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Original Article

DEVELOPMENT AND CHARACTERIZATION OF PH-RESPONSIVE POLYMERIC MICROBEADS INTERCALATED WITH MONTMORILLONITE FOR CONTROLLED RELEASE OF DOXORUBICIN

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ABSTRACT

Objective: The aim of the present study is to develop pH-responsive polymeric microbeads for controlled release of doxorubicin.

Methods: Doxorubicin-encapsulated polymeric microbeads were developed by a simple ionotropic gelation method using sodium alginate, gum ghatti, and montmorillonite (MMT). In this work, we investigate the positive benefits of MMT mineral as a drug carrier for the controlled release of DOX. X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) were used to characterize the generated microbeads. The influence of hetero-ionic concentration on drug encapsulation efficiency and drug release from microbeads was examined. *In vitro* release and swelling studies were performed at pH 2.0 and 7.4 at 37 °C. The cytotoxicity of the developed microbeads was studied using *in vitro* cultures of the human breast cancer cell line (MCF-7).

Results: FTIR confirms the generation of microbeads and also the interaction between the polymer matrix, DOX and MMT clay. XRD analysis reveals the molecular dispersion of DOX and the presence of MMT in the polymeric matrix. SEM studies reveal the developed microbeads are spherical in shape with rough surfaces. Swelling and *in vitro* release studies are dependent on the pH of the test medium, which may be favorable for intestinal drug delivery. MTT results reveal that the developed microbeads showed good *in vitro* toxicity against MCF-7 cells. The drug release kinetics of the generated microbeads are followed by both the higuchi and korsmeyer-peppas models.

Conclusion: The findings suggest that the DOX-encapsulated microbeads are promising carriers for drug delivery applications. The fabricated microbeads further needs warrant for drug delivery applications.

Keywords: Sodium alginate, Gum ghatti, Montmorillonite, Doxorubicin, Microbeads, Drug delivery

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INTRODUCTION

Hydrogels made from polymers are now essential components of current pharmaceutical dosage forms, which are employed for a wide range of biological purposes. Due to their ability to absorb water, biocompatibility, and biodegradability [1, 2]. Hydrogels have numerous benefits over other drug carriers. However, hydrogels have a number of disadvantages, including uncontrolled drug release and swelling studies, which result in a number of adverse effects. To manage the release rate and swelling properties of hydrogels, clay minerals [3], and surface coatings with other polymers such as chitosan [4] and poly-L-Lysine [5] have been introduced into the hydrogels. Controlled drug release carriers have many advantages over traditional formulations, such as the ability to control the rate of release, reduce side effects, and keep effective drug levels in the blood.

In the past few years, clay and clay minerals have played an important role in the field of biomedicine as a result of their outstanding qualities. Some of these properties are good adsorption, a large specific surface area, a high ion exchange capacity, the ability to swell, and low toxicity [6]. Ancient people in Egypt, Rome, and China used to put clay on wounds to help heal them [7-9]. It can be utilised as a component in oral drugs such as antacids, gastrointestinal protectors, dermatological protectors, and anti-diarrheal agents [10]. Due to the strong interaction between clay minerals and drug molecules, which results in high adsorption rates and a slow drug release rate, clay minerals are considered a possible choice for controlled drug release. So, clay minerals are becoming more and more useful in biomedical fields like drug delivery and regenerative medicine [11].

In the last few decades, polymeric matrices have become more popular for targeted drug delivery because they are naturally available, biodegradable, non-toxic, and work well with the human body. One such polymer, sodium alginate (SA), is a polysaccharide containing alternating blocks of β-D-mannuronic acid (M block) and α-L-guluronic acid (G-block) that is extracted from brown algae. Because of its high biocompatibility, low toxicity, and ability to gel in mild circumstances, it has found widespread use in the food and pharmaceutical industries [12, 13]. Since SA matrices are rapidly degraded and absorbed by the body during and after drug molecule release, they are widely used in the production of controlled and sustained release formulations. Therefore, alginate is a wonderful matrix for the sustained release of many pharmacological substances [14]. Another polymer, gum ghatti (GG), is an anionic polysaccharide extracted from the plant species *Anogeissus latifolia*.

GG is widely employed in the food and pharmaceutical sectors due to its exceptional emulsifying abilities. It has recently gained popularity as a controlling agent in drug delivery applications [15, 16]. In this work, we use a calcium-alginate/gum ghatti matrix and introduce multivalent cations (Mg^{2+}, Ba^{2+}, and Al^{3+}) to generate hetero-ionic SA-GG/MMT beads for controlled release of doxorubicin. When multivalent ions are incorporated into a calcium-alginate/gum ghatti matrix, the swelling behavior and release rate of drug molecules are altered. Several methods, including FTIR, XRD, and SEM, were used to characterize the generated microbeads. Furthermore, we discussed the findings of *in vitro* drug release and swelling experiments performed at 37 °C at both the intestinal and stomach environments.

MATERIALS AND METHODS

Materials

Gum ghatti (GG) and montmorillonite are purchased from Sigma Aldrich made. Sodium alginate (SA) and calcium chloride were purchased from Sd. Fine chemicals, Mumbai, India. Doxorubicin has received has a gift sample from Aspiro Pharma Pvt Ltd, India. Double distilled water was used throughout the experiment.

Synthesis of microbeads

DOX-loaded microbeads were prepared using a simple ionotropic gelationmethod [3]. Briefly, 2% aqueous dispersions of SA and GG were

prepared using distilled water. The SA and GG solutions were mixed and stirred for 5 h. After that, 100 mg of MMT and 100 mg of DOX were added to the mixture and agitated continuously until a homogeneous solution was formed. Afterward, the resultant suspension was dropped into various proportions of $CaCl_2$, $MgCl_2$, $AlCl_3$, and $Bacl_2$ solutions (as given in table 1). Instantaneously spherical microbeads were developed and kept for 40 min. The solution was decanted to collect the produced microbeads, which were washed multiple times with distilled water to remove the drug molecules on the surface and then dried at room temperature overnight.

Table 1: Composition and codes of all samples

Code	2% SA (ml)	2% GG (ml)	MMT (mg)	DOX (mg)	$CaCl_2$ (%)	$MgCl_2$ (%)	$BaCl_2$ (%)	$AlCl_3$ (%)
SG-Ca	10	10	100	100	5	0	0	0
SG-CaMg	10	10	100	100	2.5	2.5	0	0
SG-CaBa	10	10	100	100	2.5	0	2.5	0
SG-CaAl	10	10	100	100	2.5	0	0	2.5
Placebo	10	10	100	100	5	0	0	0

Characterizations techniques

FTIR spectra of DOX, Placebo, MMT, SG-Ca, SG-CaMg, SG-CaBa, and SG-CaAl microbeads were recorded between 400-4000 cm^{-1} using model Bomem MB-3000. The X-ray diffraction of DOX, Placebo, MMT, SG-Ca, SG-CaMg, SG-CaBa, and SG-CaAl microbeads were performed by a wide-angle X-ray scattering diffractometer (Panalytical X-ray Diffractometer, model-X'pert Pro) with CuKα radiation (λ= 1.54060) at a scanning rate of 10 °/min to determine the crystallinity. The morphological characterization of microbeads was observed by using SEM (JOEL MODEL JSM 840A) with an accelerated voltage of 20 kV.

Intercalation kinetics

To find the appropriate intercalation time for DOX with MMT, intercalation studies were performed by the following procedure: DOX (30 mg) and MMT (100 mg) were dissolved in 30 ml of double-distilled water at 37 °C with constant stirring. At regular intervals (0.5, 1, 2, 4, 8, and 14 h), the reaction mixture was filtered, and the DOX concentration was determined using a UV spectrophotometer with a fixed λ_{max} of 481 nm [14].

MTT assay

The MTT assay was performedto measure the *in vitro* cytotoxicity of developed compounds. A 96-well plate was seeded with 200 µl of cell suspension (2 x 104 cells per well), and the cells were allowed to develop for 24 h. Then, add test samples at various concentrations (6.25, 12.5, 25, 50, and 100 µg/ml) and incubate at 37 °C for 24 h in a 5% CO_2 environment. Incubate for 3 h after adding MTT solution (0.5 mg/ml). Afterward, the culture media was withdrawn and 100 µl of dimethyl sulfoxide (DMSO) was added to each well. The plate was then put in a gyratory shaker and agitated until the absorbance at 570 nm could be measured with a spectrophotometer.

Encapsulation efficiency

The encapsulation efficiency (EE) of microbeads loaded with DOX was calculated using a formula and procedure reported in the published literature [17]. 10 mg of microbeads were weighed and placed in 100 ml of phosphate buffer (pH 7.4, 5% absolute alcohol) for 24 h. Then the solution was sonicated for 10 min and crushed to extract the DOX from the microbeads, and the absorbance was measured with a UV-Visible spectrophotometer at a λ_{max} of 481 nm. Using the following formula, the percentage of encapsulation was calculated.

$$\% \text{ EE} = \frac{W_t}{W_i} \text{x } 100$$

Where W_t is total amount of DOX in the microbeads and W_i is total amount of DOX initially added during the preparation.

Swelling analysis

The swelling behavior of SG-Ca, SG-CaMg, SG-CaBa, and SG-CaAl microbeads was examined using a gravimetric assay at pH 2.0 and 7.4 at 37 °C. Place 30 mg of microbeads from each formulation in a phosphate buffer solution. The weight of all formulations was measured using a weighing balance at regular intervals, and the swelling degree was calculated using the following equation:

$$\% \text{ swelling degree} = \frac{W_s - W_d}{W_d} \text{x } 100$$

Where W_s is the weight of swollen beads and W_d is the weight of dry beads

***In vitro* drug release studies**

To determine the drug release behavior of the generated microbeads, dissolution analysis was performed using a dissolution apparatus (Model: DS 2000, Make: LabIndia, Mumbai, India). 30 mg of microbeads were sealed in dialysis bags and dispersed in 300 ml of phosphate buffer solution (PBS) of pH 2.0 and 7.4 at 37 °C with a rotation speed of 50 rpm. At regular intervals, 5 ml of dissolution media was taken and analyzed with a UV spectrophotometer at λ_{max} 481 nm, then the same was replenished with fresh media.

Release kinetics

The drug release kinetics was evaluated by integrating data into kinetic models, including zeroth, first order, Higuchi, and Korsmeyer–Peppas [18, 19]. The best model was also identified based on the data fit.

RESULTS AND DISCUSSION

Intercalation kinetics

As shown in fig. 1, approximately 17% of DOX was electrostatically intercalated with MMT within 90 min, and this one remained stable for the next 6 h. Therefore, in order to avoid partial interaction, a minimum of 90 min time is required to complete the interaction between DOX and MMT.

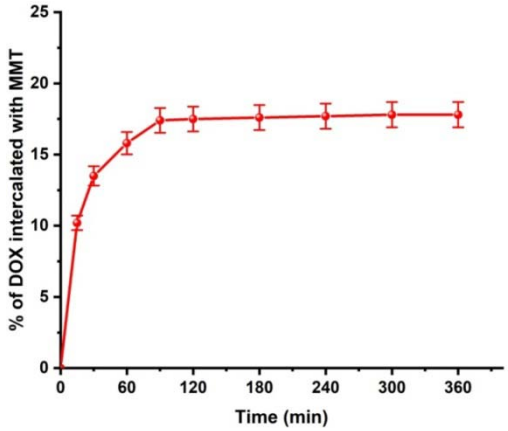

Fig. 1: The impact of time on the intercalation of MMT and CUR

Structural characterization

MMT intercalated polymeric microbeads were synthesized using different crosslinkers. Upon being loaded with DOX, SG-Ca, SG-CaMg,

SG-CaBa, and SG-CaAl are obtained. To examine possible chemical interactions among MMT, polymeric matrix and DOX in the beads, FTIR analysis is performed. The FTIR spectra of DOX (a), MMT (b), placebo microbeads (c), and DOX-loaded SG-MMT microbeads (d-g) are represented in fig. 2. The FTIR spectra of MMT (fig. 2b) show peaks at 3615-3545 cm^{-1}(O–H stretching frequency of Si–OH and Al–Al–OH), 3371 cm^{-1} (H–O–H stretching frequency of interlayer water), 1627 cm^{-1} (H–O–H bending frequency of adsorbed water), 1381, 1016 and 931 cm^{-1} (Si–O–Si stretching frequency), 794 cm^{-1} (O–Si–O bending frequency) and 509 cm^{-1} (Al–Si–O bending frequency).[20] The FTIR spectra of DOX (fig. 2a) show peaks at 3326 cm^{-1} (O-H or N-H stretching frequency), 1731 cm^{-1} (C=O stretching frequency), 1589 cm^{-1} (N-H bending frequency), 1388 cm^{-1} (O-H bending frequency), 1288 cm^{-1} (C-O stretching frequency), 1118 cm^{-1} (C-N stretching frequency), 1081 cm^{-1} (C-O stretching frequency), 995 cm^{-1} (C=C bending frequency), and 802 cm^{-1} (C-H bending frequency).[21] The FTIR spectra of placebo microbeads (fig. 2c) show peaks at 3431 cm^{-1} (O-H stretching frequency), 1596 cm^{-1} (C=O stretching frequency), and 1387 cm^{-1} (C-O-C stretching frequency). On comparing the DOX-loaded SG-MMT microbeads (fig. 2d-g) with MMT and a placebo, the peak at 3612 cm^{-1} of MMT disappeared in the spectra of the DOX-loaded SG-MMT microbeads, which confirmed an interaction takes place between the polymer matrix and MMT. Also, a peak at 6721 cm^{-1}indicates the presence of MMT in the polymeric matrix. Furthermore, new peaks were observed at 1511, 1279, and 1126 cm^{-1}which are due to the N-H, C-N, and C-O stretching frequencies of DOX. This confirms that DOX has been loaded into the microbeads.

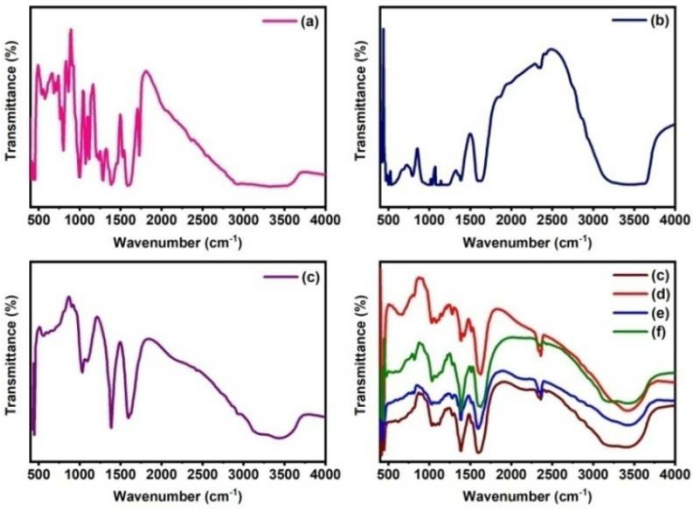

Fig. 2: FTIR spectra of (a) DOX, (b) MMT, (c) placebo microbeads, and (d-g) DOX-loaded SG-MMT microbeads

XRD analysis

The XRD diffraction patterns of MMT (a), DOX (b), placebo (c), and DOX-loaded SG-MMT microbeads are shown in fig. 2. The XRD pattern of DOX shows peaks between 10° and 25° due to crystallinity. However, these peaks are absent in DOX-loaded SG-MMT microbeads, showing that the DOX molecules are disseminated in the SA-GG matrix. The XRD pattern of MMT shows major peaks at 8.85° and 26.70°, whereas similar peaks were observed in DOX-loaded SG-MMT, which indicates that the DOX molecules are intercalated with MMT. These results are in good agreement with Reddy *et al.* [11] from their MMT-based drug delivery system.

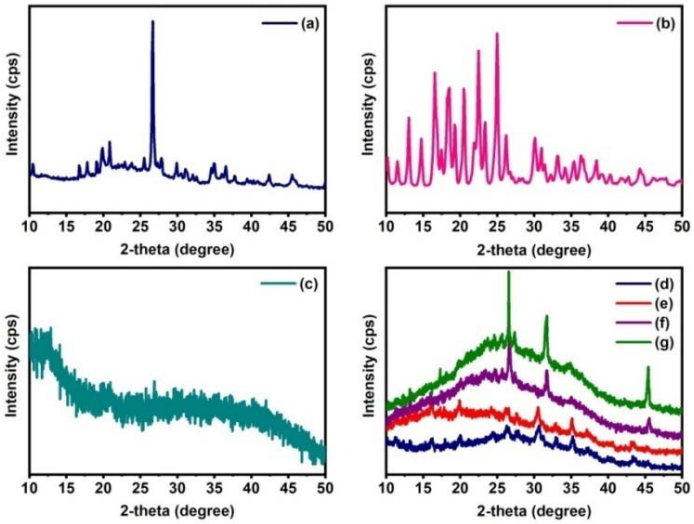

Fig. 3: XRD patterns of a) MMT, b) DOX, c) placebo, and d-g) DOX-loaded SG-MMT microbeads

SEM

The morphology of MMT-loaded microbeads was found using SEM studies, and the results are displayed in fig. 4. From fig. 4, it was observed that the microbeads are spherical in shape with a rough outer surface. On comparison of SG-CaMg and other formulations, the outer surface of SG-CaMg (fig. 4d) has a more porous nature due to the formation of a less rigid polymeric network with Mg^{2+}ions.

Consequently, a more porous nature was observed. SG-Ca, SG-CaBa, and SG-CaAl, on the other hand, form tight rigid polymeric networks with hetero ions, resulting in a less porous nature. The average size of the developed microbeads was found to be 1000-1400 µm.

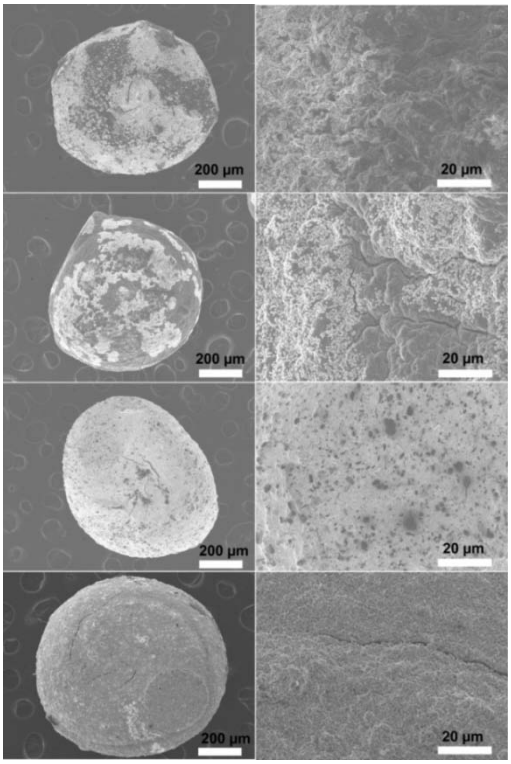

Fig. 4: SEM images of a) SG-Ca, b) SG-CaMg, c) SG-CaBa, and d) SG-CaAl microbeads

Swelling analysis

The swelling degree of polymeric beads is an important factor to consider when employing them for controlled drug release. Fig. 5 displays the results of swelling studies conducted at 37 °C in simulated intestinal fluid (pH 7.4) and simulated gastric fluid (pH 1.2). Fig. 5 shows that at pH 7.4, the swelling is much greater than at pH 2.0 [22]. The findings of the swelling behavior show that water intake is noticeably higher at pH 7.4 than at pH 1.2 for all formulations, as shown in fig. 5. In order to prevent the drugs from being released into the stomach, SA matrices can be used because of their swelling ability, which varies with the pH of the media. As a result, the developed SA beads are a viable carrier for avoiding the release of drugs into gastric fluid while transporting them to the intestine. Fig. 4 shows that the number of multivalent ions in the beads had a significant effect on the swelling degree across all formulations. At 5 h, all profiles had reached their full expanding potential. When compared to the other formulations, SG-CaMg beads show the highest percentage of water uptake. This is because the Mg^{2+}ions form a less rigid network, allowing water molecules to enter the beads via the pores and increase the swelling. However, CaBa and CaAl show less water absorption because the $Ba^{2+}$and Al^{3+}create a tight, stiff network, preventing the polymer network from allowing a high number of water molecules [20].

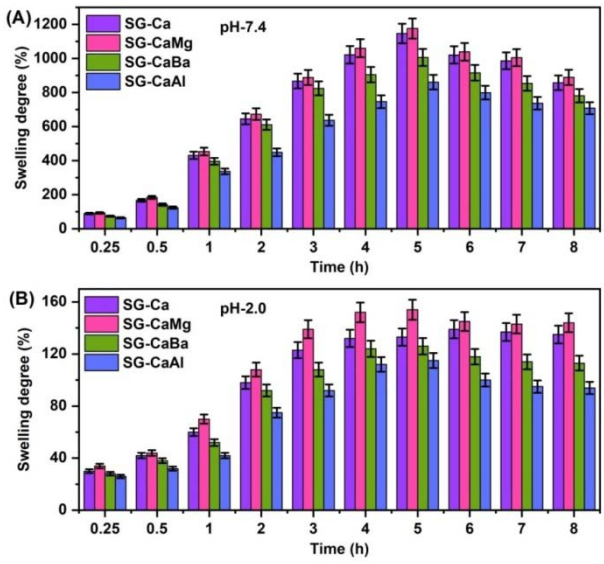

Fig. 5: Swelling studies of developed microbeads in PBS of pH 7.4 (A) and 2.0 (B) at 37 °C. (n=3, experiments were conducted in triplicate)

Encapsulation efficiency and *in vitro* release studies

The % EE of DOX in SG-Ca, SG-CaMg, SG-CaBa, and SG-CaAl microbeads is 67.5, 67.1, 61.2, and 60.5%, respectively (table 2). From the results, it was observed that the percentage EE of SG-Ca and SG-CaMg was higher compared to SG-CaBa and SG-CaAl microbeads because the network structures of SG-CaBa and SG-CaAl are more rigid, they expand to a lesser extent, resulting in low EE values. In contrast, the % EE of SG-Ca and SG-CaMg formulations increased because they form a less rigid network and swell more, resulting in high percent EE values. Fig. 6 depicts the outcomes of *in vitro* release tests conducted at 37 °C in PBS at pH 2.0 and 7.4. The overall drug release rate is observed to be greater at pH 7.4 than at pH 2.0 because the carboxylate group interacts less with the buffer at pH 7.4, making the network more permeable and increasing the network's flexibility so that the drug can be released more easily. At pH 2.0, the release rate was lower than at pH 7.4 because ionic-ionic repulsions were generated between H^+ions and the polymer matrix, preventing the entry of solvent into the polymer matrix. The release rate was consequently lowered [23]. This kind of pH response is helpful for drug delivery because it protects the drug molecules from the acidic environment of the stomach and enables their release from the carrier once they reach the intestinal regions.

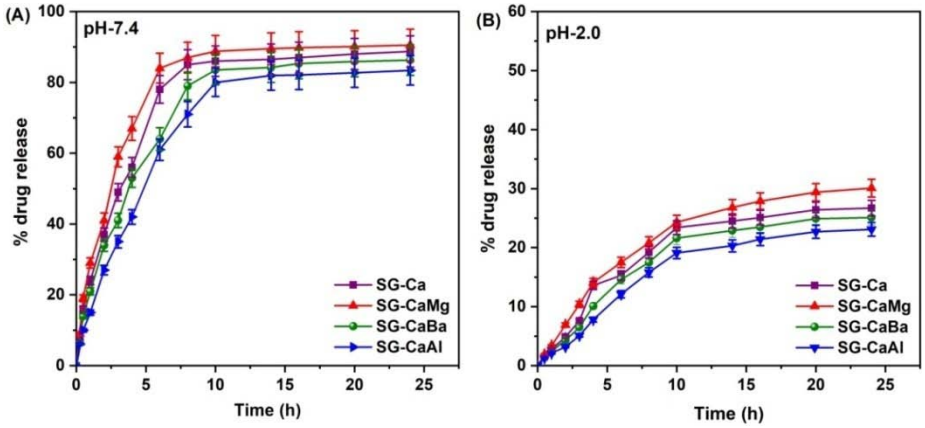

Fig. 6: *In vitro* release profiles of developed drug-loaded microbeads in PBS of pH 7.4 (A) and pH 1.2 (B) at 37 °C. (n=3, experiments were conducted in triplicate)

Table 2: Encapsulation efficiency (%EE) of all samples

S. No.	Code	% EE
1	SG-Ca	67.5±1.18
2	SG-CaMg	67.1±1.64
3	SG-CaBa	61.2±0.26
4	SG-CaAl	60.5±1.14
5	Placebo	000.000

(n=3, mean±SD; experiments were conducted in triplicate)

Release kinetics

To determine the drug release strategy, the outcomes of *in vitro* drug release tests in PBS (7.4) were fitted into various kinetic models, and the rate constant and correlation coefficient (r^2) of all compositions are shown in table 2. According to the values of the correlation coefficients, the kinetics of drug release does not behave in a zero-order or first-order fashion. The values of r^2 were close to those of the Higuchi and korsmeyer-peppas models. As a result, the Higuchi model is used to describe the rate of drug release. The Higuchi model predicts that the drug will be released from the microbeads when a liquid penetrates the matrix, dissolves the drug, and then diffuses the drug into the outside liquid through pores or intestinal channels. Based on the analysis of the Higuchi and korsmeyer-peppas models, it was clear that the basic diffusion process was involved in the process of drug release. The korsmeyer-peppas model additionally elucidated the diffusion type. A korsmeyer-peppas model fit was performed on the initial 60% of drug release data.

$$\frac{M_t}{M_\alpha} = kt^n$$

Where, M_t/M_α represents the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is the release exponent indicating the type of drug release mechanism. The values n are obtained in the range of 0.366-0.473 (table 2), which indicates the fickian type of diffusion process. P. G. Guzman *et al.* [24] has reported a similar finding from the MMT-based drug delivery system.

Table 2: Release kinetics parameters of all microbeads in PBS of pH 2.0 and 7.4 at 37 °C

Code	pH	Zero order		First order		Higuchi		Korsmeyer-Peppas	
		K_0	r^2	K_1	r^2	K_H	r^2	n	r^2
SG-Ca	7.4	5.395	0.116	0.210	0.958	22.773	0.929	0.366	0.898
	2.0	1.584	0.444	0.019	0.575	6.569	0.954	0.433	0.922
SG-CaMg	7.4	5.548	0.043	0.232	0.968	23.482	0.927	0.355	0.899
	2.0	1.766	0.489	0.022	0.632	7.298	0.964	0.443	0.937
SG-CaBa	7.4	5.308	0.189	0.195	0.957	22.342	0.931	0.379	0.897
	2.0	1.483	0.487	0.018	0.603	6.131	0.957	0.446	0.924
SG-CaAl	7.4	5.116	0.282	0.170	0.945	21.441	0.931	0.399	0.890
	2.0	1.343	0.568	0.016	0.662	5.514	0.964	0.473	0.929

In vitro toxicity studies

The *in vitro* toxicity of SG-Ca, SG-CaMg, SG-CaBa, and SG-CaAl were tested against MCF7 cells using the MTT assay, and their results are presented in fig. 7. This cell line was chosen because it is one of the cancer cell lines that has been studied the most in the scientific literature. It can be used as a model to see how our microbeads affect cancer cells. From the MTT results, it was found that the formulations SG-Ca and SG-CaMg showed higher inhibitory efficiency on MCF7 than SG-CaBa and SG-CaAl. This is due to the amount of DOX that is available in SG-Ca and SG-CaMg microbeads. These results indicate that the developed SG-Ca, SG-CaMg, SG-CaBa, and SG-CaAl microbeads had good anti-cancer activity against MCF-7 cells.

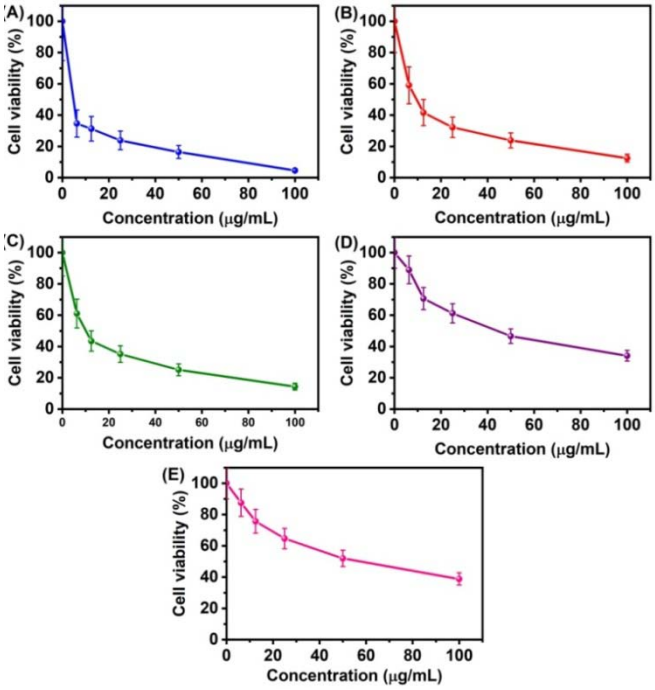

Fig. 7: Cell viability of MCF7 cell line against (A) DOX, (B) SG-Ca, (C) SG-CaMg, (d) SG-CaBa, and (E) SG-CaAl

CONCLUSION

In the present work, MMT intercalated sodium alginate/gum ghatti microbeads were developed using a simple ionotropic gelation method for the controlled release of DOX. FTIR confirmed the interaction between DOX molecules and the polymer network. XRD studies reveal the successful encapsulation of MMT and DOX in the microbeads. The produced microbeads are spherical, and their exterior surfaces are extremely rough, indicating the presence of MMT molecules. At pH 7.4, a higher degree of swelling and release rate was found, indicating that the produced microbeads may be suited for intestinal drug delivery. The Korsmeyer-Peppas equation was used to fit the *in vitro* data, and the results showed that the drug release mechanism followed a Fickian diffusion process. The developed microbeads showed well *in vitro* toxicity against the MFC-7 cell line. Based on the results, it was suggested that MMT-intercalated microbeads showed potential performance as drug carriers, and they could probably be used in many different ways to control the release of drug molecules.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that there was no conflict of interest.

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