RESEARCH ARTICLE

Development of AuNPs-Based Gel: Physicochemical Properties, Antibacterial Activity, and Skin Permeation Studies

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ABSTRACT
Gold nanoparticles (AuNPs) have raise as versatile agents in biosciences applications due to their exclusive physicochemical nature. principle of this investigation is to formulate and test a gel containing AuNPs that uses Carbopol 934 as its gelling agent. To optimise the medicinal and rheological qualities, gels were made using different concentrations of carbopol (0.5%, 1.0%, 1.5%, and 2.0%), along with various excipients such as triethanolamine and black cumin oil. The physicochemical properties of the gels were assessed, including their homogeneity, transparency, pH, spreadability, viscosity, medication concentration, and ability to gel. Effective drug delivery was demonstrated in ex vivo skin permeation and deposition tests utilising rat abdomen skin. In comparison to commercial formulations, the AuNPs gel demonstrated substantial zones of inhibition when tested against methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (PA), and *Staphylococcus aureus* (SA),. The formulations were found to be safe in skin irritancy testing. The outcomes of this study display that AuNPs gels have great promise as a safe and effective vehicle for transdermal drug administration with improved therapeutic efficacy and less discomfort.
Keywords: Gold nanoparticles (AuNPs), transdermal drug delivery, antibacterial activity, gel formulation.
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INTRODUCTION

In current years, gold nanoparticles (AuNPs) have garnered a lot of consideration due to their exceptional physicochemical features. Among these characteristics include biocompatibility, a high surface-to-volume ratio, and effortlessness of fictionalization.[1] These characteristics establish them as suitable candidates for extensivevariability of biomedical applications. A promising method for regulated drug administration, increased therapeutic efficacy, and localized treatment is the integration of AuNPs into gel formulations.[2] This is one of the applications that have emerged from these applications. A number of benefits, including ease of application, extended drug release, and increased penetration, are offered by gel-based delivery systems. These systems are especially beneficial for transdermal and topical drug delivery because of their various advantages.[3]To prepare the AuNPs gel, the synthesised gold nanoparticles were dispersed into the gel matrix with great care, so that they would be evenly distributed and retain all of their desirable characteristics. The formulations were then thoroughly tested for several physicochemical properties in order to determine their appropriateness for topical use.[4]

Wound healing, antimicrobial therapies, and cosmetic formulations are just a few of the many possible uses for the findings of this study, which seek to shed light on the manufacture of AuNPs-loaded gel systems and their potential for improving the delivery of therapeutic ingredients.[5] Improving the effectiveness and patient compliance of transdermal medication delivery systems can be achieved by the optimisation of these gels. The purpose of this research is to offer significant visions into development of AuNPs-loaded gel systems and their potential for increasing the delivery of therapeutic agents. These systems have a wide range of applications, including wound healing, antibacterial therapies, and cosmetic formulations. Transdermal drug delivery systems can be improved in terms of both their effectiveness and their patient compliance through the utilisation of a novel strategy that involves the optimisation of these gels.

MATERIALS AND METHODS

Materials

Selection and Authentication of Plant

Fresh *Brassica oleracea* (Cauliflower) leaves were handpicked from local fields, washed meticulously to remove dust and debris, and sun-dried thoroughly to eliminate moisture. A 1% plant extract solution was made by combining the finely powdered dried leaves with deionised water. Centrifuged at 5,000 rpm/30 minutes at room temperature subsequent to 30 minutes of incubation.

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Table 1: Formulation batches AuNPs gel

Ingredients	Batches (%w/w)			
	F1	F2	F3	F4
Methyl Paraben	0.2	0.2	0.2	0.2
Triethanolamine	1.0	1.0	1.0	1.0
Black cumin Oil	0.5	0.5	1	1
Carbapol 940	0.5	1	0.5	1.5
Ethanol	10	10	10	10
Guar Gum	1	0.7	0.5	0.7
Cauliflowerextract Nanoparticle	1	1	1	1
Distilled Water	Q.S.	Q.S.	Q.S.	Q.S.

Table 2: properties of AuNPs gel

Property	Result
Odour	Characteristics
Colour	Whitish
Melting Point	-50c
Appearance	Gel
Presence of solution in 95% ethanol	Clear

AuNPs were synthesized from the supernatant, which was filtered by vacuum filtration after centrifugation.[6]

Preparation of Plant Extract

Cauliflower leaves were disemboweled and left to air-dry for 2-3 days before being sliced into small segments. Ten grams of the dried, crushed material were immersed in 100 mL of ethanol and incubated for 24 hours at ambient temperature. After filtering it through Whatman No. 1 filter paper, extract was set aside at 4°C for use in subsequent investigations. A Soxhlet extraction was utilized, reducing the solvent consumption while maximizing efficiency. In this apparatus, ethanol was continuously refluxed, dissolving the organic matter from the plant, which was collected in a flask. The solvent was later recovered via distillation, leaving the concentrated organic extract behind.[7]

Synthesis of Gold Nanoparticles (AuNPs)

As per our previous work we synthesize AuNPs, a chloroauric acid ($HAuCl_4$) solution was prepared alongside the plant extract. Twenty grams of chopped cauliflower leaves were boiled in 200 mL of distilled water for 30 minutes, followed by filtered. In parallel, a 1 mM$HAuCl_4$ solution was diluted from a stock solution. The plant extract (10 mL) was slowly added to 100 mL of $HAuCl_4$ solution with continuous stirring, resulting in a visible color change, indicating the reduction of Au^+ ions. The reaction was permitted to stir at room temperature for 30 to 60 minutes.[8]

Preparation of AuNPs Gel

Carbopol 934 was used as a gelling agent and synthesised AuNPs were integrated into the gel. Carbopol in varying concentration of 0.5, 1, 1.5 and 2 % was used for formation of gel. A small amount of triethanolamine was added after weighing the carbopol and swirling it carefully into the AuNPs colloidal solution (Table 1). The physicochemical properties of the AuNPs Gel formulations were evaluated, including clarity by visual inspection and pH by means of a pre-calibrated pH meter. Using a Brookfield viscometer at 20 ± 1°C, the rheological quality of the AuNPs gel that was formed was assessed.[9]

Evaluation of AuNPs Gel

Appearance

The transparency, hue, and presence of particles in the AuNPs gel bases were evaluated visually.

Homogeneity

After the AuNPs gels were set in the containers, they were visually inspected to ensure uniformity. Their visual quality and the existence of aggregates were evaluated.

pH of Nanogel

A digital pH meter was use to monitor pH of a solution that contained two grammes of AuNPs gel dissolved in one hundred millilitres of phosphate buffer.

Determination of Spreadability

The 1.5 g sample of AuNPs gel was compressed to a consistent thickness by sandwiching it among two glass slides and then placing a 1000 g weight on top of slides for 5 minutes (Figure 1). Fifty grammes was added to pan. A measure of spreadability was time, in seconds, needed to separate two slides.[11] We used the following formula to determine it,

$S = m \times {}^{l}/_{t}$ …(1)

Table 4: Gelling time, swelling index, water vapour transmission rate of AuNPs gel formulations

Formulations	Gelling time (sec)	Swelling index (%)	WVTR (g/m2/h)
SLH1	43±1.53	18.02±0.07	7.5±0.10
SLH2	35±1.00	16.84±0.1 1	7.3±0.06
SLH3	39±1.53	17.55±0.33	7.4±0.06
SLH4	32±2.52	15.72±0.23	7.4±0.10
SLH5	27±1.53	16.44±0.12	7.3±0.10
SLH6	35±0.58	17.43±0.24	7.3±0.07
SLH7	31±1.53	17.75±0.10	7.5±0.10
SLH8	39±1.53	16.99±0.12	7.4±0.06
SLH9	27±0.58	17.75±0.1 1	7.4±0.10

Table 3: Viscosity, spredability, pH and drug content of AuNPs gel formulations

Formulations	Viscosity* (cps)	Spredability* (g.cm/sec)	pH*	Drug content* (%)
MLH1	37762.95±1.01	18.02±0.07	7.5±0.10	98.00±0.33
MLH2	41704.84±2.93	16.84±0.1 1	7.3±0.06	97.97±0.17
MLH3	39755.73±2.31	17.55±0.33	7.4±0.06	97.84±0.54
MLH4	42687.55±1.53	15.72±0.23	7.4±0.10	97.87±0.53
MLH5	42187.02±1.19	16.44±0.12	7.3±0.10	98.07±0.70
MLH6	40255.31±1.92	17.43±0.24	7.3±0.07	98.51±0.70
MLH7	38806.32±1.06	17.75±0.10	7.5±0.10	97.89±0.81
MLH8	40642.46±0.63	16.99±0.12	7.4±0.06	96.77±0.83
MLH9	38192.60±1.07	17.75±0.1 1	7.4±0.10	97.25±0.98

* Standard deviation, n=3

Where, S= Spreadability, m= weight tied to upper slide in gram, 1= length of glass slide t= time.

Drug Content

A 100 ml solution of phosphate (pH 7.4) buffer was prepared by dissolving 1 g of AuNPs gel, which is equivalent to 1% of the medication, in the solvent. We diluted a 5-milliliter sample of this solution to a volume of 25 millilitres. Next, we used a UV-visible spectrophotometer to measure the absorbance at 272 nm, which allowed us to determine the gingerol concentration. The linearity equation, Y= mx + C of pure drug, was used to determine the drug content by obtaining the intercept and slope.

Viscosity

Using a Brookfield viscosity meter, we tested and recorded the prepared AuNPs gel's viscosity at various RPMs. All speeds between 50 and 200 rpm were measured, with a 10-second interval between each setting.[12]

Ex-vivo Skin Permeation with Skin Deposition

The Institutional Animal Ethical Committee of College of Pharmacy, Pune, Maharashtra, India, gave its approval to the methods used for ex-vivo skin permeation with skin deposition research, which followed the OECD guideline notes on dermal absorption. Institutional Animal Ethics Committee (IAEC) approved the study's procedures (1197/PO/Re/S/08/CCSEA) and the care with which the animals were tested. The *in vivo* behaviour of the molecule can be learnt from the ex vivo permeation investigation.

The quantity of medicine absorbed into the blood is determined by the amount of drug that permeates. The experiment was conducted in a 22.5 ml and 2.26 cm^2 Franz diffusion cell using skin from the abdomen of rats. After the rats' skin was removed, it was rinsed with isotonic sodium chloride. The skin had to be positioned such that the donor medium touched the dermal side and SC side pointed aloft into donor compartment before procedure could proceed. The procedure was carried out with the skin placed between two donors. The next step was to apply a dialysed AuNPs gel comparable covering to the rats' skin in the donor compartment. We filled and continuously agitated receptor compartment with a Phosphate buffer (pH 7.4) that contained 30% PEG 400 (V/V). In order to replicate the skin temperature, it was maintained at 320.5°C in receptor compartment. At specific intervals [1, 2, 3, 5, 7, 9, 12 hours], portions of receptor compartment were taken out

Table 5: Drug content, Spredability, pH, Viscosity

Formulation	Spredability g.cm/sec	Drug Content	pH	Viscosity
F1	15.85±1.56	76.12±0.012	6.91	1228
F2	12.65±2.42	82.30±0.023	7.20	1831
F3	14.25±1.68	91.21±0.034	7.10	2258
F4	11.25±1.56	83.96±0.049	7.5	1069

and replaced with fresh medium that had been equilibrated at 320.5°C. This process was repeated until the sink condition was maintained. The samples were then examined using UV spectroscopy at 250nm.[13]

Antibacterial Activity of AgNPs Gel

Antibacterial activity of the AuNPs gel and the commercially available gel formulation was tested against PA (ATCC 27853), SA (ATCC 25923), and MRSA using an agar well diffusion assay, also known as antibiotic susceptibility analysis. Plates were sterilised using Mueller-Hinton agar and left to incubate at 37°C in a bacterial incubator for night before experiments were conducted to ensure that the plates were free of contamination. A homogeneous lawn of bacteria was created by seeding 100 mcl of test bacterial suspensions onto 15-minute-dry agar plates. Using the 0.5 McFarland standard, the bacterial concentration was adjusted to 1.5 X 108 CFU/mL.Wells were filled with a commercially available gel comprising 0.2% silver nitrate, 0.03% AuNPs colloidal dispersion, and 0.03% AuNPs gel. The mixture was afterward incubated at 37°C for 24 hours. Following all experiments were repeated three times, the data were displayed as the average zone of inhibition (mm) with ± standard deviation.[14]

In vitro Permeation Studies

Procurement of Rat Skin Membrane

All animal-related procedures and studies adhered to the ARRIVE Guidelines for Care with Use of Experimental Animals, as mandated through EU Directive 2010/63/EU. This study was permitted by institutional animal ethics committee (IAEC/10/19). We chose male Wistar albino rats from the shelter; they weighed 300-350 gm. Each instrument was dried and then autoclaved for 30 minutes at 121°C with 15 psi pressure before use. We weighed the rats, euthanised them with a high dosage of thiopental solution, and then dislocated their cervical spines to kill them. Then, to get rid of any hair on the back, I placed them

Figure 1: Spredability

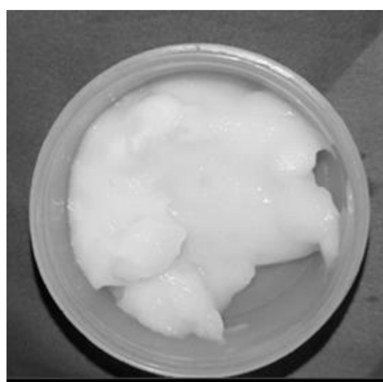

Figure 2: AuNPs gel

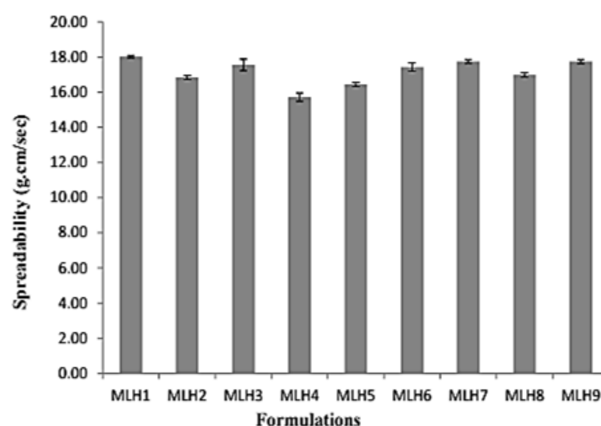

Figure 3: Spredability AuNPs gel formulations

Table 6: Antifungal activity of AuNPs gel

Test Fungi	Formulation and its Concentration			
	Control	AuNPs Nanogels (F3)		
		0.5mg	1.0mg	2.0mg
Aspergillus niger	17.5	10.5	13.5	17.5
Cladosporium species	18	10.0	14.0	18.0
Colletotricum crassipes	17	10.5	14.5	17.5
Candida albicans	19	11.0	15.0	18.5

on a dry cotton cloth and shaved them. After the rats were sterilised, the instruments were removed and the area to be sliced was marked on the previously shaved skin. Make clean incisions in the marked skin using a sterile scalpel. Only the skin, including the epidermal, layers were sliced; caution was exercised to avoid the muscle areas. After trimming the fat, wash the area by distilled water to eradicate bacteria, and then bury rats.

Method for In vitro Permeation Study

Using an in-house modified Franz diffusion cell, the resulting skin membrane was used to conduct in vitro diffusion tests using hydrogels loaded with nanoparticles. In diffusion cells, the epidermal membrane separates donor and receptor compartments. Membrane was fastened to one side of the donor compartment, a cylindrical glass tube, using a rubber band. This was done after coating it with 1 gramme of gel. The 50 mL of phosphate buffer contained in the receptor compartment hardly touched the membrane's surface once this cylinder was secured. A steady

Table 7: Groups of animal studies

Groups	Number of Animals	Description
Group 1	n = 3	Control group by injury excluding not infected with *C. albicans*
Group 2	n = 3	Rats by injury and infected through fungus but without of treatment
Group 3	n = 3	Rats by injury, infected by *C. albicans* and treated by AuNPs Nanogels cream as reference
Group 4	n = 3	Rats by injury, infected by *C. albicans* and treated by AuNPs Nanogels

temperature of 37 ± 0.5 °C was used to lock the entire unit into place. The beaker containing the receptor was kept agitated at 250 rpm using a magnetic stirrer for a period of 10 hours. To keep the sink conditions consistent, 5 mL of sample was detached from receptor compartment at regular intervals and replaced by a volume of phosphate buffer. By a UV-Vis spectrophotometer set at 299 nm, sample absorbances were measured spectrophotometrically. Using this information, we were able to determine the cumulative amount of medication that permeated the rats' skin.

Skin Irritancy Test

After obtaining approval from the institutional animal ethics council (IAEC), researchers conducted skin irritation tests on Wistar albino rats using established protocols. A 4

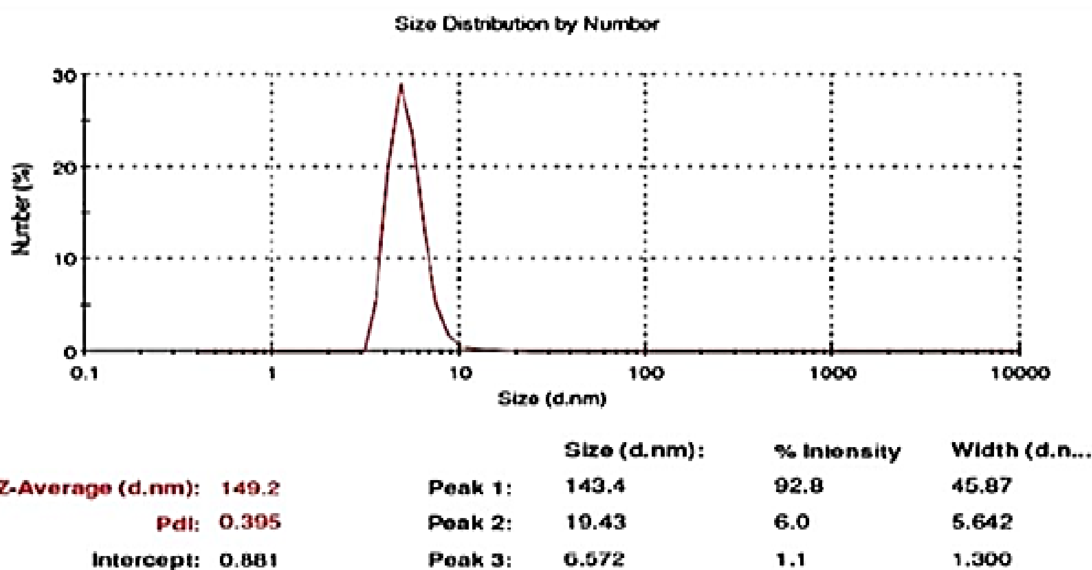

Figure 4: UV Spectra of Cauliflower extract

Table 8: Result of Skin Irritation test

Groups	Description	Wound Area (length by width) in cm2					
		0 Day	3 Days	6 Days	9 Days	12 Days	15 Days
Group 4	Rats by injury, infected by *C. albicans* + AuNPsNanogels	1.57 ±0.53	3.96 ±0.17	3.13 ±0.32	2.35 ±0.29	1.72 ±0.12	1.10 ±0.31
Group 3	Rats by injury, infected by *C. albicans* + fluconazole cream	1.71 ±0.42	2.35 ±0.24	1.92 ±0.15	1.46 ±0.18	0.78 ±0.12	0.35 ±0.26
Group 2	Rats by injury and infected by t fungus but without treatment	1.55 ±0.38	3.54 ±0.35	4.53 ±0.35	7.82 ±0.36	8.21 ±0.31	9.12 ±0.28
Group 1	Control group by injury	1.61 ±0.12	3.37 ±0.21	4.18 ±0.23	6.76 ±0.31	8.65 ±0.26	9.65 ±0.16

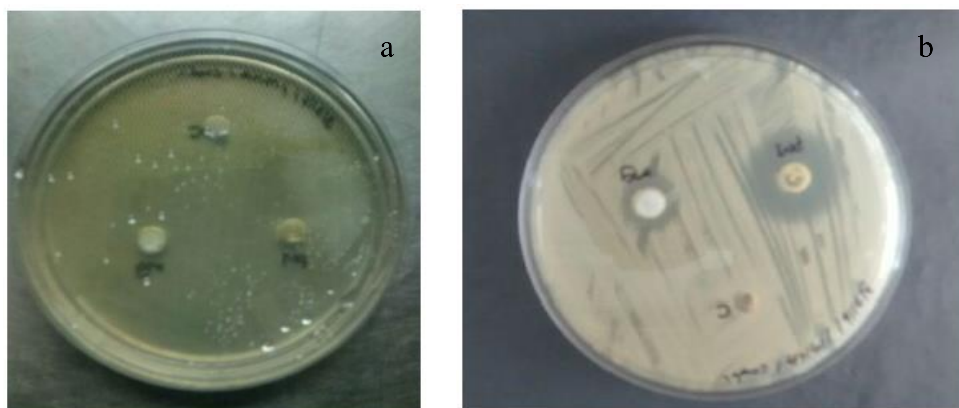

Figure 5: (a) After inoculation, 0 min growth.(b)After 48 hrs. of inoculation of AuNPsNanogels

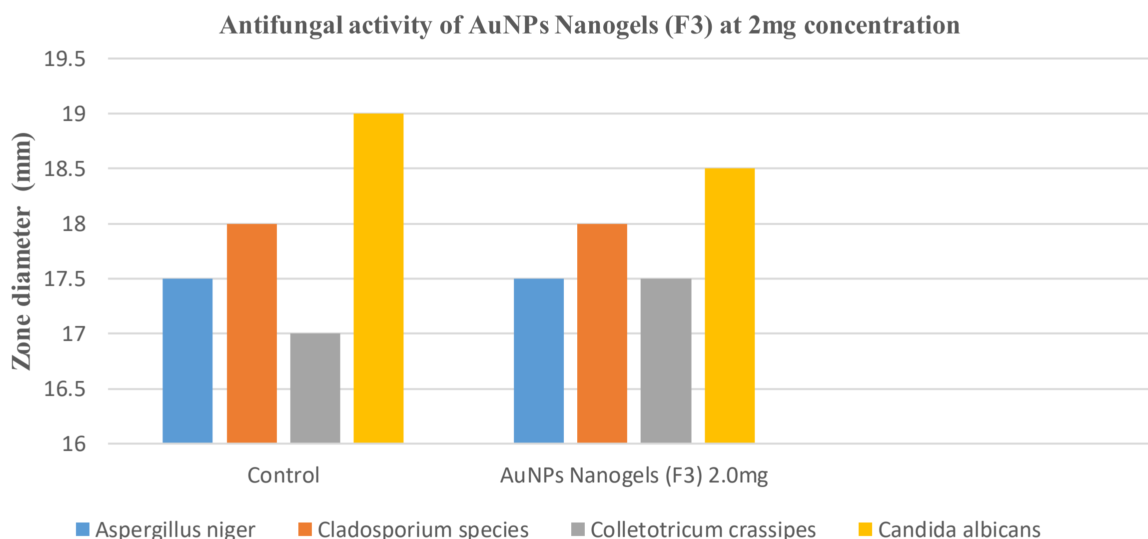

Figure 6: Antifungal activity

cm2 area was marked on dorsal side of each rat using a sterile razor and hair trimmer. The study was carried out one day following the last shave. At random, three groups of three rats were assigned to the experiment. Split into three groups: I, the control, II, and III. Each group's formulation was either optimised or commercialised. The specified region was covered with a patch after a test sample (0.5 gm) was applied to it. After 4 hours, the patch was taken off and examined for redness and swelling; subsequent assessments were made at 1, 24, 48, and 72 hours.

Group I- 20% w/v SLS solution (area A), Untreated (area B);

Group II- AuNPs gel;

It took 500mg of AuNPs gel to be administered to the designated region (A) in each group of participants. We utilised non-irritating tape (Transpore 3M surgical tape, 3M India Ltd, India) to adhere a gauze patch to the treated area. The experiment's control group was each group's B area. The experiment began with the use of a single animal. Using pure water, any leftover formulation on the skin was delicately washed off at end of 24-hour exposure time,

Table 9: Kinetic parameters of release data of AuNPs gel

Formula	r^2			Mechanism	Korsemeyer-Peppas Model		
	Zero order	First order	Higuchi		r^2	n	Mechanism
F10	0.7760	0.9412	0.9643	Diffusion	0.9765	0.579	Anomalous
F9	0.7910	0.9388	0.9489	Diffusion	0.9657	0.595	Anomalous
F8	0.3404	0.7863	0.9620	Diffusion	0.9860	0.415	Fickian
F7	0.3948	0.8719	0.9638	Diffusion	0.9798	0.429	Fickian
F6	0.4323	0.8957	0.9652	Diffusion	0.9751	0.438	Fickian
F5	0.7831	0.9401	0.9602	Diffusion	0.9742	0.585	Anomalous
F4	0.7729	0.9495	0.9674	Diffusion	0.9787	0.576	Anomalous
F3	0.5983	0.9069	0.9888	Diffusion	0.9892	0.487	Anomalous
F2	0.1883	0.8307	0.9163	Diffusion	0.9637	0.386	Fickian
F1	0.4432	0.8976	0.9664	Diffusion	0.9768	0.441	Fickian

ensuring that neither the skin's present reaction nor its epidermal integrity were altered. At1,4,48, and 72 hours, participants' skin reactions (erythema and oedema) were rated on a scale from 0 to 4.

For Erythema and Escharformation

I. Score 0: no erythema; II. Score 1: very little erythema that is hardly noticeable; III. Score 2: well-defined erythema; IV. Score 3: moderate to severe erythema; V. Score 4: extreme erythema (beef redness) to eschar formation that stops erythema from being graded.

For Edema Formation

Score0: there is no oedema; Score 1: very little oedema that is hardly noticeable; Score 2: slight oedema that is strongly defined by distinct rising in the area; Score 4: severe oedema (increased further than 1 mm and expanding outside part of exposure); Score 3: moderate oedema (raised about 1 mm) .[15]

RESULTS AND DISCUSSION

Evaluation of AuNPs Gel

Table 2 shows the results of the experiments used to authenticate the AuNPs gel (Figure 2), which included a number of criteria including organoleptic qualities, melting point, and the look of the solution.

Measurement of pH

We used a digital pH meter to find pH.dissolved in 25 millilitres of water, and weighed approximately 2.5 grammes of AuNPs gel. To make them more suitable for use on wounds without causing irritation, the pH of all hydrogel formulations ranged from 7.3±0.06 to 7.5±0.10.

Clarity Examination

All of the created formulations were visually examined against a black and white backdrop to determine their clarity, outcomes were then classified using following symbols: turbid (+), clear (++), and transparent/glassy (+++).

Spreadability

All the prepared AuNPs gel formulations using different ratios of oxidized alginate and gelatin were spreadable on the wound surface. The results of the spreadability varied from 15.72±0.23 - 18.02±0.07 g.cm/sec. The AuNPs gel were semi-stiff gels in their consistency. The results indicated that the spreadability decreased with the increase in the oxidized alginate and gelatin (Figure 3).

Figure 7: Skin Irritation test

Homogeneity

The visual evaluation of all AuNPs gel formulations was used for this testing. In this test, we poured gel into a see-through container and, once it had settled, looked for signs of air entrapment, lumps, or particles in the formulation.

Occlusion Factor

As a proxy for skin, we measured the occlusion factor by measuring the water flux through filter paper. The outcomes can be found down below. The results show that after 48 hours, the hydrogel based on nanosponges significantly prevents water loss compared to the commercially available Atonide gel.

Grittiness

The term "gelling capacity" refers to the ability to quantify the rate and volume of gelation. The formulations that have gone through the sol-gel transition are appropriate. For an extended length of time, the gel that has been created should not dissolve or erode. The outcomes for the Atonide gel and the hydrogel with the optimisedformulation are displayed below.

Extrudability

The Pfizer hardness tester was used for this assessment. Roughly fifteen grammes of gel was loaded into an aluminium tube. Next, the hardness tester's plunger was fine-tuned to securely grasp the aluminium tube. Next, a pressure of 1 kg/cm2 was applied for a duration of 30 seconds. As a result of the pressure, we measured the amount of gel that was extruded and repeated the process three times on opposite sides of the tube.

Drug Content

The drug content was insistent on to ascertain that AuNPs gel is uniformly loaded in formulation. The drug content ranged between 96.77±0.83% - 98.51±0.70%. Outcome obtained are revealed in Table 3.

Gelling Capacity

The duration required for the gel to solidify ranged from 27±0.58 to 43±1.53 seconds. Reduced gelling time was seen with an increase in oxidised alginate and gelatin concentration. Gelation is caused by an interaction between the amino groups of gelatin and the aldehyde groups of oxidised alginate, which is known as a Schiff-base interaction. The alginate molecular chain can undergo chemical crosslinking with amino functions via Schiff-base linkage when reactive aldehyde functions are generated during periodate oxidation. This process cleaves the carbon-carbon bonds of the cis-diol groups (Table 4).

UV Method

Research into the ultraviolet (UV) spectra of cauliflower extract revealed that the wavelength at which it was most absorbent was 281.8 nm (Figure 4). The λmax for the cauliflower extract was therefore determined using the absorbance at 281.8 nm. The comparison of the drug's infrared spectrum with the published spectrum of gingerol established the drug's identity. Within concentration range of 20-100 µg/ml, extract complies by Beer-Lambert's law, as indicated by regression coefficient (r2) values of 0.9995. Regression was $y = 0.0097x + 0.0132$.

Particle Size

The zeta sizer in Optimal Batch F3 was used to estimate particle size. Measured particle size was 149.2 nm. The graph clearly shows that when the concentration of excipient increases, particle size increases from 100 to 250 nm. However, after a certain concentration, the drug-to-excipient ratio causes particle size to drop. Reason behind this was the little amount of excipients available and the high drug to excipients ratio. Therefore, it was determined that the ratio of drug excipient concentration determines particle size. Batch F3, after optimization, had an average particle size of 149.2 nm.

The range of drug content in all the AuNPs gels that were created was between 76.12 and 91.21. Analysis of the F3 batch revealed a maximum drug level of 91.21%. All formulations had pH values between 6.91 and 7.20, and the drug content was discovered in following order: F3, F4, F2, and F1. The pH values were within an acceptable range and were rather near to the skin's pH since black cumin oil contains the alkaline benzoquinone ring (Table 5).

All of the formulations fell somewhere in the middle of the spreadability spectrum, with F4 having the lowest at 11.25 g.cm/sec. and F1 having the most at 15.85 g.cm/sec. The viscous nature of carbopol 940 may explain why its spreadability grew as its concentration did. Formulation F3, F4, and F1 all achieved respectable spreadability results; of the three batches, F3 had the best spreadability, slightly surpassing F2 but falling short of F1. The spreadability value of Formulation F3 is superior, and it took less time to separate the glass slides, thus the two are balanced.

Various RPMs, including 60, 100, 150, and 200, are used to measure the gel viscosity of AuNPs for each formulation. Constant viscosity is achieved at a torque setting of 95%. The F3 formulation batch outperforms the others in terms of viscosity. The formulation's viscosity was significantly affected by the Carbapol 940 concentration. The F3 formulation has a viscosity of 2258 rpm.

In-vitro Anti-Microbial Bioassay

The antifungal activity of the AuNPs gel was tested using an agar cup bioassay (Figure 5). The pre-made PDA medium (HiMedia, 39 g) was autoclaved for 20 minutes at a pressure of 15 lb./sq. in after being suspended in distilled water. The medium was inoculated with 0.5 ml of test organism cultures that were seven days old. Following inoculation, sterile cork borers measuring 8 mm were used to scoop cups out of Petri plates. Three different test solution concentrations—0.5, 1.0, and 2.0 mg—were added to each cup. The DMSO was utilised as a control, and 5 µg of Bavistin was employed as a standard. In an incubator set at 26º over a period of 24 to 78 hours, both the treatment and control groups were observed and their inhibition zones were recorded. Every treatment was accompanied by three to four independent trials. What follows is a table 6 detailing the effects of 0.5, 1.0, and 2.0 concentrations of AuNPs gel on four distinct fungi: *Aspergillus niger* (MTCC 281), Cladosporium species (MTCC 1003),*Colletotricum crassipes* (MTCC 2223) and *Candida albicans* (MTCC 183). Data was not included because, although antifungal activity was seen at all tested concentrations, it was less effective below the 1.0 mg concentration of AuNPs gel. Perhaps the AuNPs Nanogels in the test formulations are to blame for the reduced activity of the AuNPs gel at lower doses. The inhibitory zones' diameters varied from 10 to 20 mm across fungal species and doses, rising as the concentration of AuNPs gel did. A concentration of 2.0 mg of AuNPs gel was found to exhibit the highest antifungal activity (Figure 6).

In vitro Permeation Studies

All animal testing adhered to OECD and other globally acknowledged protocols for the humane treatment of test subjects. Eighteen mature Wistar rats, including both males and females, weighing an average of 220 ± 30 g, were used in the investigation. Their backs were shaved by hand after anaesthesia. Each rat had its back shaved, and then a 1.5 cm2 patch of hot copper metal was used to inflict an injury. Following injury induction, 0.5 mL of broth containing 108 Colony-Forming Units (CFU) of Candida albicans MHMR was swabbed onto each animal to inoculate it. On third day, animals were erratically assigned to six groups based on their weight. From then on, they were given various treatments. Here are the groups:

After applying sterile gauze to the rats, we secured all of the wounds with adhesive plaster. Day after day, the dressings were changed. Rat weights were recorded for each group on days 0, 3, 6, 9, 12, and 15 (Table 7), and the wound area was measured using the rule approach (length by breadth) so that we could compare the treated and untreated groups and see how the wound surface evolved.

Skin Irritation Test

After the Draize patch test, there were no erythema or edema in rats at the end of one hour and 24 hours on intact skin due to the formulations. All Primary Irritation Index (PII) scores reported at one hour, 24 hours were found to be 0.00 after the spray was applied for 24 hours. This suggested that the formulations could be said to be non-irritating. The irritation experiments showed no irritation to the skin from the topical sprays (Figure 7). There were neither any significant changes in the skin nor any liaisons or any break in the length of epithelium (Table 8).

In-Vitro Drug Release Kinetics

Kinetic models were fitted using the data acquired from the *In vitro* release experiment. Finding out how the drug was released from the AuNPs gel was the main goal of this investigation. The in vitro release data was analysed using a simplified Higuchi model in accordance with the zero-order, first-order, and diffusion-controlled mechanisms. Thanks to this, we were able to figure out the release model. For these factors, the release order was determined by favouring the mechanism with the highest coefficient of determination (r2). Table 9 displays the kinetic characteristics of the AuNPs gel. It was determined that the Higuchi model was the best fit because its r2 value was greater. A plot of % cumulative drug release against square root of time supported this, with a r2 value ranging from 0.8477 to 0.9888. This finding is consistent, but the Fickian equation doesn't always describe the mechanism of drug diffusion; in fact, it often exhibits non-Fickian (anomalous) behaviour in experiments. For these instances, the release kinetics were examined using the Korsemeyer-Peppas model. In accordance with Fick's law of diffusion,

formulations F1, F2, F6, F7, and F8 were observed, while formulation remainder exhibited unusual behaviour.

Stability Study

A 45-day stability study is conducted on the optimised formulation. The sample is kept in the conditions specified by the ICH guidelines. Data from the assessment test are compared to results from the test immediately preceding the stability study after the sample has been exposed to the stability study (Table 10).

CONCLUSION

The gels that were loaded with AuNPs were prepared to have outstanding physicochemical features, such as clarity, homogeneity, appropriate pH, and spreadability. These properties made the gels acceptable for topical application. Ex vivo skin penetration experiments demonstrated that the medication was successfully delivered, and there was a large amount of deposits in the layers of the skin. It is clear that the formulation has the potential to be used in therapeutic settings because it possesses antibacterial efficacy against both common and resistant bacterial strains. The gels were found to be non-irritating and biocompatible, as demonstrated by the results of safety testing that included skin irritancy tests and histological examinations. The pharmacokinetic investigation demonstrated that the medicine was released in a sustained manner, which provided additional evidence that the formulation was effective. Overall, the findings of this work demonstrate that AuNPs-loaded gels have the potential to serve as a promising platform for transdermal drug administration. This platform has the potential to offer improved therapeutic outcomes and a wide range of applications in the sectors of manufacturing and cosmetics. It is possible that in subsequent research, the incorporation of additional medicinal drugs and clinical investigations could be investigated in order to better validate these findings.

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