

# Development and Characterization of Span 20 Niosomes as a Nanocarrier for Sustained Oral Delivery of Nimodipine

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## Abstract

### Background

Nimodipine (NMD) is a calcium channel antagonist which is used for treatment of hypertension. Unfortunately, it suffers from first pass metabolism, short half-life and poor oral bioavailability because of its low water solubility. In this study, we intended to prepare NMD-loaded niosomes to protect the loaded drug from degradation, avoid first pass metabolism, control and sustain the drug release.

### Materials and Methods

NMD-loaded niosomes were prepared by thin film hydration method, using different ratios of cholesterol (CH) and span 20 as a non-ionic surfactant. The prepared niosomes were characterized with respect to entrapment efficiency percent (EE %), particle size (PS), polydispersity index (PDI) and zeta potential (ZP). The optimized formulations (F1, F3 and F4) were subjected to stability study and in vitro drug release. In-vivo study of F1 was also assessed in rats.

### Results

Based on the obtained experimental results, the EE% of the prepared niosomal formulations ranged from 49.6± 6.615 to 68.2± 6.129 %. They exhibited small PS (161.8 ±15.773 to 220.9± 3.417 nm), with narrow size distribution (PDI values from 0.081± 0.061 to 0.474± 0.081). High ZP values were also manifested (-38.1± 0.5567 to -52.1±1.044 mV). Stability studies elicited that there were no significant changes in percent drug retention, PS, PDI and ZP of NMD-loaded niosomes after 3 months of storage under refrigerated conditions. Almost all studied formulations displayed a sustained drug release pattern up to 24 hr. TEM of F1 formulation divulged spherical morphology. Auspiciously, the pharmacokinetic study of the optimized F1 NMD-loaded niosomes in rats revealed remarkable improvement in bioavailability, as well as sustaining the drug release when compared to free NMD suspension.

### Conclusion

In conclusion, the oral investigated NMD-loaded niosomal formulations demonstrated promising results as nanocarriers for nimodipine through accomplishing increment in bioavailability as well as duration of action.

**Key words:** nimodipine, span 20, cholesterol, niosomes.

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## I. INTRODUCTION

The oral route is the most preferable route for drug administration due to patient's acquiescence, non-invasiveness, being painless, minimal induction of immune system, easy uptake, cost-effectiveness and ease of large-scale manufacturing. Various factors affect oral drug absorption including physiological barriers like pH, enzymatic degradation, drug solubility, and stability in the gastrointestinal tract environment<sup>4,19</sup>. Novel drug delivery systems including nanocarriers, micelles, and lipid-based carriers have been explored to enhance oral drug absorption and overcome the obstacles which hinder the absorption process<sup>23</sup>.

The exclusive properties particularly small size and high surface area displayed by these systems render them more suitable for the advanced drug delivery purposes as targeting drug delivery, controlling the drug release and enhancing permeability and retention (EPR). Other than aforementioned, the nanosystems have more advantages like overcoming the pharmacokinetic and pharmacodynamic obstacles of therapeutic molecules, adjusting the dose of the drug with narrow therapeutic range and diminishing the side effects. Drug encapsulation in nanostructures can protect it from the harsh environmental degradation in the stomach and the GI tract<sup>42</sup>.

Niosomes are one of the vesicular systems, with a bilayer structure; composed of cholesterol and non-ionic surfactant<sup>32,36</sup>. They are capable of encapsulating both hydrophilic and lipophilic drugs. Niosomes are

characterized by low cost, easy formulation and controlled release of drugs<sup>18</sup>. They can improve the solubility and stability of pharmaceutical molecules; also they can shield the drug from degradation in in vivo circulation<sup>24</sup>.

Non-ionic surfactants are considered the main component in niosomal formulation. They enhance the stability and rigidity of niosome formulations with minimal in vitro and in vivo cytotoxicity. Interaction of cholesterol with non-ionic surfactants helps to obtain a stable formulation<sup>39</sup>. Niosomes are either unilamellar or multilamellar vesicles<sup>50</sup>.

Polysorbate 20 is a common non-ionic surfactant that is used in the preparation of niosomes. Span 20 is a mixture of esters formed from the fatty acid lauric acid and polyols derived from sorbitol and isosorbide. Niosomes containing polysorbate 20 show fantastic in vitro performance. They have the ability to adhere to the intestinal cells and promote the transcytosis pathway; this is owed to the PEG chains of polysorbate 20 which make the niosomal surface properties similar to PEGylated nanoparticles. Therefore, niosomes prepared by polysorbate 20 could permeate the Caco-2-cell monolayer, thus increasing the transfer of drug across intestinal epithelium to give improved therapeutic effect<sup>18, 37</sup>.

Nimodipine (NMD), a calcium channel blocker, is used in the treatment of senile dementia for prophylaxis of the vascular hemicranias, stroke, and hypertension<sup>25</sup>. Chemically, Nimodipine is isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate<sup>52</sup>. However, the oral bioavailability of NDM is about (~13%)<sup>30, 51</sup> because of its poor water solubility (2.30 µg/ml) and extensive first-pass metabolism by Cytochrome P (CYP3A4) isoenzymes and P-glycoprotein (P-gp) mediated efflux<sup>51</sup>. According to Biopharmaceutics Classification System (BCS), it belongs to class II drugs with low solubility – high permeability; it has a half-life value of 7-8 h<sup>12, 17</sup>.

Accordingly, the goal of the current study is to encapsulate NMD in new niosomal formulation using span 20 as a surfactant, aiming at improving the oral bioavailability and prolonging the duration of pharmacological action.

## II. MATERIALS AND METHODS

### 2.1. Materials

NMD and Dexamethazone acetate were gifted from Pharco pharmaceutical company (Cairo, Egypt), span 20 was purchased from SDFCL, India; cholesterol was purchased from ADVENT (India), methanol HPLC grade (Fischer), chloroform HPLC grade and (0.45 µm) Millipore filter were obtained from Cornell lab (Cairo, Egypt).

### 2.2. Methods

#### 2.2.1 Preparation of NMD Niosomes

Preparation of nimodipine loaded niosomes was assessed by thin film hydration method (TFH)<sup>8, 49</sup> using different molar ratios of span 20 and CH (total lipid used was 200 mg) as recorded in Table 1. The drug (10 mg), CH, and span 20 were dissolved in 10 ml of organic mixture consisting of methanol and chloroform (3:7 v: v) for sufficient time till complete dissolving and sonicated for ten min in an ultrasonic bath (model ss101H230, USA). The organic solvent was evaporated under reduced pressure at 60°C using rotary evaporator (USA) at 100 rpm to produce thin film on the inner flask wall. The thin film was hydrated for 20 minute at 60±1 °C with 10 ml of distilled water. The resulting niosome dispersion was exposed to sonication by ultrasonic homogenizer (Chicago, IL, USA) for 1 min (50% amplitude) to ensure size homogeneity of the produced niosomes<sup>34</sup>. The resulting dispersion was saved overnight in refrigerator for the determination of entrapment efficiency.

**Table no 1:** Amounts of cholesterol, span 20 and nimodipine used for formation of niosomes

| Formulation code | Cholesterol: span 20 weight ratio | Cholesterol (mg) | Span 20 (mg) |
|------------------|-----------------------------------|------------------|--------------|
| F1               | 1:2                               | 72 mg            | 128 mg       |
| F2               | 1:2.5                             | 62 mg            | 138 mg       |
| F3               | 1:3                               | 54 mg            | 146 mg       |
| F4               | 1:3.5                             | 48 mg            | 152 mg       |
| F5               | 1:4                               | 44 mg            | 156 mg       |
| F6               | 1:4.5                             | 40 mg            | 160 mg       |
| F7               | 1:5                               | 36 mg            | 164 mg       |

**Note:** Amount of drug used in all formulations was kept constant at 10 mg

### 2.3. Characterization of NMD niosomes

#### 2.3.1. Entrapment efficiency

Amount of NMD entrapped in niosomes was measured by direct method. Ten ml of niosomal dispersion was centrifuged using cooling centrifuge (Acculab CE16-4X100RD, USA) at 13000 rpm for 2 hours at 4°C. The niosomal precipitate was washed by adding 10ml of distilled water then centrifuged again for 0.5 hour. Entrapment efficiency percent was assessed via lysis of niosomes with chromatographic grade HPLC methanol. The precipitated vesicles were dissolved in (25ml measuring flask) and sonicated for 10 min in ultrasonic bath to make sure of complete lysis<sup>21</sup>. After that, in a 10 mL measuring flask, 100 µL of this solution were transferred and completed to volume with chromatographic grade HPLC methanol. Finally, the concentration of NMD was measured spectrophotometrically by measuring the absorbance at  $\lambda_{\max}$  239 nm using (ultraviolet/visible, JASCO, Tokyo, Japan) spectrophotometer. The %EE was estimated by Equation<sup>21</sup>.

$$\%EE = \frac{\text{Amount of entrapped drug}}{\text{Total amount added of drug}} \times 100 \quad (1)$$

#### 2.3.2. Estimation of Particle Size (PS) and Polydispersity Index (PDI)

The freshly prepared NMD-loaded niosomes were used for PS and PDI measurements after appropriate dilution with distilled water via Malvern Zetasizer by dynamic light scattering (Malvern Instruments Limited, UK). Each parameter was measured in triplicate for each formulation.

#### 2.3.3. Estimation of Zeta Potential (ZP)

The values of ZP were estimated using Malvern Zetasizer nanoseries (Malvern Instruments Limited, UK) after suitable dilution with distilled water. It was estimated in triplicate for each formulation.

#### 2.3.4. In-vitro release

Formulations F1, F3 and F4 were employed for the in vitro release study using dialysis bag method. The cellophane membrane (Mw cut off 12-14 kDa, Sigma-Aldrich membrane) was soaked in phosphate buffer PH 6.8 before use for 24 hr<sup>9, 10</sup>. NMD-loaded niosomal dispersion, equivalent to 2 mg of NMD, was placed in dialysis membranes<sup>33, 46</sup>. The bags were then sealed with clips at both ends and submerged into 100 mL of release medium (phosphate buffer and methanol in ratio 70:30). The entire assembly was kept at 37 ± 1°C with continuous stirring in horizontal GFL shaking water bath (Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) at 100 rpm<sup>35</sup> for 24 hr. Aliquots of 3mL were withdrawn at predetermined time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 24hr) and replaced with the same volume of fresh medium solution to maintain sink condition. Samples were assayed by UV spectrophotometer (JASCO, Tokyo, Japan) at  $\lambda_{\max}$  241nm in triplicate to determine amount of drug released. Release of pure NMD in distilled water was performed for comparison.

#### 2.3.5. Kinetic Analysis of the Drug Release Data

Data of drug release were applied to different kinetic models including zero order, first order<sup>31</sup> and Higuchi's model<sup>22</sup> to determine the drug release kinetics. The model with the maximum coefficient of determination ( $R^2$ ) was well thought-out the best to depict drug release kinetics. Besides, Korsmeyer–Peppas model<sup>28</sup> was operated for investigating the best mechanism of drug release through calculation of (n) value (diffusional exponent) according to the equation  $mt/m_{\infty} = kt^n$  from the slope of relation between time and fraction of drug released where n, k, t and  $mt/m_{\infty}$  express the diffusional exponent for drug release, the kinetic constant, the release time and the fraction of drug released respectively<sup>38</sup>.

#### 2.3.6. Stability Study

The stability study was performed for 3 months for F1, F3 and F4 formulations under conditions similar to those designated by ICH (International Conference of Harmonization). The optimized formulations were stored in glass bottles in a room temperature (25°C ± 2)/ (60% ± 5%) relative humidity, as well as at refrigerator temperature of 4°C ± 2. The niosomal dispersions were verified with respect to change in PS, PDI, ZP values and drug retention percent over the storage period<sup>20, 21</sup>. The data was analyzed by statistical analysis through applying unpaired t-test, followed by Tukey–Kramer tests using GraphPad Prism 8 software v8.3.0 (538) (San Diego, CA, USA) computer program.

#### 2.3.7. Transmission Electron Microscope (TEM) of the Optimized Formula F1

TEM (JEOL JEM-2100; JEOL Ltd, Tokyo, Japan) was used to scrutinize the morphology of the optimized formula F1 using an imaging viewer software and digital micrograph. After appropriate dilution of the prepared dispersion (0.1ml of the formulation in 10 ml of distilled water), on the surface of carbon-coated copper grid, one drop was placed for ten minute to allow adherence of some niosomes and the procedure was completed as reported<sup>2, 14</sup>.

### 2.3.8. In vivo Study

The in vivo procedure was accepted by the Research Ethical Committee at Mansoura University according to "The principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). Ten male rats (Sprague Dawley weighing from 250–300g) were adjusted in room with controlled breeding. The rats (two groups) (n=5) were allowed free access to water, while fasted 12 hr before the experiment. The oral dose of NMD was 10 mg/kg of the free drug or the equivalent from F1 NMD-loaded niosomes<sup>44</sup>. Each oral dose was given to the rat by oral gavage under light ether anesthesia after suspending in 0.5% w/v sodium carboxy methyl cellulose.

### 2.3.9. Plasma Sample Preparation and Extraction Procedure

After oral administration, the collected blood samples at time intervals of (0.5, 1, 2, 4, 6, 8, 10, 24, 48 hr) were stored in heparinized tubes. The plasma samples were centrifuged at 5000 rpm for 10 min by (Hettich Micro 22 R, Germany), stored in eppendorf tubes at -20°C while waiting for analysis<sup>29</sup>. After liquefying, 100 µL of every sample were added to 100 µL of dexamethasone acetate (in methanol HPLC grade as internal standard solution, 40 µg/mL) and 0.6 mL of methanol chromatographic HPLC grade. Later, the mixture was applied for mixing for five min by tube mixer (Model VM-300, Gemmy Industrial Corp.), then centrifuged for 20 min at 10000 rpm. Lastly, the filtration of supernatant was completed through 0.45 µm Millipore filter.

### 2.3.10. High Performance Liquid Chromatography

HPLC–UV analysis method was applied for determination of the plasma concentrations of NMD<sup>13</sup> with small modification. The HPLC system (Knauer, Germany) was well-appointed with an adaptable wavelength UV-VIS detector 190–750 nm (Azura UVD 2.1 L Detector), binary pump (Azura p 6.1 L) and chromatographic separation was performed with a C18 column (250 mm × 4.6 mm, 5 µm, Phenomenex Hyperclone ODS, USA). The samples (20 µL) were introduced and eluted with isocratic elution with mobile phase mixture consisting of HPLC grade of methanol/distilled water (70:30% v/v) at flow rate of 1 mL/min<sup>48</sup>. The analysis was accomplished at wavelength of 237 nm. According to ICH guidelines, the validity of the applied method was studied in terms of selectivity, specificity, linearity, accuracy and precision. Similarly, the quantification limit (QL) and the detection limit (DL) were estimated according to Equations 2 and 3<sup>13,14</sup>.

$$QL = \frac{10\sigma}{S} \quad (2) \quad DL = \frac{3.3\sigma}{S} \quad (3)$$

Where  $S$  and  $\sigma$  are the slope of plasma calibration curve of nimodipine and the SD of y intercept, respectively.

The plasma calibration curve of NMD was created by spiking 100 µL of internal standard solution with 100 µL blank plasma and 200 µL of drug solution in a concentration range of (20–80 µg/mL) by diluting the stock of NMD solution (1000 µg/mL) in methanol HPLC grade.

### 2.3.11. Pharmacokinetic Study

Plasma concentrations of NMD were evaluated by the statistical analysis using unpaired t-test through GraphPad Prism 8 software v8.3.0 (538) (GraphPad Software Inc., San Diego, CA, USA) and the pharmacokinetic parameters were estimated as informed<sup>5</sup>. The elimination rate constant ( $K_{el}$ ), elimination half-life ( $T_{1/2_{el}}$ ), maximum concentration ( $C_{max}$ ) and time of  $C_{max}$  ( $T_{max}$ ) were calculated from the experimental data. The mean residence time (MRT) and area under the concentration–time curve (AUC) were calculated by the linear trapezoidal method.

## II. RESULTS AND DISCUSSION

### 3.1 Entrapment Efficiency

The entrapment efficiency is the most important factor from the pharmaceutical point of view in the evaluation of niosomal systems. Table 2 illustrates that there is an increase in EE% with increasing the concentration of span 20 till formula F4; this may be ascribed to the increased number of vesicles formed by the increased amount of surfactant<sup>1</sup> and the interaction that occur between span 20 and CH to form rigid bilayer niosomes. Cholesterol can affect the permeability, rigidity of the vesicular bilayer and hence the entrapment efficiency. The addition of CH increases the viscosity and hence rigidity of the preparation<sup>3</sup>. In consequence, upon decreasing CH ratio (from F5 to F7), the hydrophobicity and stability of the vesicles' bilayers decrease, leading to decrease in trapping of the hydrophobic drug into bilayers<sup>7,43</sup>. Also, the high span ratio (from F5 to F7) could lead to the formation of mixed micelles together with niosomes leading to lower EE%<sup>1</sup>. Eldeeb et al., reported that high percentage of surfactant molecules, arranged within the lipid bilayer structure, increase the permeability of the vesicular membrane. This might introduce pores into the membrane and increase its fluidity leading to lower EE%<sup>16</sup>.

**Table no 2:** Characterization of NMD-loaded Niosomes

| Formulation Code | Entrapment Efficiency (%EE) | Particle Size (PS) (nm) | Polydispersity Index (PDI) | Zeta potential (mv) |
|------------------|-----------------------------|-------------------------|----------------------------|---------------------|
| F1               | 57.1 ±3.450                 | 203.633±1.41            | 0.125± 0.0587              | -46.667± 2.203      |
| F2               | 57.8± 5.739                 | 176.4 ±13.081           | 0.101±0.0814               | -38.1± 0.5567       |
| F3               | 55.6± 5.8705                | 211.5±1.752             | 0.33± 0.013                | -39.8±0.54          |
| F4               | 68.2± 6.129                 | 220.9± 3.41760          | 0.474± 0.081               | -50.233± 2.11       |
| F5               | 49.6± 6.615                 | 165.4± 16.25            | 0.132± 0.0749              | -41.5± 0.750        |
| F6               | 53.1± 6.06                  | 166.1± 16.108           | 0.081± 0.061               | -40.3±0.458         |
| F7               | 52 ±1.414                   | 161.8 ±15.773           | 0.294± 0.0827              | -52.1±1.044         |

### 3.2 Particle Size (PS) and Polydispersity Index (PDI)

The drug absorption and oral bioavailability can be augmented by small PS. The PS values of NMD-loaded niosomes vary from 161.8 to 220.9 nm as shown in table 2. The increase in particle size was positively influenced by the amount of surfactant. This may be owed to the increment in EE% of the drug resulting in larger vesicles<sup>16</sup>. PDI is an important parameter for valuation of the dispersion homogeneity. The low PDI (0.1–0.25) displays a narrow size distribution or a mono-dispersed system<sup>27</sup>; however, PDI over 0.5 suggests poly dispersion and a wide size distribution<sup>11</sup>. Values of PDI range from 0.081 to 0.474 which are considered satisfactory indicating narrow size distribution and good homogeneity (Table 2). The optimum F1 niosomal formula was represented by an appropriate size of 203.663±1.41 nm and PDI 0.125± 0.0587 as illustrated in Figure 1

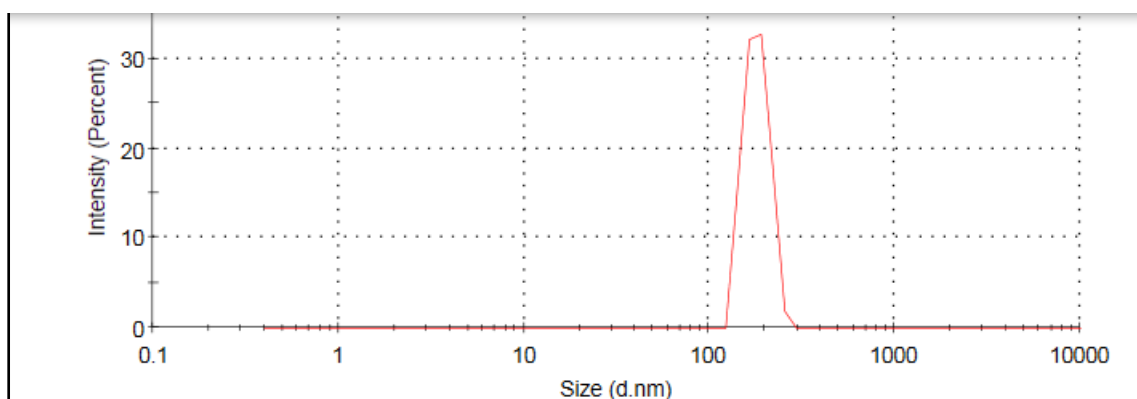


Figure 1- Size distribution curve of NMD-loaded niosomes of (F1)

### 3.3 Zeta potential

The quantity of charge on surface of niosomes is identified as zeta potential. The increase in the superficial charge of niosome leads to increment in repulsive forces that exists between the niosomes, which may prevent their accumulation. Low repulsive forces lead to particle agglomeration which causes instability due to uneven distribution in suspension. Commonly, ZP values about ±30 mV indicate steady nano-sized systems<sup>6, 15, 26</sup>. The optimum F1 niosomal formula was represented by an appropriate ZP of -46.667± 2.203 as illustrated in Figure 2.

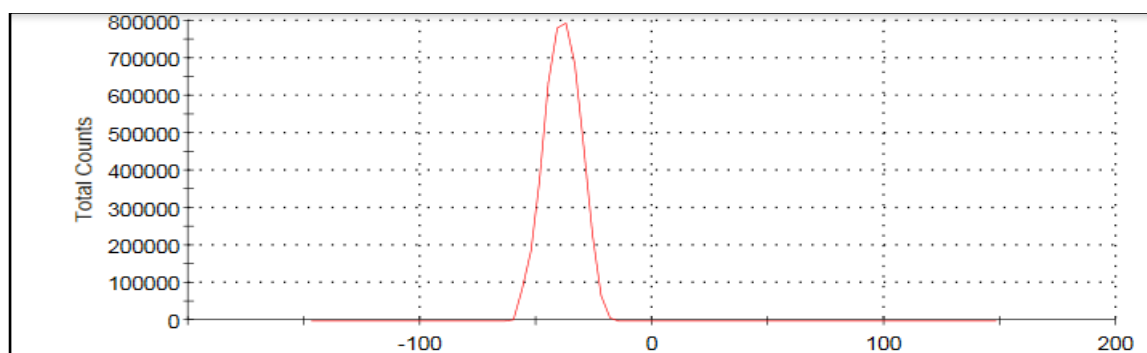


Figure 2- Zeta potential curve of NMD-loaded niosomes of (F1)

The values of zeta potential of NMD-loaded niosomes extended from -38.1 to -52.1mV, displaying that the niosomal formulations retain sufficient charge to avoid their aggregation owing to electrical repulsion. The negative charge might be as a result of the adsorbed hydroxyl ion of the medium on the vesicle's membrane<sup>45</sup>.

### 3.4 In vitro Dissolution Study

In vitro release profiles of free drug and NMD-loaded niosomes of formulations (F1, F3 and F4) in PH 6.8 were illustrated in figure 1. It is clearly illustrated that NMD niosomal formulations showed significant lower in vitro drug release profile than the drug solution (%  $Q_{8h}$  was 42.1%, 34.07%, 39% and 19.85% and %  $Q_{24h}$  was 92.28%, 44.33%, 65.36% and 31.21% for free drug, F1, F3 and F4, respectively). This might be clarified by the fact that CH markedly reduces the leakage of the drug as the inclusion of CH fills the pores in vesicular bilayers and forbids the gel-liquid phase transition of niosomal systems forming niosomes that are less leaky<sup>40</sup>. This confirms that CH acts as a membrane stabilizing agent that sustains drug release<sup>41</sup>.

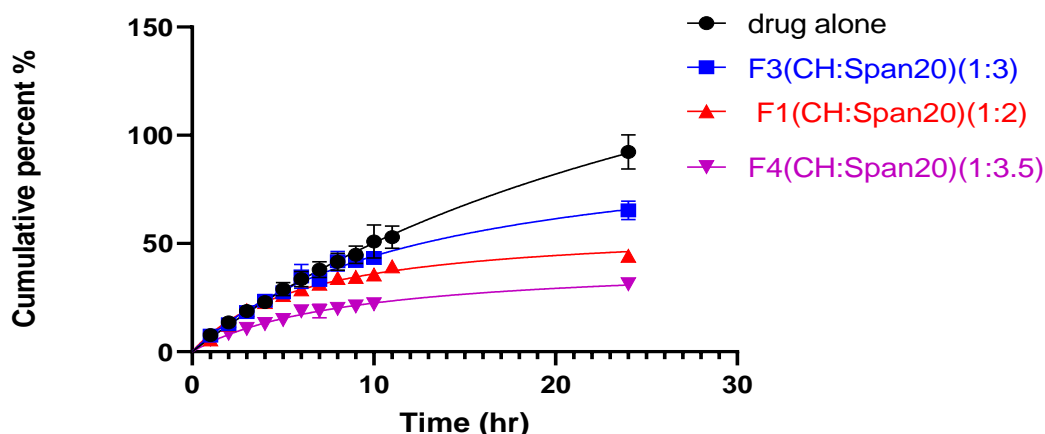


Figure 3-In vitro dissolution profiles of free NMD, F1, F3 and F4 in phosphate buffer (pH 6.8).

### 3.5 Kinetic Analysis of Drug Release Data

It is clarified from the  $R^2$  values that Higuchi's model is prevailed (Table 3), (for F1, F3 and F4), signifying that the diffusion of drug in one dimension through the bilayer structure is the model by which drug release occurs. This model postulates that the initial concentration of the drug in the matrix is higher than the drug solubility which constructs a sink condition on the surface of vesicles<sup>47</sup>; the diffusivity of the drug is constant and the release medium retains commendable sink conditions. Korsmeyer–Peppas model shed light on the drug release mechanism with n-value less than 0.5 by Fickian diffusion depends on the diffusional exponent (n) values:  $n < 0.5$  (Fickian),  $0.5 < n < 1$  (non-Fickian),  $n > 1$  (erosion-mediated release).

Table no 3: Kinetic Analysis of Drug Release Data from Niosomal formulations (F1, F3 and F4).

| Form ula Code | Zero-Order Plots Correlation Coefficient ( $R^2$ ) | First-Order Plots Correlation Coefficient ( $R^2$ ) | Higuchi's Plots Correlation Coefficient ( $R^2$ ) | Korsmeyer–Peppas Plots            |                          |                      |
|---------------|--|---|---|-----------------------------------|--------------------------|----------------------|
|               |  |   |   | Correlation Coefficient ( $R^2$ ) | Diffusional Exponent (n) | Mechanism of Release |
| F1            | 0.6919   | 0.7505  | 0.8752  | 0.8726                            | 0.04355                  | Fickian diffusion    |
| F3            | 0.8512   | 0.929   | 0.9537  | 0.9548                            | 0.02813                  | Fickian diffusion    |
| F4            | 0.8276   | 0.8600  | 0.9380  | 0.9541                            | 0.02253                  | Fickian diffusion    |

### 3.6 Physical Stability Study

Table 4, 5 and 6 represent the stability results. It was obvious that F1, F3 and F4 niosomes kept their stability with respect to the values of PS, PDI and ZP over the period of storage at refrigerator temperature.

Consequently, the optimum niosomal formula is F1 (CH: span 20 of 1:2) as represented by the appropriate size of  $203.633 \pm 1.41$  nm, PDI of  $0.125 \pm 0.0587$ , Zeta potential of  $-46.667 \pm 2.203$  mV, EE % of  $57.1 \pm 3.45$  % and percent drug released at 24 hr of 44.33%. Hence, F1 was selected for further investigations.

**Table no 4:** Drug retention percent, PS, ZP and PDI of formula F1 Stowed at Refrigerated (4±2°C) and Room (25±2°C) temperatures

| F1       |                                     |               |            |                  |                              |               |              |                  |
|----------|-------------------------------------|---------------|------------|------------------|------------------------------|---------------|--------------|------------------|
| Time     | Refrigerator temperature<br>(4±2°C) |               |            |                  | Room temperature<br>(25±2°C) |               |              |                  |
|          | Ps(nm)                              | ZP            | PDI        | % Drug retention | Ps(nm)                       | ZP            | PDI          | % Drug retention |
| Initial  | 203.633±1.41                        | -46.667±2.203 | 0.125±0.05 | 100%             | 203.633±1.4                  | -46.667±2.203 | 0.125±0.0587 | 100%             |
| 1 month  | 195.8±2.506                         | -45.367±0.328 | 0.197±0.01 | 97,36%           | 187.467±3.1                  | 44.033±1.419  | 0.236±0.042  | 90.709%          |
| 2 months | 189.367±3.80                        | -44.933±1.007 | 0.193±0.01 | 96.28%           | 184.3±3.12*                  | 44.167±0.764  | 0.245±0.040  | 82.11%           |
| 3months  | 187.967±1.82                        | -42.767±0.416 | 0.148±0.02 | 82.52%           | 183.533±3*                   | 45.033±0.283  | 0.31±0.014   | 70.11%           |

**Table no 5:** Drug retention percent, PS, ZP and PDI of formula F3 Stowed at Refrigerated (4±2°C) and Room (25±2°C) temperatures

| F3       |                                     |              |             |                  |                              |              |             |                  |
|----------|-------------------------------------|--------------|-------------|------------------|------------------------------|--------------|-------------|------------------|
| Time     | Refrigerator temperature<br>(4±2°C) |              |             |                  | Room temperature<br>(25±2°C) |              |             |                  |
|          | Ps(nm)                              | ZP           | PDI         | % Drug retention | Ps(nm)                       | ZP           | PDI         | % Drug retention |
| Initial  | 211.5±1.752                         | -39.8±0.54   | 0.33±0.013  | 100%             | 211.5±1.752                  | -39.8±0.54   | 0.33±0.013  | 100%             |
| 1 month  | 210.367±0.30                        | -44.8±0.636  | 0.31±0.042  | 87%              | 188.8±4.468                  | 44.733±0.566 | 0.261±0.035 | 93.98%           |
| 1 month  | 210.367±0.30                        | -44.8±0.636  | 0.31±0.042  | 87%              | 188.8±4.468                  | 44.733±0.566 | 0.261±0.035 | 93.98%           |
| 2 months | 214.233±1.8                         | -44.03±1.457 | 0.248±0.009 | 86.18%           | 185.8±3.46*                  | -48.667±2.46 | 0.246±0.00  | 84.5%            |
| 3 months | 209.4±1.997                         | -44.7±3.223  | 0.240±0.021 | 73.127%          | 194.1±1*                     | -47.733±4.59 | 0.207±0.00  | 81.6%            |

**Table no 6:** Drug retention percent, PS, ZP and PDI of formula F4 Stowed at Refrigerated (4±2°C) and room (25±2°C) temperatures

| F4       |                                     |               |             |                |                              |              |            |                |
|----------|-------------------------------------|---------------|-------------|----------------|------------------------------|--------------|------------|----------------|
| Time     | Refrigerator temperature<br>(4±2°C) |               |             |                | Room temperature<br>(25±2°C) |              |            |                |
|          | Ps(nm)                              | ZP            | PDI         | Drug retention | Ps(nm)                       | ZP           | PDI        | Drug retention |
| Initial  | 220.9±3.41760                       | -50.233±2.11  | 0.474±0.081 | 100%           | 220.9±3.417                  | -50.233±2.11 | 0.474±0.08 | 100%           |
| 1 month  | 246.1±1.311                         | -48.267±0.862 | 0.252±0.005 | 90.909%        | 270.8±3.85                   | 48.033±0.681 | 0.322±0.02 | 77%            |
| 2 months | 247.967±3.075                       | -51.6±2.2     | 0.245±0.035 | 95.55%         | 271.433±5                    | -55.833±0.8  | 0.3±0.035  | 70%            |
| 3 months | 234.867±1.557                       | -47.533±1.050 | 0.272±0.032 | 93.74%         | 293.167±3                    | 51.367±0.850 | 0.345±0.03 | 68%            |

**Notes:** Data are represented as the mean ±SD (n=3)

### 3.7 TEM (Transmission Electron Microscope) of the Optimum Formulation

Figure 4 clarifies TEM of F1. The vesicles seem as spherical spots with black area at the center demonstrating the core of particles which is enclosed by distinct area matching to the bilayer.



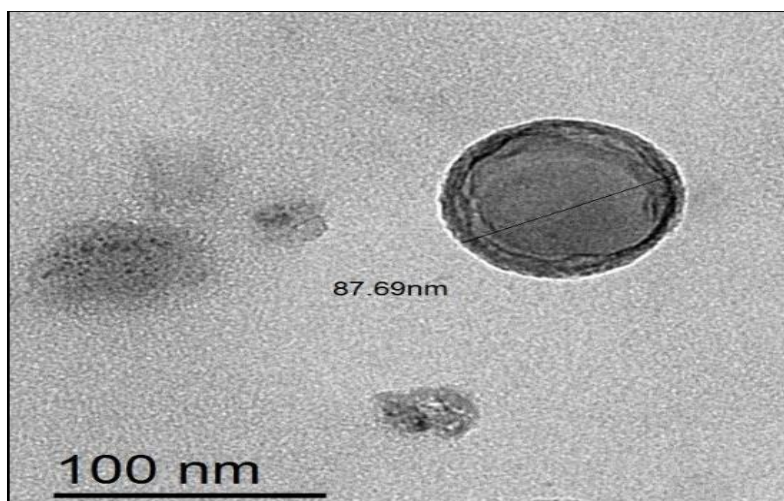


Figure 4- TMS (Transmission electron microscope) image of F1 NMD-Ns.

### 3.8 Pharmacokinetic Study

#### 3.8.1 Validation of Method

The adjusted HPLC method was estimated with respect to selectivity, specificity, linearity, precision and accuracy. The chromatogram of blank plasma, spiked with NMD and dexamethasone acetate exhibited the selectivity of this method with no interferences with blank plasma. The retention time of NMD and dexamethasone acetate appeared at  $7 \pm 0.068$  and  $4.78 \pm 0.0427$  min, respectively.

From the calibration curve of NMD in plasma, the equation of plasma concentration–time curve of NMD was  $y = (0.0235 \pm 0.001)x + (0.353 \pm 0.023)$ , with coefficient of determination ( $R^2$ ) = 0.9975, where  $y$  represents the proportion of drug peak area/internal standard peak area,  $x$  represents the plasma concentration ( $\mu\text{g/mL}$ ), the linearity was accomplished over a concentration range of (20–80  $\mu\text{g/mL}$ ). The slope of the curve was  $(0.0235 \pm 0.001)$  and intercept was  $(0.353 \pm 0.023)$ . All values were represented as the mean  $\pm$  SEM. The calculated QL was 17.3  $\mu\text{g/mL}$  and DL value was 5.7  $\mu\text{g/mL}$  as SD value of  $y$  intercept was 0.04 and the slope of the plasma calibration curve was  $0.0235 \pm 0.001$ .

As a final point, the intraday precision was  $1.953 \pm 0.022$  to  $11.9 \pm 0.850$  and accuracy was  $98.867\% \pm 3.384$  to  $109.917\% \pm 3.874$ , while the interday precision was  $1.307 \pm 0.007$  to  $13.8 \pm 0.917$ , and the accuracy was  $90.2\% \pm 2.914$  to  $108.125\% \pm 0.820$ . All values were represented as the mean  $\pm$  SEM<sup>14</sup>. Figure 5 displays the plasma profiles after oral administration of free NMD and niosomal suspension (F1) in a dose equivalent to 10 mg/kg.

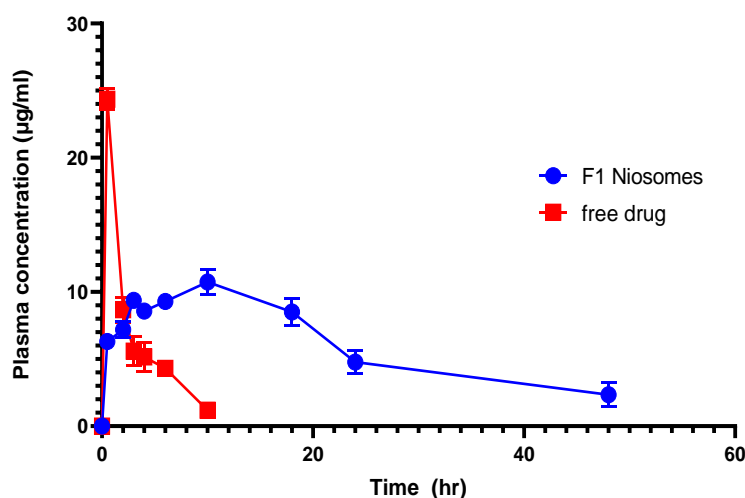


Figure 5- Plasma concentration–time curve of NMD and F1 NMD-loaded niosomes after oral administration of a dose equivalent to 10 mg/kg.

**Note:** all values were expressed as the mean  $\pm$  SEM ( $n=5$ ).



Remarkably, the oral niosomal suspension demonstrated superior MRT ( $23.533 \pm 1.660$  h) and AUC ( $330.531 \pm 15.565$   $\mu\text{g}\cdot\text{hr}/\text{mL}$ ) over oral free NMD suspension ( $6.128 \pm 0.457$  h and  $77.979 \pm 5.111$   $\mu\text{g}\cdot\text{hr}/\text{mL}$ , respectively) (Table 7). Higher MRT of F1 NMD-loaded niosomes than the free drug emphasizes the prolonged effect of niosomes. F1 niosomes were also found to be 4.24-fold higher in AUC than the free drug, reflecting the augmented bioavailability of niosomes over free drug. The relative bioavailability of F1 formulation to drug suspension was about 420%. The ability of span 20 to adhere to intestinal wall permitted the attachment of niosomal vesicles to the epithelial cells of intestine, thus enhancing drug absorption and boosting bioavailability<sup>18</sup>. The niosomes showed a  $t_{\text{max}}$  value about 20-times higher than NMD suspension indicating that NMD-loaded niosomes were successful in increasing duration of drug action. From the obtained data, it can be inferred that the incorporation of NMD in span 20 niosomes achieved a dual purpose of augmenting its oral bioavailability, as well as sustaining its effect.

**Table no 7:** Pharmacokinetic Parameters after Oral Administration of Free NMD and F1 NMD- loaded niosomes

| Pharmacokinetic Parameters                                | Free drug            | F1 NMD-loaded niosomes   |
|---|----------------------|--------------------------|
| $C_{\text{max}}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ) | $24.36 \pm 0.448$    | $10.746 \pm 0.523$       |
| $T_{\text{max}}$ (h)                                      | 0.5 hr               | 10 hr*                   |
| MRT (h)   | $6.128 \pm 0.457$    | $23.533 \pm 1.660^*$     |
| $T_{1/2\text{el}}$ (h)                                    | 8 hr                 | 12 hr*                   |
| $K_{\text{el}}$ ( $\text{h}^{-1}$ )                       | $0.084 \pm 0.014$    | $0.057 \pm 0.005^*$      |
| AUC 0-t ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )          | $69.836 \pm 8.222$   | $289.339 \pm 8.626^*$    |
| AUC 0- $\infty$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )  | $77.979 \pm 5.111$   | $330.531 \pm 15.565^*$   |
| AUMC 0-t ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )         | $172.040 \pm 2.723$  | $5065.967 \pm 305.545^*$ |
| AUMC 0- $\infty$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ) | $482.853 \pm 67.310$ | $7828.257 \pm 877.643^*$ |

**Notes:** all values are expressed as mean  $\pm$  SEM (n=5), \*represent a significant difference at ( $P < 0.05$ ) when related to free NMD.

**Abbreviations;** AUC, area under plasma concentration-time curve;  $K_{\text{el}}$  elimination rate constant;  $T_{1/2\text{el}}$ , elimination half-life; MRT, mean residence time;  $C_{\text{max}}$ , maximum plasma concentration;  $T_{\text{max}}$ , time to reach maximum plasma concentration.

## Conclusion

Niosomes are considered as an advanced drug delivery system for improving the bioavailability and prolonging the duration of action of lipophilic drugs (poorly water-soluble drugs) that belongs to class II BSC. In this study, it is clear that NMD could be loaded in niosomes by thin film hydration method. The formulation F1 containing CH: SPAN 20 (1:2) was proved to have high entrapment efficiency, high stability; low particle size, in addition to sustained drug release. Consequently NMD-loaded niosomes present a convenient and sustained release drug delivery system which, possess enhanced stability and efficacious treatment than traditional drug-delivery system.

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