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PREPARATION AND EVALUATION OF BIOCOMPATIBLE CHITOSAN NANOPARTICLES OF LEVOFLOXACIN HEMIHYDRATE

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ABSTRACT

The use of natural biocompatible polymers to deliver drugs continues to be an area of active research despite the advent of synthetic biocompatible polymers. Natural polymers remain attractive primarily because they are products of living organisms, easily available, comparatively inexpensive, and capable of a multitude of chemical modifications. In present study chitosan is used as natural polymer for encapsulation of levofloxacin. Nanoparticles are prepared and characterization is done on different parameters.

KEYWORDS: chitosan, polymeric nanoparticles, levofloxacin, biocompatible nanoparticles, antibiotic nanoparticles.

1. INTRODUCTION

The challenging and dynamic pattern of infectious diseases and the emergence of bacterial strains resistant to many currently used antibiotics demand for longer-term solutions to this ever-growing and foreseeable problem.[1] One of the recent efforts in exploring antimicrobial nanomaterials is the challenge to find which microbial pathogens may not be able to develop resistance, and novel nano sized platforms for efficient antibiotics delivery. For example, it has been suggested in recent studies that some metal nano constructs are known to possess antimicrobial activities, which are utilized in controlling infectious diseases.[2,3] Antimicrobial nanoparticulate system offer many advantages when compared to conventional antibiotics[4,5]; like in reducing acute toxicity, lowering cost and overcoming resistance. Various nano sized drug carriers are also available to efficiently administer antibiotics by improving pharmacokinetics and accumulation, while reducing the adverse

effects of antibiotics. Theoretically, nanoparticles are retained much longer in the body than small molecule antibiotics, which could be beneficial for achieving sustained therapeutic effects. On the other hand, the safety profiles of nanoparticles and nano sized antibiotics drug carriers, particularly upon long-term exposure, could be an overriding safety factor and must be considered with therapeutic effects.[6]

2. MATERIALS

Levofloxacin hemihydatre was gifted by Simpex Pharma sidgul kotdwar (Uttarakhand). Chitosan, carbopol, sodium tripolyphospahte and vanillin was provided by Department of Pharmacy, Barkatullah University (Bhopal). HPMC 4K was procured from Sigma Aldrich.

3. METHOD

3.1: Calibration curve of Levofloxacin Hemihydrate

Approximately 10 mg accurately weighed levofloxacin hemihydrate was dissolved in 10 ml of water and one ml of this solution was again diluted to 10 ml to get levofloxacin hemihydrate solution. Further dilutions were prepared from this stock solution by taking 300, 400, 500, 600 and 700 µl and diluting them to 10 ml with water. Absorbance of dilutions were recorded using UV spectrophotometer at 300 nm as this solution exhibits λ_{max} at 300 nm, although the literature states λ_{max} at 330 nm which is shown in figure no. 1.

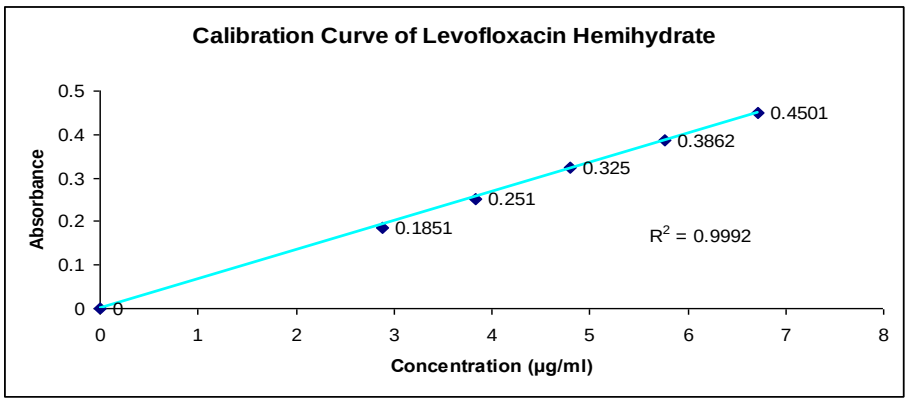

Figure No. 1: Calibration Curve of Levofloxacin Hemihydrate.

3.2: Compatibility/Stability

3.2.1: Polymer- Excipient solutions: To check the compatibility/stability of polymer-excipient solutions, solutions of polymer and excipients were prepared separately in predecided concentration in selected vehicles and were mixed. The solutions after mixing were sealed in a wide mouth plastics container (similar to final container of formulation) and

left for one month. The mixtures were observed for any precipitation or color reactions. No color reactions or precipitations was observed.

3.2.2: Drug-Polymer Solution: To check the compatibility/stability of drug-polymer solution, solution of polymers and drug were prepared separately in predecided concentrations in selected vehicles and were mixed. The solutions after mixing were sealed in a wide mouth plastics container (similar to final container of formulation) and left for one month. The mixtures were observed for any precipitation or color reactions. No color reactions or precipitations were observed.

3.3: Solubility Analysis: Solubility of levofloxacin hemihydrate and excipients was determined in water, phosphate buffer (ph 7.4) at 25 °C and in methanol. Results are shown in table 1.

Table no. 1: Solubility Analysis of Drugs and Excipients.

S. No.	Name of Drug/Excipient	Solubility in		
		Water	Phosphate Buffer (pH 7.4)	Methanol
1	Levofloxacin Hemihydrate	Slightly Soluble	Slightly Soluble	Slightly Soluble
2	Chitosan	Insoluble	Insoluble	Insoluble
3	Sodium Tripolyphosphate	Freely Soluble	Freely Soluble	Freely Soluble
4	Vanillin	Freely Soluble	Freely Soluble	Freely Soluble

3.4: Melting Point Analysis: Melting point of API and excipients were calculated by capillary method with digital melting point apparatus. Results are recorded in table 2.

Table No 2: Melting Point Determination of Drugs and Excipients.

S. No.	Name of Drug/Excipient	Melting point (in °C)
1	Levofloxacin Hemihydrate	214
3	Sodium Tripoly Phosphate	>300
4	Vanillin	81

3.5: Method of Preparation of Nanoparticles

Chitosan solutions were prepared in concentrations of 0.25, 0.50 and 0.75 % w/v in 0.1% v/v acetic acid and the drug was added in such quantity so that the final concentration will be 2% w/v. The pH of solution was maintained between 3-5. A second solution containing vanillin 1% w/v, sodium tripolyphosphate 1% w/v was prepared in water. This solution was divided in three parts and different volumes of Cween 80 were mixed so that the solution contains 0.5, 1.5 and 2.5 % v/v of surfactant. Similarly same procedure was adopted to prepare

solutions of sodium tripolyphosphate, vanillin and sodium lauryl sulfate in similar concentrations.

All the solutions so prepared were subjected to centrifugation with the help of refrigerated centrifuge at 18,000 rpm and supernatant solutions were used for further procedures. Solution of chitosan was added to surfactant solution drop-wise (20 drops/min) in a container using homogenizer. Homogenized suspension was subjected to sonication with probe sonicator for 15, 30 and 45 minutes at 750 watts using ice water (15˚C) jackets to maintain the temperature. The suspension so obtained was centrifuged at 6000 rpm for 30 min in a refrigerated centrifuge and the pellet obtained at the bottom and the supernatant were collected separately. The suspension was filtered initially through membrane of 600 nm pore size and filtrate was subjected to particle size measurements. The results indicate that $LVC_{4,}$ and LVC_{15} are having desired particle size (100 to 400 nm) depending on poly dispersitivity index and particle size. Results are shown in table no. 3.

Physical Stability: The selected four suspensions were sealed in clear air tight borosilicate glass bottles and left for 30 days undisturbed to view any precipitation or settling of particles. No formulation showed any precipitation or settling of particles.

Table No. 3: Formulations of Chitosan – Antibiotics (Levofloxacin Hemihydrate) Nanoparticles.

S.No.	Formulation Code	% w/v Antibiotic (Levofloxacin)	% Chitosan	% Surfactant	% STPP	% Vanillin
1	LVC_1	2	0.25	0.5 Cween 80	1	1
2	LVC_2	2		1.5 Cween 80	1	1
3	LVC_3	2		2.5 Cween 80	1	1
4	LVC_4	2	0.50	0.5 Cween 80	1	1
5	LVC_5	2		1.5 Cween 80	1	1
6	LVC_6	2		2.5 Cween 80	1	1
7	LVC_7	2	0.75	0.5 Cween 80	1	1
8	LVC_8	2		1.5 Cween 80	1	1
9	LVC_9	2		2.5 Cween 80	1	1
10	LVC_{10}	2	0.25	0.5 SLS	1	1
11	LVC_{11}	2		1.5 SLS	1	1
12	LVC_{12}	2		2.5 SLS	1	1
13	LVC_{13}	2	0.50	0.5 SLS	1	1
14	LVC_{14}	2		1.5 SLS	1	1
15	LVC_{15}	2		2.5 SLS	1	1
16	LVC_{16}	2	0.75	0.5 SLS	1	1
17	LVC_{17}	2		1.5 SLS	1	1
18	LVC_{18}	2		2.5 SLS	1	1

3.6: Formulation of Gel of Nanoparticles

As we were not able to separate nanoparticles from the aqueous dispersion in present laboratory conditions, aqueous dispersion of nanoparticle itself was used in the place of water to prepare gel. A total of 100 ml of nanoparticle aqueous dispersion was concentrated at -40°C and 300 *mtor* by lyophilization to 25 ml. One gram carbopol was dissolved in 75ml of water. 100 mg HPMC 4K was dissolved in 25 ml aqueous dispersion of nanoparticles. This dispersion was mixed in 75 ml of carbopol solution. To this solution 0.01 N NaOH solution was added 10 µl at a time slowly till desired consistency is achieved. The pH of gel was found to be between 7 to 8.

4. CHARACTERIZATION

4.1: Particle Size and Poly dispersitivity Index: Mean particle size and poly dispersitivity index of formulations were conducted by zeta siezer, which are shown in table no 4.

Table No. 4: Particle Size and Poly Dispersitivity Index for Chitosan-Antibiotics (Levofloxacin Hemihydrate) Nanoparticles.

S. No.	Formulation Code (Chitosan – Levofloxacin Hemihydrate Nanoparticles)	Average Particle Size (in nm)	Poly Dispersitivity Index
1	LVC_1	192	0.511
2	LVC_2	167	0.283
3	LVC_3	264	0.435
4	**LVC_4**	**228**	**0.090**
5	LVC_5	215	0.298
6	LVC_6	247	0.501
7	LVC_7	310	0.260
8	LVC_8	337	0.236
9	LVC_9	446	0.172
10	LVC_{10}	146	0.409
11	LVC_{11}	161	0.343
12	LVC_{12}	180	0.234
13	LVC_{13}	412	0.279
14	LVC_{14}	397	0.201
15	**LVC_{15}**	**291**	**0.167**
16	LVC_{16}	315	0.467
17	LVC_{17}	383	0.374
18	LVC_{18}	479	0.386

4.2: Percent Entrapment Efficiency

The pellet obtained after processing as mentioned in section 3.5 in preparation method; collected after centrifugation of primary suspension were washed with water and centrifuged

again. This process was repeated twice. Pellet was dried in vacuum and weighed accurately. The pellet was extracted with 2ml of methanol four times. The extracts were combined and evaporated to dryness. The powder was dissolved in one ml of water. One ml of the combined extract solution was diluted to 10 ml with water. The solution was filtered through whatman filter and absorbance was recorded. Percent entrapment efficiency was calculated with the help of absorbance and calibration curve. It was found to be 76% for formulation LVC_4 and 25 % for formulation LVC_{15}.

As formulation LVC_4 showed better entrapment efficiency, further characterization was then focused on LVC_4 only

4.3: Free Drug Concentration: The suspension (supernatant) collected after centrifugation at 600 rpm (as mentioned in section 3.5) was subjected to centrifugation at 18000 rpm for 30 minutes. The supernatant liquid was collected and 1 ml of this solution was diluted to 10 ml with water and absorbance was recorded at 330 nm for formulation LVC_4 with the help of UV spectroscope and it was found to be 24%.

4.4: Solid Content in Aqueous Dispersion: 50 ml filtrate obtained after passing the suspension from 600 nm pore size (as mentioned in section 3.5) with drug was kept in tarred clean and dry glass vessel for evaporation at reduced pressure at 50°C. Weight of solid content was recorded for further calculations. For formulation LVC_4 it was 0.432 gm.

5. *In-Vitro* RELEASE STUDY

5.1: Concentration of Drug that Crosses the Membrane and Reaches the Buffer Solution**.**

Franz diffusion cell was used in present study with a sample holder opening of πr^2 (1 cm radius). Fresh goat skin was obtained from slaughter house. It was depilated and was kept at 7.4 pH. The upper surface of skin was dried and placed on the sample holder. One gm gel was applied on the skin. Phosphate buffer solution of pH 7.4 was used in Franz diffusion cell of 25 ml capacity. Magnetic bead was used to allow constant stirring of phosphate buffer. One ml of aliquots were withdrawn after each hour and volume was compensated by adding one ml of phosphate buffer at each withdrawal. This process was done for the total period of 08 hours and cumulative drug content was calculated on the basis of UV absorption of each aliquot at 330 nm for formulation LVC_4.

5.2: Unabsorbed Drug Concentration

Isolated and depilated goat skin of 1 cm radius was used. It was marked for circles (total 08). This goat skin was then tied on a petri dish cover (diameter 15 cm) containing buffer solution of pH 7.4 and a magnetic bead for stirring. After arranging goat skin on petridish cover; approximately 200 mg of gel was applied on marked circles. At interval of one hour gel was wiped out with the help of methanol extracted cotton swab. After wiping swab were extracted twice with methanol. The extracts were combined and evaporated to dryness. The powder was then dissolved in one ml of water. One ml of combined extract solution was diluted to 10 ml of water. The solution was filtered through whatman filter and absorbance was recorded at 330 nm and 254 nm for levofloxacin hemihydrate and linezolid respectively. This process was done for total time period of 08 hours and data was recorded.

5.3: Drug Concentration that Resides in Membrane.

The drug in the membrane was calculated by deducting the sum of percent cumulative drug concentration that reaches to buffer solution and percent drug remaining in applied gel formulation.

Percent drug concentration in membrane = 100 – [Percent cumulative drug concentration which crosses the membrane + percent drug remaining in gel applied on skin]

Combined results for section 5.1 to 5.3 are shown in table no. 5.

Table No 5: Percent Drug Concentration That Reaches in Membrane But Does Not Get Systemically Absorbed for Formulation LVC_4.

S. No.	Time (in hours)	Percent cumulative drug concentration in buffer solution (a)	Percent unabsorbed drug concentration (b)	Percent drug concentration in membrane (c) = 100 – (a+b)
1	0	0	100	0
2	1	1.2	86.4	12.4
3	2	3.5	73.1	23.4
4	3	7.6	61.9	30.5
5	4	14.3	48.2	37.5
6	5	23.6	36.3	40.1
7	6	28.7	30.1	41.2
8	7	30.2	24.2	45.6
9	8	32.2	23.5	44.3

1. STABILITY STUDIES[7]

Accelerated stability studies were performed according to ICH guidelines Q1A-R2 at 40°C ± 2°C/ 75% RH ± 5% RH for total period of 6 months. In this period organoleptic properties, viscosity, drug concentration and pH were observed for formulations **LVC_4**, which are shown in table no. 6.

Table No. 6: Stability Study Determination.

S.No.	Observation	Formulation LVC_4 (Variation in percent)		
		0 months	3 months	6 months
1	Organoleptic Properties (smell, color change or precipitation)	No change	No change	No change
2	Viscosity	No change	No change	-0.23
3	pH	No change	No change	No change
4	Drug Content	No change	No change	0.12
5.	Moisture Loss	No change	No change	0.5

2. DISCUSSION

Levofloxacin hemihydrate showed R^2 value of 0.992 at 300 nm at concentration up to 8µg/ml used for preparation of calibration curve. API also found compatible with excipient and polymer in solution state. Except chitosan which is soluble in acidic media, all other ingredients (drugs and polymer) are water soluble. Modified homogenization method was used to prepare initially microparticles and later probe sonicator is used to reduce the particle size. Standard laboratory methods were used to separate larger particle from nanosuspension. Nano suspensions were prepared by using three concentration of polymer and few suspension of desired particle size range were selected for further studies like physical stability, entrapment efficiency, particle size distribution and in-vitro release rate. Nano suspension was concentrated with lyophillization method as filtration was not feasible with available laboratory facilities. Nanoparticles were incorporated in carbopol gel which was then subjected to in-vitro studies for release of drug. Franz's cell was used and drug retained in the skin was calculated which is responsible for local action. The final formulation was subjected to stability studies as per ICH guidelines.

3. CONCLUSION

formulation LVC4 was found to be a better formulation in comparison to other formulations prepared for present study. LVC4 with average particle size of 228 and polydispersitivity index 0.090 showed satisfactory physical properties. Keeping in mind the release rate and

percentage of drug in skin for local action. It may be concluded that chitosan may be used to prepare sustained release nanoparticles and 0.5% w/v concentration of chitosan shows satisfactory results.

4. ACKNOWLEDGEMENT

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