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Preparation and Evaluation of Darifenacin Hydrobromide Loaded Nanostructured Lipid Carriers for Oral Administration

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Abstract

Darifenacin hydrobromide is a selective M3 receptor antimuscarinic drug and it is used in the management of urinary frequency, urgency, and incontinence in detrusor instability. It is slightly soluble in water, undergoes extensive hepatic first-pass metabolism and has short elimination half-life (3–4 hours). Therefore, It has low bioavailability (15.4 % - 18.6 %). Darifenacin hydrobromide loaded nanostructured lipid carriers (NLCs) were formulated by emulsification sonication using different ratios of solid lipid to liquid lipid, different types and concentration of surfactants. Formula sixteen , containing darifenacin hydrobromide 8.9 mg , solid lipid glyceryl monostearate and olic acid in a ratio equal to 77.5:22.5 , tween $_{80}$ (0.5%) , and vitamin E that is added as an antioxidant , was considered as an optimized formula based on its particle size, polydispersity index (PDI) , zeta potential and entrapment efficiency. This formula was subjected to further characterization such as DSC, FTIR, XRD, AFM, and release study. FTIR and DSC studies indicated no interaction between drug and excipients. XRD study showed a halo pattern which is a significant pattern of amorphous form of the drug. Atomic force microscopy (AFM) study showed discrete lipid nanoparticles with no aggregation. Release study exhibited burst release in the first hour followed by sustained and controlled release up to 12 hours.

Keywords: Darifenacin hydrobromide, Nanostructured lipid carrier, Bioavailability.

الحاملات الدهنية ذات البنية النانوية المحملة بالداريفناسين هايدروبرومايد المعطاة عن طريق الفم

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الخلاصة

عقار داريفناسين هايدروبرومايد يعتبر مثبط انتقائي لمستلمات M3 ويستخدم في علاج سلس البول. هذا العقار قليل الذوبان في الماء، يتعرض الى ايض واسع النطاق بالكبد ويمتلك زمن طرح من الجسم قصير جداً من ثلاثة الى اربع ساعات لذلك التوافر الحيوي في البلازما لهذا الدواء قليل من خمسة عشر بالمئة الى ثمانية عشر بالمئة. حاملات الدهون ذات البنية النانوية المحملة بدرافيناسين هايدروبرومايد صنعت بطريقة المستحلب المنصهر مع استخدام الموجات فوق الصوتية مستخدمين نسب مختلفة من الدهون الصلبة الى الدهون السائلة، انواع مختلفة وتراكيز مختلفة من العوامل التي تقلل الشد السطحي.

التركيبة السادسة عشر تتكون من دارافيناسين هايدروبرومايد (٩, ٨ ملغم) و نسبة دهون صلبة الى دهون سائلة تساوي ٥, ٧٧ :٥, ٢٢, توين ٨٠ (٥, ٠%) , و فيتامين E يضاف كعامل مضاد للاكسدة . التركيبة السادسة عشر تعتبر أفضل تركيبة اعتماداً على حجم الجزيئات والتوزيع الحجمي للجزيئات والشحنة السطحية والقابلية على احتواء الدواء بداخلها. التركيبة السادسة عشر اختبرت باستخدام المسح الكالوري، مطيافية الاشعة تحت الحمراء (FTIR) ، حيود الاشعة السينية، اي اف ام (AFM) ، وكذلك دراسة التحرر.التركيبة السادسة عشر اختبرت باستخدام الاشعة تحت الحمراء والمسح الكالوري واظهرت عدم وجود تداخل بين الدواء والمواد الاخرى في التركيبة. اختبار حيود الاشعة السينية اظهر شكل غير متبلور , واختبار مجهر القوة الذريــة AFM اظهر وجود جزيئات منفصلة ولايوجد تجمع للجزيئات.دراسة التحرر للدواء اظهرت تحرر سريع للدواء خلال الساعة الاولى بعد ذلك تحرر بطيء الى حد اثنا عشر ساعة.اظهرت الدراسة الكلية اهمية الحاملات الدهنية ذات البنية النانوية كنواقل لزيادة التوافر الحيوي لعقار داريفناسين هايدروبرومايد مقارنة بالحبوب المعطاة عن طريق الفم .

الكلمات المفتاحية: داريفناسين هايدروبرومايد، حاملات الدهون ذات البنية النانوية، التوافر الحيوي.

Introduction

Recently, several approaches have been investigated to develop nanosized drug delivery system such as lipid nanoparticales with a solid matrix which are divided into solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). SLNs are prepared from solid lipids only. Therefore, after preparation at smallest a part of the particles crystallize in a higher energy modification (α or β). During storage, these modifications can transform to the low energy, more ordered β modification. Due to high degree of order of this modification, the number of imperfections in the crystal lattice is small and this leads to drug expulsion.

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NLCs have been developed to overcome the drawbacks associated with SLNs. They are considered to be the second generation of lipid nanoparticles. Compared to SLNs, NLCs show a higher loading capacity for active compounds by creating a less ordered solid lipid matrix, i.e. by blending a liquid lipid with the solid lipid, a higher particle drug loading can be achieved. Therefore, the NLCs have an increased drug loading capacity in comparison to SLNs and the possibility of drug expulsion during storage is less . NLCs have also a lower water content of the particle suspension and a less tendency of unpredictable gelation[1].

Darifenacin is a selective M3 antimuscarinic with actions similar to those of atropine. It has a greater selectivity for the muscarinic receptors of the bladder. It is subjected to extensive first-pass metabolism and has a short elimination half-life after intravenous and immediate release oral dosage forms (3-4 hr)[2].

The absolute bioavailability of darifenacin from 7.5 mg and 15mg prolonged release tablet was estimated to be 15.4 % and 18.6% respectively[2]. It is metabolized in the liver by cytochrome P450 isoenzymes CYP 2D6 and CYP 3A4 [2].

Darifenacin is a P-glycoprotein(P-gp) substrate. It is about 98% bound to plasma proteins . Most of the dose is excreted as metabolites in the urine and feces[2].

The objective of this study is to prepare differents darifenacin hydrobromide loaded NLCs to improve the bioavailability of darifenacin hydrobromide which undergoes extensive first-pass effect when formulated in conventional dosage form , characterization of the prepared formulas , and the selection of the best darifenacin hydrobromide loaded NLC which subjected to further characterization . After that, formulation of the best formula as a dosage form well known to the patient (capsule dosage form) was achieved in order to improve patient compliance .

Materials and Methods

Materials

Darifenacin hydrobromide and glyceryl monostearate (GMS) (hangzhou hyperchemical China) , oleic acid (central drug house company India) , tween$_{80}$, stearic acid and palmitic acid (BDH chemical England) , methanol (romil, United kingdom) and distilled deionized water was used. All other chemicals were reagent grade.

Method

Screening of components

Prior to the production of NLC formulation , lipid, oil , and surfactant screening should be performed to determine the most suitable components for the active ingredient to be incorporated in the NLC .

Solubility in solid lipid

The solubility of darifenacin hydrobromide in different solid lipids was determined by semi-quantitative method. An accurately weighed fixed quantity (8.9 mg) of the drug was taken in a series of test tubes and solid lipids were added in increments until the drug is completely solubilized. The temperature of the test tubes was controlled at 5-10 ˚C above the melting point of respective lipids [3].

The test tubes were intermittently mixed using cyclone mixer and observed for any drug residues. The amount of lipid (mg) required to completely solubilized the drug in the molten state was determined [3].

Solubility in liquid lipid

An excess amount of darifenacin hydrobromide was added to 5ml of oil in a test tube and mixed using cyclone mixer. The mixture was agitated on mechanical shaker for 24 hr at room temperature for equilibration. After equilibrium, each sample was centrifuge at 10,000 rpm for 30 min to separate the undissolved drug. Supernatant that obtained was pulled and filtered through 0.45 µm filter. The filtrate was diluted suitably with methanol and saturation solubility of darifenacin hydrobromide (mg/ml) in oil was determined by recording absorbance using UV- Vis spectrophotometer at respective λ max [4].

Preparation of nanostructured lipid carriers (NLCs)

An accurately weighed solid lipid GMS and liquid lipid oleic acid were mixed and then heated at 5 – 10 ˚C above the melting point of lipid mixture. To this lipid mixture, the drug was added to obtain a clear melting solution. An aqueous phase was prepared by dissolving surfactant in deionized water and heated to the same temperature as that of the oil phase. Then, this hot aqueous phase was added dropwise to the lipid phase at a constant rate (2 ml / min) under magnetic stirring. After that, this pre-emulsion was sonicated for 20 minutes using probe sonicator. The resulting hot nanoemulsion was cooled to room temperature to induce crystallization. Twenty-two formulas were prepared by this method as shown in table (1). Vitamin E was added to the selective formula as antioxidant. The selective formula was freeze-dried by using cryoprotectant to convert NLC to dry powder and was filled in a hard gelatin capsule of zero size [5].

Table1. Formulations of darifenacin hydrobromide loaded nanostructured lipid carriers (NLCs)

Formulas No.	Amount of drug (Darifenacin Hydrobromide) mg	Ratio of solid lipid to liquid lipid glyceryl monostearate: oleic Acid	Type of surfactant % (W / V)				Co-surfactant % (W / V)	Water
			Tween$_{20}$	Tween $_{80}$	Poloxamer$_{80}$	Span$_{80}$	Myverol	
F1	8.9	92.5 : 7.5	0.5					Q.S
F2	8.9	92.5 : 7.5	1					Q.S
F3	8.9	92.5 : 7.5	1.5					Q.S
F4	8.9	85 : 15	0.5					Q.S
F5	8.9	85 : 15	1					Q.S
F6	8.9	85 : 15	1.5					Q.S
F7	8.9	77.5 : 22.5	0.5					Q.S
F8	8.9	77.5 : 22.5	1					Q.S
F9	8.9	77.5 : 22.5	1.5					Q.S
F10	8.9	92.5 : 7.5		0.5				Q.S
F11	8.9	92.5 : 7.5		1				Q.S
F12	8.9	92.5 : 7.5		1.5				Q.S
F13	8.9	85 : 15		0.5				Q.S
F14	8.9	85 : 15		1				Q.S
F15	8.9	85 : 15		1.5				Q.S
F16	8.9	77.5 : 22.5		0.5				Q.S
F17	8.9	77.5 : 22.5		1				Q.S
F18	8.9	77.5 : 22.5		1.5				Q.S
F19	8.9	85 : 15			0.5			Q.S
F20	8.9	85 : 15			1			Q.S
F21	8.9	85 : 15		0.5			0.2	Q.S
F22	8.9	77.5 : 22.5				0.5		Q.S

Characterization and evaluation of nanostructured lipid carriers (NLCs)

Particle size and polydispersity index measurement

The particle size analysis of formulas was performed using ABT- 9000 Nano Laser Particle Size Analyzer. Before measurements, NLCs dispersion was diluted suitably using deionized water. Data was analyzed by software and values of mean particle size, polydispersity index (PDI) and particle size distribution curve were recorded [6].

Zeta potential measurement

The zeta potential analysis of formulas was performed using Zeta Sizer. Before measurements, NLCs dispersion was suitably diluted [7].

Entrapment efficiency measurement

Entrapment efficiency corresponds to the percentage of drug encapsulated within the lipid matrix. Certain volume of NLCs dispersion was accurately taken and subjected to centrifugation at 25000 rpm for 30 min at 4˚ C . After centrifugation, 1 ml of supernatant was taken and suitably diluted with methanol and the free drug concentration determined using UV-Vis Spectrophotometer and (%EE) measured using the following equation [8]:

$$EE\ (\%) = \frac{W_{initial} - W_{free}}{W_{initial}} \times 100$$

EE(%) = percentage of entrapment efficiency

$W_{initial}$ = initial drug concentration

W_{free} = free drug concentration (unentraped drug)

Differential scanning calorimetry (DSC) study

The possibility of any interaction between darifenacin hydrobromide and excipients was assessed by carrying out thermal analysis of the formulation using DSC. The analysis was performed on the pure darifenacin hydrobromide, GMS and lyophilized darifenacin hydrobromide NLCs . Each sample was weighed accurately and kept in aluminum pans and scanned between 30 ºC – 400 ºC at a heating rate of 10 ˚C/min and cooling rate of 40 ˚C/min under nitrogen gas. An empty aluminum pan was used as reference in the study [9].

FTIR spectroscopy study

FTIR helped to confirm the identity of the drug and to detect the interaction of the drug with carriers.FTIR spectral measurement for pure darifenacin hydrobromide drug, lipid glyceryl monostearate, oleic acid, $tween_{80}$, vitamin E and optimized NLCs formulation were obtained on FTIR using KBr disk method. The scanning range was 400- 4000 cm^{-1} [10].

X- Ray diffraction (XRD) study

Powder X-ray diffraction (PXRD) was performed to analyze crystalline or amorphous nature of darifenacin hydrobromide loaded NLCs. PXRD studies were performed by powder X-ray diffractometer where CUK α radiation of 1.5405˚A was used as X-ray source. For the measurements , samples were Kept in the glass sample holders followed by scanning from 2˚ to 60˚ with scan angular speed (2 θ /min) of 2˚/ min , 40 KV working voltage and 30 mA current. Samples used for study were pure darifenacin hydrobromide, glycerl monostearate, and lyophilized dariferacin hydrobromide NLC [11].

Atomic Force Microscopy (AFM) study

To study morphological changes and also the particle size of NLCs , AFM micrographs were imaged by using atomic force microscopy (AFm). The images were obtained by measurement of interaction forces between the tip and sample surface . The experiments were done in air at room temperature (25˚C) operating in noncontact mode droplets of dispersion were placed on a small mica disk. The measurements were performed in different sample locations. image data were analyzed with software [12].

In-vitro drug release and release kinetic studies

The in-vitro release of darifenacin hydrobromide from NLCs was carried out in 500 ml phosphate buffer solution (pH 6.8) by using the dissolution testing apparatus with rotating basket at 100 rpm and temperature 37∓ 0.5 ˚C [13]. This method involved placing the capsule of the selected formula inside wire basket that is rotated while immersed in the dissolution medium. Five milliliters aliquots were withdrawn at 1 , 2 , 4 , 6 , 8 , 10 , and 12hr from dissolution medium and replaced with 5 ml of fresh buffer to maintain sink condition . The aliquots withdrawn were filtered by using 0.45µm filter, suitably diluted if necessary, and analyzed by using UV-Vis Spectrophotometer. The cumulative percentage of the released drug was plotted versus time [13].

The in-vitro release profile was fitted using several kinetic models such as zero-order (cumulative percentage of drug released versus time), first – order (log cumulative percentage of drug remaining versus time), Higuchi (cumulative percentage of drug released versus square root of time), and Korsmeyer–peppas (log cumulative percentage of drug released versus log time) equations [13].

Statistical analysis

Statistical analysis of the data was carried out using one-way analysis of variance

(ANOVA) test and the level of statistically significance difference was selected as $P < 0.05$.

Results and Discussions

Selection of components

A selection of suitable lipids and other excipients was significant to develop NLCs for slightly water-soluble darifenacin hydrobromide. To keep the drug in soluble form, it was of prime importance that drug must possess higher solubility in solid lipid and oil.

Selection of solid lipid

Solid lipid was selected by checking the solubility of the drug in melted solid lipid by means of visible observation with the naked eyes under normal light. Lipids used for this study were stearic acid, palmitic acid and GMS. It was found that GMS showed highest darifenacin hydrobromide solubilizing capacity. Table (2) shows the comparative solubility of drug in different lipids.

Table2. Amount of solid lipid required to solubilize 8.9 mg of darifenacin hydrobromide

No .	Lipids	Amount of lipid
1	Stearic Acid	more than 1000 mg
2	Palmitic Acid	more than 1000 mg
3	Glyceryl monostearate	400 mg

Selection of liquid lipid

Liquid lipid was selected based on the maximum solubility of darifenacin hydrobromide in different liquid lipids.

Lipids used for this study were oleic acid, castor oil, and ethyl oleate. It was found from the result that oleic acid exhibited maximum darifenacin hydrobromide solubilizing capacity than the others as shown an table (3). Therefore, it was selected as liquid lipid to make a matrix with solid lipid GMS for the development of NLCs.

Table3. Solubility of darifenacin Hydrobromide in different oils

No .	Oil	Saturation solubility mg / ml
1	Caster oil	11.5
2	Oleic Acid	13.7
3	Ethyl oleate	12.43

Preparation of darifenacin hydrobromide loaded nanostructured lipid carriers

Emulsification sonication is a simple and popular method for preparation of NLCs and considered the method of choice for drugs showing high solubility in molten lipids [14].

Solid lipid GMS and liquid lipid (oleic Acid) were utilized to provide a core composed of highly lipophilic environment to accommodate darifenacin hydrobromide, thus becoming a suitable and optimum nanocarrier or reservoir for the drug. The incorporation of solid and liquid lipids mixture in the lipid matrix promoted imperfect crystallization, thus lowering the probability of the entrapped drug expulsion during storage. Also, the presence of liquid lipid in formulations allowed more flexibility for modulation of drug release and better drug-entrapment efficiency [15].

Characterization and evaluation of nanostructured lipid carriers particle size and polydispersity index determination

Particle size and PDI were important characteristics in the evaluation of stability of darifenacin hydrobromide loaded NLCs [16]. Four darifenacin hydrobromide formulas (F19, F20 , F21 and F22) from the prepared formulas were in microsize range , therefore they are not subjected to further characterization .

Eighteen darifenacin hydrobromide formulas in nano size range, from the prepared formulas, were successfully prepared as shown in table (4). A nanoscale particle exhibited unique physical and biological properties, making it particularly ideal for drug entrapment, and provided a large surface area for the reaction with its site of action [17]. Also, the nanoscale size minimized the probability of drug being phagocytized by macrophage of the mononuclear phagocytic system, hence decreasing the destruction of darifenacin hydrobromide NLCs in the body [18]. Particle size plays a crucial role in the gastrointestinal uptake and their clearance by the reticuloendothelial system. Therefore, the precise determination of the particle size is very important where particle size less than 300 nm is advisable for the intestinal transport[19].

Polydispersity index (PDI) is a measurement of particle size distribution that varies from 0 to 1. The polydispersity index (PDI) of darifenacin hydrobromide loaded NLCs formulas was within the acceptable range and it indicated that all the prepared NLCs were almost in monodispersity and homogeneous with narrow size distribution as shown in table (4). The closer the value of PDI to zero, the higher the homology between the particles. The PDI of less than 0.5 indicates that there was no aggregation of the nanoparticle of darifenacin hydro-

bromide-NLCs. PDI more than 0.5 is an indication of particle aggregation. The aggregates do not interact with the site of action in the way smaller individual particles do. The aggregation or agglomeration impedes the targeting efficiency of the nanoscale particle to the target organ. Also, the degree of cellular uptake might be decreased due to the presence of unwanted aggregates since , aggregation increases the particle size and lower the surface area [20-22].

Effect of concentration of surfactants on particle size

It was observed that increasing the concentration of surfactants had statistically significant effect ($p<0.05$) on particle size.The particle size was found to decrease with an increase in concentration of surfactant $tween_{80}$ and $tween_{20}$ for formulas (F1-F18) when the ratio of solid lipid GMS to liquid lipid oleic acid constant .The higher surfactants (Tween 80 and Tween 20) concentrations reduced the surface tension and facilitated particle partition. The decrease in the particle size is accompanied by a rapid and tremendous increase in the surface area . Thus, an increase in the surfactants (Tween 80 and Tween 20) concentration in the primary dispersion resultsed in rapid coverage of the newly formed particle surface [23].

Zeta potential determination

Zeta potential is essential for evaluating the storage stability of colloidal dispersions[24]. The zeta potential of the different formulas of darifenacin hydrobromide NLCs was found within the range of (- 11.78 mv to-32.44 mv) as shown in table (4). Zeta potential referred to the electrostatic charges on the surface of the nanoparticle in the dispersion, which was used to predict the long term stability of the nanoparticles [24]. Since, the zeta potentials above 30 mv or below–30 mv were required for full electrostatic stabilization [25]. Many experiments demonstrated that it is not only electrostatic repulsion had an effect on the stability of any nanoparticles, but also the use of steric stabilizer that favoured the formation of stable nanoparticle dispersion [26]. The steric hindrance from $tween_{80}$, that was used in the production of darifenacin hydrobromide-NLCs as a stabilizer , had an additional effect in increasing the particle stability [26]. Also, surface charge of the NLCs has an effect on tissue permeability and cellular up take where high positive or negative zeta potential lead to superior phagocytosis [27] .

Effect of ratio solid lipid to liquid lipid on zeta potential

The negative zeta potential value in the darifenacin hydrobromide loaded NLCs formulas related to deprotonation of carboxyl group of oleic acid. The increase in ratio of liquid lipid to solid lipid had significant effect ($p<0.05$) on zeta potential. The value of zeta potential increased when the ratio of oleic acid to glyceryl monostearte increased [28].

Entrapment efficiency determination

The entrapment efficiency of the different formulas of darifenacin hydrobromide loaded NLCs is shown in table (4) . It was consistently reported that the increase in entrapment efficiency in NLCs related to the presence of solid and liquid lipids that results in great imperfections in crystal lattice providing higher space for drug accommodation [29 - 31]. Also, higher drug solubility in liquid lipid will increase the entrapment efficiency [32].

Effect of concentration of surfactants on entrapment efficiency

It was observed that increasing the concentration of surfactants had statistically significant effect ($p<0.05$) on the entrapment efficiency of darifenacin hydrobromide. The entrapment efficiency of darifenacin hydrobromide loaded NLCs was found to decrease with an increase in the concentrations of surfactants (tween 80 and tween 20) for formulas (F1-F18) when the ratio of solid lipid glyceryl monostearate to liquid lipid oleic acid was constant. The high surfactants (tween 80 and tween 20) concentrations reduced the particle size and this decreased the amount of darifenacin hydrobromide entrapped in the core of darifenacin hydrobromide NLCs and adsorbed on the surface of darifenacin hydrobromide NLCs [33].

Table4. Particle Size, Zeta Potential, PDI and Entrapment Efficiency of Darifenacin Hydrobromide Loaded NLCs.

Formula No.	Particle size	Zeta potential	Entrapment efficiency	PDI
F1	98.9nm	-14.32	56.27%	0.22
F2	79.1 nm	-13.82	56.17%	0.22
F3	78.6 nm	-15.09	53.93%	0.37
F4	87.9 nm	-16.72	68%	0.15
F5	86.6 nm	-14.71	55.11%	0.11
F6	19.7 nm	-17.29	53.39%	0.23
F7	989 nm	-18.97	83.51%	0.03
F8	436 nm	-25.72	58.42%	0.01
F9	99.3 nm	-21.58	47.44%	0.53
F10	191 nm	-17.9	65.77%	0.1
F11	106 nm	-11.78	43.97%	0.22
F12	61.9 nm	-13.92	38.46%	0.01
F13	151 nm	-19.23	65.78%	0.04
F14	139 nm	-17.36	62.79%	0.07
F15	133 nm	-17.81	61.06%	0.02
F16	249 nm	-32.44	74.44%	0.2
F17	139 nm	-18.46	65.38%	0.49
F18	78.7 nm	-27.06	23.82%	0.33

Selection of the optimum formula

Formula sixteen regarded as the optimum formula depending on entrapment efficiency measurement which was equal to 74.44% and zeta potential which was equal to– 32.44mv in addition to the particle size which is equal to 249 nm and polydispersity index which was equal to 0.2 . Formula sixteen containing ratio of solid lipid GMS to liquid lipid oleic acid equal to 77.5:22.5 , tween 80 (0.5%), darifenacin hydrobromide 8.9 mg, and vitamin E that is added as antioxidant.

Differential Scanning Calorimetry (DSC) study

Differential scanning calorimetry was performed to characterize the polymorphism and the degree of crystallinity of darifenacin hydrobromide loaded NLCs. Figures (1 - 3) and (3) showed the DSC thermograms of darifenacin hydrobromide, GMS and darifenacin hydrobromide loaded NLCs respectively. The study showed that the melting point of darifenacin hydrobromide NLCs (69.76 ˚C) was lower than that of the bulk material GMS (76.65 ˚C) also disappearance of melting peak of darifenacin hydrobromide (235.17 °C) indicated that darifenacin hydrobromide was dissolved in the lipid matrix and encapsulated in the nanostructured lipid carrier. During the preparation , darifenacin hydrobromide was dissolved in the melted lipid phase. Following the cooling of the dispersion to room temperature, darifenacin hydrobromide melting peak was not detected anymore. The absence of this thermodynamic transition could be due to a molecular dispersed state of darifenacin hydrobromide in the mixture [34]. The decrease in the melting point of darifenacin hydrobromide NLCs (69.76 ˚C) which was below that of GMS (76.65 ˚C) is described as melting point depression [35]. The addition of oil (oleic acid) into the matrix provoked an additional shift of the melting point to lower temperature. Decrease in melting enthalpy in darifenacin hydrobromide NLCs as compared to GMS and darifenacin hydrobromide was due to less–ordered arrangement of nanoscale particles. Therefore lesser amount of energy was needed to overcome the lattice force in the NLCs than GMC . Also, incorporation of darifenacin hydrobromide inside the lipid matrix resulted in a further increase in the number of defects in the lipid crystal lattice [35].

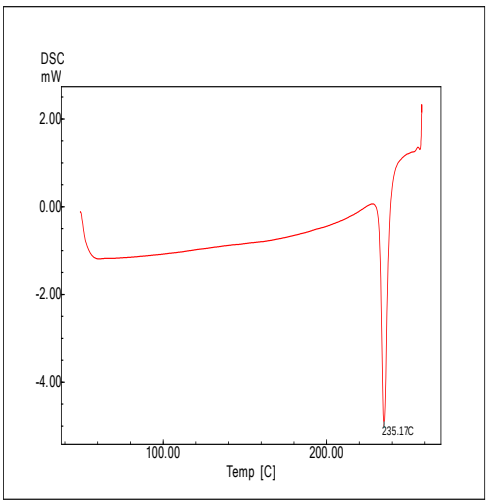

Figure 1. DSC thermogram of darifenacin hydrobromide

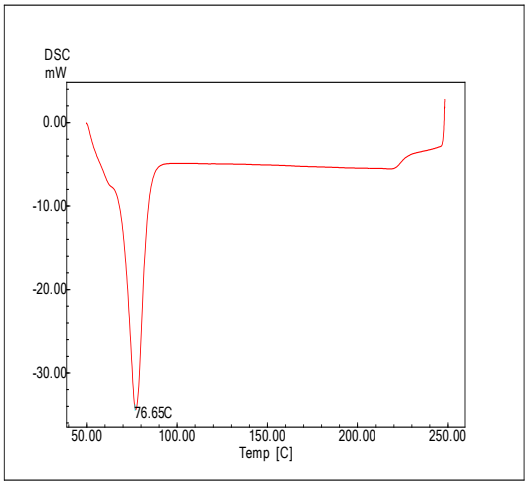

Figure 2. DSC thermogram of glyceryl monostearate.

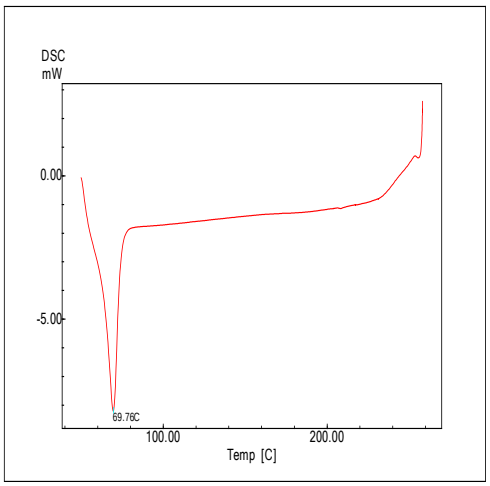

Figure 3. DSC thermogram of dariferacihydrobromide loaded NLCs.

FTIR Spectroscopy study

FTIR spectra of darifenacin hydrobromide , GMS , oleic acid, Tween 80 , vitamin E , and darifenacin hydrobromide loaded NLCs (F_{16}) are shown in figures (4 - 9) illustrated that , there was no interaction between drug and excipients since, the characteristic peaks of the drug and the major constituents are still observed in IR spectrum of the selected formula .

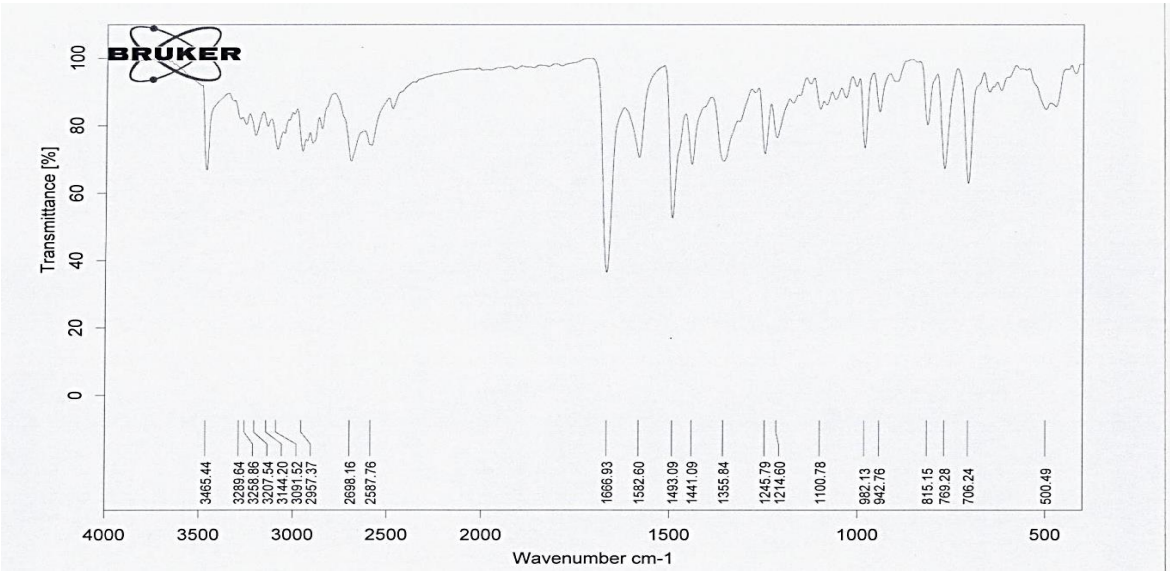

Figure 4 . IR spectrum of darifenacin hydrobromide.

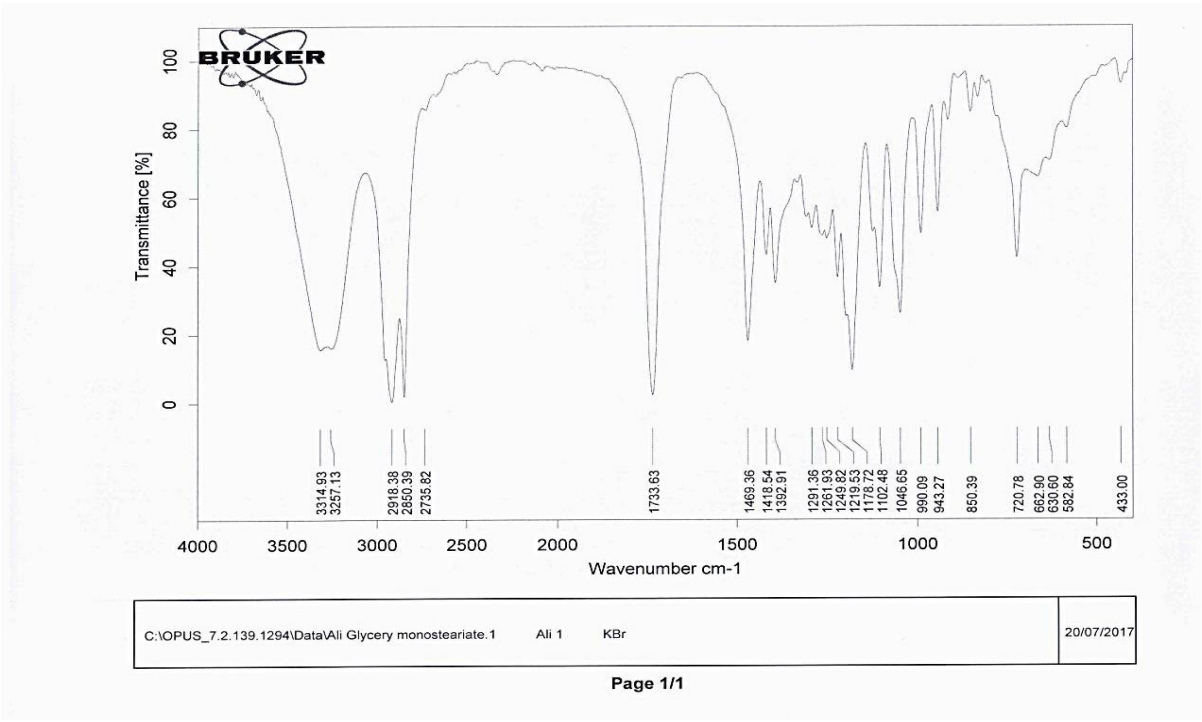

Figure 5. IR spectrum of glyceryl monostearate (GMS).

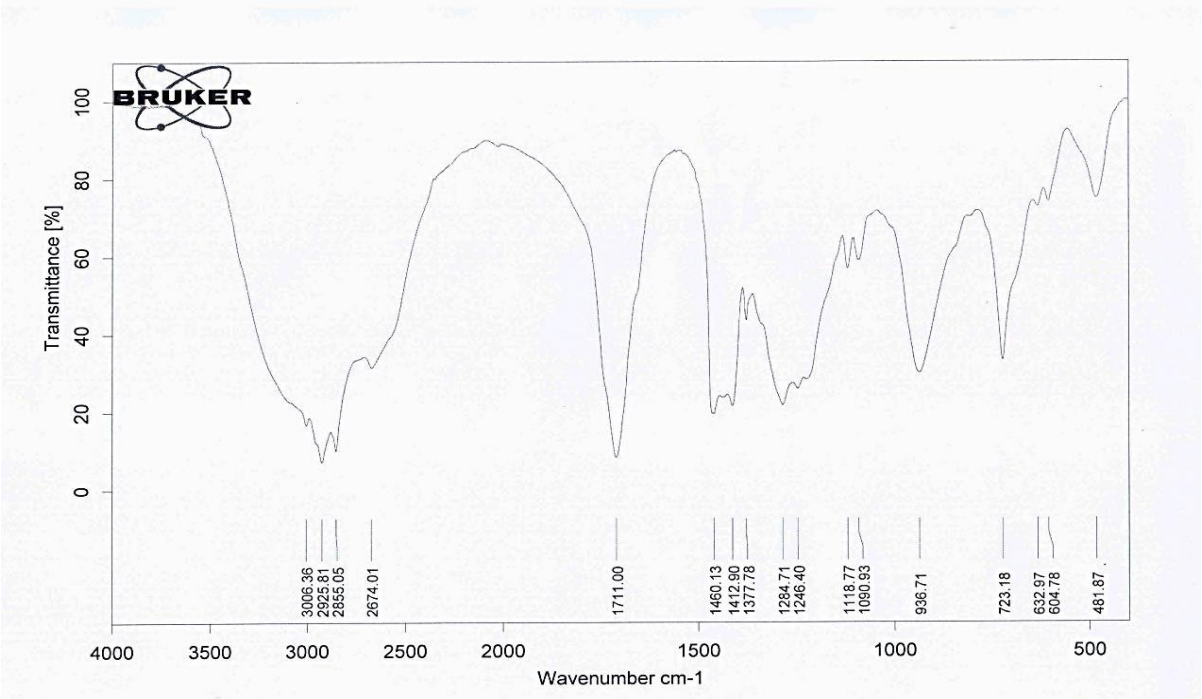

Figure 6 . IR spectrum of oleic acid

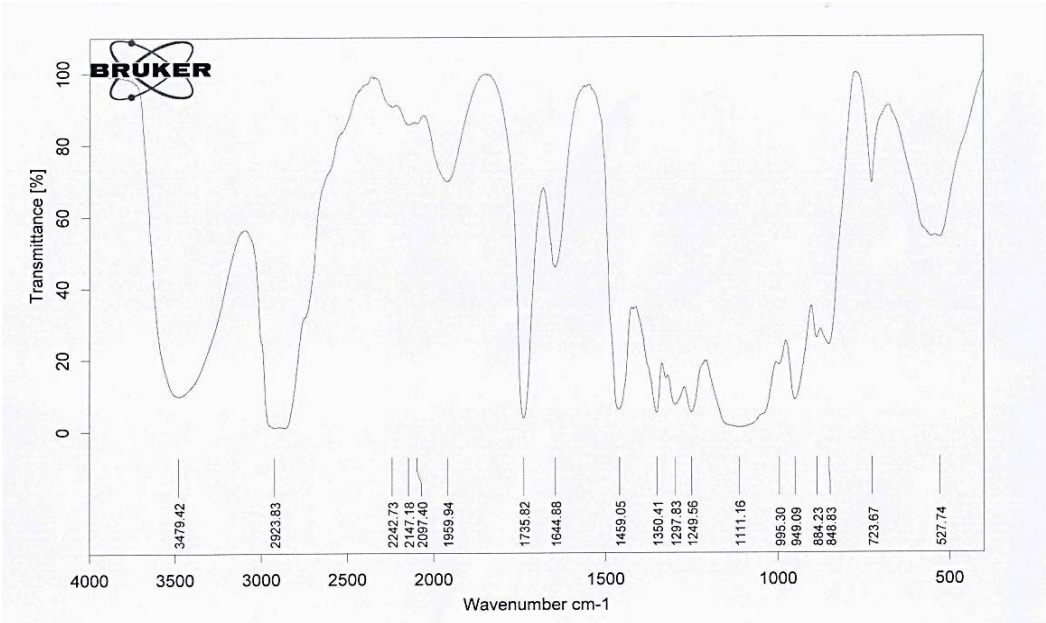

Figure 7. IR spectrum of tween$_{80}$.

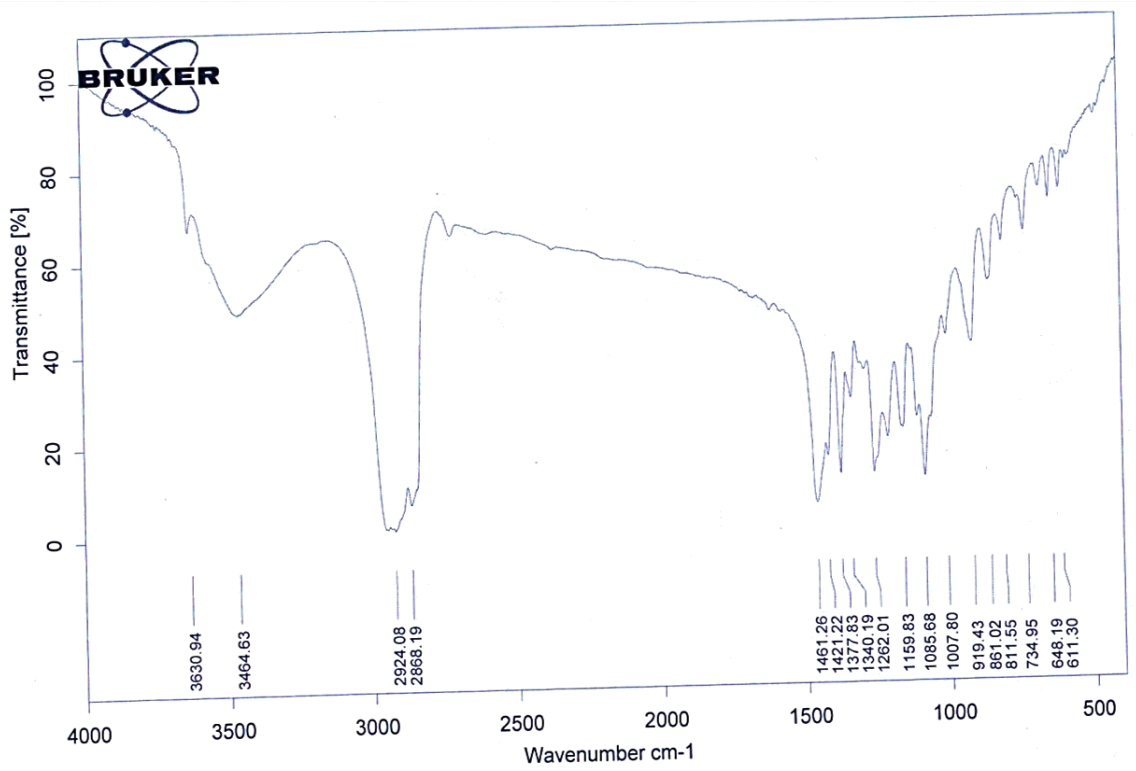

Figure8 . IR spectrum of Vitamin E.

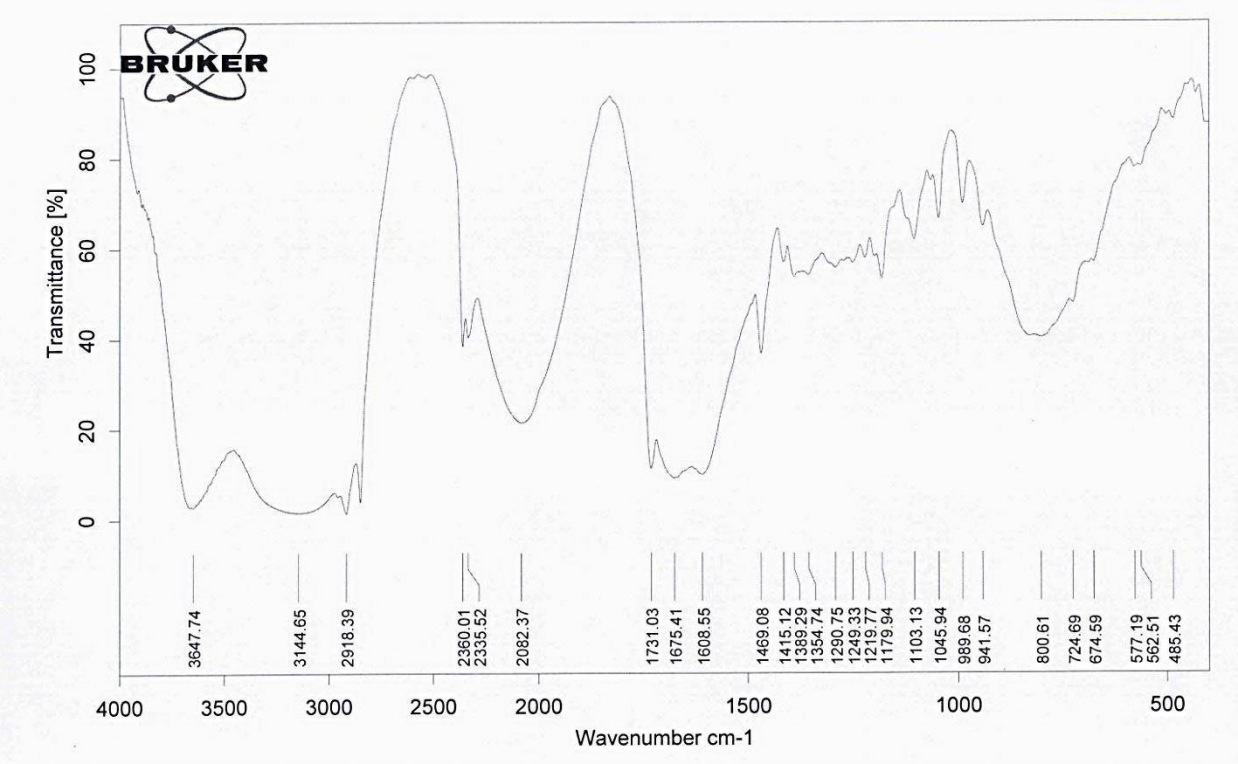

Figure 9 . IR spectrum of darifenacin hydrobromide loaded NLCs (F_{16}).

X–Ray Diffraction study

X–ray diffractograms of pure darifenacin hydrobromide , GMS and freeze dried darifenacin hydrobromide NLCs were presented in figures (10-12) . The X – Ray diffractogram of darifenacin hydrobromide exhibited sharp peaks at diffraction angles (2θ) with a typical crystalling patten. All major characteristic crystalline peaks (11.47° , 18.20° and 24.55°) disappeared in the diffractogram of darifenacin hydrobromide NLCs. This indicated that darifenacin hydrobromide converted from crystalline to amorphous form [36].

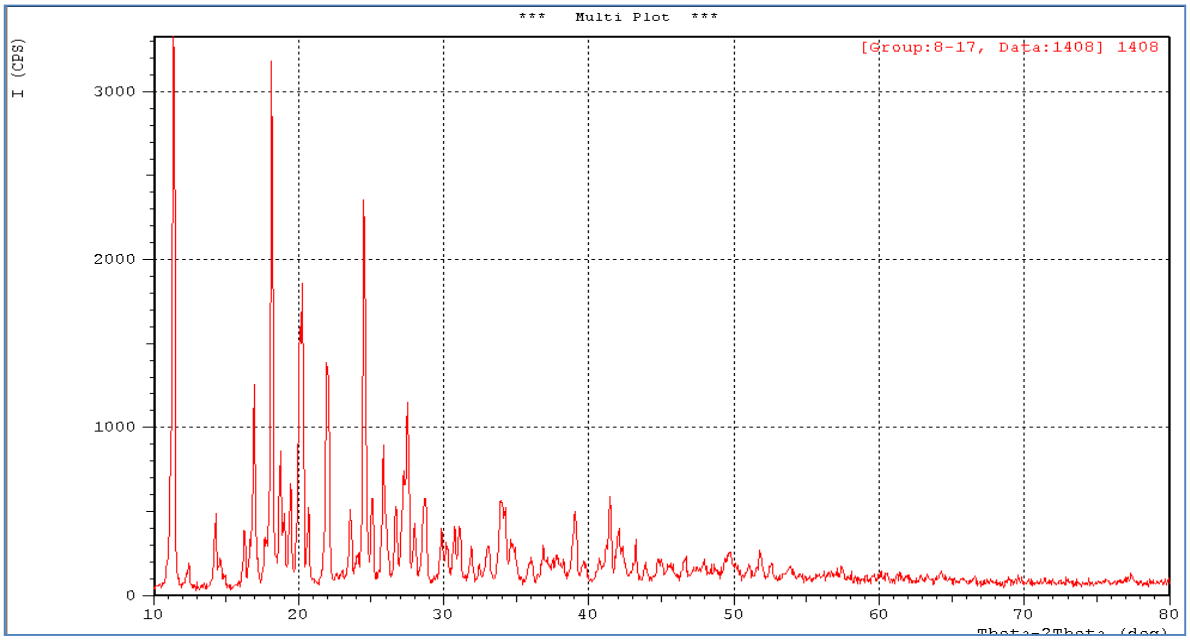

Figure 10. X – Ray diffractograms of darifenacin hydrobromide.

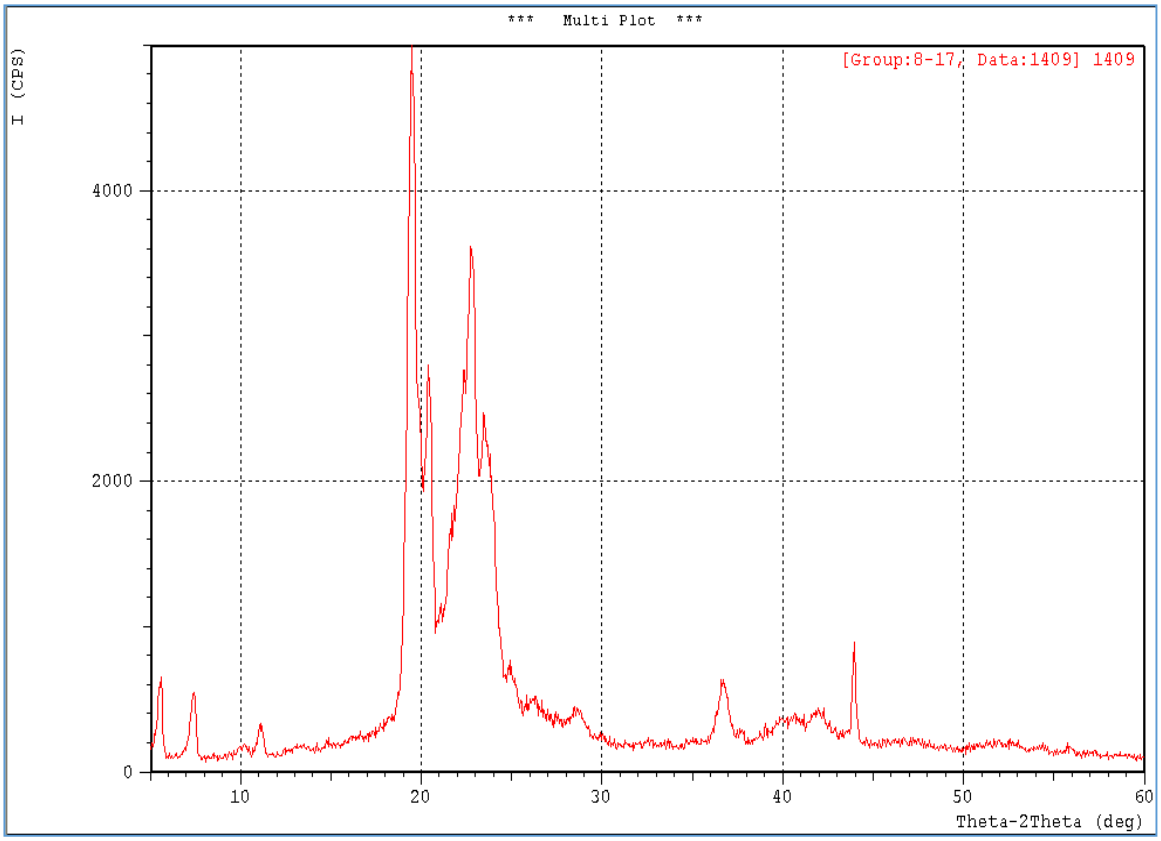

Figure 11. X – Ray diffractograms of glyceryl monostearate (GMS).

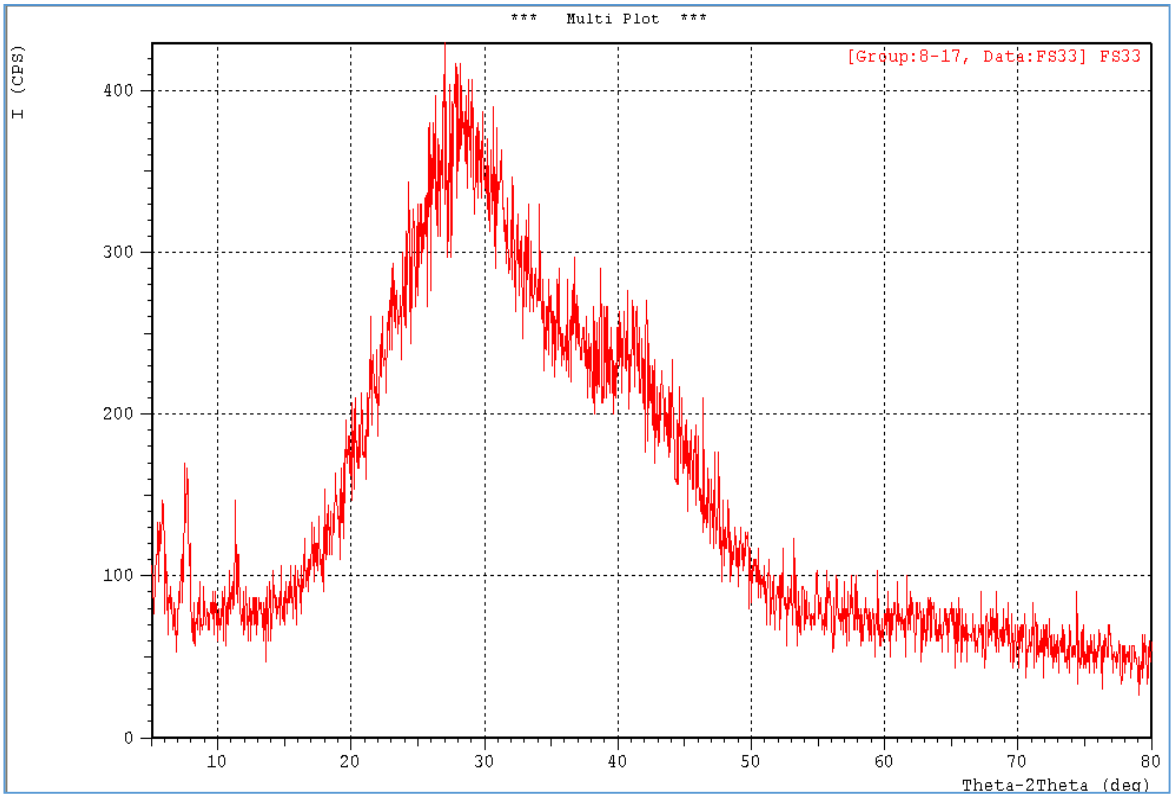

Figure 12 . X – Ray diffractograms of darifenacin hydrobromide loaded NLCs

Atomic Force Microscopy (AFM) study

The morphological analysis performed by AFM showed three-dimensional structure (figure 13) and discrete lipid nanoparticles with no aggregation. The particle size equal to 260nm as shown in the histogram of particle size distribution in figure (14) [37].

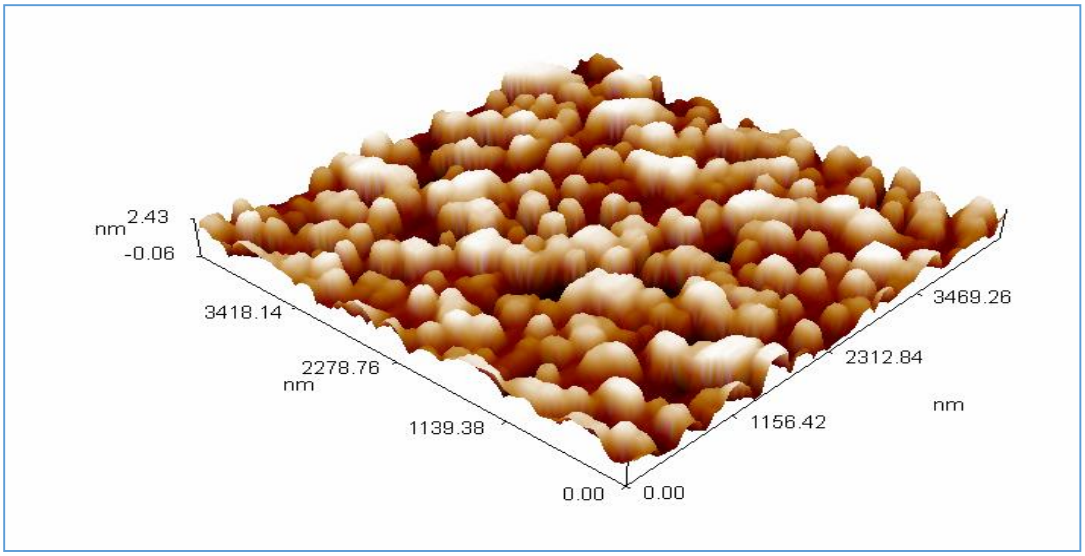

Figure 13. Three –dimensional morphology of darifenacin hydrobromide loaded NLCs (F16)

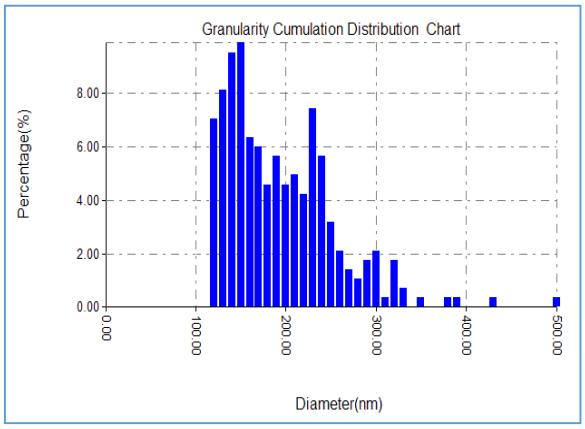

Figure 14. Granularity cumulation distribution of darifenacin hydrobromide loaded NLCs (F16)

In-vitro drug release and release kinetic studies

The in-vitro drug release of darifenacin hydrobromide loaded NLCs showed an interesting biphasic release with an initial burst effect followed by controlled and sustained release [38] as shown in figure (15). The initial burst release of darifenacin hydrobromide might be due the presence of unentrapped darifenacin hydrobromide in the NLCs [39]. Another reason might be due to most of the liquid lipid (oleic Acid) being located in the outer shell of NLCs which lead to darifenacin hydrobromide enriched shell that easily released drug by diffusion or matrix erosion [40]. The third supportive factor for the burst release that if NLCs prepared with high temperature and optimum concentration of surfactant, it may produce drug burst release (40 ,41) . At the end of first hour, 30 % of drug is released, after that the drug release follow steady pattern of controlled and sustained release up to 12 hs. The kinetic of release and the mechanism of the release from NLC was evaluated by fitting the release date into first order , zero order , Higuchi and korsemeyer – peppas as shown in table (5). The darifenacin hydrobromide loaded NLCs was fitted well with Higuchi model since R^2 value equal to 0.9425 . The n value (< 0 .5) indicated that the release behavior of darifenacin hydrobromide loaded NLCs followed fickian diffusion mechanism [41] .

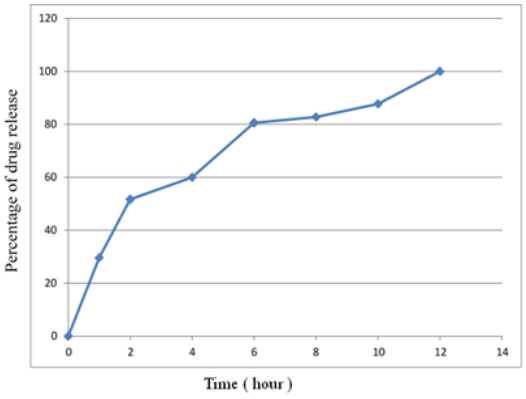

Figure 15. The percentage of drug release from formula sixteen per time at pH 6.8 and 37°C

Table5.The kinetic and the mechanism of the release data of darifenacin hydrobromide from NLC

Formula	Drug release kinetic			Krosmeyer -pepas n value
	Zero order R^2	First order R^2	Higuchi R^2	
16	0.2225	0.9298	0.9425	0.419

Conclusion

In this work, darifenacin hydrobromide loaded NLCs with sustained release for about 12 hours with biphasic profile effect was successfully prepared using solid lipid GMS and liquid lipid oleic acid in a ratio 77.5 : 22.5 in presence of 0.5 % $tween_{80}$ by using emulsification sonication method .

Future study

Stability study for the prepared darifenacin hydrobromide loaded NLCs capsules , bioavailability and clinical study are to be done

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