I. MATERIALS AND METHODS:

OBJECTIVE

Following intramuscular administration of a unit 500 mg or 1 g dose of cefotaxime injection to normal volunteers, mean peak serum concentrations were attained within 30 minutes (11.7 and 20.5) mcg/mL respectively. About 60% of the given dose was excreted from urine during the first 6 hours following the start of the infusion. Approximately 20-36% of an IV dose of cefotaxime is excreted by the kidney as unchanged cefotaxime and 15-25% as the desacetyl derivative. The desacetyl metabolites also contribute to the bactericidal activity. Two other metabolites account for about 20-25%. They lack bactericidal activity. The maximum adult dosage should not exceed 12 grams/day.

Intraperitoneal chemotherapy prolongs survival of ovarian cancer patients, but its utility is restricted by treatment-related complications and inadequate drug penetration in larger tumors. The present report describes the development of paclitaxel-loaded microspear designed for intraperitoneal treatment. These advantages may help to remove the need of permanent catheter, reduce the local toxicity, and better the compliance of patients and medical staff.

These combined features resulted in the following advantages over paclitaxel/Cremophor: greater tumor targeting (16-times higher and more sustained concentration in tumors), lower toxicity to intestinal crypts and less body weight loss, greater therapeutic efficacy (longer survival and higher cure rate), and greater convenience (less frequent dosing). Tumor-penetrating microparticles may overcome the toxicities and compliance related problems that have limited the utility of intraperitoneal therapy.

Nevertheless these formulations are quite seldom in revealing the complications associated with treatment. Basically, the complications of cefotaxime is associated with the risk of maximum dose of 12 grams/day could do some significant damage to the cells and more complicates the disease. Inversely, making use of the novel drug delivery system which reduces the dosage regimen in treating the chronic disease is a great challenge. With diversity of biodegradable polymers accessible for the object of protecting and enhancing the drug molecules to which it is used. The polymeric systems really increase the solubility, bioavailability and
safeguard the drug from gastrointestinal enzymes. Such advantageous method of polymeric system with microparticles has some remarkable benefit over the other existing methods.

The challenges are capable, particularly those related to the growth of suitable recognition layers. Useful recognition groups attached to the microparticles must be loaded to a high density while maintaining their characteristics. The potential use of nano- and microparticles from significant advantages such as:

(i) The capability to target specific locations in the body;

(ii) The reduction of the drug amount require to achieve a particular concentration in the near of the target; and

(iii) The decrease of the concentration of the drug molecules at non-target sites minimizing severe undesirable effects.

The actual purpose is to prepare the antibiotic drug cefotaxime incorporated microparticles by solvent evaporation method with selected excipients and solvent system. Further, characterization, evaluation including invitro release profile of the formulation. Besides, stability studies like DSC (Differential Scanning Calorimetry), Stress testing (ICH- Guidelines) is included in this research work.
DRUG PROFILE
CEFOTAXIME SODIUM

IUPAC Name: Cefotaxime is designated by IUPAC rules as (7R)-3-acetoxymethyl-7-[(Z)-2-(2-aminothiazol-yl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate.

Formula: C_{16}H_{16}N_{5}NaO_{7}S_{2}

Cefotaxime is a semisynthetic agent obtained from various species of the mold Cephalosporium and Streptomyces. Cephosporins inhabit bacterial cell wall synthesis and preventing cross linking of peptidoglycan [Charles, R. C. et al].

Molecular Weight: 455.47 g/mol

Category: Antimicrobial against S. aureus, Coagulase negative Staphylococci, S. pneumonia, Streptococcus spp., H. influenzae, M. catarrhalis, N. meningitides, N. gonorrhoeae, Enterobacteriaceae, E.coli

Description: Off-white crystalline powder [I. P. 2007].

Solubility: Freely soluble in water.

Storage: Stored in an air-tight light resistant amber coloured containers.

Standards: Cefotaxime Na contains the equivalent of NLT 85.5 % and NMT 96.4 % of cefotaxime, C_{16}H_{17}N_{5}O_{7}S_{2}.

Antibaterial activity: Cefotaxime is highly stable to many of the bacterial β-lactamase and active against gram-positive and gram-negative aerobic bacteria [Goodman and Gilman’s (2006)].

Mechanism of action: Cephosporins inhibit bacterial cell wall synthesis and preventing cross linking of peptidoglycan. The cephalosporins are also
considered to play a role in the augmentation of bacterial cell autolysins which may put in to bacterial cell lysis. Cefotaxime is bactericidal for both intracellular and extracellular microorganism.

**Pharmacokinetic data:**

- **Urinary excretion**: 60 % as unchanged drug and metabolites [THE MERK MANUAL (2013)].
- **Plasma protein binding**: 13-38 %
- **Clearance**: 3.5 ± 1.6 ml/min/kg
- **Volume of distribution**: 14 L
- **Half life**: 1-1.7 hrs
- **Peak time, serum: I.M.**: 30 minutes
- **Distribution**: Crosses the placenta and distributed into milk [MedlinePlus].

**Adverse Reactions:**

- **Hypersensitivity**: Maculopapular rash, Urticaria, Pruritis, Anaphylaxis/angioedema, eosinophilia
- **Hematologic**: Hypoprothrombinemia, Neutropenia, Leukopenia,Thrombocytopenia
- **GI**: Diarrhoea, C. difficile disease
- **Renal**: Interstitial nephritis
**Therapeutic uses:** Bacterial meningitis, lyme disease, bacterial arthritis, bacterial meningitis, peritonitis, septicaemia, bone and soft tissue infections, pneumoneae, gonorrhea.

**Dose:**

- **IV**: Powder for reconstitution: 500 mg, 1g, 2 g, 10 g, Intravenous Solution: 1g/50 mL, 2 g/50 ml

- **Dosing in adults**: Meningitis: 1-2 g iv/im every 8 h
  - Bone and/or joint infection: 1-2 g iv/im every 8 h
  - Gonococcus urethritis: 0.5 g im x 1 dose
  - Lower respiratory tract infection: 1-2 g iv/im every 8 h
  - Bacterial peritonitis: 1-2 g every 8-12 h
  - Dosing in pediatrics: 100-150 mg/kg/day divided every 4-6 h
FORMULATIONS OF MICROPARTICLES

Description of Chemicals

ETHYL CELLULOSE [Dahl, T. C. (2005)]

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synonym</strong></td>
<td>Ethocel, Surelease, Aquacoat ECD, Aqualon.</td>
</tr>
<tr>
<td><strong>Formula</strong></td>
<td>C(<em>{27})H(</em>{46})O</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>386.67 g/mol</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Ethylcellulose is a tasteless, free-flowing, and White to light tan colored powder</td>
</tr>
<tr>
<td><strong>Melting Point</strong></td>
<td>240 °C- 255 °C</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Ethylcellulose is practically insoluble in glycerin, propylene glycol and water. Ethylcellulose contain less than 46.5 % of ethoxy groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran and in mixtures of hydrocarbons with ethanol (95 %). Cellulose that contains not less than 46.5 % of ethoxy groups is freely soluble in chloroform, ethanol (95 %), ethylacetate, methanol, and toluene.</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>It should be stored at a temperature not exceeding not more than 32 °C in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.</td>
</tr>
<tr>
<td><strong>Applications</strong></td>
<td>Coating agent, flavouring fixative, tablet binder tablet filler, viscosity increasing agent.</td>
</tr>
</tbody>
</table>

HYDROXY PROPYLEMETHYL CELLULOSE: [Harwood, R. J. (2005)]
<table>
<thead>
<tr>
<th><strong>Synonym</strong></th>
<th>Hypromellose, 2-hydroxypropyl methyl ether cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category</strong></td>
<td>Ophthalmic lubricant, as well as excipients and controlled delivery component in oral medicaments, food additive, an emulsifier</td>
</tr>
<tr>
<td><strong>Molecular Formula</strong></td>
<td>C₃H₇₀</td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
<td>59.08 g/mol</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>White powder</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Soluble in water, most organic solvents, insoluble in hot water, alcohol</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Preserve in a well- closed container</td>
</tr>
<tr>
<td><strong>Applications</strong></td>
<td>It is an enteric film coating material or a matrix binder in solid dosage form. It is used as viscosity controlling agent, gelling agent, film former, stabilizer, lubricant binder/emulsifying agents and suspending agents.</td>
</tr>
</tbody>
</table>

**DICHLOROMETHANE:**

<table>
<thead>
<tr>
<th><strong>Synonym</strong></th>
<th>Methylene chloride, Methylene dichloride</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Formula</strong></td>
<td>CH₂Cl₂</td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
<td>84.93 g/mol</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>A colorless liquid with sweet, penetrating ether like odour</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Miscible with water, it is miscible with many organic solvents</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at a room temperature</td>
</tr>
</tbody>
</table>
**Applications** : Paint stripper and degasser. In the food industry, it has been used to decaffeinate coffee and tea as well as to prepare extract of hops and other flavorings.

**IRONOXIDE** [Galichet, L. Y. (2005)]

- **Synonyms** : Iron oxide black, iron III oxide hydrated iron oxide red, iron oxide yellow monohydrate.
- **Formula** : $\text{Fe}_2\text{O}_3$
- **Molecular Weight** : 159.68 g/mol
- **Description** : Red powder.
- **Melting Point** : 1,565 °C.
- **Solubility** : Soluble in strong mineral acids.
- **Storage** : Stored in well-closed containers stored in a cool, dry, place.
- **Applications** : widely used in cosmetics, foods, and pharmaceutical applications as colorants and UV absorbers.
### Chemicals Used:

**Table 1**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Materials</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cefotaxime Sodium</td>
<td>Orchid Chemicals</td>
</tr>
<tr>
<td>2.</td>
<td>Ironoxide AR</td>
<td>SD fine-chemicals limited</td>
</tr>
<tr>
<td>3.</td>
<td>Dichloromethane AR</td>
<td>Merck</td>
</tr>
<tr>
<td>4.</td>
<td>Ethylcellulose AR</td>
<td>Finar Reagents</td>
</tr>
<tr>
<td>5.</td>
<td>Hydroxy Propylmethyl Cellulose AR</td>
<td>SD fine-chemicals limited</td>
</tr>
<tr>
<td>6.</td>
<td>Potassium dihydrogen Ortho phosphate AR</td>
<td>Merck</td>
</tr>
<tr>
<td>7.</td>
<td>Disodium hydrogen phosphate AR</td>
<td>SD Fine-chemicals limited</td>
</tr>
<tr>
<td>8.</td>
<td>Sodium chloride AR</td>
<td>SD Fine-chemicals limited</td>
</tr>
<tr>
<td>9.</td>
<td>Sodium hydroxide AR</td>
<td>SD Fine-chemicals limited</td>
</tr>
</tbody>
</table>

### Instrument Used:

**Table 2**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Equipments Used</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Electronic Balance</td>
<td>Sartorius</td>
</tr>
<tr>
<td>2.</td>
<td>Magnetic Stirrer</td>
<td>Remi</td>
</tr>
<tr>
<td>3.</td>
<td>UV- Visible Spectrophotometer</td>
<td>Shimadzu UV-1700</td>
</tr>
<tr>
<td>4.</td>
<td>Ultra Sonic processor</td>
<td>Vibronics</td>
</tr>
<tr>
<td>5.</td>
<td>pH meter</td>
<td>Elico</td>
</tr>
<tr>
<td>6.</td>
<td>Scanning Electron microscopy</td>
<td>Carl Zeiss SEM model EVO MA 15</td>
</tr>
<tr>
<td>7.</td>
<td>Differential Scanning Colorimetry</td>
<td>NETZSCH DSC 204</td>
</tr>
<tr>
<td>8.</td>
<td>Dissolution Test Apparatus</td>
<td>Shimadzu UV-1700</td>
</tr>
<tr>
<td>9.</td>
<td>Magnetic Resonance Imaging</td>
<td>MAGNETOM_ESSENZA HFS</td>
</tr>
<tr>
<td></td>
<td>Orbital Shaker</td>
<td>Verilog</td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>10.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PREFORMULATION STUDIES:

1. Solubility:

The solubility of drug is determined by dissolving it in distilled water and phosphate buffer saline pH 7.4 using a mechanical shaker for 24 hrs. After 24 hrs absorbance were measured in Shimadzu UV-1700 spectrophotometer at a wavelength of 254 nm using distilled water and phosphate buffer saline pH 7.4 as Blank respectively and the percentage of solubilized drug was calculated. Solubility determinations were carried out in triplicate (n=3).


Weighed accurately about 0.1g of cefotaxime and dissolved it to sufficient water and make up to 100 ml with phosphate buffer. From the stock solution a series of cefotaxime solutions of known concentrations i.e. 5, 10, 15, 20, 25 and 30 µg/ml in phosphate buffer saline pH 7.4 were prepared and the absorbance were measured in order to generate a standard curve on a Shimadzu UV-1700 UV spectrophotometer at a λ of 254 nm using phosphate buffer saline pH 7.4 as Blank. The values of triplicate (n = 3) determinations are given in the Table 4 and Graph 1.

2. Preparation of Reagent:

Preparation of phosphate buffer saline pH 7.4: [IP 2007]

The phosphate buffer saline pH 7.4 was prepared by dissolving 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient distilled water to produce 1000 ml. Adjust the pH if necessary.

3. PREPARATION OF MICROSPHERES:

Ethylcellulose microspheres were prepared according to the solvent evaporation procedure. Accurately weighed quantity of ethylcellulose [M. Guyot et al (1998)] (250, 500, 750, 1000, 1250, 1500, 1750 or 2000 mg) and 500 mg of cefotaxime was dissolved in 20 ml methylene chloride [M. Guyot et al (1998)], [Shujaat Ali Khan, et al (2010)] containing iron oxide 48 mg [Beata Chertok, et al (2008)] and sonicated for 30 sec. This organic phase was then
emulsified in 250 ml of 0.5 % (w/v) hydroxy propylmethyl cellulose [M. Guyot, et al (1998)] aqueous solution, at 750 rpm. Stirring was maintained until the solvent had evaporated completely. Microspheres were then washed with deionized water, filtered and dried under vacuum for 12 hrs.

CHARACTERISTICS OF MICROSPHERES:

1. **Determination of Practical Yield:**

   The yield of microspheres was determined by comparing the total weight of microspheres formed against the combined weight of the polymer and drug. The percentage practical yield determinations were carried out in triplicate (n=3) was calculated by the following equation.

   \[
   \text{Mass of microspheres obtained} \times 100
   \]

   \[
   \text{Total weight of drug and polymer used}
   \]

2. **Determination of Angle of Repose:** [Alfred Martin, (2011c)]

   Angle of repose of the microspheres, which measures the frictional forces to microparticles flow was determined by the fixed funnel method.

   Here, the funnel is fixed at a certain height and the powder is allowed to flow through the stem to form a pile. This is the maximum angle formed between the surface of a heap of microspheres and the horizontal plane. The micromeritic determinations were carried out in triplicate (n=3) was calculated by the following equation.

   \[
   \tan^{-1} h/r
   \]

   Where \( r \) is the radius and

   \( h \) is the height of the microspheres heap.
3. **Estimation of Drug Content:** [Tuncay, M. *et al* (2000)]

Microspheres (100 mg) were sonicated in ultrasonic bath for 10 minutes in 10 ml of phosphate buffer saline pH 7.4. This suspension is then filtered through 0.22 µm Millipore filters. The filtrate was analyzed at a λ of 254 nm by Shimadzu UV-1700 UV spectrophotometer using phosphate buffer saline pH 7.4 as Blank. The absorbance gives the amount of drug present on the surface of the microspheres. To the dried and accurately weighed microspheres 5 ml of dichloromethane was added to dissolve the polymer and finally methanol was added to precipitate the polymer. The absorbance was measured at a wavelength of 254 nm by an UV spectrophotometer using phosphate buffer saline pH 7.4 as Blank. All the determinations were made in triplicate (n=3) was calculated by the following formula.

\[
\text{Drug content in microspheres} \times 100
\]

\[
\% \text{ Drug Content} = \frac{\text{Drug included in the formulation}}{\times 100}
\]

4. **Determination of Entrapment Efficiency:** [Tuncay, M. *et al* (2000)]

The microspheres (100 mg) in 10 ml phosphate buffer saline pH 7.4 after sonication, filtered through 0.22 µm Millipore filters. The absorbance of the filtrate was measured at 254 nm (Shimadzu UV spectrophotometer) using phosphate buffer saline pH 7.4 as Blank. The absorbance gives the amount of drug present on the surface of the microspheres using phosphate buffer saline pH 7.4 as Blank. To the dried and accurately weighed microspheres 5 ml of dichloromethane was added to dissolve the polymer and finally methanol was added to precipitate the polymer. The absorbance was measured at a wavelength of 254 nm by an UV spectrophotometer using phosphate buffer saline pH 7.4 as Blank. The mean entrapment efficiency values of triplicate determinations were tabulated (n=3) was calculated by the following formula.

\[
\text{Drug entrapped in microspheres} \times 100
\]

\[
\text{Percentage Entrapment Efficiency} = \frac{\text{Drug included in the formulation}}{\times 100}
\]
4. **Fourier- Transform Infra Red Interpretation:** [David, G. W. (1999)]

The drug Cefotaxime, the excipients namely Iron oxide, Ethylcellulose, Hydroxy Propylmethyl Cellulose and formulation were analyzed by Fourier- Transform I.R. Spectrophotometer for their compatibility with the help of potassium bromide disc. It is shown in the graphs.


The surface morphology and particle sizes of prepared microspheres were directly analyzed by Carl Zeiss Scanning Electron Microscopy model EVO MA 15. Because SEM has high resolution and the sample preparation is relatively easy and charging problems were avoided by depositing a thin layer of gold on the particle surface.

The sample is scanned with the help of an electron beam generated by the operation of the microscope, and the image is formed by focusing it. Here, the micro-structural characteristics of the microspheres were studied by scanning electron microscopy (SEM).

6. **Compatibility Study:** [Alfred Martin, (2011)]

The stability of formulations depends upon the compatibility of the drug with the excipients. It is of significance to detect any possible physical (or) chemical interaction, since it can affect the bioavailability and stability of the drug.

Differential scanning calorimetry is a fast and reliable to screen drug-excipients compatibility and provide maximum interactions.

**Differential Scanning Calorimetry (DSC)** [Parthiban, K. (2008)]

Differential scanning calorimetry (NETZSCH DSC 204) equipped with a monitor and a computerized Thermal Analysis System and printer were used. The instrument was calibrated with standard medium.
5-10 mg of samples was weighed and it is hermetically sealed in flat-bottomed aluminum pans. These samples were heated over a temperature range of 50-400 °C in an atmosphere of nitrogen (50 ml/mt) at a constant heating rate of 20 °C per minute, with alumina being the reference standard.

In order to avoid the need for normalization of enthalpy change on account of variation in sample weight, care would be taken to conduct DSC analysis on accurately weighed sample 5 mg or very close to 5 mg portions of the drug, as well as each excipient and each admixture in an amount (10 mg) equivalent to 5 mg of the drug. This would help not only to expedite data analysis in terms of enthalpy change (loss/gain) associated with the peak of interest but also provide the gross idea the possibility of interaction simply from visual comparison of the curves relevant to the individual excipients.


**Diffusion study**

The *in-vitro* release of Cefotaxime from the microsphere formulations were studied by dialysis bag diffusion technique.

**Diffusion Study of Cefotaxime Magnetic Microsphere**

Cefotaxime microspheres, equivalent to 10 mg of cefotaxime was taken in the dialysis bag and placed in a basket rotated at 100 rpm containing 900 ml of phosphate buffer saline pH 7.4 maintaining the temperature 37 ± 0.5 °C. Periodically 5 ml of samples were withdrawn and after each withdrawal same volume of buffer was replaced maintained at 37 ± 0.5 °C. Then the samples were assayed by Shimadzu UV-1700 UV spectrophotometer at a wavelength of 254 nm using phosphate buffer saline pH 7.4 as Blank. In-vitro diffusion studies i.e., the drug release determination was performed in triplicate (n=3).

8. **IN-VITRO RELEASE KINETICS**:

The kinetics of drug release is ascertained by plotting the values of *in-vitro* diffuse study with different kinetic models like zero order (cumulative percentage drug diffused Versus Time), first order (log cumulative percentage drug retained Versus Time), Higuchi model (cumulative
percentage drug diffused Versus $\sqrt{\text{Time}})$, Korsmeyer - Peppas model (log cumulative percentage drug diffused Versus log Time) [Paulo, C. et al (2001)]. The linear regression ($r^2$) values obtained were interpreted with the Diffusion exponent (n) of Table 3 to obtain the kinetic models.

**Table: 3**

**Types of diffusional release mechanisms from polymeric matrix:** [Goud H. et al (2005)]


<table>
<thead>
<tr>
<th>Diffusion exponent (n)</th>
<th>Drug transference mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Fickian diffusion</td>
</tr>
<tr>
<td>0.5&lt;n&lt;1.0</td>
<td>Anomalous transport</td>
</tr>
<tr>
<td>1.0</td>
<td>Case-II transport</td>
</tr>
<tr>
<td>Higher than 1.0</td>
<td>Super Case-II transport</td>
</tr>
</tbody>
</table>

9. **Stability Studies as per ICH guidelines- 2000 [ICH]**

Stability studies of drug products carried out as per ICHQ1A(R1) guidelines- 2000.

Stability Studies include analyzing of that substance of the finished product that are liable to alter during storage and which affects quality, safety, and/or efficacy. The testing covers the physical, chemical, and functionality tests (delivery systems).

The formulated microspheres were subjected to accelerated stability testing in which the microspheres were wrapped in aluminium foil and packed in screw capped light resistant amber coloured airtight containers and stored in a stability chamber at 40°C ± 2°C/75% RH ± 5% RH and they are divided in to 3x3 batches. The microsphere samples were assayed at 0, 3, and 6 months using Shimadzu UV-1700 UV spectrophotometer at a $\lambda$ of 254 nm using phosphate buffer saline pH 7.4 as Blank. Stability determinations were conducted in triplicate (n=3).

10. **IN-VIVO ANIMAL MODEL:**
The New Zealand White Rabbits were inoculated with Streptococcus pneumoniae through catheter inserted into the trachea (Test) [Damiana, C. et al (2008)], [Joseph, P. M. et al (2007)]. After 4 days of infection, Cefotaxime magnetic microparticles were administered perorally to all animals (n=3) under a magnetic field strength of 0 Tesla for control or 0.4 Tesla for test for 1 hour using 1.5 Tesla MAGNETOM_ESSENZA HFS system. MRI (Magnetic Resonance Imaging) scanning was performed before and after the administration of cefotaxime microparticles and images were taken after magnetic targeting at 6 hours [Beata Chertok. et al (2008)].