A preliminary study on chitosan/gelatin polyelectrolyte complex formation

YUJI YIN, ZONGYAN LI, YANBEI SUN, KANGDE YAO*
Research Institute of Polymeric Materials, Tianjin University, Tianjin 300072, China
E-mail: yinyuji@tju.edu.cn

Hydrogels are providing new opportunities for a variety of medical application. Examples include the use of hydrogels as skin substitutes, adhesives, matrices for drug delivery, and scaffolds for tissue engineering [1–4]. The chemical modification of natural polymers e.g. polysaccharides and proteins is a promising way for the development of such materials to make good use of their inherent biodegradability and biocompatibility.

Chitosan is a (l, 4)-linked 2-amino-2-deoxy-β-D-glucan, whose pyrone rings has reactive amimo and hydroxyl groups that can be used as modifying sites to improve their properties under mild conditions. Gelatin is a partially denatured derivative of collagen which is a well known fiber protein within the most extracellular matrixes (ECMs).

A polyelectrolyte complex (PEC) of chitosan/pectin has been useful for preparation of pH-responsive hydrogels [5, 6]. Polymer-polymer complex can be formed as a result of interchain interaction when two macromolecules are mixed in solution. The studies on the interaction between the flexible ampholytic gelatin and negatively charged linear polyanions e.g. sodium poly(styrene sulfate) or sodium poly(2-acrylamido-2-methylpropanesulfonate) have been reported [7, 8]. The results suggest that electrostatic interaction is the main driving force for these complexes formation.

Instead of gelatin interacting with synthetic polyanions, the interaction between flexible gelatin and positively charged rigid chitosan was arose more interesting recently. Our studies confirmed that the cytocompatibility of gelatin modified chitosan was improved via shielding the positively charged chitosan to a suitable charge density [9]. Moreover, bilayer chitosan-gelatin scaffolds were developed as materials for skin tissue engineering [10]. In the present work chitosan/gelatin polyledcrolyte complex formation was studied.

All conductometric measurements and pH titration were performed in a thermostated cell at 25 °C using a DDS-HA conductometer (LEICI Instrument Co., Shanghai, China). A typical formulation consisted of chitosan (CS, 720 kDa, Qingdao Institute of Pharmaceuticals, China) hydrochlorate aqueous solution (7.72×10^{-2} M) and 20×10^{-3} g/ml of gelatin (Gel, $M_w = 1.47 \times 10^5$, $M_n = 1.01 \times 10^5$, $pH_{iso} = 4.7$, Tianjin No. 3 Chemical Reagent Factory, China) aqueous solution. Titrations were followed by measuring the conductivity or pH value after each addition of an appropriate solution.

The absorbency at 510 nm was measured during the titration using a 756MC UV spectrophotometer (China), when the Gel aqueous solution was titrated with a 7.72×10^{-2} M CS hydrochloride solution. The specimen of CS/Gel PEC was obtained from the precipitate of turbidity titration and infrared (IR) spectra were measured using a Nicolet 5DX FT-IR spectrophotometer (USA).

There exist several interactions between CS and Gel macromolecules. The cationic polysaccharide CS interacts with ampholytic Gel electrostatically which mainly depends on the net charge negatively borne on Gel macromolecular chains. Therefore conductometric and pH titration could be used to follow their electrostatic interaction. Fig. 1 showed the pH and conductivity titration curves with Gel which existed initially at a Gel hydrochlorate form. There were two flex points in these curves, the former corresponded to the equivalent point of OH^- of NaOH reacting with H^+ of excess hydrochloric acid, while the latter relates to OH^- of NaOH neutralizing with proton of carboxyl groups of Gel.

From the volume between the two equivalent points and the concentration of Gel (g/ml), the apparent average molecular mass, $(\bar{M}_{eq})_{exp}$ of a charge borne on a Gel molecular chain can be expressed as following:

$$(\bar{M}_{eq})\,\exp = \frac{cv}{n_{eq}} \tag{1}$$

where c and v are the concentration (g/ml) and the volume (ml) of the Gel solution used, respectively, n_{eq} is the number of equivalent corresponding to the added volume of NaOH aqueous solution between the two equivalent points. This value can be compared to the theoretical one $(\bar{M}_{eq})_{th}$ given by:

$$(\bar{M}_{eq})_{th} = \frac{g}{n'_{eq}} \tag{2}$$

where g is the mass (g) of x amino acid moieties in the Gel, it usually chosen equal to 1000, and n'_{eq} is the number of ionizable amino acid residues present on the x moieties [11]. The data deduced from Equations 1

* Author to whom all correspondence should be addressed.

0022-2461

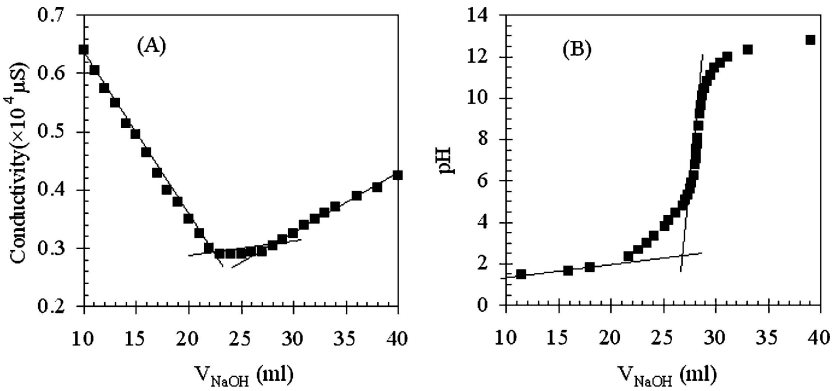

Figure 1 The conductometric (A) and pH (B) titration curves of Gel hydrochlorate aqueous solution with 0.1 M NaOH.

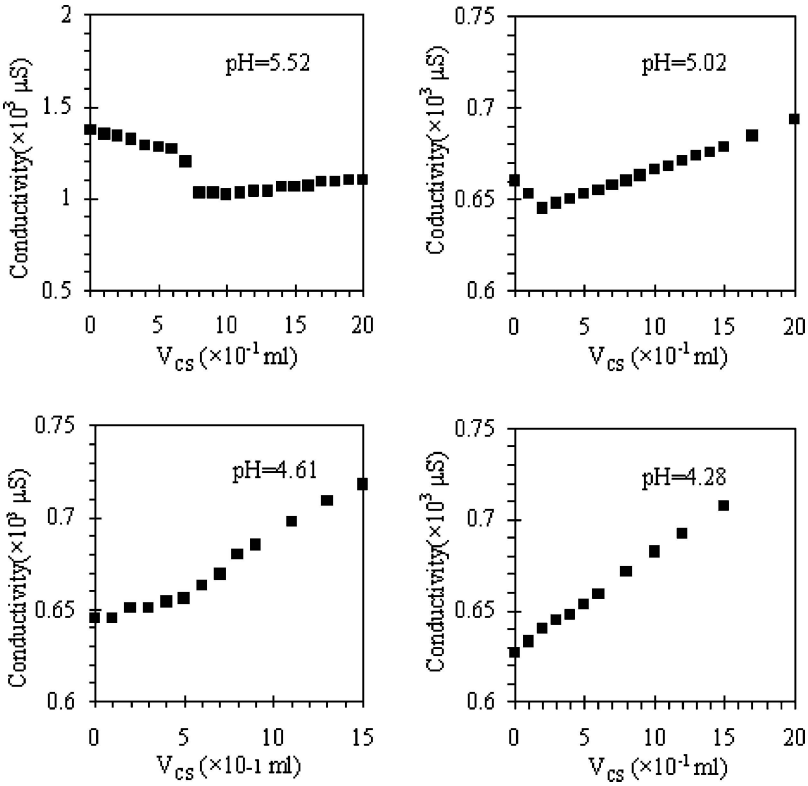

Figure 2 Variations in conductivity due to addition of CS hydrochlorate aqueous solution (7.72×10^{-2} M) to Gel aqueous solutions at different pH values.

and 2 are listed in Table I. The experimental values of $(\bar{M}_{eq})_{exp}$ obtained from pH and conductometric titrations were larger than that of $(\bar{M}_{eq})_{th}$. This means that part of the charges borne on Gel macromolecular chains were hided within the 3D hydrogen bonding network of —COOH containing Gel.

CS is a pH-sensitive polymer whose amino groups can be protonated depending on the pH of environment. When CS is mixed with ampholytic Gel in the case of pH of medium above the isoelectric point of Gel, where net charge is negative one, there occurs electrostatic interaction between the ammonium ions of CS salts and carboxylate groups of Gel.

The conductometric titration curves were shown in Fig. 2. The patterns were depending to the incorporation of CS to Gel. The equivalent point appeared at the pH > 4.7 (pH_{iso}) media (cf. Figs 2A and B), where the net charge of Gel is negative one. CS/Gel PEC can be formed via electrostatic interaction.

With the electrostatic interaction between $-NH_3^+$ and $-COO^-$ groups carried on CS and Gel, respectively in mind, one can deduce the apparent average molecular mass of the complexed anionic equivalent by means of the following relation:

$$(\bar{M}_{an,eq})_c = \frac{c' \times v'}{n''} \tag{3}$$

where c' and v' are the concentration (g/ml) and the volume (ml) of the Gel aqueous solution used, and n''

TABLE I Comparison between experimental and theoretical values of the average molecular mass of a charge borne on a Gel molecular chain, expressed in g/eq

	$(\bar{M}_{eq})_{th}$	$(\bar{M}_{eq})_{exp}$ (pH-metry)	$(\bar{M}_{eq})_{exp}$ (conductometry)
Gel	537	965	845

TABLE II Comparison between the values $(\bar{M}_{an,eq})_c$ of Gel expressed in g/eq for different pH

pH	5.52	5.02	4.61	4.28
$(\bar{M}_{an,eq})_c$	11532	47599	∞	∞

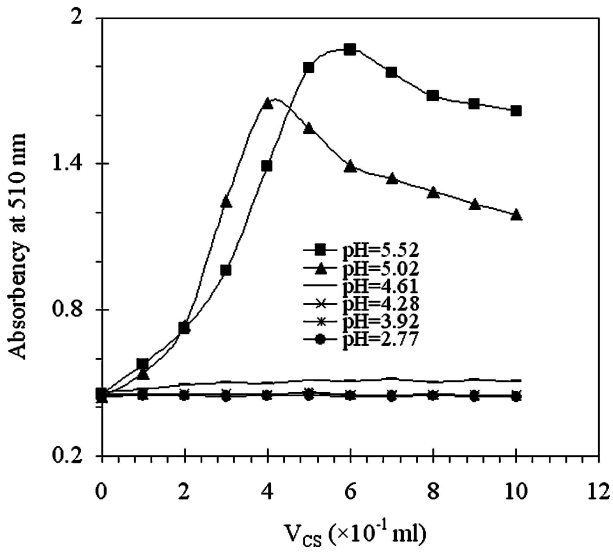

Figure 3 Absorbencies at 510 nm of Gel solution incorporated a 7.72 × 10^{-2} M CS hydrochlorate aqueous solution against its volume (V_{cs}) as a function of pH.

is the number of ammonium equivalents of CS at this state. The $(\bar{M}_{an,eq})_c$ for Gel are listed in Table II. The data reveal that the CS/Gel PEC forms in the cases of pH > pH_{iso}, again.

Absorbency at 510 nm of 20 × 10^{-3} g/ml of Gel solution against volume of CS hydrochlorate solution at various pH values was shown in Fig. 3. The absorbency of the Gel aqueous solution was pH dependent. In the low pH regions the absorbency of Gel solution were low irrespective of V_{cs} charging that implied the absence of strong interactions between Gel and CS, i.e. both the electrostatic interaction and hydrogen bonding. Fig. 3 also illustrated interaction between Gel and CS at pH higher than pH_{iso} of Gel. The absorbency increased with enhancing V_{cs}, up to a maximum value assigned to a stoichiometric association, and then decreased gradually. This phenomenon could be explained in terms of an excess of cationic CS would interfere the interaction between Gel and CS [12].

The IR spectrum of CS/Gel PEC was displayed in Fig. 4. CS was characterized by its saccharide structure at 902 and 1155 cm^{-1}, the amino band at 1589 cm^{-1} and the amide I band of the acetyl group at 1658 cm^{-1} [13]. The spectrum of Gel had characteristic peaks at 1537 cm^{-1} for N—H deformation of amide II and 1651 cm^{-1} for C=O stretching of amid I [14]. The enhancement in intensity of peak at 800–1200 cm^{-1} (saccharide structure and hydroxy groups) was observed in the spectrum of the PEC compared to that of Gel, revealing there was small amount of CS presented in the complex. This result implied that there was strong interaction between Gel and CS in the aqueous media that was enough to form PEC in situ.

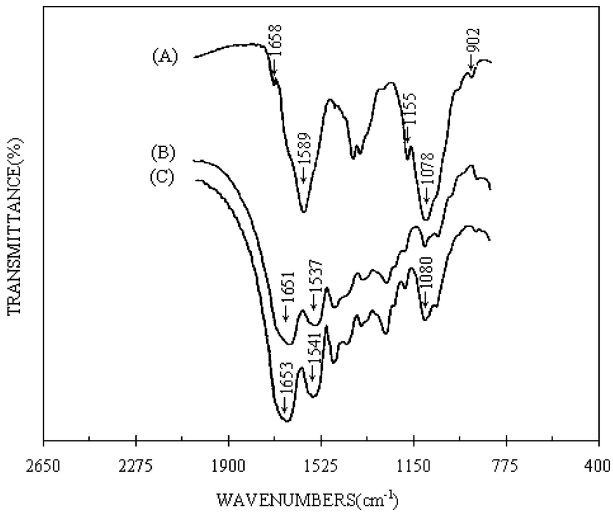

Figure 4 The IR spectra of CS (A), Gel (B) and CS/Gel PEC obtained at pH = 5.52 (cf. Fig. 3) (C).

A PEC can be formed via two ionizable polymers with apposite charges individually. This means that PEC forming reaction can only occur at pH values in the vicinity of the pKa interval of the two polyelectrolytes. The pKa of chitosan is ca. 6.5 while the pH_{iso} of gelatin is approximately 4.7. Our results indicated that the CS/Gel PEC only yielded at pH value lager than 4.7 and above pH 6.2 CS could precipitate from the solution. During complexation, polyelectrlytes can either coacervate, or form a more or less compact hydrogel. However, if ionic interactions are too strong, precipitation can occur and hinders the formation of hydrogels. Precipitaion can be avoided if electrstatic attraction is weakened by the addition of suitable salts. Their function was to reduce the attraction between the oppositely charged polyelectolytes through the counter-ions [15].

CS/Gel PEC can be reinforced by additional covalent crosslinking that lead to formation of hybrid polymer networks [16]. In general, the addition of covalent crosslinkers may influence the biocompatibility. Zhang *et al*. prepared composites films from chitosan and gelatin solution and their results suggested that the soft and flexible complex film of chitosan and gelatin, had better nerve cell affinity compared to chitosan, was a promising candidate biomaterial for nerve regeneration [17].

The properties of PEC are mainly determined by the degree of interaction between the individual polymers. This latter depends essentially on their global charge densities and determines their relative composition in the PEC. The lower the charge density of the polymer, the higher is the polymer proportion in the PEC, since more polymeric chains are required to react with the other polymers. This might be one possible reason for the existing small amount of CS in the CS/Gel PEC as confirmed by IR studies. Moreover, the mass ratio of Gel and CS in the PEC calculated from data of Fig. 2 being 4.8 and 19.4 at the pH being 5.52 and 5.02, respectively.

The relative proportion and the chemical environment were the main factors influencing swelling behavior of PEC as well. It is possible to modulate the

composition of PEC via controlling the complexation reaction degree. In addition, the pH of the solution, environmental temperature, ionic strength and feeding order of polymer solutions also affected PEC formulation [15]. During complexation, the solubility, rheology, conductivity and turbidity of the polymer solutions or the pH of the supernatant needed to be considered.

This work is the first describing the interaction between CS and Gel. It is shown that a polyelectrolyte complex is formed when pH > 4.7 (pH_{iso} of Gel). Charge density is an important feature for the polyelectrolyte in determining its extent of Gel binding.

Acknowledgment

The authors are grateful to the National Natural Science Foundation of China via Grants No. 59883002, 50203007 for their financial support

References

1. O. KIYOHAYA, I. MASAYUKI, I. TOSHIAKI and F. MASANORI, *Biomaterials* **24** (2003) 3437.
2. T. B. REECE, T. S. MAXEY and I. L. KRON, *Am. J. Surg.* **182** (2001) 40S.
3. A. S. HOFFMAN, *Adv. Drug Delivery Rev.* **43** (2002) 2.
4. J. L. DRURY and D. J. MOONEY, *Biomaterials* **24** (2003) 4337.
5. K. D. YAO, J. LIU, G. X. CHENG, X. D. LU and H. L. TU, *J. Appl. Polym. Sci.* **60** (1996) 279.
6. K. D. YAO, H. L. TU, F. CHENG, J. W. ZHANG and J. LIU, *Die Angew. Macromol. Chem.* **245** (1997) 63.
7. W. A. BOWMAN, M. RUBINSTEIN and J. S. TAN, *Macromolecules* **30** (1997) 3262.
8. J. H. E. HONE, A. M. HOWE and T. COSGROVE, *Macromolecules* **33** (2000) 1206.
9. J. S. MAO, Y. L. CUI, X. H. WANG, Y. SUN, Y. J. YIN, H. M. ZHAO and K. D. YAO, *Biomaterials*. **25** (2004) 3973.
10. J. S. MAO, L. G. ZHAO, K. D. YAO, Q. X. SHANG, G. H. YANG, Y. L. CAO and J. BIOMED, *Mater. Res.* **64A** (2003) 301.
11. M. N. TARAVEL and A. DOMARD, *Biomaterials* **14** (1993) 930.
12. C. REMUNAN-LOPEZ and R. BODMEIER, *Int. J. Pharm.* **135** (1996) 63.
13. N. SHANMUGASUNDARAM, P. RAVICHANDRAN, P. N. REDDY, N. RAMAMURTY, S. PAL and K. P. RAO, *Biomaterials* **22** (2001) 1943.
14. M. C. CHANG and J. TANAKA, *ibid.* **23** (2002) 4811.
15. J. BERGER, M. REIST, J. M. MAYER, O. FELT and R. GURNY, *Eur. J. Pharm. Biopharm.* **57** (2004) 35.
16. Y. J. YIN, K. D. YAO, G. X. CHENG and J. B. MA, *Polym. Inter.* **48** (1999) 429.
17. M. CHENG, J. DENG, F. YANG, Y. GONG, N. ZHAO and X. ZHANG, *Biomaterials* **24** (2003) 2871.

Received 21 October 2004
and accepted 11 March 2005