ORIGINAL ARTICLE

Characterization of Purified Coconut Oil Bodies as an Encapsulating Agent for Doxorubicin and Paclitaxel

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ABSTRACT

Introduction. Doxorubicin (DOX) and paclitaxel (PTX) are both widely used anticancer drugs with a broad spectrum of antitumor activity, commonly against breast, ovarian, and lung cancers. Currently, these drugs are commercially available in liposomal formulations for their use in chemotherapy. This study generally proposed coconut oil bodies (COB) obtained from *Cocos nucifera* L. as an alternative carrier for DOX and PTX rather than the currently used liposome.

Objectives. This study aimed to compare standard liposome and coconut oil bodies as drug carriers in terms of their microencapsulation efficiencies, lipid profiles, *in vitro* drug release and stability, as well as their cholesterol levels.

Methods. Coconut oil bodies (COB) were isolated and purified from *Cocos nucifera* L. by modified sucrose gradient method followed by microencapsulation of standard drugs (doxorubicin and paclitaxel) through self-assembly and freeze-thaw method. The two standard drugs were encapsulated using COB and standard liposome. Encapsulation efficiency of both materials were determined. Lipid profiles of both encapsulating materials were analyzed by Fourier-transform infrared spectroscopy, gas chromatography-flame ionization detector, and cholesterol level determination. *In vitro* drug release and pH stability of both encapsulated drugs were analyzed.

Results. Doxorubicin (DOX) and paclitaxel (PTX) were successfully incorporated in COB. Lauric acid was mainly abundant in COB and was able to lower cholesterol levels (5 mg/dL). COB incorporated with DOX and PTX showed stability at acidic and neutral pH. Drug release profile showed a rapid outburst within 3 hours compared to liposome encapsulated DOX and PTX.

Conclusion. Our study showed the encouraging potentials of using COB as wall materials that will make them attractive candidates for the formulation of pharmaceuticals for optimized drug delivery of cancer chemotherapeutics DOX and PTX.

Key Words: coconut oil bodies, liposome, microencapsulation, doxorubicin, paclitaxel, Cocos nucifera L

INTRODUCTION

Chemotherapy has been the main modality of treatment for cancer patients. However, challenges such as inadequate accessibility of drugs to the tumor tissue, intolerable toxicity, development of multi-drug resistance, and the dynamic heterogeneous biology of the growing tumors limit the success rate of cancer chemotherapeutics.[1] Among the several types of chemotherapeutic drugs, doxorubicin (Adriamycin) and paclitaxel (Taxol) are two of the first-line treatments used for advanced and metastatic cancers.[2] Doxorubicin (DOX) is an antibiotic derived from *Streptomyces peucetius* and is part of the anthracycline class of chemotherapeutic agents (Figure 1). It is mainly used for the treatment of soft

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tissue and bone sarcomas as well as cancers of the breast, ovary, bladder, and thyroid.[3] Treatment with doxorubicin is accompanied with high risk of cardiotoxicities and other side effects such as myelosuppression, alopecia, severe acute nausea and vomiting, and mucositis.[4] Paclitaxel (PTX) is an effective antineoplastic agent and has a wide spectrum of antitumor activity, particularly against ovarian, breast, and lung cancers. However, similar to doxorubicin, the delivery of paclitaxel is also associated with significant challenges. Being highly lipophilic and lacking ionizable functional groups is the drug's major limitations causing difficulties in formulation as well as clinical problems.[5]

These hindrances limit the therapeutic potential of both drugs in clinical use. An approach to solve such problems is the use of drug carriers. Numerous delivery systems are continuously being explored for further development. Drug delivery systems are means of administering a pharmaceutical compound to achieve therapeutic effect and several studies have already been conducted in the formulation of encapsulated drugs. These include use of liposomes, proliposomes, microspheres, gels, prodrugs, and cyclodextrins.[6]

Microencapsulation is the technology of packaging solid, liquid, or gaseous material within thin polymeric coatings, forming small particles called microcapsules. The coating serves as a protective film to isolate the active substance or the core and allows its release in the ideal place or ideal time. This technology has gained importance in various areas such as pharmaceutics, food, agriculture, and medicine given the value of its use and advantages.[7] As drug delivery systems, these microcapsules give material structuration, protection, controlled release of the encapsulated active agents, and more.[8]

Currently, liposomal preparations are so far the most used method in microencapsulation technology. Currently, both doxorubicin and paclitaxel are already commercially available as liposome-encapsulated drugs – Doxil® and Lipusu®, respectively. Liposomes are self-assembled spherical vesicles with one or more phospholipid bilayers separating the inner aqueous environment from the outer aqueous medium.[9] Liposomes may provide advantages, including biocompatibility, capacity for self-assembly, and the ability to carry large payloads.[10]

Although the clinical use of liposome brought significant advances in the formulation of these drugs, there are limitations which still needs further optimization. In the early stages of development of liposomal formulations, several problems associated with the use of liposomes were encountered such as a rapid reticuloendothelial system (RES) clearance and triggering of the innate immune system.[11] These challenges that come with the use of liposomes still hinder the therapeutic potential of both drugs, doxorubicin and paclitaxel, despite resolving the problems experienced with the drugs alone. Clearly, there are still much needed improvement in the formulation of the liposome as coatings and great effort must still be exerted in looking for an appropriate drug vehicle for both doxorubicin and paclitaxel.

Searching for alternative wall materials that could carry the anticancer drug can be another solution. The use of oil bodies as a stable biological microencapsulant offers a strategy in carrying active substances for application in food or pharmaceutical products.[12] Oil bodies, or oleosomes, are specialized organelles ubiquitously present in plant oil seeds as lipid storage compartments consisting of a hydrophobic core of triacylglycerol (TAGs), with a surrounding monolayer of phospholipids (PLs) where proteins, known as oleosins, with a size ranging from 0.5 to 2 μm are present.[13]

Coconut (*Cocos nucifera L.*) milk present in its plant seed could serve as an important source of oil bodies that may be used as an encapsulating agent for various materials.[14] It also possesses several other benefits such as its antibacterial, antiviral, and anti-inflammatory properties. Also, its ability to reduce cholesterol levels is an advantage over the commercially used liposome.[15]

Hence, coconut oil bodies (COB) which contains triacylglycerol and phospholipids could be a suitable candidate as drug carrier that could perform as well as liposomes. An application of COB as an alternative drug delivery system for anticancer drugs such as doxorubicin and paclitaxel would be better evaluated and characterized in this study.

METHODS

Standards, Reagents, and Chemicals

The chemicals and reagents used were 7 M urea, hexane, bromothymol blue, Folin-Ciocalteu reagent, 0.1 M HCl, 0.1 M NaOH, 10% Na_2CO_3, NaCl, buffer 1 (10 mM sodium phosphate buffer pH 7.4 with 0.4 M sucrose), buffer 2 (5 mM sodium phosphate buffer pH 7.4 with 0.2 M sucrose and 0.1% (v/v) Tween 20), buffer 3 (10 mM sodium phosphate buffer pH 7.4 with 0.25 M sucrose and 2M sodium chloride), and sodium phosphate buffer saline (pH 7.4 and pH 5.0). All reagents used in this experiment were analytical grade and purchased from Sigma-Aldrich, USA.

Isolation and Purification of COB from *Cocos nucifera L.*

Using a sucrose gradient method done in a study by Santiago & Devanadera (2016)[16], COB was isolated from the endosperm of *Cocos nucifera* L obtained from a local public market. Sucrose gradient was used for separating cell organelles, cytosolic or nuclear proteins from crude cellular extracts. Then, in a centrifuge tube, a 1:1 ratio of crude COB and buffer 1 were mixed and subjected to floatation centrifugation using a Heraeus Megafuge 8R centrifuge machine at 4°C, 10,000 x g for 30 minutes. A solid oil pad was collected, transferred to a clean tube, heated, and mixed to homogenize the sample when added to buffer 2. The procedure was repeated on oil pad for four times each with different reagents added accordingly – buffer 3, 7 M urea, 10 mM sodium phosphate buffer (pH 7.4), and hexane. Lastly, COB was washed with 10 mM sodium phosphate buffer

(pH 7.4) to remove any potentially toxic compounds such as the organic reagents used in the sample. Purity of COB was determined under microscope, wherein, the protein oleosin was clearly removed (bright field microscope) and the layer of oil body had become thinner (fluorescence microscope). The purified COB was stored at 4°C until further use.

Microencapsulation of COB and Liposome

Oil Bodies Encapsulation

An 800uL COB was sonicated for 10 minutes for complete disassembly into smaller particles or globules.[16] In a separate tube, DOX (doxorubicin HCl, Adriamycin®, West-Ward Pharmaceuticals, USA) with 2mg/mL dissolved in NSS solution and PTX (paclitaxel, Taxol®, Bristol-Myers Squibb, USA), 800 µL was mixed with COB and stored at 4°C for 10 minutes followed by thawing at room temperature. The freeze - thawing procedure was repeated thrice, after which the solution was left at room temperature for 1 hour to allow self-assembly. The solution was centrifuged at 10,000 x g, 4°C, for 30 minutes. After centrifugation, the liquid supernatant was discarded and the oil pad containing the encapsulated DOX and PTX was collected. Samples were then lyophilized. After freeze-drying for 1 day, samples were stored at -20°C freezer until further use.[16]

Liposome Encapsulation

Procedures on rehydrating and encapsulation using Liposome (Liposome kit AR grade, Sigma-Aldrich, USA) was done by mixing 0.85 mL distilled water with liposome for 30 secs. A 0.15 mL of 1 mg/mL DOX or 6 mg/mL PTX (separate from one another) was added and mixed, resulting into a homogenous milky suspension. Samples were then lyophilized. After freeze-drying for 1 day, samples were stored at -20°C refrigerator until further use.[16]

Determination of Encapsulation Efficiency (%)

A subsequent weighing was done with the following: empty tube, a tube with COB or liposome, and a tube with the encapsulated drug. COB-based drugs were centrifuged for 10 000 x g, 30 minutes at 4°C, forming an oil pad which was collected and transferred to a new weighed empty tube while the addition of liposome, drugs (DOX & PTX) was done for liposome-encapsulated drugs. To compute for the percentage of encapsulation efficiency of the wall materials, we used the formula: EE% = (weight of drug in the capsule / weight of drug introduced) x 100.

Lipid Profiling of Samples

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

FTIR for drug-loaded COB and liposome was obtained in the range of 4,000 to 400 cm^{-1} with IRAffinity-1S FTIR Spectrometer (Shimadzu) to identify the functional groups of the samples and to determine the successful incorporation of the drugs into the coconut oil bodies.

Gas Chromatography – Flame Ionization Detector (GC-FID) Analysis

COB sample was submitted and analyzed using a Dani Master Fast GC in SentroTek to determine the fatty acid content. Samples were filtered into a sample vial using Whatman No. 1 and were placed on an autosampler injector. The injector was set at 250°C and the interface temperature at 270°C while the column was set at 50°C to 150°C with 15°C/minute temperature increments and 150°C to 250°C with 3°C/minute temperature increments. The column used Capillary GC column, SP-2560, Sigma-Aldrich. The gas pump was set at 38.0 cm/minute using hydrogen gas. Sulpeco 37 component fatty acid methyl esters (FAME) Mix (Sigma-Aldrich) was used as the FAME standard for GC analysis of the samples. The identity of the fatty acids was based on the peaks of samples related to the peaks of standards fatty acids in FAME mixture in the gas chromatograph. The quantitative data (percent fatty acid composition) were based on the peak area of the sample divided by the total peak area multiplied by 100.

pH Stability Studies

COB DOX, COB PTX, Lipo DOX, and Lipo PTX were suspended in a PBS buffer (pH 7.4 and pH 5.0) for 30 days. Both initial and final absorbance of the samples were read in triplicates at 480nm (DOX) and 227nm (PTX) using a UV-VIS spectrophotometer.[16]

In Vitro Drug Release Test

Approximately 20 mg and 60 mg of DOX and PTX were weighed respectively, and each suspended separately in a 10mL PBS buffer (pH 5.0 and 7.4 for both drugs) incubated at a constant temperature of 37°C±1°C. At the pre-determined time interval (per hour for 5 hours and after 20 hours incubation), 200 uL aliquots were withdrawn from the dissolution medium and replaced with fresh PBS buffer to maintain constant volume and sink conditions. Samples were then analyzed at 480nm (DOX) and 227nm (PTX) using Multiskan GO UV-Vis spectrophotometer. All measurements were carried out three times. To calculate each sample concentration, DOX standard curve was constructed by serially diluting 25 uL of 2 mg/mL DOX stock solution in a 50 uL PBS buffer (pH 7.4) with a dilution factor of 1:2. A PTX standard curve was also constructed by serially diluting 12.5 µL of 6 mg/mL PTX stock solution in a 15uL PBS buffer (pH 7.4), and a 10 uL Folin-Ciocalteu dye with a dilution factor of 1:2. Since concentration is an intrinsic property, pH condition of the buffer is negligible. Cumulative amount of PTX and DOX was calculated and expressed as percentage in terms of the mean obtained from PTX and DOX standard curve.

Determination of cholesterol

The cholesterol level on the sample was determined based on the standard protocol of Total Cholesterol Kit (Human Diagnostics, Germany). A 1 µL of cholesterol standard was placed in three wells of a 96-well plate and were added with a reagent buffer. Then, as an initial absorbance, cholesterol standard was mixed with the reagent buffer and then incubated for 10 minutes at 37°C and measured using UV-Vis spectrophotometer at 500 nm. Thereafter, samples such as COB-DOX, COB-PTX, Lipo-DOX, and Lipo-PTX were mixed with reagent buffer, incubated for 10 minutes at 37°C, and measured using UV-Vis spectrophotometer at 500 nm. Samples were carried out in triplicates. A cholesterol standard curve was constructed by serial dilution of 200 mg/dL stock solution of cholesterol standard, with a dilution factor 1:2.

Data analysis

In this study, all data were expressed as mean ± standard deviation (n=3). Statistical analyses were performed using Microsoft Excel 2010. A paired t-test was used to determine the significant difference in the experimental method for pH stability study and cholesterol-lowering determination while one-way ANOVA ($p<0.05$) was used for the *in vitro* drug release.

RESULTS AND DISCUSSION

Drug Microencapsulation

Microencapsulation is a rapidly growing technique of novel drug delivery system which purportedly enhances the therapeutic efficacy of conventional drugs.[15] Success in drug formulation heavily relies on the chemical nature of drug microencapsulation as it will help to address the issues in drug loading and encapsulation efficiency.[17] Encapsulation efficiency is the percentage of drug molecules successfully entrapped into its drug carrier. Drug loading is regarded as a key and critical step in characterizing the quality of the formulation. Thus, to achieve a high encapsulation efficiency, factors involving the type, size, surface charge, rigidity, and method of preparation for the drug carrier are highly considered. Moreover, the characteristics and complexing agents of the drug to be encapsulated are considered as well.[18]

Currently, liposome is still widely used globally as a versatile drug carrier capable of encapsulating varying lipophilicities of drugs, in such cases a hydrophilic drug can be found in the inner aqueous phase. On the other hand, a hydrophobic drug can be encapsulated into the phospholipid bilayers.[19] However, with a hydrophobic domain, liposome can only accommodate a limited quantity of drug. These led to a low drug/drug carrier ratio since the drug has a low affinity to the phospholipid bilayer, thus supporting the 71% encapsulation efficiency of Lipo-PTX. The low affinity of PTX in the phospholipid bilayer can be attributed to the natural high cholesterol content of the liposome formulation used. High content of cholesterol reduces the penetration of PTX into the lipid bilayer due to decreased flexibility of the membrane. In addition, the low encapsulation efficiency of PTX to liposome is inherently due to lipophilicity of the drug molecule which competes with cholesterol in occupying the hydrophobic space designed for aqueous solutions into the lipid bilayer.[20]

Lipo-DOX yields 85% encapsulation efficiency because of its biocompatibility with the drug carrier and drug, being hydrophilic in nature. A liposomal DOX obtained a maximum encapsulation efficiency nearly 100% using a pH gradient technique, but a few modifications had been applied by adding 5% and 10% palm oil in the liposomal formulations through a freeze-thaw method.[21] Doxorubicin could self-associate in varying concentrations of buffers, pH and other experimental conditions. It favors assembling interactions with the hydrophobic space of the lipid bilayer of the liposome which is of high aqueous solubility conditions. However, the DOX is too sensitive to aqueous dispersal of liposome rendering it unstable upon drug release as can be seen in well-documented Lipo-DOX extravasation of capillaries and fenestrations in the cells and tissues of circulation.[22] Lipo-DOX had been a successful and perfect drug formulation for cancer therapy; a 100% encapsulation efficiency can possibly be achieved with just appropriate conditions, modifications, and methods used.

COB encapsulated drugs showed higher encapsulation efficiency compared to liposome encapsulated drugs and PTX encapsulated in COB have an approximate encapsulation efficiency of 100% due to the possibility that excess PTX and COB globules are present in the solution. In this study, an assumption was proposed that since PTX is hydrophobic in nature, it completely solubilizes into the matrix of COB containing a high lipid content, (ie, they share similar non-aqueous solubility property). The absence of cholesterol can also be considered as a key factor in the potential complete penetration of PTX in COB. Coconut oil is 90% saturated fats and 10% unsaturated fats.[23] PTX may adhere to the predominant saturated fatty acid portions of COB to strengthen its encapsulation efficiency. This structuration of COB is more helpful in the case of entrapment of DOX as it will generate a tight aqueous center of COB which mimics the physicochemical nature DOX. Nevertheless, such assumption must be further investigated.

Drug Encapsulation Analysis by FTIR Spectroscopy

FTIR is a powerful and versatile analysis tool in the pharmaceutical industry for its structural elucidation, drug formulation development, and validation. Qualitatively, FTIR identifies chemical bond functional group by their characteristic absorption in the infrared region due to its vibrational change from a ground state to an excited state.[24] FTIR identified the functional groups and confirmed incorporation of the DOX and PTX into the COB and liposomes. The representative spectra of COB exhibited

absorption peaks located at 2922, 1155 and 720 cm^{-1}. The peaks at 2900 cm^{-1} confirmed the presence of the CH_3 (alkanes) groups. The peak found in 1100 cm^{-1} represented a =C-H bend. The peak at 700 cm^{-1} confirmed the presence of the CH_2 stretch in the COB analyzed. Anthacycline are compounds of a glycoside structure, containing a sugar and aglycone moiety. The sugar is usually a daunosamine whereas the aglycone moiety consists of four six-carbon rings. The structure of DOX consists of a hydrophobic anthracycline backbone, along with several active sites, including an amine, ketone, and hydroxyl groups. The absorption peaks were located at 3355, 1742, 1638, and 687 cm^{-1}; this can be observed in Figure 1.

The broad band contour which appears in the range of 3000-3400 cm^{-1} is related to OH or NH_2 stretch. The OH absorptions are generally quite intense and smoothly curved, whereas NH_2 stretches are weaker and narrower depending on whether it is primary, secondary, or tertiary amine. The absorption peak at 1700 cm-1 can be attributed to the C=O (ketone and aldehyde) groups. Since DOX also has a benzene ring, then a characteristic absorption at about 680-900 cm^{-1} can be observed. The interaction between a drug and drug carrier can also be predicted by using FTIR. In Figure 1, the characteristic of Lipo-DOX shows all the band characteristic of DOX displayed in the figure but without any significant peak shift.

FTIR spectra of COB shows a strong correlation to the results obtained by GC-FID analysis, in which there is a high content of saturated fatty acids. Saturated fatty acid structurally consists of alkanes and carboxyl constituents. The typical absorption peak of CH_2 stretching vibrations is at 2922cm^{-1}. Likewise, a peak in the 1700cm^{-1} can be observed for carboxyl constituent. Other distinct peaks at 1155cm^{-1} and 1250cm^{-1} corresponds to =C-H and C-C bending vibrations, respectively.

Since paclitaxel consists of taxane ring with four-membered oxetane side ring and an active homochiral ester side chain, absorption peaks can be found at 3355, 1742, and

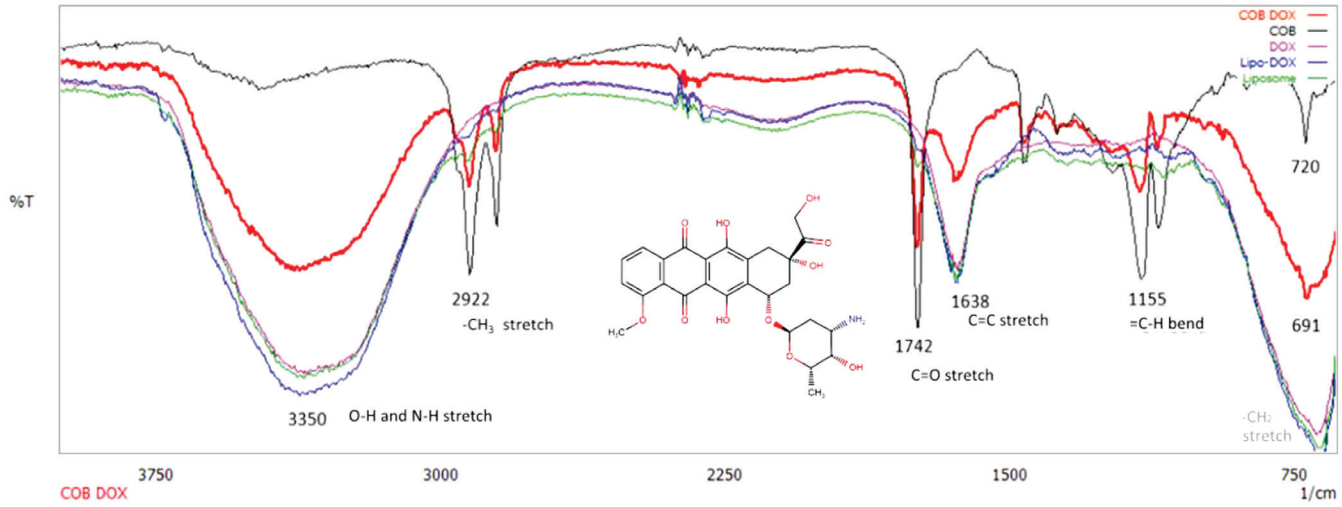

Figure 1. FT-IR absorption spectra DOX-based encapsulation using COB and Liposome.

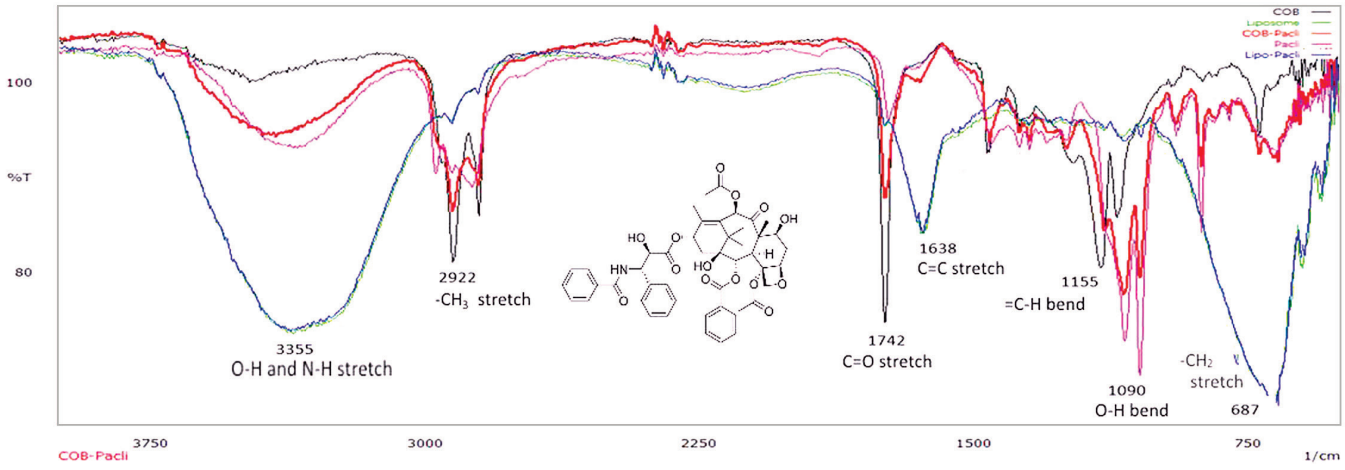

Figure 2. FT-IR absorption spectra PTX-based encapsulation using COB and Liposome.

687cm^{-1}. An absorption peak at 1638 cm^{-1} indicates aromatic ring, stretch C=C, an O-H at 3355cm^{-1}, and C=O for the ester group at 1742 cm^{-1}as shown in Figure 2. More importantly, PTX is preferentially favorable for COB than liposome since all characteristic band of the PTX is displayed, and no spectral shift is observed (Figure 2).

Analysis of Fatty Acid Composition of COB

Coconut oil bodies are rich source of medium chain fatty acids (MCFA). MCFA comprised of lauric, myristic, caprylic, capric, and caproic fatty acid[24] which was further verified on the gas chromatography (GC) data on the fatty acid composition of coconut oil bodies as seen on Figure 3. In agreement to the literature, GC analysis of COB revealed almost 50% lauric acid, followed by small amounts of myristic acid, caprylic acid, and palmitic acids as seen in Figure 3. It also conforms to the presence of about 10% unsaturated fatty acids such as oleic and linoleic acid.[25,26] This analysis further confirms our assumption on the presence of small amount of cholesterol in COB which allows for stronger adherence and penetration of DOX and PTX into the COB. MCFA allows flexible structuration of COB in the encapsulation of chemotherapeutic drugs to prevent it from rapid dispersal and dissolution both in the aqueous and lipophilic parts of the circulation. Utilization of COB formulation provides nutritional benefits and a number of pharmacological benefits of MCFA, particularly in cardioprotection and improvement of hepatic function.[27]

pH Stability Studies

Stability testing is a crucial part of drug formulation since it ensures quality, efficacy, and safety of a drug and considered by the Food and Drug Administration (FDA) to be a prerequisite for a pharmaceutical product to be sold in the market. For a pharmaceutical product to be stable, it must retain its properties and characteristics possessed within a specified limit, throughout its period of storage and use.[28] Also, it must be evaluated for numerous stressors including temperature, oxidation, UV light exposure, and hydrolysis at different pH. The drug content of COB-DOX between freshly prepared (initial day) and after storage at a room temperature for 30 days were measured by reading their absorbance at 480nm. An increase in absorbance from initial drug release until 30 days of incubation indicates the slow degradation of the COB and the slow release of DOX. The gradual release of DOX from the capsule causes relevant increase in the absorbance as portion of the drug molecule, particularly the ring structures that contain resonance, can now interact and absorb light significantly. Figure 4 clearly shows that the absorbance readings of COB-DOX initial and COB-DOX final are significantly different ($p < 0.05$) and had increased. The results indicate that the COB-DOX are stable even at 30 days incubation. On the other hand, COB-PTX's absorbance reading is not within the range of the standard curve generated.

Also, in Figure 4, there is a significant difference ($p < 0.05$) between the concentration of COB-DOX in a neutral and acidic condition. At acidic condition, hydrolysis of the coating material of COB-DOX will be favored resulting to more DOX molecules escaping the drug carrier. The acid environment gradually disrupts the quenching of COB and DOX molecules resulting to higher absorbance reading. Quenching is a type of complexation reaction where an active drug or molecule binds to its encapsulating material or carrier which loses its ability to absorb light in aqueous solution. A recent study by DebMandal M and Mandal S, suggests that the slight increase of absorbance in acidic environment may be associated to deglucosaminyl doxorubicin (A-I) – one of the degradation products of doxorubicin when exposed to acidic solutions for long time. A-I, is formed by the loss of the glucosamine moiety due to cleavage of glycosidic linkage between the tetracycline ring and the glucosamine moiety, which has the following proposed mass fragmentation product ions and m/z ratio (397.0914, 379.0824, 361.0781, and 321.0745) as determined by their LC-MS-TOF analysis.[27,28]

In Vitro Release of Encapsulated Drugs

Characterization of encapsulation efficiency and in vitro drug release are known to be a key performance parameter

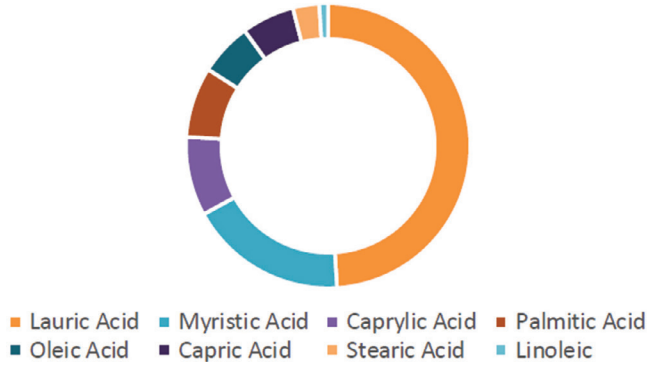

Figure 3. Fatty acid composition of coconut oil bodies.

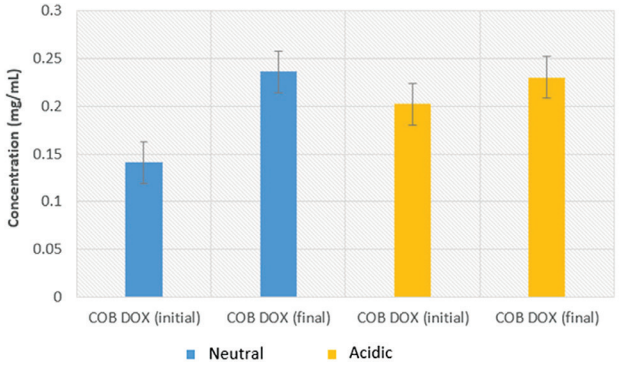

Figure 4. pH stability profile of COB DOX in an acidic *(blue)* and neutral *(yellow)* condition.

for drug formulation and product quality. These can also predict the *in vivo* performance of the encapsulated drugs. In fact, *in vitro* drug release is a process that induces drug release through either of these three mechanisms (1) erosion of the matrix to release drug from the core, (2) subsequent diffusion of drug molecules out the microparticle matrix, and (3) desorption of weakly bound drug molecule to the surface of the microparticle. Usually, a rapid initial release is attributed to the mechanism where a weakly bound drug is adsorbed only to a surface of the microparticle instead of being incorporated inside the polymer microparticle.[29]

In understanding the drug release mechanism, factors such as drug adsorption in the membrane/polymer of a drug carrier, surface-area-to-volume ratio of drug carrier, drug solubility, intermolecular interaction between the drug, and matrix of drug carrier greatly influenced drug's pharmaceutical and clinical performance.[30,31] The release rate was expressed using a zero-order kinetic model, wherein concentration is independent of time. The following formulas were used: Amount of drug = Concentration (mg/mL) x dissolution bath (mL) and CDR (%) = ((Amount of drug n+(Amount of drug N*dilution factor)) / (Total drug(mg)) x 100 wherein N represents the amount of successive drug; n represents the amount of preceding drug; CDR% = percentage of cumulative drug release.

At pH 7.4, the initial release rate of Lipo-DOX was slow and cumulative release were 13.2%, 18.5%, and 26.2% for the first 3 hours, respectively, and 17.1% for the 24th hour (Figure 5). When the pH value is slightly acidic (5.0), the cumulative releases were 10.6%, 26.5%, and 29.3% for the first 3 hours, respectively and 11.6% for the 24th hour (Figure 5). Liposomes' phospholipid bilayer and cholesterol composition might have maintained a tight and compact structure and entrapped the DOX molecules in the core, preventing its release into the acidic and neutral environment, thus a low percentage with a range of 10-63% is observed,

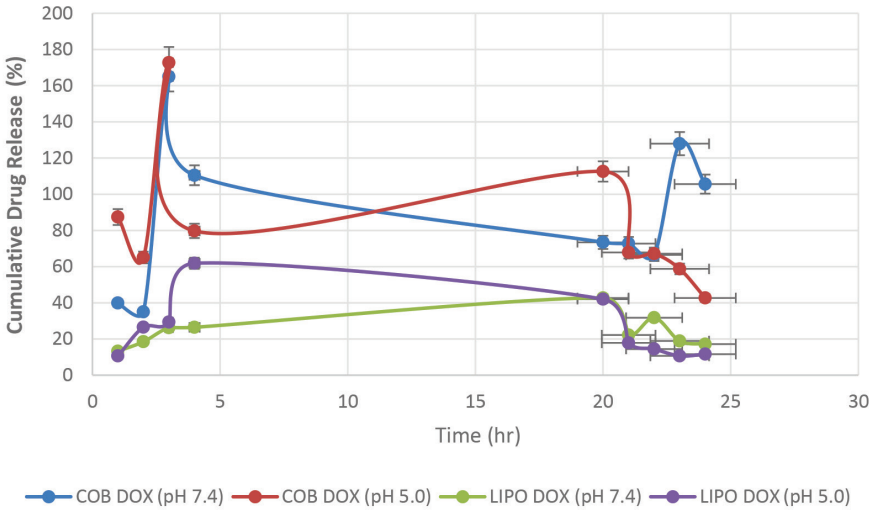

Figure 5. *In vitro* DOX release profiles from COB and liposome at pH 7.4 and 5.0.

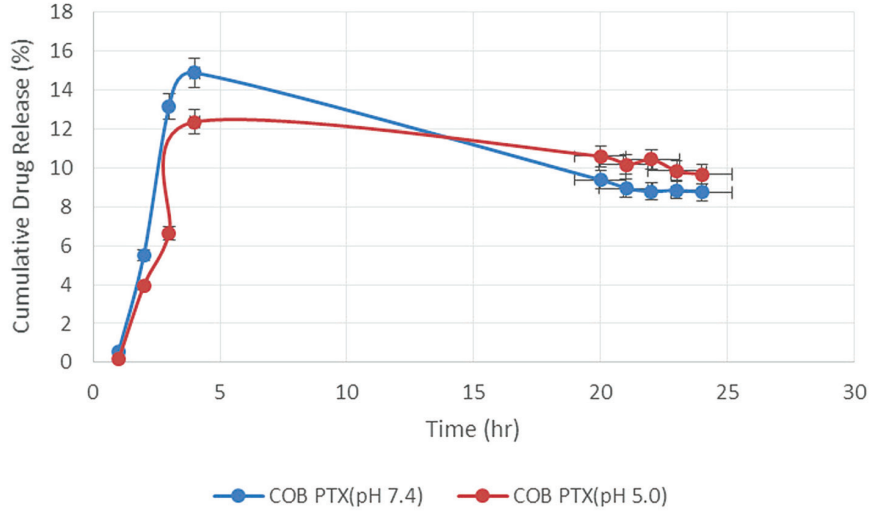

Figure 6. *In vitro* PTX release profiles from COB and liposome at pH 7.4 and 5.0.

compared to COB encapsulated drug. The DOX release from COB in vitro in PBS pH 5.0 is faster than pH 7.4 as presented in Figure 5.

Moreover, after a few hours, all drug molecules had been released in the medium. As shown in Figure 5, the percentage difference of cumulative drug release is due to not uniform preparation of drug molecules present in the original sample. Within 3 hours, 7% of PTX is released in the acidic medium while 13% is released in the neutral medium (Figure 6). Theoretically, COB-PTX should have released higher concentrations in an acidic condition than in a neutral condition since paclitaxel consists of ionizable groups which are capable of being easily degraded but the study had proposed that COB remained intact at acidic condition and kept PTX enclosed in its matrix. Overall, both liposome-encapsulated drugs and COB-encapsulated drugs exhibited a biphasic release profile with an initial burst within 3 hours followed by a sustained, plateau drug release until 24 hours.

Cholesterol Level Determination on the Encapsulating Material

Since 1961, the American Heart Association and the United States Dietary Guidelines have recommended reduction of dietary saturated fats because of its potential risk in the incidence of developing atherosclerosis and cardiovascular disease (CVD). In lieu, saturated fats were replaced by polyunsaturated and monounsaturated fats. The study is concerned on possible side effects of the COB which must be addressed and investigated. As seen in Figure 7, there is a significant difference ($p < 0.05$) between the final concentration and initial concentration of both COB-DOX and COB-PTX. The observed difference in cholesterol concentration of COB-DOX is due to biphasic system for hydrophobic interactions in an amphiphilic environment. Biphasic system accounts for doxorubicin's partitioning because the drug is present at its cation form which strongly

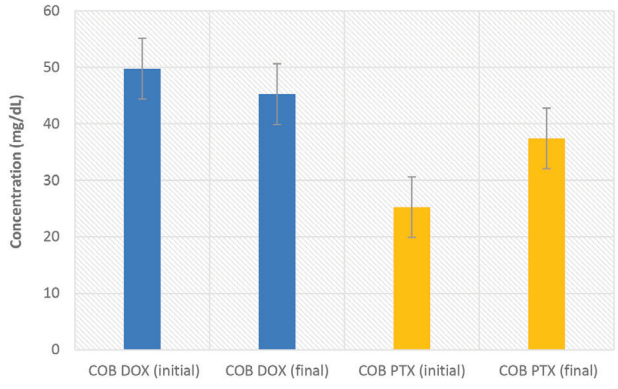

Figure 7. Comparison of cholesterol concentration of coconut oil bodies in two drugs, Doxorubicin *(DOX,blue)* and Paclitaxel *(PTX, yellow)* before and after microencapsulation.

encourages the saturated fatty acids of COB to straighten, further ordering and rigidifying the encapsulated drug with lesser cholesterol molecules.[28,32,33] The observed increase in cholesterol concentration for COB-PTX suggests medium chain and long chain fatty acids promote formation of multilamellar vesicles where cholesterol positions itself establishing electrostatic and hydrophobic interactions with PTX. It will induce fluidization mainly by cholesterol, thus, improving the accommodation and stability of incorporation PTX to COB.[34]

The process of microencapsulation using natural coconut oil bodies incorporated in these chemotherapeutic drugs has not only decreased cholesterol levels but also make the drug more stable with a drug release profile showing a rapid outburst within 3 hours. The studied physicochemical properties of COB as encapsulating agent for DOX and PTX warrants further investigation to validate its usefulness and efficacy as drug carrier of cancer chemotherapeutic agents.

CONCLUSION

Coconut oil bodies (COB) showed promising results as an encapsulating material for doxorubicin as shown by the high encapsulation efficiency exhibited. Incorporation of the drug within the wall of encapsulating material was also confirmed by the FTIR results. Both FTIR and GC-FID had validated and provided the fatty acid profile required to understand other steps of drug formulation. The stability of the COB was found to last less than 30 days and it releases DOX more favorably in an acidic environment which suggests ideal targeted release in cancer cells. However, no assurance is yet implied for paclitaxel, thus further optimization for its drug formulation is proposed. Overall, COB performs well and displays great potential as an alternative wall material for the currently used liposome.

Statement of Authorship

All authors participated in data collection, analysis, written reports, and approval of the final version of the manuscript.

Author Disclosure

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