Synthesis and characterization of a citric and lipoic acids-derived dendrimer

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Abstract: The paper describes the synthesis and characterization of two third generation dendrimers from citric acid and a variant with a dendron terminated with lipoic acid. FTIR and C NMR confirmed the presence of signals characteristic for the dendrimer functional groups OH, C-O, C=O, C-H and C-S. DSC showed that, unlike citric acid, the dendrimer is amorphous. SEM revealed changes in the structure of citric acid particles in each dendrimer generation. In addition, the structure was more homogeneous. Dendrimers are hydrophobic due to ester linkages. Contact angle > 90° confirms their hydrophobic character. Moreover, the dendrimer with a dendron terminated with lipoic acid absorbs Cu^{+2} and Fe^{+3}metal ions, so it can be used as an adsorbent for heavy metals.

Keywords: dendrimer, citric acid, lipoic acid.

Synteza i charakterystyka dendrymeru pochodzącego z kwasu cytrynowego i liponowego

Streszcznie: W pracy opisano syntezę i charakterystykę dwóch dendrymerów trzeciej generacji z kwasu cytrynowego oraz wariantu z dendronem zakończonym kwasem liponowym. Metodą FTIR i C NMR potwierdzono sygnały charakterystyczne dla grup funkcyjnych dendrymeru OH, C-O, C=O, C-H i C-S. Metodą DSC wykazano, że w odróżnieniu od kwasu cytrynowego dendrymer jest amorficzny. Mikrofotografie SEM wykazały zmiany w strukturze cząstek kwasu cytrynowego w każdym pokoleniu dendrymeru. Ponadto struktura była bardziej jednorodna. Dzięki wiązaniom estrowym otrzymane dendrymery są hydrofobowe, co potwierdza kąt zwilżania > 90°. Wykazano również, że dendrymer z dendronem zakończonym kwasem liponowym absorbuje jony metali Cu^{+2} i Fe^{+3}, dzięki czemu może być stosowany jako adsorbent metali ciężkich.

Słowa kluczowe: dendrymer, kwas cytrynowy, kwas liponowy.

Thanks to industrial progress and the excessive use of fertilizers and chemical pesticides in crops, the accumulation of metal ions in the ground has become a serious problem. The contamination of food crops by heavy metals is worrying, since these pollutants are not biodegradable, have an extended average biological life, and some of them are toxic, even at low concentrations. In addition, these heavy metals are not properly excreted from the human body, so the consumption of heavy-metal laden crops for long periods can lead to several health problems [1].

A dendrimer is commonly described as a macromolecule, characterized by an extremely branched structure, providing its surface with a high degree of versatility and functionality. The globular shape, the exclusive architectural design, the high degree of branching, the precise molecular weight, and the multivalency make dendrimers optimal and unique nanocarriers in medicine [2].

Namazi *et al.* [3] synthesized a citric acid dendrimer with a polyethylene glycol diacid nucleus and used it as a drug release agent. Moreover, they also synthesized a triblock dendrimer of citric acid, polyethylene glycol and citric acid (CPEGC) [4]. The dendrimer was used to prepare Au nanoparticles by mixing chloroauric acid

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and sodium borohydride (reducing agent) in an aqueous solution to obtain stable, isolated and homogeneous Au nanoparticles.

Ardestani *et al.* [5] synthesized a new structure of silver complexes with polyethylene glycol-citric acid-based anionic linear globular dendrimer to evaluate their effectiveness against *in vitro* HIV replication pathway. Moreover, they used FTIR, UV, and H NMR to synthesize and characterize a citric acid-polyethylene glycol-asparagine dendrimer, which they used as a theranostic biocompatible, biodegradable, and cost-effective agent [6]. Seyedeh Masoumeh Ghoreishi *et al.* [7] synthesized a polyethylene glycol and citric acid-based dendrimer with a glutamine-terminated dendron. The dendrimer was used as an agent of radiopharmaceuticals, nanoparticles, and/or small-molecule imaging biomarkers. Valikala and collaborators [8] synthesized a second-generation citric acid dendrimer with polyethylene glycol nucleus and carboxyl end groups to use obtained compound as a drug release agent.

Functional groups of dendrimers can be created to give the required properties of aqueous solubility, selectivity, and high loading capacity, among others. Dendrimers can potentially be applied to eliminate metal ions, such as Cu (II), Ni (II), Co (II), Pd (II), Pt (II), Zn (II), Fe (III), Ag (I), Au (I), Eu (III), U (VI), and Th (IV), from diluted aqueous solutions [9].

Therefore, this work aims to obtain a dendrimer from citric and lipoic acids for use as heavy metals adsorbent and will contribute to the reduction of metal ion pollution. The Fourier transform infrared spectroscopy (ATR FTIR), nuclear magnetic resonance (NMR), scanning electron microscopy with energy dispersive spectroscopy (SEM EDS), differential scanning calorimetry (DSC), contact angle and UV-Vis spectroscopy were used to characterize the dendrimers.

EXPERYMENTAL PART

Materials

The following raw materials were used in the work: citric acid anhydride (CA, Sigma Aldrich, N.CAS: 77-92-9); dimethylsulfoxide (DMSO, Sigma Aldrich, N.CAS: 67-68-5); N,N'-dicyclohexylcarbodiimide (DCC, Sigma Aldrich, N.CAS: 538-75-0); lipoic acid (LA, Sigma Aldrich, N.CAS: 1077-28-7). All reagents were purchased from Aldrich Mexico.

Methods

The chemical structure of the dendrimers was analyzed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy using a Perkin-Elmer Spectrum 2 spectrometer (USA). ATR-FTIR spectra were recorded using at least 16 scans with 4 cm^{-1} resolution, in the spectral range 4000–650 cm^{-1}, at room temperature (27°C). Nuclear magnetic resonance (C NMR) was performed on a Bruker 400 MHz (USA) device. The operating range was from 0 to 200 ppm. Deuterated dimethyl sulfoxide was used as the solvent. Scanning electron microscopy and energy dispersive spectroscopy (SEM EDS) were used to study dendrimer morphology and elemental percentages, respectively. The samples were coated with a thin layer of gold by sputtering (Denton Vacuum, model Desk V) and their morphologies were

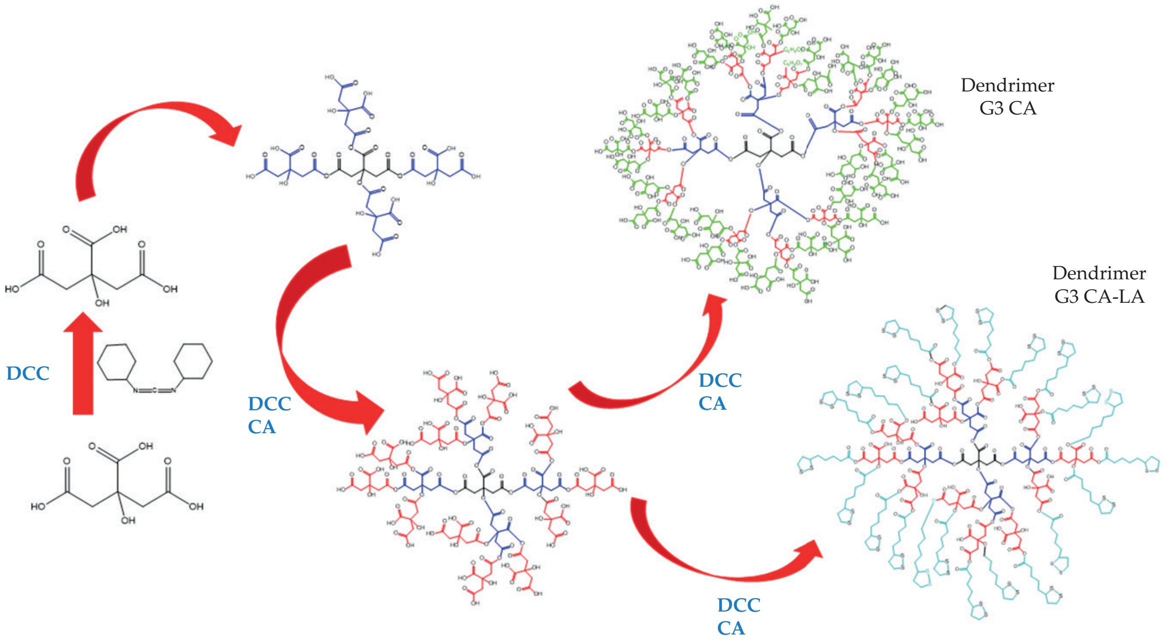


Fig. 1. Reaction scheme of the dendrimer with CA and CA-LA

analyzed by using a JEOL microscope, model JSM-6010A (Japan) operated at a voltage of 10 kV in ultravacuum. The analysis was carried out using an electron column and a tungsten cathode. Differential scanning calorimetry (DSC) was performed in the TA model Q20 apparatus in the temperature range from 40°C to 240°C with a heating rate of 10°C/min, in nitrogen atmosphere. Lonroy ASTM-D5946 equipment was used to analyze the contact angle. 20 ml of distilled water at 25°C were applied to the sample surface. A UV-Vis spectrophotometer (VE-5600UV, Mexico) was used to analyze metal ion adsorption. The presence of metals adsorbed by the dendrimer was determined in the range from 300 to 850 nm.

Dendrimer synthesis

To obtain the first generation dendrimer (G1), DMSO was placed in a beaker to act as a synthesis medium; citric acid and DCC were added and the mixture was stirred for 1 h to homogenize and activate the OH groups in the citric acid (nucleus). Citric acid was added at a 1:2 molar ratio with respect to the existing citric acid in the mixture. The sample was stirred at 600 rpm for 3 h, washed, filtered, and dried using distilled water to separate the solvent and obtain the G1 dendrimer. The same method was followed to obtain the second generation (G2) dendrimer, alternating the G1 dendrimer as starting reagent. Alamdari *et al*. [6] reported the synthesis of citric acid G2 dendrimers using DMSO and DCC.

The third generation (G3) dendrimer was obtained using the same methodology, with G2 as starting reagent for the synthesis. Additionally, the reaction time was 27 h, and either citric or lipoic acids were added to the monomers acting as terminal dendrons. Two G3 dendrimers were obtained with citric and lipoic acids as terminal dendrons (G3 CA-LA). This methodology proposes to use a 1:2 molar ratio considering the reagent utilized as monomer in each generation of the synthesis to obtain the best interaction between the chemical molecules participating in the synthesis (see Fig. 1). In addition, LA is proposed as terminal dendron in one of the dendrimers to act as an adsorbent agent of heavy metals [6, 10].

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy (FTIR)

Figure 2 shows the FTIR spectra of CA and CA-LA dendrimers. The band characteristic of the OH group is observed in 3400–3300 cm^{-1} while that of the CH group is in 2900–2999 cm^{-1}, due to formation of the dendrimer. The band characteristic of the C=O group is in the range of 1750–1700 cm^{-1} and, in 1200–900 cm^{-1}, is that of the C-O group. The bands with the characteristic signals of the functional groups increase along with the dendrimer generations since there are more functional groups in the chemical structure of the dendrimer. The FTIR spectrum of the G3 dendrimer with lipoic acid as terminal dendron shows the signals of groups characteristic of G1, G2, and G3, such as OH, C=O, CH, and C-O. Additionally, there is a key characteristic region in 601–590 cm^{-1} attributed to the C-S group in lipoic acid [11].

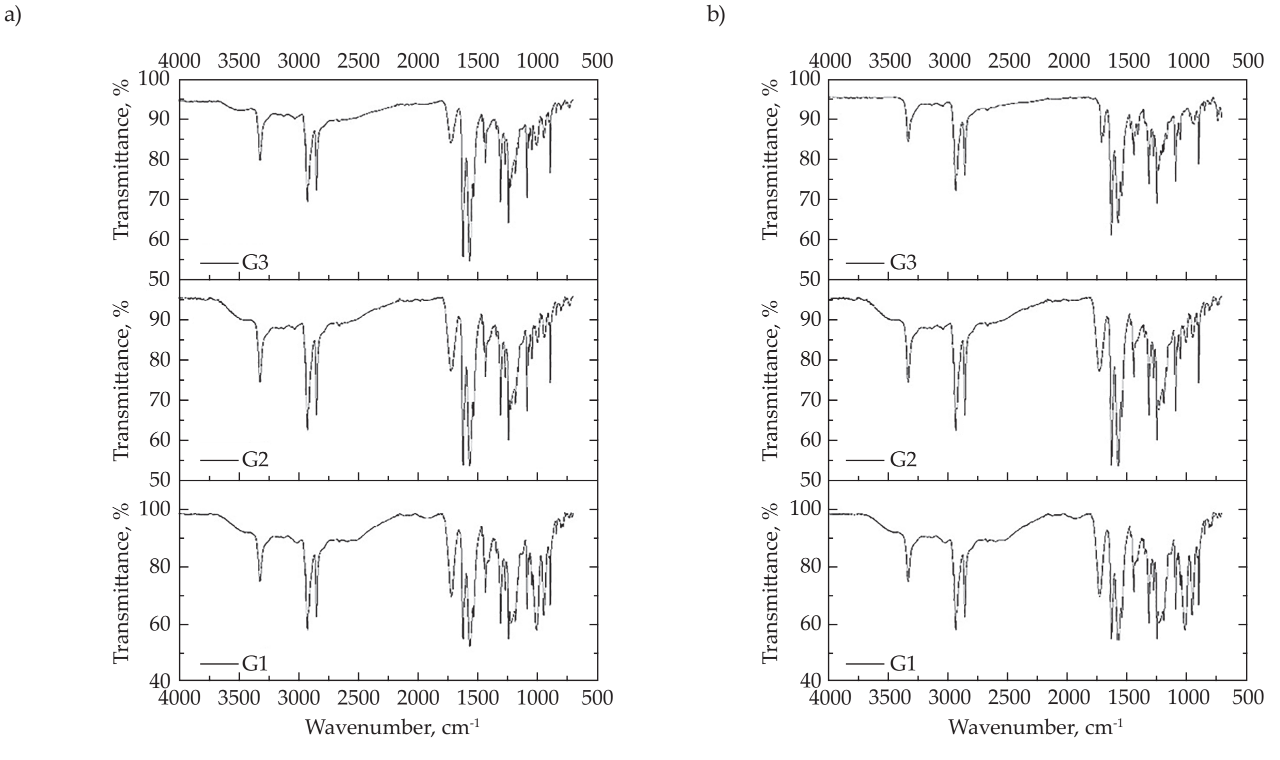


Fig. 2. ATR-FTIR spectra of a) CA, b) CA-LA dendrimers

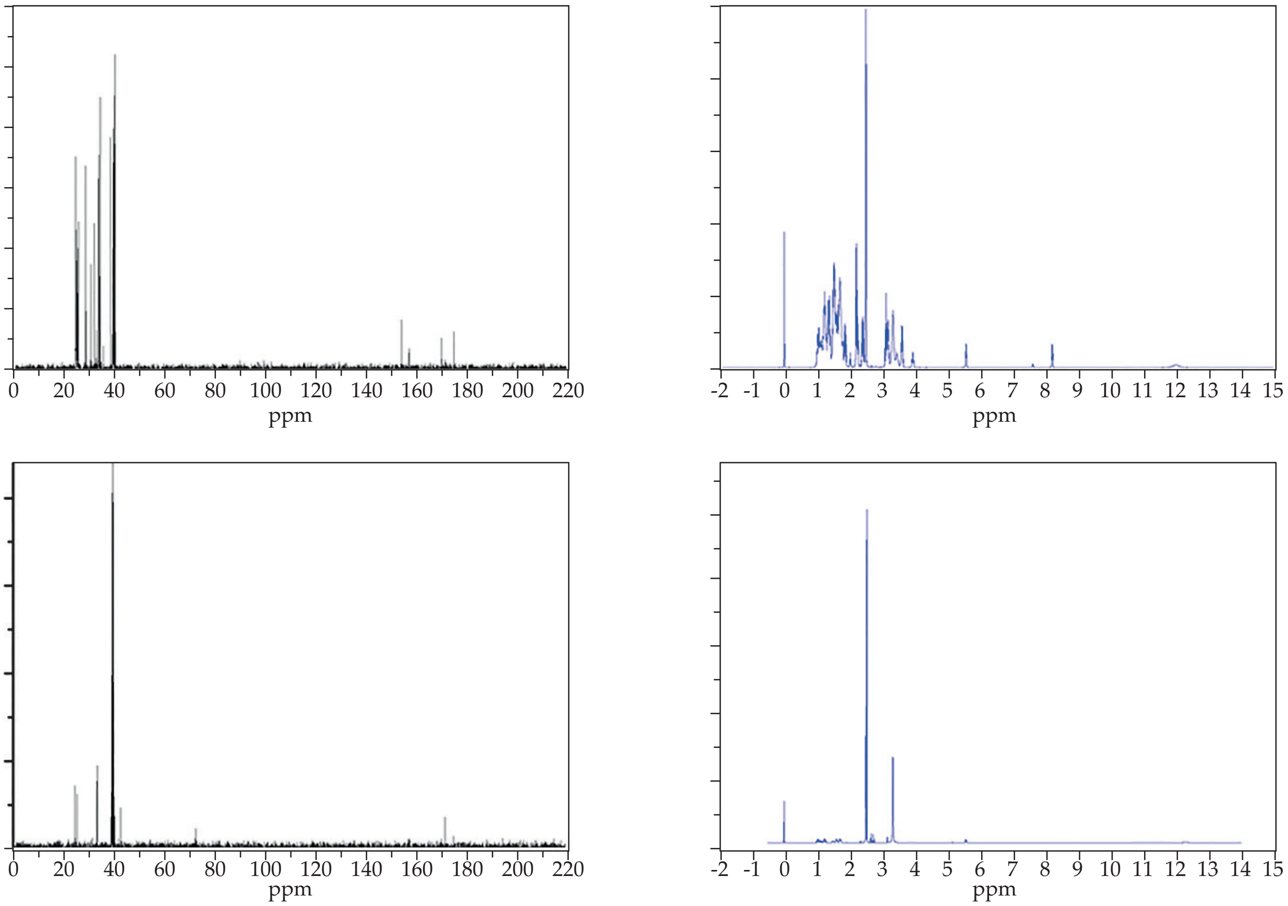


Fig. 3. Spectra of a) C NMR G3-LA, b) H NMR G3-CA-LA, c) C NMR G3 CA, d) H NMR G3 CA

Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (C-NMR) tests were performed to detect the carbon signals corresponding to the groups present in the chemical structure of the obtained polyester. NMR analysis (Fig. 3) confirms the presence of the signals described in the ATR-FTIR analysis corresponding to the G3 dendrimers. Although less intense than the rest, a distinctive CO signal is observed in the 70–80 ppm region of the C NMR spectrum. In the region of 160–180 ppm, the C=O signal is observed, and in the region of 40–45 ppm, the C-C signal [12]. H NMR of both dendrimers showed characteristic signals corresponding to the C-O group in the range of 1–3 ppm, characteristic signals corresponding to the OH group, more intensified in the lipoic acid dendrimer.

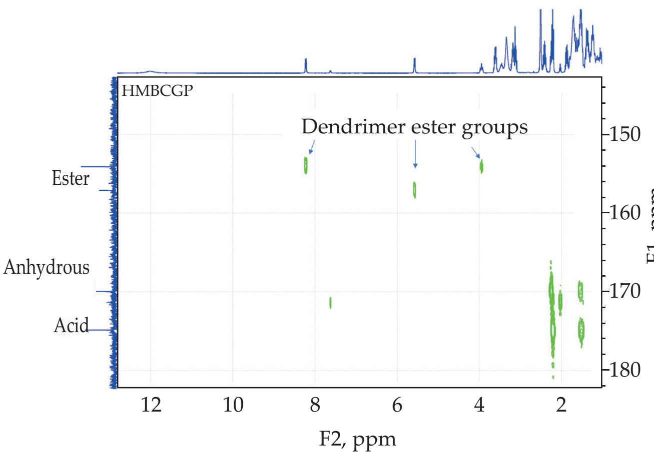


Fig. 4. Spectrum of two-dimensional NMR spectrum (2D DOSY) of G3-CA-LA

From the NMR analysis, different values of diffusion coefficients were obtained for the sample components. Fig. 4 shows a two-dimensional NMR spectrum (2D DOSY: diffusion-ordered spectroscopy), in which signals (chemical displacement and diffusion coefficients) from various functional groups, including ester groups of the dendrimer, are visible.

Scanning electron microscopy (SEM)

The SEM micrographs presented in Fig. 5 show how the structure of citric acid changes after polymerization. Changes in the rectangular prism structures were observed during the reaction leading to the formation of dendrimers. This allows for better homogenization of the dendrimer structure (see Fig. 5 c–f). The obtained dendrimers had a length of 50–96 nm, which allows them to be classified as nanostructured materials. Fig. 5f shows the structure of the G3 CA-LA dendrimer, which differs from the G3 structure shown in Fig. 5e. The large rectangular prisms exist due to the presence of lipoic acid as the

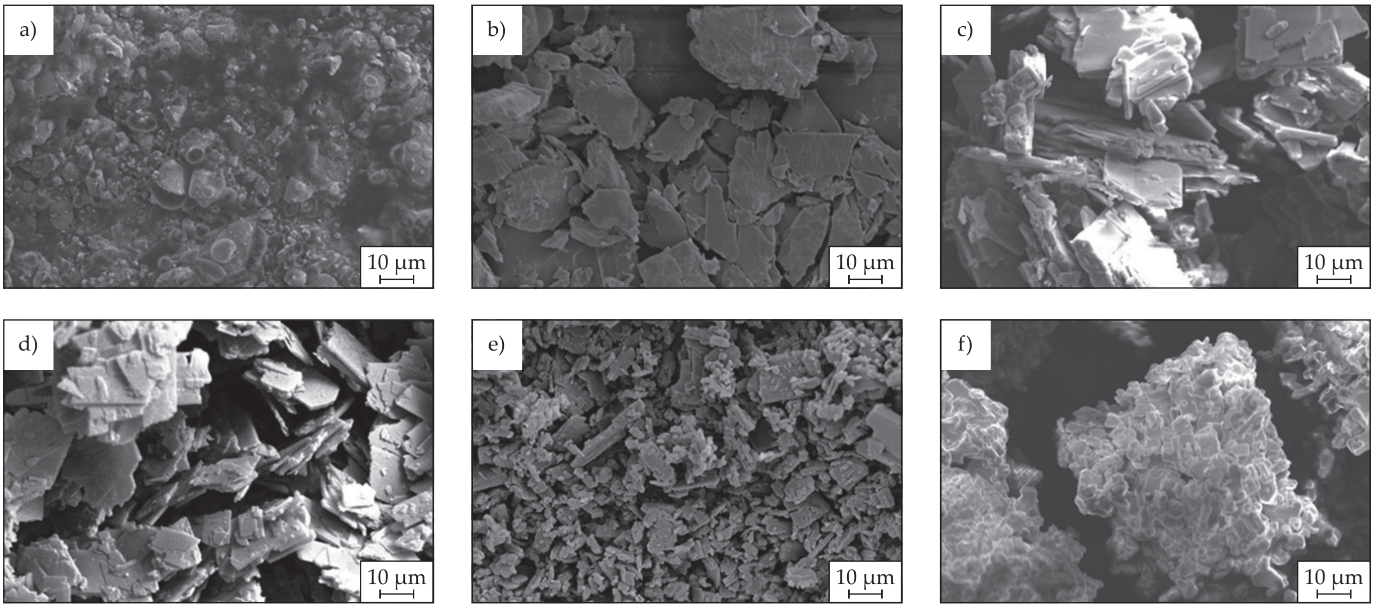
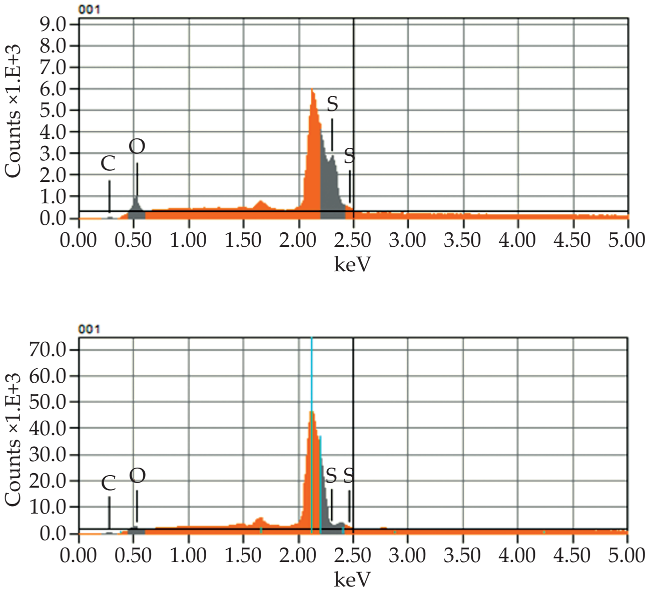


Fig. 5. SEM micrographs of a) CA, b) LA, c) G1, d) G2, e) G3, f) G3 CA-LA



Chemical formula	wt %	mol %	Sigma	Net	K ratio Line
C	19.21	31.08	4.07	2110	0.0010156 K
O	32.76	39.8	2.88	16360	0.0231613 K
S	48.03	29.12	3.98	42777	0.0845882 K
Total	100	100	–	–	–

Chemical formula	wt %	mol %	Sigma	Net	K ratio Line
C	35.45	42.25	10.58	12739	0.0061314 K
O	64.55	57.75	26.11	30246	0.0428197 K
S	nd	nd	–	–	–
Total	100	100	–	–	–

Fig. 6. EDS analysis of dendrimers: a) G3 CA-LA, b) G3 CA

terminal dendron, which gives a structure different from the synthesized dendrimer.

Energy dispersive spectroscopy (EDS)

The presence of sulfur in G3 CA-LA and G3 CA dendrimers was determined by EDS analysis. Fig. 6 shows the percentages of the elements that make up the dendrimers. No sulfur was found in the G3 CA dendrimer because it does not contain lipoic acid but citric acid. The theoretical sulfur content in the G3 CA LA dendrimer is about 22 mol%, and determined by the EDS method is about 29 mol%. The theoretical sulfur content in the dendrimer was calculated by determining the molecular weight of the G3 CA-LA dendrimer and taking into account the components percentage.

Differential scanning calorimetry (DSC)

DSC thermograms (Fig. 7) of G3 dendrimers with citric and lipoic acids show that citric acid loses crystallinity due to polymerization during dendrimer formation. In addition, in each synthesized generation, ester bonds are formed, which leads to a shift in the crystallization temperature of citric acid from 158°C to 207°C [13, 14]. The presence of lipoic acid is observed in thermogram corresponding to dendrimer G3 CA-LA at a termperature of 58°C [15]. Moreover, the peak at 210°C corresponding to the melting point of citric acid disappears, indicating that the citric acid has been consumed in the reaction.This may also suggest the formation of low molecular weight citric acid oligomers compared to dendrimers.

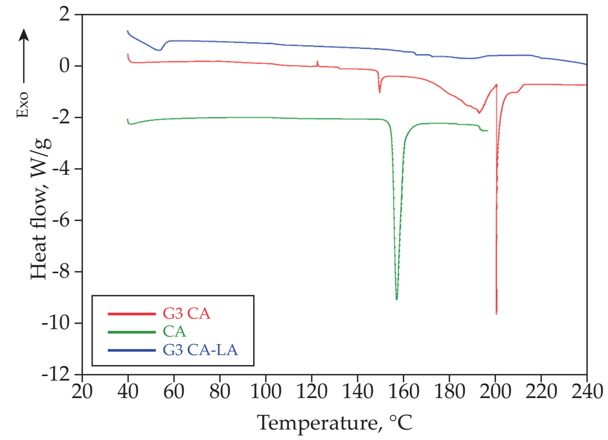


Fig. 7. DSC thermograms of CA, and G3 CA, G3 CA-LA dendrimers

Contact angle

The citric acid dendrimer is hydrophobic, as evidenced by a contact angle greater than 90° (average value 120°). In the case of the lipoic acid dendrimer, the contact angle also exceeds 90°. However, compared to the citric acid dendrimer, its hydrophobicity is lower, as evidenced by the contact angle of about 96° (see Fig. 8).

UV-Vi's spectroscopy

UV-Vi's spectroscopy shows that the G3 CA-LA dendrimer can adsorb copper ions. In Fig. 9, a hypochromic effect can be observed in the $CuSO_4$ solution, which interacts with the G3 CA-LA dendrimer. This effect is due to the adsorption of Cu^{+2} by the dendrimer, which presents lipoic acid as the final dendron. In the acid chemical structure, there are two sulfur atoms that interact with Cu^{+2}, resulting in the adsorption of this metal ion in the dendrimer. The characteristic band corresponds to the UV-Vis absorbance of Cu^{+2} in the wavelength of 810 nm [16]. Similarly, $FeCl_3$ shows a hypochromic effect in the 345 nm region of the UV-Vis spectrum [17], indicating the same phenomenon that occurs in Cu^{+2}, but Fe^{+3} is a metal ion interacting with lipoic acid. Metal absorption generates a hypochromic effect in UV analysis.

CONCLUSIONS

The lipoic acid dendrimer has the same functional groups as the G3 CA dendrimer, because until the second generation it consists of CA, which provides the C-O and C=O functional groups. The hydrophobicity observed in G3 CA and G3 CA-LA dendrimers is a consequence of the ester bonds presence. In addition, the dendrimer, thanks to its hydrophobic properties, acts as an absorber of heavy metals suspended in water. The longer time and higher mixing speed causes the particles to break, therefore with each generation of dendrimer the particle size decreases. The above is observed for both dendrimers in their last generation, the particle size decreases when they are mixed for 72 h. The smaller the particle size of the dendrimer, the larger the contact surface, which improves the usefulness of the dendrimer. The hypochromic effects observed in UV-Vis spectroscopy of $CuSO_4$ and $FeCl_3$

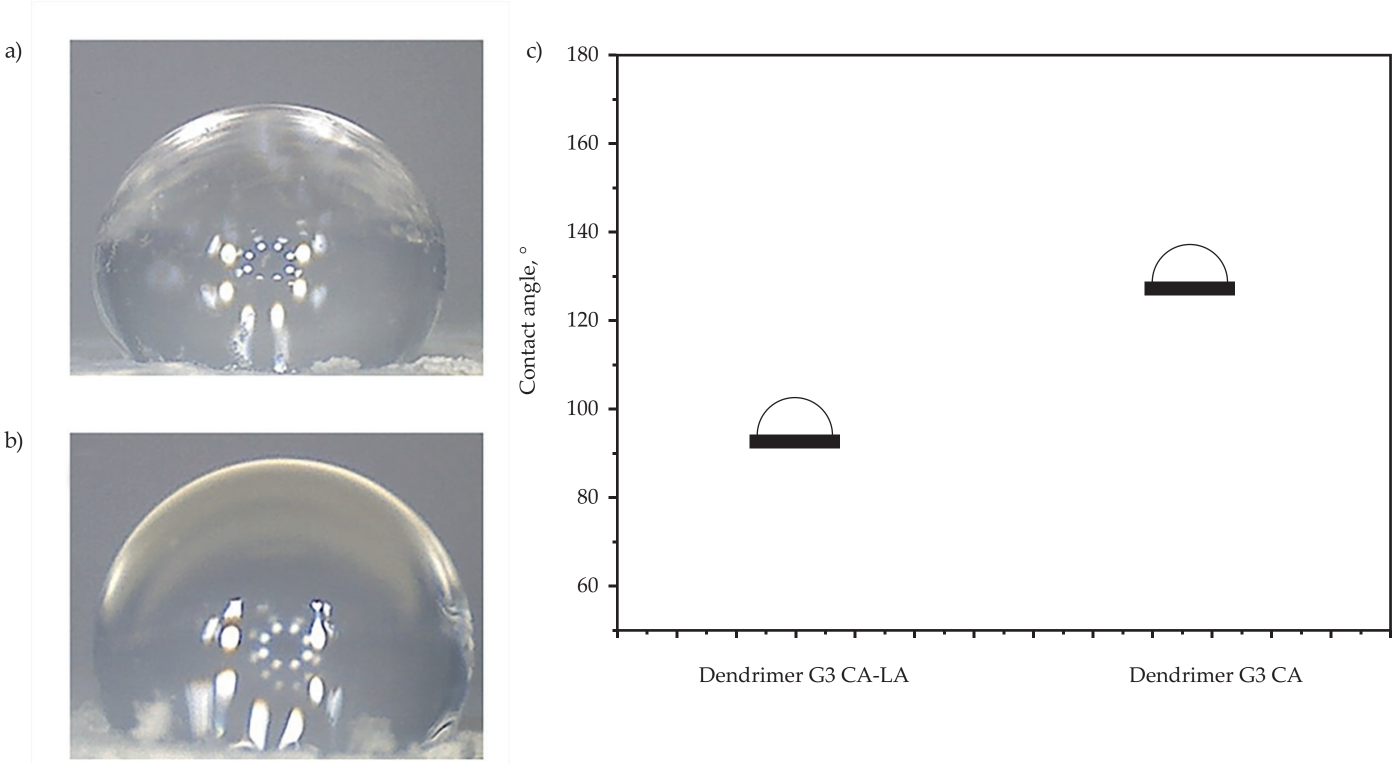


Fig. 8. Contact angle of: a) G3, b) G3 CA-LA, c) graph

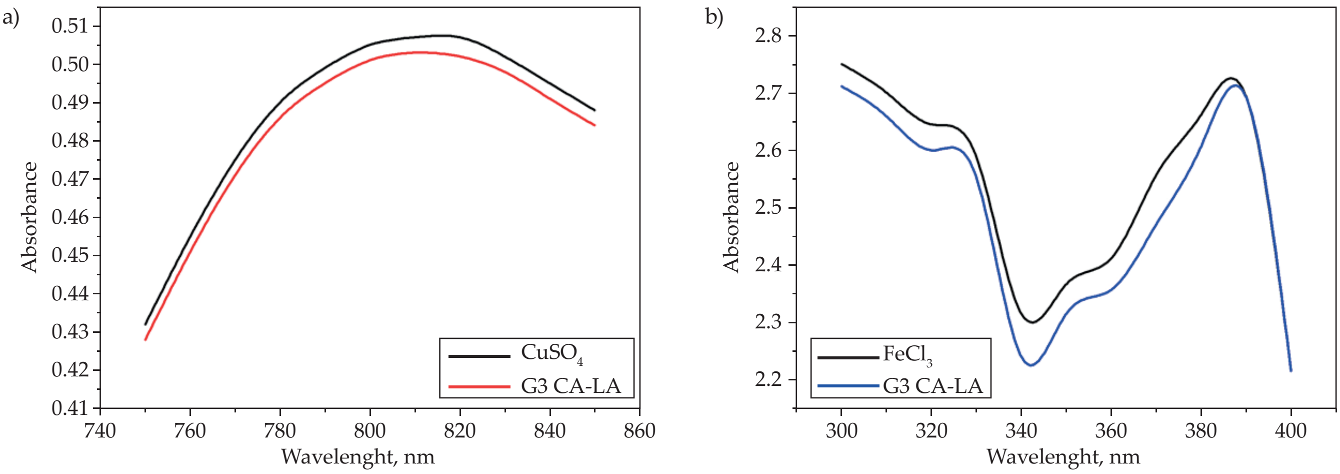


Fig. 9. UV-Vi's analysis of G3 CA-LA dendrimer with: a) $CuSO_4$, b) $FeCl_3$

solutions are the result of the dendrimer interaction with Cu^{+2} and Fe^{+3} ions. The electron cloud generated by sulfur compounds in the G3 CA-LA dendrimer has a negative charge, so the dendrimer may have an affinity for positively charged ions such as Cu^{+2} and Fe^{+3}.

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