engineering proceedings

MDPI

Proceeding Paper

Tafamidis drug delivery systems based on chitosan/polyvinyl alcohol matrix †

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Abstract: Cardiovascular diseases still keep the leading position of death globally, and according to the World Health Organization claims equal to 17.9 million cases each year. Cardiac amyloidosis caused by the formation and deposition in the myocardium of a specific protein-polysaccharide complex–amyloid is representing the main reason of death. The clinically used pharmaceutical molecules against amyloidosis are very limited: currently there are only two non-selective hydrophobic agents– diflunisal and tafamidis. In addition to the non-selective mode of action for both drugs, tafamidis, instead of its more therapeutic efficacy, is the most expensive: the yearly course costs appr. 225'000 $. One of the possible ways for the enhancing of the solubility and bioavailability, decreasing the dosage with simultaneous targeted effect is the encapsulation of the drug into polymer (biopolymer) matrixes. In contrast to the known diflunisal delivery systems, there are no any available data about the development tafamidis delivery systems for tafamidis. In this study we report for the first time the encapsulation method of tafamidis into a polymeric matrix based on the mixture of chitosan and polyvinyl alcohol (PVA). The release profile from the polymer matrix was analyzed, and no burst character was demonstrated. The obtained tafamidis-loaded polymer matrixes based on biosafe and biocompatible polymers require further investigations in vitro and in vivo to evaluate their potential for clinical application.

Keywords: Cardiac amyloidosis; Chitosan; Drug delivery system; PVA; Tafamidis

1. Introduction

Amyloidosis is systemic disease caused by the deposition of the amyloid fibril aggregates at various organs and tissues [1]. One of the dangerous forms is the cardiac amyloidosis, when the amyloids deposit in the myocardium, resulting in the death associated with cardiomyopathy [2].

Current therapy strategy, unfortunately, do not provide the prevention of the amyloidosis, and only delay the following fibrils deposition. Moreover, current therapeutics have a lot of side effects including cardiotoxicity, which negatively effects on the patient lifestyle [3]. Amyloidosis may be caused not only by transthyretin (ATTR) or immunoglobulin light chain (AL) fibrils [4], but also may be potentially associated with the deletion in the gene of giant protein titin (TTN) [5], which drastically hinder the therapy against amyloidosis.

It is well known, that one of the suitable ways to increase the solubility, bioavailability, and therapeutic efficacy is the drug encapsulation into polymer (biopolymer) systems. This process was entitled "loading" of drugs into polymer matrix. In our previous studies

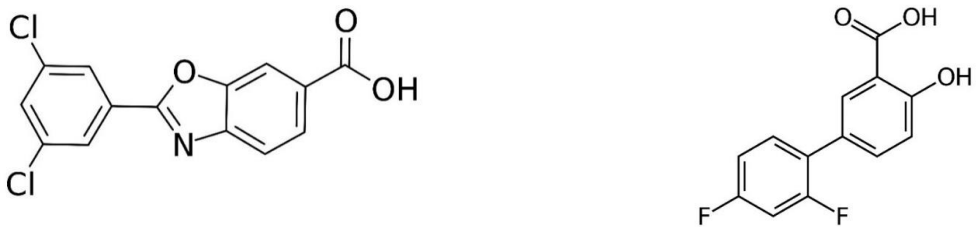
Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

we successfully loaded various natural biologically active agents into biopolymer matrixes without any chemical linkers and initiators, e.g., curcumin and usnic acid into hyaluronic acid [6,7], and mangiferin into biopolymer carrier based on high molecular chitosan and polyvinyl alcohol (PVA) [8]. Above-mentioned studies became the basis for the investigation of the polymer matrix capacity as the carrier for biologically active molecules of different chemical nature.

Tafamidis (Figure 1a) and diflunisal (Figure 1b) are only two known small molecules which represent the main therapeutic agents used to delay the amyloid deposition. These molecules have the hydrophobic nature and non-selective mode of action, which results in the low solubility, high doses and insufficient therapeutic efficacy. In addition to the non-selective mode of action for both drugs, tafamidis, instead of its more therapeutic efficacy, is the most expensive: the yearly course costs appr. 225′000 $ [9,10]. The obtained tafamidis-loaded polymer matrixes based on biosafe and biocompatible polymers require further investigations in vitro and in vivo to evaluate their potential for clinical application.



(a) tafamidis (b) diflunisal

Figure 1. Chemical structures of tafamidis (a) and diflunisal (b).

Diflunisal, as a non-steroidal anti-inflammatory drug, initially widely used as an effective medication for the treatment of rheumatoid arthritis, has possesses of effect against transthyretin (TTR) polyneuropathy.

Tafamidis, is known also as the brands Vyndaqel and Vyndamax [4], delays transthyretin cardiac amyloidosis (for both familial amyloid cardiomyopathy and familial amyloid polyneuropathy, as well as wild-type transthyretin amyloidosis, which was called senile systemic amyloidosis). The main mechanism of its action is the quaternary structure transthyretin stabilization.

The countries where Tafamidis was approved for the cardiac amyloidosis treatment include: European Union–in 2011; Japan–in 2013; United States–in 2019; Australia–March 2020. That indicates about many other possible applications of this drug, which are not yet studied.

Due to the wide use of diflunisal, a lot of drug delivery systems: nanoparticles, hydrogels, complexes, co-crystals, etc. have been developed [11]. By contrast, there are no known tafamidis delivery systems, which highlights the scientific novelty and practical soundness of encapsulated tafamidis.

Thus, the aim of this study was to develop the tafamidis loading into polymeric matrix based on the mixture of high molecular chitosan and polyvinyl alcohol (PVA). Drug release of the tafamidis from polymer matrix was analyzed and demonstrated. It can be concluded, that the obtained polymer systems should be further investigated in vitro and in vivo to be considered as the modern drug delivery systems of tafamidis for amyloidosis treatment.

2. Materials and Methods

2.1. Materials

Polyvinyl alcohol (PVA) with molecular weight (MW) equal to 75'000 Da was purchased from JSC LenReactiv (Saint Petersburg, Russian Federation). Chitosan with MW equal to 200'000 Da was purchased from LLC BioProgress (Moscow Region, Russian Federation). Glacial acetic acid (99.5% ACS, MW = 60.05 g/mol), acetonitrile (99.9% ACS, MW = 41.05 g/mol), and ethanol (98.0%, MW = 46.068 g/mol) was obtained from JSC EKOS-1 (Moscow, Russian Federation). Distilled water was prepared using laboratory distiller apparatus. All materials were used without additional purification.

2.2. Polymer solutions preparation

Polymer solutions were prepared in several stages in accordance with the previously published method [12,13]. The detailed procedure is as follows.

Firstly, PVA (0.4 g) was dissolved in distilled water (3.3 ml) using a magnetic stirrer at 90-100 °C until the polymer is completely dissolved. After PVA dissolution, a chitosan (0.3 g) was added into the prepared PVA aqueous solution, stirred for 3-5 min, and then the glacial acetic acid (4.2 ml) was added with consequently stirring at 70 °C for 1 h. Finally, ethanol (2.0 ml) was added with the following stirring at the same temperature for 30-60 min. Tafamidis (0.01 g and 0.02 g) was added into the polymer solution to obtain concentration 0.1 wt. % (sample TAF-01) and 0.2 wt. % (sample TAF-02). Final solutions were kept at 23.0 °C for stabilization and deaeration.

2.3. In Vitro Drug Release Study

For the measurement of tafamidis concentration in the prepared solutions the samples were dissolved in 10.0 mL equimolar mixture of ethanol:water, stirred, treated in an ultrasonic bath, and filtered through the membrane filter (Nylon, 0.45 μ), and analyzed.

For the drug release study, the samples (250-300 mg) were placed in a glass beaker, 50 mL of the buffer solution was added, the solutions were thermostated at 36-37°C. At intervals of time the sample aliquot equal to 500 μL were taken, mixed with 500 μl of acetonitrile, the solution (suspension) was filtered through a membrane filter (Nylon, 0.45 μ) and analyzed by HPLC method. Fresh buffer was added to the initial solutions to maintain the constant volume. The release study was done three times.

2.3. Chromatographic conditions.

The following equipment was used: liquid chromatograph Millichrome-A02, equipped with an ultraviolet detector (LLC IH "EkoNova", Russia); chromatographic column Prontosil 120-5, C18, 75×2 mm (LLC IH "EkoNova", Russia).Mobile phase PBS pH 6.8 : acetonitrile (50:50), flow rage 0,1 mL min^{-1}, column temperature 40 °C, detector UV (230, 270, 310 nm), volume injection 5 μL.

3. Results and Discussion

Very recently, the method for mangiferin loading into the polymer matrix based on chitosan and PVA has been successfully developed in our laboratory [12-14]. The ratio of chitosan-PVA has been investigated, and it was found that the only polymer concentrations equal to 4.0 wt.% for PVA and 3.0 wt.% for chitosan at final solution is optimal for mangiferin encapsulation.

The methodology for the mangiferin' loading describing the step-by-step procedure has been also developed in the details.

It became the basis for this work where we applied the mangiferin loading procedure for the clinically used molecule against cardiac amyloidosis–tafamidis. However, we met the difficulties when added tafamidis into polymer matrix. It is probably connected with the solubility and hydrophobic nature of tafamidis. Thus, the use of tafamidis in the concentrations 0.5% and 1.0% resulted into the absolutely viscous solutions where tafamidis is dissolved only very limited. We established that only 0.1% tafamidis solution may be prepared when we use the polymer system chitosan-PVA.

We also found that tafamidis should be added into the final solution, because the addition into PVA solution on the first step of the procedure resulted into absolutely insoluble matrix even at the temperature above 120 °C. The prepared polymer matrixes loaded with tafamidis are demonstrated in Figure 2.

Figure 2. The photo of tafamidis-loaded chitosan-PVA matrix.

3.1. Tafamidis Concentration in the Prepared Solutions

The tafamidis concentration in the prepared solutions are listed in the Table 1. The decrease in real content of tafamidis at TAF-02 sample is related to the subsolution of the drug–the tafamidis precipitatation was detected in the case of 0.2 wt.% tafamidis.

Table 1. The tafamidis concentration in the solutions.

	TAF-01	**TAF-02**
Tafamidis concentration, wt.%	0.10	0.17

3.2. In Vitro Drug Release Study

The tafamidis release was investigated by HPLC method under the following conditions: mobile phase–PBS pH 6.8 and acetonitrile (50:50), sample volume 5 µL, column thermostat 40°C, UV detector with wavelengths 230, 270 and 310 nm. A typical chromatogram is shown in the Figure 3.

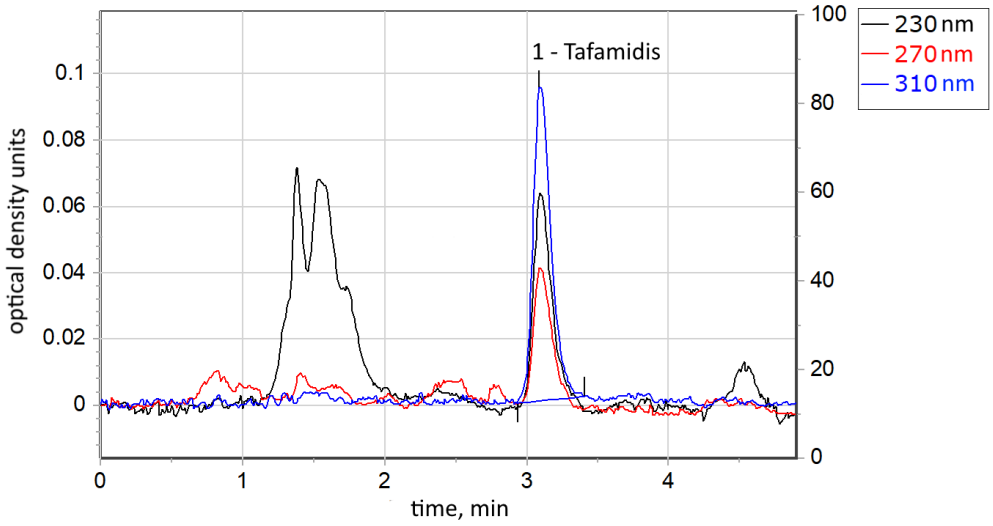
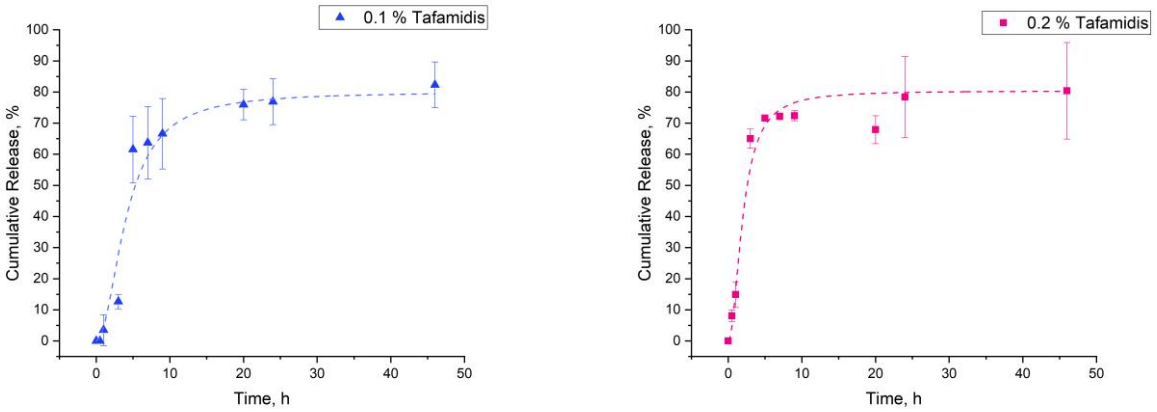


Figure 3. Typical chromatogram of tafamidis solution during the release from the chitosan-PVA matrix.

Tafamidis release profiles into phosphate buffered saline (PBS, pH 7.4) from Chitosan/PVA matrixes are demonstrated in Figure 4. The profiles have the similar kinetics regardless the drug content into polymer matrixes. Important note, that the samples demonstrate no burst release. The tafamidis concentration rapidly but gradually increases to the plateau with subsequent dosed and almost constant drug release during 46 h. This effect has a practical importance for the controlled release of the tafamidis during the therapy.



(a) (b)

Figure 3. Release profiles from tafamidis-loaded CT/PVA-based matrices: initial tafamidis concentration equal to 0.1 wt.% (a) and 0.2 wt.% (b).

Further investigations of such tafamidis drug delivery systems have to be directed on the evaluation of pH influence on drug release as well as on the investigation of the dependence of molecular mass and polymer ration on tafamidis loading and its release.

4. Conclusion

Being clinically used drug for the treatment of cardiac amyloidosis, tafamidis is quite important therapeutic molecule with the broad potential due to the fact that amyloidosis is systemic disease. However, tafamidis therapy is absolutely costly, and the drug has side-effects connected with non-selective mode of the action and quite low solubility. In the present work the drug delivery system of tafamidis based on polymeric matric chitosan–PVA has been developed for the first time. We demonstrated that tafamidis release has no burst character and may be controlled. This knowledge opens new direction in the field of smart drug delivery systems for the rarely diseases therapeutic molecules to enhance its selectivity and to decrease the doses, that finally result into cost and side effects decrease.

Author Contributions: Conceptualization, P.S., S.M., and Yu.G.; methodology, P.S., T.H.N.V., and Yu.G.; validation, S.M.; formal analysis, Yu.G., P.S., S.M.; investigation, P.S., Yu.G., and S.M.; resources, P.S., S.M., and Yu.G.; writing—original draft preparation, P.S. and S.M.; writing—review and editing, S.M.; visualization, P.S.; supervision, S.M.; project administration, S.M. and M.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, project number 21-74-20093. Link to information about the project: https://rscf.ru/en/project/21-74-20093/

Conflicts of Interest: The authors declare no conflict of interest.

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