

# Synthesis, Characterization and Antimicrobial Properties of Polyaniline Encapsulated Azo Dyes

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**Abstract:** Polyaniline encapsulated azo dyes were synthesized from polyaniline obtained by oxidative polymerization of aniline and different azo dyes obtained from 2-aminothiophene intermediates. Two different polyaniline encapsulated azo dyes were synthesized and their molecular weight ranges from 58,044- 58,128g/mol. The melting points were found to be from 286°C- 319°C. Spectroscopic assessment such as FT-IR spectroscopy and GC-MS were carried out to elucidate the structures of the synthesized polyaniline encapsulated azo dyes. The FT-IR spectra of the polyaniline encapsulated azo dyes showed absorption peaks in the range 3213.0- 3242.8cm<sup>-1</sup> due to the presence of an amino group, The benzenoid and quinoid rings for all the polyaniline encapsulated azo dyes appeared in the range 1461-1487cm<sup>-1</sup> and 1543-1595cm<sup>-1</sup> respectively while the carbon nitrogen single bond (C-N) absorption were found in the range 1263.6-1285cm<sup>-1</sup>. For the assessment of the antimicrobial properties of the synthesized polyaniline encapsulated azo dyes different microorganism such as *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus niger* were selected based on their pharmacological incidence and Agar well diffusion method was used. The result of the antimicrobial studies showed zones of inhibition ranging from 14-34mm. The strong activities of the synthesized polyaniline encapsulated azo dyes against *staphylococcus aureus*, *Salmonella typhi*, and *candida albicans* showed that it may be effective against infectious diseases which these microorganism cause. The MIC value of 12.5mg/ml and MBC/MBF value of 12.5mg/ml of the synthesized polyaniline encapsulated Dye A and Dye B had activities against *staphylococcus aureus* and *Salmonella typhi* as compared to polyaniline and the dyes individually.

**Keywords:** Polyaniline, azo dyes, antimicrobial activity

## I. INTRODUCTION

The conducting polymers (CPs) such as polyanilines, polyacetylenes, polythiophenes, and polypyrroles, are promising materials for potential industrial applications such as electrorheology (ER), electronics, and optics due to their high electrical conductivity and good environmental stability (Yılmaz *et al.*, 2011). The conductivities of these conjugated polymers are achieved through chemical oxidation or reduction reactions using a series of simple anionic or cationic species called dopants (Chandrakumar, 1999). There have been a number of reviews on conducting polymers with regard to biomedical applications. It appears that the presence of an

acidic functional group (–COOH) in the polymer chain improves the antibacterial efficacy of the copolymer. Without being bound by theory, the acidic dopants on the molecular chains of copolymers may react with the bacteria (or other relevant microbial organism) which result in their death. Alternatively, due to electrostatic adherence between copolymer molecules and the bacteria, which carry charges of different polarity, the walls of the bacteria may break and the contents of the bacteria become exposed or leak out, which cause the bacteria to die (Allen *et al.*, 2009). Marija *et al.*, (2011), has investigated the antimicrobial properties of conductive functionalized polyanilines by exploring their interaction with bacterial cells. It has been observed that lower concentrations of PANI strongly inhibited the growth of wild-type *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* as well as several other antibiotic resistant clinical pathogens (Chandravadevelu *et al.*, 2011). Nanofibre of polyaniline combined with fluconazole have been prepared by simple and cost-effective sol-gel method using d-10-camphorsulfonic acid (d-CSA) as a dopant and as a surfactant, and ammonium persulfate as the oxidant. The synthesized nano-structured material was dissolved in dimethylsulfoxide at different concentrations and tested for its antifungal properties against *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. (Gopalakrishnan *et al.*, 2011)

## II. EXPERIMENTALS

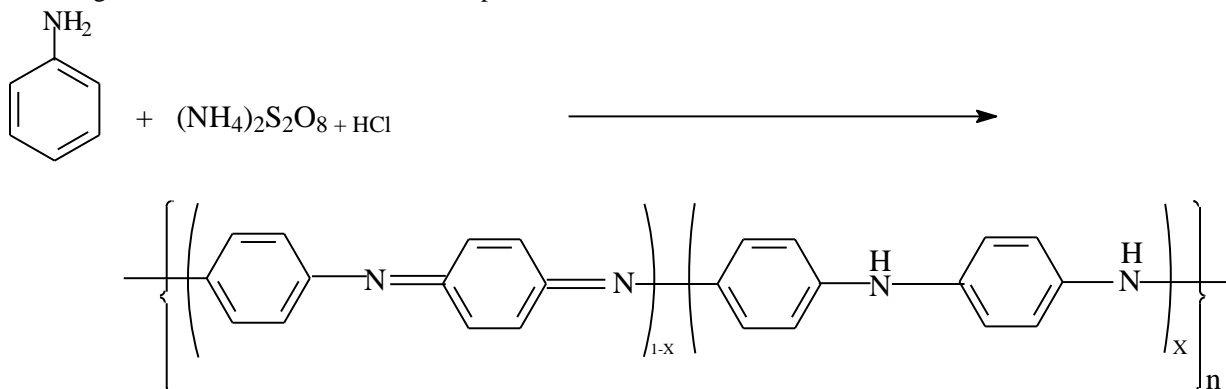
### Materials

Analytical grade reagents and chemicals from Sigma Aldrich were used for this research work, these include; acetoacetanilide, N-(2,4-dimethylphenyl)-3-oxobutyramide, N,N-dimethylaniline, p-N,N-dimethylaminobenzaldehyde, malononitrile, morpholine, propionic acid, acetic acid, dimethylformamide (DMF), dimethylsulfoxide (DMSO), sodium nitrite, concentrated sulphuric acid, mueller hinton agar, potato dextrose agar, nutrient agar, Magnetic stirrer, oven, reflux condenser, melting point apparatus, Uv-visible spectrophotometer, Fourier transform infra-red spectrophotometer, GC-M, autoclave, incubator, compound microscope, culture media.

### III. METHODS

#### Synthesis of Polyaniline

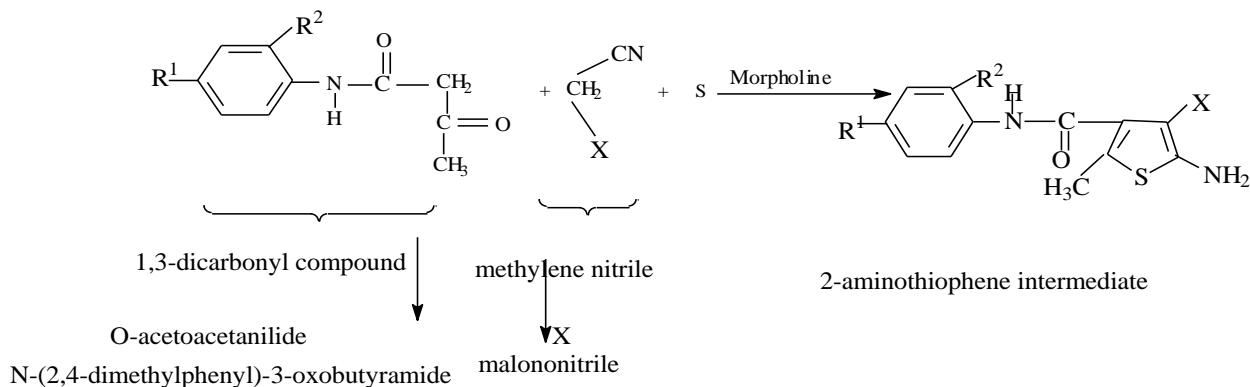
Polyaniline was synthesized according to the method of (Krzysztof, 2006) involving chemical oxidative polymerization of 1.0mol (9.3ml) aniline in an aqueous acidic medium containing 100ml of 1.0mol HCl solution in a 500ml glass bulb (equipped with a thermometer and a dropping funnel) and was cooled to 0°C. The polymerization was initiated by drop wise addition of 1.0mol (100ml) of ammonium persulfate as an oxidizing agent to the acidified solution containing the aniline monomer at room temperature



Scheme 3.1: Synthesis of polyaniline (PANI-HCl) using HCl as the dopant

#### Synthesis of Aminothiophene Intermediates

The aminothiophene intermediate was synthesized by following methods reported in the literature, as shown in the scheme 3.3 (Alaa and Tarek, 2006).



Scheme 3.3: Synthesis of aminothiophene intermediate

#### Aminothiophene Intermediate 1.

o-acetoacetanilide (21.16g, 0.1mol), malononitrile (6.96g, 0.1mol) and sulphur (3.37g, 0.1mol) in ethanol were stirred in the presence of morpholine (8.97g, 0.1mol) at 60-70°C for 3hrs. The resulting thick dark solution was cooled and stored overnight in a refrigerator, followed by filtration, washing with ethanol and then ethanol/water (1:1) solution

and was dried. The light brown powder obtained was crystallized from ethanol.

#### Aminothiophene Intermediate 2

N-(2,4-dimethylphenyl)-3-oxo-butylamide (20.72g, 0.1mol), malononitrile (6.96g, 0.1mol) and sulphur (3.37g, 0.1mol) in ethanol were stirred in the presence of morpholine (8.7g,

0.1mol) at 60-70°C for 3 hours. The resulting thick dark solution was cooled and stored overnight in a refrigerator, followed by filtration, washing with ethanol and then

ethanol/water solution and dried. The dark brown obtained powder was recrystallized from ethanol.

#### Structures of Synthesized Intermediate

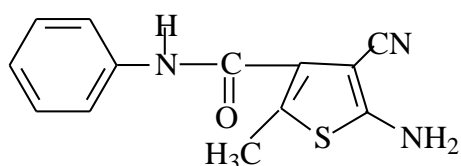


Fig 3.1: Intermediate 1

#### Procedure for Diazotization and Coupling

Sodium nitrite (1.38g, 0.02mol) was added portion wise to 10ml of concentrated sulphuric acid at 10°C and heated to 60°C with stirring for 15min. The solution was then cool to 5°C and a mixture of acetic acid and propionic acid (17:3) was

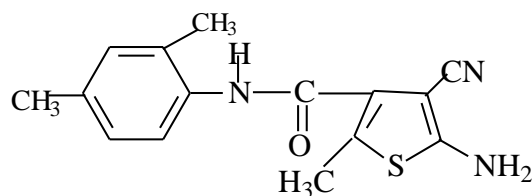
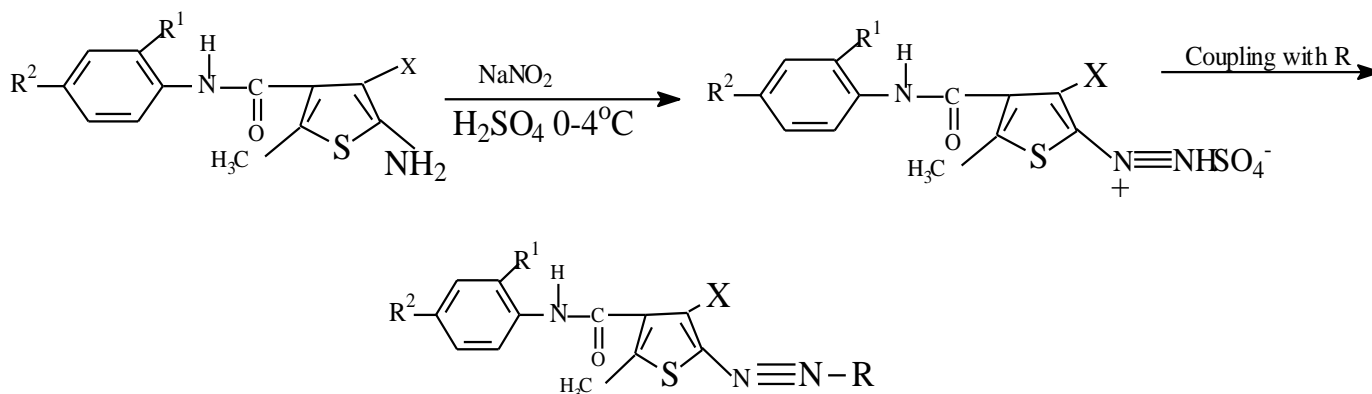


Fig 3.2: Intermediate 2

then added to the mixture below 30°C. The finely ground aminothiophene intermediate 1-2 (4.90g, 0.02mol) was added within 30min below 5°C and the whole mixture was stirred at 0-5°C for 2-4hrs. The associated steps are illustrated in the reaction scheme 3.4.



where  $R^1$  and  $R^2$  are H or methyl group and R is the coupling component

#### Scheme 3.4: Diazotization and Coupling of 2-aminothiophene.

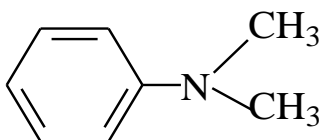


Fig 3.3: N,N-dimethylaniline

#### Coupling Reaction

The coupling component, N, N-dimethylaniline, p-N,N-dimethylaminobenzaldehyde was dissolved separately in acetic acid and cooled to 0°C by adding ice. The diazonium solution of the intermediate 1 and 2 that was prepared was then added for 30-40min with vigorous stirring (Maradiya and

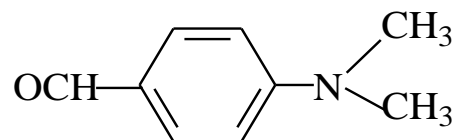
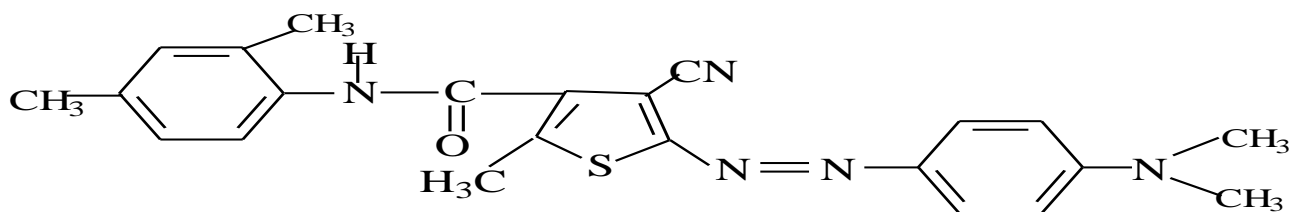
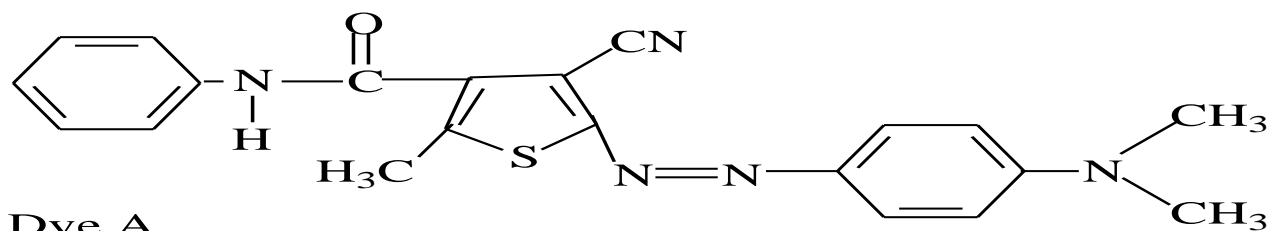


Fig 3.4: P-N,N-dimethylaminobenzaldehyde

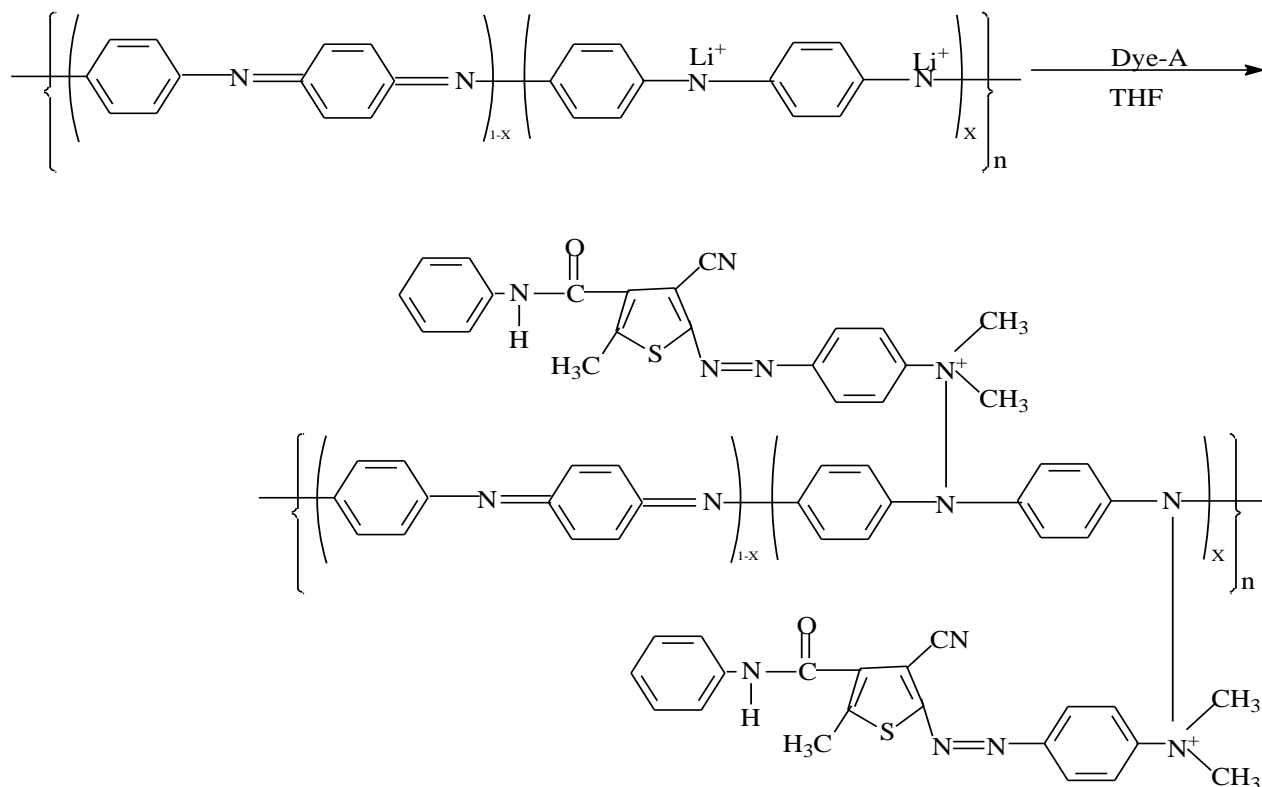
Patel, 2010). The mixture was stirred for a further 2-3hrs, under 5°C and the  $p^H$  of the solution was adjusted to 4-5 using 5ml 10% NaOH. The resulting products were collected by filtration, wash with water and dried. The crude product was purified by recrystallizing it from ethanol and DMF of ratio 9:1.



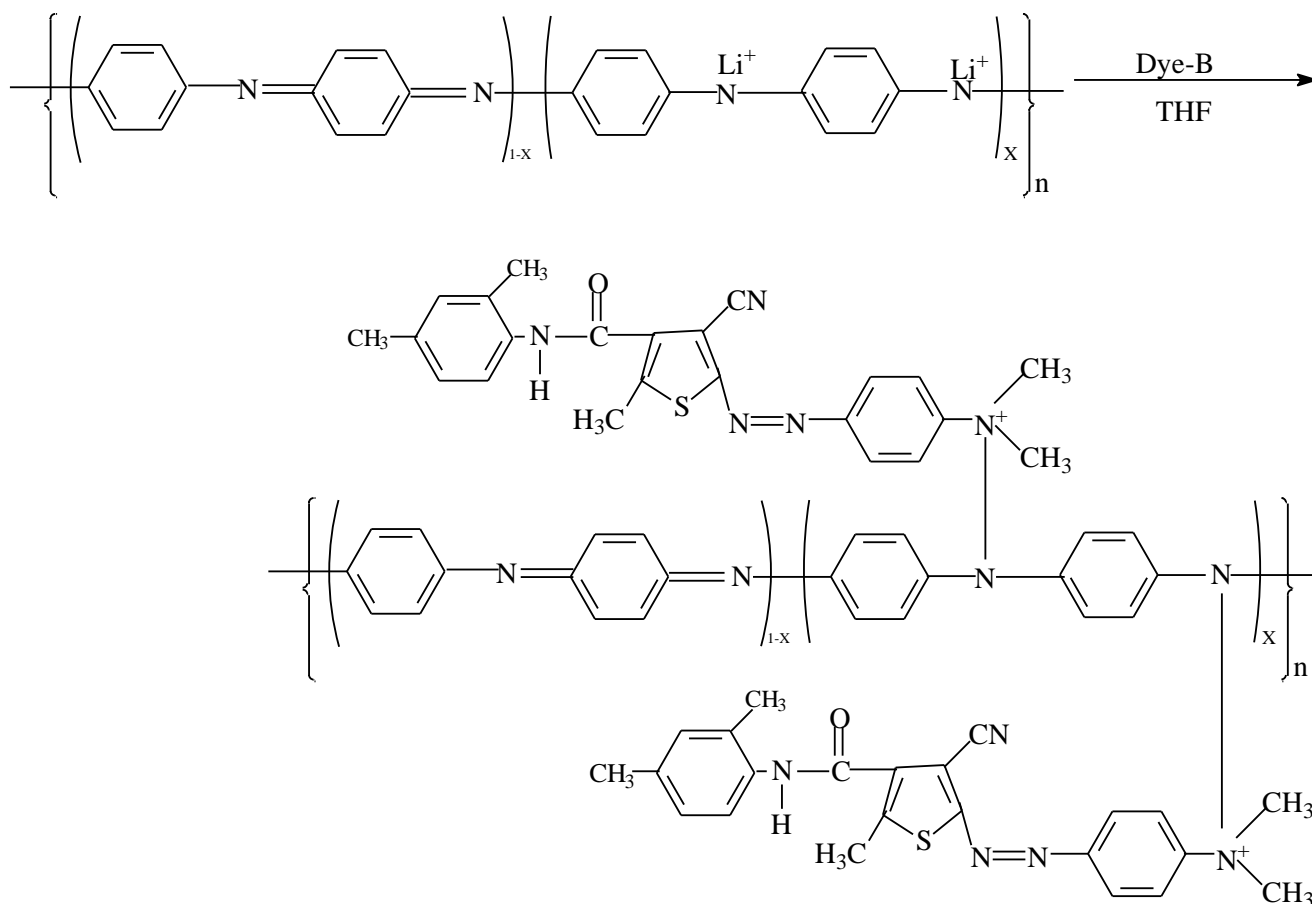
### Synthesis of polyaniline encapsulated azo dyes

The emeraldine base of hydrochloric acid doped polyaniline (1.0g) was dissolved in 30ml tetrahydrofuran (THF). The solution was allowed to cool to 0°C and a minimum amount of BuLi (2ml) was added with stirring. The color of the solution changed from dark green to green black due to the formation of lithium salt of polyaniline. In a dry conical flask Dye A

(0.025g) was dissolved in 10ml THF. The solution of the dye was then added to the reaction mixture obtained from previous step which was then stirred at room temperature for 24hrs. This procedure was then repeated with dye B, C and dye D solution. The color of the solution turned black due to the formation of black precipitate. The precipitate was filtered and washed with acetone and ethanol to remove residual amount of unreacted dye, as shown in the reaction schemes below:



Scheme 3.6: Synthesis polyaniline encapsulated dye A (PANI-DYE A)



Scheme 3.8: Synthesis polyaniline encapsulated dye B (PANI-DYE B)

#### Characterization of the Intermediates, and azo dyes substituted conducting polyaniline.

The physicochemical properties of the intermediates, synthesized dyes and the azo dyes substituted conducting polyaniline were carried out and spectroscopic properties such as FT-IR and GC-MS.

#### The Antimicrobial Screening

**Test microorganism:** The test microorganisms used for this analysis were clinical isolates of bacteria and fungi obtained from Department of Microbiology, Ahmadu Bello University, Samaru, Zaria. The isolates were: *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans* and *Aspergillus niger*.

#### Culture Media

The culture media used for the analysis includes Mueller hinton agar (MHA), Mueller hinton broth (MHB), Potato dextrose agar (PDA) and Nutrient agar (NA). These media were used for sensitivity test, determination of minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC). All media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes.

#### Determination of Inhibitory Activity (Sensitivity Test) of the Synthesized Samples Using Agar Well Diffusion Method

The standardized inocula of both the bacterial and fungal isolates were streaked on sterilized Mueller hinton and potato dextrose agar plates respectively with the aid of a sterile swab sticks. Four wells were punched on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentrations of the synthesized samples which were 100, 50, 25 and 12.5mg/ml respectively. Each well was filled up with 0.2ml of the sample. The inoculated plates with the sample were allowed to stay on the bench for about 1 hour, this is to enable the extract to diffuse on the agar. The plates were then incubated at 37°C for 24 hours (plates of Mueller hinton agar) while the plates of potato dextrose agar were incubated at room temperature for about 3-5 days. At the end of the incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (Zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimeter and the results were recorded.

*Determination of Minimum Inhibitory Concentration (MIC).*

The minimum inhibitory concentration of the synthesized samples was determined using tube dilution method with the Mueller hinton broth used as diluents. The lowest concentration of the synthesized samples showing inhibition for each organism when the sample was tested during sensitivity test which was serially diluted in the test tubes containing Mueller hinton broth. The organisms were inoculated into each tube containing the broth and the synthesized samples. The inoculated tubes were then incubated at 37°C for 24 hours. At the end of the incubation period, the tubes were examined/observed for the presence or absence of growth using turbidity as a criterion, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC). The results are shown in Table 4.7.

*Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC):*

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bacteriocidal/fungicidal concentration (MBC/MFC) of the synthesized sample. A sterilized wire loop was dipped into the test tubes that did not show turbidity (Clear) in the MIC test and a loopful was taken and streaked on a sterile nutrient agar plates. The plates were incubated at 37°C for 18-24 hours. At the end of incubation period, the plates were examined for the presence or absence of growth. This is to determine whether the antimicrobial effects of the synthesized samples are bacteriostatic or bactericidal.

## IV. RESULT AND DISCUSSION

*Physical Properties of the Synthesized Polyaniline and Polyaniline Encapsulated Azo Dyes*

The molar mass, melting point, percentage yield, and colour of the dyes azo-substituted conducting polyaniline are shown in Table 4.3

Physical Properties of the Polyaniline Encapsulated Azo Dyes.					
Dye-PANI	Molecular formular	Molecular weight (g/mol)	Melting point (°C)	% Yield	Colour of the crystal
PANI	(C <sub>24</sub> H <sub>18</sub> N <sub>4</sub> ) <sub>n</sub>	57,739	290-298	80.12	Black
A	(C <sub>24</sub> H <sub>18</sub> N <sub>4</sub> ) <sub>n</sub> . C <sub>42</sub> H <sub>38</sub> N <sub>10</sub> O <sub>2</sub> S <sub>2</sub>	58,128	286-290	87	Dark purple
B	(C <sub>24</sub> H <sub>18</sub> N <sub>4</sub> ) <sub>n</sub> . C <sub>46</sub> H <sub>46</sub> N <sub>10</sub> O <sub>2</sub> S <sub>2</sub>	58,156	314-319	63.16	Dark purple

The results from Table 4.3 shows that the percentage yield of the synthesized polyaniline is 80.12% due to the acceptable amount of monomer to oxidant ratio ( $r = 1$ ). Also, the percentage yield of 87, and 63.16 were observed for polyaniline encapsulated Dye A and Dye B respectively. The result from Table 4.3 also showed the melting point 290-298°C for polyaniline. However, polyaniline encapsulated dye B was observed to have the highest melting point of 314-

319°C, this can be attributed to the structure of the repeating unit and polymer stability with increase in relative molecular weight.

*The Infra-Red Absorption of the Synthesized Polyaniline and Polyaniline Encapsulated Azo Dyes*

The vibrational frequencies of the synthesized polyaniline and polyaniline encapsulated dyes are shown in Table 4.6

IR-Vibrational Frequencies of the Polyaniline Encapsulated Azo Dyes.				
Vibrational Mode	PANI	PANI-DYE A	PANI-DYE B	
Para-substituted	797, 764	790	790	
Benzenoid ring	1479	1461.1	1461.1	
Quinoid ring	1584	1580.4	1580.4	
C-H- Stretching	2922.2, 2780	2877.5	2877.5	
N-H- Stretching	3220.4	3213.0, 3056.4	3220.4, 3052.7	
C-N- Stretching	1289, 1118.2	1282.2	1282.2	

Table 4.6 shows the IR-vibrational frequencies of the synthesized polyaniline and polyaniline encapsulated dyes which indicates a band 797 and 764cm<sup>-1</sup> as the characteristics of the p-substituted chains for the polyaniline. The band 1479 and 1584cm<sup>-1</sup> are related to the stretching of the C-N bonds of the benzenoid and quinoid rings, respectively, while the broad band 3220.4cm<sup>-1</sup> is assigned to the N-H stretching vibration of

amine group and the peak C-H-stretching vibration is 2922.2 and 2780cm<sup>-1</sup>. However, the IR-vibrational frequencies of the polyaniline encapsulated dyes shows an absorption peaks due to stretching frequency of the NH group in the region 3213-3220.4cm<sup>-1</sup>. An absorption peak in the region 1461-1479 due to the presence of C-C stretch of the benzenoid ring while 1580-1584cm<sup>-1</sup> was due to the presence of C=C stretch of the

quinoid rings and the absorption peak in the region 1282.2-1289cm<sup>-1</sup> which is attributed to V<sub>C-N</sub> stretching. Comparing to

polyaniline some shifted band in the spectra of polyaniline encapsulated dyes were observed as shown in Table 4.6.

Table 7: GC- MS Fragmentations of the Synthesized Dyes and Intermediates

Dyes/Intermediate	Mass (m/z)
Intermediate 1	(C <sub>6</sub> H <sub>5</sub> SN <sub>2</sub> ) <sup>+</sup> = 140, (C <sub>7</sub> H <sub>7</sub> NO) <sup>+</sup> = 123
Intermediate 2	(C <sub>7</sub> H <sub>5</sub> N <sub>2</sub> SO) <sup>+</sup> = 165, (C <sub>8</sub> H <sub>10</sub> N) <sup>+</sup> = 117
Dye A	(C <sub>6</sub> H <sub>6</sub> N) <sup>+</sup> =81.1, (C <sub>7</sub> H <sub>3</sub> N <sub>3</sub> SO) <sup>+</sup> =177,(C <sub>8</sub> H <sub>11</sub> N) <sup>+</sup> = 129
Dye B	(C <sub>8</sub> H <sub>10</sub> N <sub>3</sub> ) <sup>+</sup> = 149, (C <sub>7</sub> H <sub>4</sub> N <sub>2</sub> SO) <sup>+</sup> = 179, (C <sub>8</sub> H <sub>6</sub> ) <sup>+</sup> = 91

Thus intermediate 1 and 2 showed intense molecular ion peaks at m/z 259±2 and 282±3, consistent with the molecular formulae C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>OS and C<sub>15</sub>H<sub>15</sub>N<sub>3</sub>OS respectively. The molecular ion of the intermediate 1 shown in Table 4.7 underwent fragmentations to produce peaks at m/z 139 and 123 corresponding to its molecular mass. While the intermediate 2 underwent fragmentation to produce peaks at m/z 117 and 165 respectively. However, Dye A and B showed molecular ion peaks at m/z 394.2±5 and 414.6±3, consistent

with the molecular formulae C<sub>21</sub>H<sub>19</sub>N<sub>5</sub>OS and C<sub>23</sub>H<sub>23</sub>N<sub>5</sub>OS respectively.

#### Antimicrobial Screening of the Synthesized Dyes and Polyaniline Encapsulated Azo Dyes.

The antimicrobial properties of the synthesized dyes and polyaniline encapsulated dyes against some selected pathogenic microorganism are shown in Table 4.7 – 4.9

Zone of Inhibition (Diameter) of the Polyaniline Encapsulated Azo Dyes Against

Test Microorganisms (mm)	PANI	Dye A	Dye B	Dye A-PANI	Dye B-PANI	Ciprofloxacin	Fluconazole
<i>Staphylococcus aureus</i>	14	18	16	32	29	35	0
<i>Salmonella typhi</i>	17	20	20	34	31	38	0
<i>Candida albican</i>	0	10	7	23	18	0	0
<i>Aspergillus niger</i>	10	13	10	17	20	0	31

Minimum Inhibition Concentration of the Polyaniline Encapsulated Azo Dyes Against Test Microorganism (mg/ml)

Test Organism	PANI	DYE A-PANI	DYE B-PANI	Dye A	Dye B
<i>Staphylococcus aureus</i>	25	12.5	25	25	25
<i>Salmonella typhi</i>	50	25	12.5	25	25
<i>Candida albican</i>	50	25	25	50	50
<i>Aspergillus niger</i>	50	50	50	50	50

Minimum Bactericidal/Fungicidal Concentration of the Polyaniline Encapsulated Azo Dyes Against Test Microorganism

Test Organism	PANI	DYE A-PANI	DYE B-PANI	Dye A	Dye B
<i>Staphylococcus aureus</i>	50	12.5	25	25	25
<i>Salmonella typhi</i>	50	50	12.5	25	25
<i>Candida albican</i>	50	50	25	50	50
<i>Aspergillus niger</i>		50	50	50	50

The results from Table 4.8 show that all the synthesized polyaniline encapsulated dyes have various inhibitory effect against the test organisms as compared to the dyes and polyaniline except dye c-PANI which has no effect on *Aspergillus niger*. The zone of inhibition ranges from 10-34mm. The synthesized polyaniline encapsulated dyes showed higher activity than the polyaniline and the dyes individually thus, the abstraction of the hydrogen atom from a nitrogen atom of the imine group on the polyaniline chain backbones by butyl lithium and further attachment of the dye moieties enhance their antimicrobial activities which may be further attributed to greater extent in the alteration in chemical structure and charge delocalization which is in consonant with the work of (Nirmala *et al.*, 2015) who observed that dye-containing polyaniline has an enhance antimicrobial activity as compared to polyaniline and dyes individually which was also in agreement with (Karunanithy *et al.*, 2013). From the results above in Table 4.8 the best activity was shown by the Dye A-PANI against *Staphylococcus aureus* with zone of inhibition (34mm), followed by synthesized Dye B-PANI with maximum zone of inhibition of 29 and 31mm respectively against *Staphylococcus aureus* and *Salmonella typhi*, thus bacteria causes infections that are difficult to combat due to multi drug resistant (Salie *et al.*, 1996, Afolayan and Aleiro, 2006). Also the results obtained indicated the existence of the presence of quaternary ammonium cation in various synthesized polyaniline encapsulated dyes and showed a good correlation between the reported uses of these dyes against infectious diseases. Thus, as low as MIC value of 12.5mg/l and MBC/MFC value 12.5mg/ml of the synthesized polyaniline encapsulated Dye-A had activities against *Staphylococcus aureus* and polyaniline encapsulated Dye B has MIC and MBC activity against *Salmonella typhi* at the same concentration as shown in Table 4.9 and 4-10 respectively. Hence, it is clear from the above mentioned results that the possible causes for better and enhance antimicrobial activities of polyaniline encapsulated dyes when compared to acid doped polyaniline are the delocalization of electron density in side chain groups and the presence of quaternary ammonium ion.

## V. CONCLUSION

Different polyaniline encapsulated azo dyes were synthesized by the substitution of the dyes moieties on the polyaniline backbones. Their physicochemical properties were assessed

and spectroscopic analysis such as visible spectroscopy, Fourier Transform Infra red Spectroscopy, and GC-MS were used to elucidate the structures. An agar well diffusion method was used for the assessment of their antimicrobial properties on different microorganism and the result showed zone of inhibition ranging from 10-34mm, which implies that this compound can be effective against infectious diseases which these microorganism can cause.

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