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# Use of Ion Exchange Resins as Buffers<sup>1</sup>

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**INTRODUCTION:** The pH of solutions in physiological experiments are usually maintained by the buffering capacity of weak acids or bases. There are however two shortcomings associated with these substances. Firstly, their effective buffering range is limited to one pH unit on either side of their pK, and secondly, the anions of weak acids and cations of weak bases can have physiological and osmotic effects distinct from the pH they maintain. At high buffer concentrations these physiological effects can be important and they can overshadow the effects of metabolites or inhibitors which the experiment was designed to study. This work will introduce a new use for ion exchange resins: namely, that they can be employed as "insoluble" buffers. Compared to soluble buffers, ion exchange buffers have: a wide buffering range (pH 2 to 8), fewer ion effects on physiological experiments, low osmotic pressure effects and the ability to change the pH in an experiment without changing the rest of the composition of a suspending media.

**EXPERIMENTAL. Materials and Apparatus:** The resins Amberlite CG-50 type I (carboxylic acid) and Amberlite CG-4B type I (weakly basic amine) were obtained from the Rohm and Haas Co., Washington Square, Philadelphia 5, Pennsylvania.

*Anacystis nidulans* (culture number 625) was obtained from the culture collection of algae at Indiana University (1) and was grown by the method of Kratz and Myers (2). *Saccharomyces cerevisiae* was obtained from the Dept. of Bacteriology and Immunology at this university. Growth medium for this yeast consisted of: 60 gm glucose, 2 gm NH<sub>4</sub>Cl, 1 gm KH<sub>2</sub>PO<sub>4</sub>, 2.5 gm yeast extract, 5 gm peptone and one liter glass distilled water. The cultures were grown aerobically at 23° C.

Warburg manometry was used to measure respiration and a Beckman pH meter (model H2) was used to determine pH. The volume of cells used in an experiment is the packed cell volume as determined by a cytocrit tube.

**Procedure:** The titration curve of a resin buffer is an indication of its buffering capacity and range. Such curves for a carboxylic acid and amine resin are shown in Figs. 1 and 2. These resins have an exchange capacity of about 10 millequivalents per gram resin which is higher than that observed for other resins tested. The carboxylate resin titration curve shows the presence of a

single pK value while the amine resin titration curve is characteristic of a buffer having many different pK values.

The size of the resin particles is important in determining the rate of reaction between ions in solution and the resin. For the work reported here a mesh size of 100 to 200 was found satisfactory. With this mesh size five minutes was required to achieve 95 per cent of the equilibration between added base or acid and the resin.

Resin buffers are prepared either by titrating the resins with acid or base to the desired pH, or by mixing varying proportions of the acid and base form of the resins. For example, to prepare a pH 6 buffer from the amine resins, one gram of resin is placed in a solution containing 4 ml of 1 molar HCl and 6 ml of water. The same pH is obtained by adding 0.4 gm of the base form of the resin and 0.6 gm of the acid form to 10 ml of water. To change the buffering capacity the ratio between resin and liquid phase is either increased or decreased. Unless otherwise stated resin buffers for this work were prepared by adding 0.1 gm resin to 1 ml solution.

The pH value of a resin buffer was taken as the one obtained when the resin suspension was well agitated. As the resin is allowed to settle out of the suspension the

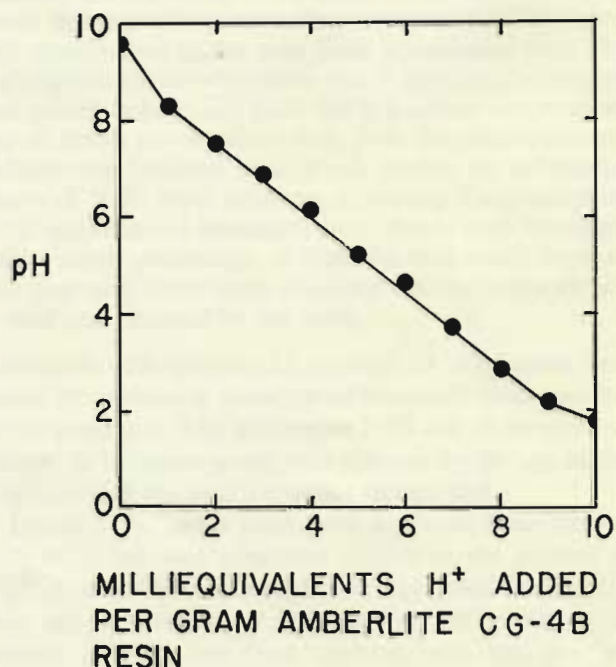


FIGURE 1. Titration curve of the amine resin Amberlite CG-4B.

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buffer is in effect being removed from the other solution constituents. The pH of resin buffers were monitored during use and were found to decrease by up to 0.1 pH units per hour.

The pH of a resin buffer depends on the concentration of ions in the external medium. (See Figs. 3 and 4.) The effects of dissociable salts is the same at any point on a titration curve so that a family of curves can be obtained by changing the salt concentration. Because resin buffers

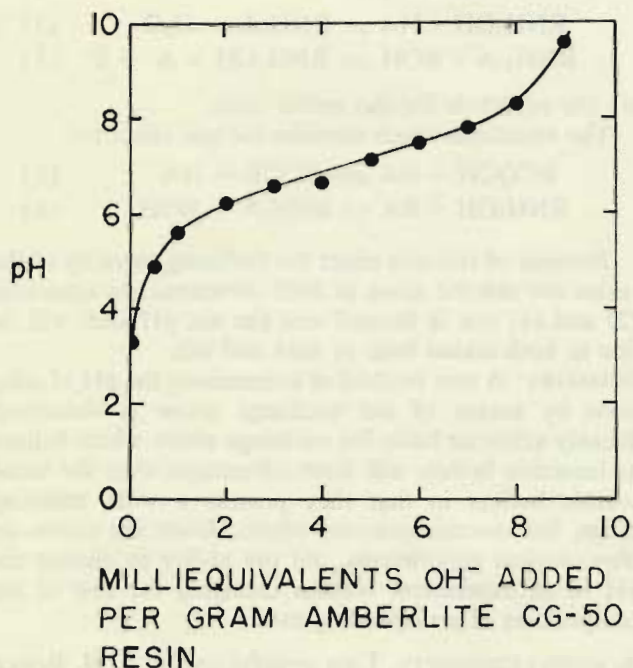


FIGURE 2. Titration curve of the carboxylic acid resin Amberlite CG-50. Resin suspended in 0.1 molar KCl.

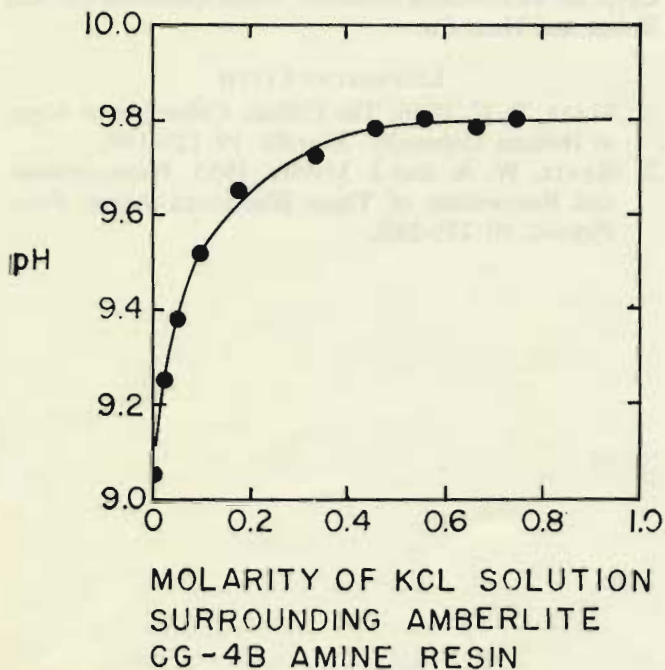


FIGURE 3. The effect of salt concentration on the pH maintained by the amine resin Amberlite CG-4B.

are sensitive to small initial changes in salt concentration it is important to consider possible ion concentration changes during an experiment.

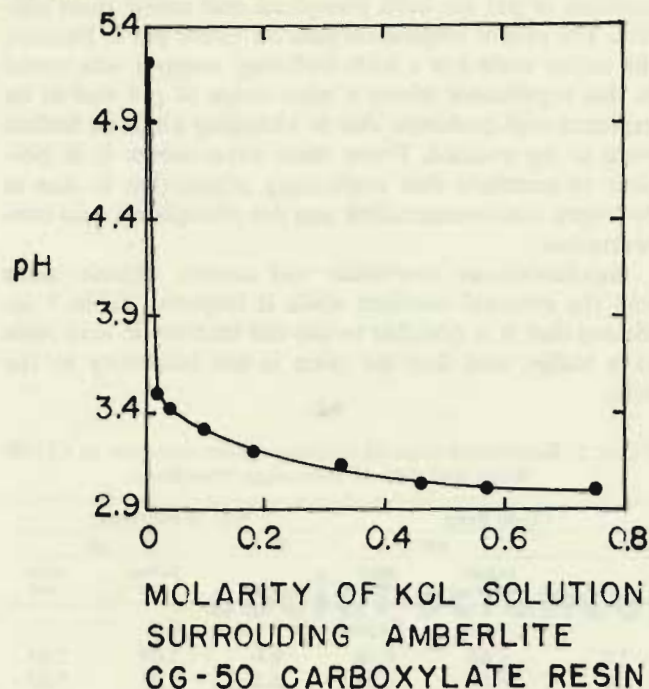


FIGURE 4. The effect of salt concentration on the pH maintained by the carboxylic acid resin Amberlite CG-50.

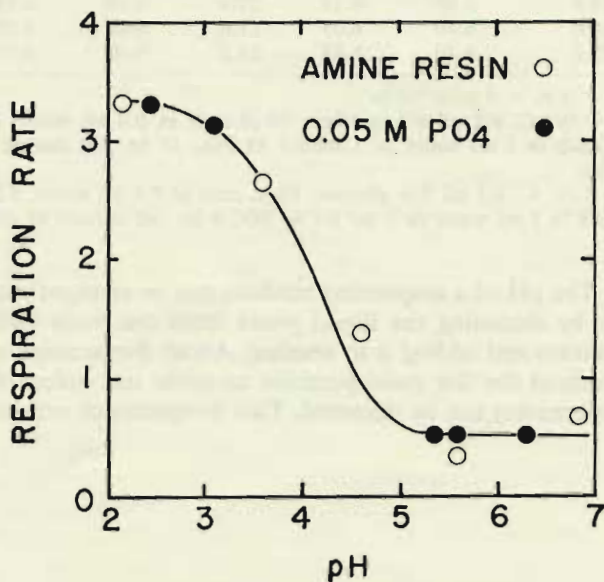


FIGURE 5. Respiration rate of *Anacystis nidulans* as a function of pH. 25 C, 0.2 ml 5% glucose, 40  $\mu$ l cells in 0.8 ml water, 0.1 g amine resin in 1 ml water or 1 ml 0.1 M phosphate buffer. Rate in  $\mu$ l O<sub>2</sub>  $\times$   $\mu$ l cells<sup>-1</sup>  $\times$  hr<sup>-1</sup>.

RESULTS AND DISCUSSION: From other studies it was shown that the respiration of *Anacystis nidulans* is increased four to five fold under acid conditions when phosphate buffers were employed for pH control. The amine resin should permit us to determine whether the effect is attributable to hydronium ion per se or to an

