H_2SO_4, and 100% of the cadmium is recovered from columns equilibrated to pH 3.4 or above, as shown in Figures 1 and 2.

Elution studies for all seven metals of interest from a column equilibrated to pH 7 using 95 ml of 1 *N* H_2SO_4 showed that all the metals except lead were eluted satisfactorily. The elution of lead averaged only 38% of the amount placed on the column. The low recovery is probably due to the formation of lead sulfate inside the resin particle. Variation of the volume and concentration of the sulfuric acid by use of 50 ml of 2 *N* and 33 ml of 3 *N* did not appreciably affect the lead or other metal elution recoveries.

Due to the trouble with cobalt and lead, the procedure finally utilized comprises a complete digestion of the entire sample with nitric acid–sulfuric acid–hydrogen peroxide catalyzed by vanadium pentoxide. The digestion time varies according to the nature of material to be oxidized; however, this step usually can be completed in less than 4 hr. The vanadium pentoxide does not interfere, since it is not retained by the resin. The digest is adjusted to pH 7, filtered to remove any precipitate, and passed through a column of Chelex 100 equilibrated to pH 7. The column is eluted with 95 ml of 1 *N* H_2SO_4 and the metals are determined by atomic absorption. The regular blank corrections ranged between 0 and 0.03 absorbance units, with the majority being less than 0.01 absorbance units. Values are higher in solutions which have been concentrated by evaporation.

Quadruplicate recoveries of standards in the range of 1 to 15 ppm were determined and averaged 99.1% for all the metals, the range being 91.1 to 103.5%. The average standard deviation was 3.55%, with a range of 1.67 to 5.88. These data are tabulated and will appear in the microfilm edition.

Recovery of added standards in the range of 1 to 15 ppm to various food commodities averaged 95.2% for all the metals, with a range of 91.4 to 100.5%. The average standard deviation was 3.03%, with a range of 1.87 to 5.25. These data are tabulated and will also appear in the microfilm edition.

The results of duplicate determinations on eight different types of foodstuffs are shown in Tables I and II. The precision indicated by the low values of the standard deviations is satisfactory for a method of this type. The relatively high values for zinc (2.1 ppm) and Mn (0.97 ppm) are caused by the large amounts of these metals present in several of the samples.

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Lactase and Other Enzymes Bound to a Phenol–Formaldehyde Resin with Glutaraldehyde

Alfred C. Olson* and William L. Stanley

A phenol–formaldehyde resin has been found to be an excellent adsorbent support for immobilizing enzymes. The enzymes can be held on the resin by treatment with the difunctional reagent glutaraldehyde. Enzymes that have been immobilized in this way include lactase (β-galactosidase), invertase, amyloglucosidase, α-chymotrypsin, and pronase. The activity of the immobilized lactase (from *Aspergillus niger*) was 200 μmol of glucose produced/min/gram of drained enzyme resin at pH 4.0 and 45°. Over 99% hydrolysis of lactose occurred when a 3% solution of lactose at pH 4.0 was passed over a 1.2 × 10 cm column of the immobilized lactase at 30 ml/hr and 45°. A lactase column was operated continuously for more than 4 weeks with no detectable loss in activity. Similar columns of the immobilized lactase were operated continuously at 60/ml/hr for more than 4 weeks with no loss in activity.

Recent advances in fixed or immobilized enzymes have been reviewed by Silman and Katchalski (1966), Guilbault (1970), Goldstein (1970), Mosbach (1971), and Orth and Brümmer (1972). By procedures such as adsorption, encapsulation, and covalent bonding it is now possible to stabilize enzymes, overcome their high initial cost by repeated reuse, and remove them from the final product.

Of the many systems described for immobilization, many are quite complex and expensive because of the cost of the chemicals, the purity of the enzyme required, and the sequence of reactions involved in the systems. This paper describes a system which avoids some of these com-

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plications. The enzyme is first adsorbed on a phenolic resin and then kept there by covalent bonding with glutaraldehyde. The phenolic resin chosen for this work was a phenol–formaldehyde which readily adsorbs proteins from dilute aqueous solutions and has been used for the purification and separation of crude enzyme preparations by selective adsorption and elution, using various buffer solutions.

Systems which depend only on adsorption to immobilize enzymes have the obvious disadvantage that the adsorbed enzyme may be washed off the support with substrate solution during use. Adsorbed enzymes also may not be as stable as covalently-bound enzymes. We found that it was possible to hold enzymes to phenol–formaldehyde resins by reacting them with glutaraldehyde after first adsorbing them onto the resin.

The reaction of glutaraldehyde with enzymes has been studied by a number of investigators. We have reported on the reaction of glutaraldehyde with papain and α-chymotrypsin to give insoluble enzymically active preparations (Jansen and Olson, 1969; Jansen *et al.*, 1971; Tomimatsu *et al.*, 1971). The physical form of glutaraldehyde-insolubilized enzymes is such that they are not readily adapted to continuous operation in column or stirred tank systems. The phenol–formaldehyde resins are highly porous, readily available in granular form, and well suited to large-volume column use. The combination of adsorption and glutaraldehyde treatment provides a workable practical system for enzyme immobilization.

Sharp *et al.* (1969) noted that the β-galactosidase system has many features to recommend it for study in immobilization systems that few other enzymes have. These investigators studied the performance of an *Escherichia coli* β-galactosidase covalently attached to porous cellulose sheets. In 1972 Woychik and Wondolowski reported on a β-galactosidase (from *Aspergillus niger*) covalently coupled by diazo-linkage to porous glass beads. For our work we also used a β-galactosidase from *Aspergillus niger* which readily hydrolyzes lactose at pH 4. Under the right conditions the successful immobilization of a β-galactosidase could be applied to the hydrolysis of lactose in milk and milk products such as whey. Such a process could be helpful in providing milk products with reduced lactose content for lactose-intolerant people (Kretchmer, 1972).

The purpose of this study, then, was to investigate the applicability of the phenol–formaldehyde glutaraldehyde procedure to successfully immobilize lactase and other enzymes.

EXPERIMENTAL SECTION

Materials. A phenol–formaldehyde resin, Duolite S-30, was obtained from Resinous Products Division of Diamond Shamrock Chemical Co. Glutaraldehyde was obtained as either a 25 or 50% aqueous solution from Union Carbide. A water-soluble, acid-stable β-galactosidase from *Aspergillus niger* was obtained from Wallerstein Co. as Lactase LP and used without further purification. A standardized liquid invertase, Sucrovert, derived from yeast, was obtained from SuCrest Corporation. A commercial liquid preparation of glucoamylase was obtained from A. E. Staley Manufacturing Co., Decatur, Ill. α-Chymotrypsin was obtained from Worthington Biochemical Corp. and Pronase grade B came from Calbiochem.

Analytical. Lactase, invertase, and glucoamylase activities were measured under conditions described later, using lactose, sucrose, and starch, respectively, as substrates and measuring the glucose produced by the glucose oxidase–chromagen procedure supplied by Worthington as Glucostat. For qualitative checks of these enzyme activities, Clinistix Reagent strips from Miles Laboratory were helpful in detecting when glucose was being produced.

Soluble lactase activity was determined by incubating aliquots of the enzyme in 10 ml of solution which was 0.40 *M* in lactose and 0.10 *M* in sodium acetate buffer, pH 4.0, at 45° and removing 1.0-ml aliquots for assay at different time intervals. Reaction was stopped by heating the aliquots at 100° for 3–5 min, after which they were analyzed for glucose produced. Resin-bound lactase activity was determined by incubating a weighed portion of resin–enzyme in a 250-ml Erlenmeyer flask with 50 ml of a solution which was 0.40 *M* in lactose and 0.10 *M* in sodium acetate buffer, pH 4.0. Incubation was at 45° with vigorous agitation, during which time aliquots of solution free of the bound enzyme were removed from the flask for glucose analysis. Reciprocal shaking at 90–120 cycles/min with a pathlength of 3 cm was found to give adequate mixing and equilibration of the solid resin–enzyme and the substrate. Aliquots of solution that did not contain any resin could be removed from the flask with automatic pipets. For both soluble and bound enzyme, initial rates were calculated from a minimum of three aliquots taken at different times within the first 10 min of reaction and before 5% of the substrate had been hydrolyzed.

For lactase activity, complete analyses for glucose, galactose, and remaining lactose were obtained by first converting freeze-dried aliquots to trimethylsilyl (TMS) derivatives, using Tri-Sil reagent (Pierce Chemical Co.). After 20 hr of silylation at 25°, the derivatized sugars were separated quantitatively, using a Varian Aerograph 1520C gas chromatograph equipped with a hydrogen flame detector. Separation of TMS derivatives was accomplished, using either a 5 or 10 ft × ⅛ in. stainless steel column packed with 3% OV-17 on 100/120 Varaport 30. The column temperature was programmed from 150 to 240° at 4°/min with helium as carrier gas and injection port temperature of 200°. Identification of the sugars was accomplished by running known sugars through the procedure and comparing retention times. Quantitative data were obtained by comparing peak areas of the samples to those of standards.

Milk samples were analyzed for the extent of hydrolysis of lactose by first acidifying with 2 ml of 25% acetic acid per 100 ml of milk, warming to 70° for 10 min, chilling in an ice bath for 10 min, and then centrifuging at 9000 rpm for 10 min. The clarified supernatant was analyzed for sugars, as described in the preceding paragraphs.

Substrate for amyloglucosidase activity was a 2% solution of Lintner's starch (Merck and Co.) in 0.01 *M* citrate buffer, pH 4.5.

Pronase and α-chymotrypsin esterase activities were determined with *N*-acetyl-L-tyrosine ethyl ester (ATEE from Mann Research Laboratories) as substrate, following a modified procedure of Balls and Jansen (1952) in which the bound enzyme was kept agitated in the incubation vessel during the analysis.

Protein was determined by the method of Lowry *et al.* (1951). Dry weights were determined by drying weighed portions of partially dry resin in an oven at 55° for 24 hr.

Immobilization Procedure. *General.* The phenol–formaldehyde resin, Duolite S-30, 10–40 mesh, was washed with distilled water, soaked overnight in 0.1 *N* NaCl, and rewashed with distilled water. In some experiments the resin was sized prior to use, using appropriate screens. A portion of wet resin was placed in a sintered glass funnel and excess moisture was removed by applying a slight vacuum. The drained resin contained from 40 to 60% water, as measured by dry-weight analysis. To a weighed aliquot of the drained resin was added a solution of the enzyme to be adsorbed. The amount of the solution added was from 1 to 2 times the weight of the drained resin and the amount of enzyme was from 1 to 20 mg (as protein) per gram of drained resin. After selected lengths of time, sufficient glutaraldehyde was added to make the final glutaraldehyde concentration in the range 1–3%. After 16 hr,

the immobilized enzyme was washed thoroughly with distilled water and assayed for enzyme activity.

Lactase. A solution of 228 mg of Lactase LP in 20 ml of distilled water was added to 10 g of drained 30–40 mesh Duolite S-30 at 5°. After 15 min with gentle agitation, 3 ml of 25% glutaraldehyde was added with swirling. After 16 hr at 5° the immobilized enzyme was washed with distilled water and analyzed for enzyme activity.

In another example, a solution of 40 g of Lactase LP in 700 ml of water was added to 750 g of drained 10–40 mesh Duolite S-30 at 25°. The flask was agitated every 15–30 min and 0.20-ml aliquots of the liquid portion free of resin were removed periodically for protein analysis. After 5 hr the mixture was chilled to 5° and 30 ml of 50% glutaraldehyde solution was added slowly with agitation. After 16 hr at 5°, the supernatant solution was drawn off and the resin containing the immobilized enzyme was washed thoroughly with distilled water and analyzed for enzyme activity.

Invertase. Sucrovert (25 ml) was added to 20 g of washed and drained Duolite S-30 (through 30 mesh) at 25°. After 16 hr at 25°, 2.0 ml of 25% glutaraldehyde was added and the mixture was held at 25° for 3 hr and then at 5° for 16 hr. The immobilized enzyme was washed with water and analyzed for enzyme activity.

Glucoamylase. Two milliliters of a commercially available glucoamylase containing about 160 mg of protein/ml was diluted to 30 ml with distilled water and added to 30 g of drained 20–30 mesh Duolite S-30 at 25° with swirling. After 16 hr at 5°, a solution of 3.2 ml of 25% glutaraldehyde diluted to 10 ml with water was added with gentle mixing. After 16 hr at 5°, the immobilized enzyme was washed with water and analyzed for enzyme activity.

α-Chymotrypsin. A solution of 102 mg of α-chymotrypsin in 20 ml of water was added to 15 g of drained Duolite S-30 (through 30 mesh) at 5°. After 16 hr at 5°, a cold solution of 2.4 ml of 25% glutaraldehyde diluted to 10 ml with water was added to the mixture. After 20 hr at 5°, the immobilized enzyme was washed with water and analyzed for enzyme activity.

Pronase. A solution of 225 mg of pronase in 40 ml of water was added to 30 g of drained Duolite S-30 (through 30 mesh) at 5°. After 16 hr, a cold solution of 4 ml of 25% glutaraldehyde diluted to 10 ml with water was added. After 20 hr at 5°, the immobilized enzyme was washed with water and analyzed for enzyme activity.

Column Operation. Resin-immobilized enzyme was packed into jacketed columns of various sizes over supporting beds of sand. Sintered glass plates were used to support the sand and resin in the columns. Substrate was passed over the columns upflow or downflow at measured flow rates, using positive displacement Cheminert Metering pumps (Chromatronic Incorporated, Berkeley, Calif.) or Masterflex tubing pumps (Cole Parmer Instrument Co.). Temperature control on the columns was maintained by circulating water at the desired temperature through the column jackets.

RESULTS

Lactase. The Lactase LP contained 12% protein. When 10–40 mesh drained resin and Lactase LP were mixed in the ratio 6.4 mg of protein/g of resin there was a 70–75% drop in protein content of the liquid phase in the first 70 min of contact, with another drop of 6% during the next 16 hr, indicating a rapid adsorption of protein by the resin. Enzyme activity of the supernatant aqueous phase decreased by more than 95% during the first 70 min of contact, which suggests the possible preferential adsorption of lactase by the resin. After fixing with glutaraldehyde, washing the resin removed 40% of the adsorbed protein, as determined by analysis of the washes for protein, and left about 5 mg of protein bound to each gram of dry resin. The activity of the soluble enzyme was 105 μmol of glucose produced/min/mg of protein under the standard conditions described in the Experimental Section. The activity of the bound enzyme was 80 μmol of glucose produced/min/mg of protein on the resin for a retention of activity of about 75%. However, due to the losses during the adsorption and washing steps, only about 30% of the activity applied to the resin was retained.

The pH during both the adsorption of the lactase and the reaction with glutaraldehyde was about 6.2. Preliminary experiments with buffers from pH 5 to 7 did not show any improvement in either the adsorption or the reaction with glutaraldehyde and subsequent experiments were done in unbuffered systems.

No significant difference in the activity of the final immobilized enzyme was observed whether contact between enzyme and resin was 15 min or 16 hr before addition of glutaraldehyde, or whether the contact took place at 5 or 25°. Glutaraldehyde treatment was done in the cold for 16–24 hr. Because of the difficulty of determining exactly how much enzyme was adsorbed to the resin, it was not possible to detect any significant differences in enzyme activity as a function of resin mesh size in the range examined.

Characteristics of Resin-Bound Lactase. The activity of the resin-bound lactase described in the preceding section was 200 μmol of glucose produced/min/g of drained resin under the conditions described in the Experimental Section. Using 1.0-g samples of the drained resin-bound lactase, the effects on activity of freezing and drying and of changing pH and temperature were studied. In these studies the same 1.0-g portion of drained immobilized lactase was used repeatedly. After an analysis the immobilized enzyme was washed with water on a sintered glass funnel to remove substrate and products, quantitatively transferred back to the 250-ml Erlenmeyer flask with water, and drained of excess water by careful decantation. By repeating the standard analysis periodically, any permanent loss in activity was detected and, in that case, a new aliquot of immobilized lactase was then used for further studies. The effect of pH on the immobilized lactase activity at 45° is shown in Table I. These results are simi-

Table I. Effect of pH on Immobilized Lactase Activity[a]

pH	Activity[b]
3.0	174
4.0	200
5.0	172
6.0	98
7.0	52
7.9	13

[a] Analyses done at 45° as described in the Experimental Section, using 1.0-g portions of the immobilized lactase. [b] μmoles of glucose produced/min/g of drained immobilized lactase.

Table II. Effect of Temperature on Immobilized Lactase Activity[a]

Temp, °C	Activity[b]
25	100
37	174
45	200
55	284
60	314

[a] Analyses done at pH 4.0 as described in the Experimental Section, using 1.0-g portions of the immobilized lactase. [b] μmoles of glucose produced/min/g of drained, immobilized lactase.

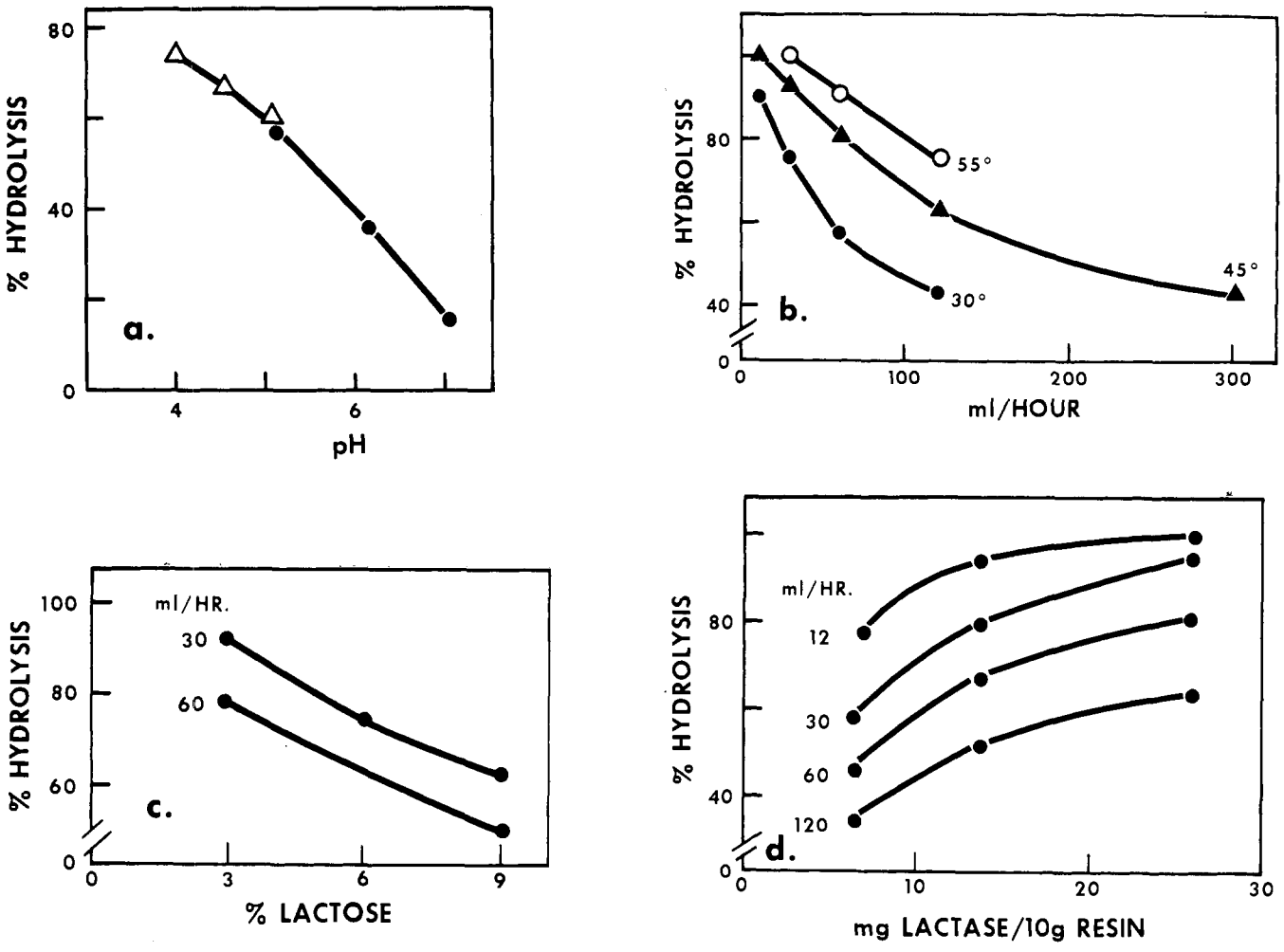

Figure 1. Performance characteristics of immobilized lactase in column operation. a. pH effect, △, sodium acetate buffer; ●, sodium phosphate buffer. b. Flow and temperature effect at pH 5.0 with 3% lactose. c. Substrate concentration effect at pH 5.2 and 45°. d. Effect of the amount of lactase on the resin using 3% lactose solutions at pH 5.2 and 45°.

lar to the effect of pH on the activity of the soluble lactase.

The immobilized lactase lost no activity when it was stored wet at 4° for 6 months. At 45° and in the pH range 4 to 6, the immobilized lactase was stable for more than 6 weeks. At 45° and pH's 3.0, 7.0, and 7.9, the immobilized lactase was stable for at least several hours. That is, rerunning the standard analysis at pH 4.0 after such treatment gave 100% of the original activity, showing that the enzyme was not inactivated or washed off of the resin during the treatment.

Activity increased as temperature was increased from 25 to 60°, as shown by the data in Table II. The stability of the bound lactase, however, decreased as the temperature was increased above 50° as shown by the data in Table III. The bound enzyme was more stable at the higher temperatures in the presence of the substrate lactose. Enzyme activity dropped 30% after the immobilized lactase was frozen for 2 days or for several weeks. All of the enzyme activity was lost after the immobilized lactase was dried at either 25 or 50°.

Immobilized Lactase Continuous Column Operation. Performance characteristics of the immobilized lactase in column operation are shown in Figure 1. Data for this figure were obtained from a 1.2 × 15 cm column packed with 10 g of Duolite S-30 which had previously been treated with 228 mg of Lactase LP and glutaraldehyde (see Experimental Section). Activity increased as pH decreased from 7 to 4 when 3% lactose solutions were passed over the column at 60 ml/hr and 30° at the designated pH's (Figure 1a). Activity also increased as temperature increased from 30 to 55° (Figure 1b). No evidence of instability was observed at 55° under these conditions for several hours. However, in view of the loss of activity observed at these higher temperatures (Table III), most subsequent long-term runs were made at 45°. As lactose concentration was increased, the percent hydrolysis decreased as expected (Figure 1c). Recycling the product from the

Table III. Heat Stability of Immobilized Lactase[a]

Temp, °C	Time of treatment, hr	0.4 *M* lactose solution	Activity at 45° pH 4.0 following treatment, % of original activity
45	24	+ or −	100
50	1	+ or −	100
55	1	+	100
55	16	+	85
55	1	−	93
60	1	+	88
60	1	−	72

[a] One-gram portions of drained immobilized lactase were treated with (+) 0.4 *M* lactose solution or with distilled water (−) at the indicated temperatures and times and then analyzed for activity.

Table IV. Activities of Several Immobilized Enzymes

Enzyme	Activity[a]
Lactase[b]	200
Invertase[c]	1200
Glucoamylase[d]	17
α-Chymotrypsin[e]	75
Pronase[f]	33

[a] Expressed as μmol of product produced per minute per g of drained immobilized enzyme. [b] 0.40 *M* lactose, pH 4.0, 45°. [c] 0.40 *M* sucrose, pH 4.5, 45°. [d] 2.0% w/v Lintner's starch, pH 4.5, 35°. [e] 0.02 *M* ATEE, pH 7.8, 30°. [f] 0.02 *M* ATEE, pH 7.8, 30°.

9% solution increased the conversion at 30 ml/hr from 61 to 72%, while a second recycle brought the overall conversion to 81%. Product inhibition (galactose) of this enzyme has previously been demonstrated by Woychik and Wondolowski (1972). As the amount of enzyme the resin was treated with was increased, the percent hydrolysis of lactose increased but not proportionally, as shown in Figure 1d. Three different columns were used to obtain these data. Three batches of 10 g each of drained resin were treated with 58, 117, and 228 mg of Lactase LP and glutaraldehyde, as described in the Experimental Section. On the basis of 12% protein in Lactase LP, this corresponds to 0.7, 1.4, and 2.8 mg of protein applied/g of drained resin. In other experiments with this lactase, it appears that the maximum amount that will stay on the resin under the conditions stated is about 3–4 mg of protein/g of drained resin.

Duolite S-30 adsorbs Lactase LP very readily from aqueous solutions, and between pH 4 and 5 it is washed off but not very readily. This is not true for the glutaraldehyde-treated preparations. The extent of these effects was determined by preparing two 1.2 × 10 cm packed columns, each with 10 g of resin which had been in contact with identical amounts of Lactase LP. The adsorbed enzyme in column 1 was not subsequently in contact with 2% glutaraldehyde, while that in column 2 was in contact with 2% glutaraldehyde solution. After 7 days continuous operation with 4% lactose solutions at 60 ml/hr, 45°, pH 4.0, the activity of column 1 had dropped 10%, while that of column 2 was unchanged. During the following 7 days of operation, addition of as much as 10% NaCl to the feed failed to drop the activity on column 2, while that on column 1 dropped another 10%. Thus, while adsorption of lactase alone to the resin provides a considerable measure of enzyme activity for continuous hydrolysis of lactose, enzyme activity was lost slowly under these conditions. This loss of activity was not observed when the adsorbed enzyme was treated with glutaraldehyde.

The stability of the glutaraldehyde-treated lactase adsorbed on resin to high salt concentrations suggests that it could be successfully used to hydrolyze the lactose in such high salt solutions as acid whey and deproteinized acid whey. A larger column with a 2.5 × 15 cm bed of immobilized lactase was also prepared. Performance characteristics of this column were similar to projected scale-up of the smaller columns, with no significant increase in pressure drop across the column. Columns of immobilized lactase were operated continuously between pH 4 and 5 at 40 to 50°, and at conversions of 70 to 80% for periods of 4 to 6 weeks with no apparent loss in activity.

When reconstituted nonfat dried milk was passed over a 1.2 × 10 cm packed column of immobilized lactase similar to the one described in Figure 1, part of the lactose was hydrolyzed. The milk contained 4.4% lactose as determined by gas–liquid chromatographic analysis. At 45° and 60 ml/hr, 20% of the lactose was hydrolyzed, while at 12 ml/hr at the same temperature, 50% of the lactose was hydrolyzed. There was a 10% drop in activity observed in the course of 17 hr of operation at 60 ml/hr. All analyses for the extent of hydrolysis of lactose in the milk samples were made by the Glucostat procedure for the glucose produced. Selected samples analyzed by the gas–liquid chromatographic procedure gave identical results for glucose and provided a material balance for glucose, galactose, and lactose present in the system.

Application of the Procedure to Other Enzymes. The activities of several enzymes adsorbed on Duolite S-30 and treated with glutaraldehyde are summarized in Table IV. The data show the applicability of the method to other enzymes.

DISCUSSION

This paper presents a simple inexpensive method in which lactase and several other enzymes can be immobilized. The resins used in the method have specific adsorptive properties for proteins and other organic compounds. They are readily available in large quantities and are inexpensive; *i.e.*, current price of the basic support material is about $32/ft^3$. The technology of the large-volume column use of these resins is well documented for other purposes and could be applied to the immobilized enzyme–resin systems. A related method for immobilizing lactase, recently reported by Wondolowski and Woychik (1972), involves binding lactase to porous glass beads with glutaraldehyde. Another related method for immobilizing enzymes, described by Haynes and Walsh (1969), involves immobilizing trypsin by adsorption to colloidal silica particles followed by intermolecular cross-linking with glutaraldehyde. According to these authors, the colloidal particles are readily sedimented and also easily dispersed to form sols. Continuous-flow, large-volume column use of such particles would appear to be difficult if not impossible.

Variations of the resin–enzyme system may have certain advantages for specific enzymes and applications. In one variation, the enzyme can be passed over a column of resin under such conditions that the enzyme is adsorbed to the resin. This can be followed by a solution of glutaraldehyde at appropriate rates and temperatures to fix the enzyme to the resin. After washing thoroughly to remove soluble enzyme and excess glutaraldehyde, the column is ready for substrate. Such a column has been successfully prepared with lactase.

In another variation, modified resins were used in place of the Duolite S-30. Duolites A1 and A6 are phenolic resins substituted with primary amino groups and mixed amines, respectively. We have successfully attached invertase to both of these resins with retention of enzyme activity.

The chemical composition and form of the resin is of considerable importance and something like a macroporous beaded polystyrene may be superior to the granular phenolic resins for some applications. Stanley and Palter (1973) prepared formylated Duolite S-30 resins and showed that a phenolic resin containing aldehyde groups may be as effective a support as a phenolic resin treated with glutaraldehyde after enzyme adsorption. Enzymes would be bound to formylated resins by both adsorption and direct covalent bonding through the aldehyde groups.

The pH, ionic strength, and ion species may be important in both the adsorption process and the reaction between enzyme and glutaraldehyde (Jansen *et al.*, 1971). Optimum values for these parameters should be investigated further, as they are probably different for different enzymes.

The potential usefulness of an immobilized lactase appears reasonable in view of the fact that most adult humans in the world do not have lactase enzyme and probably cannot utilize this component of milk (Kretchmer,

1972). The immobilized lactase described in this paper would be most applicable in special cases such as with acid wheys and deproteinized acid whey. Hydrolysis of lactose in these materials might make them more nutritionally acceptable for certain applications.

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Screening Method Based on Electric Hygrometer for Obtaining Water Sorption Isotherms

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A discontinuous gravimetric screening method with high precision has been developed for the determination of water sorption isotherms. The equipment needed is cheap and simple. A detailed analysis of the precision shows that the water activity can be determined within ±0.016 a_w units and the water content with an accuracy of ±0.1% if the temperature is kept constant. The water activity is determined by an electric hygrometer. By a strict calibration and measuring routine, the accuracy of the electric hygrometer has been improved about 30-fold. Using 20 electric hygrometers, one person can determine 160 isotherms in 15 days.

Obtaining water sorption isotherms has long been a rather elaborate method for characterizing a material and its relation to water. This is mainly due to the methods available being either expensive, laborious, time-wasting, or not accurate enough (Gál, 1967). The existing methods are based on the determination of the water content, the water activity, or a combination of both. Those determining the water content are mainly gravimetric and imply that the sample is completely equilibrated with its surroundings; *i.e.*, has attained a known water activity. The methods determining water activity are either manometric or hygrometric, depending on whether the partial pressure of the water or the relative humidity above the sample is measured. Each sample is equilibrated with a known amount of water.

Both continuous and discontinuous methods have been worked out on these principles. Continuous registrations involve complex and expensive instrumentation, compared to the discontinuous registrations, and are characterized by high precision. Their capacity is, however, very low.

The approach presented in this paper is a discontinuous screening method based on the determination of both the water activity and content. It is characterized by high precision, as well as high capacity, and is suitable for small quantities of sample. The method has been developed with special attention to the characterization of protein-rich products such as meals, concentrates, and isolates. Until now little has been known about the relation of these products to water. It is of greatest importance for the functional properties of the final product to know how this relation is affected by chemical composition and molecular structure, as well as by heat and organic solvents. It is our hypothesis that isotherms could be a useful, analytical tool in such work. Investigations in this line are now being worked out using the method described in this paper.

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MATERIALS

Chemicals and Protein Preparations. Salts of highest purity have been used to obtain well-defined atmospheres of different water activities (Robinson and Stokes, 1959) (Table I). All amino acids were of CHR quality and obtained from Fluka AG. The proteins used as application samples are: casein (nach Hammarsten), Merck AG; gelatin (Difco certified), Difco Laboratories; egg albumen (grade V, salt-free, lyophilized and recrystallized), Sigma Chem. Co.; keratin (pract.), Fluka AG.

Hygrometer (Lion, 1959; Wexler, 1957, 1965). A Pope hygrometer (Pope, 1955) numbered PCRC-55 and pur-