

## DETERMINATION AND PRECONCENTRATION OF MANGANESE USING IONIC LIQUID BASED MICROEXTRACTION TECHNIQUE IN BIOLOGICAL SAMPLES

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### ABSTRACT

*In this work, a procedure for preconcentration and determination of manganese using modified cold-induced aggregation microextraction (CIAME) based ionic liquid with the reagent 1-(2-pyridylazo)-2-naphthol (PAN) as complexing reagent was developed. In the preconcentration step, the mixture was cooled in an ice bath and manganese from 10 mL of an aqueous solution was extracted into 70 µL of ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate [Hmim][PF<sub>6</sub>]. After centrifuging the extraction phase was settled. Then, the absorbance of the complex was measured by UV-Vis spectrophotometry. In this study, the effect of matrix ions on the extraction efficiency of Mn<sup>+2</sup> were investigated. Also the optimization extraction conditions, such as: pH, PAN concentration, extraction time, addition of salt, ionic liquid volume and centrifugation rate were studied. The relative standard deviation was 1.14 % (n=4). The detection limit of 0.54 ngL<sup>-1</sup> and enrichment factor of 70 for Mn<sup>+2</sup> were achieved. The presented procedure was successfully applied for determination of manganese in environmental and biological samples.*

**Keywords:** Cold-induced aggregation microextraction Ionic Liquid, Manganese, UV-Vis spectrophotometry

### INTRODUCTION

Manganese has been called as a rare mineral. It's found in trace amounts in human body and plays a role in establishing tissue fat and cholesterol, bone, blood clotting factors and proteins. Manganese is a component of the composition of superoxide dismutase (Critchfield et al., 1993, Kazi et al., 2008). Therefore, it is important from an analytical point of view to develop sensitive methods for determination of Mn<sup>+2</sup> (Kazi et al., 2009). Different techniques have been used for measuring of manganese such as: flame atomic absorption spectrometry (FAAS) (Arpa et al., 2010), electro thermal atomic absorption spectrometry (ETAAS) (Sarzanini et al., 2001., Tokman, 2007) and inductively coupled plasma-optical emission spectrometry (ICP-OES) (Ranjbar et al., 2012). Also, UV-Vis spectrophotometry is a significant instrument on the determination of trace elements in various samples due to apart from the high sensitivity and precision, it's simple, inexpensive and available compared to other devices. The direct measurement of manganese by UV-Vis spectrophotometry is associated with the interference matrix, because the Mn<sup>+2</sup> concentrations are low in environmental and biological samples. The most effective way to avoid this problem is removal of interferences and increasing the concentration of the analyte. Therefore is requiring a separation/preconcentration step before the measurement (Vaezzadeh et al., 2010). Microextraction techniques are very efficient for the preconcentration of metal ions at trace levels in other samples (Yousefi and Shemirani, 2010). Several microextraction techniques have been suggested for the preconcentration of manganese that can be pointed to: single drop microextraction (SDME) (Manzoori et al., 2009), membrane solid phase

microextraction (MSPME) (Cui et al., 2011), dispersive liquid–liquid microextraction (DLLME) (Balogh et al., 2012). In various reports have been exhibited to the usage of ionic liquids (ILs) on preconcentration of trace metal ions (Violina et al., 2006). Ionic liquids have unique physical and chemical properties such as: negligible vapor pressure, non-flammable, good thermal stability, non-volatility, adjustable viscosity and a worthy replacement for organic and toxic solvents (Li et al., 2009, Martinis et al., 2009, Molaakbari et al., 2011).

In this work, 1-hexyl-3-methylimidazolium hexafluorophosphate [Hmim][PF<sub>6</sub>] based cold-induced aggregation microextraction (CIAME) was applied for the determination of manganese in water and biological samples. CIAME method is easy, simple and inexpensive for preconcentration of metal ions, so it can easily settle up to 40% salt (Mahpishanian and Shemirani, 2010). CIAME consist of three simple steps: (1) solubilization of the analyte in the ion liquid phase; (2) clouding; (3) phases separation for analysis (Baghdadi and Shemirani, 2008, 2009). In this method manganese was initially complexed with 1-(2-pyridylazo)-2-naphthol (PAN) reagent, so the complex is extracted into the IL- phase and is measured by UV-Vis spectrophotometry.

## EXPERIMENTAL

### Apparatus

A lambda 25 UV-Vis spectrometer was purchased from Perkin-Elmer (USA) and used recording the spectra. A Kokusan Japan centrifuge (H-11 n) was used to accelerate the phase separation process and a Jeio Tech BW-05G water bath was obtained from Hettich. A 100 $\mu$ L microsyringe (Hamilton) was employed to inject ionic liquid extracting phase to the sample solution.

### Reagents and Materials

All of the reagents used were of analytical grade. PAN, ethanol, ammonium hydroxide, ammonium chloride, acetonitrile and all salts used, were obtained from E.Merck and [Hmim][PF<sub>6</sub>] was purchased from Aldrich.

A 1000 mgL<sup>-1</sup> Mn<sup>+2</sup> stock standard solution was prepared from appropriate amounts of manganese nitrate salt (E.Merck) in deionized water, according to the standard method and diluted daily for obtaining work solutions prior to use. A solution of 1 $\times$ 10<sup>-3</sup> molL<sup>-1</sup> PAN was prepared by dissolving appropriate amount of this reagent in pure ethanol and also 0.85 mgL<sup>-1</sup> of [Hmim][PF<sub>6</sub>] was prepared in acetonitrile. A buffer solution (pH 9.5) was prepared by mixing of 50mL ammonium hydroxide solution (1 molL<sup>-1</sup>) and 50mL of ammonium chloride solution (0.18 molL<sup>-1</sup>).

### Modified CIAME Procedure

The pH of 10 mL of standard solution or sample containing Mn<sup>+2</sup> and PAN (1 $\times$ 10<sup>-5</sup> molL<sup>-1</sup>) in the screw-cap conical-bottom glass centrifuge tube was adjusted to 9.5 using ammonium hydroxide/ ammonium chloride. Then, 70 $\mu$ L [Hmim][PF<sub>6</sub>] 0.85 mgL<sup>-1</sup> was injected into the tube, and was protected in a thermostated bath at 50 °C for 4 min. Then it was placed for 15 min in an ice bath until extraction was occurred. Afterwards, the mixture was centrifuged for 5min at 4000 rpm. Therefore, the fine droplets of IL inhabited at the bottom of the centrifuge tube. Bulk aqueous phase was removed simply by pipette. So IL-phase was dissolved in 100 $\mu$ L of ethanol solution (85%) and transferred to quartz cell. The absorbance of the complex was measured at 549nm.

## Real Samples

### *Cow tail and human Hair samples*

Hair samples were washed with 1% (w/v) (DDTC), 0.1M HCl and deionized water. Afterwards, the hair samples dried in oven at 70°C for 8 hours and then digested the next day (Tadayon et al, 2012). 1g of washed hair samples were weighted and transferred to a Teflon bombs and 10mL of concentrated HNO<sub>3</sub> (65%) was added. The samples were heated on a plate about 100°C for 2h. After dissolution, the solution was allowed to cool and 5mL of H<sub>2</sub>O<sub>2</sub> (30%) was added. The mixture was heated at 70°C for 2h (S-Tehrani and Tadayon, 2009). After digestion, the sample was diluted with deionized water and was treated according to the given procedure. Mn<sup>+2</sup> was measured in the real samples prepared with UV-Vis spectrophotometry and ETAAS in order to compare.

### *Tap Water*

The sample was prepared of drinking water in Tehran city. Mn<sup>+2</sup> was measured using of spiked method in this sample.

## RESULTS AND DISCUSSION

### Effect of pH

pH plays an important role in the extraction efficiency of complex. The effect of pH on the extraction of manganese was studied by varying the pH within the ranges 4-12. As shown in Figure.1, absorbance increased in the range 4-9.5 of pH and then started to decrease. Because PAN activity decreases in the acidic qualification due to protonation of oxygen and nitrogen. Also metal settle up the form of hydroxide in strongly alkaline perimeter. Therefore, pH 9.5 was selected as the best pH.

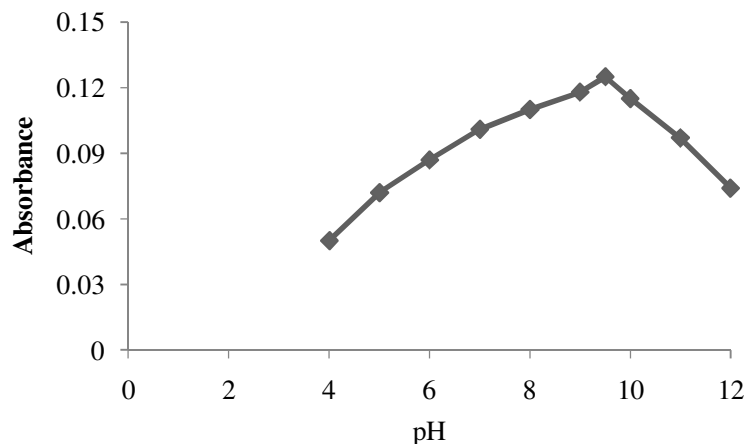


Figure 1. Effect of pH on manganese extraction: sample volume, 10mL; manganese concentration, 54 ngL<sup>-1</sup>; PAN concentration, 10<sup>-5</sup> M; IL volume, 65μL; diluting agent, 100μL.

### Effect of PAN

PAN is a azo compound which act as a ligand. In order to study the influence of PAN concentration on the absorbance of complex, different concentrations of the PAN in the range of 5×10<sup>-6</sup>-1.3×10<sup>-5</sup> molL<sup>-1</sup> were studied. The results illustrated in Fig.2. As can be seen, extraction efficiency increased with increasing ligand concentration up to 1×10<sup>-5</sup> molL<sup>-1</sup> and then remains constant. Therefore, PAN concentration of 1×10<sup>-5</sup> molL<sup>-1</sup> was selected for subsequent experiments.

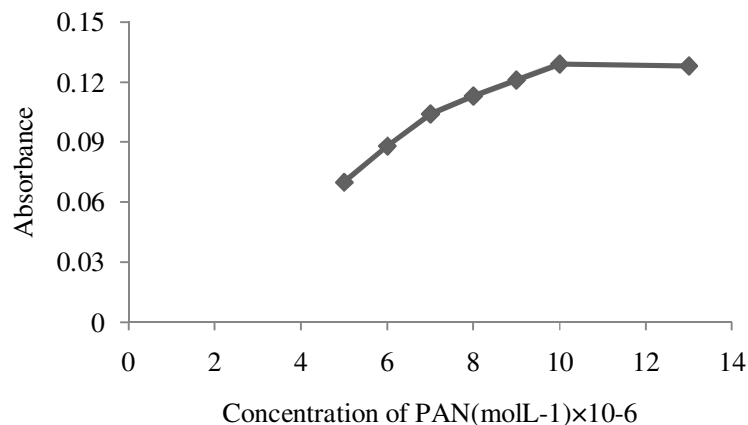


Figure 2. Effect of concentration PAN on manganese extraction: sample volume, 10mL; manganese concentration, 54 ngL<sup>-1</sup>; pH 9.5; IL volume, 65μL; diluting agent, 100μL

### Effect of NaNO<sub>3</sub>

In order to inquire into the influence of the ionic strength on the complex absorption, several experiments were performed with distinctive NaNO<sub>3</sub> concentrations (10-60% WV<sup>-1</sup>). NaNO<sub>3</sub> salt in water is only the ionic form and adding value to salt is increased the ionic strength sample solution. But if too much salt is added, displacement of PF<sub>6</sub> ion with NO<sub>3</sub> ion occurs and cause the [Hmim][NO<sub>3</sub>] salt that is completely soluble in water. This process reduces extraction efficiency. A concentration of 40% NaNO<sub>3</sub> (W V<sup>-1</sup>) was selected for this work.

### Effect of [Hmim][PF<sub>6</sub>] and Centrifuge Condition

Extraction efficiency of complex can be remarkably affected by the ionic liquid amount. Thus, the IL volume is one of the most important parameters in development of preconcentration method, because it determines the sensitivity intensification of the method. The effect of IL volume was studied in range (20-100μL). 70μL was chosen as the optimum volume. The results are depicted in Fig.3.

The final extraction performance is related to the complete separation of the ionic liquid. Thus centrifugation is necessary step to obtain two discrete phases. In order to complete sedimentation of IL phase, different rates of centrifugation (1000-6000 rpm) were studied and the rate of 4000 rpm was selected as the best spot.

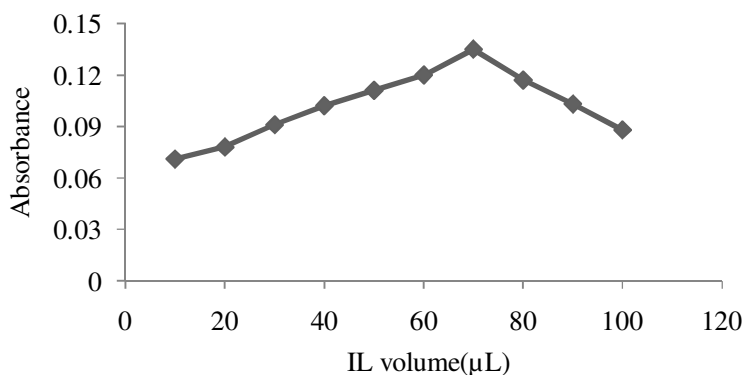


Figure 3. Effect of ionic liquid volume on manganese extraction: sample volume, 10mL; manganese concentration, 54 ngL<sup>-1</sup>; pH 9.5; PAN concentration, 1×10<sup>-5</sup> M; diluting agent, 100μL.

### Effect of Extraction Time and Temperature

Extraction time is one of the important experimental parameters in more analytical processes, particularly in microextraction methods. In M-CIAME based IL, the extraction time is defined as the interval time between finishing the disruption of [Hmim][PF<sub>6</sub>] and starting to centrifuge. Hence, extraction time plays an important role in this new procedure. Various times were studied to extracting in the range of 5-30min (Fig.4). The results explain that in 15 min, contact between extraction solvent and aqueous phase is very high.

Temperature is necessary driving force for the ionic liquid distribution and complex stability. Before shaking a solution including IL, they were heated the range of 20-50°C. At high temperature 50°C could create permanence problems for chelating agent. Therefore 50°C was selected as the most suitable temperature.

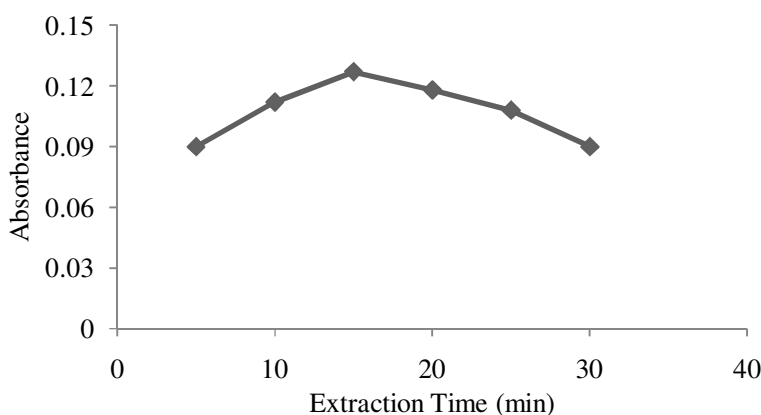


Figure 4. Effect of extraction time on manganese extraction: sample volume, 10mL; manganese concentration, 54 ngL<sup>-1</sup>; pH 9.5; PAN concentration, 1×10<sup>-5</sup> M; IL volume, 70μL; diluting agent, 100μL.

### Interferences

The effect of foreign ions in cold-induced aggregation microextraction of Mn<sup>+2</sup> using PAN were studied in the presence of several amounts of other ions under the optimized conditions. The tolerance limit for interference of each kind was set at ±5% in the analytical signal obtained for a solution including Mn<sup>+2</sup>, except for Ni<sup>+2</sup> and Pb<sup>+2</sup>.

**Table1. Effect of foreign ions the recovery of manganese (54 ngL<sup>-1</sup>)**

Foreign Ion	Molar ratio(Ion/Mn <sup>+2</sup> )	Mn recovery (%)
Cr <sup>3+</sup>	1	90.37
Ni <sup>2+</sup>	1	87.1
Mg <sup>2+</sup>	100	92.59
Co <sup>2+</sup>	1	101.4
Cu <sup>2+</sup>	10	100
Na <sup>1+</sup>	1000	97.77
K <sup>1+</sup>	100	99.25
Pb <sup>2+</sup>	1	107
Fe <sup>3+</sup>	10	94.07
Hg <sup>2+</sup>	100	99.25
Cl <sup>-</sup>	100	99.25
NO <sub>3</sub> <sup>-</sup>	100	97.77
SO <sub>4</sub> <sup>2-</sup>	100	97.03

But because of real samples are diluted 2 times and the amount of nickel and lead in these samples is very low, were found not interference from the coexisting ions for the determination of  $Mn^{+2}$ . The maximum tolerances of the examined foreign ions are given in Table 1.

### Determination of Manganese in Real Samples

The M-CIAME method was applied for the determination of manganese with UV-Vis spectrophotometry and electrothermal atomic absorption spectrometry (ETAAS) in order to compare in environmental and biological samples including (tap water, human and cow hairs). The results are shown in Table 2, as it can be seen the results obtained by UV-Vis spectrophotometry they were in good agreement with those obtained by the ETAAS.

**Table 2.1 Determination of  $Mn^{+2}$  in human and cow hairs (n=4)**

Sample	UV-Vis (Founded $Mn^{+2}$ mean $\pm$ SD <sup>a</sup> )	ET-AAS
Human hair( $\mu g g^{-1}$ )	0.51 $\pm$ 0.075	0.52 $\pm$ 0.03
Cow tail hair( $\mu g g^{-1}$ )	0.33 $\pm$ 0.051	0.35 $\pm$ 0.036

**Table 2.2 Determination of  $Mn^{+2}$  in Tap water(n=4)**

Sample	Added	UV-Vis (Founded $Mn^{+2}$ mean $\pm$ SD <sup>a</sup> )	Recovery (%)	ET-AAS
	0	0.54 $\pm$ 0.02	-	-
Tap water <sup>b</sup> (ngL <sup>-1</sup> )	54.71	55.23 $\pm$ 0.052	99.96	55.02 $\pm$ 0.041

<sup>a</sup>Standard deviation(n=3)

<sup>b</sup>From drinking water system of Tehran, Iran

### The Analytical Figures of Merit and Comparison of M-CIAME with Other Methods

According to the given procedure, the calibration graph was constructed by processing standard solutions of manganese under the optimum condition. The linear concentration range was 0.054-540  $\mu g L^{-1}$  with correlation coefficient of 0.997. The precision of the method was calculated as the relative standard deviation of 4 independent measurements for 54  $ng L^{-1}$  manganese. Likewise, the enrichment factor (EF) is defined as the ratio of the calibration curve slope for manganese before and after the preconcentration step. The limit of detection, calculated based  $3S_b/m$  (where  $S_b$  is standard deviation of the blank signal and  $m$  is the slope of calibration curve after extraction). The results are summarized in Table 3.

**Table 3. Analytical characteristics of modified CLAME method**

Parameter	$Mn^{+2}$ with preconcentration
Correlation coefficient( $R^2$ )	0.997
Limit of detection(LOD)	0.549 $ng L^{-1}$
Enrichment factor(EF)	70
%RSD(n=4)	1.14%(54 $ng L^{-1}$ )

The proposed method was compared with the other preconcentration methods used for the determination of manganese and the results are shown in Table 4. All these results indicate that in comparison with other reported methods, the M-CIAME method has low LOD and good enhancement factor. Also is a rapid, environmentally friendly and simple technique that can be used for preconcentration of metal ions in different samples. In addition, IL was used instead of volatile and toxic organic solvent as the extraction phase.

**Table 4. Comparison of modified CIAME with other methods for determination of manganese (II)**

<i>Method</i>	<i>LOD</i> ( $\mu\text{gL}^{-1}$ )	<i>RSD</i> (%)	<i>Mn(II)</i> <i>Concentration</i> ( $\mu\text{gL}^{-1}$ )	<i>Sample</i>	<i>Reference</i>
SDME(ETAAS) <sup>a</sup>	0.024	5.5	0.5	water samples	Manzoori et al, 2009
MSPME(ICP-OES) <sup>b</sup>	0.61	6.2	0.01	environmental water samples	Cui et al, 2011
CPE(FAAS) <sup>c</sup>	2.9	1.3	200	Water and food samples	Arpa et al, 2010
U-AEME(GFAAS) <sup>d</sup>	0.3	3.3	3	water samples	Mohadesi and Falahnejad, 2012
DLLME <sup>e</sup> (UV-Vis spectrophotometric)	4	1.2	55	water samples	Balogh et al, 2012

<sup>a</sup>Single drop microextraction(electrothermal atomic absorption spectrometry)

<sup>b</sup>Membrane solid phase microextraction ( inductively coupled plasma-optical emission spectrometry)

<sup>c</sup>Cloud point extraction (flame atomic absorption spectrometry)

<sup>d</sup>Ultrasound-assisted emulsification microextraction( graphite furnace atomic absorption spectrometry)

<sup>e</sup>dispersive liquid–liquid microextraction

## CONCLUSION

In this work, a novel method of cold-induced aggregation microextraction based ionic liquid was demonstrated for the preconcentration and determination of manganese in water and biological samples.

This technique acts as a selective and can be separated the analyte from interferences. Also it is simple, rapid, cheap, environment-friendly and can easily settle up to 40% salt. The use of ionic liquid (green solvent) instead of toxic and organic solvents is considered of the important characteristics of this technique. In this study good accuracy and precisions, very low detection limit was achieved for manganese. Also results obtained of this method were checked by ET-AAS spectrometry, and observed the results completely agreement with each other.

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