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Polymers Having pH-Buffering Capability for Potential Use as Solid-Phase Reagent

Koji Nakano, Isamu Moriguchi, Syunya Ohishi, and Makoto Takagi*
Department of Chemical Science and Technology, Faculty of Engineering,
Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812

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Synopsis. Macroreticular styrene–divinylbenzene copolymeric resins were functionalized with aliphatic amine moieties and carboxylato groups. Their acid dissociation properties were studied with potentiometric method. Due to the acid-base properties of the incorporated functional groups, the resins obtained showed pH-buffering abilities covering the range of pH 4—9 to be useful as solid-phase reagents.

The idea to use a reagent in a solid form is widely utilized in analytical and separation chemistry. This affords the ease of operation and often allows the reusage of the reagents. Ion-exchange resins and chelating resins are the most successful examples,[1)] and recent advances in dry reagent chemistries in clinical analysis are also remarkable.[2,3)]

In many analytical reactions, pH regulation is quite essential. However, the use of pH-buffering reagents is so far limited to a homogeneous system. If polymer resins are endowed with appropriate proton-dissociation property, such resins may be used as solid-phase, pH-buffering reagents or matrix. The ease with which they can be removed by simple filtration should find their uses like volatile buffers[4)] without retention of contaminating salts in the sample solution. On the other hand, in biological cell cultivation studies, the usefulness of polyelectrolyte pH buffer systems was pointed out by Matsumura et al.;[5)] polymeric reagents, unlike the low molecular analogs, are not expected to affect the growth factors of microorganism.

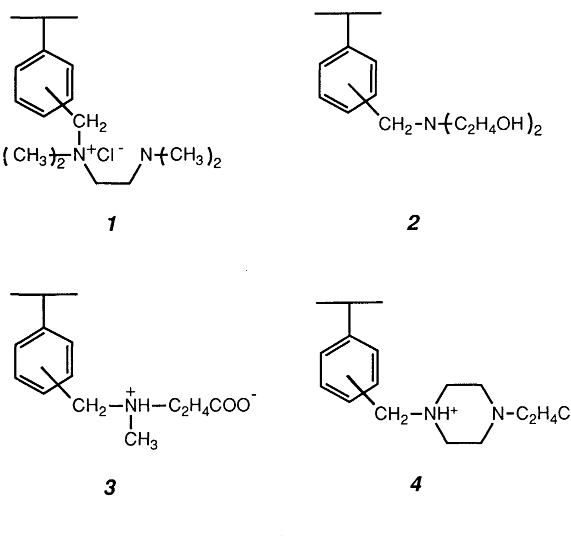
From these points of view, we have herein synthesized macroreticular styrene-divinylbenzene copolymer-based resins with acid-base properties. Since heavy metal or transition metal ions are often a target of analysis or of physiological concern, the structure of the functional groups introduced in the polymer were so designed that they do not have much complexing affinity to these metal ions. A potentiometric study on the acid dissociation properties showed that the resins have their characteristic pH-buffering region ranging from 4 to 9.

Results and Discussion

For the preparation of the solid polymer reagents, we used a chloromethylated polystyrene–divinylbenzene copolymer as the strating material. Although a variety of acid-dissociating groups can be incorporated in the solid matrix, we especially focused on aliphatic amine functions since the pK_a value of aliphatic amine largely depends on the physico-chemical nature of substituents on the nitrogen. A simple aliphatic amine shows its pH-buffering region typically at around pH 10. However, the presence of electronegative group or adjacent positive charge lowers the pK_a value by 2—3 units in general. If the protonation causes steric hindrance, the value becomes lower. As already suggested by Good,[6)] derivatizing amino group to zwitterionic form is another promising way of changing the pK_a values. On the other hand, the affinity of the bases to metal ions can be reduced by introducing cationic charges into the resin and by using tertiary amines, rather than primary and secondary amines, to increase the steric hindrance on metal coordination. Taking into account these electrostatic and steric effects, polymer reagents of structures in Scheme 1 were synthesized in the present study.

From the practical viewpoint, the amount of resin in a batch required should be as small as possible. As a measure, an ion-exchange or a chelating resin should have an effective exchange capacity of at least 1 mequiv g^{-1} on dry basis.[1)] The amino function contents in the resins of the present study as determined from elemental analysis were found to be 2—4 mequiv g^{-1}; the capacities for protonation and deprotonation seemed sufficient.

Potentiometric titration curves of polymer resins are shown in Fig. 1. In weighing the resin, the assumption was made that the nitrogen content determined by elemental analysis correctly represented all the structures contained in the resin. In other words, it was assumed that no side reactions took place in the polymer derivatization reaction. The titration curves in Fig. 1 show inflections at positions exactly expected from stoichiometry when the acids in the resin are neutralized by the added standard base. This indicates that the present



Scheme 1. Cross-linked polystyrene resins containing aliphatic amine functions.

derivatization proceeded cleanly, though in some preparations a deviation of inflection point upto 5% was observed, where some side reactions or incomplete reactions might be inferred.

As shown in Fig. 1, each polymer resin shows its characteristic pH-buffering region in fairly good agreement with the pK_a value of the monomeric parent structure. For example, resin 1 shows its pH-buffering region from pH 3 to 5. The related monomeric structural unit of this resin is *N,N,N′,N′*-tetramethylethylenediamine and its pK_{a1} value in the protonated form is 5.91 (ionic strength, I=0.1, 25 °C).[7] For other resins, the relationships of their pH-buffering regions and pK_a values of their related structural units are as follows: Resin 2 (pH buffering region, pH 5 to 8), 2,2′-iminobis(ethanol), 9.15 (I=0.1, 25 °C);[7] Resin 3 (pH 6 to 9), *N*-methylglycine, 9.75 (I=0.1, 25 °C);[7] Resin 4 (pH 6 to 10), piperazine, 5.55 and 9.81 (I=0, 25 °C).[6] The pH-buffering abilities of these resins are thus concluded to be due to the protonation and deprotonation of the amino functions incorporated into the polymer matrix. Therefore, these results offer a possible usage of these polymer resins as solid-phase, pH-buffering reagents covering the range of pH 4 to 9. In general, chemical reactions taking place in polymer matrix require a finite rate. Thus, the slow reaction rate might cause a disadvantage when faced with reaction of very rapid proton-uptake or -release in solution.

Experimental

Materials. Chloromethylated polystyrene beads were kindly gifted from Mitsubishi Kasei Corporation. The resin matrix was a macroreticular type styrene-10% divinylbenzene copolymer with a surface area and mean pore diameter of 25 m^2 g^{-1} and 900—950 Å, respectively. Other chemicals were of reagent-grade and were used as received.

Synthesis of Resins. Resin 1: A 5.0 g portion of dry resin (23 mmol of chloromethyl group) was suspended in 230 mmol *N,N,N,′N′*-tetramethylethylenediamine. The reaction mixture was heated at 50—60 °C for 4 h with gentle stirring. The resin was filtered off, washed successively with methanol, deionized water, 0.1 M (1 M=1 mol dm^{-3}) aqueous sodium hydroxide solution, methanol and finally dried at 60 °C under vacuum. The nitrogen content of the resin obtained (white to slightly yellowish) was 6.38% by elemental analysis, which corresponded to an ethylenediamine moiety content of 2.3 mmol g^{-1} (referred to dry resin).

Resin 2: By using 2,2′-iminobis(ethanol), resin 2 was prepared in the same manner as described above. The nitrogen content of the resin (white to slightly yellowish) was 5.41%, which corresponded to an amine function content of 3.9 mmol g^{-1} on dry resin basis.

Resin 3: A 5.0 g portion of resin was reacted with 230 mmol methylamine in 100 cm^3 DMF at 50—60 °C for 4 h with gentle stirring. After filtration, the resin was washed thoroughly with DMF and methanol and dried. The intermediate resin was then dispersed in 230 mmol 3-bromopropionic acid, and the reaction mixture was heated at 50—60 °C for 4 h with gentle stirring. The resin was filtered off, washed successively with methanol, deionized water, 0.1 M aqueous sodium hydroxide solution, and methanol and finally dried at 60 °C under vacuum. The nitrogen content of the resin (white to slightly yellowish) was 5.68%, which corresponded to an amine group content of 4.1 mmol g^{-1} on dry resin basis.

Resin 4: A 5.0 g portion of resin was suspended in 230 mmol piperazine in 100 cm^3 DMF. The reaction mixture was heated at 50—60 °C for 4 h with gentle stirring. The resin was filtered off and washed with DMF. The resin obtained was then suspended in 100 cm^3 DMF solution of 30 mmol 3-

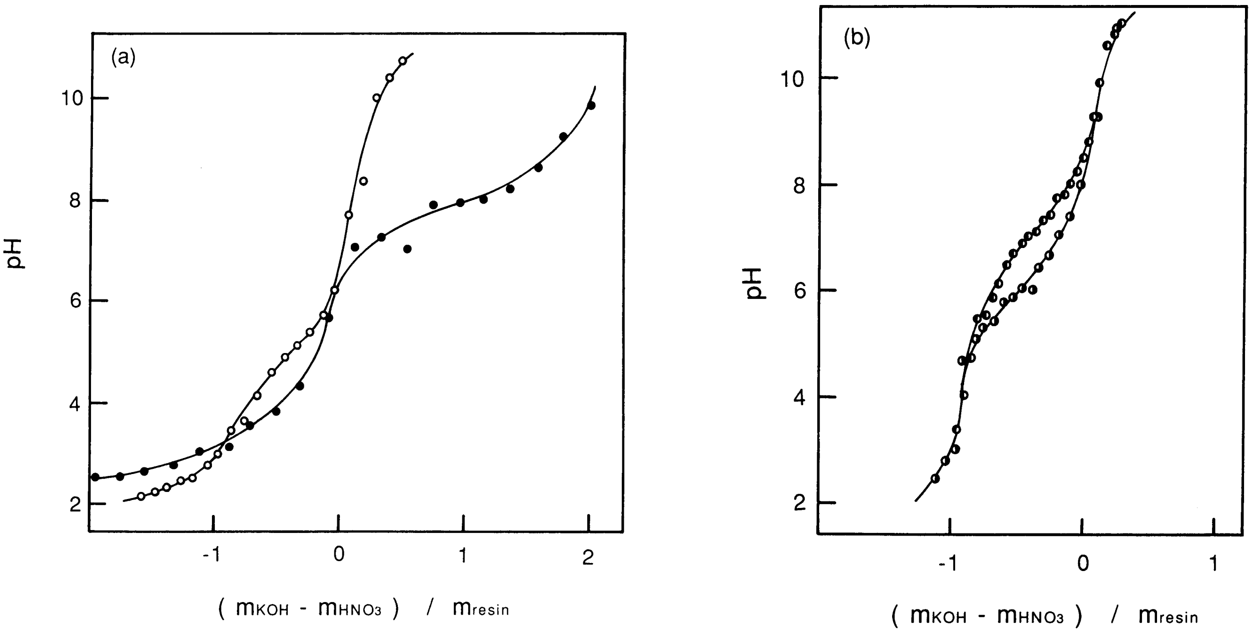


Fig. 1. Potentiometric titration curves of resins. The value m represents molar quantity of standard nitric acid, standard potassium hydroxide, and amine groups in the resin. Solid lines linking the experimental points are arbitrary. (a) (○), resin 1; (●), resin 4. (b) (◐), resin 3; (◑), resin 2. I=0.1—0.11; Temperature, 25 °C.

bromopropionic acid, and 70 mmol 1,8-diazabicyclo[5.4.6]-undec-7-ene. The mixture was heated at 40—50 °C for 4 h with gentle stirring. The resin was filtered off and washed successively with DMF, methanol, deionized water, 0.1 M aqueous sodium hydroxide solution, and methanol and then dried at 60 °C under vacuum. The nitrogen content of the resin (white to slightly yellowish) was 8.51%, which corresponded to a piperazine moiety content of 3.0 mmol g^{-1} on dry resin basis.

Acid Dissociation Properties. The polymer resin was ground and dry sieved. The 100—200 mesh fraction was collected and used for the subsequent study. A batchwise technique[8] was employed to determine the acid-dissociation properties of these resins. A 1.00 cm^3 portion of 1.0 M aqueous standard nitric acid solution was added to the weighed resin (10 mmol) in a stoppered vial. An accurately measured quantity of 1.0 M standard aqueous potassium hydroxide solution was added to attain the desired degree of neutralization. Deionized water was then added to make up the solution to 10 cm^3 and the final sample solution was deaerated with nitrogen. The ionic strength of the sample solution was adjusted between 0.1 and 0.11. Although preliminary experiments revealed that the pH of samples became constant after shaking gently for a few days, the shaking was kept over one week for complete equilibration. The temperature was kept at 25 °C all through this procedure. The pH was then measured at 25 °C under nitrogen by using a conventional pH meter (Model HM-15A, TOA Electronics Ltd., Japan) equipped with a combination pH electrode.

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