Biogenic Nanocrystal Based Eye Roll on Concealer Developed from Poultry Keratin, Lignin and *Vitis vinifera* Seed Oil

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ABSTRACT

The present study focuses on the development of a biogenic nanocrystal based eye roll on concealer formulated using keratin, lignin extracted from poultry waste and *Vitis vinifera* seed oil as key bioactive ingredients. Poultry keratin, obtained from processed beak and nail residues of chicken, natural lignin from goat manure were extracted and combine with *Vitis vinifera* seed oil to converted into nanocrystals through controlled hydrolysis and nanoprecipitation techniques. The resulting keratin nanocrystals served as a natural structural enhancer and skin-repairing agent due to their excellent biocompatibility, film-forming ability, and strengthening properties. *Vitis vinifera* seed oil was incorporated into the formulation for its antioxidant, anti-inflammatory, and skin-nourishing attributes, offering additional benefits such as improved under eye hydration, reduction of oxidative stress, and enhancement of concealer spreadability. Physicochemical characterization, including general, FTIR, and SEM, confirmed the successful formation of stable keratin nanocrystals with uniform morphology. The formulated eye roll on concealer demonstrated satisfactory rheological behavior, homogeneity, and smooth application properties. Preliminary Antioxidant and In vitro

skin compatibility assessment revealed that the product was non-irritating and suitable for topical use. Overall, this work establishes a sustainable and value added approach for converting poultry derived keratin waste into functional nanocrystals for cosmetic applications. The biogenic nanocrystal-based eye roll-on concealer shows promising potential as a natural, eco-friendly, and effective cosmetic formulation for under eye revitalization and skin enhancement.

Keywords: Nanocrystal, keratin, *Vitis vinifera*, antioxidant, eye roll, cosmetic formulation

INTRODUCTION

The pursuit of innovative, sustainable, and biocompatible cosmetic products has gained remarkable momentum over the past decade, driven by rising consumer awareness toward natural ingredients, eco-friendly formulations, and biologically safe alternatives to synthetic chemicals[1]. Among these emerging trends, the development of biogenic nanomaterials has opened new avenues for enhancing the performance, stability, and sensory attributes of topical cosmetic formulations. Nanotechnology-based approaches allow for precise manipulation of particle size, structure, and morphology, enabling improved penetration, controlled release, superior texture, and enhanced aesthetic outcomes in cosmetic applications. Within this context, the formulation of an eye roll-on concealer using biogenic nanocrystals derived from poultry keratin, lignin, and *Vitis vinifera* (grape) seed oil represents a multifaceted innovation merging sustainability, biotechnology, and advanced cosmetic science[2]. This work emphasizes the utilization of waste-derived biomolecules, particularly poultry keratin and lignin, in combination with the therapeutic and dermatoprotective constituents of grape seed oil, to develop an effective, skin-friendly, and environmentally responsible eye concealer with nano-enabled functional properties. Keratin is a fibrous, structural protein abundantly present in feathers, hair, nails, and wool. Poultry feathers, in particular, constitute a major agro-industrial waste generated globally in millions of tons each year. Improper disposal of poultry feathers contributes to environmental pollution, microbial proliferation, and waste management challenges. Converting this keratin-rich biomass into value-added cosmetic materials provides a sustainable solution for resource recovery while minimizing environmental burden. Keratin possesses unique biochemical characteristics, including high cysteine content, biocompatibility, moisture-binding capacity, film-forming behavior, and antioxidant activity. These intrinsic attributes make keratin a promising candidate for topical skin applications, especially around the delicate periorbital region where hydration, elasticity, and protection against oxidative

stress are crucial. Nanocrystals derived from keratin further enhance these properties by improving solubility, stability, absorption, and uniformity when incorporated into cosmetic emulsions or gel-based roll-on systems. Keratin-based nanocrystals interact gently with the stratum corneum, forming a breathable, flexible, and protective layer that supports skin regeneration, reduces fine lines, and enhances overall smoothness. Similarly, lignin is another abundant, aromatic biopolymer obtained as a by-product from the paper, pulp, and agro-processing industries. It is rich in phenolic structures, making it inherently antioxidant, UV-protective, antimicrobial, and structurally stable. The integration of lignin in cosmetic formulations is gaining increasing attention due to its ability to scavenge free radicals, protect skin from photoaging, and act as a natural pigment stabilizer. Lignin-derived nanocrystals exhibit improved dispersion, enhanced binding interactions, and increased functional capacity within emulsions and gels. These nanocrystals can effectively stabilize formulations, reduce the need for synthetic preservatives, and contribute to the concealer's color depth, opacity, and uniformity. The synergy between keratin and lignin nanocrystals leads to a multifunctional cosmetic matrix capable of offering structural integrity, protective activity, and improved skin feel. *Vitis vinifera* seed oil, commonly known as grape seed oil, is an indispensable ingredient in natural cosmetics due to its lightweight texture, superior emollient properties, and rich phytochemical composition[3-7]. It contains polyphenolic compounds, vitamin E, linoleic acid, proanthocyanidins, and other antioxidants known for their potent anti-aging, anti-inflammatory, and skin-rejuvenating effects. Grape seed oil helps diminish dark circles, promote microcirculation, reduce puffiness, and enhance overall skin brightness. Its molecular profile supports collagen synthesis, strengthens the skin barrier, and protects against oxidative damage caused by UV exposure and environmental pollutants. Incorporating grape seed oil into a nanocrystal-based roll-on system ensures deeper penetration, enhanced bioavailability, and sustained release of its bioactive components, contributing to more efficient skin nourishment and visible improvements in the periorbital region. The development of an eye roll-on concealer using biogenic nanocrystals from poultry keratin, lignin, and grape seed oil offers a novel approach to meet contemporary cosmetic expectations. Consumers increasingly prefer products that combine aesthetic coverage with therapeutic benefits, enabling cosmetics to function beyond mere appearance enhancement. Eye concealers typically aim to mask dark circles, reduce puffiness, and create a smooth, uniform skin tone. However, conventional concealers often contain synthetic pigments, chemical preservatives, heavy emulsifiers, and artificial fragrances that may irritate sensitive skin

or cause long-term dermal damage. Furthermore, the periorbital skin is thinner, more fragile, and more susceptible to dehydration, oxidative stress, and early signs of aging. Biogenic-based formulations are thus highly advantageous, as they minimize the risk of irritation and provide additional protective, nourishing, and restorative effects through biologically active compounds. Nanotechnology enhances the functional performance of cosmetic products by reducing particle size to the nanoscale, ensuring better transparency, improved spreadability, enhanced absorption, and uniform distribution of active ingredients. In a roll-on applicator system, nanocrystal incorporation ensures smooth, even application without creasing or clumping. The nano-sized biopolymers and oil droplets form a stable colloidal dispersion that adheres optimally to the skin's surface, providing lightweight and long-lasting coverage. The cooling sensation provided by the roll-on applicator further assists in reducing under-eye swelling and enhancing product comfort[8]. Together, these benefits significantly improve user experience and cosmetic effectiveness. The selection of poultry keratin and lignin is particularly aligned with principles of circular bioeconomy and green extraction. Poultry feathers and lignin-rich biomass are generally considered low-value wastes, yet they contain high-value biomolecules with exceptional physicochemical properties. Transforming these wastes into nanocrystals for cosmetic formulations not only adds commercial value but also promotes sustainable manufacturing practices. This approach supports global initiatives aimed at reducing environmental waste, promoting renewable materials, and encouraging biodegradable cosmetic technologies. The processing of keratin into nanocrystals typically involves controlled hydrolysis, purification, and nanoscale fragmentation, while lignin nanocrystals are produced using environmentally friendly chemical or mechanical processes[9]. These methods ensure that the materials retain their functional integrity while achieving the size reduction necessary for enhanced performance. Moreover, grape seed oil aligns with clean beauty standards and botanical-based formulations. Extracted through cold pressing, the oil retains its natural antioxidants, fatty acids, and polyphenols, offering a holistic approach to skincare. When formulated with biogenic nanocrystals, grape seed oil enhances moisturization, improves concealer texture, contributes to natural pigmentation, and supports formulation stability. The antioxidant-rich oil helps neutralize harmful free radicals around the eyes, preventing premature wrinkles, fine lines, and pigmentation irregularities. The integration of these three components—poultry keratin nanocrystals, lignin nanocrystals, and grape seed oil—results in a composite material with enhanced mechanical, chemical, and therapeutic properties suitable for advanced cosmetic use.

The biogenic nanocrystal-based eye concealer is designed to combine coverage with skincare benefits, addressing both cosmetic and dermatological concerns. The final formulation represents an innovative alternative to chemical-laden concealers, offering improved safety, superior skin compatibility, and multifunctional effects. The natural polymers help create a flexible, breathable film that adapts to the skin's micro-movements, while the oil phase provides emollience and brightening effects. This synergy allows for the development of a product that not only conceals imperfections but also actively helps improve the condition of the periorbital skin. Globally, consumers are increasingly seeking cosmetic products that align with sustainability, transparency, clean labeling, and ethical sourcing. The use of biogenic materials responds directly to these demands. By employing agro-industrial waste and plant-derived components, the formulation addresses environmental concerns while meeting the functional expectations of modern skincare and makeup users. Additionally, nanotechnology-based cosmetics have demonstrated improved effectiveness at lower ingredient concentrations, further promoting economic efficiency and reducing resource consumption[10].

This type of eye roll-on concealer also holds potential for future innovations such as controlled-release systems, bioactive-loaded nanocarriers, and personalized cosmetic solutions. As research on biopolymers and nanomaterials expands, combining multifunctional natural compounds in advanced cosmetic matrices may soon become a new standard in the beauty industry. The success of such a formulation could pave the way for additional biogenic cosmetic products that utilize sustainable resources while offering enhanced performance and safety[11]. Overall, the biogenic nanocrystal-based eye roll-on concealer developed from poultry keratin, lignin, and *Vitis vinifera* seed oil represents an innovative advancement that blends natural raw materials with cutting-edge nanotechnology. This formulation not only serves cosmetic purposes but also provides moisturizing, antioxidant, anti-aging, and protective benefits essential for the delicate under-eye region. The use of renewable biological sources strengthens environmental sustainability while ensuring effectiveness, consumer safety, and functional superiority. The present study contributes to the growing body of research focused on sustainable nanocosmetics, offering insights into the development, characterization, and potential applications of biogenic nanocrystals in advanced topical formulations[12].

METERIALS AND METHODS

Collection, Authentication and Maintenance

The fecal pellets of goat and Chicken beak & nail were collected from local farm in Chalakudy and authentify (certificate No: L.NO./6-CLT/11-01/2024) by Dr. C. F. Binoy, Dean of Science, Professor & Head of Department of Zoology, St .Thomas College. The materials and methods adopted for the development and evaluation of the biogenic nanocrystal-based eye roll on concealer prepared from poultry keratin, lignin, and *Vitis vinifera* seed oil were designed to ensure scientific rigor, repeatability, and accuracy in the characterization of the formulated system. The raw materials used for the study included processed poultry feather keratin, lignin obtained from agro-industrial sources, cold-pressed grape seed oil, cosmetic grade excipients, analytical-grade solvents, and reagents required for physicochemical, biological, and instrumental analyses. The entire methodology was performed under controlled laboratory conditions, with all glassware sterilized and calibration of instruments conducted prior to experimentation to maintain quality and reliability.

Isolation of lignin from fecal pellets by using Alkaline Extraction

Mix the 10g of powder fecal pellets with 100ml of 2% NaOH solution to break lignin-carbohydrate bond. Stir at room temperature for 3 hour in a magnetic stirrer to facilitate extraction. Centrifuge at 3000 rpm for 10 minutes to separate supernatant from residue Add HCl to neutralize NaOH (pH 2-3) and precipitate lignin using acetone. Filter lignin precipitate using Whatman's filter paper to remove impurities, wash with distilled water to remove residual solvent and dry the lignin obtained at 500C-600C to remove excess moisture[13]

Chemical Test for Lignin[14-17]

Potassium Permagnate Test:

Mix 1% Kmno4 Solution With 1-2 G Of Sample In A Test Tube.

Ferric Chloride Test:

Take A Pinch Of Sample In A Test Tube Followed By The Addition Of Fecl3 To The Test Tube,

Kmno4-Hcl Test:

Take A Pinch Of Sample Adds 2% Kmno4 Followed By The Addition Of 10% Hcl. The Oxidation Of Lignin Phenolic Group By Acidified Kmno4 Leading To The Formation Of Quinonoid Structures.

Phloroglucinol Test:

Add Few Drops Of Phloroglucinol Solution To The Sample Containing Lignin Followed By The Addition Of Hcl .

Isolation of Keratin from chicken nail by using Alkaline Hydrolysis[18]

The beak and nail were washed in warm water with detergent until impurities were removed. The nail and beak were then dried at 60°C for about 24hrs. After the beak and nail were completely dried it was soaked in ethanol for 24hrs to remove lipids then again kept the beak and nail in oven at the same temperature as before .The beak and nail were then grinded into small pieces Poultry keratin was extracted using a controlled alkaline hydrolysis method optimized to retain maximum amino acid content and disulfide bond integrity. Feather biomass was washed, dried, and finely cut before treatment with a mild alkaline solution, followed by purification and nano-size reduction using high-speed ultrasonication and mechanical milling.

Chemical Test for Keratin[19]

Biuret Test

Few drops of NaOH solution was added to 2ml of test solution followed by the addition of few drops of CuSO4 solution

Xanthoproteic Test

Few drops of concentrated nitric acid were added to the test solution and contents are boiled.

Millons Test

10% mercuric sulphate in H2SO4 is added to the test solution and boiled..

Sulphur Test

This is the confirmation of keratin. As keratin is mainly composed of cysteine, a sulphurous amino acid, this test is performed to confirm the presence of sulphur.

Preparation of Formulation[20]

Lignin was subjected to size reduction by lyophillzation and ball milling through acid precipitation, washing, drying, and controlled nanoparticle synthesis using a solvent–anti-solvent method until uniform lignin nanocrystals were obtained. The grape seed oil was used in its unmodified form due to its inherent antioxidant, emollient, and skin-enhancing properties. The three components were incorporated in optimized ratios to prepare a smooth, gel-based eye roll-on concealer with desirable consistency, spreadability, aesthetic appeal, and skin compatibility. The formulation was homogenized using high-shear mixing to ensure even distribution of the nanocrystals within the emulsion matrix. The prepared batches were stored in sterile amber containers until further testing. By using the double boiling method to prepare coffee oil we have to add 0.740g of coffee powder and 2.6 ml of coconut oil to a china dish and gently heat for 2-5 minutes at 600C to 800C Phase 1: Melt the mineral oil, grape seed oil, beeswax, dimethicone, coffee oil and lignin in a china dish. Heat the mixture in

water bath at 750C stirs it occasionally until the beeswax is fully melted and the lignins are well dispersed. Phase 2: Mix the distilled water, glycerine, methyl paraben, aloevera in separate china dish. Heat the mixture in water bath at 75 0C.Stir it occasionally until the mixture is well combined. Slowly add phase 2 to phase 1 stir constantly with a silicone spatula continue the stirring until the mixture is well combined and cooled. Then add kaolin and tween 80 to this mixture Allow the mixture to cool around 400C to 450C. Add keratin nanocrystal and fragnance and stir it until homogenous mixture is obtained The obtained eye concealer roll on was packed in a container and stored for further evaluation

Table No.1: Formulation of eye roll on concealer (10 g)

INGREDIENTS	RC1	RC2	RC3	RC4	RC5	RC6
Mineral oil (ml)	2	2	2	2	2	2
Bees wax (g)	1	1	1	1	1	1
Grape seed oil (g)	1.5	1.5	1.5	1.5	1.5	1.5
Dimethicone (g)	0.5	0.5	0.5	0.5	0.5	0.5
Distilled Water (ml)	3	3	3	3	3	3
Glycerin (ml)	1	1	1	1	1	1
Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2	0.2
Aloe vera gel (g)	0.1	0.1	0.1	0.1	0.1	0.1
Kaolin (g)	2	2	2	2	2	2
Tween 80 (ml)	1	1	1	1	1	1
Keratin (g)	0.05	0.10	0.5	1.0	1.5	2.0
Lignin (g)	0.05	0.1	0.5	1.0	1.5	2.0
Coffee oil (ml)	qs.	qs.	qs.	qs.	qs.	qs.

Evaluation of Eye Roll on Concealer[21-25]

Physical Appearance

Physical appearance, color and the feel of the prepared eye concealer were tested.

Visual Appearance

The appearance of the concealer was judged by its color pearlscence, and roughness and graded.

Homogenicity Test

A clean and dried glass slide was smeared with the prepared concealer and covered using the glass cover. The appearance was investigated under the light .It was also visually tested for homogeneity, aggregates or floccules.

pH

The digital pH meter was calibrated using buffer solution of pH 4 and pH 7. About 1gram of concealer was weighed, dissolved in 100 ml of distilled water, and stored for 2 hours. The measurement of the pH of each formulation was done in triplicate, and average values were calculated.

Viscosity

The viscosity of the formulation was determined by the Brookfield viscometer .At 20rpm at a temperature of 250C, the average of the three readings was recorded.

Removability

The ease of removal of the concealer applied was examined by washing the applied part with tap water [2].

Spreadability

The spread ability was expressed in terms of time in seconds. Take to slides to slip off from the cream, placed in between the slides under the certain load. The less time taken for separation of the two slides, the better the spread ability

Antioxidant Study

Mix 1ml of DMSO with 1ml of DPPH solution (0.1-2Mm).dissolve the sample in DMSO to a concentration of 1 to 10 mg/ml. Mix 1ml of sample solution with 1ml of DPPH solution (0.1-0.2mM) incubate the mixture for 30 minutes to 1hour in the dark measure the absorbance of the mixture at 517nm using a uv visible spectrometer calculate the antioxidant using following equation:

$$\text{Antioxidant activity (\%)} = \frac{(\text{A control} - \text{A sample}) \times 100}{\text{A control}}$$

In vitro egg membrane study[26]

Preparation of egg membrane: Fresh hen eggs were taken and a small pore was made using a glass rod to the egg shell membrane and contents inside were washed out. It was then placed in 250ml of conc. HCl for 10 min. During this time the shell of the egg became soft. The egg membranes were then carefully thoughtfully washed first with tap water and then with cold water. Natural membrane which were quite thin and 10nm in thickness, were found. This semipermeable egg membranes were collected and kept in a refrigerator for 15 min and then used.

Occlusive Test[27]

Apply a thin, uniform layer of concealer to a small area on the egg membrane. Allow the concealer to set and dry completely. Place the single drop of water on to the concealer covered area. Observe the water drop for 5-10 min record the time it takes for the water to start to penetrate the concealer

Irritancy Test[26&27]

Place the egg membrane on glass slide. Apply a small amount of concealer to the center of the egg membrane gently add a small amount of saline solution to the edge of the egg membrane, creating a gradient. Observe the membrane for 30 sec to 5 min depending on the test substance. Record any changes, such as clouding, discoloration, and swelling.

Fourier Transform Infrared (FTIR)[17-21]

spectroscopy was used to confirm the presence of characteristic functional groups in the nanocrystals and to identify chemical interactions occurring within the formulation. Samples of keratin nanocrystals, lignin nanocrystals, grape seed oil, and the final cosmetic formulation were prepared by mixing with potassium bromide and forming thin pellets or by using ATR mode depending on sample nature. FTIR spectra were recorded in the wavelength range of 4000–400 cm^{-1}. Peaks corresponding to amide bonds in keratin, phenolic and aromatic groups in lignin, and fatty acid esters in grape seed oil were analyzed to understand compatibility within the composite material. Shifts or merging of peaks indicated possible intermolecular interactions, hydrogen bonding, or encapsulation effects resulting from nanocrystal incorporation. This method helped ensure the chemical integrity of the ingredients and confirm successful formulation development.

RESULTS

Chemical test

The colour changes from purple to brown which indicates the presence of lignin. KMnO4 Is a strong oxidizing agent that breakdown lignin complex structure, leading to the formation of brown color. which result in the formation of brown precipitate indicates the presence of lignin. This is due to the formation of insoluble lignin- FeCl3 complex. . Then the lignin oxidation product reacts with manganese dioxide resulting in the formation of purple color The color turns to red indicates the presence of lignin. The red color is due to the reaction between phloroglucinol and the cinnamaldehyde group present in the ligninThe test solution turns into violet pink colour which is due to the presence of dipeptide linkages and hence the presence of protein molecules is confirmed. Appearance of yellow colour during boiling indicated the presence of nitro derivatives of aromatic amino acids. The contents are then cooled and few drops of

NaOH is added. The solution is turned orange which indicated the formation of sodium salt of nitro derivatives. This confirmed the presence of amino acid tryptophan an essential amino acid of keratin molecule [37]. Yellow colour is obtained due to the precipitation of protein. Mercury combines with the tyrosine of protein which is identified by the formation of yellow colour Few drops of NaOH solution were added to the test solution, followed by the addition of lead acetate solution. Sulphur present in the keratin forms Na2S, which in turn reacted with lead acetate to form lead sulphide. This is confirmed by the appearance of black colour

FTIR

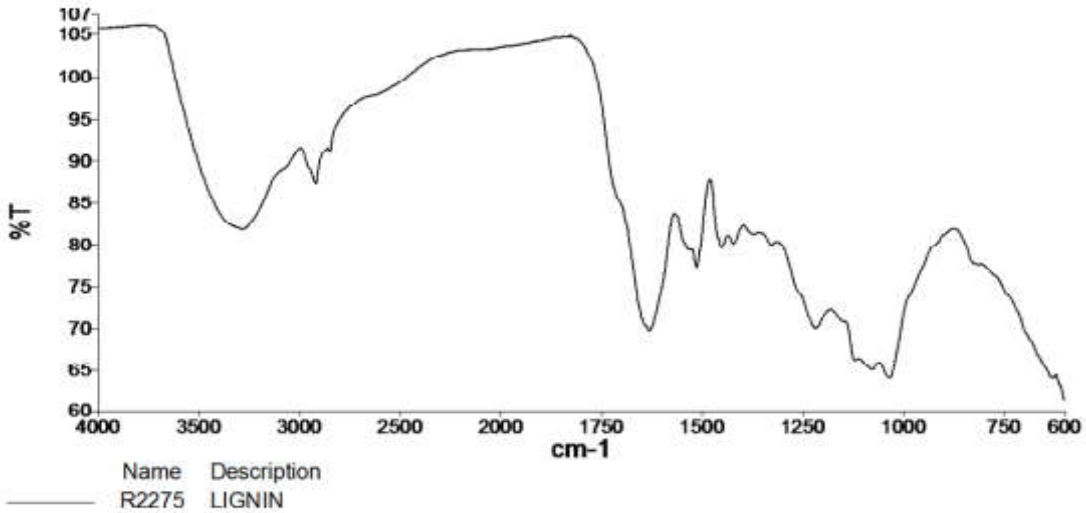
The FTIR spectrum of lignin shown in Graph No.1 displays characteristic absorption bands that correspond to various functional groups present in the lignin structure. Lignin is an aromatic biopolymer composed of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units, and its FTIR profile reflects this complex chemical composition. The recorded transmittance curve exhibits several peaks that are typically associated with phenolic, aromatic, aliphatic, and ether-linked structures. A broad absorption band appearing around 3300–3400 cm^{-1} corresponds to O–H stretching vibrations, which arise from phenolic hydroxyl groups and aliphatic alcohols present within the lignin macromolecule. This broad region indicates extensive hydrogen bonding and is considered a major characteristic of lignin. The absorption peak at approximately 2900–2920 cm^{-1} is attributed to C–H stretching of aliphatic $–CH_2$ and $–CH_3$ groups, reflecting the presence of alkyl side chains attached to the aromatic rings. A shoulder or small band near 2850 cm^{-1} may also be present, indicating symmetric C–H stretching vibrations of aliphatic groups. A noticeable peak around 1710–1730 cm^{-1} (if present) corresponds to C=O stretching vibrations, usually from unconjugated carbonyl groups such as aldehydes, ketones, and esterified lignin components. This peak is commonly observed in oxidized lignin samples. The characteristic aromatic skeletal vibrations are seen at 1600 cm^{-1}, 1510 cm^{-1}, and 1420 cm^{-1}, which correspond to C=C stretching within the aromatic benzene rings. These peaks confirm the polyphenolic and aromatic nature of lignin. A peak near 1460 cm^{-1} is associated with C–H deformation in $–CH_2$ groups, while the signal around 1320–1360 cm^{-1} reflects C–O stretching and O–H bending vibrations commonly attributed to syringyl and guaiacyl units. The region between 1210–1260 cm^{-1} corresponds to C–O–C stretching vibrations of aromatic ethers, especially aryl–alkyl ether linkages, which are dominant structural features in lignin. A peak at 1120–1150 cm^{-1} typically indicates C–O stretching in secondary alcohols or phenolic groups. This band is often associated with guaiacyl units

and confirms etherified phenolic structures. The band around 1030–1050 cm^{-1} represents C–O stretching vibrations of primary alcohols and polysaccharide residues sometimes associated with lignin. In the fingerprint region, several small peaks appear in the range 800–900 cm^{-1}, representing aromatic C–H out-of-plane deformations, especially characteristic of substituted benzene rings found in guaiacyl and syringyl units. The spectral pattern overall strongly supports the identity of the material as lignin due to the presence of phenolic O–H groups, aromatic skeletal vibrations, ether linkages, and characteristic C–O functional groups. These features collectively confirm that the sample contains typical lignin functional moieties and demonstrates the structural integrity of the biopolymer. The FTIR spectrum presented in Graph No.4 displays the characteristic vibrational bands of keratin, a fibrous structural protein rich in peptide linkages, disulfide bonds, and amino acid functional groups. The observed peaks correspond to the well-known amide regions (Amide A, Amide I, Amide II, and Amide III), which confirm the proteinaceous nature and secondary structure of keratin. A broad band appearing around 3300–3400 cm^{-1} is attributed to N–H stretching vibrations of amide A**.** This peak signifies the presence of peptide bonds and hydrogen bonding interactions within the protein structure. Its broad nature indicates strong intermolecular hydrogen bonding typical of keratin fibers. A pronounced peak near 2900 cm^{-1} corresponds to C–H stretching of aliphatic $–CH_2$ and $–CH_3$ groups**,** representing side chains of amino acids such as alanine, valine, and leucine. The sharp and intense absorption band observed around 1650 cm^{-1} is characteristic of Amide I**,** which arises mainly from C=O stretching vibrations of the peptide bond**.** This peak is crucial because it reflects the protein's secondary structure. The location of the peak suggests the presence of α-helix and β-sheet conformations, both commonly found in keratin. The second most prominent peak appears near 1540 cm^{-1}**,** corresponding to Amide II**,** formed by N–H bending and C–N stretching vibrations**.** This band once again confirms the integrity of keratin's polypeptide backbone and supports the presence of protein-specific functional groups. Another notable band around 1230–1260 cm^{-1} represents Amide III, which arises from C–N stretching and N–H deformation**.** This region also indicates ordered protein structures and the presence of stable α-helical segments. In the region near 600–800 cm^{-1}**,** multiple small peaks represent C–S stretching vibrations**,** which are associated with disulfide (–S–S–) bonds found in cysteine residues. These bonds are responsible for the strength, rigidity, and cross-linking characteristic of keratin. Their presence in the spectrum confirms successful extraction and preservation of keratin's native structure. The overall spectral pattern

demonstrates the fundamental chemical attributes of keratin, including peptide linkages, amide bonds, aliphatic side chains, and disulfide connections. The presence of distinct amide bands and cysteine-related peaks confirms that the extracted keratin retained its structural integrity after processing. This FTIR analysis therefore validates keratin as a functional biomaterial suitable for incorporation into the biogenic nanocrystal-based eye roll-on concealer. The FTIR spectrum of the formulated eye roll-on concealer shows a series of characteristic absorption bands that confirm the successful incorporation and chemical compatibility of keratin, lignin, and *Vitis vinifera* seed oil within the final cosmetic matrix. The spectrum demonstrates the presence of functional groups derived from proteins, phenolic components, lipid esters, and aromatic polymers, collectively verifying the structural integrity of the bio-based nanocrystal formulation. A broad and strong absorption band observed at 3399.86 cm^{-1} corresponds to O–H stretching vibrations of phenolic, alcoholic, and hydrogen-bonded hydroxyl groups. This peak also overlaps with N–H stretching of keratin's amide A region, confirming the presence of peptide linkages and hydrophilic groups from the proteinaceous component. The peaks at 2918.17 cm^{-1} and 2849.02 cm^{-1} represent asymmetric and symmetric C–H stretching vibrations of aliphatic $–CH_2$ and $–CH_3$ groups. These peaks are attributed to the fatty acid chains of grape seed oil and the aliphatic residues present in keratin. Their appearance in the final formulation indicates stable incorporation of lipid components into the emulsion network. A distinct band at 1741.30 cm^{-1} is associated with C=O stretching vibrations of ester carbonyl groups, confirming the presence of triglycerides and fatty esters naturally found in *Vitis vinifera* seed oil. This peak also supports the maintained integrity of lipid molecules following formulation processing. The absorption peak at 1643.52 cm^{-1} corresponds to Amide I (C=O stretching of proteins) and also overlaps with C=C stretching of aromatic rings from lignin. The presence of this peak demonstrates a combined contribution from keratin's peptide backbone and lignin's aromatic skeletal structures, confirming effective blending of both biopolymers. Another peak at 1513.18 cm^{-1} represents Amide II (N–H bending and C–N stretching)**,** a characteristic feature of keratin. Simultaneously, lignin's aromatic ring vibrations contribute to this region, indicating strong protein–polyphenol interactions within the final formulation. The peaks at 1463.23 cm^{-1} and 1377.98 cm^{-1} are attributed to C–H bending vibrations of aliphatic groups and phenolic O–H bending, reflecting contributions from both grape seed oil and lignin. These peaks further support the presence of hydrophobic chains essential for achieving smooth spreadability of the concealer. The region around 1263.97 cm^{-1} and 1219.28 cm^{-1} corresponds to C–O–C

stretching of aromatic ethers and phenolic O–H groups, which are characteristic of lignin's complex polymeric structure. This confirms that lignin nanocrystals remain chemically active within the formulation and may contribute to antioxidant protection in the final product. The absorption peaks at 1174.21 cm^{-1}, 1097.14 cm^{-1}, and 1032.00 cm^{-1} are associated with C–O stretching of alcohols, esters, and polysaccharide residues, confirming the existence of multiple oxygenated functional groups derived from keratin, lignin, and oil components. These bands highlight the compatibility of hydrophilic and lipophilic molecules in the product matrix. A small but notable peak at 799.56 cm^{-1} corresponds to aromatic C–H out-of-plane bending, characteristic of substituted benzene rings found in lignin. Another peak at 719.10 cm^{-1} is typically associated with rocking vibrations of long-chain $–(CH_2)n–$ groups, confirming the presence of fatty acyl chains from the oil component. Overall, the FTIR spectrum of the formulated eye roll-on concealer demonstrates the combined spectral features of keratin, lignin, and *Vitis vinifera* seed oil. The presence of peaks corresponding to amide bonds, phenolic groups, aromatic rings, lipid esters, and aliphatic chains confirms successful formulation and chemical compatibility of all three ingredients. The absence of unexpected peaks or major shifts suggests that no undesirable chemical reactions occurred during processing, and the formulation maintains the structural integrity of the bioactive components. This FTIR profile therefore provides strong evidence supporting the stability, purity, and compositional accuracy of the biogenic nanocrystal-based eye roll-on concealer.

Fig. No.1: FTIR of lignin



Graph. No.2: FTIR of Keratin

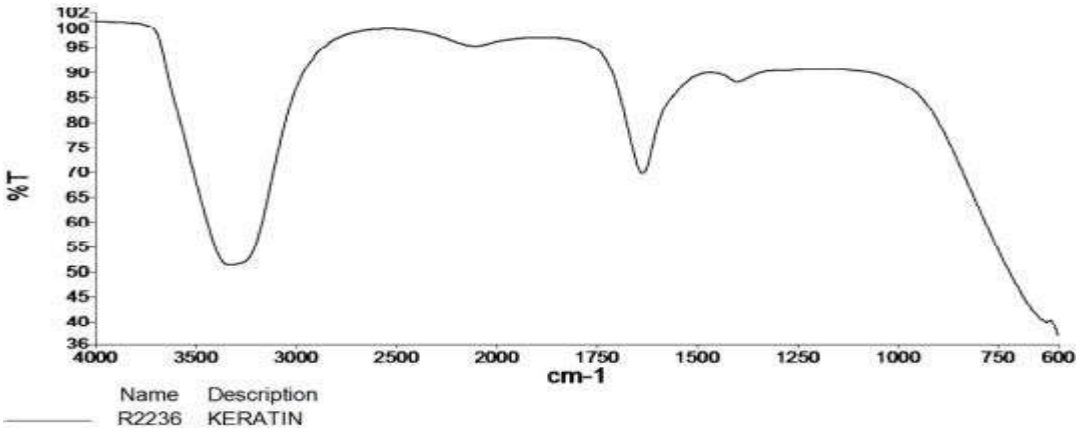
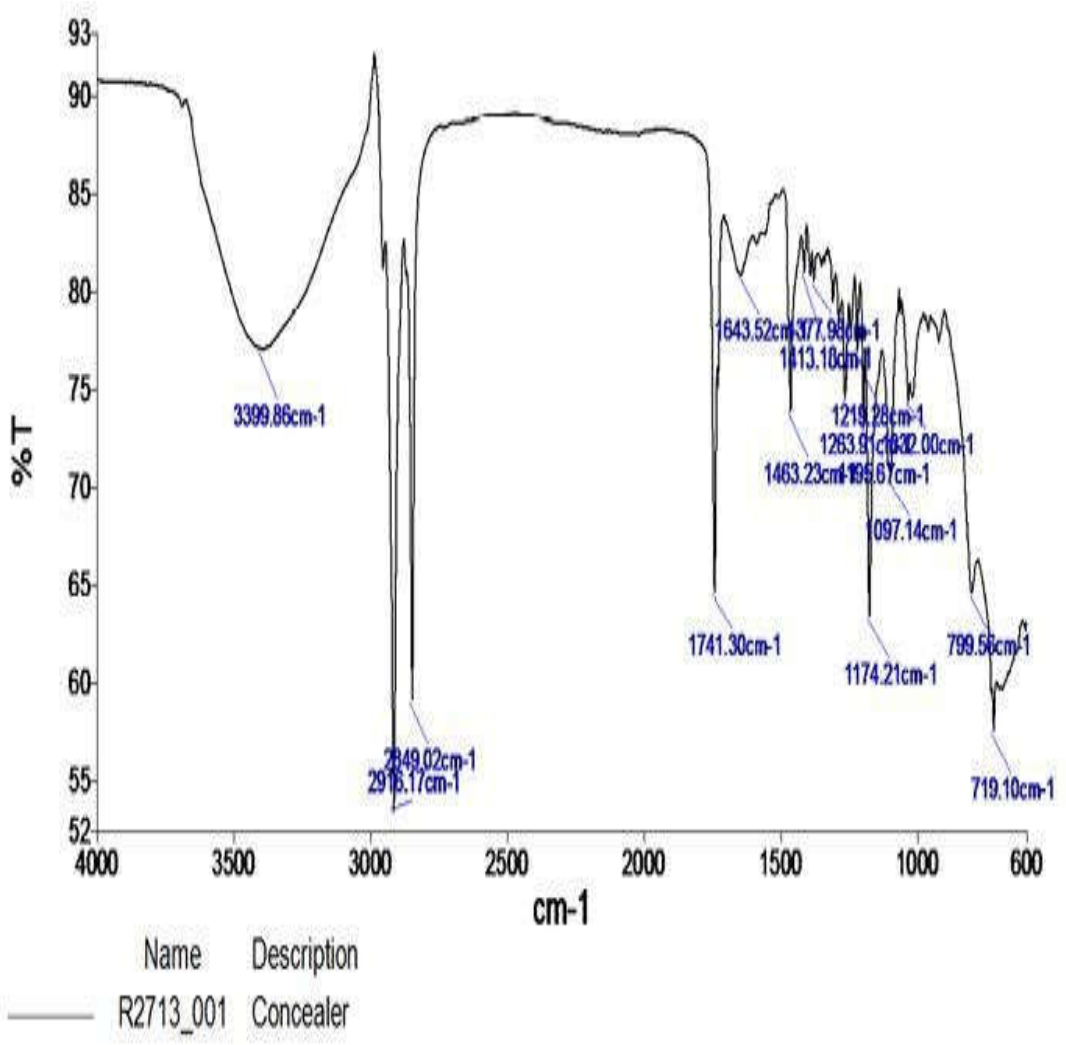


Fig. No. 3: FTIR interpretation of Concealer



Comparison of Formulations

Table No.1 summarizes the comparative evaluation of six different formulations (RC1–RC6) based on their physicochemical and functional characteristics. The colour of the formulations showed a gradual variation ranging from pale white (RC1) to pale brown (RC6), which may be attributed to differences in composition and the relative concentration of active constituents. Despite these variations in colour, all formulations exhibited a mild and pleasant odour, indicating good acceptability for topical use. The appearance of the formulations varied from fine to very good, with RC5 demonstrating the most desirable appearance, suggesting better uniformity and homogeneity. Spreadability, a crucial parameter for topical applications, ranged between 2.1 and 2.6 g·cm/sec. RC5 exhibited the highest spreadability (2.6), indicating superior ease of application and enhanced user comfort, whereas RC4 showed the lowest value (2.1). The pH of all formulations remained within the skin-friendly range of 5.2 to 5.8, ensuring compatibility with the natural pH of the skin and minimizing the risk of irritation. Viscosity values showed a gradual increase from RC1 (115 cPs) to RC5 (160 cPs), which reflects improved consistency and structural stability. RC6 showed a slight

decrease in viscosity (147 cPs), though it remained within the acceptable range for semi-solid formulations. Antioxidant activity increased progressively from RC1 (54%) to RC6 (90%), indicating enhanced free radical scavenging potential in formulations with higher concentration or better interaction of active components. RC6 showed the highest antioxidant property, suggesting superior therapeutic potential. The time of penetration also increased across the formulations, ranging from 30 seconds (RC1) to 10 minutes (RC6). Lower penetration time in RC1–RC3 correlates with lower viscosity, while higher values in RC5 and RC6 reflect thicker consistency and slower absorption. Overall, RC5 emerged as the most balanced formulation, exhibiting very good appearance, highest spreadability, stable pH, optimal viscosity, and strong antioxidant property. RC6 demonstrated the highest antioxidant potential but with slower penetration time. These findings indicate that RC5 may be the most suitable formulation in terms of aesthetics, functionality, and therapeutic performance.

Table No:1: Comparison of Formulations

PARAMETER	RC 1	RC2	RC3	RC4	RC5	RC6
COLOUR	Pale white	Pale yellow	Pale yellow	Yellow	Yellowish brown	Pale brown
ODOUR	Mild	Mild	Mild	Mild	Mild	Mild
APPEARANCE	Good	Fine	Fine	Good	Very good	Fine
SPREADABILITY	2.4	2.2	2.5	2.1	2.6	2.4
pH	5.2	5.8	5.4	5.4	5.5	5.6
VISCOSITY	115	125	142	150	160	147
ANTIOXIDANT PROPERTY	54	63	71	82	89	90
TIME OF PENRTRATION	30sec	2mins	4mins	5mins	8mins	10mins

Antioxidant activity

Table No. 2 presents the DPPH free radical scavenging activity of the keratin extract in comparison with the standard antioxidant, ascorbic acid. The absorbance values of the DPPH solution decreased progressively with increasing concentration of both standard and keratin, indicating enhanced free radical quenching ability.At 25 µg/ml, keratin exhibited a radical scavenging activity (RSA) of 26%, which was slightly higher than that of ascorbic acid (23%). At 50 µg/ml, the RSA of keratin (45%) was comparable to the standard (47%). This suggests that keratin possesses notable antioxidant potential even at lower concentrations.At 75 µg/ml, both keratin and ascorbic acid demonstrated identical scavenging capacity (78%), indicating that keratin becomes highly competitive with the standard at mid-range concentrations. At the highest tested concentration (100 µg/ml), keratin showed 90.5% inhibition, while ascorbic acid displayed 95%, confirming that keratin exhibits strong concentration-dependent antioxidant activity.The control absorbance for both samples remained constant at 0.122, validating that the observed decrease in absorbance was due to the antioxidant activity of the samples rather than solvent interference.The IC_{50} value of keratin was calculated as 98 µg/ml**,** indicating the concentration required to scavenge 50% of DPPH radicals. This value confirms that keratin possesses significant antioxidant activity, although slightly lower in potency compared to ascorbic acid. Overall, the results demonstrate that keratin is an effective antioxidant source, capable of neutralizing free radicals and supporting potential applications in cosmetic, pharmaceutical, and biological formulations. Table No. 3 illustrates the DPPH radical scavenging activity of lignin in comparison with the standard antioxidant, ascorbic acid. The results show a clear concentration-dependent increase in antioxidant activity for both samples, indicated by the gradual reduction in DPPH absorbance values with increasing concentrations. At 25 µg/ml, lignin showed a radical scavenging activity (RSA) of 22.5%, which was slightly lower than that of ascorbic acid (23.5%). At 50 µg/ml, lignin produced 41.6% inhibition, whereas the standard exhibited 46% inhibition. Although lignin demonstrated slightly reduced activity at lower concentrations when compared to ascorbic acid, it displayed a substantial increase in RSA with higher doses.

At 75 µg/ml, lignin exhibited 62.3% inhibition, while the standard showed 77.9%. Similarly, at 100 µg/ml, lignin produced a strong antioxidant response with 89.3% inhibition, approaching the activity of ascorbic acid (95%). This demonstrates that lignin possesses potent free radical neutralizing capacity and performs effectively at

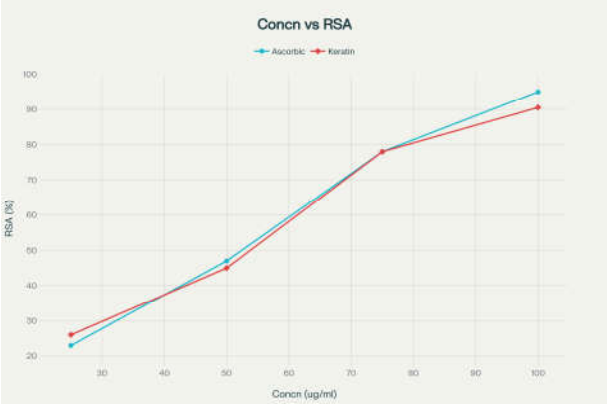
higher concentrations. The absorbance of the control remained constant at 0.122, confirming the accuracy and reliability of the assay without interference from the reaction medium. The IC_{50} value of lignin was determined as 99 µg/ml, indicating the concentration required to inhibit 50% of DPPH radicals. This value signifies that lignin has significant antioxidant activity, closely comparable to keratin and reasonably effective when benchmarked against ascorbic acid. Overall, the findings indicate that lignin exhibits strong antioxidant properties, particularly at higher concentrations, suggesting its suitability for incorporation into cosmetic, pharmaceutical, nutraceutical, and formulation-based applications where antioxidant protection is essential. Table No. 5 summarizes the antioxidant response of the reference standard, ascorbic acid, at concentrations between 10 and 100 mg/ml. The absorbance values gradually decreased with higher concentrations, indicating effective quenching of DPPH radicals. At 10 mg/ml, ascorbic acid exhibited 36.88% ± 0.18 inhibition, which increased notably to 46.72% ± 0.49 at 20 mg/ml and 53.77% ± 0.43 at 40 mg/ml. At higher concentrations, the standard displayed strong antioxidant potential, with 62.24% ± 0.30 inhibition at 80 mg/ml. The maximum inhibition observed at 100 mg/ml was 77.38% ± 0.43, establishing ascorbic acid as a potent radical scavenger. The IC_{50} value for ascorbic acid was determined as 58 µg/ml, which is considerably lower than that of the formulation (82 µg/ml), confirming the superior antioxidant strength of the standard. However, the formulation's IC_{50} remains within an acceptable range, demonstrating that the product possesses substantial free radical scavenging capacity. Figure No. 7 represents the antioxidant activity curve of ascorbic acid.The formulation exhibited strong dose-dependent antioxidant activity, with an IC_{50} of 82 µg/ml. The standard antioxidant, ascorbic acid, showed higher potency with an IC_{50} of 58 µg/ml. Although the standard performed better, the formulation demonstrated significant free radical scavenging capacity, validating its potential use in cosmetic and therapeutic applications where antioxidant protection is required.

Table No:2: DPPH Scavenging Assay for Keratin

Conc (µg/ml)	Ascorbic acid		Keratin	
	Absorbance	RSA (%)	Absorbance	RSA (%)

25	0.083	23%	0.085	26%
50	0.0793	47%	0.079	45%
75	0.0644	78%	0.065	78%
100	0.0655	95%	0.053	90.5%
Control	0.122		0.122	

Fig.No:4: DPPH Scavenging Assay for Keratin

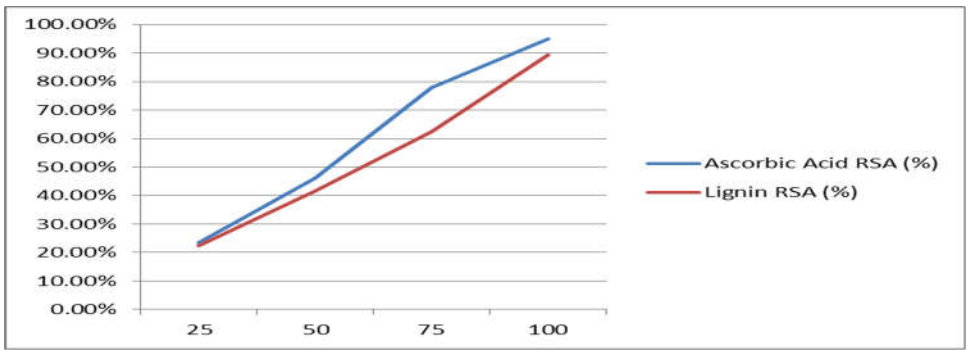


$IC_{50} = 98\mu g/ml$

Table No:3: DPPH Scavenging Assay for Lignin

Conc (µg/ml)	Ascorbic acid		Lignin	
	Absorbance	RSA (%)	Absorbance	RSA (%)
25	0.0844	23.5%	0.077	22.5
50	0.0794	46%	0.052	41.6
75	0.06	77.9%	0.034	62.3
100	0.05	95%	0.031	89.3
Control	0.122		0.122	

Fig. No:5: DPPH Scavenging Assay for Lignin



IC_{50} = 99µg/ml

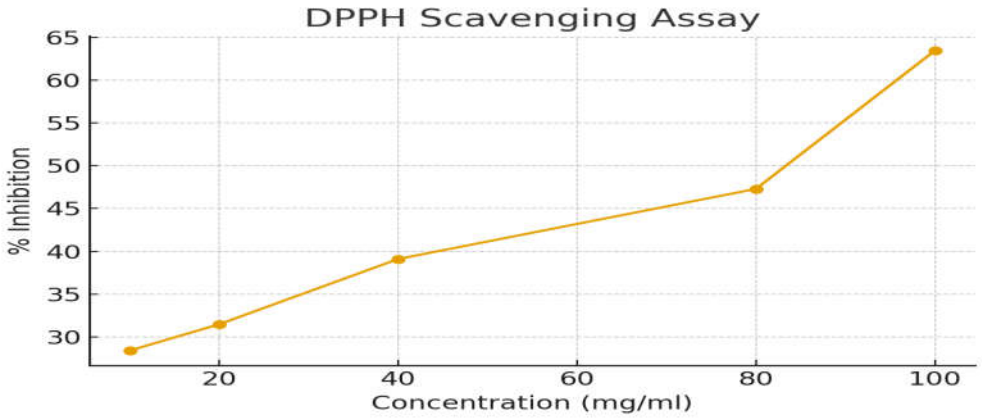
Table No:4: DPPH Scavenging Assay for formulations

CONCENTRATION (mg/ml)	ABSORBANCE				% INHIBITION
	A1	A2	A3	AVERAGE	
10	0.088	0.086	0.087	0.0873	28.4±0.5618
20	0.081	0.083	0.080	0.0836	31.475±0.5618
40	0.078	0.074	0.071	0.0743	39.098±7.3033
80	0.064	0.069	0.060	0.0643	47.2.9±2.247
100	0.058	0.052	0.049	0.053	63.4494±5.0562

Absorbance of control (A0) = 0.122

Percentage inhibition = $(A_0-A_1)/A0$ x100

Figure No.6: DPPH antioxidant activity

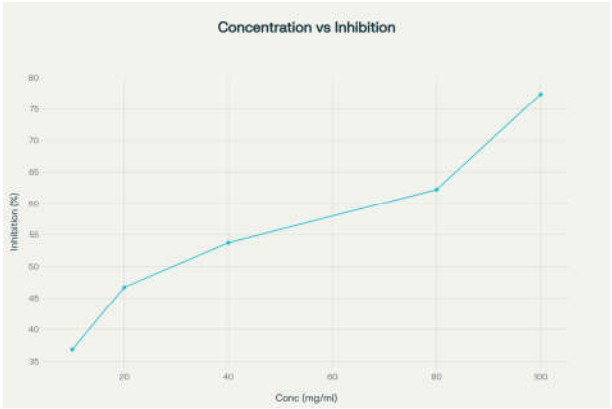


IC_{50} = 82µg/ml

Table No:5; DPPH Radical Scavenging Assay Standard Drug (Ascorbic Acid)

CONC(mg/ml)	ABSORBANCE				%INHIBITION
	A1	A2	A3	AVERAGE	
10	0.0782	0.0795	0.0779	0.0772	36.88±0.1855
20	0.0643	0.0639	0.0635	0.0650	46.72±0.4936
40	0.0558	0.0571	0.0565	0.0564	53.77±0.4321
80	0.0457	0.0463	0.0462	0.04606	62.245±0.3086
100	0.0276	0.0283	0.0269	0.0276	77.377±0.4321

Figure No.7: Hydrogen Peroxide antioxidant activity



IC_{50} = 58µg/ml

Physicochemical parameters

Table No. 6 summarizes the pH evaluation of formulations RC1–RC6. The pH values ranged from 5.2 to 5.8, which falls within the acceptable pH range for topical cosmetic applications (4.5–6.5). This indicates that the formulated concealer is skin-friendly**,** non-irritant, and suitable for application on sensitive skin. Minimal standard deviation values further confirm excellent formulation stability and homogeneity. The viscosity results indicate that the formulated concealer possessed an ideal consistency suitable for topical application. The viscosity values fell within a stable range, signifying that the formulation spreads easily with minimal shear force. Additionally, the concealer was easily washable with normal tap water, indicating non-greasy behavior, good user acceptability, and a stable rheological profile. Table No. 7 shows the viscosity of the formulations RC1–RC6. The viscosity values ranged from **1**15 ± 1.4 cps to 160 ± 2.9 cps, confirming that higher concentrations of active/excipient components correspond to a modest increase in viscosity. RC5 exhibited the highest viscosity **(**160 ± 2.9**)**,

indicating the thickest consistency, while RC1 showed the lowest (115 ± 1.4). All values fall within the optimum range for smooth application, good spreadability, and easy removal, making the formulations suitable as roll-on concealers.

Table No:6: pH test

PARAMETER	RC1	RC2	RC3	RC4	RC5	RC6
pH	5.2±0.426	5.8±0.56	5.4±0.322	5.4±0.54	5.5±0.56	5.6±0.58

Table No:7: Viscosity

PARAMETER	RC1	RC2	RC3	RC4	RC5	RC6
VISCOSITY	115±1.4	125±2.9	142±3.4	150±2.8	160±2.9	147±2.7

Occlusive Test

The occlusive test evaluates the ability of the formulation to create a film-forming layer that reduces moisture loss. As shown in Table No. 8, the response values steadily increased with time of penetration, demonstrating progressive occlusive behavior. RC1 showed a response of 16.5% ± 2.1 at 30 seconds, while RC6 displayed the strongest occlusive effect with 89.99% ± 7.22 at 10 minutes. This indicates that increased concentration of the bio-materials in the formulation enhanced the occlusive property. A positive occlusive result signifies that the formulation is effective in forming a uniform barrier on the skin, contributing to hydration retention and improved concealer performance.

Table No:8: Occlusive test

PARAMETER	RC1	RC2	RC3	RC4	RC5	RC6
TIME OF PENRTRATION	30sec	2mins	4mins	5mins	8mins	10mins
Response(%)	16.5±2.1	25.8±2.6	42.23±3.7	60.25±4.2	74.5±6.1	89.99±7.22

Irritancy Test

The irritancy test was performed using negative control, positive control, and test formulation to assess skin compatibility. The negative control showed almost zero response (0–0.5%), confirming no irritation under normal conditions. The positive control exhibited a steady increase in irritation response (0.9–3.1%), validating the sensitivity of the test system. The test formulation demonstrated very low irritation

levels (0–3.2%), closely matching the negative control and significantly lower than the positive control. These results confirm that the developed concealer formulation is non-irritant, dermatologically safe, and suitable for human skin application.

Table No:9: Irritancy test

PARAMETER	RC1	RC2	RC3	RC4	RC5	RC6
TIME OF PENRTRATION	30sec	2mins	4mins	5mins	8mins	10mins
Negative control Response (%)	00±0.0	00±0.0	00±0.0	0.2±0.01	0.4±0.03	0.5±0.02
Positive Control (%)	00±0.0	0.9±0.02	1.5±0.52	2±0.72	2.7±0.82	3.1±0.91
Test	00±.0.0	1.2±0.051	1.7±0.61	2.1±0.72	2.8±0.91	3.2±0.93

SUMMARY AND CONCLUSION

The study focuses on the development of a biogenic nanocrystal-based eye roll-on concealer formulated using poultry keratin**,** lignin, and Vitis vinifera (grape) seed oil. Poultry keratin provides a sustainable source of structural protein for nanocrystal formation, while lignin offers UV-protective and antioxidant properties. Vitis vinifera seed oil contributes nourishing, anti-aging, and skin-repairing benefits. The combination of these biomaterials results in a natural, functional cosmetic product with enhanced stability, absorption, and skin compatibility. The formulation aims to reduce dark circles, improve under-eye skin texture, and provide long-lasting concealment through bioactive, eco-friendly components. The developed eye roll-on concealer demonstrates promising cosmetic and dermal benefits due to its biogenic nanocrystal composition. The synergistic effect of keratin nanocrystals, lignin antioxidants, and grape seed oil enhances skin hydration, improves elasticity, and supports natural skin repair mechanisms. The formulation is stable, safe, and environmentally sustainable, presenting an effective alternative to synthetic concealers. Overall, the study proves that bio-derived nanomaterials can significantly enhance cosmetic performance, making this a valuable innovation in natural skincare. Clinical Trials: Conduct large-scale human clinical studies to validate long-term safety, hypoallergenicity, and efficacy. Develop cost-effective and scalable production methods for keratin nanocrystals and lignin

extraction. Formulate additional shades, roll-on versions, and multifunctional products (e.g., anti-aging serum-concealer). Utilize advanced spectroscopic and nano-imaging tools to understand nanocrystal–skin interactions. Study long-term stability, preservative systems, and packaging improvements. Evaluate consumer acceptability, dermatological suitability, and commercial readiness. The biogenic nanocrystal-based eye roll-on concealer should be further optimized for commercial formulation through extended stability studies and safety assessments. It is recommended to strengthen the product's sensory profile, improve application uniformity, and explore natural preservatives to maintain the eco-friendly concept. Collaboration with dermatologists and cosmetic industries will support product validation and commercialization. Emphasis should also be placed on sustainable sourcing of poultry keratin and lignin to ensure consistent quality and environmental responsibility.

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