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Novel peroral dosage forms with protease inhibitory activities. I. Design of capsules with fast gel-forming and fast drug-releasing properties

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Abstract

Capsules, containing the poly(acrylic acid) derivative Carbopol® 934P (C934P) with the aim of inhibiting intestinal proteolytic activities after swelling with water into a hydrated state, were designed. Erythrosin was used as a hydrophillic model drug to characterize the release properties of the dosage forms. Capsule formulations which rapidly disintegrated and released the drug quickly, were prepared because both rapid disintegration and rapid swelling of C934P and simultaneous drug release are prerequisites for the enzyme inactivating properties of the system. The capsules containing freeze-dried, neutralized C934P (FNaC934P) disintegrated quicker than the capsules containing C934P. Capsules which contained poly(glycerol ester of fatty acid) microparticles with FNaC934P released erythrosin quicker than capsules containing mixtures of FNaC934P, erythrosin and a disintegrant.

Keywords: Poly(acrylic acid) derivative; Carbomer (Carbopol® 934P); Sodium Carbopol® 934P; Freeze-dried sodium Carbopol® 934P; Poly(glycerol ester of fatty acid) (PGEF); Peroral solid dosage form; Disintegrant; Microparticle

1. Introduction

For the delivery of peptide and protein drugs, in most cases parenteral administration is necessary in order to achieve therapeutic effects. However, much effort has been expended on the development of dosage forms for non-parenteral routes such as peroral, nasal, buccal and pulmonary (Lee et al., 1991a; Morita et al., 1994; Zhou, 1994; Morimoto et al., 1995). The peroral route is particularly recognized to be the most convenient one for patients. Nevertheless, the peroral bioavailability of peptide drugs is very poor, partly because these drugs are highly susceptible to proteolytic degradation in the gastrointestinal

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fluids, the brush border of the intestinal mucosae and in the cytosol (Lee et al., 1991b; Bai, 1994). Additionally, these substances are highly polar and are high molecular weight compounds. One way to overcome the metabolic barrier to increase the bioavailability of peptide drugs, is the use of enzyme inhibitors and absorption enhancers (Zhou, 1994).

Mucoadhesive drug delivery systems have been studied to enhance the mucosal absorption of peptide drugs because of their possible, intimate contact with the underlying absorbing surfaces and their prolonged residence time (Leung et al., 1991; Morimoto et al., 1984). Morimoto et al. (1987) suggested that enhanced absorption of hydrophillic drugs by cross-linked poly(acrylic acid) derivatives was due to the increase in water absorption by the polymers and dehydration of the mucosal tissues. Lehr et al. (1992) reported that the absorption of 9-desglycinamide, 8-arginine vasopressin (DGAVP) from the rat small intestine was improved both in vitro (vertically perfused isolated gut) and in vivo (after intraduodenal bolus) when DGAVP was administered in a liquid dispersion of polycarbophil. They also reported that the degradation of DGAVP by the enzymes in a mucosal homogenate was strongly inhibited by the addition of 1% (w/v) polycarbophil. In addition, Lueßen et al. (1995) showed that polycarbophil (0.35%) and carbomer (C934P; 0.25%) have a strong inhibitory effect on the hydrolytic activity of trypsin. In this study *N*-benzoyl-L-arginine ethylester (BAEE) was used as a model compound. It was observed that the degradation of BAEE was dependent upon the presence of Ca^{2+} ions. Trypsin requires Ca^{2+} to maintain its thermodynamical stability, and has been discussed to undergo irreversible changes such as denaturation by withdrawal of Ca^{2+} (Gabel and Kasche, 1973; Bartunik et al., 1989).

However, preliminary experiments showed that it took a long time for carbomer to disperse in an aqueous medium and form a homogeneous gel. This property of carbomer makes it difficult to use it as an ingredient for fast-releasing solid dosage forms. Since most peptides are not very stable in aqueous systems, a dry solid dosage form would be essential for the design of peptide drug formulations. On the other hand, neutralized carbomer was found to swell easily and rapidly form a homogeneous gel within a short period of time. The best results were obtained when neutralized freeze-dried carbomer was used, enabling even more rapid gel formation.

The purpose of this study was to design and evaluate capsule formulations which, after disintegration of the capsules quickly release their contents of poly(acrylic acid) derivatives and other excipients such as superdisintegrants and poly-glycerol fatty acid) derivatives together with the model drug erythrosin.

2. Experimental

2.1. Materials

Carbomer (Carbopol®; C934P) and sodium salt of carbomer (Carbopol® EX-161; NaC934P) were kindly supplied by BF Goodrich (Cleveland, OH). Freeze-dried sodium salt of carbomer (FNaC934P) was prepared by freeze-drying an aqueous dispersion of 0.5% (w/v) carbomer, neutralized with 10 M NaOH (pH 7.0). Polyvinylpyrrolidone K 30 USP (Kollidon® CL; BASF Co., Ludwigshafen, Germany), croscarmellose sodium USP (Primellose®, Avebe, Veendam, The Netherlands) and sodium starch glycolate NF (Explotab®, E. Mandell Co., Carmel, NY and Primojel®, Avebe, Veendam, The Netherlands) were used as disintegrants. Tetraglycerol pentastearate (TGPS, HLB 2.6), tetraglycerol monostearate (TGMS, HLB 8.4) and tetraglycerol hexabehenate (TGHBe, HLB 1.8) were used as poly(glycerol esters of fatty acids) (PGEFs; Sakamoto Yakuhin Kogyo Co. Ltd., Osaka, Japan). Erythrosin (Food Red No. 3) was used as commercially available. All other chemicals were of reagent grade.

2.2. Preparations of capsules

2.2.1. Capsules for testing disintegration of content

Each disintegrant (Kollidon® CL, Primellose®, Explotab® or Primojel®) was mixed with C934P,

Table 1
Formulations of a mixture of Kollidon® CL, FNaC934P and erythrosin

Component	K50/50	K30/70	K10/90	K0/100	K*50/50	K*0/100
Kollidon® CL	120	72	24	—	100	—
FNaC934P	120	168	216	240	100	100
Erythrosin	10	10	10	10	10	10
Total	250	250	250	250	210	110

All values expressed as mg.

NaC934P or FNaC934, respectively, and 240 mg of the mixture were inserted into a No. 1 transparent capsule (Spruyt-Hillen, Vianen, The Netherlands). The following four disintegrant/poly(acrylic acid) derivative mixtures were prepared: 0:100, 10:90, 30:70 and 50:50.

2.2.2. Capsules for testing dissolution of content

Ten mg erythrosin were added to a Kollidon® CL/FNaC934P mixture (0:100, 10:90, 30:70 and 50:50), and 250 mg of the mixture were inserted into a No. 1 transparent capsule. TGPS, TGMS and TGHBe alone and in combination were melted at about 85°C. Thereafter, the poly(acrylates) were microdispersed within these melted poly(glycerol ester of fatty acids). Erythrosin and poly(acrylic acid) derivative (C934P, NaC934P or FNaC934P) were dispersed in the mixture. The mixture was cooled and crushed using a mortar. After sieving, 250 mg of the particles (particles diameter, < 200 μm) containing 10 mg erythrosin were inserted into a No. 1 transparent capsule. Filled capsules were tapped 100 times using a 10-g iron rod. All formulations investigated are summarized in Tables 1–3.

HLB values of the mixtures of PGEFs were calculated according to the following formula: $HLB = (xA + yB)/(x + y)$, where A and B are the HLB values of the PGEF compound and x and y are their corresponding weight fractions.

2.3. Disintegration test

The disintegration times of the constituents of each capsule were measured in a 0.05 M phosphate buffer solution of pH 7.5 at 37 ± 1°C (stroke: 32/min), except for the experiment which was done at pH 5.5 to evaluate the effect of pH on the disintegration time.

2.4. Dissolution test

Two dissolution tests were performed. Dissolution test 1 for low viscous systems was done in 500 ml of 0.05 M phosphate buffer solution, pH 6.8, at 37 ± 1°C, using the dissolution apparatus as described in USP XXII. The paddle rotation speed was 100 rev./min. Two ml of the solution taken at pre-determined time intervals were centrifuged for 10 min at 800 g, and the percentage of erythrosin dissolved was determined by measuring the absorption at 520 nm with a spectrophotometer (Shimadzu UV 190, 's Hertogenbosch, The Netherlands).

Dissolution test 2, for viscous preparations, was done in 40 ml of 0.05 M phosphate buffer solution, pH 6.8, at 37 ± 1°C. Dissolution tests were performed in 50-ml polypropylene centrifugation tubes (Greiner Labortechnik, Frickenhausen,

Table 2
Formulations of microparticles containing PGEF, poly(acrylic acid) derivatives and erythrosin

Component	ER-1	ER-2	ER-3	ER-4
TGPS	150	150	150	72
TGMS	25	25	25	28
Kollidon® CI	—	—	—	—
FNaC934P	100	—	—	100
NaC934P(EX-161)	—	100	—	—
C934P	—	—	81	—
Erythrosin	10	10	10	10
Total	285	285	266	210
HLB values	3.4	3.4	3.4	4.2

Except for HLB values, all data are expressed as mg.

Table 3
Formulations of PGEF or paraffin microparticles containing FNaC934P and erythrosin

Component	ER-5	ER-6	ER-7	ER-8	ER-9	ER-10
TGPS	100	125	150	175	—	—
TGMS	75	50	25	—	—	—
TGHBe	—	—	—	—	175	—
FNaC934P	100	100	100	100	100	100
Paraffin	—	—	—	—	—	175
Erythrosin	10	10	10	10	10	10
Total	285	285	285	285	285	285
HLB	5.1	4.3	3.4	2.6	1.8	a

Except for HLB values, all data are expressed as mg.
[a]Not calculated, « 1.8.

Germany). A specially designed propeller made of poly(ethylene) was rotated at a speed of 100 rev./min inside the poly(propylene) vessel. Samples of 200 μl were withdrawn at pre-determined time intervals, and were added to 3 ml of 0.05 M phosphate buffer (pH 6.8) and centrifuged for 10 min at 800 g. The amount of dissolved erythrosin was determined as described above.

3. Results and discussion

3.1. Screening of fast disintegrating poly(acrylic acid) derivatives

The disintegration times of capsule contents were determined to evaluate the homogeneous-gel-forming properties of the poly(acrylic acid) derivatives in combination with various disintegrants. Three types of disintegrants were investigated: croscarmellose, sodium starch glycolate and crospovidone. For freeze-dried neutralized carbomer (FNaC934P) it was found that the higher the content of the disintegrant, the faster the disintegration time of the capsule contents (Fig. 1). However, high concentrations of the disintegrants (up to 50%, w/w) were necessary to separate poly(acrylate) powder agglomerates and to disintegrate capsule contents, probably because the poly(acrylate) powder rapidly absorbed water which formed a highly viscous layer around the agglomerate surfaces. There was little difference in the disintegration times of capsules containing Explotab®, Kollidon® CL and Primellose®. The disintegration times of the capsules containing 50% C934P and 50% disintegrant (56, 56, 50 and 69 min for Explotab®, Kollidon® CL, Primellose® and Primojel®, respectively) were slower than those of the capsules containing 50% FNaC934P and 50% disintegrant (10, 8, 8 and 17 min, respectively). Rundic et al. (1985) reported that Explotab® and Primojel® differed in the degree of substitution (carboxymethylation) and cross-linkage, although both disintegrants were originated from sodium starch glycolate. Moreover, the differences between these two variables also affected the water uptake and swelling of these disintegrants. The rate of water uptake by Primo-

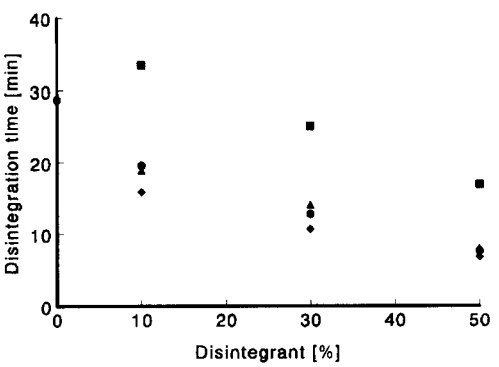

Fig. 1. Effect of disintegrant and its content on disintegration time of a capsule containing FNaC934P/disintegrant mixture in a medium of 0.05 M phosphate buffer (pH 7.5) at 37°C ($N = 2$). (◆) Explotab, (■) Primojel, (●) Kollidon® CL, (▲) Primellose.

jel® was much faster than by Explotab® (Gissinger and Stamm, 1980). It was indicated that sodium starch glycolate shows the optimal combination of degree of carboxymethylation and degree of cross-linkage (Kanig and Rundic, 1984). In another study, Rundic and Rhodes (1982) pointed out that both the rate and extent of swelling of sodium starch glycolate were dependent on the particle size, i.e. the larger particles swelled to a greater extent and a faster rate than did the finer particles. The reason for a slower disintegration time of the capsules containing Primojel® in comparison to the capsules containing Explotab® might have resulted from the difference in these variables. It is postulated that the disintegration behaviour of a sodium starch glycolate such as Explotab® and a cross-linked cellulose such as Primellose® is caused by both a rapid uptake of water and an enormous swelling of their particles. On the other hand, Kollidon® CL shows a limited swelling without formation of a gel, and wicking or capillary action plays an important role in the ability to disintegrate (Rundic and Schwarz, 1990). In spite of the difference in the mechanism of disintegration, there were little differences in the time required to disintegrate capsules containing poly(acrylic acid) derivatives. The capsules containing NaC934P and Kollidon® CL (50:50) disintegrated slower (60 min) than those containing FNaC934P and Kollidon® CL (8 min). In order to obtain an optimal gelling poly(acrylate), the capsule content needs to disintegrate into particles quickly and thereafter the particles have to disperse rapidly in the medium. A quick disintegration of the capsule content may result in the quick dispersion of the poly(acrylate) particles. Therefore, freeze-drying neutralized carbomer was found to be an efficient excipient to prepare a poly(acrylate) formulation which dispersed quickly. FNaC934P was found to be the most suitable candidate for the use of a quick gel-forming poly(acrylic acid) derivative.

3.2. Dissolution of erythrosin from poly(acrylic acid) derivatives and disintegrants

The release of erythrosin from the capsule contents increased with increasing amounts of Kollidon® CL, i.e. the release of erythrosin from the capsule containing 50% FNaC934P and 50% Kollidon® CL was the fastest (Fig. 2). Formulations are shown in Table 1.

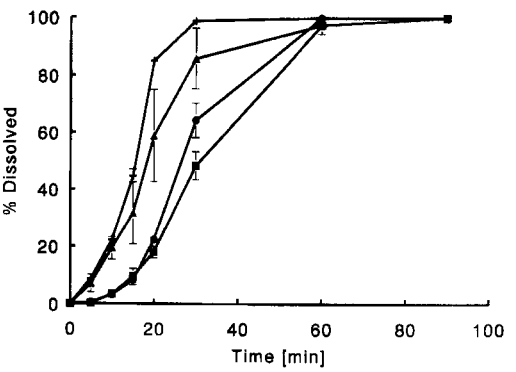

Fig. 2. Effect of Kollidon® CL content on dissolution of erythrosin from a capsule containing FNaC934P/Kollidon® CL mixture in a medium of 0.05 M phosphate buffer (pH 6.8; 500 ml); 37°C; 100 rev./min; mean ± S.D. ($N = 3$); Dissolution test 1. FNaC934P/Kollidon® CL ratio, (+) 50/50, (▲) 70/30, (●) 90/10, (■) 100/0.

When fine particles of poly(acrylic acid) derivatives (C934P, NaC934P and FNaC934P) were subjected to a large volume of neutral aqueous medium for dissolution, they swelled quickly. However, swellable polymers such as C934P or fine low-substituted hydroxypropyl cellulose (average diameter, $< 27\mu m$) were reported to be used as a base for solid controlled-release tablets (Kawashima et al., 1993). This might result from the fact that a gel-like layer was formed at the boundary between the penetrating water front and the powder particles, making it difficult for water diffusion and vice versa for drug release. It is postulated that the formation of a continuous gel-like layer on the surface of a mass composed of the particles of poly(acrylic acid) derivatives prevents water from entering and prevents erythrosin from being released. Therefore, disintegrants are not always necessary to obtain powder formulations which release erythrosin quickly, unless the particles of poly(acrylic acid) derivatives stick to each other and make a mass surrounded by a highly viscous gellayer. It is expected that capsules containing C934P, NaC934P or FNaC934P will rapidly disintegrate and release a

water-soluble drug such as erythrosin, as long as the particles are prevented from sticking together.

3.3. Dissolution of erythrosin from PGEF microparticles composed of poly(acrylic acid) derivatives

Akiyama et al. (1995) have developed mucoadhesive microspheres consisting of PGEF and C934P, and reported that these microspheres adhere to the gastrointestinal tract of rats. Modifying this system to microparticles containing C934P, NaC934P or FNaC934P and erythrosin, the profiles of erythrosin release were examined (Table 2). The release of erythrosin from the capsules containing microparticles composed of PGEF and FNaC934P (ER1) was the fastest, and release of erythrosin from the capsules composed of PGEF and C934P (ER3) was the slowest (Fig. 3). In addition, the release of erythrosin from the capsules containing microparticles of PGEF and FNaC934P (formulation ER1 in Table 2) was faster than that from the capsule containing FNaC934P and Kollidon® CL mixture (formulation K*50/50 in Table 1), whereas the release of erythrosin from a mixture of erythrosin and FNaC934P without PGEF or Kollidon® CL was the slowest as shown in Fig. 4. The release profile of erythrosin from the more hydrophillic formulation ER4 (HLB 4.2 compared to HLB 3.4 for formulation ER1) is also shown in Fig. 4. The time required for 90% of erythrosin to be released ($t_{90\%}$) for ER1, ER4, K*50/50 and K*0/100 were 14, 16, 20 and 55 min, respectively. Fig. 4 shows that addition of a disintegrant is not always necessary for the formulation containing FNaC934P in order to improve the fast release of drugs. The flaky FNaC934P particles can be separated efficiently by being mixed with melted PGEF and coated with a thin film of PGEF.

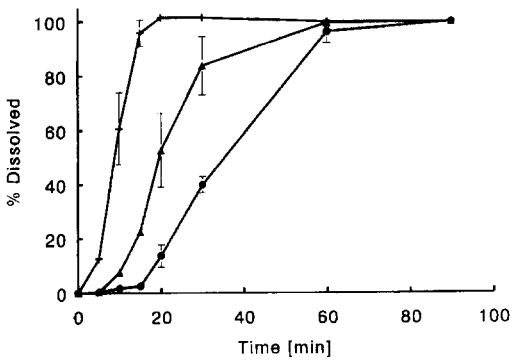

Fig. 3. Effect of formulations on dissolution of erythrosin from a capsule containing poly(acrylate)/PGEF microparticles in a medium of 0.05 M phosphate buffer (pH 6.8; 500 ml); 37°C; 100 rev./min; mean ± S.D. ($N = 3$); Dissolution test 1. (+) FNaC934P, (▲) NaC934P, (●) C934P.

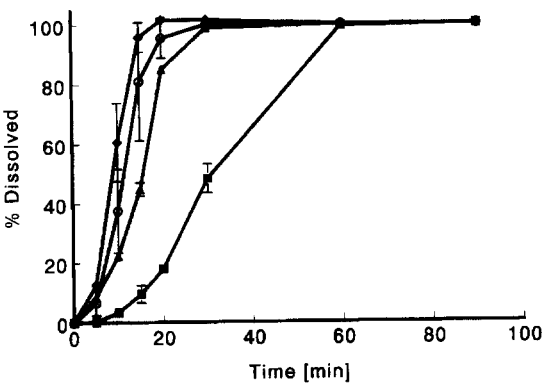

Fig. 4. Effect of formulations on dissolution of erythrosin from a capsule containing PGEF microparticles with FNaC934P, FNaC934P/Kollidon® CL mixture or FNaC934P in a medium of 0.05 M phosphate buffer (pH 6.8; 500 ml); 37°C; 100 rev./min; mean ± S.D. ($N = 3$); Dissolution test 1. FNaC934P/Kollidon® CL mixture: FNaC934P/Kollidon® CL ratio (▲) 50/50 (formulation K*50/50), (■) 0/100 (formulation K*0/100). PGEF microparticles: FNaC934P/PGEF ratio (◇) 36/64 (formulation ER 1), (○) 50/50 (formulation ER4).

Ishii and Fujii (1982) reported that sodium poly(acrylate) (PANa), with a molecular weight of 4.3-6.3 × 10^6 Da, dissolved quickly by coating PANa powder with water-permeable but insoluble high molecular weight compounds such as ethyl cellulose, whereas PANa dissolves in water at an extremely slow rate without any treatment. However, paraffin-based microparticles containing erythrosin (ER5), prepared using the same method of preparing PGEF microparticles (i.e., the microparticles are coated with a thin film of paraffin), showed the slowest release rate for erythrosin. PGEF microparticles, prepared from the very lipophilic TGHBe with an HLB value of 1.8, released erythrosin at a slower rate than those prepared with TGMS and TGPS. The times re-

quired for 50% erythrosin release ($t_{50\%}$) from ER6, ER7, ER8, ER9 and ER10 (Table 1) were 22, 12, 7, 7 and 7 min respectively, and their HLB values were 1.8, 2.6, 3.4, 4.3 and 5.1 respectively, as shown in Fig. 5. These results indicate that PGEF microparticles with higher HLB values could release erythrosin faster than those with lower HLB values, although there was little difference in the release rates of erythrosin from PGEF microparticles with HLB values ranging from 2.6 to 5.1.

From these studies it is concluded that PGEF microparticles (formulation ER4 with higher content of FNaC934P (50%) than ER1) and Kollidon® CL mixtures (formulation K*50/50; Table 1) are suitable candidates for use in formulations which have to release hydrophillic drugs very quickly.

3.4. Evaluation of the rate of erythrosin release using dissolution test 2

It was reported that trypsin activity was strongly inhibited in a medium containing 0.25% (w/v) C934P (Lueßen et al., 1994; Lueßen et al., 1995). In order to evaluate the inhibition of trypsin activity, the concentration of the poly(acrylic acid) derivative in the dissolution medium is an important factor. When a capsule containing 100 mg of a poly(acrylic acid) derivative is added into 500 ml of a dissolution medium, the concentration and viscosity would be different from those of physiological conditions, since the volume of gastric juice is in the order of 25–50 ml (Gruber et al., 1987). Therefore, the release rates were also evaluated using dissolution test 2, which uses 40 ml of dissolution medium. The release rates of erythrosin from formulations ER4 (PGEF microparticles), K*50/50 (FNaC934P/Kollidon® CL mixture) and K*0/100 (FNaC934P) were evaluated. As shown in Fig. 6, $t_{90\%}$ of ER4, K*50/50 and K*0/100 were 19, 24 and 92 min respectively, in dissolution test 2, in comparison with 16, 20 and 55 min in dissolution test 1. Thus the release rates of erythrosin were slower in dissolution test 2 than those observed in dissolution test 1 for all formulations investigated. These differences may result from the higher viscosity of the dissolution medium containing poly(acrylic acid) derivatives as used in dissolution test 2.

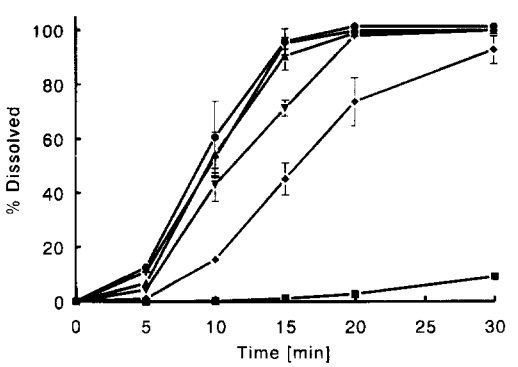

Fig. 5. Effect of HLB of PGEF on dissolution of erythrosin from a capsule containing PGEF microparticles with FNaC934P in a medium of 0.05 M phosphate buffer (pH 6.8; 500 ml); 37°C; 100 rev./min; mean ± S.D. ($N = 3$); Dissolution test 1. HLB; (+) 5.1, (▲) 4.3, (●) 3.4, (▼) 2.6, (◆) 1.8 and paraffin (■).

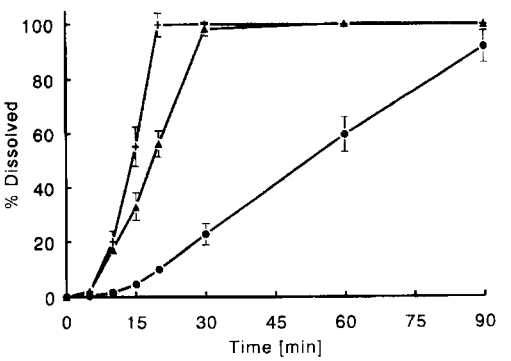

Fig. 6. Effect of formulations on dissolution of erythrosin from a capsule containing PGEF microparticles with FNaC934P, FNaC934P/Kollidon® CL mixture or FNaC934P in a medium of 0.05 M phosphate buffer (pH 6.8: 40 ml); 37°C; 100 rev./min; mean ± S.D. ($N = 3$); Dissolution test 2. FNaC934P/Kollidon® CL ratio (▲) 50/50 (formulation K*50/50), (●) 0/100 (formulation K*0/100). PGEF microparticles: FNaC934P/PGEF ratio (+) 50/50 (formulation ER4).

4. Conclusions

Capsules which quickly disintegrate and rapidly release drugs were prepared and evaluated. Rapid disintegration and quick drug release are prereq-

uisites for excipient mixtures containing cross-linked poly(acrylic acid) derivatives aimed at efficiently decreasing the activity of proteolytic enzymes. Erythrosin was used as a water-soluble model drug. The mixtures containing neutralized freeze-dried carbomer (FNaC934P) released erythrosin quicker than the capsules containing carbomer itself. Mixtures containing PGEF microparticles consisting of FNaC934P and erythrosin disintegrated and released erythrosin quicker in comparison with the capsules containing mixtures of disintegrants and erythrosin. Therefore, PGEF microparticles containing poly(acrylic acid) derivatives are promising dosage forms for quick gel-forming and fast drug-releasing purposes.

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