

Preparation, Evaluation, and Optimization of Atorvastatin Nanosuspension Incorporated Transdermal Patch

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Abstract

Background: A nanosuspension is a colloidal dispersion of submicron drug particles. Most of the new drugs are poorly soluble in both water and organic solvents. To overcome such solubility problems and bioavailability factors, many traditional approaches have been adopted. One of the most successful and easy method to enhance both solubility and bioavailability of drug was nanosuspension formulation. **Aim:** The aim was to prepare atorvastatin nanosuspension loaded matrix type transdermal patch using homogenization technique. **Materials and Methods:** Atorvastatin nanosuspension was prepared by adding 100 mg of drug with 10 mg of surfactant in an aqueous solvent to get a dispersed form, the prepared solution was homogenized at different rpm and then sonicated. Based on the particle size of the formulation, best one of F8 was selected and fabricated into transdermal patch using Hydroxypropylmethylcellulose (HPMC), dibutyl phthalate and with different concentration of dimethylsulfoxide (DMSO). Prepared patches were evaluated for various physicochemical parameters such as thickness, weight variation, folding endurance, percentage moisture loss, percentage moisture uptake, tensile strength, and analyzed its release character. **Result and Discussion:** The obtained transdermal patches were found to be thin, clear, smooth, uniform, and flexible with desired thickness for transdermal delivery of atorvastatin nanoparticles. Formulation FT-3 was selected as an optimized formulation since the release rate was 86.4% at 105 min and tensile strength was 875.6 ± 104.1 . **Conclusion:** The study concludes that atorvastatin nanosuspension of F8 with particle size of 496 nm and loaded into patch of FT3 of 100 mg HPMC, 0.75 ml DMSO, and 20 % w/w of polymer shown $86.4\% \pm 0.82\%$ of drug release at 105 min which evidence that increase in concentration of DMSO increases rate of drug release.

Key words: Atorvastatin nanosuspension, homogenization technique, transdermal drug delivery system

INTRODUCTION

Pharmaceutical nanosuspension is defined as a biphasic, dispersed, very finely colloid, solid drug particles in an aqueous vehicle with the size range below $1 \mu\text{m}$, using polymer and surfactant as a stabilizer.^[1] It is prepared by any suitable method for drug delivery applications, through various routes of administration such as topical, oral, parenteral, and pulmonary routes. Delivery of drug through the skin to achieve a systemic effect of a drug is commonly known as transdermal drug delivery, and it differs from traditional topical drug delivery. Some major advantages of transdermal drug delivery are a limitation of hepatic first pass metabolism, enhancement of therapeutic efficiency and

maintenance of steady plasma level of the drug.^[2] All statin drugs have poor aqueous solubility and lead to low oral bioavailability. To overcome such criteria, efforts have been made to improve bioavailability by preparing nanosuspension and to deliver through the transdermal system. Atorvastatin was selected for this study since it has high lipid solubility as well as high degree of first-pass metabolism but it also poorly soluble in water.

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The main components to a transdermal patch

Polymer matrix

It is considered backbone of Transdermal drug delivery system, where the drug release is controlled. Polymer should be physically stable should not decompose while storing, chemically non-reactive which should not alter the physicochemical properties of the drug and other ingredients should be non-toxic, cost should not be high.

Drug

The transdermal route is a better and perfect choice for the drugs with appropriate pharmacology and physical chemistry. Transdermal patches is an extremely attractive option for drugs which need to go extensively first pass metabolism, drugs with narrow therapeutic window, or drugs with short half-life.

Permeation enhancers

This is used to enhance the drug permeability through stratum corneum to attain higher therapeutic levels of the drug. Three types of permeation enhancer are lipophilic solvent, surface active agents, and two component systems.

MATERIALS AND METHODS

Atorvastatin was received as gift sample from Pharmafabrikon Ltd., Madurai. Hydroxypropyl methylcellulose (HPMC) K-10, dibutyl phthalate (DBP), dimethylsulfoxide (DMSO), sodium lauryl sulfate (SLS) were purchased from Nice Chemical Ltd., Cochin. All other chemicals and reagents used in this study were of analytical grade (Figure 1).

Preformulation studies

Fourier transform infrared (FT-IR) study

FT-IR spectrum of pure drug, polymer, and stabilizer were obtained using FT-IR 8400S (CE) Shimadzu spectrophotometer. The samples were prepared as KBr pellets by compressing at 6 ton/nm². The wavelength range was selected between 400 and 4000 cm⁻¹.

Solubility test

The solubility tests were performed by adding little by little to the solvent of water and methanol in a different test tube to obtain a supersaturated solution with undissolved solute particles and then centrifuged for 10 min to get a supernatant and measured at 246 nm to find out solubility.

Partition coefficient

A total of 100 mg drug was taken in the separating funnel with mixture of 30 ml pH 7.4 buffer and 30 ml octanol solution and shaken for 1 h and kept undisturbed to separate

aqueous and an organic phase. A volume of 1 ml of separated solution of both layers was taken and diluted to 100 ml with water and measured at 246 nm against buffer as a blank.

$$\text{Partition coefficient} = \frac{\text{Concentration of drug in octanol}}{\text{Concentration of drug in buffer}}$$

Preparation of atorvastatin nanosuspension

100 mg of atorvastatin was weighed and transferred into the glass beaker. To this 10 mg of SLS was added and dispersed in 100 ml of distilled water and then stirred well for 5 min. The prepared solution was then subjected to one or two cycles of homogenization with different rpm and processing time and formulation was placed in bath sonicator further for 5-10 min (Table 1).

Characterization of atorvastatin nanoparticles

Particle size analysis

The atorvastatin nanoparticles were analyzed by Malvern Zeta sizer after suitable dilution with water. The particle size was found, and it ranges between 1614 nm and 496 nm. A formulation with lower size of 496 nm was selected for further studies, and its surface charge of zeta potential was negative.

Scanning electron microscopy (SEM)

The atorvastatin nanosuspension was characterized by SEM. The prepared nanosuspension was lyophilized to form solid particles using lyodel freeze dryer. Morphological studies of atorvastatin nanoparticles were conducted by SEM. The SEM image shows that nanoparticles were within the nano range and with different shapes of particles.

Incorporation of prepared nanosuspension into transdermal system

To prepare the transdermal patch loaded with nanoformulation, 10 ml of the above-prepared nanosuspension were added dropwise to the beaker containing 100 mg hydroxyl propyl methyl cellulose polymer with continuous hand stirring. After it gets completely dispersed, add 20% w/w of DBP to polymer and with or without different concentration of dimethyl sulfoxide. The obtained solution was further stirred continuously for ½ h. The uniform dispersion was casted on petri dish and dried at room temperature overnight. After drying the patches were removed and stored in desiccator for further studies (Table 2).

Evaluation of transdermal patches

Weight variation

Prepared three patches were weighed accurately, and the average weight was calculated.^[3]

Table 1: Formulation and particle size determination of atorvastatin nanosuspension

Formulation	Homogenization 1 st cycle (min)	Homogenization 2 nd cycle (min)	Sonication (min)	Particle size (nm)
F1	22,000 (10)	–	5	1614
F2	9,000 (10)	20,000 (10)	–	812.8
F3	9,000 (10)	18,000 (10)	–	976.4
F4	9,000 (10)	18,000 (10)	5	632.2
F5	9,000 (10)	18,000 (10)	5	626.8
F6	9,000 (10)	18,000 (10)	5	732.4
F7	9,000 (10)	20,000 (15)	10	581.9
F8	9,000 (10)	20,000 (15)	10	496

Table 2: Preparation of atorvastatin transdermal patches

Formulation code	Nano solution [#] (ml)	HPMC (mg)	DMSO (%)	DBP (%)
FT-1	10	100	-	20
FT-2	10	100	0.5	20
FT-3	10	100	0.75	20

[#]10 ml of nanosolution contain 10 mg of drug, HPMC: Hydroxypropyl methylcellulose, DMSO: Dimethylsulfoxide, DBP: Dibutyl phthalate

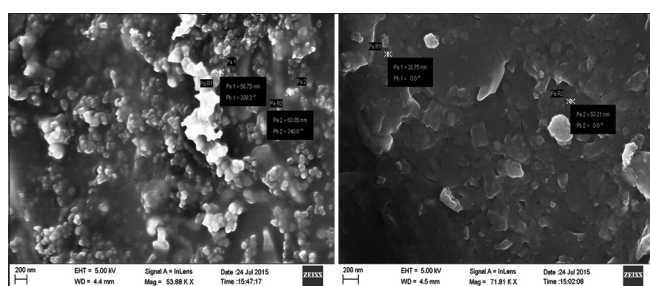


Figure 1: Scanning electron microscopy images of atorvastatin nanoparticulate

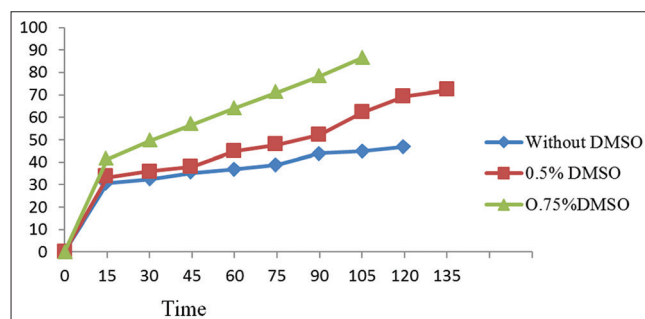


Figure 2: *In vitro* drug release of atorvastatin transdermal patches Fourier transform-1 to Fourier transform-3

Thickness

The thickness of patches was measured using screw gauze. Patch thickness was measured at three different points on the film and average of three reading was calculated.^[3]

Folding endurance

A strip of specific area was repeatedly folded at the same place until it gets broke. The number of times the

film could be folded without breaking gave the value of folding endurance and average folding endurance was calculated.^[3]

Percentage moisture loss

The patches were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature. After 24 h, the films were reweighed, and average moisture loss was calculated.^[3]

$$\text{Percentage moisture content (\%)} = \frac{\left[\frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \right] \times 100}{\text{Final weight}}$$

Percentage moisture uptake

The patches were weighed individually and to be kept in a desiccator containing a saturated solution of potassium chloride. After 24 h, the films were reweighed, and average moisture uptake was calculated.^[3]

$$\text{Percentage moisture uptake (\%)} = \frac{\left[\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \right] \times 100}{\text{Initial weight}}$$

Drug content

Patch was cut and dissolved in 100 ml of pH7.4 buffer using magnetic stirrer. Then, the solution was filtered through a filter medium. 1 ml of filtered solution was diluted with 10 ml of buffer, and the drug content was analyzed with the ultraviolet (UV) spectroscopic method (Table 3).^[4]

Table 3: Physicochemical evaluation of atorvastatin loaded transdermal patches

Formulation code	Thickness (mm)	Weightvariation (mg)±SD	Folding endurance (no)±SD	Percentage moisture loss±SD (%)	Percentage moisture uptake±SD (%)	Drug content (%)
FT-1	0.01	175±6.66	284.6±5.6	6±0.3	5.3±1.51	94±1.73
FT-2	0.013	175±3.6	292±18.6	6.06±0.01	5.1±0.3	93.3±1.52
FT-3	0.01	175.3±4.5	296.3±4.5	6.13±0.25	5.3±0.47	94.3±0.57

The above values are average of 3 findings, SD: Standard deviation

Table 4: *In vitro* drug releases of atorvastatin transdermal patches**Cumulative percentage drug release of atorvastatin transdermal patches**

Time (min)	Without DMSO FT-1 (%)	0.5% of DMSO FT-2 (%)	0.75% of DMSO FT-3 (%)
15	30.6±1.02	33.3±0.99	41.4±1.02
30	32.4±0.81	36±0.85	49.5±0.65
45	35.1±0.57	37.8±0.81	56.7±0.83
60	36.9±0.85	45±0.92	63.9±0.78
75	38.7±0.94	47.7±0.58	71.1±0.69
90	44±0.21	52.2±0.7	78.3±0.85
105	45±0.76	62±0.79	86.4±0.82
120	46.8±0.68	69.3±0.25	-
135	-	72±0.58	-

DMSO: Dimethylsulfoxide

***In vitro* drug release study**

The paddle over disc method was employed for assessment of drug release from the prepared patches. Dry patch was fixed over a petri dish at the bottom of the dissolution bowl and then placed in a 900 ml of dissolution medium phosphate buffer (pH 7.4), and the apparatus was equilibrated to $37 \pm 0.5^\circ\text{C}$. The paddle was operated at a speed limit of 50 rpm and depth of 20 mm. Samples (5 ml aliquots) were withdrawn at appropriate time intervals and analyzed by UV spectrophotometer. The experiment was performed in triplicate and the mean value calculated (Table 4 and Figure 2).^[5]

Tensile strength

Tensile strength of prepared patch was determined by using texture analyzer. Then film was cut into 10×10 mm strips. Each test strip was placed in tensile grips on the texture analyzer. Tensile strength was computed using load required to break the film. Tensile strength was the maximum stress applied to a point at which the film was broken.^[6]

Stability study

The accelerated stability study of FT3 formulation of transdermal patch of Atorvastatin was studied as per ICH Guidelines for 6 months, at $40 \pm 2^\circ\text{C}$ temperature and $75\% \pm 5\%$ RH and analyzed for drug content with sampling point of 15 days, 1-3, and 6 months.^[7]

RESULT AND DISCUSSION

In the present study, efforts are taken to prepare atorvastatin nanoparticulate loaded transdermal patch. FT-IR studies prove that excipients used and atorvastatin were compatible. Atorvastatin was soluble in methanol and poorly soluble in water, $\log P = 0.98$ also confirms the suitability of atorvastatin for transdermal drug delivery. The formulation F-8 of 9000 rpm of 10 min in 1st cycle, 20,000 rpm of 15 min and further treated in bath sonicator for 15 min has lower particle size of 496 nm compared to other nanoformulations, and it was selected to incorporate into transdermal patch. The obtained transdermal patches were found to be thin, clear, smooth, uniform, and flexible and had desired thickness for transdermal delivery of atorvastatin nanoparticles.

The physicochemical evaluation of prepared film shows that there was no change in physical characters such as appearance and color while storing at room temperature. The thickness of the patches varied from 0.01 to 0.02 mm. Average weight variation of FT-1, FT-2, and FT-3 were 175 ± 6.6 mg, 175 ± 3.6 mg and 175 ± 4.5 mg, respectively. Average Folding endurance values were 284.6 ± 5.18 numbers for FT-1, 292.6 ± 18.6 numbers for FT-2 and 296.3 ± 4.5 numbers for FT-3. Percentage Moisture loss varied between $6\% \pm 0.3\%$ for FT-1 and $6.13\% \pm 0.25\%$ for FT-3. Moisture uptakes varied between $5.1\% \pm 0.3\%$ for FT-2 and $5.3\% \pm 1.51\%$ for FT-1 and these values differed based on the concentration of DMSO. Drug content values were found to be 95% for FT-1, 93% for FT-2 and 95% for FT-3, respectively. $46.8\% \pm 0.68\%$ drug release at 120 min was found in FT-1

formulation in *In vitro* release study. To increase the dissolution rate, permeation enhancer 0.5% DMSO were introduced into the formulation FT-2 in which $72\% \pm 0.58\%$ of drug release at 135 min was found. This indicates release rate was enhanced using permeation enhancer. Hence, the release rate was induced by increasing concentration of DMSO and shown $86.4\% \pm 0.82\%$ release at 105 min with 0.75% DMSO in formulation FT-3, and this was selected as an optimized formulation. Tensile strength of optimized FT-3 formulation was determined using texture analyzer and average value was found to be 875.6 ± 104.1 and stability study of formulation FT3 proves the stability of patch with drug content of $94\% \pm 0.26\%$ at 15 days, $94\% \pm 0.72\%$ at 1 month, $97\% \pm 0.84\%$ at 2 months, $98\% \pm 0.56\%$ at 3 months, and $96\% \pm 0.03\%$ at 6 months.

CONCLUSION

In this study, atorvastatin calcium bioavailability was achieved by nanosuspension formulation techniques of formulation F8 with surfactant SLS yields 496 nm of nanoparticles and further loaded into transdermal patch of HPMC polymeric matrix with 0.75% DMSO in FT3 shown $86.4\% \pm 0.82\%$ of drug release in 105 min may useful for improvement of bioavailability through topical route of application by transdermal patches. The prepared patch is thin, flexible with good tensile strength and stability study of formulation FT-3 also confirms the suitability of method.

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