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Continuous Dialysis of Protein Solutions on a Large Scale. I. The Influence of Various Factors on the Efficiency of Dialysis

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Summary

Artificial kidneys have been found useful for dialysis of protein solutions in biochemical preparative work. Manufacturer's data for their use in clinics as artificial kidneys could not be adopted directly in the new application. Therefore investigations on the influence of various flow rates on the efficiency of dialysis have been made. In most investigations 1 *M* salt solutions have been dialyzed against deionized water, and the results are illustrated in three diagrams. A 1 *M* salt solution with a flow rate of 25 ml/min can be reduced to 0.1 *M*, which means that this procedure should be of potential value in preparative work for desalting proteins.

Dialysis remains one of the most common procedures for the coarse separation of low molecular weight compounds from high molecular weight material. Volumes of solution up to some hundred milliliters are conveniently handled in dialysis bags. The bigger the volume, the less is the ratio of the membrane area to the volume. This in turn means a lower degree of solute exchange in a certain time. Provided that the solutions on both sides of the membrane are well stirred, the time necessary for the concentration equilibrium of a solute which was present on one side at the start is directly proportional to the surface area of the membrane (*1*). The general equation describing diffusion can be written as:

$$dS = -Dq\left(\frac{dc}{dx}\right)_t dt \tag{1}$$

Here dS is the amount of transported substance, with a diffusion coefficient D, which passes in the time dt across an area q, where the concentration gradient is dc/dx. This equation can be modified to:

$$\frac{dS}{dt} = kA\frac{(C_0 - C_i)}{\Delta x} \tag{2}$$

where A is the area of the dialysis membrane, C_0 and C_i are the concentrations of the solute on either side of the membrane, Δx is the membrane thickness, and k is the permeability constant characteristic of the membrane for a given solute. The transport of a salt through a membrane can thus merely be described as a diffusion process. It is therefore important to achieve a low Δx value by using thin membranes, and also to employ efficient stirring of the solutions in contact with the membrane (*1*). If there is poor circulation stationary liquid layers with solute concentrations different from that of the circulating liquid tend to develop close to the membrane, and this results in less efficient dialysis. A satisfactory way to obtain efficient dialysis is to pump the solutions between membranes as is done in most artificial kidneys. These instruments have a comparatively large surface area per unit volume. Developments in this field is very rapid, and some types, now commercially available at a moderate price, allow for disposal after one clinical use. These instruments can be very useful in preparative work for dialysis and desalination in biochemistry and microbiology. Data are available on the properties of artificial kidneys for the continuous dialysis of blood (*2*).* The new fields of application make other data on flow rates and dialysis capacity desirable.

* After this paper was submitted for publication, an investigation on the clinical evaluation of the efficiency of the Gambro and Kiil artificial kidneys was published [A. E. Kulatilake, J. Vickers, and R. Shackman, *Brit. Med. J.*, **3**, 447 (1969)]. Recently a monograph comparing various hemodialyzers appeared (J. Holtzenbein, *Die künstliche Niere,* Ferdinand Enke Verlag, Stuttgart, 1969). However, data outside the field of hemodialysis are still very few. The most common dialyzers in clinical practice are the Kiil dialyzer (A. S. Nycotron, Drammen, Norway) and the Twin-coil dialyzers. The Gambro and the Kiil-dialyzers are about equally effective. There are several types based on the Twin-coil principle, e.g., the Ultra-Flo 145 (Travenol Laboratories, Ltd., Thetford, Norfolk, England). For comparison of the Kiil and a Twin-coil kidney, see Ref. *3*.

MATERIALS AND METHODS

Artificial kidneys (AB Gambro, Lund, Sweden) with Cuprophane membranes (Type 300 PT, 30 g/m^2, Bemberg A. G., Wuppertal, Germany) with an efficient surface area of about 1 m^2 were used. This type of membrane has a higher dialyzing capacity per unit area than the material usually used in dialysis tubings, e.g. Visking Cellulose (Union Carbide, New York) (*3*). The Cuprophane membranes also seem to be less permeable for small proteins (*4, 5*). Pumping of the solutions was done with a peristaltic pump (Sigma Co., Middleport, New York) using silicone rubber tubes. The wash water and salt solutions were pumped counter current in the dialyzer. Dialysis was performed at 20°C. The wash water was deionized in all experiments. The salt concentration was estimated from conductivity measurements (LKB Conductolyzer, LKB-Produkter AB, S-161 25 Bromma 1, Sweden) using a standard curve obtained from measurements on sodium chloride solutions of different molarities.

The Influence of Various Flow Rates on the Efficiency of Dialysis

Because many parameters influence dialysis, it was found necessary to study the effects of different flow rates of the solution to be dialyzed (a, ml/min) as well as variations of the flow rate of the washing solution (b, ml/min). In every experiment the amount of solute removed from the salt solution† is equal to the amount found in the washing solution. If c_1 is the concentration (mole/l) of the solute in the solution to be dialyzed, and c_2 is the remaining concentration (mole/l) in the retentate, and c_3 is the concentration (mole/l) of solute in the diffusate, then:

$$a(c_1 - c_2) = bc_3 \tag{3}$$

However, if the solution to be dialyzed contains a lot of solute, there will be an increase in volume during dialysis because of osmosis, provided no external pressure is applied. Because of this the input flow rate (a_1, ml/min) will have a lower value than the output flow rate (a_2, ml/min). If the salt concentration is about 1 M in the input, then a_1 will have a value about 20% lower than a_2. Of course, there will be a corresponding change in the flow rate of the wash solution,

† When the salt solution has passed the dialyzer, it can be called "retentate" as has been proposed by Craig (*6*). After the washing water has passed the dialyzer, this solution can be called the "diffusate" (*6*).

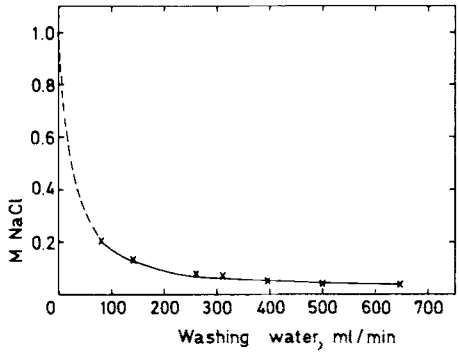

FIG. 1. Concentration of NaCl in the retentate at different flow rates of wash water (ml/min). The flow rate of the salt solution was 25 ml/min in these experiments.

but in many cases this can be neglected if b is several times larger than a. We then arrive at the equation:

$$a_1c_1 - a_2c_2 = bc_3 \tag{4}$$

Let us now see how variation of the flow rate of the wash solution influences the salt concentration in the retentate if the flow rate of this solution is kept constant (Fig. 1). A 1-M salt solution with $a_1 = 25$ ml/min can be reduced to 1/10 M when a flow rate of 150 ml/min of the wash water is used; $b = 6a_2$. (If there is no counter current effect, then b must have a value at least 9 times a_2. This figure is obtained by assuming that the salt removed is diluted in the wash water to a concentration close to that in the retentate leaving the dialyzer). As can be understood from Fig. 1, a further increase in the flow rate of the wash solution will only slightly lower the salt concentration in the retentate. This reduction in salt concentration is then obtained at the expense of high water consumption. To minimize this, it is better to decrease the flow rate of the retentate, for this will permit a corresponding reduction of the amount of the washing water needed.

When salt solutions more concentrated than 1 M are to be dialyzed, a reduction of the salt concentration to 1/10 of that in the input can still be obtained if the value of the salt solution flow rate of 25 ml/min and of the wash water is multiplied by a factor equal to the molarity. This is valid at least up to 3 M solutions of sodium chloride.

In order to obtain an efficient dialysis it is understandable that, when increasing the flow rate of the solution to be dialyzed, it is also

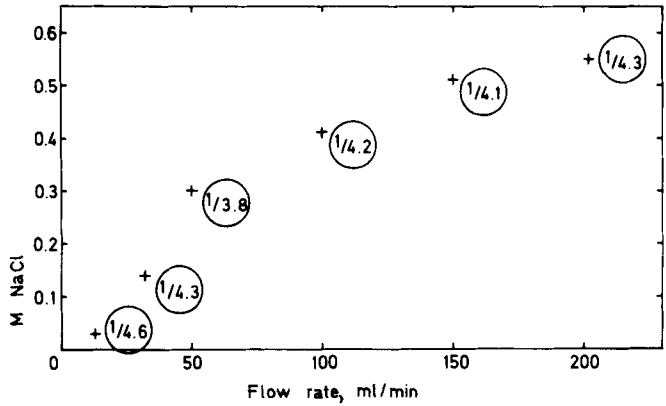

FIG. 2. Concentration of NaCl in the retentate at different flow rates of retentate and wash water (ml/min). The ratio of the flow rate of retentate to that of the wash water in each experiment is shown.

necessary to increase the flow rate of the washing solution by a certain factor. Sometimes it may be desirable to be economical with the wash water. Many experiments have been performed with a ratio of a_2 to b of about 1/4 (see Fig. 2). Figure 2 shows that the higher the flow rate of the retentate, the higher is its salt concentration. On the other hand, the total amount of salt removed per minute during dialysis increases with the flow rate (Fig. 3). At still higher flow rates than

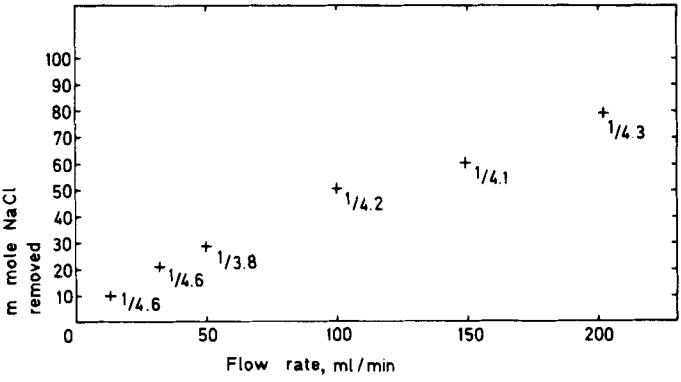

FIG. 3. The amount of salt removed from the salt solution per minute at different flow rates of retentate and wash water (ml/min). The ratio of the flow rate of retentate to that of the wash water in each experiment is shown.

shown in Fig. 3, one can expect that the amount of salt removed per minute asymptotically approaches a constant value. Of course, the flow rate to be selected in each application must be determined by the efficiency required of the dialysis. An 0.15 *M* salt solution can be reduced to 0.045 *M* at a flow rate of 200 ml/min by pumping wash water at 1500 ml/min (calculated from the data given in Ref. *2*). Although the salt concentration is only reduced to about 1/3 of the initial concentration, such a dialysis may be very valuable because a salt concentration of this order permits adsorption on ion exchanges of many proteins in biological fluids, e.g., in plasma, urine, and milk.

The dialysis characteristics of low molecular weight substances other than sodium chloride will be roughly proportional to their diffusion coefficients up to a limit where the pore sizes of the membranes cause a sterical hindrance of the molecules.

We believe that artificial kidneys can find application in preparative work on proteins and other macromolecules by decreasing salt concentration, and this is important because it permits the use of other methods, e.g., ion exchange chromatography, electrophoresis, and isoelectric focusing. It is often necessary or advantageous to have a low salt concentration or to exchange the salt ions for some volatile buffer for lyophilization of proteins. This may also be accomplished in an artificial kidney.

The use of an artificial kidney to facilitate the concentration and purification of enzymes and toxins from bacterial cultures is described in another paper (*5*). The use of such an apparatus for the concentration of protein solutions by ultrafiltration will be described in a forthcoming paper.

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