



## Research article

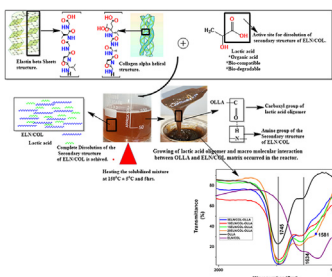
# In-situ oligomerization of lactic acid within broiler skin extracted elastin/collagen matrix for the efficacy of ointment base



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## GRAPHICAL ABSTRACT



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## ABSTRACT

Elastin and collagen were isolated from waste broiler skin and modified with *l*-lactic acid oligomer for the efficacy of substituting petroleum based ointment base matrix. Paraffin wax, which is one of the most extensively used petroleum ointment derivative is well known for its incompatibility with the skin. Chronically it clogs the skin pores, which subsequently affects the release of moisture. To mitigate this problem, a novel approach has been followed to synthesize a fully green and biocompatible ointment base matrix. The extracted Elastin and Collagen (which exists naturally in our skin) and lactic acid monomer (commonly known as a part of a body fluid) mixed at different proportion and used to synthesize a lactic acid modified elastin/collagen (OLLA-m-ELN/COL) bioconjugate matrix with lubricating characteristics via in-situ polycondensation reaction. The macromolecular interaction between Amide I group of elastin/collagen and C=O of a lactic acid oligomer was confirmed by FTIR analysis. Organoleptic analysis, spreadability, pH, and viscosity were analyzed. The six-mass loss stages which was observed in the ELN/COL matrix was changed into a single mass loss for the synthesized bioconjugate with improved thermal stability. The thermal stability improvement can be correlated with the formation of secondary macromolecular interaction.

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## 1. Introduction

An ointment is a semi-solid, homogenous, viscous system which can be formulated using various base matrixes. It consists of different active components targeting external uses such as skin, eye, stomach, nose, hands, foot, and other mucous membrane parts of the body. The protective, therapeutic, or prophylactic advantages obtained from the ointments can help to deliver the active and essential ingredients to the site where treatment is highly needed [1, 2]. It is also known to have moisturizing characteristics, making it ideal for use on the skin or mucous membrane [3].

Ointments have base matrixes as a carrier for the active components that regulate the functionality. With this ground, the choice of the base matrix needs to be given a significant care during ointment formulation. Furthermore, understanding the interaction of the skin in accordance to the active component absorption is also an important concept during the design and formulation stage [4]. Human skin is mainly composed of two important structural proteins; elastin and collagen that provide flexibility and strength [5]. These proteins deteriorate with age or with negative human intervention, such as improper use of skin care products.

Petroleum derivatives such as paraffin (soft white, yellow, soft and hard), mineral oil, and cetostearyl alcohol have been extensively used as an ointment base matrix [6, 7, 8]. Even though these conventional bases are applicable to preserving the skin moisture and suppressing the skin overexposure to sunlight, they are also known for their incompatibility with the skin [9, 10, 11]. The accumulation of saturated hydrocarbon concentration in human fat tissue has been observed to increase with age because of the extensive utilization of mineral oil-based skin care products [12]. These non-degradable ointment matrixes are toxic and cannot be metabolized, with a high possibility of clogging the skin pores [13]. Recently, various researchers are working extensively on the development of a green ointment base matrix which has biocompatibility and biodegradability characteristics [14, 15, 16, 17].

Proteins such as collagen and elastin have the promising characteristic of being used as a base matrix for skin care products [18, 19, 20, 21]. Elastin has been used in different types of biomaterials, including insoluble elastin fibers, hydrolyzed soluble elastin, recombinant tropoelastin (fragments), synthetic peptide sequence repetitions, elastin block copolymers, or in combination with other biopolymers [22, 23, 24, 25].

The other very essential protein which has been extensively utilized in the development of different new biomaterials is collagen [22, 26]. It occupies nearly 25% of the protein available in animals, which makes it the most abundant protein. It is contained in the fibrous tissue with an average molecular mass of 300 kDa. Collagen provides structure to our body parts, protects softer tissues, maintain and connect different organs with the skeleton [20, 27]. The most abundant type of collagen is type I. It is highly available in animal skin and is widely used in various medicines. It also has dual functions in the skin, which act as elastin and collagen building blocks and as a binding receptor or ligand in fibroblasts and hyaluronic acid [28, 29, 30]. Nowadays, collagen is a very essential industrial input mainly in pharmaceutical, beverage, food, and health-care industries [31]. The current worldwide collagen market is estimated to be 3.7 billion USD, which is forecasted to grow to over 6.6 billion USD by 2025 [32].

Collagen and elastin are potentially found in different parts of animals and plants forming a complex matrix with other organic molecules [21]. Due to this, the extraction and isolation become more intricate which further affect the cost [33]. Therefore, finding a cheaper raw material with high throughput and an easier alternative extraction method is a primary concern. Recently, the demand of processed poultry products increased rapidly which intern increased poultry skin as the byproduct. This waste has a significant amount of protein, which makes it a potential source of elastin and collagen [34].

The bioactivities of collagen and elastin such as, antioxidant properties, lipid-lowering activity, antihypertensive activity, as well as reparative properties in damaged skin makes it an ideal biological matrix

for the formulation of ointments [35]. Lactic acid is also one of the most important biocompatible molecules used in the production of various cosmetics products. It has a skin moisturizing ability which is often used to restore, smoothen, and fix facial deformities. The final formulated ointment quality is usually investigated and measured in terms of spreadability, pH, irritancy, diffusion and durability [5, 7, 8, 9, 10, 11].

In this article, the formulation of green, biobased, and biocompatible ointment base matrix was performed for the first time from elastin, collagen, and lactic acid. These three components can be considered as a green source for a biocompatible system. Through the control of viscosity, the polymerization of lactic acid and other essential characteristics, the replacement of petroleum derivative ointment bases to avoid chronic effects can be possible by 'Skin for the Skin' approach [14, 15, 16]. This research demonstrates the development of hybrid matrix from broiler skin proteins and lactic acid for the targeted application as ointment base matrix.

## 2. Materials and methods

### 2.1. Materials

Laboratory grade sodium chloride and sodium hydroxide from Merck; acetone (98%) and ethanol (96%) from SISCO research laboratory, and lactic acid (88 wt%) from LOBE (Netherlands) were obtained from the local chemical market in Ethiopia. Broiler skin used for the extraction of collagen and elastin was collected from Oromia region, Adama city, Ethiopia.

#### 2.1.1. Extraction of elastin/collagen matrix

Small pieces of Broiler's skin were homogenized in 1 M NaCl solution of 10 %vol for 24 h of extraction followed by multiple times washing with deionized water. Then, the pellets were defatted three times with 30 % vol acetone for 1 h followed by heating it for 15 min using water bath at constant shaking in 0.1 N NaOH solution of 30 %vol. After cooling, centrifugation, and removing the remaining fat, the residue was treated again for 45min in 0.1 N NaOH at 100 °C. The residual material of hot alkali extraction was washed several times using distilled water and dried at 80 °C in an oven [17, 36]. This method was adopted from Mehdi Nadalian et al. with some modifications as shown in Figure 1 [37] [18].

#### 2.1.2. Preparation of hybrid bioconjugate matrix (lactic acid modified elastin/collagen) for ointment base

Polycondensation reaction was undertaken to oligomerize lactic acid with in elastin/collagen matrix. As shown in Figure 2, ELN/COL (5, 10, 15, and 20 wt. %) were initially solubilized in 100 g of lactic acid at room temperature using a round-bottom flask with mild agitation. Then, the solubilized solution was transferred into a temperature controlled autoclave reactor vessel and heated up to 120 °C at atmospheric pressure to eliminate water from the solution, followed by a gradual temperature increase to 150°C ± 5 °C for 5 h. Byproduct water was collected by fitting a condenser to the outlet of the reactor. The prepared matrix was cooled, sealed in vials and labeled as 5ELN/COL-OLLA, 10ELN/COL-OLLA, 15ELN/COL-OLLA and 20ELN/COL-OLLA for further macromolecular investigation and essential ointment physiochemical characterization. A similar procedure was also used for the oligomerization of lactic acid.

### 2.2. Experimental analysis

#### 2.2.1. Proximate analysis for the extracted ELN/COL matrix

**Moisture content:** A piece of broiler skin (3 g) was weighed and spread uniformly on a glass Petri dish and placed in the oven for 3 h at 105 °C until a constant weight was obtained, followed by cooling in a desiccator. A similar procedure was also followed for the empty glass petri dish and the weight of the dish was subtracted. Afterwards, the moisture content was calculated following the formula described in Eq. (1) [20]:

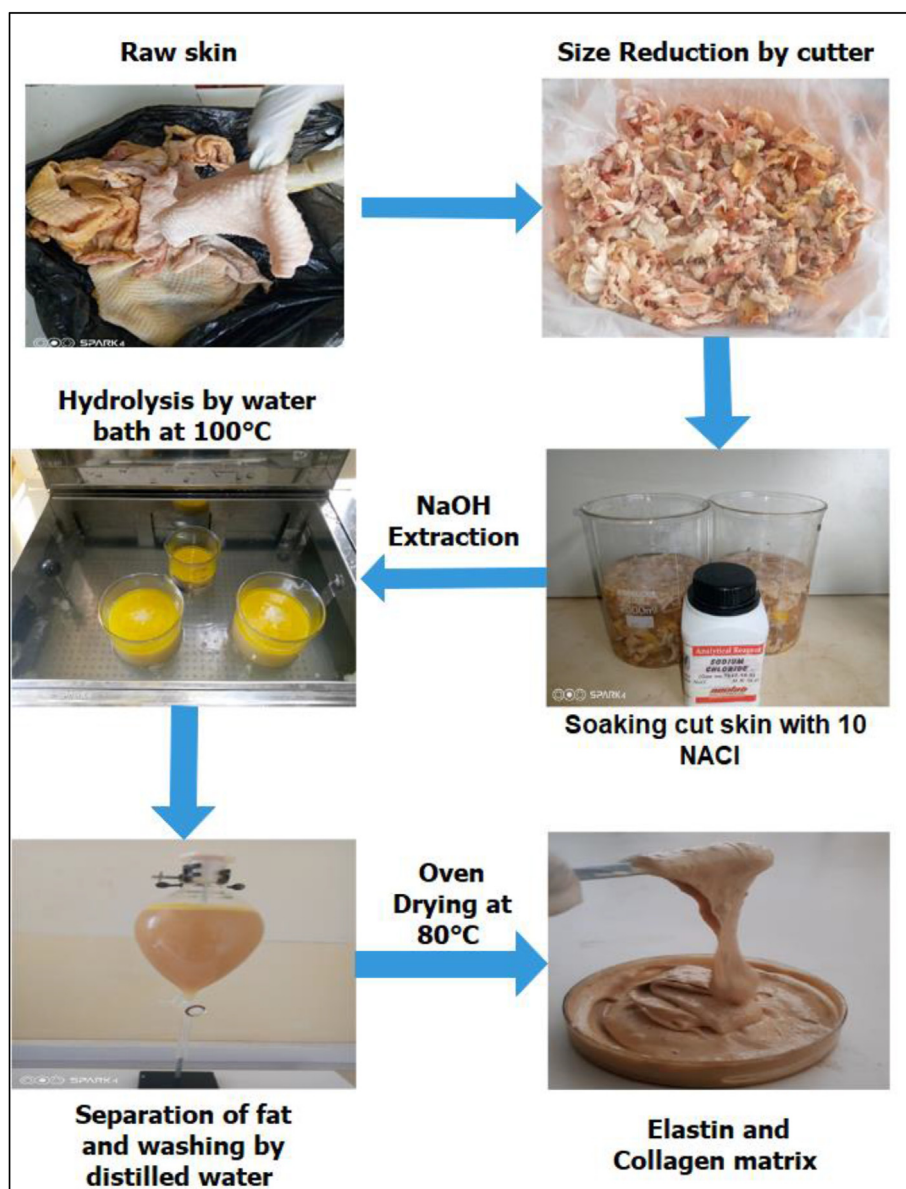


Figure 1. Extraction of Elastin and Collagen from broiler skin.

$$\text{Moisture (\%)} = \frac{W1 - W2}{W1} * 10 \quad (1)$$

where: W1 is the sample weight (g) before drying and W2 is the sample weight (g) after drying.

**Ash content:** A 5g of the broiler skin was placed into the crucible and heated overnight at 550 °C. The ratio of the ash weight to the sample weight multiplied by 100% is used to determine the sample ash content as described in Eq. (2) [20]:

$$\text{Ash content (\%)} = \frac{W2}{W1} * 100 \quad (2)$$

where: W1 is the sample weight (g) and W2 is the ash weight (g).

**Fat content:** The method used for the estimation of the fat content was adopted from AOAC, 2000. A 5g sample of broiler skin was placed in extraction thimble and transfer to Soxhlet pot to be washed with petroleum ether. After 14 h of extraction the extracted solution was transferred to a glass vial and kept in oven at  $80 \pm 5$  °C to remove the solvent. After drying, it was transferred to a desiccator for cooling followed by weighing to estimate the fat content using Eq. (3) [20]:

$$\text{Fat content (\%)} = \frac{W2}{W1} * 100 \quad (3)$$

where: W1 and W2 are the weight (g) of the sample and the fat respectively.

**Protein determination using Biuret test:** The presence of peptide bonds in the extracted sample were analyzed using a biuret reaction with alkaline copper sulfate. If the sample has two or more peptide bonds, a positive result for the test can be obtained using the Biuret reagents, which is indicated by violet or purple colors [21]. The Biuret reagent was prepared with a solution composed of sodium hydroxide (10% NaOH) and used for 1 g of the extracted sample.

#### 2.2.2. Qualitative analysis of the synthesized ointment bases

**Organoleptic parameters:** visual examination was used to characterize the physical appearance, texture, color, homogeneity, phase separation and immediate skin feel such as greasiness, stiffness and grittiness of all the prepared samples. Pressing a small quantity of prepared samples between the index finger and thumb was used to evaluate the presence of coarse particles, consistency, homogeneity and texture of the samples [38].

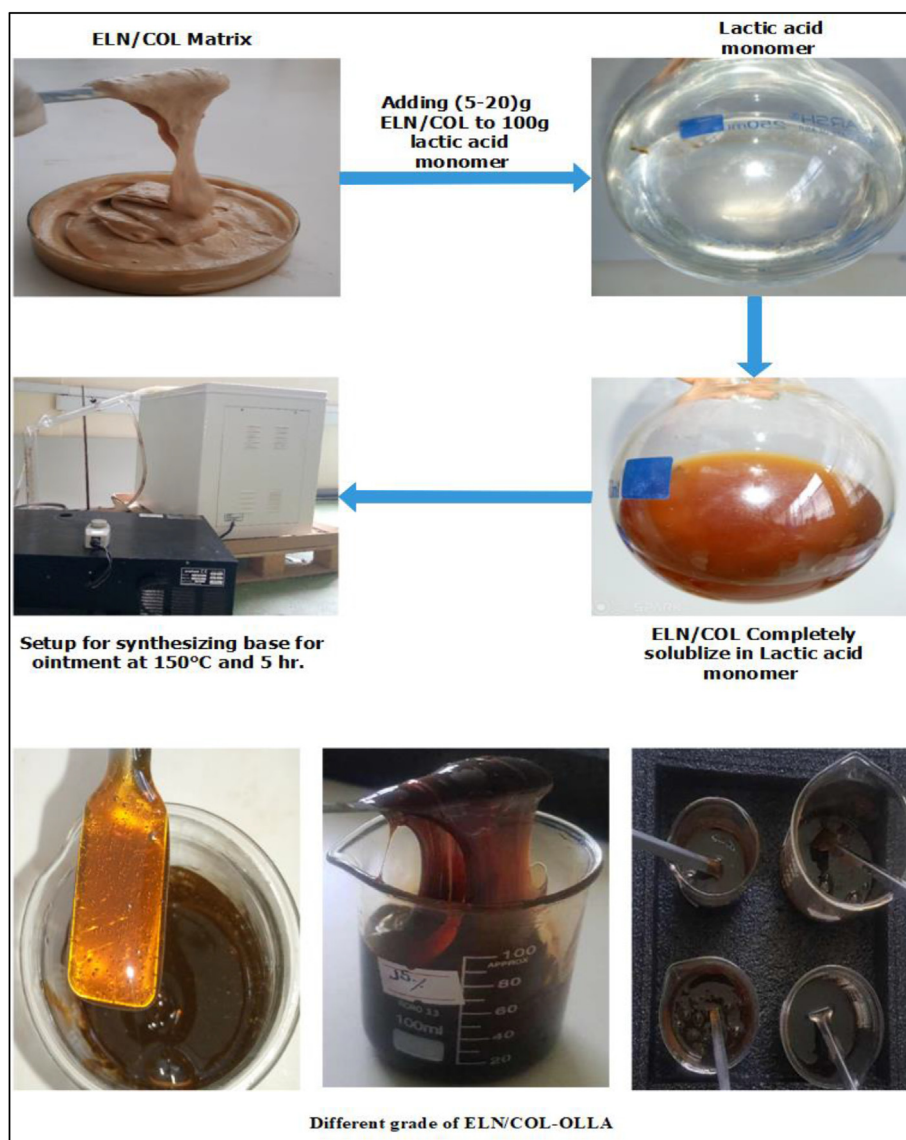


Figure 2. Synthesis procedure for the ointment base.

**pH:** one gram of ELN/COL-OLLA sample were dispersed in 100 mL of distilled water and the pH was measured using Auto digital pH meter (model number PSAW11 Avi Scientific India) [9]. Triplicate measurement was carried out for each prepared samples after calibration of the pH meter using standard buffer solution having pH value of 4, 7 and 10.

**Viscosity:** was measured by VK- 2000 viscometer (Viscotech Hispania, SL, Spain) at 25 °C with the spindle rotation of 2 RPM. Triplicate tests were carried out and average values were reported.

**Spreadability:** 1 g of a sample was placed between two horizontal plates (9.5 cm × 9.5 cm) to determine the spreading diameter at which the upper plate is subjected to 100 g standard weight. Eq. (4) was used to determine the spreadability. This method is adopted from Toppo et al. [9, 39].

$$S = (M * L) / T \quad (4)$$

where S, M, L, T refers to spreadability, applied weight, the glass slides length, and the sliding time required to separate the glasses, respectively.

### 2.2.3. Functional group analysis

The functional group analysis of extracted elastin/collagen, ELN/COL-OLLA and lactic acid oligomer was done using Fourier transform

infrared spectroscopy attenuated with total reflectance (ATR) mode (Model: type A FTIR-6600). The samples were scanned in the range of 4000 to 500  $\text{cm}^{-1}$  wavenumber with a resolution and scan rates of 4  $\text{cm}^{-1}$  and 64 respectively.

### 2.2.4. Thermo-gravimetric analysis (TGA)

TGA of the extracted and prepared samples were performed using DTG-60H model (Shimadzu instrument). Samples with a mass of 8–10 mg were thermally treated for a temperature range of 25 °C–800 °C and with 20 °C/min heating rate under nitrogen environment (20 mL/min). The mass change of each sample (wt.%) was recorded against temperature (°C) to study the thermal stability.

## 3. Result and discussion

### 3.1. Characterizations of raw broiler skin and extracted ELN/COL matrix

The hydrolyzed elastin/collagen matrix was successfully extracted with an alkaline solution from local broiler skin. The feedstock (broiler skin) and the extracted ELN/COL matrix were characterized for their physical and chemical compositions as follows.



### 3.1.1. Proximate analysis

The fat content, moisture content, and ash content (proximate analysis) of broiler skin is presented in Table 1. The reduction in moisture content and fat content were observed for the extracted ELN/COL as compare to broiler skin. Significantly small (<1%) amount of ash content was also obtained for both the broiler skin and the extracted ELN/COL. This indicates the presence of a negligible amount of inorganic residue which is in-line with previously reported articles [23].

In an alkaline solution, a complex can be formed between copper II ions (blue-colored) and peptide bonds due to the unshared electron pairs available on the nitrogen of the peptide bond. The bond between the peptide bond's amide group (=NH) and the carbonyl group (C=O) and the Cu<sup>2+</sup> ion results in the formation of a colored coordination complex, which eventually turns the blue solution purple. The amount of peptide-copper complex can be correlated with the intensity of the color formed. As indicated in the supporting information figure (W1. Protein test using Biuret Reagents), the expected deep purple color was observed after the biuret test. This is an indication of the presence of a high number of peptide bonds in the extracted elastin/collagen matrix. However, during the Biuret test for the raw skin, it was difficult to observe the color change since the skin did not dissolve in the standard reagent.

### 3.1.2. FTIR analysis of elastin/collagen

The functional groups analyzed by FTIR (Figure 3) indicates the presence of amide I, amide II, and amide III groups in the extracted elastin and collagen matrix. These functional groups are represented by respective peaks at 1605-1770 cm<sup>-1</sup>, 1488-1605 cm<sup>-1</sup>, and 1266-1360 cm<sup>-1</sup>. A similar result was also reported by Cheheltani R. *et al.*, for elastin and collagen, which were extracted from the aorta [40]. The peak observed at 1338 cm<sup>-1</sup> denotes the CH<sub>3</sub> wagging vibration of the proline side chain which is commonly happening for both collagen and elastin. As already reported in literature, the FTIR spectra of elastin and collagen are very similar and difficult to distinguish. The structural difference comes from the presence of hydroxyproline in the collagen peptide bond which makes it different from that of elastin along with the secondary structural variation. Collagen and elastin are well known for their distinguished secondary structure of  $\alpha$ -helical and  $\beta$ -sheet respectively. The peak observed at 974 cm<sup>-1</sup> designates the collagen secondary structure, while the  $\beta$ -sheet structure of elastin is clearly indicated at 1647 cm<sup>-1</sup>. Cheheltani R. *et al.*, reported that the  $\alpha$  helical and  $\beta$  sheet secondary structures of pure collagen and elastin occur at 928 cm<sup>-1</sup> and 1635 cm<sup>-1</sup> respectively. However, the amide I and amide II peaks of the collagen spectra are observed to shift into the higher wavenumbers with increasing concentration of elastin [40]. The observed higher wavenumber, which is indicated for both secondary structures imply the coexistence of elastin and collagen in the extracted protein matrix. Additionally, the amide II group of elastin and collagen shows C-H in-plane bending, C-N-C=O symmetric vibration, and CH<sub>3</sub> out of plane bending vibration at FTIR spectra of 1409 cm<sup>-1</sup>, 1493 cm<sup>-1</sup>, and 1450 cm<sup>-1</sup>, respectively.

### 3.1.3. Thermal stability analysis of ELN/COL matrix

The TGA and DTG, which are shown in Figure 4 indicates a total of six stages mass loss for the extracted ELN/COL matrix. As clearly indicated in the DTG curve, the six decomposition peaks ranges from 50 °C -120 °C, 120 °C - 217 °C, 217 °C - 446 °C, 446 °C - 557 °C, 687 °C - 713 °C, and

Table 1. Proximate analysis of Broiler skin.

Proximate analysis	Raw skin	ELN/COL Matrix
Moisture Content (%)	4.2	1.8
Ash Content (%)	<1	<1
Fat Content (%)	3.8	1.02
Biuret Test for protein	NA*	Positive (Violet color)

\* NA = Not applicable.

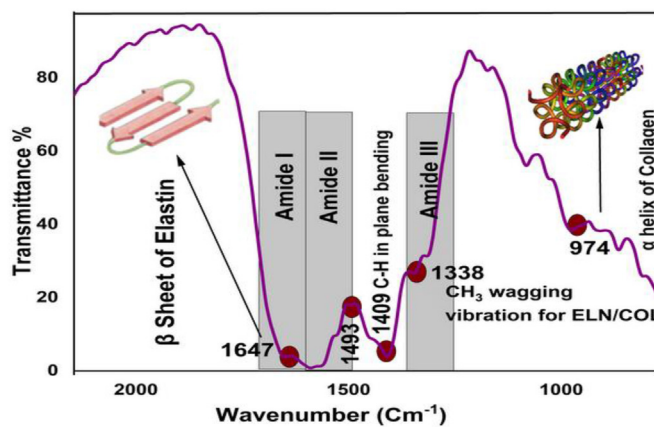


Figure 3. FTIR spectra of ELN/COL matrix.

713 °C - 775 °C with a mass loss of 34%, 21%, 30%, 6%, 1%, and 6% respectively were observed in each stages. The first and the second peaks have covered a total of 55% mass loss with a temperature range from 50 °C - 217 °C. This indicates the dehydration step and decomposition of small protein macromolecules. The macromolecules of ELN/COL matrix show a degradation above 217 °C. Since the extracted protein is composed of elastin and collagen macromolecules with variable chain length, the smallest chain decomposes relatively at lower temperature whereas higher molecular weight chains decompose at higher temperature. Moreover, the decomposition temperature of T<sub>10</sub>, T<sub>50</sub>, T<sub>max</sub>, and T<sub>90</sub> are 74 °C, 171 °C, 344 °C, and 516 °C respectively.

From the TGA result, it can be observed that the maximum ELN/COL decomposition or weight loss was approximately 30% which occurs in the temperature range from 217 to 446 °C. Therefore, for preparation of ELN/COL-OLLA matrix the modification temperature should be below the initial decomposition temperature of the third stage which is 217 °C. Zerihun Yoseph *et al.*, reported that 30% weight loss between the temperature range of 298–367 °C for elastin extracted from tannery [25, 41]. As compared to the above report, a wider mass loss peak is obtained with a lower initial temperature and a higher final temperature. This can be correlated with the coexistence of elastin and collagen in the extracted matrix. It is clearly observed that the minimum degradation temperature of the major protein matrix was 60 °C above the polymerization temperature of lactic acid which is 150 °C. This implies, the possible structural modification of the lactic acid solubilized elastin and collagen matrix during the in-situ polycondensation reaction. The selected reaction temperature (150 °C ± 5 °C) can be considered as a convenient temperature.

## 3.2. Synthesis and characterization of ELN/COL-OLLA bioconjugate targeting an ointment base matrix

### 3.2.1. Solubility of elastin/collagen in lactic acid monomer

An understanding of the solubility of proteins can provide useful information for the potential functionality and utilization, especially in foams, emulsions, and gels [26]. Figure 5 shows that the extracted protein from broiler skin (ELN/COL) was completely dissolved in a lactic acid monomer (weak organic acid) at room temperature. Solubilization of the extracted protein within the lactic acid monomer might reduce the secondary macromolecular interaction formed by the peptide bonds of the NH and C=O groups of the consecutive chains, which is responsible for the formation of  $\alpha$ -helix and  $\beta$ -sheet structure of collagen and elastin, respectively. This phenomenon can have a possibility of lactic acid oligomerization within the protein-peptide chains and could be the reason for the formation of a new secondary structure between the chains of elastin/collagen macromolecules and lactic acid oligomer. Moreover, by monitoring ELN/COL loading in lactic acid monomer during

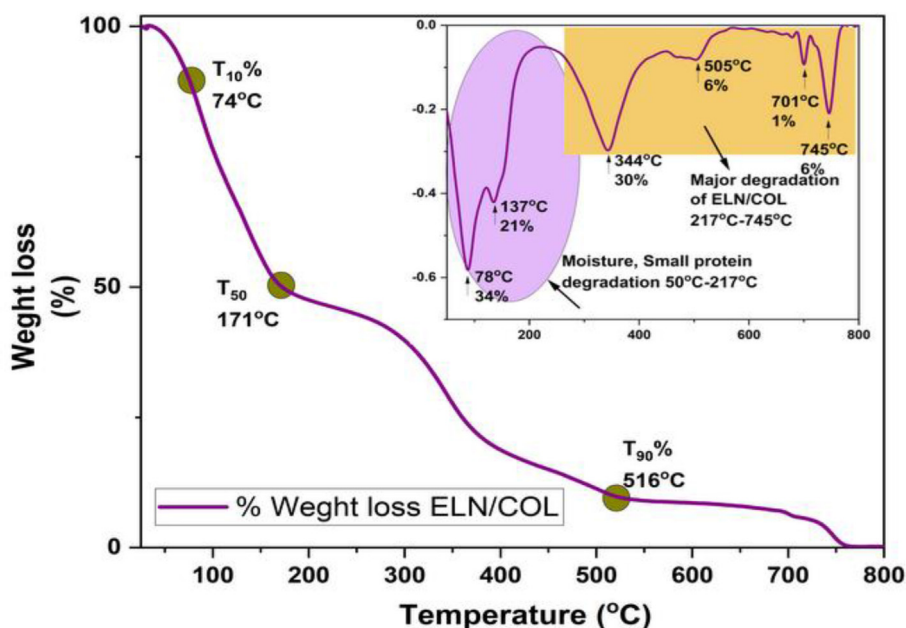


Figure 4. TGA and DTG of ELN/COL matrix.

solubilization and polycondensation reaction, the important characteristics of ointment base matrix such as viscosity and spreadability can be regulated.

3.2.2. Reaction mechanism of elastin/collagen- lactic acid

After the successful solubilizing of ELN/COL matrix in lactic acid monomer, polycondensation reactions were done at  $150\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  for five hours. The possible reactions path ways for the overall process are: lactic acid to lactic acid interaction to produce oligomer and lactic acid interaction with the dissolved ELN/COL matrix. During the dissolution step, the weakening of the protein secondary structure results in the delamination of  $\alpha$ -helix and  $\beta$ -sheet stacks leading to the lactic acid

monomers to grow within the protein chains. As the chains of the oligomers extend, intermolecular interaction with the NH group of the ELN/COL chains is expected to highly increase. This can be taken as a reason for the improvement of bulk properties observed in the prepared hybrid matrix (ELN/COL-OLLA).

Furthermore, when the lactic acid chain increases, the amount of water generated during the reaction was observed to increase. This is due to the reaction between lactic acid monomers forming the oligomer (OLLA). Oligomers are formed beside the secondary structure of the hybrid protein matrix. The macromolecular interaction between the carboxyl groups (C=O) of OLLA and the NH groups of elastin/collagen matrix produces one phase system with the ability of controlling the two

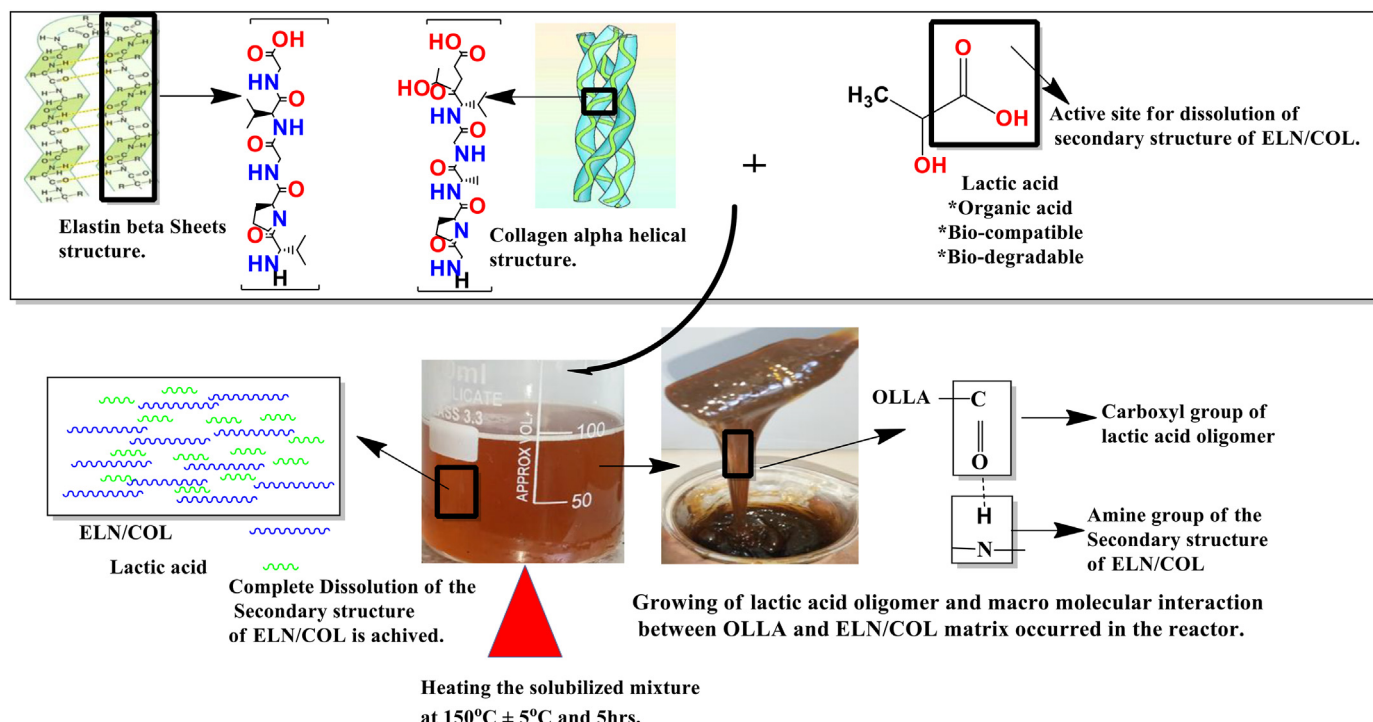


Figure 5. Solubility and Reaction mechanism of ELN/COL-OLLA Bioconjugate.

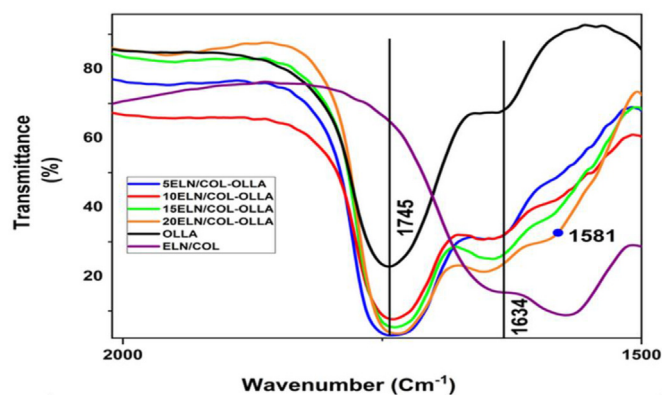


Figure 6. C=O stretching of OLLA, ELN/COL, and ELN/COL-OLLA.

essential properties of ointment matrixes, namely lubrication and spreadability. As indicated in Figure 6, the aforementioned interaction is supported by the observed OLLA major peak shift from  $1745\text{ cm}^{-1}$  to  $1737\text{ cm}^{-1}$  with the loading of ELN/COL, and this was observed due to the increased availability of amide groups ( $-\text{NH}$ ) of the ELN/COL matrix towards the carboxyl groups ( $-\text{C}=\text{O}$ ) of lactic acid. Furthermore, extended broader shoulder like peak is shown up to  $1500\text{ cm}^{-1}$  with the loading of protein matrix. This macromolecular interaction occurred due to hydrogen bonding and Van der Waals interaction.

More interestingly, a new FTIR peak is observed at  $1581\text{ cm}^{-1}$  with increasing ELN/COL concentration. The shoulder like peak observed at  $1634\text{ cm}^{-1}$  for the lactic acid oligomer was also shifted to the higher wavenumbers of  $1639\text{ cm}^{-1}$ ,  $1641\text{ cm}^{-1}$ ,  $1643\text{ cm}^{-1}$ ,  $1649\text{ cm}^{-1}$  respectively for 5, 10, 15 and 20ELN/COL-OLLA samples. This can be correlated with the formation of hydrogen bonding due to the availability of more amide groups that are responsible for improved macromolecular interactions.

### 3.2.3. Physical evaluation of synthesized ointment bases

**3.2.3.1. Organoleptic characteristics.** The physical appearance, texture, color, phase separation, and homogeneity or in general organoleptic properties of the conjugate base, were shown in Table 2. From the result, it can be confirmed that the ointment base showed a smooth texture and homogenous system (no signs of phase separation), which gives a cosmetically appealing appearance. The synthesized ointment base has a brown and dark brown color.

**3.2.3.2. Spreadability and viscosity.** The spreadability and viscosity of the prepared base were affected by increasing ELN/COL as indicated in Figure 7 and Table 3. This can be correlated with the observed macromolecular interaction discussed above. The extracted protein matrix which tends to have a semi-solid characteristic observed to dissolve in lactic acid completely which results the delamination of the secondary structures of ELN/COL matrix. When the solution is subjected to the polymerization temperature of lactic acid, the oligomer starts to grow beside the protein macromolecular chains which further promotes the interaction responsible for the modification of ELN/COL-OLLA flow properties such as viscosity and spreadability.

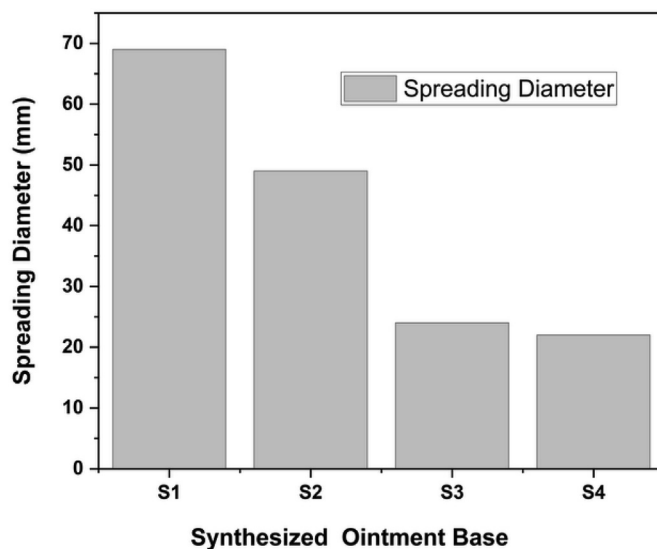


Figure 7. The spreadability values of the synthesized ointment base.

The study of spreadability for the prepared ELN/COL-OLLA viscoelastic samples gave information whether the base matrix evenly spread on the skin or not. A standard dose of medicated formulation is administered to assess the efficacy of topical on the skin, in which its spreadability is assumed to play an important role. The values indicated in Figure 7, show a decrease with the increase in the protein matrix. It shows that the prepared base matrix readily spreads with a small amount of shear while it is applied on the target surface.

From Table 3, it can easily be observed that the spreadability decreases from  $62 \pm 7\text{ g cm/s}$  to  $46 \pm 3\text{ g cm/s}$ ,  $22 \pm 2\text{ g cm/s}$  and  $20 \pm 2\text{ g cm/s}$  as the ELN/COL loading increased from 5g to 10g, 15g and 20g respectively. It was also observed that the spreadability was inversely proportional to the viscosity of the base matrix.

The synthesized ointment base matrix was observed to have spreadable and lubricant characteristics. The results indicated that the ointment base has the ability to be applied easily on the site of application without being runoff, which assures that the formulation maintains good wet contact time. Similar observations on the spreadability of various ointment bases and topical herbal formulations were reported by different researchers [9, 19, 42]. Most importantly, the obtained results indicate the possibility of controlling the spreadability by varying the ELN/COL loading. This issue needs to be addressed critically in order to design an ointment base matrix for the targeted application such as delivery of active components to the specified site. As stated in the FTIR analysis, the observed macromolecular interaction between the amide groups of the ELN/COL and the carbonyl group of the lactic acid oligomer can be considered as the main reason for the matrix to have lubrication characteristics with one phase system and controlled spreadability. The viscosity of the prepared ELN/COL-OLLA matrix was greater than  $5000\text{ Cp}$  for all matrices (S1–S4) [9].

**3.2.3.3. pH values.** The pH (Table 3) of all the synthesized ointment bases were found to be in the range of  $5.20 \pm 0.02$ – $5.76 \pm 0.02$ , which is within the normal human skin pH range [10], which helps to avoid the

Table 2. The sensory analysis of the synthesized ointment base

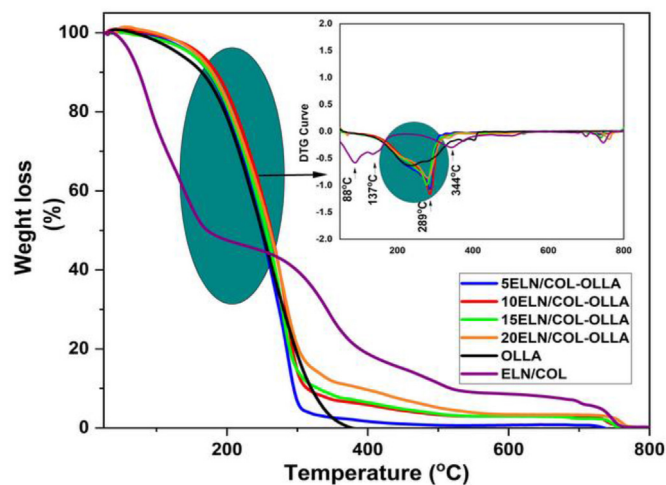
Base Synthesis	Appearance	Homogeneity	Texture	Phase Separation
S <sub>1</sub>	Light Brown	Completely homogenized	Smooth (Lubricant)	no
S <sub>2</sub>	Brown	Completely homogenized	Smooth (Lubricant)	no
S <sub>3</sub>	Dark Brown	Completely homogenized	Smooth (Lubricant)	no
S <sub>4</sub>	Dark Brown	Completely homogenized	Smooth (Lubricant)	no



**Table 3.** Evaluation parameter for the synthesized base matrix ELN/COL-OLLA.

Base Synthesis	Composition	Moisture Content (%)	Spreadability	pH	Viscosity (cp)
S <sub>1</sub>	5ELN/COL-OLLA	<1%	62 ± 7	5.20 ± 0.02	>5000
S <sub>2</sub>	10ELN/COL-OLLA	<1%	46 ± 3	5.43 ± 0.03	>5000
S <sub>3</sub>	15ELN/COL-OLLA	<1%	22 ± 2	5.56 ± 0.02	>5000
S <sub>4</sub>	20ELN/COL-OLLA	<1%	20 ± 2	5.76 ± 0.02	>5000

S<sub>1</sub>–S<sub>4</sub>: Stands for the synthesized of Ointment base.

**Figure 8.** Thermal analysis of Synthesized Ointment base.

skin irritation. The results obtained showed that the pH of the synthesized ointment base slightly increased with increasing ELN/COL concentration which can be correlated with the reduction of lactic acid oligomer.

### 3.2.4. Thermal stability analysis of ELN/COL-OLLA

Thermogravimetric analysis was performed for ELN/COL and ELN/COL-OLLA and the results were summarized using the temperature correlated to initial degradation, 50% degradation and maximum degradation. From DTG curve, it is observed that there was the formation of huge hump having thermal stability compared to the ELN/COL matrix. The ELN/COL matrix has six major degradation stages. The maximum thermal degradation temperature of the extracted protein which decomposes in different steps shifted to a higher temperature due to the reaction of lactic acid oligomer and ELN/COL. The multi-component degradation of ELN/COL changed to a single component degradation. The thermal degradation of the synthesized base showed a similar degradation with lactic acid oligomer and the six-mass loss in the ELN/COL matrix was not observed in the synthesized base. The reason for showing similar degradation characteristics can be correlated with the observed secondary interaction discussed in the reaction mechanism analysis sections.

The observed six major peaks of ELN/COL matrix (Figure 8) were changed to one major degradation peak with in the temperature range from 148–320 °C for ELN/COL-OLLA. It is clearly indicating that the 50% weight loss (T<sub>50</sub>) in ELN/COL matrix which appears up to 171 °C was not

**Table 4.** TGA analysis of ELN/COL, OLLA and synthesized ointment.

	T <sub>10</sub> (°C)	T <sub>50</sub> (°C)	T <sub>90</sub> (°C)
ELN/COL	74	171	516
OLLA	164	249	319
5ELN/COL-OLLA	178	249	295
10ELN/COL-OLLA	184	260	319
15ELN/COL-OLLA	179	256	329
20ELN/COL-OLLA	179	261	329

observed on the ELN/COL-OLLA matrix due to the macro interaction between the amide group of ELN/COL and the carbonyl group of lactic acid oligomer.

The interaction between ELN/COL and oligomer of lactic acid (OLLA) enhanced the T<sub>50</sub> (°C) degradation of extracted protein from 171 °C to 249 °C, 260 °C, 256 °C and 261 °C respectively for 5ELN/COL-OLLA, 10ELN/COL-OLLA, 15ELN/COL-OLLA and 20ELN/COL-OLLA. From Table 4, it is clearly shown that as the concentration of ELN/COL increased its thermal stability also increased.

Generally, due to the secondary force formed between the lactic acid oligomer and ELN/COL matrix the multicomponent degradation which appears in ELN/COL changed to one major peak. This shows that the synthesized ointment has higher thermal stability than the extracted protein and lactic acid oligomer.

## 4. Conclusion

In this research, development and characterization of base matrix from elastin/collagen and lactic acid (which are biobased, green, and biocompatible materials) was successfully accomplished. Poultry byproducts were used for extracting hydrolyzed elastin and collagen. The chemical structure of the extracted ELN/COL matrix was analyzed by FTIR spectroscopy and indicates the amide I, amide II, and amide III groups of elastin and collagen at 1605–1770 cm<sup>-1</sup>, 1488–1605 cm<sup>-1</sup> and 1266–1360 cm<sup>-1</sup>, respectively. CH<sub>2</sub> wagging vibration for both collagen and elastin were also indicated at 1338 cm<sup>-1</sup>.

The delamination of the secondary structure of ELN/COL can be correlated with the solubility properties of the protein within the lactic acid monomer, which is resulted in the weakening of the protein secondary structure by the formation of a new interaction between the NH groups of amides and the C=O bonds of lactic acid monomers. In-situ polycondensation was used to synthesize L-lactic acid oligomer (OLLA) and lactic acid modified elastin/collagen (ELN/COL-OLLA). The prepared lactic acid modified elastin/collagen matrix (ELN/COL-OLLA) was characterized through various physicochemical techniques targeting ointment application.

The observed six-mass loss stages in the ELN/COL matrix were superimposed into one after the modification of the protein matrix by lactic acid oligomer. The reason for showing similar degradation characteristics might be due to secondary weak interaction formed between the carboxyl group of lactic acid oligomer and the amide group of ELN/COL matrix. This phenomenon is supported by the formation of a broader shoulder peak observed at 1745 cm<sup>-1</sup>, which is increased with the addition of elastin/collagen within the lactic acid oligomer. More interestingly, with increasing ELN/COL concentration within the oligomer matrix a new FTIR peak was observed at 1581 cm<sup>-1</sup> which is attributed to the new macromolecular interaction between the protein matrix and lactic acid oligomer. From the obtained physicochemical analysis, it can be concluded that the prepared matrix can be further studied for the use of an ointment base matrix.

## Declarations

### Author contribution statement

Meseret Ewunetu Kibret: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.



Tatek Temesgen: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Melakuu Tesfaye: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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#### Data availability statement

Data included in article/supplementary material/referenced in article.

#### Declaration of interests statement

The authors declare no conflict of interest.

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