**Investigation of Drug-Excipients Compatibility of Ellagic Acid for Development of Formulation Containing Liposomes**

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### Abstract

The aim of the present study was to check the compatibility between drug: lipid and drug: excipients. Ellagic acid was selected as model drug while phospholipid and Cholesterol were considered excipients. The study was performed by Fourier transform infrared spectroscopy (FTIR), Diffraction scanning calorimetric (DSC) and High performance liquid chromatography (HPLC). The spectrophotometric graphs revealed that there was no significant changes in the position of functional groups of ellagic acid, phospholipid and Cholesterol (O-H, C=O, C-H str.) in pure drugs and excipients with respect to their physical mixtures. Further the samples were characterized by using DSC and HPLC methods and found no any interaction between drug excipients. Combination of synthetic herbal drug and pharmaceutical excipients are compatible with each other and shows no interaction. Thus this suitable combination can be employed for the successful development of novel liposomal carrier system.

### Keywords:

Ellagic acid, Compatibility, Excipients, DSC, FTIR

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### 1 Introduction

The drug and excipients compatibility study first require an aforementioned acquaintance of physicochemical properties for the development of pharmaceutical formulation\(^1\). The excipients are known to expedite administration and moderate the statement of active constituent and are considered to be pharmacologically inactive, but physical and chemical interactions with active components are possible\(^2\). Drug–excipients compatibility studies lays a base in suspicious selection of most suitable excipients which play important role to design of optimum and effective dosage form concerning ideal physico-chemical characteristic and good stability\(^3\,^4\). This compatibility study observed the incompatibility between drug and excipients and also affects their nature, bioavailability, stability and their chemical properties distressing their chemical nature in order to give affection their therapeutic efficacy and safety\(^5\). Despite the significance of the dispute, there is no universal recognized protocol for drug-excipients incompatibility testing\(^6\,^7\).

Ellagic acids have potent antioxidant activity and it is use in some various diseases like cancer, wound healing, ulcer etc.\(^8\). In recent years, nanostructured materials such as nanoparticles, liposomes etc., have been considered as potential carriers for hydrophobic drug delivery that may purpose the above mentioned complications\(^9\,\,^10\).

Novel liposomal carrier developing a significant part of research in the field of drug delivery because they have ability to distribute an extensive assortment of drugs to various parts of the body for sustained periods of time. Liposomal novel carrier systems are actively investigated as drug carriers to reduce drug toxicity and degradation, and it deliver therapeutic agents to several sites of action to promote a suitable, selective and specific targeted therapy\(^12\). DSC technique involves the application of a heating or cooling signal to a sample and a reference. When the substance goes through a thermal event, the variance in heat flow to sample and reference is observed against time and temperature. Consequently, it can be concluded that energy is associated with various thermal events including melting, glass transition temperature and crystallization etc. Apart from DSC, FTIR Spectroscopy was used as another technique to determine the hypothesized compatibility study between drug and excipients which result that same functional group was changed as compared to standard FTIR Spectra of drug and excipients due to incompatibility between drug and excipients \(^13\). The present observation and characterization of drug and excipients was aimed to check the interaction study between ellagic acid and various pharmaceutical excipients in order to formulate optimum liposomal
formulation. This interaction study was done by using DSC, FTIR and HPLC.

2 Materials and Methods

2.1 Materials

Quercetin, phosphatidylcholine and cholesterol were purchased from Hi-media chemicals Mumbai, India. HPLC grade solvents were purchased from Merck Mumbai, India. All other materials and solvents used were of analytical grade.

2.2 Sample preparation

The quercetin, phospholipid and cholesterol mixtures prepared at 1:1:1 ratio. The quercetin, phospholipid and cholesterol were individually weighed in a 10 ml glass vial and mixed on a vortex mixer for 2 min. In each of the vials, 10% of the water was added and the drug-excipient blend was further mixed. Each vial was sealed Teflon-lined screw cap and stored at 50 °C for 4 weeks. These samples were periodically examined for any change of unusual color change.

2.3 Characterization by High-Performance liquid chromatography (HPLC)

The samples after 4 weeks were withdrawn from storage and analyzed by HPLC. The drug content was determined at initial and stored samples in triplicate. An accurately weighed amount of the quercetin, phospholipid and cholesterol mixture were taken and suitably dissolved under sonication in pH 7.4 phosphate buffer and filtered through 0.45 (Millipore) filters. The sample was analyzed after making appropriate dilutions using HPLC (SDP-10A VP UV-Vis detector column RP18,250×4.6mm) at 254 nm.

2.4 Fourier transforms infrared radiation measurement (FTIR)

FTIR spectra’s were recorded on a FTIR spectroscopy using the instrument Shimadzu FT-IR 8400S in the frequency range of 400-4000 cm⁻¹ with the resolution of 4 cm⁻¹ using potassium bromide discs method. The drug and each selected excipients (1:1 w/w) were stored at 40 ± 2 °C and 75 ± 5% RH for 1 month. Individual samples as well as the mixture of ellagic acid and excipients were ground, mixed thoroughly with potassium bromide for 3-5 minute in a mortar and compressed into disc by applying a pressure of 5 tons for 5 minute in hydraulic press. The concentration of sample in potassium bromide should be in the range of 0.2% to 1%. The pellets were placed in the light path and spectrum was obtained and reviewed for evidence of any interactions.

2.5 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (Perkin Elmer Jade, California, USA, department of pharmaceutical sciences diburgarh university, Assam, India) was used for thermal analysis of drug and mixture of drug and excipients in a 1:1 w/w ratio. Individual samples (drug and excipients) as well as physical mixture of drug and excipients were weighed to about 5mg in DSC aluminum pan. The sample pan was crimped for effective heat conduction and scanned in the temperature range of 50-300°C. Heating rate of 20°C min⁻¹ was used and the thermogram obtained was reviewed for evidence of any interactions.

3 Results and Discussion

3.1 Drug content estimation by HPLC

The ellagic acid and excipients mixture was physically observed at different intervals. No Characteristic color change were observed. The assay of the drug excipients mixtures were found good. Assay value of 98.25 to 102.3 was observed at initial. There was good compatibility with the samples of drug excipients mixtures stored at 50°C for 4 weeks and showed no interaction in physical mixture (Figure 1). This clearly indicates the stable nature of the ellagic acid with excipients (Table 1).

Table1: Drug content of Ellagic acid after storage at 50 °C for 4 weeks

<table>
<thead>
<tr>
<th>Time</th>
<th>Ellagic acid</th>
<th>Ellagic acid - phospholipid</th>
<th>Ellagic acid - cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial 50 °C for 4 weeks</td>
<td>98.36</td>
<td>97.21</td>
<td>95.87</td>
</tr>
<tr>
<td>4 weeks</td>
<td>99.48</td>
<td>99.65</td>
<td>98.20</td>
</tr>
</tbody>
</table>

Fig. 1: HPLC spectrum of Ellagic acid

3.2 FTIR

FTIR technique involves the study of the different functional groups of guest and host molecules by analyzing the significant changes in the shape and position of the absorbance bands. Data obtained from FTIR spectrophotometric study clearly indicates insignificant changes in spectra obtained from physical mixture of ellagic acid and excipients. Spectra obtained from pure ellagic acid were found 3600.48 cm⁻¹ and 1700.25 cm⁻¹ for O-H str. and C=O respectively (Figure 2). The results shows by observing FTIR spectra of both the
ellagic acid and excipients physical mixture were found to be as 3241 cm\(^{-1}\), 3260.32 cm\(^{-1}\) and 3410.91 cm\(^{-1}\) for O-H str group. Spectrography of C=O str shows peak at 1680.64 cm\(^{-1}\), 1721.75 cm\(^{-1}\) for ellagic acid, phospholipid and cholesterol respectively. The findings indicate no interference between ellagic acid and excipient (Table 2). There was no major changes in peaks of ketone (C=O) and hydroxyl (O-H) in reference to the observed value of ellagic acid.

### 3.3 DSC

The DSC thermogram corresponding to quercetin, phospholipid and physical mixture are shown in Figure 3. Ellagic acid exhibits a characteristic endothermic peak at 260.44°C corresponding to its melting temperature. A broad endothermic band from 158.6°C was observed for the amorphous phospholipid, which was related to loss of water molecules i.e., dehydration process. The DSC thermogram for the solid complex of ellagic acid, phospholipid and cholesterol showed an endothermic peak at 147.5°C associated with the formation of complex in the solid state. As such there is no interaction between ellagic acid, phospholipid and cholesterol.

### Table 2: FTIR spectra of Ellagic acid and physical mixture

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Standard (cm(^{-1}))</th>
<th>Ellagic acid (cm(^{-1}))</th>
<th>Physical mixture (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-H</td>
<td>3100-3600</td>
<td>3600.48</td>
<td>3241</td>
</tr>
<tr>
<td>C=O</td>
<td>1650-1700</td>
<td>1700.25</td>
<td>1680.64</td>
</tr>
<tr>
<td>C-OH</td>
<td>1350-1400</td>
<td>-</td>
<td>1325.64</td>
</tr>
<tr>
<td>C-H</td>
<td>680-850</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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Fig. 2: FTIR spectrum of (A) Pure Ellagic acid and (B) Mixture of Ellagic acid with phospholipid

Fig. 3: DSC thermogram of (A) Pure Ellagic acid, (B) Cholesterol and (C) Physical mixture of ellagic acid and Cholesterol
4 Conclusions

The compatibility of ellagic acid with excipients was studied by different analytical techniques like Differential Scanning Calorimetry, Fourier Transform Infrared Spectroscopy and HPLC study. In the present study, results of DSC along with FTIR and HPLC (for IST) were successfully employed to assess the compatibility of ellagic acid with the excipients. No concrete evidence of interaction were observed between ellagic acid and the excipients like phospholipid, cholesterol. No characteristic colour change was observed during the storage at 50 °C for 4 weeks. The HPLC analysis results of this mixture evident the chemical stability of ellagic acid as the assay was within the acceptable range. Hence, this data’s demonstrates the potentiality of the excipients for the successful development of a novel liposomal formulation.

5 Acknowledgement

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6 References