Partitioning in Aqueous Two-Phase Systems

Theory, Methods, Uses, and Applications to Biotechnology

Edited by

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Preface

Thus God made the firmament, and divided the waters which were under the firmament from the waters which were above the firmament

–Genesis

During recent years the applications of aqueous two-phase systems to problems of separation and extraction of macromolecules, organelles, and cells have increased markedly. These systems were originally developed by Albertsson (Chapter 1) for the isolation and separation of plant organelles and viruses, and their use has been extended into most areas of biology. Currently their application in the growing field of biotechnology (Chapter 15) is attracting attention. The purpose of this volume is to provide comprehensive and critical accounts of the many applications of aqueous two-phase systems to biological problems, with the theoretical basis and the practical details of the procedures used.

The separation of the components of a mixture by distribution between two immiscible liquids, either by bulk extraction or by liquid–liquid partition chromatography, is a familiar and long-established technique in chemistry. The use of phase systems containing organic solvents for the partitioning of biological materials (and especially biological particulates) is generally precluded because of such solvents' deleterious effects. However, advantage can be taken of the phase separation that frequently occurs when solutions of two structurally different water-soluble polymers are mixed above critical concentrations. These systems spontaneously separate into two immiscible liquid phases, each phase being enriched with respect to one of the polymers. The phases have low osmotic pressure and high water content. Salts and other solutes can be included to provide the buffering capacity and the tonicity required. The liquid–liquid interface between the phases has a low interfacial tension. These physical properties make polymer phase systems very mild for labile material such as enzymes, cells, and

organelles, which, consequently, can be partitioned with no harmful effects. Aqueous phase systems containing two polymers, most commonly poly(ethylene glycol) (PEG) and dextran (Dx), have found wide application for the separation of most biological materials. Systems containing a single polymer and a high concentration of some particular salt, e.g., PEG and phosphate, have also proved useful in the separation of macromolecules.

Soluble materials added to the phase systems distribute between the bulk phases. Particulates, such as cells or organelles, generally distribute between one of the phases and the bulk interface. By manipulating the composition of the phase system, the partition of macromolecules can be made sensitive to molecular weight, net charge, molecular configuration, hydrophobicity, and the presence of specific binding sites. Similar features on organelle and cell surfaces can be made to dominate the partition behavior of such particulates. Thus aqueous polymer phase systems can be used for the analytical and preparative separation of biological material on the basis of these properties.

In order to separate two components in one or only a few steps, their partitions need to be manipulated in a manner such that one component is in one phase and the other component is in the other phase or at the interface. Successful batch extractions have been developed for plant organelles (Chapter 14), cell membranes (Chapter 13), nucleic acids (Chapter 7), and proteins (Chapter 6). For example, the partitioning of right-side-out and inside-out vesicles (obtained from fragments of thylakoid membranes) to the top and bottom phases, respectively, of a Dx–PEG system provides a simple separation. The potential of batch processes in biotechnology for the large-scale recovery of fermentation products (downstream processing) is being exploited (Chapter 15). A related application in biotechnology, extractive bioconversion, uses partitioning to extract a protein product from the phase in which it is produced by enzymes, cells, or organelles immobilized in that phase by their partition. The products can thus be removed continuously.

For materials that do not differ greatly in their partitioning behavior, single extraction steps are not sufficient to produce a separation. In such cases multiple extraction procedures such as countercurrent distribution (CCD) are required. The most widely used apparatus for CCD with aqueous two-polymer phase systems performs a discrete number of partition steps with thin layers of phase. Continuous extraction methods (e.g., using columns) are also being developed. The theory of CCD and the design and use of the thin-layer CCD apparatus are described in Chapter 4. The design of other types of apparatus for separations with aqueous phases is described in Chapter 5.

The various applications of CCD for the separation and fractionation of biological materials are described in Chapter 6 (proteins), Chapter 7 (nucleic acids), Chapter 10 (red blood cells), Chapter 11 (mammalian cells), Chapter 12 (viruses, phage, and bacteria), Chapter 13 (animal cell organelles), and Chapter 14 (plant cells and organelles). An indication of the sensitivity of the CCD method can be obtained from its ability to resolve a tumor cell population into cell subpopulations having different metastatic potentials (Chapter 11). Surface changes during the early stages of development of the slime mold *Dictyostelium discoideum* have been detected by CCD and the ameboid cells resolved into presumptive spore cells and presumptive stalk cells (Chapter 14).

Success with phase systems depends on the ability to manipulate phase composition so as to obtain appropriate partition coefficients for the materials of interest. There are three major ways in which systems can be manipulated so as to give phases with appreciably different physical properties: (1) choice of polymers (although these have, thus far, been predominantly Dx and PEG), polymer concentration, polymer molecular weight; (2) choice of salt(s) and salt concentration; and (3) chemical modification of one of the polymers by attaching a ligand for which receptors exist on the material of interest. In the last case the resulting procedure is called affinity partitioning. The experimental experience with these manipulations is discussed in each of the chapters concerned with the specific materials. General theoretical aspects are presented in Chapter 2, which also describes the properties of the polymers, phase systems, and nature of phase separation.

The partitioning of macromolecules between the two bulk phases and the partitioning of particulates between one bulk phase and the interface have both been treated theoretically (Chapter 2). Some general points are readily made. An increase in polymer concentration is associated with an increase in the dissimilarity in composition between the phases and an increase in the interfacial tension. Molecular partitioning tends to become more one-sided and, with particulates, adsorption at the interface increases. The effect of ions on partition usually arises when ions have different affinities for the two phases, giving rise to an electrostatic potential difference ($\Delta\psi$) between the phases. The $\Delta\psi$ influences the partitioning behavior of charged molecules and particulates. Such phases are "charge-sensitive." Phase systems which contain salts that do not yield an electrostatic potential between the phases are "non-charge-sensitive." Affinity partitioning provides very specific extractions of proteins, nucleic acids, membranes, and organelles, particularly when biospecific ligands are used. The base composition-dependent shifts in partition coefficients of nucleic acids that have been

obtained with base sequence-specific ligands coupled to PEG (Chapter 7) and the isolation of specific receptor-rich membrane fragments with biospecific polymer–ligands (Chapter 13) indicate the power of affinity partitioning. When the ligand coupled to the polymer interacts hydrophobically with the material, hydrophobic affinity partitioning is obtained. The esters of poly(ethylene glycol) and fatty acids have been widely used for such separations. The theory of affinity partitioning for molecules and particulates is given in Chapter 2. A description of a wide variety of polymer–ligands used in affinity partitioning as well as their synthesis is presented in Chapter 16.

The analytical applications of phase systems are diverse, and numerous examples appear in the chapters that describe the partitioning of specific materials. The general procedure is to select a phase system in which the partition is determined predominantly by one distinct property of the material being examined, e.g., charge, capacity for hydrophobic association, or interaction with a specific ligand. By measuring the partition coefficient of the material an index of these properties can be obtained rapidly and simply. CCD is often used to increase the sensitivity of analytical partitioning. The hydrophobic and charge properties, including isoelectric point, of proteins can be readily examined (Chapter 6), as can conformational changes (Chapters 8 and 9). Partitioning of nucleic acids on the basis of molecular weight and base sequence has been reported (Chapter 7). With particulates, analytical partitioning can be used to determine the isoelectric point of organelles (Chapters 13 and 14). The physicochemical surface properties (charge and hydrophobicity) of bacteria have been extensively examined by partitioning and correlations found between their partition coefficient and their tendency to adhere to other cells or to be phagocytosed (Chapter 12). Partitioning of cells in charge-sensitive phases often correlates with the cells' electrophoretic mobility (Chapter 10).

Partitioning can also be used to sensitively probe the interactions of materials such as enzyme–substrate interactions, protein–protein associations, hormone–receptor binding, and cell–cell affinities. The theory and practice of using aqueous phase systems in such studies are described in Chapters 8 and 9.

Since partitioning critically depends on the nature, concentration, and molecular weights of the polymers and on the ionic composition and concentration, the preparation of phase systems requires particular care if reproducible results are to be obtained. Chapter 3 provides practical guidelines for the preparation of phase systems and general procedures for phase system selection. Specific methods for a particular material should be sought in the appropriate chapter. Variation in the

partitioning behavior of materials as a consequence of differences in the characteristics of polymers from different lots or suppliers is a common experience. This can generally be rectified by slight, systematic modification of phase system composition (Chapter 3).

The field of aqueous phase partitioning is rapidly expanding as the comprehensive partitioning bibliography (Chapter 17) demonstrates. Currently about 70 papers appear each year, and the field comprises (as of late 1984) over 750 publications. These range over subjects as diverse as surface science, separation processes, protein purification, biotechnology, plant biochemistry, bacterial adhesion, cell differentiation and development, immunology, synthetic organic chemistry, and many others. To provide authoritative accounts of the many, diverse, and highly useful applications of partitioning in aqueous phase systems we have invited investigators who have used partitioning in various fields to critically review the areas of their expertise. Our hope is that the results obtained by use of aqueous phase systems, some of which cannot be attained by any other currently available method, will encourage others to try this uniquely versatile method.

Harry Walter
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Abbreviations and Conventions

The abbreviations used by each author are defined in the text of each chapter. However, the following short list contains some of the more common symbols as well as a few conventions used by all authors.

BSA	Bovine serum albumin
C_p	Cross-point: the pH at which two plots of partition coefficient versus pH for a material intersect, the partition usually being measured in phase systems containing different salts but the same polymer concentrations
CCD	Countercurrent distribution
CM	Carboxymethyl
Dx	Dextran
Dx-sulfate	Dextran sulfate: format for describing Dx derivatives of all kinds
Dx 500	Dextran of weight-average molecular weight approximately equal to 500,000; usually the Dx T500 fraction from Pharmacia; format for designation of nominal molecular weights of Dx fractions
FBS	Fetal bovine serum
Fi	Ficoll
G	Apparent partition coefficient derived from the location of a peak in a CCD curve. $G = r_{max}/(n - r_{max})$ where r_{max} is the cavity number of the peak of the distribution and n is the total number of transfers carried out
I_p	Isoelectric point
K	Partition coefficient; $K = c^T/c^B$ for soluble material, where c^T (or c_T) is the concentration in the top phase and c^B (or c_B) is the concentration in the bottom phase. For particulates and cells, K is given by the ratio of the number of particles in one of the bulk phases, typically the top phase in a Dx–PEG system, and the interface
K_0	K at cross-point
KPB	Potassium phosphate buffer, concentrations and pH to be defined
M_r	Relative molecular weight determined from a calibration curve generated from the behavior of a set of standards of known molecular weights

NaPB	Sodium phosphate buffer, concentrations and pH to be defined
P	Percentage of total particles partitioning into one bulk phase
$[P]$	Polymer concentration
PBS	Phosphate-buffered saline, phosphate concentration and pH to be defined
PEG	Poly(ethylene glycol)
PEG 8000	PEG of average molecular weight 8000; the fraction initially designated PEG 6000 by Union Carbide is now designated PEG 8000
PEG-palmitate	Palmitic acid ester of PEG; general format for describing PEG derivatives
PEO	Poly(ethylene oxide); polymer of the same chemical structure as PEG, but the designation is retained for molecular weight fractions greater than 20,000
TEA	Triethylamino-
TLL	Tie-line length on a phase diagram
TMA	Trimethylamino-
$\Delta\psi$	Electrostatic potential difference between the bulk phases
5 : 4 or 5/4	Designation of the polymer composition of a phase system; the first number represents % w/w of Dx, the second % w/w of PEG, followed by the total salt composition. Polymer concentrations are implied to be % w/w unless noted otherwise

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