A Recyclable Polymer-supported Glycane as pH Buffers for Urease-catalyzed Reaction

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Abstract. We utilized the PSG beads as polymer-supported buffer agents. Ammonia was measured by the Berthelot method. Buffer activity of the PSG buffers in enzyme-catalyzed reactions at neutral solution was examined for its ability to control the pH of urease-catalyzed hydrolysis of urea. The urease activity of soybean was analyzed using PSG buffers in pH 7. The recycling ability of the PSG was tested. In each cycle, the PSGs were regenerated by HCl and changed into mixed H-Na form by NaOH (H⁺ form 40% and Na⁺ form 60%). We demonstrated that PSG exchangers show high buffer activity at neutral pH. The PSG exchangers were effectively used to control the pH of urease catalyzed reactions. The new buffers are as efficient as phosphate buffers in neutral solution but more environmentally benign.

1. Introduction
Buffer action plays important roles in several aspects of biochemistry, industrial chemistry, analytical chemistry, and environmental systems. In the past several years, more than 1500 papers have been published involving buffer systems. The uses of the buffer systems in enzyme catalyzed process have received particular attention because the catalytic activity of many enzymes usually depends on pH and often has a well-defined optimum point. The buffer solution is usually prepared by mixing of a small molecule weak acid and its conjugate base, or vice versa. However, the small molecule buffer strategy generally suffers from time-consuming and laborious purification steps to separate the buffer agents from mixture after reaction. Although the purification step represents one of the ‘bottlenecks’ in the enzyme catalyzed process, little attention has been paid to simplify the workup protocols. These buffers are not consumed by the reaction, but they are generally discharged as wastewater. Moreover, buffer effluent presents an environmental hazard and potential impact. Although much effort has been exerted to reduce the wastewater 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, polymer-supported Glycane (PSG) 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 PSG beads as polymer-supported buffer agents. These agents were used to buffer the pH of urease-catalyzed reactions in a neutral pH region.

2. Experimental

2.1. Materials
PSG microbeads, Phosphate, NaCl, Canavalia ensiformis, NaOH, HCl.

2.2. Experimental methods and analysis
To investigate the buffer action of PSG microbeads, potentiometric titration of resin was studied using a conventional batch multisample technique in 0.05 N NaCl solution. The ratio between the amount of resin (the maximum capacities 9.8 mmol/g) and the amount of solution was 1:100 g/mL. The mixture was shaken for twenty-four hours at 30°C. The obtained titration curve is presented in Figure 1. It shows a step in the pH range 5.8-8.2. The step in pH correspond to the region of buffering actions, and which is assigned to transition from RCOOH groups to RCOONa. Additionally, we can find that the pH of solution will be 7.0 when adding amount of NaOH is 4 mmol/g resins, in which PSG acid in H+ form is about 40%.

Buffer activity of the PSG buffers in enzyme-catalyzed reactions at neutral solition was examined for its ability to control the pH of urease catalysed hydrolysis of urea. The PSG (2g) were placed in 100-ml Erlenmeyer flask and wetted with 50 ml 0.1 M aqueous urea solution (0.05N NaCl and 8mmol NaOH were included). The mixture was shaken for twenty-four hours at 30°C. Then urease samples from Canavalia ensiformis (purchased from Sigma and Yihe) were added carefully. The flask was stoppered and placed in an incubator. The mixture was shaken on a mechanical shaker at 30 °C. After 30 min the stopper was removed. Ammonia was desorbed from PRCOOH microbeads by adding 200 ml 0.1N HCl solution 30 min under stirring. The resulting suspensions were filtered. Ammonia 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 carried out in triplicate, and the results were compared to the results obtained from Phosphate buffers. As shown in table 1.

Figure 1. The titration curve of PSG beads in 0.05 N NaCl.
Table 1. Assaying the urease activity of urease samples buffered by PSG and Phosphate.\textsuperscript{a,b}

| Entry | buffers   | urease activity (U/mL) | pH after c |
|-------|-----------|------------------------|------------|
| 1a    | PSG       | 624.2 ± 12.4           | 7.75±0.35  |
| 1b    | phosphate | 617.6 ± 13.6           | 7.71±0.32  |
| 2a    | PSG       | 33.1 ± 2.3             | 7.47±0.16  |
| 2b    | phosphate | 31.2 ± 2.7             | 7.52±0.21  |

\textsuperscript{a}Urease samples of entry 1 and 2 from Sigma.

\textsuperscript{b}Urease activity (U/mL) in entry 1, and (U/mg) in Entry 2. cPH before : 7.0± 0.05

It is found that the results obtained from new buffers are agreement with those from the Phosphate buffers. It is worth noting that the new method does not discharge buffer effluent which presents environmental hazards.

Soybean meal is usually used in animal feeds in large amounts due mainly to its high protein concentration (44 to 48%). Nevertheless, soybean contains an unusually large number of Urease which have a negative effect on body metabolism of animals. So the urease activity of soybean is usually assayed before used in animal feeds.

We next quantified the urease activity of soybean urease samples in which solution pH was buffered into neutral solution using PSG microbeads. In which a total of 7 samples were obtained by extraction from soybean based on previous reports. 10 Soybean meal (5g) was immersed in H2O (50mL) overnight at 4 oC. Then, the mixture was centrifuged at 9700 g for 15 min. The supernatant was collected. Assaying treatments were carried out in triplicate, and the results shown in table 2.

Table 2. Assaying the urease activity of soybean urease samples buffered by PSG and Phosphate.

| Entry | Buffers   | Urease activity (U/mL) | Final pH c |
|-------|-----------|------------------------|------------|
| 1a    | PSG       | 26.1± 1.1              | 7.30 ± 0.11|
| 1b    | Phosphate | 25.4±0.7               | 7.31 ± 0.10|
| 2a    | PSG       | 22.3±0.8               | 7.26 ± 0.21|
| 2b    | Phosphate | 22.6±1.0               | 7.26 ± 0.17|
| 3a    | PSG       | 22.6±1.0               | 7.27 ± 0.15|
| 3b    | Phosphate | 21.8±1.1               | 7.25 ± 0.15|
| 4a    | PSG       | 23.1±0.9               | 7.27 ± 0.17|
| 4b    | Phosphate | 25.2±0.6               | 7.21 ± 0.23|
| 5a    | PSG       | 19.9±0.9               | 7.26 ± 0.18|
| 5b    | Phosphate | 19.8±0.7               | 7.25 ± 0.23|
| 6a    | PSG       | 24.2±0.9               | 7.30 ± 0.21|
| 6b    | Phosphate | 23.7±1.0               | 7.31 ± 0.21|
| 7a    | PSG       | 22.5±0.8               | 7.27 ± 0.21|
| 7b    | Phosphate | 22.7±1.1               | 7.31 ± 0.19|

The urease activity of soybean which analysed using PSG buffers in pH 7 is practically consistent with those by the Phosphate buffers. The urease activity of sample solution is about 18-23U/mL (equivalent to 180-230U/g soybean), which is broadly parallel to the previous reports.

Control the pH of solution play a key role in urease catalyzing process because the catalytic activity of ureases depends on the pH of solution.\textsuperscript{4} We studied the pH changes of the urease catalyzed reaction using PSG as adsorbent (initial pH 7.0). The obtained curve is presented in Figures 2. It shows that the pH represents a shift of only 0.4 units at the urease catalyzed process. The similar pH control were found when using PSG in H+ form 30% (initial pH 7.5) (see Supporting Information), and which indicates that the pH of solution remains essentially stability by the PSG adsorbing ammonia released.
Figure 2. The pH change in the urease catalyzed reaction in which PSG (H+ form 30%) as adsorbent and buffer agent.

The recycling ability of the PSG was next tested. In each cycle, the PSGs were regenerated by HCl and changed into mixed H-Na form by NaOH (H+ form 40% and Na+ form 60%). As shown in figure 3. The CPABs could be efficiently recycled in 12 consecutive assay cycles without loss of scavenging action, which demonstrate that CPAB works well as the reusing adsorbent.

Figure 3. Recyclability study of PSGs as adsorbents.

3. Conclusion
We have demonstrated that PSG beads show high buffer activity at neutral pH solution. PSG beads were used to control the pH of urease enzyme catalyzed reactions effectively. It is found that they are as efficient as the phosphate buffers in neutral solution and are more environmentally benign.

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

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