CO_2 Removal Using Immobilized Carbonic Anhydrase on Amberlite

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***Abstract*—Due to rapid emission of CO_2 gas in atmosphere, an approach has to be made to solve this issue. This paper proposed an approach which known as immobilization of carbonic anhydrase (CA) on amberlite for CO_2 removal. CA has been immobilized on amberlite by cross-linking method using glutaraldehyde. CA immobilized on amberlite was further tested in application of carbonation reaction which involved conversion of CO_2 to $CaCO_3$. It was observed that the optimum time for hydration process in carbonation reaction using immobilized CA was 10 min as compared to blank sample (15 min). The optimum mass of immobilized CA on the $CaCO_3$ precipitation was 0.4 g. The carbonate was characterized using XRD and FESEM to validate the formation of $CaCO_3$. FTIR instrument was used to determine the functional group (NH_3) present in immobilized CA enzyme structure. In conclusion, immobilized CA on amberlite support by cross-linking method could be an effective and economical in the practical conversion system of CO_2.**

***Keywords*— CO_2 removal, Amberlite, Carbonic Anhydrase, Immobilization, Carbonation reaction, $CaCO_3$ precipitation.**

I. Introduction

Carbon dioxide is one of the major gas sources that contribute to the world global warming that is coming from industrial area. It has been reported that the concentration of CO_2 in atmosphere has slightly increase from 280 ppm to 380 ppm in 2007 with accumulation rate of 1.55 ppm v per year [1]. Therefore, a research using carbon capture technology has been conducted to solve this current issue and thus save the nature for the upcoming future. In this research, carbonic anhydrase (CA) is used to capture CO_2 released in atmosphere by converting it into carbonate which will further precipitated as calcium carbonate with an addition of calcium chloride dihydrate. CA is belong to the zinc-metalloenzyme group, and it have an ability to catalyze the reversible hydration of CO_2 to bicarbonate in carbonation process [2].

CA is an enzyme which commonly was found in animals, plants and bacteria. It has been widely studied as it has an important features and high selectivity for the hydration of CO_2 [3]

Recently, CA from bovine erythrocytes is further purified to get high purity product up to 95% of CA for this purpose. The conversion reaction from CO_2 to $CaCO_3$ is shown in Figure 1[4]. The conversion mechanism for CO_2 hydration which is catalyzed by CA involved four steps as Equation 1-5 [5];

$$CO_2\ (\text{gaseous}) \longrightarrow CO_2\ (\text{aqueous}) \quad (1)$$

$$CO_2\ (\text{aqueous}) + H_2O \longrightarrow H_2CO_3 \quad (2)$$

$$H_2CO_3 \longrightarrow H^+ + HCO_3^- \quad (3)$$

$$HCO_3^- \longrightarrow H^+ + CO_3^{2-} \quad (4)$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \quad (5)$$

The reaction between gaseous CO_2 occurs rapidly in water to produce a loosely hydrated aqueous form of CO_2. In the third reaction, once the bicarbonate is formed, the carbonate ions are produced to further react with calcium chloride dihydrate solution to form calcium carbonate [1]. In the conversion CO_2 into $CaCO_3$ using CA, immobilized CA is preferable because of the enzyme stability.

Some of the techniques used for the immobilized enzyme are cross-linking with polymeric networks. The support materials includes silica, graphite and alginate have been studied for the CA enzyme immobilization. In this work, the use of amberlite as support was proposed for the enzyme immobilization. The amberlite support material provides the best combination of pore size and pore volume in order to achieve a high CA loading and activity for the enzyme immobilization [6]. Immobilization activity significantly will reduce the degradation of CO_2 hydration activity which caused by temperature and other chemical impurities that may arise during the reaction.

This paper reported the effect of mass of immobilized carbonic anhydrase towards mass of $CaCO_3$ precipitation and performance of hydration process by using immobilized CA on the amberlite. These effects were investigated for identifying an optimal condition for CA immobilization activity. To the best of our knowledge, the immobilization of CA on amberlite has not been attempted in the past for CO_2 sequestration process.

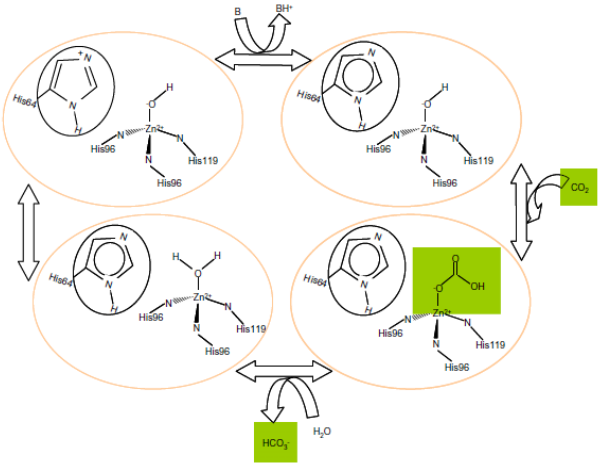

Fig. 1: Catalytic mechanism of carbonic anhydrase active site during reversible hydration of CO_2.

II. METHODOLOGY

A. Materials

Bovine carbonic anhydrase (BCA) in the form of lyophilized powder was purchased from Sigma Aldrich with a purity of 95%. Trizma base and amberlite XAD7HP were purchased from Sigma Aldrich and used without further purification. Bradford reagent and bovine serum albumin (BSA) were all purchased from ThermoFisher Scientific.

B. Preparation of cross linked

0.1% glutaraldehyde was added into 0.2 g of amberlite together with 0.6 mg CA enzyme. The reaction mixture was shaked in incubator shaker at 120 rpm for 2 h. After 2 h, CA immobilized on the amberlite was filtered out using filter paper and rinsed with Tris buffer to remove any loosely bound.

C. Immobilization method

About 0.2 g amberlite has been weighed and washed with distilled water to remove any impurities. After washing, 3 mL of Tris buffer (50 mM, pH=8.0) and 0.6 mg CA enzyme was added. The sample solution was kept in incubator shaker for 4 h at 120 rpm. For the first 2 h, the solution consists of amberlite, CA enzyme and Tris buffer. For the last 2 h, 1% Glutaraldehyde (GA) as a cross-linking agent was added into the solution mixture. After shaking, the materials were filtered out and the CA enzyme immobilized on the amberlite was left to be dried. The remaining solution (before and after immobilized) was used for the assay measured by UV-Vis. The assay system consisted of 30µL of initial solution (before immobilized) and another 30µL of final solution (after immobilized). The assay was observed in absorbance of 595 nm. Blank experiment was also conducted to estimate the self-dissociation of CA enzyme in each assay solution using distilled water and Bradford reagent. All the experiments were conducted in triplicate and the reported values are the mean of the three replicates.

D. Immobilization study

The parameters involved in the study were effect of mass of immobilized CA towards the mass of $CaCO_3$ formation and the time of hydration process for CO_2 hydration using immobilized CA. For all these studies, the experimental set up is described above.

E. Protein estimation

The concentration of protein in a CA enzyme was assayed according to the Bradford method with bovine serum albumin (BSA) as the standard protein. Protein concentration in an enzyme was determined before and after immobilized at absorbance of 595 nm.

F. Determination of hydration time

Blank CA and immobilized CA were used to determine the optimum hydration time for CO_2 gas react with calcium chloride dihydrate to produce $CaCO_3$. The experiment was began with blank CA first and continues with immobilized CA.

G. Determination of the effect of mass immobilized CA

Free CA and immobilized CA were added into Tris buffer (50mM, pH 8.0) solution containing 200 mL $CaCl_2.2H_2O$ at different mass of CA. The reaction between CO_2 and H_2O producing an amount of $CaCO_3$ was determined. The experiment was repeated for different amount of mass which were 0.2, 0.4 and 0.6 g. All the data were collected and tabulated.

H. Carbonation study

The carbonation study was done by the method reported in by [2]. In brief, 3 mL of Tris buffer (50mM, pH 8.0) was added to the beaker containing immobilized CA on amberlite and 200 mL of $CaCl_2.2H_2O$ solution. To initiate precipitation of CO_2 in forms of $CaCO_3$, freshly prepared CO_2 gas was introduced into the reaction mixture at a uniform flow rate. The mixture was kept in water bath at approximately 50°C to maintain the reaction temperature. The solution was continuously stirred by magnetic stirring bar at constant flow rate of CO_2 gas of 500 mL/min. The precipitation was determined by the changes of the solution from clear into a cloudy solution. The time taken for the precipitation to occur was recorded at 5 min. Then, the precipitated solids were collected by filtering through filter paper and the $CaCO_3$ solid powder was dried at room temperature overnight. Then, the mass of $CaCO_3$ solid powder was weighed. The experimental procedure for enzymatic precipitation of $CaCO_3$ was repeated for several times for different time taken as 10 min, 15 min and 20 min.

I. Characterization of materials

The pure of amberlite, CA enzyme and immobilized CA were analyzed using FTIR (Model no: TGA/SDTA 851 from Perkin Elmer) to determine their functional group. XRD of carbon carbonate materials was obtained by using X-ray diffractometer (Model no. D/Max 2200V/PC from Rigaku) with Cu Kα radiation (λ=0.15406 nm) operated at 30 kV and 15 mA. The samples were scanned for 2θ ranges from 10° to 80° with a scanning speed of 5°/min. The surface morphology of the materials was studied by performing Field Emission Scanning Electron Microscopy (FESEM) using SUPRA 40VP instrument. Protein determination in CA enzyme was determined by using UV-Visible Spectrophotometer (UV-Vis) (Model no. UV Line 9400 from Secomam).

III. RESULTS AND DISCUSSION

A. Protein determination of CA enzyme

The concentration of protein available in the CA enzyme was determined according to Bradford method and was characterized using UV-Vis Spectrophotometer. Standard curve was prepared first as shown in Fig. 2.

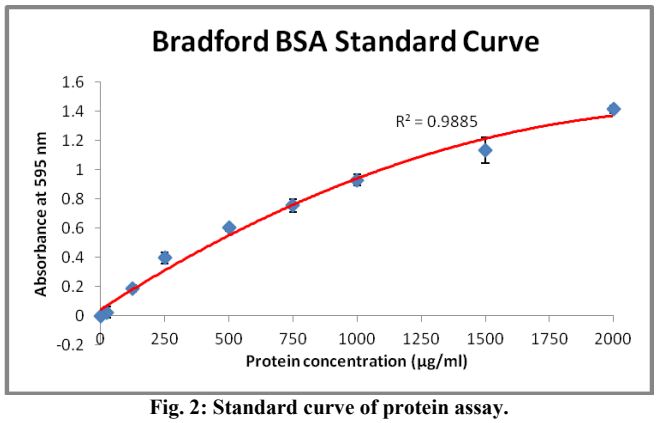

Fig. 2: Standard curve of protein assay.

Once the standard was determined, the protein concentration of enzyme was then observed for both immobilized and non-immobilized enzyme. Table 1 show the absorbance of the sample tested.

Table 1: Protein concentration in CA

Bradford	0.514
Bradford and initial (before immobilized)	0.985

Bradford and final (after immobilized)	0.522

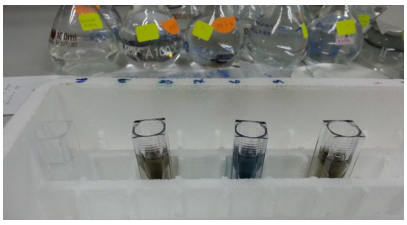

Fig. 3: Protein assay in CA using Bradford reagent.
From left; blank sample, Bradford reagent, CA solution before immobilized, CA solution after immobilized.

Based on the value obtained from UV-Vis, it shows that the protein concentration that is available in solution before immobilized was 0.471. It stated that the enzyme was already mixed with the solution and attached at the surface of amberlite. Referring to Fig. 3, it can be seen that the color of Bradford reagent changes form brown to dark blue. This is due to the presence of protein in CA solution before immobilized. The color changes were also observed in the CA solution after immobilized. It showed light brown color which indicates that the lower amount of protein concentration available in the solution. It means that, during immobilization of CA on amberlite, the percentage of binding between CA and amberlite was about 47%.

B. FESEM analysis for immobilized enzyme

The surfaces morphological of amberlite and immobilized enzyme on the surface of amberlite were determined using FESEM SUPRA 40VP. The result was illustrated in Fig. 4.

Additional information of the attached enzyme was further tested using EDX instrument to confirm the present s of Zn element on the surface of amberlite. Since CA enzyme belongs to a group of Zn-metalloenzyme, it is crucial to know whether there is Zn group attached to the surface. The EDX results on Fig. 5 show that the Zn group present on the amberlite surface containing CA enzyme.

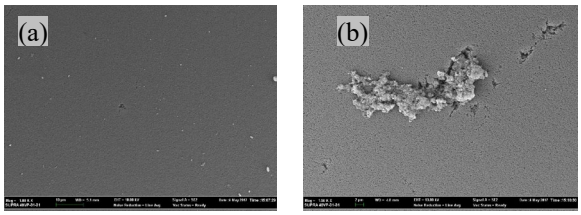

Fig. 4: FESEM image obtained in amberlite shows; (a) Morphological surface of amberlite, and (b) immobilized CA enzyme attached on amberlite. The scale represent 2µm with magnification power of 1500X for (a) image and 10µm with magnification power of 1000X for (b) image.

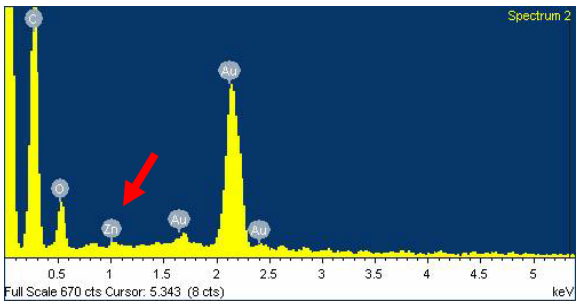

Fig. 5: Enegy Dispersive X-ray (EDX) result confirms the functional group of CA enzyme which consists of Zn-metalloenzyme.

C. FESEM for $CaCO_3$ precipitate

The morphology $CaCO_3$ precipitants were observed using FESEM. The image displayed rectangle shaped crystals of $CaCO_3$ formed by the immobilized CA enzyme which give conformations that the powder formed are in calcite structure. The image of $CaCO_3$ precipitated is shown in Fig. 6.

D. XRD

X-ray diffractometer (XRD) analysis was used to analyze the crystalline phase of calcium carbonate [7]. $CaCO_3$ has three crystalline mineral polymorphs which consists of rhombic calcite, needle-like aragonite and spherical vaterite[8]. The most common polymorph was referred to calcite because it can withstand at high pH and low temperature. Besides, calcite phase was also performed at room temperature under atmospheric conditions. For aragonite and vaterite phase, it is mostly produced at low pH and high temperature in which they can transform into stable calcite phase.

The characterization of $CaCO_3$ was performed using XRD analysis. Based on the results obtained shown by the peak pattern, there were two types of $CaCO_3$ phase forms which are calcite and vaterite. These two patterns show their own peak pattern according to JCPDS [8]. The peak patterns were then analyzed to distinguish the type of pattern at each peak refers to $CaCO_3$ crystals. It shows that at 2θ = 29.252°, 35.813°, 39.292°, 43.048°, 47.362° and 48.353° was corresponding to calcite crystal phases. Meanwhile for the diffraction peak occurred at 2θ = 22.909°, 57.254°, 60.541°, 64.548° and 65.456° was corresponding to vaterite crystal phases. The results were shown in Fig. 7.

The diffraction peak shown by $CaCO_3$ with the absence of CA enzyme was also performed using XRD analysis. This is done to determine the difference between these two types in terms of its degree of crystallinity[5]. The results obtained were given in Fig. 8.

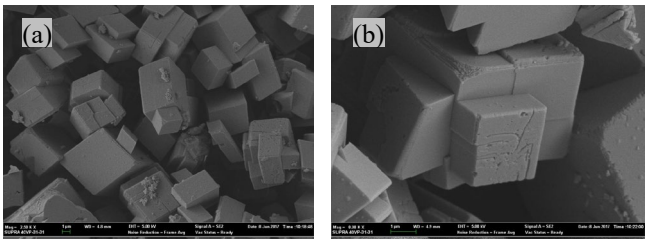

Fig. 6: FESEM image of $CaCO_3$ precipitants obtained in the presence of CA enzyme. The scales represent 10µm (2500X magnification) for (a) image and 1µm (8000X magnification) for (b) image.

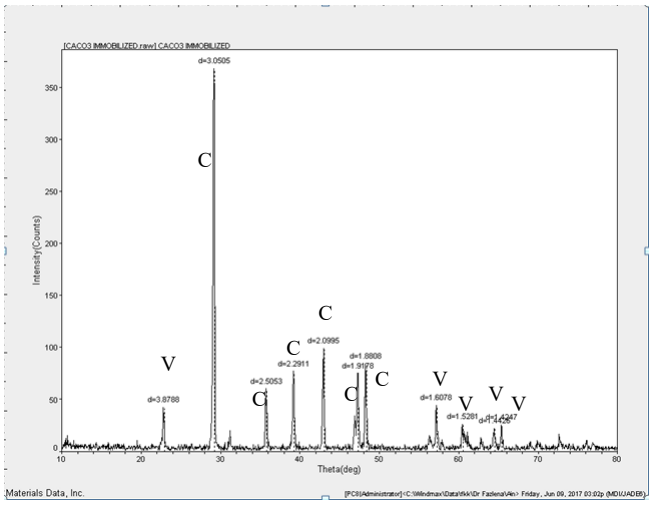

Fig. 7: XRD diffraction peak pattern of $CaCO_3$ precipitants obtained in the presence of immobilized CA; C, calcite; V, vaterite

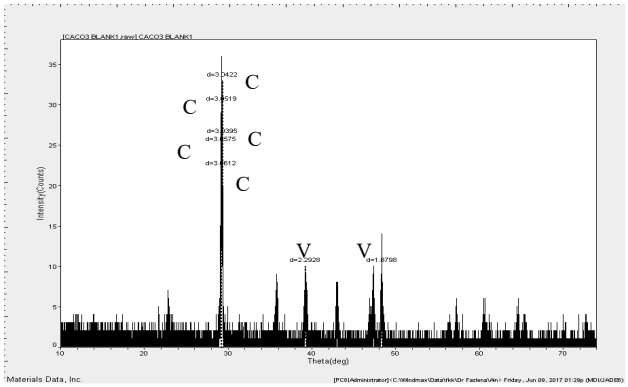

Fig. 8: XRD diffraction peak pattern of $CaCO_3$ precipitants obtained in the absence of immobilized CA; C, calcite; V, vaterite

Based on the peak pattern shown by XRD on Fig. 8, the difference in terms of peak pattern was observed. From Fig. 7, it shows that when CA enzyme was introduced into the reaction, the calcite is the major crystal morphology of $CaCO_3$. However, solution without CA enzyme shown a decreasing amount of calcite phase of $CaCO_3$. As shown in Fig. 8, 2θ = 29.148°, 29.184°, 29.238°, 29.334° and 29.36° was corresponding to calcite crystal phases. Meanwhile for the diffraction peak occurred at 2θ =39.262° and 48.380° was corresponding to vaterite crystal phases.

E. FTIR

The peaks presented at 1720.12 cm^{-1} and 1132.59 cm^{-1} were corresponds to C=O and C-O stretching vibration of ester groups from amberlite molecules as illustrated in Fig. 9. Absorption peak at 1053.51cm^{-1} was corresponds to the characteristics stretching vibration of C-N group. C=O from CA molecule presented by peaks at 1625 cm^{-1}. The analysis was validated that CA enzyme molecule was successfully immobilized on the surface of amberlite.

F. Carbonation study

Carbonation reaction was determined to study the effectiveness of immobilized enzyme. From the carbonation reaction, the precipitation of $CaCO_3$ was observed for both blank sample and immobilized CA as depicted in Fig. 10 and 11. From the experimental activity on blank sample, it was observed that precipitation of $CaCO_3$ was achieved higher at time of 15min. However, for the reaction containing CA enzyme, the time taken for the $CaCO_3$ to precipitate at higher amount was obtained at 10 min. The rapid conversion that occurred is due to the presence of enzyme which has the ability to speed up the carbonation reaction.

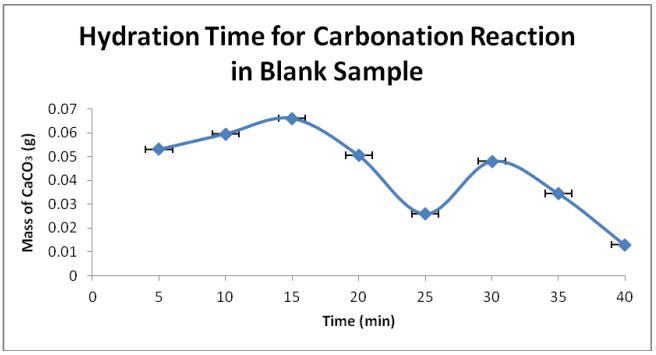

Fig. 10: Hydration time for carbonation reaction in blank sample.

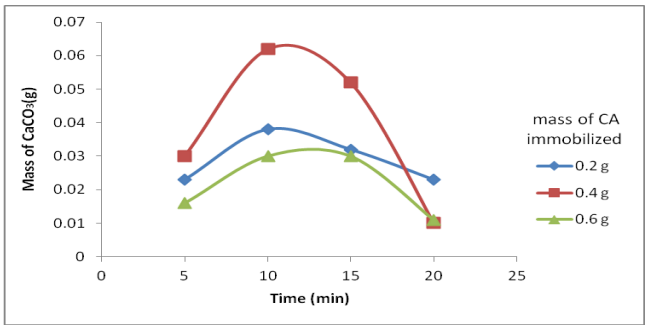

Fig. 11: Mass of immobilized CA used in the carbonation reaction to promote the mass of $CaCO_3$ precipitate.

Fig. 11 shows the trend of $CaCO_3$ mass precipitates during carbonation reaction using different mass of immobilized enzyme. The different value of optimum time was obtained due to the addition of enzyme in the reaction that enhance more rapid conversion [9]. As shown in Fig. 10, the highest amount of $CaCO_3$ mass obtained is at 15 min of the reaction. It means that the reaction of CO_2 with $CaCl_2.2H_2O$ was take place at slow reaction compared to that immobilized CA. However, it was found that the amount of $CaCO_3$ precipitation was not depends on the amount of CA used. This is because lower CA concentration gave greater ability to rapid reach a final conversion reaction to yield greater amount of $CaCO_3$ [10].

By referring to Fig. 11, 0.4 g of immobilized enzyme gave the highest amount of $CaCO_3$ precipitation as compared to 0.2 g and 0.6 g. As compared to Fig. 10, it shows that the performance of the immobilized CA on CO_2 hydration was decreased as reaction time increased. The comparison can be made for both findings which shows that hydration rate of immobilized CA was higher than that of blank buffer solution. However, this difference was only accelerated the hydration rate of CO_2 and does not contribute to any changes in the equilibrium of reaction[11].

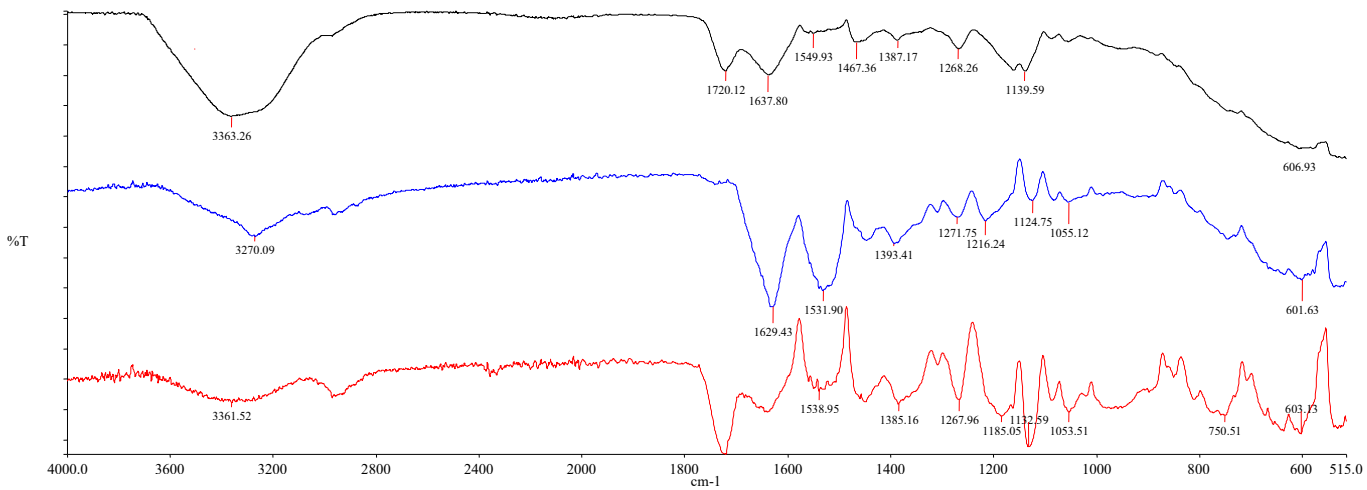

Figure 9: FTIR spectrum analysis for amberlite, enzyme and immobilized enzyme; black-line: amberlite ; blue-line: CA enzyme ; red-line: CA enzyme + amberlite

IV. CONCLUSION

Immobilization of carbonic anhydrase on amberlite was successfully carried out. The effect of mass of immobilized CA towards the formation of $CaCO_3$ mass and the performance of hydration process was determined as 0.2 g, 0.4 g, 0.6 g at time of 10 min. The carbonation reaction conducted was faster with immobilized CA as compared to blank sample.

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