

## Development of Tamarindus indica seed Derived polysaccharide and kaolin complexed Nanosponges for Hemorrhage control

L.Abinaya<sup>1</sup>, R.Sudha<sup>1</sup>, M.Pavithra<sup>1</sup>, K.S.Aswitha<sup>1</sup>

<sup>1</sup>Kamarajar College of Pharmacy, Keerapalayam, Chidambaram- 608602, Tamil Nadu, India..

\*Corresponding author:

L.Abinaya

Email ID : [abinaya77888@gmail.com](mailto:abinaya77888@gmail.com)

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### ABSTRACT

Uncontrolled bleeding continues to pose serious challenges in emergency trauma care, requiring the advancement of swift and powerful hemostatic agents. This report study investigated the development and assessment of nanosponges synthesized from Tamarindus indica seed polysaccharide complexed with kaolin, designed to achieve heightened hemostatic efficacy. The natural polysaccharide was extracted and refined, then crosslinked and combined with kaolin particles through the emulsion solvent diffusion method to fabricate porous, biocompatible nanosponges. The addition of kaolin, a recognized clot-promoting agent, enhances platelet activation and coagulation, while the polysaccharide framework offers structural support and biodegradability. Testing characterized the prepared nanosponges using FTIR, SEM, Porosity and swelling studies which provided noteworthy result. In vitro clotting experiments demonstrated considerably reduced clotting time, signifying productive hemostatic potential. Hence Tamarindus indica seed polysaccharide-kaolin nanosponges represent a promising, natural-source, cost-effective solution for hemorrhage control which will benefit in clinical setting..

**Keywords:** *Tamarindus indica*, *nanosponges*, *kaolin*, *hemostasis*, *polysaccharide*, *wound dressing*, *emulsion solvent diffusion*

### 1. INTRODUCTION

Hemorrhage remains the primary cause of death from disasters both natural and unnatural, accidents whether on the road or elsewhere, as well as general civilian trauma, additionally being the most prevalent reason for mortality during orthopedic, cardiovascular, and liver operations [1]. Excessive bleeding within the body can result in illness and even death. Bleeding amid surgery is a serious clinical dilemma that can greatly distress the patient and potentially lead to significant consequences [2]. Hemostatic agents employed surgically are recommended to better hemostasis when conventional methods like compression, sutures, or electrocoagulation prove inadequate [3]. Currently, hemostatic materials on the commercial market chiefly incorporate zeolite powder, starch hemostatic microspheres, gelatin sponges medical, chitosan bandages, fibrin dressings, oxidized cellulose, oxidized regenerated cellulose coverings, and more [4]. While existing hemostatic agents demonstrate effectiveness, limitations persist such as delayed reaction, potential side effects, and difficulties in application.

Recent progress in nanotechnology has paved the route for developing innovative remedies, for instance nanosponges to tackle these challenges. Nanosponges resemble a three- dimensional framework or scaffold whose backbone constitutes long polyester chains. Cross- linkers integrated into solution with tiny molecules acting as diminutive grappling hooks [5]. When contacting blood, haemostatic sponges can induce platelet adhesion, aggregation, and thrombosis, barring blood from escaping wounds, activating the release of coagulation factors, causing the blood to form a stable fibrous protein polymer, forming blood clots, and accomplishing the aim of wound bleeding management. Haemostatic sponges arrive in an assortment of forms and sizes [6]. This paper aims to develop nanosponges using natural polysaccharides and clay to help control bleeding during surgery. The materials used are Tamarindus indica seed polysaccharide and kaolin clay. The paper also discusses different in-vitro methods to test how the sponges affect blood clotting.

## 2. MATERIALS AND METHODS

### Materials

*Tamarindus indica* seeds were obtained from agricultural land because they are agricultural by-product. Kaolin, Citric acid and Ethyl acetate were obtained from Sisco Research Laboratories Pvt.Ltd.

#### Extraction of Polysaccharide using seeds (*Tamarindus indica*)

Cold distilled water was slowly poured into a beaker containing trisodium phosphate powder and gently mixed to form a thick slurry. This viscous mixture was then carefully transferred to a flask of rapidly boiling distilled water and left to simmer for 20 minutes with occasional swirling. The resulting clear solution was kept overnight for further settling. On the following day, it was whirled for 20 minutes in a centrifuge at high speed to remove any lingering impurities. The transparent supernatant was siphoned off and drizzled into an abundant volume of 95% ethanol while stirring briskly. The fine precipitate that emerged was collected using a fine mesh sieve and spread out on the tray of an oven. It was left to dry for 4 hours at a temperature just above 50 degrees Celsius. Once desiccated, the fluffy white polymer was stored in an airtight chamber [7].

#### Preparation of *Tamarindus indica* seed polysaccharide -Kaolin nanosponges (NS)

TSP-Kaolin nanosponges were prepared through an emulsion solvent diffusion technique. This method used two distinct phases: an aqueous solution and an organic solvent mixture. The aqueous phase consisted of polyvinyl alcohol, while the organic phase contained TSP and Kaolin dissolved in a suitable organic solvent 1:1 (Ethyl acetate). After the organic solution was thoroughly blended, it was slowly poured into the aqueous phase under vigorous stirring for 2 hrs. This process led to the formation of nanosponges, which were then harvested by filtration and thoroughly washed. Finally, the nanosponges were dried either at room temperature in air for 24 hours [8].

#### Physicochemical characterization of TSP-Kaolin nanosponges

##### Fourier Transform Infrared Spectroscopy (FTIR)

The investigation of a sample using FTIR allows for structural elucidation, especially identification of functional groups present in the structure. The sample were subjected for FTIR analysis at scan range 4000-400cm<sup>-1</sup> and KBr pellet method was used for the analysis and the spectra were obtained for further analysis. [9].

##### Scanning Electron Microscopy studies (SEM)

Surface morphology of the prepared nanosponges was investigated using SEM. Nanosponge samples were dehydrated at an oven in order to get rid off minor moisture extent previous to testing. The dried powder was accurately spread on aluminum stubs with double-side carbon adhesive tape. Blot lightly to remove surplus and ensure smooth lay. To render the samples conductive and eliminate charging under the electron beam the samples were coated with a thin gold layer (around 5–10 nm) using vacuum sputter coater. The S treated surface was characterized by SEM at several magnifications to apply surface properties, porosity and particle size distribution. [9].

##### Particle size and Polydispersity Index

The particle size of *Tamarindus indica* seed-based nanosponges, was analysed by dispersing nanosponge powder (1-2 mg) with 1-2 mL of deionized water. A 0.22µm syringe filter may be used to remove larger suspended solid contaminants. The average particle size, polydispersity index (PDI), and distribution profile are calculated from the data gathered and further processed. [10].

##### Porosity

Now, porosity study is done to see when and how many nanochannels and nanocavities are created. Nanosponges' porosity is determined with helium pycnometry because helium gas can ingress inter- and intra-particular pores of materials. Enclosed material is accurately measured by the helium displacement measurement method. Nanosponges are more porous than the parent polymer from which the polymeric system was produced owing to their porous characteristics [11]. Equation is used to calculate percent porosity

Volume fraction (porosity) is given in the following formula:

$$\% \text{Porosity} = \frac{\text{Bulk volume} - \text{True volume}}{\text{Bulk volume}} \times 100$$

##### Elemental Analysis

The EDX was simultaneously acquired with the SEM images for analysis of the elemental composition. The characteristic peaks of C, O and other inorganic elements (Si, Al or Ca originating from kaolin or additives) were also detected, indicating that the predetermined materials were incorporated into the matrix of the nanosponges

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### Water contact angle

Measurement of contact angle The prepared nanosponges wettability was evaluated by static contact angle measurement of water using sessile drop technique. A dried nanosponge powder sheet was next compaction-loaded into a thick disk or evenly cast onto a clean glass slide with the use of double sided adhesive tape to get a flat smooth surface. A deionized water droplet (2–5 µL) was carefully deposited on the surface using a micropipette. Contact angle with digital camera using a contact angle goniometer was obtained on the spot for the drop profile. The contact angle between the water droplet and the surface of nanosponge was processed via the ImageJ software. this measurement was made in various places on the surface and the average given

### Water absorption and swelling index

Optimization of water uptake and swelling index is done for the swellable polymer-based NS. This can be done by soaking NS directly in water. Equations for water uptake and swelling index severally area unit used to calculate

$$\text{Swelling Index (\%)} = \frac{W_t - W_o}{W_o} \times 100$$

Where:

W<sub>o</sub> = Initial weight of the dry sample (before swelling)

W<sub>t</sub> = Weight of the swollen sample after a specified time.

### In vitro hemostatic studies

An assay for whole blood clotting time can be used to assess the polysaccharide–kaolin nanosponges made from Tamarindus indica seeds' in vitro hemostatic activity. 100–200 µL of freshly drawn goat blood (either fresh or citrated and recalcified with 0.025 M CaCl<sub>2</sub>) is carefully added to the sample surface of a certain quantity of nanosponges (e.g., 20–50 mg) in a sterile Petri dish. Using a stopwatch, the amount of time it takes for a visible clot to form is measured. The clotting time is then compared to controls like untreated blood, plain kaolin, and a commercial hemostatic agent. Because of the nanosponges' capacity to enhance platelet adhesion, blood absorption, and coagulation factor activation, a shorter clotting time signifies improved hemostatic efficacy. [12].

### Collection of blood

Freshly collected samples: Since the samples are collected shortly after slaughter, and during the nine months of sampling, local butcher shops were selected in order to minimize the degradation of the compounds of interest. whole blood is required, the containers are already labeled and have an anticoagulant (EDTA or heparin). Samples are maintained on ice or in a cold box (4–8°C) and transported to lab within 1–2 hours. [13].

### Purity testing of blood

Visual Assessment: Cloudiness, aberrant colouring, and clots were looked at. The plasma was clear and showed no discoloration.

Microscopy: To check for anomalous cells, parasites, or microbial contamination, a stained blood smear was made. There were no signs of microbial contamination, and the blood cells were normal.

### Blood clotting assay

In each sterile test tube, 0.5 mL of the freshly collected goat blood was pipetted. The test sample tubes received 20 mg of nanosponge powder. All tubes were pre-equilibrated to 37°

A stopwatch was started as soon as mixing occurred. Every 30 seconds, tubes were tilted slightly to check for clot formation. Clotting time was registered when blood stopped flowing after tilting [14].

Negative control: blood with no additives. Positive control: blood + 20 mg kaolin.

Test sample: blood + 20mg nanosponges. The experiment was done in triplicate.

### Haemolytic activity of TSP-Kaolin nanosponges

Nanosponges (equivalent to 200 µg/ml of TSP nanosponges) were incubated at 37 °C for 90 min with 1 ml of diluted blood, Freshly pre-prepared PBS (pH 7.4) was used for all dilution purposes.

After incubation, blood containing suspensions were centrifuged at 2000 rpm for 10 min to separate plasma. The amount of haemoglobin released due to haemolysis was measured spectro-photometrically at 543 nm (Du 730, Beckman). The haemolytic activity was calculated with reference to blank and complete haemolyzed samples (induced by addition of ammonium sulfate 20% w/v). Optical microscopy was also used to see if there were any abnormalities in the blood cells after incubation. The observations were made with reference to the blank diluted blood [15].

Measure absorbance at 540nm (hemoglobin release)

Hemolysis (%) =  $\frac{A_{\text{sample}} - A_{\text{negative control}}}{A_{\text{positive control}} - A_{\text{negative control}}} \times 100$

### 3. RESULTS AND DISCUSSION

The emulsion solvent diffusion method facilitated the creation of *Tamarindus indica* seed polysaccharide-kaolin nanosponges. The analytical characterization and biological activities are discussed below.



Fig 1. Coarse nanosponges

Fig.2. Fine nanosponges

#### Fourier Transform Infrared Spectroscopy

FTIR spectrum of the synthesized nanosponges exhibited numerous characteristic absorption peaks confirming the presence of significant functional groups and successful integration of its components as follows: A large absorption band about  $\sim 3300 \text{ cm}^{-1}$  assigned due to O–H stretching vibrations, confirms that the existence of hydroxyl groups is common in polysaccharides. An absorption band at  $\sim 1700 \text{ cm}^{-1}$  is assigned to C=O stretching, which indicates the crosslinking between the polymer chains and supports the presence of ester or carboxylic functional groups. Peaks at  $\sim 1100\text{--}1200 \text{ cm}^{-1}$  correspond to C–O–C stretching vibrations, which further suggest the formation of polysaccharide backbone structure. The Si–O and Al–O vibrations respectively appear as extra peaks between  $1000\text{--}1100 \text{ cm}^{-1}$  and  $500\text{--}600 \text{ cm}^{-1}$ . This shows that kaolin (which is aluminum and silicon) was successfully incorporated into the nanosponges. The spectral results confirm the structural configuration of the nanosponges and confirm the elemental integration of organic (polysaccharide) and inorganic (kaolin) components within the nanosponges in line with their proposed use as hemostatic agents.

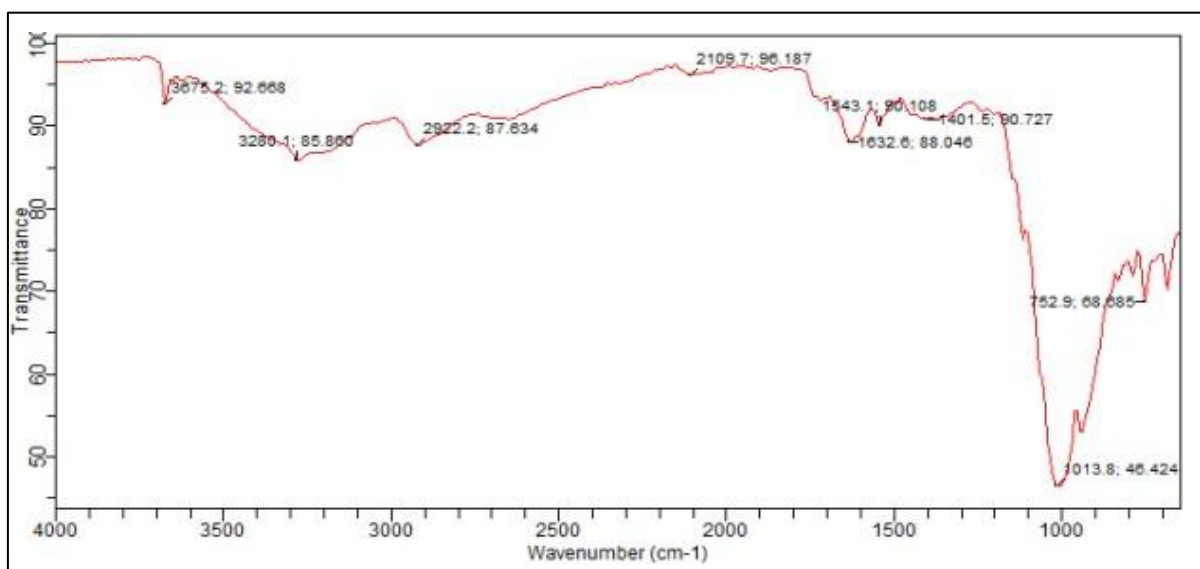


Fig.3. FTIR image of TSP-Kaolin nanosponges

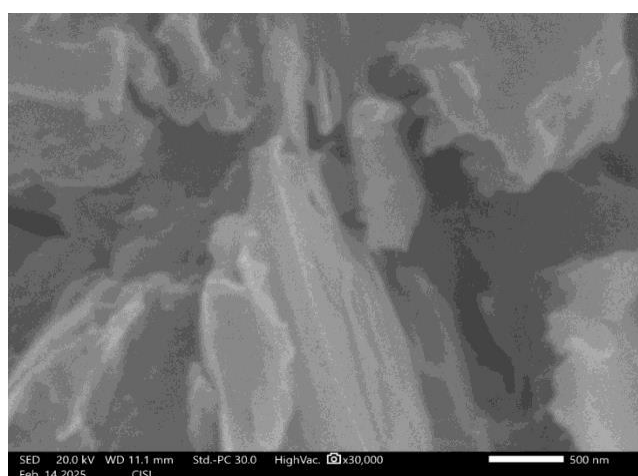
### Scanning Electron Microscope

The morphological characteristics of the Tamarindus indica seed polysaccharide- poly(ethylenimine)□kaolin composites were also studied through SEM, with an image of it presented in **Figure.4** (SEM image 30,000×). The SEM micrograph exhibited a heterogeneous and porous structure made of randomly shaped, stacked flakes in accordance with the lamellar nature of the kaolin clay. The particles seem to lie well-spread as a loosely- aggregated sponge-like net.

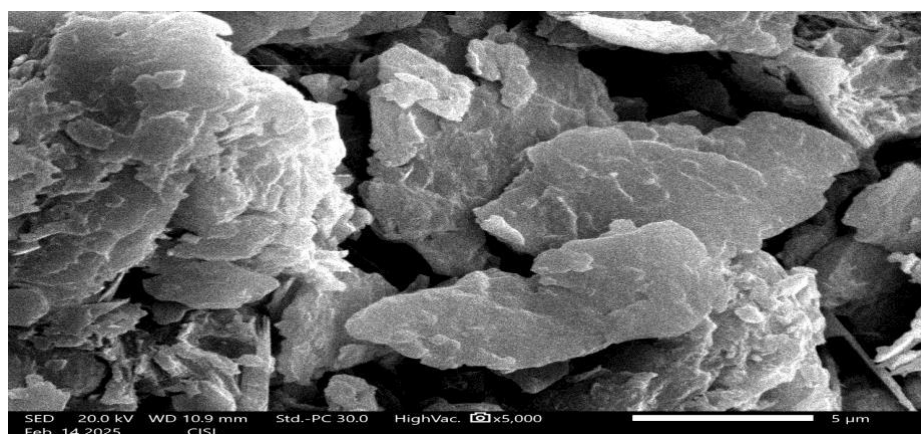
The 3D structure revealed a surface morphology with a rough and wrinkled surface with multiple interconnected pores, which indicates the actual synthesis of nanosponges, an essential feature in applications that require high surface area and porosity, e.g. applications involving drug delivery systems or adsorption matrices. The layered flaky structure observed in the image represents the kaolinite, The smooth and amorphous appearance of the matrix is due to the contribution of the Tamarindus indica seed polysaccharide, which may serves as stabilizer and binding agent during the formation of nanosponge.

Scale bar (500 nm) corroborates the nanoscale structure confirmed by high-magnification analysis revealing features with the  $< 1 \mu\text{m}$  scale. The porous architecture is believed to provide a sufficient surface area for drug loading and the sustained release of drugs, indicating that this nanosponge system has potential in pharmaceutical

and biomedical applications.



**Fig.4. SEM image of TSP-Kaolin nanosponges (30,000x)**



**Fig.5. SEM image of TSP-Kaolin nanosponges (5,000x)**

### Particle size and Polydispersity Index

The minimum particle range from 30nm to 190.5nm. The maximum particle size range from 1005.3nm to 4020.8nm. The maximum particle size is due to aggregation in the formulation. It indicates the broad distribution of particle size due to the need for process optimization such as emulsification, surfactant, stabilization on modification in the crosslinking particle parameter.



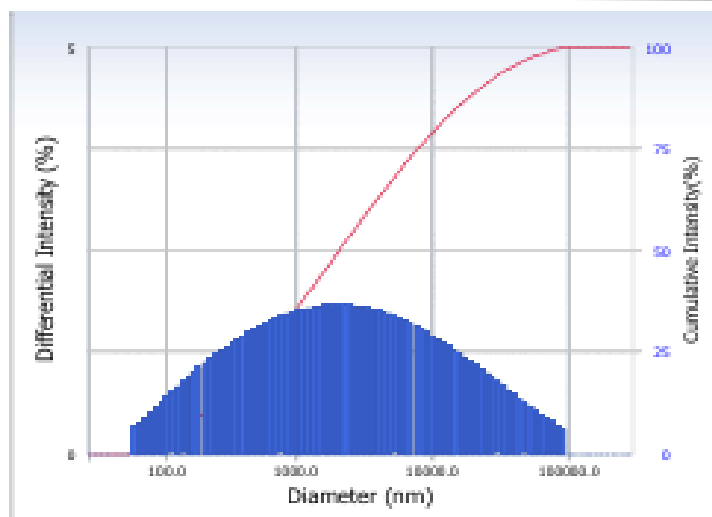


Fig.6. Intensity distribution

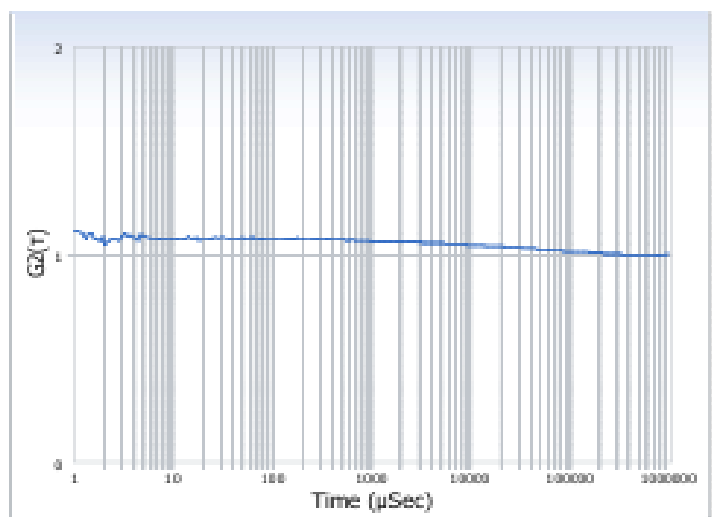


Fig.7. AC

**Fig.8** shows the detection of O, C, Si, and Al also confirmed the inclusion of kaolin in the structure of the nanosponge. Polysaccharide composition is verified through peak presence of carbon (C) and oxygen (O). Alumina and silica are the main ingredients of kaolin and support its hemostatic properties. Potassium (K) is derived from tamarind seed polysaccharide, which means that the minerals are organic. The peak appeared similar to copper (Cu), which could be an artifact from the EDX sample holder or grid and not a component of the nanosponge.

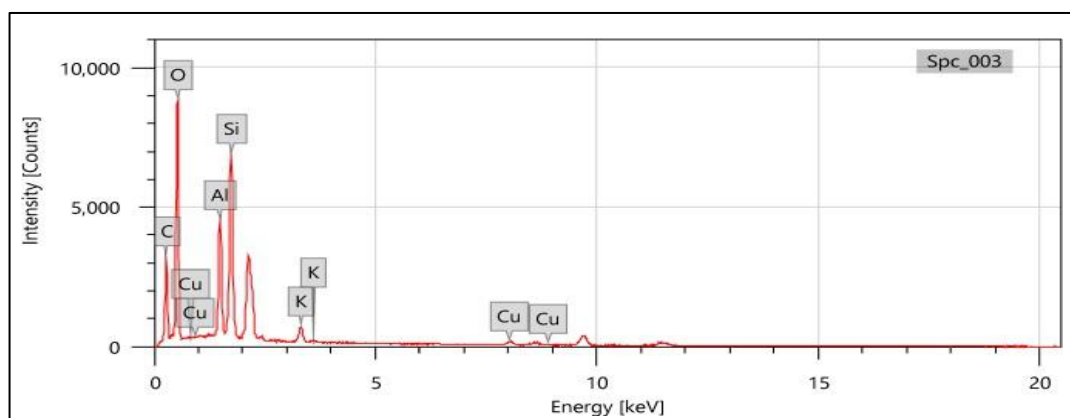
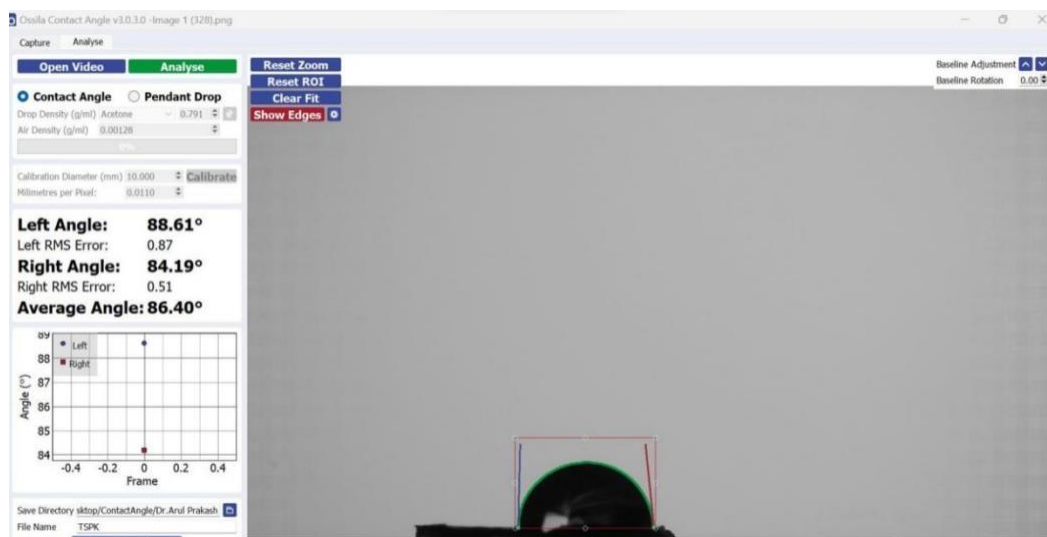


Fig.8. Elemental analysis of TSP-Kaolin nanosponges

### Water contact angle

The contact angle of  $86.40^\circ$  is measured, and indicates moderate wet ability of the surface. If this is a hydrophilicity test, the surface is borderline hydrophobic since surfaces with a contact angle  $<90^\circ$  are considered hydrophilic. If this is for biomaterial applications, a lower contact angle may be desired for greater liquid presence. This TSP-Kaolin nanosponges shows moderate hydrophilicity nature which is prerequisite for nanosponge



**Fig.9. Water contact angle of TSP-Kaolin nanosponges**

### Swelling index

The nanosponges indicate a high swelling index (~210%) having excellent fluid absorption capacity. This property is critical for promoting clot formation by concentrating blood components at the site of application. Which is favorable for hemostatic or topical delivery application.

### Invitro studies

#### Blood clotting time assay

To evaluate the hemostatic efficacy of Tamarindus indica seed-based nanosponges by measuring their ability to reduce whole blood clotting time.

Control clotting time = 210 sec Sample clotting time = 75 sec

$$= (210 - 75) / 210 \times 100 = 64.3\%$$

The nanosponges significantly reduced clotting time 60–65% faster than control, indicating strong hemostatic potential likely due to their porosity, swelling capacity, and surface interactions with blood components.

### Haemolytic test

Standard spectrophotometric hemolysis assay was applied to evaluate the hemolytic activity of Tamarindus indica seed-based nanosponge. The nanosponge sample had a calculated hemolysis percentage of -18.64%, below zero and thus non-hemolytic. Negative values can

occur as a result of Nanosponges light scattering or absorbance interference. There are small shifts on the baseline in the spectrophotometer. Potent RBC-protective or membrane-stabilizing actions from phytochemical agents.

ISO 10993-4 refers  $<5\%$  hemolysis = Non-hemolytic

These types of materials are clearly non-hemolytic, which means that they can be used in applications that contact blood. Thus, the constructed nanosponges exhibit high biocompatibility and may serve as candidates for hemostatic and wound healing application.

## 4. CONCLUSION

Herein, the nanosponges utilizing Tamarindus indica seed powder as a main polymeric fraction was fabricated to be used as a hemostatic agent and was evaluated. Formulation yielded porous, soft nanostructures that can rapidly absorb fluids and

interact with blood constituents.

Nanosponges also have a swelling ratio of ~210%, meaning they also have the ability to absorb a lot of fluid. Such a capacity is strikingly important for hemostatic substances that need to achieve effective concentration of blood cells and coagulation factors at the injury. The evidence obtained through SEM imaging with porosity and surface morphology showed a very spongy and interconnected pore structure which favors both absorption and cell adhesion. BCT assay confirmed that clotting time was extremely longer in the presence of the nanosponges than in control group. And hemostatic potential was confirmed by comparing clotting times in the control (average 210 sec) vs. The nanosponge-treated samples (clotted in 75–90 sec), a finding suggesting that the nanosponge samples clot 60–65% faster. The hemolysis test (where a value of -18.64% recorded for the nanosponges is regarded as 0% hemolysis) was used to evaluate hemocompatibility. Therefore it is concluded that *Tamarindus indica* seed-based nanosponges are biocompatible, eco-friendly

and cost-effective materials with great potential in bleeding control and wound healing. Studies will need to be conducted in vivo while the stability studies should be performed to examine the short or intermediate storage capabilities of nanosponge powder in clinical states, there should be retention time for films or sponges for these to be effectively used in emergency or surgical related applications.

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