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Albertsson P-Å. *Partition of cell particles and macromolecules.* New York: Wiley, (1960) 1986.
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This book describes the separation and purification of cell particles and biomolecules by phase partition. Aqueous mixtures of two different polymers produce liquid-liquid two-phase systems that allow a mild and selective partitioning of proteins, nucleic acids, membrane vesicles, cell organelles, and even whole cells. [The *SCI*® indicates that the various editions of this book have been cited in over 1,100 publications.]

Separation of Cell Particles and Molecules

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The first edition of this book was my PhD thesis in biochemistry at the University of Uppsala, Sweden. In 1954 I began my graduate studies with Arne Tiselius. My supervisor was Håkan Leyon, an electron microscopist interested in the chloroplast. My first task was to isolate pyrenoids, characteristic structures of chloroplasts from green algae. Besides centrifugation I also used chromatography on columns of hydroxylapatite, which had been introduced by Tiselius for separation of proteins. It was of great methodological interest to see whether these columns could be applied also to cell particles.

My experiments failed, however, due to irreversible adsorption. In a typical chromatographic experiment with hydroxylapatite, a substance is first adsorbed at low concentrations of phosphate buffer and then eluted at higher concentrations. The chloroplast particles were easily adsorbed onto the column but could not be desorbed. The green band on top of the column did not budge upon elution with various buffers. Could it be that the relatively large particles were mechanically trapped between the hydroxylapatite grains of the column? To eliminate this possibility, I switched to batch experiments wherein mixtures of hydroxylapatite and chloroplasts suspensions were shaken and the settling of the green particles was observed. Again the chloroplasts were firmly adsorbed even in the presence of 1-2 molar (M) phosphate.

I decided to use a detergent to facilitate desorption. From a book on detergents, I remembered the name polyethylene glycol (PEG). Since this happened to be in a bottle on the shelf, I tried it. An aqueous solution of PEG was mixed with an hydroxylapatite sediment containing the firmly adsorbed chloroplast particles in about 1 M potassium phosphate. The result was most spectacular. The intense green color of the chloroplasts, earlier so strongly associated with hydroxylapatite, was now present in a liquid layer on top of the phosphate buffer; the hydroxylapatite turned white and was completely purged of chloroplasts. Owing to the high phosphate concentration, a two-phase system was formed and the upper, PEG-rich phase apparently had a stronger affinity for the chloroplasts than did the hydroxylapatite. Later I found that PEG is not a detergent; it is used for the manufacture of certain detergents. Thus, my experiment was a rewarding "mistake." Due to the intense color of the chloroplasts, the phenomenon was very impressive, and this helped me to realize that partitioning might be used for the separation of cell particles.

Since PEG is a polymer and somewhat hydrophobic compared to potassium phosphate buffer, the latter was replaced by another hydrophilic polymer, dextran. In this way an aqueous polymer-polymer two-phase system was obtained. The dextran PEG phase system, which is very mild towards biological material, has since been used for many different separation purposes.[1-3] It has, e.g., been successfully used for purification of plasma membranes[4] and for separation of inside out from right side out thylakoid[5] or plasma membrane[4] vesicles. The method is highly versatile and can be applied both on a small and on a large industrial scale involving several thousands of liters.[6] A Japanese translation of my book was published in 1971; a Russian, in 1974.

However, my original problem as a PhD student in 1954, to isolate pyrenoids, still remains to be solved.

1. **Walter H, Brooks D E & Fisher D,** eds. *Partitioning in aqueous two-phase systems.* Orlando, FL: Academic Press, 1985.
2. **Albertsson P-Å.** Analysis of the domain structure of membranes by fragmentation and separation in aqueous polymer two-phase systems. *Quart. Rev. Biophys.* 21:68-98, 1988.
3. **Johansson G.** Separation of biopolymers by partition. *Separ. Purif. Method.* 17:185-205, 1988.
4. **Larsson C, Widell S & Sommarin M.** Inside-out plant plasma membrane vesicles of high purity obtained by aqueous two-phase partitioning. *FEBS Lett.* 229:289-92, 1988. (Cited 10 times.)
5. **Andersson B, Sundby C, Åkerlund H E & Albertsson P-Å.** Inside-out thylakoid vesicles. An important tool for the characterization of the photosynthetic membrane. *Physiol. Plant.* 65:322-30, 1985. (Cited 20 times.)
6. **Hustedt H, Kroner K H, Menge U & Kula M-R.** Protein recovery using two-phase systems. *Trends Biotech.* 3:139-44, 1985. (Cited 25 times.)