
ANKARA UNIVERSITY
FACULTY OF PHARMACY

ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

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# POLYMERIC MICRONEEDLES FOR NASAL DRUG DELIVERY

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## INTRODUCTION

Epilepsy is a neural disorder mostly characterized with seizures that occurs due to unbalanced production of stimulant and inhibitory signals in neurons of the brain [1]. Lacosamide increases frequency of seizures in patients with epilepsy however it has low transition through BBB [2]. Microneedles, have great potentials to overcome membrane barriers to achive sufficient doses at the targetted sites of the body. Therefore in this study, microneedles were formulated for nasal route in order to reduce the applied dose of Lacosamide to minimize the severe side effects while maintaining enhanced brain transition with the help of nasal olfactory pathway.

## METHODS

Lacosamide (gifted by Santa Farma, Turkey). Eudragit® S 100 (ES100) (Röhm Pharma, Darmstadt, Germany). All other chemicals were in analytical grade.

Microneedles were prepared by micro-molding method [3]. A modified HPLC method was used for the determination of Lacosamide [4]. Lacosamide amounts were determined and *in vitro* release, SEM (Zeiss Ultra Plus FE-SEM, Germany), FT-IR (IR Affinity-1S Shimadzu, Japan), DSC (DSC-60, Shimadzu USA) and 1H-NMR (Fourier 300 NMR Bruker, USA) analyses were performed.

## RESULTS

Compositions of the microneedles were presented in Table 1. Analyses results of SEM (Figure 1), DSC (Figure 2), FT-IR (Figure 3), $^1$H-NMR (Figure 4) parafilm puncture images (Figure 5) and Texture analyses (Figure 6) showed the structural properties of the microneedles. Lacosamide releases were extended up to 96 hours (Figure 7).

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

| Code | Lacosamide (%) | ES100 (%) | PEG 400 (%) |
|---|---|---|---|
| EL0 | - | 7.5 | 0.5 |
| EL1 | 0.6 ± 0.0 | 7.5 | 0.5 |
| EL2 | 1.1 ± 0.0 | 7.5 | 0.5 |
| EL3 | 1.5 ± 0.0 | 7.5 | 0.5 |

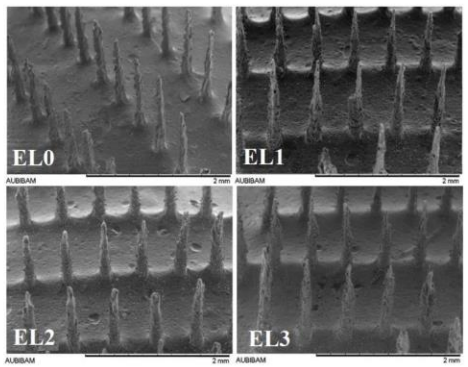


**Fig. 1.** SEM images

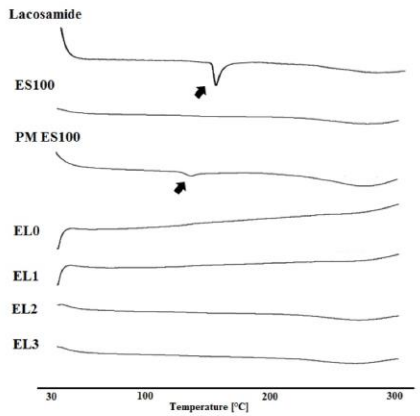


**Fig. 2.** DSC thermograms

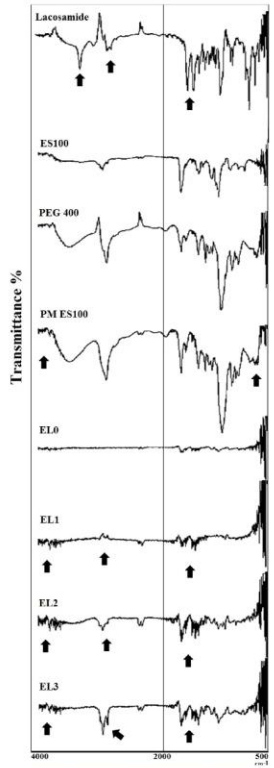


**Fig. 3.** FT-IR spectra

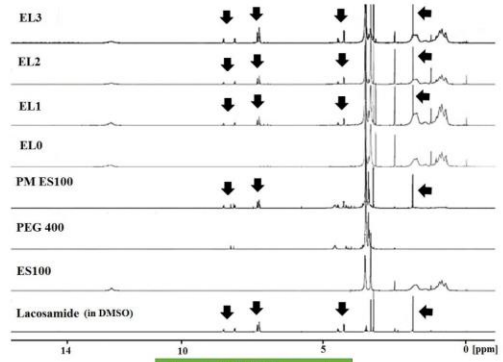


**Fig. 4.** $^1$H-NMR spectra

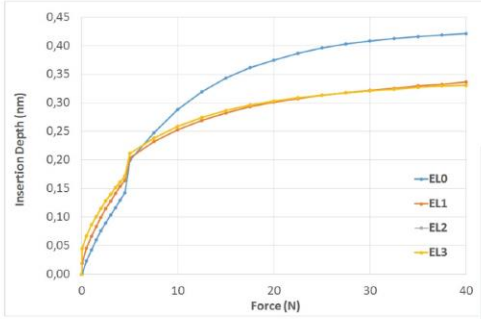


**Fig. 6.** Puncture depths (Mean ± SE, n=3)

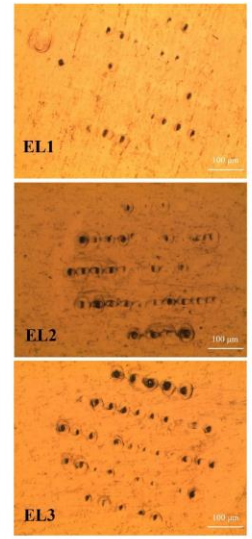


**Fig. 5.** Parafilm puncture images

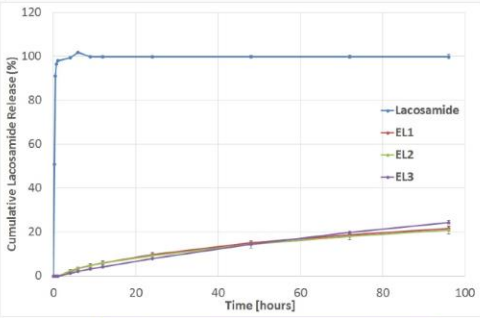


**Fig. 7.** *In vitro* Release (Mean ± SE, n=3)

## CONCLUSION

**ES100 based microneedles are promising candidates for the nasal application of Lacosamide for epilepsy treatment with less Lacosamide amount which has potential to minimize the severe side effects while maintaining enhanced brain transition with the help of olfactory pathway**

**Acknowledgements**

DOPNA-LAB for FT-IR and 1H-NMR analyses and BIBAM for SEM Analyses

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[2] Cawello W, Stockis A, et al. (2014). Ann New York Acad Scienes.1329(2014):18-32.
[3] Wang M, Hu L, et al.(2017). Lab Chip. 17(8):1373-87.
[4] Sreenivasulu V, Rao DR, et al.(2011). Res J Pharm Biol Chem Sci. 2(4):1-11.

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13th International Symposium on Pharmaceutical Sciences
22-25 June 2021

# ATORVASTATIN-ENCAPSULATED CORE-SHELL TYPE HYBRID NANOCARRIERS FOR LOCAL THERAPY OF BREAST CANCER: FORMULATION AND OPTIMIZATION STUDIES

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## INTRODUCTION

Atorvastatin is a synthetic statin commonly used in the treatment of hypercholesterolemia. Apart from this, statins appear to have pleiotropic effects, including modulation of cell growth, apoptosis. Through modulation of these pathways, statins have the potential to influence a wide range of disease processes, including cancer. However, poor aqueous solubility and poor bioavailability has limited therapeutic application of atorvastatin. Breast cancer is most commonly diagnosed cancer and the second leading cause of death among women. The major treatment strategy of breast cancer is surgical intervention followed by radiotherapy, chemotherapy or hormone therapy. Most of these strategies, especially conventional chemotherapy, can lead to a variety of undesirable side effects such as the development of cardiac or systemic toxicity in healthy tissues and drug resistance against anti-cancer drugs. These drawbacks have limited the therapeutic effectiveness of anti-cancer drugs such as Atorvastatin. To overcome these limitations, the efficacy of drug delivery strategies such as nano-sized drug carriers have been investigated with great interest in recent years (1). The aim of this study to optimize and investigate the effect of various drug/polymer ratio (X1) and phospholipid/polymer ratio (X2) on the mean particle size (Q2) of the hybrid nanocarriers of Atorvastatin by using a Design of Experiment (DoE) approach.

## MATERIALS & METHODS

### Production of Core-Shell Type Hybrid Nanoparticles

Lipid monolayer is formed through the mixture of lecithin and DSPE-PEG

Polymeric solution consist of PLGA and drug was added drop by drop to the lipid phase consist of DSPE-PEG and lecithin

Finally, sonication process was applied for the formation of lipid polymer hybrid nanocarriers spontaneously

### Characterization of Core-Shell Type Hybrid Nanoparticles

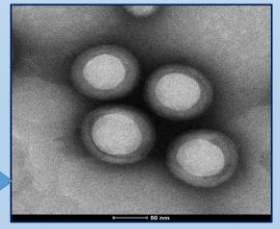
| | |
|---|---|
| Encapsulation efficiency | UV-visible spectrophotometry |
| In-vitro release studies | |
| Particle size | Photon correlation spetroscopy (Nano ZS, Malvern Ins., UK) |
| Polydispersity index (PDI) | |
| Surface charge | |
| Particle morphology | TEM (FEI Co ., USA) |

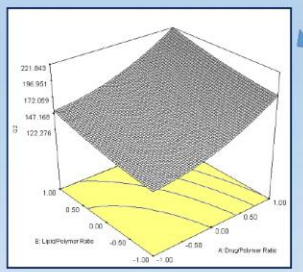
### Optimization of Core-Shell Type Hybrid Nanoparticles

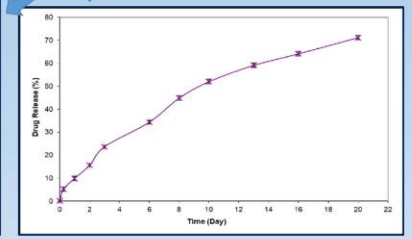
Optimization of hybrid nanocarriers was carried out using $3^2$ full factorial design through Design Expert Software to understand the influence of X1 and X2 on the response, Q2.

## RESULTS & DISCUSSION

- In this study, Atorvastatin loaded the hybrid nano-sized carriers were successfully produced through DoE approach.
- The encapsulation efficiency of the core-shell type lipid polymer hybrid nanocarriers varied widely from 33.49% to 91.27%.
- The prepared lipid–polymer hybrid systems exhibited an average particle size from 122.7 nm to 219.0 nm with polydispersity in the range from 0.070 to 0.179, which inhibited a narrow size distribution.
- AH1 coded core-shell type hybrid nanocarrier, which was located at the lower level of the design, was specified as the optimized hybrid carrier according to the model.








**Design points and response variables**

| Code | Variable coded levels | | Response Variables | |
|---|---|---|---|---|
| | X1 | X2 | Q2 (Observed) | Q2 (Predicted) |
| AH1 | -1 | -1 | 122.7 ± 1.44 | 122.3 |
| AH2 | -1 | 0 | 135.5 ± 2.67 | 132.7 |
| AH3 | -1 | +1 | 148.8 ± 1.75 | 151.9 |
| AH4 | 0 | -1 | 139.6 ± 1.81 | 140.8 |
| AH5 | 0 | 0 | 149.1 ± 2.02 | 152.3 |
| AH6 | 0 | +1 | 178.7 ± 2.64 | 172.7 |
| AH7 | +1 | -1 | 188.6 ± 1.77 | 187.8 |
| AH8 | +1 | 0 | 202.5 ± 1.43 | 200.4 |
| AH9 | +1 | +1 | 219.0 ± 1.10 | 221.8 |

| Coded levels | Actual values | |
|---|---|---|
| | X1 | X2 |
| -1 | 1/5 | 0.1 |
| 0 | 1/10 | 0.2 |
| +1 | 1/20 | 0.4 |

- Hybrid particles successfully produced with a spherical shape and negative zeta potential values ranged from -22.5 mV to -32.8 mV.

## CONCLUSION

- In this research, Atorvastatin was successfully loaded into the core-shell type hybrid nano-sized carriers for the first time.
- In conclusion, a full factorial design model was applied in order to predict the statistical effect of the important factors on the particle size of the hybrid carriers optimized using DoE approach.

## REFERENCES

1. Bakar-Ates F et al., (2020). International Journal of Pharmaceutics, 586: 119565.

# DoE BASED APPROACH FOR THE DESIGN OF PIROXICAM LOADED POLYMERIC NANOPARTICLES

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JUNE 22-25, 2021 ANKARA, TURKEY

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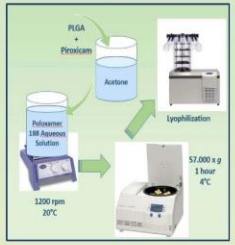
## INTRODUCTION

Hepatocellular carcinoma is the third most common solid organ malignancies cause of death from cancer and the fifth most commonly occurring cancer due to the high prevalence of chronic liver damage caused by hepatitis or cirrhosis in the world. Although surgical resection, liver transplantation, local ablation therapies, and chemotherapy which are the basic treatment strategies, it can not be obtain significant survival rate in patient with metastatic disease or advanced local disease. For this reason, there is a significant need for new treatment strategies (1). The aim of this study is to develop the polymeric nanoparticulate drug delivery systems of piroxicam which is a chemopreventive active substance and to evaluate the in-vitro characteristics for the first time. The aim of this study to optimize and investigate the effect of various drug/polymer ratio (X1) and phospholipid/polymer ratio (X2) on the mean particle size (Q2) of the hybrid nanocarriers of Atorvastatin by using a Design of Experiment (DoE) approach. For this purpose, piroxicam loaded polymeric nanoparticles were prepared as a per $3^2$ full factorial experimental design to optimize the amount of polymer (X1) and surfactant percentage ratio (X2) investigated based on the encapsulation efficiency (R1) of nano-sized systems.

## MATERIALS & METHODS

### Preparation and Optimization of Polymeric Nanoparticles

Polymeric nanoparticles of piroxicam were prepared by using nanoprecipitation technique as seen in Figure 1. Optimization of hybrid nanocarriers was carried out using $3^2$ full factorial design through Design Expert Software to understand the influence of X1 and X2 on the response, R1.

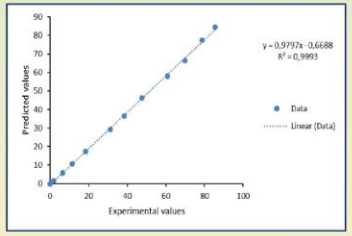


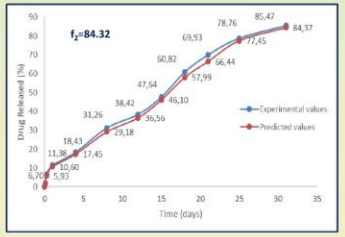

### Characterization of Polymeric Nanoparticles

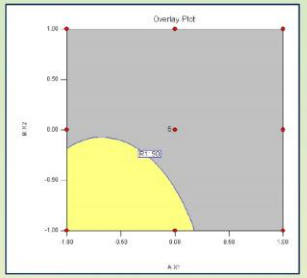
| UV-Visible Spectrophotometry | Photon Correlation Spetroscopy | Transmission Electron Microscopy |
|---|---|---|
| • Encapsulation efficiency<br>• In-vitro release studies | • Particle size<br>• PDI<br>• Surface charge | • Morphological structure |

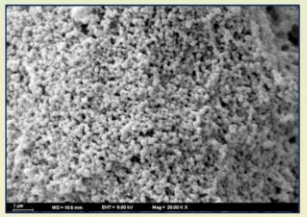
## RESULTS & DISCUSSION

- The particle size of PLGA nanoparticles ranged from 184.6 to 323.4 nm with PDI between 0.051-0.249.
- The PLGA nanoparticles obtained with a spherical shape and negative zeta potential values ranged from -9.3 mV to -32.1 mV.
- PN1 coded nanoparticle formulation, which was located at the lower level of the design, was specified as the optimized formulation according to overlaid contour plots.
- The entire time course of in-vitro release testing the correlation of experimental and predicted values resulted in a correlation coefficient of $r^2 = 0.9993$.



**Design points and response variables**

| Code | Variable coded levels | | Response Variables | |
|---|---|---|---|---|
| | X1 | X2 | R1 (Observed) | R1 (Predicted) |
| PN1 | -1 | -1 | 98.88 ± 0.87 | 96.89 |
| PN2 | -1 | 0 | 83.56 ± 1.18 | 87.59 |
| PN3 | -1 | +1 | 71.37 ± 1.20 | 69.33 |
| PN4 | 0 | -1 | 89.88 ± 0.96 | 93.55 |
| PN5 | 0 | 0 | 84.00 ± 1.51 | 82.50 |
| PN6 | 0 | +1 | 58.72 ± 2.14 | 62.48 |
| PN7 | +1 | -1 | 64.17 ± 3.42 | 62.49 |
| PN8 | +1 | 0 | 46.29 ± 3.42 | 49.69 |
| PN9 | +1 | +1 | 29.65 ± 1.65 | 27.93 |

| Coded levels | | | Actual values | |
|---|---|---|---|---|
| | | | X1 (mg) | X2 (%) |
| -1 | | | 50 | 1 |
| 0 | | | 100 | 2 |
| +1 | | | 200 | 3 |

## CONCLUSION

- The comparison between predicted and experimented data yielded in similarity factor ($f_2$) value of 84.32 indicating that these two profiles were similar with each other. This study demonstrated that the various physicochemical properties of polymeric nanoparticles loaded with piroxicam could be optimized by changing the polymer amount and surfactant concentration according to the full factorial design studies.

## ACKNOWLEDGEMENTS

This work was supported by Ankara University Scientific Research Projects Coordination (21L0237008).

## REFERENCES

1. Hu Y., et al., (2021). Process Biochemistry 100: 140-148.

# Preparation and Optimization of β-Cyclodextrin Inclusion Complexes of Atomoxetine Hydrochloride

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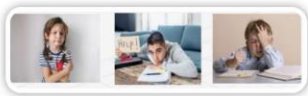
## INTRODUCTION

- Atomoxetine HCl (ATO), which is used for the treatment of attention deficit hyperactivity disorder (ADHD), has an extremely bitter taste. It is known that oral formulations of active substances with bitter taste, especially for pediatric purposes, cause patient compliance problems.
- Cyclodextrins (CDs) have also been widely used in the pharmaceutical industry due to their cavities, which can form inclusion complexes to mask the bitterness of drugs.
- The aim of this study is to prepare beta cyclodextrin (β-CyD) inclusion complexes by experimental design method in order to mask the bitter taste of ATO and formulate it in oral dosage form. In this study, with using the experimental design ATO: β-CyD inclusion complexes have been prepared and they have been optimized considering with the loaded ATO amounts and their solubility properties at 30 minutes.



## METHOD

### Phase Solubility Studies

- Aqueous solutions containing β-CyD in the molar concentration range of 0.002-0.012 were prepared.
- 5 mL of each solution was taken into colored glass vials with caps, an excessive amount of ATO was added to them and shaken in a water bath at 37 °C for 48 hours.
- According to the absorbance values determined using UV spectrophotometer, concentrations of ATO of each solutions were calculated and plotted the concentration of β-CyD versus the dissolved ATO concentrations.

### Preparation of inclusion complexes

- By kneading method, inclusion complexes were prepared by mixing ATO and β-CyD at 3:7, 2:3, 5:5, 3:2 and 7:3 molar ratios.

### Characterization studies

- IR spectrum analysis
- Differential scanning calorimetric analysis
- Dissolution studies
- Determination of ATO content

Table 1. Ingredients of inclusion complexes

| Molar ratio (ATO: β-CyD) | ATO (g) | β-CyD (g) |
|---|---|---|
| 3:7 | 0,2976 | 2,7024 |
| 2:3 | 0,3717 | 2,6283 |
| 5:5 | 0,6135 | 2,3865 |
| 3:2 | 0,7242 | 2,2758 |
| 7:3 | 1,1250 | 1,8750 |

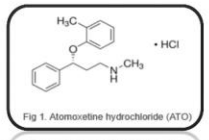


Fig 1. Atomoxetine hydrochloride (ATO)

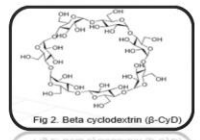


Fig 2. Beta cyclodextrin (β-CyD)

- Experimental design (DoE), as a tool of QbD, can be defined as the strategy for setting up experiments in such a manner that the information required is obtained as efficiently and precisely as possible. The potential benefits of DoE are summarized below:
  - Improved process yield and stability
  - Improved process capability
  - Reduced process variability
  - Reduced process design and development time

## RESULTS

### Phase Solubility Studies

- Since the curve obtained is an AN type curve, it was determined that ATO formed an inclusion complex that dissolves with β-CyD, but although the concentration of β-CyD increased, it would not increase more than the maximum dissolution value of ATO.

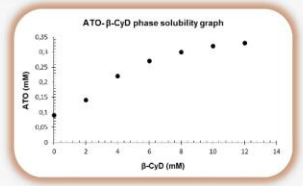


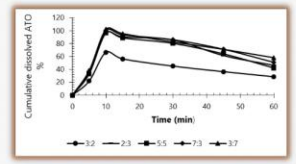

Fig 3. Phase solubility graph of ATO- β-CyD

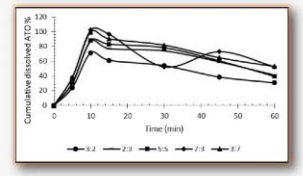
### Characterization studies

### Dissolution studies

Table 2. Cumulative dissolved ATO%

| | Time (min) | Cumulative dissolved ATO %: 3:7 | 2:3 | 5:5 | 3:2 | 7:3 |
|---|---|---|---|---|---|---|
| 20 minutes | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 38,09 | 33,89 | 33,13 | 21,95 | 37,01 |
| | 10 | 102,09 | 96,46 | 96,69 | 66,59 | 100,29 |
| | 15 | 95,58 | 90,4 | 88,9 | 58,35 | 93,59 |
| | 30 | 82,53 | 85,68 | 80,42 | 45,13 | 86,06 |
| | 45 | 71,19 | 61,53 | 64,19 | 36,57 | 71,31 |
| | 60 | 58,2 | 46,41 | 42,28 | 28,66 | 51,05 |
| 40 minutes | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 37,85 | 29,76 | 30,63 | 24,11 | 38,07 |
| | 10 | 99,61 | 86,6 | 87,85 | 71,39 | 102,08 |
| | 15 | 90,17 | 76,84 | 82,83 | 61,11 | 96,31 |
| | 30 | 81,6 | 73,84 | 78,11 | 53,83 | 52,17 |
| | 45 | 64,6 | 58,97 | 60,31 | 38,47 | 72,74 |
| | 60 | 52,61 | 41,05 | 39,4 | 30,62 | 52,36 |




### Loaded ATO amount

Table 3. Loaded ATO amount %

| | Loaded ATO amount %: 3:7 | 2:3 | 5:5 | 3:2 | 7:3 |
|---|---|---|---|---|---|
| Mixing Time | | | | | |
| 20 minutes | 98,59±0,48 | 54,88±0,65 | 85,89±0,18 | 79,23±1,2 | 82,93±0,37 |
| 40 minutes | 95,33±0,32 | 53,82±0,96 | 77,84±0,48 | 77,11±0,84 | 86,74±0,48 |

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### Experimental Design (DoE)

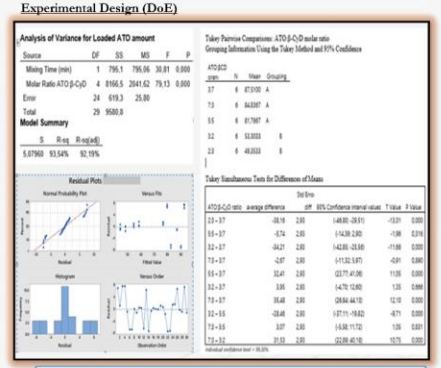


Fig 7. Variance analyze and Tukey Comparisons of ATO amount

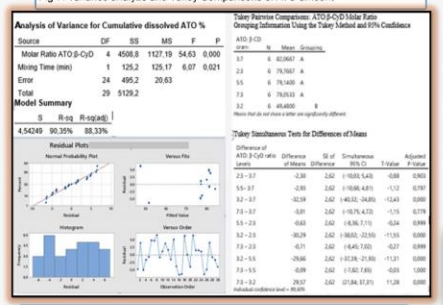


Fig 8. Variance analyze and Tukey comparisons of cumulative dissolved ATO%

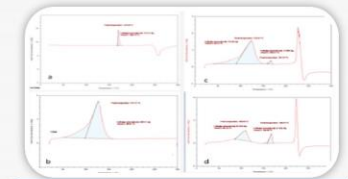

Fig 9. a: DSC analyse of ATO b: DSC analyse of β-CyD
c: DSC analyse of 2:3 ATO-β-CyD d: DSC analyse of 7:3 ATO-β-

Fig 9. DSC analysis.

| Experiment Number | ATO:β-CyD molar ratio | Mixing time (min) | Loaded ATO amount (%) | Cumulative dissolved ATO % |
|---|---|---|---|---|
| 1 | 7:3 | 40 | 86,64 | 72,32 |
| 2 | 2:3 | 20 | 55,52 | 85,68 |
| 3 | 7:3 | 40 | 87,27 | 72,04 |
| 4 | 3:7 | 20 | 100,3 | 82,52 |
| 5 | 5:5 | 20 | 86 | 80,56 |
| 6 | 7:3 | 20 | 83,14 | 85,88 |
| 7 | 7:3 | 20 | 83,14 | 85,8 |
| 8 | 3:2 | 40 | 48,14 | 53,96 |
| 9 | 5:5 | 20 | 85,68 | 80,32 |
| 10 | 5:5 | 40 | 77,11 | 78,24 |
| 11 | 2:3 | 20 | 54,89 | 85,6 |
| 12 | 3:7 | 40 | 75,89 | 81,64 |
| 13 | 3:7 | 20 | 99,02 | 82,56 |
| 14 | 7:3 | 20 | 82,51 | 86,12 |
| 15 | 2:3 | 40 | 43,6 | 73,92 |
| 16 | 3:2 | 20 | 58,7 | 45 |
| 17 | 3:2 | 20 | 58,38 | 45,24 |
| 18 | 3:2 | 40 | 48,06 | 53,68 |
| 19 | 5:5 | 40 | 77,75 | 77,12 |
| 20 | 2:3 | 40 | 44,88 | 73,8 |
| 21 | 3:2 | 40 | 46,48 | 53,84 |
| 22 | 3:7 | 40 | 74,89 | 81,56 |
| 23 | 3:7 | 40 | 75 | 81,6 |
| 24 | 5:5 | 40 | 78,06 | 78,2 |
| 25 | 3:2 | 20 | 60,06 | 45,16 |
| 26 | 7:3 | 40 | 86,32 | 72,16 |
| 27 | 2:3 | 20 | 54,23 | 85,8 |
| 28 | 5:5 | 20 | 86 | 80,4 |
| 29 | 3:7 | 20 | 99,96 | 82,52 |
| 30 | 2:3 | 40 | 43 | 73,8 |

Table 4. Optimum inclusion complex formulations

| ATO: β-CyD Molar Ratio | Mixing time (min) |
|---|---|
| 2:3 | 20 |
| 7:3 | 40 |

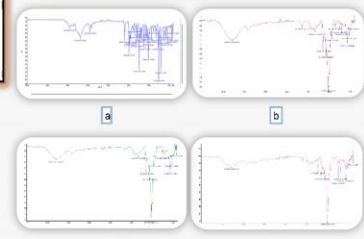


Fig 10. a: IR spectrum of ATO b: IR spectrum of β-CyD
c:IR spectrum of 2:3 ATO-β-CyD d: IR spectrum of 7:3 ATO-β-CyD

## CONCLUSION

In this study; Inclusion complexes of ATO have been prepared using the method of kneading with β-CyD.

With using the experimental design, which is the most important step of QbD, inclusion complexes have been optimized considering with the loaded ATO amounts and their solubility properties at 30 minutes. Accordingly, it has been determined that the complexes prepared with a mixing time of 2:3 (ATO: β-CyD) 20 minutes and 7:3 (ATO: β-CyD) 40 minutes have the most suitable inclusion complexes in terms of the determined properties.

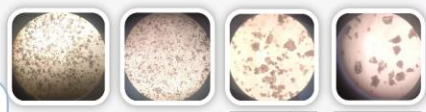


Fig 11. Optical microscope images

JUNE 22-25, 2021 ANKARA, TURKEY

13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

# A NOVEL UV/VIS SPECTROSCOPY METHOD FOR THE DETERMINATION OF ATEZOLIZUMAB: METHOD DEVELOPMENT AND VALIDATION

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## Introduction

Atezolizumab is a monoclonal antibody and has been approved by FDA for various cancer treatments (1). Regarding the atezolizumab, there is no UV spectrophotometry methodology published for the quantification of atezolizumab in pharmaceutical preparations. The aim of this study was to develop and validate a simple, fast, and reliable UV visible methodology for the determination of atezolizumab in pharmaceutical products.

## Materials and Methods

First, the maximum wavelength and the calibration curve of atezolizumab were determined using a UV/Vis spectrum. Then, validation studies were carried out to determine the reliability of the spectrophotometer method used in quantification for atezolizumab according to the criteria recommended by the FDA (2). For this purpose, linearity, accuracy, precision, durability, specificity, consistency, robustness and sensitivity were determined.

## Results

According to the experimental data, the maximum absorbance for atezolizumab was found as 280 nm (Figure 1). The method developed was linear in a range varying from 0.10 to 1.50 mg.mL$^{-1}$ determined by 6 individuals calibrations points (Figure 2). The $r^2$ value was 0.9995 indicating a 99.95% correlation in linearity and precision. The robustness showed good and similar values and the limit of detection and limit of quantification were 0.005 mg.mL$^{-1}$ and 0.018 mg.mL$^{-1}$, respectively.

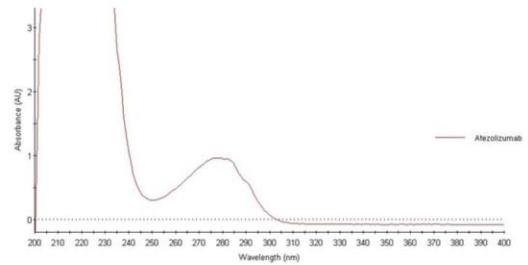


**Figure 1.** UV/Vis spectrum of atezolizumab

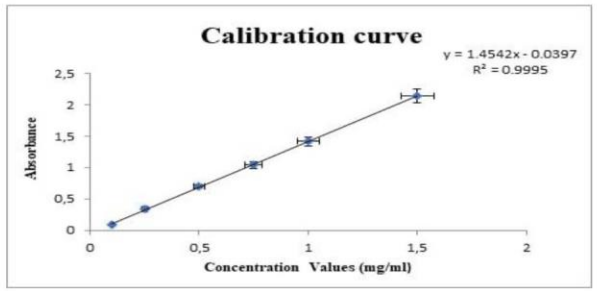


**Figure 2.** Calibration curve of atezolizumab

## Conclusions

The data corroborates the reliability as applicability of the developed UV/Vis spectroscopy method for quantitatively determining the amount of atezolizumab in pharmaceutical products.

## Acknowledgements

This study was supported by a grant of TUBITAK (SBAG-220S361) within the scope of the PhD thesis of Meliha Ekinci.

## References

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2. FDA. (2000). US Department of Health.

# Development and Optimization of Self-Nanoemulsifying Drug Delivery System of Bosentan Using Box-Behnken Design

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ISOPS 13th International Symposium on Pharmaceutical Sciences — Ankara University Faculty of Pharmacy

## INTRODUCTION

Bosentan (BOS) is an orally active endothelin receptor antagonist and Biopharmaceutics Classification System (BCS) Class II drug for the treatment of pulmonary arterial hypertension (1). Lipid-based systems such as self-nanoemulsifying drug delivery systems (SNEDDS) have been extensively investigated to improve the bioavailability and dissolution rate for poorly soluble drugs. The aim of this study was to evaluate the lipid-based formulation properties before the BOS loading. Design Expert® Version 10 was used to examine and optimize the effects of formulations.

## MATERIALS AND METHODS

According to the solubility results, Maisine and Peceol were selected as oil. In addition, Cremophor RH 40 and Labrasol were selected as surfactant and co-surfactant, respectively (2). The most appropriate combination was created using BBD and investigated the system characterization properties before drug loading. In the pseudo ternary phase diagram, the shaded area was the area in which the self nanoemulsion was formed by a system that did not contain any active substance and was determined by water titration. The ratio of $S_{mix}$ was selected as 9:1. The optimized SNEDDS was characterized with respect to dispersibility, self-emulsification time, transmittance%, droplet size, polydispersity index (PDI), dilution and pH effect, turbidity, viscosity, morphology, thermodynamic, and long-term stability studies. After these evaluations, optimum SNEDDS formulation was selected to load the drug.

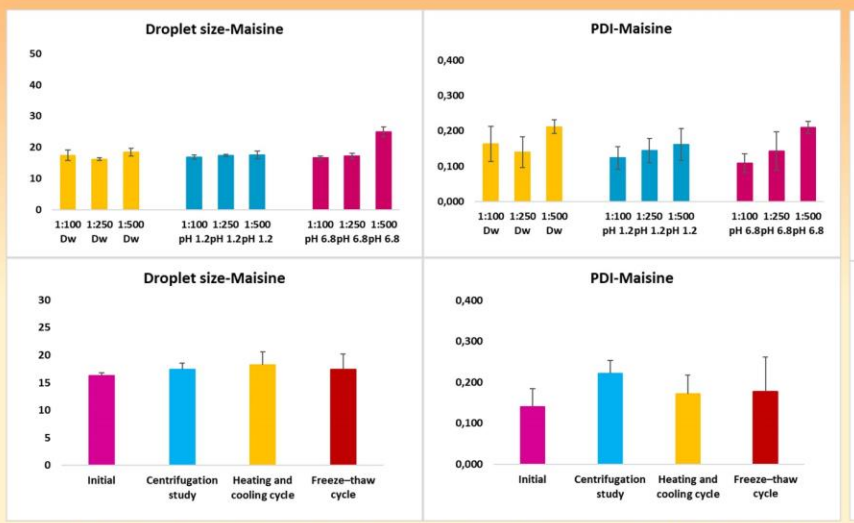


**Figure 1**. Dilution and pH effect and thermodynamic stability on droplet size and PDI for Maisine SNEDDS

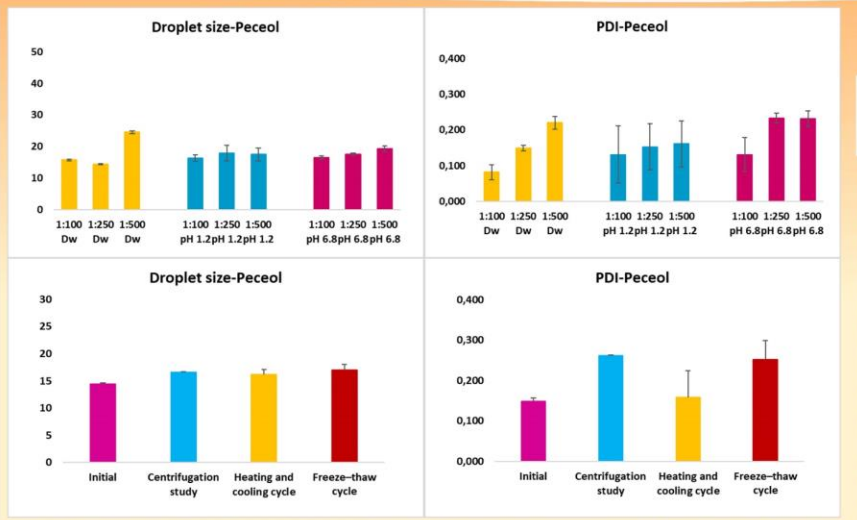


**Figure 2**. Dilution and pH effect and thermodynamic stability on droplet size and PDI for Peceol SNEDDS

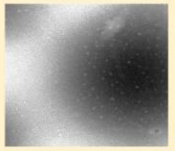

**Figure 3.** TEM image of Maisine SNEDDS

**Figure 4.** TEM image of Peceol SNEDDS

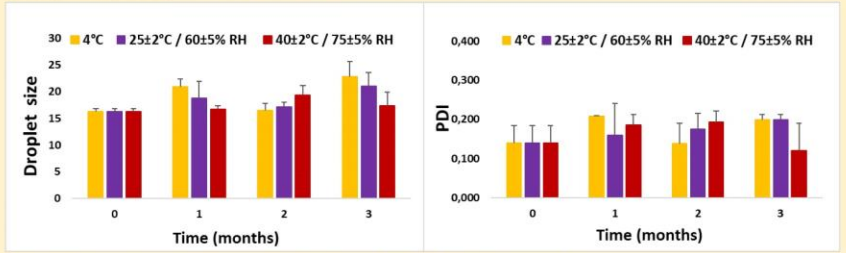


**Figure 5**. Long-term stability on droplet size and PDI for Maisine SNEDDS

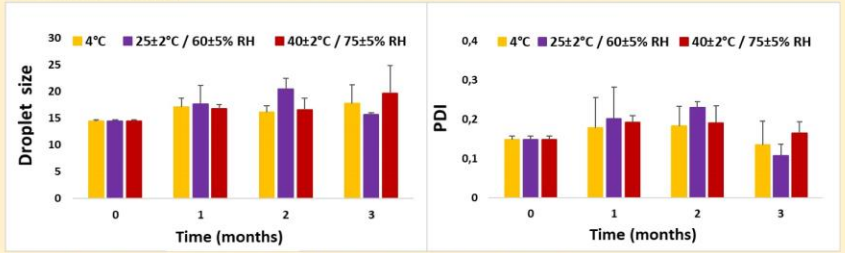


**Figure 6**. Long-term stability on droplet size and PDI for Peceol SNEDDS

**Table 1**. Characterization parameters of blank Maisine SNEDDS and Peceol SNEDDS

| | Dispersibility Studies (sec/Grade) | Self-Emulsification Time (sec) | | Percentage Transmittance | Droplet Size (nm) and PDI | Turbidity Measurements (NTU) | Viscosity Measurements (cp) |
|---|---|---|---|---|---|---|---|
| **Maisine SNEDDS** | 55.2±6.5 Grade A | 58 | Clear | 99 | 16.27±0.49 0.140±0.044 | 5.8±0.1 | 684 |
| **Peceol SNEDDS** | 58.0±1.6 Grade A | 60 | Clear | 100 | 14.44±0.21 0.149±0.008 | 5.6±0.1 | 652 |

## RESULTS

The prepared SNEDDS formulations were thermodynamically stable for Maisine-SNEDDS and Peceol-SNEDDS, respectively (Figure 1 and Figure 2). The emulsification times were <1 min for the rapid rate of emulsification and dispersibility (Table 1). TEM images illustrated the formation of a spherical shape with a size range of 10–100 nm (Figure 3 and Figure 4). The formulations exhibited no sign of precipitation, robust against dilution and effect of pH, and also showed acceptable %transmittance (≥99%), turbidity, and viscosity values (Figure 1, Figure 2 and Table 1). There were no significant differences in the stability results of various test conditions (Figure 5 and Figure 6). The prepared blank SNEDDS formulations were thermodynamically stable with a droplet size of 17.11 nm and 16.76, a PDI of 0.180 and 0.200, for BOS-loaded Maisine-SNEDDS and BOS-loaded Peceol-SNEDDS, respectively.

## CONCLUSION

The results showed that both formulations were found to be proper for the drug loading. 30 mg and 28 mg of BOS were successfully loaded to 1 g of Maisine-SNEDDS and Peceol-SNEDDS, respectively.

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## ACKNOWLEDGMENT

This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK, SBAG-217S602). The authors would like to thank Gattefossé for providing Maisine, Peceol, Labrasol, and Abdi İbrahim for providing Bosentan.

# Comparison of Biorelevant Dissolution of Medium Chain Mono and Diglycerides Based Bosentan-Loaded Self-Nanoemulsifying Formulations

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13th International Symposium on Pharmaceutical Sciences
ANKARA UNIVERSITY FACULTY OF PHARMACY

## INTRODUCTION

Self-nanoemulsifying drug delivery systems (SNEDDS) among lipid-based systems are one of the most widely used approaches for drugs with a limited dissolution rate of absorption. Bosentan (BOS), which is an endothelin receptor antagonist treatment of pulmonary arterial hypertension, is categorized as Class II in Biopharmaceutics Classification System (1). The aim of this study was to compare the biorelevant dissolution performance of BOS-loaded SNEDDS and commercial products (Tracleer®).

## MATERIALS AND METHODS

In our previous studies, the SNEDDS components were chosen and formulations were designed with Box-Behnken Design (2). It was observed that the solubility of BOS was significantly higher in the long-chain mono and diglyceride derivatives which were Maisine and Peceol comparing to the other oils (3). 30 mg and 28 mg of BOS were loaded to 1 g of blank SNEDDS formulation containing Maisine or Peceol, respectively. The formulations were filled with a hard gelatin capsule. The in vitro dissolution studies were performed USP Apparatus II at 50 rpm at 37±0.5°C in 1% SLS in distilled water, Fasted State Simulated Intestinal Fluid (FaSSIF), and Fed State Simulated Intestinal Fluid (FeSSIF). Samples were analyzed by HPLC. The dissolution profiles were compared to BOS-loaded SNEDDS versus reference tablet. The dissolution data were evaluated using DDSolver®.

## RESULTS

For both Peceol and Maisine-SNEDDS, more than 80% of the drug was released from SNEDDS and a reference tablet within 15 min and 100% release was obtained from both within 30 min in 1% SLS. Additionally, more than 80% of release was obtained within 30 min in FaSSIF and FeSSIF for SNEDDS. However, reference tablets, approximately 32% and 11% in 90 minutes were able to release in FaSSIF and FeSSIF, respectively (Figure 1). The Peceol-SNEDDS and Maisine-SNEDDS increased the percentage of cumulative dissolved by 2.98, 7.88-fold, 3.0, and 7.97-fold in FaSSIF and FeSSIF compared to the reference tablet, respectively. The similarity factor ($f_2$) was also determined (Table 1). The dissolution profiles of SNEDDS formulations and Tracleer® did not give similar dissolution curves in biorelevant media (Figure 1).

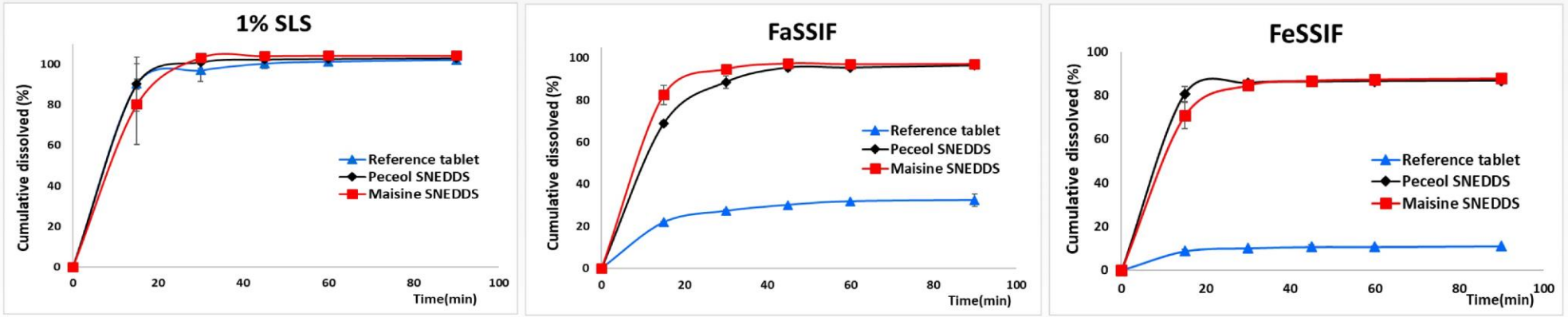


**Figure 1.** In vitro dissolution profiles of Peceol SNEDDS, Maisine SNEDDS, and reference tablet for 1% SLS, FaSSIF, and FeSSIF (n=3, mean±SD).

**Table 1.** $f_2$ values of BOS-loaded SNEDDS formulations and reference

| | BOS-loaded Peceol SNEDDS vs reference | BOS-loaded Maisine SNEDDS vs reference | BOS-loaded Peceol SNEDDS vs BOS-loaded Maisine SNEDDS |
|---|---|---|---|
| **1% SLS** | 83 | 63 | 68 |
| **FaSSIF** | 13 | 11 | 60 |
| **FeSSIF** | 8 | 9 | 69 |

## CONCLUSION

Our results showed that the in vitro dissolution profiles of SNEDDS and reference tablet was only similar in 1% SLS in distilled water which was not mimic the in vivo media. In biorelevant media that mimic fast and fed conditions, bosentan was almost completely dissolved according to the commercial product. According to the results, the bioavailability of bosentan could be enhanced in fasted and fed states compared to the commercial products because of the lipid-based formulation effect.

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1. Panigrahi, KC et al., (2018). J Drug Deliv Sci Technol. 47:31-42.
2. Yılmaz Usta D, Teksin ZŞ. The Development of SNEDDS Containing Bosentan Using Long Chain Mono and Diglyceride Mixtures, EUFEPS Annual Meeting, 2018, Athens, Greece.
3. Yılmaz Usta D, Teksin ZŞ. Evaluation of water and oil solubilities of bosentan, a BCS class IIa drug. 2nd International Gazi Pharma Symposium Series, 2017, Ankara, Turkey.

## ACKNOWLEDGMENT

This study was supported by a grant from TUBITAK (SBAG-217S602). The authors would like to thank Gattefossé for providing Maisine, Peceol, Labrasol, and Abdi İbrahim for providing Bosentan.

# EVALUATION OF BERBERINE PHYTOSOME STABILITY IN SIMULATED BODY FLUIDS BY HPLC METHOD

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## INTRODUCTION

It is increasingly common to transfer plant-based active ingredients to modern treatment. However, most of these active ingredients have long side chains and high polarity. This prevents absorption by passive diffusion from the gastrointestinal mucosa or skin (1). At this point, the new complexing technique called "phyto-phospholipid" or "phytosome" plays an important role in facilitating absorption and increasing bioavailability. Phytosomes (PSs) are structures formed by complexing plant-derived active ingredients with natural phospholipids in a suitable solvent/solvent system. PSs protect active ingredients against degradation by digestive and intestinal secretions. Berberine (BER) is a quaternary benzylisoquinoline alkaloid that can be obtained from many different plants. In recent years, antihypertensive (2), hypoglycemic (3), anticancer (4) and hepatoprotective (5) effects have also been studied by various researchers. However, the oral bioavailability of BER is low. PSs increase the oral bioavailability of BER.

In this study, BER phytosomes (BER-PSs) were prepared by a reverse phase evaporation method. The stability BER-PSs in simulated body fluids was determined. The amount of BER remaining in BPs were measured by High- Performance Liquid Chromatography (HPLC).

The stability studies of BER-PSs suggested that PSs were more stable in simule gastric fluid (SGF) pH 1.2 than simule intestinal fluis (SIF) pH 6.8.

## MATERIALS AND METHODS

### Materials

BER, soy phosphatidylcholine, acetonitrile, ammonium acetate, dichloromethane, ethanol were obtained from Sigma-Aldrich (Germany). Pepsin and pancreatin were obtained from Sigma-Aldrich (Germany). All other chemicals used are also of analytical quality.

### Preparation of BER-PSs

20 mg BER was dissolved in mixture of 5 ml ethanol and 5 ml distilled water. 40 mg of phospholipid was dissolved in 10 ml of dichloromethane. BER solution was added onto the phospholipid solution and mixed in magnetic stirrer at 60 °C and 400 rpm for 1 hour. Organic solvents were removed by rotary evaporator. BER-PSs was frozen at -20 °C and lyophilized.

### Stability tests in simulated body fluids

SGF pH 1.2 medium was prepared by dissolving 2 g sodium chloride and 3.2 g pepsin in 7 ml HCl and completing to 1000 ml with distilled water. While preparing the SIF pH 6.8 medium, 6.8 g monobasic potassium phosphate was dissolved in 250 ml distilled water, 77 ml 0.2 N sodium hydroxide, 500 ml distilled water and 10 g pancreatin were added this solution and than mixed. The pH was adjusted to 6.8 with 0.2 N NaOH and 0.2 N HCl and completed to 1000 ml with distilled water. 2 mg BER-PSs and 2 mg BER powder were incubated in different vials in SGF medium for 2 hours at 37 °C and in SIF medium for 6 hours at 37 °C. Test procedures were given in the Figure 1. At the end of this period, trifluoroacetic acid was added the test materials to stop the enzymatic activity. The amount of BER which remained in BER-PSs and BER powder which remain intact was measured by HPLC.

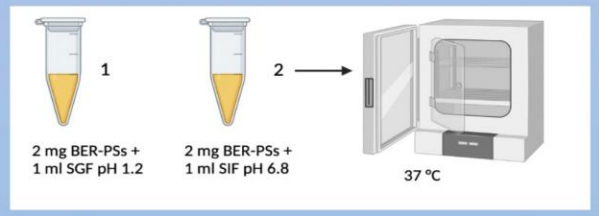


**Figure 1.** Stability test procedures in simulated body fluids

### HPLC analysis

The quantity of BER in test samples were determined using HPLC. Agilent 1260 Infinity II instrument, Zorbax Eclipse Plus C18 column (100 mm x 4.6 mm x 3.5 µm), 1 mL/min flow rate, 67:33 (v/v) as mobile phase 30 mM ammonium acetate:acetonitrile mixture, column temperature of 30 °C and a wavelength of 346 nm was used.

## RESULTS

BER-PSs were prepared by a reverse phase evaporation method. The BER-PSs showed a small particle size (236 ± 8.71 nm) and a well-dispersed structure with 76.4±3.41% encapsulation efficiency. While the amount of BER remaining without degradation in BER-PSs was measured as 79.93% in SGF pH 1.2 medium; it was measured as 70.72% in SIF pH 6.8 medium. The amount of BER powder which remain intact was measured as 91.00% in SGF pH 1.2 medium; it was measured as 79.80% in SIF pH 6.8 medium.

The stability results in simulated body fluids was shown in Figure 2.

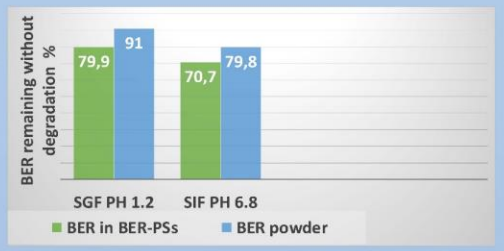


**Figure 2.** Stability results in simulated body fluids

## DISCUSSION

Rowland et al (6), demonstrated that most liposomes were little affected by the low pH during passage through the stomach. The well-organized assembly of phospholipids can protect liposomes from gastric environment disintegration. Liu et al (7), showed that the deposition of polymers on the surface of liposomes may further stabilize their structure under low-pH conditions (8). Similarly, phytosomes are also in the same situation and this study showed that their stability in SGF medium was higher than their stability in SIF medium. In our study, it was observed that the stability of BER in simulated body fluids decreased somewhat in the BER-PSs. The physical nature of phospholipids and the resulting delivery systems stability are changing a lot, depending on the sources and the degrees of purification of the phospholipids (9).

### Acknowledgement

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), grant no 215 S 664.

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# Thermodynamic Stability Testing of Ketoconazole and Caffeine Loaded Nanoemulsion Formulations for Dermal Application

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## INTRODUCTION

Ketoconazole and caffeine are shown as effective at different pathophysiological disorder belongs to hair loss. These molecules penetration through the skin is limited. Although the solubility of ketoconazole in water is very low, the solubility of caffeine in water is very high. These limitations required a new stable carrier system for their dermal application on hair loss (1,2)

Nanoemulsion formulations are recently preferred for dermal application due to their features like small droplet size, increased thermodynamic stability and enhanced drug penetration through the skin (3).

**The aim of the study** is to investigate the ketoconazole and caffeine loaded nanoemulsion formulations durability and to evaluate their capability for further studies and for dermal usage on hair loss.

## Materials:

Coconut oil, Miglyol 818 (Cremer Company) were used as the oil phase.

Cremophor RH40 (BASF Chemical Company) and Transcutol P (Gattefosse Pharmaceuticals) were used as the surfactant and the co-surfactant.

Distilled water was used as the water phase of the formulations.

## METHODS

Self-nanoemulsifying method was used for the formation of the nanoemulsion systems

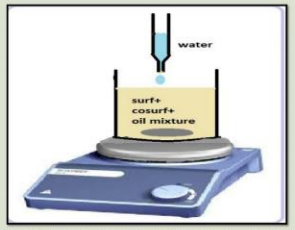


Fig1: the constraction of the nanoemulsion formulations.

Table 1: formulation compositions

| Formulation code | Formulation Composition |
|---|---|
| F1 | Cremophor RH40:Transcutol P(Km:3:1) %80, Coconut Oil-Miglyol 818(1:1) %10, Distilled water %10 |
| F2 | Cremophor RH40:Transcutol P(Km:3:1) %80, Coconut oil %10, Distilled water %10 |
| F3 | Cremophor RH40:Transcutol P(Km:3:1) %80, Miglyol 818 %10, Distilled water %10 |
| *each formulation includes %2 ketoconazole in SNEDD (surfactant+cosurfactant+oil mixture)<br>•each formulation includes %2 caffeine in water phase<br>•Each formulation was diluted with distilled water the ratio of 1:4 after drug loading | |

Table 2: Test methods

| Test | Procedure |
|---|---|
| The Centrifugation test | 3500 rpm 30 min |
| The freeze-thaw test | The freezing at -20C during 48 hours and the thawing at 25C during 48 hours per cycles/3 cycles |
| The heating-cooling test | the heating at 45C during 24 hours and the cooling at 4C during 24 hours per cycles/ 6 cycles |

## RESULTS

All the formulations were evaluated for their droplet size and polidispersity index, homogeneity, liquid crystal formation and phase separation properties before and after the tests application.

Table 3: phase separation, liquid crystal formation and homogeneity properties evaluation after tests

| | Homogeneity | | | Liquid cyristal formation | | | Phase separation | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F1 | F2 | F3 | F1 | F2 | F3 |
| **The Centrifugal test** | √ | √ | √ | X | X | X | X | X | X |
| **The freeze-thaw test** | √ | √ | √ | X | X | X | X | X | X |
| **The heating-cooling test** | √ | √ | √ | X | X | X | X | X | X |

After all tests, the all formulations were homogeneous, liquid crystals and phase separation didn't occur in any formulations.

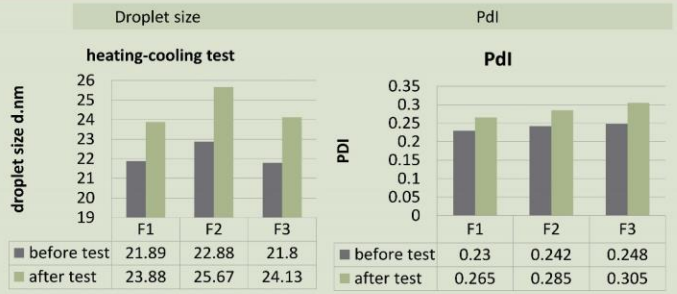


Fig2: droplet size and PdI changing with heating-cooling test application.

The droplet size remained 30 nm below in all formulations, the PdI remained 0.3 below in F1 and F2 formulations.

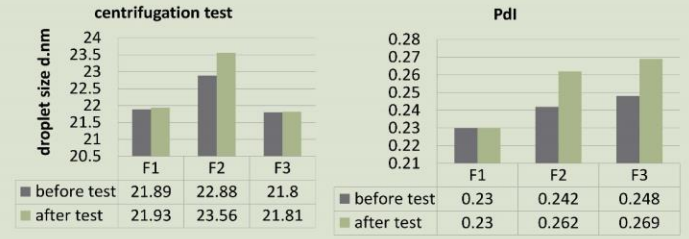


Fig3: droplet size and PdI changing with centrifugation test application.

The droplet size remained 30 nm below in all formulations after test, the PdI remained 0,3 below. There was't seen significal rising in F1 formulation.

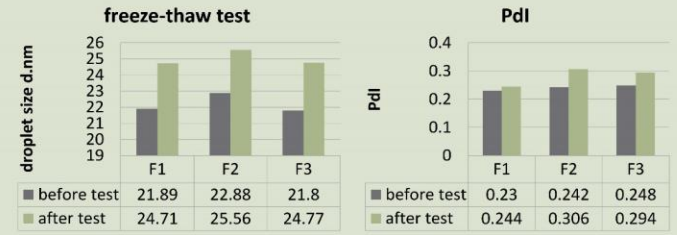


Fig4.droplet size and PdI changing with freeze-thaw test application.

The droplet size remained 30 nm below in all formulations after test, the PdI remained 0,3 below in F1 and F3 formulations.

## CONCLUSION:

- We achieved nanoemulsion systems which are droplet size below 50 nm and 0.25 pDI with natural oil and synthetic oil as drug and cosmetic carriers for dermal applications.
- Any stress tests that applied didn't change the expected droplet size and polidispersity index for intended use, F1 formulation was found most stabile with a slight difference.
- The stress tests conditions can be made harder to have significal changes.
- For this studied conditions, all the formulations were found stable and it was decided that all the formulations could be used in further studies.
- And It should be remembered that the contents and the properties of the natural oils vary according to the source.

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**Acknowledgements**
The authors are thankfull to Bilim Drug Company for providing KET and Doğacı Natural Cosmetic Company for natural oils

# Evaluation of in vitro-in vivo relationship: Bosentan-loaded lipid based formulation versus commercial product

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## Introduction

In the U.S. FDA guidance, In Vitro-In Vivo Relationship (IVIVR) is defined as a relationship between in vivo bioavailability and the in vitro release profiles (1). A stronger relation provides a prediction of in vivo results using in vitro data. Moreover, IVIVR allows a decrease in the number of in vivo studies, formulation optimization, enhance product quality, reduce costs of drug development (2). The aim of this study was to investigate IVIVR for Bosentan-loaded SNEDDS, solid SNEDDS (S-SNEDDS) tablet, and commercial product (Tracleer®).

## Methods

In previous studies, Bosentan-loaded SNEDDS and S-SNEDDS tablet formulations were developed and in vitro dissolution and pharmacokinetic studies were conducted for these formulations (3,4).

Piecewise Cubic Hermite Interpolating Polynomials (PCHIP) method was used to develop point-to-point relation between in vitro dissolution data and in vivo plasma concentration by MATLAB Version 9.10 (MathWorks, MA, USA). With this method, the unknown intermediate values (the missing points) were found at any unknown intermediate time points (5). With this method, the data set was generated by applying interpolation to 5, 10, 15, 20, 25, 30, 35, 45, 50, 55, and 60 minutes. The relationship between in vitro and in vivo data at these time points was evaluated.

## Results

The correlation coefficient ($R^2$) between fasted group plasma concentration and dissolution data in FaSSIF, FaSSIF V2, FDA-recommended media (a distilled water media containing 1% SLS), and the correlation coefficient between fed group plasma concentration and dissolution data in FeSSIF, FeSSIF V2, FDA-recommended media were shown in Fig. 1.

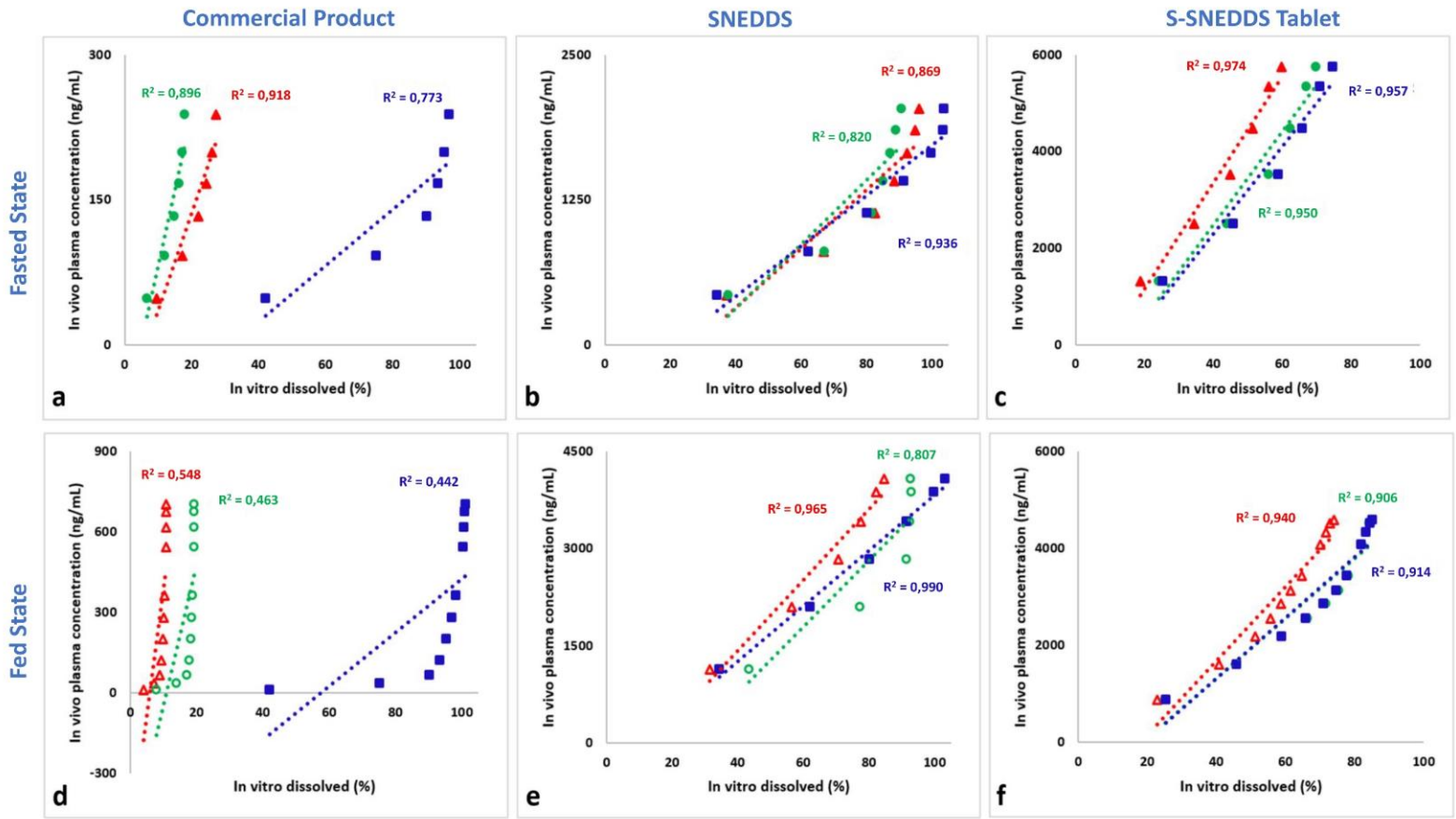


**Fig. 1.** The correlation between in vitro dissolution data (FaSSIF (▲), FaSSIF V2 (●), FeSSIF (△), FeSSIF V2 (○), FDA-recommended media (■)) and in vivo plasma concentration of (a) commercial product in fasted state, (b) SNEDDS in fasted state, (c) S-SNEDDS tablet in fasted state, (d) commercial product in fed state, (e) SNEDDS in fed state, (f) S-SNEDDS tablet in fed state.

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**Acknowledgements**

This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK) Project No: 217S602.

## Results

For evaluation of IVIVR, the dissolution curves should usually reach 100% at infinity or the last time point of dissolution study in practice (5). And also the appropriate dissolution method is the method that simulates in vivo conditions. For this purpose, the biorelevant media was also used.

Compared to biorelevant media, the lowest relationship was obtained in FDA-recommended media for commercial product (Fig. 1a-1d). In this media, the percentage dissolved reached 100%. However, bosentan is poorly water-soluble drug.

For the SNEDDS, the correlation coefficient of between the FDA-recommended media and fasted state plasma concentration was found 0.936, and the correlation coefficient of between the FDA-recommended media and fed state plasma concentration was found 0.990 which was indicated a strong relation. However, the stronger relationship was not observed in the fasted state biorelevant media (Fig. 1b-1e).

For S-SNEDDS tablet, the correlation coefficient was found 0.974, 0.950, 0.957 in FaSSIF, FaSSIF V2, FDA-recommended media and 0.940, 0.906, 0.914 in FeSSIF, FeSSIF V2, FDA-recommended media, respectively (Fig. 1c-1f). The stronger relationship was observed in the fasted state biorelevant media. Compared to bosentan-loaded lipid based formulations, the lowest relationship was obtained for commercial product.

## Conclusions

The positive relations were successfully obtained between in vitro dissolution data and in vivo plasma concentration. Since the SNEDDS and S-SNEDDS tablet increased solubility, dissolution, and lymphatic absorption of bosentan, a higher relation was observed between in vitro dissolution and plasma concentration. The lower correlation coefficient was obtained for commercial product in FDA-recommended media, since bosentan is a BCS Class IIa drug with a weak acidic property.

# DEVELOPMENT AND OPTIMIZATION OF AN ANTIHYPERTENSIVE FIXED-DOSE COMBINATION USING PLACKETT-BURMAN DESIGN

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## INTRODUCTION

Fixed Dose Combinations (FDCs) have frequently been preferred in the treatment of hypertension in cases where blood pressure needs to be reduced in the ratio of 20/10 mmHg [1]. Angiotensin Converting Enzyme Inhibitors (ACEIs) and Calcium Channel Blockers (CCBs) have been commonly prescribed together due to their additive effects. Besides, improved patient adherence by taking fewer pills make it rational to develop an FDC product containing ACEI and CCB [2].

Experimental design is the strategy of planning the effect of selected independent variables on the responses at determined levels as a result of a series of experiments. Commonly, screening designs such Plackett-Burman Design (PBD) are preferred at the earlier stages of the process to eliminate the insignificant factors[3].

In this study PBD was used in the formulation development of FDC tablets including amlodipine besylate as a CCB and enalapril maleate as an ACEI. This study aims to show how to use screening designs to determine the most effective factors on product quality while preparing an antihypertensive fixed-dose combination tablet.

## MATERIALS AND METHODS

The FDC formulation contains amodipine besylate (Hetero Drugs, India), enalapril maleate (Zheijiang Huahai, China), microcrystalline cellulose (RanQ Remedies, India), pregelatinized starch (Colorcon, England) , crospovidone (Ashland, USA), hydroxypropyl cellulose (Ashland, USA), glyceryl distearate (Gattefosse, France) and sodium bicarbonate (Solvay-Carbonate, France). Tablets were directly compressed in TDP 5 (China).

Experimental design matrix was created in Design-Expert® (Stat-Ease Inc., USA). PBD was performed with 8 factors and 12 experiments. Selected factors and their levels were given in Table 1. PBD was used to examine whether the independent variables have a significant effect on Critical Quality Attributes (CQAs).

In this study, assay (90-110% of label claim) and relative standard deviation (RSD) of content uniformity (CU) (not more than 5%), friability, disintegration, and dissolution were chosen as CQAs. Half-normal graphs and Pareto-charts were used for evaluating the PBD results.

The evaluated factors were disintegrant ratio, lubricant ratio, binder ratio, blending speed, blending time, lubrication speed, and lubrication time.

## RESULTS AND DISCUSSIONS

The factors that may affect the CQAs were gathered in an Ishikawa diagram (Figure 1) to identify Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs). The severities of the selected risk parameters were shown in Table 2. Regarding the cause and effect matrix, the parameters which were marked as “middle” and “high” were chosen as independent factors in DOE.

The results of the experiments were given in Table 3. Assay of APIs, dissolution of APIs in three different media (pH 1.2, pH 4.5, and pH 6.8), friability, and disintegration results were found to be compatible with individual reference products. Any consistent and significant model was not generated for assay and dissolution results.

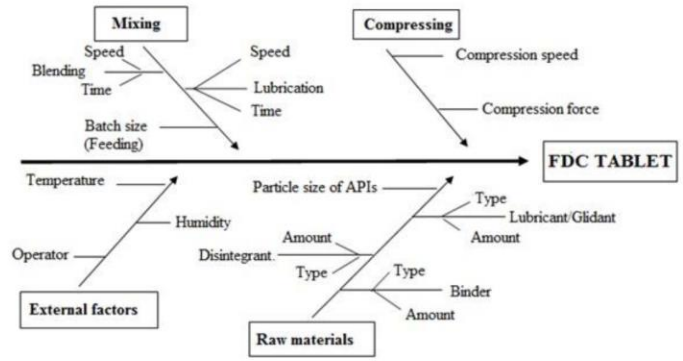


Figure 1. The Ishikawa diagram illustrating the factors which may affect the CQA

Table 1. The cause and effect matrix of the risk assessment of excipients

| | Disintegrant | Binder | Lubricant | Blending | Lubrication |
|---|---|---|---|---|---|
| **CU** | Low | Low | Low | High | Low |
| **Assay** | Low | Low | Low | High | Low |
| **Friability** | Middle | Low | Low | Low | Middle |
| **Disintegration /Dissolution** | High | High | High | Low | High |

Table 2. The factors and levels selected in PBD

| Factors | | Low level (-1) | High level (+1) |
|---|---|---|---|
| $X_1$ | Pregelatinized starch (%) | 5 | 20 |
| $X_2$ | Crospovidone (%) | 1 | 5 |
| $X_3$ | HPC (%) | 0 | 1 |
| $X_4$ | Glyceryl distearate (%) | 1 | 3 |
| $X_5$ | Blending speed (rpm) | 8 | 16 |
| $X_6$ | Blending time (min) | 8 | 20 |
| $X_7$ | Lubrication speed (rpm) | 8 | 16 |
| $X_8$ | Lubrication time (min) | 2 | 8 |

According to the Pareto chart and half-normal graphs, blending time, lubrication time, and lubrication speed had significant effects on content uniformity of enalapril ($p<0.05$) (Figure 2). Additionally, the amounts of the lubricant and disintegrant had significant effects on disintegration time ($p<0.05$). Remarkably, as blending time which was the most effective factor, increased, a higher RSD was observed. This could be caused by the segregation of EM with long-term mixing as a result of its small particle size.

The results of friability were appropriate in all formulations within 0.1-0.3%. It was seen in the Pareto chart (Figure 3) that the only significant factor in friability was the amount of glyceryl distearate ($p <0.05$).

The half-normal and Pareto graphs (Figure 4) showed that the percentages of glyceryl distearate and crospovidone had significant effects on disintegration ($p <0.05$). Disintegration time was increased as the amount of glyceryl distearate increased and decreased as the amount of crospovidone increased. It is an expected result that the disintegrants in the formulation shorten the disintegration time, while the lubricants extend it.

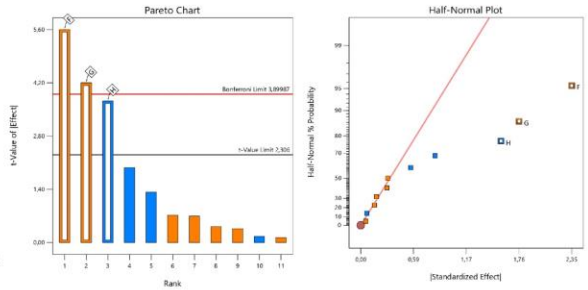


Figure 2. Half-normal (a) and Pareto (b) charts of CU of EM (F: blending time, G: lubrication speed, H: lubrication time)

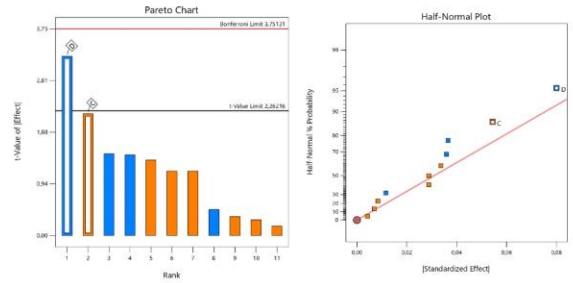


Figure 3. Half-normal (a) and Pareto (b) charts of friability (D: glyceryl distearate (%), C:hydroxypropyl cellulose (%)

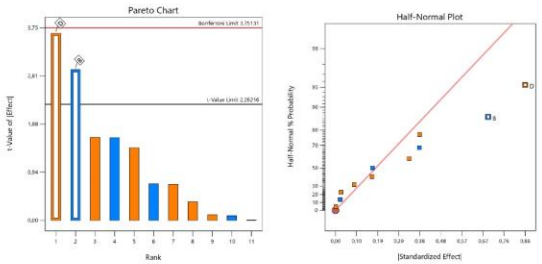


Figure 4. Half-normal (a) and Pareto (b) charts of disintegration (B: crospovidone (%), D: glyceryl distearate)

## CONCLUSIONS

This study aimed to show how to apply a screening design in order to determine the most significant factors in the development of an FDC tablet formulation. PBD was a useful tool to screen and reduce the number of the main effects of formulation parameters in the early stages

According to the PBD results, the amount of lubricant (glyceryl distearate) (1-3%) and disintegrant (crospovidone) (1-5%) were proven as the CMAs, and the blending time was exhibited as a CPPs.

Based on these results, an optimization design can be performed by surphace response methodologies and a design space can be established.

## ACKNOWLEDGMENTS

Many thanks to Nobel Pharmaceuticals, Drogsan Pharmaceuticals, Berko Pharmaceuticals, Ashland Inc. for providing raw materials used in this study.

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ISOPS 13th International Symposium on Pharmaceutical Sciences

# BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) BASED BIOWAIVER APPROACH IN TURKEY

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13th International Symposium on Pharmaceutical Sciences

## Introduction

Current legal regulations on pharmaceuticals take into account the drug properties like solubility and permeability along with biopharmaceutical characteristics such us dosage form and dissolution rate. Active ingredients for immediate release oral solid dosage forms are classified according to the Biopharmaceutics Classification System (BCS) which is accepted by health authorities like U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and World Health Organization (WHO) taking into account these pharmaceutical considerations. Under BCS, holding pharmaceuticals exempt from in vivo bioequivalence and bioavailability studies may only be possible when certain requirements are met (1).

Biowaiver applications based on BCS comprises generic drugs. In order to reduce the cost of bioequivalence studies in human, and at the same time to evaluate the performance of the active ingredient/drug, use of solubility, permeability, and in vitro dissolution studies are supported and recommended.

Pursuant to health policies of our country, manufacturing and registration of generic products are encouraged. In this study, it was aimed to evaluate the biowaiver applications in Turkey.

## Methods

The pharmaceuticals with approved biowaivers were determined, and IQVIA (a Human Data Science Company) data in terms of box numbers and prices have been evaluated and interpreted.

## Results

When the total market of drugs containing BCS Class 1 (in total 20) and BCS Class 3 (in total 8) active substances, for which a biowaiver decision has been made by the health authority of our country, was evaluated for 2010 and 2020 on a box and value basis, there was an increase of 243% on a box basis and 78% on a value basis (Figure 1).

The BCS approach to waiver of bioequivalence studies is supported by health authorities. It takes into account published FDA, EMA and WHO guidelines as well as scientific developments in this area. Until now, BCS classification and recommendation for the biowaiver according to biowaiver monographs for more than 50 drug substances have been available at FIP website at www.fip.org/bcs. Although, the monographs have no formal regulatory status, they represent the best scientific opinion currently available (Figure 2).

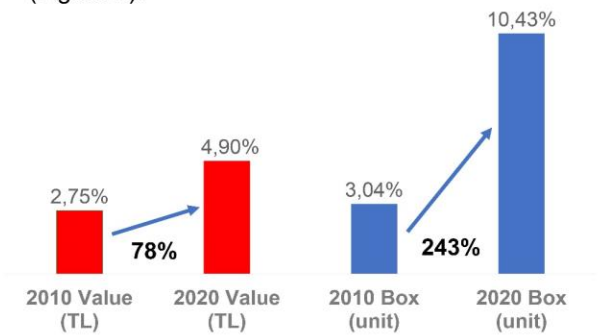


Figure 1: Total market percentage change on the basis of Value (TL) and Box (unit) in 2010 and 2020 of drugs containing BCS Class 1 and BCS Class 3 active substances (2).


Figure 2: Biowaiver suggestions in www.fip.org/bcs

## Conclusions

As a result of the biowaiver assessment, the increase in the total market percentage of BCS Class 1 and BCS Class 3 drugs on the basis of box and value contributes to the development of the generic pharmaceutical industry.

In Turkey BCS based biowaiver for BCS Class 2a generic drug products is not approved yet. As a result, based on WHO and FIP recommendations could be adopted for BCS Class 2a drugs.

In our country, health authority also takes into account the biowaiver assessments and, when the necessary criteria are met, it allows for the rapid licensing of generic drugs as a result of the biowaiver assessments. This situation positively contributes to the reduction of health expenditures, considering the budget increases that have a negative effect on the management and sustainability of public health expenditures in our country as in the world.

In addition, the increase in the number of drugs for which biowaiver decision is made, decreases the number of healthy volunteers ethically and becomes important in replacing in vivo studies with biopharmaceutical tests.

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# EVALUATIONS OF FASTED AND FED BIOAVAILABILITIES OF SELF DOUBLE EMULSIFYING DRUG DELIVERY SYSTEM OF TENOFOVIR

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## INTRODUCTION

Water-in-oil-in-water (w/o/w) double emulsions are able to enhance oral bioavailability of BCS Class 3 drugs with high solubility and low permeability. Tenofovir disoproxil fumarate (TNF) is a nucleotide reverse transcriptase inhibitör, which is used for the treatment of hepatitis B and human immunodeficiency virüs infections. It has low permeability while having high solubility, classifying as a class 3 drug in Biopharmaceutics Classification System (BCS). Permeation is the rate limiting step in the oral bioavailability of TNF. The oral bioavailability of TNF is very low and its bioavailability is influenced by fatty food intake (1). The w/o/w emulsion containing TNF was successfully developed and characterized in our previous study (2) The aim of this study was to evaluate the fasted and fed bioavailabilities of Self Double Emulsifying Drug Delivery System (SDEDDS) of TNF compared to the commercial product (Viread®) in rats.

## MATERIALS AND METHODS

Tenofovir was kindly gifted from Pharmactive from Turkey. Pharmacokinetic studies were conducted in 2 groups as fasted and fed state conditions. The suspension of Viread® tablets and SDEDDS were administered to male Sprague Dawley rats (n=5) at a dose of 61,25 mg/kg by oral gavage. Following oral administration, the blood samples (250-300 µL) were obtained from the tail vein in heparinized tubes at various time points of 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours. Blood samples were centrifuged and supernatants were separated and kept at -80°C until analysis. The LC-MS/MS method was used to determine the concentration of TNF in plasma. The non-compartmental analysis and the pharmacokinetic parameters such as $AUC_{0-24}$, $AUC_{0-\infty}$, $C_{max}$, and $t_{max}$ were calculated using WinNonlin® software.

## RESULTS

In fasted group, double emulsion formulation showed $AUC_{0-24}$ and $C_{max}$, 5673±1403 ng.min/mL and 683±130 ng/mL, respectively. In fed group, double emulsion formulation showed $AUC_{0-24}$ and $C_{max}$ 6486±3284 ng.min/mL and 847±214 ng/mL, respectively (Table 1 and Figure 1-2).

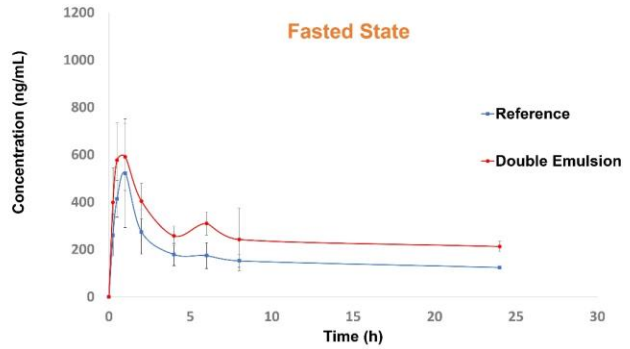


**Figure 1.** Plasma concentration-time profiles of double emulsion and reference tablet in fasted states (n=5, mean±SE).

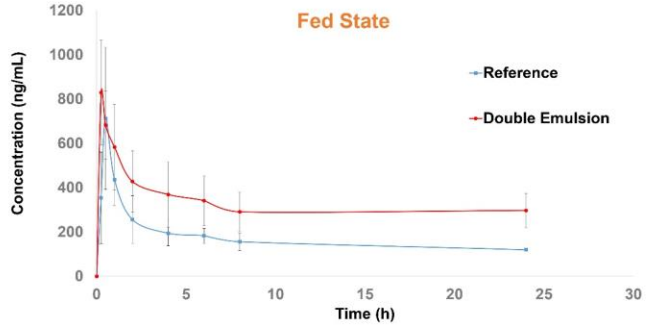


**Figure 2.** Plasma concentration-time profiles of double emulsion and reference tablet in fed states (n=5, mean±SE).

**Table 1.** Pharmacokinetic parameters of double emulsion and reference tablet in rats (mean ± SD, n=5)

| | Fasted | | Fed | |
|---|---|---|---|---|
| | Double Emulsion | Reference | Double Emulsion | Reference |
| $AUC_{0-t}$ (min.ng/mL) | 5673±1403 | 3167±1463 | 6486±3284 | 3762±956 |
| $AUC_{0-\infty}$ (min.ng/mL) | 12893±5240 | 5700±3339 | 18993±12309 | 8905±5031 |
| $C_{maks}$ (ng/mL) | 683±130 | 553±185 | 847±214 | 713±320 |
| $t_{maks}$ (min) | 0.700±0.274 | 0.875±0.250 | 0.400±0.335 | 0.50± 0.00 |

The fast and fed states $AUC_{0-24}$ values of the double emulsion formulation were found to be approximately 2-fold higher than the Viread® and $C_{max}$ values increased approximately 1,2-fold (Table 1). There was no significant difference ($p>0,05$) between the fast and fed state bioavailability of double emulsion.

## CONCLUSION

The results show that the double emulsion formulation, developed as a lipid-based drug delivery system, increases the oral bioavailability of Tenofovir and does not have fasted and fed state variations.

## ACKNOWLEDGMENTS

This study was supported by a grant from TUBITAK (Project No: 115S405). Tenofovir was kindly gifted from Pharmactive from Turkey.

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JUNE 22-25, 2021 ANKARA, TURKEY

ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

# VALIDATION OF AN HPLC METHOD FOR THE DETERMINATION OF CARFILZOMIB AND NILE RED FROM PLGA NANOPARTICLES

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## INTRODUCTION

Carfilzomib is an epoxomicin derivate that induces apoptosis and inhibits tumor growth (1). Analytical validation is an important step in formulation development. This study is aimed to develop and validate an HPLC method for the determination of Carfilzomib (CFZ) and Nile Red (NR) from poly(lactic-co-glycolic acid) (PLGA) nanoparticles for tumor inhibition.

## MATERIALS AND METHODS

The selective assay of CFZ and NR was carried out by RP-HPLC (Agilent 1200 Separations Module equipped with DAD ) according to the combined method of Lamprecht and Benoit (2) and Gopireddy et al. (3).

Table 1: HPLC conditions

| | |
|---|---|
| Mobile Phase | Water:methanol: acetonitrile(20:40:40) |
| Injection Volume | 30 µL |
| Flow rate | 0.9 mL.min$^{-1}$ |
| Detector Wavelength | DAD 200 and 560 nm |
| Column | C18 Column (300 nm x 4,6 mm x 5µm) |
| Column Temp. | 25 ℃ |

In order to determine the amount of CFZ and NR in PLGA nanoparticles, the drug-loaded nanoparticles were dissolved in 1 mL of dimethylformamide and diluted to 10 ml with acetonitrile: water (80:20). The solutions were separated and filtered through 0.45 µm membrane filters and then injected into the HPLC column. The amount of CFZ and NR in nanoparticles was calculated through the peak area values by the calibration curve.

## RESULTS

### *Specifity:*

The representative chromatograms (Fig 1) show no other peaks on the retention time of CFZ and NR. Accordingly, the proposed method could be considered selective.

### *Linearity Range:*

The peak areas of CFZ and NR were plotted against the corresponding nominal concentration to obtain a calibration graph. The method was evaluated linear in the range of 4,00-71,20 µg.mL$^{-1}$ for CFZ and 0,05-0,97 µg.mL$^{-1}$ for NR. The regression equation data are given in Tables 2 and 3.

Table 2: Linearity data of CFZ

| | |
|---|---|
| Regression equation | y = 67991,74298x + 119,0078965 |
| Correlation coeficent ($R^2$) | 0,998 |
| Lineraty range (µg.mL$^{-1}$) | 0,35 - 71,2 |
| Number of data points | 6 |
| LOD (µg.mL$^{-1}$) | 0.11 |
| LOQ (µg.mL$^{-1}$) | 0.35 |

Table 3: Linearity data of NR

| | |
|---|---|
| Regression equation | y = 234020,3068x - 0,176878752 |
| Correlation coeficent ($R^2$) | 0,999 |
| Lineraty range (µg.mL$^{-1}$) | 0,01 - 0,97 |
| Number of data points | 6 |
| LOD (µg.mL$^{-1}$) | 0.003 |
| LOQ (µg.mL$^{-1}$) | 0.01 |

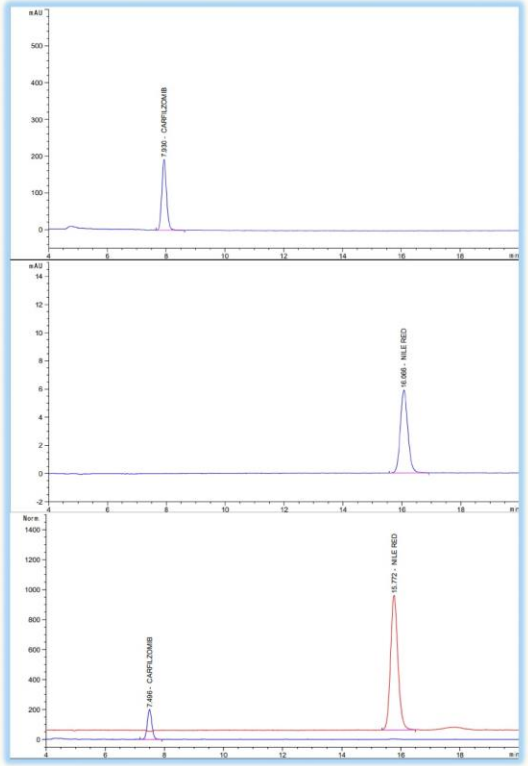


Figure 1: The chromatogram obtained in optimum condition (A: CFZ analytical standart 200 nm B: NR Analytical Standart 560 nm C: CFZ and NR loaded nanoparticle overlaid chromatogram)

### *Precision and accuracy:*

The relative standard deviation (RSD) and the bias of intra- and inter-day studies were within the acceptable range indicating that the precision and accuracy of the method were satisfactory (Intraday RSD is 1,08 and 1,96; interday RSD is 0,94 and 1,95 CFZ and NR relatively. Accuracies were within 99,51-100,77 % for CFZ and 99,35-101,08 % for NR).

### *Stability:*

During 24 h, no unexpected peak was observed which might indicate the degradation. In addition, the peak areas did not change significantly (below 2.0%) for the compound when it was compared with freshly prepared standards.

## CONCLUSIONS

The developed RP-HPLC method was successfully applied to quantitate CFZ and NR in PLGA nanoparticles.

**Melih Zeki Kaya is supported by TUBITAK 2244 grant, covering the co-operation between Hacettepe University and Novagenix Bioanalytical Drug R&D Center.**

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# DEVELOPMENT OF OROMUCOSAL FORMULATIONS - EVALUATION OF THE STRUCTURAL AND MECHANICAL PROPERTIES

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## Introduction:

Oromucosal formulations are gaining increasing interest as drug delivery products. They can be formulated e.g. as fast-disintegrating oral films (ODF) or mucoadhesive preparations intended for prolonged transmucosal absorption of an active substance. Mechanical properties of these formulations, related to their microstructure, are of a great importance according to the technological and biopharmaceutical aspects (1-3).

**Aim of the study** was to determine the physical properties of two types of formulations: orodispersible films (**ODF**) composed of hypromellose obtained during casting process and mucoadhesive oral discs (**MucD**) formulated with sodium carmellose by freeze-drying method.

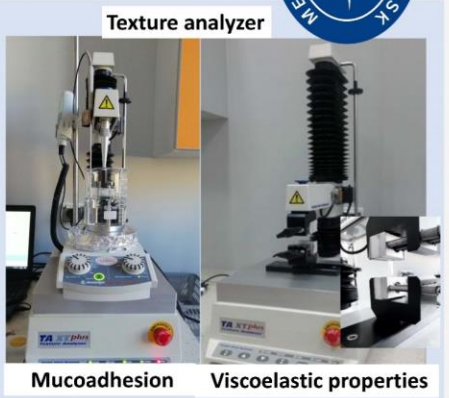
## Conclusions:

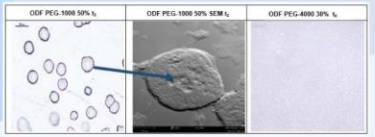
- In the development phase of ODF the measurements performed with a texture analyzer are a convenient tool for assessment how appropriate is a choice of the particular PEG type (molecular weight and concentration).
- The liquid PEGs used in concentration above 20% (PEG 400) and 40% w/w (PEG 200) significantly increased the film elasticity, but simultaneously decreased the tensile strain, also in a concentration-dependent manner. The addition of solid PEG (m.w. 4000) had no influence on these parameters.
- The hardness of freeze-dried oromucosal discs can be measured with a texture analyzer in order to adjust composition of the primary solution. The hardness was related to the polymer concentration and for the studied CMC type at least 2% solution was required to obtain satisfying hardness of MucD. Incorporation of higher doses of LID to the polymer matrix (1:1, calculated on a dry mass) caused increase in resistance of discs to compression.
- Texture analyzer can be used also to determine *in vitro* mucoadhesive properties of MucD. In our study neither the polymer concentration nor LID presence had influence on the mucoadhesion and the work of mucoadhesion measured for all tested formulations was in the narrow range of 0.27-0.43 mJ.

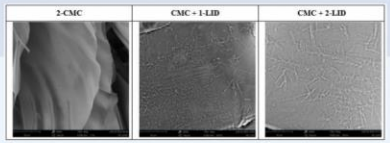
## Materials and methods:

In orded to obtain MucD the aqueous solutions of sodium carmellose (**CMC**, 1-5%) were freeze-dried in PVC blisters (15 mm diameter and 6 mm height). Formulations "placebo" and with lidocaine HCl **(LID**) were prepared. ODFs were composed of hypromellose (**HPMC**) as the matrix forming polymer and polyethylene glycol (**PEG,** m.w. 200 - 4000) used as a plasticizer (0-50% w/w of dry mass). The casting height was approx. 500 µm.

Using a texture analyzer (TA.XT plus) the viscoelastic properties of ODF were evaluated. Strips of the films of 10 mm wide were tested (initial distance of 20 mm) to the break point, with a test speed of 0.5 mm/s. Young's modulus (E) was determined from a slope of the linear region of the stress–strain curve. Mechanical examination of MucD comprised of two tests: hardness (resistance to compression to the half of disc's thickness) and mucoadhesiveness (with a gelatin disc as a substrate). Results of both tests were expressed in force ½ [N] and work [mJ] units. Moreover, microscopic observations (SEM and optic ) were performed.



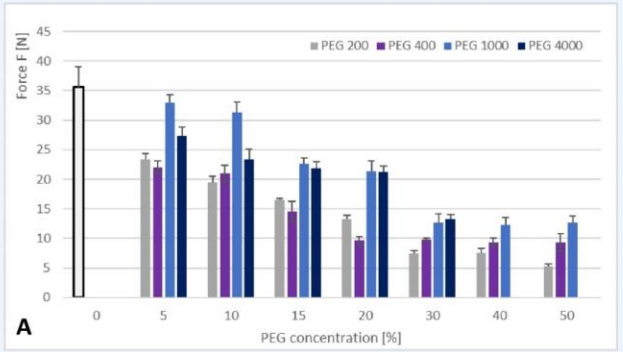



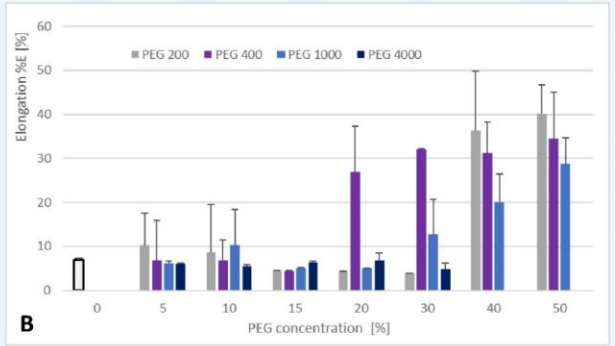



## Results:

A critical quality attribute of ODFs is the mechanical strength. Films must be also flexible enough not to crumble or crack. In order to determine the viscoelastic properties of thin films (30 – 50 µm) texture analyzer can be applied with a tensile grip attachment. The mechanical properties of ODF depended directly on PEG concentration and its molecular weight (fig. 1). Liquid PEGs 200 and 400 in dependence to their concentration increased the elasticity of the film but simultaneously diminished tear resistance in largerly. An increase of PEG concentration above 30% causes reduction of TR from 35 N to about 10 N and 8 N accordingly for PEG 400 and 200 while larger content of plasticizer (up to 50%) had no further influence on this parameter (fig. 1 A). A much higher elongation at break was observed for liquid PEGs in comparison to solid PEGs, which demonstrates that more elastic films were produced (fig. 1 B) and the highest %E value was noted for ODFs with PEG 400 content 20-30% w/w. Solid PEGs (1000 and 4000) decreased the strength (TR) of the polymer matrices in a minor degree then liquid PEGs. Due to recrystallization the recommended limit for PEG 4000 was below 30% w/w. The presence of the PEG 4000 crystals led to a decrease in the tensile strength and easily breakable structure. All examined films at low stress values showed a linear stress–strain relationship, which allowed for the determination of Young's modulus (E) (fig. 1 C). The ODFs with PEG up to 15-20% w/w have a fairly rigid structure and do not deform easily, e.g. when removed from the plates, while the increase in PEG content resulted in a certain flexibility, depending on PEG type and concentration.

In order to determine the hardness of MucD prepared by freeze-drying method a texture analyzer can be employed in the compression mode, while the measurements with a standard tablet hardness tester were unfeasible. The mechanical analysis (fig. 2A) was performed to choose the appropriate composition of the solution (polymer concentration) subjected to freeze-drying. The resistance to compression for discs obtained from 1% w/w CMC solution was too low (0.8 N, 1.3 mJ). An increase of CMC concentration to 5% caused increase in the hardness parameters to about 47 N and 74 mJ. Incorporation of a model drug substance (LID) resulted in further increase in the hardness (up to 18 N and 40 mJ). Mucoadhesive properties of pharmaceutical formulations can be analyzed with a texture analyzer, but with special A/Muc rig application. Before the study, it is necessary to select optimal test parameters and type of mucosa. In our experiments gelatin film as used as a substitute of natural mucosa. Results indicated (fig. 2B) that regardless the polymer concentration and LID presence, mucoadhesiveness of all freeze-dried discs was similar (0.27-0.43 mJ).



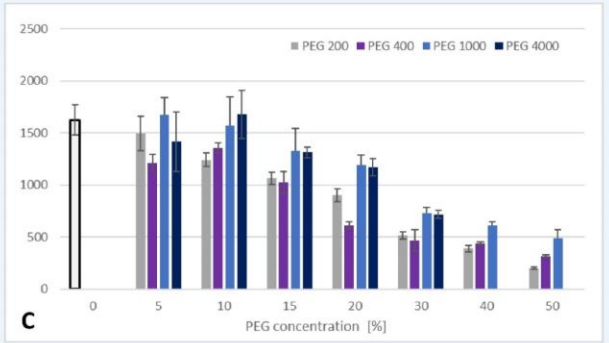
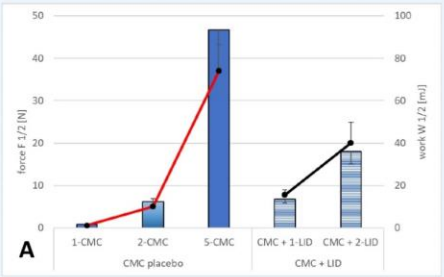




**Figure 1.** The influence of different loading (0%–50% w/w of dry mass) of liquid (Mw 200 and 400) and solid (Mw 1000 and 4000) macrogols (PEG) on mechanical behavior of ODFs: **A**- tear resistance (Force), **B** - percent elongation (%E), **C** – modul of visco-elastic deformation (Young's modulus, E).



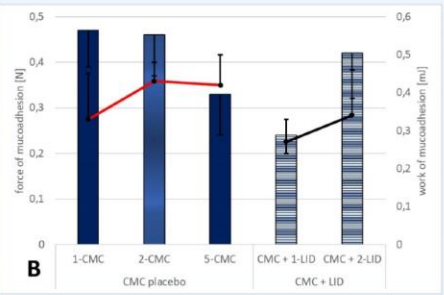




**Figure 2**. Influence of the concentration of CMC (1-5% w/w in solution) and lidocaine HCl (LID, CMC:LID ratio: 2:1 or 1:1, based on a dry mass) on the properties of MucD: A - hardness; B - mucoadhesion to a gelatin layer (mucosa substitute). Results of hardness test are expressed as force ½ [N, columns] or work ½ [mJ, lines] needed to 50% compression of discs.

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