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Extraction and Characterization of Melanin Nanoparticles from *Gallus gallus domesticus* Feathers

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ABSTRACT

Melanin, a multifunctional biopolymer naturally found in birds, has demonstrated significant potential as a reducing agent. This study reports the extraction and characterization of melanin nanoparticles derived from the waste feathers of domestic chickens (*Gallus gallus domesticus*). Melanin was extracted using an acid-base method, confirmed through UV-Vis spectroscopy, thin-layer chromatography (TLC), and transmission electron microscopy (TEM). The pigment exhibited a characteristic peak at 230 nm in UV spectroscopic analysis, an TLC exhibit the Rf value of melanin is 0.67, and Transmission electron microscopy reveals the melanin nanoparticle breadth of ~315 nm and had elongated shape with rough-surface area. Its reducing ability was validated through a Methylene Blue Reduction Test (MBRT).

INTRODUCTION

The term *melanin* is derived from the Greek word *melanos*, signifying 'black' or 'very dark', aptly describing the pigment's distinctive coloration. This designation was initially introduced in 1840 by the Swedish chemist Jöns Jacob Berzelius to identify a dark pigment isolated from ocular membranes[1]. Melanin possesses an ancient lineage as a naturally occurring pigment, with its origins tracing back to the earliest forms of life on Earth. Its presence has been identified in remarkably well-preserved dinosaur fossils, primitive avian feathers, various plant species, marine cephalopods, as well as in diverse microorganisms such as bacteria and fungi[2,3]. The incorporation of melanin within the barbules occurs through ultra-fine processes, operating at sub-micrometre to nanometre precision, which are instrumental in generating the intricate colouration observed in avian feathers[4]. Feathers exhibit remarkable coloration attributed to pigment deposition and nanostructural light scattering [5,6]. Pigmentary coloration is conferred by molecules such as melanins, carotenoids, and porphyrins etc., with melanins being the most widespread and functionally significant [5]. Melanin pigments, particularly eumelanin and pheomelanin, are biosynthesized within melanocytes and stored in organelles called melanosomes. These pigments not only produce coloration ranging from black to reddish hues but also confer structural stability, resistance to wear, thermoregulation, and protection against ultraviolet (UV) radiation [7–9].

Melanin is synthesized via the oxidation of tyrosine or L-3,4-dihydroxyphenylalanine L-DOPA and subsequent polymerization into high-molecular-weight pigments with conjugated aromatic structures. This molecular configuration endows melanin with broadband optical absorption, high thermal stability, antioxidant capacity, and metal-binding properties [10,11]. Eumelanin typically results in black or brown coloration and consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) units [12]. Pheomelanin, containing sulfur, imparts yellow-to-reddish hues and is less thermally stable [13]. Other forms include neuromelanin, present in the human brain, and allomelanin, found in plants and fungi [14,15].

Recent advances in material science have sparked renewed interest in melanin as a bioinspired nanomaterial. Melanin nanoparticles (MeNPs) derived from natural sources possess intrinsic biocompatibility, hydrophilicity, and multifunctional redox activity [16]. These properties make them excellent candidates for biomedical applications including photoprotection, drug delivery, bioimaging, and tissue engineering [17–19]. Furthermore, their environmental relevance has been highlighted in applications such as pollutant adsorption, catalytic degradation, and heavy metal remediation [20,21].

While melanin can be extracted from microbial and synthetic routes, valorizing bio-waste such as poultry feathers for melanin recovery aligns with the principles of green chemistry and circular economy [22]. The underutilized feather waste of *Gallus gallus domesticus* offers a renewable source of pigment-rich material, and its conversion into value-added nanomaterials presents a sustainable strategy for waste management and material innovation [23,24].

Despite these promising characteristics, comprehensive studies on the green synthesis and physicochemical characterization of melanin nanoparticles from poultry feathers remain limited. Recent advances have demonstrated that melanin-derived nanostructures exhibit strong biocompatibility, excellent antioxidant capacity, and unique optical properties, enabling applications in drug delivery, photoprotection, bioimaging, and pollutant remediation [25–27]. These nanoparticles have shown effective radical scavenging and light-absorbing capabilities due to their conjugated polymer structure, making them attractive for use in eco-friendly nanomaterials [28,29]. Moreover, the valorization of poultry feather waste into melanin nanoparticles contributes to circular economy strategies by converting biowaste into high-value functional materials [30]. Therefore, this work aims to extract melanin using an acid-base precipitation technique from *Gallus gallus domesticus* feathers and explore its optical, morphological, and redox properties for potential applications in nanotechnology and environmental sustainability [25–30]. for extracting melanin from poultry feather waste and evaluating its potential as a nanoscale functional material.

Melanin is a ubiquitous biological pigment found in animals, plants, fungi, and microorganisms, recognized for its distinctive black-brown coloration and multifunctional biological roles, including photo protection, antioxidation, immune modulation, and metal ion chelation [30,31]. In birds, feathers are rich in melanin, particularly eumelanin and pheomelanin, which not only contribute to coloration but also provide structural reinforcement and environmental resilience [32]. Among natural sources, the feathers of *Gallus gallus domesticus* —commonly discarded as bio-waste—represent a sustainable, underutilized resource for melanin extraction [30,33].

In recent years, melanin has emerged as a promising bioinspired nanomaterial owing to its biocompatibility, biodegradability, hydrophilicity, and redox activity [16]. Importantly, its broadband UV absorption and radical scavenging ability further enhance its utility in eco-friendly material science [34-37].

Despite growing interest, only limited studies have explored the direct conversion of feather-derived melanin into functional nanoparticles. Developing scalable and sustainable synthesis methods from avian bio-waste aligns with principles of green chemistry and circular economy [38,39]. This research aims to develop an efficient acid-base extraction protocol to obtain melanin from poultry feathers and to characterize the resulting melanin nanoparticles for their optical, morphological, and redox features to assess potential applications in nanotechnology and environmental sustainability.

1. Materials and Methods:

2.1 The methodology adopted in this study was carefully designed to maintain eco-friendly standards while ensuring reproducibility and effectiveness of melanin nanoparticle extraction and characterization. Previous research has emphasized the importance of sustainable practices for nanoparticle synthesis from natural sources, and our methods are in alignment with these standards [40,41]. Feathers of *Gallus gallus domesticus* were collected from local poultry shops. The feathers were thoroughly washed and dried.

2.2 Extraction of Melanin:

Melanin was extracted following an acid-base protocol adapted from previously published green chemistry approaches [42-45]. 50 grams of dried feathers were boiled in water and subsequently placed in 1M NaOH solution, followed by autoclaving at 121 °C for 30 minutes to solubilize the melanin. The solution was then centrifuged, and the supernatant obtained was used for the precipitation of melanin pigment. Concentrated HCl (37%) was added to the supernatant until the pH reached 1 to facilitate melanin precipitation. The precipitated melanin was collected and refluxed. The final product was washed with deionised water, followed by ethanol and water again. The purified melanin was dried, stored in an airtight container, and kept in a refrigerator until further use. This method has been shown to yield melanin of sufficient purity for Nano technological applications, while maintaining eco-sustainability and cost-effectiveness [46].

2.3 Characterization of Extracted Melanin UV-Visible spectroscopy:

UV-Visible spectroscopy was conducted in the 200–1000 nm range to identify characteristic peaks, following methods validated in prior studies on natural melanin pigments [47,48].

2.4 Thin-layer chromatography (TLC):

TLC was performed using silica gel plates with a solvent system of n-butanol: acetic acid: deionized water (70:20:10), a combination previously shown effective in distinguishing melanin's migration profile [49].

2.5 Solubility Test:

Solubility was tested in various solvents including water, ethanol, acetone, 0.1 M NaOH and 0.1 M KOH to confirm its solubility behaviour [50].

2.6 Methylene Blue Reduction Test:

To evaluate the antioxidant and redox potential of the extracted melanin nanoparticles, a Methylene Blue Reduction Test (MBRT) was conducted following modifications of previously validated protocols [51]. In test tube no.1, a mixture of 1 mL of 0.01% methylene blue and 4 mL of melanin suspension in 0.1 M KOH was prepared. In test tube no. 2 only 5 ml of 0.01% methylene blue was taken as control whereas in test tube no. 3, 1 ml of 0.01% methylene blue and 4 ml of 0.1 M KOH was taken to study the reaction of KOH and Methylene blue. The fading of blue colour was monitored visually to determine the electron-donating ability of the particles. This method, traditionally used to assess microbial respiration and electron donation, is applied here for the first time to confirm melanin identity and functionality in nanoparticle form. The adoption of MBRT as a chemical confirmation of melanin's redox properties adds a novel dimension to nanoparticle characterization and aligns with the emerging emphasis on green, functional nanomaterials [52,53]. (MBRT) To evaluate redox activity, 1 mL of 0.01% methylene blue solution was mixed with 4 mL of melanin suspension in KOH. Discoloration over time was observed as an indicator of electron-donating capacity.

2.7 Transmission electron microscopy (TEM) was performed to examine the morphological characteristics and nanoparticle size of the extracted melanin nanoparticles. The analysis was carried out at ICON Analytical Equipment Pvt. Ltd., Mumbai, India, using a high-resolution transmission electron microscope.

3. Results and Discussion:

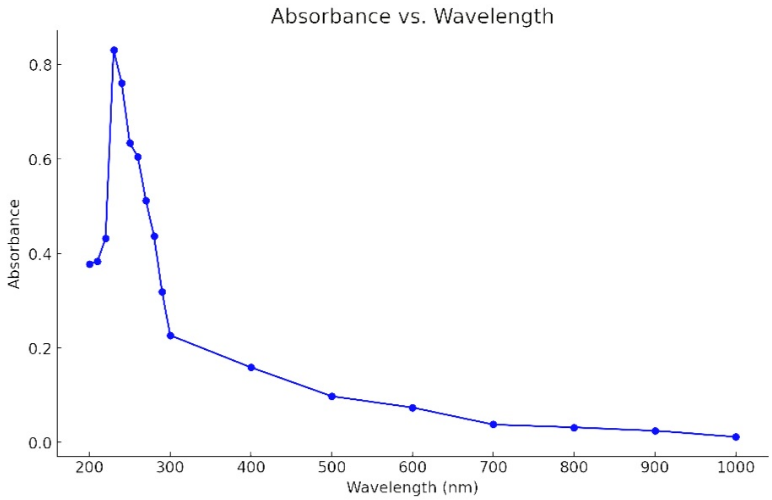

Figure 1. *UV-Visible absorption spectrum of melanin pigment extracted from Gallus gallus domesticus , manually plotted in Microsoft Excel using recorded absorbance values from 200–1000 nm. The graph exhibits a maximum absorbance near 230 nm, a hallmark of eumelanin, with a gradual decrease toward longer wavelengths—confirming broadband absorption typical of biologically derived melanin.*

3.1 UV-Vis Analysis Report of Melanin nanoparticles

The UV–Vis spectrum of the synthesized material exhibits a broad and intense absorbance peak near 230 nm, followed by a gradual decrease in absorbance extending up to 1000 nm. This spectral behaviour is characteristic of melanin, particularly eumelanin, which is known for its broadband absorption across the UV-visible spectrum, typically demonstrating high absorbance in the UV range (200–300 nm) and a monotonic decline towards the visible and near-infrared regions [54]. Several studies on both natural and synthetic melanin's—derived from sources such as *Sepia*, fungi, and avian feathers—consistently report maximal absorbance in the 220–280 nm range, further substantiating the identity of the present material as melanin [55,56]. Notably, the absence of sharp spectral features reinforces the material's heterogeneous and amorphous nature, while the long absorbance tail into the 800–1000 nm region confirms its characteristic broadband UV–visible absorption. Collectively, these observations corroborate the successful extraction of melanin nanoparticles and align with the established optical signatures of eumelanin.

3.2 The thin-layer chromatography (TLC)

To confirm the identity and purity of the extracted melanin, thin-layer chromatography (TLC) was performed. The result, shown in Fig. 4.2, further confirms the effective isolation of melanin nanoparticles from *Gallus gallus domesticus* feathers. A distinct violet-colored spot with an R*f* value of 0.67 was recorded, based on the ratio of the distance travelled by the compound to that of the solvent front. This value closely matches the R*f* of 0.68 reported by Deepthi et al. (2014) for melanin extracted from cuttlefish ink under similar chromatographic conditions [47]. The single, well-defined spot observed on the TLC plate suggests a high degree of purity of the extracted pigment.

Figure 2. *Thin-layer chromatographic profile of melanin extracted from Gallus gallus domesticus , displaying a single violet-colored spot with an Rf value of 0.67.*

3.3 Physical Appearance and Solubility

The extracted pigment was visually observed to be black in colour, which is characteristic of eumelanin-type polymers [57]. A series of solubility tests were conducted in various solvents to determine the chemical nature of the pigment. The pigment was insoluble in distilled water and common organic solvents including methanol, ethanol, and acetone. However, it demonstrated complete solubility in inorganic alkaline solutions such as 1 M NaOH and 1 M KOH. This solubility pattern is a hallmark of melanin and was consistent with previous literature on fungal and microbial melanin, which is known to be insoluble in most neutral and organic solvents but soluble in alkaline conditions due to the deprotonation of its phenolic groups [50,57]. These results strongly indicate the successful extraction of melanin with typical physicochemical characteristics.

Table 1. *Physical appearance and solubility behavior of melanin extracted from Gallus gallus domesticus .*

Sr. No.	Test	Result
1.	Colour Observation	Black

2.	Solubility test: i. Distilled water ii. Organic solvents: • Methanol • Ethanol • Acetone iii. Inorganic alkaline solutions: • 1M NaOH • 1M KOH	Insoluble Insoluble Insoluble Insoluble Soluble Soluble

3.4 Methylene Blue Reduction Test (MBRT)

Figure: 3 Right -to-Left photographic representation of the Methylene Blue Reduction Test (MBRT), highlighting the redox potential of melanin nanoparticles extracted from *Gallus gallus domesticus*. In test Tube no. 1, (rightmost) displays marked decolourization of methylene blue following the addition of melanin, indicating a pronounced electron-donating (reducing) capability. Test Tube no. 2 (centre) serves as the control and retains its original blue coloration, confirming the absence of any reductive interaction. Test tube no. 3, (leftmost) exhibits a cobalt blue hue resulting from an alkaline-induced pH shift caused by the addition of KOH to methylene blue, demonstrating the colorimetric sensitivity of the dye under basic conditions.

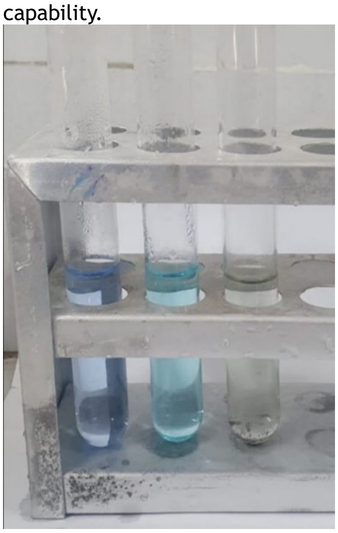

Table 2. *Methylene Blue Reduction Test results showing the redox activity of melanin extracted from Gallus gallus domesticus* .

Test No.	Components	Observation
1	Dil. Methylene Blue + Melanin + 0.1 M KOH	Methylene blue colour disappears
2	Dil. Methylene Blue	No significant colour change
3	0.1 M KOH + Dil. Methylene Blue	Colour changes from light blue to cobalt blue

In **Test Tube 1 (rightmost)**, the complete disappearance of the characteristic methylene blue colour upon mixing with melanin nanoparticles confirms their pronounced electron-donating and reducing capabilities. This behaviour is typical of eumelanin, which possesses redox-active quinone and hydroquinone moieties capable of reducing electron-accepting dyes such as methylene blue. The observed reduction is attributed to functional groups such as phenols and indoles, which facilitate electron transfer reactions through redox cycling mechanisms [58]. In contrast, **Test Tube 2 (centre)**, containing only a dilute methylene blue solution, exhibited no visible change, serving as a negative control to validate that the reduction in Test Tube 1 was specifically induced by the melanin extract. Meanwhile, **Test Tube 3 (leftmost)** presented a cobalt blue hue upon the addition of KOH to methylene blue, consistent with an alkaline-induced spectral shift rather than a redox reaction. This pH-dependent colour change underscores the sensitivity of methylene blue to environmental conditions but distinguishes it mechanistically from the reduction observed with melanin. Collectively, these findings confirm the functional identity of the pigment as melanin and demonstrate its potential utility in redox-sensitive applications, including biosensing, photodynamic therapy, and environmental remediation.

3.5 Transmission electron microscopy (TEM)

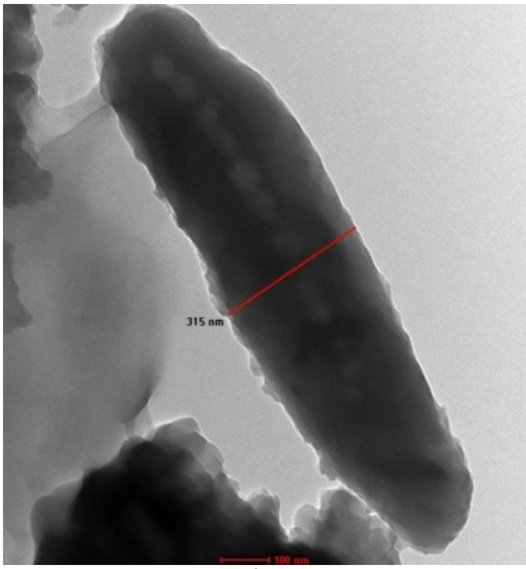

Figure 3. Transmission electron microscopy (TEM) image of a melanin nanoparticle extracted from *Gallus gallus domesticus*, revealing an elongated, rod-like morphology with an approximate diameter of 315 nm. The rough surface texture and irregular boundaries are characteristic of natural eumelanin, indicating successful particle formation suitable for nanotechnological applications.

Transmission electron microscopy (TEM) of the melanin extract revealed irregularly shaped, elongated nanoparticles with rough surface morphology. The nanoparticle shown in the image measures approximately 315 nm in width, consistent with scale dimensions typical of biologically derived melanin.

This morphology aligns with previous observations of eumelanin nanoparticles, which often exhibit non-uniform, rod-like or aggregated structures due to the inherent heterogeneity and amorphous nature of melanin biopolymers [59]. The rough surface and tendency to form compact aggregates are attributed to π–π stacking and hydrogen bonding interactions among the polymeric units [8,54].

Furthermore, the size distribution falling in the 200–400 nm range is well within the reported values for naturally sourced melanin nanoparticles, particularly those obtained from fungal, marine, and poultry waste materials [39]. The visible boundary and contrast in the TEM image also indicate a dense, carbon-rich composition — another hallmark of melanin [54,59].

These morphological traits are significant, as nanostructured melanin with such characteristics has demonstrated enhanced antioxidant, photo thermal, and drug-carrying potential, making it suitable for applications in biomedicine, coatings, and environmental remediation [60].

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