ELSEVIER

International Journal of Pharmaceutics 113 (1995) 223–229

international journal of pharmaceutics

The bioadhesive properties of a triblock copolymer of ϵ-caprolactone and ethylene oxide

L. Martini, D. Attwood *, J.H. Collett, A. D'Emanuele

Department of Pharmacy, University of Manchester, Manchester M13 9PL, UK

Received 18 March 1994; accepted 3 June 1994

Abstract

Tablets produced by compression of a novel biodegradable triblock copolymer of ϵ-caprolactone (CL) and ethylene oxide (E), $CL_6E_{90}CL_6$, have been shown to possess bioadhesive properties. The work of adhesion, the detachment force, the fracture strength, and the modulus of elasticity were calculated from force-elongation curves measured for polymer tablets in contact with mucosal tissue from rat duodenum. Environmental scanning electron microscopy was used to investigate changes in the appearance of the tablet surface during its hydration and also after adhesion to the mucosal tissue.

Keywords: Bioadhesion; Block copolymer; Environmental scanning electron microscopy

1. Introduction

Polymers with a wide range of structure have been shown to exhibit bioadhesive properties and several workers, notably Smart et al. (1984) and Park and Robinson (1984) have presented a classification of these polymers in terms of their adhesive properties, which has proved useful in the selection of suitable polymers for the fabrication of mucoadhesive controlled release systems. Although these two groups of workers used different criteria in the evaluation of adhesive properties, it has been concluded (Peppas and Buri, 1985) that polymers with strong anionic charges, as well as polymers bearing a number of carboxylic or hydroxylic groups, have good binding potential. These authors also stressed the importance of a sufficient chain flexibility to allow interpenetration of the mucosal network, favourable surface energy properties to facilitate spreading over the mucosal surface, and a sufficient macromolecular size to produce an interpenetrating layer and entanglements. As a consequence of these findings, many workers (Ponchel et al., 1987a,b; Lejoyeux et al., 1988a; Bouckaert and Remon, 1993) have chosen to include the anionic polymer poly(acrylic acid) as a component of bioadhesive controlled release systems. Other workers (Lehr et al., 1992) have cautioned about the a priori generalisation of the necessity for a negative charge and hydrogen bonding capabili-

* Corresponding author.

SSDI 0378-5173(94)00202-9

ties and have demonstrated the bioadhesive potential of high molar mass cationic polymers such as chitosan.

The adhesive properties of low molecular mass poly(oxyethylene) glycols are generally poor (Chen and Cyr, 1970; Park and Robinson, 1984; Smart et al., 1984) but could be improved by the introduction of a hydrophobic moiety in the molecule, so conferring amphiphilicity (and hence surface activity). In this study we have examined the bioadhesive properties of a matrix of a novel triblock copolymer of ϵ-caprolactone (CL) and ethylene oxide (E) with a molecular formula $CL_6E_{90}CL_6$ and a mean molecular mass of 5330 (Martini et al., 1994). In addition to the amphiphilicity arising from the presence of the caprolactone blocks, the molecular chain length is greatly increased whilst still retaining a high segmental mobility, and there is also increased secondary bond forming capacity due to the presence of ester and hydroxyl groups and ether linkages. The adhesive properties of the copolymer matrix were characterised by determination of the work of adhesion and the detachment force by analysis of force elongation curves measured for the copolymer matrix using perspex and mucosal tissue (from rat duodenum) as test substrate surfaces. Changes in the appearance of the matrix surface during its hydration and also after adhesion to mucosal tissue were examined using the technique of environmental scanning microscopy.

2. Materials and methods

2.1. Materials

The block copolymer $CL_6E_{90}CL_6$ was prepared by using polyethylene glycol 4000 [α-hydro-ω-hydroxypoly(oxyethylene), E_{90}] to initiate the polymerisation of ϵ-caprolactone at 180°C in the absence of added catalysts: the method used was that of Cerrai et al. (1989). Full details of the synthesis, purification and characterisation by ^{1}H- and ^{13}C-NMR and by GPC have been published previously (Martini et al., 1994). The mole percentage of triblock in the sample, as determined by NMR analysis, was 94%.

2.2. Methods

2.2.1. Matrix preparation

The test tablets (diameter 0.63 cm, thickness 0.40 cm, weight 145 ± 1.5 mg) were prepared from the copolymer in a compressional apparatus designed to produce low loads (750 N).

2.2.2. Measurement of adhesion

A modified ELE triaxial tensile tester was used to measure adhesive properties at ambient temperature. The upper of two cylindrical perspex supports (1 cm diameter) was connected to a calibrated strain gauge, whilst the lower support was attached to a motor driven platform. The output from the strain gauge was recorded on a chart recorder at a speed of 2 cm min^{-1}.

In the study of the adhesive properties to a perspex surface, the test tablet was fixed to the upper perspex support, using a cyanoacrylate resin. 15 μl of distilled water (sufficient to produce a uniform coverage of the surface) was placed on the surface of the tablet, using a syringe. The two perspex platforms were brought immediately together with an initial force of 0.4 N. Contact between tablet and the perspex surface was maintained for a predetermined time (the preswelling time). Measurement of the adhesion force was then commenced using an elongation rate of 0.015 cm min^{-1} and continued until the breakpoint. Experiments were repeated in triplicate.

For a typical bioadhesion experiment, a sample of tissue (mean thickness, 0.020 cm and surface area 0.312 cm^2) freshly removed from the duodenum of an adult 'non-fasted' male Sprague Dawley rat, was attached using cyanoacrylate resin to the lower support, with the mucosal surface uppermost. The test tablet was similarly attached to the upper support, and its surface was wetted with either distilled water or 0.1 mol dm^{-3} hydrochloric acid. The two supports were immediately brought together with a compressional force of 0.075 N, (the low force was used to avoid damage to the mucosal tissue). After 15

min, the measurement of adhesion force was commenced using an elongation rate of 0.015 cm min^{-1} and continued until the breakpoint. Experiments were repeated in triplicate.

2.2.3. Environmental scanning electron microscope (ESEM)

Samples of copolymer were examined in both dry and hydrated states using an ESEM (Electroscan-E3, Electroscan Corp., Wilmington, MA) at 7°C and under a vacuum of 6–8 Torr. This technique permits the direct observation of material in its wet state without the usual need for sample preparation procedures such as freezing, drying or coating, all of which may lead to modification of the sample surface (Danilatos, 1991).

3. Results and discussion

3.1. Adhesion to perspex

Preliminary experiments were performed to determine the optimum preswelling time required to achieve maximum adhesion of the tablet to the perspex surface in the absence of tissue sample. Fig. 1 shows a typical plot of force, F, against elongation, Δl, observed after a preswelling time of 15 min; similar plots were obtained at intervals of 5 min up to a maximum of 35 min. The work of adhesion, W, as calculated from the area under the F-Δl curves, is plotted as a function of preswelling time, t, in Fig. 2, which shows a distinct peak at about 15 min. The maximum force of detachment, F_{max}, was determined from the mean values of the force at the peak of the F-Δl curves. Fig. 3 shows a plot of F_{max} vs t with a maximum after a preswelling time of about 15–20 min. Since other workers (Lejoyeux et al., 1988b) have shown that the work of adhesion gives a more reliable indication of the adhesive strength, an optimum preswelling time of 15 min was selected for use in the subsequent bioadhesion experiments. This preswelling time is within the range generally considered to be desirable for a bioadhesive formulation (Peppas and Buri, 1985).

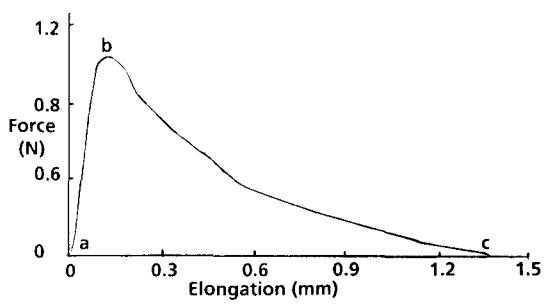

Fig. 1. Variation of the force necessary for detachment of the copolymer matrix from a perspex surface as a function of elongation after a preswelling time of 15 min.

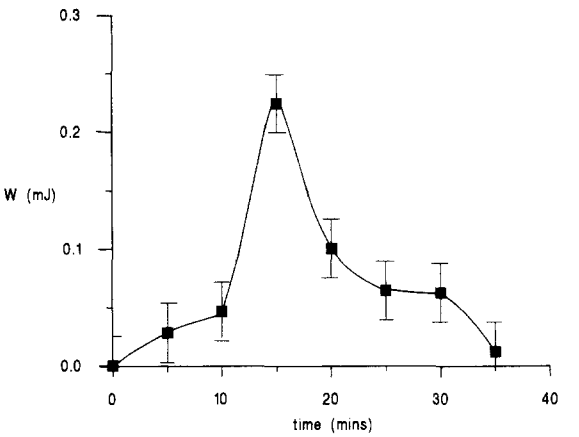

Fig. 2. Variation of the work of adhesion, W, between the copolymer matrix and the perspex substrate, as a function of preswelling time (±S.E., $n = 3$).

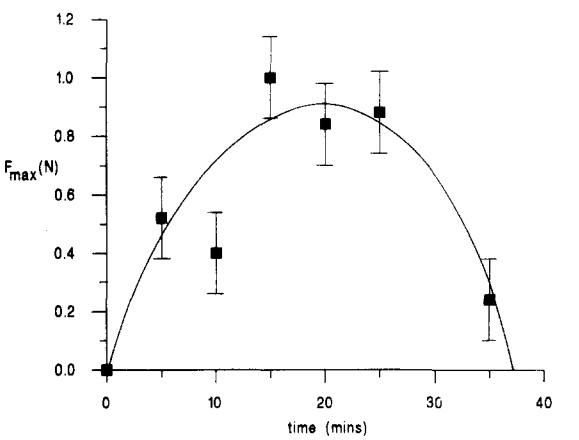

Fig. 3. Variation of the maximum force of detachment, F_{max}, between the copolymer matrix and perspex substrate, as a function of preswelling time (±S.E., $n = 3$).

The initial increase of the magnitude of the work of adhesion up to the maximum (Fig. 2) is thought to be a consequence of the disentanglement of the polymer chains and the establishment of an intimate molecular contact with the substrate surface (Lejoyeux et al., 1988b). The marked decrease of W after the maximum has been attributed to the diffusion of water away from the polymer/substrate interface into the bulk of the dry polymer matrix (Ponchel et al., 1987a). An examination of the surface of the tablet in its dry state and at intervals after its hydration, was undertaken using ESEM. Comparison of Fig. 4a, the dry tablet surface, and Fig. 4b, the surface 10 min after wetting, shows the development of a hydrated gel layer which has the appearance of undulations on the surface. It is possible that the formation of a hydrated gel layer on the tablet surface allows the flexible polymer chains within this layer to interpenetrate the substrate to a sufficient depth to create a semipermanent adhesive bond (Peppas and Buri, 1985). After a period of 30–40 min following hydration of the surface, ESEM revealed the development of channels (Fig. 4c) in the surface, leading to the interior of the matrix, which may be formed by the erosion of the matrix as water diffuses away from the surface. As a consequence, the adhesive bond is weakened resulting in the decrease of W shown in Fig. 2. These findings are in agreement with those of Chen and Cyr (1970) and Ponchel et al. (1987a) who also

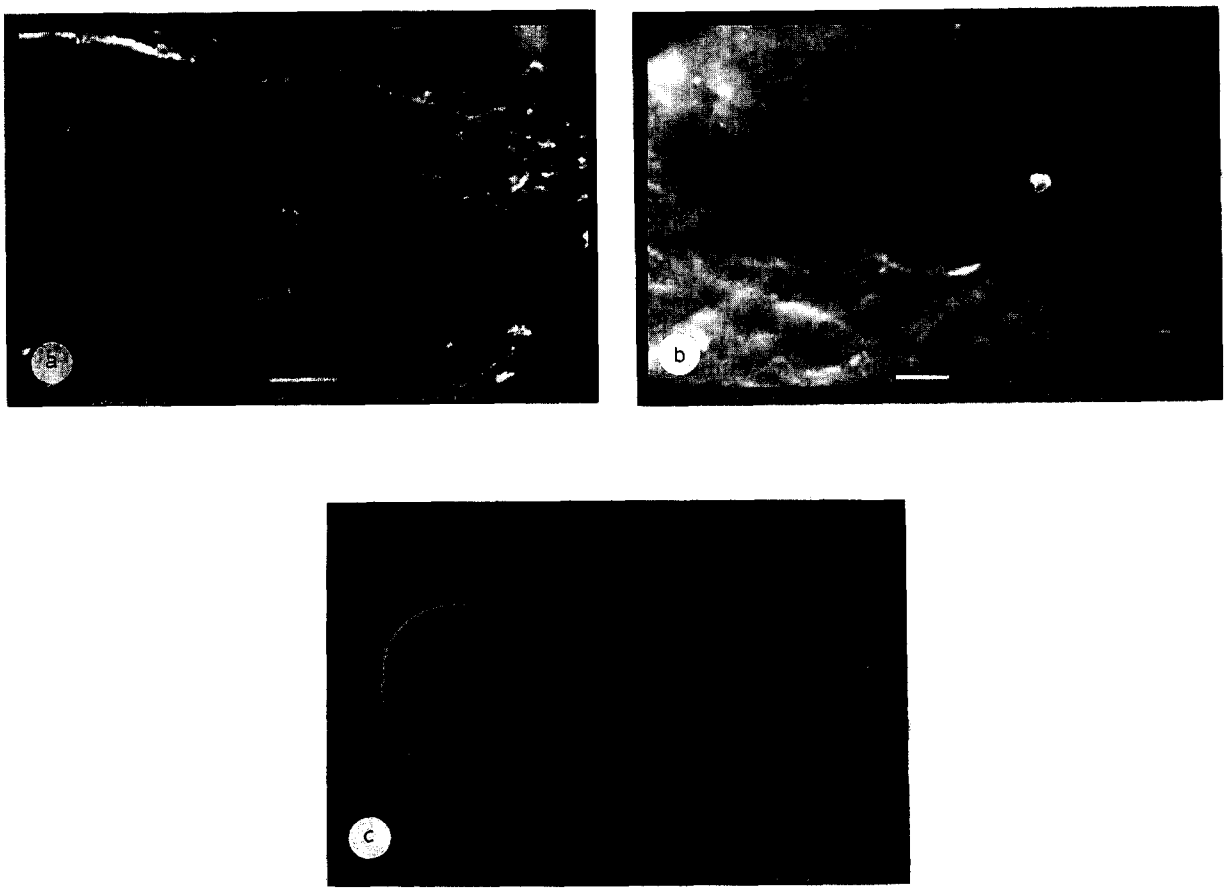

Fig. 4. Environmental scanning electron micrographs of the surface of the copolymer matrix: (a) before hydration, (b) 10 min after hydration and (c) 40 min after hydration.

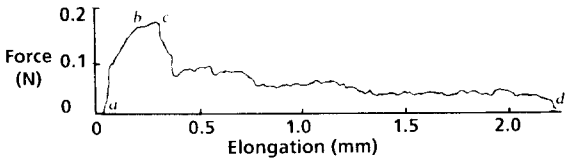

Fig. 5. Variation of the force necessary for detachment of the copolymer matrix from duodenal mucosa as a function of elongation after a preswelling time of 15 min.

reported an optimum water content for maximum adhesion.

3.2. Adhesion to mucosal tissue

Fig. 5 shows a typical force-elongation curve obtained for the adhesion of a hydrated copolymer matrix to the surface of mucosal tissue. The curve is typical of those reported for other bioadhesive systems (Ponchel et al. 1987a,b; Lejoyeux et al. 1988a) and is characterised by three regions: in the initial stages (a–b) the force increases appreciably with elongation, the contact area between the matrix and tissue (0.312 cm^2) remaining constant; the plateau b–c represents partial detachment of the matrix from the mucosal surface which gradually becomes more significant over the region c–d leading to complete detachment of the matrix at d. The differences in the appearance of F-Δl curves for adhesion to perspex (Fig. 1) and tissue (Fig. 5) are presumably a consequence of differences in the degree of interaction of the polymer chains with the substrate. The plateau seen in Fig. 5 for the tissue system and the more gradual decay of the bioadhesive force for this system suggest a greater degree of interpenetration of the polymer into the mucosal network than occurs with the tissue-free perspex substrate.

Fracture analysis of the bioadhesive experiments with water and 0.1 mol dm^{-3} HCl as wetting solvents was carried out as proposed by Ponchel et al. (1987a). The fracture strength, σ_b, of the bioadhesive bond, which corresponds to the stress of detachment at the maximum detachment force, F_{max}, was calculated from:

$$\sigma_b = F_{max}/A_o \quad (1)$$

where A_o is the contact area between tablet and tissue. The modulus of elasticity, E, which relates to the molecular rigidity of the 'composite' material at the polymer/tissue interface was calculated from:

$$E = (F/A_o)/(\Delta l/l_o) \quad (2)$$

where $F/\Delta l$ is the gradient of the region a–b of Fig. 5 and l_o denotes the sample thickness. The reversible work of adhesion, W_r, is given by:

$$W_r = W/A_o \quad (3)$$

The values obtained from the fracture analysis (Table 1) are appreciably smaller than those derived for other bioadhesive systems. For example, Ponchel et al. (1987a,b) reported W values increasing from 9.6 to 23 mJ as the poly(acrylic acid) content of hydroxypropyl methyl cellulose-poly(acrylic acid) mixtures was increased from 10 to 90%; the corresponding increase of F_{max} was from 8 to 9.5 N. However, in making comparisons with data for other bioadhesive systems it should be noted that the gels formed on hydration of the $CL_6E_{90}CL_6$ matrix are composed of closely packed micelles (Martini et al., 1994) rather than cross-linked networks as in hydrogels of poly (acrylic acid). Such structural differences will have important consequences on the relative ability of the polymer molecules to penetrate the mucus layer. Several factors which influence the bioadhesive forces should also be considered. The initial compressional force used in this present study (0.075 N) was significantly lower than that used by most workers (typically 0.5 N), in order to

Table 1
Fracture analysis of the force-elongation curves of a $CL_6E_{90}CL_6$ matrix in contact with rat duodenal mucosa

Solvent	F_{max} (N)	W (mJ)	σ_b (kPa)	E (kPa)	W_r (Nm^{-1})
Water	0.20 ± 0.07	0.16 ± 0.02	6.12 ± 2.18	5.78 ± 1.70	4.91 ± 0.32
0.1 mol dm^{-3} HCl	0.29 ± 0.02	0.14 ± 0.09	9.30 ± 0.77	–	4.60 ± 0.30

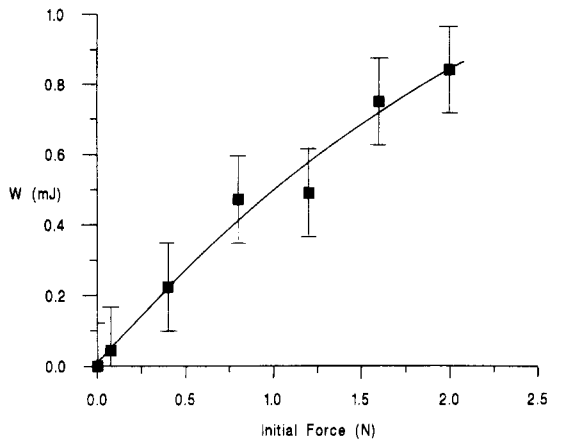

Fig. 6. Work of adhesion, W, between the copolymer matrix and perspex substrate, as a function of the initial applied force ($\pm$S.E., $n = 3$).

avoid damage to the fragile duodenal mucosa. It has been shown (Park and Robinson, 1985; Park and Park, 1990) that the bioadhesion between hydrogels and tissues increases with increase in the initial applied force. This improvement of bioadhesion was attributed to enhanced interaction with the substrate. Fig. 6 and 7 show the increases of both W and F_{max} for the adhesion of the copolymer to perspex as the initial force was increased, in agreement with the findings of these authors. The low values of the bioadhesive parameters of Table 1 may thus be partly a consequence of the very low initial force applied in this study. In this respect it is interesting to note that, in their evaluation of the mucosa-adhesive properties of a series of polymers, Mortazavi and Smart (1993) used a very low initial compression force (0.015 N) and obtained values of W of similar magnitude to those of this present study. The rate of extension used was approx. 30 times slower than rates used by other workers (Ponchel et al. 1987a,b; Lejoyeux et al., 1988a,b; Bouckaert and Remon, 1993) and this too may be a contributory cause of the low bioadhesive forces, since it has been demonstrated (Ponchel et al., 1991) that the work of adhesion increases with an increase in the rate of detachment of an adhesive from the substrate. Finally, it should be noted that different types of mucosal tissue may exhibit markedly different behaviour in bioadhesion experiments (Lejoyeux et al., 1988b) and it may not be meaningful to compare bioadhesion parameters for different polymers unless the substrates are identical.

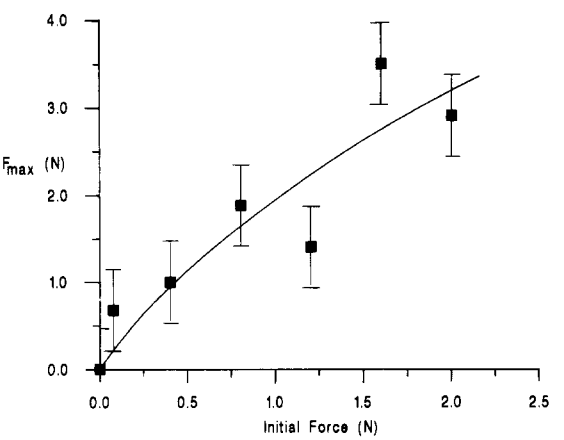

Fig. 7. Maximum force of detachment, F_{max}, between the copolymer matrix and perspex substrate, as a function of the initial applied force ($\pm$S.E., $n = 3$).

Table 1 shows similar values of the bioadhesive properties in water and 0.1 mol dm^{-3} HCl suggesting, as might be expected for this nonionic copolymer, that the bioadhesion is not significantly influenced by changes of pH over the range 1–5.5. In contrast, anionic polymers such as poly(acrylic acid) are appreciably affected by pH (Park and Robinson, 1985) and show maximum binding only when the pH is lower than the pK_a. Park and Robinson (1985) stressed the importance of mucin-mucin cohesive forces as the rate-limiting step in bioadhesion of several polymers. Similarly, Ponchel et al. (1987a) proposed a mechanism for the bioadhesion of a poly(acrylic acid)-containing tablet with bovine sublingual mucus in which bond rupture occurred in the mucus rather than at the polymer/mucus interface. Fig. 8 shows the surface of the copolymer matrix as observed by ESEM after bioadhesion to duodenal mucosa. The micrograph reveals a thin layer of tissue on the surface, indicative of rupture within the mucosal tissue. This suggests that the mechanism proposed by Ponchel et al. (1987a) applies in this system and that the strength of the bioadhesive bond is limited by the mechanical

Fig. 8. Environmental scanning electron micrographs of the surface of the copolymer matrix after bioadhesion to duodenal mucosa.

strength of bonds within the mucus and not the copolymer-mucus adhesive forces.

Acknowledgment

The authors thank the SERC for the award of a research studentship (L.M.).

References

Bouckaert, S. and Remon, J.-P., In vitro bioadhesion of a buccal, miconazole slow-release tablet. *J. Pharm. Pharmacol.*, 45 (1993) 504–507.

Cerrai, P., Tricoli, M., Andruzzi, F., Paci, M. and Paci, M., Polyether-polyester block copolymers by non-catalysed polymerisation of ϵ-caprolactone with poly(ethylene glycol). *Polymer*, 30 (1989) 338–343.

Chen, J.L. and Cyr, G.N., Compositions producing adhesion through hydration. In Manly, R.S. (Ed.), *Adhesion in Biological Systems*, Academic Press, New York, 1970, pp. 163–181.

Danilatos, G.D., Review and outline of environmental SEM at present. *J. Microsc.*, 162 (1991) 391–402.

Lehr, C.-M., Bouwstra, J.A., Schacht, E.H. and Junginger, H.E., In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int. J. Pharm.*, 78 (1992) 43–48.

Lejoyeux, F., Ponchel, G., Wouessidjewe, D., Peppas, N.A. and Duchêne, D., Assessment of a new method for the determination of bioadhesion. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 15 (1988a) 348–349.

Lejoyeux, F., Ponchel, G., Wouessidjewe, D., Peppas, N.A. and Duchêne, D., Influence of the composition of the test medium on the adhesion of a bioadhesive tablet to a biological tissue. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 15 (1988b) 460–461.

Martini, L., Attwood, D., Collett, J.H., Nicholas, C.V., Tanodekaew, S., Deng, N.-J., Heatley, F. and Booth, C., Micellisation and gelation of triblock copolymers of ethylene oxide and ϵ-caprolactone, $CL_nE_mCL_n$ in aqueous solution. *J. Chem. Soc. Faraday Trans.*, 90 (1994) 1961–1966.

Mortazavi, S.A. and Smart, J.D., An in-vitro evaluation of mucosa-adhesion using tensile and shear stresses. *J. Pharm. Pharmacol.*, 45 (Suppl. 2) (1993) 1111.

Park, K. and Park, H., Test methods of bioadhesion. In Lenaerts, V. and Gurny, R. (Eds), *Bioadhesive Drug Delivery Systems*, CRC Press, Boca Raton, 1990, Chap. 3, pp. 43–64.

Park, K. and Robinson, J.R., Bioadhesive polymers as platforms for oral-controlled drug delivery: Method to study bioadhesion. *Int. J. Pharm.*, 198 (1984) 107–127.

Park, H. and Robinson, J.R., Physicochemical properties of water insoluble polymers important to mucin/epithelial adhesion. *J. Controlled Release*, 2 (1985) 47–57.

Peppas, N.A. and Buri, P.A., Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. *J. Controlled Release*, 2 (1985) 257–275.

Ponchel, G., Lejoyeux, F. and Duchêne, D., Bioadhesion of poly(acrylic acid) containing-systems. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 18 (1991) 111.

Ponchel, G., Touchard, F., Duchêne, D. and Peppas, N.A., Bioadhesive analysis of controlled-release systems: 1. Fracture and interpenetration analysis in poly(acrylic acid)-containing systems. *J. Controlled Release*, 5 (1987a) 129–141.

Ponchel, G., Touchard, F., Wouessidjewe, D., Duchêne, D., Peppas, N.A., Bioadhesive analysis of controlled-release systems: III Bioadhesive and release behaviour of metronidazole-containing poly(acrylic acid)-hydroxypropyl methylcellulose systems. *Int. J. Pharm.*, 38 (1987b) 65–70.

Smart, J.D., Kellaway, I.W. and Worthington, H.E.C., An in vitro investigation of mucosa-adhesive materials for use in controlled drug delivery. *J. Pharm. Pharmacol.*, 36 (1984) 295–299.