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## An Innovative Gateway to Deliver Nanosized Atorvastatin by Bio-Flexy Film Approach

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**Abstract-** Atorvastatin is an antihyperlipidemic drug which is widely used to treat hyperlipidemia and lower the cholesterol level in the body, but atorvastatin has low bioavailability due to high intestinal clearance and first-pass metabolism. The main objective of our research work was to develop a formulation to increase the therapeutic efficacy of the drug. A bio-polymer was isolated from a natural edible source *Coriandrum sativum* and was subjected for screening its filmability and adhesivity. Atorvastatin was nanosized using a novel method and using the bio-polymer and other co-processing agents five bio-flexy films of different ratios (i.e. 1:1, 1:2, 1:3, 1:4, 1:5) were formulated. The isolated bio-polymer was subjected to various analytical parameters. The drug-exipient compatibility study was performed using UV and TLC method. The formulated bio-flexy films were evaluated for various parameters like weight, thickness, content uniformity, surface pH, folding endurance, and in-vitro drug permeation. The formulation AC2 (containing 1:2 bio-polymer) was found to be the best formulation having R<sup>2</sup> value 0.9989 with zero order as best fit model. The results obtained concluded that the efficacy of atorvastatin can be effectively increased by delivering it as a transdermal formulation.

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## I. INTRODUCTION

Coriander commonly known as 'dhaniya' in hindi is obtained from the seeds of *Coriandrum sativum* belonging to family Apiaceae. It is a herbal spice commonly used in culinary purposes and it possess various properties. It contains various essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and glycosides. It is highly rich in proteins, oils, carbohydrates, fibers, minerals, trace elements and vitamins. It has various pharmacological effects like anxiolytic, antidepressant, sedative-hypnotic, anticonvulsant, memory enhancement, improvement of orofacial dyskinesia, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antioxidant, cardiovascular, hypolipidemic, anti-inflammatory, analgesic, antidiabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, etc. [1,2]

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Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood. Lipid and lipoprotein abnormalities are regarded as a highly modifiable risk factor for cardiovascular disease due to the influence of cholesterol on atherosclerosis. Hyperlipidemia includes several conditions, but it usually means that you have high cholesterol and high triglyceride levels. [3]

Atorvastatin is an antihyperlipidemic drug. It belongs to the category of statins, which acts by competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. This enzyme is involved in the conversion of HMG CoA to mevalonate which is the rate limiting step in the cholesterol synthesis. The drug has low oral bioavailability due to low aqueous solubility (belongs to BCS class II) and rapid intestinal clearance and hepatic first-pass metabolism. This can be avoided by formulating a formulation for transdermal delivery. [4]

The aim of our research work was to improve therapeutic efficacy of Atorvastatin by delivering nanosized atorvastatin through trans-nabhi route. This can be attained by formulating bio-flexy film.

## II. MATERIALS AND METHODS

Atorvastatin was obtained as a gift from Mylan laboratories Ltd. *Coriandrum sativum* was procured from local market. All other reagents used were of analytical grade.

### a) Extraction of biopolymer from *Coriandrum sativum*

500 gm *Coriandrum sativum* was taken and powdered. The powder was soaked in 1000 ml of distilled water and kept in refrigerator for overnight. It was centrifuged at 3000 rpm and supernatant was collected which was treated with equal amount of propanone. It was kept in refrigerator for 24 hrs. The supernatant was centrifuged at 3000 rpm. The bio-polymer was collected and dried. The dried bio-polymer was purified by hot dialysis method. The process was repeated 6 times and the percentage yield was calculated. The purified bio-polymer was passed through 120# sieve and stored for further use. [5]

### b) Characterization of the isolated bio-polymer

The isolated bio-polymer was subjected to various physicochemical analysis like color, texture, solubility, presence of carbohydrates, proteins and starch; IR, SEM, DSC, NMR spectroscopy studies.

c) *Drug-excipient interaction study*

Drug interaction study with other excipients of the formulation was performed by dry and wet method. The drug was mixed with excipients in the ratios of 1:1, 1:3, and 3:1. The mixtures were stored at room temperature for a period of 3 days. The dilutions of the mixtures were prepared with methanol and the samples were analyzed by ultraviolet spectrophotometric method (Shimadzu 1800).<sup>[5]</sup>

d) *Preparation of nanosized atorvastatin loaded bio-flexy films*

Atorvastatin was nanosized by using a novel method. Atorvastatin was triturated with dextrose in a pestle mortar. Double distilled water was added to the solution drop by drop and triturated continuously. The solution was transferred to a beaker and was sonicated for six cycles of 3 min each. After each

sonication cycle, percentage absorbance and transmittance was observed at wavelength 200-800nm. The solution was microcentrifuged. Nanosized atorvastatin was obtained and dried. It was kept in dessicator for 24 hrs. Nanosized drug was collected and stored in cool and dry place.

Bio-flexy films were prepared by solvent casting method. Bio-polymer isolated was accurately weighed in different ratios and dissolved in 10 ml of distilled water at room temperature. Dextrose was added to this solution. Nanosized atorvastatin used as a model drug was dissolved in little amount of ethanol. The nanosized drug solution was added to the polymeric solution. It was poured in a petri-dish for natural drying. The dried bio-flexy films were obtained and packed in tightly closed container.<sup>[6,7]</sup>

Table 1: Formula for Bio-flexy films

Ingredients	AC1	AC2	AC3	AC4	AC5
Nanosized Atorvastatin (mg)	10	10	10	10	10
<i>Coriandrum sativum</i> Bio-polymer (mg)	100 (1%)	200 (2%)	300 (3%)	400 (4%)	500 (5%)
Dextrose (mg)	100	100	100	100	100
Distilled water (mL)	10	10	10	10	10

e) *Evaluation of Bio-Flexy Films*i. *Physical appearance*

The formulations were visually inspected for various factors like color, clarity, and smoothness in order to ensure the uniformity in physical appearance of the bio-flexy films.

ii. *Weight*

Three patches (1 cm<sup>2</sup>) of each formulation were taken, weighed and average weight was calculated.<sup>[8,9]</sup>

iii. *Thickness*

The thickness of the films for every formulation was measured using a micrometer screw gauge at three different places and the mean value was calculated.<sup>[8,9]</sup>

iv. *Folding endurance*

Folding endurance was determined by repeatedly folding the film at the same place till it broke. The number of times the film could be folded at the same place without breaking was recorded which is known as the folding endurance.<sup>[8,9]</sup>

v. *Surface pH*

The individual film was placed in a petridish and moistened with 0.5 ml of distilled water and kept for 30 min. The surface pH was measured by using pH meter.<sup>[5]</sup>

vi. *Drug content uniformity*

The bio-flexy film was dissolved in methanol and volume was made up to 100 ml. It was sonicated and kept for 24 hours. 0.1 mL was withdrawn from this and diluted to 10 ml. The drug content was measured by using UV Spectroscopy. This was repeated for all the formulations. From the drug content, % drug content was calculated.<sup>[8,9]</sup>

vii. *In-vitro drug release study*

The *in-vitro* drug release was carried out by using MS diffusion apparatus. This is the static method which utilizes complete replacement of the sample thus provides 100% sink condition. Egg membrane was attached on the donor compartment. A piece of formulated bio-flexy film was adhered onto the egg membrane in the donor compartment. The receptor compartment was filled with 13 ml of pH 7.4 buffer solution. Samples were withdrawn completely at regular intervals for 48 hrs and replaced completely by fresh buffer each time. The samples were analyzed by UV spectroscopy (Shimadzu 1800) at 241 nm to estimate the amount of the drug. Similarly drug diffusion study was carried out for each nanosized atorvastatin loaded bio-flexy films.<sup>[5,10]</sup>

viii. *Stability studies*

The formulated bio-flexy films were subjected to accelerated stability studies according to the ICH guidelines for six months.<sup>[11]</sup>

### III. RESULTS AND DISCUSSION

a) *Characterization of the isolated bio-polymer*

The bio-polymer isolated from *Coriandrum sativum* was found to be smooth, amorphous, odourless, and buff in color. It was slightly soluble in water. The yield was found to be 12.40±2.13 % w/w. The bio-polymer was found positive for carbohydrates and protein content. The test was negative for starch content. The color changing point was found to be 264±5°C. The IR spectra (Fig. 1) revealed the presence of aromatic phenols (3290.26 cm<sup>-1</sup>), alkanes

(2924.59  $\text{cm}^{-1}$ ), alkenes with stretching (1651.46  $\text{cm}^{-1}$ ), nitro compound (1543.89  $\text{cm}^{-1}$ ), aromatics with stretching (1455.35  $\text{cm}^{-1}$ ), sulfone (1239.50  $\text{cm}^{-1}$ ), thiocarbonyl (1151.04  $\text{cm}^{-1}$ ). These groups are responsible for bioadhesivity of the biopolymer. SEM

analysis of the bio-polymer (Fig. 2) showed that the bio-polymer has smooth surface and is amorphous in nature. It showed the morphological structure similar to the polymers which confirms that the bio-polymer is polymeric in nature.

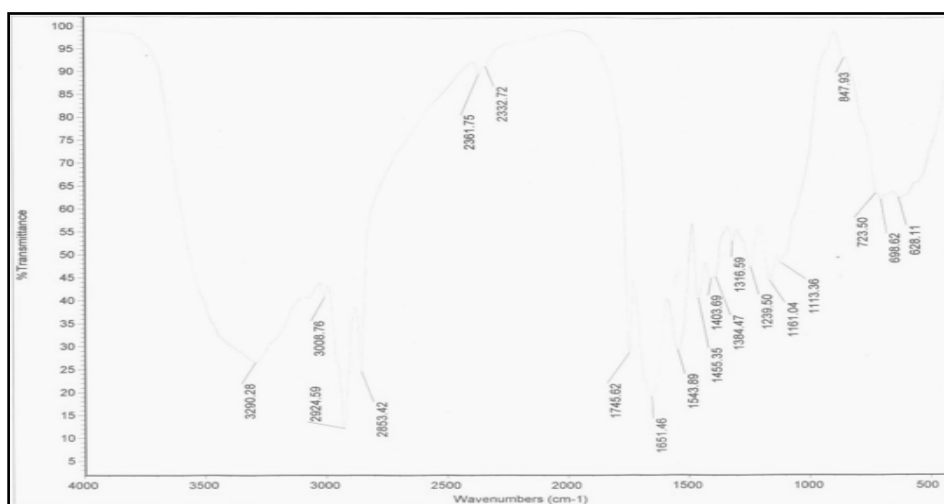


Fig. 1: IR spectrum of *Coriandrum sativum* bio-polymer

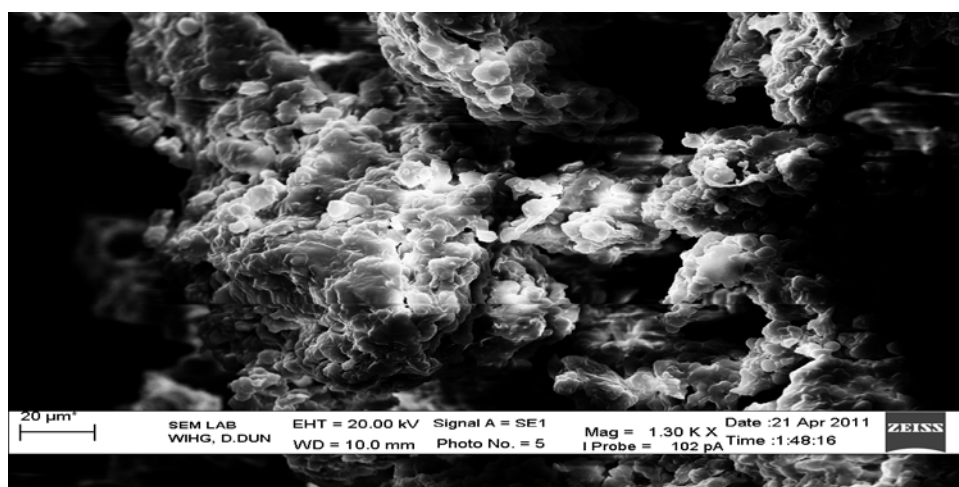


Fig. 2: SEM of *Coriandrum sativum* bio-polymer

#### b) Drug-excipient interaction study

The drug-excipient interaction studies revealed that there was no interaction between the drug and the excipients as there was no change in the wavelength of the drug.

#### c) Nanosizing of Atorvastatin

The percentage of transmittance at different wavelength represents that the light is passed through the particles which means the particle size is below that wavelength. The percentage of the particles which are present in the mixture below 400 nm. Whereas the % blockade indicates the % particle which are above 400 nm and the data was correlated with the SEM analysis. The percentage of transmittance was measured by UV spectrophotometer and after each cycle increase in the percentage transmittance was

observed which indicated that the particles may have been reduced to nano range. The effect of sonication on percentage of transmittance after each cycle is shown in fig. 2.

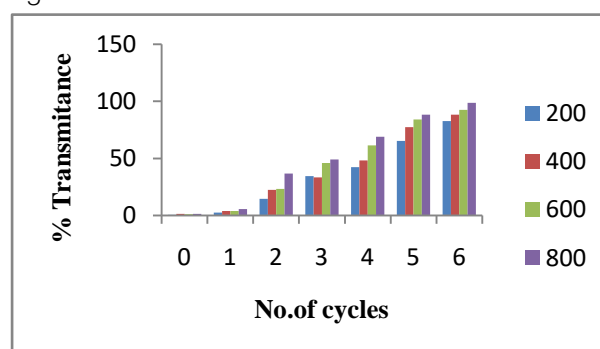


Fig. 3: Nanosizing characterisation by UV spectroscopy.

d) Thickness, weight, folding endurance and surface pH

Thickness of the bio-flexy films AC1 to AC5 containing *Coriandrum sativum* bio-penetrant ranged from  $0.35 \pm 0.15$  to  $0.39 \pm 0.11$  mm.

The weight of the bio-flexy films AC1 to AC5 containing *Coriandrum sativum* bio-penetrant ranged from  $20.62 \pm 0.12$  to  $38.43 \pm 0.25$  mg.

The micro environmental pH of the bio-flexy films ranged from 6.53 to 7.05. The pH of the bio-flexy films was found to be close to the pH of the skin. It confirms that the formulations will not cause any irritation effect.

Folding endurance of the bio-flexy films ranged from 71 to 118 (times) which indicates reasonable flexibility of the bio-flexy films.

e) Drug content uniformity

The range of drug content uniformity for the prepared bio-flexy films was found to be  $86.51 \pm 0.23$  to  $94.47 \pm 0.45$  %. No significant difference was observed in the drug content of the prepared bio-flexy films which indicated that the drug is uniformly dispersed throughout the bio-flexy films.

f) In-vitro drug release study

The drug release of bio-flexy films were analysed by using BIT-SOFT. The drug release profile was found to be in the order AC2 > AC3 > AC1 > AC4 > AC5. AC2 (1:2) was found to be the best formulation having  $t_{50}$  3.6 hrs,  $t_{80}$  26.8 hrs,  $R^2$  value 0.9989, zero order as best fit model and anomalous transport as mechanism for drug transport analyzed by BIT-SOFT 1.12.

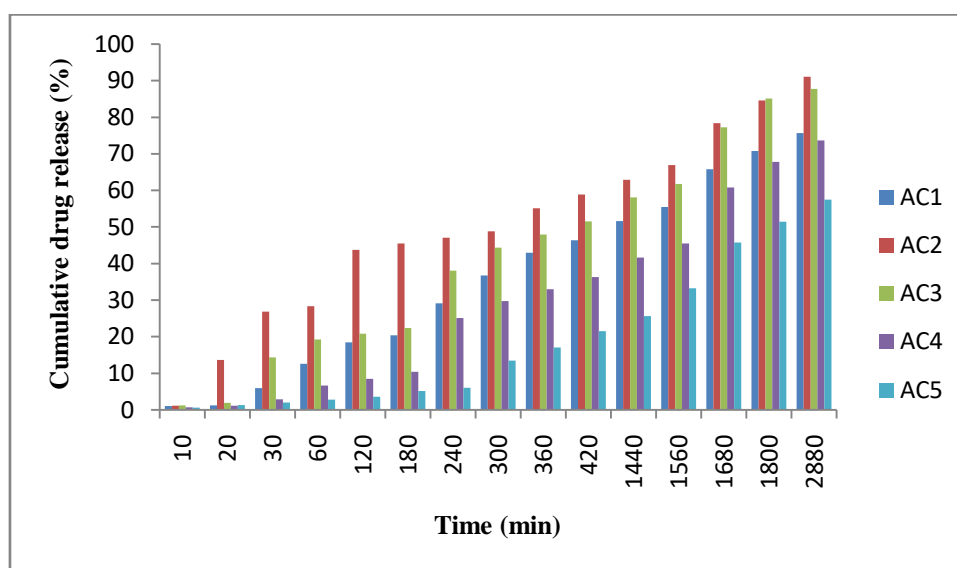


Fig. 4: In-vitro drug release of formulations.

g) Stability studies

During and at the end of stability studies, the formulations showed no change in physical appearance and flexibility. They showed insignificant difference for *in-vitro* drug release. This showed that the formulations were physically and chemically stable during the study.

#### IV. CONCLUSION

Atorvastatin is the most selling drug used for lowering the cholesterol level in the body. The problem with the drug is low bioavailability and higher risk for side effects. In this research work, an attempt has been made for formulating bio-flexy films. Bio-flexy films can act as a promising formulation for drug delivery. By nanosizing the drug, amount of the drug administered is reduced thus minimising the dose related side effects of the drug. This route by passes the first pass metabolism and thus increases the bioavailability of the drug. The bio-polymer isolated from natural edible source, *Coriandrum sativum* was found to

be biodegradable, non-toxic, and non-reactive and can be effectively isolated in large quantity. Bio-flexy films were prepared using the isolated biopolymer, nanosized atorvastatin and other co-processing agents. The isolated bio-polymer can further be used as a promising excipient for formulating various pharmaceutical formulations.

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