AN ION-EXCHANGE RESIN-CONTACT TIME METHOD FOR THE
STUDY OF INORGANIC EQUILIBRIA IN MILK

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The stability of the milk proteins as influenced by the cation-anion balance of the milk has been a subject of much consideration by investigators in the fields of dairy chemistry and technology. As a consequence, a number of different experimental approaches have been used in an attempt to clarify the chemistry of, and the phasic distribution of, the inorganic components of milk. Seekles and Smeets (12, 13) conducted an extensive study on heat-stable and unstable milks in the Netherlands. A correlation was made between the calcium and phosphorus content of the milk ultrafiltrates and the heat stability of the milk proteins as measured by the alcohol test. Ultrafiltration studies were conducted by Jenness (7) on milks subjected to heat treatments in the temperature range of 67-98°F. The ultrafilterable calcium and phosphorus were found to decrease immediately after heating but again to approach the normal values in the aged heated milk.

Other investigators used collodion membranes and dialysis experiments (6, 8, 10, 11) in studies on the colloidal and soluble phases of the inorganic constituents of heated and raw milks. In nearly all cases long time intervals were involved. Zeolite type ion exchangers were employed by Davies (2) to study the phase distribution of calcium in reconstituted milks. The batch process was used and involved contact time periods from 10 minutes to 48 hours. Verma (15) conducted a study on the colloidal and soluble polyvalent inorganic constituents in raw and heated skim milks by removing the protein with rennet precipitation.

The more recent work cited in the references has been conducted on ultrafiltrates and whey prepared from samples of raw and heat-treated skim milks by rennet action. The process of obtaining an ultrafiltrate requires a length of time varying from 30 minutes to several hours, during which time many changes may take place in the chemical equilibria of the milk. The colloidal systems that occur in milk and which may be altered during heating are also subject to the limitations of the restrictions of the membrane. The production of whey by rennet action involves the precipitation of the protein, which is capable of carrying down portions of the inorganic colloidal particles of the milk. These procedures leave much to be desired, for many changes may occur in the distribution of the inorganic constituents during dialysis, ultrafiltration, rennet precipitation,
and zeolitic contact. However, these methods were the best available, and many valuable data have been obtained.

In earlier experiments, Gehrke and Almy (4, 5) investigated the adsorption affinity to synthetic ion exchange resins of the cations and anions normal to milk. Single, binary, ternary, and quaternary systems were used of a concentration similar to that of milk. In view of these results, it was considered that an ion-exchange resin equilibration technique would be an approach of considerable merit. The use of an ion-exchange resin in the manner described below is chemically similar to the addition of a precipitating or complexing agent or similar chemical reagent. The reagent may also be recovered for study after definite time intervals, which in turn allows rate studies to be made.

Thus, the major objectives of this investigation were to develop a valid method for observing and following inorganic equilibria changes in milk and other complex biological systems as a result of various processing conditions and to make the measurements in a very short time period on the original sample.

**EXPERIMENTAL PROCEDURE**

Ion-exchange resin-contact time method (RCTM). Regenerate the ion-exchange resin in the desired form by the batch or column procedure. Wash the resin free of regenerant in a column. Remove the resin from the column and transfer the desired amount of resin (±1.0 ml. for 30 ml. or larger quantities of resin) to a 50-ml. coarse porosity fritted glass crucible. Wash the resin in the crucible thoroughly with distilled water. Transfer the resin to a 100-ml. beaker. Draw a 25-ml. aliquot of the sample test solution and place in a container from which it may be quickly poured. Then pour the sample onto the resin in the 100-ml. beaker and stir vigorously for the desired contact time. At the completion of the contact time, transfer the resin and sample to the original fritted glass crucible, which is under vacuum. Immediately wash the resin free of the sample solution with distilled water. Collect the original effluent and washings in a volumetric flask for analysis. Elute the exchanged ions from the resin with a 5% solution of the desired elutriant, using four 25-ml. portions. Allow enough time for complete elution. Wash the resin free of elutriant with distilled water. Collect the elutriant and washings and make to volume with distilled water in another volumetric flask. Take aliquots of the collections of the original effluent, and the eluate for analysis of the desired constituents.

This procedure is applicable to either the determination of anions or cations as desired. One of the major problems to be considered in the application of this technique is the choice of the proper ion-exchange resin. The resin selected for use in the preliminary investigations on the removal of calcium was made in consideration of the following points:

(a) The ability of the resin to remove Ca++ ions in preference to Mg++, K+, and Na+ ions, which are the other major cation constituents of milk.
(b) The ease with which the Ca++ ions can be eluted from the resin by elutriants which will not interfere with subsequent analysis.
(c) The ability of the resin to be placed in the regenerated condition by a
buffer system so that the milk or biological system will not be affected by a change of acidity when in contact with the resin during the removal of the various cations.

(d) A resin with a large milliequivalent exchange capacity.

The resin Amberlite IRC-50 met most of the above requirements for resin-contact time studies on removal of calcium. The resin was regenerated as completely as possible to the potassium form with a 5% potassium hydroxide solution and buffered to a pH of 6.70 by a buffer mixture containing 1.5 g. potassium hydroxide and 6.8 g. of potassium dihydrogen phosphate per liter. Calcium and magnesium are the only major cationic constituents in milk which will exchange the potassium from the IRC-50-K resin. Percentagewise, the concentration of magnesium in milk is approximately one-tenth that of the calcium. In consideration of the differences in concentration and adsorption affinities (4, 5) any change in the uptake of calcium would reflect real differences in the phasic distribution of the calcium in the sample. All of the experiments were conducted in the temperature range of 20 to 24°C.

Thirty ml. of the resin is recommended for each analysis, which has approximately 210 meq. of exchange capacity. The ratio of milliequivalents exchange capacity of resin to milliequivalents of calcium present in a 25-ml. sample of milk is then about 140:1. Five per cent hydrochloric acid was used as the elutriant. Calcium was analyzed by a modification developed in this laboratory of the dihydrogen disodium ethylenediamine tetraacetate method (3).

The anion exchange resin, Amberlite IR-4B, was chosen for the study on the removal of phosphate from raw skimmilk. This resin met most of the requirements for the removal of anions which are similar to those stipulated above in the equilibration studies for exchange of calcium. The resin was regenerated with 5% sodium hydroxide. The resin was placed in the chloride form with 5% hydrochloric acid. Thirty ml. of resin were used, which contained approximately 60 meq. of exchange capacity. The ratio of milliequivalent exchange capacity of resin to the average milliequivalents of trivalent phosphate present in 25 ml. of raw skimmilk is about 27:1. A 5% ammonium hydroxide solution was used as the elutriant. Phosphorus was analyzed by the A.O.A.C. volumetric titration procedure (1).

RESULTS AND DISCUSSION

Figure 1 presents the results of three independent determinations by the resin-contact time method for the removal of calcium from raw skimmilk. The stability numbers were measured by the acid-heat test (14), somewhat modified, and were found to be seven or eight, which is the normal range for stable milk. The removal of calcium from a true solution is also plotted on the same figure. The initial level of removal of calcium from milk at the 10-second resin-contact time was found to be 36%. A rapid and constant rate of removal was then observed up to the 70% level. From the 70% level to total removal, the rate of removal becomes smaller the longer the resin-contact time interval. The pH of the contacted samples for the complete resin-contact time range varied by ±0.2 pH units from that of the original milk samples.
A 36% removal for the 10-second time interval is comparable to the amount of soluble calcium found in rennet whey as reported by Verma (15). A definite change in the slope of the curve occurs between the 60- and 180-second contact time. The ionic calcium and the calcium in the form of dissolved complexes are probably totally exchanged previous to the 180 seconds contact time.

It has been shown by the column technique that 99.6% of the calcium in milk is available to the resin Amberlite IRC-50-K form. This supports the view that the removal of calcium approaches 100% asymptotically. At contact times of 3 minutes or greater the curve is nearly linear, and the exchange resin is removing 95% plus of the calcium in untreated milk. When a 3-minute contact time is used in comparative studies conducted on milks subjected to various heat treatment, major changes in the chemical composition of the milk may be expressed as a function of the time and the temperature of heating. Small errors in the time of contact would not cause any appreciable changes in the results of comparative studies.

A study was made to determine the reproducibility of the RCTM. This work was conducted on a single sample of milk. An average standard deviation of ±0.38 mg. was obtained from the results on three replicate analyses on 25-ml. samples of milk for contact times of 10, 20, 40, 80, and 160 seconds. The range of the standard deviations was found to be ±0.51 to ±0.16 mg. for increasing contact times. The extent of calcium removed for these contact periods ranged from 11.2 to 31.1 mg. The average per cent error for all contact times based
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Figure 2. The exchange of phosphate from raw skim milk by Amberlite IR-4B-C1. Resin-contact time studies.

(○—○) Phosphate exchanged from milk (average of two runs).

(×—×) Phosphate exchanged from a true solution of KＨ₂PO₄ and K₂HPO₄, containing 10 meq. PO₄/100 ml.

Note: Quantity of resin 30 ml.; exchange capacity 60 meq. Sample volume 25 ml.

upon all of the values of an average deviation of a single determination was ±1.64% of the amount exchanged, with a range of ±2.43 to ±0.35% for increasing contact times. The average standard deviation (±0.38) and average percent error include the results of at least 15 determinations.

Figure 2 shows the results of two independent resin-contact time studies on removal of phosphorus. The pH of the effluent gradually decreased when the milk was equilibrated with Amberlite IR-4B in the chloride form. After a 90-second or longer equilibration period, the isoelectric point was reached and the protein coagulated. The decrease in pH during the resin-contact equilibration range causes the equilibria of the phosphate systems in milk to shift to more soluble states than those normally found. The results (Figure 2) present the pattern of removal by the resin Amberlite IR-4B-C1 as affected by pH (6.7 original to 5.0 at 60 seconds contact). Experiments are under way employing buffered resins and mixtures of various anion exchange resins to obviate this pH change. These data will be published later. With a resin-contact time of 10 seconds the removal of phosphorus was 43 to 44%. This removal continued at a nearly constant rate with time to the 60-second contact period. At this contact time the per cent phosphorus removed leveled off at near 65%, the curve becoming nearly parallel to the abscissa. The extent of removal of phosphate ions from a true solution, as a function of time, is also given in Figure 2. The true solution contained equivalent amounts of the mono- and dibasic-potassium phosphates. From these data it may be concluded that about one-third of the phosphate in milk is organically bound and not available for exchange to ion-exchange resins. This is in agreement with data reported by Líng (9).
An insoluble ion-exchange resin acts as though it were in true solution and gives a direct measure of the amount of ionic calcium and phosphate available for a chemical reaction in a given sample for a definite time. The changes in the slopes of the curves (Figures 1 and 2) of resin-contact time against per cent exchanged show the comparative quantities of calcium which are available to the resin from their natural states in milk and of phosphorus which are available from the milk under the conditions of this experiment. A semi-log plot (Figure 1) of concentration versus contact time for calcium removal from the skimmilk shows a change in the rate constant in the 150-180 second contact time range. This change in the rate constant of removal can be interpreted as caused by the competition of the various components in milk and the active sites on the ion exchange resin for the calcium. The various component forms of calcium are considered as ionic, complexed and/or colloidal. The slope obtained with short resin contact times (10 to 180 seconds) is interpreted as due to exchange of calcium from the ionic and dissolved complexes, whereas the second slope with contact times of the order of 3 to 5 minutes represents exchange of ions from colloidal phases.

Under the conditions of this experiment, the curve (Figure 2) indicates a constant rate of removal of inorganic phosphate to the 65% level, but there is very limited exchange of the organic phosphate present if the assumption is made that about one-third of the total phosphorus is present as organically bound phosphorus.

Since the slopes of the equilibration curves are finite, it is indicated that an appreciable time is required to remove calcium or phosphate from the various states in which they exist in milk and that small amounts from these states are being removed, even at the 10-second time interval. However, these times are quite short and indicate that any measurement of the ionic calcium or phosphate of milk must be instantaneous, or must be of such a nature that no disturbance of the equilibria in milk is brought about.

**SUMMARY**

An ion-exchange resin-contact time equilibration procedure has been developed for the study of the inorganic equilibria of cations and anions in raw skim-milk and processed milks. With this method it is possible to study directly changes in the cation-anion components of milk and the various phases involved, as a function of processing conditions. Data are presented to show removals of calcium and phosphorus from raw skimmilk and true solutions at various contact times. Thirty-six per cent of the total calcium was removed from milk during 10 seconds contact. The rate constant for the removal of calcium was found to remain constant to the 90% level, after which the rate constant decreased.

The reproducibility of the resin-contact time method for calcium was considered good. The average standard deviation was found to be ±0.38 mg., and the average error for all contact times studied was ±1.64% of the per cent exchanged.

With a 10-second contact time about 44% of the total phosphorus was ex-
changed. A nearly constant rate of removal occurred as a function of contact
time to the 65-68% removal point. The removal leveled off at this time. These
studies indicate that about one-third of the phosphate in milk is organically
bound and not available as ions.

The slopes of the curves for contact times of 10 to 180 seconds are interpreted
as due to the exchange of calcium from the ionic and dissolved complexes,
whereas the slopes for contact times of 3 to 5 minutes are due to exchange from
colloidal phases.

The resin-contact time method does not have many of the uncertainties in-
herently present in methods such as dialysis, ultrafiltration, centrifugation, and
reunet and other precipitation techniques.

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