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Ultrasound assisted pseudo-digestion for determination of iron and manganese in citric acid fermentation mediums by electrothermal atomic absorption spectroscopy

Research Article

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Abstract: A sensitive, simple and rapid method for ultra-trace determination of iron and manganese based on ultrasound assisted pseudo-digestion in citric acid fermentation medium samples (beet and cane molasses and raw sugar based mediums) is described. Parameters influencing pseudo-digestion, such as sonication time, sample mass and solvent system were fully optimized. Final solutions obtained upon sonication were analyzed by electrothermal atomic absorption spectrometry (ETAAS). The best conditions for metal pseudo-digestion were as follows: a 25, 30 and 20 min sonication time for beet molasses, cane molasses and raw sugar based medium samples, respectively, 0.7 g sample mass of raw sugar based samples, 0.5 g sample mass of molasses based samples and an extraction mixture of concentrated HNO_3-H_2O_2, in 25 mL of solvent. Analytical results obtained for the two metals by ultrasound assisted pseudo-digestion and conventional wet digestion methods showed a good agreement. This method reduces the time required for all treatments (heating to dryness, cooling and separation) in comparison with conventional wet digestion method. The accuracy of the method was tested by comparing the obtained results with that of conventional wet digestion method.

Keywords: *Ultrasound assisted-digestion • Iron; Manganese • ETAAS • Citric acid fermentation mediums*

1. Introduction

The analytical use of ultrasound has been intensively explored for sample preparation [1,2]. Ultrasonic irradiation of aqueous solutions induces the cavitation phenomenon into liquid media, which involves the creation, growth, collapsing, and implosions of numerous gas bubbles. At the centre of each collapsed bubble, extremely high temperatures and pressures are generated as well as radicals during sonolysis of the solvent (*e.g.* hydrogen peroxide when water is the solvent). The increase of pressure favors penetration

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between insoluble interfaces which improves extractions of a number of analytes from different matrix samples. It is worth noting that the ultrasound-generated radicals accelerate the oxidation process of an organic sample and that the release of analytes is facilitated [3]. The main ultrasound devices employed for analytical purposes are the ultrasonic horn, which focuses its energy on a localized region providing more efficient cavitation, and the ultrasonic bath, which distributes its energy along the bath. Despite the lack of uniformity in the distribution in the ultrasonic bath, the regions over the piezoelectric crystals of the bath (the source of ultrasound irradiation) and their immediate vicinity provide relatively high efficiency of ultrasound irradiation [4,5]. Therefore, higher activity can be obtained exploring those regions of the bath, if the sample preparation is the determinant step. Considering a routine analysis laboratory, an ultrasound unit offers important advantages, such as higher sample throughput and the low-cost of the equipment. Compared with the traditional sample preparation methods and even microwave oven treatment, the employment of room temperature and atmospheric pressure methods makes the ultrasound-assisted (US-assisted) procedures simpler and safer for the analyst. Ultrasound-assisted extractions of metals from environmental [5-10], biological [11–13], and food [14,15] samples have been successfully performed using ultrasonic baths. Remarkable increases of efficiency and consequent reductions of time were obtained for US-assisted extractions employing ultrasonic horns [16-22].

The most widely used industrial substrates for citric acid production by *A. Niger* are beet molasses, cane molasses and raw sugar. According to Ali *et al*. [23], trace metal ions have a significant impact on citric acid accumulation by *A. Niger*. Ions such as manganese, iron, zinc, and copper affect citric acid production [24]. The process organism *A. Niger* is very sensitive to these metals, especially when cultivated in submerged fermentation [25]. High production of citric acid only occurs if a rigorous control of the availability of the trace elements is accomplished [24]. Clark *et al.* [26] observed 10% and 25% reduction in citric acid yield upon the addition of 2 ppb and 100 ppb manganese, respectively, to beet molasses fermentation medium. In fact, the presence of excess iron favors the production of oxalic acid as a secondary product, which is toxic for food applications [27]. In light of the issues described above, monitoring trace elements in citric acid fermentation medium (CAFM) is the key to assessing the influence of these elements on production of citric acid. Currently, graphite furnace atomic absorption spectrometry (GFAAS) is one of the most reliable and powerful analytical techniques for the determination of trace and ultra-trace elements in water, soil, clinical and biological samples [28-31]. It offers good sensitivity with a short analysis time, low cost (in comparison with ICP-MS [28]) and requires a low sample volume (2–100 µL) [29]. The aim of this work was to improve sample preparation performance, by the development of an ultrasound assisted pseudo-digestion method (UPM) for fast and reproducible recovery of some iron and manganese in fermentation medium samples. Parameters influencing ultrasound assisted pseudo-digestion, such as sonication time, sample mass and solvent systems were fully investigated. Heavy metal determination in the final solutions was carried out by graphite furnace atomic absorption spectroscopy and the results were compared conventional method (CM).

2. Materials and methods

2.1. Instruments and apparatus

A Neytech model 28H ultrasonic bath (USA) at a frequency of 47 kHz was used. A peristaltic pump (Ismatec IPC, Glattburg, Switzerland) was used to change the water during sonication. A Marconi model MA 4025 heating block (Pira-cicaba, SP, Brazil) was employed for wet digestion. For determination of iron and manganese, a Shimadzu 6650 atomic absorption spectrometer, equipped with a deuterium lamp background correction system, a GFA-EX7 graphite furnace and an ASC-6100 autosampler, was used in the determinations. Hallow cathode lamps (HCL) were used as the light source. All the measurements were based on integrated absorbance. The wavelength used was 248.3 nm (slit 0.2 nm) for iron and 279.5 (slit 0.2) for manganese. Pyrocoated graphite tubes, with either integrated platforms or L'vov platforms, were used for the atomization of iron and manganese. A 10 µL sample volume and 10 µL of chemical modifier solution were used. Argon was used as the protective gas.

2.2. Reagents

All chemicals were of analytical reagent grade unless otherwise stated. Deionized water (Barnstead, Dubuque, IA, USA) was used throughout. All solutions were stored in high-density polypropylene bottles. Plastic bottles, autosampler cups, and glassware were cleaned by soaking in 20% (v/v) HNO_3 for 24 h. This material was then rinsed three times with deionized water. Iron stock solutions ($Fe(NO_3)_3•9H_2O$) were prepared daily by serial dilution of commercially available 1000 mg L^{-1} standard solutions (Titrisol, Merck, and Darmstadt, Germany).

Stock standard solutions of manganese were prepared by dissolving the adequate amount of $Mn(SO_4)•4H_2O$ (Reagent grade, Merck, Darmstadt, Germany) in water. Analytical reagent nitric acid (Merck, 63%) was used after additional purification by sub-boiling distillation in a quartz still. H_2O_2 (Merck) and HCl (Reagent grade, Merck, Darmstadt, Germany) used for sample mineralization were also of analytical reagent grade. The mixed Pd and $Mg(NO_3)_2$ matrix modifier solution was prepared by placing 5 mL 0.3% $Mg(NO_3)_2$ solution and 2.5 mL10 g L^{-1} Pd solution ($Pd(NO_3)_2$, Merck) in a 25 mL volumetric flask and diluting to volume with deionized water. The final concentration of the matrix modifier solution was 0.06% $Mg(NO_3)_2$ and 0.1% Pd. High-purity argon was used to purge air from the graphite tubes.

2.3. Samples

The base fermentation medium for citric acid production had the following composition for raw sugar based medium (g L^{-1}): Raw sugar (obtained from tavakoli sugar corporation, Tehran, Iran), 140:NH_4NO_3, 0.05:KH_2PO_4, 1.0:$MgSO_4•7H_2O$, 0.25:$CuSO_4•5H_2O$, $0.6×10^{-4}$:$CaCl_2$, 3.0:$ZnSO_4•7H_2O$, 0.002. Media were sterilized at 1 kg cm^{-2} pressure (121°C) for 15 min. The composition for molasses based medium (g L^{-1}) were as follows: clarified cane and beet molasses (obtained from Islamabad beet sugar manufacture, Islamabad-e-gharb, Iran) 300.0:$K_4Fe(CN)$, 0.2 at pH 6.0:NH_4Cl, 2.0:KH_2PO_4, 1.0:$MgSO_4•7H_2O$, 0.25:$ZnSO_4$, 0.4:$CuSO_4$ 0.001. Media were sterilized at 1 kg cm^{-2} pressure (121°C) for 15 min.

3. Procedures

3.1. ETAAS detection

The samples were analyzed by ETAAS under optimum conditions (Table 1). A mixture of $Pd(NO_3)_2$ and $Mg(NO_3)_2$ was used as a chemical modifier for Fe and Mn determination. 10 µL of digested solution of real samples and 10 µL modifiers were simultaneously injected into the pyrocoated graphite tube of the furnace.

3.2. Ultrasound assisted pseudo-digestion method

For pseudo-digestion optimization, different solvent systems [concentrated HNO_3, a mixture of concentrated HNO_3–HCl (1:3, v/v) and HNO_3–H_2O_2 (2:1, v/v)], sample mass (0.1, 0.4, 0.6, 0.8 to 1.1 g, for 25 mL of solvent) and sonication times (10, 15, 20 to 50 min) were tested. To evaluate the efficiency of the process, the results obtained with the ultrasound pseudo-digestion method (UPM) were compared with those from CM. A portion (0.5 g of molasses based samples and 0.7 g of sugar based mediums) of samples was weighed into polypropylene beakers (50 mL capacity) and 25 mL of concentrated acid or a mixture of acids were added. The samples were then sonicated for 20 min (for raw sugar based medium), 25 min (for beet molasses based medium) and 30 min (for cane molasses based medium). After sonication, the liquid supernatant was evaporated to approximately 0.5 mL final volume on a heating plate. Final solution was made up to 10 mL with 1% HNO_3 and subjected to sonication for another 5 min. The solutions were then centrifuged at 3000 rpm for 5 min, and the final volume was made up to 25 mL with 1% HNO_3. The final solutions were collected in polyethylene flasks for ETAAS determinations of metals. Blanks were also treated in the same way.

Table 1. Measurement conditions for electrothermal atomization AA-6650.

Parameter	Iron	Manganese
Wave length (nm)	248.3	279.5
Lamp Current (mA)	8	6
Dry temp.(°C)/ramp/hold (s)	250/10/0	250/10/0
Ashing temp.(°C)/ramp/hold (s)	800/10/3	900/10/3
Atomization temp.(°C)/ramp/hold (s)	2500/0/2	2200/0/3
Cleaning temp.(°C) /ramp/hold (s)	2600/0/2	2500/0/2

Slit width = 0.2
Sample volume = 10 (µL)
Cuvette = Pyrocoated graphite tube
Back ground correction = D_2 lamp
Carrier gas = 200 mL min^{-1}

3.3. Conventional method (CM)

To evaluate the fast extraction method, the results obtained with this method were compared with those for wet digestion using HNO_3 and H_2O_2 as the digestion mixture. 1.0 g of each sample was placed in a beaker, followed by the addition of 2 mL of concentrated nitric acid and 1 mL of H_2O_2 (30%), and the beaker was covered with a watchglass. The beaker was allowed to stand overnight, and the contents were heated on a hot plate (100°C for 1 h). The sample was cooled until the solution became clear (about 1 h). The watchglass was removed and the acid evaporated to dryness at 100°C. The residue was completely dissolved in 1% HNO_3 and the solution was transferred to a 10 mL calibrated flask.

4. Results and discussion

4.1. Optimization of the UPM

Different citric acid fermentation medium samples (CAFM) were used for optimization purposes. Each result was the average value of three determinations performed in separate batches. Variables influencing the pseudo-digestion process were optimized within the intervals shown in Table 2.

Table 2. Operating conditions for ultrasound assisted pseudo-digestion of Fe and Mn from CAFMs samples.

Fermentation medium		Sonication time (min)	Sample mass (g)	Solvent system
Beet molasses	Studied interval	0-50	0.1-1.1	HNO_3, HNO_3-HCl, HNO_3-H_2O_2
	Best condition	25	0.5	HNO_3-H_2O_2
Cane molasses	Studied interval	0-50	0.1-1.1	HNO_3, HNO_3-HCl, HNO_3-H_2O_2
	Best condition	30	0.5	HNO_3-H_2O_2
Raw sugar	Studied interval	0-50	0.1-1.1	HNO_3, HNO_3-HCl, HNO_3-H_2O_2
	Best condition	20	0.7	HNO_3-H_2O_2

4.2. Sonication time optimization

The influence of sonication time on the digestion of samples was investigated. The results in Fig. 1 and Fig. 2 show that ultrasonic-digestion efficiency increased with increasing sonication time from 10 to 20 min (for sugar based medium), 30 min (for cane molasses based medium) and 25 min (for beet molasses based medium). There was no significant difference from 20 (in raw sugar based medium), 25 (in beet molasses based medium) and 30 (in cane molasses based medium) to 50 min sonication periods for both of the two metals; *i.e.*, 20, 25 and 30 min exposure time is enough for metals from the raw sugar based medium, beet and cane molasses based mediums, respectively.

4.3. Sample mass optimization

In this work, the 0.1 – 1.1 g mass interval was investigated with 25 mL solvent volume. The sample amount used largely depends on the procedure followed. As can be seen in Fig. 3 and Fig. 4, a significant decrease in metal recovery from molasses samples was obtained when the sample mass was larger than 0.5 g and in the raw sugar sample when the sample mass was larger than 0.7 g. There was a significant difference between 0.1 and 0.5 g sample mass for determination of both metals at 0.05 probabilities. In this work, the sample mass/solvent volume ratio was chosen as 0.5 g/25 mL for cane and beet molasses based medium and 0.7 g/25 mL for raw sugar based medium samples.

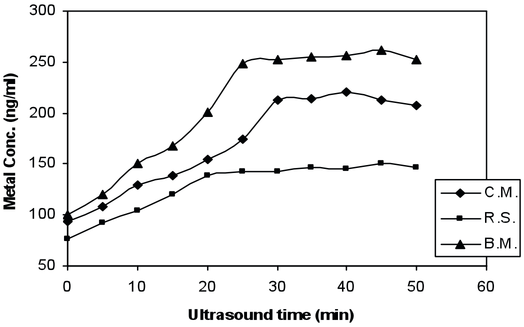

Figure. 1. Effect of sonication time on iron recovery from CAFM samples (B.M. = beet molasses, C.M. = cane molasses, R.S. = raw sugar).

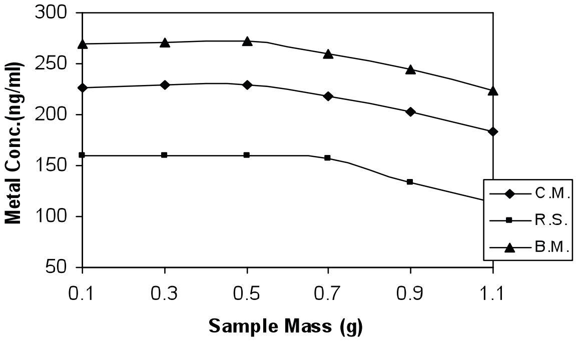

Figure. 3. Effect of the sample mass on the iron recovery from acid solvent with UPM (B.M. = beet molasses, C.M. = cane molasses, R.S. = raw sugar)

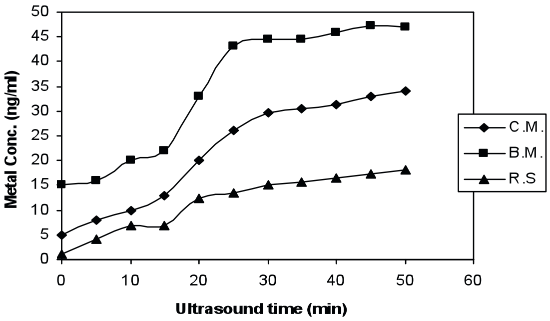

Figure. 2. Effect of sonication time on manganese recovery from CAFM samples (B.M. = beet molasses, C.M. = cane molasses, R.S. = raw sugar).

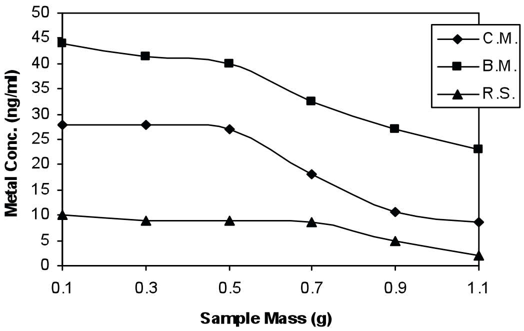

Figure. 4. Effect of the sample mass on manganese recovery from acid solvent with UPM (B.M. = beet molasses, C.M. = cane molasses, R.S. = raw sugar)

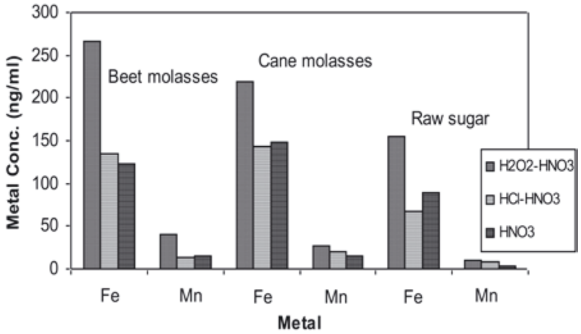

Figure. 5. The effect of solvent system on the ultrasonic assisted acid digestion of iron and manganese from CAFM samples.

Table 3. Determination of iron and manganese in different fermentation samples using ultrasound-assisted pseudo-digestion (UPM) and conventional wet acid digestion (CM) (μg g^{-1}).

Metal	Method	Metal conc. At different samples (ng mL^{-1})		
		Beet molasses	Cane molasses	Raw sugar
Fe	UPM	280.1	236.3	160.0
	CM	287.6	238.0	161.3
	Recovery (%)	97.4	99.5	99.2
Mn	UPM	47.2	28.3	9.0
	CM	49.3	28.1	9.3
	Recovery (%)	97.8	100.3	96.7

4.4. Influence of solvent systems

The influence of solvent systems, such as concentrated HNO_3, a mixture of HCl–HNO_3 and a mixture of HNO_3–H_2O_2 was studied in a univariant way by fixing the other variables at their optimal values. In the case of CAFMs, the significantly higher recoveries of iron and manganese were obtained from acid-oxidant mixtures than those values released with acid alone as shown in Fig. 5. The results obtained indicated that organic matter has an important role in controlling the release of metals. Because of heating, H_2O_2 dissociates to hydroxyl radicals (OH$^{\cdot}$) that could attack matrices in the sample. Hence, it improved the efficiency of the extraction of metals from the samples. We found that H_2O_2 in combination with HNO_3 yielded clear solutions and improved recovery and this is consistent with a previous study [32].

4.5. Analytical figures of merit

The linear range of the calibration curve reached from the detection limit up to 100 and 250 μg L^{-1}, for Fe and Mn, respectively. Characteristic masses were 0.1 and 0.3 pg for Fe and Mn, respectively. The limit of detection (LOD) was defined as 3 s/m, where s is the standard deviation corresponding to 10 blank injections and m is the slope of the calibration graph. LODs of 0.1 and 0.5 μg L^{-1} were calculated for Fe and Mn, respectively. The precision of the methods, expressed as the relative standard deviation (RSD) of a minimum of 10 independent analyses of the same sample, provided values ranging from 1.3 – 5.2% as a function of the element considered and its concentration level.

4.6. Analytical application

The optimized values for the different variables (Table 2) were applied to the analysis of samples with different matrices. All types of samples were digested with UPM and CM, and the results were compared (Table 3). With these real samples, recovery values obtained with UPM ranged 96.7 - 100.3% as compared to those values of iron and manganese obtained from CM.

5. Conclusion

The method described offers a rapid, easy and efficient sample preparation, using a low cost and easily available routine US bath, for determination of iron and manganese in citric acid fermentation medium samples by ETAAS. All parameters (sonication time, amount of sample and solvent system) were studied. The use of the UPM allowed the digestion of fermentation medium samples for iron and manganese determination in a shorter time and lower volume of acid mixtures than required by the CM, while providing similar results. The main goals achieved with the proposed method include a higher sample throughput (10 - 30 samples per hour), longer graphite tube lifetime, and lower analytical costs, when compared with other decomposition procedures prior to analysis. This method reduces the time required for all treatments (hot plate assisted digestion with high volume of acids, heating to dryness, cooling) in comparison with CM, from approximately 1-3 h to 20-30 min.

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