DISCUSSION

The report describes the treatment of pneumonia, antimicrobial activity and their ability to significantly reduce the intracellular or extracellular toxicity and replication of infectious microorganisms. The controlled release of drug and the residence of microparticles at the site of action for longer duration of time are apparent that based on the in-vitro release and in-vivo animal model. These findings are better explained by the results of different evaluation methods are as follows.

Preformulation study:

1. Solubility:

The cefotaxime solubility is determined to be very soluble in both distilled water and phosphate buffer saline pH 7.4 and do not require addition of any cosolvents to improve the solubility.

2. Standard Curve:

Cefotaxime concentration were measured UV spectrophotometrically at a wave length of 254 nm. There was a good linearity, precision, accuracy and interference in this validated process. The process obeyed the Beers law in the concentration range 5–30 µg/ml ($r^2 = 0.999$).

3. Preparation of microspheres:

Microspheres were formed without any major defect that requires no change in any of the parameters. Microspheres formed satisfactorily in all the batches by keeping all the variables at constant while the polymer concentration was alone altered. The various formulations fabricated were CM 1, CM 2, CM 3, CM 4, CM 5, CM 6, CM 7 and CM 8. Accordingly, it was reported in the literature survey that applicable concentration of 0.5 % (w/v) of hydroxy propylmethyl cellulose is sufficient to provide mechanical strength to the microspheres at the time of formulation and maintain the stability during the formulation process. It is shown in Table 5.

Characterization of Microspheres:
1. Percentage Practical Yield:

The yield of microspheres proportionately increases with increase in polymer concentration. The yield of microspheres in various formulations CM 1, CM 2, CM 3, CM 4, CM 5, CM 6, CM 7 and CM 8 was in the range 45.2, 53.2, 61.4, 76.8, 78.6, 81.5, 82.4 and 85.1% respectively. It is shown in the Table 6 and Graph 2.

2. Angle of Repose:

The angle of repose of all the formulations CM 1, CM 2, CM 3, CM 4, CM 5, CM 6, CM 7 and CM 8 were found to be 14.5 ± 0.45, 14.3 ± 0.16, 14.1 ± 0.22, 14.3 ± 0.17, 14.6 ± 0.16, 14.6 ± 0.12, 14.6 ± 0.09, and 14.8 ± 0.05 respectively. All the values were within Carr’s index (5-15 excellent flow) thus the microparticle exhibit excellent flow. The excellent flow is indicative of particles with smooth surface and there are no rough or irregularities in particle surface, which allow the particles to flow freely without interparticle friction, and the lesser, will be the angle of repose. It is shown in Table 7.

2. Drug content:

The drug content of magnetic microspheres using various concentrations of ethyl cellulose 0.5, 1, 1.5, 2, 2.5, 3, 3.5 or 4 parts with 1 part of drug was found to be 70.73 ± 0.40, 87.60 ± 0.66, 90.50 ± 0.70, 94.43 ± 0.60, 90.63 ± 0.59, 88.00 ± 0.70, 83.23 ± 0.90, and 75.47 ± 0.87 respectively and it is shown in the Table 8 and Graph 3. The higher drug content in the magnetic microspheres using ethyl cellulose excipient was present in 1:2 ratios (CM 4).

The drug content of magnetic microspheres was found to increase gradually with the increase of polymer concentration. It reaches to a maximum with increments in polymer concentration then it declines with further rising in polymer concentration. It was optimized that drug: polymer ratio 1:2 (CM 4) have a maximum content of drug and further increasing in polymer concentration above 2 g was not attempted.

3. Entrapment Efficiency:
The entrapment efficiency of cefotaxime magnetic microspheres using various concentrations of ethyl cellulose 0.5, 1, 1.5, 2, 2.5, 3, 3.5 or 4 parts with 1 part of drug were 33.6 ± 1.2, 40.5 ± 1.1, 65.2 ± 1.1, 73.6 ± 1.3, 71.1 ± 1.2, 70.8 ± 1.3, 69.8 ± 1.0, and 70.3 ± 1.6 % respectively and it is shown in the Table 9 and Graph 4. The higher entrapment efficiency in the magnetic microspheres using ethyl cellulose excipient was present in 1:2 ratios (CM 4).

As indicated in graph that percent drug entrapment increases with increasing polymer (ethyl cellulose) concentration. Since, hydroxy propylmethyl cellulose provides mechanical strength to the microspheres and makes them stable at the time of formulation. The increasing ethyl cellulose concentration reduces the percent drug entrapped after attaining a maximum at drug: polymer 1: 2 (CM 4). Hence the concentration was optimized to 1gm which holds maximum entrapped drug, and further increase in percent drug entrapment by increasing ethyl cellulose concentration above 2 gm was not attempted.

4. Fourier- Transform Infra Red Interpretation:

The characteristic spectral bands of the pure cefotaxime and excipients were compared with bands obtained from their formulation to know there is any possible chemical interaction between drug and the excipients. It is shown in the Graphs 5, 6, 7, 8 and 9 respectively.

The identical spectral bands at frequencies 3784.68 cm\(^{-1}\) (O-H stretch) divalent central atoms Fe(II) in place of octahedral atoms for clay minerals, 3400.64 cm\(^{-1}\) (O-H stretch) was due to association polymerization, to form particles, 2977.33 cm\(^{-1}\) (O-H stretch) chelation of ethyl cellulose, 1759.99 cm\(^{-1}\) (CH\(_2\)-COO-CH\(_3\) stretch) ester stretching vibration (\(\square\)- lactones) is at C-3 of cefotaxime responsible for activity against Gram negative organisms, 1574.81 cm\(^{-1}\) (C=O stretch) carbonyl ketone stretching \(\beta\)- diketone enolic signifies \(\beta\)-lactum ring of cefotaxime, 1119.03 cm\(^{-1}\) (C-N stretch) aliphatic compound stretching is at C-7 of cefotaxime, 919.10 cm\(^{-1}\) (C-H bend) alkene monosubstituted compound bending is at ethylcellulose, for formulations and drug, excipients with identical or with minor dissimilarities, at frequencies for formulation. The bands revealed no evidence of chemical interaction between the drug and excipients.

The spectral bands clearly revealed that ethylcellulose undergoes polymerization and chelation, to form microparticles by increasing length of polymer chains which involves the dispersion of active ingredients in the internal structures or pores. It is clearly evident that there
is no chemical interaction between the drug molecules and excipients that the functional groups attached to C-3 and C-7 of cefotaxime was unaltered, which are responsible for antimicrobial activity. The peaks in the formulation spectrum clearly signify the absence of HPMC in the microspheres as may be due to negligible amount in presence or non adherence of the hydrophilic polymer HPMC on the surface of hydrophobic organic droplets i.e., oil/water. It is represented in Graphs 5, 6, 7, 8 and 9 respectively.

5. Particle Size Analysis of Microspheres:

The optimized formulation (CM 4) was subjected to characterize the nature and size of microparticles. SEM examination revealed particles were spherical with no defect or any crack on the outer surface of the particle. The particles were in the size range from 30 to 150 µm and mean ± SD, (n = 3) 123.2 ± 15.9 for magnetic microspheres and it is shown in Table 10 and photos graphs were given in Figures I, II, III and IV respectively.

6. Compatibility Study:

The interaction of the active ingredient with excipients in the polymeric device is also one of the key parameter while asserting the functionality tests of a delivery system. The interaction may occur in one or more number of ways from mixing, fabrication, packing, storage, transportation, dispensing or administration of drugs.

Therefore, DSC study of Cefotaxime, individual excipients, and the optimized formulation was carried out and their thermograms were obtained and compared.

The DSC thermogram of Cefotaxime alone (Trace 1) shows that an onset of 120.8 °C and a maximum occurring at 125.3 °C.

The DSC thermogram of ethyl cellulose excipient (Trace 2) shows that an onset of 164.2 °C and a maximum occurring at 182.6 °C.

The DSC thermogram of Hydroxy Propylmethyl Cellulose (Trace 3) shows that an onset is 224.5 °C and a maximum occurring at 258.3 °C.

The DSC thermogram of Iron Oxide (Trace 4) shows that a peak at 246.9 °C.
The optimized formulation of Cefotaxime, ethyl cellulose, iron oxide and hydroxy propylmethyl cellulose shows that an onset of first peak is 124.4 °C and maximum occurring at 134.6 °C. This peak indicates that presence of Cefotaxime in the sample and the second peak onset is 156.9 °C and maximum occurring at 175.0 °C. The second peak indicates the presence of ethyl cellulose and the third peak was not appeared indicates that absence of HPMC in the sample, since it is washed immediately after the formulation process, or as may be due to negligible amount in presence or non adherence of the hydrophilic polymer HPMC on the surface of hydrophobic organic droplets i.e., oil/water. The absence was also confirmed by FT-IR studies. Since, iron oxide is clay having a very high melting point, DSC is not applicable. So there is no incompatibility between the Cefotaxime, ethyl cellulose, iron oxide, hydroxy propylmethyl cellulose from the comparative shows that Cefotaxime, ethyl cellulose, iron oxide, hydroxy propylmethyl Cellulose and the formulation. It is shown in Graphs 10, 11, 12, 13 and 14 respectively.

It was found that all the excipients are compatible with the drug.

7. **IN-VITRO RELEASE STUDY:**

**Diffusion study:**

The rate of release of cefotaxime from the magnetic microspheres was determined by various kinetic models. These findings locate the *in-vitro* release profile to fit into a particular kinetic model. A hydrophilic drug encapsulated in a polymeric matrix is mainly released by diffusion process.

Magnetic microspheres of cefotaxime are intended to deliver the drug to the lungs in pneumonia. So, the area of interest is an organ with pH 7.4. Therefore, the *in-vitro* release profile of cefotaxime magnetic microspheres was determined by conducting the diffusion studies in a simulated medium consisting of phosphate buffer saline pH 7.4.

The drug delivery from the magnetic microspheres is greatly influenced by the concentration of polymer, molecular weight of ethyl cellulose and drug loading. It is evident from the FT-IR interpretation data that association polymerization and chelation occurs in the microparticles during formulation process. This polymerization and chelation leads to increase in
length of polymer chains thereby the molecular weight of polymer increases. As a result the drug release becomes declined at higher concentration of polymer included to the formulations from CM 6 to CM 8. There was a steady and gradual increase in drug release with increase in the concentration of polymer from formulations CM 1 to CM 4 then onwards from CM 5 to CM 8 there was a decline in drug release was observed may be due to formation of pores in microspheres.

The maximum percentage cumulative drug diffused from magnetic microspheres CM 1, CM 2, CM 3, CM 4, CM 5, CM 6, CM 7 and CM 8 were found to be 96.22 % at 17 hrs, 96.77 % at 20 hrs, 97.91 % at 23 hrs, 98.46 % at 26 hrs, 97.33 % at 24 hrs 95.09 % at 22 hrs, 61.88 % at 20 hrs and 53.45 % at 20 hrs respectively. It is clearly evident from Table 19, which the percentage cumulative drug diffused was as a function of time from magnetic microsphere formulations. It is shown in Table 19 and Graph 23.

8. IN-VITRO RELEASE AND KINETIC MODELS:

The in-vitro drug diffusion profiles are fitted into various kinetic models like zero order, first order, Higuchi and Korsmeyer - Peppas.

Coefficient of correlation ($r^2$) values for Zero order plots indicates that the mechanism of cefotaxime release from the magnetic microspheres followed independent of concentration and the rate was zero order release, it was slow and steady. It is shown in the Graphs 24, 28, 32, 36, 40, 44, 48 and 52.

First order plots show that the ($r^2$) values suggest the cefotaxime release rate was concentration dependant due to nonlinearity of all the cefotaxime magnetic microsphere formulations from CM 1, CM 2, CM 3, CM 4, CM 5, CM 6 except CM 7 to CM 8. It is shown in the Graphs 25, 29, 33, 37, 41, 45, 49, and 53.

The curve of Higuchi plot represents two different release rates: first part is nonlinear then followed by a linear release. The pattern of release from the magnetic microspheres was found to be an initial- nonlinear suggests burst release and a linear- diffusion-controlled steady slow release. The initial burst release might be because of smaller size range particles with lesser surface diameter accounts for faster diffusion of drug molecules and surface adsorbed molecules
that are available at higher rates from cefotaxime magnetic microspheres to the medium resulting in nonlinear curve. Then the linear part of the curve accounts for steady slow release of cefotaxime from the magnetic microsphere was controlled and transference of cefotaxime might be by diffusion (depicts Fick’s law of diffusion) results in a linear part of the curve. It is shown in the Graphs 26, 30, 34, 38, 42, 46, 50 and 54.

The plot of in-vitro drug release values in Korsmeyer - Peppas model ($r^2$) showed good linearity where the release of the drug from polymeric matrix is governed by diffusion indicative of best fit to Fick’s law of diffusion. It is shown in the Table 23 and Graphs 27, 31, 35, 39, 43, 47, 51 and 55.

9. **Stability Studies:**

The results of accelerated storage conditions indicates that physical change, particle size, drug content, and in-vitro release of the drug did not show much variation in the magnetic microsphere formulations. They showed promising results of 94.23 ± 0.31 % of the drug retained. It is shown in the Table 21 and Graph 56. and the in-vitro release of the drug was 97.90 %. It is shown in the Table 22 and Graph 57. The mean particle size was 121.5 ± 14.5 µm did not show any significant variation. It is shown in the Table 23 and Graph V. The magnetic microparticles were almost spherical and there was no change in the colour or no crack or any defects on the outer surface of the microparticles. There was an appreciable change of 1.7 µm in the particle size was observed. The results indicated that the formulations were more stable at elevated temperature at 40 °C ± 2 °C/75% RH ± 5% RH.

Stability data clearly indicates that cefotaxime magnetic microsphere encapsulation gives protective effect at higher temperature.

10. **IN-VIVO ANIMAL MODEL:**

The formulated magnetic microspheres of cefotaxime were determined for its therapeutics effectiveness and ability to reach the site of interest to localize the drug therapy. The MR images conclude that improved cefotaxime magnetic targeting over non-targeted animals. It is shown in the Image VI, VII, VIII, IX, X, XI, XII and XIII.
These are significant refinements that further support the hypothesis that appropriate microparticular formulations can be used to effectively improve antimicrobial therapy.