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Extraction and characterization of acid soluble Collagen Type I from *Sperata Aor*

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Abstract: Collagen being the principal protein in physiological system, its demand for research, cosmetics, prosthetics, implants are always very high. The price of commercial collagen is quiet hefty and extensive delays occurs in delivery. This is the reason which has given path to extraction of nascent collagen from fish skin which will provide a base to the researcher fraternity for research on scaffolding and tissue engineering with non-commercial collagen. In this work, Indian catfish has been used for the extraction as the skin of the fish has high fat and protein content. Due to the slimy skin of the fish, the concentration and content of the collagen is high. The collagen extracted showed eminent footprints of the Type I collagen with predominant peaks of Amide A, B and Amide I, II & III observed in FTIR plots. The Electron microscopy has shown spiral structure with few open spaces which are ideal for development of composites for prosthesis, scaffolding and targeted drug delivery.

Index Terms – Collagen, FTIR, Scaffold, Drug delivery, SEM

I. INTRODUCTION

Collagen being the prime protein in our body, it's been found abundantly & predominantly in all tissues of our body. The Greek word "kolla" (glue) and the French word "collagen" were used to describe the constituent of connective tissue that yields gelatin when it is heated. Collagen was later defined as "that component of connective tissue, which generates gelatin on boiling." A significant component of connective tissue and the most prevalent protein in mammals, collagen makes up about 25% of the body's total protein content. This substance is frequently utilised to build ligaments and tendons because to its high tensile strength. All dental tissues, with the exception of enamel, include collagen as part of the extracellular matrix. In bones, cartilage, and teeth, collagen can be found. The cornea, which is present in crystalline form, is likewise filled with collagen [1-3].

Collagens are used in dental, orthopaedic, and surgical treatments to create artificial skin replacements to heal serious burns. Collagen is used in pharmaceuticals, cosmetics, and prolotherapy (strengthening the lax ligaments). Collagen may be employed in therapy in the form of blood coagulating cotton textiles, injections to treat soft tissue abscesses, dental bone filling materials, and a permeable membrane for periodontal regeneration. Collagen can be synthesised as cross-linked solids or gels with a matrix structure [4-7]. Resorbable collagen has been used in dressings, graft closure, and tooth extraction sites since the 1970s, among other applications. Collagen-based membranes have been used in periodontal and implant treatment as a barrier limiting epithelial migration and allowing cells with regeneration ability into the defect site [8,9].

Collagen can be fundamentally extracted by chemical hydrolysis and enzymatic hydrolysis. Chemical hydrolysis is all the more ordinarily utilized as a part of industry, however organic procedures that utilization the expansion of chemicals are additionally encouraging when items with high healthful esteem and enhanced usefulness are required. In addition, enzymatic procedures produce less waste and may diminish the preparing time, however they are costlier. To separate collagen, it is important to expel various covalent intra-and intermolecular cross-links, which fundamentally includes buildups of lysine and hydroxylysine, ester securities and different bonds with saccharides, all of which makes the procedure very complex.

Prior to the collagen extraction a pre-treatment is performed utilizing an acidic or alkaline process, which varies as per the inception of the crude material. The pre-treatment is utilized to expel non-collagenous substances and to acquire higher yields simultaneously. The most normally utilized extraction techniques depend on the dissolvability of collagen in unbiased saline arrangements, acidic arrangements, and acidic arrangements with included chemicals.

II. METHODOLOGY

Chemicals: All chemicals and materials that are used for the research are of scientific grade. Butyl Alcohol, NaOH pellet, NaCl powder and DeIonized water has been procured from Merck, Germany whereas, 99.9% Acetic acid has been purchased from Sigma-Aldrich.

Pre-treatment: Due to the nature of the cross-linked collagen that is available in the connective tissue of creatures, it breaks up gradually, even in bubbling water. Subsequently, a partial chemical treatment is important to break these cross-interfaces previously extraction [10]. To this end, weakened acids and bases are utilized, and the collagen is subjected to partial hydrolysis, which keeps up the collagen chains in place however the cross-links are cleaved [11]. In the acidic type of pre-treatment, the crude material is drenched in acidic solution until the point when the solution enters all through the material. As the solution infiltrates the structure of the skin at a controlled temperature it swells to a few times its underlying volume and the cleavage of the non-covalent between and intra-molecular bonds occurs [12]. The acidic procedure is more appropriate for more delicate crude materials with less interlaced collagen strands, for example, porcine and fish skins [13]. The alkaline procedure comprises of treating the crude material with a basic solution, normally sodium hydroxide (NaOH), for a period that can take from a couple of days to half a month [11]. This procedure is utilized for thicker materials that require a more forceful entrance by the treatment specialists, for example, bovine ossein or shavings [12]. NaOH and Ca(OH)2 are regularly utilized for pre-treatment, yet NaOH is better for pre-treating skins since it causes huge swelling, which encourages the extraction of collagen by expanding the exchange rate of the mass in the tissue network [14].

An examination by Liu et al. (2015) assessed the impact of antacid pre-treatment on the extraction of acid soluble collagen (ASC) from the skin of grass carp (Ctenopharyngodonidella). Concentrations of NaOH from 0.05 to 0.1 M were powerful in expelling non-collagenous proteins without losing the ASC and basic changes at temperatures of 4, 10, 15 and 20°C. Be that as it may, 0.2M and 0.5M NaOH caused a critical loss of ASC, and 0.5M NaOH brought about auxiliary alteration in the collagen at 15 and 20°C [14]. Notwithstanding the utilization of acids and bases, catalysts or chemicals may likewise be utilized to cut the cross-linked bonds to get products with various qualities [10]

Extraction Procedure: *Sperata Aor* or commonly known as Indian Catfish has a thick layer of epidermal tissue which is a reserve of basic protein, collagen and fats. The fish skin had been bought from local market and preserved in ice before further treatment. The skins were thawed and washed thoroughly first by running tap water and then by deionized water which was kept at ice cool temperature. Skins were further cleaned with 0.8 M NaCl (1:6 w/v), again in the Stephan homogenizer at 5° C for 10 min, and were rinsed with abundant running tap water. This process was repeated for atleast 2 to 3 times and excess water as removed by manual squeezing. The skin was further cut into small pieces and further treated to remove any non-collagenous parts.

For loosening the skin, i.e, for removing non collagenous parts the skin was treated with 0.1M NaOH and thoroughly mixed in a sonicator. After this vigorous mixing the mixture had been kept in normal freezer for 24 hours to enhance proper loosening of the thick fibres. After 24 hours the skin was filtered through double filtration paper and further cleaned with cold deionized water until the pH falls to 7. Following this process, the skin was treated with 15% butyl alcohol to remove fats. To make 1 litre of 15% butyl alcohol, 150 ml of the alcohol was mixed in 850 ml of deionized water. The skin was treated in two consequent batches. First 500 ml of butyl alcohol was used to treat the skin mass for 24 hours, then another 500 ml was used for another 24 hours in a normal freezer. The complete procedure has been summarized in Fig. 1.

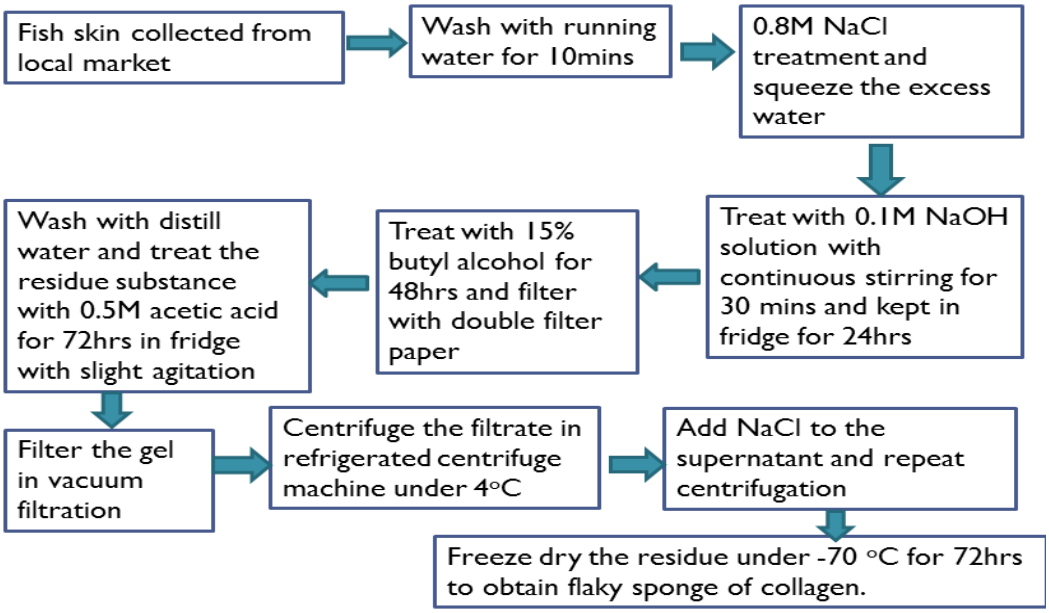

Figure 1: Schematic of the process of Collagen Extraction

After 48 hours of butyl alcohol suspension, the skin was filtered and washed thoroughly and 1 litre of 0.5M acetic acid had been added to the skin and stirred intermittently. This process is again repeated for another 24 hours. After 72 hours of acid treatment the quasi fluid mixture was filtered in a vacuum filtration unit to ensure that only soluble collagenous parts are filtered as shown in Fig. 2.

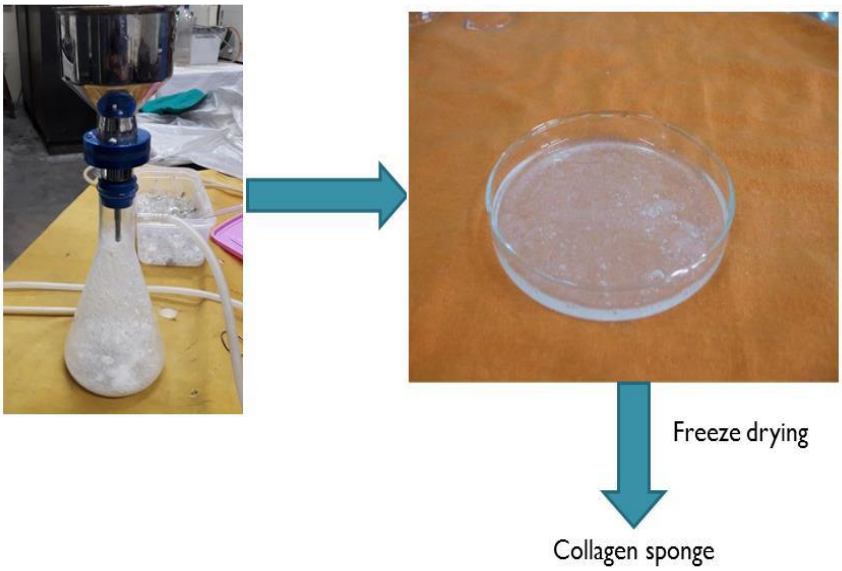

Figure 2: The vacuum filtration unit

III. RESULTS AND DISCUSSIONS

Fourier Transform Infrared Spectroscopy: The FTIR spectra of extracted collagen and silica-collagen composites are presented in Fig. 3. Amide A band corresponds to N-H stretching vibrations was found in the wave range 3419.79 cm-1. Generally, Amide A band is obtained in the range of 3400-3440 cm-1 [15] which is an indication that, hydrogen bond network has been constructed for this material. Amide I bond corresponds to carbonyl group stretching vibrations is generally found in the range of 1600-1700 cm-1, here which is found at 1668.43 cm-1 [16]. Amide II bond corresponds to CN stretching vibrations is found at 1535.34cm-1 [17]. The absorbance peak at 1238.30 cm-1 corresponds to the peak responsible for Amide III [18].

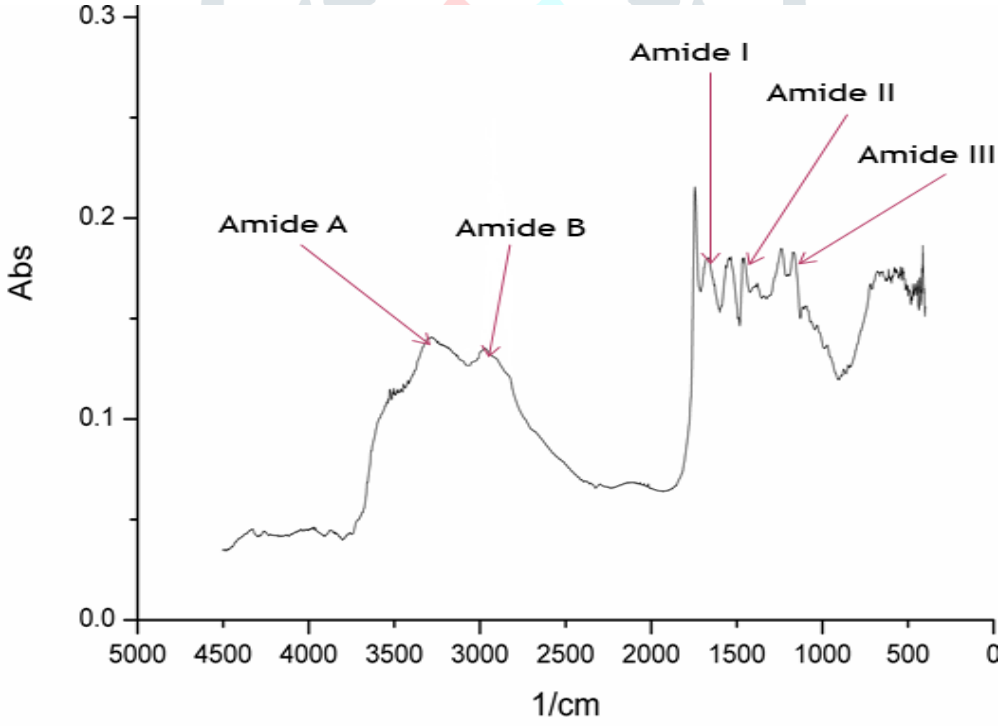

Figure 3: FTIR analysis of Extracted Collagen

Scanning Electron Microscopy: The microstructure of extracted collagen and silica-collagen composite were investigated by FESEM. The micrograph of extracted collagen in Fig. 4 shows almost spiral structure with few open spaces. The electron microscopic structure opens the way of utilizing the extracted collagen for various tissue engineering and drug delivery purposes.

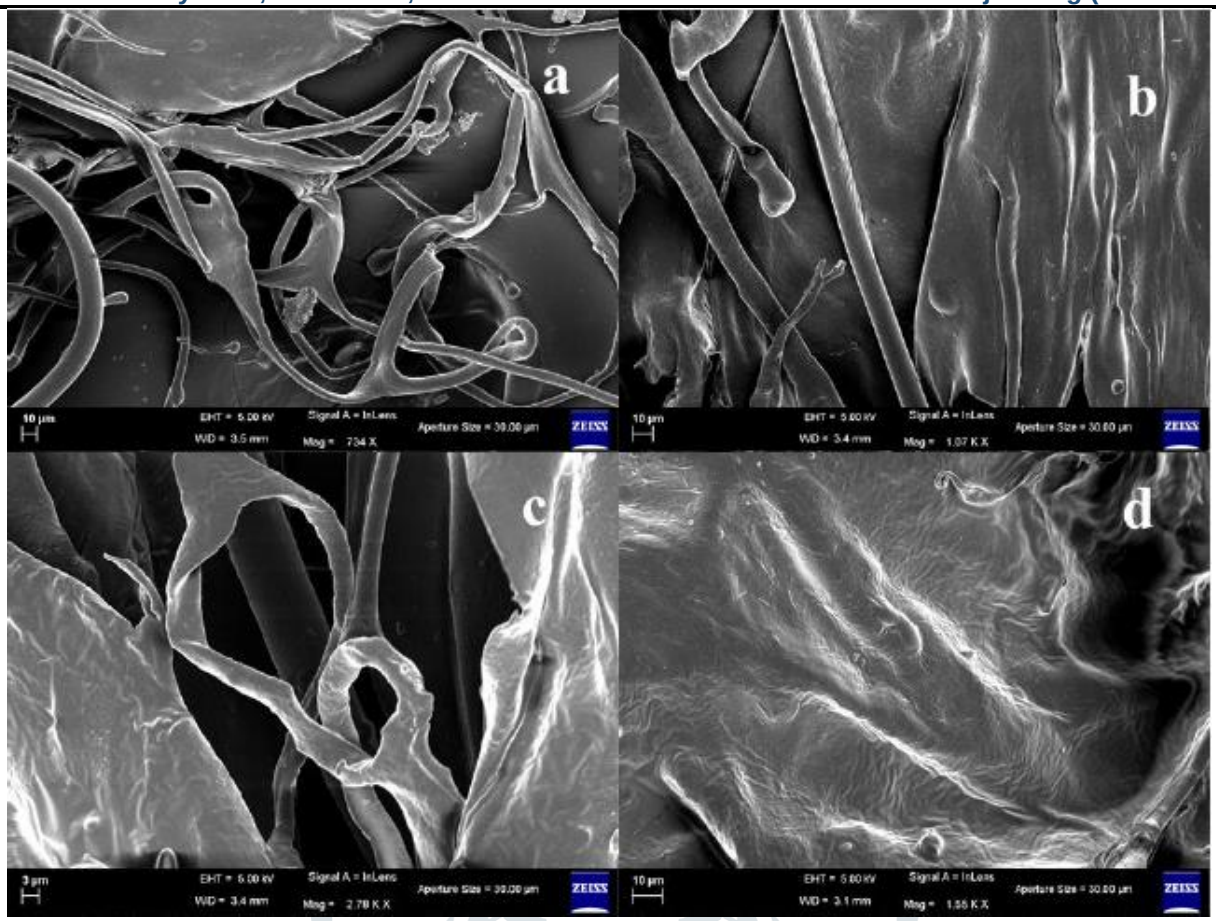

Figure 4: SEM of extracted collagen at different zooming magnitude showing knotted fibrillar structures

IV. CONCLUSIONS

The extracted material from fish skin showed characteristics absorption peaks for Amide A and Amide I, II and III which is the identification characteristics of Collagen Type I. The microstructure of the collagen is fibrillar with some knotted structure and few spaces which has determined its spongy nature. These characteristics confirm the material as predominantly collagen type I. The SEM micrograph has a porous phase which is very much likely for any scaffold for tissue growth, regeneration, cell proliferation and targeted drug delivery.

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