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ULTRAFILTRATION IN THE PIGMENT FIELD

A CRITICAL LITERATURE SURVEY

by

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INTRODUCTION

The objective in this work has been to determine, through a critical literature survey, the possible application of ultrafiltration, and other related techniques, towards the solution of certain problems encountered in the research and testing of various pigments.

Among these problems may be mentioned the following:

1. The determination of water-soluble salts in pigments, which when dispersed in water to leach out the salts, tend to peptize, thus being unfilterable through the usual filter paper. Among these pigments are the following: iron blue, certain chrome yellows, pigment green B, copper maroon, and several others. It may be added that these pigments do not give a clear supernatant liquid when subjected to ordinary centrifugation.

2. The determination of "bleed" tests in water, alcohol, and other solvents on the above mentioned pigments, as well as several others. In the normal "bleed" test on pigments, the pigment is shaken in the solvent and then filtered through ordinary filter paper. The filtrate is compared with that of a standard to determine the intensity of the "bleed". If the pigment tends to peptize at all, the filtrate will contain some of the pigment, giving an erroneous test.

3. The determination of particle size of pigments. Among the various methods of determining particle size may be mentioned the following: ultracentrifugation, ultra-

microscopy, light microscopy, electron microscopy, light scattering, x-ray scattering, nitrogen adsorption, and several others. All of these techniques are either very tedious or require the use of expensive equipment. In addition, most give only average values for particle size.

4. The determination of "over-size" particles. In many applications of pigments it is undesirable to have present, particles or aggregates larger than a certain size class. Such over-size particles may present such problemsn as poor texture in printing inks and enamels, poor tinting strength, and less transparency in inks and metallic finishes.

5. The separation of pigment from a vehicle, such as in a paint, has always been a problem for the analyst. Other techniques rarely effect a complete separation of the pigment from the vehicle or of the vehicle from the pigment.

6. The separation of one pigment from another in a mixture. Many pigments are sold as mixtures, either as a blend of two or more related colors or of a color extended with a white. A notable example would be a "chrome" green, which is a mixture of a chrome yellow and an iron blue. Ultrafiltration may be applicable in cases where the different components of a mixture are of widely different particle sizes.

In order to fully evaluate the possibilities in applying ultrafiltration to these problems, the following aspects of the technique are covered: terminology, historical

background, preparation of the various types of ultrafilters, mechanisms involved in ultrafiltration, and applications in various fields. Only those applications and techniques used in other fields, which may possibly apply to the pigment field, are included, and it is not intended that this should be considered as a comprehensive survey of the entire field of ultrafiltration.

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I. Terminology

An ultrafilter may be defined (39) as a filter whose pores or interstices are of colloidal or molecular dimensions. Filtration through it, with the object of partial or complete retention of the colloidal or molecular species present in the system filtered, is termed ultrafiltration.

For a comparison of ultrafilters with other filtering media, the following table shows the approximate applicable ranges (85).

	microns
Diameter of pores of hardened filter paper	1.5 - 2.2
Diameter of pores of Chamberland filter	0.[illegible] - 0.4
Lower limit of ultrafilters	0.001
Diameter of bacteria	0.5 - 1.2
Diameter of pigment particles (approx. range)	3 - 0.005

It is desirable, at this point, to distinguish between ultrafiltration and other closely related techniques, such as, diffusion, dialysis, osmosis, and electroultrafiltration. This distinction has been illustrated by the following familiar model (39). A vessel is divided by a membrane into two compartments, of which one is occupied by an aqueous solution, molecular or colloidal, and the other by pure water. Owing to the concentration gradient across the membrane the water will tend to diffuse into the solution, and the solutes into the water compartment. If the membrane pores are large compared with the diameters of all solute particles

present, so that no specific steric hindrance is offered to the latter, both processes take place at relative rates the same as in free diffusion. The only effect of the presence of the membrane is a reduction of the area through which diffusion can occur, and, in case the pores do not run perpendicular to the membrane surface, a prolongation of the path traversed by the diffusing molecules. If, however, the pore sizes are of the same order of magnitude as the solute particle sizes, the solute encounters a preferential resistance; this may include a simple viscous drag as represented by the Ladenburg correction to Stoke's law, specific molecular interaction, or electroviscous effects. Such diffusion may be termed impeded. Dialysis is a differential diffusion, employing a membrane impermeable to the colloidal solutes but permeable to the crystalloidal. The latter diffuse into the water, while the water diffuses into the solution. In dialysis under pressure, the solution is under sufficient pressure so that the hydrodynamical flow of water out of the solution balances the molecular diffusion into it, and the concentration of the colloidal solutes remains unchanged while the diffusible solutes escape. If the membrane is impermeable to all solutes, so that the only diffusion occurring is that of water into the solution, the process is ordinary osmosis. At osmotic equilibrium, the solution is under a hydrostatic pressure (the osmotic pressure) which causes sufficient flow out of the solution to balance the molecular diffusion of water into

it. Now, if the hydrostatic pressure is increased beyond the osmotic pressure, there is a net flow of water out of the solution compartment, with a concentrating of the solute, and we have ultrafiltration. Similarly, in dialysis under pressure, if the pressure is increased so that both water and diffusible solutes flow out of the solution, the process becomes one of ultrafiltration. Under actual conditions of ultrafiltration, the hydrodynamical flow through the membrane due to the applied pressure is usually so much greater than any diffusion effects that the latter may be considered negligible. In electroultrafiltration, the removal of electrolytes is hastened by the use of electrodes under the filtration medium, thus effecting a more complete and efficient removal of electrolytes from disperse systems.

It is also desirable to distinguish between porosity and permeability (39). The term "permeability" has been frequently used to characterize ultrafilters, as a measure of the size of the pores or interstices in the filter structure. The terminology suggested for general use designates "porosity" to describe the filter structure, and "permeability" for reference to its behavior in diffusion or filtration of a disperse system. For example, a membrane of given porosity will show varying degrees of permeability to a certain protein depending on whether the experiment is one of diffusion or ultrafiltration, and, in the latter case, on a host of physical factors including the pH, the rate of filtration, and the concentration of the solution.

II. Historical Background

The study of ultrafiltration has invariably been associated with that of dialysis, osmosis, and the problem of the semi-permeable membrane.

Experiments in dialysis through artificial membranes of collodion were recorded as early as 1855 (40). The first mention of the process now known as ultrafiltration appears to have been by Schmidt in 1856 (77), who found that, when a solution of protein or gum arabic was filtered through an animal membrane, the filtrate was less concentrated than the original solution. In 1860, Schumacher (79) described the collodion sac for dialysis, and Sanarelli (76) introduced it in 1891 for bacteriological work, including ultrafiltration of blood plasma *in vivo*. Martin (63) in 1896, used a bacteriological candle impregnated with gelatin or silicic acid as an ultrafilter to separate colloids from crystalloids. In 1905, Levy (54) ultrafiltered enzymes and showed that dialysis and ultrafiltration did not arrive at the same result.

The classic work of Bechhold (9), who coined the term "ultrafiltration" in 1906, represents the first systematic study of this subject. By impregnating filter paper with acetic acid collodion, he prepared the first series of membranes of graded porosities; he was the first to estimate critically the pore sizes in his filters, and first pointed out the role of adsorption and other physical factors in the filtration process. In the next twenty years or so, numerous

workers experimented in ultrafiltration and introduced various types of ultrafilters and methods for grading the porosity. Among these may be mentioned Bigelow and Gemberling (14) who prepared flat collodion membranes from ether-alcohol solution, and Zsigmondy and Bachman (96) who patented a graded series of membranes to be manufactured by a similar process. Meanwhile, ultrafiltration technique had been adopted by bacteriologists and physiologists, who employed it in attempts to estimate the particle size of enzymes, toxins, and viruses, and to construct models of vital processes involving membranes.

Ultrafilter membranes were regarded by some early authors as mechanical sieves, so that permeability was a function only of particle dimensions and pore dimensions. The opposite extreme in explaining semipermeability was reached by the capillary attraction theory (13,83,89) which represents the solvent as strongly adsorbed in the pore and transmitted by surface mobility of the adsorbed molecules; and by the theory of partial solubility (55), which represents the solvent as dissolving into the membrane on one side and out on the other. These theories would predict specific effects dependent on the nature of solute and solvent, whereas other workers (28) demonstrated the sieve-like behavior of membranes in the flow of various liquids through them, and it was also shown (23) that the rate of impeded diffusion of crystalloidal molecules through membranes depended principally on the molecular volume and not on the nature of

the diffusing solute. On the whole, there is adequate support for the viewpoint that the fundamental mechanism in ultrafiltration is sieving, modified by adsorption, blocking, and other effects arising from the very large ratio of pore length to pore width and of pore surface to cross-section area in all ultrafilters (39).

The most significant recent developments in ultrafiltration have been the extensive study by Manegold and collaborators (59) of the structure of collodion membranes, and the introduction by Elford (32) of the most satisfactory graded series of collodion membranes yet developed which were successfully used to estimate the sizes of particles in a number of disperse systems.

III. Present Problems of Ultrafiltration

The application of ultrafiltration to chemical and biological problems are twofold: fractionation and study of the composition of disperse systems, and estimation of the particle sizes in disperse systems.

The simplest example of the first type of problem is the preparation of a colloid-free ultrafiltrate from a sol. This is of value in the study of lyophobic colloids (68) and lyophilic colloids (64) alike, and especially in biological investigations, where much attention, has been given to protein-free ultrafiltrates of blood serum and plasma. This type would be of interest to the pigment analyst. Ultrafiltration also permits obtaining the disperse phase in

solid form, if desired. An example of the removal of particles of a different order of magnitude is the sterilization of bacteriological systems by ultrafiltration, whose methods are often superior to those involving the use of porcelain candle filters (39). Water and solutions of inorganic and organic crystalloids may be ultrafiltered to remove stray foreign particles, yielding optically clear filtrates (94). A lessimple example of fractionation is the separation of colloidal particles of different sizes. Thus, in a suspension containing bacteria, bacteriophage, and products of bacteriolysis, suitable filters have served for quantitative separation of the different constituents (20). In the case of a polydisperse colloid, successive filtration through membranes of different porosities may fractionate the large particles from the small (9).

The second type of problem, which may also have application to pigments, is the estimation of particle size. This is much more difficult and requires a more critical selection of filters and a system of calibration and standardization.

IV. Ultrafilter Membranes

A. Preparation

In any application of ultrafiltration, certain specifications must be set for the filter employed (39). The phenomena which cause the behavior of an ultrafilter to differ from that of an ideal mechanical sieve arise from the

high ratio of pore length to pore diameter; and, while it is seldom possible to reduce this ratio below a thousand, it should be limited by choosing the filter as thin as possible. On the other hand, the filter must be mechanically strong enough to withstand the pressure applied in filtration without distortion or rupture. It must be reasonably isoporous, and free from occasional pores which are much larger than the average. Of course, it must not react with or dissolve in any component of the system which is filtered through it. For a fractionation experiment, rigorous control of filter porosity is unnecessary, so long as the pores are large enough to pass the components desired in the filtrate and small enough to retain those desired in the residue. It is for this reason that so many an early experiment, in which no regulation or calibration of filter porosity was made, succeeded in the desired fractionation. It is desirable, however, to calibrate filters rigorously and to have a wide series of porosities available, in order to attain best efficiency by selecting for a given experiment the most highly porous filter which will yet perform the required separation. For estimation of particle sizes, a series of carefully calibrated filters covering a wide range of porosities is an essential requirement. For comparative experiments, groups of filters of exactly comparable porosities must be available. It may be mentioned here that high quality, carefully graded membranes are available commercially from at least one American source.

Most ultrafilter membranes are gelatinous, and in the great majority of cases the gel consists of collodion, i.e., nitrocellulose containing about 11 per cent of nitrogen (39). This gel is produced from a solution of collodion either in glacial acetic acid or in a mixture of volatile solvents including principally ether and ethyl alcohol. Preparation of artificial gel membranes of reproducible characteristics requires strict adherence to empirical rules in the minor details of technique, and is particularly true of ether-alcohol collodion membranes, but the latter are the most satisfactory if prepared with the required care.

Gelatinous membranes may be classified according to whether the gel is impregnated in a supporting structure or forms its own support. Membranes may also be prepared from non-gelatinous materials.

Collodion membranes impregnated in filter paper were introduced by Bechhold (9), in 1907, and his simple technique still represents the easiest method of preparing a graded series of ultrafilters. A piece of hardened filter paper is soaked in a solution of nitrocellulose in glacial acetic acid. The excess solution is drained from the paper, and the membrane is gelled by immersion in water. The acetic acid is removed by prolonged washing, leaving a film of nitrocellulose (with perhaps some cellulose acetate) imbedded in the filter paper. The higher the concentration of nitrocellulose in the original solution, the lower the porosity of the membrane. Retention of air by the paper, which

might result in microscopic pinholes in the final membrane, is diminished by preliminary soaking in pure acetic acid, and practically eliminated by conducting the collodion impregnation in a vacuum. Draining off the excess solution from the paper may leave a layer of irregular and excessive thickness, especially in the case of the more concentrated and hence more viscous solutions.

The chief advantage of these membranes lies in the relative simplicity of preparation and the wide range of porosities obtainable. Pore diameters may be obtained from 1 to 5 μ down to less than 10 mμ. However, in a given filter, the pore sizes vary over a considerable range, and the limited reproducibility in average pore diameter from one membrane to the next makes comparative experiments difficult, even with the rigorous control of experimental technique. Further, these filters are thicker than self-supporting collodion membranes, and, in contrast to the latter, their thickness increases with decreasing porosity, thus making the ratio of pore length to diameter doubly excessive for the densest membranes.

Impregnation of collodion in a cloth support has been patented by Duclaux (26), who has also impregnated cloth with cellulose acetate, forming a gel suitable for filtrations with some organic solvents like benzene (25). It may be possible that this type of membrane would be applicable in the separation of a pigment from a paint system.

Bargues (6) prepared membranes by dissolving

acetylcellulose in a 75 per cent solution of magnesium perchlorate, coagulating by dilution with water or dilute magnesium perchlorate solution, and washing out the salt. Within wide limits, the porosity of the precipitated filter is proportional to the thickness. The mechanical strength of these filters can be increased by forming them on filter paper, cloth or metal fabrics.

In some cases, a more rigid support for the ultrafilter gel is employed, such as porcelain, alundum, or metal. The earliest impregnated filters of this type were those prepared by Martin (63), in 1898, by filling the pores of a Chamberland candle of unglazed porcelain with gelatin or silicic acid. Laporta (63) impregnated Chamberland filters by immersing them in 4 per cent collodion. The classical "semipermeable" membranes for osmotic experiments, introduced in 1877 by Pfeffer (72), consist of copper ferrocyanide deposited in unglazed porcelain, and have a very low porosity.

The most popular porcelain impregnated filter is that of Bechhold and König (11), which is a Bechhold membrane with porcelain substituted for filter paper. Crucibles, evaporating dishes, and other vessels, with unglazed bottoms, are impregnated with acetic collodion in the usual way (39). After use, the nitrocellulose may be burned off. The porosity is varied, as above, by varying the concentration of collodion in the impregnating solution. This type of filter may also be used with non-aqueous solutions.

Ultrafilters impregnated in porcelain and the like have the advantage of mechanical convenience and strength. They are, however excessively thick, and can remove large quantities of material from filtrates by adsorption. They should be used only for filtration of large volumes of material, where rigid control of membrane porosity is not required, since accurate calibration is difficult (38).

While acetic- collodion membranes must be of the impregnated type, owing to the fragility of the acetic collodion gel, membranes made from ether-alcohol collodion have sufficient strength to be self-supporting. These are made in the form of either sacs or discs.

Collodion sacs were the first artificial membranes to be generally adopted (78), and have been used very extensively, especially in biological research. The porosity of collodion sacs is varied by adjusting the ratio of alcohol to ether in the solvent, and varying the time of draining and the duration of evaporation; also by adding small quantities of other reagents to the solution. These added substances may also effect the mechanical properties of the membrane (39).

The collodion sac is particularly popular because of ease of preparation and the large area available for filtration, and because it constitutes its own container and, unlike the disc, does not require a mechanical holder with clamp and gaskets (39). It is, however, quite unsuited for work requiring uniform and reproducible ultrafilters. In the first place, the porosity of a given sac is different

at different points, tending to be greater at the closed end than at the open end, and it is very difficult to make successive sacs of similar porosities, on account of the high viscosity of the collodion and the rapidity with which the solvents evaporate.

These difficulties may be overcome by forming the collodion into disc membranes (14,33). A thin layer of collodion solution is poured on a carefully levelled glass plate, a surface of mercury, or a glass plate floated on mercury. Regulated evaporation proceeds, either by diffusion of the solvent vapors into a fairly large draft-free enclosure, or the slow passage of known quantities of air of regulated humidity past the glass plate. Convection shields prevent irregular air currents. The temperature is carefully controlled and maintained constant. After sufficient of the solvents has evaporated, the collodion sets to a gel. The evaporation is prolonged a specified time, and is then ended by suddenly covering the collodion film with water. The remaining solvents are washed free, and the film is cut by dies into small discs. When proper attention is given to consistency of all details in technique, it is possible to prepare from the same sheet forty discs, which differ in porosity by less than 2 per cent from one another, while successive sheets poured from the same solution of collodion agree in porosity within 10 per cent. The sizes of pores in a given disc vary within comparatively small limits.

It is customary to keep a preservative, such as thymol, or formalin, in the water which covers such membranes during wash and storage, since the collodion is particularly favorable to the growth of a mold which enters the pores and completely alters the porosity. Some procedures (33), however, involve sterile techniques throughout, avoiding the presence of preservatives which might have some effect in subsequent filtrations.

The porosity of collodion disc membranes is varied by the same general methods employed for sacs. Membranes of a very low porosity are prepared by allowing the solvents to evaporate completely from a film of ether-alcohol collodion (71). Such "dry collodion" films demonstrate even a differential permeability to ions.

Duclaux and Amat (27), prepared membranes by pouring a solution of cellulose acetate in saturated aqueous magnesium perchlorate on glass, plunging into water, and removing the salts by washing with water. The porosity of these membranes varies as 1:1000 according as the concentration of the solution varies from 2 to 20 per cent. The actual thickness of the film may vary between 0.06 and 6 mm. These films undergo very slight and very slow changes in humidity. The fact that magnesium perchlorate solution dissolves other substances such as starch, gelatin, etc. enables mixed films to be prepared. A similar procedure is described by Baudouin and Lewin (8), who add 5 to 15 per cent of dry cellulose acetate to a saturated aqueous solution of magnesium

perchlorate and the mixture kept in an incubator until solution is complete. This sirupy solution is poured on glass plates surrounded by low rims and, after it has leveled off, distilled water is floated over the surface. The films obtained are stored under water and the pore size does not change with age.

Commercial cellophane is a membrane of pure cellulose, with a trace of glycerol. It was formerly possible to obtain grades of cellophane which, when swelled in water, had a porosity of about 4 mμ, and formed very convenient ultrafilters for many purposes (66). It is possible to replace the water, proceeding by way of mutually miscible liquids, by various organic solvents to yield membranes for filtration of non-aqueous solutions. Cellophane of recent manufacture is less porous, and even partially retains materials such as sucrose in ultrafiltration. Its porosity may be increased by swelling with concentrated solutions of sodium hydroxide or zinc chloride, but only with difficulty to an extent sufficient to pass sucrose in undiminished concentration (56). Several recent authors describe the use of cellophane tubing, such as that used for sausage casings (23,46, 90). The permeability of this tubing and the rate of filtration are determined by the cellulose content of the membrane.

Various animal membranes have been employed for purposes of dialysis and ultrafiltration. Among these may be mentioned fish bladder, pig's bladder, and goldbeater's skin (9,14).

A few types of ultrafilter membranes whose structure is

not gelatinous may be mentioned. Manning (62) plated nickel on 200-mesh wire gauze of nickel, and thus decreased the sizes of the interstices to give pore diameters of from 50 mμ to 300 mμ. Warrick and Mack (88) distilled the zinc out of strips of brass, leaving porous copper membranes which showed differential permeability to gases and could serve as semipermeable membranes in the osmosis of aqueous solutions of sucrose. The porosity was evidently very small. Prausnitz (74) prepared ultrafilters of sintered glass, with mean pore diameters of 1.5 μ.

Filters having the qualities of an ideal sieve have been prepared (41) by piercing the pores in a thin collodion or metallic sheet of a thickness ranging from 1 to 100 μ by means of a pencil of cathode rays.

Silicon carbide filters have been prepared (1,2,6) which are claimed to have better defined constitutions than cellulosic filters and they are nonabsorbent and nonreactive. By sorting particles as to size, pores as small as 0.02 μ can be obtained. The volume of the pores is a constant fraction of the total volume. After separating a suspension of silicon carbide in ammoniacal water by decantation, ultrafiltration, and fractional centrifugation into fractions of uniform particle size, an ultrafilter was constructed by depositing on filter paper from aqueous suspensions successive layers of particles of progressively decreasing size. Filters of this type permitted determination of micelle sizes according to the grain size of the silicon carbide used for

the filters.

B. Methods of Varying the Porosity

An adequate method for grading the porosities of ultrafilter membranes must be capable of varying the porosity continuously, maintaining satisfactory mechanical properties throughout the range.

The principal method for grading acetic collodion membranes is that originally used by Bechhold (9), - variation in the concentration of collodion in the impregnating solution. He found the membrane porosity to be related antibatically with the concentration of collodion, but results were not reproducible from one solution of collodion to the next. In later work, he was able to achieve a certain degree of reproducibility (39).

In the early experiments on ether-alcohol collodion membranes, where the porosity was graded by varying the evaporation times, no quantitative data were reported (12,14). However, it was shown that this method is incapable of producing porosities of greater than about 60 mμ. The shorter the evaporation time, the more highly porous the membrane, but the evaporation must proceed at least long enough to allow the collodion to set to a gel.

Brown (18) prepared a graded series of collodion sacs by allowing them to dry completely and then swelling them in alcohol-water mixtures of varying concentration. The higher the proportion of alcohol in the swelling solution,

the higher the porosity of the resulting membrane, but the range of variation was limited, since a concentration of over 98 per cent of alcohol in the swelling solution would dissolve the collodion. The most highly porous membrane obtained was reported impermeable to filtration of Night Blue and Congo red.

Addition of various non-solvents or precipitating agents to a collodion solution was found to increase, to a limited degree, the porosity of membranes prepared from it; among these reagents were glycerol (78), water (70), lactic acid (29), and ethylene glycol (73). The porosity increase was limited by the effect on the strength of the membrane, which became fragile if too much reagent was added, and it was impossible to prepare membranes of high enough porosity for some purposes.

As the result of a systematic study of the effect of many reagents on the porosity of membranes prepared from collodion solutions, Elford (30) found that, in general, addition of good solvents caused a decrease in membrane porosity, and non-solvents or precipitating agents, an increase in porosity. Amyl alcohol or acetone alone was a good solvent, but in the presence of each other there was an antagonistic effect which resulted in a porosity increase. On this basis, it was possible to compose mixtures of ether, ethyl alcohol, amyl alcohol, and acetone, to which were added small quantities of other reagents, for preparing a graded series of membranes of optimum mechanical properties

and with porosities covering a very wide range (2 μ to 2 mμ). Porosities were increased in steps by addition of water or amyl alcohol, and decreased by addition of acetic acid or (38) ethylene glycol monoethyl ether (Cellosolve). Fine adjustments in porosity were made by altering the evaporation time. This permitted grading of porosity on a continuous scale.

The results of adding various reagents to ether-alcohol collodion solutions, as influencing the membranes prepared from the latter, may be summarized as follows: ether-alcohol mixture (3) dilutes the collodion and makes membranes thinner, more porous, and rather brittle; ether (32) dilutes the collodion, making membranes thinner without much alteration in porosity; ethyl alcohol (32) makes membranes thicker and weaker, and decreases the porosity; methyl alcohol (32) decreases porosity; amyl alcohol (3,32) decreases porosity, but, in the presence of acetone, increases the porosity; water (32,70) increases porosity; if added in too great amounts, makes membranes brittle and non-uniform; acetic acid (29,32) decreases porosity markedly; lactic acid (29) increases porosity; ethyl acetate (3) increases porosity markedly; ethyl formate (3) increases porosity; glycerol (29,78) increases porosity; castor oil (78) increases porosity and toughness; ethylene glycol (73) increases porosity; Cellosolve (38) decreases porosity markedly.

C. Structure of Membranes

Some model of the structure of ultrafilter

membranes must be assumed for the quantitative calculation of porosity from calibration data (39). The limited means for studying membrane structure experimentally must be employed in order to select the most suitable model.

The most simple model of an ultrafilter is a sheet pierced by right circular cylinders, so that the effect in filtration is that of a bundle of cylindrical capillaries. This was the assumption made by Bechhold (10) in calibrating his acetic collodion membranes.

Manegold (57,58) has discussed possible arrangements of porous structures in some detail, distinguishing between canal structures where the solid phase is continuous, and branching structures where it is not. The latter type seems to be the more likely in the case of the gel membrane, but it is much more difficult to treat. As for canal structures, Manegold specifies six arrangements:

(a) Pores (circular cross section), all running perpendicular to the membrane surface.

(b) Pores, a third of the total number running in each of three mutually perpendicular directions, without any intersections.

(c) Pores, oriented in haphazard directions, without any intersections.

(d) Cracks or slits (rectangular cross section, all running perpendicular to the membrane surface.

(e) Slits, a third of the total number running in each of three mutually perpendicular directions,

without any intersections.

(f) Slits, oriented in haphazard directions, without any intersections.

As far as rate of flow of water through the membrane is concerned, it is impossible to distinguish between any of these structures. Manegold, after varied experimental studies on ether-alcohol membranes, concluded that structure (f) is the most likely. This was not, however, the only interpretation of his data possible (39). Most evidence points to structure (a), with slight modifications, as a satisfactory working basis. Perhaps even more important than the shape and orientation of the interstices of an ultrafilter is the degree of uniformity of their dimensions.

Elford (31) employed films from acetic collodion and ether-alcohol collodion, prepared under various conditions, for studies of their structures with the microscope and ultramicroscope. Two types of structure were distinguished. The microgel structure has interstices of microscopic dimensions, and is highly irregular, offering pores of different diameters. This type of gel is formed when membranes are prepared from dilute acetic collodion coagulated in water, or ether-alcohol collodion which is coagulated in water before the evaporation has proceeded long enough to set the film to a gel. It results from diffusion of water, a precipitating agent, into the collodion solution while the latter is fluid and the micelles are mobile. On the other hand, the ultragel has a very fine, uniform, granular structure, revealed only

by the ultramicroscope This gel is formed when ether-alcohol collodion is allowed to "set" before immersion in water, or when films of acetic collodion of high viscosity or extreme thinness are treated with water. It results from the replacement of solvent by water in a structure which is largely immobile while the replacement proceeds, the collodion micelles being held fast in a previously set gel, or oriented by surface forces in a very thin film, or behaving as if immobile in a solution of high viscosity. In ether-alcohol collodion membranes, which are in practice prepared by immersing in water only after the gel has set, the ultragel structure prevails. In acetic collodion membranes, the structure grades between microgel and ultragel according to the viscosity of the impregnating solution and the thickness of the impregnated film. This explains the lack of uniform porosity in the latter membranes. The ultragel structure is the desirable one; unfortunately, the ultramicroscope can give no information concerning its geometrical details. Von Ardenne (36), however, has shown electron photomicrographs of ultrafilter membranes from which he was able to calculate their pore diameters quite accurately.

Grabar and de Loureiro (44) prepared histological sections of various collodion filters. The more porous filters showed, under the light microscope, clusters and chains of granules, the fineness of which determines the diameter of the pores. With filters of a smaller mean pore diameter than 100 mμ, which cannot be studied with the ordinary light

microscope, but may be with the polarizing microscope, there is birefringence due to a change in structure.

One of the earliest methods of characterizing a membrane was by the proportion of empty space in its structure (87). This may be determined by obtaining the "specific water content", which is defined (35) as the relative loss of weight by removal of water from the filter pores, and is identified with the total volume of all the pores.

The specific water content of ether-alcohol collodion membranes is remarkably high and constant for porosities from 20 mμ to over 1 μ (35). The proportion of free space averages about 0.87 and is never less than 0.80 for all these membranes. Very close packing of pores in structure (a) would be required to provide this free space; hexagonal close packing, which gives a maximum of 0.90 for the circular cylinders in tangential contact, would barely suffice. Structures (b), (c), (e), and (f) are impossible for lack of room for non-intersecting pores. If pores are to be postulated running in three mutually perpendicular directions, they must be considered to intersect to an extent dependent on the value of the specific water content.

Estimation of the porosity of a membrane by measurement of the rate of flow of water through it was first suggested by Guerout (48) in 1872. The rate of flow through membranes, as a means of characterizing them, was applied by various workers, who studied its dependence on experimental conditions. It was found to be proportional to the pressure for

ether-alcohol collodion membranes (12,14,28,59), demonstrating that the flow is viscous. However, deviations from the proportionality law have been observed in both directions; the increase in rate of flow with increasing pressure may be greater than linear (59), which is attributed to distortion of the membrane at high pressure, or to bringing immobile surface layers of water into motion at high pressures; or it may be less than linear (24), which is attributed to restriction of the area through which flow occurs when the membrane is forced against a perforated support at high pressures. This last effect shows that when rate of flow measurements are made for the purpose of porosity calculations, the membrane must not be supported against a wire gauze or perforated plate, since the calculations require accurate definition of the area through which flow is occurring. In the case of a mechanical support, the area effective in filtration varies from the total area of membrane, at low pressures (24), to the limited area actually opposite the perforations, at high pressure (33). When the effective area is clearly defined, and the pressures employed do not distort the membrane, the rate of flow is almost strictly proportional to the pressure. The dependence of rate of flow on temperature has been found for collodion membranes to be non-linear, and may be entirely attributed to the temperature variation of the viscosity of the flowing liquid (28).

It is remarkable that membranes of collodion may be prepared covering a porosity range of a thousand fold, and

particularly that, in the greater part of this range, the total space occupied by the pores is independent of the size of the latter (39). Increase in porosity is evidently accomplished by redistribution of the nitrocellulose particles so that the pores become larger but also less numerous. This is probably a result of a reversible, gradual aggregation of the nitrocellulose, giving progressively larger particles (35) - particles which are not spherical, but elongated, as shown by streaming double refraction (80). Such gradual aggregation is exceptionally favored by a solvent containing amyl alcohol and acetone, explaining how the method of Elford can give such highly porous membranes with uniformity and adequate tensile strength. Presence of water favors aggregation, but it is not gradual; it produces flocking and coagulation, and the resulting membrane may be fragile and non-uniform. The difference between the action of water and that of amyl alcohol and acetone is clearly shown by titrating collodion solutions with these reagents (36).

The preparation of an ether-alcohol collodion membrane consists essentially in the evaporation of solvents until the film sets to a gel (39). The degree of aggregation of collodion at any point in the process depends in a highly specific manner on the composition of the solution. The composition of the solution depends in turn on the time elapsed and on the original proportions and volatilities of the various solvents and non-solvents in the solution. Two processes probably occur during the evaporation (36): a

gradual aggregation as the proportions of solvents and non-solvents change, and a sudden gelling when the concentration of collodion becomes sufficiently high for the aggregates to lock into a rigid structure. The membrane porosity is determined, largely, by the extent to which the aggregation has proceeded by the time gelation occurs. Continued evaporation from the set gel, accompanied by shrinkage of the gel when it is immersed in water, will decrease the eventual porosity.

According to Grabar and de Loureiro (45), the structure of the nitrocellulose membranes obtained by evaporation depends on the composition of the system at the moment of gelification. The porosity of the membrane depends on (a) its swelling (ratio liquid/solid) and (b) the dimensions of the nitrocellulose aggregates. The swelling of the gel is equal to the ratio gelifier/nitrocellulose in the initial solution; the inflation of the washed membrane is proportional to the swelling of the gel. The dimensions of the nitrocellulose aggregates depend on the nature and proportion of the solvent present in the system at the moment of gelation.

D. Calibration of Membranes

Two fundamental questions must be answered in the characterization of an ultrafilter (39). One concerns its average pore size and distribution of sizes, and the other, its behavior in filtering particulate systems. The first question is answered by measurements of specific water content, rates of flow, and bubble pressures, while the second

involves consideration of the complex mechanism of filtration. The second question is the more important in the study of ultrafiltration, as distinguished from that of the membranes themselves, but it is not convenient to characterize ultrafilters in terms of filtration of disperse systems alone. In the first place, the behavior of a filter toward one sol may differ from its behavior toward another of similar particle size. In the second place, characterization by filtration of particles of known size does not provide a continuous scale of grading. Given an ultrafilter, it is hardly possible to command a wide series of sols of different particle sizes so closely spaced as to permit selecting one which the filter retains and another, only slightly finer, which the filter passes. On the other hand, calibration by rate of flow of water assigns a specific average pore diameter to the filter. Values of average pore diameter provide a continuous scale, and, for ether-alcohol collodion membranes, are close to representing the true dimensions of the pores. The relationship between the diameter of the most highly porous filter which retains it is of a specific nature.

Calculation of the average pore diameter requires measurement of the membrane thickness, the specific water content, and the rate of flow of water through the membrane (39).

The membrane thickness may be measured by a micrometer gauge controlled by a fine spring (39), with precaution that the membrane is not compressed or deformed, or by cutting a thin strip of membrane, bending it in the form of a Z to

stand on edge, and observing it in a light microscope equipped with a micrometer ocular. More refined methods may employ an optical lever or interferometry (51).

The specific water content, S, may be determined in the following three ways (32):

(a) By the difference in weights of the membrane with its pores full of water (W_w) and then dried by heating to 60°C. or over sulfuric acid (W_d):

$$S = \frac{W_w - W_d}{dA}$$

where d is the membrane thickness and A the area.

(b) By consideration of the density of solid collodion, p; then

$$S = 1 - \frac{W_d}{dAp}$$

(c) In acetic collodion membranes, by assumption that the coagulation does not change the specific volume of the collodion, so that S equals the percentage of acetic acid in the impregnating solution.

For a convenient measure of the rate of flow of water, Elford defined an auxiliary quantity, the "R.F.W." (F), in terms which lead to the formulation

$$F = \frac{Vd}{AtP} \times 60{,}000$$

where d is the membrane thickness in millimeters, V is the volume of water which flows through an area A of the membrane in time T under a pressure P (in centimeters of water).

The average pore diameter (j) may be calculated from F

(at 20°C) and S, after introduction of dimensional constants, by the simple expression

$$j \text{ (in microns)} = 0.234 \sqrt{\frac{r}{s}}$$

E. Apparatus for Ultrafiltration

Ultrafiltration apparatus requires a liquid-tight clamp for the ultrafilter membrane, with a vessel for the filtering system under pressure and a receiver for the filtrate. For specific cases, there are many individual features of the apparatus to be considered.

For ultrafiltration at very high pressures the vessel must be constructed of metal. For corrosive solutions glass is more desirable, but limits the usable pressure (43). In sacs and filters of the Bechhold-Konig type, the filter forms its own vessel, which is closed at the top by a stopper sealed with collodion, or left open to the atmosphere for filtration under negative pressure (39).

The filter membrane may be supported on a perforated metal plate (42), a fine wire gauze (19), perforated glazed porcelain (85), or other supports. Filter paper may be used to increase the effective area by interspersing it between the membrane and the rigid support.

The filter membrane must be held securely in place in the apparatus. To effect a liquid-tight joint around the membrane, a gasket of rubber or other compressible, non-permeable material may be used.

A circular threaded ring may be used for clamping the membrane in place, as it produces an even pressure around the entire periphery. Shearing of the membrane must be avoided when assembling the apparatus. When very high pressures are used, this may not give a tight seal, so a series of individual bolts around the periphery is employed (19).

Filtration under pressures lower than atmospheric is not to be recommended for quantitative work, since the filtrate tends to concentrate by rapid evaporation (4). It is also difficult to collect successive samples of filtrate, a procedure necessary for adequate analysis of results. It is convenient, however, for filtration of large volumes of material through highly porous filters, where the upper vessel may be more easily replenished. For filtration through membranes of low porosity, it is more desirable to use very high pressures as the filtration rate is much slower.

In the more recent literature, various modifications of apparatus for ultrafiltration have been described and will be mentioned at this point.

A simple type of ultrafilter was described by Greenstein, Turner, and Jenrette (46). The framework consists of a glass tube of 1 inch inside diameter, sealed at one end and fitted with a sidearm 3 inches from the open end. The sidearm is connected through a trap to an oil pump. The closed ends of several Soxhlet extraction thimbles of 1 inch diameter are cut off and the paper cylinders inserted one on top of the other into the glass tube, reaching from the bottom of the glass

tube to well beyond the sidearm. Two or 3 glass rods, carefully polished on the ends, are inserted between the paper walls and the glass so as to cover the aperture leading to the side tube and prevent drawing the paper into the side tube when suction is applied. Then a wetted piece of 36/32 Visking cellophane tubing is tied off at one end and inserted within the paper-lined glass tube. Into the open end of the cellophane tube a No. 5 double-holed rubber stopper is inserted and open end of the glass tube sealed with the cellophane-covered stopper. A small glass funnel is set into one of the two holes of the stopper. A protein solution is then poured through the funnel into the bag and the suction started. After 4 hours of ultrafiltration of a solution containing 1.4 grams of protein in 100 cc. at 13 mm. Hg pressure, 50 cc. of water, completely free of protein, had passed through the membrane.

An ultrafilter was designed by Kuyper, Andrews and Eidt (52) which permits the collection of about 20 cc. of protein-free ultrafiltrate from 35 cc. serum during 8 hours of ultrafiltration. The filter consists of four principal parts: an upper section, a central cylinder, a funnel-shaped bottom section, and a stainless steel disk which is the support for the filtration membrane. Nitrogen at a pressure of about 100 psi. is used as the filling. All surfaces are coated with paraffin to prevent corrosion. The ultrafilter is placed in a tripod and oscillates about its vertical axis by means of an eccentric drive.

Baudouin and Lewin (7) described an apparatus in which a funnel provided with a fritted glass filter disk covered with a collodion membrane is placed in an inverted position in a beaker containing the solution to be filtered. The beaker is placed in a closed chamber with the stem of the funnel passing through a rubber stopper or stuffing box in the cover of the chamber. Compressed air or other gas is admitted to the chamber, forcing the liquid through the filter and up through the stem of the funnel to the exterior. Membranes for this apparatus are prepared by dipping the filter plate into a solution of collodion dissolved in glacial acetic acid and then hung in distilled water.

Clark (21) described an apparatus which consists of a series of rubber gaskets and cellophane sheets clamped together to provide chambers for filtration and collection of the ultrafiltrate. The fluid is conducted into the lumens of alternate gaskets through 15-gauge needles, and the filtrate escapes through similar needles. Filtration is conducted at about 1 atmosphere pressure, and the filtration rate for water is about 75 cc. per hour per 1000 sq. cm. of filtering surface.

The centrifuge was used by Wennesland (90) who employed cellophane tubes which are placed in special wire-net baskets to give them necessary support, and also by Clegg (22a) who used a cellophane sac enclosed in a nylon bag.

V. <u>The Mechanism of Ultrafiltration of Disperse Systems</u>

When a disperse system is forced through an

ultrafilter the disperse phase may be less concentrated in the filtrate because of several reasons (39). It may be (a) adsorbed on the surface of the filter and its pores (primary adsorption), (b) retained within the pores or excluded from such blocked pores (blocking), or (c) mechanically retained on top of the filter (sieving). Sieve action may result from heterodispersion in the filtered system or heteroporosity in the filter. The principal problem in selecting conditions for carrying out ultrafiltrations is to eliminate effects (a) and (b) as completely as possible, so that sieving, the desired effect, is the controlling factor. The operation of these three mechanisms may change with time as the filtration proceeds, so that, in the first place, examination of the course of filtration throws light upon the problem.

A. Experimental Procedure

The ideal method for following an ultrafiltration experiment would involve continuous measurement of the concentration of filtrate and residue during the whole process, without requiring the withdrawal of samples. This is possible in some cases by measurement of refraction or light absorption (61), but imposes serious limitations on the filtration apparatus. It is usually possible, however, to separate the filtrate into successive small samples for individual analysis, and to withdraw small samples from the residue from time to time if desired. Whenever feasible, a physical analytical method is preferable.

The relative concentration of filtrate is defined as the ratio of the concentration of a momentary small sample of filtrate to that of the original solution (34). When this is followed through the course of a filtration, a curve of one of the forms shown in figures 1 and 2 results. Curves I arise from filtration through membranes whose pores are far wider than the solute particles. When the pore sizes are of the same order of magnitude as the particle sizes, the curves make take the form of either II - IV in figure 1, or II - IV in figure 2. In each case, the initially low values of filtrate concentration are attributed to a primary adsorption of the solute in the membrane pores. This, however, becomes satisfied as soon as a sufficient volume has been filtered through. After this, the solute or disperse phase appears in the filtrate in practically undiminished concentration (curves I); or, if the pores are not large enough to permit this, the filtrate concentration levels off or slowly increases (figure 1), attributed to sieve action; or reaches a maximum and falls off more or less rapidly (figure 2), attributed to blocking.

The change with time of the concentration of the residual solution above the filter is also characteristic. When the solute appears in the filtrate in practically undiminished concentration (curves I), the concentration of the residue is also practically unchanged. True sieve action (figure I) is accompanied by an increase in the concentration of the residue, which may be manyfold if the proportion

Typical Filtration Curves (34)

Figure 1. Normal Filtration

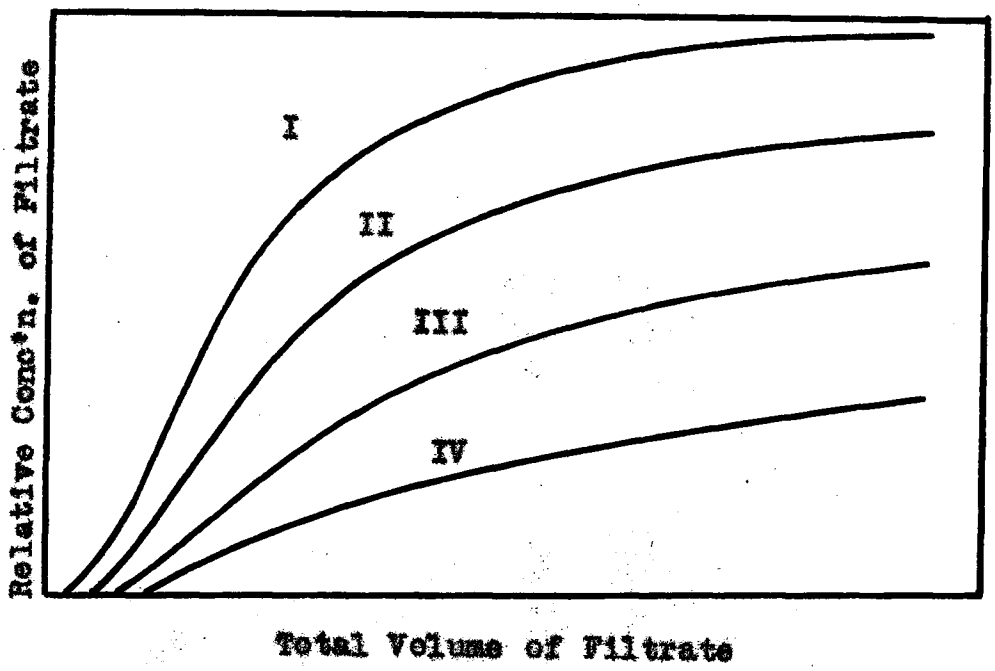
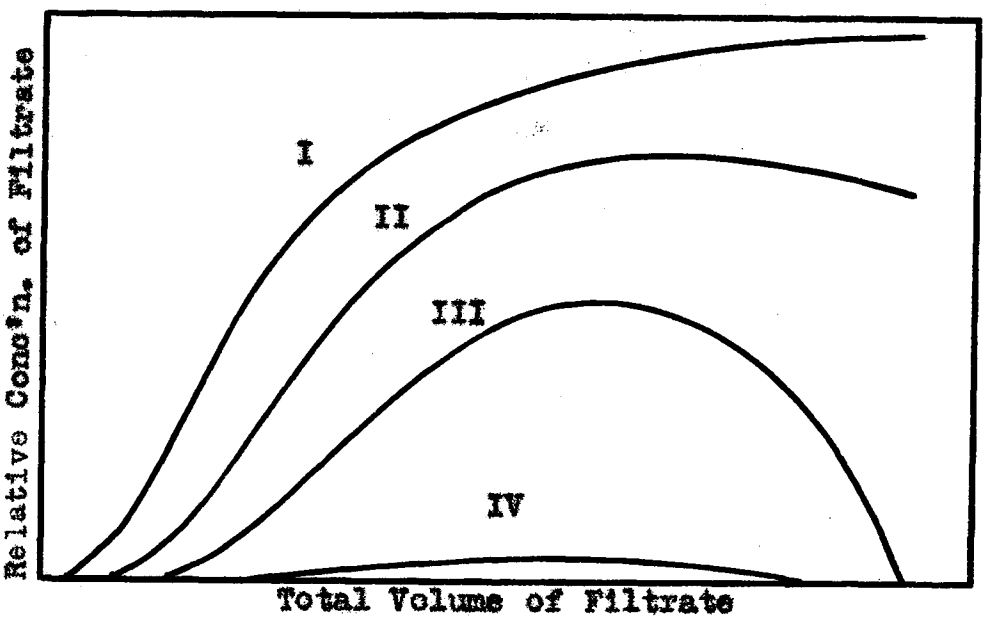


Figure 2. Abnormal Filtration



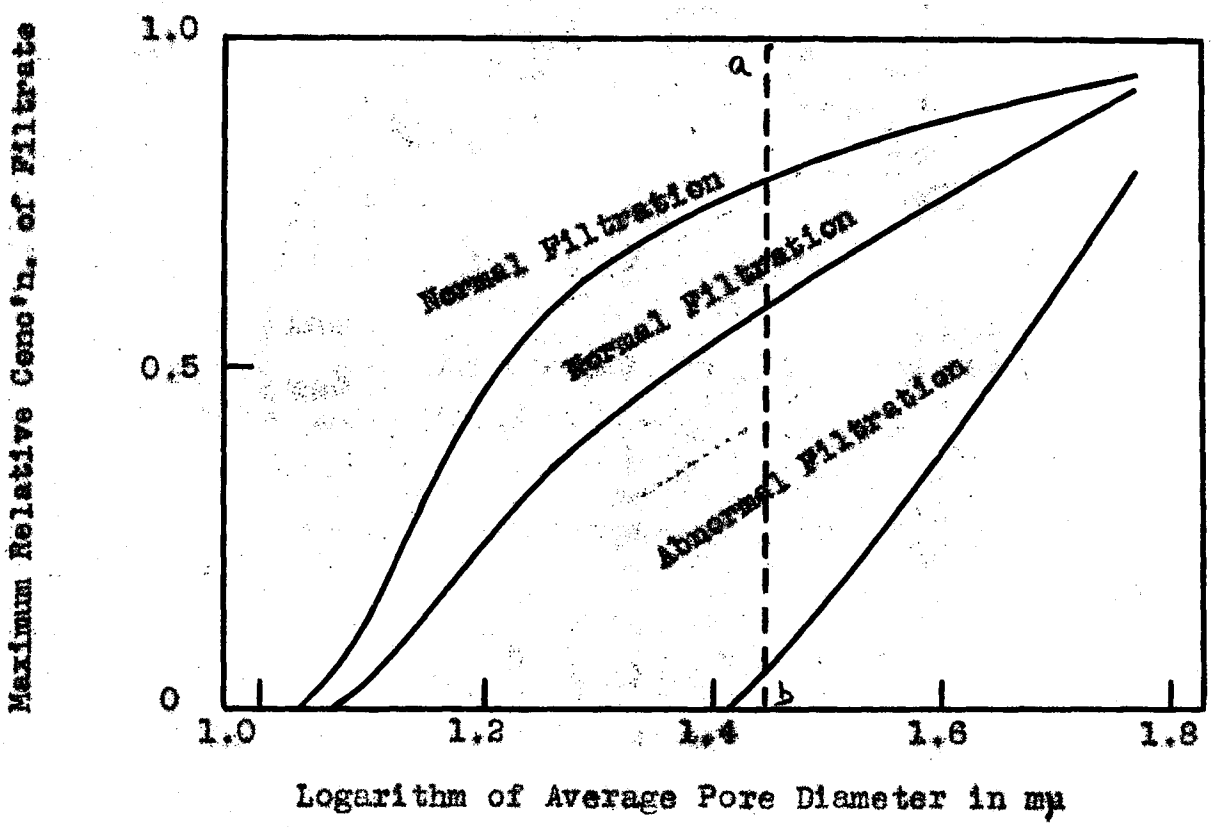
of solute passing the filter is small and a large fraction of the original volume is filtered. Blocking (figure 2) is accompanied by an increase in the concentration of the residue to some extent, but it always involves colloidal instability of the disperse phase and a tendency to flocculation and precipitation, so that the latter processes often occur in the residual solution, and the disperse phase, instead of concentrating, is precipitated on the membrane. Finally, in the case of marked primary adsorption from a limited quantity of a dilute solution, the concentration of the residue may be considerably diminished.

To obtain a quantitative measure of filterability of a given disperse system, equal volumes of the system (volumes several times that required to satisfy primary adsorption) are filtered through membranes of different porosities. The maximum relative concentration of filtrate obtained in each experiment is plotted against the membrane porosity and curves of the type shown in figure 3 result (34). The intersection of such an "end-point curve" with the porosity axis (a b) determines the end-point porosity, i.e., the highest porosity which completely retains the disperse phase. By adjusting experimental conditions, it is possible to isolate effectively the factors of adsorption and blocking, and to study means of their elimination.

B. Primary Adsorption

The effect of primary adsorption in filtration may

Figure 3. End-Point Curves (34)



be studied separately by employing filters with pores very much wider than the solute particles, so that no sieving nor blocking can occur; or by employing extremely dilute suspensions, as is possible in biological systems, so that a large volume is required to satisfy the adsorbing capacity of the membrane (39).

The more dilute the filtering solution, the greater the volume which must pass through the membrane before adsorption is satisfied and the disperse phase begins to appear in the filtrate. Elford's experiments with dyes (33) showed that, over a tenfold variation in initial concentration, the relationship was one of slightly less than inverse proportionality.

Increasing the pressure in the ultrafilter narrows the zone of primary adsorption, presumably by defining more closely against the filter support the areas through which flow can take place and by a tendency to shear away adsorbed layers in the pores (33).

The thickness of the membrane also effects the adsorping capacity. As the thickness increases, so does the volume which must be filtered before the disperse phase appears in the filtrate (81).

The presence of a capillary-active substance in the disperse system markedly decreases the zone of primary adsorption (33). The effect is presumably due to preferential adsorption of the capillary-active agent.

In summary, it is clear that the effect of primary

adsorption can always be eventually eliminated by filtering a sufficient volume through the filter membrane. The adsorbing capacity is satisfied most quickly for (a) high initial concentration of the filtering solution, (b) high filtration pressures, (c) thin membranes, and (d) presence of capillary-active substances.

C. Blocking

The effect of blocking may be studied separately by employing solutions of concentration sufficiently high to satisfy the primary adsorption at an early stage in the process. Blocking of ultrafilters is closely related to colloidal instability, and may be best studied in filtration of sols of a lyophobic tendency (39).

The more concentrated the filtering solution, the sooner blocking sets in, so that, under conditions of severe blocking, it is possible to find the maximum relative filtrate concentration related antibatically to the absolute initial concentration. Increase in pressure also favors blocking. In the extreme case, reduction of the applied pressure to zero - i.e., substituting dialysis for ultrafiltration - apparently eliminates the effects of blocking entirely.(33).

As the thickness of the membrane increases, so does the ration of pore length to pore diameter, and consequently, the more favorable are the conditions for blocking (35).

The presence of a capillary-active substance suppresses blocking, presumably by preferential adsorption with resultant "lubrication", enabling the particles to slip through the filter pores more readily (34). Foreign particles, larger than

those of the principal disperse phase, may also cause serious blocking of the pores by mechanical obstruction, even under conditions where the principal disperse phase itself does not tend to block because of colloidal instability (37). When blocking is occasioned by foreign particles, accompanied by no flocculation of the disperse phase, the concentration of the residue should increase as in true sieve action.

In summary, the effect of blocking can be diminished by (a) low initial concentrations of the filtering solution, (b) low filtration pressure, (c) thin membranes, (d) the presence of capillary-active substances, and (e) absence of gross foreign particles.

D. Capillary-active Substances

The conditions for suppression of adsorption and blocking agree in demanding the presence of a capillary-active substance, i.e., one which depresses the surface tension of the solution and is strongly adsorbed in interfaces, in particular, on the pore walls and on the surfaces of colloidal particles (35). The effect of a protective colloid in facilitating filtration of lyophobic colloids was pointed out by Bechhold (9).

The first systematic study of the effect of capillary-active substances on membrane permeability was that of Brinkman and Szent-Gyorgyi in 1923 (17). They employed collodion sacs which were ordinarily impermeable to hemoglobin in ultrafiltration at 3 atmospheres pressure. Such a membrane became permeable to this protein when a dilute

solution of sodium oleate was first passed through it. That the hemoglobin was unchanged was shown by filtration of the filtrate from a "soaped" membrane through an "unsoaped" one. The protein was retained by the latter in the usual manner. "soaped" and "unsoaped" membranes had practically the same porosity, as shown by rate of flow of water, in spite of the difference in permeability to hemoglobin. Treatment of a soaped membrane with calcium chloride rendered it again impermeable. Other capillary-active substances could be substituted for sodium oleate with varying effectiveness, - sodium linolate, sodium glycocholate, and glycerol mono-oleinate (39).

The mechanism of lubrication in filtration with a capillary-active substance is usually interpreted as a coating of the latter on both pore walls and particle surfaces. Elford found evidence of such adsorption in fine-pored membranes by means of rate of flow experiments (33).

E. Normal and Abnormal Filtration

While the conditions for suppression of primary adsorption and blocking agree in demanding thin membranes and the presence of a capillary-active substance, they disagree in their requirements for concentration and pressure. Optimum values of the latter must accordingly be chosen (34).

When blocking is effectively suppressed, so that the filtration curves have the forms of figure 1, the filtration has been termed "normal" by Elford and Ferry (34).

Filtration with blocking, giving curves like those of figure 2, is "abnormal". The latter is invariably associated with an abnormally high end point, so that filters impermeable to a given disperse phase under conditions of abnormal filtration may under normal filtration become permeable.

Occurrence of abnormal filtration apparently depends on the tendency of the particles of the disperse phase to aggregate and precipitate when they are crowded together, as when they are forced into the membrane pores. If the tendency to aggregation exists, multiple adsorbed layers will be built up within the pores, and pores ten times as wide as the particles may become completely blocked. Without this instability - i.e., when normal filtration obtains - the adsorption may be limited to a single layer, and, by selective adsorption of a capillary-active agent, even that may be suppressed (33).

F. Theory of Sieving

The desired mechanism for retaining the disperse phase in ultrafiltration is a mechanical sieving. The theoretical consequences of an idealized sieving operation are considered here.

Neglecting adsorption and blocking, Manegold and Hofmann (60) assumed the sieving to be expressible by the equation

$$\delta = \frac{C_f}{C_s}$$

where

C_f = concentration of a momentary small volume of filtrate

C_s = simultaneous concentration of the filtering solution

δ = "sieve constant", independent of C_s

The value of C_s, and concentration of the total filtrate at any point, are obtainable by integration of appropriate differential equations. For filtration in a closed system, where nothing is added or removed, the concentrations of both residue and total filtrate should increase with time, when the sieve constant is neither 0 nor 1. Such a concentration increase in both residue and filtrate has been reported by Cox and Hyde (24) for filtration of colloidal dyes through Bechhold membranes.

The significance of the sieve constant in terms of sizes of pores and solute particles remains to be discussed (39). For a perfectly monodisperse solute and a perfectly isoporous filter, it has usually been implicitly assumed that the solute would either pass in undiminished concentration, or be entirely retained, depending on the relative sizes of pores and particles. This viewpoint must be, however, erroneous. The proportions of solute and solvent which pass the membrane depend on statistical considerations, even when the solute particles are so large that the water can be considered continuous by comparison. The sieve constant should increase gradually from 0 to 1 as the pore size is progressively increased above the end-point value.

On the basis of several simplifying assumptions, it is possible to calculate ϕ as a funtion of the ratio of pore size to particle size (39). It is assumed that:

(1) The membrane structure is represented by structure (a) on page 29 and is ideally isoporous.

(2) Adsorption and blocking are absent.

(3) Every solute particle is travelling vertically downwards when its center passes the plane of the surface of the membrane, and, in order to penetrate a pore, it must be wholly within the walls of the latter; i.e., its center must lie within a circle of radius r - R, where r is the radius of the pore and R that of the particle.

(4) At the mouth of the pore there is no radial component of the hydrodynamical velocity of flow, and vertical velocity has a parabolic distribution across the capillary in accordance with Poiseuille flow.

(5) The solution above the membrane is homogeneous, thermal motion preventing any accumulation of particles at the mouth of the pore.

In this case the expression for the sieve constant is evaluated as

$$\phi = 2\left(1 - \frac{R}{r}\right)^2 - \left(1 - \frac{R}{r}\right)^4$$

where

ϕ = sieve constant

R = radius of the particle

r = radius of the pore

A plot of the sieve constant ϕ as a function of the logarithm of the ration r/R closely resembles the experimental end-point curves for normal filtration (figure 3).

A sieving effect - i.e., the partial transmission of the solute, to an extent which varies with the membrane

porosity - can thus be anticipated on purely statistical grounds. This indicates, as invalid, an assumption that a sieving effect must be due either to polydispersion in the system filtered or to heteroporosity in the filters employed.

In practice ideal isoporosity is never attained, and probably colloidal solutions are never perfectly monodisperse, although some proteins approach this condition closely (39). When the statistical frequency of pores (or particles) of different sizes is given by a distribution function which is not a sharp peak, the curves of figure 3 are less steep, and sieving occurs over a greater range of filter porosities than is the case for isoporosity and monodispersion, while the apparent end points for chemical and bacteriological systems are much farther apart.

VI. Ultrafiltration of Colloids and Crystalloids

A. Composition of Colloidal Sols

Bechhold's original investigations (9) included ultrafiltration of numerous colloidal systems. Heterodispersion of particles in a silver sol was demonstrated by filtration of two fractions which had been prepared by centrifugation; a filter was found which passed the particles of one fraction and retained those of the other. End points of sols investigated were arranged in descending order as follows:

Coarse suspensions	1 % hemoglobin
Prussian blue	Serum albumin
Platinum (Bredig)	Diphtheria toxin
Ferric hydroxide	Protalbumoses
Casein	Silicic acid

Arsenic trisulfide	Lysalbumin
Gold (40 mμ)	Deutero albumoses
Silver (20 mμ)	Litmus
Gold (1 to 4 mμ)	Dextrin
1 % gelatin	Crystalloids

Solutions made up in other ways would give different results, because colloidal gold solutions, for instance, can be made with particles of very different sizes, depending upon the technique used.

With a suitable ultrafilter it is possible to filter ordinary water so that it is absolutely sterile (5). It is also possible to obtain in this way water which can be used for work with the ultramicroscope. The behavior of mixtures is distinctly interesting. If neither of the two substances adsorbs the other, and if the particles are of different sizes, a separation can be effected. The green mixture of Prussian blue and hemoglobin filters red with a suitable filter, the fine hemoglobin going through and the coarse Prussian blue remaining behind. In a mixture of Prussian blue with arsenious sulphide or ferric oxide, there is adsorption, and everything is stopped by a filter which would let through either the arsenious sulphide alone or the ferric oxide alone. By adding a protecting colloid, sodium lysalbinate, to Prussian blue, further dispersion takes place and the Prussian blue will pass through a filter which just stopped it before. If serum albumin is added to Prussian blue and then oxalic acid, the Prussian blue particles are found to be smaller than before.

If oxalic acid is added and then the serum albumin, no marked change in the size of particles takes place. Undoubtedly, the oxalic acid is adsorbed by the Prussian blue and prevents the adsorption of the albumin to a considerable extent.

Malfitano (56) studied the composition of colloidal ferric hydroxide, stabilized with hydrochloric acid, by ultrafiltration. The ultrafiltrates (i.e., colloid-free filtrates) contained only hydrochloric acid, and repeated ultrafiltrations (adding water to make up the volume above the filter) resulted in continued hydrolysis, with eventual coagulation of the colloid, the coagulation being to some extent reversible by addition of more hydrochloric acid.

McBain and McClatchie (68), using membranes of cellophane, showed that the composition of ultrafiltrates from a ferric hydroxide sol varied greatly with the rate of filtration; the concentration of simple electrolytes in the filtrate (measured by conductivity) fell off fourfold for a sixfold increase in rate. This is the result to be expected from a blocking effect, but it is pointed out that the mutual repulsion of the colloidal particles would prevent blocking (the particles being assumably too large actually to enter the pores), and attribute the results to an internal Donnan effect. The concentration of the intermicellar fluid varies from point to point being the least in the immediate neighborhood of the micelles. The ultrafiltrate at zero pressure, or dialysate of the sol, gives the composition of the dispersing medium far from the influence of the micelles, and

is the most concentrated ultrafiltrate obtainable. In filtrations at increasingly high pressures, the micellar domains are drained to an increasing extent, and the ultrafiltrates correspondingly diluted.

McBain and Jenkins (64) using Bechhold membranes, ultrafiltered soap solutions, whose colloidal and crystalloidal composition had been previously deduced from osmotic and conductivity measurements. The concentrations of ultrafiltrates through very dense membranes (maximum pore size less than 9 mμ) represented the concentrations of the crystalloidal components, which agreed with the previous data for solutions of sodium laurate below 0.8 N and sodium oleate below 0.5 N. The ultrafiltrate concentration was independent of pressure over a wide range. Excess of pressure inhibits ultrafiltration by stopping up the pores; with insufficient pressure, ultrafiltration becomes extremely slow or ceases altogether. It is only with suitably chosen intermediate pressures that deliberately planned ultrafiltration can be carried out with trustworthy information obtained regarding diameters of pores or filtered particles. Very dilute, fresh solutions (0.01 N) of the soaps were completely ultrafilterable, showing absence of colloidal components. In sodium oleate, a fractionation was effected by membranes of maximum pore size from 15 mμ to 75 mμ, the sieving being independent of porosity over this range. This was attributed to separation of ionic micelles from neutral colloids, the former passing the filters and the latter being retained. The diameter of the ionic micelle is only a few times the length of the molecule, whereas, particles

of neutral colloid of potassium laurate are slightly less than 15 mμ in diameter and those of sodium oleate are about ten times larger. The concentrations of ionic micelle and neutral colloid thus calculated from ultrafiltration were in agreement with conductivity and freezing-point data. The alkalinity of colloid-free ultrafiltrates was a measure of the degree of hydrolysis of soap solutions.

McBain and Lucas (65) filtered 0.6 N. sodium palmitate through No. 400 cellophane at 90°C; the filtrate was 0.24 N. in sodium palmitate which agreed with the concentration of simple crystalloidal soap at this temperature and concentration deduced from conductivity and dewpoint lowering.

Wintgen and Lewenthal (93) ultrafiltered colloidal chromium hydroxide sols and found that the greater the Cl:Cr ration in the micelles, the more readily filterable were the latter. In a given sol, the colloidal Cl:Cr ration remained the same even when the suspension medium was ultrafiltered off and the residue resuspended.

Beckhold and Szidon (11), using impregnated membranes, ultrafiltered colloidal zinc sulfide, cadmium sulfide, ferric hydroxide, and collargol (silver), dispersed in benzene, toluene, petroleum ether, or linseed oil. The membranes (graded roughly in terms of the concentration of collodion in the impregnating solution) were calibrated by washing out the coagulating liquid (toluene) with alcohol and water and then determining the end points in filtration of hydrosols. The end points of the organosols (org.) and the calibrating hydrosols (aq.) are arranged in descending order:

Zinc sulfide (org.)	Hemoglobin (aq.)
Cadmium sulfide (org.)	Collargol (org.)
Prussian blue (aq.)	Ferric oleate (org.)
Ferric hydroxide (org.)	Copper oleate (org.)
Collargol (aq.)	

Bargues (6), using graded silicon carbide filters, determined micelle sizes of the following:

Arsenic sulfide	0.20 μ
Ferric hydroxide	0.05 μ
Gold	0.04 μ
Copper cobalt cyanide	0.25 μ
Prussian blue	0.15 μ

He also established the heterogeneous nature of gum arabic and egg albumin. For polystyrene (molecular weight 700,000) he found that the rate of filtration is inversely proportional to the viscosity, although a solution of cellulose acetate in dioxane did not filter according to this rule, probably because of the shape of the molecule. Thus a fraction of molecular weight 57,000 did not filter through pores of 0.1 μ, although the calculated molecular diameter is 0.0043 μ.

B. Separation of Colloids from Crystalloids

The earliest collodion membranes (75,76,77) were of low porosity, and appeared to separate colloids from crystalloids effectively. The fact that collodion membranes do not always perform this separation perfectly was noted as early as 1903 by Gerstline (47), who used membranes which permitted diffusion of peptone, albumose, starch, dextrin,

albumin, and certain enzymes. Application of suitable membranes, however, preferably under conditions of normal filtration, will permit the desired fractionation.

Wild (91) ultrafiltered 0.2 % clay suspensions in a study of the retention of phosphate by clay. The suspensions were forced through collodion membranes under pressures of 60 to 80 psi. The membranes retained 3 to 4 % of the phosphate depending upon pH but independent of the presence of the clay. Consequently, small correction factors, obtained with the use of standard solutions, must be applied.

Wintgen (92) demonstrated, in a number of examples with sols of different kinds, the normal but also the frequently abnormal course of ultrafiltration by Zsigmondy's method using collodion membranes with porcelain filter plates. Even in the normal course, conclusions about the composition of the intermicellar liquid can be drawn from the composition of the ultrafiltrate only with caution. The uncertainty arises because ordinary electrolytes may undergo changes in composition during filtration through this apparatus. For example, during filtration of dilute hydrochloric acid solution the acid accumulates in the deposit on the filter and the filtrate is correspondingly less concentrated than the original solution. In addition, part of the hydrogen ions of the sol can be replaced by calcium ions during ultrafiltration, the calcium coming from the filter plate of the apparatus. These complications are avoided if collodion sacs are used. It was shown with a ferric oxide sol that the conductivity of its ultrafiltrate by

the sac method can be calculated on the basis of the Donnan equilibrium from the potentiometrically determined activities of the hydrogen and chloride ions in the sol. From these activities and the velocities of transport of the ions in the sol, the specific conductivity of the sol can be calculated. An n-valent micellar ion is dealt with in the calculations as equivalent to n-univalent ions. In equalized ultrafiltration, in which the ultrafiltrate stands in contact with the sol residue across the membrane for several days to afford opportunity to reach equilibrium, the sol may behave differently. For example, in equalized ultrafiltration of a chromium oxide sol the conductivity of the sol can be calculated by the rule of mixtures from the volumes and conductivities of the ultrafiltrate and sol residue, and the remixing of the ultrafiltrate and sol residue gives a sol with conductivity equal to that of the original sol. With a ferric oxide sol there are distinct, though small, departures from additivity, probably because of a giving up of HCl from the ferric oxide micellar ions.

McBain (64) has advocated cellophane for the separation of colloids from crystalloids and has employed it in the study of various colloidal systems.

Boeseken and Meyer (16), not succeeding in preparing collodion membranes sufficiently tight to retain dextrin, used membranes of copper ferrocyanide impregnated in collodion; these permitted reducing sugars to dialyze, but almost completely retained dextrin of molecular weight 5500.

For a more rapid separation of crystalloidal electrolytes from colloidal sols than can be effected by dialysis, electrodialysis, or ultrafiltration, electroultrafiltration may be employed (9).

C. Sieving of Crystalloids

Collander (22) studied the impeded diffusion of many organic acids and other compounds through flat collodion membranes, prepared in three porosity grades. The rate of diffusion was related antibatically to the molecular size as measured by the molecular refractivities. Exceptions were phenol and m-nitrophenol, which diffused abnormally rapidly and were thought to be soluble in the membranes. To each membrane grade corresponded a maximum size of diffusible molecules.

Ershler (38), using rather thick ether-alcohol collodion membranes, reported that, under the same conditions of ultrafiltration, crystalloidal electrolytes might be retained to a much greater extent than non-electrolytes. The relative concentration of filtrate from a solution of a non-electrolyte was practically independent of the absolute concentration of the latter, and for electrolytes, the relative concentration of filtrate increased markedly with increasing absolute concentration of the original solution. The degree of retention of an electrolyte was the greater, the higher the valence of the ion charged unlike the membrane. This supported the explanation that the greater retention of electrolytes was due to repulsion of similarly charged ions

from the pore walls, resulting in a diminution of the effective pore diameter. He claimed that the failure to obtain complete filtration of an electrolyte with a membrane which would filter a non-electrolyte completely is not a test for colloidal constituents, as postulated by McBain (67). According to McBain, it was demonstrated, with cellophane membranes, that colloidal constituents are commonly present in non-aqueous solutions of the electrolytes silver nitrate, ammonium iodide, cadmium iodide, etc., which exhibit anomalies in electrical conductivity and osmotic behavior.

Hacker (49) determined the filtering effect of a collodion membrane for dilute aqueous solutions of hydrochloric acid, lithium chloride, sodium chloride, potassium chloride, ammonium chloride, magnesium chloride, and barium chloride. His apparatus was fitted with a stirring mechanism in order to keep in motion the layers of solution next to the membrane. The changes in concentration of the electrolyte due to the filtering effect of the membrane were determined by changes in conductivity of the solutions. The filtering effect of the membrane was too small to be measured with lithium chloride, magnesium chloride, and barium chloride. For the remaining electrolytes the measurable effect increased with the intensity of agitation of the solution on the filter. With hydrochloric acid solutions the filtering effect increased with decreasing concentration of the electrolyte. In a latter paper (50) he reported that the changes in specific conductivity of the ultrafiltrate samples during

the ultrafiltration of a ferric oxide sol are attributed to the joint effects of the Donnan equilibrium and the screening effect of the collodion membrane.

Trautmann (84) filtered 0.0017 M aqueous solutions through cellophane, using a pressure ultrafilter, and he found that potassium sulfate and disodium phosphate were retained to a marked extent by the filter; potassium iodate was retained to a lesser degree; potassium chloride, sodium chloride, and potassium iodide about one-third as much as potassium sulfate; magnesium chloride and calcium chloride were held back to a very slight extent; and urea and glucose not at all. Where there was retention of a salt, the filtrate began to have the same concentration as the original unfiltered solution only after about two-thirds of the solution had filtered through.

D. Determination of Degree of Hydration

It is possible to determine the degree of hydration of solute particles by ultrafiltration by using a reference substance which passes a filter retaining the solute in question (39). It must be assumed that the reference substance does not effect the solvation equilibrium and is itself not solvated, and that the ultrafiltrate represents the interparticulate fluid far from the influence of solvation. Then the filtrate appears to have been concentrated in the reference substance because of retention of solvated solvent.

In this way, McBain and Jenkins (64) using potassium

chloride as a reference substance, determined the hydration of potassium laurate to be approximately twelve water molecules per soap molecule. The extensive study of McBain, Kawakami, and Lucas (65) showed that this value was independent of the concentration of soap or salt at high ionic concentrations; internal Donnan effect was shown to be then suppressed. At low ionic concentrations the Donnan effect enters, and causes the apparent hydration to be two to three times as great.

VII. Quantitative Estimation of Particle Size

The remarks in the preceding sections have been confined to the behavior of ultrafilters in the filtration of different disperse systems; filtration end-points have been mentioned, expressed in terms of average pore diameter, without any attempt to deduce from these figures the sizes of the particle retained.

There are more direct procedures than ultrafiltration for determinging particle sizes (39). Among these methods, the most powerful at present is probably that of ultracentrifugation, which is applicable to both monodisperse and polydisperse systems. Accurate application of that method, however, demands elaborate apparatus and a means of analyzing the system while in the process of centrifuging (82). The optical methods employed for analysis require a substantial concentration of the disperse phase and the absence of foreign constituents of similar optical properties. These conditions are in some cases difficult to fulfill. The most

direct method of measuring the sizes of particles is by microscopy, with either the light microscope, which is applicable down to diameters of 0.2 μ, or the electron microscope which is applicable down to diameters of about 2 mμ. In either case, the process of measuring and counting individual particles is very tedious, especially in polydisperse systems.

Ultrafiltration will always be a most valuable technique for preparing homogeneous and homodisperse systems to which the more elaborate methods of particle size estimation can be applied, and may also be applied to estimation of particle sizes in cases where other methods are unsuited(39). Ultrafiltration may also be used to determine the percentage of particles above a certain size range. This would be a very desirable application in the pigment field.

The procedure, used for the estimation of particle size by means of ultrafiltration, consists of determining the filtration end-point porosity and applying to that figure an empirical correction factor representing the ratio, diameter of particle/end-point average pore diameter. The correction factor, which is itself a function of the end-point porosity, is determined by filtration of systems of known particle size.

A. Experimental Requirements

In order that the correction factor (for a given end-point porosity) be the same for the system whose particle size is to be determined as for that whose particle

size is known, experiments must be carried out under comparable conditions (39). It is especially important that normal filtration is obtained. For example, a comparison of the end points of serum albumin (81), with the known particle size (5.4 mu) shows that, in the center of the pH zone of abnormal filtration, the correction factor has the very low value of 0.12; while on either side, with normal filtration, it is as high as 0.5. Abnormal filtration is invariably associated with an abnormally low correction factor, and one which is more sensitive to slight modifications in experimental conditions, and unsuited for quantitative comparisons. The criterion of normal filtration, in terms of filtration curves (figures 1 and 2), must be established. In selecting uniform experimental conditions, it is convenient to employ a capillary-active substance in the suspensions. Hartley's brother at pH 7.4 to 7.8 has proved to be a suitable standard for proteins and all protein-like systems, while for lyophobic colloids, inorganic stabilizing agents are perhaps better. The membranes used for quantitative comparative work must have high reproducibility and be highly isoporous.

B. Determination of the Correction Factor

The empirical correction factor for converting the end-point porosity (average pore diameter), for filtration in a standard solvent, to the particle diameter of the disperse phase was determined by Elford (87) for various suspensions of known particle size. His results showed that the empirical correction factor, when plotted against the end-point porosity, passed through a minimum at about

25 mμ average pore diameter. Also, the ratio - particle size/true size of pores retaining particles - decreased uniformly with decreasing pore size. On the other hand, the ratio of the average pore size, as determined by the calibration methods of page 28, to the true pore size, fell off only slightly down to about 20 mμ, and then rapidly. The ratio of these two quantities thus estimated gives values for the ratio - particle size/end-point average pore diameter - which fall on the experimental curve, showing a minimum. The average pore diameter of calibration is always smaller than the true pore diameter, and the size of particles retained is always smaller still. Since the correction factor includes any discrepancy between the calibration porosity and the true dimensions of the pores, such discrepancy introduces no error in the estimation of particle sizes by application of the factor.

C. Experimental Results

The particle sizes of various viruses, bacteriophages, and spirochetes have been determined by Elford and other workers, using Elford's correction factors (39). The sizes of particles of bacteriological systems were estimated by correction factors which were determined, at the top of the porosity range, by filtration of other bacteriological systems, and, at the bottom of this range, by filtration of chemical systems.

The particle sizes obtained, using this method, were practically identical to those obtained by other methods of

measurement, such as, ultraviolet microscopy and ultracentrifugation. These particles covered the size range of 10 to 175 mμ.

In the future, it is likely that other physical methods will be developed to the point where they will yield more reliable determinations of particle size than untrafiltration. Ultrafiltration will probably remain the most valuable technique for preparing homogeneous and homodisperse systems to which the more elaborate methods can be applied (39).

VIII. Conclusions

After a critical investigation of the literature of ultrafiltration, the following conclusions may be drawn:

1. Although there is a wealth of literature dealing with ultrafiltration and its applications, very little mention is made of applications in the pigment field. Of course, Prussian blue, an iron blue pigment, has always been a classic example of a colloidal sol and is mentioned widely in the literature, in some cases as a standard for particle size determinations (approximate size is 40 mμ). As far as the problems concerning the analysis and evaluation of Prussian blue and other colloidal pigments are concerned, no direct reference was found.

2. The use of ultrafiltration in the determination of water-soluble salts in colloidal pigments would involve a critical experimental study of all the factors which may influence the separation of the water-soluble materials from the colloidal pigment particles. Among these are the following:

adsorption or blocking by the filtering medium, adsorption of the water-soluble material by the pigment, concentration of the aqueous phase, and the pressure and rate of filtration. Any of these factors could prevent a complete separation of the water-soluble material from the pigment, but it may be possible, through careful control of the techniques involved, to eliminate them or use correction factors obtained in a control experiment. In cases where the water-soluble materials are essentially all electrolytes, it is possible that electroultrafiltration would be much more effective.

3. In the determination of "bleed" tests of colloidal pigments in water, alcohol, and other solvents, the same potential difficulties as those mentioned above would pertain. Here too, a critical investigation of the techniques would be required.

4. The determination of the particle size of pigments by means of ultrafiltration alone would be desirable in cases where the percentage of particles in each of several size ranges is sufficient. (In most pigments, the particle size varies over a rather broad range). This could be done by the use of commercially available, carefully graded membranes having pore sizes in the proper ranges.. It would be comparatively simple and rapid to filter a dispersion of a pigment in water or other liquid through a column type filter, containing a series of membranes having progressively smaller pore sizes, much like a sieve classifier. From the weight of pigment retained on each

graded membrane, the particle size distribution could be obtained. The percentage of "over-size" particles could be determined by using a single membrane having pores in the proper size range.

5. For the separation of pigment from a vehicle, such as in an oil paint or enamel, the following types of ultrafilters may be applicable: cloth impregnated with cellulose acetate, unglazed porcelain impregnated with acetic collodion, specially treated cellophane, and graded silicon carbide. It would probably be necessary to dilute the paint system with a suitable solvent to effect a more rapid rate of filtration. In cases where water-dispersible pigments are used, such as in rubber latex and resin emulsion paints, most any type of carefully graded filter may be used.

6. In the separation of pigments of different Particle sizes in a mixture it would be necessary to prepare a dispersion in a suitable liquid and pass it through a carefully graded filter having pore sizes small enough to remove the larger particle size component and large enough to pass the smaller particle size component.

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