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PBPK Modelling of Ceftriaxone Na-loaded Starch-Sodium Alginate Polymeric Blend Prepared by Water-in-Oil Emulsification for Oral Delivery

Hina Raza [1], Yusra Ilahi [1], Zermina Rashid [2], Shabnam Nazir [3], Suryyia Manzoor [4] and Mohamed Deifallah Yousif [5]*

[1] Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[2] Department of Pharmacy, The Women University Multan, Multan, Pakistan
[3] Institute of Pharmaceutical Science, King's college London, London, UK
[4] Institute of chemical sciences, Bahauddin Zakariya University, Multan, Pakistan
[5] UCL School of Pharmacy, University College London, London, UK

Corresponding Author*

mohamed.yousif@ucl.ac.uk

ABSTRACT

Ceftriaxone is a third-generation cephalosporin antibiotic effective against many bacterial infections. However, owing to its instability in the gastrointestinal tract (GIT), it is administered by injections, which is an unfavorable route of administration. Therefore, the aim of this study was to formulate ceftriaxone into biodegradable and thermally stable polymeric blend microparticles that are suitable for oral delivery. The drug-loaded microparticles were prepared by the water-in-oil (W/O) emulsion method and consisted of starch and sodium alginate (NaAlg) as a polymeric matrix and glutaraldehyde (GA) as a cross-linking agent. Characterization of these particles using scanning electron microscopy (SEM) showed that the particles were spherical in shape with a smooth surface. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) of these particles showed no drug-polymer interactions. The highest percentage yield of particles was obtained at 3% polymer concentration. The particle size increased slightly after drug loading. The drug loading and entrapment efficiency appeared to increase with increasing polymer concentration. In vitro drug release at pH 1.2 and pH 7.4, revealed that drug release was below 20% at the acidic pH, while at pH 7.4, drug release of up to 85% was observed. The release mechanism followed first-order and Fickian diffusion patterns. Plasma concentration-time profiles were simulated for subcontinental Asian populations using commercial PBPK software, and the results

suggest that microencapsulation of ceftriaxone sodium in a polymeric blend could represent a promising approach for controlled oral delivery of the drug, with enhanced absorption and bioavailability of the drug.

KEY WORDS: Ceftriaxone sodium, sodium alginate, starch, microparticles, PBPK modelling, oral delivery

INTRODUCTION

Controlled drug delivery, which delivers the drug systemically or locally at a predetermined time for a prolonged period, ranging from days to months, has been described as one of the most effective ways of delivering poorly absorbed or gastric-unstable drugs(Debjit Bhowmik 1*, Harish Gopinath 1, B. Pragati Kumar 1, S. Duraivel 1, 2018)[1][1]. It comprises a drug carrier, shielding the enzyme and acid-labile drugs, and tailoring the drug release rates. A broad range of carriers for oral drug delivery has been developed in recent years to provide controlled release of drugs and maintain steady plasma levels for a prolonged period, with minimal adverse effects(Chien, 1992)[2][2].

Microparticles, small particles ranging from 1 to 1000 μm in diameter, are of specific interest to attain sustained/controlled drug delivery because of their versatile features, such as high drug loading and protection (Güneri et al., 2004)[3][3]. These particles are spherical and free-flowing, comprising proteins, lipids, polysaccharides, and natural and synthetic polymers(N.R, 2015)[4][4]. natural polymers are preferred over synthetic polymeric materials, because of biocompatibility, biodegradation, non-toxicity, low cost, and availability(V. Kulkarni et al., 2012)[5][5]. Starch is a natural polymer commonly used in biological applications. Owing to its complete biodegradation, low cost, and renewability, starch can produce stable products in the biological environment and is considered a promising polymer for producing sustainable materials(Lu et al., 2009; Pohja et al., 2004)[6], [7][6], [7]. Another biologically important natural polymer is sodium alginate (NaAlg), which consists of D-mannuronic acid and D-guluronic acid obtained from brown seaweeds. Sodium alginate has widespread applications in the agriculture, food, and beverage industries as a thickening and gelling agent and colloidal stabilizer(El-Zatahry et al., 2006; A. R. Kulkarni et al., 2000)[8], [9][8], [9]. NaAlg is hemocompatible and is known to form a network structure when linked with calcium ions or glutaraldehyde. This feature has been used to formulate sustained-release particulate systems from NaAlg for various drugs and proteins(FERREIRA ALMEIDA & ALMEIDA, 2004; Gombotz & Wee, 1998)[10], [11][10], [11].

Ceftriaxone sodium is a third-generation cephalosporin that belongs to BCS class-3 (biopharmaceutical classification system) and has high solubility and low permeability(Aungst et al., 1996)[12][12]. It is a semi-synthetic antibiotic that is used for the efficient treatment of numerous types of bacterial infections. It is a β lactamase – resistant cephalosporin with an

enormously long serum half-life, up to 4-10 times longer than that of other antibiotics of this class(Manimekalai et al., 2017)[13][13]. Currently, ceftriaxone sodium is administered parenterally owing to its poor absorption, as it has two negatively charged carboxylate groups and is susceptible to enzymatic degradation in the gastrointestinal tract. To overcome this problem, several studies have been conducted to deliver ceftriaxone via the oral route using different approaches, such as coupling with absorption carriers(Jeon et al., 2013; Lee et al., 2005)[14], [15][14], [15], enteric coating(Maghrabia et al., 2019)[16][16], and encapsulation of particles of different polymeric matrices[17]– [19][17]– [19][17]– [19]. A major drawback of these approaches is the high preparation cost. In addition, a high dose of the drug was needed to match the plasma i. v. concentration in some cases(Maghrabia et al., 2019)[16][16]. Chitosan nanoparticles, lipid nanoparticles, alginate beads, and microspheres have all been reported as delivery systems for ceftriaxone sodium through encapsulation; however, none of these studies have modelled the approach used for human use, especially for LMICs (low- and middle-income countries) such as Asian countries.

In this study, efforts were made to successfully develop starch and NaAlg microparticles, encapsulating the model drug ceftriaxone sodium, for oral delivery. The combination of starch and NaAlg provides the advantage of having two synergistic release mechanisms for the incorporated drug through swelling and diffusion[20]. Microparticles were prepared using the water-in-oil emulsion method, where a solution mixture of starch, NaAlg, and ceftriaxone sodium formed the aqueous phase, and liquid paraffin formed the oil phase. A nonionic surfactant (Tween 80) and emulsion stabilizer (magnesium stearate) were used to stabilize the system, whereas glutaraldehyde was used as a cross-linking agent. As the main aim of this study was to provide a formulation that is inexpensive to make an affordable in low- and middle-income countries such as Asian countries, plasma concentration-time profiles of 250 mg ceftriaxone-loaded polymeric blend after oral administration were simulated for Asian subcontinent populations using a generic physiologically based pharmacokinetic (PBPK) modelling software and compared to the *in vitro* experimental data.

EXPERIMENTAL

Materials and Reagents

Sodium alginate was purchased from Sigma-Aldrich. Starch was obtained from Duksan pure chemicals. Tween 80 and glutaraldehyde 25% glutaraldehyde solutions were obtained from Aldrich Chemicals. Liquid paraffin and magnesium stearate were purchased from Merck. Sodium hydroxide was purchased from Uni-Chem and acetone from BDH Lab Chemicals. Concentrated HCl was obtained from BDH Analar (UK). Ceftriaxone sodium was purchased from Hamaz Pharmaceuticals, Ltd. (Multan).

Preparation of Starch-Sodium Alginate Microparticles

An emulsion crosslinking system was used to prepare the starch-sodium alginate (starch-NaAlg) microparticles. Briefly, a sodium alginate aqueous solution was prepared by heating deionized water to 60 °C and then NaAlg was added slowly under continuous stirring. Aqueous starch solutions were prepared separately. Both solutions were then mixed and stirred continuously for 30 min at 1000 rpm using a high-speed stirrer (Stuart series 600, Germany) to obtain a uniform polymer blend. Ceftriaxone sodium was dispersed in the polymer blend and thoroughly stirred for 45 min. The resultant drug-polymer blend was added to liquid paraffin (100 mL) containing tween-80 (2 mL) and magnesium stearate (0.5% w/v) to form H_2O in oil (w/o) emulsion. The resulting emulsion was stirred at 600 rpm for 45 min, then 2 mL 25% glutaraldehyde solution containing 1.5 mL distilled water was added, followed by addition of 1-2 drops of concentrated HCl. The mixture was stirred for 3 h to produce microparticles. The formed microparticles were filtered, using a 0.45 µm filter, and washed frequently with methanol and acetone to remove any excess surfactant and unreacted glutaraldehyde. Finally, the particles were dried overnight at 45 °C in an oven and stored in a desiccator until further analysis. The details of the prepared microparticle formulations and their compositions are listed in Table 1.

Table 1. Composition of starch-NaAlg microparticles prepared.

Formulation code (sample)	Ceftriaxone Sodium (mg)	Polymer Loading (%)	Starch/NaAlg Ratio	Glutaraldehyde Concentration (mL)
AS-1	100	1	50:50	2
AS-2	100	1	70:30	2
AS-3	100	1	80:20	2
AS-4	100	1	60:40	2
AS-5	100	1	90:10	2
AS-6	100	2	50:50	2
AS-7	100	2	70:30	2
AS-8	100	2	80:20	2
AS-9	100	2	60:40	2
AS-10	100	2	90:10	2
AS-11	100	3	50:50	2
AS-12	100	3	70:30	2
AS-13	100	3	80:20	2
AS-14	100	3	60:40	2
AS-15	100	3	90:10	2

Characterization of Starch-Sodium Alginate Microparticles

An FT-IR study was performed to estimate the drug-polymer interactions and crosslinking to form a polymeric network. The spectra were recorded for pure starch, NaAlg, pure drug (ceftriaxone Na), blank, and ceftriaxone-loaded microparticles. Using 2 mg of the sample, KBR discs were prepared, and scanning was performed in the range of 4000-500 cm^{-1} at a resolution of 4 cm^{-1}.

XRD studies were performed using an XRD machine (Model JDX 3532, Japan) to evaluate the effect of microencapsulation on the crystallinity of the drug. XRD patterns of all the samples, including ceftriaxone sodium crystals, NaAlg, blank microparticles, and ceftriaxone sodium

loaded microparticles, were recorded using CuKa radiation, Ni-filtered, 60 kV voltage, 50 mA current and 1° per minute scanning rate over a 10° to 40° diffraction angle.

The surface characteristics of the unloaded and ceftriaxone sodium-loaded microparticles were examined using scanning electron microscopy (SEM) (*JEOL; Japan*). Microparticles were deposited on carbon tape and then coated with gold using an SPI sputter module in a high-vacuum evaporator, and the SEM images were recorded at 20 kV.

To study the phase change and weight loss of ceftriaxone sodium, starch, NaAlg, blank microparticles, and ceftriaxone sodium-loaded microparticles were studied using thermal analysis (TGA, *USA* model Q600 Series). Before loading the samples, both the reference and sample pans were tarred. After sample loading, the oven was heated to 450 °C at a rate of 15 °C/min under a nitrogen atmosphere.

All formulations were further examined for their particle size distributions using a Zetasizer instrument (*Zetasizer* Nano-series ZEN3600, Malvern *instrument* Ltd. with the software DTS-Nano, Kingdom).

Quantitative Analysis of Ceftriaxone Sodium in Starch –NaAlg Microparticles

The quantity of ceftriaxone sodium encapsulated within the microparticles was determined using an ultraviolet spectrophotometer (UV-1601, Shimadzu). Dry ceftriaxone sodium-loaded microparticles (50 mg) were ground, allowed to hydrate, and fully swell in 100 mL phosphate buffer (pH 7.4) for 24 h. After 24 h of soaking, the dispersion was filtered using a 0.45 µm filter, and suitable dilutions were made. The absorbance of ceftriaxone sodium was measured at 240 nm, and the concentrations were calculated using a calibration curve.. To calculate percent yield of the microparticles, recovered dry drug-loaded microparticles were weighed and divided by the total amount of ingredients (drug and polymer) used initially in the preparation.

Formulas below were used for calculations of encapsulation efficiency, drug loading and percentage yield respectively.

$$\text{Microencapsulation efficiency}\% = \frac{\text{Drug actual amount in microparticles}}{\text{Theoretical quantity of drug in microparticles}} \times 100 \quad (1)$$

$$\text{Drug loading}\% = \frac{\text{amount of drug in microparticles}}{\text{amount of microparticles}} \times 100 \quad (2)$$

$$\% \text{ yield} = \frac{\text{Recovered total quantity of microparticles}}{\text{Quantity of drug and polymers}} \times 100 \qquad (3)$$

Rheological (Micrometric) Properties of The Microparticles

The micrometric properties of the formed particles were evaluated using different techniques, such as angle of repose, bulk and tapped density, Hausner's ratio, and compressibility index. Details of the methods for conducting these tests are provided in the Supplementary Information.

In Vitro Drug Release and Release Kinetic Studies

The in vitro release pattern of ceftriaxone sodium from the loaded microparticles was determined using *USP* dissolution apparatus-2 (paddle apparatus) connected to an autosampler (Pharma Test PTFC 2, Germany). The specific weight of microparticles (corresponding to 250 mg of ceftriaxone sodium) was filled in a cellulose dialysis bag, tied to a paddle by silk thread, and dipped in a dissolution flask containing 900 mL of dissolution medium to sink. The dissolution studies were performed for 12 h at pH 1.2 (0.1 M HCL solution) and 7.4 (phosphate buffer solution). Both dissolution systems, at pH 1.2 and 7.4, were individually rotated at speed of 100 rpm under 37 °C temperature. At predetermined time intervals (0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 9 and12 hours), 3 mL samples were taken from the middle of the flask for UV analysis. The withdrawn volume was replaced with fresh buffer solution. All samples were filtered through a 0.22 µm membrane and then analyzed for ceftriaxone sodium content using a UV visible spectrophotometer (UV-1601 Shimadzu) at 240 nm.

For the analysis of ceftriaxone sodium release kinetics, the zero-order, first-order, and Higuchi models were applied. The Korsmeyer-Peppas model was used to determine the drug release mechanism of the controlled drug delivery system. For the detailed mechanism of the drug release, preliminary 60% of release data were fitted to the Korsmeyer-Peppas equation below:

$$\frac{M1}{M\infty} = k_3 t^n$$

where M_1 and $M\infty$ are the released drugs at times 0 and ∞. where, n is the diffusion constant. In spherical matrices, if n < 0.43, diffusion is Fickian (case -1). If 0.43 < n < 0.85, it is a non-Fickian, and if n > 0.85, the zero-order release mechanism dominates (case -2). Dissolution data were determined using DDSolver® software.

Physiologically Based Pharmacokinetic Studies

Simulations for ceftriaxone Na pharmacokinetics were performed using PK-Sim® and Mo-Bi® version 9.1 (Bayer Technology Services GmbH, Leverkusen, Germany). Physicochemical properties of the drug that were entered into PK-Sim® are molecular weight and solubility, which are 554.6 g/mol and 0.5 g/l respectively. The integration of these physicochemical properties helps creating a distribution model. First, PBPK model was developed in an individual Asian human and then extended to a population of 100 individuals. The demographic characteristics of the individuals are presented in Table 2. An oral dose of 250 mg microparticles administered once was selected as the administration protocol. Particle dissolution was selected as the formulation, and the particle size distribution was monodisperse. The thickness of the unrestricted water layer of the particles was 20 µm, whereas the particle size was 10 µm. Parameter optimization was performed in MATLAB® using the Levenberg Marquardt algorithm in the parameter identification toolbox. Clearance simulation for the drug was also conducted using a biliary clearance value of 0.070 ml/min/kg previously entered into the PK-Sim®.

Table 2. Demographic characteristics of Asian population used for pharmacokinetic modelling of Ceftriaxone Na.

Demographic characteristics (Asians)	Range	Mean
Age (years)	30-40	34
Weight (kg)	65-70	68
Height (cm)	166-170	168
BMI	24.01-24.09	24.07

RESULTS AND DISCUSSION

Synthesis and Characterization of Starch-NaAlg Microparticles

A microparticulate system comprising starch and sodium alginate was developed for the sustained delivery of ceftriaxone sodium, combining two release approaches: biodegradation and time-dependent release. Microparticles were prepared using the emulsion cross-linking method. Both polymers are anionic, hydrophilic, and biodegradable, which means they can be mixed in all fractions to prepare microparticles by the emulsion crosslinking method. Under acidic conditions, the aldehydic groups of glutaraldehyde react with both the OH groups of starch and NaAlg to form hemiacetal and acetal rings during the crosslinking reaction. Hemiacetal and acetal ring formation between polymers and glutaraldehyde has been previously reported by *El-Tahalway et al*(El-Tahlawy et al., 2007; Malafaya et al., 2006)[20], [21][20], [21]. Various factors, such as polymer and crosslinker concentrations, stirring speed, and stirring period, can significantly affect the morphology and particle size, in addition to the incorporation efficiency of the formed microparticles.

The FT-IR spectra of the starch, sodium alginate, ceftriaxone sodium, blank starch-sodium alginate microparticles, and ceftriaxone-loaded microparticles are shown in Fig. 1(a). FT-IR spectrum of starch (black band) indicates two peaks at 673.21 cm^{-1} and 1007.29 cm^{-1}, which are for C-H bend and C=O bond stretching(Vedha Hari et al., 2012)[22][22]. Sodium alginate (red band) showed an intense peak at 1582.79 cm^{-1} owing to the bending vibration of the free carboxyl group. The peak at 1560 cm −1 is due to the asymmetric stretching of carboxylate ion intermolecular interaction through the formation of hydrogen bonds between COO 2 groups in sodium alginate and OH groups in starch[24]. Peaks at 2925.04 cm^{-1} and 3500 cm^{-1} are due to the C-H stretching and OH groups of NaAlg(Çaykara et al., 2005)[23][23]. The FT-IR spectrum of the polymer microparticles without drug showed that the aldehyde groups of glutaraldehyde reacted with the OH groups of NaAlg under acidic conditions to form an acetal ring. The crosslinking of starch with glutaraldehyde is due to the nucleophilic addition of the OH group to the carbonyl group to form a hemiacetal linkage(Agarwal et al., 2015; He et al., 2012)[24], [25][24], [25]. FT-IR spectrum of ceftriaxone sodium (green band) has displayed characteristic absorption peaks around 1740.67 cm^{-1} and 1404.14 cm^{-1} belong to the β lactam C=O stretching vibration and oxime C=N stretching vibration. Other peaks at about 1609.02 cm^{-1} and 1035.23 cm^{-1} indicate the presence of C=O and C–O stretching vibrations,

respectively(Manimekalai et al., 2017; Shah et al., 2014)[13], [26][13], [26]. In ceftriaxone FT-IR spectra peaks in region of 1400 to 1450 are due to CH2 deformation and C=C stretching, but these are diminished in formulation FT-IR due to formation of intramolecular chains of polymers in the same region[29]. The spectrum of ceftriaxone-loaded microparticles (orange band in Fig. 1(a)) showed that the starch–sodium alginate mixture did not change the chemical properties of the drug. Thus, the presence of distinct peaks other than starch and alginate confirmed the presence of the drug encapsulated in the polymeric particles in a stable form(Chattopadhyay et al., 2015; Saravanan & Rao, 2010)[27], [28][27], [28]. Peaks at 3495 and 3600cm-1 in Alginate and starch are peaks of the natural polysaccharide hydroxyl groups, 3600-3200 cm-1. Disappearance of these peaks in both drug-loaded and unloaded microparticles suggest the intermolecular interaction through the formation of hydrogen bonds between COO-2 groups in sodium alginate and OH- groups in starch[32].

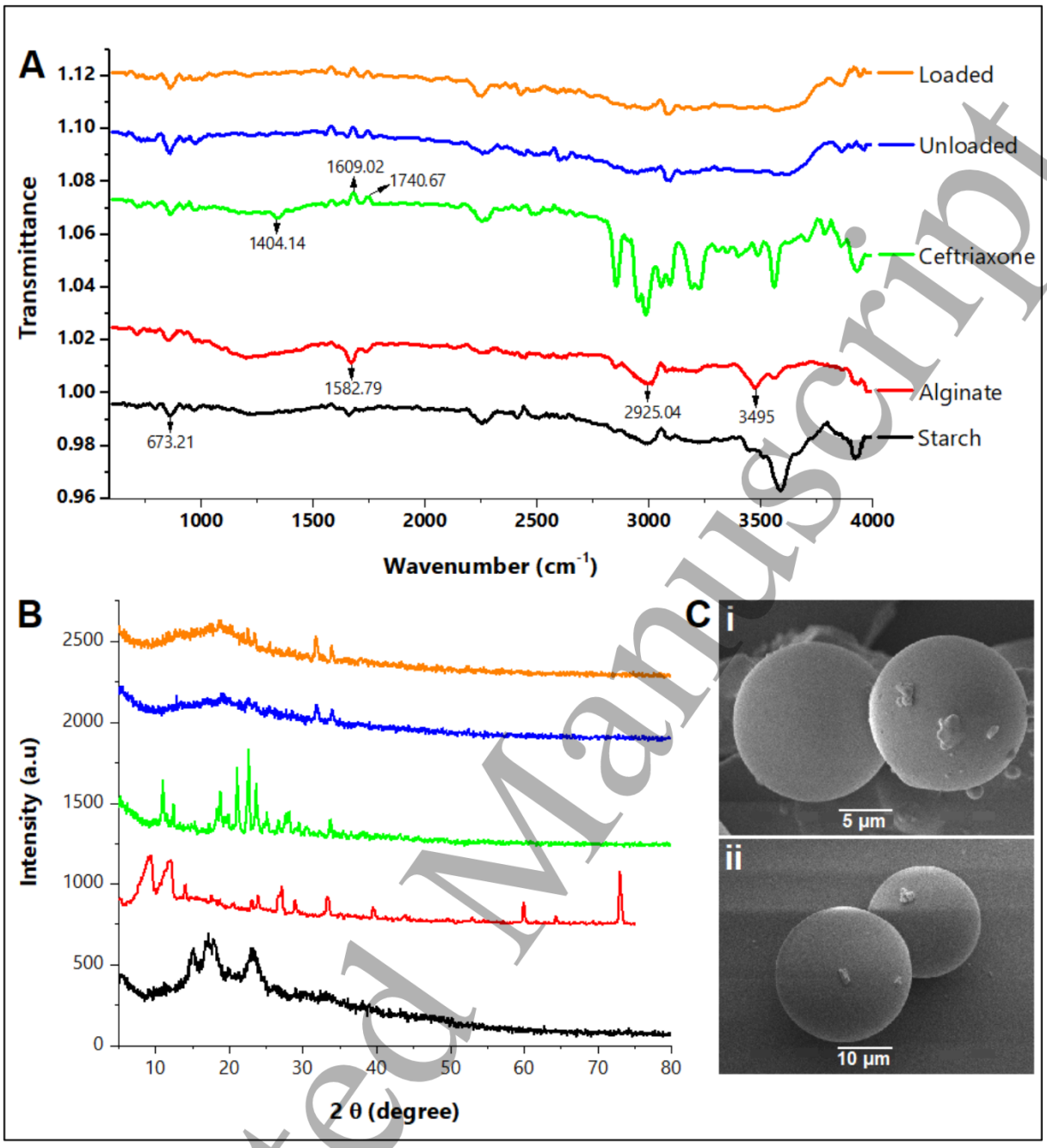

Figure 1. A is FT-IR spectra of starch (black), sodium alginate (red), pure ceftriaxone sodium (green), blank starch-sodium alginate microparticles (blue) and ceftriaxone sodium loaded microparticles (orange). B is PXRD Spectra of the same materials in A, in the same order. C, i and ii are SEM images of starch-sodium alginate microparticles before drug loading and after drug loading respectively. Scale bars for Ci and Cii are 5 µm and 10 µm respectively.

The PXRD patterns of starch, sodium alginate, ceftriaxone sodium, unloaded microparticles, and ceftriaxone Na-loaded microparticles are shown in Fig. 1(b). The diffraction peak of NaAlg at approximately 13.47° is the characteristic peak of the hydrated crystal-like structure, but its intensity decreased after the formation of the microparticles(Wang et al., 2010)[29][29]. The diffraction pattern of the unloaded microparticles indicated a wide hump in the region of

11°-24°. The consistency of the crystal structure might be due to the egg-box areas along the direction of the NaAlg chain, which is the aggregation direction. This shows complex formation between the polymers and the decreased crystalline behavior of NaAlg(Fontes et al., 2013)[30][30]. The XRD pattern of pure ceftriaxone sodium peaks was confirmed at diffraction angles of 13°, 15°, 24°, 25° indicating that it was crystalline in nature(Mio Tangea, Yusuke Hattorib, Makoto Otsukab, Miyako Yoshidaa, Jun Haginakaa & Takahiro Uchidaa, 2013)[31][31]. The PXRD pattern of ceftriaxone Na-loaded microparticles confirmed the absence of the characteristic drug peak, implying a change or variation in the crystalline nature of the drug after encapsulation into the microparticles(Yeo & Park, 2004; Yu et al., 2008)[32], [33][32], [33]. The presented PXRD is for formulation A-15 that having composition of 90 % starch and 10 % sodium alginate. From literature we come to know that Starch has semi-crystalline granular structure, so peaks at 30 and 32° in the X-ray analysis found in the microparticles can be assigned to presence of starch crystals in low fraction as the peaks are not intense[38].

The scanning electron micrographs, Ci and Cii in Fig. 1, of the unloaded and drug-loaded microparticles, respectively, show that the synthesized starch-sodium alginate microparticles are spherical in shape, with smooth surfaces and no clusters. Ceftriaxone Na-loaded microparticles appeared to have larger particles than unloaded microparticles. This is because of the incorporation of the drug into the polymeric matrix, which limits the interior shrinkage of the polymers. In previous studies, microparticles with planar and non-porous surfaces were reported to be effective for extended dissolution(Das & Senapati, 2008)[34][34]. The NaAlg microparticles synthesized in this study were also discrete (Fig. S1 in the Supplementary Information), which is a good flow criterion.

The DSC-TGA curves of the polymers, unloaded microparticles, drug-loaded microparticles, and pure drug are shown in Fig. 2. Thermal analysis of sodium alginate indicates that 1st endothermic peak at approximately 100 °C was due to the evaporation of moisture (Fig. 2(a)). The second and third exothermic peaks at 238.1°C and 368.3 °C to 394.8 °C belong to the degradation of the biopolymer and the formation of the corresponding carbonates(Devi & Kakati, 2013)[35][35]. DSC curve of pure starch is demonstrated in Fig. 2(b) where the first endothermic peak at around 27.21 °C is attributed to moisture discharge, then weight loss (25 to 50% of the original weight) occurs in the second step within the temperature range of

317.26 °C to 331.35 °C, which is an indicator of start of starch decomposition. The endothermic peak at 533.75 °C shows that the decomposition of starch is rather slow, and 23% of its weight remains. This could be due to the partial replacement of the OH groups of the starch by bulkier groups, leading to a decrease in hydrogen bonds and the formation of stronger crystals(Biliaderis et al., 1986; Thiebaud et al., 1997)[36], [37][36], [37]. DSC-TGA thermogram of unloaded microparticles is shown in Fig. 2(c), which indicates that particles remained physically stable up to 533.77°C and weight remained as a residue (P41.5%). Comparing the results of the microparticles with those of the pure polymers, it can be concluded that the polymeric matrix is more thermally stable than the individual polymers(Baimark et al., 2010)[38][38]. The DSC—TGA curve of pure ceftriaxone sodium presented in Fig. 2(e) shows that the melting point of pure ceftriaxone was greater than 155 °C. The first endothermic peak is at 25.15 °C where the weight is 100%. Ceftriaxone sodium showed 27 to 30% weight loss during the 2nd stage temperature range (between 256 and 260 °C), which is attributed to the dehydration process. The last endothermic peak appears at 533.79 °C where weight loss was 46 .71% which is an indication of complete decomposition of the drug (Manimekalai et al., 2017)[13][13]. The DSC-TGA curve of ceftriaxone sodium-loaded starch–NaAlg is shown in Fig. 2(d). In contrast to the pure drug, no sharp peak was observed for ceftriaxone sodium, indicating that the drug was uniformly distributed throughout the polymeric matrix. The endothermic peak at 335 °C was attributed to the polymorphism and melting of ceftriaxone sodium. Additionally, this matrix remained stable up to 534 °C with a total loss of weight of approximately 73.6% (Devi & Kakati, 2013; Fontes et al., 2013)[30], [35][30], [35].

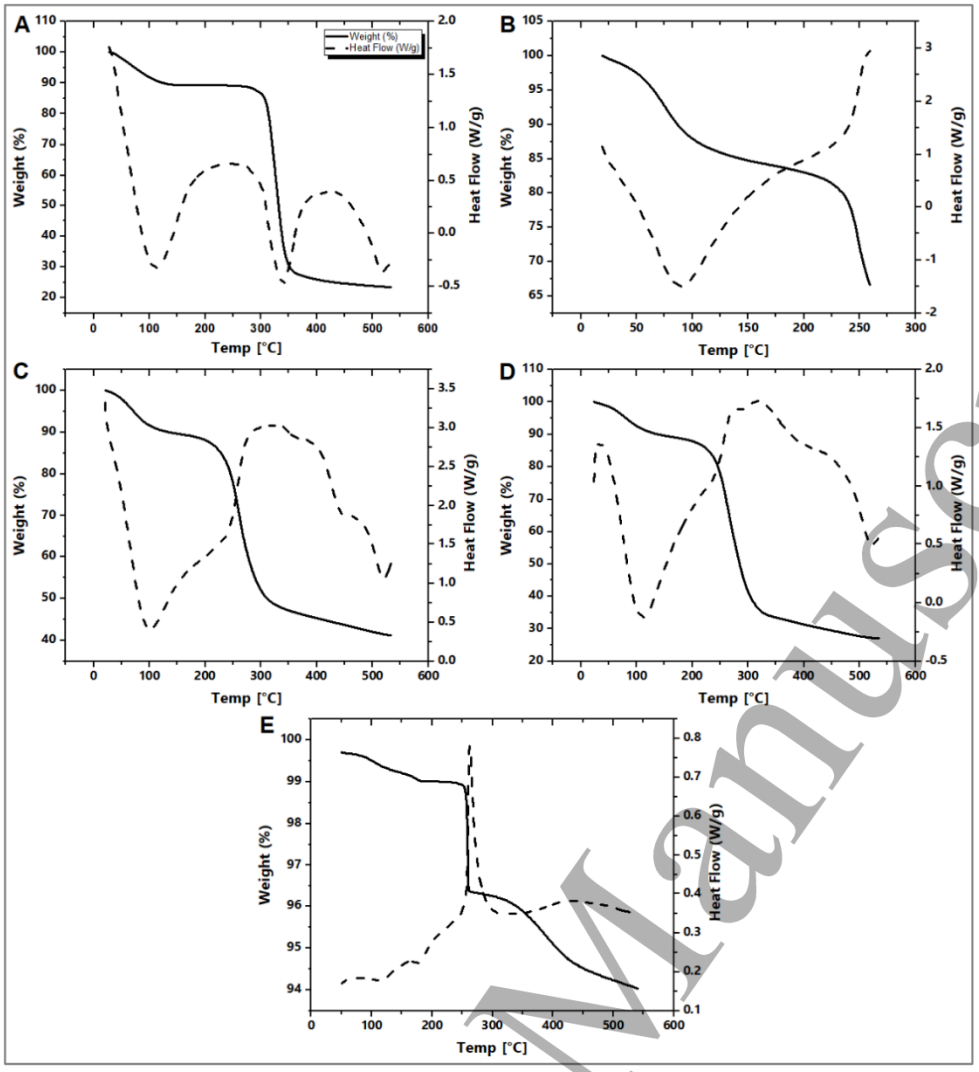

Figure 2. DSC and TGA of starch (A), sodium alginate (B), unloaded microparticles (C), Ceftriaxone sodium-loaded microparticles (D) and pure Ceftriaxone Na (E).

Analysis of particle sizes using a Zetasizer showed that the average size of blank starch–sodium alginate microparticles was smaller than that of drug-loaded microparticles (Table S.1 in the supplementary information). This means that drug loading slightly increased particle size, which can be associated with the attachment of ceftriaxone sodium molecules, which is suggested to reduce the open volume spaces in the polymer complex, hindering the interior shrinkage of microparticles(N. Patel et al., 2016a)[39][39]. Because the particle size can also be inversely affected by the stirring speed, the size of the particles decreases as the stirring speed increases. Various batches of formulations AS-1 to AS-15 (Table 1) were fabricated at various stirring speeds (500rpm, 600rpm and 800rpm), to establish the optimal stirring speed for preparing microparticles. Microparticles with optimal shape and size were formed at 600 rpm. When the agitation speed was maintained below 600 rpm, the polymer solution was not

dispersed homogenously, resulting in agglomeration(Kurkuri & Aminabhavi, 2004; Raza et al., 2016)[40], [41][40], [41].

Drug Incorporation, Loading Efficiency and Percent yield of Microparticles

The drug loading (%) and incorporation efficiency (%) of all formulations are shown in Fig. 3(a). A linear relationship was observed between the incorporation efficiency of ceftriaxone sodium and the concentration of the polymer. Drug loading (%) and incorporation efficiency (%) increased from 39% to 85% and 37% to 89%, respectively, as polymer concentration increased from 1% to 3% (Table 1). The formulation having sodium alginate and starch 60:40 ratio, showed the maximum entrapment efficiency and drug loading. A similar trend was observed in previous studies(J. Balasubramaniam et al., 2007; Liu et al., 2007)[42], [43][42], [43]. On the other hand, the obtained percent yield (%) of the ceftriaxone sodium loaded microparticles was in the range of 45 to 94% (Fig. 3(b)), where AS-1 possessed the lowest % yield (45%) and AS-15 the highest % yield (94%). The maximum yield was achieved when the polymer concentration increased. The highest % yield was obtained at polymer concentration of 3%. Similar percentages have been previously reported by *Manimekalai et al* and *Patel et al*(Manimekalai et al., 2017; N. Patel et al., 2016b)[13], [44][13], [44].

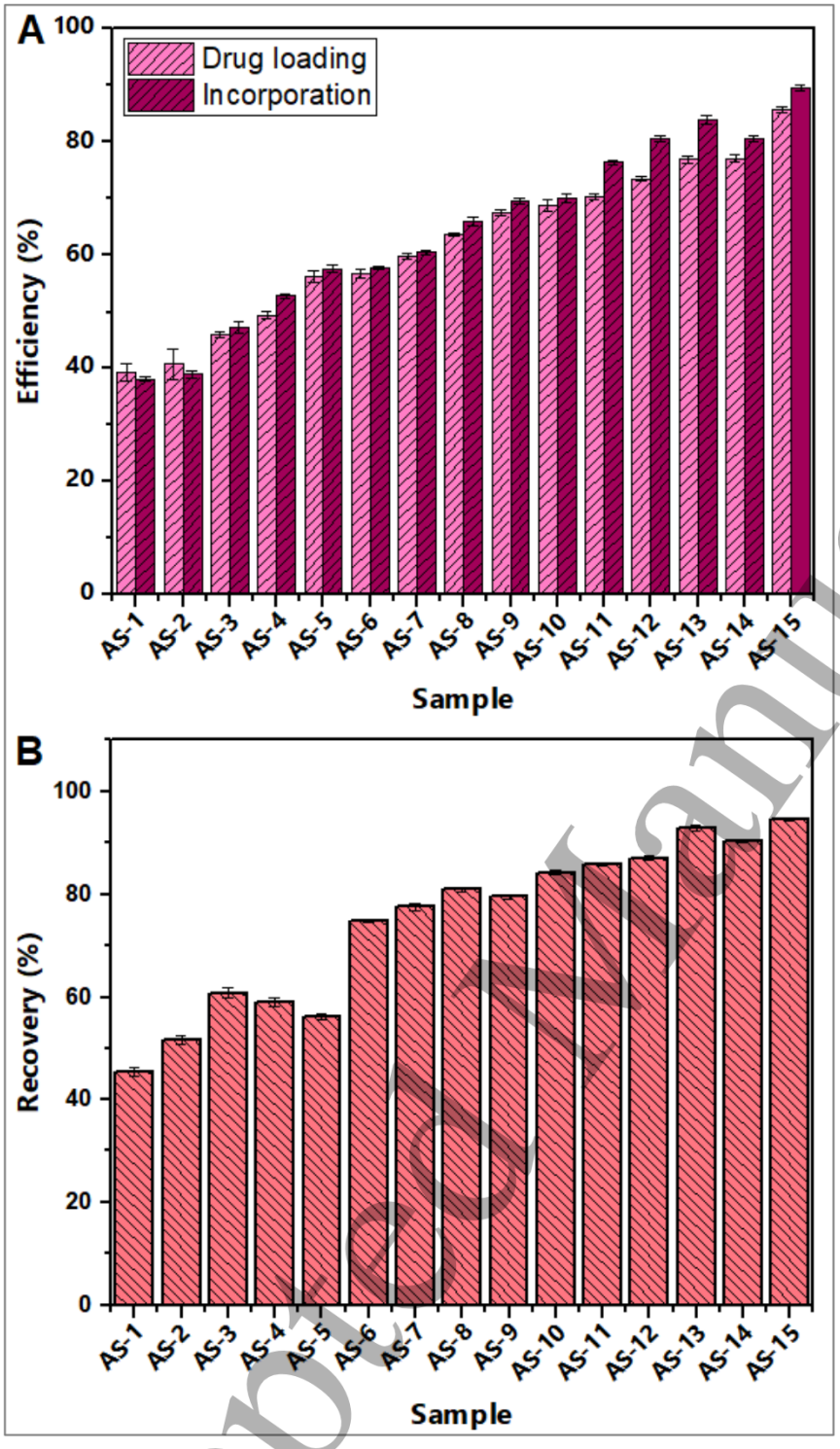

Figure 3. A is percent of drug loading and entrapment efficiency. B is percent recovery (yield) of microparticles. Error bars represent standard deviation in both A and B.

Micrometric Properties of The Microparticles

The micromeritic properties (tapped and bulk density, angle of repose, Hausner's ratio, and Carr's index) of the ceftriaxone sodium-loaded microparticles were calculated and are shown in Table S. 3 (Supplementary Information). These results show that the ceftriaxone-loaded starch-NaAlg microparticles are good flow materials. The flow properties of microparticles are essential for the homogeneity of the dosage form. AS-3 and AS-13 showed the best flow properties among all other formulations owing to their low Hausner's ratio and compressibility index(Ajay Semalty, Mukesh Pandey, 2014; Iffat, 2016)[45], [46][45], [46].

In-Vitro Drug Release and Release Kinetics

The release of ceftriaxone Na from the microparticles was evaluated at two different pH values: acidic (pH 1.2) and basic (pH 7.4). Release profile from a representative formulation "formulation AS-15'' is shown in Fig. 4. A distinct difference in the drug release data between pH 1.2 and pH 7.4 is observed. At acidic pH, ceftriaxone sodium released was below 20%. This could be ascribed to the reduced swelling of the polymeric matrix at an acidic pH, which results in decreased matrix permeability, thus leading to limited drug diffusion. An increase in the drug release to more than 85% was observed at pH 7.4 in 12 hours. These results indicate that the interpolymeric interaction that exists in the "gel" form that retards the release of the drug in the dissolution medium at pH 1.2, while at pH 7.4, microparticles swell and the gel gradually breaks down to release the drug. Thus, the characteristic feature of ceftriaxone Na release from the polymeric matrix was a biphasic release pattern. The early fast release within the first 15 minutes is stated as the 'burst' effect, which is then followed by slower 1st order release kinetics. At a basic pH, deprotonation of sodium alginate causes breakdown of the polymeric complex and completes the release of the drug as soluble ions. The in vitro release profile from all other formulations showed a similar pattern to that of AS-15 (Figure S.2 in the supplementary information). As ceftriaxone Na is acid-labile, these results suggest that it can be successfully administered orally when incorporated into the polymeric matrix used in this study, with minimal degradation.

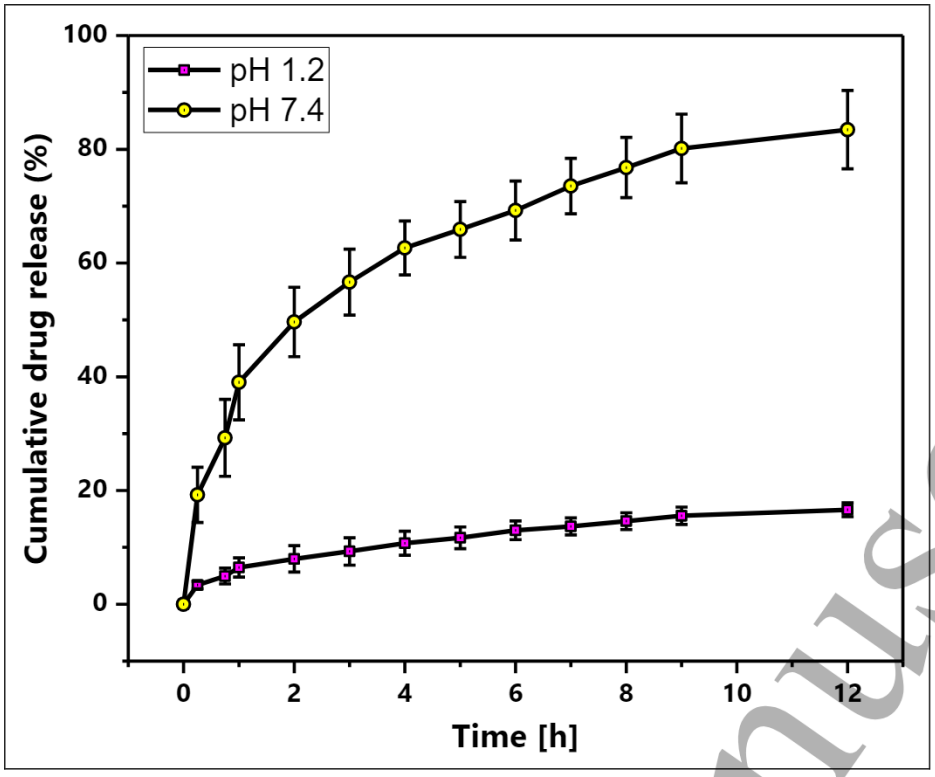

Figure 4. Release profile of ceftriaxone sodium from formulation AS-15 at different pH levels. Error bars represent standard deviation.

To evaluate the order of drug release from formulations AS-1 to AS15, various kinetic models were applied. The value of R^2 in zero-order and first order for all tested formulations ranged from 0.85 to 0.91 and 0.95 to 0.988 respectively as demonstrated in Table 3. The comparison of R^2 values for both the zero- and first-order models reveals that all the formulations follow first-order kinetics. R^2 for the Higuchi model and Hixson-Crowell ranged from 0.96 to 0.98 and 0.94 to 0.97 respectively. The AS-2 formulation exhibited the highest fit/correlation for the first-order and Higuchi models. Based on these results, it can be predicted that all formulations follow first-order release kinetics and the Higuchi model, as suggested by the diffusion processes for the drug. The value of n in the Korsmeyer-Peppas model for all formulations was lower than 0.45, which means that all formulations followed Fickian diffusion(Desai, 2007; Raza et al., 2018)[47], [48][47], [48].

Table 3. Regression coefficients of model fitting of release data.

Formulation code	Zero Order (R^2)	First Order (R^2)	Higuchi Model (R^2)	Hixson Crowell Model (R^2)	Korsemeyer-Peppas Model (n)	Release Order
AS-1	0.88	0.96	0.97	0.94	0.36	Fickian
AS-2	0.91	0.988	0.987	0.97	0.43	Fickian
AS-3	0.86	0.96	0.96	0.94	0.32	Fickian
AS-4	0.88	0.97	0.97	0.95	0.36	Fickian
AS-5	0.90	0.98	0.98	0.96	0.40	Fickian
AS-6	0.89	0.98	0.98	0.96	0.37	Fickian
AS-7	0.90	0.98	0.98	0.97	0.39	Fickian
AS-8	0.85	0.96	0.96	0.94	0.29	Fickian
AS-9	0.91	0.98	0.987	0.97	0.37	Fickian
AS-10	0.88	0.97	0.97	0.95	0.32	Fickian
AS-11	0.87	0.97	0.97	0.963	0.31	Fickian
AS-12	0.88	0.95	0.97	0.942	0.29	Fickian
AS-13	0.88	0.95	0.97	0.942	0.29	Fickian
AS-14	0.87	0.97	0.97	0.96	0.32	Fickian
AS-15	0.88	0.98	0.97	0.968	0.32	Fickian

Pharmacokinetic Analysis

Plasma concentration versus time profiles for ceftriaxone 250 mg microparticles administered orally (n =1 and n = 100) were created using the PBPK model and are shown in Fig. 5, whereas the pharmacokinetic parameters are displayed in Table 4. Plasma concentration versus time profiles showed consistent absorption of the drug in microquantities, thus supporting the micro-release pattern of the microparticles.

In both, the individual and population simulation results, ceftriaxone plasma concentration reached about 1E-11 µmol/l (1.131E-12 µg/ml) after 24 h and stayed steady at this level. This concentration is less than that achieved when the drug is administered to humans via i.v or i.m injections (Table S.4 in the supplementary information). This could be attributed to couple of reasons. First, the used ceftriaxone concentration in the simulation is less than that used in the i.v or i.m delivery reported. Second, it is well known that physiological barriers in the GIT can contribute to losing some of the drug during its passage, therefore, we suggest that the amount of ceftriaxone given orally using the formulation proposed here to be increased in order to match i.v and i.m plasma levels reached in previous reports.

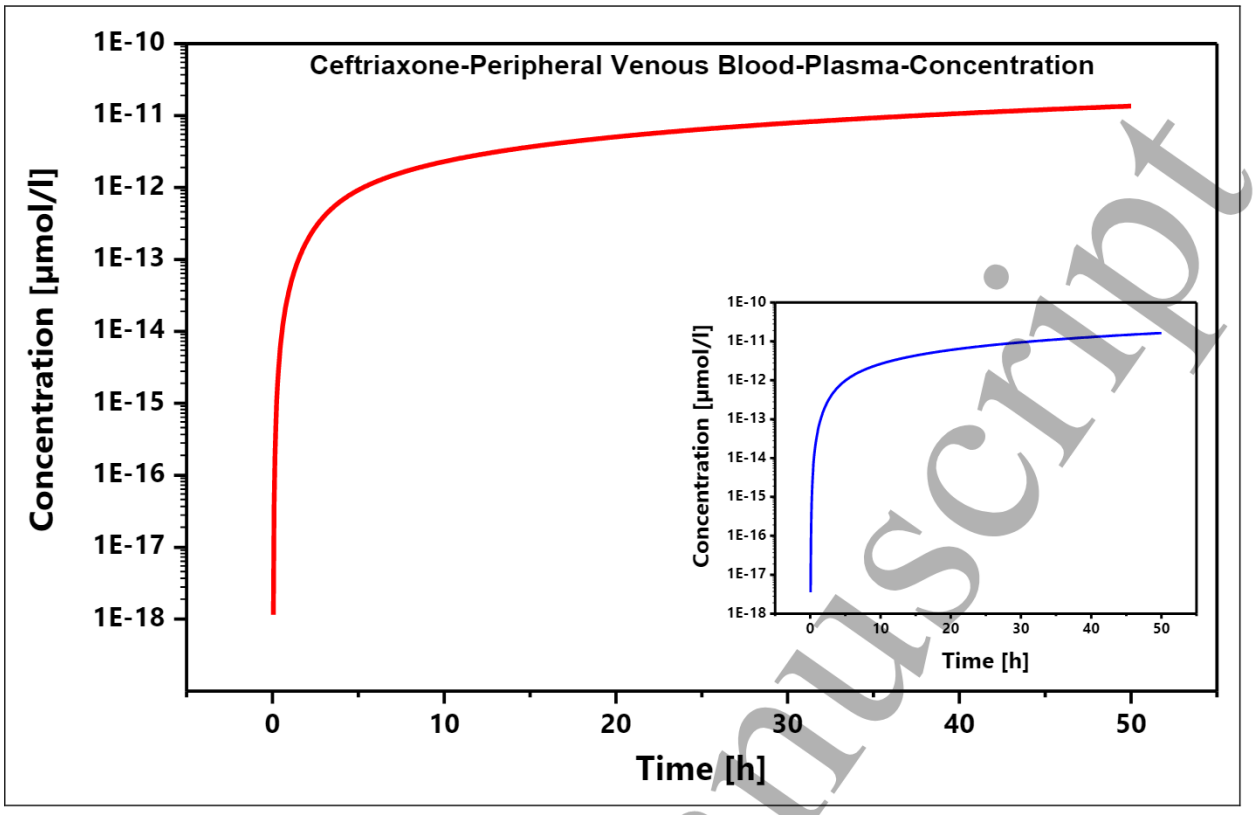

Figure 5. Plasma concentration time profile after oral administration of ceftriaxone 250mg microparticles in Asian individual (n = 1). (Inset) is plasma concentration time profile after oral administration of ceftriaxone 250mg microparticles in Asian population (n = 100).

Clearance simulation is also a part of the PBPK model, where specified values governing the clearance or metabolic aspects of drugs could be added, apart from built-in values known as the PK-Sim standard. The reported plasma clearance of ceftriaxone was 15.4 ml/min (at an IV dose of 500 mg (I. H. Patel et al., 1981)[49][49]), and the biliary clearance in PK-Sim® was 0.070 ml/min/kg(Grime & Paine, 2013)[50][50]. According to the available mass balance information in literature, 30 to 60% of administered ceftriaxone is eliminated by biliary excretion, while the rest is through renal excretion in its unchanged form. Therefore, ceftriaxone is not an ideal metabolic candidate.

Table 4. Pharmacokinetic parameters of ceftriaxone 250 mg microparticles.

PK parameter	Units	Mean (n = 1)	Mean (n =
Cmax	µmol/l	1.35×10^{-11}	1.64×10^{-11}
Tmax	hr	50	50
AUC	µg*min/l	1.98×10^{-8}	2.34×10^{-8}
C_tend	µmol/l	1.35×10^{-11}	1.64×10^{-11}
Fraction absorbed	-	3.15×10^{-12}	-

CONCLUSION

In this study, microparticles were fabricated using an emulsion cross-linking method. Microparticles were prepared using starch and sodium alginate as a polymeric blend and glutaraldehyde as a crosslinking agent. The highest % yield was obtained at 3% polymer concentration. FT-IR analysis of the microparticles indicated the formation of a polymeric network and successful entrapment of ceftriaxone sodium. XRD and DSC analyses of the microparticles indicated that the lower crystallinity of ceftriaxone Na resulted in the higher thermal stability of the prepared microparticles. A size study using a Zetasizer showed that all microparticles were in the micrometric range, and drug loading caused a slight increase in the particle size. Higher drug release in a basic medium (pH 7.4) was observed when compared to drug release in an acidic medium (pH 1.2). In vitro drug release studies indicated that microparticles can protect the therapeutic drug from the acidic environment of the stomach and can favorably deliver it at pH 7.4. The release kinetics of the ceftriaxone sodium-loaded microparticles followed first-order and Fickian diffusion. The reached plasma level through the simulated administration of ceftriaxone using PBPK modelling appeared to be less than that of the i.v and i.m administration reported in clinical research. This was attributed to different factors such as the low dose used here and the biological barriers that can aggravate drug loss when administered orally. Our results suggest that PBPK software tool combined with biorelevant dissolution tests could be suitable for formulation development and analysis of unexpected in vivo results.

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Conflict of Interest

The authors declare no conflict of interest.

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