Available online at www.sciencedirect.com
SCIENCE DIRECT

Carbohydrate Polymers 64 (2006) 473–480

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

# Synthesis of pH-sensitive PVP/CM-chitosan hydrogels with improved surface property by irradiation

Long Zhao [a,*], Ling Xu [b], Hiroshi Mitomo [a], Fumio Yoshii [c]

[a] *Department of Biological and Chemical Engineering, Gunma University, Tenjin-cho, Kiryu, Gunma 376-8515, Japan*
[b] *Department of Chemistry and Material Science, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152-0033, Japan*
[c] *Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Watanuki-machi, Takasaki, Gunma 370-1292, Japan*

Received 23 June 2005; received in revised form 11 December 2005; accepted 14 December 2005
Available online 20 January 2006

---

## Abstract

In order to develop a nontoxicity, biocompatibility, pH-sensitive hydrogel system with potential ability to immobilize and absorb proteins, the hydrogel membranes of poly(*N*-vinylpyrrolidone) (PVP) and carboxymethylated chitosan (CM-chitosan) blend were prepared with electron beam (EB) irradiation at room temperature. The FTIR spectroscopic, mechanical property, swelling behavior, and surface property of the hydrogel membrane were measured. The capacity of these gel membranes to adsorb bovine serum albumin (BSA) was investigated with varying content of CM-chitosan by static adsorption experiment at pH 7.4. The mechanical and swelling properties were improved obviously after adding CM-chitosan into PVP hydrogels. The changes of contact angles of water, glycerin and octane drops on the blend membranes surface with increasing CM-chitosan content indicated that the CM-chitosan molecules were more hydrophilic than PVP molecules, and cause a larger polar component of surface free energy. The incorporation of CM-chitosan introduced the protein adsorptive property into blend system. From these preliminary evaluations, it is possible to conclude that these materials have potential for applications in the biomedical field.

*Keywords:* Poly(*N*-vinylpyrrolidone); Carboxymethylchitosan; Hydrogels; Irradiation; pH-sensitive; Bovine serum albumin; Adsorption

---

## 1. Introduction

PVP, a synthetic polymer, has good biocompatibility and for many years has been applied as a biomaterial or additive to drug compositions, e.g. as a blood plasma expander (Altemeier, 1954) and as vitreous humor substitute (Hong et al., 1998). Under action of ionizing radiation PVP undergoes crosslinking and lead to the formation of PVP hydrogel (Rosiak, Ulanski, Pajewski, Yoshii, & Makuuchi, 1995). PVP hydrogel has excellent transparency and biocompatibility. It has been used as a main component of temporary skin covers or wound dressing. However, the hydrogel of PVP itself is of limited applicability because of its inferior mechanical properties. So a series of PVP hydrogels prepared by PVP blends play a significant role as biomedical materials (Huglin & Zakaria, 1986).

Chitosan, a copolymer of glucosamine and *N*-acetyglucosamine unites linked by 1-4 glucoside bonds, is obtained by *N*-deacetylation of chitin, which is one of the most abundant natural amino polysaccharide, and has been reported to have a variety of applications in pharmacy industry and biotechnology (Majeti & Kumar, 2000). Recently, carboxymethlated chitin derivatives such as carboxymethylchitin (CM-chitin), carboxymethylchitosan (CM-chitosan) are being found to have some beneficial medicinal and pharmaceutical application (Majeti & Kumar, 2000; Tokura et al., 1990). Compare with chitin and chitosan, CM-chitin and CM-chitosan have some special properties such as: good biodegradability, good blood-compatibility, no antigenicity, weak mitogentic activity, adjuvant activity (short life), and water-solubility (Saiki, Watanabe, Tokura, & Azuma, 1992). On the other hand, carboxymethylated chitin derivatives still keep the advantages of chitin and chitosan, which include biocompatibility, antibacterial activity, nontoxicity, adsorptive activity for

---

[*] Corresponding author. Present address: Advanced Materials Research Center, R&D Laboratories, Nissin Electric Co. Ltd, 47, Umezu-Takase-Cho, Ukyo-ku, Kyoto 615-8686, Japan. Tel.: +81 90 1848 7246; fax: +81 75861 4878.

*E-mail addresses:* Zhao_Long@nissin.co.jp (L. Zhao), long@rd.nissin.co.jp (L. Zhao).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.12.014

some special proteins. Therefore, carboxymethylated chitin derivatives might be good candidates for application in various biomedical fields.

Over the past few decades, the study of polyelectrolyte hydrogels responsive to environmental stimuli such as pH, temperature, ionic strength, electric filed, solvent composition, light, pressure, and sound or a specific chemical trigger has been a very active field of research. Among them, polyelectrolyte gels based on polysaccharides and their derivatives have attracted much attention due to their unique properties (Dumitriu, Vidal, & Chornet, 1996). Recently, the blend polyelectrolyte gels of polysaccharides and synthetic polymers are investigated extensively because of their special properties and comparatively low cost (Cascone et al., 2001; Crescenzi et al., 1997). In addition, these blend gels are also like soft rubbery membranes with relatively high elasticity, resembling the body tissue.

Some researchers reported that incorporation of polysaccharides such as kappa-carrageenan and chitosan into the PVP/water system obviously increased the gel strength and equilibrium degree of swelling of PVP hydrogel (Risbud & Bhat, 2001; Zhai, Ha, Yoshii, & Makuuchi, 2000). Comparing with chitosan, CM-chitosan, an amphoteric polyelectrolyte, which has good hydrophilicity, should exhibit good miscibility with PVP in aqueous solution and can be easily used to prepare homogenous polymer blending. In our previous work, we applied irradiation to prepare hydrogels based on PVA and CM-chitosan and found that the mechanical properties and equilibrium degree of swelling improved obviously after adding CM-chitosan into PVA. Especially, it can provide satisfying antibacterial activity against *Escherichia coli*, thus it can be widely used in the field of biomedicine and pharmacy (Zhao et al., 2003).

A series of PVP/CM-chitosan blend hydrogels were prepared by irradiation technique in this work. It was well known that the chemical structure, electric charge, hydrophilicity and hydrophobicity, swelling behavior, and flexibility of the biomedical polymers will affect their application in the biological surroundings (Tsuruta et al., 1993). In order to investigate the effects of above factors on application, the characteristics of the prepared hydrogels were studied in detail. The surface property of these samples was determined by the measurement of contact angle. Moreover, bovine serum albumin (BSA) adsorption behavior of these gels was also investigated. The aim of this study is to develop a novel hydrogel system with potential for applications in the biomedical field.

## 2. Experimental

### *2.1. Material*

PVP-K90 ($M_w$: 360,000) was supplied by Kisida Chemical Co., Ltd, Japan. CM-chitosan ($M_w$: 30,000) was obtained from Koyou Chemical Industral Co., Ltd, Japan, which has a deacetylation degree of 84% and a carboxymethyl group substitution degree of 0.91. Bovine serum albumin (BSA) used for adsorption study was obtained from ICN Biomedicals, Inc. All the other chemicals were analytical grade.

### *2.2. Preparation of blend hydrogels*

CM-chitosan powder was first dissolved in distilled water and then added to PVP solution, and continuously stirred to form homogeneous mixture with heating at 60–70 °C for 30 min. PVP in the final solution was set to 10 wt%, and the content of CM-chitosan varied from 1 to 10 wt%. The gel-like solution was poured into a Petri dish for irradiation using a beam current of 1 mA and acceleration energy of 2 MeV generated by the Cockroft Walton electron accelerator. Prior to their use, the gel membranes were immersed in distilled water for 72 h at room temperature for removing the sol, and then dried in a vacuum at 50 °C for 6 h.

### *2.3. Characterization*

#### *2.3.1. FTIR analysis*

FTIR of CM-chitosan, PVP, and blend hydrogel membranes was performed by Fourier transform infrared spectroscopy (Shimadzu FTIR-8100A spectrometer).

#### *2.3.2. Mechanical properties*

In order to estimate the mechanical properties of crosslinked material, hydrogels were examined in relaxed state (after irradiation with the original water content). The hydrogels were cut into dumbbells (ASTM D-1822-L), and then tensile strength and elongation at break were measured by Strograph-R1 Material Tester (Toyoseiki Co., Ltd, Japan) at a crosshead speed of 10 mm/min. At least five measurements for each sample were recorded and a mean value was calculated.

#### *2.3.3. Gel fraction*

After irradiation, the gel content in the sample was estimated by measuring its insoluble part after extraction in distilled water at room temperature for 72 h. The remained gel was dried to constant weight at 50 °C. Gel fraction was measured gravimetrically

$$\text{Gel fraction (\%)} = W_g/W_o \times 100 \tag{1}$$

where $W_g$ is the weight of dry gel after extraction and $W_o$ is the initial of dry gel.

#### *2.3.4. Swelling behavior*

Hydrogel samples were immersed in distilled water for different times at room temperature. After the excessive surface water was removed with filler paper, the weight of swollen gel was measured at various time intervals. The procedure was repeated until there was no further weight increase. The swelling ratio was determined according to the following equation

$$\text{Swelling ratio} = W_t - W_d/W_d \tag{2}$$

where $W_t$ is the weight of the gel after swelling and $W_d$ is the weight of dried gel before swelling. The equilibrium degree of swelling was also calculated by this equation changing $W_t$ to $W_e$ which was the equilibrium weight of swollen gel.

Effect of some factors such as acid, base, and inorganic salt on swelling behavior of the hydrogels was also performed by determining the swelling ratio of samples at above condition.

### *2.3.5. Contact angle measurement and calculation of surface free energy*

The contact angle experiments were performed using a Kyowa Kaimen Science Instrument CA-X. The contact angles of water, glycerol drops on the gel membranes were examined at room temperature. In the fully hydrated case, the octane contact angle was employed to quantify polar interactions across the polymer–water interface for assessment of surface hydrophilicity of gel. At least 10 measurements for each sample were recorded and a mean value was calculated. The surface energy of samples was calculated from the contact angle and the surface tensions of liquids by means of the Young's equation.

### *2.4. Adsorption of bovine serum albumin (BSA)*

Phosphate buffer (pH 7.4) was used to prepare BSA solution with a 1 g/l initial concentration. The gel membranes were cut into small pieces, put into 250 ml beaker containing BSA solutions. And then the mixture was agitated using a magnetic stirrer for 24 h at room temperature. BSA concentration was calculated from the absorbance at 280 nm using UV–Vis spectrometer (Shimadzu UV-265FS, Japan). By comparing the initial and final concentrations, the adsorption capacities could be calculated.

## 3. Results and discussion

### *3.1. Characteristics of PVP/CM-chitosan blend hydrogels*

### *3.1.1. FTIR spectroscopic of the hydrogels*

FTIR spectra of CM-chitosan, PVP and blend gel membrane are shown in Fig. 1. Spectra of PVP show a peak at 1288 $cm^{-1}$, a characteristic peak for C–N stretching. For CM-chitosan, the broad bands at 1587–1625 $cm^{-1}$ correspond to carboxy group and $NH_3^+$ (Liu, Yang, Li, & Yao, 2001). The peak appears at ca. 1414 $cm^{-1}$ in CM-chitosan spectrum is contributed to the –$CH_2COOH$ group. The IR spectrum of PVP/CMCTS blend hydrogel contains the characteristic adsorption bands of the two polymer components. Especially, the shoulder peak of $NH_3^+$, which appears at 1597 $cm^{-1}$, confirms the contribution of CM-chitosan to the gel. In our previous work, we reported the graft reaction between CM-chiosan and PVA in PVA/CM-chitosan blend gels prepared by irradiation, and the peak at 1597 $cm^{-1}$ for $NH_3^+$ was also clearly observed in their FTIR spectra (Zhao et al., 2003). In addition, Abad et al. reported the graft reaction in irradiated PVP/kappa-carrageenan blend gels, and also found that the major functional groups of both PVP and kappa-carrageenan appeared in their FTIR spectra (Abad, Relleve, Charito, & Dela-Rosa, 2003). The similar results of this study imply that CM-chitosan was immobilized onto crosslinked PVP molecular chain by grafting reaction.

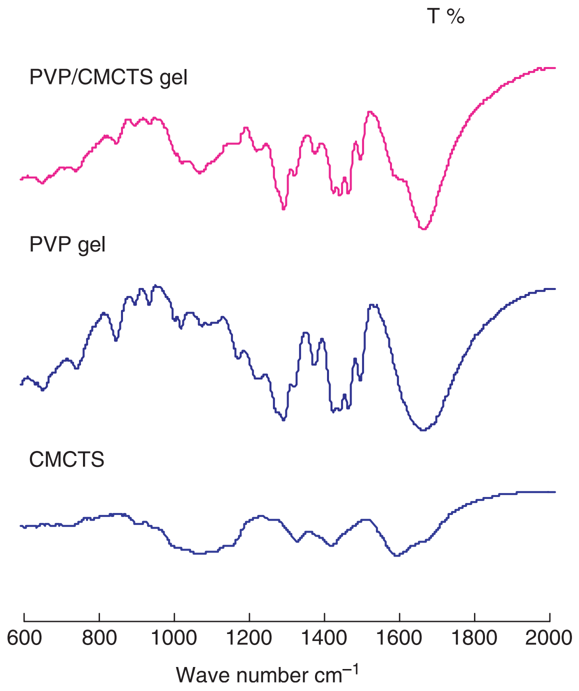

Fig. 1. FTIR spectra of PVP/CM-chitosan blend gel (gel portion), PVP gel (gel portion), and CM-chitosan. Gel samples were prepared by irradiation at 40 kGy. (PVP/CMCTS gel: 10%PVP/3%CM-chitosan; PVP gel: 10% PVP; CMCTS: CM-chitosan).

### *3.1.2. Mechanical properties of PVP/CM-chitosan blend hydrogels*

The effects of the CM-chitosan content in the PVP/CM-chitosan blend hydrogel on the mechanical properties are shown in Figs. 2 and 3. PVP/CM-chitosan had improved mechanical properties according to the results of tensile

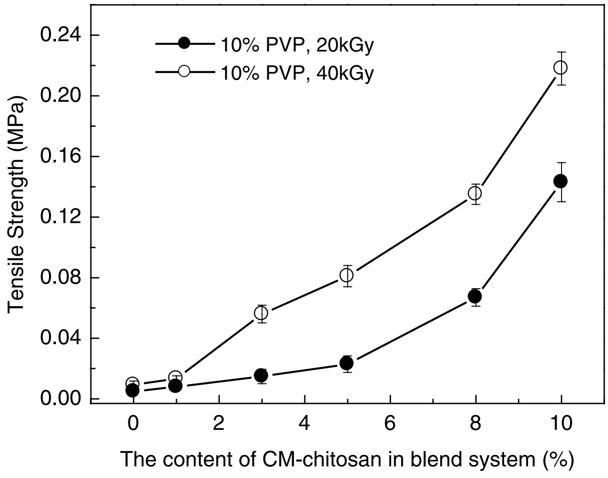

Fig. 2. Tensile strength of PVP/CM-chitosan blend hydrogels prepared by irradiation.

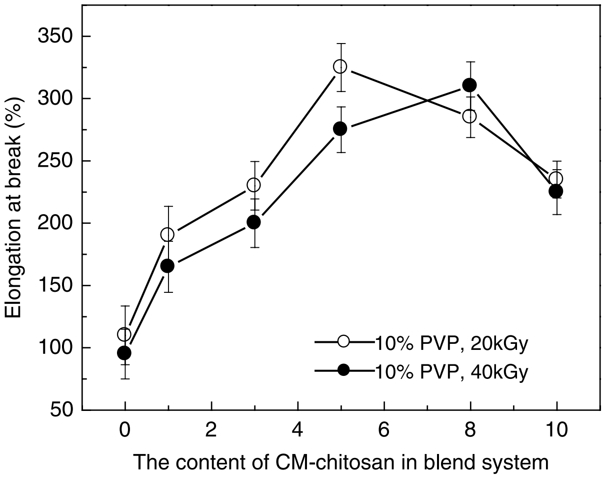

Fig. 3. Elongation at break of PVP/CM-chitosan blend hydrogels prepared by irradiation.

strength and elongation. Tensile strength increased with increasing CM-chitosan content in the blend system and dose. The elongation at break increased with the increasing amount of CM-chitosan in the blend system, up to 5% (20 kGy) or 8% (40 kGy) of CM-chitosan. Then, it decreased but still remained higher than that of pure PVP gel. Up to 5% of CM-chitosan, the elongation at break decreased with the increase dose and showed opposite behavior when compared with the tensile strength. From the results of Figs. 2 and 3, it was found that PVP/CM-chitosan blend hydrogels had improved elasticity and flexibility, so this kind of hydrogel is recommended as wound dressing material.

### 3.1.3. Gel fraction of PVP/CM-chitosan blend hydrogels

Recently, blend hydrogels of polysaccharides and synthesized polymer prepared by irradiation technical have been studied widely. Irradiation of these blends would result in the simultaneous crosslinking of synthesized polymer, degradation of polysaccharides and grafting of polysaccharides to crosslinked polymer chains, but eventually lead to the formation of hydrogel. The overall effect of these formation processes could influence the gel fraction and swelling of the hydrogels. This is clearly demonstrated in Figs. 4 and 5.

The gel fraction of PVP/CM-chitosan blend hydrogels versus the dose in the blend system with various CM-chitosan concentration are shown in Fig. 4. The gel fraction of PVP/CM-chitosan blend gels increased rapidly and then leveled off above the dose of 20 kGy. But at high dose, gel fraction decreased slightly due to the degradation of the gels. As expected, higher gel fraction is achieved in pure PVP. The gel fraction decreased with the increase of CM-chitosan content in PVP/CM-chitosan blend systems indicating that CM-chitosan is a crosslinking inhibitor whereby the PVP competes with CM-chitosan for the free radicals. The results also showed that the part of CM-chitosan could not be removed by extraction. In our previous work, we discussed the similar result occurred in PVA/CM-chitosan blend system (Zhao et al., 2003). The explanation from our previous work is also believed to fit PVP/CM-chitosan blend system. It was found that irradiation could not induced crosslinking of CM-chitosan in aqueous solutions with low concentration of the polymer (below 10 wt%). In this study, PVP/CM-chitosan/water blend system were irradiated, PVP radicals reacted easily with the other PVP molecules to form crosslinked PVP network. CM-chitosan macroradicals are considered to be separated by water and PVP molecules and placed apart from each other. However, CM-chitosan macroradicals were capable to contact with PVP chains around and then graft onto PVP network. The similar results were also observed in the hydrogels from PVP blended with kappa-carrageenan (Abad et al., 2003).

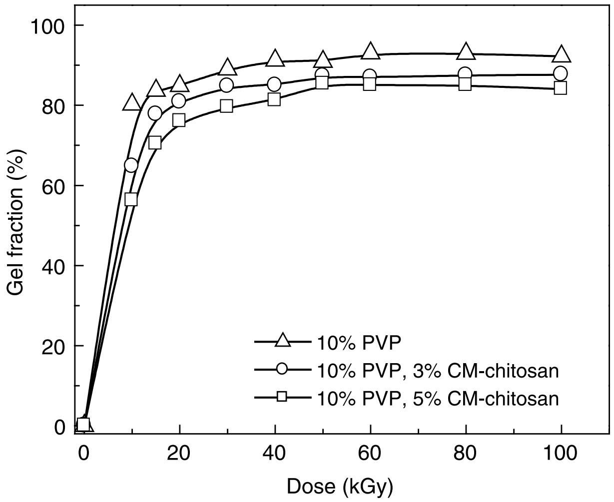

Fig. 4. Gel fraction of PVP/CM-chitosan blend hydrogels prepared by irradiation.

### 3.1.4. Swelling behavior of PVP/CM-chitosan blend hydrogels

The equilibrium degree of swelling of the PVP/CM-chitosan blend hydrogels with different CM-chitosan content as a function of dose are shown in Fig. 5. The swelling curves

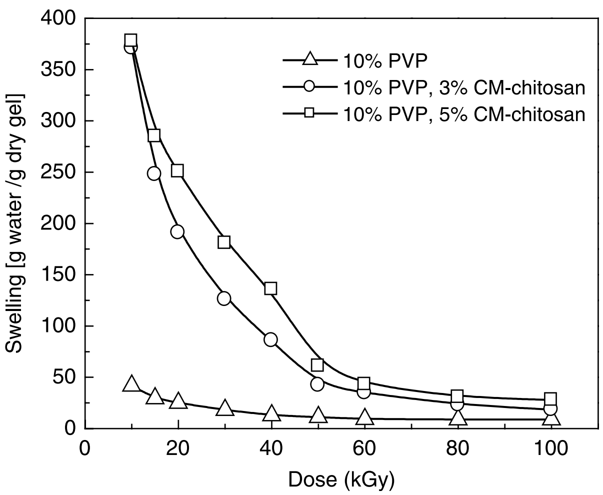

Fig. 5. Swelling of PVP/CM-chitosan blend hydrogels prepared by irradiation.

indicate a typical swelling-dose relationship. The swelling of hydrogels decreased with the dose, but increased when the CM-chitosan content was increased in the blend due to high hydrophilicity of CM-chitosan. A PVP/CM-chitosan hydrogel would in fact have a higher swelling capacity than PVP/chitosan hydrogel (Nho & Park, 2002; Risbud & Bhat, 2001). Both of them have similar structures except for the presence of carboxymethyl group in chitosan. Since, the gel fractions of these blend gels are lower than pure PVP (Fig. 4), thus as expected the equilibrium swelling is higher than that of pure PVP (Fig. 5).

Fig. 6 shows the swelling kinetics of PVP/CM-chitosan blend hydrogels in distilled water. The hydrogels reached an equilibrium swelling ratio within 2 h and then leveled off. Theoretically, when chains of polyelectrolyte forming a network contain ionizable groups, due to intermolecular coulombic repulsion, hydrogen-bonding, and polar forces, the mobile counterions present in gels develop a large swelling pressure (Rosiak & Ulanski, 1999), therefore, the polymer interactions are enhanced and should cause a very high sorption rate. However, in our work, as shown in Fig. 6, the sorption rate of blend gels was slightly slower than PVP gel. The blend gels were polyampholytic electrolytes because opposite charged groups were introduced into blend system during the fabrication procedure. The opposite charged groups from CM-chitosan enabled to keep a strong electrostatic attraction in the blend network systems and lead a higher electrostatic force at early stage of sorption. This might affect the equilibrium time.

Because the blend gels of PVP/CM-chitosan were polyelectrolyte complex, swelling of the gels showed a typical behavior of polyelectrolyte under different ionic strength of solution (Fig. 7a). The increase of ionic strength in the solution led to an increase of the osmotic pressure of the solution ions and a decrease of the excess osmotic pressure of the network ions resulted in deswelling of the gel.

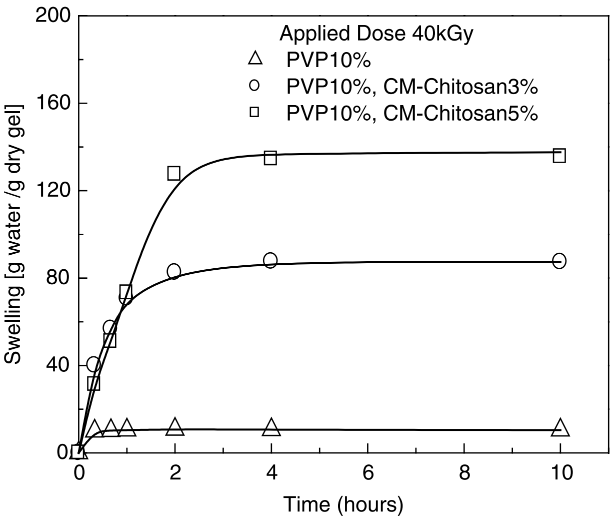

Fig. 6. Swelling kinetics of PVP/CM-chitosan blend hydrogels in distilled water.

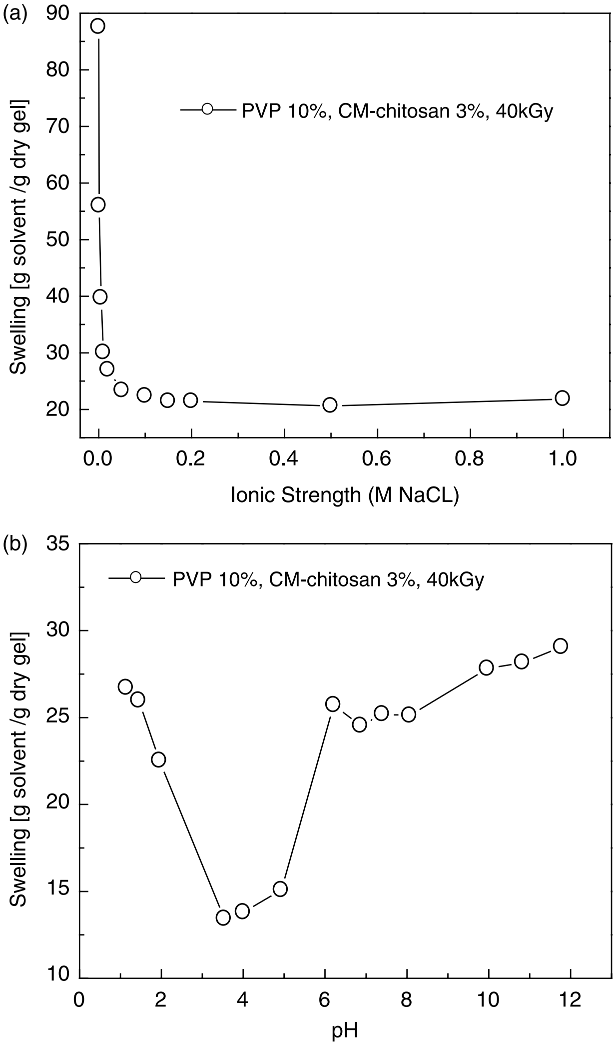

Fig. 7. (a) Swelling of a PVP/CM-chitosan blend hydrogel in NaCl aqueous solutions of various ionic strengths; (b) swelling of a PVP/CM-chitosan blend hydrogel in aqueous solutions of several pH values.

It is well known that swelling or deswelling in response to changes of pH is a typical phenomenon of polyelectrolyte gels, but non-ionic polymer networks such as PVP, PVA gel never appear the pH-sensitivity. In the present work, the pH of the external solution has been varied in the range 1–12 and the effect on the swelling ratio has been observed. The results are depicted in Fig. 7b. The blend gel swelled at low pH ($<3.5$) and also in the pH range $5.0<\text{pH}<12$. However, deswelling occurred in the range of pH 3.5–5.0. It was consistent with the hydrogel prepared from CM-chitosan, which was described in our previous work (Zhao, Mitomo, Nagasawa, Yoshii, & Kume, 2003). This can be explained that the blend gel contains both carboxyl and amino groups, and thus forms a network with oppositely charged structures, which could change the charge state of the gel varying with pH. Some researchers used CM-chitosan to synthesis blend membrane with poly(ethersulfone), and found that composite membranes possess positively charged characteristic at low pH and negatively charged characteristic at high pH by measuring the zeta potential (Zhao, Wang, & Wang, 2002, 2003). Their results confirmed that incorporation of the amphoteric CM-chitosan with the non-ionic component could improve charged characteristics of the blend system, and thus result in a pH-sensitivity. The pH sensitivity of the special gels synthesized in this work is believed to play an important role in biomedical application.

### *3.1.5. Contact angle and surface free energy of blend gel membranes*

For a material to be applied for the biomedical purpose, its bulk and surface properties are both important. Surface and interfacial behavior of biomedical materials in the aqueous environment play a distinct role in evaluating their biocompatibility with living body. The surface free energy of a material is correlated to the contact angle, which is directly related to its wettability. In addition, the contact angle measurement is inexpensive and easy to perform; this method can provide a convenient means for gaining insight into the surface properties of a biomedical material.

Many hydrogels are like soft rubbery membranes with relatively high elasticity, resembling the body tissue, possess low interfacial energy and excellent biocompatibility (Peppas, 1987). Since, hydrogels consist of substantial amount of water, from the practical point of view, it is advisable to discuss the wettability at a fully hydrated state. Therefore, the wettability of gel membranes not only in dry state but also in hydrated state is worthy of investigation.

The contact angles of water, glycerol drops on the gel membranes were investigated as a function of CM-chitosan content of the initial mixture in the blend hydrogels (xerogels) at room temperature. Water-contact angles of these blend gel membranes were below 90° and decreased with increasing the CM-chitosan content (Fig. 8). The result indicated the high hydrophilic nature of these samples. Moreover, it can be seen that the CM-chitosan molecules were more hydrophilic than PVP molecules, and may cause a larger polar component of surface free energy ($\gamma^p$).

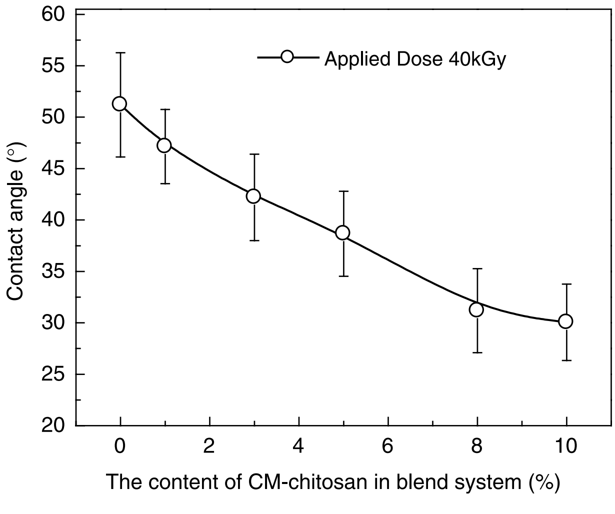

Fig. 8. Water-contact angles of PVP/CM-chitosan blend hydrogels (xerogels) at room temperature as a function of CM-chitosan content of the initial mixture.

Kaelble and co-workers have discussed surface and interfacial behavior of the materials to be applied for biomedical purpose, and developed the surface energetics criterion for analyze the surface energy to assess the bioadhesion and biocompatibility (Kaelble & Moacanin, 1977). They found that some surface energetic relations could be applied to define the dispersion and polar surface energies of biological and implant surface. Some of these surface energetic relations can be used to deduce the Young's equation. In this work, from the contact angles of test liquids, the surface free energy could be estimated by means of the Young's equation

$$\gamma_l(1+\cos\theta) = (2\sqrt{\gamma_s^d\gamma_l^d} + \sqrt{\gamma_s^p\gamma_l^p}) \quad (3)$$

where $\gamma$ is the surface free energy (or surface tension), which has two parts, namely, the dispersion (non-polar) and polar components ($\gamma = \gamma^d + \gamma^p$) (Fowkes, 1963). The superscripts of d and p mean the dispersion and polar components, respectively. The subscripts of l and s mean the liquid and solid (gel membrane), respectively. According to Eq. (3), the surface free energies of these blend gels with different CM-chitosan content were calculated, and the results are shown in Table 1.

With increasing content of CM-chitosan, the surface free energy of the gel membranes increased. It was found that the increase of the surface tension was due to the significant increase in the polar ($\gamma^p$) component for the gel membranes, although the dispersion ($\gamma^d$) component increased also. Thus, it is clear that the blend gel membranes have a high surface free energy and with high hydrophilicity. These changes were due to the existence of the amphoteric CM-chitosan in the gel membranes. From the surface tension results of these dry gel membranes, it is possible to predict the results in a hydrated state. In order to response to an aqueous environment, more hydrophilic components should migrate to the surface of the material, so contact angle on all surfaces should decrease. And after hydration, all surfaces should show higher polar ($\gamma^p$) component and higher surface tensions than dry state.

Octane is a simple hydrophobic organic solvent, which is often used to test the wettability of biomaterials in the aqueous

Table 1
Surface free energies of PVP/CM-chitosan blend gel membranes

| | Materials | Surface free energy (mJ/m$^2$) | | |
|---|---|---|---|---|
| | | $\gamma^d$ | $\gamma^p$ | $\gamma = \gamma^d + \gamma^p$ |
| Liquids[a] | Water | 21.8 | 51.0 | 72.8 |
| | Glycerol | 37.2 | 26.2 | 63.4 |
| Samples | PG[b] | 6.53 | 44.89 | 51.42 |
| | PC3G[c] | 7.29 | 50.68 | 57.97 |
| | PC5G[d] | 8.14 | 52.54 | 60.68 |

[a] Values obtained from Fowkes (1963).

[b] Degree of contact angles: water, 51 ± 5.1; glycerol, 55 ± 3.0.

[c] Degree of contact angles: water, 42 ± 4.5; glycerol, 48 ± 3.1.

[d] Degree of contact angles: water, 38 ± 4.1; glycerol, 44 ± 2.8.

Table 2
Octane-contact angles of the fully hydrated gel membranes

| Sample code[a] | PG | PC3G | PC5G |
|---|---|---|---|
| Composition (PVP/CM-chitosan/$H_2O$) | 10/0/90 | 10/3/87 | 10/5/85 |
| Water uptake (g water/g dry gel) | 13 | 88 | 138 |
| Contact angle (degree) | 126±4.4 | 143±3.1 | 150±4.2 |

[a] Gel membranes were prepared by irradiation at 40 kGy.

phase, because it is difficult to measure the water and glycerol contact angles in hydrated state. Table 2 shows the results of octane-contact angles of the fully hydrated gel membranes. The results showed that the gel membranes possess the very high hydrophilic nature and agree the prediction mentioned above.

We should note that the contact angles of test liquids were not so stable, and the deviation was relative large, which will affect the accuracy of surface tension. However, the analysis based on these results still can offer enough evidence to recognize the surface change of blend gel membranes with different CM-chitosan contents. This discussion will help us to make a good understand for their application in biomedical filed.

### 3.2. BSA static adsorption onto blend gels

Biomolecules and cells can be immobilized on and within many different supports using a variety of techniques. Some specific properties of hydrogels make them particularly suitable for this purpose (Gombotz, Hoffman, & Horbett, 1986; Hoffman, 1981; Kaetsu, 1981). Molecules and cells immobilized on or within hydrogels are more likely to retain their biological activity for longer time. Therefore, the study and application of immobilized biologically active molecules and cells have attracted much interest in both medicine and industry. Protein adsorption onto a variety of hydrogels has been studied with various methods and for many purposes. Among them, polyelectrolyte hydrogels with environmental sensitivity make them ideal materials for adsorption and enrichment of proteins and other biomolecules. In this study, CM-chitosan with carboxyl group and amino groups was introduced into non-ionic PVP hydorgels, which results in the polyamphoteric characteristic of the blend hydrogels. The effects of the content of CM-chitosan in PVP/CM-chitosan blend hydrogels on BSA adsorption are presented in Fig. 9. BSA adsorption increased with increasing CM-chitosan content in the blend hydrogel. A linear relationship was found between BSA adsorption and the content of CM-chitosan, which may due to the specific interactions between the ionized polymer and protein molecules. Castillo et al. reported that the bonding is formed between carboxyl groups in polymers and amine groups in proteins as detected by infrared spectroscopy, and such specific interactions account for the irreversible protein adsorption (Castillo, Koenig, Anderson, & Jentoft, 1986). The similar linear relationship for BSA adsorption was also found in poly(acrylonitrile-acrylamide-acrylic acid) hydrogels, the BSA adsorption increased with increasing acid and amide compositions (Hu & Tsai, 1996). Thus, such a linear correlation in Fig. 9 may indicate the specific interactions occurring to gel–protein pairs. It is well known that BSA cannot be adsorbed to pure PVP in any medium, temperature and ionic strength. It is the presence of CM-chitosan moieties, which make these gels potential adsorbents for BSA.

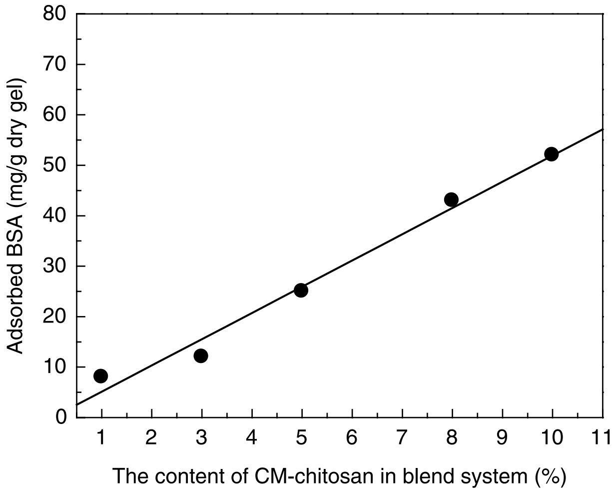

Fig. 9. BSA adsorption of PVP/CM-chitosan blend hydrogels as a function of CM-chitosan content of the initial mixture.

## 4. Conclusion

A series of novel hydrogels based on PVP and CM-chitosan could be synthesized by radiation technology. Comparing to PVP hydrogels, these blend hydrogels can provide satisfying properties such as pH-sensitivity, good swelling, good mechanical properties, improved surface property, potential adsorptive capacity for protein, etc. These properties imply that the PVP/CM-chitosan hydrogel, have potential for application in the biomedical field.

## Acknowledgements

One of the authors (Long Zhao) would like to express his gratitude to Dr Jinhua Chen (JAERI) for discussion and assistance of analysis surface property in this work.

## References

Abad, L. V., Relleve, L. S., Charito, A. T., & Dela-Rosa, A. M. (2003). Properties of radiation synthesized PVP-kappa carrageenan hydrogel blends. *Radiation Physics and Chemistry*, *68*, 901.

Altemeier, W. (1954). Studies on the possible effects of PVP on the absorption of nutrients. *Archives of Surgery*, *69*, 309.

Cascone, M. G., Barbani, N., Cristallini, C., Giusti, P., Ciardelli, G., & Lazzer, L. (2001). Bioartificial polymeric materials based on polysaccharides. *Journal of Biomaterials Science. Polymer Edition*, *12*(3), 267.

Castillo, E. J., Koenig, J. L., Anderson, J. M., & Jentoft, N. (1986). Protein adsorption on soft contact lenses. III. Mucin. *Biomaterials*, *7*, 9.

Crescenzi, V., Paradossi, G., Desideri, P., Dentini, M., Cavalieri, F., Amici, E., et al. (1997). New hydrogels based on carbohydrate and on carbohydrate–synthetic polymer networks. *Polymer Gels and Networks*, *5*, 225.

Dumitriu, S., Vidal, P., & Chornet, E. (1996). *Polysaccharides in medical application*. New York: Marcel Dekker.

Fowkes, F. M. (1963). Additivity of intermolecular forces at interfaces. I. Determination of the contribution to surface and interfacial tensions of dispersion forces in various liquids. *Journal of Physical Chemistry*, *67*, 2538.

Gombotz, W., Hoffman, A. S., & Horbett, T. A. (1986). In N. A. Peppas (Vol. Ed.), *Hydrogels in medicine and pharmacy: Vol. 1* (pp. 95–171). Boca Raton, FL: CRC Press.

Hoffman, A. S. (1981). A review of the use of radiation plus chemical and biochemical processing treatments to prepare novel biomaterials. *Radiation Physics and Chemistry*, *18*, 323.

Hong, Y., Chirila, T., Vijaysekaran, S., Shen, W., Lou, X., & Dalton, P. (1998). Biodegradation in vitro and retention in the rabbit eye of crosslinked poly(1-vinyl-2-pyrrolidinone) hydrogel as a vitreous substitute. *Journal of Biomedical Materials Research*, *39*, 650.

Hu, David S.G., & Tsai, C. E. (1996). Correlation between interfacial free energy and albumin adsorption in poly(acrylonitrile–acrylamide–acrylic acid) hydrogels. *Journal of Applied Polymer Science*, *59*, 1.

Huglin, M. B., & Zakaria, M. B. (1986). Swelling properties of copolymeric hydrogels prepared by gamma irradiation. *Journal of Applied Polymer Science*, *31*, 457.

Kaelble, D. H., & Moacanin, Jovan (1977). A surface energy analysis of bioadhesion. *Polymer*, *18*, 475.

Kaetsu, S. (1981). Immobilization of biofunctional substances. *Radiation Physics and Chemistry*, *18*, 342.

Liu, X. F., Yang, Y. L., Li, Z. D., & Yao, K. D. (2001). Antibacterial action of chitosan and carboxymethylated chitosan. *Journal of Applied Polymer Science*, *79*, 1324.

Majeti, N. V., & Kumar, R. (2000). A review of chitin and chitosan. *Reactive and Functional Polymers*, *46*, 1.

Nho, Y. C., & Park, K. R. (2002). Preparation and properties of PVA/PVP hydrogels containing chitosan by radiation. *Journal of Applied Polymer Science*, *85*, 1787.

Peppas, N. A. (Vol. Ed.) (1987). *Hydrogels in medical and pharmacy: Vol. 3*. Boca Raton, FL: CRC Press.

Risbud, M. V., & Bhat, S. V. (2001). Properties of polyvinyl pyrrolidone/beta-chitosan hydrogel membranes and their biocompatibility evaluation by haemorheological method. *Journal of Materials Science Materials in Medicine*, *12*, 75.

Rosiak, J. M., & Ulanski, P. (1999). Synthesis of hydrogels by irradiation of polymers in aqueous solution. *Radiation Physics and Chemistry*, *55*, 139.

Rosiak, J. M., Ulanski, P., Pajewski, L. A., Yoshii, F., & Makuuchi, K. (1995). Radiation formation of hydrogels for biomedical purpose. Some remarks and comments. *Radiation Physics and Chemistry*, *46*, 161.

Saiki, I., Watanabe, K., Tokura, S., & Azuma, I. (1992). Chitin derivatives as a carrier for drug delivery systerm. *Chitin Derivatives in Life Science*, 20–26.

Tokura, S., Baba, S., Uraki, Y., Miura, Y., Nishi, N., & Hasegawa, O. (1990). Carboxymethyl-chitin as a drug carrier of sustained release. *Carbohydrate Polymers*, *13*, 273.

Tsuruta, T., Hayashi, T., Kataoka, K., Ishihara, K., Kimura, Y., & Minoura, N. (1993). *Biomedical applications of polymeric materials*. Boca Raton, FL: CRC Press.

Zhai, M. L., Ha, H. F., Yoshii, F., & Makuuchi, K. (2000). Effect of kappa-carrageenan on the properties of poly(*N*-vinyl pyrrolidone)/kappa-carrageenan blend hydrogel synthesized by $\gamma$-radiation technology. *Radiation Physics and Chemistry*, *57*, 459.

Zhao, L., Mitomo, H., Nagasawa, N., Yoshii, F., & Kume, T. (2003). Radiation synthesis and characteristic of the hydrogels based on carboxymethylated chitin derivatives. *Carbohydrate Polymers*, *51*, 169.

Zhao, L., Mitomo, H., Zhai, M. L., Yoshii, F., Nagasawa, N., & Kume, T. (2003). Synthesis of antibacterial PVA/CM-chitosan blend hydrogels with electron beam irradiation. *Carbohydrate Polymers*, *53*, 439.

Zhao, Z. P., Wang, Z., & Wang, S. C. (2002). A novel *N*,*O*-carboxymethyl amphoteric chitosan/poly(ethersulfone) composite MF membrane and its charged characteristics. *Desalination*, *144*, 35.

Zhao, Z. P., Wang, Z., & Wang, S. C. (2003). Formation, charged characteristic and BSA adsorption behavior of carboxymethyl chitosan/PES composite MF membrane. *Journal of Membrane Science*, *217*, 151.