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FORMULATION AND EVALUATION OF ALLANTOIN LOADED HYDROGEL FOR SKIN REGENERATION AND REJUVENATION

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Abstract

Advancements in dermatology and cosmetics emphasize the significance of skin rejuvenation and regeneration. Hydrogels have emerged as a favorable platform for delivering active agents due to their biocompatibility, hydration potential, and controlled release characteristics. This study focused on creating and evaluating a hydrogel containing allantoin, intended to facilitate skin revitalization and renewal. The hydrogel formulation was meticulously crafted by combining natural and synthetic polymers, optimized to achieve desired viscosity, gel strength, and allantoin-loading capacity. Allantoin, recognized for its skin-calming, hydrating, and regenerative attributes, was successfully integrated into the hydrogel matrix. Comprehensive characterization of the hydrogel encompassed assessments of physicochemical attributes such as pH, rheological performance, drug release kinetics, and mechanical properties. In vitro release profiles exhibited a sustained and controlled release of allantoin from the hydrogel spanning hours, indicating its potential for prolonged topical application. Rheological analysis unveilled the hydrogel's shear-thinning behavior. The hydrogel formulation presents a pioneering approach to addressing diverse skin-related concerns and advancing dermatological and skincare treatments.

Keywords: hydrogel, allantoin, skin regeneration, rejuvenation, wound healing, hyaluronic acid, biocompatibility, cytotoxicity, fibroblasts, moisture retention.

INTRODUCTION

The demand for innovative skin care products that promote skin regeneration and rejuvenation has indeed increased. Allantoin, a natural compound derived from plants, has been widely acknowledged for its moisturizing, soothing, and wound-healing properties. Hydrogels, characterized as 3D structures. of hydrophilic polymers, have demonstrated significant promise in terms of... effective delivery systems for bioactive compounds due to the fact that of their high-water content and biocompatibility.

Allantoin:

Allantoin, also known as 5-ureidohydantoin or glyoxyldiureide, is a specific chemical compound containing the molecular formulary $C_4H_6N_4O_3$. It functions as a diureide of glyoxylic acid. This compound holds significance as a significant metabolic intermediary in various creatures, spanning animals, plants, and bacterial organisms. Its synthesis involves the transformation of uric acid, which is a breakdown product of nucleic acids, by means of the enzymatic activity of urate oxidase (uricase). Interestingly, allantoin exists naturally both as a mineral compound and in various biological contexts.[31]

FIGURE 1 ALLANTOIN

Allantoin is a natural identical molecule that was the first found in the comfrey plant. Usages are tracked allantoin containing extracts described in the 16th century used in the literature to treat wounds and appearance of burns. Today, ALANOIN is usually chemical synthesized to meet global demand. It is also used. Aside from their moisturizing attributes, these products commonly incorporate allantoin, the versatility of allantoin is evident in its extensive utilization across various beauty care applications, encompassing skincare, hygiene products, as well as sun and hair products. This compound's multifaceted benefits make it a widely favored and frequently employed ingredient in the realm of cosmetic and personal care[33].

Pharmacokinetics:

Although there is a lack of comprehensive and pertinent data to definitively establish the pharmacodynamic characteristics of the specific allantoin brand, ongoing studies indicate certain properties. Allantoin appears to possess moisturizing and keratolytic qualities. It demonstrates the capacity to enhance the moisture content within the extracellular matrix and facilitate the exfoliation process in the upper layers of deceased skin cells. These attributes collectively contribute to the potential stimulation of cell proliferation and aid in wound healing.[35]

Absorption:

In human studies, 19% and 34% of urinary allantoin were Noteworthy effects were only observed in a limited context, involving merely two subjects and specifically following the administration of elevated

doses of allantoin. This effect was witnessed subsequent to intravenous administration. urinary excretion was almost quantitative. Human model doses 75-600 mgm Excretion continued for 72 hours in humans after administration of 240 mgm, and results from subcutaneous injection were similar.[36]

Metabolism:

Uricase is an enzyme that facilitates the conversion of uric acid into allantoin. Because humans lack endogenous uricase, uric acid remains the sole end product resulting from the breakdown of surplus purine nucleotides. The presence of allantoin in human urine, therefore, arises from non-enzymatic processes where uric acid acts as the reactive component in the presence of oxygen species[37]. Given its integral role in natural metabolic pathways, the buildup of allantoin is not anticipated. Moreover, it is believed that allantoin undergoes negligible metabolic changes in both humans and animals.[38]

History:

Michele Francesco Buniva, an Italian physician, and Louis Nicolas Vauquelin, a French scientist, mistakenly assumed the amniotic fluid allantoin when they discovered it for first time in 1800. Allantoin was first identified in the fluid of the allantois by French chemist Jean Louis Lassaigne in 1821. He referred to it as "l'acide allantoique." Later, in 1837, German scientists Friedrich Wöhler and Justus Liebig synthesized allantoin from uric acid and coined its current name.[40]

Applications:

- Allantoin is present in extracts of fragrant plants and commonly found in the urine of various mammals. Chemically synthesized bulk allantoin closely resembles natural allantoin and is considered safe. Remarkably, there are over 10,000 patents associated with various aspects of allantoin.
- The majority of mammals' urine and botanical preparations of the comfrey plant both contain allantoin.
- Bulk allantoin, produced through chemical synthesis to mirror natural allantoin, is considered safe, non-toxic, and can be seamlessly integrated with cosmetic raw materials.
- It also complies with CTFA and JSCI criteria. Allantoin is mentioned in more than 10,000 patents.
- Cosmetics: Allantoin can be utilized as an ingredient in over-the-counter cosmetics by manufacturers.
- Pharmaceutical Products: Allantoin is commonly incorporated into a range of products such as toothpastes, mouthwashes, oral hygiene items, shampoos, lipsticks, anti-acne treatments, sunscreens, skin brightening lotions, various cosmetic emulsions and lotions, as well as other cosmetic and pharmaceutical formulations.
- Biomarker of Oxidative Stress: Given that uric acid is the final outcome of human purine metabolism, allantoin a suitable biomarker for quantifying oxidative stress in instances of chronic diseases and aging.[41]

Mechanism of Action:

Formal justification for the labeling action lacks well-controlled data. Nonetheless, ongoing research suggests that allantoin could exhibit a histological wound healing profile in rats, promoting normal skin

regeneration and recovery. This wound healing enhancement is backed by observations indicating that rats treated with topical allantoin preparations exhibited histological changes, including increased vascular dilation, presence of inflammatory secretions, elevated inflammatory cell count, enhanced angiogenesis, greater fibroblast proliferation, and increased collagen deposition, when compared to rats with wounds who did not receive allantoin treatment, contributing to the development of healthy tissue. Additionally, allantoin may contribute to tissue differentiation and development, particularly by fostering the growth of granulation tissue and epithelialization. Further research is necessary to comprehensively understand allantoin's mechanisms of action in the context of wound healing.[43]

Toxicity:

No studies have been provided regarding the toxicity of repeated doses and reproductive effects.
Additionally, research indicates that tumor occurrence in animals subjected to allantoin treatment did not significantly, additional evaluations for toxicity, mutagenicity, or carcinogenicity are not deemed necessary. Overall, allantoin is widely considered a safe substance for human use, given its common presence in the human diet and its natural occurrence within the human body.[44]

Skin Regeneration & Rejuvenation:

The Harvard Stem Cell Institute's (HSCI) Skin Program is committed to unraveling the reasons behind skin healing failures and scarring, as well as comprehending the mechanisms underlying the skin's progression towards thinness, fragility, and wrinkles as it ages. The ultimate objective of the Skin Program is to uncover novel therapeutic approaches for skin regeneration and rejuvenation. Despite its global impact on billions of individuals, the knowledge about preventing skin degeneration remains limited.[45]

Choices of Lifestyle

Embracing healthy choice of lifestyle can positively impact on skin regeneration process. Hurtikant advocates the following practices:

- Engaging in regular exercise
- Following a nutrient-rich diet
- Staying hydrated by consuming sufficient water
- Safeguarding the skin against environmental ultraviolet radiation, pollution, and dry
- Reducing stress whenever feasible[51]

Hydrogel:

A hydrogel can be defined as a hydrophilic polymer which links with each other and not dissolve in water. They generally come in the form of polymer compounds, which absorb readily and take precise shape in structures. The building's design allows it to hold more water while yet being well-maintained and more bloated with it. The hydrogel is synthesized in a way that it expands more when exposed to fluids.medium that behaves like water and connects each monomer with another. They primarily absorb a lot of liquid from hydrophilic functional groups connected to additional polymer supports, while network to network chains act

as their barrierand biological reaction. An antibiotic called neomycin sulphate is used to treat bacterial skin infections so treating both the underlying infection and its symptoms.

EVALUATION PARAMETER

Physical Characteristics:

The hydrogel formulations that were prepared underwent visual examination to assess the following attributes:

- pH level
- Color
- Homogeneity
- Consistency
- Grittiness
- Texture
- Presence of phase separation

Determination of pH:

By using the digital pH meter, pH of the hydrogel formulations was assessed. A gram of gel was dissolved in 25 ml of distilled water, and the gel formulation was immersed in the electrode for 30 minutes until a consistent reading was achieved. The stable reading was subsequently recorded. pH measurements were conducted in triplicate for each formulation, and the average values were computed.

Washability:

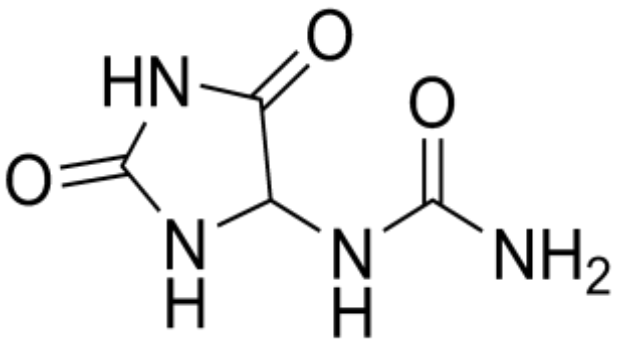
The formulations were applied to the skin, and their affluence of removal with water was evaluated through manual assessment The extent to which the formulation was removed during washing was also evaluated.

Extrudability Study:

The hydrogel formulations were loaded into collapsible metal tube or aluminum collapsible tubes. By compressing the tubes, the material was extruded, and the simplicity with which the formulation was extruded and examined.

DRUG PROFILE: -

ALLANTOIN:



(2, 5-Dioxo-4-imidazolidinyl) urea

Chemical Data: -

Formula	$C_4H_6N_4O_3$
Molecular mass	**158.117 g·mol**

Pharmacokinetic Data: -

Origin	From Bacteria, Vegetables and Animals.
Protein binding	15–20%
Metabolism	Hepatic
Half-life	1–2.5 hours
Excretion	Renal, Skin(Sweating)

Description:

Allantoin serves as a skin-active component renowned for its keratolytic, hydrating, calming, and irritation-reducing characteristics. It aids in the regeneration of surface skin cells and expedites the healing of wounds. This substance is both safe and gentle, demonstrating excellent compatibility with the skin and other cosmetic ingredients. Throughout its extensive utilization in cosmetics and topical medicinal products, there have been no indications of harmful effects or negative responses. It adheres to the stipulations of CTFA and JSCI regulations.

EXPERIMENTAL WORK

Identification of pure drug (allantoin):

Pure drug has been identified by using technique of IR. Determining the purity of Allantoin involves a combination of methods that encompass physical, chemical, and analytical approaches. Allantoin is recognized for its characteristic appearance and its ability to dissolve in water. To identify pure Allantoin

Visual Examination

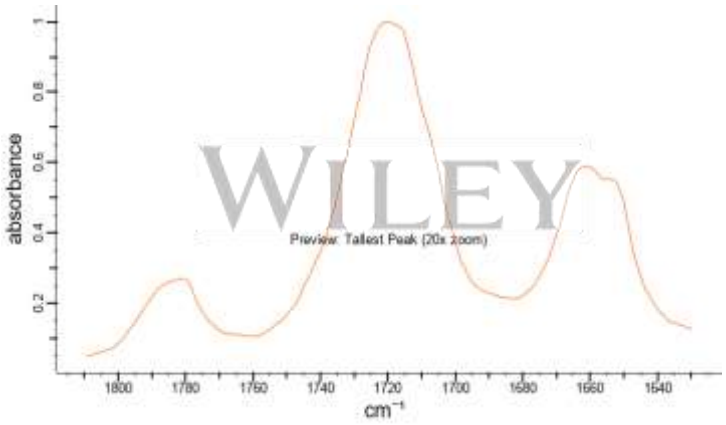


Figure 2: IR Spectroscopy

Fourier transform infra-red spectrophotometry:

- During FTIR spectroscopy, a sample is exposed to a broad array of infrared
- Wavelengths. The sample absorbs specific wavelengths corresponding to the vibrational frequencies of its constituent molecules. The absorption spectrum, portraying light absorption at different wavelengths, is then generated. By comparing these absorption patterns with reference spectra or databases, scientists can determine the makeup of the sample's molecules.
- FTIR spectroscopy has applications across diverse domains such as chemistry, biology, pharmaceuticals, materials science, environmental science, and forensics. This technique is versatile and non-destructive, offering valuable insights into the composition and structure.
- A Fourier Transform Infrared (FTIR) spectrophotometer used to capture spectra in the FTIR region comprises an optical system and a mechanism for quantifying the ratio of transmitted light intensity to incident light intensity

PREPARATION OF SAMPLE:

- For the creation of three distinct hydrogel samples, a solution containing 2% (w/v) allantoin was dispersed in deionized water while maintaining a temperature of 80°C in a water bath.
- This process was accompanied by continuous stirring until allantoin was completely dissolved.
- Subsequently, various concentrations of lyophilized Hyaluronic Acid 2% (v/v) were gradually introduced into each of the three cooled solutions at room temperature.
- To ensure uniform mixing, the solutions were stirred continuously for around 6 hours.
- The inclusion of 2% (w/v) xanthan gum prompted swift gelation of the three hydrogels.
- Ultimately, three evenly blended composite hydrogels were obtained

PREFORMULATION STUDIES:

Identification and Authentication:

UV spectrophotometric studies:

Precisely 10 milligrams of the drug were meticulously measured and subsequently dissolved in 10 milliliters of the intended solution within a 10-milliliter volumetric flask. A suitable dilution was then prepared. The spectral analysis of this prepared solution was conducted using a UV-visible spectrophotometer in the range

of 200 to 400 nanometers. The obtained spectrum was then compared to the standard for further evaluation.

Table No.1: UV Spectrophotometer Ranges

Wavelength (nm)	Interpretation	Inference
200-400 nm	Scanning range	Drug absorption maxima (λ max) at303.80 nm
303.80 nm	Highest peak	

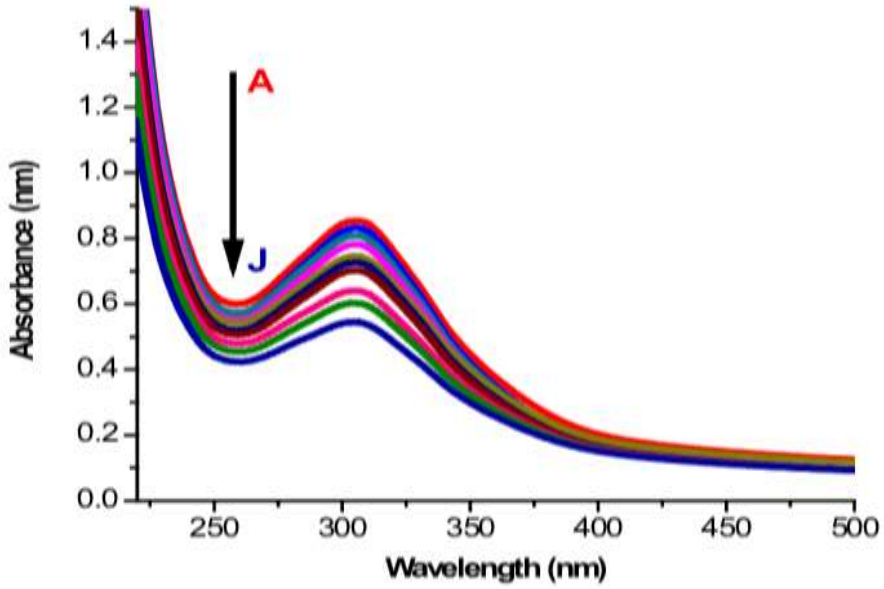


Figure 3 Standard UV spectra of drug sample in water

FT-IR spectrophotometric studies:

The infrared spectra of a compound can reveal the group that is present in that compound. KBr pellets are used to provide a medication with an infrared spectrum. One drop of the medication mixture was evenly dispersed between each KBr pellet after being combined with a small amount of oil. The pellets were put into a holder, and infrared spectra were recorded. The presence of different groups in the drug's structure was inferred from several peaks in the infrared spectrum.

EVALUATION PARAMETER:

Physical Characteristic:

The hydrogel formulations that were prepared underwent visual examination to assess their pH, color, uniformity, texture, consistency, presence of grittiness, as well as any indications of separation between phases

pH Determination:

To determine the pH of the hydrogel formulations, a digital pH meter was employed. A gram of the gel was dissolved in 25 milliliters of distilled water, and the gel formulation was immersed with an electrode for 30 minutes until a consistent reading was attained. The stable reading was then recorded. This pH measurement

process was repeated three times for each formulation, and the average values were calculated.

Washability Assessment:

The formulated hydrogel preparations were applied to the skin, and a manual assessment was conducted to gauge the ease and extent of their removal when washed with water.

Extrudability Examination:

The hydrogel formulations were filled into collapsible metal tubes or aluminum collapsible tubes. By exerting pressure on the tubes, the material was extruded, and the ease with which the formulation could be extruded was evaluated.

Table No. 2: Physical parameter of formulation

Formulation	Colour	Homogenity	Consistency	Phase Seperation
F1	White	Excellent	Excellent	None
F2	White	Good	Good	None
F3	White	Average	Average	None
F4	White	Average	Average	None

Table No. 3: Determination of Ph

Sr No	Formulation	pH
1	F1	7.05 ± 0.01
2	F2	7.02 ± 0.02
3	F3	6.95 ± 0.05
4	F4	6.98 ± 0.01
5	F5	7.05 ± 0.03

Table No. 4: Result of washability and extrudability

Sr no	Formulation	Washability	Extrudability
1	F1	+++	++
2	F2	+++	+++
3	F3	+++	+++
4	F4	+++	++
5	F5	+++	++

Spreadability Assessment:

For evaluating spreadability, two glass slides of standardized dimensions (6×2) were chosen. The hydrogel formulation under scrutiny was applied onto one of these slides.

Spreadability is calculated using the formula: $S = m \times l / t$

Where, S= Spreadability (measured in gcm/sec),

m = weight attached to the upper slide (20 grams),

l = length of the glass slide (6 cms)

t = time taken in seconds.

Table No. 5: Result of Spreadability study

Sr. No.	Formulation	Spreadability (gcm/Sec)
1	F1	12.24 ± 0.02
2	F2	13.38 ± 0.01
3	F3	14.52 ± 0.03
4	F4	13.20 ± 0.05
5	F5	14.54 ± 0.01

Viscosity:

The measurement of viscosity of the prepared hydrogel was done using Brookfield digital Viscometer.

Table No. 6 Sample of Viscosity

Sr. No.	Formulation	Viscosity (cps)
1	F1	942 ± 2.4
2	F2	987 ± 2.1
3	F3	945 ± 1.5
4	F4	940 ± 2.5
5	F5	918 ± 1.5

Table No. 7: Result of viscosity

Sr. No.	Formulation	Viscosity (cps)
1	F1	942 ± 2.4
2	F2	987 ± 2.1
3	F3	945 ± 1.5
4	F4	940 ± 2.5
5	F5	918 ± 1.5

Drug content:

Precisely weighed, equivalent to 100 mg of hydrogel, was placed into a beaker. Subsequently, 20 ml of phosphate buffer with a pH of 7.4 was added to the beaker. The mixture was thoroughly combined and then subjected to filtration using Whatman filter paper no.1. From the filtered solution, 1.0 ml was drawn and placed into a 10 ml volumetric flask. The volume was adjusted to 10 ml using phosphate buffer at pH 7.4. This resulting solution was examined utilizing a UV spectrophotometer at a wavelength of 303.80 nm, which is the λmax value.

Table No. 8: Drug Content

S.No.	Formulation	Drug content
1	F1	96.4 ± 0.2
2	F2	94.8 ± 0.1
3	F3	95.4 ± 0.2
4	F4	92.3 ± 0.1
5	F5	89.1 ± 0.3

Stability Studies:

The primary objective of stability testing is to offer evidence concerning the quality changes that occur over time in a drug substance or its product due to environmental influences like temperature, humidity, and light. This process aids in establishing recommended storage conditions, determining re-test intervals, and establishing the shelf life of the product.The International Conference on Harmonization (ICH) Guidelines, titled "Stability Testing of New Drug Substance and Product" (QIA), outlines the stability testing requirements necessary for drug registration applications in the European Union, Japan, and the United States of America. ICH specifies the duration of the study and the conditions under which it should be conducted:

Long Term Testing: 25ºC ± 2ºC / 60% RH ± 5% for 12 months

Accelerated Testing: 40ºC ± 2ºC / 75% RH ± 5% for 6 months

For the selected formulation, stability studies were executed over a span of 3 months, employing the following conditions:

25ºC/60% RH

30ºC/65% RH

40ºC/75% RH

Accelerated stability studies:

Following the International Conference on Harmonization (ICH) protocols, accelerated stability tests were performed on a fine-tuned formulation. The formulation, packaged within an aluminum tube, underwent accelerated stability testing for a duration of 3 months, aligning with ICH regulations. This testing occurred at a temperature of 40 ± 2ºC and a relative humidity of 75 ± 5%.

At consistent time intervals, samples were procured and subjected to analysis for alterations in pH, spreadability, drug content, and in vitro drug release, utilizing the procedures previously outlined. Should any modifications be detected in the evaluation parameters, they were meticulously documented for reference.

RESULTS AND DISCUSSION

Characterization of pure drug (allantoin):

Table 9: Characterization of pure drug.

Sr. no.	Characterization	Specification	Result
1	Description	Odourless White Powder	A almost white powder
2	Solubility	Freely soluble in water to 0.5%,	Complies
3	Identification By FT-IR	very slightly soluble in alcohols,	With the resolution of 4cm-1 & measurement time of 15s
4	Melting range	insoluble in oils and apolar solvent	Complies
5	Sulphated ash	Diffuse external reflectance system at the sample surface between 400-4000 cm -1	Complies
6	Losson drying	230 °C	Complies
7	Heavy Metals	Not more than 0.1%	Complies
8	Assay	Not more than 0.1%	Complies

The recently prepared composite hydrogels exhibited a smooth texture and displayed a color spectrum ranging from a pale translucent beige to a deep opaque light beige shade. As stated in pertinent literature sources the organoleptic attributes of the hyaluronic acid hydrogel composites are depicted in the provided table. Optical evaluations affirmed the steady and unchanging microstructure of these hydrogels. Additionally, these hydrogels showcased consistent swelling, elasticity, and mechanical strength

characteristics, as outlined in Section 3.8 below (average and standard deviation values were derived from a minimum of three measurements).

Table No. 10: Comparative Study

Formula e	Appearane	colour	homogeneit y	consistenc y	Allantoin Content		Phase seperation
					(W/v)%	Dried(wt %)	
A	Homogeneo us	Translucent , pale brige, neutra	Very good	Good	1%	38	No phase separation
B	Homogeneo us	Opaque, Neutral, beige	Very good	Good	2%	55	No phase separation
C	Homogeneo us	Opaque intense natural beige	Very good	Good	3%	71	No phase seperation

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