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Novel instantly-dispersible nanocarrier powder system (IDNPs) for intranasal delivery of dapoxetine hydrochloride: *In-vitro* optimization, *ex-vivo* permeation studies and *in-vivo* evaluation

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Abstract:

Dapoxetine (D) suffers from poor oral bioavailability (42%) due to extensive metabolism in the liver. The aim of this study was to enhance the bioavailability of D via preparing instantly-dispersible nanocarrier powder system (IDNPs) for intranasal delivery of D. IDNPs were prepared using the thin film hydration technique, followed by freeze-drying to obtain easily reconstituted powder providing rapid and ready method of administration. The produced nanocarrier systems were evaluated for drug content, entrapment efficiency percentage, particle size, polydispersity index, zeta potential and drug payload. The optimized nanocarrier system was morphologically evaluated via transmission electron microscopy and the optimized freeze-dried IDNPs were evaluated for ex-vivo permeation and in-vivo pharmacokinetic studies in rabbits following intranasal and oral administration. The relative bioavailability of D after intranasal administration of freeze-dried IDNPs was about 235.41 % compared to its corresponding oral nanocarrier formulation. The enhanced D permeation and improved bioavailability suggest that IDNPs could be a promising model for intranasal delivery of drugs suffering from hepatic first pass effect.

Keywords: Dapoxetine, oral bioavailability, instantly-dispersible nanocarrier powder system, freeze-drying, intranasal, Pluronics.
1. Introduction

Premature ejaculation is a common male sexual dysfunction that is treated by various therapeutic remedies including selective serotonin reuptake inhibitors (SSRIs) [1]. Dapoxetine (D), as dapoxetine hydrochloride, is the only licensed SSRI drug for treatment of premature ejaculation in adult men [2]. D acquires very rapid absorption from the gastrointestinal tract giving high serum concentrations (after \( \approx 1.29 \) h) [3] and very short elimination half-life of \( 1.31 \) h [4]. Owing to its unique pharmacological profile, it has been used as a successful “episodic”, “as-needed” or “on-demand” treatment of PE where a rapid effect of the medication is needed[2]. However, oral administration of D suffers from hepatic first pass metabolism which results in its poor bioavailability (42%) [5]. Thus, formulating intranasal systems of D would avoid the hepatic route and consequently improve D bioavailability. However, D acquires limited solubility at neutral pH of body fluids, which hampers its absorption through the nasal mucosa.

Several studies suggested different formulations that aimed to improve D bioavailability. These include a study by Srikant et al where, immediate release tablets were developed using micronized D [6]. Also, effervescent sachets of D and phosphodiesterase-5 inhibitors were formulated in a study by Ali et al. [7]. In another study by Ali et al, chewing gum formulations of D and tadalafil were produced aiming to solubilize the drug in the saliva prior to swallowing and thus increasing its bioavailability. The aforementioned studies aimed to enhance D bioavailability via using different forms of oral formulations. In our previous study, instantly soluble transmucosal matrix systems were developed that improved D bioavailability via the intranasal route [8].

Intranasal drug delivery has acquired a great attraction for systemic administration of drugs suffering from first pass metabolism in an attempt to improve their bioavailability [9, 10], for example, propranolol [11] and
metoclopramide HCl[12]. The intranasal route offers, in addition to avoidance of first pass metabolism and enhanced bioavailability, rapid onset of action due to rapid absorption [13].

Vesicular drug delivery systems and nanocarriers such as; liposomes, niosomes, transfersomes, cubosomes,….etc. hold a great promise in improving drug absorption, efficacy and hence, bioavailability[14, 15]. They play a major role in drug delivery where, they facilitate drug transfer through biological membranes, and thus improve bioavailability, Vesicular drug delivery systems, composed of phospholipids, edge activators and surface-active carriers (for example; poloxamers and polysorbates) were reported to enhance drug absorption and transmucosal permeation of several drugs [16] such as insulin [17], corticosteroids [18] and anticancer agents [19].

The success of nanocarrier formulations depends on the choice of the suitable surface-active carrier. Among several examples of surface-active carriers, Pluronic® F-127 (F127) holds the greatest promise. F127 was found to have unique characteristics such as; high solubilizing capacity and non-toxic quality, rendering it a valuable pharmaceutical vehicle for many drugs administered through different routes of administration [20, 21]. It is also known for its mucous membrane penetrating ability which enhances permeation of drugs [22].

In this study, instantly dispersible nanocarrier powder system (IDNP) was prepared by the thin film hydration technique followed by freeze drying, in order to obtain reconstituted powder for easy intranasal administration. D-optimal mixture experimental design was employed to evaluate the effect of different formulation variables on the properties of nanocarrier formulations. Transmission electron microscopic analysis was accomplished in order to examine the particle morphology of the optimized D-loaded nanocarrier formulation. The freeze-dried optimized formulation was further evaluated after intranasal administration to rabbits, compared to its corresponding oral nanocarrier formulation.
2. Materials and methods

2.1. Materials

D was kindly supplied by Marcyrl for pharmaceutical industries, El-Obour, Egypt. L-α-Phosphatidylcholine (PC) from soybean was purchased from MP Biomedicals (Santa Ana, California, USA). Pluronic® P-123 (P123) and Pluronic® F-127 (F127) were supplied from BASF Corporation, Chemical division, Parsippany N.J., USA. Tween® 80 (Polyoxyethylene sorbitan monooleate) was purchased from Sigma Aldrich Chemical Co., St. Louis, MO., USA. Aeroperl® 300 Pharma, fumed silica granulate, supplied from Evonik Industries. Mannitol (Pearlitol® 200 SD) was donated by Roquette Pharma, France. Disodium hydrogen phosphate and potassium dihydrogen phosphate were provided from Merck (Darmstadt, Germany). Distilled water was used throughout the study. All other chemicals were reagent grade and used as received.

2.2. Methods

2.2.1. Preparation of D-loaded nanocarrier systems

D-loaded nanocarrier systems were prepared according to modified thin film hydration method [23, 24]. Accurately weighted amounts of D (20 mg), PC, Tween® 80 and binary Pluronics® mixture (BPM) (F127 and P123 in the ratio of 1:2 w/w) were placed into a long-necked, round-bottom flask (250 mL) and dissolved in 10 mL ethyl alcohol using an ultrasonic bath sonicator (Crest ultrasonics corp., Trenton, USA) for 10 min. The solvent was slowly evaporated at 60 °C under reduced pressure using a rotary evaporator (Buchi R-110 Rotavapor, Flawil, Switzerland) revolving at 120 rpm for 20 min. until a thin, dry film was formed on the inner wall of the flask. The obtained dry film was then hydrated with 7 mL phosphate buffer (pH 6.8) and the flask was left to rotate at 60 °C for 90 min., under normal pressure. The volume of the formed D-loaded nanocarrier
dispersion was adjusted to 10 mL with phosphate buffer (pH 6.8) and stored in the refrigerator (at 4 °C) for further characterization.

**2.2.2. Formulation Optimization**

In order to investigate the influence of formulation variables on the nanocarrier systems' characteristics, D-loaded nanocarrier systems were prepared according to the D-optimal mixture experimental design using the Design-Expert® software version 7 (Stat-Ease, Inc., Minneapolis, Minnesota, USA). Mixture Design is commonly used when the formulation contains components whose percentage sum is 100%. D-optimal design was selected since it minimizes the variance associated with the estimates of the coefficients in the model [25]. This design, the concentration of binary Pluronics® mixture (BPM) \( (X_1) \), the concentration of PC \( (X_2) \) and the concentration of Tween® 80 \( (X_3) \) were selected as independent variables. The total concentration of the three variables was summed to 100%. The investigated responses were: entrapment efficiency percent \( (EE \%) (Y_1) \), particle size \( (PS) (Y_2) \), polydispersity index \( (PDI) (Y_3) \), zeta potential \( (ZP) (Y_4) \) and drug payload \( (PL) (Y_5) \). The optimized formulation with the desired characteristics was selected aiming to accomplish high \( EE \% \) ( > 50 %) and PL ( > 70 %), the value of ZP ( < -25 mV) together with small PS ( < 750 nm) and low PDI ( < 0.5).

**2.2.3. In-vitro characterization of the prepared D-loaded nanocarrier systems**

**2.2.3.1. Drug content**

Total drug content (free + entrapped) of the prepared formulations was determined by dissolving 0.1 mL of the nanovesicle dispersion in a suitable amount of methyl alcohol to obtain a clear solution and then measuring the UV absorbance using spectrophotometer (UV 1601, PC UV-Visible, Shimadzu, Japan) at 292 nm \( (\lambda_{\text{max}} \text{ of D in methyl alcohol}) \).
2.2.3.2. **Entrapment efficiency percent (EE %)**

In order to determine the EE %, one mL from each formulation was centrifuged using a cooling centrifuge (Model 8880, Centurion Scientific Ltd., W. Sussex, UK) at 10,000 rpm for 30 min. at -4 °C in order to separate the nanovesicles from the free (un-entrapped) drug. The supernatant containing the free drug was removed. The residue was washed well with 1 mL distilled water to remove any surface drug, re-centrifuged at 10,000 rpm for 30 min. at -4 °C. The washed separated nanovesicles were dissolved by sonication with methyl alcohol and the concentration of the entrapped drug was determined spectrophotometrically. The entrapped drug percentage was then calculated using the following equation:

$$EE\% = \left[ \frac{\text{Amount of entrapped D (mg)}}{\text{Theoretical amount of D (mg)}} \right] \times 100$$

[26, 27]

Determinations were performed in triplicate and the mean values ± S.D. were calculated.

2.2.3.3. **Particle size (PS), polydispersity index (PDI) and zeta potential (ZP)**

The mean PS, PDI and ZP were determined by Zetasizer (Malvern Instrument Ltd., Worcestershire, England) at 25 °C. Before measurements, formulations were properly diluted to assure that the dispersions were translucent and have a suitable scattering intensity [28]. PDI was measured to determine the PS distribution. ZP was measured to evaluate the physical stability of the prepared formulations. All measurements were done in triplicate and the mean values ± S.D. were reported.

2.2.3.4. **Drug payload (PL)**

PL was measured in order to determine the ability of the prepared systems to maintain the drug dissolved within the formed nanocarrier systems. The prepared nanovesicles were stored in refrigerator (at 4 °C) for one week, then one
mL of each of the nanocarrier formulations was filtered through 0.45 µm syringe filter, then 200 µL of each filtrate was dissolved in 6 mL methyl alcohol. The amount of drug was determined spectrophotometrically and calculated using the following equation:

$$PL = \left( \frac{\text{Amount of D in filtrate (mg)}}{\text{Theoretical amount of D (mg)}} \right) \times 100$$

Determinations were done in triplicate and the mean values ± S.D. were calculated.

2.2.4. Preparation of instantly dispersible nanocarrier powder systems (IDNPs) of the optimized and selected formulations

An accurate volume of each of the optimized and selected D-loaded nanocarrier systems (each equivalent to 20 mg D) were prepared. Each formulation was mixed with 5% (w/v) mannitol in a glass vial or an amount of Aeroperl® (equal in weight to the solid content of the liquid dispersion, in a ratio 1:1 w/w) in another glass vial. The samples were frozen at -22 °C for 24 h, and then placed in the lyophilizer with a condenser temperature of -45 °C and a pressure of $7 \times 10^{-2}$ mbar for 24 h. The produced samples were kept in tightly closed containers in desiccators over calcium chloride at room temperature until further use.

2.2.5. Ex-vivo permeation through rabbit’s intestine

An exact amount of each of the prepared freeze-dried optimized nanocarrier powder (IDNPs) and the selected lyophilized nanocarrier powder (both, contains Aeroperl® as a drug carrier, and each is equivalent to 20 mg D) were separately weighed and reconstituted with 7 mL distilled water, and placed in a freshly separated rabbit’s intestine (15 cm in length) tighed from both ends. The intestine was placed in 50 mL distilled water contained in glass bottles. Bottles were mounted in a shaking water bath adjusted at 37 ± 2 °C and 10 rpm. Four mL samples were withdrawn at different time intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12 and 24 h) and replaced with fresh distilled water in order to maintain the
original volume. Samples were filtered and assayed for drug content by a validated HPLC method, using a mobile phase composed of 50 % acetonitrile : 50 % 0.05 M phosphate buffer (pH 5). The detection wavelength and the flow rate were 292 nm and 1.5 mL/min, respectively. The % D permeated from each formulation was calculated and plotted versus time.

2.2.6. Transmission electron microscopic (TEM) analysis of the optimized D-loaded nanocarrier system

The particle morphology of the optimized formulation was observed using TEM analysis (JEOL (JEM-2100), Tokyo, Japan). One drop of the diluted optimized D-loaded nanocarrier system was placed on a carbon-coated copper grid and negatively stained with one drop of 2% aqueous solution of phosphotungstic acid [3] which was left to dry for 30 min. at room temperature. The grid was finally examined under the microscope.

2.2.7. In-vivo studies

The study was carried out to determine the pharmacokinetics of D after intranasal delivery from the instantly dispersible nanocarrier powder system (IDNPs) of the optimized formulation and the oral delivery of the optimized D-loaded nanocarrier liquid formulation (each contains 4 mg D) following their administration to rabbits. Eight male New Zealand white rabbits, weighing 2.5 ± 0.5 kg were housed individually in stainless steel cages and had free access to food and water. The animals were conscious throughout the experiment and were held in rabbit restrainers during blood sampling. The experimental procedures and the in-vivo study protocol was reviewed and approved by the ethical principles of the Research Ethics Committee (REC) at the Faculty of Pharmacy, Cairo University, Egypt (REC number is PI (695)).
2.2.7.1. Study design and sample collection

The study was conducted according to a parallel design. Rabbits were randomly divided into two groups, each composed of 4 rabbits. The IDNPs of the optimized formulation (equivalent to 20 mg D) was reconstituted with 10 mL phosphate buffer (pH 6.8) immediately before intranasal administration. Two mL equivalent to 4 mg D were intranasally administered to the first group where, one mL was administered to each nostril of each rabbit’s nose. The second group received 2 mL the optimized D-loaded nanocarrier liquid formulation (equivalent to 4 mg D) through the oral route [8]. The nasal dose was calculated by multiplying the human dose (mg/Kg) by correction factor (3.1) [29]. Blood samples (3 mL) were collected from the retro-orbital plexus of rabbits by a well trained practitioner where, single sample was obtained from each individual eye. The samples were collected into heparinized tubes at different time intervals after administration of each treatment. Plasma was immediately separated from the blood by centrifugation at 4000 rpm for 15 min. at room temperature, directly transferred into eppendorf tubes and stored frozen at -20 °C until drug analysis.

2.2.7.2. Development of UPLC–MS/MS chromatographic method of analysis

A volume of 50 mL of internal standard, IS (Torsemide 2 mg/mL) was added to 250 mL rabbit plasma. The samples were vortexed (Vortex mixer, VSM-3 model, PRO Scientific Inc., Oxford, England) for 30 s, precipitation technique was applied by adding 0.5 mL acetonitrile, followed by vortex for one min., then samples were centrifuged (at 3000 rpm) for 10 min. A volume of 1 mL from the clean supernatant was injected into UPLC–MS/MS system. Concentrations of D in plasma samples were calculated by referring to the prepared calibration curve.
2.2.7.3. Chromatographic conditions

Plasma samples were analyzed for D using a sensitive, selective and accurate UPLC–MS/MS method. Quantitative analysis was performed on a Waters Acquity UPLC H-Class-Xevo TQD system (MA, USA) interfaced with a Waters Quattro Premier XE triple quadrupole mass spectrometer and equipped with electrospray ionization operated in the positive ionization mode. Chromatographic separation of analytes was carried out on ACQuity UPLC HSS C18 (50 × 2.1 mm, 1.7 µm) column. The isocratic mobile phase composed of acetonitrile-0.1% formic acid (35: 65, v/v) and was delivered at a flow rate of 0.35 mL/min. The column was maintained at 35 °C and the pressure of the system was 6500 psi. The source dependent parameters maintained for both the analyze and IS were; cone gas flow, 50 L/h; desolvation gas flow, 800 L/h; capillary voltage, 3.5 kV, source temperature, 120 °C; desolvation temperature, 350 °C. The optimum values for compound dependent parameters like cone voltage and collision energy were set at 30 V and 14 eV for D and 30 V and 18 eV for IS, respectively. The mass transition ion pair, performed in the multiple-reaction monitoring (MRM) mode, of m/z 306.23 → 216.17 was followed for D and m/z 349.14 → 264.10 for IS. Mass Lynx software version 4.1 was used to control all parameters of UPLC and MS. The lower and upper limits of quantification of D in plasma were 1–2500 ng/mL.

2.2.7.4. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using program Kinetica® (version 5, Thermo Fischer Scientific, NY, USA). The maximum plasma concentration (C\text{max}, ng/mL) and the corresponding time to reach C\text{max} (T\text{max}, h) were obtained from the individual plasma concentration-time curves. The area under the curve AUC\text{0–24} (ng.h/mL) determined as the area under the plasma concentration-time curve was calculated using the linear trapezoidal rule, up to the last measured sampling time. The t\text{1/2} (h) was calculated as 0.693/K. The pharmacokinetic data obtained for each intranasal delivery were compared
with that obtained from oral treatment and analyzed for statistical significance by one-way analysis of variance (ANOVA) adopting Kinetica® software.

2.2.7.5. Statistical analysis

The data obtained from different tests were analyzed for statistical significance by one-way analysis of variance (ANOVA) adopting SPSS statistics program (version 16, SPSS Inc., Chicago, USA) followed by post hoc multiple comparisons using the least square difference (LSD). Differences were considered to be significant at p < 0.05.

3. Results and discussion

3.1. Statistical analysis

3.1.1. Effect of formulation variables on EE %

The EE % of the prepared D-loaded nanocarrier systems ranged from 19.42 % ± 0.71 to 82.70 % ± 1.06 (Table 1). Fig. (1a) shows the response contour plot illustrating the effect of varying concentrations of the different components on the EE % of drug in D-loaded nanocarrier systems (Y1). ANOVA results revealed that the terms X1X3 and X2X3 have a significant effect on decreasing the EE % of D.

It has been inferred that nanocarrier systems containing the highest amount of PC and lowest amount of Tween® 80 and BPM had the highest EE% (Fig. 1a). This may be due to the inclusion of BPM having high molecular weight (5750 Da for P123 and 12600 Da for F127), which was found to affect the EE % as it may disturb the integrity of the membrane bilayer of the formed nanovesicles resulting in an increased diffusion of D to the hydrating medium during separation of the nanovesicles by centrifugation[30]. In addition, nanovesicles produced from surfactants having longer alkyl chains had a higher EE %[31]. As previously reported, the longer the alkyl chain of the surfactant, the lower the HLB, and the higher the drug EE% [32]. This explains the lowest EE % displayed in F2, which
could be due to incorporating the highest concentration of the BPM with high HLB values together with moderate concentration of Tween® 80 (HLB = 15)[33].

It is worthy to note that nanovesicles lacking Tween® 80 had the ability to entrap 50 - 80 % of D inside the formed nanovesicles, as shown in formulations F3, F7 and F12. On the other hand, incorporation of Tween® 80 decreases the EE % of drug in the formulations. This may be due to its surfactant properties and its ability to solubilize the drug outside the formed nanovesicles through micellar solubilization and hence, only un-solubilized drug is left to be entrapped inside the nanovesicles. In addition, the molecular structure of Tween® 80 contributes to lowering the EE %. This is attributed to the presence of unsaturated alkyl chains (C_{18}) with double bond in the structure of Tween® 80 which leads to the formation of vesicles having a more permeable membrane bilayer that is not sufficiently tight due to chain bending; thus lowering EE %[31, 32].

3.1.2. Effect of formulation variables on PS, PDI and ZP

PS results of the prepared D-loaded nanocarrier formulations are presented in table 1 as z-average diameter. Das et al defined the z-average diameter as the mean hydrodynamic diameter of particles[34]. The mean particle diameters ranged from 383.8 nm ± 4.62 to 759.0 nm ± 16.25. ANOVA results showed that none of the tested formulation factors had a significant effect on the PS (p = 0.1915). Fig.(1b) shows the contour diagram illustrating the effect of different concentrations of X₁, X₂ and X₃ on the PS of the prepared nanocarriers. It is obvious that there is an optimum ratio of Tween for obtaining a nanocarrier system having small PS. In a study by Basha et al, they confirmed that the use of edge activators such as Tween® 80 produced more spherical vesicles, having low aggregation tendency and consequently smaller particle size[35]. Although the effect of Tween® 80 in our study appears to be statistically insignificant, it was noticed that the larger PS was obtained in formulations without Tween® 80. As Tween® 80 concentration increases, the PS decreases. This is due to the lowering
in the interfacial tension with increased Tween® 80 concentration, leading to formation of smaller nanovesicles[36]. Moreover, the decreased PS in the presence of high levels of Tween® 80 may be attributed to the formation of mixed micelles which are smaller in size than the formed nanovesicles[35, 37].

PDI is a dimensionless number that expresses the PS distribution of systems under investigation. As the PDI value gets closer to zero, it indicates a more homogeneous particle size distribution. The values of PDI of the prepared formulations are demonstrated in table 1. PDI values for all formulations ranged from $0.204 \pm 0.16$ to $0.795 \pm 0.06$, indicating that all formulations were homogeneous except some formulations whose PDI values were above 0.6 [38]. The effect of different concentrations of $X_1$, $X_2$ and $X_3$ on the PDI is shown in Fig. (1c). ANOVA results revealed that the sole effect of different formulation variables on the PDI of the prepared nanocarrier systems was insignificant. However, it shows a significant, synergistic effect of the three independent variables together ($p = 0.0192$). Hence, an optimum ratio of all the components for obtaining homogenous nanocarrier systems having small PDI is required.

ZP is the measure of the overall charge acquired by nanovesicles and it can be used to evaluate the stability of colloidal dispersions. Generally, the system is considered stable when the ZP value is above± 25 mV due to electrical repulsion between particles [39]. ZP values for the prepared D-loaded nanocarrier systems are presented in table 1. Results show that all of the prepared formulations exhibited acceptable ZP values ranging from $-48.1 \pm 0.92$ to $-13.7 \pm 1.06$ mV which may indicate the good stability of the prepared D-loaded nanocarrier systems. Inclusion of PC in all formulations imparts a negative charge thereby, increasing the stability of formulations by preventing aggregation of the formed nanovesicles[40]. ANOVA results revealed that all of the formulation variables had a significant effect on ZP values ($p< 0.0001$). A contour plot illustrating the effect of different concentrations of $X_1$, $X_2$ and $X_3$ on ZP [41] is shown in Fig. (1d), and
reveals that the nanocarriers having the highest concentration of PC had the highest values of negative ZP.

3.1.3. Effect on PL

PL is considered a critical parameter that is used to evaluate the physical stability of formulations. As shown in table 1, PL values (measured after one week storage in the refrigerator, at 4 °C) ranged from 29.59 % ± 1.6 to 99.90 % ± 1.8. ANOVA results showed that drug PL was significantly affected by the three formulation variables \( p = 0.0005 \). Also, it is significantly influenced by \( X_1X_3 \) \( p = 0.0241 \) as well as \( X_2X_3 \) \( p = 0.0172 \). Fig. 1e shows the contour plot illustrating the effect of different concentrations of \( X_1, X_2 \) and \( X_3 \) on the PL (%). The lowest PL was observed in nanocarrier systems having the lowest amount of Tween® 80, owing to lower solubilization capacity of D It is obvious that there are two regions of all the components for obtaining a nanocarrier system having the highest drug PL, one contains high Tween® 80 content and the other contains lower levels of BPM content. During storage, Tween® 80 and BPM were able to maintain the drug solubility owing to their high solubilizing capacities, where more drug is allowed to solubilize in the formed nanocarrier systems [42].

3.2. Formulation optimization

Optimization of pharmaceutical formulations generally aims to determine the levels of variables from which a robust product with high quality characteristics may be produced[25]. Thus, pinpointing the variables that might affect the properties of a new drug delivery system is essential. In our study, the effects of formulation variables on the EE %, PS, PDI, ZP and PL were investigated. D-optimal mixture experimental design was selected since it minimizes the variance associated with the estimates of the coefficients in the model[43]. In order to produce a product of the desired characteristics, some of the measured responses have to be minimized and others have to be maximized. The optimized formulation with the desired characteristics was chosen based on
desirability calculations aiming to accomplish high EE % ( > 50 %) and PL ( > 70 %), the value of ZP ( < -25 mV) together with small PS ( < 750 nm) and low PDI ( < 0.5). The desirability function crews all the responses into one variable to anticipate the optimum levels of the investigated factors[44]. The optimized formulation (OF-I) was then selected, prepared and evaluated in triplicate in order to assure the validity of the measured responses given by the software and then, it was subjected to further investigations. The components of OF-I are: 60.00 % BPM, 35.13 % PC and 4.86 % Tween® 80. Results revealed the high similarity between the observed and the predicted values of the optimized D-loaded nanocarrier formulation, where the optimized formulation OF-I showed EE% 63.91, PS of 501.75, PDI of 0.42, ZP of -37.5, and PL of 70.98%.

In order to evaluate the role of Tween® 80 in enhancing drug permeation, another formulation prepared without Tween® 80 (F7) was selected for comparison. F7 is composed of: 60 % BPM, 40% PC. The selected formulation was prepared and evaluated for the different formulation variables and showed an EE% of 79.90 % ± 3.96, with a PS of 523.95 nm ± 28.00, PDI of 0.31 ± 0.16, ZP of - 42.15 mV ± 8.40 and PL of 31.08 % ± 2.11. Although discarding Tween® 80 in F7 led to enhanced drug entrapment, as well as accepted values of each of PS, PDI and ZP yet, the observed PL value was very low compared to the desired target ( > 70 %). This assures that Tween® 80 incorporation is essential, in a certain concentration, to maintain the solubility of D within the formed nanovesicles upon storage, and confirms the high solubilization capacity of Tween® 80 [42].

3.3. Preparation of instantly dispersible nanocarrier powder system (IDNPs) of the optimized and the selected formulations

The optimized formulation (OF-I) and the selected formulation (F7) were lyophilized in the presence of 5% (w/v) mannitol or Aeroperl® (in the ratio 1:1 w/w). The produced freeze-dried samples using 5% (w/v) mannitol (as a carrier)
were completely dry but had a small degree of stickiness to the formulations due to the presence of Tween. On the other hand, the freeze-dried formulations mixed with Aeroperl® (as a carrier) were free flowing, non-sticky and easily reconstituted due to the high absorption capacity of Aeroperl that can overcome the stickiness imparted by Tween. Thus, the lyophilized OF-I and F7 formulations using Aeroperl® were selected for further evaluation via ex-vivo permeation studies using rabbit’s intestine.

3.4. *Ex-vivo permeation through rabbit’s intestine*

The cumulative amount of D permeated through rabbit’s intestine (µg/mL) as a function of time from the instantly dispersible nanocarrier powder systems of the optimized formulation (OF-I) and the selected formulation (F7) are illustrated in fig. 2. F7 (lacking Tween® 80) showed very slow permeation compared to OF-I (containing 4.86 % Tween® 80). Owing to its surfactant nature and its biological membrane enhancing activity, Tween® 80 is expected to enhance D biological permeation and solubility and consequently, its bioavailability [45]. Permeation results conforms well with PL assessment where, the increase in membrane permeability could be attributed to the ability of the surfactant molecules to solubilize the drug and enhance the membrane’s penetration [46].

3.5. *Transmission electron microscopic (TEM) analysis of the optimized formulation*

Fig. 3 shows the TEM micrograph of the optimized D-loaded nanocarrier formulation (OF-I). It shows well-identified spherical nanovesicles with size range 300 – 700 nm. Results are in good agreement with the results of PS measurements.

3.6. *In-vivo pharmacokinetic study*

The mean pharmacokinetic parameters are presented in table 2 (with their means and standard deviations) after single intranasal administration of the reconstituted instantly dispersible nanocarrier powder system (IDNPs) compared
to single oral administration of the optimized D-loaded nanocarrier liquid formulation (each equivalent to 4 mg D) to rabbits.

The mean plasma concentration time curves are shown in fig. 4. The mean $C_{\text{max}}$ estimate after intranasal and oral nanocarriers were 320.455 ± 85.610 ng/mL and 212.372 ± 153.047 ng/mL, respectively, with no statistical significant observed ($p = 0.2002$). Also, no significant difference was observed between the time required to reach the peak plasma concentration ($T_{\text{max}}$) after oral and intranasal administration of the nanocarrier formulations ($p = 0.2070$). Statistically significant difference ($p = 0.0143$) was found between AUC$_{0-24}$ determined after intranasal and oral administration of nanocarrier formulations. The mean half-life ($t_{1/2}$) was found to have insignificant difference ($p = 0.0940$) for the oral and the intranasal routes. This is consistent with the pharmacokinetic theory, where the increase in absorption should not affect drug elimination [47][47][45].

The relative bioavailability of D after intranasal and oral administration of the nanocarrier formulations was about 235.41%. These observations confirm that oral administration of D in nanovesicles formulation, is not efficient to improve its rate and extent of absorption compared to the intranasal administration of the corresponding IDNPs. This could be attributed to the degradation of the phospholipid based nanocarrier formulations in the GIT upon oral ingestion followed by first pass degradation of D in the liver. On the other hand, the slightly higher value of $C_{\text{max}}$ as well as the significantly higher value of AUC$_{0-24}$ following the intranasal administration of the nanocarriers compared to that of the oral route confirms the enhanced absorption of drug from the intranasal route due to the enhancement of drug dissolution in the neutral pH of body fluids and the avoidance of hepatic metabolism.
4. Conclusion

In this study, D-loaded IDNPs were prepared by thin film hydration technique followed by freeze-drying. IDNPs could be a successful approach in order to improve the bioavailability of drugs having limited solubility in neutral pH of body fluids and suffering from oral first pass effect. The bioenhanced properties of the components in the developed formulation together with the high ability to permeate the nasal mucosa are the main reasons for bioavailability enhancement. The developed IDNPs for intranasal administration exhibited improved bioavailability of D and did reduce the therapeutic dose of the drug and consequently the side effects caused by the large dose of the drug. Further clinical studies with a large number of human volunteers should be performed to prove the clinical efficacy of the IDNPs in treatment of premature ejaculation.

Declaration of interest

The authors have no declaration of interest.

References


Fig. 1. Contour plots showing the effect of different formulation variables on the dependent variables.
Fig. 2. *Ex-vivo* permeation profiles of the optimized formulation (OF-I) and a selected formulation (F7) through rabbit’s intestine.
Fig. 3. Transmission electron micrograph of the optimized D-loaded nanocarrier formulation (OF-I).
Fig. 4. Mean plasma D concentration versus time curves after single intranasal administration of the reconstituted IDNPs and single oral administration of the corresponding optimized liquid nanovesicles (each is equivalent to 4 mg D) to rabbits.
Table 1: Experimental runs, formulation variables and measured responses of the D-Optimal mixture experimental design.

<table>
<thead>
<tr>
<th>Runs</th>
<th>Independent variables</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁: BPM (%)</td>
<td>X₂: PC (%)</td>
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<tr>
<td>F1</td>
<td>69.83</td>
<td>10.17</td>
</tr>
<tr>
<td>F2</td>
<td>77.02</td>
<td>10.00</td>
</tr>
<tr>
<td>F3</td>
<td>73.92</td>
<td>26.08</td>
</tr>
<tr>
<td>F4</td>
<td>70.33</td>
<td>16.39</td>
</tr>
<tr>
<td>F5</td>
<td>60.00</td>
<td>20.84</td>
</tr>
<tr>
<td>F6</td>
<td>67.07</td>
<td>30.01</td>
</tr>
<tr>
<td>F7</td>
<td>60.00</td>
<td>40.00</td>
</tr>
<tr>
<td>F8</td>
<td>69.83</td>
<td>10.17</td>
</tr>
<tr>
<td>F9</td>
<td>80.00</td>
<td>15.08</td>
</tr>
<tr>
<td>F10</td>
<td>73.92</td>
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<td>F14</td>
<td>73.63</td>
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<tr>
<td>F15</td>
<td>80.00</td>
<td>15.08</td>
</tr>
<tr>
<td>F16</td>
<td>66.71</td>
<td>23.80</td>
</tr>
</tbody>
</table>

Each formulation contained the same amount of drug (20 mg) and the same Pluronics ratio F127: P123 (1:2) w/w (contained in the BPM).

Total drug content in each of these formulations ≈ 100 %

Data are mean values ± S.D.
Table 2: Mean pharmacokinetic parameters of D following single intranasal administration of the reconstituted IDNP and single oral administration of the optimized liquid nanovesicles (each equivalent to 4 mg D) to rabbits

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Intranasal IDNP*</th>
<th>Oral nanovesicles*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>320.455 ± 85.610</td>
<td>212.372 ± 153.047</td>
<td>0.2002</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.438 ± 0.125</td>
<td>0.313 ± 0.125</td>
<td>0.2070</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (ng.h/mL)</td>
<td>703.76 ± 268.96</td>
<td>298.94 ± 72.73</td>
<td>0.0143</td>
</tr>
<tr>
<td>$T_{\text{half}}$ (h)</td>
<td>9.67 ± 2.61</td>
<td>6.83 ± 2.55</td>
<td>0.0940</td>
</tr>
</tbody>
</table>

*Data are mean values (n = 4) ± S.D.