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## Novel use of XRF in the adsorption processes for the direct analysis of cadmium and silver in absorbent Na-alginate beads

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### Abstract:

The Na-alginate bead is commonly used in biotechnology fields such as adsorption due to ion exchange between Ca and Na with elements. Scanning electron microscopy (SEM-EDX) has proven to be a comparative method in the detections of these adsorbed elements, but the un-flat forming area of beads that can introduce impossible of the detection of element adsorbed. In contrast, X-ray fluorescence (XRF) documents analysis of elements, direct examination, which may analysis the adsorbents of elements. Here, this Study evaluated the possibility by using XRF for the direct analysis for examples of Cd and Ag in a bench stand. This Study compared this to commonly used SEM-EDX and inductively coupled plasma mass spectrometry (ICP-MS) approaches for Cd and Ag analyses. Overall, novel use of XRF provided precise analyses of the concentrations of Cd and Ag adsorbed within Na-alginate bead and revealed analyses not determined the concentrations in standard SEM-EDX or previously established in other studies of Na-alginate beads. In addition, this Study shows that XRF may be employed in different fields in biotechnology to analysis the concentrations regarding the elemental adsorption of Na-alginate beads and demonstrate the potential for XRF to monitor the procedures underpinning biotechnology sectors.

**Key words:** adsorption, biotechnology sectors, Na-alginate bead, XRF, SEM-EDX, ICP-MS.

### Introduction:

Na-alginate beads are used widely in immobilization biotechnology (1) for producing the drug in an immobilized plant (2) and hydrogen production (3), in addition to the comprehensive using in bioremediation of contaminants (4). The determination of elements in the Na-alginate beads to evaluate either the ability to use immobilized Na-alginate beads or the differences between the beads and untreated beads in the specific application such as absorption of contaminants have been repeatedly carried out by element-peak detection using SEM-EDX (5, 6). SEM-EDX can give the approximate compositions of the elements that appear in different peaks, reflecting the ratio of the elements within the selected section under SEM-EDX analysis. For example, peaks of Ca in Na-alginate beads produce a higher peak due to the sub-composition. However, the low concentrations of elements within Na-alginate beads and the complexity of these beads makes challenging the peak detection. Also, it is difficult for taking EDX analysis for the section within a bead as Na-alginate bead forms pour that makes un-flat section. The un-

flat section is not suitable for EDX analysis as EDX beam detects elements in flat and nearest section to it. However, the further process could be possible for analyses of an element with un-flat section, polish. Currently, un-flat samples are polished using a semi-automatic polishing machine with diamond abrasives to produce a flat section with a 0.25  $\mu\text{m}$  that washed with ethanol for 5 min up to three times and dried for an hour at 80 °C in an oven (7). This polished process sometimes causes surface damage to samples, therefore study the suitable and concentration of a material polishing powder is important (8) that consumes time and cost. XRF has not been used in the direct analysis of trace elements generally within Na-alginate beads, as no contribution dealing with element determinations by XRF method after using Na-alginate beads in biotechnology sectors or absorption specifically (9, 10, 11, 12, 13, 14).

In the present work, Na-alginate beads have been used for adsorption of Cd and Ag from artificial wastewater (AWW) for further determinations of absorbed Cd and Ag within the

beads by XRF approach. This hypothesis of using XRF for determining Cd and Ag within Na-alginate beads based on the hypothesis of Bull *et al.* (15) in the using XRF for also, direct analysis of trace elements in the marine algae rather than using ICP-MS or ICP-OES analyses. The preparation and characterization of Na-alginate beads were also presented here. Adsorption assays for Cd and Ag using Na-alginate beads were carried out in the batch adsorption experiments. For the comparative analysis firstly, possible independent analyses of fixative Na-alginate beads were carried out by SEM-EDX. Secondly, ICP-MS analysis was performed on beads. XRF analysis was carried out in a bench stand, and the potential using of XRF for analyzing elements within Na-alginate beads was discussed in terms of using XRF of direct monitoring of the elements within Na-alginate beads in biotechnology sectors.

### Methodology:

#### Preparation of AWW

AWW was prepared in sterilized ddH<sub>2</sub>O according to (16) and used within 24 hours.

#### Na-alginate beads preparation and adsorption experiments of Cd and Ag

3% (w/v) of sodium alginate powder was dissolved in sterilized ddH<sub>2</sub>O and stirred for an hour at 60 °C. The sodium alginate was loaded into a syringe size 1mL without its needle and dropped into 100 mL of CaCl<sub>2</sub> (2 % w/v). The formed beads were left for an hour to harden, and then removed by a sieve, washed out with 500 mL of sterilized ddH<sub>2</sub>O, kept at 4 °C and used within 48 hours (17).

#### Characterizations of Na-alginate beads

The diameters of the beads were measured by a micrometer, and the masses of beads were measured using a sensitive balance (model Pioneer, Ohaus Company).

#### Cd and Ag adsorption experiments

Adsorption experiments were carried out in a batch flask in order to get Cd and Ag adsorbed into Na-alginate beads prior investigation of the novel use of direct analysis by XRF for Cd and Ag. Experiments were performed separately for adsorption Cd and Ag in 250 mL Erlenmeyer flasks in triplicates containing 50 mL wastewater supplemented with initial concentrations 100, 200, 300, 400 and µg/L of either Cd or Ag and incubated at 37 °C for two days in a rotary shaker at 100 rpm. These beads, referred to as 'absorbent', were considered to contain a range of elevated concentrations of Cd and Ag as well as of some elements from the composition of AWW such as N, H, Cl, K, H, P, O, Na and C. After adsorption days, the supernatants of AWW were separated and analyzed by ICP-MS. Absorbent Na-alginate beads

were washed with NaCl (0.9 w/v) and stored at -20 °C prior using for either XRF analysis or fixation for SEM analysis. In terms of checking the ability of Na- alginate beads in adsorption of Cd and Ag from AWW along with its control beads, adsorption (*Q*) of determining Cd and Ag in the AWW was calculated according to de Carvalho *et al.* (18) as equation below:

$$Q (\%) = (C_0 - C_i / C_0) / 100$$

Where *C*<sub>0</sub> is the initial concentrations of Cd and Ag in the wastewater and *C*<sub>i</sub> is the concentration of Cd and Ag after two days of the adsorption experiments.

#### XRF analysis

Cd and Ag loaded Na-alginate beads which have been used in adsorption assay were analyzed by XRF using a Niton XRF analyzer (model XL3T). In terms of comparing drying and freezing methods on XRF analyses in smoothness and thickness, the loaded Na-alginate beads were divided into two parts, one part of the bead was dried at 80 °C in an oven for 24 hours, and the other part was frozen and froze -also dried, for 24 hours according to Bull *et al.* (15). For each part, the beads were filed into an XRF cup (downside was polypropylene thin film 4 µM) into the thickness < 10 mm according to Turner and Solman (19) using 10c coin fixed marker-pen, covered by polypropylene thin film (4 µM) and placed above the window of XRF. The concentrations of Cd and Ag were measured for a period counting 180 seconds at 20 KV. Two Certificated Reference Materials (CRM) of Cd and Ag in soil, low and high values (clay loam MLS) were used to verify precision and accuracy as no market CRM for the bead is available, and the accuracies were 89% and 92%, respectively.

#### Fixation of Cd and Ag loaded Na-alginate beads and analysis by SEM-EDX

For comparative analysis in the undirected analysis of adsorbed Cd and Ag by Na-alginate beads, the Cd and Ag loaded Na-alginate beads were fixed using the EDC technique (*n*-(3-dimethylaminopropyl) - *n'*- ethyl- carbodiimide) according to our protocol (20) prior for SEM-EDX analysis. The beads were cut, fixed and hydrated in serials ethanol concentrations. Then the fixative beads were dried by a critical point drier (EMTECH K850) and coated with gold prior SEM-EDX analysis using SEM analyzer (JEOL-JSM-6610LV).

#### ICP-MS analyses of Cd and Ag

The initial and final concentrations of Cd and Ag in AWW in the adsorption experiments were determined by ICP-MS analysis. Also, Cd and Ag loaded Na-alginate beads were analyzed (the

weight-adjusted to be the same in the XRF cup) after *aqua regia* digestion. The water samples were acidification directly after collecting by adding 1% (v/v)  $\text{HNO}_3$  and 50  $\mu\text{g/L}$  of indium for correction of Cd and Ag losing, in addition of using yttrium (50  $\mu\text{g/L}$ ) as an internal standard for the correction of the instrument. ICP-MS was authenticated by evaluating the limit of detection (LOD), the limit of quantities (LOQ), precision, and accuracy. The LOD of Cd and Ag were  $0.02 \pm 0.001 \mu\text{M}$ , and  $0.03 \pm 0.001 \mu\text{M}$ , respectively. While the LOQs of Cd and Ag were  $0.023 \pm 0.002 \mu\text{M}$  and  $0.03 \pm 0.001 \mu\text{M}$ , respectively. The precision (RSD) was Cd (0.21%) and Ag (0.43%). A spike recovery test was performed to check the accuracy and were  $96 \pm 0.02 \%$  (Cd) and  $94 \pm 0.01 \%$  (Ag).

## Results and Discussion:

### Characterizations of Na-alginate beads

The bead diameter of beads was almost equal; giving mean bead diameters in the range of 3.0 – 3.12 mm (Figure 1).

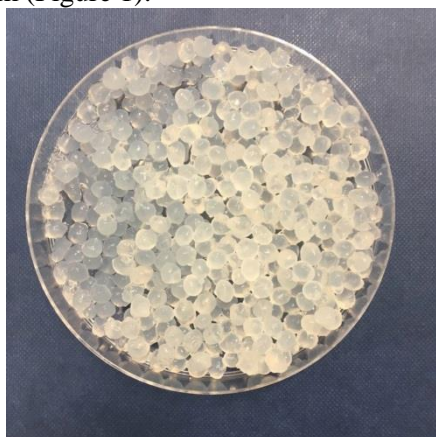


Figure 1. Na-alginate beads.

### Cd and Ag adsorption experiments

The analysis of adsorption experiments is not only useful for explaining mechanisms and amounts of adsorptions but also for evaluating the optimum method, including analytical chemistry for analysis absorbed Cd and Ag within Na-alginate beads. The batch adsorption experiments showed a higher percentage of adsorption were for Ag adsorption (**Error! Reference source not found.**). Also, the percentages of adsorption were decreased with the increases in the initial concentrations of Cd and Ag. This decline in adsorption probably due to ion exchange between Ca and Na or adsorption in the Na-alginate beads with the Cd and Ag in the AWW (21). Na-alginate beads were able to adsorb some of either Cd and Ag in batch experiments as literary has been founded Nasrullah *et al.* (22) even in low percentages in the aim of just obtaining Cd and Ag-loaded within Na-alginate beads.

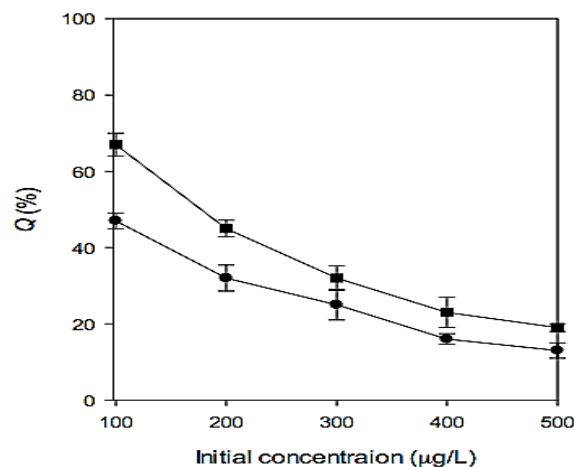


Figure 2. Adsorption percentages in batch experiments. (■) Ag and (●) Cd in 50 mL of AWW supplemented with initial nominated concentrations: 100, 200, 300, 400 and 500  $\mu\text{g/L}$  and incubated at 37 °C for two days in a rotary shaker at 100 rpm.

### XRF Analysis

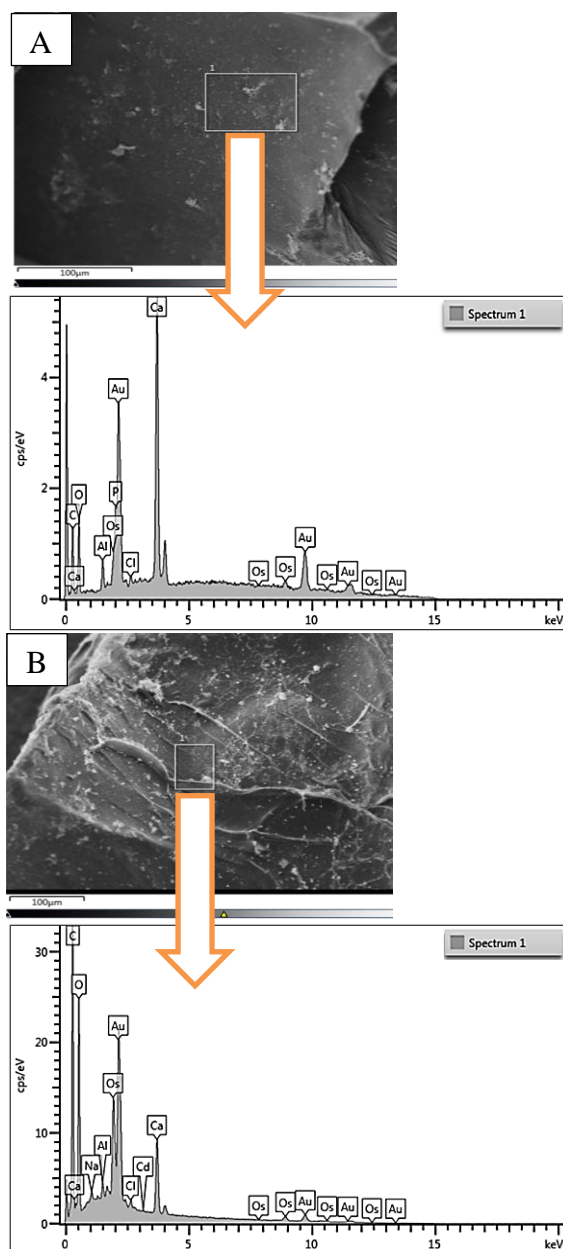
The XRF analysis was carried out of the Cd and Ag-loaded Na-alginate beads after adsorptions in the higher concentration of Cd and Ag (500  $\mu\text{g/L}$ ). The results showed that Ag recorded higher concentration in the Ag-loaded Na-alginate beads ( $88.11 \pm 2.00 \mu\text{g Ag/g}$  dry weight) than Cd concentration in the Cd-loaded Na-alginate bead ( $64.6 \pm 1.3 \mu\text{g Cd/g}$  dry weight) (**Error! Reference source not found.**). In terms of differences between the use of two dry methods of pre-measured beads by XRF analysis, the highest concentrations of either Cd or Ag were recorded in the beads, which dried at 80 °C from the beads that were first frozen and then dried, different from the results of Bull *et al.* (15) as no any differences they recorded. Perhaps due to the differences in the concentrations of Cd and Ag adsorbed into the beads, as the adsorption amounts were varied.

Table 1. The mean concentrations of cadmium and silver within loaded Na-alginate beads detected by XRF. The dried beads at -80 °C in an oven for 24 hours and the frozen and freeze-dried of Cd and Ag-loaded Na-alginate beads after adsorptions.  $\pm$  presents the standard error of the mean.

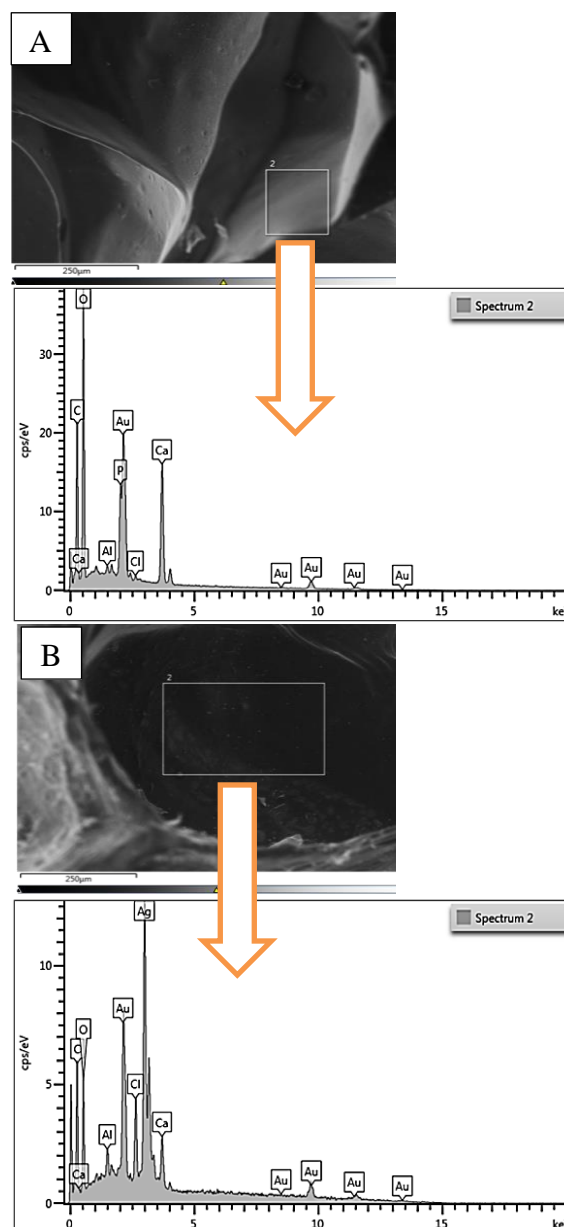
Dried at 80 °C in an oven for 24 h	$64.6 \pm 1.3 \mu\text{gCd/g}$ dry weight	$88.1 \pm 2.00 \mu\text{gAg/g}$ dry weight
Frozen and freeze-dried	$43.2 \pm 0.9 \mu\text{gCd/g}$ dry weight	$76.6 \pm 1.2 \mu\text{g Ag/g}$ dry weight

### A comparison of Cd and Ag concentrations measured by XRF, ICP-MS and SEM-EDX analysis

Undirected measurement of Cd and Ag concentrations in the Cd and Ag-loaded Na-alginate bead was subsequently fixed by EDC method, observed and analyzed under SEM-EDX. The results showed the Cd presents low peak in contrast to the peak of Ag (**Error! Reference source not found.B** and **Error! Reference source not found.B**).



**Figure 3.** SEM and SEM-EDX observations at a voltage of 12 V. (A) Na-alginate bead before the adsorption. (B) Cd-loaded Na-alginate beads after Cd adsorption of initial concentration (500 µg/L) in the AWW incubated at 37 °C for two days in a rotary shaker at 100 rpm.



**Figure 4.** SEM and SEM-EDX observations at a voltage of 12 V. (A) Na-alginate bead before the adsorption. (B) Ag-loaded Na-alginate beads after Ag adsorption of initial concentration (500 µg/L) in the AWW incubated at 37 °C for two days in a rotary shaker at 100 rpm.

Mainly this observation was due to the correlations in the results of the percentage of adsorption, the concentrations in the beads and the SEM-EDX detections. As shown previously in **Error! Reference source not found.**, the highest percentage of adsorption between Cd and Ag was of Ag adsorption, and later XRF analysis showed a higher concentration of Ag within the beads than the concentration of Cd. Also, SEM-EDX studies showed peaks of other elements in the control beads (Figure 3A and 4A), Cd-loaded Na-alginate beads (**Error! Reference source not found.B**) and Ag-

loaded Na-alginate beads (**Error! Reference source not found.**B) such as carbon (C), Na and Ca mainly from bead's composition, phosphate (P), chloride (Cl) and oxygen (O) from the composition of AWW; while the peaks of osmium (Os) were from Os that used in the fixation method and the peaks of Au was from the handling of gold in the coating samples. The ICP-MS analysis of beads showed that the total concentrations of Cd and Ag were  $51 \pm 2.6 \mu\text{g Cd/ g dry}$  and  $74.2 \pm 3.5 \mu\text{g Ag/ g dry}$ , which they were equal to the concentrations that obtained by XRF (Table 1).

### Conclusions:

Overall, although undirected, approximate estimated and complicated method (peak detection-based (SEM-EDX) has been used in the inaccurate estimation of elements generally and of Cd (23) and Ag (24) especially. The present study reported the first use of XRF in the direct analysis of an example of elements, Cd and Ag, which were absorbed within the Na-alginate beads in batch adsorption experiments. The advantages of using of XRF for direct analysis of Cd and Ag within the beads shown the concentrations of either Cd and Ag were in  $\mu\text{g/g dry weight}$  is not like the detections of Cd and Ag by SEM-EDX, which show the peaks of either Cd and Ag in cps/EV (Counts per second per electron-volt, keV: kilo electron-volt). This  $\mu\text{g/g}$  is the precise unit for measuring and concentrations of Cd and Ag or other elements rather counting the electron per second of each element by SEM-EDX. In addition to the lack of fixation method or SEM-EDX observation, where including toxic chemicals and time-consuming. Finally, digestion of the bead before analyzing by ICP-MS is sometimes not sufficient to liberate the elements from the beads. These finding of the recorded concentrations of Cd and Ag within Na-alginate beads by direct analysis of XRF providing the overall possible analysis of other elements and monitoring across an application procedure in a biotechnology sector to be directly related with other analytical chemistry methods.

### Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given permission for re-publication attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in the University of Babylon.

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## استخدام جديد لـ XRF في عمليات الامتزاز للتحميل المباشر للكاديوم والفضة في حبيبات Na-ألجينات الماصة

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### الخلاصة:

حبة النجيليات الممتازة بالعناصر كالصوديوم شائعة الاستخدام في مجالات التكنولوجيا الحيوية بسبب التبادل الأيوني بين الكالسيوم والصوديوم مع بقية العناصر. وقد ثبت الفحص المجهر الإلكتروني (SEM-EDX) طريقة تقريبية في الكشف عن هذه العناصر الممتصة في حبة النجيليات، ولكن عدم تشكيل سطوح مستوية من الثقوب في سطح حبة النجيليات يجعل استخدام المجهر الإلكتروني الماسح طريقه مستحيلة للكشف عن أي عنصر امتز على السطح. على النقيض، من استخدام الأشعة السينية (XRF) في تحليل العناصر والتحليل المباشر للعناصر الممتازة على السطوح غير المستوية. وجدت الدراسة الحالية واثبتت إمكانية استخدام XRF للتحليل المباشر كمثال الكاديوم والفضة في حبة النجيليات. وقورنت هذه الدراسة مع طريقة ICP-MS و SEM-EDX المستخدمة عادة لتحليلات الكاديوم والفضة. إذ يوفر الاستخدام الجديد لـ XRF تحليلات دقيقة لتركيزات الكاديوم والفضة الممتازة في حبيبات النجيليات، وكشفت التحليلات SEM-EDX القياسية عدم تحديد التركيزات في حبة النجيليات أو لم يتم تحديدها مسبقاً في دراسات أخرى. بالإضافة إلى ذلك، تُظهر هذه الدراسة أنه يمكن استخدام XRF في مجالات أخرى في التكنولوجيا الحيوية لتحليل التركيزات المتعلقة بالامتزاز العنصري على حبة النجيليات وإثبات إمكانية XRF لمراقبة الإجراءات التي تقوم عليها قطاعات التكنولوجيا الحيوية.

الكلمات المفتاحية: حبة النجيليات، امتزاز، XRF، SEM-EDX، ICP-MS، قطاعات التكنولوجيا الحيوية.