

## Removal of toxic metals from canola oil by newly synthesized calixarene-based resin

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**Abstract:** This study describes the synthesis, characterization, and application of a new *p*-sulfonatocalix[4]arene-based silica resin (**calix-resin 5**) for the extraction of selected metals, namely Pb, Cd, Co, Ni, Cu and Fe, from canola oil. Synthesis, morphology, purity, and elemental composition of **calix-resin 5** were analyzed by FT-IR spectroscopy, scanning electron microscopy, and energy-dispersive spectroscopy. The metal extraction efficiency of **calix-resin 5** was evaluated through the batch-wise method with a flame and graphite furnace atomic absorption spectrometer following the wet acid digestion procedure. The results obtained showed that the newly fabricated adsorbent material **calix-resin 5** is an efficient adsorbent and exhibits great potential as an alternative adsorbent for the extraction of selected Pb, Cd, Co, Ni, Cu, and Fe in canola oil metal extraction as compared to conventional clay or pure silica.

**Key words:** Calixarene, sulfonation, immobilization, adsorbent, metals, atomic absorption spectrometry

### 1. Introduction

Removal of heavy metals from oils is a major task in edible oil processing industries. Edible oils are an essential part of our daily diet. Thus, during the last few decades great interest has been focused by many researchers on the development of analytical procedures for the quantification and removal of toxic elements from edible oils.<sup>1,2</sup> Low concentrations of essential metals such as zinc, iron, copper, and cobalt are required for growth and maintenance of human health, but they are potentially toxic at elevated concentrations. Some metals, such as lead, cadmium, and nickel, are toxic even at low concentrations in edible oils and cause serious deteriorative effects on the health of consumers as well as the oxidative stability of oil.<sup>3-6</sup> Oxidative stability of edible oils is the resistance to oxidation. It is a well-established fact that oils and fats are susceptible to autoxidation and photooxidation during processing and storage. Lipid oxidation leads to undesirable flavor and taste. The nutritional quality of food products is deteriorated by lipid oxidation, which is a major issue for the food industry.<sup>7</sup> The rate of lipid oxidation depends on various factors, such as fatty acid composition (i.e. the degree of unsaturation), oil processing techniques and parameters, heat, light, and concentration of metals.<sup>8</sup> The main offenders of autoxidation in oils and fats are metals, which are powerful catalysts and generate free radicals.<sup>9</sup> Most trace metals, like Cd and Pb, are prooxidants and toxic and play a very important metabolic role.<sup>10-14</sup>

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Recently, various analytical techniques like neutron activation analysis,<sup>15</sup> potentiometry,<sup>4</sup> voltammetry,<sup>16</sup> solvent extraction,<sup>17</sup> inductively coupled plasma atomic emission spectrometry<sup>17</sup> or mass spectrometry,<sup>17</sup> and atomic absorption spectrometry (AAS)<sup>18</sup> have been applied in metal determination in edible oils. Most spectroscopic techniques involve sample pretreatments, extraction, solubilization, preconcentration, and dilution to minimize the organic matrixes in edible oil. Consequently, the majority of these techniques are tedious and time-consuming.<sup>19,20</sup> Relatively, due to its low cost, high sensitivity, easy operation, and ability to analyze element speciation, AAS is considered the most applicable technique for the determination of metal ions from edible oil.<sup>18</sup> Consequently, to ensure human health and to produce oxidatively stable oil, the precise determination and removal of toxic metals from edible oil is essential. To date, considerable efforts have been made to remediate metals from edible oil. Solid-phase extraction (SPE) is considered superior to other methods by virtue of its simplicity, smaller consumption of organic solvents, low cost, the ability to obtain a higher preconcentration factor, and greater speed.<sup>21</sup> Due to the high enrichment factor, edible oil processing industries frequently use acid-activated clay as an adsorbent. However, there are diverse drawbacks to conventional clay, such as its bulky nature, disposal problems, high residual oil content, and problems with regeneration,<sup>22,23</sup> which has produced considerable interest among researchers for the exploitation of new, reusable, and effective adsorbents.

Recently, calixarene has been given considerable importance in the field of host-guest chemistry due to the presence of phenolic hydroxyl groups. The phenolic OH groups (lower rim) of calixarene moiety can be easily functionalized/immobilized in order to produce effective ionophoric calixarene, which possesses the remarkable tendency to bind metal cations with unique size selectivity.<sup>24</sup> Additionally, the immobilization of the calixarene moiety onto modified silica enhances the adsorption capacity and makes it a robust adsorbent.<sup>25</sup> Calixarene-based silica resins for the extraction of toxic metal ions has been reported in the literature.<sup>26–28</sup> Consequently, in this study we report the preparation of *p*-sulfonated calix[4]arene-based silica resin (**calix-resin 5**). The chemical immobilization of a calix[n]arene framework onto the modified silica makes the macrocycle a versatile material that not only helps in the remediation of polluted sites but also provides chemical, physical, and thermal stability

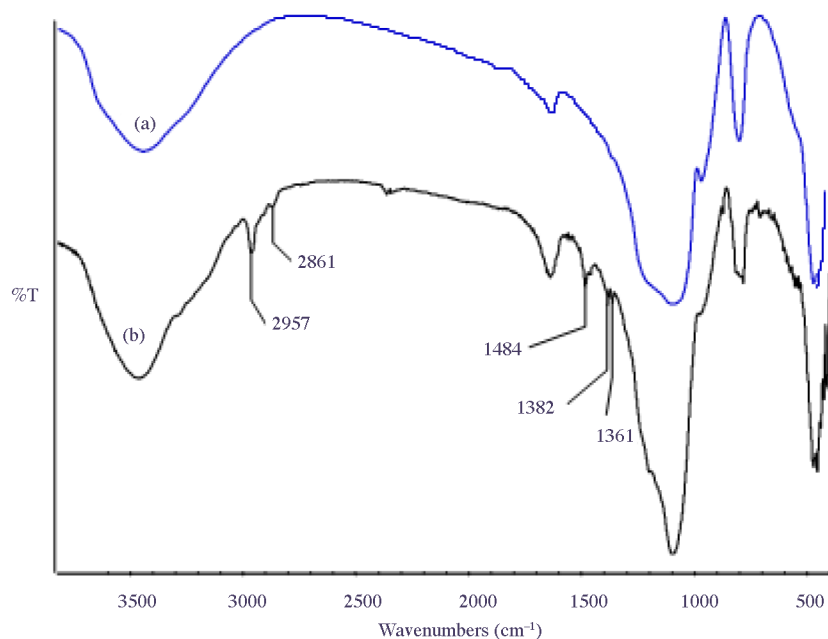
The main objective of the present study is to evaluate the extraction efficiency of newly synthesized *p*-sulfonated calix[4]arene-based silica resin (**calix-resin 5**) towards selected metals, namely Pb, Cd, Co, Ni, Cu, and Fe, from canola oil.

## 2. Results and discussion

### 2.1. Characterization

#### 2.1.1. FT-IR Study

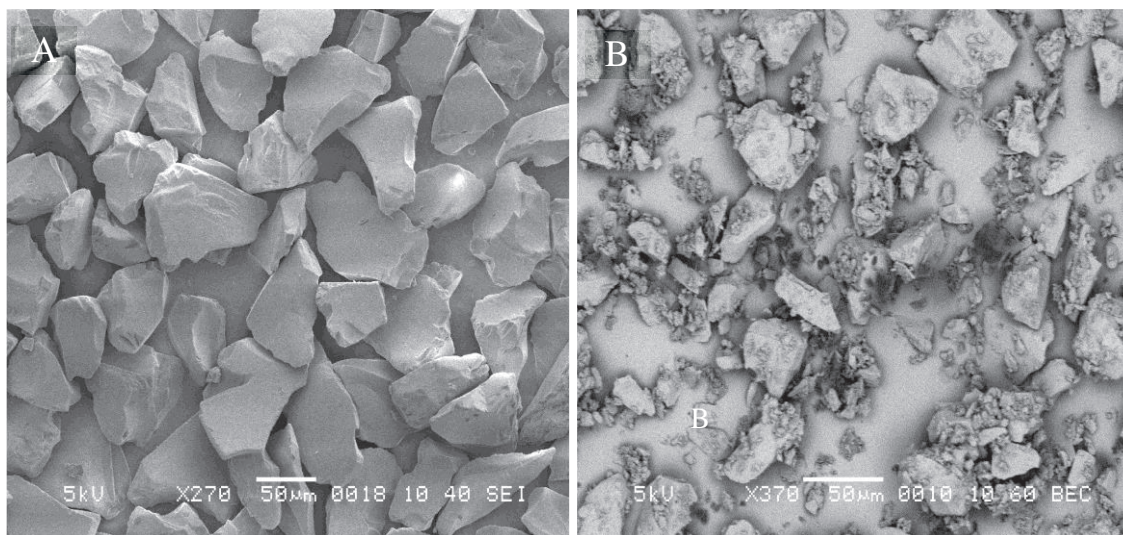
The immobilization of *p*-sulfonatocalix[4]arene (**4**) onto modified silica resin (**2**) was confirmed by FT-IR spectral analysis (Figure 1). Since the modified silica (Figure 1, line a) does not contain any carbon functionality, after immobilization with *p*-sulfonatocalix[4]arene (**4**) the resultant **calix-resin 5** shows some additional peaks at 2957 cm<sup>-1</sup>, 2861 cm<sup>-1</sup>, 1484 cm<sup>-1</sup>, 1382 cm<sup>-1</sup>, and 1361 cm<sup>-1</sup> (Figure 1, line b), corresponding to the symmetrical stretching of C-H, C=C, C-C, and C-O groups, respectively. Consequently, the appearance of some characteristic peaks of calixarene moiety offers qualitative evidence for the immobilization of *p*-sulfonatocalix[4]arene (**4**) onto the modified silica resin (**2**), as well as the formation of new *p*-sulfonatocalix[4]arene-based silica resin (**5**).



**Figure 1.** FT-IR spectra: a- pure silica, b- calix-resin 5.

### 2.1.2. Scanning electron microscopy (SEM)

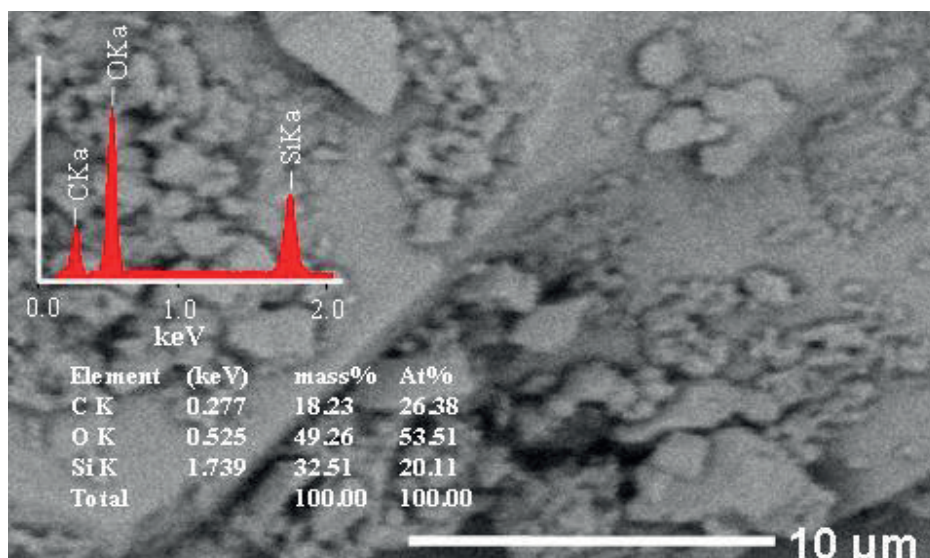
The morphological behavior of pure silica (**1**) and **calix-resin 5** was examined by SEM; the results are presented in Figures 2A and 2B. The SEM image of pure silica **1** (Figure 2A) showed a very smooth morphology while following the immobilization of **4** onto the surface of pure silica; as expected, the smooth surface of the silica was changed (Figure 2B). Thus, the deposition of foreign material, i.e. *p*-sulfonatocalix[4]arene (**4**), on the surface of modified silica (**2**) confirmed the successful formation of new **calix-resin 5** adsorbent.



**Figure 2.** SEM images of (A) pure silica 1 and (B) calix-resin 5.

### 2.1.3. Energy-dispersive spectroscopy (EDS)

In order to examine the purity and elemental composition of newly prepared **calix-resin 5**, SEM was coupled with EDS. Figure 3 provides the SEM image and EDS spectra of **calix-resin 5** with the elemental composition, which displayed approximately 26.38% of the carbon in newly prepared resin **5**. This is because modified silica is composed of hydrogen, oxygen, Si, and Cl with no carbon. The presence of carbon in newly prepared **calix-resin 5** is qualitative evidence for the successful immobilization of calix ligand **4** onto modified silica **2**.



**Figure 3.** SEM micrograph along EDS spectra of calix-resin 5 at high magnification (10  $\mu\text{m}$ ).

### 2.2. Extraction/determination of metal contents

The presence of different metals in canola oil depends on many factors, such as the species, soil used for cultivation, irrigation water, fertilizers, variety, storage tanks, road tankers, pipelines and ships' tanks.<sup>14,29</sup> Generally, edible oil refining units use the adsorption process for the removal of metals. The newly prepared **calix-resin 5** was therefore employed as an adsorbent for the extraction of selected metal ions, i.e., Pb, Cd, Ni, Co, Cu, and Fe, from canola oil. Lead, cadmium, nickel, and cobalt concentrations were determined by graphite furnace atomic absorption spectrometry (GF-AAS) equipped with pyrolytically coated graphite tubes and deuterium background correction. Iron and copper concentrations were determined by flame atomic absorption spectrometry (F-AAS). Since a diminutive quantity of lead (Pb) in edible oils may cause serious deteriorative effects for human health, the quantity of lead in edible oil is also one of the criteria for the assessment of oil quality in term of freshness and storability.<sup>16,30</sup> The obtained results, as listed in Table 1, indicate that crude canola oil contains a very high concentration of Pb, i.e. 0.85 mg L<sup>-1</sup>. Following the treatment process with conventional clay, silica, and new **calix-resin 5**, the treated canola oil samples showed lower concentrations, i.e. 0.78, 0.50, and 0.16 mg L<sup>-1</sup>, respectively (Table 1). According to CODEX, the standard maximum permissible concentration of Pb is 0.1 mg kg<sup>-1</sup>,<sup>31,32</sup> but a Pb concentration exceeding 0.85 mg L<sup>-1</sup> has been found in control canola oil; Table 1 shows that, following the adsorption process, a low concentration of Pb, i.e. 0.78 mg L<sup>-1</sup> and 0.50 mg L<sup>-1</sup>, was observed for oil treated with conventional clay and silica, respectively. A remarkable decrease in Pb concentration (i.e. 0.16 mg L<sup>-1</sup>) was observed in oil samples treated with the newly synthesized adsorbent **calix-resin 5**.

Cadmium (Cd) is known as a very potent toxic metal contaminant, responsible for both acute as well as chronic poisoning and unfavorable effects on the kidneys, liver, heart, vascular system, and neurological system in children.<sup>3,4</sup> Consequently, the presence of cadmium in edible oil adversely affects the health of consumers as well as the oxidative stability of oil. Table 1 represents the concentration of Cd in all the tested oil samples. The results indicated that crude oil contains a very high level (i.e.  $0.8 \text{ mg L}^{-1}$ ) of Cd. After the adsorption process, cadmium concentration decreased to  $0.39 \text{ mg L}^{-1}$  and  $0.42 \text{ mg L}^{-1}$  for oil treated by conventional clay and silica, respectively, as compared to the untreated crude oil. Comparatively, a low concentration of cadmium, i.e.  $0.26 \text{ mg L}^{-1}$ , was observed in the oil sample treated with **calix-resin 5**.

Nickel (Ni) is frequently used in fats and edible oil industries as a catalyst in the hydrogenation process during the conversion of vegetable oils into Vanaspati ghee. However, Ni can promote oxidative degradation, affecting the quality of these products. Due to excess nickel, some pathogenic effects such as lung cancer, oral cavity cancer, and larynx cancer have been reported.<sup>5,7</sup> Table 1 demonstrates the concentration of nickel in crude and treated oil samples. A high concentration of Ni ( $10.3 \text{ mg L}^{-1}$ ) was observed in crude oil, while comparatively low concentrations of 5.8, 7.6 and  $5.4 \text{ mg L}^{-1}$  were observed in oil samples treated with conventional clay, silica, and **calix-resin 5**, respectively. Results indicated the **calix-resin 5**-treated oil sample showed the minimum concentration of  $5.4 \text{ mg L}^{-1}$  for nickel.

Cobalt (Co) is a well-known essential element that plays a crucial role during vitamin B12 (hydroxocobalamin) formation in humans. However, excessive administration of cobalt may cause goiters and reduced thyroid activity.<sup>3</sup> Due to its catalytic nature, Co actively participates in oxidation reactions in natural fats and oils. This oxidation reaction leads to the formation of glycerides containing unsaturated fatty acids. It is well known that unsaturated fatty acids are potentially toxic to human health and alter the taste and color of edible oil, which ultimately affects its market value.<sup>33,34</sup>

Cobalt contents in oil samples before and after adsorption are listed in Table 1. The maximum and minimum concentrations of cobalt, i.e.  $0.2 \text{ mg L}^{-1}$  and  $0.11 \text{ mg L}^{-1}$ , were observed in crude oil and **calix-resin 5**-treated oil samples, respectively.

**Table 1.** Results for the determination of trace element levels in canola oil by GF-AAS.

Oils	Metal concentration			
	Pb ( $\text{mg L}^{-1}$ )	Cd ( $\text{mg L}^{-1}$ )	Ni ( $\text{mg L}^{-1}$ )	Co ( $\text{mg L}^{-1}$ )
Crude oil	0.85	0.8	10.3	0.20
Oil treated with conventional clay	0.78	0.39	5.8	0.12
Oil treated with silica	0.50	0.42	7.6	0.17
Oil treated with <b>calix-resin 5</b>	0.16	0.26	5.4	0.11

All values in the table are mean values of triplicate samples and standard deviation was less than 5%.

Copper (Cu) is an essential element in our daily diet, since it is responsible for many biological functions, such as components of enzymatic and redox systems. However, an excess of copper may cause the lipid peroxidation of lipid membranes, as well as the peroxidation of fatty acids in edible oils. The result of these oxidations is quick formation of unwanted products, which negatively affect the quality (particularly organoleptic properties) of edible oil and the consumer's health.<sup>4,33</sup> According to the World Health Organization and CODEX, the standard maximum recommended value of copper for edible oils is  $0.4 \text{ mg L}^{-1}$ , but a higher

level ( $1.11 \text{ mg L}^{-1}$ ) of Cu was observed in the crude canola oil sample (Table 2). After the adsorption process the oil samples treated by conventional clay and with showed higher levels of Cu, i.e.  $0.63 \text{ mg L}^{-1}$  and  $0.79 \text{ mg L}^{-1}$ , respectively. The lowest level of copper concentration,  $0.48 \text{ mg L}^{-1}$ , was found in the **calix-resin 5**-treated sample.

Iron (Fe) is known as an essential element and actively participates in a broad variety of metabolic processes, but elevated concentrations of Fe in fats and edible oils facilitates the catalysis of hydroperoxides to free radicals and increases the rate of peroxide formation. Several reports confirm that secondary oxidized oil products are generally toxic and that these oils are especially neurotoxic in nature.<sup>3,35</sup> Table 2 shows very interesting results: before the adsorption process crude canola oil contained  $2.67 \text{ mg L}^{-1}$  Fe, but following treatment/bleaching with conventional clay the Fe level increased from  $2.67 \text{ mg L}^{-1}$  to  $3.47 \text{ mg L}^{-1}$ , which may be due to the contamination of conventional bleaching clay.

**Table 2.** Results for the determination of trace element levels in canola oils by F-AAS.

Oils	Metal concentration	
	Cu ( $\text{mg L}^{-1}$ )	Fe ( $\text{mg L}^{-1}$ )
Crude oil	1.11	2.67
Oil treated with conventional clay	0.63	3.47
Oil treated with silica	0.79	2.55
Oil treated with <b>calix-resin 5</b>	0.48	1.94

All values in the table are mean values of triplicate samples and standard deviation was less than 5%.

### 2.3. Comparative percent extraction of metals from canola oil

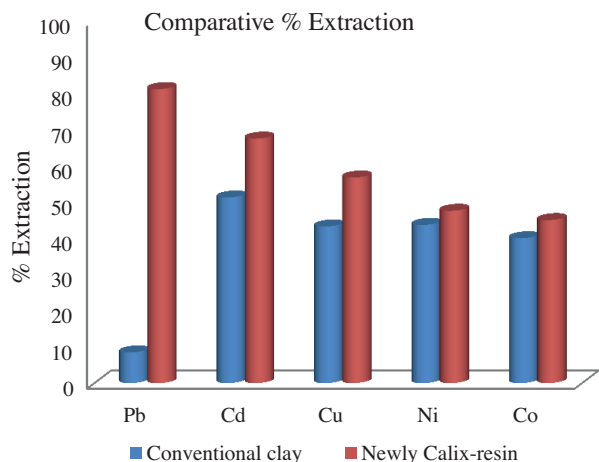
The performance of newly synthesized **calix-resin 5** in terms of metal extraction was compared with that of conventional clay and silica (Figure 4). The newly synthesized **calix-resin 5** showed a relatively higher percent extraction as compared to the conventional clay and silica adsorbents. This may be due to the greater surface area and appropriate projection of binding sites of calixarene moiety in **calix-resin 5**. The newly synthesized calix-based adsorbent showed comparatively satisfactory results for all selected metals and maximum extraction efficiency; e.g., 82% was observed for Pb, while in the case of conventional clay the extraction efficiency was found to be only 8%. The comparative results regarding the removal of selected metal ions from crude canola oil suggest the new **calix-resin 5** as an effective adsorbent and show that **calix-resin 5** has high adsorption efficiency towards the selected metals as compared to conventional clay and silica.

### 2.4. Desorption and regeneration study

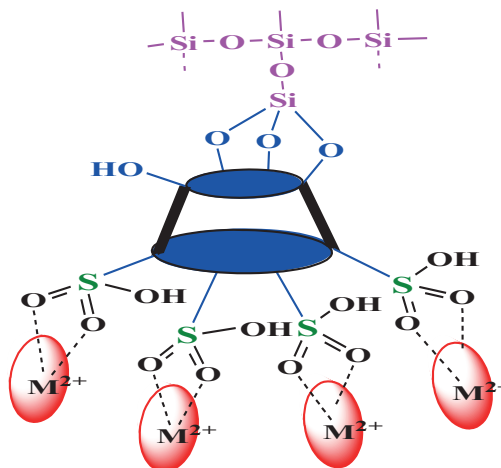
Washing the used adsorbent helps in faster recovery of material since at acidic pH functional groups undergo protonation, thus leading to desorption of metals. The dried adsorbent was then used for further cycles in order to observe the adsorptive efficiencies. **Calix-resin 5** was regenerated by using 0.1 M HCl and tested for a number of cycles for its adsorption efficacy. Results showed that adsorption ability was hardly affected after 10 uses, i.e. only 13%–15% decrease. The adsorption rate was also high due to the presence of a number of sites. It can also be justified by the presence of active binding sites such as sulfonate groups, which play an important part in binding guest molecules at suitable pH values.

## 2.5. Adsorption mechanism

The metal adsorption mechanism for the *p*-sulfonatocalix[4]arene-based silica resin (**5**) can be easily explained. The **calix-resin 5** adsorbent contains sulfonate groups ( $\text{SO}_3\text{H}$ ), and by virtue of lone pair electrons these sulfonate groups ( $\text{SO}_3\text{H}$ ) are highly reactive and anionic in nature. Consequently, these sulfonate ( $\text{SO}_3\text{H}$ ) units in **calix-resin 5** show a bonding ability towards the positively charged metal ions, as represented in Figure 5.



**Figure 4.** Comparative percent extraction of metals from canola oil.



**Figure 5.** Proposed mechanism for the extraction of metal ions with **calix-resin 5** from canola oil.

## 3. Conclusions

The new **calix-resin 5** was successfully prepared and employed as an SPE adsorbent to separate and extract the selected metal ions, i.e. Pb, Cd, Ni, Co, Cu, and Fe, from canola oil samples prior to flame and graphite furnace AAS. Compared to conventional clay and silica, the new **calix-resin 5** adsorbent has a higher extraction efficiency. The newly prepared **calix-resin 5** SPE adsorbent has high potential for Pb, Cd, Ni, Co, Cu, and Fe canola oil samples, and the method offers a faster extraction process than common extraction adsorbents, i.e. conventional clay and silica. Thus, it is concluded that newly synthesized **calix-resin 5** is a potentially more effective material as compared to conventional clay and pure silica (**1**).

## 4. Experimental

### 4.1. Chemicals and apparatus

Canola oil and conventional clay were collected from commercially available edible oil processing industry. All chemicals used were of analytical grade. Silica gel (230–400 mesh) was procured from Fluka (Germany). Analytical TLC was performed on precoated silica gel plates ( $\text{SiO}_2$ , Merck PF<sub>254</sub>). Deionized water was obtained through a Milli-Q system (Elga Model Classic UVF, UK) and used for the preparation of solutions. To prevent metal contamination, all glassware was soaked in 5%  $\text{HNO}_3$  for 24 h and washed with deionized water before use.

Elemental analyses were performed using a CHNS elemental analyzer (model Flash EA 1112, 20090-Rodano, Milan, Italy). Melting points were determined on a Gallenkamp apparatus (model MFB, 595010M, UK). IR spectra were recorded on a Thermo Nicolet 5700 FT-IR spectrometer (USA) as KBr pellets. SEM

studies were performed using a JSM-6380 instrument. The metal analysis was carried out using a PerkinElmer model A-Analyst 700 atomic absorption spectrometer (USA) and GF 3000 with deuterium background correction, equipped with a graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, and autosampler AS-800 and interfaced to a personal computer. Hollow cathode lamps (PerkinElmer) were used as radiation sources. The operational parameters are shown in Tables 3 and 4.

**Table 3.** Experimental conditions for the determination of metals through GF-AAS.

Parameters	Cd	Co	Ni	Pb
Lamp current (mA)	8.0	30	30	7.5
Wave length (nm)	228.8	240.7	232	283.3
Slit width (nm)	0.7L	0.2L	0.2L	0.7L
Ashing temperature (°C)	850	1400	1400	700
Ashing time (ramp/hold, s)	10/20	10/20	10/20	10/20
Atomization temperature (°C)	1650	2500	2500	1800
Atomization time (ramp/hold, s)	0/5.0	0/5.0	0/5.0	0/5.0

Dry temperature (°C)/dry time (ramp/hold, s) = 140/(15/5).

Cleaning temperature (°C /cleaning (ramp / hold, s) = 2600/(1/3).

Atomization site = L'vov platform of a graphite tube.

Carrier gas 200 mL/min and sample volume 10  $\mu$ L + 10  $\mu$ L modifier in each case.

**Table 4.** Experimental conditions for the determination of metals through F-AAS.

Elements	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Oxidant (air L/min)	Fuel (acetylene L/min)
Cu	325	0.7	8	17	2.0
Fe	248	0.2	10	17	2.0

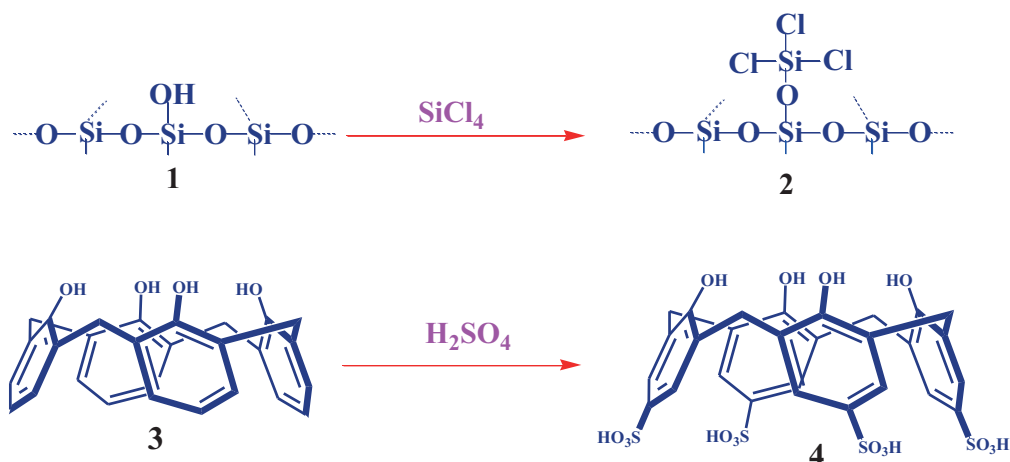
## 4.2. Synthesis

The modified silica (**2**), calix[4]arene (**3**), and 5,11,17,23-tetrasulfonato-25,26,27,28 tetrahydroxycalix[4]arene (**4**) as illustrated in Figure 6 were synthesized according to the previously published methods.<sup>36,37</sup>

### 4.2.1. Immobilization of 5,11,17,23-tetrasulfonato-25,26,27,28 tetrahydroxycalix[4]arene (**4**) onto modified silica (**2**)

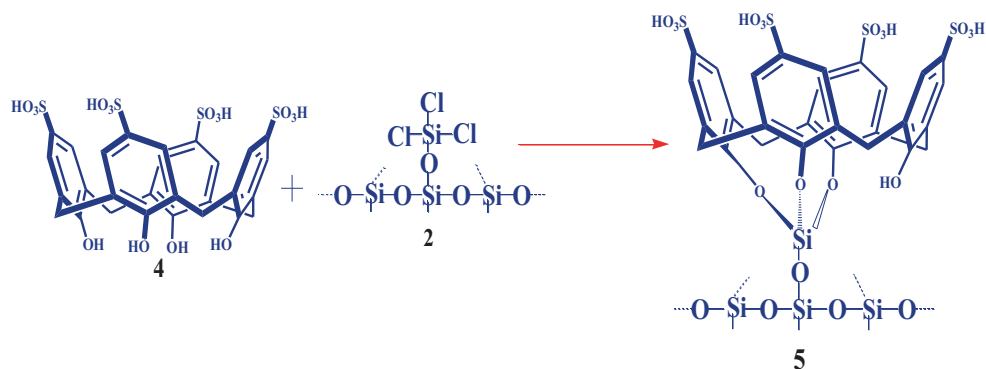
The immobilization of *p*-sulfonatocalix[4]arene (**4**) onto modified silica (**2**), as illustrated in Figure 7, was carried out as follows. A solution of *p*-sulfonatocalix[4]arene (**4**) (1.5 g, 2.016 mmol) in dichloromethane (80 mL) was added to 5 g of freshly prepared modified silica (**2**) followed by the addition of triethylamine (7 g, 9.68 mL), and the reaction mixture was refluxed for a period of 48 h. The immobilization was monitored by FT-IR spectroscopy. The resulting grayish resin was filtered off and washed with hot dichloromethane (300 mL), methanol (200 mL), water (200 mL), methanol (200 mL), and finally dichloromethane (100 mL) in order





**Figure 6.** Modification of silica (2), synthesis of calix[4]arene (3), and *p*-sulfonatocalix[4]arene (4).

to remove excess compound 4 and dried at 100 °C under vacuum. The immobilization was confirmed by evaluating the FT-IR spectrum, SEM, and EDS analysis of **calix-resin 5**.



**Figure 7.** Immobilization of *p*-sulfonatocalix[4]arene (4) onto the modified silica (2).

#### 4.3. Removal (bleaching) of metals from canola oil

First, 50 g of canola oil was taken into three conical flasks and heated at 100 °C under vacuum to evaporate the moisture content from oil. Then an equal amount (1 g) of conventional clay, pure silica, and *p*-sulfonatocalix[4]arene-based silica resin (5) was added to these flasks. The contents of the flasks were stirred at 80–90 °C for 1 h, cooled to room temperature, and filtered using Whatman filter paper. A blank sample of 50 g of canola oil (without adsorbent) was also put through the above-mentioned procedures.

#### 4.4. Sample preparation for AAS to determine the metal contents in oil samples

The conventional wet acid digestion method (Figure 8) was carried out to prepare the canola oil samples prior to flame and graphite furnace AAS determination. Each oil sample (2 mL), precisely measured into separate conical flasks, was done in triplicate. Then 5 mL of 65%  $\text{HNO}_3$  was added to each flask and subsequently the contents of the flasks were heated on an electric hot plate until semisolid masses were obtained. Once again, 5

mL of 65%  $\text{HNO}_3$  was added, followed by the addition of 2 mL of  $\text{H}_2\text{O}_2$  to each flask occasionally, continuously heated until the complete decomposition of organic matter. Following the evaporation, the rustled, semidried mass was dissolved in 5 mL of 2M  $\text{HNO}_3$  and diluted up to 25 mL with deionized water. The organic content of the oil was extracted using 25 mL of chloroform. The aqueous portion was separated with a separating funnel and filtered off through Whatman filter paper.<sup>18</sup> Metal concentration was analyzed by AAS from the aqueous portion.

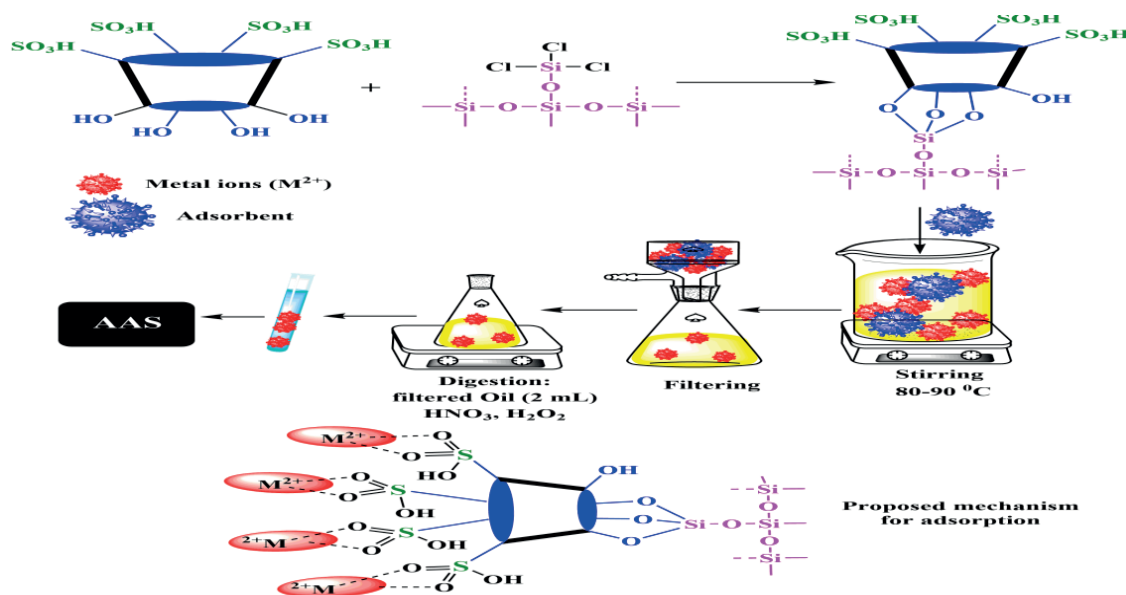


Figure 8. Schematic for the removal of toxic metals from canola oil by newly synthesized calix-resin 5.

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