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# Determination of iron in liposomal dosage forms by flame atomic absorption spectrometry after an acidic digestion

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## ABSTRACT

Improving compliance to oral iron is one of the essential goals. Iron Encapsulation into liposomes as nano-particles has provided newer opportunities for ameliorating tolerance with iron oral therapy. Several analytical techniques have been used for quantitative determination of iron. In this study, the liposomal iron has been determined quantitatively in capsules and oral drops by using flame atomic absorption spectrometry (FAAS). A new simple method for disrupting liposomal structure, dissolving and extracting entrapped iron salt (ferric pyrophosphate) in one step prior using FAAS, is achieved. This method is based on acidic digestion using boiling mineral acid for 15 minutes, it was also standardized by applying it on raw material (iron III- hydroxide polymaltose complex) and the iron concentration was determined by FAAS. Through results obtained in this study, liposomes were successfully digested with an accurate determination of liposomal iron. The percentage of iron concentration was between 96 to 104 % from the authorized content in capsules and oral drops. For iron complex in raw material the percent recovery was between 96 to 99.4 %. Thus, a versatile method was developed to facilitate determination of iron in liposomal dosage forms in short time, low requirements and lower costs.

**Keywords:** Nano-particles, Liposomal iron, Flame atomic absorption spectrometry, Acidic digestion, Mineral acid.

## 1. INTRODUCTION

Liposomes are one of the most common and well-investigated nano-carriers for targeted drug delivery, which attracted a wide attention in the pharmaceutical industry field [1].

Liposomes are biodegradable and generally considered to be pharmacologically in active with minimal toxicity, as they tend to be composed of natural phospholipids. The main advantage of systemic liposomes arise from their ability to reduce drug dosages due to the improved pharmacokinetic effect. So, they have lower side effects and better compliance [2, 3].

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Iron delivery via liposomes is an auspicious approach, as iron deficiency anemia (IDA) is one of the most prevalent nutritional deficiency disorders [4]. Iron salts such as ferrous fumarate, ferrous sulphate and others, are used to treat IDA. Low cost and wide availability of these oral iron salts are their key advantages. However, they have many limitations where GI intolerance (abdominal pain, constipation, black or tarry stool) is the most frequent side effect. Moreover, daily supplementation of oral iron salts increases hepcidin expression for nearly 24 hours which results in lower absorption of iron the next day [5].

Therefore, designing a new type of a stable iron supplementation with high absorption and lower side effects remains a challenging goal. So the importance of liposomal iron drugs becomes clear.

In addition, choosing a reliable method to specify

 

iron in liposomal dosage forms is an important issue. Several instrumental analytical techniques and different strategies for sample preparation have been used for such purpose:

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) provides the most satisfactorily sensitive and accurate technique. Unfortunately, expensive devices and strict protocols limit the prevalence of this technique in routine experiments [6]. High detection costs have also hindered the application of Inductively Coupled Plasma-Optical emission Spectroscopy (ICP-OES) and Electrothermal Atomic Absorption Spectrometry (ET-AAS), [7, 8, 9]. Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) is the appropriate atomization technique to determine concentration of iron in specimens, but its slow analysis and high cost restrict using it [10]. The photometric method is a good choice but in comparing it with Flame Atomic Absorption Spectrometry (FAAS), the latter is faster and more selective. Moreover, the determined relative standard deviations for the atomic absorption are less than those of photometric method, proving that FAAS is more accurate [11]. In addition, FAAS is one of the most practical methods with significant precision and accuracy, it is remarkable for its selectivity and speed; 10-15 seconds per specimen [12].

Choosing and applying the analytical method is not the only difficulty, sample preparation still represent the most time consuming and sensitive step. In liposomal dosage forms, it is necessary to consider disrupting the liposomal structure, dissolving and extracting the entrapped drug prior to measuring its concentration. Former methods applied on liposomal dosage forms need at least two steps for sample preparation before iron determination; one for destroying liposomes and the other for adding a material to solve and extract iron salt. Further, it is necessary to use very high speed centrifugal device or ultrafiltration technique for drug separation [13]. These former methods have many disadvantages such as many working phases, long time and more various equipment which lead to expensive costs in comparison with boiling acid disrupting technique.

Hence, based on using an acidic extraction in traditional multi-mineral preparations before measuring mineral concentrations by FAAS, this method was first applied on liposomal dosage forms containing only iron [14].

The main goal of this work is to draw attention on a very simple methodology for disrupting liposomes, solving and extracting iron salt entrapped in the liposomal structure in one step by boiling mineral acid as an alternative to surfactants or organic solvents before iron determination by FAAS.

**Materials and Methods:**

**1. Apparatus**

Flame atomic absorption spectrometry device (novAA 400, Analytic Jena), sensitive weighing scale (Kern 870) and electric heater.

**2. Reagents and materials**

Analytical grade concentrated acids: HCl (Chem-Lab Company, Belgium), $HNO_3$ (E.Merck, D-6100 Darmstadt, F.R.Germany) and $HClO_4$ (E.Merck, Darmstadt, Germany). Ferosom Forte capsules (30 mg Fe), and oral drops (7mg Fe/1 ml) of the Syrian market as a source of liposomal iron manufactured by United Pharma Canada Company which contain ferric pyrophosphate. Iron III- hydroxide polymaltose complex 34% (Chempifine Chemicals, India).

All solutions used in the experimental work were prepared by using distilled water.

**3. Sample preparation and digestion procedures**

**Capsules;** Prior to analyze three Ferosom Forte capsules, mass of the content was calculated. Total digestion of samples was carried out by mixing approximately 0.1 g of the sample with 20 ml of concentrated acid (1 M of HCl). Resulted suspension was heated for 15 minutes, after which it turned into clear

solution. Following to cooling, distilled water was added to reach 100 ml, and after an appropriate dilution, the concentration of iron was determined by FAAS.

The same procedure was repeated by changing the concentrated acid to $HClO_4$ (1 M) and $HNO_3$ (1 M) respectively.

**Oral drops;** The above mentioned steps were applied following to taking of almost 0.1 g volume of oral drops.

**Iron (III)-hydroxide poly maltose complex (raw material):**

A series of standards was prepared, each of different concentration of iron complex (1-3-6-10-15-30-50) mg/L. Standards were best prepared by a gradual dilution of a single stock solution.

The most concentrated standard was prepared following to taking an iron complex calculated mass, and being boiled it with concentrated HCl (1 M) for 15 minutes after which distilled water was added to reach the desired volume. A portion of the volume was diluted to prepare the next most concentrated standards, proceeding on with this dilutions process till standards were accomplished. After that iron concentration of standard solutions was determined by FAAS.

**Results:**

In the present work, disruption of liposomal structure and extraction of the entrapped iron salt have been accomplished in one step, using one of these boiling acids (HCl, $HClO_4$, $HNO_3$), and then concentration of iron was measured by FAAS. By applying this method some advantages such, low cost, high speed and low requirements are observed.

**Capsules;** Following to disrupting the liposomal structure via acidic digestion and the addition of distilled water to reach 100 ml, the resulted solution was diluted 10 times to be in the device measurement range where each test was repeated thrice (n=3). The resulted concentration of iron is illustrated in **Table1.**

**Table1: Concentration of Fe (mg/L) in capsules containing liposomal iron, by FAAS after an acidic digestion:**

| Acid (1 M) | Capsule 1 | Capsule 2 | Capsule 3 | Mean ± SD |
|---|---|---|---|---|
| **HCl** | 29.15± 0.22[a] | 30.53 ± 0.10[a] | 29.02 ± 0.09[a] | 29.57 ± 0.14[b] |
| **$HClO_4$** | 29.54 ± 0.63[a] | 29.9 ± 0.65[a] | 29.42 ± 0.48[a] | 29.62 ± 0.58[b] |
| **$HNO_3$** | 30.57 ± 0.12[a] | 30.01 ± 0.15[a] | 30.1 ± 0.2[a] | 30.22 ± 0.15[b] |

Values are represented as the Mean ± Standard deviation. (n=3).

[a] The average for three measurements.

[b] Mean measurements in three capsules.

**Oral drops;** Following to disrupting liposomal structure through acidic digestion and the addition of distilled water to reach 100 ml, the resulted solution was diluted 10 times to be in the device measurement range, each test was repeated thrice (n=3). The resulted concentration of iron is illustrated in **Table2.**

**Table 2: Concentration of Fe (mg/ml) in oral drops containing liposomal iron, by FAAS after an acidic digestion:**

| Acid (1 M) | Oral drops 1 | Oral drops 2 | Oral drops 3 | Mean ± SD |
|---|---|---|---|---|
| **HCl** | 6.96 ± 0.25[a] | 7.08 ± 0.10[a] | 6.85 ± 0.05[a] | 6.96 ± 0.13[b] |
| **$HClO_4$** | 6.74 ± 0.05[a] | 7.14 ± 0.20[a] | 6.93 ± 0.39[a] | 7.01 ± 0.15[b] |
| **$HNO_3$** | 6.92 ± 0.18[a] | 7.27 ± 0.11[a] | 7.27 ± 0.40[a] | 7.15 ± 0.23[b] |

Values are represented as Mean ± Standard deviation. (n=3).

[a] The average for three measurements.

[b] Mean measurements in three glasses of oral drops.

**Iron (III)-hydroxide polymaltose complex;** To standardize an acidic digestion method, standards containing known amounts of iron (III)-hydroxide polymaltose complex were used and their concentrations were measured by FAAS after an acidic digestion with HCl (1 M), each test was repeated thrice (n=3). The resulted concentration of iron is illustrated in **Table 3.**

**Table 3: Concentration of Fe (mg/L) in standard series of iron (III)-hydroxide polymaltose complex, by FAAS after an acidic digestion with HCl (1M) :**

| Concentrations of standard solutions | First measurement | Second measurement | Third measurement | Mean ± SD |
|---|---|---|---|---|
| 1 mg/L | 1.002 | 0.98 | 0.98 | 0.99 ± 0.006 |
| 3 mg/L | 2.97 | 2.98 | 2.92 | 2.96 ± 0.02 |
| 6 mg/L | 5.72 | 5.83 | 5.76 | 5.77 ± 0.04 |
| 10 mg/L | 9.53 | 9.53 | 9.70 | 9.59 ± 0.08 |
| 15 mg/L | 15.03 | 14.76 | 14.94 | 14.91 ± 0.11 |
| 30 mg/L | 29.24 | 29.44 | 29.67 | 29.45 ± 0.17 |
| 50 mg/L | 49.24 | 49.47 | 49.38 | 49.36 ± 0.09 |

Values are represented as Mean ± Standard deviation.

To evaluate the resulted concentrations of the standard series after an acidic digestion, percentage recovery was calculated. Results are illustrated in **Table 4.**

**Table 4: Percent recovery for each solution in standard series after an acidic digestion with HCl (1M) :**

| Concentrations of standard solutions | Recovered concentrations | Percent recovery |
|---|---|---|
| 1 mg/L | 0.99 mg/L | 99 % |
| 3 mg/L | 2.96 mg/L | 98.6 % |
| 6 mg/L | 5.77 mg/L | 96.2 % |
| 10 mg/L | 9.59 mg/L | 95.9 % |
| 15 mg/L | 14.91 mg/L | 99.4 % |

| Concentrations of standard solutions | Recovered concentrations | Percent recovery |
|---|---|---|
| 30 mg/L | 29.45 mg/L | 98.2 % |
| 50 mg/L | 49.36 mg/L | 98.7 % |

**Discussion:**

Results in **Table 1** indicate that the range of resulted concentrations of iron was between 97_102% of the authorized content in capsules (30 mg of Fe per one capsule), while in **Table 2,** it ranges between 96_104% of the authorized content in oral drops (7 mg Fe/1 ml). It is thus deduced that using boiling mineral acid can successfully disrupt liposomal structure, extract and dissolve the iron salt (ferric pyrophosphate) in one step and in a short time not exceeding 15 minutes.

Furthermore, there is no obvious difference between three acids (HCl, $HClO_4$, and HNO3) in the resulted concentrations of iron, so any of them can be used taking into consideration safety rules. It must be emphasized that the use of mineral acids in laboratories entails considerable health and safety risks, but with proper handling, the potential hazards may be mitigated.

In many previous studies [15, 16, 17], organic solvents were used to disrupt liposomal structure depending on that liposomes are made of lipids. These organic solvents are hydrophobic and incapable to solve ferric pyrophosphate after disrupting the lipid bilayer, so there will be need to use a high speed centrifuge or ultrafiltration to separate iron salt and the addition of another solvent to dissolve ferric pyrophosphate before determination of its concentration by FAAS.

Thus, it is clear that in many previous studies, many steps are needed before measuring iron concentration in liposomal dosage forms, long time and high costs. Besides, organic solvents have lower boiling points and can be easily evaporated at room temperature and badly affect our surroundings.

On the other hand, using surfactants previously [13] has also many limitations, not only their higher costs compared with mineral acids, moreover there is a need to use a centrifuge or ultrafiltration for the same purpose upon using organic solvents.

To standardize the digestion method with mineral acids, it was applied on raw material (Iron (III)-hydroxide polymaltose complex), the resulted concentrations of iron were very close to the prepared concentrations of standard series' solutions with percentage recovery between 96_99.4 %. As a result, it is confirmed that acidic digestion before measuring concentrations of iron by FAAS does not negatively affect iron with the possibility of following this method in case of iron salts or iron-saccharide complexes.

Consequently, acidic digestion enables reaching the goal of disrupting liposomal structure and solve iron salt in one step with no need of additional requirements such as centrifuge or ultrafiltration. This is positively reflected on time and costs.

**Conclusion:**

In this paper, an acidic digestion was performed to facilitate the quantitatively determination of iron in liposomal dosage forms. This method enabled disrupting liposomes and extracting iron salt in one step by boiling mineral acid. And it was also standardized through applying it on raw material.

Following to the acidic digestion, FAAS was applied for iron quantitative determination in raw material and liposomal dosage forms. This analytical technique is practical and not time consuming. Furthermore the operation technique is fairly easy.

Such method is a promising alternative to other methods which use surfactants and organic solvents and need two steps, one for destroying liposomes and the other for solving extracted iron salt.

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**Abbreviations:**

**FAAS:** Flame Atomic Absorption Spectrometry

**IDA:** Iron Deficiency Anemia

**GI:** Gastrointestinal

**ICP-MS:** Inductively Coupled Plasma-Mass Spectrometry

**ICP-OES:** Inductively Coupled Plasma-Optical emission Spectroscopy

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# تحديد الحديد في الأشكال الصيدلانية الليبوزومية عن طريق تقنية الامتصاص الذري باللهب بعد التهضيم الحمضي

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## ملخص

يشكل تحسين المطاوعة لأصناف الحديد الفموي أحد أهم الأهداف في المعالجة الدوائية، وقد قدّم تغليف الحديد ضمن جزيئات نانومترية (ليبوزومات) فرص جديدة وواعدة لتحسين التحمل ومطاوعة المريض للعلاج الفموي بالحديد. سابقاً تم استخدام العديد من التقنيات التحليلية للتحديد الكمي للحديد، في هذا العمل تم تحديد الحديد كمياً في مستحضرات صيدلانية ليبوزومية (كبسولات–نقط فموية) بالاعتماد على تقنية الامتصاص الذري باللهب. وقد تم تطبيق طريقة بسيطة وجديدة لم يتم استخدامها سابقاً لتحطيم البنية الليبوزومية المحيطة بملح الحديد، وحلّ واستخلاص الملح المتضمن ضمن البنية الشحمية (بيروفوسفات الحديد)، وذلك بخطوة واحدة قبل تطبيق تقنية الامتصاص الذري. يعتمد مبدأ هذه الطريقة على تحضير العينة باستخدام التهضيم الحمض بأحد الحموض المعدنية المركزة لمدة 15 دقيقة، وتم تقييس هذه الطريقة بتطبيقها على مادة أولية من معقد بولي مالتوز هيدروكسيد الحديد، بعد ذلك تم قياس تركيز الحديد باستخدام جهاز الامتصاص الذري. من خلال النتائج التي تم التوصل لها في هذه الدراسة، تبين أنه تم تفكيك الليبوزومات المغلفة لملح الحديد بشكل كامل باستخدام طريقة التهضيم الحمضي، وتراوحت تراكيز الحديد بعد تطبيق هذه الطريقة بين (96–104%) من الكمية المصرح بها على عبوات الكبسولات والنقط الفموية للحديد الليبوزومي، أما بالنسبة للمادة الأولية فقد تراوحت التراكيز التي حصلنا عليها بين (96–99.4%). وبذلك يمكن القول أننا توصلنا لطريقة جديدة تسهل تحضير عينة الحديد الليبوزومي قبل قياس تركيزه بجهاز الامتصاص الذري، وقد تم تحقيق ذلك خلال وقت قصير واستخدام معدات بسيطة وهذا انعكس بشكل إيجابي على الكلفة.

**الكلمات الدالة:** جزيئات نانومترية، حديد ليبوزومي، الامتصاص الذري باللهب، تهضيم حمضي، حمض معدني.

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