pH control in neutral solutions using polymethylacrylic acids as polymer-supported buffer agents

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Abstract. Polymethylacrylic acid beads were utilized as polymer-supported buffer agents for the pH control of neutral aqueous solutions to reduce phosphorus effluents from phosphate buffers. The agents were used to buffer the pH of urease-catalyzed reactions in a neutral PH region.

1. Introduction
Phosphate, as one of the most used buffer agents, plays important roles in several aspects of biochemistry, industrial chemistry, analytical chemistry, and environmental systems. Phosphate buffers are a type of reaction auxiliary. These buffers are not consumed by the reaction, but they are generally discharged as wastewater. Moreover, phosphate effluent presents an environmental hazard and potential impact. This waste is the main cause of eutrophication in freshwater ecosystems, thereby resulting in algal blooms and commercial fish kills. To date, almost half of all lakes and reservoirs in Eurasia and America are eutrophic. Total phosphate levels above 0.02 ppm in bodies of water accelerate eutrophication. Although much effort has been exerted to reduce the phosphate effluent from detergents, coatings, etc., few studies has been reported on reducing the effluent from phosphate buffers.

The synthesis of polymer-supported species and their applications in chemical research have received considerable attention. We recently reported polymer-supported phosphonic acids are effectively utilized as heterogenous buffer agents for the pH control of aqueous solutions in enzyme-catalyzed reactions. Their buffer regions are in the pH ranges of 4–6 and 8–10. However, most enzyme-catalyzed processes usually occur at almost-neutral solution wherein phosphate buffers are mostly used to control the pH. New buffer systems need to be developed to achieve a green and sustainable environment; these buffers should be as efficient as the phosphate buffers in neutral solution but more environmentally benign.

Thus far, polyacrylic acid beads are the most widely used solid supports in chemical synthesis and ion exchange. The pKa of these resins is approximately 6 - 7. In this study, we utilized the polymethylacrylic acid (PRCOOH) beads as polymer-supported buffer agents. These agents were used to buffer the pH of urease-catalyzed reactions in a neutral pH region.

The general strategy for the preparation of PRCOOH is described (Scheme 1) partly based on previous reports. Two steps are included. (1) Cross-linked PMMA beads were prepared via the suspension copolymerization of methyl methacrylate and divinylbenzene, and the aqueous phase was charged with stabilizers (poly(vinyl alcohol) and NaCl). (2) The resulting beads were hydrolyzed by heating in an aqueous NaOH solution to generate PRCOOH.
2. Experimental Section

2.1. General Remarks.
All reagents were purchased from Sigma-Aldrich, or Sinopharm chemical. Fourier Transform Infrared spectra were recorded on a Nicolet 380 of Thermo scientific. The sample was dried and 1 mg of powder was pressed with KBr to form a Pellet and a spectrum was recorded between 4000 and 500 cm\(^{-1}\). Scanning electronmicroscopy studies were carried out with FEI-Quanta 200. The pH measurement of the equilibrium mixture solution was made while nitrogen gas passed over the surface of the solutions to prevent dissolution of CO\(_2\) from the atmosphere.

2.2. Synthesis of PRCOOH.
(1) Polymerization experiments were performed in a 250ml reaction kettle with a glass jacket and an anchor-type stirrer (900 rpm), operated at 65 °C. In a typical experiment, the reaction vessel was loaded with NaCl (15 g), water (100 mL) with dissolved PVA (0.5 g) under nitrogen. The temperature was raised to 65 °C with stirring. The organic phase consisting of methyl methacrylate (9.5 g), divinylbenzene (0.5 g) with dissolved AIBN (0.15 g) was added. The polymerization proceeded under stirring for 10 h. The polymer beads were collected on a filter, washed with ethanol (5 × 30 mL) and hot H\(_2\)O (3 × 50 mL), and dried at room temperature for 24 h, obtaining the PRCOOCH\(_3\) beads (8.8 g).

(2) The PRCOOCH\(_3\) beads (5 g of sample) were hydrolyzed with NaOH 37% (75 mL) at 130 °C for 15 h. The beads were separated by filtration, washed with H\(_2\)O (3 × 50 mL), ethanol (3 × 20 mL) and H\(_2\)O (3 × 100 mL). The loading amount of PRCOOH was measured by assaying carboxyl content was 9.8 mmol/g.

2.3. Titration experiments.
The following procedure is a modification of previous published work.\(^{12,13}\) A number of samples (1 g each) of the ion exchanger were weighted into dry flasks. To the different samples, added successively larger amounts of standardized 0.1 N sodium hydroxide solutions that had been prepared in 0.05 M sodium chloride. NaCl solution (0.05 M) is then added as required to keep the ratio of solution volume to supports weight constant (100 mL solution per gram of supports). The stoppered flask was shaken until the equilibration was confirmed by no pH change with time in the solution.

2.4. Urease-catalyzed hydrolysis reaction with phosphate and PRCOOH as buffering agents.

2.4.1. Phosphate as buffer agents.\(^{14-16}\) Phosphate buffer (9.6 mL; 0.1 M; pH 7.5), urea (0.3 mL, 2 M) and urease enzyme solution (0.1 mL) were added to tube. The mixture was incubated for 30 min at the 30 °C with stirring. The pH was detected before and after the reaction. Aqueous HCl solution (10 mL, 0.1 M) were added to the tube, and mixture was stirred for 30 min. Urease activity was assayed by the Berthelot method.\(^{17}\) All treatments were carried out in triplicate.
2.4.2. **PRCOOH as buffer agents.** In a typical procedure, PRCOOH buffers were prepared by adding PRCOOH (0.5 g, 4.9 mmol), NaOH (0.12 g), H₂O (9.6 mL) with dissolved NaCl (0.05 M) in stoppered flasks, shaking the stoppered flasks for twenty-four hours at 30 °C. Urease enzyme (0.1 mL) and urea (0.3 mL, 2 M) were added to resulting PRCOOH buffers. The mixture was stirred at 30 °C for 30 min. The pH was detected before and after reaction. Aqueous HCl solution (10 mL, 0.1 M) was added to the tube, and mixture was stirred for 30 min. Urease activity was assayed by the Berthelot method. All treatments were carried out in triplicate.

3. **Results and discussion**

3.1. **Polymerization experiments**

The FTIR spectra of PMMA and PRCOOH (Na⁺ form) are shown in Supporting Figure 1. The peak at 1730 cm⁻¹ disappeared after hydrolysis, and new peak appeared at 1635 cm⁻¹, thereby indicating that the ester groups were changed to carboxyl groups. Scanning electron microscopy (SEM) of PRCOOH beads revealed a range of bead diameters from 70 µm to 130 µm (Figure 1). The carboxyl group loading of PRCOOH was 9.6 - 9.9 mmol/g, which was determined by acid-base titration.

![Figure 2. FTIR spectra of PRCOOH beads (Na⁺ form) and PRCOOCH₃ beads.](image)

![Figure 3. SEM of PRCOOH beads.](image)
3.2. The buffer action of PRCOOH

The buffer action of PRCOOH beads was investigated. Potentiometric titration of resin was studied using a conventional batch multisample technique in a 0.05 N NaCl solution. The ratio between the amount of resin (maximum capacity of 9.8 mmol/g) and the amount of solution was 1:100 g/mL. The mixture was shaken at 30 °C for 24 h. The obtained titration curve is presented in Figure 1, which shows a step in the pH range of 5.8-8.2. The step in pH corresponds to the region of buffering actions. This step is assigned to the transition from RCOOH groups to RCOONa. In addition, the solution pH was 7.0 when adding amount of NaOH was 6 mmol/g resins; in this solution, the polymethylacrylic acid in H⁺ form is approximately 40%.

![Figure 4. Titration curve of PRCOOH beads in 0.05 N NaCl.](image)

The buffer activity of the PRCOOH buffers in enzyme-catalyzed reactions in a neutral solution was examined with respect to its ability to control the pH during urease catalyzed hydrolysis of urea. PRCOOH (0.5 g) was placed in a Erlenmeyer flask and wetted with the 0.1 M aqueous urea solution (containing 0.05 N NaCl and 3 mmol NaOH). The mixture was shaken at 30 °C for 24 h. The urease samples from Canavalia ensiformis (purchased from Sigma) were carefully added to the mixture. The flask was stoppered and placed in an incubator. The mixture was shaken on a mechanical shaker at 30 °C. After 30 min of incubation, the stopper was removed. Ammonia was desorbed from the PRCOOH beads by adding 0.1N HCl solution for 30 min with stirring. The resulting suspensions were filtered. The ammonia level was measured by the Berthelot method. One unit of urease activity corresponds to the amount of enzyme that hydrolyzes 1 μM of urea to ammonia per minute. All treatments were performed in triplicate, and the results were compared with those obtained from phosphate buffers, as shown in Table 1.

| Entry | Buffers     | Urease activity | Final pH |     |
|-------|-------------|-----------------|----------|-----|
| 1a    | PRCOOH      | 624.2 ± 12.4    | 7.75 ± 0.35 |   |
| 1b    | phosphate   | 617.6 ± 13.6    | 7.71 ± 0.32 |   |
| 2a    | PRCOOH      | 33.1 ± 2.3      | 7.47 ± 0.16 |   |
| 2b    | phosphate   | 31.2 ± 2.7      | 7.52 ± 0.21 |   |

*Urease samples purchased from Sigma.
*Urease activity (U/mL) in Entry1, and (U/mg) in Entry 2.
*Initial pH: 7.0 ± 0.05

The results obtained from the new PRCOOH buffers agreed with those of the phosphate buffers. Notably, the new method does not discharge buffer effluent which presents environmental hazards.
3.3. Effect of pH buffer on urease activity of soybean
Soybean meal is often used in large amounts for animal feed because of its high protein concentration (44%-48%). Nevertheless, soybean contains an unusually large amount of urease which has a negative effect on the body metabolism of animals. Thus, the urease activity of soybean is usually assayed before it is used in animal feeds.

We quantified the urease activity of soybean urease samples when the solution was buffered to a neutral pH by PRCOOH beads. A total of seven samples (purchased from seven supermarkets) were obtained by extraction from soybean based on previous reports. Soybean meal (5g) was immersed in H$_2$O (50mL) overnight at 4 °C. The mixture was then centrifuged, and the supernatant was collected. The treatment assays were performed in triplicate, and the results are shown in Table 2.

Table 2. Urease activity Assays of soybean samples buffered by PRCOOH and phosphate.

| Entry | Buffers | Urease activity (U/mL) | Final pH$^a$ |
|-------|---------|------------------------|-------------|
| 1a    | PRCOOH  | 24.7±1.2               | 7.32 ± 0.15 |
| 1b    | Phosphate| 23.6±0.8               | 7.27 ± 0.21 |
| 2a    | PRCOOH  | 21.3±0.9               | 7.25 ± 0.23 |
| 2b    | Phosphate| 22.2±1.1               | 7.31 ± 0.18 |
| 3a    | PRCOOH  | 22.1±1.1               | 7.26 ± 0.15 |
| 3b    | Phosphate| 21.3±1.1               | 7.25 ± 0.21 |
| 4a    | PRCOOH  | 25.3±0.8               | 7.26 ± 0.19 |
| 4b    | Phosphate| 25.9±0.7               | 7.31 ± 0.21 |
| 5a    | PRCOOH  | 19.2±0.7               | 7.22 ± 0.17 |
| 5b    | Phosphate| 19.1±0.5               | 7.26 ± 0.21 |
| 6a    | PRCOOH  | 22.1±0.8               | 7.32 ± 0.23 |
| 6b    | Phosphate| 21.7±1.1               | 7.32 ± 0.23 |
| 7a    | PRCOOH  | 21.5±0.7               | 7.21 ± 0.19 |
| 7b    | Phosphate| 22.4±1.2               | 7.30 ± 0.16 |

$^a$Initial pH: 7.0± 0.05

The urease activity of soybean, which was analyzed with PRCOOH buffers at pH 7, is almost consistent with that by obtained with phosphate buffers. The urease activity of the sample solution was approximately 19-26U/mL (equivalent to 190 - 260 U/g soybean), which is broadly parallel to results of previous reports.  

4. Conclusion
We demonstrated that PRCOOH beads show high buffer activity at neutral pH. PRCOOH beads were effectively used to control the pH of urease catalyzed reactions. The PRCOOH buffers are as efficient as phosphate buffers in neutral solution but more environmentally benign.

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