Development and evaluation of a biopolymer based ceftriaxone sodium oral formulation

Nachiket Patel
University of Toledo

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A Thesis

entitled

Development and Evaluation of a Biopolymer based Ceftriaxone Sodium Oral Formulation

by

Nachiket Patel

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

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The University of Toledo

August 2014
Ceftriaxone sodium (CS) is a third-generation cephalosporin and belongs to BCS Class III. This antibiotic cannot be absorbed orally owing to its poor permeability through GI epithelia and its acid labile nature. The purpose of present investigation was to develop and evaluate a multiparticulate system exploiting pH-sensitive property and specific biodegradability of calcium alginate beads, for intestinal delivery of CS. Beads of CS entrapped in sodium alginate consisting of different polymers such as sodium carboxymethylcellulose, acacia, HPMC K4M and HPMC K15M as drug release modifiers were prepared by ionotropic gelation method using calcium chloride as a cross-linking agent followed by enteric coating with cellulose acetate phthalate. Sustained-release polymeric beads were then evaluated for entrapment efficiency using HPLC, in vitro drug release examined in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8), swellability, particle size and surface morphology using optical microscopy and Scanning Electron Microscopy (SEM), and surface characterization using Atomic Force Microscopy (AFM). Thermal Gravimetric Analysis (TGA) was utilized to check the strength of the polymer matrix and thermal stability of the prepared
formulations. The drug entrapment efficiency for the optimized formulation was determined to be 74.66 ± 4.99%. Swelling properties of drug-loaded beads were found to be in range from 0.859 to 3.35. Beads coated with cellulose acetate phthalate and carboxymethylcellulose aqueous polymer dispersion exhibited sustained release and followed first-order kinetics and the mechanism of diffusion was anomalous or non-Fickian (diffusion and erosion dependent swelling mechanism). The particle size of the beads was between 1.039 ± 0.197 mm to 2.146 ± 0.357 mm. TGA demonstrated the cross-linking efficiency and thermal stability of the beads. SEM images demonstrated the structure and surface of the beads. AFM images demonstrated composition and polymer dependent variations in surface structure, morphology, and roughness.
Acknowledgements

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Chapter 1

INTRODUCTION

1.1 Antibiotics

1.1.1 Background

Diseases are exceptionally common and responsible for a huge number of illnesses unfavorably influencing human well-being. The majority of the infectious diseases are caused by bacteria. Infections caused by bacteria can be avoided, controlled and treated through an antibacterial group of compounds known as antibiotics [1]. Antibiotics are the compounds which either kill or inhibit the growth of microorganisms without significant toxicity to the host. The majority of antibacterial compounds are moderately small molecules having an atomic weight of less than 2000 atomic mass unit [2].

1.1.2 History

People have used antibiotics for hundreds of years, not actually knowing their potential. In 1928, Alexander Fleming made one of the most important contributions to the field of antibiotics that led to the development of the first modern era antibiotic, penicillin. Since
the 1970s, most new antibiotics have been synthetic modifications of naturally occurring antibiotics [3, 4].

1.1.3 Antibiotics classification

Antibacterial compounds are classified based on their origin of chemical/biosynthetic in a natural, semisynthetic and synthetic [5]. They can also be classified based on their biological activity in which they are divided into two broad groups according to their biological effect on microorganisms: Bacteriostatic agents are known to slow down or stall bacterial growth and bactericidal agents kill bacteria [6]. Yet, the most widely used classification is based on their mechanism or action, spectrum of activity or chemical structure and most of these antibacterial compounds target growth processes or bacterial functions.

1.1.4 Uses of antibiotics

Antibiotics are useful for treating bacterial infections, protozoal infections and for immunomodulation. They are also used for preventing surgical wound, dental antibiotic prophylaxis as well as for the conditions such as neutropenia [7] [8].

1.1.5 Mechanism of antibiotics

Two common mechanisms by which antibiotics work can be explained in the following ways: 1) Antibiotics which are bactericidal, kills the bacteria by either interfering with
the formation of the bacterium's cell wall or its cell contents. Examples includes penicillins, metronidazole, co-trimoxazole, daptomycin, nitrofurantoin and fluoroquinolones.

2) Antibiotics which are bacteriostatic, stops bacteria from multiplying by interfering with DNA replication, bacterial protein production or other aspects of bacterial cellular metabolism. Examples include sulphonamides, lincosamides, tetracyclines, trimethoprim, chloramphenicol, macrolides and spectinomycin [9].

1.1.6 Routes of administration for antibiotics

Antibiotics are available to be administered orally, intravenously, topically in the form of ointments and creams, eye and ear drops and also in the form of suppositories. Oral antibiotics are typically prescribed for community-acquired infections, whereas intravenous administration is required for more serious infections such as deep-seated systemic infections.

1.1.7 Selection of an antibacterial agent

Due to the wide array of drugs available in the market, choosing an antibacterial agent can be challenging sometimes. To overcome those situations, these drugs are grouped into classes based upon their biochemical structure, properties and their use. Clearance, mechanism of action, absorption and side effects are some of the properties that are shared by members of the same class. Based on these classes and properties of
antibiotics, it is easier to choose the most appropriate antibiotic for a particular patient [10].

1.1.8 Generalized effect of antibiotics

Antibacterial act by binding specific targets and subsequently corrupting specific biochemical processes. They also appear to stimulate a bacterial suicide process which does amplify the effect of the antibacterial. β-lactams are the group of antibiotics which tends to work by inhibiting the cell wall synthesis [11]. Antibiotics destroy bacteria by affecting their cell structure in two possible ways. Firstly, antibiotics can cause the cell wall to burst by weakening the cell walls of the infectious bacteria and secondly they can damage the cell membranes by causing a leakage of bacterial cell wall contents.

Antibiotics also function by interfering with the metabolism of bacteria. Tetracycline and erythromycin are the antibiotics that interfere with protein synthesis whereas rifampin inhibits nucleic acid biosynthesis. Antibiotics like trimethoprim or sulfonamide have a general blocking effect on cell metabolism [4].

1.2 General characteristics of antibiotics

1.2.1 Solubility

Most of the antibiotics are freely soluble in water; soluble in ethanol and sparingly soluble in 2-propanol and acetone [12].
1.2.2 Stability

Antibiotics stability depends on its chemical structure, method of isolation and the mechanisms of inactivation. First-generation antibiotics such as penicillin, are the most unstable, followed by its semisynthetic derivatives such as carboxycylin and ampicillin. Aminoglycosides are more stable antibiotics [13].

1.2.2.1 Chemical stability

Antibiotics are prone to hydrolysis which involves the cleavage by reaction of a drug molecule with water. The rate of reaction may triple in hydrolysis reaction with a 10°C rise in temperature [14]. Hydrolysis can occur in solution, suspensions and even solid dosage forms. Antibiotics having ester bonds, amide bonds or lactam rings are more prone to hydrolysis reactions [15].

1.2.2.2 Physical stability

(i) pH

Most antibiotics are either weak acids or bases that ionize in body fluids. These antibiotics lose their effectiveness as acidity increases [16]. For example, decomposition of penicillin G is strongly dependent on the pH of the aqueous phase [17].
(ii) **Temp**

Most antibiotics are heat labile. Therefore such antibiotics should be stored between 0 °C to -20 °C to maintain its stability; which will decrease the time to 10% degradation [18].

(iii) **Light**

Some of the antibiotics are light sensitive and will degrade due to photochemical reactions. Examples include quinolones, tetracyclines, nitrofuran, sulphonamides, etc. Therefore such antibiotics should be protected from direct sunlight [19].

Rifampicin and amphotericin B are very sensitive to light and starts to degrade. Therefore those antibiotics should be stored in the dark [18].

(iv) **Moisture**

Generally, antibiotics are stored in a refrigerator or freezer. The risk of water condensation increases if the powdered antibiotics are stored at -20 °C. Aminoglycosides are hygroscopic in nature and should be stored in a desiccator [18].

1.3 **Classification of antibiotics**

Antibiotics are mostly classified based on their chemical structure. Other alternatives for classifying antibiotics includes: based on its bacterial spectrum (broad or narrow), route of administration (oral, topical or injectable) or based on its spectrum of activity (bacteriostatic or bactericidal) [20]. Broad spectrum antibiotics are effective for treating
wide range of bacterial infections, whereas narrow spectrum antibiotics are effective only for treating certain types of bacteria. Antibiotics belonging to similar class will generally show similar patterns of effectiveness, allergic potential and toxicity. Below is the schematic representation for the classification of antibiotics [21].

![Schematic representation for the classification of antibiotics](image)

**Fig. 1-1:** Schematic representation for classification of antibiotics

Main classes of antibiotics grouped on the basis of chemical structure along with mechanism of action and examples are explained in the table 1-1. Commonly used antibiotics include Penicillins, Cephalosporins, Macrolides, Tetracyclines, Fluoroquinolones & Aminoglycosides [22].
<table>
<thead>
<tr>
<th>Class based on chemical structure</th>
<th>Mechanism of action</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams</td>
<td>Inhibit bacterial cell wall synthesis</td>
<td>Penicillins</td>
</tr>
<tr>
<td>• Penicillins</td>
<td></td>
<td>• Ampicillin</td>
</tr>
<tr>
<td>• Cephalosporins</td>
<td></td>
<td>• Amoxicillin</td>
</tr>
<tr>
<td>• Carbapenems</td>
<td></td>
<td>• Oxacillin</td>
</tr>
<tr>
<td>• Monobactams</td>
<td></td>
<td>Cephalosporins</td>
</tr>
<tr>
<td>• Cefdinir</td>
<td></td>
<td>• Cefotaxime</td>
</tr>
<tr>
<td>• Ceftriaxone</td>
<td></td>
<td>• Ceftriazone</td>
</tr>
<tr>
<td>Carbapenem</td>
<td></td>
<td>Carbapenem</td>
</tr>
<tr>
<td>• Meropenem</td>
<td></td>
<td>• Doripenem</td>
</tr>
<tr>
<td>• Doripenem</td>
<td></td>
<td>• Ertapenem</td>
</tr>
<tr>
<td>Monobactams</td>
<td></td>
<td>Monobactams</td>
</tr>
<tr>
<td>• Aztreonam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td>Inhibit bacterial protein synthesis</td>
<td>Erythromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azithromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clarithromycin</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Inhibit bacterial protein synthesis</td>
<td>Tetracycline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minocycline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doxycycline</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Inhibit bacterial DNA synthesis</td>
<td>Ciproflaxacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Levofloxacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ofloxacin</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Inhibit bacterial protein synthesis</td>
<td>Gentamicin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tobramycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amikacin</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>Blocks bacterial cell metabolism by inhibiting enzymes</td>
<td>Co-trimoxazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mefanide</td>
</tr>
<tr>
<td>Others</td>
<td>Inhibit bacterial protein synthesis</td>
<td>Fusidic acid</td>
</tr>
<tr>
<td></td>
<td>Inhibit bacterial DNA synthesis</td>
<td>Mupirocin</td>
</tr>
<tr>
<td></td>
<td>Inhibit bacterial cell wall synthesis</td>
<td>Metronidazole</td>
</tr>
<tr>
<td></td>
<td>Inhibit bacterial protein synthesis</td>
<td>Bacitracin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clindamycin</td>
</tr>
</tbody>
</table>

* Note: - The information list in this table is partly based on the following references [23].
1.4 β-lactam antibiotics

Beta-Lactams (β-lactam) are antibiotics that consists of a common β-lactam ring nucleus. The ring is a four-membered cyclic amide ring containing nitrogen atom attached to β-carbon relative to the carbonyl group [24]. Structure of β-lactam ring is shown in fig. 1-2.

![Chemical structure of β-lactam ring](image)

**Fig. 1-2:** Chemical structure of β-lactam ring

1.4.1 Classification of β-lactams

β-lactam subclasses include: (i) Penicillins; (ii) Cephalosporins and Cephemycins (cephems); (iii) Carbapenems; (iv) Monobactams; and their structure containing the common β-lactam ring is shown in fig. 1-3 [25].

![Structures of β-lactams](image)

**Fig. 1-3:** β-lactams containing the common β-lactam ring
There are more than 50 different β-lactams currently available on the market. All β-lactams bind to inactivate enzymes responsible for bacterial cell wall synthesis and have common properties as such: (i) acts as bactericidal; (ii) non-toxic in nature; (iii) relatively inexpensive; (iv) are organic acids and most are soluble in water [26].

They are inactivated by bacterial-produced enzymes called β-lactamases. Some of the common β-lactamase enzymes include: (i) Penicillinases; (ii) Cephalosporinases & Cephemycinases; (iii) Carbapenemases; (iv) Monobactams

1.5 Cephalosporins

Cephalosporins are broad spectrum bacterial antibiotics that consists of a β-lactam ring fused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus which share a structural similarity and mechanism of action with other β-lactam antibiotics (e.g. penicillins, carbapenems and monobactams) [27]. These β-lactam ring structures interfere with the bacterial cell wall synthesis by inhibiting essential steps in protein binding and eventually leads to cell lysis and death of the bacterial cell [28].

1.5.1 Uses of cephalosporins

Cephalosporins have been widely used as antimicrobials because of their clinical efficiency and desirable safety profile [29]. First and second-generation cephalosporins are most commonly used for community-acquired infections, whereas the
later generation cephalosporins are used for hospital-acquired infections or complicated community-acquired infections due to their better spectrum of activity against gram-positive and gram-negative bacteria [30].

1.5.2 Basic structure of cephalosporins

The basic structure of the cephalosporin molecule consists of a two ring system which includes a β-lactam ring condensed with 6-membered dihydrothiazine ring. Chemical compounds consisting this core are relatively stable to acid hydrolysis and have greater tolerance to β-lactamases. The antibacterial activity of these antibiotics seems to be affected by the modification of side-chain in position 7 of lactam ring; whereas the pharmacokinetic and receptor binding properties are affected by the alteration of the dihydrothiazine ring at position 3 [31, 32]. The basic core structure of the cephalosporin molecule is shown in fig. 1-4.

![Basic core structure of cephalosporins](image)

**Fig. 1-4:** Basic core structure of cephalosporins
Most of the available cephalosporins available on the market are semi-synthetic
derivatives of cephalosporin C, a compound with antibacterial activity produced by the
fungus Cephalosporium [33].

1.5.3 Classification of cephalosporins

Cephalosporins have been further classified into “five generations” based upon their
chemical properties and spectrum of activity against gram-positive and gram-negative
bacteria shown in table 1-2.

<table>
<thead>
<tr>
<th>Generations</th>
<th>Spectrum of Activity</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Generation</td>
<td>active against gram-positive cocci including penicillinase-producing * * * *</td>
<td>Cefadroxil</td>
</tr>
<tr>
<td></td>
<td>* * * *  * * * *  * * *  Staphylococcus aureus * * * *  * * *</td>
<td>Cefazolin</td>
</tr>
<tr>
<td></td>
<td>* * * *  * * * *  * * *</td>
<td>Cephalexin</td>
</tr>
<tr>
<td></td>
<td>* * * *  * * * *  * * *</td>
<td>Cephradine</td>
</tr>
<tr>
<td>Second Generation</td>
<td>active against gram-positive bacteria &amp; Citrobacter, Enterobacter, * * * *</td>
<td>Cefaclor</td>
</tr>
<tr>
<td></td>
<td>* * * *  * * * *  Haemophilus influenzae, Neisseria and Serratia species * * * *</td>
<td>Cefoxithin</td>
</tr>
<tr>
<td></td>
<td>* * * *</td>
<td>Cefprozil</td>
</tr>
<tr>
<td></td>
<td>* * * *</td>
<td>Cefonicid</td>
</tr>
<tr>
<td></td>
<td>* * * *</td>
<td>Cefuroxime</td>
</tr>
<tr>
<td>Third Generation</td>
<td>less active against gram-positive bacteria but more active against gram-negative bacteria &amp; also have greater stability against beta-lactamases * * * *</td>
<td>Cefdinir</td>
</tr>
<tr>
<td></td>
<td>* * * *</td>
<td>Ceftizoxime</td>
</tr>
<tr>
<td></td>
<td>* * * *</td>
<td>Ceftriaxone</td>
</tr>
</tbody>
</table>

Table 1.2: Generations of cephalosporins with spectrum of activity
| Fourth Generation | active against wide range of both gram-positive and gram-negative organisms | • Cefepime |
| Fifth Generation | active against gram-positive and gram-negative organisms including methicillin resistant *Staphylococcus aureus* | • Ceftaroline  
• Ceftobiprole |

*Note: - The information list in this table is based on the following references [34].

Cephalosporins such as cephalexin, cepadrine, cefadroxil, cefpodoxime and cefachlor are more stable at acidic pH when administered orally. The bioavailability of those compounds range from 75-90% and are usually well absorbed within the systemic circulation. Other antibiotics are either administered via IV or IM route with plasma concentrations peaking ~30 min after injection [35].

### 1.6 Third-generation cephalosporins

Third-generation cephalosporins are widely prescribed antibiotics because of their broad spectrum of activity, proven clinical efficacy, safety profile, favorable pharmacokinetics and less adverse effects [36].

In the past fifteen years, six parenteral third-generation cephalosporins have been introduced into clinical use. Cefotaxime, ceftriaxone and ceftazidime are the three most frequently available antibacterial agents characterized by a broad spectrum of activity and increased stability to β-lactamases compared with the first and second-generation cephalosporins [37].
1.6.1 Benefits of third-generation cephalosporins

The third-generation cephalosporins, especially ceftazidime, cefotaxime, ceftriaxone and ceftizoxime penetrate effectively into the cerebrospinal fluid and are used widely in the therapy of difficult to treat gram-negative bacterial infections, including nosocomial infections, meningitis and infections caused by *Pseudomonas aeruginosa* [38].

Table 1.3: Third-generation cephalosporins with spectrum of activity

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Spectrum of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefditoren</td>
<td>Community acquired pneumonia, pharyngitis, uncomplicated skin and skin-structure infections (<em>Staphylococcus aureus</em>-not MRSA, <em>Streptococcus pyogenes</em>)</td>
</tr>
<tr>
<td>Cefetamet</td>
<td>Upper and lower community acquired respiratory tract infections</td>
</tr>
<tr>
<td>Cefixime</td>
<td>Uncomplicated UTI, otitis media, pharyngitis and tonsillitis, acute exacerbations of chronic bronchitis</td>
</tr>
<tr>
<td>Cefmenoxime</td>
<td>Common gram-positive and gram-negative pathogens</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>Respiratory tract infections, bacterial septicemia, infections of skin and skin structures</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>Bacteremia/septicemia, CNS infections, Bone or joint infections, intra-abdominal infections, gynecologic infections</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>Community-acquired pneumonia caused by <em>S. pneumonia</em>, acute uncomplicated gonorrhea caused by <em>N. gonorrhoea</em>, uncomplicated skin and skin structure infections caused by <em>S. aureus</em> or <em>S. pyogenes</em>; acute otitis media caused by <em>S. pneumonia</em></td>
</tr>
<tr>
<td>Ceftibuten</td>
<td>Treatment of acute exacerbations of chronic bronchitis, acute bacterial otitis media and pharyngitis/tonsillitis</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Treatment of lower respiratory tract infections, acute bacterial otitis media, skin and skin structure infections, bone and joint infections, intra-abdominal and urinary tract infections, pelvic inflammatory disease, uncomplicated gonorrhea, bacterial septicemia, and meningitis; used in surgical (perioperative) prophylaxis</td>
</tr>
<tr>
<td>Cefdinir</td>
<td>Treatment of community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute bacterial otitis media, acute maxillary sinusitis, pharyngitis/tonsillitis, and uncomplicated skin and skin structure infections</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>Gram-positive and gram-negative bacteria, bovine respiratory disease associated with <em>Mannheimia haemolytica, Pasteurella multocida</em> and <em>Histophilus somni</em></td>
</tr>
</tbody>
</table>
### Ceftizoxime

Lower respiratory tract infections caused by *Klebsiella spp*, *Proteus mirabilis*, *Escherichia coli*, UTI caused by *Staphylococcus aureus*, *P. aeruginosa*, gonorrhea including uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhea*.

### Ceftazidime

Treatment of documented susceptible *Pseudomonas aeruginosa* infection and infections due to other susceptible aerobic gram-negative organisms; empiric therapy of a febrile, granulocytopenic patient.

*Note:* - The information list in this table is based on the following references [39-42].

1.6.2 *Adverse effects of third-generation cephalosporins*

Adverse effects associated with use of the third-generation cephalosporins are generally similar to those that occur with other β-lactam antibiotics [43]. Third-generation cephalosporins are not currently recommended for prophylactic use in surgery except ceftriaxone [33].

1.7 *Ceftriaxone Sodium*

1.7.1 *Background*

Development of biliary colic and biliary sludge have been associated with ceftriaxone, a third-generation cephalosporin, at high doses when given parenterally [44]. Ceftriaxone is a generic name of Rocephin® which is available as a parenteral formulation that can be administered intravenously or intramuscularly for treatment of moderate to severe bacterial infections caused by susceptible organisms [45]. Ceftriaxone is considered as one of the most important medication needed in a basic health system and is also included on the World Health Organization’s list of essential medicines [46].

15
1.7.2 Dosage regimen

Typical dose regimens in adults are 1 to 2 grams given intravenously or intramuscularly in one or two divided doses daily for 7 to 14 days [45].

1.7.3 Spectrum of activity

Ceftriaxone has broad spectrum of activity against gram-positive bacteria and gram-negative bacteria compared to first and second-generation agents. Ceftriaxone is active against *S. marcenscens*, citrobacter and β-lactamase producing strains of neisseria and haemophilus. Ceftriaxone does not have useful activity against *Pseudomonas aeruginosa* and has no activity against listeria, enterococci or atypicals (Mycoplasma and Chlamydia) [47].

1.7.4 Physico-chemical properties

Ceftriaxone is a white crystalline powder and is readily soluble in water but sparingly soluble in methanol or ethanol. The pH of a 1% aqueous solution is approximately 6.7 which makes it slightly acidic. The molecular formula of ceftriaxone sodium is \( \text{C}_{18} \text{H}_{16} \text{N}_8 \text{Na}_2 \text{O}_7 \text{S}_3 \cdot 3.5 \text{H}_2\text{O} \). and has a molecular weight of 661.59 g/mol. The structural formula of ceftriaxone sodium is given in fig. 1-5 [48].
1.7.5 Pharmacokinetic properties

The half-life of ceftriaxone sodium is 7–8 hours. Ceftriaxone’s long half-life, dual route of elimination and long duration of action makes it an excellent choice of drug for outpatient antibiotic therapy with community-acquired infections. Ceftriaxone can be administered intravenously and intramuscularly. It is not available orally [37]. The intramuscular dose of ceftriaxone (Rocephin®) has been increased from 125 mg to 250 mg due to increasing resistance of the gonococcal bacteria [49].

1.7.6 Medical Uses

Ceftriaxone is widely used in treatment of serious infections that are caused by organisms resistant to most other antibiotics. It is often used for the treatment of community-acquired or mild to moderate hospital-acquired pneumonia.

It is a drug of choice for the treatment of bacterial meningitis caused by meningococci, pneumococci, susceptible enteric gram-negative rods and Haemophilus influenzae, but not Listeria monocytogenes [47]. It is also recommended for the therapy of Lyme disease.
involving the central nervous system or joints, penicillin-resistant gonorrhea and meningitis in children due to ampicillin-resistant *H. influenza* [50, 51].

Other uses include the treatment of bone and joint infections, uncomplicated gonorrhea, acute bacterial otitis media, intra-abdominal and urinary tract infections, skin and skin structure infections, pelvic inflammatory disease (PID), and bacterial septicemia. Ceftriaxone is also approved for the use in surgical (perioperative) prophylaxis [52].

1.7.7 Contraindications

(i) IV must not be co-administered with Ca-containing IV solutions in neonates ≤ 28 days because precipitation of ceftriaxone in the lungs and kidneys of neonates have been reported.

(ii) Ceftriaxone and Ca-containing solutions should not be mixed with each other or given within 48 hours.

(iii) Ceftriaxone should not be given to hyperbilirubinemic and preterm neonates because in-vitro ceftriaxone can displace bilirubin from serum albumin, potentially triggering kernicterus.

1.7.8 Side effects

Common side effects of ceftriaxone include rash, nausea, dizziness, diarrhea, blood clots, and headache. Rare adverse reactions include abdominal pain, agranulocytosis, basophilia, biliary lithiasis, bronchospasm, allergic pneumonitis,
gallbladder sludge, anaphylaxis, glycosuria, hematuria, leukocytosis, jaundice, colitis, epistaxis, dyspepsia, flatulence, lymphocytosis, monocytosis, a decrease in the prothrombin time, renal precipitations, nephrolithiasis, palpitations, seizures, and serum sickness [52].

1.8 Oral delivery of antibiotics

Antibiotics are one of the most commonly used drugs in hospital setting. One-third of patients receive antibiotic therapy, and on an average 40% of these patients will receive an intravenous (IV) agent [53]. Different routes for the administration of antibiotics includes:

(i) **Oral** – in the form of tablets, capsules or liquid for treating mild to moderate infections.

(ii) **Topical** – in the form of creams, lotions, sprays, eye and ear drops.

(iii) **Injections** – given by infusions through a drip directly into blood or muscle and typically used for more serious systemic infections [54].

Clinicians have for the most part concurred that the most ideal approach to accomplish rapid onset of drug action is to create a therapeutic blood level by administering the drug IV. However, for attaining a sustained therapeutic level, this approach requires establishing indwelling IV access, typically in a hospital setting. For patients whose infections have not made them sick enough to require hospitalization for supportive care, other routes of antibiotic administration are less intrusive and therefore attractive when
evidence supports the fact that suprainhibitory blood levels of drug can be attained by those routes [55].

1.8.1 Reconsideration of efficacy of oral antibiotics

In recent years, several factors have provoked the reconsideration of the efficacy of orally administered antibiotics. These factors include: (i) increased availability of oral agents with excellent absorption that promise to provide adequate blood levels with oral administration; (ii) increased awareness of the negative effects of prolonged IV drug administration, such as phlebitis, local and vascular infection, excess fluid administration, patient discomfort, and others; (iii) increased pressure on physicians to provide more cost-effective therapies for patients [55].

1.8.2 Benefits of switching from IV to PO

Traditionally, antibiotic agents have been administered intravenously (IV) for serious systemic infections. Patients who are in shock or have impaired intestinal absorption can be treated with IV therapy initially, but after clinical defervescence, oral antibiotics can be used for the completion of therapy [56]. Parenteral antibiotic administration can be swapped with the oral route for certain patients with brain abscess, endocarditis, meningitis, and skeletal infections, after a pathogen has been recognized and the antibiotic susceptibility determined [57]. Orally available antibiotics have been increasingly used nowadays for serious systemic infections, as more potent oral
antibiotics with high bioavailability have been introduced in the market after studying their pharmacokinetic aspects. Due to the availability of various potent oral antibiotics with high bioavailability, switching of “IV to PO” has been quite possible. This could potentially lead to far fewer hospitalizations and considerably reduce hospital and related medical costs [56, 58].

Antibiotics that consist of appropriate spectrum, high degree of activity against the presumed or known pathogen and have good bioavailability are considered ideal for oral administration. Oral antibiotics can be compared to IV antibiotics if it achieves the same serum/tissue concentration at the same dose as IV administration along with high absorption and bioavailability of ≥ 90% [56]. Drug solubility, systemic absorption, gastrointestinal permeability, tissue distribution and target tissue penetration are some of the pharmacokinetic factors that influence the outcomes in the antibiotic therapy. The Biopharmaceutics Classification System (BCS) aids in predicting clinical pharmacokinetics of a drug based on its in-vitro aqueous solubility and in-vivo “permeability”. Drug solubility and permeability influence absorption. Antibiotic absorption and distribution may be affected by factors such as intake of food, inter-patient variability and by specific drug attributes which can eventually lead to difference in efficacy and tolerability. An oral medication must be well absorbed, distributed and penetrate from the target tissue to the systemic circulation in order to show its effectiveness [59-61].
1.8.3 Factors impacting bioavailability of oral antibiotics

Bioavailability of the orally administered antibiotics is variable as it is dependent on gastrointestinal absorption, drug stability at gastric pH, co-administration of food, gastrointestinal tolerance and transit time and ileal function. Additional host factors that can impact the efficacy of oral antibiotics are neutropenia and other immunosuppressed conditions, renal dysfunction, age and patient discomfort [55].

Table 1.4: Orally administered antibiotics with their antimicrobial spectrum

<table>
<thead>
<tr>
<th>Class</th>
<th>Generations</th>
<th>Drug</th>
<th>Antimicrobial Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td></td>
<td>Ampicillin</td>
<td><em>Streptococci pyogenes</em>, <em>Streptococcus agalactiae</em>, <em>Viridans streptococci</em>, Enterococci, <em>Neisseria meningitidis</em>, <em>Pasteurella multocida</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amoxicillin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cloxacillin</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>First Generation</td>
<td>Cephalexin</td>
<td><em>Streptococci pyogenes</em>, <em>Streptococcus agalactiae</em>, <em>Viridans streptococci</em>, <em>Escherichia coli</em>, <em>Proteus mirabilis</em>, and <em>Klebsiella pneumonia</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefadroxil</td>
<td>Inadequate activity against <em>Moraxella catarrhalis</em> and <em>Hemophilus influenza</em>. Methicillin-resistant Staphylococci, Enterococci, penicillin-resistant <em>Streptococcus pneumoniae</em> are resistant.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephadrine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second Generation</td>
<td>Cefaclor</td>
<td><em>Hemophilus influenzae</em>, <em>Moraxella catarrhalis</em>, <em>Neisseria meningitidis</em>, and some Enterobacteriaceae. Inactive against methicillin-resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefprozil</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefuroxime</td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Antibiotics</td>
<td>Activities</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td>Azithromycin, Clarithromycin, Erythromycin</td>
<td><em>Streptococcus pneumoniae</em> and other streptococci, staphylococci, and <em>Corynebacterium diphtheria</em>, <em>Legionella pneumophila</em>, <em>Neisseria gonorrhoeae</em>, <em>Moraxella catarrhalis</em>, <em>Bordetella pertussis</em>. Enterobacteriaceae are resistant.</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Neomycin</td>
<td>Enterobacteriaceae, <em>Pseudomonas aeruginosa</em>, <em>Acinetobacter</em>, <em>Providencia</em>, <em>Hemophilus</em>, Staphylococci, Streptococci such as Enterococci, Group B streptococci, <em>Viridans streptococci</em></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Doxycycline, Minocycline, Tetracycline</td>
<td>Staphylococci, Streptococci, Pneumococci, Pseudomonads, Enterobacteriaceae</td>
<td></td>
</tr>
<tr>
<td><strong>Sulfonamides</strong></td>
<td><strong>Sulfisoxazole</strong>&lt;br&gt;Sulfadimethoxine&lt;br&gt;Pyrimethamine</td>
<td><strong>Streptococcus pyogenes,</strong>&lt;br&gt;<em>E. coli, Neisseria meningitides,</em> Nocardia species</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td><strong>Lincosamides</strong></td>
<td>Clindamycin&lt;br&gt;Lincomycin</td>
<td>Moderate-spectrum; they are primarily active against Gram-positive bacteria, most anaerobic bacteria and some mycoplasma</td>
<td></td>
</tr>
<tr>
<td><strong>Quinolones</strong></td>
<td><strong>First Generation</strong>&lt;br&gt;Oxolinic acid&lt;br&gt;Rosoxacin&lt;br&gt;Nalidixic acid</td>
<td>Active against some Gram-negative bacteria</td>
<td></td>
</tr>
<tr>
<td><strong>Second Generation</strong></td>
<td>Ciprofloxacin&lt;br&gt;Norfloxacin&lt;br&gt;Ofloxacin</td>
<td><em>E. coli,</em> salmonella,&lt;br&gt;Shigella, Neirrsseria,&lt;br&gt;Legionella, <em>S. aureus</em>&lt;br&gt;(systemic infections),&lt;br&gt;pseudomonas, <em>B. anthracis</em></td>
<td></td>
</tr>
<tr>
<td><strong>Third Generation</strong></td>
<td>Levofloxacin&lt;br&gt;Moxifloxacin&lt;br&gt;Gatifloxacin</td>
<td>Staphylococci, pneumococci, mycoplasma and legionella</td>
<td></td>
</tr>
<tr>
<td><strong>Fourth Generation</strong></td>
<td>Gemifloxacin&lt;br&gt;Trovafoxacin&lt;br&gt;Prulifloxacin</td>
<td><em>Staphylococcus epidermidis,</em>&lt;br&gt;<em>Staphylococcus aureus,</em>&lt;br&gt;<em>Streptococcus pneumoniae,</em>&lt;br&gt;<em>Streptococcus pyogenes,</em>&lt;br&gt;<em>Bacillus cereus,</em>&lt;br&gt;<em>Enterococcus faecalis,</em>&lt;br&gt;<em>Klebsiella pneumoniae</em> and <em>Enterobacter aerogene</em></td>
<td></td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>Bacitracin&lt;br&gt;Rifampin&lt;br&gt;Linezolid</td>
<td><em>Staphylococcus spp,</em>&lt;br&gt;<em>Streptococcus spp,</em>&lt;br&gt;<em>Enterococcus spp,</em>&lt;br&gt;<em>Bacillus cereus,</em> <em>Moraxella catarrhalis,</em> <em>Haemophilus influenza,</em> <em>Bacteroides bivius-disiens,</em>&lt;br&gt;<em>Gardnerella vaginalis,</em>&lt;br&gt;<em>Lactobacillus spp.,</em> and <em>Mobiluncus spp.,</em>&lt;br&gt;<em>Enterococcus faecium</em> and <em>Enterococcus faecalis,</em></td>
<td></td>
</tr>
</tbody>
</table>
Since a significant number of these antibiotics are well absorbed after oral administration, they are clinically valuable in the outpatient setting. Oral antimicrobial agents are generally used for community-acquired infections and parenteral antimicrobial agents are used for hospital-acquired infections [64]. Community acquired lower respiratory tract infection is a common cause of hospitalization, and intravenous antibiotics including cephalosporins are oftentimes utilized as first line treatment for serious systemic infections [65].

1.8.4 Benefits of early switch

Few advantages of switching therapy from IV to Oral route include: (i) Reduction in the likelihood of hospital acquired bacteremia and phlebitis through IV lines; (ii) Patient is more inclined to get oral antibiotics at the right time and miss less dosages; (iii) Reduction in the risk of adverse reactions; (iv) Increased patient compliance and

* Note: - The information list in this table is partially based on the following references [62, 63].

| Staphylococcus aureus, methicillin-resistant Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, the viridans group streptococci, Listeria monocytogenes, and Corynebacterium species |  |  |
possibility of earlier discharge from the hospital; (v) Reduced medical costs and nursing time [53].

1.8.5 Considerations for selecting an oral antibiotic

There are number of factors that should be taken into consideration for selection of an oral antibiotic. These include: (i) Ethical considerations (ii) Cost considerations such as per-dose cost and number of doses (iii) Indirect costs which includes patient compliance, incidence of treatment failure & incidence of adverse drug reactions, including drug interactions. (iv) Pharmacologic considerations which includes pharmacodynamics considerations, pharmaceutical considerations, pharmacokinetic considerations & high bioavailability [66].

1.8.6 Advantages of oral antibiotics

Oral cephalosporins are shown to be effective in the treatment of community-acquired pneumonias as initial therapy from the time of diagnosis or as secondary therapy followed by a brief course of parenteral third-generation cephalosporin [67, 68]. Oral administration of cephalosporins produces equally clinical results as parenteral administration and thus decreases the costs and risks associated with IV administration such as phlebitis, excess fluid administration, bacteremia, etc. Oral therapy comparatively, are of low cost, have fewer complications and causes less patient inconvenience [69].
1.9 Routes of administration for antibiotics

Typically, antibiotics are administered orally, intravenously or intramuscularly. The key point in selecting the appropriate route of administration depends on the available dosage form of the drug, patient’s condition and patient’s age [70]. Common routes of administration along with their indication and examples are shown in table 1-5.

**Table 1.5: Routes of administration for antibiotics**

<table>
<thead>
<tr>
<th>Route</th>
<th>Indication</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Whenever possible, safest and most convenient route</td>
<td>Cefadroxil, Cefalexin, Cephadrine</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>When rapid effect is desired</td>
<td>Ceftiofur, Cefovicin, Cefotetan</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>For drugs with poor oral absorption, when high blood levels are required, when rapid effect is desired</td>
<td>Cephapirin, Cephalothin, Cefazolin</td>
</tr>
<tr>
<td>Intravenous</td>
<td>In emergency situations, when immediate effect is desired, when large volumes need to be administered</td>
<td>Cefoxitin, Ceftazidime, Cefoperazone</td>
</tr>
<tr>
<td>Rectal</td>
<td>When patients are unable to take oral medications and parenteral route is not indicated, also for local effect</td>
<td>Metronidazole, Clindamycin, Cotrimoxazole</td>
</tr>
<tr>
<td>Vaginal</td>
<td>For local effect</td>
<td>Metronidazole, Clindamycin</td>
</tr>
<tr>
<td>Topical</td>
<td>For local effects on skin and mucous membrane of eye, ear, nose, mouth</td>
<td>Erythromycin, Bacitracin, Clindamycin</td>
</tr>
<tr>
<td>Transdermal</td>
<td>Provides continuous absorption and systemic effects over extended time</td>
<td>Amoxicillin, Doxycycline, Gentamicin</td>
</tr>
<tr>
<td>Inhalation</td>
<td>For local effects with respiratory tract</td>
<td>Tobramycin, Aztreonam</td>
</tr>
<tr>
<td>Intrathecal</td>
<td>Used in difficult cases where intravenous therapy failed to eradicate infection</td>
<td>Vancomycin, Gentamycin, Amikacin</td>
</tr>
</tbody>
</table>

*Note: - The information list in this table is partially based on the following references [71].
Oral: It is available in the form of tablets, capsules and liquids. It is considered as the most common and convenient route of administration.

Advantages: Liquids can be swallowed without any difficulty especially for the patients with dysphasia. Liquids can be mixed with a juice or formula in a nipple bottle for infants.

Disadvantages: Penicillins can be considered as an illustration of antibiotics that cannot be administered orally and should be preferred to be given by intramuscular or intravenous injection as they can become inactivated by stomach acid. Tetracyclines, when combined chemically with dairy foods and beverages can lead to adverse effect by formation of insoluble complex or interaction, hence due to these reasons, dairy products should be avoided while taking tetracyclines.

Parenteral: It is a type of route which can include all other routes of administration other than oral.

Subcutaneous: Liquids are injected into the muscular fatty layer of the tissue just below the dermis and above the muscle layer.

Disadvantages: There is slower absorption in subcutaneous layer especially when compared to intramuscular layer as there are very small amount of blood vessels.

Intradermal: When this injection is administered correctly, the tip of the needle will still be visible through the skin. Liquid is injected into the dermis just below the epidermis.
**Vaginal**: Creams and suppositories are most common forms used for the treatment of vaginal infections and vaginitis. Foams and gels can also be considered for vaginal route of administration. For e.g., Monistat suppositories, premarin vaginal cream.

**Topical**: Since it is directly applied to mucous membranes of ear, nose, eye, mouth and skin, this route only produces local effects and not systemic. For e.g.: erythromycin eye ointment, ciprofloxacin eye drops.

**Inhalation**: By inhaling the drug, drug will be absorbed through alveoli of the lungs and the administration by this route involves inhaling drugs in a liquid or gaseous form.

**Sublingual**: This tablet form dissolves very slowly and it is typically placed under the tongue. It is necessary to recommend that this sublingual tablet cannot be swallowed. The medication is absorbed through oral mucous membranes into large blood vessels as the absorption takes place under the tongue. It has a quicker effect when compared to oral route but cannot be used with pH sensitive drugs.

**Intramuscular**: This type of injection is given into the belly or a part of body that has large muscle mass, since they can well supply blood vessels, therefore it also provides more rapid absorption in comparison to subcutaneous injection. There are five sites for intramuscular injections which contain lowest risk of damage to adjacent blood vessels and nerves. They are rectus femoris and vastus lateralis which are located on mid-thigh, deltoid that is located on upper arm, ventrogluteal which is located on the side of the hip over gluteus muscle between the anterior and superior spines of the iliac crest and dorsogluteal located over gluteus minimus and edge of gluteus maximus muscle in upper outer quadrant. However, there are drugs that might not be acceptable to be administered
intramuscularly because they are not water soluble and they can precipitate out in muscular tissue.

**Rectal:** It is an alternatively convenient route for patients who vomit and are not able to administer the medication by any other routes, especially for conditions like constipation and hemorrhoids. However one disadvantage is that there is no predictable effectiveness and absorption is very slow via rectal route.

**Transdermal:** This route is useful in applying medication to the skin via physical delivery of medication through a porous membrane of the skin and its therapeutic effects are felt systemically. Medications for this route are available in patches; it provides sustained therapeutic blood levels by releasing the drug slowly over time.

**Intravenous:** This route has immediate therapeutic effect as the injection goes straight into the vein. There is no need for the absorption. There are three different ways by which an IV medication can be administered and it is IV piggyback in which a drug is mixed in a very small IV bag or bottle which is connected through a tubing in the existing primary IV via a port. It can also be administered by IV drip in which the drug is mixed with the fluid in an IV bottle or a bag which can be administered continuously over a long period of time. It can also be given by bolus dose which goes straight into the vein and is connected by an already establish IV line via a rubber stopper.
1.10 Oral antibiotics available in U.S. market

Table 1.6: List of few oral antibiotics available in U.S. market

<table>
<thead>
<tr>
<th>Class</th>
<th>Antibiotics</th>
<th>Brand Name(s)</th>
<th>Tablets</th>
<th>Capsules</th>
<th>Liquids</th>
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<td>✓</td>
</tr>
<tr>
<td></td>
<td>Penicillin VK</td>
<td>PC Pen VK, Pen-V</td>
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<tr>
<td></td>
<td>Ampicillin</td>
<td>Omnifen</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>Cefditoren</td>
<td>Spectracef</td>
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<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Cefprozil</td>
<td>Cefzil</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Neomycin</td>
<td>Neo-Fradin</td>
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<td>✓</td>
</tr>
<tr>
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<tr>
<td></td>
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<td>Biaxin</td>
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<td>✓</td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
<td>E.E.S., Eryc</td>
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<td>✓</td>
<td>✓</td>
</tr>
<tr>
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<td>Sulfisoxazole</td>
<td>Gantrisin</td>
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<td>✓</td>
<td>✓</td>
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<tr>
<td></td>
<td>Sulfadimethoxine</td>
<td>Albon</td>
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<td>Daraprim</td>
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<td>✓</td>
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<td>Doxycycline</td>
<td>Doryx, Vibramycin</td>
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<td>✓</td>
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<td></td>
<td>Minocycline</td>
<td>Minocin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td></td>
<td>Tetracycline</td>
<td>Adoxa</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Ciproloxacin</td>
<td>Cipro</td>
<td>✓</td>
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<tr>
<td></td>
<td>Ofloxacin</td>
<td>Floxin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin</td>
<td>Levaquin</td>
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</tr>
<tr>
<td>Nitroimidazoles</td>
<td>Metronidazole</td>
<td>Flagyl</td>
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<tr>
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<td>Vancomycin</td>
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<tr>
<td></td>
<td>Clindamycin</td>
<td>Cleocin</td>
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<tr>
<td></td>
<td>Linezolid</td>
<td>Zyvox</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

* Note: - The information list in this table is partially based on the following references [41, 72-74].

1.11 Oral delivery of cephalosporins

Oral cephalosporins are the most commonly prescribed antibiotics in the United States. Orally administered cephalosporins are β-lactamic broad-spectrum antimicrobial agents that are widely used to treat mild to moderate community bacterial infections due to
susceptible gram-positive and gram-negative bacteria. Cephalosporin generations differ widely in their \textit{in vitro} antimicrobial potency, microbial resistance, spectrum of activity, pharmacokinetic properties and cost [75, 76].

Currently, thirteen cephalosporins are available for oral use which provides a wide range of choices for the treatment of common infections based on its spectrum of activity. Some common indication of cephalosporins includes upper and lower respiratory tract infections such as bronchitis, otitis media and pharyngitis. They are also indicated for other uncomplicated urinary tract infections as well as uncomplicated skin and soft tissue infections [77–79]. Some of the oral cephalosporins available in both generic and brand formulations includes: cefadroxil (Duracef: 1st), cefaclor (Ceclor, Raniclor: 2nd), cefditoren (Spectracef: 3rd), cefdinir (Omnicef: 3rd), cefpodoxime (Vantin: 3rd), cefixime (Suprax: 3rd), ceftibuten (Cedax: 3rd), cefprozil (Cefzil: 2nd), cefuroxime (Ceftin: 2nd), cephalexin (Keftab: Apo-Cephalex, Biocef, Keflex, NovoLexin, Nu-Cephalex: 1st), cephradine (Velosef: 1st), cefetamet (Tenafet: 3rd) and loracarbef (Lorabid: 2nd) [34, 80]. Cephradine and cefuroxime are also available in parenteral forms. Other oral cephalosporins that are in development includes cefprozil, cefcanel daloxate hydrochloride and cefcapene pivoxil, in Phase II trials [81].

\textbf{1.11.1 Typical dosage regimens}

The typical dose regimens for oral cephalosporins are 250 mg to 500 mg two to four times daily for 7 to 14 days. For uncomplicated infections, oral cephalosporins are typically given for a ten day treatment course. However, shorter regimens have been shown to provide equivalent bacterial eradication and clinical cure rates as longer,
traditional treatment courses and may be more likely to result in improved patient compliance [82].

1.11.2 Side effects

Oral cephalosporins are usually well tolerated by most children. However, many children experience mild adverse effects. Despite of their wide use, severe toxicities such as drug induced liver disease from cephalosporins are very rare. Liver injury is often accompanied by rash, fever or other signs and symptoms of hypersensitivity. Rare adverse effects associated with cephalosporins include: blood dyscrasias, dizziness, elevations in liver function tests, headache, acute interstitial nephritis, pseudomembranous colitis, and lethargy [77, 78, 83].

1.11.3 Classification of oral cephalosporins

Oral cephalosporin antibiotics available in United States are shown in table 1-7. Fourth and Fifth-generation antibiotics are not included as they are not yet available in oral form.

<table>
<thead>
<tr>
<th>Generations</th>
<th>Antibiotics</th>
<th>Brand Name(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Cephalexin</td>
<td>Keflex</td>
</tr>
<tr>
<td></td>
<td>Cefadroxil</td>
<td>Duricef</td>
</tr>
<tr>
<td></td>
<td>Cephadrine</td>
<td>Velosef</td>
</tr>
<tr>
<td>Second</td>
<td>Cefaclor</td>
<td>Ceclor</td>
</tr>
<tr>
<td></td>
<td>Cefprozil</td>
<td>Cefzil</td>
</tr>
<tr>
<td></td>
<td>Cefuroxime</td>
<td>Ceftin</td>
</tr>
<tr>
<td></td>
<td>Loracarbef</td>
<td>Lorabid</td>
</tr>
<tr>
<td>Third</td>
<td>Cefdinir</td>
<td>Omnicef</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Brand Name</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Cefditoren</td>
<td>Spectracef</td>
<td></td>
</tr>
<tr>
<td>Cefixime</td>
<td>Suprax</td>
<td></td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>Vantin</td>
<td></td>
</tr>
<tr>
<td>Ceftibuten</td>
<td>Cedax</td>
<td></td>
</tr>
<tr>
<td>Cefetamet</td>
<td>Tenafet</td>
<td></td>
</tr>
</tbody>
</table>

*All these antibiotics are available in both solid and liquid/suspension formulations except cephradine and cefditoren (solid only)*

** Note: - The information list in this table is partially based on the following references [72, 84, 85]

### 1.12 Third generation cephalosporins

The third-generation cephalosporins are more active against the gram-negative bacteria than first or second-generation cephalosporins, but have less activity against *S. aureus*. Currently, there are thirteen commercially available third-generation cephalosporins available in the market.

Ceftriaxone and cefotaxime are the widely used third-generation cephalosporins for the empirical therapy of the febrile infant since they have the most activity against *S. pneumoniae* and other streptococci, including many strains that are resistant to penicillin. Ceftriaxone and cefotaxime are very similar with regards to their spectrum of antibacterial activity except the fact that ceftriaxone has much longer half-life than cefotaxime. The half-life of cefotaxime is 1-2 h whereas ceftriaxone has half-life of 7-8 h. Both ceftriaxone and cefotaxime are active against gram-positive as well as gram-negative bacteria except *L. monocytogenes*, enterococci and Pseudomonas species. Also, none of them are active against coagulase-negative staphylococci or methicillin-resistant *Staphylococcus aureus* (MRSA) [86].
Six third-generation cephalosporins are currently available for oral use; they are cefdinir (Omnicef), cefpodoxime proxetil (Vantin), cefixime (Suprax), ceftibuten (Cedax), cefditoren (Spectracef) and cefetamet (Tenafet) [87, 88].

Ceftibuten and cefixime are grouped together since they have a similar spectrum of activity against gram-positive and gram-negative bacteria. Both are active against the gram-negative bacteria which are responsible for most urinary tract infections but show limited activity against gram-positive bacteria such as *S. pneumoniae* and no activity against *S. aureus*. Since they have limited activity against *S. pneumoniae*, these agents are recommended only as 2nd-line agents for the treatment of otitis media and used in combination with other antimicrobial agents that fail the antipneumococcal activity when used alone. These agents are also used for pharyngitis as they are active against *Streptococcus pyogenes* [86].

Similar to other cephalosporins, cefdinir and cefpodoxime proxetil show high spectrum of activity against gram-negative bacteria. However, these agents also show good activity against gram-positive bacteria including *S. pyogenes, S. aureus, S. pneumonia, and* methicillin-sensitive strains. Compared to other cephalosporins, cefpodoxime achieves highest tissue concentrations in the lungs and tonsils [89].

Cefetamet and cefditoren also show high spectrum of activity against gram-positive as well as gram-negative bacteria. They show good activity against *Haemophilus influenza*, *Staphylococcus aureus*, streptococci and *Streptococcus pneumonia*, *Moraxella catarrhalis* and *B-haemolytic streptococci*. Cefetamet has marked activity against
Neisseria gonorrhoeae and possesses a broad spectrum of activity against Enterobacteriaceae whereas cefditoren has activity against S. pyrogenes [90].

1.12.1 Oral third-generation cephalosporins

Table 1.8: Orally available third-generation cephalosporins in the form of tablets, capsules and suspensions

<table>
<thead>
<tr>
<th>Oral Cephalosporin</th>
<th>Trade Name</th>
<th>Tablets</th>
<th>Capsules</th>
<th>Suspensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefdinir</td>
<td>Omnicef, Cefdiel</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Cefditoren</td>
<td>Spectracef</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Cefetamet</td>
<td>Tenafet</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Cefixime</td>
<td>Suprax</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>Vantin</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Ceftibuten</td>
<td>Cedax</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

* Note: - The information list in this table is partially based on the following references [72, 84, 85, 91, 92]

1.12.2 Medical Uses

Third-generation cephalosporins are widely used against gram-negative septicemia, gram-negative meningitis, other serious gram-negative infections, Pseudomonas aeruginosa infections and gonorrhea using ceftriaxone as the drug of choice; osteomyelitis and Lyme’s disease using ceftriaxone in home health care situations and for complicated urinary tract infections such as pyelonephritis.

There have also been some inappropriate uses yet they are widely prescribed for: (i) Surgical prophylaxis using first and second-generation agents. (ii) Otitis media & upper respiratory infections using cefixime, ceftibuten which have poor gram-positive activity. (iii) Uncomplicated upper respiratory infections [90].
1.13 References


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**139 Suppl**: p. 3s-24s.


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Chapter 2

Development and Evaluation of a Biopolymer based Ceftriaxone Sodium Oral Formulation

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Figures: 9
Tables: 9

Keywords: ionotropic gelation; beads; ceftriaxone sodium; thermal gravimetric analysis; atomic force microscopy; loose surface crystal

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2.1 ABSTRACT

Ceftriaxone sodium (CS) is a third-generation cephalosporin and belongs to BCS Class III. This antibiotic cannot be absorbed orally owing to its poor permeability through GI epithelia and its acid labile nature. The purpose of present investigation was to develop and evaluate a multiparticulate system exploiting pH-sensitive property and specific biodegradability of calcium alginate beads, for intestinal delivery of CS. Beads of CS entrapped in sodium alginate consisting of different polymers such as sodium carboxymethylcellulose, acacia, HPMC K4M and HPMC K15M as drug release modifiers were prepared by ionotropic gelation method using calcium chloride as a cross-linking agent followed by enteric coating with cellulose acetate phthalate. Sustained-release polymeric beads were then evaluated for entrapment efficiency using HPLC, in vitro drug release examined in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8), swellability, particle size and surface morphology using optical microscopy and Scanning Electron Microscopy (SEM), and surface characterization using Atomic Force Microscopy (AFM). Thermal Gravimetric Analysis (TGA) was utilized to check the strength of the polymer matrix and thermal stability of the prepared formulations. The drug entrapment efficiency for the optimized formulation was determined to be 74.66 ± 4.99%. Swelling properties of drug-loaded beads were found to be in range from 0.859 to 3.35. Beads coated with cellulose acetate phthalate and carboxymethylcellulose aqueous polymer dispersion exhibited sustained release and followed first-order kinetics and the mechanism of diffusion was anomalous or non-Fickian (diffusion and erosion dependent swelling mechanism). The particle size of the beads was between 1.039 ± 0.197 mm to 2.146 ± 0.357 mm. TGA demonstrated the
cross-linking efficiency and thermal stability of the beads. SEM images demonstrated the structure and surface of the beads. AFM images demonstrated composition and polymer dependent variations in surface structure, morphology, and roughness.
2.2 INTRODUCTION

Mainly because of patient convenience and acceptability oral route is the most convenient route of administration and oral drug delivery is the most preferable and desirable method of administering therapeutic agents for systemic effects. Traditional methods of formulation are still being employed which provide clinically effective therapy and safety to the patient by maintaining the required balance between the pharmacodynamic and pharmacokinetic profiles [93]. In order to overcome the limitations of conventional therapy, wide variety of oral drug delivery systems have been developed in recent years which provides sustained release dosing and are able to maintain steady drug plasma levels for extended periods of time with reduced drug related side effects [94].

Sodium Alginate is a hydrophilic biopolymer and naturally occurring polysaccharide obtained from brown seaweed and algae [95]. It has been widely used in controlled release applications due to its biodegradable nature. It has a unique property of gelation in aqueous media in presence of multivalent ions such as calcium and aluminum that takes place mainly at egg box junction. This unique property makes it possible to encapsulate both macromolecular agents and low molecular weight therapeutic agents. These hydrogel forming property allows sodium alginate preparations to achieve controlled release drug delivery [96].

Large number drugs currently available on the market fall under the BCS class III. Because of its poor permeation across the GIT, these drugs cannot be delivered by oral route even though they possess high therapeutic potential. Ceftriaxone sodium, a third-generation cephalosporin is highly water-soluble drug that cannot be absorbed orally due...
to its acid labile nature and poor permeability across the GI epithelia. To overcome this situation, the drug must be incorporated into delivery systems which provides greater stability in the gastric fluids and also sustained release dose [97].

Multiparticulate delivery systems are often used to obtain controlled drug delivery, stability and improve bioavailability of the acid labile drugs. The potential advantage of multiparticulate system include reduced risk of local irritation, no risk of dose dumping, increased bioavailability and less inter and intra subject variability [98]. Beads are one of the multiparticulate delivery systems employed to sustain the drug release, reduce gastrointestinal irritation by reducing side effects and improve patient compliance. Ionotropic gelation technique involves cross-linking of polyelectrolyte biopolymers such as alginates, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, etc. to counter ions such as calcium or aluminum to produce insoluble meshwork in the form of beads which would provide the sustained drug release [99-102]. Sodium alginate was chosen as the biopolymer for this research because alginates are naturally occurring anionic polysaccharides, biodegradable, non-toxic, have high biological safety and ability to incorporate acid labile drugs into the matrix formed after cross-linking [103].

Hence, an attempt was made to develop and evaluate a multiparticulate system for intestinal delivery of ceftriaxone sodium by ionotropic gelation technique using sodium alginate as the hydrophilic carrier in combination with polymers such as sodium carboxymethylcellulose, acacia, HPMC K4M and HPMC K15 as drug release modifiers in various proportions to overcome the drug related adverse effects. Cellulose acetate phthalate, a pH sensitive polymer was used to sustain the release and protect the system
from degradation of drug from the gastric fluids and to deliver ceftriaxone sodium specifically to intestine. In the proposed research, ionotropic gelation technique was used where the mixture of drug and polymer dispersion is added drop wise into the aqueous calcium chloride solution resulting in instantaneous gelation forming beads. The beads were evaluated for entrapment efficiency, loss on drying, particle size, swelling index, and *in vitro* drug release were characterized by techniques such as TGA, SEM and AFM.
2.3 MATERIALS AND METHODS

2.3.1 Materials

Ceftriaxone sodium was obtained from Apotex Corp, (Weston, FL). Sodium alginate (medium viscosity) was purchased from Sigma-Aldrich, (St Louis, MO). Sodium carboxymethylcellulose (viscosity 7MF) was purchased from Amend drug and chemical co., (Irvington, NJ). Acacia was obtained from PCCA, Houston, TX. Hydroxypropylmethylcellulose (HPMC) K4M Premium CR and K15M Premium CR was purchased from The Dow Chemical Company, (Midland, MI). Calcium chloride (anhydrous) was purchased from Fischer Scientific, (Fair Lawn, NJ) and cellulose acetate phthalate was purchased from Spectrum Quality Products, Inc., (New Brunswick, NJ). 2.06M Tetraethylammonium Hydroxide (TBAOH) was purchased from Thermo Fischer Scientific, (Sunnyvale, CA). All other solvents and reagents used were of analytical grade.

2.3.2 Methods

2.3.2.1 Preparation of beads

Micobeads of Ceftriaxone sodium was prepared using ionotropic gelation technique. In the present research, four sets of beads were prepared by using sodium alginate alone and four sets of each coated and uncoated beads were prepared by using sodium alginate in combination with other polymers like sodium carboxymethylcellulose, acacia, HPMC K4M and HPMC K15M. Calcium chloride was used as a cross-linking agent. The detailed composition of the various formulations are described in table 2-1.
Varying concentrations of sodium alginate 0.4% - 1% (w/v) was dissolved in deionized water at room temperature for 2 hours with a stirring speed of 1200 rpm. 2 mg of ceftriaxone sodium was dispersed into the sodium alginate solution and was then stirred for another 30 min for homogenous mixing of the drug-polymer solution. The dispersion was sonicated for 15 min to remove air bubbles that were formed during the drug-polymer mixing process. Bubble free drug-polymer solution was then introduced drop wise via a syringe attached with a of 22-guage hypodermic needle in to varying concentrations of aqueous calcium chloride solution (cross-linking agent) under gentle agitation at room temperature as shown in figure 2-1. The concentrations of calcium chloride ranged from 0.2% - 4% (w/v). The droplets from the drug-polymer dispersion instantaneously gelled upon contact with the calcium chloride solution which acts as a cross-linking agent [104]. The drug-loaded beads were allowed to be cure for another 15 min to enhance the rigidity of the beads. The obtained beads were harvested by filtering through a filter paper (Whatman no. 42), spread on petri dish, dried for 1-2 days at room temperature and stored in an air-tight container for further use.

![Fig. 2-1: Schematic diagram of the preparation of hydrogel beads by ionotropic gelation method.](image-url)
Beads were also prepared using polymers such as acacia, sodium carboxymethylcellulose, HPMC K4M and HPMC K15M as per table 2-1. These polymers were added to the homogenous dispersion of sodium alginate and drug as an additional step to the procedure described earlier and was stirred for 4 hours at 125-350 rpm for content uniformity. Beads were then optimized by modifying parameters such as drug-polymer ratio, combination with other polymers, concentration of cross-linking agent, entrapment efficiency, curing time and *in vitro* drug release data.

**Table 2.1:** Composition of beads

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Sodium Alginate (%w/v)</th>
<th>Calcium Chloride (%w/v)</th>
<th>Sodium carboxymethyl cellulose (%w/v)</th>
<th>Acacia</th>
<th>HPMC K4M</th>
<th>HPMC K15M</th>
<th>Cellulose Acetate Phthalate (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulations with alginate alone</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>F1</td>
<td>0.4</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>2</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>Uncoated Formulations with alginate and polymer blends</td>
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<td>F7</td>
<td>1.25</td>
<td>3.75</td>
<td>-</td>
<td>-</td>
<td>1.875</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F8</td>
<td>1.25</td>
<td>3.75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.25</td>
<td>-</td>
</tr>
<tr>
<td>Coated Formulations with alginate and polymer blends</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F9</td>
<td>1.25</td>
<td>3.75</td>
<td>4.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>F10</td>
<td>1.25</td>
<td>3.75</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>F11</td>
<td>1.25</td>
<td>3.75</td>
<td>-</td>
<td>-</td>
<td>1.875</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>F12</td>
<td>1.25</td>
<td>3.75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.25</td>
<td>5</td>
</tr>
</tbody>
</table>

* Each formulation contains 2mg of ceftriaxone sodium. Blank (without drug) formulations were also prepared for formulations F1 – F12, keeping the polymer concentration constant.
2.3.2.2 Process variables and process optimization

Variables such as concentration of sodium alginate, concentration of calcium chloride, concentration of acacia, concentration of sodium carboxymethylcellulose, concentration of HPMC K4M, concentration of HPMC K15M, curing time and stirring speed were evaluated for the optimization of the beads [105]. Various batches were prepared and analyzed for shape, size, drug loading, percent yield, entrapment efficiency and in vitro drug release. On basis of the results obtained, the process parameters were optimized as follows:

- Sodium alginate concentration: 1.25% w/v
- Sodium carboxymethylcellulose concentration: 4.25% w/v
- Acacia concentration: 2.5% w/v
- HPMC K4M concentration: 1.875% w/v
- HPMC K15M concentration: 1.25% w/v
- Calcium chloride concentration: 3.75% w/v
- Curing time: 15min
- Curing speed: - 60 rpm

The optimized process variables were used for preparing all further batches including preparation of coated beads.

2.3.2.3 Coating of beads

Formulation F5, F6, F7 and F8 were selected for enteric coating with cellulose acetate phthalate as they showed good entrapment efficiency. Cellulose acetate phthalate is a pH
sensitive polymer that dissolves above a pH of 6.2. The enteric coating solution was prepared using cellulose acetate phthalate (5% w/v) in 1:1 mixture of ethyl acetate and ethanol. This solvent system completely dissolves cellulose acetate phthalate while maintaining the integrity of the calcium alginate beads. Beads from the optimized batches F9, F10, F11 and F12 were dispersed in the enteric coating solution and then stirred at 1200 rpm for 30 minutes at room temperature. The solution was covered with parafilm in order to prevent the solvent from evaporation. Beads were harvested by filtering it with filter paper, spread on petri dish and dried for 1-2 days at room temperature and stored in an air tight container for further use.

2.3.2.4 Determination of water content in alginate beads

Formulation batches F5, F6, F7 and F8 was weighed before and after drying and the mean water loss was calculated from the following equation.

\[ W_{t}\% = \frac{(W_o - W_d)}{W_o} \times 100 \]

where, \( W_o \) and \( W_d \) represents initial weight before drying and final weight after drying respectively.

2.3.2.5 Analysis of ceftriaxone sodium by RP-HPLC

Ceftriaxone sodium was analyzed using Reversed phase-High-performance liquid chromatography (RP-HPLC) (Waters Alliance e2695 separation module, Milford, MA) equipped with a Phenomenex C18 column (Gemini 5u, 110A, 250 x 4.6 mm) and photodiode array detector (Waters Alliance 2998). The aqueous mobile phase consisted of 2.06 M tetrabutylammonium hydroxide 5.26% (v/v) (pH 7.0 adjusted with o-
phosphoric acid) and acetonitrile (ACN) in a ratio of 70:30 (v/v) respectively [106]. Isocratic separation method was utilized with a flow rate of 0.7 ml/min and column temperature set to 25°C. The injection volume was 10 µl with a run time of 10 min. The retention time of ceftriaxone sodium was found to be 7.271 min with absorption wavelength ($\lambda_{\text{max}}$) of 240 nm. Various calibration standards of ceftriaxone sodium were prepared in deionized water by serial dilutions ranged between 100 µg/ml to 0.39 µg/ml. Each standard was analyzed in triplicate for the calibration curve and the average peak area was plotted against concentration.

2.3.2.6 Determination of percent yield, drug loading and entrapment efficiency

Approximately, 200 mg of beads was completely dispersed in 50 ml of phosphate buffer solution (pH 7.4) and stirred for 3 hours at a speed of 1200 rpm. After stirring, an aliquot from the solution was taken in 2 ml eppendorf and centrifuged (Eppendorf Centrifuge 5430R, UK) for 5 min at 14,000 rpm to remove any insoluble solids. The supernatant was then analyzed for the drug content using HPLC. Observations were recorded in triplicates and percentage yield, drug loading and entrapment efficiency was calculated using the following formulas:

\[
\% \text{ Yield} = \frac{\text{Total weight of beads}}{\text{Total weight of polymers + drug}} \times 100
\]

\[
\% \text{ Drug Loading} = \frac{\text{Initial drug weight}}{\text{Total weight of beads}} \times 100
\]
2.3.2.7 Loose surface crystal study (LSC)

The purpose of this study was to estimate the amount of drug present on the surface of the beads without proper entrapment. Approximately, 200 mg of beads from formulations F9, F10, F11 and F12 were suspended in 50 ml of phosphate buffer (pH 6.8), and samples were shaken vigorously at 200 rpm for 15 min in a mechanical shaker (Thermo Scientific™ Precision Reciprocating Shaker Bath, USA) with the temperature set to 37 ± 0.5°C. The amount of drug leached out from the surface of the beads was analyzed at 240 nm using HPLC and the percentage of drug release with respect to entrapped drug in sample was recorded and shown in table 2-4 [105].

2.3.2.8 Swelling index study

This study was conducted to estimate the percentage swelling of the beads; responsible for leaching or degradation of the drug in the gastric fluid. Only batches with entrapment efficiency of more than 30% were selected for further studies. Dried ionicaly cross-linked beads increase their volume after few minutes in water or in buffers due to matrix rehydration that is dependent on the degree of cross-linking [107]. 200 mg of beads were suspended in 50 ml of simulated gastric fluid (pH 1.2) and samples were shaken at 60 rpm speed in mechanical shaker (Thermo Scientific™ Precision Reciprocating Shaker Bath, USA) and allowed to swell for 2 hours at 37 ± 0.5°C, simulating the gastric medium. After 2 hours interval, the swollen beads were carefully removed, blotted dry
and weighed. The difference between the initial and final weights of the beads were considered for finding the water sorption and the swelling index was calculated as shown in table 2-5 using the following formula:

\[
\text{Swelling index} = \frac{W_f - W_o}{W_f}
\]

Where, \( W_o \) is the initial weight of beads taken and \( W_f \) is the final weight of the beads after swelling.

2.3.2.9 In vitro drug release study

*In vitro* drug release studies were performed using a mechanical shaker (Thermo Scientific™ Precision Reciprocating Shaker Bath, USA) at 37 ± 0.5 °C and 60 rpm. The beads of formulations F9, F10, F11 and F12 were placed in 50 ml of enzyme-free simulated gastric fluid (pH 1.2) for the first 2 hours followed by enzyme-free simulated intestinal fluid (pH 6.8). 5 ml aliquots of the dissolution fluid was withdrawn at regular time intervals and replaced with the same amount of dissolution media. The aliquots from various time intervals were then centrifuged (Eppendorf Centrifuge 5430R, UK) for 15 min at 14000 rpm to remove any insoluble solids and the supernatant was analyzed for drug content using a HPLC at a wavelength \((\lambda_{\text{max}})\) of 240 nm. The tests were performed in triplicates and cumulative percentage of ceftriaxone sodium loaded beads dissolved was calculated using a regression equation generated from the calibration and is recorded in table 2-6 and 2-7.
2.3.2.10 Analysis of release kinetics and mechanism

The data obtained from in vitro drug release studies was plotted in various kinetic models to study the release kinetics. The drug release data of the in-vitro dissolution study was analyzed by fitting various kinetic models such as zero-order (% release vs time), first-order (log % retained vs time), Higuchi (% release vs square root of time) and Korsmeyer-Peppas equation. Correlation coefficient (r) values were calculated from the linear curves obtained by regression analysis of the plots. The equations used for calculating the release kinetics were as follows:

Zero Order: $C = C_o - k_o t$, where $C_o$ is the initial concentration of drug and $k_o$ is the zero-order rate constant expressed in units of concentration/time and $t$ is the time in hours.

First Order: $\log C = \log C_o - kt/2.303$, where $C_o$ is the initial concentration of drug and $k$ is the first-order constant,

Higuchi’s model: $Q = kt^{1/2}$, where $k$ is the constant reflecting the design variable of the system

Korsmeyer-Peppas model: $Mt/M_\infty = k_{kp} t^n$, where $Mt/M_\infty$ is fraction of drug released at time $t$, $k_{kp}$ is the rate constant and $n$ is the release exponent.

For finding the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model [108].
2.3.2.11 Particle size analysis

An optical microscope (Nikon SMZ800, USA) fitted with an ocular and stage micrometer having accuracy of 0.01mm was used to determine the particle size of the beads. Analysis of prepared beads was performed using a resolution of 30x to determine the diameter of 10 randomly selected beads. The instrument was calibrated at 1 unit of eyepiece micrometer equal to 1/30 mm (33.33 μm) and the average diameter of the beads was calculated using the following formula:

\[
X = \frac{\sum(X_i)}{N}
\]

where, \(X\) = Average particle diameter, \(X_i\) = individual diameter of beads and \(N\) = number of beads

2.3.2.12 Thermal gravimetric analysis (TGA)

TGA was performed by utilizing thermal analysis instrument (TA Q50, USA) under nitrogen gas. Platinum pan sample holder was initially tared and known amount of beads were transferred to it. The dynamic nitrogen flow rate was 20 ml/min and the heating rate was 10°C/min with temperature range from 25 °C – 650 °C. TGA was performed to check the strength of the polymer matrix and thermal stability by recording phase transitions and degradation patterns of various blank formulations (without drug) based on the percent weight loss and decomposition [109].
2.3.2.13 Scanning electron microscopy (SEM)

SEM was utilized to study the external morphology (size, shape and surface) of the prepared beads. Randomly selected beads were placed on double-sided copper conductive tape (NEM Nisshin EM Co. Ltd.) fixed on SEM aluminum stubs. The beads were then sputter-coated with a thin layer of gold in vacuum for 45 seconds at 20 mA using a coating unit (Cressington 108 auto sputter coater, UK) in order to make it electrically conductive and was analyzed with a SEM instrument (FEI Quanta 3D FEG Dual Beam Electron Microscope, USA) operated at 5kV.

2.3.2.14 Atomic force microscopy (AFM)

AFM was utilized to determine the variability between different batches of beads and between different beads. Parameters such as surface structure, morphology, and roughness were evaluated for the blank (without drug) and drug-loaded beads [110]. Randomly selected beads were placed on a double-sided tape fixed on an AFM stub. The beads were analyzed using AFM instrument (Nanosurf Easyscan 2 AFM) possessing a cantilever and camera affixed on top of the Easyscan 2 scan head. The scan range selected was 25um with the operating mode being dynamic force and speed of 0.1 mm/s. The results were translated by the Easyscan 2 controller and recorded by Easyscan 2 software.
2.4 RESULTS AND DISCUSSION

2.4.1 Determination of alginate beads water content

The result from table 2-2 reveals that water loss was highest in formulation F8 followed by F7, F5 and F6. Since the concentration of alginic acid and calcium chloride was identical in all the formulations, highest amount of water loss in formulation F8 was due to HPMC K15M due to its ability to retain high amounts of water when compared to other polymers [111]. F6 demonstrated least water loss due to the quick drying nature of acacia. F5 demonstrated less water less than F7 and F8; this could be attributed to the formation of a dense polymer matrix of sodium alginate and CMC and gelation with smoother surface and less pores. The results were further verified by comparing it with water loss results obtained from TGA studies.

Table 2.2: Percent of water loss from calcium-alginate beads

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Water Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>78.73 ± 1.32</td>
</tr>
<tr>
<td>F6</td>
<td>76.74 ± 0.67</td>
</tr>
<tr>
<td>F7</td>
<td>82.10 ± 1.71</td>
</tr>
<tr>
<td>F8</td>
<td>89.11 ± 1.49</td>
</tr>
</tbody>
</table>

2.4.2 Determination of percent yield, drug loading and entrapment efficiency

The entrapment efficiency for the formulation F1 to F4 was found to be in range of 8.19 ± 1.54% to 14.80 ± 1.78%. This may be attributed to leakage of the drug into the cross-linking solution due high porosity and low density of the alginate polymer matrix devoid of other polymers [112]. Entrapment efficiencies for formulations F5 to F12 were optimized by using various polymers in along with sodium alginate and also by
modulating the coating polymer ratio. The entrapment efficiency of the optimized uncoated formulations F5 to F8 were found to be in range of $38.22 \pm 1.60\%$ to $67.79 \pm 2.37\%$ with formulation F5 possessing the highest entrapment and F6 the lowest. The optimized formulations were then coated with cellulose acetate phthalate (F9 to F12) and the entrapment increased significantly for all the coated formulations. F9 possessed the highest entrapment ($74.66 \pm 4.99\%$) whereas F10 the lowest entrapment ($40.90 \pm 0.67\%$) of all coated formulations. Drug loading is directly proportional to the ratio of amount of drug used during preparation of beads to the total weight of beads produced [113]. Drug loading was found to be low and in range of $0.07 \pm 0.01$ to $1.66 \pm 1.39 \%$ in all the formulations. Low amount of the drug was used in the preparation of beads because as the drug loading was increased, the entrapment efficiency was found to decrease. Percent yield was in the range of $24.90\%$ to $93.62\%$ where F9 possessed the highest percent yield ($93.62\%$) and F1 possessed the lowest percent yield ($24.90\%$).

**Table 2.3:** Effect of polymer concentration, cross-linking agent concentration, and coating polymer concentration on percent yield, drug loading and entrapment efficiency.

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Yield (%)</th>
<th>Drug loading (% ± SD, n=3)</th>
<th>Entrapment efficiency (% ± SD, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>24.90</td>
<td>$1.66 \pm 1.39$</td>
<td>$8.19 \pm 1.54$</td>
</tr>
<tr>
<td>F2</td>
<td>63.95</td>
<td>$0.51 \pm 0.27$</td>
<td>$9.92 \pm 2.15$</td>
</tr>
<tr>
<td>F3</td>
<td>68.16</td>
<td>$0.73 \pm 0.13$</td>
<td>$12.76 \pm 0.88$</td>
</tr>
<tr>
<td>F4</td>
<td>66.15</td>
<td>$0.66 \pm 0.34$</td>
<td>$14.80 \pm 1.78$</td>
</tr>
<tr>
<td>F5</td>
<td>89.96</td>
<td>$0.12 \pm 0.02$</td>
<td>$67.79 \pm 2.37$</td>
</tr>
<tr>
<td>F6</td>
<td>35.69</td>
<td>$0.37 \pm 0.03$</td>
<td>$38.22 \pm 1.60$</td>
</tr>
<tr>
<td>F7</td>
<td>47.42</td>
<td>$0.30 \pm 0.01$</td>
<td>$42.84 \pm 1.78$</td>
</tr>
<tr>
<td>F8</td>
<td>42.81</td>
<td>$0.37 \pm 0.09$</td>
<td>$40.55 \pm 3.40$</td>
</tr>
<tr>
<td>F9</td>
<td>93.62</td>
<td>$0.07 \pm 0.01$</td>
<td>$74.66 \pm 4.99$</td>
</tr>
<tr>
<td>F10</td>
<td>45.52</td>
<td>$0.17 \pm 0.03$</td>
<td>$40.90 \pm 0.67$</td>
</tr>
<tr>
<td>F11</td>
<td>64.03</td>
<td>$0.13 \pm 0.01$</td>
<td>$48.09 \pm 2.46$</td>
</tr>
<tr>
<td>F12</td>
<td>69.01</td>
<td>$0.12 \pm 0.01$</td>
<td>$41.08 \pm 3.84$</td>
</tr>
</tbody>
</table>
2.4.3 Analysis of ceftriaxone sodium by RP-HPLC

From the chromatogram shown in figure 2-2, it is observed that ceftriaxone sodium peak was successfully separated and eluted with the retention time at 7.271 min. Since ceftriaxone is a highly polar compound, it is necessary to add an ion-pairing agent to the mobile phase to ensure retention of the drug on the column (14). So tetrabutylammonium hydroxide (TBAOH) was chosen as the ion-pairing reagent since it allows the separation of ionic and highly polar substances on reversed phase HPLC columns [114]. HPLC method validation for ceftriaxone sodium was performed and found to be linear in the range of 50 µg/ml – 0.78 µg/ml with a correlation coefficient of 1. The percentage recovery of ceftriaxone sodium ranged from 99.37% to 103.32% and intra-day precision was found to be 0.49% – 0.88% (measured using %RSD) [115].

Fig. 2-2: Chromatogram of 50 µg/ml of ceftriaxone sodium analyzed on the Gemini C18 250 x 4.6 mm, 5 µm column
2.4.4 Loose surface crystal (LSC)

LSC study was an important parameter to determine the amount of the drug present on or near to the surface of the beads. This phenomena may lead to drug degradation or leaching of the drug in gastric fluids (pH 1.2) [116]. LSC was highest in F10 formulation whereas it was lowest in formulation F11. The in vitro release data for the formulation F9 to F12 further confirmed data obtained from this study as the beads were found to release ceftriaxone when placed in simulated gastric fluid (pH 1.2) for 2 hours. This confirmed that the drug was present more near to the surface of the beads contributing to less entrapment into the beads. The reason for this might be the formation of highly porous less dense matrix or diffusion of the drug to the periphery of the particles during the curing and drying process.

Table 2.4: Percentage of Loose Surface Crystals of ceftriaxone sodium on calcium-alginate beads

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Loose Surface Crystal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F9</td>
<td>28.50 ± 4.23</td>
</tr>
<tr>
<td>F10</td>
<td>39.60 ± 2.45</td>
</tr>
<tr>
<td>F11</td>
<td>23.80 ± 6.61</td>
</tr>
<tr>
<td>F12</td>
<td>34.00 ± 2.34</td>
</tr>
</tbody>
</table>

2.4.5 Swelling index study

The “Swelling-Dissolution-Erosion” process is highly complex with the osmotic pressure gradient between the alginate gel and the environment playing an important role in the swelling process. The swelling ratio of the beads depend on the pH of the solution since under acidic conditions calcium alginate beads swelling is insignificant [117].
Table 2.5: Swelling index of calcium-alginate beads

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Swelling index</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>3.35</td>
</tr>
<tr>
<td>F6</td>
<td>1.568</td>
</tr>
<tr>
<td>F7</td>
<td>2.996</td>
</tr>
<tr>
<td>F8</td>
<td>2.379</td>
</tr>
<tr>
<td>F9</td>
<td>2.063</td>
</tr>
<tr>
<td>F10</td>
<td>0.859</td>
</tr>
<tr>
<td>F11</td>
<td>1.83</td>
</tr>
<tr>
<td>F12</td>
<td>1.506</td>
</tr>
</tbody>
</table>

The low swelling index of the calcium alginate-polymer beads (F5- F8) in acidic pH is probably due to the proton-calcium ion exchange forming insoluble alginic acid regions as the solvent penetrates into the dense gel network. The swelling behavior of the coated beads containing additional polymers (F9, F10, F11, F12) was observed to be lowest compared to formulations F5 to F8 because of the presence of pH sensitive enteric coating polymer covering the dense polymer matrix of beads. This further decreased the swelling by preventing the solvent penetration into the gel network.

For a drug to get release, the beads must absorb solvent and swell significantly. Swelling of beads is mainly due to the hydration of the hydrophilic groups of alginate and other polymers causing the improvement in the solubility of the beads membrane. The results suggests that the dried beads swell slightly in the stomach but when they are subsequently transferred to upper intestine, the particles begin to swell significantly and they behave as matrices for sustained release of the incorporated drug and finally erode into the lower intestine [105].
2.4.6 In vitro drug release study

The release profile of ceftriaxone sodium in enzyme-free simulated gastric fluid (SGF) followed by enzyme-free simulated intestinal fluid (SIF) from the calcium alginate beads prepared with addition of various sodium alginate-polymer blends are shown in figure 2-3. The drug release from the alginate beads depends on the penetration of the dissolution medium into the beads, swelling and dissolution of alginate matrix, and the dissolution of the drug subsequent to leaching through the swollen matrix [118]. Within the first 2 hours, 35% to 47% of the drug was being released in SGF for all the coated formulations. This attribute was due to the nicks on the surface of the beads which occurred by partial collapse of the polymer network due to dehydration after coating with organic solvents such as ethyl acetate [119]. The remaining part of the entrapped drug was released in the SIF, with sustained release for up to 10 hours for F10, 14 hours for F11 and F12 and 18 hours for F14. Even in the presence of cracks on the bead surface drug release continued for an extended period of time probably due to the dense gel network preventing the leaching out of the drug from the beads.

Table 2.6: In vitro % cumulative release of ceftriaxone sodium beads in simulated gastric fluid (pH 1.2).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Cumulative drug release in Simulated Gastric Fluid (pH 1.2)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F9</td>
</tr>
<tr>
<td>15</td>
<td>13.07</td>
</tr>
<tr>
<td>30</td>
<td>22.94</td>
</tr>
<tr>
<td>60</td>
<td>29.86</td>
</tr>
<tr>
<td>120</td>
<td>35.15</td>
</tr>
</tbody>
</table>

* values indicate mean %, (n=3).
**Table 2.7:** *In vitro* % cumulative release of ceftriaxone sodium beads in simulated intestinal fluid (pH 6.8).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Cumulative drug release in Simulated Intestinal Fluid (pH 6.8)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F9</td>
</tr>
<tr>
<td>135</td>
<td>53.45</td>
</tr>
<tr>
<td>150</td>
<td>61.55</td>
</tr>
<tr>
<td>180</td>
<td>70.65</td>
</tr>
<tr>
<td>240</td>
<td>78.67</td>
</tr>
<tr>
<td>360</td>
<td>85.55</td>
</tr>
<tr>
<td>600</td>
<td>92.63</td>
</tr>
<tr>
<td>840</td>
<td>95.93</td>
</tr>
<tr>
<td>1080</td>
<td>100.00</td>
</tr>
</tbody>
</table>

** values indicate mean %, (n=3).

**Fig. 2-3:** *In-vitro* drug release profile of ceftriaxone sodium loaded beads containing Sodium alginate with CMC (F9), Acacia (F10), HPMC K4M (F11) and HPMC K15M (F12). Results indicate mean % ± SD, (n=3).
2.4.7 Analysis of release kinetics and mechanism

Data from the *in vitro* release was fitted into various kinetic models to evaluate the release kinetics of ceftriaxone sodium from coated beads. The kinetic models used were zero-order, first-order, Higuchi and Korsemeyer-Peppas model. The interpretation of data was based on the value of the regression coefficients which can be found in table 2-8.

**Table 2.8:** In-vitro release kinetic parameters of ceftriaxone sodium beads coated with cellulose acetate phthalate

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F9</td>
<td>0.6824</td>
<td>0.9731</td>
<td>0.8478</td>
<td>0.8816</td>
<td>0.461</td>
</tr>
<tr>
<td>F10</td>
<td>0.7801</td>
<td>0.9794</td>
<td>0.9016</td>
<td>0.8724</td>
<td>0.2364</td>
</tr>
<tr>
<td>F11</td>
<td>0.7256</td>
<td>0.9464</td>
<td>0.8837</td>
<td>0.8961</td>
<td>0.1613</td>
</tr>
<tr>
<td>F12</td>
<td>0.6935</td>
<td>0.9683</td>
<td>0.8537</td>
<td>0.8675</td>
<td>0.1538</td>
</tr>
</tbody>
</table>

From the regression coefficients, the release kinetics for all the coated formulation (F9, F10, F11, and F12) followed first order kinetics as highest linearity was displayed for this curve. Comparing the release exponent n from the Korsmeyer-Peppas model for all the coated formulation; F10, F11 and F12 have the n value < 0.45 which indicates that the release is governed by Fickian diffusion; whereas for F9 the n value was between 0.45 to 0.89 which indicates that the release is governed by anomalous or non-Fickian diffusion. This results suggest that a combination of diffusion and erosion based drug release mechanism may be involved in release kinetics [120].
2.4.8 Particle size analysis

Particle size analysis was done by optical microscopy. The mean particle size of the beads from various formulation (F5 to F12) were obtained in the range of 1.039 ± 0.197 mm and 2.146 ± 0.357 mm. Particle size distribution of each formulation was found to be within a narrow range but the mean particle size differed among the formulations. This could be due to the increase in the relative viscosity at high concentrations of polymers thus leading to formation of large droplets during addition of drug-polymer solution into the calcium chloride solution for gelation. The results indicated that the coated formulations (F9, F10, F11, and F12) have larger particle size compared to uncoated formulations (F5, F6, F7, and F8). This could be attributed to the accumulation of the coating polymer used for preparation of beads that results in an increase in diameter of the beads.

Table 2.9: Particle size of calcium-alginate beads

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Particle Size (mm ± SD, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>1.355 ± 0.190</td>
</tr>
<tr>
<td>F6</td>
<td>1.039 ± 0.197</td>
</tr>
<tr>
<td>F7</td>
<td>1.443 ± 0.181</td>
</tr>
<tr>
<td>F8</td>
<td>1.489 ± 0.306</td>
</tr>
<tr>
<td>F9</td>
<td>1.593 ± 0.146</td>
</tr>
<tr>
<td>F10</td>
<td>1.166 ± 0.171</td>
</tr>
<tr>
<td>F11</td>
<td>1.796 ± 0.264</td>
</tr>
<tr>
<td>F12</td>
<td>2.146 ± 0.357</td>
</tr>
</tbody>
</table>
2.4.9 Thermal gravimetric analysis (TGA)

TGA thermograms of formulation F1 to F8 are shown in figure 2-4. The decomposition temperature and thermal stability of pure alginic acid and sodium carboxymethylcellulose was higher than that of cross-linked beads. Pure alginic acid and sodium carboxymethylcellulose TG curve presents an initial dehydration process followed by decomposition in two overlapping steps under nitrogen. Similar behavior was observed by Soares et al. [121]. The TGA thermograms of the cross-linked beads showed similar type of decomposition and weight loss pattern. Formulations F1 to F4, F6 and F8 showed similar type of decomposition and weight loss pattern; whereas formulations F5 and F7 showed single step decomposition and a slightly different weight loss pattern.

Formulations F1 to F4 were assessed to check the strength of the interaction between the components of the beads and results showed that F2 had greater stability (200 °C) and lower percent weight loss (58%). Similarly, when the beads containing various sodium alginate-polymer blends were compared (F5 to F8), F5 had greater stability (180 °C) and lower percent weight loss (56%) compared to formulations F6, F7 and F8. The lower percent weight loss of the beads could be indicative of strong interactions between the polymer components of the beads [122].
Fig. 2-4: Comparison of TGA thermograms of pure alginic acid, pure cmc and blank (without drug) microbead formulations of F1 – F8.

2.4.10 Scanning electron microscopy (SEM)

Based on the entrapment efficiency and in vitro results, formulation F5 and F9 were chosen for the SEM studies. Scanning electron micrographs of blank and drug loaded beads (F5 & F9), prepared by sodium alginate, sodium carboxymethylcellulose and cellulose acetate phthalate as enteric coating polymer are shown in figures 2-5, 2-6 and 2-7. SEM micrographs of the blank formulation (figures 2-5a and 2-5b) shows that the microbead was almost spherical and porous in nature with a rough outer surface. From the images (figures 2-6a and 2-6b), F5 beads was seen to possess a rough surface with a large number of pores causing the rapid release of drug into the medium. It also exhibited
a flat base and sandy appearance because of the surface-associated crystals of drug [105].

On the other hand, SEM images (figures 2-7a and 2-7b) of coated beads (F9) showed a smooth surface and smaller number of pores due to coating, which led to observation of a decrease in the drug release rate from beads. Moreover, the surface also exhibited cracks that can be attributed with the partial collapsing of the polymer network due to dehydration after coating with organic solvents such as ethyl acetate. This leads to the degradation or leaching of the drug from beads into the gastric fluid [119].

Fig. 2-5: SEM of blank (without drug) sodium alginate-cmc bead
2.4.11 Atomic force microscopy (AFM)

Atomic force microscopy (AFM) was used to obtain information about the topographical features of the beads such as morphology and roughness [110]. The images shown in
Figure 2-8 & 2-9 depicts the 3D topographical comparison between the uncoated formulation and coated formulations. From the results, it can be seen that the uncoated beads possessed greater roughness than the coated beads.

**Fig. 2-8:** Topographic images of blank (without drug) beads of (a) F5 (b) F6 (c) F7 and (d) F8 representing the surface morphology and roughness.
**Fig. 2-9:** Topographic images of (a) F9 (b) F10 (c) F11 and (d) F12 representing the surface morphology and roughness of the beads after coating.
2.5 CONCLUSION

It can be concluded that ionotropic gelation technique can be successfully used for preparation of ceftriaxone sodium beads using sodium alginate and other polymers such as sodium CMC, HPMC K4M, HPMC K15M and acacia as drug release modifiers. Parameters such as polymer concentration, calcium chloride concentration, coating polymer concentration, stirring speed and cross-linking time plays a vital role in achieving high entrapment efficiency, mean particle size, swelling behavior, surface morphology and in vitro drug release. Calcium alginate beads swelled at pH 1.2 but underwent diffusion and erosion at pH 6.8. The use of sodium alginate, carboxymethylcellulose and cellulose acetate phthalate decreased the drug release behavior in gastric conditions to some extent but sustained the drug release at intestinal pH. Further investigation using in vivo models should be carried out to substantiate the in vitro results.
2.6 REFERENCES


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