J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(2): 470-478, 2024
Doi: 10.33483/jfpau.1400158

ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREPARATION OF LIPID NANOCARRIER FORMULATIONS AND CYTOTOXICITY STUDIES OF DONEPEZIL

DONEPEZİL'İN LİPİD NANO TAŞIYICI FORMÜLASYONLARININ HAZIRLANMASI VE SİTOTOKSİSİTE ÇALIŞMALARI

Emine Selin DEMİR[1], Emre ÖZGENÇ[1]*, Evren ATLIHAN GÜNDOĞDU[1]

[1]Ege University, Faculty of Pharmacy, Department of Radiopharmacy, 35100, Izmir, Türkiye

ABSTRACT

Objective: *Our research endeavors to discover innovative formulations for the pharmaceutical component of radiopharmaceuticals, which are used to diagnose Alzheimer's disease. Our approach involves the incorporation of Donepezil, a proven active ingredient, into lipid-based nanocarrier systems. Additionally, we have conducted a comprehensive study on the cytotoxicity of Donepezil as a vital aspect of our research.*
Material and Method: *Two distinct techniques were employed in creating nanocarrier formulations: emulsion and sonication. Malvern Zeta Sizer measurements were conducted to assess the properties of the prepared formulations. In addition, the cell proliferation kit II (XTT) was used to evaluate the cytotoxicity of the active ingredient Donepezil.*
Result and Discussion: *Formulations with particle sizes ranging from 100-200 nm have been selected based on the results of characterization studies. Cytotoxicity assays have shown that amounts of Donepezil (50, 100, 500, 1000, 2000, and 5000 µg/ml) are biocompatible. These findings confirm the optimal formulation parameters for producing high-quality Donepezil-based pharmaceutical products. The characterization studies of the prepared formulations have shown that they have the potential to be used in the diagnosis of Alzheimer's disease.*
Keywords: *Characterization studies, cytotoxicity studies, donepezil, drug delivery systems*

ÖZ

Amaç: *Araştırmamızın amacı Alzheimer hastalığının teşhisinde kullanılan radyofarmasötiğin farmasötik bileşeni için yenilikçi formülasyonlar geliştirmektir. Bu hedefi gerçekleştirmek için lipit bazlı nanotaşıyıcı sistemler geliştirdik ve Donepezil aktif bileşen olarak lipid bazlı nanotaşıyıcı sistemlere dahil edildi. Ayrıca çalışmamızın önemli bir parçası olarak Donepezilin sitotoksisitesine ilişkin değerlendirmeler yapıldı*
Gereç ve Yöntem: *Nanotaşıyıcı formülasyonların oluşturulmasında emülsiyon ve sonikasyon olmak üzere iki farklı teknik kullanıldı. Hazırlanan formülasyonların özelliklerini değerlendirmek için Malvern Zeta Sizer ölçümleri yapıldı. Ayrıca Donepezil etken maddesinin sitotoksisitesini değerlendirmek için hücre proliferasyon kiti (XTT) kullanıldı.*
Sonuç ve Tartışma: *Karakterizasyon çalışmalarının sonuçlarına göre partikül boyutları 100-200 nm arasında değişen formülasyonlar seçilmiştir. Sitotoksisite analizleri, Donepezil'in 50, 100, 500, 1000, 2000 ve 5000 µg/ml aktif madde konsantrasyonlarının biyolojik olarak uyumlu olduğunu göstermiştir. Bu bulgular, yüksek kaliteli Donepezil bazlı farmasötik ürünler üretmek için en uygun formülasyon parametrelerini doğrulamaktadır.*

* **Corresponding Author / Sorumlu Yazar:** Emre Özgenç
e-mail / e-posta: emre.ozgenc@ege.edu.tr, **Phone / Tel.:** +905384361513

Submitted / Gönderilme : 04.12.2023
Accepted / Kabul : 20.02.2024
Published / Yayınlanma : 20.05.2024

Hazırlanan formülasyonların karakterizasyon çalışmaları Alzheimer hastalığının tanısında kullanılma potansiyeline sahip olduklarını göstermiştir. Formülasyonlara eklenecek Donepezil etken maddesinin optimal miktarı belirlenmiş ve biyouyumluluk açısından uygun bulunmuştur.
Anahtar Kelimeler: *Donepezil, ilaç taşyıcı sistemler, karakterizasyon çalışmaları, sitotoksisite çalışmaları*

INTRODUCTION

Alzheimer's is a neurological condition that leads to a decline in daily activities and cognitive abilities, alongside changes in behavior and neuropsychiatric symptoms. The 2019 World Alzheimer's Prevalence study revealed that dementia affects over 50 million individuals worldwide, and this number is expected to skyrocket to 152 million by 2050. Unfortunately, the disease is often discovered in its advanced stages, particularly in developing countries [1]. Currently, the Food and Drug Administration (FDA) has approved three cholinesterase inhibitors - Galantamine, Rivastigmine, and Donepezil - which are typically prescribed for mild to moderate cases [2,3]. Donepezil is a piperidine-based medication that functions by reversibly inhibiting the acetylcholinesterase enzyme. The drug is approved by the FDA for the symptomatic treatment of mild to moderate Alzheimer's disease. It is believed that Donepezil enhances cholinergic function by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by the acetylcholinesterase enzyme [4-6]. Donepezil is essentially a non-competitive inhibitor of acetylcholinesterase [7,8]. Donepezil is a medication that is utilized for the treatment of mild to moderate Alzheimer's disease. It works by inhibiting the activity of acetylcholinesterase, which elevates the amount of acetylcholine in the brain. Acetylcholine is an essential neurotransmitter involved in cognitive function. However, Donepezil and other acetylcholinesterase inhibitors can cause adverse gastrointestinal changes and hepatotoxicity [9]. Common side effects of Donepezil include diarrhea, vomiting, insomnia, fatigue, muscle cramps, nausea, and anorexia due to increased cholinergic activity in the gastrointestinal tract following oral administration [10,11]. To address these limitations, lipid-based formulations consisting of Capryol 90, oleic acid, water, and surfactants such as Span 80, Tween 80, and Soy Lecithin have been developed [12,13]. Administered orally, these formulations are available in various forms such as orally disintegrating tablets or sustained-release formulations. Nanoparticle lipid delivery systems offer a promising platform for diagnosis and treatment, which can increase the efficacy of drugs and reduce associated side effects. Lipids are administered as simple vehicles in drug administration, but some amphiphilic lipids, such as monoglycerides, self-assemble after swelling with water. This complex phase behavior is utilized to obtain nanostructured colloidal systems, and studies on it have increased recently [14,15]. In this study, researchers prepared the pharmaceutical components of the radiopharmaceutical used in the diagnosis of Alzheimer's disease. The formulations were developed, and particle size and charge were measured to study the physicochemical properties of these formulations. The emulsion and sonication methods were employed to prepare the formulations, with the sonication method being preferred due to its practicality. The composition of lipid nanocarrier systems generally includes oils or fats, surfactants, and an aqueous phase. The study evaluated different production parameters such as formulation ratios and mixing times by making changes to the ratios of the ingredients in the composition. In addition, the cytotoxicity studies of the active ingredient Donepezil were conducted using the XTT kit. The results of the cytotoxicity studies determined the correct amounts of Donepezil to be added to the formulations. The findings of this research demonstrate the potential of lipid-based formulations as an effective treatment for Alzheimer's disease.

MATERIAL AND METHOD

Preparation of Lipid-Structured Nanocarriers

During the process of developing formulations, we created different variations with varying particle sizes and loads. To accomplish this, we prepared formulations using various compositions through both emulsion and sonication methods. In the end, we found that sonication was the preferred method due to its practicality in preparing the formulations.

The composition of lipid nanocarrier systems generally includes oils or fats, surfactants, and an aqueous phase. While developing formulations;

• As the oil phase; oleic acid, Capryol 90

• Bidistilled water and water: acetone: ethanol (5:2.5:2.5 h/h) as the water phase

• Studies were carried out with Span 80, Tween 80, and Soy Lecithins (Lipoid S 70, Lipoid S 100) as surfactants.

By making changes in the ratios of the ingredients in the composition, different production parameters (formulation ratios, mixing times) were evaluated, and formulation studies were carried out.

The method is as follows; The lipids were melted at 70-80°C, and surfactant was added on top of the molten lipid. This mixture was titrated with the water phase brought to the same temperature. This mixture was first mixed in a magnetic stirrer at 1000 rpm, then this pre-emulsion was passed through a sonicator (Bandelin, GmBh, Berlin, Germany) at 500 W and 20 kHz, and thus the formulations were prepared.

Different parameters were tried in preformulation studies.

1- Formulation of ingredient and ingredient quantity changes

- Lipid type
- lipid ratio
- Surfactant type
- Surfactant ratios

2- Changes in the method used in the production phase of the formulation

- Mixing time in a sonicator

The contents and preparation conditions of the formulations are shown in Table 1.

Table 1. The contents and preparation conditions of the formulations

Formulation	Oleic acid (mg)	Tween 80 (mg)	Water (ml)	Stirring speed (rpm)	Stirring time (minutes)	Sonicator conditions
F1	300	100	5	1000	15 (F1-1) 10 (F1-2) 5 (F1-3)	500 W ve 20 kHz
F2	200	200	5	1000	15 (F2-1) 10 (F2-2) 5 (F2-3)	500 W ve 20 kHz
F3	100	300	5	1000	15 (F3-1) 10 (F3-2) 5 (F3-3)	500 W ve 20 kHz
Formulation	**Oleic acid (mg)**	**Span 80 (mg)**	**Water (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F4	300	100	5	1000	15 (F4-1) 10 (F4-2) 5 (F4-3)	500 W ve 20 kHz
F5	200	200	5	1000	15 (F5-1) 10 (F5-2) 5 (F5-3)	500 W ve 20 kHz
F6	100	300	5	1000	15 (F6-1) 10 (F6-2) 5 (F6-3)	500 W ve 20 kHz
Formulation	**Capyrol 90 (mg)**	**Span 80 (mg)**	**Water (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F7	300	100	5	1000	15 (F7-1) 10 (F7-2) 5 (F7-3)	500 W ve 20 kHz
F8	200	200	5	1000	15 (F8-1) 10 (F8-2) 5 (F8-3)	500 W ve 20 kHz
F9	100	300	5	1000	15 (F9-1) 10 (F9-2) 5 (F9-3)	500 W ve 20 kHz

Table 1 (*continue*). The contents and preparation conditions of the formulations

Formulation	Capyrol 90 (mg) (mg)	Tween 80 (mg)	Water (ml)	Stirring speed (rpm)	Stirring time (minutes)	Sonication conditions
F10	300	100	5	1000	15 (F10-1) 10 (F10-2) 5 (F10-3)	500 W ve 20 kHz
F11	200	200	5	1000	15 (F11-1) 10 (F11-2) 5 (F11-3)	500 W ve 20 kHz
F12	100	300	5	1000	15 (F12-1) 10 (F12-2) 5 (F12-3)	500 W ve 20 kHz
Formulation	**Oleic acid (mg)**	**Lipoid S 75 (mg)**	**Water:Acetone: Ethanol (5:2.5:2.5 v/v) (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F13	300	100	5	1000	15 (F13-1) 10 (F13-2) 5 (F13-3)	500 W ve 20 kHz
F14	200	200	5	1000	15 (F14-1) 10 (F14-2) 5 (F14-3)	500 W ve 20 kHz
F15	100	300	5	1000	15 (F15-1) 10 (F15-2) 5 (F15-3)	500 W ve 20 kHz
Formulation	**Oleic acid (mg)**	**Lipoid S 100 (mg)**	**Water:Acetone:Ethanol (5:2.5:2.5 v/v) (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F16	300	100	5	1000	15 (F16-1) 10 (F16-2) 5 (F16-3)	500 W ve 20 kHz
F17	200	200	5	1000	15 (F17-1) 10 (F17-2) 5 (F17-3)	500 W ve 20 kHz
F18	100	300	5	1000	15 (F18-1) 10 (F18-2) 5 (F18-3)	500 W ve 20 kHz

A total of 54 formulations were prepared. Among these formulations, F13, F14, F15, F16, F17, and F18 were canceled because aggregation was observed. Characterization studies of the remaining formulations were performed.

Characterization Studies of Prepared Formulations

Investigation of particle size properties

The prepared formulations were evaluated in terms of aggregate formation, particle size, and polydispersity index with Malvern Zetasizer (Malvern Nano ZS 90) in the particle size range of 3-1000 nm, at room temperature, with an angle of 173°. Samples were diluted with filtered, bidistilled water (pH=7) before evaluation. Dilutions were made at 1/400.

Zeta potential analysis

The formulations were evaluated with Malvern Zetasizer (Malvern Nano ZS 90) at 25°C, the dielectric constant of 78.5, the conductivity of 5 mS/cm, using DTS 1060C zeta cuvette, a field strength of 40 V/cm. Before measuring, samples were diluted with a certain amount of distilled water (pH=7). Dilutions were made at a ratio of 1/400.

Cytotoxicity

Cytotoxicity studies were conducted to determine the optimal dosage of Donepezil solution that is non-toxic to HT-22 mouse hippocampal cell lines. Various concentrations, ranging from 50 μg/ml to

5000 µg/ml, were evaluated. The primary objective of this study was to determine the optimal concentration of Donepezil that would provide maximum results while minimizing potential harm to cells. The outcome of this study would aid in establishing the recommended dosage of Donepezil for further research and clinical applications. The XTT method was used to evaluate cell viability, cytotoxicity, and proliferation. This method is a sensitive and user-friendly colorimetric assay that measures the conversion of a tetrazolium salt into a water-soluble orange formazan by mitochondrial dehydrogenases in metabolically active cells. The absorbance value at 570 nm in a microplate reader was used to measure viable cells. The cells were seeded in replicates of three at 100 µl at 1x104 cells/well in a 96-well plate and incubated for 24 hours in an incubator at 37°C and 5% CO_2. Different concentrations of Donepezil were then added to the cells. Donepezil was dissolved in a medium with dimethyl sulfoxide (DMSO) concentration of 0.1%. After a 24, 48, and 72-hour incubation period, a mixture prepared by adding activation solution to XTT reagent was added to each well and incubated for two hours. The absorbance values were measured in a microplate reader (Thermo Scientific Varioskan Flash Microplate Reader) at a wavelength of 570 nm. The CalcuSyn software was employed to determine the dosage that keeps cell viability above 70% compared to the control. Once the optimum dosage is established, formulations containing Donepezil at the appropriate dosage will be prepared for further research and clinical applications.

RESULT AND DISCUSSION

Results of Characterization Studies of Prepared Formulations

The results of the analyses measured on the Malvern zeta-sizer device are shown in Table 2. Formulations of each formulation with different mixing times (15, 10, and, 5 minutes) were prepared. Each formulation was studied as n=3, and Malvern Zeta-Sizer measurements were made.

Table 2. Malvern zeta sizer measurement results of formulations

Formulation	Particle size (nm)	PdI	Zeta Potential (mV)
F1-1	296.66 ± 5.41	0.39 ± 0.04	+12.85 ± 1.42
F1-2	135.80 ± 1.83	0.45 ± 0.04	+20.93 ± 3.30
F1-3	195.66 ± 7.41	0.52 ± 0.08	+25.41 ± 4.32
F2-1	227.80 ± 4.81	0.40 ± 0.01	-26.03 ± 7.18
F2-2	255.12 ± 3.25	0.45 ± 0.05	-25.04 ± 6.55
F2-3	229.60 ± 3.56	0.35 ± 0.03	-24.99 ± 5.18
F3-1	143.8 ± 6.24	0.53 ± 0.01	-25.60 ± 3.97
F3-2	59.89 ± 0.49	0.49 ± 0.01	-25.26 ± 1.65
F3-3	74.15 ± 2.55	0.51 ± 0.01	-25.89 ± 4.12
F4-1	114.33 ± 3.24	0.56 ± 0.03	+25.53 ± 6.31
F4-2	85.38 ± 2.16	0.52 ± 0.01	+29.01 ± 6.19
F4-3	81.02 ± 1.58	0.51 ± 0.02	+28.46 ± 2.61
F5-1	119.93 ± 2.66	0.51 ± 0.01	-20.63 ± 0.94
F5-2	91.94 ± 1.29	0.52 ± 0.01	-22.66 ± 2.25
F5-3	103.97 ± 1.17	0.53 ± 0.04	-25.91 ± 5.31
F6-1	99.29 ± 1.42	0.49 ± 0.01	+27.15 ± 2.87
F6-2	109.97 ± 3.95	0.49 ± 0.03	+23.01 ± 2.47
F6-3	103.47 ± 1.48	0.52 ± 0.02	+24.06 ± 3.79
F7-1	414.77 ± 50.79	0.59 ± 0.06	-23.99 ± 5.12
F7-2	288.90 ± 7.47	0.66 ± 0.12	-23.92 ± 2.44
F7-3	929.37 ± 39.58	0.75 ± 0.04	-24.07 ± 5.63

Table 2 (*continue*). Malvern zeta sizer measurement results of formulations

Formulation	Particle size (nm)	PdI	Zeta Potential (mV)
F8-1	764.933 ± 119.087	0.87 ±0.11	-24.77 ± 8.46
F8-2	1124.66 ± 52.54	0.93 ± 0.11	-28.53 ± 4.52
F8-3	906.80 ± 222.44	0.84 ± 0.13	-27.45 ± 3.52
F9-1	87.03 ± 1.88	0.27 ± 0.01	-26.57 ± 1.80
F9-2	72.25 ± 3.25	0.33 ± 0.02	-22.36 ± 2.45
F9-3	130.60 ± 2.74	0.43 ± 0.02	-21.17 ±0.55
F10-1	11.45 ± 0.06	0.23 ± 0.03	+25.19 ±4.76
F10-2	11.45 ± 0.06	0.14 ± 0.01	+21.66 ± 1.58
F10-3	10.31 ± 0.12	0.15 ± 0.01	+21.80 ± 1.88
F11-1	11.19 ± 0.01	0.17 ± 0.01	+24.84 ± 1.10
F11-2	14.93 ± 0.31	0.22 ± 0.01	+27.63 ± 3.21
F11-3	11.86 ± 0.09	0.18 ± 0.02	+28.56 ± 1.64
F12-1	9.72 ± 0.08	0.21 ± 0.03	-23.41 ± 2.91
F12-2	9.59 ± 0.04	0.15 ± 0.03	-26.76 ± 2.94
F12-3	10.22 ± 0.17	0.21 ± 0.01	-24.85 ± 3.95

After measurements, formulations with particle sizes in the range of 100-200 nm were selected. In the next studies, F1-2, F1-3, F3-1, F3-2, F4-1, F4-2, F4-3, F5-1, F5-2, F5-3, F6-1, F6-2, F6-3, F9-1, F9-3 formulations were continued. The graphs of the Malvern Zetasizer measurement results for the selected formulations are shown in Figure 1. Cytotoxicity studies were started.

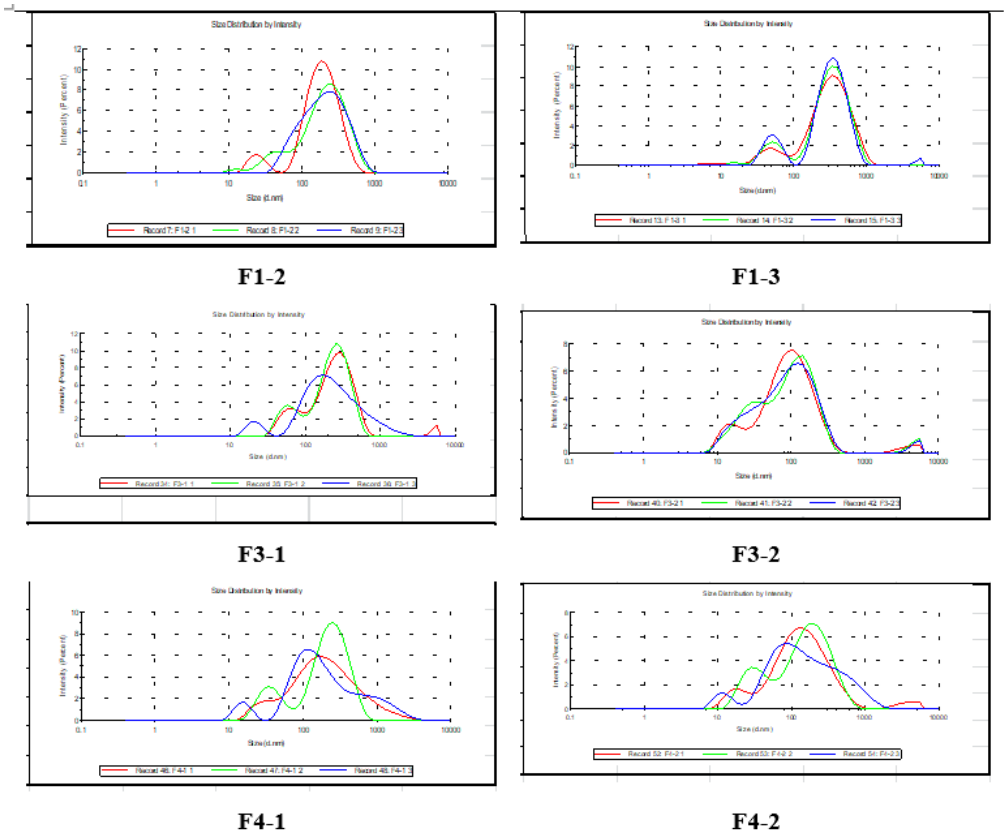

Figure 1. Graphs of Malvern Zetasizer measurement results for selected formulations

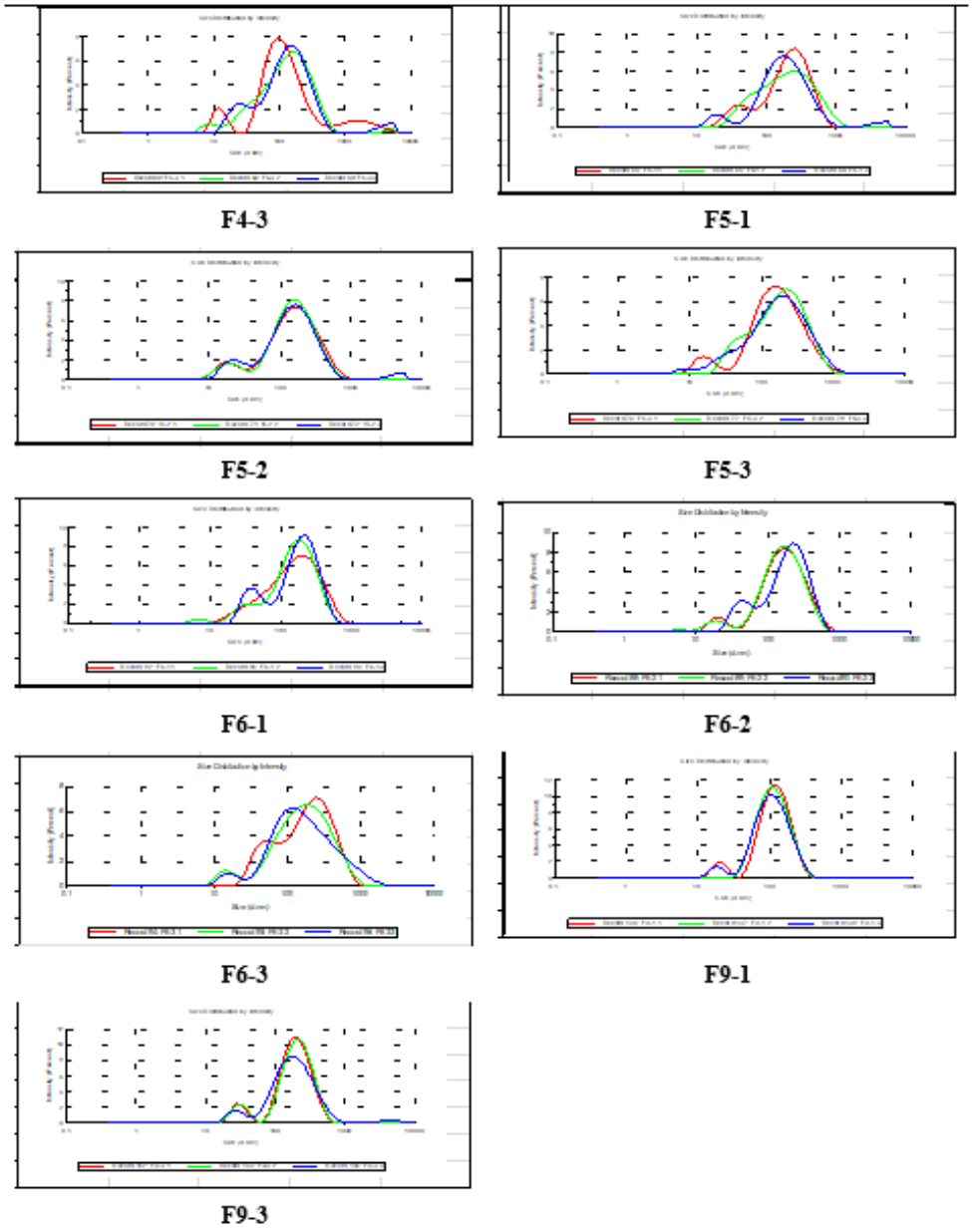

Figure 1 (*continue*). Graphs of Malvern Zetasizer measurement results for selected formulations

Results of Cytotoxicity Studies

The cytotoxicity results of different amounts of Donepezil solution are shown in Table 3 and Figure 2. Medium containing 0.1% DMSO was used for 100% viability.

Table 3. The cytotoxicity results from different amounts of Donepezil solution (n=3)

cell viability (%)	**Amount of Donepezil (ug/ml)**					
Time	**50**	**100**	**500**	**1000**	**2000**	**5000**
0	100	100	100	100	100	100
24	99.22±0.41	97.46±1.73	96.96±1.1	96.13±3.22	96.26±2.92	95.99±1.44
48	98.51±1.49	97.31±2.3	96.65±3.27	95.69±4.76	96.09±1.66	95.82±2.66
72	97.88±2.83	97.53±3.24	95.81±4.18	95.53±3.53	95.09±2.57	95.46±2.45

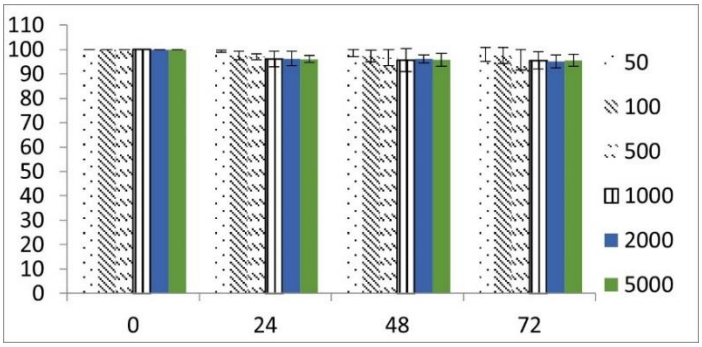

Figure 2. The cytotoxicity results from different amounts of Donepezil solution

Conclusion

The present study sought to develop nanoparticle carrier systems in lipid structure for the diagnosis of Alzheimer's disease, while also conducting cytotoxicity studies on the active substance Donepezil. Several formulations were prepared and evaluated, with a focus on selecting those that displayed optimal particle size, zeta potential, and PdI values capable of passing the blood-brain barrier. Formulations with particle sizes up to 200 nm were selected to pass the blood-brain barrier. Cytotoxicity studies have been conducted with these formulations. In addition, future studies will continue with formulations with positive zeta potential to pass the BBB and with pDI values below 0.5 in terms of homogeneity (F1-2, F6-1, and F6-2). In formulations with homogeneous distribution, this value is required to be 0.5 and below [16]. Moreover, the amount of Donepezil added to these chosen formulations was based on cytotoxicity results obtained from the active substance. Encouragingly, the study yielded promising results, suggesting that the developed formulations could prove instrumental in the diagnosis of Alzheimer's disease. Future research endeavors will continue to explore the potential of these formulations, using the selected formulations and determined amounts of Donepezil.

AUTHOR CONTRIBUTIONS

Concept: E.S.D., E.O., E.A.G.; Design: E.S.D., E.O., E.A.G.; Control: E.O., E.A.G.; Sources: E.S.D., E.O., E.A.G.; Materials: E.S.D., E.O., E.A.G.; Data Collection and/or Processing: E.S.D.; Analysis and/or Interpretation: E.S.D., E.O., E.A.G.; Literature Review: E.S.D.; Manuscript Writing: E.S.D., E.O., E.A.G.; Critical Review: E.S.D., E.O., E.A.G.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Alzheimer’s Disease International World Alzheimer Report 2019. Attitudes to dementia 2019. (2019). Retrieved January 12, 2023, from https://www.alz.co.uk/research/WorldAlzheimerReport2019.pdf.
2. Greig, S.L. (2015). Memantine ER/Donepezil: A review in Alzheimer’s disease. CNS Drugs, 29, 963970. [CrossRef]
3. Atri A. (2011). Effective pharmacological management of Alzheimer's disease. The American Journal of Managed Care, 17(13), 346-355.
4. Yasir, M., Sara U.V.S., Chauhan, I., Gaur, P.K., Singh, A.P., Puri, D., Ameeduzzafar. (2018). Solid lipid nanoparticles for nose-to-brain delivery of donepezil: formulation, optimization by Box-Behnken design, *in vitro* and *in vivo* evaluation. Artificial Cells, Nanomedicine, and Biotechnology, 46(8), 1838-1851. [CrossRef]

5. Areosa, S.A., Sherriff, F., McShane, R. (2003). Memantine for dementia. Cochrane Database of Systematic Reviews, 20, 154-160. [CrossRef]
6. Xie, Z., Liao, Q., Xu, X., Yao, M., Wan, J., Liu, D. (2006). Rapid and sensitive determination of donepezil in human plasma by liquid chromatography/tandem mass spectrometry: Application to a pharmacokinetic study. Rapid Communications in Mass Spectrometry, 20(21), 3193-3198. [CrossRef]
7. Galli, A., Mori, F., Benini, L., Cacciarelli, N. (1994). Acetylcholinesterase protection and the anti-diisopropylfluorophosphate efficacy of E2020. European Journal of Pharmacology, 270(2-3), 189-193. [CrossRef]
8. Nochi, S., Asakawa, N., Sato, T. (1995). Kinetic study on the inhibition of acetylcholinesterase by 1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]methylpiperidine hydrochloride (E2020). Biological & Pharmaceutical Bulletin, 18(8), 1145-1147. [CrossRef]
9. Agrawal, M., Saraf, S., Saraf, S., Antimisiaris, S.G., Hamano, N., Li, S.D., Chougule, M., Shoyele, S.A., Gupta, U., Ajazuddin, Alexander, A. (2018). Recent advancements in the field of nanotechnology for the delivery of anti-Alzheimer drugs in the brain region. Expert Opinion on Drug Delivery, 15(6), 589-617. [CrossRef]
10. Carvalho, F.C., Campos, M.L., Peccinini, R.G., Gremião, M.P. (2013). Nasal administration of liquid crystal precursor mucoadhesive vehicle as an alternative antiretroviral therapy. European Journal of Pharmaceutics and Biopharmaceutics, 84(1), 219-227. [CrossRef]
11. Zoeller, T., Sandra, K. (2007). Simplified biorelevant media for screening of dissolution performance of poorly soluble drugs. Dissolution Technologies, 14, 8-13. [CrossRef]
12. Sozio, P., Cerasa, L.S., Marinelli, L., Di Stefano, A. (2012). Transdermal donepezil on the treatment of Alzheimer's disease. Neuropsychiatric Disease and Treatment, 8, 361-368. [CrossRef]
13. Ruela, A.L., Carvalho, F.C., Pereira, G.R. (2016). Exploring the phase behavior of monoolein/oleic acid/water systems for enhanced donezepil administration for Alzheimer's disease treatment. Journal of Pharmaceutical Sciences, 105(1), 71-77. [CrossRef]
14. Stevenson, C.L., Bennett, D.B., Lechuga-Ballesteros, D. (2005). Pharmaceutical liquid crystals: the relevance of partially ordered systems. Journal of Pharmaceutical Sciences, 94(9), 1861-1880. [CrossRef]
15. Carvalho, F.C., Sarmento, V.H., Chiavacci, L.A., Barbi, M.S., Gremião, M.P. (2010). Development and in vitro evaluation of surfactant systems for controlled release of zidovudine. Journal of Pharmaceutical Sciences, 99(5), 2367-2374. [CrossRef]
16. Özge, A.M., Genç, R. (2016). Floresans karbon nanoparçacıkların yeşil sentezi ve pasivasyon ajanının molekül ağırlığının nanoparçacık özellikleri üzerine etkisinin incelenmesi. Sinop Üniversitesi Fen Bilimleri Dergisi, 1(2), 123-134.