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Ion-exchange chromatography by dicarboxyl cellulose gel

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Abstract

A new column packing material for ion-exchange chromatography was prepared from cellulose gel by periodate oxidation followed by chlorite oxidation to form spatially paired carboxyl groups (dicarboxyl cellulose, DCC). The carboxyl group was quantitatively introduced to spherical cellulose gel by controlling the extent of oxidation. The DCC gels were examined for their ion-exchange activity for various amines at pH of 2.5–5.5. In this pH range, aromatic amines with acid dissociation constant (pK_a) below 2.7 showed no interaction with DCC gels as expected from their lack of protonation. The amines with pK_a greater than 3.3, both aromatic and aliphatic, showed strong interaction corresponding to the amount of carboxyl introduced to the gel. However, these amines showed anomalous dependence on pH of the mobile phase, showing a maximum in retention factor at around pH 4. This is in contrast with the nearly constant retention factor of these amines on conventional carboxylated cellulose packing at pH greater than 4.0. The maximum retention factor at pH 4 of DCC gel was 4–5-times greater than that of conventional gel having a similar amount of carboxyls. Since pK_a of dicarboxyl groups ranges 3–5 as determined by acid–base titration, the pH giving maximum retention corresponds to the pH at which one of paired carboxyls is dissociated. Possible cause of this anomaly is presented in terms of dissociation state of dicarboxyl groups and its interaction with amines.

Keywords: Periodate–chlorite oxidation; pH effects; Stationary phases, LC; Dicarboxyl cellulose

1. Introduction

Cellulose is widely used as column packing material for liquid chromatography as gel material for size-exclusion separation as well as substrate for further derivatization for ion-exchange or affinity chromatography [1–7]. As such, several cellulose-based cation-exchange materials are commercially available. These products are usually prepared by introducing carboxymethyl groups under alkali-swollen conditions, and their distribution is considered to be random. Their typical ion-exchange capacity is 0.5–1.5 mmol per gram dry material, corresponding to 0.08–0.24 in degree of substitution [8].

Periodate oxidation of glycol groups is a useful method to introduce aldehyde groups to polysaccharides. This reaction for cellulose is characterized by selective cleavage of the C2–C3 bond between vicinal hydroxyl groups of the glucose unit, resulting in the formation of two aldehyde groups per glucopyranose unit (Fig. 1) [9]. Periodate oxidation proceeds under mild conditions in aqueous solution and can be easily stopped by addition of ethylene glycol. Also the degree of oxidation can be easily

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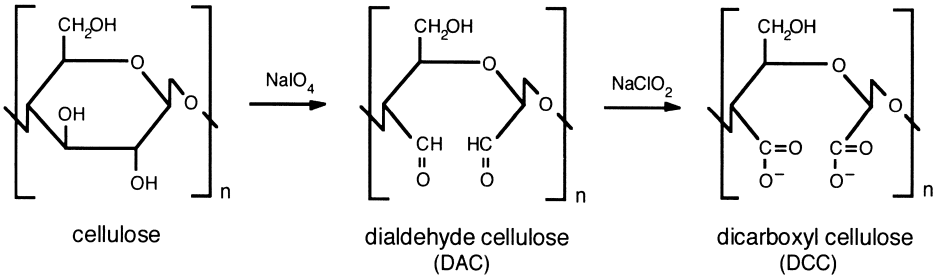

Fig. 1. Scheme of periodate–chlorite oxidation of cellulose.

controlled over a broad range by changing reaction time, temperature or amount of sodium periodate added [10]. The resulting aldehyde groups can be further converted to various substituent groups such as carboxylic acid [11–15], hydroxyls [11,13], or Schiff base with primary amines [16–20]. Therefore this reaction is potentially useful for preparing cellulose-based specialty materials such as adsorbents of heavy metals [14,16,17], proteins [18] or dyes [21] as well as column packing materials [10,22–25].

In this context, the oxidation of aldehyde to carboxyl (Fig. 1) is an attractive method for facile introduction of anionic groups. The resulting carboxyl groups arise as pairs, attached to the remaining main chain of cleaved cellulose. In this study, we applied this procedure to a commercial cellulose gel and examined its ion-exchange behavior as column packing. We paid attention to possible differences between common cation exchangers and the dicarboxyl-based exchanger due to the difference in spatial distribution of carboxyl groups.

2. Experimental

2.1. Materials

A commercial grade of spherical cellulose gel, Cellulofine GC-700sf (particle size 25–44 μm; swollen and suspended in water), was used as received (Chisso, Tokyo, Japan). Sodium metaperiodate ($NaIO_4$), ethylene glycol, benzylamine, pyridine, 2-, 3- and 4-aminopyridine, aniline, *o*-, *m*- and *p*-chloroaniline, *o*-, *m*- and *p*-toluidine, *o*-, *m*- and *p*-nitroaniline, methylamine, dimethylamine and trimethylamine were of reagent grade (Wako, Tokyo, Japan).

2.2. Periodate oxidation

A 150-ml volume of Cellulofine GC-700sf suspension containing 10 g solid was mixed with 100 ml of aqueous $NaIO_4$ containing 5% or 10% molar equivalent to glucose residue of cellulose. The mixture was stirred gently at 20°C for 24 h. After the remaining periodate was decomposed by excess ethylene glycol, the oxidized gel was washed with deionized water by repeated decantation. The products are denoted as DAC-1 (''5%'' oxidized) and DAC-2 (''10%'' oxidized). The aldehyde content was determined by elemental analysis for nitrogen of oximes formed as follows: 10 ml of DAC suspension containing 0.1 g solid was mixed 0.4 g of hydroxylamine dissolved in 100 ml of 0.1 *M* acetate buffer (pH 4.5) at 20°C for 24 h.

2.3. Preparation of dicarboxyl cellulose (DCC) gel

A 100-ml volume of DAC suspension containing 5 g solid was mixed with 100 ml of 0.4 *M* sodium chlorite in 2 *M* acetic acid and stirred gently at 20°C for 48 h. The gel was washed with deionized water by repeated decantation. The products are denoted as DCC-1 and DCC-2 corresponding to DAC-1 and DAC-2, respectively. The carboxyl content was determined by conductometric titration as follows: 45 ml of the suspension containing 0.30 g solid was mixed with 5 ml of 0.1 *M* NaCl and titrated with 0.1 *M* NaOH.

2.4. Solubility of derivatized cellulose gel

A 0.10-g amount (dry mass) of the dialdehyde or dicarboxyl cellulose gel was suspended in 12 ml of 0.18 *M* buffers of pH 2.0 (HCl–KCl), 5.0 (acetate), 7.0 (phosphate) and 9.0 (borate). After stirring the suspension for 10 days at room temperature, the gel was thoroughly washed and mass loss was determined.

2.5. Column packing procedure and evaluation of size-exclusion properties

The gel particles were packed in a stainless steel column (200×6 mm I.D.), forming a ca. 5.65 ml gel bed. Gel suspension (ca. 3 g solid in 30 ml water) was poured to the column attached to a stainless steel reservoir, to which water was pumped in at flow-rate of 3 ml/min. The obtained column showed a pressure drop of 1.0–1.5 MPa at a flow-rate of 1 ml/min.

The size-exclusion property of gels was determined by pullulan standards (Showa Denko, Tokyo, Japan) and small molecules (glucose and γ-cyclodextrin). The resulting retention volumes were used to calculate the distribution coefficient from the equation $K_{av}=(V_e-V_0)/(V_t-V_0)$, where V_e, V_0 and V_t represent solute elution volume, void volume and total bed volume, respectively.

2.6. Ion-exchange chromatography

Amines were dissolved in selected buffer solution to make 0.01–0.1% (w/w) solutions, injected from a 120 μl sample loop and eluted by the same buffer solution. The tested range of pH was 2.5–5.5. Acetate buffer (0.1 *M*) was used for pH 4.0–5.5. The buffer solutions for pH 2.5–3.5 was prepared by adding 0.1 *M* HCl to pH 4.5 acetate buffer. The flow-rate was 0.5 ml/min and the column was maintained at 25°C. Elution of solute was monitored by a refractive index detector and an ultraviolet absorption detector at optimum wavelength for each compound. For comparison, the same test was carried out with commercial cation-exchange cellulose, CM-Cellulofine C-500m (Chisso; particle size 53–125 μm).

Ethylene glycol was used as total volume marker. Retention factor was calculated using $k'=(V_e-V_t)/V_t$, where V_e is the elution volume of the compound and V_t is the elution volume of ethylene glycol. Tested solutes were *o*-nitroaniline (pK_a −0.26), *p*-nitroaniline (pK_a 0.99), *m*-nitroaniline (pK_a 2.46), *o*-chloroaniline (pK_a 2.64), *m*-chloroaniline (pK_a 3.34), *p*-chloroaniline (pK_a 3.99), *o*-toluidine (pK_a 4.57), aniline (pK_a 4.65), *m*-toluidine (pK_a 4.96), *p*-toluidine (pK_a 5.23), pyridine (pK_a 5.25), 3-aminopyridine (pK_a 6.06), 2-aminopyridine (pK_a 6.78), 4-aminopyridine (pK_a 9.17), benzylamine (pK_a 9.35), trimethylamine (pK_a 9.74), methylamine (pK_a 10.64) and dimethylamine (pK_a 10.72).

3. Results and discussion

3.1. Degree of oxidation and carboxyl content of DCC gel

Aldehyde content of DAC gels determined by nitrogen content of oxime corresponded to about 75% (molar basis) of sodium metaperiodate added. The amount of carboxyl groups introduced by chlorite oxidation was determined by conductometric titration as 0.47 mmol/g solid (DCC-1) and 0.92 mmol/g solid (DCC-2) (Table 1). These values correspond to the number of carboxyl groups of 7.7 (DCC-1) and 15.2 (DCC-2) per 100 glucose residues, agreeing with the efficiency of periodate oxidation from elemental analysis above. Carboxyl content of CM-Cellulofine C-500m was 1.21 mmol/g, being greater than those of DCC-2 above.

3.2. Chemical and mechanical stability of DAC and DCC gels

Fig. 2 shows the time course of solubilization of DAC and DCC gels at various pH values. The mass

Table 1
Aldehyde and carboxyl contents of corresponding cellulose gels

	Aldehyde content[a] (mmol/g)		Carboxyl content[b] (mmol/g)
DAC-1	0.46	DCC-1	0.47
DAC-2	0.93	DCC-2	0.92
		C-500m	1.21

[a] Elemental analysis.
[b] Conductometric titration.

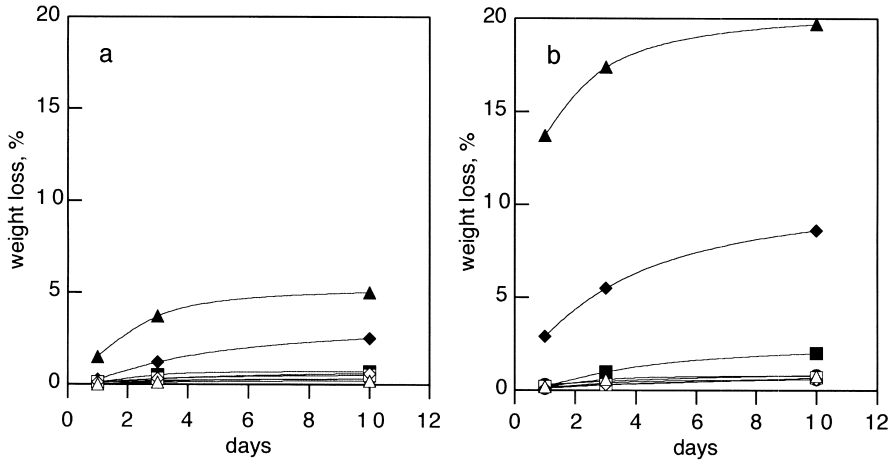

Fig. 2. Solubilization of dialdehyde and dicarboxyl cellulose gels in buffers. (a) DAC-1 (filled symbol) and DCC-1 (open symbol). (b) DAC-2 (filled symbol) and DCC-2 (open symbol). pH 2 (●, ○), pH 5 (■, □), pH 7 (◆, ◇) and pH 9 (▲, △).

loss of DAC gels was significant, especially in alkaline region. This is considered to result from scission of the main chains by alkali [26,27]. In contrast, the DCC gels were highly stable at any pH as seen in Fig. 2. Thus the conversion of dialdehyde to dicarboxyl remarkably improves the chemical stability of the gel.

The mechanical stability of the DCC gels was demonstrated by performance of the packed columns. The pressure drop of the packed columns was virtually constant for several months of chromatography operation including many exchanges to different buffer. These results show the satisfactory performance of the DCC gels as column packing material.

3.3. Size-exclusion properties of DCC gels

The size-exclusion properties of the DCC gels and CM-Cellulofine C-500m for nonionic solutes were determined by using the pullulan standards and small molecules (Figs. 3 and 4). The results show that the three gels have nearly same pore structure. That DCC-1 and DCC-2 give nearly the curve means that the oxidative conversion does not alter the porous structure of the gel. CM-Cellulofine C-500m, with similar porosity as seen in Fig. 4, is a suitable material for comparison with the present DCC gels. The figure also shows that the amines (molecular mass of 80–120) used in the following test of ion-exchange behavior are small enough to penetrate these gels. Therefore they should be eluted as void volume when there is no ionic interaction.

3.4. Ion-exchange chromatography of amines

A variety of amines were tested for ionic inter-

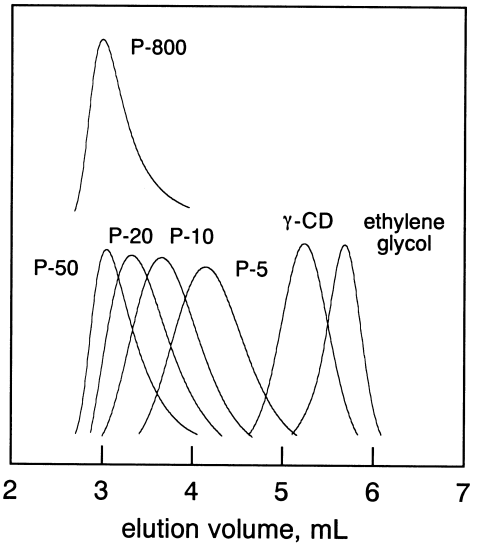

Fig. 3. Size-exclusion chromatogram of neutral solutes by DCC-2 column. Column: 200×6 mm I.D.; eluent: water; flow-rate: 0.5 ml/min; detection: refractive index. Solutes were pullulan standards (P-***), γ-cyclodextrin and ethylene glycol.

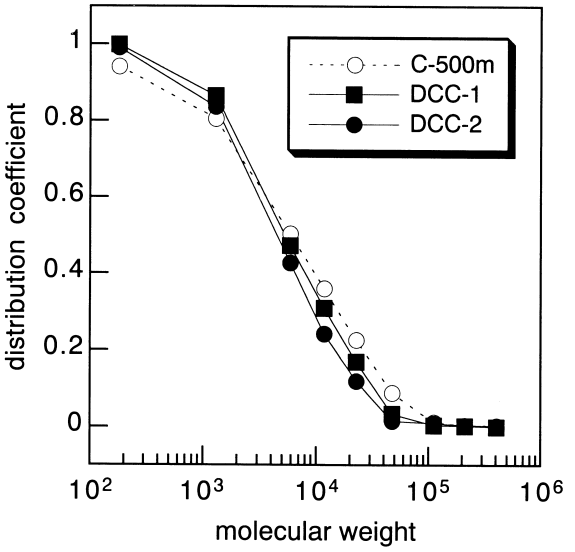

Fig. 4. Size-exclusion calibration curves for DCC and CM-Cellulofine C-500m gels.

action with the dicarboxyl cellulose gel at pH 2.5–5.5. Fig. 5 shows that *p*-nitroaniline has no ionic interaction with the DCC gels since it is not protonated in this pH range (pK_a 0.99), but slightly delayed from the standard position (retention factor between 0.4 and 0.6), probably due to hydrophobic adsorption

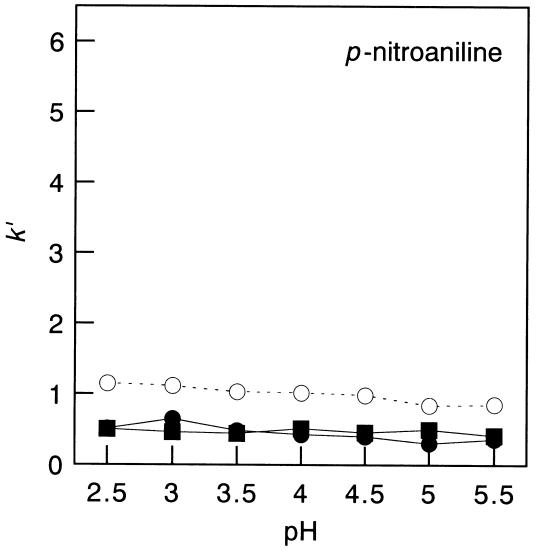

Fig. 5. Retention factor of *p*-nitroaniline (pK_a 0.99). ■: DCC-1, ●: DCC-2, ○: CM-Cellulofine C-500m.

[28]. This effect was stronger with CM-Cellulofine C-500m. All aromatic amines with pK_a values less than 2.7 showed similar behavior as in Fig. 5.

The aromatic amines with pK_a 3.3–9.4 showed significant ionic interaction with dicarboxyl cellulose gels. Fig. 6 shows the results for aniline (pK_a 4.65) and *p*-toluidine (pK_a 5.23). The pH dependence for CM-Cellulofine C-500m, with a maximum at pH 4, is explained by increasing dissociation of carboxyl combined with decreasing protonation of the amines. The retention factor of DCC gels shows similar pH dependence, with the expected difference between DCC-1 and DCC-2 from the difference in their oxidation degrees. Remarkable here, however, is that the retention factor for DCC-2 is much greater than that for CM-Cellulofine C-500m at all pH values, despite that the latter has more carboxyls (1.21 mmol/g vs. 0.92 mmol/g; the amount of solid in packed column was nearly the same for all gels). Especially at pH 4.0, the retention factor for DCC-2 is 2–2.5-times that for CM-Cellulofine C-500m. Discussion on this point will occur later.

Fig. 7 shows the pH dependence of retention factor of 2-aminopyridine (pK_a 6.78) and benzylamine (pK_a 9.35) with the tested gels at pH 2.5–5.5. In this case the retention factor for Cellulofine C-500m is nearly constant at pH 4.0–5.5 because of greater pK_a values of these amines. The retention factor for DCC gels is again significantly greater than for CM-Cellulofine C-500m and strongly dependent on pH. The retention factor for 2-aminopyridine with DCC-2 at pH 4.0 is about 3.5-times greater. These amines must be mostly present in the protonated form at these pH values. Therefore the pH dependence for DCC gels cannot be ascribed to the change in protonation degree of amines.

Because such anomaly may be caused by aromaticity of these amines, we examined behavior of aliphatic amines. Fig. 8 shows the results for methylamine (pK_a 10.64), dimethyamine (pK_a 10.72), and trimethylamine (pK_a 9.74). The pH dependences of retention factor of these amines were nearly the same to those of aromatic amines with pK_a values greater than 6. Therefore this anomalous pH dependence seems to be a general phenomenon for amines.

Thus the DCC gels, having closely paired carboxyls in contrast to randomly distributed ones in CM-Cellulofine C-500m, attracts protonated amines

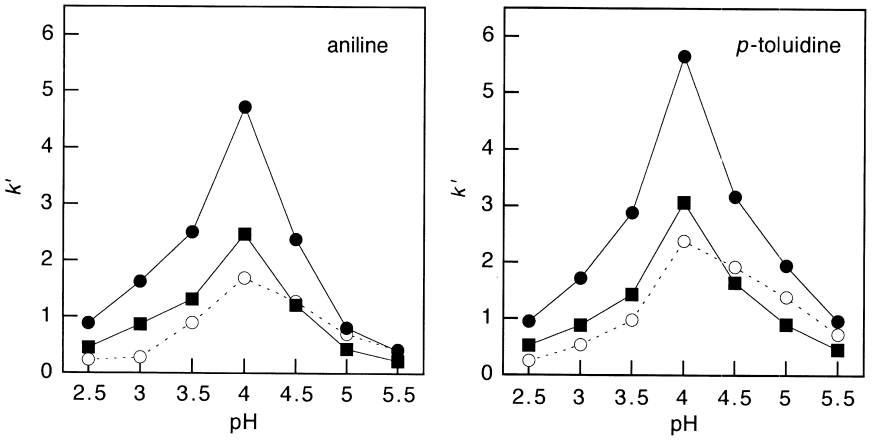

Fig. 6. The retention factors of aniline (pK_a 4.65) and *p*-toluidine (pK_a 5.23). ■: DCC-1, ●: DCC-2, ○: CM-Cellulofine C-500m.

stronger at around pH 4 than at other pH. The cause of this anomaly may lie in the dissociation state of paired carboxyl groups. Fig. 9 shows the titration curve of DCC-2 gel. The curve is a typical one for a weak dibasic acid with small difference in pK_a values. As a rough estimation, we applied the textbook method to determine pK_a values of dibasic acid with separated values (pK_{a1}>>pK_{a2}) from the titration curve, assuming starting point of pH 2.74 and end point of pH 8.02 (see legend to Fig. 9). The calculation gave pK_{a1}=3.66 and pK_{a2}=4.76. This difference is similar to that of glutaric acid (ΔpK_a= 1.1) or adipic acid (ΔpK_a=1.2), these having two carboxyls separated by trimethylene or tetramethylene groups. The pH dependence of dissociation state of carboxyls of DCC-2 gel can be calculated from the pK_a values above as in Fig. 10. The figure shows that the fraction of monodissociated state reaches a maximum at pH 4.22.

Since this value is close to the pH giving the observed maximum in retention factor, we here present a possible interpretation as indicated schematically by Fig. 11. At pH below 2, most carboxyl groups are undissociated and do not show ionic interaction (Fig. 11a). Above pH 6, most carboxyl groups are dissociated and become active ion exchanging sites, but adjacent groups are distant because of electrostatic repulsion (Fig. 11c). In this

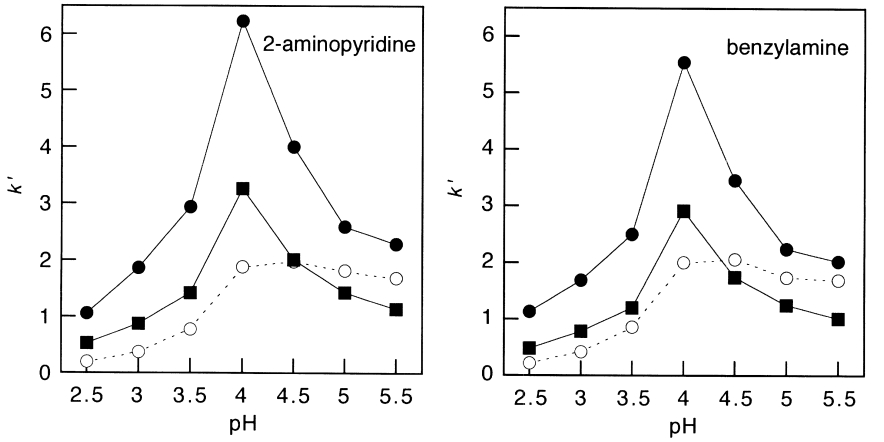

Fig. 7. Retention factors of 2-aminopyridine (pK_a 6.78) and benzylamine (pK_a 9.35). ■: DCC-1, ●: DCC-2, ○: CM-Cellulofine C-500m.

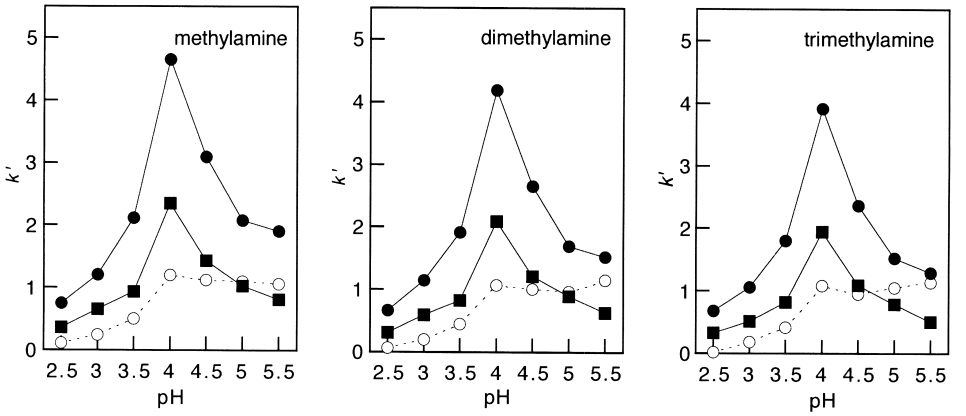

Fig. 8. Retention factors of methylamine (pK_a 10.64), dimethylamine (pK_a 10.72) and trimethylamine (pK_a 9.74). ■: DCC-1, ●: DCC-2, ○: CM-Cellulofine C-500m.

state the carboxyls would act similarly to the independent ones. In contrast, at around pH 4, most dicarboxyl groups are in monodissociated state, in which single carboxyl attracts the amine, while the undissociated carboxyl could come close to the amine. In this configuration the undissociated carboxyl might exhibit some interaction with the amine, possibly hydrogen bonding between carbonyl and amino proton (Fig. 11b). If this interaction were strong enough to compensate the scarcity of anionic sites than in fully dissociated state (i.e., some 40% less, from Fig. 10), then the amine-binding power could be stronger in monodissociated state. Verification of this mechanism might be obtained from close examination of interactions between amines and low-molecular-mass dibasic acids such as succinic or adipic acid.

Besides the pH dependence, certain steric effect

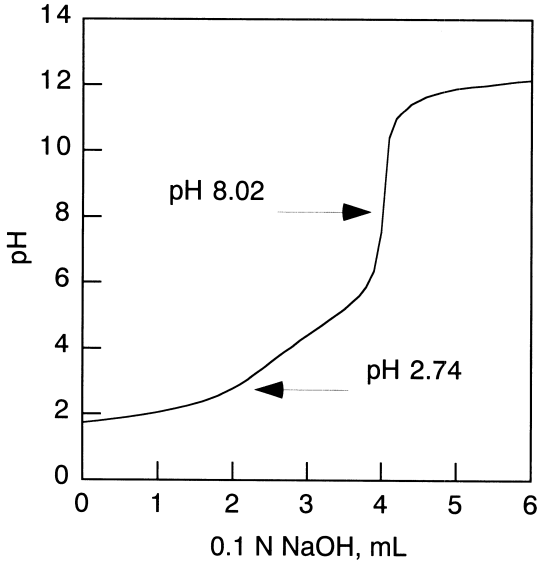

Fig. 9. The titration curve for dicarboxyl cellulose gel (DCC-2). Starting point of pH 2.74 is from the amount of added HCl and end point of pH 8.02 is from amount of carboxyl by conductometry.

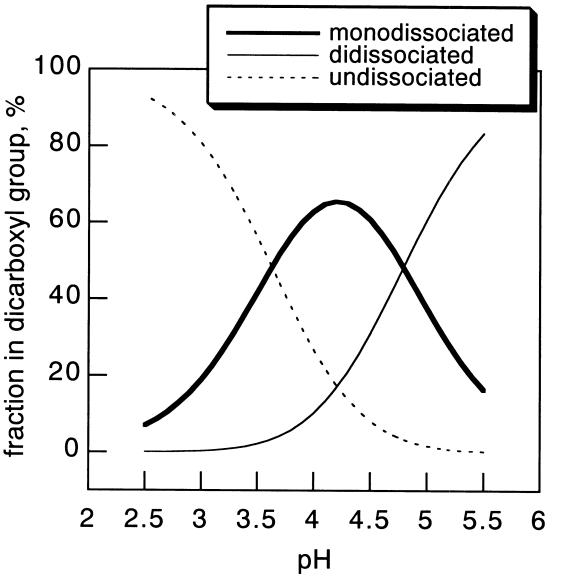

Fig. 10. The fraction of three dissociation of dicarboxyl cellulose gel calculated from pK_{a1}=3.66 and pK_{a2}=4.76.

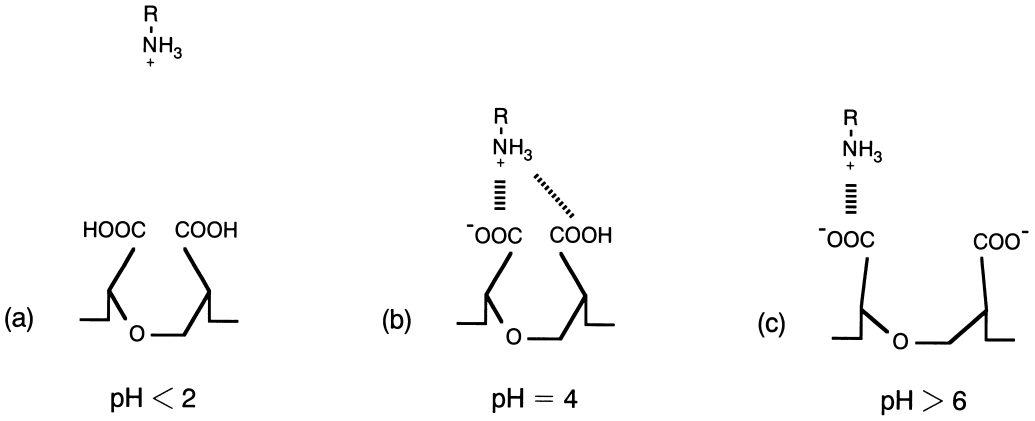

Fig. 11. Possible modes of interaction between dicarboxyl group and amine.

was noted as follows: the retention factor of these aliphatic amines with DCC gels is in the order of methylamine>dimethylamine>trimethylamine at all tested pH values (See Fig. 8). Since these amines showed no differences on CM-Cellulofine C-500m, it is likely that this difference arises from steric hindrance of methyl groups in the specific interaction with dicarboxyl groups hypothesized above.

4. Conclusion

A new type of cationic ion-exchange material was prepared by introducing carboxyls to spherical cellulose gel by controlled successive oxidations by periodate and chlorite. This material had mechanical strength and stability of packed bed suitable for chromatographic applications. The ion-exchange activity was similar to that of conventional carboxylated cellulose gel, but showed an anomalous pH dependence of retention factor for amines. We propose the cause of anomaly in terms of dissociation states of spatially paired carboxyl groups. This behavior potentially gives new means to control ion-exchange behavior of amines when the mechanism is fully understood.

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