An Alternative Nano Design Strategy by Neutralized AMPS & Soy Bean Lecithin to Form Nanoparticles

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***Abstract*—**Paclitaxel is the complex, semi synthetic, pseudo-alkaloid which is currently used in treatment of different cancer types mainly breast, ovarian, lung and Kaposi's sarcoma. It is poorly soluble in water; therefore, currently used formulations tremendously show side-effects and high toxicity. Encapsulation of the drug in a nano drug carrier which causes both reducing side effects and increasing drug activity is a desired new approach for the nano-medicine to target the site of cancer. In this study, synthesis of a novel nano paclitaxel formulation made of a new amphiphilic monomer was followed by the investigation of its pharmacological properties. UV radical polymerization was carried out by using the monomer Lecithin-2-Acrylamido-2-methylpropane (L-AMPS) and the drug spacer Methacrylated Poly(Caprolacton) (PCL-MAC), to obtain sterically high stabilized, biocompatible and biodegradable phospholipid nanoparticles, in which the drug paclitaxel (Pxl) was encapsulated (NanoPxl). Particles showed high drug loading capacity (68%) and also hydrodynamic size less than 200 nm with slight negative charge (50-200 nm, -79 mV) which has previously been reported as desired particle size and surface charge for targeting the cancer site. The drug release profile was obtained and in vitro cytotoxicity test was performed on MCF-7 cell line. Collectively, these data indicated that paclitaxel loaded Lecithin-AMPS/PCL-MAC nanoparticles can be considered as a new, safe, stable and effective nanomedicine for the treatment of breast cancer.

***Keywords*—** Paclitaxel, Nanoparticle, Drug Delivery, L-AMPS

I. Introduction

Paclitaxel molecular formula is C47H51NO14 and its structure carries the diterpenoid skeleton [1]. It promotes the polymerization of tubulin by binding with high affinity to β-subunits through the length of microtubule. This causes surface stabilization and increases microtubule polymerization, which enables conformational change of normal tubule dynamics and provides cell death. Thereby, paclitaxel has known good neoplastic activity particularly in tumor vascular cells during angiogenesis and unforgiven when compared newly synthetic ones [2, 3]. Poorly soluble hydrophobic paclitaxel can be formulated by amphipilic drug carrier molecules; for instance, liposomes, polymeric nanoparticles in order to overcome biological barriers, reduce toxicity and achieve sustained release of drug within the tumor tissue by enabling the advantage of Enhanced Permeability and Retention (EPR) effect [4-7].

Besides, one of the mostly used amphiphilic materials in synthesis of nano drug delivery systems is soy bean lecithin [8, 9]. Lecithin is naturally amphiphilic monomer and its functional derivatives have been widely used in drug delivery application [10].

In this study, nano-particle synthesis has been carried out by UV initialized polymerization of modified Lecithin which is Lecithin-2-Acrylamido-2-methylpropane (L-AMPS)monomers with each other and with the drug spacer Methacrylated Poly(Caprolacton) (PCL-MAC). Drug loaded nano particles were synthesized using the same process except that the Pxl molecules were incorporated to the reaction mixture. New amphipilic based formulation of paclitaxel was aimed to increase drug loading capacity while reducing toxicity and side effects as well as spontaneously accumulation of nanoparticle into the tumor cells in order to purpose to use EPR effect [11].

II. Materials And Methods

A. Preparation of L-AMPS /PCL-MAC Nanoparticles

Both synthesis of L-AMPS and PCL-MAC has been prepared as our previous report [11, 12]. The mixture of polymer composition was determined by L-AMPS, PCL-MAC in reported proportion and UV initiator was added. For effective loading, various initial concentrations of drug were performed with tween 80. The ratio of PCL-MAC/paclitaxel was used to pre-determine the drug loading capacity. All content have been

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dissolved in DCM, the solvent was then removed by vacuum rotary evaporation to form a dry film. This dry film was further dried under vacuum overnight to remove any traces of remaining solvent. Then, this film was equilibrated by 20 ml ddH2O at pH:8, mixture was then shaked for 30 min at 200 rpm and subjugated under UV to obtain L-AMPS/PCL-MAC paclitaxel loaded nano particles (NanoPxl). Results were evaluated by particle size analyser (DLS), and Scanning Electron Microscope (SEM).

B. Drug Loading and Release Study

Drug loading yield and release profile of paclitaxel from nanoparticles were carried out using 2 mL of performed NanoPxl in PBS solutions at a concentration of 5 mg mL-1 which were split equally into 30 Slide-A-Lyzer MINI dialysis microtubes with a molecular weight cutoff of 3500 Da (Pierce, Rockford, IL) [13]. These microtubes were dialyzed in 45 mL of PBS buffer at 37°C at 90 rpm with gentle stirring. Three milliliters of dialysis medium was withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours of the experiment, followed by renewal of the PBS buffer. At each data point, nanoparticle solutions from three microtubes were collected separately and 10 times diluted with equal volumes of acetonitrile to disassemble the particles and obtain free drug. The resulting free paclitaxel content in each microtube was assayed comparing with an already performed concentration curve obtained by measuring the fluorescence emission of paclitaxel at 440 nm excited at 390 nm using Molecular Devices Spectro Max340 PC Eliza Reader Spectrophotometer. The resulting free Paclitaxel content in each microtube was assayed using placed in 45 mL PBS, maintained at 37 ˚C and stirred at a speed of 90 rpm. The encapsulation efficiency and the loading efficiency tests were performed in triplicate [14].

C. In Vitro Studies of Nanoparticles

The cell line used to evaluate the in vitro activity of the formulations was MCF-7. The cell line was maintained in Dublecco's modified eagle medium 10% fetal bovine serum and 1.0% antibiotics (penicillin and streptomycin) in a 5% carbon dioxide humidified atmosphere at 37°C.

Optimum solutions of NanoPxl chosen from the DLS studies were used as the test solutions. A 5% dimethyl sulfoxide (DMSO) solution of Pxl also was tested as a control. All the samples were prepared and tested in triplicate. The procedure used to test the *in vitro* cytotoxic activity of the formulation is as previously described. Briefly, samples were prepared as described earlier and serial dilutions were made to obtain final Pxl concentrations ranging from 20 to 0,4 µg/ml using the respective solvent that is either PBS buffer or 10% DMSO. 190 µl of cell suspension at a density of 5×10^3 cells/well was plated in a 96-well plate. After that, 10 µl/well of the test solutions and 10 µl/well controls were added to the microtiter plates. Control groups with 10 µl of the solvents also were added. Each sample was evaluated in triplicate. The plates were then incubated for 3 days in a 5% CO_2 humidified atmosphere at 37°C.

After the incubation period, the cells were fixed to the plates by adding 100 µl/well of cold 20% trichloroacetic acid and incubating for 1 hr at 4°C. The plates were then washed, air-dried, and stained with 100 µl/well of 0.4% sulforhodamine B in 1% acetic acid for 30 min [15]. Then, the plates were washed with 1% acetic acid and rinsed, and 10 mM Tris buffer (200 µl/well) was added. The optical density was then read at 515 nm, and the readings obtained for the solvent controls were used to define 100% growth after correcting for the value obtained for the zero day control. These values were then expressed as % survival.

III. RESULTS

L-AMPS/PCL-MAC nanoparticles were first optimized before incorporation of drug. Therefore, dynamic light scattering measurements further showed that by keeping the L-AMPS weight ratio constant at 75%, we varied the PCL-MAC inherent viscosity and demonstrated that we can fine-tune particle size in a highly reproducible manner while maximum affecting nanoparticle surface charge. Results showed that preparation by using PCL-MAC:L-AMPS in 0.025X; 0.15X; 0.30 ratios concluded by achievement of particles below 200 nm without drug entrapment. Even if, 186 nm particles have been obtained by 0.025X proportion, the most stable particles have been provided by 0.30X (Fig. 1); because, the particle ζ potential highly fluctuated in the range of -51 to -79 mV by incorporation of PCL-MAC to the structure [11].

Drug adding in various ratio (Pxl:PCL-MAC; 2:1; 3:1 and 4:1) were prepared. Designated L-AMPS: PCL-MAC in ratio 0.30X was used to preparation of drug loading particles therefore of its homogenous image (Fig. 1). Maximum drug loading capacity has been noticed by Pxl: PCL-MAC ratio 2:1 (46% Pxl of total polymer content) of size as 404 nm. Upper limits of the drug, particle form damaged and jel form mount up because Pxl encapsulation yield dropped when the initial drug input was increased to 69% and 92% of total polymer content. As the result, nanoparticle is the gel system have been swell up by incorporation drug into the L-AMPS: PCL-MAC ratio 0.30X structure.

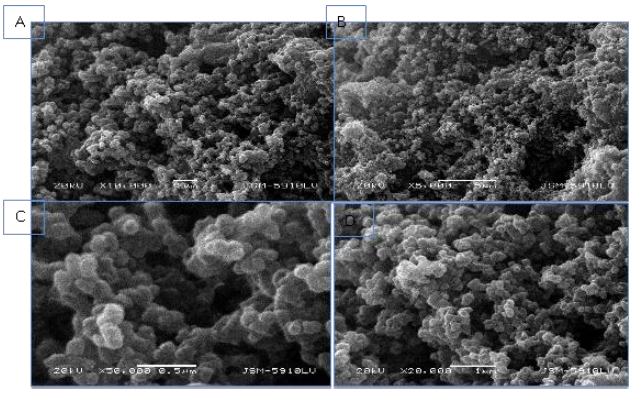

Figure 1: SEM images of 0.30X PCL-MAC:L-AMPS in a) 10.000 b) 5.000 c) 50.000 d) 20.000 magnification images .

PCL-MAC:Pxl ratio was fixed 1% to obtain optimum NanoPxl particles (OP) was designated in order to reduce particle size ≤ 200 nm. On the other hand, 1.5 % tw80 emulgator have the ability to reduce nanoparticle size by achieving more incorporation of drug into the particle by the van der walls interaction in the particle core (Fig. 2). This

showed that incorporation drug into particle reduce the nano particle size by the tw80 aid; even if, it does not affect its stability that between -70 to -79 mV (Fig. 3).

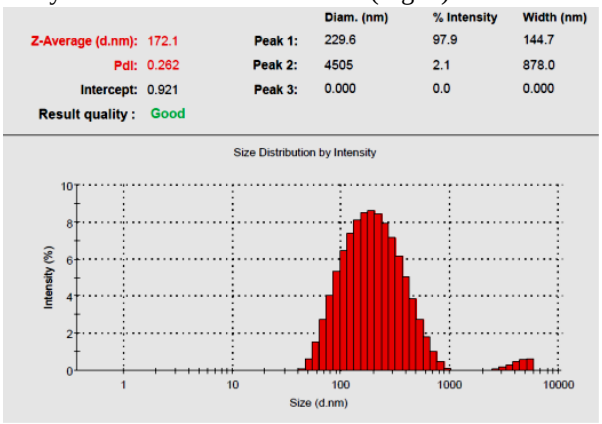

Figure 2: Size distribution of NanoPtx

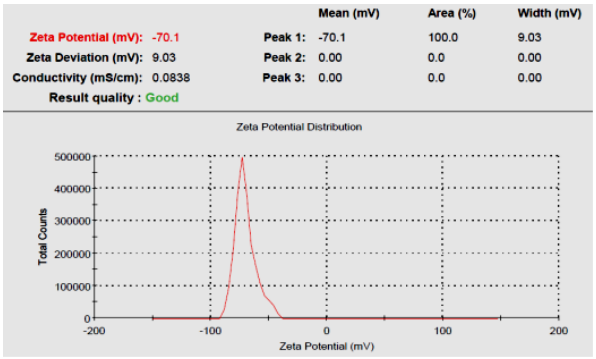

Figure 3: NanoPtx stabilization.

Results previously showed that, the percentage of drug release was plotted against time [12]. The particles possessed stable drug release rate. The release result revealed that the particle was more stable when diluted and that the drug was slowly released from the particle. In addition 115 µg/ml Paclitaxel has not been in encapsulated in particles. On the other hand, 68% encapsulation efficiency and 23% drug loading efficiency were calculated.

Finally, we investigated whether the interaction between the drug and NPs in the formulation affected the drug's bioactivity. To determine the cytotoxic activity of Pxl in NPs the formulations were tested against MCF-7, a human breast cancer cell line which has been previously used in studying the anticancer activity of Pxl [12]. The micelle formulation had cytotoxic activities toward cultured MCF-7 cells very similar to that of free drug in 10% DMSO. IC50 values in turn of NanoPxl is 18.11 µg/ml; and, Pxl in DMSO 7.3 µg/ml.

IV. Conclusions

Neutralizing of acrylamide derivate AMPS with sodium salt reacted with soy bean lecithin to form gel has been newly synthesized and used for drug carrier strategy by forming the three hydrocarbon chained L-AMPS ,which has the high drug occupy space into hydrocarbons. On the other hand, PCL have been most known synthetic molecule which was used as synthetic monomer to prepare drug nano-carrier systems.

On the other hand, synthesized PCL-MAC molecule has the good drug occupier. Thereby, this provides the overcoming the loading and stability problems of paclitaxel. UV polymerization technique provides increasing stability of high drug loaded nanoparticles by drug spacer PCL-MAC in optimum delivery size 20-200 nm. Cell culture experiment was carried out by MCF-7 human breast cancer types. Studies showed that this nano-formulation (NanoPxl) has the great promise for the treatment of human breast cancer.

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