



Preparation and characterization of baicalein loaded phytosomes: Assessment of *in vitro* parameters

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Abstract : Phytosome is a complex between natural active ingredient and a phospholipid. Further, phytosomes been applied to many popular herbal extracts or active molecules for augmenting oral dissolution. Therefore, in present investigation, orally administered Baicalein, atype of flavanoids, is poorly absorbed, and shows suboptimal dissolution. The phytosomes encapsulating baicalein (1:1 Mm) were prepared by reverse phase evaporation method followed by lyophilization. Transmission electron microscopy (TEM) analysis revealed that phytosomes were almost spherical in shape with particle size below 100 nm. The Powder ex-ray diffraction (PXRD) and differential scanning calorimetry (DSC) demonstrated that Baicalein loaded phytosomes were amorphous in nature. Amorphization of therapeutic moiety leads to improvement in dissolution. In conclusion, epigallocatechin loaded phytosomes exhibited promising results and warrant further *in vitro* and *in vivo* investigations under a set of stringent parameters for transforming in to a clinically viable products.

Key words: - Flavonoids, Phytosome and dissolution.

1. Introduction:-

Baicalein (5,6,7-trihydroxyflavone) and baicalin (syn. baicalein7-O- β -D-glucuronic acid) (1) are the principal components found among 30 other flavonoid derivatives in the roots of *Scutellaria baicalensis Georgi*. Aglycobaicalein have been attracting growing interest from pharmaceutical industries because of their excellent biological action. (2). Studies on whether baicalein has an antidiabetic effect are scarce. Baicalein was reported to have beneficial effects in diabetes-associated health complications (3-5)

It is known that 70% of all new chemical entities entering drug discovery programs are not sufficiently soluble in physiological medium to allow consistent gastrointestinal absorption of high magnitude to ensure further pharmacodynamic activities. The solubility of a drug in gastrointestinal tract is governed by multiple factors and is inherently a complex phenomenon often resulting in erratic absorption of poorly soluble drugs. Therefore, an improvement in the dissolution rate of the drug is thought to be a key factor for improving the pharmacokinetic and pharmacodynamic activities. (6).

Phytosomes are nanovesicles that combine plant extracts and phospholipids to produce fat-soluble complexes. Phytosomes are also carriers that can increase the therapeutic efficacy of the green tea leaf extract. (7). Phytosomes have better abilities than herbal extracts in terms of their absorption through the intestinal lipid membrane. (8). Reason the phytosomal suspension made into a microsphere is the long-term stability problem of suspension. Suspension stability will gradually diminish in long-term storage because crystal formation may occur due to rising room temperature, which may lead to an increase in particle size in the suspension. Changes in particle size in the suspension may affect the therapeutic effect of the dosage form. (9). In this study, phytosome of Baicalein was prepared and evaluated. The evaluation was Phase solubility analysis, Transmission electron microscopy (TEM), Differential scanning calorimetry (DSC) and Powder X-ray diffraction (PXRD).

Materials and Methods

2.1. Materials

Phospholipid was obtained from PERFECT industries Pvt. Ltd., Nagpur, M.S. and Baicalein was purchased from Sigma Aldrich, USA. All other chemicals used were of highest analytical grade and used without further purification.

2.2. Preparation of phytosome complexes of Baicalein

Phytosome was prepared using reverse phase evaporation method. First, phospholipid was diluted with dichloromethane, while the Baicalein was diluted with 90% ethanol. Then, both the dissolved Baicalein and phospholipid were poured into the round-bottom flask. The dichloromethane was evaporated using rotary vacuum evaporator at 37 °C at gradual speed started in 25 to 150 rpm, and vacuumed until an even and firm thin layer was obtained. Nitrogen gas flowed into the thin layer, and then the layer was stored in the refrigerator up to 24 h. The thin layer was then hydrated with phosphate buffer pH 5, 5 at 40°C. Once the phytosomal suspension was formed, ultrasonication was done for 2 min.

2.3. Phase solubility analysis

Phase solubility assay was implemented to assess the stoichiometry of the Baicalein phospholipid complex in the aqueous phase (10). Briefly, Baicalein (20 mg) was suspended separately in 10 ml of phosphate buffer saline (PBS, pH~7.4) containing phospholipid at concentrations ranging from 2 to 32 mM. Next, samples were stirred in an orbital shaker (150 rpm) for five consecutive days at 37±1 °C. After equilibration, the samples were passed separately through a 0.22- μm membrane filter (MDI, India), and the absorbance was read at 298 nm using an UV/visible spectrophotometer (Shimadzu, Kyoto, Japan) (11). The apparent stability constant was calculated from the slope of the phase-solubility diagram using Eq. (1):

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

Where K_c is the apparent binding/stability constant and S_0 is the solubility of the drug in the absence of cyclodextrin.

2.4 Characterization of Phytosomes

2.4.1. Transmission electron microscopy (TEM)

The particle shape and surface topography was examined by using transmission electron microscope (TEM). In brief, an aqueous dispersion of each sample was drop casted onto a carbon coated copper grid. The grid was air dried at room temperature and then loaded into the Transmission electron microscopy (TEM) (Philips Morgagni, Netherlands) already maintained at a voltage of 80 kV.

2.4.2. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to confirm the synthesis of the complex in the solid state. A characteristic endothermic peak of Baicalein, Phospholipid, Physical mixture of Baicalein and

Phospholipid and complex of Baicalein and Phospholipid was recorded using a Mettler-Toledo differential scanning calorimeter. Nitrogen was used as the carrier gas at a flow rate of 45 ml/min. Thermograms was recorded at a heating rate of 20°C/min in the temperature range of 30 - 300 °C with 10 mg of sample.

2.4.3. Powder X-ray diffraction (PXRD)

Powder X-ray diffraction (PXRD) pattern was recorded for Baicalein, Phospholipid, Physical mixture of Baicalein and Phospholipid and complex of Baicalein and Phospholipid by using RIGAKU, Rotaflex, RV 200 (Rigaku Corporation, Akishima-shi, Tokyo, Japan) with Ni filtered, Cu K α -radiation, at a voltage of 60 kV and a current of 45 mA. XRD pattern was recorded at a scanning rate of 2 °C/min over 10–60 diffraction angle (2 θ). Each sample of tailored sample was scanned for five times between 10 and 60 °C diffraction angle (2 θ) and averaged for that sample and there was three (n = 3) different samples scanned. The crystal size of Baicalein, Phospholipid, Physical mixture and complex was calculated using Scherrer equation (12).

$$D_p = 0.94\lambda/\beta_{1/2} \cos\theta$$

where D_p refers to average crystallite size, β = line broadening in radians, θ = Bragg angle and λ = X-ray wavelength.

3. Results

3.1. Phase solubility analysis

The main aim of the present investigation was to explore the ability of Phospholipid to potentiate the therapeutic index of Baicalein in Diabetic and Aphrodisiac activity by improving its solubility and dissolution characteristics. In this study we have invoked the power of inclusion chemistry to stabilize and solubilize Baicalein via the lyophilization-based cycloencapsulation technique. To this end, our first step was to determine the composition, stoichiometry, and the apparent stability constant (K_c) of Baicalein Phospholipid complex. To accomplish this we employed phase solubility analysis using a gradient concentration of complex of Baicalein - Phospholipid in solution phase as per the Higuchi and Connors system (13). The phase-solubility diagram of the complex of Baicalein with Phospholipid in solution state is shown in Fig. 1. The curve demonstrates that the solubility of Baicalein increases linearly as a function of concentration of Phospholipid. The data imply that the solubility diagram of Baicalein with Phospholipid is of the A_L type. The stability constant (K_c) of the binary system of Baicalein with Phospholipid is calculated to be 277 mM, from the linear plot of the phase solubility diagram.

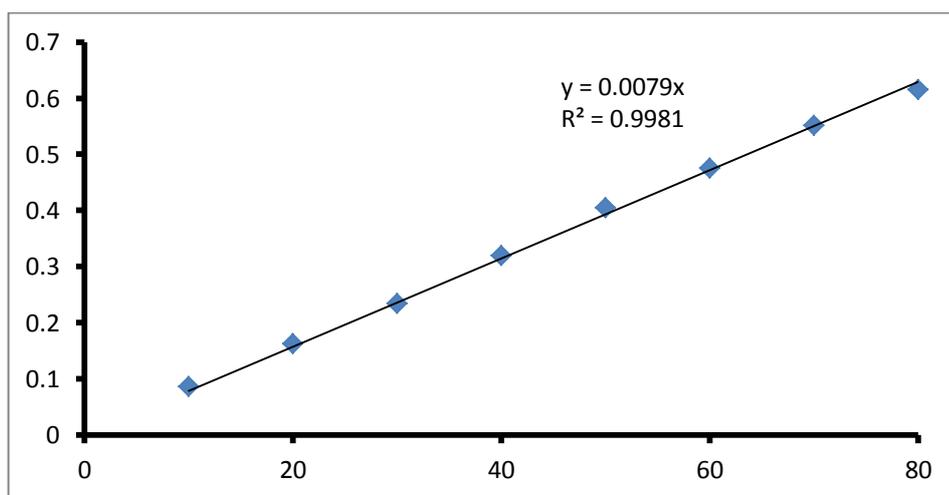


Fig 1. Phase solubility analysis of Baicalein in the presence of a gradient concentration of Phospholipid (10 – 80 mM) in phosphate buffer saline (pH ~7.4). The solubility diagram of Baicalein with Phospholipid is of the A_L type and the stability constant (K_c) was calculated to be 277 mM, from the linear plot of the phase solubility diagram.

3.2.Characterization of Phytosomes

3.2.1. Differential scanning calorimetry (DSC)

The synthesis of the complex of Baicalein with Phospholipid in the solid state was confirmed by using Differential scanning calorimetry. The endothermic peaks of the complex and the individual components as compared in Fig. 2. The data shows an endothermic peak of Baicalein at 273.25 °C near to its melting point (272 °C). The thermograms of Phospholipid exhibit a range of endothermic peaks broadly ranging from 40 to 150 °C (65 °C for Phospholipid) due to the dehydration process. The thermogram of the physical mixture of Baicalein with Phospholipid indicated the presence of identical peaks of individual components. However, the thermogram of the complex of Baicalein with Phospholipid shows a complete disappearance of the endothermic peaks characteristic of Baicalein with a significant shift in Phospholipid endothermic peaks to 101.12 °C, respectively.

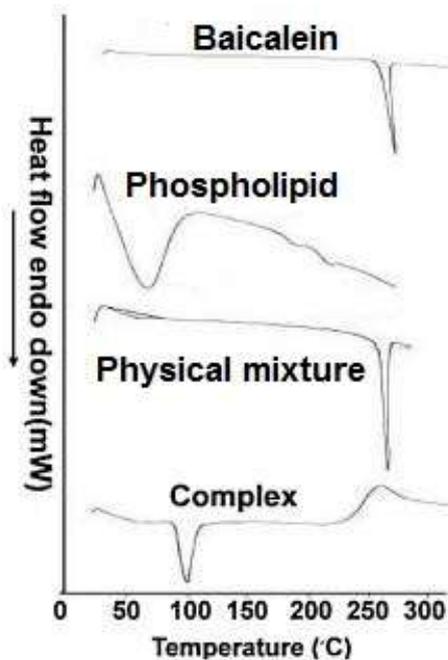


Fig.3. Differential scanning calorimetry (DSC) analysis of Baicalein, Phospholipid, physical mixture of Baicalein - Phospholipid, and complex of Baicalein – Phospholipid. The thermogram of complex of Baicalein - Phospholipid shows a complete disappearance of the endothermic peaks characteristic of Baicalein with a significant shift in the Phospholipid peak.

3.2.2. Powder X-ray diffraction (PXRD)

The amorphous state of Baicalein in the complex was defined by the Powder X-ray diffraction (PXRD) technique. The XRD pattern of Baicalein shows intense and sharp peaks indicative of its crystalline structure (Fig. 4). Although Phospholipid is crystalline in nature and shows sharp peaks in the XRD pattern however the induction of the hydroxypropyl in Phospholipid, transforms the crystalline structure into an amorphous state with its distinctive undefined broad, diffused peaks. This indicates the improved solubility of complex of Baicalein in water compared to Phospholipid. The XRD pattern of the physical mixture of Baicalein with Phospholipid manifests peaks of both compounds. Finally the complex of Baicalein with Phospholipid shows peaks of diminished intensity with some new peaks from 35° to 60°. This could be attributed to alteration in the conformational stereochemistry of Baicalein during the formation of complex (14).

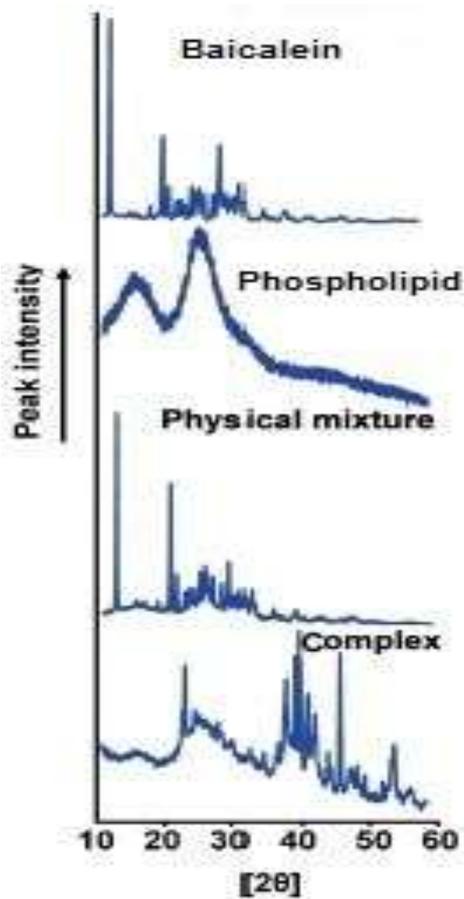


Fig.4. Powder X-ray diffraction pattern (PXRD) of Baicalein, Phospholipid, physical mixture of Baicalein with Phospholipid, and complex of Baicalein-Phospholipid. The XRD pattern of the physical mixture of Baicalein with Phospholipid reveals the presence of peaks of both compounds while the complex of Baicalein-Phospholipid shows peaks of diminished intensity with some new peaks from 35° to 60°.

3.2.3. Transmission electron microscopy (TEM)

The photomicrographs of TEM indicated that the Baicalein Phytosomes were slightly spherical and or irregular in shape. Hence, our characterization parameters pointed out that Baicalein Phytosomes were suitable for penetration into the stratum corneum layer.

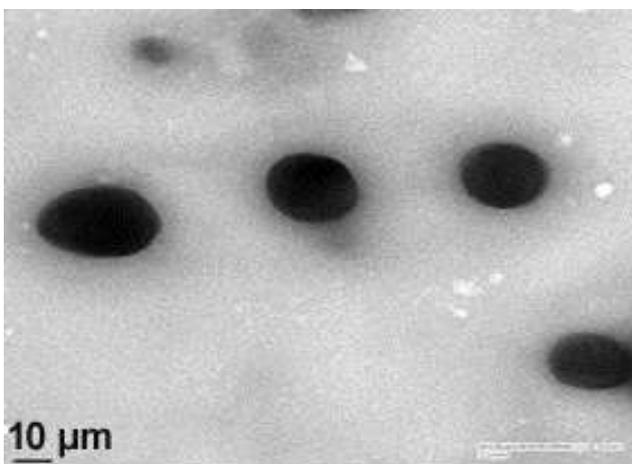


Fig 2. Transmission electron microscopy (TEM) analysis of Baicalein and Phospholipid Phytosomes. Scale in TEM. 10 μm.

4. Discussion

The phase-solubility experiments provide evidence that Baicalein forms a 1:1 complex with Phospholipid in the solution state (Fig. 1). The phase-solubility diagram can be classified as A_L type showing the formation of a water-soluble complex with a suggestive first-order kinetics for the complex formation between Baicalein and Phospholipid. Further, various spectral techniques were employed to elucidate the structure of the complex in the solid state. Differential scanning calorimetry (DSC) thermoanalysis confirms the formation of a 1:1 complex in the solid state as the endothermic peak of Baicalein disappears in the Baicalein - Phospholipid complex when compared with the endothermic peak of Phospholipid alone (Fig. 2). Furthermore, PXRD patterns of the complex of Baicalein with Phospholipid exhibits peaks of diminished intensity in comparison to the sharp peaks of Baicalein (Fig. 3). The PXRD spectroscopy substantiated that Baicalein resides in the Phospholipid cavity in a polymeric amorphous state. Typically, owing to irregular structural configurations, the amorphous phase entails minimal energy and thus offers the utmost solubility of drugs. In continuation with the results of PXRD, the photomicrographs of TEM indicated that the Baicalein Phytosomes were slightly spherical and or irregular in shape (Fig. 4). Baicalein was entrapped in the liposomal membrane due to hydrogen bonds with the polar head group of phosphatidylcholine (15). Hence, our characterization parameters pointed out that Baicalein Phytosomes were suitable for penetration into the stratum corneum layer.

5. Conclusions

In conclusion, the present study describes the use of various spectral techniques and computational studies, our data provide sufficient evidence that the Phospholipid -based complex improves the physicochemical and biological properties of Baicalein. These data are encouraging and thus warrant further in vitro and in vivo investigations under a set of stringent parameters for transforming in to a clinically viable product.

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