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The Behavior of Nanovesicles of Physicochemical Profiles: Compose of β-Cyclodextrin and Cisplatin for Anti-Tumor Activity and Decrease Toxicity Affected Normal Surrounding Tumor Tissues

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Abstract

CP (cisplatin) was entrapped in lipid bilayer and β-CD-CP (β-cyclodextrin-CP) was entrapped in hydrophilic bilayer both were prepared by chloroform film method with sonication. The entrapment efficiency of CP-LPS (CP-Liposome) and β-CD-CP-LPS were 83.44 and 82.20 %, respectively, two-tailed t-test and no significant difference at the confidence interval, $p < 0.05$. Size distribution of CP-LPS and β-CD-CP-LPS were investigated by DLS were in range 322 and 1033 nm, respectively. CP release from CP-LPS or β-CD-CP-LPS exposed to physiological conditions as PBS at 37 °C. CP from both LPS formulations have showed an initial burst release around < 17 and < 10 %, respectively. CP-LPS and β-CD-CP-LPS maintained as plateau phase of both for four days and seven days, respectively. CP-LPS showed % cumulative controlled release CP of CP-LPS and β-CD-CP-LPS were about 15 - 16 and 10 - 11 %, respectively. CP-LPS and β-CD-CP-LPS released until 83.6 % for six days and 82 % for eight days, respectively. CP released from CP-LPS and β-CD-CP-LPS were in a controlled deportment for 10 days. β-CD-CP-LPS, CP molecules bound β-CD was exhibited in dynamic equilibrium with free drug molecules which ascribes the controlled release behavior of β-CD-CP-LPS to quietly release and prolong action, diminish dose of toxicity of cancer drug that effected to normal tissues that surrounding tumor tissue. β-CD was oligosaccharides,an attracted tumor, tumor snatch themselves as nutrient consume more than the normal tissues. Thus, β-CD-CP-LPS and low dose of normal dose of CP can damage cancer cells. This synergistic effect was saved for normal cells which surrounding tumor tissue.

Keywords: Cisplatin, Nanovesicles, Hydroxypropyl β-cyclodextrin, HeLa Cells, SRB Assay, Liposomes, Anti-Tumor activity

Introduction

Nanotechnology improved and cured cancer, including nanomedicine, nanomagnetic, nanosilver, nanowires, nanotube, nano graphite and nanoscale [1]. Most nanotechnology research is concerned with medical studies to cure multiple diseases. Nano-theory in the medical survey has given potential delivery drugs which send the drugs, biologics, biosimilars, peptides, genes, Messenger RNA (mRNA), etc., to the targeted site or expect receptors or positions to cure diseases. Recently, COVID-19 has spread around the world. Recently, the pandemic of COVID-19 with the World Health Organisation (WHO). Two mRNA vaccines entrapped in lipid nanovesicles and developed by Pfizer/BioNTech and Moderna have registered in the US FDA's Emergency Use Authorization (EUA) [2]. Nanovesicles (NVs) can facilitate the intracellular delivery of the mRNA to the targeted site [3]. Nevertheless, nanotechnology has improved the delivery of cancer drugs to the targeted area. In many cases continues, cancer therapy is in advance. The concept of encapsulation of drugs in lipid-based-nano vesicular systems is modified by using lipid bilayers and hydrophilic bilayers and core, respectively. It modifies hydrophobic and hydrophilicity to diminish the improved toxicity of normal tissues surrounding cancer tissues and improve the drug's stability, circulation time and absorption. Many commercial drug delivery systems, such as Liposomes (LPSs)/niosomes, have already been stored with outstanding achievement [4]. Nanovesicle materials entrap anticancer drugs to improve potential drugs, reduce drug toxicity, sustain release, deliver the drug to the targeted site and diminish the dose of the medicine. Thus, they enhance the cancer patient's quality of life. Furthermore, nanotechnology impacts the biological area, and many materials and carrier systems exist. Non-rigid nanocarriers are many different pathways of drug delivery systems that can carry drugs to a tumor, including inorganic carriers, lipid-based carriers and polymer-carriers that utilize molecular biology [5]. Nanocarrier systems have improved controlled action because they can be externally functionalized to deliver the drug

to a specific targeted site. They are in intracellular environments till they are released to the targeted tumor cells. The main properties of organic nano materials' are their biodegradable nanocarriers and effortless elimination gets rid of the body. The modulated immunotherapies and anticancer immune responses for cancer elimination. They have been improving cancer treatments. The function of nanomaterial, various lading delivery, reactivity with ligand cells, and facilitated imaging efficient biological or chemical improvement of cell target and application of cancer therapeutics. Nanotechnology has a promising development for cancer treatment. [6] Nanocarriers can equip efficiently and reduce the dose of toxicity anticancer agents. LPSs and niosomes can employ the permeability improvement and effect of retention time for advanced extravasation from cancerous vessels [7]. Anthracyclines drugs such as daunorubicin and doxorubicin are formed in LPSs. They have completed competent drug encapsulation and significantly prolonged circulation LPS and pegylated doxorubicin LPS.

Cyclodextrins (CDs) were oligosaccharides. They are formed by glucose units bound together in crown shape, encouraging the capability to form complexes with molecules of the drug, especially hydrophobic drugs, and increase physicochemical properties absent molecular drug modifications. Composition of the water-soluble drug, CD complexes can increase drug permeation through biological membranes and have a nontoxic effect on normal cells and safety for humans [8]. In binding forces of CDs in complex formation are a) van der Waals type interactions (or hydrophobic interactions) between the lipophilic unit of the guest molecules and the CD cavity. b) The hydrogen bond between the polar functional groups of the guest molecules and the hydroxyl groups of the CD. c) Release of high-energy water molecules from the cavity in the complex formation process. d) Release of strain energy into the crown structure system of the CD [9]. e) Controlled release to optimize pharmacotherapy, drug release is controlled in conformity with the active substances' therapeutic purpose. CDs have significantly increased control of the rate or time of delivery [10]. The multifunctional characteristics of CDs allow them to be used in most drug delivery systems [11]. Mostly, cytotoxic drugs have poor hydrophiles. These molecules are joined together with poor physicochemical and biopharmaceutical properties. However, poor hydrophilic dissolution and solubility are the 2 critical point of factors that affect the formulations and modified process of drugs and limit their therapeutic application through a different route of drug administration, which is poorly soluble and belongs to class b) or e) of the biopharmaceutical classification system (BCS) [11]. Most cytotoxic anticancer drugs belong to the BCS class e), which comprises substances with low solubility in aqueous fluids and low apparent permeability. Other pharmaceutical techniques are the same solvency and solid dispersion and can enhance the drug's solubility, bioavailability, and dissolution properties. These methods suffer disadvantages, such as low drug loading and large dose. CD complexation came into living and presented great attention. Gidwani and Vyas [12] report that in the 21st century, employing double advances (CDs and nanotechnology) have appeared, which is a novel plan to tackle such formulation problems [12]. Furthermore, the extraordinary highlights of these systems (CD and nanotechnology) will concurrently propose additional ways to treat cancer successfully. Moreover, the CD is necessary to escapade the utility of these CD-based nanovesicles using *in vivo* for tumor targeting and toxicity studies of cancer-like life-threatening diseases. In the future, CDs could be employed to modify potent anticancer drugs to achieve effective treatment [9].

Cisplatin (CP) is an anticancer drug. CP requires restriction in high doses and short term for the treatment of cancer since CP-toxicity [13]. CP is used to treat a variety of tumors, but dose-limiting toxicities or intrinsic and acquired resistance limit its application in many types of cancer. The ability of cis-diamminedichloroplatinum (II), cisplatin, to inhibit the growth of cancerous cells by interfering with transcription and other Deoxyribonucleic acids (DNA)-mediated cellular functions has been evaluated over the past 30 years. CP is highly effective against several forms of cancer, most remarkably testicular tumors, and it is also ordinarily used to treat breast, ovarian, bladder, lung, head and neck cancer. Lung cancer is resistant to CP chemotherapy due to poor targeting and the development of resistance. Overwhelm, the CP dosage can be elevated, but not absent serious side effects because cisplatin is the most potent Pt anticancer member of the anticancer drug family, and its potential use in cancer [13,14].

The mechanism of action of CP adheres to DNA damage which is the reason to program cell death. It may be due to CP treating several different cancers. Therefore, CP has been chosen as the model of study. The aim of CP was selected as the model of research. CP was encapsulated in LPS, and the study behavior profiles of CP and β-CD-CP were modified to CP-LPS. β-cd-cp-LPs evaluated the physics, chemicals and stability of LPSs and the hypothesis of this research, CP-LPS and β-CD-CP-LPS treatment on cancer cells to evaluate the cytotoxicity of drug-loaded LPSs.

Materials and methods

Materials

CP (CP-Diammineplatinum dichloride) powder, L-alpha-dipalmitoyl phosphatidylcholine (DPPC, 99.6 %), Cholesterol (CHL, 99.6 %), Sulforhodamine B (SRB), DMEM medium, trypsin, L-glutamine and penicillin-streptomycin were from Sigma-Aldrich® Chemical Co., St. Louis, MO, USA. Fetal bovine serum (FBS) (Gibco BRL, Burlington, Canada). Hydroxypropyl beta cyclodextrin (HPβCD, Kleptose® HPB) was from Roquette-Freres, Lestrem Cedex, France. All solvents were from Fluka, Germany. All plastics for cell culture were obtained from NUNC™ (Denmark). HeLa, JCRB9004 (A.N.H. SCIENTIFIC, JAPAN).

Preparation of CP inclusion complex

The 1:1 molar ratio of CP: HPβCD was used to prepare β-CD-CP. CP was dissolved in deionized water, added HPβCD solution, and stirred for 25 min. The solution was lyophilized (Christ FOC-1 Model K-40 equipment, Balzers-Pfeiffer GmbH, Asslar, Germany) [8-10]. The dried product was ground, sieved through a 315-mm mesh [8], and identified by Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu 8501 spectrophotometer, Japan), Differential scanning calorimetry (DSC, Perkin-Elmer DSC-7 Model) and X-ray diffractometry (Siemens D-500 diffractometer).

Preparation of the drug-loaded NVs

Liposomes were composed of DPPC/CHL (6:4, molar ratio) 40 mM, and NVs were prepared by chloroform film-forming method with sonication [8]. Chloroform was evaporated using a rotary evaporator (R-124 Buchi, Switzerland) to obtain a thin film. Then, the film was dried overnight under a vacuum at 25 ℃. The film was then hydrated to obtain multilamellar vesicles using purified water or CP-LPS or β-CD-CP-LPS (20 mM equivalence to CP) solution with mechanical agitation at 60 ℃ for 30 min. The dispersion was sonicated at 20 kHz for 5 min using a probe-type ultrasonic generator (Vibra Celle, Sonics & Materials Inc. Newtown, CT, USA).

NVs morphology with light microscopy and transmission electron microscopy (TEM)

LPSs, CP-LPSs and β-CD-CP-LPSs were characterized by optical microscopy with transmitted light differential interference contrast (CHS 3E0245, Olympus Optical Co. Japan) and by transmission electron microscopy (TEM1200S, JEOL, Tokyo, Japan). The lamellarity of the NVs was examined by TEM using negative staining method with uranyl acetate (1 % w/v) as a contrast agent [8]. A minimum of 100 vesicles was examined for each formulation.

Physical characteristics of LPSs

The morphology and vesicular sizes of Liposomal of NVs were determined by dynamic light scattering (DLS) (Zetasizer Nano Series Nano-S, Malven instrument) [8].

Physical structure of drug-XRD

XRD patterns used obtained from a Siemens D-500 diffractometer. The source was determined at wavelength 0.70930 Å, operating with Mo-tube radiation at 50 kV and 40 mA. The samples were analyzed between 2θ angles of 5 and 60 ° at a scan rate of 1 min^{-1}. There were CP, hydroxypropyl β-cyclodextrin (HPβCD), β-CD-CP [8].

Encapsulation efficiency determination of drug-loaded NVs

The CP-LPS or β-CD-CP-LPS were separated from the not entrapped drugs by gel filtration using Sepharose CL 6 B (Fluka Chemicals, Gillingham, Dorset, UK) as a packing material and purified water as an eluent. Eluates were collected in fractions using a fractional collector (Foxy JR, Isco Inc. Lincoln, USA) at the flow rate of 10 mL/min. Then eluates were pooled, collected, and dried with a Centrivap Console (Labconco, Kansas City, Missouri) [8,16]. The percentage of drug encapsulation in bilayer vesicles was calculated from the ratio of drug entrapped in the vesicles to the total initial drug content multiplied by 100. All experiments were performed in triplicate.

Stability of CP-LPS dispersion

The liposomal NVs were kept in transparent vials and stored at room temperature 30 ± 2, 45 ± 2 and 4 ± 2 ℃ for about 3 months. The physical stability of the dispersions was observed visually. The fractions

containing the drug-loaded vesicles were analyzed with UV spectroscopy (Perkin Elmer Lambda Bio UV/Vis Spectrometer) at 301 nm [15-18].

Controlled release of CP in various formulations

For drug release experiments, 20 mM of CP (CP, CP-LPS and β-CD-CP-LPS were calculated equivalence to CP) incubated for 10 days in 100 mL of PBS at 37 ℃ whereas being constantly stirred at 50 rpm by using Dissolution Testers USP, Digital Dissolution Tester DT 950 Series (ERWEKA DT 950). All experiments were performed in triplicate. The supernatants of these samples were analyzed with UV spectroscopy (Perkin Elmer Lambda Bio UV/Vis Spectrometer) at 301 nm [15-18] to determine the amount of CP that had been released compared with a standard curve. CP encapsulation efficiency (%) was determined by the ratio of the drug encapsulated in the NVs and the initial amount of drug used in the process. The experiment selected an aliquot at the same time for 10 days.

Cytotoxicity of drug-loaded LPSs

Cytotoxicity of drug-loaded LPSs determined by SRB colorimetric assay SRB assay is a standard assay for detecting cell growth and cytotoxicity. The effect of the samples on the growth of HeLa was evaluated according to the procedure of the NCI for the *in vitro* anticancer drug screening using the protein-binding dye SRB to assess cell growth [8]. Cells were maintained as adherent cell cultures in DMEM medium supplemented with 5 % FBS, 1 % L-glutamine and 1 % penicillin-streptomycin at 37 ℃ in a humidified air incubator containing 5 % CO_2 HeLa was plated in 96-well plates and allowed to attach overnight. Cells were then exposed to serial concentrations of the samples for 24 h. After incubation, the adherent cells were fixed in situ, washed, and dyed with SRB. The bound dye was solubilized by 10 mM Tris base, and the absorbance was measured at 550 nm in a microplate reader [8,17]. The dose-response curves were generated, and the relative growth inhibition, corresponding to the concentration of the samples that inhibited the cell growth, was determined as previously described.

Results and discussion

Results

Morphology of NVs encapsulating CP

CP-LPS and β-CD-CP-LPS were prepared. There were exhibited in white translucent nanovesicular dispersions forms and white lyophilized forms, as shown in **Figures 1** and **2**, respectively. The size and morphology of all prepared LPSs were characterized by optical microscopy with transmitted light differential interference contrast, as shown in **Figure 3**, and by TEM. The lamellarity of the particles was examined by TEM using the negative staining method with uranyl acetate (1 % w/v) as a contrast agent, as shown in **Figure 4**. A minimum of 100 particles was examined for each formulation. **Figure 5** shows the morphology and estimated vesicular sizes of both LPSs. **Table 1** exhibited formulations in size and the distribution of various nano vesicular formulations by Dynamic Light Scattering, DLS (Zetasizer Nano Series Nano-S, Malven instrument). Blank LPS (DPPC/CHL 6:4) showed average vesicular size in diameter as 48.71 nm (100 % number), CP-LPS (DPPC/CHL 6:4) was 322 nm (100 % numbers) and Neutral β-CD-CP-LPS (DPPC/CHL 6:4) was 756.32 (100 of % numbers, respectively).

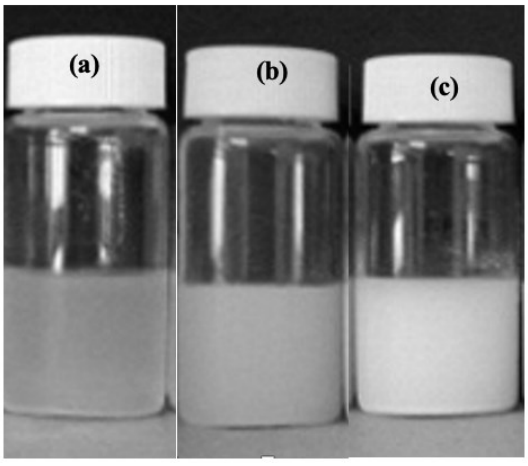


Figure 1 NVs Formulations in emulsion forms: (a) Blank LPS, (b) CP-LPS, and (c) β-CD-CP-LPS.

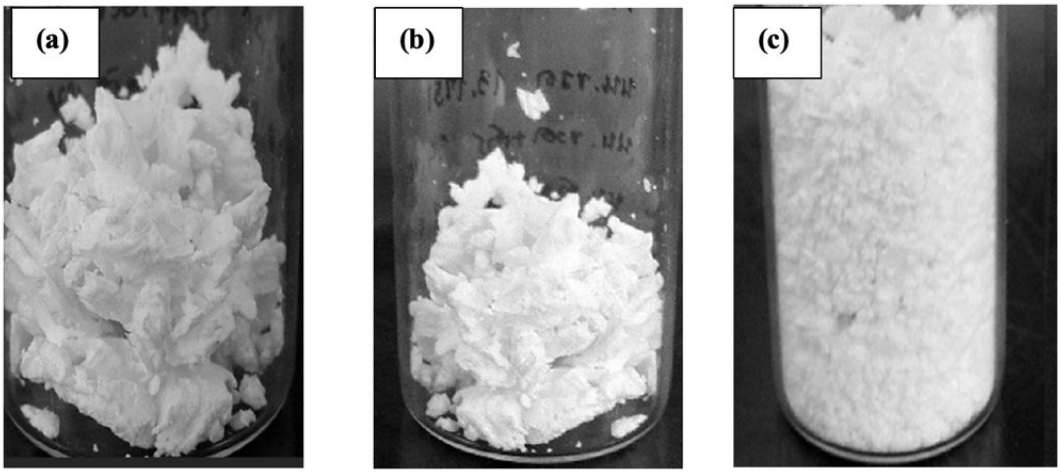


Figure 2 NVs Formulations in lyophilized forms: (a) Blank LPS**,** (b) CP-LPS, and (c) β-CD-CP-LPS.

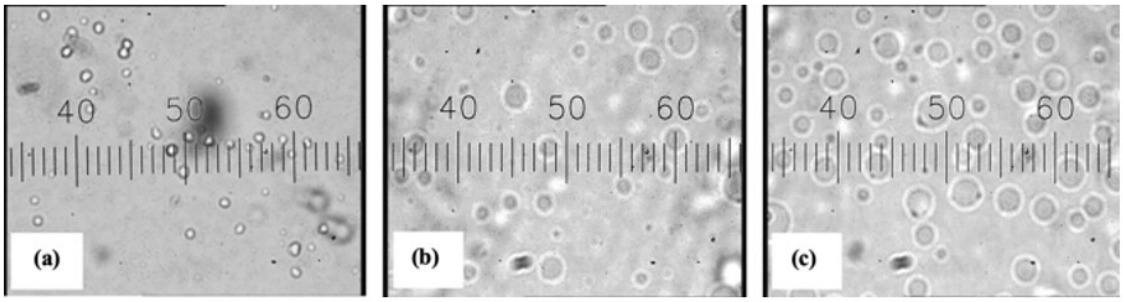


Figure 3 The size and morphology of all prepared NVs were characterized by optical microscopy with transmitted light differential interference contrast: (a) Blank LPS, (b) CP-LPS, and (c) β-CD-CP-LPS.

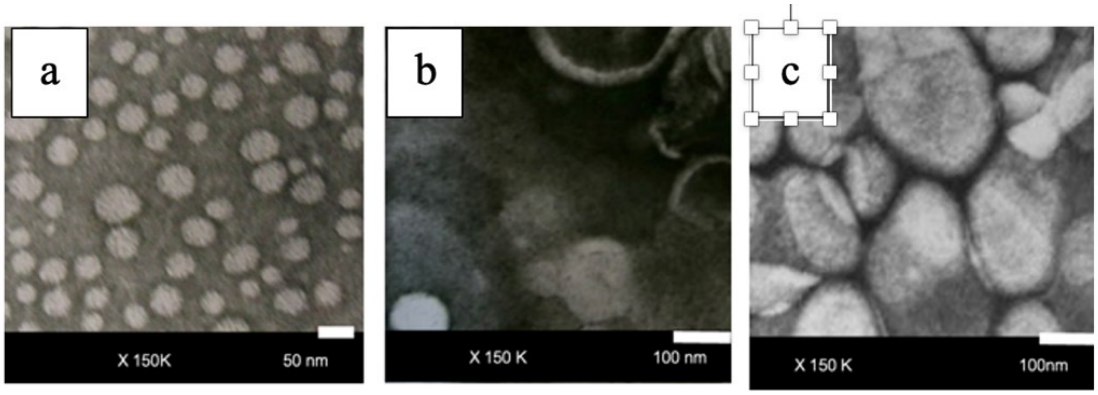


Figure 4 The size and morphology of all prepared NVs were characterized by transmission electron microscopy (TEM): **(**a) Blank LPS (×150K), (b) CP-LPS (×150K), and (c) β-CD-CP-LPS (×150K).

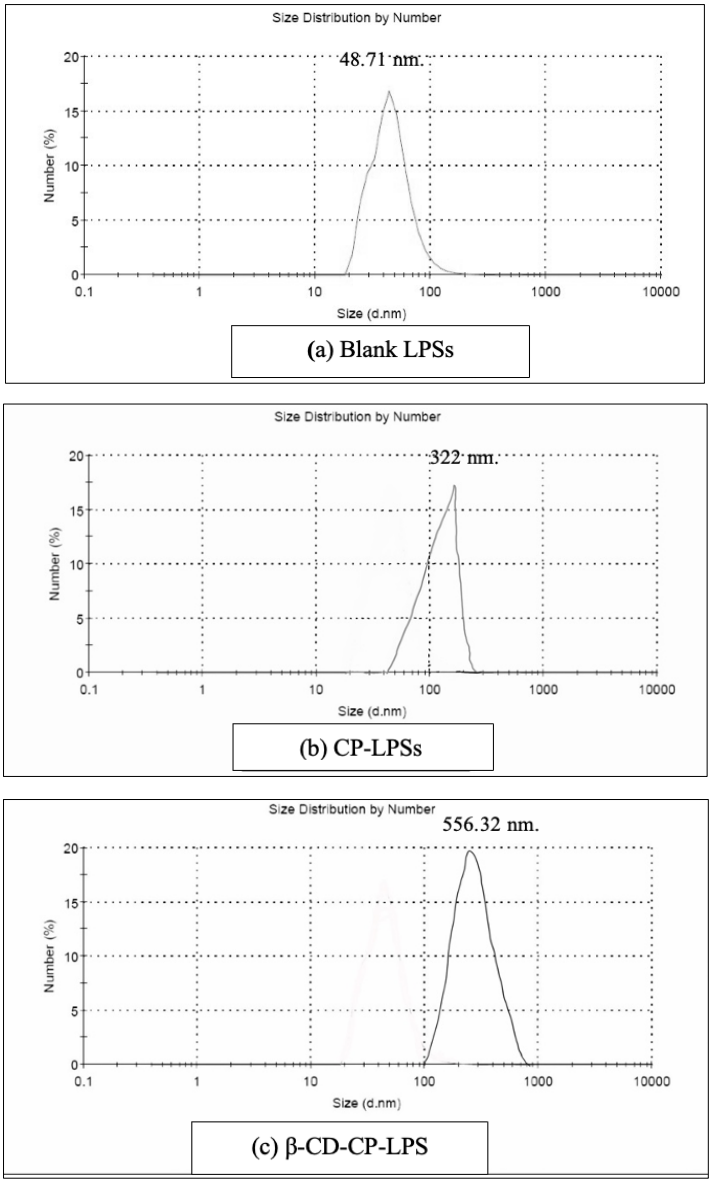


Figure 5 Chromatogram of the size distribution of various nanovesicular formulations determined by DLS (Zetasizer Nano Series Nano-S, Malven instrument).

Table 1 Size distribution of various nano vesicular formulations determined by DLS.

Formulations	Average vesicular size (Diameter, nm)	%Number
Neutral (DPPC/CHL 6:4) LPS	48.71	100
Neutral (DPPC/CHL 6:4) CP- LPS	322	100
Neutral (DPPC/CHL 6:4) β-CD-CP-LPS	756.32	100

Evidence of inclusion complex of β-CD-CP compare with HPβCD and CP into XRD

The schematic of Hydroxypropyl β-cyclodextrin (HPβCD) and the inclusion complex of CP in HPβCD has shown in **Figure 6**, which was exhibited the concept of inclusion complex between CP and HPβCD. Besides, X-ray is the widespread technique to analyze the structure of crystals of the prepared complexes-forming. The outer surfaces of the crystals showed the unique characteristic of surface originating from the different atomic or molecule structures arrangement. Compared to other complex-

forming methods, β-CD-CP showed a more similar peak to HPβCD (**Figure 7**). They indicated that this method led the CP to completely enter the cavity of HPβCD [16-18].

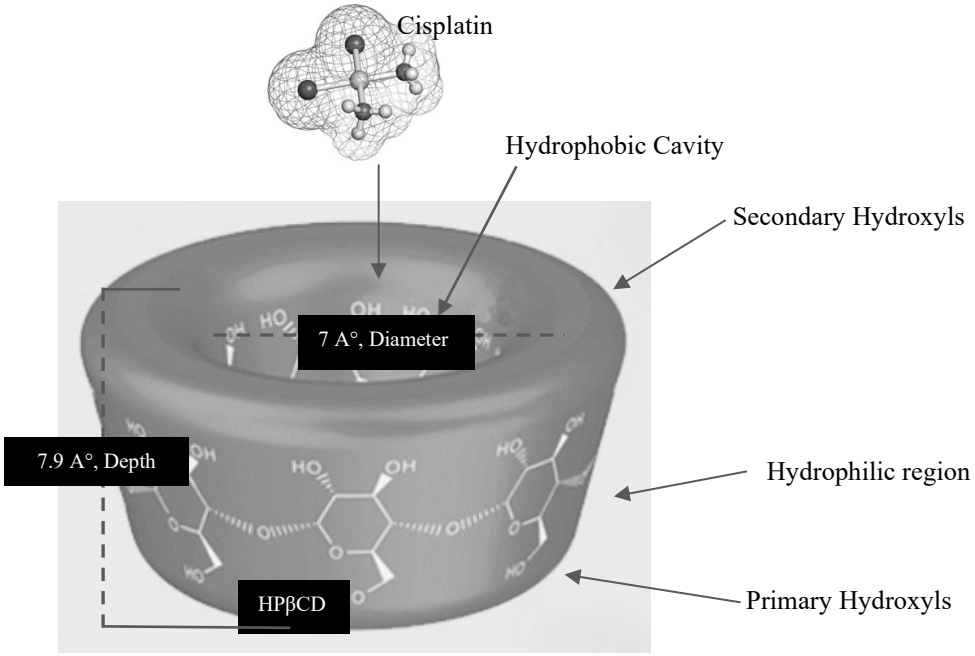


Figure 6 Schematic of Hydroxypropyl beta cyclodextrin (HPβCD) and inclusion. The complex of Cisplatin HPβCD and the tri-dimensional model.

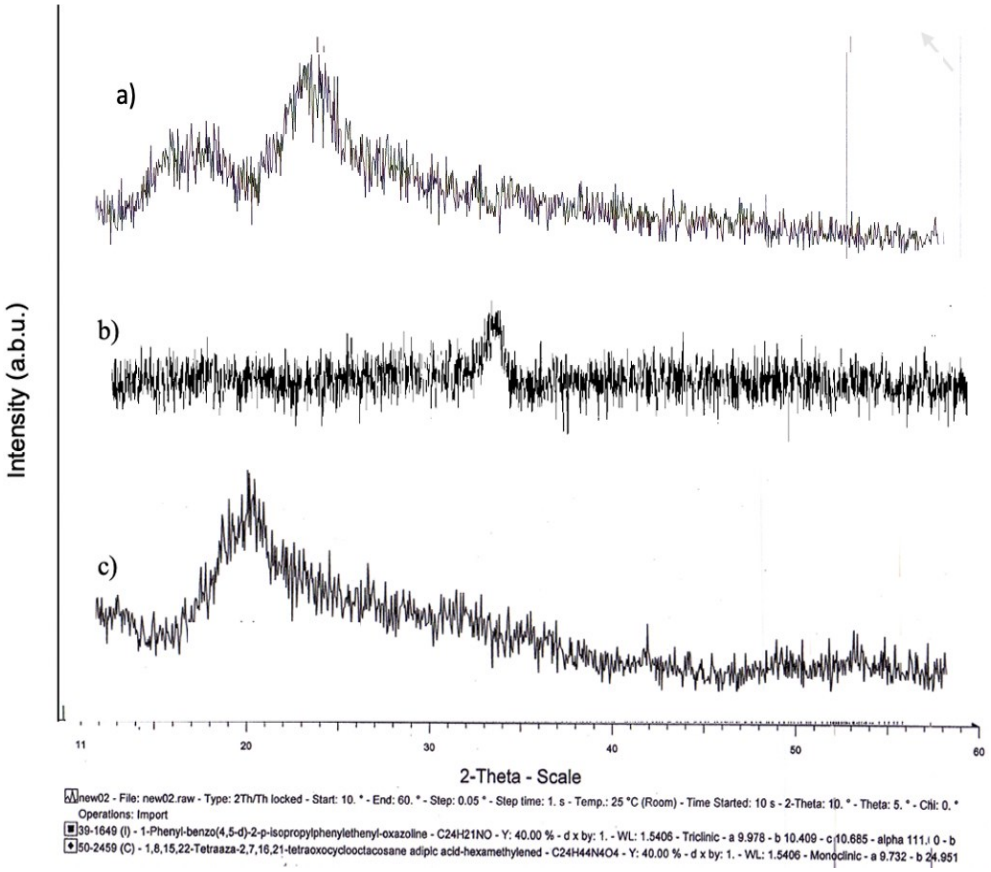


Figure 7 XRD patterns of Hydroxypropyl beta cyclodextrin (HPβCD), CP and CPC: (a) HPβCD, (b) CP, and (c) β-CD-CP.

Encapsulation efficiency determination of drug-loaded NVs

CP-LPS (20 mM) or β-CD-CP-LPS (20 mM, equivalence to CP concentration). CP-LPS, β-CD-CP-LPS, and plain DPPC/CHL (6:4 molar ratios, 40 mM, LPS) formulations were studied the physical stabilities at 3 temperatures (4 ± 2, 30 ± 2 ℃ at 75 % R.H. ± 5 %, 45 ± 2 ℃ at 75 % R.H. ± 5 %) [19] for 3 months, white dispersions, translucent, pH 6.0 ± 0.3, no sedimentation, and phase separation of both formulations exhibited at 45 ± 2 ℃ after 2 weeks (**Tables 2** and **3**). The inclusion complex was prepared by mixing CP/CD in the 1:1 molar ratio allowing one drug molecule to interact with one HPβCD molecule [9,15]. The encapsulation efficiency of β-CD-CP-LPS was calculated in the CP term, as shown in **Table 4**. Both CP-LPS and β-CD-CP-LPS were in the range of 0.42 and 0.40 mole per mole of total lipid, respectively. The percentage of encapsulation of about 80 %. The encapsulation efficiency of CP-LPS and β-CD-CP-LPS formulations appeared to be slight differences. However, statistical analysis of the encapsulation efficiency by 2-tailed t-test showed no significant difference between the 2 formulations at the confidence level of $p < 0.05$ because CP has lipophilic characteristics and can enhance its encapsulation in the lipid bilayers of the vesicles. In contrast, β-CD-CP-LPS has hydrophilic characteristics, and its increased encapsulation is from its large complex molecular size.

Table 2 Physical appearances of CP-LPS kept at different temperatures [19].

Condition	Initial appearance	Color	Appearance after 3 months	Color
4±2 °C	pH 6.0±0.3	white	pH 6.0±0.3	white
	Translucent		Translucent	
	Dispersion		Dispersion	
	Sedimentation, no		Sedimentation, no	
RT	pH 6.0±0.3	white	pH 6.0±0.3	white
30±2 °C 75±5 %RH	Translucent		Translucent	
	Dispersion		Dispersion	
	Sedimentation, no		Sedimentation, +0.5	
45±2 °C 75±5 %RH	pH 6.0±0.3	white	Phase separation after 2 weeks	white
	Translucent			
	Dispersion			
	Sedimentation, no			

Note: + number = degree of sedimentation; Degree of sedimentation of 1 is higher than 0.5

Table 3 Physical appearances of β-CD-CP-LPS kept at different temperatures [19].

Condition	Initial appearance	Color	Appearance after 3 months	Color
4±2 °C	pH 6.0±0.3	white	pH 6.0±0.3	white
	Translucent		Translucent	
	Dispersion		Dispersion	
	Sedimentation, no		Sedimentation, +0.5	

Condition	Initial appearance	Color	Appearance after 3 months	Color
RT 30±2 °C 75±5 %RH	pH 6.0±0.3	white	pH 6.0±0.3	white
	Translucent		Translucent	
	Dispersion		Dispersion	
	Sedimentation, no		Sedimentatio, +1	
45±2 °C 75±5 %RH	pH 6.0±0.3	white	Phase separation after 2 weeks	white
	Translucent			
	Dispersion			
	Sedimentation, no			

Note: + number = degree of sedimentation, Degree of sedimentation of 1 is higher than 0.5

Table 4 Encapsulation efficiency (EE) of Cisplatin (CP-LPS) and its inclusion complex (β-CD-CP-LPS).

Formulation	Encapsulation efficiency	
	% Incorporation (± S.D.)[b]	mol per mol of total vesicular concentration[a]
CP-LPS	83.44±4.27	0.42
β-CD-CP-LPS	82.20±1.07	0.40

[a] Total vesicular concentration = 40 mM
[b] Experiment data represent mean ± S.D. of three independent measurements
% incorporation of all formulations showed no significant differences ($p < 0.05$, 2-tailed t-test)

Controlled release of CP in various formulations

In vitro studies, CP release from CP was encapsulated NVs exposed to physiological conditions as PBS at 37 ℃. CP-LPS and β-CD-CP-LPS encapsulation efficiency were 83.44 and 82.20 %, respectively. For CP-LPS and β-CD-CP-LPS, CP showed an initial burst release of around < 17 and < 10 %, respectively, after the first 3 days. CP-LPS and β-CD-CP-LPS were maintained as plateau phases of CP-LPS and β-CD-CP-LPS for 4 and 7 days, respectively (**Figure 8**). This formulation showed the % cumulative controlled release of CP-LPS was about 15 -16 % and released until 83.6 % of CP for 6 days (**Figure 9**). CP-LPS removed CP level higher than β-CD-CP-LPS, about 5 - 7 %. While β-CD-CP-LPS, CP has shown an initial release was about 10 - 11 %. Moreover, % cumulative controlled release of β-CD-CP-LPS can reach extensively until 82 % of CP for 8 days (**Figure 9**).

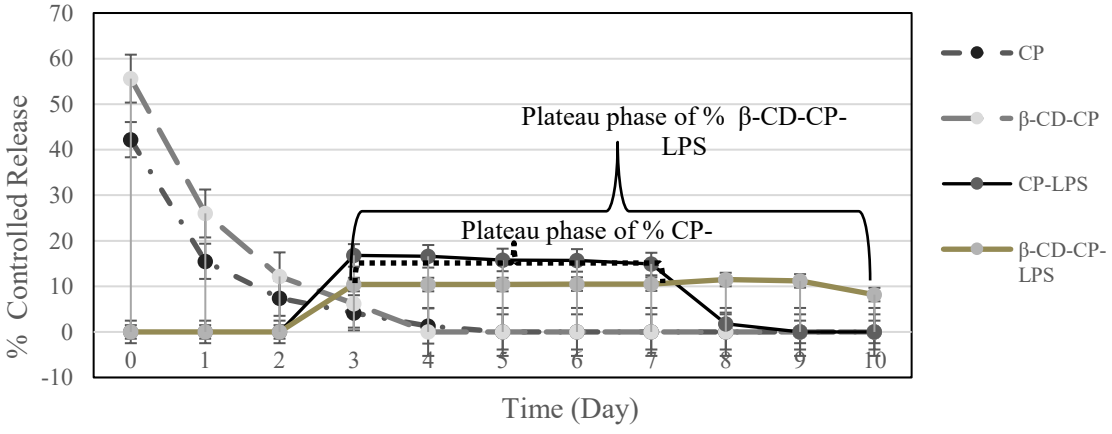


Figure 8 % Controlled Release of drugs in various formulations.

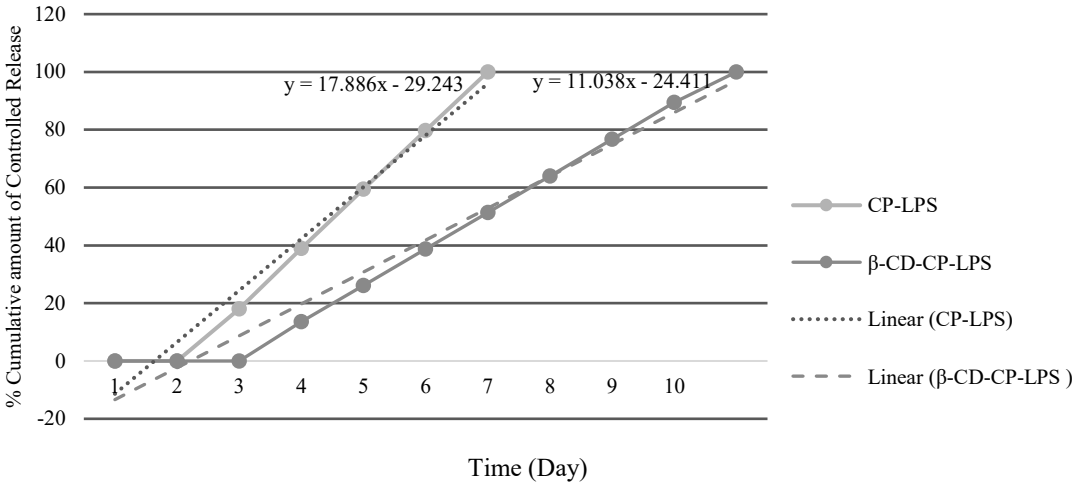
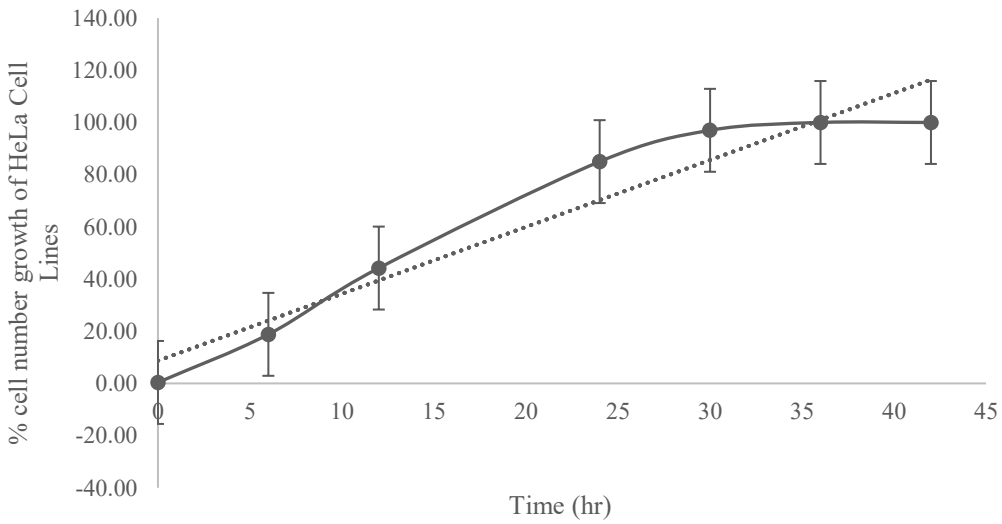


Figure 9 % Cumulative amount of Controlled Release of drugs in various formulations.
(Cisplatin (CP) release from nanoparticles. CP-LPS were dispersed in Phosphate buffer, and at predetermined times, the dispersion was centrifuged, and the supernatant analyzed by UV spectroscopy was in the range 301 nm for cisplatin content. The data shown are mean ± SD, n = 3. The dotted line represents its Higuchi model fitting of the data).

Cytotoxicity of drug-loaded nanovesicles determined by SRB colorimetric assay

Cell culture and treatment. Human cervical adenocarcinoma (HeLa) cell lines were grown in DMEM supplemented with 10 % heat-inactivated FBS and maintained in a humidified atmosphere of 5 % CO_2 at 37 ℃. Cells were trypsinized and plated in 96-well tissue culture plates. The antiproliferative effect of CP-LPS and β-CD-CP-LPS, which were entrapped and not entrapped in NVs, has been demonstrated [8]. Therefore, the SRB assay, used for cell density determination based on the measurement of cellular protein contents, has been optimized for the toxicity screening of compounds to adherent cells in a 96-well plate. The growth curve of HeLa cell lines is shown in **Figure 10**, and the dose-response plot of HeLa cells treated with blank liposomes did not show the cytotoxic effect (**Figure 11**). Dose-response plot of HeLa cells treated with various formulations from the highest to lowest concentrations was CP, β-CD-CP, CP-LPS and β-CD-CP-LPS, respectively (**Figure 11**). **Figure 12** exhibited % Relative Growth Inhibition of HeLa cells treated with various formulations and found that β-CD-CP-LPS gave the lowest dose to inhibit the growth of HeLa cells while CP gave the highest amount to inhibit the growth of HeLa cells. It can be concluded that β-CD- LPS gave the capacity to send CP to inhibit the growth of HeLa cells better than plain CP.

(a)



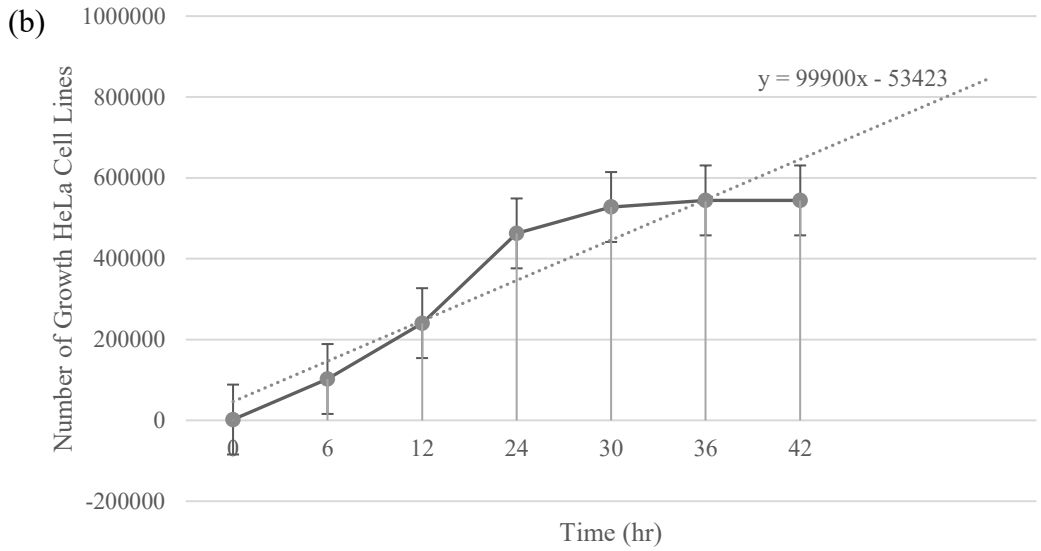


Figure 10 (a) Growth curve of % Cell number of HeLa cell lines and (b) Growth curve of Number HeLa cell lines and Linear Forecast of HeLa cells.

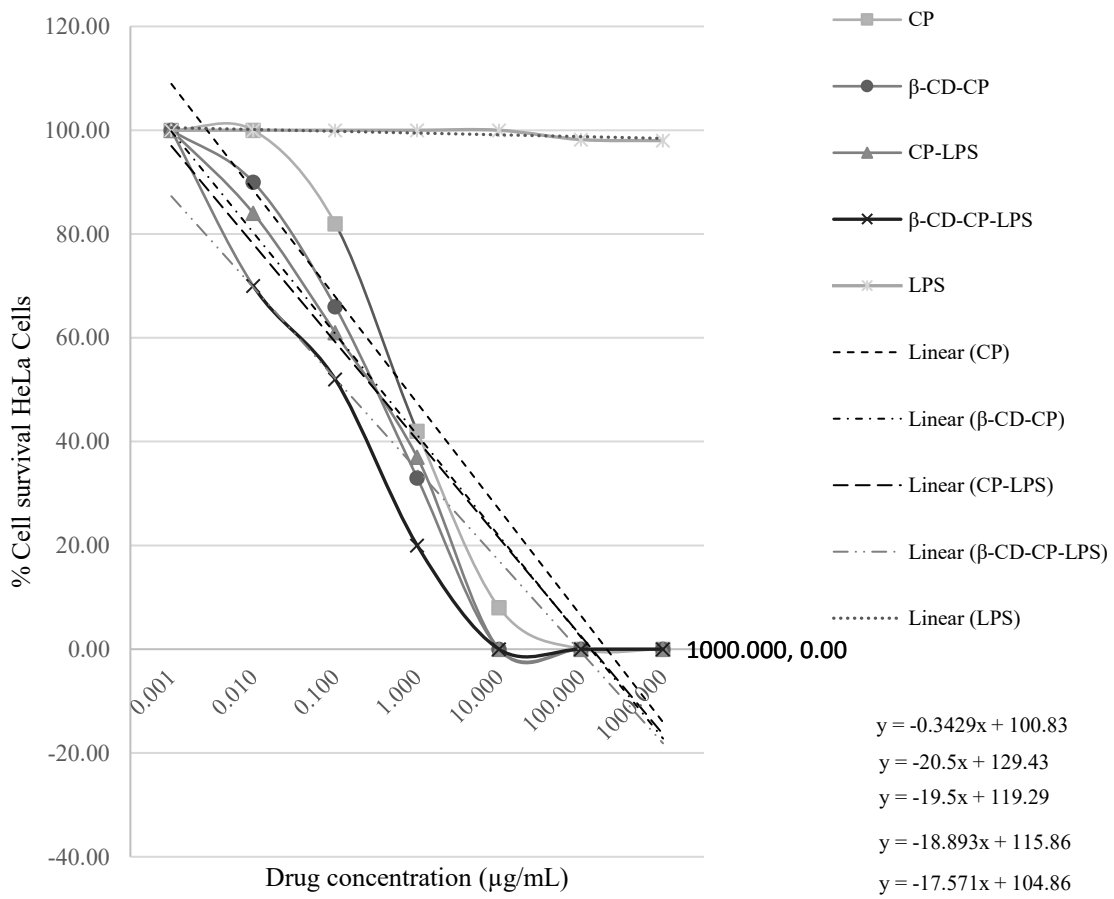


Figure 11 Cytotoxic effect of dose-response plot of HeLa cells treated with various formulations and blank LPS.

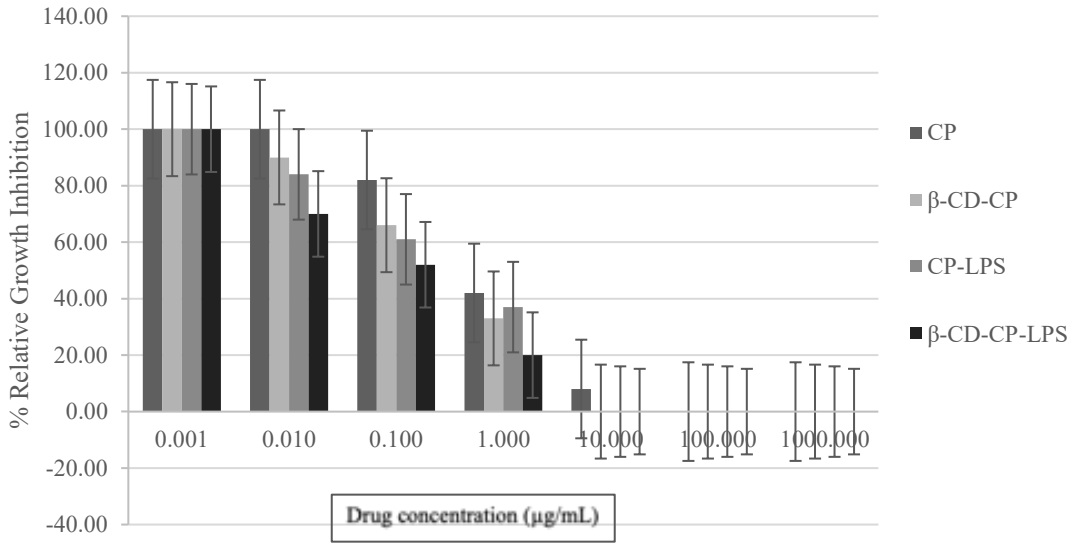


Figure 12 % Relative Growth Inhibition of HeLa cells treated with various formulations.

Discussion

This study of all procedures for CP-LPS and β-CD-CP-LPS was fulfilled in an ordinary method. The solubility of CP is 2.5 mg in 1 mL of water at 30 ℃, approximately and the solubility of CP can increase to 4 mg in 1 mL of water at 35 ℃ [15,16]. Nevertheless, in part of ultrasonic sonication, liposomal preparation increased the temperature to 40 ℃. It produced high CP solubility and formed liposomal NVs, exhibiting the stability in **Figure 1**. In tumor normalization, liposomal NVs can deliver selective CP to the tumor to overcome the immune-suppressive state, rewiring tumor signaling. Liposomes can be used to provide specific drugs to specific cells to correct or modify pathways to simplify better anti-tumor immune responses and combinational therapy [20].

The formation of β-CD-CP can increase the aqueous solubility of the drug, increase its chemical and physical stability, and enhance drug delivery through biological membranes [10-12]. Inclusion complex procedures using noncovalent bonds are formed or broken during the complex formation. In aqueous solutions, CP molecules bound within the CD inclusion complex are in dynamic equilibrium with free drug molecules [8,11,12]. The controlled release behavior of β-CD-CP-LPS ascribes to quiet release to diminish toxicity affecting normal tissues surrounding tumor tissue.

In the concept of controlled release carriers, this may be achieved by targeted delivery systems that aim to exploit the characteristics of the drug carrier and the drug target to control the biodistribution of the drug in plasma profile [20] as a comparative *in vitro* study of controlled release of CP from various formulations. The released kinetics of CP-LPS showed a biphasic profile, characterized by a rapid initial burst effect phase and reaching a plateau phase after 4 days and for CP-LPS and β-CD-CP-LPS, respectively [21-23]. CP from β-CD-CP-LPS released a rapid initial from 2 days. The immediate initial release may be ascribed to the fraction of CP content adsorbed or close to the surface of the NVs. A few studies have shown the CP-LPS and their release kinetics [22-24].

Moreover, CP from CP-LPS was released during 6 days and showed a plateau for 4 days at around 17 %. β-CD-CP-LPS exhibited a table of about 10 % for 7 days. The release actions performed were CP being entrapped in a hydrophobic multilayer, but β-CD-CP was trapped in a hydrophilic multilayer and core of LPSs. The rate-limiting step of CP-LPS was used in 1 step; CP was controlled release from hydrophobic multilamellar of NVs, while β-CD-CP was released to CP in 2 steps [21-23]. Firstly, β-CDs - CP released further om hydrophilic multilamellar of NVs. Lastly, β-CD-CP controlled the release of CP in an equilibrium pattern between β-CD-CP and to CP molecule. Thus CP from β-CD-CP-LPS exhibited prolong released for 8 days by lower concentration than CP from CP-LPS [22-24].

In part of cytotoxicity by using SRB assay of CP-LPS, CP is a cancer drug. The poor solubility of CP limits its therapeutic application, which uses high-dose treatment, and the side effect is necrosis to normal cells. In this study, the hydrophobic CP and hydrophilic β-CD-CP. They were entrapped in liposomal NVs by β-CD-CP calculated to equilibrate to CP. Although their size distribution differed, the encapsulation

efficiency of both drugs was closed. Their antiproliferative activities were enhanced. In cancer cell lines, % Cell survival of HeLa Cell lines and %, Relative growth inhibition of β-CD-CP-LPS demonstrated the most potent antiproliferative activity using the lowest concentration. At the same time, blank liposomes gave no toxic effects in the cell lines. When the considerate profile of controlled release of β-CD-CP-LPS, the result exhibited prolonged controlled release and long-term plateau phase [25,26]. Besides, cancerous cells consume food such as sugar to convert to energy to expand themselves more than normal cells. HPβCD is a cyclic oligosaccharide [27,28] that enriches nutrients and attracts cancer cells. Thus, almost β-CD-CP-LPSs killed cancerous cells in lower concentrations and controlled release more than CP-LPS. Furthermore, β-CD-CP-LPSs were saved for normal cells because they enticed cancer cells by HPβCD.

Conclusions

In this study, the experiments successfully have CP-LPS and β-CD-CP-LPS. These experiments also increased the solubility and encapsulation of CP in the lipid bilayer using slightly elevated temperatures while stirring the solution for several minutes and β-CD-CP using inclusion complex with HPβCD to encapsulate in the hydrophilic bilayer. CP-LPS and β-CD-CP-LPS are released ultimately after degradation. The results have shown that CP released from CP-LPS and β-CD-CP-LPS were in a controlled deportment for 10 days. The cytotoxic effect using SRB assay observed that % Relative Growth Inhibition of HeLa cells occurred when they were exposed to CP-LPS and β-CD-CP-LPS. This experiment found that β-CD-CP-LPS gave the best formulation. These results suggest that the CP-LPS and β-CD-CP-LPS can be used to deliver CP-LPS and β-CD-CP-LPS into lung cancer cells in a sustained action for the long term. Moreover, β-CD was an oligosaccharide that attracted tumors, and tumors snatch themselves more than the tissues surrounding them. Thus, β-CD-CP-LPS, inclusion complex, and a low dose of CP can damage cancer cells. These cooperative mechanisms of action were saved for normal cells surrounding tumor tissues.

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