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Self-Emulsifying Drug Delivery Systems: Hydrophobic Drug Polymer Complexes Provide a Sustained Release in Vitro

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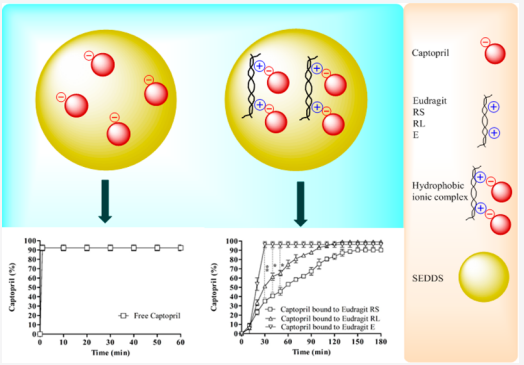
ABSTRACT: The aim of this study was to develop hydrophobic ionic drug polymer complexes in order to provide sustained drug release from self-emulsifying drug delivery systems (SEDDS). Captopril (CTL) was used as an anionic model drug to form ionic complexes with the cationic polymers Eudragit RS, RL, and E. Complexes of polymer to CTL charge ratio 1:1, 2:1, and 4:1 were incorporated in two SEDDS, namely FA which was 40% Kolliphor RH 40, 20% Kolliphor EL, and 40% castor oil and FB, which was 40% Kolliphor RH 40, 30% glycerol, 15% Kolliphor EL, and 15% castor oil. Blank and complex loaded SEDDS were characterized regarding their droplet size, polydispersity index (PDI), and zeta potential. Resazurin assay was performed on Caco-2 cells to evaluate the biocompatibility of SEDDS. Release of CTL from SEDDS was determined in release medium containing 0.2 mg/mL of 5,5′-dithiobis(2-nitrobenzoic acid) (DNTB) allowing quantification of free drug released into solution via a thiol/disulfide exchange reaction between CTL and DNTB forming a yellow dye. The droplet size of SEDDS FA and SEDDS FB were in the range of 100 ± 20 nm and 40 ± 10 nm, respectively, with a PDI < 0.5. The zeta potential of SEDDS FA and SEDDS FB increased after the incorporation of complexes. Cell viability remained above 80% after incubation with SEDDS FA and SEDDS FB in a concentration of 1% and 3% for 4 h. Without any polymer, CTL was entirely released from both SEDDS within seconds. In contrast, the higher the cationic lipophilic polymer to CTL ratio in SEDDS, the more sustained was the release of CTL. Among the polymers which were evaluated, Eudragit RL provided the most sustained release. SEDDS FA containing Eudragit RL and CTL in a ratio of 1:1 released 64.78 ± 8.28% of CTL, whereas SEDDS FB containing the same complex showed a release of 91.85 ± 1.17% within 1 h. Due to the formation of lipophilic ionic polymer complexes a sustained drug release from oily droplets formed by SEDDS can be achieved. Taking into account that drugs are otherwise instantly released from SEDDS, results of this study might open the door for numerous additional applications of SEDDS for which a sustained drug release is essential.



KEYWORDS: *hydrophobic polymer complexes, self-emulsifying drug delivery systems, captopril, log $SR_{SEDDS/release\ medium}$, sustained release, nanoemulsions*

1. INTRODUCTION

Self-emulsifying drug delivery systems (SEDDS) are homogeneous isotropic mixtures of oils, surfactants, and cosurfactants that emulsify in aqueous media forming oily droplets typically in the nanosize range. As these delivery systems offer numerous advantages in particular for mucosal delivery of various types of drugs, they are of high industrial relevance.[1,2] Poorly soluble drugs as well as hydrophobic ion pairs of drugs such as ionic liquids[3−5] can be dissolved in the oily droplets in order to reach a sufficient high bioavailability after mucosal administration. Furthermore, drugs like therapeutic peptides and oligonucleotides that are degraded on mucosal membranes by peptidases and nucleases can be protected toward these enzymes in the oily droplets.[6,7] When peptide and protein drugs are incorporated in SEDDS also, unintended thiol/disulfide exchange reactions with endogenous thiols such as glutathione or cysteine-rich subdomains of mucins can be avoided.[8,9] Moreover, as the vast majority of SEDDS contain PEG-ylated surfactants forming a muco-inert PEG-corona around the oily droplets, they are able to permeate the mucus gel barrier in a comparatively efficient manner enabling the transport of incorporated drugs to the underlying absorption membrane.[10−12]

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Despite these advantages, however, SEDDS are facing the problem of an uncontrollable drug release. Lipophilic and poorly water-soluble drugs exhibiting a much higher solubility in the oily core of the droplets formed by SEDDS are not released at all. On the contrary, hydrophilic and freely water-soluble drugs are almost instantly released since the diffusion coefficient of even large hydrophilic drugs such as proteins is relatively high and the distance from the center of the oily droplets to the surface is just in the nanometer range.[13] Trotta et al., for instance, determined the release of indomethacin from lecithin-based microemulsions using a conventional pH electrode. The release rate reported as the natural logarithm of the remaining indomethacin in the oily droplets against time was too rapid to show any sustained release.[14] To date, a sustained release can only be achieved for lipophilic drugs with a solubility ratio (SR) between the lipophilic SEDDS phase and the aqueous release medium of log SR > 3 remaining in the oily phase of the droplets because of their high solubility in this phase[13] when these oily droplets are continuously degraded at the application site releasing their payload in a sustained manner. Such a continuous degradation of SEDDS can be achieved by the incorporation of excipients exhibiting ester substructures that are degraded by lipases. However, even in the case of SEDDS containing excipients with ester substructures, the release of incorporated drugs cannot be properly controlled. In most cases this degradation process is taking place too rapidly, and even when less degradable excipients such as monoglycerides instead of triglycerides are used[15] or lipase inhibitors such as Orlistat[16] are added to slow down the degradation process, drug release is still uncontrolled as lipase activity is highly variable.

In order to overcome this substantial shortcoming of SEDDS, it was the aim of this study to develop a concept providing a sustained release from these delivery systems. The idea for this concept is based on the observation that highly lipophilic excipients such as highly lipophilic drugs remain in the oily droplets without being released at all. Binding hydrophilic drugs loosely to such excipients will likely provide a sustained release from the oily droplets. In order to provide a proof-of-concept the anionic model drug captopril (CTL) was loosely bound to the lipophilic cationic methaclylate copolymers Eudragit RS, Eudragit RL, and Eudragit E via ionic interactions. CTL was chosen because of its anionic substructure and its high solubility in aqueous media (160 mg/mL). With a comparatively low log P of 0.34,[17] it is expected to be rapidly released from SEDDS. As the oily droplets of nanoemulsions were shown to provide a protective effect toward thiol−disulfide exchange reactions in the GI-tract[9] and a sustained release of CTL is needed for therapeutic reasons because of its comparatively short elimination half-life,[18] orally administered SEDDS providing such a sustained release might be even of practical relevance for this drug. Furthermore, the thiol substructure of CTL allows a simple and accurate quantification.

2. MATERIALS AND METHODS

2.1. Experimental Materials. Captopril (CTL), glycerol, Kolliphor RH 40 (macrogolglycerol hydroxystearate), Kolliphor EL (macrogolglycerol ricinoleate), potassium phosphate dibasic, sodium phosphate monobasic, 5,5′-dithiobis(2-nitrobenzoic acid) (DNTB), sodium hydroxide, acetic acid, and ethyl acetate were purchased from Sigma-Aldrich (Vienna, Austria). The copolymers containing ethyl acrylate, methyl methacrylate, and trimethylammonioethyl methacrylate Eudragit RS 100 (1:2:0.1), Eudragit RL 100 (1:2:0.2), and Eudragit E 100 (poly(butyl methacrylate-*co*-(2-demethylaminoethyl) methacrylate-*co*-methyl methacrylate) 1:2:1) were purchased from Evonik AG (Darmstadt, Germany). Castor oil was provided by Gatt-Koller (Absam, Austria). All other chemicals and solvents used were of analytical grade and obtained from commercial sources.

2.2. Experimental Methods. *2.2.1. Preparation of Lipophilic Complexes.* Three types of hydrophobic complexes of CTL were prepared utilizing different cationic polymers as illustrated in Figure 1. The three complexes were prepared at

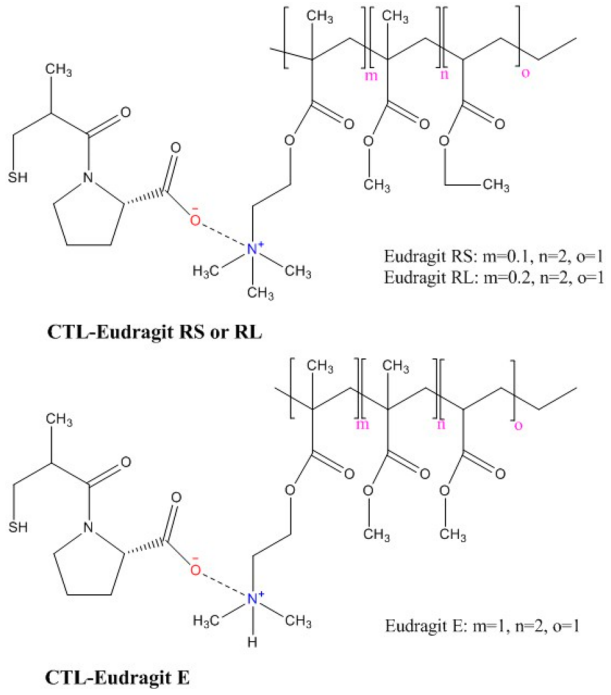

Figure 1. Target complexes of CTL with indicated hydrophobic Eudragit polymers.

three different charge ratios, 1:1, 2:1, and 4:1, and the method is summarized in Figure 2. The specified weights, namely 17.7 mg of Eudragit RL, 18.85 mg of Eudragit RS, and 10.7 mg of Eudragit E, were dissolved separately in 500 μL of methanol and 300 μL of ethyl acetate. Separately, 2.3, 1.15, and 9.3 mg of CTL were dissolved in 400 μL volumes of Kolliphor RH 40 and also in 300 μL volumes of glycerol. Subsequently, the 500 μL of polymeric methanolic solutions were added dropwise to the 400 μL of Kolliphor RH 40 under constant agitation at 300 rpm and 25 °C. In parallel, 300 μL of each polymeric solution in ethyl acetate were added dropwise to 300 μL of glycerol at 300 rpm and 25 °C. Methanol and ethyl acetate were evaporated, resulting in complex solutions in Kolliphor RH 40 and glycerol, respectively. The quantities of CTL and Eudragit polymers specified above prepared the complexes at the 1:1 charge ratio. To prepare the 2:1 and 4:1 charge ratio complexes, different weights of the CTL, as shown in the lower portion of Figure 2, were dissolved in the same volumes of Kolliphor RH 40 or glycerol, and the Kolliphor polymers were added as described above. Overall, there were 18 different combinations of CTL-complex, charge ratio, and solvent prepared, and these were used for the development of the SEDDS.

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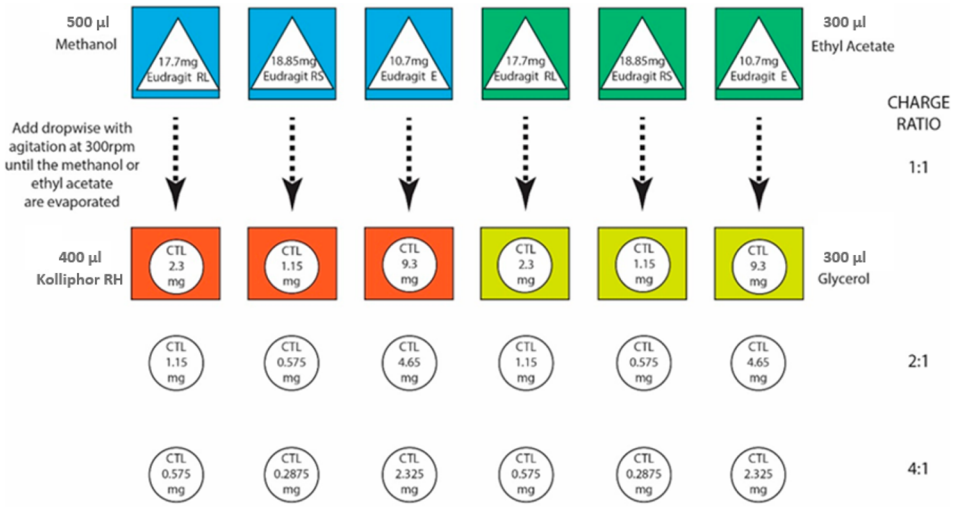

Figure 2. Illustration of the methods used to prepare the hydrophobic complexes between CTL and the Eudragit polymers.

2.2.2. SEDDS Preparation and Characterization. For the preparation of SEDDS, oils, surfactants, and solvents were mixed as shown in Table 1 using a thermomixer (Thermomixer Comfort, Eppendorf, Germany) under constant shaking at 800 rpm at 40 °C. Semisolid excipients were melted before use. SEDDS FA and SEDDS FB were loaded with hydrophobic ionic complexes as described above. The concentration of CTL−polymer complexes with a CTL to polymer charge ratio of 1:1 in SEDDS as 2% (w/v) containing 0.93, 0.23, and 0.115% (w/v) CTL featured the complexes of CTL with Eudragit E, Eudragit RL, and Eudragit RS, respectively. Thereafter, 10 μL of blank as well as of complex loaded SEDDS FA and SEDDS FB preconcentrates were emulsified in 990 μL of DNTB (0.02%) at pH 8. Droplet size, PDI, and zeta potential of blank as well as hydrophobic ionic complexes loaded SEDDS FA and SEDDS FB were determined by dynamic light scattering with Zetasizer Nano-ZSP (Malvern instruments, Worcestershire, UK).

Table 1. Composition of SEDDS Formulations[a]

Formulations	Glycerol (%)	Kolliphor RH 40 (%)	Kolliphor EL (%)	Castor Oil (%)
SEDDS FA	–	40	20	40
SEDDS FB	30	40	15	15

[a]Values are indicated in percent (v/v).

2.2.3. Evaluation of Polymer Loading. The polymer loading capacity of SEDDS FA and FB was evaluated by incorporating the polymers in the same manner as described above (section 2.2.1) but omitting CTL. In brief, increasing amounts of Eudragit RS, RL, and E in a range of 2−30 mg were dissolved in 100 μL of methanol or in 60 μL of ethyl acetate and added to 80 μL of Kolliphor RH 40 or to 60 μL of glycerol, respectively. Solution mixtures were shaken at 300 rpm until evaporation of methanol and ethyl acetate was achieved and were evaluated visually for precipitate formation. Solutions containing the dissolved polymers in Kolliphor RH 40 and glycerol without any precipitation were mixed with the other excipients as listed in Table 1 to obtain SEDDS FA and SEDDS FB, respectively.

2.2.4. Solubility Ratio (log $SR_{SEDDS/release\ medium}$). Log $SR_{SEDDS/release\ medium}$ of CTL at 37 °C was calculated by determining maximum solubility of CTL in the SEDDS preconcentrates (FA and FB) as well as in the release medium according to Pinsuwan et al.[19] Increasing concentrations of CTL were dissolved in SEDDS preconcentrate and in 0.02% DNTB pH 8 serving as release medium at 500 rpm at 37 °C for 24 h. The solutions were centrifuged at 10 500*g* for 10 min in order to remove the undissolved CTL. To determine Log $SR_{SEDDS/release\ medium}$ of CTL using eq 1, CTL solutions in SEDDS preconcentrate and release medium were separately diluted 1:100 in release medium and evaluated by measuring the absorbance at 450 nm via a microplate reader (Tecan Spark, Tecan Trading AG, Zurich, Switzerland). The same pattern described above was followed in order to evaluate the solubility of polymers in SEDDS preconcentrate. Polymers were dissolved in SEDDS FA and SEDDS FB until the saturation solubility was reached. Although the amino methacrylate copolymers are known to exhibit no aqueous solubility, it was confirmed in the release medium. Separately, polymers were dispersed in a concentration of 5 mg in 1 mL of the release medium while shaking at 500 rpm at 37 °C for 24 h. The release medium containing the polymers was centrifuged, washed with demineralized water, and lyophilized. The concentration of the polymers was evaluated by observing the weight difference of the polymers used. Log $SR_{SEDDS/release\ medium}$ was calculated according to eq 1.

$$\text{Log } SR_{SEDDS/\text{release medium}} = \log\left(\frac{\text{maximum solubility of CTL in SEDDS}}{\text{maximum solubility of CTL in release medium}}\right) \quad (1)$$

2.2.5. Cell Viability Studies - Resazurin Assay. The impact of SEDDS FA and SEDDS FB containing the CTL complexes on the viability of Caco-2 cells was evaluated by using the resazurin assay.[20] Caco-2 cells at a density of 2.5×10^4 cells per well were seeded in 24-well plates under a 5% CO_2 atmosphere and at 37 °C. Minimum essential medium (MEM) was changed on alternate days until a confluent monolayer of Caco-2 cells was attained. Test solutions of SEDDS FA and SEDDS FB containing complexes at a charge ratio of 1:1 diluted either 1% or 3% in 25 mM HEPES buffered saline (HBS) pH 7.4 were prepared. Before the experiment, cells were washed thrice with preheated HBS at 37 °C. Samples

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were added at a volume of 500 μL per well and incubated under a 5% CO_2 atmosphere and 37 °C for 4 h. HBS and 0.5% (w/v) Triton X-100 solution served as positive and negative control, respectively. Cells were washed twice with preheated HBS, and then 250 μL of resazurin (2.2 μM) solution were added and incubated under a 5% CO_2 atmosphere and at 37 °C for 3 h. Aliquots of 100 μL were transferred to 96-well plate, and fluorescent intensity was measured using a microplate reader (Tecan Spark, Tecan Trading AG, Zurich, Switzerland) at an excitation wavelength of 540 nm and an emission wavelength of 590 nm. The cell viability was calculated using eq 2.

$$\text{Cell viability (\%)} = \frac{\text{Average fluorescence of each triplicate}}{\text{Average fluorescence of positive control}} \times 100 \quad (2)$$

2.2.6. Drug Release Studies. As in situ methods provide, in contrast to membrane-diffusion and sample-and-separate methods, reliable results about the drug release behavior from SEDDS, an according method was developed for this study. At pH ≥ 8 the thiol group of CTL reacts almost instantly with DTNB forming a mixed disulfide of yellow color that can be photometrically quantified during the release process. Since the highly hydrophilic DTNB cannot diffuse into the oily droplets, only CTL being released to the aqueous phase can react with this reagent. Following this concept an aqueous 0.02% DNTB solution at pH 8 was used as a release medium. Briefly, SEDDS FA and SEDDS FB containing CTL−polymer complexes were diluted 1:100 by emulsifying 10 μL of SEDDS in 990 μL of release medium in multiple tubes utilizing a thermomixer at 300 rpm and 37 °C. Aliquots of 100 μL of each dispersion were withdrawn in triplicate, and absorbance was measured at 450 nm at time intervals of 10 min for 3 h. Between measurements, plates containing aliquots were covered and kept incubated with a shaker at 300 rpm at 37 °C. Free CTL containing SEDDS was used as control. The percentage release of CTL was calculated using eq 3. To determine reference absorbance according to this equation, free CTL dissolved as pure drug in the release medium was utilized.

$$\text{CTL (\%)} = \frac{A_t - \text{blank}}{A_r - A_e} \times 100 \quad (3)$$

A_t represents total absorbance, A_r represents reference absorbance, and A_e indicates the absorbance of an aqueous solution of 0.02% DNTB at pH 8.

In order to confirm hydrophobic ion pairing of CTL and Eudragit (RS 100, RL 100 and E 100), 5% (w/v) acetic acid solution was used. As acetic acid dissolves in the oily droplets, it can substitute CTL in the complex in a competitive manner. Briefly, a stock solution of 50 mg/mL (47.62 μL/mL) acetic acid was added to release medium as mentioned above. The release of CTL was measured at 450 nm via a microplate reader (Tecan Spark, Tecan Trading AG, Zurich, Switzerland), with free CTL used as control.

2.2.7. Calibration Curves. CTL was prepared as a 9.3 mg/mL stock solution in deionized water. Five different dilutions of CTL (i.e., 4.6, 2.3, 1.15, 0.57, and 0.28 mg/mL) were prepared from a 9.3 mg/mL stock solution in deionized water. After a further 1:100 dilution of these concentrations in the release medium, the absorbance of CTL was measured in duplicate at 450 nm in a microplate reader (Tecan Spark, Tecan Trading AG, Zurich, Switzerland). The effect of 5% (w/v) acetic acid on the absorbance readings was checked.

2.2.8. Statistical Data Analyses. Prism v 5.01 (GraphPad Software, USA) was utilized for statistical data analysis. The two-way analysis of variance (ANOVA) was used to determine the statistical significance between the time-dependent release of CTL percentages from the hydrophobic ion complexes. The degree of significance was valued as $^*p \leq 0.05$ for significant, $^{**}p \leq 0.01$ for very significant, and $^{***}p \leq 0.001$ for highly significant using 95% confidence interval (p value ≤0.05). CTL calibration curves using release medium with and without acetic acid were statistically described using the linear regression square (r^2) model. The calculated r^2 values of both curves were 0.99 ± 0.001 and 0.99 ± 0.0003. Indicated values are expressed as means ± standard deviation (SD) of at least three experiments.

3. RESULTS AND DISCUSSION

3.1. Preparation of Lipophilic Complexes. Ionic complexes of CTL were formed with the tertiary amine polymer Eudragit E as well as the quaternary ammonium polymers Eudragit RS and RL as shown in Figure 1. All three polymers formed lipophilic complexes with CTL that were

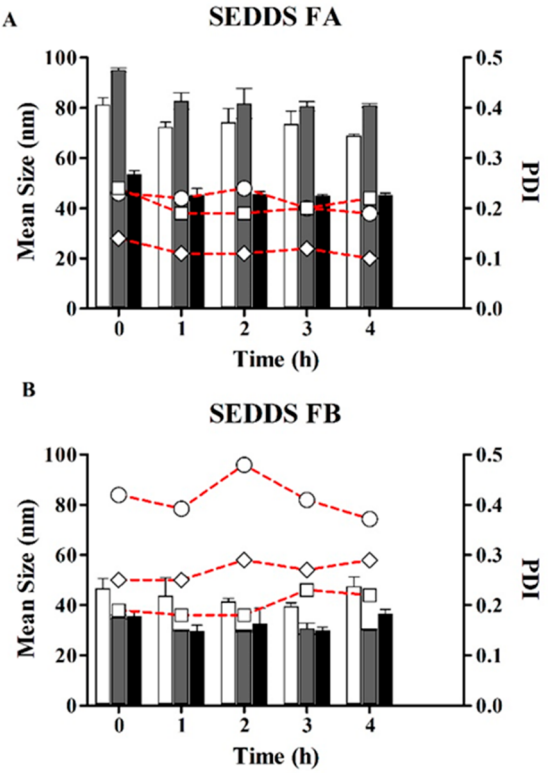

Figure 3. (A) Mean droplet size of 2% complex loaded SEDDS FA at polymer Eudragit RS (white bars), Eudragit RL (gray bars) and Eudragit E (black bars) to CTL ratio of 1:1 and PDI of SEDDS FA at Eudragit RS (○), Eudragit RL (□) and Eudragit E (◇) to CTL ratio of 1:1 at indicated time points. (B) Mean droplet size of 2% complex loaded SEDDS FB at Eudragit RS (white bars), Eudragit RL (gray bars) and Eudragit E (black bars) to CTL ratio of 1:1 and PDI of SEDDS FB at Eudragit RS (○), Eudragit RL (□) and Eudragit E (◇) to CTL ratio of 1:1 at indicated time points. Indicated values are means of at least three experiments ± SD.

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Table 2. Zeta Potential of Blank SEDDS and Complex Loaded SEDDS Diluted 1:100 in the Release Medium[a]

	Zeta potential (mV)											
	Complexes loaded SEDDS											
	Polymer to drug ratio (m/m)											
	Eudragit RS-CTL			Eudragit RL-CTL			Eudragit E-CTL					
Formulations	Blank SEDDS	1:1	2:1	4:1	1:1	2:1	4:1	1:1	2:1	4:1		
SEDDS FA	−7.98 ± 0.84	3.03 ± 0.27	4.49 ± 0.56	5.13 ± 0.49	4.75 ± 0.88	5.78 ± 0.87	7.48 ± 1.96	0.64 ± 0.14	1.19 ± 0.36	2.04 ± 0.34		
SEDDS FB	−9.0 9 ± 1.28	4.08 ± 0.61	4.61 ± 0.33	5.83 ± 0.32	5.60 ± 0.35	6.68 ± 0.21	7.67 ± 0.69	−1.20 ± 0.56	0.95 ± 0.64	2.26 ± 0.27		

[a]Indicated values are means of at least three experiments ± SD.

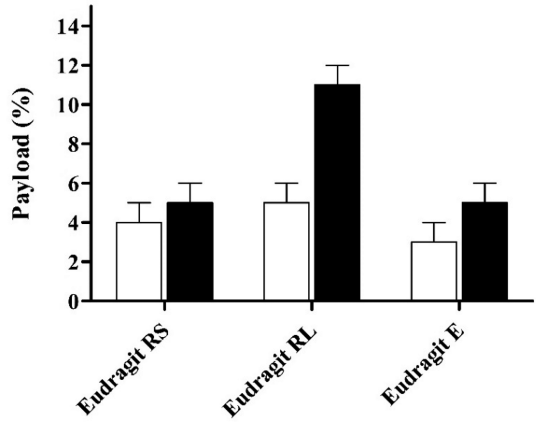

Figure 4. Maximum payload of polymers in SEDDS FA (white bars) and SEDDS FB (black bars). Values are indicated as mean ± SD (n = 3).

Table 3. Log $SR_{SEDDS/release\ medium}$ of CTL between SEDDS FA/SEDDS FB and Release Medium

SEDDS	$SR_{SEDDS/release\ medium}$	Log $SR_{SEDDS/release\ medium}$
FA (Captopril)	3.25 ± 0.12	0.51 ± 0.02
FB (Captopril)	0.85 ± 0.05	0.31 ± 0,03

sufficiently stable to provide a sustained drug release. El-Hamid et al. described that ion pair formation between negatively charged alendronate and branched positively charged polyethylenimine enhanced the lipophilicity of alendronate. Although complexation of alendronate with polyethylenimine improved drug encapsulation efficiency in nanostructured lipid carriers (NLCs) almost 9-fold, the drug release from these NLCs was significantly delayed after complexation.[21] However, NLCs (unlike SEDDS) are solid nanocarriers, and drug release is primarily controlled by a suite of complex mechanisms, this study showed nevertheless the potential of ionic drug polymer complexes to achieve sustained release. Quinteros et al. developed complexes of anionic mesalazine with Eudragit E. They observed that this complexation of the drug has a significant impact on the dissolution rate and release kinetics.[22] The solubility of methacrylate copolymers in SEDDS was a prerequisite for the design of nanoemulsions providing a sustained drug release. Methanol and ethyl acetate are among the most efficient solvents for these polymers. Moreover, the comparatively low dielectric constant of these solvents (≤30) contributed to the formation of stable drug polymer complexes. Mixing of methanolic as well as ethyl acetate polymeric solutions with Kolliphor RH 40 and glycerol containing CTL, respectively, reduced the overall dielectric constant resulting in improved ionic interactions.[23] As the carboxylic acid group of CTL has a pK_a of 3.7,[17] it exhibits a sufficiently high acidic character in glycerol and methanol.[24] Furthermore, ethyl acetate due to its aprotic nature favors the ionic association of the drug with tertiary amines of Eudragit E by means of proton transfer as a rate-limiting step.[25] In order to evaluate the impact of solvents exhibiting different dielectric constants (ε) on complex formation, CTL−polymer complexes were formed in Kolliphor RH 40 and glycerol.

3.2. SEDDS Preparation and Characterization. Two different SEDDS formulations (SEDDS FA and SEDDS FB) were developed by utilizing different ratios of oil, surfactants, and solvents according to Table 1. SEDDS FA and SEDDS FB

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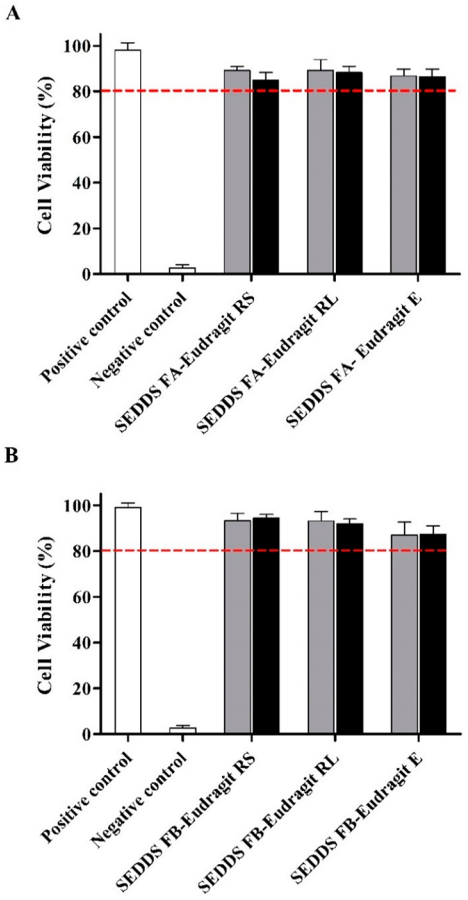

Figure 5. Caco-2 cells viability determined via resazurin assay after 4 h. (A) Influence of complex loaded SEDDS FA at a polymer to CTL charge ratio of 1:1 diluted 1% (gray bars) and 3% (black bars). (B) Influence of complex loaded SEDDS FB at a polymer to CTL charge ratio of 1:1 diluted 1% (gray bars) and 3% (black bars). Indicated values are means of at least three experiments ± SD.

spontaneously formed emulsions upon dilution (1:100) in the release medium. Using different lipophilic composition ratios modifying their emulsification properties and miscibility in the aqueous medium, both SEDDS shaped their ultimate oily droplets and differently influenced releasing CTL susceptibility for dissociation at different rates from the anchored CTL−polymer complexes. The mean droplet size of SEDDS FA was 45.58 ± 2.04 nm with PDI ≈ 0.09 whereas SEDDS FB exhibited a droplet size of 26.52 ± 0.71 nm with PDI ≈ 0.19. As shown in Figure 3A, the droplet size of complex loaded SEDDS FA was in the range 100 ± 20 nm and the PDI was less than 0.5. The size distribution of complex loaded SEDDS FB was in the range of 40 ± 10 nm as described in Figure 3B. Lam et al. also observed an increase in the size of oily droplets by incorporation of cationic surfactants as the mean droplet size of SEDDS increased from 31 to 45 nm by incorporation of 1% 1-decyl-3-methylimidazolium chloride and to 55 nm by the incorporation of 5% octylamine.[26] During the incubation period neither precipitation nor phase separation of formulations was observed. The size distribution of the oily droplets of all formulations remained constant, the and PDI remained under 0.5 indicating monodisperse emulsions during the incubation time. However, the droplet size of SEDDS was dependent on the type and concentration of the polymers used. Furthermore, incorporating CTL−polymer complexes in SEDDS FA and SEDDS FB increased the zeta potential as highlighted in Table 2. SEDDS of CTL-complexes characterizing the quaternary ammonium polymers (Eudragit RS/RL) showed greater zeta potential increases compared to the tertiary amine-based Eudragit E. In the case of tertiary amino groups exhibited by the cationic polymer Eudragit E charge is pH-dependent.[7] Lam et al. determined the zeta potential of SEDDS loaded with various cationic surfactants. The tertiary amine surfactant octylamine could not raise the zeta potential of SEDDS to a positive value even when added in a concentration of 5%, whereas the incorporation of 1% quaternary ammonium surfactants caused a dramatic shift in zeta potential to positive values.[26] To a certain extent CTL−polymer complexes seem to be located on the interface, as their incorporation in SEDDS had a significant impact on the zeta potential of the resulting oily droplets. Generally, SEDDS remained stable in the release medium despite size and zeta potential alterations caused by incorporating CTL−polymer complexes. As the mean droplet size of all formulations remained below 100 nm, they should be able to permeate the mucus layer exhibiting a mesh size in the range between 100 and 200 nm.[27,28] Furthermore, droplets exhibiting a negative zeta potential will likely permeate the mucus layer to a higher extent than positively charged droplets, as ionic interactions with anionic mucus substructures such as sialic and sulfonic acid moieties can in the case of a negative zeta potential be excluded.[10,29] The presence of a polyethylene glycol (PEG) corona being provided by Kolliphor RH 40 and EL will likely also contribute to high mucus permeating properties.[27]

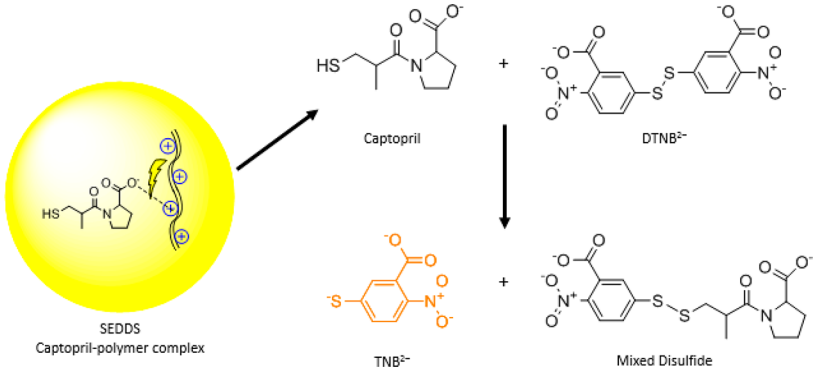

Figure 6. Schematic illustration of the concept of captopril released from SEDDS. $DTNB^{2-}$: 5,5′-dithiobis(2-nitrobenzoic acid) dianion; TNB^{2-}: 2-nitro-5-thiobenzoate dianion.

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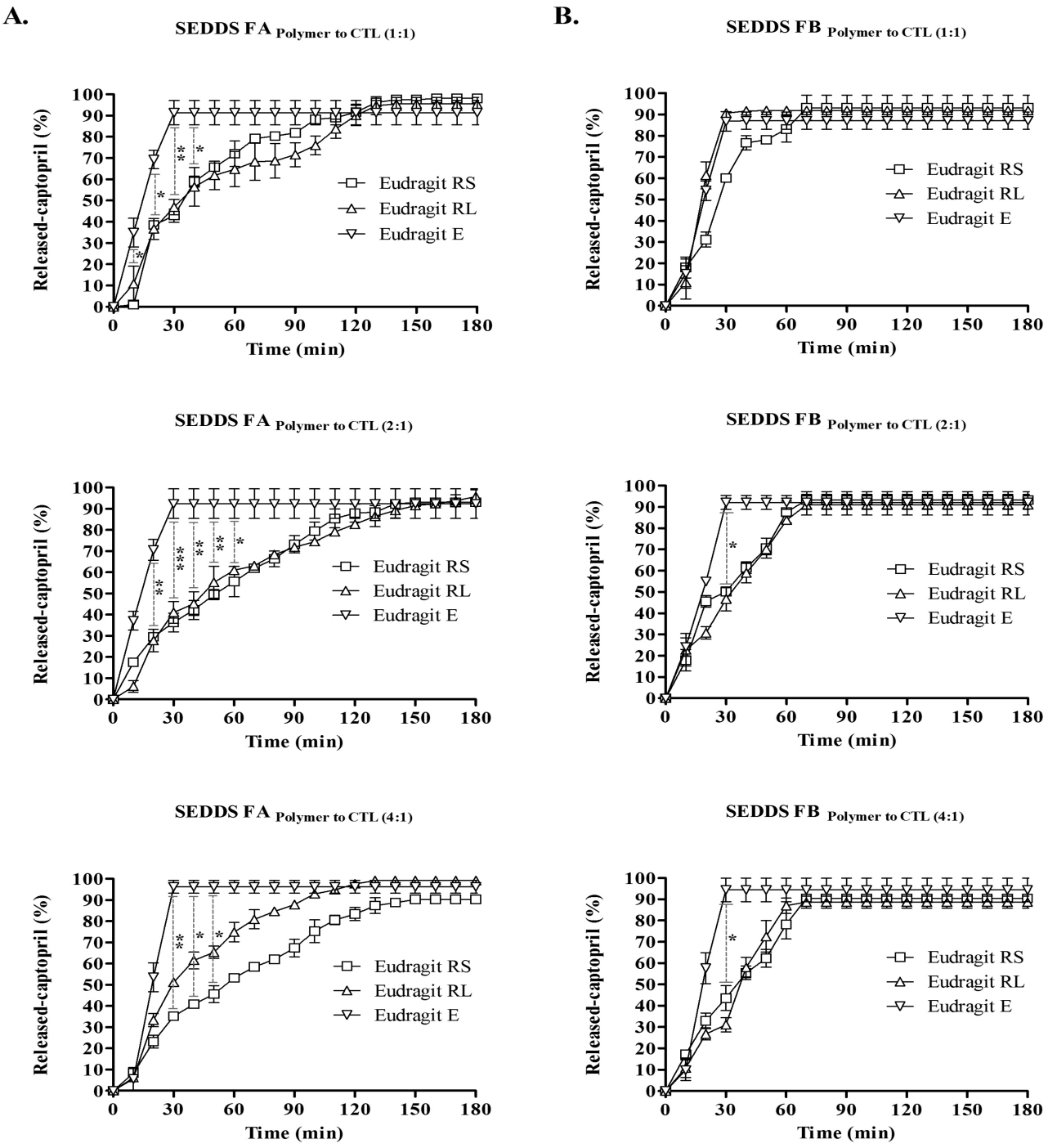

Figure 7. (A) Percentage of released CTL from SEDDS FA containing hydrophobic complexes of polymer:CTL ratios 1:1, 2:1 and 4:1 at an interval of 10 min over a time period of 180 min. (B) Percentage of released CTL from SEDDS FB containing hydrophobic complexes of polymer:CTL ratios 1:1, 2:1 and 4:1 at an interval of 10 min over a time period of 70 min. All experiments are mean ± SD of three experiments.

3.3. Evaluation of Polymer Loading. Polymers were incorporated in SEDDS via a cosolvent evaporation method. Polymers were dissolved in methanol and ethyl acetate followed by incorporation into SEDDS excipients and removing these solvents by evaporation. Soltani et al. incorporated lipophilic complexes of heparin with a cationic polymer of cyclodextrin into SEDDS by dissolving them in ethanol. The ethanolic solution containing the lipophilic complexes was added to propylene glycol used as a SEDDS excipient.[30] Pandya et al. developed polymeric micelles of simvastatin utilizing hydrophilic, low viscous grade hydroxypropyl methyl cellulose (HPMC). They developed a cosolvent evaporation method for efficient encapsulation of simvastatin into polymeric micelles utilizing a mixture of simvastatin in methanol and HPMC in water. The encapsulated drug exhibited a significantly improved dissolution efficiency compared to drug solid dispersions.[31] Results of our study showed that the incorporation of the polymers into the oily droplets has a significant impact on the release of the drug. Therefore, the maximum payload of polymers in oily droplets was evaluated. As illustrated in Figure 4, a maximum payload of 11% Eudragit RL was achieved for

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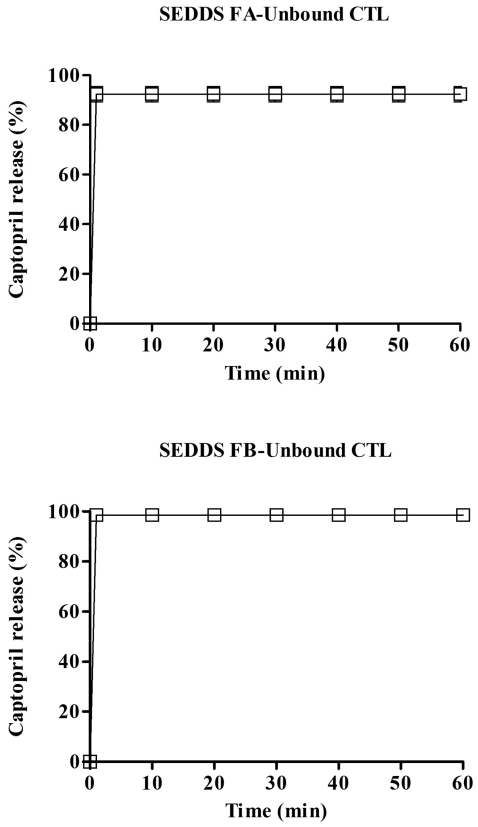

Figure 8. Percentage of unbound CTL release from SEDDS FA and FB without polymer at an interval of 10 min over a time period of 60 min. All experiments are mean ± SD of three experiments.

SEDDS FB. The homogeneous incorporation of sufficient polymer into SEDDS on one hand stabilized the drug and on the other hand decreased its release rate from the oily droplets as shown below.

3.4. Solubility Ratio (log $SR_{SEDDS/release\ medium}$). The solubility ratio between the oily phase of SEDDS and the release medium can be considered as a key parameter for the prediction of drug release kinetics from SEDDS. Drug release is simply based on a diffusion process from the oily phase into the aqueous phase. Upon dilution with aqueous media, the equilibrium of drugs between the oily droplets formed by SEDDS and release medium is reached immediately.[13] According to Table 3, log $SR_{SEDDS/release\ medium}$ of CTL was ≤0.5 indicating an immediate release of almost the entire drug from the nanodroplets. Under the assumption that 1 mL of the SEDDS FA preconcentrate is emulsified in just 100 mL of intestinal fluid, over 95% of CTL are instantly released. Taking also drug absorption from the intestinal mucosa into consideration, this release process is even further accelerated. As Eudragit RS, RL, and E exhibited a log $SR_{SEDDS/release\ medium}$ of ≥5, they remained in the oily droplets providing a sustained release of CTL having been bound to them. Song et al., for instance, investigated the release of the cationic drug AZD2811 from polylactic acid−polyethylene glycol (PLA−PEG) nanoparticles using ion pairs with several acidic counterions such as oleic and pamoic acids. They showed that ion paired AZD2811 displays sustained release in comparison to unbound AZD2811 from the PLA−PEG nanoparticles.[5]

3.5. Cell Viability Studies - Resazurin Assay. Cell viability studies were performed, as most cationic polymers are known for their interference with cell membranes and cytotoxic effect.[32] Free amino groups within the polymeric structure are responsible for pronounced interactions with negatively charged cell membranes and disturbing their metabolic pathways.[33] The impact of complex loaded SEDDS FA and SEDDS FB on the viability of Caco-2 cells was assessed by using the resazurin assay, which is based on the ability of viable cells to metabolize resazurin to its reduced form resorufin.[20] As illustrated in Figure 5, more than 80% cells remained viable proving biocompatibility of polymeric complex loaded SEDDS FA and SEDDS FB within an incubation period of 3 h. Therefore, the concentrations of the polymers ranging from 1% to 3% in SEDDS formulations can be considered as relatively safe. Zhang et al., for instance, described that a genistein encapsulated nanostructured lipid carrier coated with Eudragit RS at a concentration ranging from 0 to 100 μg/mL exhibits no cytotoxic effect on human cornea epithelial cells.[34] As these Eudragits are generally regarded as safe (GRAS) and used as coating material in numerous marketed solid dosage forms, their use in SEDDS should not be restricted by safety concerns. Nonetheless, the safety of SEDDS containing cationic polymers will have to be evaluated for each formulation on an individual basis. Lam et al., for instance, demonstrated that cationic surfactants such as benzalkonium chloride, octylamine, and alkyltrimethylammonium bromide used to enhance the lipophilicity of anionic drugs via hydrophobic ion pairing were cytotoxic at less than 0.003% and that the cytotoxicity of these cationic surfactants was even enhanced in SEDDS.[26]

3.6. Drug Release Studies. For the characterization of release kinetics from SEDDS various techniques such as membrane-diffusion methods and sample-and-separate methods are available. These methods, however, lead to erroneous results as comprehensively reviewed previously.[13,35] In contrast, in situ methods provide fast, direct, and reliable drug release profiles from SEDDS. As a sample separation is not required, drug release can be assessed on a real-time basis. For these reasons, an in situ method was developed for this study. As thiol groups rapidly and quantitatively react with DTNB at pH ≥ 8 forming TNB^{2-} that can be easily colorimetrically quantified during the release process, it was chosen for this in situ method. Because of its highly hydrophilic character as illustrated in Figure 6, DTNB cannot diffuse into the oily droplet. Consequently, only CTL that is released from the oily phase can react with this reagent. Within this study an aqueous 0.02% DNTB solution pH 8 was therefore used as the release medium.

During an ion-exchange process, Eudragit RS or RL (with permanent cationic quaternary ammonium groups) is more favored in forming stable ionic complexes.[36] On the other hand, forming ionic complexes with Eudragit E is more variable because of the highly pH-dependent tertiary amine.[7,37] Due to stronger ionic interactions, Eudragit RS/RL showed superior CTL sustained release compared to Eudragit E, as shown in Figure 7. The release of drug from the SEDDS mainly depended on the distribution coefficient and the stability of the ionic complexes.[13,38] More stable ionic complexes dissociate slowly over time, leading to a sustained drug release from the oily droplets. Drug release as a function of the drug to counterion charge ratio has also been described by Lu et al.[39] Briefly, the strength of ionic complexes depended on the charge ratio of the drug to the counterion. A strong ionic complex will be formed, if the number of charges on the

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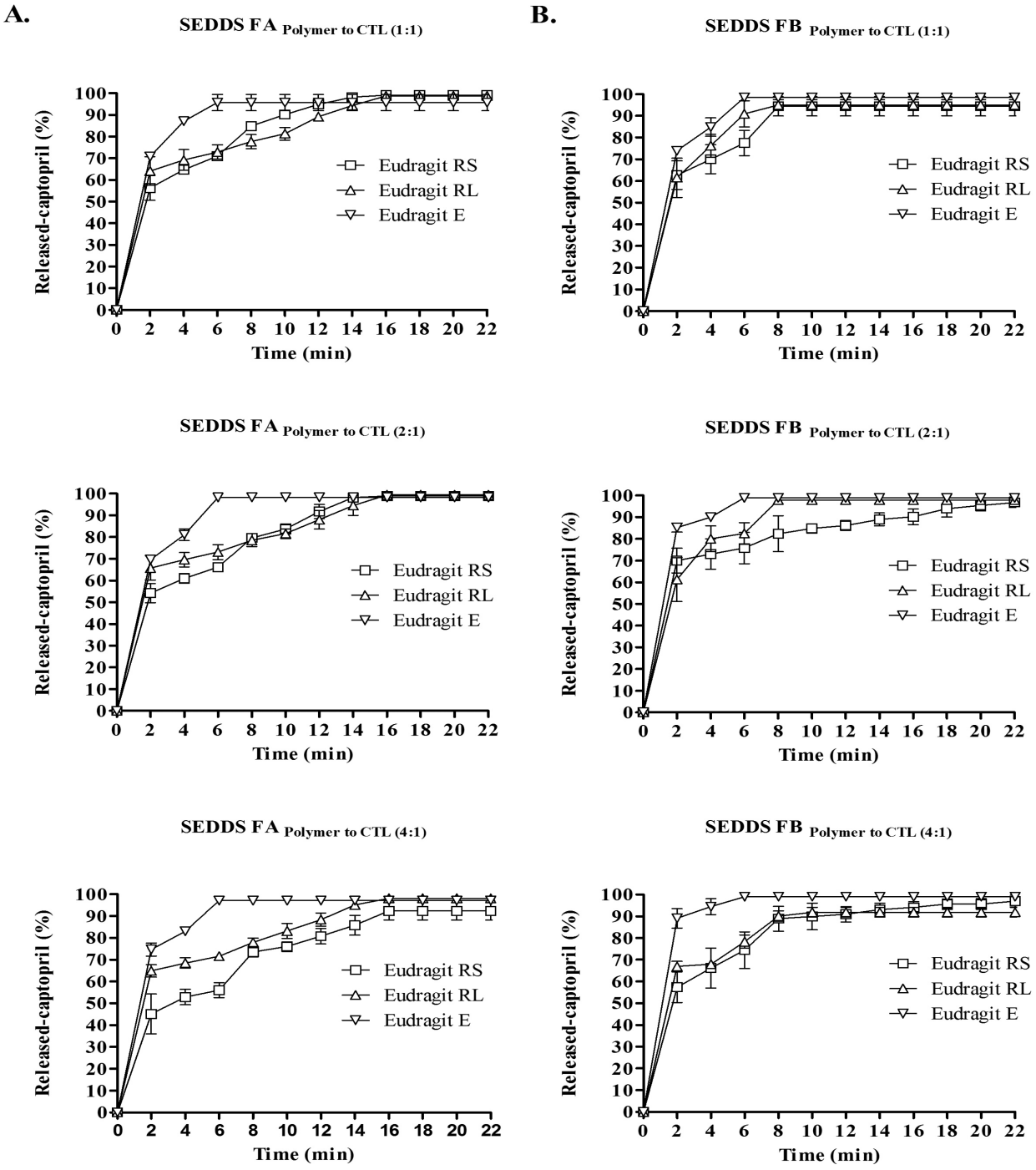

Figure 9. (A) Percentage of CTL released from SEDDS FA containing hydrophobic complexes of polymer/CTL ratios 1:1, 2:1, and 4:1 in the presence of acetic acid as ion replacer at an interval of 2 min over a time period of 22 min. (B) Percentage of CTL released from SEDDS FB containing hydrophobic complexes of polymer/CTL ratios 1:1, 2:1, and 4:1 in the presence of acetic acid as ion replacer at an interval of 2 min over a time period of 22 min. All experiments are mean ± SD of three experiments.

counterion is greater than charges on the drug and vice versa. Therefore, optimization of the polymer charge to CTL ratio led to the efficient retention of complex within the oily droplet of SEDDS formulation and caused drug sustained release.

SEDDS containing Eudragit-CTL ionic complexes displayed substantial sustained release in comparison to the unbound CTL as shown in Figure 8. This confirmed the formation of complexes between polymer and CTL and provided proof that ionic complexes play an important role in causing sustained drug release from SEDDS. Decreasing the molar ratio of CTL to polymer had no impact on the sustained release of CTL from oily droplets. This trend was observed in both SEDDS FA and SEDDS FB at all molar ratios. Moreover, the release of drug from SEDDS FA was observed to be more sustained in comparison to SEDDS FB. A possible reason for the superiority of sustained release from SEDDS FA over SEDDS FB might be the difference in dielectric constant (ε) of excipients used for complex formation. At lower dielectric constant, the dissociation of the counterion is less and more binding sites remain available, contributing toward complex

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stability.[40,41] As the composition and physical properties such as droplet size and zeta potential of FA and FB are different, of course also further effects might be involved in the more sustained release mechanism from SEDDS FA.

Sustained release of CTL from SEDDS containing polymer drug ionic complexes was compared with the release of CTL in a release medium containing 5% acetic acid. As acetic acid was added in a 2000-fold higher concentration than CTL and acetic acid with a pK_a of 4.76 which is to a higher extent ionized, CTL is likely removed from the complex by acetic acid. This strong ion replacer efficiently showed decoupling of the drug CTL bound to the polymer via ion exchange operation. Therefore, the slower release of CTL from polymer ionic complexes shifted to rapid release after addition of the acetic acid as shown in Figure 9. Comparatively, the presence of acetic acid in release medium resulted in approximately 97.07% CTL rapid release typically within ~6−22 min, whereas in the absence of acetic acid in release medium approximately 93.01% slow CTL release was typically achieved within ~60−180 min. Rapid release of CTL in release medium containing 5% acetic acid provided evidence for CTL−polymer ionic complexes. Acetic acid acted as counterion replacer for CTL resulting in immediate drug release from SEDDS. The regeneration ratio (acetic acid/CTL) was calculated using eq 4 and was shown to be about 0.27 on a weight basis.

$$\text{Regeneration ratio} = \frac{\text{Regeneration quantity (equiv)}}{\text{Ionic load (equiv)}} \quad (4)$$

This shows that acetic acid is very effective at regeneration of the CTL from the CTL−polymer complexes. In terms of the regeneration ratio, this work employed 5% acetic acid in the release medium, which by far exceeds the calculated acetic acid/CTL regeneration ratio. Therefore, the release of CTL in the acetic acid medium clearly shows that the intact complexes are responsible for the effective retention within the SEDDS.

4. CONCLUSION

In this study, complexes of captopril (CTL) were prepared using Eudragit RS, RL, and E and incorporated into SEDDS to obtain sustained release of the drug from oily droplets. SEDDS FA and SEDDS FB showed stable droplet size and non-releasing polymers indicated by a high log $SR_{SEDDS/release\ medium}$ retaining the CTL within the SEDDS. Conversely, a log $SR_{SEDDS/release\ medium}$ of free CTL below 0.5 was reflected in immediate drug release. The resazurin assay indicated that complex loaded SEDDS had no toxic effect on the viability of cells. The quaternary ammonium group based polymers (Eudragit RS and RL) resulted in a more sustained release compared to the tertiary amine polymer (Eudragit E). The findings of this study provide evidence for a sustained drug release from SEDDS, when hydrophobic drug polymer complexes are used.

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Notes

The authors declare no competing financial interest.

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