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Preface

We would like to present, with great pleasure, the inaugural volume-5, Issue-8, August 2019, of a scholarly journal, *International Journal of Engineering Research & Science*. This journal is part of the AD Publications series *in the field of Engineering, Mathematics, Physics, Chemistry and science Research Development*, and is devoted to the gamut of Engineering and Science issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with IJOER. We are certain that this issue will be followed by many others, reporting new developments in the Engineering and Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOER* readers and will stimulate further research into the vibrant area of Engineering and Science Research.



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Immobilization of Biotechnologically Important *Candida rugosa* Lipase onto Commercial Matrices

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Abstract— The continual search for alternative environmentally cleaner technologies in industrial processes has led to an increase in the use of enzymatic processes globally. However, due to their physical characteristics they require immobilization in order to remain effective. The objective of this study was to investigate the immobilization of the biotechnologically important and commercially available *Candida rugosa* lipase (CRL) by physical interfacial adsorption onto a number of matrices to act as biocatalysts. Five different types of support were tested: i) macroporous silica (synthetic inorganic), ii) polyhydroxybutyrate (natural organic), iii) polypropylene (synthetic organic), iv) polymethacrylate (synthetic organic), and v) polystyrene-divinylbenzene (synthetic organic). Results generated during this study showed that from the group of materials tested, polystyrene-divinylbenzene gave the best results with the highest amount of immobilized protein (8.10 ± 0.31 mg/g) and a good immobilization yield ($90.35\% \pm 1.53\%$). The efficiency of protein immobilization was found to be highest when carried out at pH4.5, which is close to the isoelectric point of the enzyme.

Keywords—Biocatalysts, *Candida rugosa*, immobilized protein, lipase, physical adsorption, support.

I. INTRODUCTION

Over recent decades, the search for green / environmentally friendly / cleaner technologies (balanced with the need to remain economically viable) for use in almost all industrial chemical processes has seen the increase in the use of enzymatic methodologies to achieve these targets. This has resulted in the enzyme market experiencing a high growth rate; it is estimated that in the period 2018-2023, the global enzyme market will grow at an annual rate of 4.9% and rise from US \$5.5 Billion in 2018 to US\$7 Billion in 2023 [1]. Within the global enzyme market, there are six groups of enzymes which stand out: proteases, carbohydrases, lipases, phytases, polymerases and nucleases.

The interest in these biocatalysts goes beyond their ability to simply increase the rate a chemical reaction (i.e. the role of a traditional catalyst); they offer alternatives for many chemical processes due to their unique properties of biodegradability, specificity and selectivity in reactions [1-3].

Of specific interest in this study, Lipases are extremely versatile enzymes and are used in a variety of applications: in addition to simple hydrolysis reactions they can catalyse esterification and transesterification reactions and are also frequently used in the cosmetics, food, flavouring, beverage, paper, lubricant, detergent, effluent treatment, biosensor and biodiesel industries[2-4].

The industrial application of lipases (and other enzymes) in large-scale processes is often limited by the high cost and stability issues faced under industrial conditions; they are fragile structures which are sensitive to high temperature, variations in pH and various solvents. In addition, it can be difficult to separate them from some reaction systems, which limits their recovery and re-use which in turn may also lead to the contamination of the final product [5,6]

In order to overcome some of these limitations, the use of enzymes that are immobilized is a strategy that has been adopted to protect the enzyme, however in order to remain as an efficient active biocatalyst, the selection of an appropriate support material and application protocol are key factors [7]. Support can be from various origins: organic / inorganic / natural / synthetic and may also have a variety of porosities (porous, microporous, mesoporous, macroporous). The overall success of the immobilization is dependent on the chemical, mechanical and morphological compatibility between the biocatalyst and

the surface material to which it is confined. Such compatibility factors include functional groups, hydrophobicity / hydrophilicity, internal geometry, surface area, mechanical resistance and porosity [5,8]

In the literature, lipases have been shown to be successfully immobilized through various immobilization methods including adsorption, entrapment, covalent coupling, cross-linking reversed micelles and ion pairs, with the adsorption, entrapment and covalent coupling methods being the most common [9-11]. Physical adsorption involves enzymes being physically attached to the support through weak electrostatic forces such as van der Waals forces, hydrophobic interactions or hydrogen bonding. Whereas entrapment (as the name suggests) is where the enzymes are trapped within a polymeric material (sometimes a gel) which itself is attached to the support substrate. In comparison, covalent bonding is where the enzyme is covalently bonded by some of its amino acids to a coupling group on the support substrate [12,13]

Of these methods, the physical adsorption process is attractive from an operational point of view as it has a number of benefits: i) it requires mild conditions to achieve and is a relatively easy process, ii) it generally has a low cost, iii) it eliminates the addition of chemical agents, and iv) by the desorption of inactive enzymes from the support surface of the biocatalyst it allows the reuse of the material [4,5]

Furthermore, one of the fundamental characteristics of lipases is their ability to adsorb hydrophobic surfaces through the hydrophobic pocket present in the region of the active site, which acts on the modulation of its catalytic activity [14]. This situation favours the formation of hydrophobic interactions, which are typical of the physical adsorption process.

Considering these factors, this study was designed to study hydrophobic materials of different origins to determine the best support for the immobilization of *Candida rugosa* lipase by physical adsorption. The evaluation of the influence of immobilization pH on the catalytic activity of the prepared biocatalysts was also assessed.

II. MATERIALS AND METHODS

2.1 Materials

Candida rugosa microbial lipase (type VI), polyhydroxybutyrate, polymethacrylate (Diaion® HP-2MG) and polystyrene-divinylbenzene (Diaion® HP-20) were purchased from Sigma-Aldrich® (St. Louis, USA). Macroporous silica (Immobead S60S) and polypropylene (Immobead IB-S500) were purchased from Chiral Vision (Leiden, Netherlands). Low-acid olive oil purchased locally (Carbonell). All other reagents, solvents, and gum Arabic were obtained from Synth and Vetec (São Paulo, SP).

2.2 Immobilization of Lipase by Physical Adsorption

Five different types of supports were tested: macroporous silica (synthetic inorganic), polyhydroxybutyrate (natural organic), polypropylene (synthetic organic), polymethacrylate (synthetic organic) and polystyrene-divinylbenzene (synthetic organic). Each material was tested separately by the following method which itself was adapted from the method presented by Mendes *et al* [15,16].

Firstly, the selected support was soaked in 95% ethanol for 2 hours at 25°C. After this step, the support materials were vacuum filtered and further washed with ethanol to produce “pre-treated supports”. These pre-treated supports (0.5 g) were then individually incubated in 9.5mL of enzyme solution (containing an initial protein loading of 13.6 mg protein per gram of support) in 10mM phosphate buffer at pH7.0 for 24 hours. Following the incubation period, the prepared “biocatalyst” (immobilized *Candida rugosa* microbial lipase on the support) was vacuum filtered, washed with distilled water and stored at 4°C.

2.3 Determination of Protein

The concentration of immobilized protein (IP) was determined by the method developed and published by Bradford [17]. The efficiency of the immobilization procedure was performed by determining the concentration of protein in the immobilization supernatant before and after the procedure using the calculation shown in (1).

$$IP = \frac{V \times (C_0 - C_t)}{M} \quad (1)$$

Where: IP is the mass of immobilized protein per gram of support (mg/g), V is the volume of the enzyme solution (mL), C_0 is the initial protein concentration (mg/mL), C_t is the residual protein concentration (mg/mL) after a given incubation time, and M is the support mass used for immobilization.

2.4 Determination of Hydrolytic Activity

The hydrolytic activity (HA) was determined using the Soares *et al.* hydrolysis method, which involves the hydrolysis of emulsified olive oil [18]. The substrate was treated by an emulsion containing 50g olive oil and 50g of a 3% (w/w) gum Arabic solution. The reaction solution was prepared: 5 mL of substrate, 4.9 mL of sodium phosphate buffer solution (100mM, pH7.0) and 0.1g of immobilized enzyme or 0.1 mL of enzyme in soluble form or the immobilization supernatant were added to a 125 mL Erlenmeyer flask. The flasks were incubated at 37°C for 5min in a thermostatic bath whilst shaking at 150rpm. After the incubation period, the reaction was quenched by the addition of 10 mL of a 1:1 v/v mixture of acetone and ethanol (92.5%). The fatty acids released were then quantified by titration with a 20mM aqueous sodium hydroxide (NaOH) solution, using phenolphthalein as an indicator [19].

The apparent hydrolytic activity (HA) of the biocatalysts (measured in Units/g or Units/mL) was calculated according to the methodology of Soares *et al.* [18], according to (2).

$$HA = \left(\frac{(V_t - V_c) * 10^3 * Mol}{M_e * t} \right) \quad (2)$$

Where V_t is the volume (mL) of NaOH titrant solution for the test sample; V_c is the volume (mL) of the NaOH titrant solution used in titration of the control; Mol is the molarity (mol/L) of the titrant solution; M_e is the mass (g or mL) of enzyme used (proportional to the aliquot used) whether free or immobilised; t is the reaction time (minutes).

The immobilization yield (IY) calculated as a percentage was determined using (3).

$$IY (\%) = \left(\frac{HA_0 - HA_f}{HA_0} \right) \times 100\% \quad (3)$$

Where HA_0 and HA_f are the apparent hydrolytic activity (measured in U/g or U/mL) of the enzyme in solution before and after immobilization, respectively.

The specific hydrolytic activity (HAE) of the biocatalysts (measured in Units/g or Units/mL) was calculated according to (4).

$$HAE = \frac{HA}{IP} \quad (4)$$

Where HAE is the apparent hydrolytic activity (U/g) of the prepared biocatalysts and IP (mg/g) is the concentration of immobilized protein.

2.5 Influence of pH on the Physical Adsorption of Lipase

The influence of pH on the adsorption process was determined in the range of pH4.0 to 8.0 with an ionic strength of 10mM [15,16]. In this study, sodium acetate solutions (to achieve pH4.0, 4.5 and 5.0) and sodium phosphate solutions (to achieve pH6.0, 7.0 and 8.0) were used in the preparation of the enzymatic solutions for immobilization. The suspensions (0.5g carrier in 9.5mL of enzyme solution) were incubated in sealed Duran flasks (250 mL and kept stirring (200 rpm) in a rotary incubator for a maximum period of 24 hours at 25°C. At the end of the immobilization process, aliquots (0.5mL) were removed for the experimental determination of both the hydrolytic activity and residual protein concentration of the immobilization supernatant. The hydrolytic activity of the immobilized derivative was also determined after being vacuum filtered and washed with distilled water.

III. RESULTS AND DISCUSSIONS

The properties of the commercially available support substrates selected for the *Candida rugosa* lipase immobilization study are summarised in Table 1.

TABLE 1
PROPERTIES OF THE COMMERCIALY AVAILABLE SUPPORT SUBSTRATES SELECTED FOR THE *Candida rugosa* LIPASE IMMOBILIZATION STUDY

Support	Commercial Name	Surface Area (m ² /g)	Pore Size (Å)
Polyhydroxybutyrate	Polyhydroxybutyrate	17.1 *	31.0 *
Polymethacrylate	Diaion HP2-MG	500.0 **	170.0 **
Polypropylene	Immobead IB-S500	647.8 ***	91.8 ***
Polystyrene-divinylbenzene	Diaion HP-20	500.0 **	260.0 **
Silica	Immobead 560S	74.8 #	193.0 #

*Sources: *SILVA et al.[20]; **Sigma-Aldrich; ***CHIRAL VISION; #LIMA et al.[6]*

Experimental results obtained during this study demonstrating the catalytic properties of the prepared biocatalysts are shown in Table 2.

TABLE 2
CATALYTIC PROPERTIES OF THE IMMOBILIZED *Candida rugosa* LIPASE ON THE SELECTED SUPPORTS

Support	Immobilization Yield (IY) [%]	Immobilized Protein (IP) [mg/g _s]	Apparent Hydrolytic Activity (HA) [U/g _s]	Specific Hydrolytic Activity (HAE) [U/mg _p]
Polyhydroxybutyrate	95.22 ± 5.07	4.67 ± 0.38	395.43 ± 75.41	84.69 ± 16.15
Polymethacrylate	89.16 ± 0.15	7.70 ± 0.59	50.59 ± 1.73	6.57 ± 0.23
Polypropylene	66.16 ± 1.53	6.75 ± 0.17	261.22 ± 5.20	38.69 ± 0.77
Polystyrene-divinylbenzene	90.35 ± 1.53	8.10 ± 0.31	74.20 ± 33.82	9.16 ± 4.17
Silica	73.18 ± 0.46	4.63 ± 0.47	59.33 ± 11.06	12.82 ± 2.39

Source: author data (where g_s is gram of support and mg_p is gram of protein)

As the data presented in Table 2 shows, high yields of immobilization were obtained using silica (73%), polymethacrylate (89%) and polystyrene-divinylbenzene (90%), which is probably due to the large pore size that allows high lipase retention – the crucial requirement in the preparation of active biocatalysts. These supports have been widely used in the preparation of various biocatalysts through the immobilization of several lipases in the synthesis of important esters (biolubricants and biodiesel), through esterification and transesterification reactions [6,10,11].

Candida rugosa lipase is a globular protein with a reported molecular volume of 50Å × 42Å × 33Å and a molecular diameter of approximately 51Å as reported by Gao *et al* [21]. As the data presented in Table 2 shows, the mean pore size of the supports tested was greater than the molecular diameter of the lipase except for polyhydroxybutyrate. The polyhydroxybutyrate contains pores that have a diameter smaller than that of the enzyme, which coupled with the high immobilization yield (95.22% ± 5.07%), indicates that the adsorption is preferentially on the external area of the support. Polypropylene produced a lower immobilization yield (66.16% ± 1.53%), but it also produced a high value for hydrolytic activity (261.22 ± 5.20 Units/g), which when considering how close the pore size is to the diameter of the enzyme, this suggests that the immobilization also occurs in the external region. These results indicate that *Candida rugosa* lipase can be adsorbed both on the external and internal surfaces of the support.

Due to its high immobilization yield and hydrolytic activity, polyhydroxybutyrate could be considered an adequate support, however as the immobilization occurs only on the external area of the support (resulting in a gain in hydrolytic activity) the biocatalyst effectively becomes unprotected, which means that it is vulnerable to being denatured if used under harsh industrial processing conditions, which makes the polyhydroxybutyrate a unattractive option.

The results shown in Table 2 and discussed thus far, clearly indicate that polystyrene-divinylbenzene and polymethacrylate are the two most suitable supports. Both have similarly high Immobilization Yields (IY) and quantity of Immobilized Protein (IP); however, when considering the apparent hydrolytic activities (HA) and specific hydrolytic activities (HAE), then we see a difference, which indicates that polystyrene-divinylbenzene is the better option. These differences in hydrolytic activities are most likely due to the difference in pore sizes (as shown in Table 1) where the polystyrene-divinylbenzene (260.0Å) has a larger pore size than the polymethacrylate (170.0Å). Having the larger pore size may allow better accessibility of the

substrate molecules to the biocatalytic microenvironment whilst simultaneously leaving the biocatalyst protected from external environment conditions.

Thus, polystyrene-divinylbenzene was selected as the most suitable support substrate for the second phase of testing where the effect of pH on adsorption was investigated, and the results are summarised in Table 3.

TABLE 3
INFLUENCE OF pH ON THE CATALYTIC PROPERTIES OF *Candida rugosa* LIPASE IMMOBILIZATION IN POLYSTYRENE-DIVINYLBENZENE.

pH	Immobilization Yield (IY) [%]	Immobilized Protein (IP) [mg/g _s]	Apparent Hydrolytic Activity (HA) [U/g _s]	Specific Hydrolytic Activity (HAE) [U/mg _p]
4.0	90.27 ± 6.88	6.63 ± 0.05	32.53 ± 4.52	4.94 ± 0.04
4.5	89.21 ± 0.29	6.74 ± 0.03	76.84 ± 9.62	11.40 ± 0.04
5.0	89.31 ± 1.50	7.34 ± 0.08	31.85 ± 6.34	4.34 ± 0.05
6.0	85.10 ± 1.69	1.49 ± 0.31	18.59 ± 5.37	12.78 ± 2.68
7.0	84.84 ± 1.54	2.11 ± 0.16	29.52 ± 1.96	14.02 ± 1.03
8.0	87.76 ± 1.08	4.96 ± 0.05	29.69 ± 4.14	5.99 ± 0.06

Source: author data (where g_s is gram of support and mg_p is milligram of protein)

As the data presented in Table 3 shows, there appears to be no significant influence of pH variation on the yield of immobilization (IY). However, the pH does appear to have influenced both the apparent hydrolytic activity (HA) and specific hydrolytic activity (HAE) of the biocatalyst. This is most likely to be because the active site on the *Candida rugosa* lipase has an α -helix structure which is referred to as a “cap” or “flap”. This “cap” is composed of amino acids with amphiphilic properties and the pH of the microenvironment can influence the movement of this “cap”, which are reflected in the values of enzymatic activity as reported by María *et al* [22].

With regard to the values measured for the Immobilized Protein (IP), the maximum value obtained (7.34 ± 0.08 mg/g), was recorded at pH5. However, there is a further factor which must be taken in consideration. The isoelectric point of *Candida rugosa* lipase is around pH4.2 [23]. At the isoelectric point, maximal hydrophobic interaction between lipase and the support surface (interfacial activation mechanism) which favours the enzyme adsorption process and the partitioning of substrate molecules to the surface of the biocatalyst can be expected. Additionally, it is known that the immobilization pH is an important factor in the preparation of active biocatalysts since they influence both the surface load of the supports, and the degree of ionization of the protein molecules [24].

This isoelectric point effect can be observed when looking at the hydrolytic activities recorded in Table 3. At pH4.5, both the apparent hydrolytic activity (HA) and specific hydrolytic activity (HAE) are significantly higher than the results obtained at pH5.0. As the Immobilization yield and immobilized protein content have similar values at both pH4.5 and pH5.0, the hydrolytic activity results clearly show that the most appropriate pH condition for immobilization is pH4.5 (close to the isoelectric point of lipase).

IV. CONCLUSION

The results obtained during this study indicate that polystyrene-divinylbenzene is a very good material for a support structure in the immobilization of *Candida rugosa* lipase, especially when carried out at pH4.5, which is close to its isoelectric point (pH 4.2). In addition, the physical properties of polystyrene-divinylbenzene allowed the *Candida rugosa* lipase to be immobilized preferentially within the support where the biocatalyst is protected from variations in a reaction environment, reducing the possibility of denaturation. Additional future work we focus on the stability of the prepared biocatalyst when applied in biotransformation reactions.

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Kinetic Characteristics for Reaction between Trichloroisocyanuric (TCCA) Acid with 2-Chlorobenzylidene Malononitrile (CS)

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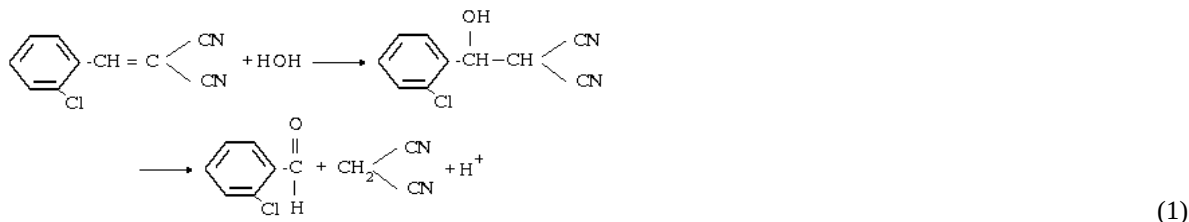
Abstract— The research experimental results determining rate constant of the reaction between TCCA and CS according to Arrhenius equation was studied. Basing the set of graphs showing the relationship between $(\ln(k) - (1/T))$ and $(\ln(k/T) - (1/T))$, the activation energy (E_a) according to the Arrhenius equation and activation enthalpy variation ($\Delta H^\ddagger$), activation entropy variation ($\Delta S^\ddagger$), free activation energy Gibbs ($\Delta G^\ddagger$) according to the Eyring equation are determined. The products of TCCA reaction and CS at pH = 9 are 2-chloro benzaldehyde oxirane-2,2-dicarbonitrile, 3-(2-chlorophenyl) so predicting the reaction mechanism consists of two reactions taking place in parallel, a hydrolysis and oxidation reactions.

Keywords— Trichloroisocyanuric acid, 2-chlorobenzylidene malononitrile, Kinetic characteristics.

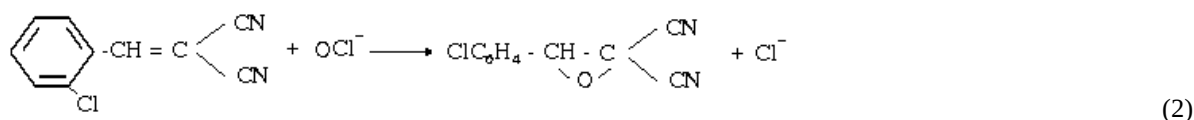
I. INTRODUCTION

2-chlorobenzylidene malononitrile (CS) is heavily used in the Vietnam War, statistics show that about 9,000 tons of CS were used on the battlefield [1]. Due to being durable, difficult to decompose, it can be found that CS existed since the war in Central and Southern Vietnam. That the reason we need to deal with the amount of residual CS found. Reactions that are often used to dissolve CS [4], [8] include:

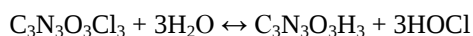
CS hydrolysis reaction takes place as follows:



and CS oxidation reaction by OCl^- takes place as follows:



Acid trichloroisocyanuric (TCCA) is hydrolyzed to form hypochlorous acid HOCl and acid cyanuric as following [3]



However, not all chlorine content in TCCA hydrolyzed into HOCl. Thus a part of chlorine still exists in TCCA called bound available chlorine [6]. TCCA is hydrolyzed to produce HOCl and depending on pH and then HOCl proceeding hydrolyze to form OCl^- [7].



So, the solution TCCA has a pH > 7 that can react with CS in two reactions (1) and (2). From the Arrhenius equation, E_a - activation energy (kJ/mol) was determined [6]; From the Eyring equation determine $\Delta H^\ddagger$ - Activated enthalpy variation (kJ/mol), $\Delta S^\ddagger$ - Activated entropic variation (kJ/mol.K) and calculate $\Delta G^\ddagger$ - Free energy variability Activated Gibbs (kJ/mol) [6]. Use GC/MS method to determine the product of TCCA reaction and CS from which to predict the mechanism of reaction.

II. EXPERIMENTAL

2.1 Apparatus and Chemicals

2.1.1 Apparatus

UV-Vis Spectrophotometer UV-VI Jasco V530, Japan

Gas chromatography/Mass spectrometry GC 6895 / MS HP 5975A, USA

2.1.2 Chemicals

TCCA (Sigma - Aldrich);

Solid CS with 99.0% purity (Vietnam);

Other used chemicals for research and analysis include: Na_2SO_4 , phosphate buffer pH = 9, NaOH, KCl, H_2SO_4 , FeCl_2 , sodium 1,2-naphthoquinone-4-sulfonate, ethanol, (Pa, Sigma- Aldrich) , acetonitrile, methanol, dichloromethane, chloroform, n-hexane (Pa, Merck).

2.2 Experimental procedure

2.2.1 Prepare the solution

Mix 50 mg of CS in 100 ml of ethanol to obtain CS solution with a concentration of 495.00 mg / L (2.652 mM / L) to obtain solution S1.

Mixing 960 mg of TCCA in 500ml of distilled water obtained TCCA solution with a concentration of 1,920.00 mg / L (8.261 mM / L) obtained S2 solution.

Take in the reaction flask 5ml of phosphate buffer pH = 9, 10mL of solution S1, 10mL of solution S2 to obtain solution S3 have: $C_{\text{CS}} = 198.00 \text{ mg / L}$; $C_{\text{TCCA}} = 768.01 \text{ mg / L}$.

2.2.2 Determine the parameters of the reaction

2.2.2.1 Determine the reaction level

After 3-minute; 5 minutes; 10 minutes; 15 minutes; 25 minutes, take sample S3 solution, analyze the remaining CS concentration. Develop a graph showing the relationship ($\ln(C_{\text{CS}}) = f(t)$). If the graph is linear, then the reaction is an apparent pseudo first order reaction.

2.2.2.2 Determine the reaction rate constant

Determine the reaction rate constant. If the reaction between TCCA and CS determined according to 2.2.2.1 is level 1, the reaction rate is expressed by the equation:

$$v = \frac{-dC}{dt} = k_A(C_0 - x) \quad (4)$$

In which: k_A is the reaction rate constant according to Arrhenius (s^{-1})

C_0 is the concentration of CS at time $t = 0 \text{ s}$ (mol/L)

x is the concentration of CS participated in the reaction

$$(C_0 - x) = C_{\text{CS}}$$

In which: C_{CS} is the concentration of CS at time t in solution (mol/L)

Transformation (4) is obtained:

$$\ln(C_{\text{CS}}) = -k_A t + CT \quad (5)$$

In which: CT is an integral constant

t is the reaction time (s)

The equation has the form of $y = ax + b$ with $y = \ln(C_{\text{CS}})$; $a = -k_A$; $x = t$; $b = CT$.

The reaction rate constant is determined as follows:

$$k_A = -a \quad (6)$$

2.2.2.3. Determination of E_a according to the Arrhenius equation

Arrhenius equation is as follows:

$$k_A = Ae^{\frac{-E_a}{RT}} \quad (7)$$

Inside:

k_A is the reaction rate constant according to Arrhenius (s^{-1})

T is the absolute temperature (K)

A is the exponential factor. According to collision theory, A depends on the frequency of collisions in the right direction and is considered to be independent of T.

E_a is activation energy (kJ/mol)

R is the ideal gas constant ($R = 8,315 \text{ J/mol.K}$)

The transformation of equation (7) is obtained:

$$\ln k_A = \frac{-E_a}{RT} + \ln A \quad (8)$$

Equation (8) has the form of $y = ax + b$ with $y = \ln(k_A)$; $a = -E_a/R$; $x = 1/T$; $b = \ln A$.

Maintain the temperature of S3 solution at the temperature respectively: 293 K, 298 K, 303 K, 313 K. Determine the reaction rate constant at the temperatures according to 2.2.2.2.

Develop a graph showing the relationship ($\ln(k_{A-CS}) = f(1/T)$). Then, activation energy is calculated according to the formula:

$$E_a = -a.R \text{ (J/mol)} \quad (9)$$

2.2.2.3. Determination of $\Delta H^\ddagger$, $\Delta S^\ddagger$, $\Delta G^\ddagger$ according to the Eyring equation:

Eyring equation:

$$k_A = \frac{k_B T}{h} e^{\frac{-\Delta H^\ddagger}{RT}} e^{\frac{\Delta S^\ddagger}{R}} \quad (10)$$

Equation transformation (10):

$$\ln \frac{k_A}{T} = -\frac{\Delta H^\ddagger}{R} \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R} \quad (11)$$

Equation (11) has the form of $y = -ax + b$, where $x = 1/T$; $y = \ln(k_A/T)$; and $a = \frac{-\Delta H^\ddagger}{R}$, $b = \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R}$

From that set of graphs shows the relationship between ($\ln(k) - (1/T)$) (11):

$$\Delta H^\ddagger = -a.R \text{ (J/mol)} \quad (12)$$

$$\Delta S^\ddagger = R(b - \ln(k_B/h)) \text{ (J/mol.K)} \quad (13)$$

Free activation energy Gibbs ($\Delta G^\ddagger$) follows the equation:

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \text{ (J/mol)} \quad (14)$$

2.2.3. Methods of analysis

2.2.3.1. Analysis of CS by UV-Vis Spectrophotometry

Sampling S3 solution in experiments, analyzing CS concentration on the Jasco-V530 UV / VIS device based on the reaction between CS and 1.2 naphthoquinon-4-sulfonate reagent in alkaline medium, CS concentration in The solution is determined by measuring the optical absorbance of the solution and comparing it with the calibration graph. Analytical

samples were dissolved in solvents; dehydration in the analytical sample by Na_2SO_4 ; Filter about 5 μl as a sample by Whatman filter [2].

2.2.3.2. Analysis of reaction products by GC/MS

Sampling solution S3 when $t = 25$ minutes, $T = 298$ K, product analysis of the reaction between TCCA and CS on HP 5975A device. Sample run program [5]: Using 03 column types with different degree of polarization are: SPB1 column size (30m*0.32mm*0.25 μm); DB5-MS column size (30m*0.32mm*0.25 μm), column OV1701 size (30m*0.32mm*0.25 μm). Temperature at the beginning of column 60 $^\circ\text{C}$, hold for 1 minute, increase 8 $^\circ\text{C}/\text{min}$ to 280 $^\circ\text{C}$, keep at that temperature for 10 minutes. Air flow rate carries (He) is 1mL/min. Scan range of 45-800M/z. Universal library NIST 2005 with about 195.000 substances.

III. RESULT AND DISCUSSION

3.1 Level of reaction between TCCA and CS

The graph shows the relationship ($\ln(C_{\text{CS}})$ -t) of CS reaction with TCCA at 298 K shown in Figure 1.

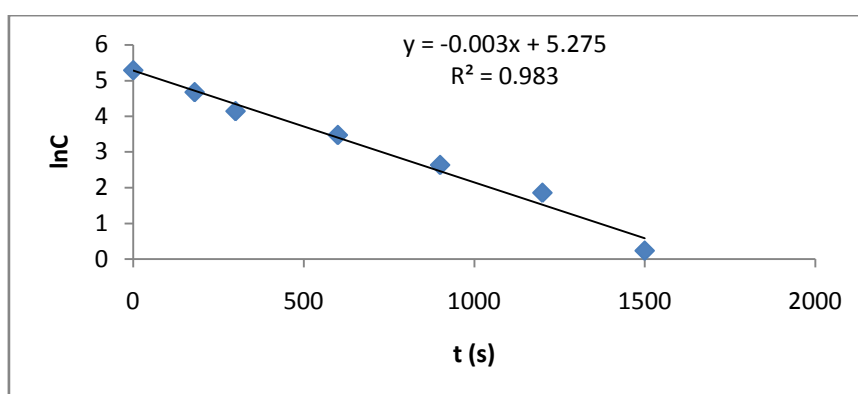


FIGURE 1. Graph of relationship representation ($\ln(C_{\text{CS}})$ -t) of CS response with TCCA

From Figure 1, the reaction between TCCA and CS has C_{CS} concentration decreased linearly over time (t), so the reaction between CS and TCCA is pseudo first order reaction with a correlation coefficient $R^2 = 0.9832$.

3.2 Kinetic study of TCCA reaction with CS

Rate constant of reaction between TCCA and CS at different temperatures in Table 1.

TABLE 1
THE REACTION RATE CONSTANT BETWEEN TCCA AND CS AND CORRELATION COEFFICIENTS AT DIFFERENT TEMPERATURES

Number order	Kind	k (s^{-1})	R^2
1	TCCA-CS-293	0.0014	0.9680
2	TCCA-CS-298	0.0031	0.9832
3	TCCA-CS-308	0.0043	0.8845
4	TCCA-CS-318	0.0069	0.9306

From Table 1, the reaction rate constant in TCCA-CS systems increases with increasing temperature, consistent with the Arrhenius equation. The correlation coefficients of the graph represent each middle relationship ($\ln(C_{\text{CS}})$ -t) at different temperatures ranging from 88.45% (TCCA-CS-308) to 98.32% (TCCA-CS-293).

Based on the value of the reaction rate constant in Table 1, plot the representation of each relationship between $(\ln(k_{CS})-(1/T))$ and $(\ln(k_{CS}/T)-(1/T))$. The results are shown in Figure 2.

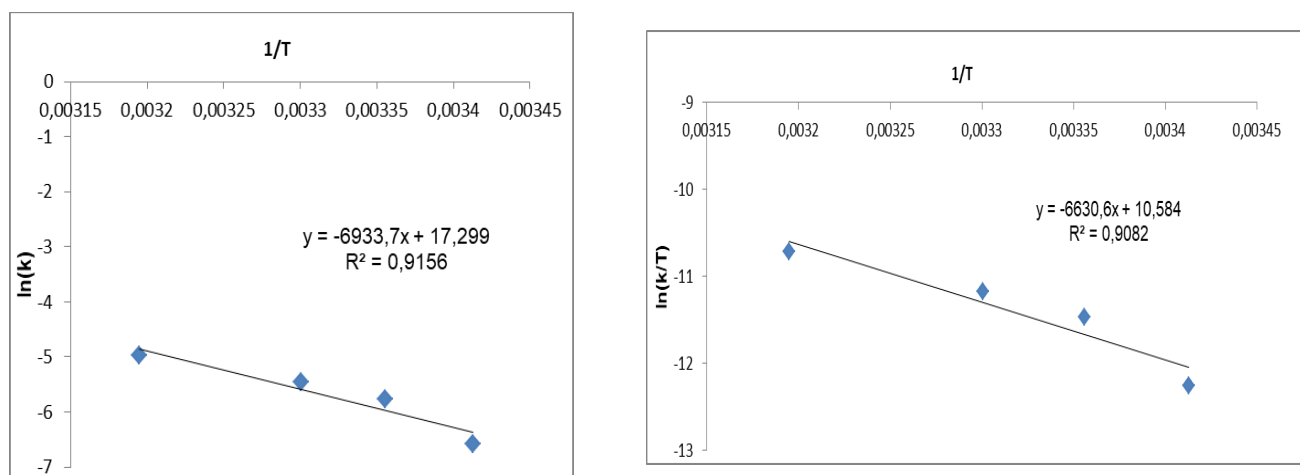


FIGURE 2. The graph shows each relationship between (a) $(\ln(k_{CS})-(1/T))$ and (b) $(\ln(k_{CS}/T)-(1/T))$ in the TCCA-CS

From Figure 2, determine the E_a value according to (9) and $\Delta H^\ddagger$, $\Delta S^\ddagger$, $\Delta G^\ddagger$ according to (12), (13), (14) as shown in Table 2.

TABLE 2
THERMODYNAMIC PARAMETERS OF TCCA REACTION WITH CS

Parameter	Value	R^2
E_a (kJ/mol)	57.65	0.9156
$\ln A$	17.299	0.9156
$\Delta H^\ddagger_{298}$ (kJ/mol)	55.13	0.9082
$\Delta S^\ddagger_{298}$ (kJ/mol.K)	-0.11	
$\Delta G^\ddagger_{298}$ (kJ/mol)	87.78	

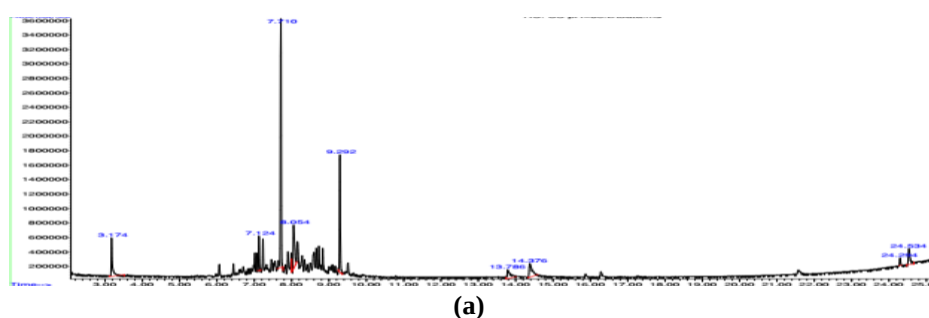
From Table 2, we see:

- E_a value of TCCA reaction with CS is consistent with experimental data in the range of 20 - 150 kJ/mol.
- Positive $\Delta H^\ddagger_{298}$ values indicate that the reaction to form the active complex is the heat recovery reaction. At the same time, the value of $\Delta H^\ddagger_{298}$ is also smaller than E_a in accordance with activated theory and activated complex theory.
- The value of $\Delta S^\ddagger_{298}$ negative indicates that the number of particles in the system reduced means the formation of active complexes, in accordance with the active complex theory.
- Value $\Delta G^\ddagger_{298}$ positive shows the process of needing energy to form an activated complex.

This is consistent with the enthalpy variation of the system.

3.3 Determine the product of the reaction between TCCA and CS

GC/MS chromatogram of post-reaction solution of TCCA-CS system in Figure 3.



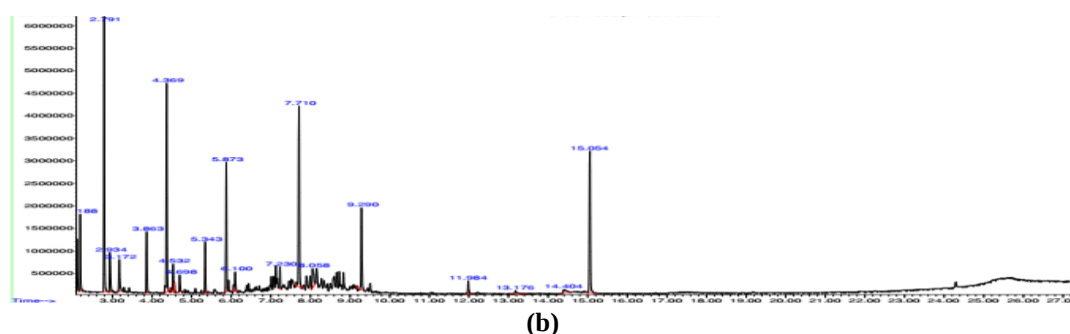


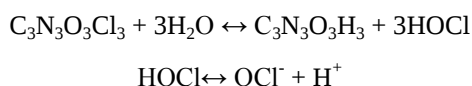
FIGURE 3. GC chromatography of (a) CS solution at pH = 9 and (b) reaction product of TCCA-CS

According to the chromatogram of the reaction product of TCCA-CS system (Figure 3. a) and the NIST spectrum of MS-library 2005, there was no pic with the time of retention in the reaction solution. It was $t_R = 14.367$ minutes for 2-Chlorobenzaldehyde (CS). This proves that there is no CS in the post-reaction solution.

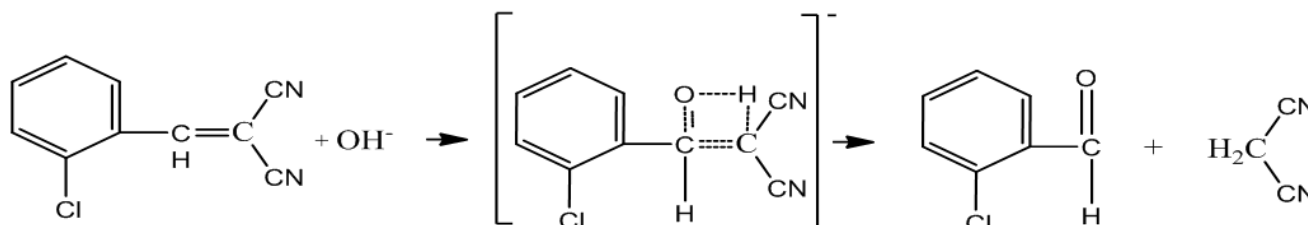
On the chromatogram of the reaction product solution (Figure 3. b), there appears pic with retention time of $t_R^1 = 8.154$ minutes corresponding to 2-chloro benzaldehyde and pic has a retention time of $t_R^2 = 12.2$ minutes corresponding to oxirane-2, 2-dicarbonitrile, 3- (2-chlorophenyl).

From the theory of CS poisoning and the results of product analysis of the toxic reaction CS by TCCA, can be predicted at pH = 9 TCCA with hydrolysis and the poisoning process consists of two reactions that occur in parallel and create products as follows:

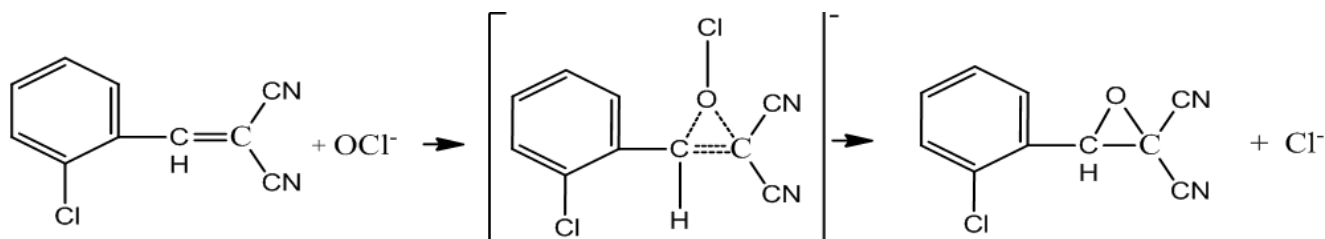
TCCA hydrolysis reaction:



Hydrolysis reaction:



Redox reaction:



IV. CONCLUSION

From the results of the study it can draw the following conclusions:

- The reaction between TCCA and CS is consistent with collision theory and activated complex theory.
- The reaction between TCCA and CS at 298 K is a pseudo- first-order reaction with $k_A = 0.0031 \text{ (s}^{-1}\text{)}$, $E_a = 57.65 \text{ (kJ/mol)}$; $\Delta H_{298}^\# = 55.13 \text{ (kJ/mol)}$; $\Delta S_{298}^\# = -0.11 \text{ (kJ/mol.K)}$; $\Delta G_{298}^\# = 87.78 \text{ (kJ/mol)}$.
- The reaction between TCCA and CS includes two parallel reactions that are hydrolysis reaction and oxidation reaction, producing two main products such as 2-chloro benzaldehyde and oxirane-2,2-dicarbonitrile, 3- (2 -chlorophenyl).

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Design Parameters and Conceptual Analysis on Gas Resource and Infrastructure Management: Trans-Nigeria Gas Pipeline Projects in Nigeria

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Abstract— Gas pipeline network and indeed gas infrastructures in the industry are liken to arteries and veins of the body. Blood circulation in the body systems travels through arteries and veins while gas resource travel through network of pipes for industrial and domestic purposes. Nigerian dependency on oil in the last 50years, has been characterised with reserves depletion and infrastructural decays. Therefore, the urgent intervention for Gas Domestication Project cannot be over emphasised. There are endless investment opportunities that abound in the natural gas sector of the Nigerian petroleum industry. At present, the annual gas production in Nigeria is about 2000 BSFC which is remarkable for an oil dominated economy (Figures 1, 2 and 3). Therefore, the initiatives for sustainable gas domestication projects such as the Calabar - Umuahia - Ajaokuta (CUA) and the Ajaokuta - Abuja - Kano (AAK) are laudable in terms of boosting economic resources for future investments. The rapid quests for industrial development of the country necessitate the conceptualization of the Trans Nigerian Gas Pipeline Projects. The project was divided into two segments the Calabar - Umuahia - Ajaokuta (CUA) trunk and the Ajaokuta - Abuja - Kaduna -Kano (AAK) trunk line respectively. The CUA is a 681km, 56" underground single-phase pipeline while the AAK is a 585km, 56" underground single-phase pipeline respectively. The spur lines were design to withstand maximum of 24"and minimum of 22"respectively. The entire project cycle from conceptualization, design and completion were undertaken within two years with about eighty-five (85%) per cent Nigerian Content. The Nigerian engineers and scientists in collaboration with the consultants, deliver the project on schedule (Table 1). The implementation strategy was predicated on the utilization of Critical Path Method (CPM) and Site Man-hour and Cost Control (SMAC) approach for project scheduling, monitoring and evaluation as well as cost control activities all through the duration,(Equations 1-6). Risk determination and safety checks were carried out at each stage for Environmental Impact Assessment (EIA), Front End Engineering Design (FEED), Detailed Engineering Design (DEED), Hazop and Hazid Safety processes, Helicopter Overfly and Right of way Mapping, Cost and Constructability Review and Invitation To Bid (ITB) documentation etc were scheduled and completed. Timely completion of the engineering design and documentation of the project was fundamental to the procurement and construction of the pipeline network. Procurement and construction work schedule are expected to increase industrial development of the country. Efficient gas utilization through gas to power projects, industrial parks along the gas pipeline routes is also affected. This study is expedient in the actual analysis and review of the initial engineering design and safety codes with a view to ensuring compliance with industry standards. The study is also significant as reference material for the actualization of the vision of designing and constructing a world class Trans-Nigerian gas pipeline projects. It will also serve asa reference material for administrators and decision makers on effective deployment and management of resources on gas pipeline projects in Nigeria.

Keywords— Conceptual Analysis, Critical Path Method, Site Man Hour and Cost Control, Infrastructure Management, Right-of-Way, Design Parameters, Environmental Impact Analysis.

I. INTRODUCTION

There is a global shift from solid and liquid fossil fuels (coal and crude oil) to cleaner and more sustainable gas and renewable energies. This is due to the global concerns about the effects of energy production and consumption on the environment (Lyons and Plisga, 2005). The Trans - Nigeria Gas pipeline projects were conceptualized to accelerate domestic gas utilization and maximization of the resources for the development of the industrial sector and enhanced economic growth. These projects were broken down into two segments "the Calabar - Umuahia - Ajaokuta" (CUA) and the "Ajaokuta - Abuja - Kaduna - Kano" (AAK) pipeline projects respectively. Fundamental to the industrial revolution of the country is gas resources which the country's proven reserve is estimated at about 198TSCF. Gas pipeline projects across the globe have been a source of massive income for the country of origin as well as those desiring to tie-in from the producer pipeline routes. The conceptual framework on the Nigeria gas projects and its expected benefits to the transformation of several

industrial parks and business pevolution across the country makes it one of the many instruments for sustainable development. Thus, the standard applied methods of project evaluation from conception to commissioning finds its usefulness in this paper using the Critical Path Method (CPM) and Site Man Hour and Cost Control (SMAC). Critical path method (CPM) determines the best period to schedule each event in a project while site man hour and cost control (SMAC) applied to project management aimed at ensuring effective utilization of time and resources for efficient production at minimal cost (Abam 1997). This approach has been adopted in several test cases to address the question of how soon the project will be completed, when is each phase of the schedule meant to start and or finish, what are the critical phases of the project that should be finished on time, what are the cost implication and, how efficient is the performance at the end of the stated period. The Trans-Nigeria Gas Pipeline Projects were designed to have a minimum of twelve gas compressor stations from the beginning in Calabar to the end in Kano.

The gas compressors needed for the projects comes in different designs and always complex to assemble for effective maintenance. The industry is also dynamic and demands competition and efficiency using sophisticated technology. However, the guiding principle is to optimize the system always. This integrated approach makes the Trans-Nigerian Gas Pipeline System design complex as one unit affects several others. The modification of the several gas compressors stations along the gas pipeline routes and or redesign of compressors to function using the pulley-chain transmission system is not a mean task. The risk level, operational and managerial expertise needed for the smooth and standard design are equally high (Chi. Ikoku, 2004). The possible workability of the project without damage to other equipment requires that key activities (operations) be identified and done, and sequence of all such activities or event phases, should be arranged in a network at a specified time interval. Besides, the question that arises on the possibility of the compressor to perform efficiently and effectively for a long time was determined. The actual cost advantage and technological gains of on-time design pump stations could be address through CPM and SMAC analysis technique. Nevertheless, the decision needs to be taken, maintenance must be carried out on the compressor and production operation must continue in the oil industries at an optimal rate and in a safe environment.

This is the focal point on which SMAC found its relevance in the project optimization of compressed gas in the oil industries. The evaluation of assigned time at the end of stated period, the determination of the efficiency of the project, the overall performance and the cost benefits are pointers to the effectiveness of this approach in the project management and optimization processes applicable on the Trans-Nigeria Gas Pipeline Projects.

II. REVIEW OF GAS INFRASTRUCTURE IN NIGERIA.

Nigeria is described more as gas endowed nation with over 198 trillion cubic feet of proven gas reserves. The potential therefore to harness our gas resources to increase export earnings and boost our domestic industrial base, through power generation and downstream petrochemicals, remain enormous. To this end the Nigerian government has initiated numerous gas infrastructure projects to ensure domestic gas supplies for industrial development. These projects, at various stages of initiation and completion, include the following: (NGMP,2012): -

- Escravos-Lagos-Pipeline-System (ELPS) Phase II
 - 36-inchx342km, 1.1 Billion standard cubic feet/day capacity
 - Targeted for completion in 2019
- Obaifu/Obrikom – Oben (OB³) Pipeline
 - 48 inches /36-inch x 127km, 2 Billion standard cubic feet/day capacity
 - Targeted for completion in 2019
- Odidi-Warri Gas Pipeline Expansion (OWEP)
 - 40-inch diameter x30Km
 - To transport 440 million standard cubic feet/day into the ELPS
 - Aimed for completion by 2019
- Ajaokuta-Abuja-Kano (AKK) Gas Pipeline
 - Part of Trans-Nigeria Gas Pipeline
 - First EPC Contractor Financing model
 - 40-inch diameter x 614km, 1.8 Bscfd capacity
 - Targeted for completion in 2020
- Qua Iboe Terminal– Obiafu/Obrikom Gas Pipeline
 - 36-inch diameter x261km pipeline

- To transport 400MMscfd from Qua Iboe Terminal
- Targeted for completion in 2020

These projects are targeted for completion, all things been equal in 2023, to increase Nigeria gas export capacity and revenue generation and domestic gas utilization in the gas value chain for industrialization.

TABLE 1
ACTIVITY BASED DESIGN PARAMETERS IN PROGRESS

	BIDDERS INFORMATION Issued by MPR on 12-Sep-09		CONCEPTUAL DESIGN STUDY Calculated by Consultant 02-Dec-09		CONCEPTUAL DESIGN STUDY MPR's Specification Issued on 05-Feb-10	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Major and Minor Components, mole%						
Methane	80.45	81.64	75	-	80.45	87.68
Ethane	5.91	6.08	-	10	6.08	6.46
Propane	3.67	3.77	-	5	0.61	3.67
Butanes	2.42	3.04	-	2	0.13	3.04
Pentanes and heavier	1.6	1.83	-	0.5	0.07	1.83
Nitrogen and other inerts	0.69	0.73	-	4	0.73	0.73
Carbon Dioxide	3.97	4.2	-	4	4.2	4.32
Other Characteristics						
Pressure psig	1,000		1,000	1,250	1,000	1,250
Gas heating value, KJ / Nm^3	37.750	42.840	35.400	42.800	34.530	39.600
Feed Gas Rate mmscfd	2,000		3,000		3,500	

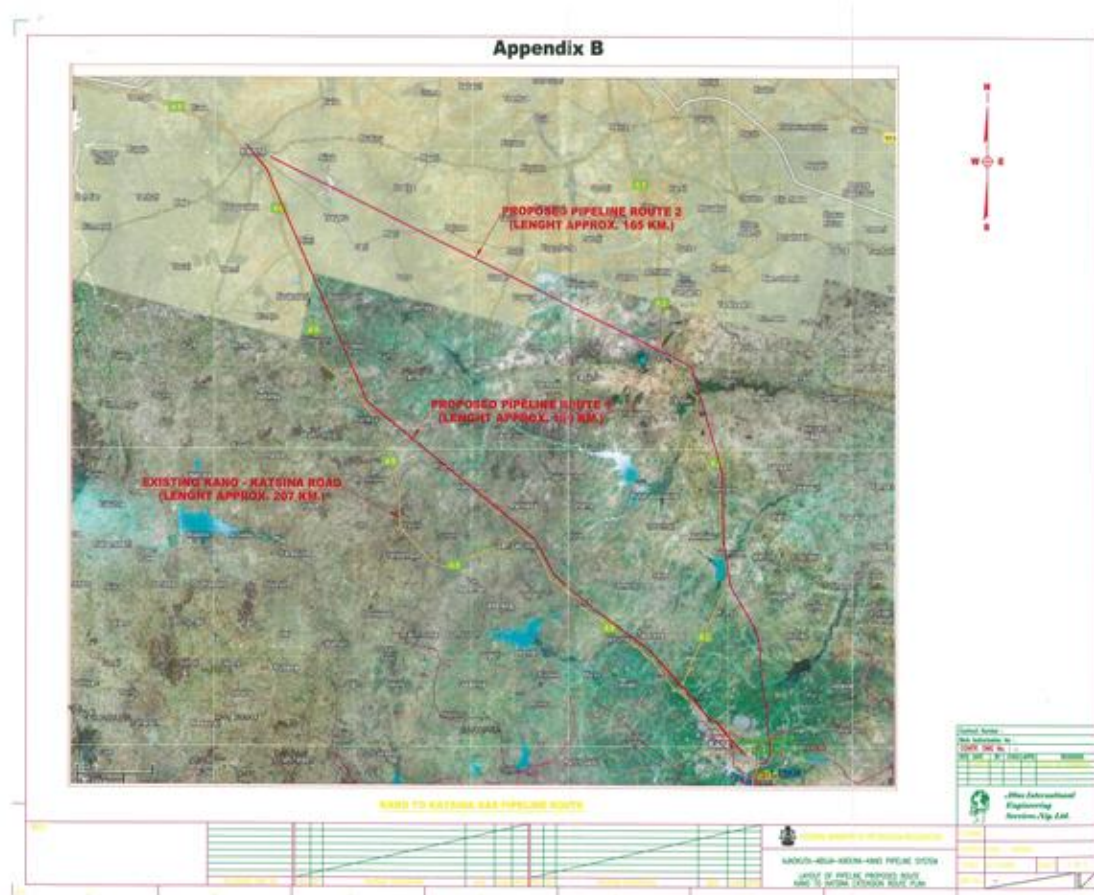


FIGURE 1: Aerial view of Right-of-Way and Mapping from GPS

III. BACKGROUND TO THE PROJECT CONCEPTUALIZATION.

The need to increase productivity through industrialization using gas resources became imperative following the privatization of NAFCON, ASCON, AJAOKUTA STEEL MILL and some of the Petrochemical plants between 1998 – 2000. Most of these plants uses natural gas as feed stocks. Besides, there was also an enormous need to increase power generation for industrial and domestic use. These and many other factors trigger the need for pipeline network that would transport natural gas from areas of abundance to areas of scarcity in the country, essentially for industrial spread of development industries. However, the field activities that must be performed at the end of the stated periods for an expected 85% completion and 90% efficiency and maintenance service of gas compressor at ALAKIRI GAS PLANT-ONNE needed to be scheduled. Such activities must be arranged to suite the CPM method and as shown in table 1. The sequence of all activities involved can be confusing and time consuming. However, since CPM is activity oriented and deterministic model, it suits the integrated approach needed to meet the target for the conceptual design to the gas pipelines. The conceptual framework was initiated.

There exist compressor designs with gearbox transmission system as a starting point. Hence, the need for a simple easy to maintain design that will use the pulley-chain power transmission system. Management decision specified a time frame within which all design, fabrication, installation test run and full operation will be achieved. High safety standard needs to be maintained and so, the safety inspectors needed to provide and ensure that personnel protective equipment (PPE) are put on for any given activity. The design engineers and maintenance crews need to have the right and adequate tools and materials for proper man-hour utilization and experience ones assigned to carry out the activities to obtain high efficiency. The management team needed to ensure regular and timely releases of funds for operation services while the technical services department needed to ensure that activities are performed in accordance with design specification and standards. The conceptual design study specification based on the job scope is as shown in table 2.

TABLE 2
COMPOSITIONAL AND PRESSURE DESIGN PARAMETERS

	BIDDERS INFORMATION:		CONCEPTUAL DESIGN STUDY:		CONCEPTUAL DESIGN STUDY:	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Major and Minor Components, mole%						
Methane	80.45	81.64	75	-	80.45	87.68
Ethane	5.91	6.08	-	10	6.08	6.46
Propane	3.67	3.77	-	5	0.61	3.67
Butanes	2.42	3.04	-	2	0.13	3.04
Pentanes and heavier	1.6	1.83	-	0.5	0.07	.83
Nitrogen and other inerts	0.69	0.73	-	4	0.73	0.73
Carbon Dioxide	3.97	4.2	-	4	4.2	4.32
Other Characteristics						
Pressure psig	1,000	1,000	1,250	1,000	1,250	
Gas heating value, KJ / Nm^3	37.750	42.840	35.400	42.800	34.530	39.600
Feed Gas Rate $mmscfd$	2,000	3,000	3,500			

IV. DESIGN CONSIDERATIONS

The need to reduced flare gases in the country harnessed and channeled it through the Trans- Nigerian Gas Pipeline Projects into the West African Energy Hub in the regional gas network for both domestic and industrial development form the main design basis for the project. The steps taken to actualize the concept is as discussed below:

4.1 Codes and Standards

The applicable Codes, Standards and Regulations for the Calabar – Umuahia – Ajaokuta, Ajaokuta – Kaduna - Kano – Katsina trunk lines were design in accordance with the Conceptual Design Specification as contained in Study Report (Ministry of Petroleum Resources, 2010. – AAK-009-SP-10-0002).

Pipelines and terminal facilities envisaged were also designed and engineered primarily in accordance with the provisions of American Standards for Measurement and Evaluation (ASME) B 31.8- 2007: Gas Transmission and Distribution Piping Systems. In addition, the following codes/standards were complied with to ensure safety and sustainable pipeline longevity in the national gas transmission scheme (Neeka, Busari and Jones 2010; Ministry of Petroleum Resources).

ASME B 31.3 Chemical Plant & Petroleum Refinery Piping

API Std. 1102 Steel Pipeline Crossing Railways & Highways

API Std. 1104 Standard for Welding Pipelines and Related Facilities

It is envisaged that the Kano tie-in point shall be an underground, welded joint most likely identical to the preceding upstream joint. The Kano Terminal Gas Station (TGS) spur line and tie-in location to be determined after the Right Of Way (ROW) survey completion. However, allowances were made for possible design variance. The fourth booster station is likely to be similar to the preceding upstream booster station; again its exact location was to be determined after the completion of the process simulation. Pipeline engineers were expected to determine the number of additional Line Break Valve Stations (LBVS). The construction of the additional LBVS are similar to the other upstream LBV stations in the country. Such categories like above ground Motor operated Valves, unmanned real time all year round with security personnel strategically stationed for surveillance. The main booster station with the Central Control Room is unchanged; the Abuja Booster Station. The proposed heavy-duty construction road alongside the proposed ROW is to be extended to link lines in Port Harcourt and Katsina respectively. It is also envisaged that two additional construction campsites with specified land dimensions will be acquired for the entire project line.

V. BASIS FOR THE ENGINEERING DESIGN SERVICES

After the initial conceptual project team meeting, personnel composition and preliminary activities, proper and adequate geographical survey to map the exact location of the old Right of Way and the proposed new Right of Way (ROW) were fundamental to the actual design process. This aspect of the overall exercise was carried out on all front and detailed reports submitted to the initiator of the project (Emmanuel, Innocent and Chukudi;2010,). Following the approval of the new ROW map, a new Environmental Impact Studies were carried out and completed, which latter form together with the ROW map the basis of the scope of work defined by the project supervisory team.

5.1 Scope of Work

Complete design for the EPC tender bid, including:

- Environmental Impact Assessment (EIA) Report
- Front End Engineering Design (FEED)
- Detailed Engineering Design (DEED)
- Construction scoping
- Hazard Operability (HAZOP) and IPF reviews
- Constructability review
- Class 3 cost estimate
- Invitation to Bid (ITB) documentation

For the construction of dual gas pipeline between Kano and Katsina.

5.2 Design Data and Parameters

Pipeline Systems for CUA and AAK have about eighteen (18) major river crossings and 20 major road crossings. Calabar → Umuahia → Ajaokuta (681km) and Ajaokuta → Lokoja → Abuja → Kano. (585km) respectively.

The design parameters are similar to all existing pipeline project data:

Feed gas condition at terminal points	pipeline quality dry gas
Maximum feed gas flow	3,000 mmscftd
Kano TGS sale gas flow	500 mmscftd
Required minimum sale gas pressure at extension	1,000 psig
Distribution pressure at all three (3) TGSs	350 psig
Maximum compressor discharge pressure at Booster stations	1,250 psig
Pipeline construction buried, twin, insulated	
Estimated length of PH – OB – OB and Kano-Katsina pipelines 168km and 160 km respectively.	
Main trunk line size	48 inch
Spur lines size at TGS	22 inch
Total No. of compressor booster stations	Nine (9)
Total No. of terminal gas stations	Seven (7)
Design life	25 years

The new pipeline is supplied with pipeline quality gas at a minimum pressure of 1,000 psig at Calabar and Ajaokuta tie-in and delivers to Kano also at a minimum pressure of 1,000 psig. In order to overcome transmission losses and enable selection of an economic pipeline diameter, a number of Compressor Booster Stations will be required at intermediate locations along the pipeline route. Spur lines will link the main pipeline with Terminal Gas Stations at Port Harcourt, Umuahia, Lokoja, Abuja and Kaduna where the gas will be discharged at a suitable pressure for consumer distribution and fiscally metered (Neeka et al 2010). The pipeline is sized for an ultimate design capacity of 3,000 mmscftd. The Odukpai, Port Harcourt, Abuja and Kaduna spurs shall be sized for 500 mmscftd each leaving up to 2,000 mmscftd available at Ajaokuta and Kano for local distribution and export to the future trans-Saharan pipeline system.

5.3 Identified constraints

It has become apparent that the Port Harcourt – Obeniyi – Obirikom (PH – OB – OB) and Kano – Katsina Extensions will necessitate alteration of the original design specifications to accommodate several Tee-offs into the expected gas plants, gas farms and fertilizer plants. Enough gas supply from the various producing companies including international oil companies must be sustained for the actualization of the projects. Most notable among these constraints are the right of way, understanding of the pipeline design concept, job scoping taking cognizance of the varied specification need at various points, projections and proper forecasting of the future utilization of gas resources from unforeseen industrial concern, environmental impact assessment studies etc. These constraints are fundamental to the actual completion work on Calabar – Umuahia – Ajaokuta and Ajaokuta – Abuja – Kaduna – Kano gas pipeline system documents to be revised and re-issued by the supervisory Ministry. The following type of documents would have to be revised for the seamless procurement and construction of the gas pipeline system to commence as planned (Busari et al 2011).

- Process engineering design documents
- Piping and pipeline engineering documents
- Control and Instrument engineering documents
- Civil engineering documents
- ROW mapping

- Cost estimation

In addition, the following submissions were expanded and resubmitted with all the relevant conceptual design provisions in accordance with the standard operational manual developed for the project (Neeka et al 2009).

- Terms of Reference document
- Environmental Impact Assessment
- Permit to survey applications

VI. MATHEMATICAL MODELS ON PROJECT NETWORK ANALYSIS

There are several pipeline designs sequences and different approaches that can be adopted to ensure effective project management and production optimization. However, to identify all events using the Critical Path Methods involving early start, early finish, late start and late finish is important on a time scale work schedule. These events were scheduled and evaluated in accordance with equations (1) and (2), and the estimated results are shown in Table 1. The fundamental assumption is that the activity items are estimated as single point estimate independent of each other. Besides, time - cost trade-off for the project and duration of one activity is assumed to be linear relative to the cost of resources applied as shown in the equations below:

$$TF = LTF - LTS - D \quad (1)$$

$$FF = ETF - ETS - D \quad (2)$$

Where; TF is the Total Float and FF is the Free Float, LTF, ETF and ETS are Late Time Finish and Early Time Finish, LTS are Early Time Start and Late Time Start respectively and D is the duration of activity.

Similarly, available literature shows that the percentage completion (% completion), predicted final hours (PTF.hrs), and performance efficiency (PERF) through the value hour method can be determined as shown in equations 3-5. Further references on the application of these principles in the determination of work break down scheduling and activity dependent sequencing in project implementation could be explored in the works of Robert S. Avies (1955), Max S. Peters (1980), Abam (1998) respectively. Also, application of estimated design values needed to apply to the entire trunk lines and the suitability in these equations were determined according to the following:

$$\text{Completion} = \frac{VA.hrs}{BT.hrs} \times 100 \quad (3)$$

$$PTF \text{ hrs} = \frac{ACT.hrs}{\%completion} \times 100 \quad (4)$$

$$PERF = \frac{VA.hrs}{ACT.hrs} \times 100 \quad (5)$$

Where; VA.hrs is the Value hours, BT.hrs is the Budget hours and ACT.hrs is the Actual hours respectively.

Activity sequencing requires the determination and documentation of the relationship between other activities with respect to completion time. The CPM and SMAC methods are typically used in the precedence diagram to structure such relationships. Sequencing usually begins with the chronological ordering of activities, based on the logical progression of events. Activity definition requires the combination of the scope document, and the utilization of the work break down structure to develop discrete activities that are unique and be associated with deliverability

$$A_D \times Q_I = O_D \quad (6)$$

Where A_D represents the Activity of Duration, Q_I represents the Quantity of an item and O_D represents the Overall Duration.

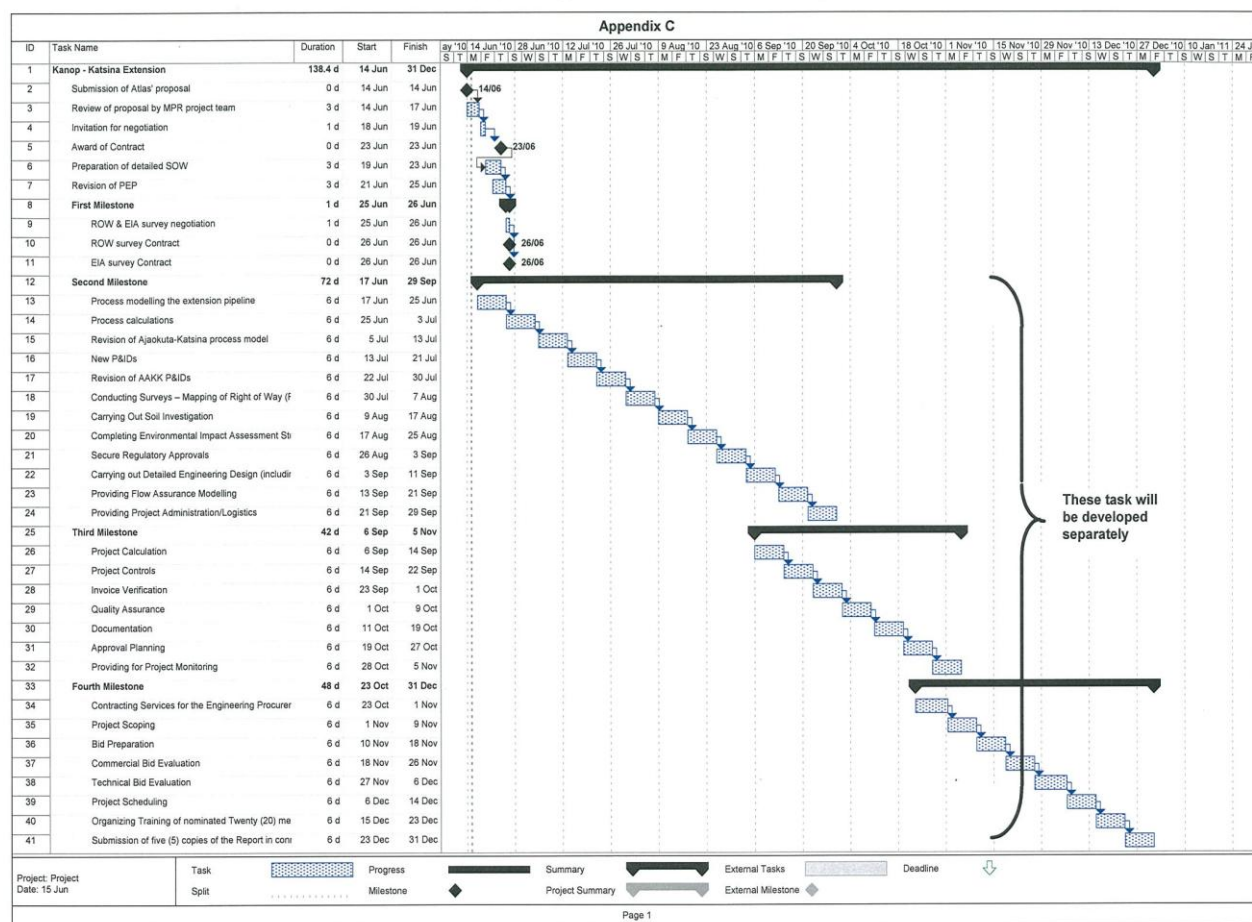


FIGURE 2: Example of the Work Breakdown Structure Analysis

TABLE 3
PIPELINE PARAMETERS

Pipeline Design Parameters	
Description	Value
Pipeline size	60 inches
Pipeline size	24 inches
Design Pressure (Psig)	1235
Operating Pressure (Psig)	1000
Max. Design Temperature, °C	
i. Above ground section	65.0
ii. Underground section	45.0
Operating temperature, °C	25-45
Economic Design Life, years	100
Corrosion Allowance, mm	3

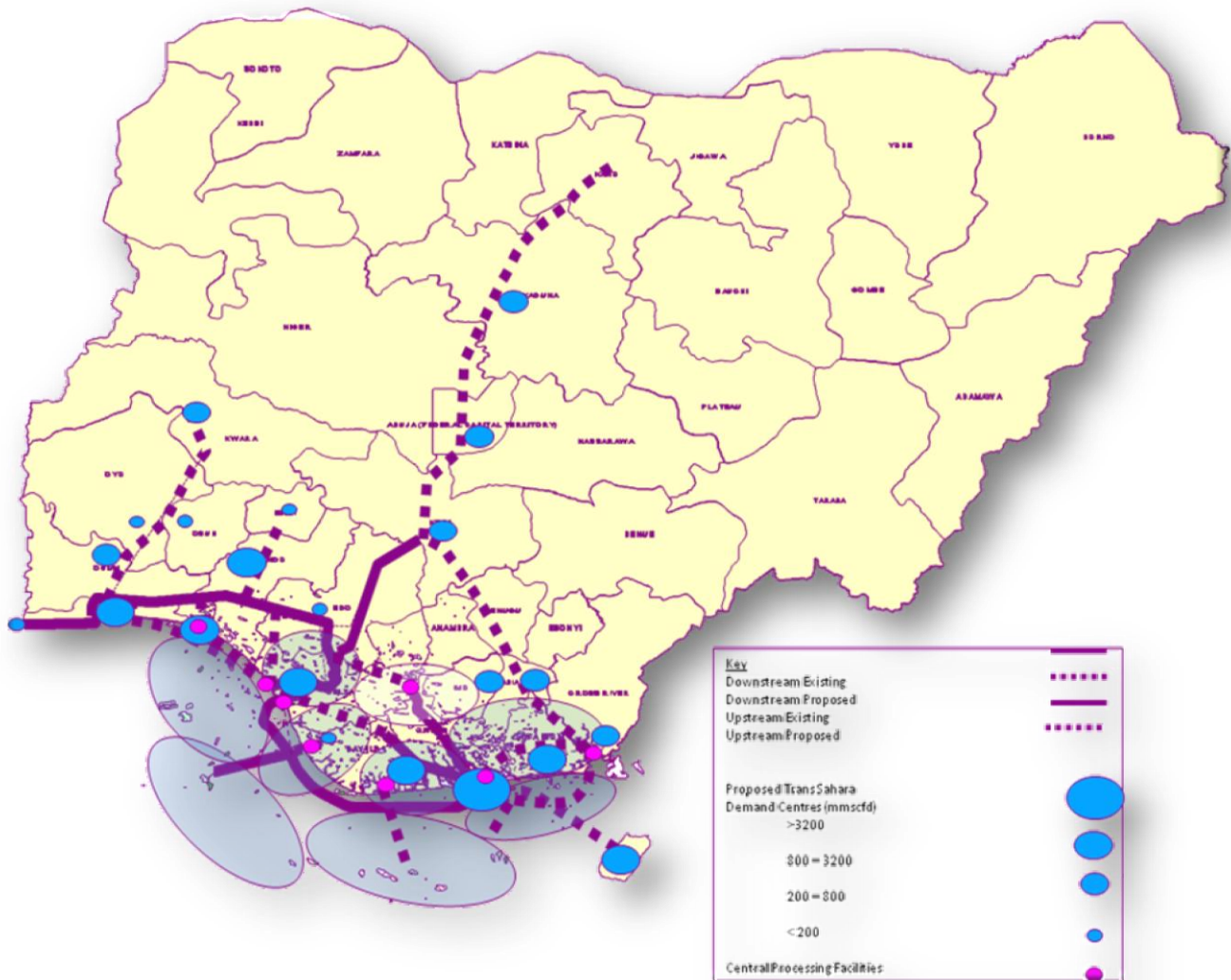


FIGURE 2: Map of Nigeria showing the gas source in Okopedi, Calabar

VII. CONCEPTUAL MANAGEMENT STRUCTURE.

Various management structures on the Trans- Nigerian Gas Pipeline Projects were design to actualize the initial conceptual plans. Some of the structures became complex and constitute sources of delay in the implementation strategy. Such structures include: the project planning and implementation team; monitoring and evaluation committee; procurement planning and implementations team; technical review and reporting team; inter-ministerial supervisory committee; stakeholders forum committee etc. These structures were subject to periodic review and were mandated to inter-phase with the appointed consultants. The office of the technical assistant to the Permanent Secretary took full responsibility for the proper and adequate reporting on the project to top ministry management and to the minister through the permanent secretary (Neeka, 2010). However, the details of the following activity-based teams are fundamental in the conceptual management structure.

7.1 Project Planning and Schedule

The project schedule is an integral part of a detail cost estimate. The duration of a project affects the cost through the period dependent cost, and the selected technology for the activity-dependent work and associated costs, affects the schedule. The activity dependent schedule is drawn from the cost estimate database to establish durations for each of the activities in the schedule. Hence, the activity duration multiplied by the quantity of an item in the planned invention list provides an estimate of the overall duration to perform the activity. The number of man hours multiplied by the quantity of an item in the same

planned inventory list equally provides an estimate of the overall manpower resources to perform that activity. The cornerstone of project planning and schedule preparation and development is a formal documented scope. The content of a formal written scope statement should spell out the expected activities and duration in the plan. Activity sequencing requires the determination and documentation of the relationship between activities. The CPM and SMAC methods are typically used in the precedence diagram to structure the relationships between activities. Sequencing usually begins with a chronological ordering of activities, based on the logical progression of events. Activity definition requires the combination of the scope document, and the utilization of the work break down structure to develop discrete activities that are unique and be associated with deliverability

7.2 Evaluation and Optimization of Critical Paths.

This is a critical decision, and if the proper level of detail is not selected it could cause the entire project team to ignore the schedule. If the schedule is prepared at too fine a level, the project runs the risk of being overwhelmed with data that inevitably the project control staff is unable to maintain. On the other hand, a schedule with too little detail is insufficient to use in tracking progress, anticipating problems or developing risk strategies. There is need to schedule activities at the level that can control the work. This may be somewhat judgemental and is dependent on the skill of the project team, its experience, the complexities of the activities and the risk involved in each activity.

The critical path is the longest sequence of activities in the work process flow chart. The critical path controls the overall length of the project. Any incremental change to the critical path activity will result in a corresponding change in the overall schedule. Hence, the critical path method was adopted to evaluate and determine what technological changes needed to be inputted into the overall project scoping. Parallel path changes or duration estimate changes can be made to shorten the critical path where necessary. The overall schedule duration is one of the major cost driven forces in a project management technique. Once adopted, it is obvious that the schedule could serve as baseline schedule to the project. It is against this schedule that project performance will be measured. It is also possible to develop these sequences into a software package, which this paper will not address now. The last phase in the development of the cost estimate and schedule is to assign the cost elements to the work breakdown structure. Typically, the work breakdown structure is used to collect and monitor costs of the program. At the owner license discretion, the baseline may be adjusted periodically to account for changes in scope of work funding constraints, or schedule changes as a function of acceleration or delays in the project.

VIII. WORK BREAKDOWN STRUCTURE AND DISCUSSION ANALYSIS

The conceptual framework on the Trans -Nigerian Gas Pipeline Projects started with the fundamental process of advertisement. For ease of planning, some activity items were executed concurrently to ensure on-time delivery. The shortlisted applications for the needed consultants were pre-qualified for bid tendering. At the end of contract award process for the consultants, formal kick-off meeting and documentation stating the project scoping is completed. Milestone project execution starting with preliminary design, conceptual design and impact assessment is carried out (see Tables 1 and 3). Several review processes are carried out simultaneously to ensure that all concepts and terms are complied with accordingly. It is also important to note that various activities slated for execution at the milestone level remained valid as planned if and only when the parties involved agreed to follow and comply with the project timetable. Thus in the execution of the Trans Nigerian Gas Pipeline project, it was the responsibility of the supervisory Ministry to ensure compliance to the project timeline with the collaboration of the project consultants' and the internally constituted project monitoring team made up of experts in the various aspects of engineering, environmental and safety management respectively. Community concerns and compliance with surveyed right of way were critical to the timely completion of the project (see Fig.1) Approval planning, invoice verification, quality assurance and control, training and manpower development as well as final report submission and close out are largely fundamental to the actual completion of the phases involved in the execution processes.

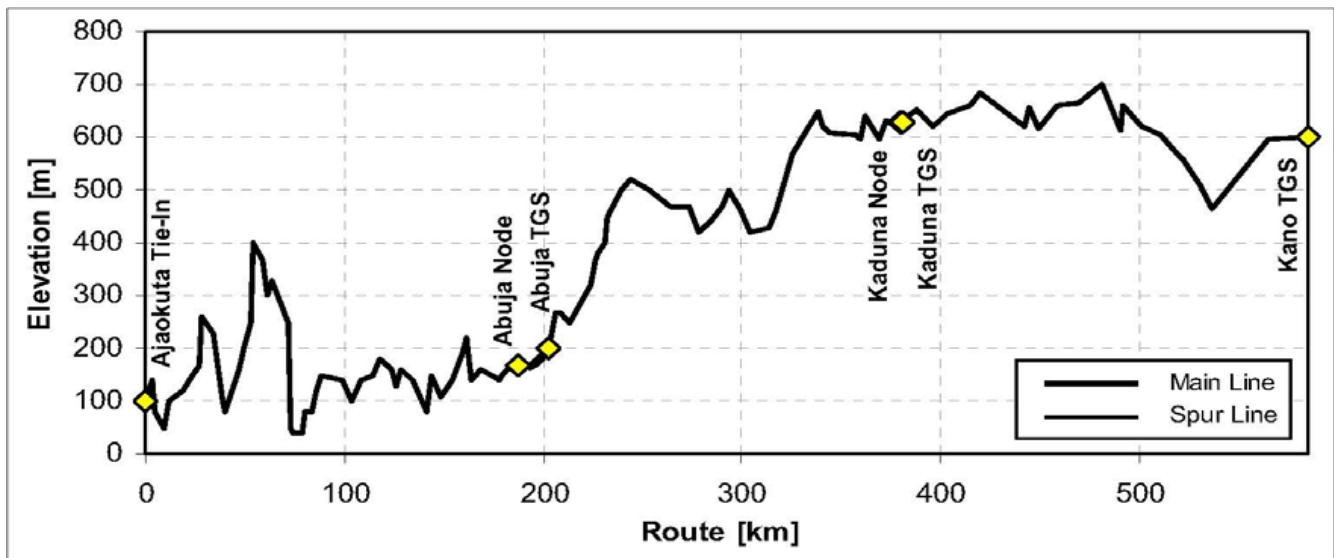


FIGURE 3: Pipe design simulated elevation

TABLE 3

ACTIVITY DEPENDENT SCHEDULE OF A PROJECT PLAN USING SMAC METHOD.

ACTIVITY OR MILESTONE	DATE	REMARKS
Project advertisement appeared	06-Apr-2009	Completed
CONSULTANT Pre-qualification	26-Apr-2009	Completed
Tender Bids issued	27-Apr-2009	Completed
Preliminary route survey completed	25-Jun-2009	Completed
Bid closing date	10-July-2009	Completed
Pre award clarification meeting	12-Sept-2009	Completed
Contract award	02-Nov-2009	Completed
Project Execution Plan submission	12-Nov-2009	Completed
Kick-off Meeting	12-Nov-2009	Completed
Submission of Terms of Reference for EIA Report	18-Nov-2009	Completed
Draft Front-End Engineering Design Report completed	03-Dec-2009	Completed
Conceptual Design Report	03-Dec-2009	Completed
Outline of EIA studies	03-Dec-2009	Completed
Milestone 1	05-Dec-2009	Completed
Detailed Engineering Design Project Execution Plan Submission	07-Dec-2009	Completed
Mapping of Right of Way Survey	12-Dec-2009	Completed
Soil Investigation	12-Dec-2009	Completed
Environmental Impact Assessment Studies	12-Dec-2009	Completed
Initiate Securing Regulatory Approvals	12-Dec-2009	Completed
Commencement of Detailed Engineering Design	12-Dec-2009	Completed
Commencement of Flow Assurance Modelling	12-Dec-2009	Completed
Milestone 2	14-Dec-2009	Completed
Revised Gas composition received	05-Feb-2010	Acknowledged
Scope of Work Revision received	06-Feb-2010	Acknowledged
HAZID Workshop	12-Feb-2010	Completed
Area I flyover of ROW	13-Feb-2010	Completed
Monitoring team from MPR working in Consultant's office	17-Feb-2010	Completed
Project monitoring by Royal Cat Int'l Ltd	02-March-2010	Completed
Project monitoring by Otis Engineering	05-March-2010	Completed
HAZAN Report	14-March-2010	Completed
Approval of Front-End Engineering Design	14-March-2010	In progress
Approval of Flow Assurance Modelling	14-March-2010	In progress

IX. CONCLUSIONS AND RECOMMENDATIONS.

Gas resources and rapid development of its infrastructures such as the Trans-Nigerian Pipeline Network are fundamental to the increasing transformational needs of the country. Industrial parks and effective domestication of gas utilization can only thrive where there are efficient and sustainable gas pipelines of high integrity. The concept of designing, procuring, constructing and commissioning a Trans-Nigerian Gas pipelines from Calabar through Ajaokuta to Kano is critical to economic and human capacity development. Several businesses and industrial concerns were anticipated with strong policy to increase job creations and reduce the number of unemployed in the labour market through new established industries and serving agencies along the pipeline network routes. For ease of administration of the complex projects and as a globally acceptable standard, critical path methods and site man-hour cost control evaluation strategy were applied. This strategic instrument applied in the process conceptual design from start to finish was fundamental to the successful determination of the key performance indicators in the project. The critical path method and site man-hour cost control applied to project management had rigorous project scheduling and milestone evaluation standards. It is the road map that outlines how the project will move from engineering concepts, to completion and site restoration. As a tool it also supported different purposes for different members of the project team. For the Engineer, it provided information on what needs to be done, when it needs to be done, and what other activities may affect the start and completion of his work. It provided a measure of performance evaluation. With the successful completion of the detailed engineering design, documentation and training of the stakeholders on the project, it is recommended that the actual processes of procurement for construction should commence without delay, knowing the overall national benefits that would ensue. Inter-agency politics should be avoided for the interest of the nation and its citizens and the need to Fast Track the development of the nation's gas resources and its critical infrastructure such as the gas pipelines. It is strongly suggested that the Ministry of Petroleum Resources should be funded to complete the procurement processes using the qualified strong workforce available in the Ministry along with selected consultants. Other stakeholders such as the Nigerian National Petroleum Corporation and the Department of Petroleum Resources should also contribute expertise and technical supports for the actualization and realization of the projects. The rapid growth of Nigerian oil and gas sector through gas utilization and sustainable assets such as pipeline network should attract tax holidays from the government in power to companies involved in construction and commissioning of the projects. Nigeria gas pipeline system document and treated as one complex publication. After the merger, terms and references to all the extensions on the gas pipeline system will be replaced with the new contractual terms. The Gas Pipeline System term in all design documentations and the Procurement and Construction Bid Packages, except for the commercial Milestone Schedule will be further reviewed. At this point, all document containing the conceptual designs and documentation had been completed for use by the supervisory Ministry. It is expected that the original contractors will continue to work with the Ministry of Petroleum Resources to actualize the contractual Milestone Schedule and deliver on the construction of the gas pipelines for the benefit of Nigerians and the stimulation of industrial growth respectively. Further submission for extension of time and additional fund to cover scope changes could also be negotiated where possible (Neeka et al 2010). With the Port Harcourt – Ob – Ob and Kano – Katsina Extension submissions, a new Milestone Schedule is proposed, and it shall be treated separately and parallel with the new Milestone Schedule. In addition, as a separate document with the details of Cost-Time-Resources estimate workout sheets developed, further review and assessment by the Ministerial Project Control Team is important to ensure quality procurement processes. Overall, critical review of the past design data and information is imperative for the actual commencement of the next phase of engineering procurement. Selected management consultants and experts should be engaged for the effective management of the projects. Gas policies should be strengthened to place more emphasis on domestic utilization rather production for exports that is currently the practice. More sectorial collaboration including gas to power, renewable energy gas utilization plan involving the power sector, environment, agriculture and petroleum etc should be worked out strategically.

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Facile fabrication of PDMS microspheres with and without fluorescent dye doping

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Abstract—Poly(dimethyl siloxane) (PDMS) microspheres are increasingly gaining importance for a wide range of applications due to their flexibility and inertness. In this paper, we have fabricated PDMS microspheres using facile emulsion formation in water by stirring with and without additional ultrasonic excitation. It was found that the particle size distribution, which can be attributed to the formation rate of cross-linked PDMS networks in the hydrophobic microspheres, depends on the temperature of the aqueous medium. Swelling of the microspheres in acetone was suggested by permeabilization as evidenced by diffusion and encapsulation of fluorescent dyes within the PDMS. Using scanning electron microscopy, the surface morphology of the spheres was confirmed to have no surface roughness or irregularity. Using fluorescence microscopy, we found that the encapsulated dyes randomly and thus uniformly distributed themselves within the cross-linked PDMS networks and retained the fluorescent properties and characteristic emission color, implying their potential for drug carrier.

Keywords—poly(dimethyl siloxane) (PDMS), microspheres, swelling, encapsulation, fluorescent dyes.

I. INTRODUCTION

Among the many important polymeric microparticles, those comprised of poly(dimethylsiloxane) (PDMS) have attracted much attention due to their biocompatibility, thermal stability, optical transparency in the UV-visible region, flexibility, non-toxicity, low polarity, low electrical conductivity, and chemical inertness [1-6]. In addition to industrial and medical applications, PDMS microspheres are also utilized in a number of new scientific disciplines, including sensors, actuators, bioanalysis and additives for polymer resins [7]. For these applications, functional compounds can be incorporated into the PDMS microspheres upon mixing them in less-polar organic solvents. With all of these properties, PDMS microspheres can be used as a matrix to deliver the functional compounds to a desired location through microcirculation. Moreover, PDMS microspheres can be suspended in solution to achieve a fluid with controllable rheological properties [8]. Such a wide range of applications has stimulated great interest in the fabrication of PDMS microspheres and in their microfluidic systems for bioanalyses and beyond. For instance, amphiphilic PDMS microspheres within a few tens to a few hundreds of microns in size have been successfully fabricated by flow-focusing and co-flowing methods [9-10], whereas for smaller PDMS spheres, with dimensions within 2.5 to 25 μm , an aqueous emulsion technique has been employed [10-11]. In this technique, the dispersed cross-linked PDMS which is immiscible in water can naturally form micro spheres by virtue of their need to reduce their hydrophobic interactions. By using this technique, the cost of fabrication can be greatly reduced and the process is environmentally friendly.

Driven by the aforementioned potential applications of PDMS microspheres, the objective of this work was focused on low cost and efficient PDMS microsphere fabrication methods. Given the hydrophobic nature of PDMS, in this work we fabricate PDMS microspheres from PDMS elastomer in an aqueous medium by creating a stirred emulsion during crosslinking, with and without sonication. The stirring and the ultrasonic waves are expected to mediate separations of agglomerates of PDMS microdroplets during and after their injection into water, keeping the particles divided and spherical as they cross link and effectively freeze out further coalescence into larger domains. Prior to the cross-linking induced freezing out of droplet fusion and coalescence processes, the small particle size is maintained due to the response of the microspheres to the mechanical or acoustic radiation force exerted on them [12]. With this method, we evaluated the effect of the temperature of the medium on the size distribution of the PDMS microspheres. This is because the rate of cross linking is expected to be strongly temperature dependent. We characterized and examined the PDMS microspheres using bright field microscopy, Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Subsequently, we also evaluated the incorporation of ionic fluorescent dyes within the PDMS microspheres. The fluorescent dyes which are used broadly as coloring and staining biological analytes can be considered as model photo-functional and ionic compounds incorporated in

the PDMS microspheres. This study would improve our understanding about dye incorporation in the cross-linked PDMS networks in the microspheres.

II. EXPERIMENTAL

PDMS (SYLGARD® 184 silicone elastomer base) and its curing agent, produced by Dow Corning Co., were supplied by Panasonic Singapore. The elastomer base and curing agent were thoroughly mixed in a Teflon dish in a 10:1 ratio by weight. This ratio was selected because it produces an optimum curing profile based on the low viscosity of cross-linker mixture and rapid rise in elasticity with time [13]. Newly prepared mixed elastomer (~500 μ L in volume) was immediately dispensed dropwise into 25 mL of aqueous medium using a small nozzle pipette. The reaction vessel was immersed in a temperature-controlled water bath and the reaction mixture was vigorously stirred or sonicated at frequency of 37 kHz. The solution rapidly turned cloudy, indicating the formation of microparticles. The suspension was continuously stirred or sonicated for 2 h. The dry product microspheres were collected by filtration and then dried. The effect of temperature on the size distribution of the PDMS microspheres was evaluated by varying the temperature of the water medium from 30 to 80 °C during the crosslinking reaction.

The chemical structure of the PDMS microspheres before and after dye encapsulation was evaluated by using an FTIR spectrometer (Cary 630, Agilent Technologies, USA). Approximately 0.1 g of the dried PDMS microspheres was directly subjected to FTIR measurement using attenuated total reflection (ATR) method. The surface morphology of the PDMS microspheres was evaluated from their scanning electron microscopic (SEM) images. The SEM image of dried PDMS microspheres, pasted onto a carbon tape, was recorded on a SEM microscope (JEOL, JSM-7610F).

The size distribution of the PDMS microspheres was evaluated from their bright-field microscopic images. For this purpose, the microspheres were cast onto a glass slide. The glass slide was then mounted on the sample stage of a microscope (Nikon, Eclipse 50iPOL) equipped with a conventional condenser lens (Nikon Achr, NA 0.40) and an objective lens (Nikon; 40 $\times$; NA 0.60). The microspheres were irradiated with white light from halogen lamp (λ =380–750 nm) through the condenser lens. The bright-field images of the microspheres in the forward direction were collected and transmitted by the objective lens into a CCD camera (Nikon; DS-Fi1C). From the bright field images, we were able to estimate the sizes of the microspheres using ImageJ software.

Ionic fluorescent dyes, including methylene blue, methyl violet 2B, crystal violet, congo red, rhodamine B, and malachite green were each individually dissolved in acetone. The concentration of the dyes was adjusted to be 5 ppm, as determined from their respective absorption spectrum which was measured using a UV-visible spectrophotometer (Shimadzu UV-1601 PC). Incorporation of the fluorescent dyes was performed by adding 0.5 mg of the dried PDMS microspheres into 5 mL of the solutions of fluorescent dyes. The mixture was shaken for 20 min and kept overnight followed by filtration to end the process of dye percolation. The filtrate was washed three times with distilled water, and then dried overnight in oven at 45 °C.

Incorporation of the fluorescent dyes in the PDMS microparticles was evidenced by fluorescence microscopic imaging using a microscope (Nikon Eclipse 50iPOL, Japan). In this measurement, a beam from a high pressure mercury lamp was utilized as an excitation light source. The excitation wavelength at 365 nm was selected by passing the beam through a band pass filter (360–390 nm). The beam then was focused by the objective lens into the microspheres mounted on the sample stage. The backward (Epi) fluorescent light from the microspheres was collected by the same objective lens, and passed through a dichroic mirror (λ >415 nm) and a long pass filter (λ >435 nm). Elastic light scattering from the excitation light was completely cut by a long pass filter. Finally, the fluorescence image was captured by a color CCD camera. By comparing the fluorescence images with that of a control measurement which was performed for bare PDMS microspheres, we can monitor the existence of the fluorescent dyes incorporated in the PDMS microspheres.

III. RESULTS AND DISCUSSION

3.1 Size Distribution of PDMS Microspheres

The particle size and surface morphology of PDMS microspheres fabricated using the two methods described above were evaluated by bright-field and SEM micrographs, respectively. Fig. 1A shows representative bright-field micrographs of the dry product retrieval of PDMS microspheres fabricated by precipitation and sonication at 30°C. This revealed that both stirring and ultrasonic wave radiation are versatile, simple, and effective methods to generate PDMS microspheres. In particular, the formation of PDMS microspheres under ultrasonic wave radiation has also been investigated by Rankin *et al.*

[14] and O'Donnell and McGinity [15]. The micrographs indicate that the microspheres show a wide particle size distribution and less agglomeration, similar to those generated by a co-flowing method [16]. With the two methods, we found that the smallest PDMS microspheres are roughly $0.8\ \mu\text{m}$, proving that smaller sized microspheres were not favored. This could be due to the high viscosity and high surface energy of PDMS oligomers in water. High viscosity would tend to hold the particles together and is connected with the internal volume free energy of the particle. Thus, the high viscosity of PDMS implies that there is a large amount of overall binding interaction between the oligomer strands. This is probably driven by entropy considerations related to chain entanglement rather than the Van der Waals forces that are dominant in such non-polar systems. A viscous particle is harder to mechanically shear and subdivide. The surface energy of the particles is expected to be high for a hydrophobic sphere in an aqueous medium. Hence surface energy considerations would also tend to drive the particle size higher. The counter to these energetic considerations are the shear forces exerted by the stirring and the acoustic waves. In other words, energy has to be provided to overcome the coalescence to form larger spheres.

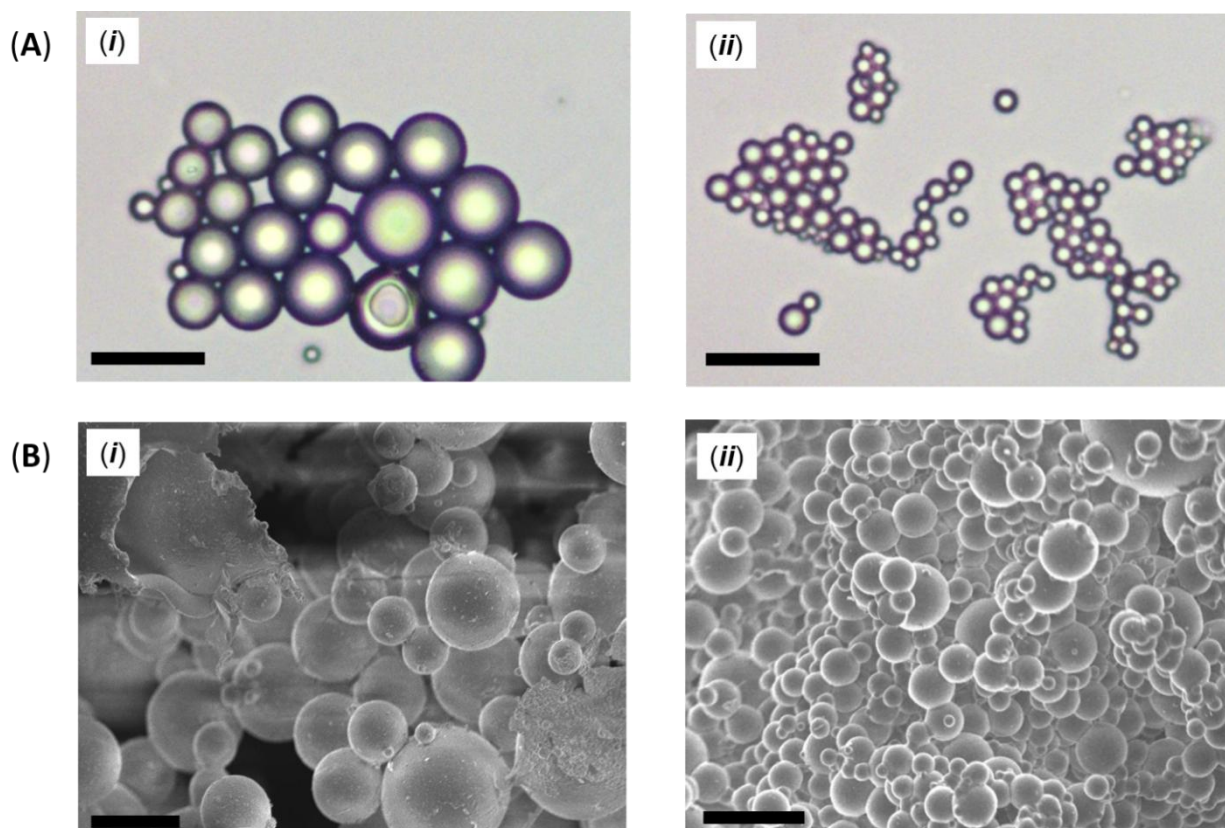


FIGURE 1. (A) The bright-field images and (B) SEM images of the dry product retrieval of PDMS microparticles fabricated by (i) stirring-shear and (ii) sonication method. Scale bars represent $10\ \mu\text{m}$.

The surface morphology evidenced by SEM micrographs of the PDMS microspheres produced by stirring shear and sonication methods are shown in Fig. 1B. The SEM micrographs of the PDMS microspheres indeed revealed that the particles were ultra-smooth and free of surface defects, although the microspheres were agglomerated with a very broad particle size distribution. The agglomeration is expected in the preparation of the dry product for microscopy analysis. It is noteworthy that, in this work, with the ratio between the elastomer base and curing agent being 10:1, the surface of the PDMS microspheres should have no significant reactive handles related to an excess of the vinyl functionalized polymers [13]. Therefore, surface impacts and imperfections should be suppressed during the microsphere formation.

By evaluating at least twenty bright-field micrographs with more than 500 microspheres, we quantified the size distribution of the multiphase microspheres to be in the range of 0.8 to $20\ \mu\text{m}$. From Fig. 2(A), it is clearly seen that the particle size distribution of the PDMS microspheres produced by the stirring-shear method is unimodal with the peak of the distribution at $1.2\ \mu\text{m}$ with coefficient of variant being 152%. The peak distribution is slightly narrower with the peak distribution at $2.2\ \mu\text{m}$ with coefficient of variant being 118% when the PDMS microspheres were produced by sonication method. Considering that the average particle size generally decreases with the strength of mechanical force which provides the initial shearing force to break-up the spheres, these results suggest that the mechanical force of ultrasonic radiation is stronger than for the

mechanical stirring. We may note that the smallest size of the microspheres was comparable to those fabricated in the presence of surfactant, though the size distribution of the latter case was trimodal with the peaks at 1, 10, and 100 μm [13]. These findings indicate that the PDMS microspheres fabricated with the two methods has a multidispersion similar to those usually observed for polymer microspheres produced by agitation, potentiometric dispersion, or sonication [13]. The unimodal distribution of the PDMS microspheres with the maximum distribution at a few tens of μm [14,15] can be attributed to low possibility of the existence of reactive functional groups on the surface to form inter-particle covalent bonds, as discussed above [13].

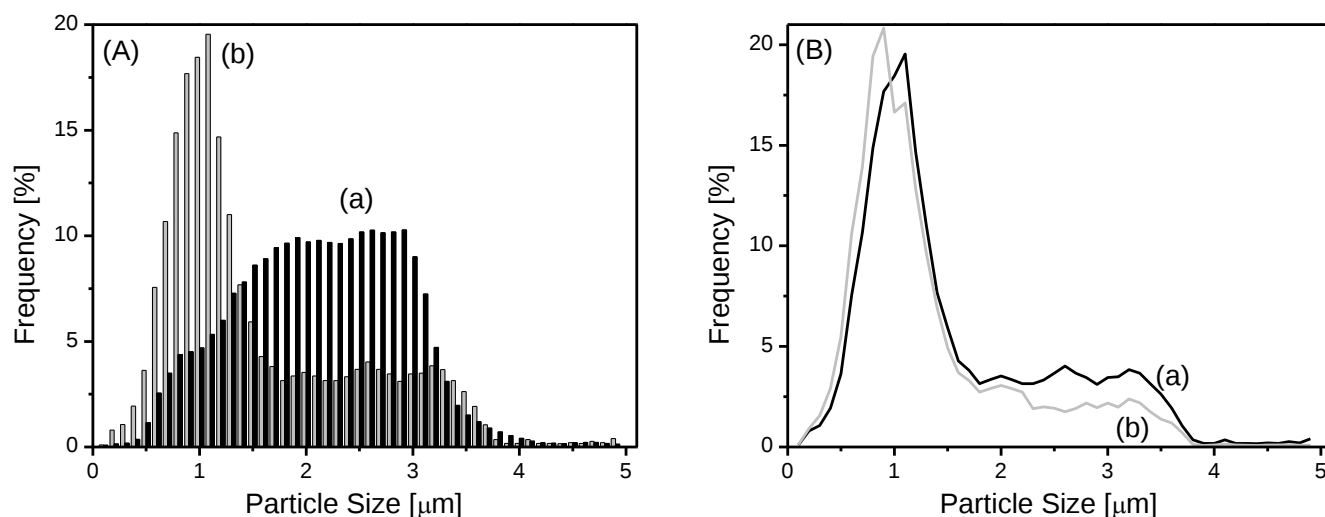


FIGURE 2. (A) The radius size distribution of PDMS microparticles fabricated by (a) stirring-shear and (b) sonication method, and (B) the representative size distributions of the PDMS microspheres fabricated using sonication method at different temperatures; (a) 30 $^{\circ}\text{C}$ and (b) 50 $^{\circ}\text{C}$.

It is known that the PDMS takes a long time to cure, and hence aggregates with inter-sphere covalent bonds leading to larger size microspheres would probably be more favorable at low temperatures. Therefore, we systematically evaluated the effect of the water medium temperature. We found that the size distribution of the microspheres becomes much narrower and the peak shifts downward as temperature increases, as shown in Fig. 2(B). This suggests that the sizes of PDMS microspheres reduces with the curing temperature most probably due to an increase in the cross-linked PDMS networks resulting in less dangling surface chains at higher temperatures [17]. The size reduction of PDMS microspheres with curing temperature has also been observed by Lee and Lee [18]. The different cross-linked PDMS networks should be evidenced by defect content, equilibrium swelling, and dye encapsulation capacity. The cross-linking of PDMS is increasingly important with recent focus on surface stiffness of the PDMS microspheres [19]. The difference in crosslinking networks, however, is not reflected in the surface morphology of the PDMS microspheres, as SEM micrographs of the PDMS microspheres fabricated at different temperatures show similar surface morphology (Fig. 1B).

3.2 Dye Encapsulation

In order to prepare fluorescent PDMS microspheres, various ionic fluorescence dyes were incorporated in the microspheres with different cross-linking networks. Incorporation of the dyes depends strongly on the swelling of PDMS microspheres in organic solvents, and we have selected acetone as the organic solvent, since this organic solvent swells PDMS microspheres to a small extent and it is miscible with water [20]. After the solvent was removed, incorporation of the dyes into the PDMS microspheres was evaluated by fluorescence microscopy. In Fig. 3, we show the fluorescence and bright-field images of PDMS microspheres incorporating methylene blue, methyl violet, crystal violet, congo red, rhodamine B, and malachite green-incorporated PDMS microparticles under irradiation with light at 365 nm. The bright field and fluorescence images of bare PDMS microspheres are also presented in Fig. 3 for comparison. It is clearly seen that, in contrast to bare PDMS microspheres, those containing the dyes show obvious fluorescence. This provides an interpretation that the chemical structures of the fluorescent dyes as well as the cross-linked PDMS networks remain intact after encapsulation. It is also proposed that the dyes are incorporated in the polymer matrix, but they do not change the cross-linked PDMS networks in the microspheres. This is because we obtained the same size distribution for bare and dye incorporated PDMS microspheres. Such an unchanged PDMS network was also demonstrated by FTIR spectrum, as discussed below. Additionally, we can

suggest that the dyes are incorporated as monomers and not as aggregates. Aggregated dyes, specifically rhodamine dyes, are well known to have greatly diminished, even zero fluorescence yields. Clearly the dyes maintain their emission properties as if they are unaggregated.

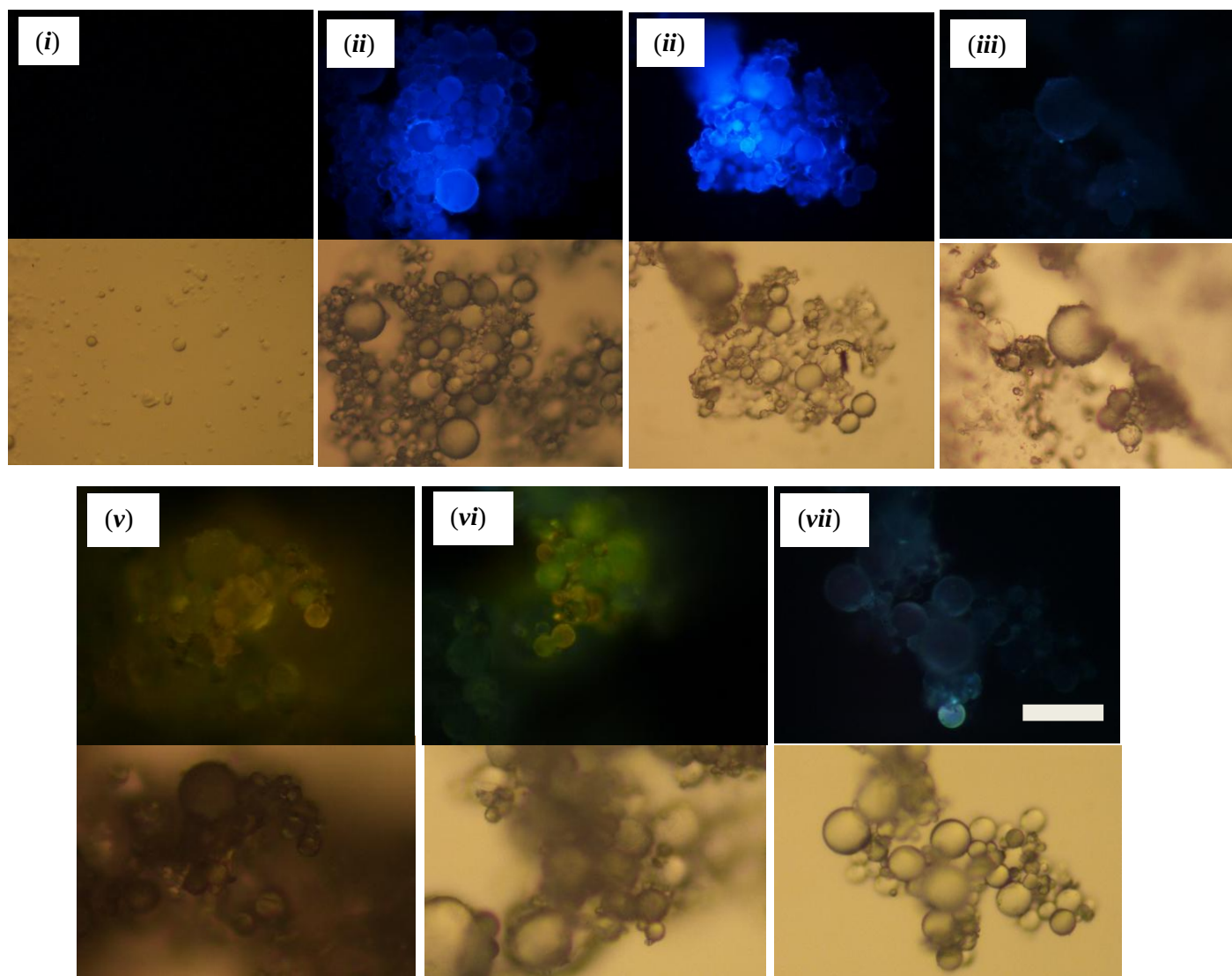


FIGURE 3. Fluorescence images of (i) bare PDMS microparticles, and (ii) methylene blue, (iii) methyl violet, (iv) crystal violet, (v) congo red, (vi) rhodamine B, and (vii) malachite green-incorporated PDMS microparticles. Their respective bright-field images are shown in the bottom row. The white scale bar represents 10 μm .

In principle, the changes in cross-linked PDMS networks should incorporate different concentrations of dyes. However, the different cross-linked PDMS networks in the microspheres upon changing the curing temperature is very tiny, thus the different concentrations of dyes is not obviously observable. Nevertheless, based on the fluorescence microscopy the fluorescent dyes are incorporated in the microspheres during the swelling-deswelling process of PDMS microspheres in acetone. It is seen that acetone is a good solvent for swelling of the PDMS microspheres. Though the degree of swelling of bulk PDMS in non-polar solvent such as cyclohexane has been found to be around eight-fold [21], the swelling kinetics of the PDMS microspheres in different organic solvents are still an open question. With the swelling-deswelling process, we consider that the fluorescent dyes are randomly contained within the microspheres because in the solution the dyes are distributed randomly and the microspheres swell isotropically. A similar feature has been observed even for encapsulation of magnetic particles by PDMS spheres [22]. The fluorescence intensity remains unchanged when measured after two weeks, revealing the stability of the fluorescence stability of the PDMS microspheres, which can be attributed to stable formation of the dyes incorporated in the PDMS structure.

3.3 ATR-FTIR of Bare and Dye Incorporated PDMS Microspheres

We conducted FTIR analyses on bare and dye incorporated PDMS microspheres to evaluate whether there were significant changes in PDMS network upon dye encapsulation. This was done to confirm the above discussion on fluorescence. As displayed in Fig. 4(a), the FTIR spectrum of bare PDMS microspheres shows vibrational peaks at 1258 and 788 cm^{-1} , attributed to the deformation and rocking vibrations of CH_3 in Si-CH_3 , and at 1065 and 1011 cm^{-1} due to Si-O-Si asymmetric deformation. In addition, the peaks observed at 2962 and 2906 cm^{-1} in the spectrum are assigned to the symmetric and asymmetric stretching vibration of CH_3 . The Si-H vibration of the cross-linking agent at 2156 cm^{-1} was not observed in the spectrum, indicating that the concentration of the curing agent in the PDMS microspheres with the ratio of silicone elastomer base and curing agent being 10:1 is not excessive [13], as this particular band would appear when either the curing agent is excessive or cross-linking of siloxane oligomer is not efficient. The peak positions of the PDMS were invariant with and without the dyes and every spectrum was identical within the resolution limit of the FTIR spectra (1–4 cm^{-1}). Furthermore there was no evidence of the dye peaks, indicating that the dye concentration was too low to register in the absorption measurement. This is not unexpected as it is well known that fluorescence is far more sensitive than absorption, since the former is dark-field and the latter is a bright-field technique.

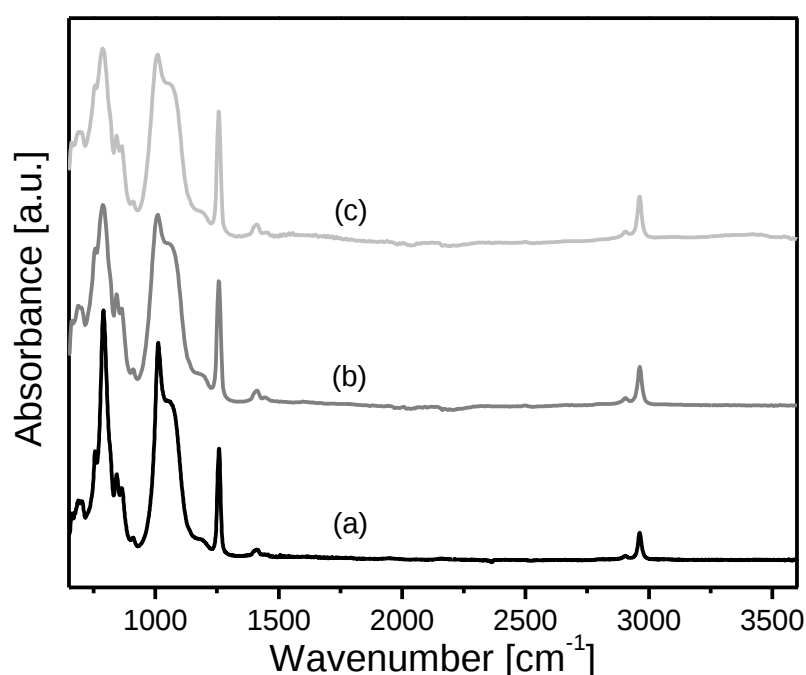


FIGURE 4. FTIR spectra of (a) bare PDMS microparticles, (b) methylene blue-incorporated PDMS microparticles, and (c) methyl violet-incorporated PDMS microparticles.

As shown in Fig. 4 (b), the dyes incorporated PDMS microspheres show similar spectral pattern to that of the bare ones, although there are slight modifications in the intensities of the peaks, which are attributable to differences in the resolution between the measurements. These changes were made to optimize the signal to noise levels. This confirms that the functional groups of PDMS microspheres remain intact upon dye incorporation, in contrast to significant changes observed after UV/ozone treatment which readily changes the chemistry of the PDMS [22-23]. Our ATR-FTIR results show that there are no new bands observable in the spectrum upon incorporation of the fluorescent dyes and no spectral shifts (see Fig. 4(c)). This implies that the dyes are weakly interacting with the PDMS matrix, or that there is simply not enough dye to be observed by any interaction effects on the matrix.

The SEM images also indicate that the dyes incorporated PDMS microspheres did not agglomerate forming their own distinct phase domains or crystal habits. Since the dyes incorporated PDMS microspheres have been washed with water, we should also consider that the dyes are encapsulated inside rather than on the surface of the microspheres. Therefore, we may expect that the cross-linking PDMS network acts as an inert matrix, instead of one in which there is chemical adsorption incorporating the dyes tightly and rigidly to the PDMS chains. This is expected because the ionic portions of the dyes should not strongly interact with the non-polar PDMS chains.

IV. CONCLUSION

In summary, we have demonstrated that PDMS microspheres can be fabricated simply by stirring induced shear and sonication methods using water as a medium. These simple methods are able to fabricate and to control the inter-sphere cross-linking of PDMS microspheres by adjusting the temperature of the water medium. We found that the particle size tends to be smaller and the size distribution is narrower with temperature, indicating that the crosslinking rate for PDMS networks in the hydrophobic microspheres is slightly increased. We also showed that the PDMS microspheres undergo swelling process, incorporating fluorescent dyes, upon replacing the medium from water to an organic solvent with dissolved dyes. Upon incorporation of the dyes, the cross-linked PDMS network in the microspheres remain intact. Thus, the PDMS microspheres can act as a matrix to encapsulate fluorescence dyes, making them fluorescent microspheres. Using scanning electron and fluorescence microscopy, we found that the fluorescent dyes are randomly distributed in the cross-linked PDMS networks and the dyes do not change either their own chemical structures or the crosslinking PDMS networks in the microspheres. This work provides and paves the way for utilization of PDMS microspheres as matrices for medicinal, surfactants, electron donor, or electron acceptor compounds for various applications.

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