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Journal of Pharmaceutical Technology

"All substances are poisons; there is none which is not a poison.
The right dose makes it either a poison or a remedy."

Paracelsus

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# JPharmTech

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# JPharmTech

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Asos Index

Eurasian Scientific Journal Index

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# JPharmTech

## Editor's Note

Dear Pharmaceutical Technology Researchers,

We are excited to be with you in this first issue of **JPharmTech**. The purpose of our journal is to inform the all pharmaceutical scientists about all latest and important research on drug development. **JPharmTech** will publish papers that focus on the research, development and evaluation of pharmaceutical delivery systems. The journal includes topics such as preformulation, drug delivery systems and targeting, formulation design, preparation, *in vitro* and *in vivo* characterization, pharmacokinetics, pharmacodynamics, biopharmaceutics and more.

**JPharmTech** is an online peer-reviewed journal following the guidelines provided by International Committee of Medical Journal Editors (ICMJE) and Committee on Publication Ethics (COPE). **JPharmTech** will be published every four months and three issues per year. Journal accepts original research and review articles on all aspects of pharmaceutical technology. Corresponding authors should submit their manuscripts through the online submission system of Dergipark (www.jpharm.tech). Processing and publication are free of charge. All published articles in the **JPharmTech** will be assigned with unique Digital Objective Identifier (DOI) provided by CrossRef; indexed in Google Scholar, Scientific Indexing Services (SIS), Eurasian Scientific Journal Index, Research Bible and ASOS index.

I want to thank you to the all of my friends who studied for those processes that are mentioned above. By the valuable contribution of our friends in the publishing team, this was a successful start for our journal.

Our main goals are having a strong international editor and referee team, to increase the recognition and index number of the **JPharmTech** and to be one of the most cited journals. That's why, your contribution and support to our journal with your papers and refereeing, are really important and valuable for us.

Thank you in advance & Best regards,

**Müzeyyen Demirel**

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# JPharmTech

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The main text should be 1000-7000 words long (not including abstract, keywords and references). All headings must be numbered consecutively and hierarchically. Please avoid using more than three levels of headings. The proposed location of figures and tables must be indicated in the main text. Within the article, avoid the use of footnotes and endnotes. Prefer to use the active voice throughout, not the passive, while explaining the study performed.

Symbols, abbreviations and conventions in papers must follow the recommended SI Units. A single space must be placed between the number and the unit. Abbreviations must be defined in brackets after their first mention in the text in accordance with internationally agreed rules.

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Research Papers should be organized as follows: Title, Author names and affiliations, Corresponding author information, Abstract, Keywords, Introduction, Materials and methods, Results and Discussion, Conclusion, Author contributions, Acknowledgments (if any), Ethics committee approval (if necessary), Conflict of interest declaration, References. These rules are a bit more flexible in review articles.

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A concise and informative title is required. Do not use any abbreviations and formulae in the title of the manuscript. The title of the manuscript must be written in sentence case except for the first word and proper nouns.

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Clearly indicate the given name(s) and family name(s) of each author. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a superscript number.

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The introduction section of the articles (particularly research articles) should include the following topics;

- establish current knowledge of the field,
- summarize previous research, providing the wider context and background and the importance of the current study,
- set the stage for the present research, indicating gaps in knowledge and presenting the research question,
- introduce present research, stating its purpose and outlining its design.

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The Methods should describe clearly how you carried out your study. Provide sufficient details like study design and data collection to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference.

### Results and Discussion

A combined Results and Discussion section is often appropriate; but optionally can be written separately. Provide a concise and precise description of the all experimental data, their interpretation as well as the experimental conclusions that can be drawn. Discussion should explore the significance of the results of the work, not repeat them. The P values and/or the specific statistical tests performed for each experiment should be included to the results and discussion section in the appropriate figure legend or main text. Results should include the name of the statistical test, followed by a colon, the test statistic and its value, degrees of freedom or sample size (depending on which is most appropriate for that test), and the P value, with indication if it is one-or two-tailed (unless you address this issue in the methods).

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The conclusion section of the articles (particularly research articles) should include the following topics;

- principles, relationships, and generalizations inferred from the results (but not a repetition of the results),
- any exceptions to or problems with those principles, relationships, and generalizations, as indicated by the results agreements or disagreements with previously published work,
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### Author Contributions

Articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used:

Concept, Design, Supervision, Materials, Data Collection and/or Processing, Analysis and/or Interpretation, Literature Search, Writing, Critical Reviews. Initials of the contributing authors (i.e. AA, BB) should be given after these specified titles and all of them must be filled.

### Acknowledgments

Acknowledgments should include sources of support, grants, disclaimers, names of those who contributed but are not authors, etc. The names of funding organizations should be written in full. If no funding or help has been provided for the research, please include the "None" statement.

### Conflict of Interest Declaration

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence their work. If no conflict exists, the authors should include the "The authors declare no conflict of interest." statement under this section.

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When reporting experiments conducted with humans indicate that the procedures were in accordance with ethical standards set forth by the committee that oversees human experimentation. Approval of research protocols by the relevant ethics committee, in accordance with international agreements.

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https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

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is required for all experimental, clinical, and drug studies. Patient names, initials, and hospital identification numbers should not be used. Manuscripts reporting the results of experimental investigations conducted with humans must state that the study protocol received institutional review board approval and that the participants provided informed consent.

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## References

Citation in text must be numbered as [1] or [1-5]. Please ensure that every reference cited in the text is also present in the reference list. References should be accurate and numbered sequentially [in square brackets] in the text and listed in the same numerical order in the reference section with the DOI link. All authors must be cited and there should be no use of the phrase et al. The abbreviations of the journal names should be used. No references sould be given for the abstract section. Date of access should be provided for online citations. Furthermore, the references can easily be edited with the **JPharmTech** EndNote Style.

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***Journal***

[1] Ates M, Kaynak MS, Sahin S. Effect of permeability enhancers on paracellular permeability of acyclovir. *J Pharm Pharmacol*. (2016); 68(6): 781-790. https://doi.org//10.1111/jphp.12551

[2] Kaynak MS, Celebier M, Akgeyik E, Sahin S, Altınoz S. Application of HPLC to investigate the physicochemical properties and intestinal permeability of ketoprofen. *Curr Pharm Anal*. (2017); 13(1): 72-79. https://doi.org/10.2174/1573412912666160422151409

[3] Başaran E, Yenilmez E, Berkman MS, Büyükköroğlu G, Yazan Y. Chitosan nanoparticles for ocular delivery of cyclosporine A. *J Microencapsul*. (2014); 31(1): 49-57. https://doi.org/10.3109/02652048.2013.805839

***Book***

[4] Fotaki N, Klein S. *In vitro* drug release testing of special dosage forms. New Jersey: John Wiley & Sons; (2019). ISBN:1118341473

[5] Wilson CG, Crowley PJ. Controlled release in oral drug delivery. New York: Springer; (2011). ISBN:1461410045

***Book Chapter***

[6] Clayton NS, Emery NJ. What do jays know about other minds and other times? In: Berthoz A, Christen Y, editors. Neurobiology of "Umwelt". Berlin: Springer; (2009). p. 109-123. ISBN:3540858962

[7] Pepperberg IM. Symbolic communication in the Grey parrot. In: Vonk J, Shackelford T, editors. The Oxford handbook of comparative evolutionary psychology. New York: Oxford University Press; (2012). p. 297-319. ISBN:0199738181

***Conference Paper***

[8] Yurtdaş Kırımlığlu G, Özer S. "Formulation and *in vitro* characterization studies of levofloxacin hemihydrate incorporated PLGA based nanoparticles." Poster. 2nd International Gazi Pharma Symposium Series, Ankara, October 11-13, 2017. p. 93.

***Patent***

[9] Wong HL, Narvekar M, Xue HY, inventors; Temple University, assignee. Nanospheres for therapeutic agent delivery. United States patent no 9724304. (2017).

***Thesis***

[10] Arora HC. Doxorubicin-nanocarriers enhance doxorubicin uptake and clathrin-mediated endocytosis in drug-resistant ovarian cancer cells [Ph.D.]. Illinois: Northwestern University; (2012).

[11] Finn NA. Role of redox systems in doxorubicin metabolism and doxorubicin-mediated cell signaling: a computational analysis [Ph.D.]. Atlanta: Georgia Institute of Technology; (2011).

***Website***

[12] Secretariat E. The agreement on the conservation of populations of European bats. (2004). EUROBATS. Retrieved April 1 2020 from https://www.eurobats.org/index.htm

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RESEARCH ARTICLE

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Journal of Pharmaceutical Technology

# Posaconazole loaded ocular inserts for antifungal activity

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ABSTRACT

Corneal and conjunctival infections are common ocular diseases; however, sometimes lead to blindness when neglected. Despite most of the ocular drug delivery systems are in eye drop form, they suffer from poor retention on the ocular surface and low ocular bioavailability leading to unsatisfactory results even with repeated treatment. Therefore, there is a need for more effective drug delivery systems for the ocular application. The present study was carried out to demonstrate that ocular inserts effectively delivers a significant concentration of drug with topical administration for the treatment of fungal infections with the help of extended residence time on the ocular surface. Chitosan-based inserts were prepared by the freeze-drying method. The prepared inserts were evaluated for various parameters. Layered structures were revealed with scanning electron microscopy analyses. Thermal and structural behaviors were analyzed by differential scanning calorimetry and Fourier-transform infrared spectroscopy with nuclear magnetic resonance analyses, respectively. Drug contents were evaluated by a validated HPLC method. *In vitro* release studies were also performed in simulated tear fluid at 34±1°C for 48 hours. Analyses results revealed that chitosan-based ocular inserts were suitable systems for posaconazole delivery for the treatment of severe ocular fungal infections.



## 1. INTRODUCTION

Ocular diseases require immediate treatment due to vision threatening critical clinical reasons [1]. Topical instillation is the most widely preferred non-invasive route of drug administration to treat diseases affecting mostly the anterior segment [2]. However, the treatment of ocular infections with traditional drug delivery systems (eye drop, etc.), especially with the topical application, is challenging due to the unique structural properties of the eye. The eye is segmented into two parts, anterior and posterior segments. The anterior segment of the eye comprises cornea, aqueous humour, iris, ciliary body, and lens, whereas the posterior segment includes retina and vitreous humour [3]. Cornea, which is regarded as the main penetration site, acts as a barrier for both hydrophilic and lipophilic drugs [4,5]. Treatment approaches differ considering the target sites of the eye; therefore, the main strategies for the enhancement of ocular bioavailability are the extension of residence time on the corneal surface and the enhancement of corneal permeability by penetration enhancers [6]. In our study, ocular inserts were formulated for the maintenance of extended duration on the ocular surface. Chitosan was used as a polymeric lattice for the structural integrity of the inserts [7]. Chitosan is a biodegradable and non-toxic biomaterial, which has excellent mucoadhesive strength and has been routinely explored for controlled drug delivery at various mucosal sites of the body [5-8]. Chitosan is a polycationic polymer due to the positively charged amino groups [9-11]. Considering the negative charge of mucin layers at mucosal membranes, cationic drug delivery systems electrostatically interact with mucosal surfaces, which results in increased bioavailability with topical application [12-16].

Posaconazole is a second-generation triazole group member like voriconazole, ravuconazole, isavuconazole, and albaconazole, has greater potency, and possesses increased activity against resistance and emerging pathogens [17]. Posaconazole has also been investigated in phase III studies and approved by the regulatory agencies for the treatment and prophylaxis of invasive fungal infections; therefore, posaconazole was selected as an active agent considering its broad-spectrum activity [18].

In this study, posaconazole loaded chitosan inserts were prepared by the lyophilization method. Besides the longer duration period of the ocular inserts, electrostatic attractions between the negatively charged ocular surface and chitosan-based cationic inserts will enhance the ocular bioavailability of the active agent. Sustained release of drugs from polymeric network gives the possibility to reduce dose and dosing frequency while maintaining the effective topical treatment of sight-threatening severe ocular fungal infections.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Posaconazole was gifted by Abdi İbrahim İlaç (İstanbul, Turkey). Chitosan (high molecular weighted; 310000-375000 Da) and Acetic acid (glacial, ≥99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol, methanol, and acetonitrile were the products of Merck (Darmstadt, Germany). All other chemicals were in analytical grade.

### 2.2. Preparation of Ocular Inserts

Ocular inserts were prepared by lyophilization method [19]. Briefly, chitosan was dissolved (40 mg/mL) in acetic acid solution (2%, v/v), and posaconazole was dissolved in ethanol:acetonitrile (5:1) mixture. Formulations were prepared by mixing these solutions with different concentrations (**Table 1**) and stirred for 24h for the evaporation of the organic solvents.

The solutions were stored at -80±5°C, and lyophilization (3L, -86°C, Operon Freeze Dryer, Gimpo-City, Korea) method was applied. A uniform film was achieved and cut into pieces and were stored in well-closed containers until being analyzed (**Figure 1**).

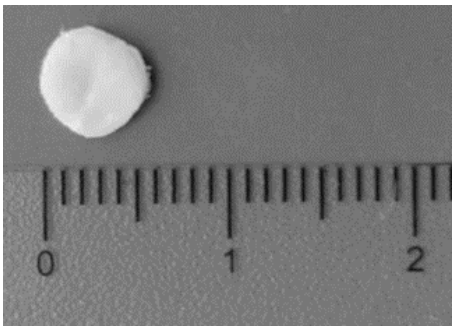


**Figure 1.** *Ocular insert prepared by lyophilization method*

### 2.3. Characterization Studies of Ocular Inserts

#### 2.3.1. Morphological analyses

The morphological properties of posaconazole loaded inserts were investigated by scanning electron microscopy (SEM) analysis (Zeiss Ultra Plus Fesem, Germany).

#### 2.3.2. Differential scanning calorimetry analyses

Structural and crystallinity changes of posaconazole and chitosan were evaluated using differential scanning calorimetry (DSC) (DSC-60, Shimadzu Scientific Instruments, Columbia, USA). Analyses were performed under nitrogen (flow rate of 50 mL/min) at 30-300°C. Thermograms of pure posaconazole and polymer were used as references.

#### 2.3.3. Fourier transform infrared spectrophotometry analyses

For the structural analyzes, Fourier transform infrared (FT-IR; IRAffinity-1S Shimadzu, Tokyo, Japan) analyses were performed. High-sensitivity DLATGS detector was used with Germanium-coated KBr Beam splitter at 7800 to 350 $cm^{-1}$ wavenumber range. FT-IR spectra of pure posaconazole and chitosan were used as references.

#### 2.3.4. Nuclear magnetic resonance analyses

For the evaluation of the interactions between the active agent and the polymer, $^{1}$H-NMR analyses were performed on Fourier 300 NMR (Brucker, Germany). Spectra of pure posaconazole and chitosan were used as references.

#### 2.3.5. Determination of posaconazole

A modified high-performance liquid chromatography (HPLC) method was used for the determination of posaconazole [20]. Shimadzu 20 A (Tokyo, Japan) with Shimadzu Shim-Pack CLC-ODS column (Tokyo, Japan; column diameter: 4.6 mm, column length: 25.0 cm, particle diameter: 5 µm, and particle size: 100 Å) was used as the instrument. Acetonitrile:distilled water (60:40, v/v) was used as the mobile phase with a flow rate of 1.0 mL/min. 20 µL constant amount of samples were injected via an autosampler (SIL-20A, Shimadzu, Tokyo, Japan) and a photodiode array detector (SPD-M20A, Shimadzu, Tokyo, Japan) was used at 262 nm. The column temperature was set to 25°C. (CTO-10AS-VP, Shimadzu, Tokyo, Japan) Validation studies were performed for data reliability [21].

#### 2.3.6. *In vitro* drug release studies

*In vitro* drug release studies were carried out by Apparatus 1 method with Pharma Test-PTWS820D (Hainburg, Germany) [22]. In this study, pH 7.4 simulated tear fluid (STF; 500 mL) was used as the release medium at 34±1°C [23]. At predetermined time intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 9, 24, and 48 hours), 1 mL samples were taken from the release medium, and the equivalent volumes of fresh medium were added back for the maintenance of sink conditions. The amount of active substance in the samples was determined with HPLC. Each analysis was repeated three times.

## 3. RESULTS AND DISCUSSION

Compositions of the formulations were given in **Table 1**. Formulations were kept in well-closed containers until being analyzed.

**Table 1.** *Compositions of the formulations*

| Ingredients | Formulation Code | | |
|---|---|---|---|
| | CPS0 | CPS1 | CPS2 |
| Chitosan HMW (mg) | 40.0 | 40.0 | 40.0 |
| Posaconazole (mg) | - | 16.7 | 25.0 |
| Ethanol:acetonitrile (5:1 (mL) | - | 2.0 | 3.0 |
| Acetic acid (2%) (mL) | 9.6 | 9.6 | 9.6 |

### 3.1. Characterization Studies of Ocular Inserts

Morphological and structural analyses were performed for the characterization of inserts.

#### 3.1.1. Morphological analyses

SEM is regarded as a reference method for the determination dimensional properties of the samples [24], therefore in this study, morphological analyses of the inserts were determined by SEM analyses, and micrographs were presented in **Figure 2**.

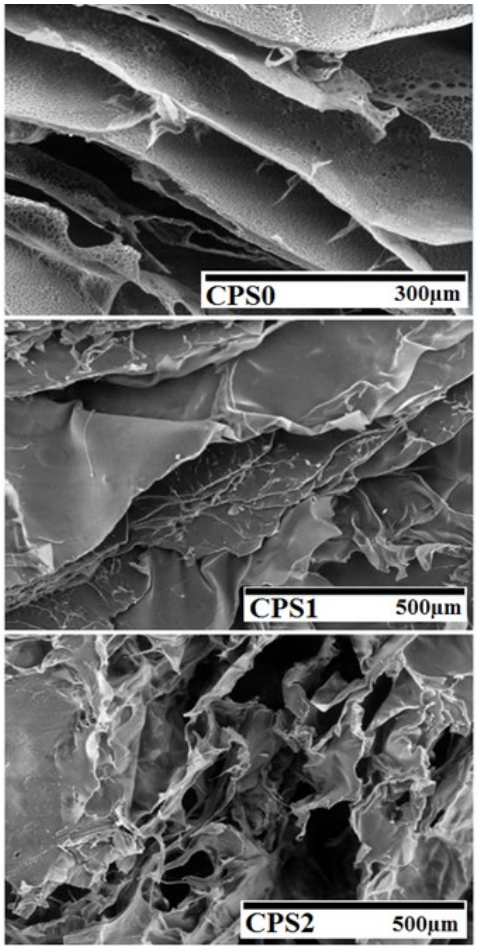


**Figure 2.** *SEM micrographs of the formulations*

Analyses results revealed that the inserts were formed in layers, which gives the possibility to enhance the tear sorption capacity of the formulations [25].

### 3.1.2. Differential scanning calorimetry analyses

DSC gives details about the thermal properties of the polymers, which is one of the most essential data for the processing of materials and also predicting the shelf life of the final product [26].

DSC thermogram of posaconazole exhibited a sharp endothermic peak at 174.59°C. Also, the melting peak was revealed in the thermogram of the physical mixture of posaconazole and chitosan, showing no chemical interaction was revealed between the active agent and polymer (**Figure 3**). No peaks were revealed in the thermograms of chitosan, showing that the amorphous structure of the polymer while the posaconazole peak was disappeared in the thermograms of formulations showing that active agent was molecularly dispersed within the polymeric layers [27]. And also, water forms intermolecular hydrogen bonding with various chitosan and posaconazole's amine and hydroxyl groups, which helps in molecular rearrangement resulting in ease of chain mobility as well as crystallinity (**Figure 3**) [25].

### 3.1.3. Fourier transform infrared spectrophotometry analyses

FT-IR spectroscopy was used to investigate the interactions between posaconazole and chitosan (**Figure 4**).

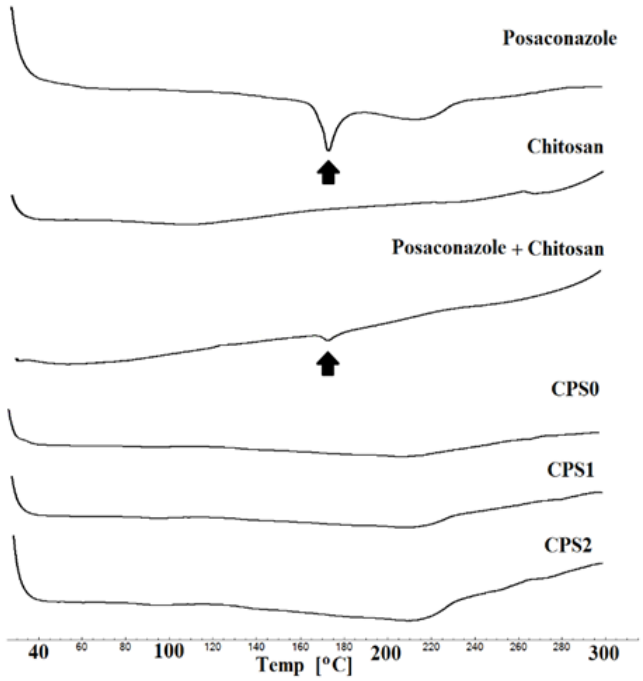


**Figure 3.** *DSC thermograms of the pure materials, physical mixture, and formulations*

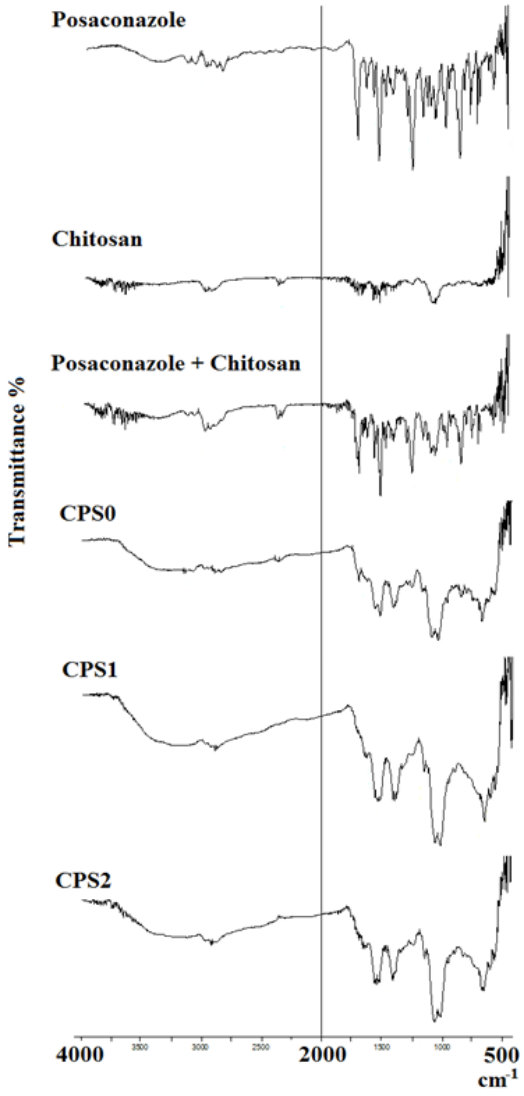


**Figure 4.** *FT-IR spectra of the pure materials, physical mixture, and formulations*

FT-IR spectrum of posaconazole shows high-intensity absorption peaks at 3061, 2966, 1685, 1508, and 1224 $cm^{-1}$ corresponding to the stretching vibrations of OH, $CH_2$, C=O, C=N, and C–N, respectively. The spectra of the physical mixtures corresponded to the spectra of individual components. Characteristic absorption peaks of posaconazole were revealed in the spectrum, indicating that posaconazole remained in the physical mixture without any interactions with chitosan [28]. However, characteristic signals of posaconazole could not be detected in the spectra of the formulations showing that active agent was molecularly dispersed within the polymeric layers in correlation with DSC analyses (**Figure 3**) [27,28].

### 3.1.4. Nuclear magnetic resonance analyses

For the evaluation of the interactions between the active agent and the polymer $^1$H-NMR analyses were performed, and analyses results were presented in **Figure 5**. Analyses results revealed that posaconazole signals were detected in the spectra of formulations with increased intensity (marked with arrows) in the range of 1-4 ppm showing that presence of posaconazole within the polymeric network without any chemical interaction [23,29].

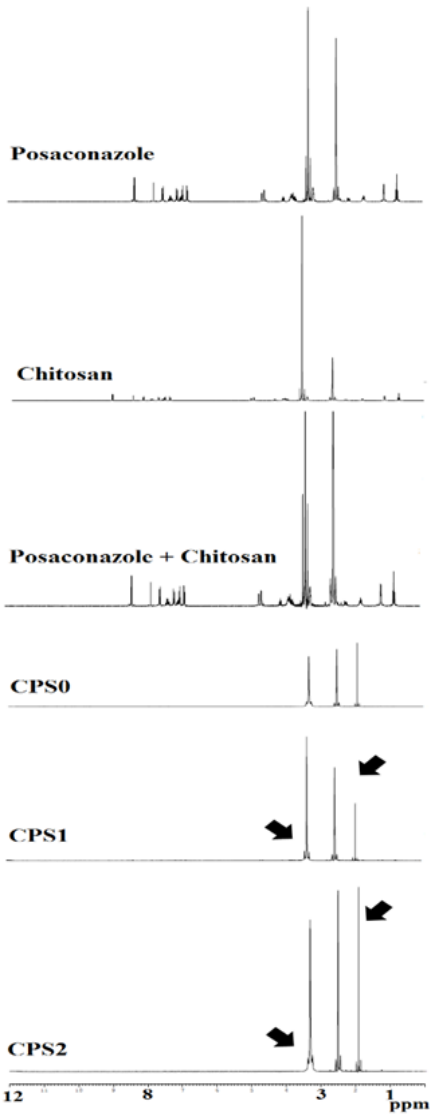


**Figure 5.** *$^1$H-NMR spectra of the pure materials, physical mixture, and formulations*

### 3.1.5. Determination of posaconazole

Validation studies of the HPLC method were carried out in accordance with the guidelines of ICH within the range of 5-200 µg/mL [21]. $R^2$ was 0.9999, accuracy values were in the range of 98.96-102.58%, while RSD value was less than 2% as a result of precision studies. The selectivity of the method was analyzed in comparison with placebo formulation (CPS0) and with all the components of the study (**Figure 6**).

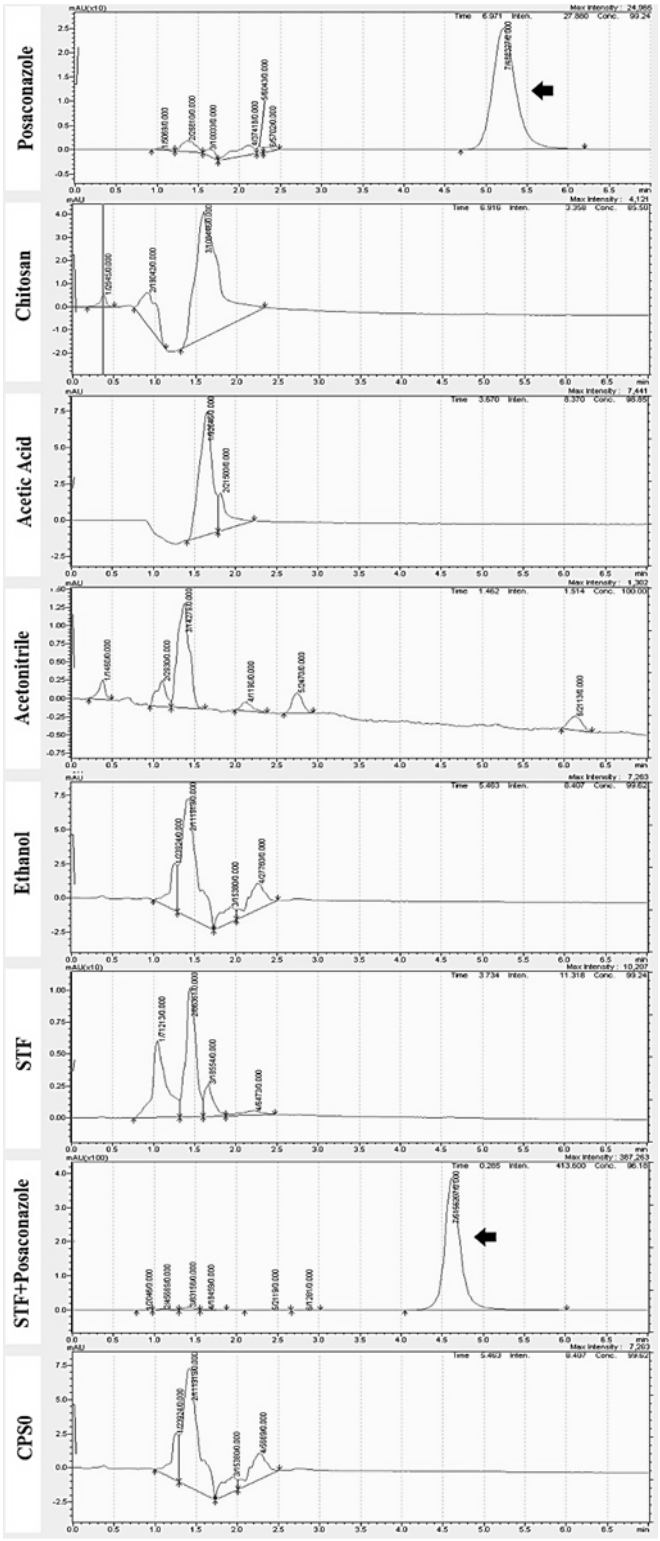


**Figure 6.** *HPLC analyses signals of posaconazole in selectivity studies*

Analyses results revealed that no other substance did give signals at the point where the active substance was determined. For the evaluation of the posaconazole amount, a constant amount of formulation was dissolved in acetic acid (2%; v/v):ethanol:acetonitrile mixture (1:1:1, v/v) and were analyzed by HPLC. Analyses results revealed posaconazole amounts were 4.01±0.02% and 5.90±0.01% (mean±SE; n=3) for the CPS1 and CPS2 formulations respectively (**Table 1**)

### 3.1.6. *In vitro* release studies

*In vitro* release studies were performed in STF at pH 7.4 for 48 hours, and analyzes results were presented in **Figure 7** [23,29,30]. According to the analysis results, the release rate of the active agent from CPS1 has reached the highest point of 40.40% while it was 49.09% for CPS2 after 48 hours. Despite the release rates could not reach 100%, the analyses were conducted for 48 hours considering the residence time on the ocular surface.

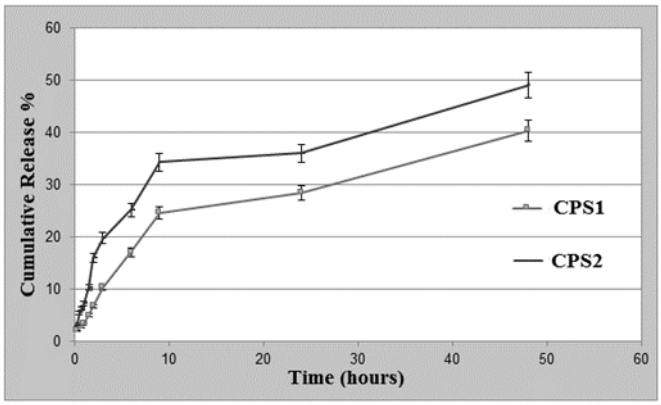


**Figure 7.** *In vitro release profiles of formulations (mean±SE; n=3)*

Posaconazole was practically insoluble in water (0.012 mg/mL); therefore, for the maintenance of sink conditions, *in vitro* release analyses were performed using Apparatus 1 (in 500 mL STF at 34±1°C) and release studies of the pure drug could not be performed with same method [23,29,31]. As the commercial products of posaconazole were in tablet, oral suspension, and injection forms, in *in vitro* release analyses comparison with commercial products could not be performed considering the results of the analyses will not contribute the ocular application of posaconazole via chitosan-based inserts [31].

Since most of the ocular formulations have poor performance due to their uncontrollable and undesirable burst releases with rapid removal from the ocular surface; biphasic release systems can maintain an initial burst release followed by a relatively steady release which enhances the ocular performance and ocular bioavailability of the applied formulation resulting ineffective treatment of severe ocular disorders [30,32].

## 4. CONCLUSION

The present study describes the development of posaconazole loaded chitosan inserts by the freeze-drying method. *In vitro* characterization analysis results revealed the characteristic properties of inserts in detail. SEM analyses revealed the layered structure of the system, which gives the possibility to increase the tear sorption of the system. Considering the cationic character of the polymeric structure enhanced ocular bioavailability with the help of electrostatic interactions between the oppositely charged ocular and polymeric surfaces as well as enhanced corneal duration period of the inserts.

## AUTHOR CONTRIBUTIONS

Concept: EY, MD, EB; Design: EY, MD, EB; Supervision: MD, EB; Materials: EB; Data Collection and/or Processing: KA, EB; Analysis and/or Interpretation: KA, EB; Literature Search: KA, EB; Writing: MD, EB; Critical Reviews: EY, MD, EB.

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## CONFLICT OF INTEREST DECLARATION

The authors declared no conflict of interest.

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# Preparation, characterization and *in vitro* evaluation of theophylline loaded microemulsion formulations

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ABSTRACT

Theophylline is widely used as a bronchodilator drug in asthma and chronic obstructive lung disease because it is effective and inexpensive. Theophylline blood concentrations should be monitored since its therapeutic index is narrow, its optimum blood levels range from 10 to 20 µg/mL; its serious side effects take place over 20 µg/mL; its efficacy falls below 10 µg/mL blood concentrations. The aim of the present study is to formulate and characterize oil-in-water microemulsion systems for oral delivery of theophylline. Microemulsion formulations represent an interesting delivery vehicle for poorly water-soluble drugs, allowing for improving their solubility and dissolution properties. A simple and reliable UV-Spectrophotometric method was developed and validated for the determination of theophylline in the concentration range of 25-150 µg/mL. *In vitro* drug release profile showed that after 1 hour, 40% of the drug released through the formulation in Simulated Intestinal Fluid at pH 6.8 as a release medium and the microemulsion formulations released more than 75% of the drug during the 5 hours study period. The globule size of drug-loaded formulations was in the range of 203.6 ± 0.2 nm. Results confirmed that the proposed microemulsion formulation containing theophylline could improve and control the drug release profile in comparison to the conventional dosage form.



## 1. INTRODUCTION

Theophylline, which is a methylxanthine derivative, is widely used in the treatment of asthma because it is effective and cheap all over the world, including our country [1]. There is evidence of the anti-inflammatory and immunomodulating effects, as well as the bronchodilator effect [2]. When theophylline was first introduced into asthma therapy, it was used as a bronchodilator, and early dose-response studies showed an increasing acute bronchodilator response. Bronchial asthma is a chronic inflammatory disease of the airways. Bronchial asthma is a state of obstructive airway obstruction that is not related to any other disease, and hyperreactivity (hyperresponsiveness) of the airways against various stimuli [2,3]. Asthma is a serious social health problem for people of all age groups in the world. Asthma is a disease that significantly restricts the quality of life when it is uncontrolled, requires constant medical care and treatment, and can even lead to death [4]. Although it is claimed that there are approximately 300 million asthma patients worldwide today, the absence of a precise and generally accepted definition of asthma makes it difficult to make a reliable comparison of reported frequency values from various parts of the world [5,6]. Until recent years, asthma was considered a disease characterized by airway smooth muscle spasm, and bronchodilator drugs were used primarily in the treatment. Today, the main goal of asthma treatment is to suppress chronic inflammation in the airways, thereby controlling and maintaining the disease [2].

Drugs used in the treatment of asthma are divided into two as controlling and relaxing drugs. Controlling medicines are mainly used daily and long-term to keep asthma under control through its anti-inflammatory effects. Relaxing drugs, on the other hand, are medicines that act rapidly, correct bronchoconstriction, relieve symptoms, and are used when necessary [6]. Theophylline preparations especially provide controlled release, and they are included in the list of controlled medicines in the treatment of asthma.

The Global Initiative for Asthma Guidelines, 2002 guidelines for asthma, recommend that theophylline can be used as an add-on therapy to patients not controlled by low doses of inhaled corticosteroids but recommend long-acting $\beta_2$-agonists as more effective and with fewer adverse effects [7]. Several clinical studies have demonstrated that adding theophylline to inhaled corticosteroids in patients with mild to moderate asthma who are not controlled gives equivalent or better asthma control than doubling the dose of inhaled corticosteroids [8,9].

Theophylline was first used in the clinic in 1907 by Minkowski and a group of doctors as a diuretic in cardiac and renal edema following extraction from tea leaves by the

German biologist Albrecht Kossel in 1888 [10]. It was added for the first time to The United States Pharmacopoeia's (USP) contents in 1918. With the development of pharmacokinetic science in the 1970s and 1980s, the pharmacokinetic properties of theophylline, the plasma level, and the relationship between bronchodilator effect and toxicity were intensively investigated. Considering this information, theophylline has become a commonly used drug in the treatment of asthma [11].

Microemulsions (MEs) are thermodynamically stable and translucent systems widely used for the systemic delivery of the drugs. In pharmaceutical fields, the interest in MEs is increasing; thus, they are applied to various administration routes. These systems form spontaneously at a certain concentration of oil, water, and surfactant. MEs often require the addition of cosurfactants to obtain an appropriate fluidity and flexibility of the interface. Due to their unique characteristics such as transparency, small droplet diameter (10-140 nm), high stability, and low surface tension, MEs are also one of the popular drug delivery systems for the controlled release of the active ingredient. Having very low inter-surface tension is essential in their formation and stability [12-14].

There are three types of MEs, including water-in-oil (w/o), oil-in-water (o/w), and bi-continuous systems. In w/o or o/w type of MEs, the droplets are surrounded by a film made of surfactant and cosurfactant molecules. The main advantages of this type of system are the simple preparation method, low energy requirement, and also high encapsulation efficacy. The critical parameter for ME preparation is the correct selection of the oil phase and the surfactant/co-surfactant component in appropriate ratios.

Recently, there has been considerable interest in the ME formulations for the delivery of hydrophilic as well as lipophilic drugs as drug carriers because of its improved drug solubilization capacity, long shelf life, ease of preparation and improvement of bioavailability. The present study deals with the progress of polysorbate-based theophylline MEs in drug delivery.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Theophylline powder, ≥99%, anhydrous; isopropyl palmitate (IPP); Tween®20 and propylene glycol were provided from Sigma-Aldrich. All other chemicals were of analytical grade and used without further purification.

### 2.2. Methods

#### 2.2.1. Analytical method validation

Method validation studies of theophylline were carried out according to the International Conference on Harmonization (ICH) guideline Q2(R1) [15]. The described method was validated with respect to linearity, precision, accuracy, the limit of detection (LOD) and quantitation (LOQ). The analysis method was developed by using a UV-Spectrophotometer, and absorbance values were obtained at 295 nm.

The stock solution of theophylline (1 mg/mL) was prepared by dissolving 50 mg of drug in 50 mL of Simulated Intestinal Fluid (SIF) (pH 6.8) prepared according to USP 29 [16]. The standard solutions were stored at 4±1°C in a clear glass volumetric flask and light protected with aluminum foil. Theophylline concentrations in the working solution chosen for the calibration curves were 25, 50, 75, 100, 125, and 150 µg/mL.

#### 2.2.2. Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were obtained with a water titration method at room temperature (25°C). For each phase diagram, the oil phase and the surfactant mixture ($S_{mix}$) were mixed and different ratios of oil:surfactant mixtures were prepared from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Water was added drop by drop to each mixture carefully until the mixture became turbid at a certain point. The mixture that remained transparent and homogeneous after stirring was assigned to the monophasic area of the phase diagram. This process was repeated in the same way for each phase diagram [17,18].

#### 2.2.3. Preparation of microemulsion formulation

The pseudo-ternary phase diagram of the largest area was selected to determine the concentration range of the components in the ME. ME formulations for further studies were selected from the weight center of these pseudo-ternary diagrams [19].

#### 2.2.4. Globule size, zeta potential and polydispersity index

The mean droplet size and size distribution (polydispersity index; PDI) of MEs were characterized by photon correlation spectroscopy (Nano ZS, Malvern, UK). Three individual runs were performed on each sample. The size of droplets is expressed as Z-average diameter and reported as the mean diameter. The surface charge of MEs was determined by measuring the zeta potential of MEs using the electrophoretic light-scattering technique (Nano ZS, Malvern, UK). For each sample, three individual runs were performed for which zeta potential was calculated as the mean value.

#### 2.2.5. Centrifugation

ME formulation was centrifuged at 3000 rpm for 30 minutes and then was examined for whether the system was monophasic or biphasic.

#### 2.2.6. *In vitro* drug release studies

*In vitro* release tests were performed using dialysis membrane methods. The medium was 50 mL of Simulated Intestinal Fluid (SIF) pH 6.8 [16], and the temperature was kept at 37±0.5°C. MEs containing the same amount of theophylline prepared based on the solubility study were directly introduced into the dissolution medium. While stirring the release medium using the magnetic stirrer at 100 rpm, 1 mL of dissolution medium was withdrawn at the predetermined period (5 min, 15 min, 30 min, 60 min, 90 min, 2 h, 3 h, 4 h, 5 h). After each sampling, 1 mL of fresh medium was added to the incubation medium. The amount of released active agent in the medium collected was determined by UV-spectroscopy, and cumulative release percentage of theophylline was calculated [20,21].

## 3. RESULTS AND DISCUSSION

### 3.1. Analytical Method Validation

The linearity between absorbance and concentration was analyzed using a calibration curve obtained from standard solutions of theophylline (25 to 150 µg/mL). Linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis. The determination coefficient ($R^2$) of 0.9991 was obtained. Linearity test results are shown in **Table 1**, and the regression curve is presented in **Figure 1**. In this study, the range was observed linearly to the highest concentration, and the $R^2$ of 0.9991 was highly significant [18].

**Table 1.** *Series and absorbance values of the linearity study*

| Conc. (µg/mL) | Absorbance | | | Mean | SD |
|---|---|---|---|---|---|
| | SET 1 | SET 2 | SET 3 | | |
| 25 | 0.077 | 0.081 | 0.076 | 0.078 | 0.003 |
| 50 | 0.172 | 0.154 | 0.158 | 0.161 | 0.009 |
| 75 | 0.271 | 0.245 | 0.233 | 0.250 | 0.019 |
| 100 | 0.333 | 0.320 | 0.295 | 0.316 | 0.019 |
| 125 | 0.421 | 0.388 | 0.366 | 0.392 | 0.028 |
| 150 | 0.513 | 0.485 | 0.429 | 0.475 | 0.043 |

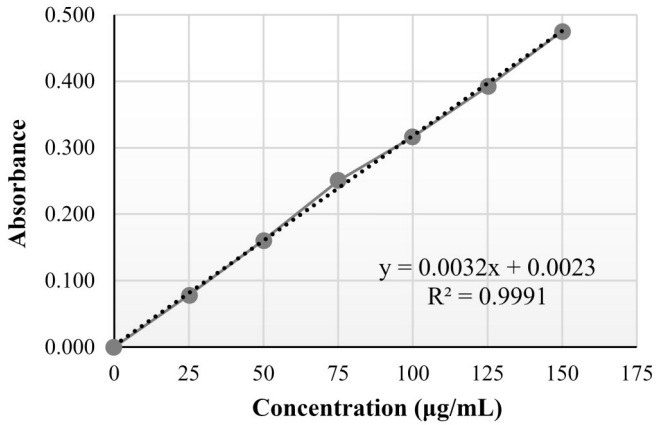


**Figure 1.** *Regression profile of theophylline*

25, 75, 125 µg/mL theophylline solutions were analyzed three times in order to evaluate method precision, standard deviation (SD), and relative standard deviation (RSD). Results of precision and repeatability tests on different concentrations are given in **Table 2**.

Since the RSD% values for both intermediate precision and repeatability were <2%, the method developed for theophylline was found to be precise according to the suggestions in ICH Q2(R1) guidelines [22].

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value and is defined recovery. The prepared three standard solutions (50, 100, 150 µg/mL) were analyzed three times to evaluate the accuracy of the method developed. As given in **Table 3**, the recoveries of theophylline at various concentrations were obtained between 97-106%. Since the percentage of recovery has been found between the acceptance criteria, which is 90-110%, the analysis system for the determination of assay is verified. Low values of standard deviation denoted good repeatability of the measurement [23].

**Table 2.** *Precision results of theophylline on three different days*

| | Absorbance | | | Concentration (µg/mL) | | |
|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 |
| 25 µg/mL | 0.076 | 0.107 | 0.076 | 23.031 | 32.719 | 23.031 |
| | 0.078 | 0.106 | 0.078 | 23.656 | 32.406 | 23.656 |
| | 0.078 | 0.108 | 0.077 | 23.656 | 33.031 | 23.344 |
| | | | **Mean** | 23.448 | 32.719 | 23.344 |
| | | | **SD** | 0.361 | 0.313 | 0.313 |
| | | | **RSD** | 1.539 | 0.955 | 1.339 |
| 75 µg/mL | 0.274 | 0.25 | 0.241 | 84.906 | 77.406 | 74.594 |
| | 0.271 | 0.245 | 0.230 | 83.969 | 75.844 | 71.156 |
| | 0.269 | 0.240 | 0.229 | 83.344 | 74.281 | 70.844 |
| | | | **Mean** | 84.073 | 75.844 | 72.198 |
| | | | **SD** | 0.786 | 1.563 | 2.081 |
| | | | **RSD** | 0.935 | 2.060 | 2.882 |
| 125 µg/mL | 0.419 | 0.389 | 0.361 | 130.219 | 120.844 | 112.094 |
| | 0.420 | 0.386 | 0.366 | 130.531 | 119.906 | 113.656 |
| | 0.425 | 0.390 | 0.370 | 132.094 | 121.156 | 114.906 |
| | | | **Mean** | 130.948 | 120.635 | 113.552 |
| | | | **SD** | 1.005 | 0.651 | 1.409 |
| | | | **RSD** | 0.767 | 0.539 | 1.241 |

Limit of detection (LOD) of an analytical procedure is the minimum amount of analyte which can be detected in a sample, while the limit of quantitation (LOQ) is the lowest concentration of an analyte in a test and can be determined with acceptable precision and accuracy under the specified test conditions. LOD and LOQ were determined using the calibration curve method, according to ICH Q2(R1) recommendations. The LOD and LOQ of the proposed method were calculated using the following equations:

$$LOD = 3.3 \times \sigma/s$$
$$LOQ = 10 \times \sigma/s$$

where,

$\sigma$ = the standard deviation of the response
s = slope of the calibration curve [24].

LOD and LOQ for theophylline were found to be 0.260 µg/mL and 0.786 µg/mL, respectively.

**Table 3.** *Accuracy results of theophylline*

| | Absorbance | Conc. (µg/mL) | Recovery % |
|---|---|---|---|
| 50 µg/mL | 0.157 | 48.344 | 96.688 |
| | 0.154 | 47.406 | 94.813 |
| | 0.163 | 50.219 | 100.438 |
| | | **Mean** | 97.313 |
| | | **SE** | 2.864 |
| 100 µg/mL | 0.333 | 103.344 | 103.344 |
| | 0.337 | 104.594 | 104.594 |
| | 0.334 | 103.656 | 103.656 |
| | | **Mean** | 103.865 |
| | | **SE** | 0.651 |
| 150 µg/mL | 0.512 | 159.281 | 106.188 |
| | 0.514 | 159.906 | 106.604 |
| | 0.512 | 159.281 | 106.188 |
| | | **Mean** | 106.326 |
| | | **SE** | 0.241 |

SE: Standard error

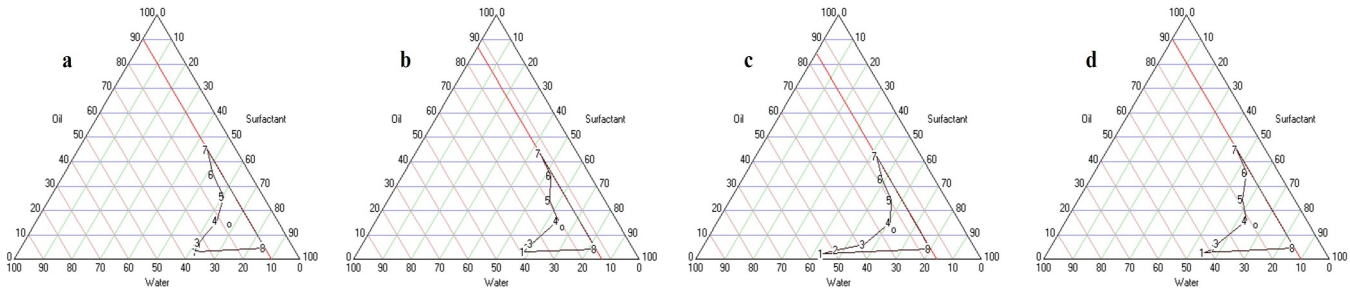


**Figure 2.** *Pseudo-ternary phase diagrams of microemulsions surfactants:cosurfactant ratio* ***a:*** *2:1,* ***b:*** *3:1,* ***c:*** *4:1,* ***d:*** *5:1*

### 3.2. Construction of Pseudo-Ternary Phase Diagrams

MEs are transparent, thermodynamically stable and isotropic oil, water, surfactant, and cosurfactant mixtures. Several studies have shown that ME formulations provide improved oral and dermal drug delivery properties [25,26]. Many ME formulations are based on short- and medium-chain alcohols, while others are based on poloxamer or propylene glycol as cosurfactants in combination with polysorbates [25].

It is known that large proportions of surfactant may cause irritation and toxicity. It is important to determine the surfactant concentration and to use the minimum concentration required for the development of the ME formulation [27].

In this study, all the ingredients were pharmaceutically acceptable and generally regarded as in the safe category. Nonionic surfactants are generally considered as having low toxicity and irritancy and, therefore, regarded to be suitable for oral administration.

Pseudo-ternary diagrams are equilateral triangles that define the compositional phase behavior of the system. The vertices represent pure ingredient or two ingredients, the edges indicate two-ingredient systems, and the interior represents all three ingredients in the system. Pseudo-ternary phase diagrams of the ME area of existence with isopropyl palmitate, Tween®20, propylene glycol, and water mixtures and the final compositions are presented in **Figure 2**. The ME formulations 5:1 ratio was selected, which showed larger ME regions. The complete composition of oil, surfactant, cosurfactant, and the aqueous phase were shown in **Table 4**.

**Table 4.** *Composition of optimum microemulsion formulation*

| Components | % |
|---|---|
| Isopropyl palmitate | 14.5 |
| Tween®20 | 57.4 |
| Propylene glycol | 9.6 |
| Water | 18.6 |

### 3.3. Characterization of Microemulsion Formulation

The final ME formulation maintained the predetermined characteristics after the addition of theophylline. Blank formulation and formulation including the active agent were clear transparent liquids with a homogenous appearance, no precipitation, good fluidity, and a yellow tinge at room temperature. When physical stability was observed, MEs showed no phase separation after centrifugation. Besides, there was no sign of turbidity or creaming.

The zeta-size analyzer was employed to determine the globule size of the ME formulation (**Figure 3**). The globule size was found to be 203.6±0.2 nm. This shows that the optimized formulation was nano-sized and could be termed as nano formulations. Smaller emulsion globule size results in lower emulsification time and a larger surface area provided for the drug absorption. The small globule size and good dispersibility of the ME indicated that the bioavailability and absorption of theophylline could be improved. There was no significant change in globule size and PDI after active agent incorporation [28-30].

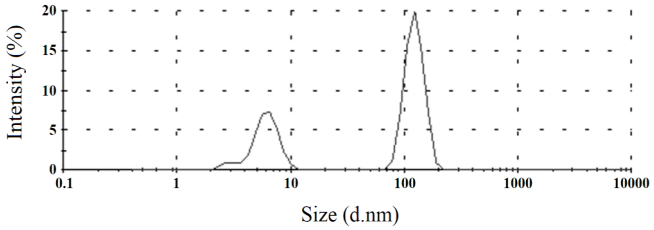


**Figure 3.** *Globule size analysis of 5:1 microemulsion formulation*

The globule size and polydispersity index have a significant impact on the physical stability of colloidal drug delivery systems when used to orally. The polydispersity index varies from 0.0 to 1.0 and is a measure of particle homogeneity. The globules are more homogeneous if the polydispersity index value is closer to zero [28,31]. The small polydispersity index of ME showed uniformity in the size distribution of globules, polydispersity index below 0.3 could be used as an indication of uniformity of globules. This result used as a determination of the stability of MEs [31].

The zeta potential is a method to measure the surface charge of particles or globules. Zeta potential is used for predicting dispersion stability, and its value depends on the physicochemical property of the drug, polymer, vehicle, presence of electrolytes, and their adsorption [29]. **Figure 4** indicates the value of the zeta potential of the optimized ME. Zeta potential is an essential property of the formed dispersion, and large zeta potential value of nanostructures was an important factor for physical stability.

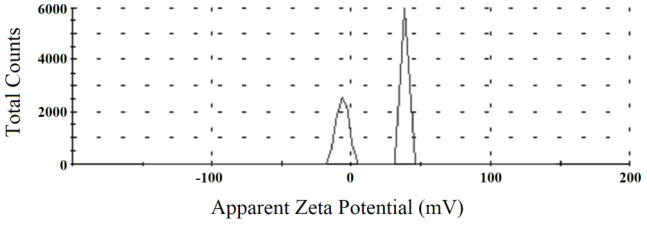


**Figure 4.** *Zeta potential of 5:1 microemulsion formulation*

The values of zeta potential indicate that the system is relatively stable. The zeta potential values of ME may approach neutral charge due to the presence of the nonionic surfactant. The ME shows positive zeta potential, which is important for achieving the interaction of the gastrointestinal system for effective and prolonged theophylline delivery [30].

### 3.4. *In Vitro* Drug Release Studies

The dialysis bag is the most commonly used technique in the literature for drug release measurements [32]. The *in vitro* dissolution study of theophylline loaded formulation was compared with the dissolution profile of the pure theophylline. The cumulative release percentages at the regular time were calculated and showed in **Figure 5**. In the figure, the cumulative drug release percentage after 1 hour was 40% and 95% for ME formulation and pure active agent solution in SIF pH 6.8, respectively.

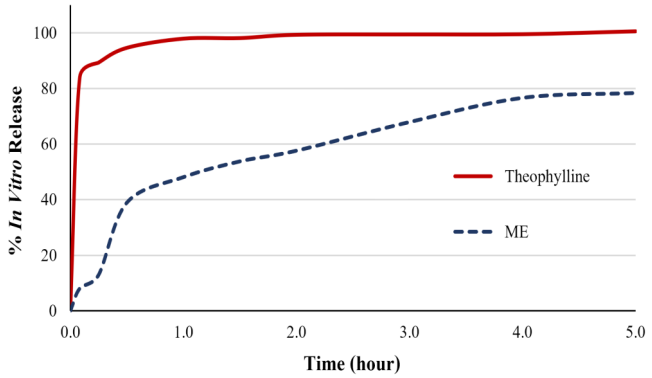


**Figure 5.** *In vitro cumulative drug release profile*

Experimental results showed that ME formulations release more than 75% of the drug during 5 hours of study period. These drug release behaviors confirm the sustained release of theophylline in the ME formulation, which enhances the drug retention time. The sustained release of this formulation is consistent with the literature [28-30].

## 4. CONCLUSION

In this study, we successfully prepared theophylline loaded ME formulation sized around 200 nm. This study shows that the o/w ME with good clarity, ideal stability, and proper characterization, can be prepared successfully using Tween®20 as the surfactant and propylene glycol as the cosurfactant. Tween®20:propylene glycol in the weight ratio of 5:1 was the surfactant providing greater area. The *in vitro* studies demonstrated the potential of developed ME for oral delivery of theophylline.

## AUTHOR CONTRIBUTIONS

Concept: TÇ, DO, UMG, ED; Design: TÇ, DO, UMG, ED; Supervision: TÇ, DO, UMG, ED; Materials: TÇ, DO, UMG, ED; Data Collection and/or Processing: TÇ, DO, UMG, ED; Analysis and/or Interpretation: TÇ, DO, UMG, ED; Literature Search: TÇ, DO, UMG, ED; Writing: TÇ, DO, UMG, ED; Critical Reviews: TÇ, DO, UMG, ED.

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## CONFLICT OF INTEREST DECLARATION

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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# Evaluation of the effect of anti-pollution & anti-aging eye cream on the collagen contraction

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ABSTRACT

This is a study of the brown algae-derived hydrolyzed algin as used in eye cream formulation. Alginic acid sodium is a gelling and non-toxic anionic polysaccharide, which is used to bone tissue engineering, preparation of alginate hydrogels, encapsulating, hydrating, protective, and de-polluting action. The skin of eye contour is thinner and less dense in support fibres than other parts of the face. Therefore, cutaneous sagging is frequently seen to appear around 40 years of age, very often aggravated by environmental factors such as pollution. The objective of the study was to evaluate the effect of anti-pollution & anti-aging eye cream on the collagen contraction. The outcomes of the characterization analysis indicate the development of successful cream formulation with optimum characteristics. No microbial growth was observed. The collagen lattices were prepared with human dermal fibroblasts, for evaluation of the effect of the cream on the collagen contraction. The lattices were treated or not (control) with the tested cream and then incubated at 37°C for 96 hours. The surface of lattices was measured by image analysis, and the lattices contraction was analyzed at 16, 24, 40, 48, 64, 72, 88, and 96 hours (the measure of lattices area). The treatment with eye cream at 0.5% decreases the surface of the lattice compared to the control (no treatment). According to the results obtained under the conditions of the test, the eye cream tends to increase the collagen contraction and a significative decrease of the lattice surface by 6.68% compared to the control at 16 hours.



## 1. INTRODUCTION

This is a study of the brown algae-derived hydrolyzed algin as used in cosmetics. The ingredient in this study is the extract of the whole or a defined part of the seaweed. “Brown algae” is a common name for seaweeds of the class *Phaeophyceae* and classified in about 265 genera with more than 1500 species [1,2].

The actual color varies depending on the proportion of brown pigment (fucoxanthin) to green pigment (chlorophyll). This algal group contains alginic acid and fucoidan in its complex cell walls. Several brown algae constituents, such as phytosterols, phytosteryl ingredients, and alginic acid, were found to be safe [3,4]. The most frequently reported function of brown algae in cosmetics is as a skin-conditioning agent; other reported functions include absorbent, antioxidant, binder, hair conditioning agent, oxidizing agent, and viscosity increasing agent [4].

Alginic acid sodium is a gelling and non-toxic anionic polysaccharide. The carboxylic acid groups on the alginic acid chain render it insoluble in water. However, converting alginic acid to its sodium form enables it to solubilize in water easily [5].

Hydrolyzed algin is used:

- in combination with chitosan, to fabricate a biodegradable porous scaffold for bone tissue engineering [6],
- to study the characteristics of a modified amphiphilic alginate derivative [7],
- to the study the impact of alginate on the rate of lipid digestion by employing an *in vitro* digestion model [8],
- in the preparation of alginate hydrogels [9],
- as an encapsulating agent of β-galactosidase microparticles [10],
- as an occlusive and hydrating agent,
- for protective and de-polluting action [11].

Brown algae have been used for wastewater/effluent treatment and removal of heavy metals (*Sargassum*, *Laminaria*, and *Ecklonia* species) [12].

Alginates, which are membrane polysaccharides taken from the skin of algae, are depolymerized to obtain a high molecular weight oligoalginate. Applied to the skin, their high degree of polymerization enables them to stay on the surface of the epidermis and form a protective mask.

Every day the skin is exposed to all sorts of impurities in the atmosphere, such as heavy metals, cigarette smoke, and

so on. These reduce the levels of hydration and oxygenation and produce free radicals, which lead to cutaneous aging. Alginates minimizes the adhesion of chelates heavy metals such as lead and cadmium to stop particles from encrusting and asphyxiating the skin. Alginates also protects the viability of cells exposed to cigarette smoke [13].

The skin of the eye contour covers 22 muscles, which are continually moving. To make this mobility possible, this area is thinner and less dense in support fibres than other parts of the face. Therefore, cutaneous sagging is frequently seen to appear around 40 years of age, very often aggravated by environmental factors such as pollution [14].

The objective of the study was to evaluate the effect of anti-pollution & anti-aging eye cream on the collagen contraction.

## 2. MATERIALS AND METHODS

### 2.1. Materials

All chemicals were of reagent and analytical grade. Double-distilled water was used throughout the study. All chemicals CAS No and functions were given in **Table 1**.

**Table 1.** *The complete formula of eye cream formulation*

| Chemical Analysis Properties | | | |
|---|---|---|---|
| **Components** | **% w/w** | **CAS No** | **Function** |
| Water | q.s. | 7732-18-5 | solvent |
| Glycerin | 5.000 | 56-81-5 | humectant |
| Cetearyl Alcohol | 4.500 | 67762-27-0 | emulsifying |
| Glyceryl Stearate<br>Ceteareth-20<br>Ceteareth-12<br>Cetyl Palmitate | 4.000 | 31566-31-1<br>68439-49-6<br>68439-49-6<br>540-10-3 | emollient<br>emulsifying |
| Caprylic/Capric Triglyceride | 2.500 | 73398-61-5 | skin conditioning |
| Dicaprylyl Carbonate | 2.000 | 1680-31-5 | emollient |
| Seawater,<br>Hydrolyzed Algin,<br>Phenethyl Alcohol,<br>Sucrose | 1.500 | -<br>-<br>60-12-8<br>57-50-1 | <br>humectant<br>skin conditioning |
| Phenoxyethanol<br>Ethylhexylglycerin | 0.765<br>0.085 | 122-99-6<br>70445-33-9 | preservative skin conditioning |
| Cera Alba | 0.600 | 8012-89-3 | emollient |
| Mica | 0.500 | 12001-26-2 | opacifying |
| Butyrospermum Parkii (Shea Butter) | 0.500 | 194043-92-0<br>91080-23-8 | skin conditioning |
| Sodium Polyacrylate | 0.300 | 9003-04-7<br>25549-84-2 | emollient<br>emulsion stabilizing<br>film forming |
| Xanthan Gum | 0.050 | 11138-66-2 | viscosity controlling |
| Tocopheryl Acetate | 0.001 | 7695-91-2<br>58-95-7 | antioxidant |

### 2.2. Preparation of Eye Cream Formulation

The complete formula is reflected in **Table 1**. All the aqueous phase materials and the oil phase ingredients were placed in two separate containers and heated to above 75°C. The water phase was then added to the oil phase using continuous agitation. Preservative, antioxidant, humectant agents and hydrolyzed algin mixture were added after cooling. The formulation was kept at 25±1°C for 48 hours to see a possible phase separation.

### 2.3. Characterization of Formulation

The formulation was evaluated for its sensorial parameters and physicochemical parameters like pH, density, and viscosity. The sensorial parameters of the formulation, such as appearance, odor, color, were determined. The pH of the formulation was detected by a digital pH-meter (Mettler Toledo S 220, Switzerland), the density was detected by a pycnometer (Mettler Toledo 30330857, Switzerland), and the viscosity measurements were performed with a vibro viscometer (AND, SV-10, Japan). The experiments were repeated five times at 25°C.

### 2.4. Microbial Contamination Tests

The microbiological contamination of formulations was evaluated by validated test kits from AFNOR using cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas Aeruginosa*, total bacteria, yeast, and mold.

### 2.5. Evaluation of The Effect of an Eye Cream on The Collagen Contraction

For technical reasons, the tested formulation was previously extracted. It was first diluted to 10% in the medium; the insoluble mixture was stirred for 1 hour to extract the active ingredients. After centrifugation, the supernatant containing the active principles (aqueous phase) was collected and diluted in culture medium at specified concentrations. This study was done with the support of ARERKO.

#### 2.5.1. Biological model

The collagen lattice model was used, which corresponds to a three dimensional (3D) reconstituted dermis, with a population of fibroblasts synthesizing new fibers and interacting with collagen fibers network (confidential composition).

#### 2.5.2. Preparation of the lattices

The cells were Normal human dermal fibroblasts from a donor of 30 years old, passage 12.

The collagen lattices were prepared with these fibroblasts in 5% of serum. The lattices contraction was analyzed after 16, 24, 40, 48, 64, 72, 88, and 96 hours of culture (photographs and measure of lattices area).

#### 2.5.3. Treatment

The lattices were treated or not (control) with the tested cream and then incubated at 37°C for 96 hours. The lattices contraction was analyzed at 16, 24, 40, 48, 64, 72, 88, and 96 hours (measure of lattices area). The surfaces of lattices were measured by image analysis at a different time of incubation (16, 24, 40, 48, 64, 72, 88, and 96 hours).

#### 2.5.4. Data expression

Results were expressed as the percentage of the surface after the contraction in the function of the initial surface (measured at $t_0$ before incubation). The more the percentage is low, the more the contraction is good.

### 2.6. Statistical Analysis

The raw data were transferred and processed using MS Excel Software. The different conditions were compared using the Student's t-test. A difference between the two groups was considered statistically significant if the p-value was less than 0.05, which was noted *p<0.05. If the p-value is lower than 0.01 and 0.001, it was noted **p<0.01 and ***p<0.001, respectively.

## 3. RESULTS AND DISCUSSION

The physicochemical and sensorial characterization parameters of the formulation are reported in **Table 2**. The density, pH, and viscosity of all formulations were found to be satisfactory. The pH of the developed cream ranged between 5.5-6.0. The density of the developed cream found 0.98 ± 0.02 g/mL. The viscosity found 14.10 ± 0.22 P. The density, pH, and viscosity of the cream was appropriate for the dermal application.

**Table 2.** *The physicochemical and sensorial characterization parameters of the eye cream formulation*

| Physicochemical Parameters | |
|---|---|
| Density (g/mL) | 0.98 ± 0.02 (25°C) |
| pH | 5.75 ± 0.10 (25°C) |
| Viscosity (P) | 14.10 ± 0.22 (25°C) |
| **Sensorial Parameters** | |
| Appearance | Cream |
| Odor | Characteristic |
| Color | White |

### 3.1. Microbial Contamination Tests

All results about microbiological contamination studies were given in **Table 3**. After the incubation period, the tests were checked for microbial growth. No microbial growth was observed. The obtained results had confirmed the microbial study of our formulation.

**Table 3.** *The microbiological study results*

| Test Microorganisms | Microbiological Parameters |
|---|---|
| Total Bacteria | < 100 CFU/mL |
| Yeast and Mould | - |
| *Escherichia coli* | - |
| *Staphylococcus aureus* | - |
| *Pseudomonas aeruginosa* | - |

### 3.2. Evaluation of The Effect of an Eye Cream on The Collagen Contraction

The treatment with eye cream (118-08) at 0.5% decrease the lattice surface compared to the control (no treatment). The cream visually decreased the lattice surface over time, which means an increase in the collagen contraction (**Figure 1-3**).

### 3.3. Discussion

This study evaluated an eye cream on the collagen contraction *in vitro* in human dermal fibroblasts cultured in a lattice of collagen that appeared to be a model close to the living dermis. Today, the use of extracts as anti-pollutant and anti-aging from various plants and marine organisms has become popular. The objective of the study was to evaluate the effect of anti-pollution & anti-aging eye cream on the collagen contraction.

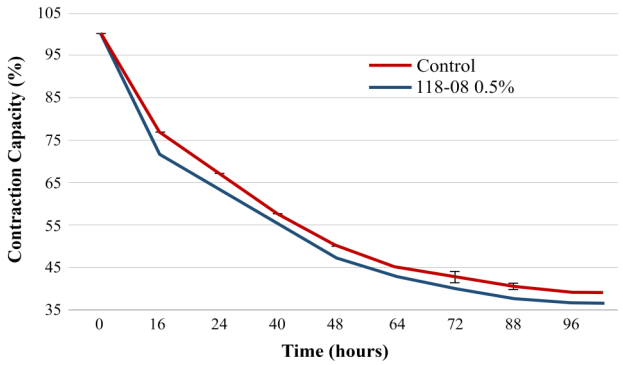


**Figure 1**. *Effect of the eye cream (118-08) at 0.5% on the lattices surface over time*

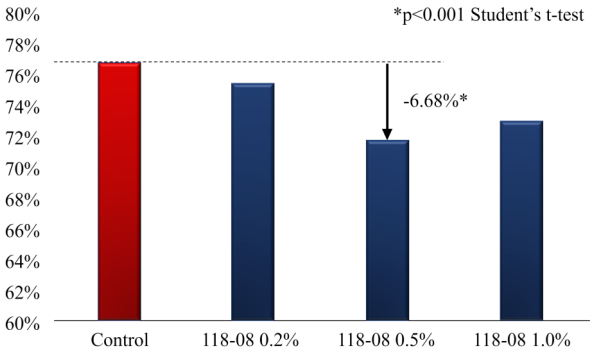


**Figure 2.** *Effect of the eye cream (118-08) at 0.5% on the lattice surface at 16 hours*

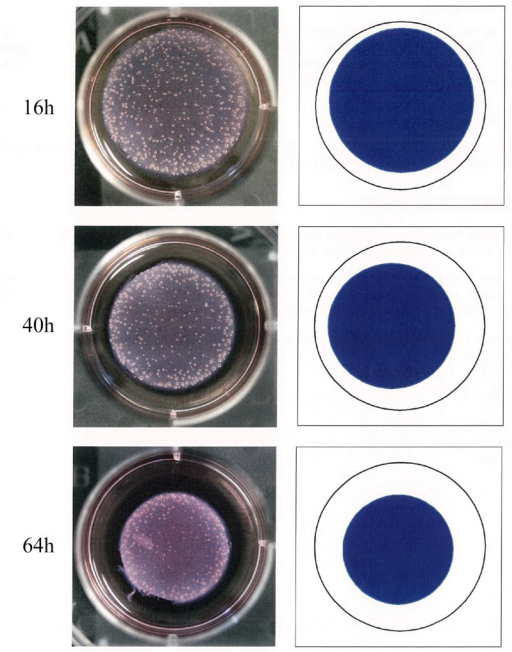


**Figure 3.** *Observation of one lattice treated with the eye cream (118-08) at 0.5% (16, 40, and 64 hours)*

Environmental pollutants have a negative effect on human health and human skin. Exposure to pollutants can cause aging, pigmentation, acne, atopic dermatitis, psoriasis, and even skin cancer, or acne [15].

Pollution has a negative impact that can be observed at the stratum corneum, which is generally colonized with residual microorganisms. In the presence of pollutants, the skin microbiome changes for the benefit of pathogenic bacteria [16]. Moreover, contamination enhances the production of reactive oxygen species and causes disturbance in the redox balance. Some pollutants also tend to permeate through the stratum corneum into deeper skin layers. There, they act as a ligand for the aryl hydrocarbon receptor (AhR), which takes part in mediating the toxic effects of pollutants. All of them cause the induction of an inflammatory cascade in the skin. The increased production of pro-inflammatory cytokines, such as interleukin1β or interleukin 8, greatly impacts the biological function of the cells, resulting in skin lesions and deterioration of skin appearance [17,18].

Vierkötter et al. showed a direct link between chronic exposure to traffic-related particulate matter and the occurrence of prominent skin aging signs, especially pigment spots, but also wrinkles in Caucasian women [19].

Li et al. then reported epidemiological evidence that indoor air pollution from cooking with solids fuels was associated with wrinkles in Chinese women [20]. A recent study has found that exposure to $NO_2$ was associated with the formation of lentigines in Caucasian and East Asians [21].

Particles can serve as carriers for organic chemicals and metals that are capable of localizing in mitochondria and generating ROS directly in mitochondria leading to collagen degradation in human skin and thereby cause wrinkle formation [22,23].

In recent years, there has been an increasing interest in products that protect us from the negative impact of pollutants, and that helps to restore the skin barrier function.

The cream was made for this purpose, and depolymerized oligoalginate of high molecular weight was used as an active agent. Applied on human skin, it protects against heavy metals, particles, air pollution, and cigarette smokes. Alginate chelate metal ions, reduce inflammatory mediators, and cytokines. Shanura et al. showed alginic acid also reduced the levels of COX-2, interleukin 6, TNF-α, and inhibited specific key molecular mediators of the NF-κB and MAPK pathways in keratinocytes. Alginic acid substantially reduced the levels of metal ions like Pb2+ and Ca2+ in keratinocytes attributable to its metal ion chelating properties. These cells presented with increased levels of NO, iNOS, COX-2, PGE2, and pro-inflammatory cytokines [24].

The cream formulation characterized based on its pH, density, and viscosity. Physicochemical characterization of the formulation is an important subject to be considered in the formulation part, especially those intended for dermal application. The cream has good appearance, color, and odor on sensorial inspection. The pH of the developed cream ranged between 5.5 and 6.0. Ideally, dermal formulations should possess pH in the range of 5.0-6.0. The basic formulation does not include the hydrolyzed algin, which served as the control for density analysis, presented a density value of 1.01 g/mL, which is similar to the values registered for the remaining formulation that 0.98 g/mL. The hydrolyzed algin incorporated into the eye cream formulation did not affect the density of the basic formulation.

Microbial contamination study is crucial to evaluate the microbial stability of formulation to ensure its safety. No microbial growth was observed for the eye cream formulation.

Cellular stiffness is significantly increased in dermal fibroblasts during aging *in vivo*. This increase in stiffness has a direct impact on cellular contraction capacity.

Human dermal fibroblasts cultured in a lattice of collagen appeared to be a model close to the living dermis. The collagen polymerizes into anchoring fibrils known to bind to cells. Then, the fibroblasts pull on these fibrils thanks to their migratory movement and thus reorganize the matrix.

The lattice decreases its diameter, which results in a phenomenon called contraction or retraction, indicative of the contractile activity of the fibroblasts [25].

The treatment with eye cream at 0.5% decreases the lattice surface compared to the control (no treatment). According to the results obtained under the conditions of the test, the eye cream used at 0.5% tends to increase the collagen contraction. The best results are observed at 0.5% with a significative decrease of the lattice surface by 6.68% compared to the control at 16 hours. The cream visually decreased the lattice surface over time, which means an increase in the collagen contraction (**Figure 1-3**). Our results are compatible with the literature. Park et al. suggested that these alginate oligosaccharides might have the potential to prevent skin aging by promoting collagen synthesis through the inhibition of collagen degrading enzyme [26].

## 4. CONCLUSION

Our study showed that the eye cream formulation obtained from hydrolyzed algin called alginic acid is a potent anti-pollution & anti-aging agent and can be used in patients with lentigines and pigment spots and wrinkles. Future controlled clinical trials are needed to evaluate the efficacy of the eye cream.

In particular, the search for anti-pollution & anti-aging agents of natural origin is progressing rapidly, which points to the need for further studies exploring the utilization of the therapeutic agents from Brown algae.

## AUTHOR CONTRIBUTIONS

Concept: GG, SA; Design: GG, SA; Supervision: GG; Materials: SA; Data Collection and/or Processing: GG, SA; Analysis and/or Interpretation: GG; Literature Search: GG; Writing: GG; Critical Reviews: GG, SA.

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## CONFLICT OF INTEREST DECLARATION

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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# An update on cyclodextrins as drug vehicles for antimicrobial applications

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ABSTRACT

Cyclodextrins belong to cyclic oligosaccharides comprised of α-(1,4) linked glucopyranose groups. Their interesting supramolecular cavity-like structure can host active molecules providing a breeding ground for drug delivery systems. Cyclodextrins, due to their unique functional structure, can produce host-guest complexes with active ingredients, such as drugs, peptides, proteins, etc.; the complexes resulted from intramolecular interactions leading to stable molecules vehicles. Moreover, cyclodextrins are already applied in pharmaceutical industry applications since they can induce the solubility of lipophilic compounds and provide bioavailability and excellent safety profile and stability. In this review, the basic background for cyclodextrins and their current applications in the antimicrobial field are discussed. Besides, the antibacterial and antifungal-applications in the pharmaceutical field attract most researchers because of microbes' resistance. Regarding this, the most recent cyclodextrin inclusion complexes with antimicrobial and antifungal drugs are summarized in this article.



## 1. INTRODUCTION

Over the last decades, solid dispersion (SD) technology has been utilized to improve oral bioavailability via the enhancement of poorly soluble drug solubility [1,2]. Besides, given that most of the active molecules are lipophilic with low aqueous solubility, their delivery in the form of amorphous SDs seems to be advantageous [3]. Except for oral bioavailability, SDs have also been applied for maximizing ocular bioavailability, as well [4]. SDs have been defined as the systems were one or more active molecules are dispersed in an inert carrier produced by various methods, melting, dissolution, or combination of them and others [5]. Solvent evaporation, electrospinning, spray drying, and hot melting, cyclodextrin complexation, as well as kneading [6-10], are commonly used techniques. Cyclodextrin (CD) complexes belong to the most handful of SDs categories [2,11].

## 2. CYCLODEXTRINS

CDs belong to the family of cyclic oligosaccharides comprised of glucopyranose units. Other names of CDs are Schardinger sugars or cycloamylose dextrins. Vielliers in 1891 was the first who discovered CDs which at that time were named as "cellulosing" [12]. Some years later, Schardinger, who has been considered as the "founding father" of CD chemistry, describes the preparation and separation methods of CDs. In contrast, recently, Loftsson and coworkers have significantly contributed to the development of the CD field [13-17].

The most common CDs are α, β, and γ, constituted by 6, 7, and 8 glucopyranose units (**Figure 1**), respectively. However, except for them, δ-, ζ-, ξ- and even η-CD (9-12 units) have been confirmed. Their structure mimics doughnut ring exists as a truncated cone (**Figure 2**); the outer part of the cone presents hydrophilic nature resulted from the hydroxyl groups of the glucopyranose units while the inner cavity is apolar. The truncated shape is the result of the rotation of the primary hydroxyl groups located at the end of the cavity reducing the size of the cavity toward the side of the secondary hydroxyls regarding the C2 and C3 carbon atoms of the glucose units located to the edges of the cavity [18]. The main property of CDs is their ability to form host-guest complexes leading to stable complexes; one or two guest molecules can be entrapped by one, two, or three CDs. The formation of the complexes can be easily detected by phase solubility studies [2,19]. It is generally accepted that

the complex formation issued by various mechanisms [20] such as;

***a.*** van der Waals interactions between the CD cavity and the hydrophobic unit of the guest molecules [21],
***b.*** hydrogen bonding between the polar functional groups of the guest molecules and the hydroxyl groups of the CD [21],
***c.*** release of high energy water molecules from the cavity during the complexation
***d.*** release of strain energy into the ring structure system of the CD [22].

Also, CDs due to the presence of the hydroxyl groups can be easily functionalized, providing derivatives such as hydroxypropyl-β-CD, sulfobutylether-β-CD [23], and others. These derivatives present more excellent solubility in water or improved properties. Asides from these, CDs can be used to graft polymers resulting in multifunctional drug carriers [24].

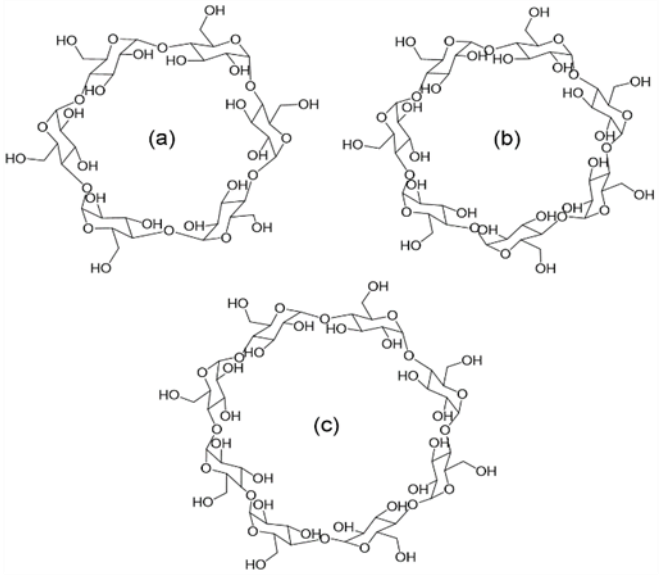


**Figure 1.** *The most common cyclodextrins structures (a) α-CD, (b) β-CD and (c) γ-CD*

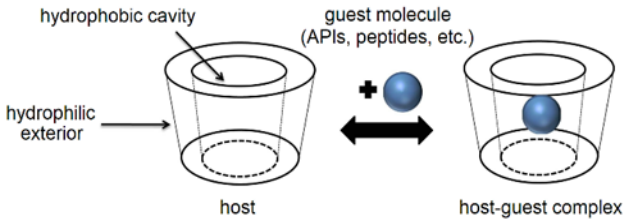


**Figure 2.** *Cyclodextrin structure and complexation mechanism (host-guest complex) APIs= Active pharmaceutical ingredients*

The natural CDs (α-, β-, γ-) are not toxic but present limited water solubility [25]. **Table 1** summarizes basic properties of natural CDs. This limitation can be overcome via their functionalization with;

***a.*** propylene oxide leading to hydroxypropylated CDs,
***b.*** monochloroacetic acid resulting in carboxymethylated CDs
***c.*** methyl iodide methylated CDs and
***d.*** 4-butane sultone resulting in sulfobutylether CDs [26].

**Table 1.** *Physicochemical properties of α-, β-, γ- CDs*

| Properties | α-CD | β-CD | γ- CD |
|---|---|---|---|
| Glucose units | 6 | 7 | 8 |
| Cavity diameter (Å) | 4.7-5.3 | 6.0-6.5 | 7.0-8.3 |
| Cavity height (Å) | 7.9 | 7.9 | 7.9 |
| Cavity volume (Å) | 174 | 262 | 427 |
| Crystal shape | hexagonal lattice | monocyclic par-allelograms | quadratic prism |
| $\log P_{oct/water}$ [1] | -13 | -14 | -17 |
| MW [2] (g/mol) | 973 | 1135 | 1297 |
| $F_{oral}$ [3] | 0.02 | 0.006 | <0.01 |

[1] *logarithm of the calculated octanol/water partition coefficient*
[2] *molecular weight*
[3] *fraction of the absorbed CD amount when orally administered to rats*

## 2.1. Preparation and Characterization Methods

For the preparation of CD complexes with various active ingredients, several preparation methods have been proposed through literature. Commonly used methods are freeze-drying, physical mixing, kneading, co-precipitation, solvent evaporation, and supercritical process [27-31]. The developed complexes to be characterized, are analyzed using Fourier-Transformed Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), scanning electron microscopy (SEM), and x-ray diffractometry (XRD) [2]. In further, the successful complexation is examined via various physicochemical methods. In detail, numerous spectroscopic methods as fluorescence spectroscopy, Ultra-violet/visible spectroscopy aqueous phase solubility studies, and nuclear magnetic resonance (NMR) spectroscopy are reported to detect the complexation between drug and CDs [32-34].

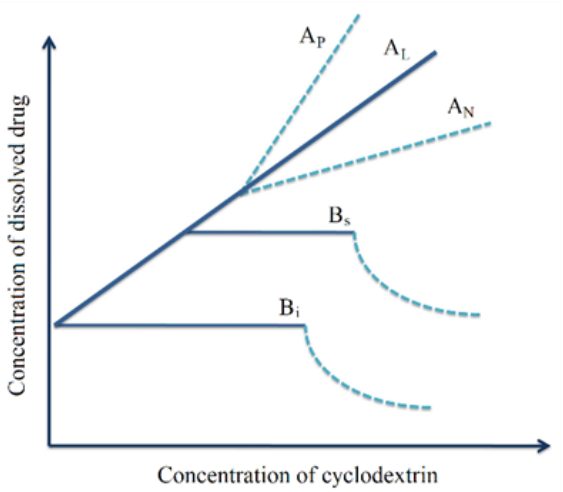


**Figure 3.** *Phase-solubility profiles acquired through Higuchi and Connors methods [33].*

The phase solubility studies, according to the molecule solubility in moles/L plotted against the molar CD concentration, is one of the most common and easy methods utilized. Although these studies do not prove the formation of the inclusion complexes, describe how the increment of CD concentration affects drug solubility. Five main profiles have been identified (**Figure 3**). Generally, A-type profiles are acquired from water-soluble CD derivatives (i.e., HP-β CD) while B-type from natural CDs. First, A-type phase (AL, AP, AN) solubility profiles are obtained when drug solubility of the drug-enhanced by enhancing CD

concentration through the complexation between the hydrophilic drug and CD. AL profile is acquired when the complex is first-order regarding CD and first or higher-order in respect of the drug resulting in 1:1, 2:1, or 3:1 drug/CD complexes. AP profile identifies the complexes with the first order of drug, but a second or higher order of CD. AN profile is difficult to be examined, but the variance from linearity could be associated with various changes, according to Jansook et al. [26]. The B-type profiles specify the formation of complexes with low solubility in the water media [17].

## 3. CYCLODEXTRIN BASED ANTIMICROBIAL SYSTEMS

Currently, physicians and researchers from the health sciences field are quite aware of the antimicrobial resistance; the ability of microbes to resist the effects of medications [35]. Both microbe and fungi resistance represents a major clinical challenge to clinicians since they can resist certain medications leading to severe complications of the patients' life [35-37]. Thus, this research field based on antimicrobial systems is a developing one. The researchers either focus on new active ingredients or other administration routes. Besides, most of the antimicrobial ingredients are lipophilic, limiting their oral use. Except, oral administration, the ocular and parenteral routes are widely utilized for the delivery of antimicrobial drugs. Consequently, the use of CDs as a matrix for the delivery of such drugs could be very promising. There are several marketed products based on CD complexes and various active ingredients. In 1976, the first marketed product was developed via prostaglandin E2 and β-CD in the form of sublingual tablets [19]. More specifically, in the case of antimicrobial pharmaceutical field, a known marketed product is the intravenous solution of Voriconazole (VRC) and Sulfobutylether-β-CD complex, supplied by Pfizer under the brand name Vfend®.

### 3.1. Cyclodextrins-Antibacterial Drug Complexes

An oral drug formulation based on tebipenem pivoxyl (TP) has been proposed. Tebipenem pivoxil-β-cyclodextrin (TP-β-CD) complex was prepared, and the physicochemical properties were changed. The inclusion of TP-β-CD was confirmed using DSC (thermal method) as well as infrared and Raman spectroscopy (spectral method). Due to the inclusion complexation, there has been an increase in solubility and chemical solid-stability. Biologically primary effects of TP and β-CD interactions decreased TP permeability through Caco-2 cell using efflux effect inhibition and enhanced antibacterial activity. The pharmaceutical formulation showed a great opportunity for the treatment of resistant bacterial infections [38].

The formation of the β-CD inclusion complex with levofloxacin was studied using fluorescence spectroscopy in pH 7.4 buffer solution. It was revealed that a 1:1 inclusion complex of β-CD with levofloxacin was developed, as the $^{1}$H NMR and IR methods depicted. The complex formation between levofloxacin and gadolinium (III) ion was examined in aqueous solutions with and without β-CD. The stability and stoichiometry constants of the complexes were reported, and the concentration distribution of some complexes has been measured as a function of pH. The effect of β-CD on dissociation constants, Ka of levofloxacin, and stability constants of levofloxacin-gadolinium (III) complexes were checked. Also, gadolinium (III) distribution in human blood was showed with computer simulation [39].

The main complication of hernia repair is mesh-infection. Bacterial infections can develop in textile structures, after knitted mesh implantation. Sanbhal et al. prepared polypropylene (PP) mesh materials, which were modified with β-CD and hexamethylene diisocyanate and then loaded with levofloxacin for the treatment of hernia mesh-infection. First, oxygen plasma was able to provide surface roughness, and then hexamethylene diisocyanate was suitably grafted onto surfaces of PP fibers. Afterward, the CD was grafted onto the hexamethylene diisocyanate modified polypropylene meshes, and levofloxacin HCL loaded into the CD. A sustained drug release was obtained between surfaces of aqueous environment and meshes. In further, samples showed sustained antibacterial activities against both Gram-positive and negative bacteria for 10 and 7 days, respectively. The complexes demonstrated a burst release after 6 hours, followed by a sustained release for 48 hours. The modified mesh was the most stable between all specimens and provided more sustained drug release, which is essential for future clinical treatments [40].

Aytaç et al. modified pharmaceutical-grade CDs (HP-β-CD, M-β-CD, and HP-γ-CD) were used for complexation with linalool. These CDs were acquired due to their higher solubility in comparison with natural CDs. Their higher water solubility could induce their successful electrospinning to nanofibrous structures. They prepared nanofibers based on the CD/linalool-inclusion complex using an electrospinning apparatus. The high mass of linalool (45-89%) was loaded into the nanofibers because of CD complexation. Besides, the thermal stability of linalool increased due to the CD inclusion complexation. The complexes demonstrated very high antibacterial activity against Gram-positive (*S. aureus*) and Gram -negative (*E. coli*) bacteria. CD complexes were dissolved entirely in water in two seconds. The produced CD based nanofibers confer high linalool loading capacity, the effective antibacterial activity of linalool, and enhanced shelf life [41].

Szabó et al. studied moxifloxacin (MOX) and β-CD complexation to enhance its antibacterial property. This inclusion complexation was examined with NMR, mass spectrometry, affinity capillary electrophoresis, DSC, and FTIR techniques. The antimicrobial test showed that the MOX inclusion complex offers slightly improved activity against *Enterococcus faecalis* and Methicillin-resistant *Staphylococcus aureus* (MRSA) [42].

Masood et al. prepared roxithromycin (ROX) encapsulated in the cavity of β-CD and HP-β-CD formulations, to enhance ROX poor solubility. In further, blank and ROX loaded poly (lactic-co-glycolic acid) nanoparticles were prepared. The nanoparticles were capable of inhibiting the growth of multidrug-resistant Gram-negative and Gram-positive bacteria compared to the HP-β-CD-ROX/PLGA NPs and β-CD-ROX/PLGA NPs [43].

He et al. produced rifampicin (RFP) HP-β-CD complexes to form a molecular inclusion complex (MRICD) with excellent stability and solubility. The complex was prepared using a solid-state grinding technique without any water revealing a greater dissolution rate of than free RFP.

Moreover, the complex showed enhanced antibacterial activity and improved the physical properties of RFP [44].

Choi et al. investigated the complexation effect of mono-6-deoxy-6-aminoethylamino-β-cyclodextrin (Et-β-CD) on the bioavailability and solubility of ciprofloxacin which is used to treat bacterial infections. The complexes were characterized using DSC, FE-SEM, FT-IR, T1 relaxation, DOSY NMR spectroscopy, 2D NOESY, and molecular modeling tests. The solubility of the ciprofloxacin complex was improved by seven-time when compared to pure ciprofloxacin. The antibacterial activity of the ciprofloxacin complex against *Staphylococcus aureus* was increased, demonstrating growth inhibition. The results of this study suggested that the induced oval-shaped cavity of Et-β-CD may be used for other guest molecules besides ciprofloxacin [45].

Taha et al. aimed to load antibiotics onto hip implants for preventing infection risk after a total hip replacement. They modified the surface of hydroxyapatite (HA) coated titanium implant material (Ti-HA) with poly CD for loading tobramycin and rifampicin. They achieved a sustained drug delivery. A strong efficacy against both Enterobacter cloacae and *Staphylococcus aureus* was achieved because of dual-antibiotic loading. The antibacterial coating (polyBTCA/ Me-β-CD) for an HA-coated titanium prosthesis provided an enlarged therapeutic spectrum [46].

### 3.2. Cyclodextrins-Antifungal Drug Complexes

Li et al. prepared fluconazole (HFlu) loaded inclusion complexes with β-CD and HP-β-CD via the co-precipitation technique. The 1:1 stoichiometry for both CD complexes was achieved according to phase solubility and fluorometric studies. These preparations were characterized by DSC-TGA and ESI-MS spectra analyses. Finally, Flu-HP-β-CD showed higher stability than for HFlu-β-CD [47].

Orgován et al. aimed to quantify acid-base and CD-complex formation equilibria of fluconazole. $^{1}$H-NMR pH titrations exhibited protonation levels in the acidic and the highly basic region (log K1= 11.96). The structure and stability of its complexes with β-CD (2-hydroxy) propyl-β-CD and sulfobutyl ether-β-CD were studied by NMR methodologies. The CD complexes of fluconazole are of average stability. Two isomeric complexes of comparable stability were formed between the β-CD and fluconazole [48].

Oral VRC is used for patients with kidney failure due to concerns about CD accumulation. Siafaka et al. compared two different preparation methods for the improvement of the dissolution rate of VRC. Poly(ε-caprolactone) (PCL) electrospun fibers were developed with an electrospinning process, and β-CD complexes were prepared with an inclusion complexation method. The formulations were loaded with various concentrations of VRC. PCL nanofibers were characterized based on morphology. β-CD complexes were evaluated for phase solubility. An improved VRC solubility was found for all formulations, whereas inhibition of fungi proliferation was also revealed [2]. Kim et al. studied the effects of IV VRC formulated using sulfobutylether β-cyclodextrin (SBECD) in patients with kidney failure. An observational study was conducted on 25 adult invasive aspergillosis patients treated with IV VRC. Even in patients with renal insufficiency after VRC treatment, no significant impairment of kidney function was observed in any patient. IV VRC formulated with SBECD did not cause an increase in the incidence of serious adverse events, including nephrotoxicity in hematological patients with CrCl<50 mL/min [49]. According to another study, VRC was incorporated in SBECD for renal function. The impact of long-term use of intravenous VRC on renal function is indefinite. Their retrospective study of data proved that the worsening of renal function was notably connected with a total dose of IV VRC (≥400 mg/kg), recommending that a higher cumulative dosage of IV VRC is a risk factor for renal dysfunction [50].

Sun et al. used the electrospinning technique to prepare VRC incorporated polyvinyl alcohol (PVA)/HP-β-CD blended nanofibers for ocular application. HP-β-CD content increased drug solubility. The nanofibers presented bead-free mean fiber diameters of 307±31 nm, and VRC was released in a sustained profile. The proton nuclear magnetic resonance was practiced to analyze the molar proportion of HP-β-CD/VRC in the nanofibers. The nanofibers remarkably improved the bioavailability and increased the half-life of VRC in rabbit tears when compared with a VRC solution. VRC nanofibers were found promising for ophthalmic drug delivery [51]. Vass et al. conducted a study to evaluate electrospinning as an alternative process of the dilution injection dosage form. High-speed electrospinning with a new continuous cyclone collection was applied to produce a formulation of VRC using sulfobutylether-β-cyclodextrin (SBE-β-CD). SBE-β-CD worked as a 'quasi-polymer,' and it could be electrospun despite its low molecular weight. The crystalline form of VRC in fibers was not detected according to DSC and XRD methods. Also, it was determined by energy dispersive spectroscopy and Raman mapping measurements that the VRC in the amorphous form in the fibers showed a proper distribution. According to Reconstitution tests with ground fiber powder, a clear solution formed later 30 seconds (similar to Vfend®). With this study, it has been proved that aqueous high-speed electrospinning, is an economically viable manufacture alternative compared to freeze-drying [52].

Herrera et al. used a co-precipitation method for preparation inclusion complexes based on β-CD and antimicrobial drugs. These preparations were characterized by entrapment efficiency (EE), thermal analysis, X-ray diffraction, 1H NMR spectroscopy, and water sorption. They also evaluated for drug release and antifungal activity. EE% was found between 66-91%. High relative humidity was affected by drug release. These complexes also showed antifungal activity on *B. cinereal*. For this reason, these preparations could be used in antifungal packaging [53].

Econazole nitrate (ECN) is a weakly basic drug with low water solubility resulting in low bioavailability. The ECN/ CD complex was used to increase the solubility of the drug in the aqueous medium. Jansook et al. conducted a study to determine the effect of CD inclusion complex and pH adjustment on ECN solubility. The solubility of this drug in acidic solutions containing α-CD was higher under the same conditions than aqueous γ-CD solutions. The presence of the ECN/CD complex was confirmed using proton nuclear magnetic resonance spectroscopy. Autoclaving increased the

drug stability of ECN/CD complexes. To create nanoparticles and microparticles, γ-CD complexes can self-assembled, while α-CD complexes are at a negligible level of self-assembly. ECN/α-CD has been shown to increase antifungal activity against filamentous fungi [54].

Eleamen et al. used a freeze-drying method for preparing a complex of HP-β-CD and 6CN10 (a poorly water-soluble 2-aminothiophene derivative). The complexes were characterized by infrared/Raman spectroscopy, thermal analysis, scanning electron microscopy, and X-ray diffraction. The water solubility of 6CN10 with HP-β-CD improved more than 29 times. The antifungal activity against *Cryptococcus neoformans* presented the better performance of the complex (46.66 µg/mL) compared to the free drug (166.66-333.33 µg/mL). This study provided useful complexation with low soluble compounds and HP-β-CD [55].

Gontijo et al. produced CDs with ellagic acid. Caco-2 cell lines cultivated in a Transwell® insert were contaminated with *Candida albicans* to promote an *in vitro* model. Characterization studies of complexes and microbial effects were evaluated. Ellagic acid exhibited the ability to defeat the *Candida albicans* invasion. Poor absorption and poor water solubility of ellagic acid probably limited this ability. Ellagic acid/hydroxypropyl-β-CD did not improve the antifungal activity. Poor water solubility was improved with HP-β-CD complexation. This formulation presented a promising antifungal activity [56].

Propiconazole nitrate incorporated inclusion complexes prepared by the freeze-drying method. The preparations were characterized by 1H-NMR, 2D Roesy NMR, and DSC. The complexes with sulfobutylether-β-CD had the highest association constant values, and the inclusion efficiency was close to 100%. Antifungal activity, in silico docking and molecular dynamics simulations, were evaluated. For all complexes showed similar results on *Candida spp*. The complexes were also evaluated for cytotoxicity, and the β-CD complex was showed higher cytotoxicity than other complexes [57].

Teodoro et al. were prepared gallic acid CDs (GA/HP-β-CD) by spray drying to enhance gallic acid (GA) solubility for the management of *Candida albicans* biofilm. Complexes were characterized by drug loading, SEM, and DSC and were tested on *Candida albicans* biofilm. The drug loading % was found approximately 10%. Inclusion complexes were confirmed with SEM and DSC tests. The developed complex kept the antimicrobial activity of the pure GA while it was found effective on *Candida albicans* biofilms of 24 and 48h. Besides, the *in vivo* results showed an anti-inflammatory activity of GA/HP-β-CD [58].

## 4. CURRENT STATUS OF CYCLODEXTRIN COMPLEXES IN PHARMACEUTICAL APPLICATIONS

CD complexes included in about 40 marketed pharmaceutical applications worldwide, in addition to many foods, toiletry, and cosmetic products [59]. CD complexes are all found in one or more pharmaceutical products in Japan, the USA, or Europe: Cefotiam hexetil hydrochloride (Pansporin T, Japan), Benexate hydrochloride (Ulgut, Lonmiel, Japan), Omeprazole (Omebeta, Europe), Piroxicam (Brexin, Europe), Cisapride (Propulsid, Europe), Itraconazole (Sporanox, Europe, USA), Mitomycin (Mitozytres, USA), 17b -Estradiol (Aerodiol, Europe), Voriconazole (Vfend, Europe, USA), Ziprasidone maleate (Geodon, Zeldox, Europe, USA), Diclofenac sodium (Voltaren, Europe) [60].

The biopharmaceutical classification system (BCS) divides oral drugs into 4 cases based on their solubility and gastrointestinal permeability. CD complexes mostly studied to improve oral bioavailability of Class II drugs (poor aqueous solubility - good permeability) and Class IV drugs (poor aqueous solubility - poor permeability) [59]. The complexes of these drugs with CDs could mask undesirable properties. CD carriers such as nanosystems (nanosponges, nanofibers, dendrimers, metallic nanoparticles, quantum dots, nanoemulsions), liposomes, micelles, micro rods, niosomes, and siRNA may hide the undesired characteristics of drugs, thus improving their bioavailability [61,62].

CD has been extensively applied in gene therapy, nanomedicine therapy, cell therapy, chemotherapy, and immunotherapy. Studies have shown that numerous anti-cancer drugs, with properties such as instability, lack of physicochemical properties, or poor water solubility, have limited application in pharmaceutical applications [62]. Also, α-CD and β-CD are not degraded by pancreatic amylases enzymes and human salivary. Thus, CD-drug complexes remain unspoiled in the upper GI tract until they reach the colon. In this state, CD complexes of anti-cancer drugs serve as a hopeful system by improving both solubility and the availability of anti-cancer drugs at the colon site [63]. Also, the versatile nature of CDs may be used to defeat the limitations of ophthalmic topical delivery systems. CDs provide an attractive way to increase the solubility of wettable and poorly soluble drugs, to enhance their permeability and retention on ocular surfaces. CD has the potential to develop conventional eye drops to offer more excellent permeability, safety, effectiveness, and stability in the topical ocular delivery of the posterior and anterior segments [64]. A review has presented regarding applications of CDs in medical textiles. It was about the release/deposition of the drug onto/from a textile underlayer to the dermis, with CD complexes. Therapeutic textile fabrics with controlled drug release give an alternative with great medical potential, given both their numerous biopharmacological advantages. The use of CD-based medical textile fabrics applies to antimicrobial, anti-psoriasis, antiallergic (atopic and contact dermatitis), or venous insufficiency at the level of the dermis [65].

## 5. CONCLUSION

In this review, an update on CDs as drug vehicles for antimicrobial applications was presented. Overall, CDs as drug vehicles promote a promising approach for antimicrobial applications due to its ability to increase stability and the water solubility of antimicrobial drugs by inclusion complexation. Antimicrobial tests in some studies have shown that the inclusion complex offers more improved activity than pure drugs. In conclusion, research over the past few years has led to the development of CD as an antimicrobial drug carrier material. The antimicrobial drug/CD complexes may have many advantages over the conventional antimicrobial drug delivery systems. Further

technological and research advancements are expected to widen the importance of CDs in antimicrobial applications.

## AUTHOR CONTRIBUTIONS

Concept: EÖB, KE, NÜO, PIS; Design: EÖB, KE, NÜO, PIS; Supervision: NÜO, PIS; Materials: EÖB, KE, NÜO, PIS; Data Collection and/or Processing: EÖB, KE, NÜO, PIS; Analysis and/or Interpretation: EÖB, KE, NÜO, PIS; Literature Search: EÖB, KE, NÜO, PIS; Writing: EÖB, KE, NÜO, PIS; Critical Reviews: NÜO, PIS.

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## CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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# Brain-targeted nanoparticles to overcome the blood-brain barrier

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ABSTRACT

The blood-brain barrier is one of the most complicated barrier to pass for therapeutic drugs. Because of the structure of the blood-brain barrier, only a few small molecules with appropriate lipophilicity, molecular weight, and charge can penetrate through the blood-brain barrier and pass in the central nervous system. Because of this unique property, blood-brain barrier is still a major problem for the treatment of central nervous system diseases. In the last decades, many strategies to overcome this barrier have been investigated. Compared to other drug delivery strategies, due to the reduced side effects and no requirement for surgical operations, brain targeted nanoparticle is one the most promising and popular strategy used do deliver drugs to the brain. Many *in vitro* and *in vivo* preclinical studies have been conducted to determine optimum brain targeted nanoparticles. These studies were reported that characteristics of nanoparticles such as particle size, zeta potential, and targeting ligand are critical to achieving the goals. In this review, first of all, the structure of the blood-brain barrier and possible causes of blood-brain barrier disruption were summarized. Later, previous strategies of brain targeted drug delivery and characteristic prosperities for optimized brain-targeted nanoparticles were evaluated. Moreover, different strategies, such as focus ultrasound, which can increase the effectiveness of nanoparticular system applications, are mentioned.



## 1. INTRODUCTION

About 100 years ago, after the intravenous injection of a proper dye, it was observed that most of the organs other than the brain were dyed. It was understood that this situation is due to a specific structure of vessels between the brain and blood, which is called the blood-brain barrier (BBB) [1]. The BBB is also one of the most complicated barrier to pass for the therapeutic drugs, and because of the structure of the BBB, only a few small molecules with appropriate lipophilicity, molecular weight, and charge can penetrate through the BBB and pass in the central nervous system (CNS). Reports have shown that as much as 98% of small molecules and nearly all large molecules (molecular weight >1kD, i.e., recombinant DNA or gene-based medicines) cannot penetrate through the BBB [2]. On the other hand, the prevalence of CNS's diseases increases with the aging population [3-5]. For the treatment of these diseases, many new compounds were developed, but most of them did not reach the market. After the evaluation to find the causes of these failures, the poor brain penetration of drugs was identified as one of the critical factors [6]. In the last decades, many strategies have been investigated to overcome this barrier [7]. When compared to other drug delivery methods, brain targeted nanoparticle is one of the most popular and critical drug delivery strategies thanks to reduced systemic side effects and no requirement of surgical operation [8]. The physicochemical, pharmacodynamics, and pharmacokinetic properties of the therapeutics could be improved by developing nanoparticular delivery systems [9].

Additionally, passing the biological barriers such as the BBB could be achieved by targeted nanoparticles [10]. These nanoparticles could be prepared with different natural or synthetic materials, and also characteristics of these nanoparticles are critical to achieving the goals. In this review, previous strategies of brain-targeted drug delivery and characteristic prosperities for optimized brain-targeted nanoparticles were evaluated.

## 2. STRUCTURE OF THE BLOOD-BRAIN BARRIER

The BBB is a selective barrier consisting of endothelial cells from cerebral capillaries, astrocytes, and pericytes (**Figure 1**) [11]. The BBB main function is to separate the brain neural environment from the blood circulation in the brain unlike the peripheral capillaries that allow the relatively free exchange of substances between blood and tissues; the BBB has the least permeable capillaries in the entire body

due to physical barriers (tight junctions) [12]. A single layer of endothelial cells forming the brain capillaries makes up the BBB, which functions as a barrier to create the proper environment for synapsis and neural function [13]. Damaging of BBB's proper function is related to Alzheimer's disease, multiple sclerosis, and Parkinson's disease onset and progression [14,15]. Due to the complexity of the BBB, our knowledge on the issue is limited. The endothelial cells making up the vessel wall form the BBB, which displays biological properties different from other cells. These unique biological properties separated them from peripheral endothelial cells. These properties include;

- A physical barrier created by tight junctions (TJs) between adjacent cells forming the BBB preventing the free transport of molecules to the brain
- Specific transporters are expressed to regulate the influx and efflux of substrates
- Transcellular transport through the cell wall is limited by low transcytosis rate
- The entry of the immune system cells is limited by low expression of leukocyte adhesion molecules in CNS endothelial cells (Glycocalyx is responsible for preventing the immunity system cell penetration into CNS) [16,17].

However, barrier features are not attributed only to endothelial cells. CNS blood vessels are neurons that are separated by pericytes and astrocytes, which serve as an interface. These whole structure formed is called neurovascular unit [18,19].

The cells comprising of neurovascular units have different functions related to BBB.

- Astrocytes
  - BBB integration
  - TJs expression, brain transporters and enzymatic systems associated with BBB regulation [20]
  - Tissue plasminogen activator (TPA) and the anticoagulant thrombomodulin regulation [21]
- Pericytes
  - Regulate by releasing growth factors for vessel formation (angiogenesis) and vessel maturation [22]
  - Crucial for barrier formation, however during adulthood barrier maintenance they can be dispensable [23]
  - Active in the clearance of amyloid aggregates which play an active role in Alzheimer's disease [24]

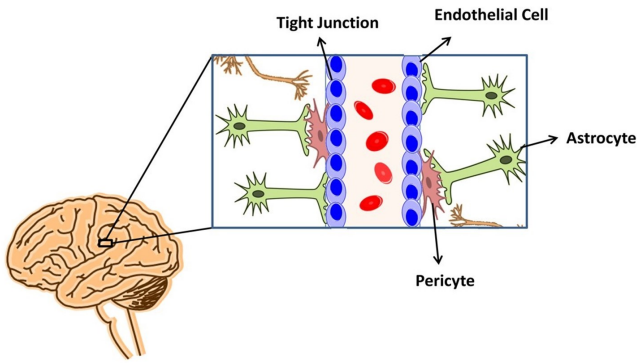


**Figure 1.** *Schematic representation the blood-brain barrier*

Mutations affecting cells which have different functions in BBB formation and maintenance lead to BBB disruption, which is the cause of many neurological diseases [14]. These mutations provide proof that BBB disruption and other vascular defects in humans contribute to the start and progression of neurological deficits.

This physiological barrier of BBB is coordinated by a series of physical, transport, and metabolic properties possessed by the endothelial cells that form the walls of the blood vessels. In peripheral vessels, molecules can pass through endothelial cells by the transcellular route, but in the BBB endothelial cells, paracellular transport is more common [11]. Specific transporters to carry specific compounds are located in luminal and abluminal sides of the endothelial cells forming a no transport barrier, facilitating or permitting the entry of necessary nutritious compounds and effluxing of harmful compounds generally large hydrophilic peptides and proteins cannot pass through the BBB, the only way to reach the CNS is using specific transportation receptor-mediated transcytosis or less specific way adsorptive mediated transcytosis (**Figure 2**) [25].

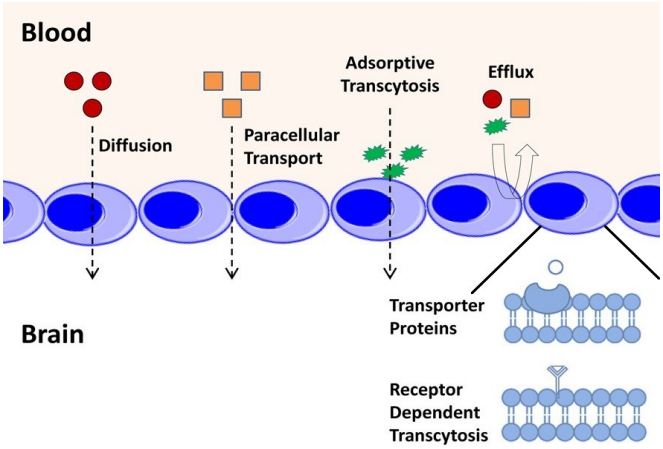


**Figure 2.** *Schematic illustration of transport mechanisms across the blood-brain barrier*

### 2.1. Diseases and Mutations Leading to BBB Disruption

#### 2.1.1. Mutations

*SLC2A1*

Mutations in endothelial cell glucose transporter GLUT1 encoding SLC2A1 genes may lead to microcephaly, seizures, and development delay [26]. In a study conducted in GLUT1 mutated mice, it was reported that BBB disruption occurred within three weeks due to glucose uptake reduction and TJs loss, which led to impaired brain perfusion, vascular regression, the onset of neurodegenerative changes and microcephaly [27].

*MFSD2A*

Mutations in encoding MFSD2A genes which is responsible for endothelial cell omega-3 transporter (Docosahexaenoic acid transporter) and caveolae-mediated transcytosis regulator across BBB, may lead to microcephaly, neuron loss and mental disability [26,28-30]. MFSD2A gene also suppresses the caveolae-mediated transcytosis; hence mutation of the gene increases the transport through BBB, promoting disruption of endothelial

barrier [31]. Decreased or diminished MFSD2A gene expression in endothelial cells in tumors leads to BBB disruption and reduced omega-3 (DHA) transport. By changing of DHA transport, this specific gene suppression promotes cancer metastasis and creates a suitable environment for cancer development. Therefore, restoration of DHA transport and metabolism functions to normal may be suggested as a method to reduce metastasis and cell growth in brain cancers.

*OCLN*

OCLN gene is responsible for encoding occludin protein, which is essential for the right function of endothelial TJs. OCLN mutations promote uncontrolled passage of blood elements to the brain leading to severe microcephaly, seizure onset, and development delay. Additionally, occludin gene silencing promoted cancer and metastasis [27].

Various gene mutations related to BBB development and maintenance may promote higher risk of Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington Disease (HD), and Amyotrophic Lateral Sclerosis (ALS).

### 2.1.2. Neurodegenerative diseases

*Alzheimer's Disease*

One of the major risk factors for AD is apolipoprotein E4 (APOE4) [32-34]. APOE4 carriers possess a high risk of BBB disruption and vascular pathology [35]. When compared to APOE4 non-carriers, APOE4 carriers can be exposed to BBB disruption, neurovascular unit dysfunction including pericyte degeneration, decreased glucose uptake and damaged cerebrovascular activity [36-41].

BBB disruption may also appear as the cause of amyloid precursor protein (APP) mutations. All these results are supported by human and transgenic animal model studies [42-45].

Some study results reported that AD pathologies appear after the BBB disruption occurs. Besides, tau transgenic animal models show that BBB disruption, leukocyte, red blood cell, and IgG infiltration before any sign of tau pathology verifying the upper statement [46].

*Parkinson's Disease*

After AD, PD is the second most common neurodegenerative disease. Dopaminergic neuron degradation in substantia nigra and filamentous and oligomeric accumulation leads to motor impairment [47].

MDR1 genes encoding ABCB1 (P-Glycoprotein) are believed to be closely related to PD. Reduced expression of MDR1 in BBB endothelial cells, is associated with the progression of PD [48].

*Huntington's Disease*

BBB disruption is present in and associated with Huntington Disease (HD). In a study performed in post-mortem HD bearing human brain and R6/2 mice, it was reported that there is a reduction of TJs protein expression (occludin and claudin-5) and increased transcytosis which leads to BBB disruption. These results confirm that vascular pathology and BBB dysfunction plays a role in HD onset and progression [49].

*Amyotrophic lateral sclerosis*

ALS is a fatal neurodegenerative disease affecting human motor systems. The main cause of the disease is not fully understood yet, and the progress in treatment has been very slow [50]. According to a study performed in ALS transgenic mice, BBB disruption, and endothelial cell damage before any symptoms of weakness and motor injury, indicating that BBB dysfunctions affect the ALS progression. Decreased expression of TJs proteins occurred after the onset of ALS symptoms [51].

*Stroke*

Ischemic and hemorrhagic stroke are closely related to BBB disruption and have a worse prognosis [52]. As a result of increased paracellular and transcellular permeability and BBB endothelial cell disruption, the blood components cross into the brain. Water and ion balance affect the brain's neural environment, and leukocyte infiltration leads to inflammation, which increases the damages to the brain tissue [53,54]. Overall, BBB disruption is one of the main causes of ischemic strokes and drastically increases the risk of a brain hemorrhage, which is a deadly condition.

*Epilepsy*

IgG leakage and TJs loss characterized BBB disruption was reported in temporal lobe epilepsy humans and transgenic rodents. The BBB disruption was located in the affected area of the seizures indicating BBB plays an important role in epilepsy. The increased permeability of the BBB was associated with the frequency of epileptic seizures, which means in chronic periods, BBB impairment is also chronic, leading to other complications as well [55].

### 2.1.3. Brain tumors

Although the neurovascular unit regulates the environment for the optimal neuronal activity, it also inhibits the delivery of the therapeutic agents through BBB into CNS for the effective treatment of brain tumors. As tumor progresses, the BBB is disrupted and named the blood-tumor barrier (BTB). When compared to BBB, BTB is more heterogeneous, has increased permeability to small and large therapeutic agents, and allows accumulation of agents in tumor regions [56-58]. Due to the condensed space inside the brain, the tumor mass can also disrupt the normal blood flow by compression of vessels in areas nearby [59]. The blood vessels in the tumor core are more permeable (leakier) compared to vessels in the periphery of the tumor, which has an intact BBB [60], which leads to a heterogeneous vasculature. The leakiness of BTB is detected by the therapeutics in the tumor area and circulating tumor cells and DNA of glioma cells in the blood. T-cells and monocytes immune cells can have been located in brain tumor areas, and the TJ protein decrease in endothelial cells indicates the leakiness of the BTB.

Together with tumor expansion, increased angiogenesis induces the formation of new vessels with the increased need for tumor nutrition. The vascular endothelial growth factor (VEGF) is deregulated during tumor expansion to create leaky and immature vasculature and a hypoxic and acidic environment that promotes tumor progression [61-63]. Anti VEGF therapies decrease the permeability and formation of

new vessels; however, it also decreases the permeability of therapeutic agents by restoring the normal function of BBB [64,65]. The anti-VEGF therapies must maintain a balance between the BBB restoration, cancer progression through hypoxia, and decreased agent delivery through BBB [66,67].

Different types of brain cancer display different permeability and BBB properties. For example, there are four subtypes of medulloblastoma displaying different permeability properties. The best treatment prognosis with antineoplastic drugs is received in the WNT medulloblastoma subtype, showing higher fenestration of vasculature, indicating more drug accumulation in the tumor area [68]. In glioma model animal studies, the tumor permeability of drug-loaded liposomes and targeted therapies is higher in BTB regardless compared to BBB as expected [69,70]. The BTB in Glioblastoma features disruptive properties by TJs reduction and glioma stem cell derived pericyte cells, which decrease the integrity of the vasculature [71,72]. Therefore, targeting stem cell pericyte cells can increase the therapeutic agent delivery to glioma improving prognosis [73,74]. In order for cancer cells to enter the brain and cause secondary brain metastasis, they must first cross through BBB. Studies show that metastatic cells can cross BBB by disrupt claudin TJ [75]. Once the metastatic cell is the brain capillaries, the cell expresses proteases and ligand to facilitate the infiltration of other metastatic cells across BBB and create an appropriate microenvironment for cell growth. The BBB properties and functionality varies for different types of breast cancer brain metastasis. In HER-2 positive breast cancer brain metastasis, and increased expression of GLUT-1 and BCRP efflux pumps can be seen. When considering the treatment of brain cancer with therapeutic agents, all these properties should be considered for an effective treatment.

## 3. GENERAL STRATEGIES TO OVERCOME THE BLOOD-BRAIN BARRIER

There are three different approaches to deliver the therapeutic agents to the brain by penetrating through the BBB; invasive, pharmacological, and physiological [76]. Firstly, in invasive approach, all the technics used to deliver therapeutics to the brain are physically based. Invasive approach by mechanically penetrating the BBB delivers the drug by intra-cerebroventricular (ICV), convection-enhanced delivery (CED), or disruption of the BBB [77,78]. The disadvantage of ICV infusion is the low drug diffusion of brain parenchyma. If the target is not located near the ventricles, then this method is not an effective one [79]. CED, in general, is the insertion of a small stereotactically guided catheter into the brain parenchyma. The drug is pumped through this catheter and penetrates to the interstitial fluid.

The limitation of this method is that in some parts of the brain, for drugs applied through infusion is hard to have a high drug concentration. Placement of the catheter is a major factor in the achieved drug amount to the targeted site [80]. Another method for delivering drugs to the brain is the disruption of the BBB. This method can be applied in different ways; disruption by osmotic pressure, MRI-guided focused ultrasound the BBB, application of bradykinin-analog [81-83]. All these methods are expensive, require hospitalization, and are non-friendly patients. In addition to this, disruption of the BBB allows harmful blood components to enter the brain and may even cause permanent damages. Secondly, in the pharmacological approach, passive transportation through the BBB depends on molecule properties like molecule charge (low hydrogen bonding), molecular weight (<500 D), and lipophilicity (for a better transport lipophilicity should increase) [84]. Using these properties, some molecules can be chemically modified to pass through the BBB by adding more lipophilic substances and increasing lipophilicity. Sometimes, chemical modification of the molecule causes pharmacological activity loss [85]. The newly formed compound by molecule modifications may have become a substrate for P-glycoprotein and ending effluxed from the brain [86]. Lastly, although there are many transport ways to penetrate the BBB, in the physiological approach, brain drug delivery is based on uptake by specific receptors for specific ligands such as low-density lipoprotein (LDL) and transferrin (Tf) [87,88]. The best way to deliver neuroactive drugs from blood capillaries into the brain is by means of specific transporters and receptors. The molecular structure of drugs can be modified, or specific ligands can be conjugated to the molecule so that the molecule is recognized by specific receptors or transporters (**Figure 2**).

## 4. OPTIMIZATION OF NANOPARTICLES FOR BRAIN DRUG DELIVERY

### 4.1. Particle Size

Today the dynamic light scattering (DLS) and nanoparticle tracking and analysis (NTA) are the most appropriate, most commonly used, and the fastest way to determine the size of nanoparticles. One of the most important characteristics of NPs is particle size and size distribution [89]. The particle size determines the biological fate, *in vivo* distribution, targeting abilities, and the toxicity of a drug delivery system [90,91]. Additionally, they also affect nanoparticles stability, drug loading, and drug releasing [92-94]. The advantages of nanoparticles over microparticles are demonstrated in many studies. Nanoparticles, according to other larger particles have a higher cell uptake of the therapeutics and can target a wider range of intracellular and cellular components because of their mobility and smaller size.

In a study performed in Caco-2 cells, 100 nm nanoparticles had 2.5 times and 6 times greater uptake rate than respectively 1 μm microparticles, and 10 μm microparticles [95]. These results indicate that particle's biodistribution can be partially arranged by controlling particle size. Drug releasing is also affected by particle size. Smaller particle size means larger area/volume ration, so most of the drugs are attached to these nanoparticles are present at the surface or close to the surface, and as a result, there is a faster drug release. Since larger particles have bigger cores more drug can be loaded, but this situation causes slower drug release due to longer distance from the core to surface. Therefore, by controlling the particle size, we can affect the drug release rate in both ways. The aggregation risk is higher for smaller particles. During redispersion, transportation and storage polymer degradation are affected by particle size too. For example, the PLGA nanoparticles' degradation rate increases when its particle size increases [96,97].

Particle size is a crucial parameter not only for the reasons listed above, but also is responsible for different amounts of drug delivery across the BBB. In a study, using an *in vitro* model of the BBB, three different sized silica nanoparticles (30, 100, and 400 nm) were compared in terms of the permeability amount through the BBB. The results of this study show that nanoparticles between 30 nm and 100 nm can pass the BBB more efficiently [98]. Although the nanoparticles' material is relevant in the BBB crossing, another study performed with different gold NPs showed similar results that 70 nm is the optimal particle size [99]. After crossing the BBB, the extracellular space (ECS) is another obstacle for the drug to be delivered to the target site, which may be relatively far from the area of drug. The diffusability of the nanoparticles should be high, and it is highly dependent on the particle size. In a study done at Johns Hopkins University, the simulation of the ECS showed that in order for the drug to penetrate through ECS it should have a particle size at least smaller than 114 nm [100]. Even though, most of the nanotechnology drugs that are market available have a nanoparticles size above 100 nm, for the BBB crossing and acceptable ECS penetration it is recommended to have a NP size smaller than 100 nm (**Table 1**).

**Table 1.** *Key points of brain drug delivery with nanoparticles*

| Parameter | Optimum Value |
|---|---|
| **Particle size** | <100 nm |
| **Zeta Potential** | Lipophilic moieties and electrically near neutral charge [101] |
| **PEGylation** | A low PEG density below %10 with mush-room configuration |

### 4.2. Surface Properties of Nanoparticles

Surface modification determines the interaction between nanoparticles and the environment, whether it is plasma protein (antibodies), cell surface (cell membrane), or another nanoparticle. Drug loading into conventional carriers shows different biodistribution profiles from the drug itself because it is targeted by the mononuclear phagocyte system (MPS) like spleen, liver, and bone marrow. After being intravenously administrated, nanoparticles are identified by the phagocytic cells of the immune system and are eliminated from the blood circulation [102]. Except the particle size, the amount of proteins (i.e., opsonins) binding to the surface of the nanoparticles is determined by the hydrophobicity of the surface [103]. Hence, the *in vivo* fate of nanoparticles is determined by the surface hydrophobicity. Biological processes like interaction with biological membranes, protein adsorption, immune response, cellular uptake, and haemolytic activity are directly affected by the hydrophobicity of nanocarriers. This parameter directly affects the distribution, stability, and immune reaction to the nanocarriers; hence it should be characterized and controlled. Therefore, if the surface of nanoparticles is not modified, it is opsonized and cleared from blood circulation by phagocytic cells.

In a study performed by Gessner et al., nanocarriers with different degrees of hydrophobicities were investigated in terms of plasma protein adsorption quantitatively. The results showed that the higher is the hydrophobicity of the nanocarrier, the more plasma proteins are adsorbed that lead to reduced blood circulation time and hindered targeting besides RES organs [104].

In a study done by Shima et al., different degrees of amphiphilic poly(γ-glutamic acid) nanoparticle hydrophobicities were investigated in terms of immune response once introduced into the blood in mice and *in vitro*. The immune response was evaluated for each increasing hydrophobic degree of NP. It was reported that immune response to nanocarriers could be controlled to a large certain extend by optimizing the surface hydrophobicity [105].

In a study reported by Zhu et al. at the University of Massachusetts, how the nanocarrier hydrophobicity and protein adsorption influence the cellular uptake. 14 different gold NP with various hydrophobicities were synthesized and investigated for uptake in the HeLa cell line. The gold NP with the most hydrophobic surface showed the higher degree of protein adsorption leading to lower cell uptake. The opposite is true for the gold NP with the lowest hydrophobic surface showing higher cellular uptake [106].

In another study performed by Saha et al., various degrees of hydrophobic gold NP were synthesized and evaluated in terms of haematolytic activity. It was observed that higher hydrophobic surface gold NP had higher haematolytic effect, and the adsorbed protein corona decreases the haemolysis of red blood cells. It was concluded that both hydrophilic and hydrophobic had no haematolytic effect after 30 minutes in the presence of plasma. However, higher hydrophobic gold NP maintained the haematolytic activity due to aggregation despite protein adsorption in the plasma environment for at least 24 hours [107].

To increase the success of targeting, it is required to lower the opsonization and increase the *in vivo* blood circulation time of drug. All these can be accomplished by coating the surface of nanoparticles with hydrophilic polymer/surfactant or hydrophilic biodegradable copolymer (i.e., polysorbate 80, PEG, poloxamine, polyethylene oxide, and poloxamer) [108-110]. In many studies, it is reported that PEGylation of the surface of the nanoparticles inhibits the opsonization from blood components. If PEG molecules have a brush-like configuration or an intermediate configuration, the complement activation and phagocytosis are reduced, but surfaces coated with PEG mushroom-like configuration favored complement activation and phagocytosis [111]. Zeta potential is used to determine the surface charge of nanoparticles, and it is affected by the content of nanoparticles and the type of medium it is dispersed in [112]. Surface modification is also important to prevent agglomeration of the nanoparticles [113]. If the zeta potential is high enough the nanoparticles, because of their opposite potentials, will repel each to reach dispersed and redispersible solution and prevent agglomeration. As zeta potential is a function of dispersion stability, if zeta potential has a value higher than ±30 mV, the dispersion is physically stable, and the aggregation between particles is inhibited. Aggregation starts at 5 mV and smaller values of zeta potential [114]. *In vitro* study performed in hCMEC/D3 BBB cell model with similar size (ranging from 105 nm to 126 nm) liposomes but different surface zeta potentials, it is concluded that a significant difference in cell uptake is determined between neutral and non-neutral surface zeta potential liposomes whereas no significant cell uptake

difference was found between -6 mV potential and larger zeta potential values [115]. The best candidates for BBB overcome are electrically near neutral and lipophilic molecules [101].

#### 4.2.1. The PEGylation of nanoparticles to overcome the blood-brain barrier

The PEGylation of the nanoparticles surface is very important in the formulation development of nanoparticles. PEG coating protects the nanoparticles from the phagocytes, and it is dependent on PEG molecular weight and density [116]. Surface-grafted hydrophilic polymers coat the NPs as a dense cloud preventing at even low concentrations the interactions with other polymers. To have a low protein adsorption, long chain and high surface density are necessary. However, the density of the surface has a greater effect on steric repulsion than the length of the chains [117]. Methoxy PEG-PLA nanoparticles were developed and compared to uncoated nanoparticles. The labeled 14C PEG-PLA nanoparticles were phagocytosed slower than F68 coated PLA nanoparticles by THP 1 monocytes cultured cells. ME-PEG PLA nanoparticles improved its half-life by 360 minutes comparing to uncoated F68 nanoparticles. Due to particle circulation, a high radioactivity was found in blood vessels and heart. After 6 hours of iv administration of nanoparticles, radioactivity was found in phagocytic organs to indicate the delaying of phagocytosis [118]. In another study, PEG-coated PLGA nanoparticles in combination with focused ultrasound (FUS), which is used to temporarily and locally open the BBB for the PEG-PLGA, without any extra conjugated ligand crossed the BBB [100]. This means that the blood circulating time of the PEGylated PLGA nanoparticles was enough for FUS to induce BBB disruption and NPs to pass to the brain. PEG density and conformation are very crucial characteristics in improving the pharmacokinetics and biodistribution of NPs. In a study performed by Sheng et al., PLA NPs were coated with different concentrations of 5%, 10%, and 20% by weight. After the preparation of PEG-coated and uncoated PLA NPs, the time needed for the macrophage cells to uptake the NPs were measured for each PEG concentration. The optimum PEG coating leading the longest blood time circulation is 10% with 34.3 hours of circulation time [119]. In another study, the effect of PEG density in targeting potential of NPs was investigated. The results show improved NP targeting with a low PEG density below 10% and mushroom configuration, which is compatible with the PEG density range in pharmacokinetics and biodistribution studies [120].

## 5. PHARMACOKINETICS AND ORGAN DISTRIBUTION

The pharmacokinetics of small molecules, large molecules like protein and drug delivery systems like nanocarriers differ very much from each other.

Pharmacokinetics is also defined as what the body does to the therapeutics; hence it is considered into four subtitles as absorption, distribution, metabolization, and elimination. For small molecules and large molecular weight molecules administered parenterally, all these processes are meaningful; however, most of the nanocarriers do not have or have very limited gastrointestinal absorption. Distribution of the nanocarriers is closely related to its design characteristics such as size, shape, surface hydrophobicity, zeta potential, and targeting moieties. The primary route of elimination is through RES organs like spleen and liver. Nanocarriers are vehicles that intent to provide better efficacy, lower side effects, and better pharmacokinetic properties for the encapsulated therapeutic agent. To achieve these goals, the particle design should consider both the physiological properties of the body and the features of the nanoparticle itself.

The nanocarriers, once they are injected, the distribution and clearance start simultaneously. The blood flow distributes them to organs of RES, targeted tissues, and mostly are cleared these organs as well. All nanocarriers administered in vein firstly pass the lungs, and then they are transported to other tissues and organs through arterial blood flow. The nanocarriers are cleared from the organism in two ways. One of them is the RES or also known as the mononuclear phagocyte system (MPS). The macrophage cells phagocytose the nanocarriers and clear them from the bloodstream in addition to retention in RES organs. The second system of nanocarrier clearance is the liver and kidneys, which function as the main clearance organs. The systems and organs active in nanocarrier are summarized below;

*Blood*

The plasma proteins bind to form a protein corona around the nanocarrier named as opsonins. The opsonins facilitate the nanocarrier clearance from the bloodstream, making it the first barrier. The reduction of opsonization is one of the strategies to consider during nanocarrier design for a longer blood circulation time.

*Spleen*

Spleen is a highly perfused organ which store blood, clears the old blood cells, filtrates the foreign particles from the blood, and produces phagocytic cells. Moghimi et al., reported that the safe limit for spherical nanocarriers to avoid spleen filtration is 150 nm, larger particles are highly prone to filtration at interendothelial cell slits of venous sinuses in the spleen, whose width is approximately 200-250 nm [121].

*Kidney*

Clearance via kidney includes tubular secretion and glomerular filtration. Particles with size less than 5.5 nm and proteins less than 3 kDa depending also from the shape can be filtered through glomerular filtration [122,123]. As the endothelial cell in glomerular filters possesses fenestrations from 50-100 nm, hence nanoparticles smaller than 100 nm in size can be filtered through the kidney [124,125].

*Liver*

The liver's function is to remove foreign particles such as bacteria, viruses, and nanocarriers from the bloodstream [126]. The fenestrations in endothelial cells similar to the EPR effect allow foreign substances to be trapped in the liver to interact with hepatocytes, Kupffer cells, and BB cells [127]. The Kupffer cell comprises 80-90% of the macrophages in the human body [128]. These cells are responsible for phagocytosis of most nanocarriers and liver accumulations [129,130]. A study by Wisse et al. reported that the fenestrae in humans, which allow the passage of particles from susoidal lumen to the surface of hepatocytes necessary for liver filtration is 107 ± 1.5 nm [131].

Another parameter that influences the nanocarrier distribution and clearance is the shape. Most of the studies cited in the literature regarding nanocarriers are shaped spherical. It is the easiest way to manufacture, and the data available is larger. It is very difficult to summarize that what kind of shape or even charge is the best for any specific tumor, as most of the nanocarriers, regardless of their shape and charge, are accumulated in the liver and spleen [132,133]. Geometric shapes play a crucial role in nanocarriers' pharmacokinetics, such as flow properties, cellular uptake, vascular adhesion, and escape from blood vessels [134]. However, there are some tendencies of specifically shaped nanocarriers toward specific organs. For example, irregularly shaped nanocarriers are accumulated mostly in spleen, and rod-shaped particles are accumulated in the lungs [135,136]. The shape also plays an important role in renal filtration. It was reported that single-walled carbon nanotubes (SWCNT) of 200-300 nm of length undergo glomerular filtration, which is a conflict with the fenestrations around 100 nm [137]. Worm-like shaped nanocarriers display different flow properties, increasing the surface of interaction with the blood component as a single, minimizing the risk of phagocytosis from macrophages as well [138].

Another parameter which is crucial for pharmacokinetics of nanocarriers is the surface modification, which is also explained in detail in the surface properties and zeta size section.

## 6. TARGETED DRUG DELIVERY

NP carrier system development as targeted drug delivery systems is being revised recently. The targeting strategy can be classified as passive and active targeting. The therapeutic agent or the carrier of the therapeutic agent should be conjugated to specific tissue or cell ligand for active targeting aim. In passive targeting, the therapeutic agent is conjugated to a macromolecule or entrapped in a NP and passively delivered to the target site. Drugs entrapped to NPs or conjugated to macromolecules can target tumors with enhanced permeability and retention (EPR) effect.

NPs can be formulated to penetrate through biologic barriers and deliver drugs. Drugs like antineoplastics, antivirals cannot penetrate through BBB to pass into the brains, which considerably limits their treatment abilities for CNS diseases. Adsorption or covalent binding of a specific ligand or monoclonal antibody (mAb) to the surface of the nanocarrier is used as means of targeted drug delivery system to the brain. The ligand or mAb interact with specific receptors located in endothelium cells of the brain capillaries to penetrate BBB as an endogenous agent. The NP application as a delivery system across BBB is a promising approach.

### 6.1. Brain Targeted Drug Delivery Using Ligand

Receptor-mediated endocytosis (RME) requires specific ligand to bind to the appropriate receptors located in the luminal side of the endothelium of BBB. Once the ligand binds to the receptors, the receptor-ligand complex is formed, and the endocytosis begins. The newly formed complex internalizes as a vesicle into the endothelial cell. After internalization of complexes four different mechanisms can occur [139];

- Ligands can be degraded by the lysosome, and the disconnected receptors return to the membrane of the cell
- Simultaneously degradation of receptor and ligand by lysosome
- Post internalization receptor and ligand are recycled (retroendocytosis)
- Receptors bound to ligands are transported inside the cell to reach another domain of plasma membrane

RME systems can use endogenous or chimeric ligands to achieve active drug targeting into the brain. For a long time, blood ligands like Tf, insulin (Ins), Ins-like growth factor (IGF1&2), leptin, IgG, folic acid, and modified low-density lipoprotein (LDL ligands as ApoE) were a focus point for brain targeted drug delivery (**Table 2**). These kinds of endogenous ligands are non-immunogenic and biocompatible; on the other hand, the main advantage is the high affinity to tumor and brain cells.

It has been shown that cerebral capillaries have a higher level of Ins receptor expression than peripheral capillaries in animals and humans [140]. The high affinity of Ins to tumor cells makes it a promising target for targeting drug delivery. However, peptidic Ins hormone has a short half-life time, and in high concentrations it may cause hypoglycemia. This side effect can be avoided if the Ins-like growth factor (IGF) was used in place of Ins because it can be administered in high concentrations without causing hypoglycemia. Most of the researchers have used the Ins receptors' properties for brain targeting because of the high density of Ins receptors on the cerebral microvessels and transcytosis triggered through them. Similar to Ins, IGF1 and IGF2 can also pass into the brain by penetrating through BBB, but there are no recent studies using this targeting ligand [141]. Tf is a monomeric glycoprotein that contains one (monoferric) or two (diferric) iron atoms. TfR is overexpressed on the brain capillary endothelium and at the surface of proliferating cells such as brain tumor cells, especially glioblastoma multiform. Besides, in healthy individuals TfR levels are low. TfR can be saturated even in the physiological state because of the high amounts of endogenous Tf found in blood [142]. Folate receptor (FR) is expressed in brain capillaries endothelial cells. Due to FR is overexpressed in several tumors; it is a tumor marker in ovarian carcinoma and brain cancer. Folates like folic acid (FA) can be carried through the membrane by three mechanisms; reduced folate transporter, FR, and FA export pump [143].

### 6.2. Brain targeted drug delivery using a monoclonal antibody

As another approach to brain targeted drug delivery are chimeric ligands like peptidomimetic mAb, which bind to specific receptors found in BBB. As another approach to brain targeted drug delivery are chimeric ligands like peptidomimetic mAb. By binding to different sites other than endogenous ligands, they do not interact or compete with these ligands unless it is administered in high doses. mAb are macromolecules and can penetrate BBB by binding to specific receptors like Tf and Ins to induce transcytosis (**Table 3**). But the main question still remains as how much of the i.v. injected amount can actually penetrate BBB and pass to the brain and what is the result compared with.

**Table 2.** *Examples in literature of drug delivery systems incorporating ligands as targeting moieties*

| Ligand | Nanocarrier | Polymer/ Coating | Drug | Results | Ref |
|---|---|---|---|---|---|
| **Tf + Folate** | Carbon dots | - | Doxorubicin (Dox) | Better Tf receptor mediated transport across BBB and better *in vitro* cell uptake compared to Dox alone | 144 |
| | Gold nanoparticles | - | - | Gold NPs bypassed the BBB *in vitro* model and *in vivo* mice model. Tf ligand conjugated NPs had better brain uptake than Tf mAb conjugated NPs. | 145 |
| | Core-shell NP | Cationic liposome | Si-RNA | SiRNA (SiEGFR) carrying, Tf (T7), which is a modified ligand, was conjugated to core shell liposome NP. According to PEGylated and not targeted core shell NP, Tf conjugated core shell NP showed a higher accumulation in the tumor site. | 146 |
| | Chitosan | TPGS | Docetaxel | C6 Glioma cell MTT assay calculated $IC_{50}$ values of TPGS-Chitosan and especially Tf-TPGS-Chitosan NP compared to commercial Docel™ are significantly lower. *In vivo* AUC values of Tf-TPGS-Chitosan values were increased referenced to Docel™. | 147 |
| | PLGA | PEG | Doxorubicin | A modified Tf was conjugated to PEG coated doxorubicin loaded PLGA NP (TPDP), which is incorporated in PLGA scaffold to control the release rate of Dox. | 148 |
| | PLGA | - | Etoposide | The efficacy of antiproliferative activity in U87MG cells was as follows in increasing order: Tf+Fa-PLGA NPs > Fa-PLGA NPs > PLGA NPs > free etoposode | 149 |
| **Folate + Des-octanoyl Ghrelin** | Polymersome | | Doxorubicin, CY5.5 | Dual ligand conjugation to the polymersome increased the crossing through BBB and inhibited the glioma tumor growth | 150 |
| | SPIO NPs | Bovine Serum Albumin (BSA) | Fluorescein Isothiocyanate (FITC) | BBB crossing was not shown. Study results performed with folic acid conjugated SPIO NPs showed the combination is biocompatible, did not affect the cell cycle and proliferation. Increased internalization in U251 cell was also observed. | 151 |
| | Cationic microbubbles (MB) + FUS | - | DNA | FUS is used to transiently disrupt the BBB to deliver the DNA loaded folat-MB to the brain tumor site enabling targeted local gene therapy. Folate targeted MB had better gene transfer than nonconjugated or DNA gene alone. | 152 |
| **ApoE + Phosphatidic Acid** | SLN | - | - | A cellular uptake study in hCMEC/D3 cells comparing SLN and ApoE-SLN uptake speed and uptake amount. ApoE-SLN showed 1.8 times higher uptake than unconjugated SLN. BBB is thought to be crossed transcellularly. | 153 |
| | BSA NPs PBCA NPs | - | Sumatriptan succinate | This is a comparative *in vivo* study in rats investigating migraine efficacy between ApoE-BSA NPs and polysorbate 80-coated PBCA NPs. LDL receptor targeting ApoE-BSA had the highest drug amount delivered in the brain. | 154 |
| | Liposome | - | - | Two ligands are conjugated to liposome for therapy of Alzheimer disease. ApoE to facilitate the BBB crossing and phosphatidic acid having a high affinity to amyloid β peptide. Results show that double targeted liposome inhibits the amyloid β aggregation and enhances the starting disaggregation of aggregates. BBB crossing amount increased 5 times according to single ligand conjugated liposome. | 155 |
| **Glutathione (GSH)** | Liposome | PEG | Amyloid targeting Ab fragments | The Ab fragment was labelled with radioisotope to follow the progress and route. GSH-liposome crossed the BBB in Alzheimer transgenic mice models. | 156 |
| | Poly(ethyleneimine) (PEI) | - | - | The ability of GSH ligand to cross through *in vitro* endothelial cell BBB model was studied. GSH-PEI NPs showed a promising approach to BBB crossing. | 157 |
| | PLGA NPs | PEG | Docetaxel | In this study cytotoxicity tests in RG2 and C6 cells and *in vitro* Transwell cellular BBB model for BBB penetration were performed for GSH-PEG-PLGA NPs. BBB permeation increased for GSH-PEG-PLGA NPs compared to free Dox solution and there is a selectivity between healthy cells and glioma cells for GSH-PEG-PLGA NPs. | 158 |

**Table 3.** *Examples in literature of drug delivery systems incorporating monoclonal antibodies as targeting moieties*

| mAb | Nano-carrier | Drug | Results | Ref |
|---|---|---|---|---|
| **Anti-Transferrin (OX26)** | PLGA NPs | iAβ5 | Anti-Aβ (DE2B4) peptide and OX26 dual conjugated PLGA NPs, were investigated for BBB penetration and toxicity in a porcine brain endothelial cells comprising BBB model. The results show better cellular uptake compared to non-targeted NPs. More studies are needed to show intracellular and intracerebral uptake increase of iAβ5. | 159 |
| **+ Chlorotoxin (CTX) Ligand** | PEGylated liposome | Plasmid DNA | BMVECs/C6 cells co-culture model of BBB confirmed penetration of BBB endothelial cells and decrease of C6 viability. By dual targeting of OX26 and CTX respectively for BBB penetration and tumor targeting resulted in tumor volume decreases in C6 glioma rats. | 160 |
| **Murine Anti-Tranferrin Receptor (Ri7)** | Quantum dots (QD) | - | Endocytosis by receptor mediated transport was investigated and confirmed in bEnd5 and N2A cells. *In vivo* studies in mice supported the *in vitro* results by showing increased internalization of Ri7-DQ in the brain. Several hours after administration of Ri7-DQ, high concentrations were determined in the brain, which means that this approach is promising to deliver drugs in therapeutical concentrations to the brain tissue. | 161 |
| **Anti-transferrin Receptor (TfR) mAb** | Polymalic acid (PMLA) PolycefinTM nanoplatform | Morpholino Antisense Oligonucleotides (AON) | AON is conjugated to PMLA nanoplatform to inhibit gene expression responsible for tumor growth. *In vivo* results showed increased animal survival confirming the internalization of TfmAb-PMLA nanoplatform to the brain. AON induced inhibition of gene responsible for tumor growth. | 162 |
| **Anti-transferrin Receptor (TfR) mAb + ApoE ligand + Curcumin** | LUV liposome | - | TfR-Mab-ApoE-Curcumin-LIPs have 3 different targeting ligands and the study investigates the potential of liposome targeting *in vitro* BBB model and *in vivo* in normal and transgenic mice. Curcumin was found to inhibit Aβ peptide aggregation and penetrate through BBB by active targeting of LDL receptor and transferrin receptor. It should be noted that different doses of liposomes demonstrated different brain targeting capabilities. | 163 164 |
| **Anti-Human Insulin Receptor Antibody (HIRMAb)** | HIRMAb-IDS protein fusion | Iduronate 2-sulfatase (IDS) Protein | HIRMAb-IDS fusion was administered to Rhesus monkeys to observe the pharmacokinetics after IV infusion of different doses. The safety of the protein fusion was investigated and with the exclusion of hypoglycemia from high HIRMAb doses, no major adverse effects were detected. Safety profiles of *in vivo* studies in monkeys confirm the possibility of clinical studies of IgG anti-receptors protein fusions in CNS diseases. | 165 |
| | - | - | After SC injection of a range of HIRMAb doses, it was concluded that the lowest dose of HIRMAb is stable and has a long blood circulating time. The lowest dose of HIRMAb, has enough for BBB penetration to deliver IgG protein fusion that can deliver therapeutical relevant dose in the brain. | 166 |
| | SLN | Saquinavir (SQV) | Different parameters like palmitic acid weight fraction, the amount of conjugated MAb and poloxamer 407 weight fraction and their effect over SLN characterization were investigated. Cytotoxicity, cell uptake in RAW264.7 cells and BBB penetration in HBMECs/Has BBB model experiments were performed for all changed parameters. 83-14 MAb/SQV-SLNs results show an increased BBB targeting efficacy. | 167 |
| **Anti-Human Insulin Receptor Antibody (83-14 Mab)** | Polymersome (PDMS-b-PMOXA) | - | HIRMAb-Polymersome was investigated in terms of BBB penetration on a human insulin expressing hCMEC/D3 cell BBB model. Endocytosis in endothelial cells and competitive inhibition uptake by inclusion of excess free 83-14 MAb were confirmed. | 168 |

There are many studies performed dedicated to this specific question. The range of BBB penetration was measured as % of drug penetrated according to injected dose over gram (%ID/g) is between 0.2-3.1% for Tf targeted liposomes [169]. As described in the recent articles, the uptake and transport of drugs should be compared to polyclonal IgG to see the difference between targeted and nontargeted drug delivery systems [170,171]. Although IgG transport to the brain is very low, it suggests that other means of transport across BBB are possible other than receptor-mediated transport. The increased transport of drug delivery systems conjugated to TfR-mAb and HIRmAb could be used to deliver therapeutical drug doses to the brain [165,171,172].

## 7. CONCLUSION

Many large molecules like peptides, proteins, genes, antisense agents, and mAb have the therapeutical potential for CNS disease treatment. Nanotechnology provides clinical advantages for drug delivery like increased drug stability and half-life, decreased side effects, and drug dose. Despite the many research on new macromolecules, drugs, and drug delivery systems, there is still the very low translation of

these studies to clinical trials for CNS diseases, and one of the main reasons is the BBB. Modification of drug delivery systems, transient disruption of BBB, and their combination are the main approaches to overcome this problem such as a tumor or Alzheimer targeted modified NPs delivery in combination with FUS to transiently disrupt BBB.

Even after many years of study in this field, many researchers have failed to acquire sufficient prove and quantitative data supporting the efficient and clinically relative doses of drugs delivered to the brain parenchyma. More mechanistic studies like investigating the intracellular sorting mechanisms after uptake of nanocarriers into the endothelial cells should be performed. Not if but when the brain drug delivery problem is solved, and with the advancement in protein and gene modification, material design, and innovations in fabrication scale-up, the rate of new drug developments will accelerate. In order to better understand the nanocarrier drug delivery system, the influence of the formulation characterization parameters like particle size, shape, zeta potential, and PDI influencing brain drug delivery should be fully understood. The optimum value of particle size is less than 114 nm, and zeta potential value is near neutral. Varies tumor-targeting moieties have been incorporated in nanocarriers, but the most effective and better studied in literature are folate, Tf, ApoE, or their corresponding mAb and HIRmAb. Although one of the most efficient brain targeting ligands like Tf has a relatively low % ID/g drug efficiency future studies should include more pharmacokinetic data regarding brain accumulative drug amounts in order to better evaluate the efficacy of the treatment and dosage.

In recent years, many studies are describing FUS as a tool to boost the transportation of nanocarriers through BBB transiently and with minor or no side effects [173,174]. In the future, if FUS technology can be more reachable and less expensive, it has the potential to cross even larger carriers into the brain.

## AUTHOR CONTRIBUTIONS

Concept: IS; Design: IS, AŞ, HT; Supervision: YÇ; Materials: IS, AŞ; Data Collection and/or Processing: IS, AŞ; Analysis and/or Interpretation: IS; Literature Search: IS, AŞ, HT; Writing: IS; Critical Reviews: YÇ.

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## CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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