

Polyamphoteric membrane study: 1. Potentiometric behaviour of succinyl chitosan aqueous solution

Keiichiro Saito and Akihiko Tanioka*

*Department of Organic and Polymeric Materials, Tokyo Institute of Technology, Ookayama,
Meguro, Tokyo 152, Japan*

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A study of the potentiometric behaviour of aqueous solutions of weak amphoteric polymers was carried out in advance of an investigation of weak polyamphoteric membrane prepared from a mixture of the amphoteric chitosan and poly(vinyl alcohol). Carboxymethyl chitosan and succinyl chitosan were used as weak polyampholytes. Succinyl chitosan is composed of amino and carboxyl groups, which were provided by substitution of the former with the latter. The portion of substituted groups from amino to carboxyl is represented by the degree of substitution (*DS*). Potentiometric titrations were performed with added salt of the 1-1 type or 2-1 type and without added salt. The behaviour could be rationalized through the dissociation mode concept based on a modified Henderson–Hasselbalch relationship. Copyright © 1996 Elsevier Science Ltd.

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INTRODUCTION

Polyelectrolyte solution has been one of the most important subjects to be studied in the fields of physical chemistry, polymer chemistry, bioscience, among others. The potentiometric behaviour of the polyelectrolyte solutions which may bear either a negative charge such as polyacrylic¹ and polymethacrylic² acids or a positive one, such as polyallylammonium³ has been studied intensively by many authors. Polyampholytes with both positively and negatively dissociative groups, such as various proteins and copolymer of dimethylaminoethyl methacrylate and methacrylic acid, are also attractive polyelectrolytes. Their potentiometric behaviour has been studied experimentally and theoretically by Katchalsky⁴ and Nagasawa⁵, respectively. Various types of charged membranes, with negatively charged groups, positively charged ones or both, have also been investigated^{6–15}. However, studies of the relationships between membrane phenomena and the behaviour of polyelectrolyte solutions, that is, membrane potential and ionic permeability through a membrane, and potentiometric and conductimetric behaviour of dilute solutions of the same polyelectrolyte or polyampholyte prepared for membrane use are rare. In this series of studies, the potentiometric investigation of a dilute solution of a polyampholyte was carried out first; the transport phenomena for a membrane composed of the same polyampholyte, as used in the first section, were also examined in order to examine the relationships between them. Succinyl chitosan was prepared from chitosan by substituting the amino group for the carboxyl group, and

employed as a polyampholyte. Chitosan^{16,17} is the deacetylated derivative of chitin found in, for example, the shells of crabs or shrimps. The uses of chitin and chitosan have spread into various fields, such as materials for medical use¹⁸, pervaporation separation¹⁹, and fixing enzymes. It is considered that the amphoteric charges are the points which could interact with other low molecular weight substances. In this series of studies, they are considered to function as fixed charged groups.

EXPERIMENTAL

Samples

The *N*-succinyl chitosans used in this study, each of which has different degree of substitution (*DS*), were supplied from Katakura Chikkarin Co., Ltd, and the chemical structure is shown in *Figure 1*. Also employed was the carboxymethyl chitosan shown in *Figure 2*, which had the same amount of carboxyl and amino groups and was supplied from Fuji Spinning Co., Ltd. The degree of polymerization was found to be about 4000, according to viscometric measurement. The degree of deacetylation was 98% for all samples, and the *DS*, which showed the portion of substituted groups from amino to carboxyl groups, was estimated using a nitrogen–carbon analyser (Sumigraph Model NC-80 Auto, Sumitomo Co., Ltd) and is summarized in *Table 1*. Before the potentiometric measurements, the samples were dialysed against water for more than 5 days using a dialysis tube made of cellulose and deionized with a mixed bed ion-exchange resin, Dowex 1 and 50, to put them in an iso-ionic state. All the reagents used were analytical grade and deionized water was used throughout the experiments.

*To whom correspondence should be addressed

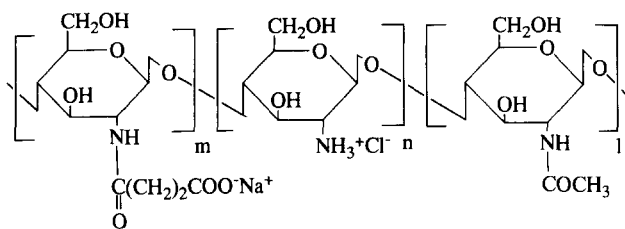


Figure 1 Schematic representation of *N*-succinyl chitosan. the degree of deacetylation is $\frac{m+n}{m+n+1} = 0.98$, and the degree of substitution is $\frac{n}{m+n}$

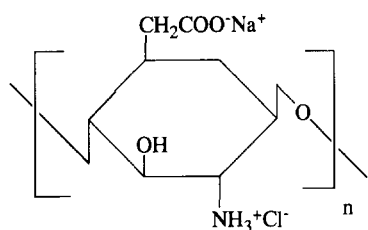


Figure 2 Schematic representation of carboxymethyl chitosan

Table 1 Degree of substitution (DS) of each sample

Sample	DS (%)
A-50	50
A-78	78

Potentiometric titration

Potentiometric titrations, with and without added salt, were performed by adding KOH (0.1M) and HCl (0.1 M) stepwise into the polymer solution (100 ml) under a N₂ gas flow. The polymer concentration ranged between 0.00057 and 0.00068 mol l⁻¹. This range of concentration was low enough to avoid any major dependency on concentration. The pH values were measured with a complex electrode (GST-5211C, TOA Electric) and a pH meter (HM-20S, TOA Electric). To clarify the titration curve, the contributions of free H⁺ and OH⁻ were subtracted from the measurements by running blank titrations for every concentration of every salt. The procedure is referred to by Kawaguchi *et al.*²⁰. The degree of dissociation, α , was determined by the amounts of KOH and HCl added, which were corrected according to the method cited above.

RESULTS AND DISCUSSION

The potentiometric titration curve for A-78 without added salt is shown in Figure 3. The point of a discontinuous change in the curve should be denoted as the iso-electric point (IEP), which has also been called the 'iso-electric jump' by Katchalsky *et al.*⁴. The signs of the net charge are positive on the left side of the IEP (Region A), zero at the IEP, and negative on the right side (Region B). Figure 4 shows an example of the potentiometric titration curves for A-78 with added salt, in which case only Region B could be obtained because the polymer solution was white and turbid when the pH of the solution was lowered. The potentiometric titration curves for A-50 are presented in Figures 5a-c. For A-50, the Region A of the curve was obtained when the

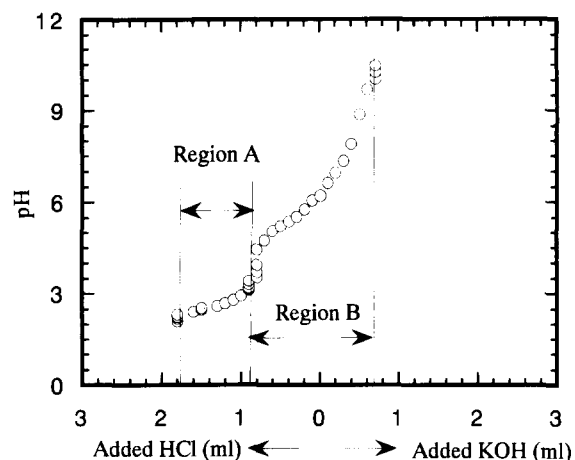


Figure 3 Directly obtained result of potentiometric titration experiment for A-78 without added salt. The polymer concentration was 0.00057 mol l⁻¹

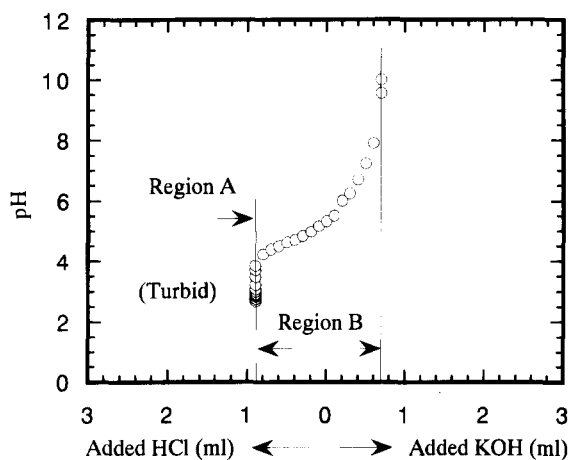


Figure 4 Directly obtained result of potentiometric titration experiment for A-78 with added salt ([KCl]=0.01 mol l⁻¹). The curve in Region A was not available because of turbidity. The polymer concentration was 0.00057 mol l⁻¹

concentration of added salt was low ([KCl]=0.01, 0.1 mol l⁻¹), as shown in Figure 5b, but was not possible when the concentration of added KCl was high ([KCl]=1 mol l⁻¹), as shown in Figure 5c. Where the potentiometric data were available over the whole range of the regions, i.e., both Region A and Region B, the dissociation constants (*K*) of the polyampholytes were calculated relative to the degree of dissociation (α), considering the state of all the amino and carboxyl groups to be undissociated when $\alpha = 0$ (-NH₃⁺, -COOH) and fully dissociated when $\alpha = 1$ (-NH₂, -COO⁻). If unavailable in Region A, *K*s were calculated relative to γ , which represents the degree of dissociation within Region B, when considering the IEP as $\gamma = 0$ and the fully dissociated state as $\gamma = 1$ (-NH₂, -COO⁻). If possible *K*s were estimated within Region A relative to β , considering the fully undissociated state as $\beta = 0$ (-NH₃⁺, -COOH) and the iso-electric point as $\beta = 1$. Calculations were performed according to the following procedure. The negative common logarithm of *K*, the p*K*, was defined by the Henderson-Hasselbalch equation (equations (1) and (2)). The p*K* is a function of the degree of dissociation

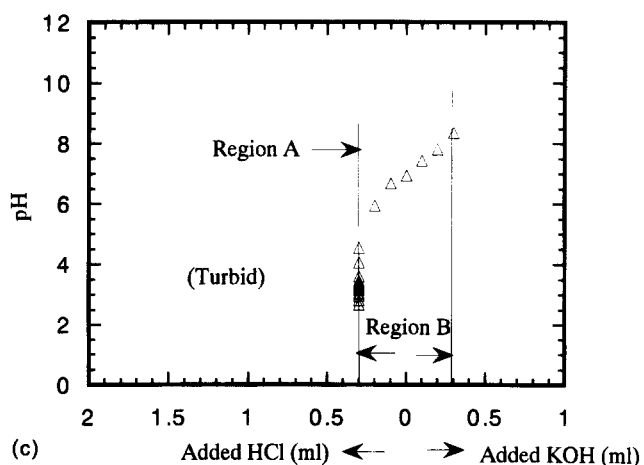
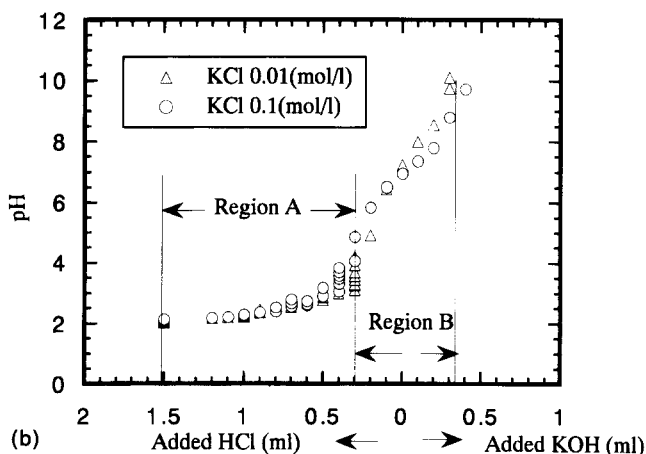
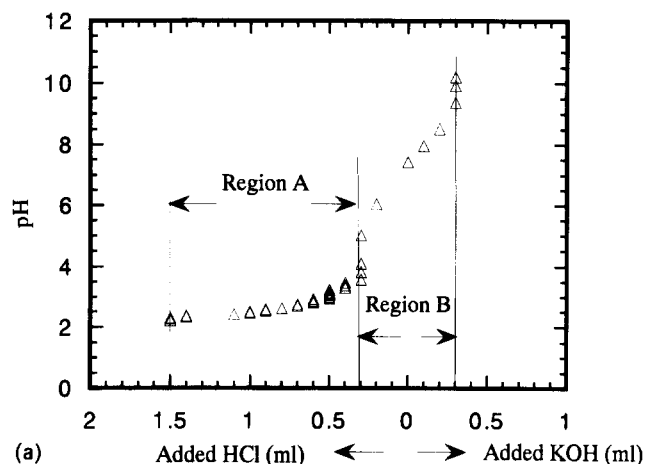


Figure 5 Directly obtained result of potentiometric titration experiment for A-50 without (→(a)) and with (→(b, c)) added salt. The curve in Region A could not be obtained because of turbidity when the concentration of added KCl is high (→(a), [KCl] = 1 mol l⁻¹). The polymer concentration was 0.00068 mol l⁻¹

(a) for the usual polyelectrolytes and amphoteric polyelectrolytes.

$$pK(\alpha) = pH + \log[(1 - \alpha)/\alpha] \quad (1)$$

$$= pK_0 + \frac{0.434}{RT} G_{el}(\alpha) \quad (2)$$

where pK_0 is an intrinsic dissociation constant, $G_{el}(\alpha)$ is the work required to remove H⁺ from the surface of polyampholyte molecule whose degree of dissociation is α . If α is replaced by β , $pK(\beta)$ express the variation in the

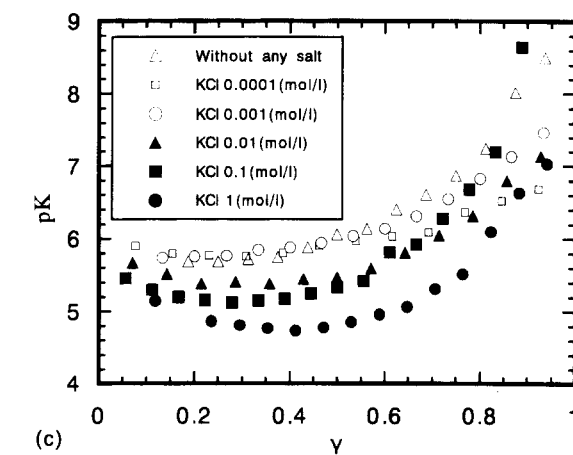
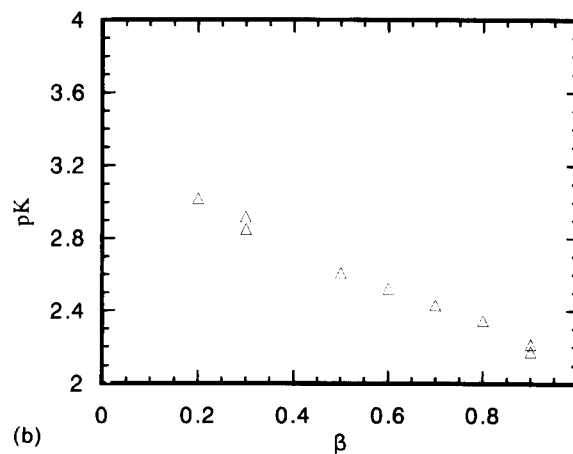
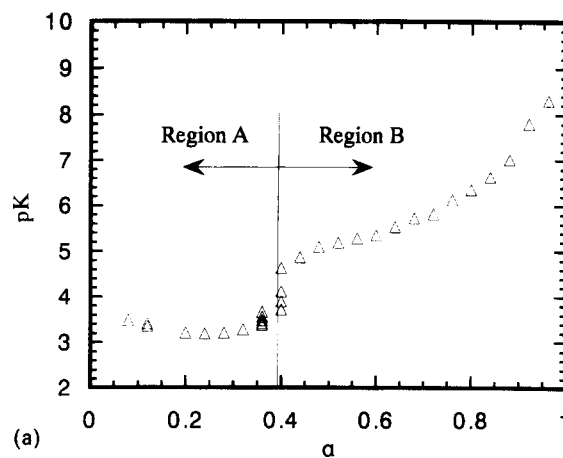


Figure 6 Potentiometric titration curve for A-78 (polymer concentration was 0.00057 mol l⁻¹) over the whole dissociation range (→(a)). The curves within Region A (→(b)) and within Region B (→(c)) are also shown. The polymer concentration was 0.00057 mol l⁻¹

dissociation constant of the polyampholyte within Region A, and if α is replaced by γ , $pK(\gamma)$ describes the value within Region B.

A series of pK values were calculated according to equation (1) and are shown in *Figures 6a-c* for A-78 and in *Figures 7a-c* for A-50 using KCl as the added salt. *Figure 6c* shows that added KCl reduced the pK of A-78 in Region B, especially for the case of low γ , which indicates an interaction between KCl and A-78 near the IEP (*Figure 8*). *Figure 9* shows the dependency of pK within Region B for varieties of salts. It can be said from *Figure 8* that 2-1 type salts, such as CaCl₂ and MgCl₂, interact with A-78 more strongly than 1-1 types, such as

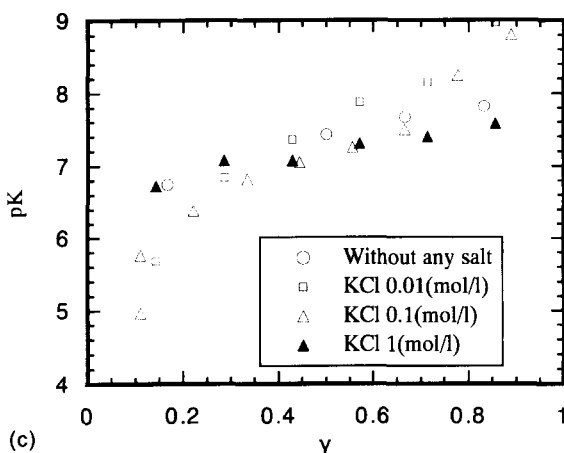
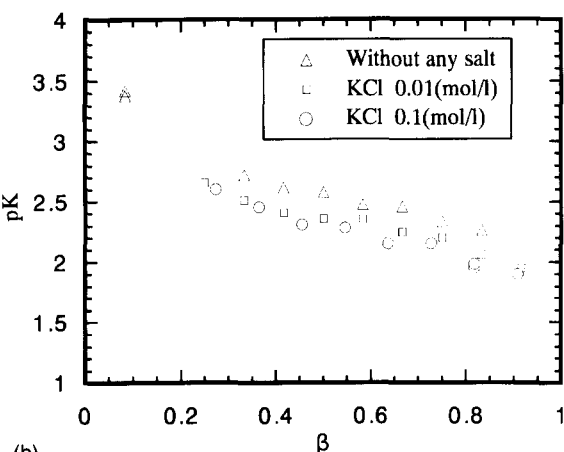
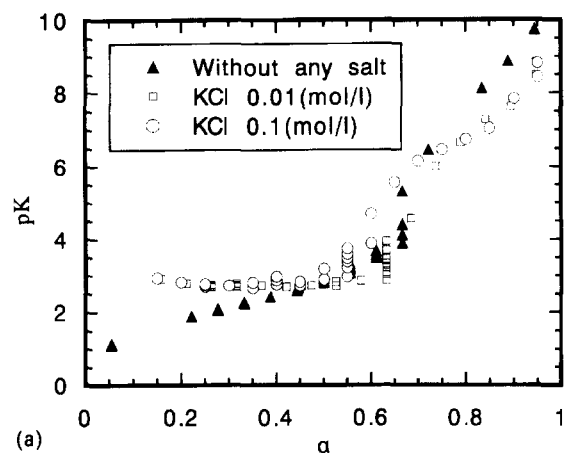


Figure 7 Potentiometric titration curve for A-50 over the whole dissociation range (\rightarrow (a)). The curves within the Region A (\rightarrow (b)) and within Region B (\rightarrow (c)) are also shown. The polymer concentration was $0.00068 \text{ mol l}^{-1}$

KCl, LiCl and tetra-n-butylammonium chloride (TBAC), especially in the low γ region. This behaviour could be attributed to the strong interaction between Ca^{2+} or Mg^{2+} and $-\text{COO}^-$ groups²¹⁻²⁴. The order of the pKs is

$$\begin{aligned} pK(\text{CaCl}_2) < pK(\text{MgCl}_2) < pK(\text{KCl}) \\ &= pK(\text{LiCl}) = pK(\text{TBAC}) \end{aligned}$$

This order is in accordance with Tasaki and Byrne's estimation^{22,23} of the preference of each ion for $-\text{COO}^-$

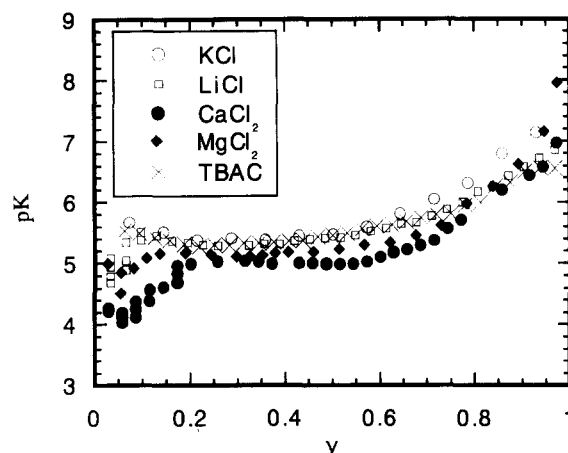


Figure 8 Potentiometric titration curve for A-78 within the Region B with added salts having the Cl^- ion in common. Concentration of the added salt in each case was 0.01 mol l^{-1} . The polymer concentration was $0.00057 \text{ mol l}^{-1}$

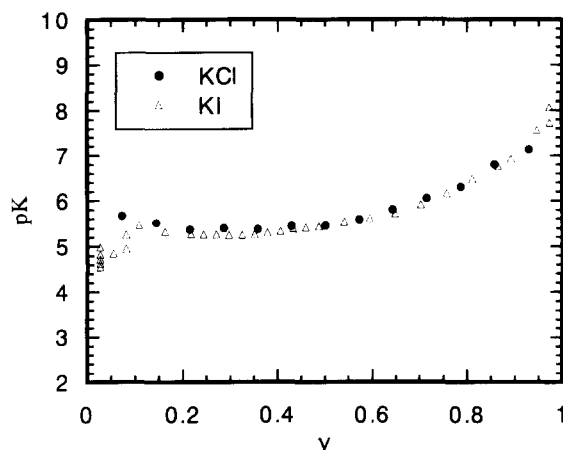


Figure 9 Potentiometric titration curve for A-78 within the Region B with added salts having K^+ ion in common. Concentration of the added salt in each case was 0.01 mol l^{-1} . The polymer concentration was $0.00057 \text{ mol l}^{-1}$

groups and the inverse order of the Stokes' radius of each ion. Figure 9 indicates that the difference in negative ions of the salts has little effect on the pK of A-78 in Region B. From the results cited above, it can be concluded that positive ions have an effect on the dissociation behaviour of A-78 in Region B and negative ions have little effect, which means that the dissociation of $-\text{COOH}$ plays an important role in Region B while that of $-\text{NH}_3^+$ does not. Nagasawa *et al.*⁵ showed that a linear polyampholyte, a copolymer of methacrylic acid and 2-dimethylaminoethyl methacrylate, displayed the same type of the potentiometric titration curve as ours and analysed it as we have in this study. They assumed that $-\text{COOH}$ groups dissociated in the Region A and $-\text{NH}_3^+$ groups dissociated in Region B and tried, unsuccessfully, to apply the model calculations by computer to explain the experimental data. They attempted to explain the molecular conformation, the molecular structure and the local dielectric constant around the charges. Although their analyses might be effective, the dissociation of $-\text{NH}_3^+$ and $-\text{COOH}$ groups should not be limited to only one region. Our sample had different amounts of amino and carboxyl

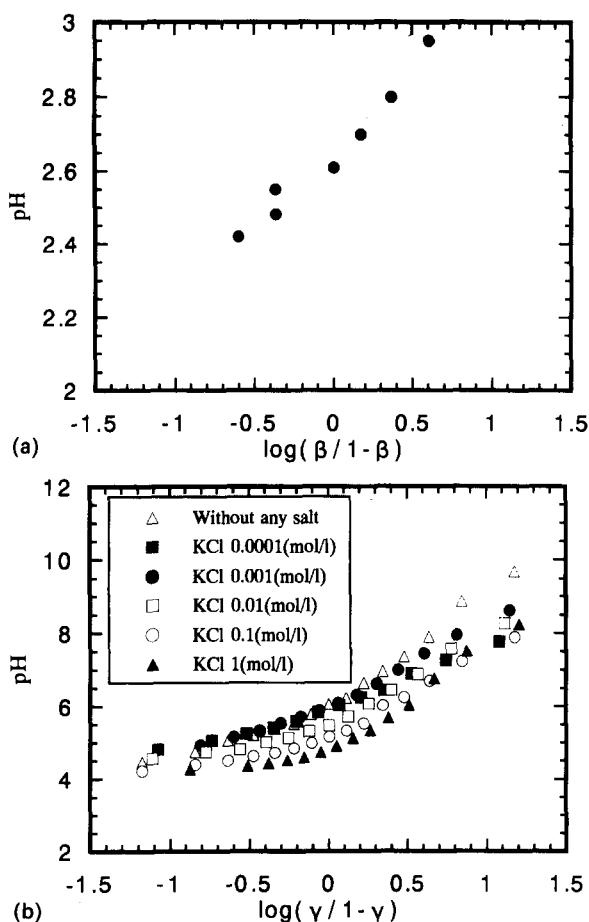


Figure 10 The modified Henderson–Hasselbalch plot of potentiometric titration data for A-78 in Region A (→(a)) and in Region B (→(b)). (a) includes a series of data without added salt while (b) includes data with added salt at various concentrations. The polymer concentration was $0.00057 \text{ mol l}^{-1}$ in all the cases

dissociative groups. Also, the dissociation constant of $-\text{NH}_3^+$ on the polymer chain should be different from that of $-\text{COOH}$. So, in our study, the concept of dissociation mode, which depends on the physical properties and the environment of the polyampholyte, was introduced. To describe this, the modified Henderson–Hasselbalch equation was suggested for use (equation (3))

$$\text{pH} = \text{p}K_{\frac{1}{2}} + n \log\left(\frac{\alpha}{1-\alpha}\right) \quad (3)$$

where $\text{p}K_{\frac{1}{2}}$ is the $\text{p}K$ at $\alpha = 0.5$, and n is a constant. Katchalsky *et al.*²⁵, Strobel *et al.*²⁶ and Fischer *et al.*²⁷ have shown that many potentiometric titration data for polyelectrolytes confirm this equation experimentally. $\text{p}K_{\frac{1}{2}}$ and n were reported to be constant, depending on the environment where titration occurred. Mandel *et al.*²⁸ determined that a conformational change in the polyelectrolyte could cause a variation in the n -parameter. So if the dissociative group from which H^+ dissociated changed, the n -parameter was assumed to change. Figure 10 shows the modified Henderson–Hasselbalch plot of the potentiometric titration data for A-78. Figure 10a shows a straight line for Region A. This means that the n -parameter is constant throughout the region. So, in Region A, dissociation of H^+ occurs according to the constant mode. In the pH range of Region A, only

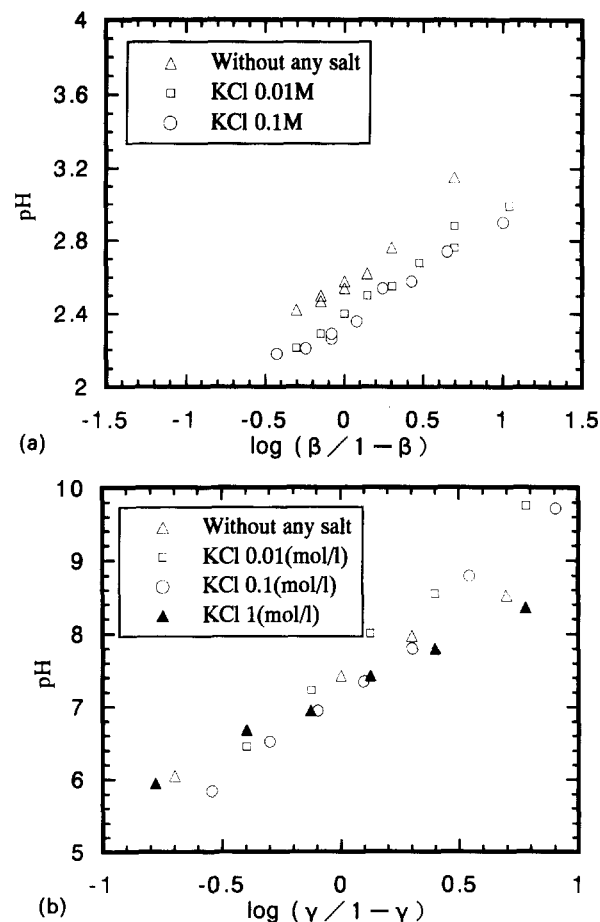


Figure 11 The modified Henderson–Hasselbalch plot of potentiometric titration data for A-50 in Region A (→(a)) and in Region B (→(b)). The polymer concentration was $0.00068 \text{ mol l}^{-1}$

$-\text{COOH}$ groups are expected to dissociate. Figure 10b which shows curves for each concentration of added KCl for Region B. This indicates that the dissociation in Region B varies gradually. In the process in which release of H^+ occurs, in the first step, near the IEP ($\gamma = 0$), a considerable fraction of the dissociative groups from which H^+ originates could be $-\text{COOH}$, which gradually decreases. The $-\text{NH}_3^+$ fraction then increases as titration proceeds towards the endpoint ($\gamma = 1$). It can also be seen from Figure 10b that the curvature increases as added KCl is increased, so that the plot shape appears to be composed of two straight lines connecting at the point $\gamma = 0.5$ ($\log \gamma / (1 - \gamma) = 0$). This means that the screening effect of added KCl emphasizes two types of dissociation mode. Thus a total of three types of dissociation mode should be considered when analysing the dissociation behaviour of a weak polyampholyte, namely a region where $-\text{COOH}$ dissociates (referred to as Region A in this study), a region where both $-\text{NH}_3^+$ and $-\text{COOH}$ dissociate in a complex manner and a region where release of H^+ from $-\text{NH}_3^+$ occurs. According to the method adopted to analyse the dissociation behaviour of A-78, that of A-50 was also discussed here in terms of the modified Henderson–Hasselbalch equation. The plot is shown in Figure 11. As presented in Figure 7, titration data without or with a low concentration of added salt were obtained in both regions. In Figure 11b, it is shown that the dissociation behaviour of A-50 followed the unique

mode through Region A. In addition to the result cited above, the n -parameter was found to be the same as for A-78 in Region A—compared with Figure 10b. It could be concluded that the dissociation behaviour of both A-78 and A-50 in Region A might be characterized by only one mode, namely the dissociation of $-\text{COOH}$ groups. Figure 11b represents the Henderson–Hasselbalch plot for A-50 in Region B. The plot line appears straight, which means that dissociation of A-50 in Region B is governed by the unique mode and is different from A-78. The reason is that A-50 has fewer $-\text{COOH}$ groups than A-78 and thus could have less influence on the dissociation behaviour of A-50 in Region B.

Although this study provides a good explanation of the dissociation behaviour of a weak polyampholyte, some subjects are still left to be solved theoretically and experimentally. The first is the fact that the polymer solution with added salt was turbid when the pH was lowered. For A-78 especially, any amount of added salt causes this phenomenon but not in the case of A-50 with a small amount of added salt. This should be attributed to the interaction between charged groups, which might be $-\text{NH}_3^+$ in this case, and low molecular weight ions. In addition, the difference in chemical configuration between A-78 and A-50 could support the results. Their physical conformation should also be an important factor. The second problem is that an amphoteric polymer with a low DS is difficult to dissolve in water, so that the method of dissolving it should be taken into account in order to investigate the potentiometric behaviour of the sample, such as A-29 whose DS is 29%, because the results for a low DS -polyampholyte should give us further information. Another possibility is using a solvent other than water. If investigated in a different solvent, however, the difference in the solvent used should be carefully considered.

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