ELSEVIER

International Journal of Pharmaceutics 218 (2001) 13–25

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Acrylic microspheres for oral controlled release of the biguanide buformin

Gheorghe Fundueanu [a], Georgeta Mocanu [a], Marieta Constantin [a], Adrian Carpov [a], Victor Bulacovschi [b], Elisabetta Esposito [c], Claudio Nastruzzi [d,*]

[a] *Petru Poni Institute of Macromolecular Chemistry, Iasi, Romania*
[b] *Gh. Asachi Technical University of Iasi, Iasi, Romania*
[c] *Department of Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy*
[d] *Department of Pharmaceutical Chemistry and Technology, University of Perugia, via del Liceo, 06100 Perugia, Italy*

Received 26 June 2000; received in revised form 11 December 2000; accepted 21 December 2000

Abstract

Spherical microparticles based on methacrylic acid–methyl methacrylate copolymer have been developed. The method chosen for the preparation of such microparticles was suspension radical copolymerization of acrylic comonomers in the presence of the ethyleneglycol dimethacrylate as crosslinking agent. The microparticles obtained were characterised by inverse size exclusion chromatography, scanning electron microscopy, swelling degree and exchange capacity. The porous volume of the microspheres ranged from 0.086 ml/g for the microparticles produced by a methacrylic acid/methyl methacrylate ratio of 1/3 and a 10% degree of crosslinking, to 8.57 ml/g for the microparticles produced by a methacrylic acid/methyl methacrylate ratio of 3/1 and 2% degree of crosslinking (in 0.1 N NaCl in phosphate buffer pH 7.4). Also the pore diameter of the swollen microparticles ranged from a few to 120 Å. Buformin tosylate — a classical hypoglycaemic drug — was included in the polymer network of the microparticles during the polymerization process. Due to the water solubility of the drug and its low solubility in the organic phase, the entrapment yield did not exceed 15%. However the amount of encapsulated drug as well as the drug released from the microparticles, was dependent on the methacrylic acid/methyl methacrylate ratio, the degree of crosslinking and solvent/comonomers ratio.

Keywords: Microparticles; Methacrylic acid; Methyl methacrylate; Buformin tosylate; Drug release

1. Introduction

The suspension polymerization technique represents a widely used procedure for the preparation of microspheres intended for different applications including imaging, agrochemicals and adhe-

* Corresponding author. Tel.: + 39-075-5855158; fax + 39-075-5847469.
E-mail address: nas@unipg.it (C. Nastruzzi).

0378-5173/01/$ - see front matter
PII: S0378-5173(01)00570-1

sives, as well as for cosmetics and pharmaceuticals (Camli et al., 1999; Lee et al., 1998).

Applications of such methods are mostly based on the use of monomers such as acrylic (Cuilliere et al., 1991; Horak et al., 1999; Jayakrishnan and Thanoo, 1990; Kriwet et al., 1998; Montagne et al., 1991) or epoxy compounds (Khanna and Speiser, 1969). In spite of the fact that the many monomers and catalysts used for polymerization are relatively toxic substances, the pure polymers obtained from these monomers are usually non-toxic and harmless to the organism. As a general rule, the first step of the suspension polymerization technique is based on the formation of an emulsion, followed by the initiation of the polymerization process that will finally result in the formation of solid microparticles. In most cases the emulsion is of the oil/water (O/W) type, and this fact constitutes one of the major limitations of the process. In fact many drugs are only scarcely soluble in the organic phase, resulting in a low percentage of encapsulation.

Polymerization techniques, in which the drug is included (e.g. ionically bound) into the microparticles during the polymerization process of three monomers (one of which acts as a crosslinking agent) have received little attention (El-Samaligy and Mahmoud, 1986; Khanna et al., 1970).

In this report we employed, as model drug, buformin, a biguanide compound used as an hypoglycaemic oral drug. The registered marketed formulation, Silubin® (Grunental, Belgium), contains the hydrochloride salt of buformin and is commercialised as a retard form, based on film coated tablets. Some pharmacological studies concerning the plasma level of buformin administered as Silubin® are reported in the literature (Beckmann et al., 1971).

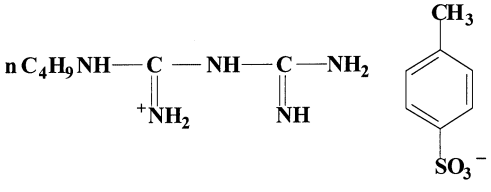

Fig. 1. Chemical structure of buformin tosylate.

In the present study, the design, production and characterisation of a controlled delivery system for buformin was investigated, that involved including the drug in crosslinked acrylic microparticles directly during the suspension copolymerization process. In particular, the influence of the co-monomer ratio, the presence of an inert solvent (other than the liquid monomers) in the organic phase and of the degree of crosslinking (D.C.) on microparticle characteristics, the drug loading and the release profile were studied.

2. Materials and methods

2.1. Chemicals

Buformin tosylate (*N*-butylimidodicarbonimidic diamine tosylate) (Bf) (see structure in Fig. 1), a strong base, freely soluble in water and ethanol, was kindly provided from S.C. Sicomed S.A. (Bucharest, Romania). Ethyleneglycol dimethacrylate (EGDM), methacrylic acid (MA) and methyl methacrylate (MM), (Fluka AG, Switzerland) were distilled under reduced pressure before their use. 2,2′-azo(*bis*-isobutyronitrile) (AIBN) (Fluka AG) was recrystallized twice from methanol, hydroxyethyl cellulose (HEC) was purchased from Hercules (Wilmington, USA), *n*-butanol and sodium chloride were provided from Fluka AG.

2.2. Microparticle production

Polymeric microparticles were prepared from MA and MM by an aqueous suspension copolymerization procedure in the presence of EGDM, as crosslinking agent. The reaction took place in a glass-reactor (500 ml volume, cylinder shape) provided with an anchor-type stirrer and a reflux condenser. The reactor was maintained at constant temperature with a thermostatic bath.

The drug was dissolved at 50°C in a butanol–MA mixture (at various ratios as a function of the studied parameters); MM was then added at the same temperature. Separately, the initiator of the radical polymerization, AIBN, was solubilized in a small amount of *n*-butanol (0.8 ml) containing

the crosslinking agent EGDM, resulting in a final concentration of 0.5% w/v with respect to the co-monomers.

The mixture of monomers and drug in *n*-butanol was dispersed, at 50°C, under vigorous stirring in the dispersing medium, which constituted 1% w/v HEC (used as stabilizer) and 20% w/v sodium chloride in water. The volume ratio between the organic phase and aqueous phase was maintained 1/3 and the stirring speed set at 400 rpm.

The reaction mixture was maintained under stirring for 1 h at 50°C to obtain complete dispersion of the monomers, afterward the crosslinker and the initiator were added and the temperature was raised to 70°C and the polymerization was continued for 10 h. After cooling to room temperature, the formed microparticles were decanted and separated by filtration using a sintered glass filter. The microparticles were then washed with water, to remove the residues of stabiliser, inorganic ions and methacrylic acid and with methanol and acetone to remove the unreacted water-insoluble monomers. Finally, the microparticles were dried under vacuum at 60°C.

2.3. Microparticle recovery

After washing and drying, the prepared microparticles were weighed and the weight compared to the initial mass of monomers plus drug and the recovery yield was calculated by the following equation:

$$\text{Recovery}(\%) = \frac{W\text{microp}}{W\text{monom} + W_D} \times 100 \qquad (1)$$

where W_{microp}, W_{monom} and W_{D}, respectively, represent the weight of the isolated microparticles, of the monomer and drug.

2.4. Dimensional and morphological analysis of the microparticles

The morphology, size and size distributions were determined by optical and electron microscopy observations. For the optical analysis an optical microscope, Diaphot (Nikon, Japan), was employed. For the electronic analysis, microparticles were metallized by gold coating (Edwards Sputter coating S150) and analysed at 15–20 kV by a scanning electron microscope (SEM) 360 Stereoscan (Cambridge Instruments, Cambridge, UK).

2.5. Exchange capacity

The microparticle exchange capacity was determined under dynamic conditions by titration of the carboxylic groups, after complete drug removal by a 0.1N NaOH solution. The results were expressed as milliequivalent carboxylic groups/g dried microspheres.

2.6. Swelling degree

The volume expansion of the microspheres was determined at equilibrium, placing the microparticles in acid buffered solutions at pH 1.2 (KCl + HCl), in 0.1 N NaCl in phosphate buffer solution (pH 7.4) or in methanol. The ratio between the volume of the swollen microparticles (V_s) and that of the dried microparticles (V_d), measured by placing the microparticles in an appropriate graduated cylinder, was defined as the swelling factor (q).

2.7. Determination of microspheres porosity by inverse size exclusion chromatography

The porosity of acrylate microparticles was determined by inverse size exclusion chromatography (ISEC). The method is based on the size exclusion principle (Porath and Flodin, 1959) with the modification that, in this case, the mobile phase is used to characterise the stationary phase, providing data about the total pore volume (ml/g) and the maximum pore size of the microparticles (R_{max}).

As molecular weight standards, deuterated water (D_2O), D(+)-sucrose ($M_w = 342.3$) and D(+)-rafinose pentahydrate ($M_w = 594.5$) were used as low molecular weight standards (for small pores) whilst dextrans with different molecular weights, namely, 10000 (DT 10), 17500 (DT 17), 40000 (DT 40), 70000 (DT 70), 500000 (DT 500), and 2000000 (DT 2000) (Pharmacia, Uppsala, Sweden), were used for large pores.

Plotting the logarithm of the molecular weight of standard molecules against elution volume (ml), the maximum radius of the pores and the total pore volume (V_p) per gram of the stationary phase were determined according to the equation:

$$Vp = (T_{D_2O} - T0)v/m \quad (2)$$

where Vp is the total pore volume, T_{D_2O} is the elution time (min) for deuterated water (D_2O); T_0 is the elution time for dextran ($M_w = 2000000$ g/mol); v is the pump flow rate (ml/min) and m is the dried mass of the packing materials (expressed in g).

The pore volume of the microparticles representing the stationary phase, can be calculated as the difference between the elution volume of the smallest probe molecule (D_2O), which diffuses within all pores in the microparticles and the elution volume of dextran ($M_w = 2000000$ g/mol), considered to be completely excluded.

Two interaction coefficients R and R_1 respectively, calculated as the ratio between Bf elution volume ($M_w = 329.4$ g/mol), D(+)-sucrose ($M_w = 342.3$ g/mol) and the elution volume of D_2O were in addition determined.

ISEC was performed using a glass column (15×0.5 cm) packed under pressure with a suspension of swollen microparticles in water or in 0.1 N NaCl in phosphate buffered solution (pH 7.4). The chromatographic equipment consisted of a peristaltic pump (Gilson, France) and a Waters differential refractometer detector (Knauer).

2.8. Drug encapsulation

The content of Bf in the microparticles was determined from elemental analysis of nitrogen (Kjeldahl method) and sulphur (Shöniger method).

2.9. In vitro drug release studies

In vitro drug release determinations were performed by the bath method, using an acidic solution (at pH 1.2) or a buffered solution (at pH 7.4) (US Pharmacopeia XXI Revision, 1985). Samples of the receiving buffer were withdrawn at different time intervals and the buformin content was spectrophotometrically determined at 220 nm (pH 1.2) or 230 nm (pH 7.4) with an UV–VIS Spectrophotometer Specord M 42 (Karl Zeiss Jena, Germany) using a previously constructed calibration curve. The same volume of fresh receiving medium was added to replace the volume of the withdrawn samples. The integrity of the drug released from the microparticles was proved by HPLC analysis using a Hewlett Packard cation exchange HPLC column (100×4.6 mm) and as mobile phase potassium dihydrogenphosphate buffer (pH 7)/acetonitrile 70/30 (v/v).

3. Results and discussion

The classical methods of drug entrapment in microparticles are mainly based on two approaches in which (a) the drug is physically entrapped in the network of a preformed polymer during the preparation of the particles or (b) the polymeric microparticles are loaded by soaking in a solution containing the drug (Bibby et al., 1999; Nastruzzi et al., 1993). This report deals with a less investigated strategy in which the drug is included into the polymer matrix directly during the copolymerization process, resulting in the microparticle formation.

Microparticles were produced by a suspension copolymerization protocol using MA and MM as starting monomers and EGDM as crosslinking agent. As a model drug, Bf, a classical hypoglycaemic drug, was used. The choice of the monomers was made on the basis of the following considerations: (a) MA has an anionic character which allows the formation of ionic complexes with basic drugs such as buformin whereas; (b) MM gives to the polymer matrix an hydrophobic character, allowing prolonged (sustained) release profiles. In addition, MA–MM linear copolymers, that are commercialised under the brand name Eudragit® (Röhm Pharma), are widely used as excipients for the production of drug formulations and in this respect are well characterised polymers both from a chemical as well as a toxicological point of view.

Finally, it is important to underline that the release of the buformin is expected to be con-

Table 1
Preparation conditions of acrylate microparticles[a]

Sample	MA/MM ratio (v/v)	D.C. (%)	Solvent/co-monomers ratio (S/C), (v/v)	Stirring speed (rpm)	Buformin in co-monomers solutions (%, w/v)
BfM no. 1	1/1	2	1/4	400	30
BfM no. 2	1/1	10	1/1	400	30
BfM no. 3	1/1	10	1/4	400	30
BfM no. 4	2/1	10	1/4	400	30
BfM no. 5	3/1	2	1/4	400	30
BfM no. 6	3/1	10	1/1	400	30
BfM no. 7	3/1	10	1/2	400	30
BfM no. 8	3/1	10	1/4	400	15
BfM no. 9	3/1	10	1/4	400	25
BfM no. 10	3/1	10	1/4	400	30
BfM no. 11	3/1	20	1/4	400	30
BfM no. 12	1/3	10	1/4	400	15

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

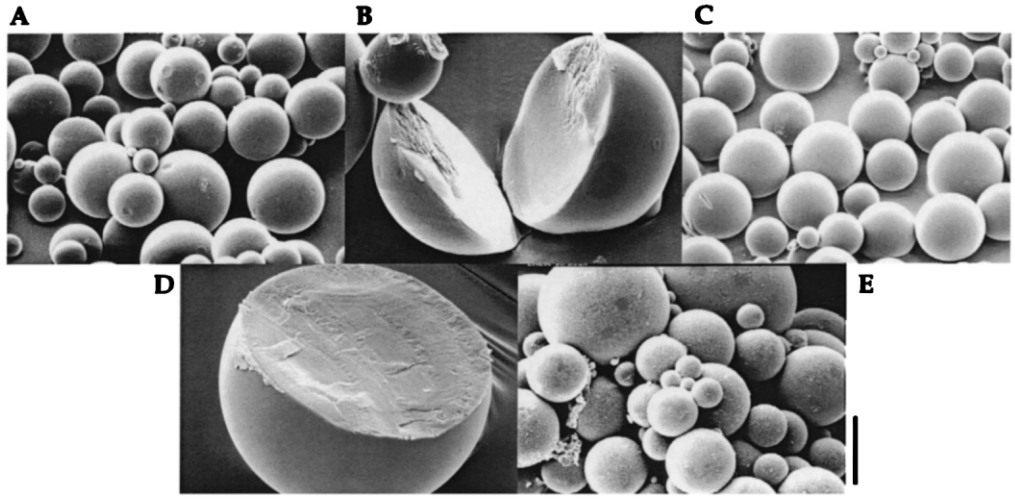

Fig. 2. Scanning electron photographs of microparticles produced by the suspension copolymerization procedure. Panels A and B: sample BfM no. 1; panels C and D: sample BfM no. 12; panel E: sample BfM no. 8.

trolled both by diffusion and by the electrostatic interactions occuring between the polymer side chains and the drug. In Table 1 the experimental conditions used for the preparation of acrylate microspheres are summarized. Each type of microparticle was prepared in three batches, and all the following experimental data represent the mean of three independent experiments.

3.1. Microparticle size and morphology

Buformin containing microparticles, produced by the suspension copolymerization procedure show, as evidenced by the SEM analysis, a spherical geometry (Fig. 2). These microparticles, produced using a small amount of crosslinker (2%), show a porous structure with small pores also

evident in the dry state (Fig. 2A and B; sample BfM no. 1). Fig. 3 shows the frequency distribution plot of buformin containing microparticles (sample BfM no. 1) showing a relatively narrow dimensional distribution and a mean diameter of 40.5 μm.

In addition, the microscopic analysis confirmed that microparticles produced with a MA/MM ratio of 1/3 (D.C. = 10%; sample BfM no. 12) are almost nonporous and with a very smooth surface (Fig. 2C and D). On the contrary, microspheres produced with a MA/MM ratio of 3/1 (D.C. = 10%; sample BfM no. 8) show a rough surface; this feature was attributed to the possible absorbtion of the stabilizer on the highly charged surface of the microspheres (Fig. 2E).

3.2. Effect of co-monomers ratio on buformin containing microparticles

Table 2 shows the results of the influence of the co-monomer ratio (MA/MM) on the characteristics of buformin containing microparticles. As is evident, increasing the proportion of MA in the co-polymerization mixture results in a decrease of microparticle recovery. During the polymerization, the hydrophilic MA has a tendency to diffuse from the organic to the aqueous phase. In order to reduce this phenomenon, NaCl was added to the aqueous phase (20% w/v) to decrease the miscibility of MA in water. In spite of this procedure, when MA is present in large amounts, some still diffuses from the organic to the aqueous layer, therefore reducing the efficiency of microparticle formation, giving a lower microsphere recovery. The increase of MA resulted, as expected, in an augmentation of the exchange capacity of the microparticles and as a consequence of the buformin encapsulation yield. In fact the drug is more efficiently bound to the microparticle matrix by ionic interactions with the acidic monomers. Nevertheless these effects were proportionally lower than the expected theoretical values. In summary, the increase of the MA/MM ratio produced a decrease in the microparticle recovery and an increase in the encapsulation drug.

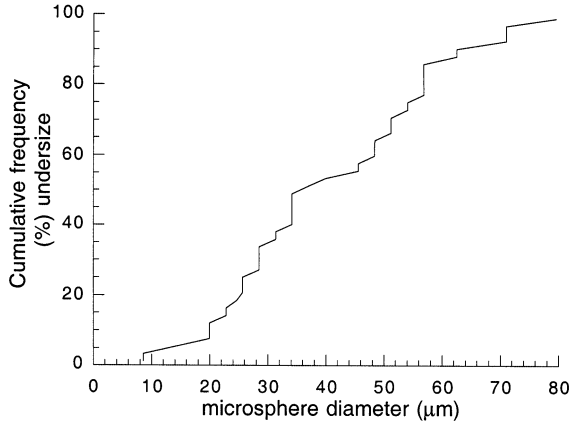

Fig. 3. Size distribution analysis of buformin-acrylic copolymers microparticles (sample BfM no. 1).

Drug entrapment was determined, on the isolated microparticles, both from nitrogen (present only on the buformin molecule) and sulphur (present only on the counterion, tosyl anion) determinations. The results obtained (reported in Table 2), indicate that during the co-polymerization process, the tosyl anion remains ionically bound to buformin, in fact the exchange between carboxylic groups and tosyl does not take place, as proved by the concordance of the buformin content in the microparticles, calculated both by sulphur and nitrogen analysis. This behaviour was tentatively attributed to the presence, in the buformin molecule, of four other free amino groups which could interact with the carboxylic groups of the microparticles, besides the amino group ionically bound to the tosyl anion.

The porosity, as well as the swelling degree of the microspheres, progressively increases with the MA proportion in the copolymer (Fig. 4A and B). This behaviour is evident when particles are placed in phosphate buffer, and even more so in methanol. Microparticles produced with a MA/MM ratio of 1/3 (10% D.C.) are almost unswellable in aqueous solutions, with a porous volume reduced at 0.086 ml/g and very small pores, inaccessible even for D(+)-sucrose ($M_w = 342.3$ g/mol).

3.3. Effect of cross-linking on buformin containing microparticles

The increase in the crosslinking agent resulted in a rise of microparticle recovery and drug en-

Table 2
Influence of the co-monomers ratio on the preparation of buformin containing microparticles[a]

Sample	MA/MM ratio		Microparticle recovery	Buformin encapsulation (%, w/w) calculated from:		E.C.(meq/g)	MA in microspheres	R_{max} (Å)	
	(v/v)	(mol/mol)	(%, w/w)	Sulphur	Nitrogen		(%, w/w)	H_2O	pH 7.4
BfM no. 10	3/1	3.82/1	60.4	13.38	14.58	6.6	60.4	28	28
BfM no. 4	2/1	2.51/1	72.5	10.29	11.29	5.8	53	28	28
BfM no. 3	1/1	1.25/1	80.2	9.12	8.94	4.26	40	28	28
BfM no. 12	1/3	1/2.41	92	9.86	10.35	2.2	21	few	few

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

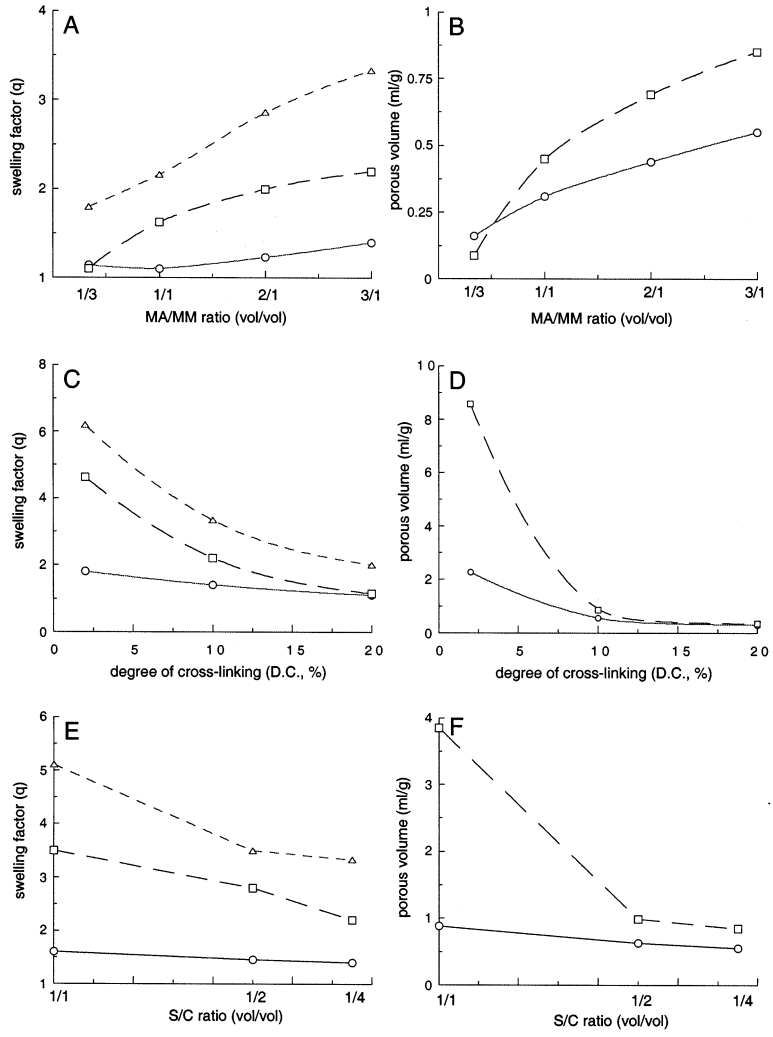

Fig. 4. Influence of MA/MM ratio (panels A and B), degree of cross-linking (panels C and D) and S/C ratio (panels E and F) on the swelling degree (q) in pH 1.2 (circles), pH 7.4 (squares), methanol (triangles) (panels A, C and E) and porous volume (V_p) in water (circles) and pH 7.4 (squares) (panels B, D and F) of acrylate microparticles. Panels A and B: Microparticles prepared with a MA/MM ratio of 1/3 (BfM no. 12), 1/1 (BfM no. 3), 2/1 (BfM no. 4) and 3/1 (BfM no. 10). Panels C and D: Microparticles prepared with a D.C. of 2% (BfM no. 5), 10% (BfM no. 10) and 20% (BfM no. 11). Panels E and F: Microparticles prepared with a S/C ratio of 1/1 (BfM no. 6), 1/2 (BfM no. 7) and 1/4 (BfM no. 10). The data represent the mean of three independent experiments.

capsulation (see Table 3) even if the differences between particles with a D.C. of 10 and 20 are minimal. This phenomenon was tentatively attributed to the co-operation between the ionically bound and the physically entrapped drug. In fact, the weakly crosslinked microparticles have a higher tendency to swell and consequently part of the entrapped drug is removed during the washing step following the preparation.

A higher D.C. results in a more rigid matrix that more efficiently retains the drug. This behaviour is evident by analysing the microparticle porosity and swelling behaviour (Fig. 4C and D), both these values decrease dramatically when

D.C. passes from 2 to 10%. We must underline that the column packing was made with microparticles kept previously for 48 h in an excess of the studied solvent until equilibrium was reached. However, during the packing of the column, microparticles with a 2% D.C. swell supplementary in phosphate buffer solution, resulting in a higher porosity (8.57 ml/g).

3.4. Effect of drug loading on microparticle characteristics

The solubility of buformin in the organic phase (co-monomers + butanol) is limited by the hydrophilic nature of the drug. In order to possibly overcome this drawback, the drug was solubilized at 50°C in a mixture of butanol and MA in which it is more soluble. The highest drug concentration that could be solubilized in these conditions was 30% (w/v) with respect to the comonomer mixture. Increasing the buformin content in the organic phase results in a higher percentage of drug encapsulation and a slight decrease of microparticle recovery (Table 4).

Adding higher amount of buformin (> 30%, w/v with respect to comonomers) in the mixture butanol–MA, led to the precipitation of the drug when MM was added. The buformin content in the organic phase can be increased using a higher amount of *n*-butanol, but in this case the drug entrapment decreases as described below.

3.5. Effect of the solvent/co-monomer ratio on buformin containing microparticles

The use of a large amount of butanol causes an increase of particle recovery (Table 5), as a result in the rise of the efficiency of copolymerization in the organic phase. On the other hand, as already mentioned, the drug entrapment decreases; this behaviour was explained with the formation of more porous microparticles (Fig. 4E and F). A highly porous matrix results in an easier escape of the drug from the microparticles (Table 5). In addition, the solvent maintains buformin solubilized until the end of polymerization resulting in an easier diffusion of drug out from the microspheres. The amount of solvent has thus two opposite effects: (a) it facilitates the copolymeriza-

Table 3
Influence of crosslinking degree on the preparation of buformin containing microparticles[a]

Sample	D.C. (%)	Microparticle recovery (%, w/w)	Buformin encapsulation (%, w/w)	E.C. (meq/g)	MA in microspheres (%, w/w)	R_{max} (Å)	
						H_2O	pH 7.4
BfM no. 5	2	50.0	7.99	7.0	63	35	60
BfM no. 10	10	60.4	14.58	6.6	60.4	28	28
BfM no. 11	20	65.7	15.52	6.6	60.0	28	28

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

Table 4
Influence of drug loading on microparticle preparation[a]

Sample	Buformin in co-monomers (%, w/v)	D.C. (%)	Microparticle recovery (%, w/w)	Buformin encapsulation	
				(%, w/w)	% from theoretical
BfM no. 8	15	10	68.0	7.95	60.82
BfM no. 9	25	10	64.2	12.8	63.87
BfM no. 10	30	10	60.4	14.58	63.03

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

Table 5
Influence of solvent/co-monomer ratio (S/C) on microparticle preparation[a]

Sample	Solvent/co-monomers ratio (v/v)	Microparticle recovery (%, w/w)	Buformin encapsulation (%, w/w)	R_{max} (Å) H_2O	pH 7.4
BfM no. 6	1/1	76	8.7	28	35
BfM no. 7	1/2	72.41	9.12	28	28
BfM no. 10	1/4	60.4	14.58	28	28

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

tion; and (b) it decreases the drug entrapment efficiency. The data reported in Tables 2–5 indicate that it is possible, by varying the reaction conditions, to prepare acrylic crosslinked microparticles with different characteristics, containing various amounts of drug.

3.6. Release characteristics

The reaction conditions under which the microparticles are formed are expected to influence their in vitro release characteristics. This feature is evident from Fig. 5A where the influence of D.C. on the release profile of buformin is shown.

Data from Fig. 5A and Table 6 demonstrate that highly crosslinked microspheres are characterised by slow release profiles. After 24 h, only 40% of buformin was released from microparticles having a D.C. of 20%, on the contrary, lower D.C. led to faster release. For instance in the case of microparticles with D.C. of 2% the drug release in 24 h was almost quantitative (97%).

As previously stated, the use of a high MA/MM ratio results in an increased drug loading due to electrostatic interactions. Therefore, the drug release mechanism from microparticles containing a higher proportion of MA is expected to be governed both by ionic interaction and diffusion from the swollen microsphere matrix. In this respect, it should be taken into account that microparticles with a high MA/MM ratio are far less hydrophobic. Two contrasting effects are expected to govern the release mechanism of the drug from such microparticles. The data reported in Fig. 5B clearly indicate that the hydrophilicity of the polymer plays a major role in modulating the drug release, in fact after 24 h, the less hydrophobic microparticles containing a higher proportion of MA released 75% of the encapsulated drug, whilst in the case of microparticles with only a third of MA, proportionally, the released buformin was 58%. In the case of the microparticles produced using a MA/MM ratio of 1/3, the released drug is almost negligible (5%). These microparticles are unswellable, with no apparent porosity (see Fig. 2A and B) with very small pores unaccesible even for a small molecule like sucrose.

Microparticles produced with a high volume ratio between solvent and monomers are characterised by a more porous internal structure (demonstrated by ISEC analysis) that influences the release of the drug. After 24 h, microparticles prepared with a 1/1 ratio released 90% of the drug, whilst in the same period microparticles prepared with a 1/4 ratio released only 75% of the drug (Fig. 5C).

The study of the defined interaction coefficients gives supplementary information concerning the release mechanism (the closer to 1 the R-value the easier the drug diffuses through all pores of the polymer matrix).

Table 6 presents the interaction coefficients R and R_1 of the two tested molecules (Buformin-charged, and D(+)-sucrose- uncharged, Mw Mw$_1$) measured in water and phosphate buffer for five selected samples.

Because R is almost equal with R_1 this explains why no electrostatic interaction between drug and matrix took place in water. On the contrary, in phosphate buffer $R \neq R_1$ which explains some electrostatic interactions between drug and matrix.

The different values of R in water for the selected samples explains the different length cov-

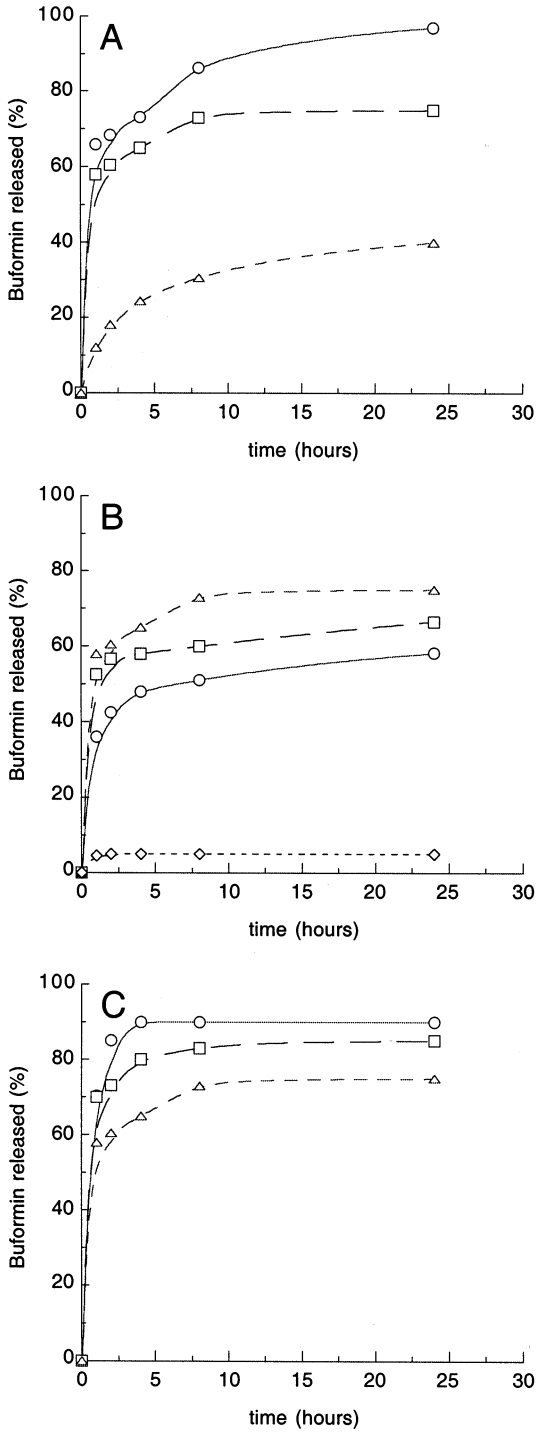

Fig. 5.

Table 6
The values of the interaction parameters R and R_1 calculated from ISEC determinations[a]

Sample	R		R_1	
	H_2O	pH 7.4	H_2O	pH 7.4
BfM no. 3	0.74	1.25	0.73	0.93
BfM no. 1	0.80	1.15	0.82	0.98
BfM no. 2	0.74	1.18	0.75	0.98
BfM no. 5	0.88	1.1	0.92	1.08
BfM no. 12	0.88	1.17	0.93	1.07

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

ered by the drug molecule during diffusion through the matrix pores.

In phosphate buffer, where the carboxylic groups are in ionic form the release of the drug is affected by the electrostatic interactions, ($R > 1$ and R_1 is almost 1 everywhere).

In the case of the microspheres produced using a MA/MM ratio of 1/3 the value of R in phosphate buffer is higher than 1 ($R = 1.17$), even if the drug is totally excluded from the pores (the same value of the elution volume with DT 2000, in water). This may be explained by the formation of hydrophobic interactions between the polymer matrix and the hydrophobic part of the drug, but this behaviour does not explain why these interactions are higher in phosphate buffer than in water ($R = 0.88$ in water). Possibly, ionic interactions between charged carboxylic groups found at the surface of the microspheres and drug could take

Fig. 5. Influence of the crosslinking degree (D.C.) (panel A), MA/MM ratio (v/v) (panel B) and solvent/co-monomers ratio (v/v) (panel C) on the release behaviour of buformin from acrylic copolymers microparticles, release experiments were performed using phosphate buffer (pH 7.4). Panel A: sample BfM no. 5 (circles), sample BfM no. 8 (squares), sample BfM no. 11 (triangles). Panel B: sample BfM no. 11 (diamonds), sample BfM no. 3 (circles), sample BfM no. 4 (squares), sample BfM no. 8 (triangles). Panel C: sample BfM no. 8 (triangles), sample BfM no. 7 (squares), sample BfM no. 6 (circles). The data represent the mean of three independent experiments.

Table 7
Effect of the experimental parameters on the release of buformin from acrylic copolymers microparticles at pH 1.2.[a]

Sample	Parameter variation D.C. (%)	MA/MM ratio(v/v)	S/C ratio (v/v)	Buformin released (%)
BfM no. 5	2	3/1	1/4	52.5
BfM no. 10	10	3/1	1/4	26.82
BfM no. 3	10	1/1	1/4	10.5
BfM no. 10	10	3/1	1/4	26.82
BfM no. 6	10	3/1	1/1	44.00
BfM no. 10	10	3/1	1/4	26.82

[a] Each batch was prepared in triplicate and all the data represent the mean of three independent experiments.

place. In water, and much more at pH 1.2, the diffusion of the drug through the pores could be the rate determining step.

The release behaviour in acidic pH (1.2), after 2 h, is summarised in Table 7. In acidic pH the buformin release is much slower than in buffer solution at pH 7.4. This behaviour could be tentatively explained on the basis of a more contracted state of the carboxylic acrylic microparticles, in fact at acidic pH the carboxylic groups are in the protonated, less hydrophilic, form, in this way the microparticles are less swollen reducing the diffusion of the drug molecules through the polymer matrix to the external fluid.

4. Conclusions

Buformin loaded microparticles were produced using a new encapsulation technology based on suspension radical copolymerization of MA and MM in the presence of EGDM as crosslinking agent. The controlled release system obtained is particularly interesting since the drug is encapsulated by ionic forces, hydrophobic interactions and physical entrapment in a crosslinked network of acrylic microparticles. It was in fact demonstrated that by appropriate variations in the reaction conditions it is possible to obtain microparticles with different characteristics in terms of porosity, exchange capacity and hydrophilic–lipophilic balance allowing the modulation of drug release profiles. Further studies will be undertaken in order to better investigate the invivo performances of microparticles.

References

Beckmann, R., Lintz, W., Schmidt-Boethelt, E., 1971. Evaluation of a sustained release form of the oral antidiabetic butylbiguanide (Silubin retard®). Eur. J. Clin. Pharmacol. 3, 221–228.

Bibby, D.C., Davies, N.M., Tucker, I.G., 1999. Poly(acrylic acid) microspheres containing β-cyclodextrin: loading and in vitro release of two dyes. Int. J. Pharm. 187, 243–250.

Camli, S.T., Senel, S., Tuncel, A., 1999. Cibacron blue F3G-A-attached uniform and macroporous poly(styrene-co-divinylbenzen) particles for specific albumin adsorbtion. J. Biomater. Sci. Polym. Ed. 10, 875–889.

Cuilliere, M.L., Montagne, P., Bessou, T., El Omari, R., Riochet, D., Varcin, P., Laroche, P., Prudí homme, P., Marchand, J., Flecheux, O., Pau, B., Duheille, J., 1991. Microparticle-enhanced nephelometric immunoassay (Nephelia) for immunoglobulins G, A and M. Clin. Chem. 37, 20–25.

El-Samaligy, M., Mahmoud, H.A., 1986. Effect of aqueous phase modifiers on drug release from polyacrylamide microbeads. Pharm. Ind. 48, 1070–1074.

Horak, D., Karpisek, M., Turkova, J., Benes, M., 1999. Hydrazide-functionalized poly(hydroxyethyl methacrylate) microspheres for imobilization of horseradish peroxidase. Biotechnol. Prog. 15, 208–215.

Jayakrishnan, A., Thanoo, B.C., 1990. Suspension polymeryzation of 2-hydroxyethyl methacrylate in the presence of polymeric diluents: a novel route to spherical highly porous beads for biomedical applications. J. Biomed. Mater. Res. 24, 913–927.

Khanna, S.C., Speiser, P., 1969. Epoxy resin beads as a pharmaceutical dosage form. I: Method of preparation. J. Pharm. Sci. 58, 1114–1117.

Khanna, S.C., Jecklin, T., Speiser, P., 1970. Bead polymerization technique for sustained release dosage form. J. Pharm. Sci. 59, 614–618.

Kriwet, B., Walter, E., Kissel, T., 1998. Synthesis of bioadhesive poly(acrylic acid) nano- and microparticles using an inverse emulsion polymerization method for the entrapment of hydrophylic drug candidates. J. Control. Release 56, 149–158.

Lee, J.H., Yoon, J.Y., Kim, W.S., 1998. Continous separation of serum proteins using a stirred cell charged with carboxylated and sulfonated microspheres. Biomed. Chromatogr. 12, 330–334.

Montagne, P., Laroche, P., Cuilliere, M.L., Riochet, D., Flecheux, O., Varcin, P., Marchand, J., Pau, B., Duhaille, J., 1991. Polyacrylic microspheres as a solid phase for microparticle enhanced nephelometric immunoassay (Nephelia®) of transferrin. J. Immunoassay 12, 165–183.

Nastruzzi, C., Fundueanu, G., Cortesi, R., Esposito, E., Gambari, R., Carpov, A., Menegatti, E., 1993. Preparation of pullulan based microspheres: entrapment of aromatic tetraamidines by ion exchange principles. Pharm. Pharmacol. Lett. 3, 51–54.

Porath, J., Flodin, P., 1959. Gel Filtration: a method for desalting and group separation. Nature 183, 1657–1659.

US Pharmacopeia XXI Revision, 1985. US Pharmacopeial Convention, Rockville, MD, p. 1420.