Development and Evaluation of Gel Incorporated with Biogenically Synthesised Silver Nanoparticles

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Abstract: The purpose of the study was to prepare gels by using polymer chitosan and carbopol. The prepared gels were incorporated with silver nanoparticles biogenically synthesized by leave extract of *Eichhornia crassipes.* The silver nanoparticles having average particle size of 21.71+/- 4.42 nm and showing antimicrobial activity against Gram positive and Gram negative bacteria were taken for preparing gels. Different characteristics like pH, spreadability, extrudability, viscosity, in-vitro drug diffusion, swelling, and antimicrobial study of the prepared gels were evaluated. The gel formulated with chitosan showed better physicochemical characteristic as compared to gel formulated with polymer carbopol. In-vitro antibacterial study of the prepared silver nanoparticle incorporated gels was carried out using microorganisms like *Staphylococcus aureus*, Bacillus subtilis, *Escherichia hermanii*, and *Pseudomonas aeruginosa.* The prepared gels showed promising activity against *Staphylococcus aureus* and *Bacillus subtilis,* moderate activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The optimized silver nanoparticle incorporated gel was having potency to be used as an antimicrobial agent.

Keywords: Silver nanoparticles, antimicrobial activity, gel, chitosan, carbopol 940.

1. INTRODUCTION

Topical preparations are formulae which are applied directly to an external body surface by spreading, rubbing, spraying or instillation. The topical route of administration has been utilized either to produce local effect for treating skin disorder or to produce systemic drug effects. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. Gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments [1]. They are highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and do not need to be removed. Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removed, emollient, non-staining, and compatible with several excipients and water soluble or miscible [2]. The U.S.P. defines gels as semisolids, either suspension of small inorganic particles or large organic molecules interpenetrated with liquid. Gels are transparent or translucent semisolid formulations containing a high ratio of solvent/gelling agent. When dispersed in an appropriate solvent, gelling agents merge or entangle to form a three dimensional colloidal network structure, which limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for gel resistance to deformation and hence, its viscoelastic properties [3]. Nanoparticles are of great interest due to their extremely small size and large surface to volume ratio, which lead to both chemical and physical differences in their properties compared to bulk of the same chemical composition, such as mechanical, biological and sterical properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point [4].

Therefore, designing and production of materials with novel applications can be resulted by controlling shape and size at nanometer scale. Nanoparticles exhibit size and shape-dependent properties which are of interest for applications ranging from biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. These particles also have many applications in different fields such as medical imaging, nanocomposites, filters, drug delivery, and hyperthermia of tumors [5]. Silver nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as integrated circuits, sensors [6], biolabelling, filters, antimicrobial deodorant fibres [7], cell electrodes [8], low-cost paper batteries (silver nano-wires) [9] and antimicrobials [10]. Silver nanoparticles have been used extensively as antimicrobial agents in health industry, food storage, textile coatings and a number of environmental applications. Antimicrobial properties of silver nanoparticles caused the use of these nano-metals in different fields of medicine, various industries, animal husbandry, packaging, accessories, cosmetics, health and military. In medical field the most important use is in burn management. The goal of the research

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was to prepare silver nanoparticles by using plant extract then formulate and evaluate various polymers with varying concentrations for the preparation of a safe, effective and stable gel containing silver nanoparticles and to evaluate the *in vitro* performance, stability and also evaluate the *in vitro* antibacterial activity for prepared formulations.

2. MATERIALS AND METHOD

2.1. Materials

Silver nanoparticles synthesized by leave extract of *Eichornia crassipie* [11], Polymers- Carbopol - 940 obtained from Bf. Goodrich, Co (USA) and Chitosan procured from Aldrich, USA., Silver nitrate from Sigma-Aldrich, Bangalore, India, Silverex gel, 0.2% w/w of silver nitrate (Ranbaxy Laboratories Ltd, India). All other chemicals and solvents were of analytical grade.

2.2. Preparation of Gel

Gels were prepared by cold mechanical method. Required quantity of polymer was weighed and sprinkled slowly on surface of purified water for 2 hrs. After that it was continuously stirred by magnetic stirrer, till the polymer soaked in the water. With continuous stirring, other ingredients like glycerol were added. Finally the drug silver nanoparticles were added to the gel with continuous stirring till drug get dispersed in gel completely. Two formulations of silver nanoparticle incorporated gels were prepared by using polymer chitosan and carbopol, the composition of each gel was shown in Table **1**. The prepared gels were packed in wide mouthed glass jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were stored in dark and cool place [12].

Table 1: Composition of Gel Formulations

Gel A	Gel B
Carbopol: 2gm	Chitosan: 2gm
Glycerol: 2gm	Glycerol: 2gm
Silver nanoparticles: 0.02gm	Glacial acetic acid: 1.5ml
Water up to 100gms	Silver nanoparticles: 0.02gm
	Water up to 100gms

2.3. Characterization of the Formulated Gels

2.3.1. pH

The pH of formulated gels was determined by digital pH meter (Make Lab India). One gram of gel was dissolved in 100 ml of distilled water and stored at 4°C for two hours. The measurement of pH of each formulation was carried out in triplicate and the average values were presented [13].

2.3.2. Spreadability

Ideal gel must have low spreadability values but good consistency. Spreadability of formulations was determined by an apparatus suggested by Multimer which was engineered in laboratory and used for slide fixed on wooden block and upper slide with one end tide to glass slide and other end tied to weight pan. A glut of gel (1 gm) was placed in between two glass slides and then 1000 gm weight was placed on slides for 5 min to squeeze the sample to a uniform thickness. Weight (80 gm) was added to pan. With the help of string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10 cm be noted. A shorter interval signifies better spreadability. The time (seconds) required to take apart the two slides, was taken as a measure of spreadability [14].

It was calculated using equation:

$$S = M. L/T$$

Where, S = spreadability, M = weight tied to upper slide, L = length of glass slide, T = time taken.

2.3.3. Viscosity

A Brookfield Rotational Digital Viscometer DV II RVTDV-II was used to determine the viscosity (in cps) of the gels. The spindle No.63 was rotated at 200 rpm. Samples of the gels were permitted to settle over 30 min at the assay temperature (25 ± 1°C) before the measurements were done [15].

2.3.4. Extrudability

The method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel extruded from tube on application of certain load. More the quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminum collapsible one-ounce tube with a nasal tip of 5 mm opening. The weight of 200gm was applied at the bottom of tube to release gel through the opening. Extrudability was determined by weighing the amount of gels extruded through the tip. The percentage of gel extruded was calculated, more extrudability indicates better application of gel [16].

2.3.5. Swelling Characteristics

Preweighed dry gel films were immersed in water and phosphate buffer solutions pH 7.4 respectively. The films were withdrawn from the solutions at different time intervals and their wet weight were determined after first blotting with a filter paper followed by blowing with a stream of air to remove the surface water and immediately weighing the films. The swelling ratio was calculated using the equation;

$$Esr(\%) = \frac{(Ws - Wd)}{Wd} \times 100$$

Where Esr was the water absorption (%wt) of the films, *Wd* and *Ws* were the weights of the samples in the dry and swollen states respectively [17].

2.3.6. Determination of Antibacterial Activity

The prepared gels were evaluated for their antibacterial activity against different bacterial strains by agar well diffusion method [18]. The discs of antibacterial agents used as reference standards were having erythromycin in a concentration of 15 µg/disc. The diameters of the inhibition zones were measured [19].

2.3.7. in-vitro Drug Diffusion Study

Egg membrane separated from egg by applying acid was used for this study. In Franz diffusion cell, 2.0 gm of gel was kept in donor compartment. The entire surface of membrane was in contact with the receptor compartment containing 100 ml of Phosphate buffer pH 7.4. The receptor compartment was continuously stirred (100 rpm) using a magnetic stirrer. The temperature maintained was 37 ± 1°C. The study was carried out for 24 hrs with the interval of 0, 0.5, 1, 1.5, and so on upto 14 hrs. The sample was withdrawn at predetermined period of time and same volume was replaced with fresh Phosphate buffer pH 7.4. The absorbance of withdrawn sample was measured at 430 nm to estimate the amount of silver nanoparticles present [20].

2.3.8. Drug Release Kinetic Study

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics. The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration. The first order Eq. (2) describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (3) [21].

$$C = k_o t \quad (1)$$

Where, k_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$\text{Log } C = \text{Log}C_0 - kt/2.303 \quad (2)$$

Where, C_0 is the initial concentration of drug and k is first order rate constant.

$$Q = Kt_{1/2} \quad (3)$$

Where, K is the constant reflecting the design variables of the system.

The following plots were made [22]:

1. Cumulative % drug release vs. time (zero order kinetic model);
2. Log cumulative of % drug remaining vs. time (first order kinetic model);
3. Cumulative % drug release vs. square root of time (higuchi model);
4. Log cumulative % drug release vs. log time (korsmeyer model).

3. RESULTS

3.1. Formulation of Gel

The formulated gels prepared by carbopol and chitosan with and without silver nanoparticles were observed to be clear with uniform consistency.

3.2. Physicochemical Characteristics of Gels

Table **2** shows the rheological characteristics of the gels. Gel B as compared to Gel A showed more viscosity at minimum and maximum rates of shear. The tested gels showed pesudoplastic flow with variable thixotropic behavior. From the results it was observed that the formulated gels were having spreadability approaching the commercial product silverex. The gel prepared by chitosan had shown better spreadability as compared to gel prepared by carbopol. From the results it was observed that the formulated gels were having extrudabilty approaching that of the commercial product silverex. The gel prepared by chitosan had shown better extrudability as compared to gel prepared by carbopol.

Table 2: Physicochemical Characteristics of the Gels

Gels	pH*	Spreadability*	Viscosity[1]	Viscosity[2]	Extrudability*
Gel A	6.77±0.058	09.63 ± 0.23	210480	2321	18.95 ± 0.08
Gel B	6.10±0.17	11.11 ± 0.30	220350	2464	20.66 ± 0.67
Gel M	7.00±0.0	11.59 ± 0.17	224076	2913	21.97 ± 0.14

*(mean ± SD, n = 3).

[1]at minimum shear rate.

[2]at maximum shear rate.

3.3. Swelling Test Study of Gel

From the swelling test data it was found that gel prepared by polymer chitosan has greater percentage swelling index as compared to gel prepared by carbopol and approaching that of the commercial gel as shown in Table **3**.

Table 3: Swelling Test Results for Different Formulation of Gel

Sample	Swelling % in PBS 7.4	Swelling %in Water
Gel A	07.00	29.00
Gel B	10.57	32.32
Gel M	10.00	34.00

3.4. Antimicrobial Study of Silver Nanoparticle Incorporated Gels

The zone of inhibition of prepared silver nanoparticle incorporated gel and marketed product was shown in Table **4**.

3.5. Drug Release Kinetic Study

The data for *in vitro* drug release of silver nanoparticles from topical gel were depicted in Figure **1**. The formulation gel B prepared using polymer chitosan was more effective, because it controls the release of drug more in comparison to the formulation gel A prepared using the polymer carbopol. The graph showing release of the drug silver nanoparticles was shown in Figure **1** for gel A and gel B.

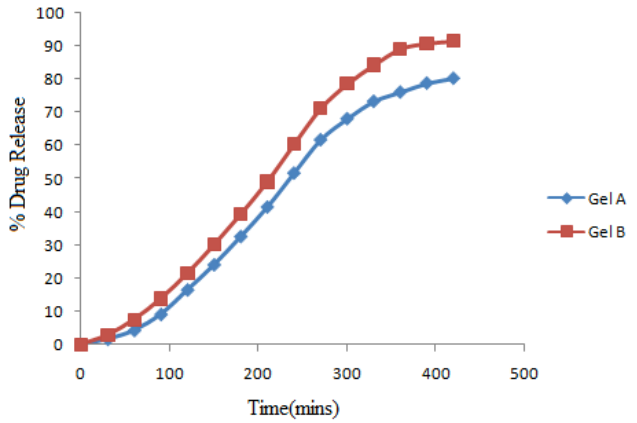

Figure 1: Percentage drug release of different gel formulations.

For formulation gel A the R^2 values for zero order plot is 0.976, first order plot is 0.962, Higuchi model plot is 0.883 and Korsmeyer model plot is 0.821. The best linearity was found in zero order release model. For formulation Gel B the R^2 values for zero order plot is 0.978, first order plot is 0.939, Higuchi model plot is 0.897 and Korsmeyer model plot is 0.891. The best linearity was found in zero order release model. From the kinetic study results shown in Table **5** it was observed that gel B was having a higher R^2 value as compared to gel A indicating release of the drug from gel B was more linear.

Table 4: Antimicrobial Activity of Different Gel Formulations

Microorganism		Mean Zone of Inhibition ± SD	
	Gel A	Gel B	Gel M
Staphylococus aureus	1.13 ± 0.08	1.15 ± 0.10	1.13 ± 0.14
Bacillus subtilis	1.09 ± 0.01	1.11 ± 0.04	1.14 ± 0.01
Escherichia heranii	1.01 ± 0.13	1.03 ± 0.10	1.03 ± 0.14
Pseudomonas aeruginosa	0.91 ± 0.07	0.93 ± 0.08	0.92 ± 0.09

Table 5: Release Parameters of Different Gel Formulations

		Zero Order Model	First Order Model	Higuchi Model	Krosemeyer Model
Gel A	R^2	0.976	0.962	0.883	0.821
	m	0.23	0.002	4.96	0.863
	c	5.98	2.40	24.90	-0.46
Gel B	R^2	0.978	0.939	0.897	0.891
	m	0.254	-0.003	5.63	0.853
	c	-4.69	2.48	26.49	-0.34

CONCLUSION

The two formulations of silver nanoparticle incorporated gels were tested for different properties. The characterization of gel had shown that pH of the formulation was approaching towards neutrality. The viscosity, spreadability and extrudability of the prepared formulations were quite closer to the marketed product. The gel formulation B prepared by polymer chitosan was observed to be a better carrier for silver nanoparticles than formulation A prepared by carbapol. Adhesiveness, stability and release of incorporated drugs are the main features that influence the applicability of gels for topical treatment, including the wound healing process. The formulated gel B can be used as an advanced drug delivery system because of its sustained release profile, water soluble nature, physical stability and good spreadability and in-vitro antimicrobial property.

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