Alginate/Montmorillonite Nanocomposites for the Delivery of Curcumin

A. R. Ramadan*, M. Ahmed**

*Department of Chemistry, The American University in Cairo,
AUC Avenue, New Cairo 11835, Egypt, aramadan@aucegypt.edu
**Department of Chemistry, The American University in Cairo,
AUC Avenue, New Cairo 11835, Egypt marwafathi@aucegypt.edu

ABSTRACT

Exfoliated montmorillonite clay (MMT) was first prepared by stirring the clay in an aqueous suspension for 4 hours, followed by filtration and drying at 70°C. Exfoliation was confirmed by X-ray diffraction (XRD) and Fourier Transform infrared (FTIR) spectroscopy. Curcumin-loaded MMT was then prepared by dispersing the exfoliated clay into an ethanoic curcumin solution. Stirring exfoliated MMT into a curcumin solution of 1 mg/ml for 1 hour and in 5% wv ratio was found to be the best condition for maximum loading which was 6.56 mg/g, corresponding to an entrapment efficiency of 25.62 %. The curcumin-loaded MMT was then encapsulated into alginate beads with different clay to alginate ratios (w/w) using the ionotropic technique.

The release of curcumin from different alginate/MMT nanocomposites was studied in different biorelevant media: fasting gastric, fasting intestinal, and fed intestinal media. Different release behaviors were found. These were influenced by the media pH, and the ratio of curcumin-loaded MMT to alginate. The percentage of curcumin released was lowest in the fasting gastric medium due to the low pH. In the intestinal media, the percentage release was higher in the fasting state than in the fed state due to the higher pH of the former medium. Sustained release was found to occur for 24 hours in the intestinal fasting medium, while curcumin release reached a plateau by 8 hours in the intestinal fed medium. The 1:20 (w/w) curcumin-MMT to alginate ratio nanocomposites showed the highest release percentage in both fasting and fed intestinal media, with the 1:2 (w/w) curcumin-MMT to alginate ratio nanocomposites showing the lowest release percentage in both media.

Keywords curcumin, alginate, montmorillonite, controlled release.

1 INTRODUCTION

Curcumin is a well-known traditional medicine with anti-inflammatory and antioxidant properties. Its pharmacological mechanism of action and safety have been studied to investigate its use in clinical and therapeutic applications [1,2]. However, its low water solubility and rapid metabolism represent challenges in this regard. Different approaches have been used to overcome these challenges, with recent attention focusing on approaches based on nanotechnology [3,4]. Clay-polymer nanocomposites are getting to play a role in nanoformulations for drug delivery. This is due to their improved rheological and mechanical properties, and controlled drug release characteristics compared to their individual components, clays and polymers. Alginate/ (MMT) nanocomposites are used as a drug delivery system for a variety of drugs due to numerous advantages of both components, such as the high loading capacity of MMT and the ability of alginate release encapsulated drugs in a controlled manner [5, 6]. The current work aims at preparing a controlled release delivery system for curcumin using alginate/MMT nanocomposites, investigating their release behavior in different biorelevant media.

2 EXPERIMENTAL

Cloisite NA+ MMT clays (Southern Clay Products, INC.) were exfoliated by stirring the clay in an aqueous suspension for 4 hours, followed by filtration and drying at 70°C. The dried powder was then ground and sieved through sieve no. 45 (355 µm mesh). Exfoliation was confirmed by X-ray diffraction (XRD) where a D8 Bruker powder diffractometer was used, operated at 40 KV and 30mA, and using a Cu target with Kα, $\lambda = 0.1542$ nm. The exfoliated MMT was then dispersed in a curcumin (Curcuma longa-Tumeric-powder) solution of 1 mg/ml of absolute ethanol (Carlo Erba), to form a 5% w/v dispersion. This was stirred at 25°C for 1 hour at 500 rpm, followed by centrifugation and decantation of the supernatant curcumin solution. The curcumin-loaded MMT was dried for 24 hours at 50°C, and then sieved through sieve no. 45. Curcumin loading was monitored by Fourier Transform infrared (FTIR) spectroscopy, using the KBr method and a Thermo-scientific Nicolet 380 spectromer. The entrapment efficiency (EE) of the loaded MMT was calculated using Equation (1), where C_i is the initial concentration of the initial curcumin (1 mg/ml), and C_f is the concentration of curcumin in the supernatant solution after the separation of the loaded MMT. Curcumin concentrations were determined through Absorbance measurements at $\lambda = 425$ nm using a Spectronic 20D+ spectrophotometer.

$$EE = (Ci - CF)/Ci * 100 \quad (1)$$

The entrapment efficiency was found to be 25.62%.

Different samples of alginate and curcumin-loaded MMT were prepared with variable ratios of clay to alginate

solution. Generally, a 2% w/v alginate (alginic acid sodium salt from brown algae - low viscosity) aqueous solution was used to disperse a known weight of the curcumin-loaded MMT. The mixture was stirred at 25°C for 1 hour at 1000 rpm to ensure homogenous dispersion. The suspension was then added to a 3% w/v $CaCl_2$ (Charlau, anhydrous, 95% purity) solution using a 1.5 mm gauge syringe and MasterFlex L/S peristaltic pump with a flow rate 1ml/min. The reaction between the alginate and the $CaCl_2$ led to the formation of beads composed of cross-linked alginate polymer containing curcumin-loaded MMT. The formed beads were allowed to cure in the $CaCl_2$ solution for 30 mins at 25°C. Four nanocomposite samples, NC1, NC2, NC3, NC4 of alginate and curcumin-MMT were thus prepared with compositions (w/w, curcumin-MMT/alginate) of 1:20, 1:10, 1:5, and 1:2 respectively. No curcumin was found to diffuse fom the curcumin-loaded MMT into the $CaCl_2$ solution during the process of bead formation, as monitored by Absorbance measurements for curcumin at $\lambda = 464$ nm.

In vitro release tests were conducted under sink conditions [7] using ready-to-use biorelevant media: fasting gastric (Biorelevant FaSSIF-original powder, dissolved in NaCl/HCl solution of pH 1.6), fasting intestinal (Biorelevant FaSSIF-V2, dissolved in maleate buffer of pH 6.5), and fed intestinal media (Biorelevant FaSSIF-original powder, dissolved in acetate buffer of pH 5.0). For these tests, curcumin-loaded MMT/alginate samples were dispersed in each of the media, and shaken for 24 hours at 100 stroke/min while thermostated at 37°C. Solution samples were drawn at different time intervals and Absorbance measured so as to determine the released amounts of curcumin. Absorbance was measured at λ values of 425 nm, 418 nm, and 424 nm for the fasting gastric medium, fasting intestinal and fed intestinal media respectively.

3 RESULTS

3.1 MMT exfoliation and curcumin loading

MMT exfoliation was confirmed by the attenuation of its basal refection peak at 7.1° 2-theta, corresponding to a d_{001} space of 12.44 Å. This allowed the loading of curcumin onto the surface of exfoliated MMT, as cationic intercalation of curcumin into MMT is difficult to achieve because of the hydrophobic and weak acidic properties of curcumin. Enhancement of the dissolution of water insoluble drugs upon adsorption on MMT surface has been reported in a number of studies [7-9].

FTIR spectra of curcumin-loaded MMT exhibited the characteristic bands of curcumin, and those of MMT. For the latter, however, changes in the relative intensities of some of its characteristic bands (notably those of Si-O-Al bending, and those assigned to the OH group) denoted the delamination of the MMT layers, suggesting an exfoliated structure. The absence of significant changes in the positions and shapes of MMT and curcumin bands in the loaded clay samples as compared to those of pristine MMT and unloaded curcumin, suggested that the interaction between curcumin and MMT in the loaded samples was primarily physical in nature.

FTIR spectra of curcumin-loaded MMT and alginate nanocomposites exhibited the characteristic bands of the different components. However, the alginate carboxylate band showed a noticeable decrease in relative intensity, possibly due to the electrostatic attraction between the negative charge of carboxylate and the positive edges of exfoliated MMT [10]. Additionally both the cyclic ether oxygen and the Si-O stretching bands of alginate and MMT respectively greatly decreased in the spectra of the nanocomposites, denoting interactions between alginate and MMT in these samples.

3.2 Curcumin release studies

The different nanocomposite samples (NC1, NC2, NC3, NC4) demonstrated different release profiles which were dependent on the type of release medium, as well as the ratios of curcumin-loaded MMT to the alginate polymer in the samples.

The release of curcumin from all samples in the fasting gastric medium was negligible. This could be attributed to the very low solubility of curcumin in the acidic gastric medium, and more significantly, to the shrinkage of alginate beads in the acidic medium due to the transformation of alginate into alginic acid, thus hindering the release of encapsulated species [11].

As for the release of curcumin in the intestinal media, two main findings are of significance: an increase in the amount of curcumin-loaded MMT in the nanocomposite sample generally decreased the percentage release of curcumin; curcumin release was higher in the fasting medium than the fed medium. This is presented in Figure 1.

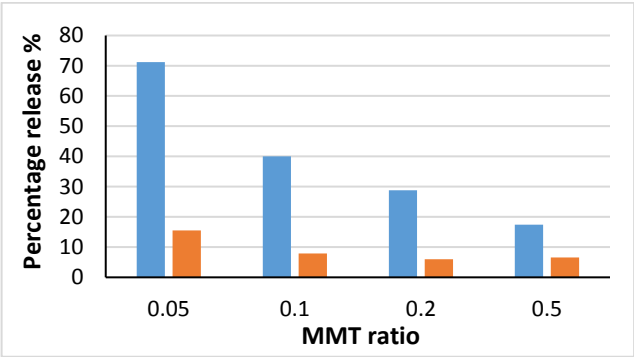

Figure 1. Percentage of curcumin released for the different relative amounts of curcumin-loaded MMT in the nanocomposite samples: ■ intestinal fasting release medium, and ■ intestinal fed release medium.

The first finding may be attributed to the increased cross-linking between the alginate chains as a result of the MMT presence [12]. This increased cross-linking reduced the alginate's swelling ability, thus decreasing the amount of curcumin released. Additionally, the presence of the exfoliated MMT in the polymer matrix provided a more tortuous pathway for the curcumin molecules to move through before being released into the dissolution media. The second finding may be attributed to the pH dependence of alginate swelling. Alginate demonstrates an increase in swelling with the increase of pH due to the loss of divalent cross-linking cations [11]. This would have led to an increase in curcumin release for the intestinal fasting state (pH = 6.5) as compared to the intestinal fed state (pH = 5.0). Additionally, the solubility of curcumin, a weak acid, could also have had a contribution to the second finding.

The release profiles of curcumin were different in the two intestinal release media, as shown in Figure 2. For the intestinal fasting medium, continued release was observed, and the time required for the release of 50% of the loaded amount ($T_{50\%}$) is more than 24 hours for all samples except for NC1 (1:20 w/w curcumin-MMT to alginate composition). On the other hand, curcumin release was found to reach a plateau after 8 hours for the intestinal fed medium. These release values are higher than values reported in the literature [13].

	Intestinal Fasting		Intestinal Fed	
	%	Amount (µg)	%	Amount (µg)
NC1	71.2	29.0	15.5	4.0
NC2	40.0	31.2	7.9	3.4
NC3	28.8	42.6	6.0	5.6
NC4	17.4	51.7	6.6	12.2

Table 1. Curcumin release in biorelevant intestinal media after 24 hours

Table 1 shows that, though the percentage release of curcumin after 24 hours is inversely proportional to the relative amounts of curcumin-loaded MMT in the nanocomposite samples, the mass released is directly proportional to these relative amounts. This is a factor that is to be taken into account for the design of controlled release systems aiming at specific dosage delivery.

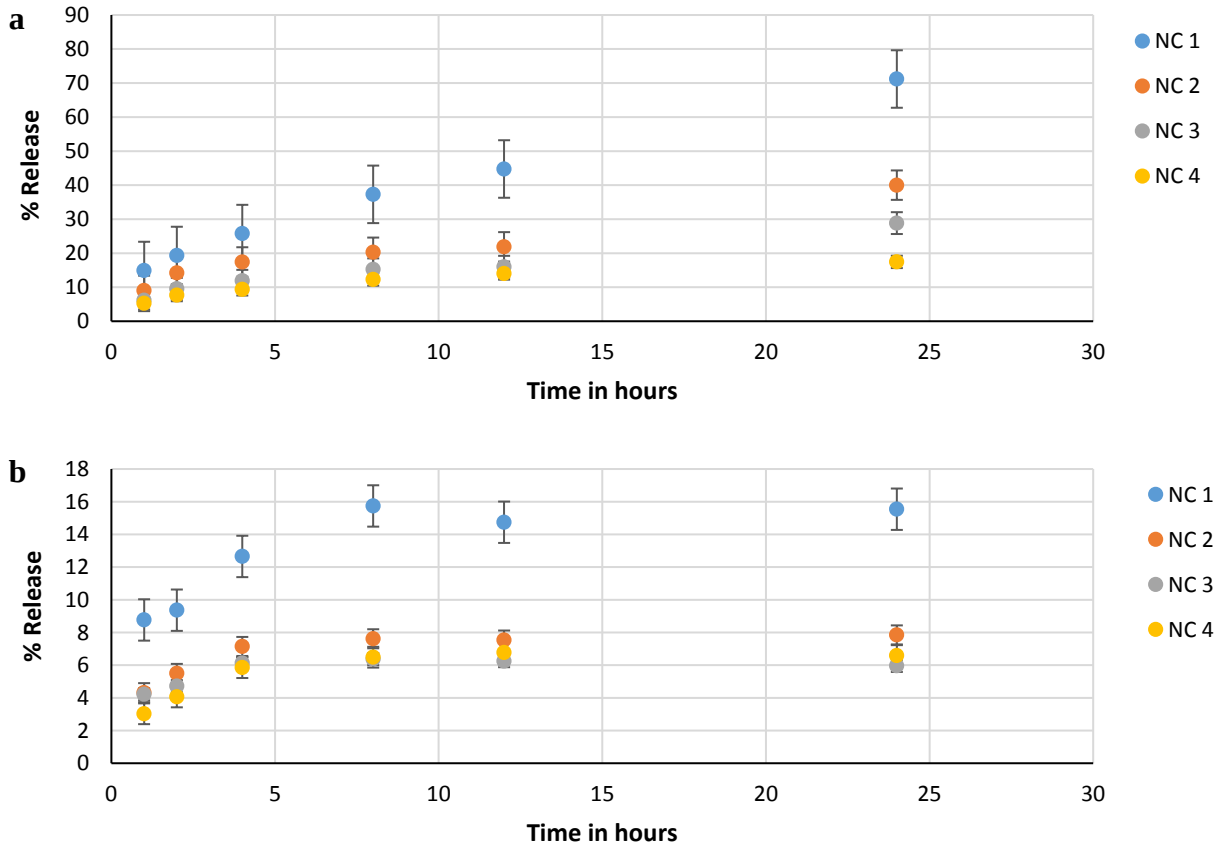

Figure 2. Curcumin release profiles for fasting intestinal medium (a), and fed intestinal medium (b), for different curcumin-MMT to alginate compositions (w/w): 1:20 (NC1), 1:10 (NC2), 1:5 (NC3) and 1:2 (NC4).

TechConnect Briefs 2017, TechConnect.org, ISBN 978-0-9988782-0-1

4 CONCLUSIONS

Prepared nanocomposite curcumin-loaded MMT and alginate polymer exhibited controlled release of curcumin in intestinal biorelevant media. The percentage release of curcumin was found to increase with a decrease of the relative amount of curcumin-loaded MMT in the nanocomposite samples. This was explained by the role of exfoliated MMT in the enhancement of the alginate polymer matrix cross-linking. Moreover, the percentage release of curcumin was found to be higher for the fasting medium than the fed medium, which was explained by the effect of the medium pH on the swelling of the alginate matrix. The curcumin release profiles were different in these two media: for the fasting medium, continued release occurred until after 24 hours, whereas for the fed medium a plateau was reached after 8 hours. No release of curcumin from the nanocomposite samples was found to occur in gastric biorelevant media. The findings reveal the promising use of alginate/MMT nanocomposites as a controlled release delivery system for curcumin.

REFERENCES

[1] R.A. Sharma, A.J. Gescher, and W.P. Steward, "Curcumin: The story so far" Eur. J. Cancer 41, 1955–1968, 2015.

[2] S.S. Bansal, M. Goel, A. Farrukh, M.V. Vadhanam, and R.C. Gupta, "Advanced drug delivery systems of curcumin for cancer chemoprevention" Cancer Prev. Res. 8, 1158–1171, 2011.

[3] M. M. Yallapu, M. Jaggi, and S. C. Chauhan, "Curcumin nanoformulations: a future nanomedicine for cancer" Drug Discov. Today 17, 71–80, 2012.

[4] O. Naksuriya, S. Okonogi, R. M. Schiffelers, and W. E. Hennink, "Curcumin nanoformulations: A review of pharmaceutical properties and preclinical studies and clinical data related to cancer treatment" Biomaterials 35, 3365–3383, 2014.

[5] C. Viseras, C. Aguzzi, P. Cerezo, and M. C. Bedmar, "Biopolymer–clay nanocomposites for controlled drug delivery" Mater. Sci. Technol. 24, 1020–1026, 2008.

[6] F. Chivrac, E. Pollet, and L. Avérous, "Progress in nano-biocomposites based on polysaccharides and nanoclays" Mater. Sci. Eng. R Reports 67, 1–17, 2009.

[7] D. J. Phillips, S. R. Pygall, V. B. Cooper, and J. C. Mann, "Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems," J. Pharm. Pharmacol. 64, 549–1559, 2012.

[8] W. R. Gombotz and S. F. Wee, "Protein release from alginate matrices," Adv. Drug Deliv. Rev. 64, 194–205, 2012.

[9] H. A. Koeleman, R. van Zyl, Steyn, N. B. Boneschans, and H. S. Steyn, "Influence of montmorillonite on the dissolution and bioavailability of phenytoin," Drug Dev. Ind. Pharm. 16, 791–805, 1990.

[10] R. I. Iliescu, E. Andronescu, C. D. Ghitulica, G. Voicu, A. Ficai, and M. Hoteteu, "Montmorillonite-alginate nanocomposite as a drug delivery system - Incorporation and in vitro release of irinotecan," Int. J. Pharm. 463, 184–192, 2014.

[11] H. Kaygusuz and F. B. Erim, "Alginate/BSA/montmorillonite composites with enhanced protein entrapment and controlled release efficiency," *React. Funct. Polym.* 73, 1420–1425, 2013.

[12] S. Hua, H. Yang, W. Wang, and A. Wang, "Controlled release of ofloxacin from chitosan-montmorillonite hydrogel," Appl. Clay Sci.50, no. 112–117, 2010.

[13] N. Suwannateep, W. Banlunara, S. P. Wanichwecharungruang, K. Chiablaem, K. Lirdprapamongkol, and J. Svasti, "Mucoadhesive curcumin nanospheres: Biological activity, adhesion to stomach mucosa and release of curcumin into the circulation," J. Control. Release 151, 176–182, 2011.