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Encapsulation of Animal Wax-Based Organogels in Alginate Microparticles

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ABSTRACT: Encapsulation of organogels is a novel perspective in the field of controlled drug delivery. This study reports encapsulation of lanolin based organogels within alginate microparticles. The microparticles were prepared by emulsification/internal gelation method. Microscopic studies suggested spherical shape of the microparticles. Fourier transform infrared, X-ray diffraction and thermal studies confirmed the presence of organogels within the microparticles. Organogels containing microparticles showed improved drug (e.g., salicylic acid and metronidazole) entrapment efficiency. The release of the drugs from the microparticles was dependent on the pH of the dissolution medium. The release was diffusion mediated. The drug loaded microparticles showed antimicrobial activity against *E. coli* and *B. subtilis*. The preliminary study suggested that the encapsulation of the organogels may help prolonging the release of the drugs and hence may be tried as vehicles for controlled drug delivery. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40910.

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INTRODUCTION

Encapsulation of the bioactive agents within polymeric matrices has gained much importance in recent years. This may be explained by the fact that encapsulation facilitates protection of the bioactive agents from the environment and helps prolonging the release by manipulating the release rate. Sodium alginate, a biopolymer obtained from brown algae, is an anionic polysaccharide and has been extensively studied for the preparation of the microparticles. Various bioactive agents encapsulated within alginate microparticles include drugs (5-fluorouracil, isoniazid, rifampicin, pyrazinamide), proteins (DNA, bovine serum albumin, horseradish peroxidase), peptides (vasoactive intestinal peptide, major outer membrane protein peptide) and vaccines (thiocyanate extract of *P. multocida*).[1–4] Microparticles encapsulating apolar liquid bioactive agents or apolar liquid containing bioactive agents have been studied extensively. Unfortunately, these microparicles suffer from the disadvantage of leaching of the internal phase under storage conditions.[2,5] This may be attributed to the high porosity of the alginate microparticles. Various researchers have proposed different methodologies, *viz.*, increasing the curing time of microparticles with $CaCl_2$ and synthesis of complex microparticles with other naturally occurring polymers (e.g., chitosan, gelatin, pectin and gums), to reduce leaching of the internal phase.[2,6] In this study, we hypothesize that gelation of the internal phase may reduce leaching of the internal phase. This is due to the fact that organogels are semi-solid at room-temperature (RT) and will reduce the flowability of the internal phase. If the hypothesis holds good, it is expected that there will be reduction in the loss of bioactive agents associated with leaching. The proposed method provides an alternate methodology to improve the drug retention capacity of the biopolymeric microparticles and may have a new dimension in the field of controlled delivery applications.

Lanolin and lanolin based organogels have been chosen as the representative organogels for this study. Lanolin is an animal wax and is abundantly available in nature.[7] The purified lanolin is non-toxic and has been used in moisturizers, cosmetics, sunscreen lotions, shaving creams and emollient products.[8–10] The chemical nature of the purified lanolin is similar to the human sebum which facilitates its biocompatibility with mammalian cells/tissues.[11] Lanolin based formulations have been studied in topical and oral drug delivery.[12,13] The organogels derived from lanolin have been found to be semi-solid at RT (25°C) and stable for longer periods of time.[14] Lanolin and lanolin based organogels were encapsulated within alginate microparticles by ionotropic internal gelation method. The

Additional Supporting Information may be found in the online version of this article.

microparticles were characterized by microscopy, X-ray diffraction (XRD), fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The *in vitro* drug delivery studies and antimicrobial studies were conducted using salicylic acid (SA) and metronidazole (MZ) as the model drugs.

MATERIALS AND METHODS

Materials

Sodium alginate, calcium chloride (fused), calcium carbonate, potassium chloride, sodium hydroxide and dialysis tubing (M_w cut-off 12–14 kDa) were purchased from Himedia, Mumbai, India. Lanolin, boric acid, potassium dihydrogen phosphate, span 80 and tween 80 were purchased from Lobachemie, Mumbai, India. Sodium acetate, glacial acetic acid and hydrochloric acid were purchased from MERCK, Mumbai, India. Sunflower oil (Sweekar, Cargill India Pvt Ltd., Haryana, India) was purchased from the local market. SA was procured from Lobachemie, Mumbai, India while MZ was received as a kind gift from Aarthi drugs Ltd., Mumbai, India. Fresh goat blood and goat small intestine were obtained from the local butcher shop. Double distilled water was used throughout the study.

Preparation of Lanolin-Based Organogels

Lanolin based organogels were prepared as per the protocol published in our previous paper.[14] In short, the organogels were prepared by varying the proportions of lanolin and water in the ratio of 2 : 1, 1 : 1 and 1 : 2. 10 g of liquefied lanolin (melted at 55°C) containing 1% (w/w) span 80 was homogenized at 1500 rpm using an overhead stirrer (RQ-126/D, Remi Motors, Mumbai, India). Water was added drop-wise to the molten lanolin under homogenization and subsequently homogenized for further 10 min after the complete addition of water. The drug containing organogels were prepared in the similar manner. 1% (w/w) of SA or MZ was dissolved in lanolin, prior to the preparation of microparticles. The drug containing lanolin was used for the preparation of organogels. The rest of the procedure was same.

Preparation of Microparticles

The microparticles were prepared by modified double emulsion/internal gelation method.[15] In short, 20 mL of 2.5% (w/w) sodium alginate solution in water was prepared. The sodium alginate solution was kept on stirring at 200 rpm using an overhead stirrer (RQ-126/D, Remi Motors, Mumbai, India). To the alginate solution, 2% (w/w) calcium carbonate was added and homogenized. To the suspension, 0.5 g of span 80 was added followed by the addition of 5 g of molten organogel. The mixture was homogenized at the same speed until the formation of a milky white emulsion. The emulsion was further stirred in an ice-bath for 10 min. The cooled emulsion was transferred to 60 mL of ice-cold sunflower oil (external oil phase), kept on stirring at 200 rpm to form a double emulsion. 5 mL of acidified oil (4.5 mL of sunflower oil + 0.5 mL glacial acetic acid) was added to the double emulsion. This resulted in the formation of the ionically crosslinked alginate microparticles. The microparticles were consolidated in 200 mL of 0.5 *M* calcium chloride solution containing 1% (v/v) tween 80. The microparticles were washed several times using water. The blank microparticles (BM) were prepared in a similar manner. BM did not contain any internal gel phase. Similarly, drug containing microparticles were prepared using drug containing lanolin or organogel. The formed microparticles were stored under refrigerated conditions for further use. The hemocompatibility of the prepared microparticles was tested as per the method reported earlier (detailed procedure is provided as supplementary information).[16,17]

Microscopy

The wet microparticles were observed under an upright bright-field compound microscope (LEICA-DM 750 equipped with ICC 50-HD camera, Germany). NI Vision Assistant-2010 software was used for particle size analysis.[18] The size distribution was estimated by calculating SPAN factor (size distribution factor) and percentage coefficient of variation (% CV).[19,20] SPAN and %CV were calculated as per the following formula:

$$\text{SPAN} = (d_{90} - d_{10})/d_{50} \quad (1)$$

$$\%\ \text{CV} = \left[\frac{\text{Standard deviation}}{\text{Mean}}\right] \times 100 \quad (2)$$

where, d_{90}, d_{50}, and d_{10} are the diameters of the 90, 50, and 10% of the microparticles population, respectively.

The microparticles were dried overnight at 40°C. The dried microparticles were coated with platinum (Pt) by sputter coating and were subsequently analyzed under scanning electron microscope (JEOL, JSM-6390, Japan). Smoothness index (SI) of the microparticles was calculated using imageJ software [imageJ 1.44P, National Institute of Health (NIH)]. SI was calculated from eq. (3):

$$\text{SI} = \left(\frac{\text{SD}_{\text{LP}}}{\text{A}_{\text{LP}}}\right) \times 100 \quad (3)$$

where, SD_{LP} and A_{LP} are the standard deviations and the means of the variation in light intensities within a particle obtained by measuring the line profiles of the microparticles.

Internal Phase Leaching Studies

The internal phase leaching was analyzed as per the previously reported literature.[18] In short, 0.5 g of the accurately weighed microparticles was wiped with a filter paper to remove the surface bound moisture.[21] The microparticles were placed over a fresh piece of dried filter paper and subsequently incubated at 37.0 ± 1.0°C. The leakage of the internal phase was monitored for 2 h.

The quantification of the leached internal phase was also performed.[22] Briefly, ~0.1 g (W_1) of microparticles was soaked in 1.0 g (W_2) of double distilled water for 1.0 h at 37°C in microcentrifuge tubes. The tubes were centrifuged at 10,000 rpm for 2 min (MC-02, SPINWIN, Tarsons). The pellet and the supernatant were separated and then dried at 55°C for 24 h. Subsequently, the dried supernatant (W_3) was weighed and the percentage of leaching from the microparticles was calculated as:

$$\%\ \text{leaching} = \frac{W_3}{W_1} * 100 \quad (4)$$

Drug Entrapment Efficiency

Accurately weighed 0.5 g of drug loaded dried microparticles were triturated in 50 mL of pure methanol and filtered through Whatmann filter paper (Sartorius stedim, grade:389) to remove the unwanted debris.[4] The amount of the drug present in the filtrate was quantified using a UV–visible spectrophotometer

Table I. Composition of Organogels

Sample	Lanolin : water (% W)	Span 80 (% W)	Salicylic acid (% W)	Metronidazole (% W)
L1	2 : 1	1.0	-	-
L2	1 : 1	1.0	-	-
L3	1 : 2	1.0	-	-
L1SA	2 : 1	1.0	1.0	-
L1MZ	2 : 1	1.0	-	1.0

(UV-3200, LabIndia, Mumbai, India) at a wavelength of 294 and 321 nm for SA and MZ, respectively. Drug entrapment efficiency (DEE) was calculated and reported as %DEE.[4,23]

$$\%\text{DEE} = \left[\frac{\text{Practical loading}}{\text{Theoritical loading}}\right] \times 100 \quad (5)$$

Molecular Interaction Studies

The microparticles were analyzed in the wavenumber range of 4000–500 cm^{-1} using an FTIR spectrophotometer (alpha-E, Bruker). The instrument was operated in the attenuated total reflectance mode. For each scanning, a set of 16 scans were performed at RT.

The raw materials and dried microparticles were subjected to XRD studies using PW3040, Philips Analytical Ltd., Holland. The source of X-rays was a monochromatic Cu Kα radiation ($\lambda = 0.154$ nm). The scan was done in the range of 5°–50° 2θ at a scanning rate of 2°/min. The measurements were carried out at RT.

Thermal Studies

Thermal properties of the microparticles were analyzed using a differential scanning calorimeter (DSC-200 F3, MAIA, Netzsch, Germany). The microparticles were analyzed at a scanning rate of 2°C/min. The analysis was performed under inert nitrogen environment. For the analysis, accurately weighed (5–15 mg) dried microparticles were hermetically sealed in aluminium (Al) crucibles with pierced Al lids. Empty Al crucible was used as the reference.

Mucoadhesivity Studies

The mucoadhesive property of the prepared microparticles was analyzed as per the method described elsewhere.[24] In short, a piece of goat's small intestine was cut open (3 × 3 cm^2) and attached to a glass slide using acrylate adhesive by exposing the internal lumen of the intestine. 0.2 g of the microparticles was weighed and placed over the exposed surface of the mucosa. A weight of 5 g was applied over the microparticles for a period of 5 min to promote mucoadhesion. Subsequently, the slides were put vertically in the United States Pharmacopeia disintegration baskets containing 900 mL of phosphate buffer (pH = 7.2) at 37.0 ± 1.0°C. The experiment was carried out for 24 h and the time needed to detach the microparticles from the mucosa was noted.

In Vitro Drug Delivery Studies

SA and MZ loaded microparticles were subjected to *in vitro* drug release studies under different pH conditions. The studies were carried out in simulated gastric and intestinal pH environments. For the study, hydrochloric acid buffer (pH = 1.2) and phosphate buffer (pH = 7.2) were prepared as per the Indian Pharmacopeia. 1 g of the dried microparticles were weighed accurately and put in dialysis membrane bag. The dialysis bag was tightened at both ends and subsequently submerged in 50 mL of buffer, kept on stirring at 100 rpm at 37.0 ± 1.0°C. The dissolution medium was replaced with fresh buffer at an interval of 30 min. The collected samples were analyzed at 294 and 321 nm using UV–visible spectrophotometer for determining the concentration of SA and MZ, respectively.

Antimicrobial Studies

The antimicrobial efficiency of the microparticles was tested against gram positive and gram negative bacteria namely *Bacillus subtilis* (*B. subtilis*) (MTCC 121) and *Eschericia coli* (*E. coli*) (NCIM 5051), respectively. The antimicrobial studies were carried out by broth dilution method.[25] Briefly, 1 g of the drug loaded dried microparticles were added to 100 mL of nutrient broth containing 1 mL of the bacterial inoculum. The concentration of the inoculated *E. coli* and *B. subtilis* was 2 × 10^4 and 1 × 10^5 CFU/mL, respectively. The broth was incubated at 37.0 ± 1.0°C with constant shaking at 120 rpm. Under aseptic conditions, 1 mL of the culture medium was collected at an interval of 1 h to measure the growth of the bacteria in the presence of microparticles. The collected samples were then analyzed at 595 nm using UV–visible spectrophotometer. Microparticles without drug were taken as negative control.

Statistical Analysis

Statistical analysis was performed by conducting Analysis of variance using trial version of MiniTab 14.0® software. The level of significance for the analysis was maintained at 0.05.

RESULTS AND DISCUSSION

Preparation of Lanolin-Based Organogels

The compositions of the organogels used in this study have been tabulated in Table I. The formation of organogel was predominantly influenced by the proportion of water. The stable organogels were formed when the lanolin : water proportion was maintained 2 : 1, 1 : 1, and 1 : 2.[14] Formation of organogels was checked by inverted tube method (Figure 1).[26,27] Upon inversion of the tubes, the formulations did not flow under the influence of gravity thereby suggesting that the developed formulations were semi-solid at RT. Incorporation of drugs (e.g., SA and MZ) into the organogels did not alter the stability and appearance of the organogels. The developed organogels (both blank and drug loaded) were found to be stable for >12 months.

Preparation of Microparticles

The microparticles were prepared by double emulsification/internal gelation method. Addition of acetic acid to the external oil phase resulted in the release of calcium ions by reacting with calcium carbonate. The released calcium ions resulted in the ionotropic crosslinking of the alginate layer.[28] Stable microparticles were formed when lanolin and L1 organogels were used as the internal phase. The use of L2 and L3 organogels did not result in the formation of microparticles. This may be

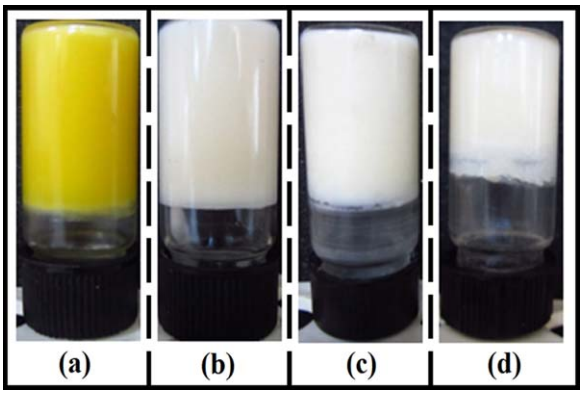

Figure 1. Photographs of lanolin and its organogels. (a) Lanolin, (b) L1 (c) L2 and (c) L3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

associated with the lower thermal stability of L2 and L3 organogels.[14] In our previous study, we found that the L2 and L3 organogels were unstable when they were tested in a cyclic thermal test (−20–37°C). In this study, addition of the molten L2 and L3 organogels to the ice cold sodium alginate solution leads to the phase separation. This resulted in unsuccessful encapsulation of L2 and L3 organogels (Figure 1c and 1d). Due to this reason, L2 and L3 containing microparticles were not investigated further. The compositions of the prepared microparticles have been tabulated in Table II. The % hemolysis due to the microparticles was found to be less than 5, suggesting their highly hemocompatible nature (Supporting Information Table S1).[17] The hemocompatibility studies indicate probable biocompatible nature of the microparticles.

Microscopy

The microstructure of the microparticles was visualized under a compound upright bright field microscope. The microscopic images of the microparticles have been provided in Figure 2. The BM microparticles were semi-transparent, which may be associated with the absence of any internal phase. Converesely, the micrographs of ML*n* and ML1 were opaque. This may be accounted to the presence of lanolin and L1 within the core of the microparticles. As lanolin and L1 are semi-solids and are opaque at RT, encapsulation of these resulted in the formation of opaque microparticles.[7]

The size distribution of the microparticles was analyzed using NI vision assistant 2010 software. The near circular particles were chosen for the particle size analysis (Heywood circularity factor = 0.9–1.1). The size distribution of the microparticles revealed that lanolin and L1 containing microparticles showed a broad size distribution compared to BM microparticles (Figure 3). The average diameter (D_{avg}) of the microparticles was found out by estimating the size of the 50% population of the microparticles. The size distribution analysis of the microparticles has been provided in Table III. The difference between the average diameter of the microparticles is statistically insignificant ($p > 0.05$) (Supporting Information Figure S1). Statistical analysis indicates that though an increase in size is observed with the addition of lanolin and L1, the increase in size is not so significant. The size distribution and polydispersity of the microparticles was expressed in terms of SPAN factor [eq. (1)]. The d_{10}, d_{50}, and d_{90} of the microparticles were calculated from the size distribution analysis. A lower SPAN factor indicates narrow size distribution and *vice-versa*.[20] In general, if the spray dried microparticles have a SPAN factor of < 2 and a d_{50} value of <10 μm, the size distribution is considered as narrow.[19,20] The developed microparticles showed a SPAN factor of <2 and the d_{50} value was >10 μm. The higher d_{50} value may be associated with the experimental conditions used for the preparation of microparticles. As a matter of fact, the average size of the microparticles prepared by ionotropic gelation method varies in the range of 10–400 μm.[29,30] Considering the method of preparation of microparticles, the size distribution of the developed microparticles may be regarded as narrow. Lanolin and L1 containing microparticles showed higher SPAN factor as compared to the BM microparticles. The variation in the % CV of the microparticles also followed a similar trend. Higher % CV was observed for ML*n* and ML1 as against BM microparticles. This suggested that the variation in the sizes of the microparticles was higher in lanolin and L1 containing microparticles and may be attributed to the physical nature of the lanolin and L1. As lanolin and L1 are viscous in nature, this might have resulted in the formation of non-homogeneous alginate mixture which in turn, resulted in the formation of microparticles of wide size distribution.[14] Apart from the physical nature of the internal phase, the homogenization speed also plays an important role in governing the size distribution of the microparticles. Usually homogenization at lower speeds exerts lower dispersive forces thereby resulting in the formation of larger sized microparticles. Increase in the viscosity of the emulsion also results in the lower dispersive force. Hence, it may be expected that the effective dispersive force during the preparation of BM microparticles might have resulted in the formation of uniform and small vesicles as compared to lanolin and L1 containing microparticles where larger sized particles were formed.[31]

The micrographs of the dried microparticles, as observed under SEM, have been shown in Figure 2. All types of the microparticles were found to be spherical and smooth in nature. The SI of the microparticles was calculated using the eq. (3). BM microparticles (SI = 5.78) was found to be smoother than ML*n*

Table II. The Internal Phase Composition of MPs

Samples	Internal phase	Observation
BM	Nil	Stable MPs were formed
ML*n*	Lanolin	Stable MPs were formed
ML1	L1	Stable MPs were formed
ML2	L2	MPs were not formed
ML3	L3	MPs were not formed
BMSA	SA	Stable MPs were formed
BMMZ	MZ	Stable MPs were formed
ML*n*SA	LnSA	Stable MPs were formed
ML*n*MZ	LnMZ	Stable MPs were formed
ML1SA	L1SA	Stable MPs were formed
ML1MZ	L1MZ	Stable MPs were formed

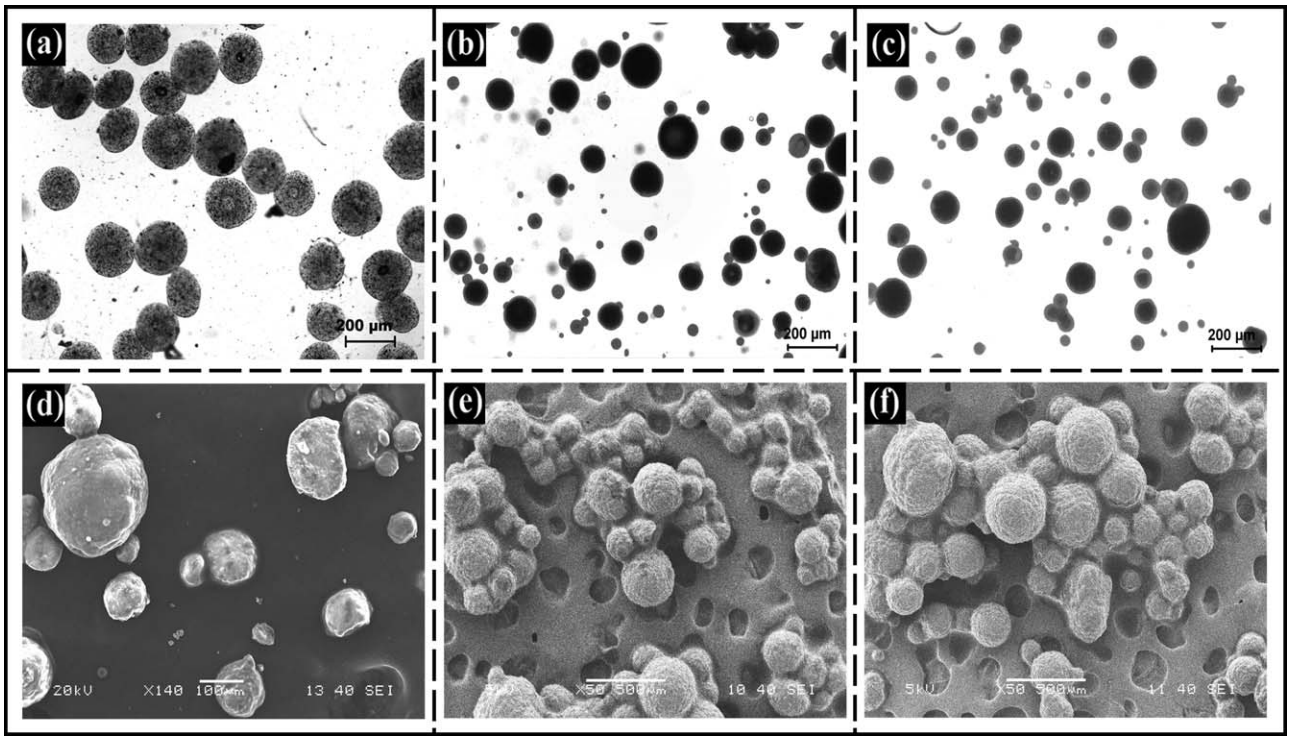

Figure 2. The bright field microscopic images: (a) BM (b) ML*n* (c) ML1 microparticles and SEM images: (d) BM, (e) ML*n* and (f) ML1 microparticles.

(SI = 8.85) and ML1 (SI = 9.34) microparticles. The results suggested that the incorporation of the organogels has resulted in the increase in the surface roughness of the microparticles.

Internal Phase Leaching Studies

The leaching of the internal phase from the microparticles was monitored (Figure 4). Lanolin and L1 containing microparticles did not show any signs of leakage at the end of the study (2 h). Leakage was checked by monitoring the wetting of the filter paper with the internal phase. Conversely in our previous study, we have observed leakage of mustard oil under similar conditions.[18] The absence of leakage of the internal phase may be associated with the gelation of the internal phase at RT. This prevented the migration of the internal phase out of the polymeric layer.[32] This ensures that the encapsulation of organogels within the microparticles prevents the leakage of bioactive agents which, in turn, can improve the DEE of the microparticles (illustrated in "DEE" section).

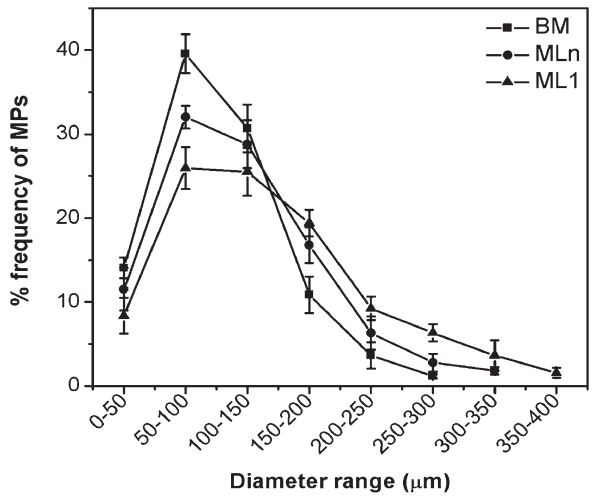

Figure 3. The size distribution analysis of microaprticles.

Leaching of the internal phase was quantified by incubating the microparticles under distilled water. The studies indicated higher leaching of the internal phase was found in BM microparticles (68%) over ML*n* (6%) and ML1 (5%). This suggests that the leaching of internal phase was insignificant from ML*n* and ML1 microparticles. Decrease in leaching of internal phase has in turn improved the DEE of the microparticles.

DEE

The dried microparticles were used to calculate the DEE. The encapsulation efficiencies of the microparticles have been tabulated in Table IV. The DEE of ML*n* and ML1 were higher than BM. For both SA and MZ, BM microparticles have shown significant difference in DEE with ML*n* and ML1 ($p < 0.05$) (Supporting Information Figure S1). This suggests that the presence of organogels has influenced the DEE of microparticles. SA containing microparticles have shown better entrapment efficiency than the MZ containing microparticles. The solubility of the drugs might have affected the entrapment efficiency of the microparticles. During the preparation of the microparticles, some amount of the drug might have diffused out of the microparticles into the external aqueous phase. As MZ possess higher solubility in water than SA, higher amount of MZ might have diffused out as compared to SA. ML*n* and ML1 have shown

Table III. Size Distribution Analysis

Sample	D_{avg}[a] ± SD[b] (μm)	SPAN factor	% CV	d_{10} (mm)	d_{50} (mm)	d_{90} (mm)
BM	94.7 ± 10.2	1.15 ± 0.7	10.7 ± 0.4	38.4 ± 0.4	98.06 ± 0.8	151.2 ± 0.6
MLn	111.28 ± 17.6	1.57 ± 1.1	14.0 ± 0.7	48.5 ± 0.4	104.1 ± 1.2	211.8 ± 1.0
ML1	130.14 ± 21.2	1.65 ± 1.2	16.3 ± 0.8	52.0 ± 0.9	127.0 ± 1.7	262.5 ± 0.8

[a] Average of >1000 particles
[b] SD: Standard deviation

%DEE >85%, which is nearly the 1.8 times the DEE of BM. The restricted movement of the drug within the organogels might have yielded higher encapsulation efficiency in ML*n* and ML1 than in BM. In other words, the semisolid nature of the organogels behaved as a barrier for the movement of the drugs. ML*n* and ML1 did not show any leaching (Figure 4). This might have also contributed in the increase DEE in these microparticles.

Molecular Interaction Studies

FTIR spectra of the raw materials and the microparticles have been shown in Figure 5. FTIR spectrogram of alginate has shown peaks for the presence of functional groups like —COO^- (1620 and 1410 cm^{-1}) and —C—OH (O—H stretching vibration at 3450 cm^{-1}, C—O stretching vibration of secondary alcohol at 1110 cm^{-1}, C—O stretching vibration of tertiary alcohol at 1150 cm—^{1}).[33] These characteristic peaks of alginate were also present in the FTIR spectra of the microparticles, indicating that the addition of lanolin and L1 did not change the chemical nature of the alginate polymer. L1 showed a narrow peak at 3400 cm^{-1} which was not present in lanolin. This may be associated with the intermolecular hydrogen bonding due to the presence of water in L1. ML*n* and ML1 have shown peaks corresponding to lanolin and L1, respectively, suggesting their presence within the microparticles. The intensity of the peak at 3400 cm^{-1} was higher in ML1SA and ML1MZ as compared to ML*n*SA and ML*n*MZ. This was expected as L1 contained more water as compared to anhydrous lanolin, which might have promoted intermolecular hydrogen bonding.[14] SA has shown peaks at ~1600 cm^{-1} (C=C bond of aromatic ring), 1666 and 1649 cm^{-1} (C=O stretching of carboxylic acid) and 756 and 719 cm^{-1} (C—H out of plane bending in the phenol substitution ring).[34] MZ has shown the peaks at 1238 cm^{-1} (ester carbonyl peak), 1747 cm^{-1} (carbonyl stretching) and 1593 cm^{-1} (asymmetric nitro stretch). Presence of peaks corresponding to the drugs within the microparticles suggested the presence of the drugs. Absence of additional peaks in the microparticles suggested that there was no chemical interaction amongst the drug molecules and the components of the microparticles.

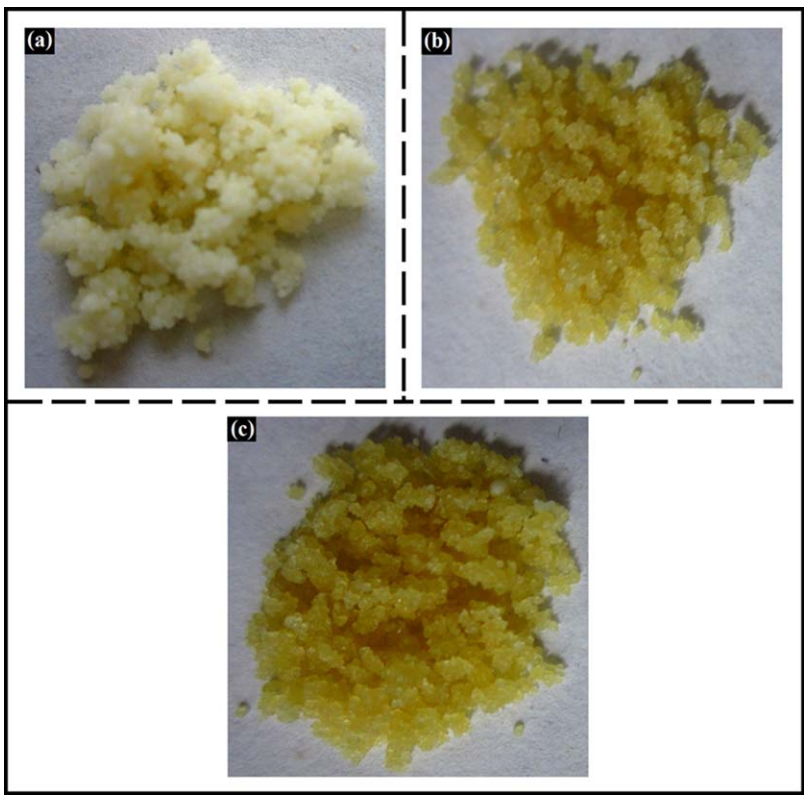

Figure 4. Photographs of the microparticles after 2 h leaching study at 37°C. (a) BM, (b) ML*n*, and (c) ML1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table IV. The % DEE of Drug Containing Microparticles

Samples	% DEE (mean ± SD)
BMSA	53 ± 2.4
MLnSA	86 ± 3.1
ML1SA	91 ± 2.4
BMMZ	46 ± 2.7
MLnMZ	82 ± 2.5
ML1MZ	85 ± 3.4

Figure 5 shows the XRD profiles of the microparticles. BM has shown a broad hump at ~12° 2θ. The profile was similar to the previously reported XRD profile of sodium alginate.[35] ML*n* and ML1 have additionally shown the characteristic peaks of their internal phase apart from the broad peak due to the alginate.[14] Microparticles have shown distinct XRD profiles, which varied on the composition of the internal phase. The characteristic peaks of lanolin and L1 (21.67° and 21.3° 2θ, respectively, as per our previous manuscript) were conserved in their respective microparticles, indicating their presence without any change in their physical properties. All the microparticles were found to be amorphous in nature as suggested by the presence of a broad peak at ~20° 2θ.[36] The change in the amorphousity was compared by calculating full-width half maximum (FWHM) of the broad peak at ~20°.[37] In general, higher degree of amorphousity is associated with higher FWHM. The FWHM of lanolin and L1 was found to be 10.76 and 10.00, respectively.[14] BM have shown highest FWHM and may be attributed to the presence of water within the microparticles.[38] ML*n* and ML1 have shown lower amorphousity than lanolin and L1, respectively. The drug containing microparticles did not show any additional peaks corresponding to SA and MZ.

Thermal Studies

The thermograms of the developed microparticles have been shown in Figure 6. The thermograms of lanolin and L1 have been reported in our previous paper[14] ML*n* and ML1 have shown endothermic peaks at ~38°C (as shoulder peaks, pointed with arrows in the figure), corresponding to the melting endotherms of lanolin and L1. This confirmed the encapsulation of lanolin and L1 within the alginate microparticles. Even though dried microparticles were used for the study, complete removal of water upon drying was not possible in alginate microparticles[39] As per the literature, polysaccharides show high affinity towards water molecules and lose/release water at different temperatures depending on the interactions with the water. Water is reported to form hydrogen bonds with the –OH groups of the alginate polymers.[40] ML*n* and ML1 have shown peaks at ~67°C. These endothermic peaks have been reported due to the evaporation of the loosely bound water.[39,41] ML*n* has shown additional endothermic peak at ~80°C. This is due to the evaporation of tightly bound water molecules. ML1 showed a single broad endothermic peak for the evaporation of the water molecules. This might have resulted due to the evaporation of the water molecules (both loosely bound and tightly bound water) in tandem. BM did not contain any internal phase which resulted in the stronger association of the water molecules with the —OH groups of alginate. This explains the occurrence of the endotherm peak at 100°C. No shoulder peak was obtained as there was no gel entrapped within the core of the microparticles.

Mucoadhesivity Studies

Mucoadhesive studies were performed by *in vitro* wash-off method. In this method, the time required for the detachment of the microparticles from the mucosal surface of the colon was checked. The detachment times of BM, ML*n*, and ML1 was

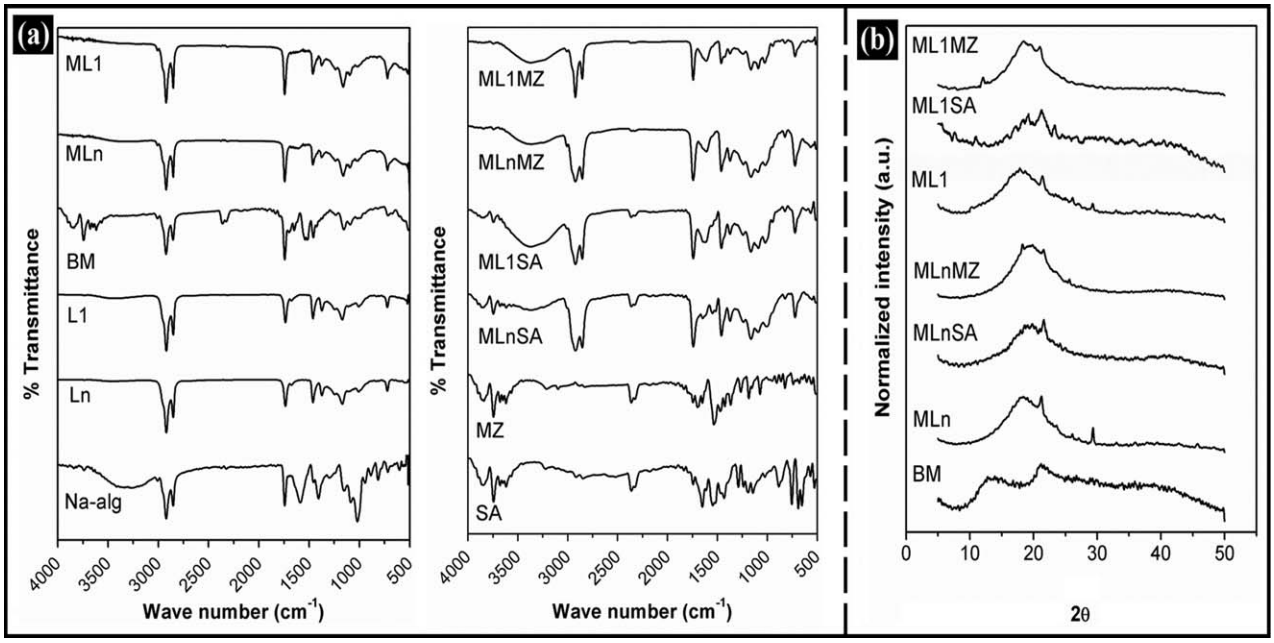

Figure 5. (a) FTIR spectra and (b) XRD profiles of the microparticles and their components.

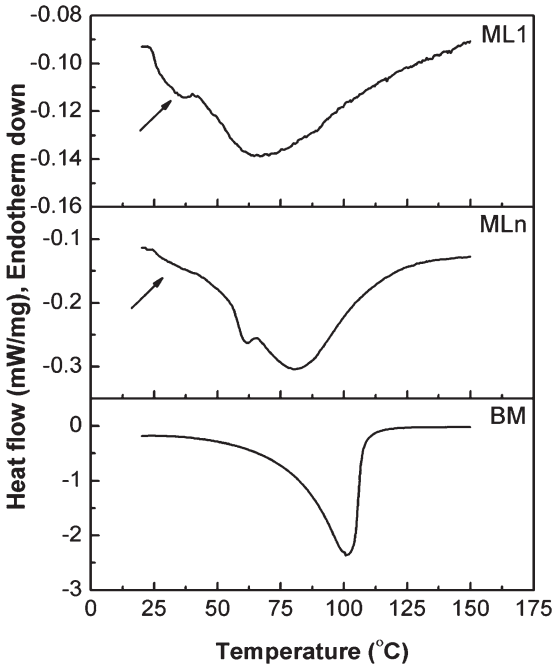

Figure 6. DSC thermograms of the microparticles.

found to be 28.25 ± 2.2 h, 24.5 ± 1.5 h, and 25.5 ± 3.25 h, respectively. All the microparticles showed high affinity towards the mucousal membrane. This may be associated with the anionic nature of the alginate molecules.[2,42] Under the given conditions, ML*n* and ML1 showed similar detaching time of ~25 h as compared to BM which showed a detaching time of ~28 h. A statistical analysis was conducted to measure the significance in the variation of detaching times. Analysis was performed by calculating the Pearson's correlation coefficient (*p* value) (Supporting Information Figure S1). The analysis suggested that the level of significance amongst the microparticles was >0.05. The coefficient of variation of BM with ML*n* and ML1 indicated insignificant variation in the detaching times. Since there was negligible leaching of the internal phase from ML*n* and ML1, this accounted for their similar mucoadhesive behavior as that of BM microparticles. In our previous study, we have found that encapsulation of oil within the alginate microparticles drastically reduces the mucoadhesive property of the microparticles.[18]

In Vitro Drug Delivery Studies

The *in vitro* cumulative percentage drug release (CPDR) profiles of SA and MZ in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) have been shown in Figure 7. The release of the drugs was affected by the physical nature of the microparticles. Under the given conditions (both gastric and intestinal environments), ML1 have shown highest *in vitro* CPDR value. The higher drug release from ML1 and ML*n* over BM can be attributed to their drug encapsulation efficiency. The drug encapsulation efficiency of ML*n* and ML1 is ~1.8 times to that of BM. As, lower amount of drugs were present in BM, lower CPDR was achieved. The release of SA was more in the basic pH than in the acidic pH. Conversely, MZ was released more in the acidic pH as compared to the basic pH.[43] The difference in the drug release behavior may be attributed to the drug solubility at different pH conditions. SA is a weak acid and is less soluble at lower pHs and more soluble at higher pHs.[44] MZ, on the other hand, has higher solubility at gastric pH environment than in the intestinal environment.[45]

The speculation of the mechanism of release of the drugs was carried out by fitting the drug release profile information in the zero-order, first order, Higuchi, Baker–Lonsdale model and Korsmeyer–Peppas models. The parameters of the best fit models have been tabulated in Table V. All the release patterns followed Higuchi, Baker–Lonsdale and Korsmeyer–Peppas models (acceptable regression coefficient was >0.95). The results suggested that the developed microparticles may be considered as swollen matrix type as the release profile can be easily described by the Baker–Lonsdale model.[46,47] Visual observation of the microparticles suggested that there was erosion of the

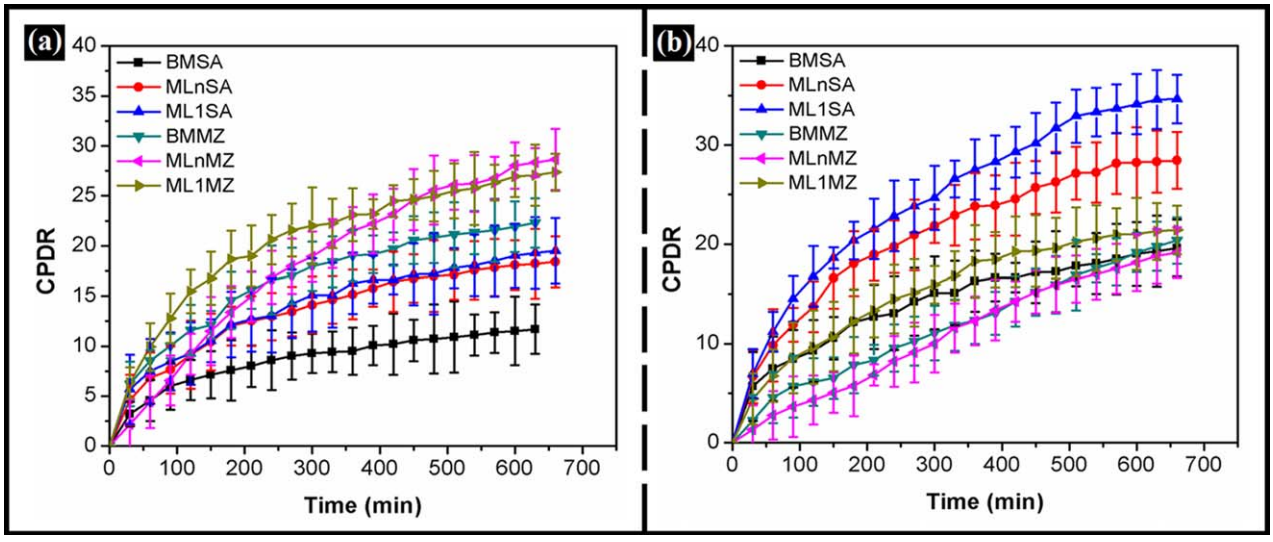

Figure 7. CPDR profiles of microparticles (a) in gastric buffer and (b) in intestinal buffer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table V. Drug Release Kinetics of the Microparticles

	Baker-Lonsdale model		Korsmeyer-Peppas model					
Sample	SGF R^2	SIF R^2	SGF R^2	'n'	Diffusion	SIF R^2	'n'	Diffusion
BMSA	0.975	0.981	0.982	0.40	Fickian	0.992	0.46	Non-Fickian
MLnSA	0.972	0.975	0.988	0.44	Fickian	0.988	0.47	Non-Fickian
ML1SA	0.980	0.989	0.992	0.41	Fickian	0.984	0.50	Non-Fickian
BMMZ	0.964	0.965	0.987	0.42	Fickian	0.992	0.67	Non-Fickian
MLnMZ	0.994	0.968	0.976	0.82	Non-Fickian	0.993	0.88	Anomalous
ML1MZ	0.951	0.976	0.960	0.52	Non-Fickian	0.991	0.52	Non-Fickian

microparticles at pH 7.2 buffer.[48] This was also evident from the erosion studies of the microparticles (procedure and results provided as supporting information). The Fickian value (n) was calculated from Korsmeyer–Peppas model. In acidic conditions, SA and MZ release followed Fickian kinetics (n value < 0.45) and non-Fickian kinetics (as n value is in between 0.45 and 0.85), respectively. Under intestinal conditions, SA containing microparticles showed non-Fickian release behavior. On the

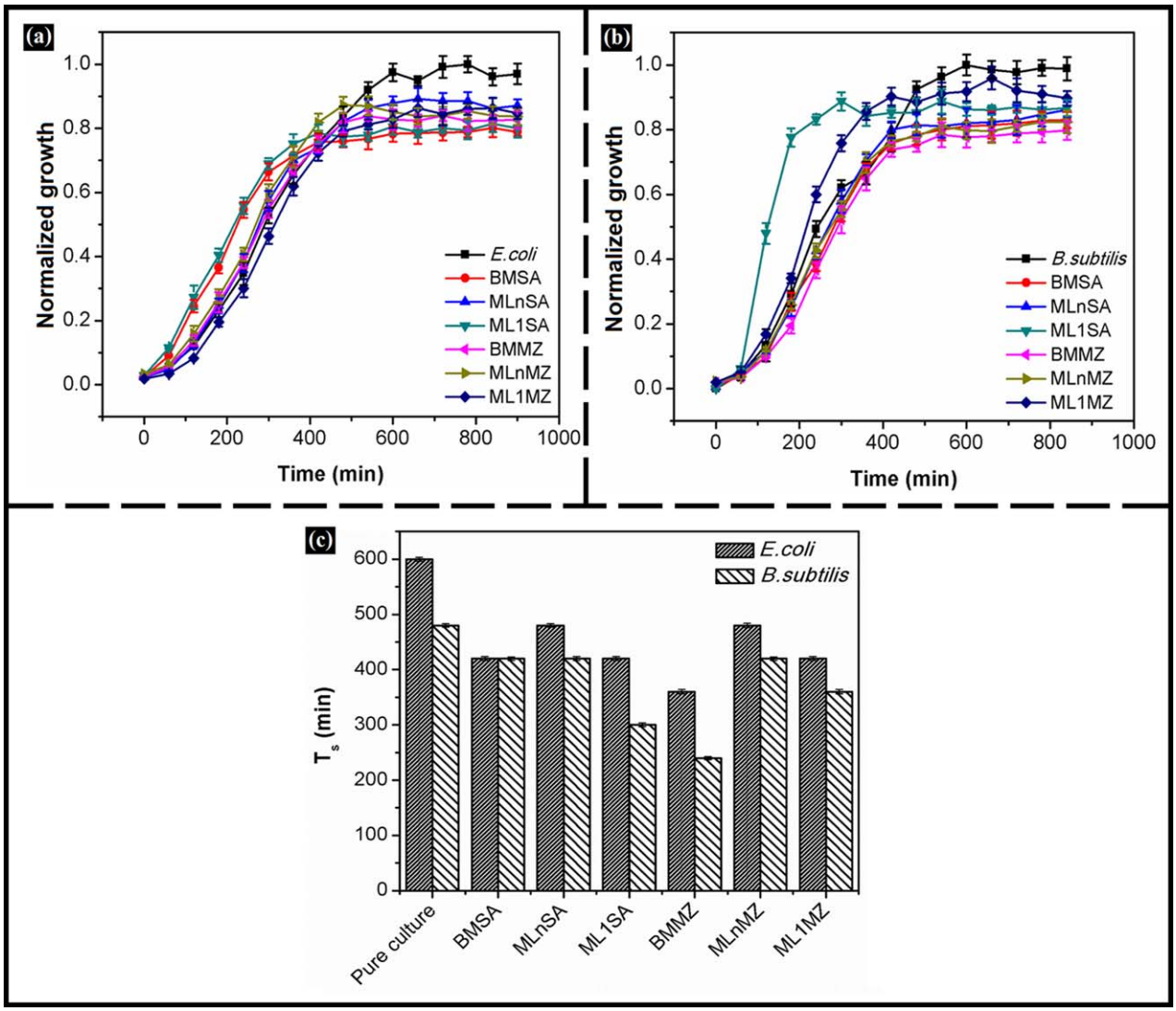

Figure 8. The antimicrobial activity of microparticles against (a) *E. coli*, (b) *B.subtilis*, and (c) The T_s data of the bacteria against microparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

contrary, ML1MZ followed non-Fickian kinetics while ML*n*MZ has followed anomalous behavior of release pattern. The non-Fickian/anomalous behavior of drug release may be due to polymer relaxation, erosion and degradation. Erosion studies also suggested that the microparticles were stable in acidic conditions and eroded under basic conditions (Supporting Information Table S1). The change of Fickian to non-Fickian release behavior of SA from the microparticles may be associated with the erosion of microparticles at higher pHs. Similar release behavior was seen with 5-aminosalicylic acid from the chitosan-Ca-alginate microparticles.[49] The non-Fickain and anomalous release behavior of the drugs from the alginate microparticles have also been reported extensively.[50,51]

Antimicrobial Studies

The antimicrobial activity of the drug containing microparticles was carried out by broth dilution method.[25] The microparticles were in direct contact with the bacteria present in the liquid broth for a period of 15 h. Microparticles without drug served as the negative control. This was done to ensure the elimination of the other factors towards the antimicrobial activity. Bacterial growth was checked by measuring the optical density of the nutrient broth at an interval of 1 h (Figure 8). The effect of drug on the growth of bacteria was analyzed by determining the time required for the bacteria to reach the stationary phase. If the bacteria, in contact with the test microparticles, reached their stationary phase in lesser time compared to the pure bacterial culture, then it can be inferred that the drug containing microparticles have antimicrobial action. The time duration required for reaching the stationary phase (T_s) of both the bacteria against the drug containing microparticles has been given in Figure 8(c).

The drug loaded microparticles have shown good antimicrobial activity against both the bacteria. BM has shown less T_s than the others due to the quick release of the drugs (evident from the drug release studies). ML1SA and ML1MZ have shown less Ts values as compared to the ML*n*SA and ML*n*MZ. This may be due to the quick release of the drugs from ML1. As the pH of the nutrient broth was adjusted to 7.0, the *in vitro* drug release profile at pH 7.2 may be compared to the testing conditions of antimicrobial studies. It was observed that at pH 7.2, ML1 showed higher CPDR for both the drugs. Higher CPDR from ML1 might have resulted in the higher antimicrobial activity.

Absence of the sudden appearance of the stationary phase suggested the absence of burst release from the microparticles. In general, alginate microparticles or, reinforced alginate and chitosan microparticles have shown burst releases of drugs under *in vitro* and *in vivo* conditions, which may lead to severe complications like inflammation and thrombosis.[52–54] This indicated that the developed formulations may be tried for the controlled delivery of drugs for prolonged periods.

CONCLUSION

Lanolin based organogels have been successfully encapsulated within the alginate microparticles prepared by ionotropic gelation method. DSC and XRD studies confirmed the encapsulation of organogels within the microparticles. SA and MZ were used as the model drugs. DEE of the organogel containing microparticles was found to be higher than the BM. Encapsulation of the organogels has enhanced the drug carrying capacity of the microparticles. The hemocompatible and mucoadhesivity studies suggested the biocompatible and mucoadhesive nature of the microparticles. *In vitro* drug release studies suggested that lanolin and L1 containing microparticles may be tried for controlled delivery applications.

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