Effect of pH and ionic strength of chitosan/casein and casein/chitosan multilayers on curcumin release

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Abstract. In the present paper the effect of pH and ionic strength on the immobilization and release of curcumin from chitosan and casein polyelectrolyte multilayers (PEMs) was investigated. The investigated PEMs were deposited on polylactic acid (PDLA) substrates. The PLA substrates were charged in a corona discharge system, consisting of a corona electrode, a grounded plate electrode and a grid placed between them. The substrate was charged for 1 minute at room temperature. Positive or negative 5kV voltage was applied to the corona electrode and 1 kV voltage with the same polarity was applied to the grid. Chitosan solutions with different pH and ionic strength were prepared. Layer-by-Layer (LbL) deposition technique was used for the multilayer build-up. For the deposition process was ensured that the first deposited layer always possessed an opposite electric charge to that of the substrate. An investigation of the water uptake properties of the deposited PEM multilayers was carried out. Curcumin was immobilized in the resulting casein layers. The release of the immobilized curcumin from the multilayers in saline buffer was investigated and the effects of the different pH and ionic strengths of the chitosan solutions were determined.

1. Introduction

The development of reliable methods for controlled drug delivery has been one of the cornerstones of modern medical research. One of the more popular methods for the creation of such delivery systems is the creation of multilayer polyelectrolyte structures by the use of Layer-by-Layer (LbL) self-assembly techniques. This method provides an easy and reliable way of immobilization of charged objects in aqueous solution on any surface [1]. Additionally it is very suitable for the creation of polyelectrolyte multilayer films, based on biodegradable polymer films.

One example of a polymer, suitable for the creation of multilayer drug delivery systems, is polylactic acid (PLA). When charged under corona discharge the surface of the PLA films undergo both chemical and physical modifications [2], which makes them suitable for the creation of multilayer structures [3]. This material is also very well suited for the creation of drug delivery systems and other medical applications as it offers a way of creating a completely biodegradable system, when used for the creation of multilayer films. Examples of polymers that can be used in the formation of multilayer structures are two abundant in nature substances – chitosan and casein.

Chitosan is one of the most popular choices for the creation of multilayer structures, not only for its antimicrobial and antibacterial properties [4, 5], but also for its ability to form stable complexes and structures at different levels of pH and ionic strength [6, 7]. Its relative abundance in nature and the potential for strong interactions with other biological substances makes it perfectly suited for the creation of biodegradable materials.
The ability of casein to form stable structures at both high and low pH levels has been shown in several studies [8, 9]. Structurally casein micelles formed at high and low levels of pH show different levels of compactness [10], which can influence any immobilization attempts. The suitability of casein for the creation of delivery systems for hydrophobic nutraceuticals, such as curcumin, has also been demonstrated [11]. This is important as one study demonstrates the effect of pH on curcumin encapsulation in whey protein nanogel particles [12]. In fact, casein is capable of forming stable structures with chitosan at varying levels of ionic strength and pH [13]. In addition, the ability of the casein/chitosan complexes to enhance the proliferation and differentiation of human stem cells [14] demonstrates its suitability for biomedical applications.

In our study, we are aiming to investigate the effects of different levels of pH and ionic strengths of the polyelectrolyte solutions on the immobilization and release of a model drug (curcumin) from a multilayer structure consisting of two different polyelectrolytes.

2. Materials and methods

2.1. Materials
Polylactic acid, chitosan (high molar mass, degree of deacetylation > 75%), casein sodium salt from bovine milk and curcumin were purchased from Sigma - Aldrich and were used without further purification. All other used chemicals were with analytical grade.

2.2. Methods

2.2.1. Preparation of casein/chitosan multilayers loaded with curcumin
Polylactic acid (PDLA) was chosen as substrate material. PDLA film with thickness approximately 40 µm was casted from 2% w/v chloroform solution and then dried at room temperature until reaching constant mass. The film was kept in desiccator at room temperature and relative humidity (RH) of 54%. Just before the LbL deposition, the substrates were charged in positive or negative corona (±5 kV corona electrode voltage and ±1 kV grid voltage) for 1 minute using a corona discharge system at T = 21÷23°C and RH = 40-60%.

Casein/chitosan polyelectrolyte multilayers (PEMs) were formulated by alternative dipping of the precharged substrate into 1% casein and 1% curcumin solution with pH 8.5 and 1% chitosan solutions with different pH – 3.0, 4.0 and 5.0. The ionic strength for both solutions was also varied from 10 mM to 1000 mM. The deposition always started with the polyelectrolyte, charged oppositely to the substrate. Each deposition step was followed by rinsing in distilled water. The casein/chitosan PEMs consisted of 8 layers. This method is a standard method, used for the creation of polyelectrolyte multilayers.

2.2.2. PEMs characterization
Atomic force microscopy (AFM) was used to investigate the PEMs surface morphology. The measurements were performed on AFM NANOSURF FLEX AFM (SWITZERLAND), in tapping mode with standard cantilever Tap190Al-G with 10 nm tip radius. The viewing field consisted of 256×256 pixels, revealing the morphology of 5 µm x 5 µm area from the sample surface. The line scan time was 1 s. Based on AFM images, the root mean square roughness $S_q$ was calculated.

The water uptake test was carried out to assess the physical stability of the PEMs at different humid conditions and to evaluate the amount of deposited polyelectrolytes. The PEMs (3 samples of each type) were placed into a desiccator with dry air at a temperature of 25°C. The samples were left to equilibrate for 72 h and then their mass was measured. After that, the samples were placed in desiccators with different relative humidity and again equilibrated for 72 h. Then their mass was again measured. The water uptake was calculated as the relative increase in the mass.

2.2.3. Yield of the preparative process
The loading of the PEMs was determined as follows: curcumin loaded PEMs (3 samples of each type) were placed into 20 ml phosphate buffer saline (pH 7.4) and stirred continuously for 72 hours on a magnetic stirrer at 37°C. Then, the samples were sonicated for 5 minutes and filtered using Chromafil® syringe filter (0.45 µm). The amount of curcumin was determined using UV/Vis spectrophotometer (Metertech SP-8001 UV/Visible Spectrophotometer, China), monitoring the band at $\lambda_{\text{max}} = 427$ nm. The drug concentration was calculated from a standard calibration curve of curcumin in phosphate buffer saline (pH 7.4).

The release study was performed using the stirred-beaker method. Each curcumin loaded PEMs (3 sample of each type) was put into a beaker containing 20 ml dissolution media (saline, pH 7.4). The temperature was maintained at 37±1°C and the rotation speed was kept at 50 rpm throughout the experiment. 3 ml of the saline solution, in which the PEMs were immersed, were collected at a specific time intervals. The liquid was analyzed spectrophotometrically at $\lambda_{\text{max}} = 427$ nm. The drug concentration was calculated from a standard calibration curve of curcumin in phosphate buffer saline (pH 7.4). The amount of 3 ml of saline solution was added to the rest of the test solution.

3. Results and discussion

3.1. AFM and water uptake measurements

The surface morphology of PEMs was investigated by atomic force microscopy. In figures 1-3 the surface morphology of PEMs obtained on positively charged PDLA substrates (a) or on negatively charged substrates (b) at different pH (3, 4, 5) and ionic strength 100 mM are presented.

![Figure 1](image1.png)

**Figure 1.** Surface morphology of PEMs obtained at pH 3 and ionic strength 100 mM

![Figure 2](image2.png)

**Figure 2.** Surface morphology of PEMs obtained at pH 4 and ionic strength 100 mM
The root mean square roughness of all investigated PEMs was calculated. The values of the root mean square roughness for type of PEMs are presented in table 1.

Table 1. Values of the root mean square roughness for all investigated PEMs

| Type of PEMs            | \( S_q \), nm | positive | negative |
|-------------------------|----------------|----------|----------|
| pH 3, 100 mM            | 1.68           | 1.36     |
| pH 4, 100 mM            | 1.81           | 1.75     |
| pH 5, 100 mM            | 2.12           | 2.06     |

The results presented in figures 1-3 and table 1 show that:

• The values of root mean square roughness of PEMs obtained on positively charged PDLA substrates are higher than those obtained on negatively charged PDLA substrates. This can be explained by a large amount of charge captured in PDLA substrate during the positive corona.

• The values of root mean square roughness increase with increasing of pH of the starting solution of chitosan and casein.

The results from the investigation of the water uptake of different configurations of PEMs (figures 4-7) demonstrate that the increase of both the pH and ionic strength of the solutions, used for the creation of the PEMs, leads to a decrease in the amount of water absorbed by the layers.
3.2. Curcumin loading and release at different pH and ionic strengths

The amount of curcumin immobilized into the PEMs films at different configurations of pH and ionic strength, for both positively and negatively charged substrates, is presented in table 2.

| Type of PEMs | Amount, µg | positive | negative |
|--------------|------------|----------|----------|
| pH 3, 100 mM | 0.391      | 0.862    |
| pH 4, 100 mM | 1.481      | 1.239    |
| pH 5, 10 mM  | 2.234      | 1.868    |
| pH 5, 100 mM | 0.983      | 0.537    |
| pH 5, 1000 mM| 0.692      | 0.850    |

The results demonstrate that the highest amount of curcumin, loaded into the PEMs is at pH 5 and ionic strength of 10 mM, with the second highest being at pH 4 and 100 mM. Decreasing the pH level to 3 and increasing the ionic strength of the solution above 100 mM at lower levels of acidity results in a smaller amount of curcumin loaded in the multilayers. This correlates to the changes in the structure of the PEMs in different conditions, which will be demonstrated further in the results.

The rate of release from PEMs, assembled at different pH and ionic strength levels, is represented in figures 8 - 11.
For all investigated configurations the rate of release remained gradual, with up to 40% of the incorporated drug being released in the initial 60 min., and up to 90% after 360 min.

The slowest rate of release was observed at ionic strength 10 mM, which can be explained by the lower roughness and the increased density of the PEM structure. An increase of the ionic strength levels results in a looser structure, which results in an increase of the release rate of the incorporated curcumin (figures 10 and 11).

Changes in the pH levels can also impact the release rate, with pH 4 having the slowest rate, making it optimal for drug retention. Any increase or decrease of the acidity results in a decrease of the rate of release. Positively charged films demonstrate a higher reduction in drug retention with a decrease of the pH level and negatively charged films demonstrating the opposite tendency (figures 8 and 9).

The release behavior of curcumin strongly correlates with the Korsmeyer-Peppas model:

$$\frac{c_t}{c_\infty} = k t^n,$$

where $c_t/c_\infty$ is the fraction of the drug release at time $t$, $k$ is the rate constant and $n$ is the release exponent. The Korsmeyer-Peppas model was chosen over other drug release models (Higuchi, 1st order kinetics) as it has the highest coefficient of determination ($R^2 > 96\%$) and a release exponent
smaller than 0.5 for all PEMs configurations. This, combined with the lack of an initial burst release, contributes to the idea that the release process is governed by diffusion, rather than dissolution [15]. Overall, the results show that the decrease of pH and ionic strength values creates a denser structure, which allows a higher level of retention of the loaded drug. In addition, the values of the loading degree demonstrate that the drug loading possesses a higher sensitivity towards pH, as a small decrease in its value can cause a big reduction in the amount of loaded drug.

4. Conclusion
In this paper, the effect of pH and ionic strength of PEMs on curcumin release was investigated. It was established that, for all investigated configurations, the rate of release remained gradual, with up to 40% of the incorporated drug being released in the initial 60 min., and up to 90% after 360 min. The slowest rate of release was observed at ionic strength 10 mM. An increase of the ionic strength levels results in a looser structure, which results in an increase of the release rate of the incorporated curcumin. Overall, the results show that the decrease of pH and ionic strength values creates a denser structure, which allows a higher level of retention of the loaded drug.

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