DEVELOPMENT AND EVALUATION OF CEFTAZIDIME LOADED MICROEMULSION FOR PARENTERAL DRUG DELIVERY SYSTEM

ABSTRACT

The goal of this study was to develop a parenteral microemulsion formulation of Ceftazidime antibiotics. The ceftazidime sparingly soluble in organic solvent like ethanol, DMSO, and dimethyl formamide were determined. For biological experiment, organic solvent- free aqueous solutions of ceftazidime (hydrate) are prepared by directly dissolving the crystalline compound in aqueous buffer. The peudo ternary phase diagrams of oil, surfactant, cosurfactants (Butanol/ Isopropyl alcohol mixture) and water were constructed to identify boundaries for microemulsion existence. Ceftazidime microemulsion particle size, solution viscosity and conductivity were evaluated. The microemulsion stability and haemolytic activity were examined after dilution in 5% dextrose solution for injection to 1 mg/mL ceftazidime. *In vitro* haemolysis studies indicated that Ceftazidime microemulsions were well tolerated by erythrocytes. The novel microemulsion formulation of Ceftazidime was developed that is suitable for parenteral administration. This new formulation could potentially have less vehicle-associated side effects that current commercial formulation of Ceftazidime based on Cremophor® EL and isopropyl alcohol solution.

KEYWORDS - Ceftazidime, Tween 40, Microemulsion, Parenteral, Phase diagram.

INTRODUCTION

Ceftazidime is a third-generation cephalosporin antibiotic consist pyridinium-1-ylmethyl and {[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-{[(2-carboxypropan-2-yl)oxy]imino}acetamido groups at positions 3 and 7, in that order, of the cephem skeleton. Ceftazidime is used as antibacterial drug, an EC 2.4.1.129 (peptidoglycan glycosyltransferase) inhibitor and a drug allergen. Ceftazidime is a class of cephalosporin and an oxime O-ether. Ceftazidime is a conjugate acid of a ceftazidime(1-).

Ceftazidime is a semisynthetic, broad-spectrum, beta-lactam antibiotic for parenteral dosage form. Ceftazidime is bacteriocidal in action exerting its effect by inhibition of enzymes responsible for cell-wall synthesis, primarily penicillin binding protein 3 (PBP3). A broad range of gram-negative organisms is susceptible to ceftazidime in vitro, including strains resistant to gentamicin and other aminoglycosides. In addition, ceftazidime has been shown to be active against gram-positive Bacteria. Ceftazidime is highly stable to most clinically important beta-lactamases, plasmid or chromosomal, which are produced by both gram-negative and gram-positive organisms and, therefore, is active against many strains resistant to ampicillin and other cephalosporins. Ceftazidime has been shown activity against the gram-negative organisms *Pseudomonas* and *Enterobacteriaceae*. ceftazidime activity against *Pseudomonas* is a distinguishing feature of ceftazidime among the cephalosporins.

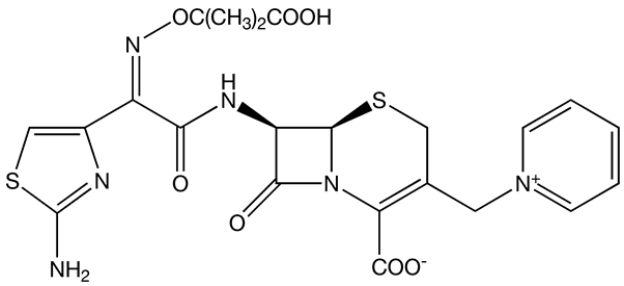

Figure no. 1. Chemical Structure of ceftazidime

SELECTION OF DRUG

Ceftazidime sample were obtained from KAPL Bangalore. Phospate buffer tablets were purchased from Sigma, USA. Butanol,glycerol monosterate, glycerine and olive oil were

purchased from Merck, Germany. Dialysis membrane (Spectro/por Dialysis Mebrane, Spectra/por 4, diameter 16 mm, molecular weight of 12-14 kDa) were purchased from Spectrum. All other chemical reagents and solvents were of analytical grade and used as received. Distilled deionized water was used for the preparation of parentral microemulsions.

1. Preparation of Microemulsion

Formulation development of ceftazidime was solubilized in butanol by vortexing. Then, oil was mixed, followed by surfactant. The resulting solution was mixed homogeneously by vortexing. Then, the specific amount of water was added and gently mixed to obtain a microemulsion. The prepared microemulsion was sterilized by the use of autoclave at 121°C and 15 psi for 15 min

2. Preparation of Pseudoternary Phase Diagram

The pseudo-ternary phase diagrams of oil, surfactant: cosurfactant [Soyabin oil: Butanol: Tween40: (16:9:1)], and water were constructed prior to optimization of the formulation. Tween 40 was mixed with cosurfactant in fixed weight ratios. Constituent of each surfactant–co-surfactant mixture were then mixed with oil phase and finally with aqueous phase. Mixtures were gently shaken or mixed by vortexing and kept at specific temperature (25°C) to achieve equilibrium. The previous solution was observed visually for their appearance, i.e., it was noted as being clear and transparent microemulsions, or crude emulsions or gels.

3. Characterization of Microemulsion

Optical Birefringence

The ceftazidime microemulsion was located between two polarizing plates and then observed for light transmittance. Following this, one of the plates was rotated relative to the other through 90° (crossed polarizer) and then examined.

Determination of Particle Size

Photon correlation spectroscopy by laser light scattering is often used to determine particle size of microemulsions of ceftazidime. A Beckman N4 Plus submicron Particle Size Analyzer was used to check particle size of the ceftazidime microemulsion. The device calculated the mean particle size of microemulsion and polydispersity as of intensity, examined spherical particles.

Polydispersity parameter is a measure of particle homogeneity and it varies range from 0.0 to 1.0. The nearer to zero the polydispersity value of the particles are more homogeneous. Prior to analyze, samples were diluted fivefold using 0.22 µ filtered double distilled water. Light scattering was examined at 90° scattering angle and temperature of 25°C or 37°C. All measurements were performed in triplicate.

4. Physiochemical characterization of microemulsion

4.1. Droplet size

The droplet size was measured using Nicomp 380 ZLS (Particle sizing systems, Santa Barbara, CA). Samples of microemulsionwere placed into 6 x 50 mm borosilicate glass tubes (Kimble Chase, Vineland, NJ) and positioned in the path of 100 mW He-Ne laser having a wavelength of 658 nm. The neutral density filter was adjusted until the scattered intensity fluctuated around 300 kHz. The light scattered was collected at 90˚and detected using a photodiode array detector. Nicomp software automatically tuned the channel width & base line and Nicomp distribution was considered for Chi-square values greater than 3 . Each sample was analyzed three times and the mean volume-weighted diameter was determined.

4.2. Zeta potential

Zeta potential was measured using by dynamic light scattering using Malvern Zetasizer, nano 90, Malvern Instruments, USA [Agrawal R and Kaur I.P., 2011]. Measurements were done in triplicate at angle of 900 maintained at 250C. Samples were prepared by dispersing the nanospheres in sufficient amount of ultra purified water (pH 7) prior to the experiment. The particle size distributions of the nanospheres were reported as poly dispersity index (PDI), a measure of the distribution broadness of the particle size. ZP is a useful parameter to predict the physical stability of colloidal systems. Each sample was analyzed three times and the zeta potential was determined from the average of the runs.

5. Accelerated Stability Testing

5.1 Centrifugation

Centrifugal methods have been used to subject the system to evaluate accelerated stability. The ceftazidime microemulsion was placed to centrifugation at 5,175×g for 30 min and examined for any separation, i.e., incompatibility (8).

5.2. Viscosity Measurements

The viscosity of the formulation was evaluated by an Oswald-type viscometer. The viscometer tube was filled with the accurate amount of the microemulsion formulation. The meniscus of the liquid solution in capillary tube was adjusted to the level of the top graduation mark with the aid of vacuum. The time in seconds was reported for liquid solution to flow from the upper mark to the lower mark in the capillary tube. The time required for water was also reported. The procedure was repeated three times and the average value was taken for calculation.

5.3. pH Measurement

The satisfactory range is pH 2–12 for intravenous and intramuscular route but for subcutaneous route, the range is reduced to range pH 2.7–9.0 when the rate of in vivo dilution is significantly reduced resulting in more probable for irritation at the injection site. The pH of microemulsion was measured using by Systronic Digital pH meter 335, standardized using pH 4.0 and 7.0 standard buffers use.

5.4 Clarity

The clarity of the samples was measured using UV-Vis Spectrophotometer at 400 nm. Deionized water was used as a blank for the analysis. Measurements were done in triplicate and the clarity was expressed as percent transmittance.

5.5. Differential Scanning Calorimetry (DSC)

Thermal and physical state properties of Cefotaxime (CEF-ME) and Ceftazidime (CFZ-ME) were determined using a Differential Scanning Calorimeter (DSC) (822e Mettler Toledo) equipped with a TS0801RO sample robot and TS0800GCI gas flow system. Samples of 5-12 mg were weighed using Mettler MT5 microbalance into a 100 µl aluminum pan, covered with lid and quickly sealed using a mechanical crimper. The study was performed at a heating rate of

10°C/min over a range of 10 - 300°C. Nitrogen was used as a standard purging gas at 20 ml/min to prevent any oxidation of the samples. Star-e software V8.10 was used to obtain the DSC scans.

5.6. Transmission electron microscopy (TEM)

The morphology of the Ceftazidime (CFZ-ME) loaded microemulsion were studied using Transmission Electron Microscopy (TEM) (model TECNAI 200 Kv TEM-Fei, Electron Optics, Japan) was used to evaluate the surface morphology. A drop of diluted samples were placed on the surface of carbon coated copper grid and stained with negative stain using a drop of 2 % (w/w) aqueous solution of phosphotungstic acid for 30 seconds. Excess staining solution was washed out by filter paper, leaving a thin aqueous film on the surface. After staining, samples were dried at room temperature for 10 minutes to carry out investigation [Bae, et.al, 2006]. The copper grids were dried overnight and TEM images were captured using Quartz PCI version 8 software.

6. Compatibility Assessment with Different Injectable Diluents

The dilutability and compatibility of developed microemulsion formulations with the 0.9% NaCl injection or 5% dextrose Table 1. Solubility Data of Ceftazidime in Oils/Surfactants/Solubilizers Oils/surfactants/solubilizers Solubility (mg/mL) Miglyol-812 4 Soyabean oil 1.5 Capmul MCM 10 Epikuron 135 F 5.5 PEG-400 110 Tween-80 35 Tween-20 30 Cremophor EL 23

Injection was evaluated by diluting the developed microemulsion formulation in different concentration ranges (0.2–1 mg/ml) with 0.9% NaCl injection and 5% dextrose injection and kept them for visual examination by means of respect to phase separation or precipitation for a period of 3 h.

7. Evaluation of *In vitro* Drug release

A synthetic membrane (Spectro/por Dialysis Mebrane, Spectra/por 4, diameter 16 mm, molecular weight of 12-14 kDa) was filled with 3 ml ceftazidime loaded microemulsion formulations. The receiver compartment (37 mL) consisted of ethanol Acta Pharmaceutica Sciencia. Vol. 55 No. 4, 2017 31 and PBS pH 7.4 (ratio of 20:80) in order to ensure sink condition. The receptor compartment was exposed to ambient temperature and covered with

parafilm to avoid evaporation. The temperature of the receptor compartment was maintained at 37±1ºC while the buffer solution was stirred at 600rpm continuously with a magnetic bar. Samples (1 mL) were withdrawn from the release medium at predetermined times (0, 2, 4, 8, 12, 16, 20 and 24h). The samples were analyzed by UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) at 261 nm. The analytical method was validated. Calibration curve was drawn.

8. Test for Sterility

The sterility testing was performed by using nutrient broth medium and microemulsion formulation incubated at 37°C for 7 days. For the evaluation of sterility testing study, groups of including developed ceftizidime microemulsion, negative control medium, and positive control medium incubated with Bacillus subtilis were observed.

9. Stability study

Stability testing is a pre-requisite for the approval of any pharmaceutical product as it ensures the product quality, safety and efficacy throughout the shelf life [129]. In order to assess the physical and chemical stability of the prepared formulation, microemulsion samples were stored at different temperatures (4°C, 25°C and 40°C) for a period of 3 months. Samples were withdrawn at regular intervals of 0, 30, 60 and 90 days and evaluated for pH, particle size, clarity/transmittance and drug content.

10. Statistical analysis

All the data obtained were expressed as mean ± SD. Analysis of variance (ANOVA) was applied to analyze the significant difference between samples. $p < 0.05$ was considered to be significant in all the cases.

11. Hemolytic study

11.1. In Vitro Erythrocyte Toxicity Study

The erythrocyte toxicity assay was examined as described by Bock et al. (10). Fresh blood serum was collected in the vial containing EDTA (anticoagulant).RBCs were isolated by centrifugation method(5,000 rpm for 5 min) and the Red blood cells were rinsed for three times with isotonic phosphate buffer pH 7.4 prior to diluting by means of buffer to prepare erythrocyte stock

dispersion (three parts of centrifuged erythrocytes + 11 parts buffer). The buffer solution composed of $Na_2PO_4 \cdot 10H_2O$ (7.95 g), KH_2PO_4 (0.76 g), NaCl (7.2 g), and distilled water (add 1,000 ml). The washing of RBCs step was repeated in order to remove debris and serum protein. A 100- µl constituent stock dispersion was added per milliliter of test sample. The obtained solution was incubated at 37°C for a period of 1 h. After incubation under shaking, debris and intact erythrocytes were detached by centrifugation. 100 milliliters of resulting supernatant was added to 2 ml of an ethanol/HCl mixture [(39 parts ethanol (99% v/v)+ 1 part of HCl (37% w/v)]. This combination dissolved all components and avoided the precipitation of hemoglobin. The absorbance of the prepared mixture was determined at 398 nm by spectrometer monitoring against a blank sample. Control sample of 100% hemolysis was employed in the experiment. The percentage of lysis caused by the test sample was obtained by following equation:

Hemolysis caused by sample (%) = (Abs of the test sample/Abs at 100% lysis) x 100

11.2. *In vitro* Cytotoxicity Analysis on Vero Cell Line

Vero cell line was maintained in DMEM medium containing 10% fetal bovine serum. Vero cells (105 cells/mL) were seeded in 96-well plate and incubated at 37°C with 5% CO2 for 24 hrs to allow the cells to adhere to the plate. Cells were treated with both aqueous and ME formulations of P60 + CAF at the optimized concentrations (corresponding to their respective MIC values) including placebo. After incubation for 24 hrs, 20 µL of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) prepared in D-PBSA (5 mg/mL) was added to each well and again incubated for 4 hrs. The media was replaced by 200 µL of DMSO to terminate the assay [20]. Absorbance was taken at 570 nm using an ELISA plate reader. The viability (%) was calculated according to the formula (100), where is absorbance obtained for cells treated with the formulation and is absorbance obtained for positive control (cells without test formulation).

RESULTS AND DISCUSSION

12. Solubility Studies

Parenteral delivery of microemulsion requires the component such oil, surfactant(s) and co surfactant(s) to be biocompatible, safe and effective. For this purpose, nonionic or zwitterionic

surfactants are used to found an appropriate pharmaceutical dosage form because they are less toxic and less affected by changes in pH and ionic strength (2).

In addition, for a lipid soluble drug, the principle intent is to achieve a formulation where the drug is dissolved in the liquid solvent. By selecting the optimum liquid vehicle composition, it is potential to minimize or eliminate precipitation of the drug. As shown in Table 1, among the limited choice of excipients, soyabin oil was selected as the oil component. However, as the dose of the drug was 20 mg, the oil component was insufficient to solubilize the drug; hence Tween40 was selected as surfactant and solubilizer components, in that order. In the process of formulation, it was observed that drug precipitated in most of the microemulsion formulation formed by varying the mixtures of mentioned excipient. For the preparation of stable ceftazidime microemulsion, complex interfacial film of combination of surfactants is favorable and therefore an amphiphilic surfactant, Epikuron®135 F having miscibility with oil, was chosen.

13. Preparation of Pseudoternary Phase Diagram

The pseudoternary Phase diagram studies were completed to investigate the effect of surfactant to co-surfactant ratio on the extent of stable o/w microemulsion region. The ceftazidime containg microemulsions in the present investigation formed spontaneously at ambient temperature when their mixturre were brought into contact. The phase of microemulsion and isotropic regions increased with raising the ratio of surfactant and cosurfactant (Fig. 2). It shows that the maximum extent of oil used in microemulsions increased extensively with increasing ratio of surfactant and cosurfactant to oil. It is recommended that ceftazidime can be administered by slow intravenous infusion over a period of 30 to 60 min. Therefore, using a constructed pseudoternary phase diagram, the most favorable ratios of the components for microemulsion which would remain stable and avoid drug precipitation over infinite dilution was selected.

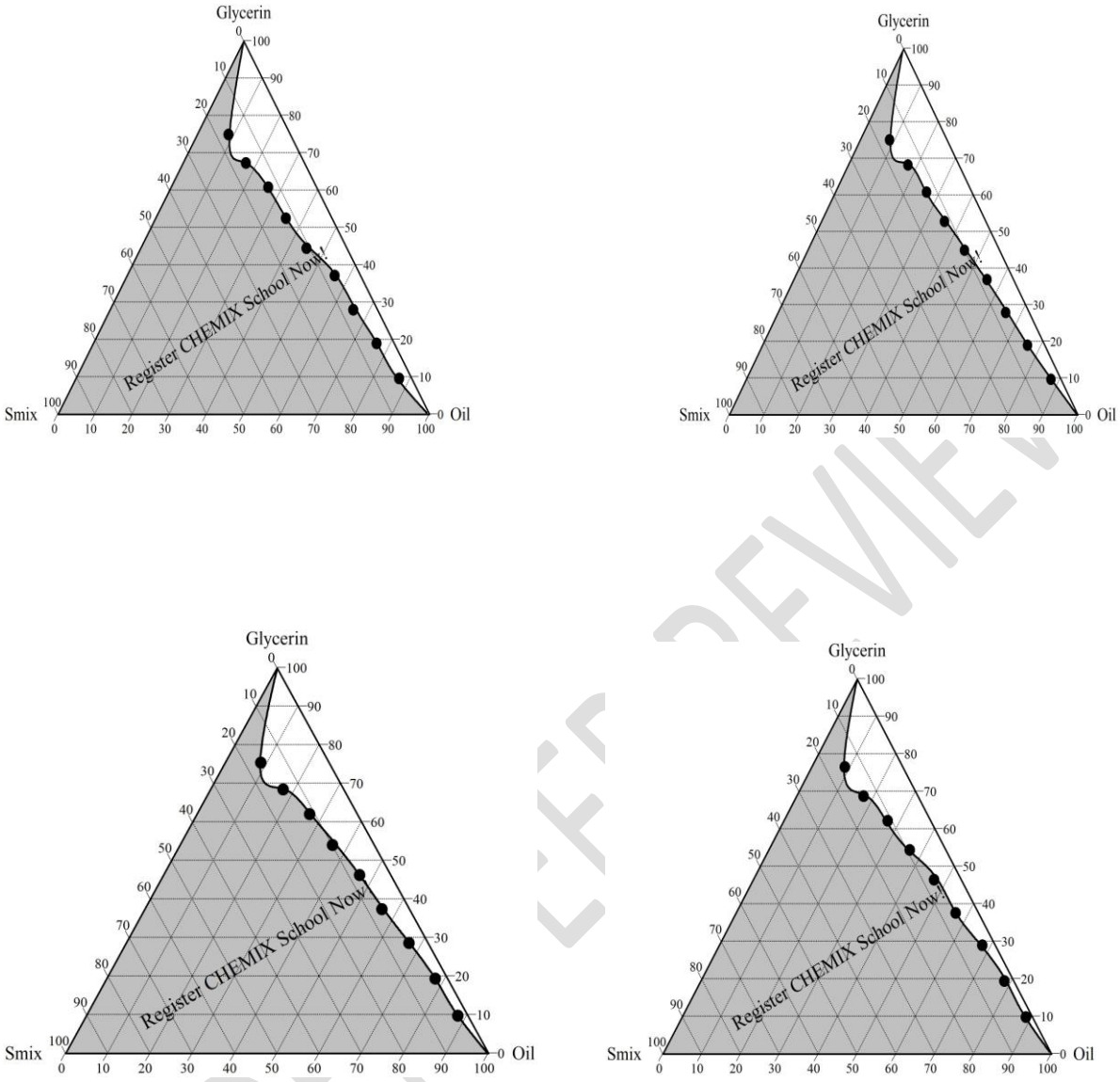

Figure no. 2. Showing Pseudo ternary phase diagrams were constructed using olive oil, GMS (surfactant) and ethanol (cosurfactant) in different mass ratio.

Table 1. Globule Size and Polydispersity Index of Developed Formulation

Sample	**Particle Size (nm)**	**Polydispersity index (PI)**
Formulation before autoclaving	55.8	0.891
Formulation after autoclaving	66.0	0.968

14. Characterization of Microemulsion

14.1. Optical Birefringence

Birefringence (6) is a light-scattering principle. It is also known as double refraction and is originating in liquid crystals and anisotropic systems. In this light- scattering system, the light passing through a material is separated into two components with different velocities and therefore different refractive index. Thus, if a liquid crystal is observed between crossed polarizer, intense bands of colors are seen which is called as birefringence. In contrast, microemulsion appears completely black. The formulated microemulsion appeared completely dark when observed between cross-polarizing plates validating that the formulation was an isotropic, colloidal dispersion.

14.2. Determination of Particle Size

It is recognized that the particle size is distribution one of the most important properties of emulsion for the analysis of its stability and also in vivo fate of emulsion. Therefore, it is also well predictable that size plays a key role in the circulation time of the particulate carriers. Various literature states, particulate carriers with smaller size can evade recognition by MPS; accordingly have longer circulation time in the bloodstream. In addition, smaller size eludes capillary blockade resulting in decrease of adverse effects frequently associated with intravenous administration of particulate carriers. Therefore, the particle size of ceftazidime microemulsion was assessed in triplicates. Table 2 shown depicts the particle size before and after autoclaving. There was marginal increase in particle size of the ceftazidime microemulsion globule; however, it was lower than 100 nm.

15. Accelerated Stability Testing

15.1. Centrifugation

Centrifugal methods (8) have long been used by emulsion technologists to induce and accelerate instability by gravitational force means. It is usually accepted that shelf life under ordinary

storage conditions can be predicted rapidly by observing the separation of disperse phase when the microemulsion is subjected to centrifugation.

In this technique used to determine the behavior of small particles in the gravitational force, i.e., their separation rates, is relatively simple and inexpensive providing a rapid perfect identification of the systems as microemulsions. Brownian motion is associated with particles smaller than 0.5 µm. The microparticles in this size range are small sufficient to absorb kinetic energy from bombardment through the molecules of dispersion medium. It has been considered that it causes such a particle to alter direction 1024 times per second. This system keeps the dispersed droplets in a condition of violent motion preventing their settling under gravitational field. consequently they do not coalesce, it is Brownian movement that keeps the droplets of ceftazidime microemulsion droplets from settling or creaming. The reason that ceftazidime microemulsion droplets do not form coalescence is due to surface free energy of a microemulsion system. At this time two droplets coalesce to form a single droplet of larger size, the interfacial tension of the new droplet becomes negative, i.e., the system has negative free-surface energy. The larger droplet size now suddenly increases its curvature to cause zero interfacial tension again and two droplets of the original size form consequence. This phenomenon appears continuously as does the bombardment of droplets by molecules of dispersion medium. It is this dynamic equilibrium that keeps the ceftazidime microemulsion systems stable.

At the end of 30 min, the developed ceftazidime microemulsion showed no phase separation and drug precipitation after centrifugation at 3,000 rpm, verifying the stability of the formulation.

Table no. 2. Drug Content Stability Data

	% Drug content				
Conditions	**Initial**	**7 days**	**1 month**	**2 months**	**3 months**
RT	101.05±0.66	98.93±0.32	97.60±0.73	98.31±0.84	97.20±0.91
30C/60% RH	-	99.04± 0.47	99.70± 0.33	99.47± 0.96	101.88 ±1.34
40 C/75%RH	-	103.74±0.29	101.34±0.51	99.12±0.88	102.86±0.73

RT= Room Temperature, RH = Relative Humidity

15.2. Viscosity Measurements

The stability of the microemulsion is also depends on its viscosity, i.e., is an expression of the resistance to flow. The viscosity defines as the tendency of the system to agglomerate. Moreover, for the developed ceftazidime microemulsion, the viscosity measurement was of utmost importance, since it had to be diluted using infusion fluids prior to administration. It is well known that the viscosity of parenteral formulation may also affect the syringeability parameter. Using this following equation, the viscosity of the formulated microemulsion was calculated to be 106.92 cP. The viscosity was calculated from the equation-

$$\eta_{1/}\,\eta_2 = \frac{\rho_{1/}\,t_1}{\rho_2\,t_2}$$

Where-

η_1 and η_2 = viscosities of the test and the standard sample,
ρ_1 and ρ_2 = Densities of the liquids
t_1 and t_2 = Respective flow times in seconds

The low viscosity property of the developed ceftazidime microemulsion ensures ease of syringeability at the same time ease of mixing with intravenous fluids with minimum mechanical agitation.

15.3. pH Measurement

The pH measurement of following microemulsion formulation was repeatedly over a period of 10 days showed that the pH of the microemulsion formulation was in an acceptable pH range for intravenous administration. For ceftazimide, pH plays a important role in the drug stability. The pH for stability of ceftazimide was found to be pH 5.4 which is considered most favorable to prevent the degradation of drug (11). Due to constant pH range, the drug content was found to be in acceptable limits over 3 months period of time.

16. Compatibility Assessment with Different Injectable Diluents

As prescribed, ceftazidime for injection required to be diluted for administration by intravenous infusion in either 5% dextrose injection or 0.9% sodium chloride injection to produce a solution containing 200 to 400 µg (0.2 to 0.4 mg) of ceftazidime per milliliter. As shown in Tables V and VI, the diluted solution was stable for sufficient time enabling slow intravenous infusion of ceftazidime in concentration range up to 1 mg/ml for 1.5 and 2 h in 0.9% sodium chloride injection and 5% dextrose injection, respectively. However, it should be noted that at the recommended concentration of 0.4 mg/ ml, there was absence of drug precipitation for 3 h in0.9% sodium chloride injection and 5% dextrose injection, respectively. This clearly reflects the superiority of the developed formulation over the existing ceftazidime injection which reports drug precipitation as its limitations.

Table 3. Compatibility of Microemulsion with 0.9% Sodium Chloride Injection

	Time in hours					
Conc. Of drug mg/ml	0.5	1	1.5	2	2.5	3
0.2	C	C	C	C	C	C
0.4	C	C	C	C	C	C
0.6	C	C	C	C	C	C
0.8	C	C	C	C	C	P
1	C	C	C	P	-	-

C clear, P Precipitation

Table 4. Compatibility of Microemulsion with 5% Dextrose Injection

	Time in Hours					
Conc. Of drug mg/ml	0.5	1	1.5	2	2.5	3
0.2	C	C	C	C	C	C
0.4	C	C	C	C	C	C
0.6	C	C	C	C	C	C

C clear, P Precipitation

17. In vitro Hemolytic Studies

The hemolytic potential of the parenteral microemulsions should be determined to prove their safety to blood components. It has been demonstrated that commonly employed parenteral cosurfactants such as glycerol or propylene glycol can cause considerable hemolysis on long term contact with the blood. Identification of hemolytic potential of a parenteral microemulsion is necessary especially when the formulation is to be administered as a continuous infusion over a long period of time. Hence, though, all the Ceftazidime (CFZ-ME) loaded microemulsions were based on the components acceptable for parenteral delivery, All the Ceftazidime (CFZ-ME) loaded microemulsions caused negligible (<1%) hemolysis on contact with human blood for 2 h.

18. In vitro Cytotoxicity Analysis on Vero Cell Line

The cytotoxicity of the microemulsions were compared with Claforan® and Zavicefta® using Vero cell line maintained in DMEM medium containing 10% fetal bovine serum. The percentage of cell viability in the presence of 20% DMSO was 10.88%. With both the formulations, the cell viability was greater than 100% at lower concentrations (0.01%, 0.1%, 1%, and 5%), whereas the cell viability was less than 15% at higher concentrations (50% and 100%). The results are presented in percentage of the control without both antibiotics (100%). After five days the same effects as on day one were observed, however on the last day of measurement no significant difference in viability compared to control was observed.

19. In vitro release studies

The samples were analyzed by UV-Visible spectrophotometer (UV-1800, Shimadzu) at 261 nm. The analytical method was used in terms of analyzing the samples. Calibration curve was created with eight-point calibration concentration with the range of 0.001-0.2mg/mL for standard solution of bulk ceftazidime. In which three independent determinations were performed at each concentration. Linear relationship between absorption and concentration of ceftazidime was observed. The standard deviation of the slope and intercept were show. The determination coefficient R2 for regression line is 0.99854 with slope of 20.624x and y + intercept of + 0.0149 for standard solution of ceftazidime. Ceftazidime loaded microemulsion formulations and CF-

ME & CF2-ME solution component were studied for in vitro release through synthetic membrane to assess and compare the performances of formulations. Figure 3 & 4 shows the in vitro release graphics. As it can be seen in Figure 3 & 4, CF-ME and CF2-ME solution shows 100% release at the end of the 24th hour.

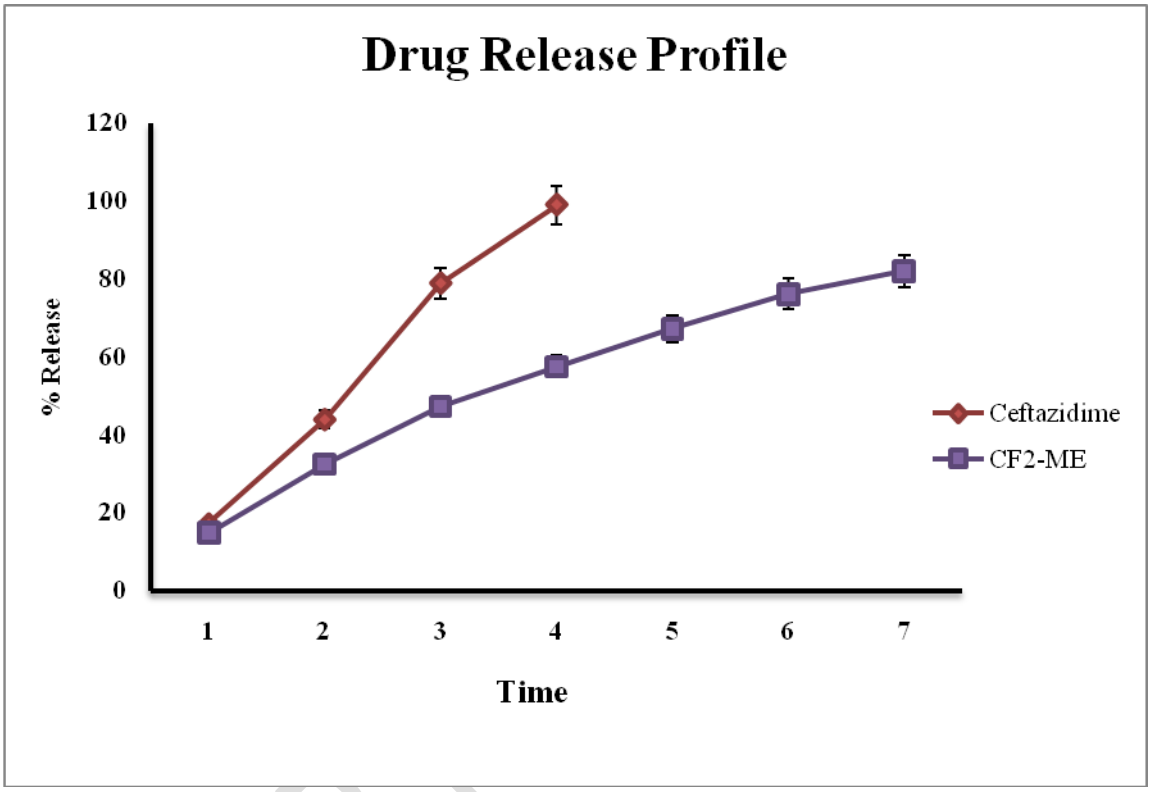

Figure No. 3. Drug Release Profile of Ceftazidime & CF2-ME

20. Test for Sterility

Sterility is one of the pivoted parameter for the parenteral preparations. However, at adequate temperatures, phase separation may take place but the microemulsions spontaneously changes to their initial state when brought back to normal temperature and on adequate mixing. While it is established, ceftazidime microemulsions can be sterilized by autoclaving (12); the developed microemulsion was sterilized by autoclaving at 121°C and 15 psi for 15 min. Even if there was phase separation after autoclaving, after shaking it gave a homogeneous microemulsion. The sterility testing of this sterile microemulsion showed absence of microbial growth representing

the effectiveness of autoclaving. In addition, this was attested by the stability of the developed microemulsion over a period of 3 months.

CONCLUSION

The parenteral ceftazidime microemulsion was successfully developed with particle size less than 100 nm. The developed microemulsion formulation was amenable to sterilization by autoclaving and was found to be robust to dilution with intravenous fluids. The *in vitro* erythrocyte toxicity study demonstrated the safety and acceptability of the formulation for parenteral administration.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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