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An oral dosage form of ceftriaxone sodium using enteric coated sustained release calcium alginate beads

Darshan Lalwani
University of Toledo

A Thesis

entitled

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by

Darshan Lalwani

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutical Sciences with Industrial Pharmacy Option

_____
Jerry Nesamony, Ph.D., Committee Chair

_____
Sai Hanuman Sagar Boddu, Ph.D, Committee Member

_____
Youssef Sari, Ph.D., Committee Member

_____
Patricia R. Komuniecki, PhD, Dean
College of Graduate Studies

The University of Toledo

May 2015

An Abstract of

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Purpose: Ceftriaxone (CTZ) is a broad spectrum semisynthetic, third generation cephalosporin antibiotic. It is an acid labile drug belonging to class III of biopharmaceutical classification system (BCS). It can be solvated quickly but suffers from the drawback of poor oral bioavailability owing to its limited permeability through gastro-intestinal epithelia. Hence, it cannot be given orally to a patient. The objective of the present research work was to develop and evaluate enteric coated calcium alginate beads of CTZ for oral delivery. **Methods**: Dispersions of CTZ and low viscosity sodium alginate with various co-polymers such as, acacia gum (AG), sodium carboxymethyl cellulose (CMC), guar gum (GG), iota-carrageenan gum (ICG), and xanthan gum (XG) were utilized to prepare CTZ entrapped beads by ionotropic external gelation method using calcium chloride as a cross linking agent. **Results**: The formulated beads were evaluated for entrapment efficiency (E.E.) using HPLC. The highest E.E. obtained was 69.3 ± 0.2% in a formulation containing sodium CMC as a co-polymer with sodium alginate. The entrapment optimized batch was further coated either with a single coat or a double coat of enteric polymers such as cellulose acetate phthalate (CAP), Opadry®

enteric, Eudragit® L-100, and Eudragit® S-100. Finally, the enteric coated polymeric beads were analyzed for entrapment efficiency, in-vitro drug release in simulated gastric fluid (pH 1.2) and simulated intestinal pH (pH 6.8), swellability, drug- polymer interaction using fourier transform- infrared spectroscopy (FT-IR), thermal stability using thermal gravimetric analysis (TGA), surface morphology using scanning electron microscopy (SEM), and surface roughness using atomic force microscopy (AFM). The entrapment optimized batch was a double coated formulation with a first coat of Eudragit® S-100 and a second coat of Eudragit® L-100 and the observed E.E. was 60.7 ± 0.5%. Swellability of the coated beads was desirable around 173% as compared to 290% of the uncoated beads. Release of drug from the beads of optimized batch followed first order kinetics. The FTIR spectral showed that there was no significant interaction between the drug and polymers. The TGA thermograms demonstrated thermal stability of the formulation. The SEM micrographs indicated smooth surface of the coated beads over uncoated ones. AFM micrographs demonstrated surface characteristics of the formulated beads and average roughness (Ra) values observed for uncoated and coated beads affirmed the SEM results.

Conclusion: In conclusion, sustained release beads of CTZ were successfully prepared using sodium alginate and sodium CMC. Parameters such as polymer concentration, calcium chloride concentration, coating polymer concentration, stirring speed and cross-linking time significantly affected entrapment efficiency, surface morphology and in-vitro drug release. The use of sodium alginate, sodium CMC and other coating polymers decreased the drug release behavior in gastric conditions to certain degree but sustained the drug release at intestinal pH

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List of Abbreviations

AA.....acacia gum

AFM.....atomic force microscopy

BCS.....biopharmaceutical classification system

CAP.....cellulose acetate phthalate

CC .....crystalline cephalexin

CEZ.....cefazolin sodium

CMC.....carboxymethyl cellulose

EE.....entrapment efficiency

FT-IR.....fourier transform- infrared spectroscopy

GG.....guar gum

ICG.....iota-carrageenan gum

MOA .....mode of action

Ra .....average roughness

Rq.....root mean square roughness

SEM .....scanning electron microscopy

TGA .....thermal gravimetric analysis

XG.....xanthan gum

Chapter 1

INTRODUCTION

1.1 β- lactam antibiotics and their stability

1.1.1 Definition

The term *antibiotic* was first used by Selman Waksman in 1941 to describe any small molecule made by a microbe that antagonizes the growth of other microbes [1]. It can also be referred as a chemical compound produced by living cells, which has the capacity to inhibit the growth, in low concentrations, or to kill other microorganisms without any significant toxicity to the host [2] & [3].

The *β- lactam antibiotics* are a broad class of antimicrobial agents which include penicillins, cephalosporins and cephamycins (cephems), carbapenems and monobactams. As the class name indicates all compounds possess a four membered lactam ring in their chemical structure. β- Lactams act by inhibiting cell wall biosynthesis in the bacteria. They are the most widely used of all the antimicrobial classes because of their high efficacy, specificity, and availability of a wide range of derivatives [4, 5].

1.1.2 History

As understanding the success and failure of anything lies in its history, it's important to look into its origins and evolution [1]. Antibiotics have had an effect not only on the treatment of infectious diseases but also on society by changing morbidity and mortality [6].

The story of discovery of the antimicrobial properties of molds goes back to 3000 BC where Chinese scribes have mentioned the treatment of infected wounds with moldy soya beans [7]. But only after the discovery of *penicillium mold* (first modern era antibiotic) by Alexander Fleming in 1928, many other antibiotics were sought out [8].

The major outburst in development of semi-synthetic penicillins was observed after the isolation of 6-aminopenicillanic acid (6-APA), a key biosynthetic intermediate of penicillin by Beecham group, which was also an intermediate in Sheehan's synthetic route [9] of penicillin development. 6-APA itself has no biological activity but its acetylation with acid chlorides gives opportunity to produce many penicillin analogues [10].

In 1948, Brotzu isolated *acremonium strain* that produced two active β-lactams: Cephalosporin-N (a new type of penicillin) and Cephalosporin-C [11]. During the investigation of cephalosporin-C structure, Abraham and Newton found that ring system in it was much more stable than that of penicillin and this led them to isolate 7-aminocephalosporanic acid (7-ACA), which is analogous to 6-APA [7] & [12]. The identification of 7-ACA subsequently led to development of the semi-synthetic cephalosporins [13].

In the late 1960s, β-lactamase-mediated resistance to β-lactam antibiotics emerged as a significant clinical threat to these lifesaving drugs. This created a need to develop antibiotics that will either evade the bacterial enzymatic inactivation caused by β-lactamase or will inhibit β-lactamase so that the β-lactam antibiotics can reach the protein binding sites [14]. By 1976, the first β-lactamase inhibitors discovered were olivanic acids produced by the gram-positive bacterium *streptomyces clavuligerus*. But owing to its chemical instability and poor penetration into the bacterial cell, no further investigation was undertaken. Around the same time, Kahan et al. isolated thienamycin from *streptomyces cattleya*. Thienamycin was the first "carbapenem" and it served as the model compound for all carbapenems developed thereafter [15] & [16].

The monobactam antibiotics are synthetic monocyclic beta-lactam compounds. Aztreonam was the first marketed monobactam and is known for its activity against most aerobic gram-negative bacilli including *pseudomonas* aeruginosa [17] & [18].

1.1.3 Classification

β-lactam antibiotics can be classified based on their structure containing the common β-lactam ring. They can also be sub-classified according to their nature of origin or based on their improved stability.

The detailed classification is in Table 1.1.

Table 1.1: Classification of β- lactam antibiotics

(1) Penicillins	Natural penicillins	Penicillin F, Penicillin K, Penicillin X, Penicillin G (benzyl penicillin).	
	Semi-synthetic penicillins	Improved oral bioavailability	Phenethicillin, Ampicillin, Amoxicillin, etc.

		Improved stability to staphylococcal penicillinase	Methicillin, Nafcillin, Cloxacillin.
		Beta-lactamase stable penicillins	Amdinocillin Carbenicillin, Piperacillin, Ticarcillin, Temocillin.
(2) Cephems	Natural cephems	Cephalosporins	Cephalosporin C
		Cephamycins	Cephamycin A, Cephamycin B, Cephamycin C.
	Semi-synthetic cephems	First generation cephalosporins	Cephaloridine, Cephalothin, Cefazolin Cefadroxil, Cephalexin, etc.
		Second generation cephalosporins	Cefamandole, Cefaclor, Ceforanide, Cefuroxime, Cefuzonam.
		Third generation cephalosporins	Cefotaxime, Ceftazidime, Ceftriaxone, Ceftibuten, Cefixime, Cefdinir.
		Fourth generation cephalosporins	Cefpirome, Cefepime.
		Anti-MRSA cephalosporins	Ceftobiprole, Ceftaroline.
		Semi-synthetic Cephamycins	Cefoxitin, Cefotetan, Cefuperazone, Cefmetazole.
(3) Carbapenems	Natural	Thienamycin	

	carbapenems	Asparenomycin	Asparenomycin A, Asparenomycin B, Asparenomycin C.
		Carpetimycin	Carpetimycin A, Carpetimycin B.
	Synthetic carbapenems	Meropenem, Ertapenem, Doripenem, Sanfetrinem.	
	Combination carbapenems (synthetic)	Imipenem/Cilastatin Panipenem/ Betamipron	
(4) Monobactams	Natural monobactams	Aztreonam, and Carumonam.	
	Synthetic monobactams	Oximonam, Tigemonam, Pirazmonam, etc.	

*****Note: The information listed in this table is based on the following references [7, 19, 20]

1.1.4 Mechanism of action of β- lactam antibiotics

β- lactam antibiotics show bactericidal effects. The targets are cell wall-synthesizing enzymes (penicillin-sensitive enzymes), which are commonly detected by their ability to bind covalently radiolabeled penicillin (hence referred to as penicillin binding proteins (PBPs)) [21]. They bind to the target and form a complex that lacks catalytic activity, and this makes organisms unable to mature and divide, which is ultimately lethal.[22]

The actual mechanism behind the disruption of the bacterial cell wall is inhibition of transpeptidase enzyme that catalyzes the cross-linking reaction of D-alanyl peptides on peptidoglycan strands which provides rigidity to the cell wall. Later, it was found that penicillins have structural resemblance to the terminal D-alanyl-D-alanine residues of the short peptides that are involved in crosslinking and hence they can easily bind to PBPs.

Cell walls of gram-negative bacilli have thin mucopeptide layer which is loosely cross-linked through adjacent diaminopimelic acid-D-alanine residues, whereas gram-positive organisms possess thick cell wall in which the mucopeptide is tightly cross-linked. Hence, it is recommended to consider these two groups separately when looking for the mode of action (MOA) of β- lactam antibiotics [23, 24].

MOA for Gram-Negative Bacilli:

In these organisms, transpeptidation reaction predominates. The bacterial cell wall synthesis is inhibited mainly by two mechanisms based on the category and the concentration of the drug employed. Drugs such as cephalexin, cephradine and low concentration of many other β- lactam agents interfere with bacterial division and gives rise to long filaments. After a particular length of 50 μm or more, these filaments stop elongating and eventually die either through rupture at discrete points in the cell wall, or by degeneration process that takes place due to unbalanced growth. On the other end, drugs like thienamycin, clavulanic acid and some other compounds produce surface changes leading to the formation of spheroplasts, which can easily lyse after osmolar pressure changes produced by osmotically active compounds like sucrose or sodium chloride [24].

MOA for Gram-Positive organisms:

Gram-positive organisms generally have fewer PBPs than gram-negative bacteria. So, though transpeptidase reaction occurs but it is a secondary event that leads to the death of cells. Several evidences have pointed out that autolysis plays a major role in death of gram-positive bacteria when exposed to most β- lactam antibiotics. According to autolysis theory, lysis is promoted by bacteria's own autolytic enzyme (autolysins).

Autolysins are bacteriolytic enzymes that digest the cell-wall peptidoglycan of the bacteria that produce them [25]. Moreover, it was observed that when gram-positive bacteria are exposed to β- lactam antibiotics, affected cells release few cell wall components like lipoteichoic acid which is a natural inhibitor of autolytic activity. Hence, loss of this inhibitor allows lysis to proceed unhindered [24].

1.2 Cephalosporins

1.2.1 Definition

Cephalosporins are semisynthetic antibacterial agents derived from cephalosporin-C, which is produced by *cephalosporium acremonium*. The antibacterial activity of cephalosporin-C was found to be very low, but modification of its hydrolysis product 7-aminocephalosporanic acid (7-ACA) has given rise to many semi-synthetic cephalosporins. They possess a β- lactam ring fused to a 6-membered dihydrothiazine ring, which is resistant to hydrolysis by penicillinase. They are among the safest and the most effective broad-spectrum bactericidal agents and hence, they are the most prescribed of all antibiotics [7, 26-28].

1.2.2 Classification of cephalosporins

Cephalosporins can be classified into various generations based upon their spectrum of activity against gram-positive and gram-negative bacteria and their ability to treat the disease as shown in Table 1.2.

Table 1.2: Use of cephalosporin antibiotics and their antimicrobial spectrum

Generation	Disease treatment	Antimicrobial spectrum
First generation	Skin and soft tissue infections, Respiratory tract infections, and Urinary tract infections.	- They show better activity against gram positive bacteria and less for gram-negative. - Active against escherichia coli, klebsiella pneumonia, proteus mirabilis, streptococcus species, methicillin sensitive staphylococcus aureus. - Inadequate activity against pseudomonas, enterococcus species, hemophilus influenza, moraxella catarrhalis.
Second generation	Respiratory tract infections and Urinary tract infections.	- Exert improved activity for H. influenza, M. catarrhalis and enterobacter than that of first generation agents. - Inactive against pseudomonas, methicillin-resistant staphylococci and enterococci.
Third generation	Lower Respiratory tract infe ctions, Acute bacterial otitis media, Skin and skin structure infections, Urinary tract infections, Uncomplicated gonorrhea, Pelvic inflammatory disease, Intra-abdominal infections, Meningitis, and Surgical prophylaxis.	- Expanded coverage for gram-negative organisms compared with first- and second-generation cephalosporins and has less gram-positive activity. - Active against S. aureus, H. influenza, klebsiella pneumonia, E.coli, enterobacter aerogenes, proteus mirabilis and serratia marcescens. - Few agents show activity for pseudomonas aeruginosa.
Fourth generation	Pneumonia (moderate to severe), Urinary tract infections (including pyelonephritis), Skin and skin structure infections, and Intra-abdominal infections.	- These exhibit activity against both gram-negative and gram-positive bacteria. - Active against E.coli, pseudomonas aeruginosa, streptococcus and klebsiella pneumonia, concurrent bacteremia, enterobacter species, proteus mirabilis, S. aureus (methicillin-susceptible strains only).

Fifth generation	Complicated skin and skin-structure infections.	- Broad spectrum with activity against resistant gram-positive bacteria especially for meticillin-resistant staphylococcus aureus (MRSA) and community-acquired pneumonia (CAP).

***** Note: The information listed in this table is based on the following references [4, 29-31]

1.2.3 Cephalosporins and their dosage forms

Cephalosporins are available in both oral and parenteral dosage form. But, both the dosage forms have developed differently. Oral cephalosporins include many molecules belonging to initial three classes, whereas parenteral cephalosporins are available in all the classes [32]. Based on the dosage forms available in U.S. market, cephalosporin antibiotics have been listed in Table 1.3.

Table 1.3 Cephalosporin antibiotics and their dosage forms available in market.

Generation	Drugs	Available dosage forms			
		Tablets	Capsules	Suspensions (reconstituted)	Parenteral
First generation	Cefadroxil	✓	✓	✓	
	Cephalexin	✓	✓	✓	
	Cefazolin				✓
Second generation	Cefaclor	✓	✓	✓	
	Cefprozil	✓		✓	
	Cefuroxime	✓		✓	✓
	Cefotetan				✓
	Cefoxitin				✓
Third generation	Cefdinir		✓	✓	
	Cefditoren	✓			
	Cefixime	✓	✓	✓	
	Cefpodoxime	✓		✓	
	Ceftibuten		✓	✓	
	Ceftriaxone				✓
	Ceftazidime				✓
	Cefotaxime				✓

Fourth generation	Cefepime				✓
Fifth generation	Ceftaroline				✓

***** Note: The information listed in this table is based on the following references [33, 34]

1.2.4 Stability of cephalosporins

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods. Thus, stability studies are widely used by pharmaceutical industry in deciding the storage conditions, suitable packaging material, shelf life, and expiration date of the product [35].

1.3 Stability of first generation cephalosporins

1.3.1 First generation oral cephalosporins

Cefadroxil and cephalexin are the first generation oral cephalosporins available in U.S. market under the brand names Duricef and Keflex respectively [36]. The antibacterial spectrum of first generation oral cephalosporins is similar to first generation parenteral cephalosporins, but their activity is moderately low. Cefadroxil is mainly available in 3 forms- amorphous anhydrous, crystalline anhydrous and monohydrous form whereas cephalexin exists in 2 forms- crystalline cephalexin (CC) and non-crystalline cephalexin (NC).

1.3.1a Stability in solid state

Letho et al. [37] determined the stability ranges of the anhydrous forms of cefadroxil at different relative humidity at 25ºC and investigated the associated solid state reactions. Thermal analysis for all the different types of cefadroxil were performed using DSC

under nitrogen flow of 50 ml/min and the heating rate of 10ºC/min with temperature range of 10 to 250ºC. Results showed that no crystallization occurs even in amorphous sample and all the forms are stable up to 90% RH. Isothermal microcalorimetry study was performed using thermal activity monitor (TAM) isothermal heat –conduction micro-calorimeter for measuring the heat flow from or to the sample. The humidity to which the sample was exposed was controlled stepwise or as a continuous ramp using Thermometric's RH perfusion unit within the range of 0% to 100% RH and the air flow was maintained at 115 ml/hr. Observations indicates that both the anhydrous forms (amorphous anhydrous and crystalline anhydrous) transfer to monohydrate form at high relative humidity.

Cephalexin capsules are recommended to be stored at 15ºC to 30ºC, whereas tablets should be stored at 20ºC to 25ºC [33].

1.3.1b Stability in aqueous solution

Diaconu et al. [38] looked in to stability of cefadroxil in aqueous reconstituted suspensions for 10 days at 2ºC to 8ºC in absence of light, and at room temperature (24ºC) in presence of natural light. Their result indicated that the suspension was stable for 9 days, when stored in the refrigerator in the absence of light and was stable for 7 days, when stored at room temperature in the presence of light.

Nascimento et al. [39] studied the stability of cephalexin in aqueous solution at 20ºC, 2ºC, and -20ºC, and their result showed that the cephalexin was stable for 14 hr, 3.4 days, and 19 days respectively.

1.3.2 First generation parenteral cephalosporins

Cefazolin sodium (CEZ) is the only first generation parenteral cephalosporin available in U.S. market under the brand names Ancef and Kefzol [36]. It crystallizes mainly in 3 forms- α form, β form, and amorphous form. The chemical stability of the drug in different solid forms varies due to their differences in physico-chemical characteristics.

1.3.2a Stability in solid state

Zhang et al. characterized the difference in stability of α-form, dehydrated α- form and amorphous form utilizing thermal gravimetric analysis (TGA) and high performance liquid chromatography (HPLC). Using TGA, they found that α form of CEZ had the best stability whereas the amorphous form had the least stability at the conversion (decomposition) level of 1%. HPLC results showed that the dehydrated α-form was relatively more stable than amorphous form [40].

1.3.2b Stability in aqueous solution

Gupta [41] attempted to check chemical stability of CEZ after reconstituting in 0.9% sodium chloride solution and storing in polypropylene syringes for pediatric use. His results showed that prepared reconstituted solution stored in polypropylene syringes, was stable for 7 days when stored at room temperature and for 22 days when stored at 5ºC. Bolos et al. [42] evaluated stability of CEZ in various commonly used intravenous infusion fluids and details are in Table 1.4. They also found that the use of buffer (pH 5.6) had only a little influence on the stability of solution.

Table 1.4: Stability of Cefazolin in different i.v. infusion fluids

Intravenous infusion fluid	Result	Reason
Mannitol Solution	Accelerated degradation of drug	Due to rate accelerating effect of the hydroxyl compounds on the drug.
Ringer's solution and lactated Ringer's solution	Accelerated degradation of drug	Due to a catalytic effect of the Ca^{2+} ions present in both solutions.
Glucose solution	At low temp: Stability decreases with increase in glucose concentration. At high temp: Reverse effect is observed.	-
Solutions containing levulose and sodium lactate	No significant changes in stability as compared to a saline solution	-

***** Note: The information listed in this table is based on reference [42]

Robinson et al. [43] evaluated the stability of CEZ in heparinized and non-heparinized dextrose containing peritoneal dialysis solution with 4 different concentrations of icodextrin at 38ºC for four days (96 hr). They found that CEZ was stable for at least 48 hr in low concentrations in both heparinized and non-heparinized dialysate fluids. When in high concentrations, CEZ is stable only in non-heparinized dialysate, and not in heparinized dialysate.

Carone et al. [44] investigated the stability of CEZ in various diluents at frozen temperatures. Their results indicated that frozen solutions of CEZ in Water for Injection USP, 5% Dextrose Injection USP and 0.9% Sodium Chloride Injection USP are stable up to 26 weeks, when stored at -10ºC or -20ºC in glass containers.

1.3.2c Stability in packaging material

Donnelly [45] determined the physical and chemical stability of CEZ reconstituted with sterile water of injection (SWFI) and stored in polypropylene syringes or dilute solutions

of CEZ prepared with 5% dextrose in water or normal saline solution and stored in polyvinylchloride (PVC) minibags. He found that CEZ when stored in polypropylene syringes or PVC minibags was stable for up to 30 days when stored at 5ºC with protection from light, followed by an additional 72 hr at 21ºC to 25ºC with exposure to light.

Xu et al. looked for the stability of CEZ in AutoDose infusion system bag. Their results indicated that it remains stable for 30 days, when stored at 4ºC and for 7 days, when stored at room temperature (23ºC) [46].

1.4 Stability of second generation cephalosporins

1.4.1 Second generation oral cephalosporins

Cefaclor, cefprozil and cefuroxime are the second generation oral cephalosporins available in U.S. market under the brand names Ceclor, Cefzil and Ceftin respectively[36]. Cefuroxime is also available in parenteral dosage form.

Cefuroxime Axetil and cefaclor exists in both crystalline and amorphous forms. Cefaclor is available in crystalline form as cefaclor monohydrate. The amorphous cefaclor is postulated to be a cause of rapid initial degradation of the formulations [47]. The degradation pathways of cefaclor in solid state might be different than in solution[48].

1.4.1a Stability in solid state

Cefuroxime tablets are recommended to be stored at 15ºC to 30ºC, whereas oral suspension powders prior to reconstitution are to be stored at 2ºC to 30ºC.

Cefprozil is recommended to be stored at 20ºC to 25ºC and excursions permitted are 15ºC to 30ºC [33].

Patel et al. [49] looked for stability of cefaclor extended release matrix tablet in different environmental conditions like 25ºC/60%RH, 30ºC/65% RH and 40ºC/75% RH.

No changes were observed in physical properties and % drug content of the tablets indicating it to be stable at different conditions.

Medenecka et al. [50] evaluated the effect of relative air humidity on the stability of cefaclor as powder for capsule, and in slow release tablets. They concluded that the degradation of cefaclor at relative air humidity (RH > 50%) is first order autocatalytic reaction dependent on the substrate concentration, while at 0% RH, it is first order reaction dependent on the substrate concentration.

1.4.1b Stability in aqueous solution

Stability of reconstituted suspension of cefuroxime is about 10 days, when stored at 2ºC to 8ºC. Whereas, reconstituted suspension of cefprozil is stable for 14 days, when stored under refrigeration [33].

Kuksal et al. [51] studied the stability of cefaclor in suspensions. In 3 months of study period, no significant changes occurred in physical and rheological characteristics in suspension reconstituted with water: propylene glycol (20:80).

1.4.2 Second generation parenteral cephalosporins

Cefotetan, cefoxitin, and cefuroxime are the third generation parenteral cephalosporins available in U.S. market under the brand names Cefotan, Mefoxin and Zinacef

respectively [36]. Cefoxitin exists in two forms- amorphous and crystalline. Amorphous form of cefoxitin sodium is considerably less stable than its crystalline form [52].

1.4.2a Stability in solid state

Cefuroxime is a mixture of diastereomers A and B. The degradation of diastereomers of cefuroxime occurring at 0% RH of the ambient air is a reversible first order reaction for both the forms, while that occurring at (RH > 25%) for amorphous form, and (RH > 50%) for crystalline form in humid air is an autocatalytic first order reaction related to substrate concentration [53, 54]. Hence, cefuroxime in intact vials is recommended to be stored at 15ºC to 30ºC and should be protected from light. Cefoxitin powder, prior to reconstitution is recommended to be stored between 2ºC to 25ºC and exposure to temperatures above 50ºC should be avoided [33].

1.4.2b Stability in aqueous solution

IV Infusion of cefuroxime in normal saline solution or 5% Dextrose solution is stable for 24 hr at room temperature, 7 days under refrigeration, and 26 weeks when frozen. After freezing, thawed solution is stable for 24 hr at room temperature or 21 days under refrigeration [33].

Hecq et al. [55] looked for stability of cefuroxime (1.5%) prepared in 5% Dextrose solution and stored in polyolefin bags frozen either individually (group A) or in one package (group B) for 98 days at -20ºC. Further, this solutions were thawed using microwaves at 270 watts (light cycle) or 800 watts (hard cycle), and finally were stored at 4ºC. Their results indicated that freezing in packaging and thawing at high wattage

decreases the stability of cefuroxime. The optimum method to conserve the solution is to freeze the individual containers followed by light cycle microwave thawing. This solution then can be stored for 23 days at 4ºC.

Stability of cefotetan reconstituted with 5% dextrose injection and stored in minibags was evaluated at different temperatures. It was observed that the reconstituted solutions were stable at room temperature, at 4ºC and at -20ºC for 2 weeks (336 hr) [56].

Gupta et al. [57] investigated the stability of cefotetan in 0.9% sodium chloride injection and 5% dextrose injection at room temperature, at 5ºC and at -10ºC. Their results showed that cefotetan was stable at room temperature for 2 days, at 5ºC for at least 41 days and at -10ºC for at least 60 days. In solutions kept at room temperature and at 5ºC, change in pH was observed. Hence, it better to follow manufacturer's recommended expiry time of 24 hr, when stored at RT and 4 days, when stored at 5ºC.

Gupta et al. [58] also studied the stability of cefoxitin at different temperatures. Their results indicated that cefoxitin solution was stable at 24ºC for 24 hr and at 5ºC for at least 13 days.

Oberholtzer et al. [52] looked for the stability of cefoxitin sodium in water (pH 5 to 7), and found that cefoxitin loses about 10% of its activity in 2 days at 25ºC.

O'Brien et al. [59] evaluated the stability of cefoxitin in common i.v. infusion fluids. They found out that cefoxitin is stable in the aqueous solutions for 40 hr at room temperature, for 30 days at 5ºC, and at least 30 weeks at -20ºC. They concluded that the stability of cefoxitin was independent of the concentrations and containers used.

1.4.2c Stability in Packaging material

Cefuroxime is compatible with ADD-Vantage vials and Premix Galaxy plastic containers. In ADD-Vantage vials, when joined but not activated, vials are stable for 14 days, but once activated, they are stable for 24 hr at room temperature or 7 days under refrigeration. Premix Galaxy plastic containers should be stored at -20ºC. Thaw container at room temperature or under refrigeration. Thawed solution is stable for 24 hr at room temperature or 28 days under refrigeration.

Cefoxitin is compatible with Duplex containers. It is recommended to store the unactivated containers at 20ºC to 25ºC and extrusions permitted are 15ºC to 30ºC, but it should not be frozen. After activation, solution is stable for 12 hr at room temperature and 7 days under refrigeration [33].

1.5 Stability of third generation cephalosporins

1.5.1 Third generation oral cephalosporins

Cefditoren, cefixime, cefpodoxime, ceftibuten and cefdinir are the third generation oral cephalosporins available in U.S. market under the brand names Spectracef, Suprax, Vantin, Cedax and Omnicef respectively [36].

1.5.1a Stability in solid state

Venugopalarao et al. [60] made an attempt to formulate hydrogel tablets of cefditoren pivoxel using sodium alginate and carbopol and checked its stability in different environmental conditions. The tablets were sealed in aluminum package and kept in humidity chambers maintained at 30 ± 2ºC, 65 ± 5% RH and 40 ± 2ºC, 75 ± 5% RH for 3

months. Their result indicated that tablets in both the conditions were stable at least for 60 days.

Paul et al. [61] formulated gastro-retentive drug delivery system for cefixime trihydrate and performed its accelerated stability studies. The formulated tablets were sealed in aluminum packaging and kept in humidity chamber at 40ºC, 75% RH for three months. The results indicated that the tablets were stable enough for various physical parameters like % drug release, friability, hardness, and % drug content.

Mooney et al. reported that the anhydrous form of cefixime was less stable than the trihydrate at elevated temperatures (56ºC to 70ºC). Koda et al. confirmed that cefixime trihydrate become unstable on storage below the critical humidity, owing to its transformation to anhydrous form by removal of its water of crystallization.

Hence, it is recommended that the humidity in package is maintained at an equilibrium level [62].

Haritha et al. [63] formulated cefixime dry emulsion using propylene glycol, organic filler, surfactant and sweetening agent and evaluated its stability at 45ºC, 75 ± 5% RH for 3 months. The emulsion was analyzed for drug entrapment and % drug release for 3 months, and no variation in results were observed.

Senthilkumar et al. [64] formulated tablets of cefpodoxime proxetil by wet granulation method using various hydrophilic and hydrophobic excipients. The accelerated stability study was conducted for the tablets at 40ºC for 6 months. Their results indicated that the tablets were physically stable, and drug content was around 85% at the end of the study.

1.5.1b Stability in aqueous solution

Cefixime powder for suspension prior to reconstitution, should be stored at 20ºC to 25ºC and after reconstitution, for 14 days at room temperature or under refrigeration, Cefpodoxime proxetil granules for suspension are recommended to be stored at 20ºC to 25ºC, and after reconstitution, suspension can be stored in the refrigerator for 14 days. Ceftibuten capsules and powder for suspension before reconstitution should be stored at 2ºC to 25ºC. The reconstituted suspension is stable for 14 days, when stored under refrigeration (2ºC to 8ºC).

Cefdinir powder for suspension, both prior and after reconstitution is recommended to be stored at 20ºC to 25ºC. The reconstituted suspension is stable for 10 days [33].

1.5.2 Third generation parenteral cephalosporins

Ceftriaxone, cefotaxime, and ceftazidime are the third generation parenteral cephalosporins available in U.S. market under the brand names Rocephin, Claforan, Fortaz/Tazicef respectively [36].

1.5.2a Stability in solid form

Ceftriaxone powder should be stored at room temperature (25ºC) or below and protected from light. After reconstitution, protection from normal light is not necessary [65]. Ceftazidime powder in intact vials should be stored at 20ºC to 25ºC, whereas cefotaxime powder is recommended to be stored at temperatures below 30ºC. Both the formulations are to be protected from light [33].

1.5.2b Stability in aqueous solution

Ceftriaxone can be reconstituted with various diluents except few containing calcium, such as Ringer's solution or Hartmann's solution, owing to its particulate forming tendency. Reconstituted solutions when stored at room temperature (25ºC) have different stability than that stored under refrigeration (2ºC to 8ºC). Stability of ceftriaxone in various diluents and concentrations at different temperatures is listed in table 1.5. Canton et al. [66] checked for stability of ceftriaxone at freezing temperatures as shown in table 1.6. The data represents shelf life (t_{90}) of ceftriaxone in various diluents at different temperatures.

Table 1.5: Stability of ceftriaxone in various diluents at different temperatures

Diluent	**Concentration (mg/ml)**	**Stored at room temperature (25ºC)**	**Stored under refrigeration (2ºC to 8ºC)**
Sterile Water for Injection	10 to 40	2 days	10 days
	100	2 days	10 days
	250, 350	24 hr	3 days
0.9% Sodium Chloride solution	10 to 40	2 days	10 days
	100	2 days	10 days
	250, 350	24 hr	3 days
5% Dextrose solution	10 to 40	2 days	10 days
	100	2 days	10 days
	250, 350	24 hr	3 days
Bacteriostatic water + 0.9% Benzyl alcohol	100	24 hr	10 days
	250, 350	24 hr	3 days
1% Lidocaine solution (without epinephrine)	100	24 hr	10 days
	250, 350	24 hr	3 days

***** Note: The information listed in this table is based on reference [65].

Table 1.6: Stability of Ceftriaxone at freezing temperatures

Diluent	Temperatures		
	-20ºC	**-40ºC**	**-70ºC**
Water	35 days	68 days	> 3 months
0.9% sodium chloride solution	34 days	60 days	> 3 months
5% dextrose solution	33 days	51 days	> 3 months

***** Note: The information listed in this table is based on reference [66].

Stability of cefotaxime sodium was checked after reconstitution with 0.9% Sodium chloride solution. It was observed that the reconstituted solution was stable for 18 days, when stored at 5ºC and was stable for 24 hr, when stored at 25ºC [67].

Gupta et al. [68] studied the stability of ceftazidime solutions in 5% dextrose injection and 0.9% sodium chloride injection. Their results indicated that the solutions were stable for 2 days, when stored at 25ºC, for 21 days in dextrose solution and for 28 days in normal saline solution, when stored at 5ºC and for 90 days, when stored at -10ºC.

Kodym et al. looked for stability of ceftazidime in 1% and 5% buffered eye drops, when stored at 4ºC and 20ºC. They observed that eye drops when stored at 20ºC, show 10% drug degradation on the 3rd day in all the 1% and 5% formulary versions. Eye drops when stored at 4ºC, 1% eye drops were stable from 18 to 27 days and 5% eye drops were stable from 12 to 21 days, depending on their composition [69].

1.5.2c Stability in Packaging material

Xu et al. [46] studied the stability of ceftriaxone sodium and ceftazidime when stored in AutoDose infusion system bags. Their results showed that ceftriaxone and ceftazidime were stable for 30 days and 7 days, when stored at 4ºC and for 5 days and 1 day, when stored at room temperature (23ºC) respectively.

Arsene et al. [70] looked for stability of ceftazidime in glass bottles and plastic containers like polypropylene bags and polyvinyl chloride (PVC) bags. Their observation indicated that glass bottles are the best option for storing the ceftazidime solution. In plastic containers, stability was found to be better in polypropylene bags than in PVC bags. Stability of cefotaxime sodium was evaluated when stored in polypropylene syringes. It was found that the reconstituted solutions stored in polypropylene syringes were stable for 18 days, when stored at 5ºC and were stable for 24 hr, when stored at 25ºC [67].

1.6 Stability of fourth generation cephalosporins

This class has drugs available only in parenteral dosage form.

1.6.1 Fourth generation parenteral cephalosporins

Cefepime hydrochloride is the only fourth generation parenteral cephalosporin available in U.S. market under the brand name Maxipime [36].

1.6.1a Stability in solid state

Cefepime powder in intact vials should be stored at 20ºC to 25ºC and should be protected from light [33].

1.6.1b Stability in aqueous solution

Cefepime is found to be compatible with 0.9% sodium chloride injection, 5% and 10 % dextrose injection, M/6 sodium lactate injection, 5% dextrose in 0.9% sodium chloride injection, lactated Ringers and 5% dextrose injection, Normosol-R and Normosal-M in

5% dextrose injection. All the solutions may be stored up to 24 hr at room temperature (20ºC to 25ºC) or 7 days under refrigeration (2ºC to 8ºC) [71].

Guyon et al. [72] looked into stability of cefepime, when reconstituted with 0.9% sodium chloride or with 5% glucose to get concentration of 8 mg/ml and stored in a polyethylene container at 24 ± 2ºC in daylight and 4 ± 2ºC in dark. The results indicated the drug was effectively stable in these solutions for 48 hr at 24 ± 2ºC in daylight, and 15 days at 4 ± 2ºC in dark.

Kodym et al. [73] evaluate the stability of cefepime in 1% and 5% buffered eye drops stored at 4ºC and 20ºC, protected from light. Results indicated that 10% degradation occurred after 21 to 28 days in 1% eye drops and after 18 to 21 days in 5% eye drops, when stored at 4ºC. Samples stored at 20ºC, and protected from light showed 10% degradation on the third day of storage regardless of composition of 1% and 5% eye drops.

1.6.1c Stability in packaging material

When stored in ADD-Vantage vials, cefepime was stable at concentrations of 10 to 40 mg/ml in 5% dextrose injection or 0.9% sodium chloride injection for 24 hr at controlled room temperature (20ºC to 25ºC) or for 7 days under refrigeration (2ºC to 8ºC) [71].

Guyon et al. [72] looked into stability of cefepime, when diluted with 0.9% sodium chloride or with 5% glucose to get concentration of 8 mg/ml and stored in a three layer laminate bag Clear-Flex® (polyethylene container) at 24 ± 2ºC in daylight and 4 ± 2ºC in dark. The results indicated the change in stability of drug over 48 hr at 24 ± 2ºC in daylight, or 15 days at 4 ± 2ºC in dark.

Trissel et al. [74] looked for stability of cefepime in different concentrations and temperature conditions, when stored in AutoDose infusion system bags and details are in Table 1.7.

Table 1.7: Stability of cefepime in AutoDose infusion system bags

Temperature condition	**Concentration prepared using 0.9% sodium chloride solution**	
	1 gm/100 ml	**4 gm/100 ml**
At room temperature (23°C)	Precipitation was observed at day-7.	Precipitation was observed at day-2.
Under refrigeration (4°C)	No precipitation was observed within 30 days of study period.	Precipitation was observed at day-7.

***** Note: The information listed in this table is based on reference [74].

1.7 Stability of fifth generation cephalosporins

Similar to fourth generation cephalosporins, this class has drugs available only in parenteral dosage form.

1.7.1 Fifth generation parenteral cephalosporins

Ceftaroline fosamil, a prodrug of the active metabolite, ceftaroline is the only fifth generation parenteral cephalosporin available in U.S. market under the brand name Teflaro [33]. It is unstable in the anhydrous form, and hence available as monohydrate form [75].

1.7.1a Stability in solid state

Ikeda et al. [76] studied the stability of both the amorphous and crystalline forms of ceftaroline. Their results indicated that the amorphous form was unstable, and the

residual % of drug was less than 90%, when stored at 8ºC for 4 weeks. It was also observed that amorphous form was stable only below -20ºC.

Generally, amorphous form of drug is preferred over crystalline form, owing to its improved solubility. Hence, they made an attempt to stabilize the drug with addition of inorganic salts, sugars and amino acids, but these were ineffective. Then, crystallization trials were performed where it was found that all crystalline batches were more stable than amorphous form. In addition to it, results indicated that increasing crystallinity improves the stability.

Moisture content analysis was conducted to check its influence on the long term stability. The obtained results showed that hydrate with approximately one molar equivalent of water was more stable then anhydrate containing more than one molar equivalent of water. In addition to this, results indicated that hydrolysis is promoted by the addition of water.

1.7.1b Stability in aqueous solution

Ikeda et al. [76] investigated the stability of ceftaroline in Britton-Robinson's buffer solutions (pH 3, 4, 5, 6, 7, 9), when stored at 25ºC. Results indicated that ceftaroline was most stable at pH 7 and its decomposition in aqueous solutions occurred under first order kinetics. The residual percentage in pH 7 buffer solution, stored at 25ºC for 24 hr was found to be more than 95%.

1.7.1c Stability in packaging material

Infusion bag: When checking for stability of constituted solution in infusion bag, it is recommended to use the solution within 6 hr, when stored at room temperature or within 24 hr, when stored under refrigeration (2ºC to 8ºC) [77].

Baxter® Mini-Bag Plus: Stability results of solutions of ceftaroline fosamil in concentrations ranging from 4 to 12 mg/ml prepared with 0.9% sodium chloride injection, and stored in Baxter® Mini-Bag Plus container indicated that the constituted solution can be stored for up to 6 hr at room temperature or for up to 24 hr, when stored under refrigeration (2ºC to 8ºC) [77].

1.8 Conclusion

In this review, an attempt was made to compile all the information on stability of cephalosporins. Based on the dosage forms available in U.S. market, each class of cephalosporins was differentiate into oral and parenteral dosage forms. As oral and parenteral cephalosporins developed differently from one another, characteristics like stability in solid state, stability in aqueous solutions, and stability in packaging materials were discussed for both of them in detail.

Overall, this review would help a researcher to choose an appropriate aqueous solution for the liquid dosage form, decide on shelf life and storage conditions for the solid and liquid dosage form, and finally select a compatible packaging material for the formulated cephalosporin product.

1.9 References

1. Clardy, J., M.A. Fischbach, and C.R. Currie, *The natural history of antibiotics.* Current biology, 2009. 19(11): p. R437-R441.
2. Fox, L.M., *Remington: The Science and Practice of Pharmacy.* 21st Edition, 2006: p. 1633.
3. *Dorland's Medical Dictionary for Health Consumers.* 2007.
4. Holten, K.B. and E.M. Onusko, *Appropriate prescribing of oral beta-lactam antibiotics.* American family physician, 2000. 62(3): p. 611-620.
5. Essack, S.Y., *The development of β-lactam antibiotics in response to the evolution of β-lactamases.* Pharmaceutical research, 2001. 18(10): p. 1391-1399.
6. Zaffiri, L., J. Gardner, and L.H. Toledo-Pereyra, *History of antibiotics. From salvarsan to cephalosporins.* Journal of Investigative Surgery, 2012. 25(2): p. 67-77.
7. Dougherty, T., *Antibiotic Discovery and Development:.... Vol. 1*. 2012: Springer Science & Business Media.
8. Alic, M., *"Antibitics" Gale Enclopedia of Cancer.* 2002.
9. Sheehan, J.C. and K.R. Henery-Logan, *The total synthesis of penicillin V.* Journal of the American Chemical Society, 1959. 81(12): p. 3089-3094.
10. Deshpande, A., K. Baheti, and N. Chatterjee, *Degradation of β-lactam antibiotics.* Curr. Sci, 2004. 87: p. 1684-1695.
11. Abraham, E., et al., *Cephalosporin N: a new type of penicillin.* 1953.
12. Foye, W.O., T.L. Lemke, and D.A. Williams, *Foye's principles of medicinal chemistry*. 2008: Lippincott Williams & Wilkins.

13. Hamilton-Miller, J., *Development of the semi-synthetic penicillins and cephalosporins.* International journal of antimicrobial agents, 2008. 31(3): p. 189-192.

14. Drawz, S.M. and R.A. Bonomo, *Three decades of β-lactamase inhibitors.* Clinical microbiology reviews, 2010. 23(1): p. 160-201.

15. Papp-Wallace, K.M., et al., *Carbapenems: past, present, and future.* Antimicrobial agents and chemotherapy, 2011. 55(11): p. 4943-4960.

16. Wise, R., *In vitro and pharmacokinetic properties of the carbapenems.* Antimicrobial agents and chemotherapy, 1986. 30(3): p. 343.

17. BREWER, N.S. and W.C. HELLINGER. *The monobactams*. in *Mayo Clinic Proceedings*. 1991. Elsevier.

18. Hellinger, W.C. and N.S. Brewer. *Carbapenems and monobactams: imipenem, meropenem, and aztreonam*. in *Mayo Clinic Proceedings*. 1999. Elsevier.

19. SHOJI, J.I., et al., *Asparenomycins A, B and C, new carbapenem antibiotics. II. Isolation and chemical characterization.* The Journal of antibiotics, 1982. 35(1): p. 15-23.

20. Kobayashi, F., et al., *Antimicrobial and beta-lactamase inhibitory activities of carpetimycins A and B, new carbapenem antibiotics.* Antimicrobial agents and chemotherapy, 1982. 21(4): p. 536-544.

21. Georgopapadakou, N., *Penicillin-binding proteins and bacterial resistance to beta-lactams.* Antimicrobial Agents and Chemotherapy, 1993. 37(10): p. 2045.

22. Cunha, B., *Third-generation cephalosporins: a review.* Clinical therapeutics, 1992. 14(5): p. 616.

23. Page, M.I., *The mechanisms of reactions of. beta.-lactam antibiotics.* Accounts of Chemical Research, 1984. 17(4): p. 144-151.

24. Greenwood, D., *Antibiotics of the beta-lactam group*. 1982: Chichester, UK; Research Studies Press, Div. of John Wiley & Sons.

25. Shockman, G. and J.-V. Höltje, *Microbial peptidoglycan (murein) hydrolases.* New comprehensive biochemistry, 1994. 27: p. 131-166.

26. DePestel, D.D., et al., *Cephalosporin use in treatment of patients with penicillin allergies.* Journal of the American Pharmacists Association: JAPhA, 2007. 48(4): p. 530-540.

27. Jindal, S.K., et al., *Textbook of Pulmonary and Critical Care Medicine Vols 1 and 2*. 2011: JP Medical Ltd.

28. Samanidou, V., E. Hapeshi, and I. Papadoyannis, *Rapid and sensitive high-performance liquid chromatographic determination of four cephalosporin antibiotics in pharmaceuticals and body fluids.* Journal of Chromatography B, 2003. 788(1): p. 147-158.

29. Edberg, S.C., *Antibiotics and infection*. 1983: Churchill Livingstone.

30. Powers, J.H., *Use and Importance of Cephalosporins in Human Medicine.* Online.

31. Zhanel, G.G., et al., *Ceftaroline.* Drugs, 2009. 69(7): p. 809-831.

32. Garcia-Rodriguez, J., J.M. Bellido, and J.G. Sanchez, *Oral cephalosporins: current perspectives.* International journal of antimicrobial agents, 1995. 5(4): p. 231-243.

33. OnlineTM, L.-C., L.-D. OnlineTM, and O. Hudson, *Lexi-Comp*. 2012, Inc.

34. Porter, R.S., et al., *The Merck manual online*. 2006: Merck Research Laboratories.

35. Behin, S., I. Punitha, and S. Krishnan, *Stability Studies of Cefepime Hydrochloride by Stability Indicating RP-HPLC Method.*

36. Hornish, R.E. and S. Katarski, *Cephalosporins in veterinary medicine-ceftiofur use in food animals.* Current topics in medicinal chemistry, 2002. 2(7): p. 717-731.

37. Lehto, V.-P. and E. Laine, *Assessment of physical stability of different forms of cefadroxil at high humidities.* International journal of pharmaceutics, 1998. 163(1): p. 49-62.

38. Diaconu, D., et al., *Studies regarding the stability of pharmaceutical formulations realized through association of beta-lactamic antibiotics 1. Stability of an oral suspension powder containing cefadroxil and cefixim.*

39. do Nascimento, T.G., et al., *Short-term stability studies of ampicillin and cephalexin in aqueous solution and human plasma: Application of least squares method in Arrhenius equation.* Journal of pharmaceutical and biomedical analysis, 2013. 73: p. 59-64.

40. Hu, C.Q., et al., *Assessment of the stability of cefazolin sodium in solids by TGA decomposition Kinetics.* Journal of Chinese Pharmaceutical Sciences, 1998. 7: p. 80-85.

41. Gupta, V.D., *Chemical Stability of Cefazolin Sodium after Reconstituting in 0.9% Sodium Chloride Injection and Storage in Polypropylene Syringes for Pediatric*

Use. International journal of pharmaceutical compounding, 2002. 7(2): p. 152-154.

42. de Bolós, J., et al., *Prediction of stability of cefazolin sodium in perfusion fluids.* International journal of pharmaceutics, 1987. 40(1): p. 175-177.

43. Robinson, R.F., et al., *Stability of cefazolin sodium in four heparinized and non-heparinized dialysate solutions at 38 C.* Peritoneal dialysis international, 2006. 26(5): p. 593-597.

44. Carone, S., et al., *Stability of frozen solutions of cefazolin sodium.* American Journal of Health-System Pharmacy, 1976. 33(7): p. 639-641.

45. Donnelly, R.F., *Stability of Cefazolin Sodium in Polypropylene Syringes and Polyvinylchloride Minibags.* The Canadian journal of hospital pharmacy, 2011. 64(4): p. 241.

46. Xu, Q.A., et al., *Stability of three cephalosporin antibiotics in AutoDose Infusion System bags.* Journal of the American Pharmaceutical Association (Washington, DC: 1996), 2001. 42(3): p. 428-431.

47. Olsen, B.A., et al., *Accelerated conditions for stability assessment of bulk and formulated cefaclor monohydrate.* Pharmaceutical development and technology, 1997. 2(4): p. 303-312.

48. Dorman, D.E., et al., *Isolation and structure elucidation of the major degradation products of cefaclor in the solid state.* Journal of pharmaceutical sciences, 1997. 86(5): p. 540-549.

49. PATEL, A., et al., *Formulation, development and evaluation of cefaclor extended release matrix tablet.* Int J Pharm Pharm Sci, 2012. 4: p. 355-57.

50. Medenecka, B., et al., *Stability of the crystalline form of cefaclor monohydrate and its pharmaceutical preparations.* Acta poloniae pharmaceutica, 2008. 66(5): p. 563-569.

51. Kuksal, K. and K. Pathak, *Use of solubility parameter to design dry suspension of cefaclor as a dual pack system.* Indian journal of pharmaceutical sciences, 2008. 70(5): p. 609.

52. Oberholtzer, E.R. and G.S. Brenner, *Cefoxitin sodium: Solution and solid-state chemical stability studies.* Journal of pharmaceutical sciences, 1979. 68(7): p. 863-866.

53. Zając, M., et al., *Evaluation of stability of cefuroxime axetil in solid state.* Journal of pharmaceutical and biomedical analysis, 2003. 32(6): p. 1181-1187.

54. Jelińska, A., et al., *The stability of the amorphous form of cefuroxime axetil in solid state.* Journal of pharmaceutical and biomedical analysis, 2006. 41(3): p. 1075-1081.

55. Hecq, J.-D., et al., *Effect of the freezing conditions and microwave thawing power on the stability of Cefuroxime in dextrose 5% infusion polyolefin bags at 4 C.* Annals of Pharmacotherapy, 2005. 39(7-8): p. 1244-1248.

56. Smith, B., *Effect of storage temperature and time on stability of cefmenoxime, ceftriaxone, and cefotetan in 5% dextrose injection.* American Journal of Health-System Pharmacy, 1983. 40(6): p. 1024-1025.

57. Gupta, V.D., et al., *Chemical stability of cefotetan disodium in 5% dextrose and 0.9% sodium chloride injections.* Journal of clinical pharmacy and therapeutics, 1990. 15(2): p. 109-114.

58. Gupta, V.D. and K. Stewart, *Stability of cefamandole nafate and cefoxitin sodium solutions.* American Journal of Health-System Pharmacy, 1981. 38(6): p. 875-879.

59. O'Brien, M., J. Portnoff, and E. Cohen, *Cefoxitin sodium compatibility with intravenous infusions and additives.* American Journal of Health-System Pharmacy, 1979. 36(1): p. 33-38.

60. Venugopalarao, G., M.S. Gowtham, and N. Sarada, *Formulation evaluation and stability studies of hydrogel tablets containing Cefditoren Pivoxil.* Journal of Pharmacy Research, 2013. 7(3): p. 230-234.

61. Paul, Y., M. Kumar, and B. Singh, *Formulation and in Vitro evaluation of Gastroretentive drug delivery system of Cefixime Trihydrate.* Int. J. Drug Dev. & Res, 2011. 3(4): p. 148-161.

62. Kitamura, S., et al., *Dehydration effect on the stability of cefixime trihydrate.* International journal of pharmaceutics, 1990. 59(3): p. 217-224.

63. HARITHA, M. and B.P. KUMAR, *FORMULATION AND EVALUATION OF CEFIXIME DRY EMULSION.*

64. C. Senthilkumar, G.R., S. Subathra, T. Suresh Kumar, S. Arun Kumar, *ENHANCEMENT OF SOLUBILITY AND DISSOLUTION RATE OF DRUG CEFPODOXIME PROXETIL.* INTERNATIONAL RESEARCH HOURNAL OF PHARMACY, 2012: p. 6.

65. Genentech USA, I., *ROCEPHIN□(ceftriaxone sodium) FOR INJECTION*.

66. Cantón, E., M.J. Esteban, and F. Rius, *Factors affecting the stability of ceftriaxone sodium in solution on storage.* International journal of pharmaceutics, 1993. 92(1): p. 47-53.

67. Gupta, V.D., *Stability of Cefotaxime Sodium After Reconstitution in 0.9% Sodium Chloride Injection and Storage in Polypropylene Syringes for Pediatric Use.* International journal of pharmaceutical compounding, 2001. 6(3): p. 234-236.

68. Gupta, V.D., C. Bethea, and M. Torre, *CHEMICAL STABILITIES OF CEFOPERAZONE SODIUM AND CEFTAZIDIME IN 5% DEXTROSE AND 0· 9% SODIUM CHLORIDE INJECTIONS.* Journal of clinical pharmacy and therapeutics, 1988. 13(3): p. 199-205.

69. Kodym, A., et al., *Stability of ceftazidime in 1% and 5% buffered eye drops determined with HPLC method.* Acta poloniae pharmaceutica, 2010. 68(1): p. 99-107.

70. Arsène, M., et al., *Comparison of ceftazidime degradation in glass bottles and plastic bags under various conditions.* Journal of clinical pharmacy and therapeutics, 2002. 27(3): p. 205-209.

71. Hospira, I., *MAXIPIME (Cefepime Hydrochloride, USP) for Injection, Reference ID: 3185289* 2012.

72. Rabouan-Guyon, S.M., et al., *Stability study of cefepime in different infusion solutions.* International journal of pharmaceutics, 1997. 154(2): p. 185-190.

73. Kodym, A., et al., *Stability of cefepime in aqueous eye drops.* Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2011. 66(1): p. 17-23.

74. Trissel, L.A. and Q.A. Xu, *Stability of cefepime hydrochloride in autodose infusion system bags.* Annals of Pharmacotherapy, 2003. 37(6): p. 804-807.

75. JANICE K POHLMAN, A.R.P., *MEDICAL REVIEW(S), CENTER FOR DRUG EVALUATION AND RESEARCH, APPLICATION NUMBER: 200327, REFERENCE ID: 2857265.* 2010.

76. Ikeda, Y., et al., *Stability and stabilization studies of TAK-599 (ceftaroline fosamil), a novel N-phosphono type prodrug of anti-methicillin resistant Staphylococcus aureus cephalosporin T-91825.* Chemical and Pharmaceutical Bulletin, 2008. 56(10): p. 1406-1411.

77. Forest Pharmaceuticals, I., *Teflaro™ (ceftaroline fosamil)-clean-labeling text-2010-11-08* 2010.

Chapter 2

Significance of the research

Ceftriaxone (CTZ) is a broad spectrum semisynthetic, third generation cephalosporin antibiotic. It is a drug in the World Health Organization's list of essential medicines and, is an acid labile drug belonging to class III of biopharmaceutical classification system (BCS).

CTZ can be solvated quickly but suffers from the drawback of poor oral bioavailability owing to its limited permeability through gastro-intestinal epithelia. Therefore, it cannot be given orally, and is available as a parenteral formulation for intravenous or intramuscular administration. The usual dose of CTZ is 1 to 2 g every 12 to 24 hours, depending on the type and severity of the infection [33].

The goal of this work was to formulate calcium alginate beads using ionotropic-external gelation technique for oral delivery of CTZ. The formulated beads can be further enteric coated to protect the drug from the harsh environment of the stomach, and also to obtain sustained release of drug in the intestine.

Chapter 3

An oral dosage form of ceftriaxone sodium using enteric coated sustained release calcium alginate beads

Darshan Lalwani[1], Steven Gollmer[2], Elisha Injeti[3], Youssef Sari[4], and Jerry Nesamony*[1]
1. Industrial Pharmacy Division, Department of Pharmacy Practice, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, OH.
2. Department of Science and Mathematics, Cedarville University, Cedarville, OH.
3. Department of Pharmaceutical Sciences, School of Pharmacy, Cedarville University, Cedarville, OH.
4. Department of Pharmacology, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, OH

Figures: 10

Tables: 8

Keywords: ionotropic external gelation; ceftriaxone sodium; enteric coated beads; atomic force microscopy; thermal gravimetric analysis; fourier transform- infrared spectroscopy

Corresponding Author*

Jerry Nesamony, Ph.D.
Department of Pharmacy Practice
College of Pharmacy and Pharmaceutical Sciences
The University of Toledo
3000 Arlington Ave. (MS1013)
Toledo, OH 43614
Phone: 419.383.1938
Fax: 419.383.1950
Email: jerry.nesamony@utoledo.edu

Purpose: Ceftriaxone (CTZ) is a broad spectrum semisynthetic, third generation cephalosporin antibiotic. It is an acid labile drug belonging to class III of biopharmaceutical classification system (BCS). It can be solvated quickly but suffers from the drawback of poor oral bioavailability owing to its limited permeability through gastro-intestinal epithelia. Hence, it cannot be given orally to a patient. The objective of the present research work was to develop and evaluate enteric coated calcium alginate beads of CTZ for oral delivery. **Methods**: Dispersions of CTZ and low viscosity sodium alginate with various co-polymers such as, acacia gum (AG), sodium carboxymethyl cellulose (CMC), guar gum (GG), iota-carrageenan gum (ICG), and xanthan gum (XG) were utilized to prepare CTZ entrapped beads by ionotropic external gelation method using calcium chloride as a cross linking agent. **Results**: The formulated beads were evaluated for entrapment efficiency (E.E.) using HPLC. The highest E.E. obtained was 69.3 ± 0.2% in a formulation containing sodium CMC as a co-polymer with sodium alginate. The entrapment optimized batch was further coated either with a single coat or a double coat of enteric polymers such as cellulose acetate phthalate (CAP), Opadry® enteric, Eudragit® L-100, and Eudragit® S-100. Finally, the enteric coated polymeric beads were analyzed for entrapment efficiency, in-vitro drug release in simulated gastric fluid (pH 1.2) and simulated intestinal pH (pH 6.8), swellability, drug- polymer interaction using fourier transform- infrared spectroscopy (FT-IR), thermal stability using thermal gravimetric analysis (TGA), surface morphology using scanning electron microscopy (SEM), and surface roughness using atomic force microscopy (AFM). The entrapment optimized batch was a double coated formulation with a first coat of Eudragit® S-100 and a second coat of Eudragit® L-100 and the observed E.E. was 60.7

± 0.5%. Swellability of the coated beads was desirable around 173% as compared to 290% of the uncoated beads. Release of drug from the beads of optimized batch followed first order kinetics. The FTIR spectral showed that there was no significant interaction between the drug and polymers. The TGA thermograms demonstrated thermal stability of the formulation. The SEM micrographs indicated smooth surface of the coated beads over uncoated ones. AFM micrographs demonstrated surface characteristics of the formulated beads and average roughness (Ra) values observed for uncoated and coated beads affirmed the SEM results.

Conclusion: In conclusion, sustained release beads of CTZ were successfully prepared using sodium alginate and sodium CMC. Parameters such as polymer concentration, calcium chloride concentration, coating polymer concentration, stirring speed and cross-linking time significantly affected entrapment efficiency, surface morphology and in-vitro drug release. The use of sodium alginate, sodium CMC and other coating polymers decreased the drug release behavior in gastric conditions to certain degree but sustained the drug release at intestinal pH.

3.2 INTRODUCTION

Cephalosporin antibiotics are among the safest and the most effective broad-spectrum bactericidal agents and hence, they are the most prescribed of all antibiotics [28]. Cephalosporins are classified into various generations based on their biological characteristics [78]. First and second generation cephalosporins are found to be active orally, but are ineffective against many forms of bacteria, typically those found in hospital infections. Though, third generation antibiotics are effective against such bacteria they are not active orally due to its poor permeability through GI epithelia [79]. Ceftriaxone sodium is a third generation cephalosporin belonging to biopharmaceutical classification system (BCS) class III, which has attributes of high solubility and low permeability. It cannot be administered orally owing to its acid labile nature and hence is available only in parenteral dosage form [80, 81].

Patient compliance plays a major role in effectiveness of the treatment. Although parenterals are valued for their speed and efficiency of delivery but, most people prefer oral dosage form because of its convenience associated with administration [82]. Therefore, finding a convenient dosage form is a recommended choice than exploiting for a new drug molecule that can satisfy the patient needs.

Choi and Lee have successfully prepared complexes for oral delivery of such antibiotics [79, 83]. Based on the similar principles, in this research an attempt was made to prepare a biopolymer bead delivery system for ceftriaxone sodium. Biopolymer beads exhibit the advantages like less inter and intra-subject variability, less likely to cause local irritation, offering controlled release and site specificity [84].

In this project, biopolymer beads were prepared using sodium alginate, sodium carboxymethyl cellulose, acacia gum, guar gum, iota-carrageenan gum and xanthan gum with calcium chloride as a cross-linking agent. The method of preparation employed was ionotropic-external gelation [79, 85-88]. Beads were further coated with various enteric coating polymers to protect the drug from the hostile acidic environment of the stomach as well as to target its release in lower GI tract [89]. The prepared beads were evaluated for entrapment efficiency, in-vitro release, and swelling ability. They were also characterized for drug-polymer interaction, thermal stability, morphological features and surface roughness.

3.3 MATERIALS AND METHODS:

3.3.1 Materials

Ceftriaxone sodium (Apotex Corp., Weston, FL.), low viscosity sodium alginate (Sigma Aldrich, St Louis, MO.), sodium carboxymethyl cellulose- viscosity 7MF (Amend drug and chemical co., Irvington, NJ), calcium chloride anhydrous (Sigma Aldrich, St Louis, MO.), xanthan gum and guar gum (Spectrum Chemicals & Laboratory Products Gardena, CA), acacia (PCCA, Houston, TX.), iota-carrageenan (Ingredients solution in., Waldo, ME.), cellulose acetate phthalate (Fluka Analytical, GmbH, CH.), Opadry® Organic Enteric (Colorcon, West Point, PA.), Eudragit® S-100 and Eudragit® L-100 (Evonik Industries, Piscataway, NJ). All other chemicals were of analytical reagent grade unless specified.

3.3.2 Methods

3.3.2.1 Preparation of beads:

Microbeads of CTZ were prepared by ionotropic-external gelation technique. Beads of sodium alginate containing five unique co-polymers were prepared using calcium chloride as crosslinking agent. The detailed compositions of the prepared formulations are described in table 3.1.

Initially, 2mg CTZ and sodium alginate were dispersed and magnetically stirred in deionized water at room temperature. Then, various co-polymers were added and stirred until they dissolved completely. These dispersions were then extruded via a 26 G syringe into aqueous calcium chloride solution. The beads were allowed to cure for 5 minutes after completely extruding the polymer dispersion to enhance the rigidity of the beads. The beads were then, recovered by filtering through a filter paper (Whatman no. 42), spread on glass petri dish, dried at room temperature for 1 to 2 days and were stored in an air tight container for further use.

Table 3.1: Composition of beads

Batch Code	Sodium Alginate (% w/v)	Calcium Chloride (% w/v)	Sodium CMC (% w/v)	Acacia gum (%w/v)	Guar gum (%w/v)	iota-carrageenan gum (% w/v)	Xanthan gum (% w/v)
Blank formulations							
F1	1.25	3.75	-	-	-	-	-
F2	1.25	3.75	4.25	-	-	-	-
Uncoated formulations							
F3	1.25	3.75	4.25	-	-	-	-
F4	1.25	3.75	-	2.5	-	-	-
F5	1.25	3.75	-	-	1.25	-	-
F6	1.25	3.75	-	-	-	2.5	-
F7	1.25	3.75	-	-	-	-	2.5

*Note: Blank formulations represent batches prepared without incorporation of CTZ.

3.3.2.2 Coating of beads

Initially, a batch from uncoated formulations was selected based on entrapment efficiency data. Then, all the coating trials were performed only on the optimized batch (F3) and have been reported in table 3.2. Various enteric coating polymers were dissolved in appropriate solvent systems. Freshly prepared beads were dispersed immediately after recovery in the enteric coating solution and were magnetically stirred at 1200 rpm for 30 minutes at room temperature. When two polymer coats were applied the harvested beads after first coating were suspended in a second coating solution and similar procedure was followed [90]. Finally, all the beads with single coat or double coat were spread on glass petri dish and dried at room temperature for 1 to 2 days.

Table 3.2: Coating polymers and solvent systems

Batch Code	Coating	Concentration (% w/v)	Solvent system
F8	CAP	5%	Ethanol : Ethyl Acetate (1:1)
F9	Opadry® Enteric	5%	Ethanol : Ethyl Acetate (4:1)
F10	Eudragit® L-100	5%	Ethanol
F11	Eudragit® S-100	5%	Ethanol
F12	(1) CAP	5%	Ethanol : Ethyl Acetate (1:1)
	(2) Opadry® Enteric	5%	Ethanol : Ethyl Acetate (4:1)
F13	(1) Opadry® Enteric	5%	Ethanol : Ethyl Acetate (4:1)
	(2) CAP	5%	Ethanol : Ethyl Acetate (1:1)
F14	(1) Eudragit® L-100	5%	Ethanol
	(2) Eudragit® S-100	5%	Ethanol
F15	(1) Eudragit® S-100	5%	Ethanol
	(2) Eudragit® L-100	5%	Ethanol

*Note: All the prepared batches were with 2 mg of CTZ.

3.3.2.3 Analysis of Ceftriaxone sodium

Reversed phase- high performance liquid chromatography (RP-HPLC) from Waters Alliance (e2695 separation module, Milford, MA) equipped with photodiode array detector (Water Alliance 2998) was utilized to analyze CTZ. A Waters Sunfire C18 column (5µm, 4.6 X 250 mm) was used as a stationary phase whereas the aqueous mobile phase consisted of a binary mixture of acetonitrile and 2.06 M tetrabutylammonium hydroxide (TBAH) 5% (v/v) (pH 7.0 adjusted with o-phosphoric acid) in the ratio of 30:70 (v/v) respectively [91]. Isocratic separation method was utilized with the flow rate of 0.7 ml/ min. The mobile phase was filtered through 0.20 µm membrane filter (Millipore) and degassed before use. The injection volume was 10 µl with a run time of 10 min and the retention time of drug was found to be 8 min at 240 nm.

3.3.2.4 Determination of entrapment efficiency of uncoated and coated beads

Approximately, 200 mg beads of formulations F2 through F6 were weighed, and magnetically stirred in 50 ml phosphate buffer solution (pH 7.4) at 1200 rpm for 3 hours. After stirring, an aliquot from these dispersions were centrifuged (Eppendorf Centrifuge 5430R, UK) at 14000 rpm for 5 min and the supernatant was analyzed for any drug content using RP-HPLC. The entrapment efficiency was calculated using the following equation:

$$\text{Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Entrapment efficiency data of uncoated formulations F3 to F7 and optimized coated batch F15 has been reported in table 3.4.

3.3.2.5 In-Vitro drug release

An aliquot known quantity of beads (formulations F8 to F15) were placed in 50 ml enzyme free simulated gastric fluid (SGF) (pH 1.2) for the initial 2 hours followed by enzyme free simulated intestinal fluid (SIF) (pH 6.8) [92]. At pre-determined time intervals, 2 ml samples were withdrawn and replaced with identical volume of dissolution media to maintain sink conditions. All samples were centrifuged (Eppendorf Centrifuge 5430R, UK) at 14000 rpm for 5 min to separate any insoluble matter and the supernatant was analyzed for CTZ using HPLC at a wavelength of 240 nm. Cumulative percentage drug release was calculated using a regression equation generated from a standard curve (y = 39753x – 4788.1). Data has been recorded in table 3.5a and table 3.5b.

3.3.2.6 Swelling studies

This study was performed for optimized uncoated batch (F3) and coated batch (F15) only. The purpose of this study was to check the suitability of the beads in gastro-intestinal delivery. 200 mg beads were dispersed in 50 ml of enzyme free simulated gastric fluid (pH 1.2) placed in a mechanical shaker bath (Thermo Scientific^{TM} Precision Reciprocating Shaker Bath, USA) maintained at 37 ± 0.5°C for 2 hours. The swollen beads were harvested using filter paper (Whatman no. 42), blot dried and weighed. The dynamic weight change was calculated using the following equation[86]:

$$\text{Dynamic weight change (\%)} = \frac{\text{(Final weight of the beads – Initial weight of the beads)}}{\text{(Initial weight of the beads)}} \times 100$$

3.3.2.7 Drug release kinetics and mechanism

Kinetic models can best describe the dissolution of solid dosage forms where the amount of drug dissolved is a function of time [93]. To analyze the release kinetics, the drug release data from the in-vitro dissolution study was fitted to different mathematical models such as Zero-order (% cumulative drug release vs time), First-order (log % cumulative drug retained vs time), and Higuchi (% cumulative drug release vs root time). For determining the release mechanism, initial 60% drug release data was fitted in Power law model (log % cumulative drug release vs log time). The equations used are shown in table 3.3 [93, 94].

Table 3.3 : Release kinetic equations

Mathematical Model	Equation	Terms
Zero-order[95]	$Mt/M\infty = k_o t$	$Mt/M\infty$ = fraction of drug released at time t. ko = zero-order release rate constant
First-order[95]	$Mt/M\infty = 1 - \exp(-k_1 t)$	k_1 = first-order release rate constant
Higuchi model[96]	$Mt/M\infty = k'\sqrt{t}$	k' = Higuchi constant
Power law model[96]	$Mt/M\infty = kt^n$	k = constant incorporating structural and geometric characteristics of the device n = release exponent, indicative of the mechanism of drug release

3.3.2.8 Drug- polymer interaction studies

Fourier transform infrared (FT-IR) spectrums were obtained for CTZ, alginic acid powder, CMC powder, blank formulation (F2) and uncoated drug-loaded beads (F3) using an IR Spectrophotometer (Thermo Scientific Nicolet iS5 with iD3 ATR).
Initially, beads were crushed with pestle and a small quantity of that was transferred to the sample chamber. Then, the spectrum was recorded over a wavenumber range of 4000 – 400 cm^{-1}. FT-IR spectroscopic analysis was performed to check the compatibility of the drug with the polymers employed.

3.3.2.9 Thermal analysis of formulation components and beads

Thermal gravimetric analysis (TGA) thermograms of alginic acid powder, alginic acid beads (F1), carboxymethyl-cellulose (CMC) powder and CMC- alginic acid beads (F2) were recorded utilizing a TA Instrument SDT Q600 at a heating rate of 10ºC/min between 25ºC to 550ºC using nitrogen as a purge gas [97]. TGA was performed to determine the polymer matrix strength and its thermal stability based on the weight losses caused by chemical reactions or physical transitions [98].

3.3.2.10 Morphological characterization of beads

Scanning electron microscopy (SEM) photomicrographs were taken for blank beads (F2), uncoated-drug loaded beads (F3) and for few coated formulations (F10, F11, F14 and F15) utilizing JSM-7500F- cold cathode analytical field emission-SEM.
Initially, few dry beads were fixed separately using double-sided carbon conductive tape on SEM aluminum stub and were then sputter-coated with gold for 20 seconds under an

argon atmosphere using Denton Vacuum Desk II Sputter Coater. Gold coating was performed to render them electrically conductive. SEM was performed to investigate the shape and surface morphology of the prepared beads.

3.3.2.11 Evaluation of surface roughness of beads

Atomic force microscopy (AFM) micrographs were obtained for blank beads (F1 and F2), uncoated-drug loaded beads (F3) and for few Eudragit® coated formulations (F10, F11, F14 and F15) utilizing Nanosurf easy Scan 2 AFM instrument with dynamic mode as an operating mode at scan range of 10 µm and speed of 0.1 mm/s.

Initially, few dry beads were fixed separately on a double sided tape on an AFM stub and this was placed under scan head. Sample was approached in two steps- (a) manual course approach till the cantilever reached within the range of the sample surface and then (b) automatic final approach was employed to prevent the tip from breaking [99].

AFM was performed to evaluate the variability in surface characteristics of the prepared beads [100].

3.4 RESULTS AND DISCUSSION

3.4.1 Determination of entrapment efficiency

Alginate beads are nontoxic orally and have high biocompatibility but, owing to its high porosity and low density, the matrix network formed is not stiff enough and hence a need of co-polymer arises [101]. In our research, ceftriaxone sodium was entrapped in beads of sodium alginate prepared with different co-polymers like carboxy-methyl cellulose (CMC), acacia gum, guar gum, iota-carrageenan gum and xanthan gum while using

calcium chloride solution as a cross-linking agent. Entrapment efficiency was calculated using the calibration curve. The calibration curve used for determination of CTZ was linear over the range of 0.78 – 50.00 µg/ml and the correlation coefficient was found to be 1 (n = 6).

Owing to the fact that entrapment efficiency depends on the physicochemical properties and the interactions between the drug and the carrier matrix, [102] the entrapment efficiency was found to be in the range of 12% to 70%. The lowest entrapment observed was around 12% for formulation F6 which consisted of sodium alginate with iota-carrageenan gum whereas highest entrapment observed was around 70% for formulation F3 having sodium alginate with CMC as a co-polymer. Hence, formulation F3 was selected as an optimized batch. This batch was further coated with different enteric polymers and from enteric coated beads a double-coated batch with Eudragit® S-100 and Eudragit® L-100 was finalized based on its in-vitro drug release data. The entrapment efficiency of finalized batch (F15) was around 61% and this decrease in entrapment may be because drug leaching into solvents employed for dissolving coating material.

Table 3.4: Entrapment efficiency data of prepared formulations

Batch code	Entrapment efficiency (% ± SD, n = 3)
F3	69.3 ± 0.2
F4	30.2 ± 1.3
F5	22.4 ± 0.4
F6	11.9 ± 1.8
F7	19.5 ± 0.3
F15	60.7 ± 0.5

3.4.2 In-Vitro drug release

Plots of percent cumulative drug release vs time for all the coated formulations are shown in Fig 3-1 and the data is in table 3.5a and table 3.5b. Ceftriaxone sodium was entrapped

in sodium alginate beads using CMC as a co-polymer. The beads were given either a single coat or a double coat with different enteric polymers. The release from the coated beads was observed in SGF pH 1.2 for initial 2 hr, followed by their transfer in SIF pH 6.8.

When given a single or double coat with phthalate based coating polymers such as cellulose acetate phthalate and Opadry® Enteric, the release in SGF was found to be 70% or more. Hence, anionic copolymers based on methacrylic acid and methyl methacrylate such as Eudragit® S-100 and Eudragit® L-100 were used. The beads when given a single coat of Eudragit® S-100 released around 42% drug in SGF and remaining drug was released in SIF in 6 hr. When the same formulation was given a second coat of Eudragit® L-100, the drug release was around 40% in SGF but it gave a sustained release of the remaining drug in SIF for 10 hr. Hence batch F15 was selected as the final optimized formulation.

The release of drug in SGF may be attributed to cracks and pores formed due to partial collapsing of the polymer network during dehydration [103].

Table 3.5a: In-vitro drug release data

Time (min)	Batch Code			
	F8	F9	F10	F11
% Cumulative drug release in SGF (pH 1.2)*				
15	31.80 ± 22.01	10.34 ± 2.00	11.63 ± 1.97	16.58 ± 1.23
30	58.26± 29.32	27.16 ± 7.41	23.46 ± 4.15	38.03 ± 5.10
60	72.40 ± 15.24	48.74 ± 2.50	35.53 ± 6.27	55.92 ± 7.98
120	87.80 ± 0.86	70.26 ± 0.68	48.01 ± 8.34	67.71 ± 5.52
% Cumulative drug release in SIF (pH 6.8)*				
135	89.20 ± 0.60	74.05 ± 0.57	56.70 ± 7.02	75.46 ± 5.97
150	90.85 ± 0.39	77.88 ± 0.66	64.70 ± 4.76	79.85 ± 5.35
180	92.44 ± 0.41	81.75 ± 0.86	72.69 ± 2.45	83.70 ± 4.44
240	94.09 ± 0.50	85.67 ± 1.13	80.67 ± 0.11	87.68 ± 3.42
360	95.87 ± 0.76	89.63 ± 1.48	88.75 ± 2.30	91.49 ± 2.27
480	97.70 ± 0.85	93.56 ± 1.75	96.68 ± 4.69	95.76 ± 1.08
600	99.45 ± 0.95	97.53 ± 2.13	100	100
720	100	100	-	-

*values indicate mean % (n =3).

Table 3.5b: In-vitro drug release data				
Time (min)	Batch Code			
	F12	F13	F14	F15
% Cumulative drug release in SGF (pH 1.2)*				
15	15.69 ± 0.78	15.82 ± 2.17	10.20 ± 2.02	9.21± 0.85
30	37.40 ± 1.22	36.78 ± 3.45	22.34 ± 1.85	19.16 ± 0.47
60	62.56 ± 1.93	63.03 ± 3.17	34.10 ± 1.14	29.87 ± 0.09
120	87.99 ± 2.22	90.98 ± 1.26	46.13 ± 0.82	40.89 ± 0.10
% Cumulative drug release in SIF (pH 6.8)*				
135	89.45 ± 2.13	91.84 ± 0.92	53.11 ± 1.21	47.43 ± 0.26
150	91.03 ± 2.08	93.10 ± 0.74	60.42 ± 1.45	53.94 ± 0.58
180	92.80 ± 2.11	94.57 ± 0.76	67.51 ± 2.15	60.74 ± 0.82
240	94.71 ± 1.97	95.69 ± 0.87	74.86 ± 2.77	67.96 ± 0.96
360	96.89 ± 1.84	97.52 ± 0.94	82.26 ± 3.30	75.89 ± 0.66
480	99.15± 1.46	99.40 ± 1.03	89.88 ± 3.73	83.91 ± 0.41
600	100	100	97.55 ± 4.24	91.96 ± 0.25
720			100	100

*values indicate mean % (n =3).

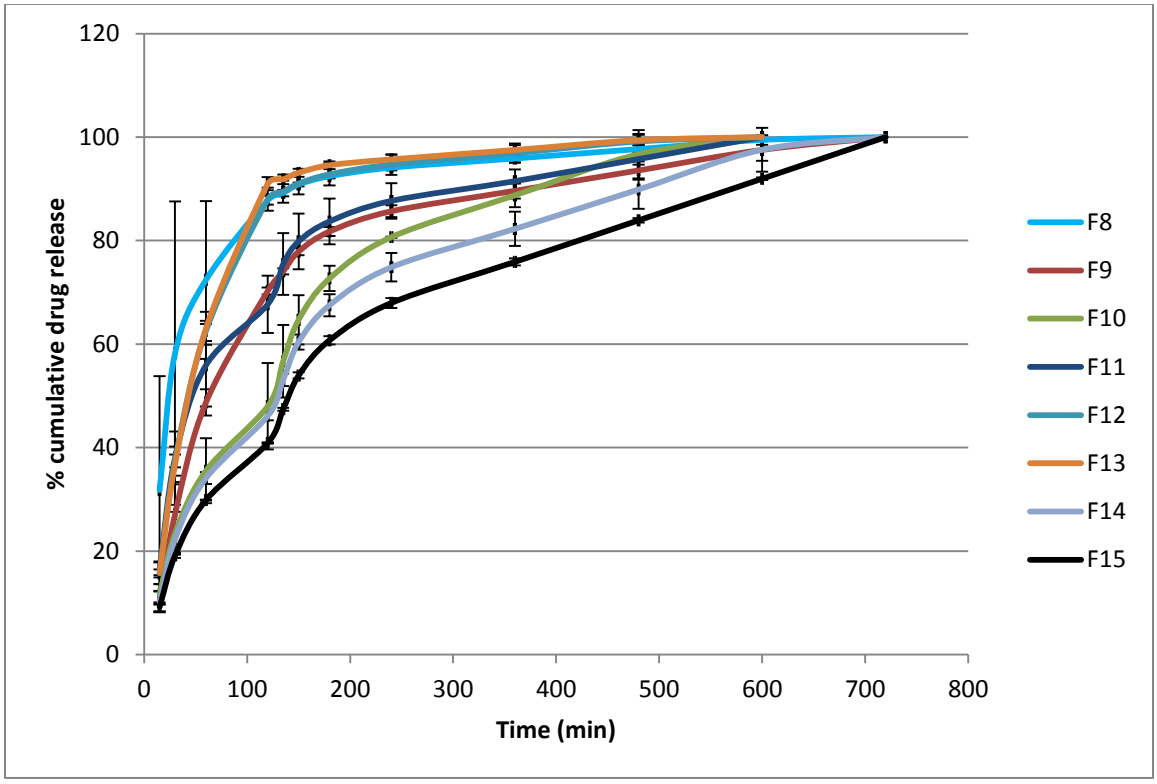


Fig 3-1: Comparative release profiles of CTZ from different coating formulations. Results indicate mean % ± SD, (n=3).

3.4.3 Swelling studies

In systems, where sodium alginate is cross-linked with calcium chloride, an osmotic pressure gradient exists between the alginate gel and the environment which comprises an important factor in the swelling process [85]. A cross-linked alginate matrix, when exposed to low pH, undergoes proton-catalyzed hydrolysis and converts into alginic acid which in-turn lowers the degree of crosslinking [87]. Dry beads when placed in water or in buffers increase their volume based on differences in pH and composition, owing to matrix rehydration based on the degree of cross-linking [104]. Fig 3-2 shows the % dynamic weight change of uncoated and coated beads in SGF. The % swelling of uncoated formulation (F3) was around 290% and for coated formulated (F15), it was around 173%. The less swelling of the coated formulation is due to the presence of enteric polymer at the surface of the beads which prevents the penetration of solvent into the gel network. The results obtained are desirable because the degree of swelling is directly related to the drug release from the formulation [105].

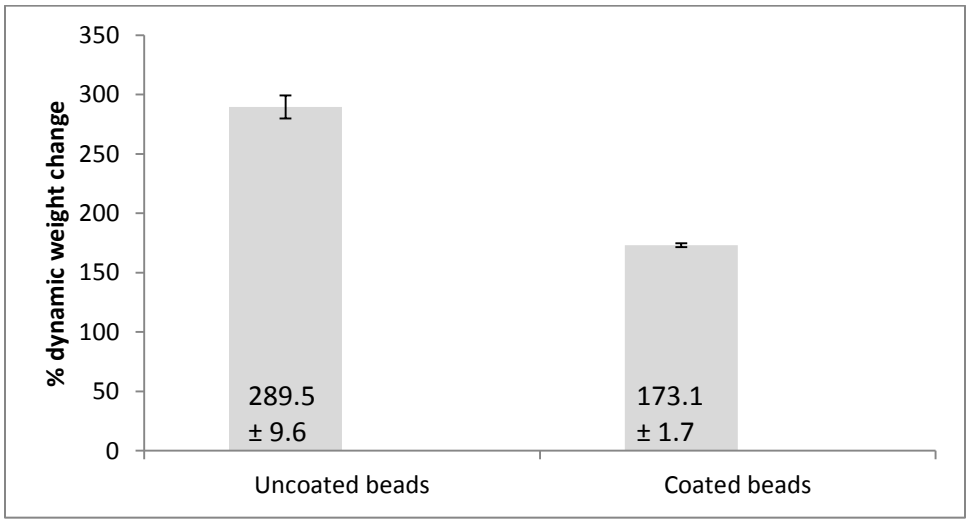


Fig 3-2: Comparison of swelling ability of uncoated and coated beads. Results indicate mean % ± SD, (n=3).

3.4.4 Drug release kinetics and mechanism

The in-vitro drug release kinetics data for all the coated formulations (F8 to F15) were fitted in to zero order, first order, Higuchi's model and Power law model and the slope values and correlation coefficient (r^2) values were calculated in each case. The values are given in table 3.6. Calculated correlation coefficients for formulations F10 and F15 (finalized batch) showed best fit for first order drug release model whereas, the remaining formulations were found to best fit power law drug release model. The values of the diffusion coefficients (n) obtained in power law model ranged from 0.5 to 1.2. Peppas (1985) used this n value for characterizing different release mechanisms, and assigned values for a slab as Fickian diffusion for n = 0.5, anomalous transport for $0.5 < n < 1.0$, case-II transport for n = 1, and super case-II transport for n > 1. When compared to the assigned values, regression coefficients of the formulations F8 to F14 (except F10) indicates that the drug release from this beads followed anomalous/ non-Fickian transport that correspond to diffusion, erosion and swelling mechanism or mixed-order kinetics [106, 107].

Table 3.6: Rate constants and regression coefficients for kinetic models

Batch Code		Zero order	First Order	Higuchi	Power law	
F8	r^2	0.4497	0.9213	0.6444	0.931	n = 0.5936
	k	0.0591	-0.0013	2.2337	6.7983	
F9	r^2	0.6044	0.9469	0.7916	0.9803	n = 1.1184
	k	0.0952	-0.0010	3.4384	1.8758	
F10	r^2	0.8144	0.9856	0.9435	0.9742	n = 0.6724
	k	0.1411	-0.0013	4.4053	2.0999	
F11	r^2	0.6452	0.9456	0.8286	0.9574	n = 0.8766
	k	0.1096	-0.0011	3.6029	1.6630	
F12	r^2	0.4631	0.9176	0.6707	0.9786	n = 0.9974
	k	0.1012	-0.0017	3.5339	1.1176	

F13	r^2	0.4346	0.896	0.6428	0.9841	n = 0.9971
	k	0.0999	-0.0018	3.5248	1.1187	
F14	r^2	0.8231	0.9645	0.9483	0.9719	n = 0.7056
	k	0.1146	-0.0010	3.8825	1.7302	
F15	r^2	0.8723	0.9862	0.9726	0.9792	n = 0.7081
	k	0.1157	-0.0007	3.8567	1.5146	

3.4.5 Drug- polymer interaction studies

FT-IR analysis was performed to investigate the molecular interactions of ceftriaxone sodium, alginic acid and carboxymethyl-cellulose in the formulated beads and spectrums are shown in fig. 3-3. The characteristic absorption peaks of pure ceftriaxone were obtained at 1648.62, 1608.64, 1228.78, 1216.81 and 1033.15 corresponding to unordered conformation, aromatic C-C bond, C-N bond, ester carbonyl and vibrations of methyl group respectively. In the spectrum of alginic acid, the absorption bands were obtained at 1300.11 for anhydride C-O and at 1596.23 and 1408.15 for symmetric and asymmetric stretching vibrations of COO^- groups respectively. IR spectrum of CMC show bands at 1228.77 and 1216.87 which are due to presence of ester carbonyl group and band at 1581.97 can be attributed to C-C stretch of phenyl. The blank formulation F2 showed the ester carbonyl band at 1216.86 but the same shifted to 1204.95 in drug loaded formulation F3. Peaks around 3015 and 2969 were observed in CMC, drug and formulation F3 spectrums which are reflective of CH (Sp^2) and CH (Sp^3) stretch respectively [95, 108, 109]. Characteristic bands of drug and other polymers were observed in the FT-IR spectrum of formulated beads, indicating the absence of chemical interactions between drug, polymers and counter-ions after production of beads.

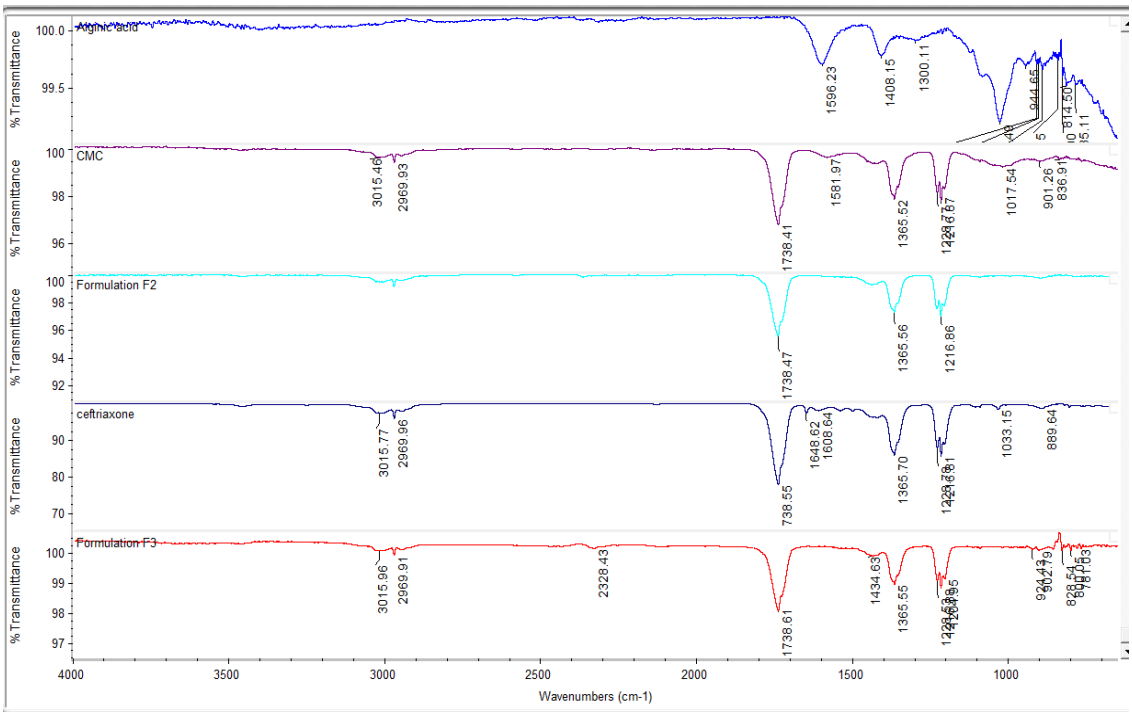


Fig. 3-3: Comparison of FT-IR spectrums of alginic acid powder, CMC powder, Ceftriaxone and formulations F2 and F3.

3.4.6 Thermal analysis of formulation components and beads

TGA thermograms of alginic acid powder, CMC powder and formulations F1 and F2 are shown in figure 3-4. The decomposition of alginic acid powder and CMC powder was lower than that of cross-linked beads of formulations (F1) and (F2). Alginic acid powder showed lower thermal stability than CMC powder. Both alginic acid powder and CMC powder represented similar type of weight loss pattern but alginic acid showed lower weight loss than CMC after decomposition. The alginic acid thermogram represented an initial dehydration process followed by decomposition in two overlapping steps as observed by Fontes et al.[110]; Similarly, CMC powder showed degradation in two steps, where the first decomposition step may be due to the evaporation of bound water and second may be attributed to the degradation of side chain and loss of CO_2 [111].

Formulation F1 and F2 showed higher thermal stability than the powdered forms of alginic acid and CMC. The higher thermal stability of beads may be due to its cross-linking which lowers the chances of elimination of small molecules like CO_2 and CO [97].

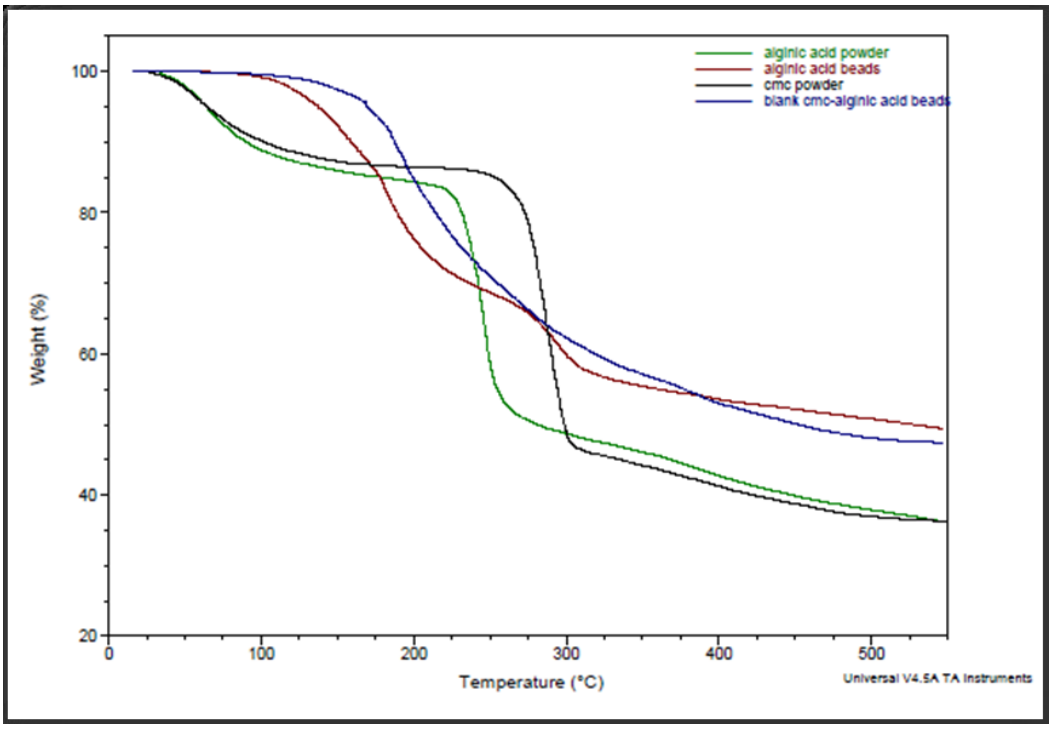
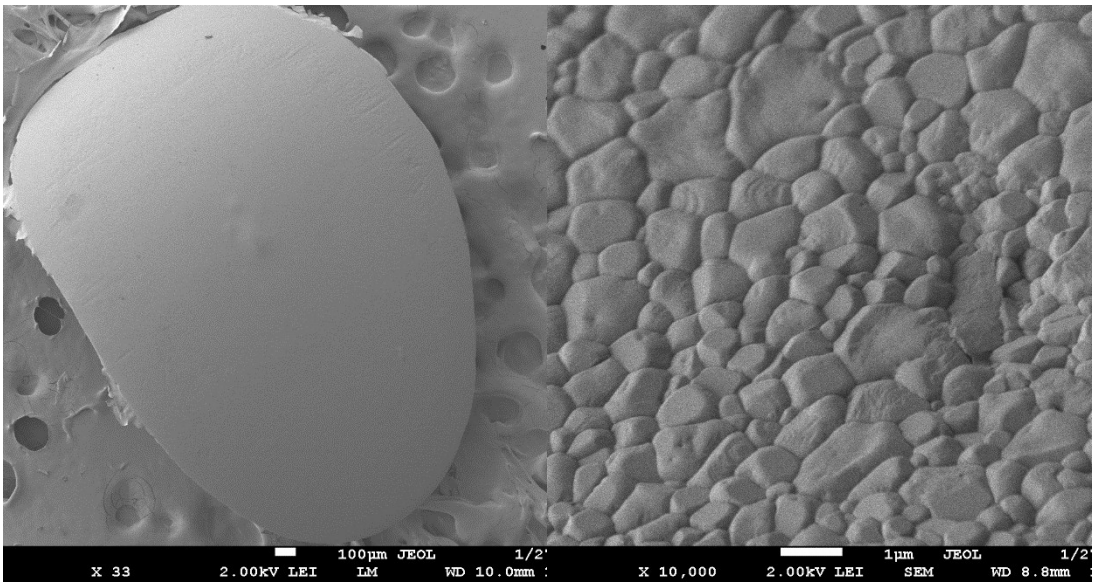


Fig. 3-4: Comparison of TGA thermograms of alginic acid powder, CMC powder, formulations F1 and F2.

3.4.7 Morphological characterization of beads

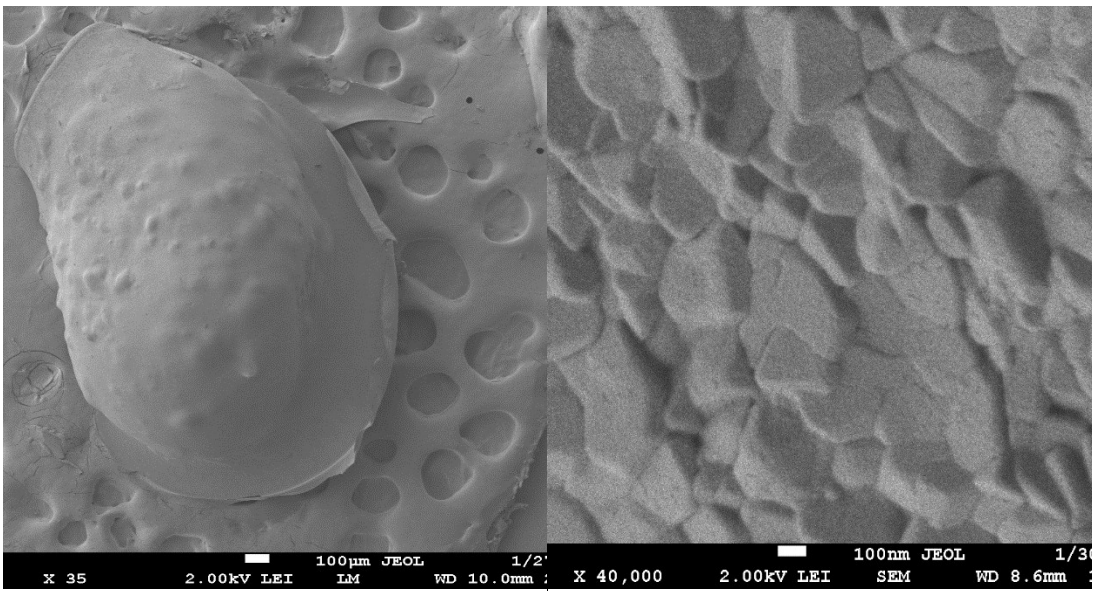
SEM studies were performed on blank formulation (F2) and uncoated drug-loaded formulation (F3), and were then compared to coated formulations (F10, F11, F14, and F15). Selection of coated formulations was based on the in-vitro drug release data obtained. SEM photomicrograph of blank bead (figures 3-5a and 3-5b) shows almost spherical shape of the bead with rough outer surface. Micrographs of uncoated drug-loaded beads (figures 3-6a and 3-6b) exhibited porous and rough surface and had a sandy

appearance due to surface associated crystals of the drug [112]. Furthermore, photomicrograph of coated formulation F15 (figures 3-7a and 3-7b) indicated the smooth surface of the bead with presence of few large wrinkles which might be attributed to collapsing of polymer network during dehydration [103].



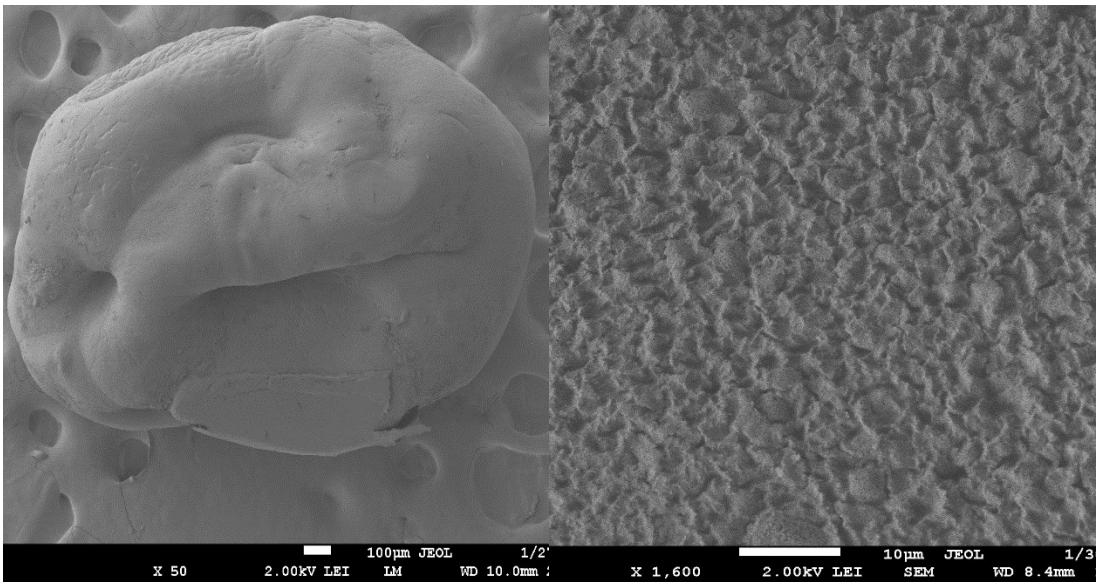
(a) **(b)**

Fig. 3-5: SEM photomicrographs of blank Alginic acid-CMC beads (Formulation F2)



(a) **(b)**

Fig. 3-6: SEM photomicrographs of uncoated CTZ loaded beads (Formulation F3)



(a) **(b)**

Fig. 3-7: SEM photomicrographs of double coated CTZ loaded beads (Formulation F15)

3.4.8 Evaluation of surface roughness of beads

AFM was utilized to examine the surface topography of the blank, uncoated and coated beads. Using easyScan 2 software average roughness and root mean square values were derived and are reported in Table 3.7. Average roughness (Ra) is the arithmetic mean of the standard deviations in height and, Root mean square (Rq) is the square root of the average of height deviations taken from the mean data plane. Rq value is often considered to be more sensitive than the Ra value, owing to its large deviation from mean line/plane [113, 114]. The obtained Rq values (figure 3-8) suggest that the coating of beads decreased the surface roughness. The 3D morphological images shown in fig 3-9 and fig 3-10 are in agreement with RMS values and show smooth surfaces for coated beads in comparison to blank and uncoated ones. The relatively smooth surface supports

the assumption that drug release from beads is caused by diffusion and matrix erosion [115].

Table 3.7: Surface roughness measurements		
Batch code	Average roughness <Ra> (nm) (Mean ± SD, n=3)	Root mean square roughness <Rq> (nm) (Mean ± SD, n=3)
F2	756.9 ± 611.3	916.3 ± 805.8
F3	956.4 ± 544.2	1102.6 ± 623.5
F10	264.2 ± 343.8	311.8 ± 408.8
F12	576.9 ± 544.6	680.0 ± 595.9
F14	582.1 ± 176.7	672.2 ± 204.6
F15	627.2 ± 307.5	712.9 ± 347.4

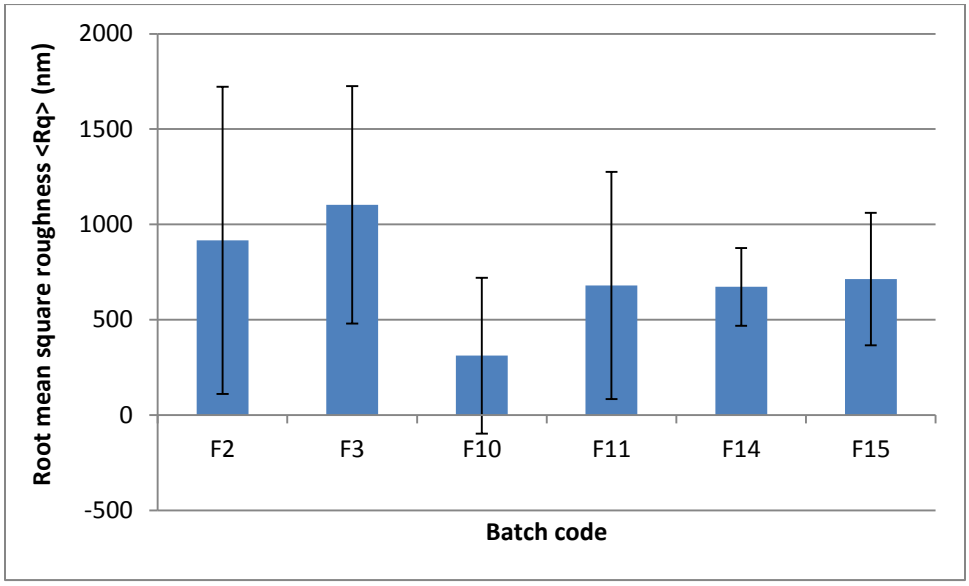
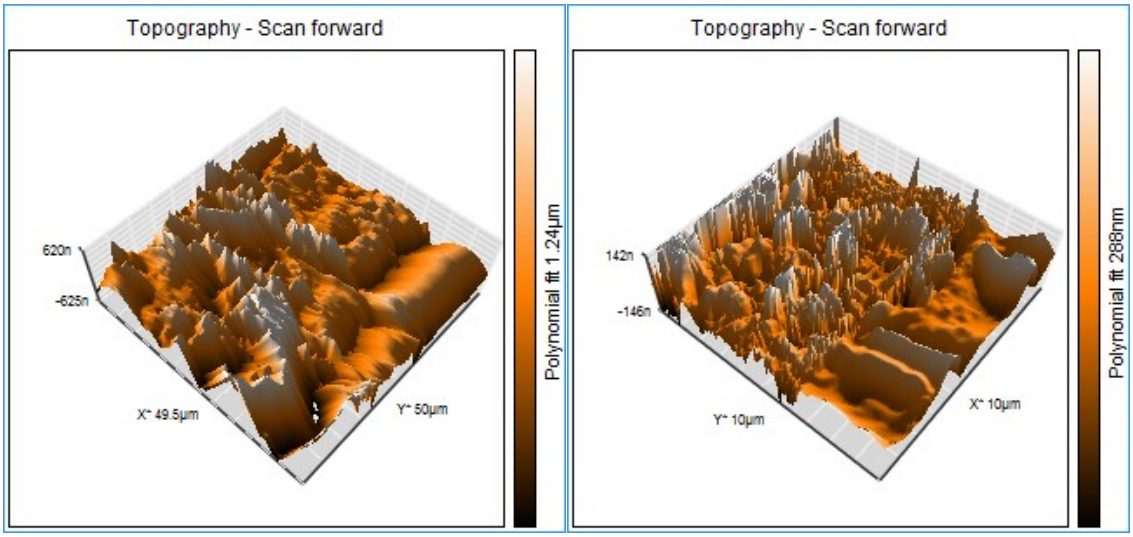
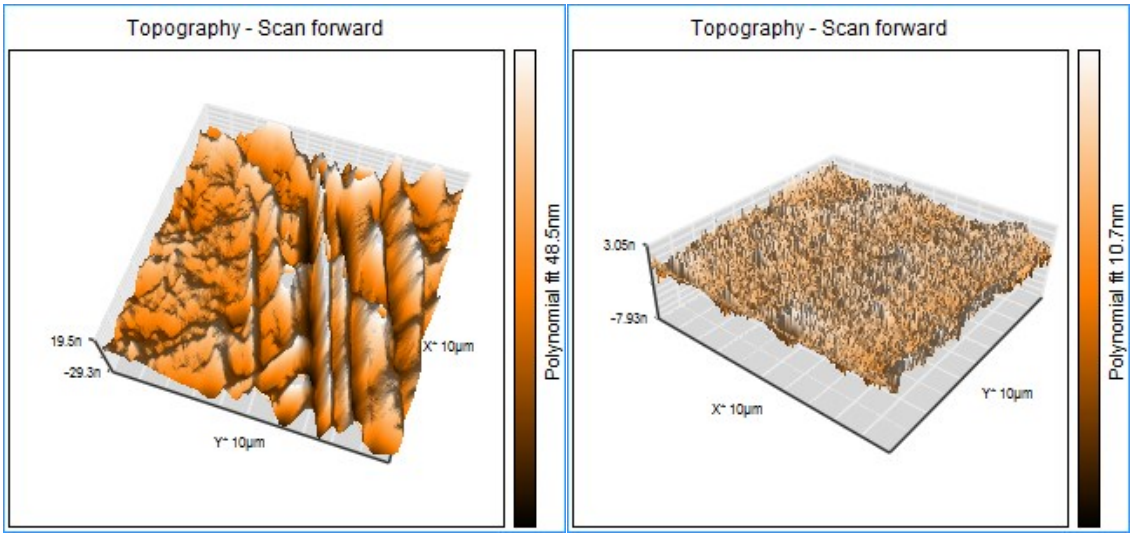


Fig. 3-8: Comparison of surface roughness of blank, uncoated and coated beads. Results indicate mean % ± SD, (n=3).

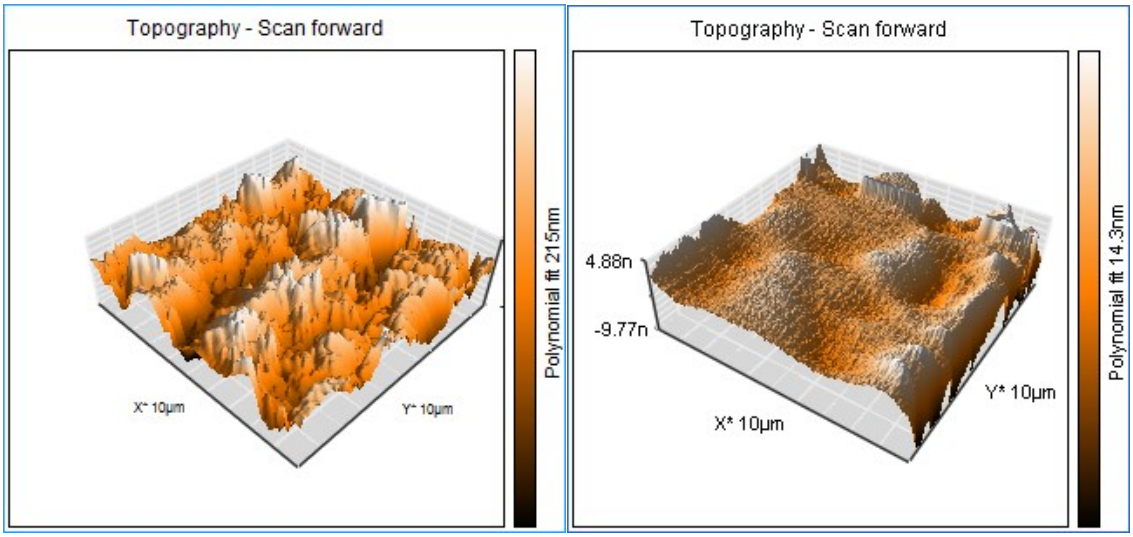


(a) **(b)**

Fig. 3-9: AFM micrographs of (a) blank formulation- F2, and (b) uncoated formulation- F3



(a) **(b)**



(c) **(d)**

Fig. 3-10: AFM micrographs of Eudragit coated formulations- (a) F10, (b) F11, (c) F14, and (d) F15.

3.5 CONCLUSION

Sustained release beads of ceftriaxone sodium were successfully prepared by ionotropic-external gelation technique using sodium alginate and CMC as polymers. FT-IR spectrums indicated the compatibility of polymers with the drug in the formulation. Prepared beads exhibited higher drug entrapment efficiency and prolonged release characteristics. To further control the drug release, enteric coat was applied where double coating with Eudragit® S-100 followed by Eudragit® L100 gave the best results. The coating polymers not only sustained the release of drug as indicated by in-vitro release data, but also prevented its release at low pH as observed in swelling results.

Future work on this project includes investigating different techniques that can be used for coating of beads and in-vivo testing in animal model to substantiate the in-vitro results.

3.6 REFERENCES

28. Samanidou, V., E. Hapeshi, and I. Papadoyannis, *Rapid and sensitive high-performance liquid chromatographic determination of four cephalosporin antibiotics in pharmaceuticals and body fluids.* Journal of Chromatography B, 2003. **788**(1): p. 147-158.

78. Cojocel, C., et al., *Nephrotoxic potential of first-, second-, and third-generation cephalosporins.* Archives of toxicology, 1988. **62**(6): p. 458-464.

79. Cho, S.W., J.S. Lee, and S.H. Choi, *Enhanced oral bioavailability of poorly absorbed drugs. I. Screening of absorption carrier for the ceftriaxone complex.* Journal of pharmaceutical sciences, 2004. **93**(3): p. 612-620.

80. Balant, L.P., P. Dayer, and R. Auckenthaler, *Clinical pharmacokinetics of the third generation cephalosporins.* Clinical pharmacokinetics, 1985. **10**(2): p. 101-143.

81. Neelam, S., G. Puneet, and B. Arundhati, *Enhancement of intestinal absorption of poorly absorbed Ceftriaxone Sodium by using mixed micelles of Polyoxy Ethylene (20) Cetyl Ether & Oleic Acid as peroral absorption enhancers.* Archives of applied science research, 2010. **2**(3): p. 131-142.

82. Shahiwala, A., *Formulation approaches in enhancement of patient compliance to oral drug therapy.* Expert opinion on drug delivery, 2011. **8**(11): p. 1521-1529.

83. Choi, S.-H. and J.-S. Lee, *Complexes to improve oral absorption of poorly absorbable antibiotics*. 2001, Google Patents.

84. Dey, N., S. Majumdar, and M. Rao, *Multiparticulate drug delivery systems for controlled release.* Tropical Journal of Pharmaceutical Research, 2008. 7(3): p. 1067-1075.

85. Manjanna, K., K.T. Pramod, and B. Shivakumar, *Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis.* Drug discoveries & therapeutics, 2010. 4(2): p. 109-122.

86. Bajpai, S., S.K. Saxena, and S. Sharma, *Swelling behavior of barium ions-crosslinked bipolymeric sodium alginate–carboxymethyl guar gum blend beads.* Reactive and Functional Polymers, 2006. 66(6): p. 659-666.

87. Bajpai, S. and S. Sharma, *Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca 2+ and Ba 2+ ions.* Reactive and Functional Polymers, 2004. 59(2): p. 129-140.

88. Ibrahim, S.M., F.I.A. El Fadl, and A.A. El-Naggar, *Preparation and characterization of crosslinked alginate–CMC beads for controlled release of nitrate salt.* Journal of Radioanalytical and Nuclear Chemistry, 2014. 299(3): p. 1531-1537.

89. Chourasia, M. and S. Jain, *Pharmaceutical approaches to colon targeted drug delivery systems.* J Pharm Pharm Sci, 2003. 6(1): p. 33-66.

90. Liu, F., et al., *A novel concept in enteric coating: a double-coating system providing rapid drug release in the proximal small intestine.* Journal of Controlled Release, 2009. 133(2): p. 119-124.

91. Shrivastava, S.M., et al., *A novel high performance liquid chromatographic method for simultaneous determination of ceftriaxone and sulbactam in sulbactomax.* International journal of biomedical science: IJBS, 2009. 5(1): p. 37.

92. Manjanna, K., K. Rajesh, and B. Shivakumar, *Formulation and optimization of natural polysaccharide hydrogel microbeads of aceclofenac sodium for oral controlled drug delivery.* American Journal of Medical Sciences and Medicine, 2013. 1(1): p. 5-17.

93. Ahad, H.A., et al., *Preparation and evaluation of famotidine high density gastro retentive microspheres with synthetic and natural polymers.* J Pharm Educ Res, 2011. 2(1): p. 110-8.

94. Arora, G., et al., *Formulation and evaluation of controlled release matrix mucoadhesive tablets of domperidone using Salvia plebeian gum.* Journal of advanced pharmaceutical technology & research, 2011. 2(3): p. 163.

95. Das, R.K., N. Kasoju, and U. Bora, *Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells.* Nanomedicine: Nanotechnology, Biology and Medicine, 2010. 6(1): p. 153-160.

96. Siepmann, J. and N. Peppas, *Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC).* Advanced drug delivery reviews, 2012. 64: p. 163-174.

97. Devi, N. and D.K. Kakati, *Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex.* Journal of Food Engineering, 2013. 117(2): p. 193-204.

98. Widmann, G., *Interpreting TGA curves.* 2001. Usercom 1.

99. NANOSURF AG, S., *easyScan 2 AFM- Operating Instructions.* 2005. Version 1.3.

100. Rokstad, A.M.A., et al., *Advances in biocompatibility and physico-chemical characterization of microspheres for cell encapsulation.* Advanced drug delivery reviews, 2014. 67: p. 111-130.

101. Sankalia, M.G., et al., *Papain entrapment in alginate beads for stability improvement and site-specific delivery: physicochemical characterization and factorial optimization using neural network modeling.* AAPS PharmSciTech, 2005. 6(2): p. E209-E222.

102. De Villiers, M.M., P. Aramwit, and G.S. Kwon, *Nanotechnology in drug delivery*. 2008: Springer Science & Business Media.

103. Pal, P. and F. Banat, *Contaminants in Industrial Lean Amine Solvent and their Removal using Biopolymers: A New Aspect.* J Phys Chem Biophys, 2014. 4(135): p. 2161-0398.1000135.

104. Crcarevska, M.S., M.G. Dodov, and K. Goracinova, *Chitosan coated Ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa.* European Journal of Pharmaceutics and Biopharmaceutics, 2008. 68(3): p. 565-578.

105. Patil, J., et al., *Ionotropically gelled novel hydrogel beads: Preparation, characterization and in vitro evaluation.* Indian journal of pharmaceutical sciences, 2011. 73(5): p. 504.

106. Jani, P., et al., *Formulation and evaluation of controlled release floating microspheres of tolperisone hydrochloride.* Asian Journal of Pharmaceutics, 2012. 6(3): p. 190.

107. Costa, P. and J.M.S. Lobo, *Modeling and comparison of dissolution profiles.* European journal of pharmaceutical sciences, 2001. 13(2): p. 123-133.

108. Adochitei, A. and G. Drochioiu, *Rapid characterization of peptide secondary structure by FT-IR spectroscopy.* Rev Roum Chim, 2011. 56(8): p. 783-791.

109. Kong, J. and S. Yu, *Fourier transform infrared spectroscopic analysis of protein secondary structures.* Acta biochimica et biophysica Sinica, 2007. 39(8): p. 549-559.

110. Fontes, G.C., et al., *Characterization of Antibiotic-Loaded Alginate-Osa Starch Microbeads Produced by Ionotropic Pregelation.* BioMed research international, 2013. 2013.

111. El-Sayed, S., et al., *DSC, TGA and dielectric properties of carboxymethyl cellulose/polyvinyl alcohol blends.* Physica B: Condensed Matter, 2011. 406(21): p. 4068-4076.

112. Manjanna, K., B. Shivakumar, and T. Kumar, *Diclofenac Sodium Microbeads for Oral Sustained Drug Delivery.* International Journal of PharmTech Research, 2009. 1(2).

113. Shahgaldian, P., et al., *AFM imaging of calixarene based solid lipid nanoparticles in gel matrices.* European journal of pharmaceutics and biopharmaceutics, 2003. 55(1): p. 107-113.

114. Oraby, S.E. and A.M. Alaskari, *Atomic force microscopy (AFM) topographical surface characterization of multilayer-coated and uncoated carbide inserts.* Microscopy (Image data type:“Friction”), 2010. 33: p. 38.

115. Mu, L. and S. Feng, *A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS.* Journal of controlled release, 2003. 86(1): p. 33-48.

REFERENCES

1. Clardy, J., M.A. Fischbach, and C.R. Currie, *The natural history of antibiotics.* Current biology, 2009. 19(11): p. R437-R441.
2. Fox, L.M., *Remington: The Science and Practice of Pharmacy.* 21st Edition, 2006: p. 1633.
3. *Dorland's Medical Dictionary for Health Consumers.* 2007.
4. Holten, K.B. and E.M. Onusko, *Appropriate prescribing of oral beta-lactam antibiotics.* American family physician, 2000. 62(3): p. 611-620.
5. Essack, S.Y., *The development of β-lactam antibiotics in response to the evolution of β-lactamases.* Pharmaceutical research, 2001. 18(10): p. 1391-1399.
6. Zaffiri, L., J. Gardner, and L.H. Toledo-Pereyra, *History of antibiotics. From salvarsan to cephalosporins.* Journal of Investigative Surgery, 2012. 25(2): p. 67-77.
7. Dougherty, T., *Antibiotic Discovery and Development:.... Vol. 1*. 2012: Springer Science & Business Media.
8. Alic, M., *"Antibitics" Gale Enclopedia of Cancer.* 2002.
9. Sheehan, J.C. and K.R. Henery-Logan, *The total synthesis of penicillin V*. Journal of the American Chemical Society, 1959. 81(12): p. 3089-3094.
10. Deshpande, A., K. Baheti, and N. Chatterjee, *Degradation of β-lactam antibiotics.* Curr. Sci, 2004. 87: p. 1684-1695.
11. Abraham, E., et al., *Cephalosporin N: a new type of penicillin.* 1953.

12. Foye, W.O., T.L. Lemke, and D.A. Williams, *Foye's principles of medicinal chemistry*. 2008: Lippincott Williams & Wilkins.

13. Hamilton-Miller, J., *Development of the semi-synthetic penicillins and cephalosporins.* International journal of antimicrobial agents, 2008. 31(3): p. 189-192.

14. Drawz, S.M. and R.A. Bonomo, *Three decades of β-lactamase inhibitors.* Clinical microbiology reviews, 2010. 23(1): p. 160-201.

15. Papp-Wallace, K.M., et al., *Carbapenems: past, present, and future.* Antimicrobial agents and chemotherapy, 2011. 55(11): p. 4943-4960.

16. Wise, R., *In vitro and pharmacokinetic properties of the carbapenems.* Antimicrobial agents and chemotherapy, 1986. 30(3): p. 343.

17. BREWER, N.S. and W.C. HELLINGER. *The monobactams*. in *Mayo Clinic Proceedings*. 1991. Elsevier.

18. Hellinger, W.C. and N.S. Brewer. *Carbapenems and monobactams: imipenem, meropenem, and aztreonam*. in *Mayo Clinic Proceedings*. 1999. Elsevier.

19. SHOJI, J.I., et al., *Asparenomycins A, B and C, new carbapenem antibiotics. II. Isolation and chemical characterization.* The Journal of antibiotics, 1982. 35(1): p. 15-23.

20. Kobayashi, F., et al., *Antimicrobial and beta-lactamase inhibitory activities of carpetimycins A and B, new carbapenem antibiotics.* Antimicrobial agents and chemotherapy, 1982. 21(4): p. 536-544.

21. Georgopapadakou, N., *Penicillin-binding proteins and bacterial resistance to beta-lactams.* Antimicrobial Agents and Chemotherapy, 1993. 37(10): p. 2045.

22. Cunha, B., *Third-generation cephalosporins: a review.* Clinical therapeutics, 1992. 14(5): p. 616.

23. Page, M.I., *The mechanisms of reactions of. beta.-lactam antibiotics.* Accounts of Chemical Research, 1984. 17(4): p. 144-151.

24. Greenwood, D., *Antibiotics of the beta-lactam group*. 1982: Chichester, UK; Research Studies Press, Div. of John Wiley & Sons.

25. Shockman, G. and J.-V. Höltje, *Microbial peptidoglycan (murein) hydrolases.* New comprehensive biochemistry, 1994. 27: p. 131-166.

26. DePestel, D.D., et al., *Cephalosporin use in treatment of patients with penicillin allergies.* Journal of the American Pharmacists Association: JAPhA, 2007. 48(4): p. 530-540.

27. Jindal, S.K., et al., *Textbook of Pulmonary and Critical Care Medicine Vols 1 and 2*. 2011: JP Medical Ltd.

28. Samanidou, V., E. Hapeshi, and I. Papadoyannis, *Rapid and sensitive high-performance liquid chromatographic determination of four cephalosporin antibiotics in pharmaceuticals and body fluids.* Journal of Chromatography B, 2003. 788(1): p. 147-158.

29. Edberg, S.C., *Antibiotics and infection*. 1983: Churchill Livingstone.

30. Powers, J.H., *Use and Importance of Cephalosporins in Human Medicine.* Online.

31. Zhanel, G.G., et al., *Ceftaroline.* Drugs, 2009. 69(7): p. 809-831.

32. Garcia-Rodriguez, J., J.M. Bellido, and J.G. Sanchez, *Oral cephalosporins: current perspectives.* International journal of antimicrobial agents, 1995. 5(4): p. 231-243.

33. OnlineTM, L.-C., L.-D. OnlineTM, and O. Hudson, *Lexi-Comp*. 2012, Inc.

34. Porter, R.S., et al., *The Merck manual online*. 2006: Merck Research Laboratories.

35. Behin, S., I. Punitha, and S. Krishnan, *Stability Studies of Cefepime Hydrochloride by Stability Indicating RP-HPLC Method.*

36. Hornish, R.E. and S. Katarski, *Cephalosporins in veterinary medicine-ceftiofur use in food animals.* Current topics in medicinal chemistry, 2002. 2(7): p. 717-731.

37. Lehto, V.-P. and E. Laine, *Assessment of physical stability of different forms of cefadroxil at high humidities.* International journal of pharmaceutics, 1998. 163(1): p. 49-62.

38. Diaconu, D., et al., *Studies regarding the stability of pharmaceutical formulations realized through association of beta-lactamic antibiotics 1. Stability of an oral suspension powder containing cefadroxil and cefixim.*

39. do Nascimento, T.G., et al., *Short-term stability studies of ampicillin and cephalexin in aqueous solution and human plasma: Application of least squares method in Arrhenius equation.* Journal of pharmaceutical and biomedical analysis, 2013. 73: p. 59-64.

40. Hu, C.Q., et al., *Assessment of the stability of cefazolin sodium in solids by TGA decomposition Kinetics.* Journal of Chinese Pharmaceutical Sciences, 1998. 7: p. 80-85.

41. Gupta, V.D., *Chemical Stability of Cefazolin Sodium after Reconstituting in 0.9% Sodium Chloride Injection and Storage in Polypropylene Syringes for Pediatric*

Use. International journal of pharmaceutical compounding, 2002. 7(2): p. 152-154.

42. de Bolós, J., et al., *Prediction of stability of cefazolin sodium in perfusion fluids.* International journal of pharmaceutics, 1987. 40(1): p. 175-177.

43. Robinson, R.F., et al., *Stability of cefazolin sodium in four heparinized and non-heparinized dialysate solutions at 38 C.* Peritoneal dialysis international, 2006. 26(5): p. 593-597.

44. Carone, S., et al., *Stability of frozen solutions of cefazolin sodium.* American Journal of Health-System Pharmacy, 1976. 33(7): p. 639-641.

45. Donnelly, R.F., *Stability of Cefazolin Sodium in Polypropylene Syringes and Polyvinylchloride Minibags.* The Canadian journal of hospital pharmacy, 2011. 64(4): p. 241.

46. Xu, Q.A., et al., *Stability of three cephalosporin antibiotics in AutoDose Infusion System bags.* Journal of the American Pharmaceutical Association (Washington, DC: 1996), 2001. 42(3): p. 428-431.

47. Olsen, B.A., et al., *Accelerated conditions for stability assessment of bulk and formulated cefaclor monohydrate.* Pharmaceutical development and technology, 1997. 2(4): p. 303-312.

48. Dorman, D.E., et al., *Isolation and structure elucidation of the major degradation products of cefaclor in the solid state.* Journal of pharmaceutical sciences, 1997. 86(5): p. 540-549.

49. PATEL, A., et al., *Formulation, development and evaluation of cefaclor extended release matrix tablet.* Int J Pharm Pharm Sci, 2012. 4: p. 355-57.

50. Medenecka, B., et al., *Stability of the crystalline form of cefaclor monohydrate and its pharmaceutical preparations.* Acta poloniae pharmaceutica, 2008. 66(5): p. 563-569.

51. Kuksal, K. and K. Pathak, *Use of solubility parameter to design dry suspension of cefaclor as a dual pack system.* Indian journal of pharmaceutical sciences, 2008. 70(5): p. 609.

52. Oberholtzer, E.R. and G.S. Brenner, *Cefoxitin sodium: Solution and solid-state chemical stability studies.* Journal of pharmaceutical sciences, 1979. 68(7): p. 863-866.

53. Zając, M., et al., *Evaluation of stability of cefuroxime axetil in solid state.* Journal of pharmaceutical and biomedical analysis, 2003. 32(6): p. 1181-1187.

54. Jelińska, A., et al., *The stability of the amorphous form of cefuroxime axetil in solid state.* Journal of pharmaceutical and biomedical analysis, 2006. 41(3): p. 1075-1081.

55. Hecq, J.-D., et al., *Effect of the freezing conditions and microwave thawing power on the stability of Cefuroxime in dextrose 5% infusion polyolefin bags at 4 C.* Annals of Pharmacotherapy, 2005. 39(7-8): p. 1244-1248.

56. Smith, B., *Effect of storage temperature and time on stability of cefmenoxime, ceftriaxone, and cefotetan in 5% dextrose injection.* American Journal of Health-System Pharmacy, 1983. 40(6): p. 1024-1025.

57. Gupta, V.D., et al., *Chemical stability of cefotetan disodium in 5% dextrose and 0.9% sodium chloride injections.* Journal of clinical pharmacy and therapeutics, 1990. 15(2): p. 109-114.

58. Gupta, V.D. and K. Stewart, *Stability of cefamandole nafate and cefoxitin sodium solutions.* American Journal of Health-System Pharmacy, 1981. 38(6): p. 875-879.

59. O'Brien, M., J. Portnoff, and E. Cohen, *Cefoxitin sodium compatibility with intravenous infusions and additives.* American Journal of Health-System Pharmacy, 1979. 36(1): p. 33-38.

60. Venugopalarao, G., M.S. Gowtham, and N. Sarada, *Formulation evaluation and stability studies of hydrogel tablets containing Cefditoren Pivoxil.* Journal of Pharmacy Research, 2013. 7(3): p. 230-234.

61. Paul, Y., M. Kumar, and B. Singh, *Formulation and in Vitro evaluation of Gastroretentive drug delivery system of Cefixime Trihydrate.* Int. J. Drug Dev. & Res, 2011. 3(4): p. 148-161.

62. Kitamura, S., et al., *Dehydration effect on the stability of cefixime trihydrate.* International journal of pharmaceutics, 1990. 59(3): p. 217-224.

63. HARITHA, M. and B.P. KUMAR, *FORMULATION AND EVALUATION OF CEFIXIME DRY EMULSION.*

64. C. Senthilkumar, G.R., S. Subathra, T. Suresh Kumar, S. Arun Kumar, *ENHANCEMENT OF SOLUBILITY AND DISSOLUTION RATE OF DRUG CEFPODOXIME PROXETIL.* INTERNATIONAL RESEARCH HOURNAL OF PHARMACY, 2012: p. 6.

65. Genentech USA, I., *ROCEPHIN (ceftriaxone sodium) FOR INJECTION*.

66. Cantón, E., M.J. Esteban, and F. Rius, *Factors affecting the stability of ceftriaxone sodium in solution on storage.* International journal of pharmaceutics, 1993. 92(1): p. 47-53.

67. Gupta, V.D., *Stability of Cefotaxime Sodium After Reconstitution in 0.9% Sodium Chloride Injection and Storage in Polypropylene Syringes for Pediatric Use.* International journal of pharmaceutical compounding, 2001. 6(3): p. 234-236.

68. Gupta, V.D., C. Bethea, and M. Torre, *CHEMICAL STABILITIES OF CEFOPERAZONE SODIUM AND CEFTAZIDIME IN 5% DEXTROSE AND 0· 9% SODIUM CHLORIDE INJECTIONS.* Journal of clinical pharmacy and therapeutics, 1988. 13(3): p. 199-205.

69. Kodym, A., et al., *Stability of ceftazidime in 1% and 5% buffered eye drops determined with HPLC method.* Acta poloniae pharmaceutica, 2010. 68(1): p. 99-107.

70. Arsène, M., et al., *Comparison of ceftazidime degradation in glass bottles and plastic bags under various conditions.* Journal of clinical pharmacy and therapeutics, 2002. 27(3): p. 205-209.

71. Hospira, I., *MAXIPIME (Cefepime Hydrochloride, USP) for Injection, Reference ID: 3185289* 2012.

72. Rabouan-Guyon, S.M., et al., *Stability study of cefepime in different infusion solutions.* International journal of pharmaceutics, 1997. 154(2): p. 185-190.

73. Kodym, A., et al., *Stability of cefepime in aqueous eye drops.* Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2011. 66(1): p. 17-23.

74. Trissel, L.A. and Q.A. Xu, *Stability of cefepime hydrochloride in autodose infusion system bags.* Annals of Pharmacotherapy, 2003. 37(6): p. 804-807.

75. JANICE K POHLMAN, A.R.P., *MEDICAL REVIEW(S), CENTER FOR DRUG EVALUATION AND RESEARCH, APPLICATION NUMBER: 200327, REFERENCE ID: 2857265.* 2010.

76. Ikeda, Y., et al., *Stability and stabilization studies of TAK-599 (ceftaroline fosamil), a novel N-phosphono type prodrug of anti-methicillin resistant Staphylococcus aureus cephalosporin T-91825.* Chemical and Pharmaceutical Bulletin, 2008. 56(10): p. 1406-1411.

77. Forest Pharmaceuticals, I., *Teflaro™ (ceftaroline fosamil)-clean-labeling text-2010-11-08* 2010.

78. Cojocel, C., et al., *Nephrotoxic potential of first-, second-, and third-generation cephalosporins.* Archives of toxicology, 1988. 62(6): p. 458-464.

79. Cho, S.W., J.S. Lee, and S.H. Choi, *Enhanced oral bioavailability of poorly absorbed drugs. I. Screening of absorption carrier for the ceftriaxone complex.* Journal of pharmaceutical sciences, 2004. 93(3): p. 612-620.

80. Balant, L.P., P. Dayer, and R. Auckenthaler, *Clinical pharmacokinetics of the third generation cephalosporins.* Clinical pharmacokinetics, 1985. 10(2): p. 101-143.

81. Neelam, S., G. Puneet, and B. Arundhati, *Enhancement of intestinal absorption of poorly absorbed Ceftriaxone Sodium by using mixed micelles of Polyoxy Ethylene (20) Cetyl Ether & Oleic Acid as peroral absorption enhancers.* Archives of applied science research, 2010. 2(3): p. 131-142.

82. Shahiwala, A., *Formulation approaches in enhancement of patient compliance to oral drug therapy.* Expert opinion on drug delivery, 2011. 8(11): p. 1521-1529.

83. Choi, S.-H. and J.-S. Lee, *Complexes to improve oral absorption of poorly absorbable antibiotics*. 2001, Google Patents.

84. Dey, N., S. Majumdar, and M. Rao, *Multiparticulate drug delivery systems for controlled release.* Tropical Journal of Pharmaceutical Research, 2008. 7(3): p. 1067-1075.

85. Manjanna, K., K.T. Pramod, and B. Shivakumar, *Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis.* Drug discoveries & therapeutics, 2010. 4(2): p. 109-122.

86. Bajpai, S., S.K. Saxena, and S. Sharma, *Swelling behavior of barium ions-crosslinked bipolymeric sodium alginate–carboxymethyl guar gum blend beads.* Reactive and Functional Polymers, 2006. 66(6): p. 659-666.

87. Bajpai, S. and S. Sharma, *Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca 2+ and Ba 2+ ions.* Reactive and Functional Polymers, 2004. 59(2): p. 129-140.

88. Ibrahim, S.M., F.I.A. El Fadl, and A.A. El-Naggar, *Preparation and characterization of crosslinked alginate–CMC beads for controlled release of nitrate salt.* Journal of Radioanalytical and Nuclear Chemistry, 2014. 299(3): p. 1531-1537.

89. Chourasia, M. and S. Jain, *Pharmaceutical approaches to colon targeted drug delivery systems.* J Pharm Pharm Sci, 2003. 6(1): p. 33-66.

90. Liu, F., et al., *A novel concept in enteric coating: a double-coating system providing rapid drug release in the proximal small intestine.* Journal of Controlled Release, 2009. 133(2): p. 119-124.

91. Shrivastava, S.M., et al., *A novel high performance liquid chromatographic method for simultaneous determination of ceftriaxone and sulbactam in sulbactomax.* International journal of biomedical science: IJBS, 2009. 5(1): p. 37.

92. Manjanna, K., K. Rajesh, and B. Shivakumar, *Formulation and optimization of natural polysaccharide hydrogel microbeads of aceclofenac sodium for oral controlled drug delivery.* American Journal of Medical Sciences and Medicine, 2013. 1(1): p. 5-17.

93. Ahad, H.A., et al., *Preparation and evaluation of famotidine high density gastro retentive microspheres with synthetic and natural polymers.* J Pharm Educ Res, 2011. 2(1): p. 110-8.

94. Arora, G., et al., *Formulation and evaluation of controlled release matrix mucoadhesive tablets of domperidone using Salvia plebeian gum.* Journal of advanced pharmaceutical technology & research, 2011. 2(3): p. 163.

95. Das, R.K., N. Kasoju, and U. Bora, *Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells.* Nanomedicine: Nanotechnology, Biology and Medicine, 2010. 6(1): p. 153-160.

96. Siepmann, J. and N. Peppas, *Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC).* Advanced drug delivery reviews, 2012. 64: p. 163-174.

97. Devi, N. and D.K. Kakati, *Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex.* Journal of Food Engineering, 2013. 117(2): p. 193-204.

98. Widmann, G., *Interpreting TGA curves.* 2001. Usercom 1.

99. NANOSURF AG, S., *easyScan 2 AFM- Operating Instructions.* 2005. Version 1.3.

100. Rokstad, A.M.A., et al., *Advances in biocompatibility and physico-chemical characterization of microspheres for cell encapsulation.* Advanced drug delivery reviews, 2014. 67: p. 111-130.

101. Sankalia, M.G., et al., *Papain entrapment in alginate beads for stability improvement and site-specific delivery: physicochemical characterization and factorial optimization using neural network modeling.* AAPS PharmSciTech, 2005. 6(2): p. E209-E222.

102. De Villiers, M.M., P. Aramwit, and G.S. Kwon, *Nanotechnology in drug delivery*. 2008: Springer Science & Business Media.

103. Pal, P. and F. Banat, *Contaminants in Industrial Lean Amine Solvent and their Removal using Biopolymers: A New Aspect.* J Phys Chem Biophys, 2014. 4(135): p. 2161-0398.1000135.

104. Crcarevska, M.S., M.G. Dodov, and K. Goracinova, *Chitosan coated Ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa.* European Journal of Pharmaceutics and Biopharmaceutics, 2008. 68(3): p. 565-578.

105. Patil, J., et al., *Ionotropically gelled novel hydrogel beads: Preparation, characterization and in vitro evaluation.* Indian journal of pharmaceutical sciences, 2011. 73(5): p. 504.

106. Jani, P., et al., *Formulation and evaluation of controlled release floating microspheres of tolperisone hydrochloride.* Asian Journal of Pharmaceutics, 2012. 6(3): p. 190.

107. Costa, P. and J.M.S. Lobo, *Modeling and comparison of dissolution profiles.* European journal of pharmaceutical sciences, 2001. 13(2): p. 123-133.

108. Adochitei, A. and G. Drochioiu, *Rapid characterization of peptide secondary structure by FT-IR spectroscopy.* Rev Roum Chim, 2011. 56(8): p. 783-791.

109. Kong, J. and S. Yu, *Fourier transform infrared spectroscopic analysis of protein secondary structures.* Acta biochimica et biophysica Sinica, 2007. 39(8): p. 549-559.

110. Fontes, G.C., et al., *Characterization of Antibiotic-Loaded Alginate-Osa Starch Microbeads Produced by Ionotropic Pregelation.* BioMed research international, 2013. 2013.

111. El-Sayed, S., et al., *DSC, TGA and dielectric properties of carboxymethyl cellulose/polyvinyl alcohol blends.* Physica B: Condensed Matter, 2011. 406(21): p. 4068-4076.

112. Manjanna, K., B. Shivakumar, and T. Kumar, *Diclofenac Sodium Microbeads for Oral Sustained Drug Delivery.* International Journal of PharmTech Research, 2009. 1(2).

113. Shahgaldian, P., et al., *AFM imaging of calixarene based solid lipid nanoparticles in gel matrices.* European journal of pharmaceutics and biopharmaceutics, 2003. 55(1): p. 107-113.

114. Oraby, S.E. and A.M. Alaskari, *Atomic force microscopy (AFM) topographical surface characterization of multilayer-coated and uncoated carbide inserts.* Microscopy (Image data type:"Friction"), 2010. 33: p. 38.

115. Mu, L. and S. Feng, *A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS.* Journal of controlled release, 2003. 86(1): p. 33-48.