EFFECTS OF pH OF MEDIUM AND MOLECULAR WEIGHT ON POLYELECTROLYTE COMPLEX FORMATION BETWEEN PECTIN AND CHITOSAN

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ABSTRACT

The effects of pH of medium and molecular weight of chitosan on polyelectrolyte complex (PEC) formation between pectin and chitosan was investigated using capillary viscometry. The intrinsic viscosity of the polymers was determined using Huggin's plot. PECs were formed between pectin and chitosan in the pH range 2.9-5.6, but no PEC was formed at pH 1.5. The proportion of pectin in pectin-chitosan PEC varied with pH of the medium due to different levels of ionisation of the polymers and also probably due to the flexibility of pectin molecule. The amount of pectin in the PEC increased with a decrease in pH while that of chitosan increased with an increase in pH of medium. Linear relationships were established between the pH of media used and the weight fraction of pectin required for optimal PEC formation with molecular weight grades of chitosan. Molecular weight of chitosan appeared to have no effect ($p>0.05$) on the optimal ratio of pectin:chitosan required for PEC formation but intrinsic viscosity of chitosan was molecular weight dependent with a rank order of intrinsic viscosities being: high molecular weight chitosan >medium molecular weight chitosan > low molecular weight chitosan.

Keywords: *Polyelectrolyte complex; Capillary viscometry; Intrinsic viscosity; Chitosan; Pectin.*

INTRODUCTION

Various types of oppositely charged polyelectrolytes interact electrostatically in aqueous media to form an insoluble solid or polyelectrolyte complex (PEC). The PEC when used to coat or encapsulate drugs, provides a greater barrier to drug release in the upper gastrointestinal tract (GIT) than the use of either material alone (Munjeri *et al.*, 1997; Yan *et al.*, 2001) and have therefore been utilised in the design of various controlled drug delivery systems (Takeuchi *et al.*, 2000; Mitrevej *et al.*, 2001; Ofori-Kwakye *et al.*, 2004).

Electrostatic interactions occurring between polyanions and polycations resulting in PEC formation are well documented in the literature. Kawashima *et al.* (1985) produced a controlled release system by film coating theophylline

granules with a PEC of chitosan and sodium tripolyphosphate. The rate of drug release was controlled by pH of the medium. At low pH values, the reduced charge of the anionic tripolyphosphate reduced the electrostatic interaction in the PEC and the network in the film loosened. Kim *et al.* (1999) prepared drug-impregnated PEC sponges composed of chitosan and sodium alginate for wound healing applications. PECs have also been formed between chitosan and other polyanions such as carboxymethylcellulose (Fukuda, 1979), sodium hyaluronate (Takayama *et al.*, 1990), sodium alginate (Takahashi *et al.*, 1990), sodium polyacrylate (Chavasit *et al.*, 1988; Takahashi *et al.*, 1990), sunset yellow (Phaechamud *et al.*, 2000) and pectin (Meshali and Gabr., 1993; De Yao *et al.*, 1997; Macleod *et al.*, 1999; Ofori-Kwakye and Fell, 2003).

Pectin and chitosan are safe, biodegradable polyelectrolytes that have been combined to form a mixed film coating for the delivery of drugs into the colon. This was achieved by utilising their ability to form PECs that protected tablet cores from drug release in the upper GIT but allowed drug release to occur in the colon through the action of pectinolytic enzymes resident in the colon (Ofori-Kwakye *et al.*, 2004).This paper investigates the possible effects of pH and molecular weight on the interactions that occur in pectin-chitosan systems in aqueous media resulting in PEC formation. The study is intended to provide a further insight into factors affecting PEC formation and help in the fabrication of PECs having the requisite physicochemical properties for use in controlled drug delivery.

MATERIALS AND METHODS

Materials

Three molecular weight grades of chitosan, namely; low molecular weight chitosan (CHL), medium molecular weight chitosan (CHM), and high molecular weight chitosan (CHH), all with degrees of acetylation (DA) of 86.2% were obtained from Sigma-Aldrich (Dorset, UK). Pectin USP was a gift from Citrus Colloids (Hereford, UK). Sodium acetate trihydrate was obtained from BDH Ltd. (Poole, UK). Hydrochloric acid (reagent grade), was supplied by Fisher Scientific (Loughborough, UK). Glacial acetic acid and ethanol 96% were general-purpose reagents from BDH Ltd. (Poole, UK). Distilled water was singly distilled and freshly prepared.

Determination of intrinsic viscosity

A minimum of 5 dilute solutions (0.2 – 1.0 % w/v) of Pectin USP, and the 3 molecular weight grades of chitosan were individually prepared in acetate buffer pH 4.8. The solutions were filtered with a Buchner funnel with filter paper (Whatman No. 1) attached to a vacuum. A calibrated, standard size A, borosilicate glass Ostwald U-tube viscometer (Technico) clamped into a levelled water bath was used to determine the reduced viscosity of the solutions from the mean flow time of five determinations of the solutions and the solvent at 30 ± 0.5 °C. The mean flow times of the polymer solutions and the reference solvent were used to determine the kinematic viscosity (v).

$$v = \eta / \rho$$

where η is dynamic viscosity and ρ is density of fluid. Since the polymer solutions were sufficiently dilute, the dynamic viscosity (viscosity) was assumed to be equal to the kinematic viscosity. The viscosities of the solvent and the polymer solutions were used to calculate the relative viscosity, specific viscosity and reduced viscosity using the following relationships:

- Relative viscosity (η_{rel}) = t/t_s
- Specific viscosity (η_{sp}) = $(t/t_s) - 1$
- Reduced viscosity (η_{red}) = η_{sp}/c
- Intrinsic viscosity ($[\eta]$) = $(\eta_{red})c \rightarrow 0$

where t is the mean flow time of polymer solution, t_s is mean flow time of solvent, and c is polymer concentration in (g/dl).

A plot of reduced viscosity against polymer concentration (Huggin's plot) on extrapolation to infinite dilution gave the intrinsic viscosity for

the individual polymers (Errington *et al.*, 1993; Tsaih and Chen, 1997; Wang *et al*, 2004).

Formation of pectin-chitosan PECs

A series of dilute solutions (0.02 – 0.1 % w/v) of Pectin USP and the 3 molecular weight grades of chitosan were prepared individually in 0.1 M HCl (pH 1.5), 0.1 M acetic acid (pH 2.9) and acetate buffer pH 3.8, 4.8 and 5.6. Twenty five millilitres (25 ml) of a given chitosan solution was mixed with 25 ml of aqueous Pectin USP prepared in the same medium in a conical flask. A minimum of 5 solutions were prepared for each pectin/chitosan mixture in a particular solvent. The total polymer concentration in all samples was fixed at 0.1 % w/v. The conical flasks were sealed with aluminium foil and incubated in a water bath at 37 °C and shaken at 40 strokes/min for 24 h. The flasks were inspected after incubation for the presence or absence of a gel-like precipitate. The content of each flask was filtered twice under vacuum through filter paper (Whatman No.1) to ensure complete removal of all precipitates formed. The specific viscosities of the supernatant solutions were determined from the mean flow times determined by capillary viscometry at 30 ± 0.5 °C.

RESULTS AND DISCUSSION

Intrinsic viscosity of polymers

Intrinsic viscosity is an important rheological parameter which is used to characterise the hydrodynamic properties of polymers and also to determine the weight average molecular weight of polymers by use of the Mark-Houwink's equation (Wang *et al.*, 1991; Errington *et al.*, 1993). Table 1 shows the intrinsic viscosities of the polymers in acetate buffer pH 4.8 at 30 ± 0.5 °C. Pectin USP had the lowest intrinsic viscosity of the polymers studied, 364 ml g^{-1}. There was a significant difference in the intrinsic viscosities of CHH and CHL, 492 ml g^{-1} and 1028 ml g^{-1}, respectively. There was, however, little or no difference in the intrinsic viscosities of CHH and CHM, 1028 ml g^{-1} and 1000 ml g^{-1}, respectively. The rank order of intrinsic viscosities for the 3 grades of chitosan was: CHH > CHM > CHL. These results are similar to those of Tsaih and Chen (1997). These workers have reported that intrinsic viscosity of chitosans was molecular weight-dependent, and decreased with a decrease in molecular weight in solutions of the same ionic strength. Launay *et al.* (1986) have shown that intrinsic viscosity is related to the shape and volume fraction of solutes in solution. Tanglertpaibul and Rao (1987) have also reported that intrinsic viscosity is related directly to the ability of polymer solutions to disturb flow and indirectly to their size and shape. Chitosans with the same DA but different molecular weights will have a similar shape factor and volume fraction at the same pH, provided there is no inter or intra-hydrogen bonding (Tsaih and Chen, 1997). Hence molecular size will be the only factor that causes the hydrodynamic volume of the different grades of chitosan to be different. Thus, intrinsic viscosity of chitosan would increase with increase in molecular weight.

Table 1: Intrinsic viscosity of pectin USP and molecular weight grades of chitosan in acetate buffer pH 4.8 at 30 ± 0.5 °C

Polymer	Intrinsic viscosity (ml g^{-1})
Pectin USP	364
Low molecular weight chitosan (CHL)	492
Medium molecular weight chitosan (CHM)	1000
High molecular weight chitosan (CHH)	1028

Formation of pectin-chitosan PECs

The pH of the media used in the preparation of pectin-chitosan PECs will have an influence on the physicochemical properties of the complexes formed. We reported previously that the difference in the surface morphology of mixed films of pectin and chitosan prepared by solvent-casting was dependent on the formation or otherwise of PEC between the polymers in the solvent used (Ofori-Kwakye and Fell, 2003). In that study, films cast at pH 1.5 had uniform, smooth surfaces while films cast at pH 2.9 showed particle aggregation and had rough surfaces due to PEC formation in the medium. De Yao *et al.* (1996; 1997) have also reported that the swelling behaviour of pectin-chitosan PECs is pH-dependent. This swelling behaviour was modulated by the weight ratio of pectin:chitosan in the PECs as well as the original concentration of pectin.

Acidic (pH 1.5; pH 2.9) and acetate buffer pH 3.8, 4.8 and 5.6 were used as media in the study because chitosan is not soluble in neutral and alkaline solutions. At the end of the incubation period, pectin/chitosan mixtures prepared at pH 1.5 were clear and showed no apparent precipitate formation. However, a cloudy solution with a gel-like precipitate was found in varying degrees in mixtures prepared at pH 2.9-5.6. Figures 1-5 show the relationship between the specific viscosity of supernatant solutions of pectin/chitosan mixtures and the weight fraction of pectin in media of varying pH values. Figure 1 shows that at pH 1.5, the specific viscosity of the supernatant solutions continuously decreased with increase in the weight fraction of pectin in the pectin/chitosan mixture. On the other hand, figures 2-5 show that the specific viscosity of supernatant solutions at pH 2.9-5.6 decreased to a minimum value and then increased as the weight fraction of pectin in the mixture was increased. The specific viscosities of the 3 molecular weight grades of chitosan and pectin, when used alone were almost proportional to the polymer concentrations (Figure 6).

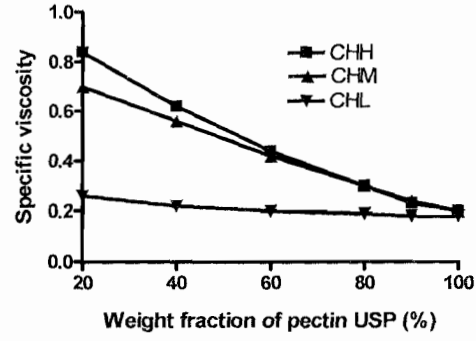

Fig. 1: Specific viscosity of supernatant solutions of pectin/chitosan mixtures in 0.1 M HCl (pH 1.5) at 37°C

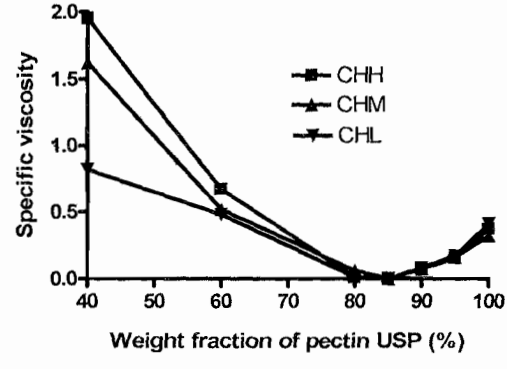

Fig. 2: Specific viscosity of supernatant solutions of pectin/chitosan mixtures in 0.1 acetic acid (pH 2.9) at 37°C

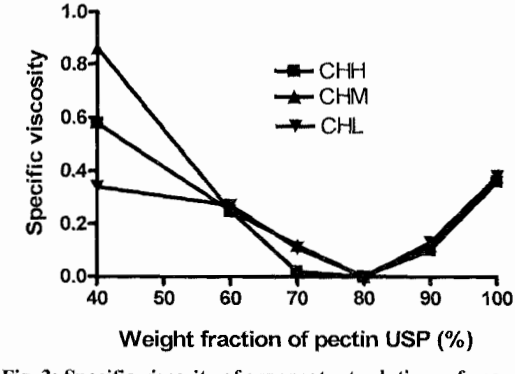

Fig. 3: Specific viscosity of supernatant solutions of pectin/chitosan mixtures in 0.1 acetate butter pH 3.8 at 37°C

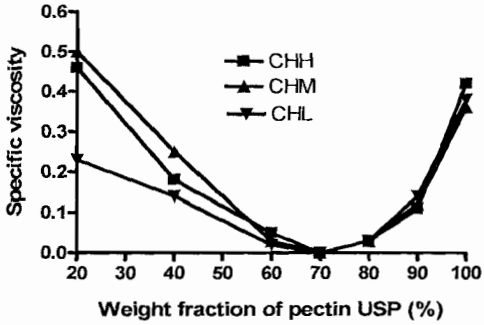

Fig. 4: Specific viscosity of supernatant solutions of pectin/chitosan mixtures in acetate butter pH 48 at 37°C

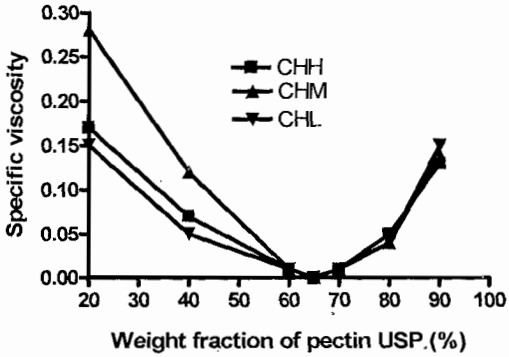

Fig. 5: Specific viscosity of supernatant solutions of pectin/chitosan mixtures in acetate buffer pH 5.6) at 37°C

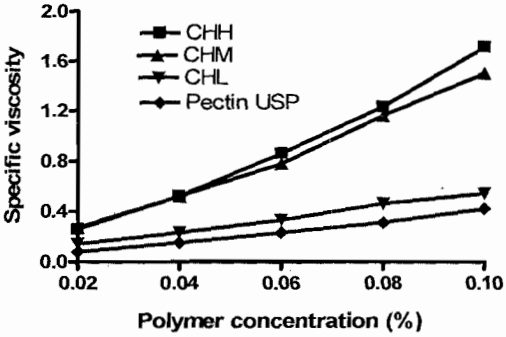

Fig. 6: Specific viscosity of pectin USP and molecular weight grades of chitosan in acetate buffer pH 4.8) at 37°C

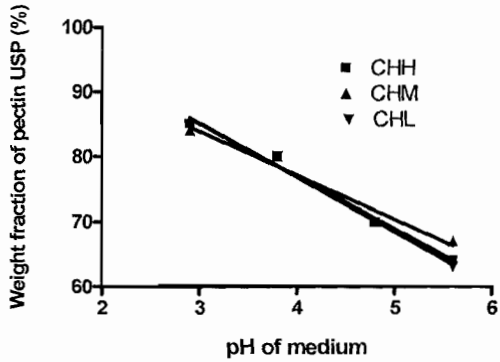

Fig. 7: The relationship between pH of medium and the weight fraction of pectin USP required for optimal PEC formation with chitosan at 37°C

The combination of solutions of two oppositely charged polyelectrolytes in a high enough concentration will result in PEC formation if the appropriate medium is used. In the current study, the mechanism of complex formation is the electrostatic interaction of the protonated amine group (NH_3^+) on chitosan molecule and the negatively charged carboxylate (COO^-) on pectin molecule (Meshali and Gabr, 1993; Rashidova *et al.,* 2004). At pH 1.5 no PEC appeared to form as the carboxylic acid groups on pectin remain totally unionised hence the pectin molecules will not have a high enough charge density to form a complex with chitosan. PEC formation were identified in the pH range 2.9-5.6 where the amine groups on chitosan would be ionised (pk_a 6.3) to interact with the carboxylate groups on pectin. The formation of a PEC results in the lowering of the specific viscosity of the supernatant solutions. Optimum conditions for PEC formation will thus occur at the minimum specific viscosity of the supernatant solutions. At this point, the specific viscosities of the supernatant solutions were observed to be almost the same as that of the pure solvents. Rashidova *et al.* (2004) used viscosity measurements to investigate PEC formation between chitosan and pectin and found that the toughness of the PEC was determined by the ratio of the amounts of the

heterogeneous polymers used. They reported that the toughest PEC was obtained by the use of equimolar quantities of pectin and chitosan and the toughness decreased with deviation of the mixture composition from equimolar.

Table 2 shows the effects of pH of medium and molecular weight on PEC formation between pectin and chitosan. The proportion of chitosan in pectin-chitosan PEC increased with increase in pH while that of pectin increased with a decrease in pH. This observation is due to the different states of ionisation of pectin and chitosan in solutions of different pH values and the flexibility of pectin molecule. At low pH, chitosan exhibits high ionisation while pectin has minimal ionisation. The formation of a PEC at low pH therefore requires a large amount of pectin to interact with a small amount of chitosan, and vice versa. Figure 7 depicts the relationships between pH of media used and the weight fraction of pectin required for maximal PEC formation with the different molecular weight grades of chitosan. All the plots were linear with R^2 values of CHH, 0.9890; CHM, 0.9689; and CHL, 0.9880. This indicates that the weight fractions of the polymers required for maximal PEC formation are pH-dependent.

The flexibility or rigidity of polymer chains has been shown by Takahashi *et al.* (1990) to have a significant role in the formation of a PEC. The flexibility of polymer chains ensures that the active sites on molecules involved in PEC formation are not greatly affected by stearic hindrance. The variation of the optimum ratio of pectin:chitosan required for PEC formation against pH is indicative of pectin being a flexible molecule. Takahashi *et al.* (1990) observed that the binding ratio of chitosan and sodium polyacrylate altered with a change in pH of medium while that of chitosan and sodium alginate remained constant and concluded that the polymer chains of sodium polyacrylate were more flexible than that of sodium alginate. Sakiyama *et al.* (1999) studied the swelling equilibra of PEC gels of dextran sulphate and chitosan in dilute NaOH or HCl of various pH values with or without NaCl. They suggested that the initial high density of the ionisable functional group as well as the flexibility of the acidic polymer chain contributed to the high pH sensitivity of the complexes.

The optimum weight ratio of pectin and chitosan required for PEC formation was almost the same ($p > 0.05$) for the 3 molecular weight grades of

Table 2: Effects of pH of medium and molecular weight on polyelectrolyte complex formation between pectin and chitosan

Medium	pH	Optimal ratio (w/w) of pectin:chitosan required for PEC formation		
		Pectin USP:CHL	Pectin USP:CHM	Pectin USP:CHH
0.1 M HCl	1.5	No PEC	No PEC	No PEC
0.1 M Acetic acid	2.9	5.67:1	5.25:1	5.67:1
Acetate buffer	3.8	4.00:1	4.00:1	4.00:1
Acetate buffer	4.8	2.33:1	2.33:1	2.33:1
Acetate buffer	5.6	1.70:1	2.03:1	1.78:1

CHL, Low molecular weight chitosan; CHM, Medium molecular weight chitosan; CHH, High molecular weight chitosan; No PEC, No formation of polyelectrolyte complex.

chitosan at the same pH (Table 2). Molecular weight thus appeared to have no effect on the optimum weight ratio of pectin to chitosan required for PEC formation in the various media used. These results are similar to that of Takeuchi *et al.* (2000). These workers investigated the formation of an alginate-chitosan PEC intended as a time-controlled release dry-coated tablet and found that an increase in the degree of acetylation in chitosan led to an increase in the amount of precipitate at the same weight fraction, while molecular weight of chitosan resulted in minimal changes in the amount of precipitate formed. Though large molecular weight chitosans are more flexible than smaller molecular weight chitosans (Chen and Tsaih, 1998) it would appear that the flexibility of chitosan chains, unlike that of pectin, have no significant effect on PEC formation.

CONCLUSIONS

The study has demonstrated that formation of a PEC between pectin and chitosan can occur in media of different pH values. The relative proportion of pectin and chitosan in the PEC was a function of the pH of the medium but appeared to be independent of the molecular weight of chitosan. A variation of the pH of medium and the use of the appropriate molecular weight grades of the polymers would produce PECs of pectin and chitosan having the requisite physicochemical properties for use in controlled drug delivery.

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