Chapter – 5

PREFORMULATION STUDIES PART - 1
Chapter 5  Pharmaceutical Profiling & Preformulation Studies

5.1 PHARMACEUTICAL PROFILE

5.1.1 PACLITAXEL

It is an Antineoplastic Agent, natural or semisynthetic diterpene from the bark of the Western (Pacific) yew (Taxus brevifolia) or needles and twigs of Taxus baccata.\(^{1,11}\)

![Molecular structure of Paclitaxel](image)

**IUPAC name:** \([2aR, 2aa, 4p, 4ap, 6p, 9a(aR', pS'), 11a, 1 - 2a, 12aa, 12ba)] - \(\beta\) - (Benzoylamino) - \(\alpha\) - hydroxybenzenepropanoic acid 6, 12b-bis(acetlyoxy)-11-
(benzoyloxy)-2a, 3, 4, 4a, 5, 6, 9, 10, 11, 12, 12a, 12b-dodecahydro-4, 11-dihydroxy-
4a, 8, 13, 13-tetramethyl-5-oxo-7, 11-methano-1H-cyclodeca[3, 4]benz [1, 2-b]oxet - 9-y1 ester

**Molecular Formula:** \(C_{47}H_{51}NO_{14}\)

**CAS Number:** 33069-62-4

**Most Popular Brands:**Abraxane, Taxol, Onxol
Pharmacological actions / Indications

a. Ovarian Cancer

In treatment of advanced carcinoma of the ovary, conventional form of paclitaxel is used in first-line therapy in combination with carboplatin or cisplatin. It is also used alone or in combination as second-line / salvage therapy in patients with advanced ovarian epithelial cancer.

b. Breast Cancer

Conventional paclitaxel is used as an adjuvant therapy in patients with evidence of axillary node tumor involvement. It is administered sequentially to standard doxorubicin-containing combination therapy. It is also used in the treatment of patients who have metastatic disease refractory to combination chemotherapy or who have experienced relapse within 6 months of adjuvant chemotherapy with anthracycline antineoplastic agent (doxorubicin). For tumors with overexpression of HER2 protein it is used in combination with trastuzumab. Breast cancer treatment also involves use of albumin bound paclitaxel for patients who have metastatic disease refractory to conventional combination therapy.

c. Non-small Cell Lung Cancer

Conventional paclitaxel is used as first-line treatment of non-small cell lung carcinoma in combination with carboplatin or cisplatin in patients for whom potentially curative surgery and/or radiation therapy are not possible. Moreover, Adjuvant therapy in selected patients with completely resected non-small cell lung cancer, used in conjunction with a platinum agent (e.g., carboplatin).
d. Small Cell Lung Cancer

Conventional paclitaxel is active in the treatment of small cell lung carcinoma.37-42

e. AIDS-related Kaposi’s Sarcoma

Conventional paclitaxel is used as second-line therapy for the palliative treatment of advanced or refractory AIDS-related Kaposi’s sarcoma (designated an orphan drug by FDA for this use).43

f. Esophageal Cancer

Conventional paclitaxel has been used for the treatment of esophageal cancer.44-47

g. Bladder Cancer

Conventional paclitaxel is active in the treatment of transitional cell bladder cancer.

h. Head and Neck Cancer

Conventional paclitaxel is active in the treatment of advanced (metastatic or locally recurrent) squamous cell carcinoma of the head and neck.48-49

i. Cervical Cancer

Conventional paclitaxel is active in the treatment of cervical cancer.50-59

j. Endometrial Cancer

Conventional paclitaxel has been used for the treatment of endometrial cancer.60-66
b. ELLAGIC ACID

Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables. The antiproliferative and antioxidant properties of ellagic acid have spurred preliminary research into the potential health benefits of ellagic acid consumption. Ellagic acid is the dilactone of hexahydroxydiphenic acid.

![Fig. 10 Molecular Structure of Ellagic acid](image)

IUPAC name: 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione

Synonyms: 4,4',5,5',6,6'-Hexahydroxydiphenic acid 2,6,2',6'-dilactone

Molecular Formula: C_{12}H_{10}O_{8}

CAS number: 476-66-4

Ellagic acid is only taken as a representative or model drug for making a nanosystem which can carry two hydrophobic moieties easily with a size being in nanorange. Although USFDA has clearly categorized it under Fake Cancer Cure, Patients should avoid. Our main intention was only to design a system which is non-interacting with Paclitaxel and has the mechanism of action similar to the herbal drugs like Pomegranate.
Chapter 5  
Pharmaceutical Profiling & Preformulation Studies

Extract, Punica granatum L. Oil; Ext; Olt which are categorized under Generally Regarded As Safe.

Reasons for selecting Ellagic acid despite of its Fake cancer Cure treatment label, being, its hydrophobicity, Purity and availability. The system is designed with an intention that can carry two hydrophobic moieties. Incorporating a hydrophilic moiety in a formulation is not an issue as it can easily solubilize i.e. go until a nanorange and can show its action. Whereas, administration of the hydrophobic drugs has always been a challenge. For this reason, we have not stressed upon taking punicalagins like moieties. Although, as we move ahead in the research work, we have designed systems keeping in mind which have the ability to carry both hydrophilic as well as hydrophobic moieties. Second reason being, the purity and easy availability.
## 187 Fake Cancer "Cures" Consumers Should Avoid

<table>
<thead>
<tr>
<th>Product</th>
<th>12611 103rd Ave Ste A, Surf City, AZ 85351</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Bone Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Breast Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Colon Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Esophageal Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Lung Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Malignant Lymphoma Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Nasal Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Prostate Therapy Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Skin Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Sponge Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Stomach Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Tongue and Oral Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Urinary and Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Ellagic Acid</td>
<td></td>
</tr>
<tr>
<td>Coral Calcium Daily</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product</th>
<th>1025 Congress Street, Suite 5, Eugene, OR 97402-6937</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Calcium Daily</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product</th>
<th>99969 Green Hill Road, Eugene, OR 97405</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Bone Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Breast Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Colon Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Esophageal Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Lung Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Malignant Lymphoma Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Nasal Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Prostate Therapy Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Skin Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Sponge Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Stomach Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Tongue and Oral Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Urinary and Cancer Tea Formula</td>
<td></td>
</tr>
<tr>
<td>Ellagic Acid</td>
<td></td>
</tr>
<tr>
<td>Coral Calcium Daily</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product</th>
<th>1025 Congress Street, Suite 5, Eugene, OR 97402-6937</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Calcium Daily</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product</th>
<th>5246 Dobson Way, PO Box 3462, Central Point, OR 97502-0360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Calcium Daily</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product</th>
<th>964 Walnut Creek Road, Franklin, NC 28734-8533</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Calcium Daily</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 11 List of Fake cancer Cures from USFDA

Generally Regarded As Safe (GRAS) Certification of Pomegranate Extract

Extracts of pomegranate are 'Generally Recognized As Safe' (GRAS) by the United States. It has been recommended to look for pomegranate ingredients that mimic the polyphenol ratio of the fruit, as potent synergistic effects have been observed in 'natural spectrum' extracts, especially pomegranate concentrate normalized to punicalagins.
Recent literature on Ellagic acid in cancer

It should be noted that Ellagic acid has not received the desired certifications due to lack of satisfactory data, as evaluated by FDA. Continuous efforts have been put in to evaluate its efficacy in different parts of the world. Research review of the year 2011 includes following articles:

a) **Influence of Ellagic acid on prostate cancer cell proliferation: A caspase-dependent pathway**

It was observed that Ellagic acid treatment of PC3 cells resulted in a dose dependent inhibition of cell growth/cell viability. This Ellagic acid mediated cell growth inhibition was found to be accompanied by induction of apoptosis, as evaluated by the cleavage of poly (ADP-ribose) polymerase (PARP) and morphological changes. Further, induction of apoptosis conveyed a decrease in the levels of antiapoptotic protein Bcl-2 and increase in proapoptotic protein Bax, thus shifting the Bax: Bcl-2 ratio in favor of apoptosis. Ellagic acid treatment of PC3 cells was also found to result in significant activation of caspases, as shown by the dose dependent decrease in the protein expression of procaspase-3, -6, -8 and -9. This Ellagic acid-mediated induction of apoptosis was significantly (80%-90%) inhibited by the caspase inhibitor N-benzyloxycarbonyl-Val-Ala-Asp (OMe)-fluoromethylketone (Z-VAD-FMK). Thus these data highlighted a caspase dependent mechanism of action of Ellagic acid in apoptosis.
b) Ellagic acid, a phenolic compound, exerts anti-angiogenesis effects via VEGFR-2 signaling pathway in breast cancer.

It was reported that ellagic acid hindered some angiogenesis-dependent pathologies. Yet, the mechanisms involved were not fully understood. Thus, its anti-angiogenesis effects and mechanisms on human breast cancer utilizing in-vitro and in-vivo methodologies were analysed. The in-silico analysis was also carried out to further analyze the structure-based interaction between ellagic acid and VEGFR-2. It was found that ellagic acid significantly inhibited a series of VEGF induced angiogenesis processes including proliferation, migration, and tube formation of endothelial cells. Besides, it directly inhibited VEGFR-2 tyrosine kinase activity and its downstream signaling pathways including MAPK and PI3K/Akt in endothelial cells. Ellagic acid also obviously inhibited neo-vessel formation in chick chorioallantoic membrane and sprouts formation of chicken aorta. Breast cancer xenografts study also revealed that ellagic acid significantly inhibited MDA-MB-231 cancer growth and P-VEGFR2 expression. Molecular docking simulation showed that ellagic acid could form hydrogen bonds and aromatic interactions within the ATP-binding region of the VEGFR-2 kinase unit. Thus, this study proved another possible mechanism of its anticancerous activity could be its effect on angiogenesis.

c) Ellagic acid inhibits melanoma growth in vitro.

In the present study, effects of ellagic acid on melanoma cells in vitro were observed. Three metastatic melanoma cell lines (1205Lu, WM852c and A375) were examined to determine the effects of ellagic acid on melanoma cell viability, cell-cycle, apoptosis, NF-κB activity, and IL-1β & IL-8 secretion. Cell viability assays demonstrated that
Ellagic acid possessed an inhibitory effect on cell proliferation at concentrations between 25 and 100 μM. In addition, ellagic acid promoted G1 cell cycle arrest, increased levels of apoptosis and decreased synthesis of IL-1β and IL-8 in melanoma cells. Ellagic acid also decreased NF-κB activity, suggesting at least one potential mechanism by which ellagic acid may exert its effects in melanoma cells. These findings support further investigation into prospective roles for ellagic acid as a therapeutic, adjuvant, or preventive agent for melanoma.

d) Chemoprevention of mammary carcinogenesis by sustained systemic delivery of ellagic acid

Many chemopreventives that show efficacy in vitro show little/no response or require bolus doses in animal models and clinical trials because of limited bioavailability. Ellagic acid has been tested in various animal models with mixed results. The efficacy of ellagic acid delivered by a subcutaneous implant compared with a dietary route against estrogen-induced mammary tumor was reported in this study. Ellagic acid delayed the first tumor appearance by 2 and 3 weeks by implant and diet routes, respectively. The tumor incidence was 75% and 69% by the implant and dietary routes when the control group had 100% palpable tumors by 26 weeks. Ellagic acid also significantly reduced the tumor burden by both implant (855±242 mm³; P=0.0375) and dietary (599±169 mm³; P=0.0133) routes compared with control (1522±299 mm³). Similar reductions were observed in tumor multiplicity (4.8±0.5; P=0.0042 and 4.5±0.4; P=0.0031 tumors/rat with implant and diet, respectively, vs. 8.9±1.2 in control). The total amount of ellagic acid administered by implant was 5.92±3.48 whereas it was 800±40 mg/rat through diet. Thus, over 130-fold dose reduction produced similar biological responses when delivered
by implant. The anticarcinogenicity effects corroborated the observed reduction in levels of pituitary prolactin. This novel approach opens new avenues to test agents individually or as mixtures for their chemopreventive potential that are discontinued, either due to lack of bioavailability or toxicity potentially associated with high doses or due to lack of availability of sufficient quantities.

e) Ellagic Acid – Chemopreventive Role in Oral Cancer

In spite of evidences for anticancer activity in various cancer cell-lines, human cancer cells, the mechanistic role of ellagic acid is not conclusive enough to be recommended for a clinical use. It was shown that ellagic acid modulates growth of tumor cells through regulation of multiple cell signaling pathways including cell proliferation pathway (cyclin dependent kinase 2, cyclin A2, cyclin B1, cyclin D1, c-myc, PKCa), cell survival/apoptosis pathway (Bcl-XL, Bax, Caspase 9/3, Akt), tumor suppressor pathway (p53, p21), inflaming Metastasis pathways (IL-1 beta, TNF-α, matrix metalloproteinases 9/3, COX-2), angiogenesis pathways (VEGF), cell immortalization (TERT), NF-κβ.

f) Ellagic Acid Modulates Antioxidant Status, Ornithine Decarboxylase Expression, and Aberrant Crypt Foci Progression in 1,2-Dimethylhydrazine-Instigated Colon Preneoplastic Lesions in Rats

Chemoprevention offers a novel approach to control the incidence of colorectal cancer (CRC), which is a fatal cause of malignancies in both Western and Asia countries. Ornithine decarboxylase (ODC) functions as a cell transition factor by regulating the biosynthesis of polyamines, which, allied with aberrant crypt foci (ACF) proliferation, cause early lesions of CRC. This study exemplifies the chemopreventive efficacy of ellagic acid (EA) in 1,2-dimethylhydrazine (DMH) initiated CRC in rats. Subcutaneous
injection of DMH (40 mg/kg body weight twice a week for 2 weeks) to the rats resulted in elevated expression of ODC, a genetic marker for CRC, and its transcription factor myelocytomatosis oncogene (c-myc). Furthermore, increased levels of lipid peroxidation and hydroperoxides with diminished levels of antioxidants including superoxide dismutase, catalase, and reduced glutathione were also observed in the tissues of DMH-intoxicated rats. Oral supplementation of EA significantly influenced maintenance of antioxidant status and transcriptional inactivation of ODC expression, reducing ACF proliferation and/or progression, thus signifying the chemopreventive efficacy of EA against CRC.

5.2 PREFORMULATION STUDIES

a. PACLITAXEL

Materials and Methods

Apparatus and Reagents

The apparatus used in the study are: UV-Visible Spectrophotometer (Shimadzu, Japan), analytical balance (Mettler AE160), pH Meter (Mettler Toledo MP220); Paclitaxel, Methanol, Ethanol, Chloroform, KBr, Hydrochloric acid, Sodium Hydroxide Pellets, analytical reagents from qualigens and SD Fine chemicals were used as materials and reagents.

Methods

Physicochemical Characterization

Paclitaxel was characterized for the following features; color, odor, Solubility, Log P/Pka values and melting point.

Solubility
1 mg of paclitaxel was added to different solvents including water. Subsequent addition of the drug was carried out to solvents until saturation, in order to determine maximum amount of the drug going into the solvent. Solutions were also checked for their stability by UV analysis at suitable time intervals.

Log P/Pkₐ

The pKₐ of a molecule is the pH at which the molecule is 50% protonated. Log P (or Log Pkₐ) is a measure of the lipophilicity of a compound. The shake flask method was used for measuring log P values. The U.V. absorbance of an aqueous solution is measured before and after being shaken with a known volume of octanol. One advantage of the method is that the appearance of compound in the octanol may be checked against the disappearance from the aqueous phase to see if any surface effects have occurred. The experiment was performed over 3 days or more to ensure equilibrium was reached, although the actual time taken in doing the experiment was about 0.5 Day.

In cases of extreme insolubility as in case of paclitaxel pKa values were measured by the solubility method. An aqueous solution of a substance is titrated in the direction of its neutral species until the free base or free acid is precipitated. pKa values were then calculated from the solubility product.

\[ S_{x} = S_{0} \left(1 + 10^{pkα-pHx}\right) \]

Where,

\( S_{x} \) = Solubility at pHx

\( S_{0} \) = Solubility of free base

d. Melting point
The MP Determination procedure as described in section <741>, p. 2033-2034 of the USP25-NF20 US Pharmacopeia was used. USP-compatible capillaries specified for MP determinations: 10 cm length, 0.8 - 1.2 mm internal diameter and 0.2 - 0.3 mm wall thickness were used.

Capillary tubes were charged with sufficient amount of the dry powder to form a column in the bottom of the tube 5.5 - 3.5 mm high when packed down as tightly as possible by tapping on a solid surface. The most common MP procedure requires inserting the capillary with the sample into the heating block 5°C below its expected MP and ramping at 1 +/- 0.5 °C/minute until the melt is complete. The MP range is recorded at the end of the melt.

Identification tests

UV spectral analysis

Absorption spectra of Paclitaxel was recorded in the methanol in the range of 200-400nm and the wavelength of the maximum absorption was determined.

Fourier-transform infrared spectroscopy (FTIR studies)

Infra red spectra was measured using Nicolet, Protege 460 FTIR spectrometer with sample being incorporated in a KBr disk.

Differential scanning calorimetry

Differential scanning calorimetric (DSC) analysis was used to characterize the thermal behavior of the pure Ellagic acid. DSC thermograms were obtained using an automatic thermal analyzer system (Pyris 6 DSC, Perkin Elmer, USA) based upon the Pyris thermal analysis software (v. 401). Temperature calibration was performed using indium calibration reference standard. Samples were crimped in a standard aluminum pans and
heated at a rate of 10°C min⁻¹ in the range of 30-200 °C under constant purging of dry Nitrogen at 30 ml min⁻¹. An empty pan, sealed in the same way as the sample was used as a reference. The peak transition onset temperature of Ellagic acid was determined.

**Stability studies**

Stability studies were performed for storage conditions and to ensure stability in the solution form. The conditions used were

- 25 °C ± 2 °C or
- 30 °C ± 2 °C

**Drug-excipient compatibility study**

In order to determine drug-excipient compatibility study, paclitaxel along with along with polymer was taken for DSC analysis.

**Results and discussion**

**Appearance (color):** white to buff colored

**Appearance (form):** Crystalline Powder

**Assay:** ≥97% as per suppliers COA

**Molecular weight:** 853.906

**Formula:** C₆₇H₁₃₁NO₁₄

**Solubility:** Paclitaxel was found to be highly lipophilic. It is practically insoluble in water. Solubility in various organic solvents is as described below.

Paclitaxel is soluble in dimethyl sulfoxide with its solubility being 50 mg/ml. 0.01M solutions can be stored as aliquots for long periods. Solubility in methanol is also similar i.e. 50 mg/ml but it undergoes hydrolysis and transesterification and solution is not stable.
for long period of time. Paclitaxel is also soluble in ethanol and acetonitrile. It is soluble in a mixture of 50% Cremophor EL and 50% anhydrous ethanol. This solution can be diluted with saline to give a final concentration of 0.03-0.60 mg/ml.

**Melting point:** 223 °C

**Boiling Point:** 957.115 °C

**Molar Refractivity:** 219.267 cm³

**Index of Refraction:** 1.637

**Surface Tension:** 68.4869995117188 dyne/cm

**Density:** 1.399 g/cm³

**Polarizibility:** 43.737 ×10⁻²⁴cm³

**Log P/Pka:** Log P value as determined by shake flask method was found to be 3.0 and pka values as determined by the solubility method was found to be 11.99.

**FTIR spectra**

Peak at 3600 cm⁻¹ corresponds to the O-H group at position 9. 2800 cm⁻¹ peak justifies C-H stretch at position 14 of paclitaxel. Peak at 1800 cm⁻¹ is due to C-H group of phenyl ring substitutions. Peak at 1600-1400 cm⁻¹ are due to C=C group of aromatic rings and Peaks between 1200-1000 cm⁻¹ are due to C-O groups of Acetyl substitutions in paclitaxel.

**UV-Visible spectroscopy**

UV spectra analysis of paclitaxel in methanol was carried out. λ max obtained was 229 nm.
Chapter 5

Pharmaceutical Profiling & Preformulation Studies

Fig. 7 UV-Vis Spectra of Paclitaxel

Fig. 8 DSC Thermogram of Paclitaxel
DSC

DSC thermogram of paclitaxel shows a small endothermic peak at 60 °C followed by marked exothermic peak at 350 °C as shown in the fig. 8.

Stability

Paclitaxel has poor solubility in water and is rapidly destroyed in weakly alkaline, aqueous solutions. The least amount of degradation in aqueous paclitaxel solutions occurred in the pH range of 3–5. Paclitaxel solutions at 0.1 and 1 mg/ml in 5% dextrose injection or 0.9% sodium chloride injection remained active for at least three days at 4, 22, or 32°C.
b. ELLAGIC ACID

Materials and Methods

Apparatus and Reagents

The apparatus used in the study are: UV-Visible Spectrophotometer (Shimadzu, Japan), analytical balance (Mettle AE160), pH Meter (Mettler Toledo MP220), Ellagic acid, Methanol, Ethanol, Chloroform, KBr, Hydrochloric acid, Sodium Hydroxide Pellets, analytical reagents from qualigens and SD Fine chemicals were used as materials and reagents.

Physicochemical Characterization

Ellagic acid was characterized for the following features; color, odor and melting point.

Methods used were same as discussed in section 5.2.1

Identification tests

a) **UV-VIS spectral characteristics**

Absorption spectra of Ellagic acid was recorded in the methanol in the range of 200-400nm and the wavelength of the maximum absorption was determined.

b) **Differential scanning calorimetry**

Differential scanning calorimetric (DSC) analysis was used to characterize the thermal behavior of the pure Ellagic acid. DSC thermograms were obtained using an automatic thermal analyzer system (Pyris 6 DSC, Perkin Elmer, USA) based upon the Pyris thermal analysis software (v. 401). Temperature calibration was performed using indium calibration reference standard. Samples were crimped in a standard aluminum pans and
heated at a rate of $10^0 \text{C min}^{-1}$ in the range of 30-200 °C under constant purging of dry Nitrogen at 30 ml min$^{-1}$. An empty pan, sealed in the same way as the sample was used as a reference. The peak transition onset temperature of Ellagic acid was determined.

Results and discussion

*Appearance (color):* cream to light yellow

*Appearance (form):* crystalline solid

*Assay:* $\geq 95\%$ as per suppliers COA

*Molecular weight:* 302.197 g/mol

*Formula:* C14H608

*Solubility:* 1 M NaOH: soluble 10 mg/mL, dark green

*Melting point:* $>360 \, ^{\circ}\text{C}$

*Boiling Point:* 796.5 °C

*Molar Refractivity:* 67.74 cm$^3$

*Index of Refraction:* 1.894

*Surface Tension:* 140.3 dyne/cm

*Density:* 1.667 g/cm$^3$

*Polarizability:* 89.52 Å$^2$

*Log P/Pka:* 2.31
UV Spectral analysis: $\lambda_{\text{max}}$ was found to be 258 nm.

Fig. 12 UV-Vis Spectra of Ellagic acid

Differential Scanning Calorimeter Thermogram

DSC thermogram of Ellagic acid two marked endothermic peaks 90$^\circ$C and 260$^\circ$C as shown below.

Fig. 13 DSC Thermogram of Ellagic acid
References:


29. Eli Lilly and Company. Gemzar (gemcitabine) prescribing information. Indianapolis, IN; 2006


56. Reviewers' comments (personal observations) on cervical cancer.


