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Effect of mole ratio on physicochemical properties of luteolin-loaded phytosome

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Abstract
Phytosome is a novel drug delivery system, containing phytoconstituents and phospholipids complex that enhance oral absorption dan bioavailability of phytoconstituents or herbal extracts. In this study, we investigate the effects of mole ratio luteolin to phosphatidylcholine on physicochemical properties of luteolin-loaded phytosome. Luteolin-loaded phytosomes (LLPs) were prepared in five different ratios by thin film hydration method. The resulting LLPs were then characterized using particle size analyzer (PSA), transmission electron microscopy (TEM), and fourier transforms infrared spectroscopy (FTIR). The LLPs that produced with mole ratio 1:1 luteolin to phosphatidylcholine gave better physicochemical properties such as particle size, PDI values and zeta potential as well as entrapment efficiency and luteolin content, compared to those of other ratios. Hence, the optimum ratio of phytoconstituents to phospholipids in phytosome preparation is 1:1.

Keywords: Luteolin, mole ratio, physicochemical properties, phytosome

Introduction
In 1989, Indena, an Italian pharmaceutical and nutraceutical company, have developed phospholipid complexation technique and patented the technology with the name PHYTOSOME®. The complex was form by chemically reacting polyphenolic plant actives with phospholipids containing phosphatidylcholine (PC) [1]. Luteolin is one of polyphenolic plant active that has enormous bioactivities but showing poor oral bioavalability. In our previous work, we used this phospholipid complex technique to develop luteolin-loaded phytosome that improve absorption of luteolin in the gastro-intestinal tract [2].
The objective of this study was to investigate the effects of mole ratio luteolin to phosphatidylcholine on physicochemical properties of luteolin-loaded phytosome.

Materials and Methods
Materials
All chemical reagents were of analytical grade and used without further purification unless otherwise stated. Luteolin (purity grade = 98% HPLC) was purchased from Acetar Biotec (Cina); *L-α-phosphatidilcholine* (soybean phosphatidilcholine, P 3644) was purchased from Sigma (United State of America); Methanol (LiChrosolv®), dimethylsulfoxide (DMSO), acetic acid glacial, and dichloromethane were purchased from Merck (Germany).

Preparation of LLP (Luteolin-Loaded Phytosome)
The LLPs were prepared by thin film hydration methods [2, 3]. LLPs were prepared in five different ratios of luteolin to phosphatidilcholine as shown in Table 1. Briefly, luteolin were dissolved in 70 mL of methanol, while soybean phosphatidilcholine were dissolved in 20 mL dichloromethane. The solution of luteolin in methanol and phosphatidilcholine solution in dichloromethane were transferred to a glass round bottom flask and refluxed (4h, 60 ^{o}C) resulting a clear yellow solution. Solvents then removed by vacuum evaporator (Rotavapor® R II, Buchi, Germany) at 337 mbar, 40^{o}C, to produce the thin film which was hydrated with 200 mL of distilled water (Figure 1).

Preparation of Standard Curve of Luteolin
1 mg of luteolin was accurately weighed and dissolved in 1 mL of methanol to give a concentration of 1 mg/mL solution. 1 mL of the above solution was pipette out into a 50 mL volumetric flask and added by distilled water to give a concentration of 20 µg/mL stock

solution. From the stock solution, dilutions of standard concentration range of 2-20 µg/mL were prepared with 10 mL methanol in 10 mL volumetric flask. Absorbance of each solution was measured at 350 nm taking methanol as blank using UV visible spectrophotometer (UV 1700, Shimadzu, Japan). Linearity test was applied to authenticate the standard curve (Figure 2).

Determination luteolin entrapment efficiency (%EE) in phytosome complex

The proportion of encapsulated luteolin was determined by centrifuging a certain volume of LLPs at 9000 rpm for 9 x 30 minutes at room temperature. The LLP complex was separated from supernatant and dissolved in methanol. The absorbance of resulting solution was measured by UV-visible spectroscopy (350 nm). The percentage entrapment efficiency (%EE) was calculated by following formula:

$$EE\ (\%) = \frac{loaded\ luteolin\ (mg)}{added\ luteolin\ (mg)} x\ 100\%$$

Determination of luteolin content in phytosome complex

25 mg of LLPs was dissolved in 25 mL of methanol and the absorbance of resulting solution was measured by UV-visible spectroscopy (350 nm). Luteolin content in LLPs was calculated based on standard curve of luteolin.

Physicochemical characterization of LLP

The average particle size, polidispersity index (PDI) and zeta potential of the luteolin-loaded phytosomes (LLPs) were determined by DLS using Malvern Zetasizer (Malvern Instruments, UK). The 2D surface morphology of the LLPs was visualized by TEM (JEM 2100, Japan). FTIR spectroscopy was used to declare the attachment of luteolin and phosphatidilcholine in pytosome complex formation.

Results and Discussion

Preparation and Characterization of Luteolin-Loaded Phytosome (LLP)

We prepared LLPs in five different ratios of luteolin to phosphatidylcholine (PC) as shown in Table 1. All formulations appeared in white-pale yellow color. In phytosome preparation, obtaining a clear solution of drug and PC in the reaction complex is a prerequisite [2]. Dichloromethane was chosen for dissolving PC, but luteolin is insoluble in this mixture. Luteolin is soluble in methanol. Dichloromethane and methanol are miscible with each other at any volume. Thus, luteolin and PC were dissolved separately in methanol and dichloromethane respectively and the two solutions were mixed and refluxed (Figure 1). Percentage of entrapment efficiency and luteolin content in LLPs were shown in Table 1.

Table 1: Mole ratio, entrapment efficiency, and luteolin content of LLP

Formula	Luteolin (mg)	Phosphatidylcholine (mg)	Mole Ratio	Entrapment Efficiency (%)	Luteolin Content in 25 mg LLP (%)
F1	60	60	3 : 1	92.62	45.85
F2	60	120	2 : 1,5	82.66	26.85
F3	60	180	1 : 1	91.12	22.22
F4	60	240	1 : 1,5	80.58	16.12
F5	60	360	1 : 2	83.17	10.34

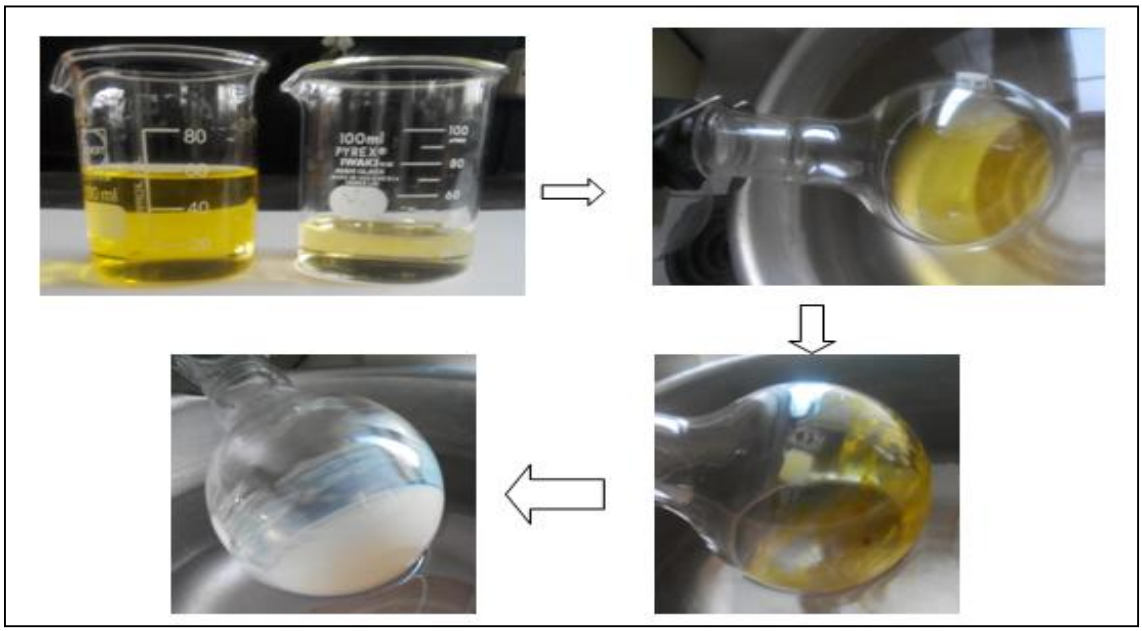


Fig 1: Preparation of luteolin-loaded phytosome

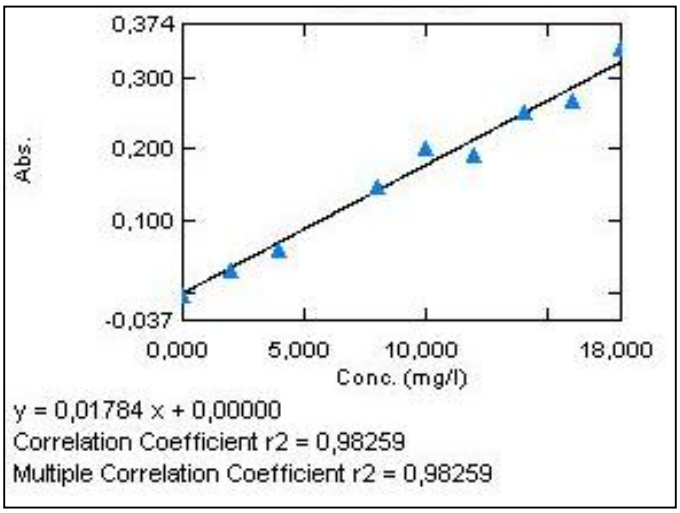


Fig 2: Luteolin standard curve

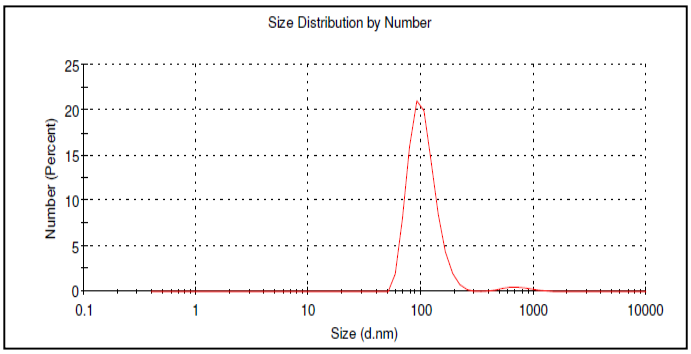
Entrapment efficiency (%EE) is an important physicochemical property in nanovesicles preparation. It shows percentage of drug entrapped in complex (phytosome) against the total drug added. The five LLPs formula showed different EE values due to different mole ratio luteolin to phosphatidylcholine. LLPs with higher ratio gave higher %EE values. This showed that the more number of luteolin molecules added the better entrapment efficiency [4]. From five formulas that has been prepared, only two formulas (F1 and F3) that have %EE values more than 90%. This result is close to the theory which states that the optimum mole ratio of luteolin-phosphatidylcholine in the preparation of phytosomes is 1: 1 [5].

Particle size, PDI value and zeta potential of LLPs were summarized in Table 2. Particle size has a major impact on the fate of a nanoparticle system as well as a size distribution. Additionally, monodisperse size distribution is essential for excellent physical stability. Figure 3 shown average particle size (as average diameter number, Av Dn) of luteolin-loaded phytosome varied from 100 – 1000 nm. From data shown in Table 2, it can be hypothesized that mole ratio luteolin to phosphatidylcholine has effect on particle size of phytosome. Phytosome with higher mole ratio (F1-F3) has smaller particle size than those with lower mole ratio (F5-F6). This can be happens since phytosomes with higher content of phosphatidylcholine tend to form aggregates [4]. However, all PDI values show wide distribution patterns.

The zeta potential values observed for the LLPs was significantly lower than -30 mV (Table 2). The high zeta potential of the produced phytosomes may be explained in that a great portion of soy phosphatidylcholine would be negatively charged in water environment with neutral pH value. Thus LLPs with higher content of phosphatidylcholine (F4 and F5) will have higher zeta potential values. This negatively charged vesicles make phytosomes dynamically stable and suggesting the potential physical stability of drug in the phytosomes in the aqueous state and optimum for drug delivery system [6-8].

Table 2: Particle size, PDI values, and zeta potential of LLPs

Formula	Average Dn (d.nm)	Polidispersity Index (PDI)	Zeta Potential (mV)
F1	122.72	0.697	-35,0
F2	108.63	0.777	-32,7
F3	105.34	0.735	-34,4
F4	1121.83	0.772	-41,4
F5	1066.18	0.428	-44,7



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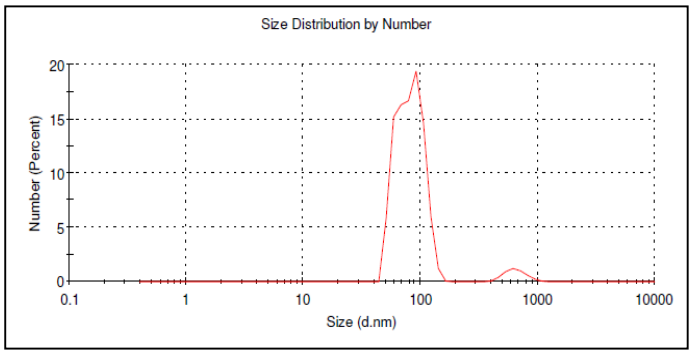

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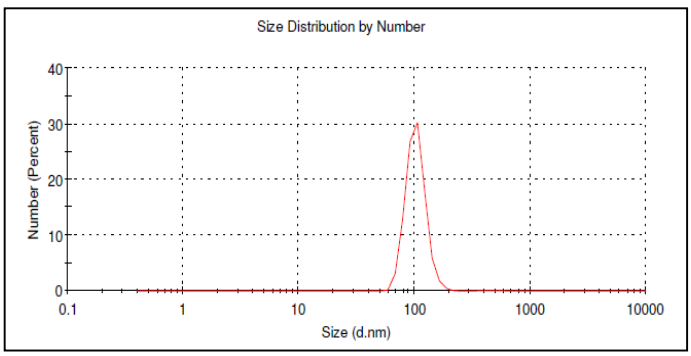

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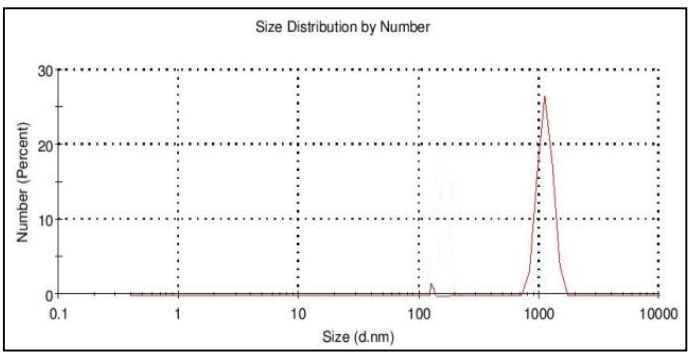

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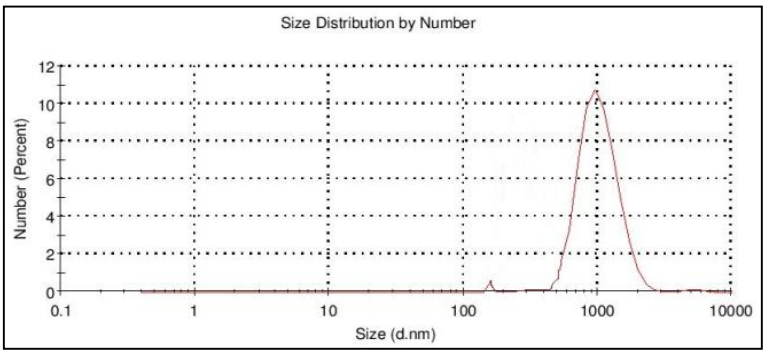
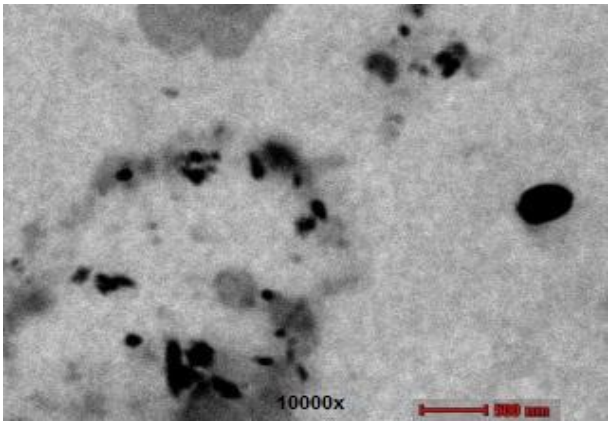


(E)

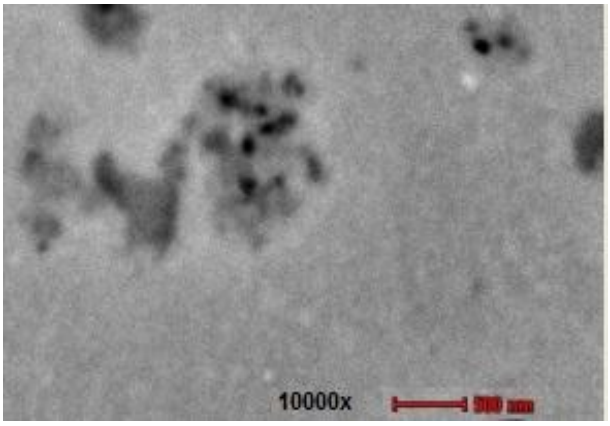
Fig 3: Particle size distribution of LLPs (A) F1, (B) F2, (C) F3, (D) F4, (E) F5

TEM images (see Figure 4A – 4E) of the luteolin-loaded phytosomes exhibited mostly the shape of a liposome-like vesicle, spheric particles, fairly uniform size, and well-distributed character dispersed in water environment. In our work, soy phosphatidylcholine (SPC) was used as the material for the delivery vehicle, and after complexing with luteolin, the polar groups in the head of SPC molecules were effectively combine with luteolin in the form of probably a hydrogen bond, obtaining a molecularly well-defined delivery vehicle. The explanation is further confirmed by FTIR spectra which will be discussed below.

The formation of the luteolin-loaded phytosome complex can be confirmed by FTIR spectroscopy comparing the spectrum of the complex with the individual components (luteolin and phosphatidylcholine) as seen in Figure 5-7. There was significant difference between the complex and each of its individual components. The spectrum of luteolin phytosome complex showed a new peak at 1360 cm^{-1}, which did not appear in luteolin and phosphatidylcholine spectra. The spectrum also showed a decreased intensity in the peak at 1730 cm^{-1}. These results suggested that hydrogen-bonding between luteolin and phosphatidylcholine play a leading role during the formation of the phytosome complex, since hydrogen-bonding will caused significant differences such as wave number shifts or intensity changes of the vibration band of functional group involved in interaction [9]. The differences were appeared at N-O band regions (1550-1290 cm^{-1}) and P-O band regions (1700-1800 cm^{-1}). These data also suggested that hydrogen-bonding occurs between polar region of luteolin and cholin moeiety of phosphatidylcholine. Thus the interaction of luteolin-phosphatidylcholine in phytosome complex can be suggested as shown at Figure 8.



(A)



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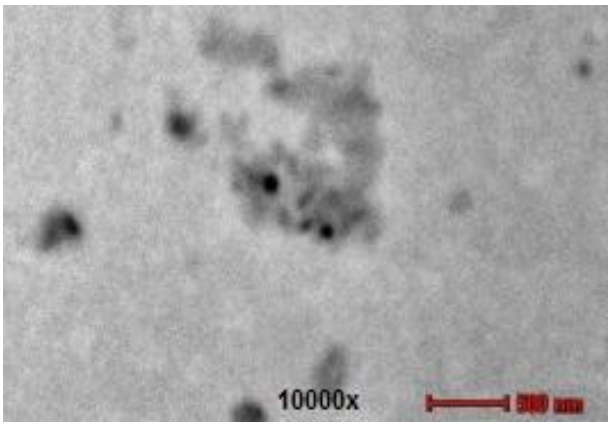

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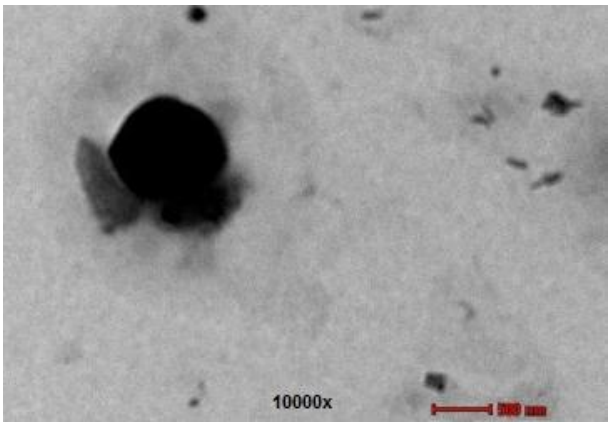

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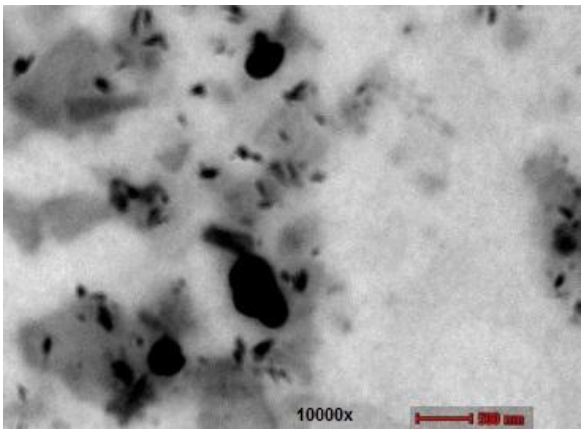

(E)

Fig 4: Morphology of LLPs

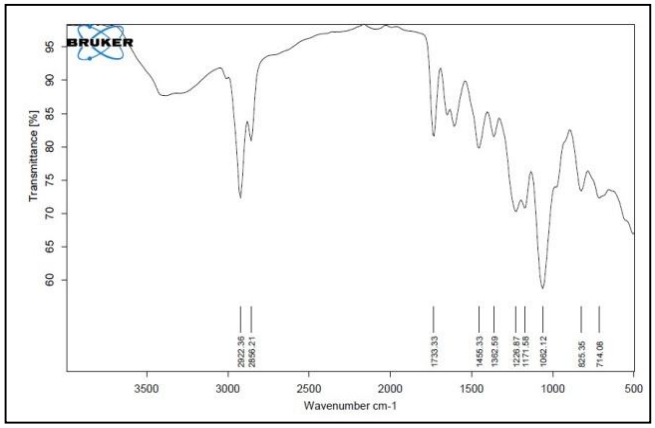

Fig 5: FTIR spectrum of LLP

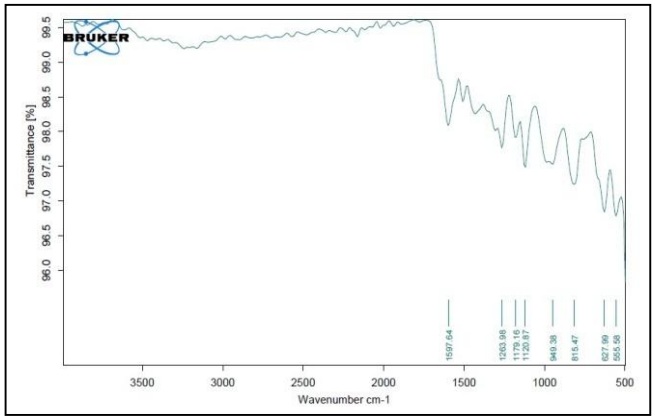

Fig 6: FTIR spectrum of luteolin

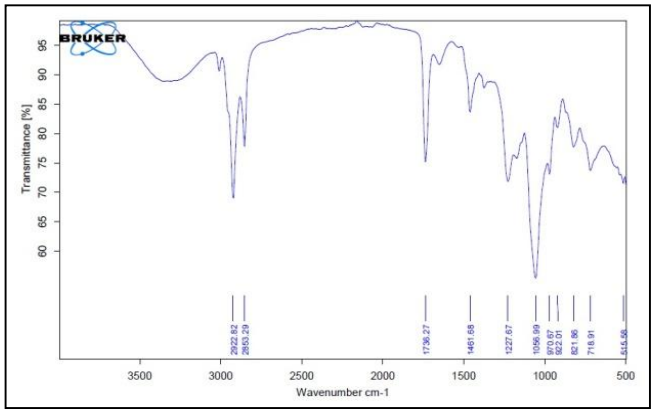

Fig 7: FTIR spectrum of phosphatidylcholine

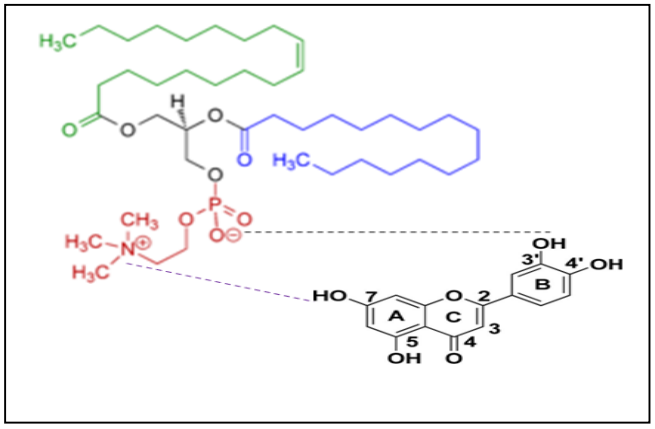

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Fig 8: Interaction between luteolin and phosphatidylcholine in LLP

Conclusion

Mole ratio luteolin to phosphatidylcholine has effect on physicochemical properties of luteolin-loaded phytosome, especially on entrapment efficiency and particle size. LLPs with higher mole ratio have better entrapment efficiency and smaller particle size. Our results showed the optimum mole ratio luteolin to phosphatidylcholine to produce LLPs was 1:1.

Acknowledgements

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References

1. Gnananath K, Nataraj KS, Rao BG. Phospholipid Complex Technique for Superior Bioavailability of Phytoconstituents. Adv Pharm Bull. 2017; 7(1):35-42.
2. Hindarto CK, Surini S, Saputri FC, Irawan C. *in vivo* Evaluation of Luteolin-Loaded Phytosome. The Pharma Innovation Journal. 2017; 6(11):347-349.
3. Sabzichi M, Hamishehkar H, Ramezani F, Sharifi S, Tabasinezhad M, Pirouzpanah M *et al*. Luteolin-loaded Phytosomes Sensitize Human Breast Carcinoma MDA-MB 231 Cells to Doxorubicin by Suppressing Nrf2 Mediated Signalling. Asian Pac J Cancer Prev. 2014; 15(13):5311-5316.
4. Singh RP, Narke R. Preparation and Evaluation Of Phytosome Of Lawsone. IJPSR. 2015; 6(12):5217-5226.
5. Kalita B, Das MK, Sharma AK. Novel Phytosome Formulations in Making Herbal Extracts More Effective.

Research J. Pharma. and Tech. 2013; 6(11):1295-1301.
6. Dang H, Hasan M, Meng W, Zhao H, Iqbal J, Dai R *et al*. Luteolin-loaded solid lipid nanoparticles synthesis, characterization, & improvement of bioavailability, pharmacokinetics *in vitro* and vivo studies. J Nanopart Res 2014; 16:2347.
7. Hou Z, Li Y, Huang Y, Zhou C, Lin J, Wang Y *et al*. Phytosomes Loaded with Mitomycin C-Soybean Phosphatidylcholine Complex Developed for Drug Delivery. Mol. Pharmaceutics. 2013; 10:90-101.
8. Tan Q, Liu S, Chen X, Wu M, Wang H, Yin H *et al*. Design and Evaluation of a Novel Evodiamine-Phospholipid Complex for Improved Oral Bioavailability. AAPS Pharm Sci Tech. 2012; 13(2):534-547.
9. Fornaro T, Burini D, Biczysko M, dan Barone V. Hydrogen-Bonding Effects on Infrared Spectra from Anharmonic Computations: Uracil–Water Complexes and Uracil Dimers. J Phys. Chem. A. 2015; 119(18):4224-4236.