cholesterol? Will the body synthesize this lipoprotein-cholesterol complex more or less independently of dietary intake—e.g., some have it and some don't?

Facts to answer these questions have not yet been published, and it will probably take several years to get sufficient evidence, confirmed by several laboratories, to answer these and similar questions accurately. In the meantime it seems unfortunate to scare the public away from the common foods that make up the bulwark of present day "good nutrition" and attempt to turn us all into California vegetarians living on wheat germ, blackstrap molasses, and a jigger of yogurt on the side.

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A Modified Method for the Determination of Anionic Surface Active Compounds

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OF the many methods proposed for the determination of anionic surface active compounds, probably the simplest and most readily carried out are those methods involving the direct titration of the anionic agent with a solution of a cationic agent, using a two-phase system. The end-point is detected by the transfer of a colored complex from an aqueous phase to an organic solvent phase, or vice versa. For the most part the relationship between the anionic and cationic agents is not claimed to be stoichiometric, the cationic solution being used merely as an intermediary and the results being expressed in terms of known weights of the same or a similar anionic compound. One method (2) however is based on stoichiometry of the reaction between the anionic and cationic agents, the cationic solution being standardized by an independent method against potassium dichromate. It is the purpose of this paper to point out certain discrepancies in data obtained by this method and to propose a simple modification which apparently leads to more precise results.

Experimental

Sodium alkyl sulphates were prepared from Eastman lauryl, myristyl, cetyl, and stearyl alcohols by the method described by Lottermoser and Stoll (3). The crude products were recrystallized repeatedly from absolute alcohol and finally dried to constant weight in vacuo at 56°C. The melting points (uncorrected) of the purified compounds were as follows:

Sodium lauryl sulphate	179°C.
Sodium myristyl sulphate	182°C.
Sodium cetyl sulphate	184°C.
Sodium stearyl sulphate	170°C.

The analytical method used was essentially that proposed by Epton (2), except that Cetavlon (alkyl trimethyl ammonium chloride, supplied by Imperial Chemical Industries) was substituted for Fixanol C (cetyl pyridinium bromide) as the cationic titrant. Details of the procedure follow.

The required aliquot of anionic solution was pipetted into a 250-ml. glass-stoppered iodine flask and the volume was made up to 50 ml. with distilled water. To this was added 25 ml. of solution containing 0.03 gm. Methylene Blue, 12 gm. conc. sulphuric acid, and 50 gm. anhydrous sodium sulphate per liter, followed by 15 ml. of chloroform. The mixture was titrated with standard Cetavlon solution (approximately 5 mM.), the flask being shaken vigorously[1] after each addition. The end-point was taken when the transfer of color from the chloroform layer to the aqueous layer was such that both layers appeared the same color when viewed by reflected light. The flask was allowed to stand for about 1 min. after each shaking before making the color comparison. The Cetavlon solution was standardized against pure potassium dichromate by the method given by Epton (2) for the standardization of Fixanol C solutions.

Solutions containing 0.05% by weight of each of the purified sodium alkyl sulphates were prepared, and 10, 20, and 40 ml. aliquots of each solution were titrated with the Cetavlon solution by the method given above, the concentration of the original solution in millimoles/liter being calculated in each case. The results are given in Table I.

Discussion

It may be noted that the experimental values for the molar concentrations of all of the solutions are lower than the corresponding values calculated from the known weight concentrations of the solutions and the theoretical molecular weights of the compounds, and furthermore the experimental values appear to depend on the size of the aliquot taken for analysis, the larger aliquots giving the higher values.

These findings suggest that the observed titration should be adjusted by the addition of a blank, and consideration of the analytical procedure confirms this conclusion. On shaking together the reagents, but substituting water for the anionic solution, all of the color remains in the aqueous phase and the chloroform phase is colorless. When anionic solution is added dropwise, with shaking after each addition,

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[1] In the Epton method it is recommended that "the bottle is shaken with just sufficient force to ensure that the phases mix thoroughly." In the present work however it was found that the data obtained were more consistent and more reproducible when the flask was shaken vigorously after each addition of titrant.

TABLE I

Concentrations of Sodium Alkyl Sulphate Solutions as Determined by Epton Method

Compound	Mol. Wt.	Calculated Conc'n* mM.	Based on Uncorrected Titrations, mM.			Based on Corrected Titrations, mM.				
			Aliquot, Ml.			Aliquot, Ml.			Aver.	Error %
			10	20	40	10	20	40		
Sod. Lauryl Sulphate	288	1.736	1.680	1.707	1.718	1.728	1.730	1.729	1.729	0.40
Sod. Myristyl Sulphate	316	1.582	1.508	1.540	1.558	1.573	1.573	1.574	1.573	0.57
Sod. Cetyl Sulphate	344	1.453	1.369	1.401	1.418	1.433	1.433	1.434	1.433	1.37
Sod. Stearyl Sulphate	372	1.344	1.310	1.334	1.346	1.358	1.358	1.358	1.358	1.04

* Based on known weight concentration of solution and theoretical molecular weight of compound.

Mean Error........................ 0.85

the color is gradually transferred from the aqueous to the chloroform phase, indicating that a certain (small) concentration of anionic agent must be present to give equal colors in the two layers. Returning again to the normal procedure, at the titration endpoint the colors of the two phases are again equal, and it is obvious that this same concentration of anionic agent must be present in excess of the amount which has reacted with the cationic titrant, i.e., the endpoint occurs before the equivalence point is reached, and the observed titration must be corrected by the addition of a blank.

The blank may be determined by direct titration of the reagents with the anionic solution followed by conversion to the equivalent volume of cationic solution. However a more convenient method, which has proved very satisfactory, is to carry out two or more titrations in the usual manner using aliquots of different size. Thus if aliquots of 10, 20, and 40 ml. are used and the corresponding titrations are T_{10}, T_{20}, and T_{40}, the blank is given by the following expressions:

$$\text{Blank} = T_{20} - 2T_{10} = T_{40} - 2T_{20} = 1/3(T_{40} - 4T_{10})$$

For example, in one case the observed titrations were:

$$T_{10} = 2.81 \text{ ml.}; \quad T_{20} = 5.74 \text{ ml.}; \quad T_{40} = 11.61 \text{ ml.}$$

$$\begin{aligned} \text{Blank} = \; & 5.74 - 2 \times 2.81 &= 0.12 \text{ ml.} \\ & 11.61 - 2 \times 5.74 &= 0.13 \text{ ml.} \\ & 1/3(11.61 - 4 \times 2.81) &= 0.12 \text{ ml.} \\ & \text{Average} &= 0.12 \text{ ml.} \end{aligned}$$

Blanks of the same order of magnitude and showing the same agreement between the three calculated values were found for all of the solutions used in the present work.

The data for the molar concentrations of the various anionic solutions, based on the corrected titration values, are given in the right hand columns of Table I. It may be seen that the corrected data are not only independent of the size of the aliquot used,[2] but approach more closely the values calculated from the theoretical molecular weights, the maximum deviation being 1.37% while the mean deviation for the four compounds was only 0.85%.

Similar determinations were carried out by each of two methods published by Barr, Oliver, and Stubbings (1). These methods are similar in principle to the above although they differ considerably in detail. The data obtained were again found to be dependent on the size of the aliquot taken for analysis, the estimated concentration of the test solutions increasing with increasing aliquot size for method A and decreasing with increasing aliquot size for method B (Tables II and III). On applying a correction as outlined above, it was found that the blank must be added to the observed titration for method A and subtracted for method B. The corrected values are given in the right hand columns of Tables II and III.

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[2] While the work herein reported covers only a four-fold range of sample weights (or volumes), consistent results have also been obtained over a twenty-fold range.

TABLE II

Concentrations of Sodium Alkyl Sulphate Solutions as Determined by Method A (Methylene Blue) of Barr, Oliver, and Stubbings

Compound	Calculated Conc'n mM.	Based on Corrected Titrations, mM.			Based on Uncorrected Titrations, mM.				
		Aliquot, Ml.			Aliquot, Ml.			Aver.	Error %
		10	20	40	10	20	40		
Sod. Lauryl Sulphate	1.736	1.664	1.680	1.691	1.701	1.700	1.700	1.700	2.07
Sod. Myristyl Sulphate	1.582	1.492	1.500	1.507	1.512	1.515	1.510	1.512	4.42
Sod. Cetyl Sulphate	1.453	1.358	1.363	1.367	1.369	1.369	1.370	1.369	5.78
Sod. Stearyl Sulphate	1.344	1.288	1.296	1.302	1.304	1.304	1.306	1.305	2.90

Mean Error........................ 3.79

TABLE III

Concentrations of Sodium Alkyl Sulphate Solutions as Determined by Method B (Bromphenol Blue) of Barr, Oliver, and Stubbings

Compound	Calculated Conc'n mM.	Based on Uncorrected Titrations, mM.			Based on Corrected Titrations, mM.				
		Aliquot, Ml.			Aliquot, Ml.			Aver.	Error %
		10	20	40	10	20	40		
Sod. Lauryl Sulphate	1.736	1.691	1.677	1.671	1.644	1.644	1.644	1.644	4.14
Sod. Myristyl Sulphate	1.582	1.519	1.516	1.515	1.512	1.512	1.512	1.512	4.42
Sod. Cetyl Sulphate	1.453	1.395	1.385	1.378	1.374	1.374	1.373	1.374	5.43
Sod. Stearyl Sulphate	1.344	1.304	1.302	1.300	1.299	1.299	1.299	1.299	3.35

Mean Error........................ 4.34

While these corrected values are independent of the aliquot size, it may be seen that the deviations from the theoretical values are considerably higher than those obtained by the modified Epton method. It should be pointed out however that in the methods of Barr, Oliver, and Stubbings the relationship between anionic and cationic agents is not claimed to be stoichiometric. The data generally are expressed in terms of known weights of a standard anionic compound.

In view of the difficulty of obtaining sodium alkyl sulphates in a state of absolute purity (1) it is considered that the mean error of 0.85% for the modified Epton method represents a comparatively high degree of accuracy. Furthermore the data obtained are based on a primary standard (potassium dichromate) which is completely independent of the material under test and which can be readily obtained in a highly purified condition.

Summary

In the determination of anionic surface active agents of the sodium alkyl sulphate type, the method of Epton (2) has been modified by the calculation of a blank to be added to the observed titration. Data obtained by this modified method indicate a mean error of 0.85%, based on the theoretical molecular weights of four purified sodium alkyl sulphates, as compared to mean errors of 3.8 and 4.3% for two other commonly used methods. Data obtained by the unmodified Epton method are shown to be dependent on the weight of sample taken for analysis whereas the modified method gave consistent results over a wide range of sample weights.

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A Modified Spinning Band Column for Low Pressure Fractionation

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THE examination of a number of fats and waxes in this laboratory has demonstrated the need for an efficient column to separate fatty acids and alcohols up to a chain length of 30 or more carbon atoms. With packed columns of high efficiency this has not been possible without some decomposition of the larger molecules, due to a considerable pressure drop between the kettle and still head. This large increase in pressure from still head to pot is not encountered in the spinning band type of column. Baker *et al.* (1) found a pressure drop of only 0.7 mm.[1] for a column 545 cm. in length and 6.7-mm. diameter when operated at 1-mm. pressure at the still head. A lower figure of 0.04 mm. is reported by Birch *et al.* for a column 85 cm. in length and 36-mm. diameter at a head pressure of 0.5 mm.

A spinning band column was therefore constructed with an internal diameter of 15 mm. and a working length of 145 cm. It had two new features which, it is believed, contribute to its high efficiency, particularly at operating pressures in the region of 1 mm. First, strips of stainless steel gauze, attached lengthwise to rotor, wiped the walls of the column and spread the descending reflux in an even film. Second, the rotor was accurately centered by means of bearings at intervals of 29 cm. to ensure even vapor and liquid conditions throughout the column.

Details of Column Assembly

The column and its accessories for operation under reduced pressure are shown diagrammatically in Figure 1 and details of the column head in Figure 2.

To reduce vibration the column and the rotor driving motor were mounted on a 2½-in. diameter steel pipe bolted to ceiling and floor. The glass tube of the column was held tightly in a 1-in. diameter aluminum tube (Figure 2-A) by a spiral of asbestos cord. This aluminum tube carried the weight of the lagging, gave an even distribution of heat, and protected the glass tube from fracture. Both the kettle (a 250-cc. flask) and the still head were sealed directly to the column. The kettle (Figure 1-A) was heated by a "Glas-Col" heater (B), separated from it by an air gap, and the temperature was controlled by a "Variac" transformer to give a constant pressure drop over the column measured by the butyl phthalate manometer (C). The need for mechanical stirring of the distilland below 2-mm. pressure has been stressed by Birch *et al.* (2), and this has been provided for by a small stainless steel impeller, 13-mm. diameter on a $\frac{1}{16}$-inch stainless steel rod attached to the bottom of the rotor and fitting loosely into a glass capillary sealed to the bottom of the kettle. The side arm of the kettle greatly facilitated charging and cleaning with solvents.

The column head is shown to scale in Figure 2. A water jacket (B) was provided to condense lower boiling materials, such as solvents. A part of the reflux was collected in a small weir at C which extended over approximately a quarter of the circumference of the tube. This weir, together with the volume of the capillary as far as the needle valve, had a capacity of 0.3 cc. The take-off was controlled by the intermittent operation of the d.c. solenoid needle valve (D) by means of a timing device (Figure 1-D), which consisted of a micro switch operated by a cam driven by a small synchronous motor (0.5 r.p.m.). The cam was made to give 10 seconds take-off in each 120 seconds. Fine control of the take-off rate could be made by adjustment of the vertical travel of the needle by means of the metal bellows attachment (F). Alternatively, changes in the take-off period could be made with different cams. This valve was well suited for

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[1] Except where stated all pressures are given in millimeters of mercury.