https://doi.org/10.36107/hfb.2022.i4.s153

УДК 502.5

Development of Cinnarizine Liposome Technology

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Declaration of competing interest: none declared

Received: 05.11.2022
Accepted: 10.12.2022
Published: 30.12.2022

ABSTRACT

Introduction. Liposomal preparations have a number of advantages: they protect the cells of the body from the toxic effects of drugs; prolong the action of the drug introduced into the body; protect medicinal substances from degradation; contribute to the manifestation of targeted specificity due to selective penetration from the blood into tissues; change the pharmacokinetics of drugs, increasing their pharmacological efficacy; allow you to create a water-soluble form of a number of medicinal substances, thereby increasing their bioavailability.

In this work, studies were carried out to develop a methodology for determining the degree of inclusion in liposomes from soy lecithin of cinnarizine, which has found wide application as a corrector of cerebral circulation disorders.

Purpose. The purpose of the study is to determine the amount of adsorption of cinnarizine with liposomes from soy lecithin.

Materials and Methods. Cinnarizine liposomes from soy lecithin were prepared by the hydration/rehydration method. To study the characteristics of the degree of incorporation of cinnarizine into liposomes, the method of equilibrium dialysis was used. The choice of this method is due to the fact that the quantitative analysis of the equilibrium concentration of cinnarizine in the dispersion medium, which is necessary to determine the amount of adsorption, is hampered by the presence of a dispersed phase, liposomes. A semipermeable membrane with a pore diameter sufficient for the penetration of cinnarizine molecules, but impermeable to liposomes, makes it possible to obtain a cinnarizine solution with a concentration close enough to the concentration in the dispersion medium of liposomes. The solution thus obtained can be subjected to quantitative analysis using spectrophotometry.

Results. A graph of the dependence of the value of adsorption of cinnarizine on liposomes on the equilibrium concentration was plotted. It was found that the value of adsorption of cinnarizine during the treatment of liposomes with ultrasound is less for all the studied concentrations. At an equilibrium concentration of cinnarizine of more than 0.0003 mol/l, the proportion of the prearat associated with liposomes stabilizes. Without sonication at the level of 24.83 ± 1.15%, with sonication at the level of 18.4 ± 1.20%.

Conclusion. It has been established that ultrasonic treatment of liposomes is expedient when cinnarizine is added to a dry lipid film, since is a factor that increases bioavailability.

KEYWORDS

liposomes, soy lecithin, cinnarizine, dialysis, inclusion degree, ultrasound

To cite: Polkovnikova, Yu. A. (2022). Development of cinnarizine liposome technology. *Health, Food & Biotechnology, 4*(4), 35–40. https://doi.org/10.36107/hfb.2022.i4.s153

HEALTH

https://doi.org/10.36107/hfb.2022.i4.s153

Разработка технологии липосомной лекарственной формы циннаризина

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Конфликт интересов:
Автор заявляет об отсутствии конфликта интересов

Поступила: 05.11.2022
Принята: 10.12.2022
Опубликована: 30.12.2022

АННОТАЦИЯ

Введение. Липосомальные препараты обладают рядом преимуществ: защищают клетки организма от токсического действия лекарственных средств; пролонгируют действие введенного в организм лекарственного средства; защищают лекарственные вещества от деградации; способствуют проявлению нацеленной специфичности за счет селективного проникновения из крови в ткани; изменяют фармакокинетику лекарственных препаратов, повышая их фармакологическую эффективность; позволяют создать водорастворимую форму ряда лекарственных субстанций, увеличивая тем самым их биодоступность. В данной работе проведены исследования по разработке методики определения степени включения в липосомы из соевого лецитина циннаризина, нашедшего широкое применение как корректора нарушений мозгового кровообращения.

Цель. Цель исследования определить величину адсорбции циннаризина с липосомами из соевого лецитина.

Материалы и методы. Липосомы циннаризина из соевого лецитина получали методом гидратации/регидратации. Для изучения характеристик степени включения циннаризина в липосомы был использован метод равновесного диализа. Выбор данного метода обусловлен тем, что количественный анализ равновесной концентрации циннаризина в дисперсионной среде, необходимый для определения величины адсорбции, затруднен присутствием дисперсной фазы – липосом. Полупроницаемая мембрана, с диаметром пор, достаточным для проникновения молекул циннаризина, но не пропускающая липосомы, позволяет получить раствор циннаризина с концентрацией, достаточно близкой к концентрации в дисперсионной среде липосом. Получаемый таким образом раствор может быть подвергнут количественному анализу с использованием спектрофотометрии.

Результаты. Построен график зависимости величины адсорбции циннаризина на липосомах от равновесной концентрации. Установлено, что величина адсорбции циннаризина при обработке липосом ультразвуком меньше для всех исследуемых концентраций. При равновесной концентрации циннаризина более 0,0003 моль/л происходит стабилизация доли препарата связанного с липосомами. Без обработки ультразвуком на уровне 24,83 ± 1,15 %, с обработкой ультразвуком на уровне 18,4 ± 1,20 %.

Выводы. Установлено, что обработка липосом ультразвуком является целесообразной при добавлении циннаризина к сухой липидной плёнке, т.к. является фактором, повышающим биодоступность

КЛЮЧЕВЫЕ СЛОВА

липосомы, соевый лецитин, циннаризин, диализ, степень включения, ультразвук

Для цитирования: Полковникова, Ю. А. (2022). Разработка технологии липосомной лекарственной формы циннаризина. *Health, Food & Biotechnology*, *4*(4), 35–40. https://doi.org/10.36107/hfb.2022.i4.s153

INTRODUCTION

Vascular diseases of the brain continue to be a thorny medical-and-social problem in contemporary society (Bertram & Tanzi, 2005; Polkovnikova & Slivkin, 2015). Pharmacological correction of the cerebral blood flow is an actual problem of modern medicine since in the basis of a lot of cerebral diseases there are factors of vascular genesis while the diseases themselves are accompanied by a loss of working ability, disability and mortality (Haress, 2015).

Various groups of medicinal drugs are applied in therapy of cerebrovascular diseases including blockage of calcium channels. One of the most applicable and economically available among them is a medicinal preparation — cinnarizine (DiSabella, 2014). Mechanism of action of this drug is exhibited in the blocking of calcium channels; in addition, under the intake of this drug antihistaminic effect is observed, moreover, cinnarizine exhibited anti-dopaminergic, anti-serotonergic and anti-cholinergic activity. Multiple studies of the medicinal preparation confirmed its efficacy for such diseases as cerebral atherosclerosis, without a coarse focal symptomatology and ischemic stroke (Sethi et al., 2018; Maghsoodi et al., 2019). It is also applied after hemorrhagic stroke and craniocerebral injury, for treating of discirculatory encephalopathy, dizziness, tinnitus, migraine, senile dementia, depression and irritability, rapid mental fatigability, impairment and loss of memory, thinking disorder, impossibility of attention focusing, in the treatment and for prevention of disorders in peripheral circulation, in cases of trophic and varicose ulcers, during supporting therapy in case of symptoms of labyrinthine disorders, including dizziness, tinnitus, nystagmus, nausea and vomiting (Erdem & Türkoğlu, 2010).

Liposomes are spherical vesicle structures composed of a uni- or multilamellar lipid bilayer surrounding internal aqueous compartments and a relatively impermeable outer lipophilic phospholipid bilayer (Szoka & Papahadjopoulos, 1980; Neuwelt et al., 2008; Abbott et al., 2006; Gregoriadis, 2008). Liposomes have gained considerable attention as drug delivery carriers because they are biocompatible, nontoxic, can deliver both hydrophilic and lipophilic drug molecules, protect their cargo from degradation by plasma enzymes, and transport their load across biological membranes and the blood brain barrier (Elbayoumi, & Torchilin, 2010; Lopalco et al., 2018; Yalçın & Türkoğlu, 2010; Xiaoli et al., 2017; Przewratil et al., 2009).

The purpose of the study is to develop a technology for obtaining liposomes of cinnarizine, and determine the degree of inclusion of cinnarizine in them.

MATERIALS AND METHODS

Preparation of liposome specimens from soya-bean lecithin

For obtaining liposomes from soya-bean lecithin method of hydration/rehydration was employed (Immordino et al., 2006). Soya-bean lecithin (Sigma) was dissolved in ethanol (0,1 g of soya-bean lecithin per 300 ml of ethanol (96%) at 38 ºC in a water bath under stirring) and filtered through the glass filter with pore size of 16 μm. Ethanol was then evaporated in the rotor vaporizer at the temperature of 38 ºC, pressure of -0,08 MPa and rotary speed of 100 rpm up to formation of semitransparent lipid film on the walls of a flask. Next, 10 ml of 0,1 M solution of hydrochloric acid or a solution of cinnarizine in 0,1 M solution of hydrochloric acid were added and agitated for 30 minutes. After that solution was subjected to ultrasound irradiation for 15 minutes.

Determination of the mass fraction of liposomes colloid solution

Watch glass was dried in the desiccator at the temperature of 60 ºC and weighed it with the use of analytical balance Radwag 220/C/2 with an accuracy up to 4-th decimal digit after the point, in grams. Then a colloid solution of liposomes was placed on the watch glass and weighed it again. Then the watch glass with a solution of liposomes was placed into the desiccator at the temperature of 80 ºC and dried its contents up to invariable mass. Mass fraction of colloid solution of the liposomes was determined by the formula (1):

$$\omega_{\%} = \frac{m_{dry} - m_{empty}}{m_{liquid} - m_{empty}} \cdot 100\ \%, \quad (1)$$

где $m_{empty.}$ — is mass of the watch glass, g;
m_{liquid} — mass of the watch glass with colloid solution, g;
m_{dry} — mass of the watch glass with a dry residue of colloid solution, g.

Preparation of cinnarizine solution

Solution A. Precise sample of cinnarizine (0,0506 g) was dissolved in 20 ml of aqueous solution of hydrochloric acid 0,1 M in the volumetric flask of 100 ml in capacity and reduced with the same solvent up to the label.

Solution B. 1,0 ml of solution A was placed into the volumetric flask of 100 ml in capacity, reduced the volume of solution with 0,1 M solution of hydrochloric acid up to the label and then agitated.

Solution C. 5 ml of solution A was placed into the volumetric flask of 50 мл in capacity, reduced the volume of solution

with 0,1 M solution of hydrochloric acid up to the label and then agitated.

Construction of a calibration graph and determination of the molar absorption coefficient for the quantitative determination of the content of cinnarizine by the spectrophotometric method

To study the binding of cinnarizine to liposomes from soy lecithin, the quantitative determination of cinnarizine in a 0.1 M aqueous solution of hydrochloric acid was performed spectrophotometrically.

Determination of the degree of cinnarizine involvement into liposomes from soya-beans lecithin

Colloid solutions of liposomes with different concentrations of cinnatizine were placed into the vials in the amount of 0,6 ml using a dispenser of Termo Scientific DPOP-1–100-1000. Then dialysis test tubes GeBAflex Mini 250 µl (Scienova, Germany) with a diameter of pores in membrane of 6–8 kDa were placed inside the vials. 0,18 ml of 0,1 M solution of hydrochloric acid were placed into the dialysis test tubes with the use of dispenser.

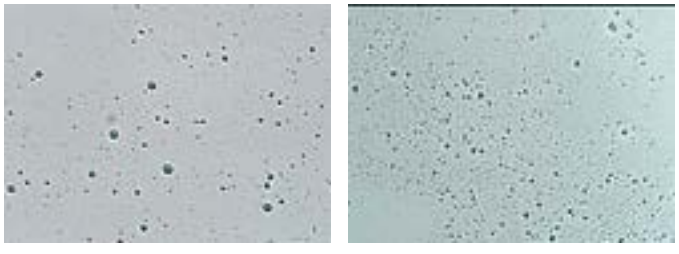
Dialyzers were placed into the thermostat at the temperature of 40 °C for 24 hours. After that, solutions in the amount of volume of 0,1 ml from the dialysis test tubes were mixed with 2,5 ml of aqueous solution of hydrochloric acid in 0,1 M concentration. Next, optical density of the obtained solutions were measured at the wavelength of 252 nm.

RESULTS AND DISCUSSION

Figure 1 shows photomicrographs of liposomes before and after sonication.

Figure 1

Micrographs of liposomes in an aqueous solution of hydrochloric acid 0.1 M (A — before ultrasound treatment, B — after ultrasound treatment)



A *B*

Mass fraction of liposomes was determined 6 times with the following determination of arithmetic mean value and standard deviation for the measured value (Table 1).

Table 1

Mass fraction of liposomes in colloidal solution

№	Mass fraction, %
1	0,2989
2	0,2490
3	0,3836
4	0,3300
5	0,2724
6	0,3014
Mean value	0,3059 ± 0,0470

Construction of a calibration graph and determination of the molar absorption coefficient for the quantitative determination of the content of cinnarizine by the spectrophotometric method

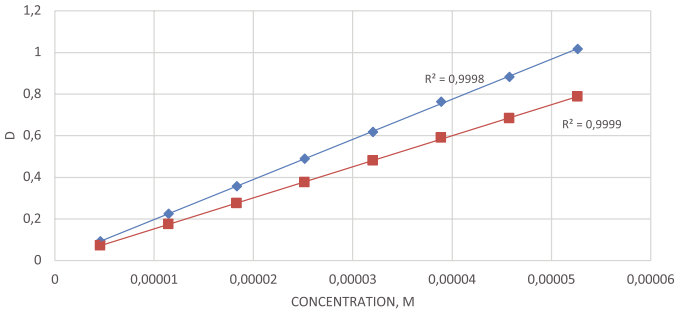
A preliminary measurement of the ultraviolet spectrum of a solution of cinnarizine B in a 0.1 M aqueous solution of hydrochloric acid was carried out.

The choice of the maximum optical density used for the quantitative determination of cinnarizine was made based on the possible effect of free lecithin in the dispersion medium of liposomes capable of penetrating through a semipermeable membrane during equilibrium dialysis (Polkovnikova, 2021). For this, a test dialysis was performed, in which 0.6 ml of a liposome solution was placed in a dialysis tube, and 0.18 ml of a 0.1 M aqueous solution of hydrochloric acid was placed in a dialyzer. Dialysis was carried out for 24 hours at a temperature of 40 °C. After that, the ultraviolet absorption spectrum of the solution in the dialyzer was measured. For a quantitative analysis of the content of cinnarizine, the absorption maximum at a wavelength of 252 nm was used, because for a given wavelength, less light absorption of free lecithin penetrating the dialysis membrane is observed.

In order to determine the linearity of the applied method of photometric analysis of the quantitative content of cinnarizine in a 0.1 M aqueous solution of hydrochloric acid, the optical densities of a series of solutions of cinnarizine of various concentrations were measured and a calibration graph was constructed in the range of concentrations and optical densities required for the study at wavelengths of 226 nm. and 252 nm (Figure 2).

Figure 2

Calibration plot for the quantitative determination of cinnarizine in a 0.1 M aqueous solution of hydrochloric acid by spectrophotometry at 226 nm and 252 nm



The linear approximation coefficients R2 = 0.9998 at a wavelength of 252 nm and R2 = 0.9999 at a wavelength of 226 nm correspond to acceptable linearity in the optical density range 0–1. For the quantitative determination of cinnarizine, the molar absorption coefficient was determined at a wavelength of 252 nm, since in this region of the absorption spectrum of cinnarizine, a lower optical density of free lecithin, which penetrates through the dialysis membrane, is observed. For this, the least squares method was used to determine the equation of the linear dependence of optical density on concentration:

$$D = 19261{,}15C + 0{,}0046679. \quad (2)$$

The free term 0.0046679 in this equation can be neglected because it is comparable to the optical density measurement error. Thus, from the equation:

$$D = 19261{,}15\ C \quad (3)$$

determined the molar absorption coefficient = 19261.15/l = 19261.15/0.998 = 19299.75 $M^{-1}cm^{-1}$ (where l is the thickness of the cuvette, cm).

Determination of the degree of cinnarizine involvement into liposomes from soya-beans lecithin

The equilibrium concentrations of cinnarizine, determined using the molar absorption coefficient and the value of drug binding to liposomes, are shown in Table 2.

Using the data obtained, a graph of the dependence of the adsorption value of cinnarizine on liposomes on the equilibrium concentration was plotted. It was found that the value of adsorption of cinnarizine during the treatment of liposomes with ultrasound is less for all the studied concentrations.

The degree of incorporation of cinnarizine into liposomes stabilizes with increasing concentration of the drug over 0,00015 mol/l and is 62, 18 ± 4,08 % without sonication of liposomes and 60,83 ± 1,23 % with sonication of liposomes.

For obtaining liposomes from soya-bean lecithin method of hydration/rehydration was employed. The molar absorption coefficient = 19299,75 $M^{-1}cm^{-1}$ is determined.

It was found that cinnarizine effectively binds to liposomes from soy lecithin in an acidic medium (the degree of binding is more than 60 %). The degree of incorporation of cinnarizine into liposomes from soy lecithin (62,18 ± 4,08%) is comparable to the degree of incorporation of cinnarizine into liposomes with the addition of hydroxypropyl-β-cyclodextrin (70%) (Jia et al., 2007).

Table 2

Values of concentration in dialysates and the value of binding of cinnarizine to liposomes in an experiment with the inclusion of cinnarizine of various concentrations in liposomes

№	Concentration of cinnarizine without liposomes, taking into account dilution in the dialyzer, mole/l	Without ultrasound treatment		With ultrasound treatment	
		Concentration of cinnarizine in dialysate, mole/l	Degree of cynnarizine binding with liposomes, mole/kg	Concentration of cinnarizine in dialysate, mole/l	Degree of cynnarizine binding with liposomes, mole/kg
0	0,0010106	0,0003723	0,2593	0,0003898	0,2522
1	0,0009430	0,0003074	0,2583	0,0003722	0,2319
2	0,0008587	0,0003356	0,2125	0,0003225	0,2179
3	0,0007191	0,0002644	0,1847	0,0002941	0,1727
4	0,0006385	0,0002796	0,1458	0,0002512	0,1574
5	0,0005389	0,0001855	0,1436	0,0001647	0,1521
6	0,0004217	0,0001678	0,1032	0,0001457	0,1122
7	0,0002099	0,0000642	0,0592	0,0001004	0,0445
8	0,0001113	0,0000328	0,0319	0,0000672	0,0179

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