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Effect of various poloxamer coatings on in vitro adhesion of isohexylcyanoacrylate nanospheres to rat ileal segments under liquid flow

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Summary

This work evaluates the bioadhesive properties of isohexylcyanoacrylate nanocapsules coated with poloxamers 407, 238, 403 and poloxamine 908 compared to nanoparticles coated with poloxamer 407. Nanocapsules (oil droplets surrounded by a polymeric wall) and nanoparticles (plain polymeric spheres) were prepared, labelled with covalently linked with tetraiodinated ^{125}I-phthalocyanine-Zn and laid on the rat ileal segment in vitro. After 0 and 10 min following transfer of nanospheres, a liquid flow was started. In all cases studied, a fraction of the nanospheres was not adherent when perfusion was initiated and was recovered in the first fraction. The other fraction, representing approx. 45% of the nanospheres, adhered firmly and was removed very slowly by the liquid flow. In addition, when nanocapsules were coated with poloxamers 238 and 407, the percentage adhesion increased between 0 and 10 min. Our results demonstrate that the greatest extent of mucoadhesion was achieved with poloxamers possessing a short central polyoxypropylene (POP) chain and long polyoxyethylene (POE) side chains. The results are discussed on the basis of diffusion of the POE groups into the mucin network and creation of secondary chemical adhesive bonds (Van der Waals type) between the nanosphere coating and mucus.

Introduction

Several approaches have been proposed for the use of bioadhesive drug delivery systems (BDDS) in nasal, ocular, and gastro-intestinal applications. Their prolonged residence time and controlled release characteristics can improve drug bioavailability and increase the duration of contact with the mucosal surface (Park and Robinson, 1984). Peptides or orally administered drugs with poor bioavailability and presystemic metabolism could benefit from their use. The work currently in progress in our laboratory is aimed at identifying the mechanism of mucoadhesion of isohexylcyanoacrylate nanocapsules with different poloxamer (407, 238, 403) and poloxamine (908) coatings in comparison with nanoparti-

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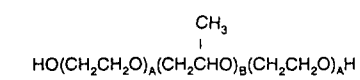
cles coated with poloxamer 407. Thus, the bioadhesive properties of these nanospheres on rat ileal segments under liquid flow in vitro were studied as a function of the degree of hydrophilicity and molecular weight of the poloxamer and poloxamine coatings.

Materials and Methods

Chemicals

Isohexylcyanoacrylate was generously donated by Dr H. Vranckx (SOPAR n.v., Zelzate, Belgium). Sulphur dioxide (Liquid Carbonic, Montréal, Canada) was dissolved in the monomer up to saturation at room temperature. Miglyol 829 (caprylic/capric diglyceride succinate) was purchased from Huls Troisdorf GmbH (Troisdorf, Germany). Cremophor (castor oil shortening) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Pure ethanol was acquired from Consolidated Alcohols Ltd (Toronto, Canada). Poloxamers are *ABA* block copolymers of oxyethylene (*A*) and oxypropylene (*B*) whereas poloxamines are *N,N',N'',N'''*-tetra[(oxyethylene)$_A$-(oxypropylene)$_B$] diaminoethylenes (Fig. 1). The following derivatives were received from BASF Canada Inc. (Montréal, Canada): poloxamer 407 (oxyethylene, 74.5%; MW, 11 500; A = 98, B = 67), poloxamer 238 (oxyethylene, 83.3%; MW, 10 800; A = 97, B = 39), poloxamer 403 (oxyethylene, 38.5%; MW, 5750; A = 21, B = 67), poloxamine 908 (oxyethylene, 80%; MW, 25 000; A = 122, B = 22). Tetraiodinated ^{125}I-phthalocyanine-Zn complex was a generous gift from Dr. J. Van Lier (MRC Group in the Radiation Sciences, Centre Hospitalier de l'Université de Sherbrooke, Canada). All other chemicals were of analytical grade.

(a)



(b)

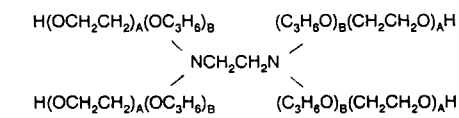


Fig. 1. (a) Poloxamer structure: *ABA* block copolymers with a central hydrophobic polyoxypropylene (POP) block and hydrophilic polyoxyethylene (POE) side chains. (b) Poloxamine structure: *N,N',N'',N'''*-tetra[(oxyethylene)$_A$(oxypropylene)$_B$] diaminoethylenes.

Preparation and purification of nanospheres

Nanocapsules were obtained by interfacial polymerization of isohexylcyanoacrylate in an oil-in-water emulsion according to the method of Chouinard et al. (1991). The oily phase consisted of 5% cremophor, 2% Miglyol, 0.5% monomer and 5×10^5 Bq/ml tetraiodinated ^{125}I-phthalocyanine-Zn complex (ZnPC-$^{125}I_4$) in ethanol and was added with stirring at a 1:2 ratio to the aqueous phase which consisted of 0.25% poloxamer or poloxamine in double-distilled water. The rate of addition was maintained at 0.5 ml/min using a peristaltic pump (Masterflex, Cole-Parmer Instrument Co., Chicago, IL, U.S.A.). Phthalocyanines were used as radiotracers owing to their very stable binding to polyisohexylcyanoacrylate nanospheres (Labib et al., 1991). Nanocapsules prepared according to this method appear as oily cores surrounded by a polymeric wall (Chouinard et al., 1991).

Nanoparticles coated with poloxamer 407 were prepared using a similar procedure but replacing the oily phase by a 0.5% monomer solution in ethanol (without addition of cremophor and Miglyol). Nanoparticles prepared according to this process appear as plain polymeric spheres.

Prior to bioadhesion experiments, the nanospheres were washed twice by centrifugation at 55 000 × g for 1 h and resuspended successively in water and sterile glucose (5%). During this process nanospheres were concentrated by resuspension to smaller volumes. The respective concentration rates were 5- and 15-fold for nanocapsules and nanoparticles. Binding rate of the radiolabeled ZnPC-$^{125}I_4$ was determined at each step during purification by gamma scintigraphy (Gamma Master 1277, LKB Wallac, Turku, Fin-

TABLE 1

Encapsulation rate (%) of radiolabeled ZnPC-$^{125}I_4$ during washing by repeated centrifugation / redispersion, and size of nanoparticles (NP) and nanocapsules coated with different poloxamers and poloxamine

	First centrifugation		Second centrifugation	
	Encapsulation rate (%)	Size ± SD (nm)	Encapsulation rate (%)	Size ± SD SD (nm)
NP	26.61	101 ± 8	80.76	102 ± 9
407	78.29	197 ± 33	97.09	179 ± 19
238	82.10	179 ± 15	97.54	172 ± 18
403	55.10	187 ± 26	97.03	184 ± 21
908	77.88	185 ± 23	98.58	185 ± 23

land) of the supernatant and the sediment. Binding percentages, as calculated from the first supernatant and sediment, are summarized in Table 1. During the second purification process, no drug was released from nanospheres. The size of the nanospheres was determined by photon correlation spectroscopy before and after purification using an N4SD nanosizer (Coulter Electronics Ltd, Hialeah, FL, U.S.A.). The results listed in Table 1 show that the purification/concentration process did not modify the size of the nanospheres.

Bioadhesion

Bioadhesion of nanospheres in vitro was evaluated according to a published method (Pimienta et al., 1990). Our goal was to study the influence of poloxamer chain lengths on in vitro adhesion of nanospheres to mucus. We observed that upper intestine offers good reproducibility and is a suitable tissue for the purpose of our study. Segments of ileum (8 cm long) from male Sprague-Dawley rats (350–400 g) were removed under diethyl ether anaesthesia and washed by 3 min perfusion with 5% glucose at 37 °C. The segments were cut lengthwise, clipped on the upper part of an inclined (12 °) silanized glass tube (1.9 cm i.d., cut lengthwise), and perfusion was initiated 7.5 cm from the lower end. The ileum was stabilized for 2 min without perfusion. A sample of nanospheres (10 μl) was laid on the upper part of the ileal segment. After different time intervals following the transfer of nanospheres onto the ileal segment, nanospheres were eluted with 5% glucose at a flow rate of 0.5 ml/min using a peristaltic pump. The liquid was collected in fractions every 60 s. The whole system was placed in a controlled environment maintained at 37 °C throughout the experiment. Radioactivity of the collected samples was measured by gamma scintigraphy. After completion of the experiments, radioactivity remaining on the ileal segments was determined. In all experiments, the total radioactivity recovered in the eluates and the ileal segment was within 95–105% of the initial amount. All experiments were performed at least in triplicate using two ileal segments from each animal. Data were analyzed by Student's t-test for pairwise comparison. Multiple treatment comparisons were studied using both one-way analysis of variance (ANOVA) and Bonferroni's method. Bonferroni's method is a correction applied when several groups are compared to a control, in which t-distribution tables are used with a significance level of p/m, where m is the number of comparisons of interest (Wallenstein et al., 1980).

Results

The elution profiles of nanospheres with different poloxamer coatings were determined at intervals of 0 and 10 min between the transfer of nanospheres to the ileum and the onset of liquid flow.

With immediate perfusion, no difference was noted between the different profiles (Fig. 2). A fraction of approx. 55% of the nanospheres was eluted immediately, corresponding to non-adherent nanospheres (Table 2). The rest was eluted at a much slower rate and after 15 min only 75% of the nanospheres were washed out. This result indicates that a fraction of the nanospheres (approx. 45%) adhered to the mucus immediately and was released quite slowly under liquid flow.

In the case of a 10 min interval, the same type of behaviour was observed (Fig. 3): an initial release of free-flowing nanospheres followed by a much slower wash-out of adhering nanospheres. Significant differences were observed between the

TABLE 2

Cumulative % of radiolabelled nanospheres ± SD [(0 min interval) NP, nanoparticles; n, number of experiments performed]

	Minute																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
NP	55.07	60.11	63.46	66.03	67.74	68.94	69.31	70.21	71.99	72.94	73.61	74.34	75.06	75.95	76.28	76.39	76.42	76.51	77.31
$n = 4$	±6.0	±7.35	±7.69	±8.08	±8.78	±8.96	±9.26	±9.46	±9.17	±9.25	±9.15	±9.22	±9.30	±8.89	±8.47	±9.11	±9.13	±9.24	±9.02
407	44.49	50.59	55.12	58.37	61.07	63.15	64.72	65.64	66.67	67.70	68.36	69.02	69.63	70.08	70.45	70.75	70.92	71.04	71.81
$n = 8$	±6.70	±6.57	±7.11	±7.82	±8.56	±9.42	±10.23	±10.61	±11.18	±11.75	±11.93	±12.28	±12.65	±12.81	±13.05	±13.21	±13.27	±13.29	±13.58
238	57.53	62.28	65.14	67.33	68.82	69.87	70.66	71.37	71.92	72.39	72.81	73.13	73.51	73.78	74.06	74.25	74.48	74.72	74.85
$n = 4$	±6.63	±6.47	±6.02	±5.78	±5.35	±5.13	±4.83	±4.85	±4.75	±4.63	±4.48	±4.44	±4.27	±4.16	±4.07	±3.95	±3.79	±3.72	±3.68
403	53.86	58.33	61.20	63.03	64.31	65.32	66.27	66.94	67.44	67.68	67.98	68.13	68.38	68.67	68.78	68.98	69.10	69.15	69.26
$n = 5$	±10.11	±9.23	±9.02	±8.88	±8.74	±8.83	±8.45	±8.64	±8.60	±8.53	±8.65	±8.62	±8.57	±8.53	±8.57	±8.46	±8.44	±8.45	±8.57
908	55.59	60.31	63.60	66.03	67.58	68.77	69.79	70.68	71.51	72.30	72.80	73.29	73.94	74.54	74.78	75.16	75.46	75.62	76.06
$n = 5$	±10.98	±11.32	±11.11	±11.14	±11.11	±11.22	±11.33	±11.50	±11.25	±11.07	±10.91	±10.84	±10.50	±10.15	±10.13	±10.04	±10.12	±10.14	±9.94

TABLE 3

Cumulative % of radiolabelled nanospheres ±SD [(10 min interval) NP, nanoparticles; n, number of experiments performed]

	Minute																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
NP	42.74	45.75	48.74	51.74	53.72	55.25	56.64	57.30	58.74	60.07	60.85	61.37	62.37	63.47	63.64	63.80	64.08	64.20	65.76
$n = 5$	±10.88	±10.91	±10.91	±10.73	±10.04	±9.79	±9.58	±9.37	±9.25	±9.46	±9.30	±9.30	±9.37	±9.44	±9.58	±9.51	±9.30	±9.40	±8.74
407	37.75	40.62	42.53	43.80	44.85	45.80	46.40	46.91	47.15	47.47	47.72	48.05	48.15	48.78	48.89	50.34	50.48	50.67	51.03
$n = 5$	±7.33	±7.97	±7.97	±7.76	±7.59	±7.77	±7.61	±7.51	±7.39	±7.30	±7.24	±7.14	±7.10	±6.91	±6.89	±6.92	±6.95	±6.93	±6.82
238	28.25	31.40	33.76	35.54	37.03	38.51	39.68	40.61	41.26	41.95	42.57	43.16	43.63	44.30	44.64	45.01	45.34	45.61	45.87
$n = 5$	±0.79	±0.77	±0.94	±0.67	±0.56	±0.59	±0.68	±0.55	±0.48	±0.44	±0.53	±0.60	±0.34	±0.68	±0.69	±0.77	±0.73	±0.71	±0.72
403	43.12	46.75	49.28	50.95	52.42	53.68	54.85	55.84	56.34	57.14	57.55	58.19	58.73	59.08	59.30	59.62	59.93	60.21	60.33
$n = 5$	±10.52	±10.00	±9.75	±9.54	±9.39	±9.17	±9.13	±8.78	±8.86	±8.74	±8.61	±8.29	±8.30	±8.32	±8.26	±8.34	±8.42	±8.51	±8.53
908	47.52	50.20	52.23	54.10	55.32	56.31	57.79	58.19	59.12	59.89	60.36	60.87	61.36	61.81	62.11	64.03	64.30	64.47	65.04
$n = 5$	±13.45	±13.55	±13.55	±13.53	±13.88	±14.47	±14.03	±15.10	±15.17	±15.07	±14.98	±15.00	±15.06	±15.09	±14.94	±13.63	±13.58	±13.59	±13.51

TABLE 4

Cumulative % of nanocapsules ± SD coated with poloxamer 407 under liquid flow started at 0, 5 and 10 min following transfer of the nanocapsules (n, number of experiments performed)

	Minute																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0 min	44.49	50.59	55.12	58.37	61.07	63.15	64.72	65.64	66.67	67.70	68.36	69.02	69.63	70.08	70.45	70.75	70.92	71.04	71.81
$n = 8$	±6.70	±6.57	±7.11	±7.82	±8.56	±9.42	±10.23	±10.61	±11.18	±11.75	±11.93	±12.28	±12.65	±12.81	±13.05	±13.21	±13.27	±13.29	±13.58
5 min	30.49	34.79	37.72	40.17	43.22	45.02	46.68	47.86	49.68	50.96	51.78	52.53	53.21	54.00	54.48	54.88	55.03	55.10	55.74
$n = 5$	±4.15	±7.07	±9.70	±10.97	±11.79	±13.16	±19.76	±15.65	±17.28	±18.58	±19.28	±19.98	±20.67	±21.64	±22.14	±22.68	±22.75	±22.72	±23.37
10 min	37.75	40.62	42.53	43.80	44.85	45.80	46.40	46.91	47.15	47.47	47.72	48.05	48.15	48.78	48.89	50.34	50.48	50.66	51.03
$n = 5$	±7.33	±7.97	±7.97	±7.76	±7.59	±7.77	±7.61	±7.51	±7.39	±7.30	±7.24	±7.14	±7.10	±6.91	±6.89	±6.92	±6.95	±6.93	±6.82

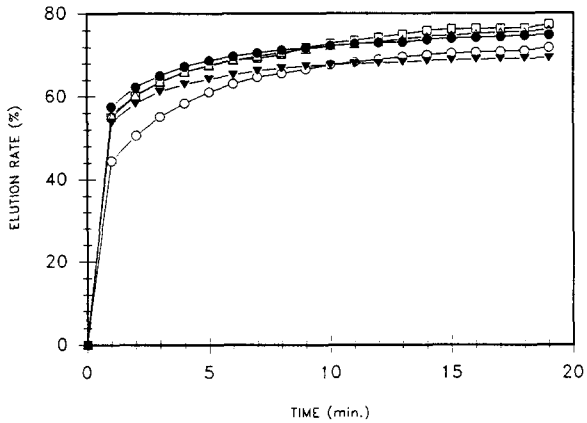


Fig. 2. Elution rate of nanocapsules and nanoparticules (NP) from rat ileal segment in vitro under liquid flow started 0 min following transfer of the nanospheres. (□) NP, (○) 407, (●) 238, (▼) 403, (△) 908.

various groups. Indeed, a greater fraction of nanocapsules coated with poloxamer 238 adhered to the mucus as compared to nanoparticles coated with poloxamer 407 and to nanocapsules coated with poloxamine 908 (Fig. 3 and Table 3). When nanoparticles and nanocapsules with the same

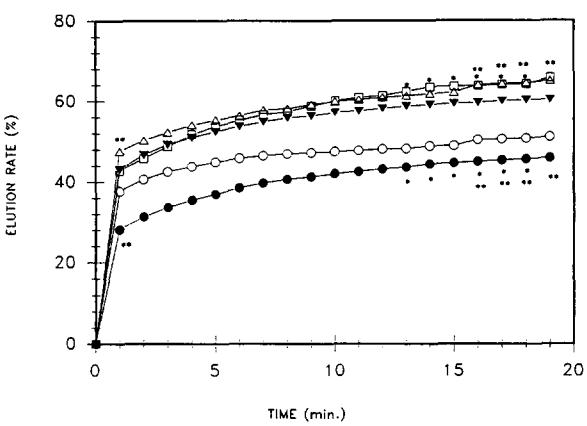


Fig. 3. Elution rate of nanocapsules and nanoparticles (NP) from rat ileal segment in vitro under liquid flow started 10 min following transfer of the nanospheres. (□) NP, (○) 407, (●) 238, (▼) 403, (△) 908. * Significant differences between elution profiles of nanocapsules coated with (●) 238 vs (□) NP. ** Significant differences between elution profiles of nanocapsules coated with (●) 238 vs (△) 908. Determined by Bonferroni's method ($p \leq 0.05$) applied to the analysis of variance.

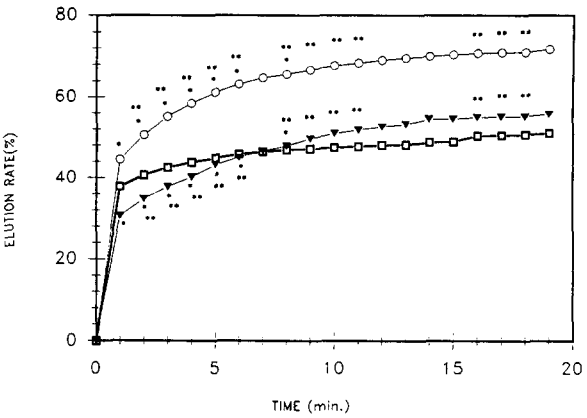


Fig. 4. Elution rate of nanocapsules coated with poloxamer 407 from rat ileal segment in vitro under liquid flow started at 0, 5, and 10 min following transfer of the nanocapsules. (○) 0 min, (▼) 5 min, (□) 10 min. * Significant differences between elution profiles of nanocapsules coated with poloxamer 407 after (○) 0 vs (▼) 5 min. ** Significant differences between elution profiles of nanocapsules coated with poloxamer 407 after (○) 0 vs (□) 10 min. Determined by Bonferroni's method ($p \leq 0.05$) applied to the analysis of variance.

coating (poloxamer 407) were compared, no difference was observed.

When the results between 0 and 10-min intervals were compared for each coating, significant differences (Student's t-test, $p \leq 0.05$) were noted for poloxamer 238 and poloxamer 407, a larger adhering fraction being noted for the longer interval. In the case of poloxamer 407, the same observation was also valid when 0 and 5-min intervals were compared (Fig. 4 and Table 4).

Discussion

In all experiments performed, it was observed that a fraction of the nanospheres was washed away immediately, whereas the rest was resistant to the liquid flow. Loosely bound material is released from the mucus during the 3 min washing preceding installation of the nanospheres. Although subsequent release is not excluded, this should not have a very significant effect on our profiles, since unbound nanospheres are released immediately whereas bound nanospheres are not washed away even after 20 min of elution. In view of this finding, one can assume that at certain

times a fraction of the nanospheres adhered to the ileum whereas the remainder was loosely bound. In an earlier study, we showed that increasing the liquid flow rate to the limit where laminar flow cannot be maintained did not significantly modify the elution profile of nanospheres (Pimienta et al., 1990). Therefore, it is not possible to compare the different formulations in terms of adhesive bond strength. A comparison based on the percentage of nanospheres adhering to the ileum at given times appears more reliable.

The adhesion of polymers has been explained as the consequence of diffusion of polymer chains across the interface above the glass transition temperature according to the model introduced by Voyutskii (1963). Later developments of this model have described the time dependency of the fracture energy (DeGennes, 1980; Prager et al., 1981). This analysis is based on a model in which polymer chains occupy a portion of space assimilated to a tube and diffuses out of this tube by reptation across one extremity.

In the case of poloxamers which are used for coating nanospheres, the POE side chain points freely into the solution whereas the central POP chain anchors the molecule to the surface of the nanospheres (Kayes et al., 1979; Klaus et al., 1990) (Fig. 5). Since POE chains are not rigid, contact with the surface of the mucus is possible without the need for the polymer to penetrate into the mucus. It is therefore likely that the immediate adhesion of the nanospheres to ileal mucus results from the development of interactions known collectively as 'secondary' forces (Van der Waals forces, hydrogen bonds, etc.), in agreement with the theory of Mikos and Peppas (1990).

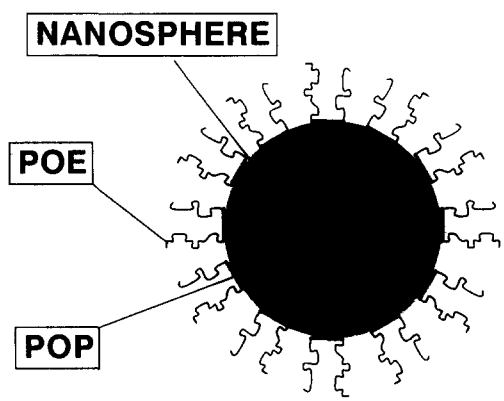


Fig. 5. Schematic representation of nanospheres coated by poloxamers, with the central POP attached to the nanosphere surface and the POE side chains pointing into the aqueous medium.

Recently, Mikos and Peppas (1988) described the molecular-weight dependency of polymer fracture properties. Their work had the merit to extend previous theoretical models to polymers in swollen gels and to differentiate between fracture by chain rupture and chain pull-out. In our case, it appears that poloxamers with long POE chains are responsible for the time-dependency of the percentage of nanospheres adhering to the mucus. In other words, when long POE chains are used, a certain fraction of initially loosely bound nanospheres develop bonds with the mucus over a 10 min period.

Such a time dependency is consistent with theories of polymer adhesion by diffusion. Furthermore, the molecular weight dependency is also consistent with the theory of Mikos and Peppas (1989) for polymer fracture by chain pull-out. It is therefore assumed that the POE chains of poloxamers are able to diffuse into mucus and to undergo molecular interactions resulting in adhesion to the nanospheres on which they are anchored by the POP fragment. For short POE chains, such diffusion does not significantly modify adhesion as evaluated through our experimental technique. In contrast, for longer chains, diffusion-dependent adhesion is demonstrated by this methodology.

A greater fraction of nanospheres adhered when coated by poloxamer 238 as compared to poloxamer 407. Since poloxamer 238 has a shorter central chain than poloxamer 407, it is assumed that a greater number of molecules are able to adsorb per unit surface area of nanospheres, thus explaining the greater degree of adhesion of nanospheres coated with poloxamer 238 as compared to 407. Poloxamine 908, although possessing long POE chains and leading to thick coatings (Klaus et al., 1990), has a different structural formula from those of poloxamers and its POE chains could have limited flexibility in solution and be subject to restricted diffusion into the

mucin network. This is somewhat different from the protective effect of poloxamers and poloxamine against phagocytosis by Kupffer cells in vitro. Indeed, in this case both poloxamer 407 and poloxamine 908 cause the greatest reduction in uptake by the liver after intravenous administration to rabbits (Illum et al., 1987a).

Nanoparticles and nanocapsules with identical coatings followed the same elution kinetics. The surface charge, densities and size of these nanospheres exhibited differences which might have resulted in different elution kinetics, thereby being factors having a significant influence on the adhesion of the nanospheres to mucosa. This observation is in agreement with the prominent role of poloxamer coatings in nanosphere adhesion assumed above.

Conclusion

Nanospheres have been tested as drug carriers for peroral delivery of poorly absorbed drug, including insulin (Damgé et al., 1988). Other authors (Illum et al., 1987b) have suggested their use for the nasal delivery of drugs. In all these cases, good adhesion to mucus is desirable. Our results show that coating nanospheres with poloxamers possessing a short central POP chain and long POE side chains allows greater mucoadhesion to be achieved. Immediate adhesion is observed probably because of the development of secondary chemical interactions between mucus and poloxamer, followed by a time-dependent increase in the number of adhering nanocapsules as a result of the diffusion of poloxamer POE chains into the mucin network. However, mucoadhesion resulting from poloxamer coating depends upon the type of nanospheres used. Therefore, the results of this study cannot be used to predict the mucoadhesive behaviour of other nanospheres coated with the same compounds. Other physico-chemical factors (charge, size, etc.) must be taken into account as well as the extent and stability of poloxamer-nanosphere binding.

Acknowledgments

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