IJPSR (2021), Volume 12, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 February 2020; received in revised form, 12 May 2020; accepted, 22 May 2020; published 01 February 2021

FABRICATION AND CHARACTERIZATION OF NANOSUSPENSION FORMULATION OF DIOSMIN FOR ENHANCED ORAL DELIVERY

Md. Ali Mujtaba [*1], Lina Eltaib [1], Naira Nayeem Akmal [2] and Khidir A. M. Hassan [1]

Department of Pharmaceutics [1], Department of Pharmaceutical Chemistry [2], Faculty of Pharmacy, Northern Border University, Kingdom of Saudi Arabia.

Keywords:

Diosmin, Nansuspension, Lyophilization, Drug delivery, Solubility

Correspondence to Author:
Md. Ali Mujtaba

Assistant Professor,
Department of Pharmaceutics,
Faculty of Pharmacy, Northern Border University, Kingdom of Saudi Arabia.

E-mail: mmujtaba@nbu.edu.sa

ABSTRACT: Nanosuspension is a biphasic system consisting of drug particles dispersed in an aqueous surfactant or polymeric solution with a particle size between 10 to 1000 nm. Nanosuspension offers the unique advantage of increasing solubility of the pure drug resulting into faster drug absorption and hence achieving faster maximum plasma concentration. Hence, the aim of this research work was to formulate and characterize surfactants/polymer-stabilized nanosuspensions of diosmin (DSN), a phytomedicine, to surpass its poor physicochemical properties and low oral bioavailability. Nanosuspensions were prepared by probe sonication with varying concentrations of different surfactants and polymers such as PVP K 25, poloxamer 188, Tween 80, and PEG 400. Nanosuspension prepared with 3% w/v PEG 400 and 3% w/v DSN, exhibited the lowest size of 203.9 nm, PDI of 0.219, and adequate zeta potential of -27.26 mV, which was subjected to solubility and *in-vitro* drug release study. The highest solubilizing capacity of DSN was manifested in orthophosphate buffer pH 12. Approximately five times increase in saturation solubility of lyophilized nanosuspension was observed than that of an unprocessed drug. DSN nanosuspensions showed a significantly enhanced release rate compared with pure DSN in orthophosphate buffer pH 12, and this was due to a decrease in particle size. Conclusively, novel DSN nanosuspension could successfully improve its solubility and dissolution characteristics with promising consequences of better drug delivery.

INTRODUCTION: Phytopharmaceuticals stand out as recent promising candidates for the treatment of chronic diseases and cancer. Fewer side effects and lower phytochemical costs from natural resources open a new approach for the treatment of various diseases and highlight the era of "back to nature".

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(2).1143-48

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).1143-48

Flavonoids like quercetin, curcumin, luteolin, rutin, and diosmin possess strong antioxidant activity as well as other interesting potential effects, including anti-inflammatory, anti-cancer, and anti-ulcer activities [1]. Diosmin (3,5,7-trihydroxy-4-methoxy-flavone 7-rutinoside) is a natural flavone occurring in the Rutaceae family and is especially abundant in the pericarp of various citrus fruits.

It is a poorly soluble flavone with outstanding therapeutic potentials and a good safety profile, and high tolerability. Diosmin (DSN) has been used for more than 30 years for its phlebotonic properties, as a vascular protector for the treatment of hemorrhoids and venous leg ulcers [2].

In the field of cancer therapy, DSN is an alternative treatment for hepatocellular, colon, and urinary bladder carcinogenesis with privileges of lower costs and adverse reactions compared to chemotherapy. In particular, DSN is characterized by poor solubility in water and most organic solvents as well. Poor drug dissolution is responsible for its poor bioavailability and high inter-subject variation following oral administration. Low drug solubility results in reduced amounts of drug absorbed; therefore, a large standard dose of DSN (500 mg twice daily) is usually required for oral dosage regimens [3].

Drug solubility is a crucial factor limiting the therapeutic advantage of many potent drugs because of low oral bioavailability. Nano-suspension has emerged as an important tool in drug delivery to rectify this solubility conflicts. Nanonization of drug and stabilization using stabilizers, termed as nanosuspension. Nanosuspen-sions are reported to increase saturation solubility due to reduced particle size and increased surface area, contributing to enhanced dissolution and eventually increased bioavailability [4]. Nano-suspensions are essentially thermodynamically unstable systems. To decrease their free energy nanosuspension tends to reduce interaction with water through flocculation, aggregation or crystal growth. Stabilizers are added to reduce the free energy of the system by decreasing interfacial tension and to prevent nanoparticle aggregation by electrostatic or steric stabilization. Stabilizer constitutes an integral part of nanosuspensions and it is important to understand their role on physical stability of nanosuspension [5]. Stabilizers may be plain surfactants, polymers or a mixture of polymer and surfactants.

Hence, nanosuspension could be an approach to enhance the aqueous solubility, dissolution, and oral bioavailability of poorly water-soluble drugs and phytoconstituents. Moreover, the nano-suspensions could be easily scaled up and transformed into more convenient dosage forms (tablets, pellets, and capsules) following some simple post-process. The above advantages provide the prerequisite for the development of nanosuspensions. Therefore, the main objective of this research work was to fabricate and characterize DSN nanosuspensions to enhance the solubility, dissolution, and stability of water-insoluble DSN in order to improve oral bioavailability.

MATERIALS AND METHODS:

Materials: Diosmin (DSN) and polaxamer 188 was obtained from Jamjoom Pharmaceutical Co. Ltd. (Jeddah, Kingdom of Saudi Arabia) as a gift sample. Tween® 80 and PEG 400 were purchased from S.D. Fine-Chem. Ltd. (Mumbai, India). The rest of the chemicals were of analytical grade and were purchased from Merck specialties private limited (Mumbai, India).

Preparation of DSN Nanosuspension: To establish the process of nanosuspension different trial batches has been prepared. Accurately weigh DSN(150 mg) was dissolved in DMSO (5 mL) then the organic solution of DSN (3%, w/v) was added dropwise into 50 mL of 2% w/v aqueous solution of different surfactants like PVP K 25, poloxamer 188, Tween 80 and PEG 400under continuous stirring which was maintained at 1000 rpm for 30 min until the homogenous suspension was obtained **Table 1**. To the resultant homogenous suspension, probe sonication (SONICS Vibra cell VC750, USA) was employed at an amplitude of 30%, pulse 30 sec for 20 min to produce nanocrystals. During this sonication, the temperature was maintained at 0°C using an ice bath. Those surfactants which showed good stabilizing properties and able to the kept the nanocrystals without any steric barrier were selected.

Only PEG 400 gave stabilized DSN nanocrystals. To optimize the formulation of nanosuspension three different batches were prepared. Accurately weighed DSN in DMSO (3%, w/v) added to 50 mL of aqueous solution of 1.0 - 3.0% w/v PEG 400 **Table 2**. The suspensions mixed by a mechanical stirrer to get homogeneous suspensions at 1000 rpm for 30 min. To the resultant homogenous suspension, probe sonication was employed at20–23 kHz for 20 min to produce nanocrystals.

Lyophilization of Nanosuspension: Accurately, 5% w/v mannitol as cryoprotectant was added into the nanosuspensions before deep freezing. 50 mL of nanosuspension filled in vials and frozen using deep freezer at −20 °C for 24 h. These frozen solids were freeze-dried using lyophilizer (Zirbus technology, Germany) at a vacuum degree of 200 Pasto produce free-flowing dry powder.

Physico-chemical Characterization of Nanosuspension:

Measurement of Particle Size, Polydispersity Index and Zeta Potential: The particle size (PS), polydispersity index (PDI), and zeta potential (ZP) of nanosuspension were measured using dynamic light scattering zetasizer (PSS NICOMP Z3000, Port Richey, FL). Prior to the measurement, the samples were diluted with double distilled water to a suitable scattering intensity and redispersed by hand shaking [6]. The nanosuspension showing the lowest particle size with acceptable zeta potential was selected for further studies. Dynamic light scattering measures Brownian motion and relates to the size of the particles. It does this by illuminating the particles with a laser and analyzing the intensity fluctuations in the scattered light. PDI obtained from the DLS measurement pretends the width of hypothetical unimodal particle size. The zeta potential values given as an indication of the stability of the particles, and a value more than ±30 mV is considered as an optimum value for the very good stability of the formulation [15].

Determination of Saturation Solubility: The saturation solubility of DSN was determined by preparing saturated solutions in water, phosphate buffer (pH 7.4), and sodium orthophosphate buffer (pH 12) following a standard shake flask method. An excess quantity of DSN was added to 10 mL of solvent in a tightly capped glass vial. To achieve uniform mixing, samples were constantly agitated at 100 rpm at 37 °C for 24 h in a reciprocating water bath. At the end of the 24 hours equilibrium at 37 °C, samples were taken out and filtered through 0.45 µ pore size filter paper and analyzed using UV-VIS spectrophotometer. These studies were carried out for bulk DSN powder and lyophilized nanosuspension. Each determination was carried out in triplicate.

***In-vitro* Release Studies:** The dissolution rate of DSN nanoparticles is measured using the dissolution test apparatus-paddle method. The rotation speed of the paddles is set to 100 rpm. About 500 mL of orthophosphate buffer (pH 12) with 0.5% SDS at 37 ± 0.5 °C used as the dissolution medium. DSN nanosuspensions equivalent to 50 mg DSN was added to the stirred dissolution medium. At predetermined time intervals, 3 mL samples withdrawn filtered through 0.45 µm membranes immediately and compensation with the same volume of fresh dissolution medium, respectively. The amount of dissolved drug was determined by spectrophotometrically at a wavelength of 266 nm using the corresponding sodium orthophosphate buffer as a blank.

RESULTS AND DISCUSSION: Stabilizers are added to nanosuspension formulations to reduce the free energy of the system by decreasing interfacial tension and to prevent nanoparticle aggregation by electrostatic or steric stabilization [7]. Stabilizers can be surfactants, polymers or a mixture of both. To establish the process optimization of nanosuspension the four different trial batches were prepared using different types of surfactants and polymer PVP K 25, poloxamer 188, Tween 80 and PEG 400 **Table 1**.

TABLE 1: PREPARATION OF NANOSUSPENSIONS OF DSN

Formulation code	Formulation Composition			
	PVP K 25 (% w/v)	poloxamer 188 (% w/v)	PEG 400 (% w/v)	Tween 80 (% w/v)
NSDSN1	2	-	-	-
NSDSN2	-	2	-	-
NSDSN3	-	-	2	-
NSDSN4	-	-	-	2

The type and amount of stabilizers also produced a significant effect on the physical stability of nanosuspension. During probe sonication, electrical energy is converted into shock waves through a series of transformations. These shockwaves produce bubbles that violently explode and collide with particles resulting in the generation of heat [8]. The stabilizer PEG 400 resulted in a smaller particle size compared to Tween 80, PVP K 25, and poloxamer 188. Based on desired particle size, PDI, and zeta potential, the trial batch NSDSN3 was selected for further development and process optimization. Thus stabilizers (PEG 400) were used in different concentrations for managing the stability of different formulations **Table 2**. After process optimization of nanosuspension, three different formulation batches NSDSN3, NSDSN5, and NSDSN6 were prepared and characterized by the particle size, PDI, and zeta potential. On the basis of particle size, PDI, and zeta potential, the formulation batch NSDSN6 was selected as an optimized formulation, which revealed that batch NSDSN6 has better stability than the other batches

and hence an appropriate concentration (3% w/v) of a stabilizing agent (PEG 400) was used for 3% w/v drug concentration which enabled total crystal surface to become covered, thus providing enough steric repulsion between the crystals.

TABLE 2: PARTICLE SIZE (PS), POLYDISPERSITY INDEX (PDI) AND ZETA POTENTIAL (ZP) OF DIFFERENT BATCHES OF NANOSUSPENSION

Formulation code	Drug% (w/v)	Conc. of PEG 400 % (w/v)	Particle size (nm)	PDI
NSDSN5	3	1.0	544.3	0.372
NSDSN3	3	2.0	409.5	0.375
NSDSN6	3	3.0	203.9	0.219

The mean particle size of three nanosuspension formulations containing different stabilizer concentration as depicted in **Table 2**. Results demonstrated that all three formulations showed a particle size in a nanometric range. A significant reduction in particle size was observed upon increasing the stabilizer ratio. The result also exhibited that the particle size reduced with the increase of PEG 400 concentration, and finally a plateau region was reached at the concentration of 3% w/v (batch NSDSN6) from where the particle size no remarkably changed. The PDI varied from 0.375 to 0.219. Out of all the selected batches, NSDSN6 exhibited the lowest PDI, which indicates a fairly narrow size distribution **Fig. 1A**. Zeta potential was determined as a function of physical stability. The stabilizer was adsorbed onto the surfaces of the generated nanoparticles, which gave the zeta potential. Batch NSDSN6 showed the best zeta potential value (-27.26 mV) **Fig. 1B**. From these results, it can be concluded that the affinity of the stabilizing agent to the newly formed crystal surface is decisive. Crystal growth is protected by the adsorbed stabilizers. Hence, during the formulation of nanosuspension, the optimization of surface-active agents has prime importance.

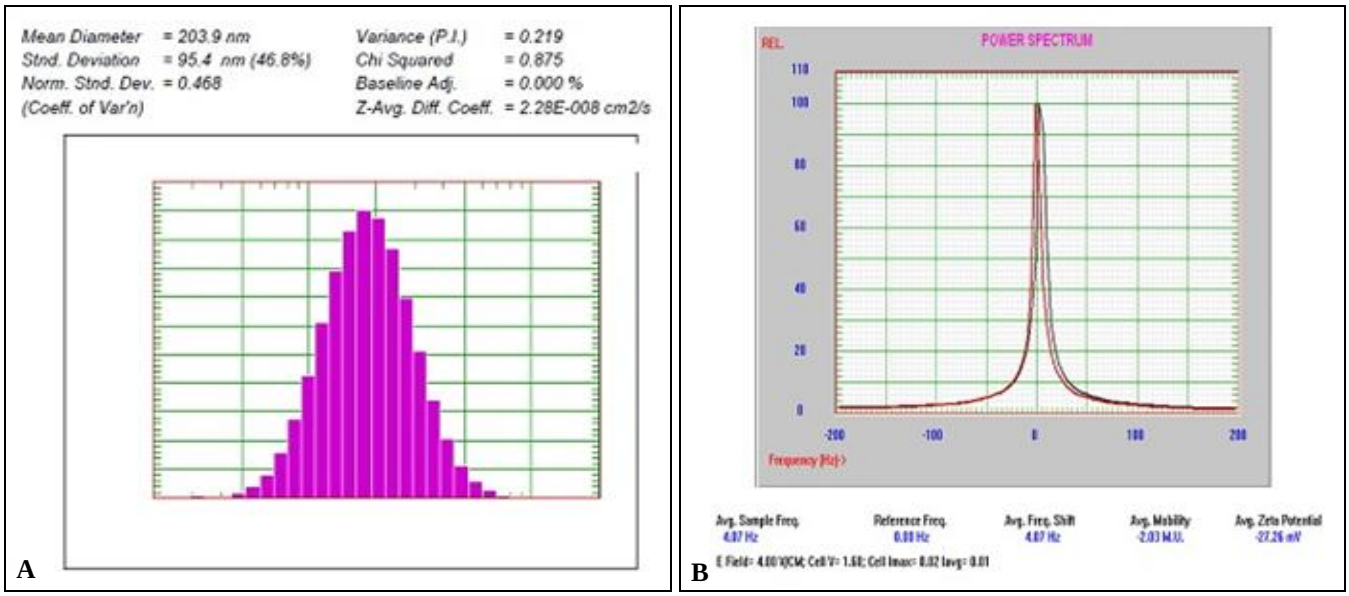

FIG. 1: A. PARTICLE SIZE DISTRIBUTION AND B. ZETA POTENTIAL OF OPTIMIZED NANOSUSPENSION (NSDSN6)

Saturation solubility data obtained for unprocessed DSN powder and lyophilized nanosuspension is demonstrated in **Table 3**. Results revealed an increase in DSN solubility as the pH was shifted to the alkaline range. The highest solubilizing capacity of DSN was manifested in orthophosphate buffer pH 12. Approximately five times increase in saturation solubility of lyophilized nanosuspension was observed than that of an unprocessed drug. Increased saturation solubility is due to the creation of high‑energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles [9].

TABLE 3: SATURATION SOLUBILITY STUDY

Solvent system	Solubility at 37±1°C (mg%) (*n*=3, mean ± SD)	
	Bulk drug (DSN)	DSN Nanosuspension
Phosphate buffer pH 7.4	0.076 ± 0.01	0.42 ± 0.15
Sodium orthophosphate buffer pH 12	24.862 ± 2.63	132.24 ± 12.67
Distilled water	1.54 ± 0.15	8.70 ± 0.21

SD: Standard deviation

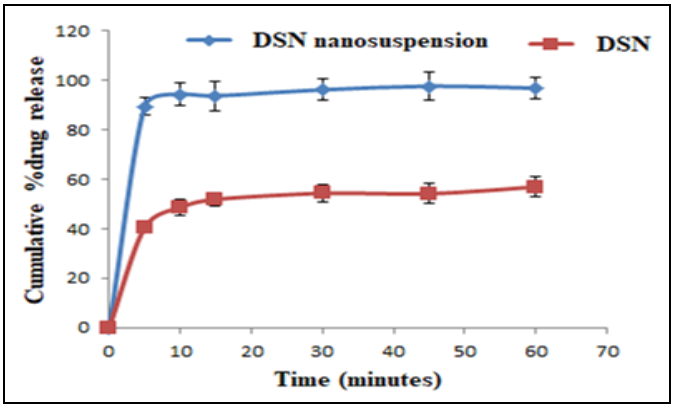

FIG. 2: DISSOLUTION PROFILE OF DSN NANO-SUSPENSION (NSDSN6) AND PURE DSN IN SODIUM ORTHOPHOSPHATE BUFFER pH 12 AT 37 °C

Fig. 2 shows the release profile of DSN from optimized batch nanosuspensions (NSDSN6) and pure drug in sodium orthophosphate buffer pH 12 at 37 °C. The *in-vitro* release of drug from nanosuspensions was studied by dialysis membrane diffusion technique, which is a very common method to estimate the % drug release from colloidal suspension. The release profile of pure drug indicates very slow diffusion of DSN with nearly 50% release in 10 min, while nano-suspensions showed a significantly enhanced release rate, 95% of the drug diffused in 10 min and 100% diffused in the 60 min test period. The slow dissolution rate of DSN in dissolution medium could be attributed to poor DSN wet ability and consequent agglomeration.

On the other hand, DSN nanosuspension formulations exhibited a high dissolution rate. Such significant enhancement in nanosuspension dissolution compared to the pure drug could be ascribed mainly to the nanometric particle size. Following Noyes–Whitney equation, progressive size reduction of the drug particles leads to an increase in the surface area resulting in an increased dissolution rate. Additionally, particle size reduction results in the decrease of the diffusion layer thickness surrounding the particles and an increased concentration gradient between the surface of the particle and bulk solution, which facilitates particle dissolution by increasing dissolution velocity [10].

CONCLUSION: In this research work, a surfactant-stabilized nanosuspension formulation of DSN was successfully developed. Nanosuspension of DSN was stabilized with varying concentration of PEG 400 (1% - 3% w/v). PEG 400 showed improvement in aqueous solubility and dissolution rate of water-insoluble DSN. The prepared nanosuspension showed the optimum particle size and zeta potential. The particle size present in nano-suspension is highly dependent on process and formulation variables.

These results indicate the suitability of the probe sonication method for the preparation of nano-suspensions of poorly soluble drugs with improved *in-vitro* dissolution rate, thus potentially capable of enhancing fast onset of therapeutic activity and bioavailability. However, pharmacokinetic and pharmacodynamic studies are required to further support the finding. The developed formulation strategy of nanosuspension could be exploited to improve the solubility and bioavailability of poorly soluble DSN and other phytomedicines.

ACKNOWLEDGEMENT: The authors gratefully acknowledge the approval and the support of this research study by grant no. 7760-PHM-2018-3-9-F from the Deanship of Scientific Research at Northern Border University, Arar, KSA.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Feldo M, Wozniak M, Wojciak-Kosior M, Sowa I, Kot-Wasik A, Aszyk J, Bogucki J, Zubilewicz T and Bogucka-Kocka A: Influence of diosmin treatment on the level of oxidative stress markers in patients with chronic venous insufficiency. Oxid Med Cell Longev 2018; 2561705.
2. Imam F, Al-Harbi NO and Al-Harbi MM: Diosmin downregulates the expression of T cell receptors, proinflammatory cytokines and NF-κB activation against LPS-induced acute lung injury in mice. Pharmacol Res 2015; 102: 1-11.
3. Li T, Zhu W, Liu G, Fang C and Quan S: Diosmin for the prevention of ovarian hyperstimulation syndrome. Int J Gynaecol Obstet 2020; 149(2): 166-70.
4. Mujtaba MA and Hassan KAM: Development and evaluation of nanocrystals of rosuvastatin for enhancement of dissolution rate. RJPBCS 2019; 10(1): 359-65.
5. Singh MK, Pooja D, Ravuri HG, Gunukula A, Kulhari H and Sistla R: Fabrication of surfactant-stabilized nanosuspension of naringenin to surpass its poor physiochemical properties and low oral bioavailability. Phytomedicine 2018; 40: 48-54.
6. Mujtaba MA, Alotaibi NM and Eltaib L: Fabrication of ciprofloxacin nanocrystals by probe sonication method for enhancement of dissolution rate. RJPBCS 2019; 10(1): 891-98.
7. Agarwal V and Bajpai M: Nanosuspension Technology For Poorly Soluble Drugs: Recent Researches, Advances and Patents. Recent Pat Nanotechnol 2015; 9(3): 178-94.

8. Jacob S, Nair AB and Shah J: Emerging role of nanosuspensions in drug delivery systems. Biomater Res 2020; 24: 3.
9. Bhalekar MR, Upadhaya PG, Reddy S, Kshirsagar SJ and Madgulkar AR: Formulation and evaluation of acyclovir nanosuspension for enhancement of oral bioavailability. Asian J Pharm 2014; 8: 110-18.
10. El-Laithy HM, Basalious EB, El-Hoseiny BM and Adel MM: Novel self-nanoemulsifying self-nanosuspension (SNESNS) for enhancing oral bioavailability of diacerein: Simultaneous portal blood absorption and lymphatic delivery. Int J Pharm 2015; 490(1-2): 146-54.

How to cite this article:
Mujtaba MA, Eltaib L, Akmal NN, and Hassan KAM: Fabrication and characterization of nanosuspension formulation of diosmin for enhanced oral delivery. Int J Pharm Sci & Res 2021; 12(2): 1143-48. doi: 10.13040/IJPSR.0975-8232.12(2).1143-48.