Preparation and optimization of media using Pluronic® micelles for solubilization of sirolimus and release from the drug eluting stents

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Understanding the in-vitro release profile of drugs from drug eluting devices such as cardiac stent is crucial in designing and optimizing the drug embedded matrices. Sirolimus (SRL), a widely used anti-inflammatory/antiproliferative/immunosuppressive hydrophobic drug undergoes irreversible changes such as hydrolysis leading to erroneous assessment of the release profile. The release profile mainly depends on the drug release medium. The present study aims to develop and optimize the aqueous medium for the solubilization of SRL and in-vitro release method from drug eluting stent (DES). In the first stage of study several release media containing different buffer compositions, pH, and a series of micelle forming PEO–PPO–PEO block copolymers (known as Pluronic®) were examined for solubility and stability of SRL by reversed phase high performance liquid chromatography (RP-HPLC). SRL showed good solubility and stability at pH 4.0 (both in acetate buffer as well as phosphate buffer) in the presence of block copolymers. Solubilization of SRL was remarkably higher in P103 and P123 micelles than more hydrophilic F68 and F127. To get further insight into the underlying drug dissolution mechanisms, critical micelle concentration temperature (CMT), and hydrodynamic size of micelles with and without drug incorporation were determined by UV-visible spectroscopy and dynamic light scattering (DLS) respectively. The micelle–water partition coefficient (P) and location of solubilized drug were also evaluated from a thermodynamics viewpoint. Finally, the optimized media were examined for the release of SRL from drug eluting stent; the data suggest that a release medium consisting of 0.1% P123 in phosphate buffer pH 4.0 is most suitable for evaluation of in-vitro release of SRL from DES.

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1. Introduction

The evolution of drug eluting stent (DES) has been a revolutionary change in the treatment of coronary artery disease. DES is very important and attractive because of its outstanding ability to reduce rate of restenosis, in-stent stenosis and repeat revascularization. DES can provide luminal scaffolding that virtually eliminates recoil and remodeling of the treated vessel [1–3]. Active pharmaceutical ingredient (API) present in the DES is the key responsible for its excellent performance.

Sirolimus (SRL) also referred to as Rapamycin, originally developed in 1975 as a macrolide antibiotic has potent anti-inflammatory, antiproliferative and immunosuppressive properties [4–6]. It has two functional molecular domains (i) left part of the molecule serves as the binding domain for intercellular cytosolic receptor and (ii) ring part as the effector domain that contributes to the specific biological activity [7,8]. SRL is practically insoluble in water and contains no ionizable functional groups. It is only slightly soluble in acceptable parenteral excipients such as ethanol, propylene glycol, glycerine, polysorbate 80 and polyethylene glycol 400 [9,10]. SRL also possesses antifungal and anti tumor activity and when used as part of a DES system it targets the very cause of in-stent restenosis, proliferating vascular smooth-muscle cells, arresting their proliferation [11,12]. A literature review explains a new generation SRL eluting percutaneous transluminal coronary angioplasty balloon catheter for restenosis therapy [13]. The solubility, stability and release kinetics are important to design the formulation and dosage of the drug.

Pharmacologically, hydrophobicity is beneficial for drug-tissue relation but solubilization and stabilization of nonpolar drugs is a problem that should be solved. An estimation of the real-time release (or dissolution) rate for the poorly water soluble drug like SRL in aqueous media is critical for characterization of the dosage forms, this process, however, consumes significant time possibly weeks or months. An accelerated (short-term) in-vitro release method is helpful for a rapid assessment of the
formulation and processing variables [14]. Also, the accelerated in-vitro release methods are desirable for the product development and quality control, particularly in the establishment of specifications for releasing product batches. Various techniques such as addition of suitable solvents [15–18], some hydrotropes [19], and surfactants [20–24] have been used to enhance the aqueous solubility of such sparingly water-soluble drugs. Various parameters like temperature, ionic strength, pH of the buffer media, enzymes, and agitation rate can also be altered to get the accelerated drug release of drugs. However, increase in temperature, ionic strength and change in pH etc. may cause degradation [25,26]. Polymeric micelles can act as nanocarrier to solubilize and release hydrophobic drugs and therefore these have gained much interest as vehicle for drug delivery systems in recent years [27].

Amphiphilic block copolymers form core–shell nano-size aggregates which can solubilize poorly soluble drugs and thus improve their bioavailability and protect from inactivation in biological media. Due to their small size (<100 nm), targeting ability, long circulation and easy production, these systems exhibit many advantages on administration and effective delivery of drugs [28–31]. Water-soluble poly(ethylene oxide)–block–poly(propylene oxide)–block–poly(ethylene oxide) copolymers are commercially available FDA approved nonionic surface active agents often known by their trade name Pluronic® (BASF). These copolymers form core–shell micelle, above a certain temperature, called the critical micellization temperature, CMT, which depends on the concentration/molecular characteristics of the copolymer and solvent condition. A hydrophobic drug can be encapsulated into micelles by chemical conjugation or physical entrapment [32–34]. Depending on steric properties and interrelations of drug and copolymer micelle core may/may not be suitable for all drugs. For this reason, in this study five copolymers with different polymer chains were used to see the factors affecting the incorporation of SRL drug during the solubilization.

The main objective of this work was to determine the optimum concentration of the suitable copolymer for adequate solubility and stability of SRL in the aqueous media which can be used in future for the in-vitro drug release analysis. Factors such as adequate solubility and stability of SRL in the aqueous media, ‘sink’ conditions under experimental set up of study were determined using reversed phase high performance liquid chromatography (RP-HPLC). Then characterization of the polymeric micelles in the media containing different concentration of copolymer was carried out for the better understanding of solubilization process of SRL. Parameters like critical micellization temperature (CMT), and hydrodynamic size of micelles with and without the loaded drug were determined by UV–vis spectroscopy and dynamic light scattering, respectively. The micelle–water partition coefficient (P) and location of solubilized drug were also evaluated and the solubilization discussed from a thermodynamics viewpoint. Finally, the optimized media were evaluated for in-vitro release profile of SRL from the DES by HPLC.

2. Materials and methods

2.1. Materials

All the HPLC grade solvents and other reagents were purchased from the Thermo Fisher Scientific, India. Pluronics® used were received as gifts from BASF Corp. Parsippany, NJ, USA and used without further purification. The drug sirolimus (molecular formula C51H79NO13, molecular weight 914.19), a semi-synthetic macrolide antibiotic was purchased from Hangzhou Zhongmeihuaodang Pharmaceuticals, China. Drug eluting stents with biodegradable polymeric coating were manufactured by Sahajanand Medical Technologies Pvt. Ltd. Surat, India.

2.2. Methods

2.2.1. High performance liquid chromatography (HPLC)

Quantification of drug was carried out using reversed phase HPLC (LC-2010 AHT; Shimadzu, Japan). The HPLC system consisted of a low pressure gradient separation module with configuration of quaternary solvent delivery pump, column heater and dual wavelength UV detector. Chromatographic system operation and recording of data were performed with the use of LC solution software. The HPLC separation was achieved using a Grace Smart C18, 4.6 mm × 250 mm, 5.0 μm particle size and 100 Å pore size HPLC column. The sample injection volume was 10 μl and flow rate was maintained at 1.0 ml min⁻¹. The mobile phase used was 80:20 (% v/v), methanol:water with Isocratic mode. The effluent was monitored with UV detector at 279.0 nm and total run time is 15 min. SRL exists in three isomeric forms [35], Iso A, Iso B and Iso C. Out of these three isomers, Iso B and Iso C can be detected and separated adequately by the HPLC method used in current research work. The quantification accomplished was based on the peak area ratio of API in sample to in-house standards (IS) using linear calibration curve with satisfactory Beer–Lambert plot (R² = 0.999).

2.2.2. Evaluation of solubility and stability

Solubility and stability of SRL was evaluated by spiking of minimum volume of highly concentrated stock solution (to minimize the organic solvent in media) prepared in methanol into the media with/without Pluronic, resulting 20 μg/ml final SRL concentration. These solutions were subjected to incubation at 37 °C at 60 rpm for 2 days in the orbital shaking incubator and subsequently analyzed at day 0 for solubility and day 1 and day 2 for the evaluation of stability in the medium. The solubility of SRL was calculated by comparing the amount of SRL recovered by HPLC at day 0 time point with theoretical amount of SRL taken. Similarly stability was found at day 1 and day 2 by comparing the amount recovered at respective time point with amount of SRL obtained at day 0.

2.2.3. Estimation of “Sink condition”

Saturated drug-loaded solutions were prepared in volumetric flask. Sink condition was estimated by dispersing about 1.0 mg of SRL in 2.0 ml of medium with and without Pluronics as well as in water. Volumetric flasks were then sonicated in the ultrasonic bath for 30 min and hand shaken the flask several times during sonication for maximum possible dissolution. Aliquots of the solution were withdrawn, filtered through 0.45 μm nylon filter using syringe and analyzed by HPLC method for the quantification.

2.2.4. Ultraviolet spectroscopy (UV)

Shimadzu (UV-2450) UV–visible double beam spectrophotometer with matched pair of stoppered fused silica cells of 1 cm optical path length was used to determine the CMT of copolymers by iodine UV spectroscopy method [36,37]. To prepare standard KI/I₂ solution, 0.5 g of iodine and 1.0 g of potassium iodide were dissolved in 50 ml triply distilled water. Foe CMT determination 25 μl KI/I₂ standard preparation was added and the UV absorbance value for varying temperature was measured. Absorbance vs. temperature profiles were measured at 366 nm using thermoelectrically controlled cell. The temperature was scanned at a heating rate of 1 °C/min. The CMT values correspond to the temperature at which the sharp increase in absorbance is observed.

2.2.5. Dynamic light scattering (DLS)

In order to determine the micelle size and polydispersity, DLS measurements were carried out at 90° scattering angle on solutions
using Zetasizer 4800 (Malvern Instruments, UK) equipped with 192 channel digital correlator (7132) and coherent (Innova) Air-ion laser at a wavelength of 514.5 nm. The average diffusion coefficients and hence the hydrodynamic size was obtained by the method of cumulants. Each measurement was repeated at least five times. All samples filtered and proper care was taken to avoid dirt.

2.2.6. In-vitro drug release

The in-vitro drug release studies were conducted using orbital shaking incubator. 8 mL of release medium was added to the DES in 10 ml volumetric flask, incubated at 37 ± 0.5 °C with an agitation of 50 rpm. The medium was changed at the end of every time points so that fresh medium was available for the next time intervals. Release media containing different copolymers and their varying concentrations were investigated; the agitation rate and temperature were maintained constant. At the end of each dripping time interval, the samples were transferred into HPLC vials and analyzed for SRL content in media by HPLC method. The in-vitro drug release analysis was carried out on 12 numbers of samples in each finalized media to evaluate and compare the profiles of SRL from DES.

The comparison of in-vitro release profiles was performed using the similarity factor, $f_2$ [38].

$$f_2 = 50 \times \log \left\{ 1 + \left( \frac{1}{n} \right) \sum \left( R_i - T_i \right)^2 \right\}^{-0.5} \times 100$$

where $n$ = time point, $R_i$ = cumulative drug release at each selected ‘n’ time interval of the reference set, $T_i$ = cumulative drug release at each selected ‘n’ time interval of the reference set.

The compared profiles were considered equivalent when $f_2$ value obtained was 50 or greater (50–100).

3. Results and discussion

3.1. Evaluation of solubility and stability of SRL

To use any media for in-vitro drug release or dissolution study, adequate solubility as well as stability should be achieved. The preliminary experiments were conducted on six different buffer compositions viz. phosphate buffers (PBS) with pH 4.0 (Media 01), 6.4 (Media 02), 7.4 (Media 03) and Acetate Buffers (AB) with pH 4.0 (Media 04), 4.5 (Media 05), 5.0 (Media 06) prepared as per the Indian Pharmacopoeias [IP]. The % recoveries of SRL at different time intervals are shown in Fig. 1.

From the above results, it can be seen that Media 01 (PBS pH 4.0) and Media 04 (AB pH 4.0) gave the better solubility as well as the stability of SRL. The instability of SRL in PBS (pH 7.4) is widely documented [10,25,39]. SRL is a 31-membered macrocycle with 15 stereocenters and multiple functional groups. The kinetics and mechanism of formation and degradation of SRL in aqueous media are not well understood. The degradation in aqueous solution at pH 7.4–8.0 results by the hydrolysis of lactone moiety. Mechanistic studies are complicated by cis–trans isomerization around the amide bond and by isomerization associated with the hemiketal formation. Very little is known about kinetics of those reactions for secobarbital in aqueous solutions.

The poor stability of the drug in PBS with pH 7.4 affects the quantification of solubilized and stabilized SRL during definite time intervals required for constructing the release or dissolution profile of SRL. Above results are in good agreement to the fact that ‘-olimus’ groups of drugs are not stable at alkaline or neutral pH [25], the dissolution medium was buffered to minimize the degradation of SRL that happens above pH 6.0. In acidic pH SRL has minimum dissociation hence slightly acidic pH are better suited. So buffering at pH 4 solves this problem and is better for minimizing the degradation of SRL. Since, SRL has minimum dissociation at this pH range (acidic pH below 6.0), pH may have little impact on elution or dissolution rate [40].

Our main purpose of this study is to develop a media that can solubilize 90% or more drug which will be released from the stent and that solubilized drug must be stable in media up to 48 h. So we assume that 0.1% concentration or lower may fulfill this requirement as CMC of Pluronics is very low. Moreover if we increase the amount of surfactant in the media, it may react with the polymers of the biodegradable film on stents during the release study of SES and also it may interfere in the chromatographic separation and quantification of SRL by HPLC. So, due to all these reasons surfactant concentrations are kept very low (up to 0.1%) but above the CMC for the initial studies. Hence the % recovery of SRL from the medium Acetate Buffer (ABS, pH 4.0) and Phosphate Buffer (PBS pH 4.0) were checked at different surfactant concentrations 0.01%, 0.05%, and 0.1% at time intervals of day 0, day 1 and day 2. The average % recovery for different copolymers at the two buffer media at pH 4 are given in Fig. 2.

Fig. 2 clearly shows that solubility in, F68 and F127 was much less as compared to F84, P103 and P123 and roughly follows the trend P123 > P84 > P103 > F127 > F68. As the % of POPO increases the hydrophobicity increases leading to increased hydrophobic interaction with drug, moreover, the number of micelles also increases at higher copolymer concentration. These two factors are primarily responsible for the micellar solubilization of this sparingly water soluble drug leads to the comparative good solubility and stability at higher concentrations [41].

Stability of drug is also equally important for the in-vitro evolution. F68 exhibits lowest stability for SRL due to its slightly hydrophilic nature while P103 and P123 showed highest stability in all the media used in the study. Fig. 2 reveals that, 0.05% and 0.1% P103 and P123 were more suitable at which SRL shows about 90% or more solubility as well as the stability up to day 2 in the medium. P123 and P103 are more hydrophobic (low HLB and CMC) than other copolymers and are therefore better for solubilization of SRL. Hence, the same concentrations as well as the little higher concentration were evaluated for the sink conditions for SRL.

3.2. Evaluation of ‘sink’ conditions

Sink condition was tested to ensure the SRL (in powder form) solubility in the medium. Sink condition in the medium at different concentration of P103 and P123 was tested with and without the copolymer. The amount of drug solubilized was determined by
Pluronics represent recovery corresponding conditions. With solubility. Their low (pH 0.5%) strong 0.5% Pluronics in PBS (pH 4.0). As solubility was found to be 0.44 μg/ml and 0.52 μg/ml in PBS (pH 4.0) and in ABS (pH 4.0) respectively at 37°C. From these results it can be concluded that solubility of SRL in water and in buffer at pH 4.0 are not much different despite of less dissociation at pH 4.0. SRL poses problem in the aqueous media for the solubilization due to very low aqueous solubility hence solubilizing agent was needed in the media to get the desired solubility as well stability.

As per the conclusion from the earlier results, 0.05, 0.1 and 0.5% concentration of P103 and P123 were prepared in the PBS (pH 4.0) and average solubility of SRL was evaluated in those sink conditions. Average solubility of SRL at different concentration of P103 and P123 in PBS is given in Fig. 3 which clearly shows strong interaction between the drug and Pluronics with corresponding increase in concentration. The solubilization enhances with increase in Pluronic® concentration (0.05–0.5%).

Pluronics® may display varied solution behavior depending of their EO/PO ratio and polymer molecular weight and thus their hydrophilic/lipophilic balance (HLB). Pluronic® P123 and P103 with low HLB values (8 and 9, respectively) show significant increase in solubility. The SRL solubility was observed higher with low HLB while no significant change in solubility was observed with those having high HLB.

Fig. 3 shows the comparison of solubilization of SRL in P103 and P123. In the entire concentration range, the amount of solubilized SRL was more for P123; which can be attributed to the fact that P123 is more hydrophobic than P103. This results in an increased hydrophobic–hydrophobic interaction, for SRL with P123 and P103. It was observed that solubilization is significantly increased with increase in concentration 0.01% to 0.5% and at 0.1% P103 and P123, SRL has adequate solubility. From the statistical evaluation of the above data using ANOVA test, p-value for solubilized drug at each concentration is 0.007982 (which is less than 0.05) shows that there is significant difference in solubilization at each concentration level but there is not significant difference between two group P103 and P123 as P-value obtained from two groups for solubilized drug is 0.152502 (which is more than 0.05) at 5% level of significance.

Hence, phosphate buffer of pH 4.0 containing 0.1% concentration of P103 and P123 were good enough for SRL solubility and stability and were further used for the evaluation of in-vitro release of SRL from DES. Before using these media for the in-vitro release profile of SRL, the solubilization process need to be understood and for these following studies were carried out to understand the micellar behavior in the optimized media.

3.3. Characterization of polymeric micelles in the media

3.3.1. Effect on hydrodynamic size of micelles

Hydrodynamic size of micelles (with and without solubilized SRL) in phosphate buffer pH 4.0 measured using DLS is shown in Table 1.

The $D_h$ of P123 and P103 are nearly same in both cases, i.e., without SRL and with SRL. This is because both the copolymers have nearly same HLB (~8–9) as P103 and P123 has high molecular
weight hydrophobic PPO blocks as well as high hydrophilic PEO blocks. It was observed that in both P103 and P123, solubilization is significantly increased with concentration 0.05–0.5% (Fig. 3). DLS results (Table 1) show the increment in size when drug is incorporated within micelle, which is complete agreement to the result showed increase in solubility of drug. Analogies to obtained results, Yang et al. [41] and Kadam et al. [42] have shown increased in solubilization of hydrophobic drug.

Table 1
Hydrodynamic size of micelles without SRL and with SRL in different copolymer concentrations (0.1% and 0.5%) at 37 °C.

<table>
<thead>
<tr>
<th>Pluronic®</th>
<th>Hydrodynamic diameter Dₚ (nm) without SRL</th>
<th>Hydrodynamic diameter Dₚ (nm) with SRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1%</td>
<td>0.5%</td>
</tr>
<tr>
<td>P103</td>
<td>18.81</td>
<td>15.13</td>
</tr>
<tr>
<td>P123</td>
<td>19.69</td>
<td>16.39</td>
</tr>
</tbody>
</table>

3.3.2. Partition coefficient and thermodynamics of solubilization

Copolymer’s ability to solubilize SRL was characterized using the following descriptors:

(i) Molar solubilization capacity, $\chi$, i.e., the number of moles of the solute (SRL drug) that can be solubilized by 1 mol of copolymer, and characterizes the ability of the copolymer to solubilize the drug.

$$\chi = \frac{S_{tot} - S_w}{C_{copolymer} - CMC}$$

(ii) Micelle–water partition coefficient, $P$, which is the ratio of the drug concentration in the micelle to the drug concentration in water (i.e. aqueous media-phosphate buffer of pH 4.0), for a copolymer concentration.

$$P = \frac{S_{tot} - S_w}{S_w}$$

(iii) Standard free energy of solubilization, estimated from the molar micelle–water partition coefficient, $P_M$ (i.e., $P$ for $C_{copolymer} = 1$ M)

$$\Delta G^0_M = -RT \ln \frac{\chi(1 - CMC)}{S_w}$$

The results shown in Table 2 clearly indicate that the solubilization capacity ($\chi$) and partition coefficient ($P$) increase as the copolymer concentration is increased. The standard free energy of solubilization, $\Delta G^0_M$, in water for SRL was calculated. All the values are negative, indicating that the migration of the drug molecules in the monomer state to the air-water interface is a spontaneous process favored by their hydrophobicity. However, more negative standard free energy of solubilization ($\Delta G^0_M$) for the copolymers with increasing concentration indicates more favored solubilization of SRL. The free energy of solubilization is negative in all cases and becomes more negative with increasing concentration of

Fig. 3. Amount of solubilized SRL vs. Pluronic® concentration in phosphate buffer pH 4.0 (●) P123; (■) P103. Data represent mean and S.D. for n = 3.
copolymers which suggests a strong hydrophobic interaction that leads to higher solubilization.

3.3.3. Effect on CMT
It is well-known that the micellar properties of copolymers like CMC, CMT, and CP vary in the presence of additives because the interfacial and micellar properties of these compounds in solution are governed by a delicate balance of hydrophobic and hydrophilic interactions. Since the micellization is strongly dependent on temperature which is usually fixed, the properties of copolymers should be optimized by the presence of additives. As drug was used in combination with copolymer, it is necessary to understand the effect of drug on the aggregation of block copolymer. With this viewpoint, CMTs were measured for Pluronics in the presence of SRL in the acetate buffer pH 4.0 media. The CMT of P123 and P103 are ∼22.0 and ∼25.5 °C respectively whereas in the presence of SRL, CMTs are ∼21.0 °C and 20 °C respectively. Decrease in CMT (Fig. 4) shows that presence of SRL induces micelle formation.

3.3.4. Locus of solubilization
The structure and polarity of the solubilate determine its locus in micelle. The location can be on the surface of a micelle, within the hydrophobic head group, on the palisade layer between the hydrophilic head group and first few carbon atoms, beyond the palisade layer, and at the core of the micelle [43]. The possible location of solubilization for drug in to micelles is significant as it is the measure of the strength of specific interactions between the solubilize and the micelle. The location of incorporated drugs within a micelle determines the extent of solubilization, the chemical reactivity of solubilizes, as well as the rate of their release from the micelles [44]. Location of drug in the micelle can be obtained by monitoring the solubility in model solvents. Comparisons of λmax of the drug in micellar solutions to that in model solvents that imitate the polarity of different regions of the micelle indicate the possible loci of the SRL in micelle.

However in this study polyethylene glycol (PEG) and polypropylene glycol (PPG) were chosen as model solvent because of similarity in structure and polarity, where PEG is to resemble polar corona and PPG to imitate micellar core. The UV–visible spectra for SRL in PEG, PPG and P103 are shown in Fig. 5.

When solubilized in copolymer, SRL absorbs at 279 nm and for PEG and PPG absorbance observed at 280 nm and 298 nm respectively. Such a shift in the λmax, towards the PEG, indicates that solubilized SRL molecules reside in the microenvironment of hydrophilic shell. This shift in λmax may be due to presence of electron donor/acceptor groups, charge transfer or interaction between drug molecule and micelle forming substance [45,46]. The reason of SRL in hydrophilic shell may be due to the SRL structure arrangement.

![Fig. 4](image-url) Plot of UV intensity of Iλ vs. temperature profile of (a) P103 and (b) P123 in presence (dark circle) and absence (empty circle) of drug.

![Fig. 5](image-url) UV–visible spectra of SRL in PEG, PPG for P123, P103.

### Table 2

SRL solubilization parameters in different copolymer solutions.

<table>
<thead>
<tr>
<th>Copolymer (%)</th>
<th>P103</th>
<th>P123</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>χ</td>
<td>Partition coefficient</td>
</tr>
<tr>
<td>0.05</td>
<td>1.19 × 10⁻⁸</td>
<td>0.81</td>
</tr>
<tr>
<td>0.1</td>
<td>3.30 × 10⁻⁸</td>
<td>2.64</td>
</tr>
<tr>
<td>0.5</td>
<td>6.54 × 10⁻⁸</td>
<td>5.61</td>
</tr>
</tbody>
</table>
3.4. In-vitro release kinetics of SRL using the developed release medium from the DES

The medium used in this study is based on the previous studies which are less hostile for SRL. The time intervals used were based on the trial and error approach. On the basis of the preliminary studies, release media containing 0.1% P103 in PBS (pH 4.0) and 0.1% P123 at 0.5, 1.5, 3, 5, 24 and 48 h were selected as suited for intended application and employed for in-vitro release studies. Fig. 6 shows the cumulative release profile of SRL from DES in both the optimized mediums.

In P123 slightly more than 90% of the drug released in 48 h while in case of P103 about 80% drug released. The residual drug content on these stents after the release period was found to be 9% in P123 and about 10% in P103. From the stents kept in 0.1% P123; about 99% of the total drug loaded was recovered whereas in the media containing 0.1% P103; about 90% of the total drug loaded was recovered. This suggests that the amount of drug transformed or degraded is slightly higher in P103 as compared to P123. The f₂ values obtained 51 indicates that the release rate profile of SRL for 0.1% P103 in PBS (pH 4.0) is not different from the profile in the 0.1% P123 in PBS (pH 4.0). But as S₃ is slightly higher than the minimum similarity factor value 50 indicating that the P123 significantly influences the release profiles. This can be explained by the fact that P123 is more hydrophobic as compared to P103 which increases the solubility of SRL in the aqueous media leading to the slightly higher drug solubility and stability. The slight decrease of SRL release in 0.1% P103 might be attributed to the promotion of hydrophobic–hydrophilic interactions at the comparatively higher hydrophilic part in its molecule. Judging by the cumulative percent release profiles, approximately 70–80% of the drug is released at a relatively rapid rate during the first three time intervals within 5.0 h, followed by slower release in P123 and almost no release in P103 over the next two time intervals up to 24 h. There was no observable increase in the rate or extent of SRL release after 24 h with both the tested release media. Overall, the initial fast release rate is commonly ascribed to the drug detachment from the polymer surface, while the later slow release results from the sustained drug release from the inner layer. Both these release media compositions ensured at least 80% of SRL release at the last time point, which is recommended as a specification for the accelerated release [47]. These results strongly substantiate that the optimized media and methodology are suitable for assessing the release kinetics of SRL in aqueous media from the DES.

4. Conclusion

Several studies describe that SRL is unstable in aqueous media such as PBS which is universally used in determining the release kinetics of the drug. To optimize the formulation using SRL a thorough understanding on the in-vitro release profile of the drug entrenched in various matrices is mandatory. We attempted here to develop a medium in which drug undergoes minimum degradation, stable for desired time interval and has adequate solubility. Through optimization of the experimental variables the current approach may be applied to evaluate drug release from a biodegradable matrix. At the earlier stage of this work, conclusions were drawn based on the chromatograms of SRL solution spiked into various media and based on those results optimization of the experimental variables such as buffer components, buffer pH and surfactant concentration was carried out because an exercise of this kind if performed on DES, is highly expensive since it requires a large number of stents. The data generated using a wide variety of media suggested that PBS with pH 4.0 containing 0.1% P123 is an appropriate medium to assess the time bound release of SRL. The efficacy of our method is demonstrated by estimating the release kinetics of SRL from a DES. Results indicate that the incorporation of copolymers in the release medium resulted in an increase in the drug solubility and stability in the aqueous media. Also, different copolymers in the same conditions and concentrations affect the release rate due to their specific molecular characteristics in the matrices. The developed short-term accelerated release method can be correlated with the real-time release. The method can be employed as a rapid quality control test during product development stage of new SRL embedded matrices or commercial manufacturing after some more refined experimental data to evaluate the method’s discriminating power. Based on the obtained knowledge, the selection of an appropriate release medium for in-vitro tests of the drug delivery systems can be facilitated, and an accelerated release method can be developed allowing for a rapid feedback on the release characteristics of a specific SRL-polymeric formulation.

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