THE ACTION OF METHYLENBLUE UPON COTTON FIBRE

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The nature of the staining act in respect of vegetable and animal fibres has not yet emerged from the region of discussion into that of ascertained fact. Owing to the desirability of making, in the light of recent research, more extended observations in this domain of enquiry, the present investigation of the amount of methylenblue taken up by cotton fibre in equilibrium with staining solutions of different concentrations was entered upon, the range of concentration employed extending over the most extreme range which the methods available permitted.

The only previous work on the staining of cotton fibre with methylenblue, which a careful search of the literature of dyeing has revealed to us, is that of Georgiewicz and Löwy (10), in 1895 (see Literature at the end of this paper). This work will be considered at some length later.

METHOD

In this investigation the methylenblue employed was the double zinc salt, $2C_{16}H_{18}N_3SCl + ZnCl_2 + H_2O$, specially purified for injection *intra vitam*. An estimation of the nitrogen present in the dried dye gave 5.36 per cent. N, the theoretical amount being 5.45 per cent. N.

The cotton wool employed was absorbent cotton wool. Before use it was thoroughly washed in distilled water, the water removed

by centrifugalisation, and drying to constant weight effected at 100°C.

All estimations of methylenblue in cotton fibre or in solution were made by means of determinations, by Kjeldahl's method, of the amount of nitrogen present, the amount of dye being calculated therefrom. The amount of dye, which had to be estimated, was small in the case of dyed cotton fibre, nevertheless fairly concordant results were obtained as a glance at column 11, Table I, shows. Colorimetric methods were not available for the whole series of concentrations employed, and were only made use of for the weakest concentrations (Series D, Table I). The temperature of experiment was 44° to 46° C., throughout.

The method of procedure adopted in these experiments was the following. A weighed quantity of cotton wool was placed in a measured amount of a solution of methylenblue of known concentration, contained in a stoppered bottle kept in an incubator at 44° to 46° C. After a lapse of from two to thirty-one days, during which the mixture of cotton wool and staining solution was from time to time agitated, the dyed cotton was removed, care being taken to avoid cooling of the staining solution. The latter was quickly removed by strong centrifugalisation, after which the dyed cotton was weighed in the moist condition (column 7, Table I), and then dried to constant weight at 100° C. (column 6, Table I). After drying, the nitrogen in the dyed cotton wool was determined by a Kjeldahl estimation of the nitrogen it contained (column 8, Table I). A suitable amount of the staining solution was also taken at the end of the staining period and its content of methylenblue similarly determined by means of a Kjeldahl estimation (column 5, Table I). With the aid of the latter a correction, representing the dye contained in the small amount of staining solution mechanically adhering to the cotton fibre after centrifugalisation (column 9, Table I), was applied to the estimation of the total amount of methylenblue in the fibre, and in this way the true amount of dye taken up by the cotton ascertained (column 8, Table I).

In addition to the above method of estimating the percentage of methylenblue taken up by cotton fibre, the same may be determined

from the loss of concentration taking place in the staining solution (column 13, Table I), but such determinations, since they represent the difference between two estimations of methylenblue (except in Series D where colorimetric estimations were made) are less accurate than the direct estimation of the amount of dye in the fibre. Nevertheless they are given, for the sake of comparison, in Table I, but are omitted in Table II.

It may be observed that the total amount of dye in the cotton fibre could not be determined with a sufficient degree of accuracy by reference to the increase in weight of the dried cotton after dyeing.

EXPERIMENTAL WORK

The results obtained by the above method are given in Table I. It will be seen that the range of the initial concentration of the staining solution lies between 4·195 per cent. and ·0046 per cent. It was

TABLE I. INTERACTION OF COTTON FIBRE AND METHYLENBLUE. TEMPERATURE 44°C. TO 46°C.

1	2	3	4	5	6	7	8	9	10	11	12	13
Series	No. of experiment	Duration of experiment	Concentration of methylenblue in staining solution : at beginning of experiment	Concentration of methylenblue in staining solution : at end of experiment	Weight of dried cotton wool at end of experiment	Weight of dyed cotton wool before drying	Amount of dye in cotton wool at end of experiment, determined by Kjeldahl estimation of N	Amount of dye in staining solution adhering to cotton wool at end of experiment calculated from columns 5, 6 and 7	Calculation from results in columns 6, 8 and 9 of : amount of dye taken up by cotton wool	Calculation from loss of concentration of dye in staining solution (columns 4, 5 and 6) of : amount of dye taken up by cotton wool	percentage of dye in cotton wool	percentage of dye in cotton wool
A1	1	2 days	4.195%	4.061%	2.9821 g.	4.2821 g.	.0712 g.	.0528 g.	.0184 g.	.61%	.0214 g.	.72%
	2	2	4.195	4.061	3.0850	4.4031	.0733	.0535	.0202	.66	.0214	.70
A2	3	4	4.195	4.070	2.9516	4.2548	.0754	.0529	.0225	.77	.0200	.68
	4	4	4.195	4.052	2.9072	4.2472	.0765	.0545	.0220	.76	.0229	.79
B1	5	6	.454	.285	3.2917	4.9222	.0275	.0030	.0245	.75	.0286	.87
	6	6	.454	.295	2.8411	4.1195	.0255	.0028	.0227	.78	.0254	.90
B2	7	6	.454	.295	2.8683	4.1810	.0244	.0029	.0215	.75	.0254	.90
	8	4	—	.247	6.4126	9.2730	.0514	.0071	.0443	.69	—	—
C1	9	4	—	.218	4.6391	7.0401	.0353	.0059	.0294	.63	—	—
	10	4	—	.248	6.6699	9.7407	.0479	.0076	.0403	.61	—	—
C2	11	6	.0473	.0282	6.0896	14.5752	.0328	.0024	.0304	.45	.0286	.47
	12	6	.0473	.0276	5.5050	13.6167	.0320	.0022	.0298	.54	.0295	.54
D	13	6	.0473	.0241	6.0231	14.4589	.0322	.0020	.0312	.52	.0348	.58
	14	6	.0473	.0266	5.8608	14.7947	.0345	.0034	.0311	.53	.0311	.53
C2	15	14	.0322	.0150	6.5878	9.8055	.0327	.0005	.0322	.49	.0273	.41
	16	14	.0322	.0157	5.5071	8.2608	.0319	.0004	.0315	.57	.0263	.47
D	17	14	.0322	.0157	5.6312	8.4652	.0327	.0004	.0329	.57	.0263	.47
	18	14	.0322	.0150	6.5634	10.3054	.0353	.0005	.0349	.53	.0273	.42
D	19	31	.0046	.0028	6.3391	9.7680	.0289	.0001	.0288	.45	.0287	.45
	20	31	.0046	.0028	6.2158	9.3720	.0285	.0001	.0284	.46	.0281	.45
21	31		.0046	.0024	6.2994	9.6573	.0344	.0001	.0343	.55	.0338	.53

determined to employ a series of concentrations forming a geometrical progression in which each member stood to the next member in the ratio of about 1 : 10, so that the total range of concentrations stood approximately in the ratio 1 : 10 : 10² : 10³. The duration of the period of staining presented some difficulty. Upon this point further observations are desirable, but it would appear, from a comparison of the results given in column 11, Table I, that equilibrium is attained by about the fourth day. The low result in Series A1, column 11, is presumably due to the staining period of two days being too short ; on this account the mean of these two estimations is omitted in Table II. The probable error of the mean of the various series (cp. column 4, Table II) lies within .05 per cent. Nevertheless, this range of error is sufficiently large to affect noticeably the regularity of the variation in the estimated percentage of dye in the fibre in the different series, as a glance at Table II shows.

The percentage of methylenblue in the dyed fibre varies but little, ranging from .48 per cent. with the lowest concentration of staining solution to .765 per cent. with the highest. If an attempt is made to apply the formula

$$C = a C^{\frac{1}{n}}$$

governing adsorption (see p. 449) it is found that the values of the two constants $a = 1.20$ and $\frac{1}{n} = .1$ agree fairly with the actual estimations as is shown in Table III, where the estimated (in brackets) and the calculated percentages of dye in cotton fibre, corresponding to the different concentrations of staining solution employed, are placed side by side.

TABLE II.—INTERACTION OF COTTON FIBRE AND METHYLENBLUE. MEAN RESULTS, TAKEN FROM TABLE I

Series	Duration of experiment	Concentration of staining solution at end of experiment	Concentration of dye in cotton wool, determined by estimation of N in dyed cotton wool
A2 ...	4 days	4.061 %	.765 %
B1 ...	6 „	.292	.76
B2 ...	4 „	.240	.64
C1 ...	6 „	.0266	.51
C2 ...	14 „	.0153	.54
D ...	31 „	.0028	.48

TABLE III.—CALCULATION, ON THE ASSUMPTION THAT $\alpha = 1.20$ AND $\frac{1}{n} = .1$, OF THE CONCENTRATION OF DYE IN COTTON WOOL IN EQUILIBRIUM WITH STAINING SOLUTIONS OF THE STRENGTH GIVEN IN TABLE II. THE FIGURES IN BRACKETS ARE THE MEAN CONCENTRATIONS GIVEN IN TABLE II.

Series		Concentration of staining solution at end of experiment	Concentration of dye in cotton fibre calculated from the formula : concentration of dye in cotton fibre =	$\sqrt[10]{\frac{\text{concentration of dye in staining solution at end of experiment}}{1.20}}$	
A2	...	4.061 %	...	.76 %	(.765)
B1	...	.292	...	.72	(.76)
B2	...	.240	...	.72	(.64)
C1	...	.0266	...	.58	(.51)
C2	...	.0153	...	.53	(.54)
D	...	.0028	...	.49	(.48)

It will be observed that the value of $\frac{1}{n}$ is smaller than that usually met with in adsorption experiments. The significance of this is considered in the next section.

The Nature of the Interaction of Fibre and Dye

1. Before proceeding to interpret, in the light of recent researches, the results obtained in the preceding section, it will be necessary to consider the various processes which may conceivably occur when cotton fibre is placed in a watery solution of methylenblue, and also to refer briefly to the nature and the cardinal characters of such processes, so far as these are known.

The interaction may conceivably assume one or more of the following three types¹: adsorption; chemical combination; the mutual action of oppositely charged bodies or particles.

2. By adsorption is meant the condensation of a dissolved substance upon a free surface.² In this condensation more energy is

1. Witt (19) suggested that in dyeing a solid solution of the dye in the fibre occurs. The distribution of the dye in fibre and staining solution does not, however, conform to the law of distribution between immiscible solvents (cp. Walker and Appleyard (18), p. 1335).

2. In such cases combination occurs in proportions which are not fixed, but depend on the concentration of the dissolved substance. For our knowledge of the quantitative relations obtaining in reactions of this type we are chiefly indebted to a series of monographs by v. Bemmelen (4), embodying his researches upon the hydrogels of ferric oxide, aluminium oxide, silicic oxide, stannic and metastannic oxides, manganic oxide, etc., and their combinations with water, hydrochloric acid, sodium chloride, barium hydroxide, etc. The subject has been studied from the microscopical standpoint by Bütschli (6), Quincke (16), and Hardy (13), and in respect also of electrical reactions by the latter author. The ultramicroscopical appearance of the solutions employed has been investigated by Zsigmondy and Liedentopf (20). Observations on the action of acids on silk fibre have been carried out quantitatively by Walker and Appleyard (18). The adsorption formula has recently been the subject of a monograph by Freundlich (8), in which further references to the literature will be found.

involved than that of simple passage from the gaseous to the liquid state.¹ Adsorption may take place at the surface of a simple solid, or at that presented by a gel or sol.² The distinctive characters of adsorption are: equilibrium between adsorbed and dissolved substance according to the relation

$$C = aC'^{\frac{1}{n}}$$

where C is the concentration of the adsorbed substance in the adsorbing body, C' is the concentration in the solvent of the substance used for adsorption, and a and $\frac{1}{n}$ are constants; a limited range of values for $\frac{1}{n}$, varying between .5 and .8;³ a lowering of tension at the solid-fluid surface, brought about by the substance used for adsorption. The last cannot be directly determined, the tension of the air-liquid surface being alone capable of measurement. As is seen in the Appendix to this article, methylenblue added to water causes an increase of the air-liquid surface tension.

3. Chemical action differs from adsorption in the circumstance that it is an interaction of atoms according to definite stoichiometrical ratios, while in adsorption molar action takes place. In consequence of this difference chemical action cannot be regarded as a limiting case of adsorption namely that in which the division of the adsorbing substance becomes progressively finer until at length it becomes molecular.⁴

In connection with the problem dealt with in this paper, the dissociation of chemical compounds is of especial interest. When salts in true solution dissociate, the relation

$$\frac{CaCb}{C} = K$$

holds, where Ca , Cb , represent the respective concentrations of acid

1. The amount of heat given out when SO_2 and NH_3 are taken up by charcoal has been found by Berthelot to be greater than would be required to condense these substances from the gaseous to the liquid state at the temperature of experiment. The same occurs when water is taken up by the hydrogel of silicic acid.

2. The amount of a substance which is taken up by a hydrogel is influenced by the mode of preparation of the latter, by temperature and, to some extent also, in the case of reversible reactions, by a change from a more concentrated to a less concentrated medium, or *vice versa*. Fuller details are to be found in v. Bemmelen's published papers.

3. Freundlich (8), 1906, p. 71.

4. In true molar action it is to be expected that solutes of different types would be adsorbed indifferently. Selective adsorption is suggestive of interatomic action.

and base, resulting from hydrolysis of the salt, and K is a constant.¹ If the undissociated salt and one of the products into which it is dissociated are insoluble, the remaining dissociation product being soluble, then so long as the phase rule holds there is a critical concentration of the latter below which complete dissociation occurs, while above this concentration complete combination takes place.² If cotton fibre and methylenblue formed such a combination, then there would be a critical concentration of methylenblue below which all the dye would be discharged from the fibre, while above this concentration complete combination of dye and fibre would occur. It is, however, expressly pointed out by Gibbs (12) that the phase rule, in its ordinary form, is valid only when capillary actions and surfaces of small curvature are excluded. Whether it holds good for non-crystalloidal substances of enormously extended surface, such as are colloids, is doubtful. It may be that such a system would act in a different manner, and that its behaviour would resemble the dissociation of soluble crystalloids.³ The investigation of such a type of dissociation, if it occurs, is not as yet practicable, since we have at present no means of measuring separately Ca and C in such heterogeneous systems. Nevertheless, the existence of solid chemical compounds of this type must be regarded as by no means excluded. If cotton fibre enters into a chemical combination with methylenblue⁴ it must be of this type, since there is no critical concentration of methylenblue below which no combination occurs.

1. This is illustrated, for example, by solutions of potassium cyanide, ethyl acetate and urea hydrochloride in which, in consequence of hydrolysis, a condition of equilibrium is exhibited in which two reacting substances (potassium hydrate and hydrocyanic acid, ethyl alcohol and acetic acid, urea and hydrochloric acid) are combined in a varying degree, depending upon the relative concentrations obtaining. In homogeneous systems formed by solutions of salts derived from feeble acids or bases, such phenomena are common.

2. The behaviour of the combination of diphenylamin and picric acid, investigated by Walker and Appleyard (18), is an illustration in point. Both diphenylammonium picrate and diphenylamin are almost completely insoluble in water. In the dissociation of the former substance all the picric acid is given off below a critical concentration of this acid. The combined picric acid resembles, in this respect, water of crystallisation, which, as is well known, is only given up below a critical vapour tension.

3. This assumes, however, that in the solid compound of dye and fibre, the dye does not exist as a solution in the fibre.

4. The formula for cellulose is consistent with the assumption that it may be regarded as a free acid, its molecule containing a terminal COOH group.

4. Both the processes referred to in sections two and three may occur together. In such a case one may predominate and influence the aspect of the interaction accordingly, or again adsorption may take place at first and later chemical action be observed. The latter has, in fact, not unfrequently been noted in experiments on adsorption.

If cotton fibre is capable both of adsorbing methylenblue and of entering into a chemical combination with it for which the phase rule in its ordinary form does not apply, then the following chemical and physical actions may conceivably occur :—

- (a) Chemical action resulting in the formation of an undissociable compound ;
- (b) Chemical action resulting in the formation of a dissociable compound ;
- (c) Adsorption ;
- (d) Adsorption, together with chemical action, resulting in the formation of an undissociable compound ;
- (e) Adsorption, together with chemical action, resulting in the formation of a dissociable compound.

The following considerations will be of assistance in attempting to distinguish between these different cases.

In case *a*, in which an undissociable chemical compound is formed, no adsorption occurring, the amount of dye taken up would be fixed, and would be independent of the concentration of the dye in the staining solution ; here there would be no difficulty.

In case *b*, the amount of dye taken up by the cotton fibre might, if the phase rule did not apply to such a combination, vary with the concentration of the staining solution. In cases of this kind the adsorption formula could, no doubt, by choosing suitable values for the constants, be made to apply more or less closely to a series of determinations of equilibrial concentrations of dye, but then the value of $\frac{1}{n}$ would not necessarily lie within the range obtaining in adsorption processes. In cases of chemical action, however, chemical affinity would be apparent, and the reacting substance would exhibit

selective action as to acid or basic dyes.¹ This criterion would fail, however, if the reacting substance were amphoteric in character.

In pure adsorption, case *c*, the distinctive character is the value of $\frac{1}{n}$, ranging between .5 and .8 (Freundlich).

The remaining two cases, *d* and *e*, would generally be characterised by the fact that the formula

$$C = aC'^{\frac{1}{n}}$$

would fit in more or less closely with the results obtained, but the value of $\frac{1}{n}$ would lie outside the range met with in adsorption processes. In such cases selective action, if recognisable, would indicate chemical action.

In the case of cotton fibre no exclusive chemical character is exhibited. The fibre stains with methylenblue, but it also stains—and far more strongly—with acid dyes, *e.g.*, congo-red. After deep staining with methylenblue it decolourises completely in running tap water, under favourable circumstances, in about a week, but after staining with congo-red decoloration is slower, being not quite complete at the end of four weeks. These observations, therefore, being merely negative, do not aid in forming an opinion as to the likelihood of cotton fibre entering, or not, into chemical combination with methylenblue.²

1. The purely mechanical or rather physical explanation offered by the hypothesis of adsorption does not account for the fact that only a restricted number of dissolved substances are taken up. It is doubtful if, in such cases, adsorption alone exists. The following illustrations may be given:—v. Bemmelen (4), who observes (1900, p. 342): ‘Doch scheint es dass in manchen Fällen die Absorption sich hervorthut als ein Vorläufer der chemischen Verbindung,’ found that the hydrogel of red manganese dioxide absorbs alkaline bases strongly, sulphuric acid and potassium sulphate slightly, and potassium chloride and nitrate not at all. Further, the hydrogel of silicic oxide takes up alkalies, including calcium hydroxide, more than acids; and the hydrogel of metastannic acid takes up hydrochloric acid strongly. The predominant or exclusive preference of fibres and tissue elements for so-called acid or basic dyes is another case in point. In a small number of cases it has been possible to ascertain that a chemical reaction takes place. v. Bemmelen observed that if hydrochloric acid is added to a hydrosol of ferric oxide, the particles of colloid collect together in the gel condition, forming a precipitate which takes up hydrochloric acid and from which ferric chloride is formed. Similarly, when barium hydroxide is added in sufficient amount to a hydrogel of silicic oxide, crystals of barium silicate having the composition $\text{BaSiO}_3 \cdot 6\text{H}_2\text{O}$ make their appearance. Barratt (2) observed that methylenblue-eosin in alcoholic solution is decomposed in the staining act, the process not being attributable to preceding hydrolysis.

2. It is not improbable that cellulose $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ contains a carboxyl group (or more than one) in its molecule. A Kjeldahl estimation shows the presence of a small amount of N in absorbent cotton wool which has been thoroughly washed in distilled water. Cotton fibre arises by a saturation of the protoplasm of vegetable cells with cellulose. It is probable that some of the original proteid material remains attached to the cellulose molecule, which would then contain one or more NH_2 groups giving it also a basic character. Owing to the large size of the cellulose molecule the presence of a single NH_2 group would have little effect on the percentage composition of cellulose.

Under these circumstances the sole guiding factor is the value of $\frac{1}{n}$. If this lies within the range met with in adsorption processes, then the interaction may be regarded as an adsorption process. If the value of $\frac{1}{n}$ lies outside this range, then the interaction must be regarded (cp. section 5) as a chemical combination attended, it may be, with an adsorption process as well. If the chemical compound formed by cotton fibre and methylenblue (assuming such to occur) is dissociated to a considerable extent, then no further analysis of the degree in which adsorption accompanies chemical combination is possible. If, however, extremely little dissociation occurs, within the limits investigated, then it is possible to determine approximately the amount of dye in chemical combination and the amount adsorbed, since, if the former were subtracted from the latter, the resulting values would represent the amount adsorbed and would conform to the adsorption formula, with a value of $\frac{1}{n}$ lying within the limits obtaining in pure adsorption processes.

Applying these considerations to the results given in Table II, it was found, by making a series of trials, that, if the amount of dye in chemical combination is assumed to be '47 per cent., the percentages of adsorbed methylenblue become those given in column 2, Table IV, while the value of $\frac{1}{n}$ becomes '67 and that of a 4'545. It will thus be seen that, in order to obtain a value of $\frac{1}{n}$ lying between the limits given by Freundlich, it is necessary to assume that the greater part of the methylenblue in the cotton is chemically combined and that only a relatively small fraction (especially so at the lower concentrations of staining solution) is adsorbed.

TABLE IV

Concentration of methylenblue in staining solution	Concentration of methylenblue in cotton wool	Concentration of methylenblue in cotton calculated on the assumption that $a = 4'545$ and $\frac{1}{n} = '67$
4'061 %	... [$'76 - '47 =$] '29 %	... '35 %
'292	... [$'72 - '47 =$] '25	... '16
'240	... [$'72 - '47 =$] '25	... '14
'0266	... [$'58 - '47 =$] '11	... '07
'0153	... [$'53 - '47 =$] '06	... '06
'0028	... [$'49 - '47 =$] '02	... '03

These figures, owing to the difficulty of making sufficiently delicate determinations of the amount of methylenblue in dyed cotton, over the range of concentrations studied, are necessarily merely approximate, but they serve to illustrate the mode in which it is suggested that the results given in Table I should be treated. It is interesting to note that if the amount of dye in chemical combination is assumed to be .47 per cent., then the molecular weight of cellulose would be about 70,000.¹

5. In the interaction of cotton fibre and methylenblue in watery solution, there is no reason to suppose that any process comparable to the mutual attraction of oppositely charged particles occurs, for a watery solution of methylenblue under the ultramicroscope is optically homogeneous (Michaelis, Biltz), and no decided movement of the dye in an electric field has been observed (Bayliss (3)).

6. Summarising the above we may say that the staining of cotton fibre by methylenblue in watery solution must, owing to the low value calculated for $\frac{1}{n}$ in Table II, be viewed as a mixed process of chemical combination and adsorption, the former predominating.

7. Reference must here be made to the observations of Georgiewicz and Löwy (10) on the action of methylenblue upon mercerised cotton fibre. Their results are given in Table V, which is taken from their paper.

TABLE V.—INTERACTION OF MERCERISED COTTON FIBRE AND METHYLENBLUE AT 14° TO 17° C., OBSERVED BY GEORGIEWICZ AND LÖWY (*loc. cit.*)

No. of experiment	Concentration of staining solution at end of experiment		Concentration of dye in cotton wool		$\sqrt[3]{\frac{\text{concentration of dye in staining solution at end of experiment}}{\text{concentration of dye in cotton wool}}}$	
1	...	.0111 %	...	.61 %	...	.365 %
2	...	.0052	...	.48	...	.356
3	...	.0023	...	.38	...	.348
4	...	.0012	...	.30	...	.356
5	...	.0007	...	.25	...	.362
Mean					...	.357

$$1. \quad \frac{\text{Molecular weight of methylenblue}}{\text{Molecular weight of cellulose}} = \frac{.317}{126 \times n} = (\text{approximately}) \frac{.47}{100}. \quad \text{Therefore } 126n = \frac{31700}{.47} = 67,490. \quad \text{A terminal COOH group would confer an acid character upon the cellulose molecule.}$$

These results show that in this case the action is an adsorption process in which $a = \cdot 375$ and $\frac{1}{n} = \frac{1}{3}$. In mercerised cotton the action of alkali has obviously produced a modification of the cellulose molecule, causing it to lose its acid character and to be no longer capable of reacting chemically with the basic dye. The amount of adsorption is greater than that taking place in our experiments with unmodified cotton fibre, as is seen from the calculated percentages in Table VI. This difference indicates that when chemical combination of fibre and dye has occurred, the capacity of the fibre for adsorption of the dye is thereby lowered. It may be that in mercerised cotton an amount of alkali is in combination with the fibre corresponding to the methylenblue which, in our experiments, appears to be chemically combined. It is to be noted that in some later experiments with picric acid as staining agent for cotton and silk, a value of $\frac{1}{n} = \cdot 1$ was obtained by Georgiewicz (II).

TABLE VI.—CALCULATION ACCORDING TO THE RESULTS OBTAINED BY GEORGIEWICZ AND LÖWY (TABLE V) OF CONCENTRATION OF DYE IN COTTON WOOL IN EQUILIBRIUM WITH STAINING SOLUTIONS OF THE END-STRENGTHS OBTAINING IN TABLE II.

Series	Concentration	Concentration of dye in cotton wool calculated from the formula :	
		$\sqrt[3]{\text{concentration of dye in staining solution at end of experiment}}$	
		concentration of dye in cotton wool =	
		.357	
A2	... 4.061 %	...	4.47 %
B1	292	...	1.86
B2	240	...	1.74
C1	0266	...	.83
C2	0153	...	.67
D	0028	...	.39

SUMMARY

The action of methylenblue in watery solution upon cotton fibre is, owing to the low value of $\frac{1}{n}$, inconsistent with a pure adsorption process. It is best interpreted as a mixed process of chemical combination and adsorption, the former being the chief process.

APPENDIX

The air-fluid tension of an aqueous solution of methylenblue

The following series of observations was made by Donnan's (7) method, T. 16.5° C.

Fluid in pipette			No. of drops	Sp. gr. of fluid	Relative surface tension
Distilled water	...	...	385	1.000	
"	...	...	387		
"	...	...	385		
"	...	...	385		
"	...	...	387		
"	...	...	387		
"	...	...	386		
			386	...	100
			mean		
Methylenblue 4 per cent.					
solution in distilled water	...	...	353	1.010	
"	...	...	352		
"	...	...	351		
"	...	...	350		
"	...	...	355		
"	...	...	351		
"	...	...	350		
"	...	...	350		
			351.5	...	110.9
			mean		

It will be seen the surface (air-fluid) tension of water is increased 10.9 per cent. by the addition of 4 per cent. of methylenblue (= .126 M).

LITERATURE

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