**RESEARCH ARTICLE**

# Preparation and *In-vitro* Evaluation of Darifenacin HBr as Nanoparticles Prepared as Nanosuspension

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**ABSTRACT**

Nanosuspension is a term that can be used to describe a colloidal dispersion of nanosized droplets of the drug in an aqueous medium with a size below 1 μm. Drug nanoparticles are one of the most significant methods to reduce the constituent part diameter and increase surface area, improving the dissolution and oral bioavailability of hydrophobic medicines, enhancing drug dissolution rate and bioavailability. Nanoparticles are produced using appropriate techniques for drug delivery applications and administered via various routes, including oral, topical, parenteral, ophthalmic, and pulmonary.

Overactive bladder (OAB) affects around 16% of adults and is more common as people become older. It causes a variety of symptoms, including urgency, incontinence, urine frequency, and nocturia. DH. It is newly drug used to treat complicated OAB. It has a higher selectivity for the bladder's muscarinic receptors. After intravenous and immediate-release oral dose forms. It suffers from extensive first-pass metabolism with a short elimination half-life and ranging between three to four hours). The current research focused on creating an extended-release dosage form utilizing Eudragit RS100.

The solvent/anti-solvent precipitation method was used to make darifenacine nanoparticles. A certain quantity of medication was dissolved in a water-miscible solvent (methanol), then poured at a specific speed into water containing stabilizer on a magnetic stirrer for a 1/2-hour; after that, the resulted product was sonicated at 37°C for 15 minutes.

The physicochemical interaction among medication with additives was explored utilizing fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetric (DSC). The particle size and zeta potential of the generated nanosuspension were calculated.

**Keywords:** Darfenacin hydrobromid, Nanoparticle, Nanosuspension, Surfactant, Polydispersity, Sustained release, Ultrasonication, Zeta potential.

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## INTRODUCTION

Reduced drug particle size is considered the most promising strategy to improve drug bioavailability and solubility, leading to a new area of nanotechnology.[1] Different method outlined in the conventional approach can be overcome by utilizing nanotechnology Nanoparticles have been explored for drug delivery to enhance the bioavailability, sustained release, and intracellular penetrability as nanomedicine continues to grow and improve.[2]

Nanoparticles are colloidal particles with sizes varying from 10 to 1000 nm. The benefits of nanotechnology include the ability to deliver effective medicine (Nanomedicine), which is expected to impact the pharmaceutical and biotechnology sectors significantly. Separation technologies, histology research, clinical diagnostic tests, and medication delivery systems are just a few of the areas that might use them.[3]

Darifenacin hydrobromide is an anticholinergic medication that is frequently used in individuals with overactive bladder who do not respond to conventional therapy. Darifenacin hydrobromide (DH) is an (S)-2-{1-[2-(2, 3-dihydrobenzofuran-5-yl) ethyl]-3-pyrrolidinyl}-2, 2-diphenylacetamide hydrobromide, which is supplied as a white crystalline solid. It is slightly water-soluble (6.03 mg/mL), and the PKA equals 9.2. Its melting point is 232-236°C. The molecular formula of DH is $C_{28}H_{31}BrN_2O_2$, and the molecular weight is 507.472 g/mol. It is a powerful muscarinic receptor antagonist, available as a hydrobromide salt. The DH oral absorption is poor due to its low solubility and poor bioavailability (15–19%), and oral bioavailability is limited by first-pass metabolism.[4,5]

The target of the current work was to study the formulation of nanosuspension of DH with polymers to improve the solubility of the drug and to prepare long-acting

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nanoparticle formulation as sustained release with high bioavailability.

## MATERIALS AND METHODS

### Materials

Darifenacine HBr, Eudragit RS, Soluplus®, P.V.A., PVP, methanol, HPMC, glycerin.

### Method of Preparation of Darifenacine HBr Nanosuspension

Nanosuspension is an easy and cost-effective approach to manufacturing a physically more stable product for low-soluble medicines. The production of nanosuspension by two techniques: "Top-down method" and "Bottom-up technology". DH nanosuspension was generated by the solvent evaporation method or known as the anti-solvent precipitation method.[6]

The generated organic drug solution (organic phase) was then introduced dropwise into a 30 mL aqueous stabilizer solution using a disposable syringe with the tip positioned directly into the aqueous medium. After that, stirring the mixture at an agitation rate of 1000 revolutions in each minute (rpm) on a magnetic stirrer for 30 minutes. to permit the evaporation of a volatile solvent.[7]

The formulation was sonicated for 15 to 30 minutes, ensuring that the formulation did not become hot. Further, the formulation was transferred to an amber-colored bottle and stored in the refrigerator.[8]

## FACTORS AFFECTING THE FORMULATION OF NANOSUSPENSION

### Effects of Concentration and Type of Stabilizer on the Particles Size of Darfenacine Nanosuspension

To reach the best formula, different types of stabilizers at various concentrations were used in the preparation of DH nanosuspensions. The formulas (F1-F9) were prepared by using dissimilar stabilizers and subjected to particle size analysis. The effect of using a single stabilizer type was studied in F3, which contains soluplus and F8-F10 contains PVA, PVP K30, and HPMC at a drug: stabilizer ratio 1:1.

### Characterization of Darfenacine HBr nanoparticles:

*Drug Characterization*

The melting point of DH was measured by capillary tube technique, recognized by the United States Pharmacopeia (USP). A sufficient quantity of pharmaceutical powder was put into a capillary glass tube sealed from one side, then the capillary tube smoothly tapped on a hard surface to form a firm, compact powder. The tube was then placed gently in the electrical melting point equipment, where the temperature was steadily increased, and monitored the range value began from liquefaction of the powder until when completely melted was recorded.[9]

*UV Spectrum*

A specific amount of DH dissolved in phosphate buffer solution pH6.8. The resulted solution was spectro-photometrically scanned from 200 to 400 nm to obtain $\lambda_{max}$ of DH. The UV scan revealed the wavelength of absorption peak ($\lambda_{max}$), after which a calibration (standard) curve was prepared on the identified wavelength of higher absorption ($\lambda_{max}$).[10]

*Particle Size (PS) and Polydispersity Index (PDI)*

A dynamic light scattering procedure was used to calculate the PS and PDI of DH nanoparticles at 25°C in a detection angle of 90° utilizing the ABT-9000 nano laser particle size analyzer. All samples were examined in triplicate and without dilution. (SD).[7,11]

*Zeta Potential Evaluation of Nanosuspension*

Zeta-potential is evaluated using zeta sizer (Zetasizer Nano ZS, Malvern instrument, Worcestershire, UK). The zeta potential describes the extent of repulsion among neighboring and identically charged particles in the dispersion media. The characteristics of surface charge were studied to assess the stability of the prepared nanosuspension. The minimum limit needed for electrostatic stabilization of nanosuspension is ± 30 mv.[12] The light scattering fluctuations caused by the Brownian motion of nanosuspension compositions were examined.[13]

*Dissolution Pattern of DH as In-vitro Nanosuspension*

Volume of nanosuspension equivalent to 15 mg DH was taken into a dialysis membrane (M.w. cutoff 12,000-14,000 Hi-media) and set to paddle of USP dissolution apparatus-Type II applying a rate of rotation reached to 100 rpm. Then a solution of buffer phosphate (pH 6.8) was utilized as a dissolution environment in a volume of 900 mL at 37 ± 0.5°C. The volume of 5 mL was withdrawn on time scheduled basis of 30 minutes from starting and then replaced by fresh media of dissolution, up to 24 hours. Samples were filtered using a 0.22 micro filter syringe (0.22 Mm). Filtrate absorbance recorded by UV analysis versus blank (phosphate buffer). The cumulative percentage release was calculated at 284 nm depending on the calibration curve.[11,14]

*Freeze Drying of Nanosuspension*

Freeze drying is used to convert the optimum formula to dry powder, later for further evaluation. Mannitol is used as a cryoprotectant at 3% w/v. About 140 mL of optimized formula was prepared and freeze-dried to yield a dry powder for evaluation. Four flasks were frozen in a deep freezer at -20°C for 24 hours. The frozen flasks were attached to the vacuum port of the device, then four flasks, each containing 35 mL of nanosuspension, instrument operated till dry powder yielded. Sublimation of solvent from frozen samples took 48 to 72 hours.[15,16]

*Scanning Electron Microscopy (SEM.)*

SEM images the surface of a solidified sample. The information recorded by the signal such as surface topography, outer layer morphology, compositional chemistry, crystallinity of the particulate, and electrical conductivity.[17]

*Fourier Transforms Infrared Spectroscopy (FTIR)*

The FTIR spectrum was documented for pure drug and optimized formulation utilizing the KBr pellet method. The

**Table 1:** Composition of darfenacin hydrobromid formulas

| *F* | *Darifenacin* | *Eudragit RS* | *Soliplus* | *PVA* | *PVP* | *HPMC* | *R* |
|---|---|---|---|---|---|---|---|
| 1 | 15 | 15 | 15 | | | | 500 |
| 2 | 15 | 15 | 15 | | | | 800 |
| 3 | 15 | 15 | 15 | | | | 1000 |
| 4 | 15 | 15 | 30 | | | | 1000 |
| 5 | 15 | 15 | 45 | | | | 1000 |
| 6 | 15 | 30 | 45 | | | | 1000 |
| 7 | 15 | 15 | | 15 | | | 1000 |
| 8 | 15 | 15 | | | 15 | | 1000 |
| 9 | 15 | 15 | | | | 15 | 1000 |

**Table 2:** Particle size with PDI of the formulations

| *Formula* | *Particle size (nm)* | *Polydispersity* |
|---|---|---|
| F1 | 152 | 0.220 |
| F2 | 118 | 0.168 |
| F3 | 68 | 0.173 |
| F4 | 58 | 0.269 |
| F5 | 83 | 0.082 |
| F6 | 170 | 0.198 |
| F7 | 139 | 0.005 |
| F8 | 264 | 0.045 |
| F9 | 184.2 | 0.346 |

pellets were made with a KBr hydraulic press that was operated under hydraulic pressure of 150 kg/cm$^2$. The measured spectra were above 3600–400 cm$^{-1}$ at ambient temperature with a resolution of 4 cm$^{-1}$, using FTIR 2500 apparatus.[18]

*Differential Scanning Calorimetric (DSC)*

DSC experiments have occurred with a DSC apparatus model DSC-6. A small amount of pure DH and the selected formula were inserted in an aluminum pan, as well as the test was performed under nitrogen atmosphere at a flow rate of 40 mL/min and scanning rate of 10°C/min in the range of 15-300°C.[19]

## RESULTS AND DISCUSSIONS

### Results

The antisolvent precipitation technique was utilized to prepare ten formulas of nanosuspension (F1-F9), and then subjected to characterization; the following parameters were estimated.

### Determination of DH Melting Point

The melting point of DH was 130 to 134°C. indicating that the drug obtained is pure.[20]

### UV Spectrum

The UV spectrum in phosphate buffer (ph 6.8) showed maximum absorption at 284 nm, which is considered an analytical wavelength, as in Figure 1.

### Analysis of Particle Size with Polydispersity Index

The mean particle diameter and polydispersity index are shown in Table 2.

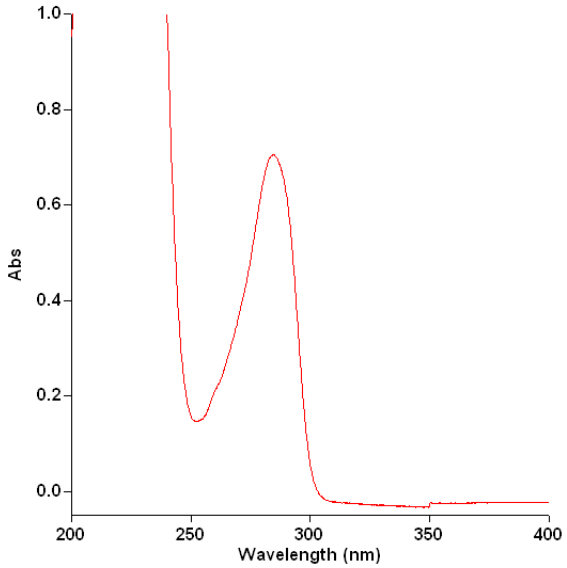


**Figure 1:** UV spectrum of DH in phosphate buffer (ph 6.8)

PDI is a factor used to clarify the particle size distribution of nanoparticles achieved from a particle analyzer and afford an indication long term stability of nanosuspension, when polydispersity index value below than 0.3 indicate narrow size distribution (homogenous), while if more than 0.3 considered a wide size distribution(heterogeneous).[21]

All formulas show good PDI with a range between 0.005 to 0.346 and particle size range between 58 to 264 nm, so all formulas have good drug particle size uniformity.

### Effect of Stirring Speed on the Particle Size

Three different speeds 500, 800, and 1000 rpm were used to formulate three formulas (F1, F2, and F3) to show the effect of stirring speed on particle size, as shown in Table 2. The optimum speed for formulas that have a drug to stabilizer ratio 1:1 was found to be 1000 rpm which produces a mean particle size 68 nm. An increasing stirring speed would result in decreased particle size. As a result, high shear stress was necessary to break down particles to the submicron range.[22]

### Types and Concentration of Stabilizer Effect

Different parameters affect particle size and stability of nanosuspension, but the type and concentration of stabilizer

are critically important. The effect of stabilizer sorts and concentrations on nanosuspension formulation is demonstrated in F1-F9. The formulas stabilized by soliplus have the smallest particle size than other formulas containing different stabilizers. This is due to the chemical nature of soliplus, which contain polyethylene glycol backbone as a hydrophilic part and vinyl caprolactam/vinyl acetate side chain as a lipophilic part, the interfacial tension of the surface particles decreases due to adsorption of soliplus onto drug particles, preventing aggregation of the freshly generated nanoparticles as a result of steric hindrance.[23]

Furthermore, the adsorption affinity of non-ionic stabilizers on particle surfaces is also influenced by stabilizer concentration; increasing the concentration of stabilizer causes a drop in particle size to a certain limit, beyond which a particle size enlarges owing to an elevated in the thickness of the coating enclosing the nanoparticles and preventing diffusion between the solvent and antisolvent phases as in (F3, F4, and F5).[24]

The formation of H-bond between DH particles and one of other stabilizer polymers like PVA, PVP, and HPMC in formulas (F7, F8, F9) may lead to the stabilization of DH nanosuspension. These polymers may efficiently adsorb onto drug particles, forming a stable barrier around the particle's surface, preventing it from growing.[25]

## Zeta Potential

The zeta potential of Darifenacin HBr nanosuspension was found to be 26 in F4, as shown in Table 3 which ensures good stability of the NS on longer storage (Figures 2 to 5).

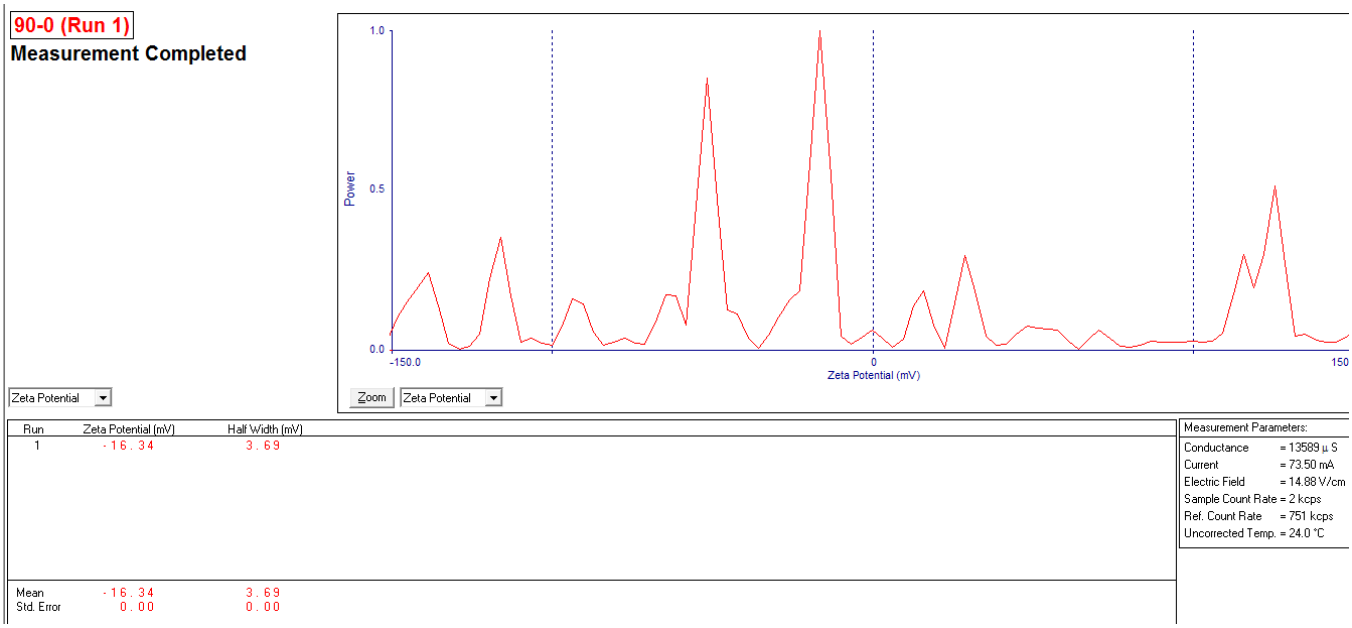


**Figure 2:** Zeta potential of formula 1

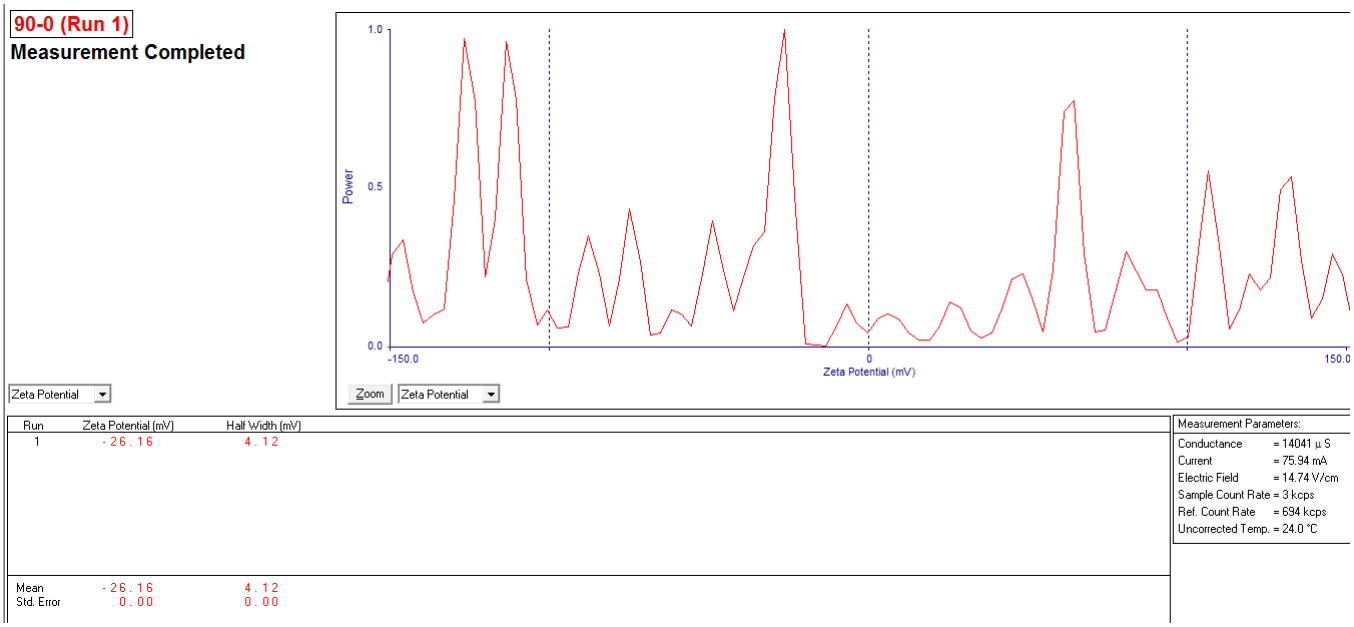


**Figure 3:** Zeta potential of formula 2

### Fourier Transforms Infrared Spectroscopy (FTIR)

The spectra of FTIR of the pure drug, polymer, and physical mixture drug: polymer at same ratio (1:1) and the selected formula was assayed and obtained characteristic peaks as in Table 4.

The main characteristic peaks of FTIR spectrum of DARFENACIN HYDROBROMIDE that at wave numbers(in cm-1) are: 3469 for N-H asymmetric stretching of amide (3500-3400), 2929 for C-H asymmetric stretching of aliphatic methyl and methylene group,1664 for C=O stretching of amide (1695 to 1630), 1581 for C=C stretching of the aromatic ring 1610 to 1500, 1438 for C-H bending of aliphatic methyl and methylene groups 1450-1400, 1353 for C-N stretching of tertiary amine (1360-1310), 1243 for C-O stretching of furan 1300 to 1000, 1099, 1060, 1033 and 1002 for in-plane C-H bending of the aromatic ring (1300 to 1000), 894, 813 and 767 for out plan C-H bending of the aromatic ring (900 to 675) and 705 for C=C bending of the aromatic ring (700–675).

According to the FTIR data, there is no chemical interaction and no differences between the peaks of the fingerprint region produced in the Darifenacin HBr spectrum and the spectra of the physical combination of Darfenacin Hydrobromide and polymers (Table 5).

**Table 3:** Zeta potential values

| *Formula* | *Zeta potential* |
|---|---|
| F3 | 16.34 |
| F4 | 26.16 |
| F5 | 36.66 |

### Differential Scanning Calorimetric (DSC) Study

The compatibility between drug and excipients can be determined by utilizing DSC, also to assess the crystalline condition of the drug when transformed to nanoparticles (Figure 5).

### Study the Release of DARFENACIN HYDROBROMIDE Nanosuspension *in vitro*

The USP dissolution test apparatus-II was used to conduct an *in vitro* dissolution investigation for three selected formulas with

**Table 4:** Characteristic Peak of Pure Drug and References

| *No* | *Pure Drug* | *Type of Peak* | *Reference* |
|---|---|---|---|
| 1 | 3469 cm-1 | N-H asymmetric stretching of an amide group | 3500–3400 cm-1 |
| 2 | 2929 cm-1 | C-H asymmetric, asymmetric stretching aliphatic methyl and methylene groups | 3000–2840 cm-1 |
| 3 | 1664 cm-1 | C=O stretching of amide | 1695–1630 cm-1 |
| 4 | 1581 cm-1 | C=C stretching of an aromatic ring | 1610–1500 cm-1 |
| 5 | 1438 cm-1 | C-H bending of methyl and methylene groups | 1450–1400 cm-1 |
| 6 | 1353 $cm^{-1}$ | C-N stretching of tertiary amine | 1360–1310 |
| 7 | 1243 $cm^{-1}$ | C-O stretching of furam ring | 1300–1000 |
| 8 | 1099,1060,1033, 1002 cm-1 | In-plane C-H bending of an aromatic ring | 1300–1000 |
| 9 | 813, 767 cm-1 | Out plane C-H bending of an aromatic ring | 900–675 cm-1 |
| 10 | 705 cm-1 | C=C bending of Aromatic ring | 700–675 cm-1 |

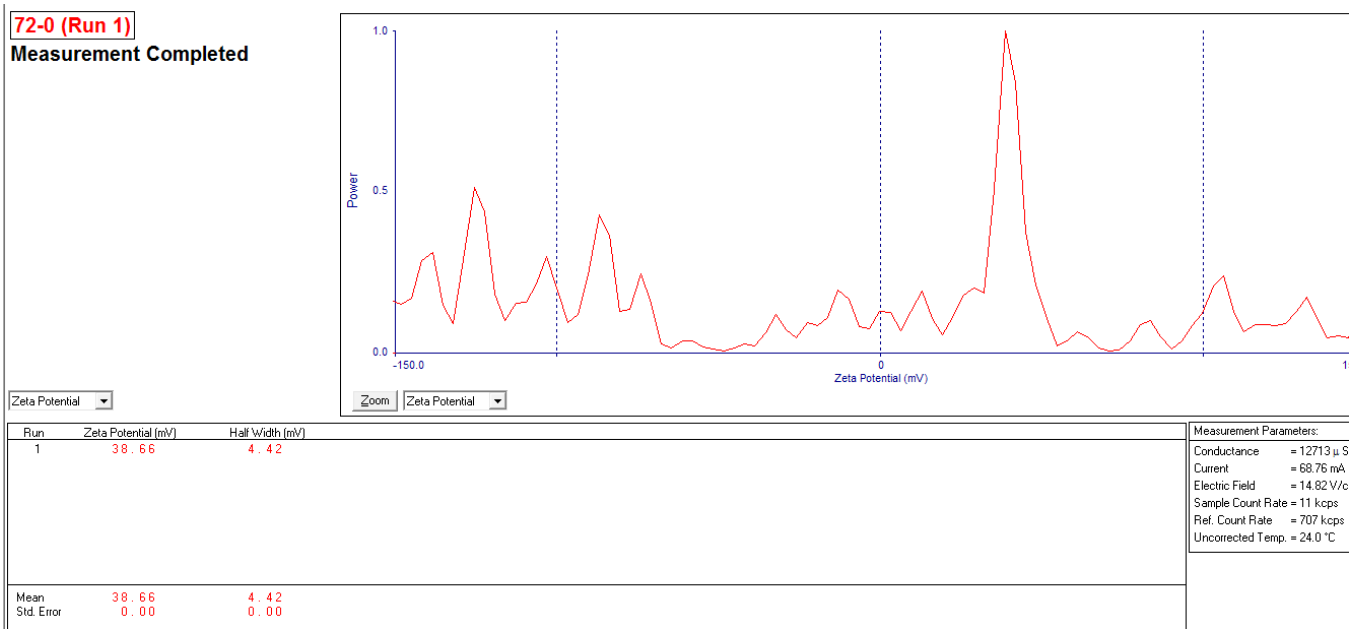


**Figure 4:** Zeta potential of formula 3

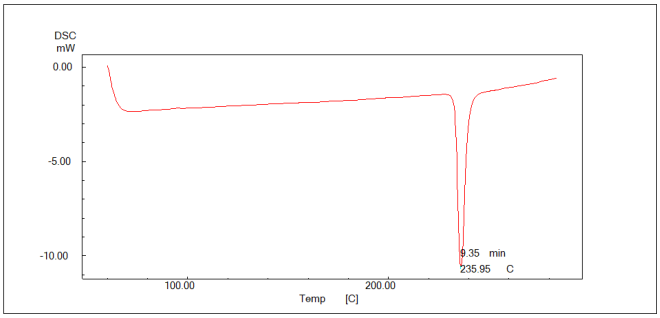


**Figure 5:** Differential scanning calorimetric of Darifenacin HBr

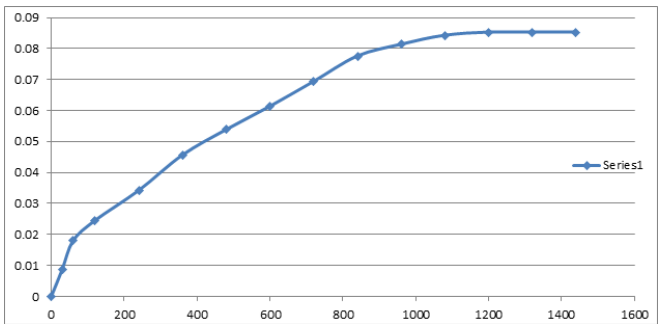


**Figure 6:** *In vitro* release profile of formula 3

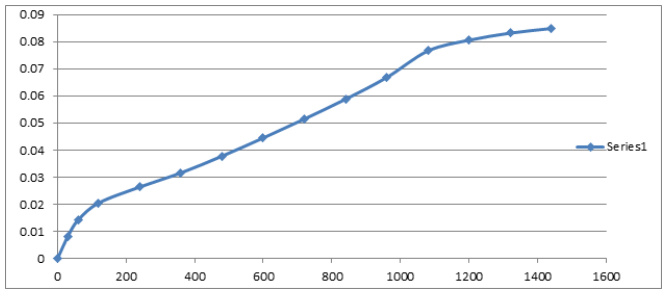


**Figure 7:** *In vitro* release profile of formula 4

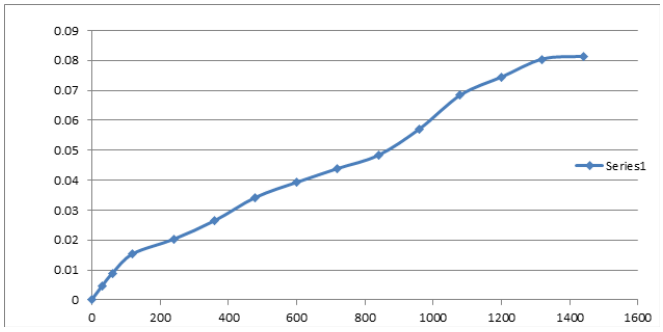


**Figure 8:** *In vitro* release profile of formula 5

lowest particle size. The media in phosphate buffer solution (pH6.8) and 37 ± 0.5°C were shown, and the results showed sustained release might reach to 24 hours. as in the Table 5 (Figures 6–9).

The rise in DR dissolving rate may be explained using the Noyes Whitney equation, which says that by lowering the particle size, especially to the nanoscale limit, the surface area increases and increases dissolution velocity. When compared F3 F4and F5, the amphiphilic stabilizer (Solupus®)

**Table 5:** *In-vitro* dissolution profiles of darifenacin HBr in F3, F4, F5

| *TIME* | *F3* | *F4* | *F5* |
|---|---|---|---|
| 30 min | 9% | 8% | 3% |
| 1 Hr. | 20% | 15% | 8% |
| 2 Hr. | 28% | 23% | 17% |
| 4 Hr. | 39 % | 30% | 22% |
| 6 Hr. | 53% | 36% | 30% |
| 8 Hr. | 63% | 44% | 39% |
| 10 Hr. | 72% | 52% | 45% |
| 12 Hr. | 81% | 60% | 51% |
| 14 Hr. | 91% | 69% | 56% |
| 16 Hr. | 96% | 78% | 67% |
| 18 Hr. | 99% | 90% | 80% |
| 20 Hr. | 101% | 95% | 88% |
| 22 Hr. | | 98% | 95% |
| 24 Hr. | | 100% | 96% |

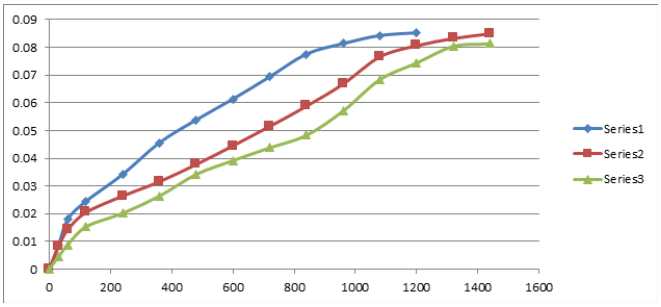


**Figure 9:** Comparison *in vitro* release profile of formula 3,4 and 5

might increase the surface wet-ability of the weakly aqueous-soluble product, resulting in better drug release through the NS formulation. The surfactant inhibited the agglomeration of particles size by supplying ionic or stearic barriers by way of which inter-particulate interactions in nanosuspensions are prevented.

## CONCLUSION

This study confirms that the antisolvent technique is suitable for preparing darifenacin nanoparticles with sustained-release efficiency. This formulation approach can be used to improve the therapeutic efficacy of poorly soluble drugs. The changes in nanoparticle size were affected by changes in polymer concentration.

We can conclude that F4 has the best characteristic and is suitable for formulation as nanosuspension for sustained release formula.

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