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Article

Potential Alendronate Sodium drug carrier by preparation and characterization of sodium alginate cross-linked Montmorillonite

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In drug therapy, it is important to provide therapeutic levels of drug to the site of action and maintain them during the treatment. This work describes the in vitro release of alendronate from sodium alginate cross-linked Montmorillonite (MMT) composite beads. Effect of crosslinking cation, concentration of montmorillonite and media on encapsulation efficiencies, and release profiles of alendronate were studied. Beads were characterized using equilibrium swelling ability study, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Energy-dispersive x-ray spectroscopy (EDX) and scanning electron microscopy (SEM). Results indicate that addition of montmorillonite increases the encapsulation efficiencies and slows down the release rates significantly.

Keywords: Alendronate Sodium. Sodium alginate. Drug delivery. Montmorillonite.

INTRODUCTION

Over the past decades, biopolymer-based delivery systems have been of great interest due to their nontoxicity, biocompatibility, biodegradability, convenience, controlled release characteristics, high encapsulation efficiency and wide application areas (Surya *et al.*, 2019). Biomaterials have traditionally been designed to be inert and not to interact with biological systems in the host (Li, Olah, Baer, 2020).

Among these, alginates and their derivatives are accepted as significant release matrices. Alginic acid is a natural polysaccharide consisting of monomeric units of 1–4 linked α-l-guluronate and β-d-mannuronate in different proportions. Its anionic form, sodium alginate, is widely used in food and pharmaceutical industries as a gelling (Shilpa, Agrawal, Ray, 2003), thickening (Sachan *et al.*, 2009), and stabilizing agent (Gombotz, Wee, 1998). The most important functionality of the alginate chain is its ability to form insoluble crosslinked gels with divalent metal ions, creating an egg-box like an egg-box like structure (Lee, Mooney, 2012), (Kaygusuz *et al.*, 2015a). The alginates were discovered by a British Pharmacist, E.C.C. Stanford; and its commercial production started in 1929 (Sachan *et al.*, 2009). However, the first drug carrier of sodium alginate was made up in 1993 by (Rajaonarivony, *et al.*, 1993). They showed alginate NPs with a wide range of particle sizes (250–850nm), formed within a sodium alginate solution by adding calcium chloride followed by poly-L-lysine. (Hamidi, Azadi, Rafiei, 2008).

The promising properties of organoclays have been proven in many areas (Rahmani, Zeynizadeh, Karami, 2020). Clay minerals (layered silicates) have been used as fundamental constituents of the Modified Drug Delivery System (MDDS) (Sarmah *et al.*, 2015), (Saxena *et al.*, 2018) with different purposes and acting through various mechanisms (Aguzzi *et al.*, 2007). Clay minerals with frequent properties governed by smectites are entitled bentonites, most of which are constituted of

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Montmorillonite (MMT). To be precise, montmorillonite is a major constituent of most bentonites (typically 80-90 wt%), and the remainder is a mixture of mineral impurities (Surya *et al.*, 2019). It is a hydrophilic mineral soil, which consists of two silica tetrahedral sheets that sandwich an edge-shared alumina octahedral sheet (He *et al.*, 2019). Clay minerals present various interesting features with active sites such, including hydroxyl groups, Lewis and Brønsted acidity, and exchangeable interlayer cations. Furthermore, the high aspect ratio of clay minerals and the small dimensions of the individual layer both make them the most exciting fields of material science (Mullassery *et al.*, 2018).

Even though, clay minerals and polymers have been often used in the primary form as a single drug carrier, they cannot meet all the needs alone (Pathania *et al.*, 2016). The insertion of organic species into layered inorganic materials provides a useful and convenient path to prepare organic-inorganic hybrids that combine the properties of both inorganic and organic agents (Wang *et al.*, 2005). Proper preparation of polymer- layered silicate composite not only improves the properties of the clay mineral particles (e.g. the stability of the clay mineral dispersion and its ion exchange behavior), but also influences the polymer traits (e.g. mechanical properties, swelling capacity, film forming abilities, rheological properties, and bioadhesion or cellular uptake) (Viseras, Cerezo, Bedmar, 2008).

Due to less reliable reports of alginate-montmorillonite intercalation, they are examined as a drug carrier in this article; further, their swelling behavior in different conditions and their drug delivery abilities are investigated.

MATERIAL AND METHODS

Material

In this experiment, Alginate ($C_6H_7NaO_6$), Sodium Hydroxide (NaOH), trisodium phosphate (Na_3PO_4), hydrochloric acid (HCl), cadmium chloride ($CdCl_2$) were purchased from Merck chemical co. Alendronate Sodium ($C_4H_{13}NO_7P_2$)and montmorillonite, respectively, were obtained from Arastoo Pharmaceutical Company and Pishgam Technology Company.

Preparation

In order to prepare alginate-loaded clay beads, MMT powder was added to a %3 alginate solution to obtain four different ratios. The mixture was stirred until all contents are homogeneously dispersed. The temperature was kept at 60˚C and the stirring speed was fixed at 600 rpm. The Beads are usually prepared by transferring drop wise in %2 Cd^{2+} solution. Beads were washed with distilled water and were kept in room temperature for 24 hour to obtain dried beads. The four samples of Alg/MMT were prepared in ratio of (1:0.25), (1:0.5), (1:1), (0.25:1) respectively.

To prepare Alg-MMT composites with drug loading, the amount of 91.363 mg of alendronate sodium trihydrate powder is mixed with 300 mg of two optimized composite samples (i.e. (1:0.25), (1:0.5)) and then 0.5ml ethanol (96%) was added to them. The resulting dough is dried at 60 °C and pressed by a press machine. Each series of six pills were placed into 6 dissolution vessels (each vial of 1 tablet), and then dissolution percentage of each tablet were measured over the periods of 0, 10, 30, 60, 120, 180, 240 and 300 minutes.

Characterization

The composition of optimized samples were determined by X-ray diffraction (XRD) using an X-ray diffractometer (Equinox 3000, Inel) with CuKα (40kV, 30mA) radiation. The surface morphology of the samples was observed with a scanning electron microscope (2100AIS, seron). Energy-dispersive x-ray spectroscopy (EDX) was also used for the elemental analysis (MIRA3TESCAN-XMU). Optical absorption for the selected samples was carried out with a PR 5000-HACH UV/Visible spectrophotometer. Infrared spectrum of the samples was recorded using a Fourier transformed infrared (FTIR) spectrophotometer (Alpha- Burker).

RESULTS AND DISCUSSION

The behavior of inflation nanocomposites in different pH, temperature and time were investigated,

however two samples were selected for drug loading due to good swelling behavior.

The swelling abilities of the beads were studied gravimetrically. A certain amount of beads was placed in distilled water and then they were accurately weighed after 1, 2, 3 and 4 hours. In the each test equilibrium swelling for different formulations was calculated as follows

$$ES = \frac{w_s - w_d}{w_d} \times 100$$

where w_s was the bead the bead weight at time t and w_d was the initial bead weight. To determine the time effect on the ES value in a near-natural condition to human body, a certain amount of beads were placed in 900 milliliters of simulated gastric fluid (SGF, a solution of 0.1 N hydrochloric acid 37%) and in 250 milliliters of simulated intestinal fluid (SIF, a solution of 0.2 M trisodium phosphate) solutions (Stippler, Kopp, Dressman, 2004). The results are shown in Figures 1 and 2.

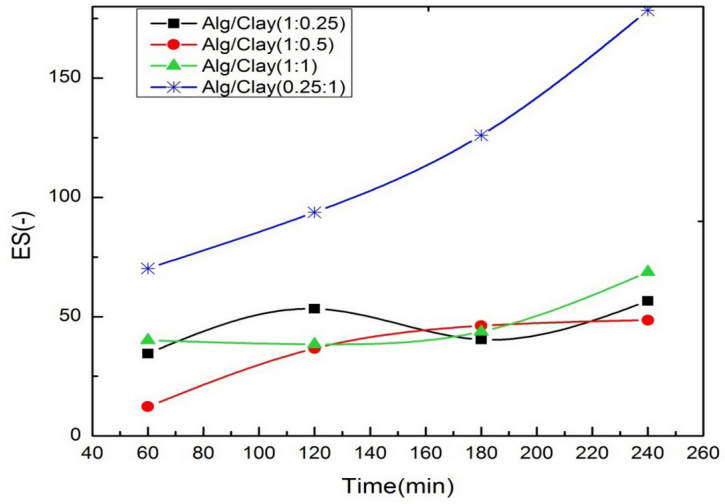

FIGURE 1 - Equilibrium swelling percent of the samples vs. time in the simulated gastric medium.

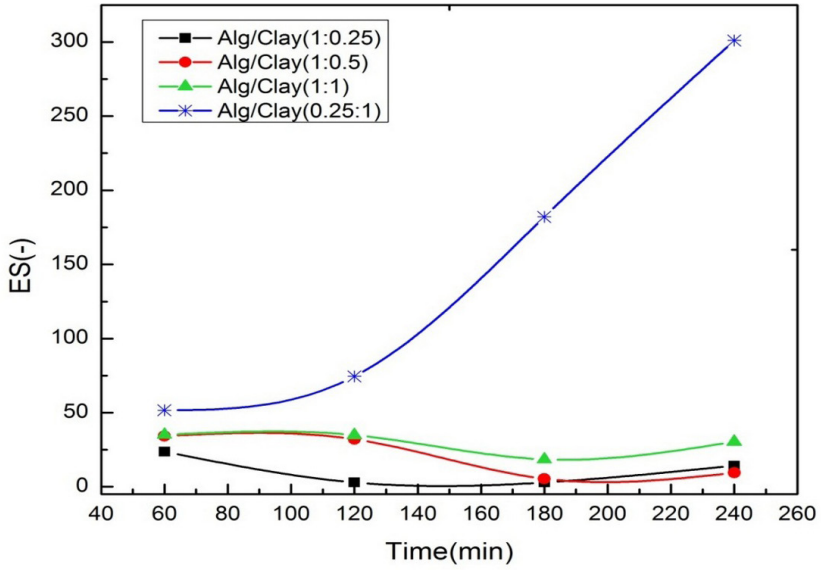

FIGURE 2 - Equilibrium swelling percent of the samples vs. time in the simulated intestinal medium.

It can be seen that the first two samples (i.e. Alg/clay (1:0.25) and Alg/clay (1:0.5)) have a more expected behavior, since, in none of them, there is an increasing absorption trend vital in drug release. It seems the increment of the clay ratio does not necessarily give a better result. In both figures, the sample Alg/clay (0.25:1) has an increase absorbing feature.

On the other hand, the interaction between MMT and alginate affected the water swelling process. With increasing relative content of MMT a further increase of equilibrium swelling was observed, possibly due to the increased porosity of the composites, which was consistent with the SEM analysis (Zhang *et al.*, 2020).

To determine the ES trend versus time at body temperature, beads were kept at body temperature (37.5 ◦C) and their ES behavior was measured in distinct different hours. In this case, unlike the SGF and SIF conditions, all of the samples reached a maximum absorption initially and then arrived at a relative decrease (Figure 3).

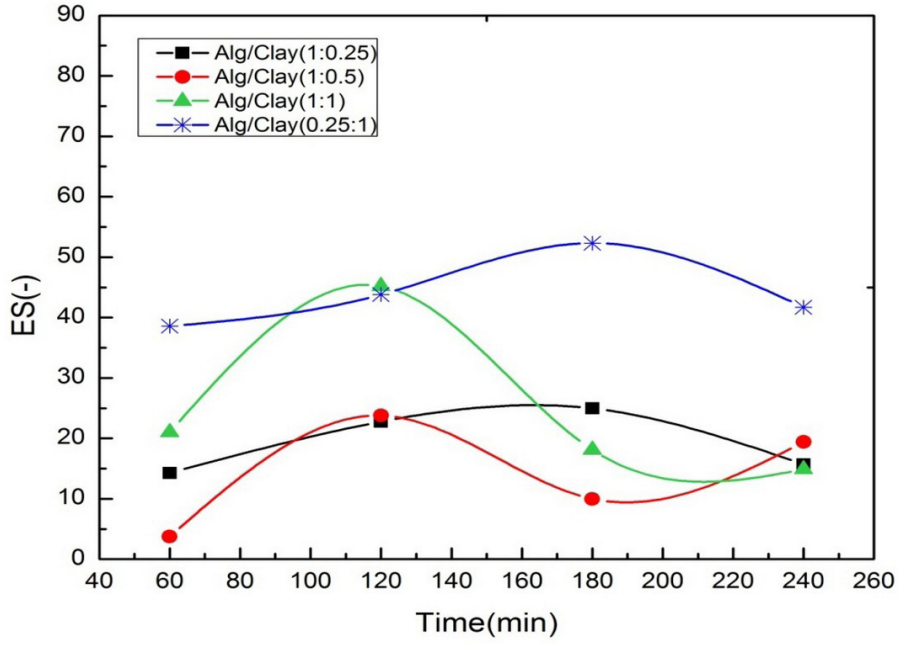

FIGURE 3 - Equilibrium swelling percent of the samples vs. time at body temperature.

One of the most important parameters affecting the drug release behavior is the surface of the carrier. Figure 4 shows the SEM micrographs of alginate and two selected samples. Comparing the SEMs of Alg and the Alg-MMT samples, the surface of the Alg-MMT beads is more rigid and with further involvement of Montmorillonite, the fracture increases. With a closer look, pure alginate hydrogel displays a smooth and homogeneous surface, showing an almost amorphous structure. More addition of MMT results in the rougher surface of the composite verifying the presence of the clay. Particles and cracks are clearly observed on the surface of the composites and the size grows by increasing clay content. The morphology of the surfaces indicates the porous structure of composite. These pores permit water to be absorbed and interacted with hydrophilic groups on the structure. As a result, the swelling ability increases (Kenawy, Azaam, El-nshar, 2019).

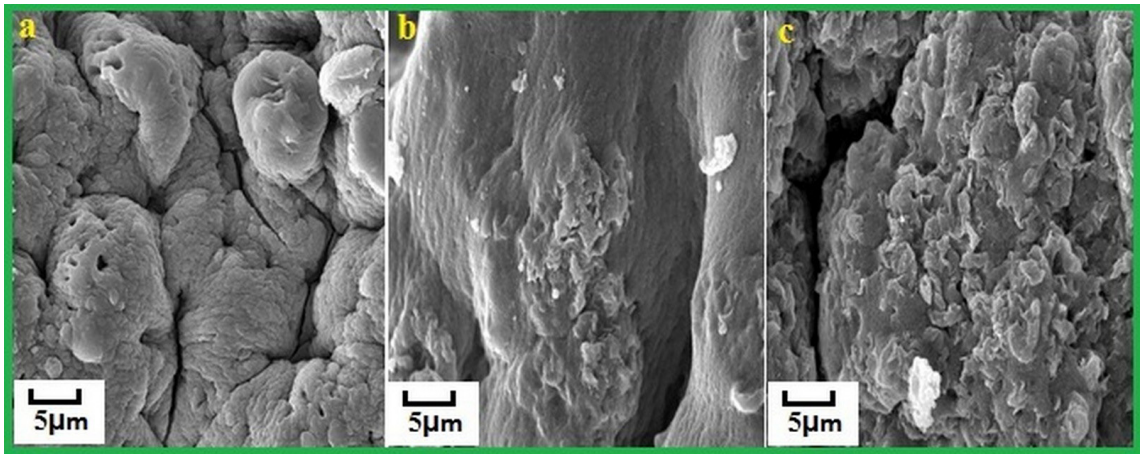

FIGURE 4 - SEM images of a) alginate (Alg), b) Alg/Clay (1:0.25) and c) Alg/Clay (1:0.5).

For better morphological comparison of two selected samples, they are shown in Figure 5 at different magnifications. According to Figure 5-a, c; by increasing MMT, the surface of the sample is associated with increased grains. The larger view (Figure 5 b, d) shows that there is a typical tangled structure. The hemispherical structure of the first sample and regular spherical structure of the second one are also impressive. Other researchers have also reported such micrographs. (Kaygusuz *et al.*, 2015b; Kevadiya *et al.*, 2010).

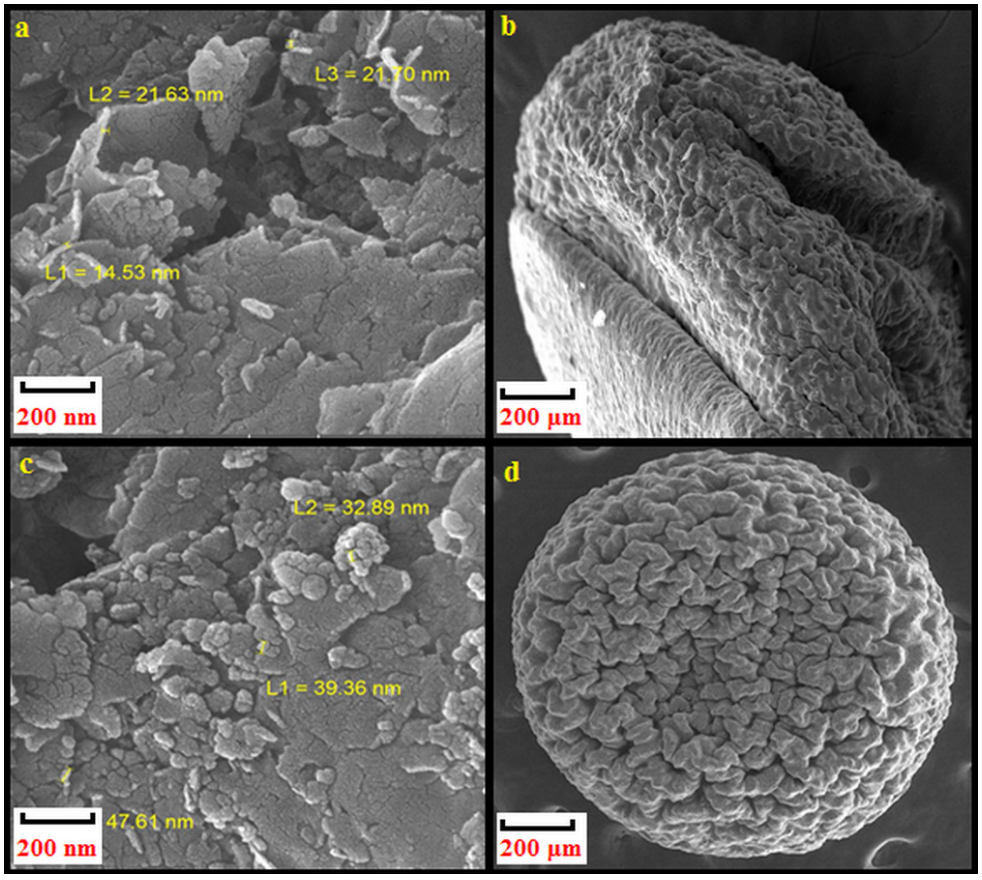

FIGURE 5 - SEM images of the alginate samples at different magnifications: a, b) Alg/Clay (1:0.25) and c, d) Alg/Clay (1:0.5).

Fourier transfer infrared spectroscopy (FTIR) is one of the simplest methods to characterize and identify functional groups present in a compound. Additional information regarding the structure and the chemical bonds between chemical species was obtained by the infrared spectroscopy. Figure 6 shows the FTIR spectra of the samples with different ratios of Alg/clay. Measurements were performed at room temperature within 400–4000 cm^{-1} wavenumber range (corresponding to middle infrared region) under ambient conditions, using KBr as a diluting agent. The following results are based on the native alginate and most of the bands in all samples are shown as justifiable shifts.

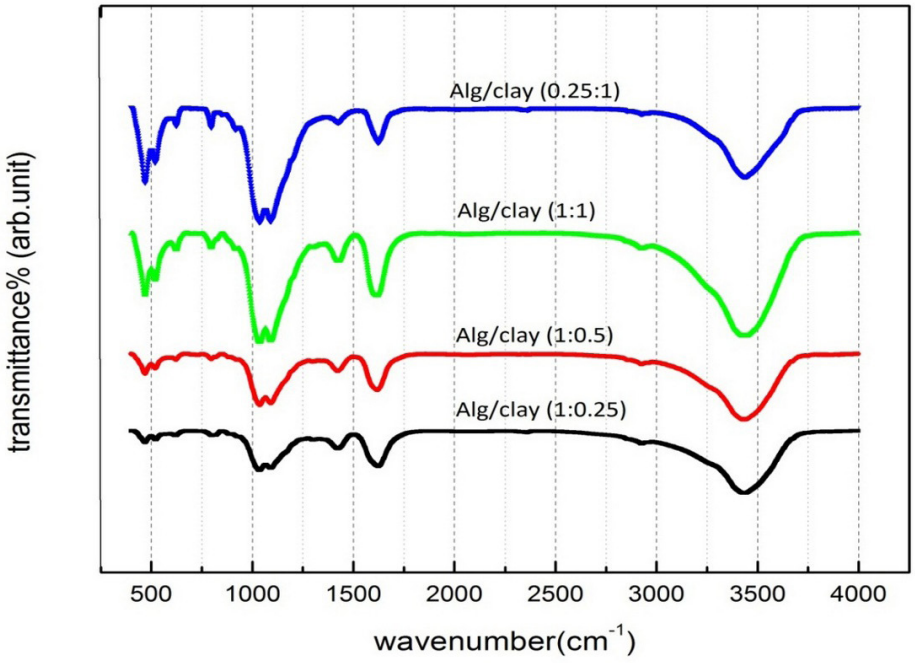

FIGURE 6 - FTIR spectra of four samples of Alg/clay.

A broad absorption band in the region of 3265–3680 cm^{-1} is assigned to the stretching vibration mode of the hydroxyl group (Bagheri Khatibani, Abbasi, 2018; Iliescu *et al.*, 2011). The -OH stretching vibration gets decreases in intensity by decreasing the clay ratio. The peak with a wave number of around 2939 cm^{-1} corresponds to the bending vibration of –CH band (Rajesh, Ravichandran, 2015). Sodium alginate shows asymmetric and symmetric stretching vibrations at about 1627 and 1415 cm^{-1}(Iliescu, Andronescu, Ghitulica, 2013). The appearance of C-O peaks at 1617, and 1419 cm^{-1} is also related to the carboxylic acid (Kevadiya *et al.*, 2010), (Rajesh, Ravichandran, 2015). Additionally, the peaks at 821 and 946 Cm^{-1} in native alginate FTIR spectra are due to the Gluronic (G) and Manuronic (M) acid functional groups respectively (Ghahramanpoor *et al.*, 2011). Further, the band around 3426 cm^{-1} corresponds to the OH stretching mode in Si-OH, and Al-OH bonds with a small shift in four samples. 1044, 621 and 520 cm^{-1} are attributed to Si-O stretching in $[SiO4]^{-4}$ tetrahedral water and in also Si-OH, and Al-OH bonds (Iliescu *et al.*, 2011). These are particular montmorillonite bonds, with decreased intensities by increasing alginate values. This might be due to a strong chemical interaction between the Si-O and -OH groups of the clay and functional groups. (Marandi, Mahdavinia, Ghafary, 2011).

A possible hydrogen bond between the carbonyl groups of alginate and hydroxyl groups of MMT and also that between hydroxyl groups of alginate and SiO_4 geometry of MMT may be effective (Shilpa, Agrawal,

Ray, 2003).The wave numbers at 798 cm $^{-1}$ corresponds to Si-O vibration in SiO_2 and 467 cm^{-1} to Si-O-Si and Na-Al-OH vibrations of MMT (Iliescu *et al.*, 2011).

The X-ray diffraction patterns for two selected samples are shown in Figure 7. Two major peaks at 13.57° and 22.75° for alginate have been reported earlier (Rajesh, Ravichandran, 2015). It seems that by increasing the value of montmorillonite, its peak gets stronger and the crystalline alginate structure gets better, i.e., the increase in montmorillonite causes a better crystallization of the nanocomposite. Based on the XRD spectrum of pure MMT, the interaction of alginate with MMT does not result in significant changes in the XRD patterns of the composites, however, the spectra are broadened due to the dispersion of clay into the polymeric matrix (Surya *et al.*, 2019).

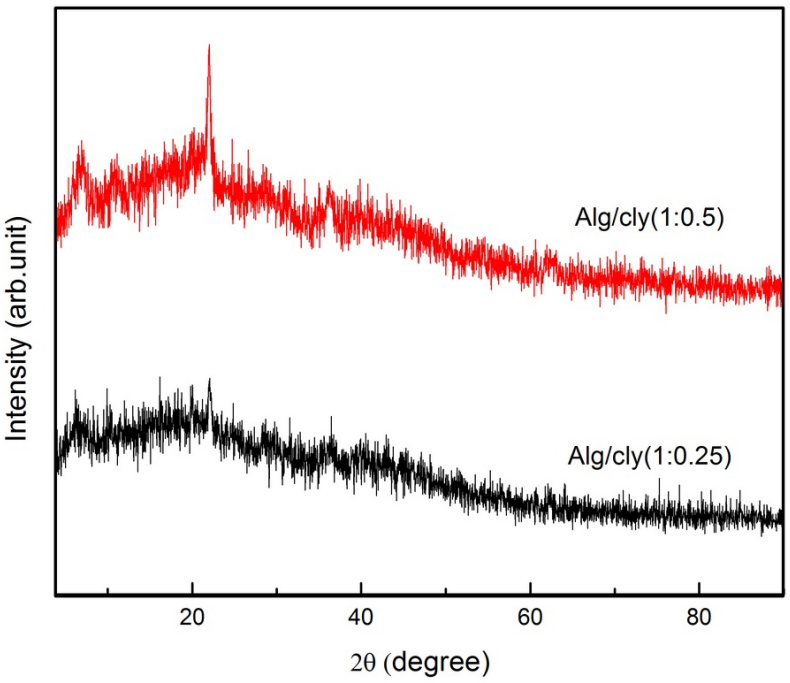

FIGURE 7 - XRD patterns of Alg/clay (1:0.25) and Alg/clay (1:0.5).

Compositional analysis can be applied to demonstrate the presence of the elemental composition of claimed compounds. In this research, it is carried out using an energy- dispersive X-ray (EDX) analysis to study the stoichiometry of Alg-MMT samples. The results are shown in Figure 8 (a, b). The high percentage of carbon is due to carbon chains (carboxylate, carbonyl and ether functional groups) in alginate. Further, a high percentage of oxygen is present since this element is commonly used as the hydroxyl functional group in alginate and montmorillonite. The aluminum element is present on the montmorillonite octahedral plates and the silicon element in the tetrahedral montmorillonite plates. The comparison of two samples shows the weight percent of aluminum and silicon in the nanocomposite with a higher percentage of montmorillonite to be higher.

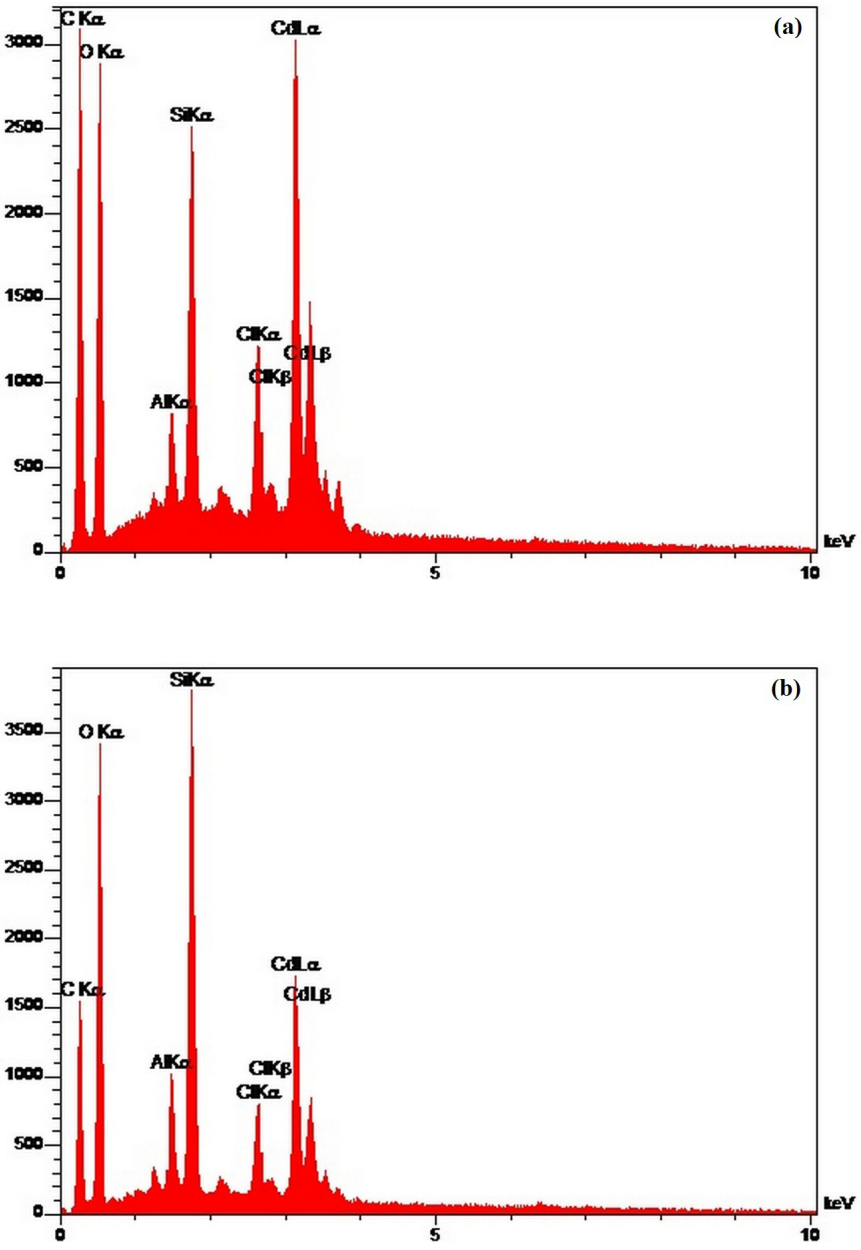

FIGURE 8 - EDX patterns of a) Alg/clay (1:025), b) Alg/clay (1:0.5).

To test the stability and quality of the samples, their absorption values (vs. time) in two different media (i.e. the acid stage like gastric position and buffer stage like intestinal position) are investigated as illustrated in Figures 9 and 10.

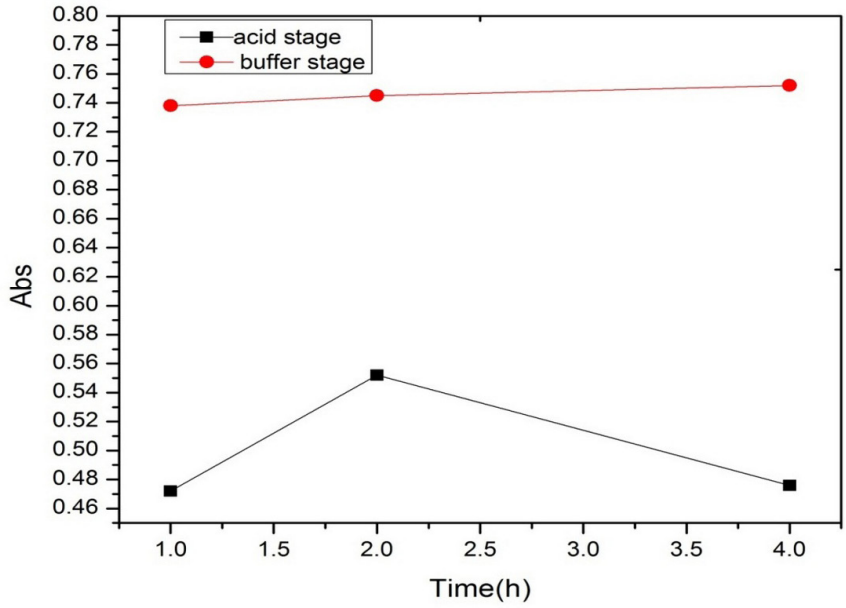

FIGURE 9 - The absorption of Alg/clay (1:025) vs. time.

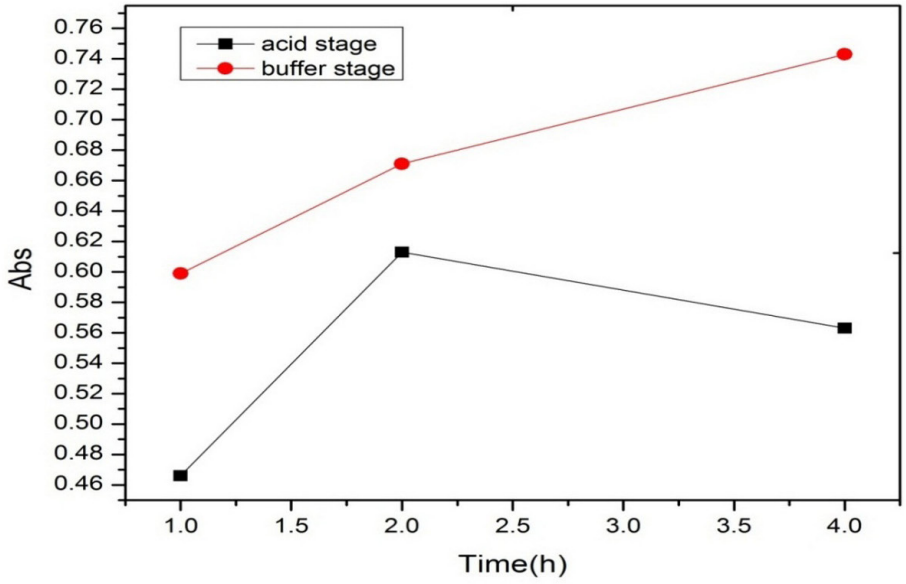

FIGURE 10 - The absorption of Alg/clay (1:0.5) vs. time.

Both samples can remain in an acidic environment for up to 2 hours, however, the descending trend shows the destruction of nanocomposites. Whereas, in a buffer environment, the samples are destroyed after the fourth hour. The slope of the graphs (especially in acidic medium) may indicate that the increase in the amount of the montmorillonite prevents the desired release of the drug.

To determine the dissolution (a criterion for drug delivery), a standard solution is prepared, and its absorption values are compared with the selected samples. To prepare the standard solution, the following steps are taken; an amount of 91.363 mg of Alendronate Sodium powder is divided into six equal parts. Then they are dissolved in distilled water to reach a volume of 1000 cc. Standard concentration is 0.015 mg/ml. The absorption

values of the standard solution are compared with those of the selected samples (average absorption of six samples at given times).

Based on literature, two mechanisms affect the release process; the Fickian diffusion and the swelling of the polymer. It seems in our cases, the release kinetics is controlled by both the diffusion and swelling process and the drug release may be due to both the swelling of alginate covering and also due to the diffusion of the drug from the modified clay (Surya *et al.*, 2019). The results showed in Figure 11. According to the figure, with increasing the time up to 180 minutes, both nanocomposites release more drugs until reaching a maximum point and then remain almost constant. By considering the slope of the graphs, it can be concluded that the release rate is constant for both; however, the sample with a higher value of clay has a relatively lower delivery rate.

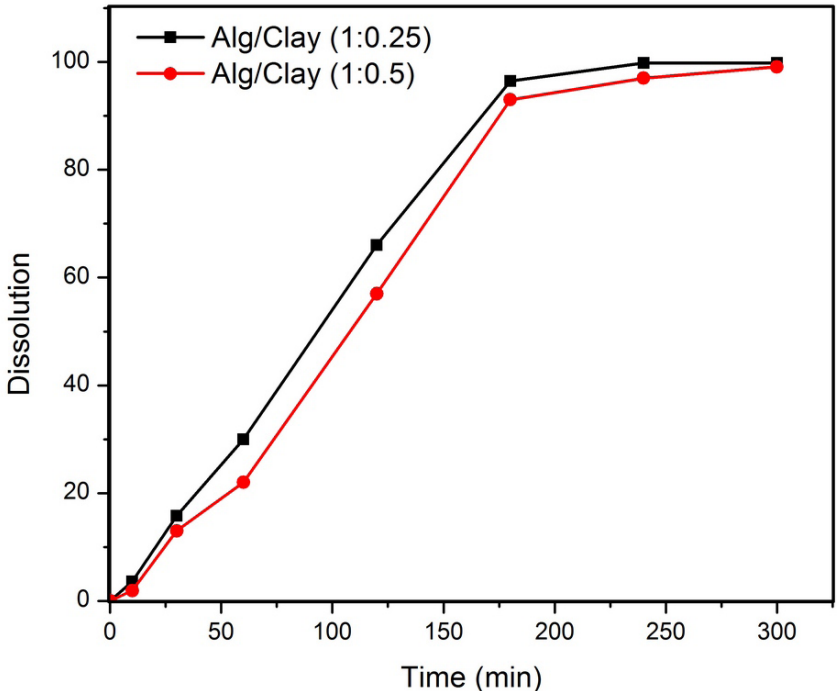

FIGURE 11 - Dissolution percent of Alg/clay (1:0.25) and Alg/clay (1:0.5) vs. time.

CONCLUSION

In this paper, powders of alginate and montmorillonite are combined and four samples with a different ratio of montmorillonite were prepared. The swelling test showed that the two samples 1 and 2 had better absorption and were used for further investigation. According to the SEM analysis, the morphological changes in the two selected samples are quite recognizable. With increasing montmorillonite, the porosity decreased and the surface of the sample is formed with more grains. In the FTIR analysis, the bands at 821 and 946 cm^{-1} of alginate and bands at 520, 621 and 1044 cm^{-1} are related to montemorillonite. The XRD analysis showed that the crystallinity of the structure was improved by increasing the amount of Montmorillonite. According to the EDX analysis, the presence of aluminum and silicon was observed in both selective samples, with the increase of the montmorillonite, the intensity of the peaks associated with these two elements was significantly different. To check the capability of drug delivery, the absorption of the selected samples were compared. It was observed

that the sample with higher value of clay has a relative lower delivery rate.

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