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Research Article

# FORMULATION AND STANDARDIZATION OF POLYHERBAL CAPSULES FOR THE MANAGEMENT OF INFLAMMATION

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## ABSTRACT

*Mangifera indica* and *Phyllanthus emblica* were extracted using 90% ethanol and 1:1, 1:2 and 2:1 ratio of the extract mixture was loaded in to liposomes. These liposomes were formulated as capsule dosage form for use in management of inflammation. The extraction yield for MI and PE were found to be 41.9 and 27.1 % respectively. The total phenolic content in MI and PE extracts were found to be 17.3±0.916 and 34.23±1.908 GAE mg/100g whereas it was found to be 49.2±2.749, 71.76±6.269 and 60.63±4.162 GAE mg/100 g for 1:1, 1:2 and 2:1 ratio mixture of MI and PE respectively. The particle size of the liposomes decreased on increasing the sonication time. The particle size of the liposome formulated using 5 min sonication time was considered optimum and was found to be 3.21 µm for blank liposome. The phenolic content in the liposomes was in harmony with the extract mixture incorporated into the liposome. EL2 exhibited the highest phenolics content. The liposomes were evaluated for stability for a period of 3 months at storage temperature of 4°C and 40°C. The liposomes were stable at both the temperature conditions and the particle size of the liposomes did not change significantly over a period of three months. The extract loaded liposomes at 5 min sonication time were blended with magnesium and calcium carbonates to prepare a prefill mixture for the capsules. The prefill blends were evaluated for bulk density, tapped density, angle of repose, Hausner's ratio and Carr's Index. All the blends possessed the capability to flow freely and may present no hindrance in capsule filling process. The weight variation of polyherbal liposome filled capsules was between 4.1-4.6 % whereas the disintegration time was found to be between 3.38 to 3.65 min.

**Keywords:** Polyherbal, Liposomes, Inflammation, Stability, Total phenolic content, Capsule.

## 1. INTRODUCTION

Inflammation is a response against stimulus initiated by foreign invaders or endogenous signals like damaged cells and tissues [1]. Acute inflammation is considered as the first line of host defense against foreign invaders. Diseased states like cancer, neurodegenerative disorder or cardiovascular conditions may slowly lead to chronic inflammation [2]. The majority of drug used in management of inflammation act on the symptoms and also precipitate several undesired effects like gastrointestinal and renal toxicities. [3] Several scientific studies on herbal medicines have been carried out for anti-inflammatory action [4-7] and it was found that flavonoids present in plants are involved in disrupting the oxidative pathways thereby causing the anti-inflammatory action of the plant extracts [8]. The use of herbal remedies carries along with it number of problems, like low solubility and associated limited absorption and bioavailability. Such biologically active compounds are also prone to *in vivo* hydrolysis, oxidation, and photolysis, urging the need for stabilization platforms [9, 10]. Several novel techniques like formulation of liquid crystal systems, polymeric and solid lipid nanoparticles, precursors systems for liquid crystals, liposomes, and microemulsions have been reported that have the capability to overcome such limitations [11]. These drug delivery systems also improve compatibility, allowing substances with different physicochemical characters to be used within the same formulation. Some even make it possible to change the drug's obvious characters and hence its behavior in the biological environment [12]. *Mangifera indica* is a medicinal plant which belongs to the Anacardiaceae family and is found to possess action in rheumatism, insomnia and toothache. Analgesic and anti-inflammatory action of *M. indica* has been reported [13]. *Phyllanthus emblica* belonging to family Phyllanthaceae is known anti-inflammatory, and antioxidant actions [14].

It was therefore decided upon to prepare a polyherbal liposomal formulation containing the ethanolic extracts of *Mangifera indica* and *Phyllanthus emblica* for the management of inflammation.

## 2. MATERIAL AND METHODS

*Mangifera indica* (MI) and *Phyllanthus emblica* (PE) plants were procured from Shubham Nursery Bhopal. High purity Soy Lecithin and cholesterol were procured from Merck Life Sciences, Mumbai. Magnesium carbonate, and calcium carbonate were purchased from Oxford Fine Chemical LLP, Mumbai. All the chemicals and reagents used were used as obtained.

### 2.1. Extraction of plant material [15]

The leaves of MI and PE were shade dried, powdered and passed through sieve no. 20. A 100 g of leaf powder (of individual plants) was extracted using 90% ethanol as the solvent using hot continuous extraction method for about 7h. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extract were allowed to dry in air and then transferred to lyophillizer for complete drying of the extracts. The dried extracts were stored in air tight containers until further processing.

### 2.2. Total Phenolic content in the extracts [16]

The extracts of *Mangifera indica* (MI) & *Phyllantus emblica* (PE) were mixed in 1:1, 1:2 and 2:1 ratio by weight respectively and each mixture was dissolved individually in ethanol to obtain a stock solution (50µg/mL) for analyses.

A 200µL of each sample was mixed with 1.4mL purified water and 100µL of Folin-Ciocalteu reagent was added. After 2 min, 300µL of 20% $Na_2CO_3$ aqueous solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (ILT-2201, Labtronics). Standard solutions of gallic acid (10-60 ppm) were similarly treated to construct the calibration curve. The control solution contained 200µL of ethanol along with the other reagents. The results are expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

### 2.3. Preparation of Liposomes [17]

Liposomes were prepared by physical dispersion method from a lipid mixture of lecithin: cholesterol (Table 1). Briefly, 240 mg of lecithin and 60 mg of Cholesterol were dissolved in 100 ml of aethanol:chloroform (1:2, v/v) solution in a round bottomed flask. Extract (200 mg) was dissolved in 5 ml of ethanol and added to the above lipid solution. The solvent was then evaporated to dryness in a rotary evaporator at 180 rpm in a 40°C water bath. When a thin film of lipid was deposited on the inner wall of the flask, phosphate buffered saline (PBS, pH 7.4, 5ml) was added and the mixture was further rotated for 30 min to obtain white homogenous dispersion of liposomes. The dispersion was then incubated in a shaker bath for 2 h at 37°C to complete the swelling process. Smaller vesicles were produced from the larger vesicles by sonicationat 40W for 1 min followed by 1 min of rest for 5, 15 or 30 min. The liposomes were incubated for another 2 h at 37°C to allow the completion of the annealing process. Control (drug-free) liposomes were prepared in a similar manner except that the extract was not added to the mixture. All liposome dispersions were characterized immediately after preparation.

**Table 1: Composition of liposome formulations**

| Formulation code | Lecithin | Cholesterol | Extract Ratio (MI:PE) |
|---|---|---|---|
| | Parts | | |
| EL1 | 4 | 1 | 1:1 |
| EL2 | 4 | 1 | 1:2 |
| EL3 | 4 | 1 | 2:1 |

### 2.4. Characterization of Liposomes [17]

The characterization of the liposomes was carried out for determination of particle size, total phenol content and stability of the liposomes.

#### *2.4.1. Size and size distribution*

The particle size of the microspheres was determined by using Malvern particle size analyzer. The liposomal sample was dispersed in water and analyzed using the particle size analyzer for average particle size of liposomal vesicles.

#### *2.4.2. Total phenolic content in liposomes*

A standard diluents solution was prepared using ethanol, acetic acid and distilled water at 50:8:42 ratios, respectively. 1mL of liposome sample was diluted at a volume ratio of 1:4 with the standard solution and filtered. After dilution, the liposome sample was agitated by vortexing for 1 min and 2.5mL Folin solution was added to 500µL diluted liposome sample. The solution was allowed to stand for 5 min in the dark and 2mL of sodium carbonate solution was added to it followed by

leaving aside in dark for another 60 min. The blank solution was prepared using 2mL sodium carbonate solution and 2.5mL Folin solution and the mixture was left in dark for 60 min. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer.

### *2.4.3. Stability of Liposomes*

The stability of the liposomal preparations was evaluated as a function of storage time. In the preliminary experiments, liposomal samples were stored in a refrigerator at 4°C and 40°C for 3 months immediately after preparation. At the end of 3 months, the size and size distribution of the samples were determined.

## 2.5. Formulation and evaluation of polyherbal capsules

The liposomes loaded with different ratio of the extracts were formulated in capsule dosage form using method reported by Azmi *et al*. [17]. The liposomes (6g) were weighed accurately and mixed with 2g each of magnesium carbonate and calcium carbonate by tumble blending in a sealed polybag. The blend was sifted through sieve no. 26 to obtain fine powder. The fine powder was subjected to characterization of powder properties and hand filled in capsules (500 mg per capsule). The capsules were evaluated for weight variation and disintegration time.

## 3. RESULTS AND DISCUSSION

### 3.1. Total Phenolic Content

The extracts of MI and PE were evaluated for quantification of the total phenolic content in them. The total phenolic content is considered to be primarily involved in the neutralization of free radicals and other mediators of several diseases including inflammation.

**Table 2: Total Phenolic content of MI, PE and extract mixtures**

| Extract | Total phenolic content (GAE mg/100g) |
|---|---|
| *Trigonella foenum-graecum* | 31.3±1.367 |
| *Zingiberofficinale* | 11.7±2.896 |
| *Piper nigrum* | 8.7±0.894 |
| Extract mixture 1:1:1 | 49.8±0.735 |
| Extract mixture 1:2:1 | 58.1±1.721 |
| Extract mixture 2:1:1 | 69.06±1.823 |

*Data expressed as gallic acid equivalent (GAE) mg per 100g of the extract, Values are mean ± SD of triplicate determinations*

The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 2. The total phenolic content in MI and PE extracts were found to be 17.3±0.916 and 34.23±1.908 GAE mg/100g.

The mixture of the extracts presented a summative increase in the total phenolic content with the highest phenolic content in the mixture containing 1:2 ratio of MI and PE respectively.

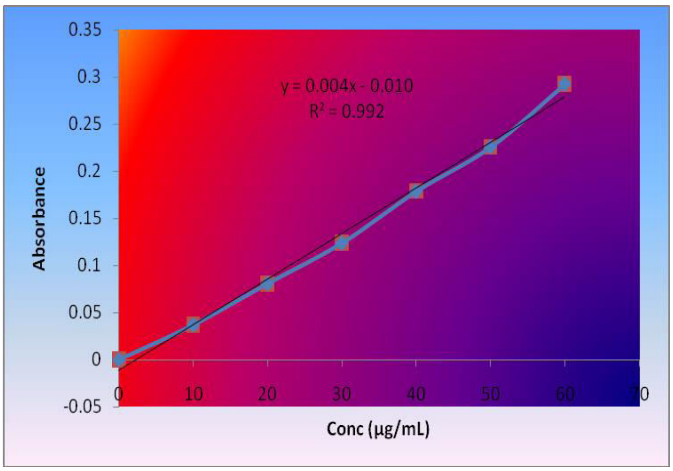


**Fig. 1: Calibration curve of gallic acid**

### 3.2. Characterization of Liposomes

#### *3.2.1. Particle size*

Table 3 presents the average size of blank vesicles and extract-loaded liposomes prepared using different sonication times. Liposomes of size range 1.89 µm were obtained (blank) when the sonication time was kept over 30 min. Decreasing the sonication time led to an increase in the vesicle size of the liposomes.

**Table 3: Particle size of liposomes**

| Formulation Code | Sonication time (min) | Particle Size (µm) |
|---|---|---|
| | | Extract loaded liposome |
| EL1 | 5 | 5.59 |
| | 15 | 4.08 |
| | 30 | 2.22 |
| EL2 | 5 | 5.03 |
| | 15 | 4.23 |
| | 30 | 2.18 |
| EL3 | 5 | 5.21 |
| | 15 | 4.23 |
| | 30 | 2.11 |
| Blank liposome | 5 | 3.21 |
| | 15 | 2.23 |
| | 30 | 1.89 |

The size range of blank liposomes obtained on sonicating for 5 and 15 min was found to be 3.21 µm and 2.23 µm respectively.

Nevertheless the smaller vesicle size is favored over the larger vesicles but it is well established that the efficiency of the liposomes is associated with the lipid content and not the particle size. This provides considerable support for using lower sonication times for formulation as the optimum formulations and subjecting them to stability study and further preparation of the capsule dosage form.

### *3.2.2. Total phenolic content in the liposomes*

The results reveal the phenolic content in the liposomes was nearly the same as the extract mixture incorporated into the liposome and the sonication time had no significant effect on the total phenolic content indicating that the encapsulation of the extract in the liposome shell was unabated by the sonication time. Although 100% of the extract could not be loaded into the liposomes as revealed from a slight decrease in the TPC of the liposomes compared to the extract mixtures. The results are presented in table 4.

**Table 4: Total phenolic content in liposomes**

| Formulation Code | Sonication time (min) | TPC |
|---|---|---|
| EL1 | 5 | 37.64±5.436 |
| | 15 | 37.21±4.938 |
| | 30 | 36.52±4.047 |
| EL2 | 5 | 57.70±3.354 |
| | 15 | 58.10±5.230 |
| | 30 | 58.15±6.307 |
| EL3 | 5 | 43.44±5.416 |
| | 15 | 41.2±4.612 |
| | 30 | 42.21±3.633 |

*Values are expressed as mean ± SD of three replicate analysis*

### 3.3. Stability of liposomes

The change in particle size over a period of three months was considered to ascertain the stability of the liposomal formulation. The stability was assessed for the optimized liposomal formulation (formed by 5 min of sonication) by storing them in three different batches for stability monitoring. The results reveal that the liposomes were stable at both the temperature conditions and the particle size of the liposomes did not change considerably over the period of three months. Fig. 1 presents a comparative representation of the change in particle size of the extract loaded liposomes stored for stability monitoring.

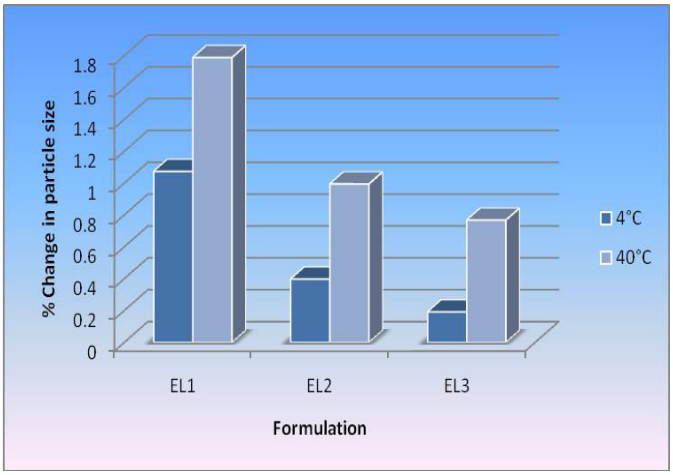


**Fig. 1: Change in particle size on storage**

### 3.4. Evaluation of blends

All the liposomes obtained from 5 min of sonication were mixed with excipients, blended and subjected to preformulation testing of the blends in order to ascertain their suitability for filling in capsules. The bulk and tapped density, angle of repose, Hausner's ratio and Carr's Index are used to determine the flow properties of the blends.

**Table 5: Prefill Parameters of the blends**

| Formulation Code | Bulk density ($g/cm^3$) | Tap density ($g/cm^3$) | Angle of repose (degree) | Carr's Index (%) | Hausner's Ratio |
|---|---|---|---|---|---|
| ELB1 | 0.315 | 0.391 | 27°89' | 19.44 | 1.24 |
| ELB2 | 0.346 | 0.428 | 29°03' | 19.16 | 1.24 |
| ELB3 | 0.361 | 0.45 | 30°01' | 19.78 | 1.25 |

*ELB- Extract loaded liposomal blend*

### 3.5. Evaluation of capsules

The capsules were evaluated for weight variation by weighing individual capsules and determining the deviation of each capsule from the average weight of 20 capsules and for disintegration time using tablet disintegration test apparatus (Table 6).

The time for disintegration of the capsules was found to be ranging from 3.38-3.65 min and the weight variation

of capsules was between 4.1-4.6 %, well within the specifications of Indian pharmacopoeia [18].

**Table 6: Evaluation parameters of polyherbal capsules**

| Formulation Code | Average Weight variation (%) | Disintegration time (min) |
|---|---|---|
| ELC1 | 4.1 | 3.4±0.309 |
| ELC2 | 4.9 | 3.65±0.327 |
| ELC3 | 4.6 | 3.38±0.365 |

*ELC- Extract loaded liposomal capsule; * Mean ± SD of 6 capsules*

## 4. CONCLUSION

Liposomes in oral drug delivery have been an investigated widely over the recent years whereas herbal drugs have been used since ages for the treatment of otherwise unmanageable ailments. The present investigation was undertaken with an objective to optimize a polyherbal formulation for the management of inflammation. Ethanolic extracts of *Mangifera indica* and *Phyllanthus emblica* were mixed in various ratios and developed as liposomes which were later incorporated into capsule dosage forms for oral delivery. The approach was found to be quite promising as the total phenolic content in the liposomes was found to be significantly at par with that of the extract mixtures indicating a good incorporation of the extracts in the liposomal shell. In future the *in vivo* assessment of the anti inflammatory action of the capsules would be undertaken to prove the efficacy of the liposome filled capsules.

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### *Conflict of interest*

The authors declare no conflict of interests

## 6. REFERENCES

1. Ghasemian M, Owlia S, Owlia MB. *Advances Pharmacol Sci.,* 2016; **41:**56-70.
2. Uttara B, Singh AV, Zamboni P, Mahajan RT. *Current Neuropharmacol*., 2009; **7:**65-74.
3. Marienfeld R, Neumann M, Chuvpilo S, Escher C, Kneitz B, Avots A, et al. *European Journal of Immunology,* 1997; **27:**1601-1609.
4. Borrelli F, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, Battista G, Orlando P, Di Marzo V, Izzo AA. *British Journal of Pharmacology,* 2015; **172:**142-158.
5. Leelaprakash G, Mohan Dass S. *International Journal of Drug Development & Research,* 2011; **3:**189-196.
6. Kohli K, Ali J, Ansari MJ, Raheman Z. *Indian Journal of Pharmacology,* 2005; **37:**141-147.
7. Achoui M, Appleton D, Abdulla MA, Awang K, Mohd MA, et al. *PLoS ONE,* 2010; **5(12):**1371-1380.
8. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. Oxford University Press, USA, 2015.
9. Ehrlich SD. Herbal medicine. [Last accessed on 2019 November 1]. Available from: http://www.umm.edu/health/medical/altmed/treatment/herbal-medicine, 2011.
10. What is Ayurveda. [Last accessed on 2019 November 1]. Available from: http://www.ashtangayurveda.com/pages/What-is-Ayurveda.asp, 2008.
11. Oreagba IA, Oshikoya KA, Amachree M. *BMC Complementary and Alternative Medicine,* 2011; **11:**117.
12. Kamboj VP. *Current Science,* 2000; **78:**35-51.
13. Bunaciu AA, Aboul-Enein HY, Fleschin S. *Appl Spectro Rev,* 2011; **46:**251-260.
14. Kumar PP, Pillai S, Michael M, Kavya K, Kumar P, Iqbal SS, et al. *J Med Sci-Tech,* 2015; **4:**55-57.
15. Sahira Banu K, Cathrine L. *International Journal of Advances Research in Chemical Sciences,* 2015; **2(4):** 25-32.
16. Chanthasri W, Puangkeaw N, Kunworarath N, Jaisamut P, Limsuwan S, Maneenoon K, Choochana P, Chusri S. *BMC Complementary and Alternative Medicine*, 2018; **18:**73.
17. Azmi AAJA, Pandey GK, Dubey BK. *Journal of Pharmacology and Biomedicine,* 2021; **5(1):**249-258.
18. Indian Pharmacopoeia. Controller Indian Pharmacopoeia Commission, New Delhi 1985.