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# Synthesis, complex formation and corneal permeation of cyclodextrin-modified, thiolated poly(aspartic acid) as self-gelling formulation of dexamethasone

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ABSTRACT

The present study aimed at developing a potential *in situ* gellable dexamethasone (DXM) eye drop. Poly(aspartic acid) (PASP) derivatives were synthesized with dual functionality to improve the solubility of DXM, and to achieve *in situ* gelation. First, amine-modified β-cyclodextrin (CD) was attached to polysuccinimide (PSI), second, thiol functionalities were added by the reaction of cysteamine and succinimide rings. Finally, the PSI derivatives were hydrolysed to the corresponding PASP derivatives to get water-soluble polymers. Phase-solubility studies confirmed the complexation ability of CD-containing PASP derivatives. *In situ* gelation and the effect of the CD immobilization on this behaviour were characterized by rheological measurements. The solubilizing effect of CD was confirmed by kinetic solubility measurements, whereas *in vitro* corneal permeability assay (corneal-PAMPA) measurements were performed to determine *in vitro* permeability and flux values. The effect of the PASP derivatives on permeation strongly depended on chemical composition and polymer concentration.

## 1. Introduction

The treatment of ocular diseases using topical formulations is a challenging task due to the complex and unique structure and the protective mechanisms of the human eye. The bioavailability of topically administered eye drops – the most commonly used formulations in the treatment of the anterior segment of the eye – can be less than 5%, as a consequence of blinking, the diluting mechanism of tears, the nasolacrimal drainage, and the transfer into the systemic circulation by local capillaries through non-corneal routes [1–3]. In the case of active pharmaceutical ingredients (APIs) with poor aqueous solubility (hydrophobic APIs), providing and maintaining a satisfactory concentration in the pre-corneal area to improve their corneal absorption is an even more challenging task. Thus, the prediction of corneal absorption of novel drug candidates and formulations is essential from the early stages of drug discovery. To this avail, several models have been developed: *ex vivo* methods using eyes or excised cornea of sacrificed animals, *in vitro* cellular methods using primary cell cultures, immortalized cell lines, or

*Abbreviations:* AcN, acetonitrile; APIs, active pharmaceutical ingredients; $C_0$, solubility; CD, cyclodextrin; DXM, dexamethasone; G', storage modulus; G", loss modulus; HPLC, high performance liquid chromatography; *J*, flux; $K_c$, complex stability constant; MA-ß-CD, 6-monodeoxy-6-monoamino-beta-cyclodextrin hydrochloride; MR, membrane retention; PAMPA, parallel artificial membrane permeability assay; PASP, poly(aspartic acid); PASP-CD, cyclodextrin functionalized poly (aspartic acid); PASP-SH, thiolated poly(aspartic acid); PASP-SH-CD, cyclodextrin-modified thiolated poly(aspartic acid); PBS, phosphate-buffered saline; PC, phosphatidylcholine; $P_e$, effective permeability; PSI, polysuccinimide.

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reconstructed tissue cultures of rabbit or human origin [4,5]. Recently we developed an *in vitro* non-cell-based method, a corneal parallel artificial membrane permeability assay (corneal-PAMPA), to support the early stages of drug development [6,7]. Our corneal-PAMPA assay is based on *ex vivo* rabbit corneal permeability data [8,9]. During the development of this method, we investigated the effects of the composition of the artificial lipid membrane, the buffer solutions, and the cosolvent content of the donor phase on the permeability of drugs. Eight commercially available eye drops in their undiluted forms and 5-, 10- and 20-fold dilutions to simulate the diluting effect of the lacrimal fluid were used as model drugs for the development of our corneal-PAMPA. The results showed that the method is sensitive to dilution, which strongly influenced the permeability. This might be explained by the decreased viscosity and relative concentration of the components of the eye drops (API, background electrolytes, other formulating agents) [10]. Higher permeability of diluted formulations is desirable for eye drops, but dilution decreases the therapeutic concentration of the APIs. However, dilution of eye drops is desirable only if the concentration of the API is kept above the therapeutic limit [11]. In order to maximize the overall absorption of poorly soluble drugs and monitor the solubility–permeability interplay [12], it is recommended to determine also the fluxes of these formulations.

Dexamethasone (DXM), a synthetic glucocorticoid drug, is often used for the treatment of ocular inflammations (e.g., uveitis), infections after cataract surgery, and corneal transplantation [13–16]. However, its poor aqueous solubility (0.16 mg/mL) makes the successful therapeutic application difficult [17]. To address this problem, several strategies can be used to increase the ocular absorption of DXM, such as the use of solubility enhancers like cyclodextrins either in their free form or immobilized to macromolecules [3,18–20], the increase of the specific surface of the formulation by microemulsions [21] or nanostructured formulations [14,15,22,23], and the increase of residence time by a bioadhesive and/or viscous or gellable formulation [24–26].

Cyclodextrin units have already been conjugated to various macromolecular structures to obtain water-soluble polymer-based formulations or hydrogels for the efficient encapsulation of hydrophobic APIs [27]. Poly(aspartic acid) (PASP) and its thiolated form as water-soluble, biodegradable, and biocompatible poly(amino acid)s are widely studied for pharmaceutical uses [28–33]. The interest in using PASP is supported by the chemical versatility of polysuccinimide – the precursor polymer of PASP – as succinimide repeating units can react with various nucleophiles and facilitate degradation under physiological conditions depending on the composition of the pendant groups [34–38]. The synthesis procedure of CD-functionalized PASP derivatives is largely determined by the chemical reactivity of the polymer and the CD used. Free radical polymerization was used to immobilize vinyl-modified CDs onto glycidyl methacrylated PASP derivatives [39], whereas vinyl sulfone functionalized CD was reacted with amine pendants of PASP derivatives to prepare hydrogel precursors [40]. Ring-opening polymerization of β-benzyl-L-aspartate in the presence of active CD was also used to prepare PASP-based formulations for plasmid DNA encapsulation [41]. Finally, the rings of PSI can be opened with 6-monodeoxy-6-monoamino-beta-cyclodextrin or mono-6-(2-aminoethylamino)-6-monodeoxy-beta-cyclodextrin without any activating or coupling reagent, resulting in CD-modified PASP derivatives [42–44]. In some cases, additional functionalities including *in situ* gelation [43] or cationic character for nucleic acid complexation [41] are simultaneously built with CD immobilization. Mucoadhesive property of several polymers (e.g., poly(acrylic acid), hyaluronic acid, chitosan, sodium carboxymethyl cellulose, poly(galacturonic acid) and poloxamer) is exploited in ocular drug delivery to prolong the residence time of APIs on the surface of the cornea [13,15,24,45–50]. The introduction of *in situ* gelling property to such formulations can result in even prolonged residence time on the eye surface, and the so-formed hydrogels can maintain a controlled drug release because of the presence of a 3D network. For ophthalmic administration, the formulations must be transparent, non-toxic, non-irritant and have to provide appropriate surface tension, pH, and refractive index. [24,51,52].

In this study, we report the syntheses of thiolated and CD-functionalized self-gelling PASP. Physicochemical properties of its formulation with DXM were investigated by phase-solubility studies and rheological measurements. *In vitro* biopharmaceutical performance (dissolution and permeability) of the CD-enabled polymeric DXM delivery system was also studied.

## 2. Experimental

### 2.1. Materials

Analytical grade solvents such as acetonitrile (AcN), chloroform, dimethyl sulfoxide (DMSO), dodecane, hexane and formic acid as well as phosphate buffered saline (PBS) powder, L-α-phosphatidylcholine (PC), dexamethasone, L-aspartic acid (extra pure, 99.5%) were purchased from Merck, KGaA (Darmstadt, Germany). Crystalline phosphoric acid (99%) was bought from Sigma-Aldrich Co., Ltd. (Budapest, Hungary). Cysteamine (95%), dibutylamine (HPLC grade, 99%), dithiothreitol (99%), *N,N*-dimethylformamide (for analysis, 99.8%) and sodium bromate (99%) were purchased from Reanal Hungary. Single isomer 6-monodeoxy-6-monoamino-beta-cyclodextrin hydrochloride (MA-β-CD hydrochloride) was provided by CycloLab Cyclodextrin Research and Development Laboratory Ltd. (Budapest, Hungary). Maxidex 1–mg/mL (lot 17I13QE) was purchased from Alcon Ltd. (Novartis Division). In the gel formulation, PBS solution of pH = 7.4 was prepared by dissolving 8 g/dm$^3$ NaCl, 0.2 g/dm$^3$ KCl, 1.44 g/dm$^3$ $Na_2HPO_4{\cdot}2H_2O$ and 0.12 g/dm$^3$ $KH_2PO_4$ in distilled water, the pH was adjusted with 0.1 M HCl. Ultrapure water (ρ greater than 18.2 MΩ cm, Millipore) was used for the preparation of aqueous solutions. Syntheses and measurements were carried out at 25 °C unless otherwise noted.

### 2.2. Syntheses

#### 2.2.1. Synthesis of poly(aspartic acid) (PASP)

Synthesis of polysuccinimide was reported in the earlier work of the group, the viscosity average molecular weight was 31.5 kDa (Table 1) [53]. 1 g PSI was dispersed in 500 mL phosphate buffer (0.5 M, pH = 8.0) and stirred for 24 h under nitrogen atmosphere to hydrolyse the succinimide rings to aspartic acid repeating units (Fig. 1). The solution was dialysed against water and freeze-dried to obtain the final product. $^1$H NMR of PASP: 2.73 (2H, CH-*CH*$_2$-CONH), 4.46, 4.65 (1H, CONH-*CH*-$CH_2$), 4.91 (1H, CON-*CH*-$CH_2$).

#### 2.2.2. Synthesis of cyclodextrin functionalized poly(aspartic acid) (PASP-CD)

Poly(aspartic acid) functionalized with 1 mol% MA-β-CD (PASP-CD) was synthesized (Fig. 1). 1 g of PSI (10.3 mmol succinimide unit) and 120.6 mg (0.103 mmol) MA-β-CD hydrochloride were dissolved in 10 mL dimethylformamide and 20.0 mg (0.154 mmol) dibutylamine was added as deprotonating agent to initiate the reaction. The solution was stirred for 24 h. The solution was poured into 150 mL 0.1 M NaOH and

**Table 1**
Degree of polymerization, degree of modification for thiol and cyclodextrin groups of poly(aspartic acid) derivatives.

| Sample code | DP [a] (–) | $X_{SH}$ [b] (%) | $X_{CD}$ [c] (%) |
|---|---|---|---|
| PASP | 320 | – | – |
| PASP-CD | 320 | – | 0.77 |
| PASP-SH | 320 | 9.9 | – |
| PASP-SH-CD | 320 | 7.6 | 0.83 |

[a] Degree of polymerization (DP) determined for PSI by viscosimetry.
[b] , [c]Degree of modification for thiol ($X_{SH}$) and cyclodextrin ($X_{CD}$) groups, respectively. Both are determined from $^1$H NMR spectra of the polymers.

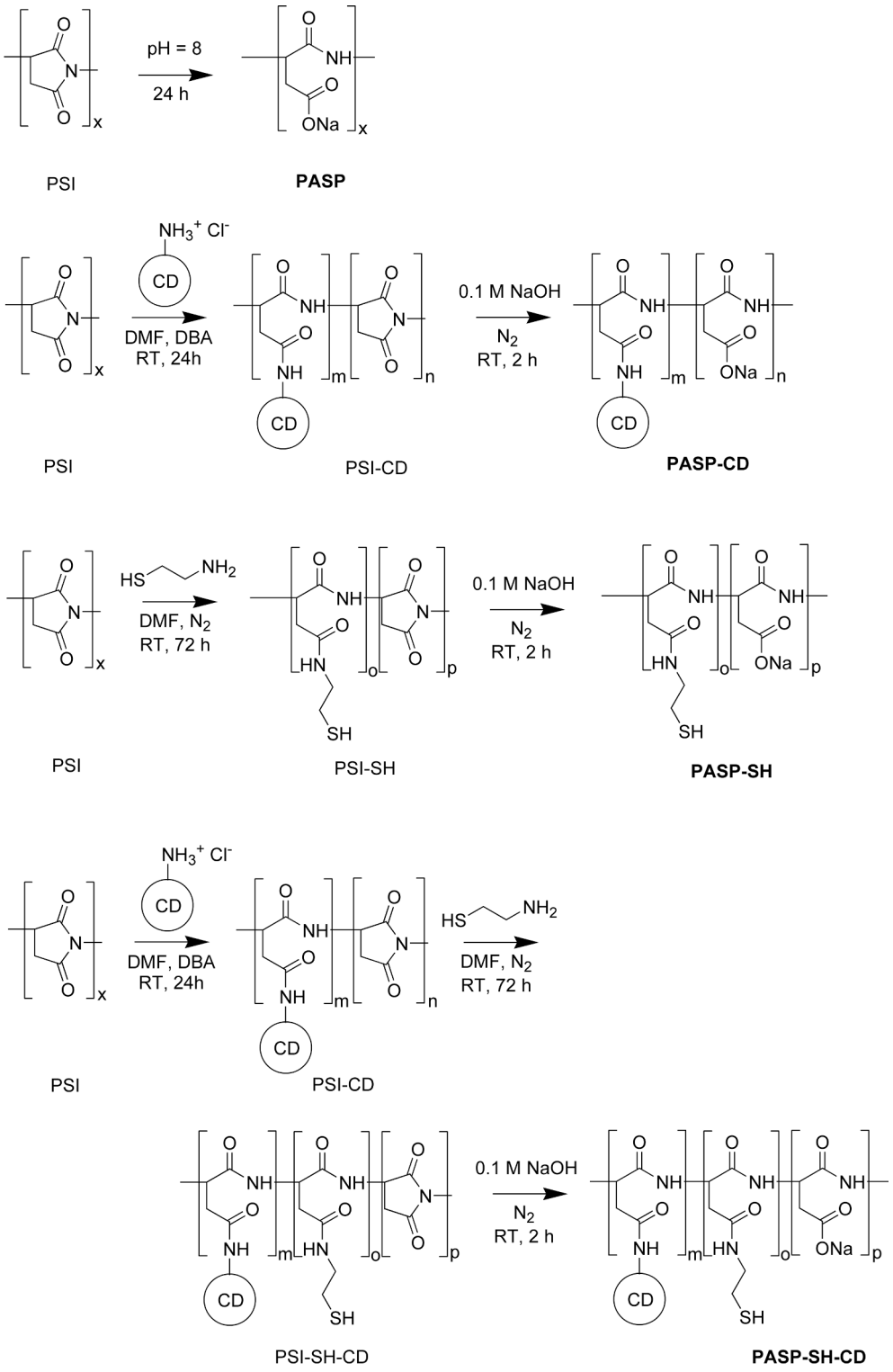


**Fig. 1.** Synthesis of PASP, PASP-CD, PASP-SH and PASP-SH-CD polymers.

stirred for 2 h to hydrolyse the unreacted succinimide rings to aspartic acid repeating units. The pH of the solution was neutralized using 1 M HCl. The solution was dialysed against water and freeze-dried to obtain the final product. $^{1}$H NMR of PASP-CD: 2.74 (2H, CH-*CH*$_2$-CONH), 3.5–4.0 (42H, O-*CH*-CH; CH-*CH*$_2$-OH; CH-*CH*$_2$-NH$_2$), 4.45, 4.65 (1H, CONH-*CH*-CH$_2$), 5.04 (7H, O-CH-O). Degree of modification was calculated to be 0.77 mol% for cyclodextrin to the total number of repeating units (Table 1).

### 2.2.3. Synthesis of thiolated poly(aspartic acid) (PASP-SH)

Poly(aspartic acid) functionalized with 10 mol% cysteamine (PASP-SH) was synthesized (Fig. 1). 1 g of PSI (10.3 mmol succinimide unit) was dissolved in 10 mL dimethylformamide and nitrogen was bubbled through the solution to remove dissolved oxygen. Then, 79.5 mg (1.03 mmol) cysteamine, and 39.7 mg (0.257 mmol) dithiothreitol was added to the mixture. The solution was stirred for 72 h under nitrogen atmosphere. The reaction mixture was poured in 150 mL of 0.1 M NaOH

solution (previously, dissolved oxygen was removed with nitrogen bubbling) and was stirred for 2 h under nitrogen atmosphere to hydrolyse the unreacted succinimide rings to aspartic acid repeating units. The pH of the solution was neutralized using 1 M HCl. The solution was dialysed against water and freeze-dried to obtain the final product. $^1$H NMR of PASP-SH: 2.62 (2H, $CH_2$-*$CH_2$*-SH), 2.73 (2H, CH-*$CH_2$*-CONH), 3.34 (2H, *$CH_2$*-$CH_2$-SH), 4.46, 4.66 (1H, CONH-*CH*-$CH_2$). The degree of modification was calculated to be 9.9 mol% for cysteamine (Table 1).

#### 2.2.4. Synthesis of cyclodextrin-modified thiolated poly(aspartic acid) (PASP-SH-CD)

Poly(aspartic acid) functionalized with 10 mol% cysteamine and 1 mol% MA-β-CD (PASP-SH-CD) was synthesized (Fig. 1). 1 g of PSI (10.3 mmol succinimide unit) and 120.6 mg (0.103 mmol) MA-β-CD hydrochloride were dissolved in 10 mL dimethylformamide and 20.0 mg (0.154 mmol) dibutylamine was added as a deprotonating agent to initiate the reaction. The solution was stirred for 24 h. Then, nitrogen was bubbled through the solution to remove dissolved oxygen, and 79.5 mg (1.03 mmol) cysteamine and 39.7 mg (0.257 mmol) dithiothreitol was added to the mixture. The solution was stirred for a further 72 h under nitrogen atmosphere. The reaction mixture was poured in 150 mL of 0.1 M NaOH solution (previously, dissolved oxygen was removed with nitrogen bubbling) and was stirred for 2 h under nitrogen atmosphere to hydrolyse the unreacted succinimide rings to aspartic acid repeating units. The pH of the solution was neutralized using 1 M HCl. The solution was dialysed against water and freeze-dried to obtain the final product. $^1$H NMR of PASP-SH-CD: 2.60 (2H, $CH_2$-*$CH_2$*-SH), 2.73 (2H, CH-*$CH_2$*-CONH), 3.14 (1H, CH-*$CH_2$*-CON), 3.33 (2H, *$CH_2$*-$CH_2$-SH), 3.5–4.0 (42H, O-*CH*-CH; CH-*$CH_2$*-OH; CH-*$CH_2$*-$NH_2$), 4.45, 4.61 (1H, CONH-*CH*-$CH_2$), 4.90 (1H, CON-*CH*-$CH_2$), 5.02 (7H, O-*CH*-O). The degree of modification was calculated to be 7.6 mol% for cysteamine and 0.83 mol% for cyclodextrin to the total number of repeating units (Table 1). Contrary to the synthesis method reported in our previous work [44], all succinimide units were successfully converted to aspartic acid repeating units making the polymer more stable due to the lack of hydrolytically labile imide rings.

### 2.3. Determination of the phase-solubility diagram

Txcess amount, 2.5 mg DXM was added to 0.5 mL aqueous solutions containing different concentrations of either MA-β-CD (0–2.5 wt%) or PASP-CD (0–15 wt%) or PASP-SH-CD (0–15 wt%). The suspensions were vortexed for 10 min, and then they were shaken for 72 h at room temperature to reach the equilibrium. Each sample was centrifuged (Jouan BR4I Multifunction Centrifuge, Thermo Scientific) at 13 500 G for 3 min and the supernatants were filtered through a 0.22 μm membrane filter. The optical absorbance spectra of the solutions were recorded and collected (Cary 60, Agilent) after sufficient dilution of the samples. As a matrix background, the corresponding MA-β-CD, PASP-CD or PASP-SH-CD solutions were used. The concentration of the DXM was calculated using a calibration curve measured on a concentration range of 6.4 – 127.4 μM (λ = 242 nm; ε = 14187 l/mol•cm; $R^2$ = 0.9966). For the phase-solubility diagram, the equivalent cyclodextrin concentration was calculated from the ratio of different repeat units of the polymer determined by $^1$H-NMR.

### 2.4. Rheological characterization of liquid and gellable formulations

Viscoelastic properties of all formulations were determined on an Anton Paar Physica MCR301 rheometer) used in oscillatory mode. The temperature was kept at 35 ± 0.1 °C using a Peltier device. Cone-plate geometry with a diameter of 25 mm (CP25–1) was used for time-dependent and frequency-dependent measurements. Each liquid formulation was first mixed with sodium bromate (c = 0.2 M), DXM (c = 1 mg/mL), and CD (the molar amount equal to that of DXM: either free or immobilized). As reference samples, DXM suspension, Maxidex formulation and suspensions containing DXM and PASP derivatives without CD units were used. The time-dependent change of storage (G') and loss modulus (G") was followed at constant strain (γ = 1%) and angular frequency (ω = 1 rad/s) over 20 min. After gelation, the frequency-dependent moduli were measured immediately at constant strain (γ = 1%) over a range of 1 to 100 rad/s recording 10 data points per decade.

### 2.5. PAMPA measurements

*In vitro* transcorneal permeability values were determined by the previously published corneal-PAMPA method (Fig. 2) [6]. Formulations of DXM and solid DXM were dissolved in PBS buffer (pH 7.4) to make solutions of 1 mg/mL nominal concentration (according to the concentration of DXM in commercially available eye drops). Then, using PBS buffer, 20-fold dilutions of these solutions and the Maxidex eye drop were made to simulate the effect of the lacrimal fluid diluting the eye drops immediately after administration [54]. All solutions were shaken for an hour at room temperature and homogenized using an Eppendorf MixMate vortex mixer for 30 s and an ultrasonic bath (Bandelin Sonorex Digiplus) for 10 min. Afterwards, the filter membranes of the donor plate (MultiscreenIP, MAIPNTR10, pore size 0.45 μm; Millipore) were coated with 5–5 μL lipid solutions (16 mg phosphatidylcholine (PC) dissolved in 600 μL solvent mixture (70% (v/v) hexane, 25% (v/v) dodecane, 5% (v/v) chloroform)). This resulted in the formation of a 10.67 w/v% lipid membrane in each well after the spontaneous evaporation of hexane and chloroform. Then, the donor plate was fit into the acceptor plate (Multiscreen Acceptor Plate, MSSACCEPT0R; Millipore) containing 300 μL of PBS solution (pH 7.4). 80–80 μLs of the undiluted solutions/Maxidex (a volume about equal to ≈2 drops, i.e., the usual prescribed dosage [55]) or 150–150 μL of the 20-fold diluted solutions were put on the membrane of the donor plate. The donor plate was covered with a sheet of wet tissue paper and a plate lid to avoid evaporation of the solvent. The plates were incubated for 4 h at 35 °C (Heidolph Titramax 1000) followed by separation of PAMPA sandwich plates. The donor solutions were filtrated (Vacuum Manifold, Millipore), and concentrations of DXM in the donor and acceptor solutions were determined by high-performance liquid chromatography with a diode-array detector (HPLC-DAD) using a calibration curve measured on a concentration range of 0.5 – 200 μM (λ = 240 nm; $R^2$ = 0.9989). The concentration of filtered donor solutions at time point zero was also determined using the same HPLC system. Test solutions from PAMPA experiments were prepared in 96-well plates and sealed before injection. For each assay, 3 replicates per compounds were measured.

The effective permeability ($P_e$, cm/s), membrane retention (*MR*) and flux (J, mol/cm$^2$ • s) of DXM were calculated using the equation (1),(2) [7] and (3) [56], respectively:

$$P_e = \frac{-2.303}{A \bullet (t - \tau_{ss})} \bullet \left(\frac{1}{1 + r_v}\right) \bullet \lg\left[-r_v + \left(\frac{1 + r_v}{1 - MR}\right) \bullet \frac{c_D(t)}{c_D(0)}\right] \quad (1)$$

$$MR = 1 - \frac{c_D(t)}{c_D(0)} - \frac{V_A c_A(t)}{V_D c_D(0)} \quad (2)$$

$$J = P_e \bullet c_D(0) \quad (3)$$

where $A$ is the filter area (0.3 cm$^2$), $V_D$ and $V_A$ are the volumes in the donor (0.15 cm$^3$) and acceptor phase (0.3 cm$^3$), $t$ is the incubation time (s), $\tau_{SS}$ is the time to reach steady-state (s), $c_D(t)$ is the concentration of the compound in the donor phase at time point $t$ (mol/cm$^3$), $c_D(0)$ is the concentration of the compound in the donor phase at time point zero (mol/cm$^3$), $c_A(t)$ is the concentration of the compound in the acceptor phase at time point $t$ (mol/cm$^3$), $r_v$ is the aqueous compartment volume ratio ($V_D/V_A$).

One-way ANOVA with Dunnett's multiple comparison test was used to determine statistically significant changes in the formulations'

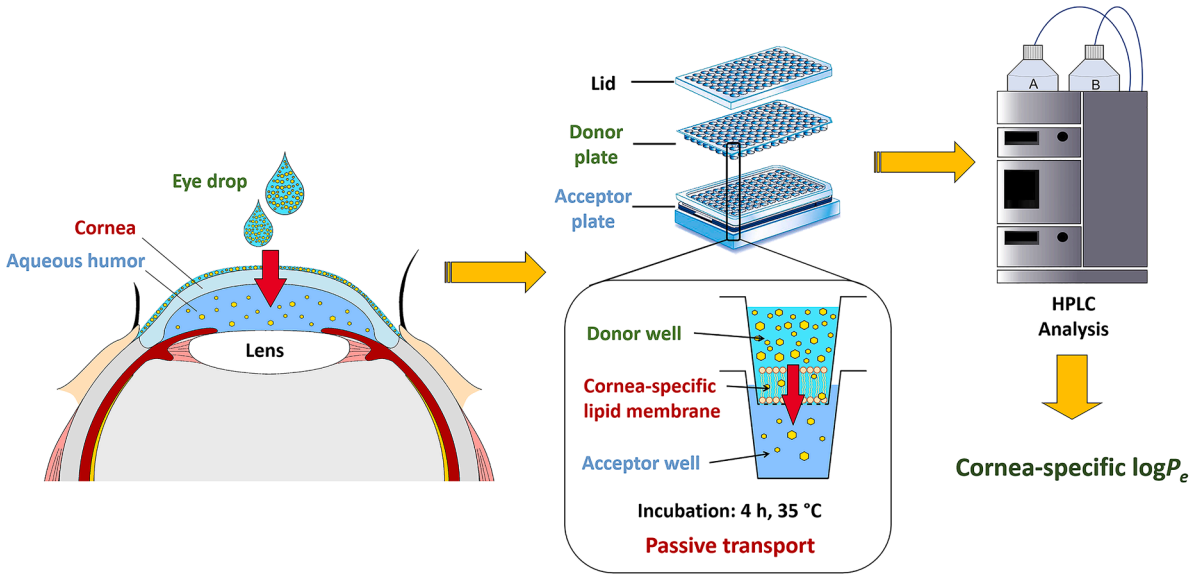


**Fig. 2.** Flowchart of *in vitro* PAMPA experiments for corneal permeability measurements.

permeability and flux values using Maxidex as control (detailed statistics are shown in Table S2-S3.).

### 2.6. Kinetic solubility measurements

1.0 mg of solid DXM and its formulations containing 1.0–1.0 mg DXM were dissolved in 1 mL PBS (nominal DXM concentration of eye drops ≈ 2550 μM). 20-fold dilutions were made with PBS, and the solutions were shaken for 4 h at 35 °C. Concentration in the solutions was determined by HPLC using the same calibration curve as in Section 2.5. One-way ANOVA with Dunnett's multiple comparison test was used to determine statistically significant changes in the formulations' solubility against the solubility of DXM in Maxidex (detailed statistics are shown in Table S1.).

### 2.7. HPLC method

Quantitative chromatographic analyses were performed using a SHIMADZU Prominence Modular HPLC system (Shimadzu Corporation, Japan) at 40 °C on a CORTECS® C18+, 90 Å, 2.7 μm (3.0 × 50 mm) column with a mobile phase flow rate of 1.0 mL/min. Composition of mobile phases: A: 0.1% (v/v) formic acid in water, B: AcN/water 95/5 (v/v) with 0.1% (v/v) of formic acid. A 5.4 min long, linear gradient program was applied: 0% B in the first 0.2 min, 0–100% B between 0.2 and 3.0 min, then 100% B was kept for another 1.1 min, and finally at 4.11 min the percentage of B was dropped to 0%. This was followed by an equilibration period of 1.3 min prior to the next injection. Chromatograms were recorded at the wavelength of 200–500 nm, integration was carried out at 240 nm. The applied injection volume was 4 μL.

## 3. Results and discussion

### 3.1. Synthesis of PASP derivatives containing CD units

The present study was devoted to synthesize PASP derivatives with CD and thiol groups and to study the physicochemical properties of their formulations as well as the potential effect of functionalities on each other. In addition to thiolated, CD-modified PASP, we also prepared PASP, thiolated PASP and CD-modified PASP as reference materials. The CD-content was chosen by considering the solubility and *in situ* gelling property of PASP derivatives. The CD-content of the liquid formulations was set to be equimolar to DXM, the latter of which was used in a concentration identical to that of a commercial eye drop, Maxidex. $^1$H NMR spectra indicated the successful completion of modification steps both for thiolation and CD immobilization with good conversion. The thiol content of PASP-SH-CD was ~ 7.6 mol% which was proven to be sufficient for aqueous *in situ* gelation in our earlier studies [32], while CD content was 0.83 mol%, thus a considerable complexation ability was expected.

### 3.2. Complex formation with dexamethasone

The solubilization of DXM by CD was characterized by the phase solubility method of Higuchi and Connors [57]. The solubility of the drug was expressed as its concentration in the homogeneous phase of the supersaturated solution and plotted against the concentration of CD units (either free or immobilized CD). The solubility isotherms are linear ($A_L$-type), indicating a favourable interaction between the host and the guest and the complete solubility of the complex within the CD concentration range investigated, while the slope values below one suggest the formation of 1:1 complexes. The immobilization of CD on a polymer chain might affect the binding stability between the CD cavity and the hydrophobic drug, as observed by Fiorica et al. [40] who obtained a significantly reduced complexation ability of immobilized CD with tamoxifen due to some steric hindrance. In contrast, Jalalvandi et al. [43] observed an increased stability constant for PEGylated PASP derivatives which was explained by a possible synergistic effect between PEG chains and CD complexes. For PASP and thiolated PASP derivatives, the complex stability was close to the one measured with free CD indicated by the similar slopes of the curves in Fig. 3. Stability constants ($K_c$) were calculated by the following equation (3):

$$K_c = \frac{a}{S_0(1-a)} \tag{3}$$

where $a$ is the slope of fitted linear to the DXM concentration – CD concentration data points, $S_0$ is the solubility of DXM in water in the absence of CD. As summarized in Table 2, the presence of PASP chains did not hinder considerably the interaction of CD cavities and DXM, neither did the thiol groups. The optimal range of stability constant for the solubilization of hydrophobic drug is 200–5000 1/M proposed by Szejtli [58] as in case of low values, solubilization is insufficient, while in case of higher values, the absorption of the drug is limited. Accordingly, water-soluble PASP formulations can be developed with immobilized CD without affecting the complexation ability of hydrophobic

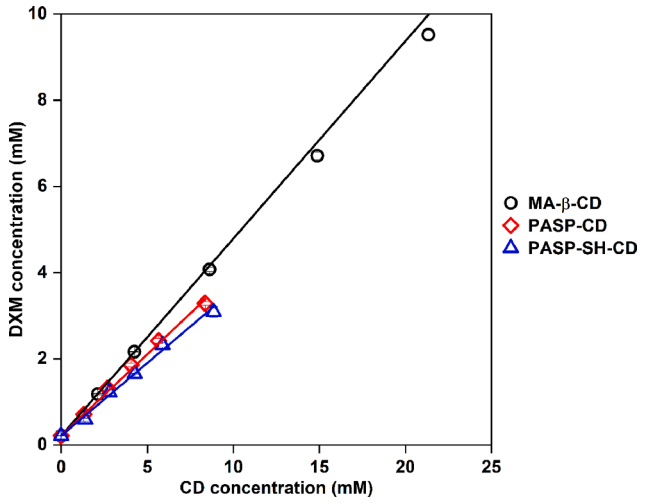


**Fig. 3.** Phase solubility diagram of dexamethasone (DXM) in different formulations. The slopes of the lines are similar for all formulations. The mean and standard deviation of each point are calculated from three parallel measurements.

**Table 2**
Slope (a) values and stability constants ($K_c$) for phase solubility data.

| Sample | a | $K_c$ (1/M) |
|---|---|---|
| MA-β-CD | 0.4579 | 3946 |
| PASP-CD | 0.3786 | 2846 |
| PASP-SH-CD | 0.3380 | 2385 |

drugs as compared to free CD, and in this way, the additional functionalities of PASP can be exploited including *in situ* gelation and bioadhesion.

### 3.3. Rheological characterization of the formulations

The efficacy of ocular treatment is strongly limited by the protective mechanisms of the eye. *In situ* gelation can increase adhesion and prolong residence time of the formulation, thus improving the bioavailability. Thiolated polymers can be converted into hydrogels by adding oxidants, as shown in our previous works [28,32]. The presence of CD units might affect *in situ* gelation, thus viscoelastic properties of all formulations were characterized by oscillatory rheology. Results of time-dependent rheological measurements are shown in the Supporting information (Figures S1). Dynamic moduli of all formulation without thiol groups is below 1 Pa and remained constant after the addition of oxidant as it was expected. The low values of storage modulus (G') indicated the liquid character of these formulations, which results in their fast elimination by tearing and blinking during topical application on the eye surface. Both thiolated PASP (PASP-SH) and CD-modified, thiolated PASP (PASP-SH-CD) displayed *in situ* gelation and the storage modulus increased more than 1000-fold after the addition of oxidant, suggesting the formation of a viscous liquid, then a gel structure. The thiolated formulations had an almost frequency-independent behaviour indicating the presence of a stiff gel structure which might ensure the prolonged adhesion of the semi-solid formulation on the eye surface. The rest of the formulations, including Maxidex, showed a frequency-dependent, liquid-like behaviour (Figure S2).

There is also a remarkable difference in the complex viscosity (Fig. 4) of the formulations with and without thiol groups after the addition of oxidant. The viscosity of all liquid formulations without thiol groups is between $10^{-3}$ and $10^{-1}$ Pa•s which is comparable to that of water. The complex viscosity of the formulations based on thiolated PASP derivatives reached 1000 Pa•s at 1 rad/s and 10 Pa•s at 100 rad/s which

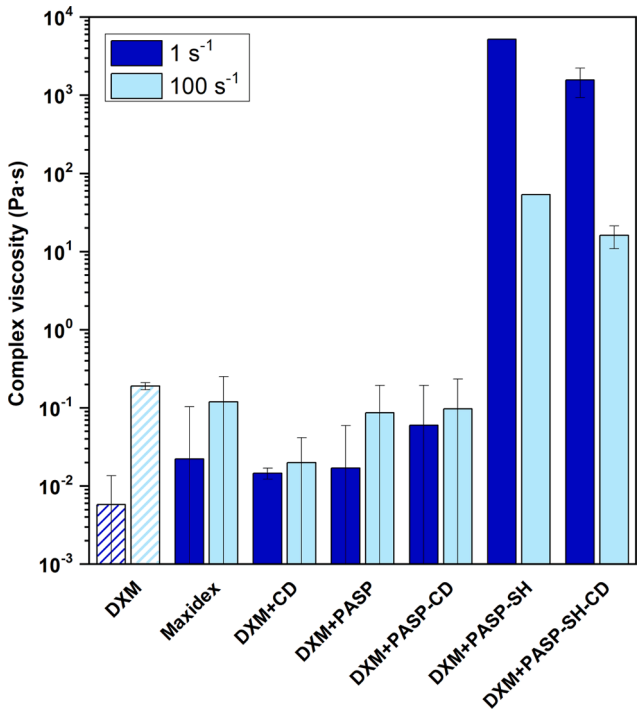


**Fig. 4.** Complex viscosity of formulations at an angular frequency of 1 and 100 rad/s. The mean and standard deviation of each point are calculated from three parallel measurements.

can help to extend the residence time on the mucosal surface. The viscoelastic properties of PASP-SH and PASP-SH-CD were similar in both time-dependent and frequency-dependent measurements, thus *in situ* gelation property via disulfide formation was not affected either by the presence of CD units, or the complexation of hydrophobic DXM. Accordingly, the dual functionality of PASP-SH-CD can be independently exploited in liquid formulations.

### 3.4. Kinetic solubility of dexamethasone

The kinetic solubility of drug substances in PBS is generally determined during the preformulation stage. As can be seen in Fig. 5, the solubility of DXM in undiluted Maxidex eye drop is more than 2 times higher than in the saturated solution of the pure API. In the cases, where CD is present in the formulations, 11 to 15-fold increase in the solubility

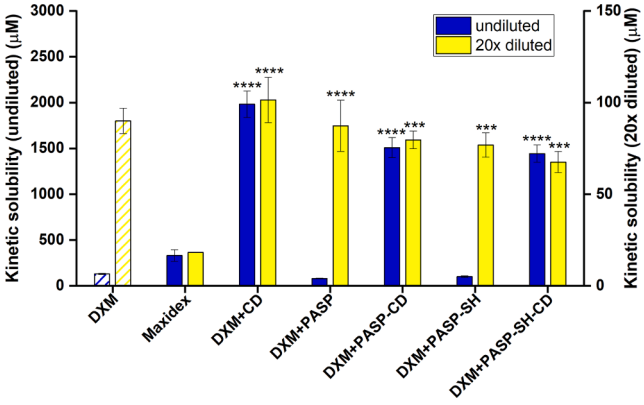


**Fig. 5.** Kinetic solubility of undiluted and 20-fold diluted solutions of DXM and its formulations (after 4 h, T = 35 °C). (Asterisks indicate the significance of changes in kinetic solubility values of formulations against the solubility of DXM in Maxidex based on p-values of Dunnett's multiple comparison test. *: p ≤ 0.05; **: p ≤ 0.01; ***: p ≤ 0.001; ****: p ≤ 0.0001).

for the undiluted forms can be observed due to the solubilizing effect of CD. The presence of PASP and its thiolated form, PASP-SH, does not improve the solubility of DXM. However, as the formulations are diluted 20-fold mimicking the diluting effect of lachrymal fluid, the solubility of CD-containing formulations is not changing upon dilution, since most likely, the whole amount of DXM is already solubilized by CD. In parallel, in the case of PASP-formulations, where CD is not present, the solubility is practically similar for the pure API (DXM) and CD-containing formulations, which finding suggests diluted system approaches the intrinsic (thermodynamic) solubility of DXM. In the case of Maxidex, the effect of the eye drop matrix seems to be diminished, resulting in less dissolved DXM. As the CD-containing formulations can be prepared as clear solutions, unlike Maxidex, they are more desirable for topical treatment of the eye since no solid particle is present to cause irritation, and no additional time is needed for the particles to dissolve.

### 3.5. Prediction of the corneal permeability and the flux of dexamethasone

Prediction of corneal permeability is of great importance in the development of a topical ophthalmic formulation. Corneal-PAMPA, an *in vitro* non-cell-based screening method, was used in this study to predict corneal permeability. The results of the corneal-PAMPA measurements show that the permeability values ($P_e$) of DXM in the case of the undiluted formulations are significantly higher for DXM + PASP and DXM + PASP-SH than that of the undiluted reference eye drop, Maxidex (Fig. 6a). On the other hand, the permeability of the formulations containing CD is significantly lower compared to Maxidex. This can be explained by the formation of DXM + CD complexes on the donor side of the membrane. Complexation significantly increases the solubility of DXM but at the same time reduces its permeation. This phenomenon has been described and discussed in detail by Loftsson's group [59,60]. For MA-β-CD the PAMPA membrane was not permeable. However, if the flux values of Maxidex and CD-containing formulations are compared (Fig. 6b), we can see that the solubility enhancing effect of CD compensates the decrease, their flux values are about the same as the eye drop's, or in the case of DXM + CD even a significant increase of flux could be measured. Quite different results can be observed in the case of the 20-fold diluted formulations. Here, DXM + PASP and DXM + PASP-CD show significantly higher permeability than Maxidex, while the flux values are significantly increased in the case of all five formulations, with the highest flux in the case of DXM + PASP-CD. In the applied PAMPA method we could not test the *in situ* gel formation of PASP-SH and PASP-SH-CD polymers, the increased adhesion and prolonged residence time of the formulation may further improve the amount of drug absorbed.

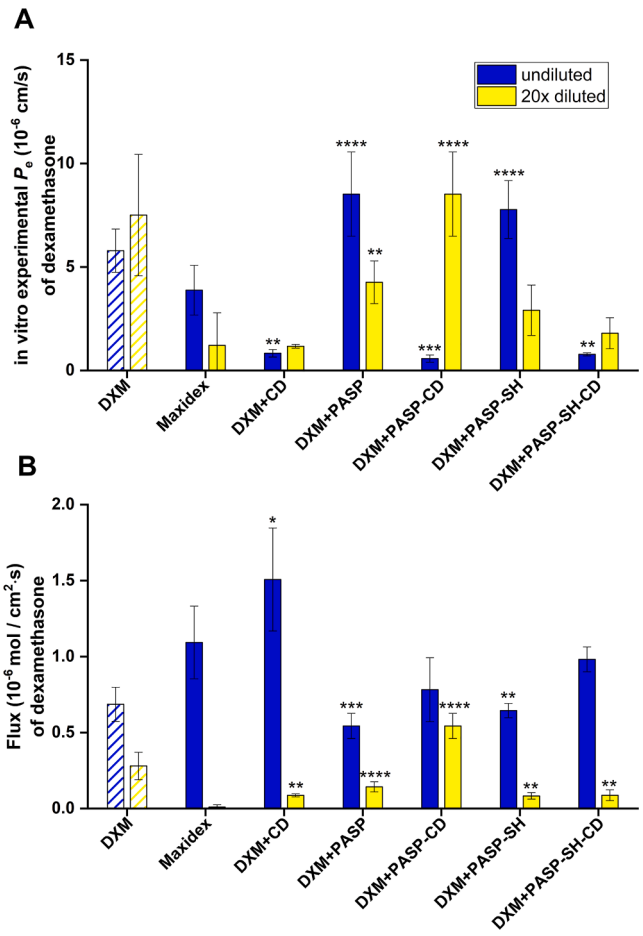


**Fig. 6.** Permeability (A) and flux (B) values of DXM from its undiluted and 20-fold diluted solutions in the case of different formulations. (Asterisks indicate the significance of changes in permeability and flux values of formulations compared to Maxidex based on p-values of Dunnett's multiple comparison test. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$).

## 4. Conclusions

New CD-modified, thiolated PASP derivatives were successfully synthesized in a facile way without the use of coupling or activating agent during polymer modification. These derivatives preserved both the complexation ability of CD towards the hydrophobic drug, DXM and the *in situ* gelation property of thiolated polymers in the presence of an oxidant. Phase solubility studies gave stability constants of PASP derivatives very close to that of the free CD. In addition, the aqueous solution of the thiolated derivatives could be converted to stiff hydrogels within a few minutes. The storage modulus and complex viscosity displayed a more than 1000-fold increase during gelation suggesting the possible increase of residence time on mucosal surfaces. Kinetic solubility studies proved the solubilizing effect of the immobilized CD units. Corneal-PAMPA measurements showed different results for different concentrations indicating the importance of dilution during topical administration on the eye surface. In the case of undiluted samples the permeability for formulations containing CD was lower than the reference eye drop because of the complex formation of CD with DXM, while PASP and thiolated PASP significantly increased permeability. Nevertheless, the flux value of DXM in the case of the DXM + CD formulation was higher than that of Maxidex due to the compensating effect of solubility. Dilution of formulations 20-fold simulating the diluting effect of tear film completely changed the order. Non-thiolated PASP-CD caused the highest permeability and flux value. In conclusion, PASP derivatives were found to be promising excipients for the development of eye drops, but several factors must be considered to achieve optimal viscosity increase or *in situ* gel formation for longer residence time, enhanced permeability, and improved bioavailability.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2022.03.008.

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