REVIEW: SYNTHESIS OF NANO BIOACTIVE GLASS OR BIOGLASS

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ABSTRACT

Currently, bioglass nanoparticles are one of the most developed nanotechnology materials due to their bioactive properties and mechanical work which can be widely applied, especially in biomedical fields such as bone tissue engineering and orthopedic implants. Writing this paper is aimed at conducting a literature review on the synthesis of bioglass nanoparticles. In this paper, we review 40 papers from 2001 to 2020. From several papers that have been reviewed, several methods can be used to make bioglass nanoparticles, including melt quenching, sol-gel, sol-gel with acid media, sol-gel using the OMC 3D template OMC, sol-gel using the DDA template, sol-gel using the P123 pluronic copolymer template, sol-gel with Cu doping, sol-gel using silver ion and fluoride doping, sol-gel from rice husk, microwave irradiation, flame synthesis, and microemulsion. The best method of nanoparticles bioglass synthesis is sol-gel using OMC 3D template for produce particles with a size of about 300 nm which is smaller than the size of the OMC 3D template, the surface area of 26 m²/g, and no agglomeration of particles observed by TEM and DLS.

Keyword:
Nanoparticle
Bioglass
Method of synthesis
Characterization
1. INTRODUCTION

Bioglass nanoparticles are one of the most widely developed nanotechnology materials because of their bioactive properties and mechanical performance which can be widely applied in bone tissue engineering [1], bone graft materials for bioactive coating on orthopedic implants [2], and as filler particles in biopolymer composites [3,4]. Bioglass nanoparticles are very important in the biomedical field because of their unique characteristics, namely osteoconductivity and osteoinductivity, and under certain conditions, they are also angiogenic and bactericidal [5]. Bioglass can react with physiological fluids to form strong bonds with the bone. The bonding will be followed by the release of ions for the formation of the hydroxycarbonateapatite (HCA) layer and the biological interaction of collagen with the glass surface so that these various reactions are very beneficial in the healing process of bone fractures [6]. The combination of bioglass nanoparticles with a polymer system is capable of producing a nanocomposite that has the potential for use in orthopedic applications, including tissue engineering and tissue regeneration. The composite modification that is biodegradable with polymers also allows the production of a substrate that can trigger the biomineralization process [7].

Several methods can be done to make bioglass nanoparticles, such as the melt quenching method, sol-gel [8], flame synthesis [9], microwave irradiation [10], and microemulsion [11]. Two main processes that can synthesize this biomaterial are the melt quenching method and sol-gel. The melt quenching method can synthesize bioglass in a short time, about several hours, by heating the initial precursors to high temperatures and following special rules. Although the melt technique is a fast method, the resulting glass usually has a low specific surface area value. According to previous research, the specific surface area value is a key factor affecting bioglass bioactivity. Increasing the specific surface area can increase the surface reaction between the artificial material and the physiological environment, thereby increasing the formation of the HA layer. The sol-gel method can synthesize bioglass at lower temperatures, has a porous structure, and a high specific surface area value which can increase the bioactivity of synthetic materials [12].

Many papers discuss the synthesis of bioglass nanoparticles, but a limited number of papers review the synthesis of bioglass nanoparticles. Therefore, this paper aims to conduct a literature review on the synthesis of bioglass nanoparticles. We review 60 papers from 2001 to 2020 regarding the synthesis of bioglass nanoparticles using the melt quenching method, sol-gel, sol-gel using acid media, sol-gel using the OMC 3D template, sol-gel using
the DDA template, sol-gel using the template, pluronic copolymer P123, sol-gel with Cu doping, sol-gel with silver ion and fluoride doping, sol-gel using rice husk, microwave irradiation, flame synthesis, and microemulsion. The comparison of these methods is presented in Table 1 below.

Table 1. Materials, research groups, methods, and research results of bioglass nanoparticle synthesis

<table>
<thead>
<tr>
<th>Material</th>
<th>Operating Condition</th>
<th>Reagents</th>
<th>Method</th>
<th>Result</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Phosphorus, pentoxide, sodium oxide, calcium hydroxide, calcium oxide, zinc oxide, titanium oxide</td>
<td>Heating time &amp; temperature Melting set: 1300°C, 40 minutes Anaeling set: 380°C, 3 hours</td>
<td>40% P₂O₅, 20% Na₂O, 10% Ca(OH)₂, 20% CaCl₂, 8% ZnO, 10% TiO₂</td>
<td>Melt quenching</td>
<td>Bioglass nanoparticles have a regular round shape and different sizes, 8.2 &lt; size &lt; 15.3 nm</td>
<td>Algarni et al., 2019</td>
</tr>
<tr>
<td>Silicon dioxide, sodium carbonate, calcium carbonate, phosphorus pentoxide</td>
<td>Heating time &amp; temperature Melting set: 1400°C, 3 hours Drying process: 80°C, 5 hours</td>
<td>53.0 SiO₂:23.0 Na₂CO₃:20.0 CaCO₃:4.0 P₂O₅</td>
<td>Melt quenching</td>
<td>Bioglass nanoparticles with a wide size distribution, 100 &lt; size &lt; 800 nm</td>
<td>Shams et al., 2018</td>
</tr>
<tr>
<td>Reagens</td>
<td>Heating time &amp; Temperature</td>
<td>Mixing Reagen</td>
<td>Drying:</td>
<td>Sol-gel</td>
<td>Bioglass nanoparticles</td>
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<tr>
<td>TEOS, ethanol, calcium nitrate tetrahydrate, phosphate pentoxide, ammonia solution</td>
<td>4.054 g TEOS; 2.372 g calcium nitrate tetrahydrate; 0.267 g phosphate pentoxide</td>
<td>30 minutes, temperature room</td>
<td>100°C</td>
<td>-</td>
<td>with different size, 200&lt; size &lt;500, and a surface area of 10.4 m²/g, but there is agglomeration</td>
</tr>
<tr>
<td>TEOS, nitric acid, alcohol, phosphoric acid, calcium nitrate, sodium hydroxide</td>
<td>Stirring: 4 hours</td>
<td>Storing the sol gel: 70 °C, 24 hours</td>
<td>Drying Powder: 600 °C, 2 hours</td>
<td>Sol-gel with acid medium</td>
<td>Bioglass with most particle sizes less than 100 nm, and there are some particles with sizes over 200 nm due to agglomeration</td>
</tr>
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Kumar et al., 2020

Durgalakshmi et al., 2014
| TEOS, TEP, calcium nitrate tetrahydrate, sodium nitrate, nitric acid | Storing the sol: 40 °C, 3 days  
Drying the xerogel: 450 °C, 3 hours | 37.81 mL TEOS; 2.863 mL TEP; 18.379 mg calcium nitrate tetrahydrate; 5.92 mg sodium nitrate; 100 mL nitric acid 1 M | Bioglass with sufficiently agglomerated and spherical morphology. The particle size is 25 < size < 50 nm. | Ray, S., & Dasgupta, S., 2020 |
|---|---|---|---|---|
| TEOS, TEP, HCl, absolute ethanol (ETOH), deionized water, calcium nitrate tetrahydrate, template 3D OMC | Immersing template 3D OMC in sol-gel: room temperature, 8 hours  
Sintering: 600 °C, 1 hour | 9.4 g TEOS; 1.1 g TEP; 0.3 g HCl 2M; 7.5 g EtOH; 0.8 g DW; 6.4 g calcium nitrate tetrahydrate | Sol-gel uses the OMC 3D template | The particle size was about 300 nm, smaller than the template size and a surface area of 26 m²/g. No particle agglomeration. The particles are well dispersed in a biopolymer matrix such as gelatin. In addition, the templates used contribute good | Ji et al., 2017 |
<table>
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<tr>
<th>Template DDA, ethanol water mixture, TEOS, TEP, CN, DW</th>
<th>Stirring: room temperature, 3 hours</th>
<th>4 g template DDA; 100 mL ethanol-water mixture (1:4); 16 mL TEOS; 1.22 mL TEP; 3.39 g CN</th>
<th>Sol-gel uses the DDA template</th>
<th>Bioglass nanoparticles have a monodisperse spherical shape with a diameter about 800 nm.</th>
<th>Chen et al., 2019</th>
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<tbody>
<tr>
<td>TEOS, TEP, water, nitric acid, calcium nitrate tetrahydrate, pluronic copolymer P123</td>
<td>Storing to form gel: room temperature, 3 days</td>
<td>Nitric acid 2 M</td>
<td>Sol-gel uses the P123 pluronic copolymer template</td>
<td>Bioglass nanoparticles have spherical morphology. The pore size and shape are fairly uniform with pore sizes 5.5&lt;\text{size}&lt;7 nm.</td>
<td>Vuong, B., &amp; My Thanh, N., 2020</td>
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<tr>
<td>Preparation</td>
<td>Heating Conditions</td>
<td>Components</td>
<td>Sol-gel details</td>
<td>Comments</td>
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<tr>
<td>TEOS, calcium nitrate tetrahydrate, copper nitrate trihydrate, TEP, ethanol-water mixture, BC aerogel</td>
<td>Heating rate 1°C/minutes with two isothermal steps of 300°C for 1 hour and 650°C for 5 hours</td>
<td>9 mL TEOS (99%); 1 g calcium nitrate tetrahydrate (99%); 0.5 g calcium nitrate trihydrate (99%); 1 mL TEP (99%); ethanol-water mixture (9:1)</td>
<td>Sol-gel with Cu doping</td>
<td>The pore sizes of the nanobioglass is in the range of 9.0&lt;size&lt;11.4 nm.</td>
<td>Luo et al., 2020</td>
</tr>
<tr>
<td>TEOS, nitric acid, TEP, calcium nitrate tetrahydrate, calcium fluoride, silver nitrate</td>
<td>Heating gel: 120°C, 24 hours Calcining: 700°C, 1 hour</td>
<td>14.8 g TEOS; 30 mL nitric acid 2M;</td>
<td>Sol-gel with silver and fluoride ion doping</td>
<td>Bioglass nanoparticles are fairly irregular in shape and less than 100 nm in size.</td>
<td>Kargozar et al., 2019</td>
</tr>
<tr>
<td>Rice husk, hydrochloric acid, sodium hydroxide, sodium silicate, calcium and phosphorus precursors</td>
<td>Drying: 80°C, 24 hours Sintering: 600 °C, 4 hours</td>
<td>HCl 10%; 100 mL NaOH 24.35%;</td>
<td>Sol-gel uses rice husks</td>
<td>Bioglass with uniform spherical particle shape and size 25 nm before sintering.</td>
<td>Durgalakshmi et al., 2020</td>
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<tr>
<td>Step</td>
<td>Details</td>
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<tr>
<td>Calcium nitrate tetra hydrate, diamonium hydrogen phosphate, sodium silicate solution</td>
<td>Drying: 80 ℃, 24 hours&lt;br&gt;Calcining: 700 ℃</td>
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<tr>
<td>37% SiO&lt;sub&gt;2&lt;/sub&gt; in NaOH solution</td>
<td>Microwav e irradiation</td>
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<tr>
<td>Bioglass nanoparticles</td>
<td>Sarkar, SK, &amp; Lee, 2011</td>
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<tr>
<th>Step</th>
<th>Details</th>
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<tbody>
<tr>
<td>TEP, TEOS, NaAc, CaAc, ethanol, water, acetic acid, P123, sulphuric acid</td>
<td>Homogenization: 32℃, 7 days and 37℃, 4 days&lt;br&gt;Drying: 40℃, 1 day&lt;br&gt;Mixing in acid treatment: 25-30℃, 3-4 hours&lt;br&gt;Calcining: 360℃</td>
</tr>
<tr>
<td>Silicon dioxide, calcium phosphate, sodium carbonate, calcium carbonate</td>
<td>Heating time &amp; Temperature</td>
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<tr>
<td>Hexamethyldisiloxane, calcium-2-ethylhexanoic acid, sodium-2-ethylhexanoic acid, and tributyl phosphate, tetrahydrofuran, oxygen</td>
<td>-</td>
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<tr>
<td>TEOS, TEP, and Ca(NO₃)₂·4H₂O, NH₃·H₂O. TritonX-100, octanol (as surfactant phase), cyclohexane (as oil phase)</td>
<td>Hydrolysis: room temperature, 3 days Drying: 100°C, 4 hours</td>
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<tr>
<td>Ammonia, calcium nitrate</td>
<td>Aging: room temperature, 48 hours</td>
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2. **NANOPARTICLES BIOGLASS SYNTHESIS METHODS**

Bioglass was firstly developed by Hench in 1969 through a mixture of melted oxide precursors at relatively high temperatures known as the melt quenching method [13,14]. The melt quenching method or melting method is the traditional one for glass preparation [15,16]. The original bioactive glass comes from (46.1 mol% SiO₂, 24.4 mol% Na₂O, 26.9 mol% CaO, and 2.6 mol% P₂O₅) and is named Bioglass. The choice of a glass composition is based on the influence of all major components on the most relevant glass properties concerning the end-use and manufacture of the product. Although extensive research has been carried out over the past 40 years, only a few glass compositions have been accepted for clinical use. Two compositions that have been approved by the US FDA for melt derivative 45S5 and S53P4 consist of four oxides: SiO₂, Na₂O, CaO, and P₂O₅ [13,14].

Besides the melt quenching method, bioglass synthesis can be carried out through the sol-gel method. The sol-gel process has a long history to use for the synthesis of other silicate and oxide systems and has become a widespread area of research with high technological relevance, for example for the manufacture of thin films, coatings, nanoparticles, and fibers. The sol-gel processing technology is carried out at low temperatures and involves the synthesis of a solution (sol), which usually consists of metal-organic and metal salt precursors followed by gelling by chemical reactions, or aggregation, and finally thermal treatment for drying, removal of organic substances, and sometimes crystallization and cooling. Ions can be also added to the bioactive glass, such as zinc, magnesium, zirconium, titanium, boron, and silver ions to improve glass functionality and bioactivity. However, usually, bioactive glass is difficult to synthesize on a nanoscale with the addition of these ions [17].

Other methods that can be used for bioglass synthesis are flame synthesis, microwave irradiation, and microemulsion. In flame synthesis or flame spray, oxygen spray dispersed the metal organic precursors present in liquid mixture [18,19]. The spray was ignited, as it
burned at above 1000℃, the organic constituents of the liquid precursors combusted to water and carbon dioxide and the metal constituents oxidized to form nanoparticles [11]. Microwave irradiation is a fast and inexpensive synthesis method in which precursors are dissolved in water, transferred to an ultrasonic bath, and irradiated [20]. Microemulsion has been shown to form a particle size in the range of nanometers, with low agglomeration [21]. However, disadvantages of the microemulsion are the production yield and the use of large amounts of oil and surfactants [11]. In water-in-oil microemulsions, the aqueous phase was dispersed to form droplets (5-20 nm in size) [22]. These aqueous droplets acted as a nanoreactor in which reactions can take place when droplets containing the suitable reactants collide with each other [23].

2.1. Melt quenching method

Algarni et al. in 2019 synthesized bioglass through the melt quenching method. The research was carried out by preparing bioglass with a composition of 40P2O5-20Na2O-10Ca(OH)2-20CaCl2-8.0ZnO-10TiO2 in mol% via the melt quenching route at the appropriate temperature. The raw materials were mixed and put in a silicate container and heated in a melting furnace for 40 minutes at a temperature of 1300℃. The resulting viscous melt was molded in a brass mold at room temperature followed by annealing at 380℃ for 2 hours [24].

Figure 1 is the TEM results showing the nano-crystalline properties of the β-TCP material at 550℃ for 30 minutes. The results showed that the regularly spherical particles had different sizes in the range 8.2-15.3 nm [24]. The Gaussian distribution of these particles is estimated in Figure 1.

![TEM result showing the nano-crystalline properties of the β-TCP material at 550℃ for 30 minutes.](image-url)
Figure 1. TEM micrograph of the BGTi bioglass prepared at 550°C for 30 minutes. Image adopted from [24]

The melt quenching method synthesis was also carried out by Shams et al. in 2018. Bioglass nanoparticles were prepared from analytical grade SiO$_2$, Na$_2$CO$_3$, CaCO$_3$, and P$_2$O$_5$ precursors. The precursors were mixed in 53.0 SiO$_2$:23.0 Na$_2$CO$_3$:20.0 CaCO$_3$:4.0 P$_2$O$_5$ molar ratios followed by milling in an agate mortar. The powder mixture was further mixed using a jar mill for 3 hours and compressed into discs with 10 mm in diameter using a hydraulic press apparatus. The discs were placed in an alumina crucible and heat treated in the furnace [25]. Figure 2 showed the furnace temperature programming.

Figure 2. The furnace temperature programming. Image adopted from [25]

After melting at 1400°C for 3 hours, the resulting molten material was quenched in distilled water to produce glass frit. The glass frit was dried in an oven at 80°C for 5 hours. The dried glass frit was milled in a Retch PM400 milling machine using zirconia cups for 6h to obtain the bioglass powder [25].

Figure 3 showed the FESEM micrograph of bioglass nanoparticles. The bioglass powder includes spherical particles with a wide size distribution from 100 to 800 nm [25].
2.2. Sol-Gel

In 2018, Kumar et al. synthesizing bioglass nanoparticles (SiO$_2$ (60%)-CaO (30%) -P$_2$O$_5$ (10%)) through the sol-gel method. The synthesis of bioglass nanoparticles was carried out by mixing TEOS (4.054 g) with ethanol using a magnetic stirrer for one hour at room temperature. In separate containers, calcium nitrate tetrahydrate (2.372 g) and phosphate pentoxide (0.267 g) were dissolved in distilled water and stirred each with a magnetic stirrer for 30 minutes at room temperature as well. After one hour, the solution containing calcium was added dropwise to the solution containing TEOS, as well as the solution containing the phosphate. After that, ammonia solution was added to the mixture to maintain pH 11. The mixture was then put in an incubator for 48 hours to obtain the gel. The obtained gel was placed in an oven at 100°C to dry [26].

The result of TEM analysis in Figure 4 shows that the shape of the bioglass nanoparticles is irregular at the nano and micro scales due to the presence of agglomeration. The particle size varies from 200-500 nm. The average surface area of the bioglass nanoparticles measured using BET with N$_2$ was 10.4 m$^2$/g. The larger the particle size, the smaller the surface area [26].
Different results were carried out by Durgalakshmi et al. in 2014. The first procedure he performed was by mixing tertraethyl orthosilicate (TEOS) and HNO₃ as an acid medium, then added alcohol to help the hydrolysis process. Gel formation occurred after 30 minutes of mixing. At 20 minute intervals, other reagents are added to the mixture such as phosphoric acid, calcium nitrate, and sodium hydroxide. The solution was stirred for 4 hours to obtain a homogeneous gel. After the hydrolysis process is complete, the sol is stored at 70°C for 24 hours, and then the dry white powder is taken at 600°C for 2 hours [27].

Figure 5 is the FESEM result which shows that the particles do not have a well-defined shape, but most of the particles are less than 100 nm in length [27]. Some particles in a size of more than 200 nm may be formed due to particle agglomeration during sintering [28].

The sol-gel synthesis method was also carried out by Ray, S. & Dasgupta, S., in 2020. To synthesize 20 grams of bioactive glass, 37.81 mL TEOS; 2.863 mL TEP; 18.397 mg of calcium nitrate tetrahydrate, and 5.92 mg of sodium nitrate are dissolved in 100 mL of 1 M nitric acid. The ingredients for the Nbg preparation are added sequentially. After adding all the precursor components, the sol formed was stored in an oven at 40°C for 3 days or until a gel was formed. The xerogel obtained was dried, crushed and transformed into powder using a mortar and pestle then calcined at 650°C for 3 hours at a rate of 3°C per minute. The obtained powder was crushed again with a mortar and pestle to obtain a finer powder [29].

The synthesized and calcined bioglass nanoparticles showed sufficiently agglomerated and spherical morphology with particle sizes ranging from 25-50 nm as shown
on the FESEM micrograph in Figure 6(a). The synthesized Nbgs was heated at 650°C to remove nitrate ions and allow calcium, phosphate, and sodium ions to diffuse in the silica network. As a result of heating at high temperatures, agglomeration and particle incorporation occurs. Figure 6(b) shows the TEM result of Nbg which has been calcined at 650°C for 3 hours where agglomeration of small spherical nanoparticles in the 8-10 nm range can be seen [29].

**Figure 6. FE-SEM of synthesized Nbg (a), HRTEM (b), Particle size distribution by intensity Image adopted from [29]**

### 2.2.1 The sol-gel method uses a template

Ji et al. in 2017 synthesized bioglass through the sol-gel method using a three-dimensional ordered macroporous carbon (3D OMC) template. Through this method, the synthesis is carried out by making the first 3D template OMC. The template was made by replicating the colloidal silica crystal template through the Stöber method. The sol-gel precursor solution of 58S-BG was prepared by dissolving 9.4 g tetraethyl orthosilicate (TEOS), 1.1 g triethylphosphate (TEP) and 0.3 g HCl (2 mol/L) in absolute ethanol (ETOH, 7.5 g) and deionized water (DW, 0.8 g). The mixture was stirred vigorously for 3 hours at room temperature to fully hydrolyze TEOS and TEP. 6.4 g of calcium nitrate tetrahydrate is added to the previous solution and stirred to give 58S BG sol-gel. The OMC template was immersed in the solution for 8 hours and the sol-gel process was carried out at room temperature. After the OMC template containing BG 58S gel was peeled off, the next process was sintering at 600°C for 1 hour in air to remove the 3D OMC template [30].

Figure 7 shows the interconnected OMC template macropores with tiny pores replicated from the silica microsphere interface of the colloidal silica crystal template. The sol-gel precursor of 58S BG infiltrated the entire OMC template through these tiny pores and formed 58S BG particles in the pores after the sol-gel process (Figure 7b). The structure of these particles remained in order after the OMC template was removed and the results showed a spherical shape (Figure 7c). TEM images show that the 58S particles were well dispersed after ultrasonication and solidified (Figure 7d). The resulting bioglass particle size was about 300 nm, much smaller than the macro size in the OMC template. This can be attributed to the volume contraction of the BG 58S particles during the sol-gel
and sintering processes. The specific surface area of the particles produced BG 58s is 26 m²/g [30].

Figure 7. (a) SEM of 3D OMC template showing macro pores with uniform pore size and small pores connecting macro pores; b) 58S particles are replicated in the macro pores of the OMC template; c) 58S particles after OMC template removal; d) The TEM of the BG 58S particles is dispersed by ultrasonication. Image adopted from [30].

The polydispersion index calculated is 0.152, indicates a narrow size distribution. No agglomerated particles were observed with TEM and DLS characterization. TGA showed that the particles did not experience weight loss when heated to 800°C in air (not shown here), assuring the macropore carbon template and organic matter in the sol-gel precursor had been removed [30].

Chen et al. in 2019 synthesized nanobioglass (nBG) using the sol-gel method using a dodecylamine (DDA) template. DDA (4 g) as a molding agent was dissolved in the ethanol-water mixture (100 mL, H₂O: C₂H₅OH = 1: 4) while stirring. After complete dissolution, TEOS (16 mL), TEP (1.22 mL) and CN (3.39 g) were added respectively at 30 minute intervals using a magnetic stirrer. Then the mixture was vigorously stirred at room temperature for 3 hours and a large amount of white precipitate was obtained. The precipitate was centrifuged, rinsed with ETOH and deionized water 3 times successively, followed by drying in vacuum at room temperature for 24 hours. Bioglass nanoparticles were obtained by sintering the dry precipitate in a furnace (CWF12/23/3216P1, Karbolit, UK) at 650°C for 3 hours to remove mold and other remaining organic components. Well controlled nano size is achieved by this method. The morphology of nBG was observed by SEM as shown in Figure 8A. The resulting bioglass nanoparticles have a monodispersed spherical shape with a diameter of about 800 nm [31].
The trilayer fibrous membrane is prepared by sequential electrospinning as shown in Figure 8B. The trilayer membrane morphology obtained was observed by SEM and fluorescent microscopy in Figure 8C. A single layer membrane consisting of chitosan, chitosan-PVA and PVAnBG was also constructed to facilitate observation. Chitosan and PVA membranes showed sub-micro ultrafine fibers with the same diameter of about 400 nm as shown in the SEM images. Chitosan fibers with red fluorescence (Rhodamin B) and PVA with green fluorescence (FITC) are homogeneously distributed on the hybrid membrane to form an interpenetration network. The spatially designed multilayer structures are further investigated with a cross-sectional view. Different layers can be distinguished in the SEM image with different microstructures. The fluorescent image also clearly shows a trilayer structure consisting of a red chitosan layer, a yellow chitosan-PVA layer, and a green PVA layer containing nBG. Both chitosan and PVA nanofibers penetrate in two layers, making the spatially layered structure stable. These results indicate that a spatially designed trilayer membrane was successfully fabricated by sequential electrospinning, with different functional components in each layer [31].

Figure 8. Making bioglass using the sol-gel method using the DDA template. Bioglass is made by combining a three-layer nano fiber membrane (nBG-TFM). (a) SEM images and EDS mapping of nBG nanoparticles. Scale bar: 2 μm. (b) The nBG-TFM scheme is made by sequential electrospinning. (c) Photograph (top), SEM image (center) and fluorescent image (bottom) of membrane electrospining. Scale bar: 1 cm (black), 10 μm (Red), 100 μm (white). Image adopted from [31]
Vuông, B. X., & Thanh, N. T. M., in 2020 synthesized bioglass through template copolymer pluronic P123. The synthesized bioglass has a composition of 58SiO$_2$-33CaO-9P$_2$O$_5$ (wt%). In short, the synthesis process, namely TEOS and TEP, are mixed in distilled water and stirred for 30 minutes with a stirring speed of 100 rpm. 2 M nitric acid is used to adjust the pH of the reaction mixture 1.5. After that calcium nitrate tetrahydrate is added and the reaction mixture continues to be stirred for 30 minutes, then a clear sol will be formed. An amount of P123 is dissolved in the above sol. The mixture was kept for 3 days at room temperature to form a gel. The gel was left to stand for 2 days at 60°C then dried at 100°C for 1 day. The dry gel was treated at 700°C for 3 hours to burn template P123 and converted to glass powder [32].

Synthesized Bioglass is analyzing by using FE-SEM observation and TEM as shown in Figure 9. Figure FE-SEM showing morphology of the ball in the nanoscale, where the individual particles tera agglomerating each other to form a porous structure that correlates. TEM analysis shows regular distances with 2D hexagonal structured porosity. In addition, the pore size and shape are fairly uniform with pore sizes ranging from 5.5 to 7 nm [32].

![Figure 9. Morphology sinte sis bioglass (a) FE-SEM and (b) TEM image. Image adopted from reference [32](image)](image)

2.2.2 Synthesis of bioglass that has been doped with other materials through the sol gel method

To improve the properties of bioglass, it can be done by adding other materials as dopants. One of which is the synthesis of bioglass by adding Cu. The synthesis was carried out by dissolving 9 mL of tetraethyl orthosilicate (TEOS, 99%), 1 g of calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$.4H$_2$O, 99%); 0.5 g (Cu(NO$_3$)$_2$.3H$_2$O, 99%), and 1 mL of triethyl phosphate (TEP, 99%). All of materials were stirred in 50 mL of absolute ethyl alcohol. After 24 hours, 125 mg of BC aerogel was added to the solution and then stirred continuously for 24 hours. The resulting BC hybrid material was rinsed with absolute ethanol then immersed in a mixture of C$_2$H$_5$OH: H$_2$O (9 mL: 1 mL) for 24 hours. The BC hybrid material was immersed in tertiary butanol for 24 hours. The samples obtained were frozen for 24 hours, then heated at a heating rate of 1°C/minute with two isothermal steps.

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of 300°C for 1 hour and 650°C for 5 hours, resulting in 5% MBG with Cu doping (named MBG-5Cu) [33].

Figure 10 (a-h) is a TEM and SEM image of the Cu doped bioglass. The figure shows that all the scaffolds show nanofibrous morphology (Figure 10a-h). The TEM results in Figure 10 (e-h) show that the four scaffolds have an ultra-fine and 3D tissue structure, which is a replication of the BC template and is beneficial for cell adhesion and proliferation. The EDS results confirmed that the molar ratios of MBG-1Cu, MBG-2.5Cu, and MBG-5Cu were 0.2; 1.9; and 3.3%, these values are lower than the theoretical values. From this research obtained that the pore size of nanofibrous bioglass is in the range of 9.0 – 11.4 nm [33].

![Figure 10. SEM image (a-d) and TEM Image (e-h) from MBG. This image is adopted from reference [33]](image)

Kargozar et al. also added other materials in 2019. Bioglass which are synthesized by the method sol-gel added silver ions and fluoride. The nanobioglass powder containing Ag⁺ and F⁻ ions was synchronized based on the 58S BG composition using the sol-gel method. 14.8 g of tetraethyl orthosilicate (TEOS) were added to 30 mL of the 2 M nitric acid solution. The mixture was allowed to react for 60 minutes for TEOS acid hydrolysis. Then, triethyl phosphate, calcium nitrate tetrahydrate, calcium fluoride and silver nitrate are added to the solution in suitable amounts. The mixing was continued for 1 hour to allow the reaction to complete. The procedure for fixing the soles was carried out using incubation at room temperature for 7 days. The resulting gel was heated at 120°C for 24 hours and the final calcination was carried out at 700°C for 1 hour to remove nitrate and stabilize some of the glass structure. The ready BG was then ground and sieved to obtain glass particles with a size below 38 µm. Then, the material is again milled to obtain fine glass particles by high-energy ball milling (Fritsch’s planetary ball mill P7) [34].

The results obtained from the Brunauer Emmett Teller (BET) analysis of the synthesized glass show a type IV isotherm of the Brunauer Deming - Deming Teller (BDDT) classification theory for all prepared
materials. From these results it can be concluded that all the glass powders that have been synthesized have pore sizes in the range of 2-50 nm [34].

The TEM micrograph in Figure 11 shows that the glass particles are at the nanoscale scale. The nanoparticle shape is quite irregular and the size is less than 100 nm [34].

![Figure 11. TEM micrograph of a nano bioglass that has been synthesized by the Sol-gel method and then added with silver and fluoride ions. Image adopted from [34]](image)

**2.2.3 Nanobioglass from rice husk synthesized by sol-gel method**

Silica was extracted from rice husk by cleaning and heat treatment methods. The dry husks were obtained from a local rice mill in Chennai and washed with 10% HCl solution and stored at 80°C for 4 hours. Rice husks that have been processed using acid are filtered and washed to a neutral pH and dried in an oven with hot air for 24 hours. The husks that have been washed and treated with acid are sintered at 600°C for 4 hours. The result is silica powder which is then used as a silica precursor for the synthesis of bioglass [BG (Husk)] in a composition similar to that of 45S5 bioglass (46.14% SiO$_2$ - 26.91% CaO-24.35% Na$_2$HAI$_2$, 6% P$_2$O$_5$) [35].

For the synthesis of BG, 45 g of silica from rice husk derivatives were dispersed in 100 mL of 24.35 mol% NaOH and stirred by heating at 100°C until a transparent solution was obtained. The transparent solution is cooled to room temperature and then the required precursors of calcium and phosphorus are added. The solution was stirred for 4 hours and kept at 60°C for 24 hours. The resulting powder was calcined at 600°C to obtain bioglass nanoparticles [35].

Sevil Yücel, in 2015 using a sample of rice husk ash obtained from the Yetiş Food Factory (Turkey). Sodium silicate solution was obtained from rice husk ash using the Kalapathy method. The method begins with burning rice husk ash at a temperature of 600°C
for 5 hours. The ash of the burned rice husk is heated with 1 M HCl solution at pH 1 to remove impurities. Acid rice husk ash was filtered under vacuum and dried at room temperature. Dry ash was heated with 1 M NaOH solution, filtered under vacuum, and washed with distilled water. Thus, a sodium silicate solution was obtained [36].

For the gelation stage, the sodium silicate solution was reacted with 1 M HCl solution to pH 9. Gel formation was carried out at room temperature for 30 minutes. Let the silica gel sit for a day. The gel was washed with distilled water 4 times to remove salt impurities and centrifuged. After that the gel was dried in an oven at 80 °C for 12 hours and ground [35].

The heat treated rice husk showed disintegration of the linear structure and segments of the structure with dimensions of about 5 μm which was observed with the many porous particles and nanostructured beads shown in Figure 12. The formation of similar nanostructures from the heat treatment of rice husks was observed by Chen et al. He performed a review on the nanostructures obtained from rice husks for energy applications [37].

Figure 12. Rice husks with heat treatment. Image adopted from [35]

Figure 13(a) showed uniform spherical bioglass nanoparticles of size ~25 nm before sintering. After sintering in Figure 13(b), there are melting and agglomeration nanostructures and hence there is the formation of micron-sized cubical arrangements with flake-like nanostructured embedded on the cubes.

Figure 13. SEM results of bioglass (a) before sintering (b) after sintering. Image adopted from [35]
2.4 Microwave irradiation method

Sarkar, SK, & Lee in 2011 synthesized bioglass nanoparticles by microwave irradiation method. The raw materials needed are calcium nitrate tetrahydrate for Ca sources, diamonium hydrogen phosphate for P sources, and sodium silicate solution (37% SiO₂ in NaOH solution) for Si sources. The precursors were dissolved in deionized water and transferred to an ultrasonic bath. The irradiation time was varied to get the optimum synthesis conditions. Microwave operation is performed in a second wave after ultrasonic irradiation. The amorphous powder obtained was washed in deionized water and filtered. After drying for 24 hours in an oven at 80°C, the powder was calcined at 700°C to develop bioglass [10]. Figure 14 shows the bioglass synthesis procedure using the microwave irradiation method.

![Bioglass synthesis procedure using microwave irradiation method](image)

**Figure 14. Bioglass synthesis procedure using microwave irradiation method. Image adopted from reference [10]**

Figure 26 is the SEM bioglass result immersed in SBF (stimulated body fluid). From Figures 15(a) and (b), it can be seen that the bioglass sintered at 1000°C forms more apatite and there is evidence of growth on the surface. Whereas the bioglass sintered at 1100°C in Figures 15(c) and (d), the surface shows a more intense formation of apatite [10].
Figure 15. SEM results of bioglass sintered without pressure (a, c) and microwave sintered (b, d) after 1 and 3 days of immersion in SBF. Image adopted from reference [10]

Different results were carried out by Kumar et al. in 2017. The first step of synthesis nanoparticles bioglass (46.1SiO2-26.9CaO-24.4Na2O-2.6P2O5 (mol %)) using irradiation microwave was the dissolution of different precursors like TEP, TEOS, NaAc, CaAc, in ethanol as a solvent in accordance with the different specific molar ratio of SiO2, CaO, Na2O, and P2O5 respectively in every one hour interval. The resultant mixture containing different precursors was stirred at room temperature for 24 hours to undergo homogenization. Additionally, 4 g P123 polymer were dissolved in 60 g of ethanol [38], before mixing the precursors keeping the constant ratio of (TEOS/TEP) and ethanol 1:4; (TEOS/TEP) and water 1:4 (in molar ratio), and water and acetic acid 1: 6 (in weight ratio) [39]. The overall solution is transferred in polystyrene petridish which is ultimately kept in the oven to undergo homogenization at 32°C for 7 days followed by the next step of 37°C for 4 days. The sample as a whole is dried at 40°C for a complete day [38].

During the acid treatment process, 1 g of resultant sample was mixed with 100 ml of sulphuric acid (48 wt%) for 3 to 4 hours in range 25 to 30°C by allowing the sample to remain in the amorphous phase. Additionally, the final product was washed with distilled water followed by acetone until sample is free from impurities. Then the final product was calcined at 360°C by using Micro Heat-High Performance Microwave Furnace [38].

Figure 16(a) is the result of FESEM analysis that showed spongy surface which is typical morphological behavior of mesoporous bioglass. Figure 16(b) is the result of BF TEM, that showed the surface of bioglass consist of wormhole-type mesoporous structure dispersed in each other in close impendency. The size of the pore particle is 2.8 nm and the surface area is 522 m2/g. [38].
2.5 Flame Synthesis Method

Flame synthesis is one of the bioglass nanoparticles synthesis methods by mixing liquid metal precursors and inserting the mixture into a fire reactor. The resulting nano powder was filtered using a filter attached to the fire [9].

The raw materials used to synthesize bioglass nanoparticles using the flame synthesis method are SiO₂, Ca₃PO₄, NaCO₃, and CaCO₃. All the ingredients are mixed and melted in a platinum dish at a temperature of 1450°C. After the melting process, the mixture is dried and ground until the desired particle size is formed [40].

The results of TEM analysis in Figures 17a) and b), show that the particles form chain-like agglomerates and are structurally amorphous. In addition, the bioglass nanoparticles are fused together and are irregular in shape, making it difficult to determine their size. However, the particle size can be estimated to have a diameter of 20-60 nm and a surface area of 60-80 m²/g [40].

Figure 16. (a) FESEM results of the bioglass (b) BF TEM results of the bioglass. Image adopted from [38]
Different result was carried out by Hild et al. in 2013. The synthesized bioglass nanoparticles composition is 45.0 wt% SiO2; 24.5 wt% Na2O; 24.5 wt% CaO; and 6.0 wt% P2O5. In the first procedure, precursors of hexamethyldisiloxane, calcium-2-ethylhexanoic acid, sodium-2-ethylhexanoic acid, and tributyl phosphate were mixed accordingly. After that, the mixture was diluted with tetrahydrofuran at a volume ratio of 2:1, dispersed oxygen (5 mL/minutes), and fed (5 mL/minutes) through a capillary (diameter 0.4 mm), into a methane/oxygen flame. As formed bioglass nanoparticles (production rate 25 g/hour) were collected on metal filters and sieved (300 μm mesh) subsequently [41].

Figure 18 is the TEM results of bioglass nanoparticles that confirmed the spherical shape and the nano-size of the particles. Bioglass nanoparticles gave a specific surface area of 40 ± 4 m²/g corresponding to a primary particle diameter of 60 nm [41].

2.6 Microemulsion

Zhao et al. in 2005 synthesized bioglass nanoparticles through the microemulsion method. The composition of the synthesized bioglass was (wt%) 36.0% CaO-4.0% P2O5-
60.0%SiO2. The materials from analytical grade TEOS, TEP, and Ca(NO3)2.4H2O, NH3.H2O. TritonX-100 and octanol (as surfactant phase), cyclohexane (as oil phase) was used to prepare the microemulsion. Two kinds of microemulsion (types A and B) in table 2 but different aqueous phases were obtained. After addition of TEOS and TEP to the microemulsion A, then the two microemulsions were mixed for 1 hour under constant stirring speed. The solubility of water in surfactant can be expressed by molar ratio of water to surfactant (γ). The value of γ was 6, 8 and 10 respectively. TEOS and TEP hydrolysis was carried out by using 12% NH3•H2O to catalyze, with a molecular ratio of (NH3•H2O+H2O)/(TEOS+TEP)=8. After 3 days reaction at room temperature, a white product was collected by centrifugal and washed repeatedly with ethanol. The solid is dried in 100℃ for 4 hours [25].

Table 2. Composition of the microemulsion system used for synthesis bioglass.

<table>
<thead>
<tr>
<th>Aqueous phase</th>
<th>Microemulsion A</th>
<th>Microemulsion B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant</td>
<td>NH3•H2O</td>
<td>Ca(NO3)2•4H2O</td>
</tr>
<tr>
<td>Surfactant</td>
<td>Triton X-100</td>
<td>Triton X-100</td>
</tr>
<tr>
<td>Oil phase</td>
<td>octanol</td>
<td>octanol</td>
</tr>
<tr>
<td></td>
<td>cyclohexane</td>
<td>cyclohexane</td>
</tr>
</tbody>
</table>

The TEM results of bioglass nanoparticles produced in microemulsion are shown in Figure 19. The bioglass nanoparticles are in form of sphericity, showing the individual nanospheres in the region of 10-40 nm in diameter. At γ=6, the mean particle diameter is 25 nm with 245.49 m2/g surface area; at γ=8, the mean particle diameter is 35 with 155.59 m2/g; at γ=10, the mean particle diameter is 50 nm with 97.52 m2/g [25].

Figure 19. TEM results of bioglass nanoparticles. Image adopted from [25]
Different results were carried out by Wang et al. in 2013. Bioglass nanoparticles with molar composition 21% SiO$_2$-60% CaO-19% P$_2$O$_5$ were prepared by microemulsion method. Firstly two different microemulsions was prepared, ammonia 25% (solution A) and 0.2 M Ca(NO$_3$)$_2$.4H$_2$O (solution B). Solution A was poured into a round bottom flask and stirred at 800r/min in a digital display electric blender. 0.45 mL of TEOS was added dropwise into solution A, and the mixing process was allowed to be continued for 30 minutes for hydrolysis of TEOS. After that, 0.3 mL TEP was dissolved into the prepared solution. After stirred for 20 minutes, solution B was added dropwise with a dropping funnel and stirred for 2 hours. Then the mixtures were aged for 48 hours at room temperature. The white precipitate was collected by centrifuge at 4000 r/minutes, washed 5 times with absolute ethanol, and dried at 50°C in a vacuum drying oven for a day. To remove the oil and surfactants, bioglass nanoparticles were calcined at 600°C for 2 hours in a muffle furnace with a heating rate of 10°C/minutes [42].

The SEM results of bioglass nanoparticles are shown in Figure 20. As shown, spherical bioglass nanoparticles with a size less than 100 nm were obtained. The TEM micrographs are shown in Figure 21, which confirmed the particle had sphere shaped and the size was less than 100 nm [42].

![Figure 20. SEM results of bioglass. Image adopted from [42]](image)

![Figure 21. TEM results of bioglass. Image adopted from [42]](image)
3. ANALYSIS

From the explanation above, we can see that there are several methods to synthesize nano bioglass such as melt-quenching, sol-gel, microwave irradiation, flame synthesis, and microemulsion. From those methods, nanoparticle which is synthesized by Vuong, B., & My Thanh, N. in 2020 has result the smallest particle which is 5.5 to 7 nm in size and nanoparticle which is synthesized by Chen et al. in 2020 has result the biggest particle which is about 800 nm in size.

Several nanoparticle which is resulted has agglomeration. Particle agglomeration can be occurred by several mechanism. Brownian agglomeration is one mechanism that leads to agglomeration; it occurs when particles collide and stick together as a result of their random, Brownian motion. Another mechanism that leads to the agglomeration of particles is known as gravitational agglomeration, which is dependent on the size of the particles and their terminal velocity. The slowly settling particles are caught by the more rapidly settling particles, leading to the formation of clusters. The clustering of particles has an effect on the pH and ionic strength [43]. Particle agglomeration can be found on the result which is synthesized by Kumar et al.; Durgalaksmi et al.; and Ray, S., & Dasgupta, S. through sol-gel method and Mačković et al. through flame synthesis.

Several methods that can produce a uniform size of bioglass nanoparticles are bioglass nanoparticles in the research of Ji et al., in 2017 with the sol-gel method uses the OMC 3D template, nanoparticles in Chen et al. in 2019 with the sol-gel method uses the DDA template, nanoparticles in research Vuong, B., & My Thanh, N. in 2020 with the sol-gel method uses the P123 pluronic copolymer template, nanoparticles in the research of Durgalakshmi et al. in 2020 with the sol-gel method uses rice husks, nanoparticles in the study Wang et al. in 2013 using the microemulsion method, and nanoparticles in the research of Sarkar, SK, & Lee in 2011 using the microwave irradiation method.

Several methods that produce different nanoparticle sizes are bioglass nanoparticles in the research of Algarni et al., 2019 and Shams et al., 2018 with the melt quenching method, bioglass nanoparticles in the research of Pawan et al., 2020 with the sol-gel method, bioglass nanoparticles in the research of Durgalakshmi et al., 2014 with the Sol-gel with acid medium method, bioglass nanoparticles in Ray, S., & Dasgupta, S., 2020 research using the sol-gel with acid medium method, bioglass nanoparticles in the research of Luo et al., 2020 with the sol-gel with method Cu doping, bioglass nanoparticles in Kargozar et al., 2019 research with the sol-gel method with silver and fluoride ion doping, bioglass nanoparticles in the research
of Mačković et al., 2012 and Hild et al., 2013 with the flame synthesis method, bioglass nanoparticles in the research Kumar et al., 2017 with microwave irradiation, and bioglass nanoparticles in Zhao et al., 2005 with microemulsion method.

From all research on making bioglass nanoparticles with several methods, it can be concluded that the best method used in the sol-gel method uses the OMC 3D template carried out by Ji et al., 2017 because it produces nanoparticles of uniform size and can be adjusted according to the template used.

4. CONCLUSION

Bioglass nanoparticle synthesis can be done through several methods such as melt quenching, sol-gel, irradiation microwave, flame synthesis, and microemulsion. The synthesis of bioglass through the melt quenching method resulting regular round shape particles and has a different size in the range of 8.2 to 100 nm. Bioglass synthesis via the sol-gel method generally produces particles of 25-300 nm in size. However, the best results are shown by the sol-gel method via the 3D template OMC. Sol-gel method with 3D template OMC produce particles measuring about 300 nm which is smaller than the template OMC and has a surface area of 26 m²/g, the particle has no agglomeration, and its well dispersible in a biopolymer matrix such as gelatin. The template contributing to excellent mechanical properties and bioactivity. Synthesis bioglass through metode flame synthesis clicking produce a particle size of 20-80 nm and a surface area of 70-90 m²/g, but there is agglomeration. Synthesis bioglass through irradiation microwave method produce a particle size of 200 nm. While synthesis bioglass through microemulsion method produce nano particle bioglass with particle size of less than 100 nm. So it can be concluded that the best method for the synthesis of bioglass nanoparticles is the sol-gel method using the 3D template OMC.

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REFERENCES


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Ray, S., & Dasgupta, S. First principle study on in-vitro antimicrobial properties of nano 52S4.6 bioactive glass. Ceramics International, 2.182, 2020


