Formulation and Evaluation of Rosuvastatin Calcium Polymeric Nanoparticles-Loaded Transdermal Patch

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ABSTRACT

Background: Transdermal drug delivery systems (TDDS) have been rapidly developed as a promising alternative to the oral delivery systems to provide controlled drug delivery and avoid the first-pass metabolism of drugs. Recently, various nanocarriers such as liposome and lipid nanoparticles have been used to enhance dermal and transdermal penetration of drugs. The aim of this study is to formulate and evaluate a polymeric nanoparticle (PNPs)-loaded transdermal patch of the antihyperlipidemic drug rosuvastatin.

Materials and Methods: Rosuvastatin PNPs were prepared by nanoprecipitation method, and evaluated for particle size, zeta-potential and polydispersity index. PNPs-loaded patch and PNPs-free patch (control) of rosuvastatin were prepared by solvent casting method using hydroxyl polymethyl cellulose K15 (HPMC K 15) as film-forming polymer and polyethylene glycol 200 (PEG 200) as a plasticizer and evaluated for physical appearance, thickness, folding endurance, surface pH, *in-vitro* release and *ex-vivo* permeability.

Results: The particle size, zeta-potential, and polydispersity index of rosuvastatin-loaded PNPs were 68.69 nm, - 7.5 mV, and 0.294, respectively. The prepared transdermal films showed acceptable appearance, thickness, and folding endurance as well as surface pH values. *In vitro* release study showed 65% and 82% cumulative release after 24 hrs for the polymeric nanoparticle-loaded patch and control patch, respectively. However. *ex vivo* permeation results showed that a significantly higher amount of rosuvastatin permeated via rat skin from the PNPs-loaded patch in comparison to the control patch (P-value < 0.05).

Conclusion: Rosuvastatin PNPs-loaded transdermal patch was successfully prepared and revealed promising releasing and permeation properties comparing to control patch.

Keywords: Polymeric nanoparticles, rosuvastatin, Eudragit L100, transdermal drug delivery, nanoprecipitation.

الخلفية: تم تطوير أنظمة توصيل الأدوية عبر الجلد (TDDS) بسرعة كبديل واعد لأنظمة توصيل الأدوية عن طريق الفم لتوفير توصيل مسيطر عليه للأدوية وتجنب التمثيل الغذائي التمريري الأول للعقاقير. في الآونة الأخيرة ، تم استخدام ناقلات نانوية مختلفة مثل الجسيمات النانوية الدهنية لتعزيز اختراق الأدوية للجلد. الهدف من هذه الدراسة هو صياغة وتقييم رقعة عبر الجلد محملة بالجسيمات النانوية البوليمرية (PNPs) لعقار روزوفاستاتين المضاد لفرط دهون الدم.

المواد وطرق العمل: تم تحضير PNPs للروزوفاستاتين بطريقة الترسيب النانوي ، وتم تقييمها لحجم الجسيمات ، وجهد زيتا ، ومؤشر التشتت المتعدد. تم تحضير رقعة محملة بـ PNPs والرقعة الخالية من PNPs (للمقارنة) من الروزوفاستاتين بطريقة الصب باستخدام هيدروكسيل بولي ميثيل السليلوز (HPMC K 15) كبوليمر مكون للفيلم وبولي إيثيلين جلايكول 200 (PEG 200) كمادة تضيف مرونة للصيغ وتقييمها من الناحية الفيزيائية، المظهر ، السماكة ، التحمل القابل للطي ، الأس الهيدروجيني السطحي ، التحرر المختبري والنفاذية خارج الجسم الحي.

النتائج: كان حجم الجسيمات ، وجهد زيتا ، ومؤشر التشتت المتعدد لـ PNPs المحملة بالروزوفاستاتين 68.69 نانومتر ، - 7.5 مللي فولت ، و 0.294، على التوالي. أظهرت الأغشية عبر الجلد المحضرة مظهر وسماكة وتحمل مقبول بالإضافة إلى قيم pH السطحية مقبولة. أظهرت دراسة التحرر المختبري إطلاقًا تراكميًا بنسبة 65٪ و 82٪ بعد 24 ساعة للرقعة البوليمرية المحملة بالجسيمات النانوية ورقعة المقارنة، على التوالي. أظهرت نتائج التخلل خارج الجسم الحي أن كمية أعلى بكثير من الروزوفاستاتين تخللت عبر جلد الجرذان من الرقعة المحمّلة بـ PNPs مقارنةً بالرقعة الخالية من PNPs .(P-value < 0.05)

الخلاصة: تم تحضير الرقعة عبر الجلد المحمّلة بالروزوفاستاتين PNPs بنجاح وكشفت عن خصائص تحرر مختبري ونفاذية واعدة مقارنة بالرقعة الخالية من PNPs.

الكلمات المفتاحية: الجسيمات النانوية البوليمرية ، روزوفاستاتين ، أيدراجيت ال100 ، توصيل الأدوية عبر الجلد ، الترسيب النانوي.

INTRODUCTION

Dyslipidemia or hyperlipidemia is a condition associated with the elevation of total cholesterol; low–density lipoprotein (LDL) cholesterol or triglyceride and low high-density lipoprotein (HDL) cholesterol or a combination of these abnormalities[1]. Stain is a group of antihyperlipidemic drugs that is effective in lowering cholesterol levels. Rosuvastatin is a fully synthetic new generation of HMG CoA reductase inhibitors[2,3], its chemical structure is shown in **figure 1**. It acts by reversible blocking of HMG CoA reductase which is the rate-limiting step in the cholesterol synthesis pathway[4]. Rosuvastatin is characterized by poor bioavailability with only 20% bioavailable after oral intake due to the first-pass effect by the liver[2]. Recently, researchers have focused to afford alternative means to oral drug delivery. Transdermal drug delivery offers a non-invasive alternative way for drug administration, bypassing the first-pass effect of gastrointestinal tract and provide sustained drug release for extended periods of time which makes the TDDS possesses superior advantages over other routes of drug delivery[5]. In this context, rosuvastatin could be a good candidate for the transdermal drug delivery system (TDDS). However, the main barrier for drug penetration through the skin is the stratum corneum layer of the skin[6]. To overcome this limitation of the TDDS, several physical and chemical strategies have been suggested[7]. Recently, nanocarriers have gained a great attention in medicine[8] and specially to enhance tissue/transdermal drug penetration for several reasons[9]. Unlike physical strategies, nanocarriers can passively enhance skin penetration without making a change in skin structure. Therefore, nanocarriers have a superiority over the physical strategies which involves the active enhancement of skin permeation by the application of an external stimulus which may cause skin irritation [9,10] or damage[11,12]. Polymeric nanoparticles (PNPs), size ranging from 10 nm to 1000 nm, have been widely explored in topical application due to their relatively small size. They possess many advantages over the other nanocarriers (e.g. lipid-based nanoacrriers) including: higher drug and carrier stability, enhancement of drugs payload, better control over drug release in case of sustained or controlled release of drugs[13]. In 2020, rosuvastatin calcium was successfully formulated as a transdermal patch by S.Satya *et al*[14]. However, the possibility of formulating

rosuvastatin as polymeric nanoparticles for transdermal delivery has not yet been discussed in the scientific research.

This study aims to prepare rosuvastatin Eudragit L100 PNPs-loaded transdermal patch. To achieve this aim rosuvastatin Eudragit L100 PNPs were prepared and loaded into transdermal film. Finally, drug release profile and *ex vivo* enhancement of transdermal rosuvastatin permeation were investigated.
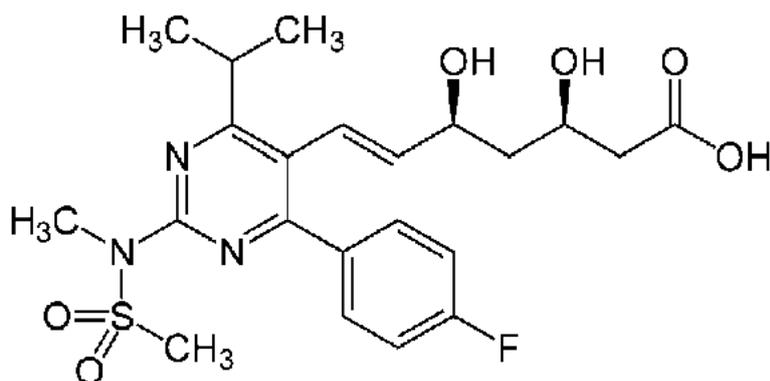

Figure 1: The chemical structure of rosuvastatin [3] .

MATERIALS AND METHODS

Materials
Rosuvastatin calcium powder was obtained from the Awa Medica factory, Erbil, Iraq. Eudragit L100 and poloxamer 188 were purchased from Hangzhou Hyper Chemicals, China. N,N-Dimethylformamide (DMF) was purchased from Sigma Aldrich, the U.S.A, and absolute ethanol was from Scharlab S.L., Spain. HPMC K15 was from Alpha Chemika, India and PEG 200 was from Riedel-de Haën, Germany. All other ingredients used were of analytical grade.

Methods

Determination of the melting point
The melting point of rosuvastatin was determined by the open capillary method[15]. The capillary tube was sealed from one side, dipped in rosuvastatin powder several times, with gentle tapping to get the powder down the tube. Thereafter, it was inserted into the melting point apparatus and a temperature being increased gradually. The temperature at which the powder was converted to liquid was considered as the melting point.

Rosuvastatin calcium calibration curve
The calibration curve of rosuvastatin calcium was constructed in phosphate saline buffer (PBS), pH 7.4. The absorbance of solutions with different rosuvastatin calcium concentrations (0.5, 1, 2, 4, 6, 8, 10, 12, 14, and 18 μg/ml) was measured using spectrophotometer at λ_{max} (242 nm). The absorbance was then plotted as a function of rosuvastatin calcium concentration to establish a calibration curve.

Solubility determination
Solubility of rosuvastatin calcium in PBS was determined. An excess amount of rosuvastatin calcium was added to 5 ml of PBS. The resulting solution was sonicated for 15 minutes to assist in the solubilization of rosuvastatin calcium.

After that, the solution was agitated in a shaking water bath at a temperature maintained at 37°C for 24 hrs to achieve equilibrium. The resultant solution was centrifuged at 4000 rpm for 15 minutes. The supernatant was taken, diluted suitably with PBS and the dissolved amount of the drug was determined by UV-visible spectrophotometer at λmax (242 nm). The experiment was performed in triplicate[16].

Preparation of rosuvastatin calcium PNPs

Preparation of rosuvastatin calcium PNPs was performed by the nanoprecipitation method that described by Fessi *et al.*[17]. rosuvastatin calcium 20 mg was dissolved in 1 ml DMF and Eudragit L100 60 mg was dissolved in 4 ml of ethanol. They mixed together to form the organic phase. Thereafter, the organic phase was added by slow, dropwise addition using a syringe into the aqueous phase which is 20 ml of distilled water contains (1% w/v) of poloxamer 188 as a stabilizer under magnetic stirring at 1100 rpm and the stirring was continued for 1 hr to ensure the evaporation of the organic phase.

Determination of particle size, zeta potential and polydispersity index

Particle size distribution, mean diameters, and polydispersity index were measured using the Dynamic Light scattering (DLS) technique by particle size analyzer (Brookhaven instruments, USA) at 25 °C. The measurement was performed after dilution by the addition of 3 drops of the dispersed PNPs into 20 ml 1mM KNO_3.

Preparation of rosuvastatin calcium transdermal patch

Rosuvastatin calcium PNPs-loaded transdermal patch was prepared by solvent casting/evaporation technique[18]. The patch was prepared by adding 300 mg of HPMC K15 polymer to 11 ml of the dispersed rosuvastatin calcium Eudragit L100 PNPs and mixed by magnetic stirrer at 1100 rpm for 2 h. After that,

polyethylene glycol 200 (PEG) was added as a plasticizer at a final concentration of 0.6% and mixing was continued for 1 h. The mixture was left overnight to remove air bubbles, and poured in the next day on a glass Petri dish, 9.5 cm in diameter, and dried in an oven at 40°C for 2 h. The yielded film was wrapped in aluminum foil and kept in the fridge for further studies. In addition, a rosuvastatin calcium-loaded patch (PNPs-free control) was also prepared as described above, except rosuvastatin calcium aqueous solution was used instead of rosuvastatin calcium PNPs dispersion. The aqueous solution of rosuvastatin calcium was prepared by dissolving 10 mg of rosuvastatin calcium in 11 ml distilled water and sonicated for 30 minutes at 35°C.

Evaluation of the patch

Physical appearance
The prepared patches were inspected visually for color, homogeneity, surface, and transparency[19].

Thickness measurement
Each patch was divided into 2 cm² pieces were randomly selected from the control and PNPs-loaded patches for thickness measurement. The thickness was measured by a micrometer caliper[20].

Folding endurance
In order to provide an idea about film flexibility, a folding endurance test was performed. A manual repetitive folding of the selected patches was performed, at the same place, until it broke. The number of times that the foldable films did not break was recorded as folding endurance value[21]. The experiment was performed in triplicate.

Surface pH
In 10 ml distilled water, three randomly selected films from each patch (2 cm²) were added for one hr. After that pH was measured using a digital pH meter, this

experiment was done in three replicates and the results were presented as mean ± SD[22].

In vitro release study

In vitro release study of rosuvastatin calcium from the control and PNPs-loaded patches was performed using paddle-over disk method in dissolution apparatus (USP type II)[23]. The patches (2 cm²) were pasted on a glass slide using double-sided adhesive tape and immersed at the bottom of the vessel which contains 100 ml of PBS release medium. The temperature was 37 °C and paddle rotation was at 50 rpm. Aliquots of 4 ml were collected at intervals of 0.5, 1, 2, 3, 4, 6, 8, and 24 hrs. Thereafter, absorbance was measured using a spectrophotometer at 242 nm. The experiment was performed in triplicate.

Kinetic modeling of the drug release

The resultant release data were fitted into four mathematical models in order to determine the mechanism of drug release. Zero order model was fitted by plotting the linear relation between the cumulative percentage of the drug released at time t versus time (Q versus time). First-order kinetic model was fitted by plotting the linear relation between the log of cumulative percentage of the drug remaining after a time t versus time (log Q_0-Q versus time), where Q_0 is the initial amount of the drug in the patch at time zero. Higuchi model was fitted by plotting the linear relation between the cumulative percentage of the drug released at time t versus the square root of time (Q versus $t^{1/2}$).

Finally, the Korsmeyer-Peppas model was performed by plotting the linear relation between the log of cumulative percentage of drug released at time t and log time (log Q versus log time). This model has an equation of ($Mt/M\infty = Kt^n$), where $Mt/M\infty$ is the fraction of drug released at the time (t) and (n) is the diffusional or drug release exponent. The n value is the slope of the linear fitting and is used to identify the exact release mechanism (type of diffusion). If n value $\leq$ 0.45, it describes Fickian diffusion; if n value 0.45 < n < 0.89, it describes non-Fickian transport; if n value= 0.89 then it describes case II transport and finally if n value > 0.89 then it describes super case II transport[24].

Ex vivo skin permeation study

Ex vivo skin permeation study of rosuvastatin calcium Eudragit PNPs-loaded patch and rosuvastatin calcium-loaded (PNPs-free control) patch was conducted using rat skin. The work was conducted with the formal approval of the Institutional Animal Care and Use Committee at the College of Veterinary Medicine, University of Mosul (Ref: UM.VET.2021.005). Male Albino rats, age (12-14 weeks) and weight (250-275 g), were obtained from the animal house of the College of Veterinary Medicine / University of Mosul. The rats were sacrificed under general anesthesia and the skin was excised by a scalpel. The hair was clipped carefully before the experiment using an electrical clipper. Thereafter, the skin was inspected for any adhering fat tissue, washed with PBS, and stored in the refrigerator to be used on the second day. The experiments were performed using dissolution apparatus (USP type II) (modified method) as shown in **figure 2**. A circular patch with an area of 2 cm² was cut and placed over the skin by using medical plaster as a backing membrane. The skin was fixed to a plastic tube using a rubber band and this system was fixed to paddle in such a way that the dermal layer faced and immersed in the medium. PBS 30 ml was used as a medium and the experiment was conducted at 37 °C and paddle rotation 50 rpm. An aliquot of 3 ml was withdrawn at 1, 2, 3, 4, 6, 8, 10 and 24 h, and replaced with fresh buffer as soon as possible. The samples were then filtered through a 0.45µm pore filter and the absorbance was measured by spectrophotometer at 242 nm.

No dilutions of samples were needed. The experiment was done in triplicate.

Permeability parameters, including cumulative amount permeated via skin after 24 h Q24 (µg/cm²), permeation flux, enhancement factor, and permeability coefficient were calculated. The permeation flux or steady-state flux (Jss) (µg/cm²/h) is the slope of the linear fitting of the curve. The enhancement factor was calculated by dividing the steady-state flux of the PNPs-loaded patch by the steady-state flux of the control patch. The permeability coefficient was calculated by dividing the steady-state flux by the concentration in the donor compartment[25].

Statistical analysis

When it is applicable, the results were expressed as mean ± SD. Statistical analysis was performed using Minitab® 18 software. Data sets were tested for statistical significance using an unpaired *t-test*. Results were considered statistically significant if $P < 0.05$.

Figure 2: The modified method (dissolution apparatus II) of *ex vivo* skin permeation study.

RESULTS AND DISCUSSION

Melting point

The melting point of rosuvastatin was 155 °C, which is closely related to that reported in many previous studies references[26,27].

Rosuvastatin calcium calibration curve

The calibration curve of rosuvastatin calcium in PBS pH 7.4 is demonstrated in **figure 3**. The calibration curve showed a linear increase in absorbance with an increase in rosuvastatin calcium concentration with a high correlation coefficient ($r^2 = 0.999$). This result indicated the suitability of the established calibration curve for determining rosuvastatin calcium concentration in PBS over the measured concentration range.
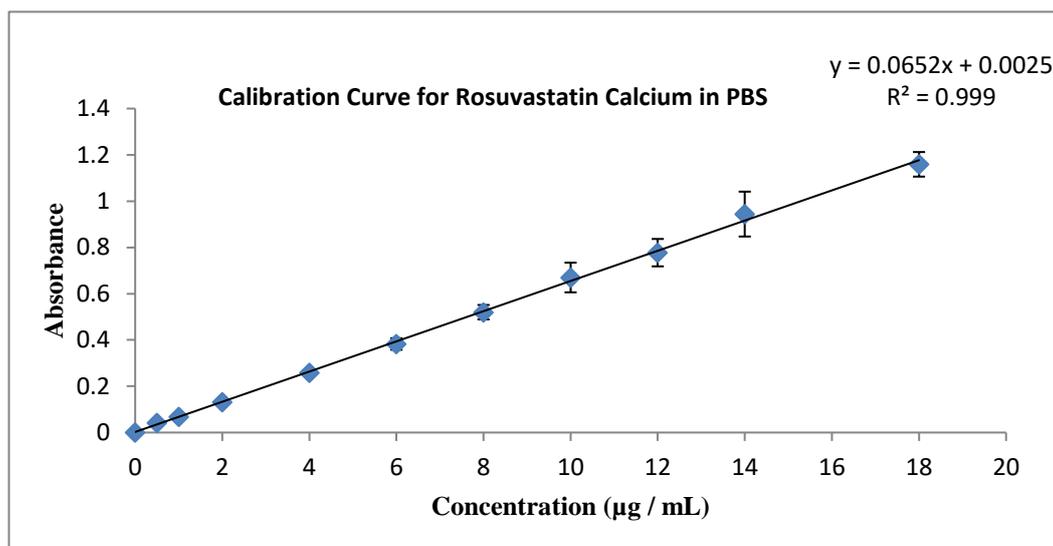
Figure 3: Calibration curve for ROC in PBS. A linear relationship between the absorbance and ROC concentration in PBS was established, r^2=0.999. Values are mean ± SD, n=3.

Solubility determination

The solubility of rosuvastatin calcium in PBS was 1.092 mg/ml. Rosuvastatin (free base) is a slightly soluble drug, its water solubility is about 18 µg/ml. Rosuvastatin is an acidic drug with a pKa value of about 4.46[26]. its solubility and ionization degree will increase with increasing pH[27]. This finding is in agreement with the results of other studies conducted by Rajput *et al.*[28] and Salih *et al.*[29]. Another study suggested 10 folds increase in rosuvastatin calcium solubility a pH 6.8[30]. This direct relationship between the solubility of the rosuvastatin calcium with the pH indicating that sink condition can be achieved in PBS pH 7.4.

Particle size, zeta-potential and polydispersity index

The formation of rosuvastatin calcium-Eudragit L100 PNPs was characterized using DLS. **Figure 4** shows the particle size distribution, and zeta-potential graphs of the prepared rosuvastatin calcium PNPs. The particle size zeta-potential and polydispersity indexes were 68.69 nm, -7.5 mV, and 0.294, respectively (**figure 4**). These results suggested the formation of small homogenous nanoparticles (less than 100 nm). Zeta potential is important to ensure the stability of the resultant dispersion. Where negative zeta potential is expected due to the presence of the anionic polymer, Eudragit L100, as a result of the appearance of free carboxyl groups at the polymer extremities[31]. In addition, the results showed acceptable polydispersity index value which is indicative of the uniformity of particle size distribution. These results were in full agreement with previous reports using nanoprecipitation method to prepare drug-loaded Eudragit L100 PNPs[31–34].
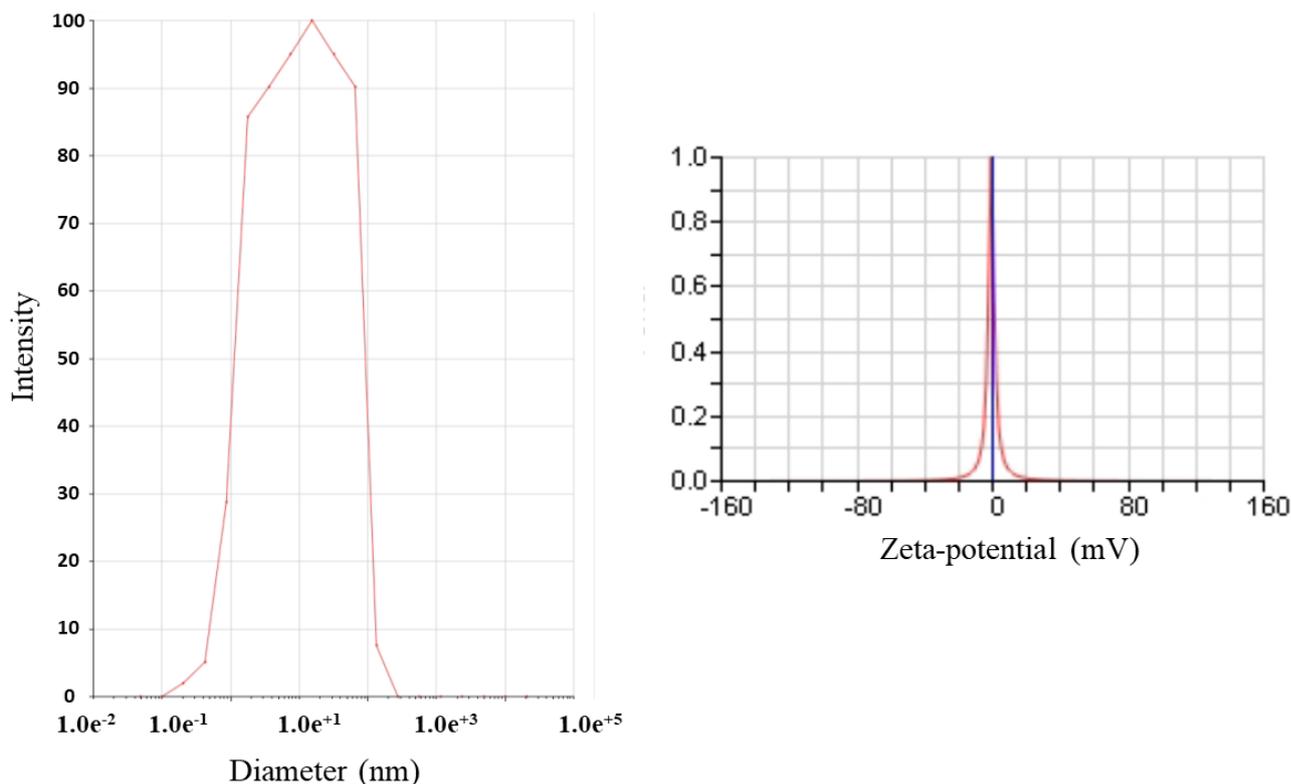
Figure 4: Particle size distribution and zeta-potential graphs of the prepared rosuvastatin calcium-Eudragit L100 PNPs.

Evaluation of the prepared transdermal patches

Physical appearance
Physical evaluation of rosuvastatin calcium-loaded (control) and rosuvastatin calcium PNPs-loaded HPMC K15 patches are shown in **table 1**. Both control patch and PNPs-loaded patch showed homogenous, smooth, translucent appearance with good flexibility and no sign of a crack, these results were in agreement with a previous study using HPMC K15 to prepare transdermal patch[35]. **Figure 5** shows the described HPMC K15 patches.

Thickness measurement
The HPMC K15 patches showed acceptable thickness values, 0.03 ± 0 and 0.021 ± 0.003 mm of control and PNPs-loaded patch, respectively (**table 1**), with minimum standard deviation, indicating that the process of preparing these films is reproducible and can produce uniform films. Thickness values were less than 1 mm which fulfilled the requirement of transdermal patch thickness[36].

Folding endurance
A folding endurance test was performed manually to confirm that the prepared film is flexible enough to tolerate handling. The resultant test values > 300 (**table 1**), indicated the effectiveness of the plasticizer (PEG 200) and suggesting that these films can successfully withstand handling during preparation and after application [22].

Surface pH
Surface pH values lied within the acceptable pH range of the skin (4.1-5.8)[37], suggesting compatibility with skin and limited risk to cause skin irritation[38].

Table 1: Physical evaluation of the prepared transdermal patches.

Patch	Thickness (mm)	Folding endurance	Surface pH
Control patch	0.03 ± 0	>300	6.23 ± 0.32
PNPs-loaded patch	0.021 ± 0.0029	>300	5.86 ± 0.057

values are mean ± SD, n=3.
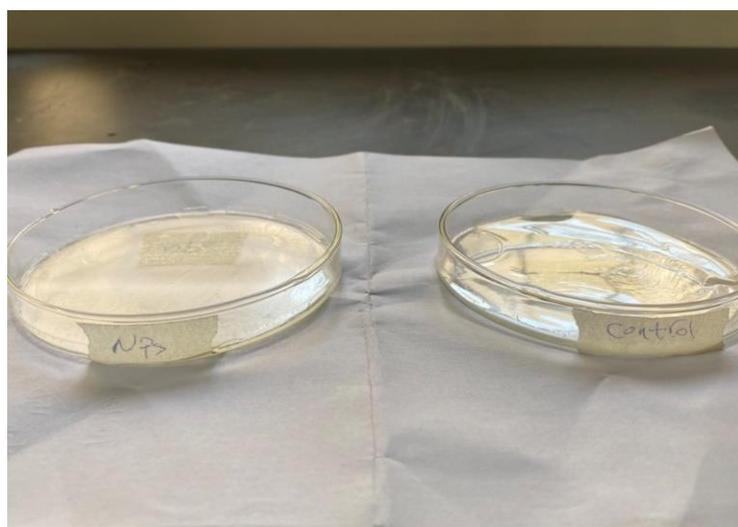
Figure 5: The control patch and PNPs-loaded patch.

***In vitro* release study**

The release profile of rosuvastatin calcium from the control patch and the Eudragit L100 PNPs-loaded patch is shown in **figure 6**. Both patches showed a biphasic release profile; an initial burst release followed by sustained release. The initial burst release of rosuvastatin calcium from PNPs-loaded patch was slightly less than that of the control patch, cumulative release percentages were 41.6 ± 0.3 % and 45.6 ± 0.3 %, respectively, which is most probably due to the rapid release of free drug presence from both patches. Thereafter, a sustained release profile of the drug was observed from both patches, with 65 ± 6 % of drug released from rosuvastatin calcium PNPs-loaded patch compared to 82 ± 3.7 % of drug released from the rosuvastatin calcium-loaded patch (PNPs-free patch) at 24 h. The slower rate of drug release from the PNPs-loaded patch is usually due to the presence of the PNPs barrier. In addition, the adsorption of the HPMC K15 polymer on the surfaces of the PNPs, and hence creates an additional barrier that impedes drug release[39]. The results of this study

were also analyzed by fitting into mathematical models for drug release kinetics to investigate the mechanism of the drug release from the above optimized patches. **Table 2** demonstrates the correlation coefficient (r^2) of the mathematical models.

Correlation coefficients of the applied mathematical models have revealed that the release of rosuvastatin calcium from the control patch was best fitted with first-order kinetic and Higuchi models (r^2 0.84 and 0.74, respectively) compared to other models. Release from the PNPs-loaded patch showed a similar trend of data fitting, however, r^2 of first-order kinetic and Higuchi models were slightly lower than their respective values in the control patch. These results suggested that the release follows the first-order kinetic diffusion-controlled release. Both patches were also moderately fitted with the zero-order kinetic model (r^2 were 0.54 and 0.58 for the control and PNPs-loaded patches, respectively), suggesting that part of the release could follow the zero-order kinetic, which represent the sustained release or linear phase of the curve.
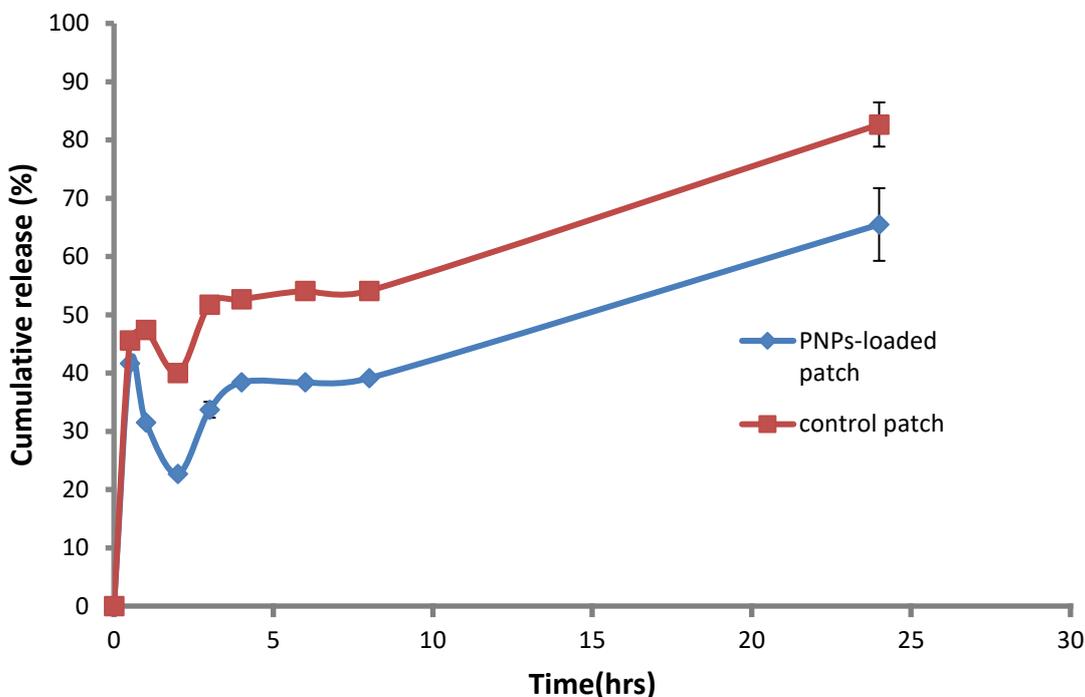

Figure 6: *In vitro* cumulative release profile of rosuvastatin calcium from PNPs-loaded patch and control patch. The release medium was PBS (pH 7.4); control patch contained rosuvastatin calcium free drug instead of rosuvastatin calcium PNPs. Values are mean ± SD, n=3.

Table 2: Mathematical models of *in vitro* release data of ROC from the control and PNPs-loaded patches

Patch	Diffusional exponent (n)	Correlation coefficient (r^2)			
		Korsmeyer-peppas	Higuchi	Zero order	First order
Control patch	0.14	0.66	0.74	0.54	0.84
PNPs-loaded patch	0.13	0.32	0.7	0.58	0.75

n *is the diffusional exponent of Korsmeyer–peppas model.*

Regarding the Korsmeyer-peppas model, the control patch was moderately fitted (r^2 0.66), however PNPs- loaded patch was inappropriately fitted with the Korsmeyer-Peppas (r^2 0.32). The diffusional exponent of Korsmeyer-peppas model (0.14 and 0.13), suggested a fickian diffusion release. It has been reported in a previous study that if the n value is less than 0.43, it still indicates a diffusion-controlled release mechanism[24].

***Ex vivo* permeability study**

Ex-vivo permeation study of rosuvastatin calcium-loaded (control) and rosuvastatin calcium PNPs-loaded patches was also carried out using rat skin. **Figure 7** shows the cumulative amount of rosuvastatin calcium permeated through rat skin as a function of time. The amount of drug permeated was slightly higher in the control patch than that of the PNPs-loaded patch for up to 2 h which could be due to

the faster release of the free drug from the matrix in the control patch compared to the rosuvastatin calcium PNPs-loaded patch. However, after 2 h the amount of rosuvastatin calcium permeated through the skin using the PNP-loaded patch has become gradually higher than that of the control patch to reach the maximum difference at time point 24h. Wherein, the amounts permeated after 24 h were 177.4 $\pm$ 2.15 µg/cm^2 and 246.4 $\pm$ 0 .66 µg/cm^2 for the control and PNPs-loaded patch, respectively, and the difference was statistically significant (at $P < 0.05$). This indicates successful incorporation of the hydrophilic drug rosuvastatin into the hydrophobic polymer Eudragit L100 which besides their small size; the hydrophobicity of the PNPs has greatly enhanced the partitioning of the hydrophilic drug into the stratum corneum. In addition, the presence of HPMC film

will prolong the contact time between the PNPs and the skin; prolong their retention and hence increase drug permeation.

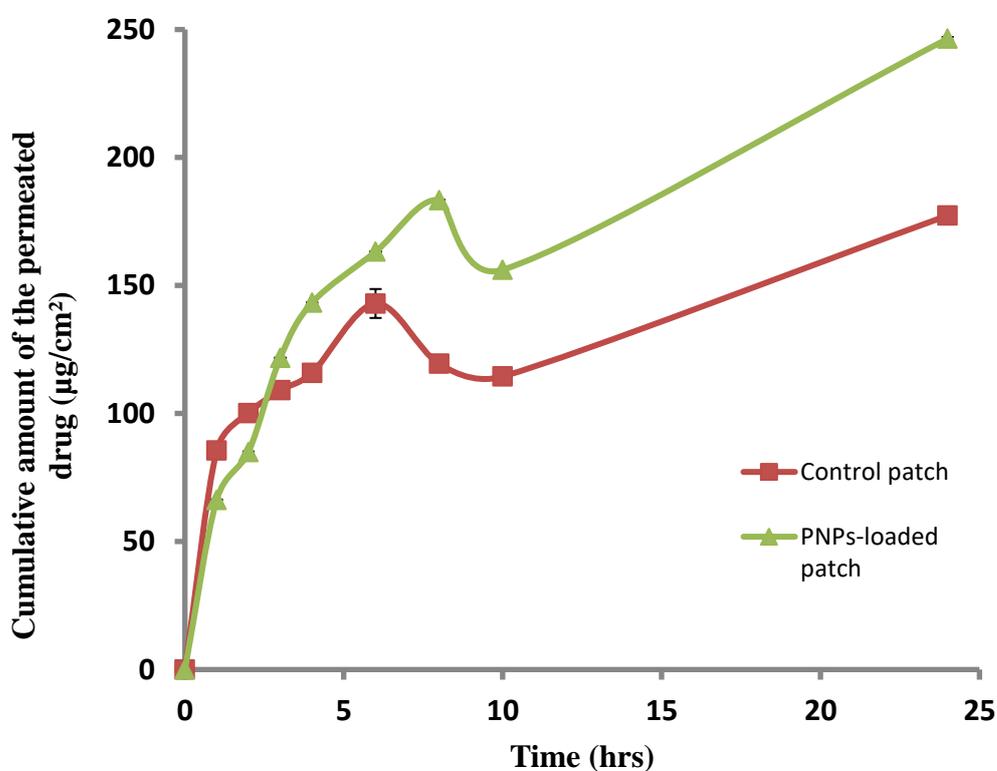Similar results were also reported by Takeuchi *et al.*[40] and Taghe *et al.*[32]

Figure 7: *Ex vivo* permeability study of PNPs-loaded patch and control patch using rat skin. Values are mean ± SD, n=3.

Table 3 shows the calculated permeability parameters of rosuvastatin calcium using the PNPs-free control patch and the PNPs-loaded patch. Permeability parameters of PNPs-loaded patch were higher than those of the control patch, suggesting the skin penetration enhancement effect of Eudragit L100 PNPs-loaded HPMC K15 film. The results of this study were in agreement with several other studies using PNPs-loaded matrix to enhance drug penetration into skin or other tissues[32,33,41,42]. Skin permeability of Ibuprofen was significantlyenhanced using ibuprofen-chitosan nanogel-loaded gellan hydrogel matrix, wherein the skin permeability enhanced with increasing chitosan concentration due to the reduction in particle size of Ibuprofen-chitosan nanogel[41].

The findings of this study also support the results of a previous study using various PNPs to enhance transdermal delivery of repaglinide. In this study, biodegradable PNPs were developed using Chitosan, poly (lactic acid) (PLA) and poly (caprolactone) (PCL) to encapsulate the antidiabetic drug, repaglinide, and loaded into transdermal patch. *In vitro* release patterns from the different polymeric nanoparticles showed sustained and prolonged release pattern with only 73.6 ± 0.6 % to 86.9 ± 1.2 % released at the end of 24 h. *Ex-vivo* permeation results demonstrated a significant percentage of repaglinide permeated via rat skin from repaglinide nanoparticles formula at the end of 24 h. For the PNPs-loaded transdermal patch, the *in-vitro* release pattern showed sustained release, and only 52.7% were released at the end of 60 h.

Table 3: Permeation parameters of ROC across rat skin from both control patch and PNPs-loaded patch.

Patch	Cumulative amount Q24 ($\mu g/cm^2$)	Steady state Flux (Jss) ($\mu g/cm^2/h$)	Enhancement factor	Permeability coefficient (cm\h)
Control patch	177.4 ± 2.15	2.9	1	61×10^{-6}
PNPs-loaded patch	$246.4 \pm 0.66*$	5.5	1.9	81×10^{-6}

Significant from control patch at P < 0.05.

This illustrates the ability of the transdermal patches loaded-PNPs to maintain steady-state concentration of the drug for a prolonged time[43]. The use of novel pH-sensitive polymers in transdermal delivery was reported in many previous studies[44,45]. Dildar *et al.* developed Flurbiprofen PNPs (Eudragit S100)-loaded transdermal patch for the treatment of rheumatoid arthritis. Results showed sustained release of Flurbiprofen from both nanoparticles and transdermal patch and higher cumulative amount permeated from PNPs containing patch compared to marketed gel[45]. Recently, Eudragit L100 PNPs-loaded HPMC ocular inserts were successfully enhanced ocular permeation of azithromycin[32] and ketorolac tromethamine (KT)[31].

CONCLUSION

In conclusion, the nanoprecipitation method was efficient in producing relatively small PNPs with great advantages in transdermal delivery. The prepared PNPs were successfully loaded into transdermal film utilizing HPMC K15 as film-forming polymer and PEG 200 as a plasticizer. The prepared films showed an acceptable appearance with thickness, folding endurance as well as surface pH values. PNPs loaded patch extended the drug release and significantly enhanced drug permeation via the skin in comparison with the control patch (P-value < 0.05).

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