Green Chemistry

Accepted Manuscript

Volume 1 | Number 1 | Jan 2013 | Pages 1–100

Green Chemistry

Cutting-edge research for a greener sustainable future

www.rsc.org/greenchem

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/greenchem

Journal Name RSCPublishing

COMMUNICATION

pH Control Using Polymer-supported Phosphonic Acids as Reusable Buffer Agents

Cite this: DOI: 10.1039/x0xx00000x

Yongsheng Li, * Yongguang Wang, ZhinanGao, Jiayue Xu, Xiaorong Liu

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

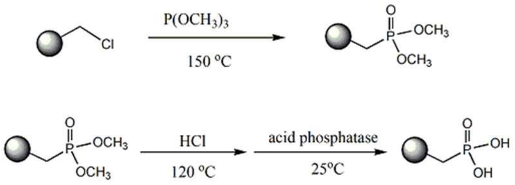
www.rsc.org/

To reduce phosphorus effluents from phosphate buffers, we report an effective protocol for pH control of aqueous solutions using polymer-supported phosphonic acids as heterogenous and reusable buffer agents. The agents were used to buffer the pH of enzyme-catalyzed reactions effectively.

The use of innocuous auxiliary substances is one of the most popular topics in green and sustainable chemistry. Large eco-benign strategies about auxiliary reagents have been reported including recyclable catalysts,[1,2] less hazard solvents,[3-6] solvent-free,[7] greener chelating agents,[8] and so on. Buffer agents, a type of reaction auxiliary, play important roles in many aspects of biochemistry, industrial chemistry, analytical chemistry, and environmental systems. Buffer agents are not consumed by the reaction, but generally discharged as wastewater. Moreover, some buffer material effluents present environmental hazards and potential impacts. For example, Phosphate effluent is the main cause of eutrophication in freshwater ecosystems, resulting to algae bloom and commercial fish kills.[9-11] To date, around 50% of all lakes and reservoirs in Eurasia and America are eutrophic. Studies have found that total phosphate above 0.02 ppm in bodies of water accelerate eutrophication.[12-14] To achieve a green and sustainable environment, a great deal of effort has been carried out to reduce the phosphate effluent. Those works include phosphate-free detergent,[15] phosphate-free synthesis,[16] phosphate-free coating, [17] phosphorus removal and recycling,[18] and so on. Although phosphate is one of the most used buffer agents, to the best of our knowledge, only one report has been published on reducing phosphate buffer effluents.[19]

Heterogenous technology in recent years has attracted significant attention in modern synthesis. It has great advantages wherein the heterogenous species can be easily isolated from the reaction mixture and reused in successive reactions. Crystalline solids of zwitterionic acids and their conjugate bases have been used effectively as heterogenous (or solid-state) buffer agents to control the pH of enzyme catalysis in organic media.[20-22] However, crystalline solids are not suitable for aqueous solutions because they are water-soluble. Polymer-supported species are usually used as heterogenous catalysts, reactants, scavengers, etc.[23-30] Nonetheless, their applications as reusable buffer agents remain less explored. Herein, we report an effective buffer protocol that the pH of the aqueous solution is buffered by polymer-supported phosphonic acids (PSPAs). The new buffers are used to enzyme-catalyzed processes. After reaction, the PSPAs can be removed easily from the mixture solutiones by simple filtration. The PSPAs can be reused effectively.

PSPAs were obtained in three steps partly based on previous reports.[31-33] (1) Polymer-supported phosphonate esters were prepared through an Arbuzov-type reaction between chloromethyl polystyrene beads (50-100 μm in diameter) and trimethyl phosphite at reflux temperature. (2) Next, they were hydrolyzed by heating in aqueous HCl solutions. (3) Then, they were subsequently hydrolyzed by acid phosphatase, resulting in PSPA beads (see Supporting Information, S2). Scanning electron microscopy (SEM) of PSPA beads reveals a range of bead diameter from 50 μm to 150 μm.



Scheme 1. Synthetic route for PSPAs.

Beads before and after hydrolysis treatments were analysed using FTIR spectroscopy (Figure 1). The P-OCH_3 absorbance at 1032 cm^{-1} disappeared after hydrolysis, and new peaks appeared at 2350 cm^{-1} and 997 cm^{-1}, which was attributed to the P-OH of phosphonic acid groups. [34-36] This indicates that phosphonic acid groups were bound to polymer supports.

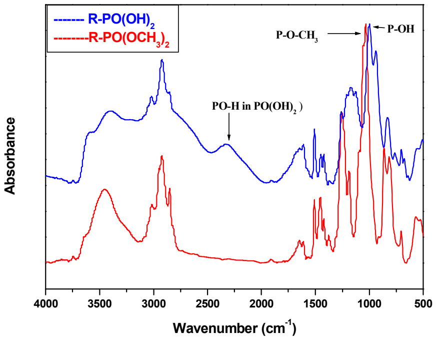


Figure 1. FTIR spectra of polymer-supported phosphonate esters (red line) and PSPAs (blue line).

To investigate buffer action of PSPAs, potentiometric titration (with NaOH) was carried out using a conventional batch multi-sample technique in 0.1 mol dm^{-3} NaCl solution.[37-40] The ratio of PSPAs to solution was 1:100 g / mL. The equilibration was confirmed by no pH change with time in the solution (see Supporting Information, S3). The titration curve obtained is presented in Figure 2. It shows two steps in pH range 4-6 and 8-10, similar to that of phenoxymethyl phosphonic acid resins. [41] The steps in pH correspond to regions of buffer actions, which are assigned to two transitions, from RPO_3H_2 to RPO_3HNa, and from RPO_3HNa to RPO_3Na_2.[36, 38] The maximum capacity of RPO_3H_2 and RPO_3HNa calculated from the curve are 3.8 mmol / g and 3.7 mmol / g, respectively.

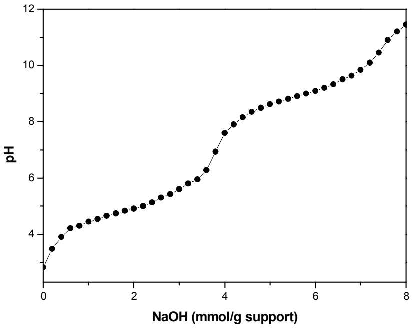


Figure 2. Titration curve of PSPAs in 0.1 mol dm^{-3} NaCl.

To reinforce the advantage of these heterogenous buffer agents, the recyclability of PSPAs was investigated, as shown in Figure 3. PSPAs were efficiently recycled in 10 consecutive buffer cycles, without loss of buffer action (about 0.1 pH change after 10 cycles). After 10 cycles, PSPAs were regenerated, demonstrating the similar pH control in another 10 cycles. These recyclability studies indicate the potential application of PSPAs as heterogenous buffer agents.

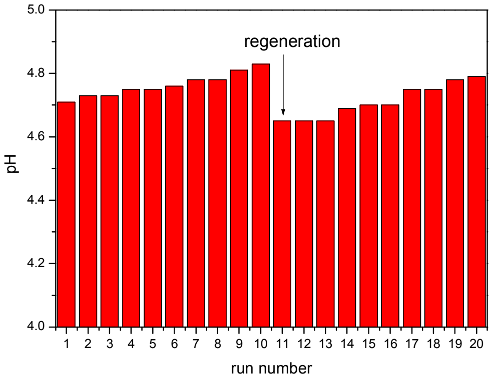


Figure 3. Recyclability study of the PSPA buffers.

To assess the effect of counterion concentration of the external solution on pH of PSPA buffer system, a series of buffer solutions were prepared with different concentrations of NaCl and their pHs were determined, as shown in Table 1. It appears that changes in counterion concentration of the external solution caused striking pH changes. As an example, upon comparison of entries 1a and 1b, we find that pH increased about 1 unit when C_{NaCl} in the external solution decreased tenfold (from 0.1mol dm^{-3} to 0.01mol dm^{-3}). This indicates that counterion concentration of the external solution plays a significant role in polymer-supported buffer systems, which is quite different from small-molecule buffer systems.

Table 1. Effect of counterion concentration in external solution on pH buffered by PSPAs.

Entry	$[RPO_3H_2]/[RPO_3HNa]$	C_{NaCl}	pH^a	pH^b
1a	3.17	0.1	4.54	—
1b	3.17	0.01	5.49	5.54
2a	1.73	0.1	5.0	—
2b	1.73	0.01	6.02	6.0
3a	1.46	0.1	5.30	—
3b	1.46	0.01	6.38	5.30
4a	1.19	0.1	5.76	—
4b	1.19	0.01	6.89	5.76

^{a}experimental results. ^{b}predicted values when C_{NaCl} in the external solution decreased tenfold.

These observations can be explained by ion-exchange equilibrium. Hydrogen-sodium exchange of PSPAs at acidic pH may be described by the following reaction scheme:

$$RPO_3H_2 + Na^+ = RPO_3HNa + H^+ \quad (1)$$

The coefficient characterizing equilibrium (K) of ion-exchange reaction (1) can be written as follows. [37,38,42,43]

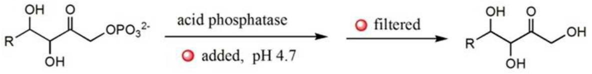
$$K = [\ RPO_3HNa\ ]\ [\ H^+\ ] / [\ RPO_3H_2\ ]\ [\ Na^+\ ] \quad (2)$$

$$pH = pK + \log [\ RPO_3HNa\ ] / [\ RPO_3H_2\ ] - \log [\ Na^+\ ] \quad (3)$$

Where K is the apparent constant of the ion exchange reaction, and the notation [ ] refers to the concentration of each species. Equation 3 shows that [H^+] and therefore pH vary depending on not only the ratio of [RPO_3HNa] / [RPO_3H_2] in the supports, but also the counterion concentration in the external solution. Approximate agreements of pH changes between the experimental results and the predicted values by Equation 3 are found (Table 1).

Buffer activity of the new buffers in enzyme-catalyzed reactions was examined for its ability to control pH of phosphate substrates dephosphorylation with phosphatase in D_2O. Acid phosphatase was elected as a model because of its well-established pH-dependent activity. [44-47] This enzyme is only active at pH 4-7 (optimal performance at pH 4.5-6). Here dephosphorylation was carried out at about pH 4.7 as reported previously,[48-50] and pH was controlled in this work by PSPAs. Reaction mixtures were stirred at 25 °C for 48 h. After the reaction, polymer-supported species were easily separated from the mixture by simple filtration (see Supporting Information, S4 and S5). Results are summarized in Table 2. Large amounts of dephosphorylation were obtained when pH was controlled by PSPA buffers (entries 1-5), and pH remained almost unchanged upon dephosphorylation. It turns out that PSPA buffers exhibit high buffer activity toward the dephosphorylation catalyzed by acid phosphatase.

Table2. Dephosphorylation of phosphate buffered by PSPAs



Entry	R-[a]	conversion (%)[c]	pH before	pH after
1	H-	>95	4.67	4.63
2	CH_3-	>95	4.69	4.67
3[b]	CH_3-	>95	4.62	4.61
4	CH_3CH_2-	>95	4.68	4.63
5	$CH_3CH_2CH_2$-	>95	4.73	4.66
6	$CH_3(CH_3)CH$-	>95	4.76	4.71

[a] Phosphate substrates prepared by condensation of DHAP and acetaldehyde in situ. [b] Using recycled PSPAs that had been used for 4 cycles. [c] Determined by ^{31}PNMR analysis after PSPAs removal.

The recycling ability of PSPAs was next tested (entry 3, in Table 2). Rates of dephosphorylation indicated that buffer agents were efficiently recycled and pH remained almost unchanged after 5 cycles, demonstrating that PSPAs work well as readily available, heterogenous, and reusable buffer agents.

Urease catalysed hydrolysis of urea was also examined, wherein the pH was controlled by PSPAs and phosphate (initial pH 6.5).[51-53] The pH changes determined are presented in Supporting Table 1 (see Supporting Information, S6). It shows that the pH buffered by PSPAs presents a shift of only 0.2 units in urease catalyzed process, which is consistent well with those by phosphate buffers. Nearly identical amounts of urease activity were obtained by the two buffer methods. It is worth noting that PSPA buffers did not create phosphorus (P) effluents in urea hydrolysis. However, P concentration of the phosphate buffer solution is 6000 times higher than P discharge limit for effluents (0.5 mg P/l) .[54]

In addition, we studied horseradish peroxidase-catalyzed reaction between hydrogen peroxide and 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) using PSPAs and phosphate as buffer agents (initial pH 6.0 as reported previously).[53,55,56] It shows that pH in the two buffer systems are remained almost unchanged upon the reactions (see Supporting Information, S7). These results suggest that PSPAs are as efficient as the phosphate buffer agents for enzyme catalysed reactions and are more environmentally benign.

Conclusions

We have demonstrated that PSPAs show high buffer activity. They were used as polymer-supported buffer agents to control pH of enzyme catalyzed reactions, separated by simple filtration, and reused effectively. Further work is underway to explore reused buffer strategy to reduce the phosphorus effluents from buffers in fine chemistry syntheses.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (51474150), the National Key Basic Research Program (2011CB612310), the Shanghai Municipal Science and Technology Commission (11JC1412400).

Notes and references

Address here. School of Materials Science and Engineering, Shanghai Institute of Technology, Shanghai 201418, China.

†

Electronic Supplementary Information (ESI) available: Experimental procedures, and ^{31}PNMR spectra. See DOI: 10.1039/c000000x/

1. D. A. Ruddy, J. A. Schaidle, J. R. Ferrell III, J. Wang, L. Moens, J. E. Hensley, *Green Chem.*, 2014, **16**, 454
2. E. A. Peterson, B. Dillon, I. Raheem, P. Richardson, D. Richter, R. Schmidt H. F. Sneddon, *Green Chem.*, 2014, **16**, 4060
3. D. Prat, J. Hayler, A. Wells, *Green Chem.*, 2014, **16**, 4546
4. P. Pollet, E. A. Davey, E. E. Ureña-Benavides, C. A. Eckert, C. L. Liotta, *Green Chem.*, 2014, **16**, 1034
5. A. Brandt, J. Gräsvik, J. P. Hallett, T. Welton, *Green Chem.*, 2013,**15**, 550
6. C. C. Weber, A. F. Masters, T. Maschmeyer, *Green Chem.*, 2013,**15**, 2655
7. M. S. Singh, S. Chowdhury, *RSC Adv.*, 2012, **2**, 4547
8. V. K. Ahluwalia, *Green Chemistry: Environmentally Benign Reaction*. CRC Press. 2010

9. P. S. Lau, N .F. Y. Tam, Y. S. Wong, *Environ. Technol.*, 1997, **18**, 945
10. C. Trépanier, S. Parent, Y. Comeau, J. Bouvrette, *Water Res.*, 2002. **36**, 1007
11. L. E. de-Bashan, Y. Bashan, *Water Res.*, 2004, **38** , 4222
12. S. Sengupta, A. Pandit, *Water Res.*, 2011, **45**, 3318
13. A. N. Sharpley, S. C. Chapra, R. Wedepohl, J. T. Sims, T. C. Daniel, K. R. Reddy, *J. Environ. Qual.*, 1994, **23**, 437
14. R. J. Seviour, T. Mino, M. Onuki, *FEMS. Microbiol. Rev.* 2003, **27**, 99
15. M. Mccoy, *Chem. Eng. News Archive*, 2011, **89**, 12
16. L. Shi, P. Yin, *Dalton Trans.*, 2013, **42**, 13607
17. R. Moore, B. Dunham, *Metal Finishing*, 2008, **106**(7,8), 46
18. J. F. Duenas, J. R. Alonso, A. F. Rey, A. S. Ferrer, *J. Hazard Mater.*,2003. **97**, 193
19. S.-J. You, N.-Q. Ren, Q.-L. Zhao, P. D. Kiely, J.-Y. Wang, F.-L. Yang, L. Fu, L. Peng, *Biosensors and Bioelectronics*, 2009, **24**, 3698
20. E. Zacharis, B. D. Moore, P. J. Halling, *J. Am. Chem. Soc.*, 1997, **119**, 12396
21. N. Harper, M. Dolman, B. D. Moore, P. J. Halling, *Chem. Eur. J.*, 2000, **6**, 1923
22. M. Quirós, M.-C. Parker, N. J. Turner, *J. Org. Chem.*, 2001, **66**, 5074
23. T. E. Kristensen, T. Hansen, *Polymer and Mesoporous Ma-terial Supported Organocatalysts,* in *Comprehensive Enantiose-lective Organocatalysis: Catalysts, Reactions, and Applications.* (ed P. I. Dalko,), Wiley-VCH Verlag GmbH & Co. KGaA, Wein-heim, Germany. 2013, PP: 651
24. G. Gelbard, *Polymer-supported Reagents*, in *Handbook of Green Chemistry and Technology* (eds: J. H. Clark, D. Macquarrie), Blackwell Science Ltd, Oxford, UK. 2002
25. P. H. Toy, Y. Lam, (eds) *Solid-Phase Organic Synthesis: Concepts, Strategies, and Applications,* John Wiley & Sons, Inc., Hoboken, NJ, USA. 2012
26. A. Kirschning, H. Monenschein, R. Wittenberg, *Angew. Chem. Int. Ed.,* 2001, **40**, 650
27. M. H. Nguyen, A. B. III. Smith, *Org. Lett.*, 2013, **15**, 4258
28. M. Benaglia, A. Puglisi, F. Cozzi, *Chem. Rev.,* 2003, **103**, 3401
29. D. E. Bergbreiter, *ACS Macro Lett.*, 2014, **3**, 260
30. P. Hodge, *Ind. Eng. Chem. Res.,* 2005, **44**, 8542
31. A. W. Trochimczuk, S. D. Alexandratos, *J. Appl. Polym. Sci.*, 1994, **52**, 1273
32. A. W. Trochimczuk, J. Jezierska, *Polymer,* 2000, **41**, 3463
33. A. Popa, C.-M. Davidescu, P. Negrea, G. Ilia, A. Katsaros, K. D. Demadis, *Ind. Eng. Chem. Res.,* 2008, **47**, 2010
34. K. D. Demadis, S. D. Katarachia, *Phosphorus, Sulfur, and Silicon,* 2004, **179**, 627
35. M. J. Danilich, D. J. Burton, R. E. Marchant, *Vib. Spectrosc.,* 1995, **9**, 22
36. G. Chaplais, J. L. Bideau, D. Leclercq, A. Vioux, *Chem. Mater.*, 2003, **15**, 1950
37. F. G. Helfferich, *Ion Exchange*. McGraw-Hill Series in Advanced Chemistry; McGraw-Hill: New York, 1962
38. A. A. Zagorodni, *Ion Exchange Materials: Properties and Applications*, 1st ed.; Elsevier: Amsterdam; Boston, 2007
39. L. S. Molochnikov, E. G. Kovalyova, I. A. Grigor'ev, A. A. Zago-rodni, *J. Phys. Chem. B* 2004, **108**, 130
40. N. J. Polyansky, G. B. Gorbunov, N. L. Polianskaya, *Methods for Investigation of Ion Exchangers*. Chemistry: Moscow, 1976
41. E. N. Walsh, T. M Beck, A. D. F. Toy, *J. Am. Chem. Soc*., 1956, **78**, 4455
42. R. Biesuz, A. A. Zagorodni, M. Muhammed, *J. Phys. Chem., B* 2001, **105**, 4721
43. E. Högfeldt, *J. Phys. Chem*. 1988, **92**, 6475
44. H. Frenkel-Mullerad, D. Avnir, *J. Am. Chem. Soc*. 2005, **127**, 8077
45. H. N. Fernley, *Mammalian Alkaline Phosphatases*. In *The Enzymes*, 3rd ed.; P. D. Boyer, Ed.; Academic Press: New York, 1971; Vol. IV, pp417
46. R. B. McComb, G. N. Bowers, S. Posen, *Alkaline Phosphatase*. PlenumPress: New York, 1979
47. K. S. Gellatly, G. Moorhead, S. Duff, D. D. Lefebvre, W. C. Plaxton, *Plant Physiol.,* 1994, **106**, 223
48. R. AlajarÍn, E. GarcÍa-Junceda, C.-H. Wong, *J. Org. Chem*., 1995, **60**, 4294
49. D. Franke, T. Machajewski, C.-C. Hsu, C.-H. Wong, *J. Org. Chem*., 2003, **68**, 6828
50. F. Charmantray, P. Dellis, S. Samreth, L. Hecqueta, *Tetrahedron Lett.,* 2006, **47**, 3261
51. B. Krajewska, W. Zaborskar. *J. Mol. Catal. B: Enzym.,* 1999, **6**, 75
52. K. Gabrovska, A. Georgieva, T. Godjevargova, O. Stoilova, N. Manolova, *J. Biotech.,* 2007, **129**, 674.
53. H. Bisswanger, *Practical Enzymology.* 2nd ed.; Weinheim: Wiley-VCH; 2011.
54. Manual for Water Environment Work. Ministry of Environmental Protection of Peoples Republic of China. 1997.
55. R. E. Childs, W. G. Bardsley. Biochem. J., 1975, **145**, 93.
56. A. Szutowicz, R. D. Kobes, P. J. Orsulak. *Anal. Biochem.,* 1984, **138**, 86.

---

pH Control Using Polymer-supported Phosphonic Acids as Reusable Buffer Agents

Yongsheng Li, * Yongguang Wang, Zhinan Gao, Jiayue Xu, Xiaorong Liu

To reduce phosphorus effluents from phosphate buffers, we use polymer-supported phosphonic acids as hetergenous and reusable buffer agents for pH control of enzyme catalysis.