Polymer Bulletin 24, 437–444 (1990)

Polymer Bulletin

Conductimetric titration of polyelectrolytes having sulfate and carboxyl groups

Tooru Akahane[1], **Sadaaki Takeuchi**[1], **and Akira Minakata**[2],*

[1]Department of Material Science, Numazu College of Technology, 3600 Ooka, Numazu, 410 Japan
[2]Department of Physics, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu, 431-31, Japan

Summary

Conductimetric titration of polyelectrolyte solutions was carried out to determine the contents of sulfate and carboxyl groups. In the mixtures of poly(acrylic acid) and poly(vinyl sulfate), and of alginic acid and dextran sulfate, the contents of sulfate and carboxyl groups observed by the conductimetric titration were in agreement with those derived from the concentration of the original homopolymers. This method was applied to a viscous polysaccharide from red algae, funoran, which contains both the sulfate and carboxyl groups. These results suggested that this method is applicable to either the samples consisting of the mixture of homopolymers or those of copolymers containing both ionic species.

Introduction

Several kinds of viscous polysaccharides such as heparin and agar are polyelectrolytes having two kinds of charged groups, i.e., sulfates and carboxyls. The content of these charged groups influences the solution properties as well as the biological function of the polysaccharides. Therefore, it is necessary to know the exact content of each charged group. There have been various works on the determination of the contents of these groups(1-8). Some works among them are based on the difference in pK values between the sulfate and carboxyl groups. Although the determination of the pK values is readily available, the reliability or reproducibility of the data seems sometimes poor. The main reason may be that the dissociation constants of the sulfate and carboxyl groups mixed in a single polymer chain are deviated from those present as a single site. In addition, a charged group in a polymer chain dissociates under an influence of other charged groups or of the electric potential of the polyion, leading to a continuous increase in the apparent pK value with increasing degree of neutralization. Owing to these facts, the change in the apparent pK values of the sulfate and carboxyl groups are overlapped with each other, and each pK value can not be determined exactly.

In the present study, we adopted a conductimetric titration as an alternative method to determine the contents of sulfate and carboxyl groups. Since the coincidence is needed between the contents determined by this method and those determined stoichiometrically, we tested it by measuring the conductance of the mixture of ionic homopolymers, one of which has sulfate groups

---

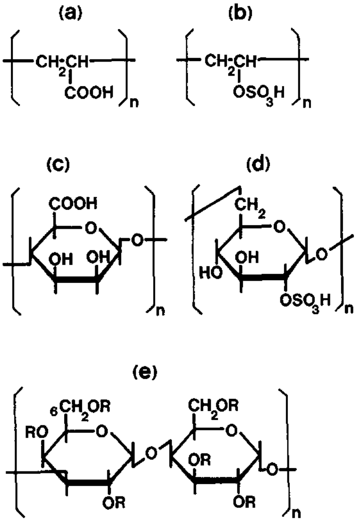
*To whom offprint requests should be sent and the other carboxyl groups. The observed values of the contents were found to be in agreement with those derived from the concentration of the original homopolymers. Then, this method was applied to a natural polyelectrolyte, funoran, an extracellular polysaccharide from a red alga, Gloiopeltis complanata, which contains both the sulfate and carboxyl groups. The content of the sulfate groups observed coincided with that found in a sample the carboxyl groups of which were methylated.

Experimental

Four kinds of ionic homopolymers were used; poly(acrylic acid)(PAA) and alginic scid(AA) as polymers having carboxyl groups, and poly(vinyl sulfate)(PVS) and dextran sulfate(DS) as polymers having sulfate groups. These structural formulas are listed in Fig.1. PAA was synthesized by polymerizing acrylic acid in ethanol as solvent and benzoyl peroxide as initiator. The product was purified by repeating precipitation in ethyl ether and dissolution in ethanol 3 times. The other polymers were commercial ones of analytical grade. These samples were dissolved in pure water and deionized by a cation exchange resin IR-120B just before use(9), because of the instability of the charged groups against deionization. The experiments were made in the absence of salts.

The method of the extraction of funoran from a red alga, Gloiopeltis complanata, has already been described(10,11). Funoran was swelled overnight in pure water and then dissolved to obtain a 0.5% aqueous solution. Since natural funoran contains Na^+ and K^+ as counterions, the solution was mixed with the cation exchange resin IR-120B and stirred slowly for 2 hrs at room temperature to convert to H-form, and freeze dried just after the ion exchange. The above procedure was done carefully, because a partial release of the sulfate groups may occur. The concentration was determined by weighing a freeze dried H-form sample.

Methylation of the carboxyl groups in funoran was carried out as follows(12). An amount of 62.5 mg of funoran was dissolved in 100ml dimethyl formamide(DMF) and 0.2 ml methyl iodide(CH_3I) was added. The mixture was heated up to 80℃ under reflux for 1 hr by attaching a reflux condenser in a draft chamber. The unreacted CH_3I was evaporated by stopping the reflux, resulting in a brown colored transparent solution.



R : H or SO_3H

C_6 site : CH_2OR or pyruvate

Fig.1 Chemical structures of the samples. (a): poly(acrylic acid)(PAA), (b): poly(vinyl sulfate)(PVS), (c): alginic acid(AA), (d): dextran sulfate(DS), (e): funoran.

Then DMF was evaporated by using a rotary evaporator. The precipitate was dissolved in pure water and purified by ultrafiltration.

The bases used were NaOH, tetraethyl ammonium hydroxide (TENOH) and tetra-n-butyl ammonium hydroxide(TBNOH) of guaranteed grade. Other chemicals were of analytical grade and used without further purification.

Conductimetric titration was carried out with a Radiometer CDM2e conductivity meter. A volume of 25ml of each H-form sample was poured into a cell for the conductimetric titration. The titrant, 0.1 N of each base was added by using a Metrohm E457 microbiuret at 25℃ in a stream of nitrogen. Potentiometric titration was made with an Orion Research 701A Ionalyzer in the same way as the conductimetric titration. All measurements were carried out in a salt-free solution to clarify the change in the conductance. Infrared absorption was measured with a Jasco A-3 infrared spectrophotometer.

Results

The conductance and pH of PAA and PVS are plotted against degree of neutralization, α in Fig.2. There is a marked difference in the titration profile between them. The conductance of PVS decreases sharply and almost linearly with α, due to the loss of freely mobile protons by the neutralization. On the other hand, the conductance of PAA rather increases with α, due

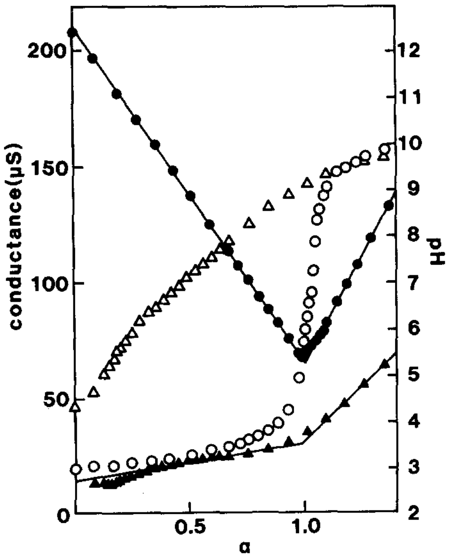


Fig.2 Conductimetric and potentiometric titration curves of PVS(● ,○) and PAA(▲ ,△) with 0.1N NaOH. Cp: 8.43×10^{-4}N(PVS), 4.25×10^{-4}N(PAA).

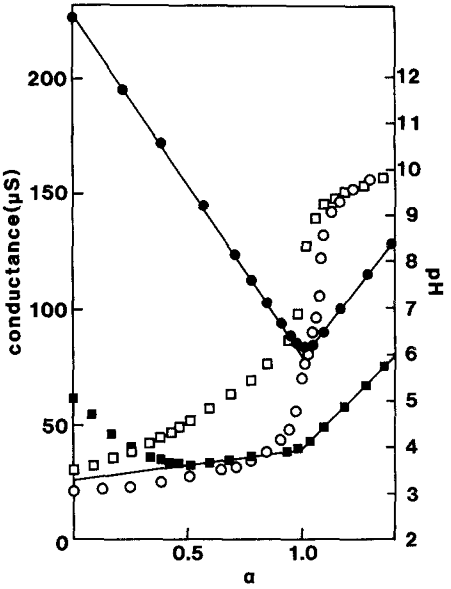


Fig.3 Conductimetric and potentiometric titration curves of DS(● ,○) and AA(■ ,□) with 0.1N NaOH. Cp: 5.54×10^{-4}N(DS), 4.21×10^{-4}N(AA).

to the replacement of a tightly bound proton by a mobile Na^+ ion. For $\alpha > 1.0$, the conductance of PVS and PAA increases linearly with the addition of free ions, Na^+ and OH^-. Similarly, difference in pH profile between PVS and PAA is understood as the difference in the state of binding of H^+ between them, that is, PVS is titrated as a strong acid but PAA as a weak acid.

The conductance of PVS at $\alpha \cong 1.0$ rather increases with α. This may be due to the depression of further dissociation of protons, resulting from an increase in the potential depth, formed by an atmospheric binding of Na^+ in the region of $\alpha \cong 1.0$. Likewise, the decrease in the conductance with α at low α in PAA is a result of the low potential depth, leading to a release of protons(13).

Figure 3 shows the results of AA and DS, similar to those in Fig.2. The difference in the titration behavior between AA and DS is not so significant as that between PAA and PVS, due mainly to the lower pK value of carboxyl groups in AA than that in PAA.

The conductimetric and potentiometric titration behaviors of the mixture of PAA and PVS, and those of the mixture of AA and DS are shown in Figs. 4 and 5, respectively. The observed curve for the mixture is found to be an intermediate of the original two curves, except that the sulfate groups are dissociated at first. The conductance first decreases, then increases slightly, and finally increases sharply with α. The linearity of the

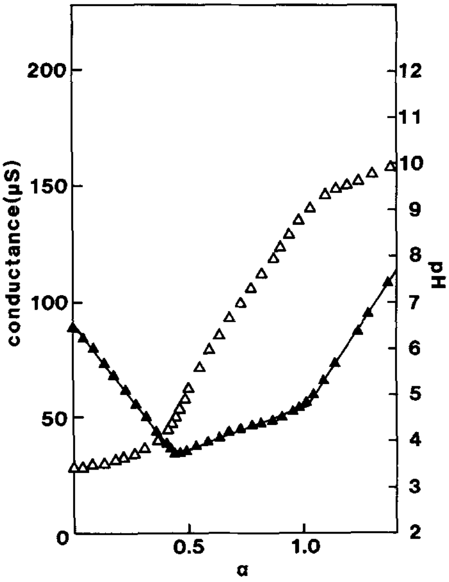


Fig.4 Conductimetric and potentiometric titration of the mixture of PVS and PAA. Cp: 3.25×10^{-4}N(PVS), 4.54×10^{-4}N (PAA).

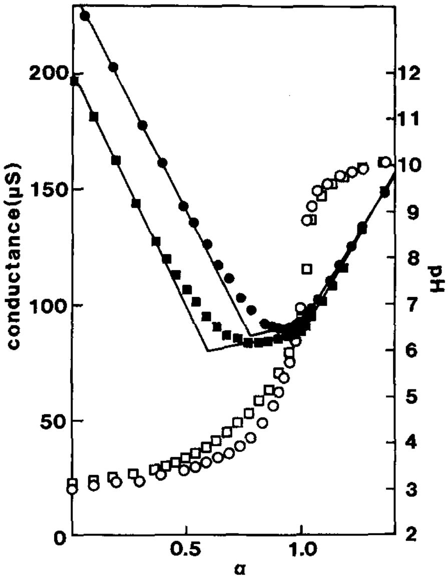


Fig.5 Conductimetric and potentiometric titration of the mixture of DS and AA. Cp: 5.71×10^{-4}N(DS), 1.61×10^{-4}N(AA) (●,○); 4.04×10^{-4}N(DS), 3.89×10^{-4}N(AA)(■,□).

conductance change with α holds at low α and at $\alpha > 1.0$, as seen in Figs. 4 and 5. If the conductance increase in the region from $\alpha \cong 0.3$ to 1.0 can be approximated as linear, this slope together with the initial and final slopes can determine the end point of titration as well as the ratio of contents of sulfate to carboxyl groups. The analysis was performed by this method tentatively called as the slope method. The results of the determination are summarized in Table 1.

As mentioned before, the potentiometric titration curve for the mixture is somewhat an intermediate one of the original two curves. The intrinsic value pK_0 of an ionic homopolymer is obtained by extrapolating the plot of the apparent pK value, $pK = pH - \log[\alpha/(1 - \alpha)]$ vs. α, to $\alpha = 0$. This method is, however, not always applicable to a mixed system or a polyelectrolyte having two kinds of charged groups, because of the gradual change in the apparent pK value with α.

As a convenient way to compare the potentiometric titration behavior with one another, we adopted here the value of pKa, tentatively defined as the value of pH at the midpoint of the neutralization. When two kinds of charged groups exist, each pKa value is defined as the pH value at half value of α between the beginning and end points of the titration of each ionizable group. We assumed here that the sulfate group dissociates at first, and its end point was determined conductimetrically. The values of pKa thus defined are listed in Table 2. The values of the mixed systems are not so different from those of the single homopolymers, suggesting no strong interaction between the sulfate and carboxyl groups.

The conductimetric and potentiometric titration of funoran

TABLE 1
Comparison of the mixing ratio of carboxyl to sulfate groups with the observed ratio.

[PAA]/[PVS]		[AA]/[DS]	
mixing ratio	observed ratio	mixing ratio	observed ratio
0.51	0.54	0.28	0.28
1.40	1.38	0.96	0.70
3.47	3.39		

TABLE 2
Values of pKa in the mixtures of two polyelectrolytes.

[PAA]/[PVS]			[AA]/[DS]		
mixing ratio	observed pKa		mixing ratio	observed pKa	
	PVS	PAA		DS	AA
0(PVS)	3.32		0(DS)	3.35	
0.51	3.41	6.92	0.28	3.31	5.06
1.26	3.61	6.78	0.96	3.33	4.57
3.47	3.77	6.85	2.96	3.24	4.34
∞ (PAA)		7.12	∞ (AA)		4.49

were carried out by using 0.0568%(wt/v) solution with NaOH, TENOH or TBNOH. The results are shown in Fig. 6. The titration behaviors are similar to those of the mixed systems. The linearity of the initial decrease as well as the final increase are clearly observed. The end point of the whole titration is observed most clearly for NaOH, but that of the sulfate group is not so apparent for all cases. The slope method was applied to determine the contents of the sulfate and carboxyl groups, although the linearity was not clearly observed in the intermediate region responsible for the titration of the carboxyl groups.

The titration behavior and infrared absorption of funoran methyl ester are shown in Figs. 7 and 8, respectively. The conductance decreases sharply and linearly with α up to $\alpha \cong 0.9$. The pH profile also resembles that of a strong acid. The conductance change is similar to that of PVS and DS, but there exists a small region near $\alpha = 1.0$, corresponding to the titration of the carboxyl groups. The ratio of the contents of $[COO^-]$ to $[SO_3^-]$ determined by the slope method is 0.13, about half the value for the original funoran, 0.29. This means that a part of methyl esters were saponified when the sample was treated with the cation exchange resin IR-120B to obtain H-form. Consequently, the brown color of the sample evaporated from DMF solution was faded to yellow in the H-form. The infrared spectroscopy of the H-form, however, indicates a clear absorption at 2900 cm^{-1}, corresponding to the absorption of the methyl group. These results indicate that at least more than half methyl groups remained and that the content of the sulfate group in the ester form was more

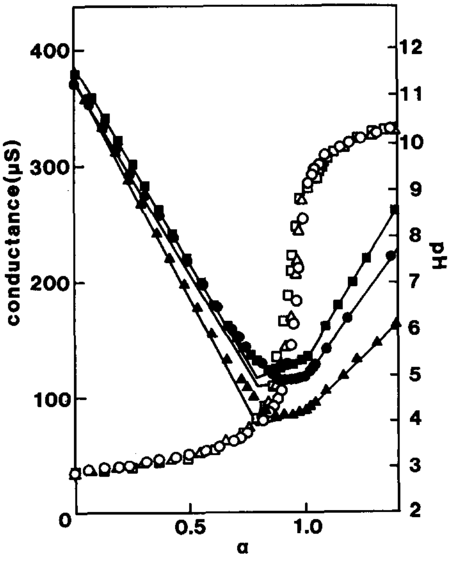


Fig.6 Conductimetric and potentiometric titration of funoran with NaOH(● ,○), TENOH(■ ,□) and TBNOH(▲ ,△).

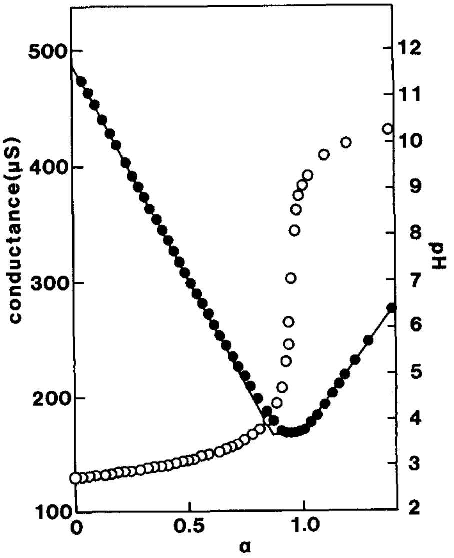


Fig.7 Conductimetric and potentiometric titration of methylated funoran with 0.1N NaOH.

easily determined than in the original funoran solution.

Discussion

As mentioned before, the conductimetric titration serves as a suitable method to determine the contents of sulfate and carboxyl groups, often encountered in natural polysaccharides. The study on the mixture of two ionic homopolymers provides a good example to clarify this problem. Namely, in the mixture of vinylic polyacids such as PAA and PVS, the determination can be performed at any ratio of both charged groups, as shown in Table 1. In the mixture of polysaccharides such as AA and DS, however, the accuracy may be lowered when the ratio of [COO^-] to [SO_3^-] becomes larger than 1.0. In the latter case, AA dissociates more easily than PAA, so that the dissociation region of AA partially overlaps that of DS. This means that the end point of the titration of the mixture of AA and DS becomes obscure as the ratio of [COO^-] increases.

The conductance change with α was linear at low α and at $\alpha > 1.0$ in most cases, which facilitated the determination of the contents by a very simple method, i.e., the slope method. A more sophisticated method such as a computer-aided best-fit method seems unnecessary at present, because the applicability of such a method depends largely on the experimental errors.

Funoran exhibits a definite conductimetric titration curve, including a long range linear portion. The reason is that the content of the sulfate is much larger than the carboxyl groups. The determination of the contents of both charged groups is easily performed.

The electrical properties as well as the biological function of the polysaccharides are known to be altered significantly by esterification of the carboxyl groups(14,15). In the present

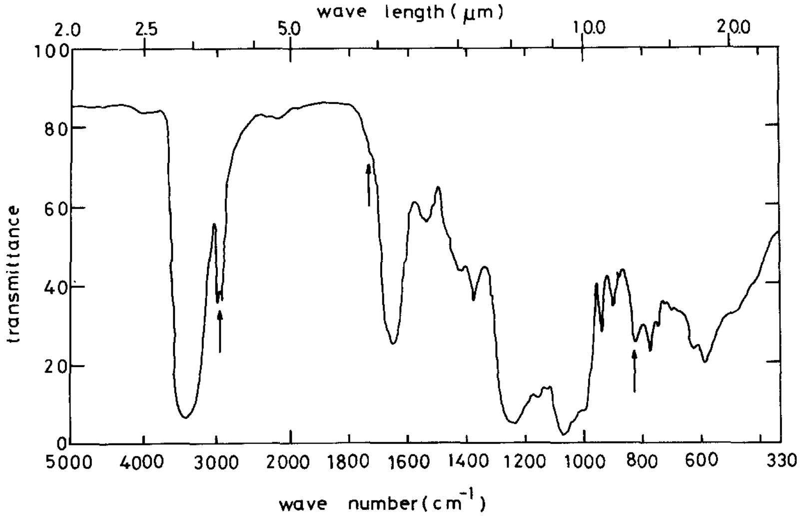


Fig.8 Infrared spectra of methylated funoran. Arrows indicate the absorption of methyl groups.

study, methylation of the carboxyl groups of funoran brought about a more definite titration curve, although a smaller portion of the carboxyl groups were not methylated. Complete methylation seems, however, very difficult because of the partial release of methyl groups during the cation exchange. Also release of the charged group itself may occur during the cation exchange, so that careful treatment is needed for the natural polysaccharides.

The end point of the titration was determined most clearly by use of NaOH among the bases used. Other bases, TENOH and TBNOH, widely used in the titration experiments because of the lack of binding to a polyion, are so large in size that these ions do not come into contact with the charged site, resulting in a broader titration curve. Especially TBN$^+$ has a low equivalent conductance, which lowers the slope for $\alpha > 1.0$, leading to another difficulty in the determination of the end point. These results are also related to the use of the salt-free solutions to clarify the conductance change. The salt-free condition is usually not adopted in the potentiometric titration, because the accuracy is lowered. The accuracy of the conductimetric titration is, on the contrary, decreased in a conductive solution.

The esterification of carboxyl groups was incomplete in the present study. If complete esterification can be performed, the method of the determination of the contents of both charged groups will be established by comparing an original sample with the methylated one. It is desirable to find a suitable method of protection of the methyl group from the treatment of ion exchange.

References

1. Okimasu S.(1958) Bull. Agr. Chem. Soc. Jpn., 22, 63-68.
2. Katsuura K.(1954) Kobunshi(Macromolecules in Japanese), 57, 382-384.
3. Casu B. and Gennaro V.(1975) Carbohyd. Res., 39, 168-176.
4. Balt S., De Bolster M.W.G., Booij M., Van Herk A.M. and Visser-Luirink G.(1983) J. Inorg. Biochem., 19, 213-226.
5. Rizzo R., Paradossi G. and Crescenzi V.(1984) Makromol. Chem., 185, 757-764.
6. Rochas C., Lahaye M. and Yaphe W.(1986) Bot. Mar., 24, 335-340.
7. Dohnal J., Vytras K., Roubal Z. and Zemanova I.(1989) J. Pharmaceut. Biomed. Anal., 7, 835-841.
8. Fernandez L.E., Valiente O.G., Mainardi V., Bello J.L., Velez H. and Rosado A.(1989) Carbohyd. Res., 190, 77-83.
9. Akahane T., Kawashima S., Hirao I., Shimizu T. and Minakata A.(1982) Polymer J., 14, 181-188.
10. Hirase S., Araki C. and Ito T.(1958) Bull. Chem. Soc. Jpn., 31, 428-431.
11. Akahane T. and Izumi S.(1976) Agr. Biol. Chem., 40, 285-289.
12. Hashimoto K.(1980) Macromolecules, 13, 786-791.
13. Minakata A.(1980) Rep. Prog. Poly. Phys. Jpn., 23, 45-48.
14. Kiyohara H., Cyong J.C. and Yamada H.(1988) Carbohyd. Res., 182, 259-275.
15. Yamada H., Ra K.S., Kiyohara H., Cyong J.C. and Otsuka Y. (1989) Carbohyd. Res., 189, 209-226.

Accepted June 2, 1990 S