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International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Development and Evaluation of Phycocyanin-Infused Hydrogel Topical Formulations for Wound Healing

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ARTICLE INFO

Article history:

Received: 24 April, 2024

Revised: 11 May, 2024

Accepted: 15 May, 2024

Published: 30 May, 2024

Keywords:

C-phycocyanin, Hydrogel, Wound healing, Drug delivery, Biocompatibility

DOI:

10.25004/IJPSDR.2024.160321

ABSTRACT

The healing of wounds is a complicated biological process impaired by several factors, and conventional treatments often result in undesirable side effects. Natural compounds have emerged as favorable alternatives due to their reduced side effects. C-phycocyanin (C-Pc), a natural phycobiliprotein derived from *Spirulina platensis*, shows promise in wound healing but is hindered by poor stability and low bioavailability. This study aimed to develop a hydrogel-based delivery system for C-Pc to enhance its stability and therapeutic efficacy in wound healing. We synthesized a grafted gum hydrogel to encapsulate C-Pc, ensuring its sustained release. The hydrogel's physical properties, including clarity, pH, spreadability, and rheological behavior, were characterized. The encapsulation efficiency, *in-vitro* release profile, antioxidant activity, and adhesion were assessed. Furthermore, the hydrogel's impact on wound healing was evaluated through *in-vivo* studies and assessments of skin irritation potential. The optimized hydrogel demonstrated excellent physical stability, appropriate viscosity, and significant bioadhesive properties, making it suitable for topical application. The encapsulated C-Pc exhibited a controlled release, enhanced antioxidant activity, and greater wound-healing efficacy than free C-Pc. *In-vivo* studies confirmed accelerated wound closure with no irritation or allergy, suggesting high biocompatibility and therapeutic potential. Developing a C-Pc encapsulated hydrogel presents a promising approach to improving wound care. This innovative approach not only stabilizes C-Pc but also enhances its healing properties, providing a safe and effective option for patients. This study paves the way for a novel formulation with translatory potential.

Introduction

A wound is characterized by tissue structure and cellular connection breakdown due to various forms of injury, including physical, chemical, thermal, infections, or immune responses. The healing of wounds involves a coordinated series of cellular and biochemical reactions aimed at restoring both the structure and function of the injured tissue.[1,2]

Several treatment choices, such as antibiotics, painkillers, and nonsteroidal anti-inflammatory drugs, are accessible for wound care, but most of these treatments come with undesirable side effects. Consequently, researchers have shifted their focus to natural compounds due to their fewer side effects. Many drugs of varying origins demonstrated significant efficacy in wound care.[3]

C-phycocyanin (C-PC) is a water-soluble phycobiliprotein that naturally occurs in *Spirulina platensis* and is biocompatible. It has garnered significant attention for its safe and non-toxic nature. Recent studies have demonstrated various properties, including antiplatelet, wound healing properties, anti-inflammation, oxidation inhibitor, hepatoprotective, anticancer, and ability to enhance immunity. However, its therapeutic application is hindered by its short plasma half-life and instability, requiring frequent doses and leading to low patient compliance.[3-5]

Hydrogels are intricate networks of hydrophilic polymers, able to retain significant amounts of biological fluids or water without dissolution. They present a promising option for various biomedical uses due to their ability

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

to interact well with biological systems, facilitate the transport of nutrients and metabolites, and mimic the native extracellular matrix (ECM).[6, 7]

In this study, we aim to develop suitable delivery systems to encapsulate C-Pc and improve its stability. We synthesized grafted gum as a potential carrier for C-Pc. This encapsulation ensures sustained release of C-Pc, potentially enhancing its protective effects and bioactivity. The encapsulation efficiency was evaluated through in vitro release studies, and in vivo research demonstrated that C-Pc-encapsulated hydrogel significantly attenuates tissue injury and improves islet functionality compared to free phycocyanin.

Materials And Methods

Materials

C-Phycocyanin (C-PC), was obtained from TCI Chemicals, Chennai, India. Locust bean gum (LBG) was provided by HiMedia Laboratories, located in Mumbai, India. Acetone and methanol were acquired from Finar Limited, Ahmedabad, India. Reagents and solvents of analytical quality were obtained from local suppliers for use in the experiment.

Animals

The study involved using male Wistar rats weighing 160 to 200 g. Before conducting the wound healing activity, the rats were accustomed to accepted laboratory settings of temperature and humidity for a week. All animals had unrestricted access to pelleted food and water throughout the study. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Methods

Preparation of hydrogel

The graft copolymer and locust bean gum (LBG) were synthesized using a method of microwave-assisted, free radical-induced polymerization. Initially, acrylamide and acrylic acid were dissolved in water and combined with an aqueous dispersion of LBG. This mixture was stirred for an hour and then exposed to microwave irradiation in alternating heating and cooling cycles for varied durations. After irradiation, the mixture was left at room temperature overnight and precipitated using acetone. Unreacted monomers were removed with methanol, and further purification was done using a 30% methanol solution to eliminate residual homopolymers and impurities. The final product, the grafted gum, was then dried at 40°C under vacuum until it attained a stable weight and was processed into fine particles.[8,9] C-Pc was incorporated into the graft polymer at a 1% wt concentration to prepare drug-loaded hydrogel. The polymer was dissolved in distilled water under continuous agitation and left overnight for complete dissolution. The solution was then manually stirred to ensure homogeneity before introducing the C-Pc solution. Vigorous stirring at 25 ± 1°C for one hour ensured thorough integration and mixing of drug and polymer.[10] The resultant C-Pc-loaded hydrogel was subsequently set aside for further characterization and analysis.

Evaluation of Hydrogel

Macroscopic analysis (Physical Examination)

The hydrogel formulation was visually inspected to evaluate its color, appearance, uniformity, texture, consistency, spreadability, and clarity.[11]

Determination of hydrogel clarity

The clarity of the developed hydrogel formulations was evaluated by visually examining them under UV light against both white and black backgrounds.[11]

pH measurement

A precise quantity (1.0 g) of hydrogel was dispersed in 100 mL of purified water. The pH of the resulting hydrogel solution was determined using a digital pH meter.[12]

Measurement of spreadability of topical hydrogel

The spreading coefficient was measured to assess the topical hydrogel's spreading characteristics, considering its 'Slip' and 'Drag' properties. This involved placing approximately 1.0 g of the hydrogel on a glass slide and covering it with another glass slide of equal length. A weight of 500 g was then placed on the upper glass slide to sandwich the hydrogel between the two slides and induce spreading over a certain distance. The time taken for the hydrogel to spread to the specified distance was documented, and the diameter of the resulting spread circle was assessed. Spreadability was determined with the formula.[11, 13]

$$S = \frac{W \times L}{T}$$

where S represents spreadability (g.cm/s), W represents the weight placed on the upper glass slide (g), L signifies the distance travelled on the glass slide (cm), and T is the time the hydrogel takes to spread (s).

Centrifugation test for hydrogel stability

About 10 grams of the hydrogel was carefully placed into a test tube with a tapering end for the centrifugation test. The test tube was then subjected to centrifugation at room temperature, with the hydrogel sample spinning at a rate of 3000 rpm for 30 minutes. The Model Centribio 80-2B was utilized to carry out this process[11] and the following equation showed the percentage of the obtained hydrogel's stability.

$$Hydrogel\ stability\ (\%) = \frac{Height\ of\ hydrogel\ syneresis}{Total\ height\ of\ hydrogel} \times 100$$

Measurement of rheological properties of hydrogel

The Brookfield viscometer equipped with a T spindle S-6 was used to evaluate the viscosity of the optimized

hydrogel. The assessment was conducted at a temperature of 25 ± 2°C. To prevent the spindle from touching the bottom of the beaker, it was carefully lowered vertically while the hydrogel was placed in a 10 mL beaker. The speed of the spindle was set at 20 rpm, and the results were noted once they stabilized. Different speeds were set between 10, 20, 50, and 100 rpm, and the assessments were made at room temperature. The corresponding dial readings were noted, and the viscosity was calculated in centipoises (cps).[12,14,15]

Texture profile analysis

The hydrogel's ability to adhere was calculated using a texture analyzer (TA.XT2, Stable Micro Systems, UK). Throughout the test, the probe was made to move towards the sample, penetrate it, and reach a depth of 5 mm from the starting point, which corresponds to the surfaces of the sample holder. The test was conducted at a speed of 1.0 mm/sec.[16, 17]

Freeze-thaw cycle

It is imperative to assess the stability of topical formulations, particularly aqueous-based ones, against extreme thermal conditions encountered during transportation and storage. This evaluation was performed through freeze-thaw cycle testing, which involves exposing the product to severe temperature fluctuations. The product is frozen at -20°C for 24 hours, thawed at ambient temperature (20–25°C) for 24 hours, exposed at 45°C for 24 hours, and then returned to ambient temperature for 24 hours. After each cycle, analytical evaluations are conducted to detect notable alterations. These evaluations should include assessments for physical changes (e.g., color, phase separation, viscosity), chemical stability (e.g., active ingredient concentration), and microbiological stability. A product is considered thermally stable and suitable for transportation if it undergoes three consecutive freeze-thaw cycles without significant changes, thus ensuring its reliability under various environmental conditions.[11, 18]

Determination of drug content

A sample of hydrogel was weighed accurately to determine the drug content. The hydrogel sample was diluted with phosphate buffer saline (PBS) of pH 7.4 and then subjected to vortexing for 10 minutes. The volume was adjusted using the same pH buffer. The resulting solution was examined at λ_{max} 620 nm using spectrophotometry.[14]

Evaluation of Drug Release from Hydrogel Using Franz Diffusion Cell

The *in-vitro* release of C-Pc from hydrogel was tested at pH 6.8 and 7.4, utilizing a method previously outlined with certain modifications, employing the Franz diffusion (FD) cell.[19,20] The release profiles were compared with C-Pc-loaded hydrogel at different pH levels. Samples were collected over time and analyzed at λ_{max} 620 nm using a UV spectrophotometer. Afterward, fresh medium was added to maintain sink conditions.

$$Release\ of\ CPc\ (\%) = \frac{Released\ CPc}{Total\ CPc} \times 100$$

Evaluation of DPPH (2-diphenyl-1-picrylhydrazyl) Scavenging Activity

The assessment of DPPH scavenging activity for pure LBG, drug C-Pc, grafted LBG, and C-Pc loaded hydrogel was conducted using a method outlined in previous studies.[21,22] Briefly, 0.2 mL of the test sample dispersed in ethanol was added to 2 mL of DPPH solution with a concentration of 0.5 nanomolar. After 30 minutes, the absorbance was assessed at 517 nm using spectrophotometry. The equation determined the DPPH scavenging ability,

$$\%DPPH\ Inhibition = \frac{Abr - Aar}{Abr} \times 100$$

Here, Abr stands for the absorption before the reaction and Aar stands for the absorption after the reaction.

Acute Dermal Irritation/Sensitization Study

The skin irritation/corrosion test operate based on principles of erythema and edema formation. The skin sensitization assessment was done following OECD guidelines 404 and modified per the Banerjee method. The study involved dividing rats into four groups, each containing three animals. One of the groups (Group 1) was given a standard skin sensitizing agent (positive control) made up of 10% propylene glycol and 0.1% w/v 1-chloro-2,4-dinitrobenzene (CDNB). Another group (Group 2) was given a standard irritant (positive control) consisting of an aqueous solution of formaldehyde at a concentration of 0.8% w/v. The third group (Group 3) was given a placebo formulation, which served as the negative control. The fourth group (Group 4) received hydrogel as the treated group. To maintain the skin intact, the fur was shaved off from the dorsal/flank region of the trunk, covering about 10% of the body surface, about a day before the test. Three sites on each animal's back were selected, and a test sample was applied to a small, shaved area (around 6 cm^2). The hydrogel was kept in loose contact with the skin during the exposure period using a semi-occlusive dressing and elastic bandages were wrapped to prevent animal access and ingestion of the test sample during the exposure periods. The signs of erythema and edema were evaluated at 1, 24, 48, and 72 hours after sample removal. Standard scoring codes were used to assess the results. The severity of erythema was measured using scores from 0 to 4. A score of 0 indicated no erythema or non-toxicity, while a score of 1 indicated very slight erythema, barely noticeable as light pink. A score of 2 indicated well-defined erythema, appearing dark pink, and a score of 3 denoted moderate to severe erythema, appearing light red. A score of 4 signified a severe erythema, appearing as beef redness. Erythema grading was hindered if the formation of an eschar occurred. The observation period

was long enough to assess the reversibility of effects. If animals continued to exhibit signs of severe pain or distress, the experiment was terminated.[23, 24]

Dermal Irritation Potential Using HET-CAM Method

The use of the Hen's egg test on the chorioallantoic membrane (HET-CAM) procedure was selected since it has proven to be effective in assessing potential irritation. The evaluation aims to determine potential irritation by examining the membrane's damage. Fertile white leghorn chicken eggs were chosen for their suitability, with the CAM serving as a model for vascularized tissue. The experimental design consisted of three distinct groups: Group 1 acted as a negative control and received a treatment of 0.9% sodium chloride (NaCl), group 2 was the test group, which was treated with an optimized formulation, and group 3 served as the positive control group and was treated with known irritants, 1N sodium hydroxide (NaOH), for comparative analysis. The embryonic development of the eggs was ensured by incubating them at 37 ± 0.5°C and 55 ± 5% relative humidity (RH) for three days prior to the experiment. Candling was used to assess the viability of the eggs. Embryo development was examined with the help of a light source to identify viable eggs, while non-viable eggs were disposed of. The viable eggs were then subjected to manual egg rotation every 12 hours for ten days to promote CAM growth. On the 10^{th} day, the air cell was marked, and the shells were removed before treating the groups with their respective solutions. The types of irritation (hemorrhage, coagulation, and blood vessel lysis) for each sign were observed for 5 minutes. After the exposure, the CAM was treated with 0.3 mL of the test formulation, with similar volumes used for positive and negative controls, and incubated under controlled conditions. Surface alterations, such as lysis, hemorrhage, and coagulation within the vascular structure, were evaluated based on HET-CAM guidelines. According to HET-CAM guidelines, these alterations were scored as follows: Absence of visible hemorrhage scored 0, minimal discoloration of the membrane scored 1, partial coverage of the structure caused by discoloration and hemorrhage scored 2, and complete coverage caused by discoloration and hemorrhage scored 3.[25] The mean scores were calculated for evaluation, taking n = 3 (Table 1).

Table 1: Evaluating irritant potential-scoring chart for HET-CAM test[25]

Scores	*Effect*	*Inference*
9–21	Structures totally covered due to membrane discoloration or hemorrhage	Severe irritant
5–8.9	Structures partially covered due to membrane discoloration or hemorrhage	Moderately irritant
1–4.9	Just visible membrane discoloration	Mild irritant
0–0.9	No visible hemorrhage	Non-irritant

Full Thickness Excision Wound Model

Animals underwent anesthesia through intraperitoneal injections of diazepam (5 mg/kg) and ketamine (75 mg/kg),[26] while their dorsal thoracic region was shaved and disinfected with 70% alcohol. Subsequently, a full-thickness wound (FTW) of about 8 mm was created. The animals were separated into five groups, each comprising six individuals (n = 6). Group I served as the untreated (control), group II received a placebo, and group III underwent treatment with API. Group IV received treatments with test formulations. All the treatments were given once daily**.** The study focused on monitoring wound contraction and the overall time taken for wound closure.[27]

Wound Size Analysis

The wound margins were outlined on a transparent sheet every three consecutive days for 14 days. The size of the healed area was determined by subtracting the initial wound area from the portion that remained unhealed. The contraction was measured as a percentage, and the time for complete epithelialization was noted after full healing.[28-30]

$$\%\ Wound\ contraction\ ratio\ (WCR) = \frac{Initial\ wound\ area - Specific\ day\ wound\ area}{Initial\ wound\ area} \times 100$$

Statistical Analysis

All experiments were executed in three sets. Calculations were depicted as mean ± SD (standard deviation), and software GraphPad Prism 8.0 was used for the statistical analysis.

Result And Discussions

Physical Appearance and Clarity

The hydrogel prepared underwent assessment for physical appearance and clarity. The optimized formulation exhibited a clear, smooth, homogeneous texture and transparency, with a subtle bluish tint and no discernible odor.

pH

The formulation showed a pH of 6.72 ± 0.09, which is considered optimal for a topical formulation. This pH value is conducive to achieving suitable viscosity and clarity of the hydrogel, suggesting minimal risk of skin irritation from the hydrogel.

Spreadability

The hydrogel exhibited high spreadability, characterized by a low spread time. The optimized hydrogel exhibited a spreadability value of 8.37 ± 0.21 cm, indicating easy spreading with minimal shear force. This suggests that the formulation can be applied smoothly without running off. Adequate spreadability is crucial for

ensuring uniform hydrogel application to the skin, thereby enhancing therapeutic efficacy. Moreover, good spreadability contributes to patient compliance with treatment regimens, making it an essential quality for topical applications.

Centrifugation Test

The purpose of the centrifugation test in the hydrogel system is to subject the system to pressure. Stirring at 3000 rpm for 10 minutes is considered equivalent to the effects of gravity over approximately one year, with no observable instability in the formulation. The stability percentage is determined based on the degree of separation in the hydrogel system, where lower separation indicates a higher stability percentage. This test influences the physical characteristics of the hydrogel system by inducing changes in molecular distribution, thereby affecting the level of separation. Higher pressure leads to increased separation levels in the hydrogel system.

Rheological Properties of Hydrogel

Achieving consistency is crucial for topical formulations of antibiotics and anti-inflammatory agents, especially when they are applied to thin layers of skin. It is important to control drug permeation by regulating hydrogel viscosity, which plays a pivotal role. Fig. 1 depicts the behavior of the hydrogel, where viscosity was tested at four different speeds - 10, 20, 50, and 100 rpm. In general, the consistency of hydrogels is reflected in their viscosity. Hydrogel viscosity is lowered as the rate of shear increases, indicating shear thinning or non-Newtonian flow. This is a desirable behavior as it reduces flow resistance when exposed to intense shear forces. The observed reduction in viscosity, which potentially indicates pseudoplastic behavior, confirms the hydrogel's high spreadability characteristic. This property enables the viscosity to decrease when subjected to a certain force while retaining the ability to remain at the application site without draining away. The optimum viscosity of the hydrogel was found to be 6500 cps at 20 rpm.

Bioadhesive Properties Using Texture Analyser

Texture analysis offers a method to evaluate hydrogels by measuring their mechanical resistance to stress.

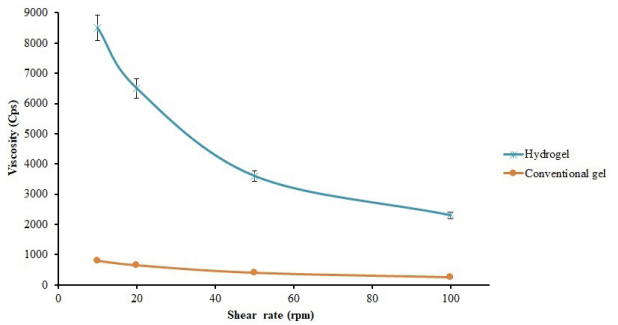

Fig. 1: Viscosity of hydrogel at varying shear rates, demonstrating shear-thinning behavior

The calculated work of adhesion was utilized to assess the bioadhesive properties of the hydrogel. A graph was plotted to illustrate the force variation over time, providing insight into the gel strength. The results indicate that the developed systems exhibit robust gel strength, ease of spreading, and adhesion, with a value of 61.3 g at 5 seconds (Fig. 2).

Freeze-Thaw Cycle

Through analytical assessments after each cycle, the formulation's resilience to thermal stress and its ability to maintain efficacy without undergoing phase separation can be determined. Successfully passing three consecutive freeze-thaw cycles indicates the product's thermal stability and suitability for transport, addressing a significant logistical challenge for these formulations.

Drug Content

The drug content was assessed to be at 94 ± 1.27% of the optimized formulation, indicating that the drug was uniformly distributed within the formulations.

Release Profile

The analysis of C-Pc release from the hydrogel was conducted in PBS at pH 6.8 and 7.4. The results indicated a higher percentage of C-Pc release at pH 7.4 (43.19 ± 2.10%) compared to pH 6.8 (37.11 ± 2.39%) (Fig. 3), potentially attributed to the heightened polymer swelling at the higher pH level compared to the lower pH of 6.8.

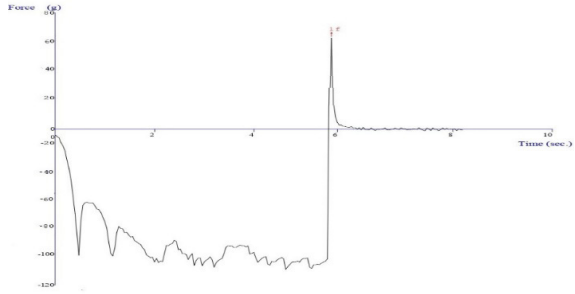

Fig. 2: Texture analysis of hydrogel formulation

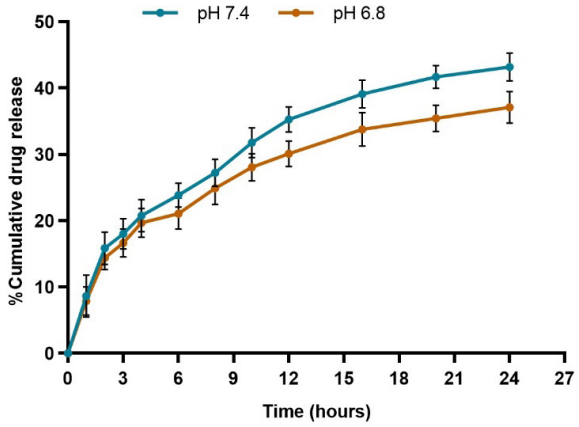

Fig. 3: Illustrates the percentage of C-Pc released from the hydrogel in phosphate buffer saline at pH 6.8 and 7.4

Estimation of DPPH Radical Scavenging Potential

Both pure gum and the drug demonstrated antioxidant activity, inhibiting DPPH radicals by 11.53 ± 2.3 and 61.22 ± 3.1%, respectively (Fig. 4). Interestingly, when the grafted gum and the drug were incorporated into the gum matrix (hydrogel formulations), there was a significant enhancement in DPPH radical suppression compared to pure gum. Specifically, the percent suppression of DPPH radicals increased to 39.02 ± 4.6% for grafted gum and 78.67 ± 4.8% for the hydrogel formulation. This improvement was statistically significant ($p < 0.05$), suggesting a synergistic effect between the drug and the gum matrix.

Acute Dermal Irritation/Sensitization Testing

Three animals were tested for skin irritation, and the total scores for redness (erythema) and swelling (edema) were calculated at both 24 and 72 hours after exposure. To find the major irritation index, divide the total score by six. Based on Draize's classification, the test sample was categorized as follows: Non-irritant (0), mild irritant (>0–2), moderate irritant (>2–5), and severe irritant (>5–8).[31] The test was validated using a positive control (formaldehyde) and showed dermal irritation and skin redness (Fig. 5B). However, no dermal responses were found in rats treated with the hydrogel formulation, indicating no irritation or corrosion. The hydrogel containing the drug showed a primary irritation index of 'zero,' indicating a non-irritant classification (Table 2). Skin sensitization experiments with positive control (CDNB) showed positive responses (Fig. 5A), while rats treated with the drug-loaded hydrogel or placebo showed no sensitization (Fig. 5C and D). Dermatological trials indicated no health hazards related to skin irritation or allergic reactions.

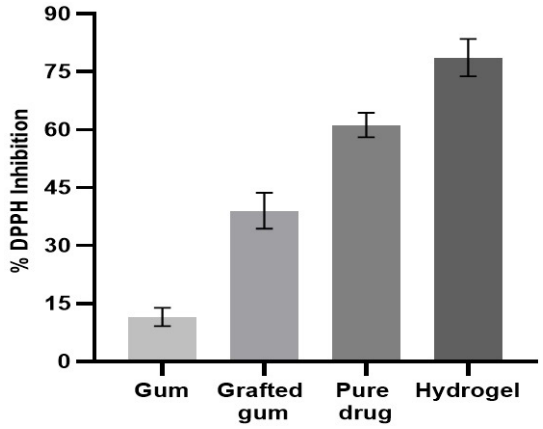

Fig. 4: Antioxidant activity of pure gum, drug, grafted gum, and hydrogel formulation

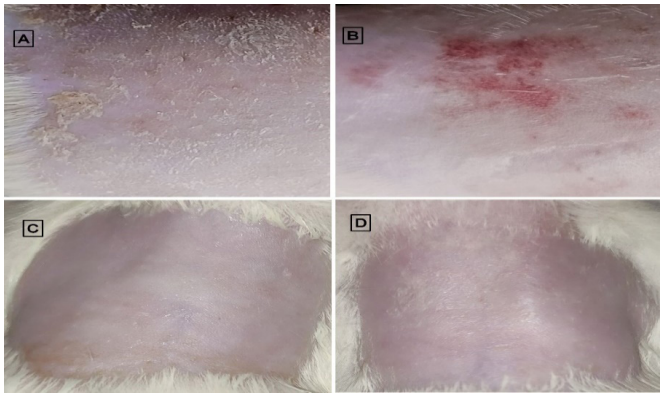

Fig. 5: Response of acute dermal irritation test and sensitization study after exposure to the test material. (A) received standard skin-sensitizing agent (positive control). (B) received standard irritant (positive control) and showed skin redness (erythema). (C) received a placebo formulation (negative control), and (D) received the drug-loaded hydrogel (treated group). Both C and D did not show erythema, edema, or other skin reactions

Dermal Irritation Potential Using HET-CAM Method

In this study, the HET-CAM method was utilized as a cost-effective and efficient means to assess the irritation potential of the hydrogel. As a positive control, we utilized 0.1M NaOH, while a negative control was established using a normal saline solution of 0.9% NaCl. The results in Table 3 consistently showed a mean score of 'zero' for the negative control, indicating negligible irritant properties over the 12 hours study period. Conversely, the positive control exhibited a mean score of 5 throughout the study, indicating severe irritation. The hydrogel formulation consistently scored 'zero' over the observation period, indicating non-irritation. Moreover, no signs of hemorrhage, coagulation, or lysis were observed, suggesting the hydrogel's suitability for topical application, as depicted in Fig. 6.

Table 2: Skin reaction observations at various time intervals resulting from the application of drug-loaded hydrogel in rats

Skin response/ reaction	*Duration of observation (Hours)*	*Number of animals*			*Mean*
		1	*2*	*3*	
Erythema/Eschar formation	1	0.0	0.0	0.0	0.0
	24	0.0	0.0	0.0	0.0
	48	0.0	0.0	0.0	0.0
	72	0.0	0.0	0.0	0.0
Edema formation	1	0.0	0.0	0.0	0.0
	24	0.0	0.0	0.0	0.0
	48	0.0	0.0	0.0	0.0
	72	0.0	0.0	0.0	0.0
Sum of erythema and edema readings at 24 and 72 hours (S)	0.0				
Primary irritation index (S/6)	0/6 = 0.0				
Classification	Non-irritant				

Table 3: Scoring of het-cam test conducted on fertile eggs using the C-Pc loaded hydrogel.

Preparations	*Number of Egg*	*Score*						
		Time (minutes)						
		0.5	*2*	*5*	*60*	*240*	*480*	*720*
0.9% NaCl (–ve control)	Egg 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Egg 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Egg 3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1M NaOH (+ve control)	Egg 1	1.0	4.0	4.0	4.0	4.0	5.0	5.0
	Egg 2	2.0	4.0	4.0	4.0	4.0	4.0	5.0
	Egg 3	1.0	4.0	4.0	4.0	4.0	4.0	5.0
	Mean	1.33	4.0	4.0	4.0	4.0	4.33	5.0
Optimized formulation (hydrogel)	Egg 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Egg 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Egg 3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0

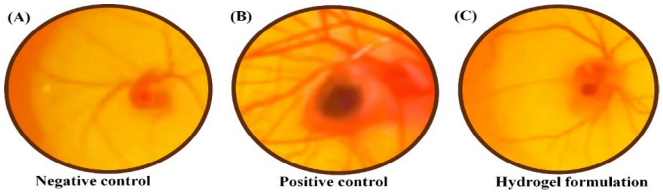

Fig. 6: Images depicting the HET-CAM test of the hydrogel of C-Pc performed on fertile eggs

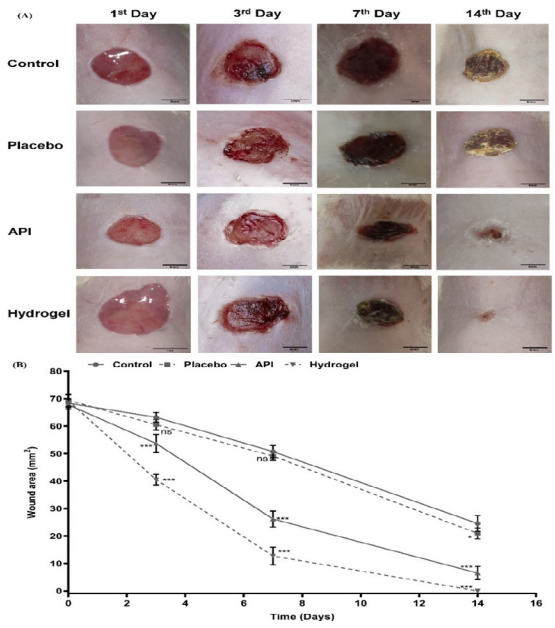

Fig. 7: Representation of wound healing progress in different treatment groups. (A) Shows photographic records taken on days 1, 3, 7, and 14 capturing the development of untreated wounds (control) in comparison to those treated with placebo, API, and hydrogel. Scale bar: 4 mm. (B) Presents quantified rates of wound closure, with mean values and standard deviations, allowing for a comparison of treatment efficacy over time

Wound Healing Activity

In the excision wound study (Fig. 7A), wound contraction showed similar progress in the groups treated with API and hydrogel formulation. Complete healing was observed in these two groups between the 10^{th} and 14^{th} day. However, animals in group I (untreated control group) and group II (treated with placebo) took more than 20 days for the wounds to heal completely.

In the wound healing study (Fig. 7B), there was noticeable progress in wound contraction in both the formulation-treated and control groups. Animals treated with the hydrogel (Group IV) demonstrated a healing rate of 0.06 ± 0.003 mm^2 by the 14^{th} day. In contrast, the untreated control group (Group I) exhibited a healing rate of 24.55 ± 2.84 mm^2 by the same day. The wound healing contraction of the treatment group (hydrogel and API) was statistically significantly higher than the negative control and placebo groups. Additionally, animals in the control group displayed signs of inflammation and septic wound formation up to the 5^{th} day of the experiment. In contrast, those treated with the formulation exhibited no observable inflammation.

Conclusion

In conclusion, our study has successfully developed a novel hydrogel-based delivery system for C-Pc, aimed at improving its stability and therapeutic effectiveness in wound healing. By meticulously characterizing the hydrogel's physical properties, we ensured its suitability for topical application. Encapsulating C-Pc within the hydrogel led to sustained release, potent antioxidant activity, and significant enhancement in wound healing compared to free C-Pc. *In-vivo* studies confirmed the promising potential of the C-Pc encapsulated hydrogel,

showing accelerated wound closure with no signs of irritation or allergy, highlighting its biocompatibility and safety. The formulation's robustness over multiple freeze-thaw cycles underscores its practical suitability. Overall, this hydrogel represents a promising advancement in wound care, offering a safe, effective, and patient-friendly alternative. Further clinical investigations are needed to validate its efficacy in humans and explore broader therapeutic applications. Optimization efforts should focus on scalability and manufacturability to facilitate its clinical translation, ultimately benefiting patients globally.

Acknowledgement

The author(s) gratefully acknowledge the support received from the Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, India, for providing the necessary facilities to carry out this work.

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HOW TO CITE THIS ARTICLE: Alka, Saraf SA. Development and Evaluation of Phycocyanin-Infused Hydrogel Topical Formulations for Wound Healing. Int. J. Pharm. Sci. Drug Res. 2024;16(3):476-484. **DOI:** 10.25004/IJPSDR.2024.160321