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# The Potential for Enhanced Systemic Delivery of Nimodipine Loaded Polymeric Nanoparticles via Bilayer Dissolving Microneedles

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## Abstract

Nimodipine (NID) is used for the prevention of ruptured intracranial aneurysms and subarachnoid hemorrhage (SAH).Nimodipine classified as class II with oral bioavailability of 5-13%. The objective of study to fabricate NID as polymeric nanoparticles (NID-NPs) loaded in a bilayer dissolving microneedle (bDMNs) patch .The MNs enhance the delivery of drugs through skin layers as a promising noninvasive and painless delivery system, improving poor solubility and lower oral bioavailability owing to first-pass metabolism. The NID-NPs formulas was fabricated with different drug to polymer(Soluplus®) w/w ratio and 0.25% polyvinylpyrrolidone K15 (PVPK15) using nanoprecipitation technique. The formulas were characterized by measuring particle size (PS), polydisperse index (PDI), entrapment efficiency (EE %). Moreover, the selected formula (FPN4) was designed as bilayer dissolved microneedles (bDMNs) using micro-molding technique in polydimethylsiloxane (PDMS) molds. Eight microneedle (MN1-MN8) patches of polymers such as hyaluronic acid (HA), polyvinylpyrrolidone (PAPK30), polyvinyl alcohol (PVA), carboxymethylcellulose (CMC), and Pullulan (Pu) employed two plasticizers, glycerin (Gly) and polyethylene glycol 400 (PEG). The optimized formula (MN2), with 10% PVA and 5% glycerin as a matrix, was evaluated by measuring surface pH, drug content, weight variation ,moisture absorbance (MA%), , and mechanical needle strength for all MNs while insertion and permeation study were measured for selected MNs .Results of optimized formula (FNP4) exhibit a PS (81.78 nm) and PDI (0.046), EE % ranging (80.37 ±1.63 -98.22 ±2.04) and zeta potential (-18.96 mV) with a spherical shape ,examined by transmission electron microscopy (TEM) ,and improved NID release (95%in25 min compare to 15.5% in 90min pure NID. The Ex-vivo permeation study displayed that 80% of NID permeated in less than 2hr with a lag time of 2-3min. Insertion and mechanical studies show high resistance. Morphological study by field emission scanning electron microscopy (FE-SEM) was studied before and after the insertion into the skin, which displayed sharp, strong, and uniform MNs. As a result, NP-bDMNs achieved an improvement in both solubility and drug release for transdermal drug delivery which may reduce dose frequency, side effects, and improved patient compliance.

**Keywords: Bilayer, Dissolving, Microneedle, Polyvinylpyrrolidone, Pullulan**

## Introduction

Stroke is the third leading cause of death globally, causing significant brain damage and cell death. Developing effective preventative strategies is challenging due to the blood brain barrier[(1)] Preventative strategies are essential in addressing diseases, but developing effective treatments remains a significant challenge due to the blood brain barrier's protective role [(1)].

In last decades, there has been an advancement in nanotechnology, through incorporating NPs as targeted drug delivery system for the treatment of neurodegenerative diseases due to their small diameters (1 -100 nm)[(2)] in which drugs either encapsulated, adsorbed or dispersed in the system [(3)].

Transdermal route is considered as encouraging approach for the administration of a wide range of pharmacological compounds like proteins, and vaccine, since skin, as a big and accessible organ, an appealing route but it acts as a barrier to many undesired and foreign chemicals (4) due to existence of stratum corneum (SC) layer of 10-15 µm approximate thickness that acts as a barrier for the molecules , preventing them from

reaching the site of action[5, 6]. The novelty of this rout for this drug should be rationale in comparison with the nose to brain route.

Microneedles as a novel delivery aid in improving delivery of several therapeutic agents via skin layers and overcome several difficulties with traditional formulations. The MNs arrays are micron-sized, minimally invasive devices with a length ranging from 250 μm to 1500 μm that can easily evade the skin's SC [5,7].They act by disrupting skin , open up micron-sized passage ways that carry the medication straight to the upper dermis, where it can enter systemic circulation without pass through the barrier [8]. Types of MNs include solid, coated, dissolving, hollow, and hydrogel-forming MNs. Several polymers have been developed to produce polymeric MNs such as, hyaluronic acid , chitosan [6],polyglycolic acid , and polyvinyl alcohol (PVA). Amidst this, PVA is becoming more popular in medical applications, including dressings, medication delivery, implantation of soft biomaterials, and targeted tissue transport systems [9].

Nimodipine a calcium antagonist (Figure.1) was originally developed by Bayer (1982) causing a dilatation of cerebral arteries, increasing cerebrovascular flow [10] ,used for the treatment of ischemic cerebrovascular disease, stroke and hypertension. However, NID has poor solubility, undergo first pass metabolism consequently in low oral bioavailability 5-13% and subsequently, limited efficacy [11]. NID is available as intravenous preparation with higher bioavailability compared to oral administration. The available injection contains a high concentration of organic solvent, 23.7% (v/v) ethanol and 17% (v/v) polyethylene glycol 400 (PEG 400) to increase drug concentration which prompts blood vessel irritation, pain, and inflammation at the injection site. According to the daily required dose, the exposure to organic solvent is 101.7 ml casing phlebitis during infusion. Besides, the crystallization of NID resulted due to dilution of injection either with glucose or saline solutions as a result of poor water solubility, which resulted in increased patient health risk [12]. Therefore, the development of safe and effective alternative NID formulations is indicated.

The aim of this study was to formulate NID as NPs loaded bDMNs as alternative route for the improvement of both lower solubility and improved patient compliance. Administration of bDMN - loaded NPs was successfully accomplished using the "poke and release" principle. The needle tips are exposed to body fluids once MN arrays are inserted into the skin. Due to the high-water solubility of the matrix material, tips will be dissolved quickly, delivered medicines to the skin. Since only needle tips of MN arrays can be injected into the skin, drugs overloaded in the base layer would not reach skin when dissolving MNs. The two-step molding technique was used to design NID-loaded bDMNs, decreased drug waste and improved drug delivery efficiency [13].

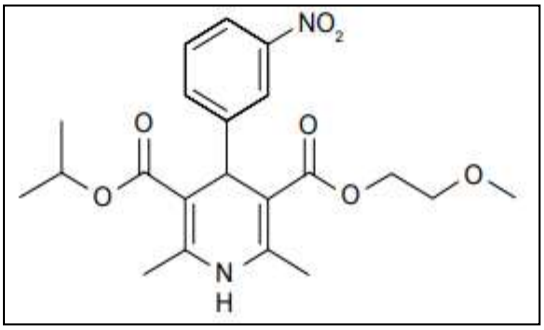



**Figure 1. Chemical Structure of NID [13]**

## Materials and Methods

### *Materials*

Nimodipine (Zhejiang Shenzhou pharmaceutical Co., LTD, china) Ethanol was purchased from Honeywell International Inc., USA. Soluplus® (BASF, Germany) polyvinylpyrrolidone K15, MW 8000 Da from Hyper Chem, China. Polyvinyl alcohol cold from CDH, China. Carboxymethyl cellulose from Central drug house, Mumbai,India. Hyaluronic acid from Hyper Chem, China, Polyethylene glycol400 was obtained from Sigma-Aldrich Co., (St Louis, MO, USA). Dialysis bag 8-14 kDa Lab Pvt. Ltd USA. 225 conical MNs (array size 15×15) with a 500 μm height, 200 μm base diameter, and 1500 μm needle pitch (Micropoint Technologies Pte, Ltd. in Singapore ) and were embedded in laser-engineered silicone templates (PDMS).

### *Method*

#### *Saturated solubility of NID*

An excess pure NID to a 10 ml tube of DW and PBS pH (7.4) brij®-35, which were shaken at 25 ± 0.5 °C and 37 ± 0.5 °C in a shaking water bath for 72 h. Then , centrifugation for 10 min at 2000 rpm (R LABNCO, USA) to remove the supernatant followed by filtration via a filter syringe (0.45 μm) and determined at λ max spectrophotometrically[15, 16].

#### *Preparation of NID-NPs*

NID-NPs were prepared utilizing nanoprecipitation method. By dissolving NID and different (w/w) ratio of polyvinyl caprolactam(PVC),polyvinyl acetate (PVA) – polyethylene glycol(PEG) graft copolymer (Soluplus®),in 3ml ethanol. Subsequently, the resultant solution was injected into the aqueous phase (27ml) containing stabilizer (0.25% PVPK15), at a rate of 0.5 mL/min under stirring speed 1000rpm at 25°C for one hr. on a magnetic stirrer (Digital Magnetic Hot Plate stirrer, Joan lab. China) to evaporate organic phase .Outcome of dispersion was explained as PS, PDI, and ZP. Furthermore, EE% was measured using an Ami-con ultrafilter with a molecular weight cut off (MWCO 10kDa) and centrifuged for 15 min at 3000 rpm, and

amount of free drug was assessed spectrophotometrically at 237 nm [17, 18].

Release behavior of NID from NID-NPs studied in 500ml phosphate buffer (PBS) pH 7.4 with 0.5% brij-35 at 37 ± 0.5 ºC and 100 rpm using cellulose membrane sac of MW cut off 8 to 14 kDa, that previously pre-soaked in PBS [19].Sink conditions was maintained in the dissolution medium by replenished with fresh dissolution medium, the collected aliquots were filtered by 0.45 µm filter syringe and subsequently analyzed spectrophotometrically [20].Furthermore, TEM was achieved for morphological study. The constituents and variable condition of the preparation of dissimilar formulas of NPs are listed in the Table 1.

**Table 1. Composition of Prepared NID-NPs**

| Formula No. | Drug (mg) | Polymer type (mg) | drug: Polymer w/w ratio | Ethanol (ml) | Stabilizer type | Stabilizer Concentrat -ion (w/v %) | O/A Ratio v/v | Speed (rpm) |
|---|---|---|---|---|---|---|---|---|
| **FNP1** | 30 | Soluplus® | 1:1 | 3ml | PVPK15 | 0.25% | 1:9 | 1000 |
| **FNP2** | 30 | Soluplus® | 1:2 | 3ml | PVP K15 | 0.25% | 1:9 | 1000 |
| **FNP3** | 30 | Soluplus® | 1:4 | 3ml | PVP K15 | 0.25% | 1:9 | 1000 |
| **FNP4** | 30 | Soluplus® | 1:8 | 3ml | PV K15 | 0.25% | 1:9 | 1000 |

***Construction of bDMNs***

Nimodipine-loaded bDMNs fabricated with selected NID-NPs using a template made from PDMS, (Micropoint Technologies Pte, Ltd. Singapore) which has the capability to create a strip of MNs, consisting of 225 pyramid-shaped needles arranged in a 15x15 pattern. The fully formed needles have a height of 500 µm, a base width of 200 µm, and interspacing 1500 µm apart.

Casting technique, employed in two steps, first 1ml of NID-NPs mixed with 0.5ml of polymeric solution used, to get a drug containing matrix solution, and added into a MN mold to allow the solution to enter the needle tips, a pressure was applied via sonication, using sonicator (Copley Scientific, UK), for 1hr and degassed for 10 min. In the second step, after drying, 2 ml solution of polymeric solution was added continuously to the mold containing NP dispersion and pressure was applied to ensure that the blank matrix solution pass into the remaining space of mold. Then, prepared MNs template was retained in a desiccator under vacuum for 10 min, consequently sonicated for 2 h. and degassed for 10min to remove any air bubbles and get optimum filling of the needle cavities and left for 48-72 h. at room temperature in the desiccator [21, 22]. Strip removed from the mold using a scalpel, sealed with aluminum foil for further investigations. Table 2 indicates the components of the various MNs formulations. Different polymers such as PVA,CMC, PVPK30, Pu and HA, and two plasticizer such as Glycerin and PEG400 were used in 5% concentration, and the prepared polymeric solution was kept overnight to ensure remove any entrapped air [21, 23].

***Characterization of NPs***

The NID-NPs were evaluated in terms of PS, PDI and ZP using zetasizer analyzer (Malvern Panalytical ,Ltd., UK) that detect the intensity of light scattered in 173º scattering angles at 25 ± 2 °C[23].Additionally , the EE %of formulation was determined by centrifuge dispersion at 3500 rpm, 25 °C for 30 min using Amicon ultrafilter with a molecular weight cut off (MWCO) 10 kDa, (Merck, sigma-Aldrich USA) to separate the NID-NPs from free drug. After collection, filtered supernatant (through 0.45 µm syringe filter) was analyzed for NID content [25].The *in-vitro* release conducted in 500ml PBS pH 7.4 at 37 ± 0.5ºC and 100 rpm at regular intervals of 5, 10, 15, 30, 45, 60, and 90 min to select optimized formula .Morphological study was achieved for optimized formula by TEM using (Zeiss-EM10C-100KV, Germany) [26, 27].

***Characterization of bDMNs***

***Microscopic and Morphology Evaluation***

Prior to testing, bDMN arrays general appearance was checking visually for any flaws where the strip was inspected below a digital microscope camera (Hitachi, Tokyo, Japan) to display shape[5]. The morphological characteristics were studied where the base radius, tip radius, and wall thickness of MNs were measured using FE-SEM model Inspect 50 FEI, Germany, in which the sample was prepared by dehydration to remove any water content and get better imaging by mounting needles to a double-side carbon conductive paper. Sample was coated with thin layer of gold as a conductive substance to prevent charring during imaging. Equipment was set in a low vacuum mode at a voltage of 15 kV [28].

**Table 2. Components of bDMNs Formulations**

| Formula No. | NID (mg) | Polymer type and amount (gm) | | | | | Plasticizer Concentration (w/w%) | | DWml | PS%* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PVA | Pu | CMC | HA | PVPK 30 | PEC 400 | Glycerin | | |
| **FMN1** | 10 | 4 | | | | | | 5% | 20 | 20% |
| **FMN2** | 10 | 2 | | | | | | 5% | 20 | 10% |
| **FMN3** | 10 | 2 | | | | | 5% | | 20 | 10% |
| **FMN4** | 10 | 1 | | | | | | 5% | 20 | 5% |
| **FMN5** | 10 | 1.5 | | | | 0.5 | | 5% | 20 | 10% |
| **FMN6** | 10 | | 1 | | | | | 5% | 20 | 5% |
| **FMN7** | 10 | | | 1 | | | | 5% | 20 | 5% |
| **FMN8** | 10 | | | | 1 | | | 5% | 20 | 5% |
| ***PS: polymeric solution** | | | | | | | | | | |

***Surface pH Measurement***

In employing bDMNs *in -vivo*, surface pH is measured to investigate any possible skin irritation due to an acidic or basic pH. Whole strip was dissolved in 10 ml of DW.The resulting pH was measured by immersing the pH meter electrode (HANNA RI 02895, Romania) in the mixture[22, 29].

***Drug Content***

Drug content of bDMNs was determined, three patches from each formulation, were quantitatively determined by cutting the needles carefully with a blade and collected, then dissolved in 50 mL of ethanol and mixed for 1 h on a magnetic stirrer at 750rpm, 1mL of the resultant dispersion was diluted with ethanol to guarantee complete dissolution of the contained NPs. The solution was filtered through 0.45µ syringe filter and analyzed spectrophotometrically at 237nm [30, 31].

***Weight Variation***

The regularity of MNs patches was confirmed by weight variation. Three randomly chosen patches from each batch were weighed separately and compared to the mean weight for deviation[32].

***Moisture Contents:*** Percent of moisture absorb (MA%) of DMNs was calculated by obtaining the initial weight of three MNs strips from each batch; after that, they were placed inside a desiccator containing a saturated solution of sodium chloride to create 75% relative humidity at 25°C temperature for 72h The patches were accurately weighed, the difference between initial and final weight represents the moisture uptake[29].

***Mechanical strength (MS)***

The MS of bDMNs was evaluated using different techniques such as compression and insertion testing. By applying a controlled axial force to the MNs, measuring their response over time, and studying how MNs deform, break, or fail under different forces and durations to assess their mechanical properties and durability. This was achieved using a Texture Analyzer (TA-XT-plus, Stable Microsystems, UK) set in compression mode[24,31].

Quantitative evaluation of mechanical property was examined by employing a needles that attached with double-side tape to the mobile cylindrical (LAP PERSPEX probe P/25 L, 25mm DIA) which designed to move vertically downward at a pre-test speed:1.00 mm/s, test speed: 5.00 mm/s and post-test speed: 10 mm/s using a force of 32 N per 30 s as the target mode[9, 34].Once the tip touched the horizontal platform, the sensor continually verified the force and distance traveled. A digital microscope was used to inspect the needles, by examining the needles' length and form, both before and after compression. When the needle broken, the force abruptly decrease; the maximum force applied immediately before dropping was interpreted as the force of needle failure [35].

***The Insertion Test***

Numerous techniques, like microscopical and histological assays, can be used to quantify the depth of penetration, diameter of the created micropores and effectiveness of MNs in delivering medications into the skin. The insertion test entails putting MN into the skin and examine penetration depth. By applying MN on rabbit's skin for two minutes using a handheld administration device with a force of 30 N. The needles create tiny or micro - channels, by penetrating the SC layer. By passing layers of epidermis and dermis, these channels enable direct transport of medications into the skin.

With the ability to see depths of up to 2000µm**,** optical coherence tomography (OCT) (Huvitz Optical Coherence Tomography HOCT-1F, Korea) was used to control whether all arrays of patch had successfully penetrated SC precisely for the chosen formula. Image-J software was used to analyze the OCT images[36].

***Histological Study***

Abdominal rabbit skin has been excised and used as a model to figure out how effectively MNs avoided impermeable SC and to discard any pathological changes or any skin irritation that might brought about due to the needle insertion. A reference skin that was devoid of MNs was utilized as a control to emphasize the variations between samples. The test was conducted after poked plain skin with optimum MN arrays using manual pressure for 3 min. The skin reaction was observed for 72 h and as soon as the skin had been removed, it was refrigerated in buffer, dried and embedded into paraffin, small pieces were cut out and positioned on a slide for examination and hematoxylin and eosin staining for pathological observations [21, 35].

***Permeation Study***

The study was performed with NID-NPs loaded in an ordinary prepared transdermal patch and with bDMNs arrays containing NID- NPs to compare different mechanisms of enhancers for same route.Using full thickness abdominal skin of adult male albino rabbit weighing 1460g ± 55g where the skin prepared, fixed between the donor and receptor compartment of 50 ml jacketed Franz diffusion cell (Perme Gear Inc, Hellertown, PA) with a diffusion area of 4 $cm^2$ maintained at 37 ± 0.5°C. PBS pH 7.4 were filled receptor chamber and kept at a rotational speed of 50 rpm using magnetic stirrer, in a way that the skin surface just flushes the diffusion fluid. The donor compartment was covered with Parafilm to avoid evaporation and contamination. Aliquots of 0.5 mL sample were taken from receiving compartments at pre-determined time intervals; 0.25, 0.5, 1, 2, 4, 6, 8 and 12 h and replaced with the same volume of receptor fluid drug content using UV spectrophotometer at λmax 237 nm was measured [30, 37].

***Statistics***

All statistics were calculated using Microsoft Excel 2019 software, Graph pad prism.8 and DD-solver software. Reported averages represented mean, SD, P-value of the tested samples [40].

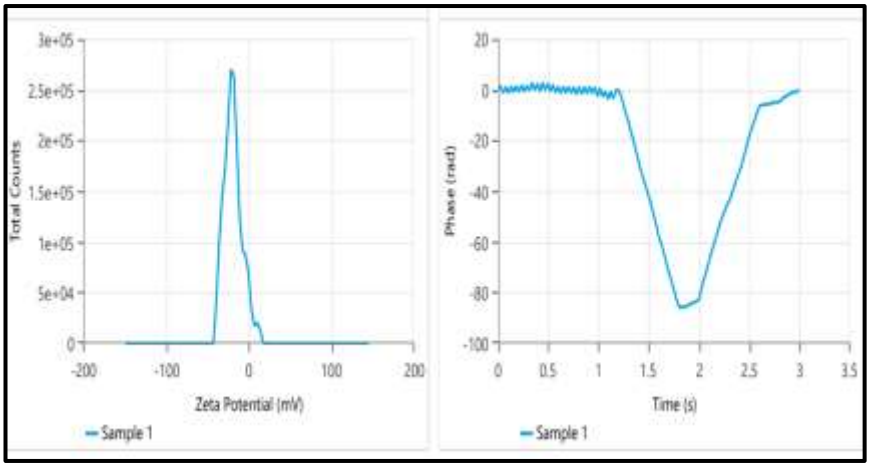
## Results and Discussion

Results of saturated solubility exhibit that NID has 31.08 ±2.3µm/ml and 99.4± 1. 3µm/ml in DW and PBS with 0.5%w/w Brij-35 (pH7.4) respectively indicating a poor solubility of NID [15]. Measurement of ***PS*** and ***PDI*** of formulated NID – NPs were screened as shown in (Table 3) indicated that most of formulations in nanosize ranging from (**249.8 ± 3.8 - 81.78 ±0.6** nm) ,PDI was deviated from (**0.44± 0.10- 0.046± 0.01)** ranging from monodispersed to polydisperse distribution which correlated to the variances in stabilizer and polymers ratio. The ***EE%*** values ranging (**80.37 ±1.63 -98.22 ±2.04**). The results exhibit that increasing soluplus concentration resulted in reducing PS since Soluplus is a bifunctional polymer that functions as both a matrix polymer and an active solubilizer through micelle formation in water. It is an excellent wetting agent that effectively reduces the interfacial tension between the hydrophobic surface of NID particles and the aqueous antisolvent due to the presence of a polyethylene glycol backbone as a hydrophilic moiety and a lipophilic vinyl caprolactam/vinyl acetate side chain, which provides an amphiphilic nature. Consequently, the prevention of aggregation of the NP as a result of steric hindrance results in the production of uniform NPs with a narrower size distribution. Formula FN4 was selected as optimized formula since it has lower PS (**81.78 ±0.6)**, PDI (**0.046±0.01),** higher **EE% (98.22 ±2.04)** and zeta potential **(-18.96mV**) that pointed out the degree of repulsion among particles which is considered acceptable due to steric stabilization of nonionic stabilizer (Figure.2).

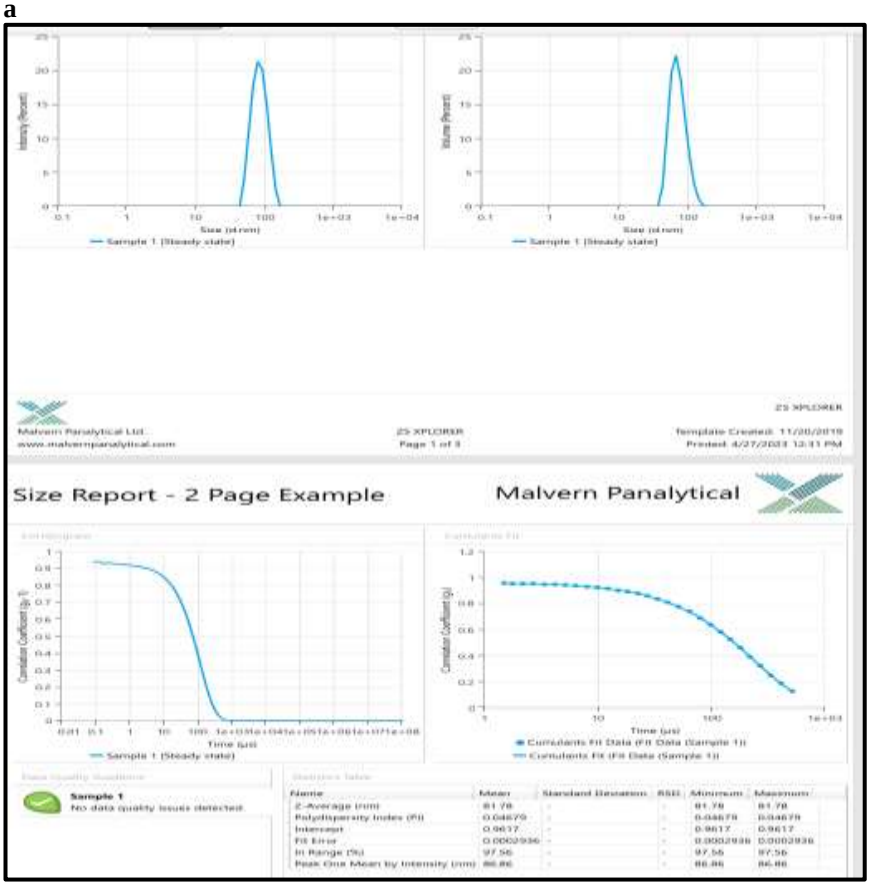
The ***TEM*** revealed regularly spherical round particles as shown in (Figure 3). Moreover, the% drug release of FN4 95%in 25 compared to 15.5% in 90min pure NID Figure.4 revealed a significant improvement of drug dissolution as a result of reducing particle size ($p<0.05$) according to Noyes–Whitney equation [41].

**Table 3. Results of NID–NP Formulation**

| Formula No. | PS (nm) | PDI | EE% |
|---|---|---|---|
| **FN1** | 249.8 ± 3.8 | 0.44 ±0.10 | 80.37 ±1.63 |
| **FN2** | 167.5 ±5.2 | 0.38 ±0.049 | 97.53 ±0.82 |
| **FN3** | 134.9 ±3.5 | 0.16 ±0.08 | 98.03 ±0.54 |
| **FN4** | 81.78 ±0.6 | 0.046±0.01 | 98.22 ±2.04 |




**a**




**b**

**Figure 2. a)ZP measurement of selected PN4, b) PS measurement of PN4**

Selected NID-NP4 formulated as bDMNs and was characterized. Results revealed that not all formulations produced flawless MNs. Most of MNs have sharp tips and uniform needles with homogenous polymer mixtures except FMN3, 6, 7,8 (Figure.5) that appear as brittle or sticky which were excluded. The optimized FMN2 (Figure.6) was selected and examine has 437-500 µm length and base width 199.7-200 µm (Figure 7) [30].

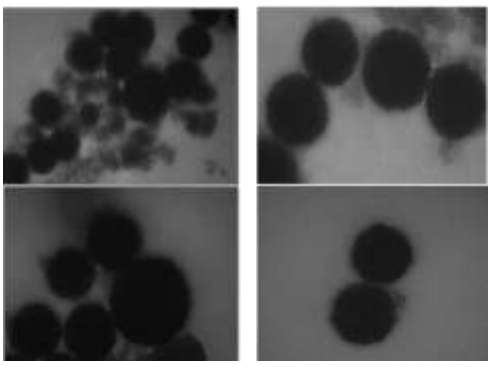


**Figure 3. The TEM of selected PN4**

In Table 4, surface pH of all successfully generated MN patches ranging from 5.7 to 6.5 ensuring absence of skin irritation when compared to the skin's pH is (5.5). Drug content in all MNs patches was determined ranging from 99-83 % which was within the acceptable range of content stated in BP (85% - 115%.) except FMN6, which can be attributed to the diffusion of the drug to the baseplate due to reduced polymer matrix viscosity indicate that the technique used in preparing DMNs indicated that the technique used in preparing DMNs patches was very efficient and NID was spread uniformly throughout the prepared patches. For ***weight variance,*** results indicated an average weight ranged from 1.2 to 0.44 gm (Table 4), demonstrating that the process utilized to prepare the DMNs patches was repeatable and produces patches with consistent weight.

**Table 4. MNs Formulations**

| Formula No. | Polymeric Solution% | Physical Appearance | Wt. (gm) | Drug content (%) | MA% | pH |
|---|---|---|---|---|---|---|
| **FMN1** | 20%PVA | Regular, firm. rigid, inflexible film | 1.2 ±0.41 | 95±1.52 | 4.21±0.052 | 5.6±0.31 |
| **FMN2** | 10%PVA | Regular, firm, sharp well-formed needles | 0.83 ±0.08 | 94 ±1.4 | 2. 3±0.11 | 6.1±0.057 |
| **FMN3** | 10%PVA | flexible film, cloudy surface, needles not | 0.53 ±0.24 | 89.5 ± 2.4 | 3.87 ± 0.43 | 6.2±0.14 |
| **FMN4** | 5%PVA | Flexible thin film, well-formed needles | 0.5 ±0.15 | 90±2.30 | 2 ± 0.01 | 6 ± 0.05 |
| **FMN5** | 10%PVA+ PVPK30 | flexible film, well-formed needles | 0.586±0.09 | 90 ±0.98 | 2.8 ± 0.34 | 5.6 ±0.32 |
| **FMN6** | 5%Pu. | Brittle, inflexible film, well-formed | 0.44 ±0.05 | 83 ±2.1 | 4.2±0.23 | 6.5 ± 0.34 |
| **FMN7** | 5%CMC | highly flexible supple film | 0.54±0.015 | 92.91 ±1.3 | 2.16 ±0.2 | 6.1 ±0.21 |
| **FMN8** | 5%HA | Irregular shape, sticky | 0.5 ±0.05 | 81.3±0.513 | 4.76±0.158 | 5.4 ±0.37 |

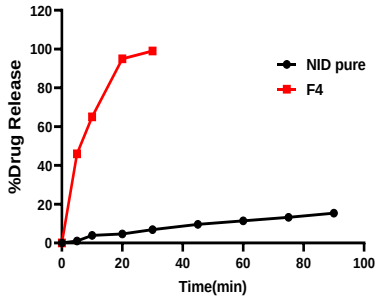



**Figure 4. Release of FN4 and pure NID**

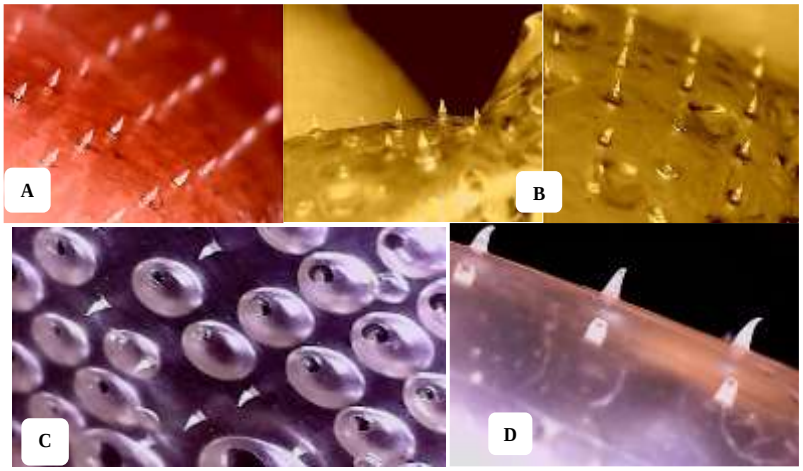



**Figure 5. Digital images of A- FMN6, B-FMN7 ,C-FMN3 ,D-FMN8**

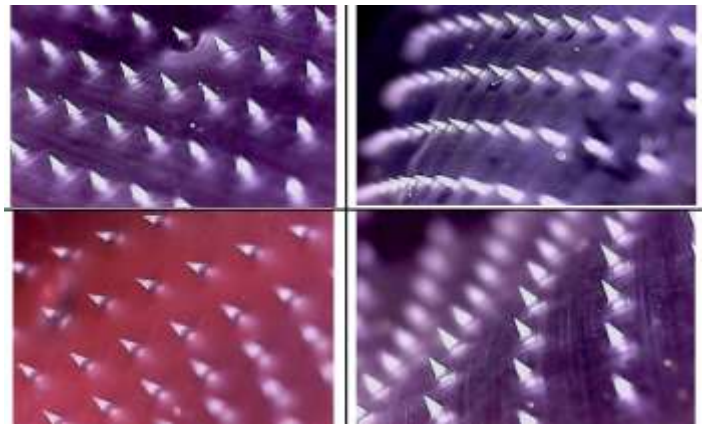


**Figure 6. bDMNs shape of FMN2**

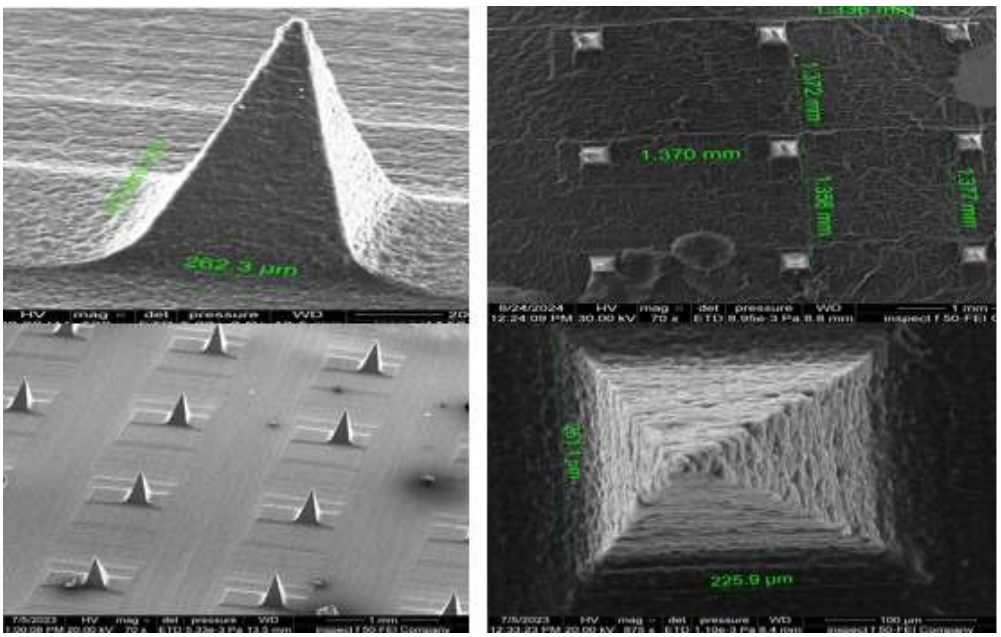



**Figure 7. DMN shape and dimensions of FMN2**

Determination of moisture uptake is essential aspect since it affects mechanical properties and stability of drugs during storage.% Moisture absorb (PMA) values is provided in Table 4 ranging from 2 to 4.27%, indicating that all of the polymers (PVPK30 , PVA,CMC,HA, Pu ) were hydrophilic in nature with strong moisture absorption properties but in a various extent[42].

Figure 8 (a, b).displayed histological findings regarding the ability of bDMNs to penetrate. Consequently, the DMN-treated skin rabbit was inspected, and intact skin was additionally examined at as a control. The findings demonstrated that DMNs able to form microchannel and cross the SC; no inflammatory symptoms or pathological alterations at the cellular level had been observed.

Figure (9 a and b ) revealed results after having applied the single MNs application , in which OCT image confirmed successful insertion with the holes created in the skin with a measured penetration depth of 598.51µm, which was closely related to the calculated depth of untreated skin (687.11 µm) previously measured . The insertion test showed that needles can reach insertion depths of 500mm.[36]

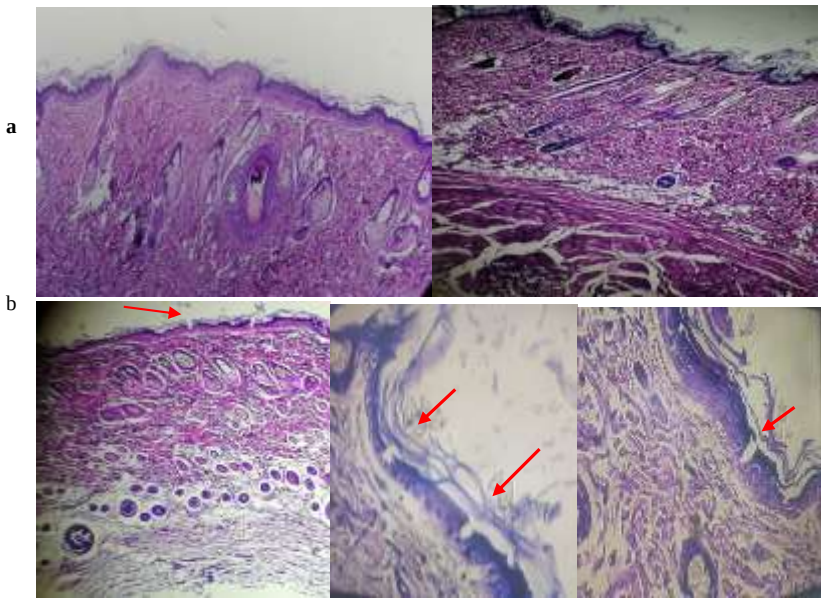



**Figure 8.a-Normal skin before insertion, b- after insertion**

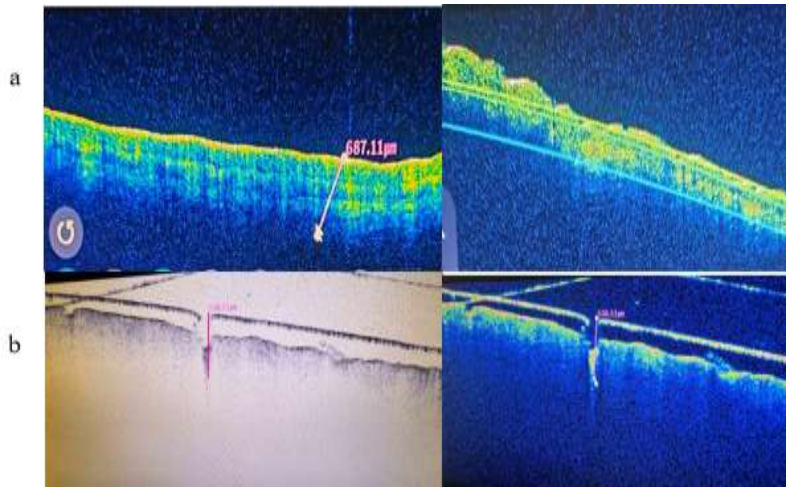
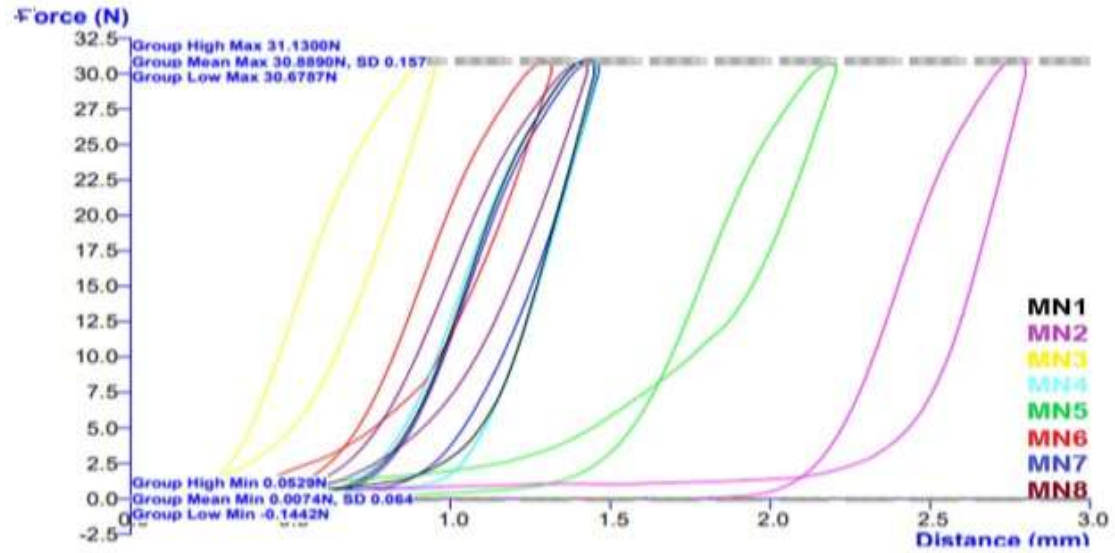



**Figure 9. OCT- Image of inserted bDMNs . (a) Normal skin layers depth measurements. (b) OCT image of bDMNs .**

***Mechanical strength(MS)***

Since MN must be inserted successfully to penetrate and bypass skin barrier and deliver drug efficiently that relying on type and amount of polymer used, tip diameter, and geometry that have an impact on the MS. comparing different batches of MN arrays and confirm consistency of manufacture. A compression test was accomplished on bDMN arrays, some of bDMNs show deformation and reduction in the height of the needles particularly under the application of 32 N force/array, part of arrays were fractured others were bended without breaking while others may not affected by force applied depending on polymer type as shown in the force-travel curves of DMNs, (Fig.10a, b1, b2, and b3)[43].It should be noted, however, that needles had been pushed against a metal block, which is far from insertion normally done. Results also showed that there was no statistically significant distinction between formulations FMN1,FMN3, FMN4, FMN6 and FMN7 ($p = 0.98$), with the exception of FMN2 and 5, which exhibits higher resistance to change while others exhibit low to moderate resistance. This was related to the polymer type used which is PVA characterized by hydrophilic nature that allows it to dissolve well in water and form a smooth, uniform solution with suitable viscosity that enables it to spread evenly when cast, leading to uniform MNs. Additionally, PVA can be cross-linked, which enhances its mechanical properties and helps maintain uniformity and it is compatible with various additives and plasticizers [33, 44].




**A**

**B1** **B2** **B3**

**Figure 10. (A) Representative chart of % reduction in the height of needles measured after application of forces (means ± SD, n = 3). (B1,2,and 3) Images of bDMNs after compression test represent changing in the shape and height of MNs [43, 44]**

***Permeation study***

To predict *in-vivo* drug absorption, permeation study was conducted for selected bDMNs (FMN2) and compared with skin treated by NID –NPs ordinary patch. Afterward, MNs' penetration-enhancing effect was assessed. By plotting the amount of NID permeated with time in min. It was found that after initiating the study, more than 80% of loaded drug permeated in 2h (Figure 11). The calculated steady-state flux, and permeability coefficient were observed to be improved significantly ($p < 0.05$) compared with simple NID patch. Additionally, lag time of bDMN was 2-3min which was less than ordinary patch 20-25min as shown in Table 5.

Concerning the disadvantages of DMN which is the incomplete penetration into the skin leading to drug wastage that may resulted from fracture of needles tip during insertion of MN, skin deposition study revealed no drug deposition which support the good performance of selected polymer formulating MN.

***Statistical analysis***

Mean ± SD data from triplicate measurements were analyzed using one-way ANOVA in Microsoft Excel 2019. Significance was determined at $p < 0.0$.

**Table 5. Parameters of Permeability Study**

| Formula | NID – bDMN | NID –Simple patch |
|---|---|---|
| **Permeability coefficient (cm/min)** | 0.124 | $1.69 * 10^{-2}$ |
| **Lag time (min)** | 2-3 | 20- 25 |
| **Flux (µg/cm$^2$.hr )** | 162.33 | 21.99 |

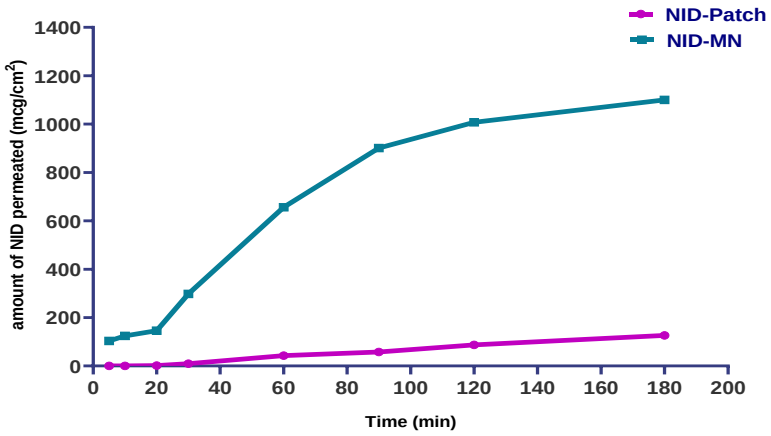



**Figure 11. Amount of NID permeated from simple patch and FMN2**

## Conclusion

Nimodipine as NPs were prepared successfully using solvent-antisolvent nanoprecipitation followed by loading into transdermal bDMNs patches which considered as a promising technological advancement to improve poor solubility. DMNs provide an alternative attitude to hypodermic injection for the delivery of drug in a painless, non-invasive manner, improve patient compliance, and safe to discard.

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## Conflicts of Interest

No conflict of interest

## Funding

Research did not receive any fund

## Ethics Statements

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## Author Contribution

A.M.R. Data collection, Investigation, Methodology, Writing –Original Draft Preparation. M.M. G. Project Administration, Supervision, Writing –Review and approve the final version of the manuscript.

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# إمكانية تعزيز التوصيل النظامي للجسيمات النانوية البوليمرية المحملة بالنيموديبين عبر الإبر الدقيقة ثنائية الطبقة الذائبة

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**الخلاصة**

النيموديبين عقار يستخدم للوقاية من تهشم الأوعية الدموية داخل الجمجمة ونزيف تحت العنكبوتية .يصنف النيمودبين على انه من ادوية الصنف الثاني استنادا لذوبانيته ويمتلك توافرا حيويا (٥-١٣٪) .كان الهدف من الدراسة هو تصييغ النيمودبين بشكل جسيمات نانوية بوليمرية محملة في رقعة إبرة مجهرية ذائبة ثنائية لتعزيز توصيل الدواء عبر طبقات الجلد كنظام توصيل واعد غير جراحي وغير مؤلم ، وتحسين الذوبانية وقلة التوافر البيولوجي الفموي( ٥-١٣ ٪) الناتج من عملية التمثيل الاولي. تم تصنيع التركيبة النانوية باستخدام نسب محتلفة (وزن / وزن) من النيمودبين الئ البوليمر (السولوبلس) و ٠,٢٥٪ بولي فينيل بيروليدونK١٥باستخدام تقنية الترسيب النانوي. وتم توصيف الصيغ بقياس حجم الجسيمات ومؤشر التشتت المتعدد وكفاءة الانحباس علاوة على ذلك تم تصميم الصيغة المختارة (FPN4) كإبر دقيقة مذابة ثنائية الطبقة (bDMNs) باستخدام تقنية القولبة الدقيقة في قوالب polydimethylsiloxane (PDMS). استخدمت ثماني بقع ميكروإبرة (MN1-MN8) من البوليمرات مثل حمض الهيالورونيك (HA) والبولي فينيل بيروليدون (PAPK30) وكحول البولي فينيل (PVA) وكربوكسي ميثيل سلولوز (CMC) وبولولان (Pu) مع نوعين من الملدنات ، الجلسرين (Gly) والبولي إيثيلين جلايكول ٤٠٠ (PEG). تم تقييم الصيغة المثلى (MN2) ، المصاغة من ١٠٪ PVA و ٥٪ من الجلسرين كمصفوفة ، عن طريق قياس درجة الحموضة السطحية ، ومحتوى الدواء ، وتغير الوزن ، وامتصاص الرطوبة (MA٪) ، وقوة الإبرة الميكانيكية لجميع الصيغ بينما تم قياس دراسة الإدخال والتغلغل للصيغة المختارة. أظهرت نتائج الصيغة المثلى (FNP4)حجم جسيمي (٨١,٧٨ نانومترومعامل تشتت (٠,٠٤٦) وكفاءة انحباس تتراوح (٨٠,٣٧ ±١,٦٣ -٩٨,٢٢ ±٢,٠٤) وجهد زيتا (-١٨,٩٦ مللي فولت) و شكل كروي والذي تم فحصه بواسطة المجهر الإلكتروني النافذ (TEM) مع تحسين إطلاق للنيمودبين(95٪ في ٢٥ دقيقة مقارنة ب ١٥,٥٪ في ٩٠دقيقة للنيمودبين النقي. أظهرت دراسة التغلغل خارج الجسم الحي أن ٨٠٪ من NID تتخلل في أقل من ساعتين مع وقت تأخر من ٢-٣ دقائق. تظهر دراسات الإدراج والميكانيكية مقاومة عالية. تمت دراسة الدراسة المورفولوجية بواسطة المجهر الإلكتروني الماسح للانبعاث الميداني (FE-SEM) قبل وبعد الإدخال في الجلد ، والذي أظهر MNs حادة وقوية وموحدة. نتيجة لذلك ، حققت NP-bDMNs تحسنا في كل من الذوبان وإطلاق الدواء لتوصيل الدواء عبر الجلد مما قد يقلل من تكرار الجرعة والآثار الجانبية وتحسين امتثال المريض .

**الكلمات المفتاحية: ثنائية الطبقة ، مذابة ، إبر دقيقة ، بولي فينيل بيروليدون ، بولولان.**