REVIEW ARTICLE

SURFACE AND COLLOID CHEMISTRY

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INTRODUCTION

SURFACE chemistry deals with the properties of the boundary or surface layers which separate two phases from one another. There are four types of surfaces (often called interfaces) of principal importance, these are the boundaries between a gas and a liquid, between two liquids, between a gas and a solid and between a liquid and a solid. If the two phases are in equilibrium with one another the interface is stable, e.g., water/hexane. If the phases are not in equilibrium, the interface is unstable and if left, diffusion or other changes will occur, e.g., water/aqueous sugar solution.

Substances present in surface or interfacial layers often have altered properties and the compositions of these layers differ from those of the bulk phases in contact with them. A substance which has a higher concentration in an interfacial layer than in one of the bulk phases, is said to be positively adsorbed at the interface.

Colloid chemistry deals with matter in a state of very fine subdivision in which each particle has a high surface/volume ratio. The principles of surface chemistry therefore largely govern the special properties of colloids. The subject of surface chemistry is thoroughly dealt with in Adam's book "The Physics and Chemistry of Surfaces"[1] while colloid chemistry is surveyed by Alexander and Johnson in their book "Colloid Science"[2]. Both these subjects are, of course, of considerable importance to pharmacists who are dealing largely with colloidal material of one form or another.

Many of the biological effects produced by drugs can be traced to mechanisms involving adsorption at interfaces within living systems and a knowledge of surface chemistry is necessary in attempting to interpret the biological activities of compounds in terms of their molecular structures and physical properties. Further studies of the adsorption of such compounds at appropriate surfaces can be expected to yield results of practical value to the synthetic chemist in the design of new drugs, and to the pharmacist in formulating the best methods of delivering drugs in the body so as to achieve maximum adsorption at the correct interface. Some direct applications of surface chemistry techniques of interest in pharmacy are the assays of surface active materials by direct measurement of the lowering of surface tension which they produce when dissolved in water, and the estimation of specific surface areas (i.e., area/g.) of pharmaceutical powders. The specific surface of a powder gives a convenient measure of the fineness of subsieve powders and is

related directly to the rates of solution and therefore the therapeutic efficiency of sparingly soluble powders. The position of surface chemistry in relation to biology has been reviewed by Rideal[4] and Alexander[5]. This review deals with some aspects of surface and colloid chemistry whose further development should be of application in pharmacy.

Surface Tension and Surface Activity

The surface tension of a liquid can be defined as the work which must be performed to produce 1 sq.cm. of new surface at constant temperature. Surface tension refers to the gas (usually air)/liquid interface, the work required to produce 1 sq.cm of new surface at a liquid/liquid interface is usually termed the interfacial tension of the pair of liquids. Both quantities are best expressed in the units of ergs/sq.cm. though they are still often given in dynes/cm. (these units are numerically equivalent, 1 erg/sq.cm.=1 dyne/cm.). Experimental methods of determining surface and interfacial tensions are summarised by Adam[1].

Many soluble organic compounds reduce the surface tension of water very considerably when present in low concentrations, particularly if the compound contains a hydrophilic group (e.g., carboxyl; sulphonate radical). This effect is due to adsorption of the solute at the air/solution interface, i.e., the solute concentrates at the surface of the solution. By determining the surface tensions (and also the thermodynamic activities) of solutions of different concentrations the actual amount of solute adsorbed at the surface can be calculated in moles/sq.cm. by means of the Gibbs adsorption equation (see Adam[1]). The adsorption layer, that is the region in which excess concentration occurs, appears to be only one molecule thick in many cases.

Substances which are strongly adsorbed at the surface of a solution are called surface active. Substances which are highly surface active at an air/aqueous solution interface are usually also strongly adsorbed at an oil/aqueous solution interface, since the hydrophobic (hydrocarbon) group in their molecule is oil-soluble. The surface activity of a compound can be rather loosely defined in terms of the molar concentration required to produce a given surface tension lowering when dissolved in water (low concentration means high surface activity). Surface activity is generally used as a comparative term within a series of compounds.

The surface activities of ionised organic compounds are very much a function of *p*H. Un-ionised molecules are adsorbed more readily at an air/aqueous solution interface than are ions, since the electrical charges on the latter repel each other and hinder the build-up of a close-packed adsorption layer. This effect is illustrated by the fact that whereas heptoic acid lowers the surface tension of water very considerably at low concentrations, ammonium heptoate shows only slight air/water surface activity (Rehbinder[6]). The relations between surface tensions and *p*H of aqueous solutions of a number of organic bases including alkaloids, have been studied by a group of Polish workers (Goslawski[7], Kamienski[8,9], Zapior[9,11], Waksmundzki[10]). Some recent

fundamental studies of the adsorption of materials at the surfaces of solutions are those of Addison[12] who has described a method for studying the rate at which adsorption onto a freshly-formed surface occurs, and Judson, Argyle, Salley and Dixon[13] who have determined surface concentrations on solutions directly by means of radioactive tracers.

The relation between air/aqueous solution surface activities and biological activities of series of chemically similar drugs, have formed the subject of a number of researches. Traube[14] reported a direct relation between surface and physiological activities of a number of narcotic substances. Weber and Legoix[15] measured the surface tensions of a large number of medicinal plant infusions, but apart from the observation that purgative solutions had exceptionally low surface tensions, they could trace no general relation between surface tension and pharmacological activity. Szende and Telbisz[16] reported that the spasmolytic activities of a series of alkaloids of the papaverine type bear a direct relation to their surface activities. Williams[17] noted the effect of changes of surface tension of the medium or the growth of some pathogenic fungi. Regnier and David[18] could find no general correlation between surface activity and anæsthetic action in aqueous solutions of procaine and its analogues. Marron and Moreland[19] found no direct relation between the surface activity of alcohols and their effects on a number of enzymes such as phosphatase and urease. Albert, Goldacre and Heymann[20] found no correlation between surface activity and antiseptic properties of solutions of acridine derivatives. Hammick and Mason[21] could find no direct connection between surface activity and antimalarial activity in the acridine series. Hirt[22] reported a correlation between spasmolytic and surface activities in a series of tertiary amines. Stacey and Webb[23] showed a simple relation between bacteriostatic power and surface tension lowering of nutrient medium by bile salts. Rideal[4] quotes a number of cases in which the germicidal action of a compound is related to its surface activity.

The rather poor correlation between air/aqueous solution surface activity and biological activity of drugs is not surprising. The surface activity of an organic compound at this interface really measures its tendency to be thrown out of aqueous solution on to a surface with a phase less polar than water and this is likely to be only one of several mechanisms governing adsorption on to surfaces in living systems. These mechanisms have been discussed by Danielli[24] who points out that the resultant adsorption of a drug at a biological surface may be made up of three main factors.

(1) Adsorption by ionic forces due to electrical charges on the surface.

(2) Adsorption of hydrophobic (hydrocarbon) groups in the drug molecule on to oily parts of the surface (or adsorption by van der Waal's forces between hydrocarbon chains already in the surface and similar chains in the drug molecule).

(3) Adsorption by interaction between polar but un-ionised groups in the drug molecule and on the surface.

Only factor (2) is related to the air/aqueous solution surface activity

of a drug and unless this is the principal mechanism of adsorption, no simple correlation with biological activity can be expected.

Albert, Goldacre and Rubbo[25] have shown that the bacteriostatic activities of a series of acridine derivatives are directly related to their dissociation constants, that is to the fraction of the total drug concentration present in the ionised form at physiological *p*H values. In this case, mechanism (1) appears to be the principal factor governing the total adsorption of drug at the bacterial surface.

In order to obtain a large enough overall adsorption of drug to produce a clear biological effect, it is necessary in many cases to have a close fit between the drug molecule and the biological surface so that all three mechanisms can contribute to the adsorption. This of course accounts for the great variation of biological activity among groups of compounds whose chemical structures differ only slightly, and effects of this type should be of value in obtaining more information about the stereochemistry of different biological surfaces.

These three mechanisms of drug adsorption can all be evaluated to some extent by physico-chemical methods. Mechanism (2) can be examined by determining air/aqueous solute or better oil/aqueous solution surface activities and also by the study of penetration of monomolecular films of lipoids (i.e., fatty or oily substances) such as cholesterol on water. Mechanisms (1) and (3) can be studied by observing the binding of ions and polar molecules by proteins in bulk solution and by observing the penetration of protein monolayers spread on water. Some of the following sections deal with these methods of studying biological adsorption.

Interfacial Tension Measurements

A number of workers have investigated the adsorption of compounds at the oil/water interface by measuring the effect they exert on the interfacial tension. Surface activity at this interface should give a better measure of the magnitude of adsorption mechanism (2) for a particular compound, than the air/water surface activity derived from surface tension measurements.

Alexander and Teorell[26] has discussed different methods for studying interfacial films. Hauser and Michaels[27], Addison and Hutchinson[28] and Adam[1] describe experimental methods for measuring interfacial tensions. Hutchinson[29] has determined the effects of a number of fatty acids and alcohols on the tension at the water/benzene interface and reports areas/molecule of solute greater than those at the air/water interface.

To produce interfaces having a closer relation to biological surfaces, a number of workers have spread films of proteins or lipoids at the oil/water interface and examined the effect of different biologically active compounds on the resulting interfacial tensions. Seelich[30] describes the effects of narcotics such as chloroform or alcohols on the tension at an oil/water interface in the presence of lecithin. He suggests that his results could form a basis for a general physical explanation of narcosis.

Tammelin and Löfgren[31] have described the effects of anæsthetics on a water/mineral oil, containing 0·05 per cent. of ergosterol, interface. They conclude that the adsorption of anæsthetic at this interface together with its solvent effect on lipoid material already present at the interface, could be considered as the source of anæsthetic action. Other recent papers dealing with adsorption at oil/water interfaces are those of Ward[32], Guastella[33] and Frazer[34]. Spread protein films at this interface have been examined by Cumper and Alexander[35].

Insoluble Monomolecular Films

A number of insoluble lipoids spread when put onto a clean water surface, to form a layer which is only one molecule thick. By studying the compression of these films and their surface potentials a number of important results have been obtained. The properties of insoluble films are summarised by Adam[1]. Proteins which are normally water-soluble can be spread to form similar films on a water surface.

The penetration of protein and lipoid films on water by substances dissolved in the water underlying the films, has been extensively studied at Cambridge. Interesting results have been obtained, particularly with penetrants which show cytolytic activity. Schulman and Rideal[36] showed a clear relation between film penetration of cholesterol and protein films and lytic activity in a number of compounds. Ruyssen and Croes[37] state that the hæmolytic activities of a series of lysins is parallel to their ability to penetrate a cholesterol film rather than to their air/water surface activities. Rideal[4] summarises results of studies of the relation between film penetration and lytic activity.

Surface Forces in Living Cells

A number of measurements of the surface forces of living cells are summarised by Harvey[38] (1937). In general low values of the order of 0·08 to 0·20 ergs/sq. cm. were found. These low values were attributed to the presence of adsorbed films of protein on either side of an oil film forming the cell wall. This simple picture of cell surface structure is not however supported by electron miscroscope and other studies of erythrocyte ghosts summarised by Ponder[39], which indicate that the surface layers of erythrocytes are heterogeneous and have a thickness of the order of 200 Å. The lipoid material appears to be contained in lenses in a colloid structure, rather than in a continuous film.

Norris[40], reported a maximum surface force of 1·4 ergs/sq. cm. for nucleated erythrocytes. In 1942 he published a review of the physical properties of living matter, including the results of cell surface force measurements[41].

These surface forces are not quite the same as interfacial tensions, as has been pointed out by Norris, they can, however, be considered roughly as cell/environment interfacial tensions. Their low values suggest that the oil/water interface with its tension of about 40 ergs/sq. cm. is not a particularly good model for studying adsorption at biological surfaces,

though if the heterogeneous nature of cell surfaces is accepted there may be patches of completely oily material in the surface at which adsorption similar to that at the oil/water interface will occur.

Coacervation

Coacervation is the separation of a colloid rich liquid phase from an aqueous solution of a colloid. Coacervates may be obtained by some salting out processes and also by mixing colloids of opposite electrical charges, for example gelatin and gum arabic solutions at appropriate *p*H. The preparation and properties of coacervates have been extensively studied by Bungenburg de Jong and his colleagues in Holland and their work has been summarised by Kruyt[42]. Coacervation has been recently reviewed by Dervichian[43]. According to Bungenburg de Jong's school, cell membranes or surfaces can be regarded as complexes of phosphatides, proteins and cations (Booij[44]) containing lipoids.

The reason for introducing coacervation at this point, is that the coacervate/equilibrium liquid interface is a stable one between two aqueous liquids, having a very low tension, and in these respects it very much resembles the surface of a cell; it should therefore provide a useful model for the study of biological adsorption.

Ruiter and Bungenberg de Jong[45] succeeded in measuring the gelatine-gum arabic coacervate/equilibrium liquid interfacial tension by a modification of the capillary rise method, they obtained values of the order of 2×10^{-3} ergs/sq. cm. At these interfaces of very low tension, ionic and other electro-chemical effects (e.g., interaction between polar groups) are likely to play a greater part in determining adsorption than the factors which determine air/water or oil/water adsorption.

Binding of Ions by Proteins in Solution

The interaction of ions and molecules with proteins in solution can be expected to give some information of use in evaluating mechanisms (1) and (3) for biological adsorption. The combination of lipoid materials with proteins formed the subject of a recent Faraday Society discussion[46] in which Luck[47] reviews the literature dealing with the binding of ions by proteins. The complexes of ions with proteins have also been reviewed by Klotz[48]. The subject has been studied by a number of techniques including dialysis equilibrium, ultrafiltration and spectrophotometry. Many of the studies have been carried out using serum albumen. According to Klotz[48], this protein shows a non-specific binding of a large variety of anions. However, some are bound more than others and an examination of the basis of these differences should lead to useful results which should perhaps give some detailed information about the configurational patterns at the surface of a protein molecule in solution.

Phase Boundary Potentials

The effects of dissolved substances on the electrostatic potential between two immiscible liquids can be used to study adsorption at such interfaces.

Beutner[49] considers that in some cases the effects of drugs on these potentials can be directly related to their pharmacological activities.

Analytical Applications of Surface Tension Measurements

Fialkov and Etinger[50] pointed out that since the surface tensions of a number of tinctures and infusions (e.g., adonis, digitalis, valerian) decrease as the concentration of active material increases, the quality of these preparations can sometimes be judged from measurements of their surface tensions. Izmailov and Shvartsman[51] suggested the use of surface tension measurements to assay the oil content of peppermint water. Izmailov and Shustova[52] studied the surface tensions of solutions of a number of alkaloids and concluded that these measurements could be used for the assay of some alkaloids in solution.

Kaunitz *et al.*[53], decided that surface tension measurements were the best method for estimating bile salts in blood serum, protein variation having little effect on this tension. Surface tensions of mixtures of serum and bile salts have been more recently studied by Tayeau and Blanquet[54]. Picon[55] reported that measurements of interfacial tensions of blood sera could be used to indicate the resistance of sera to flocculation, maximum stability being indicated by high interfacial tensions.

Etinger and Baron[56] proposed the determination of surface tensions of acidified solutions as a method for assaying acetates. The author[57] has found a differential method based on the maximum bubble pressure method, particularly suitable for quantitative estimation of small quantities of materials such as heptoic acid in aqueous solution. A further improvement of this method has been made (Russell and Saunders, unpublished), which should make it a convenient apparatus for analytical use.

Soaps

Originally the term soap was used to describe the salts of long-chain fatty acids. Alexander and Johnson[2] have proposed that this name should also be applied to the many synthetic materials of soaplike structure now available commercially, and in this sense the word is used in this review. The soaps consist of salts containing long chain surface active (hydrocarbon) groups in their molecules. On dissolving in water this group tends to be thrown out of solution and, in dilute solution, this occurs by adsorption at the air/liquid surface. In more concentrated soap solutions, the hydrocarbon groups are ejected by formation of micelles which consist of ions of colloidal dimensions formed by an agglomeration of the surface active ions so that hydrocarbon chains are out of contact with water. As a result of this effect soaps are often called colloidal electrolytes. The properties of their solutions have been reviewed by Hartley[58] and by Berry and Bean[59].

The classical soaps all contain the hydrophobic group in the anion (e.g., sodium stearate), synthetic soaps have now been prepared with surface active cations (e.g., cetyl pyridinium chloride). Another class

of synthetic soaplike materials are the non-ionic soaps formed by attaching the water-soluble but unionised polyethylene oxide grouping to a hydrocarbon chain. The surface activities of these compounds are less dependent on *p*H than are those of the anionic or cationic soaps.

The main applications of soaps depend on the wetting and detergent powers of their solutions. The relations between these properties and molecular structure of the soaps have been discussed by Price[60] and Chwala[61], while Shedlovsky[62] and Roebuck[63] outline some of the methods used for the quantitative assessment of wetting and detergent power. The latter is largely dependent on micelle formation and is discussed by Courtney-Harwood[64]. Wetting power is more a function of the surface tension lowering produced by the soap. The relation between surface tension lowering and wetting power has been studied quantitatively by Reutenauer and Sicard[65] who found good correlation for classical soaps but not for the synthetic soaps.

Due to their hydrocarbon chains, soaps are surface active at both air/water and also oil/water interfaces. In addition, owing to the electric charges which they carry, they are also strongly adsorbed onto protein surfaces at appropriate *p*H values. In consequence of this all-round surface activity, soap solutions have considerable biological activity.

The position of soaps in biology and medicine has been reviewed by Valko[66] and their bactericidal properties have been discussed by Hotchkiss[67]. As examples of the diversity of their biological activity we can quote the following effects; Goldschmidt and Koffler[68] report that yields of penicillin by biosynthesis are stimulated by anionic soaps and by the corresponding fatty acids; Hughes[69] states that the capacity of cationic soaps to accelerate glutamate decarboxylation is parallel to their capacity to form micelles; Glassman[70] has shown that two non-ionic soaps are incapable of producing hæmolysis even in concentrations at which they depress the surface tension of water to 40 ergs/cm.^{2} whereas ionic soaps are strongly hæmolytic at such concentrations, here the biological effect of lysis is clearly dependent on ionic adsorption (mechanism (1)); Cole, Hulpieu and Hopper[71] have shown that the duration of hypnosis produced in mice by barbiturates is increased by all types of surface active agents. Soaps are usually considered as bacteriostatic substances, but Williams and Rees[72] claim that at least two types of anionic soaps support the growth of some bacteria. Holmes[73] has shown that several sodium salts of fatty acids inhibit protein precipitin reactions whereas the corresponding acids have no effect, here the activity is clearly a function of ionisation rather than of surface tension lowering (mechanism (1) rather than mechanism (2)).

A number of analytical methods have been devised for the quantitative estimation of soaps. Many of these, including the purely chemical methods, are reviewed by Sexton[74]. An obvious physical method of estimation is to determine the surface tension of the soap solution and this is suitable for very dilute solutions in which the surface tension is roughly a linear function of soap concentration. In more concentrated solutions however, the surface tension becomes almost constant and inde-

pendent of concentration. Many commercial soap solutions show minima in their surface tension-concentration curves and such minima have been considered as characteristic of colloidal electrolytes (*e.g.*, see Hauser[75]). However, these minima have been shown to be due to the presence of hydrocarbon impurities in the soaps (Brady[76], Miles and Shedlovsky[77]) and are eliminated if the soap solution is extracted with ether or purified by foam fractionation. Foam fractionation is a useful method of purifying surface active substances in solution, details of the technique are given by Schütz[78] and the results of the foam fractionation of bile salts are described by Bader and Schütz[79].

Another method for determining surface active substances and soaps in dilute solution is the polarographic adsorption analysis method described by Schwarz, Schröder and Stackelberg[80] which depends on the ability of these substances to suppress current maxima in polarograms.

A third method of anlysis of ionic soaps is described by Preston[81]. This is a titration method based on the formation of complexes between anionic and cationic soaps when they are present in equimolecular proportions in solution. If the concentration of a cationic soap solution is to be determined, it is titrated with a standard anionic soap solution, continuous observation of the surface tension of the mixture during titration is made by observing the maximum pressure required to blow bubbles from the tip of a capillary immersed in the mixture, after each addition of standard solution. The end point of the titration is shown by a sharp change in the surface tension corresponding to the formation of the equimolar complex.

A similar volumetric method described by Lambert[82] is based on the relative insolubility of this equimolar complex. Titration of cationic with anionic soap is carried out as before, the end-point being the point at which the turbidity of the solution is a maximum. The turbidity can be observed continuously during the titration by a photoelectric arrangement. The use of ionic soaps as indicators in ordinary acid-base titrations was suggested by Lottermoser[83]. Since the surface activity of these soaps varies with *p*H, the end point of the titration is indicated by a sharp change in the surface tension of the solution. The well-known application of soaps as emulsifying agents is outlined by Alexander and Johnson[2]. An interesting case of spontaneous emulsification of hydrocarbons in water by means of cationic soaps, is described by Kaminski and McBain[84]. Finally, another well-known application of soaps is their use in solubilising bactericidal materials which are only sparingly soluble in water. Some of the effects of colloidal electrolytes on other biologically active compounds are described by Alexander[85]; Harkins[86] and Klevens[87] discuss the general theory of solubilisation by colloidal electrolytes. The relation between solubilisation of a phenol by aqueous soap solutions, and micelle formation, is shown by Bean and Berry[88].

Specific Surface Area of Solid Powders

The specific surface of a powder is the surface area per g. As the mean particle size of the powder decreases the specific surface increases

and so specific surface is a convenient quantity for describing the fineness of powders.

The importance of reasonably exact measurements of the fineness of powders is being realised by many sections of chemical industry (see Symposium on Particle Size Analysis[89]). Particle size analysis of coarser powders can be carried out by wet or dry sieving for powders containing particles down to 40 μ diameter (1 $\mu = 10^{-3}$ mm.), by direct microscope counts (down to 2μ), by sedimentation (down to 2μ) or by sedimentation accelerated in the centrifuge (down to 0·5 μ). If only the mean particle size of a relatively coarse powder is required, the permeability method developed by Carman[90] and Lea and Nurse[91] is experimentally convenient. However, it gives results which are not in agreement with those found by other methods for very fine powders, as the author[57] has shown in the case of very fine alumina powders. Rigden[92] and Carman and Malherbe[93] have published corrections to the equations used for calculating specific surface from air permeability data and improved apparatus for the study of permeability, but the method is still not altogether satisfactory for studying very fine materials. All these and other methods of measuring particle sizes and specific surfaces are described by Dallavalle[94]. The relation between mean particle size and specific surface of a powder is given by the simple equation. $S = \frac{6}{\rho d}$ where S is the specific surface in sq.m./g. ρ is the density of the material of which the powder is composed; d is the mean particle size in microns. This equation assumes that the powder can be treated as a set of spheres each having the mean particle size as diameter. Measurement of S and d for very fine powders is complicated by the fact that these powders tend to aggregate into clusters and the experimental result may be the mean size of these aggregates, rather than that of the ultimate particles.

The development of the electron microscope has extended the application of direct microscope counts down to powders of particle size, 50 to 100Å (1Å $= 10^{-8}$ cm.). Techniques for preparing electron micrographs of very fine powders are given by Watson[95]. The electron microscope may give aggregate particle sizes rather than ultimate particle sizes, as is shown by the work of Anderson and Emmett[96] on carbon blacks.

Methods for determining reliably the ultimate particle sizes of very fine powders depend on adsorption effects. The general principle of these methods is the adsorption of a monomolecular layer of material of known area/molecule on to the solid. From the weight of material adsorbed per g. of powder, the specific surface can be calculated, and this is, of course, simply related to the mean particle size.

The application of gas adsorption measurements for reasonably accurate determinations of the specific surface of very fine powders originated with the work of Brunauer and Emmett[97] on iron catalysts for synthetic ammonia production. The development and practice of this method is described by Brunauer[97]. Briefly, the technique is as follows. A known amount of a chemically inert gas (often nitrogen) is put into contact with a small sample of the powder which has been freed from

gas by heating in a high vacuum. The powder should be at a temperature only a few degrees above the atmospheric boiling-point of the condensed gas. As a result of adsorption of the gas on to the powder, the pressure falls, and from this pressure drop the amount adsorbed can be calculated. More gas is then admitted and the measurements repeated. The amount of gas adsorbed per g. of powder (x) is plotted against the equilibrium gas pressure (p) after each new addition of gas, to give an adsorption isotherm.

If adsorption only proceeds to the formation of a monomolecular layer of gas on the powder, the isotherm will be of the Langmuir form (I in Fugure 1), the total adsorption increases with p until the monolayer is built up, and then further increase in p produces no further increase in x.

For most gases, particularly near their boiling points, the adsorption isotherm takes the form II (Fig. 1). This is considered to represent adsorption to form, first, a monomolecular layer, then polymolecular layers, and finally condensation of gas in the capillaries between the powder particles. This isotherm has three sections, AB monolayer type of curve, BC intermediate sloping, linear portion, CD steeply sloping curve.

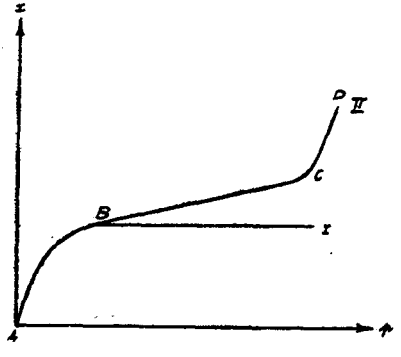

FIG. 1

The detailed theory of the adsorption isotherms of gases on solids is given in Brunauer's book[97], and has been discussed by a number of authors, including Gregg[98], Harkins and Jura[99], Tompkins[100], Everett[101] and Jones[102]. The last-mentioned writer has pointed out that the original Brunauer-Emmett adsorption isotherm equation was first developed by Langmuir[103] in 1918. There is some disagreement about the energy changes involved in multi-molecular adsorption, but it is generally agreed that for isotherms of type II it can be presumed that a complete uni-molecular layer of adsorbed gas is formed at the point B at which the adsorption curve (AB) changes into the intermediate linear portion (BC).

This result was originally obtained by Brunauer and Emmett[97], who showed that points B for a number of different gases adsorbed on to a standard powder gave almost the same value for the specific surface of the powder. If point B does represent the completion of a unimolecular adsorbed layer, then experimental measurements of x and p for a gas of known area per molecule can be used to determine the number of moles of gas required to form this layer, and from this the total surface area of the powder and its specific surface can readily be calculated. This method of determining specific surfaces from gas adsorption isotherms is often called the B.E.T. (Brunauer-Emmett-Teller) method.

Nitrogen is frequently used as adsorbate, however, since this involves measurements at very low temperatures, a number of other inert gases, boiling at more reasonable temperatures, are often used. For example, Russell and Cochran[104] have used *n*-butane vapour at 0°C. to determine the specific surfaces of alumina powders. Livingston[105] has listed the areas/molecule of a number of suitable adsorbates. Benson and Ellis[106] have used the B.E.T. method to determine specific surfaces of dried protein powders. Swintosky, Riegelman, Higuchi and Busse[107] have used low temperature nitrogen adsorption to determine the specific surfaces of a number of pharmaceutical powders. They have pointed out that the specific surfaces of sparingly soluble medicinal powders are important because they are directly related to the rates of solutions and, therefore, to the therapeutic responses of the powders. They have found that the specific surfaces of different pharmaceutical powders vary over a very wide range depending on the nature of the powder and its method of manufacture. They have also shown that some of the standard techniques for levigating fine powders such as zinc oxide have little effect on the specific surface and ultimate particle size of the powder.

The B.E.T. method for specific surface determination is rather inconvenient for routine work since it involves use of high vacuum apparatus. A number of alternative methods have been developed, based on adsorption from solution, which overcome this difficulty. The earlier solution adsorption techniques usually involved shaking a weighed amount of the powder with a solution of a dye and estimating the amount of dye adsorbed, by spectrophotometric methods. However, as the area/molecule of most dyes is rather an uncertain quantity, a number of workers have suggested the use of adsorbates such as fatty acids whose area/molecule can be presumed from the studies of insoluble films on water surfaces. Harkins and Gans[108], Ewing[109] and Smith and Fuzek[110] have used non-aqueous solutions of surface active materials to determine specific surfaces. The author[57] developed a method for determining specific surfaces of alumina powders by shaking them with very dilute aqueous solutions of *n*-heptoic acid. The amount of acid adsorbed was readily determined by the change in surface tension of the solution after shaking with the powder. The results of these measurements indicated that a stable, monomolecular film of acid is formed on the powder over quite a wide range of experimental conditions. Smith and Hurley[111] have determined the specific surfaces of carbon blacks by adsorption of fatty acids from various solvents using area/acid molecule of 20·5 sq.Å. Russell and Cochran[104] have measured the specific surfaces of alumina powders, by adsorption of stearic acid, they claim that an area/acid molecule of 17 sq.Å should be used to give results consistent with those obtained by other methods. Specific surfaces of powders can also be determined by measuring rates of solution in suitable solvents by use of radioactive tracers and by measuring heats of adsorption. These methods are discussed by Dallavalle[94]. Further application of specific

surface determinations to pharmaceutical powders should lead to better standardisation of the powders and perhaps also to improved methods of manufacture.

ELECTROPHORESIS

Electrophoresis is the study of the motion of charged particles in an electric field. The importance of electrophoresis in pharmacy and medicine is that it provides a method for separating the components of heterogeneous protein sols such as blood serum, and it can also be used to study adsorption onto colloid particles, bacteria, etc.

Micro-electrophoresis consists of observing the movement of individual colloid particles in an electric field by means of a microscope or ultra-microscope. Techniques are described by Alexander and Johnson[2]. McQuillen[112] has studied the effect of cationic soaps on the electrophoretic mobility of bacteria by this method. Dyar[113] has used micro-electrophoresis to study the nature of bacterial cell surfaces, and Alexander and McMullen[114] have used the method to examine the adsorption of a number of soaps onto bacteria and other particles of similar dimensions.

The most important electrophoretic technique is the moving boundary method developed by Tiselius[115]. In this method, a hydrophilic buffered colloid sol is put into a U-tube and istonic buffer solution is placed in either arm; special apparatus is used so that sharp boundaries between buffer and sol are obtained. An electric field is set up by immersing electrodes in the buffer solutions, and after some time the sol-buffer boundaries move in this field according to the electric charge on the colloid. The position of the boundaries is followed by means of an optical arrangement known as the " schlieren " system, which necessitates the use of rectangular section optically flat U-tubes. If a heterogeneous sol such as blood serum is put into the apparatus, then a number of boundaries appear after electrophoresis, each boundary corresponding to a component of different electrophoretic mobility. The set of boundaries gives an electrophoretic pattern from which the relative abundances of each component can be computed. By varying the *p*H, different types of separation can be achieved. Blood serum will give boundaries corresponding to albumen and α-, β- and γ-globulins. The apparatus can also be set up so that the components can be separated from one another. The moving boundary technique is fully described by Alexander and Johnson[2]. Tiselius[116] gives a general review of electrophoresis, Luetscher[117] has reviewed the position of electrophoresis in biology and medicine, and Abramson, Moyer and Gorin[118] have summarised the results of the study of the electrophoresis of proteins.

In clinical medicine, electrophoresis studies are important, since in abnormal states, characteristic patterns often appear in the serum proteins of the patient. Ewerbeck[119] claims that serum electrophoresis can be used in the diagnosis of tuberculosis in children; Rafsky *et al.*[120] have used electrophoresis to show up altered protein metabolism in liver

disease, and there are many more recent examples of the rapidly developing application of serum electrophoresis to the diagnosis and study of disease[118].

Electrophoretic methods have recently been used by Smith and Briggs[121] to study the interaction of ions with proteins.

An aspect of electrophoresis of particular pharmaceutical interest is the development of processes for the separation of antibodies, etc., from animal serum by a single simple physical process. Tiselius and Kabat[122] reported that some antibodies produced in the horse migrated electrophoretically as a new serum component between β- and γ-globulins. From rabbit sera, antibody solutions of high purity were obtained by electrophoretic separation of the γ-globulin. Van der Scheer, Wyckoff and Clarke[123] reported that the serum of horse immunised against tetanus showed large amounts of a new electrophoretic component. Newell *et al.*[124] reported that the antibodies of allergic serum were to be found in the γ-globulin fraction. Sandor[125] stated that diphtheria and other antitoxins can be separated from serum by electrophoresis. Koprowski, Richmond and Moore[126] observed that the sera of animals immunised against encephalomyelitis and other similar diseases showed no consistent change in electrophoretic pattern due to antibody formation, but the antibodies were always associated with the β- and γ-globulin fractions.

Unfortunately the Tiselius electrophoresis apparatus can only deal with relatively small amounts of material, and is, therefore, of little use for large-scale preparation of purified antibodies. The development of the process of protein fractionation by electrophoresis-convection recently reported by Cann, Kirkwood, Brown and Plescia[127] may lead to the development of larger-scale separations. In this process horizontal electrophoretic transport of proteins is imposed on a vertical convection column.

An apparatus for continuous electrophoretic separation (capable of dealing with 15-20 ml. of solution per day) has been described by Svensson and Brattsten[128]. Although many problems remain to be solved in connection with this apparatus, it offers promise for the development of a large-scale process for separating serum components.

The Tiselius apparatus is large and expensive. A much simpler apparatus suitable for analytical work has been described by Gordon, Keil, Sebesta, Knessl and Sorm[129]. In this, electrophoretic separation is carried out in agar gel, and the separated bands are revealed by ultra violet light absorption or by means of colour reagents. This method gave separations with egg white and human plasma similar to those obtained in the Tiselius apparatus. Its use in preparative work is limited by the difficulty of separating the agar from the purified protein fractions; however, it should be useful for analytical purposes.

The position of electrophoresis in colloid chemistry is very similar to that of chromatography in organic chemistry. Both provide physical techniques for separating chemically similar complex molecules without

altering the structure of these molecules. Just as chromatography has developed into a micro-method for analytical purposes (paper chromatography) and macro-methods for preparative separations (partition and ion exchange chromatography), so perhaps electrophoresis will develop into the agar gel method for analysis and the continuous process for preparative work.

Ion Exchange Resins

The ion exchange resins are a group of gels containing acidic or basic groups (called the functional groups) held in a macromolecular network. Although they are completely insoluble in water, they swell up and imbibe water when in contact with it and the functional group in the resin can exchange ions with aqueous solutions. If the resin contains an acidic functional group, it can exchange hydrogen ions and cations with solutions, if the functional group is basic, it can exchange hydroxyl ions and anions.

The four principal types of ion exchange resins now commercially available are

(a) Strong acid type. These are sulphonated resins formed by copolymerisation of materials such as styrene and divinyl benzene. The functional group is then a sulphonic acid ion, held in a hydrocarbon gel network.

(b) Weak acid type, formed by copolymerisation of acrylic acids with divinyl benzene; the functional group is carboxyl.

(c) Weak base, containing amine functional groups.

(d) Strong base, containing quaternary ammonium functional groups.

A general survey of the history, methods of preparation, structure, theory and applications of ion exchange resins is given by Nachod[130], methods of preparation and identification of resins are described by Topp and Pepper[131] and some of the possibilities of the resins in the pharmaceutical field are outlined by Winters and Kunin[132]. They should have considerable value in extracting drugs from natural sources and in pharmaceutical analysis, in addition to their chromatographic applications. A fairly large plant for extracting quinine, using ion exchange resins, was set up during the war[130]. Vota and Yufera[133] have described the extraction of several alkaloids by means of resins. The strong and weak acid resins can be prepared in a form which is completely insoluble in ethanol, which greatly increases their scope in dealing with alkaloids.

In the analytical field, Jindra and Pokorsky[134] have described the assay of a number of alkaloidal salts by running their solutions through a column of weak base resin in the hydroxyl form. The resin removes the anions from the solution and the effluent consists of an alkaloid solution which can be titrated directly with acid. A similar method for determining organic acids in solutions of their salts has been described by Vaisman and Yampol'skaya[135]. The salt solution is run through a strong acid resin column in the hydrogen form, this replaces the cations of the solution by hydrogen ions and the eluate consists of an acid solution which can be titrated directly with alkali.

Saunders and Srivastava[136] have reported quantitative absorption of

quinine on to a weak acid resin from solution in 50 per cent. ethanol and quantitative displacement of quinine from the resin by ethanolic ammonia solution, which could form the basis of a method for determining this alkaloid in mixtures.

A recent application of ion exchange resins of general interest, is the development of a mixed ion exchange column which will remove practically all the cations and anions from tap water in a single operation[137]. By this process, very pure water suitable for conductivity work ($0{\cdot}1 \times 10^{-6}$ mhos at 20°) has been prepared by Davies and Nancollas[138].

Many exchange resins show a preference for divalent ions and this effect has been utilised to separate carbonate from sodium hydroxide solutions for use in volumetric analysis. Davies[139] reports that if the caustic soda solution is passed through a column of strong base exchange resin, nearly all the carbonate is removed from the solution.

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