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PREPARATION HYALURONIC ACID NANOGEL

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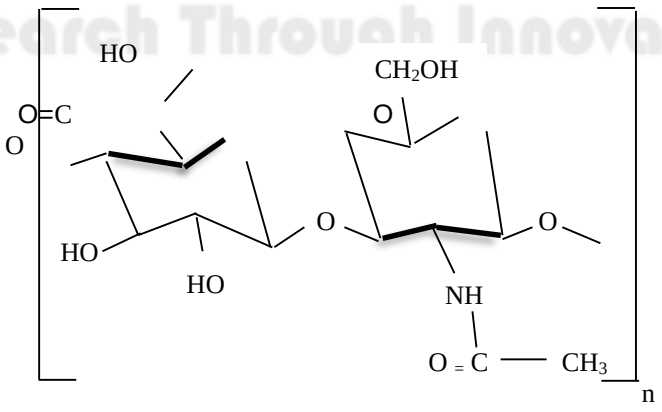
ABSTRACT: Hyaluronic acid (HA) has shown promise in managing pain. Studies have shown that high molecular weight Hyaluronan effectively reduces knee pain, synovial effusion, and improves muscular strength. The development of HA Nano-gels has opened new avenues for improving the delivery and efficacy of HA in treating knee pain. These Nano gels offer enhanced bioavailability, sustained release, and targeted delivery [9] of HA to the knee joint, thereby facilitating improved joint lubrication, reduced inflammation, and prolonged pain relief. The primary objective of this study is to investigate the transformation of hyaluronic acid (HA) into a nanogel configuration. The main focus lies in the creation of a nanogel assembly of HA, wherein the HA molecules are converted into a gel-like structure at the nanoscale through the utilization of the carbodiimide technique and the application of this nanogel assembly on bandages. With the use of medical sticky glue, this nano powder is transformed into nanogel. The goal is to create a gel-like nanogel structure on bandages and its molecular structure and crystallinity are determined through X-ray diffraction (XRD) tests and its behavior, dissolution and absorbing tendency is determined through solubility test.

KEY WORD: Hyaluronic acid, HA Nano gel –cross linking, Medical glue, EDC HCl.

INTRODUCTION

Studies have shown the efficiancy of high molecular weight hyaluronan in reducing knee pain, synovial effusion, and enhancing muscular knee strength. Hyaluronic acid is a naturally occurring substance in the synovial fluid of joints. Injections of hyaluronic acid aim to restore joint lubrication, improving joint mobility and reducing friction. [7, 8]Viscosupplementation is thought to provide pain relief by cushioning and protecting the joint surfaces. This introduction sets the stage for exploring the potential of hyaluronic acid Nanogels in effectively managing knee pain and impeding the advancement of osteoarthritis [5], thereby offering promising prospects for the treatment of this challenging degenerative joint disease.

Hyaluronic acid is a naturally occurring gel-like and slippery substance found in the human body that serves various functions, particularly in the connective tissues, skin, and eyes. HA is a polysaccharide [1] composed of D-glucuronic acid and N-acetyl-D-glucosamine linked through a glycosidic bond via β-(1□4) or β-(1□3). It is an anionic, non-sulfated glycosaminoglycan [19], and it occurs primarily in vivo as sodium hyaluronate. It is a major component found in the extracellular matrix of connective tissue. Not only that, but it's found in the synovial fluid of joints, in the vitreous body, the umbilical cord, etc. It helps things move smoothly and helps your joints work like a well-oiled machine, promoting tissue hydration and wound healing. [11]. HA has the ability to absorb water and it is non-immunogenic , biocompatible [27], biodegradable and bioactive polysaccharide. Hydrogels are utilized in various biomedical applications, including drug scaffolds, gene carriers, and tissue engineering implants.



Chemical structure of Hyaluronic acid

HA NANO PARTICLE

Hyaluronic acid is a naturally occurring gel-like and slippery substance found in the human body that serves various functions, particularly in the connective tissues, skin and eyes. Hyaluronic acid (HA) from rooster comb is purified and converted into Nano gel using various methods have been developed for cross-linked HA, which commonly result in [16,17] gel or film formation. Hyaluronic Nano-gel and microsystems can be prepared in a wide range of methods: coacervation [33], spray drying [32] or solvent evaporation are well-down techniques to produce cross-linked suspension performed in emulsion. Several studies reports on the preparation of Hyaluronan nanoparticle systems using carbodiimide technique. By using Carbodimmide technique, hyaluronic acid is cross-linked with carboxyl group.

The investigation presents a method for preparing nano-sized particulate systems using hyaluronic acid (HA) through covalent cross-linking with a diamine in aqueous media at room temperature via the carbodiimide technique **[**35-38**]**. Different molecular weights of HA were used to explore the relationship between size, cross-linking ratio, and HA molecular weight. The purified HA Nano gel is used as a layer and immobilized onto the bandage using assembly techniques. The solubility, structure, and size of these nanoparticles in dried and swollen states are discussed. Cross-linked HA nanoparticles **[**2**]** exhibit potential as delivery biosystems for various biomedical applications due to their nano-sized nature and ability to form stable colloid systems in aqueous media. In the present work, where Hyaluronic acid is being converted to Nano gel of HA assembled in layer (known as Hyaluronic acid Nano gel) were synthesized and their solubility, structure and functional group will be described and discussed. Cross-linked hyaluronan nanoparticle may dissolve in aqueous medium. Due to their appealing properties, nano-sized the delivery biosystems are appealing choices for a range of applications in medicine.

I. EXPERIMENT:

a) Materials

HA sodium salt (HA 0 h: Mw=800 kDa) was obtained from BTC lab It was a pharmaceuticalproduct.2, 2′ (ethylenedioxy)bis (ethylamine) Water-soluble 1-[3-(dimethylamine) propyl]-3ethylcarbodiimide methiodide (CDI) was applied as a condensation agent,Sodium hydroxide(NaOH), Medical liquid glue (ethyl-2-cyanoacrylate glue),diamine (100mg/ml).

Preparation of low molecular weight hyaluronic acid

The HA sodium salt was dissolved in water, adjusted to pH 2.0, and degraded at 70°C for various times. The degraded salt was precipitated with ethanol, separated, washed, and freeze-dried. The process involved various steps.

MATERIALS REQUIRED	PROPORTIONS
Distilled water	3ml
Hyaluronic acid	100mg
NaOH	0.1M
Medical liquid glue (ethyl-2-cyanoacrylate glue).	5 drops
Diamine	100mg/ml
EDC Hcl	6ml

b) Determination of molecular weight

The molecular weights of HAs were measured using size exclusion chromatography with a Waters high-performance liquid chromatography system. A column with specific dimensions was used, and absorbance was measured at 210 nm. The flow rate, mobile phase composition, and calibration curve details were provided. Lower molecular weight hAs were prepared from a commercial HA with a molecular weight of 800 kDa for nanoparticle production.

c) Synthesis of ha nano-gel

Synthesis of cross-linked hyaluronan nanoparticles HA was dissolved in water to produce 1 mg/mL solution and then adjusted to pH 5.The diamine was dissolved in water to produce 1. After the addition of water-soluble carbodiimide solution dropwise, the reaction was stirred at room temperature for 24 hr.The solution containing hyaluronan nanoparticles was purified by dialysis for 7 days against distilled water and freeze-dried.

Then create nanogels using ethyl-2-cyanoacrylate medical adhesive glue. The process involves dilution of 1% glue to 10% water, achieving the desired concentration. Then, 0.2mg and 0.4mg of the reduced concentration glue are transferred into separate petri dishes and apply over gauze swab by forming bandage

II. CHARACTERIZATION

Solubility - Solubility analysis is commonly used to study dissolution and absorbable tendency of HA Nano gel in aqueous medium. The experiment tested the solubility of Nano gel in distilled water at room temperature. Materials included Nano gel, distilled water, test tubes, petri dishes, gauze swabs, and a spatula. Two samples were prepared by placing Nano gel-applied gauze swabs into separate petri dishes and adding distilled water. The samples were left undisturbed to dissolve.

XRD - X-ray diffraction (XRD) analysis is commonly used to study the crystal structure, phase composition, and crystallinity of materials, including hyaluronic acid nano systems.

During XRD analysis, a sample is bombarded with X-rays, and the resulting diffraction pattern is recorded. This pattern provides information about the arrangement of atoms in the material and can be used to identify different phases present, assess crystallographic purity, and determine crystallite size and orientation.

III. RESULT

1. **SOLUBILITY:** It was observed from the test that the samples were left undisturbed to dissolve. The 0.2mg nanogel-applied gauze swab completely dissolved within 30 minutes, while the 0.4mg sample took 60 minutes. From the above test 0.2mg nanogel was easily soluble and it has the easily absorbable tendency.

2. **X-RAY DIFFRACTION (XRD):** The XRD was performed to identify the composition and the phase structure of the prepared materials. The obtained peak patterns for the prepared materials confirm the presence of crystalline Hyaluronic acid. The XRD patterns obtained for the Hyaluronic acid.in figure 2.1. From Fig.2.1, it was found that the prepared Hyaluronic acid were crystalline in nature with major orientation at (101) and (110**).**

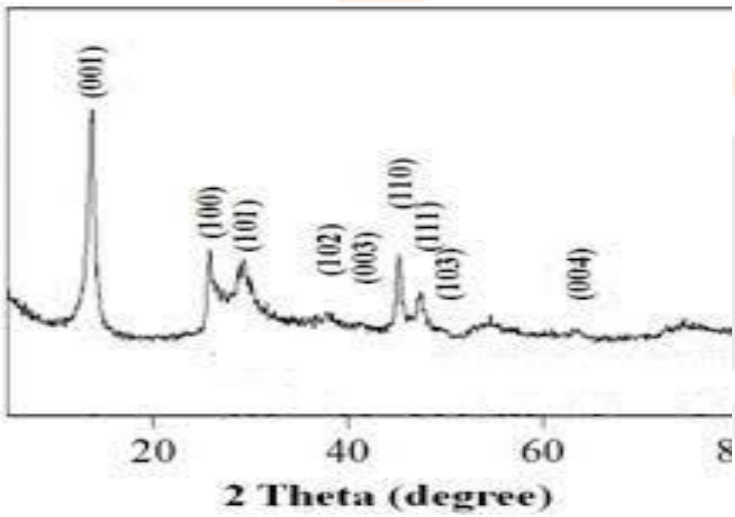


Figure 2.1: XRD pattern obtained for the prepared Hyaluronic acid powders

The % of crystallinity was found by using the following formula,

% Crystallinity = Ic/ (Ic+Ia) * 100
Where,

Ia and Ic - integrated intensities corresponding to the amorphous and crystalline phases, respectively.

Result:-The % of crystallinity was found to be 73.2.

3. FOURIER-TRANSFORM INFRARED-SPECTROSCOPY (FTIR): The FTIR was performed to confirm the pullulan and the physicochemical integrity of the pullulan composite by identifying the presence of signature functional groups. The FTIR spectrum obtained for Mg $(OH)_2$ is presented in figure 2.3. The obtained spectrum indicates the residual presence of the alcohol residues as O-H functionalization from the ethanol that was used during the synthesis process.

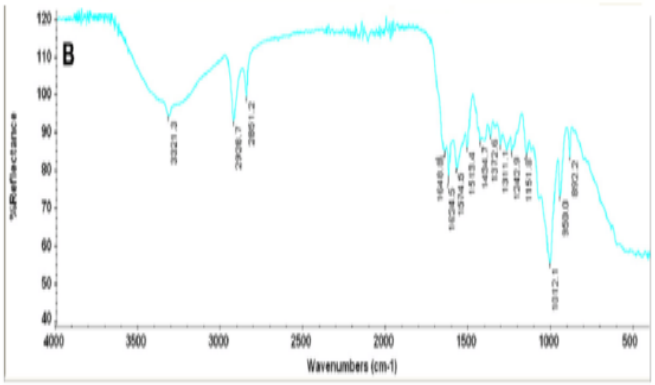


Figure 2.2: FTIR spectra obtained for the prepared Hyaluronic acid nano powders.

The FTIR spectra obtained for Hyaluronic acid nano powders is presented in figure 2.2. From fig. 2.2, A strong acetyl (-NHCOCH 3) peak was identified at 1.94 ppm along with glucosidic H at 3.05 ppm.
Peaks associated with aromatic protons of curcumin were observed at 6.67 ppm 7.12 ppm. A doublet at 7.50-7.55 ppm was also appeared for hydrogen atom adjacent to benzene ring in curcumin. Singlet peaks at 3.82 ppm was attributed to OCH 3.

IV. CONCLUSION

We have shown that nano-sized particles based on biocompatible HA has been successfully prepared by condensation reaction using 2, 2′ (ethylenedioxy) bis (ethylamine) as cross-linking agents. Transparent or opalescent stable colloid systems were fabricated in aqueous medium at room temperature. The results of characterization reveal that the Solubility, phase structure (XRD) and functional groups (FTIR) depends on molecular weight of HA and peak value.

From this XRD test we found that hyaluronic acid is crystalline in nature, in FTIR using Mg (OH) 2 strong acetyl (-NHCOCH 3) peak and solublity test it has been shown that it easily soluble and has easily absorbable tendency. Cross-linked nanoparticles have lower viscosity than the linear biopolymer due to contraction of linear chains in connection with intra chain cross-linking and entanglement coupling, and interplay between inter- and intramolecular cross-linking occurs at high biopolymer molecular weight. The low viscosity and nano-sized particles of cross-linked hyaluronic acid using carbodiimide technique could lead to development in medicinal product, and pharmaceutics.

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