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A Novel Coating of Biosynthesized Silver Nanoparticles on Orthodontic Elastomeric Ligatures

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ABSTRACT

Background: Fixed orthodontic appliances especially with orthodontic elastomeric ligatures can increase the risk for development of white spot lesions, which are often irreversible. Due to their huge surface area, silver nanoparticles are effective antimicrobials. Green synthesis employs the use of plant extracts such as Clove and Cardamom which possesses antimicrobial, antiinflammatory and antioxidant properties.

Aim: This study aims to discover a novel approach to coat orthodontic elastomeric modules or ligatures with biosynthesized silver nanoparticles using clove and cardamom as plant extract.

Materials and methods: Aqueous extract of cardamom and clove was prepared. Biosynthetic silver nanoparticles were synthesised by reacting silver nitrate ions with clove and cardamom extract (as bioreductant). Using a magnetic stirrer and a hot plate, silver nanoparticles (AgNPs) were synthesised directly on orthodontic elastomeric ligatures. Characterization of silver nanocoated elastomeric ligatures was done using Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX).

Results: At the end of third day formation of AgNPs were reported by color shifting from orange-red to dark brown. Reducing silver ions to AgNPs, as shown by UV-vis spectroscopy, generated a peak at 462 nm. SEM and EDX confirms the presence of elemental silver on silver nanocoated elastomeric ligatures.

Conclusion: An innovative coating technique incorporating magnetic stirrer along with a hot plate allows for the efficient coating of biosynthesized silver nanoparticles on orthodontic elastic ligatures. The SEM and EDX results indicate the structural integrity of the Ag nanoparticles on the biosynthetically produced orthodontic elastic ligatures is enhanced.

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INTRODUCTION

Orthodontics has evolved rapidly over the years with the significant advent of newer orthodontic materials. The development of an ideal substrate for bacterial adhesion to orthodontic materials during orthodontic treatment has remained a difficulty for the scientific community despite significant advances in orthodontic materials (1). Plaque retentive areas, where bacteria like Streptococcus mutans and Lactobacillus sp. thrive, benefit from the frequent retention of fermentable carbohydrates that are favoured by the insertion of fixed orthodontic appliances(2). Both Streptococcus mutans and Lactobacillus species increase in concentration during orthodontic therapy. White Spot Lesions (WSLs) may form around orthodontic brackets if certain bacteria induce demineralization or decalcification of tooth enamel. These lesions may proceed to dental caries if not treated.(3). WSLs are defined as "subsurface enamel porosity from carious demineralisation which appear as a milky opacity on smooth surface"(4). The prevalence has varied greatly between 2-97%(5).

One important component in dental biofilm retention is the technique used to bind orthodontic arch wires together. In the quest functional and effective orthodontic accessories, elastomeric modules have been proposed as an alternative to metallic ligatures for connecting stainless steel arch wires to brackets (6). Advantages of orthodontic elastomeric modules (OEM) over self-ligation clips include their quickness and simplicity of use, as well as the comfort and cost effective(7). The literature evaluation shows that elastomeric ligatures, despite their practical advantages, have a higher microbial count in the plaque surrounding the brackets than steel ligatures(8). Salivary proteins and biofilm quickly degrade the physical qualities of elastomeric modules in the oral cavity(9). Despite the introduction of fluoride and silver ion releasing elastomers, research has shown that the concentration of released ions is insufficient to inhibit bacterial growth (10,11).

In Dentistry, Nanotechnology has shown immense beneficial effects and is one of the most promising fields of research in the current evidence-based scenario (12,13). In the last several decades, physico-chemical and green chemistry methods have been extensively used to synthesise metallic nanoparticles (14). Green synthesis utilises high-energy renewable resources to create nanoparticles that are secure, environmentally friendly affordable, and non-toxic (15). Stable metallic nanoparticles may be synthesised by biosynthesis, which employs plant extracts with the special properties of reducing and capping agents (16). Due to their high surface-to-volume ratio, silver nanoparticles are far more effective in eradicating microbes at low concentrations than other metallic nanoparticles (17). Inhibition of WSL development by AgNPs-modified orthodontic resin against S. mutans and L. acidophilus is considerable (18). When combined with acrylic resin, they can be used to create removable dentures; when combined with composite resin, they can be used for direct restorations; when combined with irrigating solutions and obturation materials, they may be used in endodontic therapy; and when combined with a membrane, they can be utilised in guided tissue regeneration during periodontal treatment(19). Several studies have shown that the incorporation of antimicrobial silver nanoparticles into orthodontic braces, bands, wires and orthodontic adhesive materials can effectively inhibit the development of cavities and white spot lesions. Materials used in these experiments have had silver nanoparticles chemically deposited on them(20-22). Recent research has shown that silver nanocoated elastic modules outperform traditional ligatures in terms of physical characteristics(23). However, Heterotheca inuloides, a plant with minimal antimicrobial activity, was used in this research (24). Considering potent antimicrobial activity of clove and cardamom, we hypothesized that their use might be able to help reduce the prevalence of WSLs.

The plant family Mirtaceae includes the species Syzygium aromaticum (clove), which is endemic to the Maluku Islands in eastern Indonesia and is a spice that offers antibacterial, antifungal and antioxidant benefits (25). Elettaria cardamomum (Cardamom) is an aromatic perennial herb with health benefits including anti-cancer, anti-inflammatory, and antibacterial effects. Cardamom oil has been shown to have antibacterial actions against Streptococcus mutans, the bacterium responsible for most cases of oral illness (26,27).

MATERIALS AND METHODS

Plant materials

About 50 g cardamom and clove were collected, dried under ambient condition and then milled into powder using electric blender.

Preparation of clove and cardamom extracts

A total of 0.5 grammes of dried powdered clove and cardamom was combined with 50 millilitres of purified water in a heating element and boiled for 10 minutes at 80 degrees Celsius. As a consequence of the reactions, red & light-green solutions were produced.

Clove and cardamom extract solution was made, allowed to cool, and filtered using Whatman No. 1 filter paper. Thirty millilitres of each filtrate was combined and then brought to a boil for a full minute. At last an orange-red colour solution was formed. This filtrate was kept in storage before being put to use in the production of AgNPs (Figure 1).

Green synthesis of Silver (Ag) nanoparticles

A silver nitrate solution of 60 ml was made by dissolving, 1x10-2 M of the salt into 60 ml of distilled water. For three days, a combination of 60 ml of colourless silver precursor solution and 40 ml of cardamom and clove extract was stirred at 600-700 rpm on a magnetic stirrer. The mixture was also left on an orbital shaker overnight to determine if any colour changes would occur.

The gradual shift in colour was tracked over the course of three days, hour by hour. By the end of the third day, the solution had changed colour from orange-red to a dark brown. The presence of nanoparticles in the processed solution was verified using UV spectroscopy.

(Figure 2).

Characterization and purification of synthesized Silver (Ag) Nanoparticles using UV-vis spectroscopy

After the nanoparticles had been produced, characterisation of the AgNPs was carried out by taking aliquots of the solution. By the end of the third day, the solution had changed colour from orange-red to a dark brown. The presence of nanoparticles in the processed solution was verified using UV spectroscopy. After placing three millilitres of the solution inside a curette, wavelengths ranging from 300 to 700 nm were utilized to represent the findings graphically.

A Novel method of coating of orthodontic elastomeric ligatures with biosynthesized silver nanoparticles

Pretreatment of elastomeric ligatures

Injection-molded clear elastomeric ligatures (American Orthodontics, Ortho Technology, GAC). Initially elastomeric ligatures were immersed in 100ml of distilled water which was kept in ultrasonic steriliser set at 50°C for 30 minutes followed by 80 ml of isopropyl alcohol and cleaned in an ultrasonic cleaner at 40°C for 30 minutes. Then it was immersed in 100 millilitres of deionized water and placed in an ultrasonic steriliser set at 40°C for 30 minutes to rinse out or remove any residual alcohol remnants on elastomeric ligatures. Finally, it was pretreated with the main ingredient of 100 ml of 10% NaOH at 40°C for 30 minutes. Deionized water was then used to thoroughly wash the elastomeric ligatures (Figure 3).

In situ synthesis of clove and cardamom mediated silver nanoparticles on elastomeric ligatures

First, 60 ml of a silver nitrate precursor solution was mixed with 40 ml of clove and cardamom plant extract. Plant extract is used to create silver nanoparticles because of its reducing and stabilising effects. The orthodontic elastomeric ligatures were submerged in a combination of plant extract and silver nitrate precursor solution, which was contained in a 100 ml beaker. The beaker was finally left overnight on an orbital shaker at 340-350 °C, accompanied by steady stirring with a magnetic stirrer. Silver nanoparticles were synthesised and coated on elastomeric ligatures for 12 hours in the dark (to prevent photoactivation of silver nitrate).

Over the course of three days, the hue was tracked hourly. At the end of the third day, the formerly bright orange solution had become a deep brown. Using ultraviolet (UV) spectroscopy, we were able to verify that silver nanoparticles had formed in the final solution. After 12 hours of drying in an autoclave at room temperature, the orthodontic elastomeric ligatures were withdrawn from the solution (Figure 4 & 5).

Characterization of silver nanocoated elastomeric ligatures

Scanning electron microscope (SEM) observations

Scanning Electron Microscope (SEM) was performed to examine microstructural morphology of both silver nanocoated and uncoated elastomeric ligatures surface with FE-SEM, JSM-IT800, Jeol. Before subjected to SEM, both modules were sonicated with ultrasonic cleaner and ethanol to remove lose dust and debries. One elastomeric ring was isolated from both coated and uncoated modules and mounted on aluminium stubs. Mounted with carbon tape for stabilization. Sputter coating the sample using platinum sputter coating to obtain a good-quality SEM image. Observations were performed at 3.0 kV and at a working distance of 5 μm with image capturing magnification of × 2.70 K. ImageJ Software was employed for image analysis.

Energy dispersive x-ray spectroscopy (EDX) observations

Coated and uncoated elastomeric ligatures encountered qualitative elemental microanalysis of their surfaces using the same microscope and experimental settings. EDX analysis was performed for elemental and chemical analysis to confirm the presence of silver nanoparticles by Oxford XPLORE 30. Surfaces of both ligatures were analysed using EDX spectrum to assess the composition of their molecules in terms of mass (Wt) and atomic (At) percentage.

RESULTS AND DISCUSSION

Research into the causes of enamel decalcification and the best ways to prevent it during orthodontic treatment has exploded in recent years (28). In an effort to lower the prevalence of WSL among orthodontic patients, several non-compliance techniques have been devised, although with mixed results. There have been issues with the aesthetics of the material (29), the release of the active drug (30), and the effectiveness in preventing the occurrence of WSLs (31), among other things. Therefore, using nanotechnology to integrate antimicrobial elements like silver is essential. Plant extracts have been shown to catalyse the reduction of metal ions into metal nanoparticles, which might allow for the environmentally friendly manufacture of silver nanoparticles (32). A number of reports have demonstrated that silver metal ions can be bioreduced into silver nanoparticles, but this is the first research to use a unique plant extract, in this case clove and cardamom. Our research is focused on how using biosynthetic silver nanocoated elastomeric ligatures might lessen the occurrence of white spot lesions during orthodontic therapy.

Green synthesis of AgNPs: When added to the orange-red clove and cardamom extract, the silver precursor solution changed from clear to a dark brown.The formation of AgNPs was mostly extrapolated from the color changes in the reaction mixture (Figures 1 and 2 Researchers have shown that plant extracts may speed up the process of reducing metal ions into nanoparticles. Clove and cardamom extracts include secondary metabolites and phytochemicals that may be used as reducing agents in green synthesis. Here, the oxygen released during the decomposition of phytochemicals serves as a bridge between the reduced metal ions. Plants rely on these phytochemicals as stabilisers to keep their components from sticking together. The brown colour is formed due to the excitation of surface plasmon vibrations, and this colour change is used as a marker for the completion of AgNPs. This was the first study employing a new plant extract, comprised of clove and cardamom, to demonstrate bio reduction of silver metal ions to silver nanoparticles.

Characterization of biosynthesized silver nanoparticleS by UV-vis spectroscopy

The change in colour of silver nitrate from an orange-red colour to a dark brown colour provides a visible confirmation of the bio reduction of silver nitrate to AgNPs. Biosynthesized silver nanoparticle development was tracked using UV-vis spectroscopic spectroscopy. Surface plasmon resonance (SPR) absorption peaked strongly and broadly at 462 nm in the visible region of the spectrum. This peak suggests that silver salts may have been converted into AgNPs by reduction (Figure 6). Several more studies using other plant extracts support the results of these investigations (33,34).

An innovative coating of orthodontic elastomeric ligatures

Polyurethane, the thermosetting polymers used to make the elastic ligatures, is composed of a -(NH)-(C=O)-O- functional unit and is generated by step reaction polymerization. Polyurethane elastomers go through a multi-step production process. These polymers consist of relatively short, hard building blocks (aromatic rings & urea), relatively short, flexible hinges and relatively long, extremely flexible building blocks (polyether), the length of which may be adjusted (9). In order to bind AgNPs, these functional groups serve as ties.

Hydrolysis of the urethane groups on the surface may be induced by a pretreatment with NaOH. The H groups in polyurethane elastomers may take on two roles: either as a donor via the HN group or as a receptor through the C = O group. Because of the correlation between greater sensitivity and the use of 70% alcohol, it is possible that this occurrence is linked to cosmetic alterations. Studies on the elastic modules following treatments with isopropyl alcohol and sodium hydroxide, as well as the incorporation of silver nanoparticles, show that these variations do not compromise the antibacterial properties or the physical properties of AgNPs(10). The modules serve as the substrate for the Ag-Nps, which were synthesised directly on the modules and are strongly attracted to the substrate due to the Van Der Walls connections between the positively charged nanoparticles(23).

Characterization of silver nanocoated elastomeric ligatures

Scanning electron microscope (SEM) observations

Since the techniques used were very sensitive to surface contamination, the samples had to be prepared beforehand in order to eliminate the possibility of measurement mistake. We employed scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX) to get an extensive look at the surface condition and chemical composition of both untreated and silver nanocoated elastomeric ligatures. Several investigations have taken use of the high resolution chemical analysis made possible by the convergence of these approaches and the small operating distance necessary for "slice-and-view" sectioning (35,36).

According to scanning electron microscopy (SEM), uncoated translucent ligatures have the form of a bevelled ring with regularly sharp edges. The otherwise flawless surface has irregularities like tiny grains and striations here and there. Small particles are evident, suggesting that they are defects arising from the manufacturing process. Surface cracking is more pronounced in uncoated elastomeric ligatures with more surface deterioration compared to silver nanocoated elastomeric ligatures. These flaws may encourage the growth of plaque and the deposition of calculus, both of which have detrimental consequences on the supporting systems of the teeth and gums. Pathogens that induce WSLs may thrive in the tiny crevices created by calculus accumulation. Because of this, it's possible that its mechanical strength, elasticity, and force degradation would all be impacted.

SEM revealed that silver nanocoated elastomeric brown colored ligatures have the shape of bevelled ring with small irregular border on the external surface of ligatures. Surface revealed inhomogeneous coating of Ag with silver grains are much more evident on the surface of silver nanocoated elastomeric ligatures (Figure 7).

Silver nanocoated elastomeric ligatures have silver granules that are more visible on the surface, which may prevent biofilm buildup and, in turn, lead to lower plaque and calculus deposition. The chemical composition and surface reactive properties of the outer monolayer of a biofilm greatly influence its bioadhesive ability. The surface's ultrastructure flaws, gas entrapment, and adsorbate layers might affect the film's characteristics. The biofilm that forms on the coated elastomeric modules becomes less cohesive than on uncoated surfaces, making it easier to peel off in the mouth. Therefore, research provides support for the use of nanosilver-coated elastomeric ligatures as an effective, non-compliant technique of avoiding WSLs.

Energy dispersive x-ray spectroscopy (EDX) observations

Using EDX analysis, we found that the chemical composition of uncoated and silver nanocoated elastomeric ligatures differed significantly. Silver spectrum spike noted on coated ligatures confirm the presence of silver element on the coated ligatures.

The presence of carbon (C), oxygen (O), and copper (Cu) on uncoated elastomeric ligature was determined by EDX spectroscopic qualitative point analysis. The spectra show peaks of carbon, oxygen and copper in the order of high to low.

EDX spectroscopy confirmed the presence of silver (Ag) on the nano silver coated elastomeric ligatures. The presence of other elements as in uncoated ligatures such as carbon (C), oxygen(O) and copper (Cu) are also noted. The spectra show peaks of carbon, oxygen, silver and copper in the order of high to low (Figure 8). The EDX investigation confirmed that the coating procedure was the source for the silver peak in the spectrum of the coated layer.

CONCLUSION

An innovative coating technique incorporating magnetic stirrer along with a hot plate allows for the efficient coating of biosynthesized silver nanoparticles on orthodontic elastic ligatures. The SEM and EDX results indicate the structural integrity of the Ag nanoparticles on biosynthetically developed silver nanocoated orthodontic elastomeric ligatures is enhanced. Biosynthetically developed silver nanocoated elastomeric ligatures displayed to have a high potential in minimizing white spot lesions caused by orthodontic procedures.

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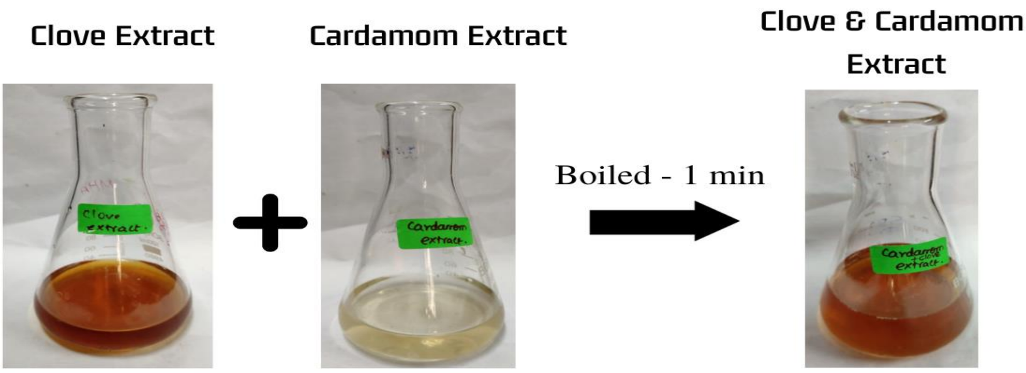

Fig.1: Preparation of plant extract: 0.5 g of dried clove and cardamom powder was mixed with 50 ml of distilled water and heated at 60-80 degrees for 5-10 minutes to make red and light green solutions. Filtration was done using Whatman filter paper no.1. Each filtrate of 30 ml was mixed together and boiled for 1 min and filtrate was stored.

Fig.2: Preparation of Silver (Ag) nanoparticles and visual observation: 60 ml of cardamom and clove extract were added to 40 ml of Silver precursor solution and mixture was continuously stirred using magnetic stirrer at 600-700 rpm and kept overnight on an orbital shaker till color change was observed from orange-red color had transformed into a dark brown color indicating formation of Ag nanoparticles.

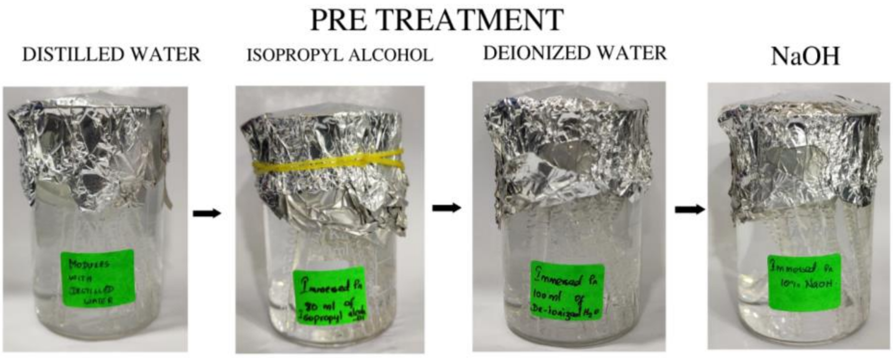

Fig.3: Pretreatment of elastomeric ligatures

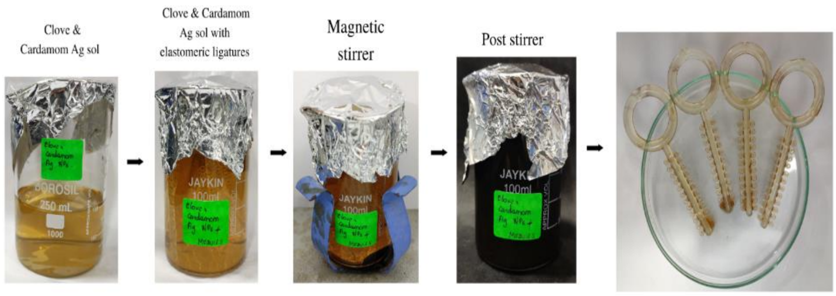

Fig.4: In situ synthesis of silver nanoparticles on elastomeric ligatures

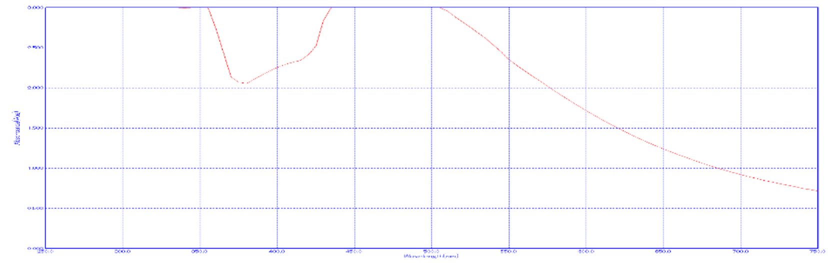

Fig.5: UV-vis spectroscopy of insitu synthesized silver nanoparticle: SPR peak at 450-460nm

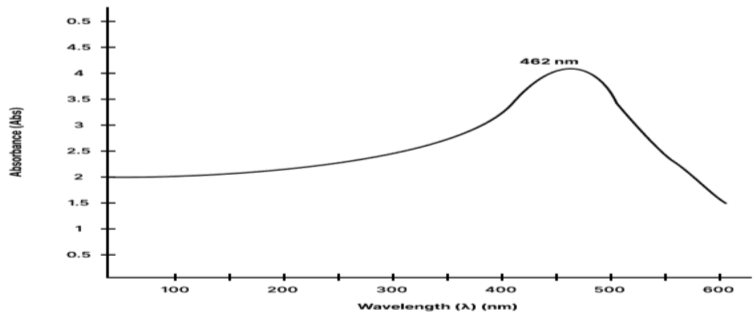

Fig.6: Characterization of AgNPs using UV-visible spectroscopy: SPR peak at 462 nm.

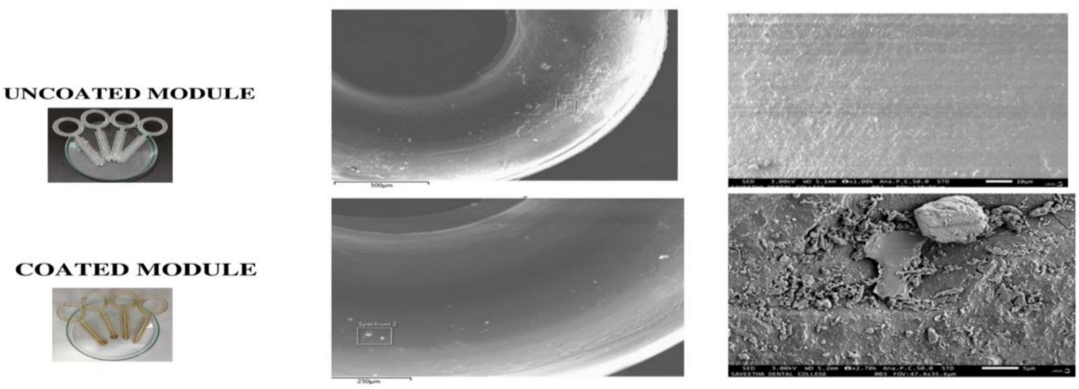

Fig.7: SEM observation of Coated vs Uncoated elastomeric ligatures- Surface cracking is more pronounced in uncoated elastomeric ligatures compared to silver nanocoated elastomeric ligatures. Surface of silver nanocoated elastomeric ligatures revealed inhomogeneous coating of Ag with silver grains are much more evident on the surface of silver nanocoated elastomeric ligatures.

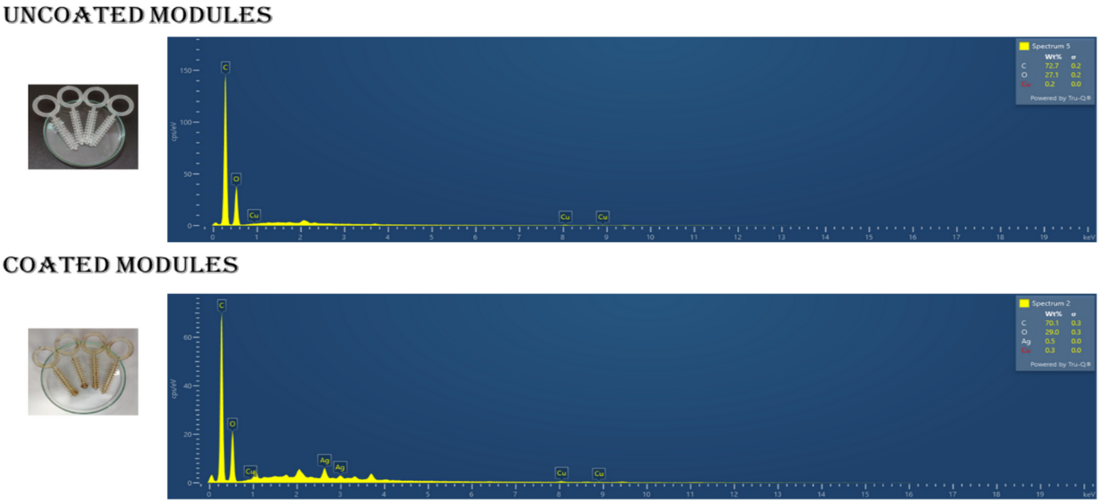

Fig.8: EDX observation of Coated vs Uncoated elastomeric ligatures- EDX spectroscopy revealed that presence of carbon (C), oxygen(O) and copper (Cu) on uncoated elastomeric ligature. Presence of silver (Ag) on the nano silver coated elastomeric ligatures is observed on silver nanocoated elastomeric modules. This suggests the silver peak in the coated-layer spectrum indeed originated from the coating process.