Fluorescence Enhancement of Levosulpiride in Presence of Metal Ions and Its Analytical Applications

INTRODUCTION

Levosulpiride, a levo enantiomer of sulpiride, chemically known as 5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy benzamide is used as antipsychotic, antidyspeptic and antiemetic medicine (Fig.1). This drug has also been used for the treatment of male sexual disorder and a dose of 25 mg/day for 60 days results in complete recovery. These studies are concerned mainly with the validation and determination of levosulpiride in human beings. The drug is fairly stable in human serum and urine which has been tested in different patients. It has been found that the drug can be detected in a concentration range of 0.25–200 ngml⁻¹ in human serum and 0.2–20 µgml⁻¹ in urine by HPLC [1,2]. The bioavailability of the drug at a dose of 100–200 mg/day is approximately 20–30% only [3]. Levosulpiride has both antiemetic and prokinetic properties because it antagonizes dopamine receptor in the central nervous system and gastro-intestinal tract [4,5]. Recently, it has been shown that, levosulpiride has moderate agonistic effect on 5HT4 receptors in the nervous system and is useful in the treatment of depression or
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Schizophrenia [6]. Patients treated for functional dyspepsia over a period of one month did not show any adverse effect except for fatigue, headache and drowsiness [7]. It is, therefore, considered as an effective and safe drug for the treatment of dyspepsia. It has been shown that the levosulpiride does not interfere with most of the drugs but, drinking during this period should be avoided. As a precaution, the drug should be avoided in pregnancy and during lactation period.

The major work done on the sulpiride thus far, concerns its identification, determination, efficacy against dyspepsia, psychosis and male sexual disorder. However, absorption and fluorescence emission spectrophotometric behavior of the drug under physiological condition and its interaction with several cations has not been studied so far.

In the present work an attempt has been made to study the interaction of the drug with metal ions by fluorescence emission, spectrophotometric and potentiometric measurements. Since enhancement in fluorescence intensity of the drug in presence of a metal ion occurs, the spectra of the drug in presence of different concentrations of several metal ions were scanned. The absorption spectra of the drug was run at different pH to see the zwitterionic condition, apparent ionization constant and the isosbestic point indicating the presence of different species in solution. Composition of the drug to metal ions and
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stability constant of the complex were determined by Job's method. In this we have developed a simple, economic and highly sensitive fluorometric method for the determination of levosulpiride, with Al(III) and Fe(III). The metal-drug interaction may result in the formation of stable complex which not only affect the bioavailability of the drug but may also deplete the blood with trace element. Both of these methods were applied for the determination of levosulpiride in dosage form.

EXPERIMENTAL

Instruments

The absorption spectra were obtained with Elico-SL-169 double beam UV-vis spectrophotometer. Fluorescence emission spectra were scanned with Hitachi-F-2500FL-spectrophotometer. All potentiometric measurements were done with Elico-LI-120 pH meter. These instruments were used throughout.

Materials and methods

Double distilled water was used throughout. Levosulpiride (Sun Pharmaceutical Industries Ltd., Jammu, India), sodium hydroxide (Merck Ltd, Mumbai, India) zinc chloride (SDH Pvt. Ltd India), and HCl (Ranbaxy Fine Chem. Ltd, India) were used as received.
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Preparation of solution

Stock solution of levosulpiride and those of metal salts (1×10^{-2}M) were prepared in double distilled water. Stock solution of drug was stored at 4 °C. Acetate buffer of pH 3.0–5.8 were prepared by mixing appropriate volume of acetic acid and 0.2M sodium acetate.

Methods for determination of interaction of drug with metal ions

Spectrophotometric method

Working solution of equimolar concentration (1×10^{-4}M) of levosulpiride and metal ions was prepared. The pH of the drug was adjusted between 2.31–10.60 by sodium hydroxide and hydrochloric acid (1×10^{-1}M to 1×10^{-2}M) to avoid interactions with buffer solution. The absorption spectra were recorded in the range 200–330 nm. The ratio of metal to levosulpiride was determined by Job's method.

Potentiometric method

For potentiometric study the following solutions were titrated against standard NaOH. The ionic strength was maintained at 0.1M by NaCl and the total volume was kept at 50 ml by adding appropriate amount of water.

(a) 5 ml (1×10^{-1}M) HCl + 5 ml (1×10^{-1}M) NaCl + 40 ml water.
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(b) 5 ml (1×10^{-1}M) HCl + 5 ml (1×10^{-1}M) NaCl + 10 ml (5×10^{-3}M) drug + 30 ml water.

(c) 5 ml (1×10^{-1}M) HCl + 5 ml (1×10^{-1}M) NaCl + 10 ml (5×10^{-3}M) drug + 20 ml (5×10^{-4}M) metal ions + 10 ml water.

Fluorescence spectrophotometric method

Solution of the levosulpiride (7×10^{-6}M) and those of metal ions (1.2×10^{-6}–8.4×10^{-6}M) were prepared. The steady state fluorescence spectra of the drug were recorded at the $\lambda_{em} = 270$–430 nm with $\lambda_{ex} = 275$ nm. This wavelength was chosen in order to avoid the inner-filter effect and to obtain the most complete emission spectrum.

Fluorescence Spectrophotometric method for the determination of levosulpiride

Calibration graph of the complexes with Fe(III) and Al(III)

A suitable aliquot containing different amounts of levosulpiride (0.310–3.414 µg/ml) and fixed amount of Fe(III) (1.0 ml of 3.6×10^{-5}M) and, similarly (0.310–2.730 µg/ml) of levosulpiride and fixed amount of Al(III) (1.0 ml of 3.6×10^{-5}M) were taken in 10 ml flasks. One ml acetate buffer of pH 5.6 was added and the volume was made up to the mark with distilled water. The fluorescence intensity of each solution was measured at $\lambda_{ex} / \lambda_{em} = 275/300$. 

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**Procedure for the assay of Tablets**

Twenty tablets were finely powdered, weighed according to the requirement and dissolved in 100 ml of water. It was filtered and stored at 4 °C and, further dilution was made from this stock solution.

**RESULTS AND DISCUSSION**

**Determination of stoichiometry and association constant of the complexes**

**Spectrophotometric study**

The absorption spectrum of levosulpiride ($1 \times 10^{-4}$M) was recorded in the region 200–330 nm. It exhibited two peaks at 216 and 295 nm (Fig. 2). Since the first peak is very strong it was selected for further absorption studies. When the spectra of the drug were run at varying pH, two isosbestic points, one at 255 and another at 275 nm were observed which indicated the presence of different chemical species in solution [8] in equilibrium with each other (Fig. 3). The apparent ionization constant ($pK_a'$) of the drug was calculated (Table 1) by the following equation.

$$pK_a' = pH + \log \left\{\frac{A_I - A_M}{A - A_M}\right\}$$

(1)

where, $A_I$ = Absorbance of drug in basic medium, $A_M$ = Absorbance of drug in acidic medium, $A$ = Absorbance of drug in aqueous medium. The metal to
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levosulpiride ratio was determined by continuous variation method (Fig.4) at their respective $\lambda_{max}$ (Table 1). It was found that two moles of the drug are bonded to one mole of the metal ion which seems quite reasonable because, of all the donor groups the amide nitrogen appears to be the most plausible site for coordination as it is the proper combination of hard acid and hard base. The stability constant of the complexes were calculated by the following equation:

$$K = \frac{A/A_{ex} C_X}{(C_M - A/A_{ex} C_X) (C_L - nA/A_{ex} C_X)^n}$$

where $K$ = stability constant, $n = X/(1-X)$ and $X$ = mole fraction of the ligand at maximum absorption. $A/A_{ex}$ is the ratio of the observed absorbance to that indicated by the tangent for the same wavelength. $C_X$, $C_M$ and $C_L$ are the limiting concentrations of the complex, metal ion and the ligand, respectively [9, 10]. The value of log$K$ is shown in Table 2.

Potentiometric study

The acid dissociation constant of the drug was first determined from the titration curve for HCl in the presence and absence of the drug (Fig.5). The $\bar{n}_A$ of levosulpiride at different pH corresponds to one dissociable proton. The formation curve was obtained by plotting the average number of ligand attached per metal ion ($\bar{n}$) versus free ligand exponent (pL) [11,12]. Since the
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maximum value of \( \bar{n} \) was about 2, it is presumed that complex is formed in 2:1 (Drug: Metal) ratio. Low concentration of metal ion solution was used to prevent the formation of polynuclear complexes in solution [13]. All calculation of stability constant were made in the low pH region to prevent the formation of hydroxo species (e.g. \([ML(OH)]\), \([MS_{x-1}(OH)]^+\) (where L is ligand, S is the solvent). The negative value of \( \Delta G \) indicates that the chelation process proceeds spontaneously. This is evaluated by both potentiometric and spectrophotometric methods.

Fluorescence enhancement study

The fluorescence emission spectrum of pure drug is markedly different from its absorption spectrum in UV region, which is attributed to its different geometry in the ground and excited states (Fig. 6). When the emission spectrum of pure drug is run between 270–430 nm at excitation wavelength of 275 nm, a strong peak at 300 nm and two weak emission peaks at 342 and 406 nm were observed (Fig. 7).

It is a ubiquitous fact that the addition of the metal ion to drug can cause either enhancement or quenching in the fluorescence intensity [14]. We have however, noted an enhancement in fluorescence intensity at 300 nm in each case (Mn-Zn). The shape and position of emission spectra of levosulpiride in
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presence and absence of transition metal ions remains unchanged which suggests that no significant change occurs in the overall electronic structure of drug the upon addition of metal ions (Fig.8). The relative enhancement in the fluorescence intensity $I/I_0$ are shown in table 3.

The enhancement in emission intensity of levosulpiride in presence of various metal ions follows the order Fe(III)$>$Ni(II)$>$Cu(II)$>$Zn(II)$>$Co(II)$>$Mn(II) (Fig.9). It does not follow any trend from Mn-Zn, although it is minimum in the case of Mn probably due to its half filled 'd' orbital. Although the transition metal ions are known to effectively quench fluorescence [15,16,17] we observed over two fold increase in intensity as a consequence of photo induced charge transfer besides the chelation of the amide nitrogen resulting in CHEF (Chelating enhancement fluorescence) effect [18,19,20]. It is known that aromatic compounds containing amide groups are strongly quenched by intersystem crossing to a triplet state and/or by the rotational relaxation linked to excited state rotation around the CO-NH and N-alkyl bonds [21]. An increase in rigidity of the system by metal ion complexation may be the reason for fluorescence enhancement. It may also be due to an increase in the redox potential of the donor so that the relevant HOMO energy decreases to a lower level than that of fluorophore. Consequently, the excited state energy of fluorophore is dumped as a visible emission [14]. The reason of this
fluorescence enhancement of levosulpiride

enhancement lies in strong perturbation of the excited state upon coordination of the metal ion. A low lying internal charge transfer state due to the presence of electron donor and acceptor group in the levosulpiride is the lowest excited state. This is however, a less emitting state and is in equilibrium with $\pi-\pi^*$ excited state of the molecule. The PCT interaction becomes weaker since the electron withdrawing group is now electron rich moiety due to the deprotonation of $-\text{NH}$ group necessary for coordination of metal ion.

**Spectrofluorimetric method for the determination of levosulpiride**

The fluorescence emission spectra of levosulpiride showed enhancement in intensity (Fig. 10) upon addition of Al(III) without any change in emission wavelength and shape of the peak. Al(III) showed maximum enhancement followed by Fe(III). Only these two metal ions are utilized for the determination of levosulpiride due to their maximum sensitivity. The influence of several variables on the formation of levosulpiride-metal complexes has also been discussed.

**Influence of metal ion concentration**

The influence of metal ion concentration was studied in the range $0.3\times10^{-6}$ to $8.0\times10^{-6}$M at 25 and 35 °C, while the concentration of levosulpiride was held constant at $7\times10^{-6}$M. It was observed that fluorescence intensity
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increases linearly with concentration of metal ions up to $3.6 \times 10^{-6}$ M and, above this concentration there is no further significant change.

**Influence of temperature and order of addition of reagents**

The influence of temperature (Fig. 11) on the formation of complexes was studied between 25 to 80 °C. Since the fluorescence emission intensity between 25–50 °C remains almost constant the subsequent experiments were done at 25 °C. The fluorescence intensity decreases above 50 °C due to dissociation of complexes at higher temperature.

In all experiment the order of addition of the reagents was the same viz, levosulpiride, metal ion and buffer solution.

**Influence of pH and buffer type**

The influence of pH (Fig. 12) on fluorescence intensity of the complexes was investigated over the pH range 2.5–8.0. The results were similar for all complexes. The fluorescence intensity of complexes increase up to pH 5.6 and remained almost constant between 5.5 and 7. For pH>7 the fluorescence intensity greatly decreased due to hydrolysis of metal ions. Maximum fluorescence intensity was observed at pH 5.6 in acetate buffer and therefore this pH was maintained in all experiments.
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Analytical performance

Validation of the proposed methods

The validity of the method for linearity, specificity, accuracy and precision according to ICH Q2B recommendations was tested (Table 4) [22]. The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected. The LOQ and LOD were calculated according to the following equation:

\[
\text{LOQ} = 10 \sigma / S \tag{3}
\]

\[
\text{LOD} = 3.3 \sigma / S \tag{4}
\]

where \( \sigma \) = standard deviation of the intercept of regression line and \( S \) = slope of the calibration curve. The proposed methods were evaluated for the accuracy as percent relative error (\% Er) and the precision as percent relative standard deviation (\% RSD) (Table 5).

Accuracy

The accuracy of both the methods was established by performing five replicate on solutions containing three different amounts (within linearity range) and calculating the percentage error (Table 5).
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Precision

The precision assay was done by replicate analysis (n = 5) 0.5, 1.5, 3.0 μgml⁻¹ (with Fe(III)) of levosulpiride while 0.8, 1.7, 2.6 μgml⁻¹ (with Al(III)) were taken for analysis. It is clearly visible from the results (Table 5) that the %RSD value for precision are lower than 0.800 for Fe(III) and 0.867 for Al(III).

Repeatability

To test the repeatability of the proposed method, five replicate analyses were done. The mean percentage recovery was found to be 100.3 ± 0.007 and 100.5 ± 0.011 using Fe(III) and Al(III) respectively.

Robustness of the method

The robustness of the method is demonstrated by the consistency of the fluorescence intensity with the deliberate minor changes in the experimental parameters such as volume of metal ions (1.0 ± 0.1) ml and those of buffers (1.0±0.1 ml). These minor changes did not affect the fluorescence intensity of the reaction product.

CONCLUSION

The effect of cation binding on the photophysical properties of
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levosulpiride has been studied. A dramatic enhancement in fluorescence intensity was observed upon binding of metal ions. This was interpreted in terms of the control of photoinduced charge transfer (PCT) and CHEF (Chelating enhancement fluorescence). The stability constants calculated spectrophotometrically and potentiometrically suggest that the complexes are fairly stable in solution. Methods for the spectrofluorimetric determination of levosulpiride were developed using its fluorescence enhancement of drug with Fe(III) and Al(III). The methods are simple, sensitive and economical with good accuracy and precision; do not require any pretreatment of the drug and tedious extraction procedure.
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Fig. 1 Structure of levosulpiride
**Fluorescence Enhancement of Levosulpiride**

![Absorption Spectrum](image)

**Fig. 2** Absorption spectrum of levosulpiride ($1 \times 10^{-4} M$) at pH 5.6 at room temperature

![Absorption Spectra](image)

**Fig. 3** Absorption Spectra of levosulpiride ($1 \times 10^{-4} M$) at different pH at room temperature
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Fig. 4(A) Continuous variation plot of levosulpiride with Mn(II) at 25 °C

Fig. 4(B) Continuous variation plot of levosulpiride with Fe(III) at 25 °C
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Fig. 4(C) Continuous variation plot of levosulpiride with Co(II) at 25 °C

Fig. 4(D) Continuous variation plot of levosulpiride with Ni(II) at 25 °C
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**Fig. 4(E) Continuous variation plot of levosulpiride with Cu(II) at 25 °C**

**Fig. 4(F) Continuous variation plot of levosulpiride with Zn(II) at 25 °C**
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Fig. 5 Potentiometric titration curve of levosulpiride with transition metal ions
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Fig. 6 Showing different ionic species in the solution at different pH.
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Fig. 7 Fluorescence Emission spectrum of levosulpiride ($7 \times 10^{-6} M$) at room temperature

Fig. 8(A) Fluorescence enhancement ($\lambda_{em} = 300$ nm) of levosulpiride with Mn(II) at room temperature
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**Fig. 8(B)** Fluorescence enhancement ($\lambda_{em} = 300$ nm) of levosulpiride with Fe(III) at room temperature

**Fig. 8(C)** Fluorescence enhancement ($\lambda_{em} = 300$ nm) of levosulpiride with Co(II) at room temperature
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Fig. 8(D) Fluorescence enhancement ($\lambda_{em} = 300$ nm) of levosulpiride with Ni(II) at room temperature

Fig. 8(E) Fluorescence enhancement ($\lambda_{em} = 300$ nm) of levosulpiride with Cu (II) at room temperature
Fluorescence Enhancement of Levosulpiride

Fig. 8(F) Fluorescence enhancement ($\lambda_{em} = 300$ nm) of levosulpiride with Zn(II) at room temperature

Fig. 9 The enhancement in emission intensity of levosulpiride in presence of various metal ions follows the order Fe(III) > Cu(II) > Ni(II) > Zn(II) > Co(II) > Mn(II)
Fig. 10 Fluorescence enhancement of levosulpiride in absence and presence Al(III)

Fig. 11 Effect of temperature on the fluorescence intensity of levosulpiride metal complexes
Fig. 12 Effect of pH on the fluorescence intensity of levosulpiride metal complexes
### Fluorescence Enhancement of Levosulpiride

#### Table 1. Apparent ionization constant (pKa') of the drug

<table>
<thead>
<tr>
<th></th>
<th>Potentiometrically</th>
<th>Spectrophotometrically</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>8.753</td>
<td>8.980</td>
</tr>
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</table>

#### Table 2. The Stability constant of metal complex of levosulpiride

<table>
<thead>
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<th>Metal ions</th>
<th>Potentiometrically</th>
<th>Spectrophotometrically</th>
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<tr>
<td></td>
<td>logK</td>
<td>- ΔG (J/mol)</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>8.82</td>
<td>50325.57</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>8.91</td>
<td>50821.39</td>
</tr>
<tr>
<td>Co(II)</td>
<td>9.03</td>
<td>51512.39</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>8.90</td>
<td>50753.51</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>9.17</td>
<td>52345.44</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>9.04</td>
<td>51609.39</td>
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Table 3. The relative intensity and limiting concentration

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>$I/I_0$</th>
<th>Limiting concentration</th>
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<tbody>
<tr>
<td>Mn(II)</td>
<td>1.24</td>
<td>3.6 μM</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>3.13</td>
<td>3.6 μM</td>
</tr>
<tr>
<td>Co(II)</td>
<td>1.52</td>
<td>3.6 μM</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>2.41</td>
<td>3.6 μM</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>2.37</td>
<td>3.6 μM</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>1.53</td>
<td>3.6 μM</td>
</tr>
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</table>
Table 4. Statistical data of calibration graph for the determination of Levosulpiride using proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>With Fe(III)</th>
<th>With Al(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation</td>
<td>$\Delta F = 182.55x + 3.8519$</td>
<td>$\Delta F = 257.58x + 1.271$</td>
</tr>
<tr>
<td>Range (µg ml$^{-1}$)</td>
<td>0.239-3.414</td>
<td>0.310-2.730</td>
</tr>
<tr>
<td>S.D. of residual ($S_{vy}$)</td>
<td>0.4187</td>
<td>0.2985</td>
</tr>
<tr>
<td>S.D. of intercept ($S_{a}$)</td>
<td>0.2820</td>
<td>0.2466</td>
</tr>
<tr>
<td>S.D. of slope ($S_{b}$)</td>
<td>0.1355</td>
<td>0.1375</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9998</td>
<td>0.9998</td>
</tr>
<tr>
<td>Variance ($S_{vy}$)$^2$</td>
<td>0.1753</td>
<td>0.0891</td>
</tr>
<tr>
<td>Limit of detection (µg ml$^{-1}$)</td>
<td>0.0051</td>
<td>0.0032</td>
</tr>
<tr>
<td>Limit of quantification (µgml$^{-1}$)</td>
<td>0.0154</td>
<td>0.0096</td>
</tr>
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Number of standard samples, $n = 7$, $\Delta F = F - F_o$, where $F_o$ is the fluorescence of levosulpiride in absence and presence of metal ions presence of metal ions and $x$ is concentration in µgml$^{-1}$
Table 5. Accuracy and precision for the determination of Levosulpiride using proposed methods

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Added amount (µgml⁻¹)</th>
<th>Found ± SD (µgml⁻¹)</th>
<th>% Er⁺</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
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<tr>
<td>Fe(III)</td>
<td>0.5</td>
<td>0.504 ± 0.004</td>
<td>0.880</td>
<td>0.800</td>
<td>100.8</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.502 ± 0.007</td>
<td>0.115</td>
<td>0.463</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.00 ± 0.010</td>
<td>0.096</td>
<td>0.351</td>
<td>100.1</td>
</tr>
<tr>
<td>Al(III)</td>
<td>0.8</td>
<td>0.804 ± 0.007</td>
<td>0.467</td>
<td>0.867</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>1.712 ± 0.014</td>
<td>0.694</td>
<td>0.810</td>
<td>100.7</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>2.606 ± 0.013</td>
<td>0.223</td>
<td>0.510</td>
<td>100.2</td>
</tr>
</tbody>
</table>

⁺Mean ± S.D. for five determinations, ⁺Percentage of relative error, ⁼Percentage relative error of S.D.
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REFERENCES


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