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Original Article

Ethyl Maltol as a New Ligand for Spectrophotometric Determination of Iron

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Abstract

In this study a new simple selective and sensitive spectrophotometric procedure for determination of Fe(III) is described. It is based on the formation of a colored complex between ferric iron and ethyl maltol, a strong and highly selective ligand for Fe(III). After mixing sample and reagent, and incubating at the room temperature, Fe(III)-ethyl maltol complex was extracted with different solvents and the absorbance was measured at 395 nm. The effect of analytical variables, i.e. amount and type of the reagents, pH, ratio of Fe(III)/ethyl maltol, presence of other ions, etc., in the determination of iron were studied. Our findings showed that the optimum wavelength for the measurement was 395 nm. The optimum condition for complex formation and determination of Fe(III) were: molar ratio of ethyl maltol/Fe(III) = 6-10; pH = 5. The best solvent for extraction was chloroform. Under the recommended conditions, formation of the complex is completed in less than 2.5 h. Limit of detection was found to be 2.5×10^{-6} M of Fe(III). Linear regression (r^2=0.9998) was observed over the range of 2.5×10^{-6} to 5×10^{-4} M of the Fe(III) with respect to the complex nominal concentration. Ions commonly associated with iron did not interfere in the present method. This is a simple, reproducible, and sensitive method for determination of Fe(III) in μmolar levels.

Keywords: Determination; Ethyl maltol; Iron; Ligand; Spectrophotometry.
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1. Introduction

The recognized importance of iron in biological and environmental systems has resulted in the establishment of many methods for its determination in a variety of environmental systems where iron is usually present at $\mu g.l^{-1}$ levels [1-4]. Several techniques have been proposed for the determination of iron species in natural samples, including spectrophotometry [5-6], atomic absorption spectrometry (AAS) [7], inductively coupled plasma-mass spectrometry [8], cathodic striping voltammetry [9], fluorimetry [10], ion chromatography [11-12], etc.

Analytical laboratories need validated

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methods of analysis for determining essential metals, such as iron, in a wide range of matrixes. Validation of an analytical method is a necessary step in controlling the quality of quantitative analysis, and can be defined as the process by which it is established that the analytical parameters of the method meet the requirements for the intended analytical applications.

Although, atomic absorption spectrometry can determine iron at ng.ml^{-1} levels, several metals were shown to interfere [1]. Among the most widely applied methods are those based on spectrophotometry, as they are experimentally rapid and simple with wide applications. Most spectrophotometric techniques involve ligands that selectively bind iron, or a particular redox state of iron, to produce a colored complex with a high molar absorptivity. The iron-ligand complex can subsequently be detected spectrophotometrically. Iron selective ligands such as thiocyanate [13-14], 2,2-bipyridyl [15-16] or 2,2,2-tripyridyl [17] were the first selective reagents used for the determination of iron. As in most of these techniques, Fe(II) is involved in color-generation reactions with an appropriate ligand [20], Fe(III) is then determined by subtracting the concentration of Fe(II) from total iron, determined either by reducing Fe(III) or by conventional non-selective methods [18-19]. The differential approach, however, often yields highly imprecise values for Fe(III) when the Fe(II) concentration is higher than that of Fe(III) [20], and most of the methods mentioned above lack sufficient sensitivity for iron determination at µM or sub-µM levels. For this reason, ferrozine has been widely used for spectrophotometric determination of Fe(II), because its use results in a sufficiently low detection limit and low blank values [4, 21].

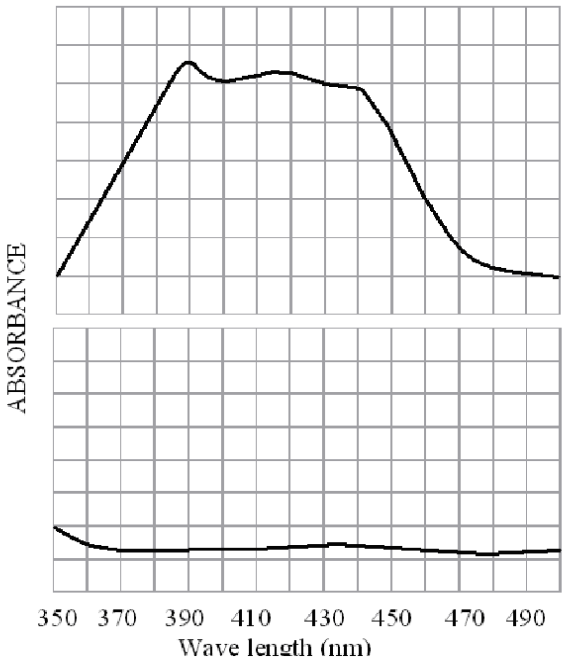

Figure 1. Absorption spectra of the ethyl maltol-Fe(III) complex (A) against reagent blank (B). The concentration of Fe(III) was kept at 5×10^{-5} M. pH=5, ethyl maltol:Fe(III) molar ratio=1:10, t = 2.5 h.

A potential problem with the classical ferrozine method is the incomplete reduction of organically complexed Fe (III) [22]. This is probably why different reducing agents (mostly ascorbic acid and hydroxylamine hydrochloride) are reported to be optimum for that purpose [23, 24]. Several studies have also demonstrated that Fe (III) in solution can also react with ferrozine, thereby interfering with the coloration of the ferrous complex [23, 25]. To overcome these problems, increasing interest has, therefore, focused on the development of methods to directly determine Fe (III).

Ethyl maltol, one of the hydroxypyranons compound group of iron chelators, shows promise as potential compounds for the treatment of iron overload by the oral route. Ethyl maltol binds Fe^{3+}, but not Fe^{2+} at acid pH to give a colored complex. The selectivity and high complex formation constant of these compounds with iron makes them good candidates for iron determination [26, 27]. The aim of this study was to propose a validated fast, sensitive and selective spectrophotometric method for the determination of Fe(III) ions using ethyl maltol as ligand.

2. Matherials and methods

2.1. Chemicals

All reagents used were of the highest available purity (at least analytical grade). Drugs used were: Iron (III) nitrate, ethyl maltol, ethanol, octanol, isopropyl alcohol, aceton, dichloromethane, chloroform, hydrochloric acid, potassium nitrate, magnesium nitrate, aluminium nitrate, sodium nitrate (Merck, Germany), magnesium sulphate, sodium chloride, sodium bicarbonate, sodium hydroxide (Aldrich).

2.2. Preparation of solutions

1. Standard Fe(III) solution (0.01 M): 404 mg of ($FeNO_3 \cdot 10H_2O$) was dissolved in 100 ml doubled distilled deionised water. The working solutions were prepared just before use.

2. Ethyl maltol (0.01 M) stock solution: 117 mg ethyl maltol was dissolved in 100 ml of double distilled deionised water. The working solutions were prepared just before use.

3. Standard solutions of KNO_3, $MgNO_3$, $NaCO_3$, $NaNO_2$, NaCl, Na_2SO_4, $CaCl_2$,

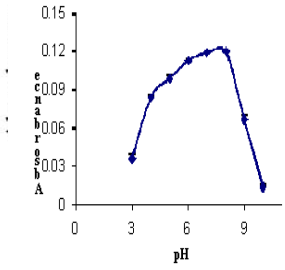

Figure 2. The effect of pH on the absorbance of ethyl maltol-Fe(III) complex. The concentration of Fe(III) was kept at 5×10^{-5} M, Ethyl maltol:Fe(III) molar ratio=1:10, t=2.5 h, λ=395 nm. Each data point plotted represents the mean absorbance value for nine replicate absorbance readings. Error bars represent the standard deviation between consecutive measurements of each sample.

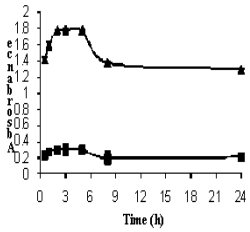

Figure 3. The effect of time on ethyl maltol-Fe(III) complex formation. The concentration of Fe(III) was kept at 2.5×10^{-5} M (square) or 1×10^{-4} M (triangle). pH=7, ethyl maltol:Fe(III) molar ratio=1:10, λ=395 nm. Each data point plotted represents the mean absorbance value for nine replicate absorbance readings. Error bars represent the standard deviation between consecutive measurements of each sample.

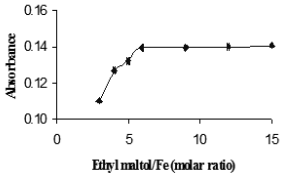

Figure 4. The effect of increasing ratio of ethyl maltol to Fe(III) on the absorbance of the ethyl maltol-Fe(III) complex. The concentration of Fe(III) was kept at 7.5×10^{-6} M. pH=7, t=2.5 h, λ=395 nm. Each data point plotted represents the mean absorbance value for nine replicate absorbance readings. Error bars represent the standard deviation between consecutive measurements of each sample.

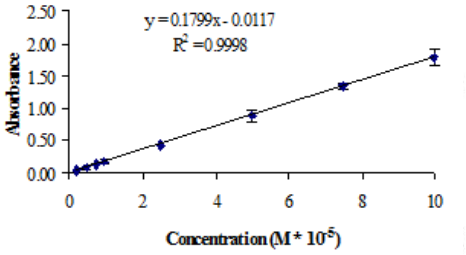

Figure 5. Standard curve for the determination of iron(III) with ethyl maltol-Fe(III) complex formation. pH=7, ethyl maltol:Fe(III) molar ratio=1:10, t = 2.5 h, λ=395 nm. Each data point plotted represents the mean absorbance value at 395 nm for six replicate absorbance readings. Error bars represent the standard deviation between consecutive measurements of each sample.

$CuSO_4$ and Al $(NO_3)_3$ were prepared by dissolving an appropriate weight of them in redistilled deionised water.

2.3. Instrumentation

The spectrophotometric analysis was performed on a double beam spectrophotometer Perkin Elmer 550S (USA) using 1 cm quartz cells with a slit width of 1 nm. Also a Perkin Elmer model 2380 atomic absorption spectrophotometer (AAS) (USA) equipped with an air: Acetylene burner was used for the iron determination. Hollow cathode lamp operating at 20 mA was employed for iron determination (air and acetylene flow rates 10 $l.min.^{-1}$ and 2 $l.min.^{-1}$, respectively; at 248 nm).

2.4. Analytical procedure

2.4.1. Calibration curves

An appropriate aliquot of studied Fe (III) 0.01 M was placed into a 10-ml calibration flask, pH was adjusted at 7 and ethyl maltol 0.01 M solution was added, and the flask was filled to the mark with redistilled deionised water. Under the experimental conditions, the absorption spectra of ethyl maltol and the Fe(III)-ethyl maltol complex were scanned at 300-600 nm. While ethyl maltol showed no absorption at 395 nm absorption maximum of Fe(III)-ethyl maltol complex was at 395 nm (Figure 1). So the absorption peak at 395 nm was chosen as determination wavelength.

2.4.2. General procedure

Into a 10-ml volumetric flask a 100 µl aliquot of the working Fe(III) solution (0.01 M) was transferred and 1 ml of ethyl maltol solution (0.01 M) was added. The absorbance of the resulting solutions was measured after 20 min. at 395 nm against control. To find out the optimum conditions, pH (3, 4, 5, 6, 7, 8, 9 and 10), time (0-24 h), ethyl maltol concentration (ethyl maltol/Fe molar ratio up

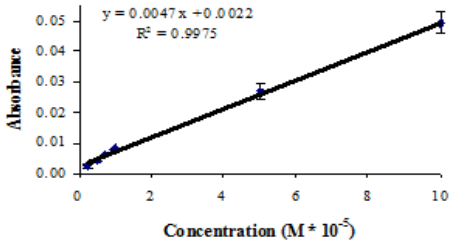

Figure 6. Standard curve for the determination of iron(III) with atomic absorption spectrometry. Each data point plotted represents the mean absorbance value at 248.3 nm for six replicate absorbance readings. Error bars represent the standard deviation between consecutive measurements of each sample.

to 15), extraction with different solvents and the effects of light were studied. The multivariate approach was used to optimize the working conditions; each optimum condition was, however, rechecked after standardizing those remaining. Each of the reported optimum conditions was established (by repeated trials), when others were kept at the optimum value. Iron concentrations in the working standard solutions chosen for the calibration curve were 2.5×10^{-6} to 1×10^{-4} M.

To increase the sensitivity of the method, solutions containing Fe(III)-ethyl maltol complex were extracted under the optimum conditions with different solvents (octanol, dichloromethane, chloroform). Also, studies were conducted to determine whether other ions interfered with the spectrophotometric determination of iron.

For the determination of Fe(III) with FAAS, the same concentrations of iron were prepared and their absorbance was measured at 248 nm.

3. Results and discussion

3.1. Effect of pH

The effect of pH on the reaction of ethyl maltol with Fe(III) is shown in Figure 2, indicating that pH 7-8 is the optimum pH for the complex formation. Therefore, pH 7 was used as the working value for all experiments.

3.2. Effects of time on complex formation and stability

Our findings from the optimization experiments showed that 2.5 h incubation time was adequate for quantitative complexation. The sample solution was examined in comparison with Fe(III) standard solution (5×10^{-5} M and 1×10^{-4} M) and no change in the absorbance was observed for up to 24 h (Figure 3). The stability of these solutions provides an indication of the method robustness.

3.3. Effect of ethyl maltol concentration

For accurate measurements, the absorbance of the complex must be independent of the concentration of the ethyl maltol. Under the optimum pH value, the effect of ethyl maltol concentration on the absorbance profile is illustrated in Figure 4. The molar ratio of ethyl maltol/Fe(III) 6-15 is sufficient for complete complex formation which gives a good safety margin. Considering the stoichiometry of the reaction between ethyl maltol and Fe(III) (1:3) [28], and that excess of ethyl maltol reagent did not affect the absorbance of the complex, its concentration was maintained in excess (molar ratio of 1:10) for experiments.

3.4. Other conditions

Although, temperature can effect complexation reactions, ambient temperature

Table 1. Changes on the absorbance after solvent extraction.

Solvent	Concentration of Fe(III) ($M\times10^{-6}$)	Absorbance±SD	
		Before extraction	After extraction
Chloroform	1	0.000±0.000	0.135±0.028
	5	0.082±0.006	0.420±0.030
	10	0.178±0.025	0.802±0.111
Dichloromethane	1	0.000±0.000	0.089±0.022
	5	0.082±0.006	0.322±0.030
	10	0.178±0.025	0.374±0.127
Octanol	1	0.000±0.000	0.014±0.007
	5	0.082±0.006	0.198±0.022
	10	0.178±0.025	0.410±0.108

pH=7, ethyl maltol:Fe (III) molar ratio=1:10, t=2.5 h, λ=395 nm. Each data point represents the mean absorbance value standard deviation of each sample for nine replicates.

Table 2. Interference of several species on the determination of iron.

Ions	Limiting concentration (molar ratio)
K^+	>1000
Na^+	>1000
Mg^{2+}	>1000
Ca^{2+}	>900
Al^{3+}	>700
NO^{2-}	>1000
SO_4^{2-}	>1000
HCO_3^-	>1000
Cl^-	>1000
NO_3^-	>1000

The concentration of Fe(III) was kept at 5×10^{-5} M, pH=7, ethyl maltol:Fe(III) molar ratio = 1:10, t = 2.5 h, λ = 395 nm.

conditions were applied throughout the experiment, enabling the *in situ* application of the method that is important for maintenance of the determination occurring in the sample. Our findings showed that light had no significant effect on absorbance up to 24 h.

3.5. Extraction with solvents

Our findings showed that extraction with chloroform, dichloromethane and octanol significantly lowered the detection limit of the employed methods (Table 1). Extraction with chloroform had the highest impact.

3.6. Interference studies

Chloride, nitrate, sulfate, and phosphate anions as well as several cations are present in natural water. Their presence can lead to a competition with the ligand (for complexation with iron), thereby reducing the overall enrichment. Studies were conducted (under the optimum conditions) to determine whether they could interfere with the spectrophotometric determination of iron. Different amounts of the potential interferences (K_3PO_4, KNO_3, NaH_2PO_4, $NaNO_2$, NaCl, $NaNO_3$, Na_2SO_4, $CaCl_2$, $MgSO_4$, $CuSO_4$ and $Al(NO_3)_3$) up to 1000 times molar ratio to that of Fe(III) were added to Fe(III) standard solutions (5×10^{-5} M and 1×10^{-4} M), and the absorbance were compared. The results from these studies are shown in Table 2. Tolerance limits were determined for a maximum error of 5 %. The tolerance limits of the electrolytes were found to be reasonably good. Ca^{2+} and Al^{3+} interfered negatively with the spectrophotometric measurements when present at 900 and 700 times the concentration of iron. The other cationic and anionic species investigated had no adverse effect on the analytical signal(s) of Fe.

Table 3. Intra-day variations.

Concentration of Fe(III) (M)	Calculated concentration ($M\times10^{-5}$)		
	Mean	SD	%CV
0.25	0.18	0.03	15.48
0.50	0.41	0.04	10.48
0.75	0.70	0.07	9.88
1.00	1.01	0.05	4.53
2.50	2.39	0.08	3.23
5.00	4.84	0.06	1.16
7.50	7.41	0.15	1.97
10.00	9.99	0.09	0.86

pH = 7, ethyl maltol:Fe (III) molar ratio = 10, t = 2.5 h, λ = 395 nm. Chloroform was used for extraction of complex. Data are presented as mean concentration for nine replicate. Standard deviation between consecutive measurements of each sample and percent of coefficient of variation are also presented.

3.7. Linearity

A linear relationship between absorbance and Fe(III) concentration was found under the described spectrophotometric conditions (2.5×10^{-6} M - 5×10^{-4} M) (Figure 5). Least squares regression equation with correlation coefficient r=0.9999 (n=9), y=0.18381x-0.0415.

3.8 Precision

To evaluate the precision of the methods, measurements were performed under conditions of repeatability and reproducibility. It was checked for the error attributable to sample handling and preparation and instrument response for a standard solution of Fe (III). The intra-day precision of the method was determined, under the optimal working conditions, by triplicate absorbance measure of the eight reconstituted preparations for measurement. For the determination of inter-day precision, repeat analyses of nine preparations over a 4-day period was carried out (Tables 3-4).

3.9. Limit of quantitation and detection

The detection limit of the proposed method was 5×10^{-6} M of Fe (III). The detection limit indicates the smallest amount of analyte that can be detected with a reasonable degree of confidence under specified conditions. This is usually defined as the concentration or mass of analyte yielding a signal equivalent to three times the standard deviation of the blank signal (signal at zero analyte) [27].

3.10. Comparison with other spectrophotometric methods

Several ligands with the ability to form a stable colored complex with Fe (III) have been used. The detection limit for Fe (III) when bitonol or 4-capril-3-methyl-1-phenyl-5-pyrazolone were used as complex forming ligands were 1.7×10^{-5} and 1.7×10^{-4} M, respectively [30, 31]. Also, using thiocyanate as ligand it is nessesary to use monoamino actyl amino banzyl phosphate to reduce inteference of some cations or anions [32]. The validity of the present method was checked with GF-AAS (Figure 6).

4. Conclusion

It can be concluded from our findings that the proposed method offers several noticeable advantages: 1. By this method, Fe (III) can be determined directly. 2. It is a fast method and the stability of colored complex is very good. The colored complex was stable up to 24 h. 3. Ethyl maltol as a new reagent for spectrophotometric determination of Fe (III) is inexpensive and available easily, it has very stable physicochemical properties and can be used to determine trace amount of Fe (III) conveniently. 4. The proposed method has very good selectivity. As nearly all the anions and cations that may coexist in samples

Table 4. Inter-day variations.

Concentration of Fe(III) ($M\times10^{-5}$)	**Calculated Concentration ($M\times10^{-5}$)**		
	Mean	**SD**	**%CV**
0.25	0.18	0.02	13.04
0.50	0.45	0.03	6.78
0.75	0.67	0.04	5.87
1.00	0.98	0.03	3.21
2.50	2.32	0.04	1.80
5.00	4.89	0.04	0.88
7.50	7.49	0.09	1.19
10.00	9.92	0.08	0.86

pH=7, ethyl maltol:Fe(III) molar ratio=10, t=2.5 h, λ=395 nm. Chloroform was used for extraction of complex. Data are presented as mean concentration for three days replicate. Standard deviation between consecutive measurements of each sample and percent of coefficient of variation are also presented.

containing iron could not interfere with the determination of Fe (III).

Acknowledgement

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