ORIGINAL RESEARCH ARTICLE

Preparation of Methotrexate loaded PLGA nanoparticles coated with PVA and Poloxamer188

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ABSTRACT

Objective(s): Nanoparticles offer an attractive platform for drug delivery through a wide variety of the body's physiological barriers. Furthermore, modification of nanoparticle surface with moeites such as Poloxamer188 can enhance their accumulation and localization at disease site. In this work, we investigated the physiochemical effect of a scavenger receptor (SR-BI) interacting moiety coated on the surface of methotrexate (MTX)-loaded PLGA nanoparticles.

Methods: Methotrexate-loaded PLGA nanoparticles were prepared by a single step nanoprecipitation technique. The prepared nanoparticles were characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM) for their size and morphology respectively. In vitro drug encapsulation efficiency (EE) and relative drug loading (DL) of nanoparticles were examined by UV-Vis spectrophotometry.

Results: The results showed that the mean diameter of nanoparticles and zeta potential increased when more poloxamer188 was added to preparation process.The DL and EE of MTX increased with increase in poloxamer188/PVA ratio. In vitro release of MTX from PLGA nanoparticle was extended by increasing poloxamer188 in preparation process.

Conclusions: MTX loaded PLGA nanoparticle modified with PVA and poloxamer 188 with suitable sizes and physiochemical properties can potentially improve drug delivery.

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INTRODUCTION

Nanoparticles are versatile nanosized structures which have found many applications in medical practice. Currently, a wide range of nanoparticles have been approved for clinical use in diagnosis(1) and therapeutics (2, 3). In addition, various research groups are persuing multifunctional nanoparticles which combine therapy and diagnostics, i.e, theranostics (4, 5). Functionality of nanoparticles can be derived from their inherent properties as a result of their sizes. For example, nanoparticles of within a specific size range can easily evade the reticulo endothelial system (RES) and increase the biological half life of their drug payload(6). Hyperthermia in cancer therapy using metalic nanoparticles (e.g gold and silver) is due to

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surface plasmonic resonance (SPR) at specific wavelengths of incident radiation(7). Magnetic nanoparticles have been investigated for drug delivery (8)and MRI imaging contrast (due to their superparamagnetism properties)(9). Semiconducting nanoparticles (quantum dots) have also generated a lot of interest for in vivo tracking and imaging(10, 11).

On the other hand, nanoparticles can have functionality imparted on them through surface modification. With regards to immune system evasion, nanoparticles can be rendered "stealth" by covering their surfaces with PEG (12). Targeting moieties attached to the surface of the nanoparticles provide homing ability to the diseased area of the body(13, 14). This strategy is called active targeting. In addition, the nanoparticle high surface area to volume ratio compounded with their large size relative to the functionalization moieties render them more pliable. As a result various moieties with different functionality can be incoperated on the surface of a single nanoparticle.

In this work, we investigated the effect of surface combination of two different surfactants on the physiochemical properties of the nanoparticles. Polyvinyl acohol (PVA) has been extensively used in the stabilization of polymeric nanoparticles in order to avoid agglomeration (15, 16). Moreover, PVA coated iron oxide nanoparticles(IONs) nanoparticles exhibited higher plasma circulation times (17). On the other hand, poloxamer188 was shown to play an important role in facilitating easier cellular uptake and reduction of drug resistance in cancer therapy (18).Animal studies demonstrated that drug loaded PLGA nanoparticle covered with poloxamer188 can easily pass through the BBB. It is further believed that poloxamer188 coated nanoparticles enter the brain by receptor mediated transcytosis scavenger receptor SR-BI(16).

Therefore, drug loaded nanoparticles with a surface combination of PVA and poloxomer188 can potentially enhance both the drugs pharmacokinetics and pharmacodynamics. However, preliminary studies of the effect of these moieties on the physiochemical properties of drug laden nanoparticles are of paramount importance. Methotrexate a commonly used anti-folate substrate as an anti-neoplastic agent(19) was chosen as a model drug for this study. Although, MTX is widely used in different chemotherapy regimens, the drug has poor biological half life after intravenous injection(20). Moreover, the potent anti-cancer drug has exhibited toxicity in different organs and systems such as gastrointestinal, dermatological and central nervous system(19). Therefore, many researchers have proposed the encapsulation of MTX in different carriers (21-23). Among them, PLGA has exhibited better physiochemical properties such as high encapsulation efficiency and drug release(23).

In our earlier work, our group reported on the comparison between emulsion and nanoprecipitation techniques in the preparation of PLGA nanoparticles. We observerd that nanoprecipitation technique was simple (single step preparation), easy, no need for toxic solvents and highly reproducible(24). Herein, we focus on the development dually coated PVA/ poloxomer188, methotrexate-loaded PLGA nanoparticles based on the nanoprecipitation technique. The developed nanoparticles were studied for their hydrodynamic diameter by DLS and zeta potential studies. Encapsulation efficiency, drug loading and in vitro release profile of nanoparticles were characterized by UV-vis spectroscopy.

MATERIALS AND METHODS

Materials

PLGA(50:50, MW 30000 g mol^{-1}) was purchased from Shenzhen Esun Industrial Co., MTX was supplied by Sigma. Acetone (99%) was bought from Carlo Erba, Polyvinyl alcohol (PVA), fully hydrolyzed (MW 30000 g mol^{-1}) and Poloxamer188 were from Merck (Germany).

Preparation of MTX loaded PLGA nanoparticles

Nanoparticles were prepared by the nanoprecipitation method. First 40 mg of PLGA and 5 mg of MTX were dissolved in 10 ml of acetone. A special amount of PVA(1% W/V) as well as poloxamer188(1% W/V) were dissolved in deionized water under magnetic stirring to get the lucid solution as an aqueous phase. The organic solution was poured in 100 ml of PVA/ poloxamer188 solution in a different ratio in the magnetic stirring condition at 500 rpm. After evaporation of organic phase, nanoparticles were centrifuged (Eppendorf centrifuge) at 12000 rpm for 30 min and washed twice to remove drugs on the surface of nanoparticles. Afterwards,

nanoparticles were prepared for freeze drier. The freeze-dried nanoparticles were stored in 4°C for other characterizations.

Characterization of nanoparticles

Particle size measurements

The hydrodynamic diameter of nanoparticles was studied by DLS. Afterwards, the morphology and mean diameter of nanoparticles were obtained by SEM and SemAfore(4.01 demo, JEOI., Finland) software.

Drug loading and encapsulation efficiency

The EE and DL of freeze-dried nanoparticles were examined by UV-vis spectrophotometry at the absorbance peak of MTX that is 303 nm. Briefly, 10 mg of freeze-dried powder of each formulation was dissolved in 3 ml of acetone. After vortexing for 10 min, the absorbance of the solutions were measured using UV-vis spectrophotometry. The DL and EE were then calculated as follows:

EE and DL were calculated using the equations in the following:

Each batch of formulations was studied in triplicate.

In vitro release investigations

The in vitro release profile of MTX was studied via dialysis bag. Nanoparticles were dispersed in phosphate buffered saline (PBS) at pH 7.4 and 1 ml of the suspension was placed in a dialysis bag (cut off 12 kDa) at 37 °C. The dialysis bag was then placed in a beaker containing 300 ml of PBS and stirred at 150 rpm. Aliquotes of 2 ml were extracted from the released medium at predetermined intervals (0, 0.5, 1, 3, 6, 12, 24, 48 and 72 h) and replaced with fresh medium. The extracted medium was then studied by UV-vis absorbance and the in vitro cumulative release profile of the formulation was plotted against time. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Size and zeta potential

Five experiments were carried out and the results of nanoparticle physiochemical properties are shown in Table 1. It was observed that addition of

$$EE\ \% = \frac{amount\ of\ drugs\ used\ to\ prepare\ nanoparticles - amount\ of\ drugs\ in\ the\ supernatant}{amount\ of\ drugs\ used\ to\ prepare\ nanoparticles} \times 100$$

$$DL\% = \frac{amount\ of\ drugs\ used\ to\ prepare\ nanoparticles - mount\ of\ drugs\ in\ the\ supernatant}{amount\ of\ drugs\ used\ to\ prepare\ nanoparticles + weight\ of\ PLGA} \times 100$$

Table 1. A brief view of the properties of nanoparticles.

Number	PVA (ml)	Poloxamer188 (ml)	Mean diameter±SD	Mean Zeta potential±SD (mv)	Relative DL (%)	Relative EE (%)
1	100	0	170±9	-15.5±0.6	4	54
2	80	20	198±13	-20.1±0.8	4.3	61
3	60	40	240±11	-23.7±0.3	4.9	68
4	40	60	287±10	-24.6±0.4	5.8	75
5	20	80	336±23	-26±1	6.4	89

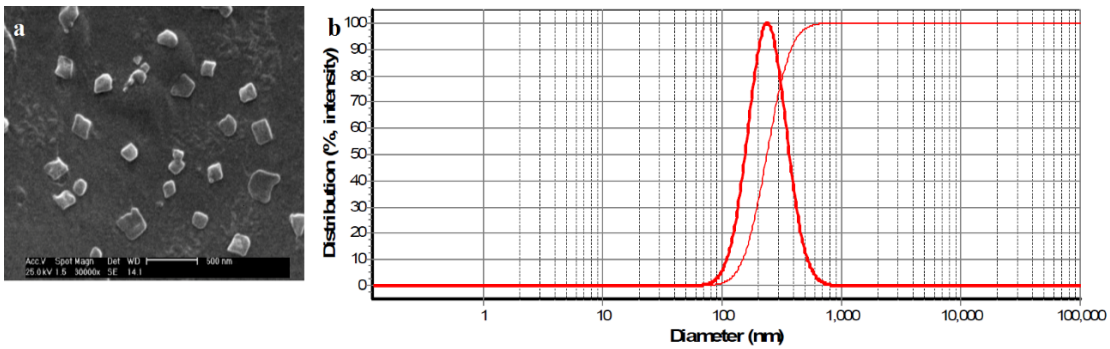

Fig. 1. a)SEM image of MTX loaded PLGA nanoparticle b)DLS result of MTX loaded PLGA nanoparticle.

poloxomer188 resulted in the increase in size of the nanoparticle from about 170 nm to 330 nm.This result is supported by the observations made by Jain *et al* (25). The researchers observed that at lower concentrations poloxomer188 adsorption is single layer whilst higher concentrations result in multi-layer adsorption. Thus the higher the poloxomer188 concentration the bigger the size of the nanoparticles.The morphology, size and size distribution of MTX loaded PLGA nanoparticles are shown in Fig. 1a and b which is related to experiment number four. The nanoparticles are not spherical in shape, rather the SEM images show irregular mostly four-sided shapes. Although this result agrees well with that of Jain *et al* (25), d'Angelo *et al* obtained spherical TEM images of poloxomer coated PLGA nanoparticles (26).

Another important parameter for nanodrug delivery colloidal systems is the zeta potential or electrokinetic potential (27). It was observed that all results had relatively large negative zeta potential which increased with increase in the amount of poloxomer188 (Fig. 2 and Table 1). The obtained zeta potential results for PVA-PLGA without poloxomer188 are similar to other work in literature (28). Wohlfart *et al* recorded a decrease of negative zeta potential when poloxomer188 was coated on PVA-PLGA nanoparticles (29). In contrast, our studies showed an increase in zeta potential with poloxomer188. This difference may be as a result of the difference in other formulation excepients, such as drugs, used in the preparation.

Drug loading and encapsulationefficiency

The amount of drug payload carried by our delivery system was assessed as drug loading efficiency and encapsulation efficiency as shown in Table 1. PVA-PLGA nanoparticles had the least DL and EE of 4 % and 54 % respectively. Our results for EE are similar to those obtained by other researchers, but our DL results are slightly lower (23, 30).We speculate that, the DL results were lower for our formulation because of the nanoprecipitation method used to in the preparation. Maleki *et al* and Afshari *et al* employed the emulsion technique which makes use of highly hydrophobic and water immiscible solvents. In comparison, nanoprecipitation technique uses water miscible solvents and thus some drug molecules may escape into the external water phase. In addition, there was an increase in both DL and EE with increasing amount of poloxomer188. This result may be explained by considering the work reported by de Oliveira *et al* (31). Their results showed that MTX tend to conjugate to the polymer surface. Hence, in this study, the amount of drug binding sites available for conjugation increased as the amount of poloxomer188 enhanced. As a result, both the drug loading and encapsulation efficiency increased.

In vitro drug release

All formulations showed extended release over a period of more of 72 hours.There was burst burst release in the first hour for all formulations (Fig. 3). This release can be attributed to the conjugatedMTX on the surface of the nanoparticles. The pattern and extent of drug release was similar to that obtained by other independent researchers (23, 30). The relationship between the amount of poloxomer188 and cumulative amount of drug release is also clearly discernable. Formulations with less amount or no poloxomer188 had higher cumulative drug release at any given point. This may be due the multi-layer adsorption of poloxomer188 on the surface of the nanoparticles which may create an extension of the nanoparticle matrix. In addition it can also be observed that formulations 1, 2 and 3 saturated after 48 hours, whilst 4 and 5 did not. This result may be explained as a vindication to the observed discrepancies in the amount of drugs encapsulated within the nanoparticles (Table 1.)

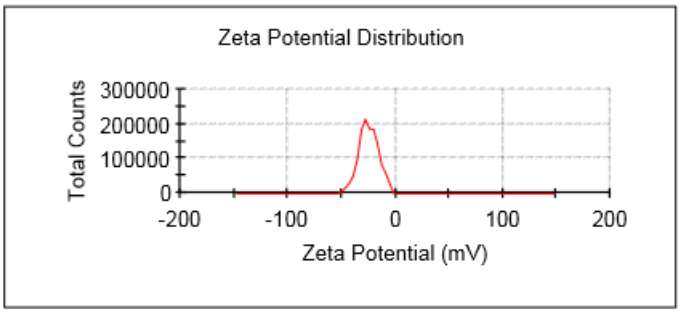

Fig. 2. Analysis of the zeta potential of the MTX loaded PLGA nanoparticle (-24.6 mV).

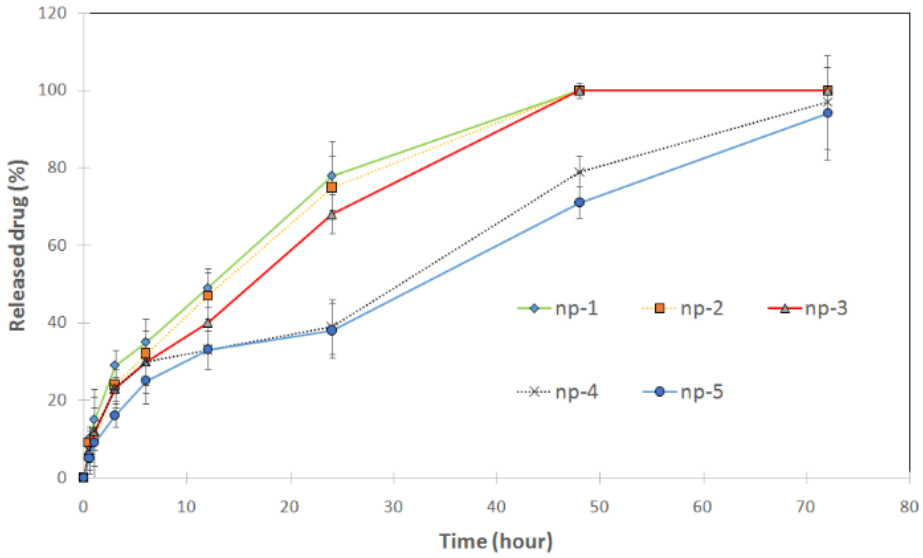

Fig. 3. Cumulative percentage of MTX release from PLGA nanoparticles in pH 7.4

CONCLUSION

We have demonstrated the impact of using two polymers, via PVA and poloxomer188, to stabilize and functionlize nanoparticles respectively. Although it is desirable to functionalize the nanoparticles for easier cellular trafficking, it is also notable that nanoparticle physiochemical properties are inevitably altered. The size of the nanoparticles increases with increasing poloxomer188, thus likely resulting in eliciting immune response. Moreover, in cancer therapy this may also result in the nanoparticles' failure to extravasate from the blood stream at tumor site by the EPR effect. However, the drug loading and encapsulation efficiency present a positive outcome that is; higher drug loading with higher amount of poloxomer188. Moreover, it has been shown that the in vitro release partten can aslo be altered for extended release by increasing poloxomer188. Thus this work presents important results which are fundamental to the development of polymer functionalized drug delivery systems.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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