Rapid Papers

Modified Methods of Dialysis

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Dialysis modifications are described that are based on the use of a novel rotating dialysis bottle in one- or two-solvent systems. Advantages include complete mixing and rapid equilibrium of diffusing substances, favourable volume changes, economy of solvent and more effective removal of diffusible materials.

Dialysis is an invaluable biochemical technique for the separation of diffusible from non-diffusible substances, but it may have certain drawbacks: (1) the time required to establish equilibrium between diffusate and dialysis residue; (2) inadequate mixing of diffusing substances that results in uneven concentrations and layering; (3) increase in volume of the dialysis residue, often to the point of bursting the dialysis membrane and increasing the load of material for further processing. Many attempts have been made to overcome these shortcomings (Laufer, 1942; Gilding & Webb, 1943; Reiner & Fenichel, 1948; Renkin, 1954; Stegemann & Toennies, 1956; Ogston, 1960; Bitter & Ewins, 1963). Generally the methods described have been cumbersome, incompletely effective and/or have involved vigorous agitation of the fluids. The last is particularly undesirable when working with solutions of certain proteins, e.g. albumins.

A simple rotation system, 'rotadialysis', has been devised which is both effective and gentle (i.e. does not require turbulent agitation). This, in turn, has led to further modifications with particular advantages: simultaneous extraction and dialysis; use of two chemically dissimilar solvents on either side of the membrane ('heterodialysis'). In the latter, the undesirable layering of solvents of disparate densities is avoided by combination with rotadialysis. Heterodialysis has been advantageously used in the preparation of solutions containing solvent mixtures, and in organic solvent fractionation of non-diffusible substances from aqueous solution.

Methods and Results

In the following investigations I used 24/32 Visking dialysis tubing obtained from the Scientific Instrument Centre, London W.C.1, U.K. Initial volume of the fluid in the tube was 100 ml, and the surface area of the tube was approx. 335 cm².

Rotadialysis

Morris & Morris (1964) state that the rate of escape of diffusible constituents in a dialysis system is increased by a factor of 4–5 by efficient stirring on

both sides of the membrane. In the present system this is accomplished without agitation by using a Rodial dialysis bottle (Luckham Ltd., Burgess Hill, Sussex, U.K.). The dialysis tube is suspended freely in the dialysis fluid in the Rodial bottle, which is rotated at approx. 14 rev./min on a slow-speed rolling machine (Rolamix RS/54; Luckham Ltd.). The principal feature of the bottle is a wall invagination that, in the course of rotation, picks up the tube and gently drops it back into the dialysis fluid. This keeps the fluid on both sides of the membrane constantly stirred, hastening the dialysis process and preventing layering of fluids passing through the membrane.

In a representative experiment an aqueous solution of 0.9% NaCl was heterodialysed against an equal volume of propan-2-ol. Analyses were carried out at intervals on the NaCl and propan-2-ol distribution, the latter being determined by specific gravity. In each case equilibrium was established within 4 h (Table 1). Comparable results were obtained with NaBr and NaI.

Heterodialysis

Consideration of the relative molecular size of commonly used solvents discloses the fact that the water molecule, despite some hydrogen-bonding, is the smallest. On this basis it was predicted that dialysis of an aqueous solution against another, miscible, solvent would result in the more rapid diffusion of the water through the membrane, thus decreasing the volume of fluid in the dialysis residue. This would considerably facilitate the further processing of the residue, e.g. filtration, centrifugation, and also avoid the danger of burst membranes. Concentration of the non-diffusible and enhanced removal of the diffusible solutes would be achieved simultaneously. Also, when the second solvent is a volatile compound, it can, if required, be removed by evaporation.

On investigation, it was determined that when 0.9% NaCl is heterodialysed against an equal volume of a sampling of organic solvents, the decrease in

Table 1. *Heterodialysis distribution of solute and solvent between diffusate and dialysis residue as a function of the time of rotation (14 rev./min)*

Time (h)	NaCl ($\mu\text{mol/ml}$)		Propan-2-ol (% v/v)	
	Diffusate	Dialysis residue	Diffusate	Dialysis residue
0	154.0	0	0	100
$\frac{1}{2}$	135.5	18.5	13	87
1	104.7	49.3	25	75
2	61.6	92.4	43	57
4	72.0	72.0	50	50

dialysis-residue volume at equilibrium is approximately as follows: 25% when dialysed against ethanol; 75% when dialysed against propan-2-ol; 80% when dialysed against 2-methylpropan-2-ol. Even with highly concentrated aqueous solutions a decrease in dialysis-residue volume is obtained: 1 vol. of 20% (w/v) sucrose heterodialysed against 1 vol. of propan-1-ol gave an 80% decrease, and 1 vol. of 40% (w/v) sucrose dialysed against 1 vol. of propan-2-ol gave a 40% decrease. Water is also lost from a saturated $(\text{NH}_4)_2\text{SO}_4$ solution when it is heterodialysed against propan-2-ol.

By proper choice of solvents, similar favourable volume change can be achieved in the replacement of a non-volatile by a volatile solvent. For example, at solvent equilibrium, the following decreases were observed: 1 vol. of propane-1,2-diol dialysed against 1 vol. of 2-methylpropan-2-ol gave a 20% decrease, and 1 vol. of ethane-1,2-diol dialysed against 1 vol. of 2-methylpropan-2-ol gave a 75% decrease.

Applications

Simultaneous extraction and dialysis

For processes in which extraction of macromolecular substances from solid materials must be followed by dialysis to remove low-molecular-weight contaminants, the two procedures can be carried out simultaneously by use of rotodialysis. The suspension of solid in extraction medium is placed in the dialysis tube and rotodialysed against the medium. Large molecules extracted in the process are retained in the dialysis residue, whereas small molecules are immediately partitioned between the dialysis residue and diffusate. The latter can be renewed as required to remove the bulk of diffusible contaminants even as extraction proceeds. Concurrently, the solid is kept in constant suspension, considerably increasing the efficiency of the extraction procedure. In one specific application the process was used in the preparation of potent grass-pollen extracts virtually free from diffusible contaminants.

Solvent mixtures

In certain cases one solvent may be added to a solution containing a different solvent to obtain some particular effect, e.g. the addition of an equal volume of glycerol to aqueous allergen extracts to produce a more stable extract for clinical use. Often the initial extract is weak, and the addition of an equal quantity of glycerol results in an undesirable halving of the allergen concentration. A study was carried out on the volume change obtained when a 0.9%-NaCl extract was heterodialysed against an equal volume of glycerol. It was found that at solvent equilibrium, the volume of 50%-(v/v)-glycerol-treated allergen extract was only one-fourth that of the starting aqueous extract, i.e. a fourfold increase in allergen concentration had been effected.

Organic solvent fractionation of non-diffusible substances from aqueous solution

This much-used procedure ordinarily results in large increases in the volume of materials to be further processed. If, for example, the recovery of a non-diffusible solute from 100ml of aqueous solution is to be accomplished by precipitation with 90% (v/v) propan-2-ol, direct addition of the latter requires 900ml of propan-2-ol.

A study was carried out to determine the optimum method for producing a 90% propan-2-ol concentration by heterodialysis. It was found that this could best be done by a two-step procedure, carrying out the dialysis in a Rodial bottle: (a) 1 vol. of aqueous solution heterodialysed against 1 vol. of propan-2-ol to yield 50% (v/v) propan-2-ol concentration; (b) 1 vol. of dialysis residue from (a) heterodialysed against 4 vol. of propan-2-ol to yield 90% (v/v) propan-2-ol concentration.

In actual practice the following results were obtained with this procedure: (a) 100ml of 0.9% NaCl solution heterodialysed against 100ml of propan-2-ol produced 22.5ml of dialysis residue; (b) 22.5ml of dialysis residue heterodialysed against 90ml of propan-2-ol produced 10ml of dialysis residue.

Whereas direct addition would have required 900ml of propan-2-ol and a final total volume of 1 litre for further processing, the two-step heterodialysis procedure used only 190ml of propan-2-ol and left only 10ml for further processing. In effect, not only had there been an 80% economy in the volume of propan-2-ol used, there had also been a 99% decrease in the volume of dialysis residue to be centrifuged or filtered.

In another experiment, to check protein recovery, 100ml of a 2% (w/v) ovalbumin solution was heterodialysed against two changes of ethanol to produce a final concentration of 90% ethanol. The final

dialysis-residue volume, including the precipitated ovalbumin, was 60ml, rather than the 1000ml which would have been obtained by direct addition of the ethanol. The precipitated protein recovered from the dialysis residue was over 98%.

Discussion

The modifications described in the present paper should increase the efficacy of dialysis and, additionally, extend its use into new areas. Avoidance of layering between aqueous solutions and organic solvents precludes denaturation of protein by contact with high concentrations of the latter. This makes feasible the introduction, by dialysis, of organic solvents into aqueous solutions, with the

several advantages described. These newer methods should find useful application both in the research and the preparatory laboratory.

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