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Formulation and Evaluation of Nanosponges Containing Murraya Koenigii Extract for Burn Wound Healing

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ABSTRACT

Murraya koenigi is a medium sized tree belonging to the family Rutaceae. The major constituents present in the Murraya koenigii leaves treat burns, bruises. Ayurveda is a traditional Indian medicinal system practiced for thousands of years. Natural remedies are more acceptable in the faith that they are safer with less side effects than the synthetic ones. The novel formulations are reported to have remarkable advantages over conventional formulations of plant actives and extracts which include enhancement of solubility, bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improved tissue macrophages distribution, sustained delivery and protection from physical and chemical degradation. Our main purpose is to treat/heal wound at faster rate with minimum side effects. Nanosponges alone are difficult to use on local tissues because they diffuse away to other parts of the body very quickly so to overcome the drawback we combined nanosponges with the herbal extract of *Murraya Koenigii.* According to literature survey it was found that ethanolic and aqueous extract shows good wound healing results. Research on curry leaves revealed that they are also effective in fighting bacterial and fungal infections. The leaf extracts from the plant have been comparable to popular main stream antibiotic drugs. During this research work, we prepared ethanolic extract of Murraya Koenigii leaves and prepared nanosponges from it for treating burn wound. The nanosponges are prepared by Quasi emulsion solvent diffusion method. The prepared nanosponges were evaluated for various in-vitro parameters and the results obtained were satisfactory.

Keywords: Nanosponges, Murraya koenigii, wound, solubility.

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INTRODUCTION

The ideal delivery systems solubilize the drug; lead the drug to the target site to fulfill the individual need of the patient and disease stage. Nanosponges are versatile drug delivery system as it can load both hydrophilic and hydrophobic drugs. Nanosponges are solid, porous, biocompatible, tiny in size with 3 dimensional structures and nanomeric cavity size which have unique ability to entrap wide variety of drugs.

Nanosponges are a novel class of nanoparticles with nanostructured hyper branched polymers and few nanometers wide cavities in which a large variety of substances can be encapsulated. The nanosponges drug delivery platform is a network of specific polymers that slowly degrades and thus releases the chosen drug. The nanosponges can be synthesized to be of specific size and to release drugs over time by varying the proportion of cross-linker to polymer.

Now regarding its mechanism of drug release, the sponge particles contain an open structure and it contains the active ingredients which are free to move in and out from the particles and into the medium until equilibrium is reached. In case of topical drug delivery, once the finished product is applied to the target tissue, the active ingredient which is already present in the vehicle will be absorbed into it, depleting the vehicle, which will become unsaturated, hence disturbing the equilibrium.

This will lead to flow of the active drug ingredient from the sponge particles into the vehicle and from it to the target tissue until the vehicle is either dried/absorbed.

The term gel was introduced in late 1800. The USP defines gel as a semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecules enclosed and interpenetrated by liquid.

A burn is a type of injury to skin caused by heat, electricity, light, radiation or friction. The wound healing process is a series of independent and overlapping stages. In these stages both cellular and matrix compounds work to reestablish the integrity of damaged tissues and replacement of lost tissues. These overlapping series are classified into 5 stages – Haemostasis, Inflammation, Migration, Proliferation and maturation.

MATERIALS AND METHOD

The raw materials like drug, polymers, excipients and chemicals required for the present work were procured from different sources. Following materials were used for the formulation and evaluation of nanosponges and gel.

Table 1: List of drug, Excipients, Polymer and Solvent

Sr.no	Drug/Polymer/Excipients/Solvent	Manufacturer
1	Leaves of Murraya Koenigii	Botanical garden of Satara College of Pharmacy, Satara
2	Eudragit RS100	Loba Chemie Pvt. Ltd., Mumbai
3	Polyvinyl alcohol	Loba Chemie Pvt. Ltd., Mumbai
4	Dichloromethane	S.D. Lab Chem. Mumbai
5	Ethanol	S.D. Lab Chem. Mumbai
6	Carbopol 934	Research –Lab Fine Chem. Industries, Mumbai
7	Methyl paraben	Loba Chemie Pvt. Ltd., Mumbai
8	Propyl paraben	Loba Chemie Pvt. Ltd., Mumbai
9	Triethanolamine	Research- Lab Fine Chem. Industries, Mumbai.

PREFORMULATION STUDIES

A)   Characterization of Murraya koenigii extract:

a.   Organoleptic properties:

Murraya Koenigii leaves extract was evaluated for its organoleptic properties such as colour, odour, and taste.

b.   Determination of pH:

The crude powder of Murraya Koenigii was dissolved in distilled water and was kept in water bath for 20 min, filtered and then pH of the filtrate was noted down with the help of pH meter.

c.   Phytochemical tests performed:

d.   Determination of total ash :

The total ash value of crude powder of Murraya Koenigii was determined by incinerating 2 g of accurately weighed crude powder in a silica crucible. It was incinerated in a muffle furnace at a temperature not exceeding 450º C until free from carbon, then cooled and weighed.

Determination of water soluble ash:

The total ash obtained was boiled with 25 ml of distilled water for 5 min. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at allowed temperature. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Determination of acid insoluble ash:

The ash obtained in the above method was boiled with 25 ml of dilute hydrochloric acid for 5 min. The residue was collected on ash less filter paper and washed with hot water, ignited, cooled and weighed. The percent of acid insoluble ash with reference to air dried drug was calculated.

Determination of loss on drying:

The loss on drying was determined by weighing 2 g of crude powder of Murraya Koenigii in an evaporating dish and then dried in an oven at 105° C till constant weight was obtained and loss on drying was calculated. The percent loss on drying was calculated on the basis of sample taken initially.

Determination of melting point :

Melting point of Murraya Koenigii extract was determined by micro controlled based melting point apparatus. The sample was inserted in capillary having one end closed. Then the capillary was inserted in bath of silicone oil which was heated in controlled manner with the help of electric heating coil. The temperature at which bubble formation occurs was noted as melting temperature.

B)   Calibration curve of Murraya Koenigii extract in Phosphate buffer pH 5.5.

Accurately weighed 100 mg (0.1 gm) Murraya Koenigii extract was taken and transferred to 100 ml volumetric flask and volume was made to 100 ml with petroleum ether (Stock I). The 10ml solution from above stock I solution was again diluted with methanol and volume was made to 100 ml (Stock II). The final solutions of stock II were then prepared in methanol. From Stock II solution aliquots of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml were transferred to 10 ml volumetric flasks and final volume was made to 10ml with methanol in the concentration range of 10-100 µg/ml. The absorbance values of these solutions were measured at 239.5 nm using double beam UV spectrophotometer (Shimadzu, PharmaspecUV-1700, Japan) against blank of methanol.

Drug – excipient compatibility studies:

Table 2: Samples used in drug-excipient compatibility studies

Pure sample	Drug + Excipient
Murraya Koenigii extract	-----
Eudragit RS 100	Eudragit RS 100 + Extract
Polyvinyl alcohol	Polyvinyl alcohol + Extract
Carbopol 934	Carbopol 934 + Extract

Visual observations:

The samples subjected to drug-excipient compatibility studies were assessed for any visual changes. The samples were observed for change in colour and nature.

Infrared Spectroscopy:

IR was determined by Fourier Transform infrared spectrophotometer (FTIR – 4100, Jasco, Japan).

A.   Formulation of Nanosponges :

Table 3: Ingredients used for formulation of nanosponges of Murraya koenigii leaves extract.

Sr. no	Ingredients	Use
1.	Murraya Koenigii extract	API
2.	Eudragit RS 100	Polymer
3.	Polyvinyl alcohol	Stabilizer
4.	Dichloromethane	Solvent
5.	Distilled water	Vehicle

Full Factorial design:

Table 4: Full factorial experimental design layout

Formulation code	Coded values	
	A	B
F1	+1	0
F2	0	+1
F3	-1	-1
F4	+1	-1
F5	-1	0
F6	-1	+1
F7	+1	+1
F8	0	0
F9	0	-1

Table 5: Design Summary

Factor	Name	Unit	Type	Coded level			Actual level		
				Low	Medium	High	Low	Medium	High
A	Eudragit RS100	gm	Numerical	-1	0	+1	0.5	1	1.5
B	Polyvinyl alcohol	gm	Numerical	-1	0	+1	0.25	0.5	0.75

Preparation of Blank Nanosponges:

Blank nanosponges were prepared by Quasi – emulsion solvent diffusion method. The inner phase was prepared by dissolving Eudragit RS 100 in a suitable solvent i.e dichloromethane. The inner phase was then poured into the polyvinyl alcohol solution in water (Outer Phase). Following 60 min of stirring (rpm 800-900), the mixture was filtered to separate the nanosponges. The nanosponges were dried in an air heated oven at 40ºC for 12 h.

Table 6: Composition of blank nanosponges

Batch no.	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eudragit Rs 100(gm)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
Polyvinyl alcohol (gm)	0.25	0.25	0.25	0.50	0.50	0.50	0.75	0.75	0.75
Distilled water (ml)	100	100	100	100	100	100	100	100	100

Preparation of drug loaded Nanosponges:

Drug loaded nanosponges were prepared by Quasi – emulsion solvent diffusion method. The inner phase was prepared by dissolving Eudragit RS 100 in a suitable solvent i.e. dichloromethane. Then drug was added to solution and dissolved under ultra sonication at 35 ℃. The inner phase was then poured into the polyvinyl alcohol solution in water (Outer Phase). Following 60 min of stirring (rpm 800-900), the mixture was filtered to separate the Nanosponges. The Nanosponges were dried in an air heated oven at 40℃ for 12 h

Table 7: Composition of drug loaded Nanosponges

Batch no.	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (gm)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Eudragit Rs 100(gm)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
Polyvinyl alcohol (gm)	0.25	0.25	0.25	0.50	0.50	0.50	0.75	0.75	0.75
Distilled water (ml)	100	100	100	100	100	100	100	100	100

Evaluation of Nanosponges:

1) Visual inspection:

The visual inspection of nanosponges was determined by optical or binocular microscopy.

2)   Determination of production yield:

The production yield of the nanosponges was determined by calculating accurately the initial weight of the raw materials and the final weight of the nanosponges obtained.

Production Yield (PY) =Practical mass of nanosponges×100/Theoretical Mass (Polymer + Drug)

 (Eq-1)

 Actual drug content and Entrapment Efficiency:

The actual drug content was determined by the amount of drug which was entrapped in nanosponges. The weighed amount of drug loaded nanosponges (50mg) was kept in 10 ml ethanol and soaked for 3 h. The samples were filtered and analyzed at 239.5nm against blank using UV spectrophotometer (Shimadzu, Pharma Spec UV-1700, Japan). Encapsulation efficiency was calculated by following formula:

Entrapment efficiency (%) = Total amount of drug ─ Free un entrapped drug× 100/ Total amount of drug -- (Eq-2)

3)   Differential Scanning Calorimetry (DSC) :

Thermal analysis is an important evaluation technique to find any possible interaction between the drug and excipient. Such interaction can be identified by any change in thermogram. Thermogram of pure Murraya koenigii leaves extract and finished nanosponges formulations were obtained using DSC instrument (Mettler Toledo DSC 821Ce, Switzerland) equipped with an intercooler. Indium

standard was used to calibrate the DSC temperature and enthalpy scale. The powder sample of nanosponges was kept in the aluminium pan and heated at constant rate of 5°C/min up to 300°C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 10ml/min.

4) Scanning Electron microscopy:

Scanning electron microscopy was used to study the microscopic aspects of the nanosponges. The morphology of nanosponges was carried out by using zeta sizer.

5) Particle size analysis:

Particle size analysis of prepared nanosponges was carried out by using zeta sizer (Particulate system nano plus).

6) Zeta potential:

Zeta potential of optimized nanosponges was measured by using zeta sizer at 25°C (Particulate system nano plus).

B.　　Formulation and evaluation of Murraya koenigii extract nanosponges gel

Preparation of Murraya koenigii extracts nanosponges gel:

1% Carbopol 934 was allowed to soak for 24 h in distilled water. On next day accurately weighed Murraya koenigii extract nanosponges were added to the gel base. Triethanolamine was added drop wise to the formulation for adjustment of required pH (5.5-5.6) and to obtain the gel in required consistency. Finally preservatives were added in the carbopol solution

Table 8: Composition of Gel containing Nanosponges of Murraya koenigii extract

Batches	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nanosponges eq. to 100mg drug	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl paraben(g)	0.015	0.020	0.025	0.015	0.020	0.025	0.015	0.020	0.025
Propyl paraben(g)	0.05	0.010	0.015	0.05	0.010	0.015	0.05	0.010	0.015
Triethanolamine(ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Carbopol 934(gm)	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

1) **Physical appearance:** The physical appearance of the formulation was checked visually.

- **Color:** The color of the formulation was checked out against white and black background.
- **Consistency:** The consistency was checked by applying on skin.
- **Odor:** The odor of gel was checked by mixing the gel in water and taking the smell.

2) **Determination of pH:**

The pH of gel was determined using digital pH meter by dipping the glass electrode completely into the gel system.

3) Determination of Spreadability:

Spreadability was determined by modified wooden block and glass slide apparatus. The apparatus consisted of a wooden block with fixed glass and a pulley. A pan was attached to another glass slide (movable) with the help of a string. For the determination of Spreadability measured amount of gel was placed on the fixed glass slide. The movable glass slide with a pan attached to it, it was placed on other fixed glass slide such that the gel was sandwiched between the two slides for 5 min. About 50gm of weight was added to the pan. Time taken for the slides to separate was noted. Spreadability was determined using formula:

$$S = M.L/T \qquad\qquad\qquad\qquad --- (Eq-3)$$

4) Determination of drug content:

The drug content of gel formulation was determined by dissolving an accurately weighed quantity (1g) of gel in 100 ml of solvent (pH 5.5-5.6). The solutions were kept for stirring up to complete dissolution of the formulations. Solutions were filtered and were subjected to spectrophotometric analysis. The drug content was calculated from calibration data.

$$\text{Drug Content} = \frac{\text{Actual conc.}}{\text{Theoretical conc.}} \times 100 \qquad ------ (Eq-4)$$

5) In vitro drug release studies:

Release of the Murraya koenigii extract from nanosponges incorporated in various gel formulations was measured through standard cellophane membrane using a Franz diffusion cell. Prior to study, cellophane membrane was soaked in diffusion medium for overnight, and then placed on the support screen of the diffusion cell assembly. Phosphate buffer pH 5.5 was used as the receptor medium and 1gm of the gel was placed on the donor side. All batches of Murraya koenigii extract (F1-F9) were used for diffusion study. At predetermined time intervals, 2ml of sample was withdrawn from the receptor compartment and replaced with same volume of phosphate buffer pH 5.5. The aliquots were analyzed by UV spectrophotometer at 239.5 nm against PBS (pH 5.5).

C. In-vivo studies for burn wound healing activity by using suitable animal model:

Burn wound healing activity:

➢ Animals: Wistar rats Male/Female (n=18)

➢ Weight: 200-300 g.

➢ Group I: Nanosponges gel containing Murraya koenigii extract.

➢ Group II: Standard (Silver sulphadiazine cream)

➢ Group III: Control (No treatment)

Procedure:

Albino Wistar rats of either sex weighing 200- 250 gm (3 months old) were randomly selected marked to permit individual identification and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory condition. Animals were not being deprived of food and water. The animals were divided into 3 groups of 6 animals each then dorsum of each rat was shaved. Immediately after the procedure anesthesia was given to animal. The burn was made by exposing the back skin to red hot beaker. The wounding day was considered as day 0. Prepared formulations were placed on the wound site. Average wound contraction was measured by a tracing paper on the wounded margin and calculated as percentage reduction in wound area. The wound contraction is calculated as percentage of the original wound size for each animal of a group. The percent of wound size induced was calculated by:

Percentage Wound Contraction (%) = Initial Wound size-specific wound size / initial wound size ×100

a. Shaved animal   **b. 0th day wound**   **c. 1st day wound**

Figure.1: Burn Wound

RESULTS AND DISCUSSION

A.   Collection of plant material:

B.   Authentication of plant material:

The authentication of seeds was done from department of Botany, Yashwantrao Chavan institute of Sciences Satara (Maharashtra) and it was confirmed that the procured leaves were of Murraya koenigii plant.

C.   Extraction of Murraya koenigii leaves:

Extract was obtained in sufficient quantity from the leaves of Murraya koenigii by soxhlet extraction method.

Figure 2: Soxhlet extraction of leaves of plant and extract of leaves

D.   Preformulation study:

A)   Characterization of Murraya koenigii extract:

a.   Organoleptic properties:

b.   Determination of pH: pH of the extract was found to be 6.7, while as per literature standard it is reported to be 6.3-6.4. As experimental values were in good agreement with official values, it could be concluded that procured extract was in pure form.

c.   Phytochemical tests performed:

Table 9: Phytochemical tests

Sr. no	Constituents	Tests	Observations
1.	Alkaloids	1. Dragendroff's test	Present
		2. Hager's test	Present
2.	Protein	Millions test	Present
3.	Saponin test	Foam test	Present
4.	Glycoside	Molisch's test	Present
5.	Protein	Millions test	Present
6.	Triterpenoids	Liberman burchard test	Present
7.	Flavinoids	Shinoda test	Present
8.	Terpenoids	Noller's test	Present

Test for alkaloids:

1. Dragendroff's test - This test was performed which showed formation of orange brown precipitate that indicated the presence of alkaloids.

2. Hager's test – This test was performed which showed formation of yellow precipitate that indicated the presence of alkaloids.

Test for glycoside:

1. Molisch's test- This test was performed which showed formation of violet ring at the junction of 2 liquids that indicated the presence of glycosides.

Test for Saponin:

1. Foam test- This test was performed which showed foam formation that indicated the presence of saponin.

Test for proteins and amino acids:

1. Millions test- This test was performed which showed formation of white precipitate. Warm the obtained precipitate which converted it into brick red colour that indicated the presence of proteins and amino acids.

Test for Triterpenoids:

1.   Lieberman burchard test- This test was performed which showed formation of first red, and then blue and finally green colour indicated presence of Triterpenoids.

Test for flavonoids:

1.   Shinoda test- This test was performed which showed formation of red coloration that indicated presence of Flavanoids.

d.   Determination of total ash: The total ash value of Murraya koenigii was found to be 12.5%, while as per literature standard it is 11.33%.

1.   Determination of water soluble ash: The water soluble ash value was found to be 2%, while as per literature standard is 1.97%.

2.   Determination of acid insoluble ash: The acid insoluble ash value was found to be 5%, while as per literature standard it is 5.33%.

e.   Determination of loss on drying: The loss on drying was found to be 10.43 gm., while as per literature standard it is 10.06±0.15gm.

f.   Determination of refractive index: The refractive index was found to be 1.5010, while as per literature standard it is 1.5021.

g.   Determination of melting point: The melting point of Murraya koenigii was found to be 99 ℃- 100 ℃, while as per literature standard it is 98°C-100°C.

h.   Determination of extractive values

1.   Alcohol soluble extractives: The alcohol soluble extractive was found to be 7.2%, while as per literature standard it is reported to be 7.75%.

2.   Water soluble extractive: The water soluble extractive was found to be 9.52%, while as per literature standard it is reported to be 9.56%.

B)   Identification of Murraya koenigii extract:

1.   Ultraviolet spectroscopy:

The λmax value of Murraya koenigii extract was found to be 239.5nm in phosphate buffer pH 5.5.This was in well compliance with the λmax value of Murraya koenigii extract in literature.

Figure 3: UV spectrum of Murraya koenigii extract in phosphate buffer pH 5.5
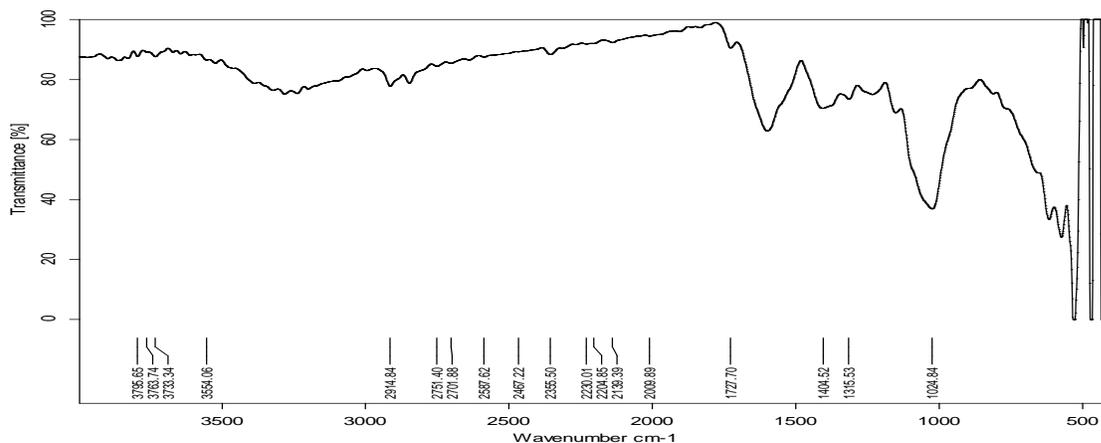
C)   Infrared spectroscopy:

Figure 4: FTIR spectrum of Murraya koenigii extract

DSC:
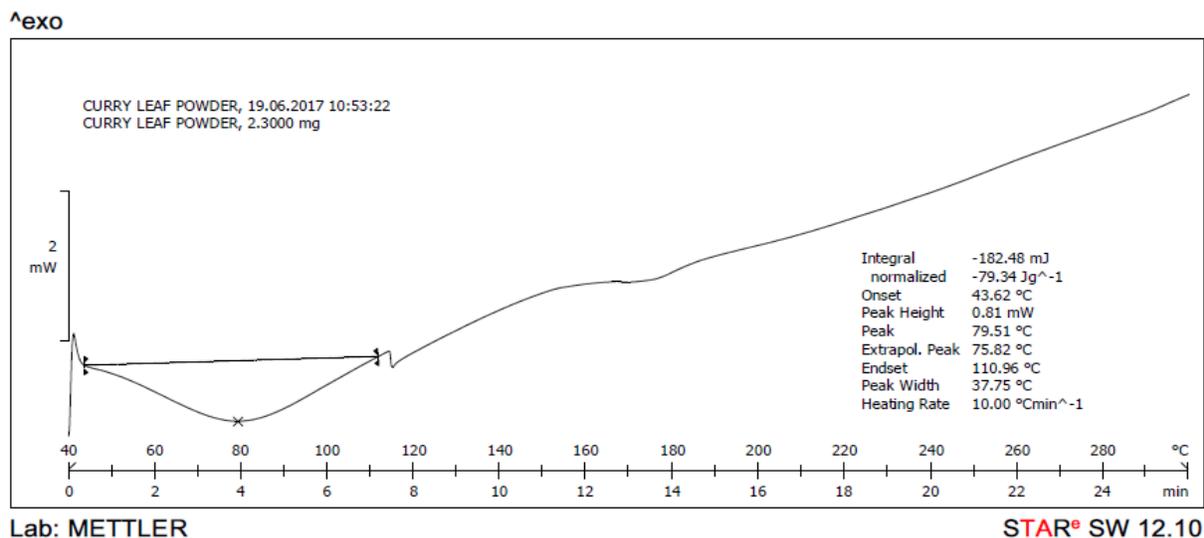
Figure 5: DSC of Murraya koenigii dried powder.

In DSC thermogram of Murraya koenigii extract endothermic peak was observed at 75.82ºC.
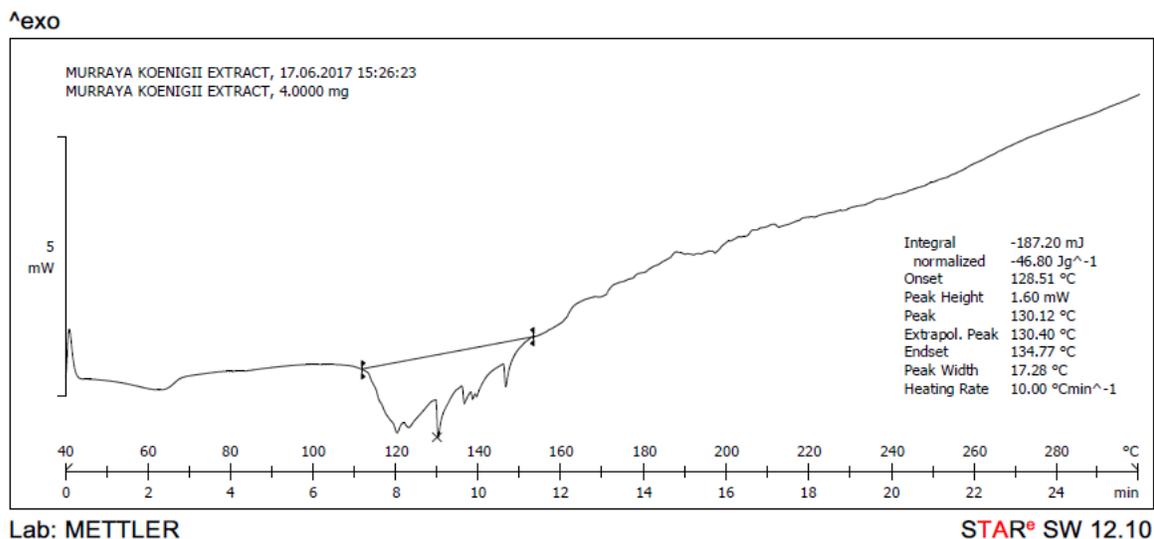
Figure 6: DSC Thermogram of Murraya koenigii extract

In DSC thermogram of Murraya koenigii extract endothermic peak was observed at 130.40ºC.

D)   **Calibration curve of Murraya koenigii extract in phosphate buffer pH 5.5:**

The calibration curve for Murraya Koenigii extract in phosphate buffer pH 5.5 was plotted by using following results of absorbance at various concentrations.

Table 10: Absorbance of Murraya koenigii extract in phosphate buffer pH 5.5

Sr. No	Concentrations (µg/ml)	Absorbance
1.	0	0
2.	10	0.0794
3.	20	0.176

4.	30	0.330
5.	40	0.472
6.	50	0.5721
7.	60	0.7021
8.	70	0.7237
9.	80	0.9212
10.	90	1.0419
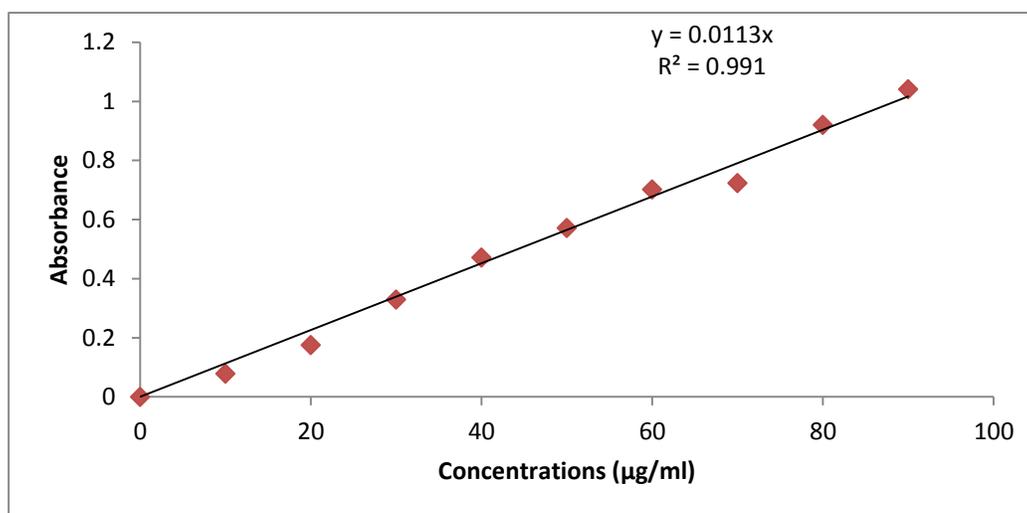
Figure 7: Calibration curve of Murraya koenigii extract in phosphate buffer pH 5.5

A.   Drug – excipient compatibility studies.

1.   Visual observations:

No notable change was observed in the sample on visual observation. There was no observable color change.

2.   Infrared spectroscopy:

FTIR spectrum of the mixture of Murraya koenigii extract and excipients was compared with spectra of individual components. An FTIR spectrum of physical mixture shows significant peaks of Murraya koenigii extract and respective excipient indicating no chemical interaction between Murraya koenigii extract and excipient.

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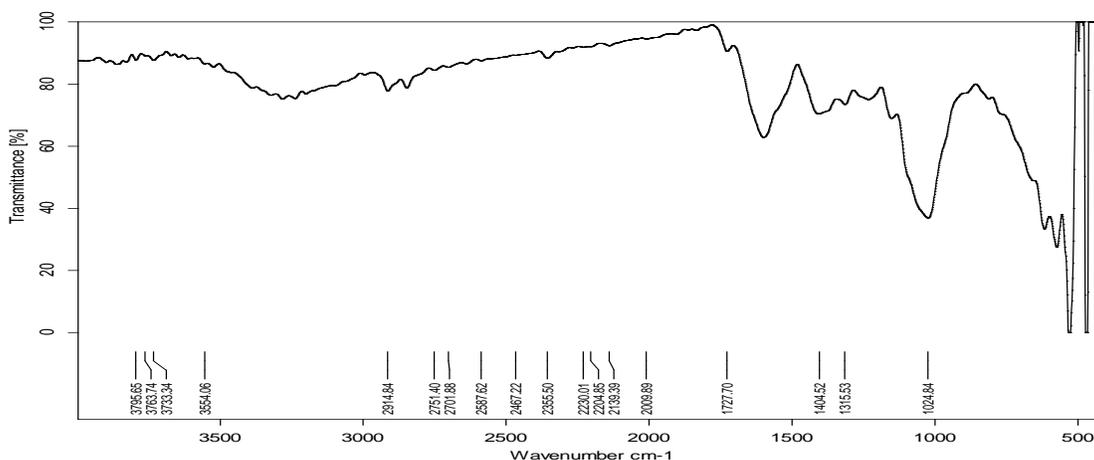
Figure 8: FTIR spectrum of Murraya koenigii
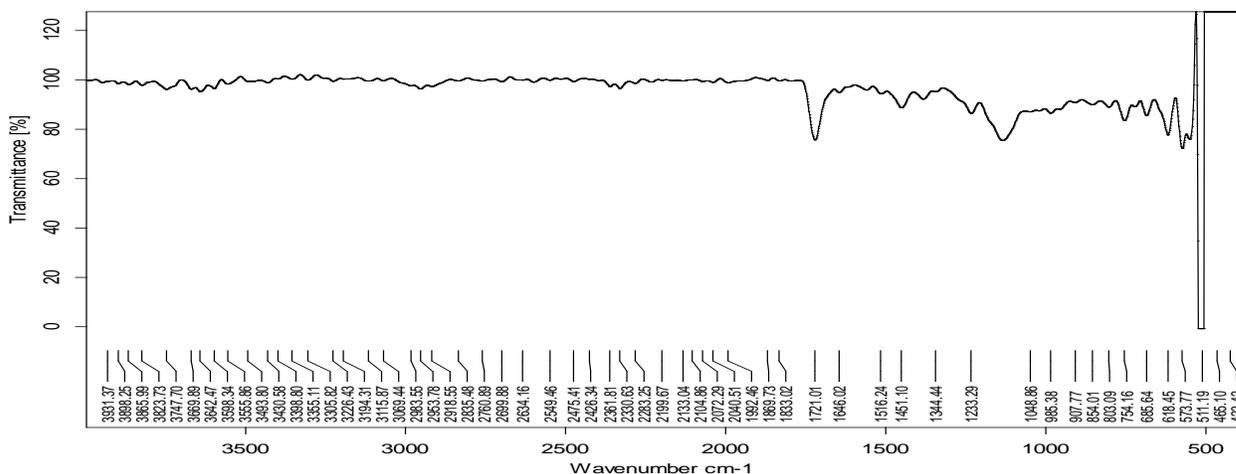
Figure 9: FTIR spectrum of Eudragit RS100

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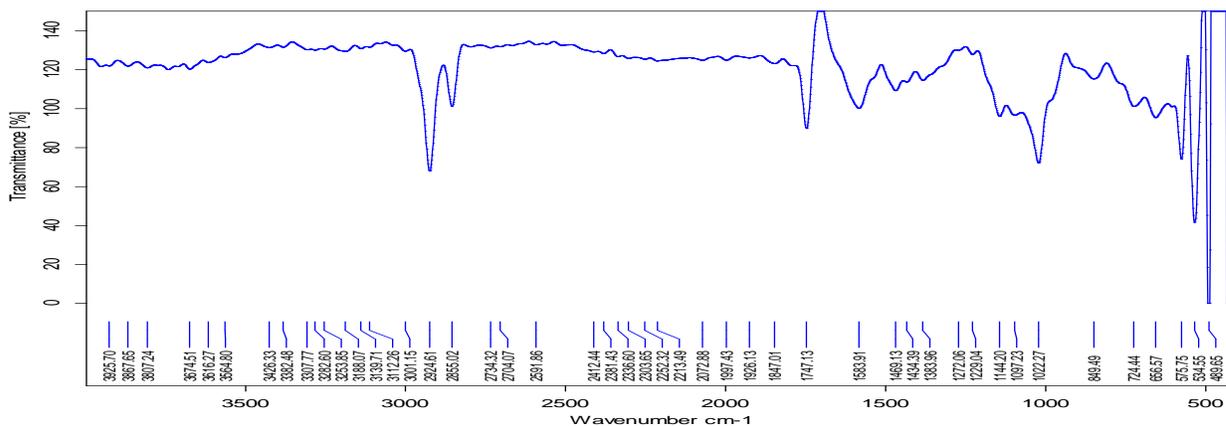
Figure 10: IR spectra of Physical mixture of Murraya koenigii extract and Eudragit RS 100

Figure 11: FTIR spectrum of polyvinyl alcohol

Figure 12: IR spectra of physical mixture of Murraya koenigii extract and PVA.

Figure 13: IR spectra of Carbopol 934
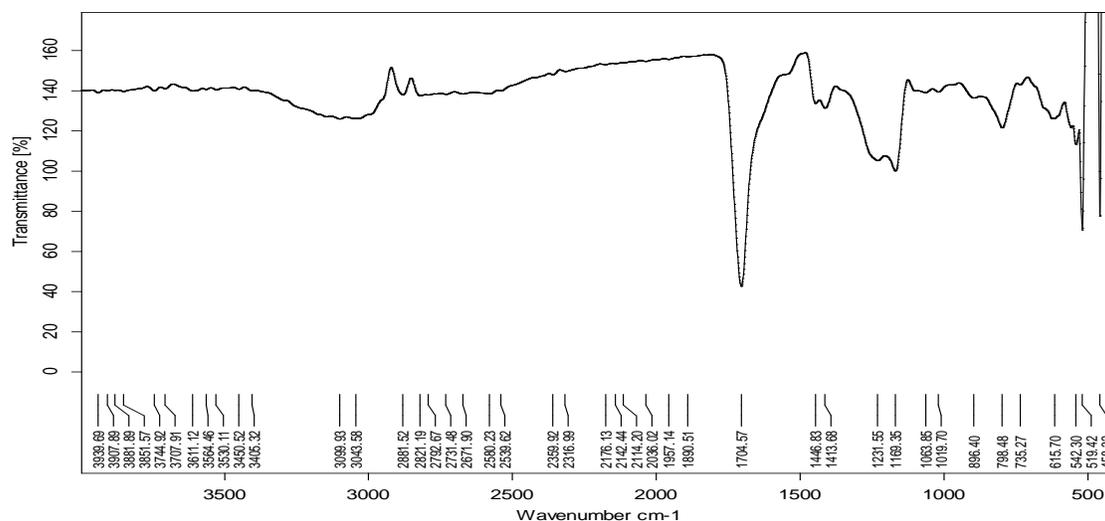
Figure 14: IR spectra of Physical mixture of Murraya koenigii extract and Carbopol 934

Evaluations of Murraya koenigii extract nanosponges:

Visual inspection:
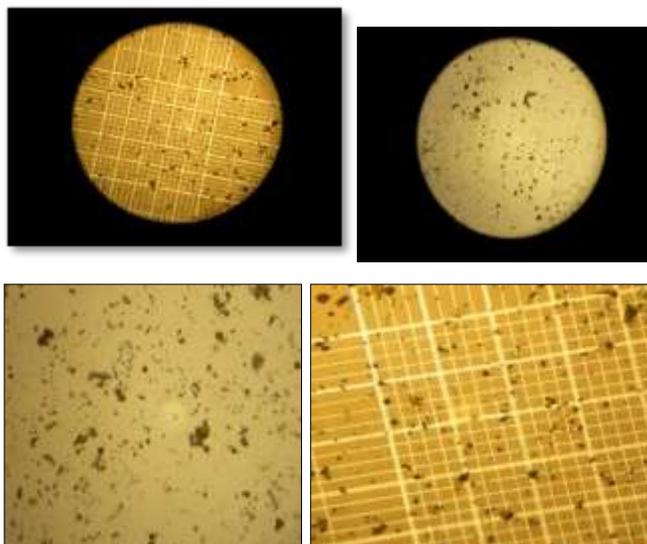
Figure 15: Optical and Binocular microscope images of nanosponges

In this figure, the nanosponges were observed in the optical microscope. The nanosponges were spread on neubauer's chamber; small cube contains two or three nanosponges. From the figure it could be concluded that the obtained product was in Nano range. The binocular image of nanosponges also exhibited the small and spherical nanosponges.

Determination of Production Yield, Entrapment Efficiency and Actual Drug Content:

Production Yield, Entrapment Efficiency and Actual Drug Content was calculated

Table 11: Production Yield, Entrapment Efficiency and Actual Drug Content of Murraya koenigii extract nanosponges

Batches	Amount of Eu RS 100 (g)	Amount of PVA (g)	Theoretical yield (g)	Practical yield (g)	Production yield (%)	Entrapment efficiency (%)	Actual drug content(%)
F1	0.5	0.25	0.95	0.4122	43.38	67.7	67.7
F2	1	0.25	1.45	0.7393	50.98	77.8	77.8
F3	1.5	0.25	1.95	1.7314	29.65	77.3	77.3
F4	0.5	0.5	1.2	0.3662	30.51	66.8	66.8
F5	1	0.5	1.7	0.7512	44.18	53	53
F6	1.5	0.5	2.2	0.6523	88.78	86.6	86.6
F7	0.5	0.75	1.45	0.7465	51.48	52	52
F8	1	0.75	1.95	0.3780	19.38	45.1	45.1
F9	1.5	0.75	2.45	1.1010	44.93	77.5	77.5

The % production yield of all batches was ranged from 19.38% to 88.78%, it was found that the production yield was greatly affected by polymer concentration as well as by concentration of polyvinyl alcohol.
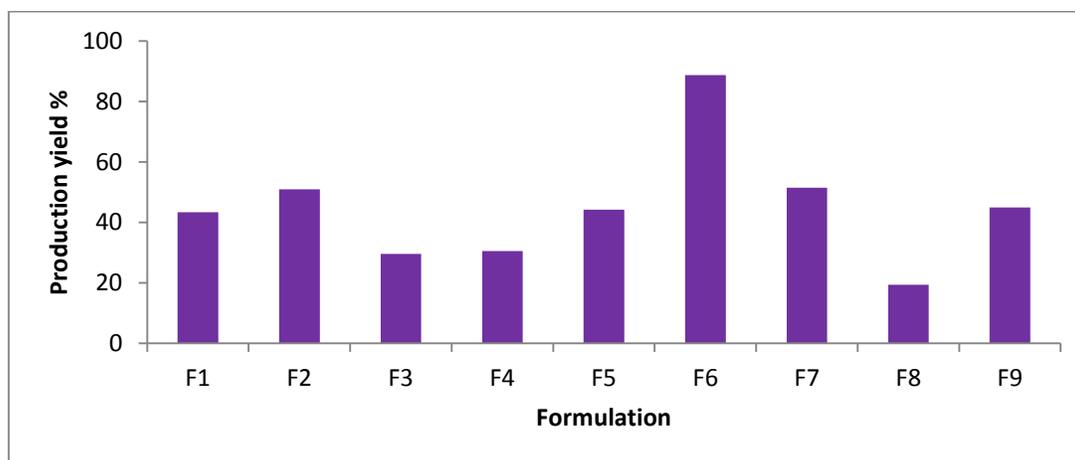
Figure 16: Percent production yield of nanosponges
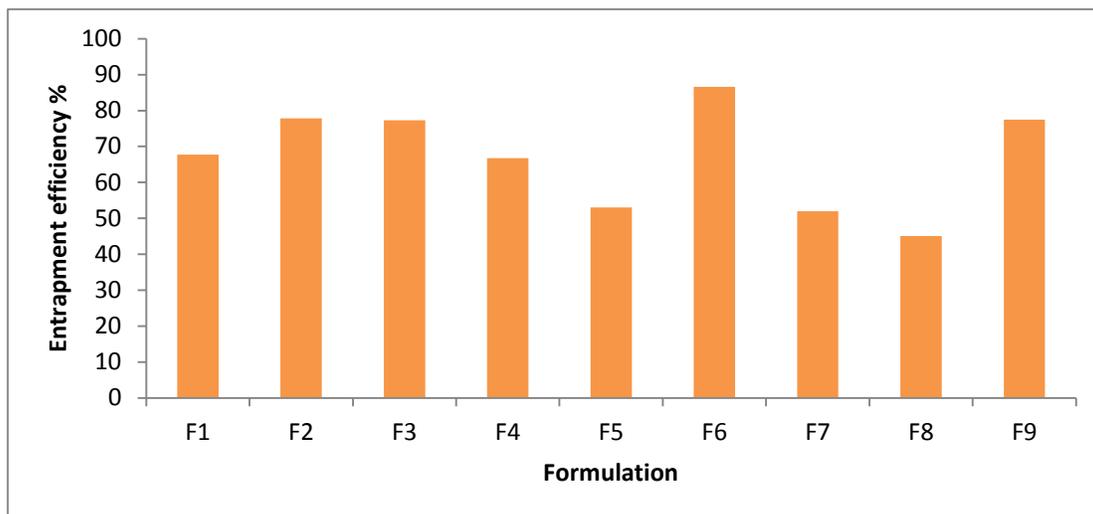
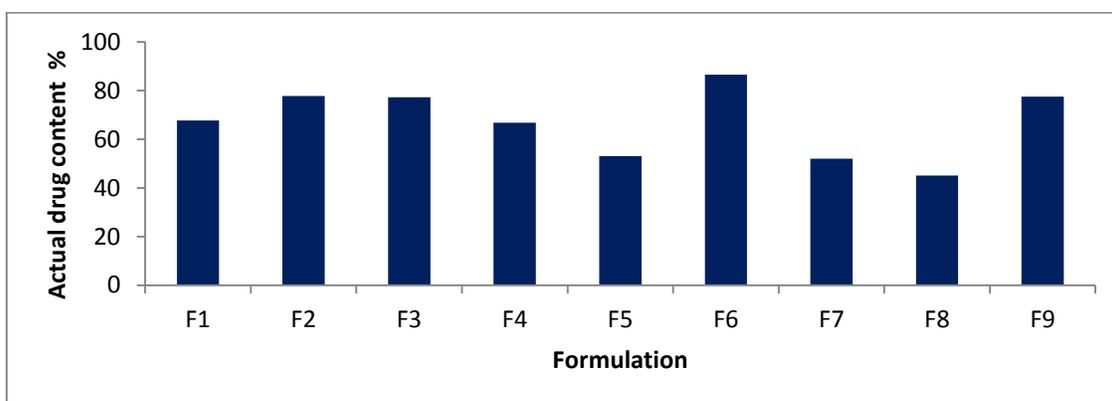**Figure 17: Percent Entrapment efficiency of nanosponges**

Figure 18: Percent Actual drug content of nanosponges

Use of higher amount of PVA while preparing nanosponges at higher amount of polymers caused slightly an increased viscosity of the dispersed phase. When solvents from inner phase diffused out, nearly all of the dispersed phase was converted to the form of solid nanosponges and separated particles appeared and also it has given higher percent entrapment efficiency and % actual drug content.

Scanning electron microscopy:
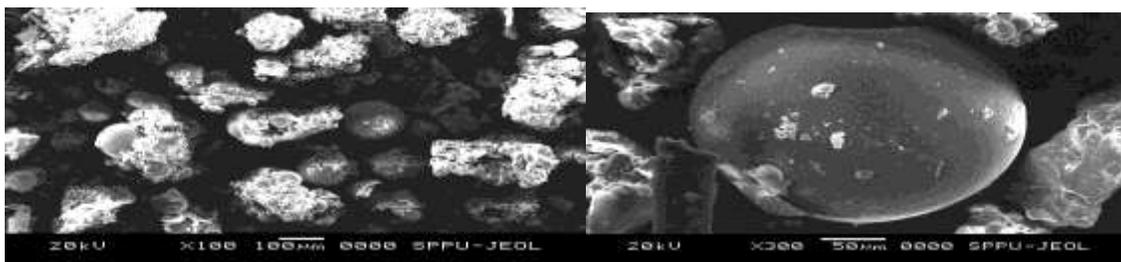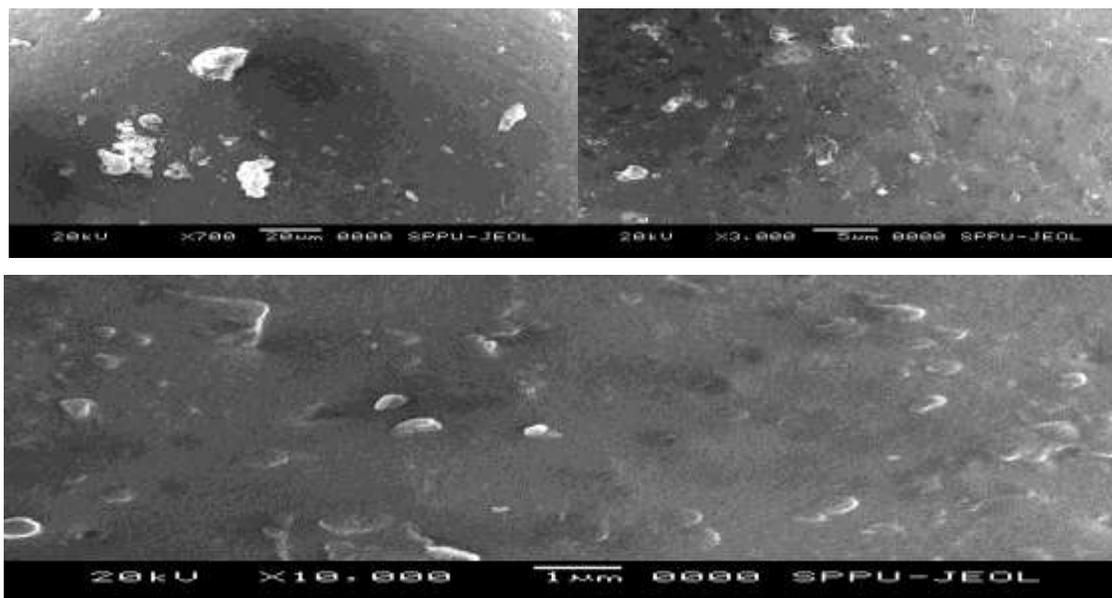
Figure 19: SEM of Nanosponges of Murraya Koenigii extract.

From SEM photographs, it was clear that the nanosponges so obtained were having the spherical shape and the spongy nature of the nanosponges was clearly evident, thus it could be concluded that the adopted method for the preparation of nanosponges was useful.

Particle size analysis:

Particle size of nanosponges should be in the range of 5 - 500 nm. The visual inspection of all batches for particle size using optical and binocular microscope revealed that the particle size was increased with the increase in the Eudragit RS 100 amount. This might be due to increasing polymer wall thickness which led to the larger size of nanosponges. The F6 batch possessed more percent of intact, uniform, spherical particles in optical microscopy; so the batch F6 was chosen for further analysis. A mean particle size of formulation F6 was found to be 55.2 nm.
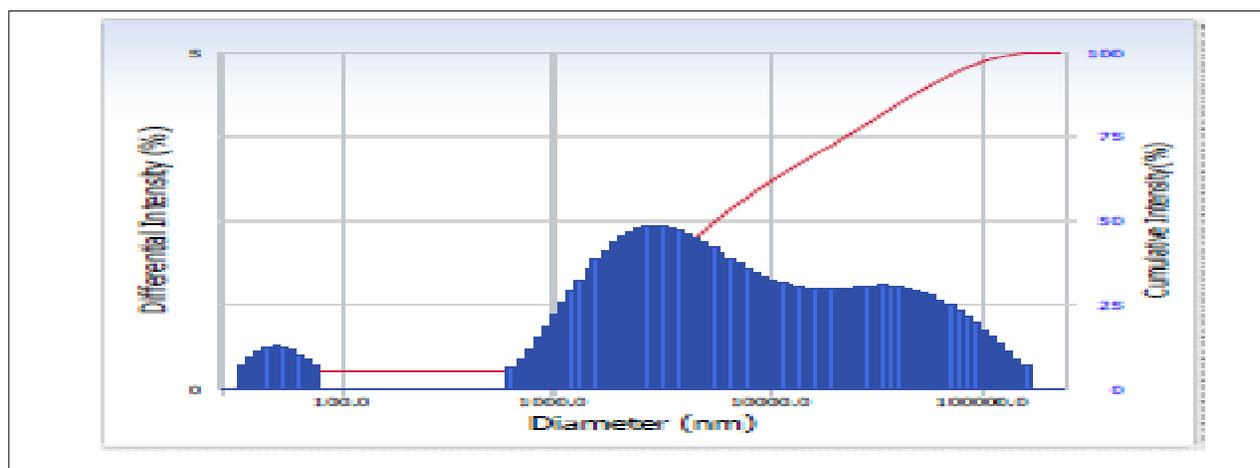

Figure 20: Particle size of nanosponges

Zeta potential:

Highly negatively or highly positive zeta potential value indicates good physical stability of the formulation. The optimised nanosponge formulation of murraya koenigii was measured by using zetasizer at 25°C, it was found to be 27.11 and it indicate the formulation to be stable.
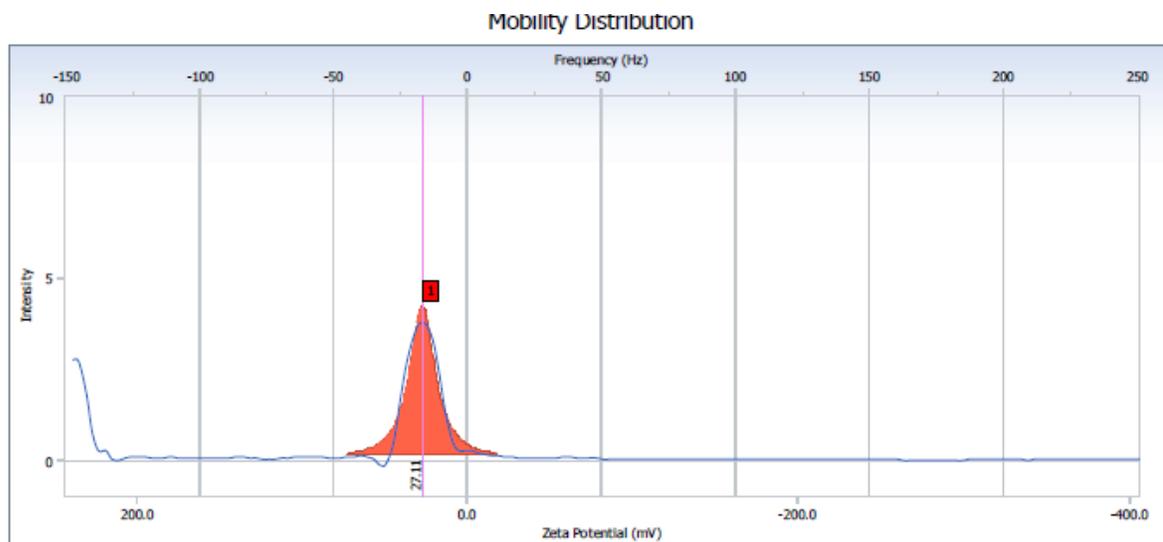

Figure 21: Zeta Potential of nanosponges

Invitro drug release studies:

In vitro drug release study was carried out using phosphate buffer pH 5.5 using Franz diffusion cell, from the results it could be revealed that % CADD was founded and ranged between 62.14% to 82.16%. Formulation F6 gave better drug release than other formulations. The cumulative percent drug release (% CADD) for all formulations was calculated. It is given in table below

Table No. 12: The cumulative percent drug release of all formulations

| Time (hr)	% CADD								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	15.38	42.18	29.5	37.24	32.85	27.42	27.69	37.81	20.15
2	28.35	47.29	36.96	42.29	39.15	37.35	35.78	44.68	28.52
3	32.54	49.65	39.16	49.73	40.72	41.47	40.87	48.71	32.54
4	42.30	51.7	42.18	55.64	42.12	59.14	52.67	65.3	36.65
5	45.76	57.12	49.72	59.16	51.79	62.42	54.35	69.73	42.39
6	52.37	65.99	52.34	63.1	56.12	69.55	56.19	73.15	49.79
7	62.55	72.31	65.16	70.12	62.14	75.21	58.78	75.79	58.92
8	68.63	81.46	80.16	72.32	69.16	82.16	62.14	79.12	68.32
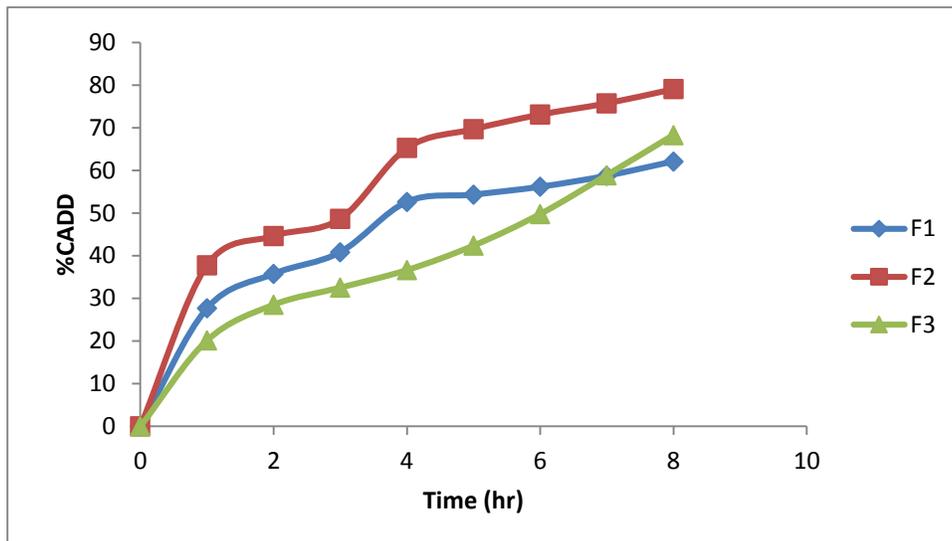
Figure 22: In vitro diffusion study of formulations F1-F3.

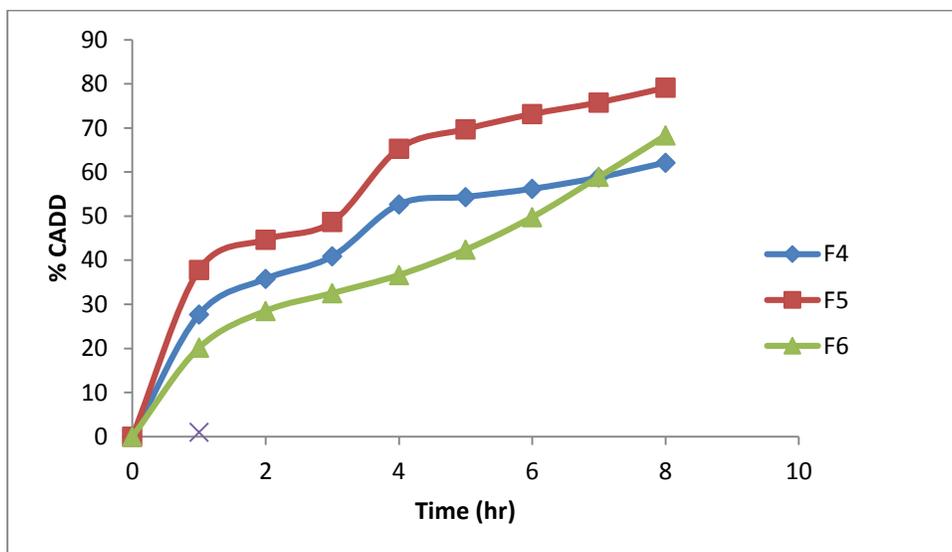**Figure 23: In vitro diffusion study of formulations F4-F6.**
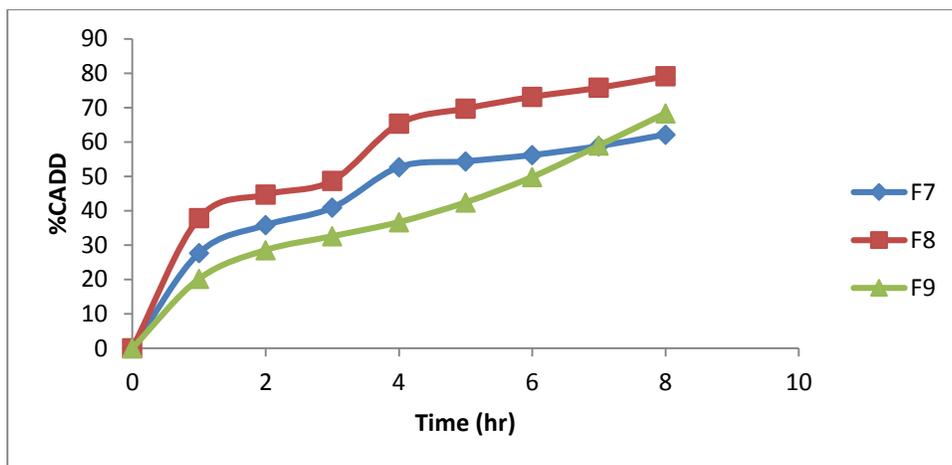

Figure 24: In vitro diffusion study of formulations F7-F9.

Infrared spectroscopy:

1.   Blank nanosponges:

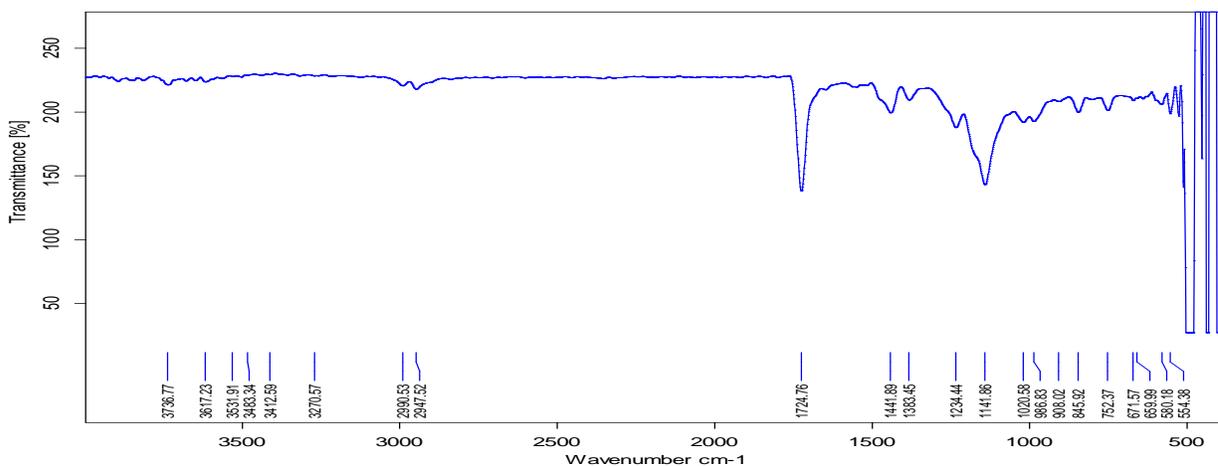**Figure 25: The IR Spectrum of blank nanosponge**
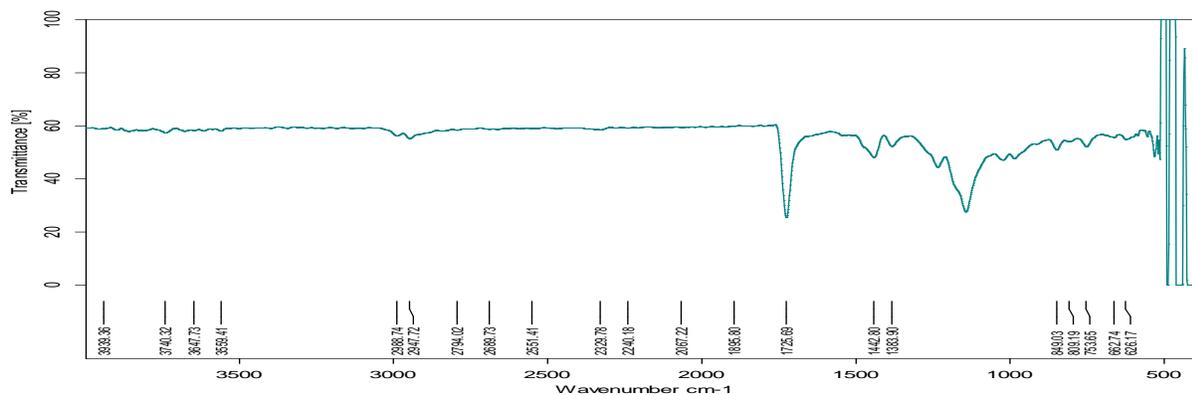
2.   Drug containing nanosponges:

Figure 26: The IR spectrum of drug containing nanosponges

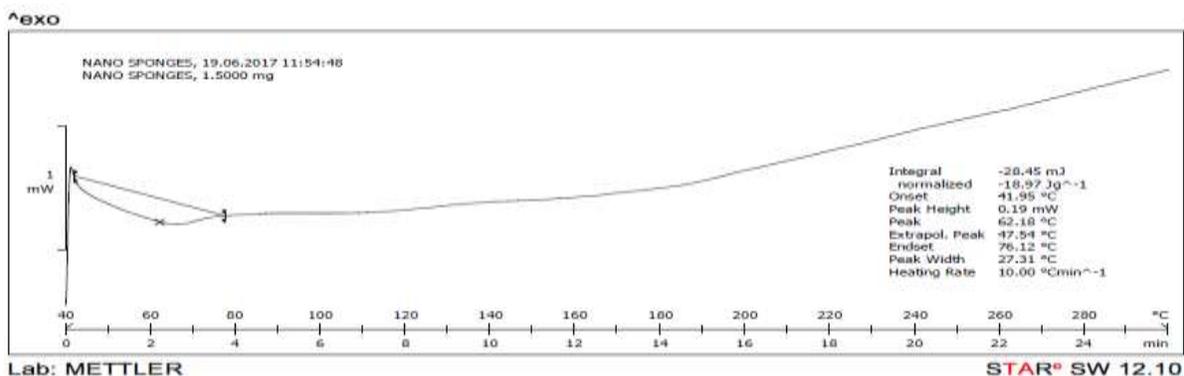**3.   Differential scanning Calorimetry (DSC)**

Figure 27: DSC of drug loaded nanosponges

Evaluations of Murraya koenigii extract nanosponges gel:

1.   Physical appearance:

The prepared gel formulations of Murraya koenigii leaves extract nanosponges were visually inspected for their colour, consistency and odour.

Colour: Pale green

Consistency: Good

Odour: Aromatic

2.   Determination of pH :

Table 13: pH of all formulations

Batches	pH			Std. Deviation
F1	5.22	5.20	5.22	5.21±0.07234
F2	6.22	6.22	6.22	6.22±0
F3	5.29	5.10	5.32	5.23±0.1193
F4	5.89	5.90	5.92	5.90±0.01528
F5	5.62	5.58	5.60	5.59±0.02
F6	5.28	5.25	5.27	5.26±0.01528
F7	6.00	6.00	6.00	6.00±0
F8	6.67	6.69	6.67	6.67±0.01155
F9	5.76	5.75	5.74	5.75±0.01
5.28	5.25	5.27	5.26±0.01528	5.28

1. Spreadability and Drug Content:

Table 14: Spreadability and % drug content of all formulations

Batches	Spreadability			Std. Deviation	Drug content (%)
F1	20	20.65	19	19.88±0.83	85.54
F2	29.16	25	27.77	27.31±2.11	89.6
F3	12.5	13.33	11.17	12.33±1.08	86.6
F4	22.36	22	19.31	21.22±1.66	81.1
F5	24.82	18.75	13.88	19.15±5.48	87.2
F6	13.87	16.30	14.81	14.99±1.22	92.5
F7	31.45	29.41	26.25	29.03±2.62	89.9
F8	18.4	21.18	17.96	19.18±1.74	82.1
F9	27.77	25	21.59	24.78±3.09	84.4

The values of spreadability of all prepared formulation were found to be in the range of 12.33±1.08 to 29.03±2.62 g.cm/sec and drug content ranges from 81.1 to 92.5. Hence, it indicates that spreadability and drug content of gel was good.

3.   In vitro drug release studies:

In vitro drug release study of gel was carried out using phosphate buffer pH 5.5 using Franz diffusion cell. From the results it could be concluded that % CADD of gel formulations gave better release. At the end of 8 h, the total amount of drug release from the formulation was found to be 82.16%.

Table 15: % CADD of optimized formulation

Time (hr)	CADD(%)
0	0
1	27.42
2	37.35
3	41.47
4	59.14
5	62.42
6	69.55
7	75.21
8	82.16

2. Stability study:

The stability studies of formulated gel were carried out at room temperature for one month. The effect of temperature, humidity and time on the physical characteristics of the gel was for assessing the stability of the prepared formulations. The stability studies were carried out when the room temperature was 25º C. The Results were shown in Table no. 32. Therefore no evidence of degradation of drug was observed.

Table 16: Appearance, pH and homogeneity of formulation.

Time interval (days)	Formulation		
	Appearance	pH	Homogeneity
0	Pale green	5.20	Very good
7	Pale green	5.20	Very good
15	Pale green	5.36	Good
30	Pale green	5.38	Good

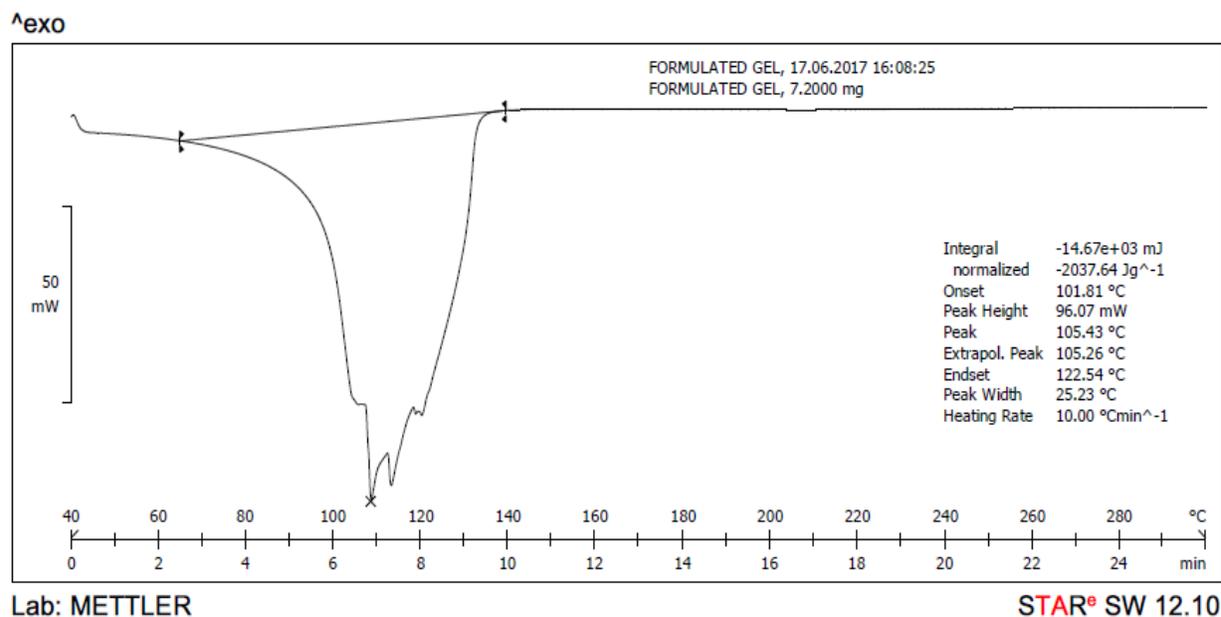## 4.   DSC of formulated gel

Figure 28: DSC of Murraya koenigii containing gel

In DSC thermogram of Murraya koenigii extract containing gel endothermic peak was observed at 105.26ºC. The DSC thermogram of drug and formulation compared with each other both shows endothermic peak. The study indicated that drug has not under go any chemical interaction with the polymer.

5.   Data analysis of formulations:

Traditional design of the pharmaceutical formulations are based on time consuming approach of changing one variable at a time which does not take into consideration the joint effect of independent variables. Thus, factorial design can serve as an essential tool to understand the complexity of the pharmaceutical formulations.

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels. The amount of Eudragit RS100 (A) and polyvinyl alcohol (B) were selected as independent variables and the dependent variables were % production yield, % CADD and % Entrapment efficiency. The data obtained was treated using Stat-Ease Design Expert software.

Table 17: Design Summary

Factor	Name	Unit	Type	Coded level			Actual level		
				Low	Medium	High	Low	Medium	High
A	Eudragit RS100	gm	Numerical	-1	0	+1	0.5	1	1.5
B	Polyvinyl alcohol	gm	Numerical	-1	0	+1	0.25	0.5	0.75

Table 18: Response Summary

Response	Name	Unit	Observations	Analysis	Minimum	Maximum

M1	Entrapment efficiency	%	9	Polynomial	45.1	86.6
M2	CADD	%	9	Polynomial	27.42	82.10
M3	PY	%	9	Polynomial	19.38	88.78

Table 19: The responses of all formulations (F1-F9)

Responses	A	B	CADD (%)	EE (%)	%PY
F1	+1	0	68.63	67.7	43.38
F2	0	+1	81.46	77.8	50.98
F3	-1	-1	80.16	77.3	29.65
F4	+1	-1	72.32	66.8	30.51
F5	-1	0	69.16	53	44.18
F6	-1	+1	82.16	86.6	88.78
F7	+1	+1	62.14	52	51.48
F8	0	0	79.12	75.1	19.38
F9	0	-1	68.32	77.5	44.93

3. **In-vivo burn wound healing:**

a.   **Weight of animals after creating burn wound**

Table No. 20: Weight of animals after creating burn wound

Time (days)	Group 1(Test)	Group 2(Standard)	Group 3(Control)
0	262.5±26.22	225±22.36	220.83±36.79
7	266.66±30.27	212.5±26.22	237.5±26.22
14	287.5±26.22	250±15.81	212.5±26.22
21	241.66±20.41	212.5±26.22	220.83±36.79
28	250±15.81	225±22.36	233.33±20.41
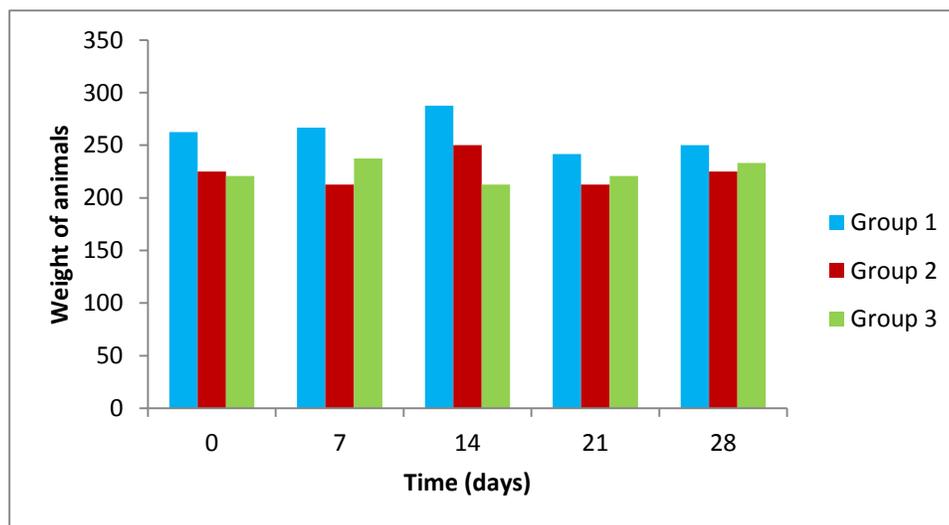
Figure 29: Weight of animals after creating burn wound

b.   **Surface area of burn wound**

Table 21: Surface area of burn wound

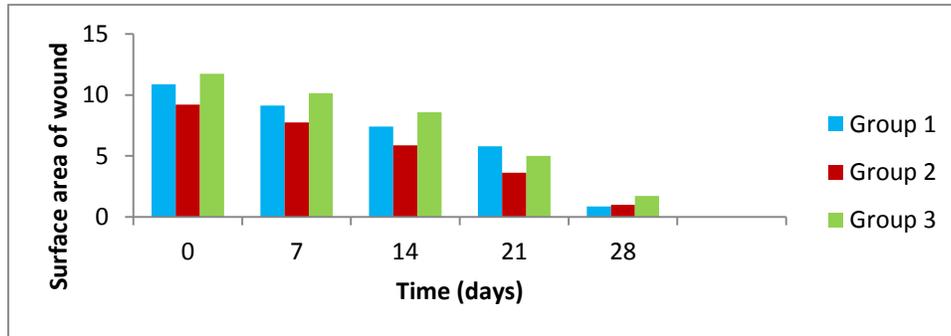| Time (days)	Group 1	Group 2	Group 3
0	10.89±2.088	9.216±1.114	11.75±1.665
7	9.142±1.579	7.738±0.999	10.14±1.659
14	7.411±1.019	5.878±1.987	8.588±1.424
21	5.79±1.289	3.615±1.717	5.015±2.245
28	0.859±0.499	1.000±0.839	1.734±1.172

Figure 30: Surface area of burn wound

c.   %of burn wound contraction

Table 22: % of burn wound contraction

Time (days)	Group 1 (Test)	Group 2(Std)	Group 3 (Control)
0	-	-	-
7	15.611±7.203	15.72±8.292	13.86±4.140
14	30.56± 19.11	32.42±20.506	26.96±5.303
21	47.87±19.105	46.235±10.848	35.57±2.106
28	89.56±8.434	85.106±9.196	78.24±9.916
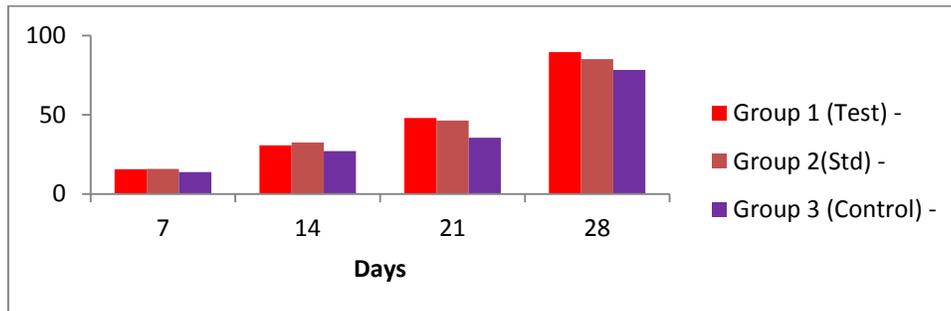
Figure 31: % of burn wound contraction

Day '0'

Group 1   **Group 2**   **Group 3**

Day '7'

Group 1   **Group 2**   **Group 3**

Day '14'

Group 1   **Group 2**   **Group 3**

Day '21'
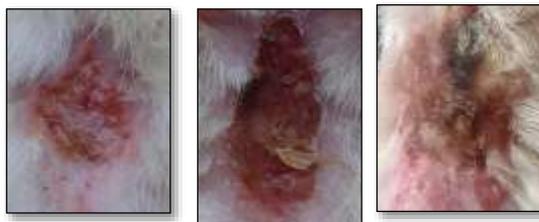
Group 1   **Group 2**   **Group 3**

Day '28'
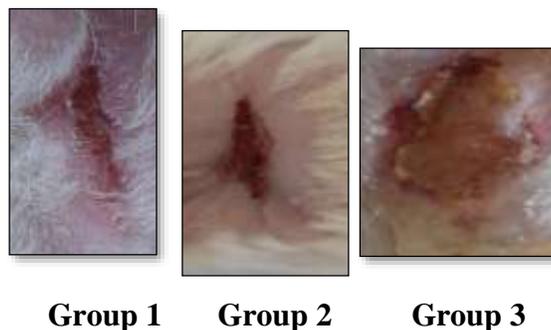
Group 1   **Group 2**   **Group 3**

Figure 32: Images of burn wound healing in rats

CONCLUSION

Burn wound injuries to the skin result in loss of its protective function as a barrier to the microorganism leading to the high risk of infection. Thus burn wound patient face high morbidity than mortality because of the large uncovered burns getting infected ; healing of wound takes place long period of dressing, leading to deformities and contractures. Managing burn injuries properly is important because they are painful and can cause disabling scarring, exclusion of affected parts or even death in severe cases. Problems such as infection, electrolyte imbalance, shock and respiratory suffering may occur.

Herbal drugs have gained more popularity during last decade throughout the world. It had been used by 80% of population in developing countries. Herbal drug contains many phytoconstituents and some of them are important active phytoconstituents. These herbals when formulated should contain similar quantity of these constituents as that of original herb.

Nanosponges are mesh like structure and due to their small size and porous nature. They can easily bind poorly water soluble drug. The F6 batch showed very fine, spherical and free flowing nanosponges. By formulating nanosponges we got free flowing drug loaded nanosponges.

Further Nanosponges having Murraya koenigii implemented in gel and *In-vivo* activity was checked. It was concluded that prepared formulation F6 showed significant burn wound healing activity.

ACKNOWLEDGEMENT

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