Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 2, 2025, pp. 807-819.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

# COMPREHENSIVE SCREENING OF STABILIZERS AND SOLVENTS FOR CURCUMIN NANOSUSPENSION

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*Poorly soluble drugs are often a challenge in product formulation. Generally, low aqueous solubility and poor bioavailability of drugs have restricted their application in various fields. Nanosuspensions enhance the solubility and bioavailability of poorly water-soluble drugs. Curcumin, a hydrophobic polyphenol, exhibits poor aqueous solubility (<1 µg/mL), and formulating it as nanosuspension can enhance its solubility. However, the optimal selection of preparation technique, solvent, stabilizer, and drug-to-stabilizer ratio is critical for formulation success for a successful formulation. This work aims to optimize the preparation process of Curcumin nanosuspension. In addition, it aims in screening of the effectiveness of different stabilizers, solvents, and ratios of drug to stabilizers. Stabilizers tested included polymers (e.g., PVP30, HPMC) and surfactants (e.g., Tween 40, Poloxamer 407) in varying proportions of drug-to-stabilizer ratios were tried to choose the best stabilizer. Solvents were used as a mixture of acetone and ethanol or as a sole solvent, ethanol alone. The preparation of Curcumin (CRN) nanosuspension was accomplished by the solvent-antisolvent method using three different techniques: magnetic stirring, ultra-sonication bath, and ultra-sonication probe. Optimization of the selected formula was established by determination of particle size, polydispersity index, and zeta potential. In addition, the investigations included measurement of entrapment efficiency, CRN loading and pH. Stability test was conducted on Curcumin nanosuspension (the selected formula) over 2 months at 25˚C and 4˚C. Concerning the solvent, ethanol as a sole solvent was better than the acetone-ethanol mixture. Magnetic stirring yielded better results than ultra-sonication methods. The best nanosuspension formula, CRN-24, was prepared using Polyvinylpyrrolidone K30 (PVP30) at a 1:2 drug-to-stabilizer ratio with magnetic stirring and ethanol as the solvent. CRN-24 exhibited a particle size of 53.42 nm, PDI of 0.18, zeta potential of -15.21 mV, entrapment efficiency of 41.12%, and drug loading of 87.19%. Stability tests showed acceptable stability at 4˚C. The main conclusion of this study is that optimal solvent, stabilizer, and drug-to-stabilizer ratio are essential for stable curcumin nanosuspensions*

***Keywords:*** *Curcumin; Nanosuspension; Polyvinylpyrrolidone 30; Solvent-antisolvent technique; Stabilizer*

## INTRODUCTION

Nanosuspensions are colloidal dispersions of drug particles typically below 1 µm, represent a promising strategy to address the challenge of poor water solubility in nearly 40% of novel chemical medications. These finely dispersed colloids consist of solid drug particles smaller than one micrometer, typically ranging from 200 to 600 nm in size[1,2]. Nanosuspension composed of pure medication and stabilizers, which prevent particle aggregation and Ostwald ripening via steric or electrostatic repulsion[2,3].

The preparation of nanosuspension involves several critical factors that influence their stability and efficacy. One of the primary challenges is the low physical stability, where the nanoparticles tend to agglomerate or to undergo crystal growth. To mitigate these

*Received : 7/2/2025 & Accepted : 13/5/2025*

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issues, stabilizers; with steric or electrostatic effects such as Polyoxyethylene sorbitan ester (Tweens), hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), and D-α-tocopheryl polyethylene glycol succinate (TPGS), are employed. These stabilizers play a crucial role in maintaining the dispersion of nanoparticles and preventing aggregation[5-7]. It is worth to mention that not only the type of stabilizer is important, but also the ratio of drug to polymer plays a significant role in formulation of nanoparticles [8, 9].

One of the common methods used for nanosuspension preparation is solvent/anti-solvent method, which involves dissolving the target material in a solvent and adding an anti-solvent, which decreases the solubility of the material and causes precipitation. This method is especially useful in pharmaceutical development, where it increases the bioavailability of poorly soluble drugs by forming nano-crystals[10].

A significant factor in solvent/anti-solvent method for nanosuspension preparation is the choice of solvents used in the preparation process. The solvents must be carefully selected to ensure the solubility of the drug and the compatibility with the stabilizers. Solvent selection must ensure drug solubility and compatibility with stabilizers to achieve a stable nanosuspension with enhanced drug saturation solubility and dissolution rate [3,10]. Curcumin (CRN) (chemical structure presented in **(Fig. 1)**, which is derived from the rhizomes of the *Curcuma longa* plant, is responsible for turmeric's yellow color, physicochemical characteristics, and the biological activity of turmeric.

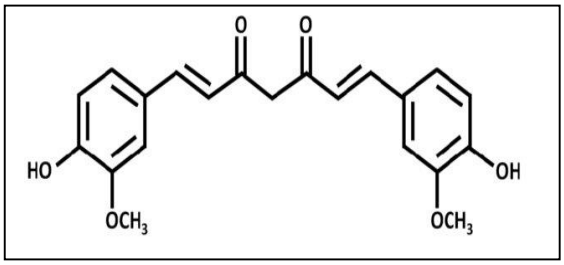


**Fig 1**: Molecular structure of curcumin[11].

A previous study explored three distinct methodologies—nano-suspension (Cur-NSM), sonication (Cur-SM), and anti-solvent precipitation (Cur-ASP)—for synthesizing curcumin nanoparticles without employing nanocarriers, aiming to enhance curcumin's water solubility. The three techniques demonstrated a significant improvement in curcumin's aqueous solubility, increasing it from 0.98 μg $mL^{-1}$ to 79.2 μg $mL^{-1}$. The results confirm that the reduction of particle size is a crucial strategy for boosting bioavailability. This research provides valuable insights into the development of curcumin formulations, emphasizing the role of innovative approaches to overcome its inherent solubility challenges and expand its therapeutic potential [12].

Beyond its culinary uses, CRN has been extensively studied for its medical applications including antimicrobial, antioxidant, hepatoprotective, antihyperlipidemic, and antiviral properties[12,13]. While traditional medicine has utilized CRN to reduce inflammation and promote tissue repair and wound healing. Interestingly, CRN exhibits anticancer properties against prostate, breast, colon, breast and lung malignancies [15–18].

In previous studies, Curcumin was evenly distributed utilizing the kneading and solvent evaporation techniques in the polymeric matrix of polyethylene glycol (PEG 6000), hydroxypropyl methyl cellulose (HPMC E5), polyvinyl pyrrolidine (PVP K30), and bovine serum albumin (BSA) to create the solid dispersion, which concluded that The best option to formulate solid amorphous curcumin dispersions that could be used as a successful delivery method was the HPMC E5 formulation[19].

Another previous study aimed to prepare a nanosuspension loaded with CRN using a statistical design approach to improve the oral bioavailability of CRN, and then to develop CRN nanosuspension coated with tannic acid to increase the mucoadhesion in the GI tract. This study stated that the investigated surfactants included sodium dodecyl sulfate (SDS), D-α-Tocopherol, Cremophor EL, Tween 20, Tween 80, Poloxamer 407, Poloxamer 188, and CTAB. Among these, SDS was selected as the surfactant for preparing CRN nanosuspension as it showed the highest solubility of curcumin[20].

Recent studies have also explored CRN potential to enhance antibody production following COVID-19 vaccination [21]. However, CRN suffered from low solubility and consequent low bioavailability that restrict their uses in many applications. Several trials were conducted to formulate CRN in nanoform to improve the solubility [22,23].

This study aims to optimize CRN nanosuspension preparation by evaluating stabilizers, solvents, and drug-to-stabilizer ratios. This could pave the way for better utilization of CRN in various therapeutic areas, addressing the limitations of its poor water solubility and enhancing its overall clinical efficacy.

## MATERIALS AND METHODS

### Materials

Curcumin was purchased from Hebei Food Additive Co. Ltd. (China), Acetone and methanol were purchased from Chem-Lab NV (Belgium), Ethanol was procured from Tedia Company, USA, Acacia, Carbopol 934 (Carb-934), Polyvinylpyrrolidone 25 (PVP25), Polyvinylpyrrolidone 30 (PVP30), Polyoxyethylene (20)- sorbitan monopalmitate (Tween 40) obtained from BDH chemicals Ltd, England. Poloxamer 407 was a gift from (Ludwigshafen, Germany). Hydroxy propyl methyl cellulose (HPMC) methocel E5 was obtained from Pioneer Company for pharmaceutical industries, Sulaymaniyah, Iraq.

### Preparation and Optimization of Curcumin Nanosuspension

### Preparation and Optimization of CRN Nanosuspension

Thirty nanosuspension of CRN were prepared using the selected stabilizer. Twenty-two of them prepared by using a mixture of solvents as the solvent for dripping while eight formula prepared by using ethanol alone as illustrated in **(Table 1)**.

10 mg of CRN was dissolved in 2 mL of solvent, whether a mixture of acetone and ethanol in (3:7 v/v) ratio or ethanol alone. Then CRN solution was dripped to 28 ml distilled water containing a stabilizer using different methods.

**Table 1:** Curcumin Nanosuspension Formulae and Preparation Conditions.

| Formula code | Stabilizer (Polymer/ surfactant) | curcumin: stabilizer ratio | Type of Solvent | Magnetic stirrer for 2.5 h | Ultra-sonication bath | Ultra-sonication probe |
|---|---|---|---|---|---|---|
| CRN-1 | Acacia | 1:4 | Ac/Eth | 1000 | - | |
| CRN-2 | PVP25 | 1:8 | Ac/Eth | 1000 | - | |
| CRN-3 | Carb-934 | 1:4 | Ac/Eth | 1000 | - | |
| CRN-4 | Poloxamer 407 | 1:4 | Ac/Eth | 1000 | - | |
| CRN-5 | Poloxamer 407 | 1:4 | Ac/Eth | - | 20 min | |
| CRN-6 | Tween 40 | 1:4 | Ac/Eth | - | 20 min | |
| CRN-7 | HPMC | 1:1 | Ac/Eth | 1000 | - | |
| CRN-8 | HPMC | 1:2 | Ac/Eth | 1000 | - | |
| CRN-9 | HPMC | 1:1 | Ac/Eth | - | 20 min | |
| CRN-10 | HPMC | 1:1 | Ac/Eth | | | 3 min |
| CRN-11 | HPMC | 1:2 | Ac/Eth | | | 3 min |
| CRN-12 | PVP30 | 1:1 | Ac/Eth | 1000 | - | - |
| CRN-13 | PVP30 | 1:2 | Ac/Eth | 1000 | - | - |
| CRN-14 | PVP30 | 1:4 | Ac/Eth | 1000 | - | - |
| CRN-15 | PVP30 | 1:1 | Ac/Eth | - | 20 min | - |
| CRN-16 | PVP30 | 1:2 | Ac/Eth | - | 20 min | - |
| CRN-17 | PVP30 | 1:4 | Ac/Eth | - | 20 min | - |
| CRN-18 | PVP30 | 1:4 | Ac/Eth | - | - | 1 min |
| CRN-19 | PVP30 | 1:4 | Ac/Eth | - | - | 2 min |
| CRN-20 | PVP30 | 1:2 | Ac/Eth | - | - | 3 min |
| CRN-21 | PVP30 | 1:4 | Ac/Eth | - | - | 3 min |
| CRN-22 | PVP30 | 1:4 | Ac/Eth | - | - | 5 min |
| CRN-23 | PVP30 | 1:1 | Eth | 1000 | - | - |
| CRN-24 | PVP30 | 1:2 | Eth | 1000 | - | - |
| CRN-25 | PVP30 | 1:1 | Eth | - | 20 min | - |
| CRN-26 | PVP30 | 1:2 | Eth | - | 20 min | - |
| CRN-27 | PVP30 | 1:4 | Eth | - | | 1 min |
| CRN-28 | PVP30 | 1:4 | Eth | - | | 2 min |
| CRN-29 | PVP30 | 1:4 | Eth | - | | 3 min |
| CRN-30 | PVP30 | 1:4 | Eth | - | | 5 min |

*CRN: Curcumin nanosuspension formula, PVP30: Polyvinylpyrrolidone 30, Ac/Eth: a mixture of 3:7 of acetone-to-ethanol ratio, Eth: ethanol alone.*

**Factors affecting the preparation of CRN Nanosuspension**

***Types of stabilizers***

The selection of the best stabilizer will maintain the physical and chemical stability of CRN by preventing aggregation and degradation. Different dispersions of CRN were prepared using different stabilizers. Stabilizers like surfactants (e.g. Polysorbates such as Tween 40 and Poloxamer 407). Polymers (e.g. HPMC, Methocel E5, Carbomer-934, PVP25, PVP30), and natural gums (e.g., Acacia) were utilized in this study to prepare CRN nanosuspension by solvent-antisolvent method.

**Factors affecting the preparation of Curcumin Nanosuspension**

***Drug-to-stabilizer ratio***

Different dispersions of CRN were prepared by accurately weighing the stabilizer in accordance with CRN at ratios of 1:1, 1:2, and 1:4 (CRN: stabilizer).

***Type of solvent***

Solvents such as acetone/ ethanol (Ac/Eth) mixture and ethanol alone were utilized based on their polarity, toxicity, and volatility to ensure adequate solubility of CRN. CRN's solubility was observed in different solvents (ethanol, methanol, and acetone). CRN is soluble in ethanol, but its solubility was enhanced enormously in the presence of acetone [24,25].

***Production Technique or Method***

***Magnetic stirring technique***

During this technique, solvent was added dropwise at 0.5 mL/min, using a 0.5 mL syringe. Magnetic stirring was conducted using the magnetic stirrer (Fisher Scientific, Korea) at 1000 rpm and at room temperature for 2-3 h. It is very important to keep the procedure of dripping protected from light and after completion, the content was kept in a dark closed container [26].

***Ultra-sonication bath***

The prepared dispersions were kept on magnetic stirrer at 500 rpm for nearly 5 min, and then the sample was sonicated in an ultrasonication bath (Power sonic 410, Korea) for 20 min at 25ºC. Light-resistant containers were used to maintain CRN stability [27].

***Ultra-sonication probe***

In this case, dripping was conducted while stirring using magnetic stirrer is continuous at 500 rpm for 15 minutes. After that the product was transferred into a 20-mL tube and a portable Ultra-sonication probe (Lab Grade Handheld with Standard Probe, China) was inserted into the tube. Each sample was treated with different on/off cycle for different duration (1, 2, 3, and 5 minutes) and all sample were cooled with iced water during the sonication [28]. Protection from light was kept in mind during all the stages of work.

**Characterization of CRN Nanosuspension**

***Drug loading and Entrapment efficiency***

A small volume of the optimized formula was diluted with methanol, then held over ultra-sonication water bath for 1.5 h, then absorbance was measured using (UV Spectrophotometer/ EMC LAB-11S, Germany) at λmax of 240 nm [29]. Calculation of drug loading was according to the following equation:

$$\text{Drug Loading\%} = \frac{\text{Actual drug loading}}{\text{Theorotical drug loading}} \times 100 \ldots . \quad (1)$$

The entrapment efficiencies (EEs) of the optimized nanosuspension **(Table 2)** were calculated by measuring the amount of drug encapsulated within or adsorbed into the nanoparticles. It was determined by measuring the concentration of the free form of the drug in the dispersion medium. A suitable amount of CRN (1.5 mL) was centrifuged at 13,500 rpm for 2.5 h at 4˚C by refrigerated Micro-centrifuge using Eppendorf test tube to separate the nanoparticles. The supernatant was taken, diluted suitably and the absorbance was measured at 240 nm against the blank (methanol) using a spectrophotometer to determine the concentration of the free form of the drug. The EE% was calculated in equation (2):

$$\text{EE\%} = \frac{\text{Initial drug loading} - \text{Free CRN concentration}}{\text{Initial drug loading}} \times 100 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2)$$

where, initial drug loading is the concentration of the drug used to prepare the formula, and Free CRN concentration is the concentration of the drug that was detected in the supernatant after micro-centrifugation [26].

***Measurements of particle size, polydispersity index, and zeta potential***

The particle size and polydispersity index (PDI) of all the prepared CRN nanosuspensions were determined using dynamic light scattering (DLS) technology using Malvern Zetasizer, ultra-red (Malvern Instruments, Malvern, UK) at 25˚C and at a scattering angle of 173˚. Size distribution analysis of the measurement accomplished based on the intensity. The zeta potential was determined for the selected nanosuspension by utilizing disposable zeta cells using the electrophoretic light scattering mobility technique (Zetasizer ZS3600, Malvern Instruments, Malvern, UK). The samples of CRN nanosuspension were sufficiently diluted with deionized water for the determination of particle size, PDI, and zeta potential [30].

***pH***

The *p*H of the optimized formula of nanosuspension was measured using pH20 Tester, APERA® Instruments, China, following the provided instructions.

***Stability Study***

Stability studies were conducted on the selected optimized formula, which showed satisfactory *in vitro* performance for various evaluation parameters. These nanosuspension were subjected to stability studies by storing this formula in refrigerator (at 4˚C) and at room temperature for 2 months. The optimized nanosuspension was examined for various physicochemical parameters, like *p*H, particle size and PDI.

**Statistical Analysis**

All the data are expressed as mean± SD of at least three independent experiments. The statistical evaluation was performed through one-way analysis of variance (ANOVA) using the minitab١٩ program for windows. Significant and highly significant difference was considered at *p*-values of 0.05 and 0.01, respectively.

## RESULTS AND DISCUSSION

**Preparation of Curcumin Nanosuspension**
**Preparation of CRN Nanosuspension**

Different stabilizers were tried as shown in (**Table1**). The samples exhibiting precipitation on the walls of their containers (example is presented in **(Fig. 2)** were excluded from further investigation. High-viscosity polymers like Carb-934 and HPMC, as well as surfactants like Tween 40, resulted in poor stabilization and large particle sizes [31].

Only PVP30 produced particles <200 nm; others exceeded 500 nm" for quantitative specificity, were excluded due to the production of large particle sizes; nearly exceeding 500 nm as tested by zeta sizer and this was not accepted as nanosuspension.

PVP30's higher molecular weight likely provides better steric stabilization than PVP25 among all the investigated polymers and surfactants. This is consistent with a work conducted to prepare CRN nanoparticle using different stabilizers, where they found that PVP30 is the most efficient stabilizer to maintain the stability of CRN nanoparticle [32]. As a non-ionic polymer, PVP, act through steric stabilization mechanism. The hydrophobic sides of PVP will cover the surface of particles and the interactions will be decreased to a level that the van der Waals forces will be less than the forces of repulsion. In addition, the hydrophilic tail of PVP will extend towards the bulk media and enhances the solubility of CRN (as a hydrophobic particle)[33].

**Factors affecting the preparation of CRN Nanosuspension**

***Drug-to-stabilizer ratio***

PVP30 was selected as stabilizers for further studies. Different dispersions of CRN and stabilizer (PVP30) at ratios of 1:1, 1:2, and 1:4 resulted in varying particle sizes across different precipitation techniques **(Table 2)**. Concerning the formula that prepared through using a magnetic stirrer technique (CRN-12, CRN-13 and CRN-14) as presented in **(Table 2)**, the results were acceptable in terms of particle size and polydispersity index (PDI). The same ratios achieved appropriate particle size reduction with the ultra-sonication bath technique (CRN-15 to CRN-17). Concerning the nanosuspension prepared with the ultra-sonication probe precipitation technique (CRN-18 to CRN-21), only the 1:4 ratio produced acceptable results. The results also indicated that the particle sizes obtained with a drug-to-stabilizer ratio of 1:4 were not acceptable for all the investigated polymers and surfactants as illustrated in **(Table 2)**. Significant differences ($p<0.05$) were observed between

nanosuspension prepared with ratios of 1:4 when compared with nanosuspension prepared with 1:2 or 1:1 ratio. Among these, a 1:2 ratio minimized particle size (53.42 nm) and PDI (0.18), yielding the smallest particle sizes and thus being the most recommended drug-to-stabilizer ratio for this study.

A comparison between CRN-23 and CRN-24, which prepared through same technique demonstrated how the change in drug to stabilizer ratio affects on the particle size. CRN-24 prepared with 1:2 ratio while CRN-23 prepared with 1:1 ratio. Increasing the polymer ratio resulted in dramatic reduction in particle size and dramatic reduction in PDI, which indicate better and uniform particle size distribution. Similar work conducted on esomeprazole indicated that the particle size shifted to lower value with the increasing of stabilizer ratio (pluronic F68) (34). The data presented in **(Table 2)** shows that nanosuspension with smaller particle sizes (e.g. CRN-24, CRN-14, CRN-16 and CRN-17) and with low PDI values (<0.36) are more uniform and likely more stable, which is advantageous for drug delivery due to their higher surface area and consistent performance. In contrast, nanosuspension with larger particle sizes (e.g. CRN-4, CRN-7 and CRN-15) with higher PDI values (>0.5) indicate significant aggregation and broader size distribution, suggesting potential stability problems and inconsistent drug release. In a study conducted by Li *et al*., CRN nanosuspensions were formulated using both PVPK30 and SDS as stabilizers to improve solubility and stability. The resulting nanosuspension exhibited particle sizes between 100 nm and 200 nm, with PDI values under 0.3, indicating a uniform particle size distribution and good stability [35,36].

**Table 2:** Particle size and PDI for all the prepared CRN formulae.

| Formula Code | Polymer name | curcumin: Polymer ratio | Z- Average (nm) | PDI |
|---|---|---|---|---|
| CRN-1 | Acacia | 1:4 | 1466 | 0.003 |
| CRN-2 | PVP25 | 1:8 | 604 | 0.881 |
| CRN-3 | Carb-934 | 1:4 | 1.238E+04 | 0.593 |
| CRN-4 | Poloxamer 407 | 1:4 | 538.4 | 0.451 |
| CRN-5 | Poloxamer 407 | 1:2 | EX | EX |
| CRN-6 | Tween40 | 1:4 | 1.013E+04 | 0.755 |
| CRN-7 | HPMC | 1:1 | 2.771E+04 | 0.602 |
| CRN-8 | HPMC | 1:1 | 2.081E+04 | 0.387 |
| CRN-9 | HPMC | 1:2 | 2.071E+04 | 0.419 |
| CRN-10 | HPMC | 1:1 | 723.2 | 0.523 |
| CRN-11 | HPMC | 1:2 | 2670 | 0.818 |
| CRN-12 | PVP30 | 1:1 | EX | EX |
| CRN-13 | PVP30 | 1:2 | 155.2 | 0.367 |
| CRN-14 | PVP30 | 1:4 | 3613 | 1.656 |
| CRN-15 | PVP30 | 1:1 | EX | EX |
| CRN-16 | PVP30 | 1:2 | 110 | 0.320 |
| CRN-17 | PVP30 | 1:4 | 481.3 | 0.498 |
| CRN-18 | PVP30 | 1:4 | EX | EX |
| CRN-19 | PVP30 | 1:4 | EX | EX |
| CRN-20 | PVP30 | 1:2 | EX | EX |
| CRN-21 | PVP30 | 1:4 | 265.6 | 0.409 |
| CRN-22 | PVP30 | 1:4 | EX | EX |
| CRN-23 | PVP30 | 1:1 | 574.8 | 0.486 |
| CRN-24 | PVP30 | 1:2 | 53.42 | 0.187 |
| CRN-25 | PVP30 | 1:1 | 53.78 | 0.114 |
| CRN-26 | PVP30 | 1:2 | 887 | 0.732 |
| CRN-27 | PVP30 | 1:4 | 39.04 | 0.441 |
| CRN-28 | PVP30 | 1:4 | 47.73 | 0.360 |
| CRN-29 | PVP30 | 1:4 | 379.5 | 0.398 |
| CRN-30 | PVP30 | 1:4 | 592.3 | 0.494 |

*CRN: Curcumin nanosuspension, PVP30: Polyvinylpyrrolidone 30, EX=Excluded.*

**Fig 2:** Example on failed CRN formulae using HPMC as stabilizer.

As a result, the positive correlation between particle size and PDI implies that as particle size increases, the uniformity decreases, highlighting the importance of optimizing both parameters for effective formula. The drug loading and entrapment efficiency are the crucial parameters in evaluating the effectiveness of a nanosuspension formula. The analysis of data reveals that the drug loading % range from 7.28% in CRN-2 to 99.85% in CRN-30. The highest (EE%) observed in CRN-13 (84.86%) and the lowest detected in CRN-15 (6.34%). The magnetic stirring technique generally resulted in high drug loading but variable EE%, while the ultra-sonication bath technique showed moderate to high drug loading with moderate EE%. The ultra-sonication probe technique achieved high drug loading with variable EE%. This may be attributed to the high energy that converted from ultrasonic wave into a microscopic vacuum bubbles. The energy produced by probe sonication is more efficient than those produced by bath sonication[37].

Different organic solvents were used for dripping, either ethanol alone or a mixture of (Ac/Eth) (ratio of 3:7 mixture). The dissolving of CRN in case of (Ac/Eth) mixture was faster than when ethanol used alone. Looking at the nanosuspension prepared with the same technique and with same ratio of stabilizer but using ethanol alone (CRN-12, CRN-13) or (Ac/Eth) mixture (CRN-23, CRN-24), yield significant difference in particle size ($p<0.05$). Comparing other nanosuspension that prepared with different techniques and different ratio of stabilizer also yield significant difference in particle size when they prepared using different solvents. The effect of solvent was also observed in other nanosuspension. This indicate that solvent selection is critical step in the formula. Significant difference ($p<0.05$) observed between the particle size of CRN-2 and CRN-24 indicate that Ethanol alone produced smaller particles (53.42 nm) than the acetone-ethanol mixture (604 nm). In addition, the PDI in CRN-24 is less than that observed with CRN-2, which indicates less heterogeneity in particle size distribution. Moreover, ethanol is less toxic than acetone and it is preferable to be used in pharmaceutical preparations.

Regarding *p*H test, the decomposition of CRN is *p*H-dependent, occurring more rapidly under neutral to basic conditions [25]. In this study, the *p*H values of the CRNs exhibit significant differences based on the solvent systems used. nanosuspension CRN-12 to CRN-22, which utilized a mixture of (Ac/Eth), maintained a narrow *p*H range (7.10 to 7.47), suggesting a consistent slightly basic environment conducive to CRN stability as illustrated in **(Table 3)**.

Conversely, nanosuspension CRN-23 to CRN-30, prepared with ethanol alone, displayed a broader *p*H range (6.01 to 8.27), indicating less effective buffering and potential stability issues. These differences confirm the significance of the solvent system on *p*H variation. This finding aligns with previous

research by Li *et al.*, where their nanosuspension of CRN with PVP30 and SDS showed consistent pH and stability [35]. Additionally, a study by Kanwall *et al.* demonstrated that CRN nanoparticles exhibited enhanced stability and solubility when prepared with appropriate stabilizers, further supporting the importance of solvent and stabilizer selection in nanosuspension formula [38]. The possible explanation for the successful use of PVP30 is the possible formation of negative charges on the particles that generate strong forces of repulsion among the nanoparticles that maintain their stability [32].

The selected formula, CRN-24, exhibited a *p*H of 6.6, which is slightly acidic. The slightly acidic *p*H of CRN-24 could influence the stability of CRN, potentially enhancing its stability when used for topical drug delivery system [38].

***Production Technique or Method***

Nanosuspension prepared using magnetic stirrer method were more acceptable than other nanosuspension prepared by other methods. Furthermore, nanosuspension with ethanol / ultra-sonication probe method (CRN-27 to CRN-30) showed that increasing the time of sonication resulted in a proportional increase in particle size and resulted in an unacceptable large size with the 5-minute formula. This is in contradiction with other researches finding where increasing the time of sonication resulted in smaller particle size [39]. Indeed, these nanosuspension (CRN-27 to CRN-30) were prepared already with high drug to polymer ratio, so the prepared particle size is expected to be large even with relatively long time of sonication.

**Selection of the Best Formula**

As an optimized formula, CRN-24 was selected due to its high loading capacity and acceptable EE%. In addition, it exhibited a very good particle size (53.42 nm) and low PDI (0.18), which might indicate homogeneous distribution of the particle sizes **(Fig. 3)**. The Zeta potential of the selected formula is very good (-15.21) as shown in **(Fig. 4)**. This reflects good stability and possible resistance to particle aggregation and agglomeration with consequent enhanced stability and prolong shelf life [33].

**Table 3:** Zeta potential, pH, Drug Loading, and entrapment efficiency of the optimized CRNs.

| Formula Code | *p*H | Z-Average (nm) | Zeta Potential (mV) mean | Drug loading% | EE% |
|---|---|---|---|---|---|
| CRN-12 | 7.47 | EX | -14.51 | 96.29 | 31.68 |
| CRN-13 | 7.32 | 155.2 | -10.1 | 7.28 | - |
| CRN-14 | 7.22 | 3613 | -16.12 | 42.11 | 84.86 |
| CRN-15 | 7.30 | EX | -0.869 | 88.16 | 17.31 |
| CRN-16 | 7.40 | 110 | -1.533 | 89.69 | 6.34 |
| CRN-17 | 7.33 | 481.3 | -15.11 | 97.65 | 34.50 |
| CRN-18 | 7.10 | EX | -9.824 | 93.56 | 30.88 |
| CRN-19 | 7.34 | EX | -17.34 | 63.17 | 34.55 |
| CRN-20 | - | EX | EX | EX | EX |
| CRN-21 | - | 265.6 | EX | EX | EX |
| CRN-22 | 7.46 | EX | EX | EX | EX |
| CRN-23 | 6.74 | 574.8 | -23.56 | 99.50 | 58.26 |
| CRN-24 | 6.60 | 53.42 | -15.21 | 87.19 | 41.12 |
| CRN-25 | 6.83 | 53.78 | -5.727 | 76.37 | 23.69 |
| CRN-26 | 6.01 | 887 | -15.21 | 99.60 | 53.177 |
| CRN-27 | 8.10 | 39.04 | -1.82 | 87.87 | 18.01 |
| CRN-28 | 7.92 | 47.73 | -1.202 | 75.00 | 7.58 |
| CRN-29 | 8.27 | 379.5 | -9.824 | 89.46 | 26.34 |
| CRN-30 | 8.12 | 592.3 | -17.34 | 99.85 | 79.95 |

*CRN: Curcumin nanosuspension, EX=Excluded, EE%: entrapment efficiency*

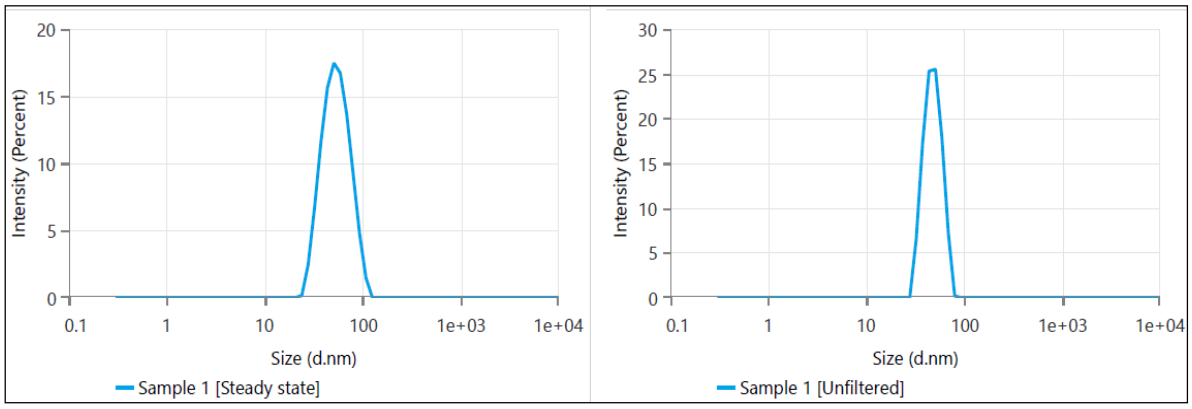


**Fig 3:** Particle size distribution analysis of the selected formula (CRN-24).

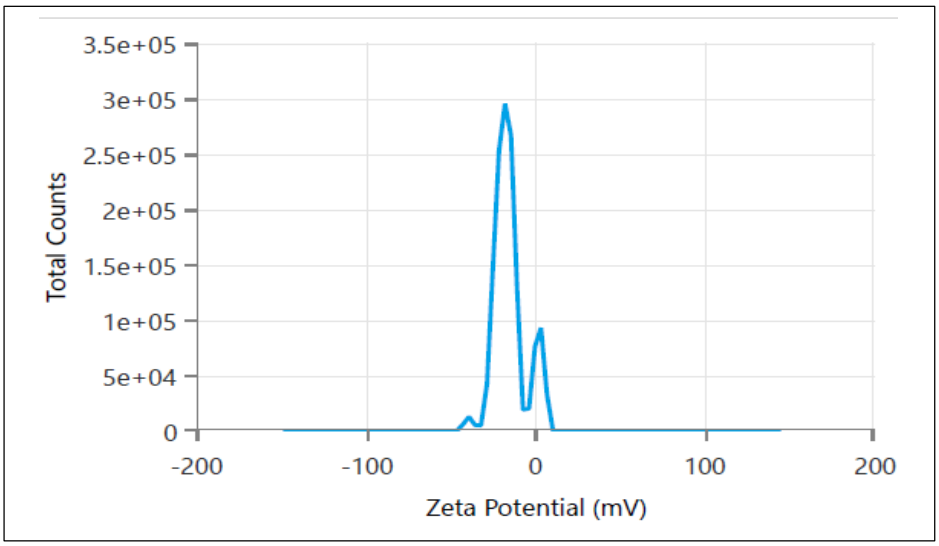


**Fig 4:** Zeta potential of the selected formula (CRN-24).

**Stability study**

Stability study was conducted on the selected formula only (CRN-24), where the formula kept at room temperature and fridge (4˚C). *p*H measurements after two months for the selected formula at room temperature and fridge were 6.5 at both conditions. **(Table 4)** presented the particle size, PDI and Zeta potential of the selected formula at room temperature (25˚C) and fridge (4˚C) at different time intervals. At 4°C, particle size increased from 53.42 nm to 103.3 nm over 8 weeks, remaining within acceptable limits than at room temperature. At fridge, although a change in particle size is observed, however the change is not very high and the formula could be considered stable and acceptable after storing at fridge for two months. Further prolong stability study is recommended to confirm these preliminary results. Regarding the stability at room temperature, there is more than doubling of particle size after two weeks and further increase in size after 4 weeks. Nevertheless, all the readings are still within the nano-scale and considered accepted values for preparation of nanosuspension.

**Table 4:** Stability study of the selected formula (CRN-24).

| | CRN-24 (R.T.) | | CRN-24 (4˚C) | |
|---|---|---|---|---|
| **Time (weeks)** | **Z-Average (nm)** | **(PDI)** | **Z-Average (nm)** | **(PDI)** |
| **0 weeks** | 53.42 | 0.1876 | 53.42 | 0.1876 |
| **2 weeks** | 148.1 | 0.1434 | 80.17 | 0.2108 |
| **4 weeks** | 160.3 | 0.1788 | 132 | 0.3873 |
| **6 weeks** | 171 | 0.2208 | 130.7 | 0.1163 |
| **8 weeks** | 119.9 | 0.2957 | 103.3 | 0.2735 |

## Conclusion

Optimal curcumin nanosuspension requires ethanol, PVP30 at a 1:2 ratio, and magnetic stirring. In this work, the selected solvent was ethanol and it was performing better than a mixture of acetone and ethanol. As a stabilizer, PVP 30 exhibit the best characteristics and a good formula prepared when 1:2 of CRN to PVP was used. The method of production had a crucial effect on the physical properties of the CRN. CRN-24, which is prepared through magnetic stirrer, was selected as the preferred formula for further stability studies due to its very low particle size (53.42 nm), low PDI (0.18) and acceptable Zeta potential (-15.21). CRN-24 remained stable at 4°C for 2 months, with minimal particle growth. However, further studies on the release of CRN were required.

## Acknowledgment

The authors express their sincere gratitude to the College of Pharmacy, University of Mosul, for providing research facilities that significantly contributed to this study. They also thank the College of Pharmacy, University of Baghdad, for their expertise and access to advanced equipment and resources, which were instrumental in our research endeavors.

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Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 2, 2025, pp. 807-819.

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# الفحص الشامل للمثبتات والمذيبات لتكوين معلق النانوكركمين

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الأدوية ذات الذوبانية الضعيفة غالبًا ما تشكل تحديًا في صياغة المنتجات.بشكل عام، قلة الذوبانية المائية وانخفاض التوافر البيولوجي للأدوية قد قيدا تطبيقها في مجالات مختلفة.تعزز النانو معلقات ذوبانية وتوافر الأدوية ذات الذوبانية الضعيفة في الماء.الكركمين، وهو بوليفينول كاره للماء، يظهر ذوبانًا مائيًا ضعيفًا 1>) ميكروغرام/مل)، وتشكيله على هيئة نانو suspension يمكن أن يعزز ذوبانه.ومع ذلك، فإن الاختيار الأمثل لتقنية التحضير، والمذيب، والمثبت، ونسبة الدواء إلى المثبت هو أمر حاسم لنجاح التركيبة.يهدف هذا العمل إلى تحسين عملية تحضير نانو تعليق الكركمين.بالإضافة إلى ذلك، يهدف إلى فحص فعالية المثبتات المختلفة، والمذيبات، ونسب الدواء إلى المثبتات.المثبتات التي تم اختبارها شملت البوليمرات (مثلPVP30 ، HPMC) والعوامل السطحية (مثلTween 40 ، Poloxamer 407) بنسب متفاوتة من نسب الدواء إلى المثبت لاختيار أفضل مثبت.تم استخدام المذيبات كمزيج من الأسيتون والإيثانول أو كمذيب منفرد، الإيثانول وحده.تم تحضير نانو تعليق الكركمين (CRN) باستخدام طريقة المذيب–المذيب المضاد باستخدام ثلاث تقنيات مختلفة: التحريك المغناطيسي، حمام الموجات فوق الصوتية، ومسبار الموجات فوق الصوتية.تم تحديد تحسين التركيبة المختارة من خلال تحديد حجم الجسيمات، ومؤشر التشتت المتعدد، وإمكانات زيتا.بالإضافة إلى ذلك، شملت التحقيقات قياس كفاءة الاحتجاز، وتحميلCRN ، ودرجة الحموضة.تم إجراء اختبار الاستقرار على تعليق الكركمين النانوي (الصيغة المختارة) لمدة شهرين عند ٢٥°م و٤°م.فيما يتعلق بالمذيب، كان الإيثانول كمذيب وحيد أفضل من خليط الأسيتون–الإيثانول.أدى التحريك المغناطيسي إلى نتائج أفضل من طرق الموجات فوق الصوتية.تم تحضير أفضل صيغة نانو معلق، CRN-24، باستخدام بولي فينيل بيروليدون (PVP30) K30 بنسبة ١:٢ من الدواء إلى المثبت مع التحريك المغناطيسي والإيثانول كمذيب.أظهرت CRN-24 حجم جزيئات يبلغ ٥٣,٤٢ نانومتر، و PDI بقيمة ٠,١٨، وإمكانات زتا بقيمة –١٥,٢١ مللي فولت، وكفاءة احتجاز بنسبة ٤١,١٢%، وتحميل دوائي بنسبة ٨٧,١٩%.أظهرت اختبارات الاستقرار استقرارًا مقبولًا عند ٤°م.الاستنتاج الرئيسي لهذه الدراسة هو أن المذيب المثالي، والمثبت، ونسبة الدواء إلى المثبت ضرورية للحصول على نانو معلقات كركم مستقرة.