



Assessing the potential of liposomes loaded with curcumin as a therapeutic intervention in asthma



Zhao Yin Ng^a, Jin-Ying Wong^a, Jithendra Panneerselvam^b, Thiagarajan Madheswaran^b, Pradeep Kumar^c, Viness Pillay^c, Alan Hsu^e, Nicole Hansbro^e, Mary Bebawy^f, Peter Wark^e, Philip Hansbro^e, Kamal Dua^{e,f}, Dinesh Kumar Chellappan^{d,*}

^a School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia

^b Department of Pharmaceutical Technology, International Medical University, Kuala Lumpur, Malaysia

^c Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

^d Department of Life Sciences, International Medical University, Kuala Lumpur, Malaysia

^e Priority Research Centre for Healthy Lungs, University of Newcastle & Hunter Medical Research Institute, New Lambton Heights, Newcastle, NSW 2305, Australia

^f Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, P.O. Box: 123 Broadway, NSW 2007, Australia

ARTICLE INFO

Keywords:

Curcumin

Liposomes

Asthma

Inflammation

Pro-inflammatory markers

Molecular modelling

ABSTRACT

Curcumin a component of turmeric, which is derived from *Curcuma longa* is used as a colouring agent and as a dietary spice for centuries. Extensive studies have been done on the anti-inflammatory activity of curcumin along with its molecular mechanism involving different signalling pathways. However, the physicochemical and biological properties such as poor solubility and rapid metabolism of curcumin have led to low bioavailability and hence limits its application. Current therapies for asthma such as bronchodilators and inhaled corticosteroids (ICS) are aimed at controlling disease symptoms and prevent asthma exacerbation. However, this approach requires lifetime therapy and is associated with a constellation of side effects. This creates a clear unmet medical need and there is an urgent demand for new and more-effective treatments. The present study is aimed to formulate liposomes containing curcumin and evaluate for its anti-inflammatory effects on lipopolysaccharide (LPS)-induced inflammation on BCI-NS1.1 cell line. Curcumin and salbutamol liposomes were formulated using lipid hydration method. The prepared liposomes were characterized in terms of particle size, zeta potential, encapsulation efficiency and in-vitro release profile. The liposomes were tested on BCI-NS1.1 cell line to evaluate its anti-inflammatory properties. The various pro-inflammatory markers studied were Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-1 β (IL-1 β) and Tumour Necrosis Factor- α (TNF- α). Additionally, molecular mechanics simulations were used to elucidate the positioning, energy minimization, and aqueous dispersion of the liposomal architecture involving lecithin and curcumin. The prepared curcumin formulation showed an average size and zeta potential of 271.3 ± 3.06 nm and -61.0 mV, respectively. The drug encapsulation efficiency of liposomal curcumin is 81.1%. Both curcumin-loaded liposomes formulation (1 μ g/mL, 5 μ g/mL) resulted in significant ($p < 0.05$) reduction in the level of pro-inflammatory marker expression such as IL-6, IL-8, IL-1 β and TNF- α compared to positive control group. Liposomal curcumin with the dose of 1 μ g/mL reduced the inflammatory markers more effectively compared to that of 5 μ g/mL. Liposomal curcumin could be a promising intervention for asthma therapy showing their efficacy in suppressing the important pro-inflammatory markers involved in the pathogenesis of asthma.

1. Introduction

Asthma is a chronic inflammatory disorder of the airways that is characterized by chronic inflammation, airway remodelling and airway hyper-responsiveness, producing variable and recurring symptoms such

as wheezing, dyspnea, chest tightness and coughing [1]. According to the World Health Organisation (WHO), the estimated number of people suffering from asthma is 235 million. In 2015, 7.8% of the United State population were suffered from asthma and the total death rate was 10.3 per million people [2]. Recent reports from the World Health

* Corresponding author.

E-mail address: dinesh.kumar@imu.edu.my (D.K. Chellappan).

<https://doi.org/10.1016/j.colsurfb.2018.08.027>

Received 22 April 2018; Received in revised form 9 August 2018; Accepted 14 August 2018

Available online 16 August 2018

0927-7765/© 2018 Elsevier B.V. All rights reserved.

Organisation (WHO) suggest that chronic respiratory diseases accounts for 7% of total deaths in Malaysia [3]. The annual mortality rate of asthma in Malaysia is 4.92543 per 100,000 people and it has increased by 14.1% since year 1990 [4]. Asthma can be controlled by the regular use of bronchodilators and other anti-inflammatory (ICS) medications [5]. Current therapies for asthma are aimed at controlling disease symptoms and prevent asthma exacerbation. However, this approach requires lifetime therapy and is associated with a constellation of side effects. This creates a clear unmet medical need and there is an urgent demand for new and more-effective treatments.

Curcumin is a component of turmeric, which is derived from *Curcuma longa* and has been used as a colouring agent and as a dietary spice. Curcumin has been known for centuries in treating several diseases in the Ayurvedic system and in the traditional Chinese medicine. Furthermore, curcumin is well tolerated in humans [6]. Extensive studies have been done on the anti-inflammatory [7,8], antioxidant [9], antibacterial [10] and anticancer [11] properties of curcumin along with its molecular mechanism in different signalling pathways. Oh et al. have found that curcumin ameliorates allergic airway inflammation and hyper-responsiveness by inhibiting the activation of NF- κ B signalling pathway in the asthmatic lung tissue [12]. Besides, Liu et al., reported that curcumin significantly attenuates the progression of airway inflammation by diminishing the production of eosinophils, white blood cells and immunoglobulin E [13,14].

The poor solubility of curcumin limits its application of curcumin in medical field. To overcome this limitation, curcumin could be incorporated into a novel delivery vesicular system such as liposomes. Liposomes have the ability to alter the pharmacokinetic characteristics and modify the bio-distribution profile of a system, thereby, resulting in an increased absorption and eventually enhanced therapeutic efficacy of a loaded drug [15]. Liposomes are reported to enhance the solubility of a drug through mechanisms namely, co-solvency, prodrug formation or by direct solubilizing effect [16].

Liposomes are synthetic vesicles which are primarily composed of phospholipid and cholesterol. Liposomes can encapsulate drugs with varying solubility, entrapped either in the aqueous core of the phospholipid bilayer or at the bilayer interface depending the nature of the drug. Liposomes are non-immunogenic and biocompatible as the materials used to formulate liposomes are naturally available in the human body [17]. Liposomal delivery also showed beneficial effect by widening the therapeutic index of encapsulated drugs, such as amphotericin, doxorubicin, levodopa and several other drugs, and hence reduced its toxicity effects [18–26]. Liposomes have also been reported for their anti-quorum sensing properties against foodborne pathogens [27]. Several studies involving liposomal curcumin have been reported recently which have shown to have anti-angiogenic, neuroprotective and anti-inflammatory potential [28–30].

There are limited studies that are reported on the safety of liposomal curcumin. Storka et al., have reported that when administered as a short term intravenous dose, the liposomal curcumin appears to be safe up to a dose of 120 mg/m² [31].

Several other cell lines namely air interface Calu-3 cells, A549 airway epithelial cell culture, airway smooth muscle cell line and rat alveolar macrophage cell line have also been used by different authors with liposomal curcumin [32–35].

2. Materials and methods

2.1. Materials

Soya lecithin, cholesterol, Tween 80 and dialysis membrane were purchased from Sigma Aldrich, USA. Cholesterol was purchased from BDH Middle East LLC, United Arab Emirates. All other solvents and chemicals used in this research were of Analytical grade.

2.2. Preparation of curcumin liposomes

Curcumin loaded liposomes were prepared using lipid hydration method [36]. 400 mg of soya lecithin, 50 mg of cholesterol, 50 mg of Tween 80 and 1 mg of Curcumin were dissolved with 10 ml of chloroform in a round-bottom flask at room temperature. The mixture solution was evaporated on a Rotavapor® R-100 (Buchi, United Kingdom) at 50 °C until a thin film was formed on the flask. The flask was left inside the fume hood overnight to allow complete evaporation of the solvent. The film was hydrated with 10 ml of distilled water and the flask was hand-shaken for 30 min. The lipid dispersion was sonicated for 3 min using a bath-sonicator (Thermo Fisher Scientific, USA).

2.3. Preparation of salbutamol liposomes

The salbutamol liposome were formulated as previously described by Shivhare et al. using 400 mg of soya lecithin, 50 mg of cholesterol, 50 mg of Tween 80 and 10 mg of salbutamol [36].

2.4. Characterization of liposomes

2.4.1. Particle size and zeta potential analysis

Prior to the measurement of size distribution and zeta potential, the liposome dispersions were diluted to the required turbidity with distilled water. The evaluation of particle size and zeta potential were done using Malvern Zetasizer Nano Series [37–39].

2.4.2. Encapsulation efficiency of liposomes

Encapsulation efficiency of liposomes was evaluated by centrifugation method. 0.1 mL of curcumin and salbutamol liposomes were diluted in 9.9 mL of distilled water and their concentration was analyzed using Perkin-Elmer UV–vis spectrophotometer at 422 nm. 1.5 mL of liposomal Curcumin and salbutamol aliquots were centrifuged on a benchtop centrifuge (Eppendorf, Germany) at 14,000 rpm for 90 min. The supernatants were withdrawn cautiously to remove the untrapped curcumin and salbutamol and their absorbance was detected at 422 nm and 276 nm, respectively. Percentage encapsulation of drugs was calculated by the following formula:

$$\text{Encapsulation Efficiency (EE\%)} = \frac{\text{Drug}_{\text{total}} - \text{Drug}_{\text{supernatant}}}{\text{Drug}_{\text{total}}}$$

where drug_{total} is the total amount of Curcumin and drug_{supernatant} is the amount of uncapsulated curcumin in the liposome solution.

2.5. In-vitro release study

This study was performed using three cells (Franz diffusion cell) per formulation. The dialysis membrane was immersed in 50 ml phosphate buffer saline (PBS) pH 7.4 for 30 min prior to experiment run. The receptor chamber was filled with degassed PBS and the membranes were placed on top of the receptor compartment. The donor compartment was placed over the membrane. 0.5 mL of Curcumin liposome was loaded in the donor compartment. The receptor media was maintained at 37 ± 1 °C with constant stirring at 400 rpm. 1 mL of sample aliquot was withdrawn from each cell at 15 min, 30 min, 1, 2, 3, 6, 9, 12 and 24 h for measurement of the drug release rate of Curcumin from formulation. The volume collected from the cell was replaced with fresh PBS. The concentrations of Curcumin in the sample aliquot were measured at 422 nm using Perkin-Elmer Lambda 25 UV–vis spectrophotometer.

2.5.1. Fourier transform infrared (FTIR) spectroscopy

The determine the compatibility of curcumin with the various excipients used to formulate liposome were carried out using FTIR machine. The wavelength was scanned between 4000 cm^{−1} and

Table 1

Size, polydispersity index (PDI), zeta potential and encapsulation efficiency of liposome preparations.

Liposome Formulations	Mean particle size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Encapsulation efficiency (%)
Curcumin	271.3 ± 3.06	0.512 ± 0.003	−61.0 ± 0.68	81.1
Salbutamol	184.2 ± 5.93	0.481 ± 0.02	−67.57 ± 0.47	88.6
Empty Vehicles	244.1 ± 4.65	0.520 ± 0.03	−56.63 ± 0.06	–

Data are expressed as the mean ± standard deviation (SD), N = 3.

500 cm^{−1}.

2.5.2. In-vitro cell culture study

BCi-NS1.1 cell line was cultured as previously described by Kedzierski et al. [40]. Briefly, BCi-NS1.1 was cultured in Bronchial Epithelial Growth Media (BEGM)-Complete (Lonza, USA), and before and during the treatment phase, the cells were cultured in Bronchial Epithelial Basal Media (BEBM)-Minimal (Lonza, USA).

2.5.3. Curcumin and lipopolysaccharide (LPS) treatment

Cells were treated with LPS (1 mg/mL) either alone or with 1 and 5 µg of liposomal Curcumin, 20 µM of liposomal salbutamol and empty liposome for 24 h. The liposomal curcumin groups were compared with the standard treatment (salbutamol).

2.5.4. Quantitative real-time PCR

RNA was extracted from treated BCi-NS1.1 cell line using RNeasy Mini Kit and Qiacube (Qiagen, USA), and 200 ng of RNA was reverse transcribed to cDNA using the high capacity cDNA reverse transcription kit (Applied Biosystems, USA). Quantitative real-time PCR was then performed using Taqman primers and probes (Applied Biosystems, USA) for IL-6, IL-8, IL-1β, and TNF-α. These are the common markers that are usually investigated in asthma [41–43].

Expression levels of target mRNA were calculated using 2-ΔΔCt relative to the reference gene (18S). This was then calculated as fold change relative to un-treated media control.

2.5.5. Molecular complexation profiling via static lattice atomistic simulations (SLAS) [44,45]

All modelling procedures and computations, including energy minimizations in Molecular Mechanics, were performed using HyperChem™ 8.0.8 Molecular Modeling Software (Hypercube Inc., Gainesville, FL, USA). The 3D structures of the molecules were constructed with natural bond angles. The models were energy-minimized using the AMBER 3 (Assisted Model Building and Energy Refinements) Force Field algorithm. The conformer having the lowest energy was used to develop the molecular complexes. A complex of one molecule with another was assembled by parallel disposition and the energy-minimization was repeated to generate the final complex molecular models. Full geometrical optimization was conducted in vacuum employing the Polak–Ribiere Conjugate Gradient method until an RMS gradient of 0.001 kcal/mol was reached. To generate the final models in a solvated system, the MM simulations were performed for cubic periodic boxes with specifically defined computational parameters. The periodic box dimensions were fixed as 30 × 15 × 35 Å³. The cut-offs were switched and the dielectric (epsilon) was set to a constant. With regards to the 1–4 Scale factors, both the electrostatic and van der Waals were set at 0.5. The outer and inner radius were measured to be 7.5 Å and 3.5 Å respectively. The solvent/polymer distance was set at 2.3 Å.

2.5.6. Molecular mechanics assisted model building and energy refinements

The MMER model for the energy factor in various molecular complexes can be written as:

$$E_{\text{molecule/complex}} = V_{\Sigma} = V_b + V_{\theta} + V_{\phi} + V_{ij} + V_{hb} + V_{el} \quad (1)$$

where, V_{Σ} is related to total steric energy for an optimized structure, V_b

corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), V_{θ} denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), V_{ϕ} represents torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolizes hydrogen-bond energy function and V_{el} stands for electrostatic energy.

In addition, the total potential energy deviation, ΔE_{Total} , was calculated as the difference between the total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, as follows:

$$\Delta E_{\text{Total(A/B)}} = E_{\text{Total(A/B)}} - [E_{\text{Total(A)}} + E_{\text{Total(B)}}] \quad (2)$$

2.6. Statistical analysis

All the values are expressed as mean ± SD. The data obtained from experiments were statistically analyzed and performed using Prism software package (GraphPad Software, Version 5.00, San Diego, CA, USA). The significance of differences between groups was analyzed by the unpaired *t*-test.

3. Results and discussion

3.1. Characterization of liposome preparations

The mean diameter size, PDI and zeta potential of the liposome preparation were determined and are summarized in Table 1. The sizes of the liposomes were in the range from 184.2 to approximate 271 nm, with a wide size distribution (PDI), ranging from 0.481 to 0.520 for all preparations. Fig. 1a–c show the results of particle size for all the liposome preparations. The sizes of the liposome preparations were heterogeneous. This is attributed to the drawback of the preparation method, lipid hydration method, which used to formulate the liposomes [46]. PDI indicates the pattern of size distribution. The size distribution is considered to be broad when PDI is greater than 0.3, whereas the narrow distribution is shown when PDI value is less than 0.3 [47]. In a previous study, it has been shown that in order for the drug to deposit at the alveoli after inhalation, the optimum size of drug must be less than 5 µm as the main mechanism for deposition is by diffusion which support our findings in terms of the particle size and distribution of prepared liposomes [48].

The zeta potentials of all formulations were measured and it ranged from −56.63 to −67.57 mV. The negative values of zeta potential were contributed by the conformation change of phospholipid head groups at the surface of liposome after the encapsulation of curcumin. In general, the absolute value of zeta potential above 30 mV could give rise to a stable dispersion in a formulation. This is due to the high electrostatic repulsion among the particles in the liposome solution and thus less aggregation will be formed.

A high encapsulation efficiency was found in both curcumin and salbutamol formulations, which showed more than 80% of encapsulated drug, in particular for salbutamol. This indicates that salbutamol has a slightly higher affinity to be entrapped compared to the curcumin. The encapsulation efficiency could be affected by the concentration of lipid in a liposome. Colletier et al., showed that there is a

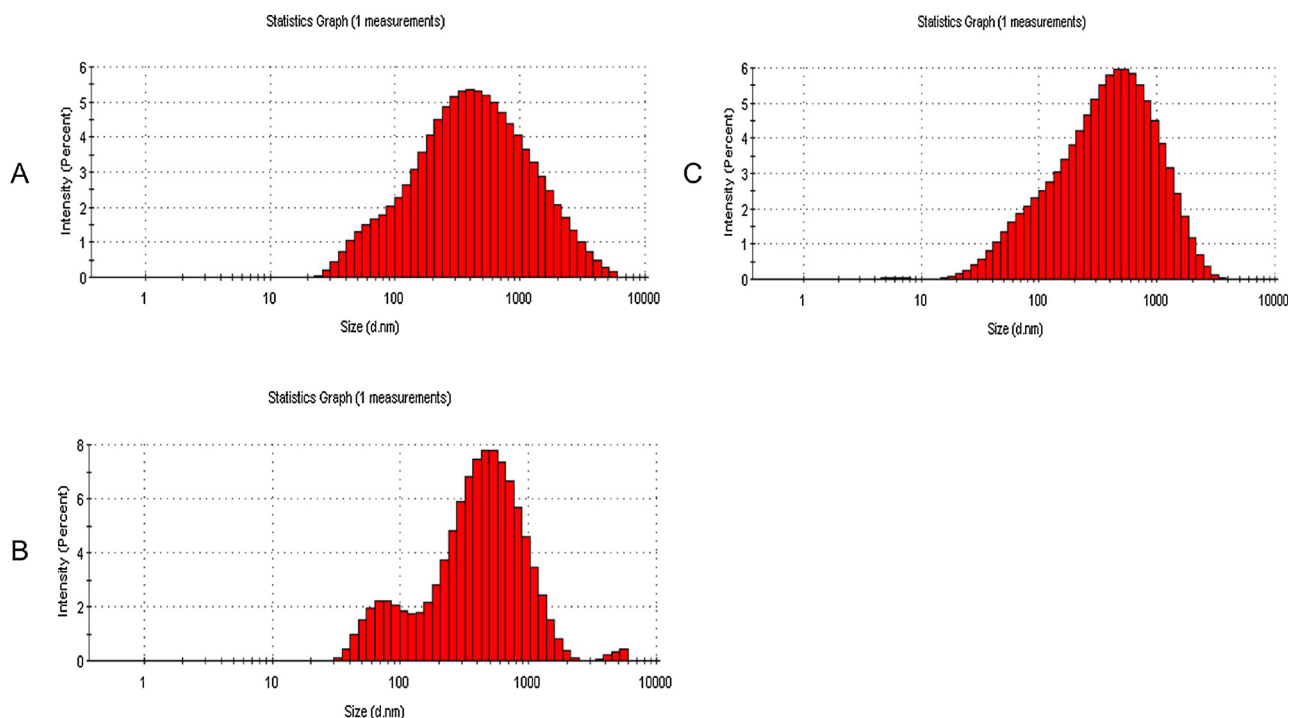


Fig. 1. Size distribution of liposome preparations. (A) curcumin liposomes (B) salbutamol liposomes and (C) empty liposomes.

linear relationship between the lipid concentration and encapsulation efficiency [49].

3.2. In-vitro release study

Fig. 2, shows the release profile of Curcumin liposome over 24 h. Results show that only 14.5% of Curcumin was released after 24 h. Release profile of curcumin from liposomes shows a prolonged manner over the longer duration of time. The curcumin encapsulated liposomes achieved sustained released pattern benefiting from the shielding effect of lipid bilayers. Chen et al also showed that Curcumin was released in prolonged manner from liposomes and less than 70% of Curcumin was released after 48 h. Another in-vitro study also showed the release of drug from small unilamellar vesicles (SUVs) which sustained for 10 days [50].

3.3. FTIR study

In the FTIR spectrum of soya lecithin, the absorption peak at 1456.26 cm^{-1} and 2852.72 cm^{-1} is attributed to the C–H bend and

stretch of the alkanes, respectively. The peak appeared at 1737.86 cm^{-1} is due to the C=O stretching vibration of the carbonyl groups. A broad peak at 3300 cm^{-1} indicates the presence of O–H functional group [51]. In Fig. 3(B), the characteristic peaks of cholesterol at 2846.22 , 2866.22 , 2929.87 cm^{-1} are contributed by the vibrations of C–H stretch from methyl group and cyclic hydrocarbons [52]. For Tween 80, the broad band at the region between 1050 – 1250 cm^{-1} is due to the C–O stretching vibration. The peak at 1647.21 cm^{-1} indicates the presence of C=C. The absorption band due to C=O stretched at 1734.01 cm^{-1} . The FTIR spectrum of curcumin is shown in Fig. 3(D). The detailed spectra analysis of Curcumin has been published earlier by Kolev et al. [53]. The region between 713.66 and 962.48 cm^{-1} showed the cis and trans–CH vibration, respectively of the benzoate. The peak at 1024.20 cm^{-1} indicates ether group while the enol C–O peak was obtained at 1271.09 . The 1504.48 cm^{-1} peak is assigned to the (C=O). A strong peak at 1600.92 cm^{-1} is attributed to the symmetric aromatic ring stretching vibrations (C=C ring). The strong peak at 1625.99 cm^{-1} has a predominantly mixed (C=C) and (C=O) character. A sharp peak at 3508.52 cm^{-1} indicates the presence of OH [54]. All the characteristic peaks of curcumin were not appearing in the FTIR spectrum shown in Fig. 3(E). The peak shown were mostly the characteristic peaks of soya lecithin. This indicates that the curcumin was successfully incorporated into the phospholipid interface [55].

3.4. Effect of Curcumin liposome treatment on the expression of pro-inflammatory markers in LPS stimulated BCi-NS1.1 cell lines (Fig. 4)

To study the effects of curcumin liposomes on LPS induced pro-inflammatory marker expression in BCi-NS1.1 cells, cells were treated with 1 mg/mL of LPS and different doses of curcumin ($1\text{ }\mu\text{g}$ and $5\text{ }\mu\text{g}$) simultaneously. Curcumin-loaded liposomes resulted in significant ($p < 0.05$) reduction in the level of pro-inflammatory marker expression such as IL-6, IL-8, IL- 1β and TNF- α compared to positive control group regardless of the dose. Liposomal curcumin with the dose of $1\text{ }\mu\text{g/mL}$ reduced the inflammatory markers more effectively compared to that of $5\text{ }\mu\text{g/mL}$. Curcumin targets multiple signalling pathways of inflammation including NF- κB and STAT3 pathways. NF- κB signalling

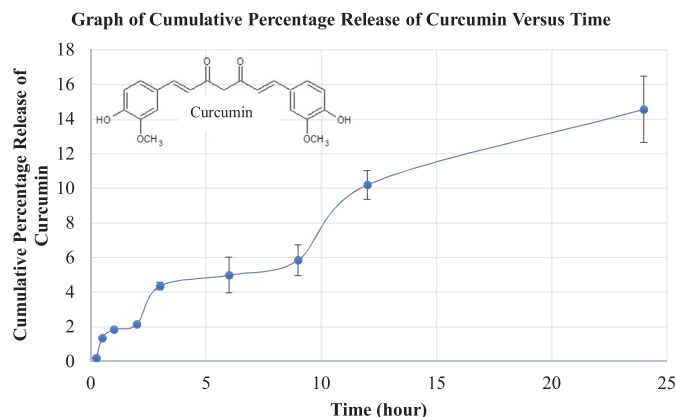


Fig. 2. Cumulative Percentage Release of Curcumin versus Time.

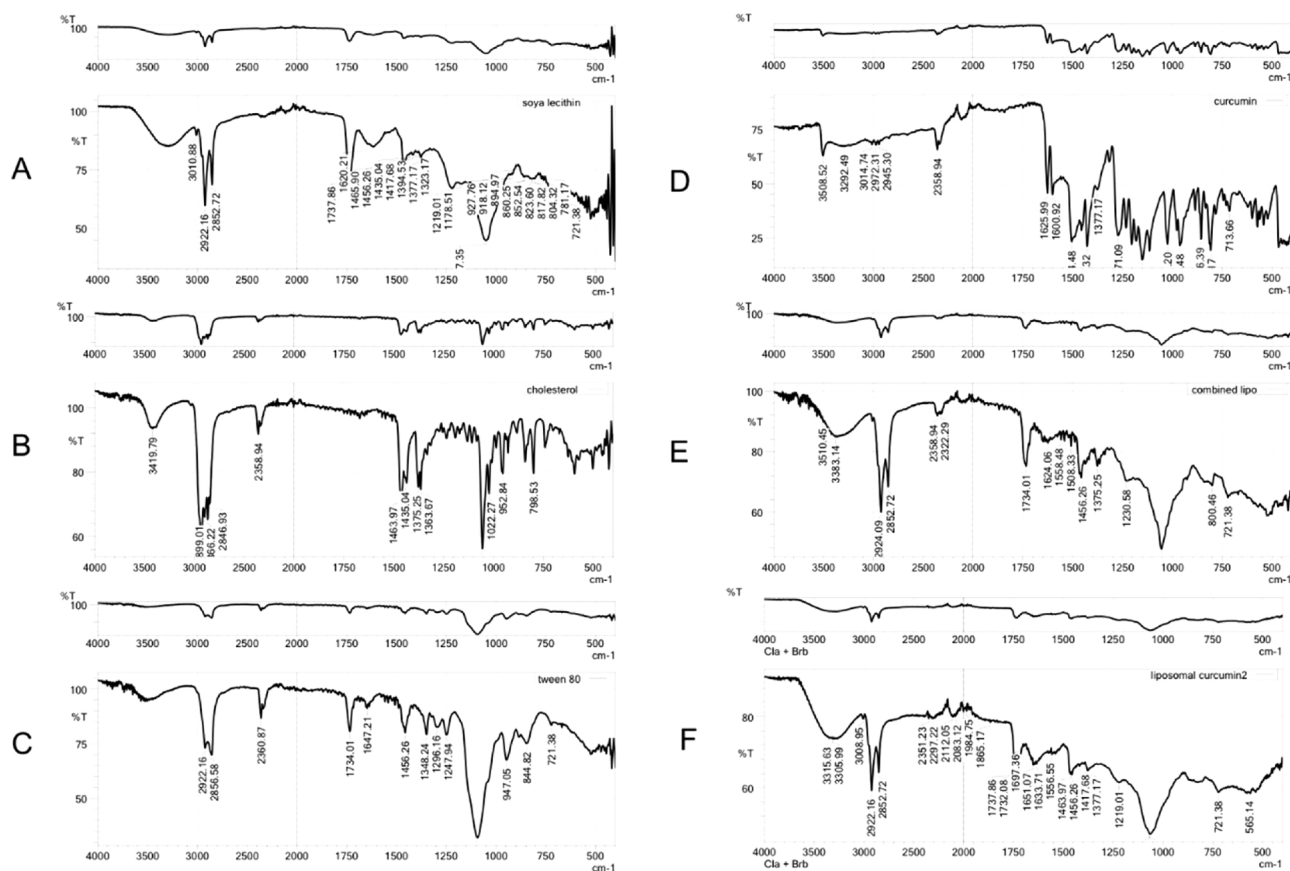


Fig. 3. FTIR spectra of (A) soya lecithin (B) Cholesterol (C) Tween 80 (D) Curcumin (E) Physical mixture of all ingredients with curcumin and (F) curcumin liposomes.

pathway appears to be important in the pathogenesis of asthma as it regulates the pro-inflammatory genes expression, leading to the production of cytokines, adhesion molecules, chemokines, enzymes and growth factors which involved in inflammation response [56]. In addition, activation of STAT3 pathways drives inflammation and remodelling of airway tissue, and accumulation of T-helper cells in a murine asthma model. Kloeasch et al., proved that liposomal Curcumin effectively suppressed the expression of pro-inflammatory markers such as IL-6, IL-8 and TNF- α in LPS-stimulated human synovial fibroblasts and mouse macrophages [57]. Another study showed that pre-treatment of curcumin in LPS-induced inflammation RAW264.7 cells noticeably reduced the level of IL-6, IL-1 β and TNF- α [13] O.

Liposomal curcumin with the dose of 1 μ g/mL reduced the pro-inflammatory markers more effectively compared to that of 5 μ g/mL. The higher concentrations of curcumin failed to demonstrate reduction of pro-inflammatory markers in a dose dependent manner as described in the literature [58].

There is a slightly reduction in the expression of IL-6 and IL-8, which was observed in the empty liposome group. The major components of soya lecithin include phosphatidylcholine, phosphatidylethanolamine, inositol phosphatides, soybean oil, sterols and free carbohydrates. [59]. Phosphatidylcholine possess anti-inflammatory property by inducing a prolonged inhibition of pro-inflammatory signaling responses via NF- κ B pathway [60].

Conditions of cells are indicated on the x-axis. Media only condition is negative control, LPS only condition is used as negative control. Bars with ‘*’ indicate significant difference at $p < 0.05$ of pro-inflammatory markers compared to negative control and bars with ‘#’ indicate significant difference of pro-inflammatory markers compared to positive control.

3.5. Molecular modelling simulations

The inherent energy attributes representing the molecular assemblies modelled using SLAS is given in Table 2.

The molecular modelling data provided important insights into the formation of liposomal structure between lecithin and the drug, curcumin. The results obtained from molecular modelling simulations can be deduced as the following:

- 1) Geometrical orientation and positioning of the drug within the lecithin molecular matrix: During the molecular simulations in vacuum, it was observed that the curcumin molecule manoeuvred around the phosphatidyl head group with two most energetically stable structures labelled as LCTN-CURCIN and LCTN-CURCOUT – corresponding to the position of curcumin within/in between the hydrophobic domain or outside the hydrophobic domain, respectively (Fig. 5). Although no hydrogen bonding was evident from the geometric optimization, it was observed and computed that the LCTN-CURCIN conformation provided a close fit geometrical structure as compared to LCTN-CURCOUT. This was further supported by a higher energy stabilization in case of LCTN-CURCIN ($\Delta E = -19.219$ kcal/mol) in comparison to LCTN-CURCOUT ($\Delta E = -14.095$ kcal/mol). The geometrical fitting of the drug within the phospholipid matrix in LCTN-CURCIN was accompanied by adjustments of bond angle contributions ($\Delta E = -2.346$ kcal/mol) and an efficient van der Waals space filling ($\Delta E = -14.461$ kcal/mol). The strong affinity and incorporation of curcumin within lecithin was further strengthened by change in the values of logP for curcumin (logP = -1.21) and lecithin (logP = 12.21) to 9.38 in case of LCTN-CURCIN. The maintenance of logP within the hydrophobic domain but reduction in the overall hydrophobicity of lecithin

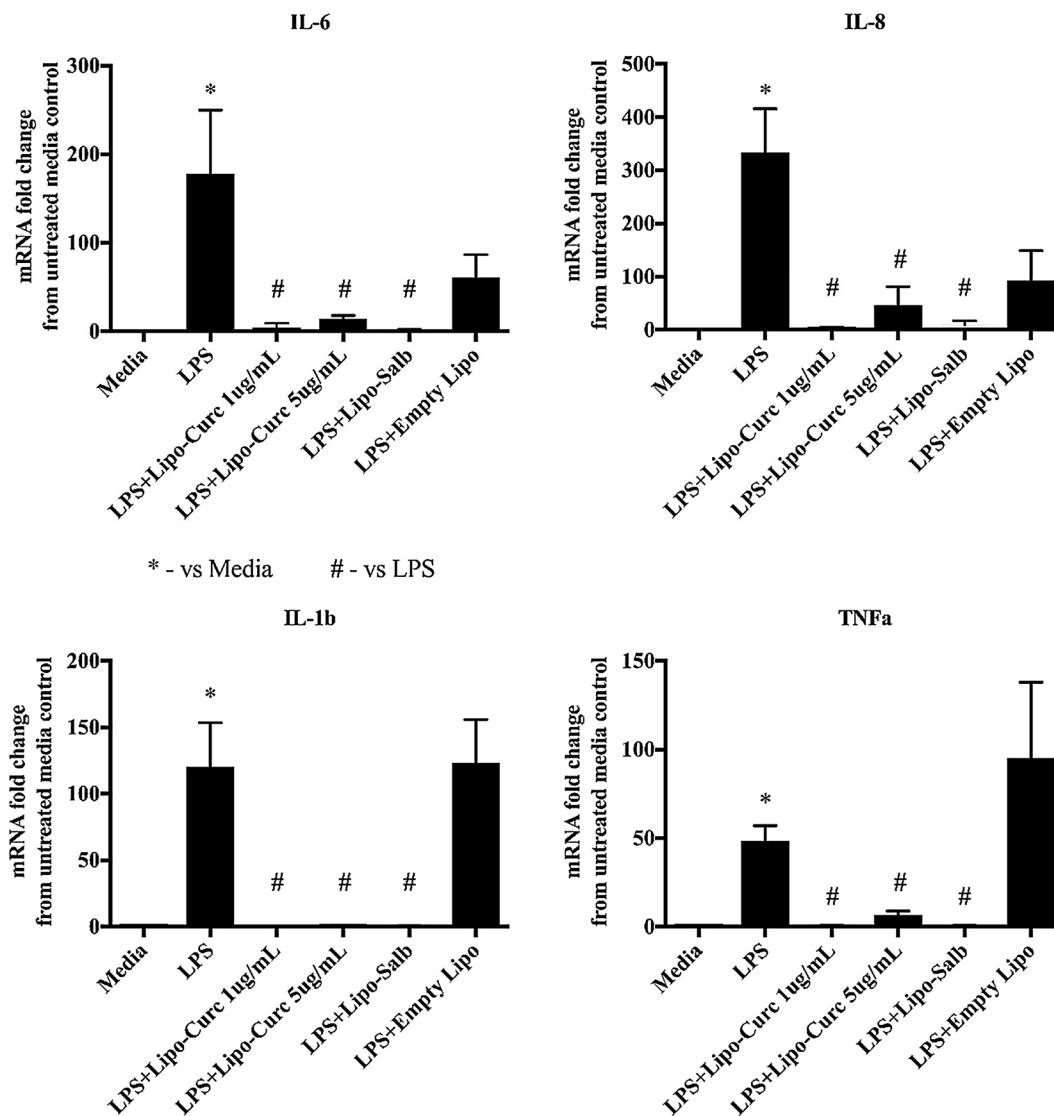


Fig. 4. Effects of curcumin liposome treatment on BCI-NS1.1 cell line. Cells were treated with curcumin liposomes at 1 and 5 $\mu\text{g/mL}$, salbutamol liposome and empty liposome for 24 h ($n = 5$).

Table 2

Inherent energy attributes representing the molecular assemblies modelled using Static Lattice Atomistic Simulations (SLAS) in vacuum.

Molecular complex	ΔE^a	$(V_s)^b$	$(V_b)^c$	$(V_\theta)^d$	$(V_\varphi)^e$	$(V_{ij})^f$	$(V_{hb})^g$	$(V_{el})^h$
LCTN	–	14.835	0.503	4.015	6.937	3.382	–0.003	0.00
CURC	–	24.900	0.938	7.953	11.154	4.920	–0.065	0.00
LCTN-CURC _{IN} ⁱ	–19.219	20.516	1.300	9.622	16.218	–6.159	–0.465	0.00
LCTN-CURC _{OUT} ^j	–14.095	25.640	1.386	12.950	15.662	–4.367	–0.194	0.00
LCTN _{H2O}	–	–6791.983	65.069	70.746	9.103	243.116	–1.910	–7178.11
CURC _{H2O}	–	–6799.062	67.826	70.759	11.725	251.675	–1.914	–7199.13
LCTN-CURC _{H2O}	–	–6786.715	66.054	81.118	20.697	227.376	–3.851	–7178.11

^a $\Delta E_{(A/B)} = E_{(A/B)} - [E_{(A)} + E_{(B)}]$.

^b total steric energy for an optimized structure.

^c bond stretching contributions.

^d bond angle contributions.

^e torsional contribution arising from deviations from optimum dihedral angles.

^f van der Waals interactions.

^g hydrogen-bond energy function.

^h electrostatic energy.

ⁱ The final geometrical confirmation has curcumin in between the hydrophobic domain.

^j The final geometrical confirmation has curcumin outside the hydrophobic domain.

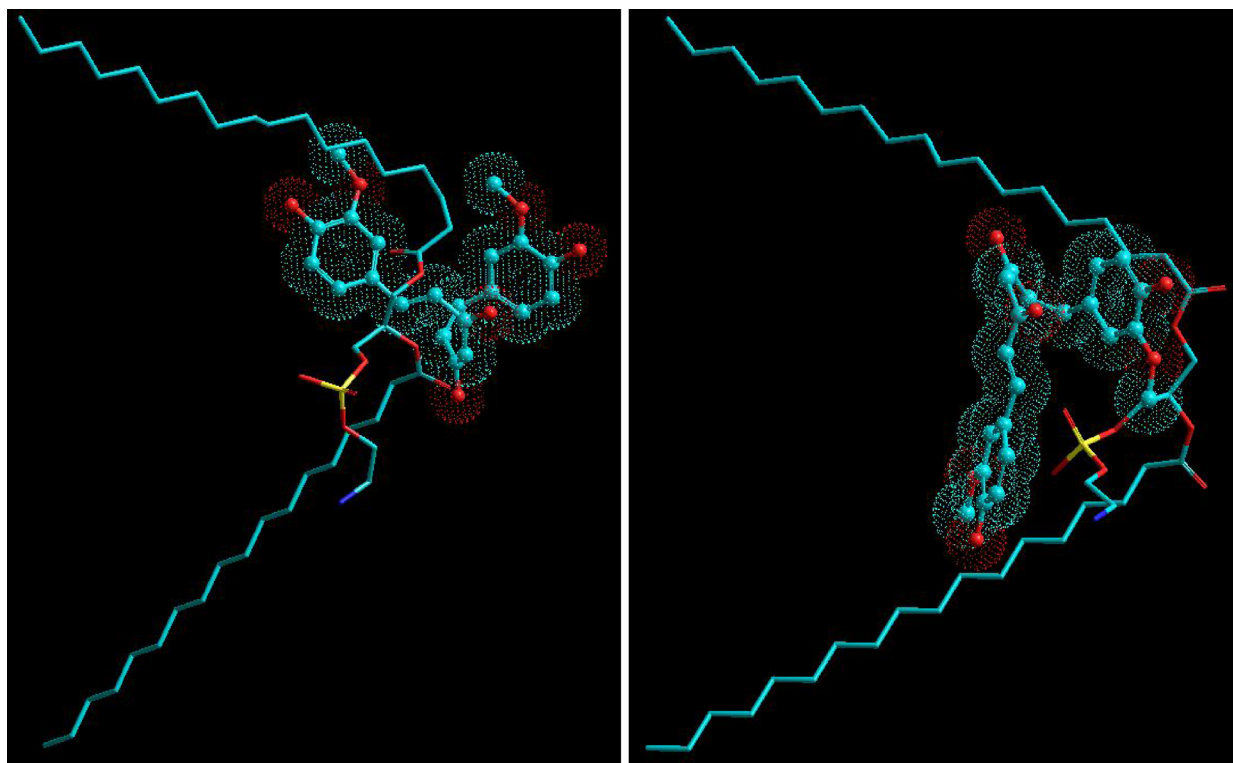


Fig. 5. Geometrical visualization of (a) LCTN-CURC_{OUT} molecular complex; and (b) LCTN-CURC_{IN} molecular complex after molecular mechanics simulations in vacuum. Curcumin is depicted in ball-and-tube rendering while lecithin is depicted as tubes. Colour coding of elements: C (cyan); P (yellow); O (red); and N (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

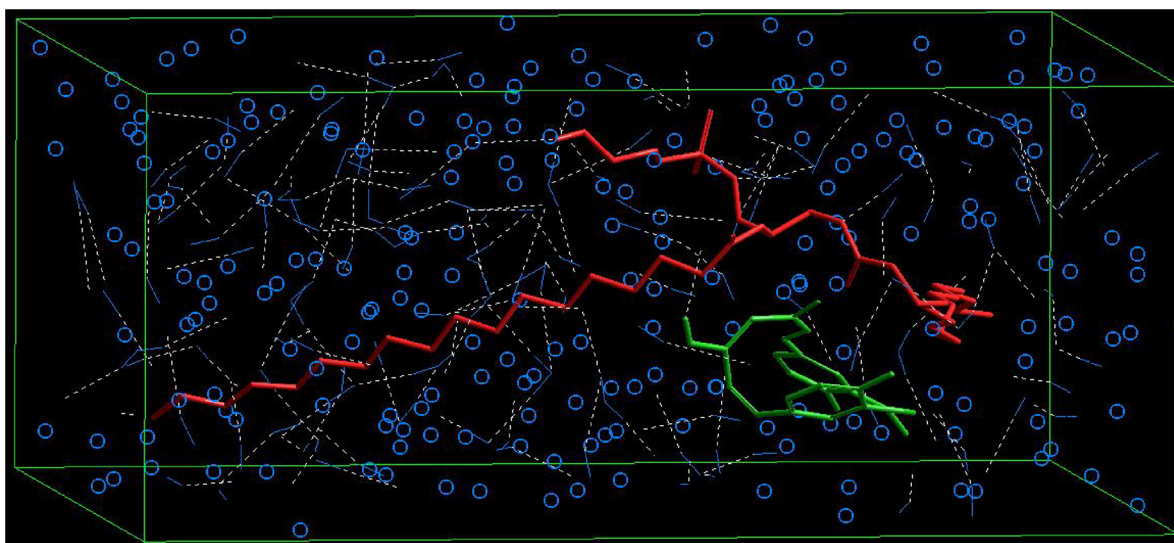


Fig. 6. Geometrical visualization of LCTN-CURC_{OUT} molecular complex after molecular mechanics simulations in aqueous phase. Colour coding of molecules: lecithin (red); curcumin (green); water (blue circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presents an interesting finding for the amphiphilic nature of the LCTN-CURCIN molecular complex.

- 2) Aqueous-phase simulation: The representative aqueous model displaying the conformational structure of the LCTN-CURCIN liposomal system is depicted in Fig. 6. The most important observation from the aqueous phase modelling was that the close curcumin fit within the LCTN-CURCIN liposomal system was now relaxed which may be due to the amphiphilic nature of lecithin showing higher affinity for water. However, the curcumin still remained in the close vicinity of the lipid chains. The final energetic profile of LCTN-

CURCIN liposomal system (−6786.715 kcal/mol) was more stabilized than the LCTN-aqueous (−6791.983 kcal/mol) and CURC-aqueous (−6799.062 kcal/mol) systems (Table 2). Interestingly, the LCTN-CURCIN liposomal system was primarily stabilised by the non-bonding interactions - van der Waals interactions, hydrogen-bond energy function, and electrostatic energy. Most importantly the H-bonding function was very prominent (−3.851 kcal/mol) which further confirmed the role of decreased logP values of lecithin when combined with curcumin. A decrease in logP of lecithin will make it less hydrophobic and hence more interactions with water

molecules and therefore more curcumin solubilisation potential.

4. Conclusion

The results of this study demonstrate that curcumin may become an attractive alternative for the treatment of asthma after being incorporated into a liposome, which will not only circumvent the low solubility of curcumin but also provides the prolonged release of curcumin leading to better patient compliance. The curcumin liposome was capable of suppressing the pro-inflammatory markers (IL-6, IL-8, IL-1 β and TNF- α) in BCI-NS1.1 cell line due to the combine effect of curcumin and phospholipid. The findings observed in our study can form the basis for future research and could be translated to other related diseases namely COPD and lung cancer. In addition, the application of nano-curcumin in the intervention of asthma, as a promising drug delivery system, would be of great interest to formulation, biological and respiratory scientists and clinicians.

Declarations of interests

None.

Acknowledgement

This research was supported by a grant from the International Medical University (IMU), Malaysia (Project ID: BPI-01/14(30)2017).

References

- [1] P.M. Hansbro, M.R. Starkey, J. Mattes, J.C. Horvat, Pulmonary immunity during respiratory infections in early life and the development of severe asthma, *Ann. Am. Thorac. Soc.* 11 (December Suppl. (5)) (2014) S297–S302.
- [2] CDC - Asthma - Data and Surveillance - Asthma Surveillance Data [Internet], (2018) [cited 2017 Oct 26] Available from <https://www.cdc.gov/asthma/asthmaadata.htm>.
- [3] Anonymous, Non-communicable Disease (NCD) Country Profile, World Health Organization, 2014.
- [4] Asthma in Malaysia [Internet], (2018) [cited 2017 Aug 13] Available from: <http://global-disease-burden.healthgrove.com/1/48209/Asthma-in-Malaysia>.
- [5] Management of Acute Exacerbations of Asthma in Adults - UpToDate [Internet], (2018) [cited 2017 Jun 10] Available from: https://www.uptodate.com.ezp.imu.edu.my/contents/management-of-acute-exacerbations-of-asthma-in-adults?source=search_result&search=asthma%20exacerbation&selectedTitle=1~150.
- [6] D. Lelli, A. Sahebkar, T.P. Johnston, C. Pedone, Curcumin use in pulmonary diseases: state of the art and future perspectives, *Pharmacol. Res.* 115 (January) (2017) 133–148.
- [7] H.-Y. Li, M. Yang, Z. Li, Z. Meng, Curcumin inhibits angiotensin II-induced inflammation and proliferation of rat vascular smooth muscle cells by elevating PPAR- γ activity and reducing oxidative stress, *Int. J. Mol. Med.* 39 (May (5)) (2017) 1307–1316.
- [8] A.K. Singh, Y. Jiang, S. Gupta, M. Younus, M. Ramzan, Anti-inflammatory potency of nano-formulated puerarin and curcumin in rats subjected to the lipopoly-saccharide-induced inflammation, *J. Med. Food* 16 (October (10)) (2013) 899–911.
- [9] Z. Fan, J. Yao, Y. Li, X. Hu, H. Shao, X. Tian, Anti-inflammatory and antioxidant effects of curcumin on acute lung injury in a rodent model of intestinal ischemia reperfusion by inhibiting the pathway of NF-Kb, *Int. J. Clin. Exp. Pathol.* 8 (April (4)) (2015) 3451–3459.
- [10] S.-Y. Teow, K. Liew, S.A. Ali, Khoo AS-B, S.-C. Peh, Antibacterial action of curcumin against *Staphylococcus aureus*: a brief review [Internet], *J. Trop. Med.* 2016 (2016) 10. Article ID 2853045, [cited 2017 Oct 10] Available from <https://www.hindawi.com/journals/jtm/2016/2853045/>.
- [11] G.-Q. Wu, K.-Q. Chai, X.-M. Zhu, H. Jiang, X. Wang, Q. Xue, et al., Anti-cancer effects of curcumin on lung cancer through the inhibition of EZH2 and NOTCH1, *Oncotarget* 7 (May (18)) (2016) 26535–26550.
- [12] S.-W. Oh, J.-Y. Cha, J.-E. Jung, B.-C. Chang, H.-J. Kwon, B.-R. Lee, et al., Curcumin attenuates allergic airway inflammation and hyper-responsiveness in mice through NF- κ B inhibition, *J. Ethnopharmacol.* 136 (July (3)) (2011) 414–421.
- [13] L. Liu, Y. Shang, M. Li, X. Han, J. Wang, J. Wang, Curcumin ameliorates asthmatic airway inflammation by activating nuclear factor-E2-related factor 2/haem oxygenase (HO)-1 signalling pathway, *Clin. Exp. Pharmacol. Physiol.* 42 (May (5)) (2015) 520–529.
- [14] M. Farazuddin, B. Dua, Q. Zia, A. Khan, B. Joshi, M. Owais, Chemotherapeutic potential of curcumin-bearing microcells against hepatocellular carcinoma in model animals, *Int. J. Nanomed.* (March (9)) (2014) 1139–1152.
- [15] T.M. Allen, C.B. Hansen, D.E. Lopes de Menezes, Pharmacokinetics of long circulating liposomes, *Adv. Drug Deliv. Rev.* 16 (1995) 267–284.
- [16] R. Panchagnula, Review: pharmaceutical aspects of paclitaxel, *Int. J. Pharm.* 172 (1998) 1–15.
- [17] M. Vashisht, P. Rani, S.K. Onteru, D. Singh, Curcumin encapsulated in milk exosomes resists human digestion and possesses enhanced intestinal permeability in vitro, *Appl. Biochem. Biotechnol.* (May) (2017).
- [18] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: from concept to clinical applications, *Adv. Drug Deliv. Rev.* 65 (January (1)) (2013) 36–48.
- [19] E. García Esteban, M.J. Cózar-Bernal, A.M. Rabasco Álvarez, M.L. González-Rodríguez, A comparative study of stabilizing effect and antioxidant activity of different antioxidants on levodopa-loaded liposomes, *J. Microencapsul.* (June) (2018) 1–33, <https://doi.org/10.1080/02652048.2018.1487473> [Epub ahead of print].
- [20] D.K. Chellappan, Z.Y. Ng, J.Y. Wong, A. Hsu, P. Wark, N. Hansbro, J. Taylor, J. Panneerselvam, T. Madheswaran, G. Gupta, M. Bebawy, P.M. Hansbro, K. Dua, Immunological axis of curcumin-loaded vesicular drug delivery systems, *Future Med. Chem.* 10 (April (8)) (2018) 839–844, <https://doi.org/10.4155/fmc-2017-0245> Epub 2018 Apr 5.
- [21] T. Zhang, Y. Chen, Y. Ge, Y. Hu, M. Li, Y. Jin, Inhalation treatment of primary lung cancer using liposomal curcumin dry powder inhalers, *Acta Pharm. Sin.* B 8 (May (3)) (2018) 440–448, <https://doi.org/10.1016/j.apsb.2018.03.004> Epub 2018 May 3.
- [22] K. Dua, T. de Jesus Andreoli Pinto, D.K. Chellappan, G. Gupta, M. Bebawy, P.M. Hansbro, Advancements in nano drug delivery systems: a challenge for bio-films in respiratory diseases, *Panminerva Med.* 60 (March (1)) (2018) 35–36, <https://doi.org/10.23736/S0031-0808.18.03402-X>.
- [23] K. Dua, S.D. Shukla, T. de Jesus Andreoli Pinto, P.M. Hansbro, Nanotechnology: advancing the translational respiratory research, *Interv. Med. Appl. Sci.* 9 (March (1)) (2017) 39–41, <https://doi.org/10.1556/1646.9.2017.1.02>.
- [24] Z.L. Li, S.F. Peng, X. Chen, Y.Q. Zhu, L.Q. Zou, W. Liu, C.M. Liu, Pluronic modified liposomes for curcumin encapsulation: sustained release, stability and bioaccessibility, *Food Res. Int.* 108 (June) (2018) 246–253, <https://doi.org/10.1016/j.foodres.2018.03.048> Epub 2018 Mar 20.
- [25] D.K. Chellappan, P.M. Hansbro, K. Dua, A. Hsu, G. Gupta, Z.Y. Ng, J.Y. Wong, J. Chellian, J. Panneerselvam, Vesicular systems containing curcumin and their applications in respiratory disorders - a mini review, *Pharm. Nanotechnol.* 5 (4) (2017) 250–254, <https://doi.org/10.2174/2211738505666170808094635>.
- [26] K. Dua, N.G. Hansbro, P.S. Foster, P.M. Hansbro, MicroRNAs as therapeutics for future drug delivery systems in treatment of lung diseases, *Drug Deliv. Transl. Res.* 7 (February (1)) (2017) 168–178, <https://doi.org/10.1007/s13346-016-0343-6> Review.
- [27] T. Ding, T. Li, J. Li, Impact of curcumin liposomes with anti-quorum sensing properties against foodborne pathogens *Aeromonas hydrophila* and *Serratia grimesii*, *Microb. Pathog.* (June (122)) (2018) 137–143, <https://doi.org/10.1016/j.micpath.2018.06.009> [Epub ahead of print].
- [28] A. Sesarman, L. Tefas, B. Sylvester, et al., Anti-angiogenic and anti-inflammatory effects of long-circulating liposomes co-encapsulating curcumin and doxorubicin, on C26 murine colon cancer cells, *Pharmacol. Rep.* 70 (2) (2018) 331–339.
- [29] L.R. Tefas, B. Sylvester, I. Tomuta, et al., Development of antiproliferative long-circulating liposomes co-encapsulating doxorubicin and curcumin, through the use of a quality-by-design approach, *Drug Des. Devel. Ther.* 11 (2017) 1605–1621.
- [30] M. Hasan, S. Latifi, C.J. Kahn, A. Tamayol, R. Habibey, E. Passeri, M. Linder, E. Arab-Tehrany, The positive role of curcumin-loaded salmon nanoliposomes on the culture of primary cortical neurons, *Mar. Drugs* 16 (2018) 218.
- [31] Angela Storka, Brigitta Vcelar, Uros Klikovic, Ghazaleh Gouya, Stefan Weisshaar, Stefan Aschauer, Gordon Bolger, Lawrence Helson, Michael Wolzt, Safety, tolerability and pharmacokinetics of liposomal curcumin in healthy humans, *Int. J. Clin. Pharmacol. Ther.* 53 (January) (2018) 54–65.
- [32] H.X. Ong, F. Benaouda, D. Traini, D. Cipolla, I. Gonda, M. Bebawy, B. Forbes, P.M. Young, In vitro and ex vivo methods predict the enhanced lung residence time of liposomal ciprofloxacin formulations for nebulisation, *Eur. J. Pharm. Biopharm.* 86 (2014) 83–89, <https://doi.org/10.1016/j.ejpb.2013.06.024>.
- [33] Pall Thor Ingvarsson, Ida Svahn Rasmussen, Michelle Viaene, Przemyslaw Jedrzej Irljak, Hanne Morck Nielsen, Camilla Foged, The surface charge of liposomal adjuvants is decisive for their interactions with the Calu-3 and A549 airway epithelial cell culture models, *Eur. J. Pharm. Biopharm.* 87 (3) (2014) 480–488.
- [34] F. Huang, H. Zhang, M. Wu, H. Yang, M. Kudo, C.J. Peters, P.G. Woodruff, O.D. Solberg, M.L. Donne, X. Huang, D. Sheppard, J.V. Fahy, P.J. Wolters, B.L. Hogan, W.E. Finkbeiner, M. Li, Y.N. Jan, L.Y. Jan, J.R. Rock, Calcium-activated chloride channel TMEM16A modulates mucin secretion and airway smooth muscle contraction, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) 16354–16359.
- [35] Kirk B. Lane, Brian Egan, Sherell Vick, Rasul Abdolrasulnia, Virginia L. Shepherd, Characterization of a rat alveolar macrophage cell line that expresses a functional mannose receptor, *J. Leukoc. Biol.* 64 (September (3)) (1998) 345–350.
- [36] Y. Chen, Q. Wu, Z. Zhang, L. Yuan, X. Liu, L. Zhou, Preparation of curcumin-loaded liposomes and evaluation of their skin permeation and pharmacodynamics, *Molecules* 17 (May (5)) (2012) 5972–5987.
- [37] P. Das, K. Kumar, A. Nambiraj, R. Rajan, R. Awasthi, K. Dua, et al., Potential therapeutic activity of Phlogacanthus thyrsoformis Hardw (Mabb) flower extract and its biofabricated silver nanoparticles against chemically induced urolithiasis in male Wistar rats, *Int. J. Biol. Macromol.* 103 (October) (2017) 621–629.
- [38] V. Velu, M. Das, N.A.N. Raj, K. Dua, H. Malipreddi, Evaluation of in vitro and in vivo anti-urolithiatic activity of silver nanoparticles containing aqueous leaf extract of *Tragia involucrata*, *Drug Deliv. Transl. Res.* 7 (June (3)) (2017) 439–449.
- [39] J.R. Madan, N.P. Ghuge, K. Dua, Formulation and evaluation of proniosomes containing lornoxicam, *Drug Deliv. Transl. Res.* 6 (October (5)) (2016) 511–518.
- [40] L. Kedzierski, M.D. Tate, A.C. Hsu, T.B. Kolesnik, E.M. Linossi, L. Dagley, et al., Suppressor of cytokine signaling (SOCS)5 ameliorates influenza infection via inhibition of EGFR signaling, *eLife* 14 (February) (2017) 6.

- [41] Z. Jevnikar, J. Östling, E. Ax, J. Calvén, K. Thörn, E. Israelsson, et al., Epithelial IL-6 trans-signaling defines a new asthma phenotype with increased airway inflammation, *J. Allergy Clin. Immunol.* (June) (2018), <https://doi.org/10.1016/j.jaci.2018.05.026> pii: S0091-6749(18)30847-9 [Epub ahead of print].
- [42] J. Ren, Y. Sun, G. Li, X.J. Zhu, J.G. Cui, Tumor necrosis factor- α , interleukin-8 and eosinophil cationic protein as serum markers of glucocorticoid efficacy in the treatment of bronchial asthma, *Respir. Physiol. Neurobiol.* (June) (2018), <https://doi.org/10.1016/j.resp.2018.06.004> pii: S1569-9048(18)30176-9 [Epub ahead of print].
- [43] Paulina Sobkowiak, Irena Wojsyk-Banaszak, Maja Kowalewska, Eliza Wasilewska, Wojciech Langwinski, Zdzisława Kycler, et al., Interleukin 1 β polymorphism and serum level are associated with pediatric asthma, *Pediatr. Pulmonol.* 52 (12) (2017) 1565–1571.
- [44] P. Kumar, Y.E. Choonara, L.C. Toit, G. Modi, D. Naidoo, V. Pillay, Novel high-viscosity polyacrylamidated chitosan for neural tissue engineering: fabrication of anisotropic neurodurable scaffold via molecular disposition of persulfate-mediated polymer slicing and complexation, *Int. J. Mol. Sci.* 13 (2012) 13966–13984.
- [45] L.C. Du Toit, T. Govender, T. Carmichael, P. Kumar, Y.E. Choonara, V. Pillay, Design of an anti-inflammatory composite nanosystem and evaluation of its potential for ocular drug delivery, *J. Pharm. Sci.* 102 (2013) 2780–2805, <https://doi.org/10.1002/jps.23650>.
- [46] S.A. Bhai, V. Yadav, Y. Mamatha, V.V. Prasanth, Liposomes: an overview, *J. Pharm. Sci. Innov.* 1 (February (1)) (2012) 13–21.
- [47] M.L.T. Zweers, D.W. Grijpma, G.H.M. Engbers, J. Feijen, The preparation of monodisperse biodegradable polyester nanoparticles with a controlled size, *J. Biomed. Mater. Res. B Appl. Biomater.* 66 (August (2)) (2003) 559–566.
- [48] N.R. Labiris, M.B. Dolovich, Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications, *Br. J. Clin. Pharmacol.* 56 (December (6)) (2003) 588–599.
- [49] J.-P. Colletier, B. Chaize, M. Winterhalter, D. Fournier, Protein encapsulation in liposomes: efficiency depends on interactions between protein and phospholipid bilayer, *BMC Biotechnol.* 10 (May (2)) (2002) 9.
- [50] J. Aukunuru, J. Sreekanth, G. Naresh, B. Madhu, R.B. Chandrasekhar, K. Prabhakar, Preparation, characterization and evaluation of hepatoprotective activity of an intravenous liposomal formulation of Bis-Demethoxy curcumin analogue (BDMCA), *Int. J. Drug Dev. Res.* 1 (1) (2009) 37–46.
- [51] N. Baran, V.K. Singh, K. Pal, A. Anis, D.K. Pradhan, K. Pramanik, Development and characterization of soy lecithin and palm oil-based organogels, *Polym.-Plast. Technol. Eng.* 53 (June (9)) (2014) 865–879.
- [52] O. Kleiner, J. Ramesh, M. Huleihel, B. Cohen, K. Kantarovich, C. Levi, et al., A comparative study of gallstones from children and adults using FTIR spectroscopy and fluorescence microscopy, *BMC Gastroenterol.* 1 (February (2)) (2002) 3.
- [53] DFT and experimental studies of the structure and vibrational spectra of curcumin - kolev, *International Journal of Quantum Chemistry*, Wiley Online Library, 2005 [Internet] [cited 2017 Oct 11] Available from: <http://onlinelibrary.wiley.com/doi/10.1002/qua.20469/abstract>.
- [54] K. Pr, G. Sreelakshmi, M. Vayalappil, R. Joseph, Water soluble complexes of curcumin with cyclodextrins: characterization by FT-Raman spectroscopy, *Vib. Spectrosc.* (September (5)) (2012) 62.
- [55] K.N. Baglale, P.G. Boland, B.D. Wagner, Fluorescence enhancement of curcumin upon inclusion into parent and modified cyclodextrins, *J. Photochem. Photobiol. Chem.* 173 (July (3)) (2005) 230–237.
- [56] M.R. Edwards, N.W. Bartlett, D. Clarke, M. Birrell, M. Belvisi, S.L. Johnston, Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease, *Pharmacol. Ther.* 121 (January (1)) (2009) 1–13.
- [57] B. Kloesch, L. Gober, S. Loebisch, B. Vcelar, L. Helson, G. Steiner, In vitro study of a liposomal curcumin formulation (LipocurTM): toxicity and biological activity in synovial fibroblasts and macrophages, *In Vivo* 30 (July (4)) (2016) 413–419.
- [58] Y. Xu, L. Liu, Curcumin alleviates macrophage activation and lung inflammation induced by influenza virus infection through inhibiting the NF- κ B signaling pathway, *Influenza Other Respir. Viruses* (June) (2017).
- [59] C.R. Scholfield, H.J. Dutton, F.W. Tanner, J.C. Cowan, Components of “soybean lecithin,” *J. Am. Oil Chem. Soc.* 25 (October (10)) (1948) 368–372.
- [60] I. Treede, A. Braun, P. Jeliaskova, T. Giese, J. Füllekrug, G. Griffiths, et al., TNF- α -induced up-regulation of pro-inflammatory cytokines is reduced by phosphatidylcholine in intestinal epithelial cells, *BMC Gastroenterol.* 9 (July (13)) (2009) 53.