

## **The influence of silanisation on the mechanical and degradation behaviour of PLGA/HA composites**

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### **Abstract:**

This study investigates the influence of silanisation on the mechanical and degradation behaviour of PLGA/HA composites. Three different silanes (mercaptopropyl trimethoxy silane (MPTMS), aminopropyl trimethoxy silane (APTMS) and aminopropyltriethoxy silane (APTES)) were applied to HA substrates in order to study the effect of head group (which binds to the polymer) and tail group (which binds to the surface hydroxyl groups in HA). A composite of hydroxyapatite (HA) and poly (D, L lactide-co-glycolide (50:50)) (PLGA) was investigated.

The influence of concentration, the reaction time, drying temperature and substrate surface on silanisation was examined. TGA was used to detect the degree of silanisation.

HA with MPTMS ( 1wt% MPTMS with reaction time of 1 hour)was used as filler in PLGA-30wt% HA composites for an in-vitro degradation study carried out in PBS. In addition, the mechanical properties of the composites were studied.

Silanisation affects the properties of the composite by improving the bonding at the interface and hence it was found to influence the plastic mechanical properties rather than the elastic mechanical properties or the degradation profile of the composite.

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**Keywords:** Hydroxyapatite;Trialkoxysilanes; poly (D, L lactide-co-glycolide (50:50)) (PLGA); silanisation; *in-vitro* degradation; plastic mechanical properties.

**Abbreviations:**

HA, Hydroxyapatite; MPTMS, Mercaptopropyl trimethoxy silane; APTMS, Aminopropyl trimethoxy silane; APTES, Aminopropyl triethoxy silane; PBS, Phosphate buffered saline; ICP-AES, Inductively coupled plasma-Atomic emission spectroscopy;

# 1 Introduction

Poly ( $\alpha$ -hydroxy acids) are biodegradable, biocompatible and bioresorbable polymers. PLGA is a copolymer of Polylactic Acid (PLA) and Polyglycolic Acid (PGA) (Middleton and Tipton, 2000). Poly ( $\alpha$ -hydroxy acids) can be used as the matrix phase in composites. Hydroxyapatite (HA) resembles the mineral component of bone (Rho et al., 1998) and has been used as filler in polymer composites (Damadzadeh et al., 2010, Zhao et al., 2012). Composites produced to mimic bone (Ignjatovic et al., 2001, Shikinami and Okuno 1999, Furukawa 2000) have resulted in the values (for the mechanical properties tested) being either too high (Furukawa 2000) or too low (Takayama et al., 2009, Zhao et al., 2012). The properties of the composite can be influenced by altering the bonding between the filler and the matrix phase at the interface (Gultekin N et al., 2004). Trialkoxysilanes are used in this study as no toxic by-product is produced when using these silanes. Recent studies have shown that silanization lowers crystallinity of treated HA when compared to untreated HA (Cisneros-Pineda et al., 2014). Modifying the surface of filler using compounds such as organosilanes can affect the bonding at the interface of the composites. Organosilanes are organic compounds that bind at the interface of the composite and can be used to improve the properties of HA composites (Gultekin N et al., 2004). More recently, Lopez-Aranguren et al., (2012) reported that silanisation results in weakly hydrogen bonded silane as well as chemically bonded silane.

In a study where HA was used as the substrate the pH was acidic for the hydrolysis of the silane prior to the addition of HA and it was increased to a basic pH after the addition of HA (Dupraz et al., 1996 Oliviera et al., 2005). The pH was altered in order to encourage the condensation and formation of silanols on the surface of HA. This has been further validated in a recent study using APTES (Russo et al., 2014).

The time allowed for hydrolysis also varies greatly and it could range from 15-50 minutes for methoxy silanes with various functional groups (pH of 3.5) (Dupraz *et al.*, 1996) to 24 hours (pH not monitored) for MPS (Domingo *et al.*, 2006)

In many cases the reaction is carried out at room temperature when HA is used as the substrate (Gultekin *et al.*, 2004, Deng *et al.*, 2010, Vasiliev *et al.*, 2008). However, as was reported in a study using MPS and hydroxyapatite as substrate the reaction temperature can be increased above room temperature (Roether and Deb, 2004). The reaction was carried out at room temperature in the present study.

Tham *et al.*, (2010) reported the silanisation of HA with 2-8wt % MPS in PMMA/HA composites. No significant differences were observed in the flexural strength or tensile modulus with change in the weight fraction. Increasing the concentration higher than 2.0wt% results in formation of oligomeric or polymeric siloxane networks that are detrimental to the overall strength of the composite (Shokoohi S *et al.*, 2008).

Apart from the Si-O-Si linkages and the Si-OH linkages produced in a silanisation reaction, silanes also form dimers, siloxane chains or three dimensional networks and these three dimensional networks have no free Si-OH reactive moieties in the structure. This means there is no chemical bond formed between the silane and the substrate (M-C B Salon, 2008).

The method commonly used to detect the silanisation of a substrate is the Fourier Transform Infrared Spectroscopy (FTIR) (Damia and Sharrock, 2006 Lopez-Aranguren *et al.*, 2012). However, TGA has been used to determine the silanisation of various substrates including HA (Karabela and Sideridou, 2011 McElwee *et al.*, 2005, Tham *et al.*, 2010).

Literature has reported two peaks observed for silanised samples one associated with physisorbed (unreacted) silane and the other due to chemisorbed (reacted) silane. *Yang et al., 2003* reported that flash vaporisation to 250°C removed any trace of physisorbed silane. The vaporisation of physisorbed and hydrogen-bonded silanol molecules (chemisorbed) was reported to occur in the temperature range of 150-375°C (*Lopez-Aranguren et al., 2012*).

The effect of silanisation on the degradation of polymer composites has not been studied in great detail. Previous studies have focussed on the effect of silanes on the solvent sorption characteristics of composites (*Karabela and Sideridou, 2008, Deb et al., 1995, Tang et al., 2008*).

The amount of water absorbed has been reported to increase with increasing filler content for composites with unmodified or silanised HA as filler which was attributed to an increase in the water-soluble impurities with increase in filler content (*Deb et al., 1995*). It has also been reported that the presence of silanised filler may result in an increase of water absorption due to a coating of silanol groups (Si-OH) that adsorbs more water onto its surface than unsilanised material (*Santos et al., 2002*).

It has been reported in the literature that silanisation of filler reduces the rate of loss of material during *in-vitro* degradation and was attributed to the hydrophobicity of silane molecules (*Rakmae et al., 2012*).

In the study presented in this paper three different silanes (mercaptopropyl trimethoxy silane (MPTMS), aminopropyl trimethoxy silane (APTMS) and aminopropyltriethoxy silane (APTES)) were applied to HA substrates in order to study the effect of head group (which binds to the polymer) and tail group (which binds to the surface hydroxyl groups in HA).

The influence of various parameters such as concentration, reaction time, and surface area on the reaction was studied. In addition, the effect of physisorbed and chemisorbed silane on the mechanical properties and degradation profile of the composite was investigated.

## 2 Materials and Methods

### 2.1 Materials:

The materials were used as received unless mentioned. Calcium hydroxide ( $Ca(OH)_2$ , 99+%) was purchased from Fisher Scientific, UK and orthophosphoric acid ( $H_3PO_4$ , 85%) was purchased from Acros Organics, UK. PLGA (50:50) was purchased from Surmodics Pharmaceuticals, USA. The three silanes used were MPTMS, APTMS and APTES from Sigma Aldrich, UK. PBS (0.01M) powder was purchased from Sigma Aldrich, UK. Standard solutions used for the detection of calcium concentration were purchased from Fisher Scientific, UK.

### 2.2 HA Synthesis:

HA was synthesised at room temperature using an aqueous precipitation reaction between 0.5 M  $Ca(OH)_2$  (Fisher Scientific, UK) and 0.3M  $H_3PO_4$  (Acros Organics, UK). The pH was maintained above 10.5 with the addition of ammonia. The HA thus synthesised was ground, milled and sieved using a 180 $\mu$ m sieve. The powder produced using this method will

be referred to as “uncalcined HA.” In addition some of the the powder was calcined at 800°C for 4 hours in an air atmosphere and will be referred to as “HA calcined in air”.

### **2.3 Silanisation:**

Silanisation was carried out using 90% ethanol as a solvent and HA as substrate in the ratio of 1:2 (w/w, HA: ethanol). APTES, APTMS and MPTMS were added in concentrations of 0.1-2 wt% of HA (as-synthesised) to the ethanol and allowed to stir for 1 hour prior to adding the HA to activate silanol groups in the solution (*Gultekin et al., 2004*). 2g of substrate was used for each reaction. The sample was dried in vacuum at 120°C for 48 hours. Using this method the silane may be bound to the surface of the HA. Silanisation was carried out without the use of the solvent so as to prevent a reaction between silane and HA. The desired concentration of silane was added directly to the HA powder and left to stir for the duration of the reaction times studied. The powders silanised using this method were not dried and were used as produced. This means that the silane was not bound to the surface of HA.

#### *2.3.1 Factors affecting silanisation*

The concentration of the silanes was varied between 0.1-2-wt%. Four different reaction times (1, 2, 3 and 24 hours) were used. The rate of hydrolysis of silane is dependent on the concentration of silane and so occurs slower for low concentration of silanes (*Abel, 2006*). These time points were chosen in order to identify the optimum reaction time.

The tail group of the silane is bonded to the HA and to compare the effect of different tail groups 2-wt% APTES, APTMS and MPTMS at 1,2, 3 and 24 hours were analysed.

The effect of calcination and hence the surface area of the substrate on the degree of silanisation was analysed by comparing the silanisation of uncalcined HA with HA calcined in air. The silanisation was carried out using 1-wt% MPTMS and a reaction time of 1 hour.

## **2.4 Characterisation of silanised powders:**

### *2.4.1 Surface Area Measurement*

The BET(Brunauer–Emmett–Teller) method was used to calculate the surface area (*Table 1*) of the different powders synthesised. A Tristar3000 from Micromeritics was used.

### *2.4.2 TGA:*

A Q500 TGA (TA instruments, USA) was used to carry out the analysis. The reaction was carried out in an air atmosphere with approximately 10-12 mg of sample used. The temperature was increased at a rate of 10°C/min from room temperature to 600°C.

## **2.5 Composite production:**

A two-step process produced the composites; the first involved the mixing of the polymer and filler in acetone; the second, injection moulding of the mixture.

### *2.5.1 Mixing*

PLGA and 30-wt%HA (1.0wt% MPTMS, 1hour) were mixed in a Polytetrafluoroethylene (PTFE) container with acetone as solvent for 1 hour using an overhead magnetic stirrer. The mix was then allowed to stand for a further hour before drying in a vacuum oven at 40°C overnight.

### *2.5.2 Injection Moulding:*

The mixture of PLGA and HA was processed into dumbbell shaped specimens (to be used for studying elastic mechanical properties), and cylindrical specimens (for the degradation study and to study plastic deformation) using a 12 cm<sup>3</sup> DSM Xplore mini injection moulder. A mould temperature of 35°C and melt temperature of 140°C was used. The injection pressure was 5.0 bar whereas the filling pressure and the holding pressure were 2.0 bar. The holding time for the uncalcined sample was 3 s for each step, however for the calcined samples the holding time was changed to 6 s in order to allow the formation of complete specimens. Dumbbell shaped and cylindrical specimens were produced. The dumbbell shaped specimens used were 35mm, in length, 4.1 mm in width and 2.1 mm in thickness. The cylindrical shaped specimens with dimensions of 12 mm (length) x 6 mm (width) were used as samples.

## 2.6 Weight Fraction of filler:

The filler content present in a composite can influence its properties (Hosseinabadi *et al.*, 2011). In order to compare the weight fraction of HA present in the composite to the intended (30wt%) weight fraction a Q500 TGA was used. The process to measure mass loss with temperature increase was carried out in an atmosphere of nitrogen.

Approximately 20-30 mg of sample was used. The samples were cut from the centre as well as the edges of the cylinders produced using the method described earlier. The temperature was increased at a rate of 40°C/min from room temperature to 500°C. Three repeats were carried out for each sample. The actual weight fraction obtained are presented in *Table 2*.

## 2.7 In-vitro degradation study

In order to assess whether the type of HA influences the degradation of the composites, hydrolytic degradation at 37°C (pH=7.4) was carried out in PBS (0.01 M) dissolved in a litre of de-ionised water. The cylindrical specimens were cut into discs (2 mm x 2 mm) using a Struers Accutom 5.

The samples once cut were weighed individually and the correct amount of PBS added in a ratio of 6 mg/ml, into the sample bottles. Each sample was tied in unbleached muslin and suspended in a closed tube, containing PBS. After the addition of the PBS the samples were maintained at 37°C, in a closed incubator, without further agitation. This was done to minimize variations due to factors such as environment, temperature and solution evaporation. The PBS was unchanged during the period of degradation. Pure polymer was used as a control. Twenty-four time points were studied in total and three replicates were made for each time point. The results presented are an average of the three repeats.

However, a total of nine pH readings were taken for the samples from day 7 to day 45 and six readings for the day 1 and day 50 samples.

The degradation of the composites was characterised by measuring water absorption, mass loss, and calcium concentration in solution and change in pH. The degradation study was carried out for 60 days. The time period is divided into three blocks in order to understand the trends better. The first block is days 1-21 the second block is days 28-34 (days 22-27 were not considered as no significant difference was expected in the properties of the samples containing HA. This is highlighted by the clear difference in the pH readings at day 21 and day 28 for the sample containing pure polymer versus samples containing filler) and the third block is days 35-60.

#### 2.7.1 *Water absorption:*

The mass of the wet samples ( $M_{\text{wet}}$ ) at each time point was measured after dabbing it with a paper towel on removal from the PBS in order to absorb the surface water. The samples were dried using a vacuum oven at 40°C and a pressure of 800 mbar for 24 hours. The samples were then weighed to measure the dry mass of the samples ( $M_{\text{dry}}$ ).

The water absorption ( $M_w$ ) of the samples was calculated using Eq (1)

$$M_w = ((M_{\text{wet}} - M_{\text{dry}}) / M_{\text{dry}}) * 100\% \quad (1)$$

#### 2.7.2 *Mass Loss:*

The samples were weighed before immersion in PBS ( $M_{\text{Initial}}$ ) and after drying ( $M_{\text{dry}}$ ). The samples were measured using an electronic balance

(AG204 Mettler-Toledo, UK) with an accuracy of 0.1mg. The mass loss ( $M_L$ ) was calculated using Eq (2)

$$M_L = ((M_{\text{dry}} - M_{\text{Initial}}) / M_{\text{Initial}}) * 100\% \quad (2)$$

### 2.7.3 Calcium concentration in solution:

Calcium concentration in the PBS after composite degradation was determined using a Liberty Ax Sequential ICP-AES (Varian Inc.). The machine was calibrated using the standard solutions (1 ppm, 10 ppm, 100 ppm, 1000 ppm) for calcium and approximately 5 ml of sample used in each run. Stock PBS solution was used as a control. As the calcium concentration in solution was expected to increase with degradation time three time points (day 21, day 30 and day 35) were chosen as representative for the study.

### 2.7.4 Change in pH:

The pH of the solution was measured during the degradation using an electronic bench-top pH meter (Orion 3 Star, Thermo Electron Corporation). Before each set of measurements, the pH meter was calibrated using standard solutions.

### Mechanical Properties

Dynamic mechanical thermal analysis (DMTA) (DMA Q800, TA Instruments, USA) was used to measure the viscoelastic properties of the injection-moulded samples. The tests were carried out in cantilever mode using a dual cantilever clamp. A multi frequency module was used at four different frequencies: 1, 10, 20 and 50 Hz. The tests were carried

out between  $-10^{\circ}\text{C}$  to  $120^{\circ}\text{C}$ , with a heating rate used of  $3^{\circ}\text{C}/\text{min}$ . Three repeats were carried out for each sample. The results presented in the paper are for  $E'$ (storage modulus) at 1 Hz. A Hounsfield (5 kN) machine was used to determine the ultimate tensile strength(UTS) of the composites, at room temperature at  $0.002\text{ mm/s}$ . A total of three repeats were carried out for each sample.

### 3 Results

#### 3.1 Silanisation:

*Fig. 1(a)* shows typical TGA graphs for bound (chemisorbed) silane with a peak at around  $320^{\circ}\text{C}$  which is associated with silane bonded to the surface of the substrate (indicated by the red arrow). (*Karabela and Sideridou, 2011*). The peak is observed for each silane type and condition (10wt% MPTMS reaction time 1 hour, 10wt% APTMS reaction time 1 hour and 10wt% APTMS reaction time 3 hours) tested. The silane bonded on the surface of the HA in this study were hydrogen bonded since the aqueous solvent method results in hydrogen bonded silane molecules .

The TGA graph for unreacted (physisorbed) silane (*Fig.1 (b)*) showed peaks of weight loss however, at  $250^{\circ}\text{C}$  (indicated by the blue arrow). The peak is observed for each silane type and condition (10wt% MPTMS reaction time 1 hour, 10wt% APTMS reaction time 1 hour and 10wt% APTMS reaction time 3 hours) tested. A temperature of  $250^{\circ}\text{C}$  is associated with the removal of any trace of physisorbed silane.

*Fig. 2(a-c)* show the thermograms from which the derivative weight loss was obtained for the chemisorbed and *Fig. 2(d-f)* physisorbed silane for

each type and condition (10wt% MPTMS reaction time 1 hour, 10wt% APTMS reaction time 1 hour and 10wt% APTMS reaction time 3 hours).

It was found that TGA used in this way was able to identify whether chemisorbed, physisorbed or both types of bonding were present. However, the technique was not sufficiently sensitive to indicate the amounts of each type of silane. The form of bonding observed C (Chemisorbed), P (Physisorbed) or M (Mixed) was recorded for each sample and is shown in *Table 3*.

The concentration of silane was varied between 0.1 and 2.0wt%. For 2.0wt% silane the sample prepared using MPTMS and APTMS showed chemisorbed peaks whereas only physisorbed peaks were observed for the sample prepared using APTES. Reaction times were varied between 1 hour and 24 hours.

Calcination did not affect the nature of the bonding as both calcined and uncalcined particles showing chemisorbed silane (*Table 3*).

### **3.2 In-vitro degradation study:**

The degradation study was carried out for 60 days. The time period is divided into three blocks in order to understand the trends better. The first block is days 1-21 the second block is days 28-34 and the third block is days 35-60. The samples for pure polymer degraded by day 21. The analyses for mass loss and water absorption however were only possible till day 14 as the polymer samples completely degraded beyond this point. 1-wt% MPTMS with reaction time of 1 hour was the type and condition of silane chosen for both chemisorbed and physisorbed silane in the results presented in the degradation study.

There were numerical differences in the percentage water absorption for the composites with and without silane (*Fig. 3(a) and 3(b)*), seen in particular between the composite containing uncalcined physisorbed HA and composites containing uncalcined HA. However, the values fell within the error limits and there was no statistically significant difference.

Similarly numerical differences were observed in the values obtained for mass loss for the composites containing silanised HA and the composites containing unmodified HA during the degradation study reported in this paper (*Fig. 3(a) and 3(b)*). However, these differences were not statistically significant.

The values for calcium concentration at different time points (day 21, day 30 and day 35) for the composites containing silanised HA were numerically different to those obtained for the composites containing unmodified HA (*Fig. 4(a) and 4(b)*). However, the values fell within the error limits and there was no statistically significant difference.

The pH readings recorded for the silanised composites were compared with the composite containing unmodified HA (*Fig.5a and 5b*).As expected, a decrease in the pH was observed with time .However the increase seen at Day 60 may be due to the complete dissolution of the HA filler from the composites . The degradation profile of the 30-wt% composites containing silanised HA was significantly delayed compared to that of the pure polymer. However, the composites containing chemisorbed and physisorbed silane showed no differences when compared to the composites containing unmodified HA.

### 3.3 Mechanical Properties:

*Fig.6* shows the DMTA curves for composites containing silanised HA from which the  $E'$  value at 20°C (*Table 4*) were extrapolated. The curves represent the middle values of the three repeats studied. These are taken directly from the DMTA, and represent the middle value (one of three readings) and therefore no error bars can be depicted.

*Table 4* shows the average storage modulus ( $E'$ ) of composites containing silanised HA. The  $E'$  values for the composite containing silanised (chemisorbed and physisorbed) uncalcined HA were similar to each other (indicated in bold) but were significantly higher than the composite containing unmodified uncalcined HA.

The  $E'$  values for the composites containing silanised (chemisorbed and physisorbed) calcined in air HA were similar to those for composites containing the calcined in air HA.

*Fig. 7* shows the UTS obtained at room temperature for composites containing silanised as well as unmodified HA. The lowest value for UTS obtained was 64.4(±2.7) MPa for composite containing uncalcined HA whereas those for the composites containing chemisorbed silanised uncalcined HA and physisorbed silanised uncalcined HA were 80.8(±0.8) MPa and 72.4(±2.9) MPa respectively. The UTS for the composite containing calcined in air HA was 69.6(±1.9) MPa whereas those for the composites containing chemisorbed silanised calcined in air HA and physisorbed silanised calcined in air HA were 90.8(±2.2) and 82.1(±0.9) MPa respectively.

## 4 Discussion:

### *Method used to detect silanisation:*

The results obtained using the TGA for the vaporisation of silane (chemisorbed or physisorbed) from the surface of HA substrate reported in this paper is in agreement with literature. The silane bonded on the surface of the HA in this study were hydrogen bonded since the aqueous solvent method results in hydrogen bonded silane molecules (*Lopez-Aranguren et al., 2012*). The vaporisation of hydrogen-bonded silanol molecules was reported to occur in the temperature range of 150-375°C (*Lopez-Aranguren et al., 2012*). The surface density of OH groups was suggested to be pivotal for chemisorption of silane.

In addition, a temperature of 250°C was reported to be associated with the removal of any trace of physisorbed silane (*Yang et al., 2003*) which is in agreement with the results obtained during this study.

### *Effect of concentration and reaction time on silanisation*

For most of the samples silanized using MPTMS, clear peaks were observed at 300-320°C, which were attributed to chemisorbed silane. However for samples silanized with APTES, clear peaks were observed only at 250°C as shown in *Table 3*. Hence samples silanized with APTES were not considered suitable for further study silanization. The TGA graphs for 10wt% shown in Fig.2 were carried out to test if it is possible to observe a difference in chemisorbed and physisorbed silane. Once the difference was established the concentration was varied between 0.1-2 wt% since concentrations higher than 2wt% have been reported to

result in formation of oligomeric or polymeric siloxane networks that are detrimental to the overall strength of the composite (*Shokoohi S et al., 2008*).

A broad decrease in chemisorbed silane was observed with increasing reaction time (*Table 3*). One of the possible reasons for the lower concentration of chemisorbed silane maybe that the available OH groups bond to the silanol groups within one hour and self-condensation (*M-C B Salon, 2008*) of silane occurs beyond the 1 hour time point. One of the possible reasons why APTES samples may not have shown any chemisorbed silane peaks is the self-condensation associated with APTES (*M-C B Salon, 2008*).

This is also the case for the physisorbed samples where no Si-O-Si or Si-OH linkages are produced and the absence of a solvent results in the formation of dimers, siloxane chains or three-dimensional networks. Shorter reaction times may be investigated.

As stated earlier different tail groups and head groups were studied. The tail group binds to the HA substrate and differences were observed between the ethoxy group and the methoxy group. HA silanised with APTES resulted in physisorbed silane for all the samples analysed.

The influence of surface area was studied however it was found that calcination did not influence the type of bonding as silanisation with 1-wt% MPTMS resulted in chemisorbed silane on the surface of both uncalcined and calcined in air HA.

Chemisorbed peaks were observed when 1-wt% MPTMS with reaction time of 1hr, as can be seen from *Table 3*, Therefore, in order to study the differences between samples containing physisorbed and chemisorbed silane on the properties of the composite, 1-wt% MPTMS with reaction time of 1hr was selected,

### *Degradation study for composites containing silanised HA*

Silanisation did not influence the degradation rate of the composites. This is different from the results reported by Rakmae et al., (2012) who attributed differences in the degradation profile observed by them to the hydrophobicity of silane. One of the possible reasons for the similar degradation profiles in our study might be the low concentration (Ramos et al., 1998) of weakly hydrogen bonded (Lopez-Aranguren et al., 2012) silane (chemisorbed), present on the surface of the HA. We also observed that the presence of physisorbed silane, which would be mainly in the form of siloxane networks on the surface of HA, did not affect the degradation rate. However, the numerical differences observed during the degradation study in the samples containing silanised HA when compared to composites containing unmodified HA maybe due to the presence of silane at the interface which influenced individual samples but did not have a statistically significant effect. It has recently been reported that silanization lowers crystallinity of treated HA when compared to untreated HA (Cisneros-Pineda et al., 2014). This decrease in crystallinity may be one of the reasons for the lack of any statistically significant difference in the composites studied.

### *Elastic and plastic mechanical properties:*

Gultekin et al., (2004) reported an increase in the  $E'$  was reported for composites containing silanised HA (10-40wt%). They attributed this to better dispersion of silanised filler within the matrix and enhancement of interfacial bonding between polymer and filler.

In contrast, the  $E'$  for the composites containing silanised (uncalcined and calcined in air) HA reported in this paper (Table 4) were similar to the composites containing unmodified HA. The similar values for the composites containing chemisorbed HA (uncalcined and calcined in air)

might be due to the lack of covalent bonding (*Lee J-H et al., 2011 Yang et al., 2009*) between silane and HA as the aqueous solution method was used for silanisation, which resulted in hydrogen bonding (*Lopez-Aranguren et al., 2012*). In the case of the physisorbed samples, similar values of  $E'$  (indicated in bold) were observed but with higher values for uncertainty. One of the possible explanations for the high values of uncertainty might be the variation in homogeneity of particle distribution observed for these composites (*Table 2*). In addition for the physisorbed samples where no Si-O-Si or Si-OH linkages are produced and the absence of a solvent results in the formation of dimers, siloxane chains or three-dimensional networks which may have resulted in the variation.

The UTS for composites containing silanised fillers depends on the enhancement of interfacial bonding between the polymer and filler (*Deng et al., 2010*) and the increase in surface hydrophobicity of the filler due to the presence of the silane (*Yang et al., 2009*).

The increase in UTS for the composites containing silanised HA in this study (*Fig. 7*) might be due to improved bonding at the interface between polymer and filler. The increase may also be due to the increased surface hydrophobicity of the silanised HA (*Yang et al., 2009*).

## 5 Conclusions

The HA (uncalcined and calcined in air) powders were silanised using APTES, APTMS and MPTMS by the aqueous solution method. The effect of type of silane and the factors affecting silanisation of HA were identified

to study the effect of silanisation on the degradation profile and mechanical properties of composites containing silanised fillers. An *In-vitro* degradation study revealed that silanisation did not influence the degradation rate of the composites. One of the possible reasons for the similar degradation profiles might be the low concentration of weakly hydrogen bonded silane (chemisorbed), present on the surface of the HA. The presence of physisorbed silane mainly in the form of siloxane networks on the surface of HA also did not affect the degradation rate. However, the numerical differences observed during the degradation study in the samples containing silanised HA when compared to composites containing unmodified HA maybe due to the presence of silane at the interface which influenced individual samples but did not have a stastically significant effect.

Silanisation had little influence on the elastic modulus of the composites produced probably due to the low concentration of silane on the surface of the HA. However, presence of silane improved the plastic mechanical properties of the composites which might be due to the improved bonding at the interface between PLGA and HA.

#### Acknowledgements

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Figures and Tables:

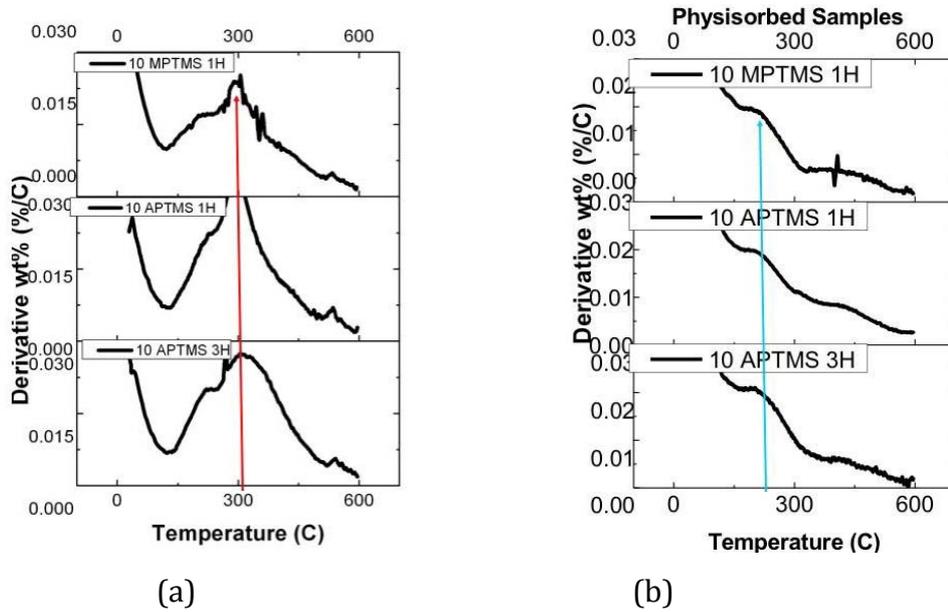
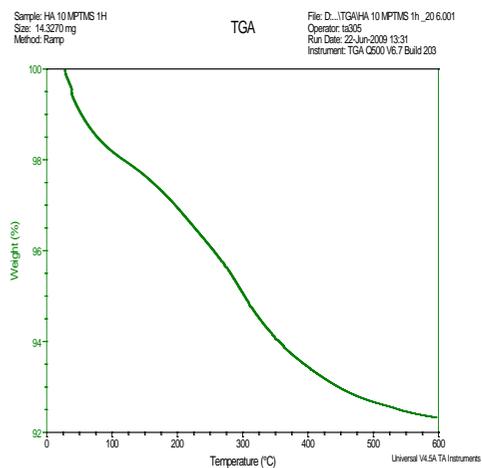
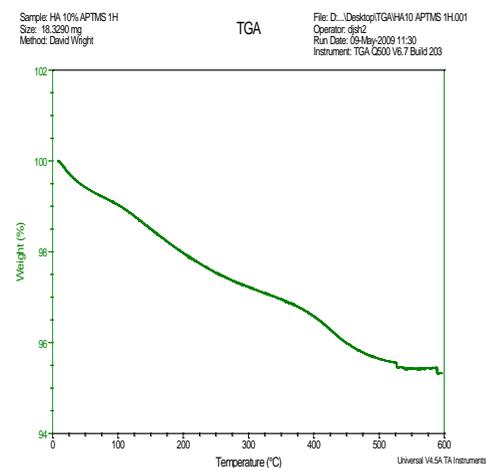


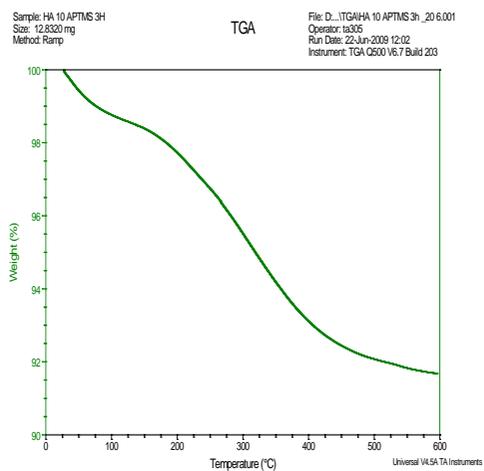
Fig.1: (a) TGA graph indicating peak associated with chemisorbed silane (red arrow indicating peak position) (b) TGA graph indicating peak associated with physisorbed silane (blue arrow indicating peak position).



(a)

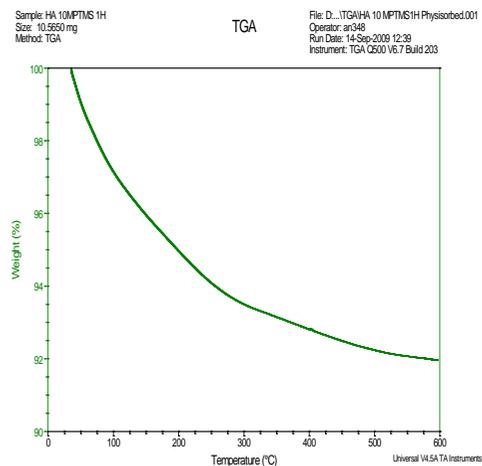


(b)

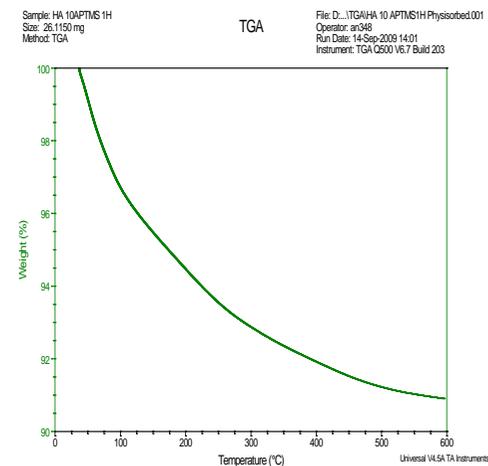


(c)

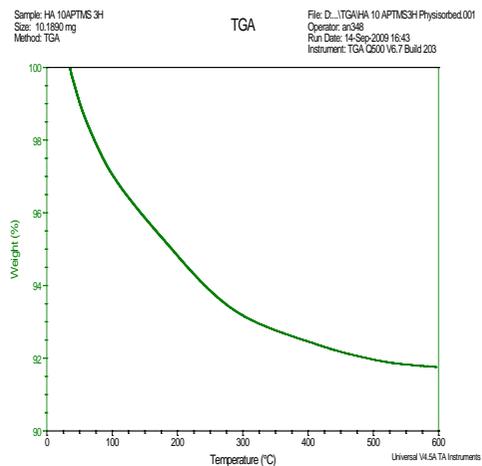
Fig.2: (a) 10wt% MPTMS reaction time 1 hour (b) 10wt% APTMS reaction time 1 hour (c) 10wt% APTMS reaction time 3 hours (Chemisorbed)



(d)



(e)



(f)

Fig.2: (d)10wt% MPTMS reaction time 1 hour (e)10wt% APTMS reaction time 1 hour (f) 10wt%APTMS reaction time 3 hours (Physisorbed)

Table 1: Specific surface area for HA powders obtained from the BET

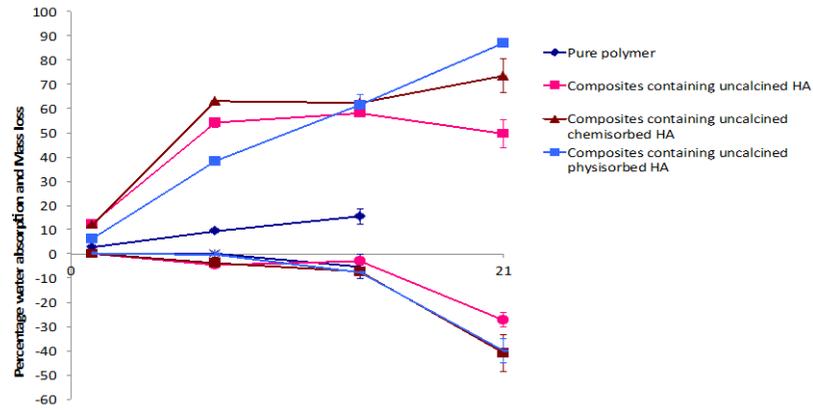
Type of HA	Specific Surface Area
Uncalcined HA	87.2 m <sup>2</sup> /g
Calcined in air HA	23.3 m <sup>2</sup> /g

Table 2: Weight fraction of filler in the composites

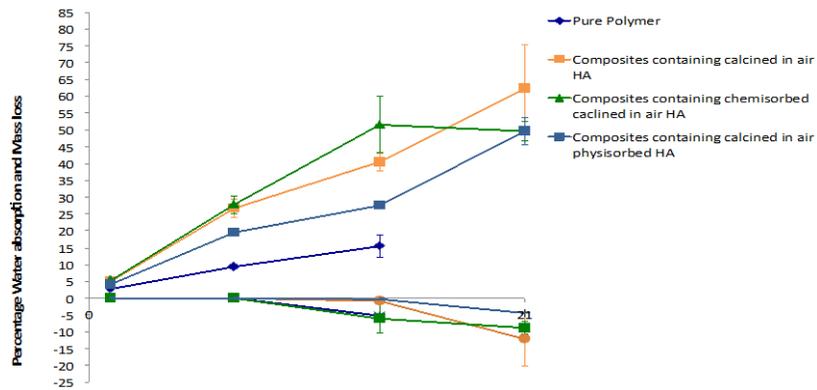
<b>Sample Name</b>	<b>Weight fraction of filler% (±S.E.)</b>
Uncalcined HA	27.7 (±0.4)
Chemisorbed Uncalcined HA	28.8 (±0.2)
<b>Physisorbed Uncalcined HA</b>	<b>27.1 (±2.9)</b>
Calcined in air	29.5(±0.6)
Chemisorbed Calcined in air HA	29.9 (±0.1)
<b>Physisorbed Calcined in air HA</b>	<b>26.0 (±2.8)</b>

Table 3: Factors affecting silanisation and type of silanisation observed (C= Chemisorbed; M=Mixed (Chemisorbed & Physisorbed); P= Physisorbed silane)

Factors affecting silanisation	Parameters held constant	Parameters Varied	MPTMS	APTMS	APTES
Effect of concentration	Substrate (Uncalcined HA), Reaction time (1 hour) No pH control	<b><u>Concentration</u></b>			
		0.1wt%	M	C	P
		0.5wt%	C	C	P
		1.0wt%	C	M	P
		1.5wt%	C	P	P
		2.0wt%	C	C	P
Effect of reaction time and silane chemistry	Substrate (Uncalcined HA), Concentration (2.0wt%) No pH control	<b><u>Reaction Time</u></b>			
		1 hour	C	C	P
		2 hours	C	M	P
		3 hours	M	M	P
		24 hours	P	M	P
Effect of calcination	Reaction time (1 hour) Concentration (1.0wt%) No pH control	<b><u>Type of HA</u></b>			
		Uncalcined HA	C	-	-
		Calcined in air HA	C	-	-

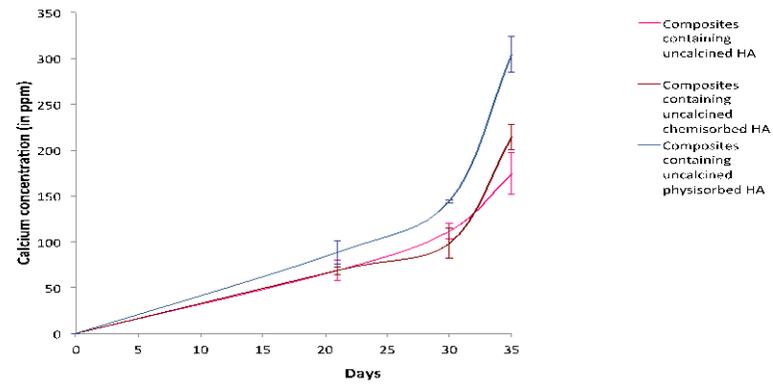


(a)

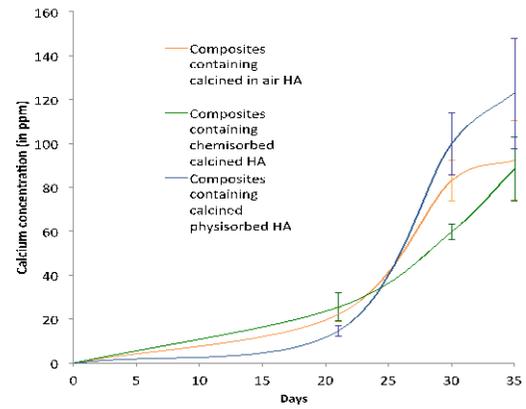


(b)

Fig. 3: (a) Water absorption and mass loss for composites containing silanised uncalcined HA (b) Water absorption and mass loss for composites containing silanised calcined in air HA up to day 21

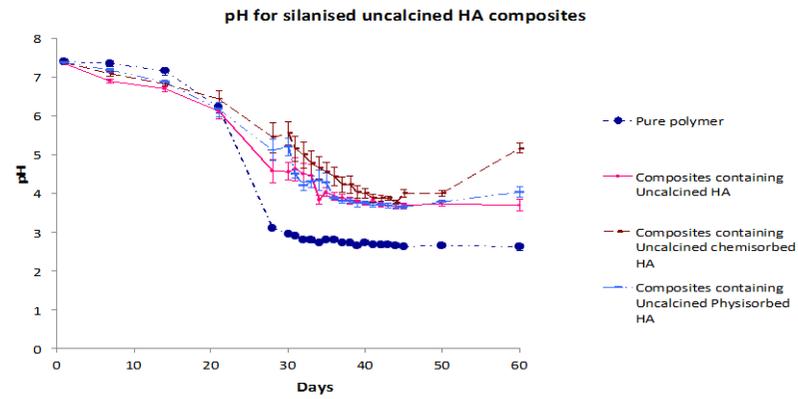


(a)



(b)

Fig.4: (a) Calcium concentration for composites containing silanised uncalcined HA  
 (b) Calcium concentration for composites containing silanised calcined in air HA  
 at three different time points



(a)

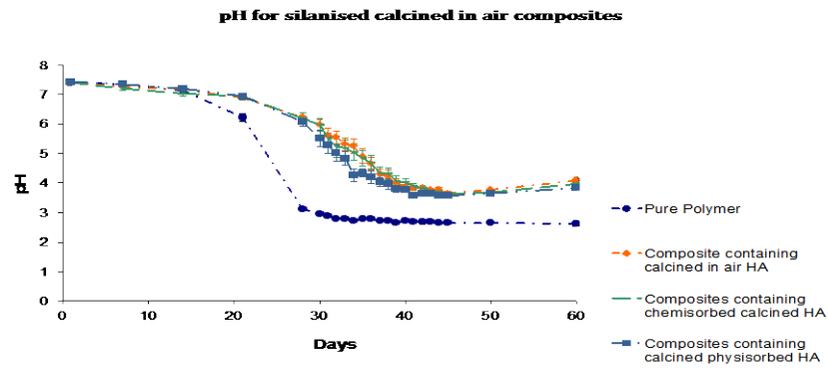


Fig.5: (a) pH readings for composites containing silanised uncalcined HA (b) pH readings for composites containing silanised calcined in air HA

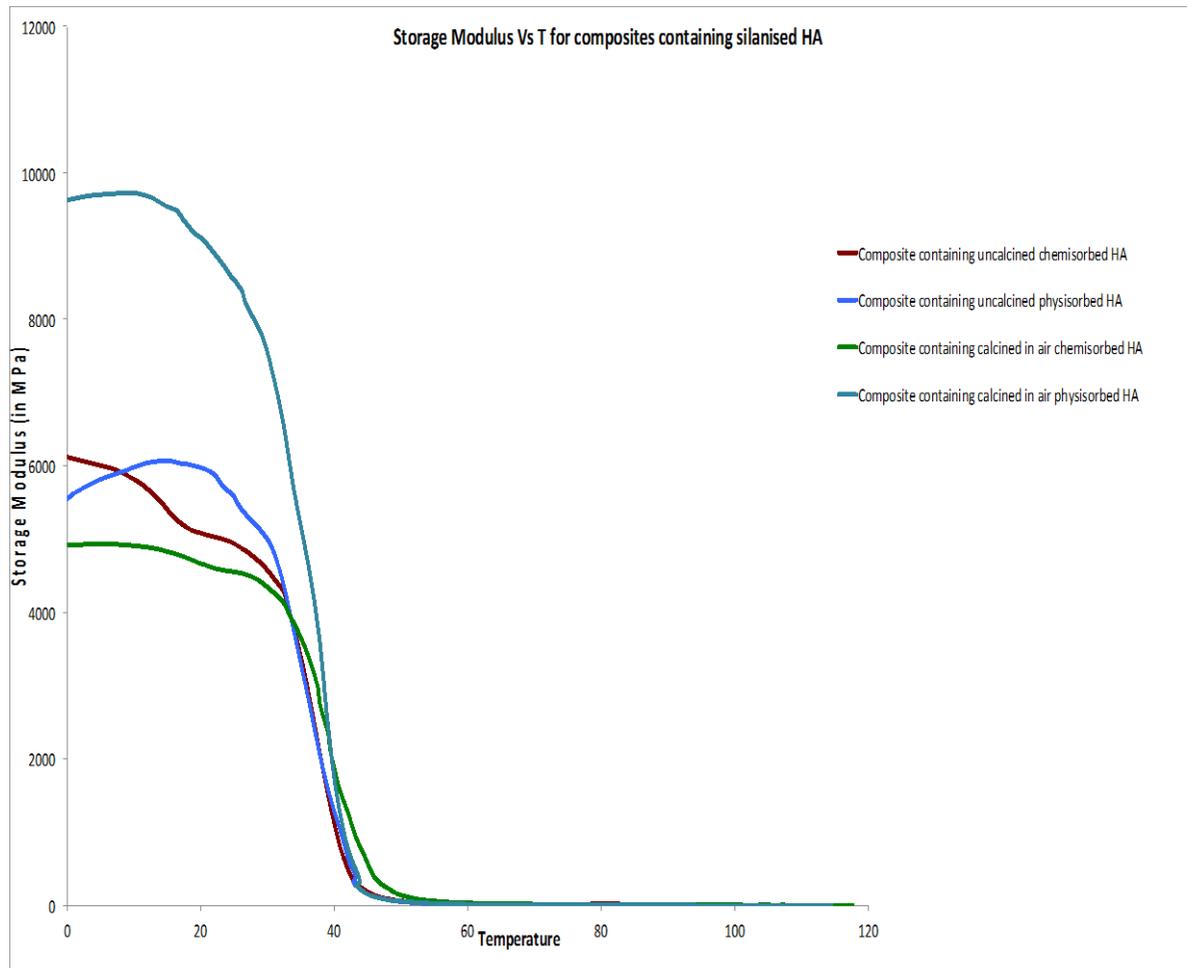


Fig.6: Representative DMTA curves for composites containing silanised HA

Table 4:  $E'$  of silanised samples

Type of Modification	Uncalcined HA ( $E'$ ( $\pm$ S.E.)) (In GPa)	Calcined in air HA ( $E'$ ( $\pm$ S.E.)) (In GPa)	Pure Polymer ( $E'$ ( $\pm$ S.E.)) (In GPa)
Unmodified	3.14( $\pm$ 0.28)	6.33 ( $\pm$ 0.54)	2.57 ( $\pm$ 0.52)
<b>Physisorbed</b>	<b>8.54 (<math>\pm</math>3.11)</b>	<b>7.51 (<math>\pm</math>1.27)</b>	-
<b>Chemisorbed</b>	<b>6.35 (<math>\pm</math>0.86)</b>	<b>5.93 (<math>\pm</math>0.89)</b>	-

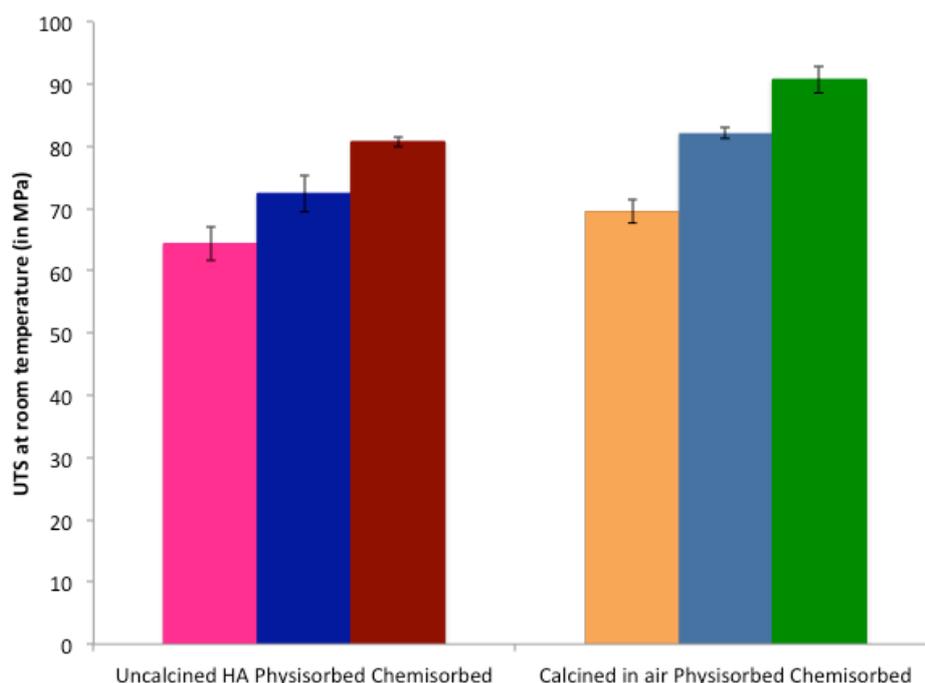


Fig.7: UTS at room temperature for composites containing unmodified and silanised (chemisorbed and physisorbed) HA

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