Mupirocin Mounted Copper Nanoparticle Offered Augmented Drug Delivery against Resistant Bacteria

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ABSTRACT

Aim: In present investigation, mupirocin coupled copper nanoparticles were synthesized to overwhelm drug resistance in Staphylococcus aureus, responsible for dermal skin infections. Materials and Methods: Mupirocin (Anclaima Pvt. Ltd. India), copper sulphate (Thermo Fisher Scientific, India), Tri sodium citrate (Nice chemicals, India) Copper nanoparticles were produced by size reduction method by using Copper Sulphate and Tri sodium citrate. CuNPs were merged into gel base of carbopol which was formed by hot method. Carbapol gel shows no phase separation. Results and Discussion: The particle size of CuNPs was found to be 413.0 ± 30.2nm. The % EE was found to be 65.2%. On description unilamellar, sphere-shaped vesicles with soft surface were detected under transmission electron microscopy. XRD of CuNPs was found out crystalline in nature. The zeta potential of nanoparticle shows less aggregation of particle with - 15.1 ± 3.69 mV value. The amount of copper content was measured 5.9 microgram/10 mg of nanoparticles. In vitro release study of Cu NPs shows 96.5% release of drug and show effective antibacterial activity against Staphylococcus aureus. Conclusion: Mupirocin copper nanoparticles were produced and show excellent antibacterial activity upon S. aureus. Keywords: Copper nanoparticle, Mupirocin, Carbopol gel, Antibacterial activity, Chemical reduction method, Copper sulphate.

INTRODUCTION

In the past decade, the cure of sickness has been consummate by administrating drugs to human body through different routes likes parental, oral, topical, sublingual, inhalation, rectal etc.1 The delivery of drug through topical denotes the application of drug onto the body employing vaginal, rectal, ophthalmic and skin as the route of administration. On human body, skin is widely used and accepted route for local application and constitutes the principal administration for local application. The term topical drug delivery means administration for medicament containing formulation to the skin to openly care for the cutaneous manifestations of a common illness (e.g. psoriasis) or cutaneous disorders with the purpose of confining the pharmacological or other effect of the medicament inside or surface of skin.2 During recent years, a report will show interest in the synthesis and applications of various metallic nanoparticles due to their outstanding optical and electronic properties, especially copper, gold and silver nanoparticles. Copper nanoparticles (CuNPs) have gradually become an active area of research because of unique chemical, physical, electrical and optical properties, low cost, ease availability and exhibit good antibacterial properties. The prime advantage of CuNPs is their low cost and its availability compared to gold and silver nanoparticles, resulting in the sample synthesis and various applications of CuNPs.3,4 Copper is easily available metal and one of the vital trace elements for mainly living
The heat in the apparatus was sequentially raised and the sealed tube was positioned in the melting point apparatus. The amount of drug was added, and tube is sealed. The determination of melting point:

Materials:

Methods

Preparation of the carbopol gel

At low concentration, carbopol 940 forms very good flexible transparent gel. The gel base of 2% was prepared by scattering 2 g carbopol 940 in 86 ml warm distilled water. Accurately weighed 0.6 g propyl paraben
and dissolved in ethanol. Accurately weighed 0.3 g methyl paraben and dissolved in 15 ml of propylene glycol. Stirred the mixture unless gelling occurred and then mixture was neutralized with the help of 50% (w/w) triethanolamine. Triethanolamine was added drop by drop to maintain the pH between 6-7. The nanoparticle formulation containing drug was slowly added in carbopol 940 gel base and mixed with the help of stirrer for 5 min continue stirring.

Evaluation of nanoparticles

Drug entrapment efficiency

Take 5 ml formulation and diluted the formulation up to 8 ml with distilled water and centrifuged the diluted formulation at 15,000 rpm at 4°C for 45 min using a cooling centrifuge. The sediment and supernatant were restored after centrifugation, their volume was calculated. Then sediment was break down through n-propanol and filtered using a 0.45 μm nylon filter. The concentration of mupirocin in the sediment and supernatant was examined by UV- spectrophotometer at 220 nm. The % entrapment efficiency was estimated:

\[
\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapped drug recovered}}{\text{Total amount of drug}} \times 100
\]

Nanoparticle shape

Transmission electron microscopy (Philips Technai electron microscope, Netherlands) were used for the forecast of nanoparticle. At room temperature, sample was dried and vesicular were forecast under microscopy working at an acceleration voltage of 200 KV for 5 min.

Particle size estimation

Dynamic light scattering method was used for the determination of copper nanoparticles, using a computerized inspection system (Malvern Zetasizer Nano-ZS, Malvern, U.K.). For the measurement of size, copper nanoparticle solution was attenuated with distilled water and implement in cuvettes of zetasizer.

Zeta potential measurement

Physical property like zeta potential which describe the net surface charge of copper nanoparticles. The stability criteria of CuNPs are measured when the zeta potential values ranges from higher than +30 mV to lower than −30 mV.

X-ray diffraction

1 ml of the copper nanoparticle solution was extend on a glass slide and dried at 40°C in an oven. The Phillips Xpert Pro Diffractometer were recorded the spectra (Cu Kα radiation, \( \lambda = 1.54 \, \text{Å} \)) running at 40 kV and 30 mA. The diffracted intensities were recorded from 10 degrees to 80 degrees 20 angles.

Copper content determination

Determination the copper (II) ions, take 200 mL of tap water in the beaker. Water is evaporated upto 50 mL. Solution is transferred into a volumetric flask and the determination is performed. To different volumes of water, the solution containing copper is added and the solution is was brought up to the mark by mixture of acetate buffer. The absorbance is measured at a wavelength of 520 nm.

Physical evaluation of nanoparticle gel

pH measurement of the nanoparticle gel

1 gm mupirocin coupled nanoparticle gel base was mixed in 100 ml beaker containing distilled water. After that pH electrode was deep in beaker and readings were reported from digital pH meter.

Viscosity study

The viscosity of copper nanoparticle was measured in Brookfield instrumentation by selecting appropriate spindle and rpm. In 50 ml beaker, 50 g of formulation was added which was set till spindle channel was drenched and set rpm. Reading pointed out over three minutes.

Spreadability

Spreadability term denote a area is required to which gel willingly fall on appliance to skin or affected part. It was calculated through formulation:

\[
S = \frac{M \times L}{T}
\]

Where

- \( T \): time taken to separate the slides
- \( L \): length of slides
- \( M \): wt. tied to upper slide

Extrudability study

The extrudability of mupirocin coupled nanoparticle gel was considered by stuffing nanoparticle gel in the foldable tubes. Determination in words of weight in grams, 10 sec required to extrude a 0.5 cm ribbon of gel.

Percentage yield

Percentage yield was calculated by the formula.

\[
\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100
\]
**Grittiness and homogeneity**

A tiny amount of nanoparticle gel was squeezed in the middle of index finger and the thumb. Uniformity of the nanoparticle gel is observed, any crude particles visible on fingers.\(^\text{19,20}\)

**In vitro release studies**

Vertical Franz diffusion cell apparatus was employed for the *in vitro* absorption studies. It contain donor as well as receptor chamber that is filled with PBS. The donor chamber is filled and the permeation of solute through the membrane is monitored at different interval of time. Episodic sampling from the receptor chamber was collected and measured. The jacketed cell personified is stirred during experiment at 500 rpm using a magnetic agitator.\(^\text{21}\)

**Drug release kinetics**

The kinetic of drug release was calculated by various kinetic models as zero order release kinetics plot, first order release kinetics plot, korsmeyer-peppas release kinetics plot and higuchi release kinetics plot. To study profile release kinetics of the copper nanoparticle figures obtained from *in-vitro* release profile were plotted for various kinetic models. The finest fit model was set by the value of $R^2$ close to 1.\(^\text{22}\)

**Antimicrobial activity studies**

Antimicrobial activity has been assayed against bacteria by using agar diffusion method. The antibiotics action of drug is as oleic of its capability to growth inhibition of bacto nutrient agar or broth. Cup-plate method shall be used for the consideration of bacterial inhibition. In experiment, discs of average diameter were prepared in the bacto agar nutrient medium, containing standard bacterial inoculums. The test samples are injected in the disc and the diameter of the zone of inhibition was evaluated. All the test samples were evaluated for antibiotics activity against *Staphylococcus aureus* (gram positive).\(^\text{23}\)

**RESULTS AND DISCUSSION**

**Melting point determination:** Melting point of pure mupirocin outcome at 78°C. Melting Point outcome three times and mean was noted.

**DSC:** The DSC thermograms showed quick endothermic peak equivalent to mupirocin melting point 77.31°C. The DSC thermogram of mupirocin is shown in Figure 1.

**Solubility studies:** Mupirocin was found to be soluble in acetone, methanol, 0.1 N HCL, distilled water, ethanol, chloroform, PBS of 6.8, 7.4

**FTIR analysis:** FT-IR analysis discovered that there was no interaction between the mupirocin and physical mixture as per given in pharmacopoeia. In the present investigation, it has been observed that there are no chemical and physical interactions because of some bond formation between mupirocin and physical mixture. Hence mupirocin drug was authentic and free from impurities. (comparison shown in Table 1) (Figure 2, 3 and 4 shown FTIR of Mupirocin, Carbapol and physical mixture of Mupirocin and Cabapol respectively).

**Calibration curve of mupirocin**

The graph obeyed beer lamberts law in this selected concentration range. The calibration equation for
straight line was observed to be \( y = 0.045x - 0.021 \) with correlation coefficient 0.998 shown in Figure 5.

**Evaluation of nanoparticles**

**Drug entrapment efficiency**

% E.E. of drug was found to be 65.2%

**Transmission electron microscopy (TEM):**

Formulation was subjected for TEM to obtain, image of nanoparticles on scale bar of 200 nm with magnification 13.0x4000. On description unilamellar, spherical vesicles with smooth surface were noticed under transmission electron microscopy (TEM) shown in Figure 6.

**Zeta potential**

The zeta potential of copper nanoparticle shows in Figure 7, less aggregation of particle with \(-15.1 \pm 3.69 \text{ mV}\) value.

**Particle size measurement:** Figure 8 shows the Dynamic Light Scattering of copper nanoparticles; the average size obtained was of 413.0 ± 30.2 nm with a narrow size distribution.

**X-ray diffraction (XRD)**

The crystal structure and phase composition of synthesized copper nanoparticles is analyzed by XRD, as shown in Figure 9. The diffraction data exhibits that the copper nanoparticles have crystalline structure.

**Copper content determination**

The amount of copper content was measured 5.9 microgram/10 mg of nanoparticles.

**Physical Evaluations of Nanoparticle Gel**

**Organoleptic characteristics**

- **Color** = pale yellow
- **Odor** = characteristic
- **Phase separation** = no
**Determination of pH of nanoparticle gel**

The pH of nanoparticle gel was recognized as 7.1

**Viscosity**

The viscosity of carbopol 940 gel base and nanoparticle gel by Brookfield instrumentation was recognized 73,200 and 72,300 cP respectively.

**Spreadability**

The spreadability of mupirocin gel coupled copper nanoparticle was recognized 13.29 g.cm²/sec. The results demonstrated that gel was effective.

**Extrudability analyzed**

The extrudability of mupirocin gel coupled copper nanoparticle was recognized positive. Positive extrudability showed the better application of gel.

**Percentage yield**

The % yield of mupirocin gel coupled copper nanoparticle was carried out 95.78%.

**Homogeneity and grittiness**

Mupirocin gel coupled copper nanoparticle was recognized homogeneous and no grittiness was indicated.

**In vitro release profile**

Franz diffusion cell apparatus was used for the in vitro release profile. The drug release profile of the mupirocin coupled copper nanoparticles is presented in Figure 10. In vitro release profile was performed to determine amount of drug release at different interval of time. The cumulative drug releases from nanoparticle reach 96.5% in 48 hr.

**Drug release kinetics**

The kinetics for drug release of mupirocin coupled Cu NPs was carried out for different models. The release profile of various models was given in Table 2.

**Zero Order release kinetics plot**

Plot the graph % cdr Vs time (Graph shown in Figure 11)

**First Order release kinetics Plot**

Graph was prepared between log % cumulative drugs remaining Vs time (Graph shown in Figure 12)

| Table 2: Drug release kinetic of mupirocin coupled Cu NPs |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Time (min.) | Log time   | Square root of time | % cumulative drug release(CDR) of formulation | Log% cumulative drug release of formulation | % cumulative remaining | Log% cumulative remaining |
| 0           | 0          | 0             | 0          | 0          | 100        | 2            |
| 30          | 1.48       | 5.48          | 5.7        | 0.76       | 94.3       | 1.97         |
| 60          | 1.75       | 7.75          | 10.1       | 1.00       | 89.9       | 1.94         |
| 120         | 2.08       | 10.95         | 19.8       | 1.29       | 80.2       | 1.90         |
| 240         | 2.38       | 15.49         | 36.3       | 1.56       | 63.7       | 1.80         |
| 480         | 2.68       | 21.91         | 53.1       | 1.73       | 46.9       | 1.66         |
| 960         | 2.98       | 30.98         | 74.2       | 1.86       | 25.8       | 1.40         |
| 1440        | 3.15       | 37.95         | 89.3       | 1.94       | 10.7       | 1.03         |
| 2880        | 3.46       | 53.67         | 96.5       | 1.97       | 3.5        | 0.58         |
**Higuchi’s Model release kinetics**

Graph was prepared between % cdr Vs square root of time (Graph shown in Figure 13).

**Korsmeyer-Peppas Model release kinetics**

Graph was prepared between log % cdr Vs log time (Graph shown in Figure 14).

Some kinetic models describing drug release from modified released dosage forms. The model release data by correlation coefficient. The correlation coefficient value was used as criteria to choose the best model to explain the drug release. From these values, it was observed that the peppas model will be fitted best model with $R^2$ value of 0.980 shown in Table 3.

**Antimicrobial activity**

Antimicrobial activity was determined by cup plate method on *S. aureus*. Antimicrobial activity of pure drug shown in Figure 15 and Antimicrobial activity of copper nanoparticles gel containing mupirocin shown in
**Table 4: Antimicrobial activity by cup-plate method.**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Pure Mupirocin</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>9 ± 1 mm</td>
<td>6 ± 2 mm</td>
</tr>
<tr>
<td>1</td>
<td>12 ± 2 mm</td>
<td>9 ± 2 mm</td>
</tr>
<tr>
<td>4</td>
<td>17 ± 1 mm</td>
<td>13 ± 3 mm</td>
</tr>
<tr>
<td>20</td>
<td>18± 3 mm</td>
<td>17 ± 1 mm</td>
</tr>
<tr>
<td>30</td>
<td>18 ± 2 mm</td>
<td>18 ± 2 mm</td>
</tr>
<tr>
<td>40</td>
<td>19 ± 2 mm</td>
<td>18 ± 1 mm</td>
</tr>
</tbody>
</table>

**Figure 16.** Comparison of pure mupirocin and formulation of antibacterial activity shown in Table 4.

**CONCLUSION**

In present investigation, mupirocin coupled copper nanoparticles were synthesized to overwhelm drug resistance in *Staphylococcus aureus*, responsible for dermal skin infections. So, prepared and evaluate the mupirocin coupled Cu NPs to get the formulation with increased antibacterial activity and suit for topical application. The Cu NPs containing mupirocin were prepared by chemical reduction method and evaluated. Based on $R^2$ value the formulation followed the Pappas model. Mupirocin containing Cu NPs based gel displayed superior efficacy against *S. aureus* owning to prolonged release as compared to pure drug.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**

Cu NPs: Copper Nanoparticle; MIC: Minimum inhibitory concentration; Vs: Versus; PBS: Phosphate buffer saline; Pvt Ltd.: Private limited; TEM: Transmission electron microscopy; S: *Staphylococcus*.

**REFERENCES**

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PICTORIAL ABSTRACT

Topical route offers several potential advantages over conventional delivery. While optimizing topical drug delivery colloidal carrier system appears as upcoming development. Recently advancement in nanoparticle was done and obtained “copper nanoparticle” which has desirable advantages like alteration in properties of drug and antibacterial activity. Cu NPs potentiate the antibacterial action of drug. Copper nanoparticles were prepared by size reduction using chemical method. Gel was prepared by carbopol 940 as gelling agent, propyl paraben and methyl paraben added as preservatives. Cu NPs shows good antibacterial activity.
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