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**ORIGINAL ARTICLE / ÖZGÜN MAKALE**

# DEVELOPMENT AND OPTIMIZATION OF INDOMETHACIN NANOSUSPENSIONS USING DESIGN OF EXPERIMENT APPROACHES

## *İNDOMETAZİN İÇEREN NANOSÜSPANSİYONLARIN GELİŞTİRİLMESİ VE FAKTÖRİYEL TASARIM YAKLAŞIMI KULLANILARAK OPTİMİZASYONU*

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## ABSTRACT

**Objective:** *In this study, it was aimed to prepare nanosuspensions that contains Indomethacin which is a BCS class II drug. To assess the cumulative impact of the chosen variables on the nanosuspension properties, a $3^4$ factorial design was applied and particle size and distributions were examined.*

**Material and Method:** *In the study, the solvent/antisolvent method was used in the preparation of the suspensions. $3^4$ factorial design. Design-Expert software was used for the evaluation of the prepared formulations in order to obtain the best formulation. PVA concentration, PVA molecular weight, solvent/antisolvent ratio, and ethanol/PEG 300 ratio were used as independent design parameters, and their effects on particle size and distribution were examined.*

**Result and Discussion:** *Nanosuspensions were successfully prepared by the solvent/antisolvent method. Particle size and polydispersity index of the nanosuspensions were found to be affected by both molecular weight and percentage of PVA in the antisolvent phase ($p < 0.05$). 0.2% (w/v) PVA; molecular weight of 31 000 for PVA and the solvent-antisolvent ratio as 3:50 were found to be the optimal parameters for the nanosuspension formulations. The particle size and polydispersity of optimum formulation were found 301.5 ± 31.1 nm and 0.159 ± 0.035, respectively.*

**Keywords:** *Factorial design, indomethacin, nanosuspension, solvent/antisolvent method*

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## ÖZ

**Amaç:** *Bu çalışmada biyofarmasötik sınıflandırma sistemine göre (BCS) 2. sınıfta bulunan indometazinin nanosüspansiyon formülasyonlarının hazırlanması ve kritik formülasyon ve işlem basamaklarının belirlenmesi amaçlanmıştır. Formülasyonların hazırlanması sırasında $3^4$ faktöriyel tasarım uygulanmış ve partikül büyüklüğü ve dağılımı incelenmiştir.*

**Gereç ve Yöntem:** *Çalışmada, nanosüspansiyonlar solvan/antisolvan yöntemi kullanılarak hazırlanmıştır. En iyi formülasyonu elde edebilmek adına Design Expert programı ile $3^4$ faktöriyel tasarım uygulanmıştır. PVA konsantrasyonu, PVA molekül ağırlığı, solvan/antisolvan oranı ve etanol/PEG 300 oranı formülasyon parametresi olarak kullanılmıştır ve bu parametrelerin değişikliğinin partikül büyüklüğü ve dağılımı üzerine olan etkisi incelenmiştir.*

**Sonuç ve Tartışma:** *Nanosüspansiyonlar solvan/antisolvan yöntemi ile başarılı bir şekilde hazırlanmıştır. Nanosüspansiyonların partikül boyutu ve polidispersite indeksinin hem molekül ağırlığından hem de antisolvan fazdaki PVA yüzdesinden etkilendiği bulunmuştur ($p < 0.05$). %0.2 PVA; PVA için 3000 molekül ağırlığı ve 3:50 çözücü-antisolvan oranı, nanosüspansiyon formülasyonları için optimal parametreler olarak bulunmuştur. Optimum formülasyonun partikül boyutu 301.5 ± 31.1 nm ve polidispersite indeksi 0.159 ± 0.035 olarak tespit edilmiştir.*


**Anahtar Kelimeler:** *Faktöriyel dizayn, indometazin, nanosüspansiyon, solvan/antisolvan metot*

## INTRODUCTION

Active substances with low water solubility have some problems with bioavailability which restrict drug development [1]. Effective gastrointestinal absorption is necessary to increase these substances' oral bioavailability. When aiming to improve the bioavailability of these kinds of compounds, efforts to improve drug solubility and dissolution rate are critical elements to take into account [2]. Therefore, it is urgent to create unique drug delivery systems that can boost the therapeutic effectiveness of these pharmacological compounds. Several unique drug delivery methods have been employed to speed up the dissolving of insoluble substances, including solid dispersion, emulsion, cyclodextrins, and nanosuspensions [3,4].

Nanosuspensions have become one of the most favourable dosage forms for the delivery of active substances that are not water soluble in recent years. They are colloidal dispersions of pure active material particles that are stabilized at the nanoscale (< 1000 nm) using the proper surfactants and/or polymers. [5,6]. In nanosuspensions, the poorly water-soluble substance is suspended in a dispersion without any matrix components. Nanosized particles and the size distribution of nanosuspensions have a considerable impact on the rate of dissolution, making them important factors in determining bioavailability. Particularly, the dissolution rate is more rapid for tiny particles with large specific surfaces [1,6]. As a result, BCS Class II and IV drugs will perform better in clinical settings due to enhanced bioavailability, quick onset of action, a decreased food effect, and other favourable pharmaceutical effects [5].

There are two ways to obtain nanosuspensions: top-down and bottom-up processes. In top-down methods, large drug particles are reduced in size by using a variety of wet-milling techniques, including media milling, microfluidization, and high-pressure homogenization. In the case of the bottom-up method, the drug is dissolved in an organic solvent and it is then precipitated by adding an antisolvent while a stabilizer is present. The solvent-antisolvent method, supercritical fluid processes, spray drying, and emulsion-solvent evaporation are a few variations of this strategy [5,6].

The solvent-antisolvent method has been generally used to form nanosuspensions recently. This method has many preparation parameters such as the selection of a suitable solvent-antisolvent ratio, optimization of sonication time and selection of polymer and surfactant [7]. These important formulation and process parameters affects the specification of nanosuspension like particle size and distribution. Optimising the formulas and proving the effects of all factors are difficult to establish a relationship between the formulation variables and their interactions, experimental design is used [9]. This strategy helps to investigate and optimize the formulation and process factors inside this design space, aiding in the creation of a predictive mathematical model. The effects can be described mathematically by a factorial design and formulations can be optimized with the fewest experiments possible. [8,9].

The safety, effectiveness, and stability of nano drug delivery systems are affected by particle size and particle size distribution. The mean particle size and the range of particle size distribution are very crucial characterization criteria as they specify the saturation solubility and dissolution rate of nanosuspensions. To create stable nanosuspension, it is crucial to maintain particle uniformity and minimize size differences to avoid differing saturation solubility and concentration gradients, which will impede Ostwald ripening. The solid-state of nanoparticles in the nanosuspension also affects how efficiently the system dissolves. As a result, particle characterisation is critical for predicting the effectiveness of nano drug delivery systems both in vitro and in vivo. Nanosuspension's in vivo pharmacokinetic and biological behaviors are highly influenced by the particle size and distribution, charge, crystallinity, and shape of the particles [4,6,10,11].

This study was aimed to evaluate the preparation process of nanosuspensions which contains Indomethacin which is BCS class II with low solubility and high permeability to determine and suggest various critical processes and formulation parameters. To assess the combined impact of the chosen factors on the properties of the nanosuspension and to optimize nanosuspension formulations, $3^4$ full factorial design was performed. Design-Expert software was used, and $3^4$ factorial design was selected to evaluate the effects of solvent ratio, the molecular weight the of stabilizer on particle size and polydispersity index (PDI) of Indomethacin nanosuspensions in this preparation method. With obtained nanosuspensions, it was purposed to enhance the solubility of Indomethacin in water and decrease the particle size and side effects.

## MATERIAL AND METHOD

### Materials

Indomethacin, Polyethylene glycol 300 (PEG 300), Polyvinyl alcohol (PVA) (MW 31 000; 30 000-70 000; 70 000-100 000), Dimethyl sulfoxide (DMSO) and Ethanol were purchased from Sigma Aldrich (Germany) All other chemicals and reagents were of analytical grade.

### Preparation of Nanosuspensions Containing Indomethacin

Nanosuspensions were prepared by using the solvent/antisolvent method which is one of the bottom-up preparation approach and nanosuspensions were obtained using the bath sonicator (Branson 5200, Spectralab Scientific, Ontario, Canada). The ratios and amounts of stabilizers, solvents and antisolvents for the formulations were decided according to similar studies [9,11-14]. Briefly, to prepare the solvent phase, a solution containing Indomethacin (20 mg) was prepared by using different ratios of PEG 300 and alcohol (v/v) mixture and the antisolvent phase was prepared using different molecular weights and concentrations of PVA in water (w/v) (MW 31 000; 30 000-70 000; 70 000-100 000) which were given in Table 1. Then antisolvent phase was added to the solvent phase (v/v) the in a bath sonicator at room temperature to obtain 50 ml formulation. Samples were taken after 5 minutes [13].

### Experimental Design

In this study, $3^4$ factorial design was used for the formation of nanosuspensions containing Indomethacin and to assess the impact of four independent variables that PEG 300/ethanol ratio (A1), PVA molecular weight (B2), PVA concentration (C3) and solvent/antisolvent ratio (D4) on particle size and PDI as dependent variables. The investigational conditions were shownin Table 1. The combinations of these parameters at the four levels were created using Design Expert 7.0 (Stat-Ease, Inc., Minneapolis, USA) software, and the statistical evaluation was applied by the quadratic model. Each variable's quantitative and qualitative impact on each response was examined. The statistical design was validated using the significant response polynomial equations produced by Design Expert software [8,15].

### Particle Size and Distribution

By using photon correlation spectroscopy, the average particle size and size distribution of the particles in nanosuspensions were determined (Z3000, Nicomp, Port Richey, FL, USA). Each sample was measured in triplicate [16].

**Table 1.** $3^4$ Factorial design and the results.

| Formulation Code | Ethanol: PEG 300 (v/v) | Surfactant (MW) | Surfactant Concentra-tion(% w/v) | Solvent: Antisolvent (v/v) | Particle Size (nm) ± SD | PDI ± SD |
|---|---|---|---|---|---|---|
| **P1** | 4:0 | PVA 31 000 | 0.2 | 3:25 | 233.70±25.60 | 1.192±16.20 |
| **P2** | | | | 3:50 | 301.50±31.10 | 0.159±0.04 |
| **P3** | | | | 3:100 | 631.30±19.60 | 0.402±10.10 |
| **P4** | | | 0.3 | 3:25 | 258.10±44.50 | 0.767±0.48 |
| **P5** | | | | 3:50 | 278.36±18.36 | 0.203±0.02 |
| **P6** | | | | 3:100 | 134.90±47.86 | 0.618±4.88 |
| **P7** | | | 0.4 | 3:25 | 616.40±110.40 | 8.468±7.80 |
| **P8** | | | | 3:50 | 63.25±21.17 | 0.717±0.24 |
| **P9** | | | | 3:100 | 9366.20±117.40 | 1.13±4.59 |
| **P10** | 0:4 | | 0.2 | 3:25 | 568.90±102.58 | 3.168±1.86 |
| **P11** | | | | 3:50 | 796.60±661.00 | 0.479±0.03 |
| **P12** | | | | 3:100 | 342.90±69.80 | 0.238±1.58 |
| **P13** | | | 0.3 | 3:25 | 219.10±82.90 | 0.887±12.30 |
| **P14** | | | | 3:50 | 322.2±20.13 | 0.39±0.17 |
| **P15** | | | | 3:100 | 327.30±88.94 | 0.326±7.84 |
| **P16** | | | 0.4 | 3:25 | 301.80±144.30 | 0.334±4.52 |
| **P17** | | | | 3:50 | 173.00±95.74 | 0.825±0.17 |
| **P18** | | | | 3:100 | 90198.30±25.20 | 6.823±54.10 |
| **P19** | 2:2 | | 0.2 | 3:25 | 712.10±58.90 | 21.031±78.80 |
| **P20** | | | | 3:50 | 253.80±15.46 | 0.162±0.04 |
| **P21** | | | | 3:100 | 1212.60±156.30 | 0.667±55.40 |
| **P22** | | | 0.3 | 3:25 | 830.75±361.54 | 39.476±52.92 |
| **P23** | | | | 3:50 | Aggregation | Aggregation |
| **P24** | | | | 3:100 | 285.40±113.02 | 0.271±4.69 |
| **P25** | | | 0.4 | 3:25 | 153.60±88.50 | 0.257±1.25 |
| **P26** | | | | 3:50 | 168.47±56.71 | 1.570±0.92 |
| **P27** | | | | 3:100 | Aggregation | Aggregation |
| **P28** | 4:0 | PVA 30 000-70 000 | 0.2 | 3:25 | 552.90±15.13 | 9.95±0.94 |
| **P29** | | | | 3:50 | 264.85±0.07 | 0.487±0.47 |
| **P30** | | | | 3:100 | 323.75±4.17 | 0.093±0.09 |
| **P31** | | | 0.3 | 3:25 | 1230.50±22.16 | 10.12±1.65 |
| **P32** | | | | 3:50 | 330.85±0.07 | 0.055±0.02 |
| **P33** | | | | 3:100 | 1757.05±15.31 | 0.589±0.09 |
| **P34** | | | 0.4 | 3:25 | 451.75±14.23 | 3.088±2.41 |
| **P35** | | | | 3:50 | 1347.10±24.84 | 10.91±0.21 |
| **P36** | | | | 3:100 | 4019.80±10.74 | 0.886±0.14 |
| **P37** | 0:4 | | 0.2 | 3:25 | 411.10±0.25 | 1.774±0.03 |
| **P38** | | | | 3:50 | Aggregation | Aggregation |
| **P39** | | | | 3:100 | 694.25±5.20 | 0.369±0.002 |

**Table 1 (*continue*).** $3^4$ Factorial design and the results.

| | | | | | | |
|---|---|---|---|---|---|---|
| **P40** | 0:4 | PVA 30 000-70 000 | 0.3 | 3:25 | 1481.20±21.82 | 75.325±12.34 |
| **P41** | | | | 3:50 | 795.80±38.01 | 35.42±9.36 |
| **P42** | | | | 3:100 | 20011.80±10.26 | 0.712±0.25 |
| **P43** | | | 0.4 | 3:25 | 390.60±116.20 | 3.423±3.30 |
| **P44** | | | | 3:50 | 597.80±121.00 | 24.325±4.90 |
| **P45** | | | | 3:100 | 479.70±15.60 | 2.484±2.80 |
| **P46** | 2:2 | | 0.2 | 3:25 | 129.40±14.07 | 0.247±0.043 |
| **P47** | | | | 3:50 | 276.93±37.70 | 1.16±0.75 |
| **P48** | | | | 3:100 | 539.20±52.90 | 1.772±56.30 |
| **P49** | | | 0.3 | 3:25 | 814.80±113.20 | 3.914±5.60 |
| **P50** | | | | 3:50 | 776.50±44.70 | 1.16±12.90 |
| **P51** | | | | 3:100 | 1287.20±47.90 | 0.828±99.10 |
| **P52** | | | 0.4 | 3:25 | 905.00±59.30 | 2.735±48.60 |
| **P53** | | | | 3:50 | 779.60±91.30 | 0.281±69.30 |
| **P54** | | | | 3:100 | 2603.30±123.50 | 0.754±63.30 |
| **P55** | 4:0 | PVA 70 000-100 000 | 0.2 | 3:25 | 893.68±48.55 | 21.76±20.41 |
| **P56** | | | | 3:50 | 2557.70±335.61 | 60.23±59.86 |
| **P57** | | | | 3:100 | 1943.10±743.13 | 8.54±11.68 |
| **P58** | | | 0.3 | 3:25 | 756.14±56.75 | 4.67±2.82 |
| **P59** | | | | 3:50 | 2230.40±75.68 | 23.79±9.58 |
| **P60** | | | | 3:100 | 1224.35±24.62 | 5.57±2.35 |
| **P61** | | | 0.4 | 3:25 | 743.70±89.00 | 2.756±0.02 |
| **P62** | | | | 3:50 | 1206.60±302.90 | 4.574±4.190 |
| **P63** | | | | 3:100 | 344.20±49.40 | 2.75±0.58 |
| **P64** | 0:4 | | 0.2 | 3:25 | 504.50±90.20 | 0.607±55.30 |
| **P65** | | | | 3:50 | Aggregation | Aggregation |
| **P66** | | | | 3:100 | 1106.20±214.10 | 0.734±26.30 |
| **P67** | | | 0.3 | 3:25 | Aggregation | Aggregation |
| **P68** | | | | 3:50 | 548.70±101.20 | 1.929±16.20 |
| **P69** | | | | 3:100 | Aggregation | Aggregation |
| **P70** | | | 0.4 | 3:25 | Aggregation | Aggregation |
| **P71** | | | | 3:50 | Aggregation | Aggregation |
| **P72** | | | | 3:100 | Aggregation | Aggregation |
| **P73** | 2:2 | | 0.2 | 3:25 | 6250.8±302.20 | 2.176±26.47 |
| **P74** | | | | 3:50 | 4135.3±250.21 | 27.479±58.20 |
| **P75** | | | | 3:100 | 10278.5±312.23 | 28.751±29.78 |
| **P76** | | | 0.3 | 3:25 | 4613.4±105.36 | 56.520±32.20 |
| **P77** | | | | 3:50 | 25372.30±191.20 | 37.308±10.65 |
| **P78** | | | | 3:100 | 5379.60±271.20 | 1.515±56.20 |
| **P79** | | | 0.4 | 3:25 | Aggregation | Aggregation |
| **P80** | | | | 3:50 | 8062.80±128.58 | 67.776±21.16 |
| **P81** | | | | 3:100 | 5647.70±2214.30 | 0.483±19.36 |

### Statistical Analysis

The results of *in vitro* data were analyzed by statistical software Design Expert 7.0 (New York, USA) using ANOVA to show statistical differences ($p < 0.05$). All results are expressed as mean ± standard deviation.

## RESULT AND DISCUSSION

### Factorial Design of Nanosuspension Formulations

Nanosuspensions that contain Indomethacin were prepared using the solvent/antisolvent method. For nanosuspension, particle size and distribution are critical parameters for nanosuspensions dissolution and this biological performance correspondingly. Many formulation and process steps can be effective on these specifications. Researchers demonstrated that suitable solvent-antisolvent ratio, sonication time and polymer and surfactant type all affect the nanoparticle formation by solvent/antisolvent method [1,17,18]. Therefore, $3^4$ factorial design was employed to calculate the impact of design factors on nanosuspension preparation and optimization. In 81 formulations, just 10 formulations precipitated. Other formulations obtained successfully. It shows that Indomethacin nanosuspensions can be prepared by this method, successfully. By excluding factors from the design model that had a p-value greater than 0.05 and calculating the model for independent variables, significant variables were found [8,19].

### Effect of the Independent Variables on Nanosuspensions Containing Indomethacin

#### *Particle Size*

The mean particle size of all the batches of Indomethacin nanosuspensions were shown in Table 1. The mean particle sizes of all the formulations were found in the range of 63.267±21.17 to 90198.3±25.2 nm based on the variables of solvent/antisolvent ratio, PEG 300/alcohol ratio, PVA concentration and molecular weight. Parameters of the response surfaces obtained from a $3^4$ factorial design for particle size were presented in Table 2.

The mathematical equation for particle size was:

$$= 808.34 - 2892.77\,A + 463.89\,B + 2272.33\,C + 2520.64\,D + 864.08\,AB - 2423.89\,AC - 2646.54\,AD - 2434.81\,BC - 2956.75\,BD + 2841.16\,CD + 25.88\,A^2 + 3260.07\,B^2 + 592.63\,C^2 + 1127.47\,D^2$$

ANOVA analysis indicated that ethanol/PEG 300 ratio had a significant effect on particle size (Table 2).

When the effect of independent variables on dependent variables was evaluated, it was found that particle size increased with the higher PEG 300 ratios used in formulations; however smaller particles were observed when PVA-MW 30 000-70 000 was used (Figure 1a).

Particle size was increased with the higher PEG 300 ratios used in formulations; however smaller particles were observed when the PVA concentration was 0.3 %(w/v). As the percentage of the PVA concentration increases, the particle size increases. Also, as PEG 300 ratio was higher, smaller particles were observed when the solvent: antisolvent ratio was decreased. It was shown that the particle size increased as the solvent:antisolvent ratio increased (Figure 1b-1c). PEG 300 and similar agents decrease the interfacial tension, so agglomeration become harder for the particles. Because of this reason, it was expected as the PEG 300 ratio increases, particle size decreases; but in our study we showed bigger particle size with higher PEG 300 ratio conversely. The formation of micelles above the optimal critical micel concentration (CMC) may be the cause of this increase in particle size, which becomes drug particles vulnerable [9,20]. The stabilization of nanosuspensions depends mostly on the stabilizer concentration. The concentration of surfactant should be utilized under CMC. Using insufficient stabilizer will lead to prevent the drug molecules' surface from being completely covered, which is necessary to create steric repulsion between the suspended nanoparticles. Micelles, on the other hand, will form at concentrations higher than the CMC. The produced nanosuspensions' thermal instability is

significantly influenced by the micelle formation. A concentration above CMC may actually result in less surfactant adsorption, further destabilizing the nanosuspensions and contributing to the growth of the particle size [21].

Particle size was increased with the higher PVA concentrations used in formulations; however smaller particles were observed when PVA MW 30 000-70 000 was used (Figure 1d) as the solvent:antisolvent ratio decreases, the particle size decreases with higher PVA concentration (Figure 1e). When higher PVA concentrations were used, because of the high viscosity, the input energy that we used for the formation of nanosuspension may not be enough to obtain smaller particles [5]. Also the particle size can grow as a result of the existence of a thick coating on the particle surface and diffusion between the solvent and the antisolvent is prevented during precipitation if the polymer concentration is continuously raised [18,22].

As the selected solvent:antisolvent ratio decreased, the particle size decreased and when low molecular weight of PVA was selected, the decrease in the solvent:antisolvent ratio had a greater effect on the decrease in the particle size (Figure-1f). In a similar study, researchers found similar results for solvent: antisolvent ratio. This effect might be brought on by the stabilizer's ability to bind to the drug's surface. Because stabilizer adsorbed polymer molecules which leave the surface of the drug nanocrystals and travel toward the bulk of the liquid at high volumes of antisolvent (water), it may not be able to provide enough steric stabilization or assembly for the higher number of core [11].

**Table 2.** Parameters of the response surfaces obtained from a $3^4$ factorial design for particle size.

| **ANOVA for Response Surface Quadratic Model** | | | | | | |
|---|---|---|---|---|---|---|
| **Analysis of variance table [Partial sum of squares - Type III]** | | | | | | |
| | **Sum of** | | **Mean** | **F** | **p-value** | |
| **Source** | **Squares** | **df** | **Square** | **Value** | **Prob > F** | |
| **Model** | 2.62E+09 | 14 | 1.87E+08 | 1.857926 | 0.0480 | significant |
| **A-Ethanol:PEG 300** | 4.52E+08 | 1 | 4.52E+08 | 4.494372 | 0.0378 | significant |
| **B-PVA (MW)** | 11620343 | 1 | 11620343 | 0.115576 | 0.7350 | |
| **C-PVA Concentration** | 2.79E+08 | 1 | 2.79E+08 | 2.773222 | 0.1006 | |
| **D-Solvent:Antisolvent** | 3.43E+08 | 1 | 3.43E+08 | 3.412428 | 0.0692 | |
| **AB** | 26878759 | 1 | 26878759 | 0.267335 | 0.6069 | |
| **AC** | 2.12E+08 | 1 | 2.12E+08 | 2.103669 | 0.1517 | |
| **AD** | 2.52E+08 | 1 | 2.52E+08 | 2.507882 | 0.1181 | |
| **BC** | 2.13E+08 | 1 | 2.13E+08 | 2.12266 | 0.1499 | |
| **BD** | 3.15E+08 | 1 | 3.15E+08 | 3.130251 | 0.0815 | |
| **CD** | 2.91E+08 | 1 | 2.91E+08 | 2.890281 | 0.0938 | |
| **$A^2$** | 12054.06 | 1 | 12054.06 | 0.00012 | 0.9913 | |
| **$B^2$** | 1.91E+08 | 1 | 1.91E+08 | 1.902719 | 0.1724 | |
| **$C^2$** | 6321790 | 1 | 6321790 | 0.062876 | 0.8028 | |
| **$D^2$** | 22881306 | 1 | 22881306 | 0.227577 | 0.6349 | |
| **Residual** | 6.64E+09 | 66 | 1.01E+08 | | | |
| **Cor Total** | 9.25E+09 | 80 | | | | |

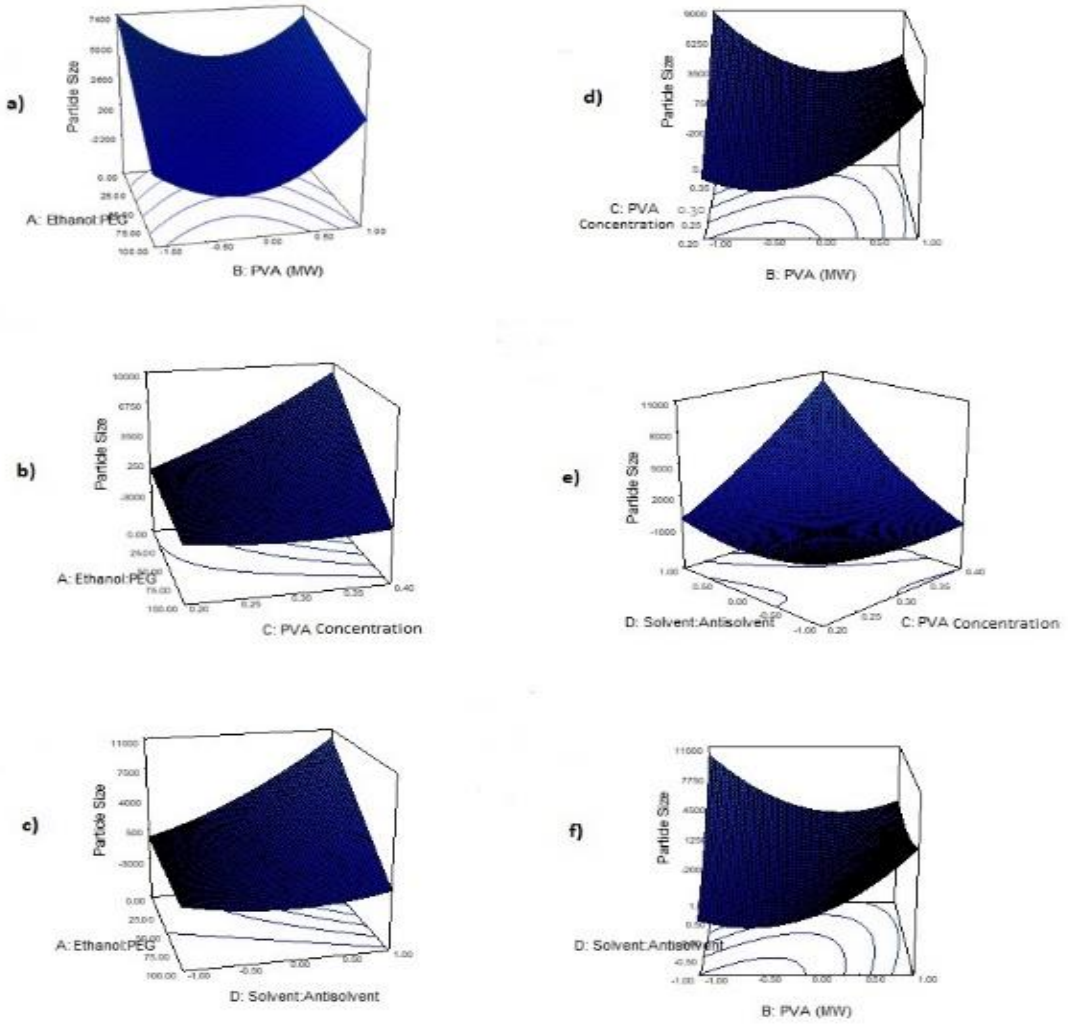


**Figure 1.** 3D surface response plots showing effect of factors A, B, C, and D on response on particle size.

### *Polydispersity Index (PDI)*

The crucial characteristic known as PDI provides data on the physical stability of nanosuspensions. The PDI must be extremely low to create the perfect nanosuspension formation. When the PDI value is near to zero, the sample is said to as monodisperse. When the PDI value is less than 0.2, a limited size distribution is considered [17]. In Table 3, the PDI of each formulation is displayed. However, a polydisperse distribution is thought to exist when the PDI value is greater than 0.2. The PDI of all the batches of Indomethacin nanosuspensions were shown in Table 1. The PDI of all the formulations were found in the range of 0.159±0.035 to 75.325± 12.34 nm based on the variables. Parameters of the response surfaces obtained from a $3^4$ factorial design for particle size were presented in Table 3.

The mathematical equation for particle size was:

$$= 3.76 + 0.82\ A + 3.47\ B - 0.78\ C - 1.20\ D + 1.59\ AB - 2.64\ AC - 0.93\ AD - 2.14\ BC - 0.47\ BD + 0.62\ CD + 1.36\ A^2 + 2.14\ B^2 + 1.11\ C^2 - 2.72\ D^2$$

ANOVA analysis indicated that molecular weight of PVA had a significant quadratic effect on PDI (Table 3).

The PDI was increased with the raise of the ethanol/PEG 300 ratio. Moreover smaller PDI was observed when PVA MW 31 000 was used and also when PVA concentration was 0.2% (w/v). This increase in PDI, which makes drug particles vulnerable, may be caused by the formation of micelles above the optimal CMC.

In addition that, it was observed the PDI decreased as the solvent:antisolvent ratio increased conversely high ethanol ratio. Shariare et al. found similar results in their study with furosemide nanosuspensions in 2019. It can be a result of enhanced agglomeration of drug nanoparticles at higher solvent ratios. This may be due to different mixing power between solvent and antisolvent because of the different densities of solvents used for the formulations due to different ethanol concentrations. It could result in an increased nucleation rate for crystal growth. Different mixing situation due to different density could lead to grow in crystals. [12].

The PDI was increased with the increase in the PVA concentration. As PVA concentration increases up to a certain value, PDI increases, then decreases. Similar results were found by some researchers in their studies. Researchers found higher PDI and particle size by using higher stabilizer concentration for their nanosuspension formulations [9,17]. It could be a result of formation of micelles at concentrations greater than the CMC. The nanosuspensions may become even more unstable at concentrations above CMC and may experience less surfactant adsorption, which would lead to an increase in particle size and PDI [11,21]. Another result that we foundwas smaller PDI was observed when PVA (MW 31 000) was used with higher PVA concentration.

Different molecular weights of PVA significantly affect PDI ($p<0.05$). As PVA molecular weight increases, PDI increases. In formulations prepared with large molecular weight PVA, PDI increases as the PVA concentration decreases. When using PVA (MW 70 000-100 000), the PDI increases as the percentage of PVA used decreases. When using PVA (MW 31 000), the PDI decreases as the percentage of PVA used decreases. The molecular weight and concentration of PVA have a significant impact on particle size and PDI, which may be related to the high viscosity and interfacial tension of the aqueous phase. PVA grades with significant levels of hydrolysis have reportedly been shown to be poorly soluble in water. PVA's solubility, viscosity, and surface tension are all influenced by the material's molecular weight, concentration, hydrolysis percentage, and temperature. As a result, PVA's high molecular weight and concentration formed particles with a wider size distribution [23]. It can be explained that larger molecular weight of PVAwhich formed more dense solutions are better at protecting the drug moiety than smaller molecular weight of PVA formed less viscose solution. In addition, compared to larger MW, stabilizer molecules migrate at a slower rate at the drug-polymer interface at less viscose polymer solution. The solution's viscosity increases as the number of collisions, which further slows the rate of mass transfer from the solution to the solid-liquid interface through diffusion [9,17].

**Table 3.** Parameters of the response surfaces obtained from a $3^4$ factorial design for PDI.

| **ANOVA for Response Surface Quadratic Model** | | | | | | |
|---|---|---|---|---|---|---|
| **Analysis of variance table [Partial sum of squares - Type III]** | | | | | | |
| | **Sum of** | | **Mean** | **F** | **p-value** | |
| **Source** | **Squares** | **df** | **Square** | **Value** | **Prob > F** | |
| **Model** | 1627.153 | 14 | 116.2252 | 2.46472 | 0.0073 | significant |
| **A-Ethanol:PEG 300** | 36.09344 | 1 | 36.09344 | 0.765412 | 0.3848 | |
| **B-PVA (MW)** | 649.2131 | 1 | 649.2131 | 13.76748 | 0.0004 | significant |
| **C-PVA Concentration (% w/v)** | 32.71646 | 1 | 32.71646 | 0.693799 | 0.4079 | |
| **D-Solvent:Antisolvent** | 77.3286 | 1 | 77.3286 | 1.639862 | 0.2048 | |
| **AB** | 91.48444 | 1 | 91.48444 | 1.940057 | 0.1683 | |
| **AC** | 250.5203 | 1 | 250.5203 | 5.312637 | 0.0243 | significant |
| **AD** | 31.29843 | 1 | 31.29843 | 0.663727 | 0.4182 | |

**Table 3 (*continue*).** Parameters of the response surfaces obtained from a $3^4$ factorial design for PDI.

| | | | | | | |
|---|---|---|---|---|---|---|
| **BC** | 165.3089 | 1 | 165.3089 | 3.505608 | 0.0656 | |
| **BD** | 7.821344 | 1 | 7.821344 | 0.165863 | 0.6851 | |
| **CD** | 13.82724 | 1 | 13.82724 | 0.293226 | 0.5900 | |
| **$A^2$** | 33.11804 | 1 | 33.11804 | 0.702315 | 0.4050 | |
| **$B^2$** | 82.80201 | 1 | 82.80201 | 1.755934 | 0.1897 | |
| **$C^2$** | 22.1593 | 1 | 22.1593 | 0.469919 | 0.4954 | |
| **$D^2$** | 133.4615 | 1 | 133.4615 | 2.830239 | 0.0972 | |
| **Residual** | 3112.266 | 66 | 47.15555 | | | |
| **Cor Total** | 4739.419 | 80 | | | | |

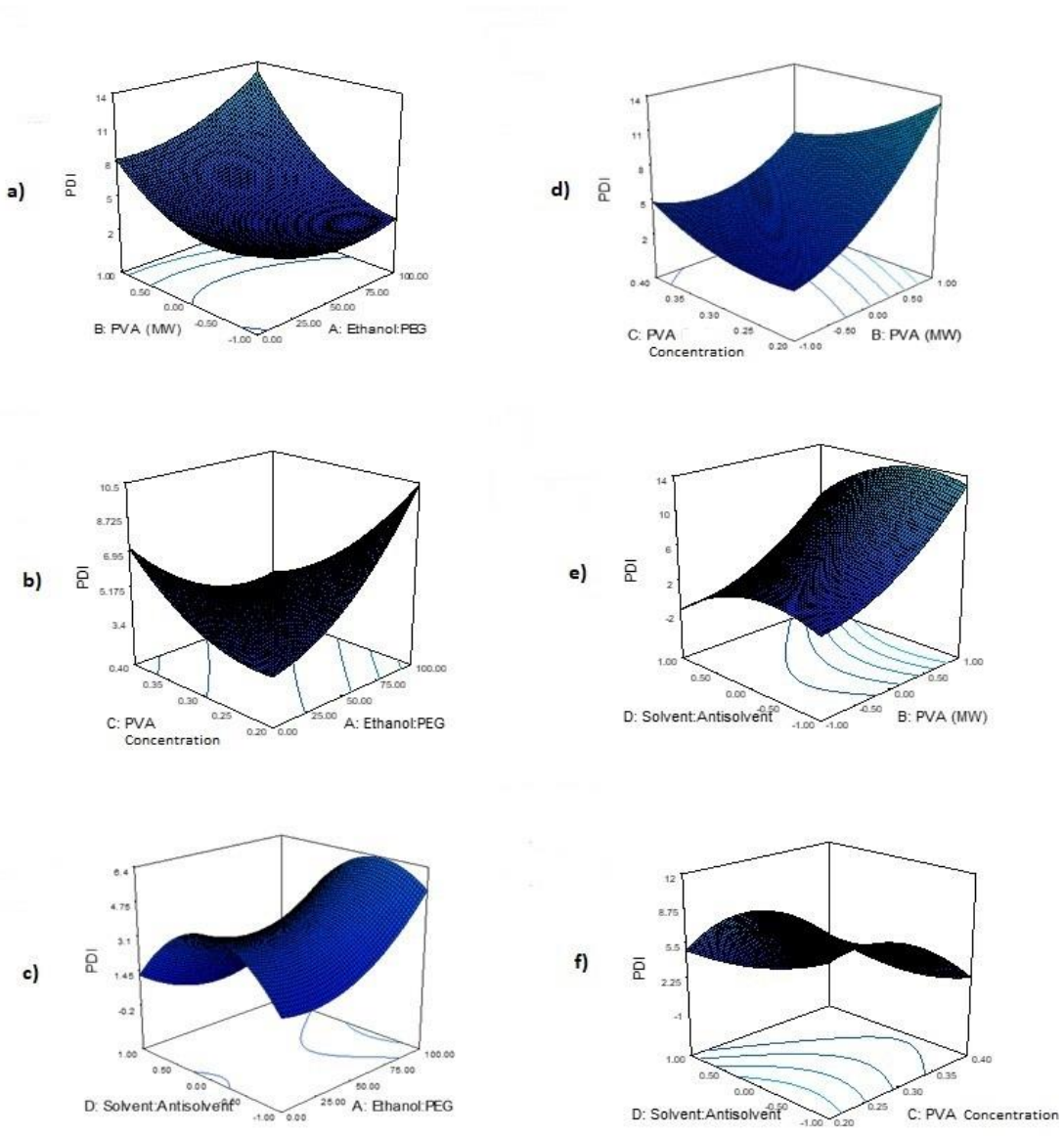


**Figure 2.** 3D surface response plots showing effect of factors A, B, C, and D on response on PDI

### Determination of Optimal Formulation

ANOVA was used to investigate the ideal Indomethacin nanosuspension formulation, and the F test was used to evaluate each parameter. Accordingly, the Design Expert analysis of nanosuspensions was optimized based on the criteria of desired particle size and low PDI values. Table 4 shows the results according to experimental design.

**Table 4.** The optimum parameters according to experimental design results.

| Parameters | Value |
|---|---|
| PVA concentration | 0.2 % (w/v) |
| PVA molecular weight | 31 000 |
| Solvent/antisolvent ratio | 3:50 |

In this study, our aim was to develop nanosuspensions including Indomethacin to overcome water-solubility problem of drug, so to increase the oral bioavalaibility. To obtain the optimum formulation Design-Expert program was used and $3^4$ factorial design was planned for the preparation of formulations. Ethanol/PEG 300 ratio, PVA molecular weight, PVA concentration and solvent/antisolvent ratio were used as independent parameters for design and the effect of these parameters on particle size and distribution were evaluated.

Both molecular weight and concentration of PVA in the antisolvent phase were found to affect the particle size and polydispersity index of the nanosuspensions ($p<0.05$). The optimum parameters were found to be 0.2% (w/v) PVA (MW. 31 000) with a solvent-antisolvent ratio of 3:50 (particle size: 301.5 ± 31.1 nm, polydispersity index: 0.159 ± 0.035). As a result, nanosuspension formulations were successfully prepared using the solvent/antisolvent method. Therefore, it can be concluded that both the type and percent of stabilizer is important to obtain stable nanosuspensions.

## AUTHOR CONTRIBUTIONS

Concept: G.R.T, C.K.Ö.; Design: G.R.T, C.K.Ö.; Control: G.R.T, C.K.Ö., Y.Ö.; Sources: G.R.T, C.K.Ö., Y.Ö; Materials: G.R.T, C.K.Ö., Y.Ö; Data Collection and/or Processing: G.R.T.; Analysis and/or Interpretation: G.R.T.; Literature Review: G.R.T, C.K.Ö., Y.Ö; Manuscript Writing: G.R.T.; Critical Review: G.R.T, C.K.Ö., Y.Ö.; Other: -

## CONFLICT OF INTEREST

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

## ETHICS COMMITTEE APPROVAL

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

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