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Screening of stabilizing agents to optimize flurbiprofen nanosuspensions using experimental design

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ABSTRACT

Although flurbiprofen (FB), as one of non-steroidal anti-inflammatory drugs, has various pharmacological applications, it shows low dermal bioavailability due to its low water solubility. To overcome this solubility problem, FB nanosuspensions were developed and the effects of stabilizers were investigated with regard to critical quality attributes. While PVP K30 and HPMC 3 cps were used as non-ionic polymeric stabilizer, Tween 80 and Plantacare 2000 (PL) were used as non-ionic surfactants. The influence of these types of stabilizers and their different ratios were tested. The selected formulations according to results of experimental design were also characterized by SEM, FTIR, XRPD, DSC, and the stability studies were performed. According to results of characterization studies, PL was selected as the appropriate stabilizer. The determined nanosuspension stabilized with PL had the nanosized, spherical, and homogenous dispersed particles. There is no polymorphic change on the crystalline state of FB while producing nanosuspension stabilized with PL. It also retained its stability compared with PVP stabilized nanosuspensions for one month. It was concluded that the design of experimental approach is a useful tool to determine the effect of stabilizer on quality attributes of nanosuspensions and to select the optimum type and ratio of stabilizer for obtaining more stable nanosuspensions.

1. Introduction

Flurbiprofen (FB) is one of the non-steroidal anti-inflammatory drugs and has various pharmacological applications, such as gout and osteoarthritis [1]. It is a lipophilic molecule and poorly soluble in water. The drug substances that are poorly water soluble have delivery problems such as low dermal bioavailability [2]. To overcome this solubility problem, many approaches have been applied, such as using cosolvent [3,4] and preparing in the forms of solid dispersions, liposomes, emulsions, and nanoparticles [5–8]. Because possible toxicity is related with the use of large amounts of organic solvents and excipients, their advantages are limited.

To increase solubility of lipophilic drug substance, another promising approach is the preparation of nanosuspension systems. Nanosuspensions can be defined as nano-sized (10–1000 nm) drug particles (crystalline or amorphous) covered by minimum amount of suitable surfactants, polymers or combination of them [9]. By means of decreasing particle size and increasing surface area, nanosuspensions enhance the physicochemical properties, such as dissolution, saturation solubility, biological performance, and physical stability of formulations [10]. Moreover, nanosuspensions have such advantages as high amount of drug content, low excipient side effects, ease of scale up and low cost of production [11]. Thus, nanosuspensions bring benefits in regard to applicability to various dosing routes, such as oral, parenteral, ocular, pulmonary, and dermal delivery [12].

Nanosuspension can be produced by using top down and bottom up techniques [13]. High pressure homogenization (microfluidization) is one of the top down techniques to produce nanosuspension, which can be easily adapted to industrial production [14]. The critical process parameters of this method are homogenization cycle, homogenization pressure, chamber type and size, and temperature. During the application of high level of pressure and homogenization cycles at this technique, it generates cavitation forces, which may lead to a reduction in particle sizes and an increase in Gibbs energy. Because of the increased Gibbs free energy of the system, surface area and interfaces are formed together [15]. This formation of high-energy surfaces may increase saturation solubility, dissolution velocity and improves bioavailability [16]. Also, this high surface energy may cause particle size growth which is known as Ostwald ripening effect [17]. For this reason, nanosuspensions, which have tendency for crystal growth and agglomeration, are thermodynamically unstable colloidal systems [18]. In order to protect the nanosuspension system against stability problems, the use of a stabilizer is needed. Stabilizers prevent the aggregation and agglomeration of nanosuspensions by surrounding the nanosized drug

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particles. Therefore, beside the process parameters, the formulation parameters such as stabilizer type, amount of stabilizer, drug substance to stabilizer ratio and content of drug substance are very effective parameters on the characteristics of final formulation [19,20].

Selection of suitable type and amount of stabilizer provides electrostatic and steric stabilization which may be obtained by means of ionic surfactants and non-ionic polymeric stabilizers/surfactants, respectively [21]. Electrostatic stabilization is obtained by adsorbing the ionic molecules onto the surface of the particles. In steric stabilization, non-ionic stabilizers were commonly utilized. Non-ionic stabilizers have multiple binding sides and tail. Hydrophobic sides of stabilizers cover the surface of particles and minimize the interactions to a level that the van der Waals attractive forces are less than the repulsive forces. On the other hand, hydrophilic tail of a non-ionic stabilizer extends towards the bulk media and increases the water solubility of hydrophobic drug particles [22].

The selection of stabilizer is specific to each drug substance and the type and amount of stabilizer affect the physical stability of the nanosuspension systems [23]. The specific interaction between the drug substance and the stabilizer plays a critical role in the formation of nanosuspensions. Hardness, hydrophobicity, and chemical structure of the drugs and the surface tension, viscosity, molecular weight and functional groups of the stabilizers are very important factors to understand the effect of the interaction between the stabilizers and drug substances [24,25].

Besides the type of the stabilizer, the amount of the stabilizer is another important parameter to optimize nanosuspension system. The use of an inadequate amount of stabilizer will not completely cover the surfaces of the drug particles, which is required to provide the steric and ionic barrier between the particles in the nanosuspensions [26]. The amount of the stabilizer can vary over a wide range [27]. Thus, the appropriate amount of stabilizer should be optimized. In addition to conventional experimentation, Design of Expert (DoE) was used in this study to investigate the effect of the Critical Formulation Attributes (CFA), such as stabilizer type/amount and Critical Process Parameters (CPP) and their interactions on the critical quality attributes (CQA) of the formulations [28]. Particle size, particle size distribution and zeta potential were identified as CQAs which define the solid state stability and quality of nanosuspensions and DoE approach was utilized to predict the required quality attributes of an optimum formulation.

In this study, FB nanosuspensions were prepared with different types and amounts of stabilizers. To determine the suitable type and amount of stabilizer, characterization studies were performed. Two levels full factorial design was employed for obtaining a prediction of an optimized FB nanosuspension. The effects of percentage of FB, FB: stabilizer ratio, type of stabilizer, and homogenization cycle on the quality attributes of FB nanosuspensions were investigated. HPMC and PVP K30 as polymeric stabilizers and Tween 80 and Plantacare 2000 (PL) as surfactants were selected to investigate the effect of stabilizer. Characterization studies of FB nanosuspensions were carried out by measuring particle size (PS), polydispersity index (PDI) and zeta potential (ZP) values. Scanning electron microscopy (SEM), differential scattering calorimetry (DSC), X-ray powder diffraction (XRPD) and Fourier transform infrared (FTIR) measurements were also performed for the selected formulations which were prepared with suitable type and amount of stabilizer.

Here we placed emphasis on the role of most critical properties of stabilizer and drug substance on the nature of stabilization required to produce successful nanosuspensions. Because, unfortunately, there are no systematic principles to select the suitable type of stabilizer and optimize the amount of the stabilizer. Experimental knowledge about the effect of stabilizer on the stability and efficacy of nanosuspensions are also limited. Moreover, as far as we know, there has been no previous detailed publication related to the comparison of non-ionic polymeric stabilizers and surfactants on the basis of critical quality attributes of nanosuspensions by using quality by design. Therefore, this study aims to show the advantages of DoE approach by which the optimum stabilizer type and amount can be selected and to time can be saved by decreasing the number of experiments [29]. Also, it gives insight into the mechanism of stabilization as a function of critical stabilizer and drug substance properties.

2. Materials and methods

2.1. Materials

Flurbiprofen was kindly supplied by Sanovel Pharma (Istanbul, Turkey). Plantacare® 2000 UP (decyl glucoside), Tween® 80 (Polysorbate 80), HPMC 3 cps, PVP K30 were provided from BASF (Germany).

2.2. Preparation of FB nanosuspensions

FB nanosuspensions were prepared using high pressure homogenization (HPH) method with Microfluidics LV1 (Microfluidizer® Processors, USA) [30]. To start the HPH method, stabilizer was first dissolved in distilled water under magnetic stirrer. Then coarse powder of FB was sufficiently suspended in this stabilizer solution. This macro suspension was stirred for completely wetting of FB particles. After the preparation of stirred macro suspension, high speed homogenizer ((Ultraturrax (Heidolph® –Silent Crusher M)) was applied at 10.000 rpm for 10 min. Finally, coarse suspension of FB was processed via the high pressure homogenizer at 30.000 psi pressure, which was determined in our previous study [31] and under controlled temperature conditions to produce nanosuspensions (Fig. 1).

2.3. Screening of surface stabilizers

The type and amount of stabilizers were determined by measuring the PS, PDI, and ZP of FB nanosuspensions. In this study, HPMC and PVP as the polymeric stabilizers and Tween 80 and Plantacare 2000 as the surfactants were utilized. 2^4 -full factorial design were performed for both polymeric stabilizers and surfactants with their different amount, separately. According to interaction and surface graphs, the optimum stabilizer type and amount for both polymeric stabilizer and surfactants were determined. Optimum formulation, which is prepared with appropriate stabilizer type and amount, was evaluated by means of SEM, FTIR, DSC and XRPD measurement and stability studies.

2.4. Optimization of FB nanosuspensions

Experimental design studies were applied to evaluate the effect of process and formulation parameters on FB nanosuspensions and its stability. DoE approach was also used to reduce the number of experiments and to obtain optimum nanosuspension formulation according to statistical calculations. For this purpose, PS, PDI and ZP values were determined as dependent variables. The formulation and process parameters were selected as independent variables. While the stabilizer type, stabilizer ratio and amount of FB were determined as critical formulation parameters, the homogenization cycle was selected as critical process parameter for HPH method.

2^4 (2 levels, 4 factors) full factorial design using Design Expert ® Software Version 9 was performed to optimize the FB nanosuspension. The four independent variables and their two levels evaluated in this study were percentage of FB (1%–4%) FB: stabilizer ratio (1:4, 4:1), type of stabilizer (HPMC-PVP K30 or Tween 80-Plantacare 2000), homogenization cycle (10 cycle-30 cycle). Each experiment was randomly performed twice. Moreover, three center points were added to enhance the predictability of the model, a total of 38 experiments. Effects of the process and formulation parameters were evaluated according to main effects and interactions. Then the optimum stabilizer type was selected for further studies.

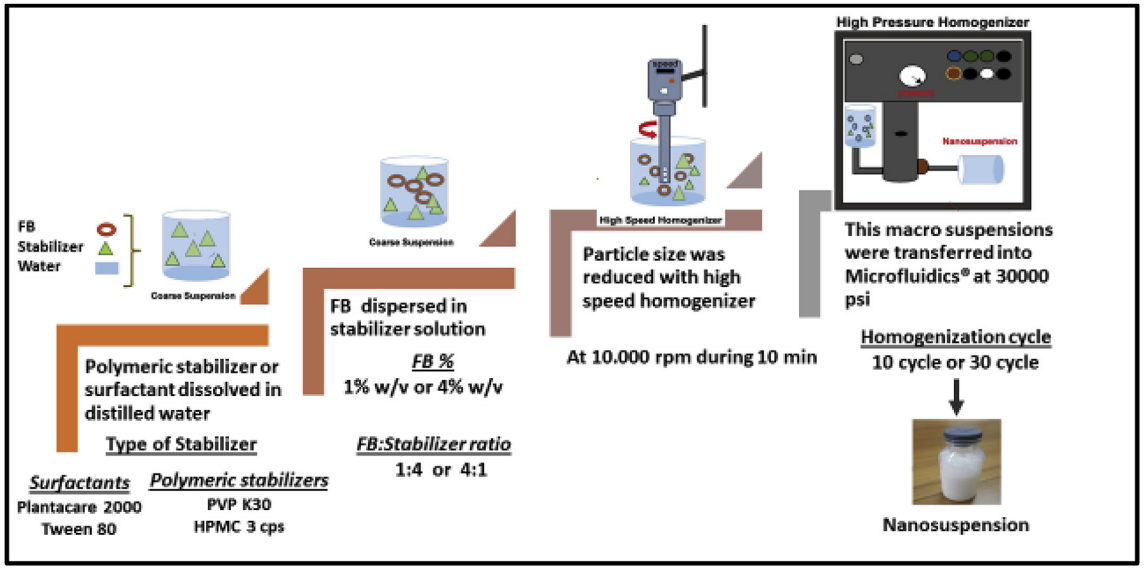

Fig. 1. Flow chart of production method of nanosuspensions.

2.5. Lyophilization of nanosuspensions

To characterize the optimum nanosuspension with DSC, XRPD and SEM; lyophilization process was applied. Nanosuspensions were put inside of the vials and they were frozen at −80 °C for 3 h. Then freeze-drying process was performed at optimized 50 °C, 0.021 mbar for 48 h using Christ Alpha 1–2 LD® Freeze Dryer. The PS, PDI and ZP values of nanosuspensions were also found suitable after the lyophilization process.

2.6. Characterization of nanosuspensions

2.6.1. Determination of particle size (PS), polydispersity index (PDI) and zeta potential (ZP) values

Coarse FB powder and the FB macro suspensions were characterized by the laser diffraction method using Symphatec HELOS (Symphatec GmbH, Clausthal-Zellerfeld, Germany). To determine the PS, PDI and ZP values of nanosuspensions, the photon correlation spectroscopic method was used (Malvern Instruments, UK).

2.6.2. Surface morphology of nanosuspensions

The morphological properties of the coarse FB, physical mixtures and nanosuspensions were analyzed with scanning electron microscopy (SEM) (Quanta 400F Field Emission), respectively. The samples were directly fixed on a metal stub using double sided adhesive tape. The samples were coated with gold-palladium in vacuum before scanning and observed by SEM at a voltage of 5–20 kV.

2.6.3. Fourier transform infrared (FTIR)

In FTIR study, the infrared spectra of the FB in the isotropic mixture of excipients were obtained. FTIR analysis of FB coarse powder, the physical mixtures (4:1 as drug: stabilizer (PVP or PL)) and nanosuspensions were carried out to evaluate the presence of drug-stabilizer interaction. The samples were dried under vacuum to remove the residual moisture. All samples were scanned for absorbance over the range from 2900 to 500 cm^{-1} at the resolution of 4 cm^{-1}.

2.6.4. X-ray powder diffraction (XRPD)

X-ray powder diffractometry (XRPD) patterns of the FB powder, physical mixture and nanosuspensions were collected with X-ray diffractometer (Rigaku Ultima-IV Powder Diffractometer). The patterns were recorded by sample scan from 5° to 120° 2θ at a scan rate of 1°/min and a voltage of 40 kV.

2.6.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry measurement was performed using Shimadzu DSC 60 with heating rate of 20 °C/min in the temperature ranging from 25 °C to 300 °C. Powders (FB, physical mixture and the nanosuspensions) were approximately weighted (2 mg) in an aluminum pans, crimped and then sealed. DSC thermograms were acquired under flow rate of nitrogen (100 mL/min) using Indium as temperature calibrator. Three different measurements were performed for each sample and average of maximum peak and extrapolated onset temperature were reported.

2.7. Physical stability

Short term stability studies were carried out to evaluate the physical stability of FB nanosuspension formulations which are prepared with PVP and PL as a non-ionic polymeric stabilizer and a non-ionic surfactant, respectively. Formulation was stored at room temperature (25 °C) during one month and followed by measuring PS, PDI and ZP values of nanosuspensions at 1st, 7th, 14th, 30th day.

2.8. Statistical analysis

Data analysis was carried out with ANOVA tests/the SPSS software, Version 16. Results were expressed as the mean ± standard deviation. Statistically significant differences between PS, PDI and ZP values were evaluated with the 0.05 level of probability ($p < 0.05$).

3. Results and discussion

3.1. Preparation of nanosuspensions

Nanosuspensions of FB were successfully prepared with HPH technique using two non-ionic polymeric stabilizers (HPMC and PVP K30) and two non-ionic surfactants (Tween 80 and Plantacare 2000). Our major aim was to prepare FB nanosuspensions which have PS below 1000 nm and it was achieved. The reduction of PS of nanosuspensions are very effective to increase the solubility, permeability and thus bioavailability of formulations by means of increasing surface area [32]. Also, there are many studies compatible with this theory [33–35]. Thus, in this study, firstly it was aimed to lower PS below 1000 nm in

Table 1
Particle size (PS), polydispersity index (PDI) and zeta potential (ZP) values of NS formulations prepared with polymeric stabilizers.

FB% (w/v)	Type of Stabilizer	FB:Stabilizer	Cycle Number	PS	PDI	ZP
4	PVP	4	10	837.7 ± 14.6	0.154 ± 0.229	−23.4 ± 1.8
2.5	PVP	2.125	20	1098.3 ± 97.3	0.518 ± 0.059	−20.9 ± 0.4
2.5	HPMC	2.125	20	1087.6 ± 37.2	0.882 ± 0.057	−8.0 ± 1.6
4	HPMC	4	10	1151.7 ± 36.8	0.665 ± 0.021	−11.4 ± 0.3
1	PVP	0.25	10	3532.3 ± 188.7	1.000 ± 0.000	−13.6 ± 0.5
1	HPMC	0.25	30	2255.3 ± 166.4	0.476 ± 0.183	−7.1 ± 0.3
4	PVP	0.25	10	1414.7 ± 45.6	0.227 ± 0.071	−9.1 ± 0.3
4	HPMC	4	30	986.1 ± 68.0	0.358 ± 0.059	−11.6 ± 0.6
2.5	HPMC	2.125	20	805.9 ± 30.0	0.125 ± 0.033	−6.5 ± 0.3
1	PVP	4	30	1807.3 ± 134.5	0.783 ± 0.102	−9.6 ± 0.6
4	PVP	0.25	30	2960.3 ± 675.9	0.573 ± 0.156	−9.3 ± 0.1
4	HPMC	0.25	30	957.0 ± 28.9	0.474 ± 0.019	−2.9 ± 0.2
1	PVP	0.25	30	1480.7 ± 9.7	0.536 ± 0.103	−13.1 ± 0.4
4	HPMC	0.25	10	682.9 ± 16.0	0.218 ± 0.048	−1.1 ± 0.5
2.5	PVP	2.125	20	1225.3 ± 50.3	0.606 ± 0.014	−17.1 ± 1.3
1	HPMC	4	30	864.9 ± 48.7	0.328 ± 0.055	−18.9 ± 1.2
1	HPMC	0.25	30	1931.3 ± 205.2	0.292 ± 0.178	−5.7 ± 0.3
2.5	PVP	2.125	20	1442.0 ± 92.7	0.875 ± 0.103	−17.4 ± 1.4
4	PVP	4	10	879.1 ± 13.8	0.340 ± 0.090	−22.7 ± 0.4
4	HPMC	4	10	1099.3 ± 65.1	0.490 ± 0.110	−11.3 ± 0.3
1	HPMC	4	10	1043.0 ± 114.0	0.479 ± 0.180	−10.0 ± 0.1
1	HPMC	0.25	10	1335.7 ± 48.3	0.381 ± 0.353	−7.6 ± 0.5
1	PVP	4	10	1196.0 ± 18.7	0.677 ± 0.030	−10.3 ± 0.3
4	HPMC	4	30	874.1 ± 29.5	0.292 ± 0.070	−12.7 ± 0.3
4	HPMC	0.25	30	1050.9 ± 66.9	0.525 ± 0.037	−2.3 ± 0.2
4	HPMC	0.25	10	662.5 ± 31.8	0.884 ± 0.200	−1.5 ± 0.3
4	PVP	0.25	10	1514.0 ± 55.0	0.367 ± 0.396	−9.2 ± 0.3
4	PVP	0.25	30	2381.0 ± 47.3	0.730 ± 0.020	−9.4 ± 0.1
2.5	HPMC	2.125	20	714.6 ± 35.9	0.441 ± 0.036	−7.1 ± 0.1
1	PVP	0.25	10	3531.3 ± 293.7	1.000 ± 0.000	−13.7 ± 0.6
1	PVP	0.25	30	1535.0 ± 46.9	0.353 ± 0.133	−13.5 ± 0.4
4	PVP	4	30	1049.6 ± 58.1	0.478 ± 0.236	−23.1 ± 0.3
1	PVP	4	10	1158.0 ± 30.3	0.471 ± 0.300	−10.6 ± 0.3
1	HPMC	4	10	1050.0 ± 10.8	0.528 ± 0.033	−10.5 ± 0.7
1	HPMC	4	30	858.0 ± 34.4	0.349 ± 0.110	−18.5 ± 1.1
4	PVP	4	30	1067.0 ± 12.8	0.497 ± 0.069	−23.1 ± 0.4
1	HPMC	0.25	10	1243.3 ± 23.6	0.246 ± 0.219	−7.6 ± 0.6
1	PVP	4	30	1236.0 ± 129.6	0.732 ± 0.152	−11.0 ± 0.7

other words to obtain nanosuspensions, secondly to reach the lowest PS of FB nanosuspensions by using different types of polymeric stabilizers and surfactants. For this purpose, all prepared nanosuspensions were characterized on the basis of PS, PDI and ZP values, as shown in Table 1 and Table 2. At the beginning of the study, the PS of coarse suspension was 29.43 ± 3.25 μm (d_{50}) and it was lowered to 13.05 ± 2.37 μm (d_{50}) after the high speed homogenizer process with 10 000 rpm speed for 10 min. After that HPH method was applied and PS was lowered to approximately 805 nm and 593 nm with polymeric stabilizers and surfactants, respectively.

The PDI is the measure of size distribution of the nanoparticles and it is important to identify if a drug substance can achieve homogenous and nano range size distribution following the production method. PDI values of the nanosuspensions are critical to the stability of the product. The results of PDI measurement of formulations prepared with polymeric stabilizers and surfactants were also measured and the values varied from 0.15 to 1.00 for polymeric stabilizer and from 0.17 to 1.00 for surfactants (Tables 1 and 2). Nanosuspensions which have PDI values less than 0.5 were considered acceptable CQA of nanosuspensions because this indicates narrow and monodisperse size distribution [36].

The ZP also indicates the physical stability of nanosuspension and is defined as the potential difference between the stationary layer of fluid attached to the particle and the dispersion medium [37]. In other words, it is a measurement of the electric charge at the surface and the thickness of the diffusion layer which plays a critical role for predicting the short and long term stability of colloidal systems. The ZP values of FB nanosuspensions prepared with surfactants were found to be higher than polymeric stabilizers in this study. ZP values for surfactants ranged from −18.5 to −38.6 mV and these values are suitable to obtain stable nanosuspension systems (Tables 1 and 2). It is recommended that ZP between −20 and + 20 mV are considered desirable [10] whereas between −30 and + 30 mV are considered strongly stable [38].

3.2. Screening of stabilizer agents

A stabilizer is essential in the nanosuspension formulation and can suppress agglomeration, and maintain the stability of the whole nanosuspension system [39]. The selected stabilizer should have high affinity to surface of specific drug particles. FB is a non-polar drug substance; therefore, the ionic stabilizers are not suitable and enough to provide high affinity to surface of FB particles. However, the non-ionic stabilizers show high affinity to hydrophobic groups of FB by means of their hydrophobic domains at the surface [40]. Therefore, in this research, two types of non-ionic stabilizers (polymeric stabilizers and surfactants) were investigated at different ratios to gain insight about the effects of non-ionic stabilizers on the quality attributes of FB-NS formulations. While HPMC and PVP K30 were selected as macromolecular polymeric stabilizers, Tween 80 and Plantacare 2000 were selected as small molecular weight surfactants and their effect on the CQAs of nanosuspensions were evaluated separately.

3.2.1. Polymeric stabilizers

Hydroxypropyl methylcellulose (HPMC) is one of the most common types of cellulose in pharmaceutical nanosuspension applications and is known as semisynthetic non-ionic polymer [9]. It has a molecular formula of $C_{56}H_{108}O_{30}$. Polyvinylpyrrolidone (PVP) is used in the pharmaceutical industry as a synthetic polymer vehicle for suspending drugs and has the molecular formula of $(C_6H_9NO)_n$. In this study, the PS of

Table 2
Particle size (PS), polydispersity index (PDI) and zeta potential (ZP) values of NS formulations prepared with surfactants.

Run	FB% (w/v)	Stabilizer	FB:stabilizer	Cycle Number	PS	PDI	ZP
1	1	Plantacare 2000	0.25	30	1576.7 ± 102.3	0.526 ± 0.051	−30.5 ± 0.9
2	1	Tween 80	0.25	10	3228.0 ± 292.1	0.896 ± 0.180	−18.5 ± 1.7
3	4	Tween 80	0.25	10	4536.0 ± 114.6	0.084 ± 0.054	−22.9 ± 0.3
4	1	Tween 80	4	30	2229.0 ± 172.4	0.725 ± 0.045	−21.9 ± 0.7
5	4	Tween 80	4	30	2630.0 ± 206.2	0.136 ± 0.072	−20.9 ± 0.1
6	4	Tween 80	0.25	10	4080.0 ± 613.0	0.367 ± 0.198	−22.9 ± 0.3
7	4	Plantacare 2000	0.25	10	3665.0 ± 508.2	0.907 ± 0.162	−35.7 ± 1.0
8	1	Plantacare 2000	4	30	750.8 ± 34.5	0.787 ± 0.105	−28.7 ± 1.6
9	1	Plantacare 2000	4	30	749.3 ± 41.2	0.692 ± 0.111	−29.4 ± 0.7
10	1	Plantacare 2000	4	10	1442.0 ± 29.7	0.173 ± 0.129	−36.3 ± 0.9
11	1	Tween 80	0.25	30	1502.0 ± 473.4	0.927 ± 0.113	−18.8 ± 2.1
12	1	Plantacare 2000	0.25	30	1585.0 ± 79.0	0.635 ± 0.127	−30.2 ± 1.2
13	4	Tween 80	4	10	3418.0 ± 322.1	0.456 ± 0.071	−21.9 ± 0.4
14	2.5	Tween 80	2.125	20	3146.0 ± 119.9	1.000 ± 0.000	−21.7 ± 0.9
15	4	Plantacare 2000	0.25	10	3895.0 ± 834.8	1.000 ± 0.000	−34.6 ± 1.1
16	4	Plantacare 2000	4	30	614.4 ± 22.9	0.376 ± 0.023	−30.1 ± 1.4
17	1	Tween 80	4	30	2424.0 ± 89.1	0.641 ± 0.229	−21.3 ± 0.4
18	4	Tween 80	0.25	30	1424.0 ± 32.5	0.407 ± 0.242	−22.4 ± 0.6
19	1	Plantacare 2000	0.25	10	2503.0 ± 523.3	1.000 ± 0.000	−39.0 ± 0.8
20	1	Tween 80	4	10	7269.0 ± 2607.0	1.000 ± 0.000	−16.0 ± 2.1
21	1	Plantacare 2000	4	10	1378.0 ± 26.0	0.507 ± 0.348	−34.5 ± 0.9
22	1	Plantacare 2000	0.25	10	2321.0 ± 33.5	0.715 ± 0.032	−39.0 ± 0.8
23	4	Plantacare 2000	4	10	1429.0 ± 18.9	0.385 ± 0.086	−30.7 ± 0.3
24	4	Tween 80	0.25	30	150.4 ± 62.4	0.626 ± 0.024	−23.1 ± 0.2
25	2.5	Plantacare 2000	2.125	20	1049.6 ± 61.5	0.65 ± 0.044	−31.6 ± 0.6
26	1	Tween 80	0.25	10	3108.0 ± 166.3	0.827 ± 0.159	−19.8 ± 0.6
27	2.5	Tween 80	2.125	20	2686.0 ± 279.8	1.000 ± 0.000	−21.7 ± 0.9
28	4	Tween 80	4	10	3525.0 ± 401.5	0.547 ± 0.226	−21.1 ± 0.3
29	4	Tween 80	4	30	2998.0 ± 42.5	0.662 ± 0.552	−21.5 ± 0.6
30	4	Plantacare 2000	4	10	1460.0 ± 28.5	0.662 ± 0.171	−30.7 ± 0.6
31	2.5	Tween 80	2.125	20	3459.0 ± 423.5	1.000 ± 0.000	−18.9 ± 2.2
32	4	Plantacare 2000	4	30	593.1 ± 5.3	0.375 ± 0.023	−30.1 ± 0.6
33	4	Plantacare 2000	0.25	30	2449.0 ± 328.5	0.888 ± 0.097	−37.8 ± 7.2
34	4	Plantacare 2000	0.25	30	2635.0 ± 231.0	0.907 ± 0.094	−38.6 ± 0.6
35	1	Tween 80	4	10	6845.0 ± 1135.3	1.000 ± 0.000	−18.5 ± 1.1
36	1	Tween 80	0.25	30	1932.0 ± 126.5	0.668 ± 0.124	−19.9 ± 0.4
37	2.5	Plantacare 2000	2.125	20	1193.0 ± 85.3	0.729 ± 0.037	−31.6 ± 0.6
38	2.5	Plantacare 2000	2.125	20	1069.0 ± 51.2	0.510 ± 0.017	−35.1 ± 0.4

nanosuspension was lowered to 650–850 nm using HPMC and PVP. The ZP values of nanosuspensions stabilized with PVP were found higher than HPMC. While the PS of nanosuspensions stabilized with PVP were higher than HPMC, ZP values were also higher than HPMC. These results can be explained with the fact that total surface area available for adsorption in PVP stabilized nanosuspensions was larger compared to prepared with HPMC. Therefore, the higher amount of stabilizer was needed for fully covering of the surface of drug particles in the case of larger particles as compared to smaller particles. Hence, the higher amount of stabilizer at the per unit of surface may lead to negatively larger ZP values for nanosuspensions with larger particles [28].

As a result of increasing homogenization cycle, PS and PDI values generally decreased (Table 1). These results were found to be correlated with a previous research [15], which improved the nitrofurazone nanosuspensions using different stabilizers. The PS and PDI of PVP K30 stabilized nanosuspensions were found smaller than HPMC stabilized nanosuspensions. Choi et al., investigated the role of polymeric stabilizers for drug nanocrystal dispersions. In the study, PVP stabilized nanosuspensions were found better than HPC stabilized nanosuspensions and the researchers suggested that this situation can be related with the chemical structure of PVP [41].

3.2.2. Surfactants

Plantacare® 2000 UP is a decyl glucoside nonionic surfactant, with good dermatological compatibility, that is suitable for use as a base surfactant in pharmaceutical industry [42]. Tween 80 is a polyethylene sorbitol ester, non-ionic surfactant and an emulsifier derived from polyethoxylated sorbitan and oleic acid. Pyo et al., produced the miconazole nitrate nanocrystals for dermal application with six different skin-friendly surfactants, such as Poloxamer 188, Poloxamer 407, Plantacare 810UP, Plantacare 2000 UP, Tween 80, and Miranol Ultra C32. As a result, smaller PS were obtained by using Tween 80, Plantacare 2000 and Poloxamer 407 [43]. Kobierski et al., produced resveratrol nanosuspensions with four types of surfactants (Tween 80, Poloxamer 188, Plantacare 2000 and Inutec SP1) for dermal application. The researchers determined that nanocrystal sizes were smaller for Poloxamer, Plantacare stabilized nanosuspensions compared with the Tween, Inutec stabilized nanosuspensions [44]. Also Mishra et al., prepared the hesperetin nanosuspensions with the same four types of surfactants and it was determined that Tween was slightly less efficient to preserve the nanocrystal size directly after production [45]. In our research, the results were correlated with these previous studies. The PS and PDI values of nanosuspensions stabilized with PL were found to be lower than those stabilized with Tween 80. Moreover, ZP values of all nanosuspensions stabilized with PL were higher than Tween 80 (Table 2).

3.3. Optimization of FB nanosuspensions

3.3.1. Experimental design for polymeric stabilizers

2^4 (2 levels, 4 factors) full factorial design was performed to determine the effect of formulations and process parameters on PS, PDI and ZP values of nanosuspensions stabilized with two types of polymeric stabilizers. The statistical results of experimental design are shown in Table 3. On the basis of PS and ZP values, the main effects of percentage of FB, type of stabilizer and FB:stabilizer ratio were found significant. While two-way interaction between type of stabilizer and FB:stabilizer ratio was found significant on the PS; interaction between

Table 3
Analysis of variance for the FB nanosuspensions prepared with polymeric stabilizers on the basis of particle size (PS), polydispersity index (PDI) and zeta potential (ZP) values.

PS			PDI		ZP	
Source	F Value	p-value	F Value	p-value	F Value	p-value
Model	4.39	0.0013	1.41	0.2344	5.10	0.0003
A- Percentage of FB	5.10	0.0328	1.20	0.2844	1.11	0.3021
B- Type of Stabilizer	11.02	0.0028	2.42	0.1323	9.34	0.0051
C– FB: Stabilizer Ratio	15.50	0.0006	0.28	0.5994	25.24	< 0.0001
D- Homogenization Cycle	0.11	0.7402	0.080	0.7799	0.089	0.7680
AB	7.965E-003	0.9296	5.88	0.0229	11.03	0.0027
AC	1.89	0.1810	0.40	0.5322	3.42	0.0760
AD	3.28	0.0820	1.49	0.2341	0.40	0.5344
BC	5.81	0.0236	0.27	0.6071	5.286E-007	0.9994
BD	0.51	0.4809	1.00	0.3267	2.72	0.1110
CD	0.011	0.9159	0.092	0.7641	2.63	0.1167

percentage of FB and type of stabilizer was found significant on the ZP values. For PDI, all main effects were insignificant while two-way interaction between percentage of FB and type of stabilizer was significant.

The effects of all significant interactions on PS, PDI and ZP values are shown in Fig. 2. As can be seen in Fig. 2, type of stabilizer, FB: stabilizer ratio and percentage of FB affect the quality attributes of nanosuspensions. On the basis of type of stabilizer, stabilization and PS reduction ability of PVP and HPMC depend on the molecular weights, including surface energies and specific interactions. Specific interactions between drug and polymeric stabilizers are related with the presence of functional groups. These parameters affect the stabilizers' efficiency on the PS, PDI and ZP values of nanosuspensions. These effects of type of stabilizer and ratio on the PS are presented in Fig. 2A. At the constant FB % and homogenization cycle, the PS of nanosuspensions stabilized with HPMC was found similar to PVP for 4:1 ratio. However, HPMC was found better than PVP for 1:4 ratio (Fig. 2A).

These results can be related with a high degree of substitution as methoxy or hydroxypropyl groups of HPMC [9]. FB have hydroxyl functional groups (–OH), as HPMC does. Thus, HPMC can form hydrogen bonds with the FB and inhibit the crystal growth. PVP cannot form any strong hydrogen bonds because it does not have any hydroxyl groups. At the constant FB:stabilizer ratio and homogenization cycle, due to increasing FB content, the PDI decreased (Fig. 2B) and ZP values increased (Fig. 2C). At the constant FB %, PDI and ZP values of nanosuspensions stabilized with PVP were found better than HPMC (Fig. 2B and C). These results can be related with the hydrophobic surface of FB. The hydrophobic surface without polar functional groups may be ideal for physical adsorption and steric stabilization by PVP [18]. PVP can adsorb to the hydrophobic surface of FB and increase the stability of nanosuspensions. According to all these results, PVP was found better than HPMC in obtaining stable nanosuspension systems.

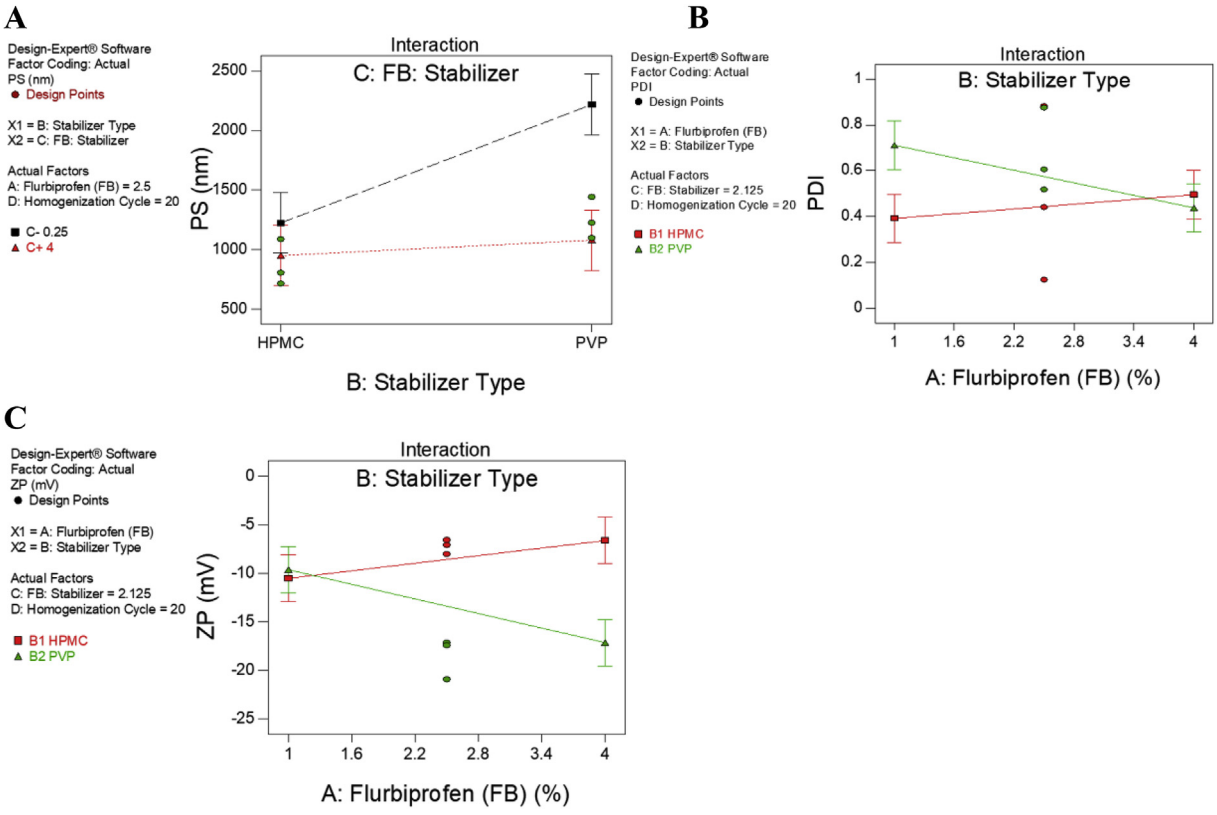

Fig. 2. Interaction graph showing the effect of process and formulation parameters on FB-NS prepared polymeric stabilizer **A)** Particle size (PS) **B)** Polydispersity index (PDI) and **C)** Zeta potential (ZP) values.

Table 4
Analysis of variance for the FB nanosuspensions prepared with surfactants on the basis of particle size (PS), polydispersity index (PDI) and zeta potential (ZP) values.

PS			PDI		ZP	
Source	F Value	p-value	F Value	p-value	F Value	p-value
Model	17.10	< 0.0001	2.57	0.0250	43.71	< 0.0001
A- Percentage of FB	1.306E-005	0.9971	5.63	0.0250	4.12	0.0524
B- Type of Stabilizer	53.62	< 0.0001	0.16	0.6890	389.27	< 0.0001
C– FB: Stabilizer Ratio	0.35	0.5600	3.33	0.0791	12.85	0.0013
D- Homogenization Cycle	51.10	< 0.0001	0.20	0.6611	2.28	0.1424
AB	5.71	0.0242	9.77	0.0042	3.63	0.0676
AC	12.02	0.0018	0.55	0.4642	3.83	0.0606
AD	1.27	0.2689	0.15	0.6974	4.96	0.0345
BC	35.97	< 0.0001	5.83	0.0228	7.05	0.0131
BD	10.87	0.0027	0.032	0.8588	8.86	0.0061
CD	0.080	0.7795	0.010	0.9209	0.22	0.6394
Curvature						
Residual						
Lack of Fit	40.37	< 0.0001	5.91	0.0008	10.99	< 0.0001
Pure Error						
Cor Total	17.10	< 0.0001	2.57	0.0250	43.71	< 0.0001

3.3.2. *Experimental design for surfactants*

2^4 (2 levels, 4 factors) full factorial design was performed to determine the effect of formulation and process parameters on PS, PDI and ZP values of nanosuspensions stabilized with two types of surfactants. The statistical results of experimental design are shown in Table 4. The main effects of homogenization cycle and type of stabilizer were found significant on the basis of PS.

The effects of significant two way interactions on the PS are shown in Fig. 3. PS of all nanosuspensions stabilized with PL were found lower than PS of formulations stabilized with Tween 80 (Fig. 3A). As shown in Fig. 3B and C, at the constant homogenization cycle and FB:stabilizer ratio, due to increasing FB % and FB:stabilizer ratio, PS decreased for nanosuspensions stabilized with PL. As a result of increasing homogenization cycle, PS decreased at the constant FB% and FB:stabilizer ratio.

The effects of significant two way interactions on the PDI are shown in Fig. 4. At the constant homogenization cycle and FB:stabilizer ratio, PL leads to obtaining similar PDI values for 1% FB and 4% FB content. As a result of increasing FB:PL ratio, PDI of formulations decreased when FB% and homogenization cycle were constant.

The effects of significant two way interactions on the ZP are shown in Fig. 5. At the constant homogenization cycle and FB%, ZP values of

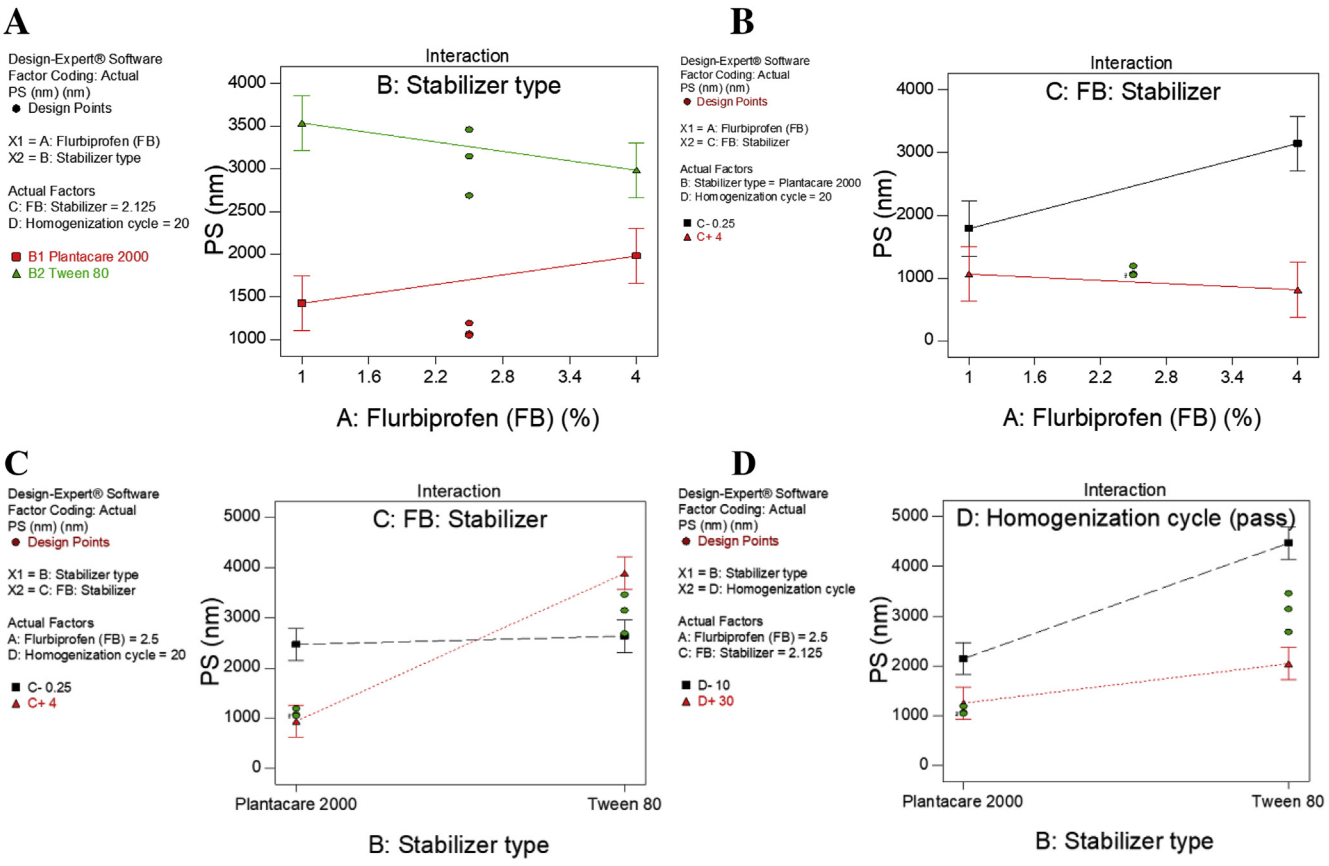

Fig. 3. Interaction graphs showing the effect of process and formulation parameters on particle size (PS) values of FB-NS prepared surfactants **A)** AB (FB% & Stabilizer type) **B)** AC (FB% & FB:Stabilizer ratio) **C)** BC (Stabilizer type & FB:Stabilizer ratio) and **D)** BD (Stabilizer type & Homogenization cycle).

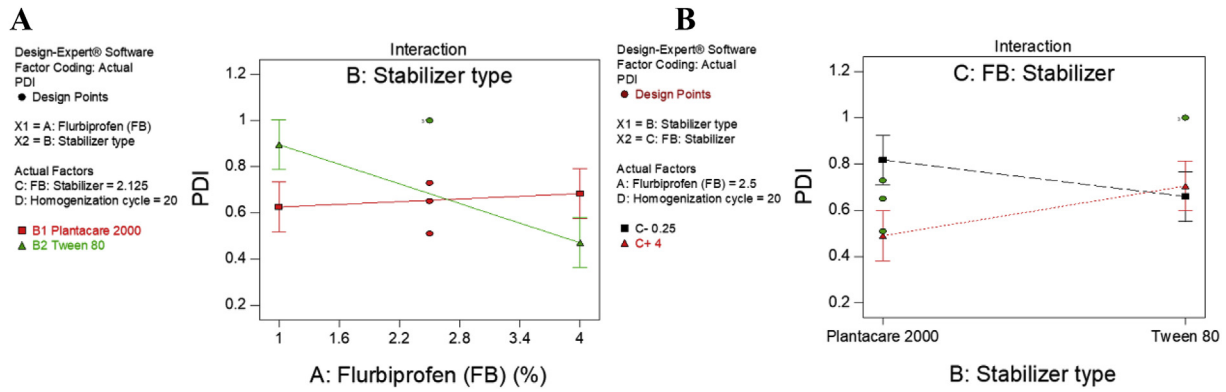

Fig. 4. Interaction graphs showing the effect of process and formulation parameters on polydispersity index (PDI) values of FB-NS prepared surfactants **A)** AB (FB% & Stabilizer type) **B)** BC (Stabilizer type & FB:Stabilizer ratio).

nanosuspensions stabilized with PL were found higher than Tween 80 regardless of FB:stabilizer ratio (Fig. 5A) and homogenization cycle (Fig. 5B). As a result of increasing homogenization cycle from 10 cycle to 30 cycle, ZP values decreased in terms of friction at the constant FB:PL ratio (Fig. 5C). All these results showed that the PL was found better than Tween 80 as a stabilizer. The PS and PDI values of nanosuspensions decreased and ZP values increased by means of using PL. This situation can be related with the molecular structure and weight of surfactants. The molecular weight of PL is lower than Tween 80. Molecular weight is related to viscosity. In the current study, the smallest particles were obtained by PL due to low dispersion viscosity. High viscosity caused aggregation and crystal growth because of increasing friction. Moreover, the molecular structure of PL contains effective hydroxyl groups and hydrophobic surface to interact with FB. Tween 80 had also hydroxyl groups, but the number of them were lower than PL.

The results of experimental design for polymeric stabilizers and surfactants, suitable type of stabilizer and stabilizer ratio were determined. The stabilizer ratio should be optimized for all types of stabilizers because the coverage of the particle surface is not sufficient at low stabilizer ratio and causes lower sterically unstable nanosuspensions. At the higher ratio of stabilizer, flocculation and aggregation can occur. Thus, the optimal ratio of drug:stabilizer should be investigated for all types of stabilizers.

In this study, the optimum parameters were determined as 4% FB, 4:1 FB:stabilizer ratio, 10 homogenization cycle for PVP and 1% FB 4:1 FB:stabilizer ratio 30 homogenization cycle for HPMC. According to PS,

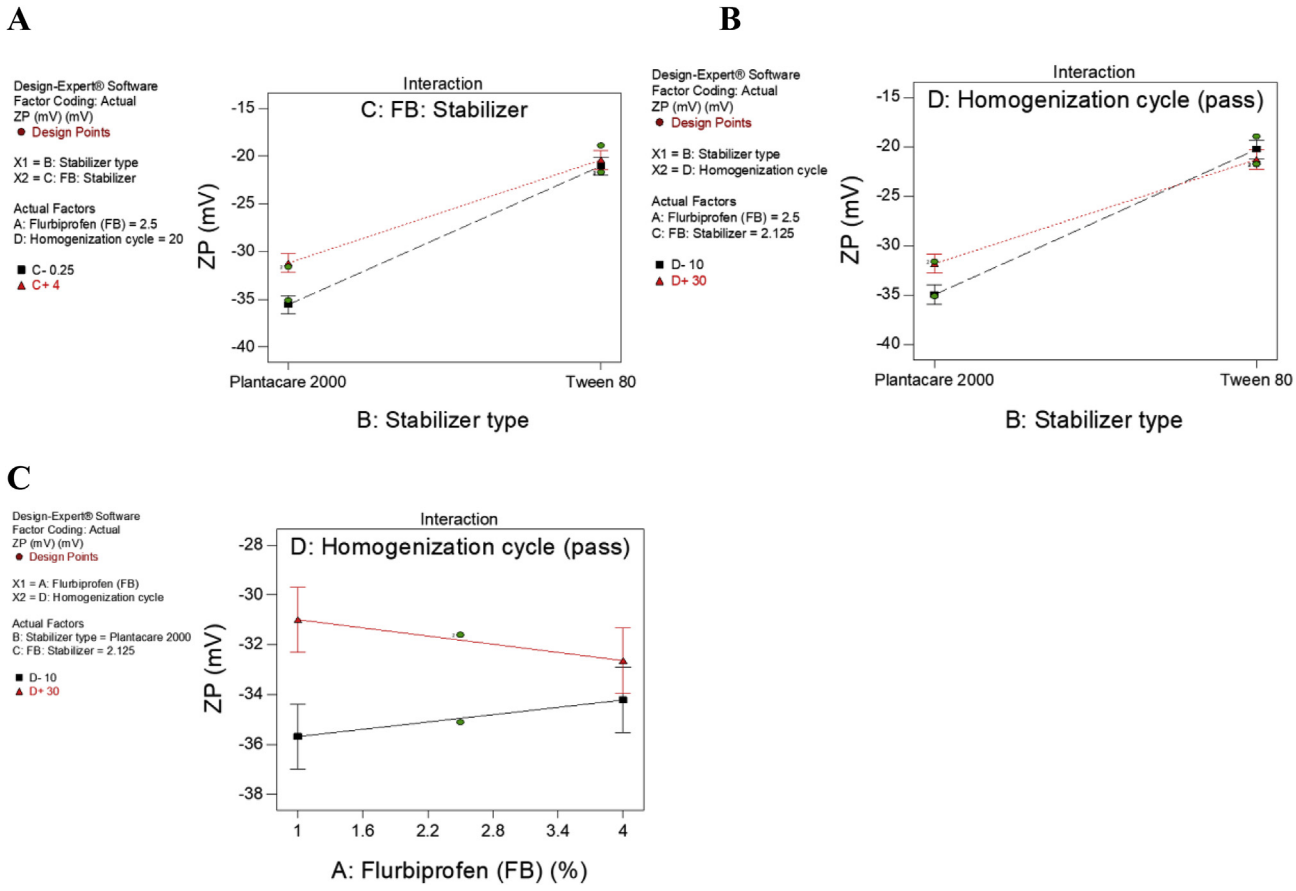

Fig. 5. Interaction graphs showing the effect of process and formulation parameters on zeta potential (ZP) values of FB-NS prepared surfactants **A)** BC (Stabilizer type & FB: Stabilizer ratio), **B)** BD (Stabilizer type & Homogenization cycle) and **C)** AD (FB% & Homogenization cycle).

PDI and ZP values PVP were found better than HPMC as a polymeric stabilizer. The optimum parameters were determined as 4% FB, 4:1 FB:stabilizer ratio, 30 homogenization cycle for PL and 4% FB 1:4 FB:stabilizer ratio 30 homogenization cycle for Tween 80. Compared with other stabilizers, PL, as a non-ionic surfactant, was found more efficient in obtaining small PS and stable nanosuspension systems. These results demonstrated that both the PS and the ZP of FB nanosuspensions are affected by the types and amount of stabilizers and this observation is inconsistent with previous viewpoints that the PS is dependent on the preparation process and the ZP is mainly dependent on the type and amount of stabilizers [46,47]. From these results, it can be seen that the types and amount of stabilizers were critical in determining both the PS and ZP of nanosuspensions and these results were similar with those of a previous study in the literature, which is about the effect of stabilizing agents on the properties of myricetin nanosuspensions [48]. The researchers indicated that the ZP values of nanosuspensions stabilized with surfactants were higher than those with polymeric stabilizers. Based on these results, in our another study, FB nanosuspensions were optimized using just Plantacare 2000 as a surfactant with 3^3 full factorial design [49]. Moreover, curcumin, hesperetin and resveratrol nanosuspensions were successfully produced using Plantacare 2000 as a suitable stabilizer [44,45,50]. To continue with other characterization studies, PL was selected as an efficient stabilizing agent in this study, which is elaborated in the following section.

3.4. Characterization of nanosuspensions

3.4.1. Surface morphology of nanosuspensions

Scanning electron microscopy (SEM) images show the differences in the morphological structure of formulations as a function of different stabilizers. The morphology of the coarse powder of FB, physical mixtures and nanosuspensions were investigated in the present study. The nanosuspensions were prepared with four different non-ionic stabilizers using HPH process. It can be observed that the SEM images of coarse powder FB exhibits irregular and needle shaped crystals at micrometer size with broad size distribution (Fig. 6A).

Fig. 6 shows the SEM images of physical mixture and nanosuspensions prepared with polymeric stabilizers. In physical mixtures, it was observed that the HPMC and PVP covered the surface of FB particles (Fig. 6B and D). While the PVP converted the coarse FB particles into the nanosuspensions with a relatively narrow size distribution (Fig. 6E and G), the nanosuspensions were stabilized with HPMC tended to agglomerate (Fig. 6C and F).

The effects of two types of surfactants (PL and Tween 80) on the morphological properties of FB nanosuspensions were also investigated and the images were compared with the coarse powder of FB and physical mixtures. According to images, Tween 80 caused the rod shaped FB particles in physical mixture and nanosuspensions and showed the tendency to agglomeration (Fig. 7A, Fig. 7B and E). In physical mixture of FB and PL, PL adsorbed to the surface of FB powder and increased the wettability of FB (Fig. 7C). Nanosuspensions stabilized with PL showed nanosized spherical shaped particles with homogenous PS distribution without agglomeration (Fig. 7D and F).

The collusion and cavitation forces of the HPH process transformed the particles into a more spherical shape [51]. It can be said that reducing PS into nanometer range under high pressure leads to shape transformation of the particles [52]. Also, the visual observation was found in correlation with the PS results. All the SEM results showed that the PVP leads to obtaining more homogenous systems compared with the HPMC as a polymeric stabilizer. PL provided the production of the spherical shaped and nanosized FB particles. Moreover, nanosuspensions stabilized with the PL showed more homogenous PS distribution compared with the Tween 80. As a result, to prevent the aggregation of nanosuspension, PVP and PL were selected as a polymeric stabilizer and surfactant, respectively. On the basis of SEM analysis, PL exhibits

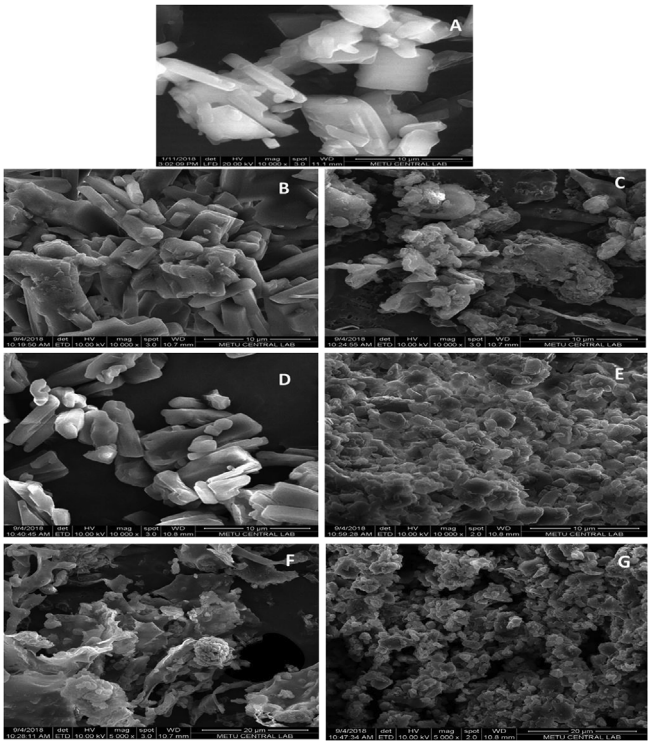

Fig. 6. SEM images of FB coarse powder (mag. 10.000x) **(A)**, physical mixture of FB and HPMC (mag. 10.000x) **(B),** FB nanosuspension stabilized with HPMC (mag. 10.000x) **(C)**, physical mixture of FB and PVP (mag.10.000x) **(D)**, FB nanosuspension stabilized with PVP (mag. 10.000x) **(E),** FB nanosuspension stabilized with HPMC (mag. 5.000x) **(F),** FB nanosuspension stabilized with PVP (mag. 5.000x) **(G).**

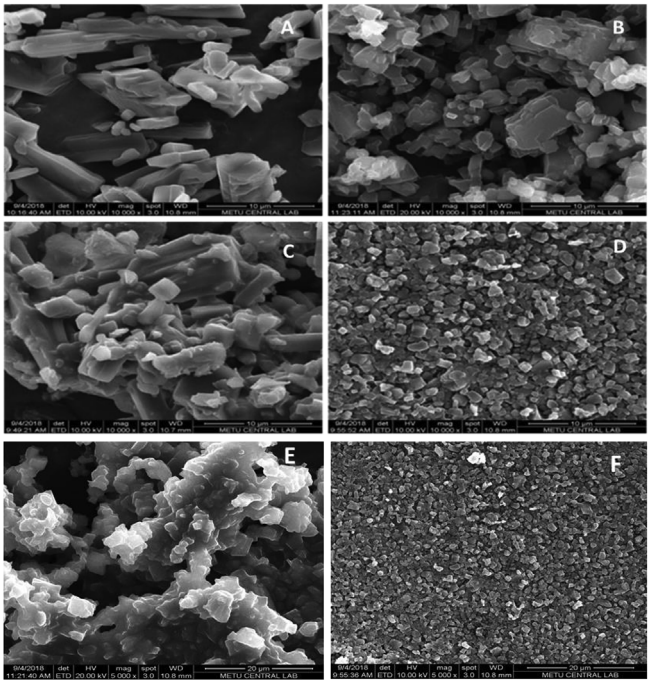

Fig. 7. SEM images of physical mixture of FB and Tween 80 (mag. 10.000x) **(A)**, FB nanosuspension stabilized with Tween 80 (mag. 10.000x) **(B)**, physical mixture of FB and PL (mag. 10.000x) **(C)**, FB nanosuspension stabilized with PL (mag. 10.000x) **(D)**, FB nanosuspension stabilized with Tween 80 (mag. 5.000x) **(E)**, FB nanosuspension stabilized with PL (mag. 5.000x) **(F)**.

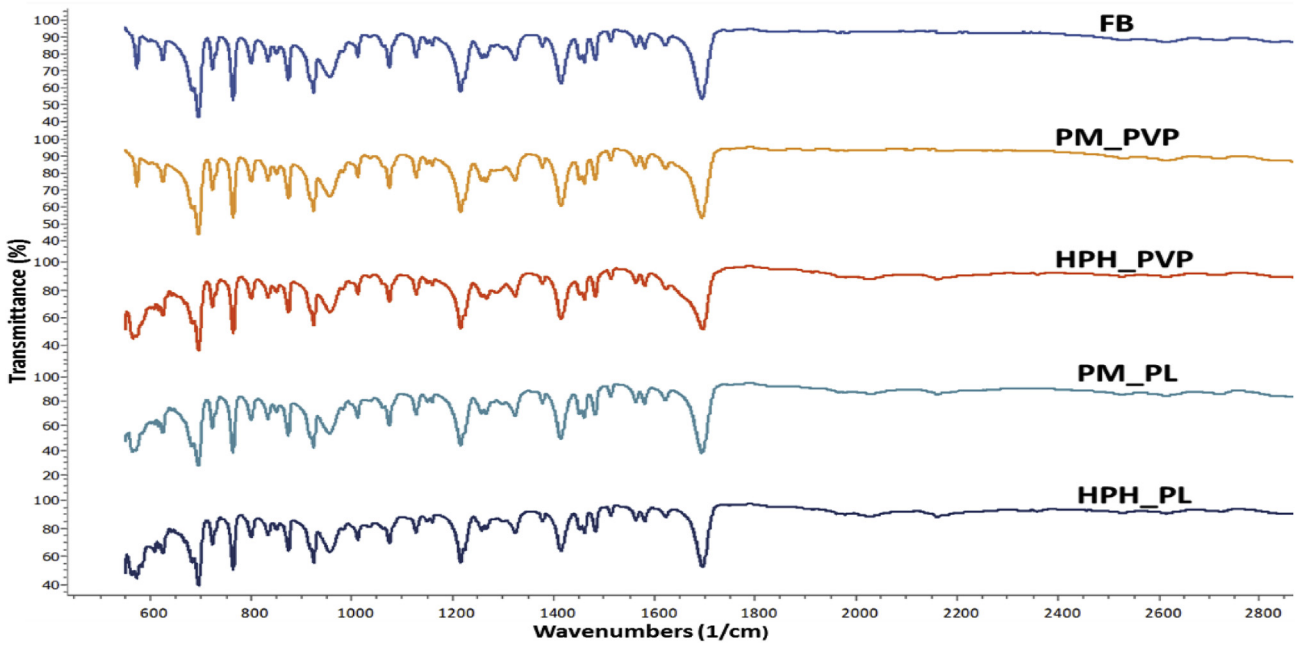

Fig. 8. FTIR spectra of FB, physical mixture and nanosuspensions prepared with PVP or PL.

smaller, spherical shaped and homogenously dispersed FB particles than PVP. Also, other characterization studies (FTIR, XRPD, DSC and stability studies) were performed to compare PL with PVP.

3.4.2. Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of FB, physical mixtures and nanosuspensions of FB with PVP or PL are shown in Fig. 8. The FTIR is one of the most important characterization studies to evaluate the interaction between drug and excipient and also to observe drug stability in formulation after HPH and lyophilization processes [53]. The spectra of all samples, such as the C=O stretching of acid at approximately 1740 cm^{-1}, C–O stretching of acid at 1210 cm^{-1} and C–H bending of methyl group at 1420 cm^{-1} showed that there was not any different peaks appeared in the spectra of FB coarse powder. Also, the intensities of all peaks of all samples were seen to be similar. The FTIR peaks are consistent with different studies [53–55] These results mean that there is no interaction between the FB and the stabilizers (PVP and PL). The chemical structure and drug stability were kept during homogenization and lyophilization processes.

3.4.3. Evaluation of crystalline state

Crystalline state and thermal analysis of the nanosuspension powders were performed for two types of stabilizers (PVP as a polymeric stabilizer and PL as a surfactant) which were selected according to SEM results. Fig. 9 exhibits XRPD scattering and Fig. 10 exhibits the DSC thermograms of coarse powder of FB, physical mixtures and nanosuspensions powder. To investigate the crystalline state of nanosuspensions stabilized with different types of stabilizers, the melting points were compared with coarse powder of FB and physical mixtures. The crystalline change can be observed after some physical treatments or due to formulation factors such as using unsuitable stabilizers. Therefore, the investigation of crystalline state of formulations provide insight into the polymorphic changes related with nanosizing and lyophilization process or using stabilizers for this research.

3.4.3.1. X-ray powder diffraction (XRPD). The inner crystalline structure of FB, physical mixtures and nanosuspensions produced using HPH process were investigated by XRPD. The XRPD pattern of them can be visualized in Fig. 9. Low peak intensities in physical mixtures of FB and PVP were obtained, and this could be explained as the interactions with drug or dilution of the FB powders with stabilizers [34,56]. The diffractograms showed that there were no different peaks for nanosuspensions stabilized with PVP or PL compared with the physical mixture and coarse powder of FB. All of the peaks were verified as finger print of FB. These results confirmed that the HPH and lyophilization process did not affect the crystalline state of FB.

3.4.3.2. Differential scattering calorimetry (DSC). As shown in Fig. 10, the sharp endothermic peak (melting point) of pure FB powder was indicated at 114 °C whereas no such characteristic peak of FB was observed in physical mixture (PVP and FB) or FB nanosuspensions stabilized with PVP, suggesting that FB transformed into the amorphous form. However, specific sharp melting point of FB powder was observed in both physical mixture and nanosuspensions prepared with PL. The melting point of physical mixtures (FB and PL) was also observed at 114 °C and this means that stabilizer (Plantacare 2000) did not change the crystalline state of FB and there was no incompatibility between PL and FB. The melting point of FB nanosuspensions produced with HPH process was also found similar to coarse powder and physical mixture of FB. The melting point of FB changed just approximately 3 °C with preparing FB nanosuspension (at 112.14 °C). The nanosizing of the particles may lead to a decrease in the melting point [57]. It means that there is no polymorphic change of FB during HPH and lyophilization process.

According to all these XRPD and DSC results, PL was found better than PVP as PVP changes the crystalline state of FB and leads to the transformation of the amorphous form of FB. The amorphous forms can lower the bioavailability during shelf life of drugs because of affecting stability compared with the crystalline state [47]. To avoid these stability problems, nanosuspensions should be in crystalline form. These results were supported with stability studies.

3.4.4. Physical stability

The alterations in the mean PS and PDI values of the PVP or PL stabilized nanosuspensions during a month are shown in Fig. 11. While there was no change on the PS and PDI values of PL stabilized nanosuspensions, PS and PDI values of PVP stabilized nanosuspensions increased. As shown in Fig. 12, it was indicated that the ZP values of PVP stabilized nanosuspensions decreased from −23 mV to −14 mV. There was no significant change for PL stabilized nanosuspensions and at the

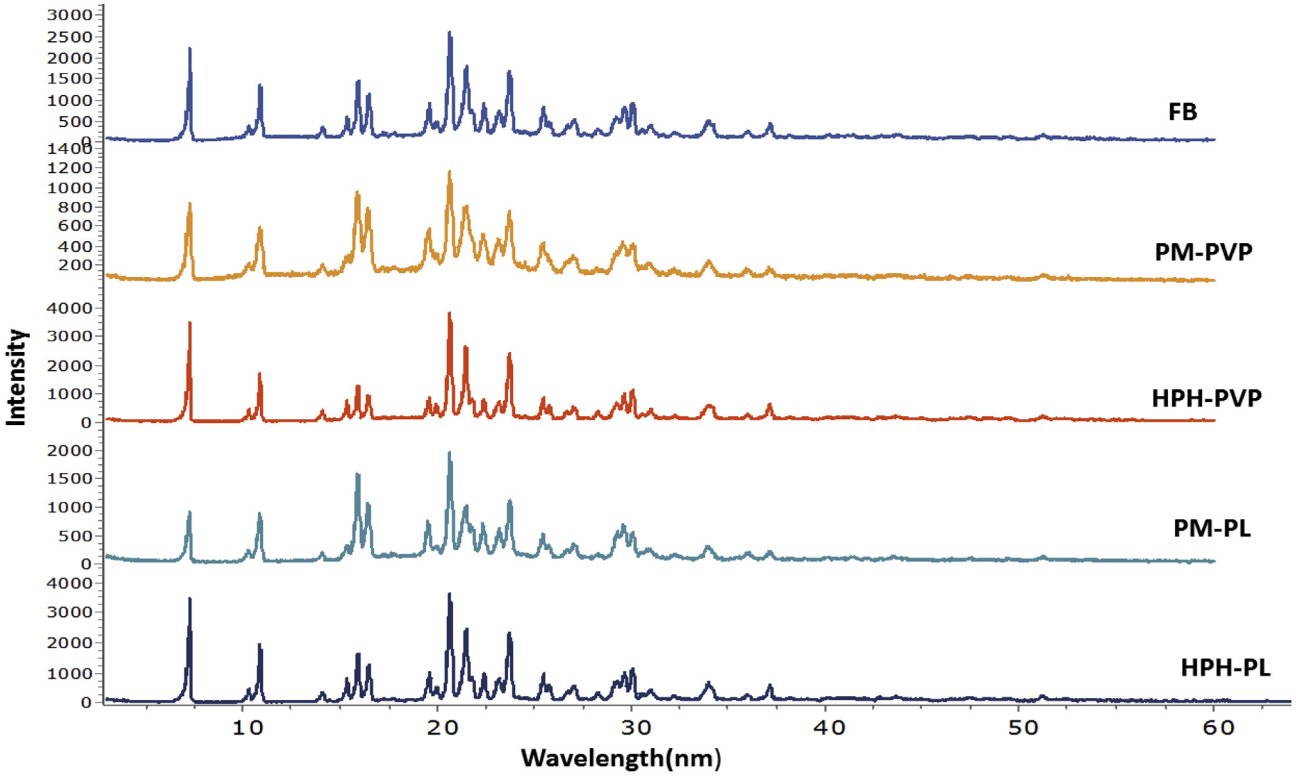

Fig. 9. XRPD scattering of FB, physical mixture (PM) of FB and PVP, FB nanosuspension stabilized with PVP, physical mixture of FB and PL, FB nanosuspension stabilized with PL.

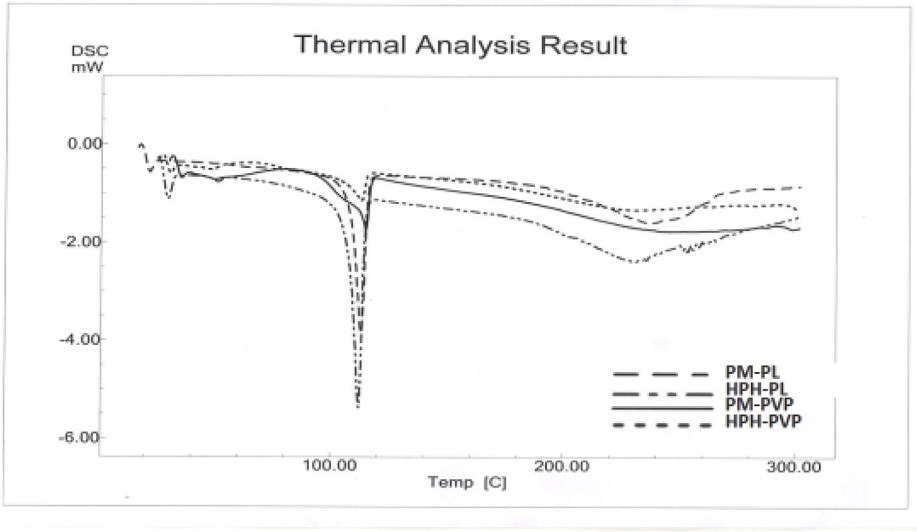

Fig. 10. DSC thermograms of FB, physical mixture of FB and PL (PM-PL), FB nanosuspension stabilized with PL (HPH-PL), physical mixture of FB and PVP (PM-PVP), FB nanosuspension stabilized with PVP (HPH-PVP).

end of one month, its ZP values (approximately −30 mV) were still suitable to obtain stable nano systems. These results are in line with a previous study. Mishra et al., investigated the stability of hesperetin nanosuspensions and nanosuspensions prepared with Plantacare 2000 and they were found stable with no change on the PS. However, the PS of Poloxamer and Tween stabilized nanosuspensions slightly increased [45].

4. Conclusion

FB nanosuspensions were successfully prepared with two types of polymeric stabilizers and two types of surfactants. These nanosuspensions were investigated by means of an experimental design. The DoE approach is useful for investigating the effect of stabilizer type and ratio while preparing nanosuspension formulations and to optimize the final formulation. According to PS, PDI and ZP values, optimum parameters of nanosuspensions were determined and the optimum formulations for all types of stabilizers were characterized. The SEM results showed that the PL and PVP provided better morphology than others. Then the optimum formulation, which was stabilized with PL and PVP, was characterized using XRPD, FTIR and DSC. The DSC results showed that PVP stabilized FB nanosuspensions transformed into the amorphous

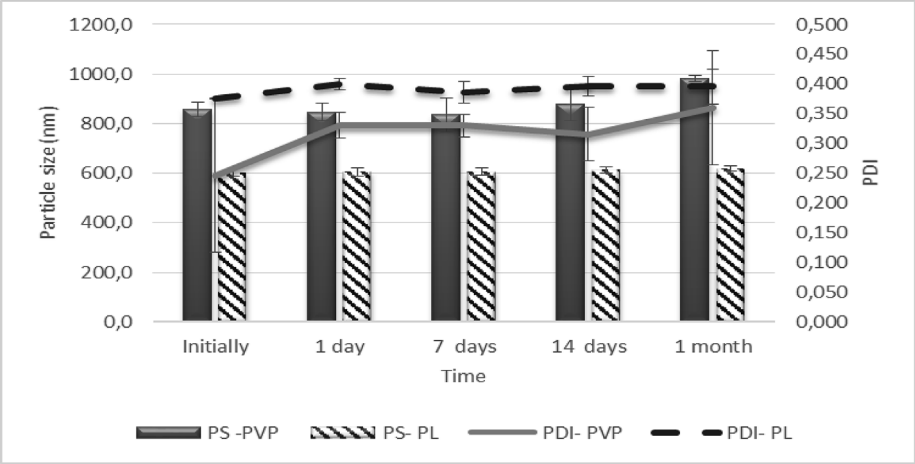

Fig. 11. PS and PDI values of nanosuspensions stabilized with PVP or PL at 1st day, 7th days, 14th days and 1st month at 25 °C temperature (Mean ± S.D.).

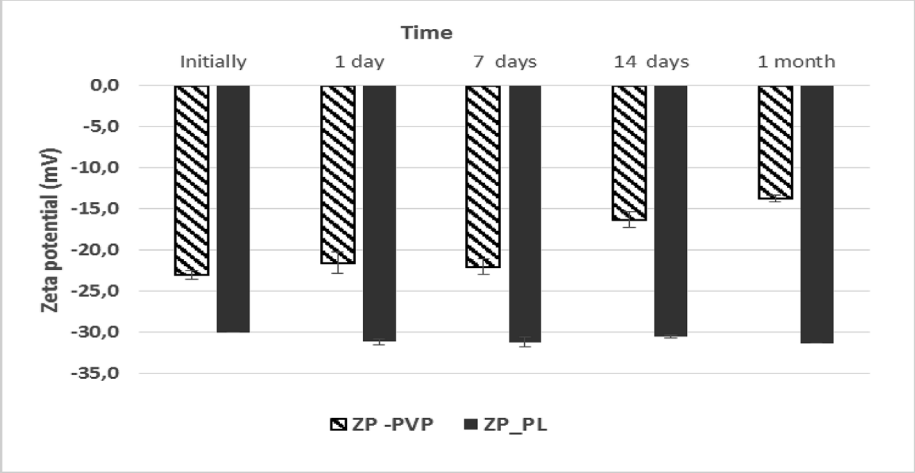

Fig. 12. ZP values of nanosuspensions stabilized with PVP or PL at 1st day, 7th days, 14th days and 1st month at 25 °C temperature (Mean ± S.D.).

form and PL stabilized FB nanosuspensions protected the crystalline state. At the end of stability studies, just PL stabilized nanosuspensions were found stable. These studies suggested that the PL is a more efficient stabilizer to obtain smaller PS and more stable nanosuspension systems.

This study demonstrated the importance of the stabilizer (type and amount) determination, which is a critical step to prevent agglomeration and crystal growth of nanosuspensions, and its effect on the stability of formulations. Besides providing insight about stabilization of nanosuspensions, this study also focused on the experimental design for determining the effect of critical formulation parameters on the CQA of nanosuspensions and decreasing the number of experiment by studying in design space.

CRediT authorship contribution statement

Ayse Nur Oktay: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Sibel Ilbasmis-Tamer:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Alptug Karakucuk:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Nevin Celebi:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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