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Box Behnken Design Optimization for Synthesis of Dexibuprofen Nanocrystals Using Anti-solvent Precipitation Method and Oral Bioavailability Testing

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Abstract

Dexibuprofen (DXI) is the S(+) enantiomer of Ibuprofen [(±)-a-methyl-4–(2-methylpropyl) benzeneacetic acid. It has poor physical solubility of less than 1mg/mL. Due to its solubility, it causes low bioavailability in the gastrointestinal tract. One way to improve its solubility is to reduce its size to form nanocrystals. The objective of this study is to synthesize stable nanocrystals of dexibuprofen (DXI) through the use of the anti-solvent precipitation method. Furthermore, the study aims to optimize the synthesis process using the Box-Behnken method and evaluate the oral bioavailability of the nanocrystals. DXI nanocrystals were synthesized using an antisolvent precipitation method. Optimization formula using an experimental design by Box Behnken to determine the formula for DXI nanocrystals with three-factor variations in magnetic stirring speed (400-800 RPM), the concentration of PVP K90 (1-3%), and Tween 80 (1-3%), as stabilizers for DXI nanocrystal synthesis. The optimum DXI nanocrystal was dried by Freeze-drier and further characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM). The nanocrystals were evaluated by solubility and in vitro dissolution tests. The oral bioavailability test of DXI nanocrystal was conducted on Wistar male rats and compared to raw DXI. The optimized DXI nanocrystal was produced from manufacturing under conditions of magnetic stirrer speed at 400 RPM, 3% of PVP K90, and 3% of Tween 80. The resulting DXI nanocrystal has a size of 118.83nm, with polydispersity index and zeta potential values of

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0.422 and -186.6 mV, respectively. The DSC characterization found DXI nanocrystal powder to peak at 45.41°C. Furthermore, the results of the diffractogram in the PXRD test of the DXI nanocrystal powder showed a sharp intensity indicating that the DXI nanocrystals did not change into other physical forms. Finally, the solubility, dissolution, and oral bioavailability of DXI nanocrystals were higher than raw DXI. The optimized DXI nanocrystal was successfully manufactured using the anti-solvent precipitation method with conditions optimization results from Box-Behnken Design. The resulting optimal formula shows better solubility and oral bioavailability than raw DXI.

Keywords: DXI, nanocrystals, precipitation, Box-Behnken, oral bioavailability.

INTRODUCTION

Dexibuprofen (DXI) is the S(+) enantiomer of Ibuprofen [(±)-a-methyl-4–(2-methylpropyl) benzeneacetic acid] with a solubility of less than 1mg/mL. Several clinical trials and post marketing surveillance studies have been conducted to expand on the findings of dexibuprofen (Phleps, 2001). In the last five years, 4,836 patients have taken dexibuprofen in clinical. Only 3.7% of the patients experienced adverse reactions. The efficacy of dexibuprofen has been shown to be at least comparable to that of diclofenac, naproxen, and celecoxib, as well as having demonstrated good tolerability. Research also shows that dexibuprofen processed in a special crystal form is a safe and effective treatment for various pain treatments (Kaehler, 2003; Wu et al., 2020). Efforts to improve the physical properties of an active substance can be done by reducing the particle size. Reducing drug size to the nanoscale can increase the surface area to volume ratio, thereby increasing solubility and dissolution rate, and improving the in vivo performance of drugs with poor solubility (Khan et al., 2018; Liu, 2013; Sánchez-López et al., 2020).

This study was carried out with the aim of synthesizing DXI nanocrystals. The antisolvent composition in the synthesis of DXI nanocrystals must have the right composition. This is related to the supersaturation conditions of the active substance solution. The next problem in the synthesis of nanocrystals is their stability. Nanocrystals may agglomerate during manufacture or storage. This happens because of an agglomeration event that produces agglomerates. Agglomerates are the main particle clusters that interact physically by binding with each other (Bruinink et al., 2015; Jenkins et al., 2015). The polymer polyvinylpyrrolidone (PVP) is the stabilizer chosen because it is a polymer used as a stabilizer in various studies on nanoparticle synthesis (Sutarna et al., 2022). PVP is also known as a polymer that can increase solubility (Tehrani et al., 2019). Apart from PVP, Tween

80 is also added as a surfactant. In this study, it is necessary to optimize each composition of PVP K90, Tween 80 and magnetic stirrer speed in the formation of DXI nanocrystals. Optimization of a research will take a lot of time and costs which of course is not cheap, therefore we need a tool to determine the optimization of a research.

In optimizing the DXI nanocrystal formula, the addition of PVP K90, Tween 80 and magnetic stirrer speed was carried out using a statistical design of experiment (DOE) approach consisting of factorial design and response surface methods (RSM) to obtain more effective and efficient results. Experimental design with Minitab software ver.19 results in polynomial equations from experimental data as mathematical and statistical modeling known as the response surface method (RSM) (Almeida et al., 2008; Nursal et al, 2019). The Box-Behnken RSM experimental design is more efficient than other designs because it has the same variables but a few trials, thereby reducing test costs (Nursal et al, 2019).

The purpose of this study was to synthesize DXI nanocrystals and optimized using the Box-Behnken 7 design. The resulting DXI nanocrystals are evaluated by testing their characterization, solubility, and bioavailability.

MATERIALS AND METHODS

Materials

Materials for the study include DXI (Batch Number: A 001-202001004) purchased from Beijing Mesochem Technology Co., Ltd., Beijing; Polyvinyl pyrrolidone (PVP) K-90 (Batch number: 08297052G0); Tween 80; Mannitol (Batch Number: 201305062); trial version of Minitab.19; and 200-280 g male Wistar Rats purchased from the Animal Laboratory of the Bandung Institute of Technology.

Methods

DXI Synthesis

The DXI nanocrystals were synthesized using the antisolvent precipitation method with 3g of DXI dissolved in 5 mL of ethanol. The DXI solution in ethanol was dripped with water contained PVP K90 and Tween 80 while stirring on a magnetic stirrer. Optimization formula using DOE Box Behnken to determine the formula for DXI nanocrystals with variations in the speed of magnetic stirrer rotation at 400-800 RPM and the addition of PVP K90 (1-3%) and Tween 80 (1-3%) as stabilizers for DXI nanocrystal synthesis. The experimental factor used in Box Behnken design shown in Table 1. The optimum conditions for the synthesis of DXI nanocrystals were carried out based on response optimization of zeta potential in formulas with particle sizes below 1000 nm.

Table 1. Experimental Factor used in Box-Behnken Design

Factor	Name	Range & Level		
		-1	0	+1
A	Stirring Speed (RPM)	400	600	800
B	PVP K90 (%)	1	2	3
C	Tween 80 (%)	1	2	3

Drying of DXI Nanocrystal

Drying of DXI nanocrystal was carried out on only one of the most optimal formulas based on response optimization analysis of zeta potential. The process of changing phase from liquid to powder form was conducted out using the freeze-drying method. DXI nanocrystals was added with 5% w/w mannitol of the DXI weight. The resulting DXI powder was used for further test.

Characterization Study

Powder X-ray diffraction studies (PXRD).

The DXI raw and the synthesized nanocrystals was determined by a powder diffractometer (D8 ADVANCE, Bruker, Germany). The scanning of the samples was carried out in triplicate in the range of 2θ is 0-50° (0.05° step size, and 2 seconds of time lapse between the steps) with copper Kα as a radiation source, 1 mm slit at 1.542 Å wavelength.

Scanning electron microscopy (SEM)

Images of DXI were taken at various magnifications with the sputtered particles coated with gold prior to morphological study using a Joel JSM5910 scanning electron microscope at an operating voltage of 30 mA for a duration of 2 min and an accelerating voltage of 20 kV. The examination was carried out at the Geological Survey Center Laboratory, Bandung, Indonesia.

Differential scanning calorimetry (DSC)

The fusion heat and the melting point of DXI samples (nanocrystals and unprocessed drug substance) and PVP K-90 were tested using a Mettler Toledo Differential Scanning Calorimeter (Mettler Toledo®, USA). Samples from each of the raw and optimized polymer DXI nanocrystals (2–3 mg) were placed separately on blank and sealed aluminum plates, then heated at a rate of 10°C min−1 from 25 to 120°C, with all samples analyzed in triplicate.

Recovery Test

The recovery test was carried out using the comparison method. This method was carried out by measuring the level of analyte in the sample and then comparing the results obtained with a reference standard in which the levels are known with certainty. The comparison used must have the same characteristics as the sample (Araujo, 2009). Considering the comparison method being used, the first thing to do was to create a DXI calibration curve to obtain a linear regression

equation. A total of 212.7 mg of DXI nanocrystal powder was weighed and then dissolved in a volumetric flask and added with water up to 1000 ml. Then, 50 mL of DXI nanocrystal solution was added to the volumetric flask using a 5 mL pipette. The diluted solution was measured using a UV-Vis spectrophotometer at a wavelength of 220nm. The results were then analyzed.

Solubility Test

The solubility test was carried out by preparing a vial containing 10 ml of water, then adding the nanocrystalline powder while weighing it. The addition of powder was done while stirring occasionally until a precipitate was observed. After settling, the vials were shaken using an orbital shaker for 24 hours. After 24 hours, it was filtered and then measured using a UV-Vis spectrophotometer at a wavelength of 220nm.

Dissolution Test

Dissolution testing was conducted on DXI nanocrystal and DXI Raw based on the USP reference. The DXI nanocrystal or raw DXI was weighed equivalent to 400 mg and put into a vessel containing 900 mL of phosphate buffer pH 7.4 at 37 ± 0.5°C and 50 rpm of paddle rotation speed. The samples were taken at 5, 10, 15, 30, 45 and 60 minutes. Then the samples were filtered and analyzed by ultraviolet spectrophotometry at 220 nm.

In Vivo Oral Bioavailability Test

Collection of Rats Plasma

The experimental procedure was approved by the Animal Ethics Committee of the Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, West Java Province (Ethical Approval No. 9023/KEP-UNJANI/XI/2022. The experimental animals were divided into two groups (raw DXI and DXI nanocrystal) each consisting of six mice. Each group was given suspension containing raw DXI and nanocrystal DXI at a dose equivalent to 400 mg/70 kg BW with dispersion in 0.5 w/v sodium carboxymethyl cellulose in water. Animal blood was taken through a lateral tail vein before and after at 5, 10, 15, 30, 60, 120, 180, 300, and 480 minutes after orally administered. Then, the blood plasma was process by protein precipitation and measured using HPLC undercurve 220nm.

HPLC Analysis for Estimation of DXI in Rat Plasma

The produced plasma was processed by centrifuging each sample using a centrifuge tube at a speed of 12000 rpm for 3 minutes. Then, 100 microliters of the plasma were pipetted and added with 200 microliters of acetonitrile in a 1.5mL Eppendorf tube. The mixture was centrifuged at a speed of 12000 rpm for 3 minutes. Next, 20 microliters of the clear part were taken and injected into an HPLC column. The

chromatographic separation was achieved using a Pursuit XRs 100Å C18 column (150 × 4,6 mm id, 5 µm) with mobile phase comprising of acetonitrile, and acetic acid 1% (25:75, v/v), delivered at a flow rate of 1,0 mL/min. The column temperature was maintained at 25°C. The chromatographic peak-area ratio, based on UV absorbency at 220 nm, was used for quantitative analysis.

Pharmacokinetic Analysis

This study is considered to assess the comparative bioavailability of DXI raw and DXI nanocrystals, consequently the pharmacokinetic profiles (grade and drug levels) of the DXI raw and DXI nanocrystal products measured based on the drug concentration measured in rat plasma collected from the vein in the tail. A wide range of relative oral bioavailability values for AUC0-t was found using the formula AUC0-t from DXI raw / AUC0-t from DXI nanocrystals. Pharmacokinetic analysis it was carried out using concentration vs time profile estimation using DXI nanocrystals. The parameters, AUC0-t, Cmax and AUC0-∞ considered as the primary pharmacokinetic parameters and tmax, t½, and Kel were considered as secondary pharmacokinetic parameters.

RESULT AND DISCUSSION

DXI Nanocrystal Synthesis

From the design formula obtained from the device, DXI nanocrystals were synthesized, and response data were obtained in the form of particle size, polydispersity index, and zeta potential, as shown in Table 2.

Table 2. Formula Design and Results of Dexibuprofen Nanocrystal 3-Factor 10

RPM	PVP K-90 (%)	Tween 80 (%)	Particle Size (nm)	PI	Zeta Potential (mV)
400	1	2	105,00±**9,22**	0,54±**0,05**	111.3±**1,18**
400	3	2	101,40±**23,51**	0,41±**0,03**	226,10±**3,70**
600	2	2	95,27±**1,11**	0,51±**0,00**	134,60±**2,88**
800	1	2	100,07±**0,70**	0,57±**0,01**	54,80±**2,72**
600	2	2	93,83±**1,11**	0,49±**0,01**	-130,00±**1,73**
600	1	1	121,67±**54,89**	0,49±**0,17**	60,60±**1,18**
600	3	1	98,83±**6,70**	0,38±**0,02**	-0,97±**0,01**
600	2	2	95,83±**1,68**	0,52±**0,02**	120,60±**1,56**
800	3	2	69,77±**0,35**	0,51±**0,01**	-156,60±**1,91**
400	2	3	81,17±**5,32**	0,56±**0,06**	144,30±**1,15**
600	1	3	94,47±**0,12**	0,55±**0,00**	-0,57±**0,03**
400	2	1	96,93±**0,93**	0,51±**0,01**	-92,10±**0,95**
800	2	3	87,43±**1,14**	0,49±**0,00**	53,91±**1,00**
800	2	1	118,73±**9,72**	0,43±**0,01**	22,47±**0,99**
600	3	3	83,43±**10,65**	0,46±**0,06**	256,40±**1,83**

The mean particle size analyzed by Particle Size Analysis (PSA), the results show the size of the particle formed is 69-121nm. This shows that the composition of variations between stirrer speed, PVP K90 and Tween 80 succeeded in producing DXI nanocrystals. It corresponds to the definition of nano size thatDrug nanocrystals are nanometer-sized crystals that exhibit a crystalline structure. The definition of a nanoparticle varies across disciplines, such as colloid chemistry, where particles below 100 nm or even 20 nm are considered nanoparticles. In the pharmaceutical field, nanoparticles are typically defined as particles ranging from a few nanometers in size. Based on the size unit, in the pharmaceutical area nanoparticles should be defined as having a size between a few nanometers and 1000 nm (≤1µm) (Gao et al., 2008; Mirza et al., 2017).

Polydispersity index (PI) or the breadth of the particle size distribution) obtained from research that has been conducted show results between 0.3-0.5. The PI value ranges from 0 (indicating monodisperse particles) to 0.5 (indicating a broad distribution). It is a crucial parameter that influences the physical stability of the particles. In order to achieve long-term stability, it is desirable to have a low PI value (Gao et al., 2008). Finally, it's important to note the Zeta potential measurement in this study showed a size variation of 0.57-156mV. The measurement of zeta potential provides valuable insight into the storage stability of submicron colloidal dispersions (Li et al., 2006). Some potential zeta results obtained from this study are in accordance with the existing literature. In general, the occurrence of particle aggregation is less likely when particles possess an adequate zeta potential, which enables sufficient electric repulsion, or when there is a sufficient steric barrier to provide steric repulsion between particles. Based on the literature, it is recommended to have a zeta potential of at least -30 mV for electrostatically stabilized systems and -20 mV for systems stabilized through steric interactions in order to achieve physical stability in nanocrystal suspensions (Gao et al., 2008).

The results of the regression response surface analysis show that with a P value (<0.05) the results of the experiment using three factors at Box-Behnken are acceptable. this can be seen in the constant value of 0.000 which shows the results of the study have regular measurement values. It can be seen in Table 3.

Table 3. Response Surface Regression Synthesis Nanocrystal DXI

Term	Coeff	SE Coeff	T-Value	P-Value	VIF
Constant	128.4	11.1	11.57	0	
RPM	-35.88	6.79	-5.28	0.003	1
PVP K-90 (%)	51.47	6.79	7.58	0.001	1
Tween 80 (%)	34.88	6.79	5.14	0.004	1

RPM*RPM	3.6	10	0.36	0.736	1.01
PVP K-90 (%)*PVP K-90 (%)	5	10	0.5	0.639	1.01
Tween 80 (%)*Tween 80 (%)	-53.8	10	-5.38	0.003	1.01
RPM*PVP K-90 (%)	-3.5	9.61	-0.36	0.731	1
RPM*Tween 80 (%)	-5.2	9.61	-0.54	0.612	1
PVP K-90 (%)*Tween 80 (%)	78.87	9.61	8.21	0	1

Parameters that statistically affect the stirrer speed of PVP K90 and Tween 80 concentrations statistically affect the preparation of DXI nanocrystals. This can be seen from the P-Value in the Box-Behnken experimental design. If the P-Value is less than α (≤ 0.05) then the hypothesis H0 is accepted, which means that the independent variable affects the value of the fixed variable. Vice versa, if the P-Value ≥ of α (0.05) then the hypothesis H0 is rejected, which means that the independent variable does not really affect the value of the fixed variable (Box, 1960).

The results of the Anova data from the Box-Behnken experimental design, Analysis of variance in optimizing the manufacture of DXI nanocrystals can be accepted using the Linear model. This is shown by the results that can be seen in table 4.

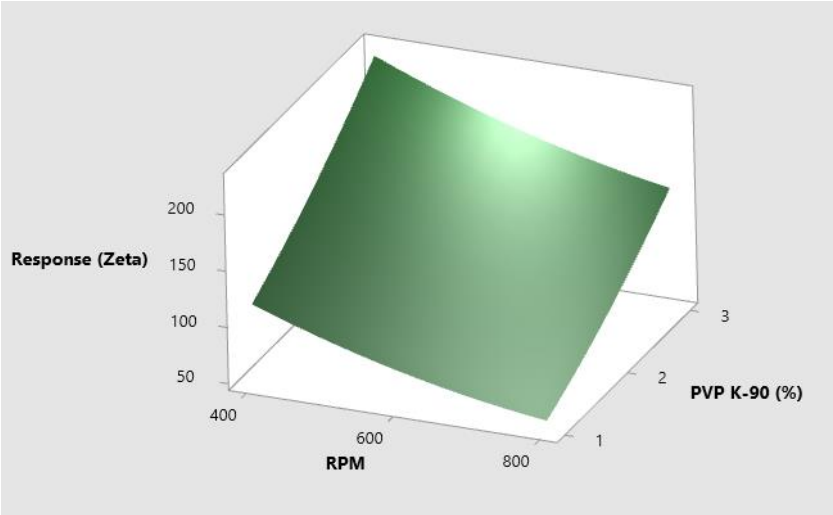
Table 4. Analysis of Variants Synthesis Nanocrystal DXI

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	77433.7	8603.7	23.31	0.001
Linear	3	41233	13744.3	37.23	0.001
RPM	1	10300.2	10300.2	27.9	0.003
PVP K-90 (%)	1	21197.1	21197.1	57.42	0.001
Tween 80 (%)	1	9735.7	9735.7	26.37	0.004
Square	3	11164.4	3721.5	10.08	0.015
RPM*RPM	1	46.8	46.8	0.13	0.736
PVP K-90 (%)*PVP K-90 (%)	1	92	92	0.25	0.639
Tween 80 (%)*Tween 80 (%)	1	10670	10670	28.9	0.003
2-Way Interaction	3	25036.3	8345.4	22.61	0.002
RPM*PVP K-90 (%)	1	49	49	0.13	0.731
RPM*Tween 80 (%)	1	108.1	108.1	0.29	0.612
PVP K-90 (%)*Tween 80 (%)	1	24879.3	24879.3	67.39	0
Error	5	1845.9	369.2		
Lack-of-Fit	3	1744	581.3	11.42	0.082
Pure Error	2	101.8	50.9		
Total	14	79279.6			

To ensure the appropriateness of the model, two tests were conducted: a lack of fit test and a coefficient of determination test (R2). The lack of fit test examines the suitability of the first-order linear

model by assessing any discrepancies. Its purpose is to determine whether the model adequately represents the data. If the lack of fit test yields non-significant results, it can be concluded that the first-order linear model is suitable for the data. The P-value is a factor of significance to the response that explains the approximate suitability of the results of the Pareto analysis with the responses obtained. The lack of fit number of 0.082 indicated that the error factor > 0.05 modeling is not different from the experimental results (Box, 1960; Nopiani Y, 2013). Additionally, the data also shows a contour response, which illustrates that the zeta potential below -40 would be obtained in the synthesis of nanocrystals with a composition of 3% PVP K90 and 3% Tween 80. This is illustrated in Figure 1.

Figure 1. Contour Plot Response Zeta of Potential Values to PVP K-90 and Tween 80 hold values stirring speed at 400 RPM



The equation obtained for the effect of a factor on the response Y is as follows:

Response (Zeta) = 209 - 0.305 Stirring speed (RPM) - 124.1 PVP K 90 (%)
+ 95.7 Tween 80 (%)
+ 0.000204 Stirring speed (RPM)*Stirring speed (RPM)
+ 7.1 PVP K90 (%)*PVP K-90 (%)- 49.2 Tween 80 (%)*Tween 80 (%)
- 0.0175 Stirring speed (RPM)*PVP K-90 (%)
- 0.0385 Stirring speed (RPM)*Tween 80 (%)
+ 78.87 PVP K90 (%)*Tween 80 (%)

The regression results show a correlation between the RSM statistical values and the experimental values. The data function determines the negative or positive effect based on the results of the response, which

is an indication of suitable (+) and unsuitable (-) (Sadrnia et al., 2021). The optimum formula is determined by first deriving equation for zeta potential response is -297.58 with the formula of 3gr DXI, 5ml ethanol, 3% PVP K-30, and 3%Tween 80. The optimum formula is presented in Table 5.

Table 5. Prediction of DXI Nanocrystal Optimum Formula at Zeta Potential Values

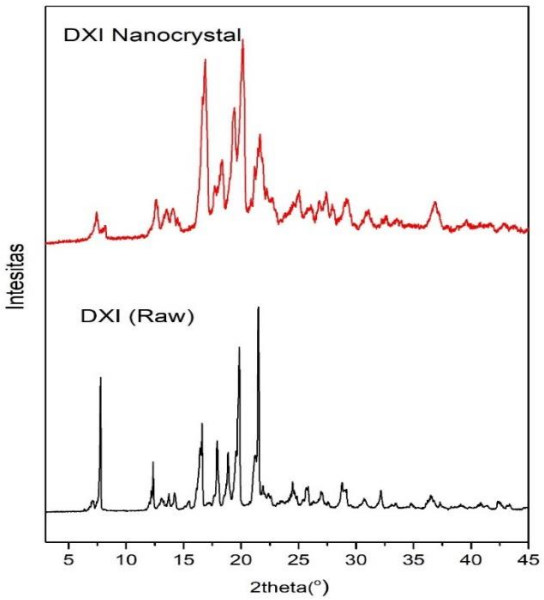
Variable	**Setting**			
RPM	400			
PVP K-90 (%)	3			
Tween 80 (%)	3			
Response	**Fit**	**SE Fit**	**95% CI**	**95% PI**
Response (Zeta)	297,58	23,5	(237,2; 358,0)	(218,4; 376,7)

Based on the multiple responses obtained, the next step was to synthesize DXI nanocrystals based on the predicted formula, namely with a composition of 3gr DXI, 5ml ethanol, 3% PVP K-30, and 3% Tween 80, and a stirring speed of around 400 RPM. The results of nanocrystal preparation based on the response prediction are particle size of 118.83±53.19 nm, polydispersity index of 0.422±0.014, and potential zeta value of 186.60±24.637. The result of this preparation was dried using a freeze-dry tool with the addition of mannitol to prevent aggregation by protecting the nanocrystals through a matrix mechanism and the hypothesis of particle separation in the frozen phase (Mohammady et al, 2020; Tang et al, 2004). For the study, mannitol was used as a cryoprotectant because it is widely used as a cryoprotectant in various studies of changing nanocrystalline forms from liquid to solid using the freeze-drying method (Bejrapha et al., 2011; Eerdenbrugh et al, 2008). Of the active substance, 5% mannitol was added to the nanocrystal preparation prior to the drying process. The drying process resulted in 2.96g of DXI powder.

Characterization Test

The raw DXI and DXI nanocrystal tests using XRD produced sharp X-ray diffractograms, indicating that the DXI crystalline properties in the DXI nanocrystals persist without transformation to another physical form after the synthesis. The difference in peak intensity and sharpness between raw DXI and DXI nanocrystals can be observed (Figure 4). Furthermore, the XRD test on PVP K90 was also carried out as a control to evaluate its impact on the crystal structure produced from DXI nanocrystals. However, the effect of PVP K90 on the resulting nanocrystalline diffractogram is not observed.

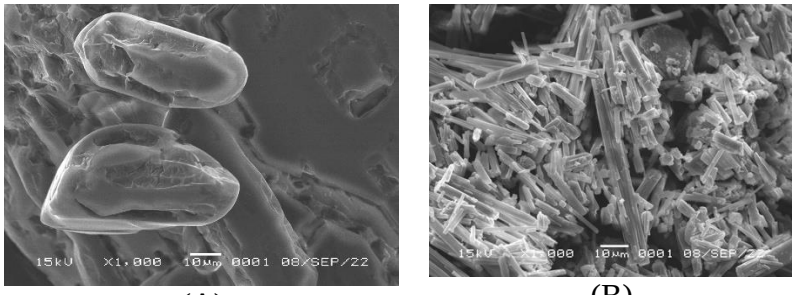
Figure 2. XRD Test Results of Raw DXI (A), PVP K90 (B), and DXI Nanocrystals (C)



The appearance of the peak for DXI nanocrystals is shown in Figure 4, indicating a reduced intensity. Calculations crystal size are performed using the Scherer formula. As seen in the crystalline grain on DXI raw is 115.35±112.78 nm and on DXI Nanocrystal is 31.83±40.02 nm. A decrease in crystal intensity can occur due to reduced particle size, which can impact X-ray reflection at a small angle (Ullah et al., 2018). The smaller the particle size, the lower the sharpness, which may cause some peaks to disappear (Martins et al., 2011).

The morphological test of the DXI that has been processed into solid form was carried out using SEM. The SEM DXI micrograph of the solid form shows that the particles underwent a change in shape from rod-like shapes to smaller crystal forms, which can be seen in Figure 2. This change in shape could be due to changes in smaller particles causing a change in morphology. In addition, the particles are found to be homogeneously distributed with limited agglomeration indicating that all particles are homogeneously distributed, with rectangular and cuboidal crystal shapes.

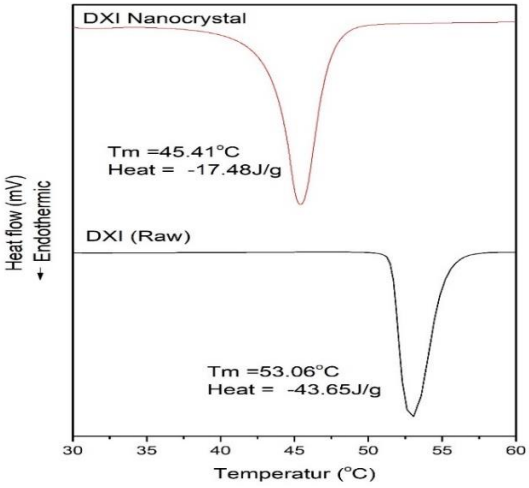
Figure 3. Profile of SEM Results for Untreated Raw DXI (A) and DXI Nanocrystals (B)



(A) (B)

The comparative DSC and XRD tests demonstrate that the resulting DXI nanoparticles are crystalline in nature and retain the physical shape associated with unprocessed drug substances. Sharp melting endotherms are observed in both unprocessed and processed raw DXI (Figure 3). However, the melting temperature of unprocessed DXI is observed to have a slightly higher melting temperature (53.06°C) compared to the DXI nanocrystals (45.41°C).

Figure 4. DSC Spectrogram Results for Raw DXI, PVP K90, and DXI Nanocrystals



The difference in the melting temperature of raw DXI and DXI nanocrystals could have occurred due to the smaller particle size so that the melting temperature of the DXI nanocrystals decreased. The reduced particle size of the DXI also causes a slight widening of the neck of the endothermic peak (Figure 3). In addition, the endothermic peak of the DXI nanocrystals appear with slightly widened necks, which is due to the small particle size and the synthesis process. This can also occur due to the effect of polymers adsorbed onto the surface

of the DXI nanocrystals (Martins et al., 2011; Ullah et al., 2018; Valleri et al., 2004).

Recovery and Solubility Test

The percent recovery test of DXI nanocrystal was calculate using a linear regression equation of Y = 0.0403x+0.0556 and the result is 81.140%. Percent recovery refers to the quantity of a product obtained after undergoing synthesis and purification processes. It serves as a measure of the efficiency of the synthesis reaction. In the field of organic chemistry, this term is commonly employed to assess the yield achieved through recrystallization (Mariana et al., 2018; Naeger, 2015). The recovery results can be used as the basis for dose calculations in dissolution tests and blood profiles.

Table 6. Calculation Results of Dexibuprofen Nanocrystal Recovery

Absorbent (Y)	b	a	x	Content (mg)	Recovery (%)
0.250	0.0403	0.0556	4.8	24.119	80.314
0.252	0.0403	0.0556	4.9	24.367	81.140
0.254	0.0403	0.0556	4.9	24.615	81.967
				ẋ	81,140

The solubility test of DXI nanocrystals showed a significant increase in solubility from 0.33 mg/mL to 14.719 mg/mL. The results were filtered and measured using a UV-Vis spectrophotometer. The results of the solubility test can be seen in Table 5, which shows that the solubility of DXI nanocrystal has 45.5-times more soluble than raw DXI.

Table 7. Solubility Calculation Results of DXI Nanocrystals for Raw DXI

Sample	Solubility (mg/mL)
Raw DXI	0.330±0.060
DXI Nanocrystals	14.719±0.040

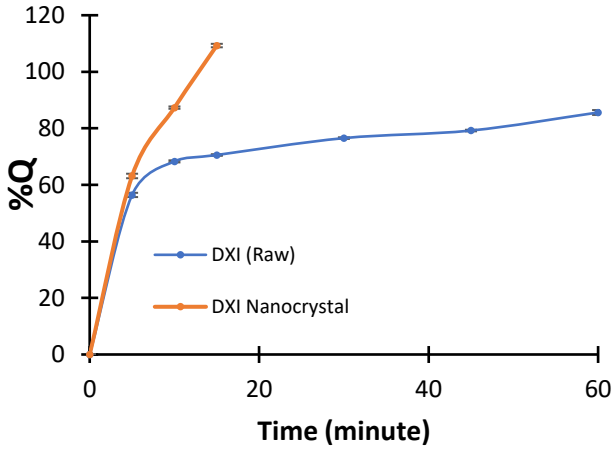
The solubility of a substance in the form of nanocrystals is generally higher, which is evident in the increased solubility of DXI nanocrystals. The increase in the solubility of DXI nanocrystals is also thought to be due to the change in particle size that becomes smaller than the raw DXI particles and may not be due to the addition of polymers and surfactants. The changing of particle to nano size which has an impact on increasing the surface area of the particle is the main thing for the purpose of nanocrystals formation, so as to increase the solubility and dissolution rate of drugs (Gigliobianco, 2018).

Dissolution Test

The result of dissolution test is shown in Figure 6. at 10 minutes, the amount of solute reached 80% for DXI nanocrystals and 68% for raw DXI. In addition, at 15 minutes, the amount of solute reached 100% for DXI nanocrystals and 71% for raw DXI. This was done to prove that DXI nanocrystals have a different dissolution profile than raw DXI.

Dissolution is defined as the amount of drug dissolved per time unit under standardized conditions, temperature, and medium composition. The increased solubility causes the dissolution profile of the DXI nanocrystals to show a greater amount of solute at a given time compared to raw DXI (Figure 6). Smaller particle shapes will cause an increase in solubility, thereby affecting the dissolution results (Ullah et al., 2018).

Figure 6. Dissolution Results of Raw DXI and DXI Nanocrystals in Phosphate Buffer pH 7.4



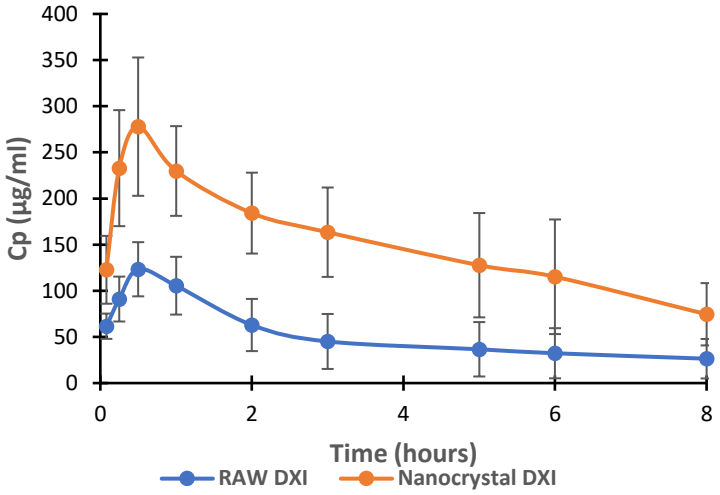
In Vivo Bioavaibility Test

The pharmacokinetic profiles DXI nanocrystal compare with DXI (raw) shown that the median tmax of raw DXI and DXI nanocrystals are 0.58 and 0.5, this shows that the two substances are the same. The results showed the Cmax (µg/ml) are 103.93 µg/ml and 320.43 ml respectively. The Cmax of raw DXI is lower than that of DXI nanocrystals this shows that the level of DXI nanocrystals in the blood is higher than DXI (raw). Furthermore, the AUC0–t for raw DXI is 245.59 µg/mL.hours and DXI nanocrystals 1194.76 µg/mL.hours indicating that the value of DXI nanocrystals in blood is higher than raw DXI. With these results it is possible to carry out further research regarding reducing the dose given to patients with the same indication.

Table 8. Calculation Results of Oral Raw DXI and DXI Nanocrystal Bioavailability Tests in Wistar Strain Male Rats

Sample	t_{max} (hours)	C_{max} (µg/ml)	AUC 0-8 h (µg/mL.hours)	AUC 0-∞ (µg/mL.hours)
Raw DXI	0.58±0.20	103.93±23.40	245.59±100.95	369.52±133.85
DXI nanocrystals	0.50±0	320.43±124.60	1194.76±224.00	1622.68±269.60

Figure 7. Comparison of the Pharmacokinetic Profiles of Raw DXI and DXI Nanocrystals in Wistar Male Rats



CONCLUSION

The optimized DXI nanocrystal was successfully manufactured using the anti-solvent precipitation method with conditions optimization result from Box-Behnken Design which consists of magnetic stirrer speed at 400 RPM, 3% of PVP K90, and 3% of Tween 80. The resulting DXI nanocrystal has a size of 118.83nm, with polydispersity index and zeta potential values of 0.422 and -186.6 mV, respectively. The DSC characterization found DXI nanocrystal powder to peak at 45.41°C. Furthermore, the results of the diffractogram in the PXRD test of the DXI nanocrystal powder showed a sharp intensity indicating that the DXI nanocrystals did not change into other physical forms. Finally, the oral bioavailability of DXI nanocrystals was better than raw DXI. The AUC0–t for raw DXI is 245.59 µg/mL.hours, and DXI nanocrystals are 1194.76 µg/mL. Hours indicate that the value of DXI nanocrystals in the blood is higher than raw DXI. With these results, it is possible to conduct further research regarding reducing the dose given to patients with the same indication.

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