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Simvastatin In-Situ Forming Implants: Preparation and Characterization

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ABSTRACT

Biodegradable injectable *in-situ* forming implants (ISFIs) emerged as a parenteral depot system to offer a sustained-release effect of many drugs. The aim of the present study was to prepare and evaluate simvastatin (SMV) loaded ISFIs. The ISFIs were formulated by applying the phase inversion technique using a 25% w/w concentration of PLA, PLGA 50:50 and PLGA 75:25 polymers with 20%w/w drug. N-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO) and triactin were used as solvents. The influence of the solvent on the viscosity of the polymer solution and the morphology of the formed implants was investigated. Moreover, the effect of the solvent and polymer on the release of SMV was determined. ISFI prepared using triacetin showed the highest viscosity followed by those prepared using N-methyl-2-pyrrolidone (NMP) and dimethyl sulfoxide (DMSO). Only NMP and DMSO could form intact implants within 5-15 minutes. The *in-vitro* dissolution study indicated that ISFIs could sustain SMV release up to 21 days with a total drug released ranging from 48.37 to 78.73% depending on polymer and solvent type. ISFI prepared using DMSO showed higher rate of drug release than those prepared using NMP. Increasing polymer hydrophilicity by increasing glycolide ratio resulted in increasing SMV burst effect and release rate. The obtained results indicated that SMV implants can be considered as a promising system for site-specific controlled delivery of SMV.

INTRODUCTION

Parenteral delivery of drugs represents main route of drug administration to achieve a fast action. Although the injection of suspensions and emulsions was extensively used, these dosage forms do not allow sustained or controlled release of the drug. Several systems, such as microemulsions, vesicular systems and nanoparticles have been developed that can achieve a sustained release of the drug. The major drawbacks of these systems include the migration of the drug from the site of injection, the time needed for their formulation and scale up production (1). Thus, liquid formulations developing a solid depot after injection, also known as *in-situ* forming implants (ISFIs), emerged as an attractive parenteral delivery system. ISFI can deliver drugs at a controlled rate over an extended period of time, and hence reducing the dose and frequency of administration (2).

ISFI offers many advantages for both systemic and local drug delivery. Among them, the ease of manufacture and application, the ability to incorporate a wide range of molecules including proteins and hydrophobic drugs (3, 4). They can be localized at the site of injection, reducing the distribution of the drug to other body organs, and hence reducing the side effects (5).

Generally, ISFI preparation depends on the use of biodegradable, water insoluble polymers, which are dissolved in water miscible solvents. Upon injection into body, water penetrates the system and the organic solvents diffuse outside, resulting in polymer precipitation and implant formation (6). The most commonly investigated polymers include poly (lactic acid; PLA), co-poly (lactic: glycolic acid; PLGA) and poly (€-caprolactone; PCL) (7). Whereas, the solvents used may differ from hydrophilic solvents as NMP, DMSO, glycol furol and tetraglycol to the more hydrophobic solvents such as triacetin, ethyl acetate, and benzyl benzoate (8). However, NMP and DMSO are favored owing to their excellent water solubility and highly acceptable safety and toxicology profiles.

Statins, the potent inhibitors of 3-hydroxy- 3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), were mainly used for their cholesterol-lowering effect. They protect against many cardiovascular and cerebrovascular diseases. However, several therapeutic effects were recently reported, which include bone regeneration, antithrombotic, antioxidant, anti-inflammatory, and immunosuppressive actions (9, 10).

The therapeutic benefits of simvastatin (SMV) have been extended in the last decade to involve the prevention and the treatment of many diseases. Among them, it could enhance the process of fracture and bone healing (11-15). Moreover, the antitumor activity of SMV was investigated in many types of research. The IC_{50} of the combined simvastatin and tocotrienol nanoparticles was lower than the reference α-tocopherol nanoparticles, which confirmed the potential of the simvastatin in cancer therapy (16). Same results were obtained upon combined administration of tocotrienols and simvastatin by lipid nanoemulsions (17).

However, the delivery of SMV to the target site is considered a challenge for the pharmaceutical industry. SMV is poorly soluble in aqueous phase with a short half-life (about 2 h). Oral delivery of SMV resulted in extensive first-pass effect with low oral bioavailability of about 5% (18). Therefore, one of the main obstacle of SMV formulation is to control and sustain its release rate.

In light of the above, the goal of the present study was to formulate a long acting SMV loaded ISFI for controlled and localized delivery, thus reducing SMV distribution to other body organs. In this study, the effect of solvent and polymer type on the viscosity of the polymer solution, morphology of the formed ISFI and *in-vitro* dissolution was investigated.

MATERIALS AND METHODS

Materials

Simvastatin with 98% purity (SMV) was kindly supplied by Amoun Pharmaceutical Company, Cairo, Egypt. PURASORB PDLG 7507(PLGA; lactide/glycolide ratio is 75:25, with an inherent viscosity midpoint of 0.7 dl/g) and PURASORB PDLG 5004 (PLGA; lactide/glycolide ratio is 50:50, with an inherent viscosity midpoint of 0.4 dl/g) were kindly supplied by Corbion Purac Biomaterials, Netherlands. Poly (D, L-lactide) (PLA; Mw=75,000–120,000), N-methyl-2-pyrrolidone (NMP) and 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co., USA. Dimethyl sulphoxide (DMSO) was purchased from Alpha Chemika, Mumbai, India. Triacetin (TA) was purchased from BDH chemicals Ltd, Poole, England. Disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and sodium chloride (NaCl) were purchased from El-Nasr Pharmaceutical Co, Adwic, Egypt. All other chemicals used were of analytical grade.

Methods

Determination of simvastatin solubility in phosphate buffer saline and organic solvents

The solubility of SMV in different organic solvents used for preparing the ISFIs and in the phosphate buffer saline (PBS) release medium was determined (7). Excess amount of SMV was added to each solvent and sonicated for 5 min to get a clear solution. The obtained solution is centrifuged for 15 min at 15,000 rpm. 10µl of clear supernatant was withdrawn and appropriate dilutions were performed. Drug concentration in PBS was determined at λ_{max} 238 nm using UV spectrophotometer (Jenway-6800, Japan).

Preparation of polymer solution

The appropriate amounts of either PLA, PLGA50:50 or PLGA 75:25 were dissolved in the selected organic solvents; NMP, DMSO and TA so as to prepare ISFIs containing 25%w/w of the selected polymer. The mixture was vortexed till complete dissolution of the polymer. Desired amount of SMV was then added to the prepared solutions and then mixed. All polymer solutions were prepared with 20% drug loading.

Drug loading (DL%) was calculated using the formula below:

Drug loading % = [Weight of drug/ Weight of (drug + polymer)] x 100.

Syringibility test through 23G needle was performed for all formulations (7). To check the ability of the polymer solution to form implant, they were injected into PBS.

Rheological Measurements

The viscosity of the prepared polymer solution using PLGA 50:50 with different solvents was obtained using cone and plate programmable viscometer (Brookfield Engineering Laboratories Inc., Model HADV-II, USA) connected to a digital thermostatically controlled circulating water bath (Polyscience, Model 9101, USA). Steady shear measurement was conducted where the rheograms of the prepared solutions was performed at 25±0.1°C with spindle 52, the shear rates range from 50 to 400 s^{-1} corresponding to 25 to 200 rpm with 10 s between each two successive speeds. Following loading of the viscometer, sample was left to achieve equilibrium for 5 min. Ramp time for each viscosity stage was reading after 20 s. All studies were performed in triplicates and the average was taken (19).

Morphological study on ISFIs using real-time optical microscopic

In order to study the sol-gel transition process and the effect of solvent type and its diffusion on the implant formation, samples were observed using a real-time optical microscopic (8). A specified amount of 1µl of drug-free polymer solution was prepared using PLGA 50:50 and was placed on two glass slides, drops of fresh PBS were added closely on the polymer solution from one side, while excess PBS was absorbed by a piece of filter paper from the other side, optical pictures were recorded in 0, 5, 10, 15, and 30 min under 10x and 40x magnifications power using ordinary light microscope (Carl Zeiss, Berlin, Germany) and photograph was taken by means of a fitted camera (Panasonic, Japan).

Determination of simvastatin *in-vitro* release rate

The *in-vitro* release was performed using shaking water bath (GFL, Germany), using 20-ml screw cap vials, filled with 10ml PBS (pH 7.4) with 30% ethanol to maintain sink condition (20). A sample of 0.5g of the prepared polymer solution was injected into the vials to form a compact mass. The sample vials were shaken at 37°C±0.1°C with a constant agitation (100 oscillation/min). One-milliliter samples were withdrawn and replaced with equal volumes of fresh buffer solution. Concentration of SMV in the withdrawn samples was determined using HPLC method described below.

Determination of simvastatin content using HPLC method

The amount of SMV released into the dissolution media was determined by high performance liquid chromatography using a $C_{18,}$ RP-column (HPLC; 1200 series, Agilent Technologies, Inc., USA). The mobile phase of acetonitrile–phosphate buffer–methanol (5:3: 1, v/v/v) was delivered at a flow rate of 2 ml/min. The obtained samples were filtered by 0.45µm membrane into a vial and a 20 µl of the samples was injected with an autosampler (Agilent 1100/1200). The column effluent was detected at λ_{max} 238 nm by diode array detector. The calibration curve for the quantification of SMV was at 12-100 µg/ml with a correlation coefficient of R^2= 0.994.

RESULTS AND DISCUSSION

Determination of simvastatin solubility in phosphate buffer saline and organic solvents

The solubility of SMV was determined in the different organic solvents used for preparation and in the PBS (pH 7.4) as a release medium. It was observed that SMV has a low solubility

in PBS media (1.5mg/ml). However, it showed a higher solubility in both NMP, and DMSO (≈1 g/ml) while less solubility in TA (≈0.33 g/ml). Generally, ISFIs can form homogeneous or heterogeneous system depending on whether the drug is dissolved or dispersed in the polymer solution respectively (21). Thus, it could be concluded that the injectable formulation of SMV in all used solvents would form a homogeneous polymer solution of SMV.

Preparation of polymer solution

It was observed that all the used solvents had a good solvating power for the PLA and PLGA polymers forming injectable polymer solutions. Besides, NMP and DMSO were able to dissolve the tested polymers faster than TA that required more time to form a homogenous solution. Moreover, all the polymer solutions pass through 23G needle, although ISFIs prepared using TA need greater force for injection.

The formulated systems were evaluated in PBS to observe their behaviors upon coming in contact with aqueous media. It was observed that the formulations prepared by the NMP and DMSO solutions start precipitating into a mass as soon as they come in contact with aqueous phase. ISFI prepared using NMP formed a rigid compact depot, which was formed within 5 minutes after injection (Figure 1a). However, this transition takes a shorter time with the formation of a suspended film with the drug-polymer matrix prepared using DMSO (Figure 1b). In contrast, the injection of the polymer solution prepared using TA into PBS results in no solution-gel transition till half an hour and only turbid solution was obtained (Figure 1c).

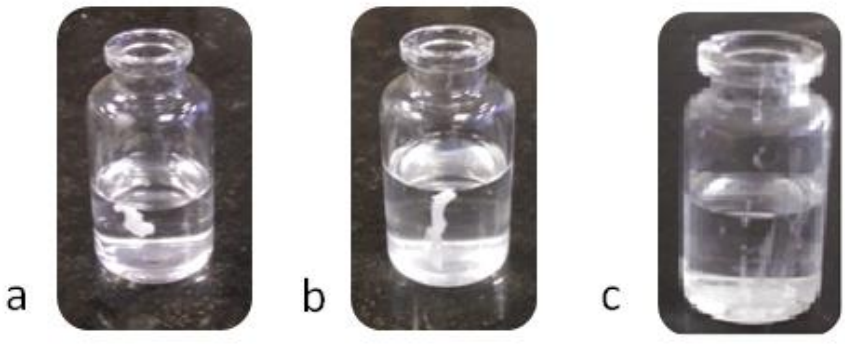


Figure 1: The effect of solvent type on the shape of the formed implant prepared using PLGA 50:50; (a): polymer solution prepared using NMP, (b): using DMSO; (c) using triacetin

The different behavior of the formed implants is attributed to two main factors, which are the miscibility of the solvent used in PBS and the viscosity of the formed polymer solutions (7, 8,

22). In case of water-miscible solvents as NMP and DMSO, the injection of polymer solution into PBS resulted in rapid diffusion of these solvents into the aqueous medium, with fast sol-gel transition. This caused water insoluble polymer to precipitate forming a mass. TA possesses the lowest miscibility in PBS than NMP and DMSO resulting in slower diffusion into the aqueous phase. As a result, the top layer did not solidify so fast and therefore only a turbid solution was obtained.

In addition, the viscosity is an important parameter to get pharmaceutically accepted ISFIs. The viscosity of the formed solutions using the different solvents can be arranged in the order of TA > NMP > DMSO as seen in the rheograms (Figure 2). The high viscosity of the polymer solution results in less spreading throughout the aqueous media and the faster formation of a compact mass of depot, which was observed with ISFI prepared with NMP. Lower viscosity of ISFI prepared using DMSO resulted in faster spreading with the formation of suspended film. Although ISFI prepared using TA had the highest viscosity, it could not form a mass due to its slow diffusion into PBS and inability to form a depot. Viscosity of formulation should be controlled during injection to avoid leakage of the medication before the formation of the depot. High viscous polymer solution needs less time for precipitation, when it interacts with physiological fluid in the body, as phase inversion occurs in a brief time. This also helps to decrease lag time between injection of solution in the body and solidification process (7).

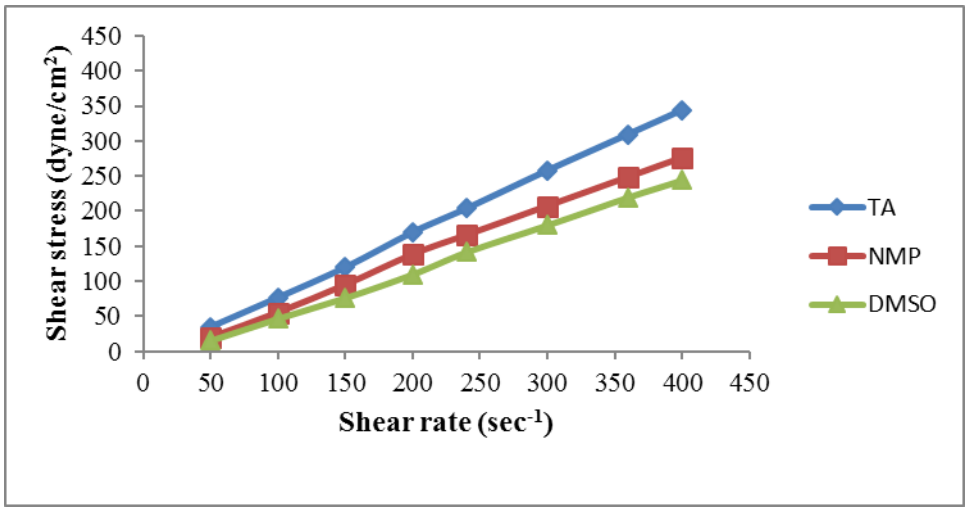


Figure 2: Rheograms of polymer solution prepared using PLGA 50:50 using different solvents

Morphological study on ISFIs using real-time optical microscopic

The effect of solvent type on the sol-gel transition and the morphology of the solid implants were also assessed. The study was carried out on the drug-free polymer solutions to prevent the effect of dispersed drug particles. The different behavior of the ISFIs prepared using NMP and DMSO was observed as soon as PBS added to the drop of the formulation. The images are shown in Figure 3.

For the ISFI prepared using NMP (Figure 3a), a solidified polymer layer was formed immediately upon the addition of PBS. This was followed by the formation of rigid structure with sharp edges within 5 min with complete formation of the darker matrix after 10 min. It was found that there was no variation in the hardness of structure after 15-30 min. This means that a complete depot was formed within 10 min. By examining the morphology of the formed film as shown in Figure 3b, more transparent droplets was observed in the middle of the film, which corresponds to the organic solvent (NMP) used in the preparation. This indicates that the rapid formation of the observed film entrapped some of the NMP within the formed matrix. The entrapment of NMP slowed down its diffusion into the aqueous medium with subsequent slowing down of the inner polymer solidification. This delayed the formation of the complete homogenous matrix till 10 minutes. By observing images of ISFI prepared using DMSO (Figure 4a), it was found that the immediate addition of PBS resulted in the formation of more homogenous matrix. No significant change in the formed structure was observed within 5 to 30 min after PBS addition. However, it can be observed in Figure (4b) that some of the precipitated polymers together with the organic solvent dissipated out of the formed film. Results of study are in accordance with Wang et al. (8).

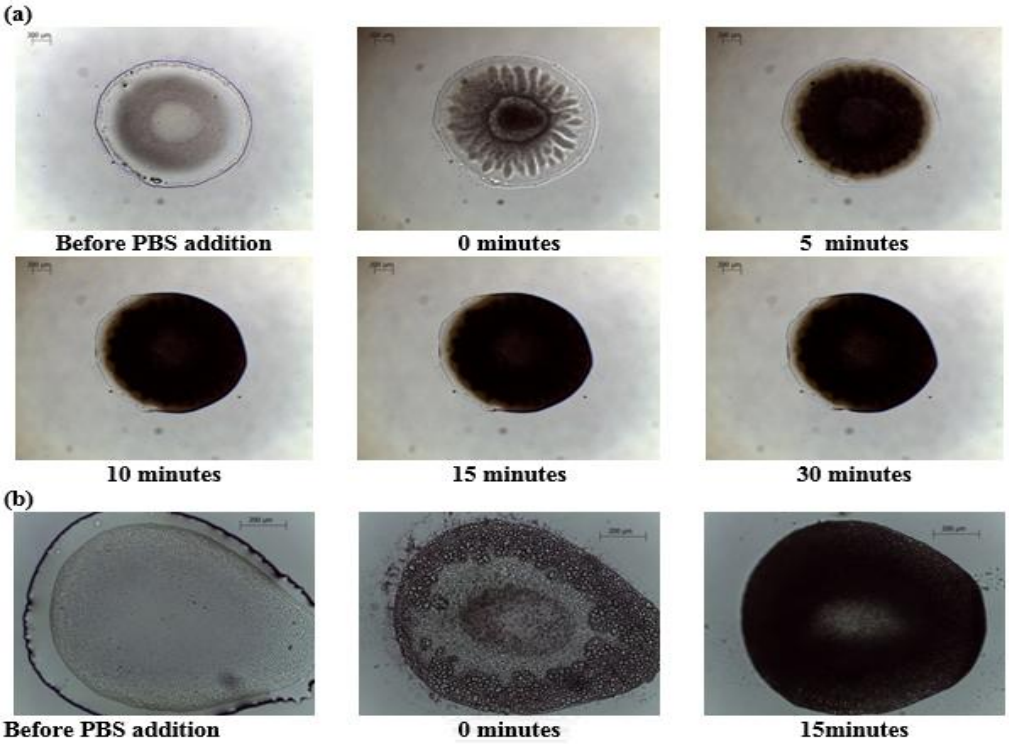


Figure 3: Real time optical microscope of implants prepared using NMP; (a): at magnification 10x and (b); at magnification 40x

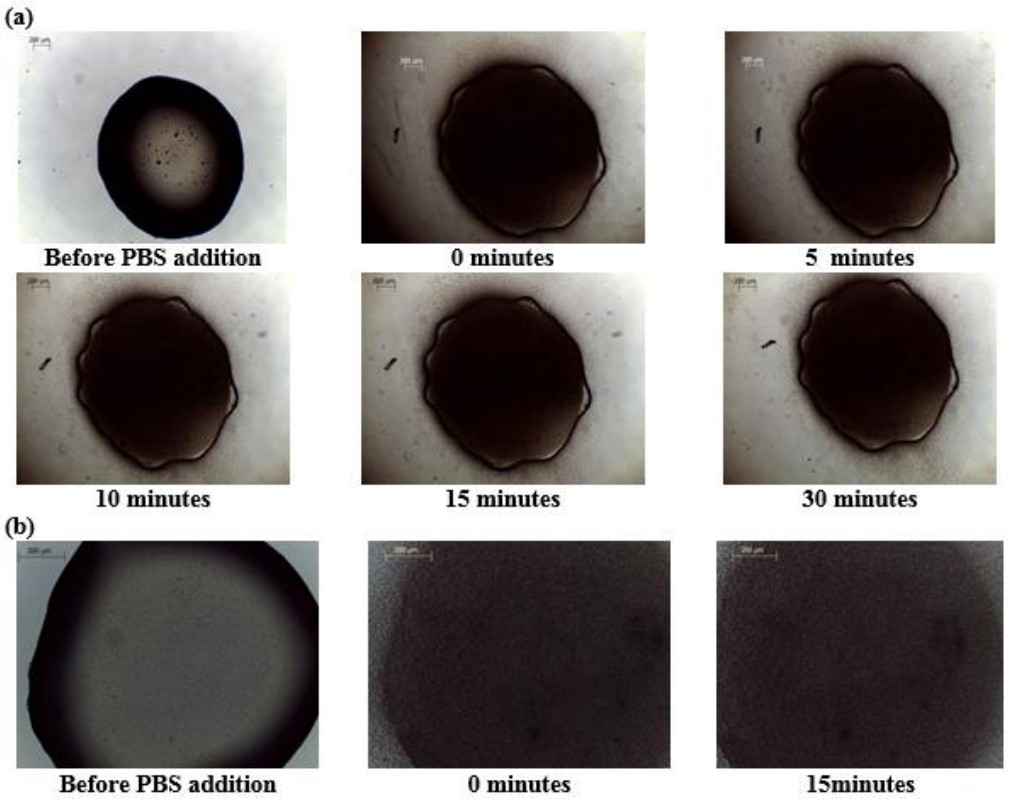


Figure 4: Real time optical microscope of implants prepared using DMSO; (a): at magnification 10x and (b); at magnification 40x

Simvastatin *in-vitro* release study

In-vitro release study was performed using the HPLC technique (SMV retention time was observed at 19.78 min as shown in Figure 5) in order to study the effect polymer and solvent type on the release profile. It can be observed that all ISFIs prepared using DMSO regardless of the polymer type show a higher initial burst effect and faster rate of drug release in comparison to ISFIs prepared using NMP (Figure 6). That could be attributed to the viscosity of the polymer solutions. As mentioned above, the viscosity of the ISFI prepared using NMP show a greater viscosity than those prepared using DMSO. Increasing the viscosity of the polymer solution resulted in fast solidification, which could hamper the drug particles from diffusion and slow down the removal of drug into the medium. In addition, the fast diffusion of the DMSO to the surrounding aqueous medium led to rapid dissipation of the drug during the lag time between the injection of polymer solution and formation of the solid implant with high burst effect and a rapid release of the dissolved drugs.

On the other hand, in NMP ISFIs, the slower solvent diffusion and slower phase inversion as revealed by optical microscope images resulted in slower rate of drug release. Another explanation was investigated by Kranz and Bodmeier (23), they reported that NMP is a superior solvent for PLA and PLGA than DMSO. The greater the ability of the used solvent to solvate the polymer, the more amount of the non-solvent required for precipitating the polymer solution and the slower the precipitation rate. A slower polymer precipitation led to a less porous implant surface, thus decreasing the initial drug release (4).

It can be observed that ISFIs formed from PLA showed the slowest rate of release followed by PLGA 75:25 and finally PLGA 50:50 (Figure 7). Results are in a good agreement with our previous study, which revealed that PLGA 50:50 implants showed faster rate of release than PLA implants (19). PLGA polymers containing different proportion of lactic and glycolic acids undergo hydrolysis faster than those containing higher ratio of either of the two monomers. Polyglycolide acid is highly crystalline due to absence of the methyl side groups of the PLA, however, when it was prepared with D, L PLA it became amorphous. Both PLA and PLGA are amorphous but PGA is more hydrophilic so it absorbs more water and degrades more quickly. The slower rate of SMV release from ISFIs prepared from PLGA 75:25 in relation to those prepared from PLGA 50:50 is due to the higher molecular weight of PLGA 75:25 which usually increases the viscosity of the polymer solution, and slows drug release rate.

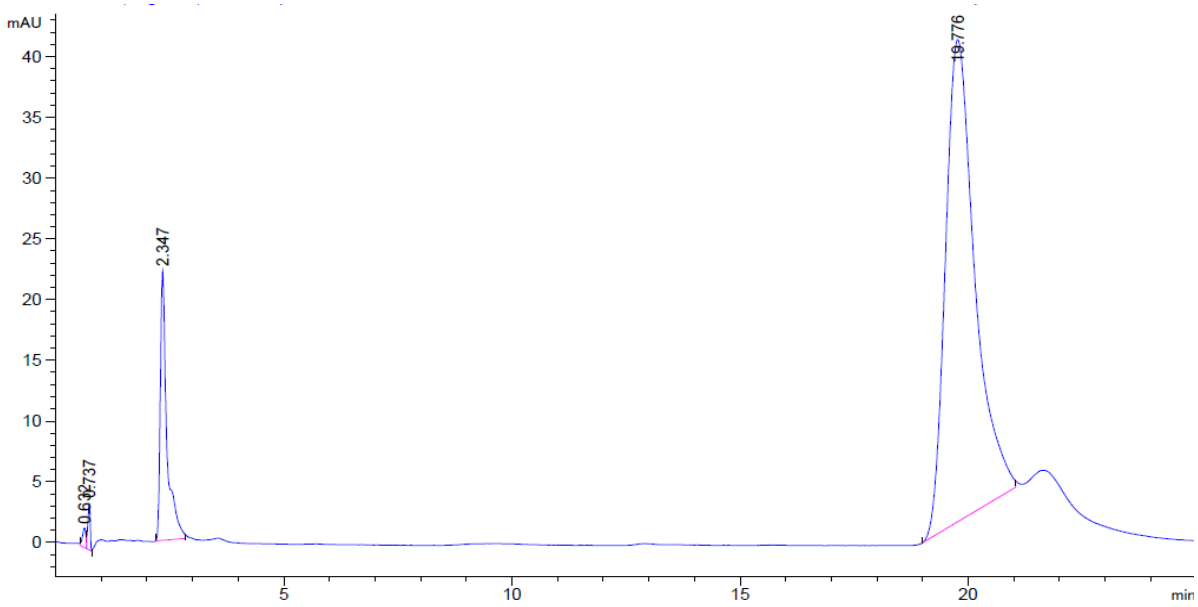
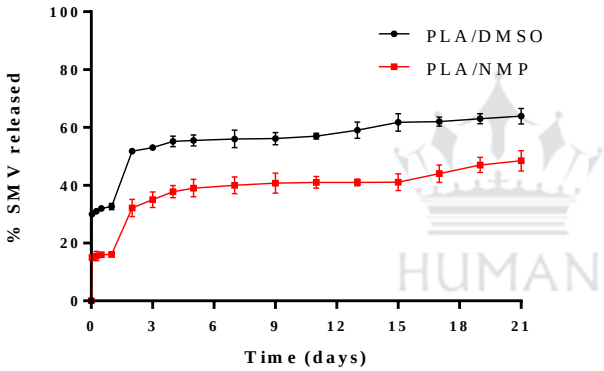
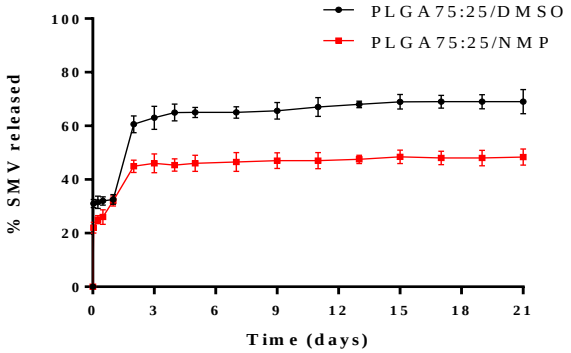


Figure 5: Chromatogram of pure simvastatin

(A)



(B)



(C)

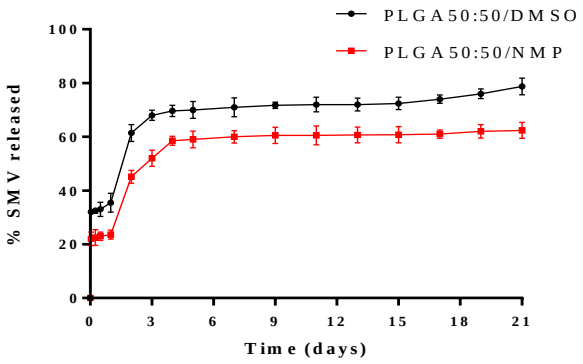
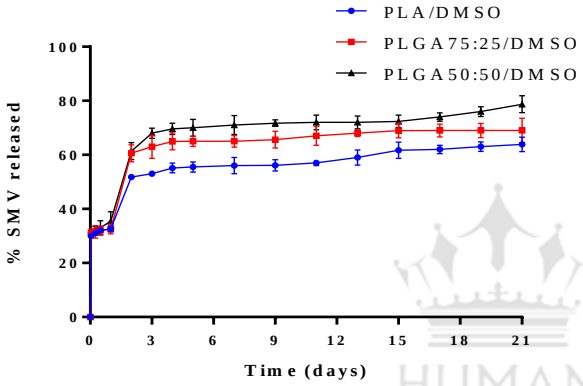


Figure (6): Effect of solvent type on the *in-vitro* release of simvastatin from *in-situ* implants

(A)



(B)

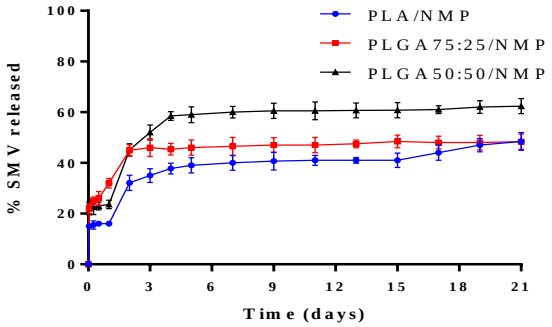


Figure 7: Effect of polymer type on the *in-vitro* release of simvastatin from *in-situ* implants

CONCLUSION

Study concluded that both NMP and DMSO are good solvents for the feasible preparation of in-situ forming implants. Solvent type affected the viscosity of the prepared polymer solution, with subsequent effect on the morphology of the formed implants. Both solvent and polymer type have a significant effect on simvastatin *in-vitro* release rate. NMP together with PLA

polymer resulted in slowest rate of simvastatin release. ISFIs can be considered as a promising system for the controlled release of simvastatin.

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