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The preparation of cellulose nitrate derivatives and their adsorption properties for creatinine

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Abstract

As the sorbent of creatinine, cellulose nitrate (CN) with low degree of nitrification was synthesized from cellulose nitrate by denitration; secondary amine CN (ACN) was synthesized by the reaction of CN, epichlorohydrin and ethylene diamine, and quaternary ammonium CN (CCN) was synthesized by the reaction of CN and 3-chloro-2-hydroxyl-propyltrimethyl ammonium chloride (CHPA). The structures of these three sorbents were studied by elemental analysis and ^{13}C NMR. The adsorption properties for creatinine were evaluated for dialysates with different pH values. At a temperature of 37 °C and a dialysate pH 7.0, the adsorption capacities of ACN, CCN and CN for creatinine were 2.04, 1.47 and 1.08 mg/g, respectively. The adsorption properties of ACN, CCN and CN were not influenced by the content of urea.

Keywords: Cellulose; Derivative; Creatinine; Sorbent; Synthesize

1. Introduction

Creatinine (2-amino-1-methyl-2-imidazoline-4-one) is the metabolic product of creatine which can storage energy in muscle (Robert, Micheal, & George, 1980). The methylcarbamidine group of creatinine can reduce kidney function and accelerates renal decline. Creatinine usually accumulates in the blood of chronic renal failures (Carrey et al., 2003; Spierto, Hannon, Gunter, & Smith, 1997; Torremans et al., 2003). Creatinine is removed by dialysis but at the cost of undesirable cardiovascular tolerance because of high rates removal of sodium and water (McIntyre, 2004). Creatinine not only exists in serum, but also in the alimentary canal (stomach and intestine). Oral sorbents such as active carbon and ion exchange resin can adsorb creatinine in the alimentary canal, and reduce the concentration of creatinine in serum, which has been shown to attenuate the progression of chronic renal failure in humans (Sato, Miyazaki, Sakemi, & Mohri, 2000). They are all broad-spectrum sorbents and have some remedial effects; however, they have the disadvantage of adsorbing non-selectives and show poor biocompatibilities, so their clinical effects are not perfect (Koide et al., 1991). There are few reports of a creatinine sorbent with highly adsorption capability. A new oral sorbent with highly adsorption capability would be of value for the treatment of chronic renal failure.

Cellulose is a linear polysaccharide consisting of β-1,4-linked glucose. It is an abundant natural material which is nontoxic, non-immunogenic characters, biocompatible and degradable. So it has a broad application in food (Nakaji et al., 2004), medicine (Song et al., 2002) and the chemical industry (Ass, Frollini, & Heinze, 2004).

It is proved in our laboratory that cellulose nitrate, a derivative of cellulose, has the ability of sorption for creatinine, but the saturated adsorption capacity is rather low and the optimal pH value needed for the dialysate is about 10. In fact, the pH value in the human intestine is 7.0–8.5.

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In order to increase the saturated adsorption capacity and increase the optimal pH range, we introduce secondary amine group and quaternary ammonium group to modify the molecule of cellulose nitrate and improve the adsorption microenvironment of cellulose nitrate and creatinine. The modified CNs are shown to be efficient and specific sorbents for creatinine.

2. Experiments

2.1. Materials

Cellulose nitrate (the content of nitrogen was 12.15–12.20%, substitution degree was 2.32) was purchased from the Shanghai Fuel Chemical Factory in China. The standard solution of creatinine (15 mg/L) was obtained from Beihua Fine Chemical Limited Company in Beijing, China. AST-120 (officinal oral active carbon sorbent) was obtained from KUREHA Chemical Company in Japan. Active silica gel (the average granularity is 0.063 mm) was purchased from Hangu Haizhong Chemical Factory in Tianjin, China. 3-Chloro-2-hydroxyl-propyltrimethyl ammonium chloride (analytical grade) was obtained from Guofeng Fine Chemical Limited Company in Shandong, China. All reagents but creatinine (biochemistry reagent) were of analytical grade.

2.2. Preparation of CN

Cellulose nitrate was dried to a constant weight in the oven at 57 °C, and then cooled in a desiccator. The dry cellulose nitrate 7.8 g and the appropriate HNO_3 (65–68%) solution were placed in a 250 ml conical flask. The conical flask was put into a shaker, and the temperature and time of reaction carried out during shaking were selected for denitration. The mixture in the conical flask was cooled to room temperature after the reaction, and then separated by centrifugation. The crude product was boiled in distilled water, then filtered through a vacuum filter and simultaneously washed with hot water until the filtrate pH value reached 6. The product was boiled in Na_2CO_3 (0.03–0.06%) solution then filtered through a vacuum filter and at the same time washed with distilled water until the filtrate pH value reached 6, and then dried under vacuum. Finally, cellulose nitrate with a low nitrification degree was obtained in the form of a white floc or powder. Sorbents with different nitrification degrees of 1.07, 1.15, 1.76 and 2.32 were obtained by controlling different temperatures and times of the reaction, and named as CN-1.07, CN-1.15, CN-1.76 and CN-2.32.

CN-1.15 was demonstrated to be the best adsorption effect by comparing the results of the adsorption experiment. So CN-1.15 was used as the raw material to prepare the following derivatives. The structure of CN-1.15 is shown in Fig. 1.

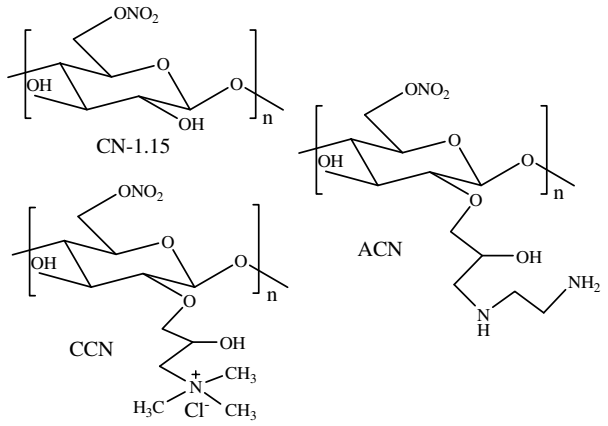

Fig. 1. Structures of CN-1.15, ACN and CCN.

2.3. Preparation of ACN

1.0 g (4.7×10^{-3} mol) CN-1.15, 40 ml ethanol, 0.79 g (8.6×10^{-3} mol) epichlorohydrin and 3.5 ml distilled water were placed in four-necked round bottom flask equipped with a mechanical stirrer, a reflux condenser with drying column filled with $CaCl_2$, an immersion thermometer, and dropping constant press funnel. The mixture was stirred adequately and simultaneously heated to 75 °C, followed by slow addition of 7.7 g (7.7×10^{-5} mol) $HClO_4$ and 3.4 ml (0.19 mol) water. The reaction was kept in 3 h. Ammonification was carried out by dropping the temperature to 70 °C and adding 0.60 ml (9.0×10^{-3} mol) ethylene diamine into the mixture. The mixture was cooled after 3.5 h, and then water was added to deposit the product. The deposit was filtrated through a vacuum filter and simultaneously washed with water until the pH value of the filtrate reached 6, and then the water and the unreacted CN-1.15, which were soluble in ethanol, were washed away by ethanol. 0.65 g ACN was obtained by torrefaction at 60 °C. The yield was 65%. The structure of ACN is shown in Fig. 1.

2.4. Preparation of CCN

1.0 g (4.7×10^{-3} mol) CN-1.15, 40 ml ethanol and 0.0303 g (7.57×10^{-4} mol) NaOH were placed in a three-necked flask equipped with a mechanical stirrer, a reflux condenser and an immersion thermometer. The mixture was stirred until CN-1.15 dissolved, followed by the addition of 1.02 g of the solution containing 38% CHPA (2.56×10^{-3} mol). After that, the temperature was elevated to 50 °C. The reaction was maintained for 2 h. The mixture was cooled, followed by the addition of hydrochloric acid to neutralization. Acetone was added to deposit the product after 1-h-stirring. The deposit was filtered through a vacuum filter and at the same time washed with water and ethanol. Pure CCN as a light-yellow powder was obtained by vacuum drying at 60 °C. The yield was 51%. The structure of CCN is shown in Fig. 1.

2.5. Determination of nitrogen content

The total quantity of nitrogen (N%) was determined by element analysis (YANCO CHNCORER MF-3, Germany). The quantity of nitrogen in amino-group (N_{NH2}%) and quaternary ammonium group (Nc%) were determined by Kjeldahl determination.

$$N_{ONO2}\%(ACN) = N\%(ACN) - N_{NH2}\%(ACN)$$

$$N_{ONO2}\%(CCN) = N\%(CCN) - Nc\%(CCN)$$

N_{ONO2}% (ACN), quantity of nitrogen in nitro group of ACN; N_{ONO2}% (CCN), quantity of nitrogen in nitro group of CCN; N% (ACN), total quantity of nitrogen of ACN; N% (CCN), total quantity of nitrogen of CCN; N_{NH2}% (ACN), quantity of nitrogen in amino group of ACN; Nc% (CCN), quantity of nitrogen in the quaternary ammonium group of CCN.

2.6. Determination of adsorption properties for creatinine

The dialysate which were similar to the physiological condition in human body contained Na^+, K^+, Ca^+, Mg^+, Cl^- and Ac^- whose concentration were respectively 132.50, 1.00, 1.75, 0.50, 98.00, 40.00 mmol/L. Then right amount creatinine was introduced into the dialysate to keep the concentration at 60 mg/L. Sorbents (0.5 g) were added into the above solution of 25 ml to test the adsorption ability at 37 °C in the microbe incubator. The change in concentration of creatinine was determined by Jaffe' reaction (Falco' et al., 2001). Each sample was measured in triplicate and the average was used.

$$AC = (C_0 - C) \times 0.025/m$$

AC, adsorption capacity (mg/g); C_0, concentration of creatinine before adsorption (mg/L); C, concentration of creatinine after adsorption (mg/L); 0.025, volume of dialysate (L); m, quantity of adsorbents (g).

2.7. X-ray diffractometry

Measurement was carried out using a BDX3300 X-ray diffractometer, (Germany, 40 kV, 100 mA) equipped with a Ni-filtered Cu radiation and a curved graphite crystal monochromator at a scanning rate of 2°/min. The diffractometer was equipped with 1° divergence slit, a 0.16 mm raster, a 0.2 mm receiving slit and a 1° scatter slit. Radiation was detected with a proportional detector.

3. Results and discussion

3.1. Elemental analyses

The result of elemental analysis (YANCO CHNCORER MF-3, Germany) of complexes is shown in Table 1.

By calculation using the formula:

$$r_{ONO2} = (r_c N_{ONO2})/Nc; \quad (1)$$

Table 1
The data of elemental analysis of complexes

Complexes	Mass ratio/% exper. (calcd.)				
	C	H	N_{ONO2}	N_{NH2}	Nc
CN-1.15	32.79 (33.68)	3.05 (4.14)	7.53	0	0
CCN	36.83 (37.36)	4.03 (5.37)	5.42	–	2.00
ACN	37.51 (37.84)	5.48 (5.55)	5.76	5.40	–

$$r_c = (162Nc)/(1400 - 151.5Nc - 45N_{ONO2}) \quad (2)$$

$$r_{ONO2} = (2r_{NH2} \times N_{ONO2})/N_{NH2} \quad (3)$$

$$r_{NH2} = (162N_{NH2})/(2800 - 116N_{NH2} - 90N_{ONO2}) \quad (4)$$

r_{ONO2} was the substitution degree (the average number of substituted groups per glucose unit) of nitro groups; r_c was the substitution degree of quaternary ammonium groups; r_{NH2} was the substitution degree of amino-groups.

According to formula (1) and (2) and the data of elemental analysis, we got the results: $r_c = 0.38$, $r_{ONO2} = 1.03$. Here, $r_{ONO2}(1.03)$ was lower than $r_{ONO2}(1.15)$, the original substitution degree of nitrification. It showed that denitration had happened during the preparation of CCN.

According to formula (3) and (4) and the data of elemental analysis, we got the results: $r_{NH2} = 0.53$, $r_{ONO2} = 1.13$. Here, $r_{ONO2}(1.13)$ is close to $r_{ONO2}(1.15)$, the original substitution degree of nitrification. This showed that there was little denitration during the preparation of ACN.

3.2. ^{13}C NMR spectrum analyses

^{13}C NMR spectrum (Varian Unity-Plus 400 Spectrometer, America) is shown in Fig. 2. The hydroxyl group connected with C-6 of CN-1.15 was partly replaced by the nitro group. The chemical shift of C-6 shifted from original δ (60.3 ppm) to δ (70.6 ppm) because the electron-attracting induced effect of the nitro group on C-6 was stronger than that of hydroxyl group on C-6. In addition, the peak at δ (70.6 ppm) was much larger than the peak at δ (60.3 ppm). This indicated that most of the hydroxyl groups of C-6 were esterified. At the same time, hydroxyl groups connected with C-3 and C-2 were also replaced by nitro groups, and their peaks appeared at δ (82.4 ppm) and δ (83.7 ppm), but the strength of their peaks was weaker than the original peaks at δ (78.0 ppm) and δ (79.4 ppm), where no esterification happened. This indicated that the hydroxyl groups on C-3 and C-2 were only partially esterified, and esterification mainly occurred on the hydroxyl groups on C-6.

As shown in Fig. 3, peaks at δ (70.5 ppm), δ (82.4 ppm) and δ (84.1 ppm), respectively, represented esterified hydroxyl groups on C-6, C-3 and C-2 of ACN's glucose residue, the chemical shifts of which were almost same to that of CN-1.15. This indicated that there was almost no effect on esterified hydroxyl groups on C-6, C-3 and C-2 of CN-1.15 in the ammonification.

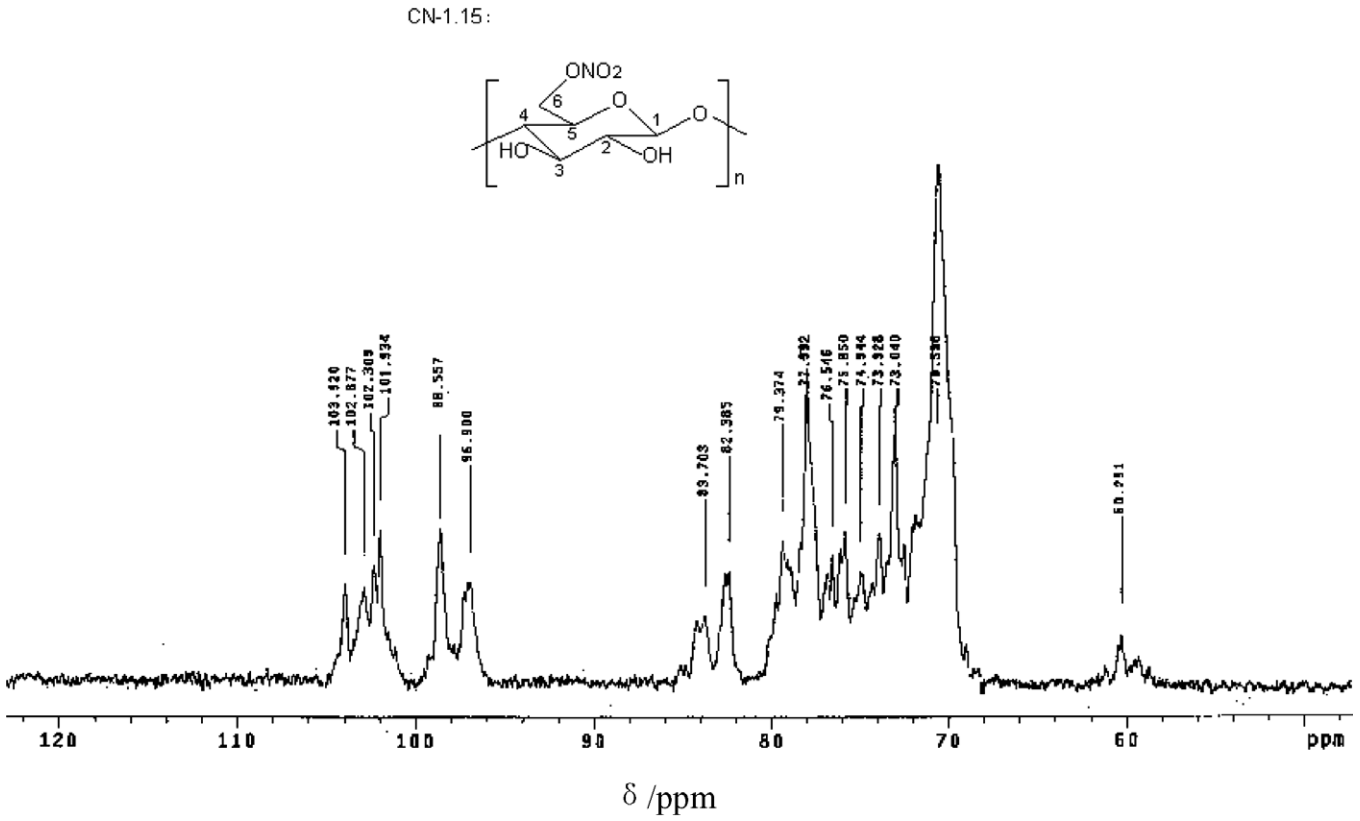

Fig. 2. The ^{13}C NMR spectrum of CN-1.15.

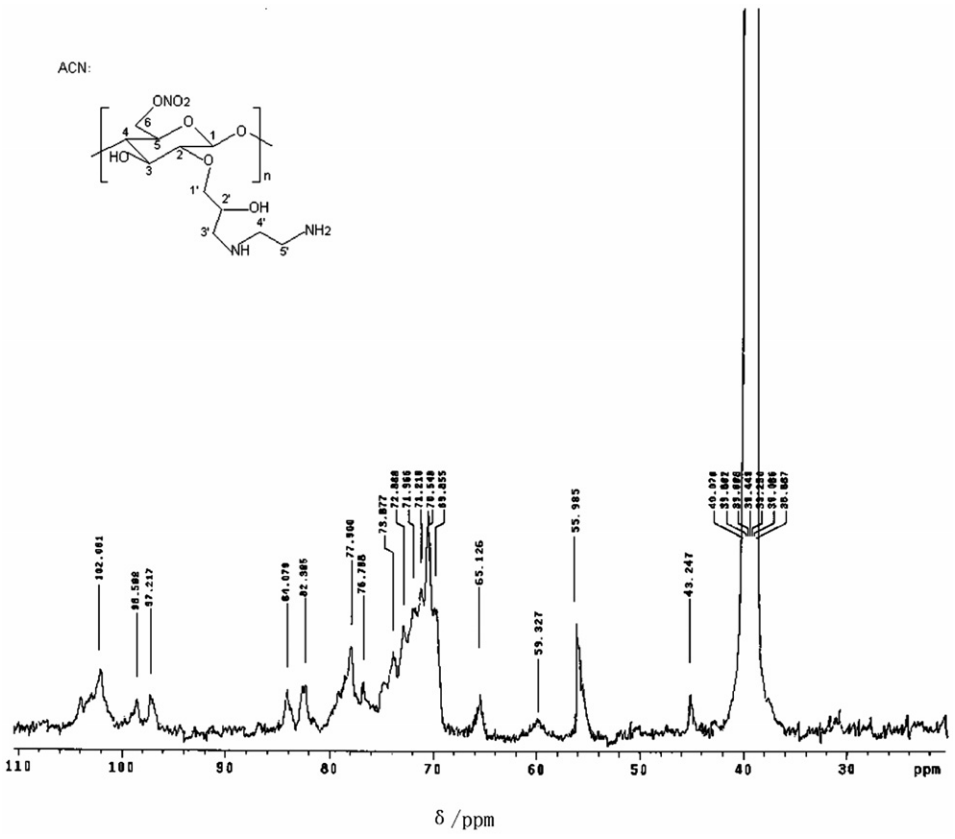

Fig. 3. The ^{13}C NMR spectrum of ACN.

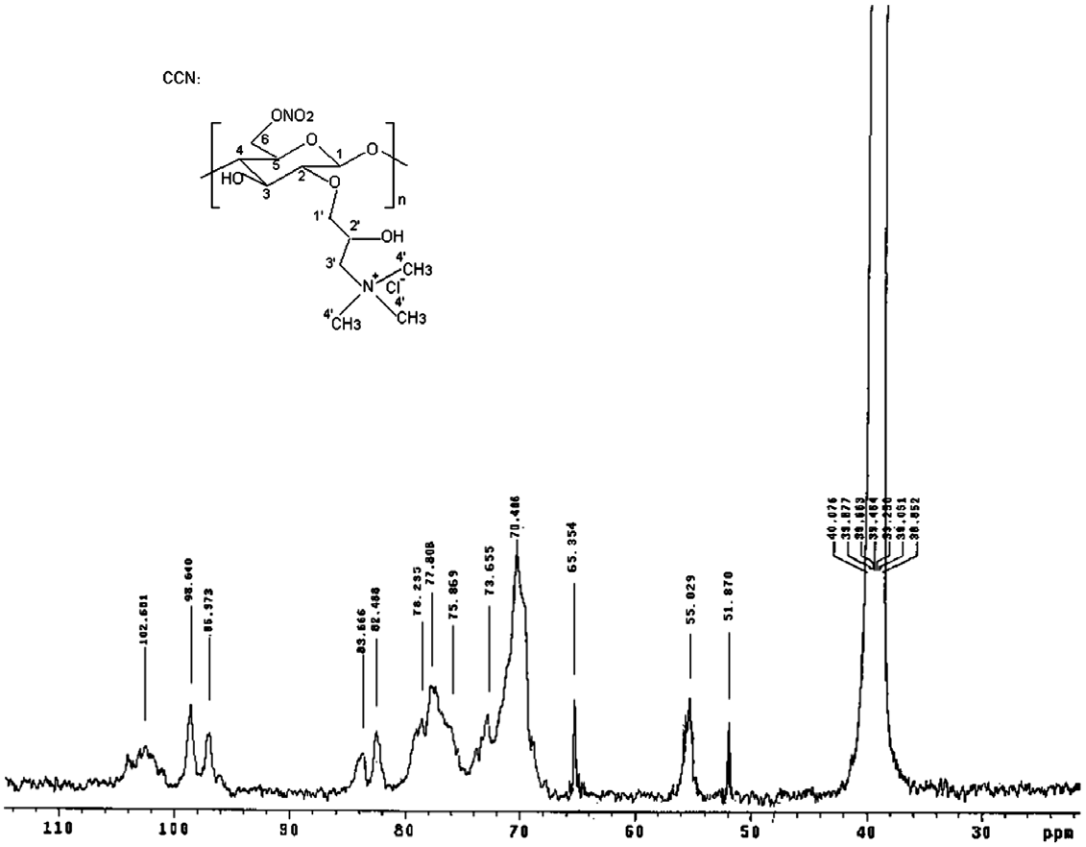

Fig. 4. The ^{13}C NMR spectrum of CCN.

As shown in Fig. 4, peaks at δ (70.4 ppm), δ (82.5 ppm) and δ (83.7 ppm), respectively, represented esterified hydroxyl groups on C-6, C-3 and C-2 of CCN's glucose residue, the chemical shifts of which were close to that of CN-1.15. This indicated that there was only a little effect on esterified hydroxyl groups on C-6, C-3 and C-2 of CN-1.15 in the quaterisation.

3.3. Adsorption properties for creatinine

As shown in Fig. 5, the times which sorbents with different substitution degrees spent on adsorption saturation for creatinine were approximately similar (4 h). That was to say, the time sorbents spent on adsorption saturation for creatinine was independent of the substitution degree. However, with increasing degree of substitution the saturated adsorption capacity of sorbents increased and then decreased (as shown in Fig. 6). The optimal saturated adsorption capacity was shown by CN-1.15. The creatinine adsorption of CN was directly related to active group (–O–NO_2), which improved the saturated adsorption capacity with the increasing degree of substitution. At the same time, the higher the substitution degree of CN, the more similar the glucose structural unit was. The improvement in the structural regularity increased the degree of crystallization of the CN molecule chain (as proved in Fig. 7). The crystallization embedded the partial active

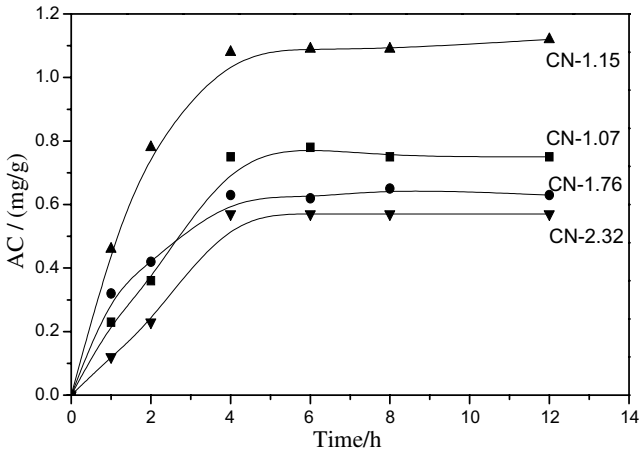

Fig. 5. Adsorption curve of CN with different substitution degrees creatinine concentration in the dialysate 60 mg/L, pH value 7.0, adsorption temperature 37 °C.

groups (–O–NO_2) of CN and decreased the water-solubility of CN. This limited the saturated adsorption capacity of CN for hydrophilic creatinine. Therefore, a proper degree of substitution degree was necessary for the sorbent to reach the best saturated adsorption capacity.

Fig. 8 showed that the adsorption capacity of ACN and CCN were higher than CN-1.15 at any time. The introduction of secondary amine group and quaternary ammonium

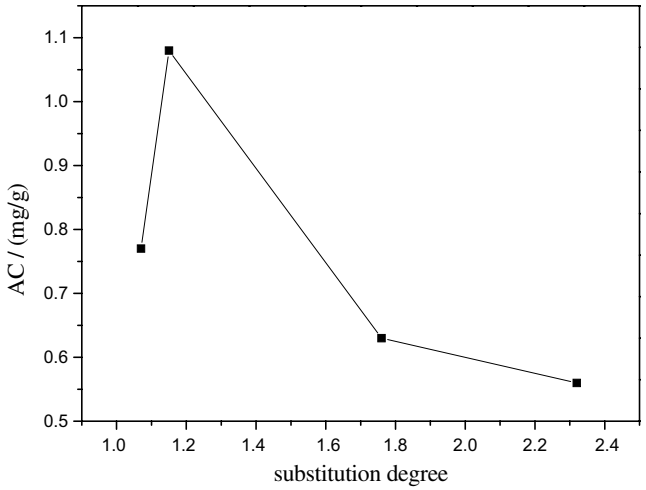

Fig. 6. Adsorption capacity of CN with different substitution degrees creatinine concentration in the dialysate 60 mg/L, pH value 7.0, adsorption temperature 37 °C, adsorption time 4 h.

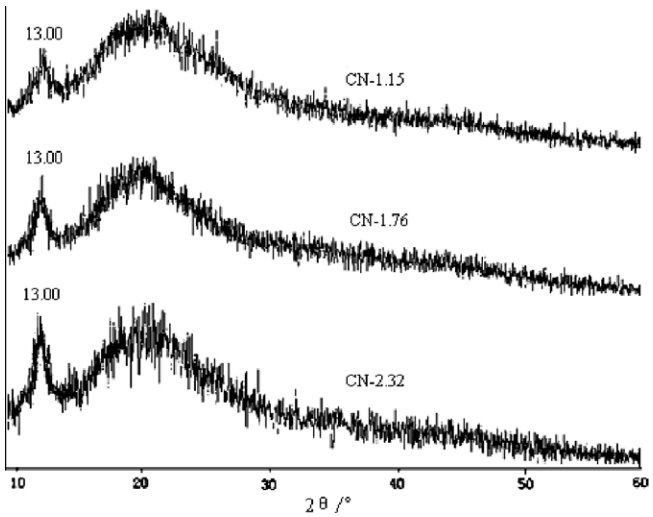

Fig. 7. X-ray spectra of CN with different substitution degrees.

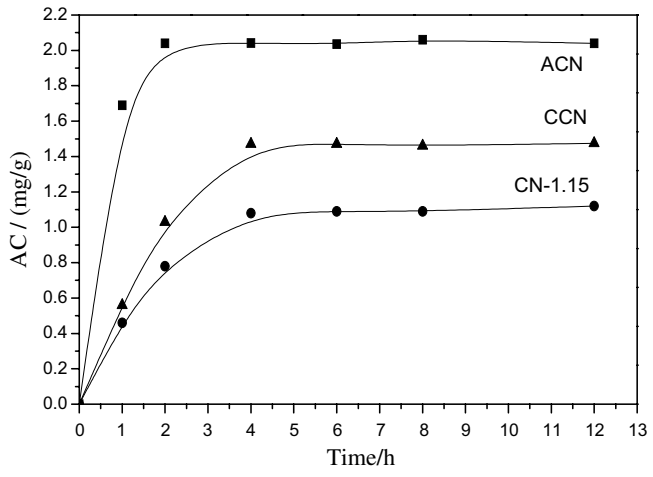

Fig. 8. Adsorption curve of different sorbents creatinine concentration in the dialysate 60 mg/L, pH value 7.0, adsorption temperature 37 °C.

group reduced the number of hydroxyl groups, which contributed to intramolecular and intermolecular hydrogen bonding. Moreover, the steric effect of secondary amine group and quaternary ammonium group made it difficult to form intermolecular hydrogen bonds. So the crystallization degrees of ACN and CCN molecule chains were lower than CN (as proved in Fig. 9), and creatinine was easier to diffuse into the ACN and CCN molecule. But adsorption rate and capability of CCN were lower than ACN. This might be related to the denitration in the preparation of CCN. It was in accordance with the conclusion from the elemental analysis mentioned above.

3.4. Influence of pH value of dialysate on adsorption properties

As shown in Fig. 10, the adsorption capacities of CN-1.15, ACN and CCN were independent of pH value in

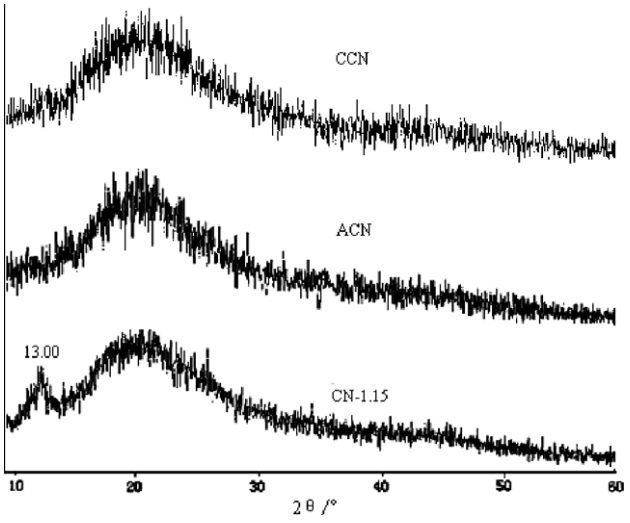

Fig. 9. X-ray spectra of different sorbents.

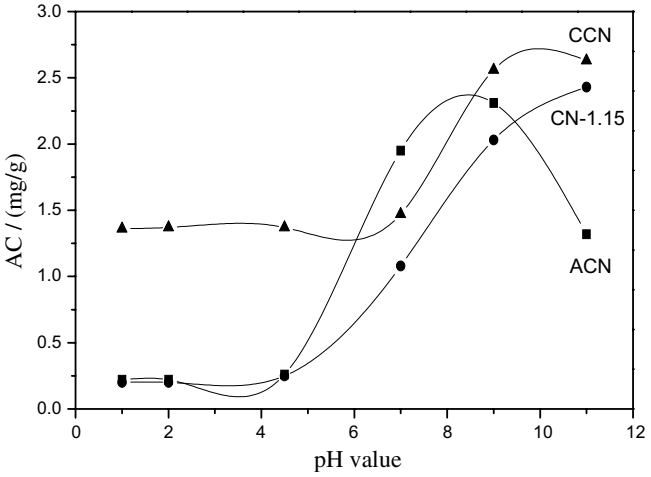

Fig. 10. Influence of pH value in dialysates on adsorption for creatinine concentration in the dialysate 60 mg/L, adsorption temperature 37 °C, adsorption time 4 h.

Table 2
The influence of urea on AC (mg/g) of different sorbents creatinine concentration in the dialysate 60 mg/L, pH value 7.0, adsorption temperature 37 °C, adsorption time 4 h

Urea content (mg/L)	AST-120	Activated silica gel	ACN	CCN	CN-1.15
0	2.34	0.56	2.04	1.47	1.08
142	0.85	0.23	2.02	1.45	1.03

the range of 1–4.5 and increased along with the rise of the pH value from 4.5 to 8.5. The OH^- in solution was advantageous to the creatinine adsorption, because the increase of OH-concentration improved the swelling of cellulose molecule, accordingly, creatinine could diffusion into the molecule of cellulose easily; moreover, OH^- in solution could enhance the polarity of the nitro group in the CN molecule. However, superfluous OH^- in the solution would result in the denitration of sorbents, which would go against the adsorption capacity. So the adsorption capacities of CN-1.15, ACN and CCN increased very slowly or declined when the pH value was over 9.

The pH value which was appropriate for CN-1.15 was too high for the human intestine (7–8.5), fortunately, the adsorption capacity of ACN and CCN were better than CN-1.15 in the range of 7–8.5. The modification of substituted groups to the molecule of cellulose was contributing to ACN and CCN. Furthermore, ACN were more efficient than CCN because of the denitration in the preparation of CCN.

3.5. Influence of urea on adsorption capacity

Compared with the healthy human, the quantity of urea in the intestine increased in subject with chronic renal failures. So the influence of urea on the adsorption capacity of sorbents should be tested. As shown in Table 2, although the adsorption capacity of AST-120 was optimal without urea, the introduction of urea obviously decreased the adsorption capacity of AST-120 from 2.34 to 0.85 mg/g. And the adsorption capacity of activated silica gel also declined by nearly 60% when urea was introduced. However, urea hardly influenced the adsorption capacity of ACN, CN-1.15 and CCN, when, the adsorption capacity of ACN was kept above 2 mg/g whenever there was urea in the dialysate or not. So ACN was the most satisfied adsorbent for creatinine in the presence of urea.

4. Conclusion

Three derivatives of cellulose, CN-1.15, ACN and CCN, were reported in this paper. They all had activities of adsorption for creatinine; ACN and CCN showed optimum adsorption at the pH value in the human compared with CN-1.15. Moreover, the adsorption capacity was not influenced by urea, which normally existed in the human intestine. In views of urea existence, CN-1.15, ACN and CCN were more efficient than commonly used oral sorbents like active carbon and AST-120.

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