Bentonite –Lactic Acid- Tranexamic Acid Composite as a Sustainable Drug Releasing Haemostatic Agent

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***Abstract*— Fast-acting topical hemostatic agents are essential for effective bleeding control in emergency and surgical settings. Beyond rapid clot formation, the inhibition of fibrinolysis plays a crucial role in enhancing coagulation and improving survival outcomes. In the present study, intercalated composites of tranexamic acid–lactic acid–bentonite (TA–LA–bentonite) were synthesized to achieve sustained release for hemostatic applications. A simple, rapid, and reliable method was employed for the synthesis of these composite materials. The resulting compounds were comprehensively characterized using Fourier-transform infrared spectroscopy (FTIR), ultraviolet–visible (UV–Vis) spectroscopy, thermogravimetric and differential thermal analysis (TGA/DTA), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The loading efficiency and pH-dependent release behavior of the active ingredient were quantitatively assessed using a colorimetric assay, in which ninhydrin served as the chromogenic reagent.**

***Index Terms*— Bentonite, lactic acid, drug delivery, tranexamic acid, haemostatic agent**

I. INTRODUCTION

Tranexamic acid (trans-4-(aminomethyl)cyclohexane-1-carboxylic acid), a synthetic derivative of lysine, is a potent antifibrinolytic agent that functions by reversibly binding to plasminogen, thereby inhibiting its conversion to plasmin—the enzyme responsible for fibrin degradation. This mechanism stabilizes fibrin clots and effectively reduces bleeding [1–4]. Owing to its proven efficacy, tranexamic acid (TA) is included in the World Health Organization's list of essential medicines for hemorrhage management.Hemophilia, a hereditary bleeding disorder characterized by a deficiency in clotting factors, poses significant challenges in achieving effective hemostasis. In such cases, TA has been shown to enhance clot strength, elasticity, and stability when used in combination with clotting factor therapy. Moreover, it can serve as a monotherapy in mild to moderate cases [5]. Beyond its antifibrinolytic role, TA has also been reported to reinforce fibrin networks and promote collagen synthesis within clots, thereby improving clot integrity and reducing the likelihood of re-bleeding [6–9].

Hemostatic agents (HAs) encompass a range of materials developed to control bleeding, including scaffolds, sealants, adhesives, and topical biologics such as fibrin or thrombin. However, scaffold-based and biological HAs often suffer from limitations including high cost, delayed action, poor biodegradability, and potential viral transmission. Among inorganic topical hemostatic agents (THAs), bentonite clay is a promising alternative due to its unique crystallographic and physicochemical properties [10]. Its primary constituent, montmorillonite, is a layered aluminosilicate composed of alternating Si–O tetrahedra and Al–O octahedra. This 2D structure imparts high cation exchange capacity and substantial absorbency. Additionally, bentonite's swelling capability and interlayer tunability via ion exchange render it highly suitable for drug loading and controlled release applications [11–14].

In the present study, hybrid composites of tranexamic acid–lactic acid–bentonite (TA–LA–bentonite) were synthesized via an intercalation technique. The composites were characterized using UV–Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and thermogravimetric/differential thermal analysis (TGA/DTA). The release behavior of TA was investigated under various pH conditions using a colorimetric assay based on the ninhydrin reaction. Under alkaline conditions (pH 8), TA reacts with ninhydrin to form a bluish-purple chromophore, with maximum absorbance at 565 nm. This spectrophotometric method enabled the quantitative analysis of TA loading and its pH-dependent release kinetics over time

II. MATERIALS AND METHOD

2.1.Purification of bentonite

100 g of raw bentonite were dispersed in 1 L of 1 M NaCl solution and stirred for 12 h. The dispersion was reacted three times with 1 M NaCl solution. After centrifugation, the sodium-rich bentonite was washed with de-ionized water until free of chloride ion as tested by $AgNO_3$ solution. Purified bentonite was dried and ground to powder.

2.2. *Lactic acid treatment*

Lactic acid and purified bentonite were treated in a ratio of 1:2 by vigorous stirring. For this purpose, 3 g of sodium-induced bentonite and 6 g of lactic acid were stirred for 12 hours. Excess lactic acid was washed with distilled water. It is then dried and pulverized.

3.*Intercalation of Tranexamic acid on lactic acid treated bentonite* (Bent LA) *under various pH conditions (Bent-LA-TA)*

The effect of pH on the intercalation of TA into lactic acid incorporated Na-bentonite (Bent LA) was studied. For this study 0.6g of TA is treated with Bent LA at different pH 4,7 and 9.5 at constant temperature, time and concentration. Centrifuged supernatant was collected and TA in the supernatant analysed by UV – visible spectroscopy using ninhydrin [17].

4. *Colorimetric detection of TA*

Two sample solutions of different concentration were prepared by dissolving 600mg and 100mg of TA in two 100ml flasks separately using deionized water. Buffer 7 was prepared by adding 10mg of phosphate buffer (pH-7) in 10ml of deionized water. In a boiling tube take 1, 2, 3, 4 and 5ml of sample solution and to each solution add 1ml buffer and 0.5ml ninhydrin, heated in water bath for 20 minutes. Absorbance measurements were made at 565 nm against a sample blank due to formation of Ruhemann's purple. Caliberation plot for higher and lower concentrations were made, and from the linear regression equation the amount of TA intercalated to and released from bentonite under different pH was calculated [17].

5. Characterisation of Bent-LA-TA composites

FTIR analysis- Functional group analyses of prepared composite materials were carried out using Schimadzu FTIR spectrometer between the range 400cm-1to 4000cm-1.KBr disc method is used for analysis. XRD- The crystalline structure of the bentonite composites were investigated using GE X-ray Diffraction system – Seifert XRD 3003 TT, France, with CuKα1 radiation of wavelength 1.5406 Å.TG-DTG- Thermal analysis of Bentonnite and Ta-La-Bentonite composite were carried out using Hitachi TG-DTG instrument from a temperature range room temperature to 800^oC at a heating rate of 10$^oCmin^{-1}$.Scanning Electron Microscopy (SEM)-Morphological analysis of prepared composite materials were carried out using Jeol 6390LA/ OXFORD XMX N.Zeta Analysis- Surface charge of the composite materials was studied using Malvern zeta analyser. The composites were dispersed in different pH buffer solution to study the variation of surface charge.

III. RESULTS AND DISCUSSIONS

Bentonite clay exhibits a gel-like appearance when dispersed in water, primarily due to its strong interactions with water molecules. The hydroxyl (–OH) groups located in the interlayer regions of bentonite facilitate hydrogen bonding with water, promoting both swelling and dispersion. In the present study, a tranexamic acid–lactic acid–bentonite (TA–LA–bentonite) intercalated composite was synthesized for potential use as a hemostatic agent. A green and environmentally friendly synthesis route was employed to intercalate tranexamic acid (TA) into the bentonite layers. Initially, lactic acid (LA) was introduced to expand the interlayer spacing of bentonite, thereby enhancing the efficiency of subsequent TA intercalation. Different pH conditions were explored to optimize the incorporation of TA into the clay matrix.

The release behavior of TA from the composite was evaluated using a colorimetric assay with ninhydrin as the chromogenic reagent. Figure 1 displays the synthesized TA–LA–bentonite composite along with a visual representation of TA detection using the ninhydrin method. Figure 2 presents calibration plots corresponding to both low and high TA concentrations (see Supporting Information, Figure S1). Ninhydrin is widely used for the quantitative detection of amino and imino acids due to its reaction with primary amines to form Ruhemann's purple, a bluish-purple chromophore that exhibits a strong absorbance peak at 565 nm.

Under alkaline conditions, ninhydrin is transformed into o-carboxyphenylglyoxal, which further reduces to 2-hydroxyindan-1,3-dione [18,19]. Tranexamic acid, containing a primary aliphatic amine, reacts with ninhydrin in the presence of phosphate buffer (pH 8.0) through oxidative deamination. This leads to the formation of Ruhemann's purple via the condensation of reduced ninhydrin intermediates. The resulting purple complex enables a sensitive, accurate, and cost-effective spectrophotometric method for TA quantification (see *Scheme 1* in Supporting Information).

Figure1- Ta-La-Bentonite composite and colorimetric detection of TA using ninhydrin reagent.

The linear regression equations for the lower and higher concentrations of tranexamic acid (TA) were determined to be A = 0.08515 + 993.9 C and A = 1.947 + 875.13 C, respectively (see Supporting Information). These equations were employed to quantify the amount of TA adsorbed and subsequently released from the TA–LA–bentonite composite system. Lactic acid was used to expand the interlayer spacing of bentonite through interactions between its carboxyl group and the hydroxyl groups present in the clay. This interaction facilitates the intercalation of amino acids or therapeutic agents, rendering the modified bentonite an effective carrier for drug delivery applications.

Figure 3 shows the UV–Vis spectra of the supernatant solutions after TA adsorption under various pH conditions. The black spectrum corresponds to the initial TA concentration (1.2 mM). Based on the calibration plots, the amount of TA adsorbed at different pH levels was calculated to be 0.3 mM at pH 4, 0.9 mM at pH 7, and 1.0 mM at pH 9. The highest intercalation efficiency, approximately 83%, was observed at pH 9. This behavior can be explained based on the acid–base properties of TA, which has a pKa of 4.22 for the –NH_2 group and 10.22 for the –COOH group. Within this pH range, TA exists primarily in its zwitterionic form [20]. At pH 9, partial deprotonation of the carboxyl group of lactic acid occurs, which enhances electrostatic interactions and promotes maximal TA intercalation into the expanded bentonite layers.

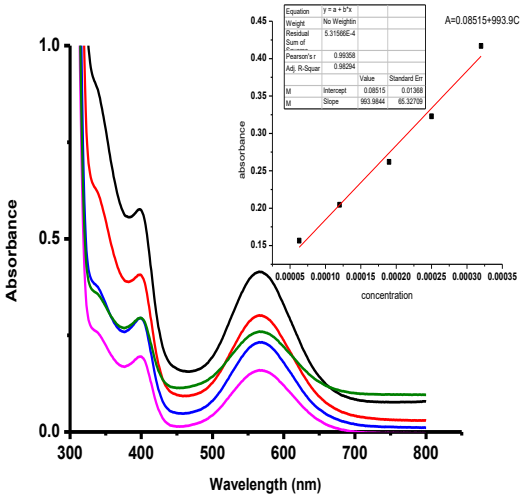
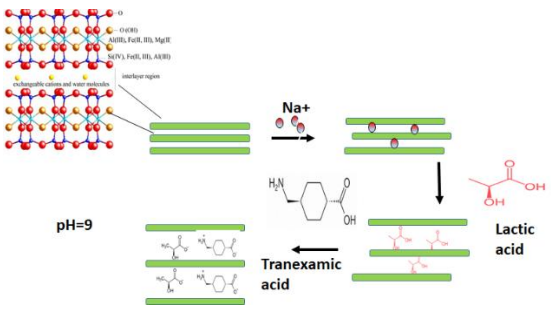


Figure 2 - Calibration plot for TA concentration ranges from 0.3mM to 5µM



Schematic representation of Tranexamic acid incorporation into Bentonite – lactic acid composite

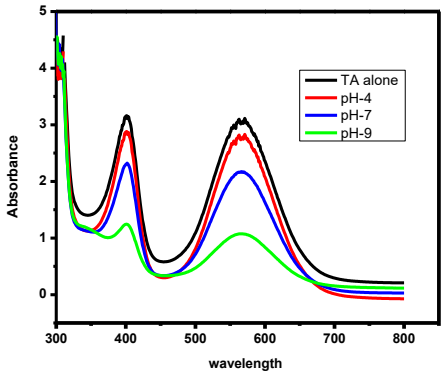


Figure 3- UV-Vis spectrum for supernatant solution after TA absorption in various pH conditions.

Fig.4 showed the FTIR analysis of bentonite, Na-bentonite and Ta-La-Bentonite. We found that the absorption band of bentonite at 1639cm^{-1} was caused by the vibration of the Al-OH and hydroxyl groups in adsorbed water, and the absorption band at 1034 cm^{-1} was caused by the plane vibration of Si-O. Absorption bands corresponding to Si–O, Al–O and Ca–O vibrations (3629, 3437, 1638, 1429, 1083, 1052, 917, 798, 620, 525, and 468 cm−1) (fig3a).Lactic acid after intercalation with clay a shift in CO stretching towards lower frequency 1690cm^{-1} due to hydrogen bonding. The FTIR intensity at ~3312 cm^{-1} is associated with O-H stretching. The C-H stretching bands are located at 2983, 2935 and 2883 cm^{-1} (fig.3b)· The absorption band of Ta-La-Bentonite at 1637cm^{-1} was caused by the bending vibration of N-H, and the absorption band at 2925cm^{-1} and 2862cm^{-1} was caused by the stretching vibration of -OH on the -COOH. The infrared spectra of the composites showed that the absorption band at 1630cm^{-1} was shifted relative to the absorption band of bentonite at 1639cm^{-1}, which could be related to the bending vibration of N-H after bentonite was intercalated by TA (fig. 3c). The two new absorption band at 1719cm^{-1} corresponds to CO stretching frequency of COOH group of TA. Vibration of C-H on saturated carbon at 2945cm^{-1}.

Figure 5 shows the SEM image of Bentonite and the composite prepared. SEM was used to observe the changes of micromorphology of the materials before and after modification. Comparing the electron microscope images of Ta-La-Bentonite and simple bentonite demonstrates that Ta-La-Bentonite is more compact than bentonite , which may be due to the replacement of cations such as Na^+ and Ca^{2+} by TA, which optimizes the crystal structure. SEM images indicated that tranexamic acid could intercalate into layers of bentonite successfully.

Figure-6 corresponds to the XRD analysis. XRD analysis was carried out to study the intercalation of TA on Bentonite. From the figure it is clear that d(001)plane increases with sodium ion intercalation, an increase in peak intensity at 20 due to the enlargement of inter-planar spacing. But after treatment with TA shows a decrease in peak intensity, due to the conversion crystalline nature to amorphous. New peaks at 22.2 and 29 corresponding to TA was appeared in the composite confirms the successful incorporation of TA via hydrogen bonding or replacing the inter-ions like Ca, Na and Mg to form new composites. XRD analysis clearly indicates the intercalation of tranexamic acid within in the planes of bentonite.

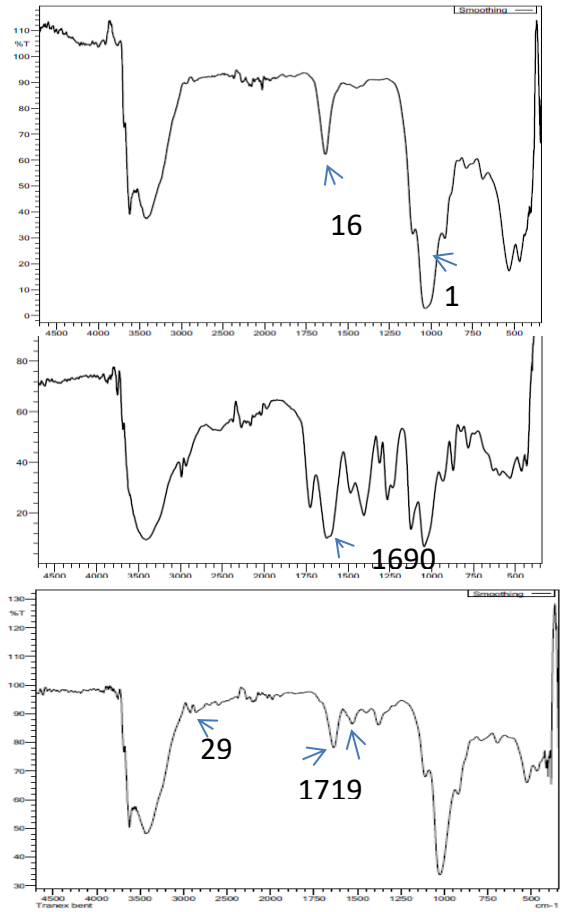


Figure 4-FTIR of Bentonite, Bent-Na, Ta-La-Bentonite

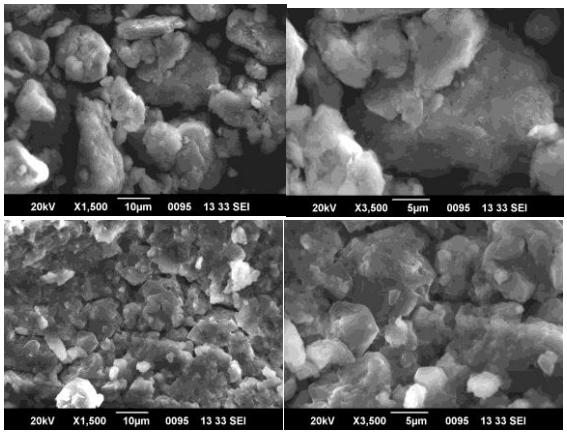


Figure-5 SEM image of Bentonite and Bent-LA-TA composites

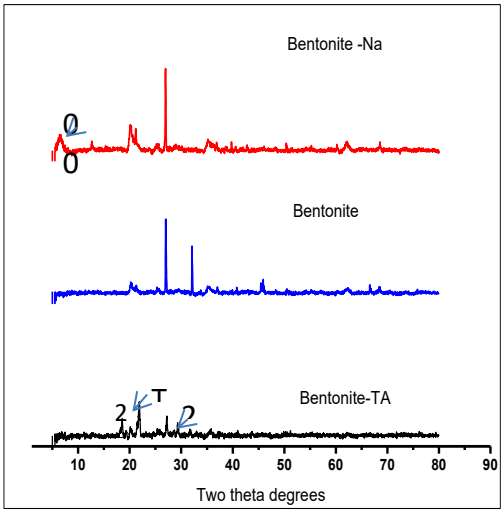


Figure- 6 XRD of Bentonite, Na-Bent, and Bent-LA-TA

Figure 7 shows the TG/DTA analysis. Based on the thermogravimetric analysis of the sample, the analysis diagram of bentonite reveals that the weight loss before 100°C was due mainly to the free hydrothermal decomposition of the surface of Bentonite. The secondary weight loss was mainly due to the crystal phase transformation of bentonite losing bound water. Bare LA have a decomposition temperature at 251°C, but Bent-LA-TA shows a weight loss at 258, 355, 446 and 492°C weight loss at 258°C corresponds to the loss of LA which shifts towards higher region confirms the composite formation. The boiling point of TA is about 300°C. The distance between the molecules of TA increased at this temperature, causing considerable weight loss due to the volatilization of gas. The weight loss between 350-500 °C corresponds to the TA shows the interaction of TA with Bent-LA. About 880 µg of weight loss is observed for 7mg of sample taken. The thermal stability of TA is poor, and its structural functional groups—such as the six membered ring and carboxyl—will decompose, resulting in further loss in mass.

The amount of TA released was quantified calorimetrically using Ninhydrin as reagent. Release studies were conducted at different pH conditions -2, 4 and 7.4. A sustained release for 48 hrs was observed at pH 7.4. This might be due to weakening of hydrogen bond interaction of drug from clay interstitial sites. At acidic conditions a fast release within 1hour was observed, due to protonation of amino group. Fig 8 shows the UV-Vis spectra recorded at pH7.4 at different time intervals. Fig 9 shows the plot of concentration vs time , the amount of TA release was calculated from the calibration plot.

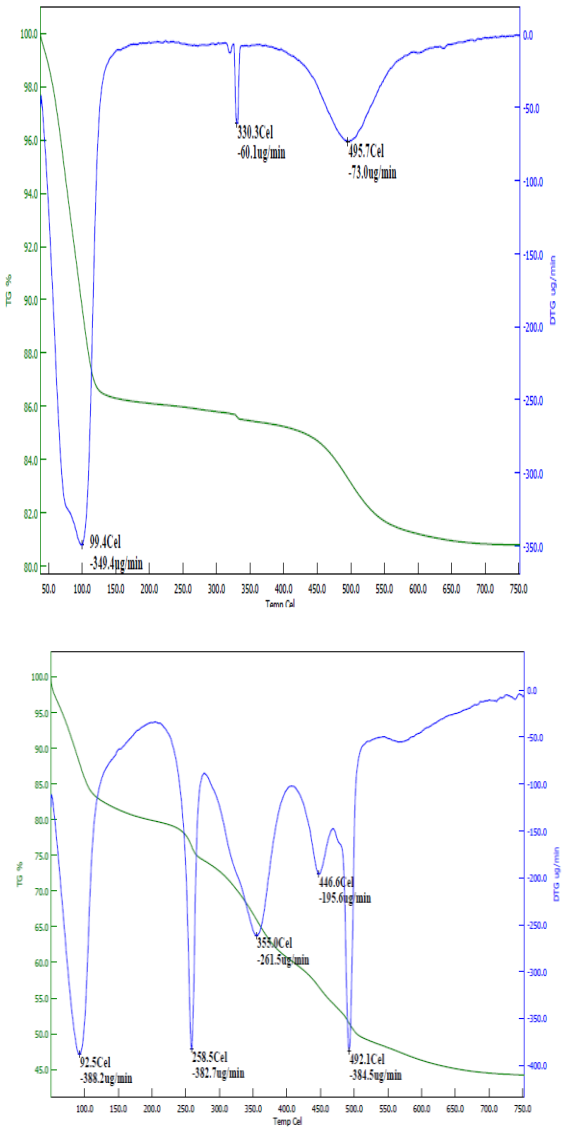


Figure 7- TG/DTA of bentonite and Bent-LA/TA

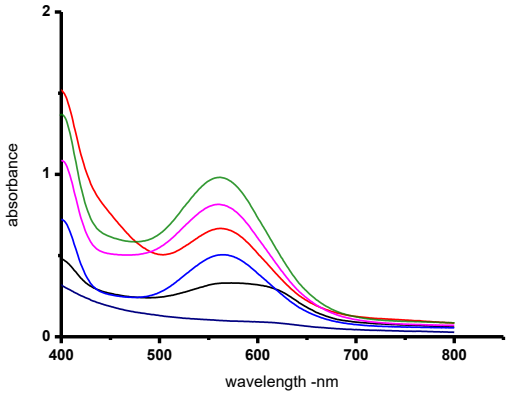


Figure -8 UV-Vis spectrum showing TA release at pH-7.4 for various time intervals.

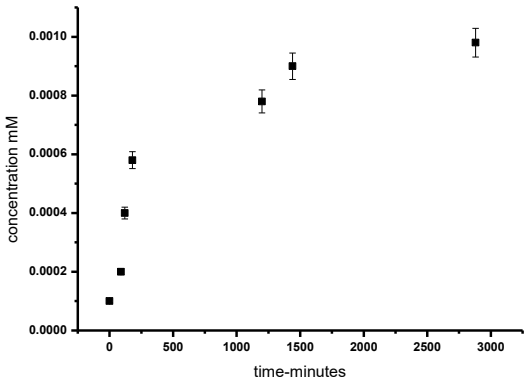


Figure-9 Plot of concentration vs time

Sample	Zeroth-order $C = K_0 t$	Higuchi Model $Q = K_H t^{1/2}$	First-order $C = \ln C_0 - Kt$	Hixson-Crowell $Q_0^{1/3} - Q_t^{1/3} = K_{HC} t$
-----	0.75112	0.93599	0.7898	0.93491

Regression coefficient, R^2 calculated from the linear form of different kinetics.The best fitted model is Higuchi model which predicts swelling mechanism of drug release. From this studies it can be concluded that, the developed material is a bio-compatible one and can be effectively used for the treatment of bleeding disorders. From the mechanism studies it is clear that the model follows a swelling mechanism for drug release.

IV. CONCLUSIONS

Tranexamic acid (trans-4-aminomethyl cyclohexane carboxylic acid) is an inhibitor of fibrinolysis and thus has been used as a hemostatic agent for the treatment of severe hemorrhage. TA,is a cheap, non-patented drug, has been included in the list of essential drugs by the WHO and is widely used all over the world. Present work we have synthesised Tranexamic acid/Lactic acid/Bentonitecomposite material for drug delivery application. Prepared composite material was characterised using different FTIR, XRD, UV-Vis, TG/DTA, SEM and Zeta analysis. Its release studies were studied at different pH conditions and were found that a sustainable release is observed at pH 7.4. The amount of Drug intercalated and released was quantified by colorimetric method using Ninhydrin as coloring agent. The release mechanism was best fitted with Higuchi model shows the release mechanism follows swelling process for drug release.

V. ACKNOWLEDGEMENTS

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Competing Interest

Authors declare that they have no conflicts of interest.

Author contribution

All authors contribute to the study conception and design. Material preparation, data collection and analysis were equally contributed by all.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The experiments comply with the current laws of the country.

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SUPPORTING INFORMATION

BENTONITE –LACTIC ACID- TRANEXAMIC ACID COMPOSITE AS A SUSTAINABLE DRUG RELEASING HAEMOSTATIC AGENT

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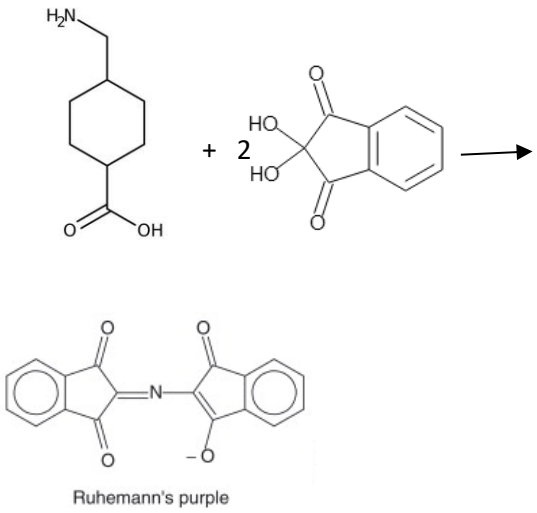
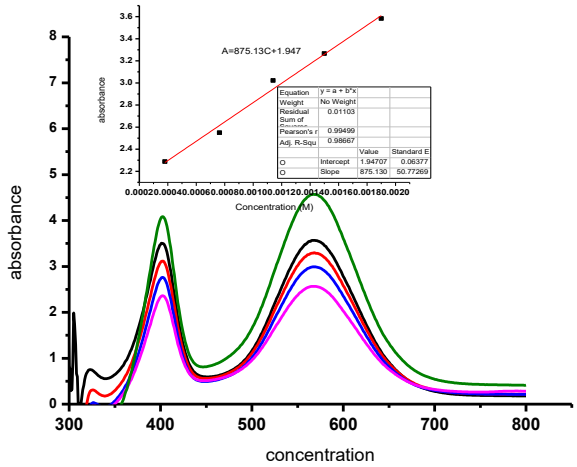
Supporting Information



Scheme1



FigS1-Calibration plot for TA (2mM to 0.2 mM) higher concentration.