CCCIV.—*Studies in Adsorption by an Optical Method. Fixation of Methylene-blue by Yeast-phospho-protein Sol within the Disperse Phase.*

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IN a previous paper on this subject (this vol., p. 102), Fodor and Riwlin showed that, working with sols of such proteins as casein, natural white of egg, purified albumin and gelatin, no difference was detectable spectrophotometrically between simple solutions of methylene-blue and solutions of this dye containing also any of these proteins. Our further researches on phospho-protein sol derived from yeast macerates have led to positive results.

The yeast phospho-protein was prepared by the method of Fodor (*Fermentforsch.*, 1921, **4**, 209), and contained 15·69% N and 5·95% S. It was insoluble in water, but readily formed a milky, colloidal suspension which wandered to the anode with the cataphoretic fall of potential. For its preparation from macerates of yeast, the fluid was diluted with water as required and fractionally precipitated with dilute hydrochloric acid.

The different fractions, after being thoroughly washed and dried, gave products which proved to be of the same composition on analysis but which, before drying (*i.e.*, in the freshly-precipitated condition), showed differing colloidal properties. The first fractions when rubbed with water gave a suspension—a stable, milky sol which kept as such for many days without sedimentation occurring. The fractions obtained with the more concentrated acid began to show sedimentation very soon.

The stable sols serve very well for adsorption experiments, particularly because of the fact that they contain slightly solvatised particles, which are clearly detectable ultra-microscopically and can be salted out. No other albuminous substance can be converted into ultra-visible sols in such a simple manner as can yeast-phospho-protein.

We determined by the spectrophotometric method previously described, the light-absorption of the following three mixtures:

1. Yeast-phospho-protein sol + water (transmissive power = a).

2. Yeast-phospho-protein sol + methylene-blue (transmissive power = b).

3. Methylene-blue + water (transmissive power = c).

When, in the case of the second mixture, no methylene-blue is withdrawn from the solution (as a result of adsorption by the phospho-protein particles), the transmissive power (b) is calculable from that of the two other solutions (a and c), *i.e.*, $b = ac$.

In all our determinations on phospho-protein it was found, however, that $b > ac$. This indicates that the mixture of methylene-blue and phospho-protein sol is more permeable to light than would be expected from the turbidity of the two separate solutions.

FIG. 1.

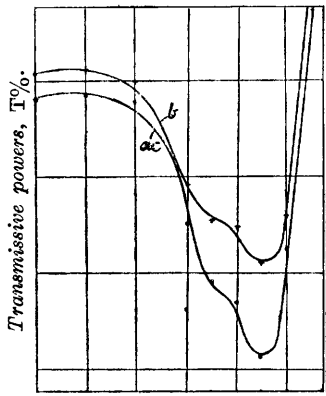
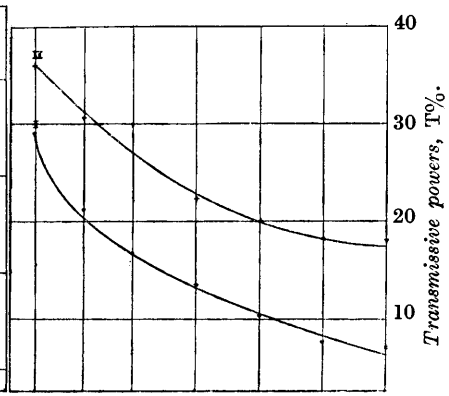


FIG. 2.



I = ac *and* II = b *for mixtures of methylene-blue + phospho-protein sol.*

Consequently, methylene-blue has disappeared from the solution, having accumulated on the surface of the phospho-protein particles.

From the fact that the transmission curve (Fig. 1) obtained from the calculated values of ac has the same form as that obtained from the values (b) actually found, it follows that we are dealing here with a non-chemical adsorption phenomenon. Moreover, the minima of the two curves occur at exactly the same wave-length. These facts, according to our previous work, indicate that we are dealing with true lyosorption.

From a very large number of observations, we quote here only one series which concerns mixtures of yeast-phospho-protein sol with increasing quantities of methylene-blue (Table I).

TABLE I.

Transmissive powers of mixtures of methylene-blue and phospho-protein sol.

% Conc. of methylene-blue.		6×10^{-4}.		8×10^{-4}.		10×10^{-4}.		$12{\cdot}5 \times 10^{-4}$.	
$\lambda(\mu\mu)$.	*a.*	*ac.*	*b.*	*ac.*	*b.*	*ac.*	*b.*	*ac.*	*b.*
521	50	47·5	52·5	47·5	50	45	46·5	45	45
542	54	49	51	48	50·5	46	46·5	43·5	45
564	59·5	49·5	50	47·5	49·5	41·5	42	40·5	37
590	64	39·5	45	35	39	28·5	34	25	27
604	65	34	39·5	29	35	23	32	19·5	25
620	68·5	31	37	26·5	34·5	20·5	30	17	24
637	70	29	36	21	30·5	16·5	27	13·5	22
655	70	29·5	40	27	35	21	30·5	18	25
674	70	59·5	60	56	57	52·5	50		

% Conc. of methylene-blue.		15×10^{-4}.		$17{\cdot}5 \times 10^{-4}$.		20×10^{-4}.	
$\lambda(\mu\mu)$.	*a.*	*ac.*	*b.*	*ac.*	*b.*	*ac.*	*b.*
521	50	43	45	41·5	44·5	38	41·5
542	54	43	43	40·5	42·5	38	40·5
564	59·5	35·5	36·5	33	35	30	33
590	64	19	27·5	16	25	13	19·5
604	65	15	25	12·5	20·5	9·5	18·5
620	68·5	13·5	22	10	19	9	18
637	70	10·5	20	7·5	18·5	7	18
655	70	17·5	24·5	14·5	21	14	19·5
674	70			42	47	38·5	41·5

In order to determine the fraction of the methylene-blue adsorbed we made use of the following graphic method. The values of *b* were plotted as a function of the methylene-blue concentration for a definite wave-length, *e.g.*, $\lambda = 637$ $\mu\mu$, and so also were the *ac* figures. From this graph (Fig. 2) the amount of adsorption for the various concentrations could be found.

The transmissive power, *b*, found for the methylene-blue (*e.g.*, for concentration 0·001%) has the same value as the calculated *ac* for the methylene-blue of concentration 0·00065%. This therefore indicates that 35% of the methylene-blue has been adsorbed by the yeast-phospho-protein particles and does not play any part in the light-absorption.

A source of error which must not be overlooked when working with methylene-blue in presence of products which have been isolated from living cells and tissues is the possible presence of fermentative substances capable of reducing the dye. In order to determine whether positive results (*i.e.*, disappearance of methylene-blue) were due to this cause we studied the change in the reduction of absorption with time.

The results showed (see Table II) that the change is not due to

TABLE II.

Influence of time on the adsorption.

Phospho-protein sol + Methylene-blue (0·0015%).

Time after mixing (hrs.).	0.		3.		4.		24.	
$\lambda(\mu\mu)$.	*ac.*	*b.*	*ac.*	*b.*	*ac.*	*b.*	*ac.*	*b.*
564	14·5	22·5	15·5	24	15·5	24·5	15·5	25
590	13·5	25	14·5	26	14·5	27	14·5	27
604	11	23·5	11·5	25	11·5	25·5	11·5	26
620	18·5	28	19·5	28·5	20	29·5	20	30

a fermentative reduction of methylene-blue, for the final values of the absorption are attained almost immediately after the solutions have been mixed. The subsequent change of absorption (*b*—*ac*), of the order of about 10% of the total absorption, is a phenomenon with which one has to reckon in all adsorption phenomena, and used to be described as static adsorption as contrasted with dynamic.

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