



# Adsorption and Conformations of Starch at Solid–liquid Interfaces Using Spectrophotometry and Turbidity Techniques

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## Authors' contributions

*This work was carried out in collaboration between all authors. Author FE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AI and K. Edbey managed the analyses of the study. Authors K. Elabdily and NM managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The adsorption behaviour of starch on silica surface, which is mimic active sites existing on the surface of kaolinite, was investigated by a combination of spectrophotometry and turbidity techniques. It was demonstrated that the conformational behaviour of starch chain in aqueous solution is temperature and pH sensitive. Starch solution showed a rapid increase in turbidity, with decreasing temperature. This suggested that collapse is occurring causing the biopolymer chains to aggregate. The decrease in turbidity indicated that an open coil starch was formed at higher temperature. The starch with negatively charged alkoxides (R-O<sup>-</sup>), due to the deprotonating of hydroxyl groups at mild basic pH values, showed a larger decrease in turbidity than the starch at low pH values, which indicates that chain aggregation is favored by a neutral starch chain and that the formation of an expanded shape is favored by charged starch macromolecules. The point of zero charge results suggested that electrostatic interactions are mainly responsible for the

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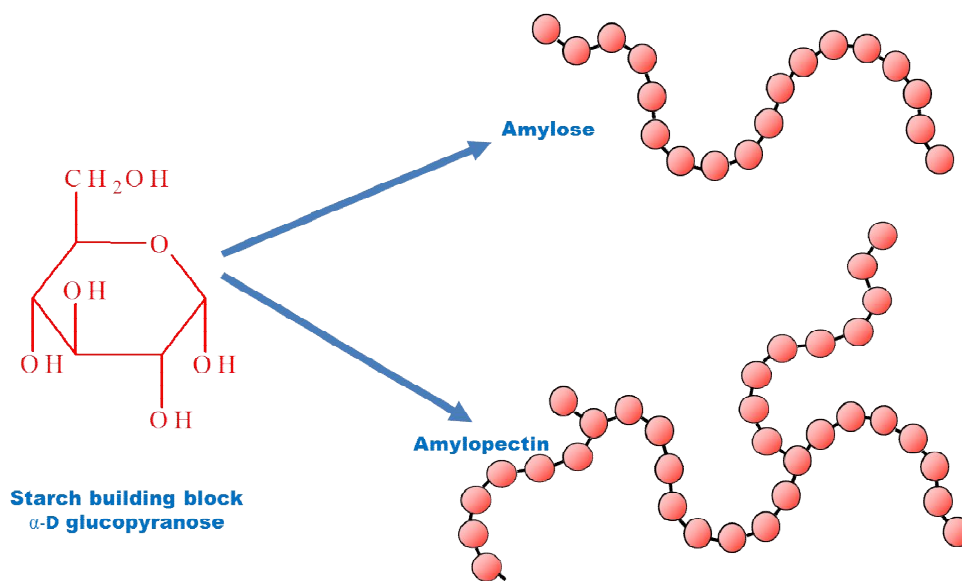
adsorption of starch on  $\text{SiO}_2$  suspension molecules. The UV-absorption spectra of anthracene labelled-starch proposed that the adsorption density of starch on silica is pH dependent. Starch adsorption on silica is extensively increased by decreasing pH to 7, as the electrostatic attraction between the  $\text{R-C-OH}_2^+$  groups in starch and the negatively charged silica ( $-\text{Si-O}^-$ ) was enhanced. The flocculation of silica suspensions by starch chains depends on the adsorption behaviour of starch on the silica particles. The bridging flocculation of silica particles by starch is rather weak at pH 11, since starch shows extremely low affinity towards silica. When starch shows high affinity towards silica at pH 9 and 7, it starts to play a significant role in the flocculation of silica particles.

**Keywords:** Silica; starch; anthracene-labeled-starch; adsorption; flocculation; turbidity.

## 1. INTRODUCTION

Kaolinite is a common clay mineral, mainly composed by alumina and silica of general chemical formula  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$  [1]. The adsorption of starch on kaolinite plays a vital role in the elimination of kaolinite from iron ore in selective flocculation and froth flotation processes [1]. A major problem with the iron ore is their kaolinite content [1]. The high kaolinite content in iron ore results in a highly viscous slag and high coke rate, which is causing in sinter plant operations and an explosion of furnace [1]. For charged polysaccharide-adsorbent systems, mainly strong electrostatic interaction works and influences adsorption according to their charges. For uncharged polysaccharides, only H-bonding and solvation forces are important [1-3]. The understanding of starch behaviour in aqueous solution begins with an understanding of the

basic chemistry. Starch granular organization as well as amylose and amylopectin structure depends on the botanical source. Amylose, the linear D-glucose chain (see Fig. 1), has on average between 500 and 6,000  $\alpha$ -D glucopyranose units that are distributed among 1 to 20 chains. Each chain has shown an average degree of polymerization (DP) of 500 [4]. Some spaced branching points were detected in amylose; however, it presents linear polymer characteristics. The properties of amylose might be explained as its diverse molecular conformations. For example, in neutral solutions the conformation is a random coil [4]. In present work, maize starch provides the focus of the study. Maize starch is a natural, nontoxic and biodegradable biopolymer. Chemically, starch is made up of basically two macromolecules, amylose and amylopectin. Specifically, it contains 80% of amylose and 20% amylopectin.



**Fig. 1. Linear and branched starch biopolymers**

Starch has a unique physicochemical behaviour, which is affected by its composition and the environment surrounding [1,2,4-8]. Several studies [1-3,5,6] have reported that the interaction behaviour of starch with minerals is influenced by factors such as concentration and pH of the medium. In fact, the molecular interactions of an aggregated starch solution are not fully understood. However, it has been known that starch chain can expand at pH higher than its pKa value (pKa = 12.6 [8]), due to electrostatic repulsion between ionized starch hydroxyl groups [4,8]. The starch hydroxyl groups may interact with mineral surfaces, these moieties could interact via weak hydrogen bonding or strong chemical interaction [3]. The stability of natural polysaccharides, such as starch, when in presence of solid surfaces is influenced by the conformation adopted by these natural polysaccharides [6]. However, the conformational behaviour of adsorbed starch is not well understood. In this work, we aim to use the spectrophotometry and turbidity techniques to study the conformational behaviour of the diluted aqueous solution of starch and photophysically probe-labelled starch. Turbidimetric technique was applied to study the effect of temperature and pH on the conformational behaviour of a starch chain, both in bulk solution and at the adsorbed state. The interaction of this natural polysaccharide with silica, which is mimic active sites existing on the surface of kaolinite [9] was also, studied using UV-spectroscopy technique. This study delivers a well understanding of the starch-kaolinite interactions, which is of critical importance for the flotation of iron ore and the selective flocculation of iron.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

Colloidal silica, 30 wt. % suspension in H<sub>2</sub>O with a particle diameter of 34.7±1.4 nm, surface area of 225 m<sup>2</sup>/g, pH of 9.2 (1 wt% in water), e.m.f

of -123 mv (1 wt% in water), Density of 1.2100 g/cm<sup>3</sup>, and molecular formula of SiO<sub>2</sub> was purchased from Sigma-Aldrich, UK.

### 2.2 Labeling Reaction

The photophysically probe-labeled starch (anthracene labeled-starch) was prepared based on method adapted from Horinaka et al. [10], with slight modification, see Fig. 2. Starch (from Riedel-De Haenag Seelze-Hannover) was purified thrice before the labeling reaction by reprecipitation from water to 2-propanol. Purification was also carried out for 9-anthracenemethanol (An-MeOH) (Aldrich) by recrystallization using an equivalent-volume mixture of water and methanol. The purified starch (1.03 g) was dissolved in water (100 ml) of pH 2, which is adjusted using hydrochloric acid along with An-MeOH (0.236 g) and 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDPCH) (Wako) (0.327 g). EDPCH was used without further purification. Doubly distilled water was used in this study. The aqueous reactant was stirred for 4 h at 40°C to allow the hydroxyl groups of An-MeOH to etherify with the hydroxyl groups of starch. The product was poured into 2-propanol, and the precipitate obtained was purified thrice by reprecipitation from water to 2-propanol before being used as the anthracene labeled-starch. The viscosity-average molecular weight  $M_v$  of pure starch and labeled-starch, with low loading of the anthracene (0.1mol%), were determined to be 3.5 x 10<sup>6</sup> and 3.1 x 10<sup>6</sup> Da using viscometry, values of 0.59 and 0.29 for  $K$  and  $a$ , respectively, in water at 25°C [11].

### 2.3 Determination of Turbidity for Starch Solutions

Starch solutions were prepared by dispersing a specific amount from starch in about 1000 g distilled water. The solution was heated to 70°C while being stirred. The starch suspensions were held at 70°C for 30 min while stirred. The pH

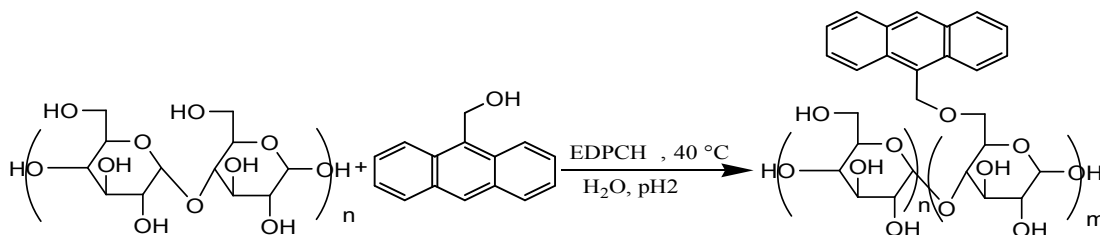


Fig. 2. Labeling reaction of starch with anthracene methanol

was 8 for all the starch solutions. No salt was added to any solution. The transmittance of the solution was measured on a conventional turbidity meter (Lamotte 2020 We Turbidimeter). Each sample was run in a cycle from 70°C to room temperature and back to 15°C, with duration of 1 h for each run. During the pH study the temperature was fixed at 25°C and the pH was varied by adding HCl or NaOH. Calibration was done once every day meter is used prior to beginning analysis.

## 2.4 Point of Zero Charge Measurements

Point of zero charge for starch and silica solution was carried out via potentiometric titrations according to previously published protocol [12]. The titrations of samples were carried out at 25°C using NaCl as the background electrolyte. The solutions were prepared by dissolving a known amount of material in double distilled water. The 0.1 M NaOH and 0.1 M HCl solutions were prepared from NaOH and HCl using pure water, and the exact concentration was determined prior to the titration against a primary standard  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ . The samples were dissolved in 25 mL of NaCl electrolyte (0.1 M), and the solution was hold in a closed glass vessel, yielding a constant pH value. The sample solutions were acidified to  $\text{pH} \approx 2$  using 0.1 M HCl and then titrated to  $\text{pH} \approx 11$  using 0.1 M NaOH. A blank without desired sample was also titrated. To assess reversibility and protonation behaviour, a reverse acidimetric titration was applied following the base titration. Each experiment was done 3 times. All titrations were performed at 25°C. The titrator was set to add successive acid or base every 30 seconds. The electrode was standardized on a proton concentration scale,  $[\text{H}^+]$ , and the slope deviation from the theoretical Nernst value was always within 1%.

## 2.5 Adsorption Experiment

The correct proportions of each unlabelled and labelled starch was measured and mixed with 1 gram silica, and then the pH of the sample adjusted to the required value. The mixture was shaken overnight to ensure that adsorption had reached equilibrium. The solution was then left for 6 hours to deposit the solid phase from the aqueous phase. The supernatant was then carefully withdrawn from the top of the sample tube. To quantify the amount of adsorption, ultraviolet (UV) spectra were recorded with a Perkin–Elmer spectrophotometer of labelled polysaccharide mineral solution before and after

separation. The scan was done from 290 nm to 400 nm with a scan speed of 400 nm/min. The adsorbed amount was calculated by the difference in the concentration of labelled starch solutions before and after adsorption by the Beer-Lambert law (maximum absorption intensity at 365 nm) using the following equation [13]:

$$\Gamma = \frac{(C_i - C_e)V}{mA} \quad (1)$$

Where  $\Gamma$  is the starch adsorption density ( $\text{mg}/\text{m}^2$ ),  $C_i$  the initial starch concentration ( $\text{mg}/\text{mL}$ ),  $C_e$  is the equilibrium starch concentration ( $\text{mg}/\text{mL}$ ),  $V$  is the volume of sample (L),  $m$  is the mass of  $\text{SiO}_2$  (g), and  $A$  is the specific surface area of  $\text{SiO}_2$  ( $\text{m}^2/\text{g}$ ).

On the other hand, the turbidity analysis was carried out of unlabelled starch mineral solution before separation. Absorption intensities were expressed in arbitrary units (a.u), while turbidity values are expressed in nephelometric turbidity units (NTU).

## 2.6 General Isotherm Equation

The experimental adsorption data were mathematically fitted by general isotherm equation (Equation 2) that was firstly derived by taking into the account that adsorption on solid-liquid interface occurs in two steps [13-16].

$$\Gamma = \frac{\Gamma_{\infty} k_1 C (1/n + k_2 C^{n-1})}{1 + k_1 C (1 + k_2 C^{n-1})} \quad (2)$$

Where  $\Gamma$  is the amount of starch adsorbed,  $\Gamma_{\infty}$  is the maximum adsorbed amount,  $k_1$  and  $k_2$  are the equilibrium constants for first layer and multilayer, respectively, and  $n$  the number of clusters of starch molecules.  $C$  represents the equilibrium starch concentration.

## 3. RESULTS AND DISCUSSION

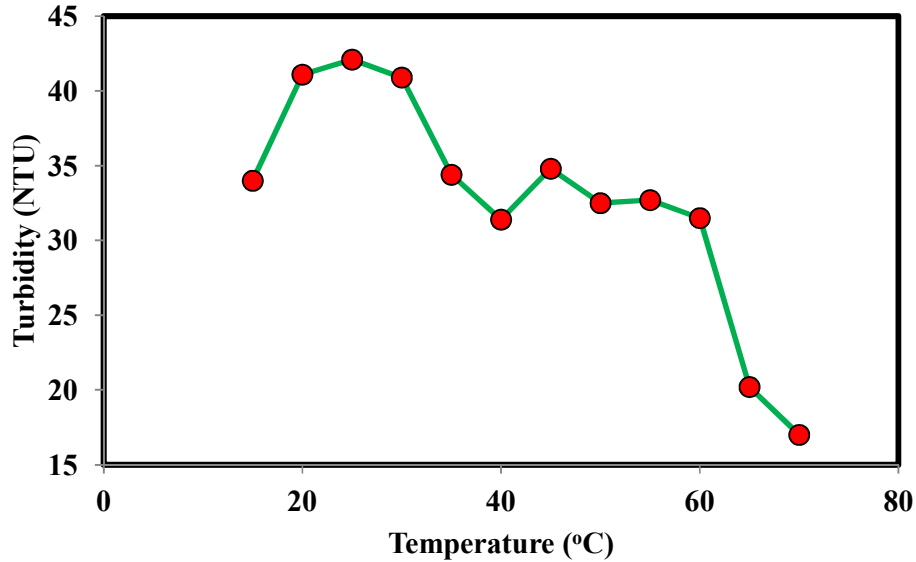
### 3.1 Turbidity of Starch Aqueous Solution as a Function of Temperature and pH

Fig. 3 shows the turbidity of starch aqueous solution as a function of temperature. Aggregation of starch chains in water creates a turbid system [4,8]. The scattering of light and the turbidity depend strongly on the starch conformations [4,8]. Starch solution showed a rapid increase in turbidity, with decreasing temperature. A relatively large increase in the turbidity value was observed when the biopolymer solution was cooled to 15°C. This suggests that collapse is occurring causing the

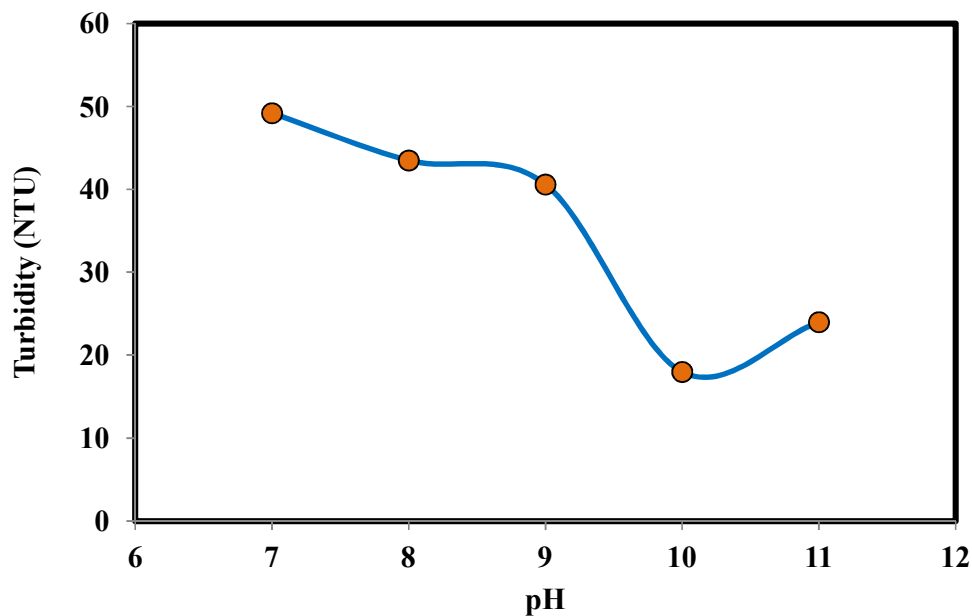
polymer to aggregate and decreasing the amount of light reaching the detector. Turbidity analysis indicated that an open coil starch was formed at higher temperature (70°C).

turbidity values could be attributed to deprotonating of hydroxyl groups at mild basic pH values [8], which leads to form negatively charged alkoxides (R-O<sup>-</sup>). Consequently, an expanded starch chain formed given transparent solution. On the contrary, at low pH values (pH 7 and 8), the starch chain aggregation is favored by a neutral starch chain, hence turbid solution formed.

Fig. 4 displays the turbidity of 0.15 g/L of starch solution as a function of pH. The decrease in the turbidity was recorded when the pH of solution was increased from 7 to 11. This reduction in



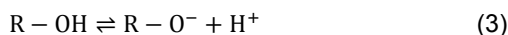
**Fig. 3.** The turbidity of 0.15 g/L of starch solution as a function of temperature at pH8, (Data represent mean values of triplicate measurements)



**Fig. 4.** The turbidity of 0.15 g/L of starch solution as a function of pH at 25°C, (Data represent mean values of triplicate measurements)

### 3.2 The Point of Zero Charge of Starch and Silica Solutions

Unlike a simple electrolyte, a polyelectrolyte undergoes conformational change both in a solution and in the adsorbed state [17]. Due to the multifunctional groups that exist within biopolymer backbone, the adsorption of macromolecules onto minerals is different from that of small molecules. In other words, the chemistry of biopolymers is a key factor governing their conformational behaviour [17]. In this section, the potentiometric technique is applied to study the acid-base behaviour of polymers and the effect of pH on the conformational behaviour of the semi-diluted aqueous solution of unlabelled starch. As shown in Fig. 1 starch chain bears a hydroxyl (R-OH) repeating unit. In this work about 58333 available hydroxyl groups exist in macromolecule backbone ( $M_v = 3.5 \times 10^6$  g/mol). A fraction of R-OH groups dissociates to R-O<sup>-</sup> when the pH of the solution is changed [1, 4, 8] (Equation 3).



The variation in the relative charge density of silica suspensions in the absence and presence of adsorbed starch is illustrated in Fig. 5. The point of zero charges ( $pH_{pzc}$ ) equal  $\sim 3.5$  for silica and  $\sim 9$  for starch,  $pH_{pzc}$  is known as the pH value at which the whole material charge is equal to zero [18]. For example, when the pH value is

lower than the  $pH_{pzc}$  number, the surface of the starch exhibits a global positive charge due to the protonation of the hydroxyl groups. In contrast, when the pH value is higher than  $pH_{pzc}$  value, the biopolymer backbone acquires a negative charge because of the deprotonating of the hydroxyl groups [1,4,8]. As it can be concluded clearly from Fig. 5, the adsorption of silica on the polysaccharide decreases the positive charge considerably from  $\sim 9$  to  $\sim 5$  and lowers the  $pH_{pzc}$  of the starch from  $\sim 9$  to  $\sim 7$ . These results propose that electrostatic interactions are predominantly responsible for the adsorption of starch on SiO<sub>2</sub> suspension molecules.

### 3.3 UV-spectroscopy Analysis of Anthracene Labeled-starch at Silica Suspensions as a Function of pH

The UV-absorption spectra of 0.1 wt% of anthracene labelled-starch at pH 7 and 11 in 10 wt% silica are displayed in Figs. 6 and 7. As mentioned before in the experimental part of the adsorption experiment the absorption intensities were recorded before and after separation. The results showed that the absorption intensity of the supernatant (after separation) is importantly reduced at pH7 compared to a minor reduction at pH11, suggesting that, a large quantity of biopolymer attaches to silica at pH7 while a smaller quantity is adsorbed at pH 11.

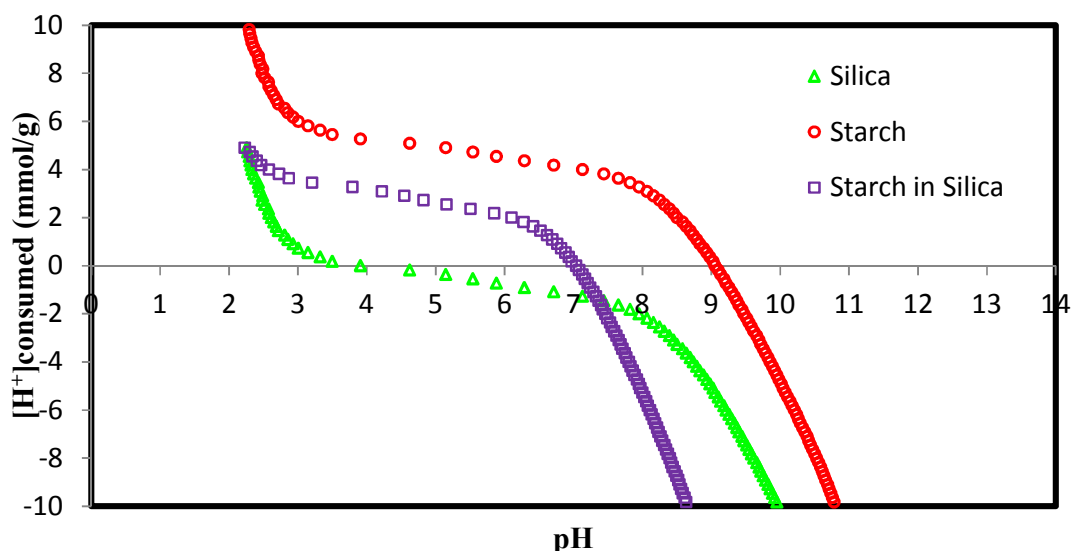


Fig. 5. Relative charge versus pH for silica ( $\Delta$ ), starch ( $\circ$ ) and Starch in silica ( $\square$ ) solutions in 0.01 M NaCl

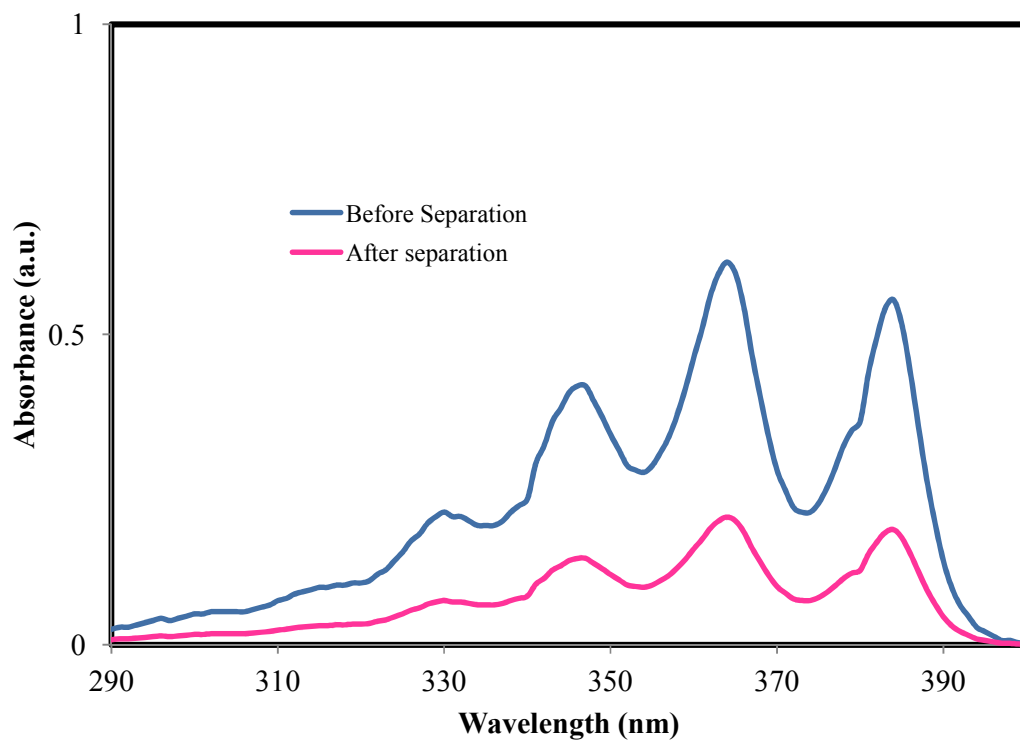


Fig. 6. UV-absorption spectra of aqueous anthracene-labelled starch (10<sup>-1</sup> wt%) and silica (10 wt%) solutions before and after the separation at pH 7

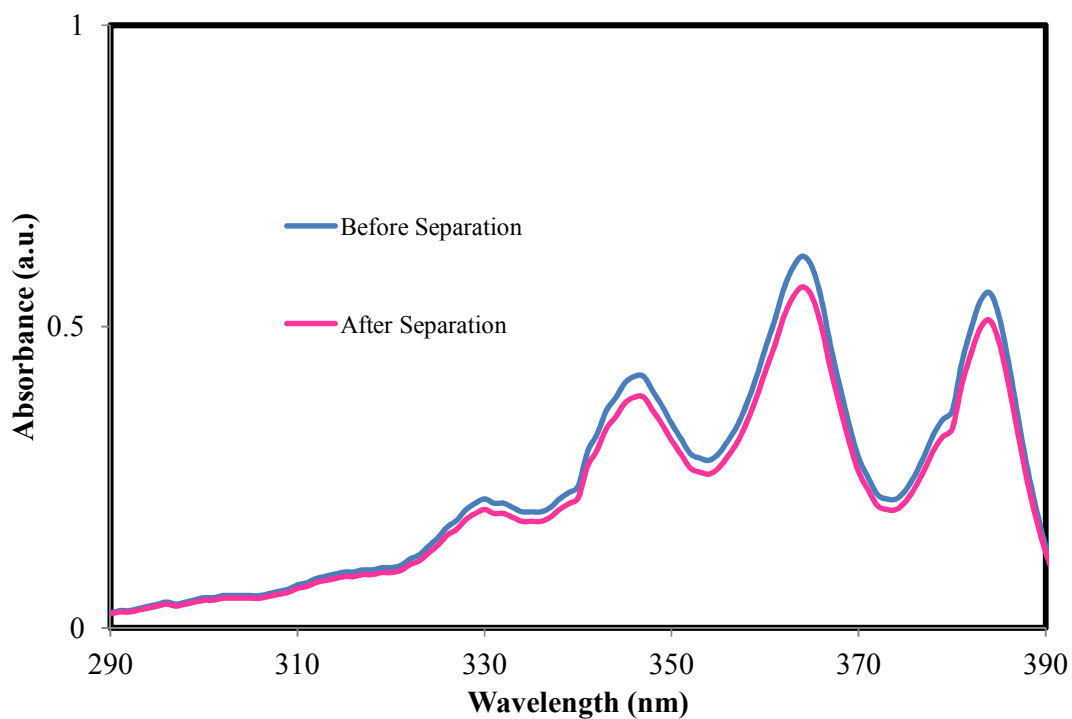


Fig. 7. UV-absorption spectra of aqueous anthracene-labelled starch (10<sup>-1</sup> wt%) and kaolinite (10 wt%) solutions before and after the separation at pH 11

Fig. 8 presents the amount of adsorbed starch as a function of pH and equilibrium starch concentration. In general, the amount of adsorbed starch increases when the pH drops from 11 to 7. The pH dependence of the adsorption could be attributed to the biopolymer

and mineral  $pH_{pzc}$  values, which are 3.5 and 9, respectively. It means that the silica surface is negatively charged at all studied pH values, while the starch is negatively charged at pH 11, neutral at pH 9 and positively charged at pH7. As a result, the electrostatic attraction between starch

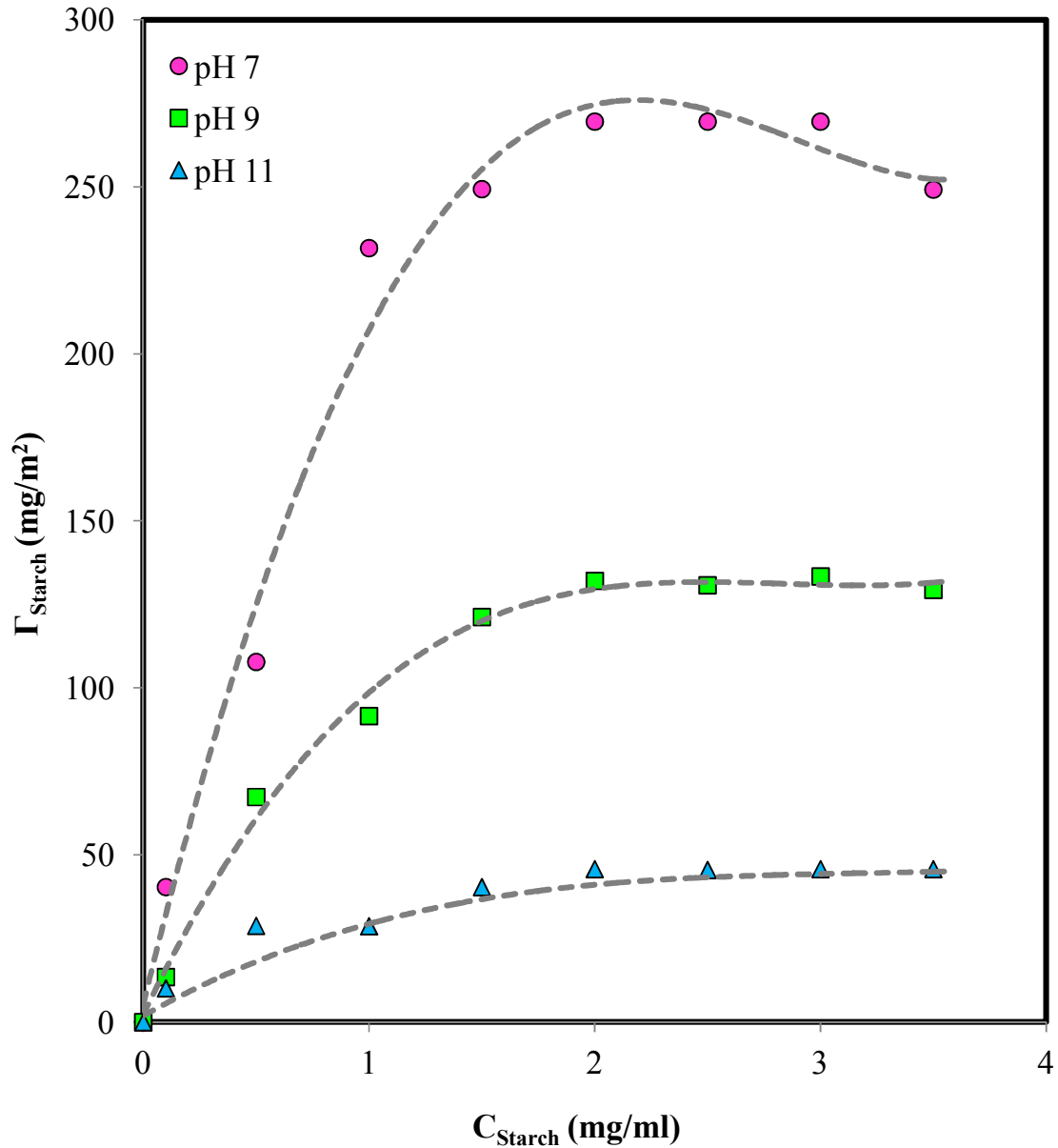


Fig. 8. Adsorption isotherms of starch onto silica at different pH values in aqueous solution as a function of the equilibrium starch concentration, isotherms quantified using steady UV-absorption spectroscopy of anthracene-starch. The points (•,■,▲) are the experimental data, while the dashed lines (--) are the mathematical fitting by general isotherm equation.



chains and the silica suspensions takes place at pH7. The increase of starch adsorption at pH7 could be attributed to the electrostatic attraction between the R-C-OH<sub>2</sub><sup>+</sup> groups in starch and the negatively charged silica (-Si-O<sup>-</sup>). When both the biopolymer and silica have the same kind of charge (i.e. at pH 11). The adsorption of starch on the silica suspensions may be because of that non-electrostatic forces appear between biopolymer and the mineral particles. Otherwise, the interaction of similarly charged materials should not occur [19]. At pH9 besides the non-electrostatic forces, the hydrogen bonding could be responsible for adsorption. On contrary, the starch adsorption increases by increasing the quantity of added starch from 0.1 to 3.5 mg/ml. Exclusively, a steady increase in the adsorbed starch at pH values of 9 and11. While, a marked increase was observed at pH7.

The parameters for the adsorption of starch on silica surfaces can be calculated from the general isotherm equation fitted to the data in Fig. 8. Table shows that the adsorption density reaches to its maximum value (294 mg/m<sup>2</sup>) when the pH of solution decreased from 11 to 7. Similarly, the equilibrium constants  $k_{1,Starch}$ ,  $k_{2,Starch}$  for first layer and multilayer, and the number of clusters of starch molecules  $n$  are also increased by decreasing pH. This suggests that the monolayer adsorption isotherm of starch can be elucidated by  $k_{1,Starch}$ , whereas the multilayer adsorption isotherm of starch can be explained by  $k_{2,Starch}$ . It was reported that [14]  $k_1$  values could be used to estimate the electrostatic and non-electrostatic interactions between polyelectrolyte and mineral. If the adsorption is promoted by only electrostatic force, the first layer adsorption or the value of  $k_{1,Starch}$ , will be decreased when the polyelectrolyte and mineral are similarly charged, this can be achieved by changing the pH of solution. On the contrary, when non-electrostatic interaction happens, the adsorption of similarly charged polyelectrolyte

onto mineral is increased. At pH 9 and 11, starch does not adsorb on silica until a certain equilibrium concentration of starch is obtained, probably caused by the electrostatic repulsion between the anionic starch molecules and negatively charged silica particles. As the pH decreases to 7, the global charge on starch becomes partially positive which provides electrostatic attraction to the anionic silica particles. Thus, adsorption amounts increase with decreasing the pH of solution.

The adsorption free energy ( $\Delta G_o$ ) of the starch adsorption process on the silica surfaces can be calculated using the  $k_1$  value obtained from the isotherm fitted to the data in Fig. 8. A general adsorption isotherm model was fitted to the data to produce adsorption free energies given in Table. The negative values for  $\Delta G_o$  indicates a favourable binding of starch to the silica surface, with the polysaccharide binding more strongly to the surface at lower pH values, when less functional groups are positively charged.

### 3.4 Turbidity Analysis of Starch at Silica Suspensions as a Function of pH

Fig. 9 shows the effect of pH on the flocculation of the silica suspensions by starch in aqueous system. The high turbidity values at pH 11 propose starch does not act as a flocculant for silica suspensions at pH, this is because of the low adsorption density of starch chains. On the other hand, since the pH decreases to 9 and 7, where starch shows higher affinity to silica suspensions, starch particles are powerfully flocculated at low silica content, this is indicated by a decrease in the turbidity values. At high content of silica, the repulsion forces generated between negatively charged silica particles start to rise and ultimately the suspensions are re-dispersed, a gradual increase in turbidity values is noted.

**Table 1. The fit parameters for the adsorption of starch at different pH values in aqueous solution which are maximum adsorbed amount  $\Gamma_{\infty, Starch}$ , the equilibrium constants  $k_{1, Starch}$ ,  $k_{2, Starch}$  for first layer and multilayer, respectively, and  $n$  the number of clusters of starch molecules**

pH	$\Gamma_{\infty,Starch}$ (mg/m <sup>2</sup> )	$k_{1,Starch}$ (m <sup>2</sup> /mg)	$k_{2,Starch}$ (m <sup>2</sup> /mg) <sup>n-1</sup>	$n_{Starch}$	$\Delta G_o$ (J/mol)
7	294	2.6	1667	5.6	-16472
9	156	1.8	834	5.4	-14017
11	50	1.5	567	5.1	-12445

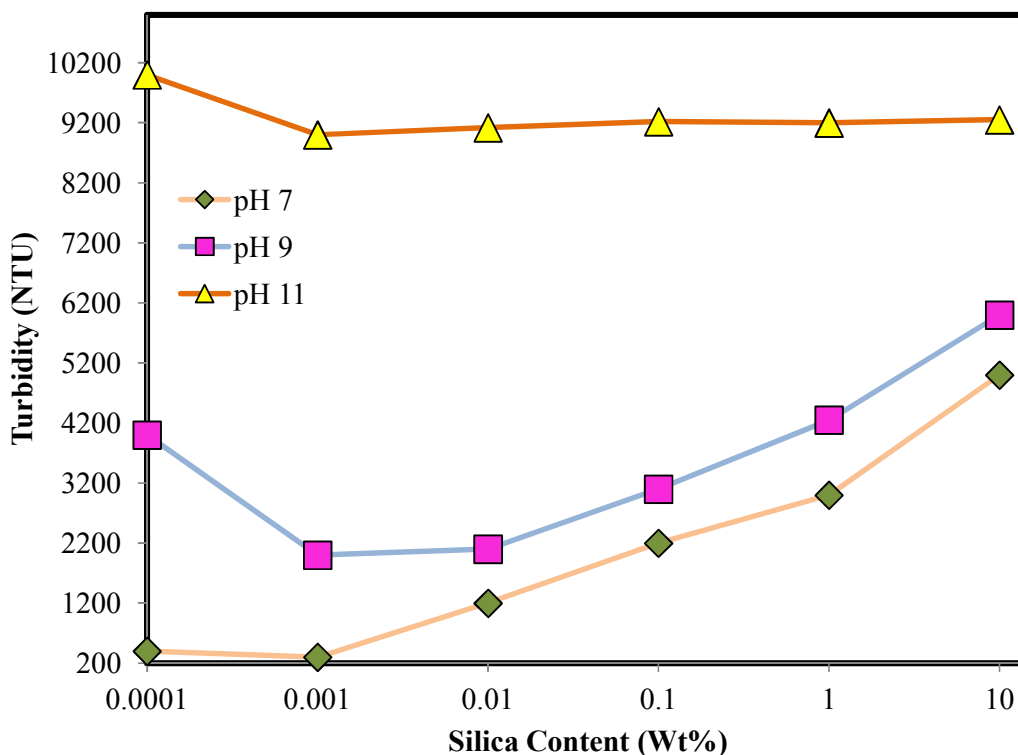


Fig. 9. Effect of pH on the stability of the silica adsorption at starch in aqueous system

#### 4. CONCLUSIONS

The conformational behaviour of starch chain in aqueous solution is temperature and pH sensitive. Starch solution showed a rapid increase in turbidity, with decreasing temperature. This suggested that collapse is occurring causing the biopolymer chains to aggregate. The decrease in turbidity indicated that an open coil starch was formed at higher temperature. The starch with negatively charged alkoxides, due to the deprotonating of hydroxyl groups at mild basic pH values, showed a larger decrease in turbidity than the starch at low pH values, which indicates that chain aggregation is favored by a neutral starch chain and that the formation of an expanded shape is favored by charged starch macromolecules. The point of zero charge results suggested that electrostatic interactions are mainly responsible for the adsorption of starch on  $\text{SiO}_2$  suspension molecules. The UV-absorption spectra of anthracene labelled-starch proposed that the adsorption density of starch on silica is pH dependent. Starch adsorption on silica is extensively increased by decreasing pH to 7, as the electrostatic attraction between the  $\text{R-C-OH}_2^+$  groups in starch and the negatively

charged silica ( $-\text{Si-O}^-$ ) was enhanced. The flocculation of silica suspensions by starch chains depends on the adsorption behaviour of starch on the silica particles. The bridging flocculation of silica particles by starch is rather weak at pH 11, since starch shows extremely low affinity towards silica. When starch shows high affinity towards silica at pH 9 and 7, it starts to play a significant role in the flocculation of silica particles.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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