 N HCl, temperature $37 \pm 1°C$. 5 ml samples were removed at various times over a period of 12 h (0.08, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10 and 12). The samples were filtered (Millipore) and assayed. The volume loss after sampling was made up with prewarmed 0.1 N HCl.

Scanning electron microscopy

Surface morphology of the microparticles was examined before and after dissolution using a Hitachi scanning electron microscope (Model S-520).

In vivo study protocol

Six male dogs (greyhounds) weighing between 20 and 30 kg were given doses of approximately 14.2 mg/kg of sulphamethizole powder, $< 53\ \mu m$, in capsule form or a capsule containing the equivalent dose in microparticle form (Product A(11)). Each preparation was administered in a randomized crossover design with 1-week interval between administration. The dogs were fasted overnight and for 12 h following administration of the dose. 5 ml blood samples were collected at regular intervals up to 12 h and then at 24 h, centrifuged and the plasma was removed and stored at approximately 4°C until analysis. The total amount of sulphonamide in a plasma sample was determined using the Bratton and Marshall procedure.

Results and Discussion

The method reported for producing microparticles was easy and reproducible. Particles of selected size ranges could be prepared by varying the grinding times.

Scanning electron microscopy (SEM)

SEM revealed that the products were very irregular with rough and smooth areas present (see Fig. 1A and B). This irregularity is presumably due to the method of preparation and the crystalline nature of the PHB used. At higher magnification the surfaces of the particles contained pores and cracks and in some cases exposed surface crystals (see Fig. 1B–E). The latter may be a result of poor deposition of the polymer in the matrix and/or the high drug loadings employed (50% ω/ω). It may also, however, have resulted from damage to the brittle matrix during grinding. The product that was overcoated with L(-)PLA had no apparent surface crystals which were probably shed during the coating procedure or actually overcoated. Pores were present, however, on the surface due to solvent evaporation (Fig. 1F). Post-dissolution photomicrographs (Fig. 2A–C) indicated that most of the products examined remained intact throughout the dissolution time (12 h) but became much more porous. However, the scanning electron photomicrograph of product A(1) in Fig. 2D would indicate that there may have been some erosion as a result of the dissolution or due to mishandling during sample preparation.

Effect of product modifications on in vitro release

Fig. 3 shows the effect of variations in particle size on the release rate in vitro (Products A(1), (11) and (111)). As expected, the smaller the particle size the greater the release rate. For example, the $t_{50\%}$ release for the three products tested (A(1), (11) and (111)) was 6 h, 1.2 h and 0.5 h, respectively. Smaller particle sizes have greater surface areas per unit weight, a higher concentration of drug at the surface and comparatively less depth in the matrix compared with larger particle sizes. These factors jointly give rise to increased drug release rate.

In vitro dissolution can also be altered by changing the drug loading in the matrix (see Fig. 4). The cumulative percent release after 12 h for products A(1), B and C was 60%, 50% and 45%, respectively. Lower drug loadings mean that less pores and channels will be formed in the matrix during leaching of the drug with a consequent reduction in the release rate.

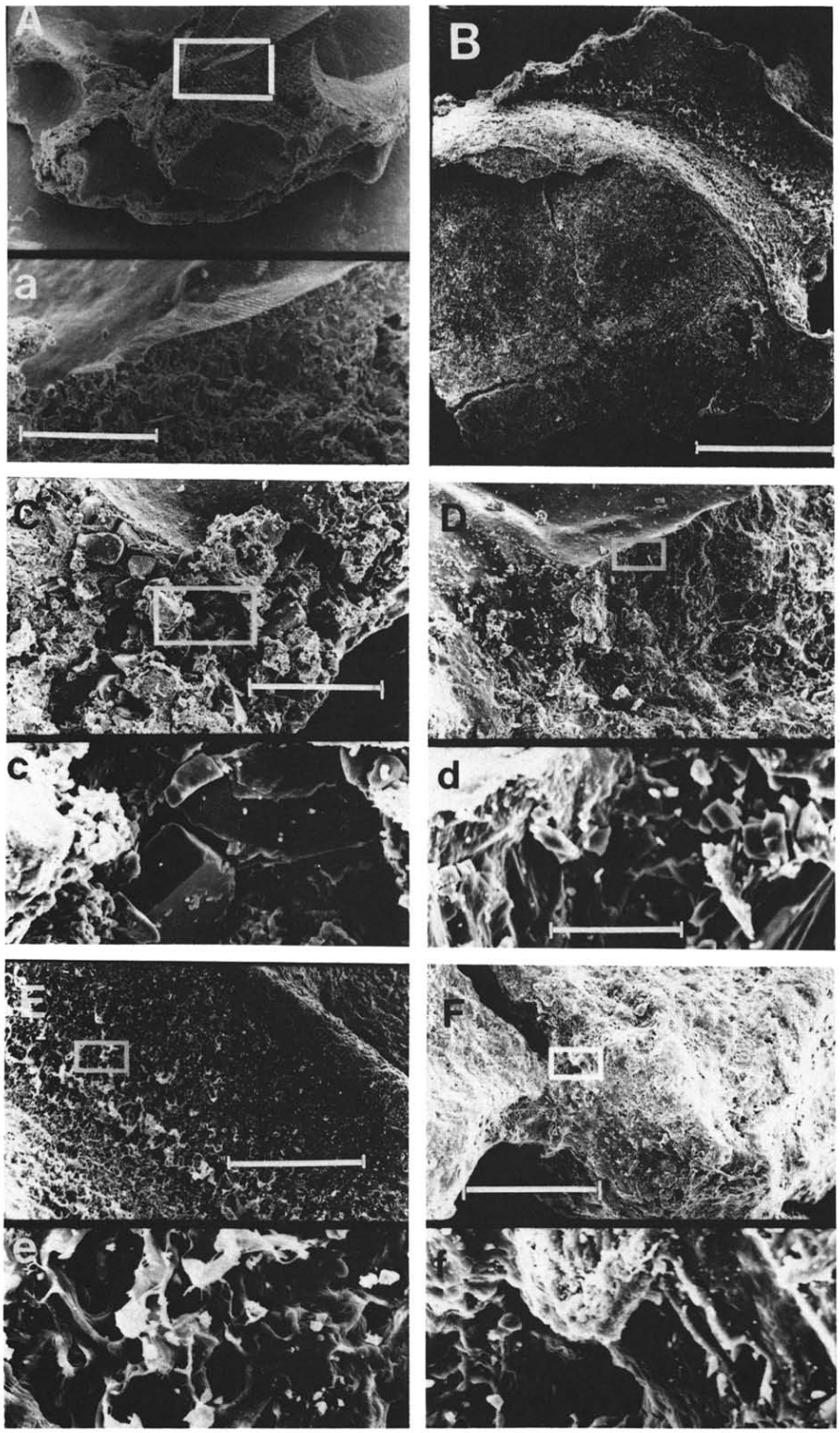

Fig. 1. Pre-dissolution scanning electron micrographs of product A(1) [A, 18 ×, a, 90 ×, bar, 600 μm; C, 132 ×, c, 660 ×, bar, 81.6 μm], of product G [B, 30 ×, bar, 360 μm; D, 90 ×, d, 150 ×, bar, 120 μm], of product E [E, 90 ×, e, 900 ×, bar, 120 μm] and of product H [F, 90 ×, f, 900 ×, bar, 120 μm].

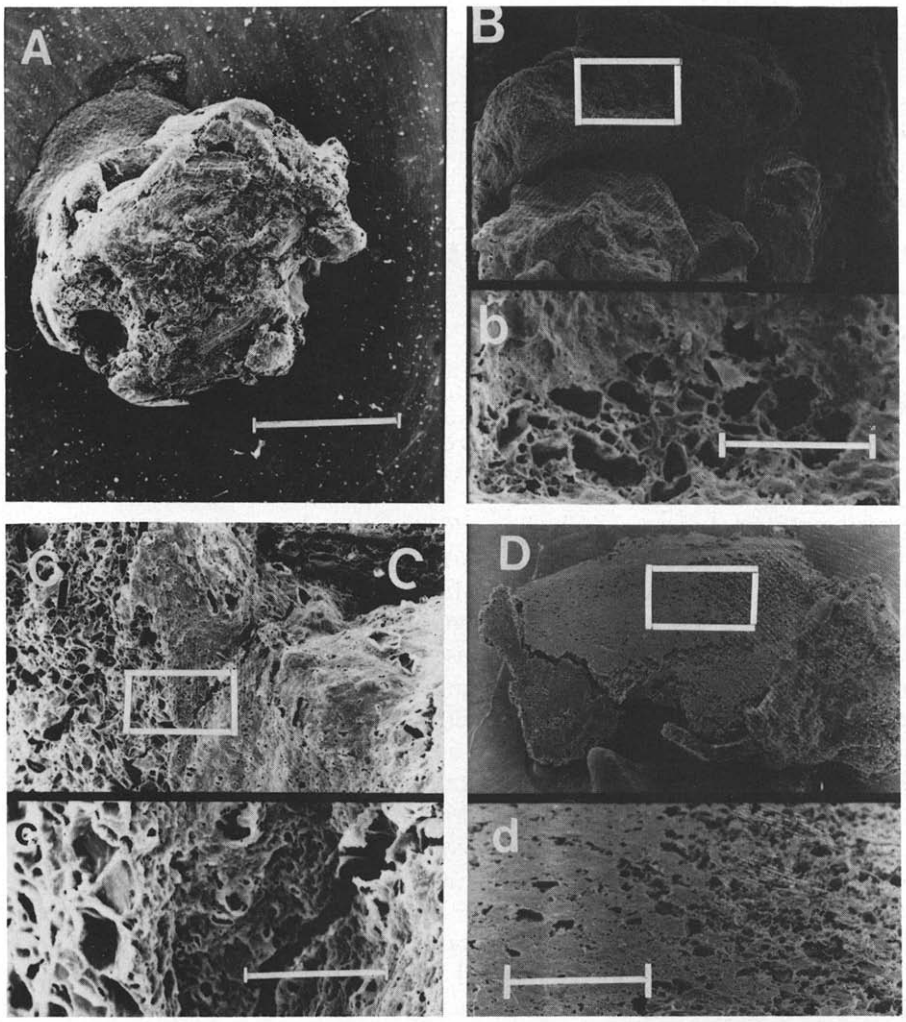

Fig. 2. Post-dissolution scanning electron micrographs of product C[A, 18 ×, bar, 600 μm], of product H[B, 48 ×, b, 240 ×, bar, 228 μm], of product F[C, 90 ×, c, 450 ×, bar = 120 μm] and of product A(1) [D, 18 ×, d, 90 ×, bar, 600 μm].

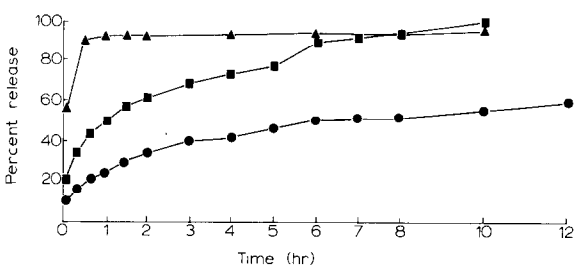

Fig. 3. In vitro release of sulphamethizole from Products: A(1), ●; A(11), ■; and A(111), ▲.

Generally, alteration of the molecular weight of a polymer should have little effect on drug diffusivity. However, for the three molecular weights tested (Products A(1), D and E), there was a significant difference in the release rate of the drug, sulphamethizole, between the lower molecular weight polymer and the two higher molecular weights (see Fig. 5). The latter two polymers released the drug at a faster rate than the former. PHB is a highly crystalline polymer and may well give rise to poor and uneven deposition in the matrix. Increasing the molecular weight of the

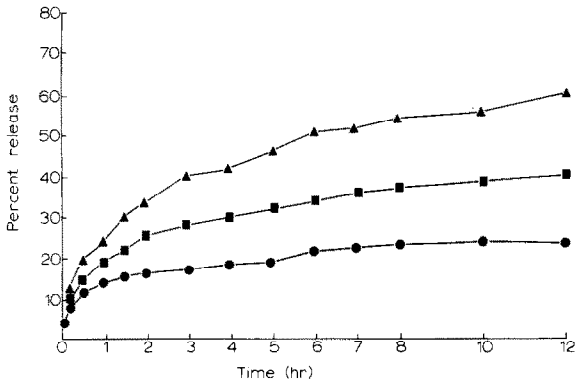

Fig. 4. In vitro release of sulphamethizole from Products: A(1), ▲; B, ■; and C, ●.

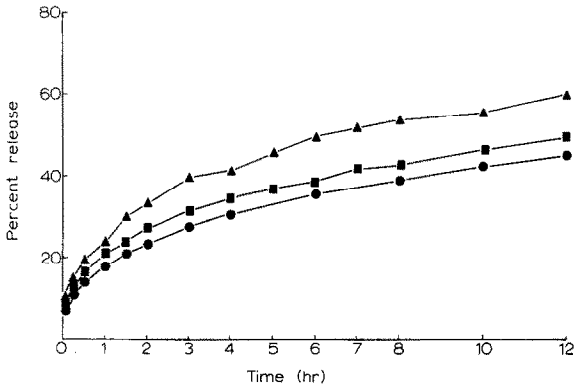

Fig. 6. In vitro release of sulphamethizole from Products: A(1), ▲; F, ■; and G, ●.

polymer may have enhanced this problem. Indeed Bissery et al. (1984) found that PHB microspheres were often misshapen and attributed this to the high crystallinity of the polymer. In contrast, they found that poly(D,L)-lactide (DL-PLA), an amorphous polymer, consistently formed spherical microparticles. Copolymers of PHB are more amorphous than the homopolymer PHB and would be expected to show less resistance to the diffusing drug molecules with a resulting faster release. In the present study, however, the two copolymers of PHB/PHV (Products F and G) had a $t_{50\%}$ approximately twice that of the homopolymer (Products A(1), $t_{50\%} = 6$ h; F, $t_{50\%} = 12.2$ h; and G, $t_{50\%} = 13.2$ h) which was significantly different even allowing for the small differences in drug loading and/or molecular weights of the products (see Fig. 6). This unexpected result may be attributed to better matrix formation with the more homogeneous amorphous copolymer. Copolymerization causes internal plasticization giving rise to better alignment and deposition in the matrix thus reducing diffusivity. In contrast, the drug may be deposited as large clusters in the crystalline homopolymer rather than evenly deposited and may therefore lose the full protection of the polymer. It is also possible that the grinding procedure may have had a more damaging effect on the more crystalline homopolymer than the amorphous copolymers.

A small burst effect was observed for most of the products examined and this is due to the surface (cf. Fig. 1C and D). Overcoating of product A(11) with approximately 8% ω/ω L (L)PLA reduced the burst effect presumably due to loss of

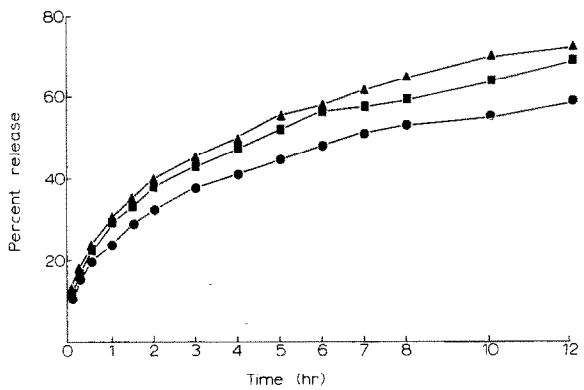

Fig. 5. In vitro release of sulphamethizole from Products: E, ▲; D, ■; and A(1), ●.

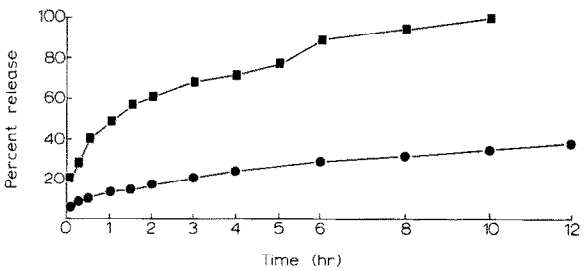

Fig. 7. In vitro release of sulphamethizole from PHB, Product A(11) ■, and overcoated with 8% ω/ω L(-)PLA, product H ●.

surface crystals during the coating procedure and/or due to overcoating of the crystals (Product H, Fig. 7). However, the PLA also had a substantial effect on the overall release rate resulting in a cumulative percent release up to 12 h of 37.6% compared with 100% release for the uncoated product. Similarly, Bissery et al. (1984) found that 1-(2-chloroethyl)-3-cyclohexylnitrosurea was extracted much more quickly from PHB microspheres than DL-PLA microspheres.

Release characteristics

It is difficult to assign any mathematical equation to describe the in vitro release of such a collection of imprecise geometrical particles. However, a reasonably good agreement was found between the theoretical release curves computed from the equation below and the experimental release of the drug from the microparticles:

$$\frac{3}{2}\left(1-\left[1-\frac{M_t}{M_\infty}\right]^{\frac{2}{3}}\right)-\frac{M_t}{M_\infty}=\frac{3DC_st}{r_o^2\,C_o}$$

where M_t/M_∞ = fraction of drug released up to time t, D = diffusion coefficient of the drug in the particle matrix, C_s = solubility of drug in the particle matrix, t = time, r_o = radius of the particle, and C_o = initial concentration of the drug in the particle.

This equation was derived by Baker and Lonsdale (1974) to describe drug release from dispersed spherical matrices and was based on the previous approach of Higuchi (1963). Differential scanning calorimetry of the microparticles indicated a dispersion of sulphamethizole in the polymer matrix. For example, Fig. 8 shows a comparison of the theoretical release versus the experimental release for product A(1). The higher initial experimental release of the drug compared with the theoretical release is presumably due to the greater surface area of the irregular particles compared with a spherical particle of equivalent mean diameter.

In vivo studies

Product A(11) when administered in vivo to 6 dogs showed a sustained release compared with a conventional product of a similar dose (See Fig. 9; Product A(11), 13.4 mg/kg and a conventional dose (C.D.), 14.2 mg/kg). Over a 12 h period, the bioavailability of the two products tested was similar, allowing for the 5% difference in the dose administered (C.D.) AUC = 45 $\mu g \cdot h/ml$; A(11), AUC = 40 $\mu g \cdot h/ml$). Intravenous dosing of 2 dogs (14 mg/kg) indicated that the data obtained could be adequately fitted to a one-compartment model. In order to compare the in vivo absorption with the in vitro release, the plasma concentration versus time plot was converted to the fraction of drug absorbed versus time using the Wagner-Nel-

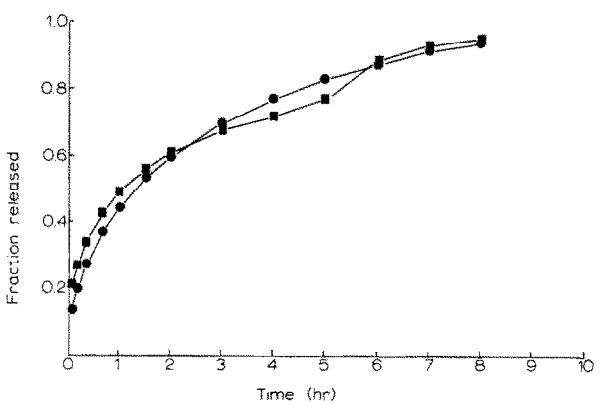

Fig. 8. Comparison of the theoretical (●) versus experimental (■) release of drug for particles mean size 1590 μm containing 50% drug loading. Product A(1).

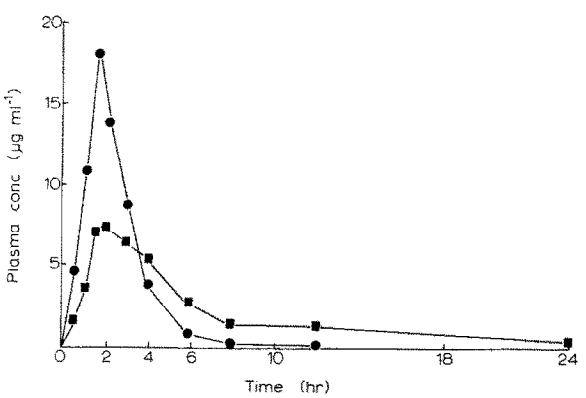

Fig. 9. Mean plasma sulphamethizole concentration (n = 6) of a conventional dose (●) and Product A(11) (■).

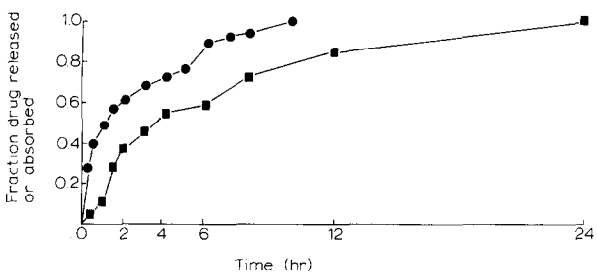

Fig. 10. In vitro release (●) and in vivo absorption (■) of Product A(11).

son method (1963). Fig. 10 shows the comparison between in vitro and in vivo profiles and would indicate that the in vivo absorption was controlled by the in vitro dissolution. The slight increase in the release rate in vitro at approximately 5 h may have resulted from sudden weakening or erosion of the particles. This was not, however, evident with the release from the larger particle size of the same product (cf. Fig. 8, product A(1)).

Kaneniwa and Watari (1978) found that because sulphonamides are weak acids, they showed a change in the absorption rate due to a change of dissolution environment with movement of the drugs from a low pH to a high pH in the gastrointestinal tract. Sulphamethizole, pK_a of 2.0 and 5.45, should show increased solubility at pH above and below pH 3.83 with a slightly higher solubility at pH 7.4 than pH 1.0 (Nakano et al., 1979). However, the in vivo absorption rate did not apparently increase as expected with the passage of the particles into the higher pH surroundings of the small intestine presumably due to the effect of the polymer. Itoh et al. (1980) found that as they increased the PLA content of their coating, they lost the in vitro pH-dependent release of sulphamethizole seen with lower concentrations of PLA.

Product H was also administered to two of the dogs under study. The resulting plasma levels were constant over a period of 24 h at a concentration of approximately 3.5 μg/ml compared with a maximum of 15 μg/ml for the conventional dose and 10 μg/ml for the uncoated product. All three products had similar bioavailability. The low plasma levels obtained with the coated product H were most likely as a result of the slow release from the product (cf. Fig. 7). Overall, it would appear that the in vitro dissolution studies of these products may be useful guidelines for predicting their in vivo behaviour.

Conclusions

These results collectively indicate that the method reported is capable of producing irregular drug loaded microparticles that can sustain the in vitro and in vivo release of sulphamethizole. The release rate may be decreased by increasing the particle size used, by decreasing the drug loading in the matrix or by using copolymers of PHB/PHV instead of the homopolymer PHB. Increasing the molecular weight of the homopolymer tended to give faster drug release. Overcoating of the homopolymer PHB with L(-)PLA reduced the initial burst effect and further sustained the overall release. The in vitro release profiles may be modelled approximately using an equation for the release of dispersed drug from spherical matrices. A good correlation was obtained between the in vitro release and the in vivo absorption for one of the products examined.

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