A study on equilibrium and kinetics of carbocation-to-carbinol conversion for di- and tri- arylmethane dye cations in aqueous solutions: Relative stabilities of dye carbocations and mechanism of dye carbinol formation

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Arylmethane dye cations form a structurally interesting set of stable carbocations. A detailed study on rate-equilibria of carbinol formation from two diarylmethane and nine triarylmethane dye carbocations in aqueous solutions has been carried out using spectrophotometric measurements. The conclusions reached are: (i) The stability order found (auramine O > crystal violet > methyl violet > victoria blue R > victoria pure blue BO > ethyl violet > pararosaniline > brilliant green > malachite green > carbocation form of Michler’s hydrol > methyl green), seems to be determined by an interplay of dye carbocation / carbinol conformation and stereoelectronic effects of substituents; and (ii) carbinol formation is general base catalysed and occurs by the rate determining attack of a H₂O molecule on the dye carbocation centre via two kinetic pathways one mediated by another H₂O molecule and the other by a OH⁻ ion.

Rate-equilibrium parameters and structure-reactivity correlations for carbocation-to-carbinol conversion provide a valuable basis for the analysis of mechanisms of reactions involving carbocation intermediates. One structurally interesting class of carbocations is provided by 4-amino or alkylamino arylmethane dye cations. Highly electron donating 4-aminoaryl groups endow a methyl cation remarkable stability in aqueous solutions. Further, steric crowding by aryl groups around the central carbon makes reactions of these methyl cations with a nucleophile considerably slower than those of most of other cations of similar stability. In fact, the rate of conversion of 4-amino or alkylamino di- or tri-arylmethane dye carbocation to the carbinol is sufficiently slow for monitoring the kinetics by conventional spectrophotometric techniques. Moreover, a significant number of structural effects due to carbocation structure, stereoelectronic influences of aryl ring substituents, hydration of the substituent and the carbocation centre etc. are encountered in the reaction. Arylmethane dye cations are well-known reagents for solvent extraction-spectrophotometric determination of a number of metals and non-metals. Their carbinol forms are sufficiently basic and potential reference bases for studying proton transfer from various acids in apolar aprotic solvents. Despite such importance no detailed study on rate-equilibrium of arylmethane dye carbocation-to-carbinol conversion in aqueous media was reported excepting the instances of crystal violet and malachite green and of some preliminary studies on carbinal formation from several such dyes; whereas carbinal formation from various other carbocations has been the subject of many systematic investigations. Towards bridging this gap a detailed investigation on rate-equilibrium of dye carbocation-to-carbinol conversion in aqueous solutions was undertaken with the objectives of determining stability scales, structure-reactivity relationships and carbinal-formation mechanisms for a set of eleven di-/tri-arylmethane dye cations.

The selected dye cations are: Carbocation form of Michler’s hydrol (MH; 4,4’-bis NMe₂ Ph₂C⁺H), auramine O (AR; 4,4’-bis NMe₂ Ph₂C⁺NH₂), pararosaniline (PR; 4,4’,4”-tris NH₄ Ph₃C⁺), malachite green (MG; 4,4’-bis NMe₂ Ph₃C⁺), brilliant green (BG; 4,4’-bis NEt₂ Ph₃C⁺), methyl violet (MV; 4,4’-bis NMe₂ - 4’”,NHMe Ph₃C⁺), crystal violet (CV; 4,4’,4”-tris NMe₂ Ph₃C⁺), methyl green (MG; 4,4’-bis NEt₂ - 4’”,NHMe Ph₃C⁺), ethyl violet (EV; 4,4’,4”-tris NEt₂ Ph₃C⁺), victoria blue R (VBR; 4,4’-bis NMe₂ Ph₄-4’”,NHEt 1-NaphC⁺), and victoria pure blue BO (VBP; 4,4’-bis NEt₂ Ph₄-4’”,NHEt 1-NaphC⁺).
Materials and Methods

The di- and tri- arylmethane dye samples employed were from Aldrich, BDH, E. Merck or Sigma. Ten of the eleven samples were obtained in their cationic (R\(^+\)) form viz. auramine 0, pararosaniline, malachite green, brilliant green, methyl violet, crystal violet, methyl green, ethyl violet, victoria blue R and victoria pure blue BO. However, the remaining sample was obtained in its colourless carbinol (ROH) from Michler's hydro 1. It was completely converted to its coloured cationic form (MH) by dissolving in a HCl solution of pH ~5 and adjusting the pH for the maximum absorbance of the cationic form of Michler’s hydroxyl (MH) at its \(\lambda_{\text{max}}\) 604 nm.

The buffers used for carbocation-carbinol conversion for the above dyes were phosphate (KH\(_2\)PO\(_4\)-NaOH) buffers for pH, 5.50-9.00 and borate (Na\(_2\)B\(_4\)O\(_7\)-NaOH) buffers for pH, 9.20-11.60. pH was measured in a 335 Systronics digital pH-meter.

Equilibrium and kinetics of carbocation-to-carbinol conversion for a dye cation (R\(^+\)) were studied by measuring absorbances of the dye cation in buffer solutions of appropriate pH range each maintained at the ionic strength of 0.01 M, at 35.0 ± 0.1°C for an initial dye cation (R\(^+\)) concentration (~4 \(\times\) 10\(^{-5}\)M) corresponding to the absorbance of 1 Abs. at its measuring wave length (\(\lambda_{\text{max}}\)) in a 160 A Shimadzu UV-vis recording spectrophotometer with cuvet thermostatting facility. The absorbance of a dye cation (R\(^+\)) falls with increase in pH due to increase in conversion to the colourless carbinol (ROH). The initial absorbance (Ao) for the calculation of equilibrium and rate constants (Eqs 3 and 6) was then the limiting maximum absorbances at its \(\lambda_{\text{max}}\) on lowering the pH of the medium. The pHs chosen for the study were not on the strongly acidic side so that R\(^+\)-ROH equilibration was freed from participation of species like RH\(^2+\), ROH\(^+\). Absorbances were measured at 604 nm for MH, 540 nm for PR, 615 nm for MG, 630 nm for BG, 580 nm for MV, 590 nm for CV, 632 nm for MeG, 595 nm for EV, 610 nm for VBR and 615 nm for VBP. However, for AR which undergoes conversion to both carbinol and imine forms, measurements of absorbance were made simultaneously at three wavelengths : 315 nm, 370 nm and 430 nm corresponding to imine form, carbinol form and cationic form of the dye AR respectively (discussed in Appendix). The molar absorption coefficients (10\(^4\) dm\(^3\) mol\(^{-1}\) cm\(^{-1}\)) for the dyes were : 9.53 for MH, 2.86 for AR, 6.46 for PR, 6.24 for MG, 5.07 for BG, 5.46 for MV, 5.19 for CV, 6.13 for MeG, 6.03 for EV, 5.27 for VBR and 2.70 for VBP at their respective \(\lambda_{\text{max}}\).

Results and Discussion

Dye carbocation – carbinol equilibration

The equilibrium constant \(K_{\text{eq}}\) for the dye carbocation (R\(^+\))-to-carbinol (ROH) conversion in aqueous solutions:

\[
R^+ + H_2O \rightleftharpoons ROH + H^+ \quad \ldots (1)
\]

\[
K_{\text{eq}} = \frac{[ROH][H^+]}{[R^+]^2} \quad \ldots (2)
\]

where the activity of water being defined as unity in dilute aqueous solutions was determined as:

\[
pK_{\text{eq}} = pH + \log \left( \frac{A_R}{A_H - A_L} \right) \quad \ldots (3)
\]

The Debye-Hückel correction for activity coefficients, \(-|z_1^2||z_2^2|/\mu\) at the ionic strength (\(\mu\)) of 0.01 M for the buffer media used was not significant enough to be incorporated in Eq.3. Values of \(pK_{\text{eq}}\) for the select dye carbocations are reported in Table I.

As seen from the data in Table I, stability of the dyes measured by their \(pK_{\text{eq}}\) varies considerably spanning a range of 4.4 \(pK_{\text{eq}}\) units and depends significantly on the type and number of aryl rings contiguous to the carbocation centre as well as on the nature and number of \(\pi\)-donor substituents at the 4-position of the aryl rings.

The order of stability among the dye carbocations in aqueous solutions is thus:

\[
\text{AR} > \text{MY} > \text{MG} > \text{BG} \quad \text{or} \quad \text{MeG} \quad \text{or} \quad \text{BV} \quad \text{or} \quad \text{CV} \quad \text{or} \quad \text{EV}\]

The effects of structure on dye carbocation stability

Stability of a dye cation towards a nucleophilic attack should depend primarily upon the degree of direct resonance interaction between its cabocation centre and the lone pair of electrons on the nitrogen atom of 4-amino/alkylamino substituents on the aryl rings attached to it. A perusal of the data on \(pK_{\text{eq}}\) and \(\sum \sigma^*\) (sum of the Brown substituent constants\(^{14,13}\) expressing exalted resonance properties for the 4-substituents over all the aryl rings) in Table 1 shows clearly that there is no overall general correlation between \(pK_{\text{eq}}\) and \(\sum \sigma^*\) e.g. although the set...
Table 1– Equilibrium and kinetic parameters for dye carbocation-to-carbinol conversion at 35±0.1°C

<table>
<thead>
<tr>
<th>Dye carbocations</th>
<th>Chemical name</th>
<th>4-aryl ring substituents</th>
<th>( \sigma^+ )</th>
<th>pH range</th>
<th>( pK_{a+} ) ((\pm 0.03))</th>
<th>Log ( k_{OH} ) ((M \cdot l \cdot min^{-1}))</th>
<th>Log ( k_{H^+} ) ((min \cdot l))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cation form Michler’s hydrol (MH)</td>
<td>4,4'-bis dimethylenediphenyl carbocation</td>
<td>Two-NMe(_2)</td>
<td>-3.40</td>
<td>5.72-7.20</td>
<td>5.92(\pm 0.03)</td>
<td>7.34(\pm 0.01)</td>
<td>-0.76(\pm 0.01)</td>
</tr>
<tr>
<td>Auramine O (AR)</td>
<td>4,4'-bis dimethylenediphenylamino carbocation</td>
<td>Two-NMe(_2)</td>
<td>-3.40</td>
<td>9.80-11.00</td>
<td>9.88(\pm 0.03)</td>
<td>1.13(\pm 0.01)</td>
<td>-2.10(\pm 0.01)</td>
</tr>
<tr>
<td>Pararosaniline (PR)</td>
<td>4,4',4''-tris amino triphenyl carbocation</td>
<td>Three-NH(_2)</td>
<td>-3.90</td>
<td>7.00-9.06</td>
<td>7.27(\pm 0.03)</td>
<td>2.64(\pm 0.01)</td>
<td>-1.70(\pm 0.01)</td>
</tr>
<tr>
<td>Malachite green (MG)</td>
<td>4,4'-bis dimethylenetriphenyl carbocation</td>
<td>Two-NMe(_2)</td>
<td>-3.40</td>
<td>6.50-8.02</td>
<td>6.55(\pm 0.03)</td>
<td>2.66(\pm 0.01)</td>
<td>-1.69(\pm 0.01)</td>
</tr>
<tr>
<td>Brilliant green (BG)</td>
<td>4,4'-bis diethylenetriphenyl carbocation</td>
<td>Two-NEt(_2)</td>
<td>-4.14</td>
<td>6.32-8.34</td>
<td>6.87(\pm 0.03)</td>
<td>2.50(\pm 0.01)</td>
<td>-1.87(\pm 0.01)</td>
</tr>
<tr>
<td>Methyl violet (MV)</td>
<td>4,4'-bis dimethyleno 4''-monomethylenetriphenyl carbocation</td>
<td>Two-NMe(_2), One-NMe(_2) &amp; -5.21</td>
<td>8.70-10.20</td>
<td>9.02(\pm 0.03)</td>
<td>1.42(\pm 0.01)</td>
<td>-3.02(\pm 0.01)</td>
<td></td>
</tr>
<tr>
<td>Crystal violet (CV)</td>
<td>4,4',4''-tris dimethylenetriphenyl carbocation</td>
<td>Three-NMe(_2)</td>
<td>-5.10</td>
<td>8.70-10.10</td>
<td>9.06(\pm 0.03)</td>
<td>1.40(\pm 0.01)</td>
<td>-3.04(\pm 0.01)</td>
</tr>
<tr>
<td>Methyl green (MeG)</td>
<td>4,4'-bis dimethyleno 4''-dimethylammonium triphenyl carbocation</td>
<td>Two-NMe(_2), One-NMe(_2), One-NEt(_2) &amp; -2.99</td>
<td>5.50-7.50</td>
<td>5.48(\pm 0.03)</td>
<td>3.31(\pm 0.01)</td>
<td>-1.51(\pm 0.01)</td>
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<tr>
<td>Ethyl violet (EV)</td>
<td>4,4',4''-tris diethylenetriphenyl carbocation</td>
<td>Three-NEt(_2)</td>
<td>-6.21</td>
<td>8.20-10.00</td>
<td>8.56(\pm 0.03)</td>
<td>1.68(\pm 0.01)</td>
<td>-2.80(\pm 0.01)</td>
</tr>
<tr>
<td>Victoria Blue R (VBR)</td>
<td>4,4'-bis dimethyleno diphenyl, 4''-monoethyleno 1-naphthyl carbocation</td>
<td>Two-NMe(_2), One-NHEt(_2) &amp; -5.21</td>
<td>9.30-10.60</td>
<td>8.67(\pm 0.03)</td>
<td>1.18(\pm 0.01)</td>
<td>-2.86(\pm 0.01)</td>
<td></td>
</tr>
<tr>
<td>Victoria Pure Blue BO (VBP)</td>
<td>4,4'-bis diethyleno diphenyl, 4''-monoethyleno 1-naphthyl carbocation</td>
<td>Two-NEt(_2), One-NHEt(_2) &amp; -5.95</td>
<td>9.60-11.00</td>
<td>8.57(\pm 0.03)</td>
<td>1.73(\pm 0.01)</td>
<td>-2.76(\pm 0.01)</td>
<td></td>
</tr>
</tbody>
</table>

\( a \): \( \sigma^+ \) for \(-\text{NH}_2\) = -1.30, \(-\text{NHMe}\) = -1.81, \(-\text{NMe}_2\) = -1.70, \(-\text{NEt}_2\) = -2.07 (vide Ref. 15.).
\( \sigma^+ \) for \(-\text{NMe}_2\text{Et}\) being not available taken equal to \( \sigma^+ \) for \(-\text{NMe}_3\text{Et}\) = +0.41 (vide Ref. 15.).
\( \sigma^+ \) for \(-\text{NHEt}\) being not available taken equal to \( \sigma^+ \) for \(-\text{NHMe}\) = -1.80 on the basis of nearly equal dipole moments (-1.64D) of N-methyl and N-ethylanilines (vide Ref. 16). The substituent constant would be more negative for a 1-naphthyl ring, its conjugating power being 1.6 times that of a phenyl ring (vide Dewar, M J S, *The molecular orbital theory of organic chemistry*, McGraw Hill, New York, 1969).

\( b \): Maximum variation
of dye cations (MH, AR and MG) or (MV and VBR) have same \( \Sigma \sigma^+ \), their \( pK_{R^+} \) values differ considerably pointing to significant effect of dye carbocation structure (\( \text{-Ph}_2 \text{C}^+ \text{H}_2, \text{-Ph}_2 \text{(NH}_2 \text{)}\text{C}^+ \text{H}_2, \text{-Ph}_3 \text{C}^+ \text{H}_2, \text{-Ph}_2 \text{(Naph)}\text{C}^+ \)) on \( pK_{R^+} \). However, within a set of four triphenylmethane dye cations PR, MG, MV and CV (i.e. those having \(-\text{H}, -\text{NH}_2, -\text{NHCH}_3\) or \(-\text{N(CH}_3)_2\) group only as 4-substituents ) there exists a linear \( pK_{R^+} \) versus \( \Sigma \sigma^+ \) correlation ( \( r = 0.996 \) ) :

\[
pK_{R^+} = -1.41 \Sigma \sigma^+ + 1.76 \quad \ldots (5)
\]

Fig. 1 - The norm \( pK_{R^+} \) versus \( \Sigma \sigma^+ \) relationship (Eq.5) for dye carbocation-to-carbinol equilibration; and the points in \( pK_{R^+} \) versus \( \Sigma \sigma^+ \) plot for eleven di-/tri-/arylmethane dye cations(1:MH, 2: AR, 3: PR, 4: MG, 5: BG, 6: MV,7: CV, 8: MeG, 9: EV, 10: VBR, 11: VBP)

Since of all 4-substituents in triarylmethane dye cations, only \(-\text{NH}_2, -\text{NHCH}_3\) and \(-\text{N(CH}_3)_2\) do not interact sterically with their ortho- or peri- hydrogen and would exert their characteristic direct resonance effect for a triarylcabocation centre, the correlation line (Eq. 5) for the dyes PR, MG, MV and CV can justifiably be regarded as the norm for dye carbocation-to-carbinol equilibration. Figure 1 displays this norm and shows further that the remaining five triphenylmethane dyes (i.e. BG, EV, MeG, VBR and VBP) and one diarylmethane dye (MH) have too low \( pK_{R^+} \) whereas the other diarylmethane dye (AR) has too high \( pK_{R^+} \) than what be predicted from the norm. These five triarylcabocations have one or more 4-NHCH\(_2\)H\(_2\), 4-N(CH\(_3\))\(_2\), 4-NHC\(_2\)H\(_2\) substituents each in their phenyl or 1-naphthyl rings. However, these 4-substituents are not coplanar with their aryl rings owing to steric interactions with the ortho- or peri- hydrogen\(^+\) and thus would exert their resonance effect on the carboxionic site at a considerably reduced level as compared to a 4-NH\(_2\), 4-NHCH\(_2\) or 4-N(CH\(_3\))\(_2\) substituent. Too low \( pK_{R^+} \) for BG, EV, MeG, VBR and VBP can thus be attributed to lack of coplanarity of their 4-ethylamino substituents with the respective aryl rings.

The markedly lower \( pK_{R^+} \) of the diphenylmethyl cation MH as compared to its norm value which is coincident with \( pK_{R^+} \) of its phenyl derivative MG (Fig. 1) is, however, difficult to rationalize. On going from MH to MG, distortion of a planar structure to a propeller-type conformation enforced by severe steric interactions between ortho protons\(^{12}\) would give rise to significant differences in electronic\(^{12,18,19}\), steric\(^{11}\), hydration\(^{11}\) etc. effects between these two carbocations and their carbinols. The net result cannot be predicted \textit{a priori} without detailed knowledge of conformations of the carbocations and the carbinols.

The other diphenylmethane dye cation AR unlike the above six dyes shows a positive deviation from the norm (Fig. 1). AR has the peculiarity of having a \(-\text{NH}_2\) group bonded to its central carbon. Its too high \( pK_{R^+} \) can thus be attributed to delocalization of the carbocationic charge by strong resonance effect of the \(-\text{NH}_2\) group.

**Kinetics and mechanism of dye carbocation-carbinol equilibration**

The dye carbocation \((R^+)\) –carbinol \((ROH)\) equilibration in a buffer of given \( pH \) for each of the eleven select dyes was found to follow first order kinetics. The overall pseudo-first order rate constant \((k)\), determined using Eq. (6)

\[
k = (2.303/t) \log (A/A_0) \quad \ldots (6)
\]

where \( A_0 \) and \( A \) are absorbances of the dye carbocation at its \( \lambda_{max} \) initially and at time \( t \), was found to increase linearly with [\( \text{OH}^- \)]. The overall rate constant \((k)\) for the equilibration is

\[
k = k_f + k_h \quad \ldots (7)
\]

where \( k_f \) and \( k_h \) are the individual pseudo-first order rate constants for the forward step, the formation of ROH from \( R^+ \) and the reverse step, the decomposition of ROH back to \( R^+ \), respectively of the \( R^+\text{-ROH} \) equilibration.

At equilibrium,

\[
k_f[R^+] = k_h[ROH] \quad \ldots (8)
\]

and applying Eq. (2),

\[
k_f/k_h = K_{R^+}/[\text{H}^+] \quad \ldots (9)
\]

It follows,
The results on pH-dependence of pseudo-first order rate constant \(k_r\) for carbinol formation from each of the dye carbocations were found to fit the expression:

\[ k_r = k' \left/ \left( 1 + [H^+]/K_{eq} \right) \right. \]

... (10)

Thus, dye carbinol formation is a general base catalysed and appears to occur by two parallel kinetic routes. The first one distinguished by the pseudo-first order rate constant \(k_{H2O}\) is interpretable as the rate-determining attack of a \(H_2O\) molecule on the dye carbocation mediated by another \(H_2O\) molecule as the general base catalyst. The other one distinguished by the second order rate constant \(k_{OH}\), the predominant route in basic solution, is interpretable as the \(OH^-\) (general base catalyst)-mediated attack of a \(H_2O\) molecule on the carbocation. The kinetic parameters \(k_{H2O}\) and \(k_{OH}\) can reasonably be employed as measures of relative electrophilicities of dye carbocations. The values obtained for \(log k_{H2O}\) and \(log k_{OH}\) of the dye cations are compiled in Table 1 along with \(pK_{eq}^+\) and \(\Sigma \sigma^e\).

A perusal of the data in Table 1 shows that there is no overall general correlation between stabilities \((pK_{eq}^+)\) and electrophilicities \((log k_{H2O}\) or \(log k_{OH}\)\) of the dye cations: e.g., although MH and MeG differ in stability only by 0.4 \(pK_{eq}^+\) unit, the difference in their electrophilicities is as large as 3.9 \(log k_{OH}\) units. However, among the nine triarylmethane dye cations, there do exist fairly linear correlations between \(log k_{H2O}\) or \(log k_{OH}\) and \(pK_{eq}^+\) : \[ log k_{H2O} = -0.52 pK_{eq}^+ + 1.68 \quad (n = 9, r = 0.882) \]

... (12)

\[ log k_{OH} = -0.51 pK_{eq}^+ + 6.18 \quad (n = 9, r = 0.847) \]

... (13)

The equality in the regression coefficients of the Eqs (12) and (13) and the near unity regression coefficient of \(log k_{OH}\) versus \(log k_{H2O}\) correlation:

\[ log k_{OH} = 0.90 log k_{H2O} + 4.22 \quad (n = 9, r = 0.853) \]

... (14)

lend strong support to the inference drawn on the close similarity between the two carbinition formation mechanisms characterised by \(log k_{H2O}\) and \(log k_{OH}\). This is further supported by Ritchie et al.'s finding that entropies of activation for carbinition formation from two triarylmethane dyes MG and CV are highly negative by both the pathways \((log k_{H2O}\) and \(log k_{OH}\)). The solvent water thus must be more ordered at the transition state than at the reactant. It appears that on the approach of a nucleophile, on one hand water in the cybotactic region around the dye carbocation gets more electrostricted as the positive charge on its central carbon increases due to accompanying attenuation of direct conjugation in the carbocation, and on the other hand 'highly disordered' water in the shell exterior to the cybotactic region is released to 'less disordered' bulk water both contributing to the negative entropy of activation. The body of evidence so obtained suggests interpretation of \(k_{OH}\) in terms of a transition state in which \(OH^-\) instead of attacking directly the dye cation, acts as a general base catalyst for the attack of a \(H_2O\) molecule on the carbocation, essentially a solvent-separated ion-pair, the transition state postulated by Ritchie\(^9\) for cation-anion combination reactions; and of \(k_{H2O}\) in terms of a transition state where the nucleophilic attack of one \(H_2O\) molecule instead of occurring simply, is assisted by general base catalysis from a second \(H_2O\) molecule. Very similar conclusions about the transition states of the reactions of \(H_2O\) and \(OH^-\) with quaternary heterocyclic cations were reached by Bunting and his coworkers\(^21\).

**Acknowledgement**

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**Appendix**

The case of auramine O – Auramine O (AR), 4,4’-bis NMe_2Ph_2C=NMe \((\lambda_{max} = 340 \text{ nm})\) owing to its –NH_2 group bonded to the central carbon undergoes simultaneous conversion to its imine form (AR_{imine}), 4,4’-bis NMe_2Ph_2C=NH \((\lambda_{max} = 315 \text{ nm})\) called Homolka base\(^10\) and its carbinol form \((AR_{carbinol})\), 4,4’-bis NMe_2Ph_2C(NH_2)OH \((\lambda_{max} = 370 \text{ nm})\) in basic buffers, the first order plot for decolorisation of AR monitored at 430 nm was bilinear, the initial one of higher slope corresponding to AR_{imine} formation supported by simultaneous monitoring at 315nm, reaching equilibration within 80-100 s, depending on pH, and the second slower one to AR_{carbinol} formation supported by monitoring at 370 nm. Thus if \(A_{1}, A_{2}\) and \(A_{3}\) refer to absorbances of AR at 430 nm for a medium of given pH, corresponding to commencement of decolorisation of AR, completion of imine equilibration – the intersection point of the above bilinear plot and final equilibration for both AR_{imine} and AR_{carbinol} formation respectively.

\[ pH + \log A_{i} = (\log A_{i})_{\text{ equilibrium}} \]

... (15)

\[ (\log A_{i})_{\text{ equilibrium}} = \log K_{eq} \times \left( [H^+] ight)^{[AR_{mix}]} / [AR] \]

\[ \left( [H^+] ight)^{[AR_{mix}]} \] would measure \(pK_{eq}^+\) with \(K_{eq}^+\) defined as \( \left( [H^+] ight)^{[AR_{mix}]} / [AR] \)
and $pH + \log A_0(\text{Ag}_0\text{A}_0)$ would measure $pK_{\text{imine}}$ with $K_{\text{imine}}$ defined as $\langle [\text{AR}_{\text{imine}}] / [\text{AR}] \rangle$.

$K_{eq}$ as defined by Eq. 2 was then $(K_{eq} - K_{\text{imine}})$ and reported as $pK_{eq}$ in Table 1. Further, the value of $pK_{\text{imine}} - pK_{eq}$ which equals log $[\text{AR}_{\text{carbinol}}] / [\text{AR}_{\text{imine}}]$ showed that the total colourless base of auramine O in equilibrium with the dye cation is 89% in the form of the carbinol base, $\text{AR}_{\text{carbinol}}$ and 11% in the form of the imine base $\text{AR}_{\text{imine}}$ (values of $pK_{\text{imine}}$, $pK_{\text{eq}}$, and $pK_{eq}$ being 10.81, 9.88 and 9.85 respectively).

References