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A FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPIC STUDY ON OVINE SCAPULAR CARTILAGE

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Abstract: The paper deals with FTIR spectroscopy of ovine scapular cartilage. The study reports that the constituents of ovine scapular cartilage are mainly collagen and proteoglycans; Carbonate ions and Phosphate ions are in a very small quantity.
Keywords: FTIR spctroscopy; Scapular Cartilage; Collagen; Ovine.

1. Introduction

Infrared spectroscopy is a potential tool to provide valuable information in the study of biological systems. It is very much helpful in the study of structure of various macromolecular components and their conformations within tissue. In most of the cases, infrared spectroscopy gives rapid qualitative and quantitative identification of organic and inorganic constituents and their combinations in mineralized biological tissues.

Louisfert and Pobbequin [1] studied a variety of natural and synthetic samples of $CaCo_3$, both individually and in mixture by infrared spectroscopy. Posner and Perlof [2] based on IR data suggested that the mineral portions of the bone and tooth tissues are calcium deficient hydroxyl apatite. Posner et al [3] carried out infrared studies on synthetic hydroxyl apatite Ca_{10} $(PO_4)_6$ $(OH)_2$, containing low calcium, have shown the presence of fairly strong hydrogen bonds in proportion to the missing number of calcium ions.

Compere and Bates [4] made infrared analysis of two hundred specimens comprising two species of marine molluscan shells and determined calcite-aroganite ratios. They concluded that infrared analysis is easier and less expensive than any other method for macroscopic analysis of the structures and calcium carbonate depositions in molluscan shells.

Ramaswamy et al [5] studied the mineralogical form of $CaCo_3$ deposition in some marine molluscan shells through infrared spectroscopy. They computed the percentage composition

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of calcite and aroganite using the characteristic frequencies of the two polymorphs and results were correlated with the different environments of the species.

Srinivasa Rao and Srinivasa Manja [6] studied the infrared spectrum of human bone powder and ash with a view to understand the nature of hydrogen bonding in these biological apatites. The infrared spectrum revealed bands characteristic of CO_3 ion and this observation suggests that bone material consists of carbonate containing apatites. The average hydrogen bond lengths in bone powder and bone ash are estimated to be $2.76A^0$ and $2.80A^0$ respectively.

Watabe [7] presented a review article on crystal growth of calcium carbonate in invertebrates, which covers a wide ground. He examined the crystalline characteristics of calcareous structures, their formation, nucleation and growth of crystals, the organic matrix and other factors controlling the crystalline patterns in the invertebrates. Samata and Krampitz [8] studied Ca^{++} binding polypeptides in oyster shells. They reported that the basic architecture of the amino acid sequence of all Ca^{++} binding polypeptides is the same.

Bills et al [9] made the orientation and micro structural studies on the cortical components of various bovine bone. Although the function and the structure of the bone differed widely, the orientation texture of the apatite crystals were predominantly uniaxial and very similar. The orientation function of the collagen component was found to be similar to that of the mineral, thus demonstrating that their intimate relationship exists in all bone structure and function.

Dowker and Elliot [10] investigated the formation of molecular Co_2 in synthetic apatites, dental enamel, dentine and various apatite rock phosphates by infrared spectroscopy. After heating the samples in the range of temperature 120^0 to 900^0C, the Co_2 band at 2340 cm^{-1} was observed in the synthetic samples, enamel, and some of the rock phosphates, but not in dentine or bone. It was suggested that the absence of this band in dentine and bone is caused by the small crystal size of their apatites.

Khromova and Lazarev [11] investigated the conformational transition of triple helix-unordered chains of a collagen like polytripeptide by infrared spectroscopy and microcalorimetry. It was shown that transition is of a one- stage character.

Camacho et al (2001) analysed FTIR absorption spectra of pure type II Collagen and aggracan (PG) separately and in mixtures with different concentrations of these two pure compounds and concluded that collagen content correlates best with the areas under the amide 1 and amide 2 regions.

Saarakkala et al [13] observed carbohydrate region absorbance, quantified with and without normalization with amide 1 region. Rieppo et al [14] investigated the changes in the collagen network during growth and maturation of pigs. IR studies were made on molecular conformation and permeation in skin [15].

A search of literature reveals that inspite of extensive investigations on infrared spectroscopy of human skin, hair, nail, bone and collagen on different aspects and their constituents such as lipids and proteins, no information is available on qualitative and quantitative identification of organic and inorganic constituents and their combinations of ovine scapular cartilage. In view of this, in the present investigation, infrared spectroscopic study has been made on ovine scapular cartilage, in order to assess its molecular composition.

2. Materials and Methods

The cartilage samples of scapula of the animal Ox were collected from beef shop after 4 to 5 hours of slaughtering. The samples were cleaned, flesh attached to them was removed completely and then Sun dried for a day or two. FTIR spectra of powdered samples were recorded with Thermo - Nicolet Nexus 670 FTIR spectrometer and depicted in Fig. 1. Thedata obtained from FTIR spectra is presented in Table 1.

3. Results and Discussion

Fig.1is FT – IR spectrumof Ovine scapular cartilage, which reveals a series of bands with different intensities. Table 1 presents data on wave numbers and corresponding Transmittance (%) obtained from FTIR spectra along with characteristic vibrations of functional groups.

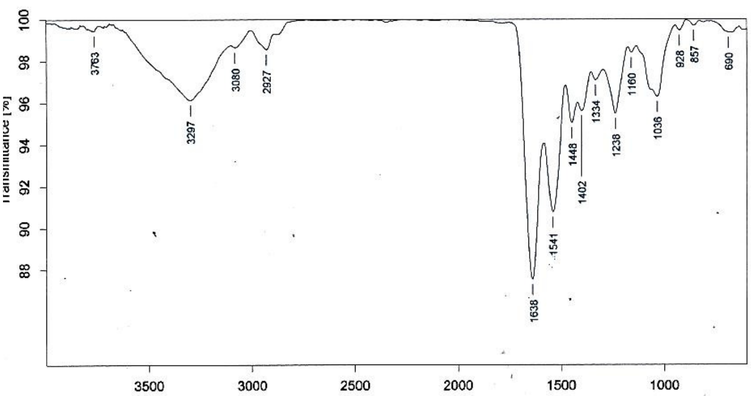

Fig. 1. FT IR spectra of Ovine scapular cartilage

For the systematic analysis, IR spectrum is divided into three regions. Region I is from 4000 to 3000 cm^{-1}, concerned with water and hydroxyl group. This region is of considerable interest, because it reveals the nature of hydrogen bonding. Region II is 3000 to 1500 cm^{-1}, wherein bands for functional groups are observed. In this region, major IR absorption pertaining to collagen occurs. Region III is 1500 – 200 cm^{-1}, which has significant importance in the context of biological minerals and their combinations. The spectra of cartilage powder indicate the presence of bands characteristics of phosphate ion, carbon ion, water molecule and also of some functional groups concerned with protein – the collagen.

The IR bands at wave number 3080 cm^{-1} is related to Amide B. The dominating bands at 1638 cm^{-1} and 1541 cm^{-1} may be originated due to collagen and proteoglycans and collagen dominates the absorption (Fig. 1). These bands are related to the stretching vibrations of C=O bonds and N-H bonds respectively. A band around 1238 cm^{-1} is due to the C-N stretch with N-H bending vibrations. These bands are concerned with proteins and the strongest band at 1638 $cm^{-1}$is characteristic of collagen [16].

The bands at 1160 cm^{-1}, 1036 cm^{-1} and 928 cm^{-1} are related to stretching vibrations of C-O and C-OH as well as C-C ring vibrations in carbohydrates. These bands are believed to be more specific to proteoglycans [17].

The bands at 1448 cm^{-1} and 1402 cm^{-1}, originated from P-O anti symmetric stretch and from P = O asymmetric stretch, are related to calcium phosphate ions. The strongest absorption band at 887 $cm^{-1}$is specific to calcium carbonate ions. The band 712 cm^{-1} characteristic of calcium carbonate is missing. Hence calcium carbonate is not present in Cartilage.

Table 1 – FT IR data on Ovine scapular cartilage

Wave Number (cm^{-1})	*Transmittance (%)*	*Characteristic vibrations of functional groups*
3763	99.46	Carbonyl C=O stretching
3297	96.12	H-O-H stretching
3080	98.64	Amide B
2927	98.58	Asymmetric CH_2 stretching
1638	87.52	Amide 1 of pleated sheet structures of protein
1541	90.74	Amide II band of protein; N-H stretching
1448	95.03	P-O anti symmetric stretch
1402	95.56	P=O asymmetric stretching of phosphodiesters
1334	97.08	O-C-O stretching
1238	95.46	Amide III band components of proteins C-N stretch with N-H bend
1160	98.39	C-O-C, C-O dominated by ring vibrations in Carbohydrates
1036	96.29	
928	99.46	
887	99.93	Carbonate ion
690	99.35	C-O-C deformation

The study concludes that the constituents of ovine scapular cartilage are mainly collagen (maximum portion) and proteoglycans (relatively in small quantity); Carbonate ions and Phosphate ions are in a very small quantity.

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