Adsorption of Cationic (Basic) Dyes by Fixed Yeast Cells

CHARLES H. GILES AND ROBERT B. MCKAY

Department of Pure and Applied Chemistry, The University of Strathclyde, Glasgow, Scotland

Received for publication 19 September 1964

Abstract

Giles, Charles H. (The University of Strathclyde, Glasgow, Scotland), and Robert B. McKay. Adsorption of cationic (basic) dyes by fixed yeast cells. J. Bacteriol. 89:390-397, 1965.—The adsorption of 10 typical cationic dyes on formalin-fixed yeast cells has been studied by determining isotherms, and the results are consistent with an ion-exchange mechanism. The adsorption on this complex substrate is similar to that on the simpler substrate, alumina. The dyes are probably aggregated when adsorbed, and the size of the aggregates increases with increase in the molecular weight of the dye ion. After considering the possible adsorption sites, and comparing the data with adsorption on simpler substrates, we suggest that the most important adsorption sites may be phosphate or other strongly acidic groups.

The solution adsorption mechanisms of a variety of organic solutes on organic and inorganic surfaces have been investigated here in recent years by determining adsorption isotherms. Adsorption from solution is quite distinct from adsorption of gases or vapors. A solution is a phase of at least two components, whereas the gases or vapors used in adsorption tests are usually phases of one component.) An adsorption isotherm is the plot at a given temperature of the weight of solute adsorbed on unit weight of substrate (millimoles per kilogram) against the weight of solute remaining in solution when equilibrium has been established (millimoles per liter).

The substrates so far used have been essentially homogeneous. Some are highly porous, e.g., anodic alumina (Giles et al., 1959), carbons (Giles and Naksha, 1962), and natural fibrous materials (Chipalkatti, Giles, and Vallance, 1954; Giles and Hassan, 1958; Giles et al., 1958), and others are nonporous, e.g., powdered alumina, silica, and graphite (see Giles and Naksha, 1962, and earlier papers in the present series). In all these cases, the same general principles relating the nature of adsorption isotherm to the adsorption mechanism have been found to apply. Usually the nonporous substrates reach equilibrium more rapidly than the porous ones, but, except in a very few cases, the nature of the isotherm appears to be independent of the degree of porosity of the surface.

A system of classification was proposed (Giles et al., 1960) whereby all solution adsorption isotherms are divided into four main classes, according to the shape of the initial part of the curve, and then further into several subgroups. This classification enables the adsorption mechanism to be identified, in many cases, from the isotherm shape. In this paper we shall be concerned with S, L ("Langmuir"), and H ("high affinity") classes of isotherm (Fig. 1). For further details and references, see Giles et al. (1960).

The aim of the present work, which is a quantitative investigation of the adsorption of cationic dyes on formalin-fixed yeast cells, is to determine whether the general principles established for essentially homogeneous substrates can be used to interpret adsorption mechanisms on biological substrates. Adsorption of dyes is the basis of most histochemical techniques; cationic dyes, in particular, are especially important for staining microorganisms.

Briefly, in an aqueous suspension of fixed yeast cells, each cell may be regarded as a highly porous solid particle, ovoid in shape, and of average diameter of about 4 μ. The cell wall, a completely permeable mucopeptide framework, on which are supported other constituents, such as teichoic acids, is about 20% of the dry cell weight (Trevelyan, 1938); the cell membrane, a polysaccharide-protein-lipid complex, is probably <1% of the cell weight and completely per-
meable in fixed cells; the cytoplasm and nucleus, rich in nucleoprotein, together comprise about 89% of the dry cell weight. Clearly, the cytoplasm and nucleus will have the greatest effect on the adsorption properties, whereas the effect of the cell membrane will be almost negligible.

It has long been known that cationic dyes are strongly adsorbed by the dead cells of microorganisms, including yeast. Yeast is a gram-positive organism, and, since cationic dyes are used in the Gram staining test, their adsorption properties in this case have special interest. There is evidence to suggest adsorption depends on the liquid-solid ratio (Borzani and Vairo, 1958; 1959; Vairo and Borzani, 1960; Finkelstein and Bartholomew, 1960) and that the mechanism of adsorption is ion exchange (McCalla, 1940, 1941a, b; Bartholomew, Roberts, and Evans, 1950; James, 1957).

Materials and Methods

Purification of dyes. The dyes used were Crystal Violet (C.I. 42353), Etsyl Violet (C.I. 42600), Magenta P (C.I. 42910), Malachite Green (C.I. 42000), Methylen Blue BP (C.I. 52015), Rhodamine B (C.I. 45170), Rhodamine 3B (C.I. 45175), Safranine (C.I. 50240), Victoria Blue BN (C.I. 44940), and Victoria Pure Blue BO (C.I. 42510). Normal types of cationic dye were used, and all except Victoria Blue BN, Victoria Pure Blue BO, and Rhodamine 3B were purified by leaching diluent-free “batch” grade samples with hot 10% HCl solution, filtering the hot liquor, and allowing the dyes to crystallize from them. The crystallization was repeated at least once, and the crystals were dried first at 40 to 50 C for several hours and then in a vacuum desiccator over potassium hydroxide overnight (high-temperature drying was considered to be undesirable). Victoria Blue BN and Victoria Pure Blue BO could not be purified satisfactorily, because of their low solubility in the acid solution; Rhodamine 3B formed an unfilterable gel with ethanol (probably the best alternative solvent; recrystallization from hydrochloric acid was considered to be undesirable on account of possible hydrolysis of the —CO₂H₃ group); and untreated batch grade (diluent-free) samples were therefore used. The purity of the dye was determined by combustion microanalysis for C, H, and N; by flame analysis (flame spectrophotometer; Evans Electroelenium Ltd.) for Na (a rough measure of salt impurity), and, in the case of Victoria Pure Blue BO and Victoria Blue BN, by potentiometric titration of chloride ion with silver nitrate in neutral solution. All the dyes had only trace amounts of Na; Rhodamine B had no detectable amount (confirmed by ashing; 0.04% ash). The elementary analyses suggested that the impurity present in the recrystallized samples was mainly water, with a little HCl in some cases.

Preparation of dye solutions. Distilled or de-mineralized water was used in all experiments. All the dyes were dissolved cold, except Victoria Blue BN and Victoria Pure Blue BO, which were dissolved with careful warming. In all cases, the solution concentrations were corrected for purity of the samples. All dye solutions were prepared immediately before use, and exposure to light was minimized.

Fig. 1. Three typical solution adsorption isotherms, classified (Giles et al., 1960) as S.2, L.2, and H.2.

Preparation of glassware for dye solutions. Basic dyes are strongly adsorbed by glass. To minimize this effect, all glassware to be used in contact with dye solutions was steeped before use for at least 1 hr (usually overnight) in a solution (1 to 2 g per liter) of a cationic surface-active agent, etyl trimethylammonium bromide, which is preferentially adsorbed. The glass surfaces were then thoroughly rinsed with water before use.

Analysis of dye solutions. The dye solutions were analyzed at the long wavelength absorption peak on a Unicam SP 600 spectrophotometer before and after the adsorption tests.

Tests of stability of basic dyes at elevated temperatures. Some basic dyes in solution are susceptible to decomposition on standing at elevated temperatures (greater than ca. 50 C.). It was found, by observing the optical densities of the present dye solutions before and after rotation in sealed glass tubes at 50 C (highest temperature used in the adsorption tests) in a thermostatically controlled water bath, that no significant change occurred over a period of 2 hr. It was concluded that no significant decomposition had occurred.

Substrate. The substrate used in all the adsorption experiments was brewer’s yeast, a strain of Saccharomyces cerevisiae, obtained from the Distillers Co., Ltd. The yeast was fixed and stored in a 4% formaldehyde solution, such that the yeast to liquid ratio was ca. 1:4 by weight. Preliminary tests showed that the adsorption characteristics of basic dyes on this substrate stored for 18 months were the same as on freshly fixed yeast.

For all the experiments, a small sample of yeast was removed from storage, washed three times, and suspended in distilled or demineralized water.

Standardization of yeast suspension. A yeast
suspension (5 ml) was pipetted into a siliceous crucible previously dried to constant weight. The sample was carefully evaporated to dryness at 120°C. The dry weight of yeast, and hence the concentration of the suspension, was then found. The experiment was performed in triplicate. A series of dilute suspensions was prepared from the original, and their optical densities were measured at a suitable wavelength (5,000 Å, arbitrarily chosen). A calibration graph of optical density against yeast suspension concentration (dry weight basis) was then drawn for use in subsequent experiments.

**Adsorption procedure.** For determining adsorption isotherms, a series of standard dye solutions was prepared and 5-ml samples of each were mixed with 5-ml samples of a standard yeast suspension in oven-dried test tubes. The tubes were sealed in a Bunsen flame and rotated mechanically at ca. 35 rev/min in a thermostatically controlled water bath for 2 hr. The tubes were then removed, broken, and the contents were centrifuged. The supernatant solutions were decanted for analysis.

**Preliminary experiments (Table 1).** The adsorption of the basic dyes used was found to be 80 to 90% complete in 2 to 3 min; the remaining adsorption in some cases required up to 1 hr (rate curves of adsorption, 1963). In the isotherm determinations, the solutions were therefore agitated for at least 2 hr.

Isotherms for several of the dyes used were determined (i) over a wide range of solid-liquid ratio, i.e., yeast suspension concentrations, buffered; (ii) at several temperatures, unbuffered; and (iii) in systems buffered at pH 9. Although the amount of dye adsorbed was dependent under the foregoing conditions (the higher the solid-liquid ratio (being high pH values), on the temperature, and on the pH of the test solution, the shapes of the isotherms were, in all cases, found to be independent of these variables.

Further, a few control experiments were performed by use of unfixed yeast cells (the live proportion of the cells being killed during the experiment by the dyes) and on yeast cells fixed by boiling a concentrated aqueous suspension for 20 min. The shapes of the isotherms obtained for the adsorption of the dyes on these substrates were essentially the same as those on the formalin-fixed cells.

The above experiments show that the type of isotherm is determined by the adsorption on a basic dye on yeast cells is a characteristic of the yeast and is unaffected by the conditions of the experiment and by the pretreatment given to the yeast.

**pH value of adsorption solutions.** The pH values of the equilibrium solutions corresponding to the beginning of the isotherm plateau lay within the narrow range 4.42 to 5.10, with one exception: Rhodamine B, pH 3.63. The concentration of this dye was higher than that of the others (Fig. 2). It was therefore not necessary to buffer the solutions. Buffering would be undesirable because buffer salts would tend to alter the aggregation properties of the dyes in solution, and so perhaps interfere with the adsorption. Moreover, in biological staining, pH control is not normally used.

Some preliminary tests (Table 1) showed that, when the pH is raised as high as 9.0, adsorption is increased, as expected for adsorption at predominately anionic sites.

**Selected conditions for the main series of experiments.** The main series of experiments, the results of which are discussed below, were performed under the following conditions: The solid-liquid ratio was maintained at the same value for all the isotherm determinations on yeast. This was achieved by using yeast suspensions of concentration 0.246 ± 0.006 g per liter in all the test mixtures. The tests at room temperature were controlled at 20 ± 0.5°C; those at elevated temperatures were controlled at 50 ± 1°C. As stated

### Table 1. Preliminary isotherm determinations with yeast cells subjected to various treatments, and at various suspension concentrations

<table>
<thead>
<tr>
<th>Dye</th>
<th>Yeast suspension conc</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Isotherm type</th>
<th>Maximal adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/liter)</td>
<td>(g/l)</td>
<td>(°C)</td>
<td></td>
<td>(mg/liter)</td>
</tr>
<tr>
<td>Crystal</td>
<td>F1</td>
<td>0.110</td>
<td>W</td>
<td>22</td>
<td>L2</td>
</tr>
<tr>
<td>Violet</td>
<td>F1</td>
<td>0.440</td>
<td>W</td>
<td>39</td>
<td>L2</td>
</tr>
<tr>
<td>F1</td>
<td>0.384</td>
<td>W</td>
<td>30</td>
<td>L2</td>
<td>335</td>
</tr>
<tr>
<td>F1</td>
<td>0.083</td>
<td>B9</td>
<td>20</td>
<td>L1</td>
<td>400</td>
</tr>
<tr>
<td>U</td>
<td>0.100</td>
<td>W</td>
<td>19</td>
<td>L2</td>
<td>370</td>
</tr>
<tr>
<td>U</td>
<td>0.279</td>
<td>W</td>
<td>16</td>
<td>L2</td>
<td>308</td>
</tr>
<tr>
<td>U</td>
<td>0.158</td>
<td>W</td>
<td>40</td>
<td>L2</td>
<td>364</td>
</tr>
<tr>
<td>B</td>
<td>0.241</td>
<td>W</td>
<td>20</td>
<td>L2</td>
<td>230</td>
</tr>
<tr>
<td>B</td>
<td>0.341</td>
<td>W</td>
<td>50</td>
<td>L2</td>
<td>230</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>F2</td>
<td>0.312</td>
<td>W</td>
<td>17</td>
<td>L2</td>
</tr>
<tr>
<td>BP</td>
<td>F2</td>
<td>0.184</td>
<td>W</td>
<td>21</td>
<td>L2</td>
</tr>
<tr>
<td>F2</td>
<td>0.222</td>
<td>W</td>
<td>49</td>
<td>L2</td>
<td>272</td>
</tr>
<tr>
<td>F1</td>
<td>0.208</td>
<td>W</td>
<td>45</td>
<td>L2</td>
<td>312</td>
</tr>
<tr>
<td>F1</td>
<td>0.213</td>
<td>B9</td>
<td>23</td>
<td>L2</td>
<td>354</td>
</tr>
<tr>
<td>F1</td>
<td>0.321</td>
<td>W</td>
<td>18</td>
<td>L2</td>
<td>194</td>
</tr>
<tr>
<td>F1</td>
<td>0.391</td>
<td>W</td>
<td>17</td>
<td>L2</td>
<td>133</td>
</tr>
<tr>
<td>B</td>
<td>0.475</td>
<td>W</td>
<td>49</td>
<td>L2</td>
<td>171</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>F1</td>
<td>0.151</td>
<td>W</td>
<td>14</td>
<td>L2</td>
</tr>
<tr>
<td>Victoria</td>
<td>U</td>
<td>0.175</td>
<td>W</td>
<td>19</td>
<td>H2</td>
</tr>
<tr>
<td>Pure Blue</td>
<td>U</td>
<td>0.208</td>
<td>W</td>
<td>40</td>
<td>H2</td>
</tr>
</tbody>
</table>

* The following symbols are used: F1, formalin-fixed yeast cells (sample 1); F2, formalin-fixed yeast cells (sample 2); U, untreated yeast cells; B, boiled (20 min) yeast cells.

* W, water; B9, buffer solution at pH 9.

* See Giles et al., 1960.

* Maximal adsorption not reached.
previously, all the tests on yeast were performed within the pH limits of 4.42 to 5.10 (except with Rhodamine B).

Reproducibility of results. The isotherms determined at room temperature by use of suspensions prepared from any one formalin-fixed yeast sample could be reproduced to within ca. ±5% accuracy (estimated at the plateau). The margin of error in the isotherms at 50°C was found to be greater than this, probably owing to a fall in temperature between the removal of the tubes from the thermostat bath and the end of the centrifuging step. There are two main types of error: (i) those affecting the whole isotherm, i.e., making all points too high or too low, and (ii) those affecting isolated points. The former includes errors in the suspension analysis, which involves reading from an experimentally determined graph; the latter includes errors in pipetting samples of yeast suspensions and errors due to incomplete separation in centrifuging. Bad centrifuging, however, was found to produce very serious and obvious errors which could be eliminated by careful manipulation.

The adsorption properties of formalin-fixed samples of yeast remained constant over long periods of time.

Adsorption experiments on other substrates. A few adsorption tests were made on other substrates, i.e., silk and wool (cleaned and scoured fabrics; wool, specific surface area, 56.6 m²/g, by p-nitrophenol from water (Giles and Nakha, 1962); silk, not determined), graphite (Acheson Colloids Ltd., 0.28% ash, specific surface area by nitrogen adsorption, 125 m²/g), chromatographic alumina (May and Baker, specific surface area, 4.6 m²/g), and deoxyribonucleic acid (DNA). In the latter case, 5-ml portions of a 0.4% aqueous solution of the sodium salt of DNA (L. Light and Co.) were acidified with 0.1 ml of 50% (v/v) hydrochloric acid. The free DNA was thus precipitated out; the final pH of the suspension was 2.5. To each 5-ml portion thus treated, a 5-ml portion of standard dye solution was added, and the mixtures were rotated mechanically for 24 hr in the thermostatic bath at 20°C. The supernatant liquor was separated by centrifuging and analyzed spectrophotometrically.

RESULTS AND DISCUSSION

The results of the main series of experiments are given in Table 2 and Fig. 2. The extremely high rate of adsorption is in accordance with the ion-exchange mechanism suggested by other authors (McCalla, 1940, 1941a; b; Bartholomew, Roberts, and Evans, 1950; James, 1957). Also, it is well known that yeast cells are stained throughout by cationic dyes, and the present results are consistent with this fact.

Adsorption isotherms on yeast (Fig. 2). Crystal Violet, Magenta P, Malachite Green, Methylene Blue BP, Rhodamine 3B, and Safranine give isotherms of type L (Giles et al., 1960) and thus show "normal" Langmuir adsorption (see Borzani and Vairo, 1958, 1959; Vairo and Borzani, 1960; James, 1957). Victoria Blue BO, Victoria Blue BN, and Ethyl Violet give isotherms of type H. These three dyes are completely removed from dilute solutions by the yeast and clearly have a high affinity for it. Rhodamine B on yeast gives an isotherm of type S (Fig. 2H); this anomalous behavior is discussed below.

Adsorption of the rhodamines on yeast. The adsorption of Rhodamine B (Fig. 2H) and Rhodamine 3B (Fig. 2G) is similar to their adsorption on chromatographic alumina, where they were also adsorbed by ion exchange and gave S- and L-type isotherms (Fig. 4E), respect-

<table>
<thead>
<tr>
<th>Table 2. Adsorption data for cationic dyes on yeast</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Dye</th>
<th>Isotherm type</th>
<th>Probable orientation</th>
<th>Maximal equilibrium adsorption</th>
<th>Projected area of dye adsorption</th>
<th>Calculated specific surface area</th>
<th>Coverage factor</th>
<th>Cationic wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mmoles/kg</td>
<td>A²</td>
<td>m²/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>L2</td>
<td>Aggregated</td>
<td>264</td>
<td>224</td>
<td>356</td>
<td>5.41</td>
<td>372</td>
</tr>
<tr>
<td>Ethyl Violet</td>
<td>H2</td>
<td>Aggregated</td>
<td>316</td>
<td>206</td>
<td>509</td>
<td>7.75</td>
<td>456</td>
</tr>
<tr>
<td>Magenta P</td>
<td>L2</td>
<td>Aggregated</td>
<td>103</td>
<td>168</td>
<td>115</td>
<td>2.51</td>
<td>302</td>
</tr>
<tr>
<td>Malachite Green</td>
<td>L2</td>
<td>Flat</td>
<td>61</td>
<td>181</td>
<td>66.5</td>
<td>1.01</td>
<td>329</td>
</tr>
<tr>
<td>Methylene Blue BP</td>
<td>L2</td>
<td>Aggregated</td>
<td>153</td>
<td>120</td>
<td>111</td>
<td>1.89</td>
<td>284</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>S2</td>
<td>Edge-on</td>
<td>88</td>
<td>124</td>
<td>65.7</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>Rhodamine 3B</td>
<td>L2</td>
<td>Aggregated</td>
<td>ca. 130</td>
<td>184</td>
<td>144</td>
<td>2.19</td>
<td>471</td>
</tr>
<tr>
<td>Safranine</td>
<td>L2</td>
<td>Aggregated</td>
<td>176</td>
<td>148</td>
<td>157</td>
<td>2.39</td>
<td>315</td>
</tr>
<tr>
<td>Victoria Blue BN</td>
<td>H2</td>
<td>Aggregated</td>
<td>300</td>
<td>251</td>
<td>453</td>
<td>6.80</td>
<td>470</td>
</tr>
<tr>
<td>Victoria Pure Blue BO</td>
<td>H2</td>
<td>Aggregated</td>
<td>308</td>
<td>268</td>
<td>505</td>
<td>9.00</td>
<td>478</td>
</tr>
</tbody>
</table>

* See Giles et al. (1960).
† Area of smallest enclosing rectangle (in most probable orientation).
‡ Ratio of maximal amount of dye adsorbed to calculated monolayer capacity (for stated probable orientation); see text.
 approach with a vertical orientation. At very low concentrations, the dye is scarcely adsorbed, but, as the concentration is increased, the dye is forced to the surface of the alumina by the internal pressure of the solution, and the adsorption is aided by interaction between adjacent vertically oriented adsorbed molecules. The fully esterified Rhodamine 3B, which is not dipolar ionic in character, has a higher affinity for the substrate and the adsorption follows the normal L-type isotherm. The fact that these two dyes, indeed, also the other dyes, behave the same way on yeast as on alumina suggests that the complex structure of yeast and the dipolar ionic character of many of the constituent macromolecules have little effect on the adsorption mechanism, and that the isotherms on yeast may be interpreted in the same way as has been described for the isotherms on alumina. The following section lends further support to this view.

Aggregation of cationic dyes adsorbed by ion exchange. It has previously been shown that cationic dyes are aggregated when adsorbed by ion exchange on chromatographic alumina (Giles, Easton, and McKay, 1964). An estimate of the size of the aggregates of each dye was made by calculating the coverage factor: the ratio of the specific surface area of the substrate estimated from the dye adsorption (the product of the amount of dye adsorbed at the isotherm plateau, the projected area of the dye molecule at the surface, and the Avogadro number) to the true specific surface area (estimated by nitrogen adsorption and by the adsorption of well-characterized solutes which give S isotherms). For simplicity, the dye molecules were assumed to be in their most probable orientation, i.e., lying flat on the surface, and the projected area of each molecule was estimated from Catalin molecular scale models. It was shown that the coverage factor was a linear function of the logarithm of the molecular weight of the dye cation. [This relationship has been dealt with more fully in recent work (Easton, Giles, and McKay, 1964). Coverage factors of monovalent dyes (both anionic and cationic) on a variety of simple substrates vary linearly with the logarithm of the molecular weight of the dye ion. Further, all the plots can be superimposed to give a line of high statistical significance. The present results with yeast conform to this general relationship.] In other words, the size of the adsorbed aggregates increased with the size of the dye cation.

With yeast, although the concept of a specific surface area is difficult to comprehend fully, it is significant that a similar relationship can be demonstrated (Table 2, Fig. 3). The "specific
surface area” of the yeast (65.7 m²/g, Table 2) is estimated from the adsorption of Rhodamine B, which gives an S-type isotherm on yeast, as on alumina, where it forms a close-packed monolayer of vertically oriented molecules (Giles, Easton, and McKay, 1964), as do all solutes giving an isotherm of this type (Giles et al., 1960). The data for the other dyes are given in Table 2. It may reasonably be concluded that cationic dyes are aggregated when adsorbed on yeast. The possible effect of aggregation on staining processes has been discussed in a previous paper which is intended for physical chemists (McKay, 1963).

Malachite Green and Rhodamine 3B are exceptional in that they give much lower coverage factors than would be expected from the molecular weights of their cations. They are not included in Fig. 3. They are also exceptional, however, in that the ionic charge distribution (Lewis et al., 1943) is highly unsymmetrical about an axis perpendicular to the plane of the molecule, and it may be that this reduces their ability to form aggregates. It remains possible, though, that they are involved in some specific interaction with yeast.

The dyes which have the largest coverage factors, i.e., Ethyl Violet, Victoria Pure Blue BO, and Victoria Blue BN, give isotherms of type H2 which have been observed in several other systems where large ionic micelles are adsorbed (Giles et al., 1960).

Nature of the adsorption sites. On a substrate of such complex chemical constitution as yeast, the dye cations are clearly subjected to many types of attractive forces. Nevertheless, the present results and those of other authors (McCalla, 1940, 1941a, b; Bartholomew, Roberts, and Evans, 1950; James, 1957) suggest that the adsorption mechanism is predominantly ionic in character. This implies that the most probable adsorption sites are carboxyl groups in polypeptide side chains, phosphoric acid residues in nucleic acids (and perhaps teichoic acids), and perhaps even sulfonyl residues in polysaccharides. It seems reasonable to suppose that the powerful attraction of the strongly acidic phosphoric acid residues will predominate over that of the weakly acidic carboxyl groups.

There is convincing experimental evidence from histochemical studies of sectioned animal cells (Baker, 1962) that the nucleic acids in the cytoplasm (RNA) and especially the nuclei (DNA) are strongly stained by basic dyes, much more so than the other cellular parts when diluted dye solutions are used (with concentrated solutions, other substances such as acidic proteins and acidic mucopolysaccharides are often strongly stained as well). If this is so in yeast cells (it should be remembered that in yeast cells, the nucleic acid-rich nucleus and cytoplasm together comprise about 80% of the dry cell weight), then the effect of the phosphoric acid residues will have the greatest influence on the overall adsorption characteristics and thus on the shapes of the adsorption isotherms. To give experimental support to this view, a study of the adsorption of Rhodamine B on DNA and various other selected substrates was made (Fig. 4).

Adsorption of Rhodamine B on other substrates.

Nature of the adsorption sites. On a substrate of such complex chemical constitution as yeast, the dye cations are clearly subjected to many types of attractive forces. Nevertheless, the present results and those of other authors (McCalla, 1940, 1941a, b; Bartholomew, Roberts, and Evans, 1950; James, 1957) suggest that the adsorption mechanism is predominantly ionic in character. This implies that the most probable adsorption sites are carboxyl groups in polypeptide side chains, phosphoric acid residues in nucleic acids (and perhaps teichoic acids), and perhaps even sulfonyl residues in polysaccharides. It seems reasonable to suppose that the powerful attraction of the strongly acidic phosphoric acid residues will predominate over that of the weakly acidic carboxyl groups.

There is convincing experimental evidence from histochemical studies of sectioned animal cells (Baker, 1962) that the nucleic acids in the cytoplasm (RNA) and especially the nuclei (DNA) are strongly stained by basic dyes, much more so than the other cellular parts when diluted dye solutions are used (with concentrated solutions, other substances such as acidic proteins and acidic mucopolysaccharides are often strongly stained as well). If this is so in yeast cells (it should be remembered that in yeast cells, the nucleic acid-rich nucleus and cytoplasm together comprise about 80% of the dry cell weight), then the effect of the phosphoric acid residues will have the greatest influence on the overall adsorption characteristics and thus on the shapes of the adsorption isotherms. To give experimental support to this view, a study of the adsorption of Rhodamine B on DNA and various other selected substrates was made (Fig. 4).
On wool and silk, isotherms of type L2 are obtained (Fig. 4A, B, C), and, on graphite, one of type H2. The amount of adsorption on graphite corresponds very closely to a monolayer of dye cations oriented parallel to the surface (area of the dye molecule flat is ca. 230 Å², as estimated from the model). On chromatographic alumina, however, an S2-type isotherm (Fig. 4E) is obtained. This indicates a vertical orientation of the dye cations (cross-sectional area, 124 Å²), and the amount of adsorption does, in fact, correspond to a close-packed monolayer so oriented.

On substrates with a high proportion of hydrocarbon residues, e.g., protein fibers, or with condensed aromatic systems, e.g., graphite, adsorption is due largely to van der Waal's attraction between the mainly hydrophobic surface and the aromatic ring system of the dye ions (Chipalkatti, Giles, and Vallance, 1964; Vickerstaff, 1954). This mechanism favors flatwise orientation (Fig. 5), and so accounts for the L-type isotherm. On chromatographic alumina, which has a negatively charged ionic surface and no hydrocarbon residues or aromatic systems, ion-ion attraction is predominant, and the vertical orientation is favored, as discussed above and by Giles, Easton, and McKay (1964).

On the above argument it is indeed unlikely that the principal adsorption sites in yeast cells are located in the protein constituents; they are more likely to be in the strongly acidic nucleic acids, or in teichoic acids. To check this view, the isotherm for Rhodamine B on solid DNA was determined. This isotherm (Fig. 4F) is, in fact, of the S-type, like that on yeast, and quite unlike that obtained with the protein fibers. (Isotherms for wool were determined by use of both unacidified dye solutions, for comparison with the yeast experiment, and solutions acidified to pH 2.5 for comparison with the DNA experiment. There is no difference in isotherm type, though; as expected, adsorption is less from the acid solution, because of the increased positive charge on the fibers. Note that the scales on the X and Y axes of the isotherm on DNA are both 100X.

![Fig. 5. Suggested orientation of Rhodamine B cations on graphite or protein surfaces. Here the surface attracts preferentially the aromatic nuclei of the dye cations, and thus there is flatwise orientation.](http://jb.asm.org/)

There is evidence that RNA is less permeable to cationic dyes than DNA (Goldstein, 1961). With a mixture of two cationic dyes, one of low and the other of high molecular weight, DNA is stained by both dyes, but RNA is preferentially stained by the dye of low cationic weight. In the present experiments with yeast, however, the dyes are applied separately and competition for the substrate does not occur. There is therefore no reason to suppose that the lower permeability of RNA will prevent its being stained here. Indeed, there is evidence that the very large anionic dye, Methyl Blue (anionic weight, 745), which stains chromatin strongly (Baker, 1962), reacts with both the DNA and RNA constituents (by a nonionic mechanism) in preference to the protein constituent (McKay, 1962). It is clear that this form of RNA at least is permeable to much larger molecules than those used in the present study.

Effect of temperature. Adsorption is essentially an exothermic process and hence should be favored by low temperatures. If, however, the heat of adsorption is low (e.g., in ion exchange or van der Waal's adsorption), then the effect of temperature is less marked. For example, it has been shown that the ion-exchange adsorption of anionic dyes by acidified alumina (Cummings et al., 1959) and by proteins (Vickerstaff, 1954; Kiotta and Urquart, 1949) is virtually unaffected by substantial changes in the ambient temperature.

Isotherms for several of the dyes used have been determined at two temperatures, i.e., 20 and 50°C (Fig. 3). In each case, the adsorption is anomalous in being greater at high than at low temperature. This type of anomalous adsorption has been observed with some inorganic substrates and appears to be due to aggregation of the dyes in solution (Giles, Nakhwa, and Greezek, 1961; Giles, Easton, and McKay, 1964).

It is well known that high temperatures favor the disaggregation of dye molecules in solution (Vickerstaff, 1954), and it has been suggested (Giles, Nakhwa, and Greezek, 1961) that the dye molecules are reaggregated on adsorption. In the present case, in which the adsorption is extremely rapid and occurs throughout the highly porous substrate, the monodisperse dye cations will tend to be preferentially withdrawn from solution, since their smaller size will enable them to pass more easily and more quickly than micelles through the pores. The action of high temperature will accentuate this effect by greatly increasing the proportion of the monodisperse form in
solution. Nevertheless, the high coverage factors show that at equilibrium the species present at the adsorption sites is a micellar form. It is therefore suggested that aggregation of the monodisperse dye cations to form ionic micelles occurs at the moment of close approach to the adsorption sites. Such aggregation will be favored by the increased dye concentration near the sites, and perhaps by loss of solvated water.

Acknowledgments

We thank P. D. Ritchie for his interest; Allied Colloids (Bradford) Ltd., Badische Anilin-und Soda-Fabrik A. G., L. B. Holliday and Co. Ltd., and Imperial Chemical Industries Ltd., Dyestuffs Division, for the gift of dyes; The Distillers Co. Ltd., for gifts of yeast; the Department of Scientific and Industrial Research for a Scholarship (to R. B. McK.); A. C. Syme for analyzing the purified dye samples; and I. A. Easton and A. H. Tolia for some of the tests illustrated in Fig. 4.

Literature Cited


McCalla, T. M. 1941a. The adsorption of hydrogen ions by bacteria as measured by the glass electrode. J. Bacteriol. 41:775-783.


