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Investigations of Surface Interactions between PEGDA-Polymer and Drug using Surface Plasmon Resonance

Abstract: For the development of combination products, the determination of the release kinetics of polymer-based drug delivery systems (DDS) is a central and often time-consuming investigation. Classical methods are often unsuitable for large-scale screening of potential polymers for combination products. We present a rapid method based on surface plasmon resonance spectroscopy (SPR), using PEGDA₇₀₀ (Poly(ethylene glycol) diacrylate, average molecular weight (M_n) 700) as an example. This method is capable of determining the kinetics of drug association and subsequent release within minutes. The proportion of desorption and diffusion can be determined separately.

Surface plasmon resonance spectroscopy (SPR) is a label-free and very sensitive optical technique, which allows for real-time observation of surface interactions. The prepared SPR chips were spin-coated with PEGDA₇₀₀ and crosslinked via photoinduced polymerization.

The association and dissociation kinetics of dexamethasone phosphate in swollen PEGDA₇₀₀ have been studied at different concentrations. The maximum loading of the surface was also obtained by this method.

The study of PEGDA presented here identified the well-established SPR-based spectroscopy as a potential tool in the development of combination products.

Keywords: SPR, PEGDA, Surface interaction, Association, Dissociation.

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1 Introduction

For the development of combination products, the determination of the release kinetics of polymer-based drug delivery systems (DDS) is a central and often time-consuming investigation.

One option to achieve a faster release and reduce the experiment length is to increase temperature. However, this creates unrealistic conditions, often rendering these methods unsuitable for large-scale screening of potential polymers for combination products.

In order to determine the kinetics of surface interactions, optical methods have been used for decades, which allow label-free and real-time determination. Surface plasmon resonance (SPR) spectroscopy is a well-established method that measures small changes in film thickness based on refractive index. It is often used to study antigen-antibody, ligand-receptor or protein-DNA interactions.

Therefore, one substance is immobilised on the surface as a ligand and the other is flowed over the surface as an analyte. Due to the high sensitivity, kinetic rate [1], equilibrium constants [2] and the maximum loading can be determined with only small amounts of analyte.

We present a rapid method based on SPR spectroscopy using as an example PEGDA₇₀₀. This method is suitable for the determination of the kinetics of drug association and subsequent release within minutes. The SPR screening method can accommodate variable loading conditions, concentrations and drug types. The proportion of desorption and diffusion can be determined separately.

2 Material and methods

2.1 Chemicals

1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), Allylamine, 2-Hydroxy-4'-(2-

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hydroxyethoxy)-2-methylpropiophenone (IRGACURE) and PEGDA₇₀₀ (Poly(ethylene glycol) diacrylate, average Mn=700) are purchased from Sigma-Aldrich (Merck, Germany). Dexamethasone phosphate was purchased from Dr. Mann Pharma (Berlin, Germany). Dimethylsulfoxide, >99.8% was purchased from Carl Roth, Germany.

2.2 Sample preparation

An SPR chip coated with carboxymethyl dextran hydrogel was used. (SPR-Sensorchip CMD 500D, XanTec bioanalytics GmbH, Duesseldorf, Germany). The freshly opened SPR chip was used directly and was immersed in 2 mL of an aqueous solution containing 75 mmol/L EDC and 32.5 mmol/L NHS for 2.5 h to activate the carboxyl groups on the surface.

The resulting surfaces were rinsed with ultra-pure water and air-dried before immersed in a solution with 10% allylamine in water (v/v) for 1.5 h. Afterwards the SPR chip was rinsed again with ultra-pure water, air-dried and spin-coated with a 75 μ L of a 0.5% PEGDA₇₀₀ solution (KLM spin coater, SCE). For this, a solution of 10 mg IRGACURE in 20 μ L dimethyl sulfoxide was dissolved in 0.48 mL water, mixed with 0.5 mL PEGDA₇₀₀ and diluted with water to a concentration of 0.5%.

First, the speed was increased to 50 rps over 60 s and held for 5 s. Then the speed was increased to 200 rps over 15 s and held for another 5 s. Finally, the SPR chip was dried at 40 °C at 10 mbar for 1 h and photopolymerised under UV for 1 h.

2.3 Contact angle measurement

The surface hydrophilicity of the coatings on the SPR chip was analysed by contact angle measurement of ultra-pure water sessile drop (2 μ L) on different positions of the SPR chip using a contact angle system (OCA 20, Dataphysics Instruments GmbH, Filderstadt, Germany). Mean values and standard deviation of the contact angles were calculated (n = 10 duplicates).

2.4 SPR measurement

The sensorgrams were monitored using an SPR Biosensor SR7500DC (Xantec, Duesseldorf, Germany) while rinsing the surface with ultra-pure water at a flow rate of 25 μ L/min. The sample volume was 38 μ L and the injection was carried out over 1.5 min so that equilibrium could be reached. The

dissociation phase was 15 min long so that the surface could re-calibrate and no further washing procedure was necessary.

The measured changes in surface density were subjected to kinetic modelling (TraceDrawer v. 1.6) to assess the association (k_{ass}) and dissociation (k_{diss}) kinetic constants and the B_{max} value, which indicates the maximum loading of the surface. The fitting was done with the one-to-one (2-states) model [3] (see eq 1). In this model, the adduct AB is formed after an initial association. In a further reaction step, AB* is formed. The subsequent dissociation can only take place after the back reaction of AB* to AB.

3 Results

3.1 Contact angle

At each step of the surface modification, the contact angle was measured to observe the progress. (Table 1). Due to the hydrogel coating, no contact angle could be determined on the freshly opened SPR chip. Activation with EDC/NHS made the surface more hydrophobic. The expected increase of the contact angle by allylamine treatment could only be observed after 90 min.

Table 1: Change of the contact angle during the process of the surface modification (n=10).

Treatment	Time	Contact Angle $\pm$ SD [°]
Freshly opened SPR chip	---	Not measurable
EDC/NHS	60 min	23.0 $\pm$ 4.2
	120 min	41.4 $\pm$ 3.8
	150 min	57.7 $\pm$ 5.2
Allylamin	45 min	57.6 $\pm$ 2.2
	90 min	79.1 $\pm$ 3.45
PEGDA (polymerized)	---	64.3 $\pm$ 2.4

3.2 SPR Spectroscopy

After obtaining a stable baseline, samples of dexamethasone phosphate in ultra-pure water were measured at different concentrations.

Selected sensorgrams are shown in Figure 1. The model one-to-one (2-states) was fitted globally to all courses. The parameters are listed in Table 2. Compared to related systems association and dissociation of the first step (k_{ass1} and k_{diss1}) are in similar range, while k_{diss2} with $4.81 \cdot 10^{-9} \text{ s}^{-1}$ is quite low [4,5].

Figure 1: Sensorgram with fitted model (one-to-one, 2-states).

Table 2: Parameter of the model fit (model: one-to-one, 2-states)

Parameter	Value
B_{max} (uRIU) ⁺	72.14
k_{ass1} ($\text{M}^{-1} \text{ s}^{-1}$)	$1.65 \cdot 10^4$
k_{diss1} (s^{-1})	$6.46 \cdot 10^{-2}$
k_{ass2} (s^{-1})	$6.97 \cdot 10^{-3}$
k_{diss2} (s^{-1})	$4.81 \cdot 10^{-9}$
χ^2 (uRIU) ²	3.96

⁺1uRIU=1 ng/mm²; B_{max} =maximum load of the surface
² χ^2 =value for accuracy of the fitting.

The χ^2 gives a measure of the accuracy of the fitting. It is quite low, indicating a good fit, and supports the validity of the chosen model.

Comparative measurements with an unmodified dextran SPR chips show a B_{max} value of 162.5 uRIU. Comparison to a PEGDA₇₀₀ modified SPR chip showed that there was no influence from the dextran layer ($B_{\text{max}} = 72.14 \text{ uRIU}$).

4 Discussion and Conclusion

The procedure developed enables PEGDA to be covalently bound in a sufficiently thin layer on an SPR chip by polymerisation. By monitoring the contact angle on a regular basis, the progress of the modification could be easily followed.

The values of the association and dissociation constants (k_{ass1} , k_{ass2} , k_{diss1} , k_{diss2}) show that after the association of the dexamethasone phosphate on the PEGDA surface there is another process. This is probably the diffusion into the bulk. The low value of k_{diss2} is the reason for the slow decrease of the signal and shows that the dissociation of the substance is limited by the diffusion within the layer. Furthermore, B_{max} was determined to be 72.14 uRIU. This corresponds to an approximate maximum loading on the SPR chip surface of 72 ng/mm².

Taken together these data support the use of SPR spectroscopy to investigate near surface processes that occur during drug release.

Author Statement

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