ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1055–1061

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

The effect of Fe^{3+} ion on benzylpenicillin in micellar and nonmicellar aqueous solutions[1]

N. Carp[a,*], V. Tufoi[a], M. Carp[a], V. Aldea[b], V. Badescu[c]

[a] *Romanian Academy, Institute of Physical Chemistry, Spl. Independentei 202, Bucharest 77208, Romania*
[b] *University of Medicine and Pharmacy, Faculty of Pharmacy, Traian Vuia 6, Bucharest, Romania*
[c] *ICECHIM, Spl. Independentei 202, Bucharest 77208, Romania*

Received for review 13 September 1995; revised manuscript received 5 December 1995

Abstract

The effect of Fe^{3+} ions on the hydrolysis of benzylpenicillin in aqueous solution below and above the critical micellar concentration (c.m.c. = 0.26 M) has been studied. The catalytic effect of Fe^{3+} ions on hydrolysis of this drug has been demonstrated; the reaction rate constant, calculated from UV absorption data of non-micellar solutions, decreases with concentration. It is likely that Fe^{3+} ions form complexes with the products of degradation. The bright brown slightly soluble product formed in micellar solutions was analyzed by UV–Vis reflection, IR and mass spectroscopy and, in agreement with the results of elemental analysis, is postulated to be an octahedral binuclear complex, $Fe_2BP_4(OH)_2$. This study has been linked with the clinical observation that prolonged treatment with penicillin leads to anaemia.

Keywords: Anaemia; Aqueous solutions; Benzylpenicillin potassium salt; Fe^{3+} effect; Micellar solutions

1. Introduction

The catalytic effect of traces of heavy metals (Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}) upon hydrolytic degradation reactions of beta-lactam antibiotics is widely known. For antibiotics such as benzylpenicillin, ampicillin or oxacillin in aqueous solution, evidence has presented that the formation of complexes with the ions mentioned above corresponded to the following metal–ligand ratios: = 1:1; 1:2; 1:3; and 2:3 [1–4]; for Cu^{2+} a slightly soluble compound of 1:1 molar ratio was obtained [2]. The action of Fe^{3+} upon benzylpenicillin has not been previously reported. The clinical findings of anaemia which appears after prolonged treatment with this antibiotic suggested to the authors the need for a study of the effect of trivalent iron upon the stability of aqueous solutions of benzylpenicillin potassium salt (BPK). The capacity of alkaline BP salts to self-associate in aqueous solution and to form anionic micelles at concentrations higher that its c.m.c. (0.26 M) has been studied [5].

* Corresponding author. Fax: (+40) 31-21-147.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

PII S0731-7085(96)01765-7

2. Experimental

2.1. Reagents

BPK, Romanian commercial product; anhydrous chemically pure ferric chloride (Merck); and freshly boiled doubly distilled water were used.

2.2. Method of investigation for nonmicellar solutions

The effect of Fe^{3+} ions on BPK aqueous solutions was studied by measurements by differential UV–Vis absorption spectrophotometry; use has been made of a Specord M-400 (C. Zeiss, Jena, Germany) spectrophotometer. The spectra were measured within the 215–400 nm range in a series of aqueous solutions containing BPK and iron ions in various molar ratios; comparison was made with BPK of the same concentration as that of the sample and the effect of time was examined.

2.3. Methods of analysis

Various methods of analysis of the slightly soluble product resulting from the interaction of Fe^{3+} ion with BPK in micelles were used. IR spectroscopy was used in the range 4000–400 cm^{-1} in KBr pellets and in the 800–200 cm^{-1} range in CsI pellets. The spectra were recorded with an IR Specord M-80 (C. Zeiss) spectrophotometer. Mass spectrometry with a 70-SE VG analytical double-focusing spectrometer was used under the following conditions: 70 V electron impact; response time 0.1 ms; acceleration voltage 8 kV; ion source temperature 180°C; solid samples were introduced directly in the ionization source; the rate of temperature increase was 1°C s^{-1} in the 50–150°C range and 5°C s^{-1} at temperatures higher than 150°C. UV reflection spectroscopy was carried out using the second-order derivative program. Elemental analysis was carried out with a 1106 Carlo-Erba elemental analyzer.

3. Results and discussion

3.1. The effect of Fe^{3+} on the micellar solutions

Unlike the divalent iron ion, which yields a slightly soluble product in micellar solutions, the trivalent iron ion reacts instantaneously with the BPK self-associated molecules to form a beige product.

3.2. Characterization of the solid product

3.2.1. UV–Vis spectra

Fig. 1 shows the reflection spectrum of the solid product compared with those of BPK and ferric chloride. The spectrum of the product (a) preserves only the wide band with a maximum at about 320 nm from the BPK spectrum, which is specific to the beta-lactam-thiazolidinic chromophore (βLT) and the penicillinic chromophore; (b) takes over the large background of ferric chloride. Additional information was supplied by second-order derivatives which allow, between certain limits, the solving of the zero-order spectrum. Thus, the changes of the second derivative spectrum of the slightly soluble product compared with that of BPK prove that the most affected zones are those where the βLT nucleus, beta-lactamic (βL) carbonyl, thiazolidinic sulphur or penicillinic chromophore groups absorb. In the spectral region below 280 nm where the contribu-

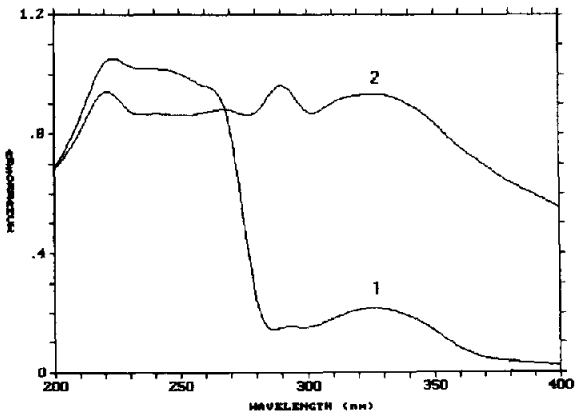

Fig. 1. UV–Vis reflection spectra of (1) BPK and (2) the slightly soluble product.

tions to absorption of the βL carbonyl, the amidic carbonyl on the side-chain and the α band of the benzenic chromophore with a fine vibrational structure are superimposed, the peak amplitude at 262 nm decreases; this is as a consequence of the reaction undergone by one of the carbonyl groups, either by the opening of the βL cycle or by interaction between iron ion with the carboxyl group of penicilloic acid, the primary degradation product. For wavelengths >300 nm where the βLT nucleus and derivatives resulting from opening of the cyclic structure with sulphur absorb, the negative peak at 330 nm of the solid product does not change its amplitude with respect to that of BPK; however their positions are slightly shifted hypsochromically. This shift demonstrates the increase of molecular energy as a consequence of the interaction between iron ion and the nitrogen atom of the thiazolidinic nucleus.

3.2.2. IR spectra

The IR spectra in the 4000–400 cm^{-1} range provide the following information on the interaction of trivalent iron ions with the BPK: (1) the narrow band corresponding to the stretching vibration ν_{NH} asymmetrically associated with a maximum at 3320 cm^{-1} [7] broadens in the solid product owing to the superimposition with the band corresponding to ν_{OH}; (2) the bands of ionized carboxyl $\nu_{ass.(COO-)}$ at about 1600 cm^{-1} [7] and $\nu_{sym.(COO-)}$ at about 1400 cm^{-1} [7] are considerably widened and intensified, emphasizing the implication of the carboxyl group in achieving a metal–oxygen bond; (3) the attenuation of the absorption of βL carbonyl (1670 cm^{-1}) and of the amidic bands on the intensified spectral background in the 1000–1700 cm^{-1} range due to the vibration bands of ionized carboxyl over which they are superimposed also suggests the hydrolytic degradation of penicillin. The spectrum modification over the 800–200 cm^{-1} range (Fig. 2) provides evidence for the degradation of penicillin as well as for the formation of an interaction complex with iron ions. The amide VI band at 520 cm^{-1} (ν_{NCO}) of penicillin loses individuality in the case of the slightly soluble product; in its spectrum a new broadened vibrational band (δ_{C-COO} out-of-plane bend) appears with a maximum at

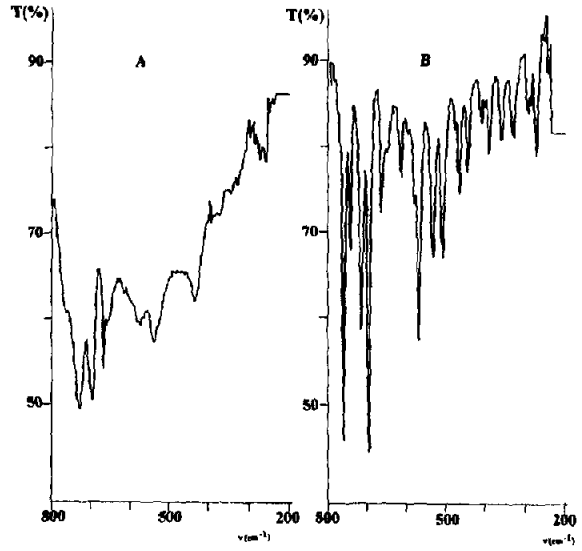

Fig. 2. IR spectra in CsI of the solid product (A) and BPK (B).

540 cm^{-1}. The band at 575 cm^{-1} (γ_{OCO}) widens in the case of the product discussed on the same increased background as the more intense band at 540 cm^{-1}. The intensifying of the band at 725 cm^{-1} assigned to the in-plane vibration δ_{OCO} together with the diminishing of the intense band at 760 cm^{-1} (amide V) of penicillin and the appearance of the band at 540 cm^{-1} confirms the breaking of the βL ring and the formation of the penicilloic acid and also the immobilization of thiazolidinic nitrogen in a bond with the trivalent iron ion. The changes in the range of the skeletal bending of the βLT ring 400–350 cm^{-1} support the above statements.

3.2.3. Mass spectra

Mass spectra of the solid product and BPK (Figs. 3 and 4) emphasise the difference between these compounds. In the mass spectrum of BPK the signal for benzylpenicillin ion (BP^+) corresponds to the weight 334 and that for the alkali metal (K^+) to the weight 39; from the splitting of BPK (weight 373) or the BP^+ molecule arise the peaks at weight 91, corresponding to the tropilium ion ($C_7H_7^+$), and at weights 282 and 256 respectively. The mass spectrum of the solid product shows, besides the peak of the fragment corresponding to the tropilium ion, another origi-

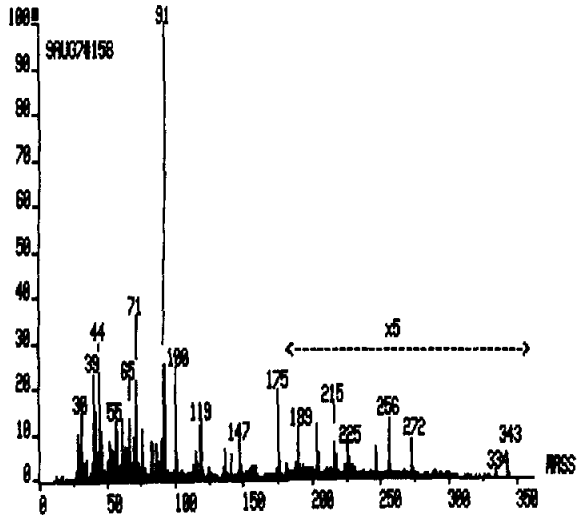

Fig. 3. Mass spectrum of BPK.

nating from decarboxylation, at weight 44 (COO^+).

Splitting by decarboxylation confirms the previous data about the involvement of the carboxyl in a Fe–O bond. The breaking of this bond is emphasized by the mass fragments of iron ion (weight 56) and by one of the carboxylate forms

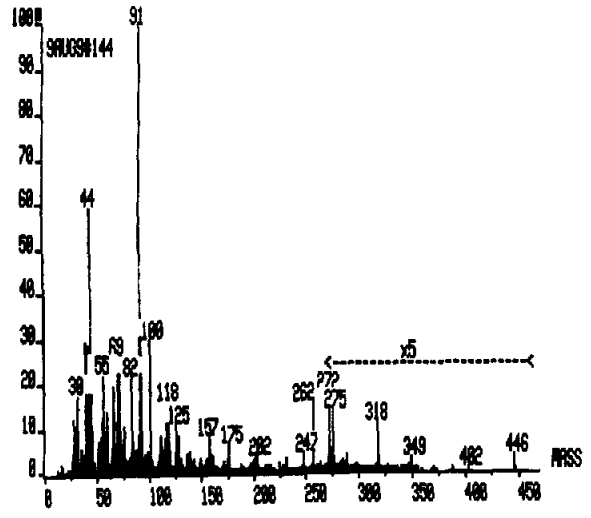

Fig. 4. Mass spectrum of solid product.

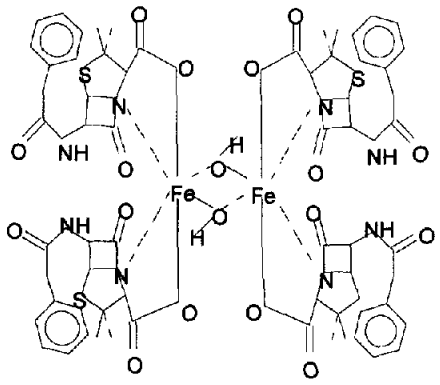

Fig. 5. The proposed model of the molecular structure of the binuclear complex $Fe_2BP_4(OH)_2$.

of degraded penicillin, e.g. the ion of penicilloic ion of weight 349. The fragment whose weight is 446 [apparently the sum of BPK weight (373) with that of iron ion (56) and that of a hydroxyl group (17)] could originate from the interaction of the penicilloic degradation product with metal which corresponds to a 1:1 molar ratio. The formation of the primary hydrolytic degradation product under the effect of iron ion also accounts for the existence of the signal corresponding to penicillin dimer of weight 664. The heavy fragments suggest the formation of high degree polymerization products ($n = 2$) and of the complexes of trivalent iron with the degradation products of BP. The performance of the equipment employed has not shown evidence of masses higher than 1000.

3.2.4. Elemental analysis

Elemental analysis indicates a molar reaction ratio BPK–Fe^{3+} = 4:2. Taking into account that trivalent iron exists in solution in hydroxyhydrated form it should be possible to form an octahedral complex of general formula $[(BP)_xFE_y(OH)_z]$. The solid product may correspond to the formula $[(BP)_4Fe_2(OH)_2]$ (Fig. 5). A mononuclear complex 1:1, may be formed first (Fig. 6).

3.2.5. The effect of Fe^{3+} ions upon benzylpenicillin in nonmicellar solutions

The effect of Fe^{3+} upon aqueous solutions of BPK over the concentration range 10^{-4}–10^{-2} M

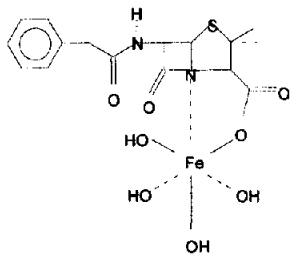

Fig. 6. The proposed model of the molecular structure of the mononuclear complex $FeBP(OH)_6$.

was studied by UV–Vis differential spectrophotometry as functions of the molar ratio of the reactants and time. The difference spectra, obtained under the previously mentioned experimental conditions (Fig. 7), show the development with time of a band with a maximum at about 241 nm in parallel with the diminishing of absorption at wavelengths lower than that corresponding to the isosbestic point ($\lambda = 225$ nm). The second derivative of the 10^{-4} M solution shows a negative peak with the highest amplitude at 238 nm even after 60 min, although after a longer time passes, the peak is intensified at 243 nm. The peak at 238 nm could represent proof of the existence of hydrated iron ion [6] which is not consumed during the reaction and which allows the development of the peak at 243 nm. This indicates the role of the catalyst of the degradation reaction in diluted aqueous solution. The absorbance of the difference spectrum in the zero-order spectrum varies linearly during the first 60 min. The equations which describe this dependence for aqueous solutions of 10^{-4} M BPK concentration are:
(a) molar ratio $BPK/Fe^{3+} = 2:1$;

$$\Delta A(250\text{ nm}) = 0.00682t + 0.220:$$

$$\text{variance} = 2.61 \times 10^{-5}:$$

$$\text{max. deviation} = 0.00324 \quad (1)$$

$$\Delta A(260\text{ nm}) = 0.00453t + 0.196:$$

$$\text{variance} = 6.32 \times 10^{-6}:$$

$$\text{max. deviation} = 0.00540 \quad (2)$$

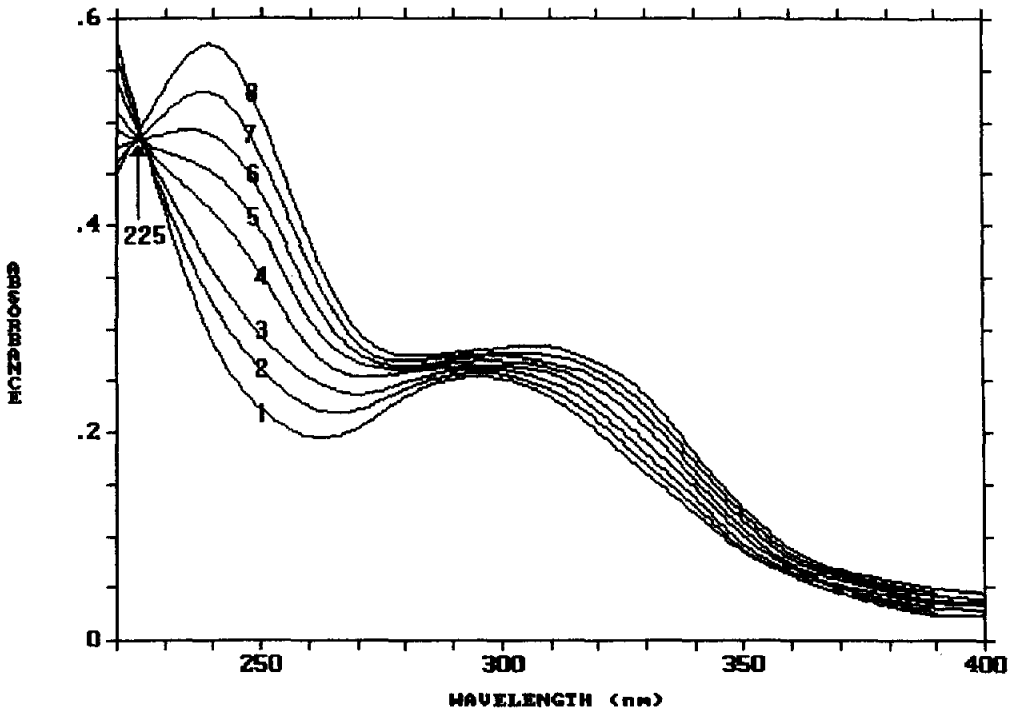

Fig. 7. The effect of iron ions upon BP in non-micellar aqueous solution ($c = 10^{-4}$ M) shown by the time dependence of difference UV-Vis absorption spectra for a molar ratio $BP–Fe^{3+} = 2:1$; $\Delta t = 6$ min and curve 1 corresponds to $t = 0$.

These equations in fact describe the general variation of absorbance. If a better correlation is sought, a change of slope is noted after 24 min. For example, for a wavelength of 260 nm the following linear equations are obtained:

$y'_1 = 2.251x + 0.408$:

variance = 0.00124:

max. deviation = 0.00353

and

$y'_2 = 0.736x + 0.793$:

variance = 4.89×10^{-4}:

max. deviation = 0.00437

The intercept indicates the time when the reaction between the degradation product of BP and iron ions begins. The same intercept is obtained for solutions of concentrations 10^{-3} M.
(b) BPK/Fe^{3+} molar ratio = 3:2;

$\Delta A(260\text{ nm}) = 0.00117t + 0.032$:

variance = 4.58×10^{-2}:

max. deviation = 0.00180 (3)

For the 10^{-3} M solution the slopes of the variation for the two intersecting straight lines are about four times smaller than in the case of 2:1 molar ratio, which suggests a limitation of hydrolytic degradation.

In conclusion, in dilute solution the trivalent iron ion can be considered to be present in one of the hydrohydrated forms: $FeCl_3 \rightarrow Fe^{3+} + 3Cl^-$; $Fe^{3+} + 6H_2O \rightarrow [Fe(H_2O)_6]^{3+}$; $[Fe(H_2O)_6]^{3+} \rightleftharpoons [Fe(H_2O)_5OH]^{2+} + H_3O^+$ and $[Fe(H_2O)_5OH] \rightleftharpoons [Fe(H_2O)_4(OH)_2]^+ + H_3O^+$. In such forms, the iron ion attacks the βL carbonyl to which it is bonded by breaking the βLT ring to form penicilloate; this is confirmed by the results of the analyses of the slightly soluble product. It was expected that the iron ion would be bonded to the carboxylic oxygen and form a co-ordinated covalent bond with the thiazolidinic nitrogen atom. This becomes more likely in the concentration range where the self-associated molecules appear and especially over the micellar range; a prominent role is played by the molecular conformation. The

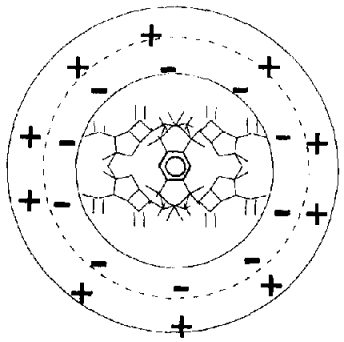

Fig. 8. The model of a BPK micelle.

folded conformation on the part of the side-chain characteristic to the solid state is retained in solution. While in dilute aqueous solution the BP molecule can rotate freely about the amide group on the side-chain, the micellar state restricts the rotational degrees of freedom; the molecular conformation foreshadows that of the solid state [5]. In the micelle (Fig. 8), the molecule is oriented with the aromatic ring towards the hydrophobic core and with the ionized carboxylic group of the βLT nucleus on its periphery, it makes up the negatively charged cover. The groups with this character, the βLT carbonyl and the amidic groups on the side-chain, lie inside the hydrated zone of the micelle, where the reduced number of water molecules in contact with the biologically active group (βL carbonyl) limits the hydrolytic degradation. The micelle cover consists of an electric double layer created by the potassium ions and by the ionized carboxylic groups. When $FeCl_3$ is added to BPK micellar solution, the iron ions are oriented towards the positive layer and settle among the potassium ions. Because these ions can bond the ionized carboxylic groups from the immediate neighbourhood and accept on a d orbital the lone pair of the thiazolidinic nitrogen atom, the highly organized system is perturbed and in consequence a slightly soluble product is formed. If the iron ions penetrate with their hydroxyhydrated part into that part of the micelle, they form contacts with the βL carbonyl group and catalyze the hydrolytic degradation reaction; the penicilloic acid formed is finally complexed by the iron ion; a metal–oxygen bond with its car-

boxyl group and a coordinated bond with the amidic nitrogen are formed. The confirmation of both mechanisms is supported by the experimental data provided by the methods of analysis utilized in the study of the slightly soluble product. The assertion that as a result of the perturbation of the self-associating system with a high degree of organization, the iron ion can also interact with the βL carbonyl group, is supported by the hydrolytic degradation demonstrated by spectrophotometric measurements in non-micellar solutions.

4. Conclusions

The effect of Fe^{3+} ions upon aqueous solutions of benzylpenicillin depends on the degree of their self organization. In non-micellar solutions, depending on the metal–ligand molar ratio, either soluble complexes formed with the primary hydrolytic degradation product (penicilloic acid), or, in competition, complexes of the type mentioned in the case of micellar solutions (by Fe–O carboxylic bond and $N_{\beta LT} > Fe$ covelent coordinated bond) are formed, following a reaction conditioned by the dissociation of BPK, a salt of a weak acid with a strong base. The effect of trivalent iron ions upon BPK in micellar and non-micellar aqueous solutions is important from a technological and especially from pharmacological viewpoints. Thus for average doses of 5 400 000 UI day^{-1}, taking into account that 3 h after administration, 1.2 $\mu g\ ml^{-1}$ remains in the body and that the treatment lasts 6 days, the accumulation of the antibiotic in the blood amounts to about 1.15×10^{-3} M, a concentration at which the first associated forms begin to form, and which anticipates the premicellar and micellar states [5]. For a body with normal renal functioning, the antibiotic has a half-life of 0.65 h. In the case of a patient with renal insufficiency the half-life is 7–10 h; therefore the accumulation of the antibiotic in the blood is much greater and consequently the incidence of anaemia will probably be higher.

References

[1] R. Hay and A.K. Basak, J. Chem. Soc., Dalton Trans., (1989) 197–201.
[2] W. Sher, M. Veber et al., Vestn. Slov. Kem. Drugs., 35 (1988) 297–306.
[3] A. Tiwari, H. Sharma et al., Indian J. Chem., Sect. A, 19A (1980) 83–84.
[4] N.P. Gensmantel, P. Nigel and P. Proctor, J. Chem. Soc., Perkin Trans. 2 (1980) 1725–1732.
[5] N. Carp, V. Tufoi and M. Carp, Analyst, 119 (1994) 1087–1091.
[6] A.B.P. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, 1968, p. 295.
[7] J. Dziegielewski, J. Hanuza and B. Jezowska-Trzebiatowska, Bull. Acad. Pol. Sci., Ser. Sci. Chim., 21 (1973) 643–656.