INTRODUCTION

The therapeutic applications of traditional phytomedicines have gained popularity for the management of human disease efficiently since ancient time. The recent advancement of the herbal drug technology comes into force with an objective to improve bioavailability of polar bio-active constituents such as phenolic, glycosides, flavonoids etc. In the field of herbal drug delivery system several approaches, such as ensnare the lipophilic carrier, incorporation of solubility enhancer, physical/chemical or structural modification have been adapted to enhance bioavailability and therapeutic indices of drugs thus achieving better results compared to conventional herbal extracts.

The bioactive ingredients from the whole plant are being extracted, isolated and examined for specific therapeutic applications but there is a chance of partial or total loss of pharmacological activity during isolation and purification process. The traditional herbal formulations usually consist of pharmacologically active constituent along with toxic compound depending on the nature of the plant. Plant extracts often possess low potency in vivo or in animal model though exhibiting excellent therapeutic activity in vitro. The multi-ring molecular structure and lipid solubility of plant extract retards the absorption process by passive diffusion and limits their ability to cross biological membrane respectively resulting poor bioavailability. Further orally administered bioactive herbal constituents are destroyed by or lost to the gastric juice or they become ineffective due to interaction with other drugs or nutraceuticals. 

Herbosome, a revolutionary model, has been successfully developed to achieve remarkable bioavailability of herbal drug and target significant pharmacological response with balanced nutrient safety. The term “Herbo” means plant whereas “some” means cell-like. Herbosome, a compatible macromolecular complex, is prepared by entrapping standardized plant extracts or polar bio-active phytoconstituents into phospholipids. These complex is formed via the interaction of stoichiometric quantity of phospholipids with herbal constituent in an aprotic solvent. Herbosome structures (size range: 50 nm- few hundred μm) are comprised of pharmacologically active phytoconstituents surrounded by natural or synthetic phospholipids like phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine. The lipophilic herbosomes are found to be high and moderate solubility in non-polar solvents and fats respectively with definite melting point. Herbosomes are assumed to be micelle being treated with water resembling liposomal structure but possess fundamental difference. Their ease of manufacture, better stability, prolonged therapeutic effect, bio-membrane penetration potential and enhanced bioavailability offer great advantages. In-vitro characterisations of herbosome include study of microscopical properties, entrapment efficiency, percentage yield, zeta potential, particle size, analysis of surface texture, drug release profile. Some of the instrumental techniques employed are FT-IR spectroscopy, NMR, XRD, DSC, DLS, TEM, PCS etc. Diffusion mediated drug release from vesicle structured herbosome provides efficient barrier and permits design of oral as well as topical drug delivery systems for achieving systemic effect.

Keywords: Aprotic solvent, Bioavailability, Bio-membrane penetration, Herbosome, Phospholipids.
METHODOLOGY

Herbosomes, a molecular complex, are prepared via the interaction of bioactive phytoconstituents with 3-2 moles of phospholipids (preferably with one mole of phospholipids) with or without aprotic solvents like dioxane or acetone and followed the isolation process by either precipitation or lyophilization or spray drying. For the complex formation, the ratio between these two moieties should be maintained in the range of 0.5-2.0 moles. It was reported that the most suitable ratio of phospholipids to flavonoids is 1:1.10, 13 The different formulation methodologies of herbosomes are described below.

**Anti-solvent precipitation technique**

1. The specific amount of plant extract + phospholipid (into a 100 ml round bottom flask)
2. Reflux with 20 ml of dichloromethane for 2 h (temperature < 60°C)
3. Concentrate the mixture to 5-10 ml
4. Add dissolving agent (Hexane) (20 ml) carefully
5. Continuous stirring
6. Filter and collect the precipitate
7. Crush dried precipitate in a mortar
8. Sieve the powder through #100 mesh
9. Store the powdered complex in an amber coloured glass bottle (at 25°C)

**Rotary evaporation technique**

1. Dissolve the specific amount of plant extract + phospholipid in 30 ml of tetrahydrofuran (into a rotary round bottom flask)
2. Temperature < 40°C Continuous stirring for 3 hrs
3. Add n-hexane to thin film of the sample oooof the-
4. Continuous stirring
5. Collect the precipitate
6. Store the powdered complex in an amber coloured glass bottle (at 25°C)

**Solvent evaporation technique**

1. The specific amount of plant extract + phospholipid (into a 100 ml round bottom flask)
2. Reflux with 20 ml of acetone for 2 h (temperature: 50-60°C)
3. Concentrate the mixture to 5-10 ml
4. Filter and collect the precipitate
5. Store dried powdered complex in an amber coloured glass bottle (at 25°C)

**Ether-injection technique**

1. Dissolve the drug-lipid complex in an organic solvent
2. Inject this mixture slowly into a heated aqueous agent
3. Formation of amphiphilic vesicles having different structures (depending on concentration)

Working Principle

The amphiphilic nature of phospholipids consists of both polar head and non-polar tail regions. A weak hydrogen bond is formed via the interaction of amine or phosphate group containing polar head of phospholipids with hydrophilic ends of substrates results the formation of a complex imparting lipophilic properties by twisting of non-polar tail of phospholipid itself. Therefore, herbosome becomes suitable for both oral as well as topical delivery of drug. 6, 14

CHARACTERISATION

Organoleptic evaluation

The organoleptic properties of herbosomes are found on the basis of colour, odour, and transparency. Solubility and wavelength maxima of the drug are also determined by organoleptic evaluation. Keerthi et al reported that Ashwagandha extract was found to be soluble in phosphate buffered saline (pH 7.4) and dimethylsulphoxide and the standard solution showed linearity at λ max 226nm. Jain et al found that Mangiferin–soya phosphatidylcholine (MF–SPC) complex was soluble in dichloromethane and insoluble in methanol, hexane.15, 16

Microscopic study

Several structural characteristics of herbosomes such as morphology, crystallization, stress or magnetic domain and internal structural network are visualized by transmission electron microscopy (TEM) whereas scanning electron microscopy (SEM) provides the information regarding surface texture of herbosome. 17 Rathee et al observed spherical structure of Citrullus colocynthis (L.), Momordica balsamina and Momordica dioica extract containing phytosome (Polyherbal phytosome) without aggregation by TEM and digital microscopy. 17 Nazeer et al also found a spherical shaped phytosome containing methanolic extract of Allium sativum with the help of SEM micrographs (at 20KV for the magnification range of 7500). 18

Particle size and zeta potential

Dynamic light scattering (DLS) in combination with computerized inspection system and photon correlation spectroscopy (PCS) are employed for the determination of particle size and zeta potential. Ittadwar et al reported that the average particle size and zeta potential of umbelliferone phytosome was found to be 1139 nm and -
0.05mV respectively. 19 Keerthi et al observed the average particle size of Ashwagandha phytosome was 98.4nm by using by particle size analyzer (Horiba scientific nanopartica S2100). The value of zeta potential (-28.7mV) indicated good stability of Ashwagandha phytosome. 15

**Percentage yield and Entrapment efficiency**

The entrapment efficiency provides information about the percentage of drug entrapped into the herbosome by ultracentrifugation technique. 7 Entrapment efficiency and percentage yield of drug are determined by following equation. 15, 17

\[
\text{Entrapment efficiency} (\%) = \left\{ \frac{\text{Weight of total drug} - \text{weight of free drug}}{\text{Weight of total drug}} \right\} \times 100
\]

Percentage yield = (weight of herbosomes formed) ÷ (weight of drugs and non volatile excipients) × 100

Keerthi et al found the entrapment efficiency of Ashwagandha phytosome (100mg Ashwagandha extract and 300mg Soylecithin) was 90.1%. If the lipid quantity exceeds 300mg, the entrapment efficiency was found to be decreased indicating the more lipid concentration is not suitable for ensnaring drug into the matrix. 15 The entrapment efficiency of Allium sativum and Polyherbal phytosome were found to be 97.306 % and 92.1% respectively. 17, 18 Studies revealed that greater entrapment efficiency of phytosome (greater than 90%) than conventional dosage forms was due to higher lipid solubility of garlic oil. 20 The percentage yield of Polyherbal extract was found to be 72%. 17

**X-ray diffraction study**

The crystalline behaviour and the diffraction pattern are determined by X-ray powder diffraction method. X-ray diffraction pattern of the umbelliferone phytosome showed diffraction peaks of crystallinity at a diffraction angle (2θ) 15.681 to 27.618 by operating Bruker AXS D8 Advance at 35 mA tube current and 40 kV tube voltages. 19

**Drug content**

Suitable methods for the determination of drug content such as UV spectroscopy, HPLC, GC-MS are adapted to quantify the amount of drug. 7 The drug content of optimised umbelliferone phytosome (1:2 stoichiometric molar ratios of umbelliferone and phospholipon 90H) was found to be 97.40% by UV spectrometer at 324nm. 19

**Thermal analysis**

Differential scanning calorimetry (DSC) determines the transition temperature, onset temperature, enthalpy of the vesicle shaped herbosome. It is another approach of drug- excipient compatibility study. Similar endothermic melting transition of anti-diabetic polyherbal extracts and phospholipids indicated the compatibility of phytosome formulation. 17 Ittadwar et al observed the exothermic peak and onset temperature of umbelliferone phytosome at 75.17 °C and 63.55 °C respectively. A little chemical interaction was also detected by DSC thermogram. 19

**Spectroscopic evaluation**

The spectroscopic study provides the confirmation of complexity between bio-active herbal constituents and phospholipids moiety therefore helps in the assessment of physical and chemical interaction between them. 17

**Nuclear Magnetic Resonance**

In characterization of herbosomes, 1H and 13C-NMR are most commonly used. 1H-NMR spectra of the evodiamine-phospholipid complex indicated some intermolecular interactions whereas intramolecular hydrogen bonding was observed in Mangiferin–soya phosphatidylcholine complex. 21, 16 13C-NMR study of umbelliferone revealed the signal for carbon at δ 155.57 and 138.59 ppm with a hydroxyl and a carbonyl group respectively whereas the umbelliferone phytosome showed chemical shift from δ 155.57 to 160.39 ppm due to shifting of signals on the higher side. 19

**FT-IR spectroscopy**

FTIR spectroscopy plays a significant role to determine the stability of herbosomal topical gel as well as herbosomal dispersion system. 7 FT-IR spectrum revealed the interference of resveratrol-phospholipid complexes at polar end group of the Phospholipon 90G. Board band of Phospholipon 90G and trans-resveratrol complex, an indication of intermolecular hydrogen bonding, was employed as a confirmation tool of the complexation. 22

**In vitro drug release and drug release kinetics**

In-vitro drug release study is carried out by using dialysis bag or Franz diffusion cell and different kinetic modelling (zero, first, Higuchi, Korsmeyer–Peppas model) are adapted to detect the release kinetics and diffusion mechanism of herbosome. Rathee et al revealed 92% biphasic drug release from the polyherbal phytosome in 12 hrs. 17 Chauhan et al reported that Aegle marmelos herbosomes (Aegle marmelos: Soylecithin-1:2) showed 91% and 92% drug release in 8 and 12 hrs respectively. 23 The capsules of Ashwagandha phytosomes showed time and concentration dependent 76.8% drug release. 15 Nazeer et al found zero order time dependent 85.35% drug release at the end of 3 hrs from phytosomal complex of Allium sativum indicating the reliability for dosage delivery and the drug release followed non-fickian diffusion mechanism. 14 Sikarwar et al revealed first order release profile of Marsupsin–phospholipid complex. 24

**Vesicle stability**

The stability study of herbosome follows the measurement of mean size and structural configuration of herbosome over time by DLS and TEM respectively following ICH guidelines. 7 Ashwagandha phytosomes were found to be stabilized at room temperature and refrigerated temperature for 3 months. 15 Aegle marmelos herbosomes showed higher stability at a frozen temperature (2-4°C). 21
Rathe et al observed no significant changes of polyherbal phytosome on the basis of consistency, phase separation, entrapment efficiency and drug content after 2 months of stability study.17

APPLICATION9, 25-31

The applications of herbosomes are summarized in Table 1.

<table>
<thead>
<tr>
<th>Phytoconstituent</th>
<th>Application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procyanidolic oligomers from Grape seeds extract</td>
<td>Systemic antioxidant</td>
</tr>
<tr>
<td>Epigallocatechin 3-O-gallate from <em>Camelia sinensis</em></td>
<td>Systemic antioxidant, Anti-cancer, Hypolipidemic</td>
</tr>
<tr>
<td>Silybin from Sili marin</td>
<td>Skin protective and Hepatoprotective</td>
</tr>
<tr>
<td>Ginkgo flavonoids from <em>Ginkgo biloba</em></td>
<td>Cerebro-protective</td>
</tr>
<tr>
<td>Anthocyanosides from <em>Vaccinium myrtillus</em></td>
<td>Antioxidant, Capillary tone enhancer</td>
</tr>
<tr>
<td>Fatty acids, alcohols &amp; sterols from <em>Serenoaepens</em></td>
<td>Anti-oxidant, Benign Prostatic hyperplasia</td>
</tr>
<tr>
<td>18-β glycyrrhetinic acid</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Anthocyanosides from an extract of <em>Bilberry</em></td>
<td>Potent antioxidant, Capillary tone enhancer, Maintain blood vessel permeability, Effective against venous insufficiency</td>
</tr>
<tr>
<td>A standardized extract of <em>Melilotus officinalis</em></td>
<td>Effective against chronic venous insufficiency of the lower limbs.</td>
</tr>
<tr>
<td>Polyphenols from olive oil</td>
<td>Anti-inflammatory, Antioxidants, Capacity to reduce the oxidation of low density lipoprotein, cholesterol, and also have</td>
</tr>
<tr>
<td>Echinacosides from <em>Echinacea angustifolia</em></td>
<td>Improve immunity</td>
</tr>
</tbody>
</table>

CONCLUSION

Herbosomes, a promising herbal drug technology, can overcome the barrier to formulate the dosage forms with hydrophilic therapeutically active phytoconstituents and show better bioavailability than conventional herbal extracts. Their simple formulation methodology and characterisation techniques pay attention to the reseachers. Moreover, many patents are endorsed for these novel formulations. Herbosome offers a great future by providing permission in the design of oral and topical drug delivery systems.

Author’s Contributions: Authors contributed equally to the design and implementation of the review and the writing of the manuscript.

REFERENCES


20. Wencui Z, Ying QiW, Di W, Preparation of solid lipid nanoparticles loaded with garlic oil and evaluation of their in vitro and in vivo characteristics, European Review for Medical and Pharmacological Sciences, 19(19), 2015, 3742-50.


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