Preparation of Drug Carrier from Glycosides and Polymer: Determine the Encapsulation Efficiency

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***Abstract*—Glycosides-polymer as drug carrier is used widely in pharmaceutical industry because of its pharmacological action and therapeutic effect. The drug carrier was formulated using thin film approach and the properties of the vesicle which the surface charge, size and encapsulation efficiency was studied using dynamic light scattering and fluorescence spectroscopy. Glucoside and Galactoside vesicles is formulated with the volume ratio of 1:0.4 to DCP with the effect of the presence of cholesterol to the vesicle stability and encapsulation efficiency of drug methylene blue (MB) was determined. The findings show the presence of cholesterol in the glucoside vesicle produce the highest encapsulation efficiency at 1.73% and the lowest at 1.14% for galactoside vesicle with the cholesterol absent. The loading efficiency is find to be low for all formulation at no more than 3.7%.**

Keywords*— *encapsulation efficiency, drug carrier, vesicles, glycosides, cholesterol, loading efficiency, polymer, colloid

I. INTRODUCTION

Liposomes is widely used as the drug carrier [1], however there is a resurgence in recent times in study of niosomes as the drug carrier that uses non-ionic surfactants. Niosomes is a versatile drug carrier where it accommodate both hydrophilic and hydrophobic drugs. There are various types of niosomes such as multi-lamellar vesicles (mlv), large uni-lamellar vesicles (luv) and small uni-lamellar vesicles (suv) [2]. The self-assembly vesicles are formed from surfactants molecules such as glycosides to encapsulate the drugs. The vesicles that are formed is from surfactants that is renewable, biodegradable and has low inherent toxicity and immunogenicity which is alkyl glycosides [5]. Studies also proven noisome as drug carrier can enhance drug delivery to reach the skin's impermeable barrier.

The development of the glycosides based drug carrier is the topic of many extensive studies that demonstrated the ability of glycosides as the drug carrier. Glycosides is used widely in pharmaceutical industry because of its pharmacological action. For example, cardiac glycosides which the effectiveness depends on the aglycone and the sugar that attached increase the fixation of the glycoside to the heart muscle [5].

It is reported glycosides-polymer drug carrier is more efficient on a few specific target organ such as liver, lung and kidney. Alkyl glycosides from palm kernel oil (PKO) was obtained with the disaccharides used are glucose and galactose. The polymer in the polymeric vesicles used are *p*-ethylene-glycol (PEG) that enhances the physical and chemical stability of drugs that is administered *in-vivo*. PEG is good for biological applications because of its low intrinsic toxicity, aside from soluble in water.

Encapsulation efficiency has always been considered to be a major factor during pharmaceutical study on the new drug in order to have a drug carrier that is not only working, but that does not harm the body that the drugs is administered on. This paper focuses on the encapsulation efficiency for drugs that is synthesized from glycosides and polymer.

In this research, one of the pharmacokinetics properties of drug carrier which is encapsulation efficiency is investigated. Vesicular formulation of PKO based glycosides was prepared to study the encapsulation efficiency as the drug carrier. The vesicle was prepared using thin film hydration method [6]. Comparison of encapsulation efficiency with the presence of cholesterol during formulation is also studied.

II. METHODOLOGY

A. Vesicle Preparation

Polymeric glycoside vesicle which consisted glycosides, Dicetyl Phosphate (DCP), *p*-ethylene-glycol (PEG120) with the introduction of cholesterol was investigated. The glycosides and *p*-ethylene-glycol was dissolved in methanol: chloroform with ratio (4:1, v/v), with DCP was dissolved in tetrahydrofuran (THF) and cholesterol was dissolved in chloroform. Dicetyl phosphate was introduced to minimize the aggregation between uncharged particles by inducing negative charge. The volume ratio of glycosides and DCP was set at 1:0.4. Rotary evaporation was used at temperature 55 ^{0}C to remove the organic solvents which a thin film can be observed at the bottom of round-bottom flask. Evaporation process was further continued for another 15 minutes to ensure complete removal of the organic solvents. The thin film was hydrated by Phosphate Buferr Saline (PBS) (pH = 7.4) with the introduction of 80 µM methylene blue at 70 ^{0}C in water bath for half an hour. The vesicle formed is the sonicated to get small uni-lamellar vehicle (SUV) using ultrasonicator machine for 10 minutes (30 sec on, 10 sec on) at 150 W. The formulation of the vesicle is shown in table below.

Vesicle	GPKO (4.4×10^{-2} M)	GalPKO (4.4×10^{-2})	PEG12 (4.00×10^{-3})	DCP (2.4×10^{-3})	Cholestrol (3.2×10^{-3})	Ratio (v/v)
GPKO w/o cholesterol	2.3 ml	N/A	0.5 ml	0.9 ml	N/A	1:0.2:0.4
GalPKO w/o cholesterol	N/A	2.3 ml	0.5 ml	0.9 ml	0.9 ml	1:0.2:0.4:0.4
GPKO with cholesterol	2.3 ml	N/A	0.5 ml	0.9 ml	N/A	1:0.2:0.4
GalPKO with cholesterol	N/A	2.3 ml	0.5 ml	0.9 ml	0.9 ml	1:0.2:0.4:0.4

Table 1: *Formulation component of glycoside vesicle*

B. Zeta potential and size

Malvern ZEN 3600 Nano Series apparatus is used to determine the average size of the vesicle, zeta potential and polydispersity index (PDI) via quasielastic laser light scattering where the measurements temperature is maintained at 25 ^{0}C.

C. Encapsulation and loading efficiency

The free MB is separated from the vesicle using Gel Permeation Chromatography (GPC) with Sephadex G-50 spin columns. The vesicles of encapsulated MB were disrupted using methanol with ratio (1:1, v/v) to extract the methylene blue and being analyzed by fluorescence detector. Different concentrations of MB in PBS were used to construct the calibration curve.

Encapsulation efficiency is calculated using eqn. 1:

$$\text{Encapsulation efficiency, \%} = \frac{\textit{Encapsulated Methylene blue}}{\textit{Total Methylene blue}} \text{ X } 100 \quad \textbf{(1)}$$

Loading efficiency of the drug can also be determined from the ratio of volume of the MB in vesicle to the mass of vesicles. (Eqn. 2):

$$\text{Loading efficiency, \%} = \frac{\textit{volume}_{mb}\ \textit{in vesicle}}{\textit{volume of vesicle}} \text{ X } 100 \quad \textbf{(2)}$$

III. RESULTS AND DISCUSSION

A. Zeta potential

The stability of the colloid was indicated by zeta potential and since the particle is small unilamellar vesicle, high zeta potential indicate stability. Zeta potential observed for the drug carrier was on the negative side (< -30), GPKO with and without cholesterol formulation showed zeta potential of -28.9±17 mV and -23.27±13 mV respectively, for GalPKO with and without cholesterol, the zeta potential was -25.83±1 mV and -26.52±1 mV indicating that the colloid is stable but has slight tendency to be aggregated. The negative value could be contributed by the use of anionic surfactants. It is generally accepted that high zeta potential, $\zeta > -30$ mV is electrically stable.

B. Drug encapsulation and loading efficiency

Both hydrophilic and hydrophobic drugs is widely used in pharmaceutical industry. The drug is prepared with the aim to have high encapsulation efficiency, low in toxicity, release at target site and in general high therapeutic effect.

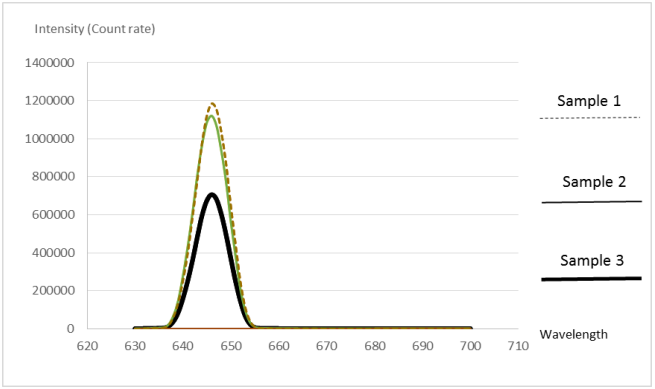
The performance of the drug carrier vesicle can be determined through looking at one of its parameter. In this paper the encapsulation efficiency of glycoside surfactant drug vesicle. There are various factors that can influence the encapsulation efficiency of the drug vesicle, in this particular paper the formulation component factor is studied to understand the encapsulation efficiency. MB which is famous in clinical application especially in cancer treatment [3], is encapsulated within the vesicle through passive loading approach.

	Combination of alkyl Glycoside to form vesicle			
	GPKO/DCP/CHL	GPKO/DCP	GalPKO/DCP/CHL	GalPKO/DCP
Preparation method	Thin film hydration			
Self-assembly	Small Unilamellar Vesicle (SUV)			
Size (nm)	173±6	125.1±0	247.5±9	178.2±34

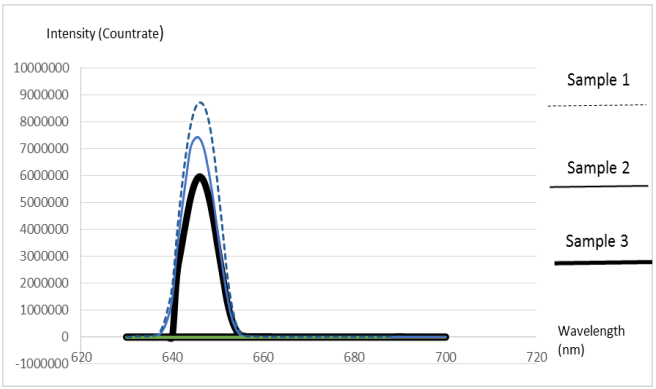
Table 2: *Properties of vesicle formulation*

The concentration of encapsulated MB is measured through its fluorescence intensity and compare it with the calibration curve of $R^2 = 0.9648$ that was created through 4-step dilution of MB in PBS solution. The presence of cholesterol was also studied where cholesterol was reported to be able to improve the encapsulation efficiency of drug vesicle since it reduces the permeability of the vesicle [25]. Apart from cholesterol, dicetyl phosphate was also added into all four formulation to increase the stability of the vesicle and prevent aggregation.

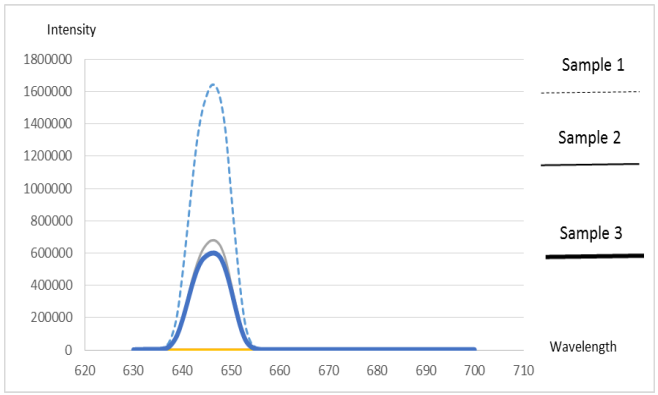
The fluorescence detectors was used to know the intensity of MB in the sample. The result of fluorescence detector is shown in figure 1.



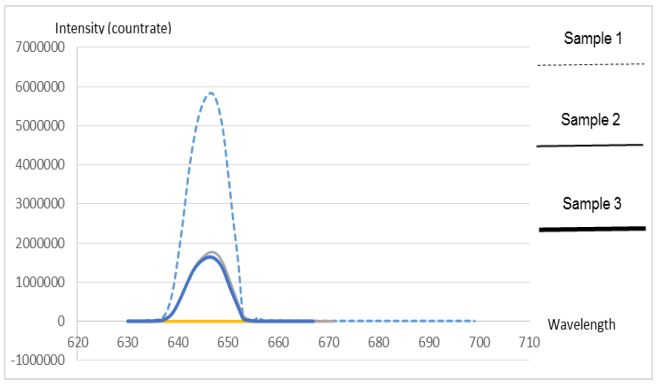
(a)



(b)



(c)



(d)

Figure 1: *The fluorescence intensity reading (a) C8G/DCP (b) C8Gal/DCP (c) C8Gal/DCP/CHL (d) C8G/DCP/CHL*

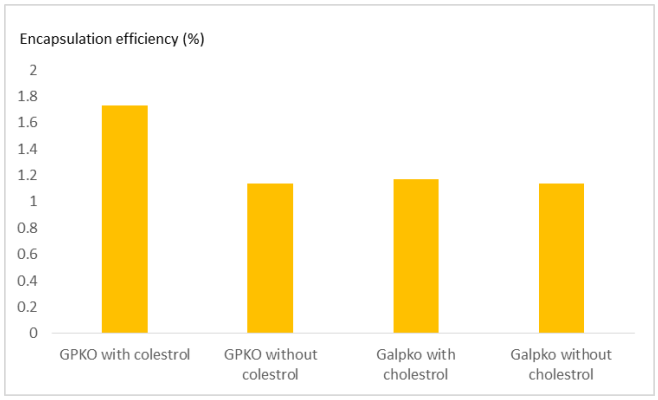


Figure 2: *Encapsulation efficiency based on sample*

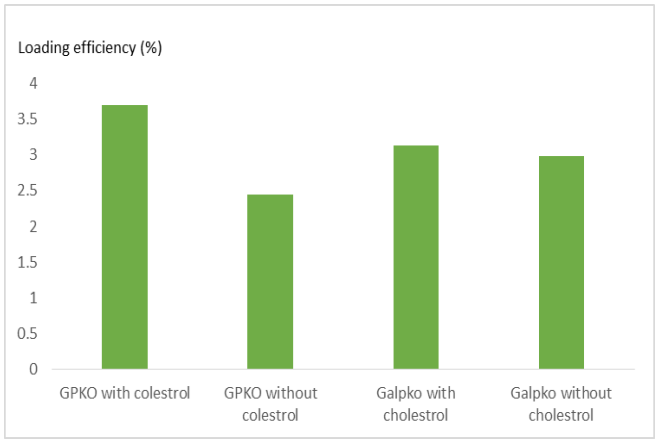


Figure 3: *Loading efficiency based on sample*

To calculate the encapsulation efficiency, reading is taken at peak of the wavelength range set at the calibration curve which is 678-685 nm

The encapsulation efficiency in both GPKO and GaPKOl reportedly higher with the introduction of cholesterol. The encapsulated MB for each of the formulations is as follow (GPKO with cholesterol: 52.3 µM, GPKO without cholesterol: 34.53 µM, GalPKO with cholesterol: 35.496 µM, GalPKO without cholesterol: 34.452 µM).

The formulation of GPKO/DCP/CHL is the highest at 1.73 % EE which can be considered high. The lowest encapsulation of MB is the vesicle with formulation GalPKO/DCP at 1.14 % EE, this can be contributed due to the fact that GalPKO is a hydrophobic surfactant that can cause MB to compete for packing space in the vesicle (ref). The loading efficiency is also low with the highest is the formulation of GPKO/DCP/CHL at 3.7 %. There is significant difference in the loading efficiency in the GPKO/DCP with the presence of cholesterol as compared to the same formulation without cholesterol which confirm the findings from Malinda et.al [3], which reported the increase of the physical stability of the vesicle [4]. For formulation involving the use of surfactant galactoside, loading efficiency do not differ much from the addition of cholesterol which may suggest limited flexibility in the vesicle environment.

IV. Conclusion

The behavior of the vesicle is affected by the formulation of components which results in the varying encapsulation efficiency. The presence of cholesterol show improving stability of the vesicles which in turn increase encapsulation efficiency. Alkyl glucoside and galactoside is formulated to have high encapsulation efficiency with the initial total MB is 3.03 mM. The behavior of hydrophobic surfactant (GalPKO) used in drug carrier is also compared with the hydrophilic (GPKO) with the finding shows that hydrophilic surfactant responds better to the formulation in which the encapsulation efficiency is higher than that of GalPKO. The formulation of GPKO (C8G/DCP/CHL) with adding the cholesterol achieved the highest encapsulation efficiency.

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