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Journal of the Society of Cosmetic Chemists

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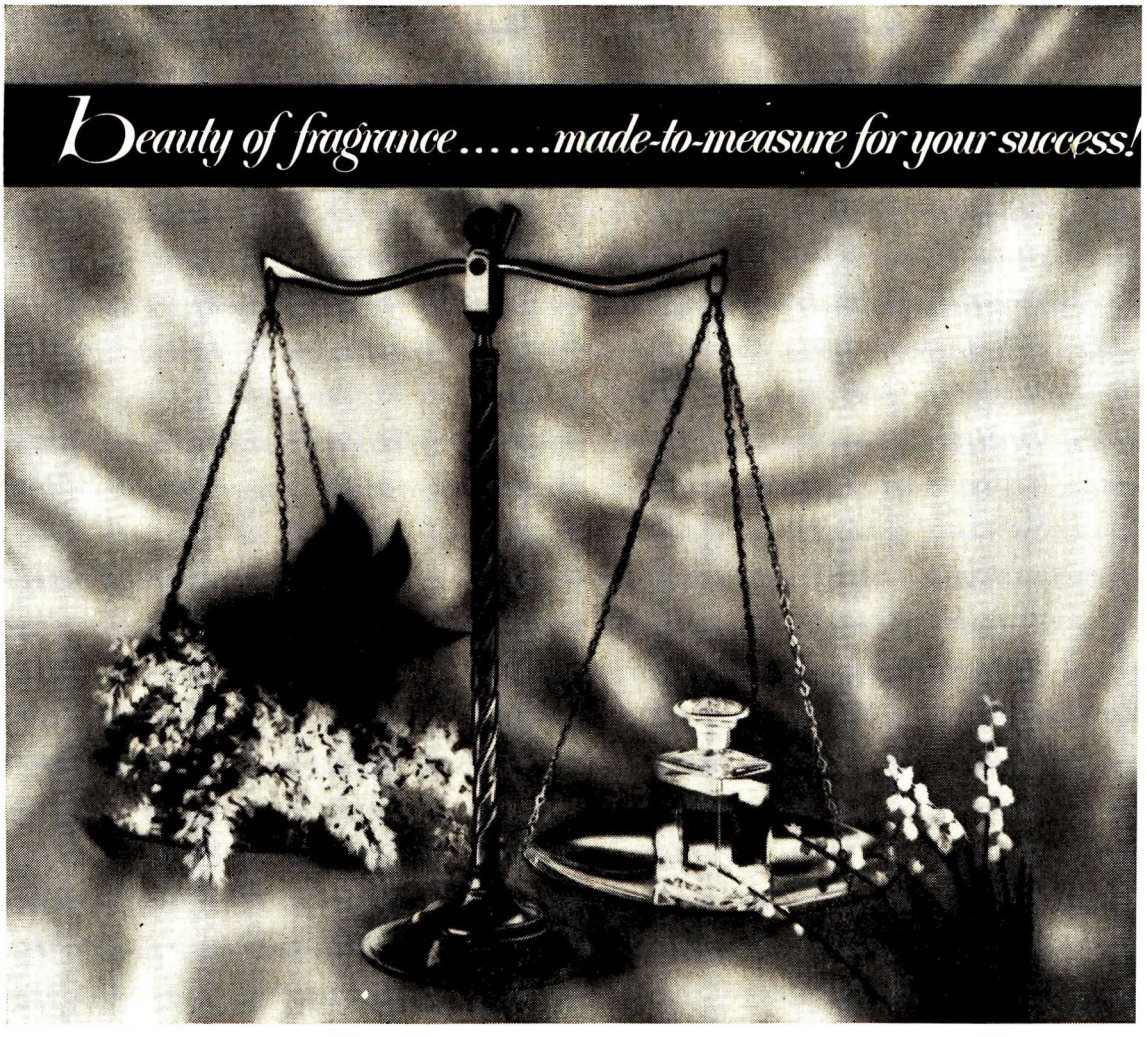

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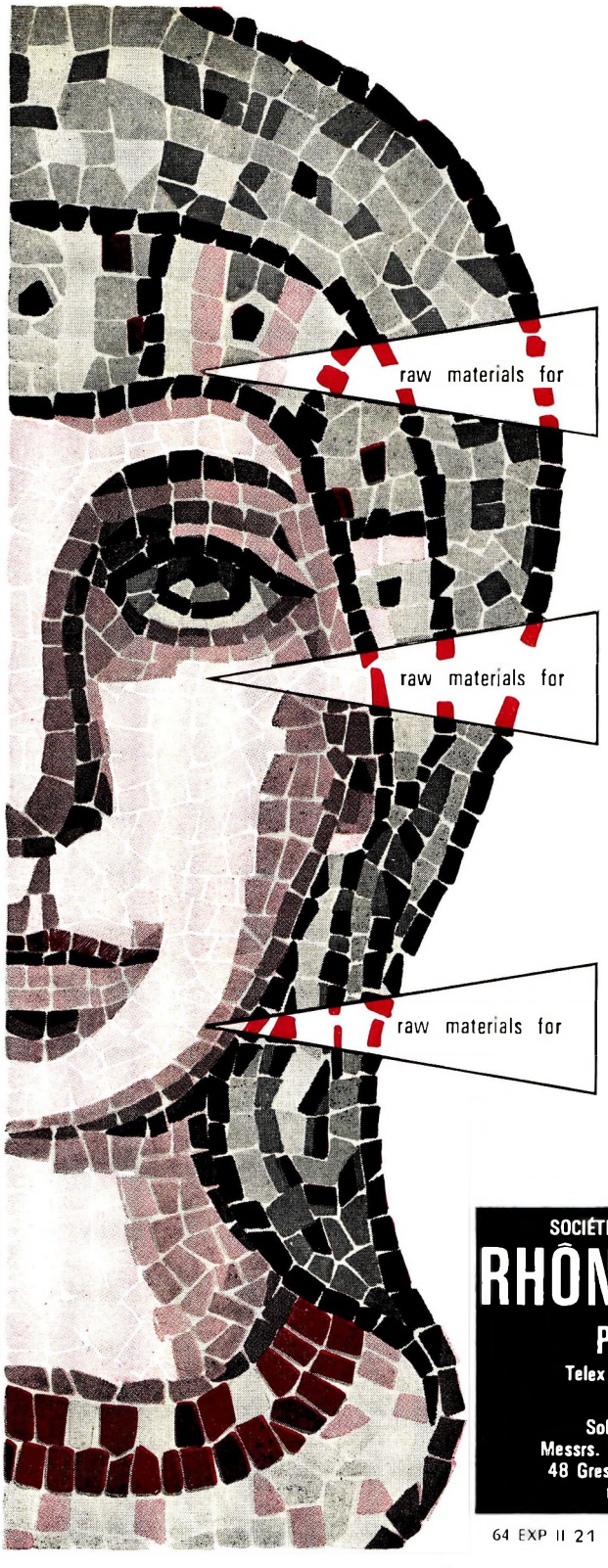

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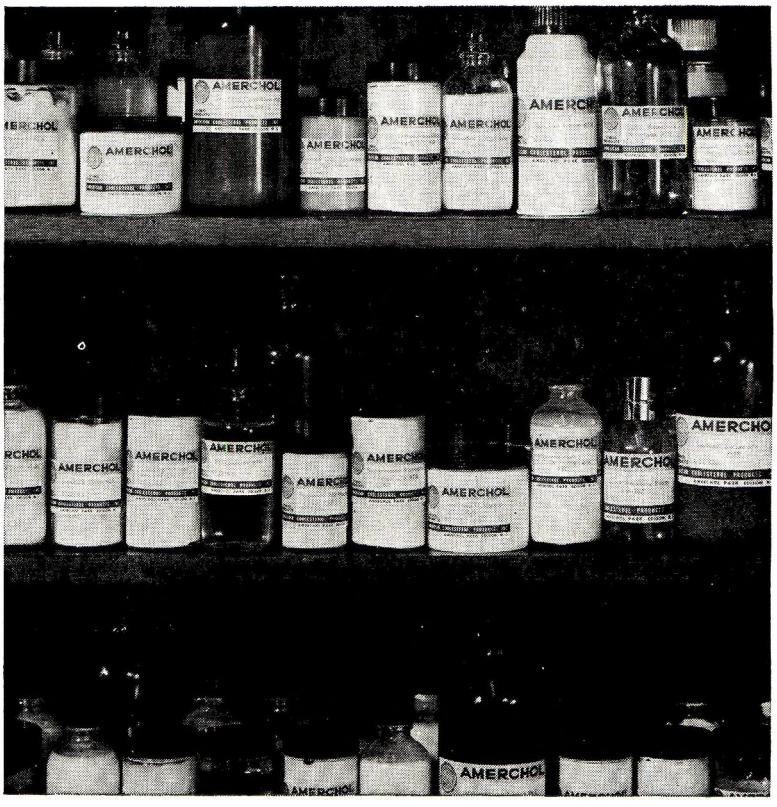

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Rapid method for the determination of hydroxyl values: J. FLEMING
Journal of the Society of Cosmetic Chemists **17** 625-630 (1966)

Synopsis—A rapid method is described for the routine determination of hydroxyl values on a wide range of hydroxy compounds commonly used in the cosmetic industry. The procedure is to acetylate the compound at 60°C by means of a single reagent containing acetic anhydride and pyridine in toluene. At the end of the reaction period the unreacted anhydride is hydrolysed with water and titrated. The difference between this titration and a blank gives the amount of anhydride absorbed. The method is rapid, simple and accurate and has been applied to a wide range of oils, waxes and solvents. As it requires no specialised techniques or equipment, it can be carried out by non-technical staff in the smallest of laboratories.

Fluorescent antibody techniques in dermatology: N. R. ROWELL
Journal of the Society of Cosmetic Chemists **17** 631-640 (1966)

Synopsis—An outline of the principles underlying fluorescent antibody techniques is given. In dermatology, these methods have been used as histological markers in studies of the antigenicity of connective tissue and in the investigation of the possible role of auto-immune factors in diseases involving the skin. They have also been used to demonstrate the serological abnormalities which are a characteristic feature of many auto-immune diseases.

Spectral slit width and other sources of error in uv spectrophotometry:
A. R. ROGERS. *Journal of the Society of Cosmetic Chemists* **17** 641-655 (1966)

Synopsis—Errors caused by slit width effects, unbalanced reflection losses in the cuvettes, stray light and electrolyte effects are reviewed. Slit-width errors are small with *p*aminobenzoates, aminophenols, camphor, methyl and propyl *p*hydroxybenzoates, methyl and butyl phthalates, and salicylates, but may be appreciable with hexachlorophane, expecially in non-polar solvents. The need is stressed for the analyst to have sufficient understanding of the optical principles of spectrophotometry to be able either to avoid the principal sources of error or to minimize their effect on measurements of light absorption.

Preparative gas chromatography: G. R. FITCH
Journal of the Society of Cosmetic Chemists **17** 657-667 (1966)

Synopsis—The main aspects of this technique are discussed, including the newer developments in continuous gas chromatography. Some possible applications in the field of cosmetic chemistry are given.

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J. Soc. Cosmetic Chemists **17** 625-630 (1966)

Rapid method for the determination of hydroxyl values

J. FLEMING*

Synopsis—A rapid method is described for the routine determination of hydroxyl values on a wide range of hydroxy compounds commonly used in the cosmetic industry. The procedure is to acetylate the compound at 60°C by means of a single reagent containing acetic anhydride and pyridine in toluene. At the end of the reaction period the unreacted anhydride is hydrolysed with water and titrated. The difference between this titration and a blank gives the amount of anhydride absorbed. The method is rapid, simple and accurate and has been applied to a wide range of oils, waxes and solvents. As it requires no specialised techniques or equipment, it can be carried out by non-technical staff in the smallest of laboratories.

Preliminary work

It was thought necessary to devise a rapid but accurate method for the routine determination of hydroxyl values of raw materials used in the manufacture of cosmetics.

The B.P. procedure (1) is claimed to give the most accurate results, but this method was thought too long for the routine analysis of a large number of samples.

The recommended method by the Toilet Preparations Federation (2) involves the use of a solution of acetic anhydride in pyridine with a reflux time of 1 hr. The noxious nature of the solvent made the use of a fume cupboard essential; and the very dark colour of the reagent, making the end point difficult to see, precluded its use as a routine method. The method described by the T.G.A. (3) using a solution of acetyl chloride in toluene, with pyridine added as a catalyst, was found to give inconsistent results. Finally, the method described by Sully (4) appeared to be the best, but it was found that the stearic anhydride was quite expensive to buy and its preparation in the laboratory is not a simple task. The method also

*Gala Cosmetic Group Ltd., Surbiton, Surrey.

involves a reflux procedure and it was thought that this, too, was not practicable for the routine analysis of a large number of samples. The experiments described below show that the use of a single reagent–solvent system of acetic anhydride in toluene, with pyridine as a catalyst (A.P.T. reagent) acetylated a wide range of hydroxylic compounds in less than 60 min at 60°C. The reagent is colourless, and in use the smell of pyridine is not particularly noticeable if some care is taken. The acetic anhydride is readily hydrolysed by the addition of water, and the acetic acid titrated with aqueous potassium hydroxide solution. Some doubts were at first expressed of the danger of loss of acetic anhydride, but this has not been found experimentally.

Experimental

Initial work was done at room temperature, but it was found that the reaction was not complete at the end of 2 hr. However, it was found that the majority of materials reacted quantitatively in less than 60 min at 60°C and subsequent determinations were carried out at this temperature (*Table I*). Some materials were found to be only slowly acetylated, e.g. castor oil, cholesterol, etc., and this was possibly due to some steric effects (*Table II*). All the results shown are the average of at least two determinations.

Table I
Use of new reagent

Material	Reaction time (min)	Temperature	Hydroxyl value
Cetyl alcohol (3 g of sample)	30	20°C	175
	60	20°C	202
	120	20°C	210
	15	60°C	195
	30	60°C	222
	60	60°C	221
Oleyl alcohol (3 g of sample)	30	20°C	148
	60	20°C	180
	120	20°C	191
	15	60°C	185
	30	60°C	206
	60	60°C	206

An excess of reagent must be present as with the Sully method, and the difference between the blank and the titration should never be greater than about 14 ml of N sodium hydroxide (*Table III*).

This method has been in use in these laboratories for over 10 months and has been found to give consistent results for a wide range of materials (*Table IV*).

Table II
Reaction times of materials at 60°C

Material	Time (min)	Hydroxyl value
Castor oil (4 g of sample)	30	84
	60	115
	120	154
	180	153
Glycerol (0.3 g of sample)	30	1804
	60	1809
	120	1815
	180	1815
Cholesterol (4 g of sample)	30	90
	60	118
	120	138

Table III
Effect of sample weight on results

Sample weight (g)	Hydroxyl value	ml N KOH used
2.0	221	7.8
3.0	221	11.7
4.0	220	15.5
4.5	211	16.7
5.0	206	18.1
These results were obtained on the same sample of cetyl alcohol		

Table IV
Results on successive samples of materials

Material	Sample wt. (g)	Reaction time (min) at 60°C	Results	Specification
Oleyl alcohol	3	30	211	
	3	30	208	
	3	30	210	207 – 211
	3	30	208	
	3	30	209	
Sorbitan sesquioleate	3	30	195	
	3	30	198	185 – 205
	3	30	195	
Cetyl alcohol	3	30	222	
	3	30	221	
	3	30	219	
	3	30	219	218 – 232
	3	30	227	
	3	30	222	
	3	30	218	
	3	30	221	

Table IV (contd.)

Material	Sample wt. (g)	Reaction time (min) at 60°C	Results	Specification
Polyoxyethylene sorbitan monolaurate	4 4 4	30 30 30	109 114 113	105 – 115
Ethoxylated castor oil	10 10 10	120 120 120	68 67 70	57 – 80
Hydrogenated castor oil	4 4	120 120	148 146	Greater than 140
Wool wax alcohols	4 4 4	30 30 30	127 118 117	115 – 125
Polyoxyethylene stearyl ether	3 3 3 3	30 30 30 30	146 149 148 147	145 – 155
Ethoxylated cetostearyl alcohol	3 3 3 3 3 3 3	30 30 30 30 30 30 30	182 178 184 179 175 179 182	175 – 185
Lauryl alcohol (94%)	2.5 2.5 2.5 2.5	30 30 30 30	299 297 300 298	295 – 305
Castor oil	4 4 4 4 4 4	120 120 120 120 120 120	154 148 152 156 148 152	145 – 155
2-Octyl-dodecanol	3 3 3 3	30 30 30 30	172 179 174 174	170 – 180

The method has also been applied to several materials for which no specification for the hydroxyl value is quoted by the suppliers. In these cases the theoretical values were calculated, and the hydroxyl values were determined experimentally. In all the samples thus examined, the experimental values were close to those calculated (*Table V*).

Table V
Experimental results on successive samples

Material	Sample weight (g)	Reaction time (min) at 60°C	Result	Theory
Cholesterol	4	120	138	145
	4	120	141	
	4	120	143	
Menthol	1	120	363	359
	1	120	369	
	1	120	363	
	1	120	358	
Benzyl alcohol	1.2	30	492	519
	1.2	30	498	
	1.2	30	504	
2-Phenyl ethanol	1.5	30	453	459
	1.5	30	459	
	1.5	30	461	

Method

Reagents

A.P.T. Reagent. Mix 200 ml toluene, 40 ml pyridine and 60 ml acetic anhydride (Analar). Store in a stoppered, amber glass bottle.

Potassium hydroxide solution. N aqueous potassium hydroxide solution, standardised against potassium hydrogen phthalate.

Phenolphthalein. 1% alcoholic solution.

Procedure

Weigh the specified weight of material into a 250 ml conical flask (W g). Pipette in 10 ml of the A.P.T. reagent and warm on a water bath until solution is complete. Lightly stopper the flask and place in an oven (or water bath) at 60°C for the specified time – normally 30 min. Add 25 ml distilled water, stopper and shake vigorously for a few seconds to hydrolyse the excess anhydride, and titrate with N KOH solution, using phenolphthalein as indicator (A ml). Shake the flask vigorously near the end point to remove any acid from the upper toluene layer. Perform a blank, as above, but omitting the sample (B ml).

Calculation

$$\text{Hydroxyl value} = \frac{(B-A) \times N \times 56.1}{W}$$

where N is the normality of the KOH solution.

(*Received: 27th May 1966*)

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(1) British Pharmacopoeia, p. 1057 (1963).
(2) British Standard 684 : 1958.
(3) Toilet Goods Assoc. Analytical Method No. **24**.
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J. Soc. Cosmetic Chemists **17** 631-640 (1966)

Fluorescent antibody techniques in dermatology

N. R. ROWELL*

Presented at the Symposium on "Physical Methods," organised by the Society of Cosmetic Chemists of Great Britain, in Bristol on 17th November 1965.

Synopsis—An outline of the principles underlying fluorescent antibody techniques is given. In dermatology, these methods have been used as histological markers in studies of the antigenicity of connective tissue and in the investigation of the possible role of auto-immune factors in diseases involving the skin. They have also been used to demonstrate the serological abnormalities which are a characteristic feature of many auto-immune diseases.

In recent years fluorescent antibody techniques have been used in many fields of medicine, both as research tools and as diagnostic aids. Immunological reactions are useful because of their specificity and sensitivity. Coons *et al* (1,2), who introduced the fluorescent antibody technique, found that antibody globulin can be chemically combined with fluorescent dyes such as fluorescein *iso*cyanate without losing its biological or immunological properties. Such conjugates could be used as histological markers to indicate the sites of reactions between antibody and antigen in tissue sections or cell smears. The choice of a fluorescent label allows the site of such reactions to be demonstrated when sections are examined in UV light. Fluorescent dyes are used because they can be detected in a lower concentration than ordinary dyes.

The Coons' fluorescent antibody technique has been used in the study of antigens in bacteria, viruses, protozoa, fungi and in animal and human tissues. It is also suitable for investigation of the presence of antibody in tissue and in serum.

*Consultant Physician, Department of Dermatology, General Infirmary at Leeds, Yorks.

The technique is simple in principle but certain precautions must be taken in order to obtain valid observations. An excellent account of the technical aspects has been published by Nairn (3).

Principles of fluorescent antibody methods

Direct staining method

In this technique conjugated specific antibodies are used for the detection of antigens in the direct staining of material. Obviously specific antisera have to be prepared in laboratory animals. This method is used for the identification of bacteria, viruses and protozoa and also for the detection of antigenic material in tissue sections. For the latter the constituents of tissue have to be separated and used as antigens for raising antisera in animals. These specific antisera are labelled with fluorescent dyes and then applied to the histological sections. Such investigations of tissue components have been used for a variety of tissues such as kidney, lung, skin, leucocytes and megakaryocytes. Antisera against hormones and enzymes have also been studied in this way. Localization of blood group substances in human tissue also relies upon the direct method of fluorescent protein tracing.

Direct staining for gammaglobulin

Antiglobulin and anti-gammaglobulin sera can be conjugated with fluorescein and used to detect the presence of globulin or gammaglobulin in tissues. This has been useful for the investigation of lymph nodes, and in the study of rheumatoid factor in lymph nodes and synovial membranes, in patients suffering from rheumatoid arthritis. Similarly, gammaglobulin has been found in the glomerular tufts of kidney in patients suffering from systemic lupus erythematosus. We have studied the distribution of gammaglobulin in various skin lesions as well as in the internal organs of patients who have died from certain auto-immune diseases.

Indirect staining

In this technique, a serum containing antibody is applied to a frozen section of tissue and if a specific reaction occurs with some antigen in the tissue, the antibody will become firmly fixed to the antigen and will not be removed by washing. If the tissue is now stained with an antiglobulin conjugate, the conjugate will unite with the globulin and this also is not removed by washing. If the section is now examined under UV light the areas of specific fluorescence will indicate the presence and distribution of

the antigen in the tissue. Obviously this so-called "sandwich technique" has an infinite variety of applications. It may be used to characterize the antigens in tissues or to determine the antibodies which circulate in various auto-immune and other diseases.

What are the snags which may be encountered in these apparently simple procedures?

Firstly, there is the problem of non-specific staining. This results from the presence of unconjugated fluorescent dye and heavily labelled protein molecules which may directly stain the tissues. All of the former can be easily removed from the conjugated serum by careful dialysis, whereas the heavily labelled protein molecules have to be removed by absorption with mouse liver or by fractionation chromatographically on Sephadex columns. Moreover, the globulin fraction is usually crude and really contains a variety of globulin fractions. If tissue sections are used, autofluorescence, that is, a bluish-silver appearance of certain tissues may be confused with, or obscure, the apple-green fluorescent fluorescein *iso*cyanate. More recently, lissamine rhodamine B200 which fluoresces a salmon-pink colour, has been used as an alternative. Sometimes it is necessary to use more than one label if examinations of tissue using more than one antibody are required. In 1958, Riggs *et al* (4) introduced fluorescein *iso*thiocyanate which fluoresces green as does fluorescein *iso*cyanate, but is easier to conjugate.

Uses in dermatology

Fluorescent antibody techniques have been used in three main ways in dermatology. Firstly, as a research measure to determine the types of antigen in the connective tissue in the skin and other organs; secondly, in order to determine whether globulin and other evidence of auto-immune reaction is present in sections of skin and other organs; and thirdly, in the investigation of various antibodies circulating in the blood of patients suffering from presumed auto-immune diseases.

Studies of connective tissue in the skin and other organs

The connective tissue may become involved in a variety of diseases and many investigations have been carried out to determine the changes which take place. The fluorescent antibody technique has been used in only a limited way, so far, either to determine the presence of antibodies fixed to pathologically altered connective tissue or for studies of the antigenicity of connective tissue. The initial investigations on this aspect of the technique have been described by Scott (5,6).

For the study of antigens in connective tissue it is necessary to prepare antisera by injecting human tissue homogenates into rabbits. The rabbit serum globulin is labelled with either fluorescein or with lissamine rhodamine B200. When this fluorescent conjugate antiserum is applied to frozen sections of human tissue any reaction between the antiserum and a specific antigen can be detected by fluorescence under uv light. It is possible to use more than one antibody provided it is conjugated with a different contrasting label. Scott has used anti-human glomerulus antisera and anti-human synovial antisera conjugated with fluorescein and lissamine rhodamine B200 respectively. It is most important to eliminate non-specific staining by absorption and inhibition tests. Specific staining (that is fluorescence) should be prevented by pretreatment of tissue sections by the corresponding unconjugated antibody. When two such conjugates are used, in addition to the areas stained green with fluorescein and orange/red with lissamine rhodamine, many intermediate colours in the yellow/orange range can be seen in the sections. It is, however, possible to separate broad groups of tissue antigens. By using two contrasting conjugates against renal glomeruli and synovium, Scott was able to recognize three groups of connective tissue antigens, which he considers to be antigens to basement membrane, to reticulin and to fibrous tissue. The situation is not quite so simple as it sounds as all three groups react with antisera prepared against human glomeruli, but only reticulin and the fibrous tissue antigen react with human synovium antisera.

Obviously the finding of materials of different antigenicity in connective tissue is of great interest, but the next stage is to determine the chemical nature of the antigenic components, and this has not been done so far. The technique has been used by Dr. D. G. Scott, who now works in the Department of Dermatology at Leeds, on several types of connective tissue disorder, but no consistent staining pattern has been found and the problem is complicated because the antigenicity of connective tissue probably alters with age and, possibly, with sex. We are about to begin studies of normal human skin to establish these points. In pathological conditions in connective tissue disorders, such as systemic lupus erythematosus and systemic sclerosis, the pattern of staining of blood vessels by anti-connective tissue antibodies appears to be altered and abnormal material may be seen in the dermis. It must be pointed out that unstained tissue shows auto-fluorescence of the elastic tissue and it has been noticed that the pattern of auto-fluorescence may be altered in these diseases. We are in the process of evaluating these changes.

Investigation of skin lesions for the presence of globulin and other abnormalities

Arteritic lesions

Dr. Scott and I have been interested in the histology of various types of skin lesions, a common feature of which has been arteritis, that is, inflammation of the small blood vessels of the skin (7). Briefly, the method of investigation was to expose frozen sections of tissue to globulin fractions of antisera labelled with fluorescein-*iso*thiocyanate or with lissamine rhodamine B200. The types of lesions which we investigated were those of so-called necrotizing arteriolitis, reticulate livedo, nodular vasculitis, erythema nodosum, systemic and discoid lupus erythematosus and other lesions in which blood vessels might be expected to be abnormal.

Our observations are of interest in that there was no convincing relationship between the appearances of the lesions, clinically, and the presence or absence of globulin in them. Globulin occurred less frequently than other evidence of histological changes, such as perivascular cuffing with lymphocytes or increased cellularity of the dermis. We concluded from our observations that perivascular cuffing of lymphocytes appeared as an early feature before the development of globulin and that it might be inferred that perivascular lymphocytes are the initiating factors in the lesions and that globulin may be concerned either in the progression or regression of the lesions. In addition, there did not appear to be any consistent relationship between the distribution of globulin and the clinical appearances, but in the main the globulin did occur in areas of major pathological change.

As an extension of this type of investigation Parish and Rhodes (8) examined by the indirect technique, nine biopsies from cases of nodular vasculitis for fixation of streptococcal complexes using rabbit anti-streptococcal serum and tubercle antigen using rabbit anti-human tubercle serum. In only one case was fluorescence seen with streptococcal group A antigen, and this patient had had a quinsy two weeks earlier. Tubercle antigen was demonstrated in two out of three patients who had active tuberculosis of the cervical nodes. In only two biopsies could gamma-globulin be demonstrated, one of which was that from the patient with the quinsy.

Lupus erythematosus

Globulin could be demonstrated in lupus erythematosus in the form of oval structures (cytoid bodies) in the region of the dermo-epidermal

junction, and by using contrastingly labelled mixtures of conjugates including anti-human glomerular conjugates, the cytoid bodies were confirmed as being in the area of altered dermo-epidermal basement membrane. Some workers (9,10) have noticed a broad band of fluorescence in this area, but in our experience this band is rather narrow. However, the appearances vary in sections in different patients and the discrepancy may only be the result of examination of lesions of different age. Globulin was frequently, but not invariably, found in the walls of arterioles and capillaries, but there did not appear to be any consistent reason for the variability. What does this globulin represent? It might be thought to indicate the site of a specific antibody-antigen reaction, but this cannot be assumed with any certainty. We are proceeding now to determine, by using anti-human complement conjugates, whether complement, which is probably a better marker of an antibody-antigen reaction, is present in the same areas.

Other dermatological conditions

The fluorescent antibody technique has been used by Raskin (11) to investigate the possible role of auto-immune factors in other dermatological conditions. She was unable to demonstrate any specific antigen-antibody reaction in the skin of patients with psoriasis, erythema multiforme, pemphigus vulgaris and dermatitis herpetiformis.

The demonstration of humoral antibodies in dermatological diseases

It is now well established that the serum of patients suffering from diseases considered to be auto-immune, such as systemic lupus erythematosus, contain a variety of humoral auto-antibodies. It has more recently been established that these antibodies are not confined to systemic lupus erythematosus but are found in a variety of conditions including discoid lupus erythematosus, systemic sclerosis and rheumatoid arthritis. These conditions all have dermatological features, but it is important to realize that the conditions dealt with by dermatologists include those which are of a widespread nature and involve all tissues of the body. These auto-antibodies can be demonstrated by a variety of techniques including complement fixation, precipitation tests, etc., but here I am only concerned in dealing with their demonstration by fluorescent antibody techniques.

The indirect sandwich technique is used. In this technique the patient's serum is applied to frozen sections of any mammalian tissue for half an hour. The serum is then removed by washing, leaving any anti-nuclear antibody globulin attached to the nuclear material in the tissue.

Fluorescent labelled rabbit antibody to human gammaglobulin is then applied to the tissue for a further period after which the conjugate is removed by washing. If the antibody has become attached to the nuclei in the first stage, the conjugate will show up when the section is examined under a fluorescence microscope. There are several antinuclear factors in the blood and these can be distinguished by the characteristic staining patterns (12).

In the first type of staining pattern, the homogeneous pattern, the nucleus is stained all over, although in some areas there may be more intense staining than in others. The second type of staining pattern is the so-called "speckled" type, in which the nuclei show points of fluorescence. In the third type of staining, the nucleoli are intensely stained. There is a fourth type of staining in which there is a narrow band of more intense fluorescence at the surface of the nucleus. The latter type of staining is uncommon and has only been seen in sera containing anti-DNA antibody. These staining patterns represent reactions between specific antibodies and antigenic material in the nucleus.

The test is carried out with quadrupling dilutions of serum and a titre of 1 in 16 or more is taken as positive. The incidence of antinuclear factor in the normal population, by this criterion, is 4%. Antinuclear factor is found in 82% of patients suffering from systemic lupus erythematosus, 35% of patients with discoid lupus erythematosus, 78% of patients with systemic sclerosis and 24% of patients with rheumatoid arthritis. In each condition homogeneous factor is present twice as frequently as speckled factor. Nucleolar factor is much less frequently found, its incidence varying from 0.67% in discoid lupus erythematosus to 19% in progressive systemic sclerosis. None of these factors are specific to any particular condition and they remain remarkably constant in individual patients, often over many years, and do not vary markedly with progress of the disease. They are helpful diagnostically, but only if considered in conjunction with the clinical features and other laboratory investigations.

The homogeneous type of staining is produced by anti-nucleohistone. The speckled type of staining is produced by an antibody to saline extract of nuclei. The nucleolar antigen has not yet been determined. These antibodies are not the cause of the diseases in which they are found. They are not present in all cases, their titres are not related to the progress of the disease nor are they specific to any particular type of disease. Antinuclear factors cross the placenta but they do not have any harmful effect on the infant and they decay at the same rate as other globulin antibodies such as those to tetanus and diphtheria (13).

In addition to the above connective tissue disorders, Dr. J. S. Beck and I have also investigated serum from many other conditions. In the main, antinuclear factor has not been demonstrated in patients with polyarteritis nodosa and various other types of cutaneous arteritis. It is also absent from patients suffering from Raynaud's disease, dermatomyositis, various types of purpura, toxic erythema, erythema multiforme, actinic dermatitis, exfoliative dermatitis, erythema nodosum, Reiter's syndrome and sarcoidosis. Usually patients with an obscure illness and high titres of antinuclear factor turn out eventually to develop some form of connective tissue disease. The occasional finding of antinuclear factor in low titre is probably due to the fact that 4% of the normal population have antinuclear factor in their serum.

Comment

The fluorescent antibody technique has considerable potentialities in all branches of medicine including dermatology. Dermatological lesions are particularly suitable for study by this method because of the ease with which biopsies may be obtained. Fresh frozen material is required and retrospective studies are not possible on formalin-fixed material. On the other hand, the investigation of sera for the presence of antibodies can be done in main centres, and if sera are kept frozen at —20°C it is possible for them to be stored for at least two years without loss of activity. This paper is only a brief outline of some of the ways in which this technique has been used up to now in dermatology.

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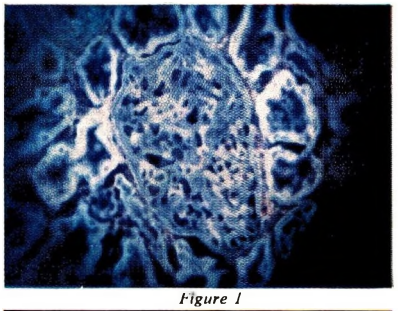
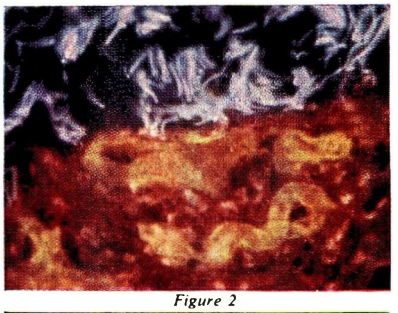


Figure 1



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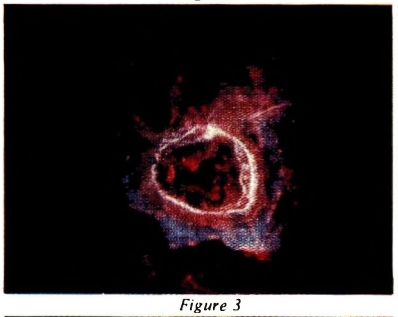

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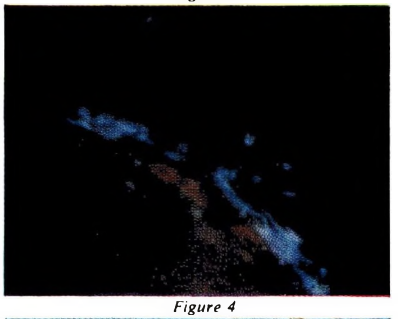

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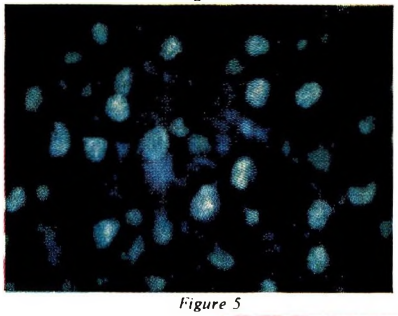

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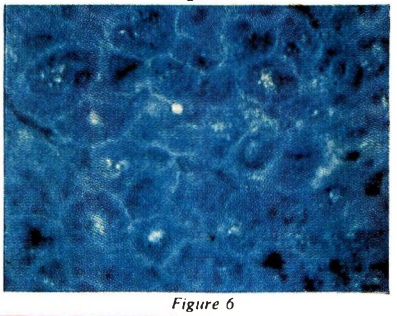

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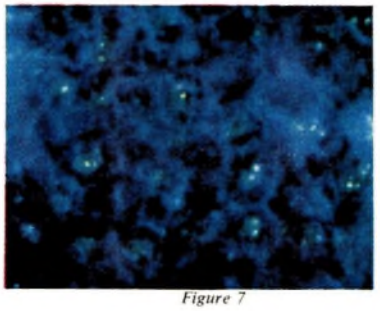

Figure 7

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Introduction by the lecturer

Fluorescent antibody techniques are now used in many fields of medicine, and it is possible that some of you may find them useful in your own work. In recent years dermatology has taken its rightful place as a branch of general medicine and much basic, as well as clinical, research is now going on.

The principles underlying the techniques have already been described above. Basically they depend on the *specific* relationship of antibody to its corresponding antigen. An antibody is a globulin, usually a gamma globulin, produced by the body and capable of reacting with a particular chemical group normally forming part of an antigen. An antigen is a substance capable of reacting specifically with an antibody. Antibodies, against human tissue components, can be prepared in experimental animals. These antibodies can then be labelled with fluorescent dyes, and used as histological markers on human tissues.

Figs. 1—7 illustrate aspects in which my colleagues, Dr. Scott, in the Department of Dermatology at Leeds, Dr. Beck, of the University of Aberdeen, and I have been interested in.

Fig. 1 shows a human renal glomerulus stained by two contrastingly labelled antibodies. The capillary walls are stained a yellowish-green, the epithelial cells yellow (showing the presence of both antigens), and the interstitial tissue red. *Fig. 2* shows a blood vessel in the papillary layer of the epidermis. The silvery blue auto-fluorescence of the elastic tissue under uv light is well shown. *Fig. 3* is a transverse section of an artery in polyarteritis nodosa. Granules of globulin in the media are shown up by a lissamine rhodamine conjugated anti-human globulin antibody.

Sometimes unsuspected histological features may be found. In discoid lupus erythematosus cytoid bodies, which are globules of globulin, are shown up in the region of the epidermo-dermal junction (*Fig. 4*).

In certain systemic diseases, considered to be auto-immune in nature, several antibodies against nuclear material are present in the serum in high titre. Some of these can be demonstrated by fluorescent antibody techniques. *Fig. 5* shows the commonest type of staining pattern, the so-called homogeneous type. *Fig. 6* shows the speckled type, and in *Fig. 7* the nucleoli are stained a brilliant yellow.

The techniques are simple in principle, but very time-consuming in practice if non-specific staining and other snags are to be avoided.

DISCUSSION

MR. N. J. VAN ABBÉ: I should like to refer to the work that we have been attempting on dandruff. We would like to be able to consider the role of *Pityrosporum ovale or obiculare* as possible pathogens by assaying the antigen that they produce in terms of antibody, either in tissue or circulating. We have not progressed very far owing to the difficulty of producing satisfactory antigenic extracts from these organisms.

However, if we were successful, would the demonstration of the presence of a high level of antibody be an indication of the pathogenic role of the organism?

THE LECTURER: The demonstration of antibody in the serum would not necessarily indicate that the organism was concerned in the aetiology of the condition. Non-pathogenic organisms can produce antibodies. On the other hand, the demonstration of a higher incidence of antibodies in subjects with dandruff as compared with a control group matched for age and sex, would be very interesting, although not conclusive.

MR. N. J. VAN ABBÉ: I think you did mention that you have examined psoriasis by your techniques. I think the analogy between psoriasis and dandruff is rather far fetched; none the less it would be interesting if you would comment on what you found in psoriasis.

THE LECTURER: Although Dr. P. R. J. Burch and I (14) recently put forward a new hypothesis to explain the aetiology of psoriasis, we have not investigated psoriatic patients or lesions using fluorescent antibody techniques. Investigative work on psoriasis in recent years has mainly failed to demonstrate any immunological abnormality. Nevertheless, I feel that different methods may eventually be successful.

MR. C. D. MOORE: Has this work any bearing on the problem of dermatitis created by excessive use of surfactants?

THE LECTURER: The fluorescent antibody technique is only one of many methods for demonstrating immunological abnormalities. Work is being done on various types of dermatitis but the results have been rather disappointing. Parish *et al* (15) have been able to demonstrate skin specific cytotoxic antibodies in only about 3–4% of patients with widespread autosensitization dermatitis. However, it must be realized that antibodies may be either humoral or cell-bound, and the latter are difficult to demonstrate.

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J. Soc. Cosmetic Chemists **17** 641-655 (1966)

Spectral slit width and other sources of error in uv spectrophotometry

A. R. ROGERS*

Presented at the Symposium on "Physical Methods," organised by the Society of Cosmetic Chemists of Great Britain, in Bristol on 17th November 1965.

Synopsis—Errors caused by slit width effects, unbalanced reflection losses in the cuvettes, stray light and electrolyte effects are reviewed. Slit-width errors are small with *p*amino-benzoates, aminophenols, camphor, methyl and propyl *p*hydroxybenzoates, methyl and butyl phthalates, and salicylates, but may be appreciable with hexachlorophane, especially in non-polar solvents. The need is stressed for the analyst to have sufficient understanding of the optical principles of spectrophotometry to be able either to avoid the principal sources of error or to minimize their effect on measurements of light absorption.

UV spectrophotometers are now so widely used and on the whole so reliable that the analyst can easily forget that the extinction reading taken from the instrument scale, or from the chart of the recorder, may not be correct. Even if it is correct, it may not be directly proportional to the concentration of the substance being analysed.

In this paper, attention is directed to some of the errors that may arise in spectrophotometry, with emphasis on the instrumental factors responsible. Although the title mentions only uv spectrophotometry, much of the discussion applies also to the visible region and some also to the ir region of the spectrum. The more obvious causes of wrong results, such as fingerprints on the optical faces of the cuvettes or insufficient sample in the cuvette or measurements of turbid samples, will be mentioned only briefly or not at all.

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Useful reviews of spectrophotometric errors are given by Beaven and Johnson (1), Gibson (2), Jaselskis (3), Lothian (4), Meehan (5), Mellon (6), West (7) and White (8), and reference should be made to these works, and to issues of the *Photoelectric Spectrometry Group Bulletin,* for much additional information.

The various errors will be discussed, where appropriate, in relation to Beer's law, $E \propto C$, and Lambert's law, $E \propto d$, where E is the extinction (or absorbance or optical density), C the concentration of the absorbing solute, and d the path length of the cuvette. The extinction E is related to the transmittance (or fraction of light transmitted) T by the identity $E \equiv -\log T$ or $T \equiv 10^{-E}$.

Testing Beer's law

Since Beer's law is the usual basis of quantitative spectrophotometry, tests of the law should be made during the development of an assay procedure. There are advantages in plotting E/C *vs.* C as well as the usual graph of E *vs.* C, because the former will reveal more clearly whether or not the extinction coefficient $(dE/dC)_d$ varies with C (*Fig. 1*). On the other hand, the E *vs.* C plot shows more clearly whether there is a "blank" value, absorption of light even in the absence of the ingredient being analysed.

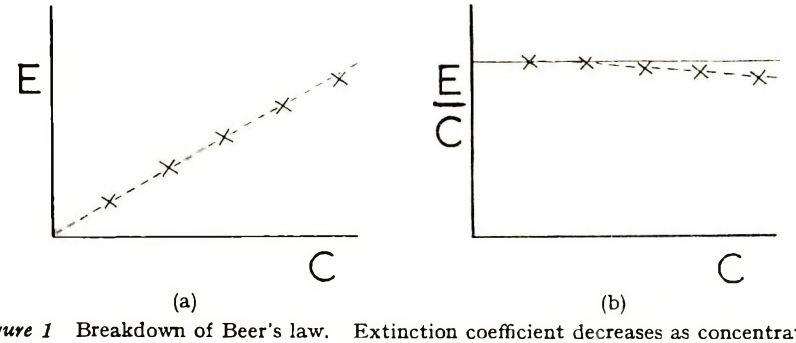

Figure 1 Breakdown of Beer's law. Extinction coefficient decreases as concentration increases.

Higgins (9) has shown that if there is a blank value it is better to use the uncorrected extinction values in plotting the calibration curve, rather than to subtract the blank value from each and to plot the corrected values. However, the mathematical model used assumes that the error of an extinction measurement is independent of the extinction, and it is not certain whether the same conclusion could be drawn if the more

realistic assumption is made that the error of a transmittance measurement is independent of the extinction.

If Beer's law is not valid at higher extinctions (or higher concentrations), a choice has to be made between (a) accepting the error, (b) using a more complicated mathematical expression for the relationship between E and C, (c) confining the measurement to a range where Beer's law is valid with sufficient accuracy, and (d) attempting to find the cause of the breakdown and altering the assay procedure accordingly. One should always try to find the reason for lack of direct proportionality between E and C, because it may be due to faults of technique or in the instrument and because the errors may increase with time. For example, if the curvature of the graph in *Fig. 1* (*a*) is due to stray light (see below), then the curvature will increase and the errors will become greater as the spectrophotometer light source ages and as the optics become poorer.

What is regarded as an acceptable departure from Beer's law depends in part upon the context of the measurement and in part upon subjective considerations. In a colorimetric procedure for the determination of vanadium at the ppm level, it has been stated by Geary and Larsson (10) that "the complex obeys Beer's law over the optical density range of 0 to 1.0 unit" when in fact E/C falls from 0.602 to 0.515 optical density units/ μg/ml over this range, but this is probably an extreme case.

Slit width errors

The monochromator of a spectrophotometer must necessarily allow a finite range of wavelengths of light to pass through the sample. The narrower the entrance and exit slits of the monochromator, the smaller the range of wavelengths transmitted, but there is a limit to closing down the slits, set by the minimum amount of energy that must reach the detector to allow the measurement to be made in a satisfactory manner. In other words, only an approximation to monochromaticity can be achieved in practice.

It may be mentioned in passing that it is wise to pay attention to the "signal strength meter" of a spectrophotometer such as the Perkin-Elmer 137UV or to the "check voltage" of the Hilger and Watts *Uvispek*, to ensure that no attempt is made to use the instrument in conditions where the detector is receiving only a very low signal.

How do observed extinctions depend upon the wavelength spread of the radiation used for their measurement? Is it possible from measure-

ments using heterochromatic radiation to derive what the extinction would be in monochromatic radiation? (5).

Extinction measurements for quantitative analysis are usually made at absorption maxima and it is easily shown that the measured extinction will be less than the true value in the limit of zero slit width. The sharper the absorption maximum of the sample, the more important this slit effect will be, and in an extreme case (procyclidine hydrochloride in aqueous solution at 263.5 mμ) opening the slits of the Uvispek from 0.4 to 0.6 mm. causes a fall of 2% in the measured extinction (11).

Some makers of spectrophotometers (for example, Hilger and Watts) recommend operating the instrument at a fixed spectral slit width, for example at 0.5 mμ. This probably represents a reasonable compromise between ensuring sufficient energy to operate the spectrophotometer and avoiding loss of accuracy, but for quite a number of pharmaceutical substances appreciable errors are introduced if this slit width is much exceeded (11–13). Other manufacturers (for example, Beckman, Unicam) recommend operation of the instrument at a fixed "gain" or "sensitivity" setting, and with this technique little attention may be paid to the slit width needed to balance the spectrophotometer on the reference cuvette. As the spectrophotometer ages, the slit settings will become wider and increasingly low assay results may be obtained.

The effect of spectral slit width errors on the spectrophotometic analysis of a number of materials used in the cosmetic industry has been investigated. The substances examined include *p*aminobenzoates, aminophenols, camphor, hexachlorophane, methyl and propyl *p*hydroxybenzoates, methyl and butyl phthalates, and salicylates. As expected, the slit effect is quite small for most of the substances, because the absorption maxima are broad and well-rounded, and spectral slit widths of up to 1.0 mμ can be used without appreciable error. However, hexachlorophane shows quite a sharp maximum near 300 mμ, and care should be taken to use sufficiently narrow slits in the direct spectrophotometric analysis of this compound, especially in a non-polar solvent (*Fig. 2*). On the Uvispek, the mechanical slit width should not exceed 0.4 mm., which corresponds to a spectral slit width of about 0.6 mμ if a petroleum solvent is used. The effect of change of solvent has been noticed also with the antihistamine chlorpheniramine, which shows a much sharper absorption maximum in plain aqueous solution than in dilute acid (12). The solvent in which the substance shows the broader absorption maximum should be used.

It might be thought that slit-width errors could be avoided by the

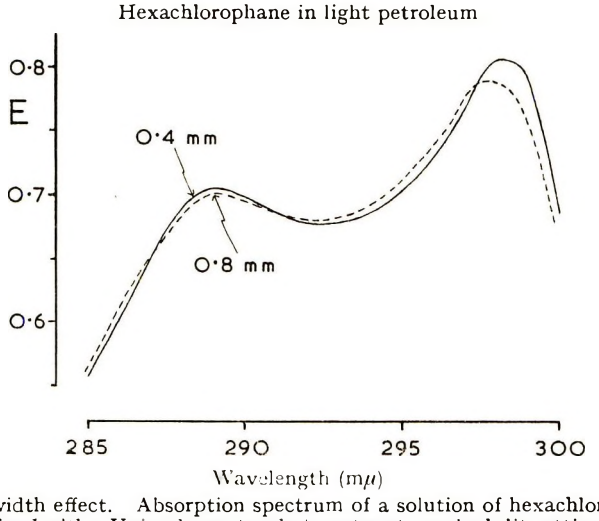

Figure 2 Slit width effect. Absorption spectrum of a solution of hexachlorophane in light petroleum, obtained with a Uvispek spectrophotometer at nominal slit settings of——0.4 mm and ---- 0.8 mm.

adoption of a procedure in which the extinction of the sample is compared with that of a reference substance in the same conditions, and this has been recommended by Andersen (14). Small slit width errors do not invalidate Beer's law (15), but at wider slit widths not only has a graph of E *vs.* C too small a slope (that is, too low an extinction coefficient), but it is curved over at higher concentrations, becoming concave to the C axis (*Fig. 3*). The extinction coefficient decreases with increasing concentration of the sample. This means that if the expected relationship $E_{sam}/E_{ref} = C_{sam}/C_{ref}$ is to hold with accuracy, not only must the slit settings, wavelength settings, etc., of the spectrophotometer be identical, but the concentrations of sample and reference solutions must be identical.

The extent to which the extinction coefficient varies with the concentration, and therefore the extent to which the concentrations of sample and reference may differ without affecting the assay result, remains to be determined in practical cases. From some preliminary experiments, the author believes that the curvature of the Beer's law plot will not be sufficient to prevent use of the reference solution technique, provided that the concentrations are within about 20% of one another, and provided that the extinctions do not exceed unity.

Reflection errors

When a beam of light passes from one medium into another of different refractive index, some of the light is reflected. When the dividing surface

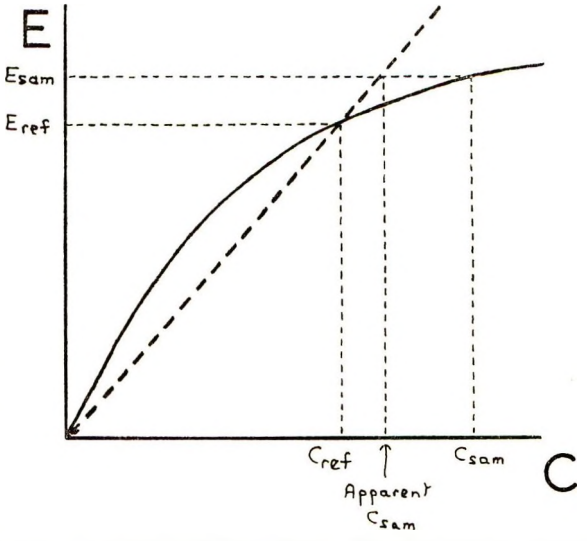

Figure 3 Breakdown of Beer's law. Use of reference substance.
—— True dependence of E upon C.
- - - - Assumed dependence of E upon C.

is plane and smooth, the media transparent, and the light incident perpendicular to the surface, the fraction reflected is $f = (n_1 - n_2)^2/(n_1 + n_2)^2$, where n_1 is the refractive index of medium 1 and n_2 that of medium 2.

For example, the fraction reflected when light passes from air (n = 1.00) into flint glass (n = 1.58), or from glass into air, is $f = (0.58/2.58)^2 = 0.0505$ or 5.05%. The fraction transmitted is $(1 - f) = 0.9495$ or about 95%. For the surface between glass and water (n = 1.33), $f = (0.25/2.91)^2 = 0.0074$ or 0.74%. In transmissions through a glass plate, two reflecting surfaces are involved, air-glass and glass-air, and if only a single reflection at each surface is considered, the fraction transmitted is $(1 - f)^2 = 0.9016$. This is not the observed transmittance, however, because multiple reflections must be taken into account, and the correct value of the transmittance is $(1 - f)/(1 + f) = 0.9039$, or an extinction of about 0.044. When light is transmitted through a glass cuvette containing a liquid, two glass-air and two glass-liquid surfaces are involved. For a glass cuvette containing water, the transmittance is about 0.891 or an extinction of about 0.050.

In quantitative spectrophotometric analysis, the assumption is often made that the extinction of the sample solution is equal to the sum of the extinction of an identical cuvette containing pure solvent and the contribution made by the absorption of the sample itself. To what extent

is it possible to replace the contents of the reference cuvette by some other transparent solvent?

If the solvent for the sample is fairly volatile, for example chloroform (n = 1.45), it may be experimentally convenient to put the much less volatile and equally transparent water into the reference cuvette. The reflection losses will be different, however, and in fact the transmittance will be about 0.891 with water and 0.901 with chloroform. The extinction of the "blank" will be 0.050 with water, instead of 0.045 with chloroform, and a Beer's law plot of E *vs.* C will show a small negative blank value. Water appears to be less transparent than chloroform.

Although this effect is quite small for such a drastic change of solvent, and it is smaller still if crown glass (n = 1.51) or silica (n = 1.46) cuvettes are used, it must be appreciated that all of the calculations in this section have been based on the refractive index values of the substances in visible light. The refractive index differences can be greater in the uv region, and so reflection errors can become more important.

Another effect must be considered. The refractive index of the solvent will be altered when sample is dissolved in it. The refractive index of the contents of the sample cuvette will always differ from that of the liquid in the reference cuvette. Spectrophotometric measurements are naturally made in regions of absorption of light by the sample, and it is in these regions of the spectrum that "anomalous dispersion" effects are observed. It follows that a part of what is normally considered to be the absorption of light by a sample in a particular solvent may really be loss of light by reflection due to lack of refractive index matching of solvent and sample solution. This source of error has been mentioned (3) but has apparently not been studied experimentally. Indeed, few measurements have been made of the refractive index of absorbing solutions, especially in the uv, and apparatus is being constructed to study these and related phenomena in our laboratories.

Stray light errors

"Stray light" is a convenient term for that part of the light leaving the monochromator that is not part of the band of wavelengths nominally transmitted at the instrument settings (slit, wavelengths, etc.) used. Some of this stray light is due to imperfections of the optical parts, and has wavelengths quite close to the nominal wavelength. Its effect is on the whole similar to a slight increase in the slit width, and it will not be considered further here. Much more serious is stray light of totally

unrelated wavelengths, which originates from the scatter of light and from unwanted reflections within the monochromator. In a grating instrument, stray light may come also from orders of the spectrum other than that nominally used.

Although the actual amount of stray light will normally be very small, its effect will be greatly increased if the light source of the spectrophotometer is emitting little light of the desired range of wavelengths, and if the photocell or other detector is more sensitive to the stray light than to the wavelength nominally transmitted. Figures quoted for the percentage of stray light from a monochromator are normally "working values" based on the instrument response. Thus, "1% of stray light" means that the electrical signal produced by the detector as a result of the stray light is 1% of that due to light of the wavelengths nominally transmitted.

Consider measurements made on a sample at a wavelength where the true extinction of the sample is high, but in the presence of stray radiation of wavelengths to which the sample is transparent. If I_o is the incident intensity of light of the nominal wavelengths, I the intensity of light transmitted by the sample at the same wavelength, and I_s the intensity of the stray light (all I values being expressed as instrument response), then the extinction measured will be $E_m = \log (I_o + I_s)/(I + I_s)$ instead of the true value, $E = \log (I_o/I)$ with no stray light. (*Fig. 4.*)

As the stray light becomes greater, the measured transmittance rises from (I/I_o) towards unity, and the measured extinction falls from $\log (I_o/I)$ towards zero.

The effect of a given level of stray light, I_s, will be least at low extinctions, for then I tends towards I_o and $(I_o + I_s)/(I + I_s)$ and (I_o/I) become identical in the limit when $E = 0$. At high extinctions, as I tends towards zero, E_m tends towards a maximum value, namely $\log (I_o + I_s)/I_s$ or $\log (1 + I_o/I_s)$, but of course this maximum value varies with wavelength because I_o is a function of the wavelength.

Stray light effects are greatest at the ends of the useful range of wavelengths of a particular combination of light source, monochromator and detector.

Consider an attempt to measure the extinction of a 0.05 M aqueous solution of glycine at wavelengths below about 225 mμ. An ordinary uv spectrophotometer, capable of measuring extinctions up to 3 (transmittances down to 0.1%) should show no maximum, but only a steeply rising absorption curve at the shorter wavelengths. However, when the

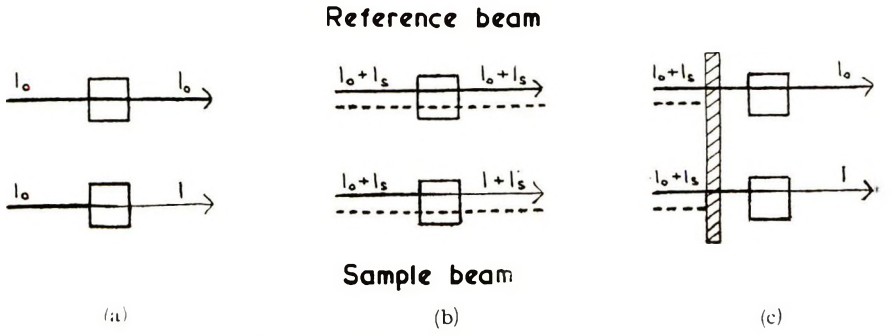

Figure 4 Stray light effect.

(a) No stray light. $E = \log \frac{I_o}{I}$.

(b) Stray light. No filter. $E = \log \frac{I_o + I_s}{I + I_s}$.

(c) Stray light. Filter. $E = \log \frac{I_o}{I}$.

monochromator is set to transmit a wavelength of, say, 210 mμ, any stray light from longer wavelengths will also reach the sample, and since the output of the hydrogen or deuterium lamp and the sensitivity of the photocell or photomultiplier are both greater at the longer wavelengths, the effect of the stray light may be detectable as a reduction of the measured extinction. At a shorter wavelength, say 205 mμ, the stray light level will be about the same, but the signal due to light of 205 mμ will be less, because the true extinction of the sample is greater at 205 mμ than at 210 mμ, and because I_o itself is greater at 205 mμ than at 210 mμ. The relative effect of the stray light will therefore be greater at 205 mμ, and the reduction of the extinction will be greater than at 210 mμ. If the relative effect of the stray light is large enough, the spectrum measured will show a completely spurious maximum.

This is illustrated in *Fig. 5*, where the spectrum recorded by the Unicam SP.700 spectrophotometer (which is almost new) may be taken as correct down to about 205 mμ, whereas both the other spectrophotometers show low extinctions in this region. The Perkin-Elmer instrument is about three years old, and the Uvispek about nine years old. The effect of deterioration of the optics is clear, for all three spectrophotometers behaved equally well when new.

It should perhaps be mentioned that although the Perkin-Elmer spectrophotometer cannot record directly extinctions greater than 1.5, measurements at higher extinctions can be made indirectly if the reference

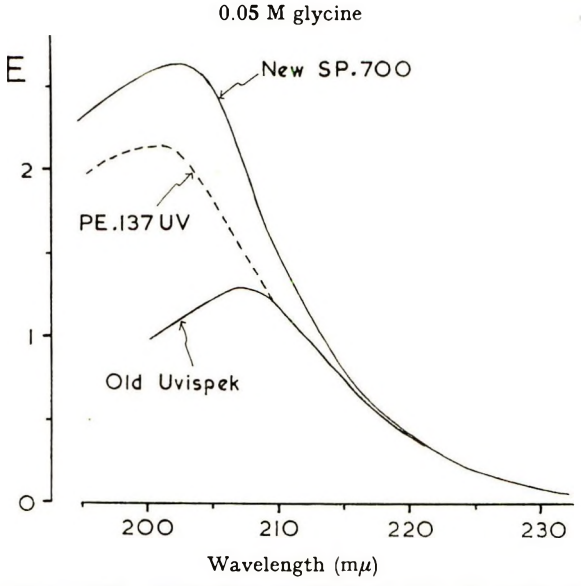

Figure 5 Stray light effect. Absorption spectrum of 0.05 M glycine in water obtained with a new Unicam SP.700, a Perkin-Elmer 137UV and an old Hilger and Watts Uvispek.

beam is attenuated by the insertion of a grid of known extinction, substantially independent of wavelength. The SP.700 extinctions were calculated from transmittances recorded on the 0 to 11% and the 0 to 110% ranges, for the absorbance accessory did not allow direct recording of extinctions greater than 2.

It is important to realise that different percentages of stray light are measured by different techniques, and that stray light effects depend not only upon the state of the spectrophotometer, but also upon the shape of the (true) absorption spectra of the sample and the solvent. It is reassuring to be reminded that if a spectrophotometer is satisfactory below 230 mμ, it will be very much better at longer wavelengths (16).

At the other end of the uv spectrum, incorrect readings may be made if a tungsten light source is used in the region below 390 mμ. The intensity of light of the desired wavelength in the near uv region is very much less than that of visible light of longer wavelengths, and so even a very small true percentage of stray light may have a large effect in terms of the signal from the detector, which also is more sensitive to visible light than to uv light. The measurements can be improved in two ways. A Chance OX.7 or similar filter can be inserted into both reference and sample beams; this will transmit the desired uv light but be opaque to

the visible, stray light (*Fig. 4*). Alternatively, a hydrogen or deuterium source can be used instead of the tungsten lamp; the output of visible light by a hydrogen lamp is very small and so the stray light effect will largely disappear (*Fig. 6*).

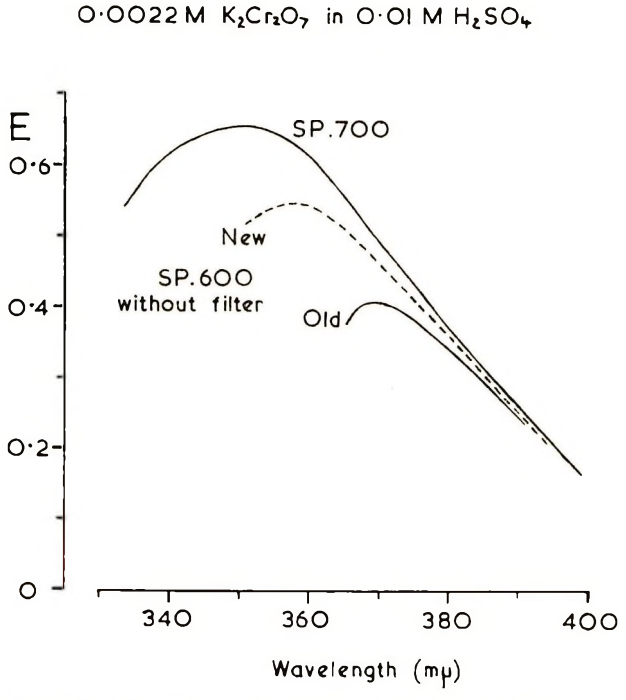

Figure 6 Stray light effect. Absorption spectrum of 0.0022 M potassium dichromate in 0.01 M sulphuric acid, obtained with a new Unicam SP.700 (deuterium lamp), a new Unicam SP.600 (tungsten lamp; no filter) and an old Unicam SP.600 (tungsten lamp; no filter). The spectra obtained with both SP.600 spectrophotometers with use of an OX.7 filter could be superimposed on the SP.700 curve.

Electrolyte effects

Junejo and Glenn (17) have found that neutral salts such as sodium chloride can have an appreciable effect upon the uv absorption of aromatic compounds such as benzyl alcohol in aqueous solution (*Fig. 7*). They have pointed out that this has important implications in spectrophotometric analysis, not only because the extinction coefficient of an aromatic compound may (erroneously) be assumed to be the same in the presence of electrolytes as in plain aqueous solution, but especially in differential spectrophotometry by the "$\Delta\varepsilon$" technique (18), where the erroneous

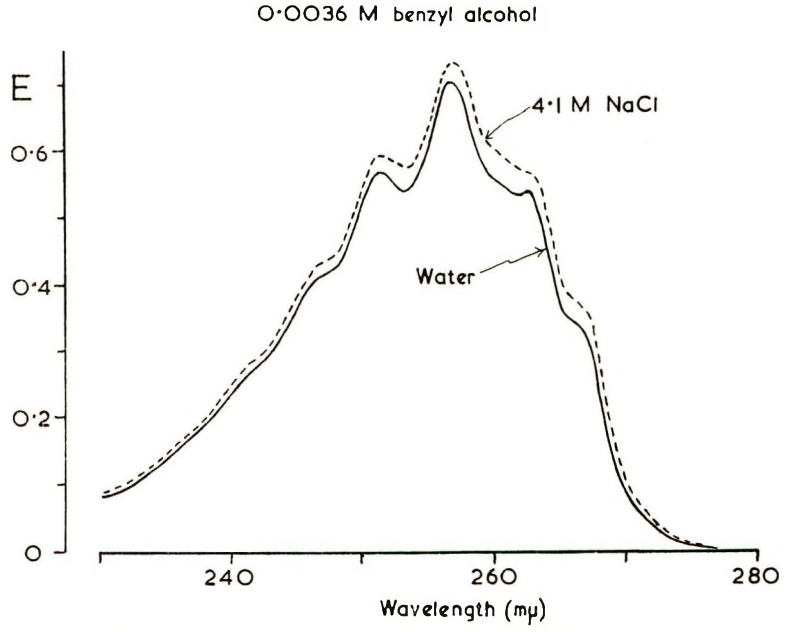

Figure 7 Electrolyte effect. Absorption spectrum of 0.0036 M benzyl alcohol —— in water and - - - - in 4.1 M sodium chloride.

assumption may be made that the background or irrelevant absorption is unaffected by the electrolyte concentration (17,19).

This electrolyte effect is shown whether or not the aromatic substance has acidic or basic properties, and it is not due to an effect upon the position of an acid-base equilibrium. The explanation may be in terms of donor-acceptor complexes between the electrons of the aromatic ring and the cation of the electrolyte (17,20) or perturbation of the electronic transition by the electrical fields of the ions (21).

Conclusion

Spectrophotometric errors due to slit width effects, unbalanced reflection losses, stray light or electrolytes can usually be avoided by attention to the instrument or by modification of the experimental procedure, provided that the possibility of error is considered. At the worst, one may be able to use a non-spectrophotometric method to acquire the desired information. The difficulty often lies in the lack of knowledge that something may be wrong, and the author can recommend the institution of control charts, filled in every day or week after simple tests (*e.g.*, of a glass

filter) have been made, to warn when there is a likelihood of "things going off the rails" (22).

Finally, a reminder should be given that the actual measurement of an extinction is only one stage of a complete analysis, which may have involved a number of separation and dilution stages. Poor accuracy or lack of reproducibility of the final result may be due to errors of weighing or pipetting (23) or extraction or development of colour (24) rather than to the spectrophotometer.

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DISCUSSION

MR. D. H. DORKEN: What routine control tests do you use to make sure that the instrument is in satisfactory condition for use?

THE LECTURER: For the manual instruments, the wavelength calibration and the extinction of a test filter at a single wavelength are checked every day of use, and the slit width required to balance the spectrophotometer is recorded, as recommended in (22). Every few months, a more extensive series of tests is made, such as those by the service engineer on his annual maintenance visit or when the instrument is new.

MR. D. C. CULLUM: The errors listed are of two kinds – those that may change with the age of the instrument, such as slit-width and stray-light errors, and those that are part of the analytical procedure. How important are the latter for the practical analyst who is interested in getting a percentage content of an ingredient, rather than an absolute extinction coefficient?

THE LECTURER: In my view, use of a reference or standard solution is very desirable in any spectrophotometric assay where accuracy is important, and this will usually take care of many types of error, since the errors will tend to cancel in the sample and reference solutions. This is not always adequate as shown in *Fig. 3*, but at least the accuracy should be improved by this technique. Spectrophotometers as usually operated are not really capable of high absolute accuracy, but their accuracy in comparative measurements is usually good.

DR. N. A. PUTTNAM: I should like to emphasize this last point, bearing in mind the results that have been published by the Photo-electric Spectrometry Group for their collaborative tests of manual and recording uv instruments.

THE LECTURER: I agree that the P.S.G. tests revealed variations as great as 5% from one instrument-user combination to another, but it would be fair to say that there were certain technical difficulties in preparing and handling the samples. Many of the collaborators were not analysts but chemists who normally use their spectrophotometers only to get the overall shape of the spectrum, rather than an accurate extinction coefficient.

DR. J. J. MAUSNER: Can the slit-width effect be reduced by taking the total integral area under the spectral curve instead of the extinction at the wavelength of maximum absorption?

THE LECTURER: I am not sure that the answer is known with certainty for uv measurements, although this approach has certainly been used in the ir region. An improvement can probably be effected, but one must decide whether to integrate extinctions or transmittances with respect to wavelength or wavenumber. Since it is usually easy to eliminate the slit-width effect in uv spectrophotometry by narrowing the slits, I doubt if use of an integrator would be worth the effort; I have recently obtained an integrator for our SP.700 spectrophotometer and this is something I plan to examine.

MR. R. CLARK: Are there any particular problems in measuring refractive index in the uv region?

THE LECTURER: Suitable commercial instruments are not available, but I have been fortunate in obtaining the support of the Science Research Council in getting one built. Although a differential uv refractometer is marketed in the U.S.A., I wish to make direct absolute measurements, and initially the instrument will be manually operated. The precise temperature control of the sample may be one difficulty, and absorption of light by the sample may be another. So little work has been published in this field that it is rather difficult to predict anything.

DR. N. A. PUTTNAM: Do you intend to measure the refractive index at wavelengths inside the absorption bands of the samples?

THE LECTURER: Yes. It is the "anomalous" dispersion in this region that is of major interest, and I expect that the measurements within the absorption bands will be more rewarding than those where the sample is transparent.

J. Soc. Cosmetic Chemists **17** 657-667 (1966)

Preparative gas chromatography

G. R. FITCH*

Presented at the Symposium on "Physical Methods," organised by the Society of Cosmetic Chemists of Great Britain, in Bristol on 17th November 1965.

Synopsis—The main aspects of this technique are discussed, including the newer developments in continuous gas chromatography. Some possible applications in the field of cosmetic chemistry are given.

Introduction

The widespread application of gas chromatography stems mainly from the work of Martin and James (1) on gas-liquid chromatography. The majority of publications since then have dealt with analytical and theoretical aspects of the subject, whilst the primary function of the technique, namely the ability to separate and purify individual components of a mixture, has tended to play a minor role.

The scope of the method as a preparative tool ranges from the normal analytical scale of several microlitres suitable for identification of unknown peaks by spectrophotometric analysis, to a kilogram per day scale for high purity materials.

Since no substances have partition coefficients of zero or infinity, components are never completely separated and the limits of impurity can be fixed according to requirements. The more stringent these are, the greater the process time and hence the operating costs increase. The technique does not necessarily replace other separation techniques, but is particularly useful with gaseous or liquid mixtures (boiling up to about 400°C), which are otherwise difficult or impossible to separate, and where, on a large scale, an azeotropic or extractive distillation plant would be uneconomic and inflexible.

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It has been shown (2) that a chromatographic column is most efficient when a very small sample is used. In order to handle larger quantities, analytical columns can be overloaded with a consequent loss of efficiency. Alternatively, the separation may be repeated many times, either manually or automatically until samples of a suitable size are obtained. This process can be reduced in time by running several columns in parallel.

Finally, the process can be made continuous. One method of achieving this is by making the column material move countercurrent to the gas phase and continuously injecting the mixture into the centre of the column–so called "moving bed chromatography."

Difficulties as to whether such a process is truly chromatographic were discussed at the London Symposium on Gas Chromatography (3) and the justification for discussing such a process in chromatographic terms were given quite clearly by Benedek (4) who describes continuous methods of operation analogous to all conventional methods.

The choice of method depends on the sample size, difficulty of separation and, of course, the apparatus available. Some of the points which follow may help to solve this problem. Instrumental aspects will not be dealt with here and are fully covered in most standard textbooks (5).

Batch processes

A typical analytical column of say $\frac{1}{2}$ cm diameter operates most efficiently with a sample size of the order of 1μl. If it can be assumed that the capacity is proportional to the square of the diameter, a 1 ml sample would require a 7.5 cm column to achieve the same quality of products.

If some loss of separating power can be tolerated, overloading the column permits the handling of larger quantities than this by at least a factor of 10, and in fact, many 2.5 cm preparative columns have been described, and are available commercially, that can handle 1 ml samples. It should be noted that losses in column efficiency arising from causes other than overloading will occur. These are mainly due to difficulties in packing large diameter columns. The introduction of constrictions into the column by Frisons (6), following a suggestion by Golay (7) appears to be very effective in minimizing these effects.

Some of the first reported preparative units were described at the London Symposium in 1956. Whitham (8) gave details of a 1.27 cm diameter column for 1 ml samples and Evans and Tatlow (9) discussed the separation of 10 ml samples on a 3 cm column. Bayer (10) reported

that the sample size could be increased from 0.1 to 10 g on corresponding columns of 1 to 10 cm without serious loss in efficiency. Many other units ranging from 2.5 to 7.5 cm in diameter have since been described, some of them up to 25 ft long (11–14).

The advantages of such systems are that they are fairly easy and cheap to construct since the very close temperature control associated with analytical units is not necessary. Many commercial units can be readily adapted for large scale work with the addition of a column and a stream splitter to ensure that the detector is not overloaded.

The main disadvantages lie in the length of time required to run a sample, and the relatively high cost of column materials and carrier gas. The elution of slowly moving components can be facilitated by progressively raising the temperature, so-called "programme temperature operation," but this is not usual practice on a large scale column.

It is often more economic to repeat the separation on a high speed analytical column and bulk the collected fractions together until a sufficiently large sample is obtained, than to scale up the column dimensions. This can be done manually but is obviously suited to some automatic injection and collection system such as that described by Ambrose and Collerson (15) and refined by Atkinson and Tuey (11). The latter workers used a 2 cm × 30 cm column and were able to prepare 50–100 g of 99.8% purity material per 24 hr. Even larger quantities result from the automatic injection system of Bayer's (10), using larger columns.

A saving of time, without the loss in efficiency associated with large scale columns results from using analytical columns in parallel. The practical difficulty of ensuring that the components are eluted from each column at exactly the same time was overcome by Johns *et al* (16) who used eight 6 ft × $\frac{5}{8}$″ columns each containing the same weight of stationary phase and having separate pressure drop controls.

Continuous processes

A very effective continuous unit has been described by Dinello (17). This consists of a set of 100 columns, each 0.6 cm × 120 cm mounted on a rotating drum, such that the columns move transversely to the carrier gas flow. The speed of rotation of the drum is controlled so that a particular component is collected in the same sample tube from each column. This eliminates the need for a complex sample switching system and any detection and recording system. They describe separations of 1/1 *n*heptane/toluene at 60 ml/hr and suggest that the upper limit for

the separation of a benzene/cyclohexane mixture at 99.9% purity should be about 220 ml/hr.

An extension of this technique has been described by Giddings (18) following an earlier suggestion by Martin (19). Here the column packing is contained between two concentric cylinders which can be rotated about a vertical axis. Continuous injection of the solute mixture to one side of the top of the column should result in the individual solutes following a spiral path down the column and emerging at fixed points. However, lateral diffusion gave products of poorer quality than expected.

The first truly countercurrent process was described by Freund *et al* (20) in 1956 for the resolution of industrial gases following earlier work by Berg (21). Although this process is one of gas-solid chromatography, and given the name "hypersorption," it is a direct parallel with continuous gas-liquid chromatography. A plant is described capable of separating 10^5 m^3/day of acetylene from the products resulting from the partial oxidation of methane. More recently Husband *et al* (22) have used active carbon to continuously separate ethyl alcohol/benzene mixtures at a rate of 720 ml/hr on a 2.5 cm column.

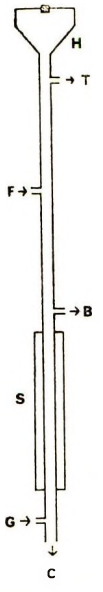

Figure 1 Moving bed column (H–hopper, T–top product, B–bottom product, F–feed, S–stripping section, G–carrier gas, C–solid flow controller).

The earliest continuous gas-liquid unit was described by Scott (23) for the separation of benzene from coal gas on a 5 cm column. A similar

unit has been described by Schulz (24) for the separation of cis and trans butene on a 1 cm^2 column at 78 ml/hr. Both these units use a gaseous feed and the capacity on a weight basis is not very high.

Barker (25) has studied the process in terms of transfer units using a liquid feed of benzene/cyclohexane at 30 ml/hr and the process has been discussed in terms of plate theory by Fitch *et al* (26). The operation of such a process can be described by reference to *Fig. 1*. The column packing, consisting of say 10-30 B.S.S. Chromosorb P impregnated with a suitable stationary phase is fed into the hopper and its rate of movement down the column controlled with a suitable device, such as a rotating drum, at the bottom of the column. The carrier gas, usually nitrogen, is fed into the bottom of the column and its passage counter-current to the packing is ensured by maintaining a slight vacuum on the exit ports. The top and bottom products are taken off at the ends of the extraction column as shown, while the feed enters at the centre of this portion. Complete stripping of the solutes is ensured by heating the stripping section.

A suitable arrangement for gas flows is shown in *Fig. 2*. The liquid feed can be fed into the column with a micropump of appropriate size.

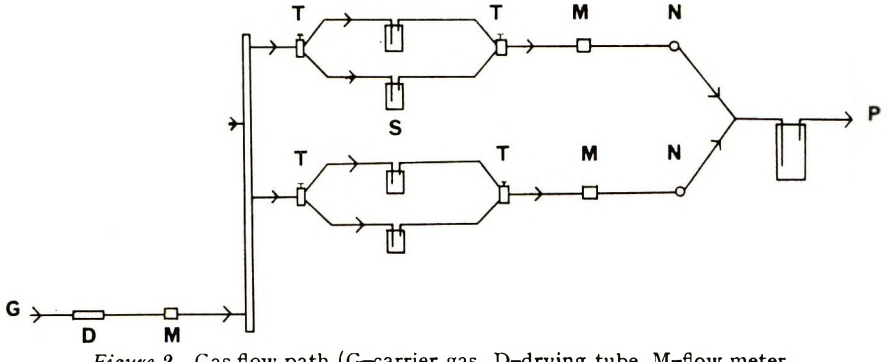

Figure 2 Gas flow path (G–carrier gas, D–drying tube, M–flow meter, T–tap, S–sample tube, N–needle valve, P–pump).

The conditions for separation in terms of gas and solid flow rates are such that at the temperature of the column, the component(s) with the higher volatility move with the gas phase and those with the lower volatility move in the direction of the solid packing, these being removed in the stripping section. The gas and solid flow rates necessary to bring about the desired fractionation can be calculated directly from the appropriate chromatograms obtained on an analytical column at the same temperature and on the same stationary phase. Full details of this are

given in (26). Similarly, investigational work on the choice of a suitable stationary phase and operating temperature can be obtained using a normal analytical column.

The capacity of the column is a function of the partition coefficients and their ratio, as well as of the gas and liquid flow rates and column temperature. The relative retention time is probably the most important single factor in determining the capacity of the column and is analogous to the relative volatility factor in distillation units. A theoretical analysis of this problem is being undertaken. Obviously the column must never reach the stage of visible "wetting," and the upper limit of gas flow will correspond to the onset of fluidization of the moving bed. An interesting complication in the case of azeotropic systems has been noted by Tiley (27) which would set an upper limit on the feed rate due to a change in the relative volatility from greater to less than unity as the concentration increases.

A further variant on the basic process has been described by Bradley and Tiley (28) in which the usual microporous type of chromatographic support is replaced by a packing of "knitmesh" and the liquid phase percolates over this countercurrent to the gas phase. It appears that while this process is easier to control, the plate height is greater than with the normal microporous type packing.

Injection and collection of samples

In the conventional batch type units, the thermal capacity of the column packing is not usually sufficient to ensure rapid volatilization of the large sample and some form of vaporizer is desirable, operating slightly above the column temperature. Care must be taken to ensure that no thermal degradation takes place, and incidentally, that the column temperature itself does not give rise to any appreciable bleed of the stationary phase. Introduction of the sample is usually accomplished with a syringe, but by-pass injectors have been described (29).

The positions at which cuts must be made to yield samples of a given impurity level have been treated by Said (30). The actual method of collection of the samples has received much attention and depends very much on the boiling points of the constituents and their concentration in the gas stream.

If one only requires a sample for further examination by a spectrophotometry method it is possible, using an ordinary analytical column, to condense the fraction directly on to potassium bromide, as described by

Leggow (31). Alternative methods include absorption in benzene, followed by evaporation on to a KBr disc (32) or in cooled dioxan (33). Thomas and Dwyer have absorbed the vapour directly on to a "millipore" filter made out of cellulosic membrane, and mounted this in the sample beam of an IR spectrophotometer (34).

For efficient recovery of larger samples, the shape and temperature of the trap appears to be most important, particularly for components boiling up to 100°C. Verzule (35) has made an analysis of the variables affecting trapping efficiency and has shown that a drop in the gas flow rate during peak elution helps to increase the percentage recovery.

The formation of "mists" is often very troublesome and suggested methods of overcoming this include the addition of glass wool to the trap and the direct adsorption on to carbon. Thompson (36) has used an electrostatic precipitator to give up to 98% recovery from a 2.5 cm column at a rate of 2 g/5 min. However, this method has to be used with care since degradation of the sample is possible under these conditions (11).

For the collection of several components from one sample, a wide variety of manual and automatic fraction collectors have been described (37,38). In any apparatus involving several collection ports on a manifold, it is imperative that the temperature of the apparatus should be maintained above the boiling points of the constituents to prevent condensation prior to the traps.

Applications

In the cosmetic industry, the main applications of this technique are the isolation of both major and minor constituents from raw materials for use as standards, and for assessment of flavour and odour characteristics. The possibility of continuous preparation of key constituents on a kilogram scale may become important in the formulation of products where undesirable characteristics are exhibited by some constituents of the raw material. In some cases, it may be sufficient to isolate fractions of similar type or molecular weight.

The comparative costs of continuous chromatography compared with fractional distillation are difficult to assess, and, in general, a distillation process would be preferred, if suitable. If, however, the desired separation is not possible by distillation, a thorough literature search followed by evaluation of stationary phases, temperatures and flow rates are essential to ensure that the components will be eluted in the desired purity range and in the shortest time. Once optimum conditions are

established, the changeover to either larger columns or to a continuous unit is relatively quick and cheap. From that point onwards, the running costs are made up mainly of carrier gas costs (high purity gases are not usually necessary) and normal supervisory costs. Preliminary trials soon indicate the output of the unit for the separation in question; this may differ widely with various starting materials.

An example would be the isolation of major constituents of essential oils such as citral a and citral b which constitute some 60–90% of lemongrass oil. At a temperature of about 100°C on a P.E.G. 400 column, these isomers are eluted much later than the other constituents and are themselves fairly well separated, with a relative retention ratio of about 1.3. On a continuous basis, flow conditions could be adjusted to give as a bottom product either the mixed isomers, which could then be separated on a second column, or pure citral a, the citral b leaving with the impurities as a top product.

Likewise, l-carvone is readily separated as a bottom product under similar conditions from spearmint oil.

Some applications of the continuous process may be covered by patent restrictions and references to these are given (39).

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Introduction by the lecturer

Two publications have recently been distributed by manufacturers of conventional preparative scale chromatographic units. One of these advocates the use of short, wide columns and the other favours long, narrow ones. It is clear that either type has advantages over the other in particular circumstances.

Our special interest is in continuous chromatography. One of the main virtues of this type of apparatus is its simplicity and cheapness–a total outlay of about £100 should suffice. All the initial investigational work on gas and liquid flow rates and on choice of a partitioning liquid can be carried out on a conventional G.L.C. unit. The details of the calculations of flow conditions from specific retention volumes are given in (26).

Continuous chromatography would appear to be the preferred method of separation where large quantities of purified material are required. It is particularly useful in cases where it is desired to split the feed mixture into two fractions. Although multi-component fractionation is possible, it is probably easier to handle this type of sample on an automatic injection batch type instrument.

Continuous chromatography does not appear to be very satisfactory if the relative retention ratio of the components is lower than 1.2, though this depends on the purity required.

The capacity of any gas chromatographic unit, batch or continuous, depends very much on this relative retention ratio; any quoted values for the capacity of a particular unit are meaningless without this information.

We are currently following two lines of research on the continuous process. In

one unit we are using conventional chromatographic column materials, and these are allowed to fall at a controlled rate countercurrent to the gas phase. In the second unit, the column is packed with knit-mesh and the chosen stationary liquid phase is allowed to percolate over this, again the gas phase flowing in an opposite direction. The former type gives a smaller plate height and hence for the same length of column a better quality product results, whereas the second type of unit is easier to control.

The estimated number of theoretical plates in the 1m extraction stage of the moving bed apparatus is about 80 and while this may seem low compared with values usually quoted for conventional G.L.C. columns, it is in fact equivalent to about 6,000 plates in a batch process.

Most of the work proceeding at the moment is concerned with the prediction of the maximum capacity under given operating conditions. The equations relating the various factors are suitable for computer programming but this needs appropriate physical data, such as the variation of activity coefficients with concentration, and this is also being studied.

Some of the theoretical results so far produced indicate that there is a critical operating temperature for a particular system operating at a given capacity. Above or below this temperature, the purity of the product decreases. Binary azeotropic mixtures also set a limit on the capacity of a given system, the relative retention ratio changing from greater than unity to less than unity, as the concentration increases.

The cost of running a continuous unit is very low, and would be approximately the same as a conventional preparative G.L.C. unit. Commercial grade nitrogen, or even compressed air in certain cases, is the main consumable and so a high purity material could be prepared at about 5–10% more than the cost of the raw material.

One final word of warning to anyone contemplating the use of preparative G.L.C. High temperatures must be used with care since the products can readily be contaminated by solvent "bleed"; in addition many of the materials of interest in the cosmetic industry are heat-sensitive.

DISCUSSION

MR. J. D. CHESHIRE: Could you give us any information on how this technique might be applied to continuous process control? If one is looking for one particular component in a cosmetic product, it may be possible to continuously monitor for this if it is volatile.

THE LECTURER: This is interesting but I think there would be difficulties. The system itself often takes several hours to reach equilibrium, and sufficient control of the flow rates for analytical purposes would be difficult. In continuous preparative work, the flow rates can fluctuate about set values without affecting the overall quality of the products, but in your case, the concentration of the component of interest in the carrier gas stream would fluctuate with the gas flow fluctuation without any change in the feed composition.

A conventional high speed analytical unit would be more suitable for this type of process monitoring.

MR. J. D. CHESHIRE: I was thinking of using a much smaller column than is used for preparative work.

THE LECTURER: Scaling down the column would decrease the hold-up time but

we have found that the solid flow is very difficult to control if the column is much less than 1″ in diameter.

Mr. R. J. Brook: We have found on a "Wilkens" preparative unit that a $\frac{3}{8}$″ diameter column, 20′ long gives about the same resolution as a $\frac{1}{4}$″ diameter column, 6′ long. With short fat columns, "F. and M" fit special discs to correct wall effects and prevent band spreading, and this increases the resolution. Without these discs, I think $\frac{1}{2}$″ diameter is the maximum of practical use.

The Lecturer: It is important that some form of flow distributor is introduced into the column if the diameter is to be increased for larger samples. These may be in the form of capillary connectors or distributor discs. Without these, a large loss in resolution occurs.

Dr. A. W. Middleton: Can you expand on moving stationary phase?

The Lecturer: In continuous chromatography, the same liquids are used as partitioning agents as with conventional G.L.C. The stationary phase on the solid support moves down the column under the influence of gravity. It is obviously incorrect to refer to it under these conditions as a stationary liquid phase, though it is not a free flowing liquid. We use commercial grade dinonyl phthalate, which appears to have the same properties as the more expensive chromatographic grade.

Book reviews

DERMATOLOGY: A FUNCTIONAL INTRODUCTION.

A. Jarrett, R. I. C. Spearman and P. A. Riley. Pp. x + 246 + Ill. (1966). *English Universities Press, London.* Paperback: 20s. Boards: 30s.

This opens with a delightful comparison between textbooks of dermatology and horticultural catalogues; lists of strange diseases are said to look remarkably like a nurseryman's account of his stock. Both, of course, have tended to use dog–Latin in the same manner. impressively rather than informatively. However, with Dr. Jarrett among the list of authors, one would not expect to find the traditionally unscientific approach and the volume indeed attempts to pioneer a different route.

The basis of dermatology is unfolded in 17 chapters, many of which deal primarily with a morphological aspect of the subject (the epidermis, the dermis, the hair follicle, sweat glands, etc.) along with associated abnormalities or pathological conditions. A number of chapters, however, start from a more general grouping of allied problems such as those on "metabolic diseases of the skin", "skin sensitivity and associated disorders" and "genetics and the skin". Thus the approach tries to be logical and scientific, whilst at the same time complying with its title indicating that it is an "introduction" and not a massive work of reference.

As cosmetic chemists, we are conscious of the hazy borderline dividing our field from the undeniable province of therapeutics; not only should we encompass a sound knowledge of our own field, but we need also to be able to recognize where trespassing begins. For example, whilst dandruff is invariably considered a justifiable target for a medicated toiletry product, we should have some understanding of other scalp disorders less appropriate for lay diagnosis and treatment. Whilst we may not read this book from cover to cover like a novel, there is certainly useful information for the cosmetic scientist on nearly every page; a particularly relevant chapter is, of course, the one dealing with skin sensitivity.

Perhaps the most noticeable weakness is in the section on skin medication. This does little more than reiterate the usual list of weary old mystery ingredients, such as tar, ichthammol and dithranol; the authors have little to say about these, in fact, for hardly anything is known for sure about their true activity (if any). There is surprisingly scant attention to the corticosteroids and antibiotics or to systemic medication for skin disorders. Some of the more pertinent information on treatment is given under the individual disorders but the empiricism governing the problem of skin medication is, on the whole, only too evident.

Bearing in mind that there is a great deal of research to be done before anyone can write a wholeheartedly scientific textbook of dermatology, we can at least suggest that this publication is the best introduction to the field yet made available to the cosmetic scientist. N. J. VAN ABBÉ.

DE STRUCTURA ET FUNCTIONE STRATORUM EPIDERMIS S. D. BARRIERRAE. (The structure and function of epidermal barriers). Pp. 635 + Ill. (1965). *Purkyne University Press, Brno.* Kc. 47. Also *Swets & Zeitlinger, Amsterdam.*

Perhaps the most disturbing feature of the "information explosion" is the nagging suspicion that one may be wasting time exploring avenues to which research workers in another country have already devoted considerable attention. International symposia and conferences aim to destroy the barriers of distance and language, but we cannot attend every such gathering that might be useful. Publication of the proceedings of international meetings is therefore probably the most effective way of disseminating information of the kind in question – at least, in theory; in practice, the problem still arises in the choice of languages for publication. In the present instance, we have the reported proceedings of the 2nd Dermatological Symposium with international participation, held in Brno, Czechoslovakia, during October 1964. The volume uses three official languages (German, Russian and English); each paper is given in the author's chosen language, with summaries printed in the other two. Rather less than 20% of the material is given in English, so the reader must decide whether he can adequately cope with the remainder.

The field of basic dermatological research is certainly not adequately covered by reference only to work in the U.K.; this is, indeed, still on a very restricted scale. Much more has been going on in the U.S.A., which is fortunate for English language readers. But probably too little is heard of German work in this field (though such work has an extensive historical background) and hardly anything is known in the Western World about skin investigations in Eastern Europe. An interesting exception to this ignorance is that those who attended the 1962 Congress of the I.F.S.C.C. in London will remember the particularly good paper on the measurement of insensible perspiration, by Hybasek and Serak, from whom another communication appears in the work under review; Prof. Hybasek is, in fact, one of the symposium editors.

The book reprints over 80 papers dealing with various aspects of the epidermal barrier, including such a practical topic as the influence of detergents on permeability. Other topics of special interest deal with pH and with water regulating mechanisms. There is no doubt at all that cosmetic scientists should take a serious interest in the subject–matter of this volume, though many readers will doubtless be deterred by the language problem which it has not fully overcome. N. J. VAN ABBÉ.

MASS SPECTROMETRY. Editor: R. I. Reed. Pp. x + 463 + Ill. (1965). *Academic Press, London and New York.* 105s.

Dr. Reed organised a NATO sponsored international summer school on the theory, design and applications of mass spectrometry at Glasgow University in August 1964. Although the primary emphasis of the "Advanced Study Institute" was on the practical extensions, he considered it desirable to publish a permanent record of the 23 formal lectures, which included a variety of novel aspects of this rapidly developing field of instrumentation. Dr. Reed largely left the text in each lecturer's own individual style: this sometimes has led to quaint constructions from the overseas contributors but nevertheless the general result is a remarkably readable

and interesting collection of papers with surprisingly little unnecessary overlap – a tribute to the thoroughness with which the course was originally planned.

The first contribution constitutes a detailed description of the principles, design, construction, uses and limitations of single – and two types of double–focussing mass deflexion spectrometers. The second paper is concerned with the more esoteric radio-frequency ion separation systems: time of flight (TOF) and potential well, following both linear and circular paths. A short lecture describes technical developments in TOF spectrometry, particularly with regard to energy control of ion source. Analytical applications of the relatively new branch of field ion mass spectroscopy are reviewed by one of its leading exponents, H. D. Beckey. There follow three theoretical papers devoted to, respectively, quasi–equilibrium mechanisms, ionisation efficiency and electronic transitions in macrostructures: the latter includes a useful appendix containing a worked example of an algorithm to assign a molecular formula from an accurate knowledge of the main isotope mass peak. Primarily of interest to physicists are three chapters on ion optics, the determination of ionisation and appearance potentials, and the physical meaning of photon and electron impact ionisation. The few inorganic applications discussed include some rather telegraphic notes on the quantitative determination of elemental impurities using a spark source and a general paper on the mass spectrometry of volatile halides, carbonyls and hydrides. Two papers on high temperature mass analysis are concerned with thermochemical calculations for hot gas streams and the determination of gas phase equilibria. Beynon discusses the mass spectrometry of large complex and relatively non–volatile organic compounds and also boron halides. Other novel applications include the mass analysis of ions in flames, measuring the stability of carbonium ions from appearance potentials and coupling a mass spectrograph to high and low pressure reactors to study the kinetics of heterogeneous systems. An interesting paper examines differences in the nature and relative abundance of fragments from pairs of geometrical isomers and then seeks to explain the results thermodynamically. Dr. Reed's own contribution is to discuss four specific problems: he analyses reasons for discrepancies in relative abundances of ion fragments from different designs of mass spectrometers, the use of a close proximity probe for introducing solid samples, and distinction of competing structures for cis/trans isomers, and for two related dipeptides. Other papers include a review of ion–molecule collision reactions, the effect of variation in ionisation chamber temperature on reproducibility of fragment intensities, and finally advice on the choice of combinations of glc separation with subsequent mass analysis.

Dr. Reed is to be congratulated on assembling a distinguished panel of experts: the record of their lectures on such a variety of specialist techniques and applications constitutes a valuable addition to the literature of mass spectrometry.

G. F. PHILLIPS.

TREATISE ON ANALYTICAL CHEMISTRY, PART I, Vol. 5.

Editors: I. M. Kolthoff and P. J. Elving. Pp. xx + 2707–3346 + Ill. (1964). *Interscience Publishers, New York–London–Sydney.* 120s.

This volume represents the first half of twentyone subject review chapters on 'Optical Methods of Analysis', which are themselves only a small section of the treatise. The chapters are not clearly classified, but this shortcoming is somewhat counteracted by the comprehensive index. Each chapter has ample references to further literature.

The first chapter serves as an introduction to optical methods of analysis and discusses the characteristics of radiant energy and the processes involved when it is emitted or absorbed.

There are four chapters on various aspects of spectroscopy, dealing with principles, practice and instrumentation. The chapters entitled "Fundamentals of spectrophotometry" and "Ultraviolet and visible spectrophotometry" provide valuable information for the applied chemist. The former, after introducing the laws and nomenclature of spectroscopy, discusses those more practical aspects, such as sources of error and limits of accuracy, which are so often ignored. The other chapter named, almost a textbook in itself, deals with principles, applications, instrumentation and techniques. Readers of the sections on applications and techniques will find that numerous applications to their own work will be suggested. Specialists in spectroscopy would find the whole work to be a valuable review, and prospective purchasers of spectrophotometers would be advised to consult first the section on instrumentation.

In contrast, the reviewer was not convinced of the value of "Microwave spectrophotometry". The author quotes examples of analytical problems which could easily be solved with a gas chromatograph which is needed, in any case, to purify the sample prior to microwave analysis. He suggests that unfamiliarity with the technique explains its lack of use, yet does little to sell his speciality to the practising analyst.

A full account of the principles and apparatus for analysis by X-ray methods is followed by applications including the routine analysis of crystalline powders and amorphous materials. A related speciality, "X-ray microanalysis by means of electron probes", is described in which a fine electron beam is used to excite local characteristic X-radiation with a resolution of 0.2 to 100 cubic microns. Besides the obvious applications to metallurgy, geology and ceramics, biological studies have included calcium distribution in bone, copper in eye cornea and iron in animal intestine.

Cosmetic chemists concerned about anomalous results so often obtained with polymeric raw materials should read the chapter on "Principles of light scattering". The author treats various types of dispersed system theoretically and continues with a description of particle-size and molecular-weight determinations. "Nephelometry and turbidimetry" are reviewed in another chapter that emphasises the sensitivity and versatility of these techniques if properly understood. A large section on applications includes such uses as clarity determination, continuous-stream analysis, particle size studies, and a number of inorganic and organic applications. Biochemical uses include amino-acid, vitamin and antibiotic assay by measurement of microbiological growth.

The chapter on "Fluorimetry" is disappointingly short, but the review is sufficient to indicate the scope of this technique, although details and applications are left to the many references. The value of this method for studying fluorescent dyestuffs and optical brightening agents is not mentioned.

Systems for colour measurement are compared in the chapter dealing with the "Specification and designation of colour", which pays particular attention to the anomalies found between measurement and visual impression of colour.

This volume of the treatise will prove of value to all seeking to apply modern techniques to their work. It has areas of interest not only for the analyst, but also includes information for instrumental, basic research and applied chemists.

J. D. CHESHIRE.

ESSAYS IN BIOCHEMISTRY. Vol. 2. Editors: P. N. Campbell and G. D. Greville. Pp. xiii + 227 + Ill. (1966). *Academic Press, London and New York.* 25s.

Following the success of Volume 1, the Biochemical Society has produced a second in this series of essays suitable for advanced students.

H. L. Kornberg (Anaplerotic sequences and their role in metabolism) deals with some less obvious aspects of the metabolic map. The cyclic pathways of intermediary metabolism, the best known of which is the tricarboxylic acid cycle, are used as sources of raw material for other metabolic pathways. When quantities of material are removed from the cycle, other mechanisms must come into play to replace the cycle intermediates and keep the cycle in existence. These mechanisms have been termed 'anaplerotic' sequences, the term being derived from the Greek for 'filling up'. Personally, being anti-jargon, I would prefer the term 'filling-up sequences'. Professor Kornberg describes the reaction paths which have been worked out, and discusses mechanisms of their control.

D. G. Walker (The nature and function of hexokinases in animal tissues) discusses the enzymes responsible for phosphorylation of hexoses by ATP. Aspects discussed include methods of study, mechanism of action, and the physiological role in various mammalian tissues.

R. M. C. Dawson (The metabolism of animal phospholipids and their turnover in cell membranes) deals with materials which are not usually thought of in dynamic terms. The biological function of phospholipid is predominantly as a structural component of cell membranes. Although little structural specificity can be demonstrated for this function, phospholipid is none the less essential for much cellular activity. Its removal from the mitochondrion results in loss of coordinated enzyme activity, and from the erythrocyte results in lysis of the cell. The significance of its appreciable rate of turnover in tissues has not been decided, but the possibilities are discussed of its relationship to cell wall function, cell wall movement, and cell reproduction.

V. Petrow (Steroidal oral contraceptive agents) writes an essay designed to provide an integrated picture of the newer methods of fertility control, popularly known as 'The Pill', which are derived from the natural mechanisms of contraception during pregnancy. It seems likely that the effects of the progestin-oestrogen mixtures approved in this country are achieved primarily by inhibition of the production of follicle-stimulating hormone and luteinising hormone, with consequent inhibition of ovulation. The historical development, structural requirements, and biochemical side effects, are dealt with succinctly in a very readable essay, finishing with a consideration of other methods of fertility control and possible future developments.

G. H. Dixon (Mechanisms of protein evolution) discusses the slowly accumulating evidence that large classes of enzymes occurring in present day organisms may be derived from relatively few primitive genes originally specifying proteins which catalysed a few fundamental reaction types. This evidence comes from comparisons of amino acid sequences of proteins having the same function in different species. The differences may be explained in terms of base sequences in the nucleic acids, and it is interesting to speculate as to how these differences arose. This is a fascinating topic, but the essay is perhaps overlong and detailed for the general audience at which the book is aimed.

This volume maintains the high standards set by the first. It is to be hoped that

the temptation to produce full-length review articles will be resisted, and that future volumes may continue to be readable by scientists wishing to obtain a picture of topics outside their speciality. B. G. OVERELL.

BROMINE AND ITS COMPOUNDS. Editor: Z. E. Jolles. Pp. xxvii + 940 + Ill. (1966). *Ernest Benn, London.* 210s.

Dr. Jolles has succeeded in collecting together authoritative reviews by a number of authors and shaping them into a very readable monograph on bromine. When the volume of information in the book is considered, the title seems almost inadequate.

An account of the early difficulties which beset would-be bromine manufacturers is very well written and traces developments up to the present day methods of production. The advent of the motor-car created a demand for ever increasing quantities of bromine in the form of ethylene dibromide, which was used as a fuel additive. How this was instrumental in the growth of the present day bromine industry provides light and very entertaining reading.

The first part of the book is set out on classical lines. A very full discussion of the physical properties and general chemical reactions of bromine is followed by a consideration of its inorganic and organic compounds. Information is easy to find in these concisely written sections and the reader's task is further simplified by an excellent system of cross reference throughout the whole book. Also included are some preparative details which it could be argued are out of place in a book of this kind. Nevertheless, since these details, in some cases, are previously unpublished their inclusion is probably justified.

Further sections of the book deal at some length with the analytical and radiation chemistry of bromine. In common with the remainder of the book, these sections are very detailed and provide the expert and the student with a comprehensive survey of information and practical techniques.

Biological aspects of bromine compounds are covered by a very full discussion of their toxicity and metabolism in plants and mammals. A large part of this section is concerned with bromide therapy and the toxicity hazards as well as the advantages of this form of treatment are fully discussed. This is followed by a detailed consideration of the pharmacology of organic bromo compounds, comparisons being made with the corresponding chloro derivatives where appropriate.

The remainder of the book is devoted to the numerous industrial applications of bromine. It is very difficult for any one person to review such a diversity of topics as are contained in this section. All that one can say is that the same high standard of presentation is maintained and there is no doubt that the reader will find plenty to interest him in these pages.

As a whole, the book should prove of value to student and expert alike. An abundance of general chemical principles is contained within its pages and these should be enough to set any student thinking. However, the obvious intention is to provide the academic and industrial research worker with a book of reference on bromine. One can say without doubt that this has been achieved.

H. R. J. WADDINGTON

THE CHEMISTRY OF ALKENES. Editor: S. Patai. Pp. ix + 1315 + Ill. (1964). *John Wiley, New York, London and Sydney.* 315s.

Professor Patai has planned a series of advanced treatises that exhaustively examine the chemistry of specific organic functional groups: this 1200 page, multi-author, compendium is the first. He does not aim to present to research chemist and graduate student a critical appraisal of all theoretical, physical, mechanistic and practical features of alkene chemistry. Instead 19 authors deal with a definite aspect, either directly concerning formation of the carbon/carbon double bond or reactions specifically at or adjacent to its site.

Most appropriately the first contribution, which occupies almost 150 pages, is a dissertation by Professor *C. A. Coulson* and a co-author, on wave mechanics and the alkene bond. They rightly anticipate surprise that more than half of this chapter constitutes an extended monograph on basic principles of wave mechanics. The reader is taken successively through simple functions for hydrogen-like atoms, analysis and some attempt at visualization of atomic orbitals, manipulation of mathematical operators, simple 2-electron systems, successive approximations to the hydrogen molecule, use of the overlap integral, introduction of molecular orbitals with determinants for the multi-electron system and LCAO treatment of simple diatomic molecules, with fluorine and HF as examples. It is p.88 before an organic molecule, acetylene, is encountered, and on p.95 ethylene at last appears. Hybridization, complex π electron systems and use of the Hückel approximation, lead into consideration of conjugated polyenes and delocalisation energy. Finally, electronic spectra of polyenes and substituted alkenes are analysed.

Subsequent chapters are just as specialised in their own way but there is no further risk of mathematical indigestion! In 50 pages, *W. H. Saunders* reviews the mechanism and stereochemistry of elimination reactions in solution and the key factors affecting reactivity, including variation in alkyl and leaving groups and in base and solvent. There is also a brief discussion of the classical orientation rules. In another relatively short chapter *A. Maccoll*, of the UC (London) kinetic school, analyses alkene formation in the gas phase by homogeneous elimination reactions, with appropriate reference to catalysis and relevant equilibria. Olefin forming condensations are somewhat telegraphically examined by *T. I. Cromwell* (University of Virginia): he includes aldol, Knoevenagel, Wittig, Perkin and similar nucleophilic triggered reactions. This is a very useful summary for the research cosmetic chemist concerned with synthesis. Kinetic mechanisms for correlation of structural features are also given for these reactions. *E. J. Kuchar* comprehensively reviews the detection and determination of the alkenes. Chemical quantitative methods include halogenation, hydrogenation and oxidation. Valuable advice is given on the use, as well as an outline of general principles, of quantitative and qualitative methods of gas-solid and gas-liquid chromatography and spectro-metric techniques include ir and mass and, more briefly nmr, Raman, uv and visible. The π complexes formed by acyclic and homocyclic alkenes with metals, especially iron, are adequately described by *M. Cais* of the Haifa Institute of Technology. A wide field of alkene rearrangements is covered by *Dr. R. McKenzie*, whose interest in transformation of diene adducts stems at least from his days as a Civil Service Research Fellow. In little more than 70 pages he manages to cram a vast amount of information on cis-trans conversions, prototropic and anionotropic shifts and several miscellaneous rearrangements including those of Cope and Claisen.

The editor himself is a co-author of a remarkably long chapter devoted to the rather forcing conditions required for nucleophilic attack on the C=C system; they decide to avoid the superficially attractive proposition of a unified mechanistic approach and instead emphasise correlations when separately discussing individual reactions. It is, however, a serious criticism of this book that the ubiquitous and facile electrophilic attack on the alkene function is not examined. Another long chapter explores the more specialised topic of the reaction, at the C=C, of systems yielding carbenes (RR'C:) and free radicals. Two University of California authors review nucleophilic substitution and isomerization of the allylic function; they include a special section on the structure – but not the practical implications – of allylic metal (Grignard type) reagents.

Despite rigidly restricting their own definition, three German writers supply monographic coverage (about 200 pages) to the vast field of inter- and intra-molecular cycloaddition reactions of olefins. The formation of 3- and 4-membered carbo- and hetero-cyclic ring systems, 1-3 dipolar addition and normal and retro Diels-Alder condensations are all assessed. The editor has even more carefully restricted the topics covered in each chapter and there is surprisingly little overlap in the next, which – quite logically – is concerned with the synthesis, properties and rearrangements of conjugated dienes; adequate weight is given to the important spectroscopic features of this grouping. A seemingly unnecessarily long chapter (over 120 pages) then examines minutely the relatively esoteric subject of odd and even cumulenes; with characteristic Teutonic thoroughness the theoretical implications are not neglected. Finally, the cumulene-like system present in ketens is discussed by (the late) *Dr. R. N. Lacey*; a wide variety of preparations, addition reactions and polymerisations are described for this important family of intermediates in synthesis.

Whilst one could question the balance given to the topics, and the arbitrary nature of the division between them, it is beyond question that Professor Patai has produced a most authoritative and monumental reference text. It is hoped that in future editions he will succeed in recruiting chapters on electrophilic reactions and possibly something on the vital significance of alkene biochemistry It is understood that similar treatises devoted to the carbonyl group and the ether linkage are in course of preparation: if the high standard of this volume is maintained the literature of organic chemistry will be notably enhanced. G. F. PHILLIPS.

ADVANCES IN HETEROCYCLIC CHEMISTRY. Vol. 5.

Editor: A. R. Katritzky. Pp. xiv + 395 + Ill. (1965). *Academic Press, New York and London.*

Volume five in this series includes four reviews devoted to the chemistry of particular groups of compounds: pyrrolizidines, aromatic quinolizines, 1,2,4-thiadiazoles and aminochrome pigments; and two reviews concerned with the application of molecular orbital theory to heterocyclic chemistry, specifically to sulphur heterocycles and to azines.

R. Zahradnik in "Electronic structure of heterocyclic sulfur compounds" (67 pages) gives a theoretical discussion of electronic structure and its influence on reactivity, physical properties and aromaticity. R. Zahradnik and J. Koutecky then present "Theoretical studies of physiocochemical properties and reactivity of azines" (50 pages).

In "1,2,4-Thiadiazoles" (86 pages), F. Kurzer gives a comprehensive review covering history, nomenclature, structure, synthesis, chemical, physical, physiological and pharmacological properties, and uses. "The aminochromes" (86 pages) by R. A. Heacock includes details of their formation, physical and chemical properties and their significance to biology and medicine. B. S. Thyagarajan then surveys the syntheses, reactions and spectra of "Aromatic quinolizines" (24 pages). To conclude the volume N. K. Kochetkov and A. M. Likhosherstov review the several aspects of "Advances in pyrrolizidine chemistry" (53 pages) which is of especial importance because it contains a number of alkaloids.

Each review includes numerous references, many of which are to very recent work, and comprehensive author and subject indexes complete the volume. The presentation and production of this book continues the usual high standard.

R. P. REEVES.

TEXTBOOK OF ORGANIC CHEMISTRY. L. N. Ferguson. 2nd edn. Pp. xii + 755 + Ill. (1965). *D. Van Nostrand Company, Princeton, Toronto, New York, London.* 92s.

It is important at the outset to recognise the ambit of this book. This is an elementary introduction to organic chemistry within a rational framework for a first year undergraduate course. For this reason it is unreasonable to criticise the very superficial treatment given to a number of specialised topics that are properly dealt with in a second or even third year. Nevertheless it comes as a shock to see how restricted is the vision permitted of heterocyclic families, with a few alkaloid structures to whet the appetite, only monoterpenes are dealt with, steroids receive two-and-a-half pages – mainly formulae, and so the criticism might continue. Indeed one almost wonders why these were included at all! To be fair, then, this textbook must be judged by the strength of the grounding in the principles of organic chemistry that it gives and trust that the later years of the undergraduate course extend in considerable depth the vast and fascinating chemistry of natural products.

This new edition has a number of worthwhile improvements such as the inclusion of physical methods of determining structure and – in common with most contemporary organic texts – a much wider use of reaction mechanisms and electronic interpretation of structure and a general updating of the content of the more conventional chapters. The author adopts a position intermediate between the classical functional group treatment and a purely mechanistic approach. Also in keeping with modern texts, references to original papers are frequently cited.

The first chapter – the structure of organic molecules – starts at a very primitive level but leads into IUPAC (anachronistically referred to as IUC) nomenclature, with passing mention of several linear code notations. It is noticed that the "best answers" to some of the set questions are not always these favoured by IUPAC – or the American Chemical Society. One is glad to see that the concept of conformation and stereospecificity are introduced early into the text, as a logical development from acyclic to alicyclic hydrocarbon elimination and addition reactions. Chapter six – physical methods of structure determination – is to be recommended. It deals briefly with the application of X-ray diffraction measurements and dipole moments and gives a simple introduction to electronic and vibration absorption spectra, whilst for mass spectrometry and nmr the principles are mentioned, the latter with brief

examples of coupling constants and chemical shifts – which curiously are only given as delta values and yet elsewhere in the text numerous references to nmr measurements are expressed in tau notation without any definition. More seriously, one might complain that the nmr test questions are unfair in that they can not be tackled strictly on the data presented but need supplementary correlation tables. The discussion, in chapter seven, of aromaticity, as far as it goes, is a logical exposition and the empirical introduction to aromatic substitution is clear and concise: both leave plenty of scope for theoretical lectures later in the course. Not all readers would accept the statement that the symbol phi "is used to donote any aryl group without regard to what substituents are present", a more general custom is that "Ar" is used in this broad context whereas ø or Ph are reserved explicitly either for C_6H_5 or for C_6H_{5-n} where n substituents have been specifically cited.

Having carped at the limitations elsewhere in the text, it was a pleasant surprise to find, in the section on optical activity and configuration, a clear exposition of the R/S ("steering wheel") notation originally due to Cahn *et al*: this convenient nomenclature is perhaps not as widely appreciated as it might be. In addition to the carefully planned exercises and consolidated questions which probe the assimilation of each chapter, and the "tests" intended to evaluate the students' grasp and integration of all material presented to that point, the tutorial utility of this book is notably enhanced by a review chapter that explores and consolidates the general knowledge gained from the first 19 chapters. There follows a first class chapter that discusses the elucidation of structure by functional group analysis: qualitative chemical tests are throughout correlated with generalised diagnostic uv, ir and nmr spectroscopic assignments. Finally there are the already criticised derisory chapters on natural products and, in contrast, a most effective introduction to the (literally) vitally important topic of our age – chemotherapy: the mechanism of activity of various drug families is briefly examined and a few structural formulae are given by way of example but deliberately no attempt is made to provide a general classification of drugs. The style, as elsewhere in the book, is chatty and informative; occasionally unexplained terms (e.g. radiography) are used or there is a confusion of terminology (e.g. teratogenesis is equated with Mongolism).

This book is a curious mixture: excellent in its examples of function or mechanism, with interesting reflexions on practice in the USA and Europe (e.g. in industrial polymers) and with frequent interpolations (set in small type) of historical or cultural significance; yet contrasting with its superficial or downright poor coverage of even the parent substances in families of natural products. It is an innovation to see ir and nmr spectra illustrating structural points in an elementary textbook. More attention should have been given to proof reading the exercises as well as the main text; thus, the first line has been transposed and another is missing in questions on p.457, the answers to Set *6* include one question that does not even appear on p.176, and answer 13 on p.665, wrongly refers to a methyl substituent. And yet when all is said, this is a most readable and stimulating book. In British practice it is perhaps unusual to find one large (750 pp.) book devoted to the first year of a course only – but within this (expensive) limitation it must be commended. G. F. PHILLIPS.

Society of Cosmetic Chemists of Great Britain

1966 DIPLOMA EXAMINATION

Brunel College

PAPER I

(Monday, 20th June 1966.)

Candidates should answer FIVE questions from *not less than FOUR* sections. Each answer must be commenced on a separate sheet of paper.

SECTION A

1. What are the properties and composition of
   (i) Temporary hair colourants
   (ii) Semi-permanent colourants and
   (iii) Permanent hair colourants.
   To what extent do existing products satisfy the requirements of an ideal hair colourant?
2. Describe the effect of hair waving products containing thioglycollate on the structure of human hair. Comment on the performance tests which are available for the assessment of such products.

SECTION B

3. Discuss the importance of tinted foundations in modern make-up. Describe the main classes of tinted foundations giving a typical formula where possible and relate each foundation to the appropriate skin type.
4. What are the basic requirements of a good nail lacquer? Discuss the ingredients of a nail lacquer describing the function of each ingredient.

SECTION C

5. What are the factors that affect the efficiency of the steam heated jacket of a mixing vessel? Discuss these factors, describing how the

transfer of heat can be made more efficient. How can the type of stirrer used help this heat transfer?

6. Describe the procedure you would use for a storage test of a permanent waving lotion. The packs to be tested are a glass bottle, and a new type of plastic which has been suggested as possibly suitable. Give the reasons why you choose to do the test in the way you have chosen and indicate what you would look for during the test.

Section D

7. Give an account of the structure, chemical composition and function of the cell wall and cytoplasmic membrane of a typical bacterial cell indicating the part these structures play in controlling passage of solutes into and out of the cell.

8. You are presented with an antiseptic emulsion. Describe techniques you would use to determine the antiseptic properties of the emulsion and indicate clearly the limitations of each technique you describe.

Section E

9. Describe the structure and function of eccrine and apocrine glands. Distinguish between the action of anti-perspirants and deodorants in the prevention of body odour. Indicate which active ingredients you would use for each of these two product types and give reasons for your selections.

10. What are the principal requirements for an effective dentifrice? Name the classes of ingredient used in dentifrices and give specific examples of each class. Indicate how the proportions vary in the different types of dentifrice and discuss the relative popularity of the different types.

Paper II

(Wednesday, 22nd June 1966.)

Candidates should answer FIVE questions from *not less than FOUR* sections. Each answer must be commenced on a separate sheet of paper.

Section A

1. Describe in detail the practical and theoretical aspects of TWO of the following experiments:-
   (i) Maximum bubble method for the determination of surface tension.
   (ii) Adsorption of acetic acid on charcoal.
   (iii) Colloids – the precipitation of lyophobic sols by electrolytes.
2. Write short notes on:-
   (a) Wetting and Spreading
   (b) Cloud point of nonionic surface active agents
   (c) The structure of micelles.

Section B

3. Write notes on *two* of the following:-
   (a) Isomerism in fatty materials and its influence on physical properties.
   (b) The relative properties of oils and waxes for use in cosmetic preparations.
   (c) The chemical products, derived from oils and fats, which are useful to the cosmetic chemist.
4. Describe the physical and chemical methods that are used to establish the composition of fatty acids and glycerides. Briefly mention some of the chief analytical characteristics of value in assessing the composition of fatty materials.

Section C

5. Discuss the importance of chromatography as a separation method in analytical chemistry. Indicate applications of importance to the cosmetics industry.
6. Give a short account of the theory of the infra-red absorption spectra of simple molecules, indicating why such spectra can be of value in elucidating molecular structure. Outline the principles of an instrument designed to measure an infra-red spectrum.

Section D

(Candidates should answer all parts of these questions.)

7. What do you understand by the word "perfume"?
   Give a simple basic formula for a rose, jasmin, or Eau de Cologne perfume, illustrating by the ingredients you have used in your perfume the terms "top note", "end note", "blender", "modifier", "fixation". Briefly discuss the stability of your perfume for:-
   (i) white toilet soap
   (ii) talcum powder.

8. (i) Write brief notes on the fields of chemistry associated with the natural odorous products used in perfumery.
   (ii) Give examples of two chemicals of each field and the products in which they naturally occur.
   (iii) Show how linalol can be made synthetically by two different processes.
   (iv) What chemical composition differences exist between citronella oil Ceylon and citronella oil Java?

Section E

9. Define viscosity. Distinguish between the following types of rheological behaviour using flow diagrams:-
   Newtonian
   Plastic
   Pseudoplastic
   Thixotropic
   Dilatant
   Indicate how the different types of flow behaviour are related to the internal structure of the material. Describe one method for the quantitative specification of thioxotropy.

10. Given a sample of a cosmetic emulsion, describe in detail the methods you would use to obtain the following information:-
    Emulsion type
    Particle size distribution
    pH
    Water content
    Nature of oil phase (solid or liquid)
    Melting point of oil phase (if solid)
    Emulsifier type.

Successful candidates

Eleven out of eighteen candidates were successful. Diplomas were awarded to the following:

Z. A. Allawala	V. Choksi	*E. J. Pilcher
Miss P. A. Barnes	Mrs. P. M. Hagarty	R. S. Shanbhag
P. R. Brace	G. S. Hinds	M. P. Sharp
Miss H. Brown	C. A. Kilgour	

*Hibbott Memorial Prize

1966-67 PROGRAMME

Lectures will be delivered on the following Thursdays:
Venue: The Royal Society of Arts, John Adam Street, London, W.C.2.
Time: 7.30 p.m.

6th October 1966
Modern frontiers in aerosols
H. R. Hearn (Metal Box Company)

1st December 1966
Lucent syrops tinct with cinnamon . . .
Dr. V. L. S. Charley (Beecham Food & Drink Division)

5th January 1967
Group discussion on the subject of the needs and future trends in cosmetic legislation
Dr. R. Allen, Dr. G. Carriere, C. Williams, and Prof. A. N. Worden

2nd February 1967
Cosmetic raw materials '67
W. Harding (Croda Ltd.)

4th May 1967
The examination of hair by new physical techniques
Dr. A. W. Holmes (Unilever Research Laboratory, Isleworth)

MEDAL LECTURE: Wednesday, 22nd March 1967
The inter-action of detergents and skin
Professor M. K. Polano (Professor of Dermatology, Leiden University)

1967 DINNER AND DANCE: Saturday, 4th February 1967, at the Europa Hotel, Grosvenor Square, London, W.1.

ANNUAL GENERAL MEETING: Monday, 22nd May 1967, at the Washington Hotel, Curzon Street, London, W.1.

SYMPOSIUM ON PRODUCT TESTING

A Symposium on Product Testing will be held at the Grand Hotel, Eastbourne, Sussex, on 14th and 15th November 1966. Participation is permitted only when application has been made on the appropriate form, and the fee duly paid. This is £5.5.0. for each participant who is a member of one of the Societies of Cosmetic Chemists affiliated to the I.F.S.C.C. The registration fee for non-members if £8.8.0. Registration forms giving all details are available from the General Secretary, Mrs D. Mott, 18 Warner Close, Harlington, Middx. **The closing date for registration is 17th October 1966.**

Monday, 14th November 1966

13.00 Symposium Lunch.

Afternoon

14.15 "The importance of consumer testing in product development"
R. W. ARTINGSTALL, F.S.S., M.M.R.S. (*Beecham Group Ltd., Brentford*).

14.45 "The role of consumer studies in research"
MRS. S. M. LUDFORD, B.Sc. (*Unilever Research Laboratory, Isleworth*).

15.15 "The evaluation of prophylactic dentifrices"
W. H. BULL, B.Sc., F.R.I.C. (*Unilever Research Laboratory, Isleworth*).

15.45 "The measurement and interpretation of the abrasiveness of dentifrices"
DR. K. H. WRIGHT (*National Engineering Laboratory, Glasgow*).

19.30 Reception

20.30 Symposium Dinner

Tuesday, 15th November 1966

Morning

9.30 "The quantitative estimation of detergency and allied properties of shampoos in practice"
S. V. BRASCH, B.Sc., Text. Chem. and MISS J. AMOORE, L.I.Biol. (*Beecham Toiletry Division, Brentford*).

10.00 "The potential irritancy to the rabbit eye mucosa of commercially available cream shampoos"
K. H. HARPER, PH.D., A.R.I.C. and R. E. DAVIS, B.Sc., M.I.Biol. (*Huntingdon Research Centre, Huntingdon*).

11.00 Coffee.

11.15 "Evaluation of aerosol products"
A. HERZKA, B.Sc., F.R.I.C. (*Pressurized Packaging Consultants Ltd., London*).

11.45 "Evaluation of placebos in clinical trials"
P. MACDONALD, M.Sc., A.R.I.C., A.I.S., A.I.M.A. (*Dept. of Mathematics, Brunel College, Acton*).

12.30 Symposium Lunch.

Afternoon

14.15 "Contact dermatitis from cosmetics"
E. CRONIN, M.R.C.P. (*Institute of Dermatology, St. John's Hospital for Diseases of the Skin, London*).

14.45 Brains Trust.

Hector W. Hibbott
1908-1966

Facing page 685

OBITUARY

Dr. H. W. Hibbott

It is with sadness that the death is recorded on 23rd June 1966 of Hector Whittington Hibbott, M.Sc., Ph.D.(Sheff.), F.R.I.C., a Founder Member of the Society.

Apart from enforced absence due to statutory requirements Dr. Hibbott served on the Council of the Society from the inaugural meeting in 1948 until a month before his untimely passing. During that period he occupied the Vice-Presidency from 1958–1960 and was elected President in 1960, an honour which he well deserved and a position which he held with distinction. Further recognition of his work for the Society was made when he became an Honorary Member in 1963.

He served on various committees and in particular he was actively interested in the Diploma course held at Brunel College, London, W.3, he, himself being one of many lecturers. He valiantly undertook the task of editing the "Handbook of Cosmetic Science" which was based on the lectures. This was published in 1963 and is truly a monument of his career.

On many occasions he was also the Society's delegate to the Council of the International Federation of Societies of Cosmetic Chemists. He served on numerous BSI Committees and on the Standards Committee of the Toilet Preparations Federation.

As a man he was greatly respected, not only because he was a chemist of high standing, but also because he was of quiet demeanour and placid yet possessed great courage and firmness. His gentle smile, his ability to understand the varying points of view of those around him and his readiness to help – oft'times to guide – towards a satisfactory solution of many of the problems of the Society, endeared him to all his colleagues.

Everyone who was fortunate enough to know him admired his character, and that he should die at the early age of 58 adds to the sorrow that must be felt at his passing. Perhaps it could best be said of him, *well done*.

Our deepest sympathy is extended to his wife, Constance, and his children.

ROBERT H. MARRIOTT

H. J. Holmes

It is with regret that we have to record the death of Mr. Herbert Holmes, who died suddenly on 12th June 1966, at the age of 55.

Mr. Holmes was a Founder Member of the Society, and its first Honorary Secretary, a post which he held for four years and to which he devoted a lot of time and energy. He also served on the Council from 1952–1954, and from 1955–1957.

Before the war he worked in the laboratories of British Drug Houses, Reuter (4711) and Pepsodent Ltd. but during the war he branched out as a technical consultant to the cosmetic industry. He founded Laboratory Facilities Ltd.

He is survived by his widow, three sons and a daughter.

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