

Adsorption of Methylene Blue by Potato Starch: Effect of Methanol, Dioxane, Sucrose, and Urea in Aqueous Systems

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Abstract □ Methylene blue is adsorbed by potato starch from aqueous solution as a monomer or dimer, depending upon its concentration. The adsorption phenomenon can be described by the Langmuir equation. The effect of methanol, dioxane, sucrose, and urea on methylene blue adsorption has been examined. These water-miscible additives shift the monomer-dimer equilibrium of the dye in the direction of the monomer and reduce the extent of methylene blue adsorption without significantly affecting the maximum possible adsorption. This appears to indicate that their effect is primarily on the effective concentration of the dye. At high concentrations, methanol and dioxane apparently further reduce adsorption by affecting the surface of potato starch.

Keyphrases □ Methylene blue—potato starch adsorption □ Starch, potato—methylene blue adsorption □ Methanol, dioxane, sucrose, urea effects—methylene blue adsorption □ Sucrose effect—methylene blue visible spectrum □ Colorimetric analysis—spectrophotometer

Enhanced adsorption of water-soluble certified dyes to various solid dosage form ingredients has been suggested as a means of overcoming the problems of dye migration during the drying of tablet granulations (1). During a study of anionic dye adsorption on various starches, significant adsorption was noted for all types of starch except potato starch (1, 2). Schoch and Maywald (3) observed also that Congo Red, a negatively charged dye, is not adsorbed by potato starch, presumably because of the presence of phosphate groups not ordinarily found in the other starches (4). In view of this negative charge, potato starch would be expected to adsorb cationic substances significantly. Schoch and Maywald (3) found that the cationic dye, methylene blue, strongly stains potato starch granules, but no quantitative studies on the adsorption of cationic substances by potato starch have appeared.

In the present study, methylene blue was chosen as a model dye in view of the information available concerning its state in solution (5-8). In dilute aqueous solutions, the dye exists principally in its monomeric form, while in concentrated solution the dimeric and multimeric forms predominate as a result of hydrophobic self-association. Since the monomer-multimer equilibria are sensitive to various polar additives (5), the effect of such substances on methylene blue adsorption was also investigated. The additives examined were dioxane, methanol, sucrose, and urea.

EXPERIMENTAL

Materials—Methylene blue USP and potato starch (purified powder)¹ were used as received. Water contents of the trihydrate

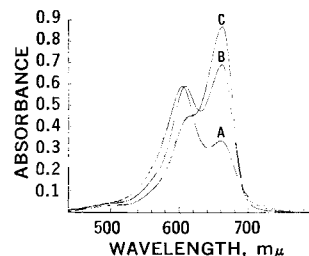


Figure 1—Effect of concentration upon the visible spectrum of methylene blue at room temperature. Key: A, 1.2×10^{-3} M, 0.1-mm. cells; B, 1.2×10^{-4} M, 1-mm. cells; and C, 1.2×10^{-5} M, 10-mm. cells.

dye and the starch were determined and were taken into consideration when recording their weights. Reagent grade dioxane, methanol, and sucrose and urea USP were used without further purification. Dye solutions for adsorption studies, with or without additives, were always freshly prepared using double-distilled water.

Spectral Studies—To illustrate the effect of concentration upon the visible spectrum of methylene blue, the following concentrations were employed: 1.2×10^{-3} M, 1.2×10^{-4} M, and 1.2×10^{-5} M; the cells used were of 0.1-, 1-, and 10-mm. light pathlengths, respectively. The effect of additives upon the spectrum of the dye was examined at constant dye concentration (1.2×10^{-4} M) and various additive concentrations, using 1-mm. cells. Spectra were recorded at room temperature on a spectrophotometer (Cary 15).

Adsorption Studies—All adsorption experiments were done at a controlled room temperature of $26 \pm 1^\circ$. Approximately 0.5 g. of potato starch was accurately weighed into each of a series of vials. Ten milliliters of a dye solution was added to each vial, and the vials were stoppered with butyl rubber closures. It was initially ascertained that these stoppers adsorbed no methylene blue. The adsorption vials were shaken occasionally over 4 hr. Preliminary experiments indicated that this time was more than sufficient for the various systems to attain equilibrium.

Upon equilibration, 1 ml. of clear, granule-free, supernatant dye solution was withdrawn from each vial. It was then suitably diluted with water, and its concentration was determined spectrophotometrically at 660 mμ. A spectrophotometer (Cary 15) and 1-cm. glass cells were used. When the systems contained additives, it was necessary to make sure that the dye solutions were diluted to a point where the additive concentrations were too low to interfere with the spectral procedure. This precaution precluded adsorption studies at relatively low equilibrium dye concentrations in the presence of additives. From the concentration of dye solutions before and after contact with starch, the amount of dye adsorbed was determined.

RESULTS

Spectral Properties of Methylene Blue—The changes in the visible spectrum of methylene blue, reflecting changes in the state of aggregation with increasing dye concentration, are shown in Fig. 1. The peak at ~ 660 mμ has been assigned to the monomeric form, which predominates in dilute aqueous solutions. The dimeric form absorbs at ~ 590 mμ; the population of this form increases as the dye concentration is increased. Possibility of higher multimers has been proposed for some time; recently, existence of a trimer was conclusively proven and a rather broad peak at ~ 580 mμ (not shown in Fig. 1) was ascribed to the trimer (8). Because of these spectral properties, the Beer's law relationship is applicable only in a limited concentration range, up to $\sim 1 \times 10^{-5}$ M, when absorbance cells of 1-cm. light pathlength are used.

Figure 2 illustrates the effect of increasing additive concentrations upon the spectrum of the dye. Relative effectiveness of these

¹ Fisher Scientific Co., Pittsburgh, Pa.

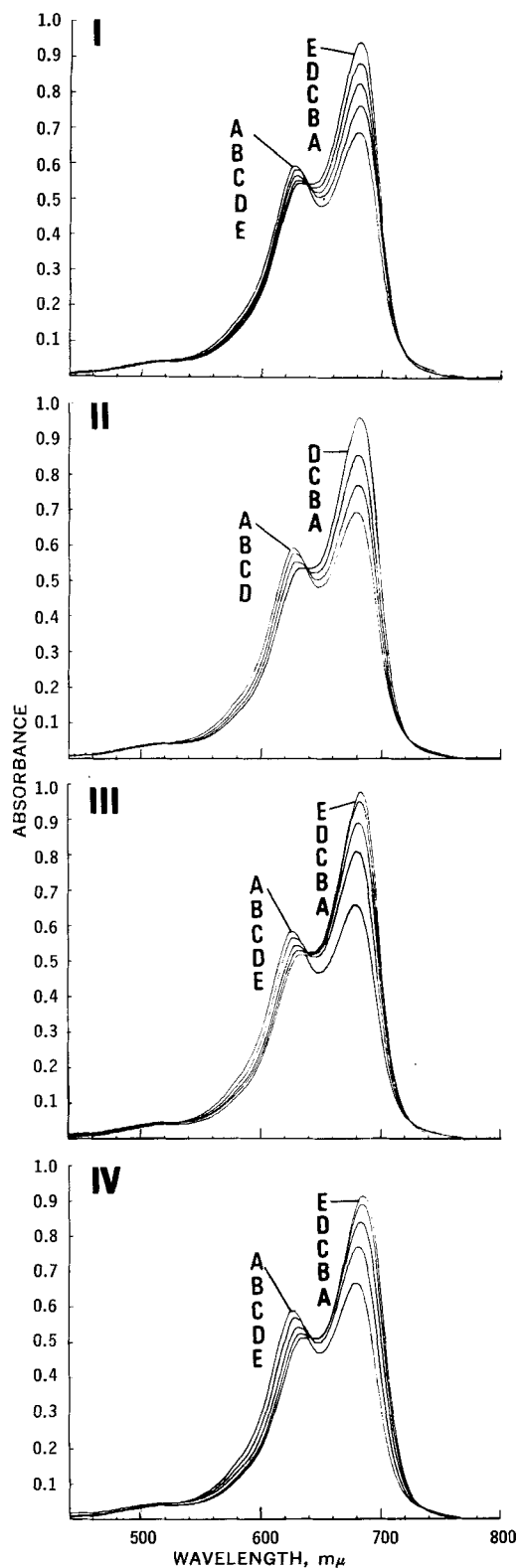


Figure 2—Effect of additive concentration upon the visible spectrum of methylene blue at room temperature. I: Methanol, A, 0%; B, 5%; C, 10%; D, 15%; and E, 20% v/v. II: Dioxane, A, 0%; B, 2.5%; C, 5%; and D, 10% v/v. III: Sucrose, A, 0 M; B, 0.5 M; C, 1 M; D, 1.5 M; and E, 2.0 M. IV: Urea, A, 0 M; B, 1 M; C, 2 M; D, 3 M; and E, 4 M. Dye concentration: 1.2×10^{-4} M; 1-mm. cells.

additives in diminishing the self-association of methylene blue may be examined by considering the ratio of the absorbance of the monomer peak to that of the dimer peak. Plots of monomer/dimer ratio (M/D ratio) versus the concentration of additives are shown in

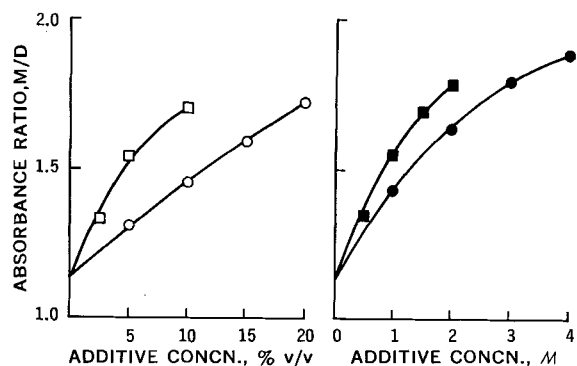


Figure 3—Relationship between additive concentration and absorbance ratio of the monomer and dimer peaks. Key: O, methanol; □, dioxane; ■, sucrose; and ●, urea.

Fig. 3. Sucrose is more effective than urea, on a molar basis, in reducing the self-association of the dye. This observation has an interesting parallel in the work of Emerson and Holtzer (9) who found that at equimolar concentrations, sucrose is more effective than urea in raising the CMC of dodecyltrimethylammonium bromide. Of the two organic solvents employed, dioxane is more effective than methanol at equal volume concentration.

Adsorption Studies—The Langmuir equation for adsorption from solution may be written in its linear form as follows:

$$\frac{C}{x/M} = \frac{C}{k_2} + \frac{1}{k_1 k_2} \quad (\text{Eq. 1})$$

where C is the concentration of the solute at equilibrium, x/M is the amount of solute adsorbed per unit weight of the adsorbent, and k_1 and k_2 are constants. The constant k_1 represents the tendency for adsorption to occur and may be considered as an equilibrium constant for the adsorption process; k_2 represents the maximum value of x/M at a given temperature determined by the number of available sites or the surface area of the adsorbent. If a system follows the Langmuir adsorption equation, then plots of $C/(x/M)$ versus C should be linear. The constants k_1 and k_2 may be calculated from the values of the slope and intercept of such Langmuir plots.

Figure 4 is a Langmuir plot for the adsorption of methylene blue on potato starch from water. The plot shows two linear regions of significantly different slopes. The lower region corresponds to dilute solutions in which methylene blue exists predominantly as the monomer; the higher region corresponds to solutions in which the dimers predominate. These regions are referred to hereafter as the monomer

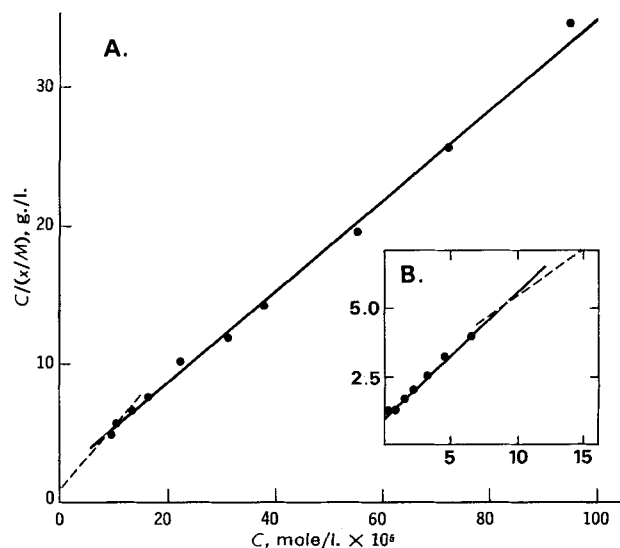


Figure 4—Langmuir plots for adsorption of methylene blue on potato starch from water. A: Solid line and data points are for dimer range; dashed line is for monomer range. B: Solid line and data points are for monomer range; dashed line is for dimer range. The lines drawn are calculated least-squares lines.

Table I—Langmuir Constants for Adsorption of Methylene Blue on Potato Starch from Aqueous Solutions at 26°

Dye Concentration Range	Langmuir Constants		Correlation Coefficient of Linear Fit
	k_1 , l./mole	k_2 , moles/g.	
Low, predominantly monomer	0.466×10^4	2.18×10^{-5}	0.996
High, predominantly dimer	1.54×10^4	3.04×10^{-5}	0.998

and dimer regions, respectively. Since the trimer is formed at relatively high concentrations, it was assumed that within the dye concentration range employed in this study the fraction of trimer present was negligible.

The Langmuir constants calculated from the linear plot of Fig. 4, along with the correlation coefficients of the linear fits, are listed in Table I.

Effect of Additives—Figures 5–8 show, respectively, the effect of methanol, dioxane, sucrose, and urea upon the adsorption of methylene blue by potato starch. Over the dye concentration range employed, adsorption decreases with an increase in the additive content of the system. This finding is in agreement with previous observations made with dye–starch systems containing water-miscible organic solvents (2, 10, 11). In the experiments reported here, it was not possible to obtain valid results at low dye concentrations because the presence of additives gave rise to difficulties in the analytical procedure, as indicated in the *Experimental* section. The data obtained in the presence of additives, therefore, refer to relatively high dye concentrations (dimer range).

All the systems examined followed the Langmuir equation. Representative Langmuir plots, for the systems containing sucrose, are shown in Fig. 9. The Langmuir constants and the correlation coefficients for all the systems examined are listed in Table II. For comparison, the values of M/D ratio for these systems are also listed. The values listed in Table II make the following points apparent: (a) k_1 decreases with an increase in the additive content of the system; (b) k_1 decreases as the M/D ratio increases (or as the relative amount of dimer decreases); and (c) k_2 remains relatively unchanged (except at 10% volume concentration of methanol or dioxane).

DISCUSSION

Effect of Additives upon Self-Association of Methylene Blue—

All four additives employed in this study shift the monomer/dimer

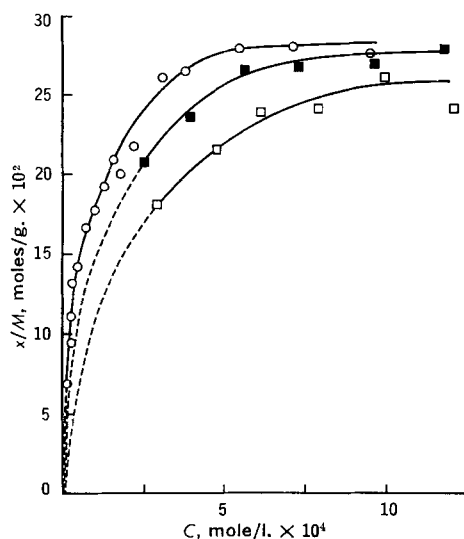


Figure 5—Isotherms for adsorption of methylene blue on potato starch. Key: O, water; ■, 5% methanol; and □, 10% methanol.

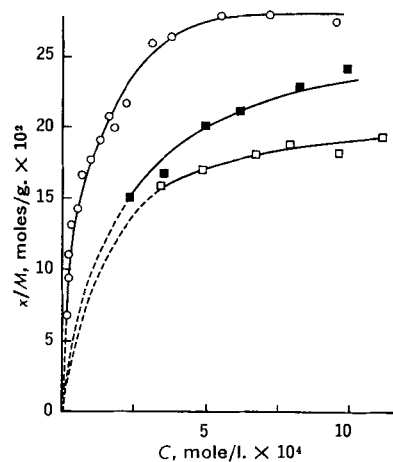


Figure 6—Isotherms for adsorption of methylene blue on potato starch. Key: O, water; ■, 5% dioxane; and □, 10% dioxane.

equilibrium of methylene blue in aqueous solution toward the direction of the monomer. The organic solvents, methanol and dioxane, probably act this way by interacting with water and by lowering the dielectric constant of the system (12). However, it seems unlikely that the same mechanism is operative with urea and sucrose; urea increases the dielectric constant of water (13), whereas sucrose decreases it (14–16). Mukerjee and Ghosh (5) suggested that urea affects the monomer/multimer equilibrium of methylene blue by disrupting the structure of water. Uedeira and Uedeira (17) suggested that sucrose also diminishes self-association in some aromatic azo dyes by a similar mechanism.

Additionally, urea and sucrose are said to have opposite effects upon water structure (18); urea is believed to be a “structure breaker” and sucrose a “structure maker.” The terms structure maker and structure breaker, as applied to the action of urea on water, have been the subjects of recent controversies (19, 20) of both a mechanistic as well as a semantic nature. In view of such controversies, it is difficult to say what specific mechanisms are responsible for the actions of the four additives used in this study. However, these additives all have the potential for interacting with water and they all diminish self-association of methylene blue in aqueous solution.

Adsorption of Methylene Blue from Water—Giles *et al.* (21) and Giles (22) suggested that methylene blue is adsorbed as the dimer² on several nonporous adsorbents. The results of the present study show that, although at relatively high concentrations the dimer may be preferentially adsorbed, at very low concentrations, in which the monomer predominates, an appreciable amount of the dye is adsorbed on potato starch. It would appear that if adsorption experiments were performed with only relatively high concentrations of methylene blue, the monomer region might remain undetected. Additional evidence for the possibility of monomer adsorption comes from the

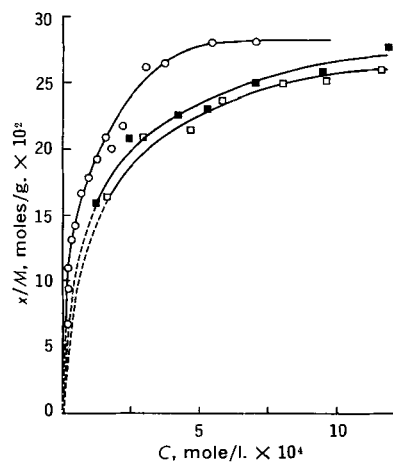


Figure 7—Isotherms for adsorption of methylene blue on potato starch. Key: O, water; ■, 0.5 M sucrose; and □, 1.0 M sucrose.

² The work of Giles *et al.* (21) and Giles (22) suggests that dimer adsorption of methylene blue involves adsorption of one molecule upon the adsorbent with the second molecule stacked lengthwise on top of the first. Dimer adsorption is not believed to involve side-by-side arrangement of two molecules on the surface of the adsorbent.

Table II—Langmuir Constants for Adsorption of Methylene Blue on Potato Starch from Aqueous Systems Containing Additives and M/D Ratios for These Systems

System	Langmuir Constants ^a			M/D Absorbance Ratio ^b
	k_1 , l./mole $\times 10^{-4}$	k_2 , moles/g. $\times 10^5$	Correlation Coefficient of Linear Fit	
Water (dimer range)	1.54	3.04	0.998	1.129
5% (v/v) Methanol	0.76	3.11	0.980	1.316
10% (v/v) Methanol	0.67	2.87	0.997	1.458
5% (v/v) Dioxane	0.40	3.07	0.997	1.545
10% (v/v) Dioxane	0.39	2.47	0.983	1.796
0.5 M Sucrose	0.74	3.07	0.996	1.350
1.0 M Sucrose	0.36	3.08	0.994	1.545
0.5 M Urea	0.77	2.97	0.995	1.279
1.0 M Urea	0.47	3.08	0.997	1.429

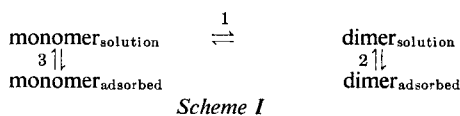
^a Determined at experimental temperature of $26 \pm 1^\circ$. ^b At methylene blue concentration = 1.2×10^{-4} M.

work of Imura (23), who found that more methylene blue was adsorbed by some clays when the initial dye concentration in the system was high than when methylene blue was added in small successive amounts until the same total concentration was reached in the system. This observation may be interpreted as follows: When the initial concentration is high, methylene blue exists principally as the dimer, and it is adsorbed as a monolayer predominantly of dimers; when small successive amounts of methylene blue are added, it exists in the unaggregated (monomer) form which is adsorbed.

If the two regions of the Langmuir plot (Fig. 4) corresponded strictly to monomers and dimers, then one would expect that k_2 (dimer) should be twice as large as k_2 (monomer). Experimentally, such is not the case; k_2 (dimer) is only 1.39 times k_2 (monomer). This finding suggests that in neither the "monomer" nor the "dimer" region is there adsorption of only one dye species. The two regions of the Langmuir plot represent the predominant species.

Additionally, the specific surface area calculated from both of the k_2 values and a molecular area of 120 \AA^2 for methylene blue (22) do not agree with the specific surface area determined by porosity measurements (24). These values (in m^2/g .) are: 15.6 [from k_2 (monomer)], 11.0 [from k_2 (dimer)], and 0.2 (from porosity measurements).³ These much higher values from methylene blue adsorption measurements may be due to penetration and diffusion of the dye solution into the starch grain. Thus the actual area available for adsorption would be much greater than the outer grain surface. A similar lack of correlation between the specific surface area values obtained by different methods was found in a study of the adsorption of various anionic certified dyes by several starches (2). The structure and porosity of the starch grain are important factors to be considered in the study of adsorption by the grain; both the external and the internal surfaces of the grain probably participate when dissolved substances are adsorbed.

Adsorption in the Presence of Additives—The equilibria between various forms of methylene blue can be represented as shown in Scheme I:



Equilibrium 3 comes into the picture, apparently, only at very low dye concentration. Under the conditions employed in this study, dimer adsorption is predominant. The Langmuir constants listed in Table II refer to adsorption characteristics of methylene blue dimer.

The decreasing value of k_1 when the systems contain increasing amounts of additives indicates essentially what Figs. 5–8 show,

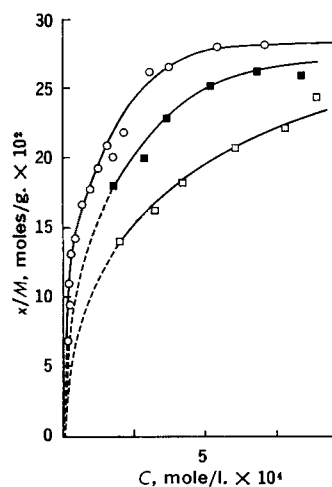


Figure 8—Isotherms for adsorption of methylene blue on potato starch. Key: ○, water; ■, 0.5 M urea; and □, 1.0 M urea.

i.e., the extent of adsorption decreases with an increase in the amount of additive. All the additives employed in this study shift Equilibrium 1 in the direction of the monomer, resulting in a decreased effective concentration of the dimer. The relative amount of dimer is still much greater than that of monomer and, since dimer is the predominant adsorbing species, decreased adsorption is reflected in lower k_1 values.

The relatively unchanged values of k_2 (or maximum possible amount of dye adsorbed) with increasing additive contents of the system indicate that the number of sites available for adsorption remains essentially unaltered. This observation indicates that the additives primarily affect the dye rather than the starch. However, at organic solvent concentrations of 10% (v/v) or higher, the starch is apparently affected. This is reflected in diminished values of both k_1 and k_2 . When the systems contained 50% or higher volumes of methanol or dioxane, dye adsorption was virtually eliminated. Starch is known to contain water of hydration as part of its grain structure. It is possible that organic solvents in excess shrink or dehydrate this hydrophilic polymer.

Apparent adsorption, as noted in this study for methylene blue, can also occur with a variety of cationic substances including drugs.

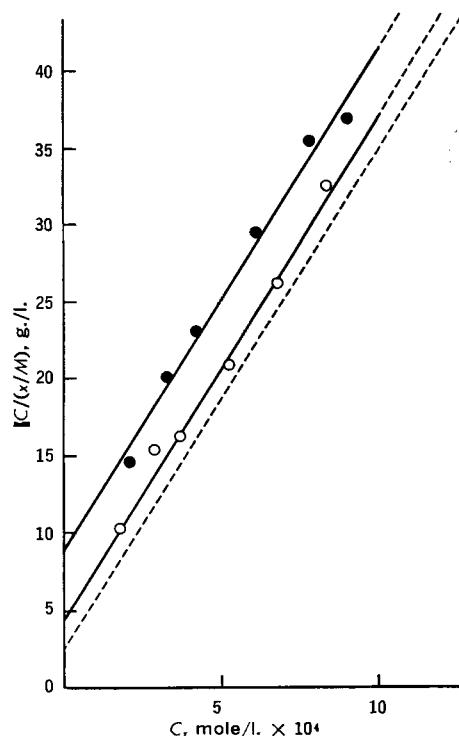


Figure 9—Langmuir plot for adsorption of methylene blue on potato starch from aqueous sucrose solutions. Key: ---, water (dimer range); ○, 0.5 M sucrose; and ●, 1.0 M sucrose.

³ The cooperation of H. M. Rootare of the Department of Dental Materials, University of Michigan, in specific surface area determination is gratefully acknowledged.

In the case of dyes, enhanced adsorption is desirable for uniform coloring of solid dosage forms. However, in the case of drugs, adsorption to a seemingly inert excipient such as starch may affect their release and availability. Problems due to drug adsorption by excipients may also arise during *in vitro* evaluation of the solid dosage forms and during quantitative analysis for the active ingredient. This would be particularly true for potent drugs that have low effective doses. Adsorption studies during the preformulation stages in the development of a drug product can provide clues to such problems.

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Spectrophotometric Determination of Diphenhydramine Hydrochloride in an Antiallergic Cream

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Abstract □ A specific method for the quantitative determination of diphenhydramine hydrochloride in a cream formulation has been developed. The method entails the extraction of diphenhydramine by chloroform, further purification of the extracted base by column chromatography using alginic acid, and its spectrophotometric determination in the eluate at 258 m μ .

Keyphrases □ Diphenhydramine cream—analysis □ Column chromatography—separation □ UV spectrophotometry—analysis

The isolation of a pharmacologically active ingredient from a pharmaceutical formulation containing surfactants is often difficult. Various authors have followed different procedures for the removal of unwanted ingredients. Jones (1) described a method for the determination of diethylstilbestrol in a water-dispersible suppository using column chromatography followed by TLC. Gottlieb (2) used refluxing with an organic solvent to break down the emulsion and subsequently recovered the active drug (which was also diethylstilbestrol) using an aluminum column. More recently,

Forman (3) developed an assay for dienestrol in a cream¹ using urea-inclusion chemistry to remove the excess of monostearin. The information obtained from any of these studies is valuable and may indicate a general approach to analysis employing the two basic steps of extraction and cleanup. However, each of them is a specific case which depends upon the physical and chemical properties of the active component.

Therefore, it is reasonable to assume that different methods of separation may be required for differing formulations containing the same active ingredients and for differing active ingredients contained in similar formulations. Diphenhydramine hydrochloride is found in various combinations in commercially available pharmaceutical preparations, and various assays for its determination have been reviewed (4-6). No procedure, however, has been reported involving the quantification of diphenhydramine hydrochloride in an

¹ The analysis was applied to a product containing 2% of diphenhydramine hydrochloride and marketed as "Allergin Cream."