ELSEVIER

International Journal of Pharmaceutics 127 (1996) 135–145

international journal of pharmaceutics

Interactions between bioadhesive poly(acrylic acid) and calcium ions

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Received 13 March 1995; accepted 2 May 1995

Abstract

Polycarbophil (PC) is a weakly crosslinked poly(acrylic acid) used for bioadhesive delivery systems. Complexation of calcium ions by this polymer depends on the accessability of carboxylate functions in the polymer. Therefore, the calcium binding capacity increased linearly with the degree of neutralisation. Crosslinking rendered 20% of the carboxyl inaccessable to calcium whereas in water-soluble non-crosslinked poly(acrylic acid) (PAA), all carboxylate groups bound calcium. Addition of ionic substances reduced the fraction of calcium ions bound due to a dehydration of the polymer and a competition between the ions for binding. In a physiological buffer, PC chelated maximally 80% of the total calcium concentration, and water-soluble PAA bound 95%. In comparison to calcium chloride solution, the binding constant decreased from 51400 l/mol to 1800 l/mol in Tyrode's solution, moreover, the number of binding sites in the polymer was reduced.

A dispersion of PC in water yielded swollen particles with a size of ca. 2.5 μm, depending on the degree of neutralisation and on the concentration of electrolytes, especially calcium ions. Addition of electrolytes caused a decrease of the particle size due to dehydration of the polymer. The reduction was more pronounced with calcium.

Chelation of calcium by PC could be an explanation for the increase in bioavailability of drugs observed with bioadhesive PC delivery systems. The function and structure of epithelial tissue could be affected by chelation of extracellular calcium.

Keywords: Poly(acrylic acid); Calcium chelation; Bioadhesion; Microspheres

1. Introduction

Bioadhesive drug delivery systems, which adhere to the mucus layer of epithelial surfaces, have attracted some interest during recent years because they may provide a solution for bioavailability problems (Jimenez-Castellanos et al., 1993). Poly(acrylic acid) (PAA) has been shown to facilitate the absorption of drugs from mucosal tissues: PAA increased the absorbed fraction of peptide drugs (Morimoto et al., 1984; Morimoto et al., 1985; Ryden and Edman, 1992), prolonged the transit time of the drug delivery system through the gastro-intestinal tract (Harris et al., 1989) and possibly affected the metabolism

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SSDI 0378-5173(95)04098-U

of drugs in the gut lumen (Lehr et al., 1992). Polycarbophil (PC), a weakly crosslinked PAA, has especially shown excellent bioadhesive properties (Ch'ng et al., 1985; Ranga Rao and Buri, 1989). Due to crosslinking, the polymer is insoluble in water, but swells rapidly, yielding turbid dispersions with a viscosity slightly higher than water (Carbopol Resins Handbook, 1991). On neutralisation, the dispersion becomes clear and the viscosity increases dramatically forming a hydrogel. This gel network is destroyed by ions affecting the hydration of the carboxylate groups, thereby generating turbid dispersions of low viscosity (Ünlü et al., 1992; Lin et al., 1993). Divalent cations, e.g. calcium ions in physiological fluids, are very effective in destroying the hydrogel network (Carbopol Resins Handbook, 1991). They serve as a cross-linker reacting simultaneously with two carboxylic groups of the same or different PAA-chains.

However, the influence of PC on the concentration of calcium ions has also to be taken into account: the polymer may bind calcium and acts as weak ion exchanger (Charman et al., 1991). Carboxylate functions of PAA interact with calcium ions in an equilibrium reaction:

$$P + Ca^{2+} \rightleftharpoons PCa^{2+}$$

Neglecting the difference between concentration and activity, the binding constant is defined as follows:

$$(1)\quad K = \frac{c(PCa^{2+})}{c(P) \times c(Ca_f^{2+})}$$

$c(P)$ is the concentration of free polymeric groups, $c(Ca_f^{2+})$ the free calcium concentration and $c(PCa^{2+})$ the concentration of the chelate. The concentration of free polymeric groups is calculated from the total polymer concentration $c(P_0)$, multiplied by n, the number of active binding sites per polymer unit, reduced by the concentration of the chelate:

$$(2)\quad c(P) = n \times c(P_0) - c(PCa^{2+})$$

The concentration of the chelate $c(PCa^{2+})$ is equal to the concentration of bound calcium $c(Ca_b^{2+})$. After rearrangegement, one obtains:

$$(3)\quad c(Ca_f^{2+}) + \frac{1}{K} = \frac{c(Ca_f^{2+})}{c(Ca_b^{2+})} \times n \times c(P_0)$$

The scatchard equation, a special function which can also be derived from equation (3), gives the binding constant and the number of binding sites:

$$(4)\quad \frac{r}{c(Ca_f^{2+})} = n \times K - K \times r \quad (r = c(Ca_b^{2+})/c(P_0))$$

Equation (4) can be plotted as the ratio $c(Ca_f^{2+})$ versus r. The function yields a straight line if the binding sites have the same structure and affinity. The binding constant K is derived from the slope while the number of binding sites n is obtained from the intercept.

Chelation of calcium and other metal ions by PAA could be the explanation for several biological effects, e.g. the higher rate of absorption of drugs and the inhibition of enzymes. Depletion of extracellular calcium may affect the integrity of epithelial cells, causing higher permeability (Gumbiner, 1987). Many enzymes require metal ions as cofactors or as central ions. The chelation of these ions by PAA could also lead to enzyme inhibition. It is still unknown if the presence of calcium ions affect the adhesion of bioadhesive drug delivery systems (Lejoyeux et al., 1989; Smart, 1992). Calcium depletion in the mucus gel PAA could also influence the barrier function (Forstner and Forstner, 1975).

To correlate these biological effects with the chelation behaviour of PC, it is necessary to determine the interactions between the polymer and calcium ions, especially the chelation under physiological conditions.

2. Materials and methods

2.1. Materials

Polycarbophil AA-1 was a gift from BF Goodrich GmbH (D-Neuss). Water-soluble PAA was produced by solution polymerization of acrylic acid in water (Markert, 1987). Lanthan diluent was purchased from Dr. W. Ingold AG (CH-Urdorf). All other materials of analytical

grade were purchased from Merck (D-Darmstadt).

Tyrode's solution consisted of (in g/l): 8.00 NaCl, 0.20 KCl, 0.20 $CaCl_2$, 0.10 $MgCl_2$, 0.065 $Na_2HPO_4^*2H_2O$, 1.00 $NaHCO_3$, 1.00 glucose.

2.2. Determination of carboxylic acid groups

PC or PAA (400 mg) was dispersed in 400 ml distilled water and titrated with 0.2 M sodium hydroxide solution. The end-point of the neutralization was determined by potentiometric measurement.

2.3. Calcium chelation by PC

2.3.1. Dependence on the degree of neutralisation

Calcium chloride (200 mg/l ≅ 1.80 mM) and PC (270 mg/l ≅ 3.60 mM carboxylic groups) were dissolved either in distilled water or in isotonic saline (9000 mg/l sodium chloride) and the carboxylic acid groups of the polymer were neutralised by sodium hydroxide solution in the range from 0 to 100% in steps of 10%. The molar ratio of polymer groups (carboxylic acid and neutralized carboxylate groups) and calcium ions was 2 to 1.

The turbid dispersions were centrifuged (3000 rpm; 30 min) and 200 μl of the clear supernatant were diluted with 5000 μl lanthanum diluent to suppress other ions. The calcium concentraction was determined by atomic absorption spectroscopy (AA Spectrophotometer IL 551, Instrumentation Laboratory, D-Kirchheim). Each sample was measured tenfold and the concentration of the free ion was determined using a calibration curve of five standard solutions.

2.3.2. Dependence on the ionic strength concentration

To dispersions of PC (135, 270 or 540 mg/l) in calcium chloride solution (200 mg/l ≅ 1.80 mM calcium), sodium chloride was added in the range from 0 to 9000 mg/l in steps of 1800 mg/l. The free calcium concentration was determined as described above.

2.3.3. Dependence on the polymer concentration

Neutralised PC was added to a solution of calcium chloride (200 mg/l) and magnesium chloride (100 mg/l $MgCl_2$ = 214 mg/l $MgCl_2^*6H_2O$) in water, isotonic saline or the physiological Tyrode's solution. In the last case, the pH value was corrected to 7.4 by hydrochloric acid. The free calcium concentration was determined as described above.

2.4. Calcium chelation by water-soluble PAA

PAA, produced by solution polymerisation, was dissolved either in calcium chloride solution (200 mg/l) or in Tyrode's solution. In the first case, the polymer was neutralised by sodium hydroxide; in the other case, the pH value was corrected to 7.4.

Twenty millilitres of the solution were dialyzed against 2.0 ml distilled water (calcium chloride solution) or saline (Tyrode's solution) in a dialyis tube Spectrapor with a molecular weight cut-off of 20 000 Dalton (Roth, D-Karlsruhe). After 24 h, while stirring the dispersion, 200 μl of the solution in the tube was removed, diluted with 5000 μl lanthanum diluent and the calcium concentration was determined (see above).

2.5. Particle size

PC dispersed in water (concentration 270 mg/l, see above for procedure) and neutralised to a degree of 0, 50 or 100% and sodium chloride (9000 mg/l), calcium chloride (200 mg/l), or a combination of both were added. The particle size of the different dispersions was determined by laser light diffraction (Mastersizer X, Malvern). The presentation 2GDD of the computer programme Mastersizer X release 1.1 was used to calculate the mean of the volume distribution. This presentation has a diffractive index of the dispersed particles of 1.35 which takes the swelling of the polymer into account.

2.6. Zeta potential

The electrophoretic mobility of the PC dispersions in calcium chloride solution (200 mg/l) was

measured by laser doppler anemometry (Zetasizer 4, Malvern). Each sample was measured fivefold for 30 s at 293 K, the applied voltage was 150 V. The computer programme Malvern Zeta Mode release 31 calculated the zeta potential.

3. Results and discussion

3.1. Calcium chelation

Fig. 1 shows the calcium chelation of PC depending on the degree of neutralisation in water or in isotonic solution of sodium chloride. In these solutions, the molar concentration of carboxylic acid groups, determined by potentiometry, was twice the concentration of calcium. This ratio of 2 to 1 was chosen assuming that two carboxylic groups bind one calcium ion. When only calcium ions are present, the amount of free calcium decreased linearly with the degree of neutralisation of PC (linear regression: $f(x) = 98.63 - 79.86 \times x$; $r = 0.998$).

This function represents the fraction of free calcium in the solution depending on the degree of neutralisation. Un-neutralized PAA bound almost no calcium ions. However, at full neutralisation of PC only 80% of the original calcium was absorbed and the ratio of neutralised carboxylic groups and bound calcium ions was about 2.5 to 1. This means that 2.5 carboxylate groups of the polymer bind one calcium ion, instead of 2.0 as expected according to the charges of the substances, or that 20 percent of the neutralised groups remained free and did not bind calcium.

At a low pH, the number of dissociated acid groups in the polymer is small, as expected from theoretical considerations. Charman et al., 1991 have reported a similar result with the polymer carbopol, which is also a slightly cross-linked PAA. At pH 3.0, carbopol did not chelate calcium ions, however, at pH 9.0 the polymer was an effective chelator.

When sodium chloride is added to a solution of calcium chloride a the slope of the curve was totally different (Fig. 1) and the rate of chelation was reduced. Up to 40% neutralisation of the polymer, almost no calcium was withdrawn from the solution; at higher degrees of neutralisation, calcium was absorbed by the polymer, but the amount was always smaller than in calcium chloride solution. At full neutralisation of PC, the concentration of calcium bound only 40% of the starting value in contrast to 80%, when no sodium chloride was present.

Fig. 2 shows the effects of polymer and sodium chloride concentration on the fraction of free calcium. In this and the following cases, PC was fully neutralised. Sodium ions displaced calcium from the polymer at all polymer concentrations.

Fig. 3 demonstrates the effects of ionic strength and of polymer concentration, and therefore of the molar ratio of carboxylic groups to calcium ions, on the chelation of calcium. Clearly the chelation depended on the concentration of electrolytes. The higher the ionic strength, the smaller the percentage of calcium that was chelated by PC, and similar results were found for magnesium ions (data not shown). From a ratio of 0 to 3, a linear relationship between the bound ions and polymer concentration was found, the functions of the linear regression are shown in Table 1. Both calcium and magnesium ions were chelated. In each of the three media, the extent of calcium chelation was higher than that of magnesium.

The addition of sodium chloride and other ions to dispersions of PC has two effects, which both reduce the amount of bound calcium: on one hand it leads to a polymer dehydration, on the other hand monovalent cations like sodium ions are capable of binding to carboxylate groups and displacing calcium ions, since the other electrolytes compete with calcium. Although PAA has a high affinity for divalent cations (the effect of chelation), but only moderate affinity for monovalent ions, the calcium ions are preferentially removed until competition arises from alkaline ions.

Non-crosslinked PAA is less sensitive to electrolytes. The hydrogels did not collapse to a turbid dispersion after addition of ions. Therefore, the amount of free calcium ions was determined by equilibrium dialysis. In calcium chloride solution, water-soluble PAA shows similar binding properties for calcium ions as PC (Fig. 4). At low polymer concentrations, a molar interaction of 2 carboxylate groups with one bound calcium ion

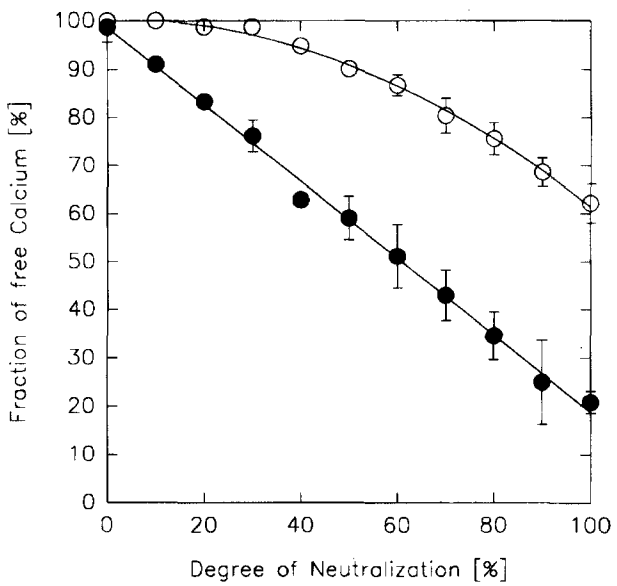

Fig. 1. Influence of neutralization degree on binding of calcium ions by polycarbophil. 200mg/l $CaCl_2$ in (●) distilled water or (○) saline. Fraction of free calcium is expressed as percentage of the the original concentration. Mean ± S.D. (n = 3).

was detectable. This ratio of 2 to 1 indicates that every neutralised acid group took part in the chelation, two carboxylate groups bound one cation. In contrast, PC showed a ratio of 2.5 to 1, which indicates that some of the carboxylate groups of PC do not participate in the chelation. This phenomenon might be caused by steric hindrance of the interactions between the crosslinked polymer chains and the hydrated calcium ion. The large ions require enough space to penetrate the crosslinked polymer chains and react with the carboxylate groups.

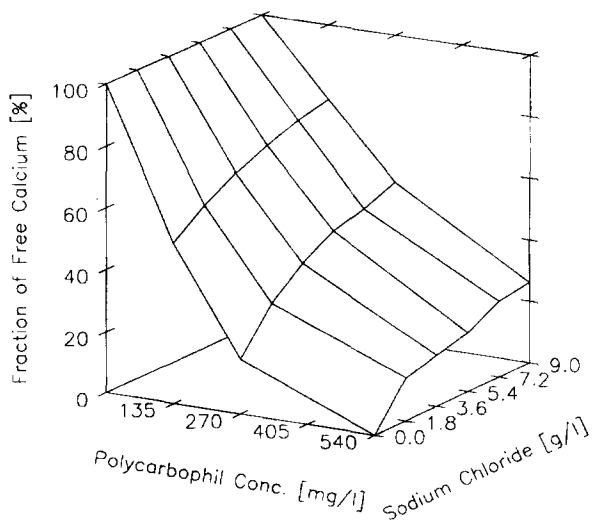

Fig. 2. Influence of sodium chloride concentration on binding of calcium ions by polycarbophil in calcium chloride solution (200 mg/l). Mean ± S.D. (n = 3).

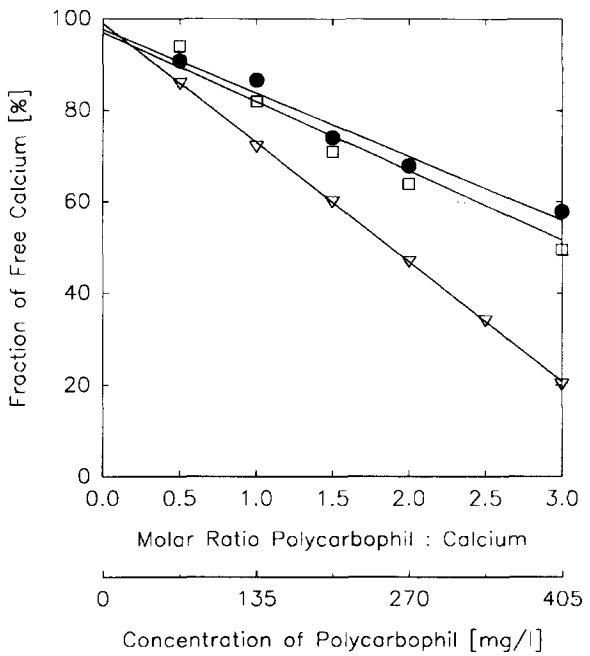

Fig. 3. Influence of polymer: calcium ratio on binding of calcium ions by polycarbophil. 200 mg/l $CaCl_2$ and 100 mg/l $MgCl_2$ in (▽) distilled water, (□) saline or (●) Tyrode's solution. Mean ± S.D. (n = 3).

On neutralisation, the polymer/calcium ratio increased to 3 or more and the solution contained only calcium as cations. Both PC and water-soluble PAA bound the whole amount of calcium, so free calcium could not be detected. A similar result was again found by Charman et al., 1991, the calcium content was below the sensitivity of the ionselective electrode.

In Tyrode's solution, we have to consider the additional effect of pH (7.4) in which both PC and water-soluble PAA were not fully neutralised. In this solution, it was not possible to chelate all the calcium and magnesium ions, although the polymer concentration increased: the fraction of unbound calcium decreased and approached a plateau at higher concentrations of PC. At molar ratios above 10, the fraction of free cations present in the dispersion was approximately 20% of the initial value (Fig. 5). For water-soluble PAA, the slope of the curve of free calcium was comparable to the curve of PC dispersion; however, water-soluble PAA was more efficient in binding calcium and absorbed 95% at most.

At low polymer concentrations a linear relationship between the polymer content and the fraction of bound calcium is observed, whereas at high polymer concentrations the fraction of free calcium remains constant.

This observation can be explained according to equation (3):

Table 1
Linear regression of calcium and magnesium binding by polycarbophil in dependence on the molar ratio polycarbophil: calcium 200 mg/l $CaCl_2$ and 100 mg/l $MgCl_2$ in distilled water saline or Tyrode's solution (see Figs. 3 and 4)

Solution	Calcium	r	Magnesium	r
$CaCl_2$ + $MgCl_2$	98.94% - 26.03%*x	0.999	100.70% - 11.69%*x	0.997
$CaCl_2$ + $MgCl_2$ + NaCl	96.96% - 15.10%*x	0.985	102.99% - 7.47%*x	0.994
$CaCl_2$ + $MgCl_2$ in Tyrodelsg.	97.63% - 13.89%*x	0.993	97.83% - 5.70%*x	0.998

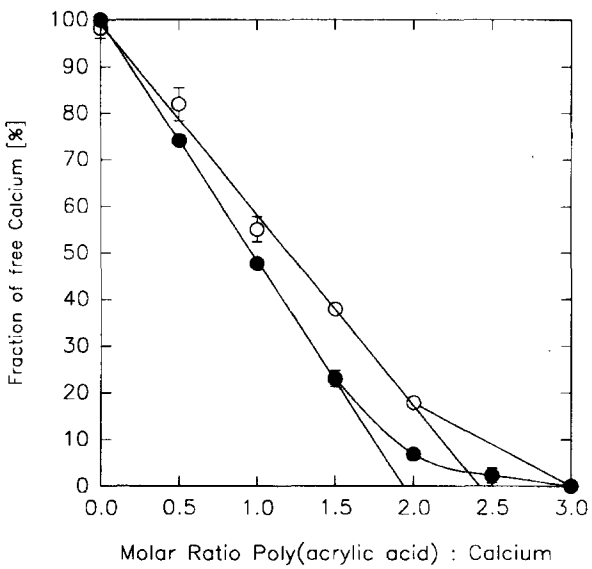

Fig. 4. Influence of polymer: calcium ratio on binding of calcium ions by (○) polycarbophil or by (●) water-soluble poly(acrylic acid) in calcium chloride solution (200 mg/l). Mean ± S.D. (n = 3). Linear regression: polycarbophil f(x) = 99,17-40,95*x, r = 0.997; water-soluble poly(acrylic acid) f(x) = 99,82-51,50*x, r = 0.999.

$$(3)\ c(Ca_f^{2+}) + \frac{1}{K} = \frac{c(Ca_f^{2+})}{c(Ca_b^{2+})} \times n \times c(P_0)$$

For low polymer concentrations, the free calcium concentration is clearly higher than 1/K. In this case, the ratio of bound calcium to the polymer is constant and equal to number of binding sites:

$$(5)\ c(Ca_f^{2+}) = \frac{c(Ca_f^{2+})}{c(Ca_b^{2+})} \times n \times c(P_0)$$

$$\Leftrightarrow \quad \frac{c(Ca_b^{2+})}{c(P0)} = n$$

At high polymer concentrations calcium, is almost completely bound to PAA and the free calcium concentration is lower than 1/K:

$$(6)\ \frac{1}{K} = \frac{c(Ca_f^{2+})}{c(Ca_b^{2+})} \times n \times c(P_0)$$

$$\Leftrightarrow \quad \frac{c(Ca_b^{2+})}{c(Ca_f^{2+})} = n \times c(P_0) \times K$$

In that case, the ratio between bound and free calcium is constant and independent on the polymer concentration. If the polymer concentration rises, the number of binding sites decreases. The ratio between calcium and other electrolytes, which compete for binding, becomes unfavourable, so that these ions will be bound by the polymer and no further calcium ions.

Fig. 6 presents the scatchard plot of water-soluble PAA in Tyrode's solution. The binding constant derived from the linear regression is 1810 l/mol, the dissociation constant is the reciprocal value of the binding constant: 5.52×10^{-4} mol/l.

Table 3 shows the constants and the proportion of active binding sites in calcium chloride or Tyrode's solution. In calcium chloride solution, the binding constant is much higher than in a solution with physiological concentrations of electrolytes; moreover, the number of active sites is enlarged: 0.526 calcium ions are bound by every carboxylate group of the polymer compared to 0.304 in Tyrode's solution. In both cases, the maximum chelation capacity of the polymer can be calculated from these values when they are multiplied with the molecular weights of the monomeric unit and calcium; the capacity is 0.321 g calcium/1 g PAA in calcium chloride solution and 0.185 g calcium/1 g PAA in Tyrode's solution.

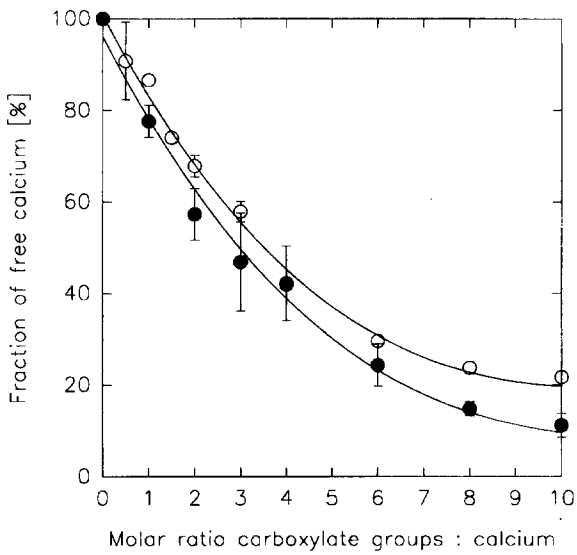

Fig. 5. Influence of polymer: calcium ratio on binding of calcium ions by (○) polycarbophil or by (●) water-soluble poly(acrylic acid) in Tyrode's solution. Mean ± S.D. (n = 6-9).

If the polymer is cross-linked, like PC, the affinity is almost unchanged, but the number of binding sites is reduced to 0.195 per unit.

3.2. Particle size

Dispersions of PC clearly showed a particulate structure, the mean of the volume distribution was about 2.5 μm (Table 2). In distilled water the particle size of PC increased, when the polymer was neutralised. The addition of sodium chloride reduced the size of the polymer particles compared to the dispersion in distilled water.

If calcium chloride was dissolved in the saline solution, differences could be seen depending on the degree of neutralisation: at 0% there was no effect of calcium on the dimension of the swollen particles, in saline 1.848 μm, in saline plus calcium 1.844 μm; at 50% a slight reduction and at 100% a significant reduction of the particle size could be observed.

Addition of calcium chloride reduced the particle size of the dispersion effectively, but this effect was only obvious when the carboxylic groups were neutralised, e.g. at 50% neutralisation, the particle size was 0.717 μm.

If the polymer particles in the dispersion are neutralised, their size increases. The ionised monomer subunits repel each other, which lead to an increase in the hydration of the polymer and in the particle size. Sodium chloride causes a dehydration of the polymer and, if the polymer is neutralised, a compensation of the negative charges. Hence, the particle size declines at all degrees of neutralisation in comparison to the dispersions in distilled water. This result is in agreement with the observations of Park and Robinson, 1985 who reported that the equilibrium swelling of PC increases with the pH, a sharp rise occurs at pH 5-6 where the acid groups were neutralized. When they added sodium chloride, the swelling was significantly reduced. Chitnis et al., 1991 described that the swelling of cross-linked PAA decreased by sodium chloride.

If sodium and calcium ions are added to the dispersions, the calcium ions have no effect without neutralisation. This fact correlates with the chelation behaviour of PAA: no calcium is bound and no interaction between the polymer and the calcium is visible, whereas at 50 or 100% of neutralisation calcium ions are chelated and lessen the particle size. Consequently, the addition of

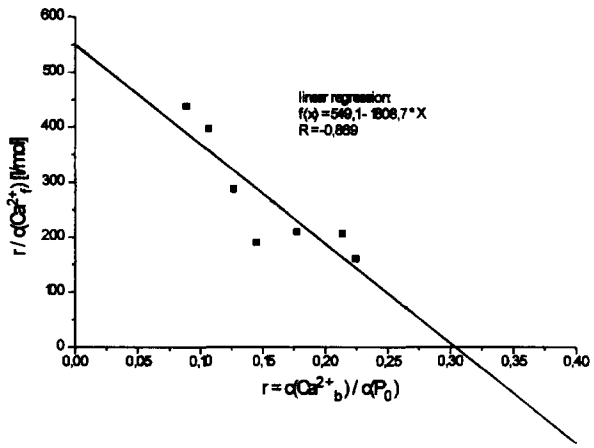

Fig. 6. Scatchard plot: binding of calcium by water-soluble poly(acrylic acid) in Tyrode's solution.

calcium chloride without sodium chloride more clearly shows this influence on the particle size of the PC dispersion, that is dependant on the pH - or rather the degree of neutralisation - and related to an ionic interaction with the ionised acid groups. The polymer is able to bind calcium ions by its carboxylate groups. The binding interaction of calcium with ionised functional groups within the polymer reduces the repulsive effect that the neutralization has upon the neighbouring groups. The calcium ions serve as cross-linkers between the polymer chains. In accordance with this mechanism, the swelling of the polymer is more distinctly reduced by calcium chloride compared to sodium chloride (Leung and Robinson, 1990; Chitnis et al., 1991).

3.3. Zeta potential

The zeta potential of PC particles in distilled water was − 32 mV, the addition of calcium chloride increased the potential to − 10 mV, but it decreased with the degree of neutralisation (Fig. 7).

The addition of calcium chloride reduces the negative zeta potential of PC dispersions due to the compensation of negative charges of the polymer by calcium ions. However, when the polymer

Table 2
Particle size [μm] of polycarbophil dispersion: Effect of degree of neutralisation and of added electrolytes. Mean ± S.D. (n = 6)

Neutralisation degree	Distilled water	NaCl (9000 mg/l)	NaCl + $CaCl_2$	$CaCl_2$ (200 mg/l)
0%	2.491 ± 0.168	1.848 ± 0.052	1.844 ± 0.082	1.995 ± 0.061
50%	3.415 ± 0.557	2.727 ± 0.312	2.417 ± 0.152	0.717 ± 0.069
100%	3.910 ± 0.604	2.873 ± 0.349	2.185 ± 0.156	0.852 ± 0.204

Table 3
Constants and number of binding sites of calcium ions to poly(acrylic acid) in dependence on the polymer and the medium

	Binding constant K [l/mol]	Number of binding sites per monomer unit n	Dissociation constant 1/K[mol/l]
PAA in $CaCl_2$ solution	51450	0.526	1.944×10^{-5}
PAA in Tyrode's solution	1810	0.304	5.525×10^{-4}
Polycarbophil in Tyrode's solution	2090	0.195	4.785×10^{-4}

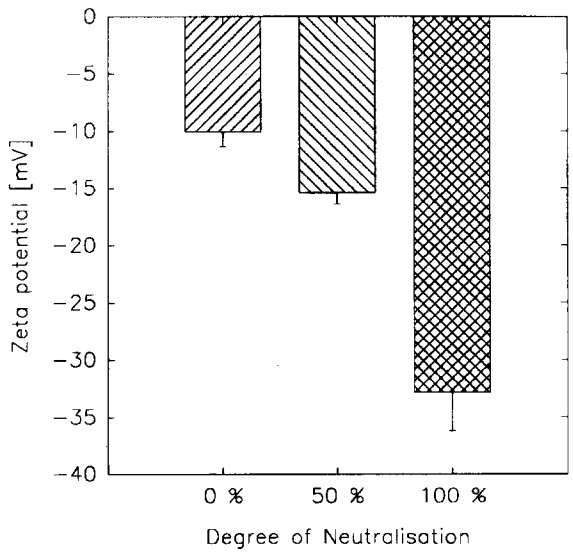

Fig. 7. Influence of neutralisation degree on zeta potential of polycarbophil dispersion in $CaCl_2$ solution (200 mg/l). Mean ± S.D. (n = 6).

is fully neutralised, the number of negative charges on the surface of the particles is high. The surface density of free carboxylate groups grows with the degree of neutralisation, leading to a more negative zeta potential, although the polymer binds more calcium ions.

The addition of sodium ions to dispersions of PC has two effects: on the one hand, it reduces the particle size through an polymer dehydration mechanism; on the other hand, sodium is capable of binding to carboxylate groups and displacing calcium ions. Both reduce the amount of bound calcium. Other electrolytes compete with calcium, e.g. magnesium or, to a lower extent, sodium.

PC is capable of binding calcium and magnesium and removes these ions from a physiological solution. It has clearly been shown by several investigators (Artursson and Magnusson, 1990; Bhat et al., 1993; Noach et al., 1993) that the function and structure of epithelial monolayers are disrupted by the chelation of extracellular calcium. The withdrawal of calcium may be the explanation for the increased junction permeability.

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