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Gelatin/$CaCl_2$ based cellulose nonwoven for haemostat applications

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This study investigates the controlled delivery of wound healing and clotting agents in wound dressings to enhance haemostatic efficiency. Conventional methods such as exhaustion and padding result in excessive consumption of these agents, leading to inefficiencies. To address this, electrospraying of polymer is carried out on the wound contact site. A wet-laid hemostat is developed, followed by the electrospraying of gelatin and calcium ions to improve haemostatic performance. The prepared hemostats exhibit enhanced blood clotting efficiency and phosphate buffer solution (PBS) uptake, demonstrating their potential for advanced wound dressing applications.

Keywords: Blood clotting, Cationisation, Electrospraying, Gelatin, Wet laid, Wound dressing

1 Introduction

Uncontrollable bleeding causes adverse physiological conditions and leads to death in combat, surgery and accidents. The blood loss from the injured site has to be controlled with the aid of a haemostat when the natural blood coagulation cascade fails. The ideal haemostat should absorb body fluids, thereby concentrating blood coagulation components and promoting blood clotting. Natural (chitosan, starch, gelatin, alginate, and cellulose), synthetic (polyacrylic acid, zeolite, kaolin) and biologically derived materials (collagen, fibrinogen, and thrombin) are usually deployed as haemostats. Among these, cellulose, gelatin and calcium play an essential role in haemostasis due to their biocompatibility and ability to facilitate clot formation. Cellulose, in the form of short fibres, is known to be hydrophilic with better liquid retention properties, making it a suitable candidate for wound dressing applications by wet laid technique[1]. Gelatin and calcium are chosen in the present study to augment haemostasis. Gelatin, a water-soluble denatured collagen protein, can absorb five to ten times its dry weight in water, making it highly effective for haemostatic applications[2]. Additionally, calcium plays a pivotal role in initiating the blood coagulation cascade by facilitating the conversion of prothrombin to thrombin, which is the precursor for the formation of insoluble fibrin clots from soluble fibrinogen[3]. Gelatin also contributes to haemostasis by forming a mechanical barrier restricting blood flow while naturally resorbing within the body over two to six weeks[4].

Recent studies have demonstrated that calcium-incorporated gelatin nanofibrous webs, prepared using electrospinning techniques, exhibit lower haemolysis rates, reduced haemostasis time, and enhanced platelet adhesion, ultimately improving blood clotting[5]. Furthermore, gelatin-calcium polymer microfibres electrospun in an ethanol bath have been shown to form a fibrin-like mesh structure that effectively promotes coagulation[6]. To enhance the binding affinity of cellulose fibres to haemostatic agents, surface modification through cationisation using 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) has been employed[7]. The wet laying technique offers advantages over other nonwoven manufacturing techniques, as it utilises an aqueous solvent and short fibres to form a fibrous substrate suitable for haemostatic applications[8].

One of the major challenges in the preparation of wound dressings is the effective utilisation of agents deployed for haemostasis, as many of these materials are expensive. Mostly, all wound dressings are prepared either through exhaustion or a doping process wherein the polymer and additives are spread through the matrix. However, to be cost-effective and optimal performance, these agents must be concentrated at the wound-contacting surface to facilitate targeted haemostasis while ensuring sustained release[9]. In this study, gelatin and calcium ions were electrospray onto the wound-contacting surface of a wet-laid

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cellulose substrate to enhance haemostatic efficiency. Additionally, the cellulose fibres underwent cationisation to improve the binding of haemostatic agents, thereby ensuring better agent retention and haemostasis performance. The prepared samples were subsequently evaluated for their haemostatic efficacy.

2 Materials and Methods

2.1 Materials

Wood pulp was purchased from the South Indian Textile Research Association (SITRA) Coimbatore. Sodium hydroxide (NaOH), trifluoroethanol and sulphuric acid were obtained from Sisco Research Laboratories, Mumbai. CHPTAC (3-chloro 2- hydroxy propyl trimethyl ammonium chloride), calcium chloride ($CaCl_2$) and gelatin polymer (molecular weight ~ 60 kDa) were purchased from Sigma Aldrich Corporation, USA.

2.2 Methods

Fibrous wood pulp was treated with 18 wt % NaOH to increase the softness of the material (Na-wood pulp). The treated wood pulp was surface modified with CHPTAC to impart cationic charges on the surface by treating the fibres with 10 wt % NaOH at room temperature (26±2 ºC), followed by treatment with 65 wt % cationising agent at 80 ºC. The surface-modified fibres were then thoroughly washed, neutralised with 1 wt % sulphuric acid, and further subjected to aqueous washes to remove residual chemicals.

A wet-laid web comprising control and cationising fibres was formed using laboratory-scale wet-laying equipment. The haemostat dressing was developed by electrospraying gelatin (0.3 g) and a gelatin/ $CaCl_2$ solution (9 mM) in trifluoroethanol onto the wet-laid nonwoven web using Gamma High Voltage Research machine (Ormond Beach, USA), with machine parameters such as flow rate of 0.5 mL/h, the distance between needle tip to a collector of 15 cm and voltage of 20 kV.

2.3 Characterisation

The fibrous haemostat was characterised for the determination of functional groups using Fourier Transform Infrared (FTIR) (Bruker Tensor 27, Japan) spectroscopy. The thermal stability of the prepared samples was studied using a thermogravimetric analyser. The deposition of nanoparticles on the nonwoven surface was examined using a scanning electron microscope (S-3400 SEM, HITACHI, Japan). Phosphate buffer solution (PBS) uptake was evaluated by immersing the samples in PBS for 30 min. The weight change was measured, and the PBS uptake was calculated using Eq.1:

$$\text{PBS Absorbency (\%)} = \frac{W_1 - W_0}{W_0} \times 100 \quad \ldots (1)$$

where W_0 and W_1 are the weights of the samples before and after immersion in PBS, respectively.

The blood clotting time was calculated using a modified Lee and White method in which the blood was added to the samples in a test tube, and the clotting time was recorded as the duration required for the blood to resist flow[9]. The results were obtained from three independent samples, and the data were expressed as mean values with standard deviation.

3 Results and Discussion

3.1 Structural and Morphological Analysis

The functionality of wound dressings is largely determined by their base materials and incorporated haemostatic agents. In the present work, cellulose serves as the base substrate due to its excellent absorption and retention properties. During trauma/surgery, effective blood absorption is essential to initiate mechanical clotting under applied pressure. Although cellulose fibres can easily be used for the application as they can absorb through swelling, the major challenge is to prepare as a web using a wet laying process. Uniform wet-laid can be obtained if better dispersion of fibres is achieved. To address this, cationisation of the fibres is performed, enhancing dispersion and absorption properties. The wet-laid fibrous web exhibits a thickness of 6±5 mm and a mass of 250±9 g/m^2. Upon electrospraying with gelatin and $CaCl_2$, the weight and thickness of the fibrous nonwoven web remain unchanged, indicating minimal material deposition.

Gelatin with the lowest possible concentration is electrosprayed onto the prepared wet-laid web along with $CaCl_2$. The deposition of polymeric nanoparticles and calcium ions onto the substrate is confirmed using SEM (Fig. 1). SEM images reveal a higher deposition density on cationised nonwoven webs compared to control samples, likely due to the enhanced interaction between the cationic surface charge on the substrate and the sprayed particles. The electrosprayed particles are uniformly deposited throughout the fibrous web, increasing the surface area and reactivity, both of which are essential for hemostat applications.

3.2 Chemical and Thermal Characterisation

The control and cationised samples are characterised by FTIR spectroscopy [Fig. 2 (a)]. The peak around 2955 cm^{-1} corresponds to the stretching of CH_2 groups, and 3415 cm^{-1} corresponds to the stretching of the hydroxyl group in the cellulose polymer chain. A reduction in the broadness of the hydroxyl peak in cationised and calcium-incorporated samples suggests an interaction between the hydroxyl groups of wood pulp and the reactive groups of CHPTAC and Ca^{2+} ions. However, gelatin-incorporated control samples show a broad spectrum, which may be due to the hydrophilic nature of gelatin. Additionally, the characteristic carbonyl (C=O) stretching frequency at 1600–1700 cm^{-1} corresponds to the polypeptide backbone of the gelatin polymer, while the peak around 1000 cm^{-1} is associated with NH_2 vibrations in the substrate[11].

The thermogram of the developed samples is shown in Fig. 2 (b). The weight loss took place in three stages as the onset degradation occurs around 100 ºC, which was due to the removal of surface-bound water. The second stage, starting around 350 ºC, represents cellulose degradation. In cationised samples, degradation begins at a lower temperature (~300°C), likely due to the substitution of cationic groups on the hydroxyl groups of cellulose. The third onset degradation observed around 400 ºC is attributed to the degradation of the cellulosic polymer backbone. The incorporation of gelatin and $CaCl_2$ does not significantly alter the thermal stability of the material.

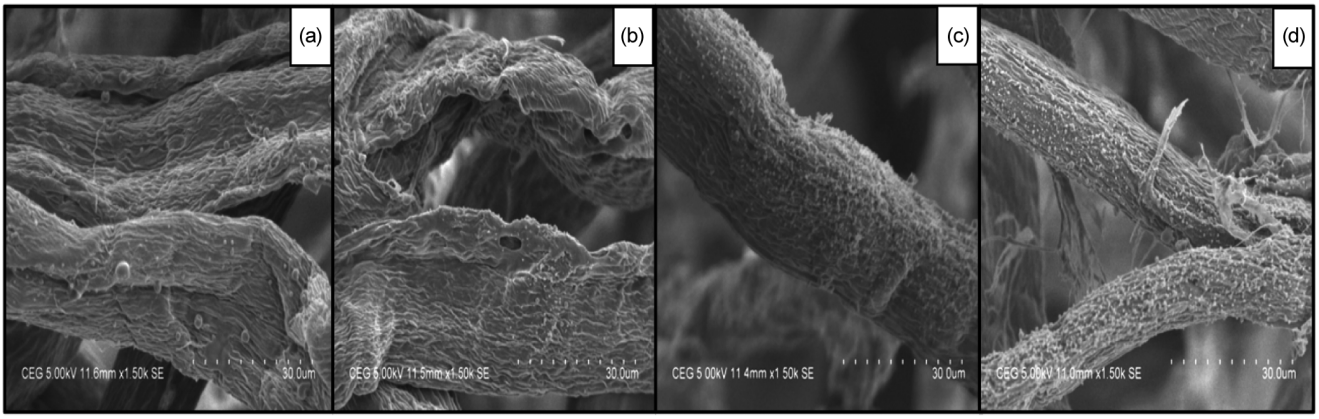

Fig. 1 — SEM images of developed wet-laid samples (a) gelatin sprayed, (b) gelatin-$CaCl_2$ sprayed, (c) cationised gelatin sprayed, and (d) cationised gelatin-$CaCl_2$-sprayed

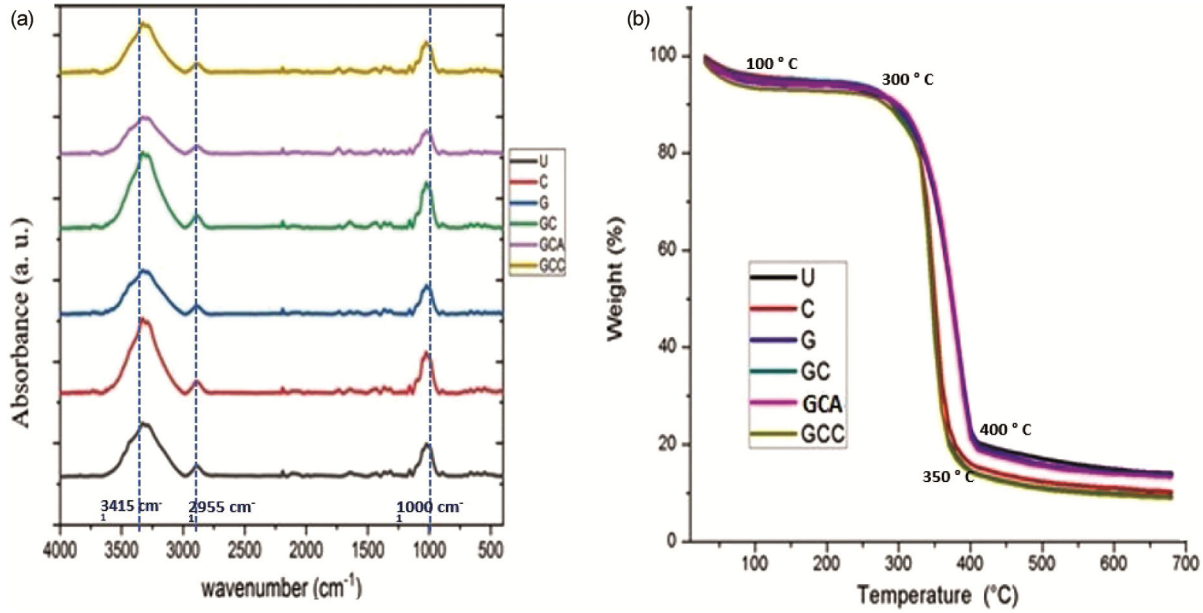

Fig. 2 — FTIR and TGA spectra of developed wet laid samples (U – untreated, C - cationised, G- untreated gelatin, GC - cationised gelatin, GCA - untreated gelatin with $CaCl_2$, GCC - cationised gelatin with $CaCl_2$)

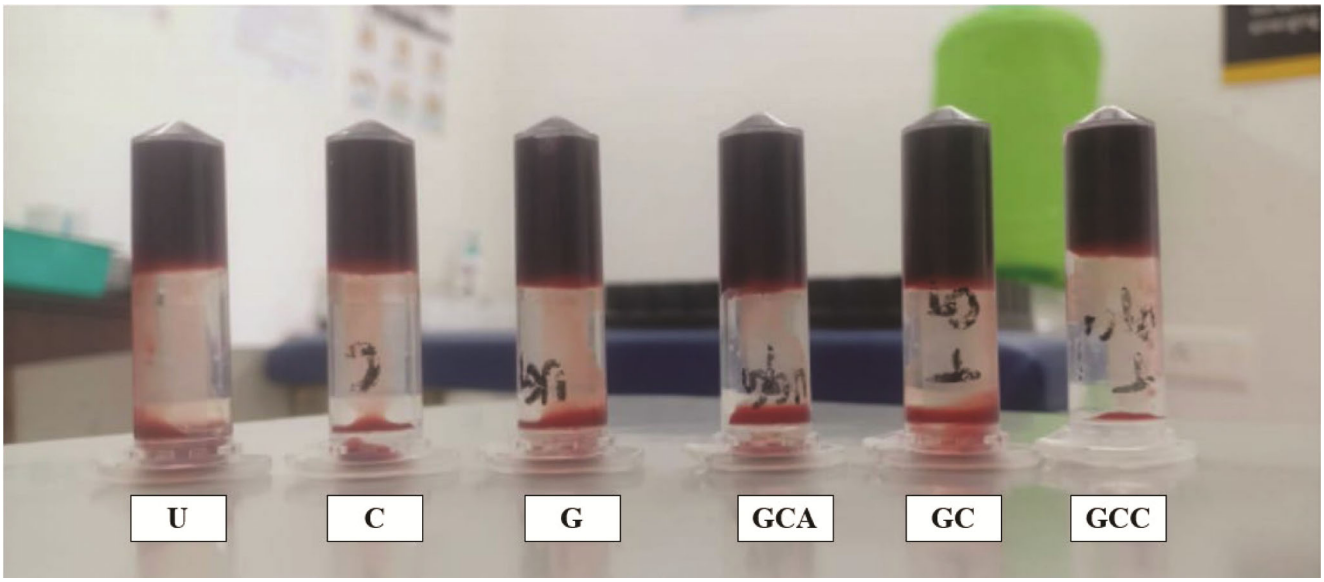

Fig. 3 — Blood clotting test for developed samples; U – untreated, C - cationised, G- untreated gelatin, GCA- untreated gelatin and $CaCl_2$, GC - cationised gelatin, GCC - cationised gelatin and $CaCl_2$

Table 1 — PBS uptake and blood clotting time of developed samples

Sample	PBS uptake, %	Blood Clotting time, s
Untreated	980 ±5	144±5
Cationised	954 ±3	120 ±3
Untreated gelatin	1025 ±2	90 ± 4
Untreated gelatin-$CaCl_2$	960±7	84 ± 5
Cationised gelatin	1044 ±2	66 ± 2
Cationised gelatin-$CaCl_2$	952 ±4	45 ± 2

3.3 PBS Uptake

The absorption capacity of the developed fibrous samples is evaluated through PBS uptake studies, as summarised in Table 1. An ideal haemostatic dressing should efficiently absorb blood while simultaneously promoting clot formation. The untreated control sample exhibits the highest PBS uptake (980 ± 5%), owing to the presence of free hydrophilic groups in cellulose. However, cationisation slightly reduces absorption (954 ± 3%), likely due to the masking of hydroxyl groups by the cationic agent. The incorporation of gelatin increases the absorbency of both control (1025 ± 2%) and cationised (1044 ± 2%) samples, attributed to the hydrophilic nature of denatured collagen. However, the addition of calcium ions through electro spraying technique leads to a 9% decrease in absorbency, suggesting a possible interaction between calcium ions and polymeric chains, reducing free sites for water absorption.

3.4 Blood Clotting Efficiency

Blood clotting time is a critical parameter for assessing haemostatic efficiency. The clotting times for different samples are presented in Table 1 and illustrated in Fig. 3. In the absence of haemostatic agents, normal blood forms a clot within 210 s. Cationised samples enhanced clotting efficiency (120 ± 3 s), possibly due to the cationic-charged surface, which interacts with negatively charged blood components to accelerate coagulation. The inclusion of gelatin further reduces clotting time (90 ± 4 s), as gelatin absorbs and concentrates blood components, promoting clot formation. The most significant improvement is observed in cationised samples containing both gelatin and $CaCl_2$ (45 ± 2 s), where calcium ions facilitate the conversion of prothrombin to thrombin, thereby enhancing the clotting cascade.

4 Conclusion

The study demonstrates that cationisation enhances fibre dispersion and improves haemostatic performance by reducing clotting time. Electrosprayed gelatin and $CaCl_2$ significantly improve blood clotting efficiency by concentrating blood components and accelerating thrombin formation. The cationised samples with gelatin and $CaCl_2$ reveal a clotting time of 45 s. Moreover, the developed samples exhibited good absorbency. While cationisation slightly reduces PBS uptake, the haemostatic benefits outweigh this limitation, making cationised gelatin-$CaCl_2$-treated nonwovens a promising material for advanced haemostatic wound dressings. The work can be extended to wound dressing applications incorporated with additives and has the potential for scale-up.

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