Ohkawa et al (1979)\textsuperscript{16} studied the assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. The reaction of lipid peroxides in animal tissues with thiobarbituric acid was dependent pH of the reaction mixture as was the case for linoleic acid hydroperoxide. The optimum pH was found to be 3.5. Taking this fact into consideration, a standard procedure for the assay of lipid peroxide level in animal tissues by their reaction with thiobarbituric acid was developed as follows. Ten percent (w/v) tissue homogenate was mixed with sodium dodecyl sulfate, acetate buffer (pH 3.5), and aqueous solution of thiobarbituric acid. After heating at 95°C for 60 min, the red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532 nm. As an external standard, tetramethoxypropane was used, and lipid peroxide level was expressed in terms of nmol malondialdehyde. Using this method, the lipid peroxide level in the liver of rats suffering from carbon tetrachloride intoxication was investigated.

Bolognia et al (1990)\textsuperscript{17} studied hairless pigmented guinea pigs, a new model for the study of mammalian pigmentation. A stock of hairless pigmented guinea pigs was developed to facilitate studies of mammalian pigmentation. This stock combines the convenience of a hairless animal with a pigmentary system that is similar to human skin. In both human and guinea pig skin, active melanocytes are located in the basal layer of the interfollicular epidermis. Hairless albino guinea pigs on an outbred Hartley background (CrI:IAF/HA(hr/hr)BR; designated hrlhr) were mated with red-haired guinea pigs (designated Hr/Hr). Red-haired heterozygotes from the F1 generation (Hr/lhr) were then mated with each other or with hairless albino guinea pigs. The F2 generation included hairless pigmented guinea pigs that retained their interfollicular epidermal melanocytes and whose skin was red-brown in color. Following UV irradiation, there was an increase in cutaneous pigmentation as well as an increase in the number of active epidermal melanocytes.

Palumbo et al (1991)\textsuperscript{18} studied the mechanism of inhibition of melanogenesis by hydroquinone. Hydroquinone (HQ) is one of the most effective inhibitors of melanogenesis in vitro and in vivo, and is widely used for the treatment of melanosis and other hyperpigmentary disorders. We have investigated the effect of HQ on the tyrosinase catalysed conversion of tyrosine to melanin. Incubation of 0.5 mM tyrosine with 0.07 U/ml tyrosinase in phosphate buffer at pH 6.8 in the presence of 0.5 mM...
HQ led to no detectable melanin formation, clue to the preferential oxidation of HQ with respect to tyrosine. These results suggest that the depigmenting activity, of HQ may partly be related to the ability, of the compound to act as an alternate substrate of tyrosinase, thereby competing for tyrosine oxidation in active melanocytes.

Barber et al (1995)\(^{19}\) studied the percutaneous absorption of hydroquinone (HQ) through rat and human skin in vitro the rates of percutaneous absorption of HQ through human stratum corneum and full-thickness rat skin have been measured in vitro using 5% aqueous solutions of HQ as the donor solutions. The studies were performed using infinite doses of aqueous solutions containing "C-labeled HQ in Franz-type diffusion cells. The measured absorption rate (mean \(\pm\) SD.) of HQ through human stratum corneum was 0.52 \(\pm\) 0.13 \(\mu\)g/cm\(^2\)/h. HQ would be classified as ‘slow’ with respect to its absorption through human stratum corneum.

Bolognia et al (1995)\(^{20}\) studied the enhancement of the depigmenting effect of hydroquinone by cystamine and buthionine sulfoximine. In this study, we examined the possibility that the effect of hydroquinone (HQ) on pigmentation could be potentiated by inhibiting the production of GSH. In vitro studies using melanoma cell lines demonstrated that both cystamine and BSO could potentiate the inhibitory effects of HQ on tyrosinase activity and melanin content. A synergistic decrease in hair pigmentation was observed when a combination of HQ (2-4%) and BSO (5%) was applied to the dorsal skin of C57BL mice. In black hairless guinea-pigs, the application of HQ plus either BSO or cystamine resulted in a significant decrease in epidermal pigmentation when compared with any of the agents alone. The possibility exists that in the future a combination of HO plus cystamine or BSO could be used to treat disorders such as melasma and post-inflammatory hyperpigmentation.

Majmudar et al (1998)\(^{21}\) studied the in vitro method for screening skin-whitening products, Melanoderm is an in vitro model of the human epidermis consisting of well-differentiated, cultured human keratinocytes and melanocytes. We utilized this model to evaluate the efficacy, stability, and cytotoxicity of whitening agents. Magnesium ascorbyl phosphate(eMAP), kojic acid, and lactic acid in aqueous or anhydrous base were applied to Melanoderm. Following incubation, tyrosinase activity was measured using L-dihydroxyphenylalanine(L-DOPA). Melanocytes staining was observed under
the microscope. Melanoderm treated with either MAP, kojic acid, or lactic acid showed 33%, 48%, and 46% reduction, respectively, of tyrosinase activity. Microscopic examination of treated Melanoderm clearly showed the dendritic nature of melanocytes and normal morphology of keratinocytes and MTT assay suggested that the test materials were not cytotoxic.

Spiclin et al (2001) studied Ascorbyl palmitate and sodium ascorbyl phosphate are derivatives of ascorbic acid, which differ in stability and hydro-lipophilic properties. They are widely used in cosmetic and pharmaceutical preparations. In the present work the stability of both derivatives was studied in microemulsions for topical use as carrier systems. The microemulsions were of both o/w and w/o types and composed of the same ingredients. The stability of the less stable derivative ascorbyl palmitate was tested under different conditions to evaluate the influence of initial concentration, location in microemulsion, dissolved oxygen and storage conditions. High concentrations of ascorbyl palmitate reduced the extent of its degradation. The location of ascorbyl palmitate in the microemulsion and oxygen dissolved in the system together significantly influence the stability of the compound. Light accelerated the degradation of ascorbyl palmitate. In contrast, sodium ascorbyl phosphate was stable in both types of microemulsions. Sodium ascorbyl phosphate is shown to be convenient as an active ingredient in topical preparations. In the case of ascorbyl palmitate, long-term stability in selected microemulsions was not adequate. To formulate an optimal carrier system for this ingredient other factors influencing the stability have to be considered.

Gopinath et al. (2004) studied the formation, characterization and applications of Ascorbyl palmitate vesicles (Aspasomes). Ascorbyl palmitate (ASP) was explored as bilayer vesicle forming material. It formed vesicles (Aspasomes) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Aspasomes were prepared by film hydration method followed by sonication in which aqueous drug solution was encapsulated in aqueous regions of bilayer. Aspasomes were obtained with all compositions containing 18–72 mol% cholesterol. Differential scanning calorimetric data of aspasome dispersion and anhydrous mixtures of ascorbyl palmitate, cholesterol and dicetyl phosphate confirm the formation of bilayered vesicles with ascorbyl palmitate. The antioxidant potency of Aspasomes was assessed.
by measuring the protection offered by this preparation against quinolinic acid induced lipoperoxidation of whole human blood in vitro, where in the lipoperoxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS) levels. Aspasome rendered much better antioxidant activity than ascorbic acid.

**Huang et al (2004)**24 studied the simultaneous determination of magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin and hydroquinone in skin whitening cosmetics by hplc. A high performance liquid chromatographic method was developed for simultaneous determination of 5 whitening ingredients: magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin and hydroquinone decomposed from arbutin in cosmetics.

**Abramovits et al (2005)**25 studied the practical comparison of hydroquinone containing products for the treatment of melasma. It is an acquired hyperpigmentation due to an increase in the amount of melanin within melanocytes. Most often it affects the forehead, malar eminences, upper lip, and chin. The hyperpigmented patches are usually symmetrical and can have a sharp irregular border. Histologically, three forms exist (epidermal, dermal, and mixed epidermal and dermal) with the hue of the pigmentation correlating to the histologic type: brown (epidermal type), blue-gray (dermal type), or brown-gray (mixed epidermal and dermal type). The depth can be clinically estimated with the help of a Wood’s lamp. The epidermal type is most responsive to treatment.

**English et al (2005)**26 studied were conducted to determine the absorption, tissue distribution, excretion, and metabolism of 14C-hydroquinone (HQ) in male and female rats following single oral, repeated oral, or 24-h dermal administration. The concentration of parent compound in blood was also determined following a single 50-mg/kg gavage administration. Absorption into the blood was rapid after oral dosing; the maximum concentration of parent compound was attained within 20 min after dosing, and the maximum concentration of total 14C was attained within 30 min. Parent compound represented 61% of total 14C in blood, indicative of extensive first-pass metabolism. Excretion was primarily via the urine within the first 8 h of gavage. Typically, 87–94% of the 14C was excreted in urine. Dermal application of 14C–HQ
(20 lCi) as a 5.4% aqueous solution resulted in near background levels of 14C in blood; the maximum mean blood concentration was 0.65 lg HQ equivalents/g in females and not quantifiable in males. The majority (61–71%) of the 14C was recovered from the skin surface by washing at 24 h. HQ was extensively metabolized following oral dosing with typically <3% of the dose excreted as parent compound. The major urinary metabolites of HQ were glucuronide and O-sulfate conjugates, which represented 45–53% and 19–33%, respectively, of an oral dose. A <5% metabolite was identified as a mercapturic acid conjugate of HQ.

**Gad et al (2005)** studied therapeutically used topically for depigmentation to treat skin blemishes, for example, hypermelanosis. Hydroquinone occurs naturally, as a conjugate with b-D-glucopyranoside, in the leaves, bark, and fruit of a number of plants, and its presence may be an important factor in fire-blight resistance in the pear. It may also play an important part in the defense mechanisms of some insects.

**Parvez et al (2006)** reviewed the mechanism of skin depigmenting and lightening agents. The type and amount of melanin synthesized by the melanocyte, and its distribution pattern in the surrounding keratinocytes, determines the actual color of the skin. Melanin forms through a series of oxidative reactions involving the amino acid tyrosine in the presence of the enzyme tyrosinase. Tyrosinase catalyses three different reactions in the biosynthetic pathway of melanin in melanocytes: the hydroxylation of tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone; furthermore, in humans, dopaquinone is converted by a series of complex reactions to melanin.

**Yarosh et al (2008)** studied hydroquinone composition for skin whitening and lightening skin color (or for reducing skin darkening) and for repairing DNA damage are provided. Skin lightening also called whitening, lightening, clarifying, fading, bleaching is a major skin care product category around the world. High pigments deposit in skin, may be congenital which we recognize in some cases as mole, or they may develop during growth and development as areas of uneven pigmentation.
Teeranachaideekul et al (2008)\textsuperscript{30} studied the development of ascorbyl palmitate nanocrystals applying the nanosuspension technology. Ascorbyl palmitate (AP) is an antioxidant used in both cosmetics and food industry. Owing to its poor solubility and instability caused by oxidation having been observed in several colloidal systems, the aim of this study was to investigate the feasibility of applying the nanosuspension technology by high-pressure homogenization (HPH) (DissoCubes® technology) to enhance the chemical stability of AP, followed by lyophilization. Sodium dodecyl sulfate (SDS) and Tween 80 were chosen as emulsifying agents to stabilize the developed AP nanosuspensions. After 3 months of storage at three different temperatures (4 °C, 25 °C and 40 °C), the photon correlation spectroscopy (PCS) analysis of AP nanosuspensions revealed that the mean particle size of those stabilized with SDS significantly increased compared to those stabilized with Tween 80.

Uddin et al (2010)\textsuperscript{31} studied the highly sensitive spectrometric method for determination of hydroquinone in skin lightening creams. The newly investigated method is very useful for HQ determination in dilute samples of creams or cosmetics. The major advantages of the developed method are as follows: economical, simpler, faster and highly sensitive. The method could be equally helpful for HQ analysis in aqueous samples. The method is based on using ammonium meta-vanadate as an oxidizing catalyst for conversion of HQ to p-benzoquinone (BQ) in the presence of oxygen. As a result of higher absorption of UV light by BQ than by HQ, its signal has been utilized for determining HQ at the trace level.

Anstey et al (2010)\textsuperscript{32} studied the normal skin colour by a number of chromophores, the most important of which is melanin. Besides melanin, other chromophores that contribute significantly to skin colour include haemoglobin (in both the oxygenated and reduced state) and carotenoids. Racial and ethnic differences in skin colour are related to the number, size, shape, distribution and degradation of melanin-laden organelles called melanosomes. These are produced by melanocytes and are transferred to the surrounding epidermal keratinocytes. Two types of melanin pigmentation occur in humans. The first is constitutive skin colour, which is the amount of melanin pigmentation that is genetically determined in the absence of sun exposure and other influences. The other is facultative (inducible) skin colour or ‘tan’,
which results from sun exposure. Increased pigmentation can also be due to endocrine, paracrine and autocrine factors the darkest pigmented human subjects are deep brown or black-brown in colour. Most peoples of the world fall between these two extremes and are moderate brown or yellow-brown in colour.