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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_4Cl, 1 gm KH_2PO_4, 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

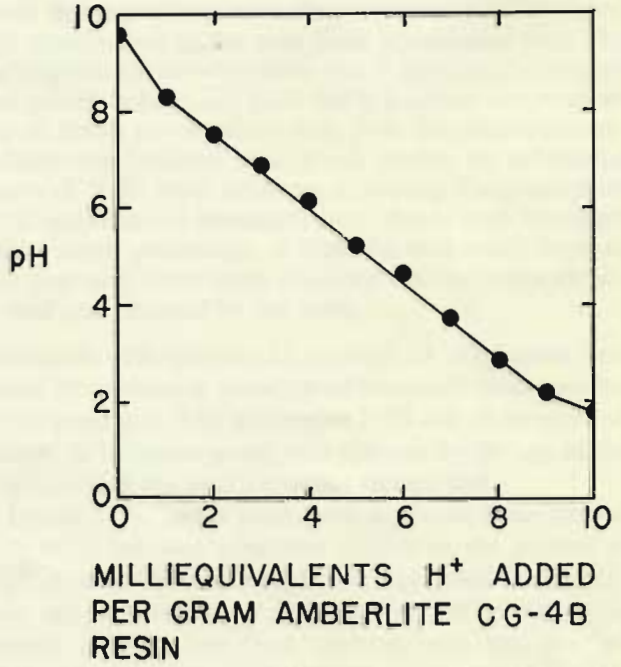

FIGURE 1. Titration curve of the amine resin Amberlite CG-4B.

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

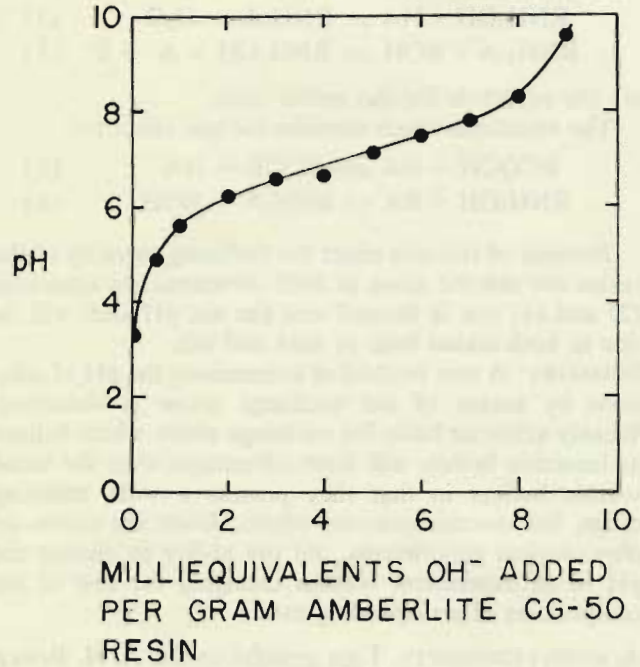

FIGURE 2. Titration curve of the carboxylic acid resin Amberlite CG-50. Resin suspended in 0.1 molar KCl.

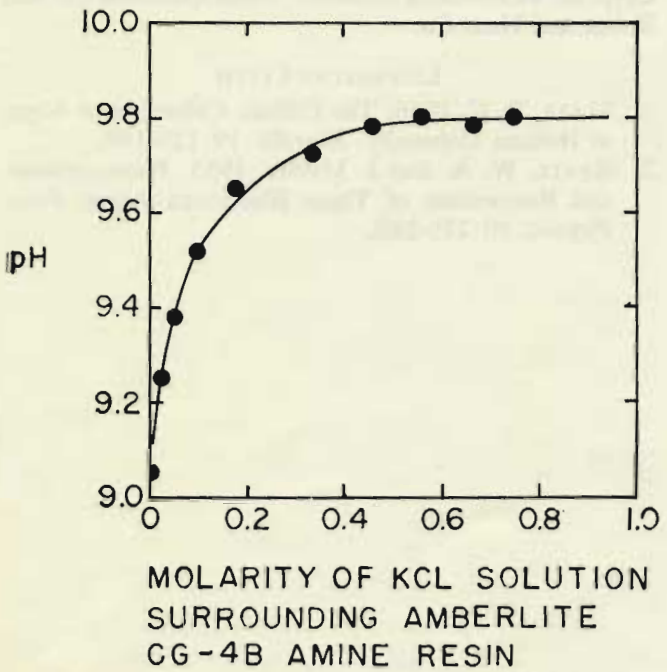

FIGURE 3. The effect of salt concentration on the pH maintained by the amine resin Amberlite CG-4B.

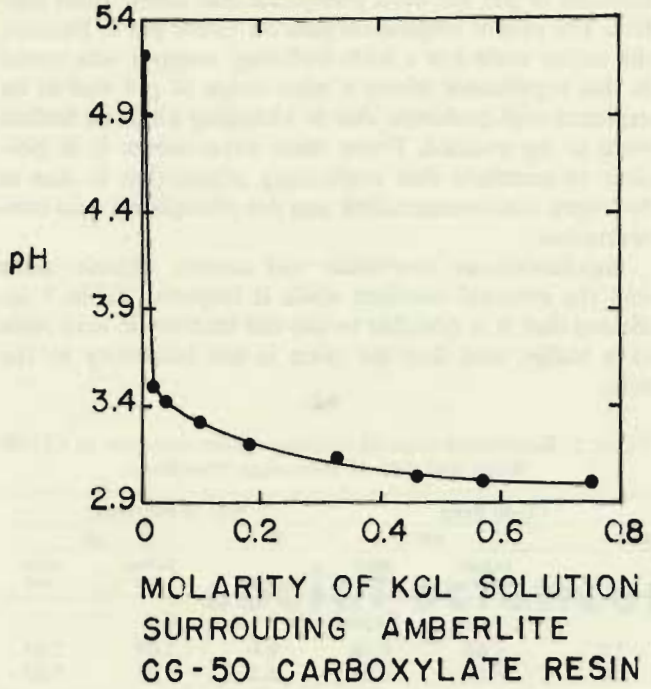

FIGURE 4. The effect of salt concentration on the pH maintained by the carboxylic acid resin Amberlite CG-50.

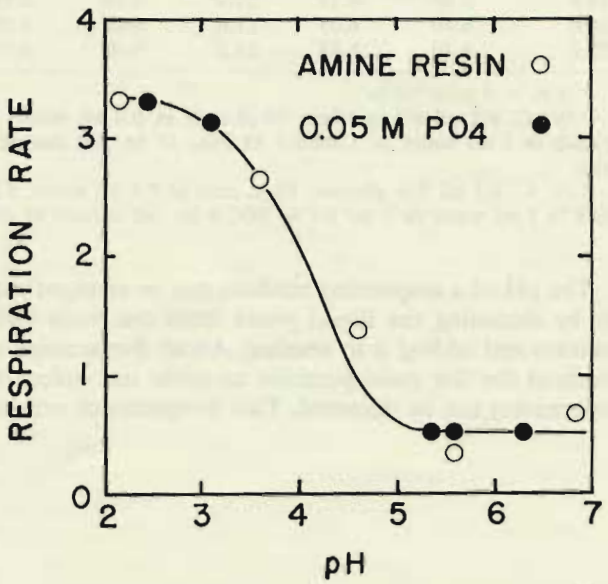

FIGURE 5. Respiration rate of Anacystis nidulans as a function of pH. 25 C, 0.2 ml 5% glucose, 40 μl cells in 0.8 ml water, 0.1 g amine resin in 1 ml water or 1 ml 0.1 M phosphate buffer. Rate in $\mu l\ O_2 \times \mu l$ cells$^{-1} \times$ hr^{-1}.

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

effect of phosphoric acid concentration. With a resin buffer only the hydrogen, hydroxyl and chloride ions are in solution. Fig. 5 shows the rate of respiration as a function of pH for both phosphate and amine resin buffers. The rate of respiration falls off below pH 2. Because the amine resin has a wide buffering range it was useful in this experiment where a wide range of pH was to be explored and problems due to changing kinds of buffers were to be avoided. From these experiments it is possible to conclude that respiratory stimulation is due to hydrogen ion concentration and not phosphoric acid concentration.

Saccharomyces cerevisiae will secrete organic acids into the external medium while it respires. Table 1 indicates that it is possible to use the carboxylic acid resin as a buffer, and that the resin is not inhibitory to the cells.

TABLE 1. Respiration rates of *Saccharomyces cerevisiae* in CG-50 Resin and 0.05 M Potassium Phosphate

CG-50 Resin			0.05 M Phosphate		
Rate *	pH		Rate *	pH	
	Before run	After run		Before run	After run
		Experiment 1 †			
7.0	6.60	6.08	6.6	7.05	7.05
8.0	6.60	6.08	6.8	7.05	7.05
		Experiment 2 ‡			
21.0	6.40	6.00	21.8	6.40	6.32
19.6	6.40	6.15	21.8	6.40	6.35
20.0	6.40	6.05	21.6	6.40	6.37
20.2	6.40	6.05	21.2	6.40	6.37

* $\mu l\ O_2 \times \mu l\ cells^{-1} \times hr^{-1}$.

† 30° C, 0.2 ml 5% glucose, 10 μl cells in 0.8 ml water, 0.1 g resin in 1 ml water or 1 ml 0.1 M PO_4, 12 hr. old culture of cells.

‡ 30° C, 0.2 ml 5% glucose, 10 μl cells in 0.8 ml water, 0.2 g resin in 1 ml water or 1 ml 0.1 M PO_4, 4 hr. old culture of cells.

The pH of a suspending medium can be changed readily by decanting the liquid phase from one resin buffer mixture and adding it to another. About five seconds are required for the resin particles to settle out before the supernatant can be decanted. This procedure of exposing the suspending medium to different resin buffer mixtures can be repeated many times.

The equations used to describe the buffer action of resins are similar to that of weak acids and bases. Where R represents the resin polymer. A any anion and B any cation:

$$RCOOH + BOH \rightleftharpoons RCOOB + H_2O \quad (1)$$

$$RCOOB + HA \rightleftharpoons RCOOH + A^- + B^+ \quad (2)$$

are the equations for the carboxylic acid resin and

$$RNH_3OH + HA \rightleftharpoons RNH_3A + H_2O \quad (3)$$

$$RNH_3A + BOH \rightleftharpoons RNH_3OH + A^- + B^+ \quad (4)$$

are the equations for the amine resin.

The equations which describe the salt effect are:

$$RCOOH + BA \rightleftharpoons RCOOB + HA \quad (5)$$

$$RNH_3OH + BA \rightleftharpoons RNH_3A + BOH \quad (6)$$

Because of this salt effect the buffering capacity of the resins are not the same in both directions. In equations (2) and (4) salt is formed and the net pH shift will be due to both added base or acid and salt.

SUMMARY: A new method of maintaining the pH of solutions by means of ion exchange resins is described. Weakly acidic or basic ion exchange resins which behave as insoluble buffers will have advantages over the usual soluble buffers in that they possess a wide buffering range, low osmotic pressure effects, fewer ion effects on physiological experiments and the ability to change the pH in an experiment without changing the rest of the composition of a suspending media.

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