TOTAL COLOURING MATTERS CONTENT (VOLUME 4)
(TENTATIVE)

Requested information:
Information on the wavelength of maximum absorbance, absorptivity and/or specific absorbance (including information on the solvent used) for the 17 synthetic colours for which the specifications monograph indicates that the colour is used to form a lake. Currently available data are included in the draft table attached to the revised method. The objective is to provide single values for each synthetic colour, thus recommendations to allow for the establishment of consensus values would be welcomed.

Total colouring matters content
Two general methods are used for determination of total colouring matters: ‘Colouring Matters Content by Spectrophotometry’ and ‘Colouring Matters Content by Titration with Titanous Chloride.’

When using the spectrophotometric method, all colours present in the sample that absorb in the same region as that of the main colour will contribute to the absorbance figure used to calculate the results; subsidiary colouring matters of markedly different hue will not be accounted for by this method.

The titanous chloride reduction method assumes that isomers and subsidiary colouring matters have the same titanous chloride equivalent as the main colouring matter.

Colouring Matters Content by Spectrophotometry
Three experimental procedures are described. Procedure 1 is used for water-soluble colouring matters. Procedure 2 is used for organic solvent-soluble colouring matters. Procedure 3 is used for lakes. Information pertaining to the wavelength of maximum absorbance, absorptivity or specific absorbance necessary for determination of percent colouring matters for the lakes of synthetic colours is included in Table 1.

Principle
The absorbance of a solution of the colouring matter is determined at its wavelength of maximum absorption and the total colouring matters content is calculated using the standard absorptivity or specific absorbance value provided in Table 1.

Apparatus
- UV-visible range spectrophotometer capable of accurate (± 1% or better) measurement of absorbance in the region of 350 - 700 nm with an effective slit width of 10 nm or less
- Spectrophotometer cells, 1 cm path length

Procedure 1 – Colouring matters content of water-soluble colouring matters
Accurately weigh 0.25 g (± 0.02 g) of the sample (W). Transfer to a 1-liter volumetric flask. Add the solvent prescribed in Table 1 and swirl to dissolve.
Make up to volume and mix. Dilute the solution with the same solvent in order to obtain an absorbance between 0.3 and 0.7. Measure the absorbance (A) at the wavelength of maximum absorption in a 1 cm path length cell, using the prescribed solvent as the blank.

**Calculation**

Calculate the total colouring matters content of the sample using either of the following equations:

\[
\text{% total colouring matter} = 100 \times \frac{A \times F}{a \times W}
\]

\[
\text{% total colouring matter} = 1000 \times \frac{A \times F}{A_{1\%}^{1\text{cm}} \times W}
\]

where

- A is the absorbance of the sample solution at the wavelength of maximum absorption;
- \(A_{1\%}^{1\text{cm}}\) is the specific absorbance given in Table 1;
- a is the absorptivity of the standard in liter/(g·cm) given in Table 1;
- W is the weight of the sample in g; and
- F is the dilution factor.

**Procedure 2 – Colouring matters content of organic solvent-soluble colouring matters**

**Reagents**
- Chloroform, reagent grade, acid free
- Cyclohexane, reagent grade

Accurately weigh 0.08 g (± 0.01 g) of the sample (W) into a 100-ml volumetric flask (V1). Add 20 ml of chloroform and dissolve by swirling briefly. Make sure that the solution is clear. Make up to volume with cyclohexane and mix. Pipet 5.0 ml of the solution (v1) into a second 100-ml volumetric flask (V2) and make up to volume with cyclohexane. Pipet 5.0 ml of this diluted solution (v2) into the final 100-ml volumetric flask (V3) and make up to volume with cyclohexane. Measure the absorbance (A) of the twice-diluted solution at the wavelength of maximum absorption in a 1 cm cell, using cyclohexane as the blank.

Perform this procedure promptly, avoiding exposure to air insofar as possible and undertaking all operations in the absence of direct sunlight.

**Calculation**

Calculate the total colouring matters content of the sample using either of the following equations:

\[
\text{% total colouring matter} = 100 \times \frac{A \times V_1 \times V_2 \times V_3}{a \times 10 \times v_1 \times v_2 \times W}
\]

\[
\text{% total colouring matter} = 1000 \times \frac{A \times V_1 \times V_2 \times V_3}{v_1 \times v_2 \times W \times A_{1\%}^{1\text{cm}} \times 10}
\]
where
A is absorbance of the sample solution at the wavelength of maximum absorbance;
A_{1\%}^{\text{1 cm}} is the specific absorbance of the standard indicated in the specification monograph;
V_1, V_2, and V_3 are the volumes of the three volumetric flasks (each 100 ml);
v_1 and v_2 are the volumes of the two pipets (each 5 ml);
a is absorptivity of the standard in liter/(g·cm); and
10^{-3} is the conversion factor.

Procedure 3 – Colouring matters content of lakes

Reagents
- Potassium dihydrogen phosphate, reagent grade
- Sodium hydroxide, reagent grade
- Phosphoric acid, reagent grade
- Hydrochloric acid, reagent grade

Prepare 0.1 M phosphate buffer pH 7 as follows: Weigh 13.61 g of potassium dihydrogen phosphate into a 2000-ml beaker and dissolve in about 900 ml of water. Add about 90 ml of 1 N sodium hydroxide. Measure the pH using a pH-meter and adjust the pH to 7.0 using 0.1 N sodium hydroxide or diluted phosphoric acid. Make to volume in a 1-liter volumetric flask.

Accurately weigh a quantity of lake which will give an absorbance approximately equal to that of the parent colour when the latter is tested according to Procedure 1, above. Transfer to a 250-ml beaker containing 10 ml hydrochloric acid previously diluted with water to approximately 50 ml. Heat with stirring to dissolve the lake, and then cool to ambient temperature. Transfer to a 1-liter volumetric flask, make up to volume with pH 7 phosphate buffer, and mix. Proceed as detailed in Procedure 1, above, and in the specification monograph, using the values for wavelength of maximum absorbance and absorptivity or specific absorbance included in Table 1, and using the phosphate buffer as the spectrophotometric blank.
Table 1. Values for synthetic colours for use in performing test for Colouring Matters Content by Spectrophotometry

<table>
<thead>
<tr>
<th>JECFA Colour</th>
<th>Wavelength of Maximum Absorbance (nm)</th>
<th>Absorptivity (l/(g·cm))</th>
<th>Specific absorbance</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allura Red AC</td>
<td>500 (FDA¹), 504 (EU²), 502 (FCC), 497-501 (Japan)³</td>
<td>52.0 (FDA, FCC)</td>
<td>540 (EU)</td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>520 (EU), 518-522 (Japan)</td>
<td>44.0⁴</td>
<td>440 (EU)</td>
<td></td>
</tr>
<tr>
<td>Azorubine</td>
<td>510 (EU)</td>
<td>51.6⁴</td>
<td>516 (EU)</td>
<td></td>
</tr>
<tr>
<td>Brilliant Black PN</td>
<td>570 (EU)</td>
<td>53.0⁴</td>
<td>530 (EU)</td>
<td></td>
</tr>
<tr>
<td>Brilliant Blue FCF</td>
<td>630 (FDA &amp; EU), 628-632 (Japan)</td>
<td>164 (FDA)</td>
<td>1630 (EU)</td>
<td></td>
</tr>
<tr>
<td>Brown HT</td>
<td>460 (EU)</td>
<td>40.3⁴</td>
<td>403 (EU)</td>
<td></td>
</tr>
<tr>
<td>Erythrosine</td>
<td>527 (FDA), 526 (EU), 524-528 (Japan)</td>
<td>110 (FDA)</td>
<td>1100 (EU)</td>
<td></td>
</tr>
<tr>
<td>Fast Green FCF</td>
<td>625 (FDA), 622-626 (Japan)</td>
<td>156 (FDA)</td>
<td>1560⁵</td>
<td></td>
</tr>
<tr>
<td>Fast Red E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green S</td>
<td>632 (EU)</td>
<td>172⁴</td>
<td>1720 (EU)</td>
<td></td>
</tr>
<tr>
<td>Indigotine</td>
<td>610 (FDA &amp; EU), 610-614 (Japan)</td>
<td>47.8 (FDA)</td>
<td>480 (EU)</td>
<td></td>
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<tr>
<td>Patent Blue V</td>
<td>638 (EU)</td>
<td>200⁴</td>
<td>2000 (EU)</td>
<td></td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>505 (EU), 506-510 (Japan)</td>
<td>43.0⁴</td>
<td>430 (EU)</td>
<td></td>
</tr>
<tr>
<td>Quinoline Yellow</td>
<td>415 (JECFA)</td>
<td>86.5 (JECFA)</td>
<td>865 (EU)</td>
<td></td>
</tr>
<tr>
<td>Red 2G</td>
<td>532 (EU)</td>
<td>62.0⁴</td>
<td>620 (EU)</td>
<td></td>
</tr>
<tr>
<td>Sunset Yellow FCF</td>
<td>484 (FDA), 485 (EU), 480-484 (Japan)</td>
<td>54.0 (FDA)</td>
<td>555 (EU)</td>
<td></td>
</tr>
<tr>
<td>Tartrazine</td>
<td>428 (FDA), 426 (EU), 426-430 (Japan)</td>
<td>53.0 (FDA)</td>
<td>530 (EU)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Values based on information from the United States Food and Drug Administration (FDA)
² Values based on information obtained from the European Union (EU)
³ Values based on information obtained from Japan
⁴ Calculated from specific absorbance
⁵ Calculated from absorptivity