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Review Article

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OVERVIEW OF MELANIN PIGMENTS AND THEIR ANALYTICAL DETECTION: A REVIEW

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ABSTRACT

The highly molecular weight melanin pigments which are widespread in living organisms are usually formed due to the oxidation and polymerization of phenolic compounds into eumelanin, pheomelanin and neuromelanin. Almost of these pigments are stable, insoluble and biochemical substance resistance. They have a strong ability to absorb the ultraviolet radiation and scattering the light. There properties facile their photoprotective activity against skin damage. The distribution of melanin pigments in the body of living organisms not only gave the bright and attractive color but also have impact role in certain disorders such as melanoma or skin cancer. In this review, we introduced a general overview for melanin pigments, including their definition, physico-chemical properties, biological action, the possible biosynthesis pathways and the relation of melanin with certain disorders. Finally, we also provided the important analytical methods that used for the quantification of melanin in diverse samples.

KEYWORDS: Melanin; Biosynthesis pathway; Melanin Chemistry; Analytical quantification of melanin; Skin pigmentation.

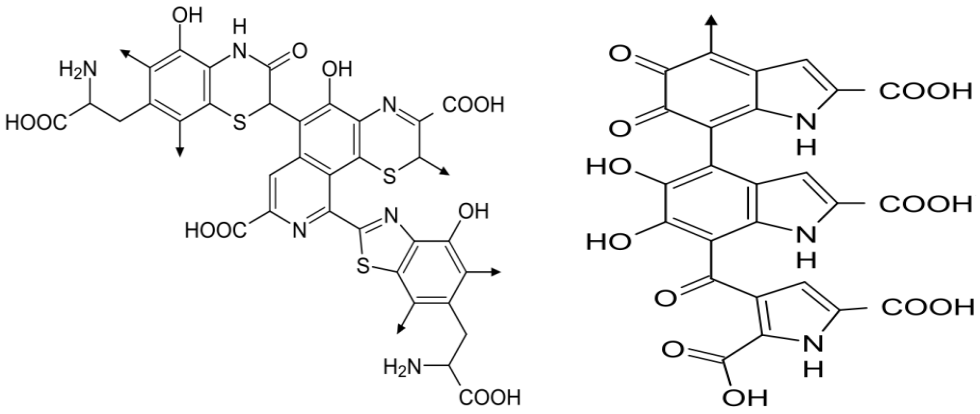
INTRODUCTION

What is melanin?

Melanin is a class of natural compounds act as pigments which produced due to the oxidation and polymerization of tyrosine. They are found in specialized cells in the most organisms called melanocytes.[1-4] There are at least three naturally occurring kinds of melanin, the basic types of them are eumelanin, which is found in two types, dark brown and black.[5,6] It is responsible for the color of skin, eyes and hair and the color are primarily controlled by melanin content.[7,8] It provides the color of pupils and irises of the eyes.[9] Also, it protects the human body from skin damage as a result of sun exposure.[10] Pheomelanin is a diametric pigment which is a cysteine with a benzothiazine red polymer. Most of the pigmented tissues are mixed with both eumelanin and pheomelanin.[11] Neuromelanin is the third type of melanin; it is a dark insoluble pigment which presents in central nervous system, particularly in sunstantia nigra as well as the internal tissues of brain, medulla and zona reticularis of the adrenal gland.[12] Also, it is widely found in animals [13,14] and plants.[15]

Chemistry of melanins

Despite of many researchers worked hardly for many decades to communicate the biochemistry, biophysics with cell pigmentation. We still have not got a clear picture of the macromolecular structure of melanin pigment in its synthetic state or naturally occurring protein bounded state. But, there is a primary agreement that melanins have unique macromolecules with undefined chemical structure and molecular weight. Eumelanin polymer is characterized as a heterogeneous macromolecule which obtained from the oxidation states of 5,6-dihydroxyindole and 5,6- dihydroxyindole-2-carboxylic acid units with pyrrole units.[16] Eumelanin has a general absorption ranging from 200 to 2400 nm and IR absorption in the range of 2.5 to 10 μm.[17] Pheomelanin is also, a heterogeneous macromolecule derived from the sulfur containing cysteinyldopa (Figure 1).



Pheomelanin **Eumelanin**

Figure 1: Chemical Structure of Pheomelanin and Eumelanin.

Biosynthesis pathway of both eumelanin and pheomelanin

The biosynthesis of melanin is located in a specialized skin cells in the basal layer of epidermis in humans, mammals and birds.[18] These cells produce the two black and dark brown types of eumelanin as well as yellow to reddish brown pheomelanin.[19] Dopaquinone is the same precursor to drive both melanin types. They are produced by initial reaction involved the hydroxylation of L-tyrosine amino acids to 3,4-dihydroxyphenylalanine (DOPA) by the aid of tyrosinase enzyme which is considered for the synthesis of all types of melanin.[20] Then DOPA extensive series of oxidation reactions biopolymer melanin is produced.[21-25]

The main biosynthesis pathway of both eumelanins and pheomelanins is based on the catalytic activity of the tyrosinase enzyme. Firstly, tyrosine by the catalytic action of tyrosinase enzyme produces DOPA which converted into dopaquinone. Then, the formation of pheomelanin can produce by two ways; when the dopaquinone combined with cysteine to form 5-S-cysteinyldopa then benzothiazine as an intermediate compound then finally give pheomelanin. Another way is the combination of dopaquinone with cysteine to form 2-S-cynteinyldopa and produce benzothiazine intermediate to form pheomelanin (Figure 2).

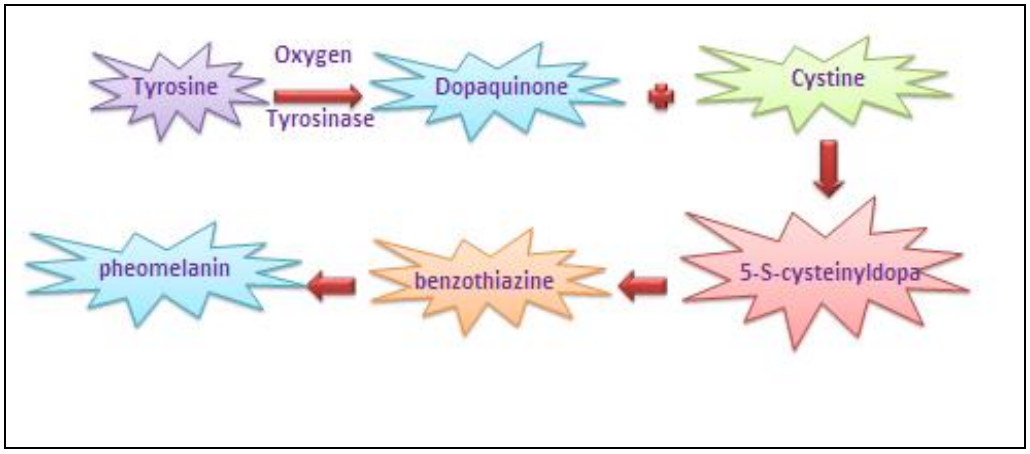


Figure 2: Schematic diagram for pheomelanin biosynthesis.

Melanin can be formed by another biosynthesis pathway which is the conversion of dopaquinone into leucodopachrome. The first step of eumelanin formation is based on the conversion of dopaquinone into leucodopachrome then to dopachrome which gives 5, 6-dihydroxyindole-2-carboxylic acid and by oxidation converted into quinone then eumelanin. The second pathway is the conversion of dopaquinone into leucodopachrome then dopachrome which gives 5,6-dihydroxyindole then by oxidation gives quinone and finally eumelanin (Figure 3).

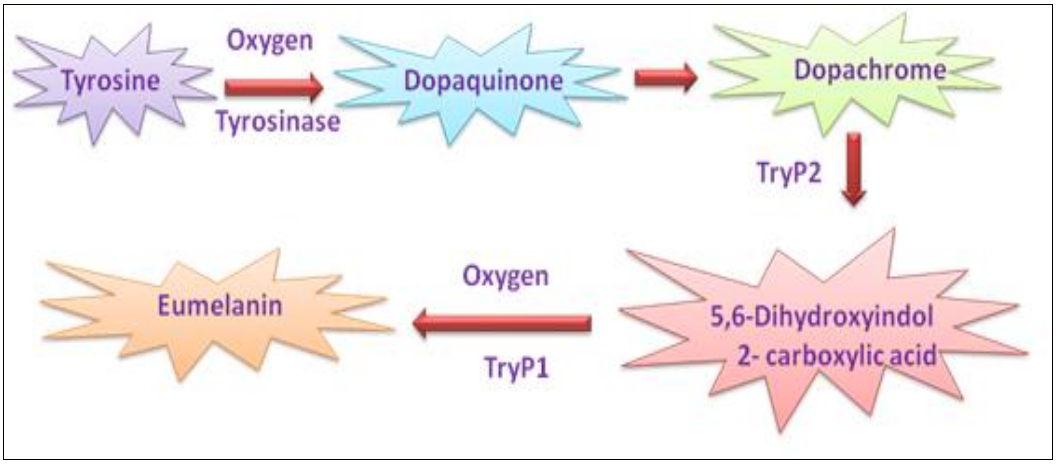


Figure 3: Schematic diagram for Eumelanin biosynthesis.

Physico-chemical properties of melanin

Melanin has two key functions, pigmentation and photoprotection of the skin cells from the harmful UV radiation.[26] It is generally accepted that there is a significant correlation between the human skin resistance to UV radiation and the amount of pigments in the skin.[27] Armstrong and Kricker [28] are believed that the incidence of UV radiation is closely related to the skin cancer. In addition the individuals who are genetically have low skin ability to tan and pigmentation had a high susceptibility to skin malignant melanoma. The photoprotective properties of melanin are attributed to its highest ability to absorb and scatter the higher energy photons from the ultraviolet (UV) and the blue part of the solar spectrum. By ultrafast photodynamics, the energy of the absorbed photons will be converted quickly into heat.[29] Furthermore, the photoprotective action of melanin is also due to its ability to quench the excited state of certain molecules that produced in pigmented cells. Also melanin was recognized by its powerful activity as antioxidant due to its activity as a reactive oxygen species (ROS).[30] It has the ability to inhibit the free radical reactions and hence shielding the polyunsaturated phospholipids against oxidative modification.

Biological properties of melanin

Biologically, melanin has various functions including skin pigmentation, hair and feather coloration, strengthening plant cell walls and insect cuticle. It has the ability to absorb ultraviolet and visible light and responsible for the rmoregulation, photoprotection and camouflage of skin. Commercially, melanin is recommended as an active component of photoprotective creams and it is also used as a potential target for anti-melanoma therapy.[31]

In the animal kingdom, the role of pigmentation is clearly noticed as it provides the bright and attractive colors of animals. On the other hand it is used for camouflage as demonstrated by certain species such as cuttlefish which secreted a melanin ink to distract and confuse its predators. It is also believed to be a defense system of certain fungi and bacteria against ionizing UV radiation,[32] chemical strain and biochemical strain. Also, it is important to note that melanin is a radical free polymer presenting a high-conjugated structure that enables the transformation of UV-VIS radiation into heat.[33] Additionally, the disorders in melanin formation can cause melanoma, which is the most aggressive type of skin cancer.[34,35] Also, various depigmentation disorders can be occurred, such as ocular albinism. These disorders are due to the deficiency in lysosomal functions.[36]

Melanin pigmentation

The dramatic function of melanocytes is widely affected the visible pigmentation of the skin, eyes and hair. Melanocytes are found in dermal-epidermal tissues which give raise the skin color and in hair follicles which responsible for hair color. The process of coloration depends on critical and complex steps due to the development, proliferation and differentiation of melanocytes precursors should be produced with very high fidelity to cause uniform pigmentation.[37] Melanin pigment is produced in specialized organelles called melanosomes which are produced by melanocytes. The synthesis and deposition of melanin are affected by several types of proteins; including tyrosine (TYR), monoclonal tyrosine antibody (TYRP1) and discrete cosine transform (DCT). Relatively high quantities of melanins are produced and distributed uniformly in keratinocytes to increase the ability to absorb the light and hence give the visible dark skin and hair. Whereas, a small amounts of melanins are found in the lighter color skin and hair with low ability to absorb the light and hence minimize the visible coloration of them.[38]

Relation of melanin and the skin cancer

The extensive exposure to UV has an adverse action on the skin, causing photocarcinogenesis especially in light skin, which has a dramatic risk for skin cancer approximately (15-70) fold more than the darker skin.[39-42] The scientists believed that the rate of malignant melanoma and cell carcinoma is higher in white peoples than those black.[43] Genetically skin pigmentation is

regulated by the melanocortin 1 receptor (MC1R) that its gene is susceptible to skin melanoma.[44] As we knew, skin pigmentation was based on the amounts of melanin, which is distributed in keratinocytes. The response of keratinocytes to extensive UV absorption in various types of skin showed an inverse relationship between the DNA damage and melanin content.[45] The harmful response of human skin to UV absorption referred to the production of skin to oxidative cellular damage and two types of DNA damage.[46] An impact role of melanin distribution in the upper part of the skin was detected on its photoprotective effect of the cell. The photocarcinogenesis due to the UV-DNA damage in lower skin layer is more crucial than in the upper layer. This was attributed to the presence of melanocytes as well as keratinocytes cells and transient amplifying cells, which are not lost through the desquamation process and therefore cause different types of skin cancers.[47]

ANALYTICAL DETECTION OF MELANIN

Several spectroscopic and chromatographic methods have been addressed for the determination of melanin in pure synthetic form, in natural samples and in bio-samples.

UV and Visible spectrophotometric methods

Hunold and Malessa[48] investigated 18 melanin pigmentation subjects (in vivo detection) by recording the light absorption of these subjects using λ_{max} = 675 nm. The obtained results revealed that the mean of about 16 caucasian fundi was found to be 0.79 ± 0.17, while the value of albino and Negro fundus were found to be 0.06 and 1.36 E, respectively. These two values are found outside the range of caucasian values. The results were of good agreements with those performed using enucleated eyes. Dwyer et. al.[49] used a spectrophotometric method for the detection of the density of cutaneous melanin in skin that protected it from light radiation damage. They could detect the density of cutaneous melanin by evaluating the reflectance of visible light by the skin. The biopsy of 3 mm of skin was taken and the detection was carried out at 650 - 700 nm. The melanin skin reflectance was measured at 680 nm (r = 0.33). The method of detection can provide the discrimination of individual susceptibility to epidermal tumors. Furthermore, Ito et al.[50] suggested a simple spectrophotometric method to assay eumelanin in tissue samples. A hot hydroiodic acid was used to hydrolyze the hair and melanoma samples solubilized eumelanin pigment and hot sodium hydroxide was used to dissolve the eumelanin pigments in the presence of hydrogen peroxide. The absorbance spectrum was recorded at 350 nm. Another spectrophotometric method was proposed by Ozeki et al.[51] to quantify both eumelanin and pheomelanin by the detection of pyrrole-2,3,5-tricarboxylic acid (PTCA) and aminohydroxyphenylalanine (AHP). The proposed method was based on dissolving melanin in soluene-350 plus water and the detection was at an absorbance range of 500-600 nm. The relative ratio of both melanins was detected using the absorbance ratio.

Verschoore et al.[52] investigated the distribution of melanin and its effect on the patients of idiopathic cutaneous hyperchromia at the orbital region (ICHOR). The authors applied the spectrophotometric technique to perform this investigation and the study revealed that the deposition of melanin in dark circles may play a role in ICHOR pathogenesis and cause the darkening of skin under eyes.

Photoacoustic spectroscopy methods

Watanabe et. al.[53] suggested a very sensitive photoacoustic spectroscopy for the estimation of melanin in human hair. The detection was mainly based on the treatment of both human hair and synthetic melanin with 50 % sulfuric acid. Then the collection of the melanin is performed using membrane filter. Then the photoacoustic signals were measured by 10 mW He-Ne laser beam. A linear relationship was fitted between the photoacoustic signal vs. weight of samples in the ranges of 0.1-20 and 10-800 μg with a lower detection limit of 5 *n*g and 0.38 μg for synthetic melanin and human hair, respectively. In 2004 Viator et. al.[54] developed a new photoacoustic probe to detect the epidermal melanin distribution by using Q-switched, frequency-double neodymium, yttrium, aluminum, garnet laser at 532 nm. The detection was carried out using ten human subjects with skin phototype I-VI. The results indicated that the melanin is completely absent in vitilligo human subjects.

Miscellaneous spectroscopic methods

Katritzky et. al.[55] utilized a 1H nuclear magnetic resonance (1H NMR) to study Sepia melanin, Sepia melanin free acid and human hair melanin. The investigation was performed under alkaline medium using the deuterium oxide solution at pH 10 − 11. To suggest the possible monomeric unit profile of the Sepia melanin polymeric chain, the empirical formula for the Sepia melanin free acid was calculated and used to estimate the number of protons in the aromatic region of it.

A sensitive flow-injection (FI) electrogenerated chemiluminescence method was introduced by Alarfaj et al.[56] for the determination of melanin. The developed method was based on the electrogenerated chemiluminescnce reaction of $Ru(bpy)_3^{2+}$ with melanin in an acidic medium. The method provides the determination of 0.01 − 2.0 μg mL^{-1} and 3.0 − 35 μg mL^{-1} of melanin with correlation coefficients (*r*) > 0.9999. The lower limit of detection (LOD) was 0.0005 μg mL^{-1} (S/N = 3) and the lower limit of quantitation (LOQ) is 0.01 μg mL^{-1}.

Chromatographic methods

Chromatography is the fastest growing analytical technique for the analysis of samples. Its simplicity, high

selectivity and wide range of sensitivity make it ideal for the analysis of many samples.

High performance liquid chromatography with electrochemical detection

Wakamatsu et. al.[57] developed a liquid chromatographic method to measure levels of eumelanin in urine samples and evaluated its clinical significance. The authors addressed the chromatographic detection by producing pyrrole-2,3,5-tricarboxylic acid from permanganate oxidation of eumelanin, then detecting it using liquid chromatography. The quantified level of pyrrole-2,3,5-tricarboxylic acid was in melanoma patients was elevated 2.1-fold compared with control subjects.

Kolb et. al.[58] were interested to determine pheomelanin in biological samples using high performance liquid chromatography. The detection was carried out after chemical degradation of melanin and detects its degradation products using an electrochemical detector. This method displayed good reproducibility and precision. The linear concentration range was 490 ng and 850 ng. The detection method provides the facility to understand the formed melanin in cell lines cultured in different growth media.

Another chromatographic detection method was developed by Wakamatsu and Ito[59] to quantify both eumelanin and pheomelanin in biological samples. Chemical degradation of melanin followed by chromatographic analysis by high performance liquid chromatography was suggested. This method proved to be a useful tool in studying melanogenesis.

An accurate and selective chromatographic method to determine both 4-amino-3-hydroxyphenylalanine and 3-amino-4-hydroxyphenylalanin in human urine was provided by Takasaki et. al.[60] The melanin polymer was treated with hydroiodic acid and subjected to analysis using reversed phase high performance liquid chromatography in conjunction with electrochemical detection. Ion pairing mobile phase containing 25 mmol L^{-1} ammonium acetate and sodium octanesulfonate was used. Well defined peaks with linear detection over the range of 0 - 2 ng for both compounds were obtained.

A further chromatographic method based on the hydrolysis of pheomelanin was studied by Nezirevic et. al.[61] The chromatographic detection was performed under optimal conditions, including, mobile phase of acetonitrile: 0.1 M ammonium acetate buffer, pH 4.5 (82:18, v/v) and detection at 400 mV the detection was carried out showing good linear relationship over a concentration range of 0.05-0.5 µg L^{-1}. Excellent results were recorded for the detection of pheomelanin in human urine of melanoma patients.

A simple high performance liquid chromatographic method based on the direct injection of hydroiodic acid reduction products of pheomelanin was proposed by Wakamatsu et. al.[62] The two aminohydroxyphenylalanine isomers were studied separately using human hair samples with various colors.

The quantification of both melanin pigments eumelanin and pheomelanin in hair and melanoma tissues using high performance liquid chromatography with electrochemical detector was carried out by Ito and Fujita.[63] The degradation process was performed using samples of 0.5 mg of melanin, 5 mg melanoma tissues and 2 mg hair. The developed method facilitates the analysis of approximately 20 samples through 3 working days.

High performance liquid chromatography with UV detection

Magarelli et. al.[64] introduced a simple extraction and purification process of sepia melanin using hydrochloric acid 0.5 – 3.0 mol L^{-1}. The obtained high degree purity of melanin was subjected to chemical degradation and HPLC method was applied for the detection of the degraded products.

The analysis of both eumelanin and pheomelanin in melanin tissues was introduced by Panzella et. al.[65] The authors used high performance liquid chromatography coupled with an ultraviolet detector for recording the results after the degradation of the melanin pigmented tissues using hydrogen peroxide in the presence of alkaline medium. The used mobile phase was 1% formic acid: methanol. This method was employed to determine eumelanin and pheomelanin in mammalian hair, skin and irises.

A simple and accurate high performance liquid chromatography with ultraviolet detection for the analysis and quantifying the amount of melanin pigments in the hair and melanoma tissues was suggested by Ito and Jimbow.[66] In this method the authors used permanganate as oxidizing agent to eumelanin and hydroiodic acid for the hydrolysis of pheomelanin. The chromatographic method was applied to detect the degradation products with respect to the reference standards of melanosome-melanin and synthetic melanin for eumelanin and pheomelanin, respectively.

High performance liquid chromatography with fluorimetric detection

Yang et. al.[67] in 2007 described new high performance liquid chromatographic method coupled with fluorescence detector to detect pheomelanin in biological samples. The developed method displayed high accuracy and sensitivity of 0.11 $n$mol L^{-1} (2.2 fmol per injection) at signal-to-noise ratio of 3 for each product and linearity range of 0.02-10 µmol L^{-1}, r > 0.995. The authors employed this method to method quantify the pheomelanin in various biological samples such as melanoma cell lines of human and rat, melanoma tissue and hair samples.

Capillary electrophoresis (CE)

Zhang et. al.[68,69] reported two sensitive micellar electrokinetic capillary chromatographic methods with laser-induced fluorescence detection for the determination of pheomelanin in diverse biological materials. In the first method the authors chosen 5-carboxyfluorescein succinimidyl ester as derivatizing reagent to the two markers aminohydroxyphenylalanine isomers produced after reductive hydrolysis of pheomelanin with hydroiodic acid. This method was successfully applied to detect pheomelanin in two human melanoma cell cultures, black hair, melanoma tissue and urine samples of human melanoma patients. While in the second method the chosen reagent was 4-(1-pyrene) butyric acid *N*-hydroxysuccinimide ester. The separation was carried out using a ground electrolyte consisting of 20 mmol L^{-1} phosphate buffers of pH 7.4, 30 mmol L^{-1} cholate, and 30% methanol. This method was successfully applied to monitor the pheomelanin in diverse biological samples with the spiked recoveries in the range of 94–101%, at a signal-to-noise ratio of 3.

Gravimetric method

In 1978 Jan Borovansky[70] provided new simple gravimetric method based on the resistance of melanin to acid attack. The method was applied using animal tissues and the author removed the lipids by solvent extraction after samples hydrolysis by acid.

CONCLUSION

Considering the melanin as a regulatory factor for pigmentation in various organisms as well as a photoprotective factor against the harmful ultraviolet radiation, will encouraging the researchers to focus their attention to communicate between the melanin pigments and the presence of various characteristic features of living organisms. Furthermore, many articles were published to correlate the relation between the distribution of melanin pigments in the body of living organisms and the risk of melanoma pathogenesis. Despite of the impact role of melanin pigments on the life of microorganisms, the analytical methods which developed to isolate, characterize and quantify these pigments still limited and the workers in the analytical chemistry field need to develop and suggest more simple and accurate techniques to facilitate the detection of such pigments in diverse samples.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Anderson H, Bulow FA, Mollgard K. The histochemical and ultrastructural bases of the cellular function of the human fetal adenohypophysis. Prog Histochem Cytochem, 1970; 1: 153-184.
2. Westerhof W. The discovery of the human melanocyte, Pigment Cell Res, 2006; 19(3): 183–193. doi: 10.1111/j.1600-0749.2006.00313.x.
3. Prota G. Melanins and Melanogenesis; Academic Press, San Diego, CA, 1992. See also: G. Prota, Fortschr Chem Org Naturst, 1995; 64: 94-148.
4. Tsatmali M, Ancans J, Thody AJ. Melanocyte function and its control by melanocortin peptides. J Histochem Cytochem, 2002; 50(2): 125-133. doi: 10.1177/002215540205000201.
5. Koshy S, Vettivel S. Melanin pigments in human pineal gland. J Anal Soc India, 2001; 50(2): 122-126.
6. Thody AJ, Higgins EM, Wakamatsu K, Ito S, Burchill SA, Marks JM. Pheomelanin as well as eumelanin is present inhuman epidermis. J Invest Dermatol, 1991; 97(2): 340-344.
7. Van Neste D, Tobin DJ. Hair cycle and hair pigmentation: dynamic interactions and changes associated with aging. Micron, 2004; 35(3): 193-200.
8. Yamaguchi Y, Brenner M, Hearing VJ. The Regulation of skin pigmentation. J Biol Chem, 2007; 282(38): 27557-27561.
9. Wang H, Lin S, Liu X, Kang SB. Separating Reflections in Human Iris Images for Illumination Estimation. Tenth IEEE International Conference on Computer Vision, 2005; 2: 1691–1698. doi*:*10.1109/ICCV.2005.215.
10. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. Photochem Photobiol, 2008; 84: 539–549. doi: 10.1111/j.1751-1097.2007.00226.x.
11. Ye T, Pawlak A, Sarna T, Simon JD. Different molecular constituents in pheomelanin are responsible for emission, transient absorption and oxygen photoconsumption. Photochem Photobiol, 2008; 84: 437-443.
12. Zucca FA, Basso E, Cupaioli FA, Ferrari E, Sulzer D, Casella L, Zecca L. Neuromelanin of the human substantia nigra: An update. Neurotox Res, 2014; 25(1): 13-23. doi: 10.1007/s12640-013-9435-y.
13. McGraw KJ, Safran RJ, Wakamatsu K. How feather color reflects its melanin content. Fun Ecol, 2005; 19: 816-821.
14. Prota G, Searle AG. biochemical sites of gene action for melanogenesis in mammals. Annales de génétique et de sélection animale, 1978; 10(1): 1-8. doi*:*10.1186/1297-9686-10-1-1.
15. Meng TX, Zhang CF, Miyamoto T, Ishikawa H, Shimizu K, Ohga S, Kondo R. The Melanin Biosynthesis Stimulating Compounds Isolated from the Fruiting Bodies of Pleurotus citrinopileatus. J Cosm Erm Sci Appl, 2012; 2: 151-157.
16. Ito S, Wakamatsu K. Chemistry of Melanins. In: The Pigmentary System. Physiology and Pathophysiology, 2nd edn. Nordlund JJ, Boissy RE, Hearing V J, King RA, Oetting WS, Ortonne JP, eds (Oxford, UK: Blackwell Publishing), 2006; 282–310.

17. Jimbow K, Quevedo WC, Prota G, Fitzpatrick TB. Biology of Melanocytes. In: Fitzpatrick TB, Freedberg IM, et al. (Eds), Fitzpatrick's Dermatology in General Medicine, 5th international ed., McGraw-Hill, New York 1999; 1: 192-219.
18. d'Ischia M, Napolitano A, Pezzella A, Meredith P, Sarna T, Chemical and Structural Diversity in Eumelanins – Unexplored Bio-Optoelectronic Materials Angew. Chem Int Ed Engl, 2009; 48(22): 3914-3921. doi: 10.1002/anie.200803786.
19. Ito S, Wakamatsu K. Review. Photochem. Photobiol, 2008; 84: 582–592.
20. Ito S, Wakamatsu K. Review. Chemistry of mixed melanogenesis-pivotal roles of dopaquinone. Photochem Photobiol, 2008; 84: 582-592. doi: 10.1111/j.1751-1097.2007.00238.x.
21. Raper HS, XIV. The tyrosinase-tyrosine reaction. VI. Production from tyrosine of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid-the precursors of melanin. Biochem J, 1927; 21: 89-96.
22. Mason HS. Structure of melanins. In: Gordon M, ed. Pigment Cell Biology. Academic, New York, NY, 1959; 563-582.
23. Mason HS. The structure of melanin. In: Montagna, W.; Hu, F. eds. Advances in Biology of the Skin. Vol VIII, The pigmentary system. pergamon, Oxford, UK, 1967; 293-312.
24. Riley PA. The evolution of melanogenesis. In: Zeise L, Chedekel MR, Fitzpatrick TB, eds. Melanin: Its role in human photoprotection. Valdenmar, Overland Park, Kansas, 1995; 1-10.
25. Land EJ, Riley PA. Spontaneous redox reactions of dopaquinone and the balance between the eumelanic and phaeomelanic pathways. Pigment Cell Res, 2000; 13: 273-277.
26. Takeuchi S, Zhang W, Wakamatsu K, Ito S, Hearing VJ, Kraemer KH, Brash DE. Melanin acts as a potent UVB photosensitizer to cause an atypical mode of cell death in murine skin. Proc Natl Acad Sci U. S. A. 2004; 101: 15076–15081. doi: 10.1073/pnas.0403994101.
27. Meredith P, Sarna T. The physical and chemical properties of emulanin. Pigment Cell Res, 2006; 19(6): 572–594. doi:10.1111/j.1600-0749.2006.00345.x.
28. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B, 2001; 63: 8-18.
29. Ye T, Simon JD. Comparison of the ultrafast absorption dynamics of eumelanin and pheomelanin. J Phys Chem B, 2003; 107: 11240–11244.
30. Goncalves RCR, Pombeiro-Sponchiado SR. Antioxidant Activity of the Melanin Pigment Extracted from Aspergillus Nidulans. J Pharm Bull, 2005; 28(6): 1129-1131.
31. Riley PA. Melanin. Int J Biochem Cell Biol, 1997; 29(11): 1235-1239.
32. Meyskens FL, Farmer PJ, Fruehauf J. Redox regulation in human melanocytes and melanoma. J Pigment Cell Res, 2001; 14: 148-154.
33. Cesarini JP. Photo-induced events in the human melanocytic system: photoaggression and photoprotection. Pigment Cell Res, 1988; 1: 223-233.
34. Gidanian S, Mentelle M, Meyskens FL, Farmer PJ. Melanosomal damage in normal human melanocytes induced by UVB and metal uptake--a basis for the prooxidant state of melanoma. Photochem Photobio, 2008; 84: 556-564.
35. Oliveria SA, Saraiya M, Geller AC, Heneghan MK, Jorgensen C. Sun exposure and risk of melanoma. Arch Dis Child, 2006; 91: 131-138.
36. Sulzer D, Mosharov E, Talloczy Z, Zucca FA, Simon JD, Zecca LJ. Neurochem, 2008; 106(1): 24-36.
37. Bennett DC, Lamoreux ML. The color loci of mice a genetic century. Pigment Cell Res, 2003; 16: 333-344.
38. Kushimoto T, Basrur V, Valencia J, Matsunaga J, Vieira WD, Ferrans VJ, Muller J, Appella E, Hearing VJ. A new model for melanosome biogenesis based on the purification and mapping of early melanosomes. Proc Natl Acad Sci, 2001; 98: 10698-10703.
39. Matsumura Y, Ananthaswamy HN. Molecular mechanisms of photocarcinogenesis. Front Biosci, 2002; 7: 765-783.
40. Wei Q, Lee JE, Gershenwald JE, Ross MI, Mansfield PF, Strom SS, Wang LE, Guo Z, Qiao Y, Amos CI, Spitz MR, Duvic M. Repair of UV light-induced DNA damage and risk of cutaneous malignant melanoma. J Natl Cancer Inst, 2003; 95: 308-315.
41. Gilchrest BA, Eller MS, Geller AC, Yaar M. The pathogenesis of melanoma induced by ultraviolet radiation. N. Engl J Med, 1991; 340: 1341-1348.
42. Landi MT, Baccarelli A, Tarone RE, Pesatori A, Tucker MA, Hedayati M, Grossman L. DNA repair, dysplastic nevi, and sunlight sensitivity in the development of cutaneous malignant melanoma. J Natl Cancer Inst, 2003; 94: 94–101.
43. Preston DS, Stern RS. Nonmelanoma cancers of the skin. New Eng J Med, 1992; 327: 1649-1662.
44. Robinson SJ, Healy E. Human melanocortin 1 receptor (MC1R) gene variants alter melanoma cell growth and adhesion to extracellular matrix. Oncogene, 2002; 21: 8037-8046.
45. Tadokoro T, Kobayashi N, Zmudzka BZ, Ito S, Wakamatsu K, Yamaguchi Y, Korossy KS, Miller SA, Beer JZ, Hearing VJ. UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin and photosensitivity. FASEB J, 2003; 17: 1177-1179.
46. Agar N, Young AR. Melanogenesis: a photoprotective response to DNA damage. Mutat Res, 2005; 571: 121-132.
47. Halder RM, Bridgeman-Shah S. Skin cancer in African Americans. Cancer, 1995; 75: 667-673.
48. Hunold W, Malessa P. Spectrophotometric Determination of the Melanin Pigmentation of the

Human Ocular Fundus in vivo. Ophthal Res, 1974; 6: 355-362. doi:10.1159/000264723.
49. Dwyer T, Muller HK, Bkizzard L, Ashbolt R, Phillips G. The use of spectrophotometry to estimate melanin density in Caucasians. Cancer Epiderminol Biomarkers Prev, 1998; 7(3): 203-206.
50. Ito S, Wakamatsu K, Ozeki H. Spectrophotometric Assay of eumelanin in tissue samples. Anal Biochem, 1993; 215: 273–277. http://dx.doi.org/10.1006/abio.1993.1586.
51. Ozeki H, Ito S, Wakamatsu K, Thody AJ. Spectrophotometric characterization of eumelanin and pheomelanin in hair. Pigment Cell Res, 1996; 9: 265–270.
52. Verschoore M, Gupta S, Sharma VK, Ortonne JP. Determination of melanin and haemoglubin in the skin of idiopathic cutaneous hyperchromia of the orbital region (ICHOR): A study of Indian patients. J Caut Aesth Sur, 2012; 5(3): 176-182. doi: 10.4103/0974-2077.101371.
53. Watanabe T, Tamura A, Yoshimura Y, Nakazawa H. Determination of melanin in human hair by photoacoustic spectroscopy Anal Biochem, 1997; 254(2): 267–271.
54. Viator JA, Komadina J, Svaasand LO, Aguilar G, Choi B, Nelson JS. A comparative study of photoacoustic and reflectance methods for determination of epidermal melanin content. J Invest Dermatol, 2004; 122(6): 1432-1439. http://dx.doi.org/10.1111/j.0022-202X.2004.22610.x.
55. Katritzky AR, Akhmedov NG, Denisenko SN, Denisko OV. 1H NMR spectroscopic characterization of solutions of Sepia melanin, Sepia melanin free acid and human hair melanin. Pigment Cell Res, 2002; 15(2): 93-97.
56. Alarfaj NA, Abdalla MAE, Al-Hamza AM. A Sensitive electrogenerated chemiluminescence assay for determination of melanin in natural and biological samples. Int J Electrochem Sci, 2012; 7: 7888-7901.
57. Wakamatsu K, Takasaki A, Kagedal B, Kageshita T, Ito S. Determination of eumelanin in human urine. Pigment Cell Res, 2006; 19(2): 163-169.
58. Kolb AM, Lentjes EG, Smit NP, Schothorst A, Vermeer BJ, Pavel S. Determination of pheomelanin by measument of aminohydroxyohenylananine isomer with high-performance liquid chromatography. Anal Bio chem, 1997; 252: 293–298.
59. Wakamatsu K, Ito S. Advanced chemical methods in melanin determination. Pigment Cell Res, 2002; 15: 174-183.
60. Takasaki A, Nezirevic D, Arstrand K, Wakamatsu K, Ito S, Kagedal B. HPLC analysis of pheomelanin degradation products in human urine. Pigment Cell Res, 2003; 16: 480-486.
61. Nezirevic D, Arstrand K, Kagedal B. Hydrophilic interaction liquid chromatographic analysis of aminohyroxyphenylalanines from melanin pigments. J Chromatogr A, 2007; 1163(1-2): 70-79.
62. Wakamatsu K, Ito S, Rees JL. The usefulness of 4-amino-3-hhydroxyphenylalanine as a specific marker of pheomelanin. Pigment Cell Res, 2002; 15(3): 225-232.
63. Ito S, Fujita K. Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical degradation and liquid chromatography. Anal Biochem, 1985; 144: 527–536.
64. Mario Magarelli M, Passamonti P, Renieri C. Purification, characterization and analysis of sepia melanin from commercial sepia ink (Sepia Officinalis). Rev CES Med Veter Zoo, 2010; 5(2): 18-28.
65. Panzella L, Manini P, Monfrecola G, d'Ischia M, Napolitano A. An easy-to-run method for routine analysis of eumelanin and pheomelanin in pigmented tissues. Pigment Cell Res, 2007; 20: 128-133.
66. Ito S, Jimbow K. Quantitative analysis of eumelanin and pheomelanin in hair and melanomas. J Invest Dermatol, 1983; 80: 268-272.
67. Yang Q, Zhang XL, Ma M, Huang KJ, Zhang JX, Ni WZ, Fang CX, Zheng Y. New high-performance liquid chromatographic method for sensitive determination of pheomelanin in biological materials by precolumn fluorescence derivatization with naphthalene-2,3-dicarboxaldehyde. J Chromatogr A, 2007; 1146(1): 23-31.
68. Zhang XL, Yang Q, Jiao BH, Dai J, Zhang JX, Ni WZ. Sensitive determination of pheomelanin after 5-carboxyfluorescein succinimidyl ester precapillary derivatization and micellar electrokinetic capillary chromatography with laser-induced fluorescence detection J Chromatogr B, 2008; 861(1): 136-139.
69. Zhang X, Yuan B, Yang Q. Selective and sensitive determination of pheomelanin in biological samples using MEKC with laser-induced fluorescence detection based on intramolecular excimer-forming fluorescence derivatization. Chromatographia, 2011; 73: 527–534.
70. Borovansky J. Quantitative determination of melanin. Micrichim Acta, 1978; 70(5): 432-429. doi:10.1007/BF01197094.