Original Article

Formulation, Characterization of Baclofen Nanoliposome Vesicles by Nylon-66 Nanofiber Membranes, and Evaluation of Their Effect on Lactate Dehydrogenase and Creatine Kinase Enzymes as Inhibitors

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Abstract

Background: Formulation of baclofen in lipid-based nanoparticles may be helpful to overcome its difficulties in order to reach the site of action in the central nervous system. This study reports production, characterization, and evaluation of baclofen nanoliposome vesicles (BLV_2). This way makes drugs more stable for delivery systems design. **Methods:** Baclofen nanoliposomes were prepare by the method of wetting thin films, vesicles in the solution filtered by passing them through nylon 6.6 fibers to improve homogeneity and ensure the passage of small unilamellar lipid vesicles. The formation of the vesicles was tested by spectrophotometric assay Fourier transform infrared spectroscopy (FT-IR), dynamic light scattering (DLS), and morphology method field emission scanning electron microscopy (FESEM). In addition *in vitro* release assay, inhibition activity for lactate dehydrogenase (LDH), creatine kinase (CK) enzymes, and half maximal inhibitory concentration 50 were measured. **Results:** Baclofen nanoliposome vesicles were demonstrated by FTIR, DLS, Zeta potential, and FESEM. Moreover, ultraviolet and visible absorption was revealed at 218 nm, ζ potential rate at -65.6 mV, and DLS values were recorded at (36.67–172.6) nm.. The polydispersity index value was ranged (0.369–0.663), indicating good monodispersity and stability. Also, an effective formulation showed inhibition activity of both LDH and CK enzymes at IC50 (86.359, 72.480) ppm, respectively. **Conclusion:** *In vitro* study of BLV_2 application opened new horizons aimed to use the drug more accessibility by targeting the muscles. New baclofen vesicular formulations containing nanoparticles have been developed and prescribed to improve the effectiveness of the drug.

Keywords: Baclofen, drug delivery systems, liposomes vesicles, nylon 6.6

Introduction

Baclofen is an analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) found in the central nervous system (CNS). Baclofen is a racemic mixture of the R-(+)- and S-(−)-enantiomers. The R-enantiomer has been reported to have a higher CNS activity. Baclofen binds to the GABA receptors preventing the release of other inhibitory neurotransmitters like glycine and thus decreasing the excitatory input. The drug also binds to GABA receptors, and the action of the drug is relating to it.[1] *In vitro* studies shown that baclofen had bind with GABA receptors. Baclofen acts postsynaptic ally and inhibits the influx of calcium by resting membrane depolarization. It inhibits the release of excitatory neurotransmitters such as glutamate and aspartate. There are no published reports of the pharmacokinetics and toxic kinetics of baclofen.[2] The metabolites formed are expected to be excreted in urine. After oral administration of baclofen, it shows good absorption, a low volume of distribution, and a short half-life in plasma, around 4 h. Only 15% of the biologically active R-(−)

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enantiomer administered doses are excreted not metabolized in urine. Biotransformation of the drug is considered an oxidative process but it has not been characterized. The drug is not removed by hemodialysis because the drug has a high volume of distribution, a low renal clearance at therapeutic levels, and it has a low protein binding. Baclofen is using to treat spasticity of spinal origin due to spinal cord lesions such as traumatic and demyelination.[3] It is the better tolerated than other drugs used to treat spasticity because its action is on the spinal cord not in the brain. In spasticity associated with lesions above the spinal origin like cerebral palsy and stroke, the drug is often intrathecal used, not orally in solution to minimize systemic action.[4] The pharmacokinetic of baclofen has not been studying in the current study, but the drug is expected to behave like other lipophilic compounds. There are only two papers on the absorption of baclofen and no papers on the pharmacokinetics of baclofen after intoxication appear in the literature review.[5,6]

Liposomes are globular vesicles of amphiphiles with an aqueous core concealed in lipophilic lamellae. The dual-compartment structure enables them to entrap both hydrophilic and lipophilic drugs in the core and the bilayer, respectively.[7] Classification of liposomes based on morphology, dimensions, surface charge, and function.[8] Liposomal dosage forms have different administration-transmitted enhanced stability and biodistribution of entrapped drugs.[9] However, the quality elements including size, morphology, surface charge, and ligand, if attached, essentially affect the pharmacokinetic behavior of entrapped therapeutics. A few techniques give it to provide the preferred configuration and the properties, augmenting the therapeutic roles of the featured drug. Nevertheless, the present techniques require the use of expensive materials or complicated procedures.[10] Nanotechnology/nanosciences comprises a synthesis and application of nano-sized particles with an average size of 1–100 nm and is widely applied in the field of energy, chemical, health care, and cosmetics. In scientific research, the most fastest-growing field is nanotechnology, which was first proposed by the scientist Richard Feynman in the year 1959.[11]

In view of this, developed three new methods, chloroform injection (CJ), chloroform-water dissemination (CWD), and spontaneous phase transition (SPT) methods, to fabric liposomes consisting of just ampholipids (Zwitterionic Lipids) and cholesterol, or/and Phosphatidylcholine(PC), using the simplest equipment. The idea of this work led to the development of a straightforward novel method and found morphological features with the versatile compositions of cholesterol and soyalecithin.

Methods

Baclofen from Baoji Guokang (China), Soya Lecithin and Phosphate Buffer Saline (PBS) from Himedia (India), Cholesterol from Avonchem (UK), Various surfactants (Span-80, Tween-80, Sodium deoxycholate) from Lobachemie (India), Nylon 66 from Mackin (China), Formic acid, Chloroform, and ethanol from Chemlab (UK), Lactate Dehydrogenase (LDH) Assay kit from Biolabo (France), Creatin kinase (CK) Assay kit from Biosystem (Spain).

Ethical consideration

The current study adhered to the ethical standards set by the College of Science, and the study was approved by the Scientific Research Ethics Committee in the Department of Chemistry/ University of Maysan, ID no. 1023 dated October 1, 2024.

Inclusion criteria

This paper was organized such that the new method and the results are described. Development of three new methods, chloroform injection (CI), CWD, and SPT methods, to fabric liposomes consisting of just ampholipids and cholesterol, or/ and soyalecithins, using the simplest equipment. This study may lead to the development of a novel, direct morphological features discovery with diverse compositions of cholesterol and soy lecithin. This paper was organized such that the new method and the results are described in separate sections.

Exclusion criteria

Any baclofen liposome vesicle formulations that failed to meet the desired physicochemical properties or stability criteria were likely excluded from the study. The study comprehensively evaluated the physicochemical properties and inhibitory activity of the baclofen nanoliposome vesicles (BLV2). Formulations that did not undergo complete characterization or biological activity assessment were likely excluded.

Characterization of baclofen

Determination of λ_{max} wavelength of baclofen

Fifty milligram of baclofen was dissolved in 100 ml of PBS (pH 6.8) to prepare (0.5 mg/ml) a stock solution. The maximum wavelength (λ_{max}) of baclofen was then determined by preparing a dilute solution of 25 µg/ml of the stock solution, and its spectral scanning was performed in the wavelength range (200–400 nm) using a ultraviolet-visible (UV-vis) spectrophotometer.[12,13]

Determination of calibration curve of baclofen

To determine the calibration curve, a series of diluted solutions with different concentrations (5, 10, 15, 20, and 25 µg/ml) were prepared from the previously prepared (0.5 mg/ml) stock solution. The absorbance of each diluted solution was then measured at the maximum wavelength (λ_{max}) of baclofen using an UV-vis spectrophotometer. The absorbance of each diluted solution was then plotted against its concentration.[12,13] Types of samples, experimental repeats, and data statistical analysis were presented as mean value ± standard deviation (n = 3).

Preparation of nano baclofen liposome

Liposomes are prepared by the thin-film hydration method, the most common method.[3] In this method, lecithin, cholesterol, and surfactants were dissolved in an organic solvent (chloroform) in a round-bottomed flask continuous stirring using a magnetic stirrer. The solution was evaporated

using a rotary evaporator to remove the organic solvent, resulting in a thin film lipid. To ensure the removal of any solvent residue, the layer was left in a vacuum. This thin film is then hydrated using 10 ml of PBS (pH 6.8) containing the baclofen to be loaded into the liposomes. The mixture was heated at 58°C ± 1°C for 10 min to ensure the thin film lipid is integrated with the prepared, preheated aqueous solution. The solution was stirred using a vortexes for 10 min, resulting in multilamellar lipid vesicles forming in the solution. These vesicles were subjected to ultrasonication using sonicator bath for 30 min at power of 400 watts obtain unilamellar vesicles, which may be heterogeneous.[14,15]

These vesicles in the solution were filtered by passing them through nylon 6.6 fibers to improve homogeneity and ensure the passage of small unilamellar lipid vesicles. This filtration process was performed using the Buchner funnel technique. Stability studies: Stability is examined by evaluating the composition and size of the formulation over time. Optimized formulations are stored at various temperatures in tightly sealed amber bottles. Pharmaceutical products intended for long-term refrigerated storage should be stored at 5°C ± 3°C for 1 month, and accelerated testing should be performed at 25°C ± 2°C/60% ± 5% relative humidity for 1 month. This method uses different ratios of lecithin and surfactants while maintaining a different ratio for baclofen and cholesterol, as shown in Table 1.[12]

Characterization techniques for liposomes formulation

Fourier transform infrared spectroscopy

A Fourier transform infrared spectroscopy (FTIR) spectrophotometer was used to get the FTIR spectra of Baclofen and the Baclofen-loaded liposome vesicles to anticipate and explore any physicochemical intuitive between different components in a detailing were analyzed of the samples at wave numbers (400–4000 cm^{-1}).[12]

Field emission scanning electronic microscope

The sample was examined using a scanning electron microscope to determine the size and shape of the particles in the sample at an accelerating voltage of 15 kV and under a high vacuum. Baclofen liposome vesicles were frozen using liquid nitrogen; then, the sample was mechanically crushed. After crushing, the surface of the sample was coated with a thin layer of carbon, which allowed the surface structure of the liposomes to be clearly seen when examined by a scanning electron microscope.[16]

Dynamic light scattering and zeta potential analysis

Using dynamic light scattering (DLS) analyzer and Zeta potential, the average particle size and the polydispersity index are determined. When measuring liposomes using DLS, they were diluted with PBS to increase the accuracy of the measurement, and the temperature is fixed at 25°C because it affects the movement of the particles.

Manufacturing of nylon 6,6 nano-fibers using electrospinning

Solution preparation

In this study, the materials used were nylon 6,6 and formic acid to prepare nylon 6,6 solution at room temperature. 25 ml of formic acid was used to dissolve 3.6 g of nylon 6,6 granules. The solution continuously stirred with a magnetic stirrer for 90 min for homogeneous solution. The solution was kept in a refrigerator for 15 min before electrospinning.[17]

Electrospinning

The electrospinning device consists of a syringe (5 ml) and a flat collector. The electric voltage was (16 kV) and the flow rate was 0.5 ml/h. The distance between the needle tip and the flat collector was maintained at 15 cm.[17] To form nanofibers made of nylon 6,6, the previously prepared solution is poured into a 5 ml syringe. Aluminum foil was fixed on the flat collector and the nanofibers are deposited on the aluminum foil using electrospinning technique for 10–20 h. After the deposition process, the Nanofibers are heat treated at 50°C to remove the remaining solvent and to separate the fibers from the aluminum foil. The fibers are then stored in suitable conditions away from contamination.[18]

Application of nylon 6,6 nanofibers

In this study, nylon 6,6 nanofibers are used as membranes to filter liposomes, ensuring the removal of large molecules and obtaining a homogeneous solution of the final product.[19]

In vitro release assay

Slow-release study of BLV_2 loaded encapsulated with different ratios of carbopol gel (0.5, 1, and 1.5%) in buffer fluid. The release of baclofen was investigated at different times (1, 2, 3, 4, 5, 6, 7, 8, 9, 10) h;[12] the amount of BLV_2 released from the (BLV_2 carbopol gels) was assessed using UV-Vis spectroscopy at (218) nm. A standard curve was used to determine the concentration of released baclofen and the percentage of release was calculated using the following equations [Table 1],

Table 1: Formulation code for preparation of liposomes

Formulation	Baclofen (mg)	Soya lecithin (mg)	Cholesterol (mg)	Surfactants (mg)
BLV_1	50	80	10	Tween 80 (10) Span 80 (10)
BLV_2	50	85	10	Tween 80 (15)
BLV_3	50	80	10	Span 80 (20)
BLV_4	50	85	10	Sodium deoxycholate (15)

BLV: Baclofen liposome vesicles

Amount of baclofen released (mg/mL) = Concentration × dissolution bath volume × dilution factor/1000

Percentage of baclofen release = (amount of baclofen released/ weight of nanogel liposomes vesicle) ×100.

Enzyme inhibition mechanisms and methods assay

Effect of baclofen liposomes on lactate dehydrogenase enzyme

According to the manufacturer's instructions (BIOLABO SAS, France), the estimation of LDH enzyme activity was assessed using tris pH 7.2 (80 mmol/L) as a buffer, pyruvate (1.6 mmol/L) as a substrate, and NADH (0.2 mmol/L) as a coenzyme.

The mechanism mentioned is the reaction of the enzyme LDH which catalyzes the conversion of pyruvate to lactate.[20] The LDH enzyme, which generates one µM of lactate per minute at 37°C, is the particular indicator of enzyme activity [See Scheme 1]. As LDH enzyme inhibitors, diluted solutions (15, 30, 45, 60, 75, and 100 ppm) of baclofen liposome vesicles were added. Twenty µL of the inhibitor and serum pool (for patients with muscle spasticity) were added after 1 ml of the working reagent had been in a water bath for 5 min at 37°C. At 340 nm, the activity was measured after 30 s, 1 min, and 2 min by UV-Vis Spectrophotometer.[21] Equation (1) was used to estimate the enzyme activity rate.

$$UI\backslash L = (\Delta Abs/min) \times 8095 \quad (1)$$

While Equation (2) was used to measure the percentage inhibition.

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Activity}(IU\backslash L)\text{with inhibitors}}{\text{Activity}(IU\backslash L)\text{whiout inhibitors}}\right) \times 100 \quad (2)$$

Effect of baclofen nano liposomes vesicles on creatine kinase enzyme

According to the manufacturer's instructions (Biosystem, Spain) See Scheme 2 , the estimation of CK enzyme activity was assessed[21] using Buffer/Glucose/NAC. The reaction mixture contained imidazole buffer (IMI buffer, 100 mmol/L, pH 6.7), glucose (GLC, 40 mmol/L), N-acetyl-L-cysteine (NAC, 30 mmol/L), magnesium acetate (MgAc, 15.3 mmol/L), nicotinamide adenine dinucleotide phosphate (NADP, 2.5 mmol/L), and hexokinase (HK, ≥4000 U/L).

To inhibit the CK enzyme, several diluted concentrations of drug-loaded liposomes vesicles (15, 30, 45, 60, 75, and 100) ppm were prepared. Thus, 1 ml of the CK enzyme the working reagent put in a water bath for 5 min at 37°C, then 20 µL of each of the inhibitor and serum (from people suffering from severe muscle spasms) were added. The absorbance of the enzyme activity was measured after and before adding the inhibitor at 1, 2, and 3 min using UV Spectrophotometer at a maximum wavelength of 340 nm.[21] The enzyme activity rate estimated using Equation (1).

$$UI\backslash L = (\Delta Abs/min) \times 8095 \quad (1)$$

An inhibition percentage is measured by Equation 2.

$$\% \text{ Inhibition} = 100 - \left(\frac{Activity(IU\backslash L)\,with\ inhibitors}{Activity(IU\backslash L)\,whiout\ inhibitors}\right) \times 100 \quad (2)$$

Half-maximal inhibitory concentration calculations:

The half-maximal inhibitory concentration values were calculated using the % of inhibition versus concentration curve (BLV_2) for both the enzymes Lactate dehydrogenase (LDH) and creatine kinase (CK).

Data availability statement

Data are available from the author upon reasonable request.

Results

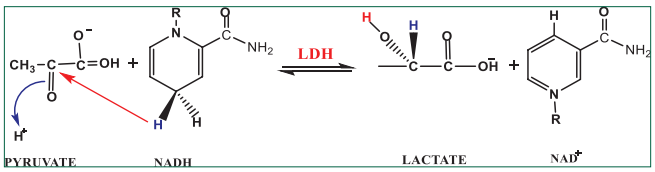
Characterization of baclofen

Wavelength maximum (λ_{max}) of baclofen

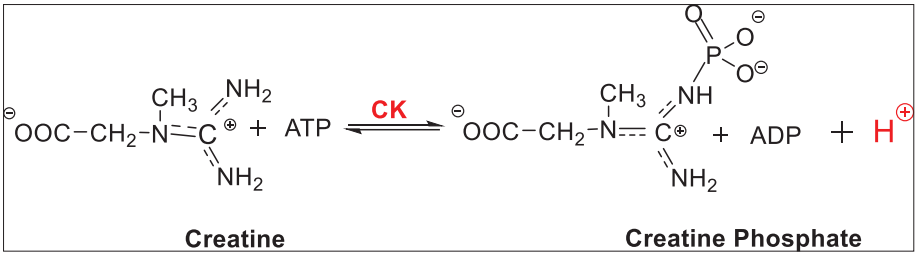
Using a UV-Vis spectrophotometer, a spectral scan was performed in the range (200–400 nm) for a solution containing 25 µg/ml of baclofen in PBS. The maximum absorption wavelength (λ_{max}) was identified at 218 nm, demonstrating close agreement with previously reported values in the literature[14], as summarized in Table 2 and Figure 1.

Calibration curve

As shown in Figure 1, a linear correlation (R = 0.9993) was observed between absorbance and concentration, indicating



Scheme 1: Enzymatic Reduction of Pyruvate to Lactate by Lactate Dehydrogenase (LDH)



Scheme 2: Phosphorylation of Creatine by ATP Catalyzed by Creatine Kinase (CK)

a strong linear relationship consistent with the Beer-Lambert law and confirming the reliability of the calibration curve.

Fourier transform infrared spectroscopy analysis

In this study, Fourier transformation infrared spectroscopy was used to identify the chemical and physical interactions between baclofen and baclofen-loaded liposomes. The FTIR spectral analysis of baclofen revealed a prominent absorption band at 3522 cm^{-1}, corresponding to the N–H stretching vibration, and another characteristic peak at 3381 cm^{-1}, attributed to the O–H stretching vibration of the hydroxyl functional group. The aliphatic C-H group exhibited a medium absorption band extending between 2926 and 2895 cm^{-1}. A peak at 1647 cm^{-1} was also identified, attributed to the vibrational stretching of the carboxylic C = O bond, and a peak at 1508 cm^{-1} was attributed to the vibrational stretching of the aromatic C = C bond. A peak at 785 cm^{-1} was observed, attributed to the bending stretching of the C-Cl bond.[22]

Dynamic light scattering and zeta potential analysis

DLS measurement results showed that the average hydrodynamic diameters of all samples ranged between 36.67 and 172.6 nm. DLS measures the diameter of particles in solution based on Brownian motion, meaning it measures the size of particles including the wetting layer of water molecules. PDI values were calculated for all samples, and the values were within an acceptable range (0.369–0.663), as shown in Table 3. In order to examine the surface charge and stability of BLV_2, a zeta-potential investigation was conducted. The results revealed that the synthesized BVL2 has good stability, as evidenced by the emergence of a distinct peak at roughly (– 30 to -105)mV or in -65.6 [Figure 2].

Table 2: Graph data of baclofen in phosphate buffer saline pH6.8

Concentration (ppm)	Absorbance
0	0
5	0.235±0.001
10	0.459±0.037
15	0.653±0.002
20	0.869±0.0014
25	1.101±0.068

Table 3: All formulation hydrodynamic diameters and polydispersity index

Formulation	Hydrodynamic diameters	PDI
BLV_1	173.6 nm	0.486
BLV_2	36.67 nm	0.359
BLV_3	167.2	0.514
BLV_4	169.8	0.663

PDI: Polydispersity index, BLV: Baclofen liposome vesicles

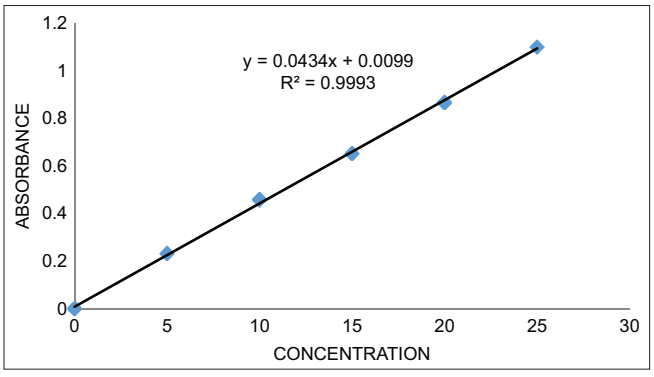


Figure 1: Calibration curve of baclofen in phosphate buffer saline

Field emission scanning electron microscopy results for nylon 6,6 nanofibers

Figure 3 illustrates the morphological characteristics of nylon 66 nanofibers synthesized using an electrospinning device. Based on the field emission scanning electron microscopy (FESEM) images, Figure 3 shows that the fibers have an average diameter of 57.29 nm.[23]

Enzyme inhibition mechanisms

An inhibiting activity of BLV2 at lactate dehydrogenase with 0.15 mmo l/L, while inhibition dose on creatine kinase enzyme 90 mmol/L.

Determination of the effect of baclofen nano liposomes vesicles on inhibition of (lactate dehydrogenase) enzyme

Table 4 shows the relationship between substrate concentration [S] and the rate of the enzyme reaction in the absence of an inhibitor.

Michaelis–Menten curve for the enzyme LDH in the absence of an inhibitor is shown in Figures 4 and 5.

Table 5 shows the percentage inhibition of LDH enzyme activity estimated at 356 using liposomes at six dilute concentrations.

While Table 6 shown Substrate concentrations values and enzymatic reaction rate of LDH after adding baclofen liposomes vesicles as an inhibitor.

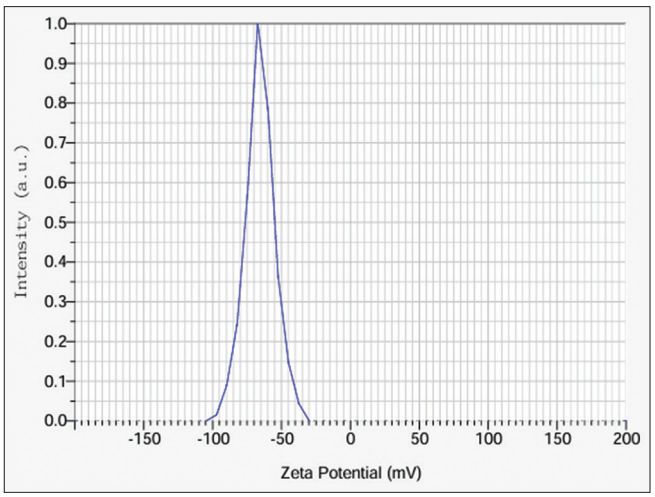


Figure 2: Zeta potential spectra of the obtained of baclofen nanoliposome vesicle

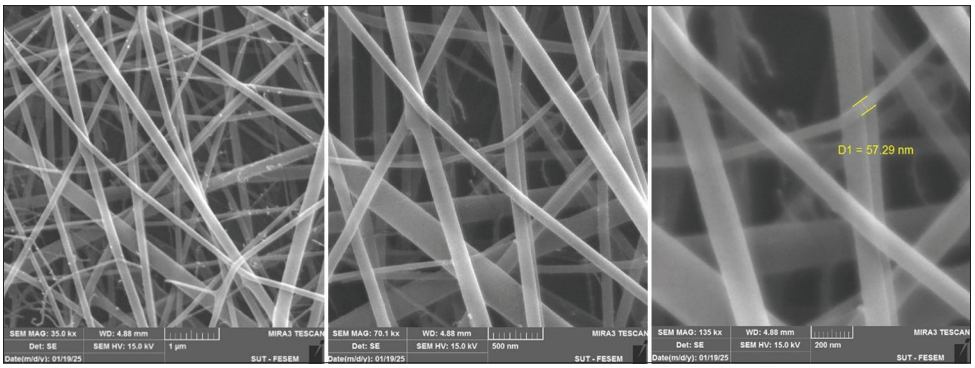


Figure 3: Field emission scanning electron microscopy images of nylon 66 nanofibers

Table 4: Substrate concentration values and enzymatic reaction rate of lactate dehydrogenase before adding baclofen liposome vesicles2 as an inhibitor

V (UI/L)	S (mmol/L)
130	0.05
170	0.07
259	0.11
332	0.15
348	0.18
356	0.2

S: Substrate concentrations, V: Enzymatic reaction rate

Table 5: The estimated effectiveness of baclofen liposomes vesicles$_2$ in inhibiting the lactate dehydrogenase enzyme was 356 UI/L

Inhibitor concentration (ppm)	Enzyme activity (U/L)	Inhibitor (%)
15	300	16
30	275	27
45	227	36
60	219	38
75	194	46
100	170	52

In vitro release study

As the results of the synthesis and characterization of BLV2 loaded encapsulated with different ratios of carbopol gel (0.5, 1, and 1.5%) in buffer fluid see Table 7. This study includes measuring the release of (BLV2). The results refer to the effect of carpapol is most commonly stable and nontoxic when used as plasticizers, which can increase the flexibility and permeability of water and reduce stiffness. It has been observed that the release is rapid at the minimum ratio of the carbopol 934 used and from the first hr. of immersion in buffer . Moreover, the release has been more restricted with increasing carbapol ratio against the duration of release see Figure 6.

Determination of the effect of liposomes baclofen vesicles on inhibition of (creatine kinase) enzyme

Data in the Table 8 were shown the relationship between substrate concentration [S], and the rate of the enzyme reaction

Table 6: Substrate concentration values and enzymatic reaction rate of lactate dehydrogenase after adding baclofen liposomes vesicles$_2$ as an inhibitor

V (UI/L)	S (mmol/L)
117	0.05
137	0.07
162	0.11
202	0.15
227	0.18
267	0.2

S: Substrate concentrations, V: Enzymatic reaction rate

Table 7: Percentage release of baclofen liposomes vesicles$_2$ gel

Time (h)	BLV$_2$ with 0.5 (%) carbopol	BLV$_2$ with 1 (%) carbopol	BLV$_2$ with 1.5 (%) carbopol
0	0	0	0
1	13.3±0.02	9.46±0.015	6.75±0.02
2	19±0.0264	16.81±0.15	15.06±0.1
3	29.58±0.102	25.76±0.019	23.55±0.09
4	43.87±0.210	39.83±0.054	38.25±0.05
5	49.78±0.07	45.71±0.801	43.27±0.016
6	58.423±0.489	52.56±0.3	48.50±0.02
7	65.52±0.453	57.58±0.2	54.77±0.61
8	71.65±0.300	64.29±0.005	60.43±0.5
9	75.45±0.736	71.94±0.2	66.25±0.12
10	80.75±0.11	74.38±0.1	70.25±0.89

BLV: Baclofen liposome vesicles

in the absence of an inhibitor. In addition, Figure 5 shows that the curve takes a typical shape for enzymes that follow the Michaelis–Menten equation curve in the absence of an inhibitor. Therefore, the substrate concentration increases, and enzymatic reaction rate increases. This is due to the availability of binding sites in the enzyme.

Table 9 shows percentage inhibition CK enzyme active estimated at 440 UI/L Using six diluted concentrations of baclofen-loaded liposomes, the highest inhibition rate reached 66% at the highest diluted concentration. This supports the inhibitor's ability to affect the activity of the CK enzyme by binding to ES and E via hydrogen bonds believed to form

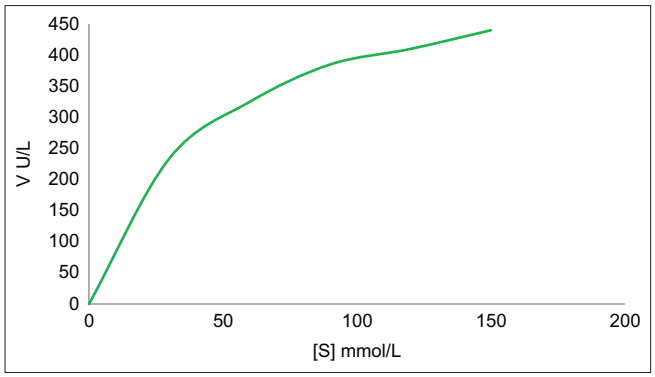


Figure 4: Michaelis–Menten equation's curve between the rate of enzymatic reaction V and Substrate concentration [S]

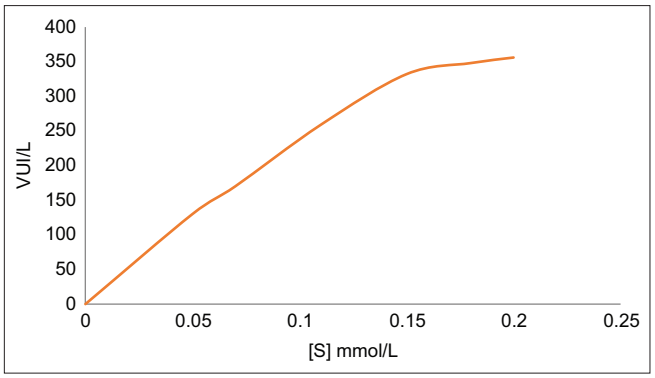


Figure 5: Michaelis—Menten equation's curve between the rate of enzymatic reaction V and substrate concentration [S]

between the functional groups in baclofen and the active binding sites.[21]

Hance, Table 10 were recorded Substrate concentrations values and enzymatic reaction rate of CK after adding baclofen liposomes vesicles as an inhibitor.

As noted in Table 11, the values of Km and V_{max} are shown without an inhibitor (45,588) respectively. When an inhibitor was added, the V_{max} value decreases to (500) and the Km value remains constant.

Discussion

FTIR spectrum of the liposomes loaded-baclofen, there was a slight change in the main baclofen peaks. So that were indicated to a very minor chemical interactions occurred that did not affect baclofen's effectiveness. It generally known that liposomes do not interact significantly with the active ingredient, but rather surround it. Some minor interactions may occur, indicating the successful loading of baclofen into the liposomes. This confirms the good compatibility between baclofen and the additives.[24] Moreover, the appearance of DLS and PDI values in the nanoscale field and a PDI value of <0.7 indicates a uniform distribution of nanoparticles and the absence of nanoparticle agglomerates.[25-27] It is well known that a nanomaterial's physicochemical properties, such as its size, shape, surface area, zeta potential, and composition, have a substantial impact on the results of their cytotoxicity. The biological activity of baclofen nanoliposome vesicles is significantly influenced by their shape, size, and concentration. In order to examine the surface charge and stability of BLV_2, a zeta-potential investigation was conducted. The results revealed that the synthesized have good stability, as evidenced by the emergence of a distinct peak at roughly −65.6 mV.

The FESEM images in Figure 3 show a dense network of regular, intertwined fibers belong to nylon 66 nanofibers which appear to be free of beads. This is due to the good balance of the polymer solution and its compatibility with the parameters of the electrospinning device.[23,28] It was be noted that the fiber structure exhibited high interlocking with good porosity, demonstrating the success of the electrospinning device in producing nylon 66 nanofibers. These optical properties confirm the fibers' efficiency in filtering liposomes. The results of this study are consistent with previous studies,[16,29] which demonstrated that the balanced composition of the polymer solution and the absence

Table 8: Substrate concentration values and enzymatic reaction rate of creatine kinase before adding baclofen liposomes vesicles2 as an inhibitor

V (UI/L)	S (mmol/L)
234	30
325	60
385	90
410	120
440	150

S: Substrate concentrations, V: Enzymatic reaction rate

Table 9: The estimated effectiveness of baclofen liposome vesicles in inhibiting the creatine kinase enzyme was 440 UI/L

Inhibitor concentration (ppm)	Enzyme activity (U/L)	Inhibitor (%)
15	400	10
30	345	21
45	305	31
60	243	45
75	213	52
100	152	66

Table 10: Substrate concentration values and enzymatic reaction rate of creatine kinase after adding baclofen liposomes vesicles$_2$ as an inhibitor

V (UI/L)	S (mmol/L)
202	30
265	60
310	90
382	120
401	150

S: Substrate concentrations, V: Enzymatic reaction rate

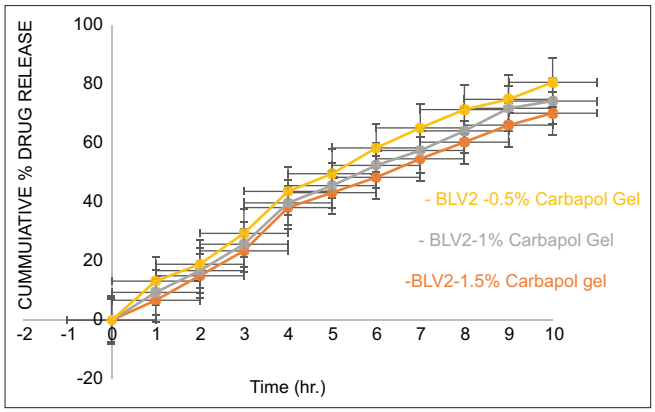


Figure 6: *In vitro* release for BLV_2 with different concentrations of Carbopol 934 in (phosphate buffer saline). BLV: Baclofen liposome vesicles

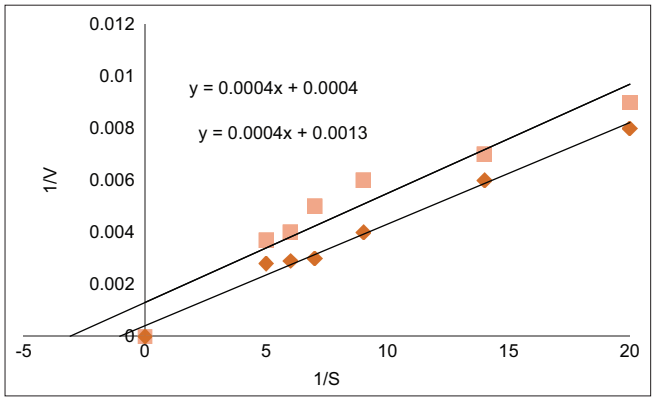


Figure 7: Line Weaver Burk Plot: Equation of lactate dehydrogenase enzyme without and with inhibitor (V^{-1} with inhibitor V^{-2} without inhibitor)

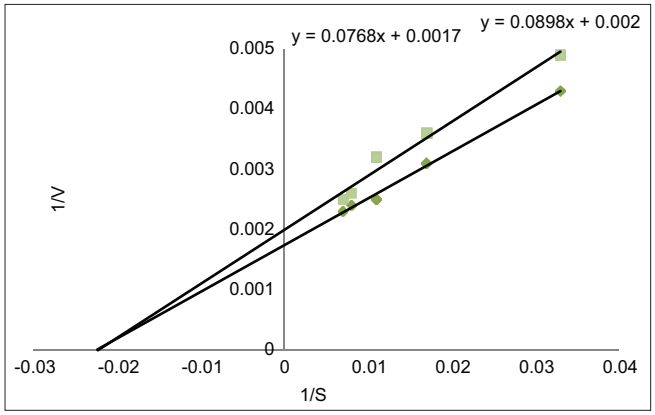


Figure 8: Line Weaver Burk Plot: Equation of creatine kinase enzyme without and with inhibitor (V^{-1} with inhibitor, V^{-2} without inhibitor)

of other materials in the solution led to the formation of nanofibers that were ideally free of beads.

Notably, *in vitro* release data of BLV_2 study includes measuring the release of BLV2 with different ratio of Carbopol 934 gel in PBS solutions. Accordingly, the results refer to the effect of 0.5% of carbopol 934 gel is most commonly stable and nontoxic, which can increase the flexibility and permeability of water and reduce stiffness. Hence, the release has been more restricted with increasing carbopol ratio against the duration of release see Figure 6.

In vitro study, Figure 4 refers to that: any rise in substrate concentration does not lead to an increase in the rate of the reaction because of enzyme binding saturation sites.[30] Both of V_{max} and Km values were recorded without, and with (baclofen nanoliposomes) as inhibitor (2500, 1) (769, 0.3) respectively [Table 12]. Wherefore a type of inhibition was uncompetitive. In this type of inhibition, the inhibitor binds to the enzyme-substrate (ES) complex rather than to the free enzyme, leading to a reduction in the activity of the LDH enzyme about 52%. This interpretation supported by the graph shown in Figure 7 where the lines are nearly parallel, a behavior characteristic of noncompetitive inhibition.[31] The inhibitor's role had created hydrogen bonds with ES that believed to form between the functional groups in baclofen and the active binding sites.[21]

Forther, CK enzyme data can be interpreted as an increasing substrate value with a gradual decrease in an enzymic reaction rate until it reaches a maximum, where all binding sites in the enzyme are saturated [Table 9].[30] As well as the changes indicated that the type of inhibition was noncompetitive inhibition about 66% using baclofen nanoliposomes. In this type, the noncompetitive inhibitor binds to the ES complex as well as to the free enzyme, leading to a reduction in the activity of the CK enzyme. The Line Weaver Burk diagram shown in Figure 8 supports this interpretation. This diagram shows the behavior characteristic of noncompetitive inhibition.[32]

Conclusion

Nanocomposites help stabilize components. Recently baclofen, a muscle relaxant of the GABA derivative family, began to be innovatively used for the symptomatic treatment of spasticity due to brain or spinal injury. Novel vesicular formulations of baclofen containing nanoliposomes were developed and characterized to improve the drug effectiveness. In order to develop nanoliposome vesicle formulations of baclofen and evaluate their potential application as more effective drug carriers in the treatment of spasticity, different formulations of them were developed and their physical–chemical and technological properties were assessed. All these were demonstrated through spectroscopic and morphological characterization tests and biological investigation of the resulting BLV_2.

Outcomes of the study

Baclofen liposomes vesicles were formed using a nylon 66 membrane network. The inhibitory activity of BLV_2 was also studied *in vitro* study.

Rationale of the study

Baclofen, a drug used to treat muscle spasms, has disadvantages when taken orally. Research is needed to develop a drug that

Table 11: The kind of inhibition for nano baclofen liposome vesicles affecting the creatine kinase enzyme

Name of compound	Kind of inhibition	K_m	V_{max}
BLV	Noncompetitive inhibition	Without inhibitor: 45 With inhibitor: 45	Without inhibitor: 588 With inhibitor: 500

BLV: Baclofen liposome vesicles

Table 12: The kind of inhibition for nano baclofen liposomes vesicles affecting the lactate dehydrogenase enzyme

Name of compound	Kind of inhibition	K_m	V_{max}
BLV	Uncompetitive inhibition	Without inhibitor: 1 With inhibitor: 0.3	Without inhibitor: 2500 With inhibitor: 769

BLV: Baclofen liposomes vesicles

can be used as an immediate treatment for muscle spasms by targeting the inhibition of the relevant enzymes.

Limitation of the study

Raw materials and characteristic assays are expensive, and there is no financial support.

Author contribution

I.Q.F. conceived of the presented idea, developed the theory, and performed the computations. H.K.T. carried out the experiment and I.Q.F. verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ranjan A, Kumar A, Sinha N, Kumar A. Leprosy presenting as distal acquired demyelinating symmetric variant of chronic inflammatory demyelinating polyneuropathy: An atypical manifestation of Hansan's disease. Int J Mycobacteriol 2024;13:218-20.
2. Gunjal R, Gajbhar A, Vichare V, Sutar A, Deshmukh M, Choudhari V. Development and validation of chemometric-assisted spectrophotometric method for the simultaneous estimation of aceclofenac, paracetamol, and chlorzoxazone with impurities. Biomed Biotechnol Res J 2022;6:458-65.
3. Mat Luwi NE, Kadir R, Mohamud R, Garcia-Santana ML, Acevedo R, Sarmiento ME, *et al.* Liposomes derived from *Mycobacterium* smegmatis promote immune activation of mice bone marrow-derived dendritic cells. Int J Mycobacteriol 2020;9:261-7.
4. Mujwar S. Computational bioprospecting of andrographolide derivatives as potent cyclooxygenase-2 inhibitors. Biomed Biotechnol Res J 2021;5:446-50.
5. Romito JW, Turner ER, Rosener JA, Coldiron L, Udipi A, Nohrn L, *et al.* Baclofen therapeutics, toxicity, and withdrawal: A narrative review. SAGE Open Med 2021;9:20503121211022196. - PMC - PubMed.
6. Dudek CM, Yee CH, Greenberg M, McCarver SC. Baclofen overdose with unique cardiovascular effects. BMJ Case Rep 2024;17:e260869.
7. Khattak MI, Ahmed N, Umer MF, Riaz A, Ahmad NM, Khan GM. Chloroform-injection (CI) and spontaneous-phase-transition (SPT) are novel methods, simplifying the fabrication of liposomes with versatile solution to cholesterol content and size distribution. Pharmaceutics 2020;12:1065.
8. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: Structure, composition, types, and clinical applications. Heliyon 2022;8:e09394.
9. Sahoo SK. Formulation and characterization of liposomes. In: Anandharamakrishnan C, Dutta S, editors. Liposomal Encapsulation in Food Science and Technology. sciencedirect: Academic Press; 2023. p. 39-63.
10. Abbasi H, Kouchak M, Mirveis Z, Hajipour F, Khodarahmi M, Rahbar N, *et al.* What we need to know about liposomes as drug nanocarriers: An updated review. Adv Pharm Bull 2023;13:7-23.
11. Bharathy MS, Jeyaleela GD, Vimala JD, Aranganathan A, Margrat SS. Comparison of anti-inflammatory activities of biogenic *Gymnema sylvestre*- and paricum sumatrense-mediated titanium dioxide nanoparticles. Biomed Biotechnol Res J 2021;5:405-11.
12. Fatima N, Ilyas N, Babu S. Formulation and *in-vitro* evaluation of baclofen loaded transferosomal gel. J Drug Deliv Ther 2023;13:4-14.
13. AbdulKhaleq NM. Formulation and Evaluation of Baclofen as a Model Gastroretentive Solid Drug Delivery System Using FDM 3D Printing Technique, Ph.D. Dissertation, Dept of Pharmaceutics, Coll. of Pharmacy, Univ of Baghdad, Baghdad, Iraq; 2023.
14. Large DE, Abdelmessih RG, Fink EA, Auguste DT. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. Adv Drug Deliv Rev 2021;176:113851.
15. Mura P, Maestrelli F, Cirri M, Nerli G, Di Cesare Mannelli L, Ghelardini C, *et al.* Improvement of butamben anesthetic efficacy by the development of deformable liposomes bearing the drug as cyclodextrin complex. Pharmaceutics 2021;13:872.
16. Taher MK, Falih IQ, Abdullah YJ. Design of delivery systems (nanoemulsions and biopolymer nanoparticles) of cloves essential oil: Preparation, characterizations, study the release, and antioxidant activity. Prev Diagn Treat Strat Med 2024;3:163-70.
17. Kanu NJ, Chavan SS, Jadhav GV, Hude NS, Ganesh RP, Patil JT. Manufacturing of nylon 6, 6 nanofibers using electrospinning. Int J Anal Exp Finite Elem Anal 2019;6:67-9.
18. Bairagi S, Khandelwal G, Karagiorgis X, Gokhool S, Kumar C, Min G, *et al*. High-performance triboelectric nanogenerators based on commercial textiles: Electrospun nylon 66 nanofibers on silk and PVDF on polyester. ACS Appl Mater Interfaces 2022;14:44591-603.
19. Kumar S, Kumar S. Transfersomes based dermal gel of baclofen for management of chronic pain. Eur Chem Bull 2023;12:8417-40.
20. Khan AA, Allemailem KS, Alhumaydhi FA, Gowder SJ, Rahmani AH. The biochemical and clinical perspectives of lactate dehydrogenase: An enzyme of active metabolism. Endocr Metab Immune Disord Drug Targets 2020;20:855-68.
21. Neamah SA, Falih IQ, Albukhaty S, Sasikumar P. Phytochemical characteristic analysis and biological activity for *Capparis spinosa* L. Fruit extract. Rafidain J Sci 2024;33:68-77.
22. Abdulkhaleq NM, Ghareeb MM. Development of gastro-floating drug delivery system by 3D printing: Impact of formulation and design on the release profile of baclofen. J Contemp Med Sci 2022;8:413-9.
23. Nguyen TN, Lee DH, Kim JJ. Incorporation of silica particles attached to nylon 66 electrospun nanofibers with cement. Materials (Basel) 2022;15:7011.
24. Lombardo D, Kiselev MA. Methods of liposomes preparation: Formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. Pharmaceutics 2022;14:543.

25. Perrie Y, Ali H, Kirby DJ, Mohammed AU, McNeil SE, Vangala A. Environmental scanning electron microscope imaging of vesicle systems. Methods Mol Biol 2010;606:319-31.
26. Alkhatib MH, Bayoumi HM. Topotecan incorporation into avocado oil-based nanoemulsion potentiates its inhibitory effect on the cellular growth of various human cancer cells. Biomed Biotechnol Res J 2024;8:474-86.
27. Neamah SA, Albukhaty S, Falih IQ, Dewir YH, Mahood HB. Biosynthesis of zinc oxide nanoparticles using *Capparis spinosa* L. fruit extract: Characterization, biocompatibility, and antioxidant activity. Appl Sci 2023;13:1-14.
28. Sriyanti I, Agustini MP, Jauhari J, Sukemi S, Nawawi Z. Electrospun nylon-6 nanofibers and their characteristics. Scientific Journal of Physics Education 2020;9:9-19.
29. Jauhari J, Wiranata S, Rahma A, Nawawi Z, Sriyanti I. Polyvinylpyrrolidone/cellulose acetate nanofibers synthesized using electrospinning method and their characteristics. Mater Res Express 2019;6:064002.
30. Sriyanti I, Edikresnha D, Rahma A, Munir MM, Rachmawati H, Khairurrijal K, "Correlation between structures and antioxidant activities of polyvinylpyrrolidone/ Garcinia mangostana L. extract composite nanofiber mats prepared using Electrospinning," J. Nanomater., vol. 2017, doi: 10.1155/2017/9687896.
31. Roskoski R. "Michaelis-Menten kinetics," in Reference Module in Biomedical Research, Elsevier, 2015, doi: 10.1016/B978-0-12-801238-3.05143-6.
32. Waldrop GL. "Protein function: Kinetics of enzyme inhibition," in Encyclopedia of Biological. Chemistry,Elsevier; 3rd ed., vol. 3, p. 14-20, 2021, doi: 10.1016/B978-0-12-819460-7.00027-X.