trans-2,4,5-Tris-(4-nitrophenyl)-4,5-dihydro-1 H-imidazol (**10**). Gelbe Kristalle, Schmp. 202 °C (Zers.) (EtOH). Ausbeute: 93%. IR (KBr, cm^{-1}): $\tilde{\nu} = 3420$ (NH), 1590 (C=N), 1550, 1350 (NO_2). UV (MeOH, nm): λ_{max} (lg ε) = 203 (4,55), 270 (4,51). ^{1}H-NMR ([D$_6$]DMSO): δ = 5,85 (s, br., + D_2O: scharf, 4-H, 5-H), 7,30 (″d″, 4 H, 2-H, 6-H, N = 8.7), 7,95 (″d″, 4 H, 3-H, 5-H, N = 8.7), 8,32–8,34 (m, 2′-H, 6′-H), 8,41–8,44 (m, 3′-H, 5′-H, NH). EI-MS: m/z (%) = 433 ([M]$^{+\bullet}$, 12), 283 (100). $C_{21}H_{15}N_5O_6$ (433,4)
Das Schmelzverhalten von **5** in Lit. [11, 12] dürfte auf Schmp.-Depressionen durch mehr oder weniger große Anteile an **10** hervorgerufen worden sein.

[5] Die Darstellung erfolgte nach der Methode von Surrey [14].

[6] Wegen Substanzmangels konnten keine weiteren analytischen Daten ermittelt werden.

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Reactions in imipramine-pyrocatechol violet and imipramine-iron(III) ion systems

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Two methods for the determination of imipramine hydrochloride are presented. One method employs the liability of the imipramine tertiary amine group nitrogen atom to form ion-association complexes with organic compounds. The formed complex is insoluble in water but well soluble in organic solvents. This property is the base of a new extractive-spectrophotometric method for imipramine determination. The second method is based on the oxidation of imipramine by iron(III) ions. Imipramine hydrochloride is oxidized by Fe(III) ions at 75 °C with the formation of coloured products. Both reactions were followed spectrophotometrically by measuring the absorbance at 445 nm (extractive method) and at 670 nm (oxidative method). The methods have been successfully applied to the determination of imipramine in a liquid for injection. The results were compared with those obtained by the official procedure.

1. Introduction

Dibenzoazepine derivatives are chemically active substances [1, 2]. They react with some organic compounds and with halide and thiocyanide complexes of metals [3–7]. They also undergo oxidative reaction with the formation of coloured products [8–12]. The oxidation reaction is a 4-electron process, which leads, via cation-radical intermediates, to an intensely coloured dimer [13]. The properties mentioned can be used as a base of spectrophotometric and extractive-spectrophotometric methods for the determination of dibenzoazepines.
The most popular tools used for dibenzoazepine determination are various chromatographic techniques [14–16], electrochemical [17–20] or immunochemical methods [21, 22]. The number of spectrophotometric methods is very limited, so the authors have taken the task to elaborate new and simple methods for the determination of imipramine (IMP). Here, the reactions of imipramine with pyrocatechol violet (FPK) and iron(III) ions are described.

2. Investigations, results and discussion

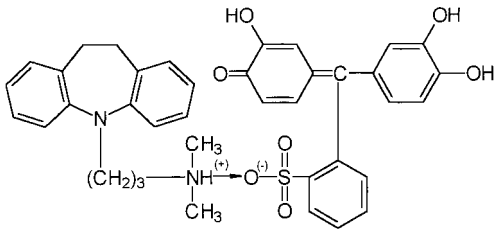
2.1. Imipramine-pyrocatechol violet system

IMP forms with pyrocatechol violet an yellow-orange precipitate insoluble in water, but very well soluble in organic solvents. Among the solvents tested, the mixture of chloroform-butyl alcohol (2:1) appeared to be the most efficient. The visible spectrum of the coloured extract showed a maximum at 445 nm.
Acidification of the aqueous phase stimulated the formation of the compound and its extraction. The use of hydrochloric, phosphoric or acetic acid decreased the absorbance of extracts but the presence of sulphuric acid increased the absorbance of the reaction product. The intensity of colour is stable in the range $2 \cdot 10^{-4}$–$3 \cdot 10^{-4}$ mol/l of sulphuric acid. A concentration of sulphuric acid of $2.5 \cdot 10^{-4}$ mol/l^3 was selected for further work.
It was found, that absorbance was stable when a 2–10-fold excess of FPK to IMP was used. A 15–20-fold excess caused slight decrease of extracts absorbance.

The composition of the complex was studied by Job's method of continuous variation and by spectrophotometric titration. The obtained results showed that the composition of the associate was equimolar (1:1). Coloured extracts were stable for about 5 h.

The absorption spectrum of the complex showed maxima at 212 nm, 276 nm and 332 nm in UV-region and at 445 nm in the VIS-region. The obtained spectrum was compared with the UV-VIS spectra of IMP and FPK. It was found that the characteristic for FPK bands at 276 nm, 332 nm and 445 nm and the IMP hydrochloride band at 212 nm were preserved in the complex spectrum. The IMP band at 260 nm was shifted to longer wavelengths and one maximum with the FPK band at 276 nm was formed. Furthermore, no new absorption bands were observed. These observations seemed to suggest that the investigated compound was formed by electrostatic interaction between IMP cations and anions of FPK. This type of compound can be classified as an ion-association complex. The ion-association character of the investigated compound was confirmed by IR-spectroscopy. In the IMP-FPK spectrum the absorption band characteristic for IMP hydrochloride (700–1600 cm^{-1}) was preserved, proving that the IMP structure remains unchanged. The absorption bands characteristic for the benzodiazepine nitrogen (2850–3000 cm^{-1}) also were preserved in the spectrum, which means that the aromatic nitrogen atom was not engaged in the compound formation. The bands attributed to the stretch vibration of pyrocatechol violet –OH group were found in the complex spectrum. On the other hand, bands at 2400–2600 cm^{-1} attributed to ternary amines N–H group disappeared. This suggested that the compound was formed with participation of the nitrogen atom from the tertiary amine group in the aliphatic chain of IMP.

From the IR spectra it could be supposed that the complex was produced as the result of exchange of the chloride anion of IMP hydrochloride and a FPK anion. The proposed structure of the complex (**1**) is presented below.



1

The investigated complex is very well soluble in methanol, ethanol and acetone. It has a melting range of 182–183 °C. The properties described above can be the base of a new extractive-spectrophotometric method for IMP determination. Table 1 shows the analytical appraisals of the method. It allowed to determine IMP in range 5–40 μg/ml. The extractive-spectrophotometric method was characterised by the molar coefficients $7.7 \cdot 10^3$ l mol^{-1} cm^{-1} with a correlation coefficient of 0.9995. The calibration curve was described by the equation $y = 7.7 \cdot 10^3 x + 0.0387$.

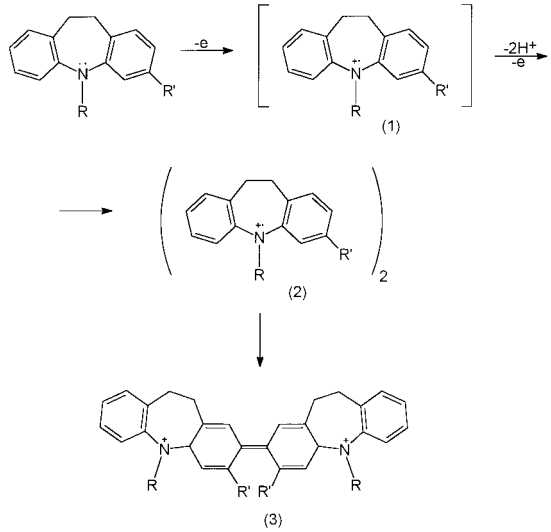
2.2. Oxidative reaction of imipramine hydrochloride with Fe(III) ions

IMP hydrochloride reacts with iron(III) ions at higher temperatures with formation of a blue product. The run of reaction depends on the concentration and type of the acid used, concentration of oxidant, temperaure and time of heating. The measurements were done spectrophotometrically at 670 nm, using a mixture of reagents without IMP as a blank. According to the literature [6] the following mechanism of reaction can be proposed (Scheme):

Table 1: Results of imipramine hydrochloride determination by the pyrocatechol violet method

Amount of imipramine (μg/ml)	Mean absorbance (X) n = 5	Interval	SD	$\mu = X \pm t_{95} S_r$
4.75	0.140	0.015	0.011	0.140 ± 0.013
7.9	0.220	0.030	0.012	0.220 ± 0.0140
12.7	0.345	0.020	0.008	0.345 ± 0.009
15.8	0.427	0.020	0.076	0.427 ± 0.009
19.0	0.506	0.020	0.008	0.506 ± 0.009
22.2	0.585	0.025	0.009	0.585 ± 0.011
23.8	0.625	0.030	0.012	0.625 ± 0.014
26.9	0.697	0.010	0.007	0.697 ± 0.009
30.0	0.769	0.015	0.007	0.769 ± 0.008
31.7	0.807	0.020	0.008	0.807 ± 0.009
34.9	0.880	0.025	0.008	0.880 ± 0.009
38.0	0.960	0.020	0.007	0.960 ± 0.0082
39.6	0.990	0.030	0.015	0.990 ± 0.017

Scheme



The run of oxidation reaction of imipramine. 1 – cation radical of imipramine: 2 – dimerization of two cation-radicals: 3 – coloured dimer: (R′ = –H: R= $-CH_2-CH_2-CH_2-N(CH_3)_2$)

The signal was low at 18–65 °C but it increased distinctly at higher temperatures (65–85 °C). Under such conditions the stability of the coloured product also increased. The temperature of 70 °C was selected as optimal. The optimum reaction time was determined at 75 °C. Heating for 5 min was recommended as optimal.

Keeping all other parameters constant, the influence of concentration of various acids on absorbance and stability of colour was studied. It was noticed that the presence of strong inorganic acids like sulphuric or hydrochloric acid decreased the solution absorbance. The formation of a coloured product was most efficient in the presence of acetic acid (1.5 M).

The effect of iron(III) concentration on the colour intensity was maximal and constant when a 15–25-fold excess of iron in ratio to IMP was used.

These conditions were used for IMP quantification. The Beer law was obeyed between 0.7 and 6.5 μg IMP/ml.

The equation of calibration graph is $y = 2.2 \cdot 10^4 x + 0.0119$ with a correlation coefficient of 0.9998. The results are presented in Table 2.

Table 2: Results of imipramine hydrochloride determination by the iron(III) method

Amount of imipramine (μg/ml)	Mean absorbance (X) n = 5	Interval	SD	$\mu = X \pm t_{95} S_r$
0.70	0.069	0.006	0.022	0.069 ± 0.003
1.58	0.121	0.006	0.026	0.121 ± 0.003
2.38	0.183	0.006	0.019	0.183 ± 0.002
3.17	0.233	0.005	0.019	0.233 ± 0.002
3.96	0.293	0.005	0.019	0.239 ± 0.002
4.75	0.342	0.004	0.017	0.342 ± 0.002
5.55	0.386	0.029	0.012	0.386 ± 0.015
6.50	0.442	0.009	0.030	0.442 ± 0.004

2.3. Application to a commercial product

The elaborated methods were applied for analysis of pharmaceutical preparations. Commercial ampoules containing IMP hydrochloride were analysed by the proposed methods. The results were compared with those obtained by official methods and found not to differ significantly (Table 3).

Table 3: Determination of imipramine in solution for injection by the described methods

Sample	Method	Found by described method (mg)	Found by pharmacopoeal method [23] (mg)	Relative error (%)
Injection ampoules containing 25 mg of imipramine	Pyrocatechol violet method	25.3 ± 0.058	25.1	0.8%
	Iron(III) method	25.2 ± 0.033	25.1	0.4%

The presented procedures are simple and sensitive. The extractive-spectrophotometric method requires about 20 min for one determination, the second method about 10 min. Both methods are precise. The relative error of determination does not exceed $\pm 1\%$.

3. Experimental

3.1. Reagents and apparatus

3.1.1. Reagents

All reagents were of analytical grade and the solutions were prepared with deionized distilled water. The aqueous imipramine (IMP) hydrochloride stock solution (10^{-2} mol/l) was prepared from a commercial product (Sigma Chemical Co). The solution was stable for at least half of a month and was kept in an amber coloured bottle at 4 °C.
Working solutions (10^{-3} mol/l) were prepared just before use.
Pyrocatechol violet (FPK) solution, (10^{-2} mol/l), was prepared from commercial product (P.O.Ch.-Gliwice) by dissolving an appropriate amount in 100 ml of H_2O. $FeCl_3$ stock solution, ($4 \cdot 10^{-1}$ mol/l), was prepared from a commercial product (P.O.Ch.-Gliwice) by dissolving the required amount of salt in H_2O. The working solution (10^{-3} mol/l) was prepared by dissolving stock solution in 0.5 M H_2SO_4.
Solutions of 5 M H_2SO_4, H_3PO_4, HNO_3 and HCl were prepared by dissolving an appropriate volume of concentrated acids in distilled deionized H_2O.

3.1.2. Apparatus

Hewlett-Packard 8452A Diode Array UV-VIS Spectrophotometer; Spectrophotometer Spekol 11, Carl Zeiss Jena; FTIR-spectrometer Magna 550, II serie, Nicolet.

3.2. Recommended procedures

3.2.1. Pyrocatechol violet method

Into a 25 ml separatory funnel were placed variable volumes of $5 \cdot 10^{-4}$ mol/l aqueous solution of IMP hydrochloride, 2.5 ml of 10^{-3} mol/l H_2SO_4 and 5 ml $5 \cdot 10^{-4}$ mol/l of FPK aqueous solution. The total volume of aqueous phase was adjusted to 10 ml with distilled H_2O. A $CHCl_3$ and butyl alcohol (2:1, (10 ml)) mixture was added and the mixture was shaken for 3 min. The two phases were allowed to separate and the organic phase was transferred into 10 ml calibrated test tubes. The absorbance was measured at $\lambda = 445$ nm against $CHCl_3$/butyl alcohol mixture as a blank.

3.2.2. Iron(III) chloride method

Into 10 ml calibration test tubes were placed 1 ml of $4 \cdot 10^{-2}$ mol/l $FeCl_3$ solution, 1.5 ml of 10 mol/l CH_3COOH volume of $1 \cdot 10^{-4}$ mol/l IMP hydrochloride solution. The total volume was diluted to the mark with distilled H_2O, mixed well and then heated at 75 °C. The absorbance was measured after cooling the solution to room temperature at 670 nm against a mixture of reagents without IMP as a blank.

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