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Research paper

The formation and permeability of drugs across free pectin and chitosan films prepared by a spraying method

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Abstract

This study has investigated the permeation of drugs through free films made of pectin and chitosan. The background for this study is the intended use of the films as coating material in a colon-specific drug delivery device. The factors that varied when making the films were the pectin source and grade of the pectin, degree of deacetylation of the chitosan and ratio between pectin and chitosan. The permeability of the model drug in 0.1 M HCl was low with an average drug release of 1.3×10^{-3}%/cm. The films containing high content of chitosan showed exponential kinetics while the films containing high content of pectin showed 0-order kinetics. The release of drug in phosphate buffer pH 6.8 showed 0-order kinetics. The lowest permeability was obtained for a film consisting of a high content of pectin to chitosan, chitosan with a high degree of deacetylation and non-amidated low methoxylated citrus pectin. The permeation of paracetamol for this combination was 9.4×10^{3}%/cm. This film combination had a combined diffusion of only 0.046%/cm after 1 h in 0.1 M HCl and 4 h in phosphate buffer pH 6.8.

Keywords: Permeability; Pectin; Chitosan; Free films; Multivariate analysis; Colon delivery

1. Introduction

Targeting drugs to the colon via oral administration has attracted much attention, and different approaches for colon-specific drug delivery have been reported in the literature [1]. One of the possibilities is using polymers degradable by the enzymes primarily present in the colon [2–4]. Pectin and chitosan are biopolymers that may be suitable for a colon drug delivery device utilising this principle. The potential of pectin as a carrier for colon drug delivery has been previously demonstrated [5,6]. Pectin is a non-toxic water-soluble gel-forming polysaccharide containing carboxylic acid groups. It is extracted from different sources, e.g. apple or citrus [7]. Chitosan has also been reported to be valuable in colon drug delivery, for example, as enteric-coated chitosan capsules to enhance uptake of insulin [8].

Chitosan is a non-toxic polysaccharide soluble in acidic media containing amino groups. It is extracted from crabs, among other sources. A mixture of the two polymers has been studied as press coating on tablets [9], as hydrogel beads [10] and as film-coating excipients in combination with HPMC [11]. A mixture of pectin and chitosan will form a polyelectrolyte complex (PEC) at pH values in the range of 3–6, hence, this combination is expected to yield a less soluble, more pH-resistant barrier than each of the two polymers alone [12,13]. This is expected to reduce the release of drug in the upper GI tract.

Although, films of pectin and chitosan have been reported, to our knowledge, no study has systematically investigated the influence on the complex by indigenous properties of the polymers like source along with degree and type of substitution.

To uncover parts of this area, the aim of this study was to evaluate the effect of some formulation factors on the permeability of free pectin/chitosan combination films prepared by a spray method.

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2. Materials and methods

2.1. Materials

Four types of pectin were used: amidated low methoxylated citrus pectin, pectin type 920 batch no. 6753/01 from Citrus Colloid (UK), amidated low methoxylated apple pectin, batch no. 0903188, non-amidated low methoxylated apple pectin, batch no. 0903187 and non-amidated low methoxylated citrus pectin batch no. 0903185 all from Herbstreith and Fox (Germany) (see Table 1 for characteristics of the pectins). The raw materials were used for the formation of films while purified pectin was used for the intrinsic viscosity measurements.

Two types of chitosan were used, Seacure CL214 batch no. 607-783-08 (degree of *N*-deacetylation, DdA 89%) and Seacure CL211 batch no. 707-771-10 (DdA 60%) Pronova Biopolymer (Norway). The molecular weight of the chitosans were 205 000 ± 15 000 (information provided by the manufacturer).

Glycerol NMD (Norway) was used as plasticizer. The model substances used for the permeability tests were erytrocin Merck (Germany), anionic in phosphate buffer pH 6.8 and paracetamol NMD (Norway) neutral in 0.1 M HCl and phosphate buffer pH 6.8.

2.2. Experimental design

The effects of four different formulation factors on the permeability of the pectin and chitosan films were investigated in a full factorial 2^4 design. The variables and their levels are presented in Table 2. The mixture ratio between pectin and chitosan is defined as $r = m_{pectin}/(m_{pectin} + m_{chitosan})$. Three center points were included in the study. These films were made from amidated low methoxylated citrus pectin in combination with chitosan (DdA 89%) at $r = 0.5$.

2.3. Intrinsic viscosity and estimation of the molecular weight of the pectins

Intrinsic viscosity measurements of the different pectin types were performed with a Micro-Ostwald capillary viscometer at 25 ± 0.1°C. The solvent was 1% sodium hexametafosfate ($(NaPO_3)_6$) pH 4.5. To remove impurities, the pectins were centrifuged at 3800 rpm for 12 h and dialyzed against water for 7 days and freeze-dried prior to use. The molecular weights of the pectin types were calculated from the intrinsic viscosity η by the use of the Mark–Houwinks relationship, $[\eta] = KM^a$. For this particular system, Christensen has calculated $K = 4.7 \times 10^{-5}$ (100 ml/g) and $a = 1$ [14]. Anger and Berth have for a similar system calculated $K = 9.55 \times 10^{-2}$ (ml/g) and $a = 0.73$ [15]. For the amidated and non-amidated citrus pectins, the intrinsic viscosity and Huggins constant in water were also determined. Huggins constant k' is determined from the equation $\eta_{sp}/C = [\eta] + k'[\eta]^2 C$ where η_{sp}/C is the reduced viscosity, η_{red}.

2.4. Preparation of polymer solutions

Separate pectin and chitosan solutions (100 g) were prepared. The appropriate type and amount of polymer and 20% plasticizer was dissolved in distilled water. The solutions were stirred for 2 h. The sum of pectin and chitosan was kept constant at 4 g.

2.5. Preparation of free films

The films were prepared according to a modified version of the method described by Arwidsson [16], employing two parallel nozzles for simultaneous spraying of the two polymer solutions onto the rotating cylinder. Atomizing air pressure was 3 bar, flow rate of the solutions was 3.0 ml/min and spraying distance was 20 cm. The films were continuously dried at 60°C during spraying and for an additional 30 min at 60°C after spraying. The films were kept in a container at room temperature with a relative humidity of 22.5% for at least 1 week prior to the permeability studies.

2.6. Permeability studies

A test unit was prepared by dissolving the model substance (40 and 15 mg in HCl and buffer, respectively) in 8 ml of the appropriate test medium in a glass vial. Film thickness was measured at 3–6 different places with a micrometer. Film samples measuring 1.22 cm^2 were cut and put on top of the vial. The test unit was sealed with a rubber ring and an aluminium capsule [17]. The test unit was

Table 1
The molecular weight of the different pectin types derived from capillary viscometry measurements

Pectin type	DM[a]	DA[a]	Huggins constant k' in $(NaPO_3)_6$	Huggins constant k' in water	Mw
Amidated citrus pectin	25	23	0.51	0.17	$\sim 5 \times 10^4$
Non-amidated citrus pectin	35	–	0.58	0.42	$\sim 5 \times 10^4$
Amidated apple pectin	31	–	0.68	–	$\sim 6 \times 10^4$
Non-amidated apple pectin	35		0.64	–	$\sim 5 \times 10^4$

DM, degree of methoxylation; DA, degree of amidation.
[a] Given by producer.

Table 2
The experimental design generated by Unscrambler and results from the permeability tests

Film no.	Chitosan type (degree of deacetylation)	Grade of the pectin (amidation) (coding)	Pectin source (coding)	Ratio r (coding)	% Paracetamol permeated in 0.1 M HCl after 1 h (%/cm at 1 h) average $\times 10^{-3}$ ($n = 3$)	P in phosphate buffer paracetamol model drug (%/cm/h) average $\times 10^{-3}$ ($n = 3$)	P in phosphate buffer erytrocin model drug (%/cm/h) average $\times 10^{-3}$ ($n = 6$)
1	DdA 89% (+1)	NA (−1)	Citrus (+1)	0.25 (−1)	8.0 ± 6.9	11.6 ± 2.5	6.3 ± 2.0
2	DdA 89% (+1)	AM (+1)	Apple (−1)	0.75 (+1)	18.3 ± 7.5	14.7 ± 4.1	10.8 ± 3.7
3	DdA 60% (−1)	AM (+1)	Citrus (+1)	0.25 (−1)	9.7 ± 5.0	33.5 ± 8.4	13.6 ± 1.7
4	DdA 89% (+1)	AM (+1)	Apple (−1)	0.25 (−1)	7.3 ± 4.3	17.9 ± 2.6	6.3 ± 1.5
5	DdA 89% (+1)	AM (+1)	Citrus (+1)	0.25 (−1)	4.8 ± 3.6	21.5 ± 2.8	5.5 ± 0.8
6	DdA 60% (−1)	NA (−1)	Citrus (+1)	0.25 (−1)	4.4 ± 1.0	14.7 ± 0.3	6.4 ± 1.0
7	DdA 89% (+1)	AM (+1)	Citrus (+1)	0.75 (+1)	18.3 ± 4.0	12.2 ± 1.1	5.9 ± 1.0
8	DdA 89% (+1)	AM (+1)	Citrus (+1)	0.50 (0)	16.6 ± 5.3	Not tested	8.3 ± 2.3
9	DdA 89% (+1)	NA (−1)	Citrus (+1)	0.75 (+1)	8.6 ± 4.8	9.4 ± 1.4	4.4 ± 1.3
10	DdA 89% (+1)	NA (−1)	Apple (−1)	0.25 (−1)	Dissolved	21.4 ± 3.9	5.5 ± 2.3
11	DdA 89% (+1)	AM (+1)	Citrus (+1)	0.50 (0)	17.5 ± 10.9	16.9 ± 5.2	6.1 ± 1.2
12	DdA 60% (−1)	NA (−1)	Citrus (+1)	0.75 (+1)	7.5 ± 6.8	12.0 ± 2.3	6.7 ± 1.6
13	DdA 89% (+1)	AM (+1)	Citrus (+1)	0.50 (0)	Not tested	Not tested	8.9 ± 2.1
14	DdA 60% (−1)	AM (+1)	Apple (−1)	0.25 (−1)	Dissolved	61.6 ± 16.7	15.5 ± 2.6
15	DdA 89% (+1)	NA (−1)	Apple (−1)	0.75 (+1)	19.9 ± 3.8	22.4 ± 1.1	6.8 ± 2.0
16	DdA 60% (−1)	NA (−1)	Apple (−1)	0.25 (−1)	19.3 ± 15.9	31.5 ± 18.2	11.3 ± 2.1
17	DdA 60% (−1)	AM (+1)	Apple (−1)	0.75 (+1)	21.3 ± 2.4	22.1 ± 8.0	10.4 ± 1.3
18	DdA 60% (−1)	AM (+1)	Citrus (+1)	0.75 (+1)	21.1 ± 5.9	17.2 ± 3.4	9.4 ± 4.2
19	DdA 60% (−1)	NA (−1)	Apple (−1)	0.75 (+1)	10.9 ± 6.6	16.1 ± 3.0	10.4 ± 3.2

NA, non-amidated low methoxylated pectin; AM, amidated low methoxylated pectin; dDa, degree of deacetylation.

placed in a USP paddle apparatus 37°C at 50 rpm, for 3 h in 1 l 0.1 M HCl and at least 5 h in 1 l phosphate buffer pH 6.8, respectively. Samples of 3 ml were drawn every 15 min. Due to the constant pH inside the test unit, continuous changes in the pH of the dissolution medium could not be accomplished. The amounts of model substances (paracetamol or erytrocin) released through the films were determined spectrophotometrically ($\lambda = 525$ nm for erytrocin and 243 nm for paracetamol in phosphate buffer pH 6.8 and 280 nm for paracetamol in 0.1 M HCl). Each permeability test was repeated 3–6 times. Based on the diffusion profile of the different films, the permeability (P) in phosphate buffer pH 6.8 was calculated according to the equation: $P = (K_{app}H)/A$. K_{app} represents the slope of the release profile, H, the film thickness (cm) and A, the surface area of the film (cm^2) [18]. The permeability of the films in 0.1 M HCl was calculated as percent paracetamol permeated after 1 h, corrected for film area and thickness.

2.7. Multivariate analysis

Partial least square regression (PLS) was applied (The Unscrambler, Camo ASA, Norway) to evaluate the data and to identify the most important factors for producing a film with low permeability in both 0.1 M HCl and phosphate buffer pH 6.8. The variation of each variable was scaled to unit variance (1/SD). The models were calculated using systematic cross-validation blocking individual replica. Jack-knifing was used to estimate the uncertainty of the PLS regression coefficients [19]. The interpretation of PLS values and plots (graphs, score plots and loading plots) can be found elsewhere [20,21].

3. Results

3.1. Intrinsic viscosity and estimation of the molecular weight of the pectins

The results from capillary viscometry studies of pectin in $(NaPO_3)_6$ showed that there were only small differences in the molecular weight of the different pectin types (Table 1). The Huggins constant, k' varied from 0.51 to 0.68. This indicates that $(NaPO_3)_6$ is a marginal solvent for this polymer (close to theta conditions, $k' = 0.5$) In order to characterise the association tendency in aqueous solutions of pectin, intrinsic viscosity was measured for the citrus pectins. For concentrations of non-amidated citrus pectin in water below 0.5% wt/wt, a non-association system was formed with a Huggins constant k' of 0.42 and an intrinsic viscosity of 347 ml/g. It was not possible to increase the concentration of polymer further because the polymer started to aggregate. Amidated citrus pectin in water also formed a non-association system with k' of 0.17 and an intrinsic viscosity of 386 ml/g. Increasing the polymer concentration to 1% did not promote aggregation and k' was unaffected.

3.2. Formation of the free films

Preparation of free films consisting of pectin and chitosan

was possible for all tested combinations with the spray method used.

3.3. Permeability of paracetamol in 0.1 M HCl

Most of the films showed low permeability in 0.1 M HCl, but two of the films dissolved completely (Table 2). Since some of the films showed deviating diffusion kinetics (non-0-order), evaluating the diffusion rate by calculation of the slope was not possible (Fig. 1). Instead, the amount of paracetamol permeated after 1 h, corrected for film area and thickness (%/cm) was used to describe the properties of the films.

The films that dissolved (Films #10 and 14) were excluded from further statistical evaluation. Average permeation (±SD) of drug through the remaining films was $1.3 \times 10^{-3} \pm 0.4 \times 10^{-3}$%/cm. This corresponds to a permeation of 4% paracetamol after 1 h for films with an average thickness of 40 μm.

A PLS analysis showed that a low permeation value was favoured by a high content of chitosan in the film (low *r*). The main differences in the diffusion profile were observed between films containing low and high content of chitosan. Films with a low content of chitosan (high *r*) showed 0-order kinetics, while films with high content of chitosan (low *r*) showed exponential kinetics (Fig. 1a, b).

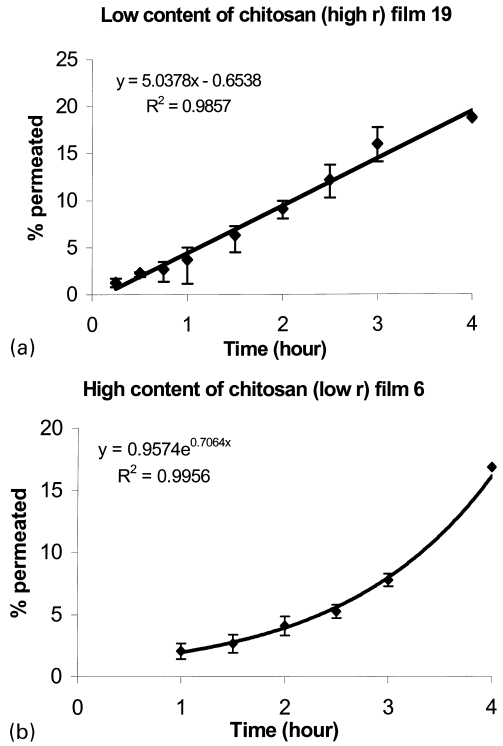

Fig. 1. The diffusion profiles in 0.1 M HCl was dependent on the content of chitosan ($n = 3–6$). (a) A typical diffusion profile when the content of chitosan was low (high *r*) showing 0-order kinetics. (b) A typical diffusion profile when the content of chitosan was high (low *r*) showing exponential kinetics.

3.4. Permeability of paracetamol in phosphate buffer pH 6.8

None of the films dissolved in phosphate buffer pH 6.8 but large variations with respect to the permeability were observed (see Table 2). All the diffusion profiles of the films showed 0-order kinetics.

A PLS analysis was performed. The regression coefficients (Fig. 2) showed that all the investigated main factors were important for controlling the permeability in phosphate buffer pH 6.8 ($p < 0.05$) in addition to some interactions. It was decided to split the matrix and determine separate models for the pectin types (amidated and non-amidated pectins).

A PLS of the non-amidated pectins showed two groups of samples (Fig. 3) with citrus pectin displaying the lowest *P* values and the apple pectins, the highest values. Further modelling of the non-amidated citrus pectin separately revealed that a low *P* was favoured by using the chitosan with high degree of deacetylation and having a high content of pectin to chitosan (high *r*).

For the amidated pectins, the source of the pectin did not have any effect on *P*. Also, for this pectin type, a low *P* was favoured by using the highly deacetylated chitosan and a high content of pectin to chitosan (high *r*).

In order to decide whether an amidated or non-amidated pectin type was preferable, a model was made from the non-amidated and amidated citrus pectin. A PLS analysis showed that amidation was positively correlated to *P* and as expected, the degree of deacetylation, ratio and interaction between chitosan and amidation and amidation and ratio were negatively correlated (Fig. 4).

To summarise, the lowest *P* was obtained by using non-amidated citrus pectin in combination with highly deacetylated chitosan and a high content of pectin to chitosan (high *r*).

3.5. Permeability of erytrocin in phosphate buffer pH 6.8

When erytrocin was used as the model drug, the value of *P* was significantly reduced compared to paracetamol. All the films showed 0-order kinetics (Fig. 5). The most striking difference was, however, the presence of a lag-time of 2–5 h. A PLS2 analysis showed that a long lag-time was negatively correlated to *P*, which means that a small *P* is obtained when the lag-time is long. The most important factors for obtaining a long lag-time were the use of a high chitosan content (low *r*) (Fig. 5) and chitosan with a high degree of deacetylation. The permeability (*P*) was shown to be governed by the same factors as when paracetamol was used as the model substance.

4. Discussion

Preparation of the free films was a complicated process since the polymers were not mixed prior to the spraying.

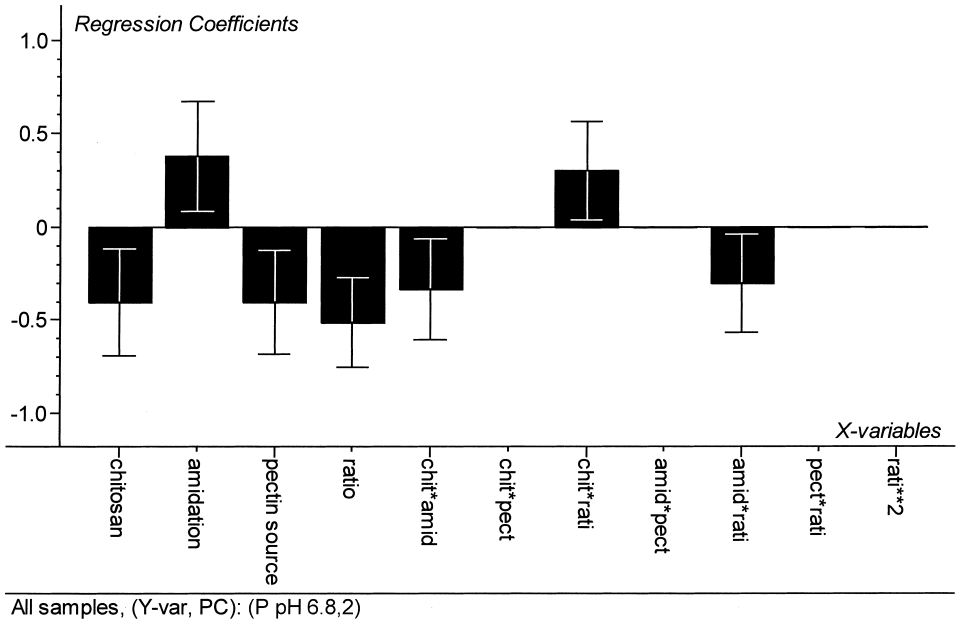

Fig. 2. Significant regression coefficients ($p < 0.05$) for a PLS of the permeability in phosphate buffer pH 6.8 when all the samples were included and paracetamol was used as the model drug. The explained *X*-variance was 28% and explained *Y*-variance 75% using two PCs.

Compared to casting, a film prepared by spraying would probably be less homogenous. The homogeneity of the films may be evaluated by comparing the standard deviation of *P* for replications of the center points. The standard deviation observed in this study was higher than values reported in other studies where films have been prepared by casting [18].

A possible explanation for the difference is that by mixing the polymers prior to spraying/casting, a homogeneous solution is produced and the film will consist of an interpenetrating polymer network of similar structure as in the solution. Casting may, therefore, be preferable when the polymers are mixable in the solution media. But when making films consisting of pectin and chitosan using water as a solvent, mixing of the polymers is not possible due to a phase separation. Spraying from separate polymer solutions, a possible outcome is a film composed of a mixture of small homogeneous but individual chitosan and pectin droplets

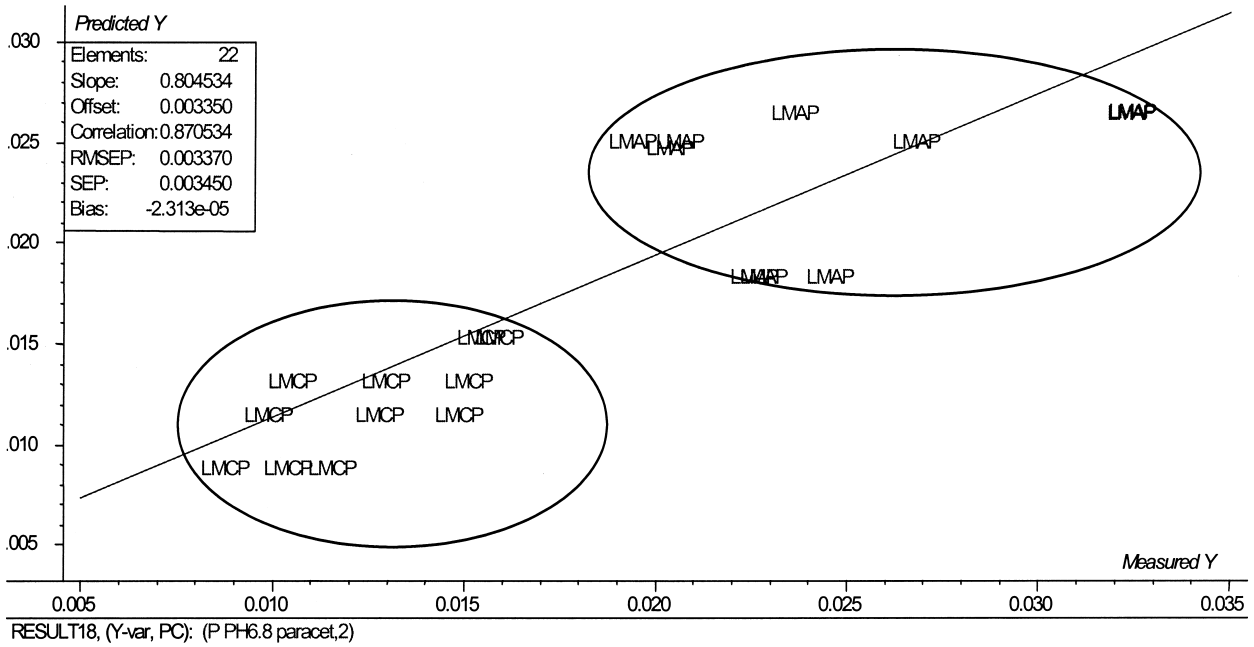

Fig. 3. Grouping of the non-amidated low methoxylated pectin, the citrus pectin (LMCP) down left showing the lowest *P* and the apple pectin (LMAP) up right showing the highest *P* of paracetamol in phosphate buffer pH 6.8. The explained *X*-variance was 63% and the explained *Y*-variance was 83% using two PCs.

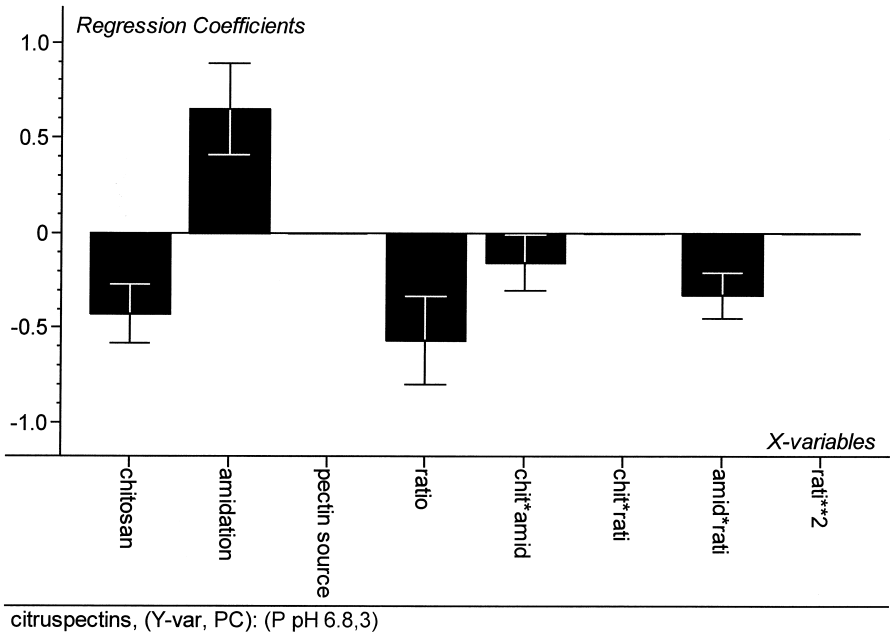

Fig. 4. Significant regression coefficients ($p < 0.05$) for a PLS of the permeability in phosphate buffer pH 6.8 of the non-amidated and the amidated pectin types when paracetamol was used as the model drug. The explained *X*-variance was 26% and the explained *Y*-variance was 77%.

leading to a more heterogeneous film. If this is the case, an interpenetrating polymer network will not be formed until exposing the film to the dissolution medium. Regardless of the mechanism for film-formation, the permeability properties of the film will be determined by attractions between the two polymers and possible attractions between the polymers and model drug.

All films were found to have a low permeability in 0.1 M HCl except films 10 and 14. But the results from the permeability studies in 0.1 M HCl which showed that a low *P* was favoured by a low value of *r* were, however, surprising. The permeability properties in 0.1 M HCl were expected to be dependent on the content of pectin. Pectin is only slightly soluble under these conditions and has no charge to promote swelling of the film. In addition, there are not any possible ionic interactions between the two polymers. However, pectin (DM 35) and chitosan have been shown to make a thermoreversible gel in 0.1 M HCl, which demonstrate that pectin and chitosan do interact, probably through hydrogen bonding [22]. For the pectin and chitosan types, in this study, all the combinations probably have a gel point temperature higher than 37°C or just below.

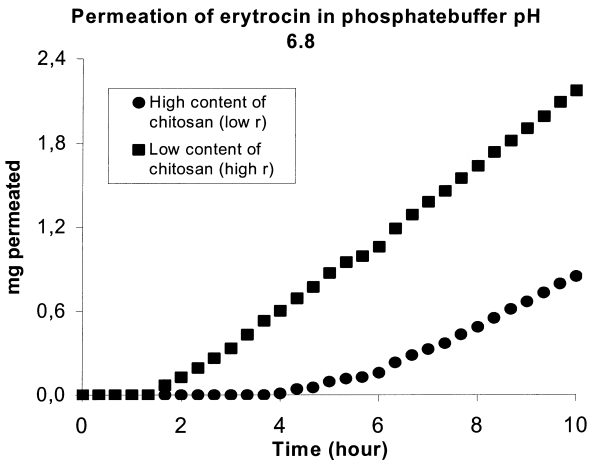

Fig. 5. The release profile (0-order) in phosphate buffer pH 6.8 when erytrocin was used as model drug of two different films differing only in the content of chitosan. The higher the content of chitosan the longer the lag-time.

Generally speaking, after 1 h, the permeability of the pectin and chitosan films containing high content of chitosan (low *r*) was lower than in films containing a high content of pectin (high *r*). This may be related to differences in cross-linking density of the systems. It has been argued [22] that at high chitosan contents, a fairly "tight" network is formed composed of many long chitosan chains, where both entanglements and pectin associations can contribute to the cross-link junctions. At low chitosan contents, a more "open" network is expected with a longer average strand length between cross-links [22]. The permeability of the films after 1 h may be related to the feature discussed above, but when the testing time is increased from 1 to 4 h, the permeation through the films is altered and the films with low values of *r* (high content of chitosan) display a dramatic increase in the permeability. Chitosan in 0.1 M HCl is positively charged and will, therefore, cause increased swelling of the film [23]. Initially, the films with high chitosan contents will probably swell slowly but in the course of time, the swelling will open the network and promote the permeability of the model drug. On the other hand, films with high pectin contents have a more open structure, which permits the drug to permeate from the start,

but since the films do not swell, the release rate of the drug will remain constant.

Irrespective of the constituents and release mechanisms, the differences between the observed permeation values were rather low after 1 h in 0.1 M HCl.

Due to this finding, factors affecting the permeation through the films in buffer pH 6.8 will determine the overall permeability of the combination films, especially since, the period the formulation will stay in the small intestine is much longer than the residence time in the stomach. In phosphate buffer pH 6.8, the polymers will be oppositely charged leading to the formation of a PEC due to ionic interactions between the polymers.

The molecular weights of the pectins were assumed to be equal ($\sim 5 \times 10^4 - \sim 6 \times 10^4$) and not included as a factor during the modelling. The PLS analysis of P in phosphate buffer pH 6.8 showed that the most important factors for obtaining a low P was a high ratio of non-amidated citrus pectin in combination with chitosan with a high degree of deacetylation. It has been proposed that there is an optimum ratio preferable for making a tight PEC between pectin and chitosan [13]. The optimum ratio found in this study ($r = 0.75$) is in good agreement with the study given above.

For chitosan with a high degree of deacetylation, the interaction with pectin may be facilitated resulting in a stronger PEC. Highly deacetylated chitosan contains more amine groups: the more protonated groups present, the higher the possibilities for ionic interactions with the carboxylic acid groups of pectin. Another possible explanation for the increased stability of the PEC is that chitosan, with an increased amount of protonated groups, could be more flexible than chitosan with lower amount of charge [24]. However, different views exist concerning the effect of acetylation on chain stiffness [25]. The difference in the degree of deacetylation may also affect the thermodynamic properties of the system. The success of non-amidated citrus pectin is probably due to its many free carboxylic groups. Non-amidated pectin has more free groups to be ionised than the amidated pectin (lower degree of total substitution). Or, as for chitosan, it may be the flexibility of the polymer chains controlling the tightness of the polymer network. Another reason for the deviation observed may be differences in thermodynamic conditions of the solution. The viscosity studies showed that the amidated citrus pectin had a lower Huggins constant k' in water than the non-amidated citrus pectin, both polymers had a k'-value that proved water to be a solvent that prevented associations. However, the tendencies for the polymers to aggregate were higher for the non-amidated citrus pectin indicated by a change in k' when increasing the concentration. This self-aggregation may have a positive effect for building a tight film-network [26].

The only difference between erytrocin and paracetamol, as model drugs, was the lag-time observed prior to the release of erytrocin. A PLS analysis showed that this lag-time was correlated to a high content of chitosan (low r). The retention of erytrocin is probably caused by ionic interactions between the positively charged chitosan and the negatively charged erytrocin. The size of the model drug may also contribute to the longer lag-time for erytrocin compared to paracetamol.

5. Conclusion

This study has shown that the permeability constant P in phosphate buffer pH 6.8 determines the overall permeability of the films and is highly dependent on the investigated formulation factors. The optimum film combination had a combined diffusion of only 0.046%/cm after 1 h in HCl and 4 h in phosphate buffer. The most promising films were formulated with non-amidated low methoxylated citrus pectin in combination with a chitosan with a high degree of deacetylation. The content of pectin should be higher than the content of chitosan (high r). A charged model substance will be retained to a greater extent than a neutral substance.

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