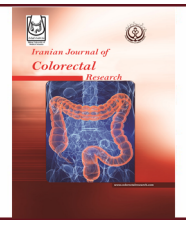
Original Article

Iran J Colorectal Res 2021;9(4):149-157.

Iranian Journal of Colorectal Research



Development of a Novel Niosomal Formulation for Gabapentin

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Received: 10-10-2021
Revised: 20-12-2021
Accepted: 20-12-2021

Abstract

Introduction: Gabapentin is an anticonvulsant drug prescribed to treat partial seizures and neuropathic pain. Niosomes, as lipid-based drug carriers, can improve the pharmacokinetic properties of therapeutic agents. In this study, we developed an optimal niosomal formulation for gabapentin and assessed its cytotoxicity effect on normal cells and a colon cancer cell line.
Methods: Several niosomal formulations were developed and analyzed regarding physicochemical properties. For the G_3 and G_4 formulations, the release profiles complied much better with the Korsmeyer-Peppas model, suggesting the Fickian diffusion mechanism in gabapentin release. The effect of the optimized niosomal formulation of gabapentin on the SW48 colon cancer cell line was assessed using the MTT assay.
Results: The niosomal formulation of G3 showed 60% drug release in 48 hours, and the G4 formulation showed 52%. The cytotoxic effect of the optimized formulation (G3) on the colon cancer cell line resulted in an IC50 of 45 µg/ml (200 µM) after 48 h, compared to 0.2 mg/mL (1.17 mM) for free gabapentin. Hence, the niosomal formulation of gabapentin was more cytotoxic for the colon cancer cell line than pure gabapentin.
Conclusion: The optimal niosomal formulation of gabapentin exhibited good storage stability and provided slow, prolonged release. This formulation showed cytotoxic effects on colon cancer cells without significant toxicity for normal fibroblasts.

Keywords: Gabapentin, Drug delivery, In vitro, Niosomes, Span

Please cite this paper as:
Akbarzadeh I, Tabarzad M, Khazraei H, Ostovan VR. Development of a Novel Niosomal Formulation for Gabapentin. *Iran J Colorectal Res*. 2021;9(4):149-157. doi: 10.30476/ACRR.2022.93824.1120.

Introduction

Gabapentin, first approved in 1993, is a structural analog of gamma-aminobutyric acid (GABA). GABA is an inhibitory neurotransmitter, and its analog was approved as an anticonvulsant medication. According to the increased number of diabetic patients suffering from complications like neuropathic pain, gabapentin holds significant value in the drug market in developing countries (1, 2).

The oral bioavailability of gabapentin is less than 60%. Accordingly, novel drug delivery systems can

improve the pharmacokinetic properties of gabapentin for oral delivery while introducing other ways of administration for particular indications, including efficient topical administration for neuropathic pain of diabetic patients. In such situations, a novel drug delivery route might reduce the severity and number of adverse reactions following gabapentin administration (3, 4). Newly developed delivery systems have many advantages like enhanced bioavailability, diminished side effects, and lower dosing frequency. For example, it was reported that a novel delivery system of gabapentin that had a one-layer expandable gastroretentive controlled system could extend its release in the gastrointestinal system by six hours (5). Another report explained the delivery of gabapentin by floating microspheres, prolonging the drug delivery and improving the release and analgesic activity (6). The microemulsion formulation for the transdermal flux of gabapentin was studied in vitro, suggesting potential vehicles for transdermal delivery of gabapentin (7).

The use of nanosystems to transport drug molecules toward the desired site of action represents an attractive and valuable field of pharmaceutical research. Novel drug delivery systems, especially nanosized ones, have significantly improved the therapeutic efficacy and safety of conventional drugs. They also can provide sustained or controlled release systems to minimize the rate of adverse effects (8).

For orally administered drugs, optimized delivery systems are necessary for protection against gastric acid and enzymes. Such systems can improve drug bioavailability and minimize the impact of food consumption on drug absorption. In this regard, nano delivery systems show promising capabilities, particularly for oral administration of highly polar drugs, macromolecular therapeutic agents, and highly sensitive drug molecules (9-11).

Liposomes, microspheres, niosomes, and other nanoparticulate systems for drug delivery have been extensively studied. Niosomes were introduced in 1985 as an improved version of liposomes. They are more stable than liposomes and are efficient delivery systems and non-toxic nanostructures for medical applications. Niosomes are produced from non-ionic surfactants and can enhance the uptake of payloads into the cell cytoplasm. In addition to non-ionic surfactants, a steroidal molecule (cholesterol) is also incorporated into the niosome structure, improving the rigidity of the bilayer and its similarity with the cell membrane. Niosomes nanosystems can protect the drug before reaching the site of action while improving the cell permeation for reaching intracellular targets (12, 13). Niosome nanostructures can be administered via the ophthalmic (14, 15), intramuscular (16), intravenous (17, 18), intrathecal (19), oral (20, 21), and transdermal (22-24) routes.

Various drug niosomal formulations have been studied in targeted, controlled, and sustained release systems (25-27). Niosomal formulations of gabapentin may improve its pharmacokinetic properties following oral administration, enhance its permeation through the blood-brain barrier, and make it a potential topical formulation for neurological pain. This study developed novel niosomal formulations of gabapentin and subjected them to physicochemical *in vitro* analysis studies. Subsequently, the cytotoxicity of the optimal niosomal formulation was evaluated on normal and colon cancer cells.

Methods

Materials

Gabapentin was tenderly donated as a gift sample by Tehran Daru Co. (Tehran, Iran). Cholesterol, Tween™ 60 (Polysorbate 60), Span™ 60 (Sorbitan monostearate), and dialysis membranes (MWCO 12000 Da) were supplied by Sigma Aldrich (USA), while phosphate-buffered saline (PBS) was obtained from BIO BASIC (Canada). Methanol, chloroform, Amicon® ultrafiltration units (Ultra-0.5-Membrane, MWCO 30000 Da), and the Milli-Q® water purification system (Millipore, Darmstadt, Germany) were also used. The colon cancer cell line (SW48) and human fibroblast cells were purchased from Pasture Institute, Tehran, Iran. Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA, and fetal bovine serums (FBS) were supplied by Gibco (Thermo Fisher Scientific, Germany). MTT materials were purchased from Sigma-Aldrich Chemicals (Bornem, Belgium).

Compatibility Study

The interaction of drugs with niosomes excipients was studied by Fourier transform infrared (FTIR) spectroscopy using the NICOLET 6700 FTIR (USA) device in solid-state. The potassium bromide pellets were scanned at room temperature between 4000 cm^{-1} to 400 cm^{-1} at a constant resolution of 4 cm^{-1}.

Preparation of Niosomes

The thin layer hydration approach was appropriated to prepare the niosomal formulations (28). For the first step, cholesterol and the surface-active agent (1:2 molar ratios) were dissolved in chloroform and methanol (10 mL, 2:3 v/v); the organic solvent was separated in a rotary evaporator (Heidolph Instruments, Germany) over 30 min at 150 rpm and 60 ºC. Next, to achieve the niosomal formulations, the residual dried thin films were hydrated for 30 min utilizing gabapentin solution (in PBS, pH 7.4) at 60 ºC and 120 rpm. Eventually, the samples were sonicated (Hielscher up50H ultrasonic processor, Germany) for 7 min to accomplish equal niosome size distribution and were cached in a refrigerator (4 °C). Different molar ratios of cholesterol, Tween 60, and Span 60 were examined to explore the effect of vesicle composition on niosomal properties (Table 1).

Table 1: Different niosomal formulations for encapsulation of gabapentin.

Niosomal formulation	Surfactants:cholesterol % molar ratio	Surfactant blend	
		Tween 60 (%)	Span 60 (%)
G1	1:1	0	100
G2	1:1	30	70
G3	1:1	50	50
G4	1:1	70	30

Size, Morphology, and Polydispersity Index (PDI)

Dynamic light scattering (DLS) was used to determine the average size and the size distribution of niosomes (Malvern Instrument, U.K.). Concisely, 1:100 dilutions of niosome formulations were prepared using deionized water to evade the multiple scattering phenomenon because of antiparticle interactions. In due course, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were also used to study the morphology of niosomal particles (Zeiss EM900 Transmission Electron Microscope, Germany).

In Vitro Release Studies

The gabapentin release profiles were studied in PBS buffer (pH 7.4) along the dialysis membrane (MWCO 12 KD); 2 mL of gabapentin-loaded niosomes and free gabapentin were dialyzed into 50 mL of PBS buffer solution for 72 h at 37 °C. Subsequently, 1 mL of PBS was replaced with an equal volume of PBS at specified time points (0, 0.5, 1, 2, 4, 8, 24, 48, and 72 h). Released gabapentin concentrations were determined with UV-visible spectrophotometry. The niosomal samples' release kinetic mechanism was analyzed by adopting zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models.

Stability Studies

The optimized niosome formulation was stored under two different storage conditions for stability assessment. Each formulation was classified into two assortments kept at 25±1 °C (room temperature) and 4±1 °C (refrigeration temperature) for one month. The mean particle size (nm) and entrapment efficiency (EE) were enumerated at definite time intervals (0, 14, and 30 days).

Cell Culturing and Experimental Model

The SW48 colon cancer cell line and human fibroblast cells (normal) were studied. The cell lines were cultured in DMEM media with 5% FBS at 37 °C with 5% CO2. The cells grew in flasks within 48 h before being trypsinized and seeded at the concentration of 5×10^5 cell/well in 96-well plates. They were then incubated for 24 h with the same media and conditions. After 24 h, the cells were observed by microscopy, and if the morphology was normal, niosome and pure gabapentin were added to the plates ahead of 48 h of incubation.

Cell Cytotoxicity Study

We used the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay to quantitatively assess cell viability and monitor the mitochondrial activity of viable cells. The cells were treated with gabapentin at different concentrations, blank niosome (without gabapentin), and different concentrations of optimally formulized gabapentin-loaded niosomes. Each sample was added to wells in triplicates and incubated for 48 h (optimum time in our study) at 37 °C. Then, MTT reagent (20 µL) was added ahead of 4 h of incubation. The absorbance was taken on a multi-detection microplate reader at 570 nm wavelength. 5-fluorouracil (5-FU) was used as an anti-cancer drug and positive control. This pyrimidine analog is the most common chemotherapeutic agent prescribed in solid cancers like colon cancer. Therefore, we used 5FU in our experiments on the SW48 colon cancer cell line as a standard antiproliferative agent.

Statistical Analysis

Data were expressed as the mean values±standard deviation (SD). All experimental data were analyzed by Mann-Whitney U test and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS 11.5 for Windows. The level of significance was kept at $P<0.05$. The IC50 of gabapentin niosomes was calculated using the Sigmaplot (Ver. 8) linear regression curve.

Results

Characterization of Niosomes

As demonstrated in Table 2, various niosomal formulations of gabapentin led to different polydispersity index (PDI), sizes, and entrapment efficiencies (EEs). Vesicle size was measured by the dynamic light scattering method (Table 2). As evident, a limited increase in the percentage of Tween 60 in the niosomal formulation of gabapentin led to decreased vesicle size. The minimum size was obtained in the ratio of 50:50 of Span 60:Tween 60 (135.3 nm). With a further increase in Tween 60, the niosomal formulation size increased (163.1 nm). The highest encapsulation efficiency (76.34 %) of the drug-loaded niosomal formulation was related to the 50:50 ratio of Span 60 to Tween 60, representing a significant difference to other prepared niosomal formulations.

Shape and Size of Gabapentin Niosomes

As shown in Figure 1, the morphology of the optimized niosomal gabapentin (G_3) formulation

Table 2: Vesicle size, polydispersity index (PDI), and encapsulation efficiency (EE) of niosomal formulations.

Formulations	Vesicle size	PDI	EE (%)
G_1	238.8±18.25	0.250±0.035	61.77±1.35
G_2	199.5±12.25	0.130±0.017	62.13±0.75
G_3	135.3±5.85	0.203±0.015	76.34±2.49
G_4	163.1±14.55	0.198±0.021	68.45±1.84

*Data are represented as mean±standard deviation (SD), n=3

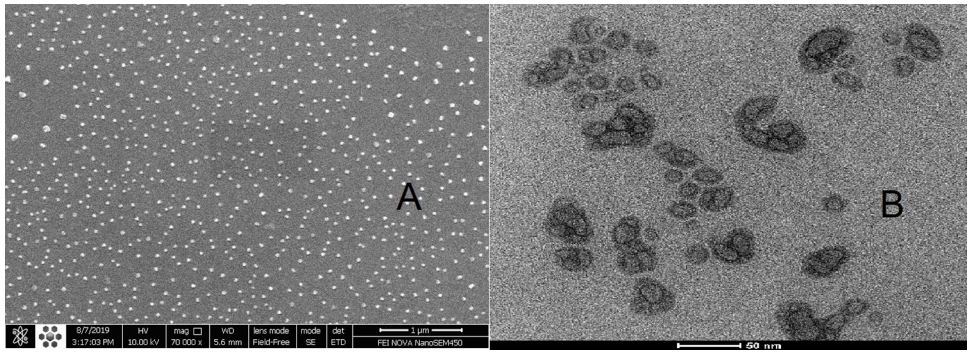

Figure 1: Scanning electron microscopy (A) and transmission electron microscopy (B) analysis of niosome encapsulated gabapentin.

was spherical (Figure 1A (SEM), 1B (TEM)). A uniform spherical morphology was seen with an average diameter of <50 nm and a smooth surface. No aggregation of niosomes was observed. Rigid boundaries of niosomes were established.

Fourier Transform Infrared (FTIR) Analysis

The FTIR spectra explicated the distinct components of the niosomal formulations. The optimum formulation without the drug (i.e., empty noisome) included Span 80, DCP, and cholesterol (Figure 2, Table 3).

In Vitro Drug Release and Kinetics

The profile of gabapentin release (Figure 3) from the optimum niosomal formulation showed fast initial release for the first 8 hours before gentle release for up to 48 hours. However, 95% of the drug load of free gabapentin was released in the first 8 hours, after which the curve showed an almost steady state. In comparison, drug release over 48 hours was 60% in the G_3 formulation and 52% in the G_4 formulation.

Several kinetic release models were fitted on the G, G_3, and G_4 samples (Table 4). A figure with a higher linear regression coefficient is the best-fitting sample diffusion kinetic model (close to 1). For the G_3 and G_4 formulations, the release profile complied much better with the Korsmeyer-Peppas model, suggesting the Fickian diffusion release mechanism for gabapentin.

Stability Studies

To evaluate the stability of the optimal niosomal formulation (G_3), we determined its particle dispersion index (PDI), mean vesicle size, and entrapment efficiency (EE) at various time intervals and temperatures. According to the results, the size and PDI of the niosomal formulation loaded with gabapentin (G3) increased after increasing the

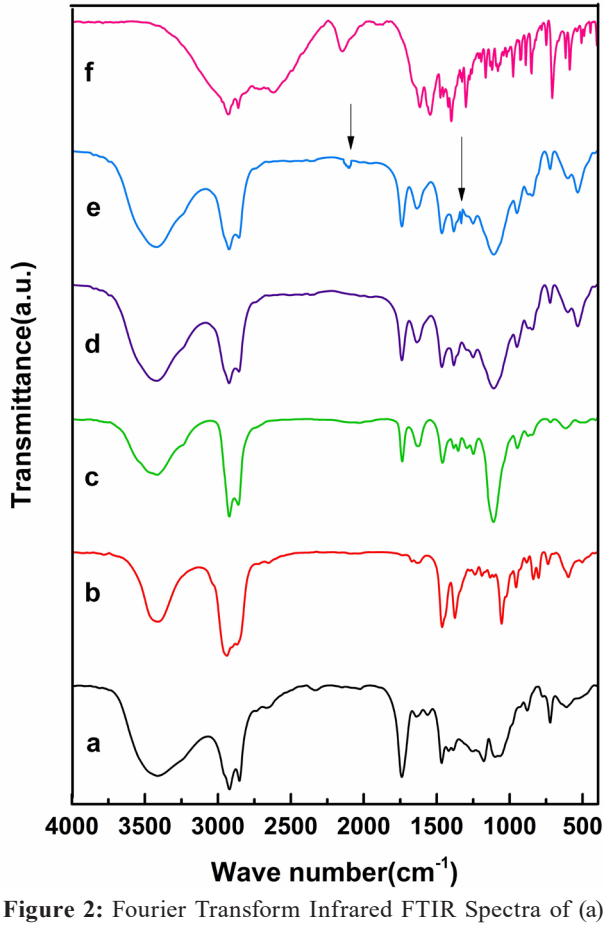

Figure 2: Fourier Transform Infrared FTIR Spectra of (a) Cholesterol, (**b**)Span™ 60, (c)Tween60, (d) Niosome, (f), Gabapentin loaded niosome (g) Gabapentin.

temperature or storage time. Figure 4 demonstrates that the EE decreased with increasing temperature or storage time, although it was less sensitive than other features.

Cell Cytotoxicity Assay

The cytotoxic effect of the novel formulation

Table 3: The main characteristic peaks in the Fourier transform infrared (FTIR) spectra of different samples/chemicals.

Sample/chemical	Peak cm^{-1}	Description
Tween™ 60	1125	C–O stretching
	1747	C=O stretching
	2800-3000	C-H stretching
	3452	OH stretching
Span™ 60	1125	C–O stretching
	2800-3000	C-H stretching
	3452	OH stretching
Cholesterol	1747	C=O stretching
	2800-3000	C-H stretching
	3452	OH stretching
	1035-1378	CH_2 bending and CH_2 deformation
	1506	C-C stretching in the aromatic ring
	1674	C=C stretching
Niosomes	1125	C–O stretching
	1747	C=O stretching
	2800-3000	C-H stretching
	3452	OH stretching
Gabapentin	2857 - 2935	–NH3+ stretching vibration
	2152	Distinct side chain and/or CN stretching vibration
	1618	Ionized asymmetric carboxylate
	1578and 1526	NH3+deformation vibration
	1344-1500	Asymmetric carboxylate band and/or CH2 deformation band
	1344	Fingerprint of gabapentin
Gabapentin-loaded niosomes	1330	Fingerprint of gabapentin
	2112	Distinct side chain and/or CN stretching vibration

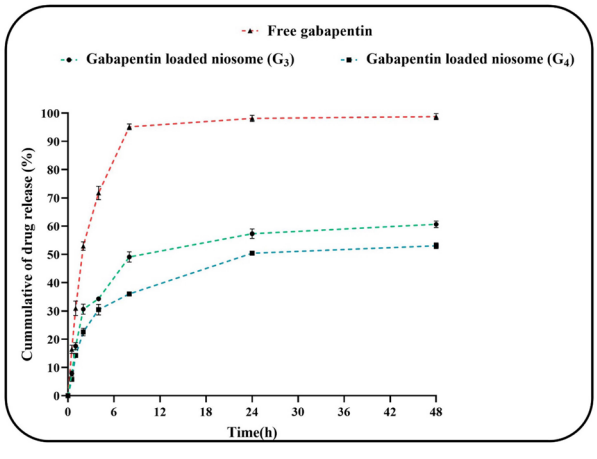

Figure 3: In vitro drug release profile of gabapentin solution (G) and gabapentin from different niosomal formulations (G3 & G4).

Table 4: The kinetic release models and the parameters obtained for niosomal formulations.

Release model	R^2
Zero-Order $Q_t = Q_0 + K_0 t$	G: R^2=0.4862
	G_3: R^2=0.6364
	G_4: R^2=0.7059
First-Order $\text{Log } Q_t = \text{Log } Q_0 + K_1 t$	G: R^2=0.8258
	G_3: R^2=0.7250
	G_4: R^2=0.7743
Higuchi $Q_t = Q_0 + KHt_{0.5}$	G: R^2=0.6836
	G_3: R^2=0.8145
	G_4: R^2=0.8726
Korsmeyer-Peppas $\frac{M_t}{M} = kt^n$	G: R^2=0.7245, n=0.3988
	G_3: R^2=0.9437, n=0.4331
	G_4: R^2=0.9682, n=0.4682

Table 5: Cell viability in different concentrations of 5-FU in different cell lines (%)

Cell lines		SW48	Fibroblast
5-FU concentrations	250 µg/ml	58.1%	92.1%
	100 µg/ml	75.4%	82.3%
	10 µg/ml	81.5%	83.6%
	1 µg/ml	100.0%	86.2%

was assessed on the SW48 colon cancer cell line and fibroblast cells as normal cells. As a positive control, the effect of 5-FU was also studied (Table 5, Supplement 1). The cytotoxic effect of pure gabapentin on SW48 cells was assessed using the MTT assay (Figure 5), revealing an IC50 of 0.2 mg/mL (1.17 mM) after 48 h. In terms of the cytotoxic effect of the optimized formulation (G3) on SW48 cells (Figure 6), the IC50 was 45 µg/mL (200 µM) after 48 h. Therefore, the niosomal formulation of gabapentin was more cytotoxic to the colon cancer cells than pure gabapentin.

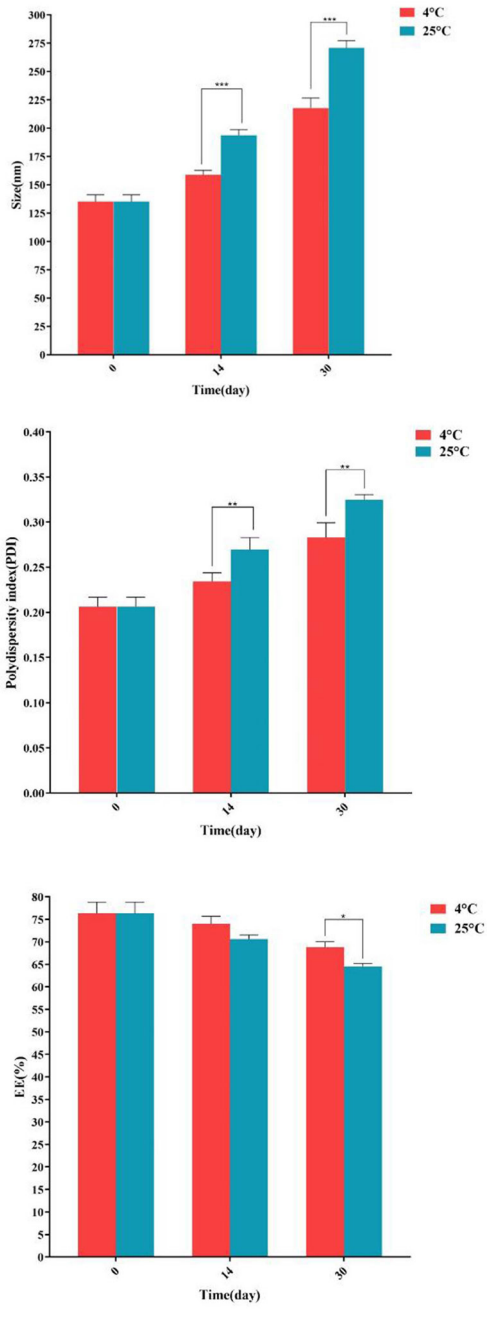

Figure 4: The effect of time and temperature of storage on the average size (A), the polydispersity index (B), and the encapsulation efficiency (C) of gabapentin loaded niosomal formulation, (*P<0.05, **P<0.01, and ***P<0.001).

Discussion

The present study sought to develop and optimize a novel niosomal formulation of gabapentin before assessing its cytotoxicity on normal and colon cancer cells. The maximum encapsulation efficiency (76.34 %) of the drug-loaded niosomal formulation was related to the sample containing a 1:1 ratio of Span 60 to Tween 60. This optimal formulation (G_3) demonstrated anti-cancer activity, showing a significantly higher IC50 for SW48 colon cancer cells (0.2 mg/mL; 1.17 mM) than normal fibroblasts (45 µg/mL; 200 µM).

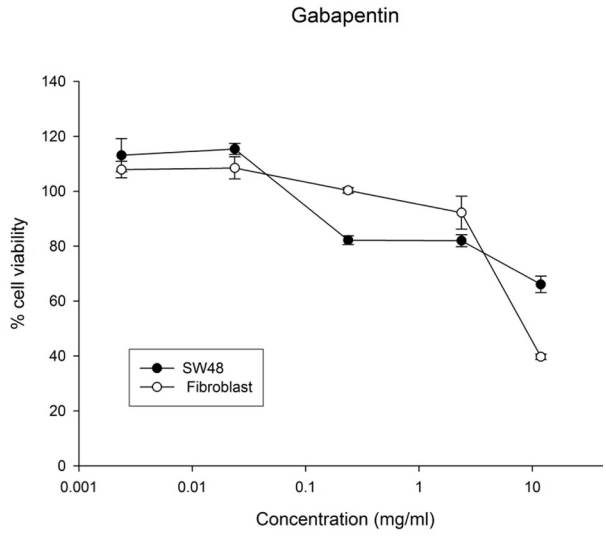

Figure 5: The toxicity of different concentrations (mg/ml) of gabapentin on SW48 and fibroblast cell lines after 48 hours of incubation

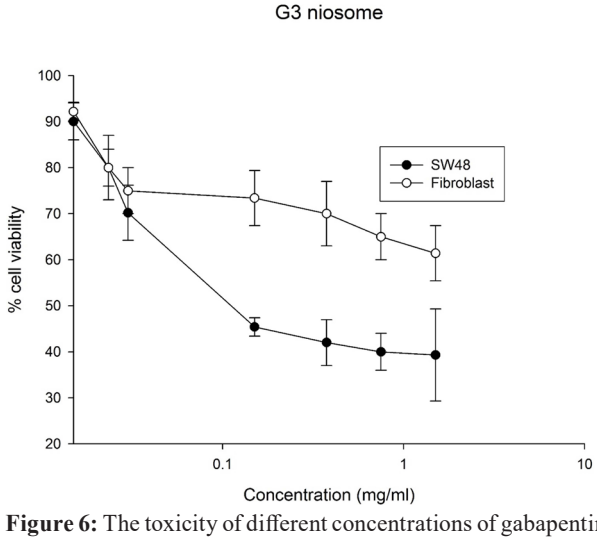

Figure 6: The toxicity of different concentrations of gabapentin niosomes (mg/ml) on SW48 and fibroblast cell lines after 48 hours of incubation

In general, the ratio of Span 60 to Tween 60 had a significant effect on the size of the niosomal particles. However, since the PDI values of the niosomes were less than 0.3, there was a narrow distribution of particle size. The size of the niosomal particles initially decreased with the addition of Tween 60 but then increased. Hence, the smallest particle size was achieved with a 1:1 ratio of Span 60 to Tween 60. Tween 60 is a non-ionic surfactant with hydrophobic properties, though Span 60 is more hydrophobic (29). The dissimilarity in Span 60 to Tween 60 ratios could control the hydrophilic-lipophilic balance of the surfactants and its effect on the interactions with drugs. The high hydrophobicity of Tween 60 decreases the rigidity of the niosomal membrane. Therefore, the addition of Span 60 can improve the niosomal rigidity and accompanying cholesterol with surfactants (Span 60:Tween 60) at a 1:1 molar ratio, resulting in condensed niosomal films (30).

In the present study, different ratios of spans 60 and tweens 80 led to a broad range of hydrophilic-

lipophilic balances (HLBs) in the prepared niosomes. Higher phase transition temperatures of Span 60 and Tween 60 result in greater entrapment of drugs (31, 32). Cholesterol is the most well-known additive agent found in niosomal formulations because of its capacity to influence membrane rigidity and enhance the stability and vesicular integrity of niosomes. Similar to the previous reports, increasing the cholesterol concentration led to the development of larger vesicles (33, 34).

The morphology of the optimized gabapentin niosomes (G_3) was spherical, and no aggregation was observed. Furthermore, the niosomes established rigid boundaries. SEM and TEM analysis showed smaller particles than those obtained by the Nano Zetasizer, probably due to the drying process in SEM and TEM imaging, which revealed the exact diameter of each particle. Also, through DLS measurement, the hydrodynamic diameter was recorded (30, 35-37).

In this work, FTIR spectroscopy was used to explicate the distinct components of the niosomal formulations. The peaks at 1674 cm^{-1} associated with C=C stretching in cholesterol were obscure in the FTIR spectra of the niosomes (28, 38, 39). Two characteristics peaks of the drug molecule (gabapentin), including 1330 cm^{-1} and 2112 cm^{-1}, were detected in the optimal niosomal formulation. However, the C=C stretching peaks in cholesterol disappeared in the FTIR spectra, confirming the entrapment of cholesterol molecules in the lipid bilayer shell and the formation of niosomes (28, 39).

Drug release is an important factor affecting medicinal formulations. In this study, 95% of the drug load of free gabapentin was released in the first 8 hours, after which the curve showed an almost steady state. In comparison, drug release over 48 hours was 60% in the G_3 formulation and 52% in the G_4 formulation. The consequences of the gabapentin release profile from two formulations (G3 and G4) during 48 h proved that the cumulative release profile was biphasic (30, 40). The initial rapid release phase may result from drug desorption from the outer surface of the niosomes, and the second passive release phase might rely on gradual drug diffusion through the membrane (41). Table 3 shows the R^2 of each model, indicating that diffusion and erosion control the mechanism of drug release (28, 42, 43). With the Fickian diffusion model, n values of between 0.43 and 0.85 indicate Fickian drug release (30, 43). For G_3 and G_4 formulations, the release profile complied much better with the Korsmeyer-Peppas model, suggesting the Fickian diffusion release mechanism for gabapentin.

Our study showed that the size and PDI of the niosomal formulation loaded with gabapentin (G3) increased by increasing the temperature or storage time. Figure 4 records that encapsulation efficiency decreased with increasing temperature or storage time, although it was less sensitive than other features. Stability experimentations explicated that niosomes did not change for at least two weeks at 4±2 °C, probably due to the low mobility of the bilayer at this temperature (44). Nevertheless, particles fusion or aggregation resulted in increased vesicle size during storage (45). Moreover, the encapsulation efficiency may decrease at high temperatures as the fluidization of the lipid membrane increases, and the drug could leak into the external matrices (46). Besides, high temperatures increase the rate of drug diffusion (44).

In this study, the cytotoxic effect of pure gabapentin on SW48 cells was shown by an IC50 of 0.2 mg/mL after 48 h, compared to an IC50 of 45 µg/mL for the optimized niosomal formulation. Therefore, the niosomal formulation enhanced the toxic effect of gabapentin on SW48 colon cancer cells. However, the cytotoxic effect of the niosomal formulation on normal fibroblast cells was low. Previously, the cytotoxicity of gabapentin on SKOV3, JURKAT, and U87 cancer cell lines was reported with IC50 values of 87.9, 60.82, and 48.35 µM, respectively, and the Cu complex of gabapentin had higher cytotoxicity on these cancer cell lines (47). Another study on human glioma cell lines (U-87 MG and T98G) showed that 20 µg/mL gabapentin had less than 30% inhibitory activity on cell proliferation (48), similar to what was reported for the effect of gabapentin on the M5 glial cells, which did not influence the viability of cells (49). Moreover, a study on astrocytes showed that gabapentin at low concentrations (about 10 µg/mL) could not significantly be toxic and did not alter metabolic activities (50). Here, a high dose of gabapentin was analyzed compared to previous reports, and at 200 µg/mL, gabapentin showed toxicity on the SW48 colon cancer cells. This toxicity shifted to a lesser dose in the niosomal formulation of gabapentin. As a result, the niosomal formulation of gabapentin may be considered as a promising delivery system for oral administration of gabapentin. Furthermore, it may be a good therapeutic system for rectal treatment of colon cancers and regional neurogenic pain.

Conclusion

In this study, niosomal formulations of gabapentin were designed and optimized to achieve more efficient oral delivery of this anticonvulsant agent. The optimized formulation of gabapentin niosomes had good stability and exhibited sustained drug release after an initial burst, which can help develop a controlled release delivery system. The optimized formulation had a low cytotoxic effect on normal fibroblast cells. Moreover, the cytotoxic effect of this formulation on the SW48 colon cancer cell line was also not high, though it was significantly more than pure gabapentin.

Acknowledgments

The authors would like to thank Sharif University,

Department of chemistry laboratory's staffs for their laboratory assistance. The authors would like to thank the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for their assistance in editing this article.

Conflicts of interest: None declared.

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