Effect of pH, Urea, EDTA & Deoxycholate on Metachromasia Induced by Heteropolyanions

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Phosphomolybdic acid, phosphotungstic acid and silicotungstic acid as chromotropes, have been found to induce metachromatic effect in toluidine blue. These chromotropes produce stronger metachromatic effect than that by some well known chromotropes including isopolyacids excepting inorganic polyphosphates. The metachromatic effect increases with increasing [heteropolyanion]l[dye] ratio. The formation of the metachromatic complexes has been found to be suppressed by urea, EDTA and deoxycholate. Amongst these, EDTA is the most potent reagent followed by deoxycholate and urea. Effect of pH on the metachromatic effect has also been studied.

Metachromasia produced in toluidine blue by various types of chromotropes including inorganic isopolyacids and polyphosphates has been studied earlier. Metachromatic property of this dye with heteropolyanions has been also reported. The present investigation deals with the effect of pH, urea, EDTA and deoxycholate on the metachromatic complexes formed by toluidine blue with phosphomolybdic acid (PMA), phosphotungstic acid (PTA) and silicotungstic acid (STA).

Materials and Methods
PTA, STA, PMA (E. Merck), urea (BDH), EDTA and deoxycholate (IDPL) were used as such without further purification. Toluidine blue (E. Merck) was purified by a known method and its purity was checked chromatographically.

An aqueous solution containing a heteropolyanion (PTA, PMA or STA) was added to 3 ml of the dye solution (3.3 \times 10^{-4} M). The total volume of the solution was made up to 7.0 ml by the addition of doubly distilled water. At higher ratio of heteropolyanion to dye, the complex tended to change gradually from blue to pink-violet. All the observations were made immediately after preparing the solution and a fixed dye concentration was used in all the experiments. For studying the effect of pH, urea, EDTA and deoxycholate on metachromatic complex, the dye and heteropolyanion concentrations corresponding to maximum complex formation were taken. To this was added a definite amount of urea (0.05 M, 0.1 M) or EDTA (0.5 M) or deoxycholate (0.5 M) and the final volume was made up to 7.0 ml with doubly distilled water. The spectra were recorded using a Perkin-Elmer spectrophotometer model-24.

Results and Discussion

The visible spectra of the dye, STA-dye, PMA-dye and PTA-dye complexes are shown in Fig. 1. For a given dye concentration of 1.4 \times 10^{-4} M, the concentrations of heteropolyanions mentioned in the caption to Fig. 1 give maximum spectral shifts. Further increase in the concentrations of heteropolyanions does not bring about any further spectral shifts.

It is evident that the heteropolyanions STA, PMA and PTA are effective in inducing metachromasia in toluidine blue, the effect being hypochromicity coupled with hypsochromic shifts.

The effect of pH on metachromasia induced by STA, PMA and PTA is presented in Table 1. It is evident that the maximum metachromatic effect for PTA and STA is induced at the pH 5.6 whereas the pH value of 4.6 induces maximum metachromatic effect in the case of PMA.

Fig. 2 shows the urea titration profiles of the metachromatic complexes of PMA, PTA and STA with toluidine blue. The metachromatic complexes with PTA and PMA are completely suppressed by 5 \times 10^{-2} M and 4.2 \times 10^{-3} M urea respectively whereas 5 \times 10^{-2} M urea is not sufficient to suppress the metachromatic complex formed by STA. This indicates that STA-dye complex is the most stable against urea followed by PTA-dye and PMA-dye complexes in that order.

The urea titration curves in Fig. 2 depict three
Fig. 1—Visible spectra of (1) toluidine blue in water (1.4 x 10^-4 M), (2) STA (1 x 10^-6 M) + toluidine blue, (3) PMA (1.5 x 10^-5 M) + toluidine blue, (4) PTA (9.7 x 10^-6 M) + toluidine blue

Fig. 2—Urea titration of metachromatic complexes of toluidine blue and heteropolyanions [-O- STA; - ▲ - PTA; - ● - PMA]

Fig. 3—EDTA titration of metachromatic complexes of toluidine blue and heteropolyanions [-O- STA; - ● - PTA; - ▲ - PMA]

Table 1—Effect of pH on Toluidine Blue
d Metachromasia
Induced by Heteropolyanions in the pH Range 3.6-7.0

<table>
<thead>
<tr>
<th>Chromotropes</th>
<th>Conc. of chrom trope (M)</th>
<th>Buffer</th>
<th>pH Shift (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphotungstic acid</td>
<td>9.7 x 10^-6</td>
<td>Acetate 3.6</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetate 4.6</td>
<td>42</td>
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<td></td>
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<td>Acetate 5.6</td>
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<tr>
<td></td>
<td></td>
<td>Phosphate 7.0</td>
<td>43</td>
</tr>
<tr>
<td>Silicotungstic acid</td>
<td>1.0 x 10^-6</td>
<td>Acetate 3.6</td>
<td>48</td>
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<td></td>
<td></td>
<td>Acetate 4.6</td>
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<td></td>
<td>Acetate 5.6</td>
<td>50</td>
</tr>
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<td></td>
<td></td>
<td>Phosphate 7.0</td>
<td>40</td>
</tr>
<tr>
<td>Phosphomolybdic acid</td>
<td>1.5 x 10^-5</td>
<td>Acetate 3.6</td>
<td>35</td>
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<tr>
<td></td>
<td></td>
<td>Acetate 4.6</td>
<td>43</td>
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<tr>
<td></td>
<td></td>
<td>Acetate 5.6</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phosphate 7.0</td>
<td>40</td>
</tr>
</tbody>
</table>

*Phosphotungstic acid, PTA; silicotungstic acid, STA; phosphomolybdic acid, PMA.
Acetate buffer: acetic acid/sodium acetate buffer = 0.01 M.
Phosphate buffer: disodium hydrogenphosphate/sodium hydrogen phosphate = 0.01 M.
[Toluidine blue] = 1.4 x 10^-4 M.

sections in the case of STA-dye and PTA-dye complexes implying three types of bindings between the dye and the heteropolyanions. However, the metachromatic complex formed by the interactions of dye and PMA is too weak to demonstrate the three sections; only two sections are observed in this case.

Fig. 3 shows EDTA titration profiles of the metachromatic complexes of PMA, PTA and STA with toluidine blue. The EDTA titration profile of STA-dye complex depicts three sections implying three types of bindings between the heteropolyanion and the dye. PMA- and PTA-dye complexes are very sensitive towards the suppression by EDTA and only two sections are observed in the titration curves of their complexes.

The titration profiles of deoxycholate with PTA-dye and STA-dye complexes are shown in Fig. 4. In this case also, STA-dye complex exhibits three sections in the titration curve indicating three types of binding between the dye and the heteropolyanion. PTA-dye complex depicts only two sections indicating its sensitivity towards suppression by deoxycholate. PMA-dye metachromatic complex is too sensitive to demonstrate the titration curve.

The metachromatic shifts observed in these studies are of higher order than those previously reported for several other inducers1-3,8,9.

The suppression of metachromatic reactions by
urea is not due to disruption of the hydrogen bonds only which may or may not be involved in the formation of metachromatic complexes; the primary forces responsible for inducing metachromasia are electrostatic in nature\textsuperscript{1-3}. The non-ionic/hydrophobic forces are likely to get involved in this phenomenon subsequently. The instability of these metachromatic complexes towards urea may be interpreted to mean that hydrophobic forces are playing a cardinal role in this phenomenon and once they get weakened by urea, the primary ionic forces are not able to maintain metachromasia.

EDTA dissociates the metachromatic complexes formed by the macroionic interactions of heteropolyanions and toluidine blue. It may be inferred, in analogy to behaviour of urea, that EDTA also acts by suppressing both ionic and hydrophobic forces involved. This property of EDTA is unrelated to its chelating action\textsuperscript{10}.

Deoxycholate also suppresses the metachromasia induced by heteropolyanions. This anionic detergent has already been shown\textsuperscript{11} to dissociate a large variety of binary macroionic complexes including the suppression of metachromasia. It is more potent in comparison to urea. Such selective action against metachromasia has not been reported hitherto and such studies may have analytical potential of differentiating biomolecules.

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