Liposomes for the delivery of Tissue Plasminogen Activator

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Introduction

Tissue plasminogen activator (tPA) is well-known for its fibrin-targeting properties and using its liposomal form can reduce side effects and improve thrombolytic activity [1, 2]. In this study liposomal tPA (L-tPA) was obtained and characterized for size, zeta-potential, entrapment efficiency, *in vitro* release and activity.

L-tPA was prepared using sonication-lyophilization-rehydratation method from the mixture of soy phosphatidylcholine (PC), cholesterol (Ch) 1.5:1 mol. and tPA (1:1 wt.).

Results

According to atomic force microscopy (AFM, NanoScope MultiMode, Veeco), liposomes had round shape and its size was about 60 nm (fig.1). The size of liposomes is a crutial parameter for their applications in case of using an injectable form (less than 200 nm).

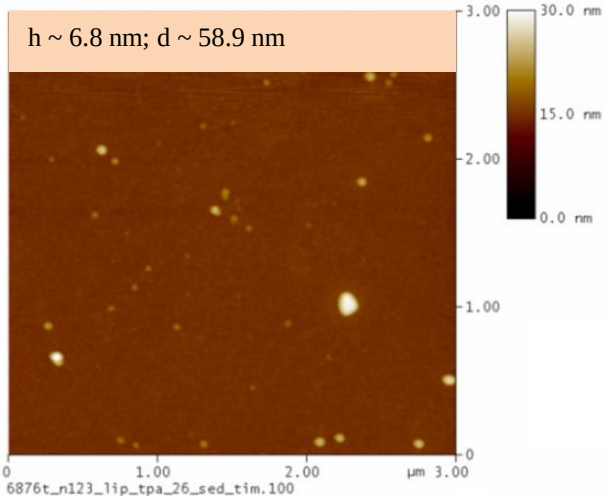


Fig.1. AFM-image of L-tPA on silica surface (tapping mode)

The mean hydrodynamic diameter and zeta potential (ZP) of L-tPA, measured using dynamic light scattering (Zetasizer NanoZS, Malvern), were ~130 nm and -57 mV respectively (table 1). Zeta potential is a critical parameter for the stability of liposomes. The higher zeta potential, the higher the surface charge and repulsive forces between vesicles into solution that lead to its stability. It is important for applications to have ZP higher than |30| mV.

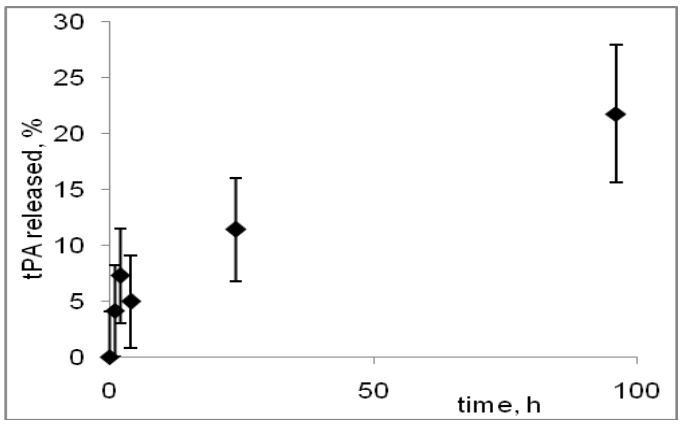
The entrapment efficiency of liposomes, determined by Bradford method, was 87-89 %.

Table 1. Characteristics of L-tPA

Hydrodynamic diameter	~130 nm
Zeta potential	-56,6±0,4 mV
Entrapment efficiency	87-89 %
Activity	~60,8 %
Activity after 24 hours	~66,0 (+5,2) %

According to the data (fig. 2), ~4 % of the drug was released within the first 1 h and ~22 % over a period of 96 h. Initial tPA release profile indicates low leakage of liposomes which allows to store and use them during 1 hour after the reconstitution.

Fig.2. The in vitro release of tPA from liposomes (water, 37 C)



Activity of tissue plasminogen activator determined using Chromogenic substrate (S-2288, Chromogenix) was about 61 % after liposome recontitution and increased up to 66 % after 24 h incubation (37 C), which shows slight release of tPA and mantaining its activity.

Thus, developed liposomal form of tissue plasminogen activator due to its characteristics (small size, stability, high entrapment efficiency, low leakage of the drug and high activity) can be used for treatment cardiovascular diseases.

References:

1. Koudelka S. Mikulik R., Masek J. et al. Liposomal nanocarriers for plasminogen activators// J. Control. Rel. -2016.-**227**.- P.45–57.

2. Absar S., Nahar K., Kwon Y.M., Ahsan F. Thrombus-targeted nanocarrier attenuates bleeding complications associated with conventional thrombolytic therapy// Pharm Res. – 2013. –**30.** – P. 1663–1676.