

SPECTROPHOTOMETRIC METHOD

Using a suitable spectrophotometer according to the manufacturer's instructions, determine the transmittance (T) at least over the range 400–700 nm, at intervals of not greater than 10 nm. Express the result as a percentage. Calculate the tristimulus values X , Y , and Z and the colour co-ordinates L^* , a^* and b^* .

DETERMINATION OF COLORATION

Calibrate the instrument according to the manufacturer's recommendations. Carry out system performance tests prior to each measurement or at regular intervals, depending on the use of the apparatus. For this purpose, use certified reference materials within the measurement range.

Operate the apparatus according to the manufacturer's instructions and test the sample solution and reference solution(s) under the same conditions (e.g. path length of the cuvette, temperature).

For transmittance measurements, use *water R* as the standard, assigning it a transmittance of 100.0 per cent at all wavelengths in the visible spectrum. Then the weighting factors \bar{x}_λ , \bar{y}_λ and \bar{z}_λ for CIE standard illuminant C are used to calculate the tristimulus values corresponding to colour co-ordinates $L^* = 100$, $a^* = 0$ and $b^* = 0$.

Reference measurements can be made using the colour co-ordinates of *water R* or freshly prepared pharmacopoeial reference solutions, or using the respective colour co-ordinates stored in the instrument manufacturer's database, provided the latter have been obtained under the same testing conditions.

If the test solution is turbid or hazy, it is filtered or centrifuged. If the test solution is not filtered or centrifuged, any haziness or turbidity is reported with the results. Air bubbles are to be avoided or, where applicable, removed.

The instrumental method is used to compare 2 solutions in terms of their colour or colour difference, or a deviation from a defined colour. Calculate the colour difference (ΔE^*_{tr}) between the test solution (t) and a reference solution (r) using the following equation:

$$\Delta E^*_{tr} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences in colour co-ordinates.

The CIE *LCh* colour co-ordinates may be used instead of the CIE *Lab* colour co-ordinates.

Assessment of location within the $L^*a^*b^*$ colour space.

Instruments may provide information on the actual location of the test solution within the $L^*a^*b^*$ colour space. Using appropriate algorithms, correspondence to pharmacopoeial reference solutions (such as 'test solution equals reference solution XY', 'test solution close to reference solution XY' or 'test solution between reference solutions XY and XZ') can be obtained.



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2.2.3. POTENTIOMETRIC DETERMINATION OF pH

The pH of an aqueous solution is defined as the negative logarithm of the activity of its hydrogen ions, expressed conventionally as the hydrogen ion concentration of the solution. For practical purposes, its definition is an experimental one. The pH of a solution to be examined is related to that of a reference solution (pH_s) by the following equation:

$$\text{pH} = \text{pH}_s - \frac{E - E_s}{k}$$

in which E is the potential, expressed in volts, of the cell containing the solution to be examined and E_s is the potential, expressed in volts, of the cell containing the solution of known pH (pH_s), k is the change in potential per unit change in pH, expressed in volts and calculated from the Nernst equation.

Table 2.2.3.-1. – Values of k at different temperatures

Temperature (°C)	k (V)
15	0.0572
20	0.0582
25	0.0592
30	0.0601
35	0.0611

The potentiometric determination of pH is made by measuring the potential difference between 2 appropriate electrodes immersed in the solution to be examined; 1 of these electrodes is sensitive to hydrogen ions (usually a glass electrode) and the other is the reference electrode (e.g. a silver-silver chloride electrode). They are often combined as 1 compact electrode, together with a temperature probe.

Apparatus. The measuring apparatus is usually a voltmeter with an input resistance at least 100 times that of the electrodes used. It is normally graduated in pH units and has a sensitivity such that discrimination of at least 0.05 pH unit or at least 0.003 V may be achieved.

Recent pH meters are microprocessor-controlled and are operated using the manufacturer's firmware or software, according to given instructions.

Management of electrodes. The electrodes are stored appropriately and according to the manufacturer's recommendations (e.g. in an electrolyte solution or a suitable storage solution). Before measurement, the electrodes are visually checked. Refillable electrodes are checked for the absence of air bubbles in the glass bulb and to ensure that the inner electrolyte solution level is satisfactory. The filling orifice has to remain open during the measurement. It is also recommended that the diaphragm of the reference electrode is checked. Before first use, or if the electrode has been stored out of electrolyte solution, it is usually necessary to condition it, according to the recommendations of the manufacturer. If pH stabilisation is too slow (i.e. a long response time), or a zero point shift, reduced slope or any other difficulties in calibration are observed, the electrode will probably need to be cleaned or replaced. The cleaning is performed depending on the type of sample and as prescribed in the manufacturer's manual. Regular cleaning is recommended.

Calibration and measurement conditions. Unless otherwise prescribed in the monograph, all measurements are carried out at the same temperature as that used for calibration (± 2.5 °C), usually between 20 °C and 25 °C. Table 2.2.3.-2 shows the variation of pH with respect to temperature of a number of reference buffer solutions used for calibration. Follow the manufacturer's instructions for temperature correction.

The calibration consists of the determination of the slope (e.g. 95–105 per cent) and the offset of the measuring system. Most commercial pH meters offer a "self test" or "start-up test" where, for example, the slope and asymmetry potential are tested and compared to the manufacturer's specifications. The apparatus is calibrated using at least 2 buffer solutions chosen so that the expected pH value of the solution to be examined lies between the pH values of the buffer solutions. The range must be at least 2 pH units. The pH of another buffer solution of intermediate pH, read from the scale, must not differ by more than 0.05 pH units from the value corresponding to that solution.

Reference buffer solutions are preferably commercially available certified reference materials. Alternatively, buffer solutions can be prepared in-house according to Table 2.2.3.-2.

These solutions must be traceable to primary standards. Calibration has to be performed on a regular basis, preferably each day of use or before each series of measurements. Immerse the electrodes in the solution to be examined and take the reading in the same conditions as those applied for the reference buffer solutions.

If suspensions, emulsions or solutions of non-aqueous or partially non-aqueous character are measured on a system calibrated as described above, the pH reading can only be considered to be an approximation of the true value. Suitable electrodes have to be used for pH measurements of such mixtures.

Table 2.2.3.-2. – pH of reference buffer solutions at various temperatures

Temperature (°C)	Potassium tetraoxalate 0.05 M	Potassium hydrogen tartrate saturated at 25 °C	Potassium dihydrogen citrate 0.05 M	Potassium hydrogen phthalate 0.05 M	Potassium dihydrogen phosphate 0.025 M + Disodium hydrogen phosphate 0.025 M	Potassium dihydrogen phosphate 0.0087 M + Disodium hydrogen phosphate 0.0303 M	Disodium tetraborate 0.01 M	Sodium carbonate 0.025 M + Sodium bicarbonate 0.025 M	Calcium hydroxide, saturated at 25 °C
	$C_4H_3KO_8 \cdot 2H_2O$	$C_4H_5KO_6$	$C_6H_7KO_7$	$C_8H_5KO_4$	$KH_2PO_4 + Na_2HPO_4$	$KH_2PO_4 + Na_2HPO_4$	$Na_2B_4O_7 \cdot 10H_2O$	$Na_2CO_3 + NaHCO_3$	$Ca(OH)_2$
15	1.67		3.80	4.00	6.90	7.45	9.28	10.12	12.81
20	1.68		3.79	4.00	6.88	7.43	9.23	10.06	12.63
25	1.68	3.56	3.78	4.01	6.87	7.41	9.18	10.01	12.45
30	1.68	3.55	3.77	4.02	6.85	7.40	9.14	9.97	12.29
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93	12.13
$\frac{\Delta pH^{(1)}}{\Delta t}$	+ 0.001	– 0.0014	– 0.0022	+ 0.0012	– 0.0028	– 0.0028	– 0.0082	– 0.0096	– 0.034

(1) pH variation per degree Celsius.

PREPARATION OF REFERENCE BUFFER SOLUTIONS

Potassium tetraoxalate 0.05 M. Dissolve 12.61 g of $C_4H_3KO_8 \cdot 2H_2O$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Potassium hydrogen tartrate, saturated at 25 °C. Shake an excess of $C_4H_5KO_6$ vigorously with *carbon dioxide-free water R* at 25 °C. Filter or decant. Prepare immediately before use.

Potassium dihydrogen citrate 0.05 M. Dissolve 11.41 g of $C_6H_7KO_7$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent. Prepare immediately before use.

Potassium hydrogen phthalate 0.05 M. Dissolve 10.13 g of $C_8H_5KO_4$, previously dried for 1 h at 110 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Potassium dihydrogen phosphate 0.025 M + Disodium hydrogen phosphate 0.025 M. Dissolve 3.39 g of KH_2PO_4 and 3.53 g of Na_2HPO_4 , both previously dried for 2 h at 120 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Potassium dihydrogen phosphate 0.0087 M + Disodium hydrogen phosphate 0.0303 M. Dissolve 1.18 g of KH_2PO_4 and 4.30 g of Na_2HPO_4 , both previously dried for 2 h at 120 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Disodium tetraborate 0.01 M. Dissolve 3.80 g of $Na_2B_4O_7 \cdot 10H_2O$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent. Store protected from atmospheric carbon dioxide.

Sodium carbonate 0.025 M + Sodium hydrogen carbonate 0.025 M. Dissolve 2.64 g of Na_2CO_3 and 2.09 g of $NaHCO_3$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent. Store protected from atmospheric carbon dioxide.

Calcium hydroxide, saturated at 25 °C. Shake an excess of *calcium hydroxide R* with *carbon dioxide-free water R* and decant at 25 °C. Store protected from atmospheric carbon dioxide.

STORAGE OF BUFFER SOLUTIONS

Store buffer solutions in suitable chemically-resistant, airtight containers, such as type I glass bottles or plastic containers suitable for aqueous solutions.



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2.2.4. APPROXIMATE pH OF SOLUTIONS

Determine the approximate pH using a *pH indicator strip R*. Alternatively, pH indicators such as those described in Table 2.2.4.-1 can be used.

Table 2.2.4.-1

Reaction	pH	Indicator
Alkaline	> 8	Red litmus paper R
Slightly alkaline	8 – 10	Phenolphthalein solution R Thymol blue solution R
Strongly alkaline	> 10	Phenolphthalein paper R Thymol blue solution R
Neutral	6 – 8	Methyl red solution R Phenol red solution R
Acid	< 6	Methyl red solution R Bromothymol blue solution R1
Slightly acid	4 – 6	Methyl red solution R Bromocresol green solution R
Strongly acid	< 4	Congo red paper R