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Design, development and evaluation of herbal extract loaded nanogel of *wrightia tinctoria* for diabetic wounds

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Abstract
The project aimed to formulate and evaluate a nanogel loaded with *wrightia tinctoria* (w. tinctoria) leaves extract for its potential diabetic wound healing activity. W. tinctoria, commonly known as the Sweet Indrajao or Pala Indigo Plant, is known for its medicinal properties, including wound healing. Nanogels have gained attention in recent years as a promising drug delivery system due to their small size, high stability, and ability to encapsulate various bioactive compounds. The project began with the extraction of wrightia tinctoria leaf extract using a suitable solvent. The extract was then loaded into a nanogel formulation using a specific technique. The formulation process involved optimization of various parameters such as polymer concentration, surfactant type, and stirring speed to achieve a stable and effective nanogel. The formulated nanogel was subjected to various characterization tests to assess its physical and chemical properties, such as particle size, zeta potential, drug loading efficiency, and *in vitro* release profile. These tests confirmed the successful encapsulation of wrightia tinctoria leaf extract within the nanogel and its controlled release behaviour. The findings of this project suggest that wrightia tinctoria leaf extract-loaded nanogel holds great promise as a potential therapeutic option for diabetic wound healing applications. The nanogel formulation can provide controlled and sustained release of active compounds, improving the efficacy and duration of the wound healing process.

Keywords: *Wrightia tinctoria* leaf extract, nanogel, partical size, zeta potential, diabetic wound

Introduction

Novel drug delivery system

Recent discoveries into the pharmacokinetic and Pharmacodynamic behavior of drugs have made the creation of the idealdrug delivery system easier. The carriers known as innovative drug delivery systems (NDDS)help keep medication doses within therapeutic ranges for extended periods of time. Novel medication delivery systems have a number of benefits over traditional drug delivery methods.

- For an extended length of time, the ideal therapeutic medication retention in the bloodor tissue may be sustained.
- Extended periods of time at pre-determined release rates may be achieved.
- For drugs with a short half-life, the duration could be extended
- It may be possible to eliminate side effects by targeting the location of action.
- Frequently dosage and medication waste

With the goals of minimizing drug degradation or loss, preventing harmful side effects, improving drug bioavailability, and encouraging and facilitating the accumulation of the drug in the necessary bio- zone (site), different kinds of drug delivery systems have been developed, and some are currently under development. There aren't any number innovative carriers that have been proven effective in delivering drugs in a targeted and regulated manner. It is crucial to assess the terminologies used under the many main categories of innovative drug delivery systems effectively.

- Drug action is provided at a predetermined pace by sustained or controlled drug –- delivery systems, which offer a continuous (zero-order) release of the medication atlevels in the blood that are therapeutically efficacious.
- Drug action is achieved by localized drug delivery systems through either spatial or temporal regulation of drug release (typically at a rate that is rate-limiting) in the target area [1].

Nanogels

- Nanogels are three-dimensional hydrogel materials formed by networks of cross-linked swellable polymers.
- They are nanoscale in size and can hold water without dissolving into the aqueous medium.
- Nanogels can be synthesized using naturally occurring or synthetic polymers, or a combination of both.

Properties and Design

- Nanogels can be designed to be compatible with various molecules, including drugs, fluorophores, peptides, proteins, nucleic acids, and inorganic nanoparticles.
- Their characteristics (charge, size, porosity, amphiphilicity, degradability, and softness) can be adjusted by modifying their chemical composition.
- Nanogels are mostly spherical and can have core-shell or core-shell-corona structures.

Advantages for Drug Delivery

- Nanogels offer targeted drug delivery due to their softness, stimuli-responsive behavior, and swelling properties.
- They protect cargo from elimination and degradation.

- Nanogels have a large surface area for bioconjugation and stable interior networks for biomolecule incorporation.
- They provide controlled particle size and biodegradability for sustained drug release.

Drug Release and Specificity
- Nanogels allow controlled drug release, overcoming limitations of macro drug delivery vehicles.
- Their high loading capacity surpasses liposomes and polymeric micelles.
- Nanogels can hold biological molecules via electrostatic, van der Waals, or hydrophobic interactions.

Applications
- Nanogels are used for targeted drug delivery, protein therapeutics, combination drug delivery, and oligonucleotide delivery.
- Nanogels are promising carriers for efficient drug delivery, offering versatility and overcoming challenges associated with chemotherapy.

Materials
- Eudragit s-100
- Tween-80
- Glycerol
- Carbopol-940
- Triethanolamine
- *Wrightia tinctoria* leaf extract

Methodology
- Collection of medicinal plant
- Authentication for collected medicinal plant
- Drying
- Grinding of crude drugs
- Solvent selection
- Extraction of crude drug
- Formulation of nanogels
- Evaluation of formulated nanogels

Collection of Medicinal Plant
- Leaves are collected from wild plants in and around the Coimbatore.
- It's known that the active constituents of medicinal plants are affected by many factors and may vary during the course of plant growth.

Proper time of collection is very important to obtain a drug of good quality.

Plant Authentication
Plant authentication is the process of verifying the identity of a plant. This is often done in research to ensure that the correct plant is being studied. The leaves *Wrightia tinctorial (Roxb) R*.Br. (BSI/SRC/5/23/2023-24/Tech/111) were collected from in and around Coimbatore, India in the month of januvary 2024 and it was identified and authenticated. The taxonomical identification and authentication of the plant was done by Dr. M.u. Sharief, scientiest "f" & head of office, Botanical survey of India, southern regional centre, Coimbatore.

Shade Drying of Plants
Reasons for dryin
- To facilitate preservation of contents.
- To fix their constituents, by preventing reactions that may occur in presence of water.
- To prevent the growth of microorganisms such as bacteria and fungi.
- To facilitate their grinding.
- To reduce their size and weight.

Insufficient drying favours spoilage by micro-organisms and makes it possible for enzymatic destruction.

Natural drying
This is accomplished by natural air in sun or shade.

Lyophilization (Freeze drying)
Frozen material is placed in an evacuated apparatus which has a cold surface maintained at -60 to 80˚c. Water vapour from the frozen material passes rapidly to the cold surface. It is used for drying heat it is used for drying heat sensitive substances E.g. Antibiotics and protein.

Chemical drying using desiccator
An absolutely dried drug that completely freed from water, when exposed to air it absorbs 8-10% of moisture and is called air-dry drug.

Stabilization
- On long storage, enzymatic reactions will slowly destroy the constituents, because the last traces of water can never be removed.
- In order to avoid this degradation, the enzymes should be destroyed before drying, a process usually called stabilization.

Grinding of crude drugs
- Regardless of whether the crude drug is to be used for isolation of a pure compound or manufacture of a simple preparation the first operation that must be performed is grinding of the plant material to a powder of suitable particle size.
- It is important that the particles are of as uniform size as possible
- Large particles take a longer time for complete extraction than small ones.
- Large differences in particle size thus slow down the extraction process.

Solvent Selection
The ideal solvent for a certain pharmacologically active constituent should:
- Be highly selective for the compound to be extracted.
- Have a high capacity for extraction in terms of coefficient of saturation of the compound in the medium.
- Not react with the extracted compound or with other compounds in the plant material.
- Have a low price.
- Be harmless to man and to the environment.
- Be completely volatile.

Preparation of plant extract

The dried powder of wrightia tinctoria (100mg) was extracted successively with 700ml of ethanol in a Soxhlet extractor for 18 hours. The solvent was evaporated at 30-35˚c. The yield was a dark brownish residue of about 50 ml.

Fig 1

Formulation of Nanogel

Accurately weighed quantity of Drug, Eudragit S-100 (polymer) and Tween-80 as stabilizer are dissolved in glycerol while stirring. Prepared aqueous phase containing Carbopol-940 dissolved in water with continuous stirring and heat. This drug containing phase is sonicated on Ultra sonic bath sonicator. The drug phase is added drop by drop into the aqueous phase during homogenization to form emulsion. The emulsion converted into nanodroplets by homogenizer which formed O/W emulsion. Homogenization was continued for one hour. Triethanolamine added to form the gel with continuous stirring to nanogel.

Fig 2

Table 1: Formulation table of *Wrightia Tinctoria* Nanogel

Composition	F1	F2	F3	F4	F5	F6
Wrightia tinctoria herbal plant extract(ml)	2	2	2	2	2	2
Eudragit-s-100(mg)	10	15	10	25	15	25
Tween-80(ml)	5	5	10	5	10	5
Glycerol(ml)	10	10	5	10	10	10
Carbapol(mg)	5	15	5	10	15	30
Water(ml)	15	50	10	20	30	50
Triethanolamine(ml)	1	1	1	1	1	1

Evaluation of Nanogel

Appearance

The prepared gel bases were inspected visually for clarity, colour and presence of any particles.

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

pH measurement

The pH measurement was carried out by using calibrated digital type pH meter by dipping the glass electrode and the reference electrode completely into gel system so as to cover the electrodes.

Measurement of particle size of formulation

The mean size of the selected nanogels were determined by using Malvern Mastersizer 2000 MS. The mean particle size was recorded.

Zeta potential

The zeta potential indicates the overall charge a partical acquires in a specific medium stability of the nano gel during storage can be predicted from ZP value.ZP indicates the degree of repulsion between close and similarly charged particals in the dispersion the suitable dilutions of the dispersion are made using distilled water and it is scanned by using disposable sizing cuvette.the electrophoretic mobility (zeta potential) measurement of nanogel was done by using zetasizer (Nano ZS90,Malvern instruments).the samples were placed in polystyrene cuvette(at 25C) combined with zeta dip cell was used to measure the potential

Drug content

For the estimation of the drug in gel, Wrightia tinctoria was extracted from 1 gm of gel formulation with 50 ml of acetate buffer (pH 5.5) and mixture was filtered through membrane filter (pore size 0.45 µm). From this, 2 ml was pipette out and made upto 10 ml. The absorbance of the sample was determined spectrophotometrically at 270 nm. The concentration of Wrightia tinctoria was estimated from the calibration curve.

***In vitro* release studies**

The drug release from the formulation was determined by using the apparatus known as Franz Diffusion Cell, which consist of a cylindrical glass tube which was opened at both the ends. 1 gm of gel was spread uniformly on the surface of cellophane membrane (previously soaked in medium for 24 hrs) and was fixed to the one end of tube. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touches (1-2 mm deep) the surface of diffusion medium i.e. 100 ml of pH 5.5 acetate buffer contained in 100 ml beaker. The assembly was placed on thermostatic hot plate with magnetic stirrer and maintained at temperature 37°±2° the contents were stirred using magnetic bar at 100 rpm for a period of 8 hrs, 2 ml of samples were withdrawn at different time intervals. This 2 ml was diluted upto 10 ml of fresh acetate buffer (pH 5.5) and sample were analyse at 270 nm in UV-Vis.

Spreadability

Spreadibility is determined by wooden block and glass slide apparatus. Weights about 20g were to the pan and the time were noted for upper slide (movable) to separate completely

from the fixed slides. Spreadability was then calculated by using the formula:

S=M.L/T

Where, S=spreadability, L=Length of glass slide, M=weight tied to upper slide, T=Time taken to separate the slide completely from each other.

Extrudability

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting plug flow. The method adopted for evaluating nanogel formulation for extrudability is based upon the quantity in percentage of nanogel and nanogel extruded from lacquered aluminium collapsible tube on application of weight in grams required at least 0.5cm ribbon of nanogel in 10 second and the extrudability of formulations was checked.

Extrudability = Applied weight to extrude the nanogel from tube (in gm)/ Area (in cm2). Viscosity:

The viscosity of the formulations (Nanogel) was determined at 250C by using Brookfield viscometer. The measurement of each formulation was done in triplicate and average values are calculated.

Result and Discussion

Table 2

Evaluation parameters	F1	F2	F3	F4	F5	F6
Appearance	Clear	Clear	Clear	Clear	Clear	Clear
Homogeneity	Homo genous	Homo genous	Homo genous	Homo genous	Homo genous	Homo genous
Ph	5.2	5.4	5.43	5.39	6.2	6.8
Viscosity in cp at 50(rpm)	7500	8585	9063	8000	9400	9577
Partical size(nm)	630.4	580.1	560.2	480.7	457.4	437.5
Drug content	87.12	89.71	92.29	94.55	96.11	98.5
In vitro drug relese (%)	80.21	79.56	83.27	85.51	88.13	91.47
Spreadbility (g.cm/)	3.73	4.1	5.05	6.29	6.8	7.8
Extrudability (g)	219	268	270	254	266	270

Particle Size and Pdi

Particle size of optimized formulation is 476.5nm and PDI is 0.170 as the smaller particle size may in turn bring about more bio-availability.

Zeta Potential

The reduced zeta potential value of -31.2 mV indicated that the prepared nanogel possess a higher degree of long- term stability

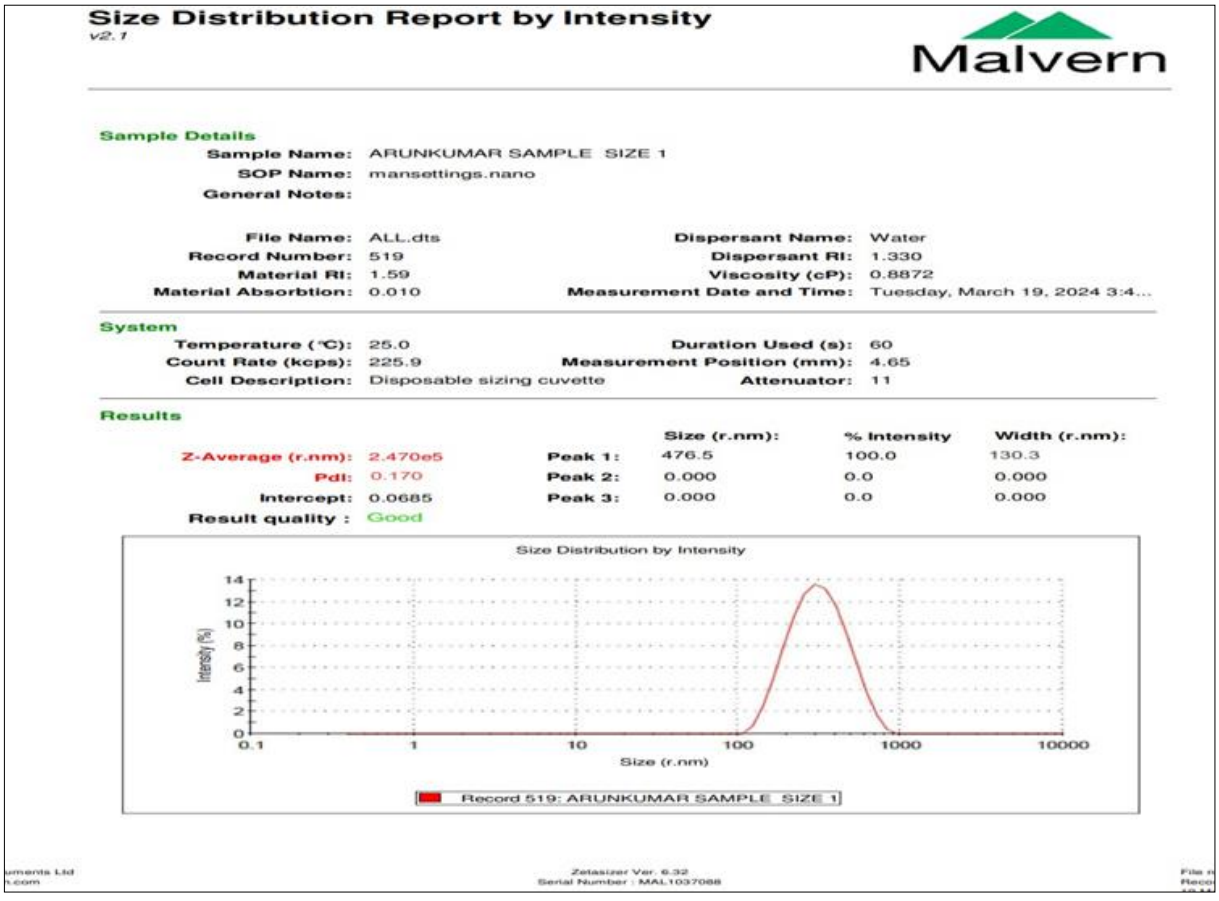

Fig 3: Partical Size Report

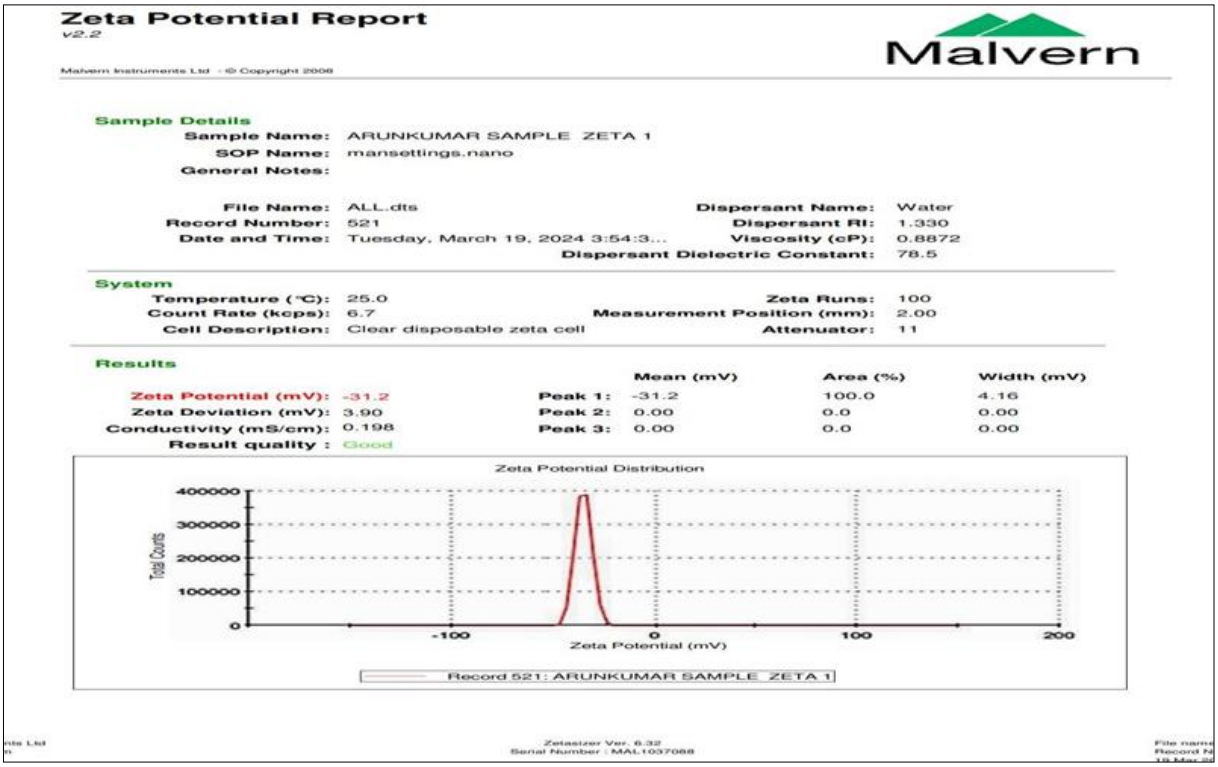

Fig 4: Zeta Potential Report

In vitro Drug Release Studies

Table 3: *In vitro* drug release profile for formulation f6

Time (In hrs)	Cumulative % Drug Release
1	3.95
2	17.14
3	42.26
4	57.66
5	67.49
6	78.54
7	89.15
8	92.97

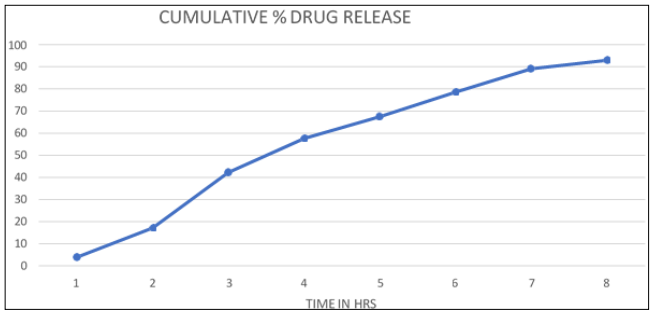

Fig 5: *In vitro* Drug Release Graph of F6

Table 4: Cumulative Drug Release Values of Different Formulations Of wrightia tinctoria

Time (in hrs)	Cumulative % drug release					
	F1	F2	F3	F4	F5	F6
1	0.68	0.89	1.23	2.10	3.10	3.95
2	3.35	5.27	7.12	6.25	13.15	17.14
3	6.54	14.21	14.21	16.34	31.53	42.26
4	13.21	20.28	25.31	31.38	43.26	57.66
5	47.97	36.51	43.59	47.12	56.45	67.49
6	64.1	52.25	56.82	59.10	71.68	78.54
7	71.98	68.78	67.61	70.67	79.71	89.15
8	78.18	79.39	86.74	84.42	88.13	92.97

Cumulative Profile for Wrightia Tinctoria Nanogel

- At the end of the 8 hours, the *in vitro* release of the different formulations were found to be in the range of 78.18% to 92.97%.
- The reason for release of formulated nanogel is good.

From the above *in vitro* release table the optimized formulations were found to be F6 because of higher *in vitro* release of 92.97% when compared to the other formulations. As it is more hydrophilic it enhances the release of the drug.

Summery and Conclusion

The project successfully formulated a nanogel loaded with wrightia tinctoria leaf extract for diabetic wound healing. Here are the highlights:

1. **Nanogel Formulation**
   - Wrightia tinctoria leaf extract was incorporated into a nanogel using specific techniques.
   - Parameters like polymer concentration and surfactant type were optimized.

2. **Characterization Tests**
   - The nanogel's properties were assessed:
   - Particle size: Small and stable.
   - Zeta potential: Surface charge.
   - Drug loading efficiency: Effective encapsulation.
   - *In vitro* release profile: Controlled release of active compounds.

3. **Promising Findings**
   - Wrightia tinctoria-loaded nanogel holds promise for diabetic wound healing.
   - Controlled release enhances efficacy and duration of wound healing.

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