

Sustained Release of 5-Fluorouracil from Polymeric Nanoparticles

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Abstract

The use of biodegradable nanoparticles loaded with 5-fluorouracil was investigated as a potential means to sustain the release of this drug. Nanoparticles prepared from four biodegradable polymers were loaded with 5-fluorouracil using three loading concentrations of drug and three different concentrations of added polymer.

Washing particles using a centrifugation/re-suspension with ultrasound protocol was found to dislodge the majority of drug, resulting in an over-estimation of incorporation efficiency and low levels of strongly entrapped drug. Increasing the initial 5-fluorouracil concentration before polymer/monomer addition increased the drug loading in both washed and unwashed particles. Increasing the amount of polymer used to make nanoparticles did not increase loadings, but did produce increased amounts of unusable polymer waste. Drug release from nanoparticles was evaluated using a Franz cell diffusion apparatus, which showed an initial burst effect followed by a slower release phase over 24 h. Indeed, nanoparticles prepared from poly(lactide-co-glycolide) released 66% of their 5-fluorouracil payload over this period.

It was concluded that 5-fluorouracil-loaded nanoparticles could be readily included into a hydrogel-based delivery system to provide sustained drug release for trans-epithelial drug-delivery applications.

Nanoparticulate delivery systems, such as those based on poly(alkylcyanoacrylate) polymers, have been studied extensively for many years as a potential means for drug delivery and targeting (Douglas et al 1987; Couvreur et al 1995; Crommelin et al 1995). These nanoparticles are prepared using procedures that are well established, such as emulsification polymerisation (Couvreur 1988), solvent dispersal with interfacial deposition (Fessi et al 1989) and interfacial polymerisation (Krause et al 1986). These techniques involve the sequential addition of one phase to another, with one phase containing the dissolved payload for the particle. During emulsification polymerisation, the drug is dissolved in the aqueous polymerisation medium before the addition of monomer whereas, during

interfacial deposition, it is generally dissolved, along with the polymer, in the water-miscible solvent.

5-Fluorouracil, a pyrimidine analogue, displays a broad spectrum of activity against several solid tumours by interfering with thymidylate synthesis (Heidelberger 1965). There have been several attempts to modify the delivery of 5-fluorouracil using either polymer conjugates (Nichifor et al 1996) or incorporation into a particulate carrier (Kreuter & Hartmann 1983). The ultimate aim of these strategies was to reduce associated side-effects and improve the therapeutic index. Improving efficacy using a particulate platform will, of course, require a useful loading of 5-fluorouracil in the particles. Because of the hydrophilic nature of this compound, it is to be expected that loading from an aqueous polymerisation phase into a hydrophobic particulate core is low. Furthermore, loading into a particulate core will depend on the polymer used and on experimental

factors used during the preparation procedure (McCarron et al 1999). Most of the polymeric materials used to prepare 5-fluorouracil-loaded nanoparticles are synthetic in nature, such as poly (cyanoacrylate) (Sawant & Murthy 1993) and D,L-lactide/coglycolide copolymer (Niwa et al 1993). Other more novel carriers include cyclic core dendritic polymers (Zhuo et al 1999). Incorporation levels using these polymers have been found to be variable, ranging from 1.2% for cellulose nanoparticles (Mukherji et al 1990) to 14.3% for poly(glutaraldehyde) nanoparticles (Mukherji et al 1989).

The aim of this study was to investigate the use of biodegradable nanoparticles loaded with 5-fluorouracil as a potential means to sustain the release of this drug. A specific objective was to identify a candidate polymeric carrier that can achieve a useful loading with minimal drug loss, which occurs during washing procedures. Another objective was to evaluate the release profiles from 5-fluorouracil-loaded particles, which, as a continuation of this work, could be embedded throughout the matrix of a secondary delivery device, such as a bioadhesive patch system. Such a system may have potential application for delivery of 5-fluorouracil to mucosal epithelia, such as that found on the external uterine cervix. The novelty of such a system is that the 5-fluorouracil-loaded particles may exert an additional sustained-release action, over and above that produced by the patch alone. On hydration, and after the initiation of bioadhesion to the cervical tissue, the patch swells into a hydrogel matrix that allows embedded drug to freely diffuse out. Previous work using 5-fluorouracil-loaded patches (Woolfson et al 1995; McCarron et al 1997) has demonstrated that drug penetration into, with subsequent clearance from, cervical tissue is excessively rapid for ideal neoplastic control. This problem may be overcome by sustaining the delivery of 5-fluorouracil by using loaded particles suspended within the bioadhesive platform.

Materials and Methods

Materials

Nanoparticles were prepared from poly(isobutylcyanoacrylate) (PBCA, Loctite Ltd, Ireland), poly(lactide-co-glycolide) (PLGA, Resomer RG505, Boehringer Ingelheim, Germany), poly (caprolactone) (PCL, CAPA 640, Solvay Interlox Ltd, Cheshire, UK) and Eudragit (L-100, Röhm Pharma, Darmstadt, Germany). All solvents, such

as acetone, ethanol and tetrahydrofuran were of HPLC grade (Labscan Ltd, Dublin, Ireland) and reagents used to prepare buffered solutions were of AnalaR grade (BDH, Poole, UK).

Particle preparation

PBCA particles were prepared by emulsion polymerisation of monomer in an acidic aqueous phase. The monomer was added drop-wise to 25 mL of buffer (pH 2.5) containing 5-fluorouracil and 0.5% w/v Lutrol F68 and allowed to polymerise for an hour. Nanoparticles made from polymeric precursors were prepared by polymer deposition with subsequent solvent evaporation. Polymer and 5-fluorouracil were dissolved in 25 mL of a suitable polymer solvent. The water-miscible solvent chosen depended on the polymer type. With the aid of brief sonication, PLGA was dissolved in tetrahydrofuran, PCL dissolved in a 50:50 mix of acetone in ethanol and Eudragit was dissolved in ethanol. This was added at a constant flow rate using a peristaltic pump through a 26G syringe needle at 1.0 mL min⁻¹ into 50 mL of phosphate buffer pH 2.5 containing 0.5% w/v Lutrol F68. The suspension was concentrated down to 25 mL using rotary evaporation under vacuum.

An estimate of the efficiency of particle preparation was determined using Equation 1.

$$\begin{aligned} \text{Efficiency of particle recovery (\%)} \\ = (\text{mass of particles } (\mu\text{g}))/ \\ (\text{mass of added polymer } (\mu\text{g})) \times 100 \end{aligned} \quad (1)$$

Drug analysis

5-Fluorouracil concentration was determined using reversed-phase HPLC (Shimadzu SCL-10A system controller, SPD-10A VP UV/Vis Detector, SIL-10AD VP Auto Injector, LC-10AT VP Liquid Chromatograph) comprising a Waters Spherisorb 5 μm ODS2 4.6 \times 250 mm analytical column. The mobile phase consisted of 0.1% phosphoric acid (pH 2.0) in methanol (96:4) running at 1.0 mL min⁻¹. Detection was at 266 nm, with a lower limit extending down to 20 ng mL⁻¹.

Effect of washing conditions on drug loading

Particles were prepared, as detailed above, using a constant loading concentration of 2.5 mg mL⁻¹ of 5-fluorouracil in either the polymerisation medium (PBCA) or the polymer solvent (PCL, PLGA and Eudragit). Three samples of each nanoparticle suspension were centrifuged at 56 000 g for up to

10 min, the supernatants decanted carefully and analysed using HPLC. The concentration of drug in the supernatant was used to determine the percentage loading in unwashed particles.

Drug loading in washed particles was evaluated by firstly re-suspending the three pellets separately using ultrasound at two different power densities. Particles were re-suspended using either 2.500 w cm^{-3} from an ultrasonic probe (Jencons Vibra-cell, Danbury, CT) or 0.061 w cm^{-3} while submerged in an ultrasonic bath (Decon FS200, Decon Ultrasonics Ltd, Hove, UK). The suspension was re-centrifuged at $56\,000 \text{ g}$, the washed pellet dissolved in tetrahydrofuran (THF) and the 5-fluorouracil concentration determined using HPLC.

The percentage loading of 5-fluorouracil in both washed and unwashed particles was determined using Equation 2.

$$\text{Loading (\%)} = \frac{\text{amount of 5-fluorouracil } (\mu\text{g})}{\text{(mass of particles } (\mu\text{g})) \times 100} \quad (2)$$

Effect of 5-fluorouracil and polymer concentration on particle loading

Nanoparticles of PLGA, PCL, PICBA and Eudragit were prepared as described above, keeping the polymer and surfactant concentrations constant, but varying the concentration of 5-fluorouracil in the aqueous phase. Three concentrations of 5-fluorouracil were used, namely 50, 500 and $5000 \mu\text{g mL}^{-1}$. The particle suspension was centrifuged and the supernatant analysed by reverse-phase HPLC to determine the drug loading in the unwashed particles. Pellets were re-suspended, washed in distilled water, re-centrifuged and freeze-dried for 24 h before determination of the loadings in washed particles using HPLC. This procedure was repeated a further two times for each concentration of 5-fluorouracil to gain a measure of the statistical spread.

Similarly, the amount of polymer in the non-aqueous phase was varied to investigate the effect of polymer concentration on the drug loading in particles prepared using the solvent displacement procedure. Particles were prepared as detailed above with a varying polymer content (0.5, 1.0 or 2.0% w/v), while holding the concentration of 5-fluorouracil constant at $5000 \mu\text{g mL}^{-1}$. Again, this procedure was repeated a further two times.

Particle characterisation

Approximately 0.5 mL of nanoparticle suspension was added to 50 mL of filtered 0.02 M phosphate

buffer at pH 7.3. The Z_{ave} diameter and polydispersity of the nanoparticles prepared at each drug and polymer concentration were characterised by photon correlation spectroscopy (PCS) and zeta potentials were measured by laser anemometry (Malvern Zetasizer 4, Malvern Instruments Ltd, Malvern, UK). Particle characterisation was carried out three times for each sample.

Diffusion cell experiments

A Franz cell dissolution apparatus was used to monitor 5-fluorouracil release from the nanoparticles. The receptor phase was phosphate-buffered saline (PBS, pH 7.4) thermostatically maintained at 37°C , with each release experiment run in triplicate. Cuprophane membrane (Medicell International, Liverpool, UK) with a molecular cut off 10 000 Daltons, was used to separate receptor and donor phases. The latter consisted of a 1-mL suspension of nanoparticles (10 mg), sonicated for 5 s to aid re-suspension, in a 1% w/v Tween 80 solution in PBS. Samples ($180 \mu\text{L}$) from the receptor phase were taken at time intervals with a pre-calibrated filter straw and an equivalent volume of PBS replaced into the receiver compartment. Diffusion of 5-fluorouracil into the receptor phase was evaluated by HPLC.

Scanning electron microscopy

Particles were visualised using a Jeol JSM-6400 scanning electron microscope (15 kV accelerating voltage). Dry particulate samples were dusted on to a double-sided adhesive pad applied previously to an aluminium stub. Excess sample was removed and the stub sputter-coated with a 30-nm layer of gold. Particles not subjected to freeze-drying procedures were visualised by adding the suspension directly to the aluminium stub and allowing it to air dry before sputter-coating.

Results and Discussion

The polymers used in this study have been investigated extensively as precursors for nanoparticle-based delivery systems, particularly the alkylcyanoacrylates and lactide-co-glycolides. They offer ideal biodegradation profiles associated with low toxicity. However, for such carriers to be therapeutically useful, either as intravenous carriers or as a component in a mucoadhesive system, they must achieve a useful loading of drug. 5-Fluorouracil has posed considerable problems in this respect and produces poor loading, particularly in

alkylcyanoacrylates and lactide-co-glycolide particles. This study compared the loading and release profiles in both these polymers and the lesser-used PCL and Eudragit in an attempt to identify a suitable polymer for inclusion in a mucoadhesive, patch-based system.

Drug loading is generally determined by analysing the supernatant, once the particles have been formed into a pellet using centrifugation. Free drug remains in solution, whereas the incorporated drug sediments down with the nanoparticle. Table 1 shows that the 5-fluorouracil loading achieved using the four polymers in this study was approximately 4% w/w, with PCL and PLGA achieving the highest loading. However, when the particles were subjected to only one wash cycle, this loading dropped to below 1% w/w. This indicates that the majority of 5-fluorouracil is held loosely to the particle surface and is displaced readily. Thus, the use of ultrasound, which is frequently required to re-suspend the particulate pellet during nanoparticle preparation, could dislodge further amounts of drug. Table 1 also shows a comparison of re-suspension using ultrasound of two intensities. Single factor analysis of variance reveals that there is no significant difference between the three washing procedures ($P=0.198$), indicating how effective a single wash, without the aid of ultrasound, is at removing a hydrophilic drug from these types of delivery system. Although Eudragit and PBCA showed no noticeable loss of drug by using more intense ultrasound, the sonic probe could dislodge almost all the drug from PLGA and PCL nanoparticles. These results demonstrate that 5-fluorouracil probably exists as a combination of both tightly incorporated and loosely bound drug. Determination of hydrophilic drug loading based on the analysis of the supernatant alone with no wash cycle, or determination based on filtration (Mukherji et al 1989) will possibly lead to an over-estimation of drug that is tightly associated with the particle.

The concentration of 5-fluorouracil in the aqueous phase was raised from $50 \mu\text{g mL}^{-1}$ to $5000 \mu\text{g mL}^{-1}$ to achieve greater loading. Table 2 shows the loading in both unwashed and washed particles as determined by analysing the freeze-dried pellet. Increasing the 5-fluorouracil concentration increases the loading for all polymer types. Indeed, all polymer types show a similar loading, even though the PBCA particles had been made using a different procedure from the other three. One wash removed a considerable amount of drug, again indicating that the majority was loosely bound. Therefore, by using the highest concentration of 5-fluorouracil, a loading of approximately 5% w/w could be achieved, but one wash was sufficient to reduce this to less than 0.5% w/w.

The effect of increasing the drug concentration on the physicochemical parameters of the particles is shown in Table 2. Particle size was not influenced by the concentration of the drug, except for Eudragit, which showed an initial increase followed by a drop. The PBCA particles were the least polydisperse, which did change as the drug concentration increased. In contrast, the PLGA particles were more disperse, which was not influenced noticeably by 5-fluorouracil concentration. Zeta potential measurements did not show any distinct pattern, except for Eudragit, which became more negative on increasing the 5-fluorouracil concentration.

The amount of polymer added to the polymer nonsolvent was also investigated as a possible way to also increase loading of 5-fluorouracil in the particles, using the solvent disposition procedure. Table 3 shows that increasing the amount of PLGA allowed more drug to be loaded into the particle. However, this must be considered in combination with the efficiency of particle preparation, which showed that more unusable polymeric agglomerates were formed and the recovery of suitable nanoparticles fell markedly. Increasing the amount of PCL and Eudragit did not produce increased

Table 1. Comparison between drug loading in unwashed nanoparticles and nanoparticles washed using either no ultrasound or ultrasound at two different power densities.

Polymer type	5-Fluorouracil loading (% w/w)			
	Unwashed particles	Washed (no sonication)	Washed (sonication at 0.061 w cm^{-3})	Washed (sonication at 2.500 w cm^{-3})
PLGA	5.49 ± 0.64	0.12 ± 0.01	0.64 ± 0.00	not detected
PCL	5.44 ± 0.71	0.92 ± 1.03	0.81 ± 0.94	not detected
Eudragit	4.06 ± 0.18	0.29 ± 0.01	0.48 ± 0.01	0.37 ± 0.01
PBCA	3.18 ± 0.48	0.51 ± 0.09	0.88 ± 0.23	0.82 ± 0.02

Values are means \pm s.d., $n=3$.

Table 2. Effect on 5-fluorouracil loading and nanoparticle characterisation of increasing the drug concentration in the polymerisation/precipitated phase.

	Concn of 5-fluorouracil in aqueous phase (% w/v)		
	50 $\mu\text{g mL}^{-1}$	500 $\mu\text{g mL}^{-1}$	5000 $\mu\text{g mL}^{-1}$
5-Fluorouracil loading (% w/w) in unwashed particles			
PBCA	0.0324 \pm 0.0008	0.6058 \pm 0.0567	4.8336 \pm 0.1642
PLGA	0.0505 \pm 0.0054	0.5212 \pm 0.0077	4.5381 \pm 0.3200
PCL	0.0541 \pm 0.0038	0.6134 \pm 0.0293	4.1499 \pm 0.3472
Eudragit	0.0308 \pm 0.0007	0.6072 \pm 0.0128	3.5528 \pm 0.1497
5-Fluorouracil loading (% w/w) in washed particles			
PBCA	0.0074 \pm 0.0004	0.0618 \pm 0.0039	0.2959 \pm 0.0490
PLGA	0.0164 \pm 0.0014	0.0355 \pm 0.0017	0.1693 \pm 0.0147
PCL	0.0243 \pm 0.0033	0.0624 \pm 0.0129	0.4047 \pm 0.0477
Eudragit	0.0056 \pm 0.0000	0.0263 \pm 0.0018	0.0967 \pm 0.0072
Particle diameter (nm)			
PBCA	160.9 \pm 3.1	163.8 \pm 0.9	191.2 \pm 5.7
PLGA	186.4 \pm 11.8	178.3 \pm 26.8	194.3 \pm 17.5
PCL	190.3 \pm 15.4	219.5 \pm 40.2	194.6 \pm 2.1
Eudragit	865.9 \pm 55.5	1470.5 \pm 52.9	249.5 \pm 20.1
Polydispersity			
PBCA	0.049 \pm 0.041	0.041 \pm 0.004	0.097 \pm 0.063
PLGA	0.480 \pm 0.040	0.517 \pm 0.059	0.421 \pm 0.113
PCL	0.359 \pm 0.112	0.796 \pm 0.300	0.289 \pm 0.056
Eudragit	0.392 \pm 0.117	0.579 \pm 0.365	0.321 \pm 0.347
Zeta potential (mV)			
PBCA	-17.5 \pm (-1.2)	-21.3 \pm (-5.8)	-22.2 \pm (-3.7)
PLGA	-13.6 \pm (-0.3)	-16.2 \pm (-0.6)	-11.8 \pm (-0.4)
PCL	-2.7 \pm (-0.7)	-5.4 \pm (-8.9)	-2.2 \pm (-1.3)
Eudragit	-9.7 \pm (-0.7)	-11.3 \pm (-0.9)	-24.4 \pm (-1.2)

Values are means \pm s.d., n = 3.

Table 3. Effect of increasing the polymer concentrations used to prepare particles on the 5-fluorouracil loading, particle recovery rates and particle characterisation.

	Concn of polymer in the aqueous phase (% w/w)		
	0.5	1.0	2.0
5-Fluorouracil loading (% w/w)			
PLGA	0.5982 \pm 0.0033	0.6067 \pm 0.0355	3.0626 \pm 0.0942
PCL	1.8806 \pm 0.0507	1.1507 \pm 0.0711	1.2845 \pm 0.2191
Eudragit	0.4289 \pm 0.0290	0.1255 \pm 0.1255	0.1896 \pm 0.0048
Particle recovery rate			
PLGA	42.90	31.63	5.07
PCL	15.69	23.37	14.51
Eudragit	62.39	59.08	11.06
Particle diameter (nm)			
PLGA	194.3 \pm 17.5	314.4 \pm 6.3	328.0 \pm 20.1
PCL	194.6 \pm 3.12	224.7 \pm 4.2	295.3 \pm 13.6
Eudragit	249.5 \pm 3.1	387.5 \pm 7.8	235.3 \pm 6.3
Polydispersity			
PLGA	0.421 \pm 0.113	0.318 \pm 0.062	0.421 \pm 0.016
PCL	0.289 \pm 0.056	0.102 \pm 0.087	0.212 \pm 0.094
Eudragit	0.321 \pm 0.347	0.075 \pm 0.030	0.369 \pm 0.102
Zeta potential (mV)			
PLGA	-11.8 \pm (-0.4)	-12.0 \pm (-0.3)	-9.7 \pm (-4.5)
PCL	-2.2 \pm (-1.3)	-1.8 \pm (-0.9)	-2.3 \pm (-4.3)
Eudragit	24.4 \pm (-1.2)	-12.5 \pm (-1.0)	-10.4 \pm (-5.5)

Values are means \pm s.d., n = 3.

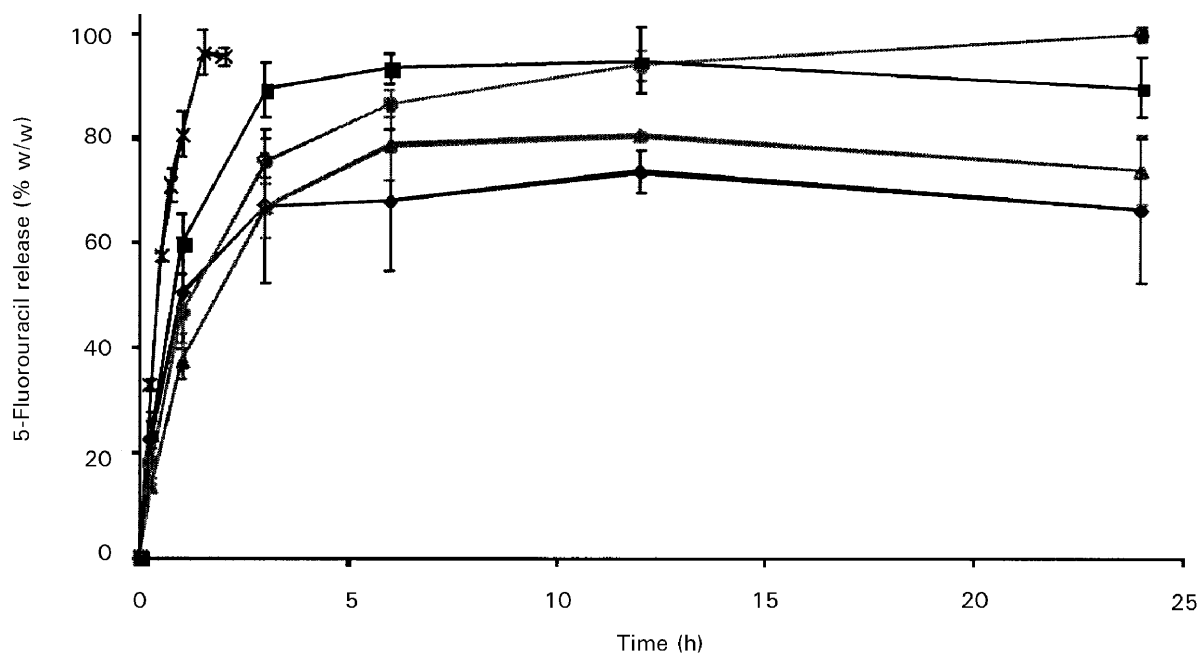


Figure 1. Release of 5-fluorouracil from nanoparticles prepared using the different polymeric carriers (◆, PLGA; ▲, Eudragit; ●, PBCA; ■, PCL) over 24 h compared with the equilibration profile of free 5-fluorouracil (×) in solution.

loading, with the maximum loading achieved by using the lowest polymer concentration. The recovery of Eudragit particles also fell as the amount of polymer added was increased.

The effect of polymer concentration on physico-chemical parameters is shown in Table 3. The particle size remained at around 250 nm for all polymers, indicating that excess polymer led not to bigger particles, but to more unusable aggregates. Both polydispersity and zeta potential were not strongly influenced by additional polymer. Drug release was evaluated using a Franz cell dissolution apparatus. A Cuprophane membrane, with a molecular weight cut-off point at 10 000 Daltons, was chosen to separate both compartments, allowing free drug to equilibrate across into the receptor phase. This method was used as an alternative to the more commonly used centrifugation method, where samples from a nanoparticulate suspension are withdrawn at time-intervals and centrifuged to allow analysis of free drug in the supernatant. However, prolonged periods of time were used to clarify the supernatant from all nanoparticulate matter. During the centrifugation run time, drug release was still occurring. 5-Fluorouracil release was most rapid during this initial part of the release profile (Figure 1), particularly if there was evidence of a burst effect. It is thus possible that information during the initial release could be lost. For this reason, it was felt that the Franz cell-based method represented a more responsive procedure with no lag time between sampling and free-drug determination.

Figure 1 shows the release of 5-fluorouracil over 24 h from washed particles prepared using the highest concentration of 5-fluorouracil in the aqueous phase. When incorporated into each type of particle, the appearance of 5-fluorouracil in the receptor phase was considerably slower than that from free drug in solution. An initial burst effect was seen over the first three hours, followed by a slower release phase up to 24 h. A similar biphasic release pattern has also been reported for PLGA nanoparticles (Niwa et al 1993) and chitosan microspheres (Akbuga & Bergisadi 1996). PLGA and Eudragit displayed the slowest release pattern with 34% and 26% of the drug remaining in the particles after 24 h, respectively (Table 4). Only 10.3% of the drug remained in the PCL particles and the PBCA particles had released their entire payload. A closer examination of the release profile during the first hour of the burst phase is shown in Figure 2. Even over this short period of time, the

Table 4. Percentage of 5-fluorouracil released from particles after 1 h and 24 h.

Polymer	Percentage of 5-fluorouracil released	
	1 h	24 h
PBCA	46.7	99.99
PCL	59.96	89.72
PLGA	50.48	66.29
Eudragit	37.34	73.89

Table 5. Short-term release data fitted to either a root-time or exponential model with corresponding regression coefficient

Polymer		Q/\sqrt{t} ($\mu\text{g h}^{-0.5}$)	Regression coefficient, r^2	Exponential rate constant	Regression coefficient, r^2
Eudragit	0.5%	1.165 ± 0.071	0.949 ± 0.087	0.023 ± 0.002	0.904 ± 0.061
	1.0%	0.139 ± 0.001	0.991 ± 0.001	0.025 ± 0.004	0.905 ± 0.008
	2.0	0.286 ± 0.006	0.966 ± 0.059	0.023 ± 0.002	0.933 ± 0.011
PCL	0.5%	26.426 ± 2.199	0.996 ± 0.003	0.021 ± 0.001	0.934 ± 0.014
	1.0%	7.604 ± 0.729	0.996 ± 0.002	0.021 ± 0.002	0.926 ± 0.011
	2.0%	15.350 ± 2.244	0.995 ± 0.003	0.023 ± 0.005	0.924 ± 0.028
PLGA	0.5%	2.541 ± 0.523	0.977 ± 0.016	0.017 ± 0.001	0.885 ± 0.028
	1.0%	3.233 ± 0.313	0.984 ± 0.020	0.022 ± 0.005	0.889 ± 0.053
	2.0	61.608 ± 5.231	0.991 ± 0.009	0.022 ± 0.004	0.901 ± 0.885

Q is the amount of 5-fluorouracil (μg) released and t is time (h). Values are means \pm s.d. of three release experiments.

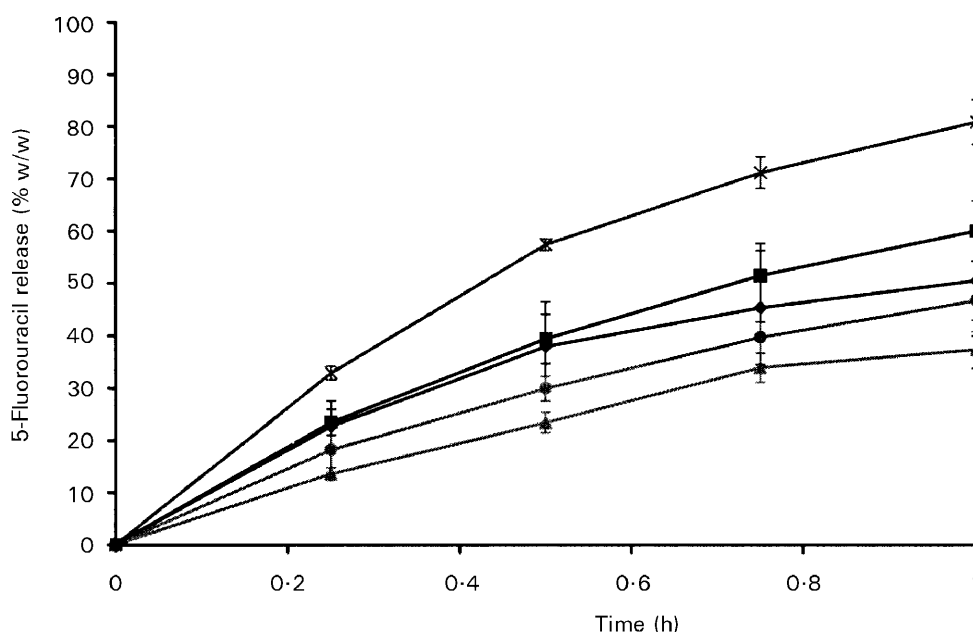


Figure 2. Release of 5-fluorouracil from nanoparticles prepared using different polymeric carriers (\blacklozenge , PLGA; \blacktriangle , Eudragit; \bullet , PBCA; \blacksquare , PCL) during the first hour, compared with the equilibration profile of free 5-fluorouracil (\times) in solution.

Eudragit nanoparticles displayed a clear sustained-release profile.

The effect of increasing the amount of added polymer on the release rate was studied for PLGA, PCL and Eudragit. Attempts have been made to model the release rate of drug from a simple monolithic device with spherical geometry to either a square-root (Guy et al 1982) or exponential (Illum et al 1986) function of time. These functions are especially applicable to the short-term release profile. As the majority of drug is released during the burst phase, the release data over the first hour was fitted to both these models and the goodness of fit estimated from the regression coefficient. A simple exponential model did not fit the release data as well as the root-time relationship (Table 5), the latter giving better linear regression. Some formulations, in particular those made from PLGA

and PCL, gave rapid release over the burst phase (Table 5). However, Eudragit particles displayed some evidence of sustained release of 5-fluorouracil and, therefore, is the polymer of choice from the four used in this study for controlled-release applications.

The initial burst effect is a clear characteristic of these types of particulate carrier. Attempts to lessen its effect have included surface coating with poly(saccharide) (Ohya et al 1994) and imparting a thermo-sensitive release profile by surface coating with dipalmitoylphosphatidyl choline (Ohya et al 1992). In this work, the concentration of polymer added initially was used to alter this burst effect, possibly by making the particles larger. Table 5 shows that there is no clear relationship between the amount of added polymer and the effect of sustained release. Furthermore, Table 3 shows that

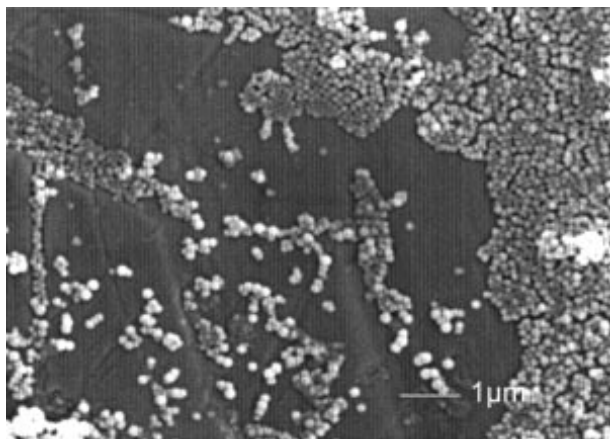


Figure 3. Scanning electron micrograph of PBCA particles taken before freeze-drying and drug-release studies ($\times 10\,000$). Particles are spherical and approximately 200 nm in diameter.

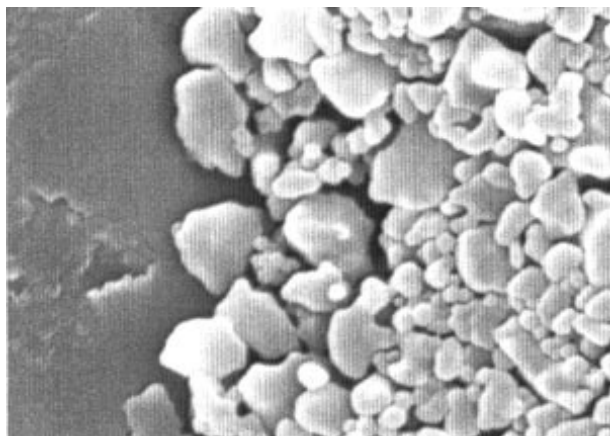


Figure 4. Scanning electron micrograph of PBCA particles after drug release in the donor phase for 24 h ($\times 19\,000$). Particles have lost their spherical appearance possibly by surface erosion.

marked increases in size are not observed as a greater concentration of polymer is added.

Scanning electron microscopy (SEM) was used to verify the particle diameter, as reported using PCS. All particles prepared using the different polymers appeared similar and of smooth and spherical topography, with the exception of PCL particles, which appeared less spherical. Diameters were approximately 200 nm and in good agreement with PCS data (Figure 3).

Particles were also visualised after freeze-drying. Under low magnification, all particle samples, irrespective of polymer type, appeared to resemble large agglomerated flakes. However, on closer examination, these flakes revealed a network of adhered nanoparticles. Again, these multi-particulate flakes were a feature of all types of freeze-dried polymer used in this work.

After a 24-h release study, particles were withdrawn from the donor phase and examined using SEM. Figure 4 shows PBCA particles which appear to have lost their spherical shape and have become more irregular in their circumference. After 24 h, PCL, PLGA and Eudragit showed no appreciable signs of surface erosion and retained their initial appearance.

Conclusions

A sustained release of 5-fluorouracil was achievable from the four polymeric carriers studied. The burst effect was a dominant feature of all release profiles. This possibly originated from the loosely attached drug on the particle surface. Therefore, although drug loading in the particle, as determined by analysing the supernatant, approached 4% w/w this was reduced to below 1% w/w using mild washing conditions. Increasing the initial concentration of drug or polymer did not affect the release profiles. Particles prepared from Eudragit sustained the release most and represent a candidate formulation for inclusion into an overarching delivery system, such as the bioadhesive patch, to further retard the release. Further studies will be undertaken to load particles into such a matrix and to evaluate drug release into both aqueous receptor phases and excised mucous tissue.

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