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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 size below 1µm. Drug nanoparticles are one of the most significant methods that reduce constituent part diameter and increasing surface area, leading to improve dissolution and oral bioavailability of hydrophobic medicines, which is enhances drug dissolution rate and bioavailability. Nanoparticles produced using appropriate techniques for drug delivery applications and administered via a variety of 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.

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 half hour, after that the resulted product sonicated at 37°C for 15 minutes.

The physicochemical interaction among medication with additives was explored utilizing F.T.I.R and D.S.C, and the particle size as well as zeta potential of the generated nanosuspension was calculated.

Keywords: Darfenacin hydrobromid • Nanosuspension • Nano particle • Surfactant • Polydispersity • Sustained release • Ultrasonication • Zeta potential

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 methods 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 size varying from 10 to 1000 nm. The benefits of nanotechnology including the ability to deliver effective medicine (Nanomedicine), which is expected to have a significant impact on the pharmaceutical and biotechnology sectors. Separation technologies, histology research, clinical diagnostic tests, and medication delivery systems are just a few of the areas in which they might be used [3].

Darifenacin hydrobromide is an anticholinergic medication that is frequently used in individuals with overactive bladder who do not respond to conventional therapy [4]. 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 [5]. It is slightly water soluble (6.03mg/ml), and the pka equals to 9.2. Its melting point is 232-236 °C [6].The molecular formula of DH is C28H31BrN2O2, and the molecular weight is 507.472 g/mol [7]. 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 [8].

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The target of the current work was to study the formulation of nanosuspension of DH with polymers to improve the solubility of drug and to prepare long acting nanoparticles formulation as sustained release with high bioavailability [9].

Materials and Methods

Materials

Darifenacine HBr, Eudragit RS, Soluplus®, PVA, 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 [10]. The production of nanosuspension by two techniques: "Top-down method" and "Bottom-up technology" [11]. DH Nanosuspension was generated by the solvent evaporation method or known as the anti-solvent precipitation method [12].

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 [13]. After that; stirring the mixture at an agitation rate of 1000 revolutions in each minute (rpm) on a magnetic stirrer for 30 min. to permit the evaporation of a volatile solvent [14].

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 [15].

Factors Affecting the Formulation of Nanosuspension and 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 [16]. The formulas (F1-F9) prepared by using dissimilar stabilizers and subjected to particle size analysis. The effect of using single stabilizer type was studied in F3 which contain soluplus and F8-F10 contains PVA, PVP K30, and HPMC at drug: stabilizer ratio 1:1 [17].

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) [18]. 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 [19]. 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 the powder until when completely melted was recorded [20].

UV spectrum (Varian Cary 100)

A specific amount of DH dissolved in phosphate buffer solution pH6.8. The resulted solution spectro-photometrically scanned from 200-400 nm to obtained λ max of DH [21].

The UV scan revealed the wavelength of absorption peak (λ max), after which a calibration (standard) curve was prepared on the identified wavelength of higher absorption (λ max) [22].

P.S and PDI:

Dynamic light scattering procedure was used to calculate the P.S and PDI of DH nanoparticles at 25 ℃ in a detection angle of 90o utilizing the ABT-9000 Nano Laser Particle Size Analyzer. All samples were examined in triplicate and without dilution. (SD).

Zeta potential evaluation of nanosuspension

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

Dissolution pattern of DH as In-vitro nanosuspension

Volume of nanosuspension equivalent to 15mg DH was taken into dialysis membrane (M.w.cutoff12,000-14,000 Hi-media), and set to paddle of USP dissolution apparatus-Type II applying rate of rotation reached to 100 rpm. Then solution of buffer phosphate (pH 6.8) was utilized as dissolution environment in a volume of 900 ml at 37± 0.5°C. Volume of 5ml was withdrawn on time scheduled basis of 30min from starting, and then replaced by fresh media of dissolution, up to 24 hr. Samples were filtered using 0.22 micro filter syringe (0.22 Mm). Filtrate absorbance recorded by UV analysis versus blank (phosphate buffer). Depending on a calibration curve, percentage cumulative release was calculated at 284nm.

Freeze drying of nanosuspension

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

Scanning electron microscopy (SEM)

SEM images the surface of solidified sample. Information recorded by the signal such as surface topography, outer layer morphology, compositional chemistry, crystallinity of the particulate and electrical conductivity .

Fourier transforms infrared spectroscopy (FTIR)

The (FTIR) spectrum was documented for pure drug and optimized formulation utilizing KBr pellet method. The pellets were made with a KBr hydraulic press that was operated under hydraulic pressure of 150 kg/cm2. The measured spectra was above 3600-400

cm−1 at ambient temperature with a resolution of 4 cm−1, using FT-IR 2500 apparatus

Differential Scanning Calorimetric (DSC)

DSC experiments were occurred with a DSC apparatus model DSC-6. A little amount of pure DH and selected formula are 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.

F	DAREFENACIN	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 1: Composition of Darfenacin hydrobromid formulas.

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 130-134°C. indicating that the drug obtained is pure

Analysis of Particle Size with Polydispersity Index:

The mean particle diameter and polydispersity index are shown in table (2).

Polydispersity index 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).

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

Table 2: Particle Size with Polydispersity Index of the Formulations.

All formula show good PDI with range between 0.005- 0.346 and particle size range between 58-264nm so all formula have good uniformity of drug particle size.

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 lowest particle size than other formulas contained different stabilizer, this is due to the chemical nature of Soliplus which contain polyethylene glycol backbone as 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.(22)

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 enlarge owing to an elevate in the thickness of the coating enclosing the nanoparticles and preventing diffusion between the solvent and antisolvent phases as in(F3, F4 and F5).(23)

the formation of H-bond between DH particles and one of other stabilizer polymers like PVA,PVP,HPMC in formulas(F7,F8,F9) may lead to 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.(24)

Zeta potential

Zeta potential of DARFENACIN HYDROBROMID nanosuspension was found to be 28.03 in F4 as shown in table (3) which ensures good stability of the NS on longer storage.

Formula	Zeta potential
F3	-44.97
F4	28.03
F5	38.66

Table 3: Zeta potential values.

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 selected formula was assay and obtained characteristic peaks as in table 4.

The main characteristic peaks of FTIR spectrum of DARFENACIN HYDROBROMID 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 (1695-1630), 1581 for C=C stretching of aromatic ring (1610-1500), 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 (1300-1000), 1099, 1060, 1033 and 1002 for in-plane C-H bending of aromatic ring (1300-1000), 894,813 and 767 for out plan C-H bending of aromatic ring (900-675) and 705 for C=C bending of 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 DARFENACIN HYDROBROMID spectrum and the spectra of the physical combination of DARFENACIN HYDROBROMID and polymers (table4)

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

Table 4: Characteristic Peak of Pure Drug and References.

The compatibility between drug and excipients can be determined by utilizing DSC, also to assess the crystalline condition of drug when transformed to nanoparticles (Figure 1).

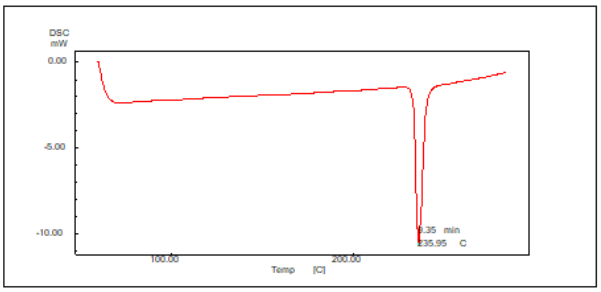

Figure 1: Differential scanning calorimetric (DSC) study.

Study the Release of DARFENACIN HYDROBROMID Nanosuspension in vitro

The USP dissolution test apparatus-II was used to conduct an in vitro dissolution investigation for 3 selected formulas with lowest particle size. The media in phosphate buffer solution (pH6.8) and 37 ± 0.5°c were shown, and the results showed sustained release may reach to 24hr. as in table below.

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%

Table 5: In-Vitro Dissolution profiles.

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 increasing dissolution velocity. When compared F3, F4and F5, the amphiphilic stabilizer (Solupus®) 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 supply ionic or stearic barriers by way of which inter-particulate interactions in nanosuspensions are prevented (Figure 2).

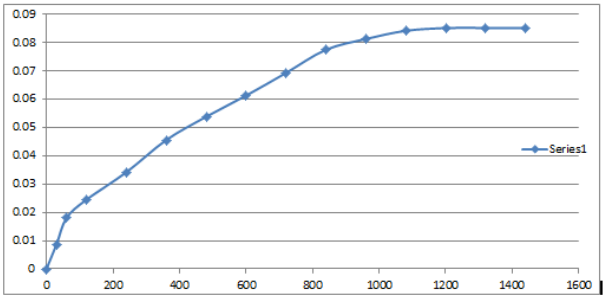

Figure 2: In vitro release profile of formula 3.

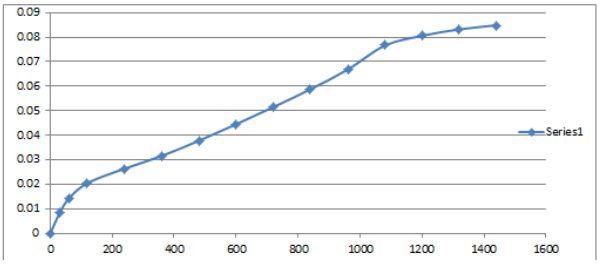

Figure 3: In vitro release profile of formula 4.

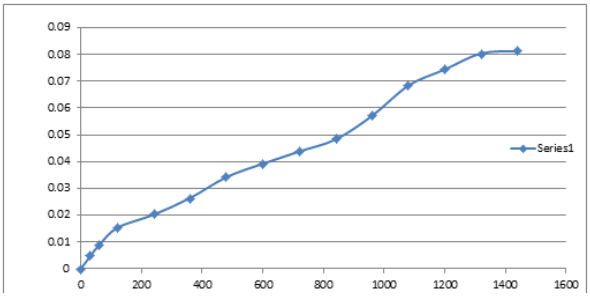

Figure 4: In vitro release profile of formula 5.

Conclusion

This study confirms that the antisolvent technique is suitable for the preparation of 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.

From the above, can conclude F4 has the best characteristic and suitable for formulation as nanosuspension for sustained release formula.

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