Chapter – 2

REVIEW OF LITERATURE
2.1 **Major Constituents in Tobacco Leaf**

Tobacco contains a wide spectrum of thousands of chemical compounds broadly classified as alkaloids, nitrogenous constituents, acids, phenolics, lipids and inorganic substances (Stedman, 1968 and Narasimha Rao *et al.*, 2007).

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Nor-nicotine</td>
<td>Nicotine</td>
</tr>
<tr>
<td>Anabasine</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Fructose</td>
<td></td>
</tr>
<tr>
<td>Nitrogenous substances</td>
<td></td>
</tr>
<tr>
<td>Nitrate Nitrogen</td>
<td>Proteins</td>
</tr>
<tr>
<td>Ammonical Nitrogen</td>
<td></td>
</tr>
<tr>
<td>Non volatile Aliphatic Acids</td>
<td></td>
</tr>
<tr>
<td>Citric Acid</td>
<td>Malic Acid</td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td></td>
</tr>
<tr>
<td>Poly phenols</td>
<td></td>
</tr>
<tr>
<td>Rutin</td>
<td>Chlorogenic Acid</td>
</tr>
<tr>
<td>Inorganic Constituents</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>Chloride</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>Structural Constituents</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>Cellulose</td>
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<tr>
<td>Pectin</td>
<td></td>
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**LIPIDS**

<table>
<thead>
<tr>
<th>Hydrocarbons</th>
<th>Neophytadiene</th>
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<tbody>
<tr>
<td>Fatty Acids</td>
<td></td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>Linolinic Acid</td>
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<tr>
<td>Linoleic Acid</td>
<td></td>
</tr>
<tr>
<td>Sterols</td>
<td></td>
</tr>
<tr>
<td>Stigmasterol</td>
<td></td>
</tr>
<tr>
<td>Campasterol</td>
<td></td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td></td>
</tr>
<tr>
<td>Terpenes</td>
<td>Solanesol</td>
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</tbody>
</table>

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2.1.1 Nicotine

Nicotine is the principal alkaloid in tobacco having commercial value since a very long time. Nicotine is the source for the botanical pesticide, nicotine sulphate. Nicotine in the form of sulphate is used as pesticide to harmful insects. Nicotine sulphate is eco-friendly and is easily bio-degradable and as such leaves no hazardous residues on vegetables and fruits bound to market, and nicotine could be starting material for synthesis of nicotinic acid, nicotinamide, nikethamide used in pharmaceutical industry. The average nicotine content of tobacco in India ranges from 1-3% (Anonymous, 1976a). According to (Narasimha Rao et al., 2005) there is considerable variation in the levels of nicotine content in various types of tobacco produced in different agro-ecological situations [FCV tobacco: 2.50%; Bidi tobacco (Gujarat): 9.71%; HDBRG tobacco: 3.89%; Natu tobacco: 2.79%; Chewing tobacco (Tamil Nadu): 2.93%; Chewing tobacco (Bihar): 3.70%; Motihari-Bitri (West Bengal): 6.64%].

2.1.2 Lipids

The term lipids will be defined here as those substances which are
A. Insoluble in water
B. Soluble in organic solvents such as Chloroform, Ether or Benzene
C. Containing long chain hydrocarbon groups in their molecules and
D. Present in or derived from living organisms

This definition covers a wide range of compounds and includes long chain hydrocarbons, alcohols, aldehydes, fatty acids and derivatives such as glycerides, wax esters, phospholipids, glycolipids and sulpholipids. Also included in this group are substances which are usually considered as belonging to other class of compounds like fat-soluble vitamins A, D, E and K and their derivatives as well as carotenoids and sterols and their fatty acid esters. Solanesol is a compound belonging to this class, known as terpenols or polyrenols. The topic of lipids in tobacco has been reviewed by Ellington et al. (1978a, 1978b).
A large number of compounds identified in tobacco leaf and smoke, constitute the broad group of lipids which may be polar or non-polar. They received a good deal of attention as they are related to the leaf quality & aroma and they are also shown as important precursors in the generation of carcinogenic smoke components on pyrolysis, such as smoke PAH (Schlotzhauer et al., 1969), (Chortyk et al., 1973). It was shown that pyrolysis of hexane extract of tobacco yielded nearly two-thirds of benzo-a-pyrene (BaP) obtained in tobacco pyrolysis (Schlotzhauer et al., 1969). Davis (1976) reviewed the contribution of individual components of tobacco lipids and waxes to smoking quality and aroma. Quantitatively the non-polar lipids as extracted by petroleum ether exhibit a wide genetic variability and they range from 6.51 to 15.30% in FCV tobacco germplasm and 1.11 to 7.93% in burley cultivars (Chaplin et al., 1980). In addition to genetic factors, many production factors like cultural practices, weather conditions and curing methods determine the qualitative and quantitative composition of different classes of components comprising the non-polar lipids.

Generally, the overall quality of tobacco seems to be inversely related to the amount of petroleum ether extractables (PEE). The qualitative composition of the PEE was investigated in detail by various workers by employing sophisticated analytical techniques. The qualitative composition of 90% methanol soluble fraction of PEE was reported to include aliphatic paraffin hydrocarbons, neophytadiene, cyclic paraffins, steryl esters, fatty acid esters, polyenes, phthalates, solanesol, sterols, tocopherols, acid salts, higher fatty acids, bases, resins and unidentified acidic substances (Stedman et al., 1962). Working on the PEE of Bulgarian tobacco, Ivanov and Ognyanov (1966) reported the presence of paraffins, neophytadiene, carbonyls, alcohols, fatty acids, sterols, solanesyl esters, solanesol and sterol esters. Employing gel permeation chromatography Cook et al. (1969) isolated glycerides, solanesyl esters, sterol esters, solanochromene, α-tocopherol, β-amyrin and sterols in the hexane extract of FCV tobacco.
Reid (1974) isolated the cuticular and cytoplasmic lipids from green leaves of tobacco and examined their composition. While the former contained alkanes, sterols, steryl esters and complex mixtures of di-terpenoids, the latter was composed of carotenes, plastoquinones, solanesol and steryl esters.

Nagaraj and Chakraborty (1978) studied the lipophilic composition of Indian natu tobacco and reported the quantitative composition of various fractions obtained from 90% methanol solubles, neutrals and water solubles.

Ellington et al. (1978b) studied the lipid distribution in various parts of flue-cured tobacco plant with individual data on total solanesol, neophytadiene, hydrocarbons, total major fatty acids and total sterols. Severson et al. (1978a) determined the transfer rate of various lipid constituents from tobacco leaf to smoke which include C25 to C34 paraffin hydrocarbons, neophytadiene, phytol, fatty alcohols, sterols, major fatty acids, squalene, tocopherol, β-amyрин, cycloartenol, 24-methylene cycloartanol and solanesol.

2.1.2.1 Terpenes

Terpenes (or isoprenoids, or terpenoids), represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products. Terpenoids can be described as modified terpenes, where methyl groups are moved or removed, or oxygen atoms added. Inversely, some authors use the term "terpenes" more broadly, to include the terpenoids. During the 19th century, chemical works on turpentine led to name "terpene", the hydrocarbons with the general formula C_{10}H_{16} found in that complex plant product. These terpenes are frequently found in plant essential oils which contain the "Quinta essentia", the plant fragrance. They are universally present in small amounts in living organisms, where they play numerous vital roles in plant physiology as well as important functions in all cellular membranes. On the other hand, they are also accumulated in many cases, and it is shown that
the extraordinary variety they display can be due to ecological factors playing an evolutionary role (Ourisson, 1990). They may be defined as a group of molecules whose structure is based on a various but definite number of isoprene units (methylbuta-1, 3-diene, named hemiterpene, with 5 carbon atoms).

A rational classification of the terpenes has been established based upon the number of isoprene (or isopentane) units incorporated in the basic molecular skeleton:

![Isoprene](image)

**Table 2.1.2.1a: Classification of terpenes**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Terpenes</th>
<th>Isoprene units</th>
<th>Carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Monoterpenes</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Sesquiterpenes</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Diterpenes</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>Sesterpenes</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>Triterpenes</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>Carotenoids</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>Rubber</td>
<td>&gt; 100</td>
<td>&gt; 500</td>
</tr>
</tbody>
</table>

Mono-, sesqui-, di-, and sesterpenes contain the isoprene units linked in a head to tail fashion. The triterpenes and carotenoids (tetra terpenes) contain two C\textsubscript{15} and C\textsubscript{20} units, respectively linked head to head. More than 1000 monoterpenes, 7000 sesquiterpenes and more than 3000 diterpenes have been described. Many terpenes are hydrocarbons, but oxygen-containing compounds such as alcohols, aldehydes or ketones are also found. These derivatives are frequently named terpenoids. These isoprenoid alcohols are also known as...
terpenols. Search for polyisoprenoid alcohols was initiated with the accidental discovery of solanesol in tobacco leaves (Rowland et al., 1956) and isolation of several polyprenols (C_{30}-C_{45}) in cellulose pulp extracts (Lindgren, 1965). As a rule, the chain of these polyisoprenoid alcohols has a number of prenyl units in the range 5 to 25. Some important members of the series are as follows:

**Table 2.1.2.1b: Important isoprenoid constituents**

<table>
<thead>
<tr>
<th>n</th>
<th>Number of isoprene unit</th>
<th>Number of carbons</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>10</td>
<td>Geraniol</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>15</td>
<td>Farnesol</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>20</td>
<td>Geranylgeraniol</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>25</td>
<td>Geranyl farnesol</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>45</td>
<td>Solanesol</td>
</tr>
<tr>
<td>8-11</td>
<td>10-13</td>
<td>50-65</td>
<td>Castaprenols-Ficaprenols</td>
</tr>
</tbody>
</table>

Wahlberg et al. (1977) have comprehensively reviewed the subject of tobacco isoprenoid constituents and provided comments on some of the more important materials. Solanesol is a major component of tobacco generally ranging in quantity from 0.4 to 4%. While this polyprenol was first isolated from tobacco, it is now considered to be a ubiquitous leaf component in the plant kingdom.

### 2.1.3 Solanesol

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... al., 1962). However, tobacco is the richest source of this chemical. The presence of a long chain of repeating isoprene units (nine) in the solanesol molecule makes it a valuable source material for synthesizing metabolically active quinones and other drugs. Considering the importance of solanesol in the pharmaceutical industry, utilization of tobacco or tobacco waste as a source of this chemical has attained significance. Thus, solanesol has excellent prospects in the future as drug intermediate, which has generated considerable interest in this compound. Since the first report on its isolation, extensive research has been carried out providing voluminous information on the analytical techniques, influence of growing conditions and agronomic practices on solanesol content in the leaf at various growth stages of the plant and extraction processes. Thus, the patent literature on solanesol amplifies the significance of this compound. Solanesol concentrate of a higher order of purity will definitely have an enhanced economic value.

Considerable work was done by the Japanese scientists on various aspects of solanesol, viz., biological source material, improved analytical techniques and various recovery processes. Fukusaki et al. (2004) described the biosynthetic pathway for solanesol. The biosynthetic pathway experiments were performed with [1-\textsuperscript{13}C] deoxy-D-xylulose triacetate and (RS)-[2-\textsuperscript{13}C]mevalonolactone in the tobacco plant. The labeling pattern for solanesol was elucidated to reveal that the isoprene moiety of solanesol would be derived from deoxy-xylulose. The result strongly suggests that tobacco solanesol is biosynthesized via the 2-\textsuperscript{C}-methyl-D-erythritol 4-phosphate (MEP) pathway. The feeding of two \textsuperscript{13}C-labeled biosynthetic precursors to tobacco plants to prove the isopentenyl diphosphate (IPP) biosynthetic pathway for solanesol.
2.2 Isolation and Characterization of Solanesol from *Nicotiana tabacum*.L.

2.2.1 Solanesol in tobacco

It is one of the important precursors of the tumorigenic poly nuclear aromatic hydrocarbons (PAHs) of tobacco smoke. Reduction of its levels in tobacco, leads to safe smoking products due to reduced PAH levels in cigarette smoke. It is also the starting material for many high value bio-chemicals, including coenzyme Q$_{10}$ and Vitamin-K analogues (Hamamura *et al.*, 2002). As a starting material for Q$_{10}$, it is used in treatment of different cancers. Coenzyme Q$_{10}$ is well known not only to reduce the number and size of tumors but also improve cardiovascular health (Yalcin *et al.*, 2004), (Singal *et al.*, 1999). Solanesol itself could be used as an antibiotic, cardiac stimulant and lipid antioxidant. At present clinical trials are under progress to explore its use as an anticancer drug. It is also stated that solanesol possess antibacterial, anti-inflammation and antiulcer properties (Khidyrova *et al.*, 2002). There is a great demand for solanesol for production of Q$_{10}$ and other uses. Thus, its isolation not only reduces the risks of PAH from tobacco smoke but also makes use of it as a starting material in synthesis of several value-added products such as Q$_{10}$ and other analogues. Therefore isolation of solanesol from tobacco is gaining a lot of importance in recent years. Solanesol is present in the lamina of tobacco leaves while absent in stems and stalk (Severson *et al.*, 1977). Various sections of the tobacco plant are shown Fig.2.2.1. The content of solanesol in tobacco depends upon a number of factors. It varies from 0.3-3.0 % according to the type and variety of tobacco, duration of growth and method of curing (Zhang *et al.*, 2005). A substantial portion of solanesol exists in the form of fatty acid esters due to which proper curing and saponification play an important role in converting them in to free solanesol (Scholtzhauer *et al.*, 1976). Tobacco also contains several other organic compounds that can be easily co-extracted with solanesol which interfere with subsequent separation and purification processes (Troje *et al.*, 1997).
**SOLANESOL**

- An acyclic trisquiterpenoid alcohol with 9 isoprene units

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>$C_{45}H_{74}O$</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>631.08</td>
</tr>
<tr>
<td>Melting point</td>
<td>41.5 – 42.5 °C</td>
</tr>
<tr>
<td>Analysis</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td></td>
<td>High Pressure Liquid Chromatography</td>
</tr>
</tbody>
</table>

- Ubiquitous – occurs in many botanical & biological species

**Botanical**

- *Aesculus hippocastanum* (Horse chestnut – tree)
- *Morus alba* (Mulberry – leaves)
- *Solanum tuberosum* (Potato – leaves)
- *Nicotiana tabacum* (Tobacco – leaves)
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Biological

*Bombyx mori* (Silk worm – feces)

Butter fat & Cream fat

- Tobacco is the principal source
- Important precursor of PAH compounds
- Identified as market in Environmental Tobacco Smoke (ETS)
- Chemurgic value: Starting material for high value pharmaceutical compounds - Coenzyme Q9, Coenzyme Q10, vitamin K2, vitamin E.

### 2.2.2 Chemical and physical properties of solanesol

Solanesol is a white waxy solid soluble in organic solvents but insoluble in water and optically inactive. It was originally characterized as pentatertpene but latter works indicated the presence of 9 isoprenoid groups (Erickson *et al.*, 1959; Rowland *et al.*, 1960). It has an UV absorption band at 197 nm (CRC Atlas, 1975) and IR absorption bands at 3300 (s), 2850(vs), 1660(s), 1440(vs), 1380(s), 1320(w), 1210(w), 1145(m), 1090(m), 990(s), 835(m) and 740(w) cm⁻¹ (Mold *et al.*, 1957). Solanesol crystallizes in to two forms having significantly different IR spectra (Rowland *et al.*, 1959).

**Chemical name** : (2E, 6E, 10D, 14E, 18E, 22E, 26E, 30E) – 3, 7, 11, 15, 19, 23, 27, 31, 35 - Nonamethyl 2, 6,10,14,18, 22, 26, 30, 34 – Hexatriacontanoal

**Common name** : Solanesol, Nonaisoprenol

**Mol. formula** : C₄₅H₇₄O

**Mol. weight** : 631.08

**Mp** : 41.5 – 42.5 °C

**Appearance** : Light yellow or white solid

**Solubility** : Soluble in organic solvents but insoluble in water
2.2.3 Analysis of solanesol

Unlike many other tobacco constituents, the quantitative analysis of solanesol presents several problems, thus requiring more sophisticated techniques. Absence of selective UV absorption precludes UV spectrophotometry. TLC densitometry offers a most convenient and rapid method for solanesol determination. Though TLC technique ensures satisfactory separation of solanesol from other PMA-sensitive lipid components, the succeeding densitometry imposes many limitations unless machine coated plates are used and the chromogenic reagent is impregnated as a preceding step to TLC development. TLC densitometry can best give semi-quantitative estimates because of the above reasons and also due to the decomposition of solanesol on TLC plates, making GLC or HPLC techniques imperative for accurate and reliable analysis of solanesol. Low volatility, poor Flame Ionization Detector (FID) response and formation of solanescenes by the thermal dehydration of solanesol under the operating conditions of GLC, the compound requires derivitization either as corresponding saturated alcohol by dehydrogenation. However, HPLC appears to be a preferred technique because of the ease of analysis, particularly the avoidance of derivitization. Different HPLC conditions
are reported in the literature for analysis of the compound: Zorbax-Sil column, hexane: isopropyl ether (70:30) as eluent; Resolve C18 column, gradient elution with methanol: acetonitrile; Partisil 5 column, hexane; diethyl ether (93:7) as mobile phase; C18 column, methanol as mobile phase. Narasimha Rao et al. (2000) developed a method for extraction of solanesol from tobacco and subsequent reverse-phase HPLC, employing a C18 column and isopropyl alcohol: methanol (60:40) as mobile phase, which has the flexibility to analyze green/cured leaf samples and crude/enriched extracts.

Stedman et al. (1962) reported relatively higher but variable value of solanesol content (1-2%) in flue-cured tobacco by gravimetric estimation of column chromatographic eluate. An improved infra-red spectrophotometric method of estimation of solanesol in flue-cured tobacco was developed by Stedman et al. (1962).

The method gave a cumulative estimate of “solanesol like substances” (SLS) since the observed absorbancy ratio 10.1µ / 12.0 µ was not specific for solanesol alone but included either interfering olefinic analogs which accompany solanesol in the leaf. The tobacco sample was extracted with skellysolve B and the residue, after solvent removal, was chromatographed on alumina column and developed with 60% benzene in petroleum ether followed by benzene alone. The eluate with benzene was rich in solanesol and the residue after solvent removal was further purified by crystallization from acetone at -25 °C. The method of estimation was based on the extinction of the sample at 10.1µ and 12.0 µ as compared to a standard. The authors found that burley and bright tobacco had levels of SLS greater than 1%. Maryland, Perique, fire-cured, cigar-filler and cigar binder tobaccos contained less SLS.

A thin-layer chromatographic method for the separation of major lipids found in the hexane extract of tobacco was reported by Woollen et al. (1972).
The three different solvent systems viz., petroleum ether (60-80°C): acetone: acetic acid (65:35:2, v/v); petroleum ether: diethyl ether: acetic acid (70:30:5, v/v); petroleum ether: diethyl ether: acetic acid (98:1:1, v/v) and visualized by spraying with phosphomolybdic acid. The intensities of the bands produced were determined by densitometry to produce a lipid profile. They observed that the profile varied with leaf position and underwent several changes during curing. Solanesol was positively correlated with ascending leaf position.

Woollen and Jones (1971) reported a thin layer densitometric method wherein they stressed excellent solanesol specificity of phosphomolybdic acid in their work but they did not present any specific value for flue-cured tobacco.

Severson et al. (1977) developed a method of estimation of total and free solanesol based on high temperature gas liquid chromatography after converting the alcohol into its trimethyl silyl (TMS) derivative. They reported significantly higher quantities of solanesol in leaf (up to 3% of leaf weight) by this method.

Deki (1977) reported a thin-layer densitometric method of estimation of solanesol in tobacco with a detection limit as low as 0.2 µg.

Severson et al. (1978a) reported a rapid and reproducible method for the analysis of major tobacco lipids including solanesol, which involved silicic acid column chromatography followed by gas chromatography. They reported 1.99 - 3.11% solanesol in flue-cured tobacco grown according to conventional methods and a very low level of solanesol (0.09-0.12%) in close grown tobaccos.

A number of gas chromatographic methods for determination of solanesol in tobacco were reported (Wellburn et al., 1966; Chamberlain et al., 1990; Severson et al., 1978b; Sheen et al., 1978; Woolen et al., 1971). The low volatility and poor FID response of solanesol render the technique unsuitable (Wellburn et al., 1966). The sample preparation also involves a number of time-
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consuming derivatization steps (Chamberlain et al., 1990; Severson et al., 1978a). Sheen et al. (1978) reported a packed column GC method involving lengthy extraction procedures and hydrogenation of solanesol. The other methods include gravimetry and thin layer chromatography (TLC) (Woolen et al., 1971), coulometry (Zhao et al., 2002). HPLC with various detectors including UV, RID, ELSD and MS was used to determine solanesol. Most of the methods reported were in normal phase mode with UV detection (Zhao et al., 1997). Zhoua et al. (2006) reported a RP-HPLC method using ELSD as a detector for determination of solanesol in tobacco. ELSD is not only a specialized detector but also requires a large volume of nebulizer gas of high purity. It makes the method unsuitable for routine analysis solanesol because of the cost ineffectiveness. However, reversed phase HPLC with UV detection is often preferred not only because of its higher sensitivity but also wide availability and suitability. Recently, Chen et al. (2007) reported RP-HPLC with UV and ESI-TOF/MS determination of solanesol in the crude extracts of tobacco leaves. The major drawback of this method lies in detection. The analytes were monitored at 202 nm by PDA where the acetonitrile used as one of the mobile phase and the acetonitrile solvent generally interferes. The use of such a short wavelength UV also produces baseline artifacts. The C4 column was selected to elute the solanesol peak, retention time 10min. Due to the increasing demand for solanesol, herein, we report for the first time, a convenient and rapid method for determination as well as isolation of solanesol from tobacco in laboratory experiment. The details are presented.

2.2.4 Solanesol content in different types of tobacco

Though, the initial report of Rowland et al. (1956) mentioned only 0.4% solanesol in tobacco, later reports suggest even higher > 3% solanesol in some varieties (Severson et al., 1978b).
Narasimha Rao et al. (1978) assessed the potentialities of various types of Indian tobacco waste as sources of useful phytochemicals like solanesol, nicotine and organic acids and concluded that flue-cured tobacco scrap is the best source for solanesol.

Phani Kiran et al. (2008) estimation of solanesol in tobacco and non-tobacco plants.

2.2.4.1 Solanesyl esters
Rowland et al. (1959) have reported the presence of fatty acid esters of solanesol in tobacco. The esters were isolated by chromatographic separation of a hexane extract of aged flue-cured tobacco. Three ester fractions were obtained by chromatography using silicic acid. The esters were subsequently crystallized from acetone at \(-27^\circ\)C. The solanesol esters amounted to about 0.03\% of the leaf, the principal ester being solanesyl palmitate (0.011\% dry weight of leaf). As 15 - 20\% of total solanesol occurs as esters of higher fatty acids like myristic, lauric, palmitic, oleic, linoleic and linolenic (Rowland et al., 1960), the total solanesol content is higher than the free solanesol. Solanesyl acetate was isolated from this extract by Rowland and Latimer (1959). Free solanesol content of tobacco depends on many factors like type and variety of tobacco, stalk position, type of curing, period of storage etc. Nagaraj (1976) has reported that solanesol and solanesol acetate occurred at a lower concentration in Natu tobacco (0.05\% of lamina).

2.2.5 Extraction of Solanesol
Rowland et al. (1956) extracted tobacco with methanol followed by ether. The combined residues were treated with ether and the water solubles were removed by water extraction. The ethereal fraction was extracted with a buffer solution (pH: 11.8). The ethereal solution was then concentrated and purified by either precipitation from acetone at \(-27^\circ\)C or partitioning between 90\% methanol
and hexane. Further purification, achieved by chromatography on alumina, silicic acid and florisil, yielded pure solanesol.

Carruthers and Johnstone (1960) isolated a specimen of solanesol by chromatography of the neutral fraction of an alcoholic extract of green tobacco leaf and crystallizing to constant melting point 37.5 - 38.5 °C.

Considering the importance of solanesol in pharmaceutical industry for the manufacture of synthetic drugs, Billinsky and Stedman (1962) suggested the utilization of cheap tobacco including waste material as a source of this chemical.

Grossman et al. (1963) employed a new technique, which involved initial petroleum ether extraction, concentration of the neutral fraction after removing acids and bases under vacuum. The waxes were removed from acetone solution at 40°C and the de-waxed solution was subjected to molecular distillation at 300°C and 100 – 200 µ pressure. Solanesol was isolated from unsaponifiables of silk worm feces and mulberry leaves (Asahina et al., 1970) by adopting the techniques like solvent extraction, fractional crystallization, adsorption chromatography and molecular distillation.

A petroleum ether extract of flue-cured tobacco leaf was separated by silicic acid column chromatography into eight major fractions for pyrolysis studies. The solanesol rich fraction was purified by chromatography on neutral alumina and the residue of benzene: chloroform (1:2, v/v) eluate was dissolved in petroleum ether and cooled at -70 °C; the precipitate was again chromatographed on a micro-column of florisil which yielded 0.71% solanesol and 0.10% solanesol ester (Ellington et al., 1978b).

Narasimha Rao and Chakraborty (1979) developed a process involving selective extraction of solanesol with methanol followed by cooling the extract to
precipitate solanesol and followed by cooling the extract to precipitate solanesol and recrystallisation using methanol: chloroform (2:1) yielding a fraction containing approximately 75% solanesol. Takemura and Amano (1979) treated the unsaponifiable fraction of tobacco extract with mixture of acetone: methanol (2:1), insoluble matter was removed, solution was concentrated, dissolved in acetone: methanol (2:100), insoluble matter was removed, treated with charcoal and solanesol was precipitated at low temperature. The product was of high purity with 70% recovery. The patented process for recovery of solanesol involved centrifugal liquid partition chromatography using hexane as a stationary phase and a hydrous alcohol such as methanol, ethanol etc. with 5–10% water content as mobile phase. Subsequently, the liquid flow direction was reversed, followed by fractionation with hexane to obtain solanesol, which was further purified by HPLC (Takahiro, 1988). According to Zhang (1991), n-hexane was used as extracting solvent and a pasty solanesol was obtained after two extractions followed by concentrations. In another process, solanesol was extracted from moldy or broken tobacco by extraction with solvents such as n-hexane, ultra filtration and concentration by membrane evaporation (Zhang et al., 1994a). As per the Chinese Patent (Zhang et al., 1994b), extraction of unsaponifiable matter of solanesol involved addition of solvent and catalyst for saponification reaction, filtering, mixing, separation, ultra-filtration and concentration. In the technique of sprinkling extraction, pretreated tobacco was sent into a rotating grid, repeatedly and cyclically sprinkled and extracted by a solvent; finally the extract was purified and concentrated to obtain solanesol (Fu et al., 1995). In another process (Wu, 1996) tobacco after nicotine extraction was dried, crushed, extracted several times with C\textsubscript{5}-C\textsubscript{12} alkane as solvent. Extracts mixed and concentrated, saponified with alcohol and alkali solution, after removing water and solvent, treated with mixed ketone and alcohol liquid I & II separately and cooled to separate pure solanesol crystal. In the process developed by Zhang et al. (1996) for solanesol recovery involved continuous extraction and twice setting process and tail-gas adsorption process. Recently, supercritical
fluid extraction was adopted for extraction of solanesol (Wu, 2001). It is reported that compared with hexane and other non-polar oil solvents, the CO₂ solvent has better ability to extract solanesol. According to the Chinese Patent (Ma et al., 2002), the method of purifying solanesol from extract containing solanesol involves, dissolving solanesol extract having purity not less than 70% in n-hexane, adopting adsorption chromatography, using a solvent mixture of n-hexane, 1, 2-dichloroethane and acetone for elution and subsequent gel permeation chromatography with n-hexane as solvent, concentrating and cooling to obtain solanesol of purity above 95%.

For extraction of solanesol on an industrial scale, there are two obvious routes viz., 1) hexane extraction followed by enrichment methods and 2) methanol extraction and fractional crystallization. Solanesol and its esters can be extracted with hexane along with other lipids but enrichment of the hexane extract containing 10-15% solanesol presents many problems and is tedious. Similarly, methanol extraction and cooling of the extracts, results in a fraction containing about 50% solanesol which can be enriched to about 75% by re-crystallization. As solanesol is utilized in the pharmaceutical industry for preparation of drug intermediates, much higher purity (>95%) is desirable. The main advantages of the process developed by Narasimha Rao et al. (2007) for the recovery of solanesol of purity more than 95% from tobacco green leaf/tobacco cured leaf/tobacco waste and crude extracts are as follows. Extraction with a polar solvent and subsequent enrichment by cooling makes further purification easy as solanesol of purity more than 95% obtained in the process can be utilized in drug development. Economic viability of the present process is enhanced by the adsorbent used, substantial reduction in adsorbent requirement, as compared to conventional column chromatography, single solvent employed to elute the compound, as against solvent mixtures reported in the literature and regeneration of the adsorbent for reuse, thus, avoiding techniques like centrifugal liquid partition chromatography, molecular distillation, ultra filtration followed by
membrane evaporation, gel permeation chromatography etc. Adoptability for processing tobacco green leaf or cured leaf or waste or crude extracts for extraction of pure solanesol, thus enlarging the raw material base.

One of the key problems in extraction of solanesol from tobacco is the selection of a suitable solvent for maximum yield and purity. As solanesol lies in the cellular chloroplast of the tobacco leaves, not only the solubility but also penetrability of the solvent is very important for complete extraction. Further, its separation from the crude extract and purification pose several problems because of the presence of closely related fatty acids, alcohols, alkaloids, tobacco pigments and other organic impurities. The food and pharmaceutical grade solanesol has to be of highest purity for quality, safety and efficacy of the finished products. Therefore it is quite important to develop processes that can selectively separate and purify solanesol from the crude extracts of tobacco leaves. Several techniques were described for extraction, isolation and purification of solanesol from tobacco in the literature (Duan et al., 2000). Most of the methods involve multiple step procedures, which are non-specific, quite tedious and time consuming. Generally, maceration, percolation, ultra sonication, soxhlet and bubble column were used for extraction of phytochemicals from the plant materials (Keca et al., 1997; Doig et al., 2005). The first two techniques are not only time consuming but also give low yields of the desired products. Soxhlet extraction has been the most versatile among all other conventional techniques. It serves as an (i) extraction step for the isolation of phyto-constituents and (ii) used as a bench mark against which any new extraction technique is compared. The basic extraction apparatus consists of a reservoir, an extraction body, a heating source and a reflux condenser. As the extracted analyte will normally have a higher boiling point than the solvent, it is preferentially retained in the flask and fresh solvent circulates. This ensures that only fresh solvent is used to extract the analyte from the sample in the thimble. One of the major significant shortcomings of soxhlet extraction is the lengthy extraction time that can be 8, 16, 24 hours or more (Pastor et al., 1997). It has limited analytical applications
and not suitable for handling of bulk quantities of tobacco. Tang et al. proposed an extraction procedure with petroleum ether under reflux at 50°C followed by silica gel column chromatography for isolation and purification of solanesol from tobacco leaves (Tang et al., 2007). However, the heat reflux processes involve lengthy operations, bulk amount of solvents and ultimately thermal decomposition of the target compounds. Microwave assisted extraction (MAE) coupled with saponification was reported to be effective for extraction of solanesol from tobacco leaves (Zhou et al., 2006). It has the advantages, such as shorter time, less solvent consumption, higher extraction rate, better products with lower cost. Saponification alleviates emulsification in this process. Zhou et al. (2006) compared different extraction procedures to recover solanesol from tobacco leaves and the data are presented in figure 2.2.5.

![Graph showing the comparison of different extraction procedures for recovery of solanesol from tobacco leaves.](image)

**Fig. 2.2.5:** Comparison of different extraction procedures for recovery of solanesol from tobacco leaves.

The MAE was found to be faster as can be observed in Fig.2.2.5. Ultrasonic extraction (Chen et al., 2008) combined with saponification was also reported (Chen et al., 2007) for extraction of solanesol from tobacco leaves. Highest yields were obtained using 20mg/ml KOH in ethanol at 60°C for 4 hrs. However, both MAE and ultrasound sonication consume high energy and are not
suitable for commercial production. Supercritical fluid extraction (SFE) is one of the environment friendly processes for separating the active ingredients from plant materials. It uses supercritical fluids (CO$_2$) as one of the extraction solvents. The supercritical fluids are less expensive and extract the analytes faster when compared to organic solvents. By adding modifiers to supercritical fluids, the polarity could be changed for increasing the selectivity. However, the technique is sensitive to process control and the phase transitions. SFE was used as a refining method to produce solanesol from crude cream (Qunly et al., 2005). It was also employed as a method for extracting high percentage of purity solanesol from waste tobacco leaves. However the methods described involve several pretreatment procedures and extraction steps (Guan, 2006). Recently, Ruiz-Rodriguez et al. (2008) have reported extraction of solanesol with supercritical CO$_2$. The average yields of solanesol were 18.8% of the extract. Nicotine was also co-extracted which could be hazardous. Recently high-speed countercurrent chromatography (HSCCC) for isolation of solanesol from the crude extracts of tobacco was reported (Du et al., 2006). Here, the crude extract, instead of raw tobacco was used as a feedstock for purification of solanesol. The purity of solanesol thus obtained was less than 95%. A slow rotary counter current chromatography (SRCCC) involving a non-aqueous two-phase solvent system of sunflower oil-ethanol was also used to produce food grade solanesol in a commercial scale (Zhao et al., 2007). However, the process is not cost effective and the purity of solanesol was only 26%.

The countercurrent extraction which features a continuous relative movement between solvent and feed materials offers high yields of extractable material with comparatively less solvent requirement and time. It is widely employed in the food industry and its extension to the pharmaceutical industry requires a better understanding of the extraction process.
2.2.6 Solanesol in Drug Development

Different derivatives starting from solanesol were evaluated for various biological activities. Among the substituted benzoate esters, the solanesyl-(5-amino, 4-nitro)-benzoate is as effective as chlofibrate in reducing serum cholesterol and also has anti-cholestimic activity. Substituted solanesyl sulphates and their salts are useful as anti-inflammatories, antiallergics and are described as reverse transcriptase inhibitors for treatment of AIDS. N-solanesyl-N,N1-bis (3,4-dimethoxybenzyl) ethylenediamine (SDB-ethylenediamine) inhibits the colony formation of multi-drug resistant mutant cell lines derived from Chinese hamster V 79(v79/ADM) and human hepatoma PLC/PRF/5 (PLC/COL) cells to greater extent than that of the parental cells when combined with other anti-tumor agents. It potentiated the cytotoxic activity of almost all kinds of drugs tested, including Adriamycin (ADM), Actinomysin D, Vincristine, Cytoxine arabinoside and 5-fluorouracil urea (5;FU) and potential ratios were higher against V 79/ADM cells than V 79/S cells. Among the anti-tumor agents tested, the activities of bleomycin group of antibiotics were more strongly enhanced by SDB – ethylene diamine and the potentiation was higher in parental cells than in V 79/ADM cells, SDB – ethylene diamine enhanced the uptake of ADM and daunorubicin into V 79/ADM and its parental cells, but it did not increase the uptake of 5-FU or peplomycin, indicating that different mechanisms operate for potentiation in the case of the latter drugs, i.e. not simply an increase of intracellular drug uptake. Two fragments of SDB – ethylene diamine, solanesol and the diamine component (Verpamil – like moiety), showed neither cytotoxic activity nor potentiator activity, even if they were mixed together, indicating that steric conformation of intact SDB – ethylene diamine molecule is more important for these two activities (Suzuki et al., 1990; Tomida et al., 1990; Chida et al., 2005). Isomeric solanesol (2-Z) prepared from solanesol was reported as antihypertensive, antihyperlepidimic and anti-tumor agents, (S) 2,3 – dihydrosolanesol and (S) 2,3–dihydro solanesyl mono phosphate are used in inhibiting metastasis of cancers. Some substituted solanesyl amine and ether derivatives have been synthesized
and tested for anti-viral and anti-tumor activities in rats and interferon inducing activity in humans. Preparation of anti-ulcer isoprenoids having solanesol moiety has been reported in the literature. The long chain of solanesol which imparts the lipophilic nature to the molecule makes it an attractive candidate for chemical modification and evaluating the biological activity. A compound unable to reach inside the cell due to its hydrophilic nature and cross the membrane can be made to do so, if it is attached to an active pharmacophore / molecule so as to exert biological pressure. Important commercial products from solanesol are presented in Figure. 2.2.6.

**Fig. 2.2.6: Important commercial products from Solanesol**

![Chemical structures of Solanesol-related compounds](image)

**SOLANESOL ACETATE**

**HOMEPHENONE**

**SOLANESNES**

**Co Q0**

**PLASTOQUINONE**

**2-SOLANESYL 1,4 NAPHTHQINONE**
2.3 Coenzyme Q\textsubscript{10} (Ubiquinone)

Coenzyme Q\textsubscript{10}, also known as ubiquinone, is a vitamin-like substance used by the human body to help produce ATP in the electron transport chain and is found throughout the body, with the highest concentrations in the heart and liver (Ernster \textit{et al}., 1995). Coenzyme Q\textsubscript{10} is currently being studied as a supplement which can possibly provide relief for migraine headache sufferers (Sandor \textit{et al}., 2005), protect people from Parkinson’s disease and other neurodegenerative diseases (Matthews \textit{et al}., 1998), and lower blood pressure. These studies have helped develop coenzyme Q\textsubscript{10} into a high value product thus increasing its demand. Coenzyme Q\textsubscript{10} is currently available as a dietary supplement, as well as being added to products like skin cream and toothpaste (Kaneka Nutrients, 2008). Coenzyme Q\textsubscript{10} is currently fermented from yeast (Chokshi, 2011). There are established protocols for developing coenzyme Q\textsubscript{10} from solanesol (West, 2004)) as well as studies that have found that using solanesol can increase the yield of coenzyme Q\textsubscript{10} derived from the fermentation of yeast (Liu \textit{et al}., 2008).

CoQ\textsubscript{10} was first isolated from beef heart mitochondria by Dr. Frederick Crane of Wisconsin, U.S.A (Crane \textit{et al}., 1957). The same year, Professor Morton of England defined a compound obtained from vitamin a deficient rat liver to be the same as CoQ\textsubscript{10} (Morton \textit{et al}., 1957). Professor Morton introduced the name ubiquinone, meaning the ubiquitous quinone. In 1958, Professor Karl Folkers and coworkers at Merck, Inc., determined the precise chemical structure of CoQ\textsubscript{10}: 2,3-dimethoxy-5 methyl-6 decaprenyl benzoquinone (Fig.2.3), synthesized it, and were the first to produce it by fermentation. In the mid-1960's, Professor Yamamura of Japan became the first in the world to use coenzyme Q\textsubscript{7} (a related compound) in the treatment of human disease: congestive heart failure. Mellors and Tappel (1966) showed that reduced CoQ\textsubscript{6} was an effective antioxidant. Gian Paolo Littarru of Italy along with Professor Karl Folkers (1972) documented a deficiency of CoQ\textsubscript{10} in human heart disease. By the mid-1970's,
the Japanese perfected the industrial technology to produce pure CoQ$_{10}$ in quantities sufficient for larger clinical trials. Peter Mitchell of England received the Nobel Prize in 1978 for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory, which includes the vital proton motive role of CoQ$_{10}$ in energy transfer systems (Michell, 1976; Mitchell, 1979; Mitchell, 1988; Mitchell et al., 1991).

![Chemical structure of CoQ$_{10}$: 2,3-dimethoxy-5 methyl-6 decaprenyl Benzoquinone](image)

**Fig. 2.3: Chemical structure of CoQ$_{10}$: 2,3-dimethoxy-5 methyl-6 decaprenyl Benzoquinone**

Clinical trials have established the important role of CoQ$_{10}$ in the following areas: Heart ischemia, Liver injury, Heart failure prevention, alopecia, dysacousis, mysathenia, pulmonary emphysema, encephalosclerosis, bronchial asthma, promoting pancreatic functions and secretions, aregenerative anemia, skin synthetic function, promoting healthy gums, high blood pressure and sugar metabolism problems (Blinakov et al., 1986; Folgers et al., 1974, 1985, 1986, 1991, 1993; Greenberg et al., 1990; Hanaki et al., 1991; Hans, 1998; Judy et al., 1993; Lockwood et al., 1994).

In the early 1980's, there was a considerable acceleration in the number and size of clinical trials. These resulted in part from the availability of pure CoQ$_{10}$ in large quantities from pharmaceutical companies in Japan and from the capacity to directly measure CoQ$_{10}$ in blood and tissue by high performance liquid chromatography. Lars Ernster of Sweden, enlarged upon CoQ$_{10}$'s importance as an antioxidant and free radical scavenger (Ernster, 1977). Professor Karl Folkers went on to receive the Priestly Medal from the American Chemical Society in 1986 and the National Medal of Science from President Bush in 1990 for his work with CoQ$_{10}$ and other vitamins.
2.3: Photograph of Coenzyme Q<sub>10</sub>

Significant milestones in Coenzyme Q<sub>10</sub> research and in the evolution of CoQ<sub>10</sub> products.

**Table 2.3: Coenzyme Q<sub>10</sub> timeline**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1957</td>
<td>CoQ&lt;sub&gt;10&lt;/sub&gt; was first isolated from beef heart mitochondria - Dr. Frederick L. Crane, University of Wisconsin.</td>
</tr>
<tr>
<td>1958</td>
<td>Chemical structure of CoQ&lt;sub&gt;10&lt;/sub&gt; determined - Dr. Karl Folkers and colleagues at Merck, Sharpe and Dohme</td>
</tr>
<tr>
<td>1960</td>
<td>Patients with CHF successfully treated with CoQ7 for the first time - Dr. Y. Yamamura in Japan.</td>
</tr>
<tr>
<td>1975</td>
<td>“Protonmotive Q cycle” in the mitochondrial electron transport chain proposed by Dr. P. Mitchell in England.</td>
</tr>
<tr>
<td>1978</td>
<td>Dr. P. Mitchell awarded the Nobel prize for elucidating its mechanism of action.</td>
</tr>
<tr>
<td>1980</td>
<td>Renewed interest in the use of CoQ&lt;sub&gt;10&lt;/sub&gt; for heart disease. Several double-blind studies in the US and elsewhere led by Dr. K. Folkers and his colleagues documenting its efficacy. Exploration of the potential usefulness of CoQ&lt;sub&gt;10&lt;/sub&gt; for various other indications including mitochondrial cytopathies.</td>
</tr>
<tr>
<td>1990</td>
<td>Increased awareness of the potential health benefits and soaring popularity of CoQ&lt;sub&gt;10&lt;/sub&gt; as a dietary supplement.</td>
</tr>
<tr>
<td>1994</td>
<td>Passage of Dietary Supplement Health and Education Act (DSHEA) by the US Congress. CoQ&lt;sub&gt;10&lt;/sub&gt; grandfathered as a dietary supplement.</td>
</tr>
<tr>
<td>1996</td>
<td>Introduction of Q-Gel, the solubilized CoQ&lt;sub&gt;10&lt;/sub&gt; with enhanced bioavailability.</td>
</tr>
<tr>
<td>1998</td>
<td>Numerous studies documenting the superior performance of Q-Gel and other solubilized CoQ10 products. Publication of findings in several peer-reviewed journals and presentations at various scientific meetings.</td>
</tr>
<tr>
<td>1999</td>
<td>Orphan Drug Designation awarded by the FDA for CoQ&lt;sub&gt;10&lt;/sub&gt; for the treatment of mitochondrial cytopathies. Introduction of solubilized and stabilized ubiquinol.</td>
</tr>
</tbody>
</table>
2001  |  Publication of USP monographs on CoQ₁₀.
2003  |  Two additional Orphan Drug Designations awarded by the FDA for CoQ₁₀ as ubiquinol (Q-Nol for the treatment of Huntington’s Disease and Pediatric Congestive Heart Failure.
2004  |  Introduction of CoQ₁₀-cyclodextrin complex as HydroQSorb with superior bioavailability for solid dosage form applications.
2005  |  Introduction of Liquid Q a nanodispersion of CoQ₁₀ in water – with superior bioavailability.
2006  |  Introduction of Ubiquinol QH by Kaneka

### 2.3.1 Chemical nature of Coenzyme Q₁₀

The chemical structure of CoQ₁₀, elucidated by Dr. Karl Folkers and his group, is 2,3–dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone. Coenzyme Q₁₀ is liposoluble and has 10 isoprene units. It exists in nature and in the body in two forms: the oxidized form, called ubiquinone, and the reduced form which is named ubiquinol. The structures of ubiquinone and ubiquinol are shown in Fig.2.3.1.

![Chemical structure of Coenzyme Q₁₀](image)

**Fig. 2.3.1: Structure of Coenzyme Q₁₀, Ubiquinone (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone) and ubiquinol (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone).**
2.3.2 Chemical properties of Coenzyme Q₁₀

The oxidized structure of CoQ₁₀ is shown in Fig. 2.3.2. The various kinds of Coenzyme Q can be distinguished by the number of isoprenoid side-chains they have. The most common CoQ in human mitochondria is Q₁₀. The 10 refers to the number of isoprene repeats. The image below has three isoprenoid units and would be called Q₃ (Wolf et al., 1958).

![Oxidised structure of Coenzyme Q₁₀](image)

**Fig. 2.3.2: Oxidised structure of Coenzyme Q₁₀**

The physicochemical characteristics of CoQ₁₀ (Ubiquinone and Ubiquinol) are shown in Table 2.3.2 (a) and 2.3.2(b).

<table>
<thead>
<tr>
<th>Table 2.3.2(a): Properties of Ubiquinone (CoQ₁₀)</th>
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</thead>
<tbody>
<tr>
<td>Appearance</td>
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<tr>
<td>Empirical formula</td>
</tr>
<tr>
<td>Molecular weight</td>
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<tr>
<td>Melting point</td>
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<td>Solubility</td>
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<table>
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<tr>
<th>Table 2.3.2(b): Properties of Ubiquinol (CoQ₁₀H₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
</tr>
<tr>
<td>Empirical Formula</td>
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<tr>
<td>Molecular Weight</td>
</tr>
<tr>
<td>Melting Point</td>
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<tr>
<td>Solubility</td>
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</tbody>
</table>
2.3.3 Biochemical role of Coenzyme Q$_{10}$

![Biochemical role of Coenzyme Q$_{10}$](image)

Fig. 2.3.3: Biochemical role of Coenzyme Q$_{10}$

Electron transport chain ("UQ" visible in green near center.) CoQ is found in the membranes of many organelles. Since its primary function in cells is in generating energy, the highest concentration is found on the inner membrane of the mitochondrion. Some other organelles that contain CoQ$_{10}$ include endoplasmic reticulum, peroxisomes, lysosomes and vesicles (Mayo Clinic Drugs & Supplements, 2008; Berbel-Garcia et al., 2004).

2.3.4 Occurrence in nature

CoQ compounds are widely distributed in nature, from microorganisms to plants to animals including humans. In humans and several other species, the side chain is comprised of 10 isoprene units and hence the name CoQ$_{10}$. Animal products such as beef, pork and chicken are relatively good sources of CoQ$_{10}$. Organ meats such as heart and muscle are the best sources. As a general rule, tissues with high energy demands contain relatively high amounts of CoQ$_{10}$. Among foods of plant origin, broccoli and spinach contain significant amounts of CoQ$_{10}$. Unrefined vegetable oils such as soybean oil and palm oil are also good sources of CoQ$_{10}$. 
2.3.5 Inhibition by statins and beta blockers

Coenzyme Q\textsubscript{10} shares a common biosynthetic pathway with cholesterol. The synthesis of an intermediary precursor of CoQ\textsubscript{10}, mevalonate, is inhibited by some beta blockers, blood pressure-lowering medication and statins, a class of cholesterol-lowering drugs. Statins can reduce serum levels of CoQ\textsubscript{10} by up to 40% (Ghirlanda et al., 1993). Some research suggests the logical option of supplementation with CoQ\textsubscript{10} as a routine adjunct to any treatment that may reduce endogenous production of CoQ\textsubscript{10}, based on a balance of likely benefit against very small risk (Sarter, 2002; Thibault et al., 1996).

2.3.6 Absorption and metabolism

CoQ\textsubscript{10} is a crystalline powder that is insoluble in water due to its low polarity. It has a relatively high molecular weight (863 g/mol) and its solubility in lipids is also limited so it is very poorly absorbed in the gastrointestinal tract (Bhagavan et al., 2007). Absorption follows the same process as that of lipids and the uptake mechanism appears to be similar to that of vitamin E, another lipid-soluble nutrient. Emulsification and micelle formation is required for the absorption of fats (Bhagavan et al., 2006). For CoQ\textsubscript{10}, this process is chiefly facilitated by secretions from the pancreas and bile salts in the small intestine. A general rule is that the higher the dose orally administered, the lower the per cent of the dose absorbed. Data on the metabolism of CoQ\textsubscript{10} in animals and humans are limited. A study with \textsuperscript{14}C-labeled CoQ\textsubscript{10} in rats showed most of the radioactivity in the liver 2 hours after oral administration when the peak plasma radioactivity was observed, but it should be noted that CoQ\textsubscript{9} is the predominant form of coenzyme Q in rats. It appears that CoQ\textsubscript{10} is metabolised in all tissues, while a major route for its elimination is biliary and fecal excretion. After the withdrawal of CoQ\textsubscript{10} supplementation, the levels return to their normal levels within a few days, irrespective of the type of formulation used (Kishi et al., 1964; Ozawa et al., 1986).
2.3.7 **Pharmacokinetics and bioavailability**

Some reports have been published on the pharmacokinetics of CoQ\textsubscript{10}. The plasma peak can be observed 2–6 hours after oral administration, mainly depending on the design of the study. In some studies, a second plasma peak was also observed at about 24 hours after administration, probably due to both enterohepatic recycling and redistribution from the liver to circulation. Tomono *et al.* (1986) used deuterium-labelled crystalline CoQ\textsubscript{10} to investigate pharmacokinetics in human and determined an elimination half-time of 33 hours (Bhagavan *et al.*, 2006).

2.3.8 **Life span**

One study demonstrated that low dosages of CoQ\textsubscript{10} reduce oxidation and DNA doublestrand breaks and a combination of a diet rich in polyunsaturated fatty acids and CoQ\textsubscript{10} supplementation leads to a longer lifespan in rats. Coles and Harris demonstrated an extension in the lifespan of rats when they were given CoQ\textsubscript{10} supplementation. Another study demonstrated that CoQ\textsubscript{10} extends the lifespan of *C. elegans* (nematode) (Coles *et al.*, 1996; Ishii *et al.*, 2004).

2.3.9 **Biosynthesis of Coenzyme Q\textsubscript{10}**

The benzoquinone portion of CoQ\textsubscript{10} is synthesized from tyrosine, whereas the isoprene sidechain is synthesized from acetyl-CoA through the mevalonate pathway. The mevalonate pathway is also used for the first steps of cholesterol biosynthesis (Kishi *et al.*, 1977).
Fig. 2.3.9: Biosynthesis of Coenzyme (CoQ₁₀)

2.3.10 Coenzyme Q10 synthesized in our body

CoQ₁₀ is present in almost all the cells in our body and also in circulation (in lipoproteins). Practically every cell has the ability to synthesize CoQ₁₀. The endogenous synthesis of CoQ₁₀ happens to be a very complex process requiring numerous vitamins such as vitamin B₆, vitamin B₁₂, folic acid, niacinamide, pantothenic acid and vitamin C, and also certain trace elements. The quinone ring structure is derived from the amino acid tyrosine, the methyl groups on the ring supplied by methionine, and the isoprenoid side chain coming from the mevalonate pathway (the same pathway shared by cholesterol). Thus the production of CoQ₁₀ is dependent on an adequate supply of numerous precursors and cofactors, and a deficiency of one or more of these essential components can adversely affect the production of adequate amounts of CoQ₁₀.
2.3.11 Commercial production of Coenzyme Q\textsubscript{10}

Commercial production of CoQ\textsubscript{10} is largely by way of yeast fermentation, and to a smaller extent by bacterial fermentation. There is also a semisynthetic process for producing CoQ\textsubscript{10} using solanesol, a tobacco byproduct, that provides the phytol side chain, and the amino acid tyrosine for the ring structure. At this time, most of the world supply of CoQ\textsubscript{10} comes from Japan, with smaller quantities coming from China, India, South Korea and Italy.

2.3.12 Difference between natural and synthetic Coenzyme Q\textsubscript{10}

CoQ\textsubscript{10} occurs in two isomeric forms, namely the “trans” and the “cis” forms. The natural CoQ\textsubscript{10} is in the trans form whereas the synthetic CoQ\textsubscript{10} contains a mixture of both trans and cis isomers. The USP limits the presence of other CoQ analogs and the cis-isomer and related impurities to less than 1.5%.

2.3.13 Assessment of CoenzymQ10

In humans, plasma or serum CoQ\textsubscript{10} concentrations will serve as a good indicator of status. The best way to assay CoQ\textsubscript{10} is by HPLC by UV or electrochemical detection. However, it should be noted that plasma CoQ\textsubscript{10} may not always reflect tissue status. Localized deficiencies of CoQ\textsubscript{10} may exist such as in the skeletal muscle or myocardial tissue while plasma concentrations may show “normal” values. If biopsy material is available, tissue CoQ\textsubscript{10} analysis can yield more useful information (Steele \textit{et al.}, 2004).

2.3.14 Coenzyme Q\textsubscript{10} functions

The primary function of CoQ\textsubscript{10} in our body is in cellular energy production. It is a critical component of mitochondria that are present in practically every cell in our body. Mitochondria may in fact be considered as fuel cells where biological energy called ATP (adenosine triphosphate) is produced. CoQ\textsubscript{10} is also a potent antioxidant and it helps protect the tissues and the cellular components in the body from free radical damage. In addition, CoQ\textsubscript{10} has other important functions in the body (Ernster \textit{et al.}, 1995; Crane, 2001).
2.3.15 Working of Coenzyme $Q_{10}$ in our body

The body survives on the energy that is created when cells break down sugars, fats and amino acids (proteins). Much of this breakdown happens within tiny enclosures in the cells called mitochondria. Co$Q_{10}$ is the substance in the mitochondria that carries electrons involved in producing energy from the food that you eat. Co$Q_{10}$ is believed to be one of the first antioxidants that are depleted when LDL (the good cholesterol) is subjected to oxidation. In other words, it prevents the oxidation of lipoproteins in the blood, reducing the risk of harmful plaques that narrow arteries and contribute to heart disease. Co$Q_{10}$ also seems to have anti-inflammatory properties, which may account for its reported benefits in treating migraines. There is also research to support its role in bolstering the immune system and helping the body to fight off infection and deal with the many disorders that have been connected to autoimmune system dysfunctions (diabetes, in particular) (Kishi et al., 1977). The concentration of Co$Q_{10}$ in the body decreases year by year, indicating that it has a close relationship with aging.

![Co$Q_{10}$ distribution in the body](image)

Co$Q_{10}$ is a crucial component of the electron transport chain (respiratory chain) in the mitochondria where energy derived by a process called oxidative phosphorylation from the products of fatty acid, protein and carbohydrate metabolism is converted into biological energy called adenosine triphosphate (ATP) that drives cellular machinery and all biosynthetic processes. Co$Q_{10}$
functions as an essential cofactor for the activities of the enzyme systems called complexes I, II and III in the electron transport chain. It shuttles electrons from complex I (nicotinamide adenine dinucleotide dehydrogenase) and Complex II (succinate dehydrogenase) to complex III (ubiquinone-cytochrome c reductase) by virtue of its redox (reduction-oxidation) properties. It is during this process of electron transfer along the electron transport chain that vital biological energy as ATP is generated (Fig. 2.3.15). Thus, CoQ_{10} plays a critical role in cellular bioenergetics (Ernster et al., 1995; Crane, 2001; Rauchova et al., 2001; Nohl et al., 2001).

**Coenzyme Q10 Energy Production**

![Diagram of CoQ10 and energy production](image)

*Fig. 2.3.15b: CoQ_{10} and energy production*
Animal-based products and in particular organ meats such as heart are relatively good sources of CoQ\textsubscript{10}. But in reality, it is not likely that one could consume large quantities of any organ meat needed to obtain a reasonable amount of CoQ10 from dietary sources. CoQ\textsubscript{10} supplements are therefore desirable and bioenhanced formulations of CoQ\textsubscript{10} are available that can provide adequate amounts in a readily absorbable form.

CoQ\textsubscript{10} is oil soluble synthetic vitamin like substance used in prevention and cure of most life threatening diseases. It also works as immuno modulator and hence proved as a gift for one suffering from immuno deficiency disorders. It also helps in management of pain and depression.

Because of its fundamental role in cellular bioenergetics and also as an important antioxidant, CoQ\textsubscript{10} plays a vital role in our well-being. Since it is involved in the pathophysiology of numerous disease states listed in the next section, assuring adequate CoQ\textsubscript{10} status is essential for maintaining good health and preventing or reducing the risk for numerous chronic degenerative and metabolic diseases.

2.3.16 The clinical conditions and health benefits associated with Coenzyme Q\textsubscript{10}

There is a large body of data on the beneficial effects of CoQ\textsubscript{10} supplementation in various disease states (Bliznakov \textit{et al.}, 1987; Sinatra, 1998; Pepping, 1999; Overvad \textit{et al.}, 1999; Fuke \textit{et al.}, 2000). The following list shows health problems that are associated with impaired CoQ\textsubscript{10} status, and also numerous disease states and conditions where CoQ\textsubscript{10} supplementation has been found to be beneficial.
The Potential Beneficial Effects of Coenzyme Q\textsubscript{10} Supplementation:

*Cardiovascular disease, Cardiomyopathy, Congestive heart failure, Angina pectoris, Arrhythmias, Mitral valve prolapse, Hypertension, Atherosclerosis, Cardiotoxicity (drug-induced), Neurodegenerative diseases, Huntington’s Disease, Parkinson’s Disease, Alzheimer’s Disease, Amyotrophic lateral sclerosis (Lou Gehrig’s Disease), Neuromuscular diseases, Mitochondrial cytopathies (MELAS, MERRF, etc.), Muscular dystrophy, Ataxias, Diabetes, Cancer, Chronic obstructive pulmonary disease, Asthma, Migraine, Immune disorders, HIV/AIDS, Periodontal disease, Chronic fatigue syndrome and Male infertility,

*Cardiovascular disease tops the list of disorders, and there is substantial evidence for the therapeutic role of CoQ\textsubscript{10} supplementation in heart failure.

CoQ\textsubscript{10} has also been found beneficial in various other conditions related to the heart and the cardiovascular system (Sinatra, 1998; Pepping, 1999; Overvad et al., 1999; Greenberg et al., 1990; Soja et al., 1997). In addition to its basic function in cellular bioenergetics, CoQ\textsubscript{10} has an important role as an antioxidant in maintaining cardiovascular health by way of protecting LDL from oxidation (Alleva et al., 1997). The role of CoQ\textsubscript{10} in neuro degenerative diseases has received a great deal of attention in recent years (Beal, 2002; Beal et al., 2003; Beal, 2004). Preliminary evidence for a beneficial effect of high doses of CoQ\textsubscript{10} supplementation particularly in the case of Parkinson’s and Huntington’s diseases is indeed promising (Kieburtz, 2001). The importance of CoQ\textsubscript{10} in the treatment of mitochondrial diseases that involve multisystem disorders is also well recognized (DiMauro et al., 1998; Fosslien, 2001; Gold et al., 2001).

**2.3.17 Coenzyme Q\textsubscript{10} uses:** (Abe et al., 1983; Eisai Co., 1982; Weber et al., 1994; Witte et al., 2001; Singh et al., 1999; Khatta et al., 2000; Mortensen et al., 1997; Henriksen et al., 1999; Jolliet et al., 1998; Marz et al., 2000).
1. Improves bioenergetics in heart muscle
2. Improves haemodynamics
3. Normalizes diastolic function
4. Improves exercise tolerance in angina
5. Improves functional classification in congestive heart failure
6. Reduces need for medications
7. Improves clinical outcomes
8. Reduces cardiac mortality
9. Reverses CoQ\(_{10}\)-lowering side effect of cholesterol lowering drugs (“stanins”)
10. Produces modest blood pressure reductions in hypertension
11. Reduces oxidation of LDL cholesterol
12. Regenerates vitamin E
13. Prevents pro-oxidant side effects of vitamin E
14. Improves the course of AIDS, Cancer, Parkinson’s & Gum disease
15. Boosts immune system
16. Effect of aging
17. Increases energy and athletic performance
18. Assists weight loss
19. Medical food in diabetics
20. Treatment of alopecia, myasthenia, bronchial asthma, preliminary emphysema and dycacusis
21. Constituent of eye ointments and cosmetics

2.3.18 The future of Coenzyme Q\(_{10}\)

CoQ\(_{10}\) is an essential element of food that can now be used medicinally to support the sick host in conditions where nutritional depletion and cellular dysfunction occur. Surely, the combination of disease attacking strategy and host supportive treatments would yield much better results in clinical medicine. All metabolically active tissues are highly sensitive to a deficiency of CoQ\(_{10}\). It is reasonable to assume that optimal nutrition (which would include optimal levels of CoQ\(_{10}\)) is generally beneficial in any disease state, including cancer. Another interesting topic is the relationship between the immune system and CoQ\(_{10}\). In
keeping with the free radical therapy of aging, the antioxidant properties of CoQ\textsubscript{10} have clear implications in the slowing of aging and age related degenerative diseases. New evidence shows that CoQ\textsubscript{10} is present in other cell membranes. In the other membrane it may contribute to the control of cell growth, especially in lymphocytes. The clinical experience with CoQ\textsubscript{10} in heart failure and till today, 12 million people have used it in Japan as a prescription medicine, without any toxicity or harmful effects. This food supplement is inexpensive and easy to use. Clearly people will try it and see if it works for them. With the information spreading through grass-root level organizations and informal publications, the importance of CoQ\textsubscript{10} is spreading rapidly among general public.

2.3.19 Synthesis of CoQ\textsubscript{10} - Reported Procedures

Various methods have been reported in the literature for the synthesis of Coenzyme Q10 from solanesol. Several approaches known for synthesizing the Ubiquinones have been developed over the past 3-4 decades. The reported procedures for building polyprenyl chains and coupling are discussed below.

**Work Carried out by other Research Groups on Coupling**

**a) Muruyama’s approach** (Muruyama *et al.*, 1978)

In this approach (Scheme-1) the allyl tributyl tin reagents react with quinones in the presence of BF\textsubscript{3}.Et\textsubscript{2}O to give allyl substituted hydroquinones. Oxidation of these hydro quinones gave corresponding quinones.

Scheme-1
Review of Literature

Reagents and conditions:
a) Bu$_3$Sn, BF$_3$OEt$_2$, CH$_2$Cl$_2$, –78°C, b) Aq. FeCl$_3$.

b) Tabushi’s approach (Tabashi et al., 1978):
Quinones were prepared by regiospecific oxidative prenylation in presence of β-cyclodextrin (Scheme-2).

![Scheme-2](image)

Reagents and conditions:
a) Phenyl Bromide, β-cyclodextrin, Borate buffer, MeOH, r.t, 48h.

c) Sueda’s approach (Sueda et al., 1978):
In this approach the protected hydroquinone was reacted with n-BuLi and geranyl bromide in presence of CuI to give protected allyl substituted compound. In the second attempt they have done Grignard reaction (Scheme-3).

![Scheme-3](image)

Reagents and conditions:
a) n-Buli, CuI, THF, -60°C, 1.5h then r.t, overnight or MgI$_2$, THF, HMPA, CuI.
d) **Aoyagi’s approach** (Aoyagi *et al.*, 1979):

In this method Aoyagi used (Scheme-4) a resin for coupling hydroquinone with solanesol or decaprenol to give ubiquinone after oxidation.

Scheme – 4

Reagents and conditions:

a) Amberlyst 15, cyclohexone – benzene, 25-30°C, 2h; b) 10% Aq.FeCl₃.

e) **Naruta’s approach** (Naruta *et al.*, 1979):

Naruta’s approach (Scheme-5) deals with coupling of hydroquinone with isodecaprenol in presence of BF₃.OEt₂ and nitromethane-hexane at 43°C which after oxidation gave Ubiquinone (COQ₁₀).

Scheme – 5

Reagents and conditions

a) BF₃.OEt₂, MeNO₂ and Hexane, 43°C, 10min;
b) FeCl₃.6H₂O, Isopropyl alcohol, rt, 1h.
f) Mitsui’s approach (Mitsui, 1980):

The hydro quinone (Scheme–6) reacted with the corresponding allyl alcohol in presence of BF$_3$.Et$_2$O, urea, which after oxidation gave the desired quinones.

Reagents and conditions:
a) BF$_3$.OEt$_2$, Solanesol, urea, C$_6$H$_6$-hexane, r.t. 2h;
b) Aq. FeCl$_3$.

g) Toyokichi’s approach (Toyokichi et al., 1982):

2,3-dimethoxy-5-methyl-6-polyprenyl quinones (Scheme-7) were obtained selectively by the BF$_3$.OEt$_2$ catalyzed polyprenyl rearrangement followed by oxidation with MnO$_2$. 
**Reagents and conditions:**

a) NaH, DMSO, rt;  

b) \(\text{Br}\left(\begin{array}{c}\text{H} \\ \text{H} \\ \text{H} \\ \text{H} \\ \text{H} \\ \text{H} \\ \text{H} \\ \text{H} \\ \text{H} \\ \text{H} \end{array}\right)\), THF, rt;  

c) 30% KOH/MeOH, 0\(^0\)C;  

d) BF\(_3\).OEt\(_2\), CCl\(_4\), -15\(^0\)C; e) MnO\(_2\).  

**h) Lipshutz’s approach** (Lipshutz *et al.*, 2002):  

Lipshutz’s approach (scheme-8) carried out the ubiquinone synthesis using nickel catalyst in standard fashion. The oxidation was done by using catalytic amounts of Jacobsen’s Co (salen) complex.

Scheme -8

Reagents and conditions:  

a) PCl\(_3\), DMF, rt; b) TMS=CH\(_2\)Li, THF, -78\(^0\)C, 6h;  

c) NaO\(_2\)C\(_2\)H\(_5\), C\(_2\)H\(_5\)OH, 50-60\(^0\)C; d) AlCl\(_3\), TsCl; e) DIBAL, HCl;  

f) Me\(_3\)Al, Cat.CP\(_2\)ZrCl\(_2\), cat. Ni(O), rt; g) n-BuLi, C\(_2\)H\(_5\)O, 0\(^0\)C, 4h;  

h) Cat. Co(Salen), DMF, PhMe, O\(_2\), 24h, rt.
i) **Lipshutz’s approach** (Lipshutz *et al.*, 2005):

Lipshutz’s approach (Scheme-9) a new route to the key coupling partner, chloromethyleted COQ0, allows for direct formation of COQ_{10} via nickel-catalyzed cross-coupling with the side chain in the form of an in situ-derived vinyl alone.

**Scheme - 9**

Reagents and conditions:

a) Cat. Ni(O)

j) **Ravada’s approach** (Ravada *et al.*, 2009):

Ravada’s approach (Scheme-10), This process utilized inexpensive isoprenol as a precursor for the synthesis of an early intermediate with a single isoprene unit. Another key step was the selective oxidation of trans methyl of isoprene unit as a prelude to the expansion of the side chain to decaprenyl group using solanesol.

**Scheme -10**
Reagents and conditions:

a) CCl₄, 2-methyl-3-buten-2-ol, BF₃, rt, 96.1%;
b) NaOH, DMS, 90°C, 78%;
c) SeO₂, EtOH;
d) EtOH, NaBH₄, rt, 65%;
e) THF, Pyridine, PBr₃, 0°C, 92%;
f) CH₂Cl₂, TEA, Sodium p-toluenesulphinate, rt, 83%;
g) t-BuOK, THF, Solanesol bromide, -20-30°C, 74%;
h) THF, EtOH, Na, rt, 79%;
i) CH₃CN, CH₂Cl₂, CAN, 0°C, 72%.

2.4 Determination of Ubiquinone (CoQ₁₀) and its Process Related Impurities by HPLC

2.4.1 Assay of Coenzyme Q₁₀ in biological fluids

The plasma levels of CoQ₁₀ are useful for monitoring the bioavailability of orally administered CoQ₁₀ and it correlates with clinical efficacy. Several methods were described in the literature for assaying CoQ₁₀ or its reduced (ubiquinol-10) and oxidized (ubiquinone-10) forms in plasma (Tomasetti et al., 1999; Lang et al., 1987; Finckh et al., 1999; Podda et al., 1999; Finckh et al., 1995; Okamoto et al., 1988; Edlund et al., 1988; Lagendijk et al., 1996; Yamashita et al., 1997). Mosca et al. (2002) have developed a single step dilution method for determination of CoQ₁₀ in plasma, along with reduced and oxidized form of CoQ₁₀. A ESI-LC-MS-MS method was reported (Ruiz-Jimenez et al., 2007) for simultaneous determination of CoQ₁₀ and the reduced form ubiquinol-10 (CoQ₁₀H₂) in human serum. It was applied to serum samples from healthy middle-age women, in which the CoQ₁₀H₂/CoQ₁₀ ratio was used as marker of oxidative stress. Rodriguez-Estrada et al. (2006) carried out a preliminary study by using HPLC to determine the presence of CoQ₁₀ in cortical and medullary normal human kidney tissues, as well as in neoplasms of different malignancy degrees. Barshop et al. (2007) reported three methods of HPLC-coupled with UV
or electrochemical detection, and tandem mass spectrometry to determine CoQ\(_{10}\) concentrations in various human fluids and tissues. Jiang et al. (2004) have developed a method of determination of CoQ\(_{10}\) in human plasma by column-switching high performance liquid chromatography. CoQ\(_{10}\) was quantitatively extracted with 1-propanol, centrifuged and the supernatant was cleaned on an octadecyl-bonded silica column and transferred to reversed-phase column by a column-switching valve and detected at 275 nm using a mobile phase containing 10% (v/v) isopropanol in methanol at a flow-rate of 1.5 ml/min. Karpinska et al. (2006) proposed a HPLC-UV method for the simultaneous determination of retinol, \(\alpha\)-tocopherol and CoQ\(_{10}\) in human plasma at 324, 292 and 276 nm. The mobile phase consisted of methanol – \(n\)-hexane (72:28, v/v). CoQ\(_{10}\) was isolated from the lipoproteins of plasma by treatment with methanol followed by extraction with \(n\)-hexane. Subsequent clean-up on silica and \(C_{18}\) solid-phase extraction cartridges produced a clean extract. The method was applied to determine the levels of lipophilic vitamins in plasma of healthy children. Grossi et al. (1992) proposed three different methods for determination of CoQ\(_{10}\) in plasma. HPLC- UV detection at 275 nm, a microanalysis utilizing a three-electrode electrochemical detector on a \(C_{18}\) column. The minimum detectable plasma CoQ\(_{10}\) level was 0.05 and 0.005 mg/l for UV and electrochemical detection, respectively. The methods correlate very well with classical ethanol – \(n\)-hexane extraction with UV detection. The methods were applied for determination of CoQ\(_{10}\) plasma values in health subjects, athletes, hyperthyroid, hypothyroid and hypercholesterolaemic patients. Li et al. (2008) developed a UPLC–MS method for determining CoQ\(_{10}\) levels in rat serum. The separation of CoQ\(_{10}\) was performed on a Waters Acquity UPLCTM BEH \(C_{18}\) column (1.7\(\mu\)m,1.0 mm\(\times\)50 mm) with the mobile phase containing acetonitrile, 2-propanol, and formic acid (90:10:0.1) over 5 min. CoQ\(_{10}\) was quantitatively extracted into 2-propanol using a fast extraction procedure. This procedure provided a precise, sensitive and direct assay method for the determination of CoQ\(_{10}\) in rat serum after oral administration.
2.4.2 Analysis of \(\text{CoQ}_{10}\) in bulk drugs and pharmaceuticals

Xia and Xu (2006) have demonstrated a UV spectrometry method for determination of total and the encapsulated \(\text{CoQ}_{10}\) content in liposomal \(\text{CoQ}_{10}\) formulations. This study demonstrated a method to release encapsulated \(\text{CoQ}_{10}\) from liposomal suspension using moderate amounts of the non-ionic surfactant Tween 80 solubilization and UV spectrometry, in which 1 mL of the reducing agent NaBH\(_4\) (7 mg mL\(^{-1}\)) was added. The authors found difference in results between classical organic solvent extraction and the tween 80 solubilization method. Tween 80 method was applied to determine \(\text{CoQ}_{10}\) content quantitatively. The relative standard deviation was lower than 5%. Ratnam et al. (2006) reported to first UV spectroscopic and HPLC methods for the simultaneous analysis of ellagic acid (EA) and \(\text{CoQ}_{10}\) in pharmaceutical preparations. The wavelengths identified for UV method were 223 and 383 nm for \(\text{CoQ}_{10}\) and EA respectively. In HPLC method, poly ethylene glycol (PEG) bonded phase column provided hydrophilicity to the reverse phase C\(_{18}\) column and thus resulted in relative rapid elution of \(\text{CoQ}_{10}\) and increased the retention of the poorly retained EA. Mobile phase consisting of acetonitrile - ethanol-5mM potassium dihydrogen orthophosphate buffer (pH 2.5) was used in a gradient elution mode. Wavelengths 254 and 275 nm were selected for EA and \(\text{CoQ}_{10}\) detection, respectively. The derivative spectroscopic method was compared for the linearity, accuracy and precision with the HPLC method. Karpinska et al. (2004) have developed a second derivative UV spectrophotometry method for simultaneous determination of retinol acetate, tocopherol acetate and \(\text{CoQ}_{10}\) in synthetic mixtures and pharmaceuticals without involving any sample preparation. Derivative UV method required a large number of preliminary tests to select the convenient order, polynomial degree, derivative window width and wavelength. The wavelengths at 212, 220 and 351 nm for tocopherol acetate, \(\text{CoQ}_{10}\) and retinol acetate, respectively were chosen. The results were discussed.
in relation to applicability of derivative technique in analysis of lipid soluble vitamins. A FT-IR spectrometric method was developed for the rapid, direct measurement of CoQ$_{10}$ in different pharmaceutical products. Conventional KBr spectra were compared for the best determination of active substance in drug preparations. Lambert-Beer's law and two chemometric approaches, partial least squares and principal component regression methods were used in data processing (Bunaciu et al., 2007). A simple and rapid voltammetric method (Michalkiewicz, 2008) was developed for the quantitative determination of CoQ$_{10}$ in pharmaceutical preparations. Studies with differential pulse voltammetry (DPV) were carried out using a glassy carbon electrode (GCE) in a mixed solvent containing 80:20v/v acetic acid : acetonitrile. A well-defined reduction peak of CoQ$_{10}$ was obtained at $-20$ mV vs. Ag/AgCl. The voltammetric technique applied provided a precise determination of CoQ$_{10}$ using the multiple standard addition method.

Derivative UV spectrophotometry, FT-IR and HPLC were used for analysis of CoQ$_{10}$ in pharmaceuticals and human plasma. US Pharmacopoeia (2007) described a normal phase HPLC method using L3 column (silica) and the mobile phase composition of n-hexane and ethyl acetate (97:3) for assay of CoQ$_{10}$ in bulk drugs. However, none of the methods address the problem of separation and determination of process related impurities, which are most likely to be present in the finished products of CoQ$_{10}$.

Further, to the best of the knowledge, no method for determination of its impurities was reported either in bulk drugs or pharmaceuticals. Thus, there is a great need for analytical methods, which will be helpful to monitor the levels of impurities in the finished products of CoQ$_{10}$ during process development.
2.5 Objectives of the Present Research Work

The objective of the present research work is extracting the high value bio-chemicals from tobacco and tobacco waste for use in for pharmaceutical industry. Solanesol has many medicinal benefits and being starting material for many high-value biochemicals. Solanesol is a naturally occurring sesquiterpene alcohol present in tobacco (Nicotiana tabacum L).

The work involves purification of crude solanesol (15-20%) by different methods i) silicagel column chromatography  ii) saponification followed by silica gel column chromatography  iii) saponification followed by recrystallization with different solvents viz., acetone, methanol and acetonitrile. The work involves determination of solanesol by a rapid RP- HPLC method and validation of the method with respect to a accuracy, precision, linearity, limits of detection and quantification.

The presence of a long chain of repeating isoprene units (nine) in the solanesol molecule makes it a valuable source material for synthesizing metabolically active quinones and other drugs. Solanesol is the starting material for synthesis of Vitamin K₂, Vitamin-E, Coenzyme Q₉, Coenzyme Q₁₀ and anti-cancer potentiating agent like N-solensyl-N₁,N₁-bis (3,4-dimethoxy benzyl) ethylene diamine. Considering the importance of solanesol in the pharmaceutical industry, utilizing of tobacco or tobacco waste as a source of this chemical has attained significance. Thus, solanesol has excellent prospects in the future as drug intermediate, which has generated considerable interest in this compound, more particularly being the starting material for the production of Coenzyme Q₁₀ (CoQ₁₀).

The present research work involves a new synthetic route for the preparation of coenzyme Q₁₀ from solanesol with appreciable yield.
To optimize the analytical conditions for monitoring of Co enzyme Q_{10} and its synthesis related substances using RP-HPLC. The work also involves characterisation of the synthetic impurities using modern spectroscopic techniques viz., ^1H NMR, APCI-MS. Validation of the developed RP-HPLC method and applicability of the method for assay of Coenzyme Q_{10} and determination of its process related impurities in drug substances and formulations.