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ORIGINAL ARTICLE

Synthesis of protein-coated biocompatible methotrexate-loaded PLA-PEG-PLA nanoparticles for breast cancer treatment

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Abstract

Background: PLA-PEG-PLA triblock polymer nanoparticles are promising tools for targeted dug delivery. The main aim in designing polymeric nanoparticles for drug delivery is achieving a controlled and targeted release of a specific drug at the therapeutically optimal rate and choosing a suitable preparation method to encapsulate the drug efficiently, which depends mainly on the nature of the drug (hydrophilic or hydrophobic). In this study, methotrexate (MTX)-loaded nanoparticles were prepared by the double emulsion method.

Method: Biodegradable polymer polyethylene glycol-polylactide acid tri-block was used with poly(vinyl alcohol) as emulsifier. The resulting methotrexate polymer nanoparticles were coated with bovine serum albumin in order to improve their biocompatibility. This study focused on particle size distribution, zeta potential, encapsulation efficiency, loading capacity, and *in vitro* drug release at various concentrations of PVA (0.5%, 1%, 2%, and 3%).

Results: Reduced particle size of methotrexate-loaded nanoparticles was obtained using lower PVA concentrations. Enhanced encapsulation efficiency and loading capacity was obtained using 1% PVA. FT-IR characterization was conducted for the void polymer nanoparticles and for drug-loaded nanoparticles with methotrexate, and the protein-coated nanoparticles in solid state showed the structure of the plain PEG-PLA and the drug-loaded nanoparticles with methotrexate. The methotrexate-loaded PLA-PEG-PLA nanoparticles have been studied *in vitro*; the drug release, drug loading, and yield are reported.

Conclusion: The drug release profile was monitored over a period of 168 hours, and was free of burst effect before the protein coating. The results obtained from this work are promising; this work can be taken further to develop MTX based therapies.

Keywords: *PEG*; *PLA*; *biodegradable nanoparticles*; *particle size*; *encapsulation efficiency*; *double emulsion method*; *poly(vinyl alcohol)*

Dr. Salam Massadeh is a research scientist at King Abdullah International Medical Research Center. She established the Therapy Development Lab under the Developmental Medicine Department. Dr. Massadeh earned her PhD degree in the field of bionanotechnology at the University of East Anglia, through a funding award from the Engineering and Physical Sciences Research Council, United Kingdom. In addition, she holds a master's degree in the field of drug delivery systems from the University of Reims Champagne-Ardenne, France. She also holds a BSc degree in pharmacy.

Dr. Massadeh is a registered member of the Royal Pharmacists Society, the British Society of Biology, the Royal Society of Chemistry, the British Biochemical Society, the British Society for Nanomedicine, the Institute of Nanotechnology (UK), and a member of the International Federation of Pharmacists, a worldwide association.

Dr. Manal Alaamery, Head of the Developmental Medicine Department at (KAIMRC) and was an instructor at the Harvard Medical School and Children's Hospital Boston, earned her Postdoctoral Fellowship training in Clinical Genetics form Harvard Medical School Genetics Program/Boston Children's Hospital and a Doctoral (Ph.D.) degree in Molecular Genetics & Biology from Boston College and a Master of Science in Clinical Microbiology from Thomas Jefferson University. She worked for several years as a Research Fellow at the Harvard Medical School Genetics Program where she completed her clinical training at the Laboratory of Molecular Medicine (LMM) and her Postdoctoral training at the Walsh Laboratory at Children's Hospital Boston.

Researchers have devoted significant efforts to designing and developing therapeutic agents in biodegradable biocompatible nanocomposites to be used as drug carriers. Carrier technology offers an intelligent approach to site-specific drug delivery by coupling the drug to carrier particles such as nanospheres, nanocapsules, and micelles. The biodistribution of such carriers depends on their surface properties (1–4). The major goal in designing polymeric nanoparticles for drug delivery includes realising the controlled and targeted release of the active agent to the specific site of action at the therapeutically optimal rate and choosing a suitable preparation method to encapsulate the drug efficiently, which depends mainly on the nature of the drug (hydrophilic or hydrophobic) (1, 2). One of the most important concerns is gaining a clear understanding of the nature of the nanoparticles as drug carriers including particle size, potential surface charge, drug encapsulation efficiency, and release profile. All these properties may play significant roles in the *in vitro* behaviour. In regard to the synthesis of biodegradable polymeric nanoparticles, the double emulsion method is one of the most widely used techniques in the synthesis of drug-carrier nanoparticles, and poly(vinyl alcohol) (PVA) is the most commonly used emulsifier in this process. The water-in-oil-in-water (w/o/w) double emulsion method consists of dispersed oil globules containing small aqueous droplets. To form a w/o/w emulsion, first a hydrophobic surfactant is dissolved in oil, and then aqueous solvent or water is added to form the first emulsion, w/o. The second emulsion is formed when an aqueous surfactant is added to produce w/o/w. The addition of emulsifier is a necessity to stabilise emulsions. The choice of the emulsifier is crucial in the formation of the emulsion and its long-term stability (5). The most effective emulsifiers are non-ionic surfactants that can be used to emulsify o/w or w/o. In addition, they can stabilise the emulsion against flocculation and coalescence.

Methotrexate (MTX) (4-amino-10-methylfolic acid) is an anticancer agent. It is also known as *amethopterin* and is classified as an antimetabolite chemotherapy. It is given in the early stages of breast cancer, as well as in the advanced stages of this disease. MTX kills cancer cells by acting as false building blocks in a cancer cell's genes, causing the cancer cell to die as it prepares to divide. It is currently given as an IV treatment, which causes several side effects due to unspecific binding to undesirable sites (6–8). The delivery of MTX has been studied and investigated by some scientists, where it has been shown that the cytotoxicity of MTX against cancer cells increases when combined with Nanoparticles (NPs). However, most of the reported studies have focused on the use of magnetic nanoparticles (9), carbon nanotubes (10), dendrimers (11), chitosan (12–15), and poly(lactic-co-glycolic acid) (PLGA) nanoparticles (16).

Shatha Al-Qatanani worked as a Research technologist in King Abdullah International Medical Research Center located in Riyadh, Saudi Arabia. Shatha is a graduate of Jordan University of Science and Technology, where she received a master's degree in Applied Chemical Sciences. Her professional interests focus on drug improvement, nanomedicines, and physical chemistry. Her current projects include synthesis of nanoparticles, protein coating and bio-imaging. In addition, she served as a researcher at Jordan University of Sciences and Technology and was trained at Royal Scientific Society.

Mr. Saqer Alarifi is a Teaching assistant at KSAU-HS; he earned his B.Sc. degree in pharmacy with honors from King Saud University. He has worked as a Researcher in Riyadh Pharma, where he was involved in the development of a new formula for an API. In addition, he has worked on several research projects directly related to nano-drug delivery systems, polymer nanoparticles, and solid lipid nanoparticles. He has helped in maintaining ongoing research projects, analyzing and interpreting data. He is currently pursuing his higher education in North America.

Ms. Shahad Bawazeer, a junior researcher, earned her Bachelor degree with first-class honors in Genetics from the University of Manchester in 2013. She was trained at King Faisal Specialist Hospital & Research Centre in various molecular and genetic laboratory techniques. In 2014, Ms. Bawazeer joined the Developmental Medicine Department at King Abdullah International Medical Research Center (KAIMRC) as a Research Laboratory Technologist, where she was exposed to a large number of challenging research techniques.

Ms. Yusra AlYafee, is a junior researcher, earned her B.Sc. and M.Sc. degree with honors in Clinical Biochemistry from King Saud University. She worked as a Research Technologist in the Neurogenetic Department at King Faisal Specialist Hospital and Research Centre (KFSHRC) in Riyadh. In addition, she worked in an autism research project directed by King Abdulaziz City for Science and Technology (KACST). Recently, Ms. Ahmed played a vital role in setting up the infrastructure of the Developmental Medicine Department at King Abdullah International Medical Research Center (KAIMRC), and she helps is maintaining ongoing research projects. Her current research interest is studying the basic pathway that contributes to several genetic disorders.

In this study, we synthesised a drug delivery system of MTX-loaded PLA-PEG-PLA NPs using the double emulsion method as a promising tool to deliver MTX. PLA-PEG-PLA triblock polymer was used in this study because it is biodegradable, has low immune responses *in vivo*, and has high physical strength to form stable

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nanoparticles. It has been approved by the US Food and Drug Administration for many applications, including drug delivery. Moreover, its amphiphilic nature makes it a suitable carrier of anticancer lipophilic drugs (17). In this work, we were able to control the particle size by varying different parameters. An *in vitro* analysis of the synthesised MTX drug delivery system is presented in this paper; nanoparticle yield, drug loading, and entrapment efficiency are included. The MTX release was monitored over a period of 168 h.

Results and discussion

Synthesis of MTX-loaded PLA-PEG-PLA NPs

The preparation of the PLA-PEG-PLA triblock polymer nanoparticles was achieved by applying the double emulsion method. In this case, the triblock polymer was dissolved in an oil phase (chloroform), with the addition of dimethyl sulfoxide (DMSO), and high-power ultrasonication of the first emulsion forms was performed. Then, PVA was added as an emulsifying agent to form the second emulsion with the polymer/DMSO solution. PVA was added in variable concentrations (1, 2, and 3% respectively). The MTX-loaded PLA-PEG-PLA NPs were synthesised using the same method; however, the MTX was added in the first step, forming the first emulsion. Figure 1 illustrates the synthesis method used to obtain the MTX-loaded PLA-PEG-PLA NPs. The different batches synthesised were characterised using dynamic light scattering (DLS) for particle size determination.

The PVA solution stabilises the emulsion between polyethylene glycol-polylactide acid (PEG-PLA) with MTX dissolved in DMSO. PEG-PLA is soluble in chloroform, while MTX is insoluble in chloroform due to differences in density and polarity. An emulsion forms when the polymer and the drug solution are homogenised together using high-speed rotation by employing a sonicator, causing them to appear in a single phase. The resulting emulsion is unstable. Adding PVA overcomes this obstacle; the hydroxyl group will interact with DMSO, and the vinyl group will interact with chloroform to enrich the stability of the formed emulsion refer to the schematic illustration in Figure 1 (17).

BSA coating of the MTX-loaded PLA-PEG-PLA NPs

The MTX-loaded PLA-PEG-PLA NPs were coated with bovine serum albumin (BSA) to increase their biocompatibility. BSA was used here as a model protein due to its unique properties and its high surface-binding affinity. The BSA coating was applied by incubating the PLA-PEG-PLA NPs with a BSA solution at 60°C; the BSA attached to the surface of the PLA-PEG-PLA NPs *via* physical adsorption. Samples of pure PLA-PEG-PLA, MTX-loaded PLA-PEG-PLA, BSA, and BSA-coated-MTX-loaded NPs in a physical mixture of 1:1 were characterised by Fourier transform infrared (FTIR) spectroscopy. The obtained spectra are illustrated in Fig. 2a–c, which shows the characteristic peak at 3,505 cm^{-1} is assigned to the terminal hydroxyl groups in the copolymer. The bands at 2,995; 2,950; and 2,880 cm^{-1} are due to C–H stretch. The strong absorption at 1,760 cm^{-1} is assigned to C=O

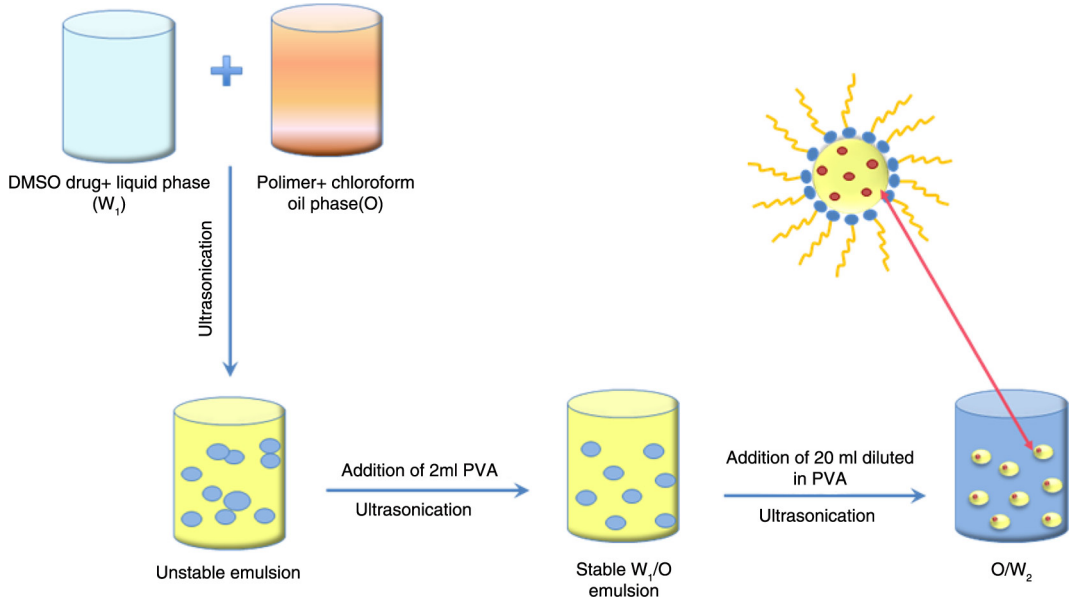

Fig. 1. Schematic illustration of the synthesis method of methotrexate PLA-PEG-PLA NPs. Before adding the poly(vinyl alcohol) (PVA), large interfacial tension is formed at curved surfaces (causing formation of unstable emulsion); after the addition of the PVA, the interfacial tension decreases by making the interfacial surface flat.

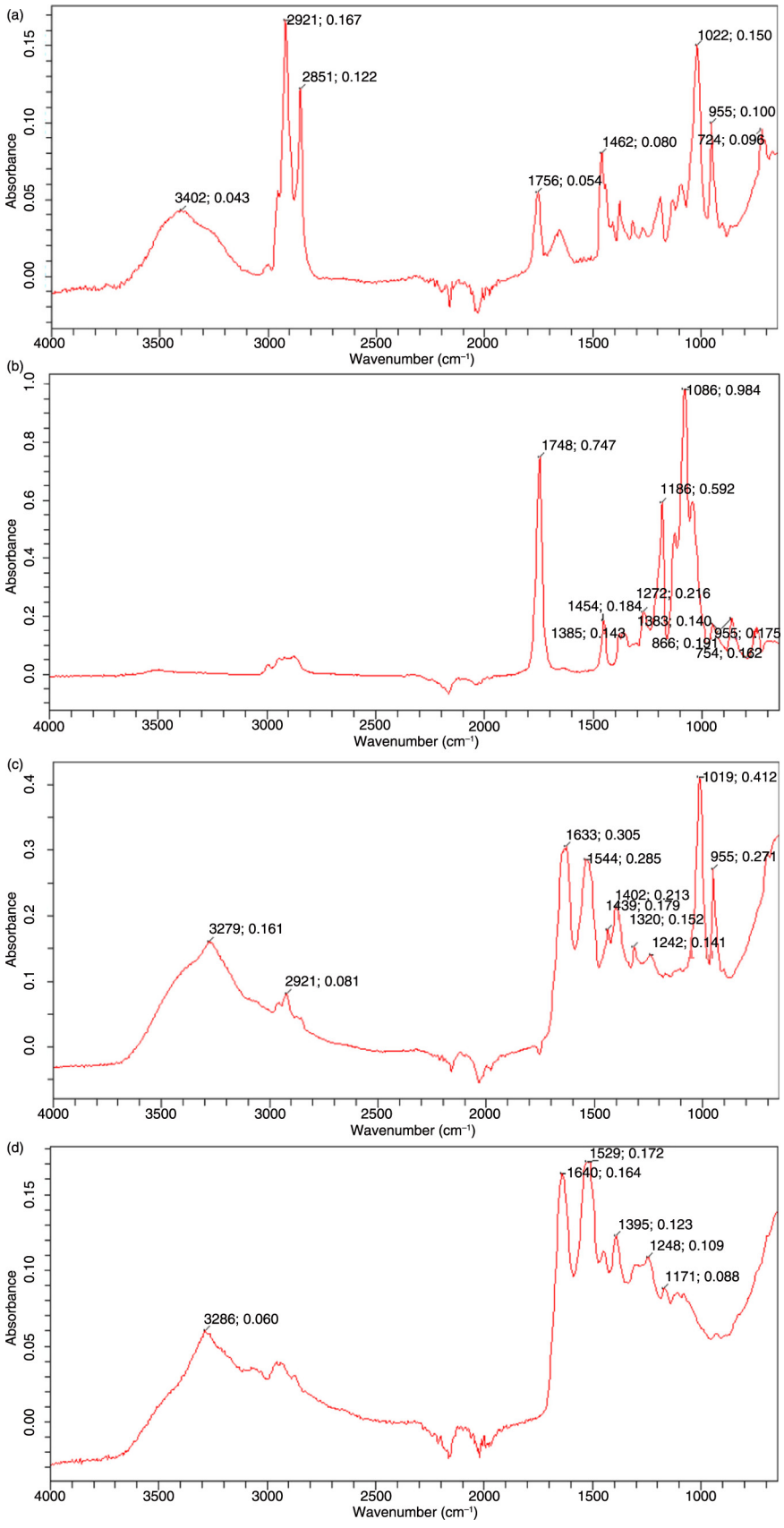

Fig. 2. Fourier transform infrared spectra of (a) methotrexate (MTX)-loaded PLA-PEG-PLA NPs; (b) PLA-PEG-PLA NPs; (c) bovine serum albumin (BSA)-coated, MTX-loaded PLA-PEG-PLA NPs; and (d) BSA.

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Table 1. Median particle size and potential of free, letrozole (LTZ)-loaded, and MTX-loaded nanoparticles at different PVA concentrations

Formulation	PVA%	Particle size (nm)	Poly dispersity index (PDI)	Zeta potential (mV)	Mobility (μ/s) (V/cm)
(PLA-PEG-PLA) NPs	3	157.01 ± 0.98	0.212 ± 0.020	−12.36 ± 2.84	−0.97 ± 0.22
MTX-loaded NPs		172.28 ± 2.97	0.118 ± 0.030	−11.71 ± 4.01	−0.91 ± 0.31
(PLA-PEG-PLA) NPs	2	145.43 ± 1.55	0.155 ± 0.018	−28.47 ± 2.62	−2.22 ± 0.21
MTX-loaded NPs		111.31 ± 0.95	0.211 ± 0.006	−22.67 ± 1.19	−1.77 ± 0.09
(PLA-PEG-PLA) NPs	1	100.41 ± 2.13	0.209 ± 0.006	−31.43 ± 2.90	−2.46 ± 0.230
MTX-loaded NPs		79.3 ± 0.52	0.225 ± 0.006	−21.65 ± 0.93	−1.69 ± 0.007
(PLA-PEG-PLA) NPs	0.5	128.07 ± 0.50	0.165 ± 0.009	−3.51 ± 2.73	−0.27 ± 0.21
MTX-loaded NPs		103.69 ± 0.63	0.156 ± 0.012	−28.49 ± 0.48	−2.23 ± 0.04

stretch, while the band at 1,175–1,095 cm^{-1} is due to C–O stretch. In addition, Fig. 2c and d shows the spectra of the BSA-coated MTX-loaded PLA-PEG-PLA and the free BSA, where similar characteristic bands are present in both spectra, illustrating successful BSA coating of the MTX-loaded PLA-PEG-PLA NPs.

Particle size and size distribution of PLA-PEG-PLA NPs and MTX-loaded PLA-PEG-PLA NPs

The mean particle size of the developed NPs measured by DLS at different PVA concentrations is presented in Table 1.

The particle size and the size distribution were measured by DLS. The mean diameter of the (PLA-PEG-PLA) NPs varied depending on the emulsifier used. It was observed that the mean particle size is directly related to the concentration of PVA used in the formula. For instance, the results presented in Table 1 show a direct correlation between the particle size and the percentage of PVA used. The zeta potential and the electrophoretic mobility were measured using a ZetaPALS potential analyser (Brookhaven instruments, Holtsville, New York, USA); the results are displayed in Table 1. The surface charges of the (PLA-PEG-PLA) NPs and the MTX-loaded NPs were all in the negative range, indicating the presence of negatively charged functional groups. This finding is in agreement with the results obtained from the FTIR analysis. Figure 3 shows the variation of PVA concentrations and its effect on particle size.

Particle size and size distribution of BSA-coated MTX-loaded nanoparticles

The mean particle size of BSA-coated, MTX-loaded PLA-PEG-PLA NPs measured by DLS at different PVA concentrations is presented in Table 2. The BSA-coated, MTX-loaded PLA-PEG-PLA NPs had a size range of approximately 218–168 nm. However, examining the

Table 2. Particle size of BSA-coated, MTX-loaded nanoparticles at different PVA concentrations

Formulation	PVA%	Particle size (nm)	PDI
BSA–MTX-loaded NPs	3	168.75 ± 1.10	0.254 ± 0.012
BSA–MTX-loaded NPs	2	196.35 ± 1.63	0.324 ± 0.006
BSA–MTX-loaded NPs	1	218.67 ± 2.70	0.332 ± 0.10
BSA–MTX-loaded NPs	0.5	274.36 ± 4.86	0.306 ± 0.012

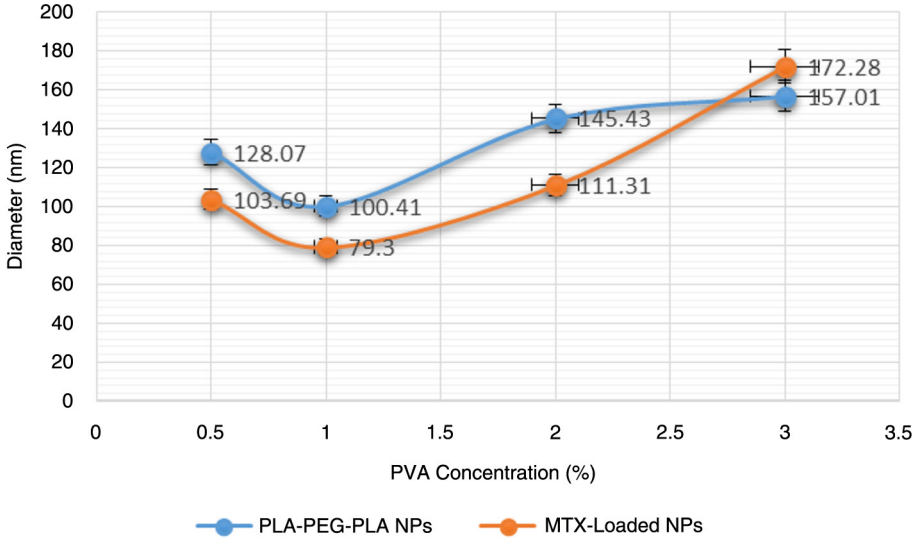

Fig. 3. The effect of varying PVA concentration on (PLA-PEG-PLA) NPs and MTX-loaded PLA-PEG-PLA NP particle size.

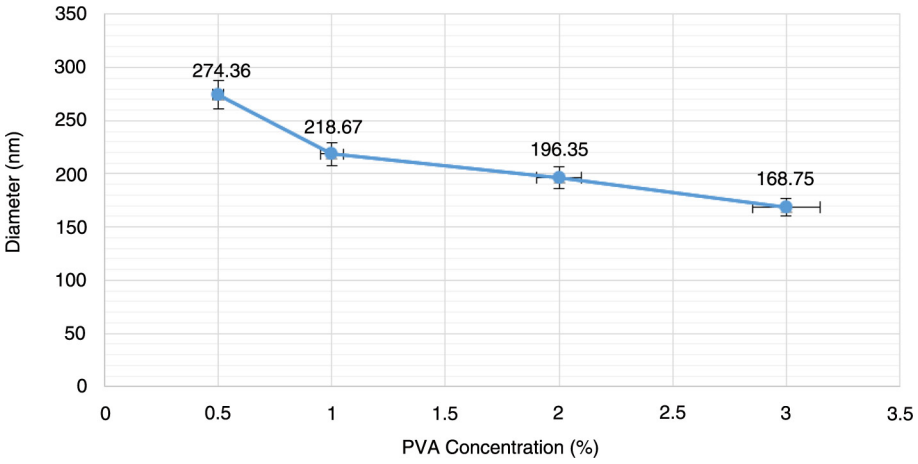

Fig. 4. MTX-loaded NP coating at different PVA concentrations.

results displayed in Table 1, the MTX-loaded PLA-PEG-PLA NPs had a size range of 79–157 nm in diameter. In both formulations (BSA-coated and non-BSA-coated) of MTX-loaded PLA-PEG-PLA NPs, the particle size was directly related to the ratio of PVA used during the synthesis. When the concentration of PVA was high in the formula, a decrease in particle size was observed.

In the case of the BSA-coated, MTX-loaded PLA-PEG-PLA NPs, the BSA coating had an effect on the particle size. The BSA attached to the surface of the NPs by adsorption. BSA is adsorbed onto the surface of the MTX-loaded PLA-PEG-PLA NPs, where it also tends to be desorbed due to reversible weak adsorption. Figure 4 shows that the BSA-coated, MTX-loaded PLA-PEG-PLA NPs tend to have a decrease in particle size with different PVA concentrations in the formula.

The MTX-loaded PLA-PEG-PLA nanoparticles synthesised were within the size range of 120–200 nm; they showed a relatively homogeneous size distribution as revealed by the polydispersity index values. In order to avoid the elimination of NPs in spleen sinusoids and liver fenestrae and to effectively deliver drug to the targeted tumour tissue, the size of loaded nanoparticles should not exceed 200 nm and should not be 6 nm or smaller to avoid rapid elimination from the bloodstream (9). As a result, this work provides a suitable method to control and optimise the size of NPs.

Evaluation of nanoparticle yield, drug loading, and entrapment efficiency

Drug entrapment efficiency was determined by the direct method. The drug entrapment and the entrapment efficiency depend on many factors including the composition of the copolymer, the drug used, and the method of preparation. Table 3 lists the encapsulation efficiency of MTX at each concentration of PVA used for the synthesis of MTX-loaded PLA-PEG-PLA NPs. From Table 3, it can be seen that the micelle yield was high, having a maximum of 80.4% when 1% of the PVA was used, and the yield was at a minimum of 59.2% when the PVA was 2% of the formula. In addition, the entrapment efficiency was 47.8% when 1% of the PVA was used, and the entrapment efficiency was at a minimum of 23.4% when the PVA was 2%. Similarly, for the loading capacity, the highest loading capacity was obtained at 1%. Therefore, 1% PVA in the formula gave the optimal parameters in terms of yield, entrapment efficiency, and loading capacity.

In vitro *drug release studies*

The release of drugs from biodegradable nanoparticles is a process that is affected significantly by many factors – for instance, the type of polymer used, polymer degradation rate, crystallinity, melting temperature, and the binding affinity between the drug and the polymer.

Another crucial factor that plays a major role in the release profile is the ability of the polymer to integrate the drug. It has been reported that hydrophobic drugs tend to have a lower release rate from nanoparticles. Water insoluble drugs, in this case MTX, tend to stay within the hydrophobic core of the nanoparticles, having an effect on the release profile (18–23).

Figure 5 shows the release profile of different batches of MTX-loaded PLA-PEG-PLA NPs synthesised with different concentrations of PVA (0.5, 1, and 2%). The MTX release was measured at different time intervals over a period of 72 h. As illustrated in Fig. 5, the release

Table 3. Encapsulation efficiency, loading capacity, and percent yield of MTX-loaded NPs at different PVA concentrations

PVA %	0.5	1	2	3
% LC	0.4167	0.7774	0.5207	0.5860
% EE	24.4	47.8	23.4	39.3
% yield	76.5	80.4	59.2	86.6

LC, loading capacity; EE, encapsulation efficiency.

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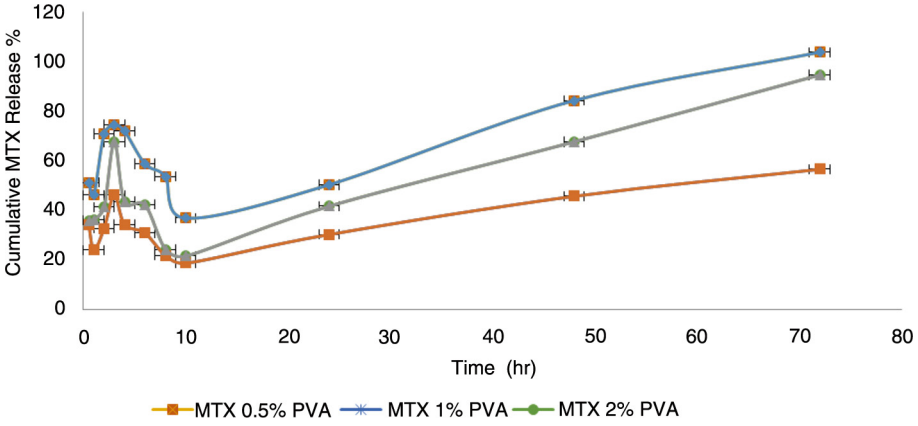

Fig. 5. Cumulative drug release of MTX-loaded PLA-PEG-PLA NPs over a period of 72 h. The three lines represent different batches of MTX-loaded PLA-PEG-PLA NPs prepared with 0.5, 1, and 2% PVA.

profile of the three batches was quite similar. All three release profiles exhibited some degree of burst effect in the first few hours, and the MTX was then released slowly over 72 h, which makes these MTX-loaded PLA-PEG-PLA NPs candidates for sustained release formulations.

Furthermore, Fig. 6 shows the MTX release from MTX-loaded PLA-PEG-PLA NPs and BSA-coated, MTX-loaded PLA-PEG-PLA NPs. The MTX release was measured over a period of 168 h. Figure 6 shows that the BSA-coated NPs display a rapid release onset, demonstrated by the burst release observed during the first hour of release. In addition, both the MTX-loaded PLA-PEG-PLA NPs and the BSA-coated, MTX-loaded PLA-PEG-PLA NPs have an extended release profile over a period of 168 h, which also indicates that these formulas may be suitable formulations as sustained release therapy.

Materials and methods

Materials

Polylactide-block-poly(ethylene glycol)-block-polylactide triblock (PLA average Mn = 1,500; PEG average Mn = 900), MTX (Mw = 454.44 g/mol anhydrous basis), chloroform, PVA (Mw = 89,000–98,000; 99% hydrolysed), and BSA (lyophilised powder ≥ 96%) were obtained from Sigma-Aldrich, USA and were used as received.

Preparation of 3, 2, 1, 0.5% PVA

To prepare 3, 2, 1, and 0.5% PVA, 3.0, 2.0, 1.0, and 0.5 g, respectively, were weighed separately and dissolved in 100 mL distilled water (Table 4). The weighed amount was added gradually to cold water while stirring. After adding the full amount, the solution was heated to 140°C for 2 h. The solution container was covered with aluminium foil

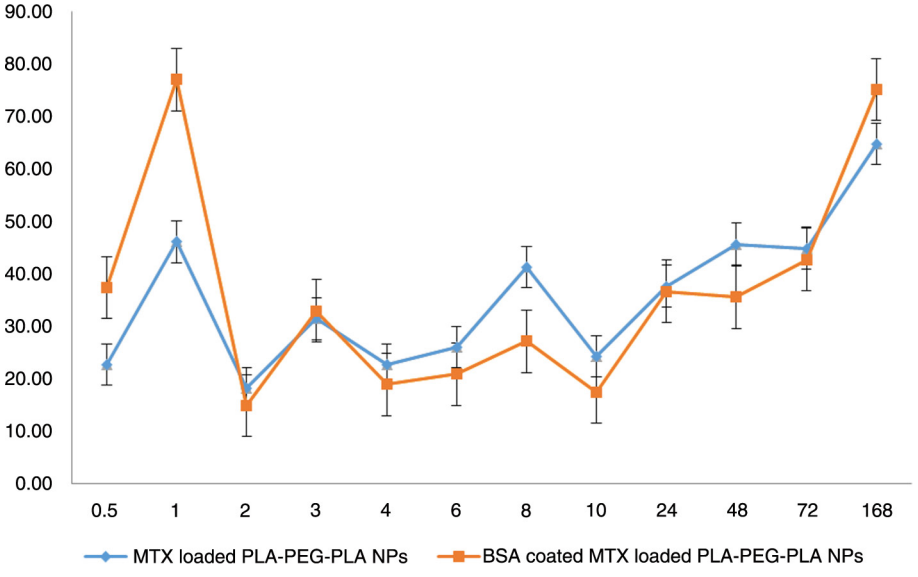

Fig. 6. Drug release of MTX-loaded PLA-PEG-PLA NPs and BSA-coated, MTX-loaded PLA-PEG-PLA NPs.

Table 4. Concentration variations of PVA used for synthesising PEG-PLA nanoparticles

PVA concentration (%)	Added volume of concentrated PVA (mL)	Dilution of each PVA solution	Added volume (mL) of diluted solution of PVA
3	2.0	0.3	20.0
2	2.0	0.2	20.0
1	2.0	0.05	20.0
0.5	2.0	0.05	20.0

to distribute the heat evenly over the container. After 2 h, a clear solution was obtained.

Synthesis of PLA-PEG-PLA nanoparticles using different concentrations of PVA

The nanoparticles were prepared by the double emulsion method (7). In 1.0 mL chloroform, 15.0 mg of PEG-PLA was dissolved. 200 µL of DMSO was emulsified by ultrasonication using a 2.0 mm probe (VCX 130 ultrasonic processor, Sonics, CT, USA) (30 s, 100% power) in the polymer solution; in this way, the first emulsion was formed. Separately, 2.0 mL of 3, 2, 1, and 0.5% PVA were emulsified in the polymer/DMSO solution by ultrasonication using a 2.0 mm probe (30 s, 100% power). The resulting emulsion was then diluted by adding 20.0 mL of 3%, 2%, 0.5%, and 0.5% PVA solution. The obtained white emulsion was then centrifuged for 1.0 h at 16,000 rpm.

Synthesis of MTX-loaded nanoparticles using different concentrations of PVA

The nanoparticles were prepared by the double emulsion method (7). In 1.0 mL chloroform, 15.0 mg of PEG-PLA was dissolved. 200 µL of MTX dissolved in 1.0 mL DMSO was emulsified by ultrasonication using a 2.0 mm probe (VCX 130 ultrasonic processor, Sonics) (30 s, 100% power) in the polymer solution; in this way, the first emulsion was formed. 2.0 mL of 3, 2, 1, and 0.5% PVA were separately emulsified in the polymer/(drug + DMSO) solution by ultrasonication using a 2.0 mm probe (30 s, 100% power). The resulting emulsion was then diluted by adding 20.0 mL of 0.3, 0.2, 0.05, and 0.05% PVA solutions. The white emulsion obtained was then centrifuged (Thermo Scientific, Massachusetts, USA) for 1.0 h at 16,000 rpm. The pellets were washed three times to remove any excess drug and were centrifuged after each wash for 1.0 h at 16,000 rpm.

Synthesis of BSA-coated MTX-loaded nanoparticles

A solution of MTX-loaded NPs was prepared by dissolving 1.2 mg (±0.1) in 2.00 mL distilled water. To 1 mL of the MTX-loaded NP solution, 100 µL of 1% BSA solution was added. The solution then was incubated for 2 h at 38°C with shaking at 350 rpm. The sample was then collected and dried using a Rotavapor rotary evaporator (BUCHI, Flawil, Switzerland), weighed, labelled, and stored at 4°C.

The obtained white solid of (MTX-loaded) NPs was purified by dialysis. A 100-µm membrane was placed on each side of a 1,000-µL double-sided reservoir and was filled with 1 mL of NP solution (MTX-loaded NP solution); this was placed in 250 mL of 0.06 M Phoshphate Buffer Saline (PBS) with stirring. The PBS solution was changed every 3 h, a total of three times.

Characterisation of void and loaded nanoparticles

FTIR characterisation

FTIR (Cary 630 FTIR spectrometer, Agilent, Santa Clara, USA) analysis was conducted to verify the possibility of chemical bonds between drug and polymer. Samples of pure PLA-PEG-PLA; MTX-loaded nanoparticles; pure BSA; and BSA-coated, MTX-loaded NPs were scanned in the IR range from 400 to 4,000 cm^{-1}.

Particle size analysis

The particle size distribution (mean diameter and polydispersity index) of nanoparticles were determined using a Brookhaven ZetaPALS analyser. Each nanoparticle preparation was analysed with five readings per nanoparticle sample. Mean particle size and polydispersity index were calculated for each sample. For a monodisperse system, the polydispersity index should range between 0.03 and 0.06 (8), and the zeta potential was determined by phase analysis light scattering using a ZetaPALS zeta potential analyser (Brookhaven, New York, USA) at 658 nm, with scattering angle of 90° at 25°C. All samples were diluted in water to a suitable scattering intensity, and measurements were performed with three independent batches of NPs at each PVA concentration duplicate.

The encapsulation efficiency and loading capacity

The encapsulation efficiency and drug-loading capacity were determined by calculating the ratio between the encapsulated drug (MTX) and the total amount of added drug. This was achieved by dissolving the loaded nanoparticles in DMSO and measuring the absorbance of MTX-loaded nanoparticles at a λmax of 360 nm using a SpectraMax Plus384 UV spectrophotometer (Molecular Device, Silicon Valley, California, USA) to determine the amount of encapsulated drug in each batch (direct method).

In vitro release study of MTX-loaded NPs at different PVA concentrations

Known amounts of MTX/PLA-PEG NPs were dispersed by a bath sonicator (Branson 3800, Connecticut, USA) for 20 min with the release media (1.0 mL of phosphate buffer, pH = 7.2). An amount equivalent to 690 mcg of the NPs in 1.0 ml of release media was placed inside sealed cellulose dialysis tubing (Carolina, North Carolina, USA) with a cut-off of 12,000–14,000 Da. The dialysis tubing was placed in a

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screw-cap bottle with 19.0 ml release media and kept in a shaking water bath (GFL 1083, Burgwedel, Germany) at 37°C and medium speed. At different time intervals, aliquots of 3.0 ml were withdrawn and immediately replaced with the same volume of fresh release media. The amounts of MTX released were assessed by double beam UV spectrophotometer (Thermo-Evolution UV 60 S, Thermo Scientific, Massachusetts, USA), which was set up at 310 nm for MTX *versus* a calibration curve prepared in the same buffer.

Conclusions

The PLA-PEG-PLA triblock polymer nanoparticle is a biocompatible polymer that shows great promise in the field of drug delivery. MTX was successfully loaded within the nanoparticles, and the drug showed sustained release over a period of 168 h. Moreover, varying the emulsifying agent PVA had a clear effect on the particle size and did not affect the release profile. In addition, the MTX-loaded PLA-PEG-PLA nanoparticles were coated with BSA to improve their biocompatibility. The BSA coating of the MTX PLA-PEG-PLA NPs has been shown to affect the release profile of the MTX; it has been observed that there was a burst release in the first hour of the release.

We monitored the drug release of different batches of MTX-loaded PLA-PEG-PLA nanoparticles. The release profile showed sustained release over a period of 168 h. It has been demonstrated that the BSA-coated, MTX-loaded PLA-PEG-PLA nanoparticles had a burst effect in the release when compared with the MTX-loaded PLA-PEG-PLA nanoparticles. The overall results indicate that the synthesised biocompatible MTX-loaded PLA-PEG-PLA nanoparticles have significant potential to be used as sustained release anticancer therapies, overcoming the side effects of the current IV treatments.

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Conflict of interest and funding

The authors declare no conflict of interest.

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