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The use of visible absorption spectra to evaluate different color reactions for the detection of cyclic ureides

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Modifications of the Zwikker- and Parri color detection tests were investigated and compared according to their ability to distinguish between nine different barbituric acids and hydantoins. Solutions of the resulting complexes in 50% DMSO were analyzed spectrophotometrically. 350 spectra have been analyzed and criteria for their assessment have been defined. The evaluation based upon the occurrence of a peak in the visible absorption spectra, in comparison with the spectrum of the blank solution. The results were in accordance to those obtained in the visual assessment using a color palette formerly introduced. Cobalt(II) nitrate and methanolic solution of piperidine, or cyclohexylamine, respectively, were the suitable components to get unmistakable results.

1. Introduction

The detection of cyclic ureides *via* complexation with heavy metal salts has long been used in the analysis of pharmaceutical important heterocycles, and different conditions have been applied with sometimes contradictory results. Zwikker- and Parri color tests are also part of several pharmacopoeias, e.g. identification reactions of barbital, phenobarbital, phenytoin, and ethosuximide.

In a previous paper, we have described a method to compare different color reactions for the detection of barbituric acids and hydantoins (Meusel et al. 2005). Several modifications of the Zwikker- and Parri color tests have been used to determine a suitable composition with respect to the organic base and the heavy metal salt (cobalt or copper). In order to evaluate the different compositions, nine test compounds were selected (for structures, see Table), including seven compounds for which a positive color reaction was expected (**I–III**, **V**, **VII–IX**) and two that should fail to react (**IV**, **VI**). The evaluation of the tests was conducted on the basis of the simple decision whether the color of the blank differed from that of the test. Colors were visually determined according to a newly introduced color palette of seven defined colors. Herein we report on the evaluation of Zwikker- and Parri color detection tests by means of visible absorption spectra.

Bult and Klasen have spectrophotometrically characterized complexes of test compounds with cobalt(II) salts and organic amines, where cyclohexylamine and a 6 : 4 mixture of ethanol and methanol were found to be particularly suitable for the detection of 5,5-diethylbarbituric acid (barbital) (Bult and Klasen 1974a, b, c).

The aims of the present study were (i) to demonstrate whether the maxima of the recorded spectra correlate with the visually observed colors, (ii) to show whether it is possible to evaluate the quality of the tests by means of visible-light spectra, (iii) to compare the results of the evaluation based on the spectrophotometric method with those of the visual observation.

2. Investigations, results and discussion

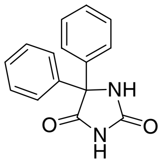
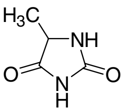
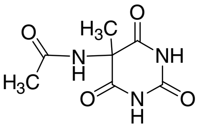
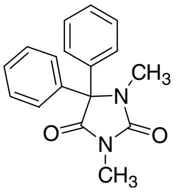
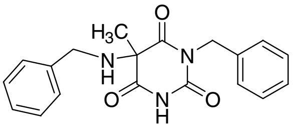
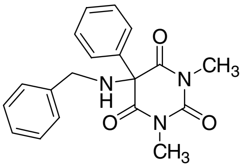
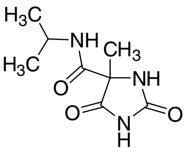
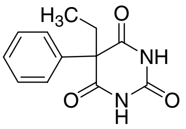
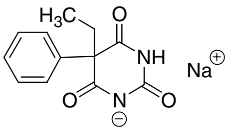
Thirty six different tests (see Experimental) were evaluated using nine test compounds, and the resulting complexes were analyzed spectrophotometrically. As an example, the documentation list for one of the tests is given in the Table.

It was found that precipitation occurred in several test mixtures after the addition of the corresponding amine. The precipitate was removed by filtration, and both the resulting filtrate as well as the clear test solutions were examined by recording the visible spectra. When analyzing these data, no correlation between the absorption maximum and the observed color was found in the case of the filtered mixtures. It could be deduced, that the color-causing complex precipitated and disappeared more or less in the filtrate (data not shown). However, when the clear solutions were recorded, such a correlation was evident.

Therefore, it was intended to dissolve the test mixtures, and the same amount of DMSO was added. Thus, clear solutions were produced in nearly all test modifications (except for test 9). The visible absorption spectra of these 350 solutions were then recorded. The spectra possessed either one or no peak. A peak was defined as a local maximum of the spectrum, and the threshold, the minimum difference between a peak and a valley, was set to 0.01.

The correlation between the wavelength of the peak and the color visually determined prior to the addition of DMSO was examined. It was considered that the wavelength of the peak should be within the wavelength range of that color which is complementary to the observed one.

Table: Documentation list for test 1

Compd.	Structure	Visual color determination	Wavelength of the peak	Absorbance coefficient	Appearance of a peak
Blank	–	yellow	400 nm*	0.723	no
I		hotpink	400 nm*	0.174	no
II		hotpink	523 nm	0.078	yes
III		darkviolet	529 nm	0.099	yes
IV		yellow	400 nm*	0.648	no
V		hotpink	400 nm*	0.398	no
VI		yellow	400 nm*	0.612	no
VII		hotpink	519 nm	0.088	yes
VIII		darkviolet	555 nm	0.157	yes
IX		darkviolet	559 nm	0.215	yes

* maximum absorbance at 400 nm, no peak occurred

This assumption was confirmed. For example, in 124 cases the color ‘hotpink’ was visually determined and the peaks of the corresponding solution were observed in the range of 495–536 nm. In 112 cases the color ‘darkviolet’ came along with a wavelength range of 526–613 nm. The color ‘blue’ corresponded to 563–631 nm (13 cases), ‘deepskyblue’ to 628–719 nm (10 cases), and ‘lightgreen’ to 628–697 nm (22 cases). The color ‘mediumslateblue’ was not observed. Spectra with a maximum absorption at 400 nm and no peak at a higher wavelength were not considered. The observed color in these 69 cases was mainly ‘yellow’.

Next, it was analyzed whether the quality of the tests could be assessed using the spectral data, and the following criteria were defined. A test for a given compound was set ‘positive’, when a peak was detected in the spectrum. On the other hand, a test for a given compound was set ‘negative’, when no peak appeared. As a matter of course, the corresponding blank must not exhibit a peak. For example, from the documentation list for test 1 (Table), it can be seen that test 1 gave correct results for compounds **II**–**IV** and **VI**–**IX**, but incorrect results for compounds **I** and **V**.

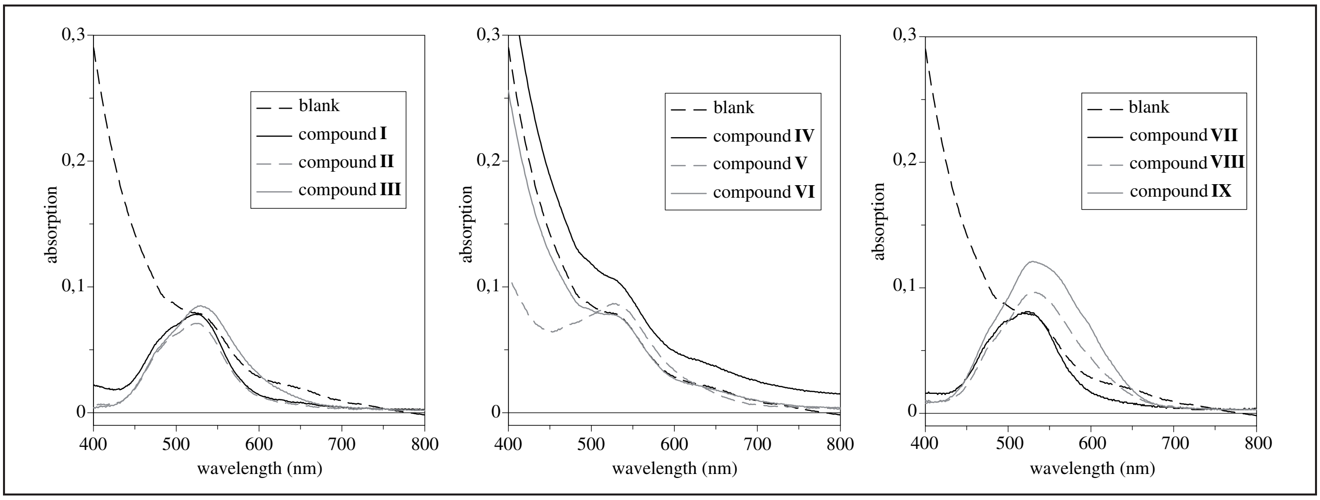


Fig.: Visible absorption spectra of the solutions of the complexes in 50% DMSO after performing test 2

The analysis of the whole data set gave the following results. In tests 1–8, the blanks did not have a peak and thus for compounds **I–IX** the decision positive/negative could be made. When performing test 9, a clear solution could not be obtained and therefore the absorption measurement was not carried out. Tests 10–36 did not meet the criteria to distinguish between 'positive' and 'negative' in the spectrophotometric analysis because their blanks gave spectra with a peak.

In the visual color determination, tests 1–8 were found to be well suited, since they gave 'positive' results for the right seven compounds and 'negative' results for **IV** and **VI** (Meusel et al. 2005). We have now examined that two tests (i.e. 2 and 4) gave correct results for the nine compounds based on the criteria noted above. It is worth mentioning, that these two tests were among the suitable tests of the previous study.

The components of test 2 are cobalt(II) nitrate (10% m/V methanolic solution) and piperidine (5% m/V methanolic solution), those of test 4 are cobalt(II) nitrate (10% m/V methanolic solution) and cyclohexylamine (5% m/V methanolic solution). Absorption spectra of the complexes resulted from test 2 with the nine compounds, as well as the blank, are depicted in the Figure.

In summary, several modifications of the Zwikker- and Parri color tests to detect cyclic ureides were examined on the basis of 350 visible absorption spectra. It was found, that the heavy metal complexes could be dissolved after addition of DMSO and thus, nearly all reaction mixtures could be subjected to the spectrophotometric analysis. The spectral data were in accordance with the visually observed colors. Therefore, the recording of absorption spectra represents an objective method to support the analysis of this detection reaction. Two favorable compositions, both containing cobalt(II) nitrate, were selected, and a procedure to analyze the color tests by means of visible spectra was proposed.

3. Experimental

The test compounds **I–IX** were available from the previous study (Meusel et al. 2005). $Co(CH_3COO)_2$ was purchased from Grüssing (Filsum, Germany), $Co(NO_3)_2 \times 6\,H_2O$ from Alfa Aesar (Heysham, UK), $Co(SCN)_2$ from Aldrich (Steinheim, Germany), $Cu(CH_3COO)_2 \times H_2O$ and $CuSO_4$ from Merck (Darmstadt, Germany). For the spectral measurements a Varian Cary 50 Bio UV-Vis spectrometer and quartz cuvettes (1 mL) were used.

The test compounds were dissolved in methanol at a concentration of 1.67 g/L. The composition of the different tests was as follows, test 1–10: methanolic $Co(NO_3)_2$ solution (10% m/V); test 11–20: methanolic $Co(CH_3COO)_2$ solution (10% m/V); test 21–30: methanolic $Co(SCN)_2$ solution (10% m/V); test 31–33: aqueous $Cu(CH_3COO)_2$ solution (5% m/V); test 34–36: aqueous $CuSO_4$ solution (5% m/V); test 1, 11, 21: methanolic piperidine solution (10% m/V); test 2, 12, 22: methanolic piperidine solution (5% m/V); test 3, 13, 23: methanolic cyclohexylamine solution (10% m/V); test 4, 14, 24: methanolic cyclohexylamine solution (5% m/V); test 5, 15, 25, 31, 34: methanolic isopropylamine solution (10% m/V); test 6, 16, 26: methanolic isopropylamine solution (5% m/V); test 7, 17, 27, 32, 35: methanolic *tert*-butylamine solution (10% m/V); test 8, 18, 28: methanolic *tert*-butylamine solution (5% m/V); test 9, 19, 29: methanolic morpholine solution (10% m/V); test 10, 20, 30: methanolic morpholine solution (5% m/V); test 33, 36: methanolic pyridine solution (10% m/V).

To 1.5 mL of the solution of the test compound (or 1.5 mL methanol in case of the blank) 50 μL of the solution of the heavy metal salt and 50 μL of the solution of the base were added. The mixture was shaken, and immediately the color was observed visually as described (Meusel et al. 2005). One minute after addition of the corresponding base the mixtures were diluted with 1.6 mL of DMSO. It was mixed using a pipette (1 mL) and 1 mL of the obtained solution was transferred into a cuvette. A visible absorption spectrum ($\lambda = 400$–800 nm, data interval 1 nm) was recorded 30 s after addition of DMSO.

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