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Prolonged Release Evaluation of an Injectable Anticancer Drug using Human Serum Albumin Nanoparticle

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ABSTRACT

Human serum albumin nanoparticles (HSA-NPs) were synthesized using the modified desolvation method. Fourier transform infrared spectroscopy (FT-IR), electronic absorption spectroscopy (UV-Vis), Zeta Sizer as well as field emission scanning electron microscope (FE-SEM) of the sample confirmed the formation of HSA NPs with an average size of 68 nm. The obtained results shown that HSA-NPs was successfully synthesized. The cytotoxic study of HSA-NPs in the HFFF2, normal cell lines was conducted and cell viability percentage demonstrated more than 90% within 24 h. Therefore, the synthesized NPs were nontoxic compared to the control samples. Furthermore, the release of the oxaliplatin as an anticancer drug incorporated in HSA NPs was also investigated in physiological conditions. The drug loading (DL) and drug entrapment efficiency (DEE) were enhanced and the DL of 0.9% and DEE of 51% are achievable. The Higuchi model was shown the best fitting compared to the different kinetically release model. This result and result of cyclic voltammetry indicated that the drug release mechanism followed by diffusion manner. Therefore, our present study showed that the biocompatible HSA NPs could improve prolonged release of oxaliplatin as anticancer drugs.

Keywords: Prolonged release, Human serum albumin, Nanoparticles, Anticancer

INTRODUCTION

Cancer is the uncontrolled growth of cells with loss of differentiation and commonly with metastasis. Anticancer drugs are used to control the growth of cancerous cells. Oxaliplatin is a third-generation platinum-derived antineoplastic agent that has been proved effective mainly against advanced colorectal cancer (CRC). Oxaliplatin administration has an acute excitatory and sensitizing effect, including unpleasant cold allodynia in the distal extremities, mouth, and throat and usually associated with muscle cramps. However, the major side effect, a dose-limiting toxicity of the peripheral nerves, affects the sustainability of the planned treatment. Although these acute symptoms resolve within 1 week, severe chronic sensory neuropathy develops with the increasing cumulative dose and is characterized by distal paresthesia and numbness [1,2].

Drug delivery systems aim to deliver the drugs over an extended duration or at a specific time during treatment. Therefore, the improvement of the efficiency and convenience of a drug release system is of paramount importance. In the past two decades, numerous nanoparticles (NPs) have been extensively explored in order to achieve precisely controlled drug delivery [3]. However, the application and development of high-efficiency drug release system are still limited due to a lacking of simple, stable and efficient drug delivery vehicle [4].

Human serum albumin (HSA) is a highly water-soluble globular monomeric plasma protein with a relative molecular weight of 67 KDa, consisting of 585 amino acid residues, one sulfhydryl group and 17 disulfide bridges. Among nanoparticulate carriers, HSA nanoparticles have long been the center of attention in the pharmaceutical industry due to their ability to bind to various drug molecules, great stability during storage and in vivo usage, no toxicity and antigenicity, biodegradability, reproducibility, scale up of the production process and a better control over release properties. In addition, significant

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amounts of drug can be incorporated into the particle matrix because of the large number of drug binding sites on the albumin molecule [5].

Interaction between oxaliplatin as injectable anticancer drugs and human serum albumin was indicated that van der Waals forces and hydrogen-bond were played predominate in the binding process [6,7]. Therefore, these binding suggested that release of drug from the bloodstream, and also increase drug solubility in plasma, decrease its toxicity, protect from oxidation and prolong its *in vivo* half-life [8-10] but their efficacy is largely constrained by their lack of ability to achieve high concentration, due to their limited loading capacity and low degree of functionalization capability. Drug delivery systems using composites and polymers were demonstrated to improve this deficiency [11]. Therefore, developing an injectable delivery system of oxaliplatin is essential for an effective treatment with increased the efficacy and reduced side effects.

Here, we have prepared colloidal drug carrier HSA nanostructures using a modified desolvation method and then Oxaliplatin was loaded in bio-nanoparticle. The *in vitro* drug release was evaluated and the efficient loading and entrapment Oxaliplatin in HSA-NPs was determined. In addition, the mechanism of drug release was investigated. We hope the modified protocol of HSA nanoparticles preparation verifies the complications in a prolonged release of drugs.

MATERIAL AND METHODS

Reagents and Apparatus

Human serum albumin (HSA), phosphate buffer saline tablet (PBS), glutaraldehyde solution were purchased from the Sigma Aldrich Company (USA). Oxaliplatin were purchased from Merck Chemicals CO (Germany). The human foreskin fibroblast normal cell line, HFFF2 for the cytotoxicity studies were obtained from the Cell Bank of the Pasteur Institute in Tehran (Iran). All other reagents and solvents were of analytical grade and double distilled water was used for all experiments.

Field Emission Scanning Electron Microscopy measurements carried out on a Mira 3-XMU model in order to provide topographical and elemental information at specific magnifications with a virtually unlimited depth of field. The Fourier transform infrared (FT-IR) spectra were recorded using a Jasco-460 Plus FT-IR spectrometer in KBr disk in the range of 4000-400 cm^{-1}. Electronic absorption spectra (UV) were recorded on a Spectrophotometer (T 80+ UV-Vis spectrometer). The zeta-potential measurements were performed on a Malvern Instruments Zeta Sizer 3000 HSA using a standard rectangular quartz cell. Cyclic voltammetry measurements were performed using Parstate 2273 Potentiostate. The three-electrode system consisted of a glassy carbon working electrode; calomel electrode and platinum wire were used as reference and counter electrodes.

Preparation of Human Serum Albumin Nanoparticles

Human serum albumin nanoparticles (HSA-NPs) were prepared in the production process by the desolvation technique with a slight change [12]. Initially, 50 mg HSA in 2 ml of deionized water was stirred at 500 rpm using a magnetic stirrer at the room temperature for 10 min. This is followed by the addition of 8 ml ethanol (desolvating agent) gradually at the rate of 0.5 ml min^{-1}. Then, glutaraldehyde 8% has been added to the solution and has been stirred continuously (500 rpm) at the room temperature for 20 h. The prepared nanoconjugate was purified by 5 cycles of centrifugation at 20,000 × g for 40 min. Finally, the pellet was resuspended in 2 ml of deionized water.

Cytotoxic Studies

Cell proliferation was evaluated by using a system based on the tetrazolium compound [3-(4,5-dimethylthia-zol-2-yl)-2,5-diphenyltetrazolium bromide, MTT] which was reduced by living cells to yield a soluble formazan product which can be assayed colorimetrically. The MTT assay is dependent on the cleavage and the conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells. The human foreskin fibroblast normal cell line, HFFF2, was maintained in a RPMI medium supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine, streptomycin and penicillin (5 μg ml^{-1}), at 310 K under a 5% CO_2/95% air atmosphere. Harvested cells were seeded into 96-well plates with the concentration of the sterilized nanoparticles (6.25 mg ml^{-1}) and incubated for 24 h. At the end of the four hour

incubation period, 25 µl of MTT solution (5 mg ml^{-1} in PBS) was added to each well containing fresh culture media. The insoluble produced formation was then dissolved in a solution containing 10% SDS and 50% DMF (under dark condition for 2 h at 310 K) and optical density (OD) has read against reagent blank which was measured at 570 nm by Microplate reader. Absorbance has read as a function of concentration of converting dye. The OD value of study groups was divided by the OD value of untreated control and presented as a percentage of the control (as 100%). Results were analyzed for statistical significance using a two-tailed Student's t-test. Changes were considered significant at $p < 0.05$.

Loading of Anticancer Drug on to HSA

Loading of Oxaliplatin on the HSA nanoparticles performed by mixing of 0.1 ml (5 mM) with 1 ml of as-prepared protein and 2.9 ml deionize water for 24 h in a dark room, followed by sonication for 4 min. Then, the mixture was centrifuged at 11000 rpm for 10 min to remove the unloaded drug from the mixture. The drug loading (DL) and drug efficiency entrapment (DEE) of the HSA were measured by UV-Vis spectrophotometer and calculated by the following equations:

$$DL (\%) = (\text{weight of drug NPs/weight of NPs}) \times 100 \quad (1)$$

$$DEE (\%) = (\text{weight of drug NPs/Total weight of drug}) \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Characterization of HSA-nanoparticles

HSA nanoparticles were produced by a modified desolvation process. Two main groups can be noted in the FT-IR spectra of the HSA-NPs, at 1655 cm^{-1} for C=O bonds and at 1536 cm^{-1} for C-N stretching coupled with N-H bending modes). The changes in the intensity of the band and slight shifts in the FT-IR spectra of HSA-NPs compared with HSA confirm the formation of HSA-NPs. FT-IR measurement results showed in Fig. 1.

The UV absorption spectra of HSA shows a strong absorption peak at about 220 nm resulted of the $n \rightarrow \pi^*$ transition for the peptide bond of α-helix and a weak peak at 278 nm assigned to $\pi \rightarrow \pi^*$ transition of the phenyl rings in aromatic acid residues (Trp, Tyr and Phe) [13]. Through comparison between observation UV-Vis spectra of HSA and HSA NPs, it can be indicated that the structure was slightly altered in preparation of HSA-NPs (Fig. 2).

Measurement of the aqueous suspensions of HSA was performed by Zeta Sizer (DLS). Average size of Hydrodynamic diameter of HSA-NPs were 161 ± 8 nm (Fig. 3).

The surface characterization of HSA was examined by Field Emission Scanning Electron Microscope (FE-SEM). This method used to verify spherical shape and narrow size distribution of HSA. The structure of HSA NPs was approved by FE-SEM images as can be seen from Fig. 4. The obtained average size of HSA NPs was 68 ± 4 nm.

Cytotoxic Activity

Toxicity effect of synthesized HSA-NPs was investigated on HFFF2 normal cell lines. Cell viability was measured after 24 h according to mitochondrial dehydrogenase enzyme activity rate that was obtained by MTT assay. The average of normal cells viability shows that HSA-NPs were non-toxic in comparison with a control sample (Fig. 5).

Characterization of Oxaliplatin-loaded HSA-NPs

Drug loaded HSA was created through a simple mixing procedure and the removal of excess drug. The quantity of Oxaliplatin bonded was determined by UV-Vis spectroscopy analysis by measuring the absorbance percentage of the characteristic peak (247 nm) of Oxaliplatin. In loading process as described, solution was centrifuged and aqueous phase use for calculating unloaded drug. The drug loading rate on HSA was determined by standard curves of the drug in 247 nm. Drug loading rate on HSA was determined by standard curves of the drug in 247 nm:

$$y = 3.9484 x - 0.0035$$

By this formulation amount of unloaded drug obtained and percentage of loaded Oxaliplatin were calculated following to the Eqs. (1) and (2).

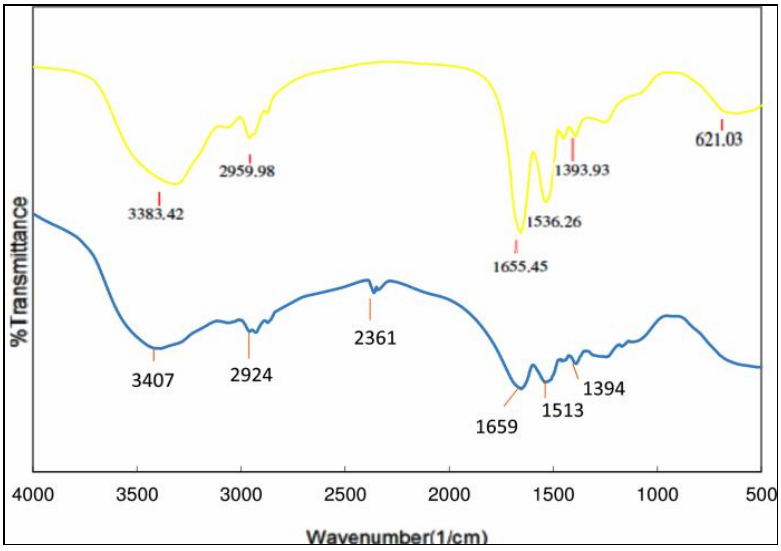

Fig. 1. FT-IR of HSA (blue line) and HSA-NPs (yellow line).

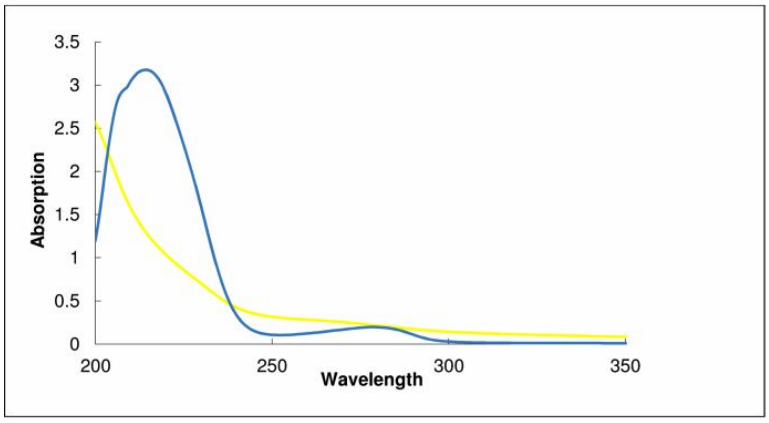

Fig. 2. Typical UV-Vis spectra of HSA (blue line) and HSA-NPs (yellow line).

By this formulation it can be concluded that HSA nanoparticles loaded the drug almost 51 ± 3 % and the drug loading was 0.9 ± 0.04 % that was better than previous studies [14]. The obtained results suggested that the current process is favorable for the design and development of biocompatible nanoscale systems.

Release Behavior of Oxaliplatin-loaded HSA-NPs

To investigate the release behavior all the prepared solutions (4 ml) of HSA-NPs were sealed in a dialysis tube. The dialysis tubes were immersed in phosphate buffer solution (PBS) separately. The tube was under sterile conditions and rotated with 300 rpm. 5 ml of released media is taken every 30 min for 2 h and thereafter every 1 h until 6 h after that in 20 h and 72 h. The release behavior of the loaded drug to HSA-NPs was monitored and measured by UV-Vis spectrometer. The obtained results showed that the release of Oxaliplatin from the HSA-NPs was 7 h (Fig. 6). The results demonstrated a more sustained release behavior was observed from HSA-NPs in comparison to the other matrix polymers such as β-Lactoglobulin and bovin serum albumin [15,16].

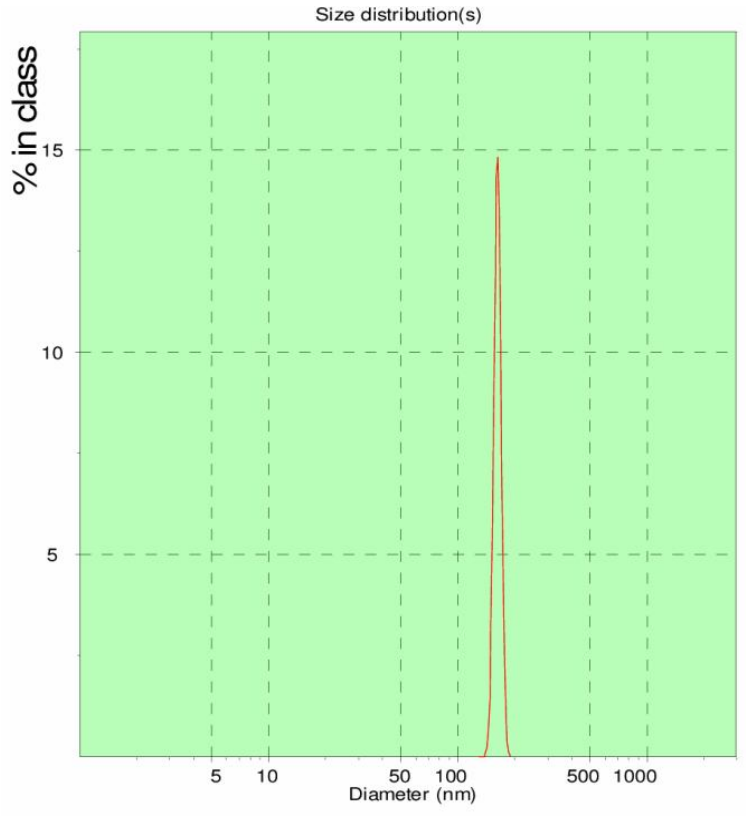

Fig. 3. Size distribution of HSA-NPs.

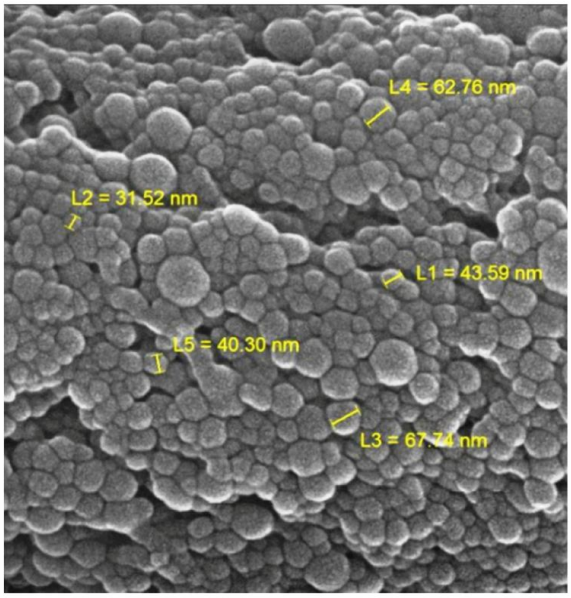

Fig. 4. FE-SEM image of HSA-NPs.

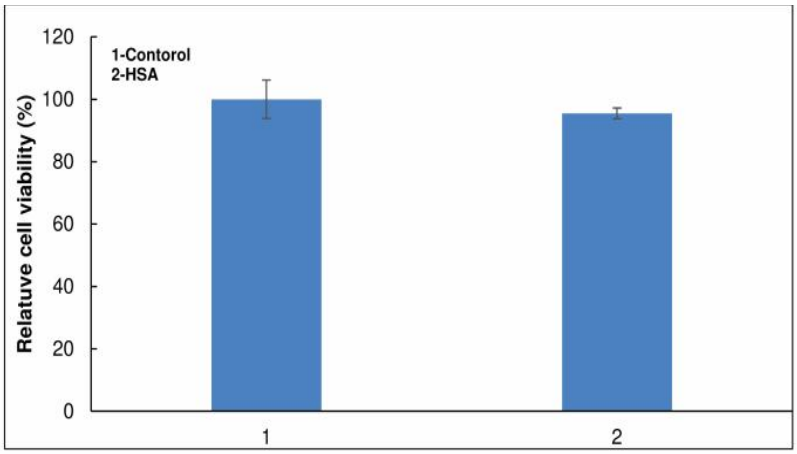

Fig. 5. The cell viability of HSA-NPs in in the HFFF2 1) control 2) HSA-NPs.

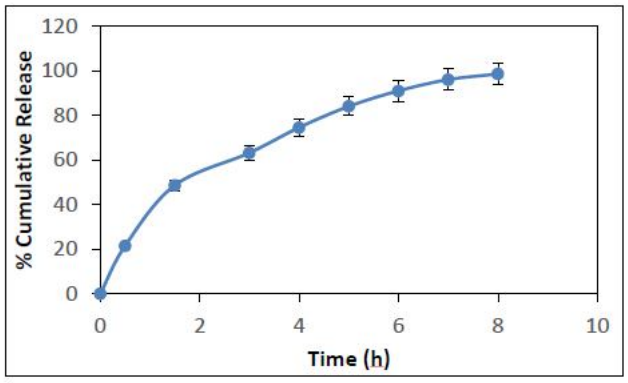

Fig. 6. Cumulative release curve of oxaliplatin loaded with HSA-NPs.

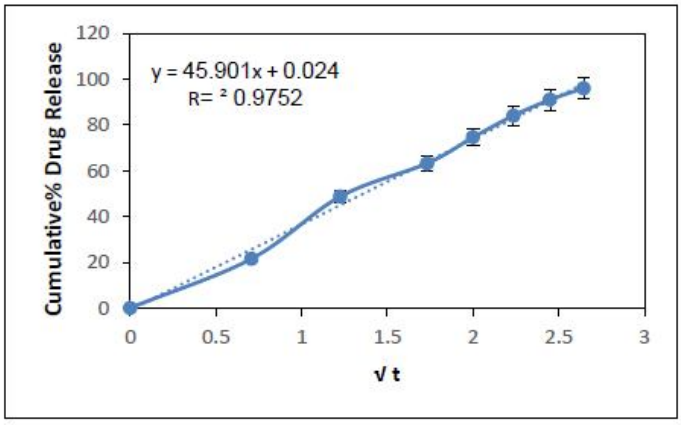

Fig. 7. The amount of oxaliplatin released in √t (Higuchi Diagram).

Table 1. The Kinetic Parameters of Various Mathematical Models

Zero order		First order		Higuchi model		Korsmever-pepas model	
k	R^2	*k*	R^2	*k*	R^2	n	R^2
20.26	0.84	0.18	0.96	45.9	0.97	0.45	0.93

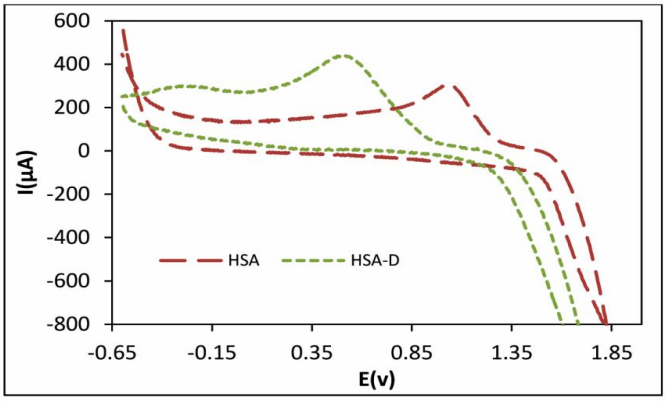

Fig. 8. Voltammogram of HSA-NPs and HSA-NPs with Oxaliplatin at 150 mV s^{-1} scan rate.

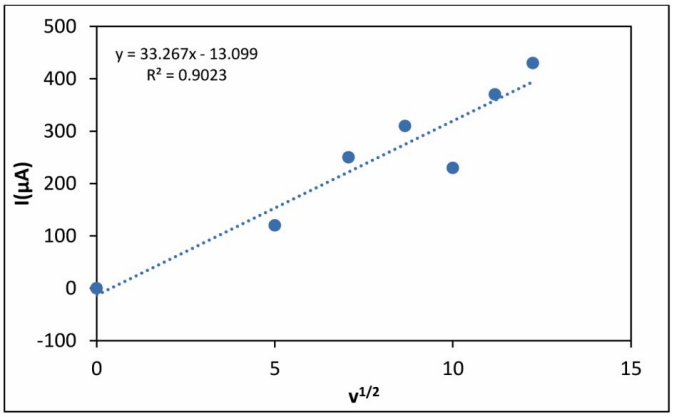

Fig. 9. I_p *vs.* $v^{1/2}$ plots of HSA-NPs with Oxaliplatin.

Release Mechanism of Oxaliplatin-loaded HSA-NPs

The mechanism of the drug release was investigated using mathematical models [17]. These approaches shown that release behavior of drug followed by Higuchi release model based on the correlation coefficient value (Table 1, Fig. 7). The equation of the Higuchi release model is:

$$Q = A\sqrt{D(2C - C_s)C_s t} \quad (3)$$

where, where Q is the amount of drug released in time t per unit area A, *C* is the drug initial concentration, *Cs* is the drug solubility in the matrix media and *D* is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance [17,18]. It is possible to simplify the Higuchi

model as:

$$Q = K \times t^{\frac{1}{2}} \quad (4)$$

where, K is the Higuchi dissolution constant.

The obtained result confirmed to the drug release by diffusion mechanism. Therefore, the anticancer water soluble drug, Oxaliplatin, incorporated in to polymer matrix, HSA-NPs.

CV Investigation of Oxaliplatin and HSA-NPs Interactions

Cyclic Voltammetry (CV) is one of the most important and useful electrochemical analytical techniques. Due to the existing resemblance between electrochemical and biological reactions it can be assumed that the redox mechanisms taking place at the electrode and in the body share similar principle. CV behavior of HSA-NPs in the absence and presence of Oxaliplatin at a glassy carbon electrode were studied in different scan rates (25, 50, 75, 100, 125 and 150) which is shown in Fig. 8. Based on the resulted from the I_p *vs.* $\nu^{1/2}$ plots (Fig. 9), I_p was increased. According to the equation reported in literature [19] diffusion coefficient can be calculated, that is 4.9×10^{-6} cm^2 s^{-1}. It showed that release mechanism is diffusion, which is compatible with the result from UV-Vis experiment.

CONCLUTIONS

Human serum albumin nanoparticles with a mean diameter of 68 ± 4 nm were prepared for the delivery of the anticancer drug oxaliplatine by using the modified desolvation method. Nanoparticles were characterized by FT-IR, UV-Vis spectroscopy, Zeta Sizer and FE-SEM analysis. Cell viability of HSA-NPs was performed which showed nontoxic properties. *In vitro* drug release studies demonstrate that the DL of 0.9 ± 0.04% and DEE of 51 ± 3 % are achievable. In addition, the release behavior studies shown that the release of Oxaliplatin from the HSA-NPs was 7 h by diffusion mechanism. Therefore, this approach provides an effective route for the production of biocompatible prolonged release systems, containing anticancer drugs and the other biomedical applications.

REFERENCES

[1] P. Kokotis, M. Schmelz, E. Kostouros, N. Karandreas, M.A. Dimopoulos, Clin. Colorectal. Cancer 15 (2016) 133.
[2] A.V. Krishnan, D. Goldstein, M. Friedlander, M.C. Kiernan, Muscle Nerve 32 (2005) 51.
[3] I.P. Kaur, H. Singh, J. Controlled Release 184 (2014) 36.
[4] M.E. Davis, Z. Chen, D.M. Shin, Nat. Rev. Drug Discovery 7 (2008) 771.
[5] H. Kouchakzadeh, S.A. Shojaosadati, F. Shokri, Chem. Eng. Res. Des. 92 (2014) 1681.
[6] T. Lemma, J. Pawliszyn, J. Pharm. Biomed. Anal. 50 (2009) 570.
[7] Y. You, X. Chen, J. Qin, X. Yao, Colloid. Surf. B 69 (2009) 51.
[8] M.M. McCallum, A.J. Pawlak, W.R. Shadrick, A. Simeonov, A. Jadhav, A. Yasgar, D.J. Maloney, L.A. Arnold, Anal. Bioanal. Chem. 406 (2014) 1867.
[9] F. Li, M. Feterl, J.M. Warner, A.I. Day, F.R. Keene, J.G. Collins, Dalton Trans. 42 (2013) 8868.
[10] Z. Kazemi, H. Amiri Rudbari, M. Sahihi, V. Mirkhani, M. Moghadam, S. Tangestaninejad, I. Mohammadpoor-Baltork, G. Azimi, S. Gharaghani, A.A. Kajani, J. Photochem. Photobiol. 163 (2016) 246.
[11] J. Liu, L. Cui, D. Losic, Acta Biomat. 9 (2013) 9243.
[12] S. Mahanta, S. Paul, Colloid. Surf B. 134 (2015) 178.
[13] I. Khosravi, F. Hosseini, M. Khorshidifard, M. Sahihi, H.A. Rudbari, J. Mol. Struct. 1119 (2016) 373.
[14] S. Zhou, X. Deng, H. Yang, Biomat. 24 (2003) 3563.
[15] S.C. Chen, Y.C. Wu, F.L. Mi, Y.H. Lin, L.C. Yu, H.W. Sung, Controlled Release 96 (2004) 285.
[16] B. Ghalandari, A. Divsalar, A. Komeili, M. Eslami-Moghadam, A.A. Saboury, K. Parivarh, Biomacromol. J. 1 (2015) 204.
[17] S. Dash, P.N. Murthy, L. Nath, P. Chowdhury, Acta Pol. Pharm. 67 (2010) 217.
[18] J. Siepmann, N.A. Peppas, Inter. J. Pharm. 418 (2011) 6.
[19] A. Shah, E. Nosheen, S. Munir, A. Bashah, Photochem. Photobiol. B 120 (2013) 90.