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Design of Cefuroxime Loaded Bio-Nano Gels For Brain Specificity via Ear

N.V.Satheesh Madhav, Deepika Raina and Mukta Mala

Abstract: Encephalitis is usually the result of an infection. In many cases this is caused by a virus, but often no cause is found. Encephalitis causes high rates of illness and death, yet its epidemiology remains poorly understood. Cefuroxime is a semi-synthetic cephalosporin antibiotic, chemically similar to penicillin. Cefuroxime axetil is a Class IV drug as per the Biopharmaceutical Classification System. It is a prodrug of Cefuroxime which is practically insoluble in water with a log P of 3.8. The aim of the research was to target the drug to the brain to treat encephalitis and localized effect to the middle ear otitis media via ear. Bio-polymer was isolated from *Cucumis sativus* and process was optimized. Drug-polymer interaction study, physico-chemical characterization and acute toxicity study of isolated bio-polymer were performed. Five formulations were formulated by double emulsion solvent diffusion method by using bio-polymer and cefuroxime as a model drug. Prepared nanoparticles were subjected to pH study, viscosity, Entrapment efficiency, Spreadibility, *In-Vitro* Release, *In-Vivo* Release study and stability study. Our research work revealed that the formulated nano-gels showed promising stability. On the basis of % Release, T50 % and other parameters FN5 was found to be the best formulation showing drug release profile maximum at 76 %. The conclusion was drawn that the ear can be used as novelistic platform for delivery of cefuroxime to brain region in the formulation of bio-nanogel.

Keywords: Biopolymer, Nanoparticle, Double Emulsion Solvent Diffusion method.

1. INTRODUCTION:

Encephalitis is viral inflammation of the brain. Encephalitis may cause flu-like symptoms, such as a fever or severe headache, confused thinking, seizures, or problems with senses or movement. Severe cases of encephalitis, while relatively rare, can be life-threatening as case of encephalitis is unpredictable, it's crucial to get a timely diagnosis and treatment (1). The cause of encephalitis is often unidentified, but the most commonly diagnosed reason is a viral infection(2). Tablet and a suspension (liquid) tablet that are available in market has a strong bitter taste (3). Cefuroxime is a semi-synthetic cephalosporin antibiotic, chemically similar to penicillin.

Prof. (Dr) Satheesh Madhav, Director –Faculty of Pharmacy, DIT University, Dehradun India
Email Id:satheesh_madhav@yahoo.com

Cephalosporins stop or slow the growth of bacterial cells by preventing bacteria from forming the cell wall that surrounds each cell. The cell wall protects bacteria from the external environment and keeps the contents of the cell together. Without a cell wall, bacteria are not able to survive. Cefuroxime is effective against a wide variety of bacteria, such as Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, E. coli, N. gonorrhea, and many others(4).Crystalline cefuroxime axetil, however, does not exhibit adequate bioavailability upon oral administration. It is important that cephalosporin compounds for oral administration should be in a firm which provides high bioavailability whereby absorption into the blood stream is maximized and the amount of antibiotic remaining in the gastrointestinal tract is minimized. Any antibiotic which is not absorbed will be therapeutically ineffective and by remaining in the gastrointestinal tract may cause side effects (5).

2. MATERIAL AND METHODS

2.1 Isolation of biopolymer: *Cucumis sativus* was purchased from the market and peeled out with the help of knife. Pulp portion was separated from the *Cucumis sativus* and crushed in the mechanical shaker for 20 minutes. The crushed portion was sieved through muslin cloth to collect the juice. 50 ml of *Cucumis sativus* juice was taken and then 50 ml of non-aqueous solvent viz. propanone was added to it. The above solution was kept for refrigeration for two hours. Then it was centrifuged at 3000 rpm for 10 minutes to remove the residual matter. The polymeric solution was taken and dried. The biomaterial was collected and % yield was calculated. Purification of isolated biomaterial was done by hot dialysis method by replacing hot water every 4 hours until dialysate showed negative test with barium chloride and silver nitrate. The process of bio-material extraction was optimized by repeating the process of extraction 5 times, practical yield was calculated. Each time 100 gm of pulp of *Cucumis sativus* was used for extraction & yield was compared.

2.2 Physicochemical properties of isolated biopolymer: Physicochemical properties of the isolated biopolymer such as color, odor, color changing point, taste, texture, solubility along with the chemical test was determined.

2.3 Preparation of Bio-nanoparticles by double emulsion solvent diffusion method (w/o/w): Primary emulsion was prepared by adding 5 mg of *Cucumis sativus* bio-polymer (primary emulsifier), 10 mg of Cefuroxime drug, 5 ml of Methylene Chloride and 2.5 ml of water. Then above solution was sonicated for 10 minutes. 1% solution of *Lagenaria siceraria* bio-polymer (secondary emulsifier) was prepared. Then 10 ml of 1% *Lagenaria siceraria* bio-polymer solution was added drop wise in to the primary emulsion. The above solution was kept for sonication, micro-centrifuged, and evaporated in order to achieve bio-nanoparticles.

Bio-nanoparticles were dispersed in to the 0.5 % xanthum gum solution to achieve nanogel. Five formulations loaded with the varying concentration of *Cucumis sativus* bio-polymer (FN1-FN5) were prepared by similar procedure.

FORMULATION TABLE:

Table 1: Formulation Details

Formulation	FN1	FN2	FN3	FN4	FN5
***Cucumis sativus* biopolymer (mg)**	5	10	15	20	25
Cefuroxime (mg)	10	10	10	10	10
Methylene Chloride (ml)	5	5	5	5	5
Water (ml)	2.5	2.5	2.5	2.5	2.5
1% *Lagenaria siceraria* Biopolymer solution (ml)	10	10	10	10	10

3 EVALUATION OF NANOGEL:

3.1pH: of nanogel formulations were measured using a pH meter of a glass electrode. Texture of Nanogel was determined by applying the nanogel on the skin surface for observing its texture and grittiness.

3.2 Viscosity of Nanogel was performed by Ostwald Viscometer apparatus. 1 % (w/w) nanogel solutions were prepared in water.

3.3 Drug Content of Nanogel: 10 mg drug was added in to the 100 ml of methyl alcohol to achieve the stock solution of conc. 100µg/ml. Then different concentrations (2, 4, 6, 8, 10 µg/ml) were prepared from the stock solution. And absorbance was noted down at λ 279 nm then graph was plotted and slope value (Y) was calculated. 1 ml of all the formulations (nanogel) were taken and dissolved in methyl alcohol. The prepared solutions were analyzed by using UV –Visible spectrophotometer at λ 279nm then graph was plotted and slope value (Y) was calculated.

Concentration= Abs/Slope

Drug Content Uniformity =Concentration * 10

$$\text{Drug Entrapment} = \frac{\text{Amount of drug reelased from nanogel}}{\text{Amount of drug initially taken to prepare the nanogel}} 100$$

3.4 Spreadibility of Nanogel: Spreadibility of nanogel was performed by Shear Stress apparatus. 0.1 ml of nanogel was placed on the lower slide of the shear stress apparatus. After that another slide was kept over it. Then the nanogel was spread out and radius of the circle was measured, and area was calculated by using given formula:

Area of circle = πr^2

3.5 *In-Vitro* Drug Release Study of Nanogel:

Franz Diffusion Method: Release kinetics of cefuroxime from nanogel was determined by Franz Diffusion Method, in this method egg shell membrane which serve as natural bio-mucous membrane and mimic the ear mucosal surface was removed from the egg and it was clamped between the donor and the receptor chamber of vertical diffusion cell with an effective diffusion area of 2.8 cm2 and a 7 ml cell volume. In this egg shell membrane was used to mimic the mucosal surface of ear. The receptor chamber was filled with freshly prepared buffer solution. The diffusion cell was maintained at 37 ◦C using a re-circulating water bath and the solution in the receptor chambers was stirred continuously at 300 rpm. The formulation (1ml) was gently placed in the donor chamber. 2 ml sample was withdrawn with pipette at time intervals of 5, 10, 15, 20, 25, 30min, 1, 2, 3, 4, 5, 6, 7, 8, 24 hr and replaced by the 1 ml of fresh 7.4 pH buffer. The samples were diluted with 2 ml of 7.4 pH buffer. These samples were filtered through 0.45µm membrane filter and the drug was estimated by using UV spectrophotometer at λ 279 nm. Then graph was plotted between % CDR and Time and R^2, T30 % and T50 % were calculated. On the basis of R^2 and T50 % best formulations were selected and used for *In-Vivo* studies.

Release kinetics: In order to investigate the mechanism of Cefuroxime release from different nanogel formulations, the release data were analyzed with bit software for the following mathematical models, zero-order, first-order, Higuchi matrix, Peppas and Hix. Crow.

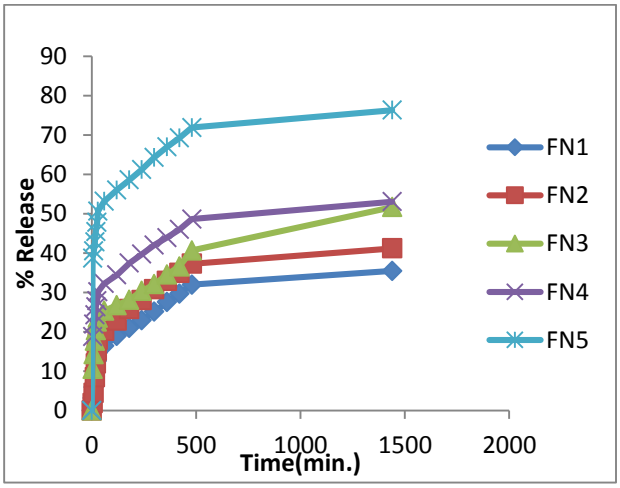

Figure 1: *In Vitro* Release

3.6 *In-Vivo* Drug Release Study:

In-Vivo Release study was done to determine how much amount of drug is released in to blood as it help to calculating amount of drug targeted to brain. Rat was taken and weighed on the weighing balance. Dose for the rat was calculated (2gm/kg body weight). 0.32 ml of best formulation (FN5) was inserted in to the ear of rat. Then blood sample was withdrawn after 1, 4, 8, 24, and 48 hours. Blood sample was centrifuged at 4000 rpm for 15 min. Serum was collected and diluted with methyl alcohol and drug was estimated by using UV spectrophotometer at λ 279 nm.

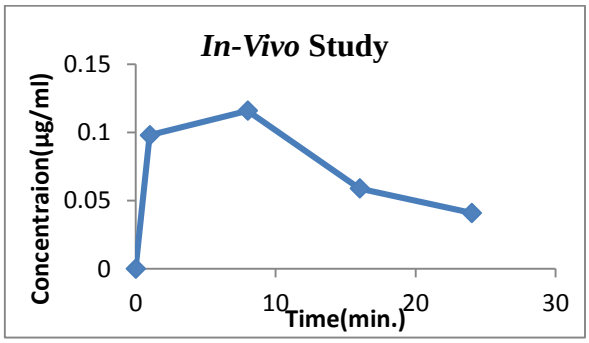

Figure 2 *In-Vivo* Study of Bio-nanogel (FN5)

3.7 Stability studies: Stability studies were carried out for 3 months as per ICH guidelines. Formulations were kept in an amber color vials in an elevated temperature at 40ºC, 25% RH, in refrigerator and at room temperature. Weekly observation was carried out for color, odor, taste, its entrapment efficiency and *In-Vitro* drug release. The stability of drug loaded nanoparticles was evaluated in terms of change in color, odor, taste, its entrapment efficiency, and *In-Vitro* drug released.

Stability studies showed that the formulations *Cucumis sativus* bio-polymer (FN1-FN5) were stable at room temperature, 45 ◦C and 4 ◦C as no change in the color, odor, and taste was observed and no significant changes were was observed in % release and drug content. No aggregation and no oozing were found.

4. RESULT AND DISCUSSION:

4.1Physicochemical properties of the isolated bio-polymer were determined.

4.1a. Bio-polymer was found to be light green in color, having characteristic odor, tasteless and non sticky in texture.

4.1b. Color changing point: Was found to be between 150°C- 160°C.

4.1c. Solubility: Biopolymer was found to be soluble in water.

4.1d.Chemical Tests: carbohydrate and protein were present in the isolated biopolymer.

4.1e. Prepared nanoparticles were subjected to pH study, viscosity, Entrapment efficiency, Spreadibility.

Table 2: Evaluation Parameters

Formulation	pH± S.D.	Viscosity± S.D(cps)	E.E± S.D (%)	Spreadibility± S.D
FN1	6.31±0.07	0.96±0	39±0.005	0.87±0.05
FN2	6.40±0.25	0.986±2	42.1±0.007	0.69±0.02
FN3	6.43±0.32	1.021±0	46.1±0.008	0.60±0.03
FN4	6.38±0.10	1.056±2	46.7±0.005	0.58±0.03
FN5	6.26±0.03	1.06±3	63.7±0.007	0.56±0.02

In-Vitro Release, In-Vivo Release study and stability study were also performed. Research work revealed that the formulated nano-gels showed promising stability. On the basis of % Release, T50 % and other parameters FN5 was found to be the best formulation showing drug release profile maximum at 76 %. The conclusion was drawn that the ear can be used as novelistic platform for delivery of cefuroxime to brain region as well as local action in the formulation of bio-nanogel.

Table 3: Evaluation Parameters

Formulation	pH± S.D.	Viscosity± S.D(cps)	E.E± S.D (%)	Spreadibility± S.D
FN5	6.26±0.03	1.06±3	63.7±0.00	0.56±0.02

5 CONCLUSION:

A wide types of viruses have been notified to cause encephalitis, specific antiviral therapy for viral encephalitis is generally restricted to disease caused by the herpes viruses, especially herpes simplex virus In all cases of acute encephalitis, befitting examination and ancillary care form the inbuilt part of the management strategy This research work revealed that the isolated novel biopolymer was found effective in the formulation of nano-gels by minimizing side effects. The conclusion was drawn that the novel bio polymer can be found effective for formulating Nano-gels. Proposed mechanism based on the research out coming, for drug targeting to brain from ear may achieve via neural pathway which is located ear and brain through vestibular ganglion to verve VIII and from nerve VII to left cochlear neuron which is located in brain. This route can be used for drug targeting to the brain**.** Different drugs were given to the rats and reduced activity of rat indicates that drug was entered in to the brain of rat through ear via neural pathway. The best formulation was selected on the basis that further increase in the concentration of the biopolymer showed no significant change in t_{50} and t_{80}.Further the dose of the cefuroxime was also reduced to many folds as targeted to the brain. and

so can be used to treat various brain diseases including encephalitis and local ear diseases.

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