engineering proceedings

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Proceeding Paper

Tafamidis Drug Delivery Systems Based on Chitosan/Polyvinyl Alcohol Matrix †

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† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/.

Abstract: Cardiovascular diseases retain their position as the leading cause of death globally, and according to the World Health Organization, there are 17.9 million cases of these diseases each year. Cardiac amyloidosis caused by the formation and deposition of a specific protein–polysaccharide complex–amyloid in the myocardium represents the main cause of death. The pharmaceutical molecules clinically used 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 of both drugs, tafamidis, with greater therapeutic efficacy, is the most expensive: the yearly course costs appr. USD 225,000. One of the possible ways of enhancing its solubility and bioavailability, decreasing the dosage with the simultaneous targeted effect, is the encapsulation of the drug into polymer (biopolymer) matrixes. In contrast to the known diflunisal delivery systems, there are no available data on the development of tafamidis delivery systems. In this study, we report, for the first time, a method for the encapsulation of tafamidis into a polymeric matrix based on the mixture of chitosan and polyvinyl alcohol (PVA). The release profile of the polymer matrix was analyzed, and no burst characteristic 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

Citation: Snetkov, P.; Generalova, Y.; Vu, T.H.N.; Morozkina, S.; Uspenskaya, M. Tafamidis Drug Delivery Systems Based on Chitosan/Polyvinyl Alcohol Matrix. *Eng. Proc.* **2023**, *56*, 260. https://doi.org/10.3390/ASEC2023-15905

Academic Editor: Marco Annunziata

Published: 7 December 2023

1. Introduction

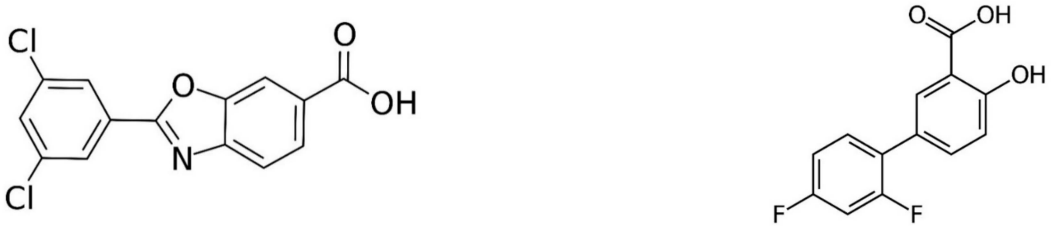
Amyloidosis is a systemic disease caused by the deposition of amyloid fibril aggregates in various organs and tissues [1]. One of the most dangerous forms is cardiac amyloidosis, when the amyloids deposit in the myocardium, resulting in death associated with cardiomyopathy [2,3].

Current therapy strategies, unfortunately, cannot prevent amyloidosis, and only delay further fibril deposition. Moreover, current therapeutics have a lot of side effects, including cardiotoxicity, which negatively affects patient lifestyle [4]. Amyloidosis may be caused not only by transthyretin (ATTR) or immunoglobulin light-chain (AL) fibrils [5] but also potentially a deletion in the gene of giant protein titin (TTN) [6], which drastically hinders the therapy against amyloidosis.

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

studies, 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 [7] and mangiferin into a biopolymer carrier based on high-molecular-weight chitosan and polyvinyl alcohol (PVA) [8]. The above-mentioned studies became the basis for the investigation of the polymer matrix's capacity as a carrier for biologically active molecules of different chemical natures.

Tafamidis (Figure 1a) and diflunisal (Figure 1b) are the only two known small-molecule therapeutic agents used to delay amyloid deposition. These molecules have a hydrophobic nature and non-selective mode of action, which results in low solubility, high doses, and insufficient therapeutic efficacy. In addition to the non-selective mode of action for both drugs, tafamidis, with its greater therapeutic efficacy, is the most expensive: the yearly course costs appr. USD 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 [11], was initially widely used as an effective medication for the treatment of rheumatoid arthritis and possesses an effect against transthyretin (TTR) polyneuropathy.

Tafamidis, also known as the brands Vyndaqel and Vyndamax [12], delays transthyretin cardiac amyloidosis (for both familial amyloid cardiomyopathy and familial amyloid polyneuropathy, as well as wild-type transthyretin amyloidosis, which is called senile systemic amyloidosis). The main mechanism of its action is quaternary structure transthyretin stabilization.

Countries where tafamidis has been approved for cardiac amyloidosis treatment include the countries of the European Union in 2011; Japan in 2013; the United States in 2019; and Australia in March 2020. This indicates many other possible applications of this drug which have not yet been studied.

Due to the wide use of diflunisal, a lot of drug delivery systems, e.g., nanoparticles, hydrogels, complexes, co-crystals, etc., have been developed [13]. 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 a method of loading tafamidis into a polymeric matrix based on a mixture of high-molecular-weight chitosan and polyvinyl alcohol (PVA). Drug release of tafamidis from the polymer matrix was analyzed and demonstrated. It can be concluded that the obtained polymer system should be further investigated in vitro and in vivo as a modern drug delivery system of tafamidis for amyloidosis treatment.

2. Materials and Methods

2.1. Materials

Polyvinyl alcohol (PVA) with a molecular weight (MW) equal to 75,000 Da was purchased from JSC LenReactiv (Saint Petersburg, Russia). Chitosan with a MW equal to 200,000 Da was purchased from LLC BioProgress (Moscow Region, Russia). 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) were obtained from JSC EKOS-1 (Moscow, Russia).

Distilled water was prepared using a laboratory distiller apparatus. All materials were used without additional purification.

2.2. Polymer Solution Preparation

Polymer solutions were prepared in several stages in accordance with a previously published method [14]. 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 completely dissolved. After PVA dissolution, a chitosan (0.3 g) was added into the prepared PVA aqueous solution and stirred for 3–5 min, and then the glacial acetic acid (4.2 mL) was added with constant stirring at 70 °C for 1 h. Finally, ethanol (2.0 mL) was added with continued 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 concentrations of 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 of an equimolar mixture of ethanol/water, stirred, treated in an ultrasonic bath, 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, and the solutions were thermostated at 36–37 °C. At various intervals of time, sample aliquots equal to 500 μL were taken and mixed with 500 μL of acetonitrile, and the solution (suspension) was filtered through a membrane filter (Nylon, 0.45 μ) and analyzed by the HPLC method. Fresh buffer was added to the initial solutions to maintain a constant volume. The release study was conducted three times.

2.4. Chromatographic Conditions

The following equipment was used: liquid chromatograph, Millichrome-A02, equipped with an ultraviolet detector (LLC IH "EkoNova", Novosibirsk, Russia); chromatographic column, Prontosil 120-5, C18, 75 × 2 mm (LLC IH "EkoNova", Novosibirsk, 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 a polymer matrix based on chitosan and PVA was successfully developed in our laboratory [14]. The ratio of chitosan to PVA was investigated, and it was found that polymer concentrations equal to 4.0 wt.% for PVA and 3.0 wt.% for chitosan in the final solutions were optimal for mangiferin encapsulation.

The methodology for mangiferin loading with a step-by-step, detailed description of the procedure was also developed.

It became the basis for this work, where we applied the mangiferin loading procedure for a clinically used molecule against cardiac amyloidosis—tafamidis. However, we encountered difficulties when adding tafamidis into the polymer matrix. This 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 absolutely viscous solutions where the dissolution of tafamidis was very limited. We established that only 0.1% tafamidis solution is feasible when we use the polymer system chitosan-PVA.

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

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

3.1. Tafamidis Concentration in the Prepared Solutions

The tafamidis concentrations in the prepared solutions are listed in Table 1. The decrease in the actual content of tafamidis in the TAF-02 sample was related to its precipitation in the solution, which was detected in the case of 0.2 wt.% tafamidis.

Table 1. The tafamidis concentrations 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 the HPLC method under the following conditions: mobile phase, PBS pH 6.8 and acetonitrile (50:50); sample volume, 5 µL; column thermostat, 40 °C; and UV detection at wavelengths of 230, 270, and 310 nm. A typical chromatogram is shown in Figure 3.

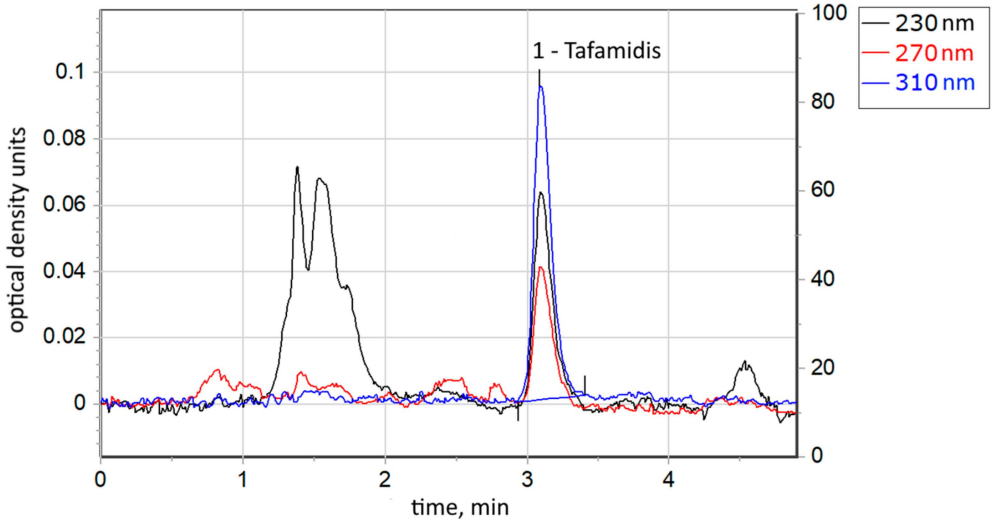
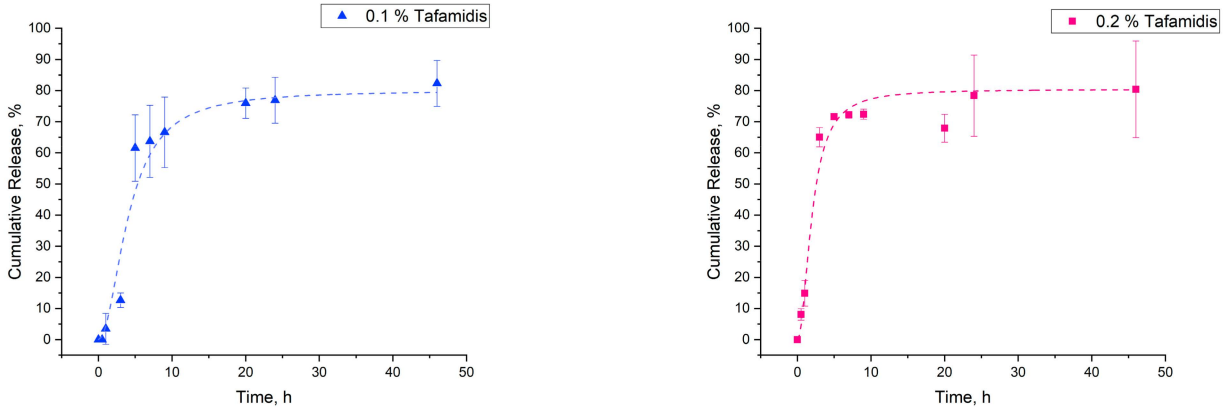


Figure 3. Typical chromatogram of tafamidis solution during the release from the chitosan-PVA matrix.

Tafamidis release profiles into phosphate-buffered saline (PBS, pH 7.4) from chitosan/PVA matrixes are demonstrated in Figure 4. The profiles have similar kinetics regardless of the drug content in polymer matrixes. It is important to note that the samples demonstrated no burst release. The tafamidis concentration rapidly increased until reach-

ing a plateau with subsequent doses and exhibited almost constant drug release over 46 h. This effect has practical importance for the controlled release of tafamidis during therapy.



(**a**) (**b**)

Figure 4. Release profiles from tafamidis-loaded CT/PVA-based matrixes. Initial tafamidis concentrations equal to 0.1 wt.% (**a**) and 0.2 wt.% (**b**).

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

4. Conclusions

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

Author Contributions: Conceptualization, P.S., S.M. and Y.G.; methodology, P.S., T.H.N.V. and Y.G.; validation, S.M.; formal analysis, Y.G., P.S. and S.M.; investigation, P.S., Y.G. and S.M.; resources, P.S., S.M. and Y.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/ (accessed on 30 June 2023).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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

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