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Leaching of pectin from mixed films containing pectin, chitosan and HPMC intended for biphasic drug delivery

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Abstract

Mixed films containing pectin, chitosan and HPMC, prepared by solvent casting from 0.1 M HCl (pH 1.5) and 0.1 M acetic acid (pH 2.9) were evaluated for their morphological and leaching properties. Films cast at pH 1.5 were uniform with smooth surfaces while films cast at pH 2.9 showed particle aggregation and had rough surfaces due to polyelectrolyte complex (PEC) formation between pectin and chitosan in the medium. The leaching of pectin was higher from films cast at pH 1.5 due to the absence of PEC formation. Pectin leaching was controlled in simulated upper gastrointestinal conditions but was accelerated in the presence of pectinolytic enzymes. The leaching of pectin from the mixed films was a function of the pH of the film casting solvent, pH of the incubation medium, PEC formation and HPMC content.

Keywords: Colonic delivery; Pectin; Chitosan; Mixed films; Polyelectrolyte formation; Leaching of pectin

1. Introduction

The use of mixed films in drug delivery is for optimising the physicochemical and permeability properties of the resultant films. Chitosan may form a complex with pectin and reduce the aqueous solubility of the individual polymers. HPMC enhances the film-forming properties such as toughness, elasticity and tensile strength. Hydrophilic materials such as pectin, chitosan and HPMC may, however, leach from mixed films in gastrointestinal fluids and can affect the integrity of film-coated products as they traverse the gastrointestinal tract (GIT). Hydrophilic materials may therefore act as drug release regulators in mixed film coatings. By altering the leaching characteristics of films, biphasic drug delivery, where some of the dose in a dosage form is released in the upper GIT, and the remainder in the colon, may be achieved. This can be valuable in a range of therapeutic areas (Turner-Warwich, 1988; Willich et al., 1992; Maggi et al., 1999).

The leaching of hydrophilic materials from films is well documented in the literature. Bodmeier and Paeratakul (1991) have described the rapid leaching of dibasic calcium phosphate from films consisting of aqueous acrylic latex and dibasic

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calcium phosphate into simulated gastric fluids to form a rate-controlling, micro-porous membrane. Guo (1994) has described the leaching of a water soluble plasticiser (PEG-600) from cellulose acetate films. This created aqueous channels in the film matrix through which encapsulated drugs could diffuse during dissolution. Semde et al. (1998) investigated the leaching of pectin from mixed films prepared from Aquacoat ECD 30, Surelease clear, Eudragit RS30D or Eudragit NE30D and 5–15% w/w of pectin HM or 10% w/w calcium pectinate. They found that pectin HM or calcium pectinate were rapidly released from the films into 0.05 M acetate–phosphate buffer (pH 4.5), except from the mixed pectin/Eudragit RS films. Rao and Diwan (1998) evaluated ethylcellulose/polyvinyl pyrrolidone (PVP) films containing diltiazem HCl and indomethacin for controlled drug delivery to the skin. They found that the release rates of both drugs increased linearly with increased drug concentration and PVP fraction in the film and concluded that the increase in release rate may be due to leaching of PVP which resulted in the formation of pores in films.

This paper investigates the leaching of pectin from mixed films of pectin, chitosan and HPMC into simulated gastrointestinal fluids. This paper presents data that is essentially different from those generated in earlier studies in that pectin complexation with chitosan is intended to control the rate of leaching. The work is intended to provide a further insight into earlier studies (Ofori-Kwakye and Fell, 2001) assessing the permeability properties of mixed films of pectin, chitosan and HPMC.

2. Materials and methods

2.1. Materials

Pectin USP was a gift from Citrus Colloids (Hereford, UK). High molecular weight chitosan (Chitosan HM) was obtained from Sigma-Aldrich (Dorset, UK). HPMC was received as Methocel E4M Premium grade from Colorcon (Kent, UK). Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate and glycerol were general purpose reagents from BDH Ltd (Poole, UK). Pectinex® Ultra SP-L with a standard activity of 26 000 PG (pH 3.5) was supplied by Novo Nordisk Ferment Ltd (Neumatt, Switzerland). Pectinex® Ultra SP-L is a highly active pectinolytic enzyme preparation produced by a selected strain of *Aspergilus aculeatus* and was used to simulate colonic pectinolytic enzymes. Carbazole and D-galacturonic acid were obtained from Sigma Chemical Company (St. Louis, USA). Sodium tetraborate was supplied by Fisons Scientific (Loughborough, UK). Absolute ethanol, acetic acid (glacial), Tween-80, ConvoL® concentrated sodium hydroxide VS (NaOH) and ConvoL® concentrated hydrochloric acid VS (HCl) were supplied by BDH Ltd. Sulphuric acid (technical grade) and hydrochloric acid were supplied by Fisher Scientific (Loughborough, UK). All other chemicals used were of analytical reagent grade.

2.2. Film manufacture

Mixed films consisting of pectin USP and high molecular weight chitosan in a fixed ratio of 6:1 containing 0–20% HPMC were prepared by solvent casting in 0.1 M HCl (pH 1.5) or 0.1 M acetic acid (pH 2.9) as described elsewhere (Ofori-Kwakye and Fell, 2001).

2.3. Scanning electron microscopy studies

The surface morphology of the mixed films cast from the two media were studied by scanning electron microscopy (SEM). Dry film samples were stored at room temperature in a desiccator for at least a week before use. Samples were cut with a scalpel and mounted on 1.27 cm aluminium pin stubs (Agar Scientific Ltd, Essex, UK) with double pressure sensitive adhesive tape and lubricated with Silver dagg (topped-up with butyl acetate). Samples were coated with a thin layer of gold (Sputter Coater, SC 500 Emscope, UK) to improve conductivity and observed with a Cambridge Stereoscan 360 SEM (Cambridge, UK).

2.4. Galacturonic acid content of pectin USP

The galacturonic acid content and the degree of methoxylation of pectin USP were determined according to the US Pharmacopoeia XXIII (1995) assay method.

2.5. Leaching studies

Film samples of dimensions 2×2 cm^2 were cut from the different film formulations and accurately weighed. Film thickness was measured at six different places with a micrometer (Moore and Wright, England) and the mean thickness calculated. Samples with thicknesses in the range 100–166 mm were selected and incubated in a water bath under conditions chosen to mimic the transit times and pH conditions pertaining in the upper GIT and the pH in the colon. The film samples were incubated in 100 ml 0.1 M HCl at 37 °C for 2 h; immediately followed by pH 7.4 Sorensen's phosphate buffer for 3 h. The effect of pectinolytic enzymes on the release of pectin from the mixed films was investigated by incubating the films in 100 ml pH 6.0 Sorensen's phosphate buffer at 37 °C with and without pectinex® Ultra SP-L enzymes (0.2 ml/100 ml) for 5 h. The test solutions included 0.05% Tween-80 (Semde et al., 1998) in a concentration of 0.1 ml/100 ml solution to improve the hydration of the film surfaces.

At specified time intervals 1 ml samples were taken from the solutions and replaced with fresh solution and the amount of pectin USP or its degradation product released was determined by employing the method of Bitter and Muir (1962) which is a modification of Dische's carbazole reaction for uronic acids (Dische, 1947). The main advantage of the carbazole reaction is its specificity and simplicity, the major constituent of pectin USP being D-galacturonic acid which is an uronic acid. A 1-ml sample of the test solution containing galacturonic acid was treated with 5 ml of 0.025 M sodium tetraborate prepared in concentrated sulphuric acid. Upon heating for 10 min in a boiling water bath, galacturonic acid was converted to a furfural-type chromogene. Addition of 0.2 ml of 0.125% carbazole (in ethanol) and heating the resulting solution for 15 min at 100 °C formed a coloured product upon cooling. The absorbance of the coloured product was then read at 530 nm (Corning colorimeter 253, Essex, England).

Calibration graphs with D-galacturonic acid as a standard in 0.1 M HCl, pH 6.0 and 7.4 Sorensen's phosphate buffer solutions in the concentration range 4–40 μg/ml were obtained by employing the above method. From these, the amount of pectin released into the various media was determined. The galacturonic acid contents of the initial film samples used in the leaching study were determined from the proportion of pectin in a particular film formulation and the weight of the sample used. The percentage of pectin released from the film samples (mean $\pm$ SD, $n = 3$) into solution was calculated and plotted against time.

3. Results and discussion

3.1. Scanning electron microscopy studies

Films cast from 0.1 M HCl had relatively smooth surfaces while those of 0.1 M acetic acid were rough and uneven. The difference in surface morphology is attributable to the formation or otherwise of a polyelectrolyte complex (PEC) between pectin and chitosan in the different casting solvents used in film fabrication. There will be no formation of a PEC when pectin and chitosan gels prepared in 0.1 M HCl are mixed to form the films. This will lead to the formation of homogenous films having relatively smooth surfaces. However, a PEC will form in situ as aqueous gels of pectin and chitosan prepared in 0.1 M acetic acid are mixed. The instantaneous formation of a PEC upon mixing of pectin and chitosan gels resulted in the formation of heterogeneous films made up of PEC-rich areas (particle aggregates) and areas made up mostly of physical mixtures of pectin and HPMC or chitosan and HPMC. The SEM studies have shown that 0.1 M HCl is more suited (as compared to 0.1 M acetic acid) for the fabrication of pectin/chitosan/HPMC films having appropriate physicochemical properties.

3.2. Leaching of pectin

A study of the kinetics of pectin leaching from mixed films consisting of pectin, chitosan and HPMC under simulated GIT conditions is an important means of evaluating film integrity and stability as it traverses the GIT. Also, the leaching of pectin from mixed films of pectin, chitosan and HPMC films can have a significant effect on the release of drugs from film-coated dosage forms. This is more so where pectin is the major component of the films. The evaluation of the leaching of pectin into simulated gastrointestinal fluids involved the assay of galacturonic acid which is the main constituent/degradation product of pectin. The galacturonic acid content of pectin USP was high (92.4±0.6%, $n=3$) with a low degree of methoxylation (9.8±0.1%, $n=3$). These values are well within the US Pharmacopoeia XXIII (1995) specification that 'pectin yields not less than 74% of galacturonic acid and not less than 6.7% of methoxy groups calculated on the dried basis'.

Tables 1 and 2 are the release data of pectin from films cast from 0.1 M HCl and 0.1 M acetic acid, respectively, into simulated upper GIT conditions (pH 1.5, 2 h; pH 7.4, 3 h) at 37 °C. The mean amount of pectin released from the five film formulations cast from 0.1 M HCl into simulated upper GIT fluids was 29.2±4.7%, with the mean gastric release (pH 1.5, 2 h) being 19.9±5.3%. The mean amount of pectin released from films cast from 0.1 M acetic acid into simulated upper GIT fluids was 22.8±4.3%, with the mean gastric release being 3.1±0.9%. Thus, a much smaller percentage of pectin was leached from films cast at pH 2.9 than films cast at pH 1.5. Also, for films cast at pH 2.9, a much smaller proportion of pectin was leached in gastric conditions than for films cast at pH 1.5. In films cast from 0.1 M HCl, an increase in HPMC content of films from 0 to 10% resulted in an increase in pectin leaching. Further increases in HPMC content caused a reduction in pectin leaching. These results correlate directly with the permeability profiles of the pectin/chitosan/HPMC films to the drug, paracetamol (Ofori-Kwakye and Fell, 2001). The inclusion of HPMC in films cast from 0.1 M acetic acid also caused an increase in pectin leaching. This, however, did not follow any consistent pattern. For both sets of films, the lowest amount of pectin was released in films without the inclusion of HPMC. HPMC itself may leach from the films (Lindstedt et al., 1991; FrohoffHulsmann et al., 1999) to create a porous structure which may allow more pectin to leach. Higher concentrations of HPMC would, however, reduce the free water volume and increase the viscosity causing a reduction in the amount of pectin leaching.

Table 1
Leaching of pectin from mixed films of pectin/chitosan/HPMC cast from 0.1 M HCl into simulated upper gastrointestinal fluids (pH 1.5, 2 h: pH 7.4, 3 h) (mean±SD, $n=3$)

% HPMC	% Pectin released (2 h)	% Pectin released (5 h)
0	13.89±0.60	23.23±2.04
5	19.39±0.85	26.96±2.64
10	28.03±0.54	36.12±0.86
15	21.37±1.80	29.33±0.06
20	16.95±0.31	30.14±0.76

Table 2
Leaching of pectin from mixed films of pectin/chitosan/HPMC cast from 0.1 M acetic acid into simulated upper gastrointestinal fluids (pH 1.5, 2 h: pH 7.4, 3 h) (mean±SD, $n=3$)

% HPMC	% Pectin released (2 h)	% Pectin released (5 h)
0	2.49±0.08	18.73±1.86
5	1.95±0.76	24.05±0.76
10	3.91±0.35	22.63±2.59
15	3.50±0.35	29.23±2.06
20	3.82±0.83	19.23±2.97

Pectin and chitosan, in the correct pH, would form a PEC through electrostatic interaction between the carboxylic acid groups in pectin and the amine groups in chitosan (Meshali and Gabr, 1993). There will be no PEC formation between pectin and chitosan in films cast from 0.1 M HCl as the carboxylic acid groups in pectin will remain practically unionised. Films cast from this medium may, however, form PECs in situ when they are exposed to the varying pH values in the GIT. Films cast from 0.1 M HCl in simulated upper GIT fluids became easily hydrated leading to increased leaching of pectin and increased film

porosity. In contrast, a PEC was formed between pectin and chitosan in films cast from 0.1 M acetic acid. This reduces the aqueous solubility of the films and accounts for the minimal amount of pectin which leached from the films into simulated upper GIT fluids as compared to that of films cast from 0.1 M HCl.

Figs. 1 and 2 depict the leaching of pectin from films cast from 0.1 M HCl and 0.1 M acetic acid into pH 6.0 Sorensen's phosphate buffer with and without pectinex® Ultra SPL enzymes at 37 °C. In the absence of enzymes, the amount of pectin which leached into pH 6.0 Sorensen's phosphate buffer in 5 h was higher from films cast from 0.1 M HCl (26.3 ± 5.2%) than from films cast from 0.1 M acetic acid (19.8 ± 3.3%). The extent of pectin leaching from films into pH 6.0 Sorensen's phosphate buffer within 5 h in the absence of pectinolytic enzymes contrasts with the results reported by Semde et al. (1998). These workers prepared mixed pectin/cellulosic or acrylic polymer coatings as possible carriers for colonic drug delivery. They observed, from leaching studies, that almost all the pectin in films consisting of pectin HM and Aquacoat® ECD30; calcium pectinate and Aquacoat® ECD30; pectin HM (10% w/v) and Surelease® clear, and pectin HM and Eudragit® NE30D were released within 30 min into pH 4.5 acetate–phosphate buffer (without pectinolytic enzymes). They found, however, that films of pectin HM or LM and Eudragit RS30D were suitable carriers for colonic drug delivery as the extent of pectin leaching from these films was minimal.

In the current work, the leaching of pectin was accelerated in the presence of pectinolytic enzymes in films cast from 0.1 M HCl (45.2 ± 4.1%) and in films cast from 0.1 M acetic acid (47.18 ± 5.88%) The results indicate that pectin in the film matrix was accessible to the pectinolytic enzymes. The increased degradation of pectin by the enzymes and its subsequent migration out of the films will

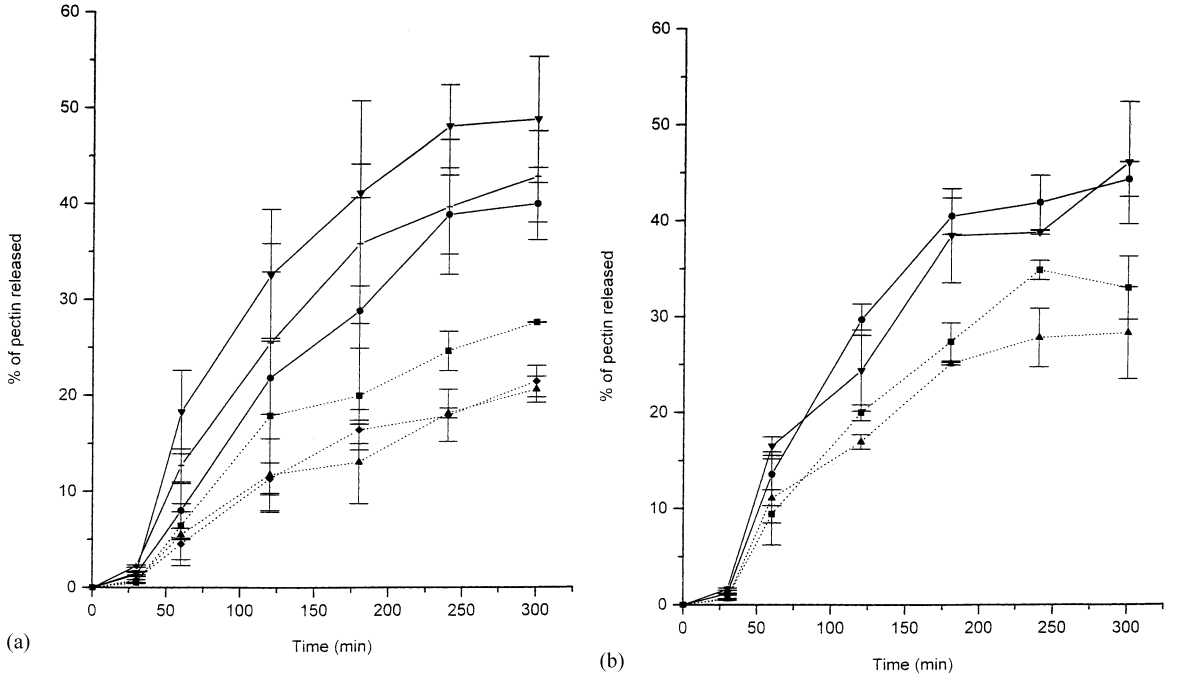

Fig. 1. (a) The effect of pectinolytic enzymes on the leaching of pectin from films cast from 0.1 M HCl (mean ± SD, $n = 3$). Dotted line = absence of enzymes, complete line = presence of enzymes. (■), (●) = 0% HPMC; (▲), (▼) = 5% HPMC; (◆), (+) = 10% HPMC. (b) The effect of pectinolytic enzymes on the leaching of pectin from films cast from 0.1 M HCl (mean ± SD). Dotted line = absence of enzymes, complete line = presence of enzymes. (■), (●) = 15% HPMC; (▲), (▼) = 20% HPMC.

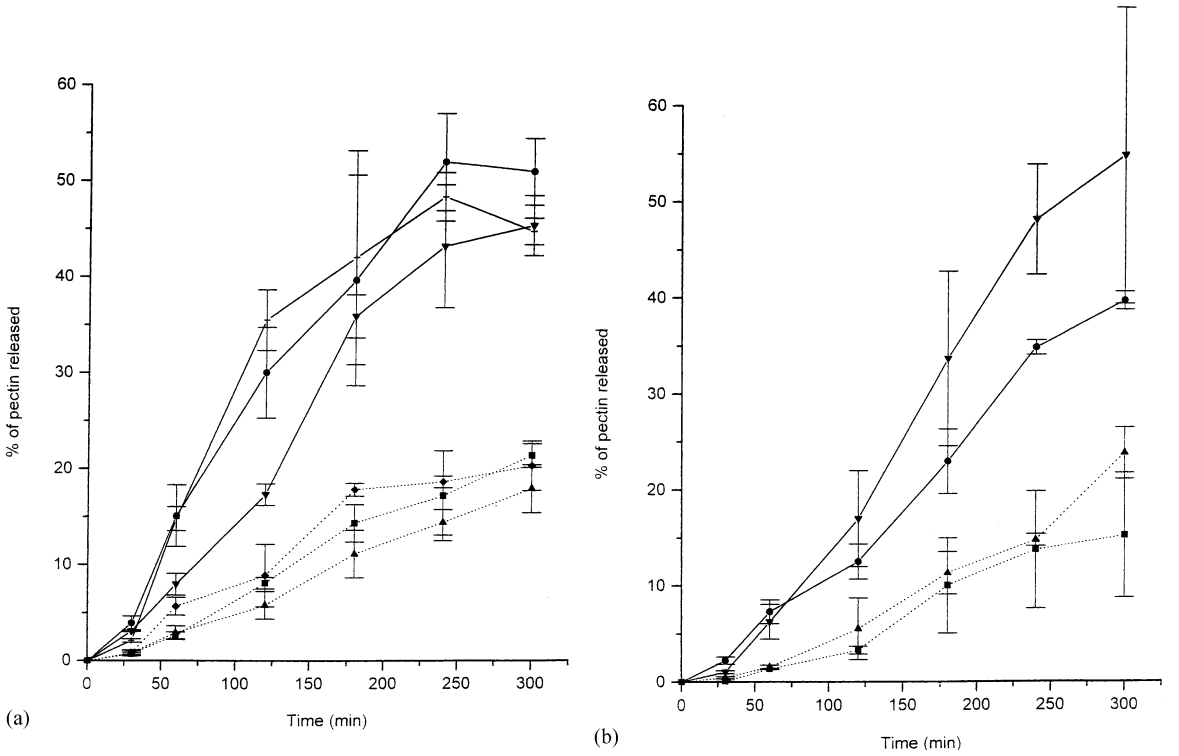

Fig. 2. (a) The effect of pectinolytic enzymes on the leaching of pectin from films cast from 0.1 M acetic acid (mean ± SD). Dotted line = absence of enzymes, complete line = presence of enzymes. Key as Fig. 1a. (b) The effect of pectinolytic enzymes on the leaching of pectin from films cast from 0.1 M acetic acid (mean ± SD). Dotted line = absence of enzymes, complete line = presence of enzymes. Key as Fig. 1b.

increase the free volume in the polymeric film matrices and enhance the drug release properties of film-coated dosage forms.

The kinetics of pectin leaching indicates the potential of the film coating system consisting of pectin, chitosan and HPMC for biphasic drug delivery. This is because the leaching of pectin exhibited a biphasic pattern with a controlled leaching in the upper GIT which was accelerated in the presence of pectinolytic enzymes. Drug release from dosage forms coated with such films may replicate the biphasic leaching pattern of pectin to achieve biphasic drug release. Pectin may thus act as a drug release regulator in pectin/chitosan/HPMC coated dosage forms. The mixed films consisting of pectin, chitosan and HPMC act as matrix polymeric systems from which pectin can diffuse out into aqueous media creating aqueous channels or water-filled pores and through which drugs may diffuse.

4. Conclusions

The leaching studies have shown that pectin can diffuse out of films consisting of pectin, chitosan and HPMC into simulated gastrointestinal fluids. The rate of pectin leaching was controlled in upper gastrointestinal fluids and was accelerated in colonic fluids. The film coating systems thus have the potential for use as a carrier for the biphasic delivery of drugs. The leaching of the large molecular weight pectin will create aqueous channels or water-filled pores which may allow the diffusion of drug molecules through the film coatings of dosage forms. The rate of pectin

leaching was a function of the pH of the film casting solvent, the pH of the incubation medium, interaction between pectin and chitosan (formation or otherwise of a PEC) and the HPMC content of the films.

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