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Green Synthesis of Strontium Fluorapatite Nanoparticles Using Extracts of Equisetum Arvense and Laminariales

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ABSTRACT

Introduction: White spot lesions (WSL) are a common consequence of orthodontic treatment. Remineralization of these WSLs can take place when calcium and phosphate ions are supplied from an external source to the tooth to promote ion deposition into crystal voids in demineralized enamel, producing a net mineral gain.

Materials And Method: Strontium fluorapatite was synthesised by green synthesis of Equisetum arvense and Laminariales. Extracts were mixed with hydroxyapatite and strontium chloride to produce strontium and hydroxyapatite nanoparticles (NPs). UV-vis spectroscopy was used to detect the formation of NPs. Post spectroscopic analysis, the two mixtures were then added together and 0.090g of fluorine was added. FTIR spectroscopy and SEM-EDX analysis for surface characterisation and elemental analysis of the NPs were performed.

Result: Colour change was observed after stirring the mixture on a magnetic stirrer indicating the formation of NPs. UV – Vis spectroscopic analysis showed sharp peaks at 375 nm wavelength which corresponds to the surface plasmon resonance band of the strontium fluorapatite NPs. FTIR spectroscopy showed peaks resonating with the different elements of the NPs. The SEM-EDX analysis shows rod-like particles depicting the presence of strontium. Some spherical shaped agglomerates corresponded to the hydroxyapatite NPs. The composition obtained from EDAX analysis was Strontium (7.6 Wt%), Fluorine (5.4 Wt%), Calcium (9.4 Wt%) and Phosphorus (4.2 Wt%).

Conclusion: Within the limitations of this study, we can conclude that strontium fluorapatite NPs can be effectively produced by green synthesis using plant extracts of Equisetum arvense and Laminariales. Future scope for the study includes investigating the biocompatibility of the NPs to be incorporated into consumer products which would prove to be beneficial in remineralising white spot lesions.

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INTRODUCTION

Dental caries is a multifactorial infectious disease of the teeth characterised by demineralization and cavitation of the hard tissue. (1)(2) When the pH is less than 5.5 which is the critical pH, the tooth mineral acts as buffer and loses calcium and phosphate ions into the oral cavity, leading to the formation of White spot lesions (WSL). White spot lesions frequently occur as a consequence of orthodontic treatment.(3) Remineralization of these WSL can take place when calcium and phosphate ions are supplied from an external source to the tooth to promote ion deposition into crystal voids in demineralized enamel, producing a net mineral gain.(4)

Strontium fluorapatite, green synthesis, nanoparticle, horsetail, kelp, white spot lesions

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Synthetic hydroxyapatite (HA) holds exceptional bioactive characteristics such as osteoconductivity and cytocompatibility due to its maximum correlation to the mineral portion of bone tissue. (5) Nevertheless, synthetic HA which is stoichiometric, decomposes into distinct phases at high sintering temperatures which makes it unstable. HA nanoparticles however, have ultrafine structure and high surface reactivity similar to the mineral found in bones. In addition, demineralization may be hindered when particle size decreases to nano-size level.

The non-stoichiometric HA can be synthesised with incorporation of various ionic substitutions into the crystal lattice such as strontium (Sr) and fluorine (F), which can result in improvement of biomaterial characteristics, such as the crystallinity and dissolution rate. Fluorine is a substantial trace element among hard tissues, which can advance HA crystallisation and mineralization in the bone formation process, and further increases the differentiation and proliferation of osteoblasts and consequently induces bone regeneration. Strontium is one of the most significant cations in hard tissues, stimulating cell growth and preventing bone resorption as well as inhibiting osteoporosis. Thus, it is rational to conclude that incorporation of fluorine and strontium into HA could be desirable for the advancement of the bioactivity. (6)

Efforts are being made to develop environmentally friendly synthesis techniques to fabricate nanoparticles. (7) Techniques of green synthesis are achieved through bio-organisms, plants , algae, fungi and bacteria (8). Cost efficiency with low risks of toxicity and minimization of waste are the important advantages of green synthesis.(9)

The plant extracts of Equisetum arvense (Horsetail) and Laminarales (Kelp) have been used in this study for the green synthesis of strontium fluorapatite nanoparticles. Horsetail contains a special form of absorbable silica that strengthens and restores teeth, bones, connective tissues, hair, and nails.(10) Kelp is good for the teeth as it contains enzymes that protect against tooth decay by stripping bacteria away from plaque. They also possess a high potential for pharmaceutical and nutraceutical applications and are also rich in meroterpenoids that have antioxidant properties. (11) Hence, the aim of this study was to employ green synthesis method for production of strontium and fluorine nanoparticles to form strontium fluorapatite nanocomposite to help in remineralisation of white spot lesions occurring after orthodontic treatment.

MATERIALS AND METHODS

Study setting

This was an in vitro study conducted between March 2022 to April 2022 on a laboratory platform in Gold Lab, Saveetha Dental College and Hospital, Chennai, Tamil Nadu. Ethical clearance was obtained from the institutional review board prior to the commencement of this study.

Preparation of extract

Strontium fluorapatite was synthesised by a novel green synthesis method. Leaf extracts of Equisetum arvense and Laminariales were purchased from a local herbal garden. All the chemicals used in the production were of analytical grade and are of above 99% purity. 2.4gm of dried horsetail leaves and 2.4gm of dried kelp was weighed on a digital balance and mixed thoroughly with 100 ml of distilled water in a 250-ml beaker. This solution was heated at 50 - 60 ºC until vapours were seen emerging from the beaker kept in a heating mantle. The extract obtained was cooled and purification of solution was done by filtration using a Whatman filter paper no.1. Residue collected in the filter paper was discarded and the supernatant was collected in a conical flask. The filtrate was collected in a 250-ml Erlenmeyer flask and stored in a refrigerator for further use.

Preparation of hydroxyapatite nanoparticles

0.2g of hydroxyapatite powder was mixed with 30ml of the leaves extract and kept overnight on an orbital shaker for homogenous mixing of all particles. The reaction mixture was stirred continuously on a REMI 2MLH magnetic stirrer set at 600 RPM for 72 hours and was monitored for colour change.

Preparation of strontium nanoparticles

0.525 of strontium chloride was mixed with 30ml of the leaves extract and kept overnight on an orbital shaker for homogenous mixing of all particles. The reaction mixture was stirred continuously on a REMI 2MLH magnetic stirrer set at 600 RPM for 72 hours and was monitored for colour change.

The UV - vis spectroscopy was then used to determine the rate of absorption of light and thereby detect the formation of NPs. The scanning range of the samples was between 250 to 650nm. All UV -Vis absorption spectra were read against distilled water. The UV - vis spectroscopy was first switched on and kept for 20 minutes to let the lamp heat sufficiently. A 5ml sample of both the HApNps and SrNps was taken and kept in the first chamber. A standard control was kept on the other chamber to act as the control, to compare the formation of NPs. At 1 hour, 5 hour and 24 hour intervals, the particulates were monitored using UV spectroscopy to see when the NPs were formed. When the graph reading reaches a sharp peak, it is indicative of NPs formation. Colour change of HApNPs and SrNps indicated its formation at a certain wavelength that was measured on a UV – vis spectrophotometer.

Post spectroscopic analysis, the two mixtures were then added together. To this mixture. 0.090g of fluorine was added. The mixture was collected in 2 test tubes and the precipitate was separated from the reaction solution by centrifugation at 8000 rpm at 60 °C for 10 min and pellets were collected. Pellets were dried using a hot air oven operating at 80 °C for 2 h and preserved in air-tight bottles for further studies.

Fournier Transform Infrared spectroscopy

FTIR is a very versatile tool for surface characterization of nanoparticles. FTIR spectroscopy is a technique used to identify the characteristic functional groups from the spectral bands that allow us to know the conjugation between the nanomaterial and the adsorbed biomolecules.(12) Fournier transform infrared spectroscopy was done using Alpha Bruker's ll FT-IR in the range of 400-4000cm-1.

Scanning electron microscopy-energy dispersive X-ray analysis

SEM-EDX provides a quick nondestructive determination of the elemental composition of the sample. A thin layer of the centrifuged pellet containing Sr-FApNPs is applied on an aluminium foil and autoclaved at 60 °C for 10 mins. The foil is then sent for surface characterization and elemental analysis using SEM-EDX.

RESULTS

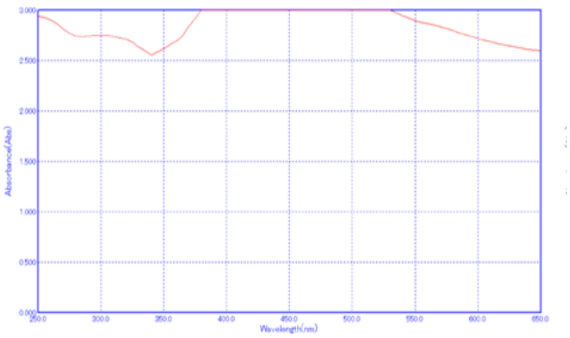
Visual observation of nanoparticles

Colour change was observed after stirring SrNps and HApNp's continuously on a magnetic stirrer. The change in colour from pale brown to dark brown indicated the formation of strontium and hydroxyapatite nanoparticles. Colour change was also observed after fluorine was added to the mixture to form strontium fluorapatite nanoparticles.

Figure 1: Visual observation showing the colour change from pale brown to dark brown indicating the formation of strontium and hydroxyapatite nanoparticles

UV-vis spectroscopy

UV – Vis spectroscopic analysis was used initially to monitor the formation of SrNp's and HApNps at 0 hours, 1 hour, 5 hours and 24 hours intervals. Sharp peaks were seen at 420 nm and 410 nm wavelengths, which corresponded to the surface plasmon resonance (SPR) band of the Strontium nanoparticles and hydroxyapatite nanoparticles respectively. UV – Vis spectroscopic analysis was then used later to monitor the formation of Sr-FApNps at 1 hour, 5 hours and 24 hours intervals as well. A sharp peak was seen at 375nm wavelength which correspond to the surface plasmon resonance band of the Strontium fluorapatite nanoparticles. This confirmed the formation of the nanoparticles.



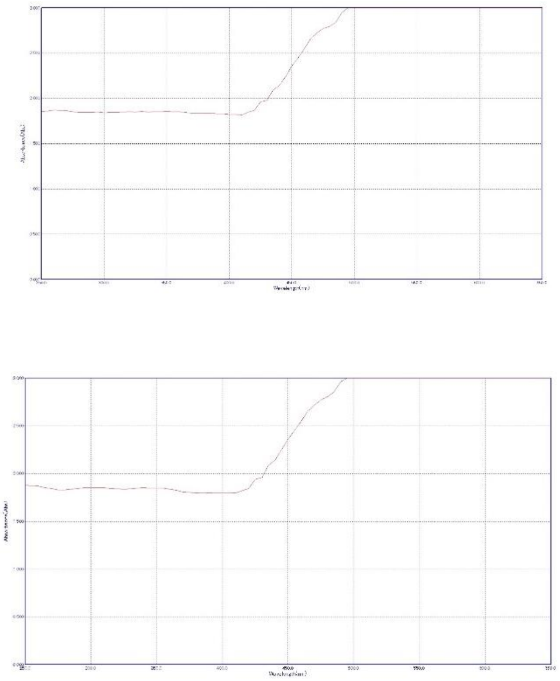


Figure 2: UV – Vis spectroscopic analysis showing sharp peak at 375 nm wavelength.

Fournier Transform Infrared spectroscopy

FT-IR spectra and functional groups involved in Sr-FAp NP synthesis illustrated peaks in the range of 500 – 4000cm−1 which falls under the mid-infrared spectrum. The broad peak at 3328.88 corresponds to polymeric OH stretch, NH stretch of aliphatic primary amino and imino compounds. The small peak at 1612.2o corresponds to NH bends of primary and secondary amines and open chain imino and azo groups. A weak peak at 1452.37 corresponds to methyl and methylene C-H bends and carboxylic acid salts. A sharp peak at 1028.13 demonstrates cyclohexane ring and skeletal C-C vibrations, aromatic C-h bend, aliphatic fluoro compounds and phosphates. Small peak at 601.37 denotes aliphatic bromo compounds and disulphides and a small peak at 562.11 denotes aliphatic iodo compounds.

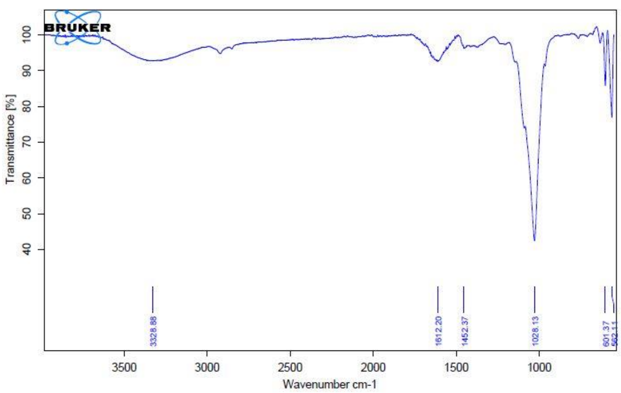


Figure 3: FTIR analysis

Scanning electron microscopy-energy dispersive X-ray analysis

The analysis shows rod-like particles ranging from 10-40 nm in size. The rod-like particles depict metallic elements. This shows the presence of strontium. Some spherical shaped nanoparticles ranging from 5-25 nm in size were observed in SEM analysis. The spherical agglomerates correspond to the hydroxyapatite nanoparticles.

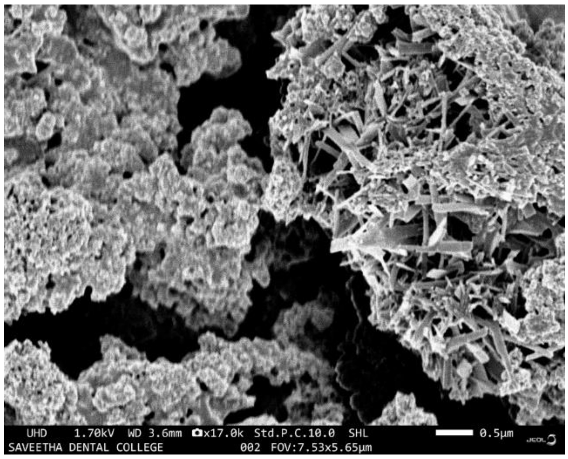


Figure 4: SEM analysis showing sharp rod like metallic elements

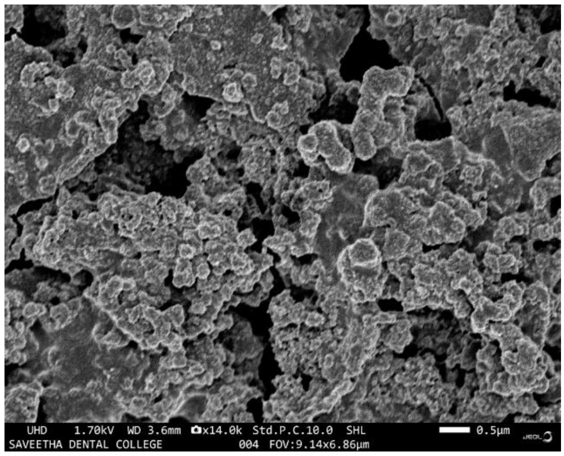


Figure 5: SEM analysis showing spherical agglomerates resembling HA.

The composition obtained from EDAX analysis was Strontium (7.0 Wt%), Fluorine (5.4 Wt%), Calcium (9.4 Wt%) and Phosphorus (4.2 Wt%). Traces of Oxygen (25.3 Wt%) and Carbon (40.4 Wt%) seen. The presence of carbon in trace amounts indicates the involvement of plant phytochemical groups in reduction and capping of the synthesised Sr-FApNps.

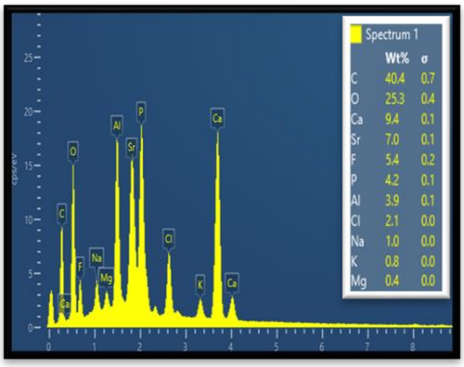


Figure 6 : Elemental analysis of the nanoparticle

DISCUSSION

The present study involved using extracts of Horsetail and Kelp and hydroxyapatite precursors for the synthesis of Sr-FApNps. This novel formulation of nanoparticles is the first of its kind and has not been reported in previous literature. Visual examination showed a colour change from pale brown to dark brown, which was indicative of the formation of the nanoparticles. This was confirmed by means of a UV -Vis Spectroscopy. SEM analysis revealed rod-like structures ranging between 10-40 nm showing presence of Strontium and spherical shaped structures which were ranging between 5-25nm in size indicating hydroxyapatite agglomerates. This was in accordance with other studies which saw similar spherical shaped nanoparticles ranging within the size ranges of our observed result. The elemental analysis showed increased levels of strontium, calcium and phosphate particles with fluorine. Both FTIR and SEM EDX analyses show development of Strontium fluorapatite nanoparticles.

Green synthesis has gained wide recognition as a clean synthesis technique in recent years. Most of these conventional physical and chemical nanoparticle synthesis techniques result in the usage of toxic chemicals and are expensive. Efforts are being made to develop environmentally friendly synthesis techniques to fabricate nanoparticles. Basic principles of "green synthesis" include prevention or minimization of waste, reduction of pollution, and the use of safer (or non-toxic) solvents as well as renewable raw materials. Among the available green methods of synthesis for metal/metal oxide nanoparticles, utilisation of plant extracts is a rather simple and easy process to produce nanoparticles at large scale relative to bacteria and/or fungi mediated synthesis. These products are known collectively as biogenic nanoparticles. (13) Effective phytochemicals in various plant extracts act as reducing or capping agents and are capable of reducing metal salts into metal nanoparticles. Green synthesis is eco friendly as toxic chemicals are not used and there is an overall reduction in the cost of production.

Smaller sized nanoparticles have an extended half-life period, longer circulation time and carry increased drug concentration.(14) Nanoparticles have accelerated adsorption rate due to their high surface to volume ratio. Their non-toxic properties make them excellent vehicles in drug delivery systems. They also eradicate the possibility of developing antibiotic tolerance. Ultra-small size (1-100 nm) and large surface area-to-mass are major advantages of using Sr-FApNps. The biological advantages that the Horsetail and Kelp extract possesses, can be effectively incorporated into the nanoparticles.

The developed strontium fluorapatite nanoparticles possess properties of fluorine and hydroxyapatite. If proved to be biocompatible possessing remineralising properties, it can be advantageous in remineralising white spot lesions which are common unfavourable consequences of fixed orthodontic appliances. Further development could lead to restoration of lost enamel surfaces. (2)

CONCLUSION

Within the limitations of this study, we can conclude that strontium fluorapatite can be effectively produced by green synthesis using plant extracts of Equisetum arvense and Laminariales. The strontium fluorapatite nanoparticles were synthesised without utilising any toxic chemical as a reducing agent. Future scope for the study includes investigating the biocompatibility of the nanoparticles to be incorporated into consumer products. Such products would prove to be beneficial in remineralising white spot lesions which is a common unfavourable outcome of fixed orthodontic appliances.

REFERENCES

1. Elkassas D, Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. J Dent. 2014 Apr;42(4):466–74.
2. Sari M, Ramadhanti DM, Amalina R, Chotimah, Ana ID, Yusuf Y. Development of a hydroxyapatite nanoparticle-based gel for enamel remineralization -A physicochemical properties and cell viability assay analysis. Dent Mater J. 2022 Feb 1;41(1):68–77.
3. Willmot D. White Spot Lesions After Orthodontic Treatment. Semin Orthod. 2008 Sep 1;14(3):209–19.
4. Lei J, Guo J, Fu D, Wang Y, Du X, Zhou L, et al. Influence of three remineralization materials on physicochemical structure of demineralized enamel. Journal of Wuhan University of Technology-Mater Sci Ed. 2014 Apr 16;29(2):410–6.
5. Darroudi M, Hossein EH, Housaindokht MR, Youssefi A. Synthesis of nano-fluorohydroxyapatite thin films by sol-gel method [Internet]. Vol. 29, Malaysian Journal of Science. 2010. p. 252–61. Available from: http://dx.doi.org/10.22452/mjs.vol29no3.9
6. Synthesis and characterization of strontium fluor-hydroxyapatite nanoparticles for dental applications. Microchem J. 2020 Mar 1;153:104485.
7. Hubenthal F. Noble Metal Nanoparticles: Synthesis and Optical Properties [Internet]. Comprehensive Nanoscience and Technology. 2011. p. 375–435. Available from: http://dx.doi.org/10.1016/b978-0-12-374396-1.00034-9
8. Ghodake GS, Deshpande NG, Lee YP, Jin ES. Pear fruit extract-assisted room-temperature biosynthesis of gold nanoplates [Internet]. Vol. 75, Colloids and Surfaces B: Biointerfaces. 2010. p. 584–9. Available from: http://dx.doi.org/10.1016/j.colsurfb.2009.09.040
9. Sanghi R, Verma P. Biomimetic synthesis and characterisation

of protein capped silver nanoparticles. Bioresour Technol. 2009 Jan;100(1):501–4.

10. Boeing T, Tafarelo Moreno KG, Gasparotto Junior A, Mota da Silva L, de Souza P. Phytochemistry and Pharmacology of the Genus (Equisetaceae): A Narrative Review of the Species with Therapeutic Potential for Kidney Diseases. Evid Based Complement Alternat Med. 2021 Mar 5;2021:6658434.
11. Azam MS, Choi J, Lee MS, Kim HR. Hypopigmenting Effects of Brown Algae-Derived Phytochemicals: A Review on Molecular Mechanisms. Mar Drugs. 2017 Sep 24;15(10):297.
12. Metal and metal oxide nanoparticles: An integrated perspective of the green synthesis methods by natural products and waste valorization: applications and challenges. In: Comprehensive Analytical Chemistry. Elsevier; 2021. p. 433–69.
13. Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P. “Green” synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnology. 2018 Oct 30;16(1):1–24.
14. Shukla AK, Iravani S. Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier; 2018. 548 p.