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Properties of colour reference solutions of the European Pharmacopoea in CIE L*a*b* colour space

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The coordinates of CIE L*a*b* uniform colour space have been acquired from the transmitance spectra of colour reference solutions of European Pharmacopoeia (Ph.Eur.). Calculation of colour differences of these solutions from purified water ΔE^* gave their values in the range between 0.7 (B_9 solution) to 36 (Y_1 solution) CIE units. Excluding red colour reference soulutions, ΔE^* values did not depend on concentrations of colour compounds linearly. Small ΔE^* values founded by the brown and brownish-yellow colour reference solutions of the lowest concentrations can possibly cause some problems of visual examination of the degree of coloration of liquids according to Ph.Eur.

Colour reference solutions are used by Ph.Eur. for visual examination of the degree of coloration of liquids in the range brown-yellow-red. Colorimetric properties of these colour reference solutions have only rarely been reffered to (Ali and Castle 2003). An older paper deals with colour reference solutions of the Hungarian Pharmacopoeia (Stampf and Jelinekné Nikolics 1989). There are colour reference solutions mixed in eight concentrations from four primary colour solutions to four colour series (yellow, pink, green, brown) described here, while Ph.Eur. uses different solutions mixed to five colour series from three primary colour solutions in seven (yellow, Y, red, R, greenish-yellow, GY, brownish-yellow, BY) or nine respectively (brown, B) concentrations. The aim of this study was to acquire information about properties of colour reference solutions Ph.Eur. in CIE L*a*b* colour space. Spatial colour difference in this space can be expressed as a single numerical value ΔE^* calculated according to the formula $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Wyszecki and Stiles 2000; Krishna Prasad et al. 1996). Colour differences in CIE units in this paper were calculated against purified water and are summarized in the Table.

It is apparent from the data in the Table that the range of colour changes ΔE^* in the series of red colour reference solutions Ph.Eur. is 24 units, while in other colour reference solution series it is greater and ranges from 31 to 36 CIE units. A change of colour corresponding to a value of $\Delta E^* > 1.5$ can be perceived by the human eye (Stark et al. 1996). The values determined in colour reference solutions

Table: Colour differences (ΔE^* CIELAB) of colour reference solutions Ph.Eur. against water ($\bar{x}$ from $n = 5$)

Colour reference solution	ΔE^* (CIE units)
$B_9 \rightarrow B_1$	$0.7 \rightarrow 31$
$BY_7 \rightarrow BY_1$	$1.4 \rightarrow 34$
$Y_7 \rightarrow Y_1$	$1.8 \rightarrow 36$
$GY_7 \rightarrow GY_1$	$2.0 \rightarrow 35$
$R_7 \rightarrow R_1$	$2.8 \rightarrow 24$

Ph.Eur. show and our practical experiences acknowledge that namely the brown and brownish-yellow solutions of the least concentrations can cause some complaints of their visual comparison with a sample resulting from too small values of their colour differences. Additional measurement of B_7 solution led to the value $\Delta E = 1.5$. Therefore it would be advantageous for more objective recognition and decision to substitute visual comparison with measurement and limitation of solutions absorbance in the visual spectral area by suitably chosen wavelenghts, or to perform a comparison directly in parameters of CIE $L^*a^*b^*$ colour space, which can be for colour reference solutions stored in computer for future comparison (Ali and Castle 2003). Regression analysis of dependence of ΔE^* values on a volume of a colour standard solution in 100 ml of colour reference solution (thus on a concentration of colour components in a solution) confirmed that, excluding the red colour reference solution, regression using second-degree polynomial is a better model than simple linear regression. Both models mentioned were significant ($\alpha = 0.05$) by the red solution, correlation coefficients were 0.9949 for linear regression and 0.9968 respectively, criterion F value was in the first case 293.9, but in the second one only 154.3 and application of Scott's multicollinearity criterion showed this model incorrect. Other colour reference solutions reached by using second-degree polynomial regression always not only higher values of correlation coefficiets and lower values of regression coefficient a_0 in the equation $y = a_1x + a_2x^2 + a_0$, but also essentially higher criterion F values. Values of correlation coefficient ranged for these solutions from 0.9881 to 0.9995 and the relationship between concentration of colour components of a solution (x) and ΔE^* (y) value was expressed e.g. for the series of yellow colour reference solutions by the equation $y = 0.5997x - 0.002468x^2 + 0.3037$, while the regression coefficient was not statistically significant ($\alpha = 0.05$) in (similarly as for brown, brownish-yellow and greenish-yelow colour reference solutions).

Experimental

Colour reference solutions were prepared from ferric chloride, cobalt(II)chloride, copper sulfate and hydrochloric acid of p.a. purity and from purified water according to Ph.Eur. therewith the concentrations of yellow, red and blue primary solutions were not corrected. All the colour reference solutions except the sixth and the eigths brown solution and the fifths and sixth other ones were prepared and evaluated. Transmittances of colour reference solutions were measured during about 30 min after their preparation in the range 380–770 nm with a Helios Beta spectrophotometer (ThermoSpectronic) in 1 cm Plastibrand® plastic cells cat. No. 7591 50 (Brand) in colour mode, 600 nm/min, against purified water. Values of the coordinates L^*, a^*, b^* of CIELAB colour space were calculated with Chroma v. 2.0 Colour Measurement software, Part No. 9423 UV8 50910E (ChromSpec). From these values, differences ΔE^* of colour in CIE units against purified water were calculated. Coordinates L^*, a^*, b^* were calculated for normalized C light and for 10° standard observer. Results of five single measurements were statistically processed by testing for outliers from the range, calculation of arithmetic mean and by regression analysis (Sachs 1974). Regression analysis was performed using QC.Expert calculation software (TriloByte® Ltd., Pardubice, CZ).

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