Technical Papers

Use of Three-Component Liquid Systems for Countercurrent Distribution

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If two solutes are to be separated effectively by the countercurrent distribution method without the use of a large number of transfers, the following conditions are necessary: (i) the values of the partition ratios for the two solutes must be sufficiently far apart; (ii) the two partition ratios must not both be too much greater than unity, or both be too much smaller than unity (*1*). Many times a solvent pair cannot be found to meet the second of these requirements when the first has been met. Sometimes in such cases a third liquid component that makes the solvent pair more miscible in one another is added to a system to lower (or raise) both partition ratios. This third liquid component brings both partition ratios nearer to unity, and the three-component solvent system may be satisfactory if there is a composition giving two phases for which the values of the partition ratios of the solutes will satisfy the two afore-mentioned conditions.

It should be pointed out, however, that there are factors other than conditions (i) and (ii) that must be considered in choosing a solvent system (*2*). Also, the importance of conditions (i) and (ii) can be reduced by the use of automatic equipment that makes thousands of transfers practical (*1*). It is the purpose of this note to describe and explain a method for finding the composition of a liquid system of three given liquids that will give the best separation of two given solutes at a given temperature (*3*).

Consider two partially miscible liquids A and B and a third liquid C, which when added to a system of A and B increases their miscibility, thus producing a three-component system with a critical composition of complete miscibility (plait point). This three-component system can, in principle, be represented on the conventional triangular diagram showing a two-phase region with tie-lines joining the pairs of compositions that can exist as phases in contact. Points on the same tie-line are not distinguishable for our purpose, since all such points represent systems having the same two phases and differing only in the relative amounts of these two phases. Our problem, therefore, is reduced to finding the proper tie-line. In most cases the triangular phase diagram and, therefore, the tie-lines are not known beforehand.

Consider any variable α that is in a one-to-one correspondence with the tie-lines. The partition ratios K_1 and K_2 of the two solutes are functions of a single variable α, that is, at a constant temperature. There is an infinite number of ways of choosing α, and these are all equally acceptable in principle, but some are more convenient to use in practice. A convenient choice of α is the volume percentage of C under the condition that the volume of the two coexisting phases be equal or in some other constant ratio. A method of using this choice of α is described here.

When C is added to the system of A and B that has its two coexisting phases in equal volumes, C goes preferentially into one of the two phases, thus causing the volume of this phase to become greater than the volume of the other. Suppose the phase that tends to grow the faster is the A-phase. If C and B are added in the proper ratio, the volumes of the two phases remain equal, and the system follows a path that crosses each tie-line once, and only once, until the critical composition is reached. A determination of K_1 and K_2 at various compositions along this path gives graphs of K_1 and K_2 as functions of α, the volume percentage of C. Both partition ratios are unity at the plait point if the effect of the solute on the system is negligible. The best value of α according to some criterions of separation can now be obtained. Several criterions for effectiveness of separation have been proposed (*1, 2, 4*). Suppose a criterion has been chosen, and according to it the separation S has been expressed as a function of K_1 and K_2. A graph of S as a function of α can then be obtained, and if there is a composition that will give the best separation according to the criterion used, then the graph of S as a function of α should have a maximum at the corresponding value of α. The value of S at that value of α is a measure of the best separation.

It should be noted that paths other than the equal-volume path would give the maximum of S at different percentages of C, but all these percentages correspond to points on the same tie-line.

The advantage of this method results from the fact that the separation is expressed as a function of one variable. The number of experiments required to determine the optimum composition and the best separation possible with a given system is thus reduced to a minimum. Preliminary work in this laboratory dealing with the separation of triglycerides indicates that an estimate of the best composition and the best separation can be obtained from the composition at the plait point and the values of the partition ratios at two other compositions.

It should be noted that the method described here gives the best composition of the solvent system when the two phases have equal volumes. If it is desired to use countercurrent distribution with the volumes of the phases in some other ratio r, then it may be more convenient to obtain the best composition by the use of a path along which the ratio of the volumes of the two phases is kept equal to r.

References and Notes

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Isolation of Toxic Crystals from Sweet Peas (*Lathyrus odoratus*)

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Schilling (*1*) has reported the isolation from sweet peas (*Lathyrus odoratus*) of crystals that are active in the production of skeletal changes in rats. Active crystals that appear to be identical to these have been isolated also in this laboratory (*2*). The procedure most recently used in this laboratory is simple and seems to give crystals of good purity.

The first active crystals that I prepared were obtained with the aid of ion-exchange resins. In this isolation, the residue from an alcoholic sweet pea extract was partitioned between ether and water. The aqueous phase was purified by treatment with basic lead acetate followed by passage through the strongly basic anion-exchange Amberlite IRA-400 (hydroxyl form). The toxic factor was then adsorbed on the strongly acidic cation-exchange resin Amberlite IR-105 (H) (*3*), from which it was eluted with 5 percent H_2SO_4. Excess H_2SO_4 was removed with barium hydroxide. After concentration of the solution to a small volume, the addition of alcohol caused the precipitation of an unidentified alkali sulfate, which was removed by filtering the warm solution. Fine, white needles separated on cooling. These sintered and decomposed at about 225°C and consisted of the sulfate of an amine. When they were fed to rats, they gave rise to the typical skeletal changes seen in odoratism (sweet pea lathyrism).

More recently, active crystals have been isolated from sweet peas by means of the procedure used in the following isolation.

Coarsely ground sweet peas (1400 g) were placed in a large Soxhlet extractor and were thoroughly extracted with *n* hexane (Skelly Solve B) to remove lipids. The peas were then dried and extracted continuously with 95 percent ethyl alcohol for 7 hr. The alcoholic extract was allowed to cool and to stand overnight. During this time, dense rosettes of yellowish crystals formed in the extract. These crystals, which represented the impure toxic factor, were purified as follows.

The supernatant solution was decanted, and the crystals were washed several times with ethyl ether to remove small amounts of oily material. The crystals were dissolved in 90 ml of water and were treated with a solution of basic lead acetate until no more precipitation occurred. Following filtration, excess lead was removed with hydrogen sulfide, and the lead sulfide filtrate was evaporated to dryness under reduced pressure. The white, solid residue was dissolved in boiling alcohol with the gradual addition of small amounts of water. On cooling, crystals formed which were filtered with suction, washed with 95 percent alcohol, and dried. The yield was 1.6 g; mp 197°C with decomposition.

Recrystallization from alcohol-water mixtures gave very fine, long, colorless needles melting at 209° to 210°C, uncor. (Fig. 1). The nitrogen content by semimicro-Kjeldahl determination was 21.3 percent (21.25 percent; 21.29 percent) (*4*).

When fed to rats at a level of 0.2 percent of the diet, the crystals were somewhat less effective in producing skeletal changes in the animals than were sweet pea diets containing 25 percent sweet peas.

The crystalline material has the following properties: it is very soluble in water, is insoluble in ether, and has limited solubility in alcohol. It gives a positive ninhydrin reaction. During the isolation studies, it was determined that the toxic factor dialyzes readily through Visking cellulose and that it is not precipi-

Fig. 1. Toxic crystals isolated from sweet peas (×100).