Supporting Information

for

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

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Content

S1. General Remarks.

All reagents were purchased from Sigma-Aldrich, or Sinopharm chemical. ^{31}P NMR spectra were recorded at 500 MHz on a Bruker instrument and referenced using external 85% aqueous H_3PO_4. Solid-state ^{31}P magic-angle sample-spinning (MAS) NMR spectra were obtained using a Agilent-NMR-vnmrs 600 MHz instrument. All 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.

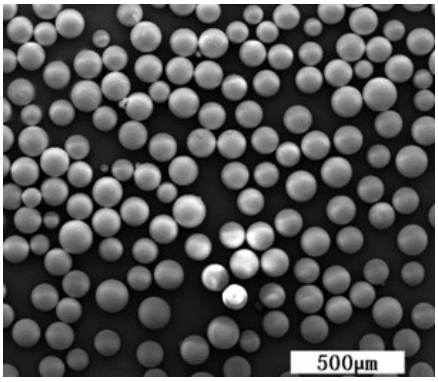
S2. Synthesis of PSPAs.

The following procedure is a modification of previous published work.[1,2]

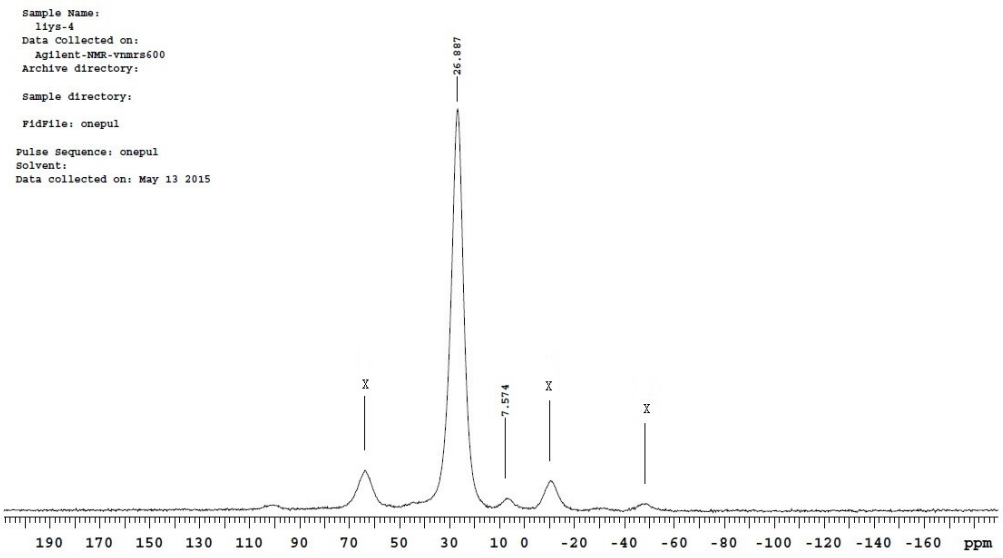
(1) A sample of chloromethyl styrene-divinybenzene copolymer (5 g) and trimethylphosphite (50 mL) was maintained under stirring for 48 h at the trimethylphosphite reflux temperature. The polymer beads were separated by filtration, washed with ethanol (5 X 30 mL) and H_2O (3 X 30 mL), and dried at 60 ^oC for 24 h, obtaining the Polymer-supported phosphonate esters (7.5 g).

(2) The Polymer-supported phosphonate esters (5 g of sample) were hydrolyzed with hydrochloric acid 37% (75 mL) at a reflux temperature for 15 h, resulting beads with two groups: R-PO_3H_2 and intermediate R–P(O)(OH)(OCH_3). The beads were separated by filtration, washed with H_2O (3 X 50 mL), ethanol (3 X 20 mL) and H_2O (3 X 100 mL).

(3) For hydrolyzing intermediate R–P(O)(OH)(OCH_3) to R-PO_3H_2, the resulting samples were hydrolyzed again by acid phosphatase (200 U) in H_2O (100mL) at 25 ^oC for 24 h, and dried at 60 ^oC for 24 h, obtaining the beads (3.7 g). Elemental analysis results: C, 61.8%; H, 6.27%. The loading amount of PA was measured by assaying phosphorus content[1] was 3.91 mmol / g. Solid-P NMR spectra of PSPA beads was shown in Supporting Figure 2.



Supporting Figure 1. SEM micrograph of PSPA beads.



Supporting Figure 2. Solid-P NMR spectra of PSPA beads (X: sidebands).

Reference:

(1) A.W.Trochimczuk, J. Jezierska, *Polymer* 2000,**41**, 3463–3470.

(2) A. Popa, C. -M. Davidescu, P. Negrea, G. Ilia, A. Katsaros, K. D. Demadis, *Ind. Eng. Chem. Res*. 2008, **47**, 2010-2017.

S3. Titration experiments.

The following procedure is a modification of previous published work. [1,2]

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 solution that had been prepared in 0.1 M sodium chloride. NaCl solution (0.1 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.

Reference:

(1) F. G. Helfferich, *Ion Exchange*; McGraw-Hill Series in AdvancedChemistry; McGraw-Hill: New York, **1962**.

(2) A. A. Zagorodni, *Ion Exchange Materials: Properties and Applications*, 1st ed.; Elsevier: Amsterdam; Boston, **2007**.

S4.The dephosphorylation buffered by PSPAs.

In a typical procedure, the dephosphorylation was obtained by 3 steps:

(1) PSPAs buffered agents were prepared by adding PSPAs (5 g, 19 mmol), NaCl (0.3 g) and NaOH (0.3 g, 7.5 mmol) and D_2O (50 mL) in stoppered flasks, shaking the stoppered flasks until The equilibration was established.

(2)Phosphate substrates were prepared by condensation reaction of dihydroxyacetone phosphate (DHAP) and Acetaldehyde in situ as previously published work modified slightly (ref.1-3). An D_2O solution (10 mL) of DHAP (1 mmol), Acetaldehyde (2 mmol), NaCl (60 mg) and aldolase (20 U) was incubated until a turnover of 100% had been achieved. The pH was adjusted to 4-5 with HCl.

(3) The PSPAs buffered agents (0.8 mL, 0.5 g dry, came from (1)) was added to the resulting Phosphate substrates (10 mL), and the mixture was stirred at 25 ^{o}C for 48 h. The pH was detected before and after reaction. PSPAs were separated by filtration, and the filtrate was checked directly by ^{31}PNMR.

Reference:

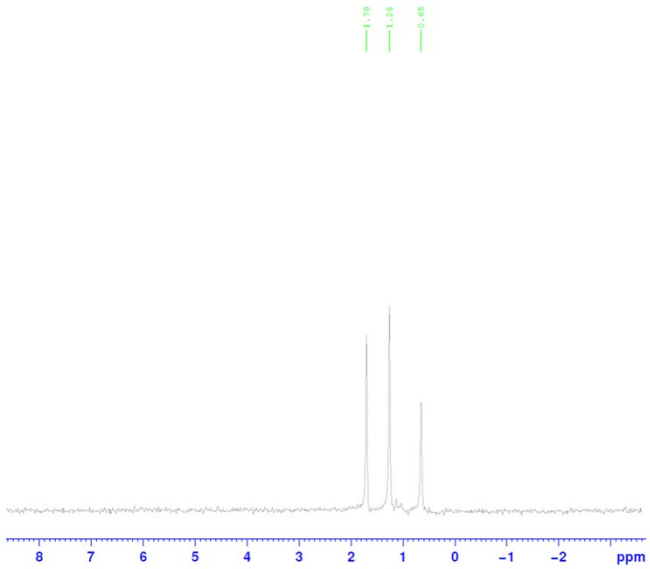
(1) D. Franke, T. Machajewski, C.-C. Hsu, C.-H. Wong, *J. Org. Chem*. 2003, **68**, 6828-6831.

(2) F. Charmantray, P. Dellis, S. Samreth, L. Hecqueta, *Tetrahedron Lett*.2006**, 47**, 3261–3263.

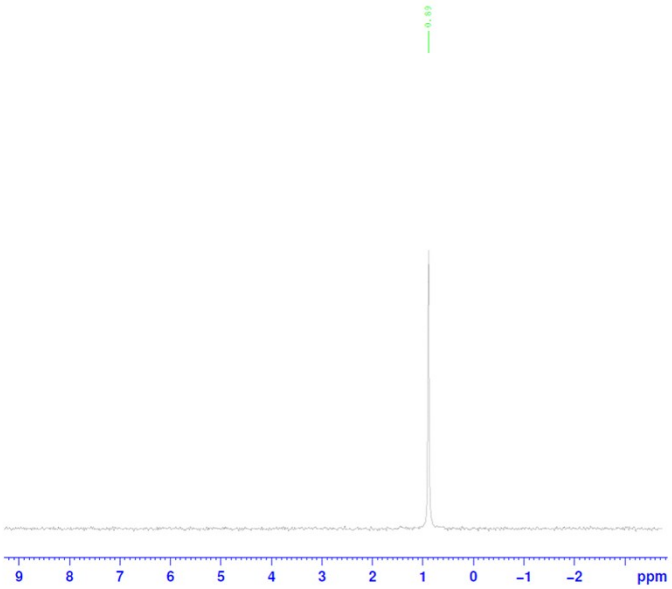
(3) DHAP was assayed with acoupled-enezme system as described by Bergmeyer. (H. U. Bergmeyer, *In Methods of Enzymatic analyses,* vol. 3rd ed., Verlag Chemie: Mannheim, **1984**; pp342-350.)

S5. ^{31}PNMR spectra of reaction mixture.

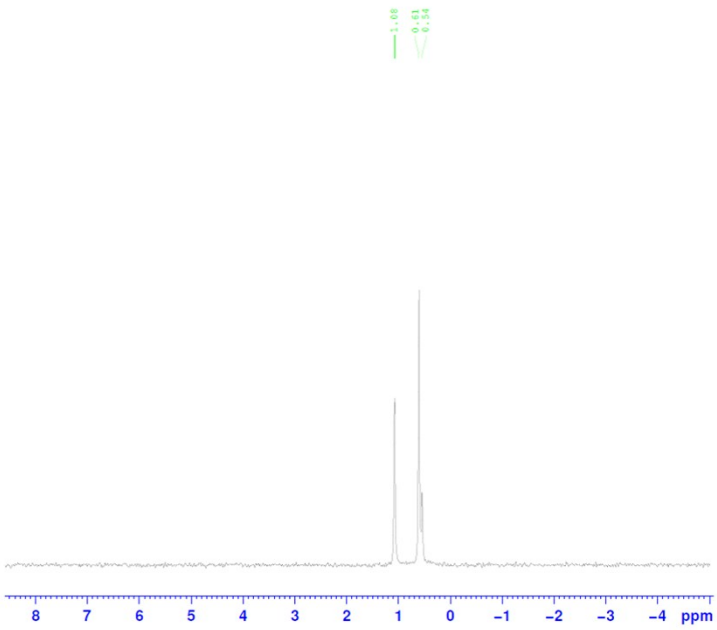
Supporting Figure 3: Before dephosphorylation of $CH_2(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 1 of table 2)



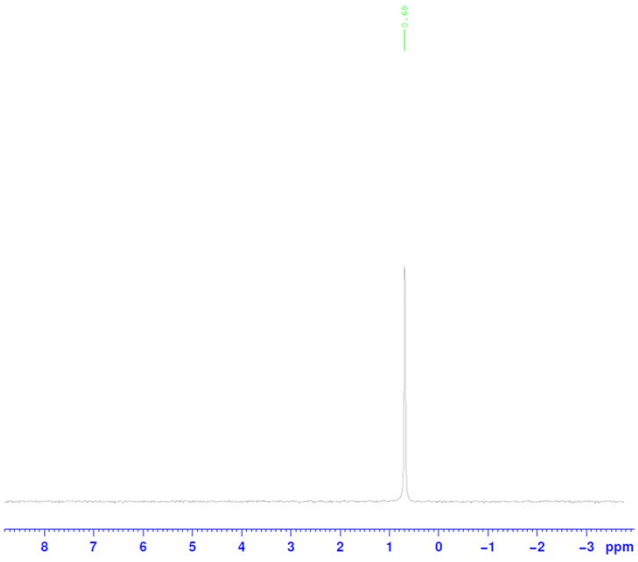
Supporting Figure 4: After dephosphorylation of $CH_2(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 1 of table 2)



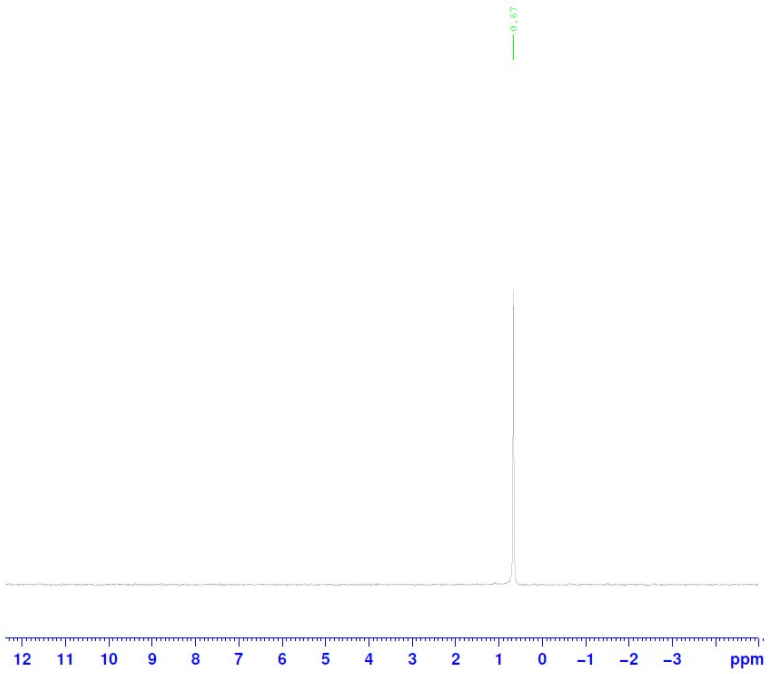
Supporting Figure 5: Before dephosphorylation of $CH_3CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 2 of table 2)



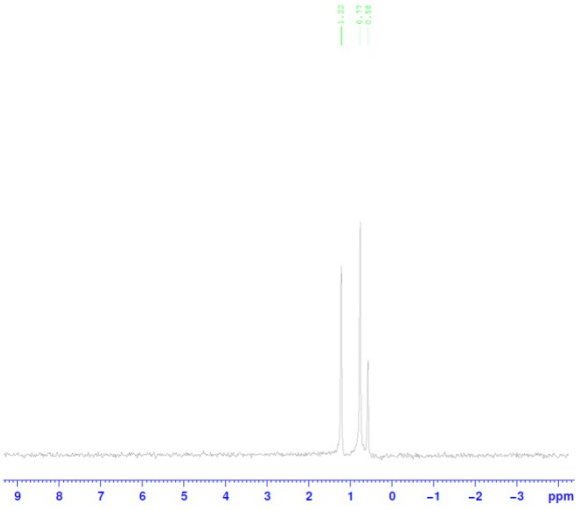
Supporting Figure 6: After dephosphorylation of $CH_3CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 2 of table 2)



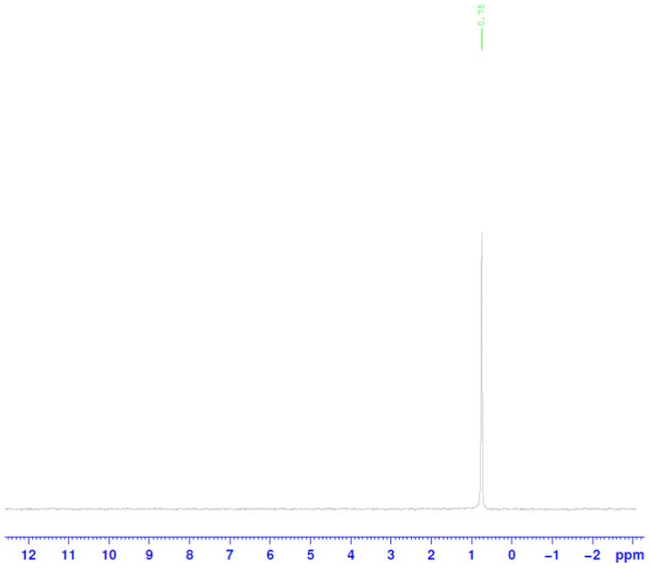
Supporting Figure 7: After dephosphorylation of $CH_3CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 3 of table 2)



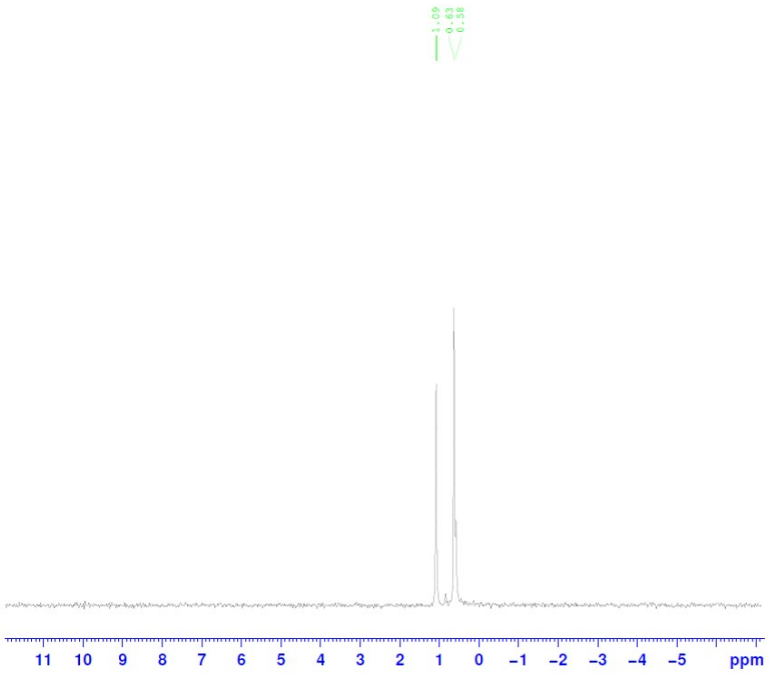
Supporting Figure 8: Before dephosphorylation of $CH_3CH_2CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 4 of table 2)



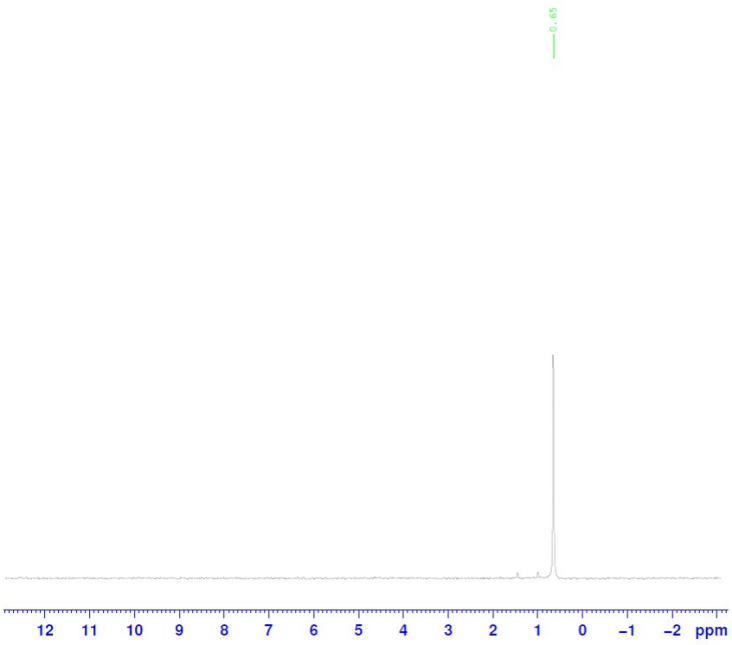
Supporting Figure 9: After dephosphorylation of $CH_3CH_2CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 4 of table 2)



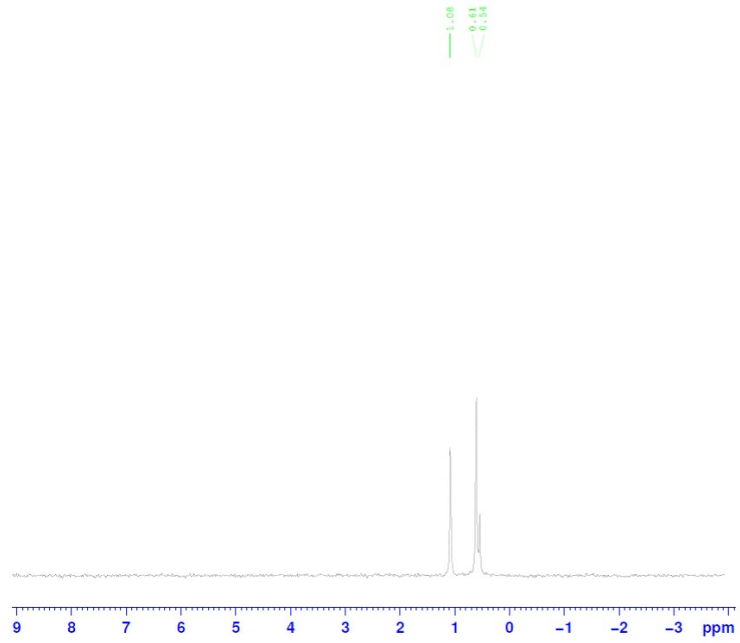
Supporting Figure 10: Before dephosphorylation of $CH_3CH_2CH_2CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 5 of table 2)



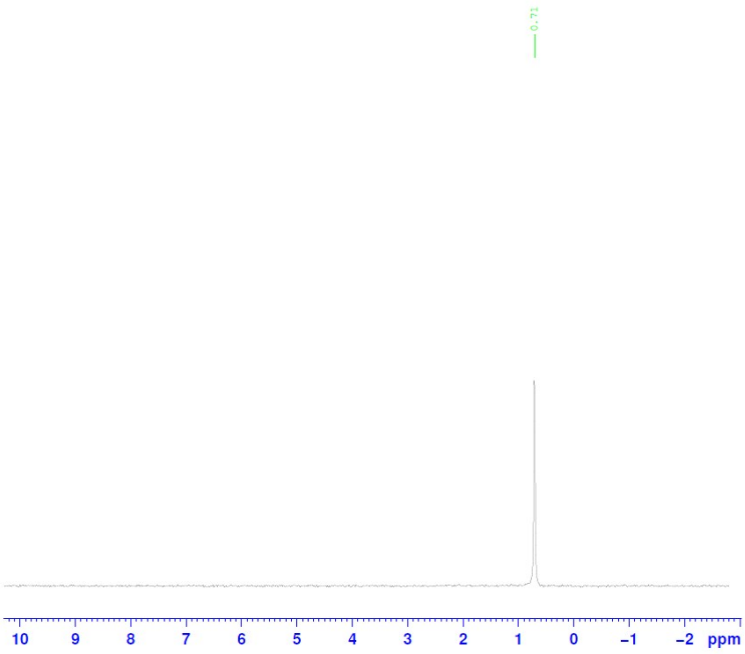
Supporting Figure 11: After dephosphorylation of $CH_3CH_2CH_2CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 5 of table 2)



Supporting Figure 12: Before dephosphorylation of $CH_3CH(CH_3)CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 6 of table 2)



Supporting Figure 13: After dephosphorylation of $CH_3CH(CH_3)CH(OH)CH(OH)COCH_2OPO_3^{2-}$ (Entry 6 of table 2)



S6. Urease-catalyzed hydrolysis reaction with Phosphate and PSPAs as buffering agents.

1) Phosphate as buffer agents.[1-3]

Phosphate buffer (9.6 mL; 0.1 M; pH 6.5), 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 25 °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 10 min at 25 °C. Urease activity was assayed by the Berthelot method.[4] All treatments were carried out in triplicate, as shown in Supporting Table 1.

2) PSPAs as buffer agents.

In a typical procedure, PSPA buffers were prepared by adding PSPAs (0.5 g, 1.9 mmol), NaCl (0.03 g), NaOH (0.055 g, 1.35 mmol) and H_2O (9.6 mL) in stoppered flasks, shaking the stoppered flasks for twenty-four hours at 25 °C. Urease enzyme (0.1 mL) and urea (0.3 mL, 2 M)were added to resulting PSPA buffers. The mixture was stirred at 25 °C for 30 min. The pH was detected before and after reaction. Aqueous HCl solution (10 mL, 0.1 M) were added to the tube, and mixture was stirred for 10 min at 25 °C. Urease activity was assayed by the Berthelot method. [4] All treatments were carried out in triplicate, as shown in Supporting Table 1.

Supporting **T**able 1. Urease catalyzed hydrolysis buffered by PSPAs and phosphate.

entry	buffers	Urease activity (U / mg)	pH before	pH after
1	PSPAs	2.32 ± 0.21	6.50 ± 0.05	6.67 ± 0.11
2	Phosphate	2.41 ± 0.18	6.50 ± 0.03	6.63 ± 0.12

Reference:

1. B. Krajewska, W. Zaborskar. *J. Mol. Catal. B: Enzym.* 1999, **6**, 75.
2. K. Gabrovska, A. Georgieva, T. Godjevargova, O. Stoilova, N. Manolova, *J. Biotech.* 2007, **129**, 674.
3. H. Bisswanger, *Practical Enzymology.* 2nd ed.; Weinheim: Wiley-VCH; 2011.
4. T.T. Ngo, A.P.H. Phan, C.F. Yam, H.M. Lenhoff, *Anal. Chem.* 1982, **54**, 46.

S7. Horseradish peroxidase-catalyzed reaction using Phosphate and PSPAs as buffering agents.

1) Phosphate as buffer agents.

Phosphate buffer (7.8 mL, 0.1 M, pH 6.0), 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) (ABTS, 1 mL, 20 mM), H_2O_2 (1 mL, 10 mM) and enzyme solution (0.2 mL) were added to tube. Mixture was stirred for 5 min at 25 °C. The pH and absorbance were detected before and after reaction. All treatments were carried out in triplicate, as shown in Table 2.

2) PSPAs as buffer agents.

In a typical procedure, PSPA buffers were prepared by adding PSPAs (0.5 g, 1.9 mmol), NaCl (0.03 g,) , NaOH (0.055 g, 1.35 mmol) and H_2O (7.8 mL) in stoppered flasks, shaking the stoppered flasks for twenty-four hours at 25 ^oC. ABTS (1 mL, 20 mM), H_2O_2 (1 mL, 10 mM) and enzyme solution (0.2 mL) were added to the PSPA buffers. The mixture was stirred for 5 min at 25 °C. The pH and absorbance were detected before and after reaction. All treatments were carried out in triplicate, as shown in Supporting Table 2.

Supporting Table 2. Horseradish peroxidase-catalyzed reaction buffered by PSPAs and phosphate.

entry	Buffer agent	peroxidase activity	pH before	pH after
1	PSPAs	77.3 ± 5. 8	6.0 ± 0.05	6.0 ± 0.11
2	Phosphate	80.1 ± 3. 9	6.0 ± 0.02	6.0 ± 0.12

Follow absorption change at 414 nm, 25^{o}C, ε_{414} = 24.6 x10^3 L mol^{-1} cm^{-1}. One unit of peroxidase activity corresponds to the amount of enzyme that oxidizes 1 μM of ABTS per minute at 25 °C at pH 6.0.

Reference:

1. H. Bisswanger, *Practical Enzymology.* 2nd ed.; Weinheim: Wiley-VCH; 2011.
2. R. E. Childs, W. G. Bardsley. *Biochem. J.*, 1975, **145**, 93.
3. A. Szutowicz, R. D. Kobes, P. J. Orsulak. *Anal. Biochem.,* 1984, **138**, 86.