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Preparation of Bioglass/Chitosan Composite Incorporated with Dexamethasone by Sol-gel Method.

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IN THIS STUDY, we used the sol-gel method to prepare a composite of Bioglass/chitosan doped with dexamethasone (Dexa) as an anti-inflammatory drug. A solution of 1 wt.% chitosan was prepared with 1% acetic acid (v/v), and was added to a mixture of tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP)and Ca $(NO_3)_2.4H_2O$ as arrow materials of ($65\%SiO_2$-$25\%CaO$-$10\%P_2O_5$). Finally, different percentages of the drugs (5,10,15 wt.%) were added as follows BG/CH5D, BG/CH10D and BG/CH15D respectively. Thermal behavior and surface structure were investigated with different techniques. Bioactivity of all samples was determined after investigate in the simulate body fluid (SBF). Both the pH and the concentration of some ions such as calcium and phosphorus were measured. The results showed a layer of hydroxy apatite (HA) was deposited on the surface of all samples, but this layer decreased as the dexamethasone concentration increased due to cross-linking between the drug with (BG/CH) composite which confirms its biological activity despite carrying the drug used Dexa.

Keywords: Bioactive glass, Chitosan composite, Drug delivery system, Dexamethasone.

INTRODUCTION

Chitosan (CH) has been extensively used in different biomedical applications, such as carrier of controlled drugs, in the manufacture of artificial membranes and orthopedic applications[1]–[3]. Chitosan based bone graft substitutes are biocompatible, biodegradable, osteo-inductive, osteo-conductive and structurally similar to bone, with excellent cost effectiveness and mechanical strength. Chitosan based hydrogels and wound healing bandages have also establish a great market in the field of medicine. More recently, chitosan has gained popularity for its use as a matrix molecule for drug delivery and also finds an upcoming utility in the area of dentistry[4]. Chitosan and its derivations have broad wide properties such as antibacterial, antimicrobial, coagulating, and drug delivery which have gained numerous attentions. Disadvantages of chitosan including weak mechanical properties, and low chemical and thermal stability[5]–[7].Polymers used in bioactive glass/polymer composites can improve the mechanical and physical properties of bioactive glasses and can also modify drug release profiles [3]. Hydroxyapatite (HA) can be chosen as biocompatibility symbol and its chemical composition is similar to the mineral part of bone and tooth tissue. Weak mechanical properties limit hydroxyapatite's application[8]. The main aim of this study was to prepare drug delivery system composed of bioactive glass/ chitosan composites loaded with different concentration of dexamethasone and evaluate bioactivity of different samples.

Material and Methods

We prepared and investigated the bioactivity of bioglass/chitosan biocomposite loaded with different concentration of dexamethasone. Where

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the first solution (A) of bioglass (65%SiO_2-25%CaO-10%P_2O_5) which prepared with sol-gel method was added to second solution (B)of chitosan polymer 1%wt. dissolved in 1%(v/v) acidic acid. Dexamethasone with different concentrations (5,10 and 15 %wt.) was added to the above BG/CH composite(A+B). Bioactivity of samples were investigated with FTIR, TGA, DSC, SEM, and EDX before and after immersion in (SBF). Ca^{+2}, PO_4^{-3}ions and pH was examined with UV-Vis spectroscopy after (1,2,4,8,16,21 and 33) days.

Preparation of bioglass / Chitosan solution.

A solution of bioactive glass was prepared with chemical Composition 65%SiO_2, 10% P_2O_5 and 25% CaO, for which tetraethyl orthosilicate (TEOS; Si $(OC_2H_5)_4$ (Assay 99.0%) purchased from **sigma aldrish Company, Canada)** and triethyl phosphate (TEP; $C_6H_{15}O_4P$ (Assay 99.%٢) purchased from **Alfa Aesar Company, Germany)**, and distilled water in ethanol (**Merck**). and Ca $(NO_3)_2.4H_2O$ **(Panreac PRS),** were mixed as defined below: 24.43ml of TEOS was added into 4.22 mL of nitric acid (HNO_3) the mixture was allowed to react for 60 minutes to promote the acid hydrolysis of TEOS. Then, 2.38ml of P_2O_5 was added and mixed during 60 minutes, and finally, 10.74gm of $Ca(NO_3)_2.4H_2O$ was added to the mixture and kept under stirring for 1.5 hour until hydrolysis perfected and polycondensation, following the protocol proposed by sol-gel method as shown in Fig. (1)[9].

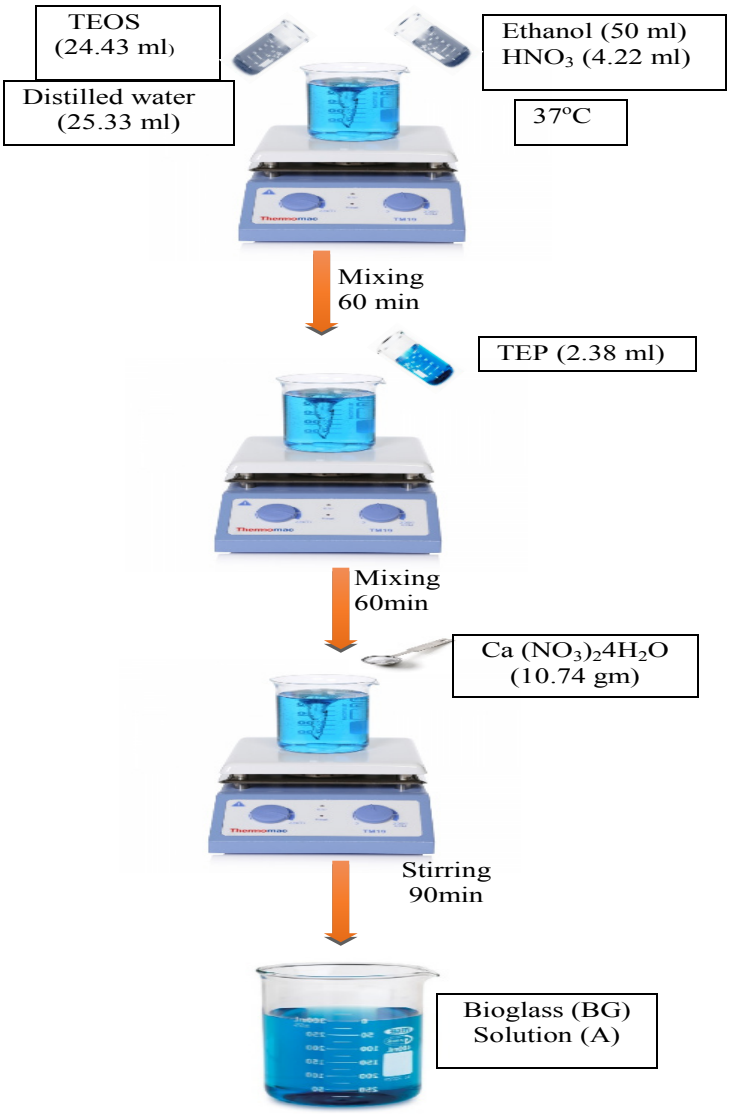

Fig.1. Flowchart for Bioglass preparation steps.

Chitosan solution was prepared with a concentration of 1 % wt./v, by dissolving 1 gm of chitosan powder in a solution of 1% (v/v) acetic acid with stirring during 3 h. Then, TEOS/ HNO_3, P_2O_5 and Ca $(NO_3)_2.4H_2O$ as precursor compounds for bioactive glass production was added to the chitosan solution. The BG/CH precursor's solution was mixed thoroughly for 1.5 hour until fully homogenized. TEP as Crosslinking agent's solutions were prepared to be 1% wt., seen in Fig. (2)[9].

Dexamethasone were found in Three samples with different wt.% of (5, 10, and 15) were added to solution of TEOS/HNO_3, TEP, and Ca (NO_3) as precursor of Bioglass. Subsequently added to a chitosan solution which prepared previously in 1 wt.% of acetic acid. The obtained mixture was stirred for 6 hours and dried for 2 days/120° C as illustrated in Fig. (3).

The prepared composites loaded with dexamethasone are named according to dexamethasone concentration as BG/CH5D, BG/CH10D, and BG/CH15D. All composites were examined with FTIR, SEM, EDX before and after invitro test in (SBF) and characterized with TGA, DSC. as well as calcium and phosphorous ions were evaluated in (SBF) after (1,2,4,8,16,21 and 33) days with UV-Vis spectroscopy.

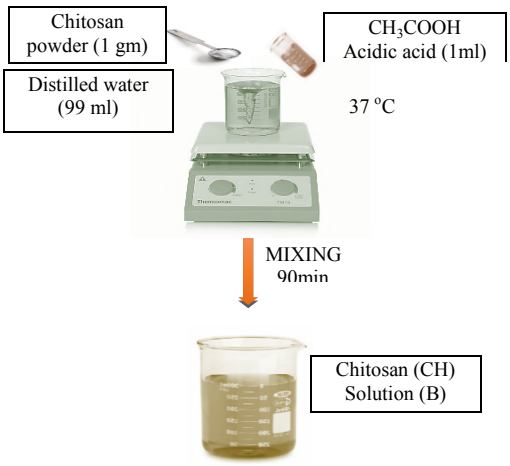

Fig.2. Flowchart for Chitosan preparation steps.

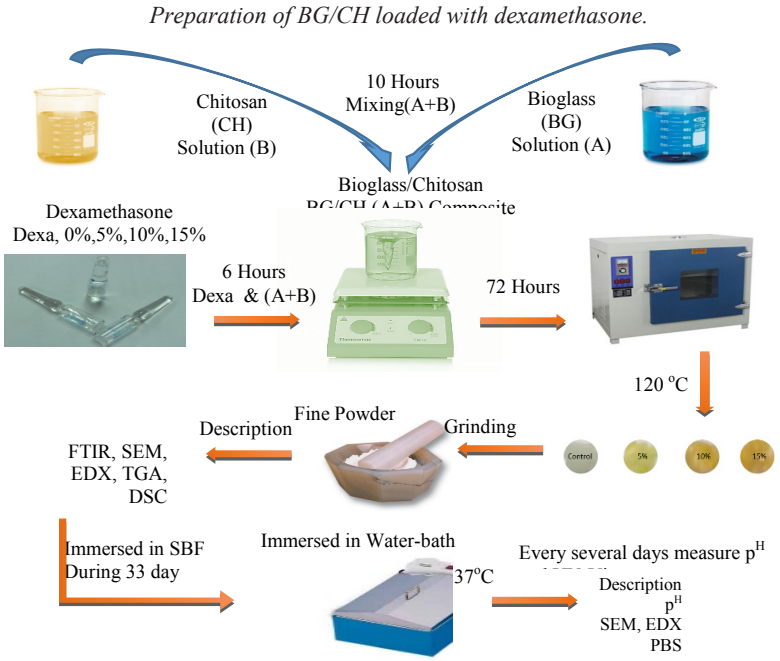

Fig.3. Flowchart for preparation of the BG/CH composite with dexamethasone loaded and investigate steps.

Standard Operating Procedure of (SBF) preparation.

0.5g of each BG/CH composite and BG/CH with dexamethasone was immersed in 50 ml of **(SBF)** with ion concentrations nearly equal to those of human blood plasma **Table (1**) under fixed conditions at 37°C for 33 days, in order to get a homogeneous apatite layer. SBF solution was prepared by adding concentrated solutions of KCl, NaCl, $NaHCO_3$, $MgSO_4.7H_2O$ and KH_2PO_4 to distilled water buffered with tris(hydroxymethyl) aminomethane and HCl to pH 7.4 at 37° C [10].

Characterization techniques:

Thermo Gravimetric Analysis (TGA) / Differential Scanning Calorimeter (DSC)

Thermogravimetric analysis of the various **BG/CH** dexamethasone in a **SETARAM DTA-TG labsys™**Evolution-1750 equipment), with α-Al_2O_3 powders as a reference material. Samples of 5 mg crushed powder were put into an Al_2O_3 crucible and the measurements were performed in the temperature range 23–1000˚C (heating rate 10 ˚C/min) in air. The data was baseline corrected by carrying out a blank run and subtracting this from the plot obtained instrument. The sample was heated in flowing Argon gas atmosphere at a heating rate of 10ºC/min. The weight loss measurements were also done in the same instrument and both the graphs were merged into one for comparative analysis. The surface morphology of the resultant composite before and after immersion in SBF, was performed by using **Philips XL 30** scanning electron microscope (SEM), with an accelerating voltage of 30 kV. Specimens were placed on a stub using a carbon sticker and examined under the microscope.

A 2 ml sample of SBF is taken from each test tube after 1, 2, 4, 8, 16, 21 and 33 days from the beginning of the immersion. And stored frozen until analyzed for determining the concentration of Ca, P and dexamethasone release as a drug concentration increase by a time by using UV-VIS spectroscopy (**JASCO v-630**).

The dissolution (Cation release) of Bioglass (BG), bioglass/chitosan (BG/CH) and various ratio of (BG/CH) dexamethasone were tested by soaking in 50 ml of (SBF). The specimens within plastic containers were immersed in a thermodynamic (shaking–water bath at constant separate then incubated for 33 days at 37˚C). All the test materials were present in triplicate. Thus, there were altogether 3 test tubes for each material. The pH of the SBF solution was measured using **Jenway 3510** pH meter. The change in pH of the SBF with and without the modified by dexamethasone was recorded at pre-determined time intervals.

Results and Discussion

Thermo Gravimetric Analysis (TGA)

The knowledge of the thermal behavior of organic-inorganic materials is of great importance, in determining their best processing conditions. Thermo gravimetric analysis (TGA) is an important tool for thermal stability studies of materials. It allows determining the temperature range at which a heated sample undergoes a major conformational change by means of monitoring the thermal weight loss profile.

TGA analysis was employed in the evaluation of the thermal stability of Bioglass/chitosan Composite that obtained by sol–gel process (10 ˚C /min in nitrogen atmosphere).

Fig. (4): Shows TGA curves (25-1000 ˚C temperature range) of BG/CH composite as a function of TEOS content. In order to follow some significant effects, the weight loss was studied in three temperature intervals: 0-147˚C (I), 148-235˚C (II), 236-555˚C (III).

The initial weight loss (Region I), in Bioglass/Chitosan and bioglass/Chitosan with dexamethasone is considered to be the result of the elimination of ethanol and water [11]. The weight loss in BG/CH sample occurs at 50 up to 148˚C (31.14% weight loss), whereas in the BG/CH with dexamethasone, this loss starts from 50˚C to 148˚C. The quantity of water and ethanol that lost from the samples are losing, increases with the decreasing of dexamethasone ratio. This result agree with more tendency to hydrolysis with decreasing dexamethasone content.

TABLE 1. Comparison of the ionic concentrations in blood plasma and the theoretical one in SBF (mmol l^{-1}).

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Bloodplasma	142.0	3.6–5.5	1.0	2.1–2.6	95.0–107.0	27.0	0.65–1.45	1.0
SBF	142.0	5.0	1.0	2.5	126.0	10.0	1.0	1.0

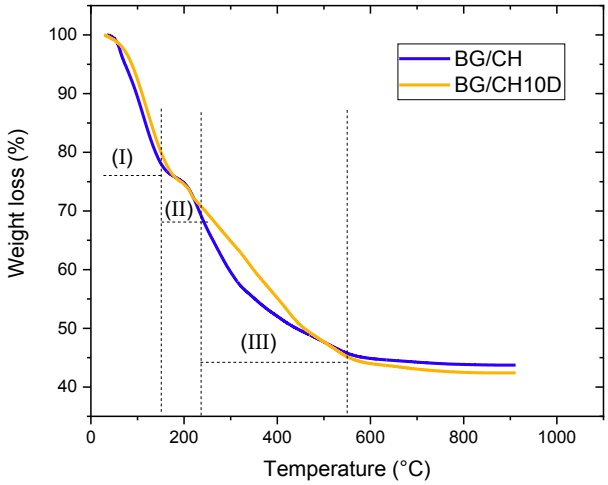

Fig. 4. TGA curves of BG/CH and BG/CH10D samples.

The second weight loss (Region II), is due to the partial thermal degradation of organic matter (The Chitosan) in the BG/CH composite. The elimination of organic matter in BG/CH sample occurs from 148 up to 235 ˚C with (8.5% weight loss), whereas in the Bioglass/chitosan with dexamethasone, this loss starts from 145 ˚C to 235 ˚C. The elimination of organic components from the hybrids start at 150 ˚C, this indicates that, in order to avoid the elimination of organics from the samples, the composite should be cured while being prepared at a temperature which does not exceeds 150 ˚C. In other words, 120 ˚C is a suitable temperature for curing these hybrids[12], [13].

The third weight loss stage (Region III) at 236-555˚C (23.5% weight loss) is due to the complete burning of organics in the hybrid [14]. The TGA curves became flat at 600-1000˚C because the organic component had been completely removed, leaving behind only an inorganic component as SiO_2 and Ca $(NO_3)_2.4H_2O$.

From the TGA data, it is clear that, as the hydrocarbon chain content increases, the weight loss increase. In addition, 120˚C is a suitable temperature for curing these hybrids.

Differential Scanning Calorimeter (DSC).

It is important to study the structure of bioactive glasses to understand its properties. Many of the physical properties, such as the crystallization temperature and solubility are intimately related to the alkali metals content and to their contribution in the structure.

Thus, a better knowledge of the structural role of the alkali metal components in particular will lead to an improved understanding of the relationship between structure and properties. So that, thermal analysis was performed on the synthesized Bioglass/Chitosan (BG/CH) composite and BG/CH composite with dexamethasone (BG/CH10D) to find out the major phenomena occur during sintering. DSC trace is a plot of heat changes of the composite as a function of temperature and used to determine temperatures at which phase transitions occur.

Fig. (5) shows that the DSC trace exhibited two endothermic peaks and three exothermic peaks for the two samples.

The first broad endothermic peak, which initiated nearly at room temperature to reach at 135°C and 149°C for BG/CH and BG/CH10D respectively, consistent with the consumption of heat energy to release the physisorbed water and the pore liquor (water and alcohol by-products from the polycondensation reaction) that were not removed during drying[15]**.** The observed endothermic peaks in the range of 85–149˚C is attributed to the loss of the residual solvent (water and ethanol)[16]**.** All the DSC curves show endothermic peaks in the range of temperature of 50 to 149˚C. The presence of such peaks was attributed to the loss of volatile components or the possibility of chain relaxation. Furthermore, in this temperature range is also verified the breakdown of hydrogen bonds which are present in chitosan structure and other molecular associations [17]**.**

TABLE 2. Thermogravimetric Results Obtained fof Bioglass / Chitosan Free and Dexamethasone

	TGA	
Sample	**Weight loss % at 600 °C**	**Residue 50 % at °C**
BG/CH	65.2	440.9
BG/CH10D	65.01	457.5

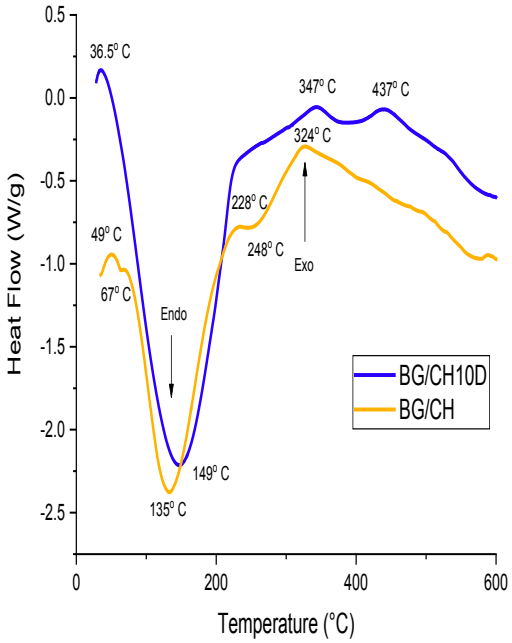

Fig. 5. DSC thermo analysis curves of BG/CH and BG/CH10D samples.

The second endothermic peak, observed at 248°C corresponds to the pyrolysis reaction of free organic species (ethoxy group) and/or the release of the resulting water from the two reactions: the first is the further condensation of silanol groups (Si---O--- Si) between (Si---OH) and (OH---Si), the second is also condensation of phosphate group (P---O---P) between (P---OH) and (OH---P*)* [18]**.** This silanol group of the colloidal silica gel is thought to be changed to SiO_2 via two steps like the evaporation of water and ethanol and the decomposition of organic residues like $SiO–C_2H_5$ derived from the freest reactions of silica gel network surfaces with ethanol solvent [19].

The exothermic peaks, curve of pure Bioglass/ Chitosan exhibits a weak exothermic peak at 324°C, which is attributed to the degradation of that Chitosan and the curve of BG/CH10D with dexamethasone there is another peak appeared at 347°C to collapse the chemical bond between the BG/CH and dexamethasone.

Two exothermic peaks are also observed at 347 and 437°C related to the thermal decomposition for amine and acetyl residues, characteristic for BG/CH10D sample [20].

Biological Protein adsorption

The chemical composition and surface charge of biomaterial surfaces strongly affect the protein adsorption behavior[21]. This is perhaps through interaction between the functional groups on the samples surfaces and those of the protein itself. After immersion of the samples in 10 mg/ml Bovine Serum albumin solution in PBS, adsorption was allowed to proceed in an incubator for 2 h at 37 ˚C.

Fig. (6) shows spectral decomposition of amide I band and amide II band for Bovine Serum Albumin (BSA) adsorption through the surfaces of samples, (a) β-sheet at 1680 cm-1, (b) α-helix at 1653 cm-1, (c) β- sheet at 1627 cm-1, (d) β-sheet at 1555 cm-1 (e) β-sheet and random at 1524 cm-1, (a, b, c peaks for amide I band, d, e peaks for amide II band) [22].

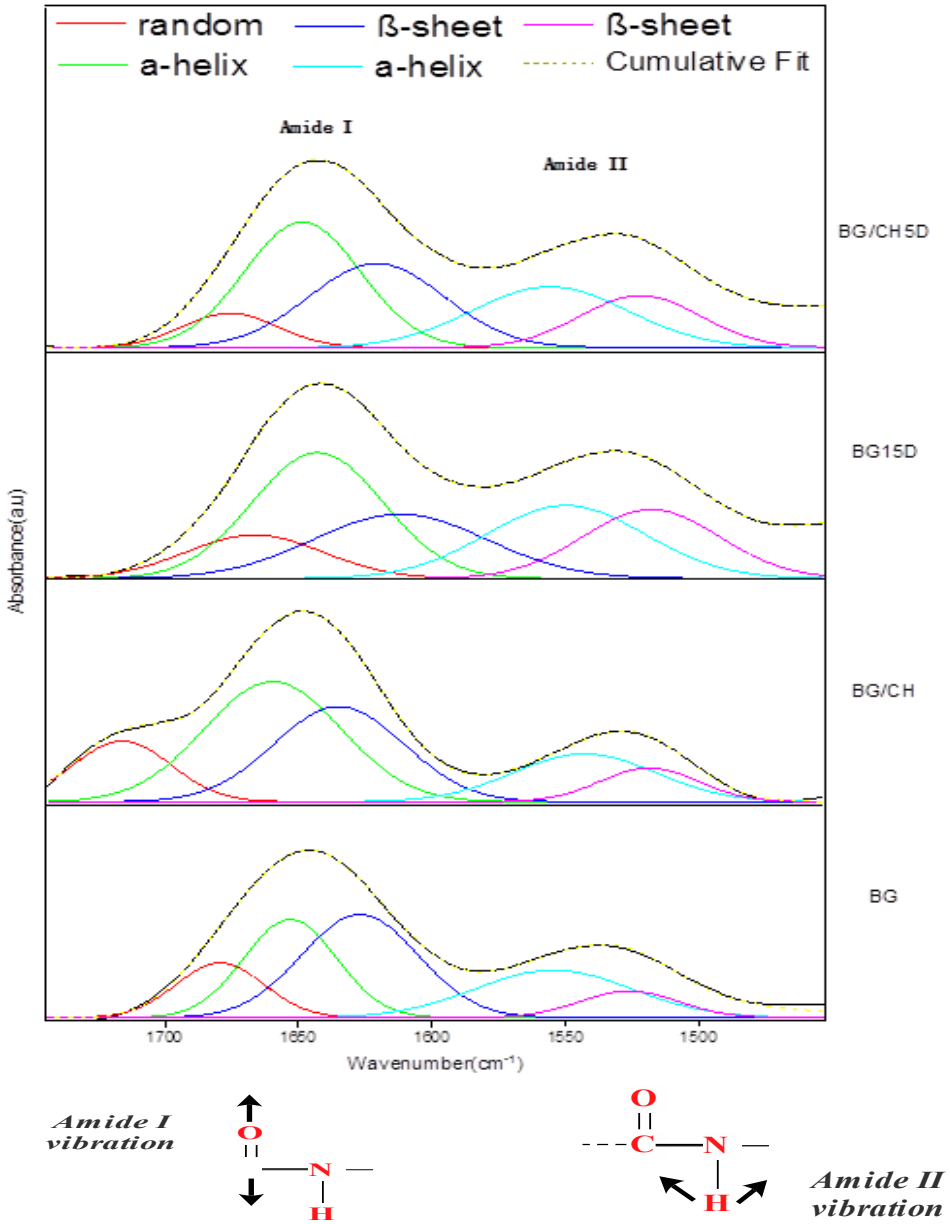

Fig. 6. FTIR spectra of the Amide I and Amide II bands of BSA adsorbed on the surfaces of the samples.

There was an increase in the ratio of the Amide I to the Amide II on all the surfaces of bioglass sample (BG) due to adsorption. According to the popularly accepted understanding, electrostatic interactions are very important for protein adsorption [23].

The ratio between Amide I and Amide II respectively increased in BG sample and decreased gradually in BG/CH5D, BG/CH10D and BG/CH15D samples.

The increased of surface negativity of the Bioglass (BG) due to the amount of hydroxyl group enhanced the reactivity of the BG indicating that strongly protein adsorption due to the interaction between the amine group NH_2 of the bovine serum albumin (BSA) with the OH group of Bioglass surface and the interaction between Chitosan and Dexamethasone (Dexa) by nucleophilic addition reaction[24]. For Dexa immobilization, the amino groups of Chitosan surface were reacted with carbonyl groups of Dexa to form the Dexa-Chitosan formation shown in Fig (7).

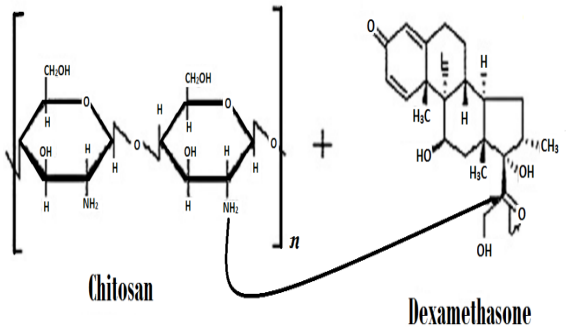

Fig. 7. Interaction between Chitosan and Dexamethasone [24].

Negativity of the surface of BG/CH and BG/CH loaded with dexa decreased due to the reactivity with carbonyl group of dexamethasone with Bioglass and chitosan, which blocked the negative sites on the surface. From the results in Fig (4.11) it can be concluded that the reactivity of protein with BG/CH5D, BG/CH10D and BG/CH15D decreased gradually due to decreasing of negativity of the surface, which decreased by interaction with carbonyl group of Dexamethasone.

Morphological analysis of samples (SEM & EDX).

Dexamethasone does not seem to produce detectable changes in the morphology of the polymeric matrix. Also, from EDX analysis results, the presence of Si, Ca and P indicating that BG was conducted to chitosan matrix. The bone-bonding capability of a composite is determined by its ability to formation of apatite layer on its surface upon immersion in SBF solution for (33 day), a part from some exception where as it is possible to a material to be directly bonded to living bone without the formation of apatite on their surface [25].

The EDX data reveals that the silicon (Si) ions in the composite surface showed slightly decreasing after 33 days in SBF, followed by an increase in calcium (Ca) and phosphorous (P). The increases in Ca and P, accompanied by a decrease in the concentration of Si on the surface of the composite sample, prove the development of apatite. As it is observed in EDX spectra of samples after immersion along 33 days in SBF [26].Also, the association of chitosan with bioactive glass improved the formation and the crystallization of hydroxyapatite layer.

It is considered that the porous structure of BG/CH biocomposite microsphere should be responsible for the formation of a dense apatite layer. From the EDX analysis results, the presence of Si, Ca and P indicating that BG was conducted to chitosan matrix and on the surface of composite it can be demonstrated, as shown in Fig (8).

The BG/CH biocomposite with a high surface area to volume can facilitate the transport of Ca^{2+} and PO_4^{3-} ions from the physiological fluid onto the surface of BG/CH biocomposite to form a dense apatite layer. These porosity of BG/CH deceased as dexamethasone concentrations increased in BG/CH10D and BG/CH15D than BG/CH5D, which decrease the formation of apatite layer shown in Fig (9).

Calcium concentration

The change in concentration of Ca ions can reflect the competition condition between the rate of dissolution and precipitation processes. At first, the concentration of calcium in SBF solution is approximately 100 mg/l. The Ca concentration was found to be increase more than 100 mg/l this means that the dissolution rate is faster than the precipitation rate, while the Ca concentrations showed values less than 100 mg/l.

Fig (10) shows the change of concentration of Ca ions after soaking of BG/CH loaded with Dexamethasone in SBF solution. The increase in the Ca concentration observed for all specimens from the first day up to the eight day in SBF is attributed to dissolution of the bioglass phase. The rate of dissolution started to be gradually decreased from day 8 up to 33 days of immersed in SBF.

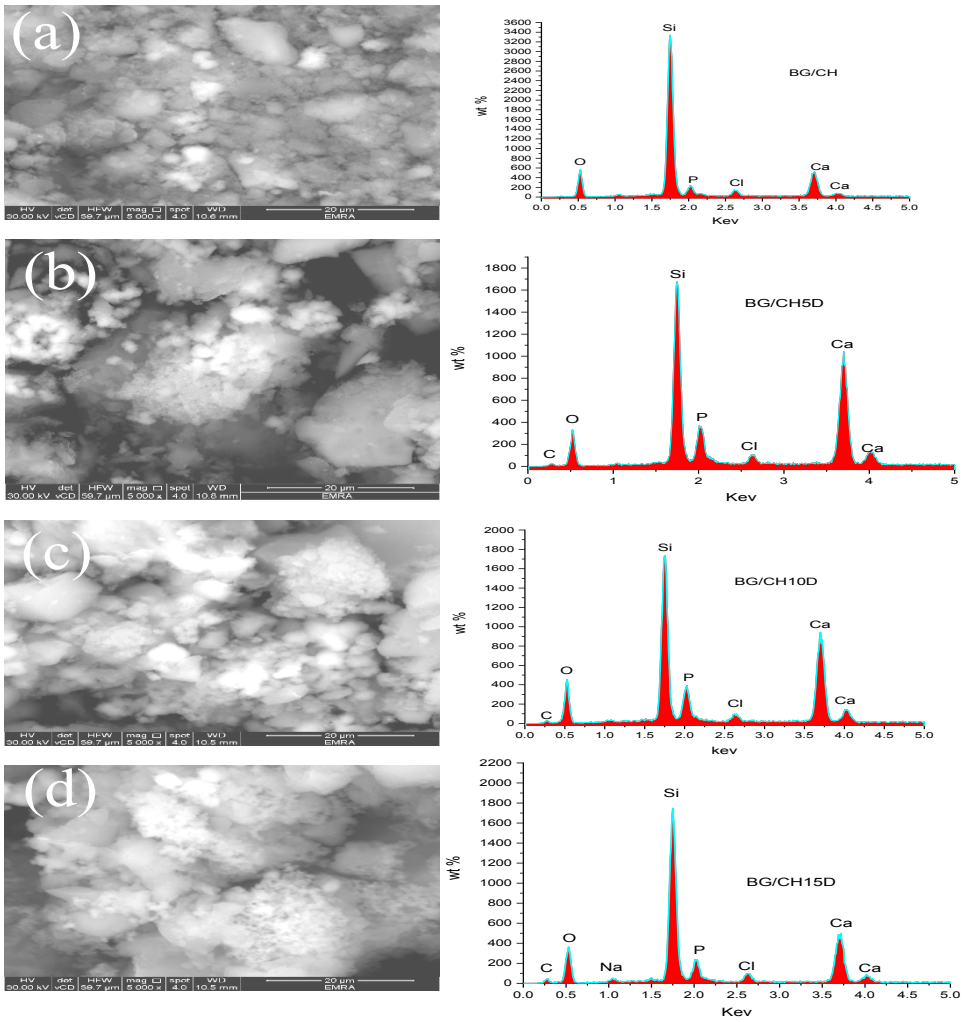

Fig. 8. SEM image – EDX spectrum of (a) BG/CH (b) BG/CH5 (c) BG/CH10D (d) BG/CH15D after immersion in SBF for 33 days.

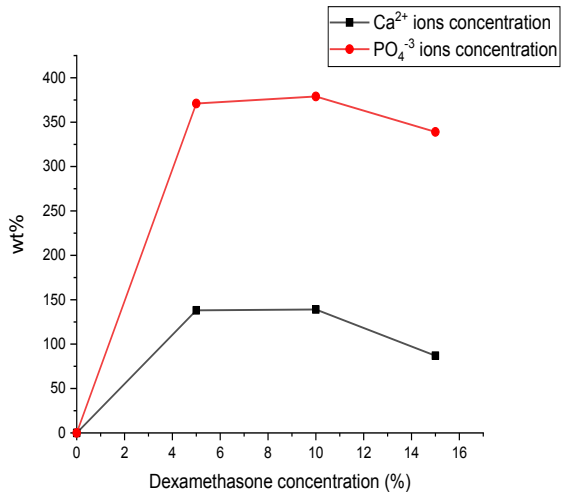

Fig. 9. Release profile of dexamethasone in terms of the percentage (%) as a function of Ca and P concentration.

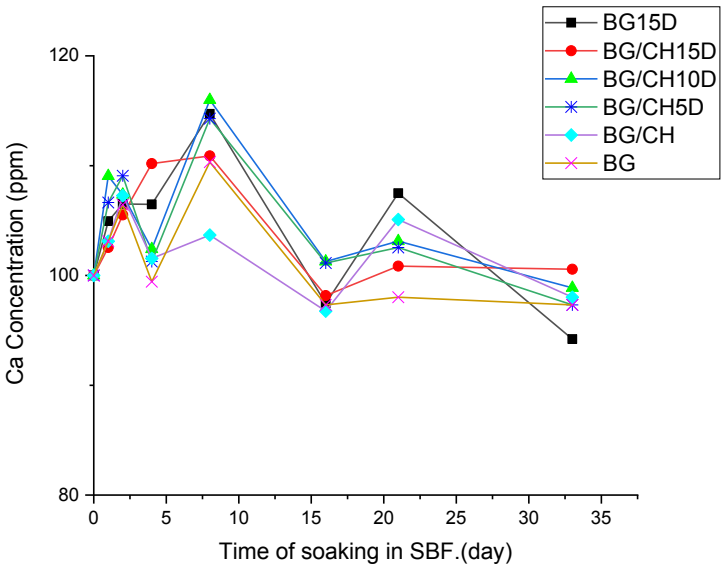

Fig. 10. The concentration of Ca2+ ions after soaking in SBF solution for 33 days.

The calcium concentration seems to increase at the initial stage for all the BG/CH composites. This increase in calcium concentration is induced by the release of calcium ions from the BG/CH composites. The decrease of calcium concentration from 8 up to 33 days may be due to the consumption of calcium ions through apatite formation on their surfaces[27].

The decreasing in the Ca^{2+} ions concentration in SBF is attributed to the rapid growth of the apatite nuclei formed on the surface of the BG/CH composite that overcome the release rate of calcium ions to the solution. The pH varied according to Ca^{2+} concentration variations, because of Ca^{2+} ions in the BG/CH composite exchanged with H^+ or H_3O^+ in the SBF[28]. This means that a layer of apatite formed in all samples, but the surface area of it decreased by increasing the dexamethasone concentration.

Phosphorus concentration

Fig (11) shows the concentration of P^{5+} ions after 33 days soaking of the composites in SBF solution. Before immersion phosphate ion concentration in SBF is 30 ppm only. After immersion of the samples in SBF for 33days, at 24 h it is noted that phosphate concentration in all BG/CH composites increased up to high values due to release of phosphate from these samples to the solution, but it was gradually decreased after 24h up to the fourth day to reach low values indicating the consumption of phosphate in the formation the layer of crystalline HCA on the surface of BG/CH composites.

After soaking in SBF phosphorus concentration increased rapidly from 4 up to 10 days, then it showed approximately a constant value from 10 up to 33 days. This decrease in phosphorus concentration maybe due to the consumption of phosphate ions through the formation of apatite on the surfaces of BG/CH composites with chitosan and dexamethasone [29].

pH analysis

The variation of pH values relative to soaking time in SBF of BG/CH and BG/Dexa composites are shown in Fig (12) The dissolution of Ca^{2+} ions from the samples maybe lead to the locally increase of the pH value of the surrounding fluid. In the start and during the four days, pH decreased until reach a value of 7.420 Bioglass (BG), 7.383 (BG15D), 7.302 (BG/CH), 7.316 (BG/CH5D), 7.405 (BG/CH10D) and 7.392 (BG/CH15D).

The pH increased after 4 to 16 days in SBF immersed as a consequence of partial dissolution that gives an idea about the high reactivity of these materials. These facts agree with the formation mechanisms of the apatite layer on BG/CH composite, i.e., in early stages, an interchange takes place between Ca^{2+} and H_3O^+ ions from the solution. Such interchanges provoke an increase in pH that accordingly favors the formation of apatite nuclei on the silanol groups in the BG/CH composites surface.

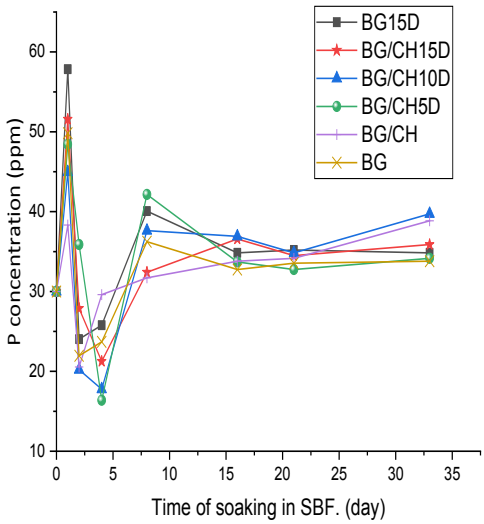

Fig. 11. The concentration of P^{5+} ions in SBF solution after soaking for 33 days.

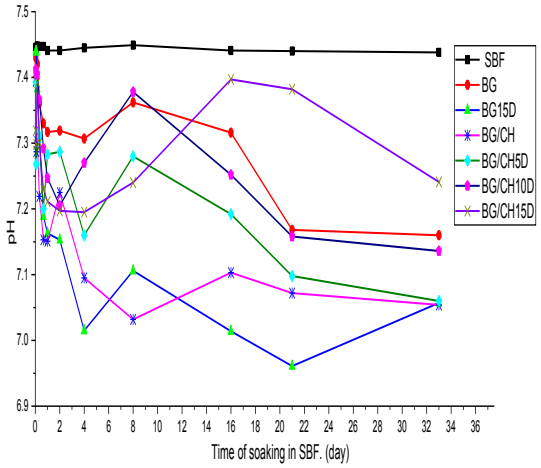

Fig. 12. The change in pH results of BG, BG/CH and BG/CH loaded with Dexamethasone after immersion in SBF for 33days.

The formation of apatite in SBF is strongly pH dependent; the increase in pH actually signifies for the reduction in the concentration of H+ due to the replacement of metal ions in the BG/CH composites and subsequent production of OH- groups due to breaking of siloxane bond. [30]

From the 16 day up to 33-days of immersion in SBF the pH decreased gradually until reach 7.16 Bioglass (BG), 7.057 (BG15D), 7.054 (BG/CH), 7.06 (BG/CH5D), 7.136 (BG/CH10D) and 7.241 (BG/CH15D). The fluctuation in pH values of all samples may be explained when considering the result of two opposite processes:

i. The release of Ca^{2+} from the BG/CH composites.

ii. The consumption of Ca^{2+} due to the formation of apatite layer.

Therefore, when the releasing rate of Ca^{2+} is higher than its consumption rate, the pH will be changed ether by increase or decrease.

It is found from the results that substitution of Ca ions in the BG/CH composites increase the solubility of the BG/CH composites, which subsequently increase the interchange Ca^{2+} and H_3O^+ ions from the solution and increase the pH value.

The pH changes within the body can be used to induce a response since different organs or tissues have different and specific pH value. Table (3) shows the different pH of some organs or tissues within the human body. The presence of ionizable weak

TABLE 3. pH in the different tissues (adapted from [31])

Tissue/ organ	pH
Blood	7.35–7.45
Stomach	1.0–3.0
Duodenum	4.8–8.2
Colon	7.0–7.5
Early endosome	6.0–6.5
Late endosone	5.0–6.0
Lysosome	4.5–5.0
Tumor	6.5–7.2

Conclusion

- Bioactive glass/Chitosan Composite incorporated with Dexamethasone was prepared with sol-gel method.
- Thermal behavior and surface structure were estimated with different techniques (TGA, DSC, SEM and EDX).
- Bioactivity of prepared samples were evaluated in vitro by simulated body fluid
- The results indicated that a layer of hydroxy apatite (HA) was formed on the surface of all samples, but this layer decreased as the dexamethasone concentration increased due to cross-linking between the drug with (BG/CH) composite which confirms its biological activity despite carrying the drug used Dexa.
- Bioactive glass / chitosan composites is low cost and effective biomaterial.

In an upcoming study we will study the exit of the drug dexamethasone with ultraviolet light (UV-Vis spectroscopy) technique.

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إعداد مزيج من الزجاج الحيوي والكيتوزان المحضر بطريقة السول جل مدمج معه ديكساميثازون

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يتألف العظم من هجين من مواد عضوية مثل الياف االكولاجين وغير عضوية مثل مادة الهيدروكسى اباتيت. لذلك واحدة من السمات المهمة والمطلوبة فى تصميم بدائل العظام النشطة بيولوجيا هو أن تتألف من مادة غير عضوية والتى يمثلها الزجاج الحيوى ومادة عضوية مثل البوليمرات والتى تعمل علي تحسين الخصائص الميكانيكية للبديل الحيوي مما يزيد من مرونتها بجانب أنها نشطة حيويا وبالتالي تكون أفضل من السيراميك الحيوي.

والمتطلبات الأساسية لعمل مادة صناعية بديلة للعظام أن يكون لها خصائص صنع روابط مع العظام أي تكوين طبقة من الهيدروكسي أباتيت شبية بالعظام علي سطحها. حيث ان ظهور مادة الهيدروكسي أباتيت يكون مرتبط بوجود مجموعة(Si–OH) علي سطح العينة ويسرع علي تحرير أيون الكالسيوم من العينة إلي السائل المشابة لبلازما دم الانسان (SBF). وبالتالي فإننا قمنا بتطوير جيل من خليط محب للماء من مادة عضوية وغير عضوية بإستخدام TEOS كمصدر غيرعضوي مع بوليمر الكيتوزان كمصدر عضوي و أضفنا الي ذلك الخليط نسب مختلفة من عقار مضاد الالتهابات (Dexamethasone) .تم في هذه الدراسة تحضير العينات كالتالى :

[١] بإستخدام طريقة السول-جيل تم تحضير عينة ضابطه Control Sample فى التركيب من الزجاج الحيوي](Bioglass) (BG)[بالإضافة إلي خمس عينات اخري حيث يضاف العقار ديكساميثازون بنسب مختلة مع نسبه ثابتة من البوليمر الكيتوزان في ثلاث عينات وذلك بنسب مولارية ٥٪,١٠٪ و ١٥٪ بالاضافة الي عينتان اخرتان هذان العينتان احداهما خاليه من العقار ديكساميثازون وبها نفس نسبة البوليمر الكيتوزان والاخري هي عبارة عن الزجاج الحيوي مضافا اليه العقار ديكساميثازون وذلك بنسبة مولارية ١٥ ٪ ولكن بدون البوليمر الكيتوزان واسمائهم كما يلي./(BG/CH, BG/CH5D, BG/CH10D, BG/CH15D)

هذا لفهم التفاعل بين العقار والزجاج الحيوي ومعرفة الي اي منهم كانت تنتمي المركبات المتكونه .

[٢] تم التخلص من الرطوبة والايثانول الموجود بالعينات التى تم تحضيرها وذلك بالمعالجه الحرارية عند ١٢٠م° لمدة يومين في الفرن .

[٣] تم دراسة النشاط الحيوى لهذه العينات باختبارها خارج جسم الكائن الحى (In-vitro test) حيث تم غمس ٠,٨ جرام من كل عينة لمدة ٣٣ يوم فى ٨٠ ميلي لتر من سائل محضر كيميائيا بتركيزات مقاربة لتركيب الأيونات الموجودة في بلازما دم الإنسان (SBF) داخل حمام مائي عند ٣٧م° وعمل بعض القياسات للعينات قبل وبعد الغمر مثل TGA, XRD, FTIR, SEM و EDX وبدراسة المنحنى التثاقلى الحرارى (TGA curve) فى العينات وجد ان الفقد فى الوزن يحدث عند درجات حرارة تبدأ من ١٥٠ درجة مئوية نتيجة الى التخلص الجزئى من المواد العضوية (Organic Materials) المستخدمة فى التحضير، أي أنه لكي نتجنب حرق المادة العضوية من جميع العينات لابد ان يكون التجفيف عند درجة حرارة لا تتجاوز ١٥٠ درجة مئوية بمعني آخر ١٢٠ درجة مئوية هي درجة حرارة مناسبة لتجفيف العينات في الفرن.