Dispersion of Bioactive Glass using cetyltrimethylammonium bromide

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ABSTRACT

The dispersion of bioactive glass, (BG), sample was studied using cetyltrimethylammonium bromide (CTAB). An array of experimental techniques like adsorption, zeta potential and turbidity were used to achieve this goal. Turbidity was used as a parameter to reflect the degree of dispersion where a higher value of turbidity represents higher dispersion efficiency. Dispersion of the BG particles was largely affected by CTAB concentration and the solution pH. Dispersion of the BG particles was increased with increasing of CTAB concentration till the CMC of CTAB is reached. Beyond the CMC, the BG particles become hydrophilic causing a decrease in glass dispersion. On the other hand, dispersion of the BG particles was affected with the solution pH. It was increased with increasing pH till pH 9. At higher pH, dispersion was decreased where the formation of micelles is favored.

Introduction

Many kinds of polymers have been widely used as scaffold biomaterials in bone tissue engineering research, [1-3].However, these kinds of polymers have a few disadvantages such as the lack of bioactivity, [4] and poor mechanical properties, [5]. In order to ameliorate these problems, bioactive inorganic fillers, such as hydroxyapatite, [6] wollastonite, [7] or β-tricalcium phosphate, [8] and bioactive glasses (BGs) [9] were introduced into biodegradable polymers to fabricate filler/polymer composites. BGs exhibit especially high bioactivity through the release of dissolution ions such as Ca, P and Si, which may also affect both gene expression in osteogenic cells and vascularization, and subsequently promote a high rate of bone formation [10] and [11]. Bioactive glasses react chemically with the body fluids. The reaction product is an apatite, which, with the intervention of biological drivers, assists the generation of bone matrix and bone growth. The main application of these bioactive glasses in the clinical field is the filling of osseous cavities, manufacture of small parts for middle ear bone replacement and maxillofacial reconstruction and dental applications. However, one of the most problematic issues for these polymer/inorganic composites is the tendency for agglomeration of the inorganic particles in the polymer matrix, [7]. Particle aggregation in composite materials tends to increase susceptibility to chemical and mechanical failure. On the contrary, well dispersed particles could increase the contact area between the polymer matrix and the inorganic reinforcing phases for interfacial bond development. Therefore, it is critical to improve the dispersion of inorganic particles in preparing polymer/bioactive glass composites. The mostly used method to improve the dispersion of inorganic particles in polymeric matrix were mechanical stirring, [13] and ultrasonic energy, [14] which, only, showed temporary effects since the particle aggregation would occur due to the colloid stability. Chemical modification of the surface of inorganic particles may be a solution to this problem, [15]. The aim of this study is to improve the homogeneous dispersion of BG particles in aqueous solution, by surface modification using CTAB to enable biological applications. An array of experimental techniques like adsorption, zeta potential and turbidity were used to achieve this goal.

Materials and Methods

Materials: The synthesis of the glass was carried out by hydrolysis and polycondensation of 68 ml of tetraethyl orthosilicate,$C_8H_{20}O_4Si$ (TEOS),50 ml of distilled water,8.6 ml of triethylphosphate,$C_6H_{15}O_4P$,(TEP) , 41.3g of calcium nitrate, $Ca(NO_3)_2.4H_2O$ and 1N HCl. The synthesis was carried out in a hermetically sealed cylindrical Teflon container at room temperature for 3 days. The gel was then kept at 60°C for another 3 days where a hole, of one mm in diameter, was made in the lid to let the gas escape and the gel is dried at 130°C for 2 days more. The produced dried gel was crushed and calcined at 700°C for 3 h in Muffled electric oven.

Characterization of the prepared bioactive glass

The XRD pattern of the prepared BG is shown in Fig. (1).It did not show any evidence of crystalline phase and confirmed the amorphous character of glass. It presents a diffraction halo between 20 and 40° (2θ) with center at 325°. This diffraction halo is characteristic of the diffusion phenomena in amorphous materials and the absence of long range order in the matrix of BG. It consists of an amorphous material.

Fig. (2) presents the IR spectrum of BG. It shows several characteristic bands of silica network. The band at 503cm^{-1} is attributed to an angular deformation vibration of Si-O-Si between SiO_4 tetrahedrons. Three other bands at 745, 932 and 1036 cm^{-1}, respectively, are characteristic of stretching vibration of Si-O chemical bond in SiO_4 tetrahedron. The last band at 590cm^{-1} is characteristic of bending vibration of O-P-O liaison. Its slight intensity highlights the presence of a small amount of phosphate linked to the vitreous matrix.

Methods:

The adsorption density of CTAB, on the glass surface, was determined by adding one gram of ground sample in 100 ml of double distilled water with the desired CTAB concentration. The pH was adjusted using HCl and NaOH. The suspension was shaken for 1 h at controlled temperature of 25 ± 1ºC. Then, the samples were centrifuged at 15000 rpm for 15 minutes. The total organic carbon,TOC, (residual concentration) in 40 ml of

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supernatant was determined using a 'Phoenix 8000' Total Carbon Analyzer" instrument. The average of three readings was taken as a measure for the residual concentration of organic carbon. The adsorbed amount was then calculated as the difference between initial and residual concentrations.
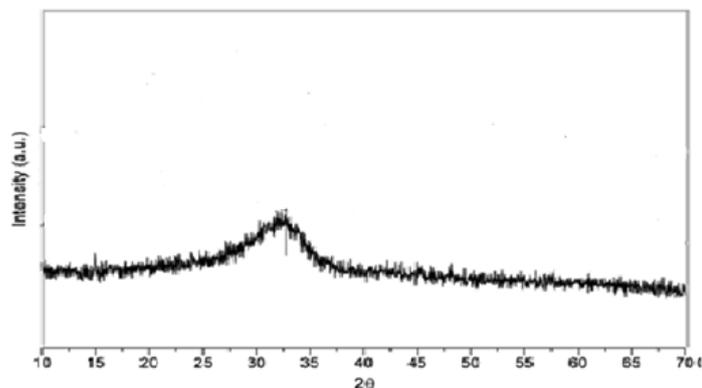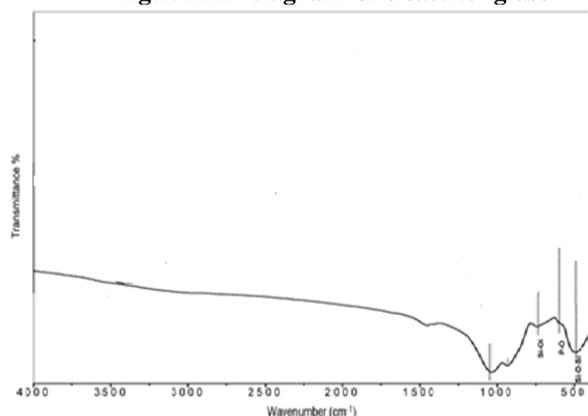

Fig.1. XRD diagram of bioactive glass

Fig. 2. IR spectra of bioactive glass

The solution turbidity was measured using Turbidity Meter, HI 93703, Microprocessor, HANNA Instruments. A 0.5 gram of BG was stirred in 100 ml double distilled water with and without CTAB at a pulp density of 1% solid. After pH adjustment, the pulp was stirred for 20 minutes with magnetic stirrer. The suspension was, then transferred to the measuring cell. The suspension was inverted 10 times at $180°$, left for settling and readings of turbidity are recorded with time.

A laser Zeta-meter, Malvern Instrument model Zeta Sizer 2000, was used for zeta potential measurements. In these measurements a 0.01 gm of BG was placed in 50 ml double distilled water with a known concentration of CTAB at ionic strength of 2×10^{-2} M KNO_3 as an indifferent electrolyte.

Results and Discussion

Adsorption of CTAB at the bioactive glass-water interface

Effect of CTAB concentration

A series of quantitative adsorption measurements were carried out over a wide range of CTAB concentrations, fig.(3). The adsorption density increases sharply reaching a maximum around the critical micelle concentration, CMC, of CTAB (≈ 1 mM) [16]. Beyond the CMC the adsorption density declines rather to lower values at high concentrations of CTAB. The maximum adsorption density is 8.7 μmole/m^2 . This value amounts to 263.6% of the monolayer coverage which points to multi-layer-adsorption on bioactive glass sample. The monolayer capacity is 3.3 μmole/m^2 of the BET area based on a cross-sectional area of 49 $A°^2$ for CTAB molecule, [17]. The area occupied by each CTAB molecule at the maximum adsorption density is 19.33 $A°^2$.

Inspection of the above results reveals a high affinity of the BG sample towards CTAB. This was due to the fact that CTAB is a strongly ionized surfactant with a positive charge on its inorganic head group. This group will be attached to the negatively charged BG surface, fig. (5), under the influence of the electrostatic attraction forces. It was suggested that CTAB adsorption was driven by electrostatic force at low concentrations and as the surface excess increases and lateral hydrophobic interactions between long alkyl chains of adsorbate come to play, led to the formation of surface aggregates (termed hemi micelles), Figure (4). This was followed by the fast increase in surface excess, [18], till reaching CMC of CTAB. Beyond this point, the adsorption density decreases with increasing CTAB concentration. This was due to the formation of CTAB micelles, progressively, consumes the CTAB species adsorbed on the bioglass surface. Besides, association of surfactant species on the solid surface begins to occur before the CMC, leading to the formation of hemimicelles which are physically adsorbed onto the glass surface. As the forces of physical adsorption are relatively weak, these species may be desorbed again taking the originally adsorbed species to the bulk solution. At and beyond the CMC these hemimicelles are consumed in forming micelles in the electrolyte. Both factors results in a decrease in the adsorption density on the glass surface.
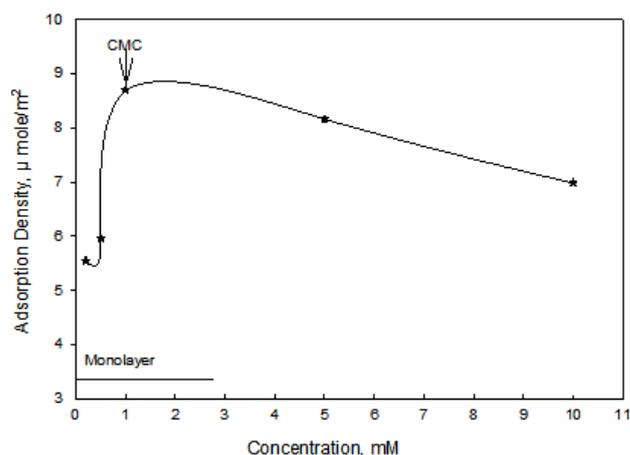

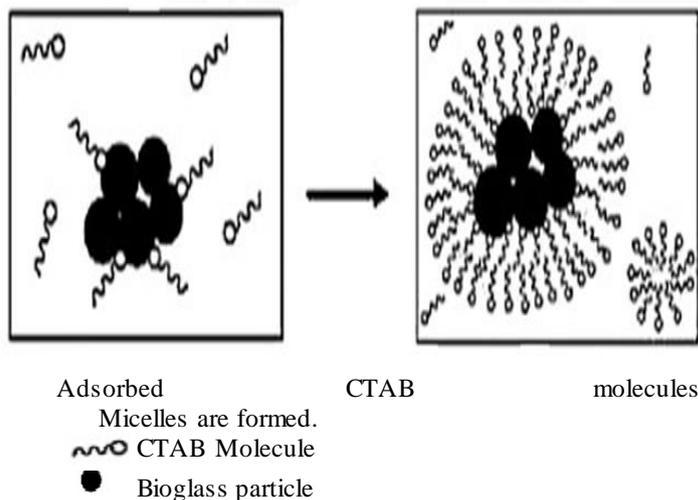**Fig.3. Adsorption Isotherm of CTAB on Bio-active Glass**

Adsorbed   CTAB   molecules Micelles are formed.

CTAB Molecule

Bioglass particle

Fig. 4. A schematic diagram represents the adsorption of CTAB molecules on the BG particles
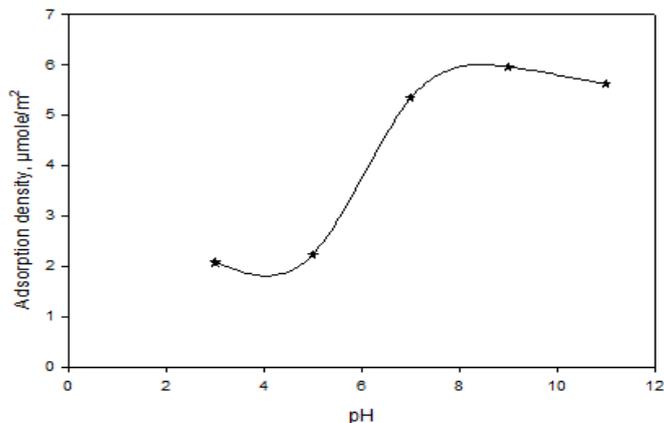
Fig. 5. Adsorption of CTAB on bioglass as a function of solution pH using 0.5 mM CTAB solution.
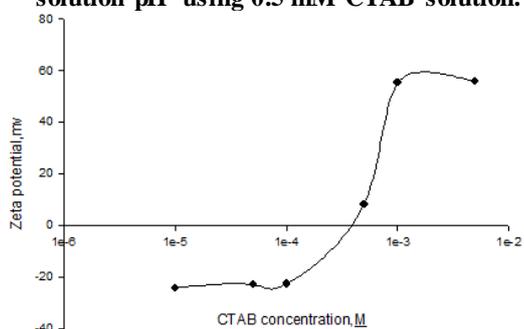

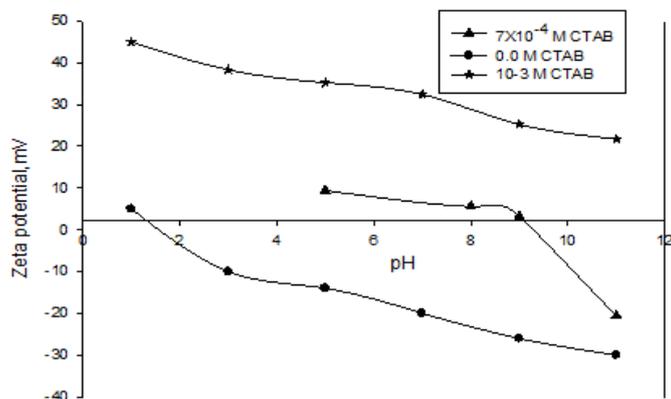**Fig. 6. Effect of CTAB concentration on zeta potential of bio-glass at pH 9**

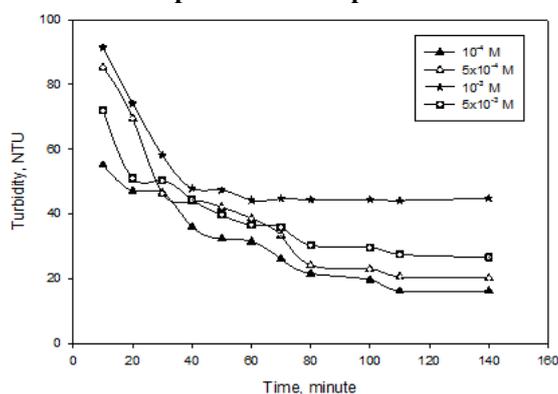**Fig. 7. Effect of solution pH on the zeta potential of the BG particles in the presence of CTAB**

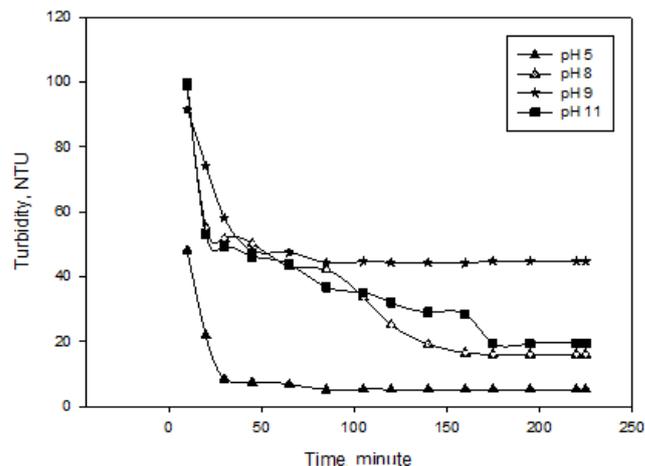**Fig. 8 Effect of CTAB concentration on bio-glass turbidity at pH 9**

Fig.9. Effect of solution pH on bio-glass turbidity using 7×10^{-4} M CTAB

Effect of pH

Surfaces possessing highly charged groups in aqueous solvents are especially sensitive to environmental conditions such as pH. This usually causes marked changes in the adsorption of ionic surfactants onto charged solid adsorbents. As the pH of the aqueous phase is lowered, a solid surface will usually become more positive due to the adsorption of protons from solution onto the charged sites. This will decrease the adsorption of cationic surfactants. The reverse is true when the pH of the aqueous phase is raised, [19].

CTAB consists of a long hydrocarbon chain bonded to a head group where the charge of this head group plays an important role in the dependence of adsorption of surfactants on pH. Since CTAB is a cationic surfactant will require negative sites, to be present on a mineral surface, before adsorption can occur. Adsorption would be expected to increase by increase of negative sites and vice versa. Fig. (5) shows the adsorption of CTAB on glass as a function of pH which is related to the ionic species present in the system. At low pH values i.e. pH< 6, there is no significant increase in adsorption density. In this case, the mineral surface has excess positive sites. Hence, the adsorption density would be low as a result of the repulsive forces between these sites and the CTAB species which are positively charged. As the pH increases, the acidity decreases and so also does the density of positive sites on the mineral surface. Consequently, the adsorption density of CTAB cations increases. At high pH values the adsorption of CTAB from aqueous solution is almost doubled. For example, at aqueous phase concentration of 0.5mM CTAB, the adsorption density increases by a factor of 2 from pH=5 to pH=7.This may be attributed to specific adsorption of negatively charged OH⁻ ions on the glass surface. However, the glass surface becomes more favorable towards the adsorption of positively charged CTAB surfactant molecules.

Zeta Potential Measurements

Surface charge is an important property of a solid, since it can determine what can adsorb, penetrate or adhere. Zeta potential measurements are an indicator of the charge of the particle's surface.

Effect of CTAB concentration

Fig. (6) shows the zeta potential of glass to which CTAB has been sorbed as a function of solution concentration. It clearly shows that there are specific binding between bio-glass particles and surfactant. It is seen that zeta potential changed from -22 mV to +60 mV when CTAB concentration was increased from 10^{-5} M to 10^{-3}M. At low CTAB coverage (< 7×10^{-4} M), glass particles are negatively charged. As the

aqueous phase concentration increases, the glass reaches an iso-electric point. This point corresponds to monolayer coverage of CTAB on the glass. As the aqueous phase concentration increases further, a bi-layer of CTAB is formed and the glass becomes positively charged. These results indicate that CTAB is bound preferentially to bio-glass particles in a way that the particles surface charge is changed. This can be explained as follows. When the glass particles are dispersed at concentration <7 X 10^{-4} M of CTAB solution, the positive head groups of CTAB are selectively adsorbed onto the negatively glass surface due to ionic interactions. Such a process yields net surface charge of CTAB coated glass = zero. On increasing the concentration of surfactant molecules, a monolayer is formed with hydrophobic tails away from the surface. The remaining surfactant molecules form a double layer through hydrophobic interaction between tails and the positive head groups facing the aqueous phase which gives net positive charge to the final structure.

Effect of solution pH

Variation of zeta potential with equilibrium pH of glass suspensions in CTAB solution prepared in 10^{-3}M NaCl solution is show in fig.(7). As seen from this fig. the glass surface has negative zeta potential without addition of CTAB and the iso-electric point is located at around pH 2. On the other hand, in the presence of 10^{-3} M and 7×10^{-4} M CTAB the charge of the glass surface turns to positive. However, the positive value of zeta potential can be attributed to charge neutralization of the negative sites on the glass surface with CTA^{+} ions. The fact that the zeta potential of the glass is negative at pH >10, on using 7×10^{-4} M CTAB concentration, may be due to the existing of high negative charge sites on glass surface. In this case CTAB concentration is not enough to neutralize such negative sites.

Turbidity measurements

The stability of particle and colloidal slurries is an important phenomenon in many industries such as paint, printing ink, pharmaceutical, etc. Particle settling, which destabilizes the suspension, is often caused by the shielding of surface charges on the particles which would result in coagulation and subsequent settling. It has been found that the effects of addition of conventional stabilizing agents (e.g. ionic surfactants, polymers) increase the stability of the particle, [20].In this research turbidity will be used as a parameter to reflect the degree of dispersion where a higher value of turbidity represents higher dispersion efficiency.

Effect of CTAB concentration

Dispersion of bioglass sample, with CTAB, was determined using turbidometry measurements. As seen from fig. (8) dispersion of the bioglass depends on CTAB concentration. The dispersion, increases with concentration and reaches a maximum value at 7×10^{-7}M of CTAB. A sharp decrease in the dispersion is observed at a concentration of 5×10^{-3} M . If we combine the results of the dispersion measurements with those of corresponding adsorption and zeta potential, the dispersion determination may be explained as follows. At low CTAB concentrations, before the formation of surface aggregates on the bio-glass surface, it is suggested that the adsorbed CTAB molecules distribute randomly on the surface with their hydrophobic tails lying parallel to the substrate plane. In this case, the surface free energy is relatively low with moderate dispersion values of the particles. As the surfactant concentration increases, more surfactant molecules are adsorbed at the water/bioglass interface with their hydrophobic tails facing toward the aqueous phase. This causes further decrease of surface free energy and increasing dispersion until the formation

of a compact surfactant-adsorbed monolayer. Consequently, the hydrocarbon chains of the adsorbed CTAB molecules may gather the hydrocarbon radicals found in solution forming a second layer of CTAB with the positively charged head groups protruding out into the solution. In this case, the BG particles become hydrophilic causing a decrease in glass dispersion as can be noticed on using 5×10^{-3}M CTAB. Also, it is seen from the zeta potential measurements, fig. (6), that the concentration of CTAB at which the BG surface charge changes sign from negative to positive coincide with low dispersion of BG particles implying that the BG surface become strongly hydrophilic again.

Besides, for CTAB concentrations equal to 1mM, where it is postulated hemimicelle adsorption is dominant, the zeta potential is about 60 mV.This high potential results in substantial electrical double-layer repulsion and stable suspensions. When the surfactant concentration is 5×10^{-3}M, poor dispersion obtained despite both significant adsorption and zeta potential values. This suggests that the steric repulsion due to adsorbed CTAB is insufficient to overcome the van der Waals attraction.

Effect of solution pH

The effect of solution pH on bioglass dispersion, using 7×10^{-4}M of CTAB, is shown in fig. (9). This figure shows that at low pH the BG surface is highly hydrophilic. A comparison of fig. (9) and (5) shows that this portion corresponds to the region where CTAB adsorption is < monolayer and the surface free energy is relatively low with moderate dispersion values of the particles. As pH increases dispersion increases and go through a maximum at pH 9 corresponding to the i.e.p. of the BG coated surface with CTAB. As the BG surface becomes negatively charged, i.e. high pH, turbidity was decreased where the formation of micelles is favored, [21]. Micelles, by virtue of their structure, tend to be hydrophilic where particles dispersion decreases as found at pH 11, fig. (9). Besides, at high pH values, the thickness of the solvation layer around the ionic head group of CTAB decreases. This will increases the hydrophilicity of the BG particles results in decreasing the solution turbidity.

Conclusions

Dispersion of bioactive glass particles was studied in the presence of CTAB. The dispersion of the particles was examined using turbidity, zeta potential and adsorption. Dispersion of the particles was largely affected by CTAB concentration and the solution pH. Adsorption of CTAB was achieved through, mainly, ionic interactions and consequent bilayer (or multilayer) formation on particle surface. However, at low CTAB concentrations a monolayer is formed, due to interaction of opposite charges on the glass surface and the surfactant ions. At higher concentrations a different mechanism (probably hydrophobic bonding) causes a bilayer to be built up. The obtained results would be of ultimate importance in selecting a given reagent at a given solution pH and equilibrium concentration yielding a stable suspension to enable biological applications.

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