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Development and evaluation of Rosuvastatin loaded bio-flexy film using a novel flexy film former from *Cleome viscosa*

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Abstract
The main objective of the study was the formulation and evaluation of bio-adhesive flexy films to deliver nanosized rosuvastatin. The bio-flexy films were aimed to deliver through the nabhi. A novel bio-film former was isolated from the seeds of a natural edible source, *Cleome viscosa*. The isolated biopolymer was screened for its film forming ability by preparing bi-flexy films of the isolated biopolymer by solvent casting method. Five formulations (RC1-RC5) were prepared by varying the polymer: drug ratios (1:1, 1:2, 1:3, 1:4, 1:5) by solvent casting method. Rosuvastatin was nanosized, and used as the model drug. The formulated bio-flexy films were tested for surface pH, thickness, weight variation, content uniformity, folding endurance. *In-vitro* study showed that formulation RC2 (1:2) was the best formulation having R^2 value 0.9286, and Higuchi-Matrix as best fit model. The conclusion was drawn that the isolated biopolymer has good film forming capacity, and can be utilised for designing various pharmaceutical formulations.

Keywords: *Cleome viscosa*, biopolymer, film former, bio-flexy films, rosuvastatin

1. Introduction
Cleome viscosa is known as dog mustard or wild mustard, and in local language this is also known as 'jakhiya'. It is an annual plant which belongs to the family capparacae or cappardaceae. This plant is highly rich in a number of clinical constituents which are useful for various pharmacological and medicinal activities. It shows antimicrobial, analgesic, antiemetic, antidiarrheal, hepatoprotective, anticonvulsant, psychopharmacological, antitumor, antifibrotic activities. It is also useful in piles, eyesore, earache, lumbago, skin diseases, malarial fever, jaundice etc.[1,2,3]
Hyperlipidemia is considered as one of the main cause of cardiovascular diseases. It is a condition of increase in the amount of one or more plasma lipids including phospholipids, cholesterol, triglycerides, and plasma lipoproteins. Hyperlipidemia leads to atherosclerosis and atherosclerosis associated diseases [4]. Statins are the first line choice for the treatment of hyperlipidemia.
Rosuvastatin is the seventh drug in the category of statins and got approved in 2003. The drug acts by competitively inhibiting HMG CoA reductase enzyme. This enzyme is located in hepatic tissue and produces mevalonate. The production of mevalonate is the major step in the synthesis of cholesterol. Thus by inhibiting HMG CoA reductase enzyme, it reduces the production of cholesterol in the body, which in turns decreases the amount of low density lipoproteins. The oral bioavailability of the drug is low, which is only 20% [5]. The reason for its low bioavailability may be due to the low solubility of the drug. An attempt has been made to avoid this problem by formulating bio-flexy films for trans-nabhi drug delivery.

2. Materials and Methods
Rosuvastatin was obtained as a gift from Mylan Laboratories Ltd, Delhi, India. *Cleome viscosa* was procured from local market of Dehradun, Uttarakhand, India. All other reagents used were of analytical grade.

2.1 Extraction of biopolymer from *Cleome viscosa*
200 gm *Cleome viscosa seeds* were taken and soaked in distilled water. The seeds were ground and soaked in CCl_4. The upper layer of the mixture was decanted and treated with 2-propanone in the ratio (1:2). It was refrigerated for 24 hrs. The biopolymer was collected by centrifugation at 3000 rpm for 15 minutes. The biopolymer was kept for drying in the desiccators. The dried biopolymer was further purified by using hot dialysis method. The procedure was repeated six times, and the percentage yield of the biopolymer was calculated. The purified biopolymer was passed through 200# sieve and stored in a closed container for further use. [6]

2.2 Characterization of the isolated biopolymer
The isolated biopolymer was checked for various physicochemical parameters like texture, color, solubility, the presence of carbohydrates, proteins and starch; IR, SEM, DSC, NMR spectroscopy studies, etc.

2.3 Drug-excipient interaction study
The drug interaction study was performed to check the compatibility of the drug with other excipients used in the formulation. The study was performed by the wet and dry method. The nanosized drug was mixed with excipients in three ratios (1:1, 1:3, and 3:1). The mixtures were kept at the room temperature for three days. The mixtures were diluted with methanol, and the samples were analyzed by ultraviolet spectrophotometric method (Shimadzu 1800).[6]

2.4 Preparation of nanosized Rosuvastatin loaded bio-flexy films
Rosuvastatin was nanosized by using a novel method. Rosuvastatin 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 were observed at wavelength 200-800nm. The solution was micro centrifuge. Nanosized Rosuvastatin was obtained and dried. It was kept in a desiccator for 24 hrs. The nanosized drug was collected and stored in a cool and dry place.

Bio-flexy films were prepared by the 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 Rosuvastatin used as a model drug was dissolved in a 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 a tightly closed container [7].

Table 1: Formula for Bio-flexy films

Ingredients	RC1	RC2	RC3	RC4	RC5
Nanosized Rosuvastatin (mg)	10	10	10	10	10
Cleome viscosa 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

2.5 Evaluation Of bio-flexy Films

2.5.1 Weight

Weight uniformity study of bio-flexy films was performed by taking a piece (1 cm^2) from three films of each batch. All the patches were weighed individually on an electronic balance. The average weight was calculated and noted [8, 9].

2.5.2 Thickness

The thickness of the films was determined by using a micrometre screw gauge. The thickness was measured at three different places of each film. Average was calculated and reported with standard deviation [8, 9].

2.5.3 Folding endurance

The bio-flexy film was subjected to folding endurance by repeatedly folding the film at the same place till it broke. The number of times, the film could be folded at a place without cracking was recorded, and reported as the folding endurance. The same was repeated with randomly selected three films from each batch [8, 9].

2.5.4 Surface pH

A piece (1 cm^2) of ach formulation was cut, and placed in a Petri dish. The film was moistened with 0.5 ml of distilled water and allowed to swell. After 1 hr, the surface pH of the film was measured by using pH meter. The procedure was repeated three times and the average value was calculated [6].

2.5.5 Drug content uniformity

The bio-flexy film (1 cm^2) was taken and dissolved in methanol. The volume was made up to 100 ml with phosphate buffer of pH 7.4. The solution was stirred on magnetic stirrer, and kept for 24 hours. From this solution, 0.1 mL sample was withdrawn and diluted to 10 ml with the buffer. The absorbance was measured by using UV Spectrophotometer. The same procedure was performed for all the formulations. From the absorbance, % drug content was calculated [8, 9].

2.5.6 *In-vitro* drug release study

The *In-vitro* drug release study was performed by using MS diffusion apparatus. A thermostatically controlled environment was created and the temperature was maintained at 37ºC using orbital shaker incubator. Egg membrane was tied to the donor compartment. A piece of bio-flexy film has adhered onto the egg membrane in the donor compartment. Phosphate buffer of pH 7.4 buffer solution was filled in the receptor compartment. 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) [5, 10].

2.5.7 Stability studies

The accelerated stability studies were performed as per the ICH guidelines for six months for all the formulations. After every 15 days, the films were checked for any change in the physical appearance, and *In-vitro* drug release [11].

3. Results and Discussion

3.1 Characterization of the isolated biopolymer

Table 2: Physico-chemical properties of isolated biopolymer

Yield	10.04±0.65 %
Color	Buff
Texture	Smooth powder
Odour	Odourless
Color changing point	171±4ºC
Test for carbohydrate	Positive
Test for protein	Positive
Test for starch	Negative

The IR spectra (Fig. 1) revealed the presence of O-H stretch of alcohol (3410.9 cm-1), C-O stretch of carboxylic (1149.15, 1256.2, 1040.12 cm-1), NH of amide (1621.11 cm-1). SEM analysis of the biopolymer (Fig. 2) showed that the biopolymer has a smooth crystalline surface. It showed the morphological structure similar to the polymers which confirm that the biopolymer is polymeric in nature.

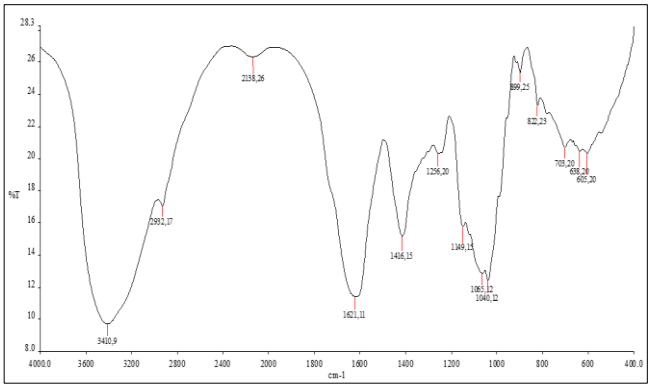

Fig 1: IR spectrum of *Cleome viscosa* bio-polymer

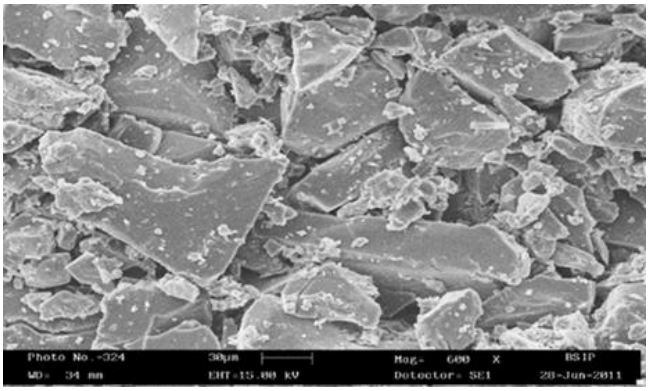

Fig 2: SEM of *Cleome viscosa* bio-polymer

3.2 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.

3.3 Nanosizing of Rosuvastatin

The percentage of transmittance at different wavelength represents that the amount of light passed through the particles, which means that the particles present in the solution are below that wavelength. Whereas, the % blockade indicates the % particle which are above that wavelength. The percentage of transmittance was measured by UV spectrophotometer and after each cycle increase in the % transmittance was observed which indicated that the particles may have been reduced to nano range. The effect of sonication on the percentage of transmittance after each cycle is shown in fig. 2.

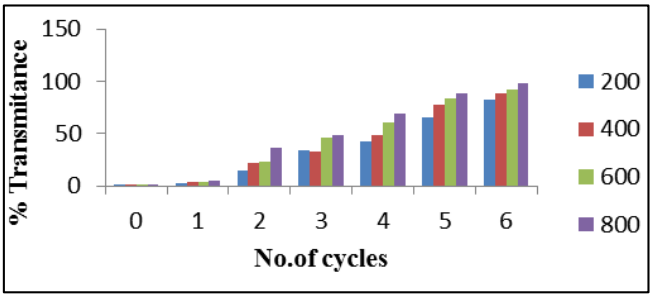

Fig 3: Nanosizing characterization by UV spectroscopy.

3.4 Thickness, weight, folding endurance and surface pH

The thickness of the bio-flexy films RC1 to RC5 containing *Cleome viscosa* biopolymer ranged from 0.26 ± 0.06 to 0.58 ± 0.04 mm.

The weight of the bio-flexy films RC1 to RC5 containing *Cleome viscosa* biopolymer ranged from 11.22 ± 0.08 to 37.61 ± 0.47 mg.

The microenvironmental pH of the formulations ranged from 6.54 to 6.85. The pH of the bio-flexy films was found to be close to the pH of the skin. It indicates that the formulations will have no irritation effect on the skin when applied.

Folding endurance of the bio-flexy films ranged from 164 to 187 (times) which indicates rationale flexibility of the bio-flexy films.

3.5 Drug content uniformity

The drug content uniformity of the formulations was found to be in the range of 91.66±0.06 to 96.21±0.05 %. This indicates the uniform distribution of the drug in the formulations.

3.6 *In-vitro* drug release study

The *In-vitro* drug release study data was interpreted by using BIT-SOFT 1.12. It was observed that the drug release profile was in the order RC2> RC1> RC3> RC4> RC5. RC2 (1:2) was found to be the best formulation having t_{50} 7.2 hrs, t_{80} 32.4 hrs, R^2 value 0.9286, Higuchi-Matrix as the best fit model. The mechanism for drug transport was observed as Fickian diffusion.

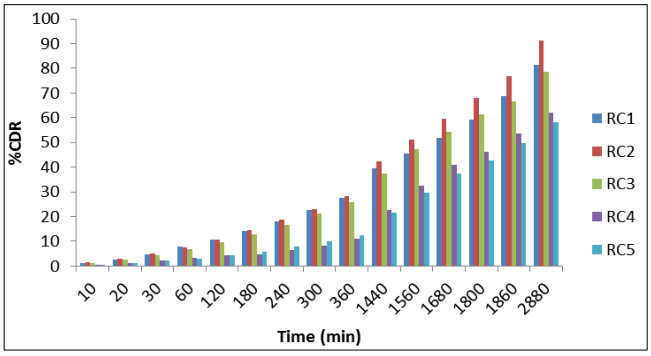

Fig 4: *In-vitro* drug release of bio-flexy films.

3.7 Stability studies

The bio-flexy films showed no physical change and flexibility. No significant change in the content uniformity and *In-vitro* drug release was observed. The study revealed that the formulations were stable.

4. Conclusion

Rosuvastatin is the most effective drug for the treatment of hyperlipidemia. But, the problem with this drug is its low bioavailability due to less solubility, and risk of several side effects. In this research work, we have developed and evaluated nanosized Rosuvastatin loaded bio-flexy films. Formulation of bio-flexy films results in the elimination of step involving the dissolution of the drug to deliver it in the systemic circulation, hence the problem related to the low solubility of the drug can be avoided. By nanosizing the drug, amount of the drug administered is reduced thus minimizing the dose-related side effects of the drug. Bio-flexy films can act as a promising formulation for drug delivery. The biopolymer isolated from the natural edible source, *Cleome viscosa* was found to be biodegradable, non-toxic, and non-reactive. The biopolymer showed good film forming ability. It was found to be an effective bio-excipient for designing the formulations. The formulation RC2 was found to be the best formulation among all. The results revealed that controlled drug release can be achieved for up to 48 hrs of the delivery. The isolated biopolymer can further be used as a promising excipient for formulating various pharmaceutical formulations.

5. References

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