XVIII.—*Studies in Adsorption by an Optical Method. Fixation by Dispersoids of Methylene-blue within the Disperse Phase.*

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ADSORBENTS in a comparatively coarse state of division, and therefore readily separable from the solution, have hitherto been used in investigations on adsorption. When one comes to deal with colloidal, finely divided adsorbents—and there is no doubt that the micelle of a sol also can act as an adsorbent—the difficulty of separating the phases from one another becomes almost insurmountable. Scarcely any case of this kind has been thoroughly examined because of the experimental difficulties. Dynamic methods, which cause a displacement of the equilibrium, and ultrafiltration are useless. The applicable static methods are limited in number. Potentiometric and conductivity methods, useful as they are in the case of proteins, which adsorb relatively large quantities of electrolytes, can be employed to measure the adsorption of only a few definite ions, and the method of compensation-dialysis has many disadvantages, one of which is the disturbance due to the membrane. For these and other reasons, it seemed to the authors that an optical method would be the most suitable for measuring adsorption, and, since the polarimeter and the refractometer cannot deal with opaque or turbid liquids, it should be based upon the absorption of light.

The concentration of a dye in solution will diminish on addition of a colloidal solution, owing to adsorption of the dye by the micelle. The absorption curves of the dye solution before and after the addition will therefore not be the same; and the difference between them will be a measure of the adsorption, if other losses of light, *e.g.*, by diffusion, do not affect the result.

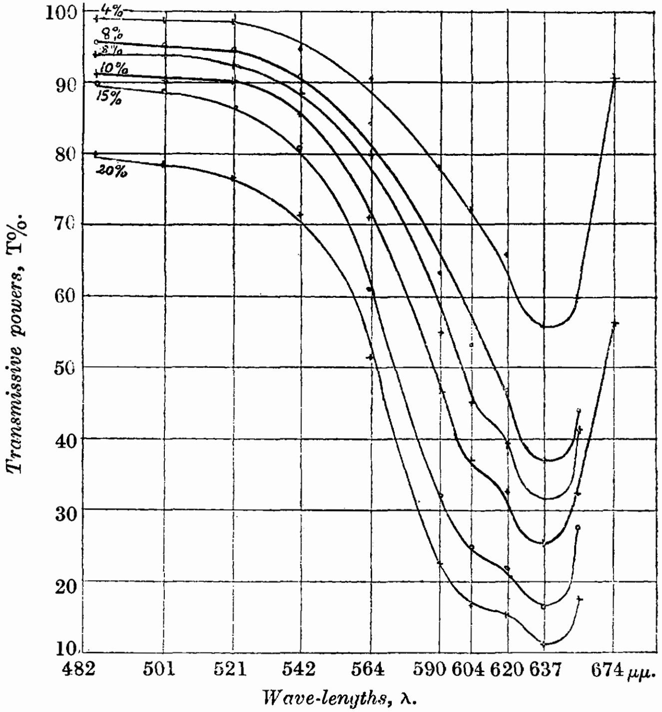
A suitable dye solution must comply with the following requirements: 1. It must be stable. 2. Its absorption spectrum must (*a*) fall within the visual region and not be too extended, and (*b*) be continuous and consist of one band. The curve will then have only one maximum or minimum, in the neighbourhood of which slight changes can be very conveniently measured.

Method.—The transmissive powers, T, of a dye solution for light of different wave-lengths were measured directly in percentages with Vierorat's improved form of the Schmidt-Haensch spectrophotometer. The transmission curve of the solution was then obtained by plotting the values of T as ordinates and wave-lengths (λ) as abscissæ (Fig. 1).

The concentration of a dye solution may be determined by this method in the following way, which obviates the application of Beer's law. The absorption curves of a series of solutions of the dye of known concentrations are drawn. The minima of these curves will all lie on one and the same ordinate, *viz.*, that drawn through the wave-length corresponding to minimum transmissive power. A second graph is drawn, having the concentrations of the solutions as abscissæ and the corresponding minimal transmissive powers as ordinates. The minimal transmissive power of the solution of unknown concentration is now determined from its transmission curve, and the corresponding abscissa in the second graph will then give the concentration.

FIG. 1.

Transmission curves of methylene-blue in water.



EXPERIMENTAL.

Preparation of the Dye Solutions.—A series of solutions was prepared containing 4, 6, 8, 10, 12½, 15, 17½, 20, 25, 30, 40, and 50% *

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* For convenience' sake, a true concentration of 0·000n% is written as n%.

of a standard solution of methylene-blue (0·1 g. in 1000 c.c. of water). Ten c.c. of each of those solutions were mixed with 10 c.c. of water, and the transmission curves of the mixtures were plotted (Fig. 1). From time to time, the transmissive powers were redetermined to ensure that they still agreed with those of the freshly prepared solutions, and therefore that the concentrations of methylene-blue were unchanged.

Methylene-blue is adsorbed by many substances, including filter-paper. The solutions, therefore, were not filtered and the parts of the vessels used that would come into contact with the liquid were specially polished.

Solutions of methylene-blue are not very stable. Under the influence of light and air they slowly lose their colour, and much more quickly in presence of alkali. The mixture of methylene-blue and a sol under investigation was therefore compared with a solution having the same hydrogen-ion concentration. Wherever possible, for the preparation of the comparison liquid the same acid or alkali was used as that present in the sol.

In many cases the sol itself was cloudy. The effect of the consequent scattering of light was ascertained in the following way. The transmissive power (a) of a mixture of 10 c.c. of the sol with 10 c.c. of water was measured. If c is the transmissive power of the methylene-blue solution (10 c.c. of the diluted standard solution + 10 c.c. of water) and b that of the experimental mixture (10 c.c. of the diluted standard solution of methylene-blue + 10 c.c. of the sol), then, provided that there was no interference due to chemical reaction or adsorption, for each wave-length, $ac = b$.

If the sol transmitted light to the same extent as water, $a = 1$; and to find any deviation from the 'mixture' law, it was only necessary to compare c with b.

TABLE I.

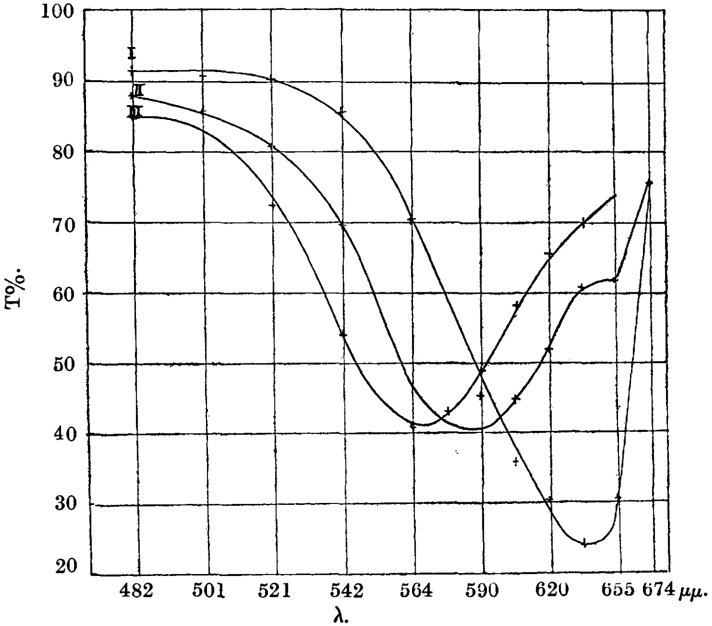
Transmissive powers of methylene-blue + silica sol.

	% Conc. of methylene-blue.						
λ ($\mu\mu$).	4.	6.	8.	10.	12·5.	15.	17·5.
482	95	93	89·5	87·5	85	83	80
501	95·5	92·5	89	85·5	83	80	75
521	94	89·5	84·5	81	79	75	69·5
542	84	79·5	74·5	69	64	58	52
564	70	60	53	45	40	35	30
590	*65·5*	*55*	*47·5*	*40*	*34·5*	*25*	*22*
604	74·5	61	53·5	44·5	40	30	26·5
620	80·5	69·5	60	51·5	45·5	40	35
637	85	77	68	60	55	46	42
655	86	79	70	61	57·5	49	44
674	90	85	82	75	—	65	—

1. *Silica Sol.*—This was prepared by Ostwald's method ("Kleines Praktikum der Kolloidchemie," 3rd ed., p. 8, expt. 23). $a = 1$ for all wave-lengths.

The transmissive power of the reddish-violet mixture of the methylene-blue solution with the silica sol (Fig. 2, curve II) is quite different from that of its blue mixture with water (curve I); the minimum has shifted from λ 637 μμ to λ 590 μμ and is of about twice the magnitude. Moreover, the two transmission curves have different forms. These facts all indicate the production of a compound.

FIG. 2.



I, *Methylene-blue+water.* II, *Methylene-blue+silica sol.*
III, *Methylene-blue+silica sol+sodium hydroxide.*

To prove that the effect of the sol on the methylene-blue was not due to the presence of hydrogen- or hydroxyl-ions, the transmission curves were determined of methylene-blue solutions containing these ions. It will be seen (Table II) that the addition neither of acid nor of alkali caused any considerable alteration in the position of the minimum of the curve for the methylene-blue solution during the time of observation. If the solutions were kept for some time, the minimum shifted towards the blue end of the spectrum (see last two columns). Acid was without appreciable effect

TABLE II.

Effect of acid or alkali on the transmissive powers of methylene-blue.

	12·5% Methylene-blue.			17·5% Methylene-blue + N/10-NaOH.		
λ (μμ).	+ Water.	+*N*/10-HCl.	+*N*/10-NaOH.	Soon after mixing.	After 2 hrs.	After 24 hrs.
482	91·5	91	90·5	79	79	61·5
501	92	91·5	89·5	75	75	55
521	91·5	90	89·5	69	67·5	45
542	85	85	83	55	55	35
564	70	68·5	70	40	39·5	29·5
590	46	39·5	51	29	29	*25*
604	36	30	41	25·5	*25*	48·5
620	31·5	25	35·5	24·5	25·5	65
637	*25·5*	*20*	*29·5*	*23·5*	29	75
655	32	26	35	28·5	37·5	80

on the transmissive power of the mixture of methylene-blue solution and silica sol, whilst the addition of alkali caused the point of inflexion in the region of λ = 637 μμ (Fig. 2, curve II) almost to disappear and the minimum to shift to λ = 564 μμ (curve III).

When a mixture of methylene-blue solution and silica sol was kept in an unpolished vessel, there was little if any adsorption of the dye on the walls. It seems as if the dye is so strongly held by the particles of sol that it is indifferent to any other influence.

An experiment with kaolin gave somewhat similar results: Mixtures of 10% methylene-blue solution with (*a*) water, (*b*) silica sol, and (*c*) 1% sodium silicate solution were each shaken with 100 mg. of kaolin, which was then allowed to settle. The supernatant liquid was (*a*) colourless, (*b*) violet-blue, (*c*) blue.

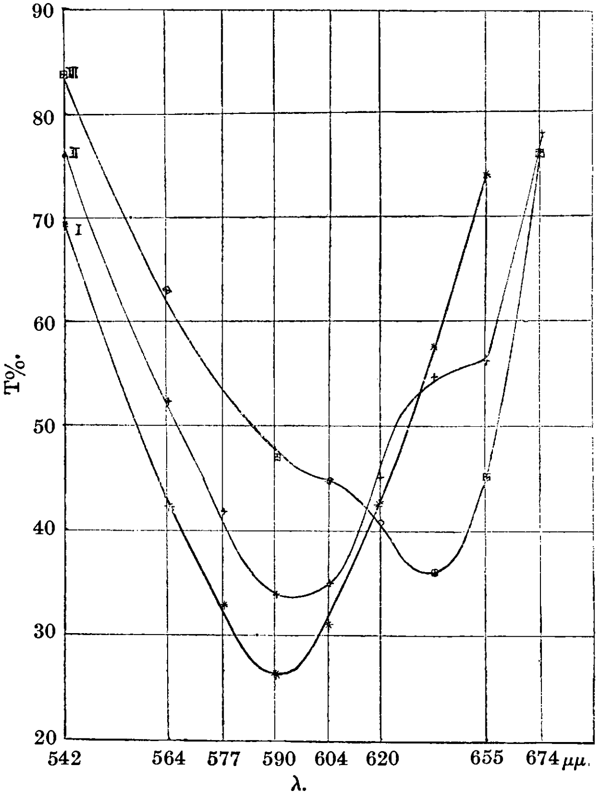
In another experiment, 2, 3, 5, 10, 30, and 50% solutions of methylene-blue were each mixed with 10 c.c. of silica sol and shaken with 100 mg. of kaolin. After settling, the sediment was coloured in every case, the liquors from the 2% and 3% solutions were colourless, and the others were slightly coloured.

Sodium Silicate (Merck's commercial solution).—When the last experiment was repeated with a 1% solution of sodium silicate instead of the silica sol, even the liquors from the 2% and 3% solutions of methylene-blue were coloured. Sodium silicate seems to retain the dye better than silica sol.

On mixing solutions of methylene-blue and sodium silicate a blue precipitate was obtained or not according as the former or the latter was in excess. A similar behaviour was not observed in the case of the silica sol.

The transmission curves of mixtures of solutions of methylene-blue and sodium silicate were determined; the solutions were of course alkaline. As in the case of the silica sol (Fig. 2, curve II), a minimum was found at λ 590 μμ (Fig. 3, curve I). On addition of insufficient hydrochloric acid for neutralisation the minimum was slightly displaced and a bend appeared in the region of λ 637 μμ (curve II). When the mixture was made acid, the minimum shifted to λ 637 μμ, its place being taken by a slight bend (curve III). If the acid was added very carefully until the mixture was neutral, the whole mass coagulated.

Fig. 3.



I, *Methylene-blue + sodium silicate + water.* II, *Methylene-blue + sodium silicate + hydrochloric acid (still faintly alkaline).* III, *Methylene-blue + sodium silicate + hydrochloric acid (faintly acid).*

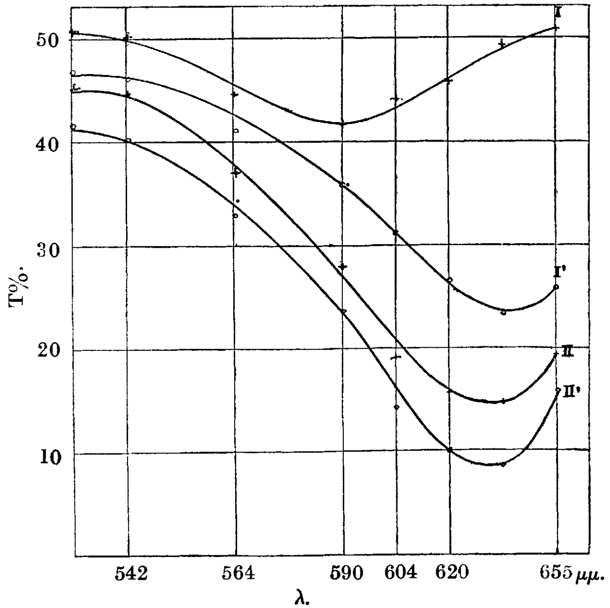
Kaolin.—A suspension of 2 g. of kaolin in 100 c.c. of water was kept for 18 hours, and the turbid liquor was then syphoned off. This liquor, containing only the finest particles of the kaolin, was used in the following experiments and was well shaken before each determination.

Table III.

Transmissive powers of mixtures of methylene-blue and kaolin.

	Me-b (6%) + kaolin.			Me-b (10%) + kaolin.			Me-b (15%) + kaolin.		
λ (μμ).	*a.*	*ac.*	*b.*	*a.*	*ac.*	*b.*	*a.*	*ac.*	*b.*
482	48	46	50	50	46	49	47	42	45·5
501	47·5	45·5	50·5	49·5	45·5	49	47	42	46
521	48·5	46	50·5	49	44	49	48·5	41·5	45
542	51	46	50	51	44·5	48	50	40	44·5
564	51·5	42	45	52	38	42·5	53·5	33	37·5
590	57	36	41·5	58	26	37	54·5	18	22
604	58	30·5	44	59	21	36	55	14	19·5
620	60	27	45·5	61	18	35	57	11·5	17
637	63	23·5	49	62	15·5	35	60	9	15
655	63	27	50·5	64	19	39	60	16·5	20

Fig. 4.



I=b *for the mixture* 6% *methylene-blue+kaolin.* I′=ac *for the mixture* 6% *methylene-blue+kaolin.* II=b *for the mixture* 15% *methylene-blue+kaolin.* II′=ac *for the mixture* 15% *methylene-blue+kaolin.*

The transmissive powers of the kaolin suspension (*a*), of the methylene-blue solution (*c*), and of the mixture of the two (*b*) were determined and *b* was compared with *ac* (Table III and Fig. 4). The *b* transmission curves and the calculated *ac* curves are almost parallel for the more concentrated solutions of methylene-blue, but diverge in the case of dilute solutions. The *b* curve, however, invariably lies above the corresponding *ac* curve, and therefore it

TABLE IV.

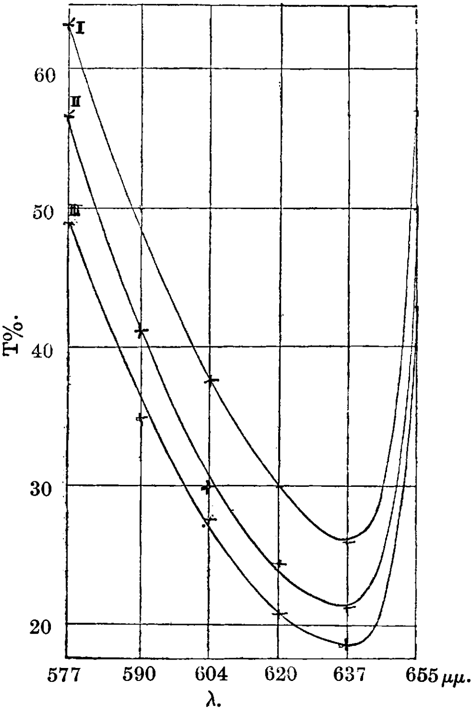
Transmissive power of methylene-blue (10%).

λ ($\mu\mu$).	+ Water	+N/50-Acetic acid.	+ Aluminium hydroxide sol.
577	63	57	49
590	50	41	35
604	37·5	30	28
620	30	24·5	21
637	*26*	*21*	*18·5*
655	57	50	42·5

may be concluded that the methylene-blue is partly adsorbed by the kaolin.

Aluminium Hydroxide Sol.—The sol was prepared by Ostwald's method (*op. cit.*, p. 12, expt. 30). A mixture of methylene-blue solution and the sol was less transparent than the corresponding mixture of methylene-blue and water. This result was due to the presence in the sol of acetate ions, which diminish the transmissive power of methylene-blue (Table IV and Fig. 5). Almost identical transmission curves were obtained for the methylene-blue solution + the aluminium hydroxide sol and methylene-blue solution + an acetate buffer the p_H of which was the same as that of the sol (3·84).* No adsorption of methylene-blue, therefore, can be demonstrated in the case of aluminium hydroxide sol.

FIG. 5.



I, 10% *methylene-blue+water.* II, 10% *methylene-blue+*N/50*-acetic acid.* III, 10% *methylene-blue+aluminium hydroxide sol.*

Natural Protein.—A solution of white of egg in water (20 vols.)

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* Compare A. Fodor, "Determination of the affinity constants of weak acids and bases" ("Handbuch der biologischen Arbeitsmethoden," E. Abderhalden, p. 474).

was filtered. The filtrate was turbid, and therefore *a* had to be determined. On comparing *ac* (where c = transmissive power of methylene-blue + buffer solution) with *b*, no difference was observed (Table V). Natural protein, therefore, does not adsorb methylene-blue.

TABLE V.

Transmissive power of white of egg + methylene-blue (10%).

λ ($\mu\mu$).	*a*.	*c*.	*ac*.	*b*.	λ ($\mu\mu$).	*a*.	*c*.	*ac*.	*b*.
482	92	93	85·5	86	590	95	47·5	45	44
501	93·5	93	87	86	604	95	35	33	34
521	93·5	92·5	86·5	85·5	620	95	29	27·5	28·5
542	94	84·5	79·5	78	637	95·5	24	23	24
564	95	70·5	67	69·5	655	96	33·5	32	34·5

An experiment was also made with the protein at the isoelectric point. Acid was added in increasing quantities to solutions of the protein, and the concentration was thus found (*viz.*, 0·6 c.c. of *N*/10-hydrochloric acid for 10 c.c. of protein solution) at which the protein coagulated most rapidly on heating. The above experiments were repeated with such a protein mixture, but again there was no difference between *ac* and *b*.

Finally, the protein was finely dispersed by the addition of sodium hydroxide (1 c.c. of *N*/10-solution to 10 c.c. of protein solution), and the mixture with methylene-blue examined in the usual way, the comparison liquid being a solution of sodium hydroxide of the same p_H as the protein mixture. No adsorption of methylene-blue was detected.

Casein.—A solution of 2 g. of casein (Hammersten) in 60 c.c. of water and 2 c.c. of *N*-sodium hydroxide was diluted to 100 c.c. with water, a slight excess of hydrochloric acid added, and the solution kept for 12 hours. The precipitated casein was washed free from chloride, and dissolved in 100 c.c. of *M*/15-disodium hydrogen phosphate or *M*/15-sodium carbonate.

The p_H of the phosphate solution was 7·45. A buffer solution of the same p_H was made by mixing 84 c.c. of *M*/15-disodium hydrogen phosphate with 16 c.c. of water.

Mixtures of either solution of casein (phosphate or carbonate) with methylene-blue were examined, but no difference was found between *ac* and *b*, and therefore no adsorption occurred.

Pure Albumin.—A 10% solution of white of egg was mixed with an equal volume of saturated ammonium sulphate solution, the precipitated globulin removed, and the filtrate dialysed for several days until it was free from sulphate. The solution of albumin thus obtained was used in experiments with methylene-blue, but no adsorption was detected.

Saponin.—The transmission curve of a mixture of methylene-blue with a 10% solution of saponin (for which $a = 1$) was the same as that of its mixture with water. There was, therefore, no adsorption. Kobert (Freundlich, "Kapillarchemie," 3rd ed., 1923, p. 889) dialysed a saponin solution against a solution of methylene-blue and showed that the dye accumulated in the sol so that its concentration there exceeded that in the external solution. We tried to repeat this experiment; but the whole of the methylene-blue was adsorbed by our dialysis membrane ("Dialysierhülse" of Schleicher and Schüll) and both liquids became colourless.

Gelatin.—A 1% solution of gelatin, made by warming 1 g. to 70° with 100 g. of water, becomes more opalescent on standing, but if it is prepared under the proper conditions, reproducible values of its transmissive power can be obtained. The calculated and the experimental values of the transmissive power of the mixture methylene-blue + gelatin were the same. Hence gelatin does not adsorb the dye.

Discussion.

It has been shown that sols of proteins, such as casein, natural white of egg, purified albumin and gelatin, do not adsorb methylene-blue; the substances remain mutually uninfluenced. This result is very remarkable, because, in the precipitation of a solution of protein containing methylene-blue by means of electrolytes, the dye always comes down with the coagulum and colours it blue. Possibly in the latter case, the particles of protein may be completely dehydrated; and the activities of the hydrated and the dehydrated particles may be different.

Also aluminium hydroxide sol and methylene-blue in solution are without influence on each other. Flocculating aluminium hydroxide, however, may carry down methylene-blue. Since both substances are basic, the reason for the carrying down must be sought in some physical cause—an explanation which might be applicable to coagulating protein also. The conditions of this mechanical adsorption are of course not yet known. It must, however, be kept in mind that methylene-blue is mechanically retained by substances of many different kinds, *e.g.*, glass, filter-paper, different kinds of membranes, etc. The adsorption of methylene-blue by finely divided kaolin is also a mechanical one, as may be seen from the absorption curve. The experimental curve runs almost parallel to the calculated one and lies above it. Consequently the transmissive power observed is greater than that calculated, but here, unlike the case of silica sol or sodium silicate, no new absorption minimum results, so that there is no new specific absorption curve of the mixture to be observed. Indeed the calculated curve for the kaolin–

methylene-blue mixture has an absorption minimum, namely, that of pure methylene-blue, at $\lambda = 637\,\mu\mu$, and this does not appear in the experimental curves for very low concentrations of methylene-blue (Table III). This, however, is because the kaolin adsorbs the whole of the methylene-blue.

The absorption curve of the system silica sol + methylene-blue, on the other hand, is characteristic in that it has a new absorption minimum (quite removed from that of the methylene-blue). This and the production of a violet colour, appreciable by the naked eye, indicate chemical combination between the silica and the dye.

The same violet coloration and the same absorption minimum are obtained when a dilute solution of water-glass mixed with methylene-blue is used. The same compound is produced with its own absorption minimum at $\lambda = 590\,\mu\mu$, near which the absorption minimum of methylene-blue also is to be found.

This investigation shows that it is possible by spectrophotometric methods to determine (1) whether a dye is not adsorbed at all by colloidal adsorbents (*e.g.*, albumin, casein, gelatin,* saponin, aluminium hydroxide sol), (2) whether a mechanical adsorption takes place, *e.g.*, kaolin suspension (*lyosorption*), or (3) whether there is chemical combination between the adsorbent and the dye, *e.g.*, water-glass solution, silica sol (*chemosorption*). (See Fodor, *Koll.-chem. Beih.*, 1923, **18**, 77; "Grundlagen der Dispersoid Chemie," Dresden u. Leipzig, 1925.)

In case 1 the curves calculated from the values of *a* and *c* agree with the experimental curves.

In case 2 there is a parallel displacement upwards of the experimental curve.

In case 3 the curve has a special form with one (or perhaps several) new absorption minimum.

It is often very difficult to distinguish between chemosorption and lyosorption. In case 3, however, we believe that the appearance of a new absorption minimum indicates chemosorption. An example of the capacity of colloidal solutions to form reproducible definite stoicheiometric compounds is to be found in the case of the albumin solutions investigated by Sörensen and Höyrup (*Z. physiol. Chem.*, 1918, **103**, 104).

We have to thank Miss S. Rubinstein for help in the experimental part of the work.

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* Recent researches have shown that the phospho-protein sol from yeast-macerate is able to fix methylene-blue fairly strongly. The results will be published later.