Characterisation and Assessment of Antimicrobial Activity of a Novel Nano Bioactive Glass-Based Intracanal Medicament using Fourier Transform Infrared Spectroscopy: An In Vitro Study

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ABSTRACT
Introduction: Calcium hydroxide is considered as one of the first preferences in multiple-visit root canal medication. Bioglass, which is composed of 45% SiO2 , 24% Na2 O, 24.5% CaO and 6% P2O5, developed in the year 1971 as a novel material with antimicrobial properties. Antimicrobial nanoparticles, due to their unique physio-chemical properties, show effects opposing resistant pathogens in pharmaceutical science.
Aim: The aim of the present study was to characterize a novel nano bioactive glass (BAG-np) based intracanal medicament and assess its antimicrobial activity.
Methodology: To analyse the functional groups, the synthesised nano bioactive glass and calcium hydroxide were subjected to Fourier Transform Infrared Spectroscopy (FTIR).With the resolution of 4 cm^{-1} the FTIR spectra were recorded from 400 to 4400 cm^{-1}. The antimicrobial activity of BAG-np, calcium hydroxide, and TAP was evaluated using agar medium to find the lowest inhibitory concentration, use the diffusion and broth dilution techniques against *E. faecalis* and *C. albicans*.
Results: The FTIR spectrum of synthesised BAG-np demonstrated peaks at 509.99, 552.14, 665.14, 697.42, 785.40, 866.09, 1007.77, 1257.8, 1409.72, 2904.50 and 2962.54 cm^{-1} and The FTIR spectrum of calcium hydroxide demonstrated peaks at 3461.32, 2867.50, 1644.08, 1460.36, 1347.95, 1285.14, 1246.20, 1191.86, 1096.15, 943.73, 882.43, 842.05, 636.84, 608.05 and 530.00 cm^{-1}.The NBG-based intracanal medicament exhibited presence of antimicrobial activity against both *E. faecalis* and *C. albicans*. The MIC indicated that the novel medicament was effective at lower concentrations. The zone of inhibition tests corroborated these findings, showing clear zones of microbial growth inhibition.
Conclusion: The study demonstrated the presence of silica and phosphate bonds as well as the existence of oxygen groups that do not bridge in the synthesised bioactive glass nanoparticles and the presence of hydroxyl groups and calcium ions in calcium hydroxide. Nano bioactive glass (NBAG) demonstrated superior antimicrobial efficacy against the *Enterococcus faecalis* and *Candida albicans* microbial colonies, outperforming calcium hydroxide and rivaling triple antibiotic paste (TAP). Unlike TAP, NBAG avoids drawbacks like discoloration and antimicrobial resistance while promoting periapical healing and tissue regeneration.

Key Words: Bioactive glass, Calcium Hydroxide, Intracanal Medicament

INTRODUCTION

The predictability of success of endodontic therapy relies on understanding the endodontic pathology and the ability to combat the same. Endodontic infections are multi-microbial in nature and predominantly dominated by obligate anaerobic bacteria when it is a primary endodontic infection (Narayanan & Vaishnavi, 2010). Microorganisms that are resistant to antimicrobial treatment and survive the biomechanical preparation of canals include a span of gram negative anaerobic rods, gram positive bacteria and sometimes yeasts in small amounts (Narayanan & Vaishnavi, 2010). Among these, the ones that have been repeatedly identified in failed root canal cases are E. faecalis and C. albicans (Kumar et al., 2015; Love, 2001). Microorganisms have a tendency to form microbial communities by adhering to surfaces, thus forming biofilms. An endodontic infection consists of a loose collection of filaments, rods, cocci and spirochetes suspended in moist canals spaces as well as dense aggregates that stick along the canal walls and form thick layers of bacterial condensation. The inter-bacterial spaces contain extracellular matrices of bacterial origin. Biofilms afford the resident microorganisms protection and resistance against exogenous influences such as antimicrobial agents (Svensäter & Bergenholtz, 2004). It has been well established that pulpal disease and endodontic failures are a result of endodontic microflora which justifies the need for effective biomechanical procedures for their eradication (Gomes et al., 1996). Single visit endodontic treatment involves the use of hand and rotary instruments for the mechanical removal of infected debris along with the use of bactericidal solutions such as sodium hypochlorite for chemical disinfection. Intra canal medicaments are commonly used in multi visit endodontic therapy. Calcium hydroxide is thought to be among the initial options for a root canal treatment that requires several visits (Kawashima et al., 2009). The emergence of resistant bacterial species is one issue pertaining to the effectiveness of the irrigants and medications that are currently in the market. Antimicrobial nanoparticles, due to their unique physio-chemical properties, show effects against pathogens that are resistant in pharmaceutical science. Several in vitro studies have evaluated the efficacy of using nanoparticles as a treatment modality against endodontic pathogens. Bioglass was developed in the year 1971 as a new material with antimicrobial properties that could also bond to bone structures. It consisted of 45% SiO2 , 24% Na2 O, 24.5% CaO and 6% P2O5. The mechanism of action of bioactive glass involves, release of ions when its in contact with aqueous environment, increasing the neighbouring pH, Calcium and phosphate ions precipitate in the bacterial cell membrane, disrupting its functions and inhibiting bacterial development as the osmotic pressure surrounding the bacterial cell rises.(Ibrahim et al., n.d.). The aim of this present study was to study the characterization of the novel nano bioactive glass based intracanal medicament and calcium hydroxide using Fourier Transform Infrared Spectroscopy (FTIR) and assess its antimicrobial activity using disc diffusion method.

MATERIALS AND METHOD

Sample preparation

Standard bioactive glass 45S5 (Perioglass™ US Biomaterials Corp., Alachua, FL, USA) was obtained from a commercial source. According to the manufacturer, this material includes 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5. Bioactive glass nano powders (BAG-np) were prepared utilising the sol gel technique. Silicon and phosphorus alkoxides, sodium salt (sodium hydroxide), and calcium salt (calcium hydroxide) were used to prepare the oxide composition. Ethyl alcohol and deionized water were employed as solvents. After preparing the gel at 70 degrees Celsius and pH 2, it was aged for a week to finish the reaction before being heated to 800 degrees Celsius. FTIR was used to analyze the particles.

Calcium hydroxide (98% extra pure, ACROS Organics™, Fisher Scientific) was sourced for this study, Using a macrogol-propylene glycol base, ciprofloxacin, metronidazole, and minocycline were combined in a 1:1:1 ratio to create triple antibiotic paste (TAP).

Characterization of BAG-Nanoparticles and Calcium Hydroxide-

The synthesised BAG-np and calcium hydroxide 98% extra pure ACROS Organics™ (Fisher Scientific) were subjected to FTIR analysis.The purpose of the investigation was to determine which functional groups were present in the synthesized nanoparticles. For FTIR measurement, the nanoparticles were compacted into a pellet after being combined with potassium bromide (KBr). With a resolution of 4 cm−1, the FTIR spectra were captured between 400 and 4400 cm−1. To eliminate any adsorbed molecules, the powders were dried overnight at 100°C prior to measurements.

Antimicrobial activity assessment-

The disc diffusion method was employed to ascertain the antibacterial activity of three extracts and a negative control (saline). The bacterial cultures of E. faecalis and C. albicans tested were spread over Mueller Hinton agar (MHA) (Hi Media, Mumbai, India) using a swab moistened with the bacterial suspension. Eventually, a 4 sterile disc was placed on MHA agar and filled in the concentration of 40μL (20mg/mL). After that, the plate incubated at 37°C for a day. After incubation, a vernier calliper was used to measure the wells' zone in millimeters in order to look for antibacterial activity.

Evaluation of the Minimum inhibitory concentration-

The broth microdilution technique was used to figure out the minimum inhibitory concentration (MIC), ranges between 20 and 0.039 milligrams per milliliter were seen. Using the method described by Sybiya Vasantha Packiavathy et al. (2012) (Sybiya Vasantha Packiavathy et al. 2012) MIC of nano BAG samples was determined.In summary, 20 µL of broth culture containing E. faecalis and C. albicans with cell mass comparable to 0.5 McFarland turbidity standard (equivalent to 1.5 × 10^8 CFU/mL) was added to tubes containing the BHI broth. Following a further dilution with nano BAG extract, all tubes were incubated for 24 hours at 37°C. Following the incubation period, each tube received 30μL of 2,3,5-triphenyl tetrazolium chloride (TTC)., and a 30-min waiting period was ensued for a colour change, confirming the results. The MIC is recognized as the point at which there was no growth (no colour).

RESULTS

FTIR spectra revealed the diverse functional groups present in the synthesised BAG-np and calcium hydroxide. The FTIR spectrum of synthesised BAG-np demonstrated peaks at 509.99, 552.14, 665.14, 697.42, 785.40, 866.09, 1007.77, 1257.8, 1409.72, 2904.50 and 2962.54 cm^{-1} [Fig 1]. The analysis's peaks identified the two transverse optical modes (TO) of the Si–O–Si group. The bending vibrations of Si–O-Si bonds were identified by the peaks between 500 and 800 cm^{-1} which is characteristic of the silica network in bioactive glass. The asymmetric stretching mode of Si–O was indicated by the peak at 1007.77 cm^{-1}.The bending vibration of the phosphate groups (P-O) of the bioactive material was indicated by the peak at 866.09 cm^{-1}. The asymmetric stretching vibrations of phosphate (P-O) bonds was indicated by the peak at 1257.8 cm^{-1}. The peak at 1409.72 cm^{-1} demonstrated the bending vibrations of carbonate (C-O) groups due to the sol-gel process' dissolution of CO2 from the atmosphere. The FTIR spectrum of calcium hydroxide demonstrated peaks at 3461.32, 2867.50, 1644.08, 1460.36, 1347.95, 1285.14, 1246.20, 1191.86, 1096.15, 943.73, 882.43, 842.05, 636.84, 608.05 and 530.00 cm^{-1} [Fig 2]. The OH stretch of the hydroxyl group in the compound was denoted by the peak at 3461.32 cm^{-1}. The FTIR peak at 1460.36 cm^{-1} in calcium hydroxide ($Ca(OH)_2$) is typically associated with bending vibrations of the O-H bond in the hydroxyl group. The peaks observed in the range of 600-650 cm^{-1} could be attributed to the lattice vibrations or metal-oxygen vibrations in calcium hydroxide ($Ca(OH)_2$). This could indicate stretching or bending motions of the Ca-O bond in the solid structure. The FTIR peak at 530.00 cm^{-1} in calcium hydroxide ($Ca(OH)_2$) is associated with metal-oxygen stretching vibrations, specifically related to the Ca-O bond in the lattice vibrations of the calcium hydroxide crystal structure.

Minimum inhibitory Concentration (MIC)-

The antibacterial activity of A two-fold serial dilution approach was used to analyze the nano BAG sample, covering concentrations ranging from 20 mg/mL to 0.039 mg/mL. Notably, at the end point of 2.5 mg/mL nano BAG extract inhibited *E. faecalis.* Additionally, at the end point of 5 mg/mL nano BAG sample and *C. albicans* growth.

Antimicrobial susceptibility -

The zone of inhibition was measured for both *E. faecalis* and *C. albicans* for nano BAG and recorded diameter of 20 mm in *E. faecalis and* 14mm in *C. albicans*.

Similar findings were recorded for Triple antibiotic paste with dimensions of 24 mm for E. faecalis and 20 mm in C. albicans.

Calcium hydroxide group showed the weakest zone of inhibition measuring 12 mm for E. faecalis and 10 mm for c. albicans.

To perform the statistical analysis for the zone of inhibition data, one-way ANOVA followed by Tukey's HSD post-hoc test was used to compare the means of the zones of inhibition for nano bioactive glass (BAG-np), triple antibiotic paste (TAP), and calcium hydroxide against *E. faecalis* and *C. albicans*.

One way ANOVA-

Table 1 -

Source	Sum of squares	dF (Degree of freedom)	Mean Square	F value	P value
Between groups	192	2	96	24.0	0.001
Within groups	12	3	4		
Total	204	5			

Interpretation: The p-value (0.001) is less than 0.05, indicating significant differences among the groups.

Table 2 -

C Albicans -

Source	Sum of squares	dF (Degree of freedom)	Mean Square	F value	P value
Between groups	200	2	100	25.0	0.001
Within groups	12	3	4		

Total	212	5			

Interpretation: The p-value (0.001) is less than 0.05, indicating significant differences among the groups.

DISCUSSION

Nanoparticles are described as solid particles with a dimension of 1-100 nm. Their amplified antibacterial efficacy can be attributed to their enhanced surface area to mass ratio and high charge density that leads to their interaction with the negatively charged surface of bacterial cells. Furthermore, due to their small size, they can easily penetrate the dentinal tubules to greater depths (Gupta et al., 2023). Literature has demonstrated the use of chitosan, zinc oxide and silver nanoparticles for disruption of biofilm and reducing the bacterial load within the biofilm. Prior studies have demonstrated that copper oxide nanoparticles (CONPs) and chitosan nanoparticles (CHNPs) have more antibacterial activity than traditional intracanal medications like calcium hydroxide and oxide (Kaukab et al., 2023).

Bioactive glass is a synthetic material that was developed in the late 1960s (Jones, 2013). Its osteoconductive, osteoinductive and angiogenic properties facilitate its application in bone regeneration and repair(Baino et al., 2016; Miguez-Pacheco et al., 2015). In recent times there is an increased interest in studying the antibacterial properties of bioactive glass. Various mechanisms have been proposed for the same. Bioactive glass contains sodium ions, which when exchanged with the protons in bodily fluids leads to an increase in the local pH. The alkaline environment causes a change in the morphology and ultrastructure of bacteria which alters the expression pattern of numerous genes and proteins (Ran et al., 2013). The bacterial cytoplasm contains solutes which are at a higher concentration than the surrounding environment. The cell membrane experiences positive pressure as a result. An increase in the concentration of external solutes results in a rapid efflux of water which causes a drop in the pressure across the cell membrane, as a result of which there is an alteration in the size and shape of the cell and increase in the stress levels of the membrane. The release of silica, calcium, and phosphate ions by bioactive glass thus results in disruption of bacterial cell membrane (Drago et al., 2018; Hupa, 2011; Jones, 2013). In vitro studies have also highlighted using bioactive glass to lessen the amount of biofilm that multidrug-resistant Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus create (Coraça-Huber et al., 2014; Drago et al., 2014). Studies have indicated that decreasing the particle size of BAG can enhance its antibacterial effectiveness (Stoor et al., 1998). Obeid et al observed that BAG-np was superior to that of BAG and calcium hydroxide(Obeid et al., 2021).

In the present study, the sol-gel technique was used in the synthesis of BAG-nps which is a frequently used technique in the preparation of silica nanoparticles.The method entails creating networks by arranging gelation and colloidal suspension (sol) to create a continuous liquid phase system (gel) (Singh et al., 2014).To create nanoparticles with more predictable morphology and size, the precursors are combined and allowed to react in a controlled manner under liquid circumstances.The method is more convenient and adaptable because it doesn't require the use of complex equipment and the produced nanoparticles are stabilized at a reasonably low temperature (Zheng & Boccaccini, 2017). FTIR is the analytical technique that was used to detect the functional groups in the synthesised nanoparticles. Molecules have varying levels of rotational and vibrational energy. The vibration and rotation of molecules affected by infrared light at a specific wavelength are measured by FTIR. The peaks observed in the FTIR analysis are characteristic of different functional groups (Eid, 2021). The bending and stretching vibrations of Si-O bonds are indicative of the presence of silicate groups which are crucial for the structural integrity and bioactivity of bioactive glass. At lower binding energies, the well-resolved peaks of Si-O-Si vibrations are accompanied by a shoulder that is linked to Si-O-Na or Ca, also known as the non-bridging oxygen groups (NBO) [Fig 1]. The breakdown of Si-O-Si bonds and the creation of Si-O-NBO groups enable the biological reaction seen at the interphase of bioactive glass material when exposed to physiological fluids (Serra et al., 2003). The presence of phosphate phases are important for its bioactivity and can enhance the interaction of the glass with biological tissues. The incorporation of carbonate ions into the glass structure enhances its bioactivity and mimics the composition of natural bone. The results of this present study is in agreement with FTIR observations made in previous research where the functional groups present in bioactive glass were analysed (Hong et al., 2009; Luz & Mano, 2011).

Using a solvent carrier, calcium hydroxide dissolves Ca2+ and OH-. The extremely alkaline character of $Ca(OH)_2$ is caused by the hydroxyl ions.$Ca(OH)_2$ has a pH of approximately 12.5; bacteria in the diseased root canal are destroyed when they come into direct touch with this substance because pathogens cannot thrive in such an alkaline environment.The hydroxyl ions are free radicals that are extremely oxidizing and hence demonstrate high reactivity with biomolecules resulting in denaturation of proteins, bacterial cytoplasmic membrane damage, and/or DNA damage (Kim & Kim, 2014). Two hydroxyl groups and the O-H bond's stretching vibration are present in calcium hydroxide, typically appearing in the region of 3400-3500 cm^{-1}, often with varying intensities depending on factors like the hydrogen bonding and the specific form of the hydroxyl group (e.g., free or hydrogen-bonded). The bending of the O-H group in hydrated or hydroxide-rich materials like calcium hydroxide causes bending vibrations of the O-H bond which appears in the range of 1400-1500 cm^{-1}. The lattice vibrations in calcium hydroxide which was observed in the study typically arises from the motion of calcium ions (Ca^{2+}) and oxygen atoms (O^{2-}) within the crystal lattice of the compound. This region is characteristic of many metal-oxygen bond vibrations in inorganic solids. C-O vibration modes of carbonate groups were identified at 1347.95, 1285.14, 1246.20, 1191.86, 1096.15, and 882.43 cm^{-1} absorption bands. This C-O presence might be specified as a small contaminant from the CO2 atmosphere (Zakaria et al., 2018).

FTIR primarily provides information on the molecular vibrations of bonds within the sample. One of the limitations of this is that many compounds have overlapping absorption bands which makes it difficult to distinguish between them. This is a preliminary study of characterization of the novel compound. To get around some of its limitations, future research could combine FTIR with methods like nuclear magnetic resonance (NMR), mass spectrometry (MS), or gas chromatography (GC).

CONCLUSION

The current investigation concludes that the synthesized bioactive glass nanoparticles contain non-bridging oxygen groups in addition to silica and phosphate bonds. The biological response displayed by bioactive glass nanoparticles, which in turn makes it a potentially effective intracanal medication, is greatly influenced by these functional groups. The study also confirms the presence of hydroxyl groups and calcium ions in calcium hydroxide and its dissociation which is mainly responsible for its antimicrobial activity.

REFERENCES

Baino, F., Novajra, G., Miguez-Pacheco, V., Boccaccini, A. R., & Vitale-Brovarone, C.

(2016). Bioactive glasses: Special applications outside the skeletal system. *Journal of Non-Crystalline Solids*, *432*, 15–30.

Coraça-Huber, D. C., Fille, M., Hausdorfer, J., Putzer, D., & Nogler, M. (2014). Efficacy of antibacterial bioactive glass S53P4 against S. aureus biofilms grown on titanium discs in vitro. *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society*, *32*(1), 175–177.

Drago, L., Toscano, M., & Bottagisio, M. (2018). Recent Evidence on Bioactive Glass Antimicrobial and Antibiofilm Activity: A Mini-Review. *Materials*, *11*(2). https://doi.org/10.3390/ma11020326

Drago, L., Vassena, C., Fenu, S., De Vecchi, E., Signori, V., De Francesco, R., & Romanò, C. L. (2014). In vitro antibiofilm activity of bioactive glass S53P4. *Future Microbiology*, *9*(5), 593–601.

Eid, M. M. (2021). Characterization of Nanoparticles by FTIR and FTIR-Microscopy. In *Handbook of Consumer Nanoproducts* (pp. 1–30). Springer Singapore.

Gomes, B. P. F., Lilley, J. D., & Drucker, D. B. (1996). Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *International Endodontic Journal*, *29*(4), 235–241.

Gupta, A., Singh, A., & Aggarwal, V. (2023). Effect of nanoparticles on antibacterial efficacy of intracanal medicament: A scoping review. *Endodontology*, *35*(4), 283–289.

Hong, Z., Reis, R. L., & Mano, J. F. (2009). Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles. *Journal of Biomedical Materials Research. Part A*, *88*(2), 304–313.

Hupa, L. (2011). Melt-derived bioactive glasses. In *Bioactive Glasses* (pp. 3–28). Elsevier.

Ibrahim, A. I. O., Petrik, L., Moodley, D. S., & Patel, N. (n.d.). *Use of antibacterial* *nanoparticles in Endodontics*. South African Dental Journal. Retrieved September 21, 2024, from https://journals.co.za/doi/10.10520/EJC-7e9a1fb9a

Jones, J. R. (2013). Review of bioactive glass: from Hench to hybrids. *Acta Biomaterialia*, *9*(1), 4457–4486.

Kaukab, A., Gaur, S., Agnihotri, R., & Taneja, V. (2023). Silver Nanoparticles as an Intracanal Medicament: A Scoping Review. *TheScientificWorldJournal*, *2023*, 9451685.

Kawashima, N., Wadachi, R., Suda, H., Yeng, T., & Parashos, P. (2009). Root canal medicaments*. *International Dental Journal*, *59*(1), 5–11.

Kim, D., & Kim, E. (2014). Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part I. In vitro studies. *Restorative Dentistry & Endodontics*, *39*(4), 241–252.

Kumar, J., Sharma, R., Sharma, M., Prabhavathi, V., Paul, J., & Chowdary, C. D. (2015). Presence of Candida albicans in Root Canals of Teeth with Apical Periodontitis and Evaluation of their Possible Role in Failure of Endodontic Treatment. *Journal of International Oral Health : JIOH*, *7*(2), 42–45.

Love, R. M. (2001). Enterococcus faecalis--a mechanism for its role in endodontic failure. *International Endodontic Journal*, *34*(5), 399–405.

Luz, G. M., & Mano, J. F. (2011). Preparation and characterization of bioactive glass nanoparticles prepared by sol-gel for biomedical applications. *Nanotechnology*, *22*(49), 494014.

Miguez-Pacheco, V., Hench, L. L., & Boccaccini, A. R. (2015). Bioactive glasses beyond bone and teeth: emerging applications in contact with soft tissues. *Acta Biomaterialia*, *13*, 1–15.

Narayanan, L. L., & Vaishnavi, C. (2010). Endodontic microbiology. *Journal of*

Conservative Dentistry: JCD, *13*(4), 233–239.

Obeid, M. F., El-Batouty, K. M., & Aslam, M. (2021). The effect of using nanoparticles in bioactive glass on its antimicrobial properties. *Restorative Dentistry & Endodontics*, *46*(4), e58.

Ran, S., He, Z., & Liang, J. (2013). Survival of Enterococcus faecalis during alkaline stress: changes in morphology, ultrastructure, physiochemical properties of the cell wall and specific gene transcripts. *Archives of Oral Biology*, *58*(11), 1667–1676.

Serra, J., González, P., Liste, S., Serra, C., Chiussi, S., León, B., Pérez-Amor, M., Ylänen, H. O., & Hupa, M. (2003). FTIR and XPS studies of bioactive silica based glasses. *Journal of Non-Crystalline Solids*, *332*(1-3), 20–27.

Singh, L. P., Bhattacharyya, S. K., Kumar, R., Mishra, G., Sharma, U., Singh, G., & Ahalawat, S. (2014). Sol-Gel processing of silica nanoparticles and their applications. *Advances in Colloid and Interface Science*, *214*, 17–37.

Stoor, P., Söderling, E., & Salonen, J. I. (1998). Antibacterial effects of a bioactive glass paste on oral microorganisms. *Acta Odontologica Scandinavica*, *56*(3), 161–165.

Svensäter, G., & Bergenholtz, G. (2004). Biofilms in endodontic infections. *Endodontic Topics*, *9*(1), 27–36.

Zakaria, M. N., Sidiqa, A. N., Artilia, I., & Cahyanto, A. (2018). Synthesis and Characterization of Calcium Hydroxide from Indonesian Limestone as Endodontic Intracanal Medicament. *Key Engineering Materials*, *782*, 268–272.

Zheng, K., & Boccaccini, A. R. (2017). Sol-gel processing of bioactive glass nanoparticles: A review. *Advances in Colloid and Interface Science*, *249*, 363–373.

FIGURES

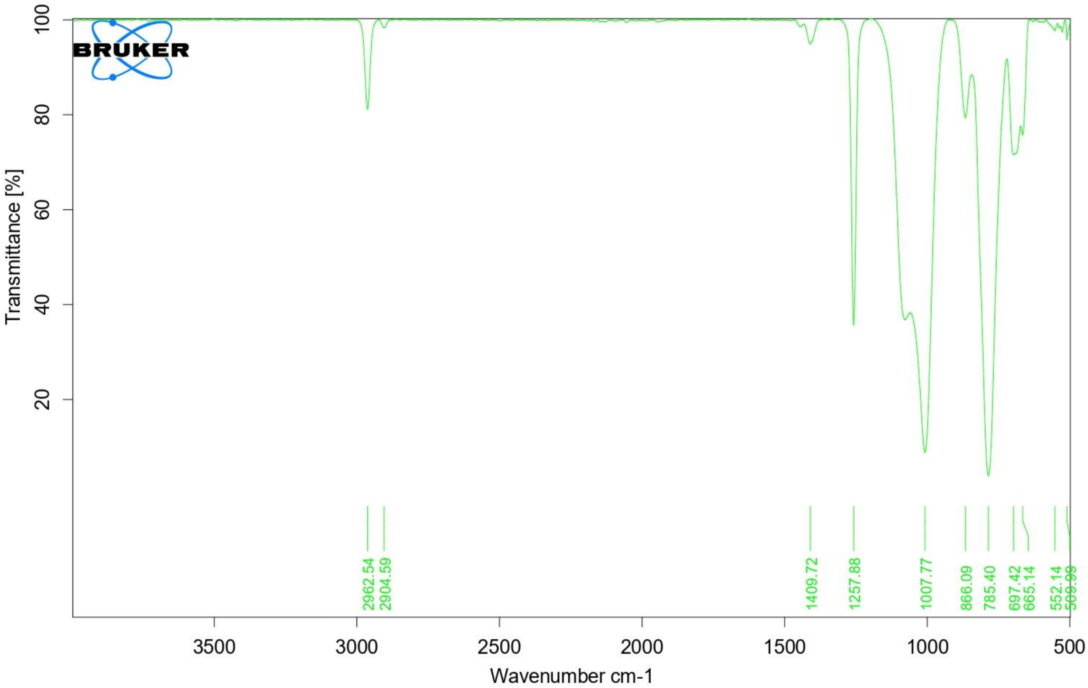

Fig1: Fourier Transform Infrared Spectroscopy analysis of bioactive glass nanoparticles demonstrating the various peaks that represent the presence of functional groups.

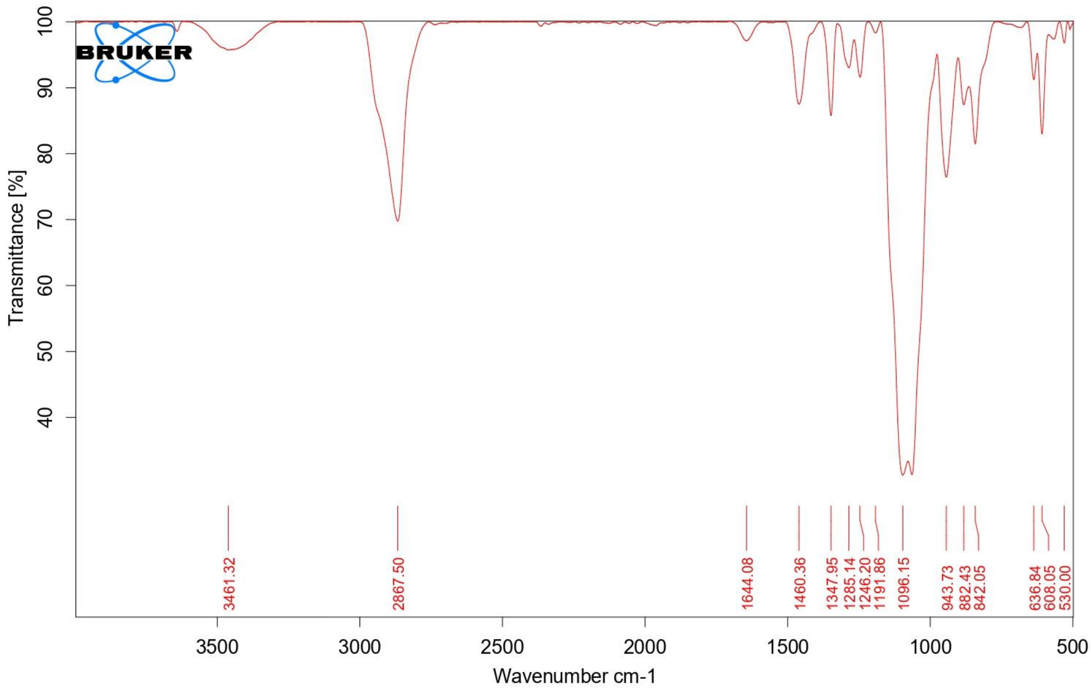

Fig 2: Fig1: Fourier Transform Infrared Spectroscopy analysis of calcium hydroxide demonstrating the various peaks that represent the presence of functional groups.