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# **Formulation and evaluation of floating-mucoadhesive alginate beads for targeting H.pylori**

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## **Abstract**

**Objectives:** There are various obstacles in the eradication of *Helicobacter.pylori* (*H. pylori*) infections, including low antibiotic levels and poor accessibility of the drug at the site of the infection. This study describes the preparation and characterisation of novel floating-mucoadhesive alginate beads loaded with clarithromycin (CMN) for delivery to the gastric mucosa to improve the eradication of this micro-organism.

**Methods:** Calcium alginate beads were prepared by ionotropic gelation. The formulation was modified through addition of oil and coating with chitosan in order to improve floating, mucoadhesion and modify drug release.

**Key findings:** SEM confirmed the sphericity of the beads with X-ray microtomography (X $\mu$ MT) showing the 3D structure of the beads with the layered internal structure of the bead and the even distribution of the drug within the bead. This formulation combined two gastro-retentive strategies and these formulations produced excellent in vitro floating, mucoadhesive and drug release characteristics. Enhanced stability of the beads in phosphate buffer raises a potential for the modified formulations to be targeted to regions of higher pH within the gastrointestinal tract with a higher pH. Drug release from these beads was sustained through an unstirred mucin layer simulating in vivo conditions under which the *H. pylori* resides in the gastric mucosa.

**Conclusions:** This novel formulation will ensure retention for a longer period in the stomach than conventional formulations and control drug release, ensuring high local drug concentrations, leading to improved eradication of the bacteria.

## **Introduction**

The development of conditions such as chronic active gastritis, peptic ulcer disease, gastric mucosal-associated lymphoid tissue lymphoma and gastric carcinoma has been linked to *H. pylori* infection [1-3]. Infection with this Gram-negative micro-aerophilic bacterium is silent and a percentage of the infected population will develop conditions such as chronic gastritis, peptic and duodenal ulcers [4]. About half a million new cases/year of gastric cancer, about 55 % of the total cases worldwide, have been linked to *H. pylori* and it has been predicted to be one of the top ten leading causes of death worldwide by 2020 [5, 6]. *H. pylori* infection is considerably higher in developing countries (80 – 90 %), than in developed countries (10 – 50 % of the total population) [7]. *H. pylori* attach to the gastric epithelial cells causing

progressive injury to the gastric mucosa and its function [8, 9]. *H. pylori* is sensitive to many antibiotics in vitro; however no single agent is effective alone in vivo [10], therefore, a combination therapy is required to effectively eradicate the bacterium. Infections are difficult to eradicate because the bacterium resides below the gastric mucus adherent to the gastric epithelium; therefore, access of drugs to this particular site is limited. In addition, the bacteria can acquire resistance to the commonly used antimicrobial drugs [11], therefore a combination of two antibiotics including CMN, amoxicillin and metronidazole with a gastric acid inhibitor remains the first line therapy regimen for the eradication of *H.pylori* [12, 13]. However, the persistent rise in resistance of this bacterium to these antibiotics; the hostile environment of the stomach which reduces antibiotic bioavailability at the site of action [14] and the formation of biofilms by *H.pylori* on the gastric mucosa epithelium can cause treatment failures [15]. Another major problem is limited gastric residence of conventional antibiotic formulations (0.5 - 2 h), which even if designed to ensure release of drug over a longer period of time may not be retained in the stomach for that long. This has encouraged research into producing alternatives to the commonly used therapies [16]. Gastroretentive dosage forms (GRDFs) may prolong the residence time of dosage forms in the stomach [17] and must be strong enough to withstand peristaltic waves of the stomach and be easily removed from the stomach upon drug release [18]. GRDFs such as floating systems have a bulk density lower than that of gastric fluid and therefore remain buoyant on stomach contents without affecting gastric emptying for a prolonged time [19]. Mucoadhesive systems can provide an intimate contact between the delivery system and the underlying target mucosal surface, thereby improving the bioavailability [20] and therapeutic performance of the drug [21] by plugging and sealing the mucosal cell in the gastrointestinal (GI) tract [22] whilst controlling drug release. The use of a combined floating and mucoadhesive drug delivery system will explore any synergy between the mechanisms, thereby overcoming the shortcomings of the individual system [23]. This approach may extend the duration of retention in the stomach [24] and this, in addition to a sustained drug release profile, may lead to an enhancement in activity and reduced dosing frequencies [25]. In this study, sodium alginate (SAL), a natural hydrophilic polymer was used to prepare novel CMN-loaded calcium alginate beads as a potential gastroretentive floating-mucoadhesive drug delivery device. The SAL beads were modified to improve the floating and mucoadhesive properties by including olive oil (an oil with reported anti-*H.pylori* activity ([26, 27] and inclusion of with chitosan (a mucoadhesive polymer [28] forming a polyelectrolyte complex membrane respectively, both of which control CMN drug release. This formulation method has been

explored separately with either modification with oil or polymer coating but not as a combination of both in the delivery of CMN from alginate beads. These formulations should have the advantage of supplying high local concentrations of CMN into the gastric mucosa, where *H. pylori* is resident, leading to more effective targeting of this bacteria and a reduction in dose [29] thereby, reducing the risk of drug resistance.

## **Materials and Methods**

### **Materials**

SAL, chitosan, olive oil, calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), Mucin Type (III) and CMN were obtained from Sigma Aldrich (UK).

### **HPLC assay**

HPLC was performed on a Shimadzu system equipped with a SPD-20 AV Prominence UV/VIS detector, an LC 20 AT pump, and SIL-20A Prominence auto-sampler. Data acquisition was carried out on a LC solution software integrator with separation performed using a SphereClone 5 $\mu\text{m}$  ODS (2) column (150 x 4.6  $\mu\text{m}$ ) (Phenomenex). Mobile phase was  $\text{KH}_2\text{PO}_4$  (50 mM): ACN, pH 4.6 (50:50 v/v) containing 5 mM 1-Octanesulphonic acid (1-OCTS) [30], at a flow rate of 1.5 ml/min determined at 50 °C with an injection volume of 50  $\mu\text{l}$  and a run time of 5 minutes.

### **Preformulation studies**

Stability of CMN and determination of degradation rate constant

Solubility was assessed by adding excess CMN in buffers (pH= 1.2 - 8) and the solution was agitated continuously for 1 h. Stability of CMN was assessed by dispersing CMN (50 mg) in 100 ml buffers (pH = 1.2 - 7) maintained at  $37 \pm 1$  °C and stirred at 100 rpm. Sampling was done at different time intervals after adjusting the pH to 5.0 to prevent further degradation and analysed. The half life ( $t_{1/2}$ ) of CMN was determined from the pseudo first order degradation rate constant (k).

### **Preparation and optimisation of beads**

SAL suspension (10 g) was extruded drop-wise into CaCl<sub>2</sub>. The beads formed were collected, washed, frozen in liquid N<sub>2</sub> and freeze dried for 24 h. Optimisation for the ideal concentrations of SAL, CaCl<sub>2</sub> and curing times required for formation of discrete spherical beads in our laboratory have been detailed in a previous article [31]. Drug loaded beads were prepared by dispersing CMN evenly in the SAL solution before extrusion into the CaCl<sub>2</sub> solution (Table 1).

### **Preparation of modified beads**

Olive oil was added to 10 g of SAL solution and homogenised for 10 min to form a stable emulsion. CMN was added to the emulsion and the beads prepared as detailed in the previous section. Some of the beads were coated with chitosan solution to enhance the mucoadhesion and control the drug release (Table 1). Chitosan was dissolved in 1 % v/v glacial acetic acid and the beads were immersed in the coating medium for 30 min. The beads were washed, filtered and dried as before.

### **Characterisation of beads**

Study of morphology and physical characteristics of alginate beads

Bead sizes (n = 50) were measured with an electronic digital calliper [32]. The three dimensional structure, surface and internal pore structure of the beads were obtained using X-ray microtomography (X $\mu$ MT) (Nikon XTH225 X-Ray Microtomography) and scanning electron microscopy (SEM) (Stereoscan 90 Scanning Electron Microscope (Cambridge, UK).

Density measurements and porosity

Dry beads were filled to the mark of a 10 ml volumetric cylinder ( $W_1$ ) which was weighed ( $W_2$ ). The bulk density ( $P_b$ ) of the beads was calculated using:

$$P_b = \frac{(W_2 - W_1)}{10} \quad [33],$$

where,  $W_2$  is the weight of the beads and the cylinder, and  $W_1$  is the weight of the cylinder only. The true densities ( $P_t$ ) of the beads were determined using a (Quantachrome multipycnometer (Model MVP-D160-E)) with a  $5 \text{ cm}^3$  micro sample cup. The porosity  $P$  of the beads was determined using the formula

$$P = 1 - \frac{P_b}{P_t} \quad [34, 35]$$

Drug loading and drug entrapment efficiency (DEE)

Beads (100 mg) were digested in 100 ml PBS (phosphate buffered saline) under agitation at  $37^\circ\text{C}$  over 24 h, assayed and parameters calculated using the following equations.

$$\text{Drug loading } \left( \% \frac{w}{w} \right) = \frac{\text{amount of drug in sample (mg)}}{\text{sample weight (mg)}} \times 100$$

$$\text{DEE (\%)} = \frac{\% \text{ drug loading}}{\% \text{ theoretical drug loading}} \times 100$$

Calcium content of beads

Beads (100 mg) was dissolved in 10 ml concentrated  $\text{HNO}_3$  by heating at  $50^\circ\text{C}$ . This solution was diluted and analysed for  $\text{Ca}^{2+}$  ion at 422.7 nm by flame Atomic absorption spectroscopy (Perkin Elmer AAnalyst 100).

Loose surface crystals (LSC)

Beads (100 mg) were agitated for 5 minutes in 100 ml PBS (pH 7.4) using a mechanical shaker and the leached drug was assayed using HPLC. The % LSC was calculated using the following equation:

$$\% \text{ LSC} = \frac{\text{amount of leached drug (mg)}}{\text{amount of entrapped drug (mg)}} \times 100$$

### Differential scanning calorimetry (DSC)

Samples (5 - 10 mg) were held at 25 °C for 1 min and then heated from 25 °C to 300 °C at a rate of 10 °C / min under N<sub>2</sub> atmosphere using a DSC-1 Mettler Toledo (Mettler-Toledo, Switzerland).

### Powder X-ray diffraction analysis (P-XRD)

Powdered samples were placed and levelled in a stainless steel holder and analysed using a Bruker D2 Phase diffractometer (Bruker, UK). The samples were scanned between 5 and 40 of  $2\theta$  with a step size of 0.019° and a step time of 32.5 s.

### FTIR

Samples were scanned from 400 - 4000 cm<sup>-1</sup> using a Thermo Nicolet 380 FTIR with Diamond ATR. The characteristic peaks of IR spectra were recorded.

### Zeta potential ( $Z_p$ )

Suspensions of the beads in 0.1N HCl (pH 2.0) and phosphate buffer (pH 6.8) were sonicated and analysed using Zetasizer Nano Z (Malvern Instruments Ltd., UK). Each sample was analysed six times to obtain an average value and a SD.

### Determination of in vitro buoyancy lag time and duration

Beads (n = 50) were placed in 0.1N HCl (pH 2.0) containing 0.02 %w/v Tween 20 maintained at 37 ± 1 °C and agitated at 100 rpm for 24 h using a USP Type II dissolution apparatus. The floating lag time and the duration of floating were recorded.



## Swelling studies

Beads were immersed in 100 ml of 0.1N HCl (pH 2.0) or PBS (pH 7.4) and, at fixed time intervals; the beads were removed, dried and weighed. Dynamic weight change of the beads was calculated according to the formula:

$$\% \text{ weight change} = \frac{W_s - W_i}{W_i} \times 100$$

where,  $W_s$  is the weight of the beads in the swollen state

$W_i$  is the initial weight of the beads.

## In vitro drug release and release kinetics

Drug release was studied from beads equivalent to 100 mg CMN using USP Type 1 dissolution apparatus in 0.1N HCl (pH 2) or PBS (pH 7.4) agitated at 100 rpm and maintained at  $37 \pm 1$  °C. Release kinetics was assessed using various kinetic models [36-40] and  $f_2$  analysis [41].

## Drug release in mucin suspension

In vitro diffusion of encapsulated drug through a 3 % w/v mucin suspension at pH 2 and pH 5, were carried out using vertical Franz diffusion cells. The receptor compartment contained 30 ml buffer maintained at  $37 \pm 1$  °C and agitated at 400 rpm. Dialysis membrane (cut off MW 14,000) was mounted between the donor and receiver cells with the mucin suspension representing an unstirred layer and 1 ml of receptor fluid was sampled and analysed.

## Ex vivo wash off mucoadhesion tests

A 5 cm wide and 3 cm long piece of porcine gastric mucosa was mounted onto a Perspex mounting block and 30 beads were spread evenly on the tissue surface, and allowed to incubate for ~ 20 min. The block was positioned at an angle of 30 ° in a humidity chamber (> 90 % RH) and maintained at  $37 \pm 1$  °C. Buffers at pH 2.0 (0.1 N HCl) and pH 7.4 (PBS) were

circulated over the tissue at a rate of 1 ml/min for 8 h. Beads remaining on the tissue after each hour were counted and the percentage of the remaining microspheres was calculated using the formula:

$$\% \text{ Mucoadhesion} = \frac{N_0 - N_i}{N_0}$$

where,  $N_0$  = number of beads applied initially and  $N_i$  = number of beads rinsed from the tissue.

#### Storage stability of beads

Beads were sealed in vials and stored at 4° C and room temperature (20 °C) over a period of three months. The drug loading,  $Z_p$ , mucoadhesion and in vitro release were determined at the end of days 30, 60 and 90.

#### Statistical analysis

Data were expressed as mean  $\pm$  SD (standard deviation). Student t tests and one way analysis of variance (ANOVA) were used to determine statistical significance. Probability values  $p > 0.05$ .

## Results

### Preformulation studies

#### HPLC assay

CMN was detected at 210 nm with a retention time of  $\sim 2.5$  min. The limit of detection (LOD) and limit of quantification (LOQ) were  $1.85 \pm 0.01$   $\mu\text{g/ml}$  and  $5.63 \pm 0.05$   $\mu\text{g/ml}$ , respectively. The intra-day and inter-day relative standard deviation (RSD) were  $< 5\%$ .

#### Solubility and stability profile of CMN

Solubility and stability of CMN is pH dependent (Figure 1a and 1b) with the highest solubility ( $\sim 17$  mg/ml) and lowest stability observed at the lowest pH studied.  $k$  values at pH 1.2, 2.0, 3.0 and 5.0 were  $1.45 \pm 0.13$   $\text{h}^{-1}$ ;  $0.45 \pm 0.01$   $\text{h}^{-1}$ ,  $0.055 \pm 0.007$   $\text{h}^{-1}$  and  $0.0028 \pm 0.0003$   $\text{h}^{-1}$  respectively with CMN being more stable as pH increased and the  $t_{1/2}$  were  $0.47 \pm$

0.04 h,  $1.53 \pm 0.005$  h,  $12.65 \pm 1.72$  h and over 100 h respectively. These results correspond to results obtained by earlier studies [42].

#### Characterization of beads

Beads were spherical as shown in Figure 2. X $\mu$ MT was used to visualise the bead structure without any prior treatment (Figure 2 a-f) unlike SEM (Figure 2 g- i). Diameter and bulk density increased with drug - polymer ratio ( $p < 0.05$ ) (Table 2) with porosity  $> 75$  %. The calcium content was reduced with an increase in drug content ( $p < 0.05$ ).

#### Drug loading and DEE

DEE was  $> 80$  % (Table 2), even at low drug-polymer ratios and this was due to the low solubility of CMN in 1% CaCl<sub>2</sub> solution which was determined to  $2.1 \pm 0.82$  mg/ml. Olive oil increased the DEE of the beads by  $\sim 6$  % with coating having no effect on DEE. Drug loading had no effect on LSC, which was reduced by at least 75 % following coating of the beads (Table 3).

#### Buoyancy profile

E1 - E4 samples (unmodified beads) floated initially with a lag time  $< 3$  min, however 40-50 % of the beads sank after the first hour. After 24 hours, only 15 - 45 % of the beads remained floating. Beads loaded with 10 %w/w oil retained buoyancy lag times were  $< 1$  min ( $p < 0.05$ ) (Table 2).

#### DSC

CMN showed a single endothermic peak with an average onset at  $227.5 \pm 0.32$  °C ( $n=3$ ), which corresponds to its documented melting point. The physical mixture of CMN and SAL and drug loaded beads (both modified and unmodified beads) had similar endothermic peaks at  $226.74 \pm 0.78$  °C ( $n = 3$ ) and  $226.56 \pm 0.89$  °C ( $n = 3$ ) respectively (Figure 3).

## P-XRD

P-XRD profiles of the beads showed characteristic peaks observed in CMN. However, there was a significant reduction in peak intensities in the drug loaded beads due to the encapsulation process (Figure 4).

## FT-IR

Characteristic bands of E1 (blank beads) were at  $3381.5\text{ cm}^{-1}$  representing the O-H band,  $1606.9\text{ cm}^{-1}$  and  $1428.4\text{ cm}^{-1}$  representing the asymmetric band of the carboxylate, the band at  $1032\text{ cm}^{-1}$  is given by the guluronic units [43], and  $819.6\text{ cm}^{-1}$ , identified in the literature as the combination of three possible vibrational modes (tCO+dCCO+dCCH) [44]. IR spectra of CMN showed the characteristic band of hydrogen bonds between –OH groups vibration at  $3479.5\text{ cm}^{-1}$  (Figure 5). The characteristic band C=O vibration of the lactone group at  $1732.9\text{ cm}^{-1}$  and strong absorption band at  $1692\text{ cm}^{-1}$  belonging to the carbonyl ketone peak for N-CH<sub>3</sub> stretching of aromatic ring at  $1457.7\text{ cm}^{-1}$  (Figure 6). Drug loaded CMN beads (both modified and unmodified) showed similar peaks with reduced intensities.

## Swelling studies

Swelling of E1 (blank) in HCl was about 350 % w/w and this was achieved after ~ 100 min. The presence of drug and modification of the beads led to a reduction in swelling (Figure 7a). In PBS, E1 exhibited maximal swelling of about 2500 % w/w, which was achieved between 100 and 150 min. The beads subsequently started to disintegrate after this time leading to a reduction in weight (Figure 7b). Oil-modified beads both coated and uncoated showed maximal swelling of ~ 250 % with an increase in time for complete dissolution of the beads in PBS. The coated oil modified beads remained stable till the end of this study at 6 h.

## Zeta potential (Zp)

Zp increased positively and there was an inversion of Zp on inclusion of chitosan in the bead formulation (Figure 10a). This was observed at both pH levels; however, the degree of change was larger at pH 2.0 because at this pH, chitosan is highly positively charged. E1 beads were negatively charged and this negative charge increased negatively with an increase in pH due to the presence of ionized carboxylic groups of the alginic acid

### In vitro drug release

Drug release was biphasic with an initial rapid release followed by a subsequent slower release. Drug release from E4 (10 % w/w CMN) was more sustained than E2 (1% w/w CMN) where release was rapid ( $p < 0.05$ ) (Figure 8a). This is as a result of the drug content as beads with higher drug loading exhibited slower release rates. Although the addition of oil did not impact the release rate ( $p > 0.05$ ) ( $f_2 = 62.7$ ); coating with chitosan reduced the release rate ( $p < 0.05$ ) ( $f_2 = 30.1$ ) (Figure 8b), however the molecular weight of chitosan used had little effect on drug release ( $f_2 = 57.4$ ). E2 – E6 (unmodified and oil modified) formulations fitted well to Higuchi kinetics ( $R^2 > 0.99$ ), while the coated beads exhibited zero order kinetics ( $R^2 > 0.99$ ). This was followed closely by Higuchi kinetics ( $R^2 > 0.98$ ). The release exponent,  $n \leq 0.45$  indicates Fickian transport drug release mechanism (Table 4) with an initial rapid release followed by tailing over time.

The release of CMN from these beads in PBS was rapid when compared with release in the acidic media (Figure 8c). After 2 h, the unmodified beads started to disintegrate and complete release was achieved after this time. However, in the coated oil-modified beads, drug release was more sustained and complete drug release was achieved at ~ 8 h with the beads completely dissolving at ~ 9 h. The release exponent on fitting to the Korsmeyer-Peppas model was  $0.46 \leq n < 0.53$  also indicating non-Fickian diffusion.

### Drug release in mucin suspension

Drug diffusion from beads through mucin solution was sustained over a period of 12 h at both pH levels. There was no burst release from the beads and release from E10 was reduced compared to E4. Drug fluxes were  $1020.13 \pm 30.12 \mu\text{g cm}^{-2} \text{h}^{-1}$  (saturated CMN solution);  $355.3 \pm 5.78 \mu\text{g cm}^{-2} \text{h}^{-1}$  (unmodified beads, E4) and  $221.1 \pm 9.43 \mu\text{g cm}^{-2} \text{h}^{-1}$  (coated oil modified bead, E10) at pH 2.0 (Figure 9a). At pH 5.0, the fluxes were significantly reduced ( $p < 0.05$ ) compared with those observed at pH 2.0 with flux of saturated solution being  $734.8 \pm 46.31 \mu\text{g cm}^{-2} \text{h}^{-1}$ ;  $198.7 \pm 23.52 \mu\text{g cm}^{-2} \text{h}^{-1}$  (E4) and  $87.8 \pm 6.41 \mu\text{g cm}^{-2} \text{h}^{-1}$  (E10) (Figure 9b). This change in flux is due to the differences in solubility of CMN at both pH levels.

### Mucoadhesion tests

In acidic media, > 50 % beads detached from the porcine mucosa after the first hour and the presence of drug reduced the mucoadhesion in unmodified beads. However, more than 80 % beads remained attached within the same time interval for the coated oil modified beads and up till the end of the study at 8 h. The adhered beads were between  $13.3 \pm 6.6$  and  $43.3 \pm 9.9$  at pH 7.4 (Figure 10b) after 8 h.

### Storage stability

After 3 months of storage at both 4 °C and 20 °C, there were no significant differences in the DEE,  $Z_p$ , buoyancy, mucoadhesion and drug release.  $f_2$  values for comparison of formulations at the beginning and end of the stability study were all > 50, indicating similarity of the profiles.

## Discussion

Stability of CMN at different buffer pHs was assessed due to its instability in acidic pH [45, 46] and without this, drug release would be underestimated [47]. Also, due to the fact that CMN is used in combination with other stomach acid reducing drugs, it was important to assess its stability. X $\mu$ MT and SEM images showed the macroporous internal structure of the hydrogel, with concentric layers with voids between the layers (Figure 2). The contour structure is associated with the cross-linking direction of the external gelation process from the surface to the core of the beads. The pores occur in areas of former ice crystals and are unique to freeze dried alginate beads and this aids buoyancy [48] producing beads with a bulk density less than 1 g/cm<sup>3</sup>. The homogenous distribution of CMN in the beads was observed in Figure 2e, which also demonstrates the crystalline nature of the drug in the beads. This is an important property of drug loaded alginate beads and this even distribution is attributed to the fine particle size of CMN and its tendency not to sediment in the polymer solution. This is in contrast to the observation of the uneven distribution of drug in beads observed by other researchers [49]. Drug rendered the beads less porous with the CMN occupying the interstitial spaces between the layers. Also, oil acts as a hydrophobic floating aid and as a dispersed phase to prepare a stable emulsion and create multiple tiny pockets in the matrix to

enhance its buoyancy (Figure 2c, 2f and 2i) and Table 2. 100 % buoyancy of the beads was achieved with the addition of 10 % w/w olive oil with a floating lag time of < 1 min compared with the lag times of unmodified beads which were ~ 3 min. The presence of Olive oil may provide a synergistic effect against the bacteria due to its reported anti *H. pylori* activity. Solid state characterization of the oil modified/ coated oil modified beads (E6 /E10) by DSC, P-XRD and FTIR showed no significant change in the scans indicating crystalline nature and stability of the drug in the formulation. This further confirms the presence of the crystalline drug observed in X $\mu$ MT images (Figure 2e). In acidic media, CMN release from the unmodified beads was biphasic, fast (due to the solubility of CMN at this pH and the presence of dispersed drug) and was complete within 6 h with drug release rate reducing with an increase in drug-polymer ratio ( $p < 0.05$ ). Modification of the beads with oil and further coating led a significant reduction in drug release rate extending drug release from an initial 6 h (unmodified beads) to ~ 12 h (Figure 8). When the unmodified calcium alginate beads are exposed to media with a pH > 4.0, Ca<sup>2+</sup> ions are displaced from the alginate network and the carboxyl residues are protonated to form alginic acid [50, 51] due to an ion exchange with displacement of Ca ion by Na from PBS, leading to chain relaxation and gel swelling. The formation of calcium phosphate renders the calcium alginate structure loose and soluble [52]. However, for the oil modified and coated oil modified beads, this ion exchange was affected with the maximum swelling being significantly reduced and complete dissolution of the unmodified; oil modified and coated oil modified beads being ~ 240 min, ~ 360 min and ~ 600 min respectively. CMN release in PBS was still overall faster than that observed in acidic media due to the progressive increase in pore volume in the swollen beads. Higuchi kinetics has previously been reported to describe drug release from alginate beads [47]. Coating of the beads reduced the porosity at the bead surface leading to a more sustained drug and by varying the molecular weight of the chitosan used; there was a further reduction in the drug release.

The negative charge of alginate beads allows ionic interaction with positively charged chitosan and the Z<sub>p</sub> inversion confirmed successful incorporation of chitosan within the formulation. The *ex-vivo* mucoadhesion tests demonstrated improved mucoadhesive properties of the oil modified formulations compared with the lower mucoadhesion exhibited with SAL formulations. This suggests the likely modification of the bead surface on addition of chitosan. Drug transport rate through mucus can be an important determinant of the efficacy of a formulation. Mucus is the primary barrier with which drugs must interact and diffuse through for drugs to be absorbed. The mucus layer of the stomach and the intestine

are reported to be 50 - 600  $\mu\text{m}$  and 15 - 450  $\mu\text{m}$  respectively [53-55]. Interspecies variations in mucus thickness limit the suitability of common laboratory animals for in vivo studies [56]. Drug release through mucin suspension demonstrated there was a sustained and adequate release of drug from the beads and the presence of chitosan on the surface of the beads did not hinder drug release in the presence of mucin. This method represents a more realistic in vitro drug release model as it better represents the in vivo environment. There was ~ 40 % reduction in flux with coated oil modified beads (E10) versus unmodified beads (E4) as a result of reduction of surface associated drug (no burst release); the interaction of mucin with the chitosan surface of the bead, thereby making the bead surface less available for outward drug diffusion and the presence of oil. Therefore this formulation will release its drug content when adhered to the stomach mucosa and have the potential to penetrate through the mucus where the *H. pylori* is resident.

The decreased mucoadhesion of the beads in PBS may be as result of the loss of the calcium ion from the beads [57, 58] and reduced interaction between chitosan and the mucus at this pH. Mucoadhesion occur with an initial wetting process causing an intimate contact between the mucus and the swelling mucoadhesive polymer. The polymer strands relax, followed by penetration of the polymer chains into the mucus network and finally the formation of secondary chemical bonds. A combination of the results from the ex vivo wash-off test and the buoyancy tests indicate that the coated oil modified beads containing CMN have a potential for mucoadhesion to the stomach mucosa and floating in the stomach. Storage of the beads at both 4 °C and 20 °C over 90 days did not show any significant change in the physical characteristics such as the color and texture. In addition, there was no significant difference in all DEE,  $Z_p$ , buoyancy, mucoadhesion and drug release therefore, the formulations were stable and could be stored at these temperatures without any requirement for special storage conditions.

## **Conclusion**

Emulsion gelation method was successfully used to produce floating CMN beads. The beads produced were spherical as confirmed by SEM and X $\mu$ MT with over 80 % DEE. Buoyancy was enhanced by modification with oil and by coating the beads with chitosan a mucoadhesive surface layer was added. The coated beads adhered to the porcine gastric mucosa and sustained drug release. The enhanced buoyancy and mucoadhesion of the beads



will be useful for gastroretentive applications. Beads that were both modified with oil and coated provided the best combined buoyancy, mucoadhesion and release profile, with the beads floating for at least 24 h; over 75 % beads adhering to pig gastric mucosa beyond 8 h and ensuring drug release beyond 8 h in gastric pH. The stability of the modified beads was also improved in PBS. The coated beads best fitted zero order release kinetics and they showed good storage stability at both 4 °C and 20 °C. Therefore, the modification of calcium alginate beads with oil and further coating of the beads with chitosan can ensure the development of effective sustained release floating-mucoadhesive drug delivery devices and provides a potential for a formulation to deliver drugs to the stomach and other parts of the GI with higher pH due to its improved stability in alkaline medium.

### **Declaration of Interest**

The authors report no declarations of interest.

## References

1. Peterson WL. Helicobacter pylori and Peptic Ulcer Disease. *New England Journal of Medicine*. 1991;324(15):1043-8.
2. Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. *Clin Microbiol Rev*. 1997;10(4):720-41.
3. Suerbaum S, Michetti P. Helicobacter pylori Infection. *New England Journal of Medicine*. 2002;347(15):1175-86.
4. Ernst PB, Gold BD. The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. *Annu Rev Microbiol*. 2000;54:615-40.
5. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet*. 1997;349(9061):1269-76.
6. Kawahara Y, Mizuno M, Yoshino T, Yokota K, Oguma K, Okada H, et al. HLA-DQA1\*0103-DQB1\*0601 haplotype and Helicobacter pylori-positive gastric mucosa-associated lymphoid tissue lymphoma. *Clin Gastroenterol Hepatol*. 2005;3(9):865-8.
7. Rothenbacher D, Brenner H. Burden of Helicobacter pylori and H. pylori-related diseases in developed countries: recent developments and future implications. *Microbes Infect*. 2003;5(8):693-703.
8. Suzuki H, Ishii H. Role of apoptosis in Helicobacter pylori-associated gastric mucosal injury. *J Gastroenterol Hepatol*. 2000;15(Suppl):D46-54.
9. Lehmann FS, Terracciano L, Carena I, Baeriswyl C, Drewe J, Tornillo L, et al. In situ correlation of cytokine secretion and apoptosis in Helicobacter pylori-associated gastritis. *Am J Physiol Gastrointest Liver Physiol*. 2002;283(2):G481-8.
10. Bazzoli F, Bianchi Porro G, Maconi G, Molteni M, Pozzato P, Zagari RM. Treatment of Helicobacter pylori infection. indications and regimens: an update. *Digestive and Liver Disease*. 2002;34(1):70-83.
11. Iijima K, Sekine H, Koike T, Imatani A, Ohara S, Shimosegawa T. Long-term effect of Helicobacter -pylori eradication on the reversibility of acid secretion in profound hypochlorhydria. *Aliment Pharmacol Ther*. 2004;19(11):1181-8.
12. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut*. 2007 June 1, 2007;56(6):772-81.
13. Georgopoulos SD, Papastergiou V, Karatapanis S. Helicobacter pylori Eradication Therapies in the Era of Increasing Antibiotic Resistance: A Paradigm Shift to Improved Efficacy. *Gastroenterology Research and Practice*. 2012;2012:9.
14. Batchelor H, Conway B, Williams RO. Targeting the infections within the gastro- intestinal tract. In: R.O. IW, Taft D.R, J.T. M, editors. *Advanced drug formulation design to optimize therapeutic outcomes (Drugs and the Pharmaceutical Sciences)*. New York:: Informa Healthcare; 2007. p. 217-44.
15. Cammarota G, Sanguinetti M, Gallo A, Posteraro B. Review article: biofilm formation by Helicobacter pylori as a target for eradication of resistant infection. *Alimentary Pharmacology & Therapeutics*. 2012 Aug;36(3):222-30.
16. Adebisi A, Conway BR. Gastroretentive microparticles for drug delivery applications. *Journal of Microencapsulation*. 2011;28(8):689-708.
17. Miyazaki Y, Yakou S, Takayama K. Comparison of gastroretentive microspheres and sustained-release preparations using theophylline pharmacokinetics. *J Pharm Pharmacol*. 2008 Jun;60(6):693-8.
18. Anilkumar SJ. Gastro retentive Drug Delivery Systems: An Overview. *Pharminfonet*; 2008.

19. Umamaheshwari RB, Jain S, Bhadra D, Jain NK. Floating microspheres bearing acetohydroxamic acid for the treatment of *Helicobacter pylori*. *J Pharm Pharmacol*. 2003;55(12):1607-13.
20. Kohri N, Naasani I, Iseki K, Miyazaki K. Improving the oral bioavailability of sulpiride by a gastric-retained form in rabbits. *J Pharm Pharmacol*. 1996 Apr;48(4):371-4.
21. Boddupalli BM, Mohammed ZN, Nath RA, Banji D. Mucoadhesive drug delivery system: An overview. *J Adv Pharm Technol Res*. 2010 Oct;1(4):381-7.
22. Akiyama Y, Nagahara N, Nara E, Kitano M, Iwasa S, Yamamoto I, et al. Evaluation of oral mucoadhesive microspheres in man on the basis of the pharmacokinetics of furosemide and riboflavin, compounds with limited gastrointestinal absorption sites. *J Pharm Pharmacol*. 1998 Feb;50(2):159-66.
23. Ahuja A, Khar RK, Ali J. Mucoadhesive Drug Delivery Systems. *Drug Development & Industrial Pharmacy*. 1997;23(5):489-515.
24. Singh B, Garg B, Chaturvedi SC, Arora S, Mandsaurwale R, Kapil R, et al. Formulation development of gastroretentive tablets of lamivudine using the floating-bioadhesive potential of optimized polymer blends. *Journal of Pharmacy and Pharmacology*. 2012;64(5):654-69.
25. Park K, Robinson JR. Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion. *International Journal of Pharmaceutics*. 1984;19(2):107-27.
26. Romero C, Medina E, Vargas J, Brenes M, De Castro A. In vitro activity of olive oil polyphenols against *Helicobacter pylori*. *J Agric Food Chem*. 2007;55(3):680-6.
27. Castro M, Romero C, de Castro A, Vargas J, Medina E, Millan R, et al. Assessment of *Helicobacter pylori* eradication by virgin olive oil. *Helicobacter*. 2012;17(4):305-11. doi: 10.1111/j.523-5378.2012.00949.x. Epub 2012 Apr 17.
28. Gåserød O, Jolliffe IG, Hampson FC, Dettmar PW, Skjåk-Bræk G. The enhancement of the bioadhesive properties of calcium alginate gel beads by coating with chitosan. *International Journal of Pharmaceutics*. 1998;175(2):237-46.
29. Ishak RA, Awad GA, Mortada ND, Nour SA. Preparation, in vitro and in vivo evaluation of stomach-specific metronidazole-loaded alginate beads as local anti-*Helicobacter pylori* therapy. *J Control Release*. 2007 Jun 4;119(2):207-14.
30. Erah PO, Barrett DA, Shaw PN. Ion-pair high-performance liquid chromatographic assay method for the assessment of clarithromycin stability in aqueous solution and in gastric juice. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1996 6/28;/682(1):73-8.
31. Adebisi AO, Conway BR. Preparation and characterisation of gastroretentive alginate beads for targeting *H. pylori*. *J Microencapsul*. 2014;31(1):58-67.
32. Murata Y, Sasaki N, Miyamoto E, Kawashima S. Use of floating alginate gel beads for stomach-specific drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*. 2000;50(2):221-6.
33. Bai YX, Li YF. Preparation and characterization of crosslinked porous cellulose beads. *Carbohydrate Polymers*. 2006 May 30;64(3):402-7.
34. Gal A, Nussinovitch A. Hydrocolloid carriers with filler inclusion for diltiazem hydrochloride release. *J Pharm Sci*. 2007 Jan;96(1):168-78.
35. Smrdel P, Bogataj M, Zega A, Planinsek O, Mrhar A. Shape optimization and characterization of polysaccharide beads prepared by ionotropic gelation. *Journal of Microencapsulation*. 2008 Mar;25(2):90-105.
36. Najib N, Suleiman MS. The Kinetics of Drug Release from Ethylcellulose Solid Dispersions. *Drug Development and Industrial Pharmacy*. 1985;11(12):2169-81.
37. Desai SJ, Singh P, Simonelli AP, Higuchi WI. Investigation of factors influencing release of solid drug dispersed in inert matrices. 3. Quantitative studies involving the polyethylene plastic matrix. *J Pharm Sci*. 1966;55(11):1230-4.
38. Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J Pharm Sci*. 1963;52:1145-9.

39. Hixson AW, Crowell JH. Dependence of Reaction Velocity upon surface and Agitation. *Industrial & Engineering Chemistry*. 1931 1931/08/01;23(8):923-31.
40. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics*. 1983 5//;15(1):25-35.
41. Moore JW, Flanner HH. Mathematical comparison of curves with an emphasis on in vitro dissolution profiles. *Pharmaceutical Technology*. 1996;20:64-74.
42. Chun MK, Sah H, Choi HK. Preparation of mucoadhesive microspheres containing antimicrobial agents for eradication of *H. pylori*. *Int J Pharm*. 2005 Jun 13;297(1-2):172-9.
43. Pereira L, Sousa A, Coelho H, Amado AM, Ribeiro-Claro PJA. Use of FTIR, FT-Raman and <sup>13</sup>C-NMR spectroscopy for identification of some seaweed phycocolloids. *Biomolecular Engineering*. 2003;20(4-6):223-8.
44. Dupuy B, Arien A, Perrot Minnot A. FT-IR of membranes made with alginate/polylysine complexes. Variations with the mannuronic or guluronic content of the polysaccharides. *Artif Cells Blood Substit Immobil Biotechnol*. 1994;22(1):71-82.
45. Nakagawa Y, Itai S, Yoshida T, Nagai T. Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, in comparison with erythromycin. *Chem Pharm Bull (Tokyo)*. 1992;40(3):725-8.
46. Erah PO, Goddard AF, Barrett DA, Shaw PN, Spiller RC. The stability of amoxicillin, clarithromycin and metronidazole in gastric juice: relevance to the treatment of *Helicobacter pylori* infection. *J Antimicrob Chemother*. 1997;39(1):5-12.
47. Rajinikanth PS, Mishra B. Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate *H-pylori*. *Journal of Controlled Release*. 2008 Jan 4;125(1):33-41.
48. Stops F, Fell JT, Collett JH, Martini LG. Floating dosage forms to prolong gastro-retention--the characterisation of calcium alginate beads. *Int J Pharm*. 2008 Feb 28;350(1-2):301-11.
49. Whitehead L, Collett JH, Fell JT. Amoxicillin release from a floating dosage form based on alginates. *Int J Pharm*. 2000 Dec 4;210(1-2):45-9.
50. Østberg T, Lund EM, Graffner C. Calcium alginate matrices for oral multiple unit administration: IV. Release characteristics in different media. *International Journal of Pharmaceutics*. 1994;112(3):241-8.
51. Pasparakis G, Bouropoulos N. Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. *Int J Pharm*. 2006 Oct 12;323(1-2):34-42.
52. Bajpai SK, Sharma S. Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca<sup>2+</sup> and Ba<sup>2+</sup> ions. *Reactive and Functional Polymers*. 2004 5//;59(2):129-40.
53. Lee SP, Nicholls JF. Diffusion of Charged Ions in Mucus Gel - Effect of Net Charge. *Biorheology*. 1987;24(6):565-9.
54. Norris DA, Puri N, Sinko PJ. The effect of physical barriers and properties on the oral absorption of particulates. *Adv Drug Deliv Rev*. 1998 Dec 1;34(2-3):135-54.
55. Khanvilkar K, Donovan MD, Flanagan DR. Drug transfer through mucus. *Advanced Drug Delivery Reviews*. 2001 Jun 11;48(2-3):173-93.
56. Varum FJ, Veiga F, Sousa JS, Basit AW. Mucus thickness in the gastrointestinal tract of laboratory animals. *J Pharm Pharmacol*. 2012;64(2):218-27. doi: 10.1111/j.2042-7158.2011.01399.x. Epub 2011 Nov 18.
57. Ikeda K, Murata K, Kobayashi M, Noda K. Enhancement of bioavailability of dopamine via nasal route in beagle dogs. *Chem Pharm Bull (Tokyo)*. 1992;40(8):2155-8.
58. Nayak AK, Hasnain MS, Beg S, Alam MI. Mucoadhesive beads of gliclazide: Design, development, and evaluation. *ScienceAsia*. 2010;36(4):319-25.

Code	CMN (w/w) %	Olive oil (w/w) (%)	Chitosan (w/v) (%)
E-1	-	-	-
E-2	1	-	-
E-3	5	-	-
E-4	10	-	-
E-5	5	10	-
E-6	10	10	-
E-7	10	10	0.5 (LMW)
E-8	10	10	1 (LMW)
E-9	10	10	0.5 (HMW)
E-10	10	10	1 (HMW)

**Table 1: CMN loading of unmodified beads, modified beads with olive oil and modified beads with olive oil and coating with low molecular weight (LMW) chitosan and high molecular weight (HMW) chitosan**

<b>Code</b>	<b>% Drug loading</b>	<b>% DEE</b>	<b>Diameter (mm)</b>	<b>Bulk density (g/cm<sup>3</sup>)</b>	<b>Porosity (%)</b>	<b>% Buoyancy</b>	<b>Floating lag time (min)</b>
<b>E-1</b>	-	-	1.62 (0.4)	0.13 (0.03)	84.98	35 (10)	<3
<b>E-2</b>	22.0 (1.2)	88.0 (4.6)	1.78 (0.2)	0.20 (0.02)	81.29	30 (10)	<3
<b>E-3</b>	53.5 (0.8)	85.5 (1.2)	2.15 (0.1)	0.23 (0.01)	80.97	20 (5)	<3
<b>E-4</b>	66.5 (1.3)	86.5 (1.5)	2.31 (0.3)	0.27 (0.03)	79.91	25 (5)	<3
<b>E-5</b>	24.3 (2.5)	90.0 (9.3)	2.33 (0.4)	0.25 (0.02)	80.68	100	<1
<b>E-6</b>	40.2 (1.3)	92.5 (3.1)	2.42 (0.2)	0.29 (0.02)	80.44	100	<1
<b>E-7</b>	35.4 (2.4)	81.2 (5.5)	2.49 (0.3)	0.30 (0.04)	80.19	100	<1
<b>E-8</b>	37.5 (2.7)	86.3 (6.2)	2.51 (0.4)	0.29 (0.03)	80.74	100	<1
<b>E-9</b>	36.2 (1.3)	83.3 (2.9)	2.47 (0.2)	0.28 (0.02)	82.34	100	<1
<b>E-10</b>	40.3 (1.9)	92.7 (4.4)	2.55 (0.3)	0.29 (0.03)	80.78	100	<1

**Table 2: Physical characteristics of CMN loaded beads. Results are presented as mean (standard deviation)**

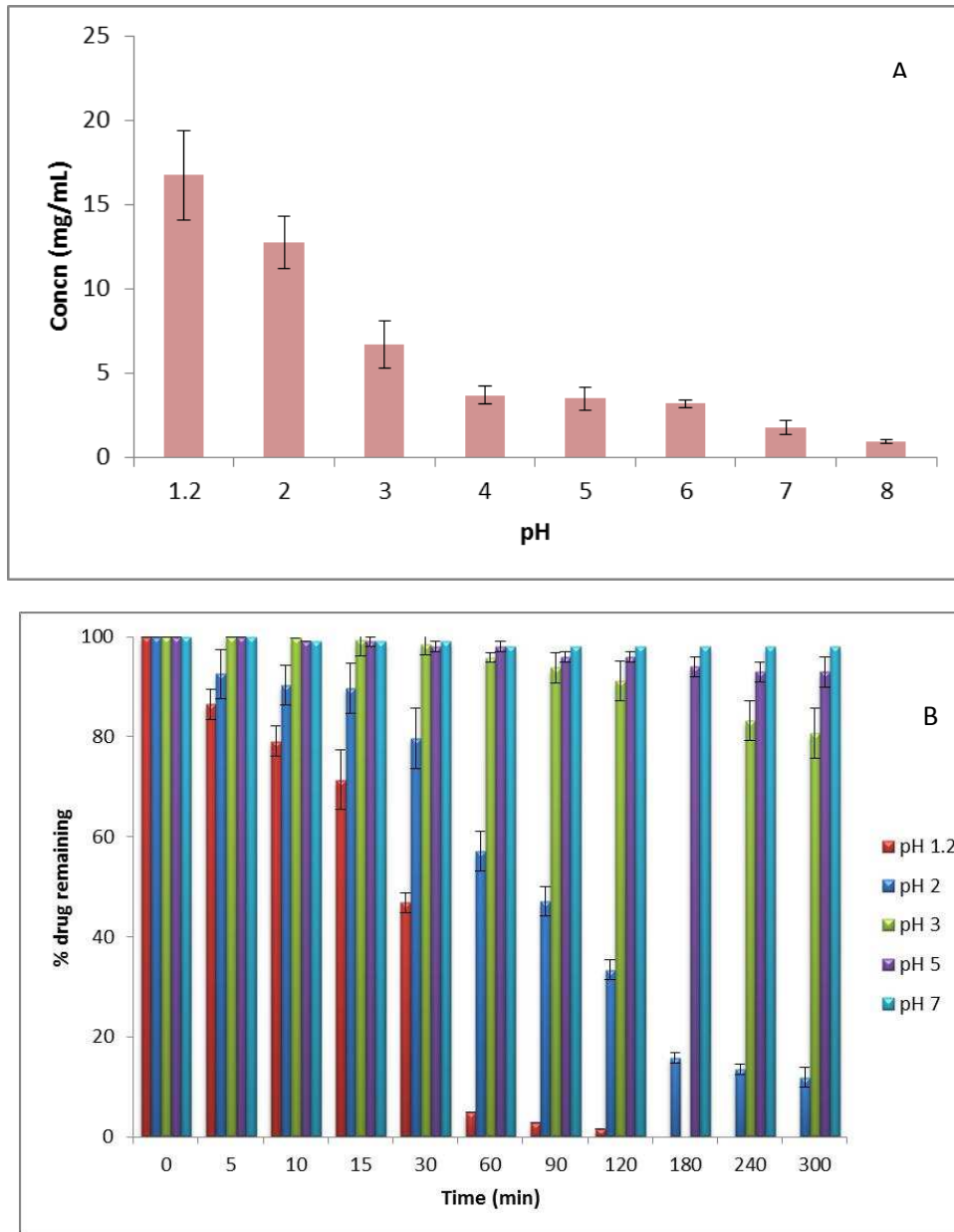
<b>Code</b>	<b>Calcium content (mg/10mg bead)</b>	<b>LSC %</b>
<b>E-1</b>	0.61 (0.08)	-
<b>E-2</b>	0.41 (0.06)	10.71 (2.92)
<b>E-3</b>	0.32 (0.04)	9.56 (3.18)
<b>E-4</b>	0.29 (0.04)	8.98(1.73)
<b>E-5</b>	0.12 (0.02)	5.22(3.74)
<b>E-6</b>	0.14 (0.02)	6.22(2.18)
<b>E-7</b>	0.15 (0.02)	1.98(0.42)
<b>E-8</b>	0.14 (0.01)	2.64(1.52)
<b>E-9</b>	0.16 (0.03)	2.11(1.92)
<b>E-10</b>	0.14 (0.01)	2.25(0.73)

**Table 3: Characteristics of CMN loaded beads. Results are presented as mean (standard deviation)**

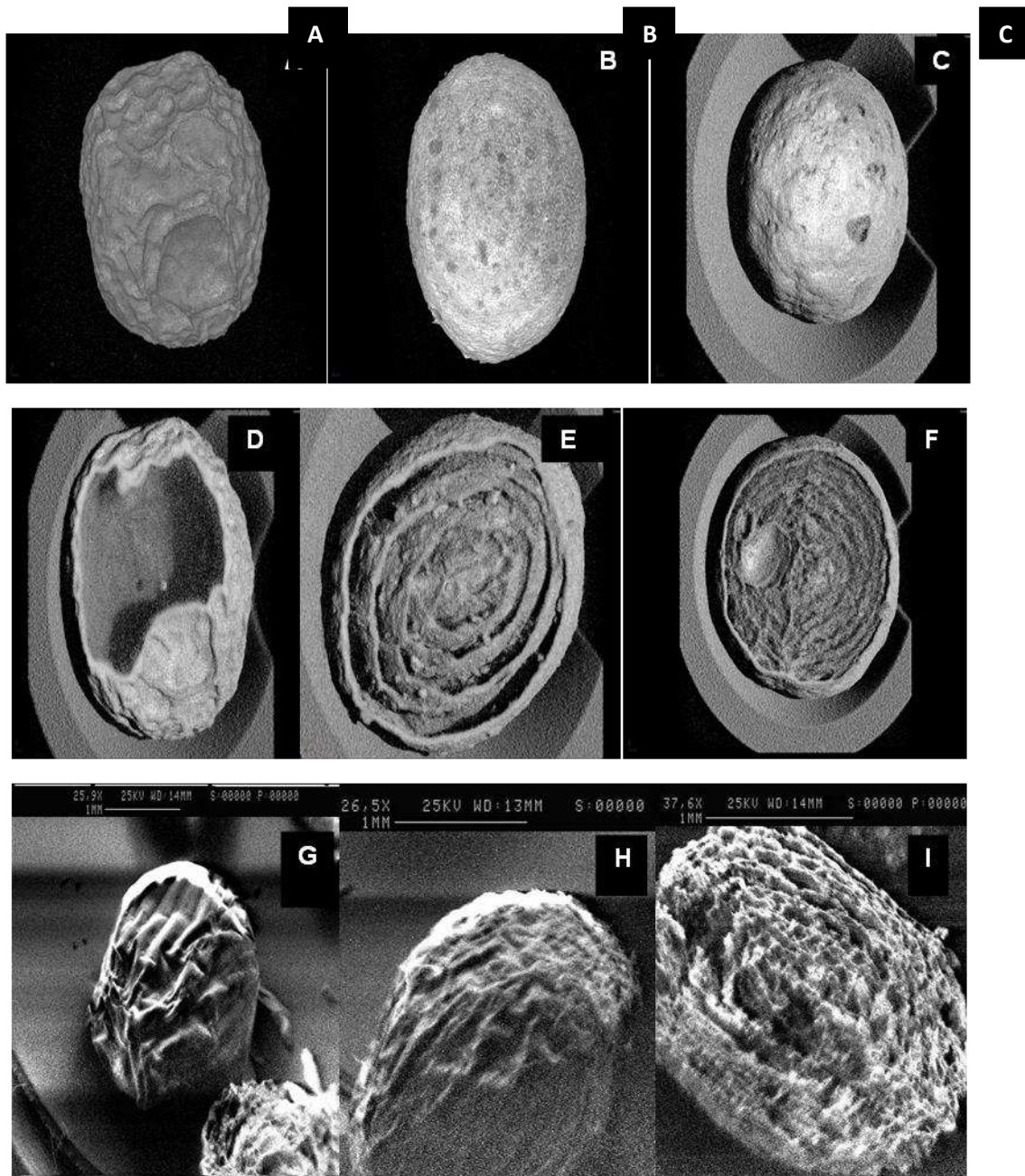
Code	Zero order		First order		Higuchi model		Korsmeyer-Peppas	
	R <sup>2</sup>	K <sub>0</sub> (h <sup>-1</sup> )	R <sup>2</sup>	K <sub>1</sub> (h <sup>-1</sup> )	R <sup>2</sup>	K <sub>h</sub> (/h <sup>-1/2</sup> )	R <sup>2</sup>	n
<b>E2</b>	0.9976	117.12	1.099	0.9965	0.9993	130.4	0.9133	0.41
<b>E3</b>	0.9841	24.21	0.9791	0.21	0.9937	40.71	0.9829	0.37
<b>E4</b>	0.9791	20.68	0.9889	0.18	0.9987	38.36	0.9823	0.38
<b>E6</b>	0.9677	20.69	0.9737	0.16	0.9984	41.19	0.9829	0.45
<b>E8</b>	0.9969	17.26	0.9229	0.07	0.9914	26.86	0.9796	0.43
<b>E10</b>	0.9928	15.73	0.9114	0.05	0.9832	24.29	0.9721	0.45

**Table 4: Dissolution parameters of the beads (pH 2.0) (E2-E4 – unmodified beads); (E6 – oil modified beads) and E8 /E10 – chitosan oil modified beads.**

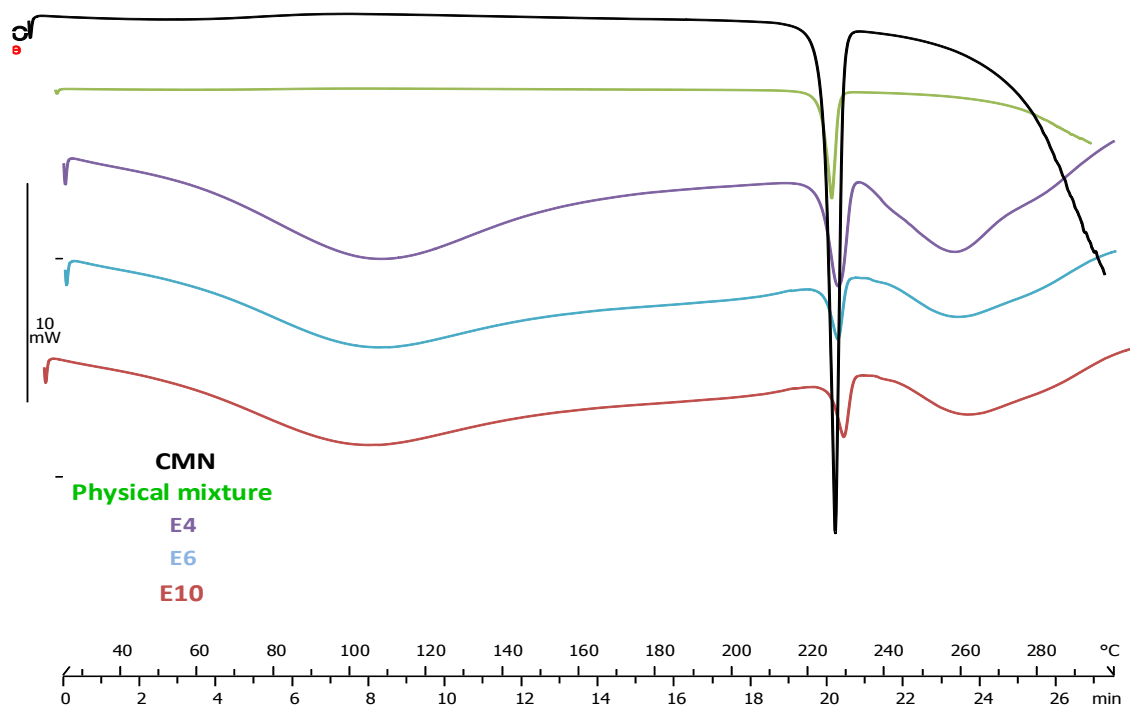




**Fig 1: A) Solubility profile of clarithromycin, B) pH-stability profile of clarithromycin at 37°C; Bar presented as mean  $\pm$  SD (n=3)**

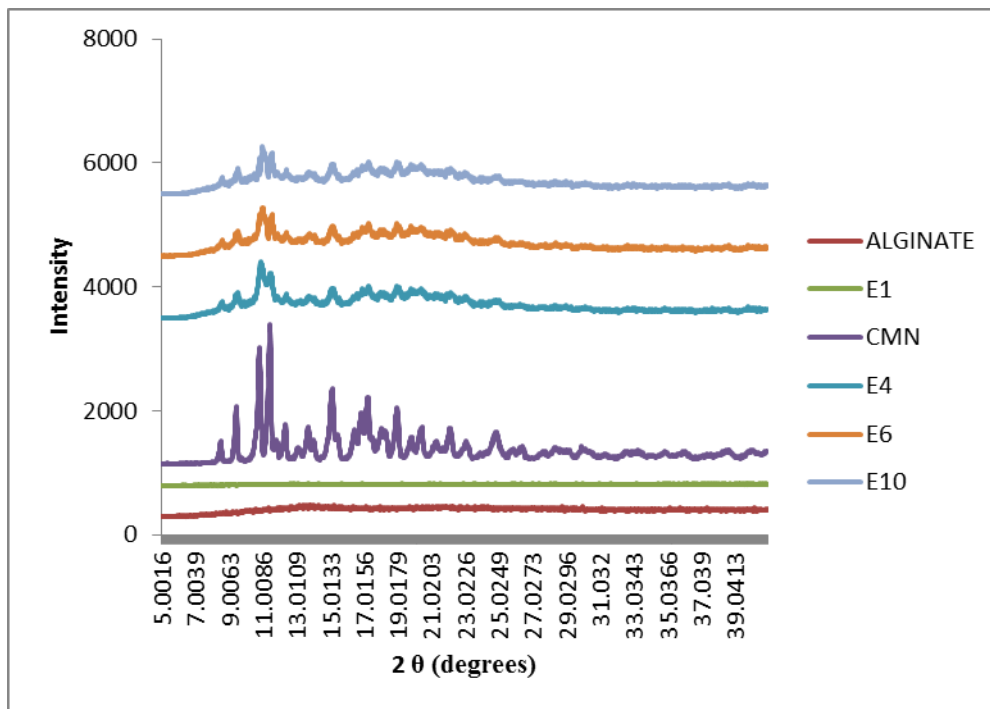


**Figure 2: 3D X-ray tomography images of E1 (blank beads) (A&D), E2 (CMN loaded beads) (B & E), E10 (coated oil-modified beads), (C&F); SEM images of E1 (G) and E10 (H & I).**



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Figure 3: DSC scans of pure CMN, physical mixture of SAL and CMN, CMN loaded beads (E4); oil-modified beads (E6) and coated oil-modified CMN beads (E10).



**Figure 4: P-XRD scans of alginate polymer, pure CMN, blank (E1), CMN loaded beads (E4); oil-modified beads (E6) and coated oil-modified CMN beads (E10).**

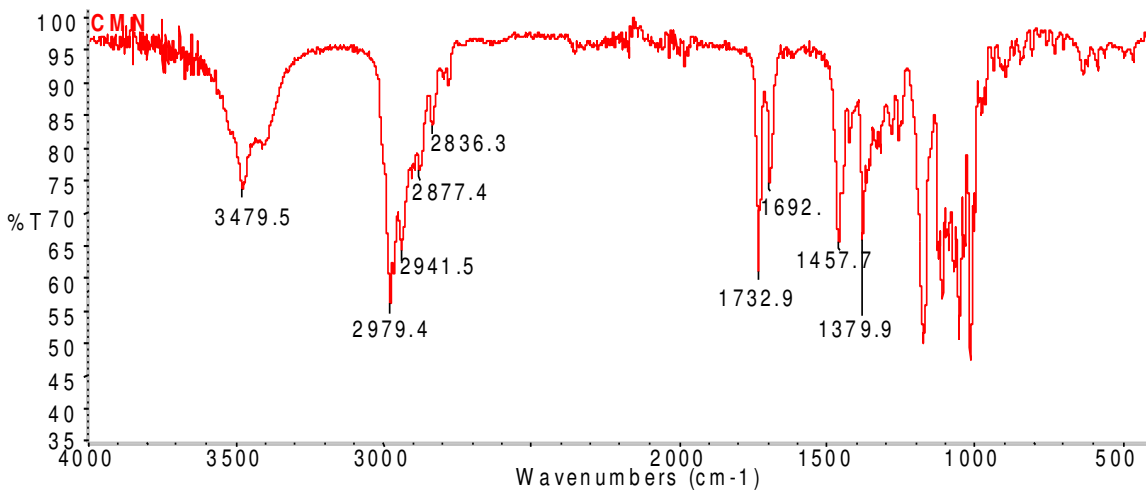
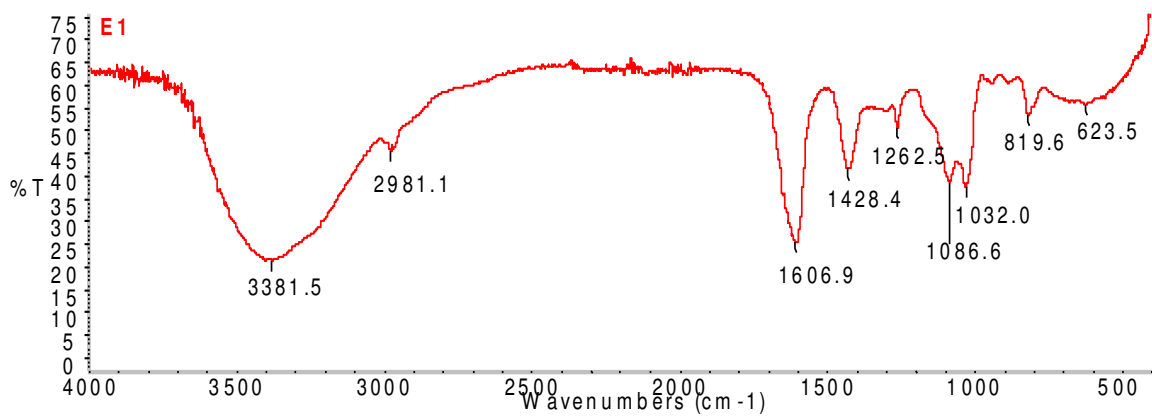
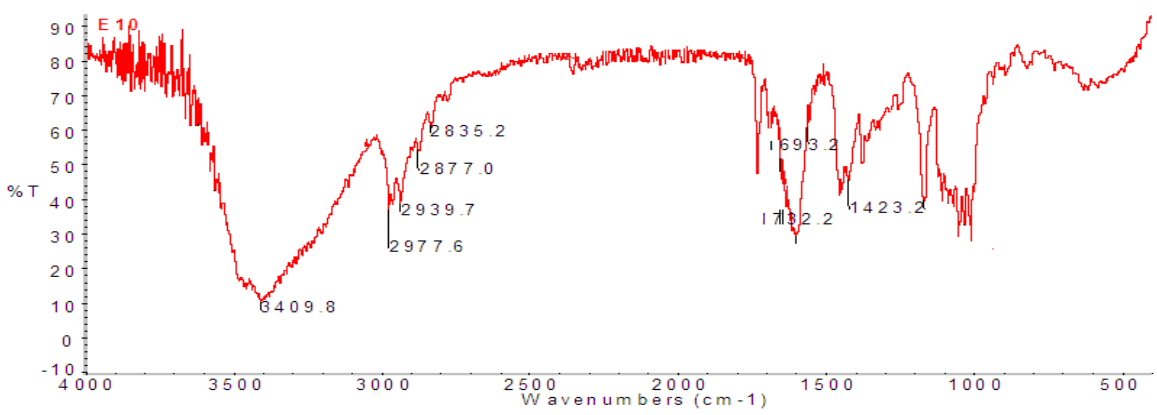
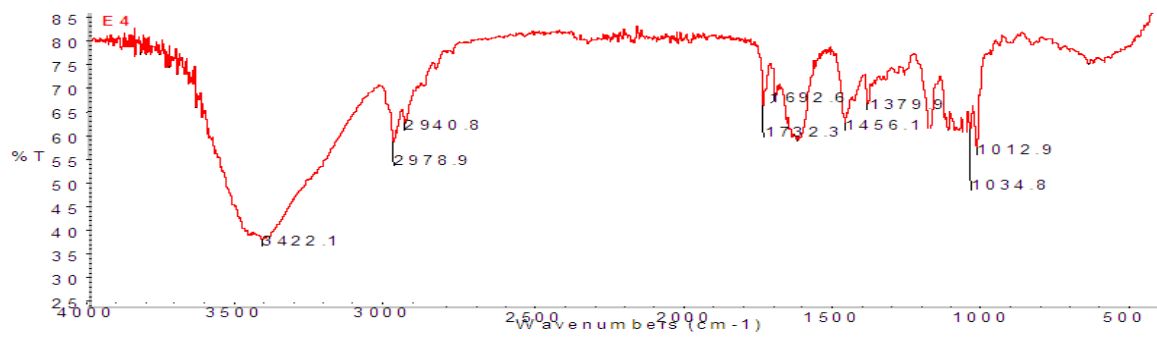
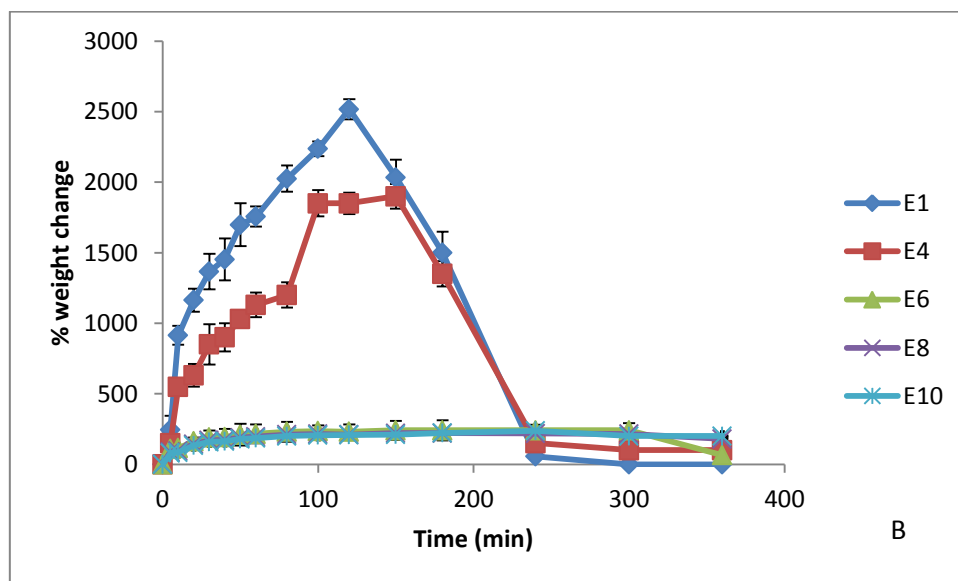
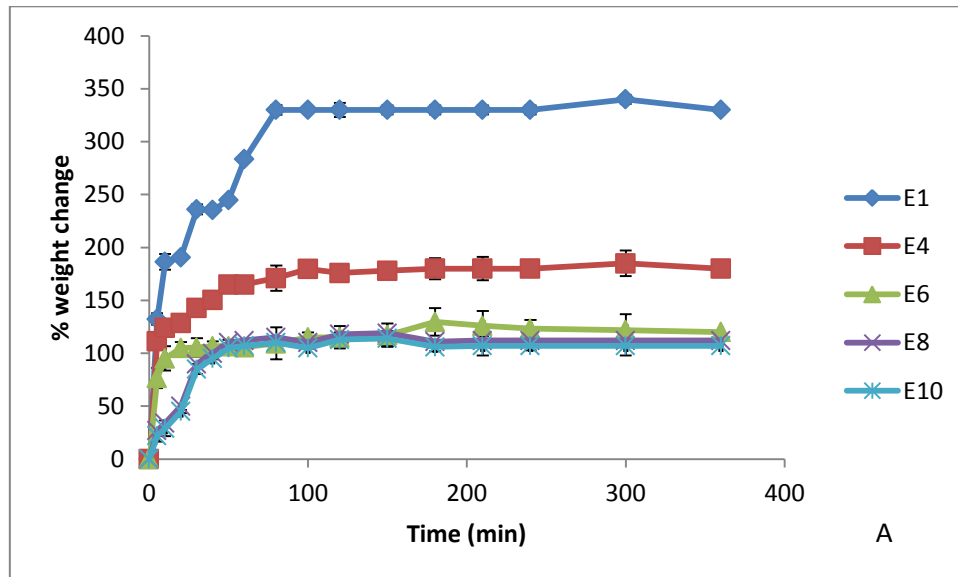


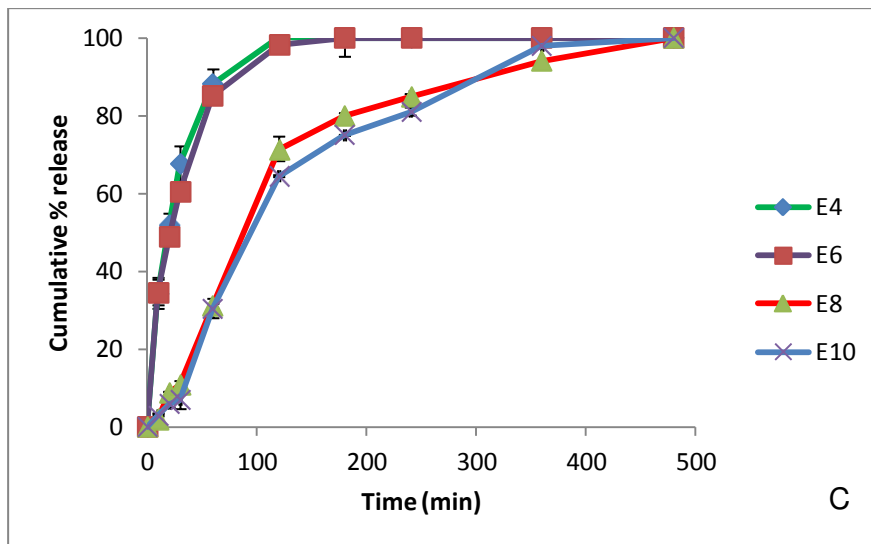
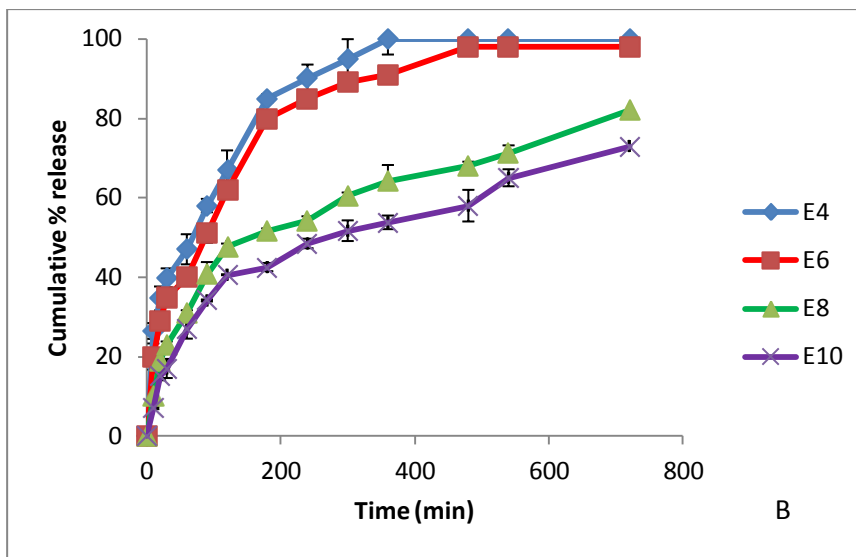
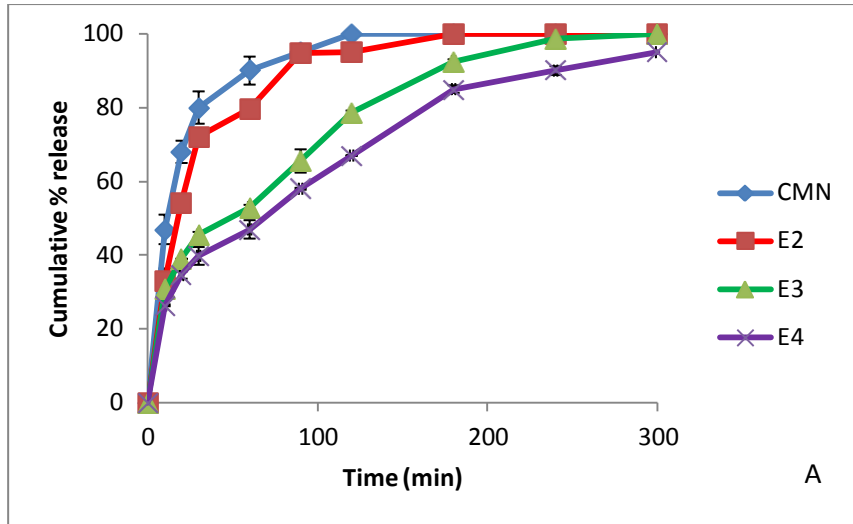
Figure 5: FTIR scans of pure CMN and blank alginate beads (E1)



**Figure 6: FTIR scans of unmodified (E4) beads and coated oil-modified (E10) beads**

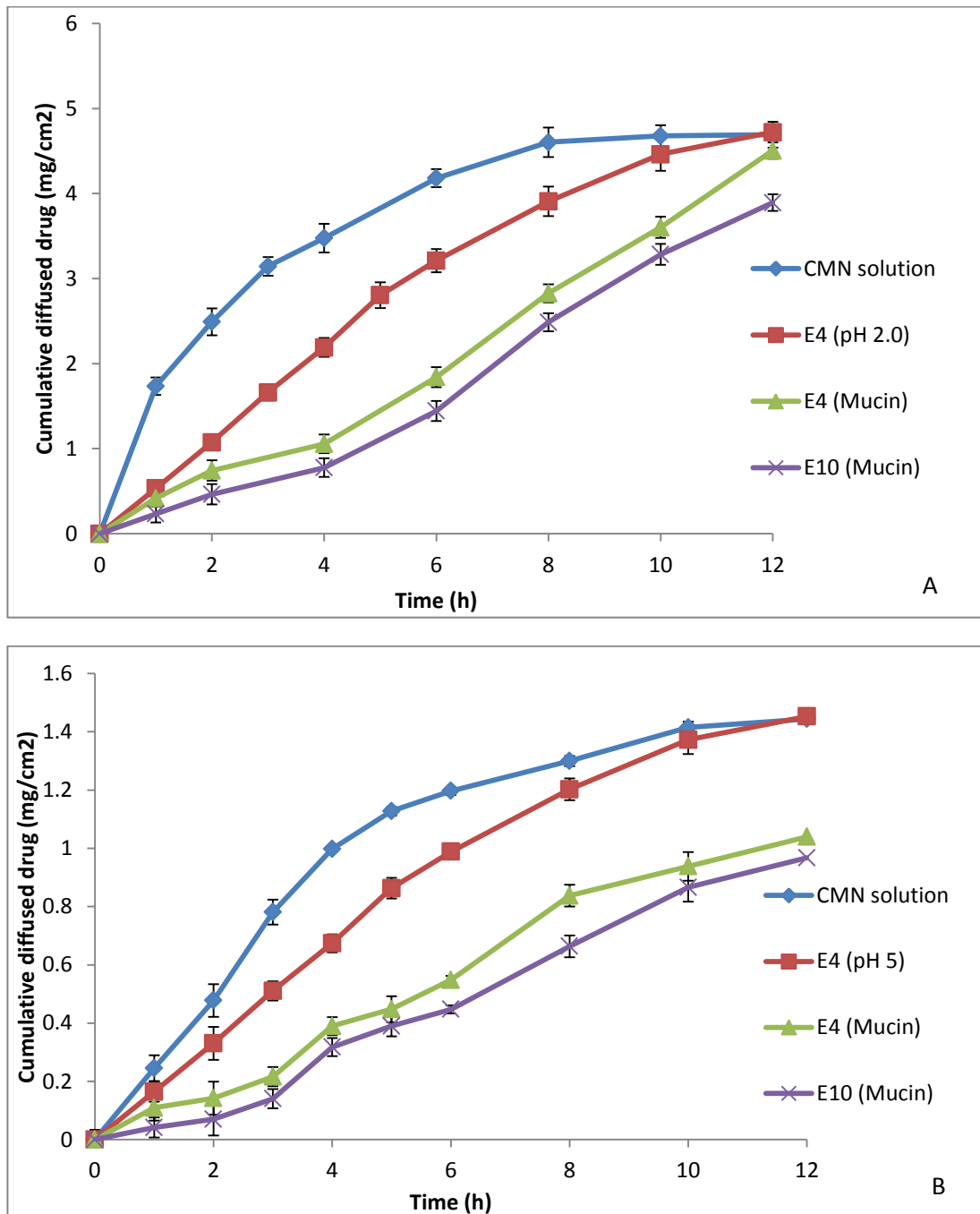


**Figure 7: Swelling profile of blank (E1), CMN loaded beads (E4); oil-modified beads (E6), 1 % LMW chitosan coated oil-modified CMN beads (E8) and 1 % HMW chitosan coated oil-modified CMN beads (E10) in A) 0.1 N HCl (pH 2.0) and B) PBS (pH 7.4)**

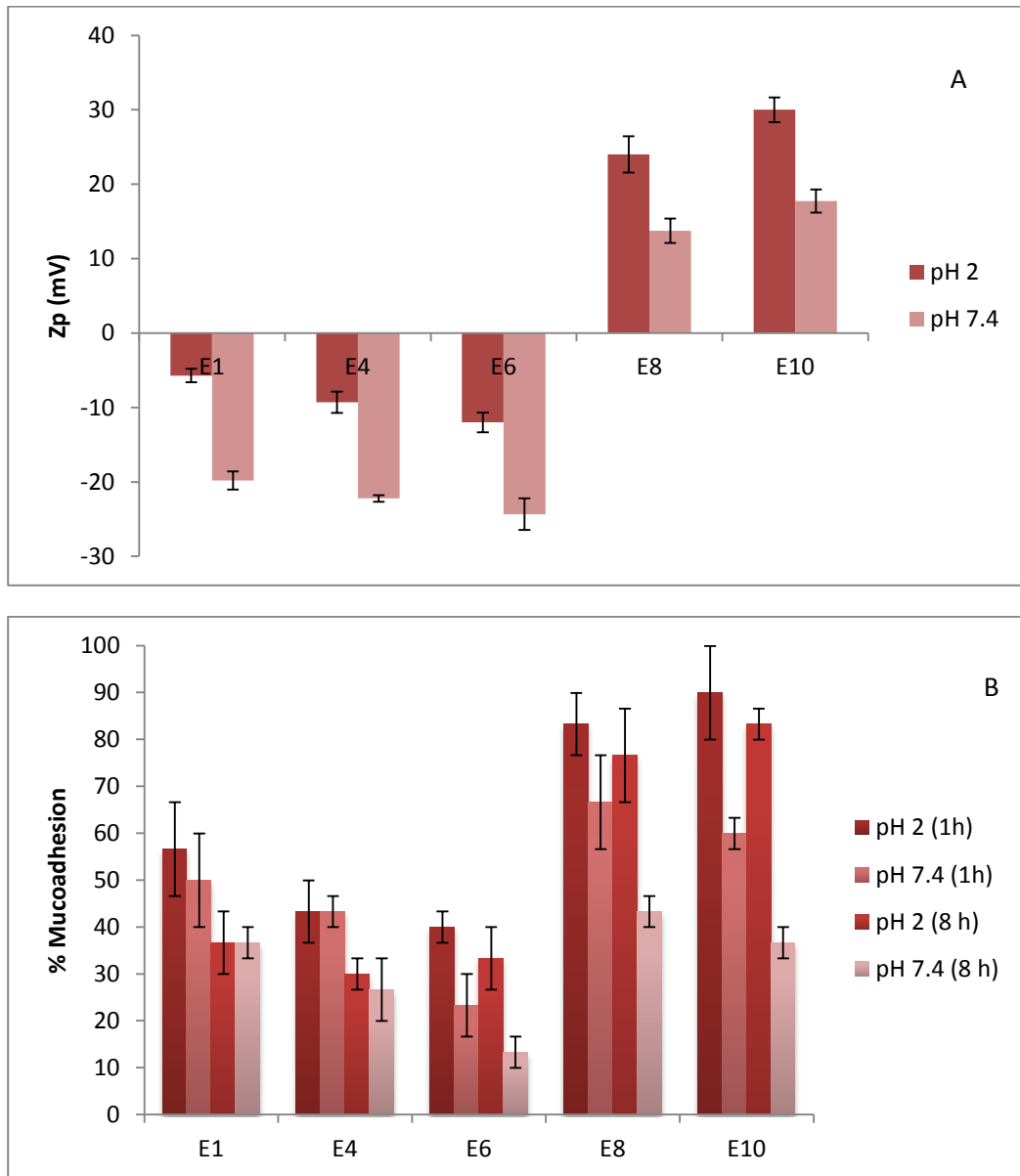


**Figure 8: A) Dissolution profiles of pure drug versus drug release from drug loaded beads (E2-E4); Comparison of release profiles of CMN loaded beads (E4); oil-modified beads (E6), 1 % LMW chitosan coated-oil modified CMN beads (E8) and 1 % HMW chitosan coated oil-modified CMN beads (E10) in B) 0.1N HCl (pH 2.0) and C) PBS (pH 7.4).**





**Figure 9 : Franz cell diffusion studies of the saturated CMN solution, drug loaded beads (E4) and 1 % HMW chitosan coated oil-modified CMN beads (E10) in mucin suspension at (A) pH 2 and (B) pH 5.0. Results presented as mean  $\pm$  SD (n=3).**



**Figure 10: A) Zeta potential and B) mucoadhesion of blank (E1), CMN loaded beads (E4); oil-modified beads (E6), 1 % LMW chitosan coated oil-modified CMN beads (E8) and 1 % HMW chitosan coated oil-modified CMN beads (E10) beads determined at pH 2.0 and pH 7.4.**