CHAPTER 5 MATERIALS AND METHODS

Extracts of seeds of *B.lanzan* family Anacardiaceae and extracts of seeds of *Simmondisia chinesis* family Simmondsiaceae

Sample preparation

The seeds were washed, dried and stored each in an air tight cooled bottle prior to use for analysis.

Phytochemical analysis

The analysis for tannin, saponins, cardiac glycosides and alkaloids were carried out according to standard methods.

Mineral analysis

Minerals were determined by digesting the ash with 3M Hydrochloric acid using the atomic absorption spectrophotometer for Calcium, Magnesium, Manganese and Iron and the flame photometer for potassium and sodium.

Vitamin analysis

The composition of the water-insoluble vitamins, riboflavin and thiamine, were determined by the method of Scalar, while ascorbic acid content was determined by the method of AOAC,

PHYTOCHEMICAL STUDIES

The Phytochemical investigation of a plant involves authentication and extraction of plant material; qualitative and quantitative evaluations; separation and Parallel to this may be the assessment of pharmacological activity.

PHYSIO-CHEMICAL CONSTANTS

Shade dried powdered plant materials for used for the determination of the physio chemical constants in accordance with the WHO guidelines.

DETRMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incarnation is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or edibility added to it, as a form of adulteration. Ash value of a crude drug is defined as the inorganic residue remaining after incineration, which compiles of inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Hence it is used for the determination ofthe quality and purity of the crude drug in the powdered form.

TOTAL ASH

Total ash method is designed to measure the total amount of material remaining after

ignition. They include both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot for 30 minutes and cooled in the desiccators Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred silica dish at a temperature not exceeding 450^0C until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

WATER SOLUBLE ASH:

The difference in weight between the total ash and the residue after treatment of the total ash in water

Procedure:

Total ash obtained is boiled for 5 minutes with 25 ml of water, insoluble matter were collected in an ashless filter paper, washed with hot water and ignite for 15 min at a temperature not exceeding 450^0. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

$$\textbf{Water soluble ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

ACID INSOLUBLE ASH:

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25 ml of dilute hydrochloric acid is added. The insoluble matter is collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible dry on hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air dried drug is calculated.

$$\textbf{Acid insoluble ash} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$$

SULPHATED ASH

Sulphated ash test is used to measure the amount of residual substance not volatilized from a

sample. These tests are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible was heated to redness for 10 minutes, allowed to cool in a desiccator and weigh. 2 g of sample were accurately weighed, ignited gently then thoroughly hared. Cool, moistened the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25^{o} until all black particles have disappeared. Crucible is allowed tocool, add few drops of sulphuric acid and heat. Ignite as before, allow cooling and weighing. This process is repeated until two successive weighing differ by more than 0.5 mg.

$$\text{Sulphated ash} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$$

DETERMINATION OF MOISTURE CONTENT:

LOSS ON DRYING

10 g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Uses of high speed mill in preparing the samples are avoided. The samples in the tarred evaporating dish were placed in the drying chamber (105℃) for 5 hours and weigh. Drying and weighing is continued every one hour interval until the difference between the two successive weights is not more than 0.25 percent. Constant weight is reached when the two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in desiccators, show not more than 0.001 g difference. Percentage moisture content is compared with respect to the air dried sample.

$$\textbf{\% Moisture content} = \frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

Extract preparation

Soxhlet extraction Method

It is a simple and effective method. It has been used for a wide range of samples like soils, sediments, and animal and plant tissues. A wide variety of solvents like dichloromethane (DCM), pure or mixed with acetone or hexane, and acetone–hexane mixtures can be used. The use of non-polar solvents only is not recommended. The minimum time needed for a regular Soxhlet extraction is normally ∼8 hours. Sulfur present in the sediment and soil samples is also extracted, and must be removed by a later cleanup step.

The Soxhlet extraction is a combination of both, percolation and maceration methods. The extraction is carried out in a special apparatus known as Soxhlet apparatus that was designed by Franz von Soxhlet in 1879

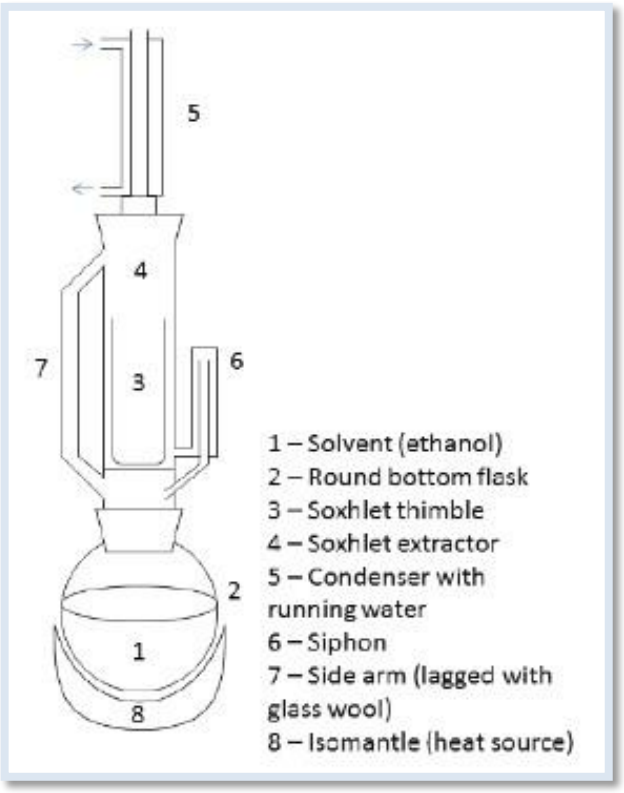


Figure No 5.1: Soxhlet apparatus

Extracts of seeds of *B.lanzan* family Anacardiaceae and extracts of seeds of *Simmondisia chinesis* family Simmondsiaceae

Method

- seeds of *B.lanzan or Simmondisia chinesis* (2.5 kg) were extracted with methanol (3x24 h) at 20-250C (Room temperature).
- The solvent was subsequently evaporated under reduced pressure at 50 to produce a concentrated extract.
- 280g of methanol extract was fractionated by n-hexane and water to gain an 86g n-hexane extract and the layer of water.
- The water layer then extracted with ethyl acetate to gain a 120g ethyl acetate fraction and 90g water fraction.
- Ethyl acetate fraction, which was the most active fraction, was chromatographed by Wakogel C200 (Wako Pure Chemical, Japan) with a mixture of n-hexane, ethyl acetate and methanol with raised the polarity. Main compounds were then isolated and purified by silica G60 with sulfuric acid-ethanol (1:9) and were

identified by spectroscopic methods consist of mass spectroscopy (MS), ultraviolet (UV), infrared spectrometry (IR), and nuclear magnetic resonance (NMR).

Preparation of Gel:

Carbopol 940 gel base, after optimization was formulated by first hydrating required quantity of carbopol in propylene glycol and distilled water q.s. for 24hr The mixture was stirred by keeping it on mechanical stirrer at 50rpm for 30mins at 25^0C, until all carbopol was dispersed and then other excipients like methyl paraben (0.2%w/w) and propyl paraben (0.02%w/w) and triethanolamine were added in order to increase stability of the gel.

Screening of Influential Variables Regular 3^2design was followed for screening of significant formulation and process variables involved in the gel development. Table 6.1 presents the description of high to low levels of various variables screened for their impact in the development of gel of colchicine by including BLO and SCO

Table 5.1: List of variables employed in Regular 3^2Design

Factors	Levels		
	(-1)	**0**	**(+1)**
Viscosity	0.7	1.7	2.7
Amount of PE(ml)	0.1	0.5	0.9

Table 6.1 contains List of Variables which was employed in Study with their low, medium and high levels. 2 variables Amount of carbopol 934, Amount of PE were select as independent variables under 3 levels (low, medium and high).

Factorial Batches of gel formulations

7a.Coded levels translated in actual quantities.

Factors	Levels		
	-1	**0**	**+1**
X1 Concentration of gelling agent	0.7%	1.7 %	2.7%
2 Concentration of PE	0.1 %	0.5%	0.9%

Independent Variables:

X1 = Concentration of gelling agent

(Carbopol 940). X2 = Concentration of

Permeation enhancers.

Dependent Variables:

Y1 = Viscosity

Y2 = % Cumulative drug release

at 8hrs

7b. Factorial design layout.

Batch code	Coded values		Actual values	
			Concentration of Gelling agent in %	Concentration of Propylene glycol
F1	0	0	0.7	0.1
F2	0	-1	0.7	0.5
F3	0	+1	0.7	0.9
F4	-1	0	1.7	0.1
F5	-1	-1	1.7	0.5
F6	-1	+1	1.7	0.9
F7	+1	0	2.7	0.1
F8	+1	-1	2.7	0.5
F9	+1	+1	2.7	0.9

Table 5.2: Composition of gel on the Basis of Regular 3^2Design

Name of Ingredient	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9
Drug (%)	1	1	1	1	1	1	1	1	1
Carbopol 940	0.5	0.5	0.5	1.2	1.2	1.2	1.9	1.9	1.9
JJBO (ml)	0.1	0.5	0.9	0.1	0.5	0.9	0.1	0.5	0.9
Polyethylene glycol (ml)	5	5	5	5	5	5	5	5	5
IPA (ml)	5	5	5	5	5	5	5	5	5
Methyl Paraben (gm)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

(gm)									
TEA (ml)	qs	qs	qs	qs	qs	qs	qs	qs	qs
Distilled water (ml)	Upto 100	Upto 100l	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100

Table 5.2 shows the BL9 and SC9 array for the three factors, two levels design adopted in the current studies. When the data of table 5.2 put into the DoE, it will provide combinations of 9 formulations as listed in table 5.2. Viscosity and penetration efficiency were the key response variables which was investigated thoroughly for selecting the significant formulation and response variables.

Evaluation of gel

Viscosity determination: Viscosity size was determined using the Brookfield viscometer

Penetration efficiency: prepared gel was estimated for its penetration efficiency.

Formulated gel was taken for its ex-vivo drug release studies. Accordingly optimized formulations were separate out. The formulations in a required dose were spread on goat skin and snake skin. Sampling was done as 5ml sample withdrawn at specific interval. It was then diluted and prepared solution was taken to make further study of drug release. The samples were analyzed on UV spectrophotometer (Spectrascan2600).

Transmission Electron Microscopy: Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after15min was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan)

On the basis of results obtained from the study was selected as optimized formulation.

Table 5.3: Composition of optimized gel formulation

Formulation	**Carbopol 940(g)**	**BLO(ml)**
BL5	1.7	0.5

Formulation	**Carbopol 940(g)**	**SCO(ml)**
SC4	1.7	0.1

Preparation of Gels

Preparation of Carbopol gel base: Carbopol 934 (0.5 g) was weighed and dispersed in 100ml distilled water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerin was added to the gel for maintaining consistency. Preservatives (methyl Paraben and Propyl Paraben) also added into the gel. Similarly 1 and 2% Carbopol gels were prepared

Table 5.4: Composition of different gel base

Formulation	Carbopol (%)
BL4	0.7
BL5	1.7
BL6	2.5

Formulation	Carbopol (%)
SC4	0.7
SC5	1.7
SC6	2.5

Preparation of gels: Carbopol 940 gel base, after optimization was formulated by first hydrating required quantity of carbopol in propylene glycol and distilled water q.s. for 24hr. The mixture was stirred by keeping it on mechanical stirrer at 50rpm for 30mins at 25^0C, until all carbopol was dispersed and then other excipients like methyl paraben (0.2%w/w) and propyl paraben (0.02%w/w) and triethanolamine were added in order to increase stability of the gel.

Valuation of Gel

Determination of pH: Weighed 50 gm of each gel formulation were transferred in 10 ml of beaker and the pH was determined using digital pH meter. pH of the topical gel formulation should be between 3–9 to treat the skin infections

Spreadability: The Spreadability of colchicine gel formulation was measured as slip and drag characteristics of the gels. A apparatus was designed and fabricated which consisted of two glass slides, the lower one was fixed to a wooden plate and the upper glass slide one was attached by a hook to a balance. The Spreadability evaluation was done by using the formula: S=ml/t, where S, is Spreadability, m is weight in the pan tied to upper glass slide and t is the time taken by slide to travel a specific distance and l is the distance traveled. For the practical

purpose the mass, length was kept constant and 't' was determined

Skin Irritation study:

The test was performed on three volunteers by applying 1gm formation on the wrist area. The observation was done for 1hr and data was prepared based on the observation.

Draize's skin irritation study: for primary skin irritation test, three volunteers were selected and then colchicines gel of different batches were applied on the area of 2 square inches of the wrist for 2 hrs and observed the skin for irritation/lesion REF

Score	Description
0	no
0.5	Faint, barely perceptible erythema or slight dryness
1	Faint but definite erythema, no eruption or broken skin or no erythema but, definite dryness and may have epidermal fissuring.
1.5	Well defined erythema or faint erythema with definite dryness, may have epidermal fissuring.
2	Moderate erythema: may have few papules or erythema in the cracks
2.5	Moderate erythema with barely perceptible edema
3	Severe erythema (beet redness) may have generalized papules or moderate to severe erythema with slight edema (edges well defines by raising).
3.5	Moderate to severe erythema with moderate edema (confined to patch area).
4	Generalized vesicles or Escher formation or moderate to severe erythema and/or edema extending beyond the patched area.

Table No. 5.5 Skin irritation score

Measurement of viscosity: The viscosity of gels was evauated by using a Brookfield viscometer (DV-II model). A T-Bar spindle in combination with a helipath stand was used to measure the viscosity triplicate to get accurate readings

(a) Selection of spindle: The procedure started on trial and error started from T95 spindle. The goal was to obtain a viscometer dial or display (% torque) reading between 10 and 100, the relative error of measurement improves as the reading approaches 100. Spindle T 95 was used for the measurement of viscosity of all the gels.

(b) Spindle Immersion: The T –bar spindle (T95) was lowered perpendicularly in the centre taking care that spindle does not touch bottom of the beaker containing gel.

(c) Measurement of Viscosity: The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors such as temperature, pressure and sample size etc. which

affect the viscosity were maintained during the process. The torque reading was always greater than 10%. Five readings taken over a period of 60 sec. at 10 rpm were averaged to obtain the viscosity.

(d) Preparation of Analysis of Gel Formulation

Preparation of Standard Curve of Colchicine

100 mg each of Colchicine was accurately weighed and dissolved in PBS (pH 7.5) in 100 ml volumetric flask the volume was made upto 100 ml. The obtained solution is ≈1000 µg/ml of each drugs and suitable dilution to make 5µg/ml of Colchicine. The absorbance was taken on double beam U.V. spectrophotometer using λmax at 350.0 nm for colchicine. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve.

Determination of drug content:

After preparing batches, all the formulations were subjected to determine drug content. 10gm of gel was diluted with 100ml buffer and obtained solution was estimated by Spectrascan UV 2600 (double beam).

Method

Validation

Linearity

Linearity of analytical procedure is its ability to obtain test within a given range, which is directly proportional to area of analyte in the sample. The calibration plot was prepared after analysis of five different (from 1 to 5µg/ml and 10 to 50 µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve) and the standard calibration curve of the drugs are shown From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard

dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

- IntermediatePrecision
- Day toDay
- Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Selection of formulations for Drug release study:

Based on the studies like viscosity, Spreadability, Skin irritation study formulation those had shown acceptable results were selected for ex-vivo diffusion study.

Following formulations were selected for diffusion study:

Formulation	**Carbopol 940(g)**	**BLO(ml)**
BL4	0.5	0.1
BL5	1.7	0.5
BL6	2.9	0.9

Formulation	**Carbopol 940(g)**	**BLO(ml)**
SC4	0.5	0.1
SC5	1.7	0.5
SC6	2.9	0.9

Table No 5.6: formulations for diffusion study

These formulation have shown good result when they were evaluated for pH, Drug content, Viscosity, skin irritation study and Spreadability.

***Ex-vivo* diffusion study:** An *ex-vivo*drug release study was performed using modified Franz diffusion cell. Skin of goat and snake was placed between receptor and donor compartments. Gel equivalent to 1 mg of colchicine was placed in the donor compartment and the receptor compartment was filled with PBS pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5℃ with stirring at 50rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer

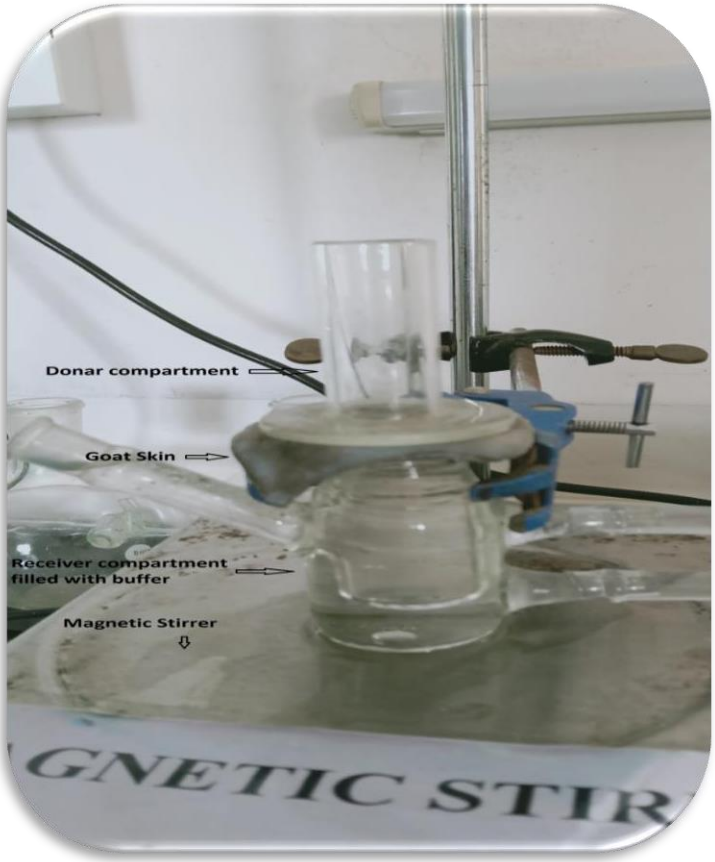


Figure 5.2: Franz diffusion cell with goat skin

Mathematical treatment of *in-vitro* release data: The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage form characteristics areused.

1. Zero-order kinetics: The pharmaceutical dosage forms which follow this profile, release the same amount of drug by unit of time and it is the ideal method of drug release in

order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express thismodel:

$$Q_t = Q_o + K_o\, t$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initial amount of drug in the solution (most times, Q_o=0) and $K_o$is the zero order release constant

2. First-order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initial amount of drug in the solution and $K_1$is the zero order release constant.

In this way a graph of the decimal logarithm of the released amount of drug versus time is plotted which will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in such a way that is proportional to the amount of drug remaining in its interior and amount of drug released by unit of time diminishes.

3. Higuchi model: Higuchi model was developed by several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

$$Q = K_H . t^{1/2}$$

Where Q is the amount of drug released in time t and K_H is the Higuchi dissolution constant. Higuchi model describes release of drug as a diffusion process on the basis of Flick's law, square root time dependent. This relation can be used to describe the dissolution of drug from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drugs

Korsmeyer-Peppas model: Korsmeyer*et al.* used a simple empirical equation to describe release behaviour of general solute from controlled release polymermatrices:

$$\frac{M_t}{M_\infty} = a\, t^n$$

Where $M_t/M_¥$is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log $M_t/M_¥$ versus log time curve **(Korsmeyer*et al.* 1983)**. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism (Peppas*et al.*, 1985). Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of n=0.5 for Fickian diffusion and higher values of n, between 0.5 and 1.0, or n=1.0, for mass transfer following a non- Fickian model (Table 6.9). In case of a cylinder n=0.45 instead of 0.5, and 0.89 instead of

This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent (Pappas, 1985). To the determination of the exponent n the portion of the release curve where $M_t/M_¥ < 0.6$should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (l) in the beginning of the drug release from the pharmaceutical dosageform:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and b value would be zero and only $at^n$is used. This mathematical model, also known as *Power Law*, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms.

Oven Stability studies: The determination of the physical and chemical stability of the optimized formulations was conducted. Accordingly, the formulation was subjected to centrifugation test. For this purpose, the samples were centrifuged at 3000 rpm for 30 minutes. For the next test, which lasted ninety days, the samples were subjected to heating in an oven (50 ± 2 °C), cooling in refrigerator (5 ± 2 °C), and to room temperature (25 ± 2 °C). During this period, the kept formations were observed for their organoleptic characters like color, pH, and homogeneity.

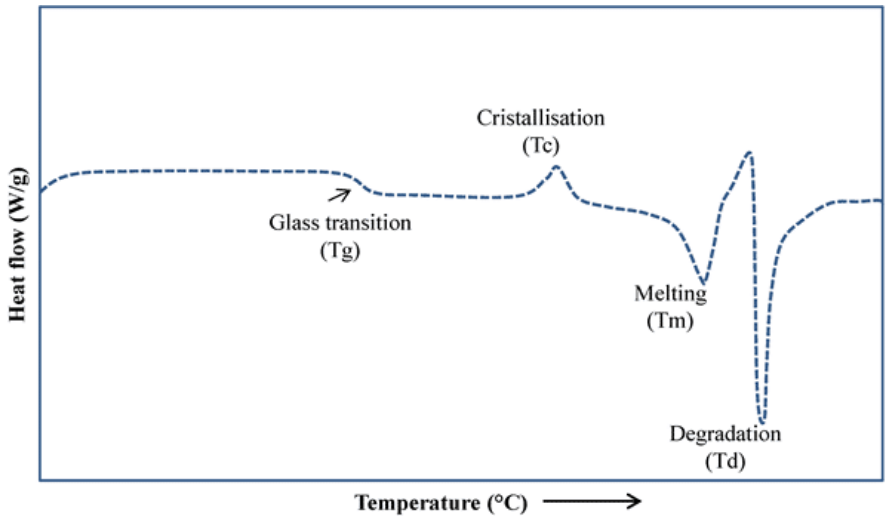
(a) Effect of storage temperature on drug content: After storage for a specified period

of time of 7, 15, 21, 28 and 60 days, the drug content of the formulations was determined. Drug content in gel was determined spectrophotometrically as discussed previously (6.4.5.).

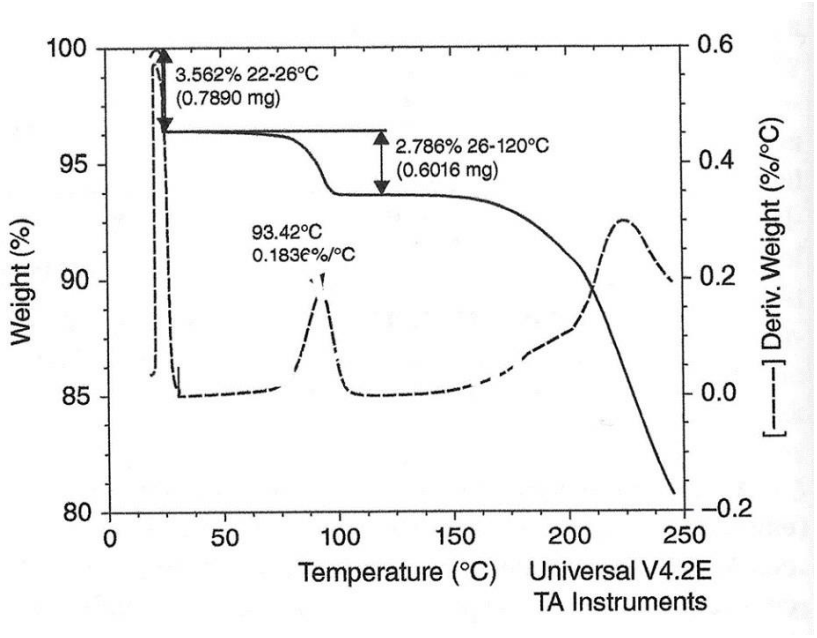
DSC and TGA

Differential scanning calorimetry (DSC) is a thermal analysis technique that measures the difference in heat flow between a sample and a reference as they are subjected to a controlled temperature program. The heat flow is measured in watts (W) and corresponds to the transmitted power. The change in enthalpy of the sample is determined from the heat flow curve. Endothermic reactions absorb heat, which is indicated by a negative heat flow. Exothermic reactions release heat, which is indicated by a positive heat flow.

TGA thermal curves are generally displayed with time or temperature as the X axis and weight or weight percent as the Y axis. A typical TGA thermal curve for the dehydration of a sample of SAR474832 hydrate Form 1 is shown in the figure where there are two steps of weight loss, one due to 3.6% dehydration over the range of 22-26 ˚ C, and another one with 2.8% weight loss from 60 – 120 ˚ C. DSC and TGA are usually combined together to more thoroughly understand the thermal behavior of a pharmaceutical material



DSC



TGA

AFM (Atomic Force Microscopy (AFM) analysis)

Surface topography can be measured using pictures with near-atomic resolution obtained through the use of Atomic Force Microscopy (AFM) analysis. Another name for AFM is scanning probe microscopy. Sample surface roughness can be measured by Atomic Force Microscopy down to the angstrom scale. AFM analysis can offer quantitative measures of feature sizes, including step heights and other dimensions, in addition to surface images. Furthermore, qualitative mapping of other physical properties as adhesion, modulus, dopant distribution, conductivity, surface potential, electric field, and magnetic domains can be achieved by sophisticated atomic force microscopy measurement modes.

Measurements of contact angles

Using the sessile drop method, the contact angles between water and diiodomethane were measured against tablets of formulations 1, 4, and 7. SCA20 software (Dataphysics, Germany) was used to analyze the results under ambient settings after the contact angle system OCA15plus (Dataphysics, Germany) was used to get the results. A 2 ml drop of diiodomethane and 2 ml of water were poured onto tablet surfaces at a 1 ml/s dosing rate using a micro-syringe. The internal camera was used to record the contact angle of the drop at 0 and 1.2 s. The software built into the device calculated the contact angles that were seen during this time. With both solvents, three determinations of each formulation were made. The angle was used to calculate the polar surface

energy and dispersive energy.

Acute toxicity studies

Acute oral toxicity studies of gel formulations were carried out in Swiss mice using the limit test or main test (Up and down procedure) as per OECD guideline 425. formulation was dissolved in DMSO and a single oral dose of 2000 mg/kg was used for limit test in overnight fasted animals. The animals were observed continuously for first 6 h after dosing and thereafter for 14 days for toxicity signs, morbidity and mortality. The dose for main test was selected from the default progression factor on the basis of onset, duration and severity of toxic sign, morbidity and time of death in limit test. When the main test was performed, the high dose at which animal showed mortality and low dose at which animal survived were used to calculate LD_{50} by using AOT software.

No signs of lethality or morbidity were detected in the rats given different doses up to 5000 mg/kg of extract for two weeks. Therefore, the median lethal dose (LD_{50}) of extract was higher than 5000 mg/kg for formulations.

The Experiment in Detail:

Nine trial runs total were carefully organized, with designated levels made using the Design Expert® software. These runs were documented in the provided table. The formulations, as dictated by the design, were meticulously prepared and subjected to scrutiny for their % drug release (Y1) it was considered as response or dependent variables. The observed responses were fitted into various mathematical models, such as linear, two-factor interaction (2FI), and quadratic models, in order to reveal the underlying dynamics. The ensuing analysis of variance (ANOVA) helped determine the created model's statistical significance as well as the statistical importance of the terms that made up the model.

Visualizing Relationships:

To enhance comprehension of the intricate relationships between the independent and dependent variables, 3D response surface plots, 2D contour plots, and perturbation graphs were generated using the Design Expert® software. These graphical representations provided an insightful depiction of how changes in variables influence the responses. Characterization of the Atomic Force Microscopy (AFM), contact angle and TGA was performed using Molecular Imaging (FastScan Dimension, Bruker) in tapping mode to describe the thin film surface morphology. Silicon cantilevers (Fastcsan-A) with a typical tip radius of 5 nm were used. The cantilevers had a resonance frequency of

around 1.25 kHz. The 2D AFM images were taken on a 400 nm scale. The 2D images were converted to 3D images using WSxM 5.0 Develop 9.1 software

Optimization Strategy:

The pinnacle of the process involved optimizing the colchicine formulation. This was pursued by striving for desirable attributes, The optimized formulation's coordinates were distinctly marked on the overlay plot, highlighting its location.

Validation and Assessment:

The validation phase encompassed preparing the optimized formulation as determined by the optimization process. This formulation was then subjected to assessment, measuring vesicle size and percentage entrapment efficiency. To gauge the efficacy of the model, a comparison between predicted and observed responses was carried out, yielding the percentage error.

In essence, this comprehensive experimental approach utilizing advanced software and analytical techniques allowed for a systematic exploration of the relationship between variables and their impact on the formulation attributes. It facilitated the optimization process, ensuring the final formulation met the desired criteria while offering a validated model for future applications.

Estimation of Pharmacokinetics parameters of stand colchicine, topical formulation BL6 and SL06 after topical skin application in wistar rats

Procedure:

Wistar Rats of either sex weighing 200-300g were acclimatized to laboratory conditions for 7 days. Animals were divided into 3 groups (n=2). The furs of the rats were closely shaved avoiding any abrading to skin. Group I was administered orally with colchicine standard 0.5 mg tablet dissolved in distilled water. Group II and Group III was applied with test formulation BL6

and SL06 respectively. The blood samples were withdrawn under light anesthesia from retro orbital plexus from each animal. The blood was withdrawn after the test compound administration at various time intervals mainly at 0.5, 1, 4, 6, 12, 24, and 48 hours. The blood samples were collected in the heparinized tubes and plasma was separated and used for estimation of concentration of test and stand compound. The HPLC analytical method was adopted for the estimation of plasma concentration