RESEARCH ARTICLE

Preparation and Evaluation of Rizatriptan Benzoate Loaded Nanostructured Lipid Carrier Using Different Surfactant/Co-Surfactant Systems

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ABSTRACT

Migraine is the second most common neurological illness, affecting around one billion people each year; triptans are commonly used in the treatment; rizatriptan benzoate is a member of the triptan class, it's available as a tablet dosage form. Unfortunately, it undergoes first-pass effect after oral intake. The intranasal route of administration represents a solve to such a problem. Because it has a limited permeability, an effort has been made to circumvent this hurdle. Nanotechnology's innovation has offered a useful answer to several issues in the pharmaceutical field in which it could improve drug permeability. The aim of the present work involved loading rizatriptan benzoate on a nanostructured lipid carrier as an attempt to resolve adversity affiliated with the intended drug. The high-speed homogenization technique prepares six formulas. The formulations' particle size, polydispersity index, zeta potential, entrapment efficiency, loading capacity, and *in-vitro* drug release were studied. Differential scanning calorimetry (DSC), Fourier transforms infrared spectroscopy (FTIR), and powder X-ray diffraction (PXRD) was investigated to exclude drug excipient incompatibility and to evaluate the crystallinity state of rizatriptan benzoate before and after formulation. Successful formulations were obtained with an acceptable nanostructured parameter *In-vitro* drug release profile illustrates a biphasic pattern in which an immediate followed by a persistent phase over a 6 hours. release period, with an estimated percent of the medication being released with an anomalous release mechanism. The compatibility and crystallinity investigation revealed that rizatriptan benzoate was compatible with the other excipients used in the research, and the drug molecule was found to be in an amorphous state within the lipid matrix. In conclusion, nanostructured lipid carriers might be a potential delivery approach for improving intranasal administration of rizatriptan benzoate.

Keywords: Migraine, Nanostructured lipid carrier, Rizatriptan benzoate, Tween 80.

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INTRODUCTION

In recent years, nanoparticulate carriers have shown considerable promise as delivery systems due to their nanoscale size and unique characteristics. The remedies are protected from moisture, physiological pH and enzymes, improved bioavailability, dose reduction, controlled drug release, prolonged circulation time, improved intracellular penetration, and targeted delivery to specific sites or organs through the carriers' surface modifications.[1] Several nanocarriers have been developed for drug delivery and diagnostic applications, including nanocrystals, nanotubes and nanowires, liposomes, polymeric nanoparticles, hydrogels, dendrimers, and lipid nanoparticles.[2] Lipid nanoparticles are a carrier system with many appealing characteristics, including the potential to encapsulate medicines in a solid particulate matrix, biodegradability, low toxicity, and industrial scalability.[3] The development of a new lipid nanoparticulate drug delivery system began with the manufacture of solid lipid nanoparticles (SLNs). Incorporating the medication into a variety of biocompatible lipids produced in the nano range has shown to be a potential mechanism of drug delivery through lipid nanocarriers. Depending on the drug's thermal stability, it was produced using a cold or hot homogenization process. SLNs have been shown to have several drawbacks, such as drug leakage via the matrix during storage and decreased drug loading efficiency. These restrictions of SLNs were noticed by researchers, prompting Muller (a German scientist) to design new lipid carriers known as nanostructured lipid carriers (NLC) in 1999/2000. The NLC formulation was founded on

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incorporating the medicine into a combination of solid and liquid lipid in various ratios. To circumvent the limits imposed by the SLN core's crystallinity, NLCs were constructed to produce a less/no crystalline matrix with a solidified core.[4]

Based on the physical properties of solid and liquid lipids, three kinds of NLC are created. Imperfect type NLC includes mixing spatially distinct lipids made up of multiple fatty acids, resulting in crystal order defects. An amorphous type was generated by combining specific lipids like hydroxyoctacosanyl hydroxystearate or iso-propyl myristate with the solid lipid, as a consequence, the NLC is amorphous rather than organized, preventing drug ejection due to modification during storage. While multiple types of NLC contain numerous nanosized liquid oil pockets scattered throughout the solid matrix. Because drug solubility is better in these nanosized compartments, drug loading is enhanced.[5]

Migraine without aura, migraine with aura, and chronic migraine are the three main categories, the symptoms and duration of migraine depend on the category. The major is headache (lasting from 4 to 72 hours) which is unilateral location, pulsating quality, moderate or severe pain, and aggravated by or causing avoidance of routine physical activity (e.g., walking or climbing stairs). Nausea, vomiting or both, photophobia, and phonophobia are mainly accompanied symptoms.[6] Rizatriptan benzoate is a potent and selective serotonin (5-$HT_{1B/1D}$) receptor, agonist. It is a second-generation triptan and is used in the acute treatment of migraine attacks with or without aura and cluster headaches. It is reported that the oral bioavailability of rizatriptan benzoate is only 45% due to the hepatic first-pass effect and the half-life is 2–3 hours.[7] Hence, the main aim of this study was to develop a suitable nanostructured lipid carrier system for rizatriptan benzoate with maximum loading capacity intended to be administered through the nasal pathway.

MATERIALS AND METHODS

Rizatriptan benzoate (RNB), glyceryl monostearate (GMS), lecithin, and cremophore LE were supplied from Baoji Guokang Bio-Technology CO., LTD, China. Tween 80 was obtained from SCRC, India. Oleic acid was purchased from Thomas baker India. Deionized water was bought from al Rafidain technological company Iraq. All of the other materials are of analytical grade.

Preparation of RNB - NLC

Rizatriptan benzoate nanostructured lipid carrier (RNB-NLC) can be formulated using the high-shear homogenization approach, which is commonly used for nanoparticle production. The lipid phase, which contains (glyceryl monostearate, oleic acid, and RNB), is melted to about 78°C (10°C above the solid lipid's recorded melting point), and then gradually added to the aqueous phase, which contains deionized water and the surfactant system, which had previously warmed to the same lipid phase temperature under continuous stirring with a hot plate magnetic stirrer for 5 minutes. The pre-emulsion is then homogenized to an extreme using a high-speed homogenizer for 15 minutes at 20000 rpm. The lipid nanoparticles were prepared and the nanoemulsion is placed aside at room temperature to rigidify.[8] The fabricated formulas are mentioned in Table 1.

Evaluation of the RNB-NLC

Particle Size and Polydispersity Index (PDI)

The droplet size and PDI of RNB-NLC was determined by analyzing the fluctuations in light scattering due to the Brownian motion of the particle using the dynamic light scattering (DLS) technique (Zetasizer, Malvern, UK). RNB-NLC sample was diluted with deionized water (10-fold) and gently stirred (to increase the homogeneity) before measurement.[9]

Determination of Zeta Potential

The zeta potential of the formulated RNB-NLC was measured by using the DLC technique (Zetasizer, Malvern, UK). A sample was placed in the electrophoretic cell and measured at 25 ± 1°C, and the average values were recorded. Zeta potential gives information about the surface charge properties of the prepared formula in which a prediction of the stability character can be done.[10]

Drug Entrapment Efficiency and Loading Efficiency

The entrapment efficiency (EE%) and loading efficiency (LE%) were determined indirectly by measuring the concentration of free RNB in the NLCs. Test tube containing 5 mL of the sample

Table 1: Preparation of Rizatriptan Nanostructured Lipid Carrier

Formula	*FR 1*	*FR 2*	*FR 3*	*FR 4*	*FR 5*	*FR 6*
Rizatriptan benzoate (mg)	5	5	5	5	5	5
Glyceryl monostearate (mg)	37	37	37	37	37	37
Oleic acid (mg)	3	3	3	3	3	3
Tween 80 (w/w)	2.5	3	----	----	2.5	3
Cremophore LE (w/w)	----	----	2.5	3	2.5	3
Lecithin (w/w)	----	----	----	----	1	1.5
Volume of aqueous phase (mL)	2	2	2	2	2	2
Homogenization rate (rpm)	20000	20000	20000	20000	20000	20000
Time of homogenization (min.)	15	15	15	15	15	15

was centrifuged at 6000 rpm for 10 minutes, after that the supernatant layer was taken and filtered through a 0.45 millipore filter and analyzed utilizing UV spectrophotometer at 225 nm. The (EE%) and (LE%) were calculated using the following equations:[11]

$$\textbf{Entrapment effeciecy} = \frac{Total\ drug - Free\ drug}{Total\ drug} x100$$

$$\textbf{Loading efficiency} = \frac{Total\ drug - Free\ drug}{Total\ lipid} x100$$

In-vitro Release Profile

The RNB-NLC formulas having the smallest particle are subjected to *in-vitro* drug release studies, it was carried out through the dialysis bag diffusion technique over 6 hours, phosphate-buffer solution (pH 7.4) was used as a release medium. Briefly, a specified volume of NLC equivalent to 5 mg of rizatriptan benzoate has filled in a dialysis bag (cellulose membrane with molecular weight cut-off (8000–14000)), clamped, and immersed in a glass container containing 250 mL of release medium at 37 ± 0.5˚C to similar *in-vivo* conditions, then stirred at 50 rpm using a hot plate magnetic stirrer. At predetermined time intervals of (0, 0.5, 1, 2, 4, and 6 hours), the sample of 1-mL was taken. The release medium was replaced with the same volume of freshly milieu to guarantee sink conditions. After that, the amount of RNB was measured by a UV spectrophotometer at 225 nm. The cumulative amount of rizatriptan benzoate released was calculated and plotted versus time.[12]

Fourier Transform Infrared Spectroscopy (FTIR)

The compatibility of RNB with another excipient in RNB-NLC was assessed using FTIR spectroscopy (Shimadzu, Japan) at the range 4000–400 cm $^{-1}$ to check possible chemical interaction between their functional groups.[13] FTIR was performed for pure RNB, glyceryl monostearate, and the optimum RNB-NLC formula.

Differential Scanning Calorimetry (DSC)

DSC experiments were carried out through the use of DSC-60plus (Shimadzu, Japan). In an aluminum pan, samples of around 5 mg of pure drug powder, glyceryl monostearate, and a physical combination of the chosen formula were placed, and the testing was performed under a nitrogen environment at a flow rate of 40 mL/min and a scanning rate of 10°C/min in the range of 15–300°C.[14]

Powder X-ray Diffraction (PXRD)

The crystallinity of pure RNB, GMS, and the selected formula was investigated using PXRD. The target metals Cu, filter K, 45 kV voltage, and 30 mA current were evaluated under the following circumstances: At a step size of 0.02°, samples were scanned throughout a 2° range of 5–80°.[15]

Statistical Analysis

The experimental results are presented as a mean of three triplicate models with standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA), statistically significant at the level of ($p < 0.05$) and non-significant at the level of ($p > 0.05$).

RESULTS AND DISCUSSION

Effect on the Particle Size and PDI

Surfactants are essential in obtaining NLCs and influence the lipid particles' size or stability and the encapsulated substance's release profile. They act to reduce the interfacial tension, facilitating the partition of the particles during the emulsification.[16] The magnitude of this effectiveness largely depends on its nature. Consequently, the evaluation of various surfactants is generally useful to find the desired results for each system. The ability to stabilize the system, and thus keep the size distribution more homogeneous depends strongly on the structure and other properties of the surfactant.[17]

Surfactants have a significant influence on the quality and particle size of NLCs. The emulsification process is anticipated as taking place in two stages during the manufacture of NLCs. To minimize particle agglomeration during the early pre-emulsion stage, the emulsifier must cover freshly generated surfaces as quickly as possible before reaching the crystallization stage. Moreover, during the emulsification process, it produced the most persistent layer between the lipid and aqueous phases, minimizing the coalescence between them and resulting in a more homogeneous population of NLCs.[18] Tween 80 was utilized because it can prevent particle aggregation and because its long-saturated alkyl chains slow the polymorphic transition into the stable modification, enhancing the stability of NLCs. Their low toxicity is also well-known and is regarded as safe pharmaceutical excipients.[19]

A reduction in the particle size in a significant manner ($p < 0.05$) was observed upon an increase tween 80 concentration from 2.5 to 3% as shown in Figure 1. These results could be attributed to the surface-active properties of the surfactants, which reduced the interfacial tension between the aqueous and lipid phases, resulting in the formation of smaller sized emulsion droplets during the first stage of the production of NLCs (the pre-emulsion formation stage). The particles' steric stabilization, which reduces their ability to assemble, may also be responsible for this impact.[20]

The PDI is a measure of the particle size distribution, as described in the literature, a monodisperse sample has PDI values near 0, but values between 0.1 and 0.3 indicate a tight size distribution.[21] It was obvious from the obtained data that the PDI values were less than 0.3; this indicates a homogenous particle size distribution of all formulas.

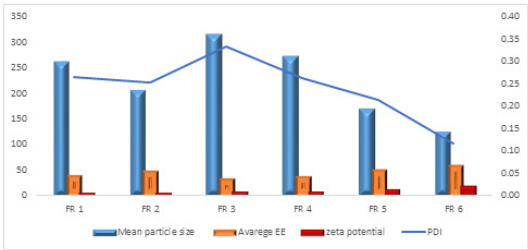

Figure 1: Effect of different type and concentration of surfactant and co surfactant on the particle size, PDI, zeta potential, EE% of RNB-NLC (FR1-FR6)

Concerning the effect of another type of surfactant (Cremophor EL), formulas (FR3 (2.5% w/w), FR4 (3% w/w)) are designed, it was seen that the particle size was significantly (p <0.05) enhanced undesirably as compared to the first type. Moreover, the PDI also increase but to a little value. Unlike Tween 80, Cremophor is organized in a branching fashion. In several studies, researchers found that a more linear shape of the surfactant encourages packing among the lipids, retaining the particles' physicochemical and thermodynamic stability while also decreasing the interfacial tension. As a result, the combination of these two factors (linear structure of tween 80 and the appropriate HLB value) stabilized this lipid system and favored a smaller diameter and a more homogenous particle size distribution in the final product.[22]

It was hypothesized in the previous studies that the addition of co-surfactant has a good role in improving the criteria of NLC, so formulas (FR5 (lecithin 1% w/w), FR6 (lecithin 1.5% w/w), are fabricated to investigate the behavioral work of these compound in different type and concentration. The addition of lecithin has shown a desirable negative effect on the particle size of the prepared NLC in a statistically significant state ($p < 0.05$). The reason behind these results may be due to the fact that lecithin is a kind of emulsifier helper that assist in stabilizing the small particles formed, a similar observation was seen by Wu *et al.*[23]

Effect of Zeta Potential (ZP)

Concerning ZP which reflects the electric potential and the surface charge of nanoparticles in preparation and is a predicting factor of long-term stability. When ZP values are higher, the electrostatic repulsion of the attractive Van der Waals forces stops nanoparticle aggregation from occurring. Lipids arrangements on the surface of the nanoparticle, surfactant surface charge, and the charge of the encapsulated drug interfere with ZP.[24] From the measurement of zeta potential, unluckily, tween 80 gave a less negative charge value, which might explain the tendency of the prepared NLC to agglomeration after a small period of production, an interesting story claimed that NLC system using a steric stabilizer does not need a high value of ZP to maintain their stability during the storage period, this result is similar to that obtained by Eh Suk *et al.*[25]

Likewise, lecithin showed to impart a more negative charge of the formulated RNB-NLC resulting in an increment in the ZP with statistical significance ($p < 0.05$), this might also be linked to the existence of hydroxyl and hence boosts the stability of the nanodispersion system, a similar outcome was obtained by Czajkowska.[26]

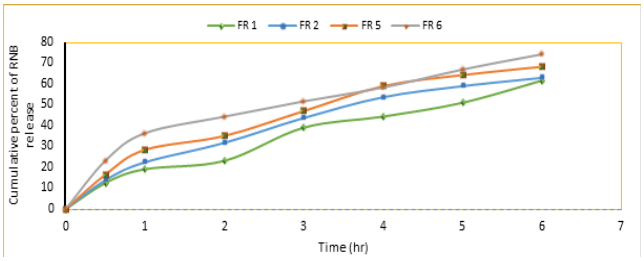

Figure 2: Cumulative release profile of RNB – NLC (FR1, FR2, FR5, and FR6) in phosphate buffer solution pH (7.4).

Effect on the Entrapment Efficiency and Loading Efficiency

In terms of the effect of tween 80 on entrapment efficiency and loading capacity, the results showed that EE was higher when the lipid and surfactant ratios were at medium levels, which could be explained by the creation of more available space between lipids to accommodate RNB molecules, as found by Cunha *et al.*.[27] Moreover, Kang *et al.* state that an adequate surfactant amount might emulsify the melting mixture of lipids and medicines adequately, preventing pharmaceuticals from leaking into the exterior water phase.[28] Moreover, it was seen that lecithin can enhance the amount of drug-loaded in NLC since the hydrophilic group can offer some spaces where the drug molecule can be localized and reduce leakage into the outside.[29]

In-vitro Release Profile

The basic condition for the success of each pharmaceutical formulation is the drug released from it in a streamLined manner that aligns with the goals to be achieved. Therefore, the most important test for the validity of the RNB-NLC is the drug release profile. The test was carried out on four samples of the drug loaded on NLC (FR1, FR2, FR5, and FR6). It was performed using phosphate buffer solution pH 7.4 for 6 hours using the dialysis bag method. The release profile obtained was seen in Figure 2.

A predominant biphasic release modern was seen clearly through the investigation of the release pattern of RNB from all the prepared NLC formulas. An early burst of drug release of a high amount was followed by a period that lasts for a long time. The release behavior may be a possible explanation, including the drug-enriched shell of the RNB incorporation model, which involved a significant amount of the drug being entrapped in the outermost shell of the particle during lipid recrystallization, resulting in an extremely short RNB diffusion pathway, as well as the high stabilizer concentration used in the formulation, which may have increased the amount of drug released into the dissolution medium.[30,31]

Moreover, emulsifiers with high HLB value yield a faster release pattern. In a nutshell selection of emulsifiers, their concentration is significant in the fabrication of NLC with effective drug delivery, reduced particles size, and narrow size distribution assuring predictable drug release. Over and above, Girotra *et al.* propose that the cumulative percent of drug release improved dramatically with the presence of surfactant and co-surfactant which may be related to solubilization enhancement of the drug and, as a result, makes it easier for the drug to leach off the NLC and enter the release media.[32] A slow-release model was observed in which the drug incorporated within the lipid template was liberated either through diffusion or after erosion of the lipid particle. This biphasic release pattern may be explained by the model of drug incorporation, which predicts that a drug-enriched shell will be formed, and the higher rate of drug release may be related

to the small particle size, which is characterized by a large surface area for drug diffusion to the surrounding dissolution medium.[33]

FTIR Spectroscopy

The FTIR spectroscopic analysis is useful in establishing the compatibility of medicine with other excipients, which is a crucial rule in the selection of the best one. Additionally, the FTIR analysis is a good tool for investigating any structural changes that may occur in the drug due to exposure to severe and demanding circumstances throughout the formulation process.

FTIR of pure RNB, GMS, and RNB- NLCs were illustrated in Figures 3(a-c), respectively. The FTIR of rizatriptan benzoate show characteristic peaks are 3113 cm^{-1} for N-H stretching, 2939, 2858 cm^{-1} for CH_3, CH_2 stretching, 1604, 1500 cm^{-1} for C=C, C=N stretching, 1446,1377 cm^{-1} for CH_2, CH_3 bending, NH bend at 1280 and 887, 825, 756 cm^{-1} for CH and C-N stretch at 1134 cm^{-1}, CN out of plane bend.[32] The IR spectrum of GMS exhibited peaks at three positions 3113.3, 2915, and 2848.5 cm^{-1}. These peaks are due to (C-H) stretching of alkane and the carboxyl group (C=O) stretching peak is observed at 1728 cm^{-1}.[34]

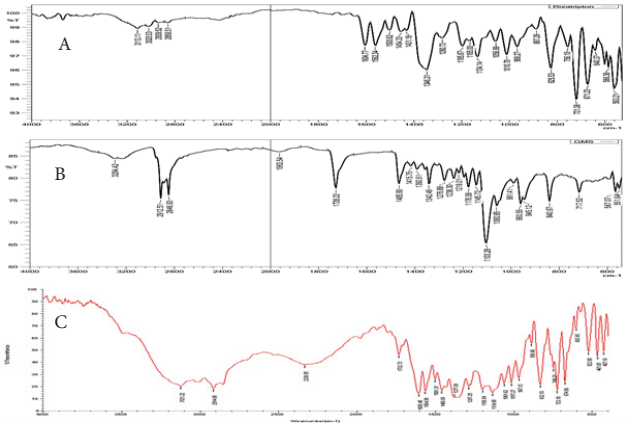

Figure 3: FTIR spectra of the (a) rizatriptan benzoate, (b) glyceryl monostearate, and (c) selected RNB-NLC (FR6).

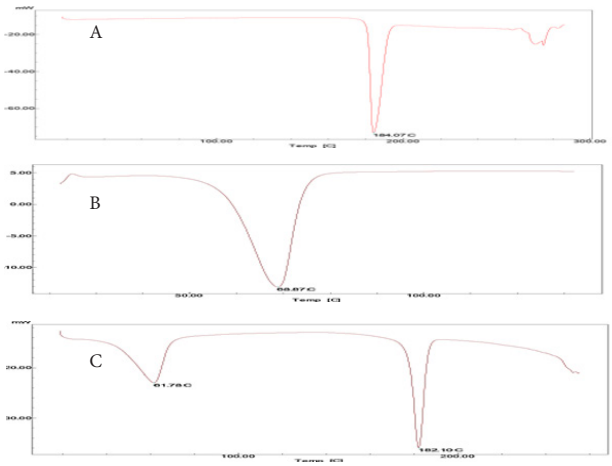

Figure 4: DSC thermogram of (a) rizatriptan benzoate, (b) glyceryl monostearate, and (c) optimized formula (FR6)

Fortunately, when the accurate investigation of the Figure 3(c), the major peaks of the drug to be formulated are present, and this categorically guarantees that the chemical integrity and molecular structure of RNB are not tampered with throughout the preparation procedure. Even after the formulation, FTIR spectroscopy confirmed the compatibility of RNB with lipids and surfactant systems.

DSC

DSC enables the quantitative detection of all processes in which energy is required or produced. DSC is an effective tool for investigating the melting behavior and crystalline state of nanocarriers and raw materials. Since crystallinity significantly impacts some properties of lipid nanoparticles such as drug loading, drug release, and stability of drugs during storage, studying it can be useful for judgment about nanocarriers features.[35] The DSC study of the thermal behavior of RNB, GMS, and the physical mixture of the same formula component (FR6) were represented in Figures 4(a-c), respectively. The drug thermogram shows a sharp endothermic peak at 184.4ºC corresponding to its melting point indicating RNB purity and anhydrous crystalline structure. There is an endothermic peak at 68.87ºC as declared in Figure 4b, representing the thermogram of GMS and indicating that the molecular organization is a very well-organized crystalline structure. DSC thermograms of the physical mixture (Figure 4c) revealed that no considerable change was observed in the melting peak of rizatriptan benzoate with GMS, which indicates that no interaction occurs between the drug and the polymers used in the formulation.

X-Ray Diffractometry

Through the utilization of X-ray scattering, it is possible to assess the length of the long and short spacings of the lipid lattice. Measuring the NLC dispersions themselves is recommended because solvent removal will lead to modification changes.[36]

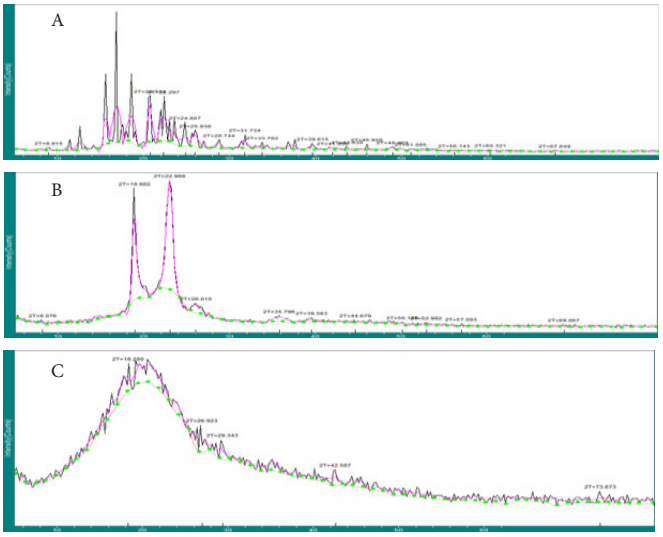

Figure 5: XRD pattern of (a) Rizatriptan benzoate, (b) Glyceryl monostearate, and (c) Optimized formula (FR6)

Figures 5(a-c) illustrated XRD patterns investigations of the crystalline behavior of pure drug (RNB), GMS, and optimized formula (FR6), respectively. XRD patterns of RNB show a high-intensity diffraction peaks at 2θ values of 20.73°, 25.11°, 44.60°, and 51.25° which indicate the crystallinity of the structure drug molecule, similar observation was obtained by Giotra *et al*.[32] GMS XRD pattern revealed diffraction peaks at 2θ values of 5.5°, 7.4°, 19.5°, 20.6°, and 23.4. However, XRD pattern of the optimized formula exhibits the disorganization of the crystalline feature of GMS with small peak intensity. Moreover, most of the constructive peaks relating to the drug RNB were disappeared, which gives a hint to the conversion of crystal state to the disorder amorphous or molecularly dispersed form. Possible inclusion of drug molecule into the NLC structure and consequently, it may prove the reported high entrapment efficiency.[37]

CONCLUSION

Last but not least, the most significant component of an NLC formulation is lipids, and one or more surfactants (preferably amphiphilic) are usually required to aid in the solubilization and increase dispersion. To emulsify the lipid phase in water, a suitable HLB for the surfactant is necessary, which is significant from the standpoint of formulation design. A combination of hydrophilic-lipophilic surfactants/co-surfactants were very useful in obtaining desired preparation. It is well known that using surfactants of both lipophilic and hydrophilic nature improves the stability of a dispersed system, hydrophilic surfactants (tween®80), and lipophilic co-surfactants (lecithin) were used to prevent nanoparticle agglomeration due to surface tension reduction and to facilitate droplet division during homogenization.

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