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Chemistry

Banana Peels Powder

Hydrophobic Carbazole Ring

Highlights

Water Quality of River

Phenyl Sulfonyl Pyrazoline

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 1 VERSION 1.0

GLOBAL JOURNAL OF SCIENCE FRONTIER RESEARCH: B

CHEMISTRY

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Transition of Morphology between Nanosheet and Nanosphere of Ternary Comb Copolymers with Hydrophobic Carbazole Ring Induced by Changes in Surface Pressure

By Yohei Kaneko, Takahiro Kikkawa & Atsuhiro Fujimori
Saitama University, Japan

Abstract- Transition behavior from monolayer on the water surface to single particle layer of ternary comb copolymer containing carbazole ring have investigated by surface pressure-area isotherm, atomic force microscopy, X-ray diffraction. This polymer monolayer is able to form the "polymer nanosheet" with amorphous layers and strong interaction between main-chains fabricated by a Langmuir-Blodgett (LB) technique. Further, single particle layer is able to form the multi-particle layered organization of "polymer nanosphere" by LB method. This ternary comb copolymer synthesized by radical copolymerization with hydrogenated and fluorinated long-chain vinyl compounds. Poly(N-vinylcarbazole) homopolymer formed single particle layer on the water surface after spreading, immediately. Spontaneous "particulation" behavior is accelerated by incorporation of carbazole units in ternary copolymer films. Monolayer of these copolymers on the water surface formed below 10 mN m–1, and this monolayer transited to single particle layer above 15 mN m–1.

Keywords: organized molecular films, ternary comb copolymer, fluorocarbon, hydrocarbon, n-vinyl carbazole.

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Abstract- Transition behavior from monolayer on the water surface to single particle layer of ternary comb copolymer containing carbazole ring have investigated by surface pressure-area isotherm, atomic force microscopy, X-ray diffraction. This polymer monolayer is able to form the “polymer nanosheet” with amorphous layers and strong interaction between main-chains fabricated by a Langmuir-Blodgett (LB) technique. Further, single particle layer is able to form the multi-particle layered organization of “polymer nanosphere” by LB method. This ternary comb copolymer synthesized by radical copolymerization with hydrogenated and fluorinated long-chain vinyl compounds. Poly(N-vinylcarbazole) homopolymer formed single particle layer on the water surface after spreading, immediately. Spontaneous “particulation” behavior is accelerated by incorporation of carbazole units in ternary copolymer films. Monolayer of these copolymers on the water surface formed below 10 mN m–1, and this monolayer transited to single particle layer above 15 mN m–1.

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I. Introduction

Organized molecular films1 fabricated using functional polymers with precisely controlled structures can be developed as candidates for biomimetic models2 and molecular electronic devices,3 which have attracted considerable interest in fundamental science, as well as for their potential applications. Generally, it is well known that crystalline polymers construct hierarchical structures from lamellae on the nanometer scale to spherulite on the mesoscopic scale. 4 Crystals in these crystalline polymers are generally formed by the folding of the main-chain, although there main-chain, although there are two types of structural characteristics for side-chain crystalline ternary comb polymers. It is known that ternary comb polymers, which have long n-alkyl side-chains with crystallization ability attached to an amorphous main-chain, are packed into a layer structure along the c-axis and sub-cell of the side-chains in the ab-plane.5 This “sub-cell” structure6 of the side-chain is formed as a two-dimensional lattice, and the “layer structure” along the c-axis often reflects the long spacing between the main-chains in the accumulated double layer structures. With regard to the industrial application of these type of ternary comb polymers, poly(octadecyl)-based materials that use co-crystallization with another hydrocarbon to incorporate the material into the same crystalline lattice have found numerous applications such as pour-point depressants for lubricating oils or fuels, rheological modifiers, additives in petroleum products, and smart gels.7

On the other hand, many researchers have attempted to utilize poly-N-vinylcarbazole (Poly-NVCz) as organic light emitting diodes, organic transistors, and host compounds for organic electroluminescence8 in recent years, because it is well known that Poly-NVCz exhibits properties identical to those of organic semiconductors.9 Further, it is expected that Poly-NVCz can be used to realize a hologram memory material because of the high refractive index (greater than 1.68) of its monomer.10

As mentioned above, it is essential to control the molecular orientation and the arrangement at the monomolecular level in order to drive the functionality of these organic molecular devices in an efficient manner. However, in many cases, it is difficult to control the molecular arrangement and the homopolymer packing because Poly-NVCz itself easily forms an amorphous polymer.11

In previous study, we have investigated the control of solid-state structures and the formation of organized films for the newly synthesized comb copolymers containing hydrogenated and fluorinated side-chains.12 These comb polymers form a side-chain crystal in the bulk state by van der Waals interaction between the side-chains and the extremely stable condensed monolayers at the air/water interface. If bulky and flat functional groups are incorporated in the main-chains of side-chain crystalline polymers, it will be possible to control the arrangement of bulky groups at

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Authors α σ ρ : Graduate School of Science and Engineering, Faculty of Engineering, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama, Japan. e-mail: fujimori@fms.saitama-u.ac.jp

the molecular level, regardless of the low crystallinity of the polymers.

Recently, the fine structures in the solid-state of the synthesized ternary comb polymers containing N-vinylcarbazole (NVCz) in the main-chains obtained by co-polymerization with both hydrogenated and fluorinated long-chain compounds were investigated by wide-angle X-ray diffraction (XRD). Further, the molecular arrangements of the organized molecular films in ternary comb copolymers containing NVCz units were estimated by in-plane XRD.13

In this study, transition behavior from monolayer on the water surface to single particle layer of ternary comb copolymer containing carbazole ring have investigated by surface pressure-area (*π*-A) isotherm, atomic force microscopy (AFM), out-of-plane XRD.

II. EXPERIMENTAL

a) Materials

The ternary comb copolymers used in this study were obtained by the copolymerization of NVCz with octadecyl acrylate (OA) and 2-(perfluorodecyl)ethyl acrylate (FF10EA) at various monomer ratios. Copolymerizations were carried out in an acetone solution at 50 °C for 48 h using azobisisobutyronitrile (AIBN) as an initiator. These monomers and the initiator were purchased from Tokyo Kasei Co. Ltd. and Daikin Fine Chemicals Co. Ltd., and were used without further purification. The precipitated polymers were washed with acetone until they were free of monomers. The ternary comb copolymer compositions were determined by 1H NMR (Nihon Densi Co. Ltd. EX270 NMR) spectroscopy. The tacticity of fluorinated homopolymer obtained by 1H NMR analysis according to the reference was found to be almost syndiotactic (Diad: 58 %). In this study, we synthesized ternary copolymers with two series of molecular weights estimated to be approximately Mw = 4.43 × 104 and 4.93 × 103 (Mw/Mn ≈ 2.14 and 1.17 respectively) on the basis of GPC (JASCO-860-CO) measurements.

According to the theory of the Q-e scheme proposed by T. Alfrey and C.C. Price,14 these ternary comb polymers form alternating copolymers in the view of relation of NVCz and long-chain acrylates. In this case, the e values of NVCz, OA and FF10EA are –1.40, +1.12 and +0.66 respectively. Thus, it appears that the NVCz : long-chain acrylates copolymers almost form ideal alternating copolymers, especially for the 2:1:1 monomer ratio.

b) Formation of copolymer monolayers on water surface and estimation of molecular arrangement in the films

The monolayers of the ternary comb copolymer with NVCz were spread from the chloroform or chloroform/trifluoroacetic acid = 90/10 (v/v) mixed solutions (about 10–4 M) onto the distilled water (about 18 MΩ•cm). The *π*–A isotherms were measured by a USI-3-22 film balance (USI Co. Ltd.) at 15 °C. These monolayers were transferred onto glass (XRD sample), quartz (UV-vis and fluorescence spectroscopy), and mica (AFM) substrates by the Langmuir-Blodgett (LB) method to obtain alternating Y-type film at 15 °C.

The long spacing between the layer structures along the c-axis estimated by an out-of-plane X-ray diffractometer (Rigaku, Rint-Ultima III, CuKα radiation, 40 kV, 40 mA) equipped with a graphite monochromator.

III. RESULTS AND DISCUSSION

Figure 1 shows *π*-A isotherm of “single particle layer” on the water surface and corresponding AFM image of single particle layer on solid substrate of Poly-NVCz. This homopolymer formed single particle layer on the water surface after spreading, immediately. This

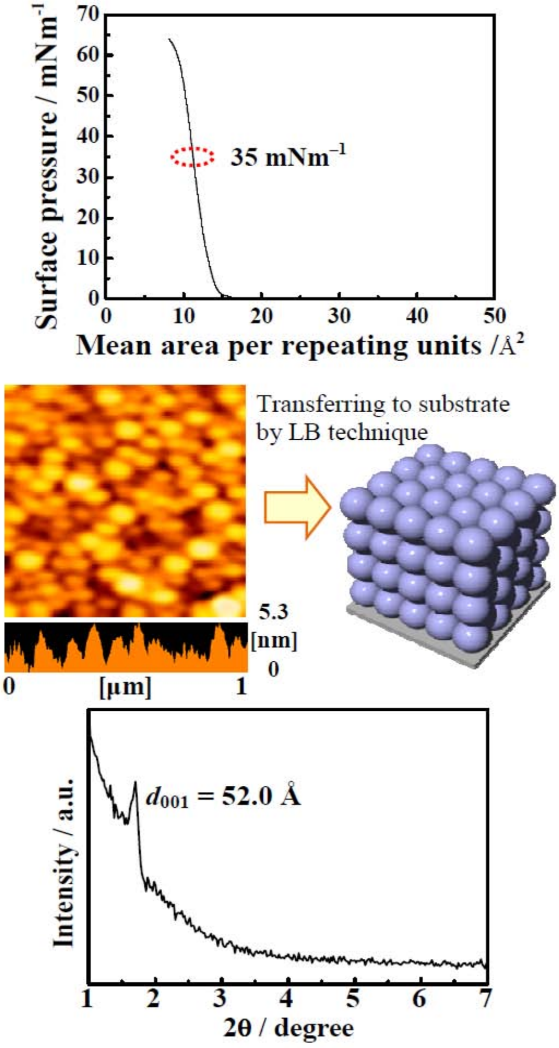

Fig.1 : *π*-A isotherm of single particle layer on the water surface and AFM images of single particle layer on solid of PNVCz homopolymer, and out-of-plane XRD of multi-particle layers of Poly-NVCz.

figure also shows out-of-plane XRD of multi-particle layers of Poly-NVCz. From the result of this measurement, it is found that inter-particle spacing along the c-axis correspond to 52 Å.

Figure 2 shows π-A isotherm of "monolayer" on the water surface and corresponding AFM image of monolayer on solid substrate of NVCz:FF10EA:OA = 2:1:1 copolymer. This figure also shows out-of-plane XRD of multilayers of this copolymer. This profile clearly indicates higher order reflection of (00l) plane. Therefore, it is found that this LB multilayer formed highly ordered layer structure.

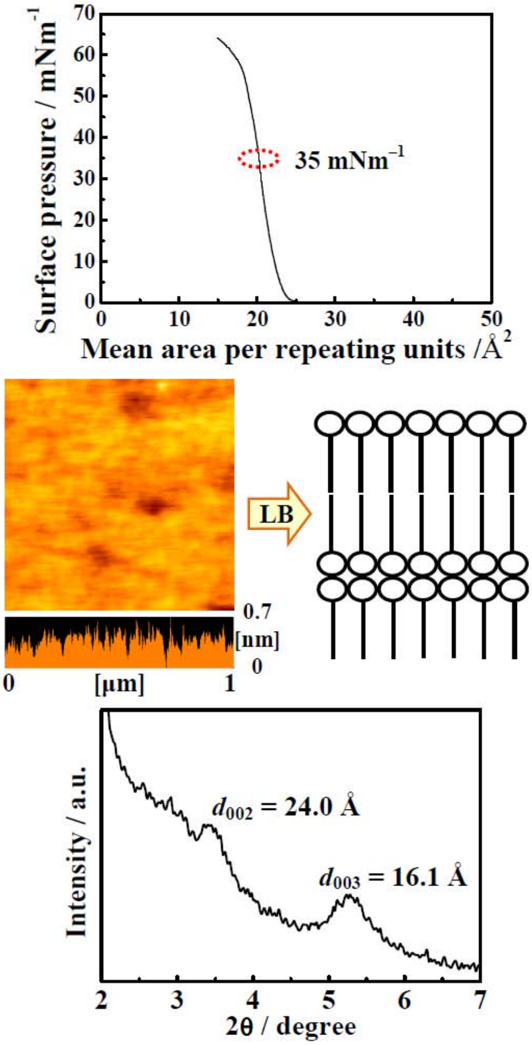

Fig. 2 : π-A isotherm of monolayer on the water surface and AFM images of monolayer on solid of VCz:FF$_{10}$ EA:OA = 2:1:1 copolymer, and out-of-plane XRD of multilayers of this copolymer

Figure 3 shows π -A isotherm of monolayer on the water surface of NVCz:FF10EA:OA = 3:1:1, 4:1:1, and 6:1:1 copolymers. All isotherms indicate an existence of plateau region based on the two-dimensional phase transition.

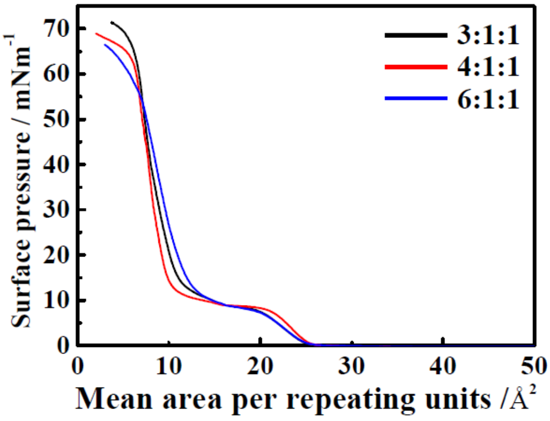

Fig. 3 : π-A isotherm of monolayer on the water surface of NVCz: FF$_{10}$EA:OA = 3:1:1, 4:1:1, and 6:1:1 copolymers

Figure 4 shows AFM images of Z-type monolayer and single particle layer on the water surface NVCz:FF10EA:OA = 3:1:1, 4:1:1, and 6:1:1 copolymers transferred at before and after transition. This figure indicates monolayer of these copolymers on the water surface formed below 10 mN m–1, and this monolayer transited to single particle layer above 15 mN m–1. This figure also means that spontaneous "particulation" behavior is accelerated by incorporation of carbazole units in ternary copolymer films.

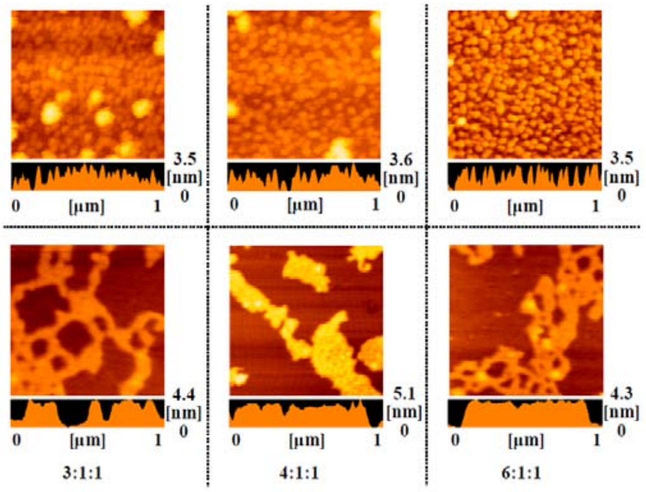

Fig. 4 : AFM images of Z-type monolayers and single particle layers on the water surface of NVCz: FF$_{10}$EA: OA = 3:1:1, 4:1:1, and 6:1:1 copolymers transferred at before (5 mN m^{-1}, lower column) and after (35 mN m^{-1}, upper column) transition.

Figure 5 shows out-of-plane XRD profiles of LB multilayers of NVCz:FF10EA:OA = 4:1:1 copolymer transferred at 35 and 5 mN m–1. It is found that both organizations commonly formed ordered layer structure along the c-axis.

Figure 6 shows schematic illustration of polymer nanosheet and polymer nanosphere layered organization, and their formation mechanism. It seems that polymer particles are formed by piled up molecular chain, like a bilayer structure in high surface pressure region.

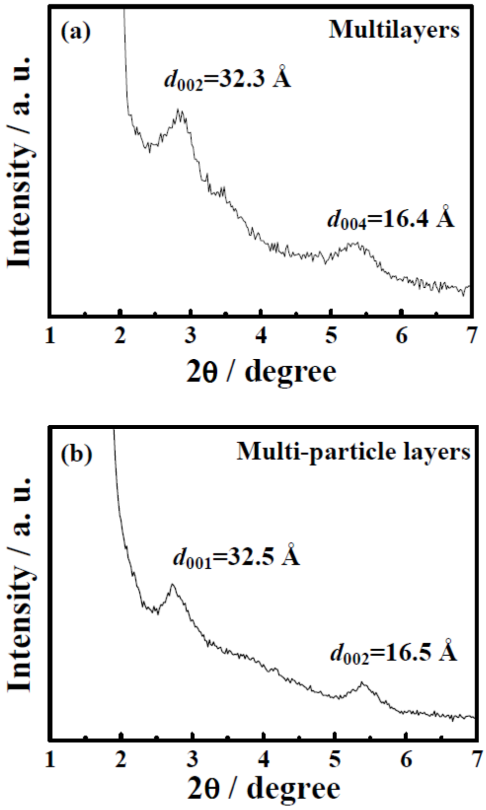

Fig. 5 : Out-of-plane XRD profiles of LB multilayers of NVCz:FF$_{10}$EA:OA = 4:1:1 copolymer transferred at (a) 5 mN m^{-1} and (b) 35 mN m^{-1}.

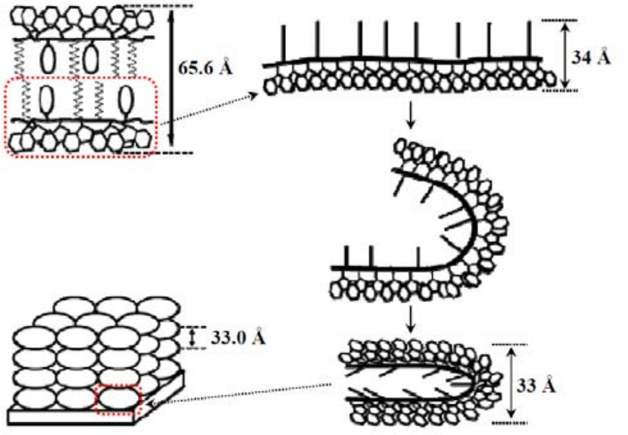

Fig. 6 : Schematic illustration of polymer nanosheet and polymer nanosphere layered organization, and their formation mechanism

Figure 7 shows UV-vis and fluorescence spectra of Poly-NVCz and NVCz:FF10EA:OA = 4:1:1 copolymer in solution. Further, fig. 8 shows ones of

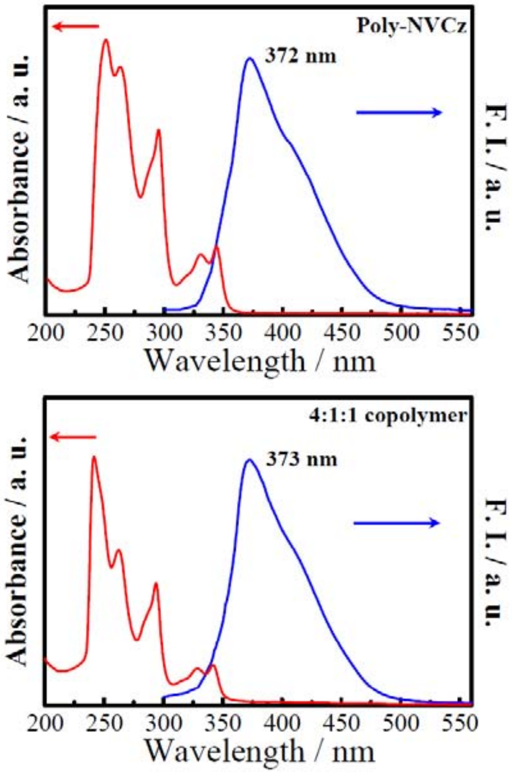

Fig. 7: UV-vis and fluorescence spectra of Poly-NVCz and NVCz:FF$_{10}$EA:OA = 4:1:1 copolymer in solution

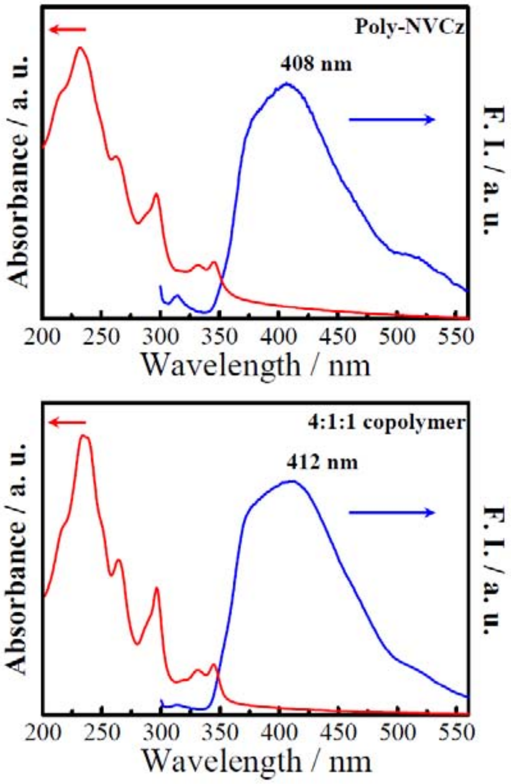

Fig. 8 : UV-vis and fluorescence spectra of multi-particle layered organization of Poly-NVCz and NVCz: FF$_{10}$EA:OA = 4:1:1 copolymer.

multi-particle layered organization of same materials.

The "polymer nanosphere" of these ternary comb copolymers showed the absorption band around 250 nm and emitted fluorescence at 400 nm. The emitted band in fluorescence spectrum showed red-shift to the one of their solution. It is speculated that this result means formation of aggregated structure by π-π stacking of NVCz rings.[15-17]

IV. CONCLUSION

Formation behavior of single particle layer of ternary comb copolymer containing NVCz have investigated. This copolymer monolayer is able to form the "polymer nanosheet" fabricated by a LB technique. Further, single particle layer is able to form the multi-particle layered organization of "polymer nanosphere". This ternary comb copolymer synthesized by radical copolymerization. Spontaneous particle formation is attained by incorporation of hydrophobic carbazole. Monolayer of these copolymers on the water surface transited to single particle layer above 15 mN m–1.

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Analysis by GC-MS of an Aza-Michael Reaction Catalyzed by CALB on an Orbital Shaker and under Microwave Irradiation

By Sandra S. Ribeiro, Marciana P. Uliana, Timothy J. Brocksom & André L. M. Porto

Universidade De São Paulo, Brazil

Abstract- In this study, aza-Michael reactions between 1-phenylmethanamine and α,β-unsaturated cyclohexenones were investigated, using lipase from *Candida antarctica*. The reactions were performed in various organic solvents (CH_2Cl_2, hexane, MeOH, toluene, THF) under mild conditions, with orbital shaking and microwave irradiation. The reactions showed good results in the presence of CALB, yielding the Michael adducts and imines. The reaction products were analyzed by GC-MS and in some cases it was found that the reverse aza-Michael reaction had occurred.

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Analysis by GC-MS of an Aza-Michael Reaction Catalyzed by CALB on an Orbital Shaker and under Microwave Irradiation

Sandra S. Ribeiro [α], Marciana P. Uliana [σ], Timothy J. Brocksom [ρ] & André L. M. Porto [ω]

Abstract- In this study, aza-Michael reactions between 1-phenylmethanamine and ***α,β***-unsaturated cyclohexenones were investigated, using lipase from *Candida antarctica*. The reactions were performed in various organic solvents (CH_2Cl_2, hexane, MeOH, toluene, THF) under mild conditions, with orbital shaking and microwave irradiation. The reactions showed good results in the presence of CALB, yielding the Michael adducts and imines. The reaction products were analyzed by GC-MS and in some cases it was found that the reverse aza-Michael reaction had occurred.

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I. Introduction

Chiral amines are an important class of organic compounds, on account of their utility in the preparation of pharmaceutical and industrial products of considerable interest [1,2]. These compounds are intermediates in the synthesis of a large number of organic compounds and are widely used to prepare derivatives of natural products, antibiotics, chiral auxiliaries and lactams. Owing to their vast range of applications, the modification and improvement of techniques for synthesizing ***β***-amino carbonyl compounds has been a research objective in recent years [2, 3].

The conjugate addition of the nucleophilic nitrogen in compounds such as amines to ***α,β***-unsaturated carbonyl or nitrile compounds, namely the aza-Michael reaction, constitutes a key reaction for the construction of C-N bonds in the preparation of ***β***-amino esters and nitriles [4]. This reaction is highly versatile as it can occur between various *N*-nucleophiles (aliphatic and aromatic amines, amides, carbamates and azides) and Michael acceptors (enones, acrylates, unsaturated nitriles, amides, sulfones, phosphonates, trifluoromethylalkenes and nitroalkenes) [5].

Recently, an efficient enzymatic protocol was reported for the synthesis of ***β***-amino esters via aza-Michael addition of primary and secondary amines to acrylates; CALB (*Candida antarctica* lipase B) was the biocatalyst and the corresponding ***β***-amino esters were produced in good yields [6]. Green chemistry, in which harmful organic solvents and extreme conditions are avoided, is growing in importance; furthermore, the catalyst can be recycled. An efficient and simple protocol for aza-Michael addition of aliphatic and aromatic amines to electron-deficient alkenes has been established, using TMG-based ionic liquids as catalyst under solvent-free conditions [7]. Solvent-free aza-Michael reactions between a variety of amines and ***α,β***-unsaturated carbonyl compounds under microwave irradiation, catalyzed by perchloric acid impregnated on silica gel ($HClO_4{=}SiO_2$), produced the corresponding adducts [8]. Another method has been developed for the aza-Michael addition of acrylonitrile to 2-aryloxymethylbenzimidazole derivatives, in the presence of anhydrous potassium carbonate under microwave irradiation, to synthesize a novel series of 1-cyanoethyl-2-aryloxymethylbenzimidazole derivatives [9]. The present paper summarizes the results of our investigation into the conjugate addition of nitrogen-containing nucleophiles to electron-deficient ketones, in which environmentally-friendly enzymatic catalysis was carried out in an orbital shaker and under microwave irradiation, yielding aza-Michael imine adducts and reverse aza-Michael compounds.

II. Materials and Methods

a) *General procedure*

The Michael addition reactions were carried out on a Tecnal TE-421 orbital shaker. The performance of the enzymatic reactions was measured by analyzing the products in a Shimadzu GC 2010 gas chromatograph equipped with an AOC 20i auto injector, a flame ionization detector (FID) and a J&W Scientific DB-5 column (30 m x 0.25 mm x 0.25 µm). The conditions employed in the gas chromatograph were as follows: carrier gas: nitrogen (60 kPa); injector temperature: 250 °C; injector split ratio: 1:20; detector temperature: 300 °C; initial oven temperature: 80 °C for 2 min; final oven temperature: 250 °C for 3 min; heating rate: 10 °C min^{-1}, and total time of analysis: 22.0 min (Supplementary Information – SI). Gas chromatography–mass spectrometry (GC-MS): a Shimadzu GC2010 Plus

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Authors ω α: Instituto de Química de São Carlos, Universidade de São Paulo, Av. João Dagnone, 1100, Ed. Química Ambiental, J. Santa Angelina, 13563-120, São Carlos, SP, Brazil.
e-mail: almporto@iqsc.usp.br
Authors σ ρ: Universidade Federal de São Carlos, Rod. Washington Luís, km 235, 13565-905, São Carlos, SP, Brazil.

gas-chromatography system coupled to a mass-selective detector (Shimadzu MS2010plus) was used in the electron ionisation (EI) mode. The GC-MS oven was fitted with a DB-5 fused silica column (J&W Scientific 30 m x 0.25 mm x 0.25 μm). The oven temperature was programmed from 50 °C to 270 °C at a heating rate of 10 °C/min, and a total time of analysis: 32.0 min. The injector and detector temperatures were maintained at 300 °C; injector split ratio was 1:20 and helium was used as the carrier gas at a pressure of 60 kPa. The compounds were identified from the fragmentation ions (70 eV) detected in the GC-MS spectra (Supplementary Information – SI). The structures were confirmed after comparison with Mass Spectral Database (NIST 5.0) and by co-injection of authentic standards. Reactions were purified by column chromatography on silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and EtOAc (8:2). Column effluents were monitored by TLC, using aluminium-backed pre-coated silica gel.

b) *Enzyme and chemicals*

Novozym 435® (component B of the lipase from *Candida antarctica* immobilized on macroporous poly-acrylate resin, with 10,000 propyl laurate units per gram), was a gift from Novo Nordisk (Curitiba-Paraná, Brazil). Solvents (EtOAc, CH_2Cl_2, *n*-hexane, MeOH, toluene, THF) were commercially available and of analytical grade. Cyclohex-2-en-1-one **1**, 1-phenylmethanamine **2** and 3-methylcyclohex-2-en-1-one **5** were purchased from Sigma-Aldrich and 2,5-dimethyl-*para*-benzoquinone **8** was synthesized from 2,5-dimethyl-phenol [10, 11].

c) *Lipase-catalyzed aza-Michael reactions under conventional conditions (orbital shaker)*

CALB lipase (80 mg), the unsaturated ketone [2-cyclohexen-1-one **1** (40 μL, 0.41 mmol), 3-methylcyclohex-2-en-1-one **5** (40 μL, 0.35 mmol) or 2,5-dimethyl-*para*-benzoquinone **8** (40 μL, 0.33 mmol)] and the amine [1-phenylmethanamine **2** (40 μL, 0.36 mmol)] were separately added to 10 mL of the organic solvent (*n*-hexane, toluene, dichloromethane, tetrahydrofuran or methyl alcohol) in 50 mL Erlenmeyer flasks. The flasks were sealed with a rubber stopper and the reaction mixture was shaken in an orbital shaker at 33 °C and 133 rpm. The reaction progress was monitored by collecting samples (0.5 mL) at intervals, between 2 and 48 hours from the start, and analyzed by gas chromatography GC-FID and GC-MS. The enzymatic aza-Michael reactions were carried out in triplicate.

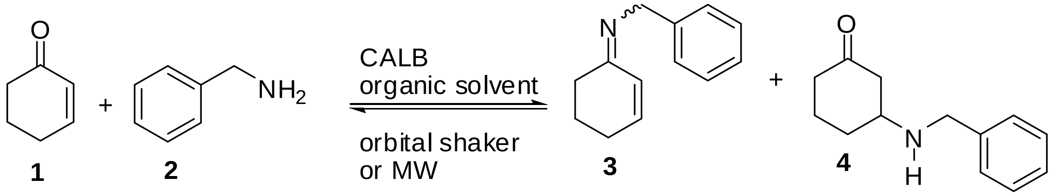
d) *Lipase-catalyzed aza-Michael with microwave irradiation*

The microwave irradiation (MW) experiments were performed with a Discover System from CEM Corporation. Organic solvents (10 mL, EtOAc, CH_2Cl_2, *n*-hexane, MeOH, toluene, THF), primary amine **2** (40 μL, 0.36 mmol), Michael acceptors **1** (40 μL, 0.41 mmol), **5** (40 μL, 0.35 mmol) and **8** (40 μL, 0.33 mmol) and CALB (80 mg) were separately added to a 50 mL flat bottom flask. The whole reaction mixture was placed in the microwave oven at a frequency 2.45 GHz and irradiated at 33, 40, 50 and 60 °C, power output of about 70 W. The reaction progress was monitored by collecting samples (0.1 mL) and analyzed by gas chromatography with a J & W Scientific DB-5 (30 m x 0.25 mm x 0.25 μm). After 3 hours of reaction, the flask was removed and the immobilized lipase was filtered off. The organic solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with 8:2 hexane and ethyl acetate as eluent. The reactions were carried out in triplicate and the results are presented and discussed next.

III. Results and Discussion

The lipase from *Candida antarctica* (CALB) is often used to promote asymmetric reactions via transesterification of racemic alcohols and amines by chemo-enzymatic resolution [12-15]. The enzymes used to catalyze unusual reactions, commonly named promiscuous enzymes, such as the Michael addition reaction catalyzed by lipases, are being investigated with interest [6, 16-17].

The aim of this study was to carry out Michael additions with the immobilized lipase from *C. antarctica*, on account of its proven catalytic efficiency and thermal stability, with high activity at 70-90 °C [18]. Our study began with cyclohex-2-enone **1**, due to the absence of steric hindrance in the 1,4-addition, and the achiral primary amine, 1-phenylmethanamine **2**. Under these conditions, the reaction can favor two products, the imine *N*-(cyclohex-2-en-1-ylidene)-1-phenylmethanaminne **3** and the chiral aza-Michael adduct 3-(benzylamino) cyclohexanone **4** (Scheme 1).



Scheme 1 : Aza-Michael reactions using CALB on an orbital shaker and microwave irradiation

Initially, this reaction was tested in five organic solvents (*n*-hexane, dichloromethane, methanol, tetrahydrofuran and toluene). It was also performed in the absence of CALB, since primary amine **2** is a strong nucleophile and *α,β*-unsaturated ketone **1** is a good Michael acceptor, which could favor the spontaneous formation of products **3** and **4**.

Under orbital shaking, in the presence of CALB, this reaction gave the aza-Michael adduct **4** at low conversion rates, varying with the solvent used (c 9-37%), and imine **3** at conversions of 5-23%, in 48 h. The non-enzymatic reaction gave similar results for adduct **4** (c 3-29%) and imine **3** (4-34%) in 48 h. The reaction in the presence of CALB and *n*-hexane produced adduct **4** at 25% conversion and imine **3** at 23% in 48 h, while under the same conditions without CALB lower yields of both adduct **4** (c 10%) and imine **3** (c 9%) in 48 h were observed. In this case, there is a visible influence of lipase in the formation of the aza-Michael adduct **4** (SI-1). In methanol, there was good conversion to the adduct **4** (c 38%) and imine **3** (c 14%) in 48 h. In the absence of CALB, a decrease occurred in the conversion of adduct **4** (c 29%) and an increase in conversion to imine **3** (c 35%) in 48 h (SI-2).

It is reported in the literature that CALB exhibits good catalytic activity in hydrophobic solvents [12, 14, 19]. In our studies the reactions in *n*-hexane and methanol were not efficient. Factors such as the polarity of the substrates (ketone **1** and amine **2**) may have influenced these results, and Michael addition reactions may be occurring spontaneously and reversibly, yielding unstable products, without being promoted by CALB [17].

In dichloromethane, with CALB, the reaction showed low conversion to adduct **4** (c 10%) and imine **3** (c 6%) after 48 h. In the absence of lipase we observed similar values to those obtained enzymatically for adduct **4** (c 7%) and imine **3** (c 4%), at 48 h (SI-3).

In toluene, adduct **4** showed low conversion (c <14%), as did imine **3** (c 11%), after 48 h. In the non-enzymatic reaction, adduct **4** was obtained at a conversion of less than 3% and the imine at 2% in 48 h (SI-4). The choice of solvent depends strongly on the solubility of the catalyst and of the donor and acceptor in the Michael addition, as well as the ability of the solvent to prevent the occurrence of side reactions. For example, if the reactant or products are susceptible to alcoholysis (ester hydrolysis), transesterification, autocondensation, reverse Michael reactions, non-hydroxyl solvents are desirable [20].

Aiming to improve the conversion of aza-Michael addition in the presence of CALB, the reactions with ketone **1** and amine **2** were promoted, in methanol, THF, dichloromethane and *n*-hexane, by microwave irradiation (40 °C, 70 W). Under these conditions, the aza-Michael adduct **4** showed 0.3-42% conversion and imine **3** of 2-40% conversion, depending on the solvent used. The reaction in methanol gave the best result, the adduct **4** was produced at 42% conversion and imine **3** at 4%, in 3 h.

In *n*-hexane and dichloromethane, only imine **3** (c 12%) was formed in 3 h. In THF, there were low conversions of adduct **4** (0.3-6%) and imine **3** (1-3%) in 3 h. The reactions in dichloromethane and hexane, without CALB, yielded the adduct **4** and imine **3** in small amounts (c<10%) by microwave irradiation for 3 h.

The regioselectivity of the reactions depends on the conditions in which they are carried out, the type of catalyst, the solvent and the nucleophile [21]. In these studies, the reaction both in the presence and absence of CALB favored the formation of products via 1,2-addition and 1,4-addition with similar conversions.

Dhake et al. (2010) conducted aza-Michael additions between various primary and secondary amines and acrylates, in the presence of lipase, to obtain the corresponding *β*-amino esters. In this study, methyl acrylate and CALB in toluene showed the best results in the synthesis of the Michael adduct. It was observed that the activity of the enzyme increased with the temperature, up to 60 °C. However, at higher temperatures (> 80 °C), the yield of the reaction decreased to 75%, possibly due to inactivation of the enzyme [6].

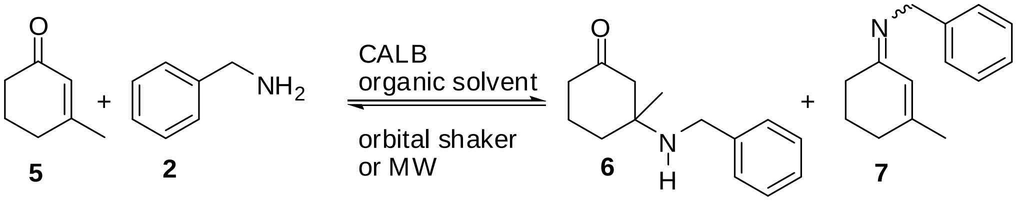
All the reactions in this first part were monitored by GC-FID and GC-MS analysis, which showed the formation of the aza-Michael adduct **4** and imine **3** (Supplementary Information - SI). During the isolation of the adduct **4** by silica gel only the starting reactants, namely enone **1** and the amine **2**, were found. Thus, it was concluded that a reverse Michael reaction occurred during the purification process. The instability of these products was confirmed by the main fragments obtained in the mass spectra, which were compared with data from the Nist 5.0 library.

In the GC-MS chromatogram of the reaction, in *n*-hexane under orbital shaking for 4 days (SI-5), a signal with a retention time of 14.5 minutes was observed, which provided the spectrum of a molecular ion at *m/z* 185 $[M]^+$. The mass spectrum of imine **3** was comparable to the Nist 5.0 mass spectral database. The fragmentation of the molecular ion at *m/z* 185 provided the tropylium ion, at *m/z* 91, as the base peak. On the basis of these fragmentations it was possible to suggest the formation of imine **3** (SI-5 and SI-6).

Another signal in the chromatogram, with retention time 15.9 minutes, showed the molecular ion at *m/z* 203 that corresponds exactly to the aza-Michael adduct **4**. The mass spectrum was similar to that reported for adduct **4** in the Nist 5.0 mass spectral database, and is consistent with the literature [22, 23]. The fragmentation pattern of the molecular ion at *m/z* 203 again provided the tropylium ion (*m/z* 91) as a base peak, which confirmed the formation of adduct **4** (SI-5 and SI-6).

Thus, we conclude from the spectral evidence that it is possible to obtain the compounds **3** and **4** from enone **1** and amine **2**. However, due to the instability of the products, they were not isolated, but were characterized by mass spectrometry.

The next step was to evaluate the regioselectivity of the Michael reaction by using the substituted acceptor, 3-methylcyclohex-2-en-1-one **5** and the achiral donor, 1-phenylmethanamine **2**. The reactions were performed in the presence and absence of CALB in the orbital shaker, with hexane and methanol, and under microwave irradiation, with methanol, dichloromethane and hexane (Scheme 2).



Scheme 2 : Aza-Michael reactions using CALB on an orbital shaker and microwave irradiation

The reactions under orbital shaking were similar, with or without CALB, in methanol: the imine **7** was produced at 27-38% conversion in 24 h, whereas the adduct **6** was not detected under these conditions (SI-7).

In hexane and with CALB, adduct **6** was not observed after 24 h, but only imine **7** at 30% conversion (SI-8). In the absence of lipase, imine **7** was produced at less than 5%. In this case, CALB influnced greatly the product obtained by 1,2-addition. In these reactions, the methyl group at position 3 of enone **5** hinders the Michael 1,4-addition, probably by steric and electronic effects. The larger the substituent group on the ***α,β***-unsaturated ketone, the more unfavorable is the 1,4-addition [20].

Initially, good yields were expected for adduct **6** with lipase in hexane, because the enzyme acts very well in nonpolar solvents. However, ketone **5** and amine **2** have polar groups that may influence the low conversion to imine **7**.

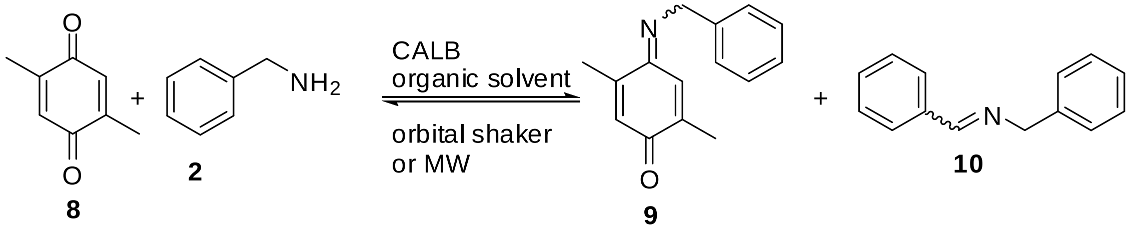
The reactions under microwave irradiation were performed at 33 and 50 °C and 70 W. The enzymatic reaction in methanol and dichloromethane at 33 °C did not form adduct **6** and imine **7** showed conversion < 5%.

Yu et al. studied the effects of temperature on the CALB in the transesterification of (±)-2-octanol by microwave irradiation. In that study the temperature was assessed from 40 to 100 °C, and the activity of the enzyme increased with temperature up to 80 °C, while there was a fall in enzymatic activity at higher temperatures [24]. The elevated temperature in a microwave reactor increases the probability of collision between enzyme and substrate molecules, which can promote the formation of the enzyme-substrate complex and increase the rate of reaction and the final conversion [24].

Given the low conversion of reaction in methanol and dichloromethane, the temperature was raised to 50 °C and 70 W, resulting in a better performance of the reaction in methanol in the formation of the ***α,β***-unsaturated imine **7**, but with low conversion (c = 10%). The results of these experiments were similar in the enzymatic and non-enzymatic reactions (c<11%, 50 °C, 70 W), yielding only the imine **7** at low conversion. There are several factors that may influence the low performance of these reactions. Amine **2** may be interacting with other parts of the lipase, hampering the Michael addition under these conditions. Also these reactions are subject to the reverse aza-Michael pathway; thus, the instability of imine **7** was confirmed by the main fragments obtained in the mass spectra by GC-MS, compared with data from the Nist 5.0 library (SI-8).

Analysis of the chromatogram of the reaction performed in hexane by orbital shaking, showed the presence of a signal with a retention time of 15.9 minutes. Its mass spectrum showed the ion fragment at *m/z* 199, corresponding to imine **7** (SI-8 and SI-9). To confirm the formation of imine **7**, the reaction was performed in the orbital shaker with methanol. After the reaction was complete and the presence of imine **7** confirmed by GC-MS, 10 mL of distilled water was added and the reaction was maintained under shaking for one hour. After extraction, the reaction was analyzed by GC-MS and showed the reacting species **5** and **2**.

Finally, we carried out the reaction with primary amine **2** and 2,5-dimethyl-*para*-benzoquinone **8**. The experiments were conducted in hexane with CALB on an orbital shaker and under microwave irradiation for 96 h. After this time, two products with retention times of 15.0 minutes (c 43%) and 19.0 minutes (c 34%) were detected by GC-MS analysis (Scheme 3) (SI-10).



Scheme 3 : Aza-Michael reactions using CALB on an orbital shaker and under microwave irradiation

The reactions were conducted in hexane and microwave reactor at 60 °C (70 W) in the presence of CALB. After 5h, the reactions yielded two products with retention times of 15.0 minutes (c 21%) and 19.0 minutes (c 18%) with complete consumption of amine **2** by GC-MS analysis (SI-11).

Analysis of the mass spectrum of the compound with retention time 15.0 minutes showed the presence of a molecular ion with the base peak at *m/z* 225. There was an intense peak at *m/z* 148 (75%) corresponding to the loss of a fragment at *m/z* 77. These fragments are consistent with the structure of the compound 4-(benzylimino)-2,5-dimethylcyclohexa-2,5-dienone **9** (SI-11 and SI-12).

The compound with retention time 19.0 minutes provided a mass spectrum for a molecular ion at *m/z* 195 and base peak at *m/z* 91. Analysis of the mass spectra with reference to the Nist 5.0 library and in comparison with the literature [25] suggested the formation of *N*-benzylidene-1-phenylmethanamine **10** (SI-11 and SI-12).

The formation of **10** could occur by nucleophilic attack of amine **2** on imine **9**. The non-enzymatic reaction does not occur under microwave irradiation at 3 h. However, the reaction under microwave irradiation promoted the formation **10** in face of the reactivity of the species involved (SI-11 and SI-12).

Finally, to confirm the production of imines **9** and **10**, the reaction mixture was subjected to hydrolysis at 24 hours, then extracted with ethyl acetate and analyzed by GC-MS. After hydrolysis, products **9** and **10** were all converted to the starting reagents **2** and **8**, confirming the synthesis of unstable imines in aqueous medium (SI-13 and SI-14).

In the chromatogram of the hydrolysis reaction, no amine **2** was observed, but the corresponding *N*-benzylacetamide **11** with retention time 11.3 minutes was formed by acetylation of amine **2** during extraction with ethyl acetate. The compound was confirmed by synthesis of the authentic standard (SI-15).

IV. CONCLUSION

In this paper we present studies on the regioselectivity of aza-Michael addition reactions between amine **2**, and enones **1**, **5** and **8** under various experimental conditions, such as types of organic solvent, presence and absence of lipase from *Candida antarctica*, orbital shaking and microwave irradiation. All the reactions yielded products via the formation of 1,2- and 1,4-additions. Owing to the poor stability of the compounds formed, GC-MS analyzes were carried out and showed the formation of aza-Michael adducts and several imines. Reverse aza-Michael reactions and hydrolysis of imines allowed us to verify the formation of all the products. In some cases, it was also possible to observe the influence of lipase and microwave irradiation on the performance of the reactions. The compounds were confirmed by detailed analysis of their mass spectra and the corresponding ions formed.

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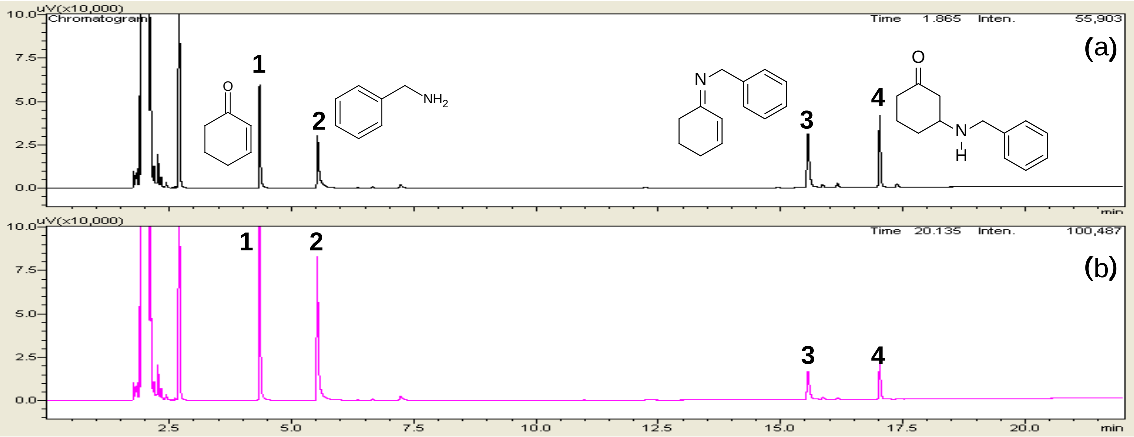
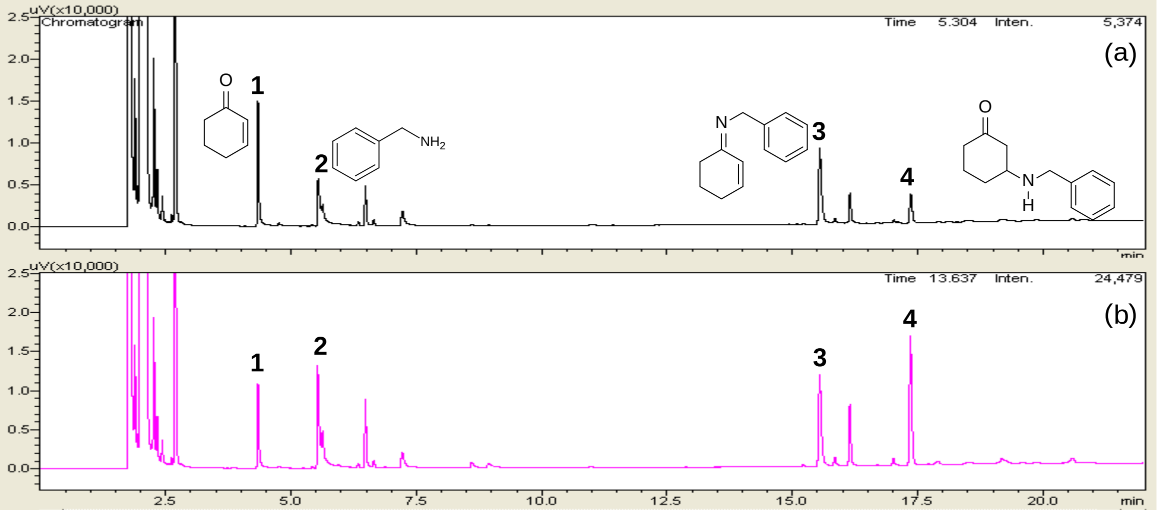
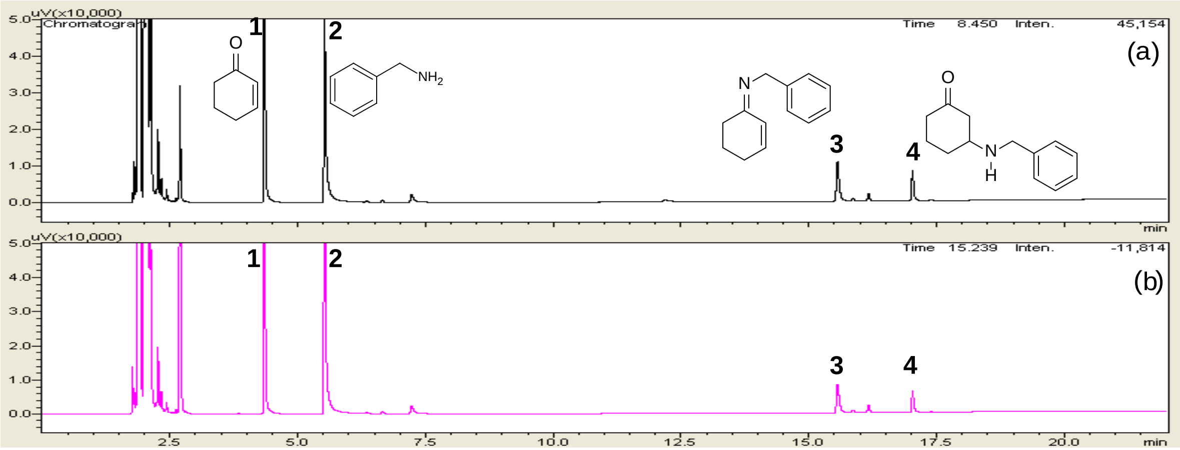
Supplementary Information



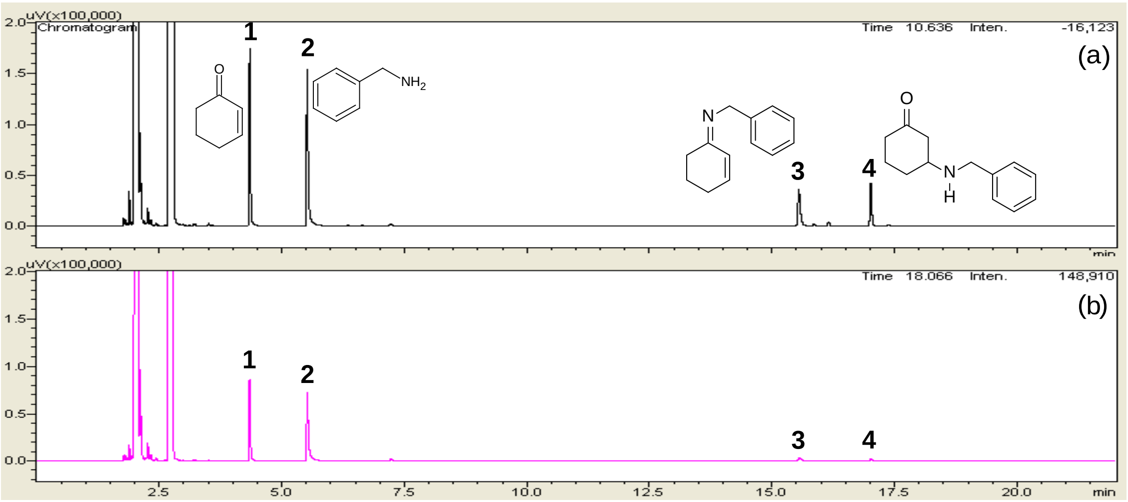
SI-1 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in hexane under orbital shaking (48 h, 33 °C, 133 rpm). (a) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



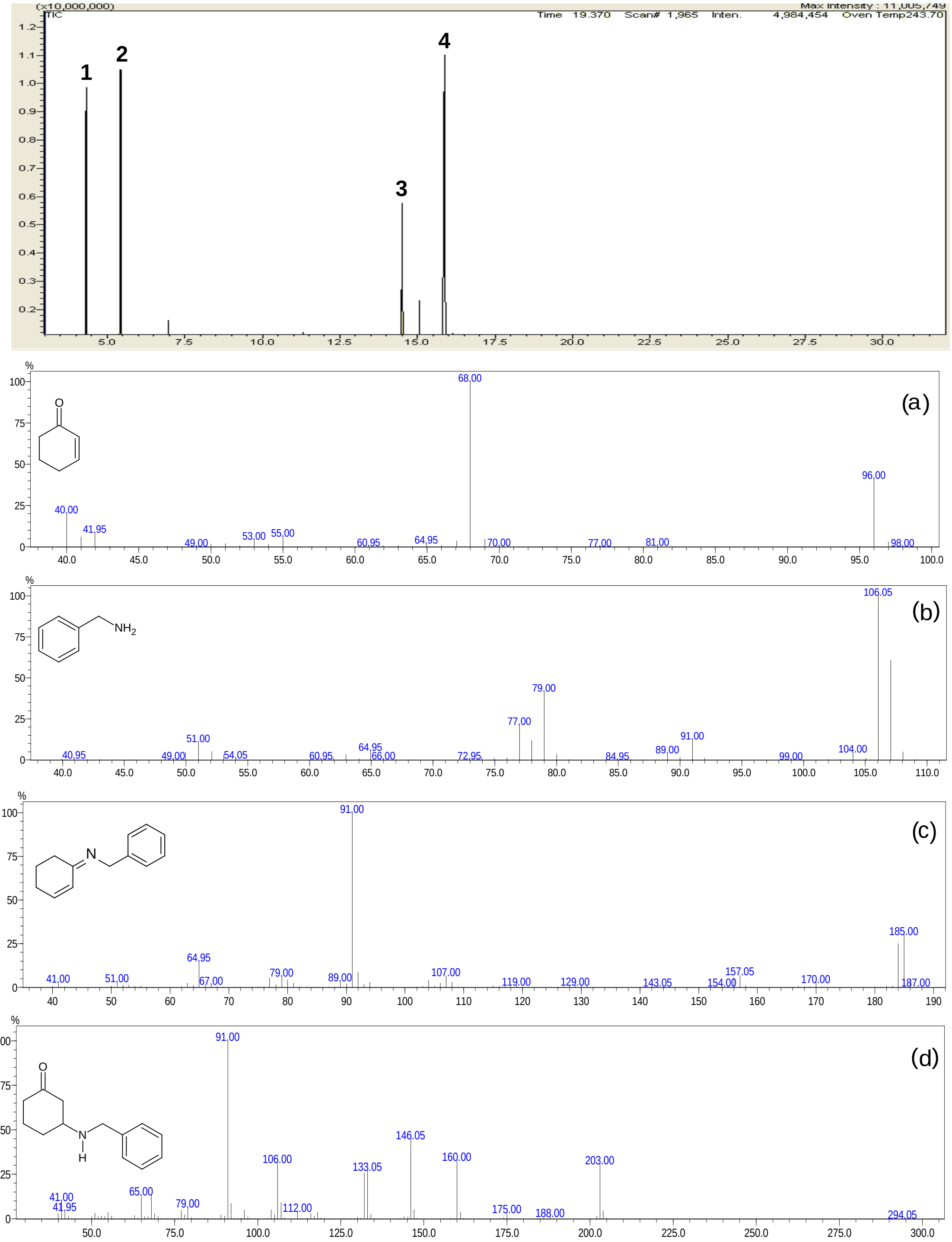
SI- 2 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in methanol under orbital shaking (48 h, 33 °C, 133 rpm). (b) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



SI- 3 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in dichloromethane under orbital shaking (48 h, 33 °C, 133 rpm). (a) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



SI- 4 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in toluene under orbital shaking (48 h, 33 °C, 133 rpm). (b) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



SI- 5 : Chromatogram and mass spectrum obtained by GC-MS (EI, 70 eV) for the reaction of ketone **1** with amine **2** in hexane under orbital shaking for 4 days (33 °C, 133 rpm). (a) Ketone **1**. (b) Amine **2**. (c) Mass spectrum obtained from a signal with a retention time of 14.5 min. (d) Mass spectrum obtained from a signal with a retention time of 15.9 min. Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min

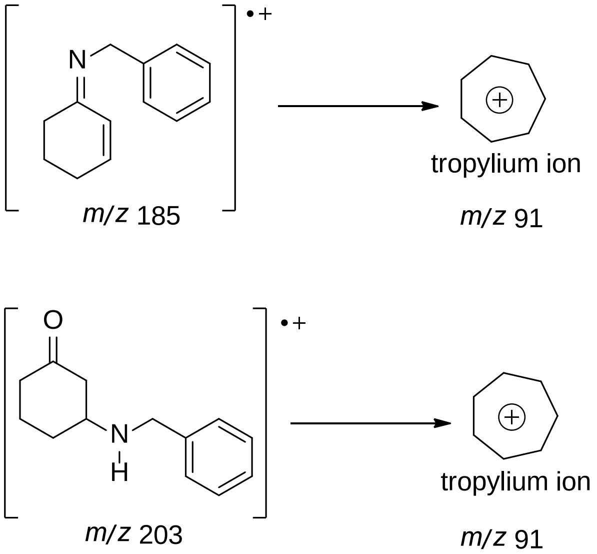
GC-MS (EI, 70 eV):

2-cyclohexen-1-one **1**, C_6H_8O, *m/z* (%): 68 (100), 96 (M^+, 41), 40 (21), 55 (6), 81 (0.5).

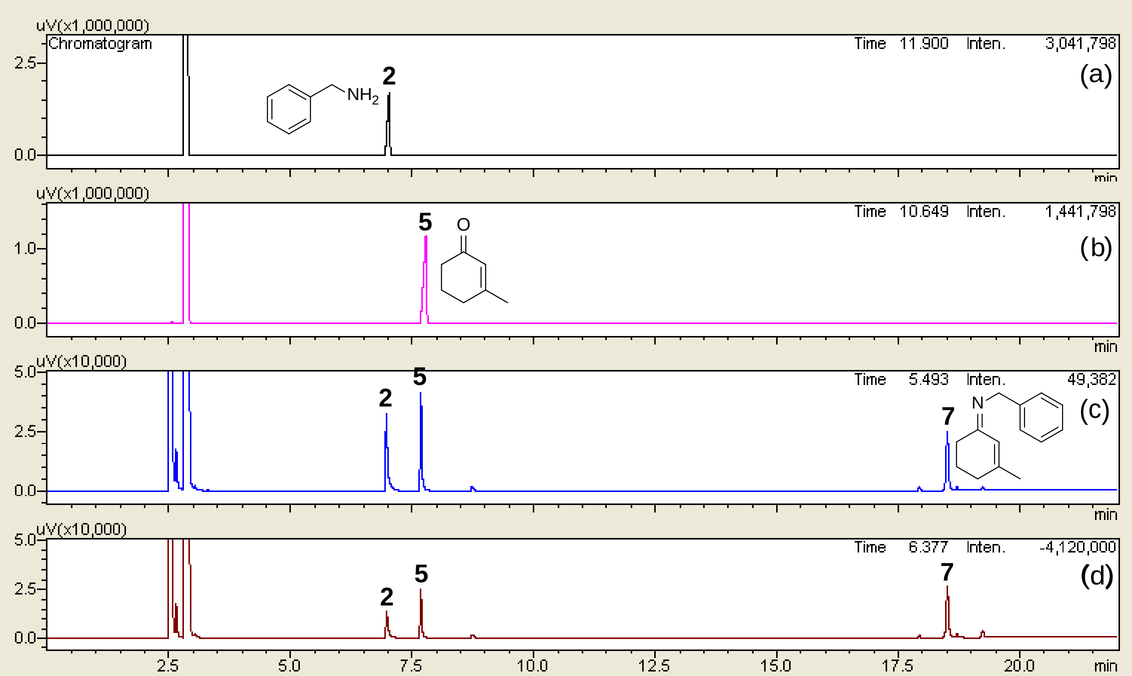
1-phenylmethanamine **2**, C_7H_9N, *m/z* (%): 106 (M^+-1, 100), 107 (M^+, 61), 79 (42), 91(12), 51 (11), 65 (6).

N-(cyclohex-2-en-1-ylidene)-1-phenylmethanamine **3**, $C_{13}H_{15}N$, *m/z* (%): 65 (15), 91 (100), 185 (M^+, 30), 157 (7).

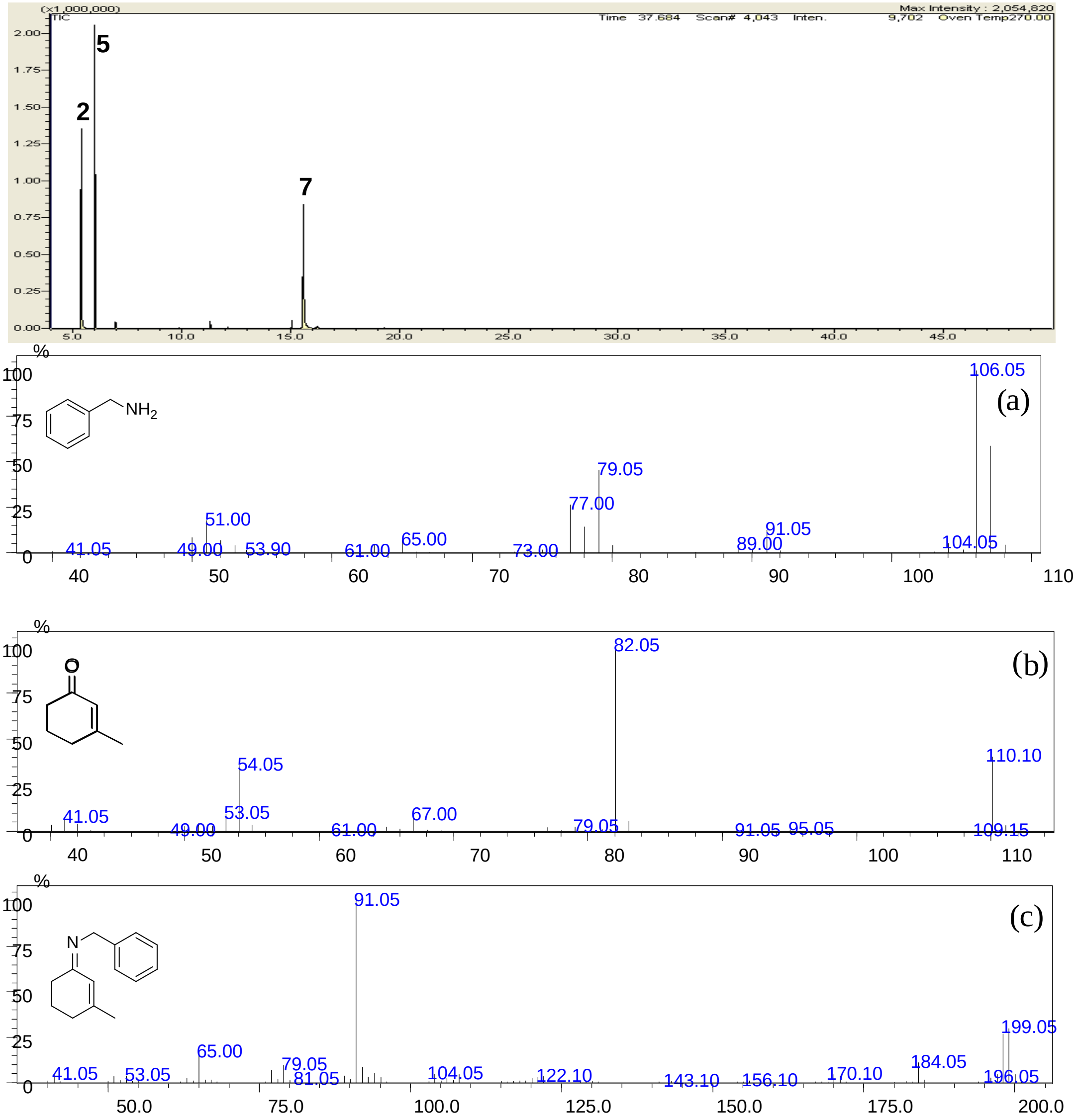
3-(benzylamino)cyclohexanone **4** $C_{13}H_{17}NO$, *m/z* (%): 91 (100), 146 (44), 160 (32), 203 (M^+, 30), 133 (27), 106 (31), 65 (13).



SI-6 : Cleavage probable at the bond β to the ring, giving the tropylium ion



SI- 7 : Chromatograms obtained by GC-FID. (a) Standard of amine **2**. (b) Standard of ketone **5**. (c) Reaction of amine **2** with ketone **5** in methanol under orbital shaking in the presence of lipase (24 h, 33 °C, 133 rpm). (d) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 μm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



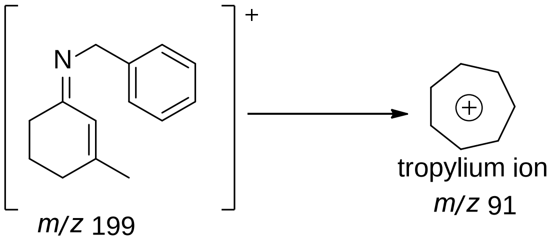
SI- 8 : Chromatogram and mass spectrum obtained by GC-MS (EI, 70 eV) for the reaction of ketone **5** with amine **2** in hexane under orbital shaking (24 h, 33 °C, 133 rpm). (a) Amine **2**. (b) Ketone **5**. (c) Mass spectrum obtained from a signal with a retention time of 15.9 min. Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min

GC-MS (EI, 70 eV):

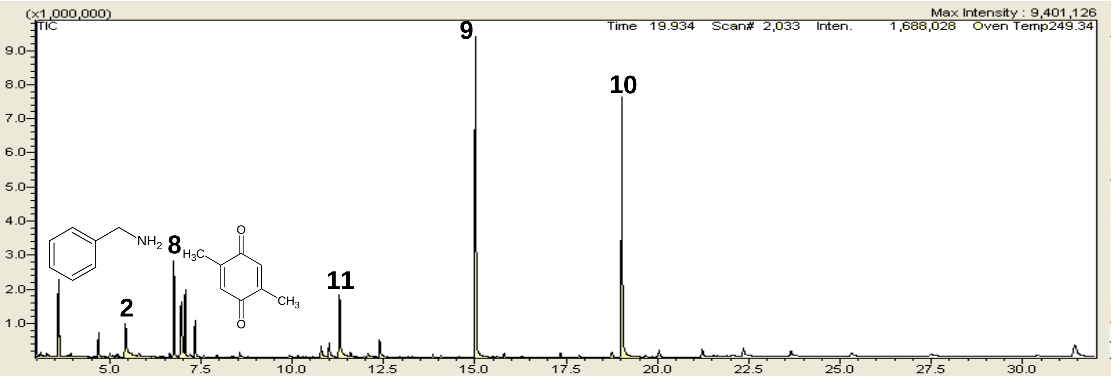
3-methylcyclohex-2-en-1-one **5**, $C_7H_{10}O$, *m/z* (%): 82(100), 110(40, M^+), 54(36), 41(8).

1-phenylmethanamine **2** C_7H_9N, *m/z* (%): 106 (M^+-1, 100), 107 (M^+, 61), 79 (42), 91(12), 51(11), 65 (6).

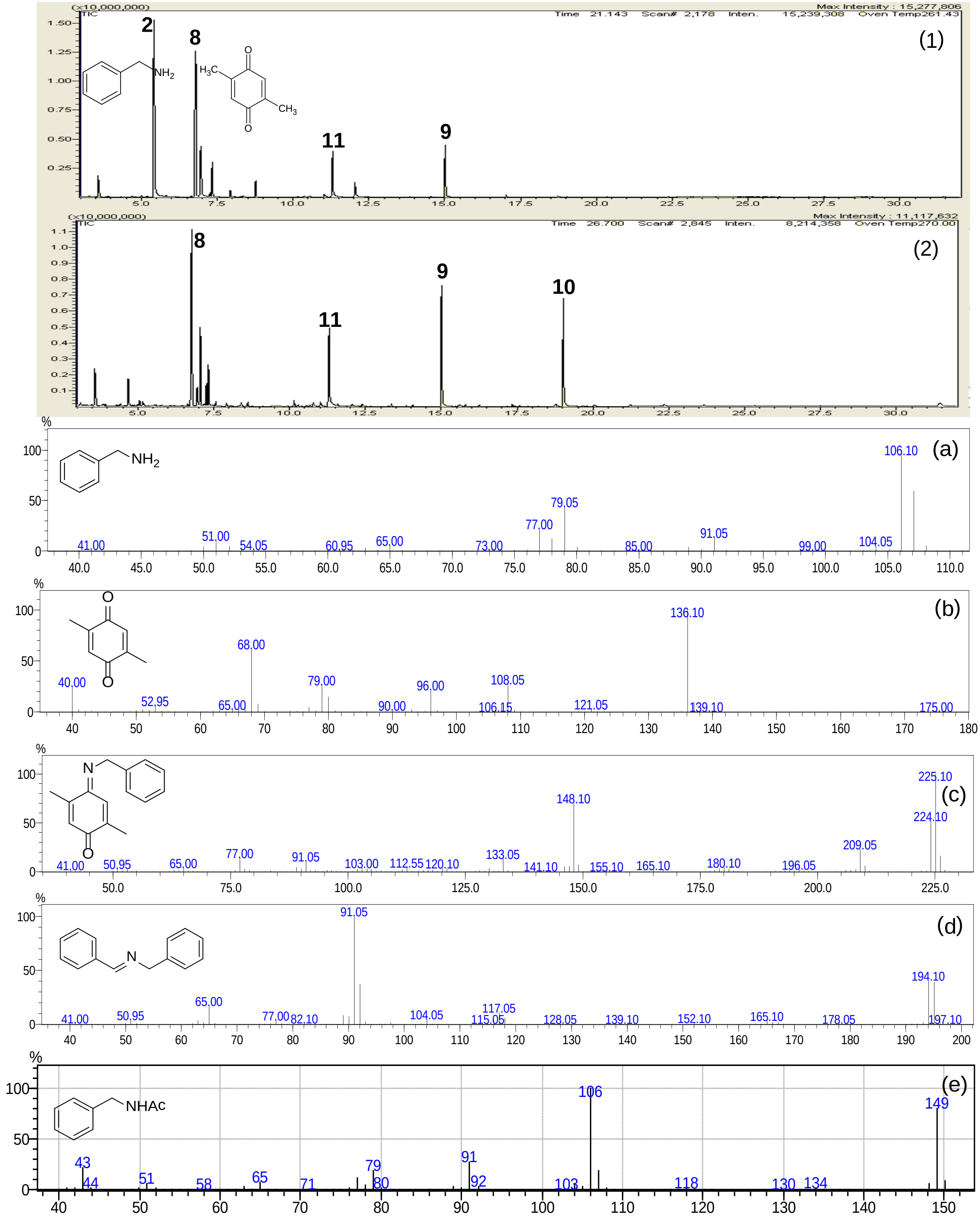
N-(3-methylcyclohex-2-en-1-ylidene)-1-phenylmethanamine **7**, $C_{14}H_{17}N$, *m/z* (%): 91(100), 199 (M^+, 30), 65(17), 184(11), 79(10).



SI-9 : Cleavage probable at the bond β to the ring, giving the tropylium ion



SI-10 : Chromatogram obtained by GC-MS. Reaction of amine **2** with ketone **8** in hexane under orbital shaking (96 h, 33 ^{o}C, 130 rpm). Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 50 ^{o}C, Tf = 270 ^{o}C, r = 10 ^{o}C/min, t = 22.0 min



SI-11 : Chromatograms and mass spectrum obtained by GC-MS (EI, 70 eV). (1) Non-enzymatic reaction of amine **2** with ketone **8** in hexane under microwave irradiation (3 h, 60 °C, 70 W). (2) Enzymatic reaction of amine **2** with ketone **8** in hexane under microwave irradiation (5 h, 60 °C, 70 W). (a) Amine **2**. (b) Ketone **8**. (c) Mass spectrum obtained from a signal with a retention time of 15.0 min. (d) Mass spectrum obtained from a signal with a retention time of 19.0 min. (e) Mass spectrum obtained from a signal with a retention time of 11.3 min. Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min

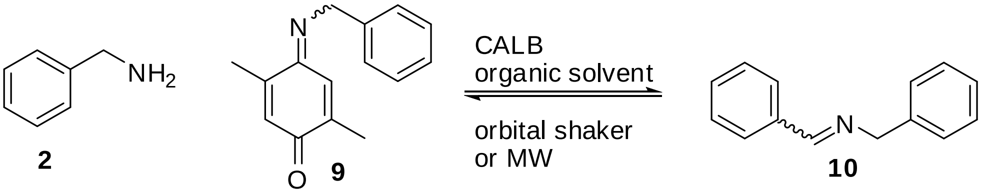
GC-MS (EI, 70 eV):

2,5-dimethyl-*para*-benzoquinone **8**, $C_8H_8O_2$, *m/z* (%): 136(M$^+$, 100), 68(66), 79(28), 108(29), 40(28), 96(22), 121(3).

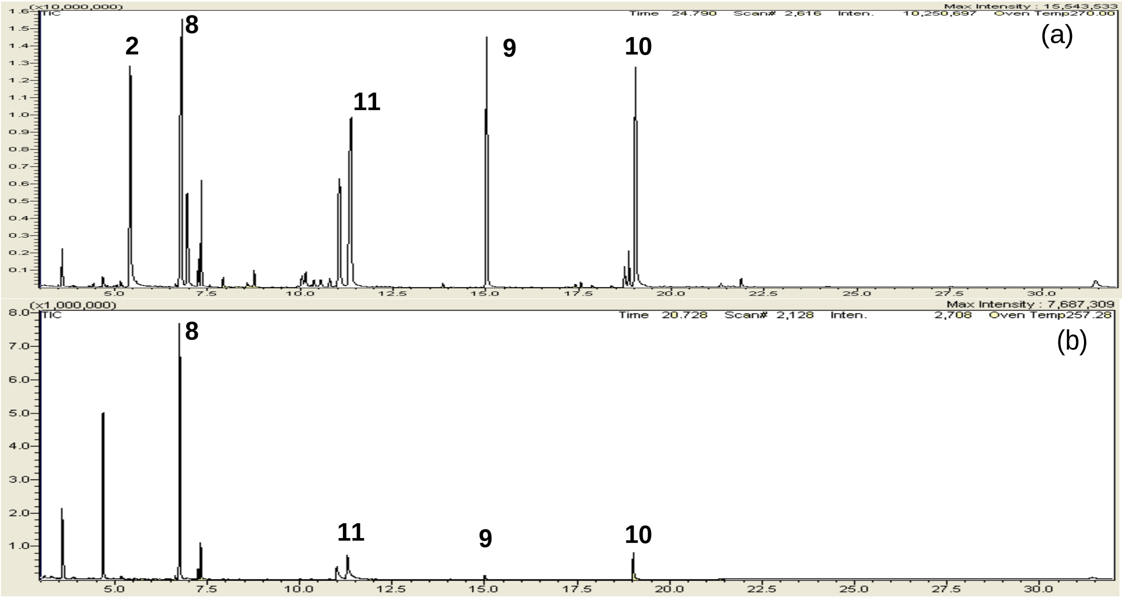
4-(benzylimino)-2,5-dimethylcyclohexa-2,5-dienone **9**, $C_{15}H_{15}NO$, *m/z* (%): 225(M$^+$, 100), 148(70), 224(52), 209(23), 77(14).

N-benzylidene-1-phenylmethanamine **10**, $C_{14}H_{13}N$, *m/z* (%): 65 (17), 91(100), 92 (37), 117 (11), 194 (40), 195 (M$^+$, 38).

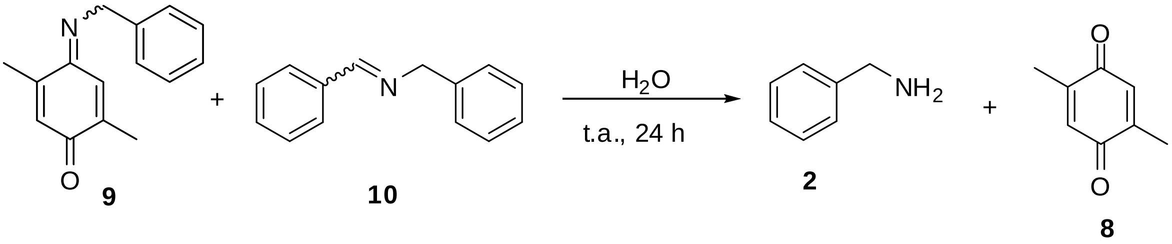
N-benzylacetamide **11** $C_9H_{11}NO$, *m/z* (%): 106 (100), 149 (M$^+$, 79), 91(27), 79(18), 43(21).



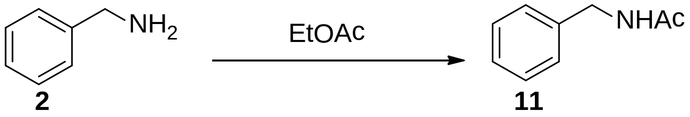
SI-12 : Aza-Michael reactions using CALB on an orbital shaker and microwave irradiation



SI- 13 : Chromatograms obtained by GC-MS. (a) Enzymatic reaction of amine **2** with ketone **8** in hexane under orbital shaking in triplicate (48 h, 33 °C, 130 rpm). (b) Enzymatic reaction of amine **2** with ketone **8** in hexane after extraction into ethyl acetate and water after 24 hours on an orbital shaker (33 °C, 133 rpm). Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min



SI- 14 : Extraction of aza-Michael reaction with ethyl acetate and water after 24 h on an orbital shaker (33 °C, 133 rpm)



SI-15 : Acetylation of amine **2** by EtOAc

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Water Quality of River Tungabhadra Due to the Discharge of Industrial Effluent at Harihar, District Davanagere, Karnataka State, India

By Basavaraj M. Kalshetty, Shobha. N & M. B. Kalashetti

Bharathier University, India

Abstract- Water is a prime mover of all activities and essential feature for all modern developments. Water is distributed in different forms, such as rain water, river water, spring water and mineral water. Rain water is considered to be the purest form, however, is associated with dissolved gases such as CO_2, SO_2, NH_3 etc., from atmosphere. Water used for Industrial development and municipal purposes, it is better to ensure the quality of water for these purposes. There is influence of Industrial waste. Sewage on the water quality, the waste products can change the water Chemistry. Water gives life to Industries, but, Industries kill the water Chemistry. The waste water which emerges out after uses from industries have no definite composition, the pollutants associated with industrial effluents such as organic matter, inorganic dissolved solids, fertilizer materials, suspended solids, heavy metals from toxic pollutants and micro organisms and also pathogens. The industrial wastes are responsible for water color, turbidity, odour, hardness, toxic elements, bacteria and micro organisms. Industrial wastes contain poisonous chemicals which are difficult to remove from its homogeneous solution state.

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WATER QUALITY OF RIVER TUNGABHADRA DUE TO THE DISCHARGE OF INDUSTRIAL EFFLUENT AT HARIHAR, DISTRICT DAVANAGERE, KARNATAKA STATE, INDIA

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Basavaraj M. Kalshetty [α], Shobha. N [σ] & M. B. Kalashetti [ρ]

Abstract- Water is a prime mover of all activities and essential feature for all modern developments. Water is distributed in different forms, such as rain water, river water, spring water and mineral water. Rain water is considered to be the purest form, however, is associated with dissolved gases such as CO_2, SO_2, NH_3 etc., from atmosphere. Water used for Industrial development and municipal purposes, it is better to ensure the quality of water for these purposes. There is influence of Industrial waste. Sewage on the water quality, the waste products can change the water Chemistry. Water gives life to Industries, but, Industries kill the water Chemistry. The waste water which emerges out after uses from industries have no definite composition, the pollutants associated with industrial effluents such as organic matter, inorganic dissolved solids, fertilizer materials, suspended solids, heavy metals from toxic pollutants and micro organisms and also pathogens. The industrial wastes are responsible for water color, turbidity, odour, hardness, toxic elements, bacteria and micro organisms. Industrial wastes contain poisonous chemicals which are difficult to remove from its homogeneous solution state.

I. INTRODUCTION

Rivers are life line of living being and constitute an integral part of both rural and urban community as a source of drinking and cooking purposes. Water used by the public must be free from disease causing bacteria; toxic chemicals, excessive amount of minerals and organic matter1. Fresh water becomes polluted due to three major reasons, excess nutrients from sewage, industries, mining and agriculture. According to recent investigations ground water contamination also increasing due to anthropogenic activities like disposal of waste, sewage, industrial waste2. Due to rapid Industrialization, Urbanization, therefore, pollution of water resources needs a serious and immediate attention through periodical check up of water quality.

Keeping in view the main objective of the present investigation is the evaluation of physico-chemical aspects of Tungabhadra River water from the selected locations at Harihar Polyfibre Industries, to specify accurately and timely information regarding the quality of river water at industrial effluent disposal point and both at upstream and downstream flow of water.

The present findings may be helpful to shape sound public policy and to implement water quality improvement programme effectively as well as efficiently.

Study area: The two Major Industries are discharging their effluents in the river Tungabhadra at Kumarpattanam near Harihar town, District Davanagere, Karnataka State.

1. Harihar Polyfibres Factory (HPF) is the largest Rayon grade pulp manufacturing unit in India (1972). Daily manufacturing capacity approximately 200 tones, generating more than 30 thousands liters of industrial effluents per day.
2. Grasilene Rayon Factory (GRF) 1977 produces about 850 tones of Grasilene fiber per month generating about 10 thousand liters of highly polluted effluents.

Both factories locating on the left bank of river Tungabhadra on NH-4 at Kumarpattanam. The area selected for studies are up stream flow of water from discharge point, discharge point and downstream flow of water from the effluent disposal point. The water quality of upstream investigated for the comparative study of effluent effect on water body. The partially treated effluent run in one canal for a distance of half km through a small village called Nalavagal, before joining the river Tungabhadra at only one location. The waste water was colored before mixing with river water, carrying lot of foam along the downstream, recently the treatment of effluents from these two factories are adapted little new technology or discharging into river without any proper treatment before discharging.

People residing in these areas utilize river water for their daily needs. Sampling locations were selected on the basis of the detailed survey of the study area and discussion with local public, the details regarding the locations is given in Table 1.

Author α: B.L.D.E'S Science College Jamkhandi, Bagalkot District, Karnataka State, India. e-mail: drkalshetty@gmail.com

Author σ: ITC Limited, ITC Life Science & Technology Centre, Peenya Industrial Area, 1st Phase, Bangalore-58. & Research Scholars, Department of Chemistry, Bharathier University, Coimbatore.

Author ρ: P.G. Studies in Department of Chemistry, Karnataka University, Dharwad.

Table 1: Details of sampling locations

No.	Stream	Sampling location	Distance from discharging point.
1	Upstream	Harihar side (Makanur)	0.5 km.
2	Discharge point	Factory point	50 feet
3	Down stream	Nadiharalahalli	0.5 km.
4	Down stream	Airani	5 km.

a) *Experimental*

All the samples were collected in clean polythene bottles with necessary precaution and brought to the research Laboratory without the addition of any preservatives and subjected to the physico-chemical analysis within 24 hours after collection, in order to assess their quality and portability water samples were analyzed using standard methods2, 3, and 4. All the chemicals used were A.R. Grade; Double distilled water was used for the preparation of various solutions. The physico-chemical parameters such as Temperature, pH, EC, TDS, DO, TH, TA, Calcium, Magnesium, Sodium, Potassium, Chloride, Nitrate and Sulphate were determined by standard procedures5. The pH and EC were carried out by using pH-meter and Electrical conductivity meter respectively. Sodium (Na+) and potassium (K+) were determined by using flame photometer; sulphate (SO4’’) ion concentration was determined by using visible spectrophotometer.

II. RESULTS AND DISCUSSION

The assessed physico-chemical parameters after the analytical determination given in Table 2 to Table 5. The values were compared with the standard values given by WHO6, ISI7 and with ICMR8 as given in Table 6. The water samples collected at upstream (spot 1) were not having any objectionable color appearance, taste or odour during the monitoring periods (August-2013, September- 2013, October- 2013 and November-2013). The samples collected at the industrial disposal point the water is found in thick dark brown coffee colored before mixing with river water, carrying lot of foam along the downstream. The water samples at the downstream from the discharge point were collected and the analyzed. The results indicate the river water under goes natural purification during its flow along the stream.

Table 2 : The average values of Physico-chemical parameters and Trace elements in the study locations:

Location: upstream (Spot 1) 0.5 km. from discharge point towards Harihar- side

Date	Temp.	pH	Ec	TDS	COD	BOD	DO	Cl-	CO3’’	HCO3’	Calcium
25.08.13	26.6	7.3	298	97	24	6.4	6.0	36.8	ND	2.1	22
22.09.13	27.8	7.4	162	96	16	6.3	6.6	28.0	ND	2.2	15
26.10.13	27.5	7.3	181	88	19	3.5	6.8	26.5	0.7	2.6	26
24.11.13	26.6	7.2	149	78	10	3.1	6.9	34.0	0.6	2.8	18
Date	Mg	Na	K	SO4’’	TH	Zn	Cu	Fe	Mn	Cobalt	Nickel
25.08.13	5.0	34	7	08	65	0.189	0.03	0.85	0.14	0.04	0.10
22.09.13	3.2	20	4	12	68	0.281	0.06	1.04	0.16	0.06	0.09
26.10.13	4.0	24	5	17	45	0.314	0.02	0.98	0.09	0.06	0.02
24.11.13	3.2	21	6	14	68	0.201	0.09	0.92	0.12	0.06	0.13

Table 3 : Location: Discharge point (Spot 2) 50 feet away from the factory

Date	Temp.	pH	Ec	TDS	COD	BOD	DO	Cl-	CO3’’	HCO3’	Calcium
25.08.13	28.0	7.2	2896	1388	172	74	1.8	123	0.5	13.4	152
22.09.13	28.0	7.3	1099	1622	122	107	2.9	127	0.8	9.92	172
26.10.13	27.9	7.2	798	898	130	112	2.8	226	1.36	6.67	95
24.11.13	26.9	7.0	787	1012	179	105	3.8	276	1.80	12.8	87
Date	Mg	Na	K	SO4’’	TH	Zn	Cu	Fe	Mn	Cobalt	Nickel
25.08.13	39	89	30	330	21	4.95	0.07	1.56	0.34	0.12	0.86
22.09.13	54	110	28	194	194	2.98	0.09	2.08	0.25	0.84	0.92
26.10.13	18	92	27	230	330	1.50	0.07	1.58	0.21	0.18	0.20
24.11.13	22	59	26	270	250	3.15	0.09	1.71	0.42	1.98	0.19

Table 4 : Location: Downstream (Spot 3) Near Nandihalli village 0.5 km. from the discharge point

Date	Temp.	pH	Ec	TDS	COD	BOD	DO	Cl-	CO3''	HCO3'	Calcium
25.08.13	29	7.6	1950	486	49	31	4.8	41.2	0.3	9.8	86
22.09.13	29	7.8	799	589	18	36	4.9	33.0	0.4	6.4	44
26.10.13	29	7.7	678	414	27	27.5	4.8	37.4	0.9	3.2	36
24.11.13	26.8	7.9	317	465	29	27.0	4.3	47.0	0.8	6.7	61
Date	Mg	Na	K	SO4''	TH	Zn	Cu	Fe	Mn	Cobalt	Nickel
25.08.13	28	48	27	390	99	1.96	0.06	0.95	0.30	0.14	0.38
22.09.13	27.2	55	24	129	95	2.92	0.09	1.24	0.21	0.49	0.64
26.10.13	7.2	61	21	276	178	1.96	0.06	0.31	0.14	0.12	0.09
24.11.13	5.2	60	18	165	132	0.99	0.06	1.24	0.24	1.04	0.46

Table 5 : Location: Downstream (Spot 4) Near Airani village 5 km from the discharge point

Date	Temp.	pH	Ec	TDS	COD	BOD	DO	Cl-	CO3''	HCO3'	Calcium
25.08.13	29.1	7.9	1546	461	47	27.0	5.6	54.5	0.2	6.8	91.0
22.09.13	29.0	8.3	825	612	14	28.5	5.8	31.1	0.1	3.7	51.4
26.10.13	29.1	7.6	640	220	23	20.1	6.2	54.2	0.5	2.7	41.0
24.11.13	26.4	8.2	402	312	18	31.0	6.6	51.0	0.5	4.2	54.0
Date	Mg	Na	K	SO4''	TH	Zn	Cu	Fe	Mn	Cobalt	Nickel
25.08.13	23.3	47	25	280	65	0.98	0.07	0.86	0.21	0.09	0.40
22.09.13	16.3	54	19	101	89	1.86	0.06	1.36	0.14	0.62	0.32
26.10.13	5.8	31	18	176	92	0.85	0.06	0.27	0.17	0.09	0.08
24.11.13	4.9	26	14	94	122	0.82	0.03	0.94	0.18	0.59	0.24

a) *Temperature*

All physiological activities and life processes of aquatic organisms are generally influenced by water temperature[9], it is an important parameter because effects bio-chemical reactions in aquatic organisms. A raise in temperature of water leads to the speeding up of chemical reactions in the water body. Temperature reduces the solubility of gases and amplifies the tastes and odours. In the present investigation the temperature range was found in between 26.9oC to 28.30oC at the industrial effluent discharge point. The temperature of upstream water found in between 26.6oC to 27.8oC where as downstream water temperature at spot S3 and S4 were in between 26.4oC to 29.1oC.

Table 6 : Prescribed legal limits and guidelines

Parameter	WHO	ISI	ICMR
pH	7.0 – 8.5	6.5-8.5	6.5-9.2
EC	1.4 ds/m	1400µs/cm	250
TDS	500	500	1500-3000
BOD	5.0	5.0	---
COD	10.0	10.0	---
DO	5.0	5.0	5.0
Cl'	250	250	1000
Calcium	75-100	45-75	200
Magnesium	30-150	30	50
Sodium	200	200	---
Potassium	1.4	1.4	---
Sulphate	150-250	150-200	400
TH	300	300	600
TA	120	200-600	200-600
Zinc	0.75	---	---
Copper	0.60	---	---
Iron	<1	0.3	---
Manganese	2.0	0.3	---
Nitrate	40-100	40-100	40-100
Fluoride	1.0-1.5	1.0-1.5	1.0-1.5
Color	5 HU	5 HU	5 HU
Odour	Unobjectionable	Unobjectionable	Unobjectionable
Turbidity	5 NTU	5 NTU	5 NTU

b) Taste and Odour

Disagreeable odour and taste in water may be because of the presence of vegetation, inorganic constituents and organic matters, the discharge of waste water and industrial effluents in water bodies. WHO and BIS limit: unobjectionable or agreeable due to aesthetic condition. But, at the present studies water samples were found to be odourless, whereas the water chemistry was spoiled at the discharge disposal point. Downstream water under gone the natural purification possessing tasteless and odourless characters.

c) Turbidity

Turbidity in water is caused by suspended matter finely divided organic and inorganic matter, soluble colored organic compounds are responsible for the developing the turbidity in water. Plankton and other microscopic organisms make the water turbid. Turbidity was found to be O NTU. WHO and BIS limit: 5-10 NTU; aesthetic consideration and harmful bacteria may be associated with particles. In the present study the water sample at industrial effluent disposal point was found to be in coloured due to industrial waste and finely divided organic and inorganic matter.

d) pH (Power of Hydrogen)

The pH of natural water is important index of hydrogen ion activity and it is resulting value of the acid - base interaction of a number of mineral and organic components in water. pH is an important ecological factor and is a term used and universally to express the intensity of the acid and alkaline condition of the water samples. Most of the water samples were slightly alkaline due to the presence of carbonates (CO3'') and bicarbonates (HCO3'). pH-value determines the equilibrium between free CO2, HCO3' and CO3''. The present study reveals that the pH of upstream water found in between 7.2 to 7.4, where as in downstream water was found to be in between 7.6 to 8.3 indicates more alkaline in nature and may reflect contamination of OH- ions by strong bases such as sodium hydroxide and calcium hydroxide10. At the disposal point the pH of water was ranged from 7.0 to 7.3 (WHO and BIS limit: 6.5 to 8.5; health reflect problem, may affect mucous membrane and problem in water supply system).

e) Electric Conductivity(EC)

Electric conductivity is caused due to presence of electrolytes which dissociate in to cations and anions, It is a measure of water capacity to convey electric current. It is an indicator of the degree of mineralization of water. The EC is correlated with total dissolved solids11. It signifies the amount of total dissolved salts. Presence of high amount of dissolved inorganic substances in ionized form.

In the present investigation the EC-values of water sample at the industrial effluent point during monitoring periods ranged in between 789 to 2896 Micromhos/cm, and indicate the presence of some ionic matter such as Ca,Mg,Cl,SO4,CO3,HCO3 and some trace elements.

The EC-values of upstream and downstream water samples were in the legal limit, most of the water sample content substantial amounts of dissolved CO2, HCO3' and hydroxides, these constituents are the results of dissolution of minerals in the soil and atmosphere12.

f) Density, Surface Tension and Viscosity

Density of the water samples at the disposal point was 0.9982 gram/cm3, while surface Tension was 71.812 dyne/cm. Viscosity of water sample was calculated as 0.0091 poise/cm/sec. which was in the comparable ranges.

g) Total dissolved solids (TDS)

TDS is an index of solid present in dissolved form and estimated as being 0.5 to 0.9 times the conductivity in ms/cm. It indicates the salinity behavior of ground water. Water containing more than 500 ppm of TDS is not considered desirable for drinking water supplies, but in unavoidable case 1500 ppm is also allowed13 for domestic use. In the present investigation the TDS values of upstream water samples at various monitoring months found in between 78 ppm to 97 ppm. The downstream water samples are also found in the normal prescribed legal ranges ie., in between 129 ppm to 589 ppm, where as the TDS values of water sample at the industrial effluent point was ranged in between 787 ppm to 2896 ppm, more than the proscribed value, this indicates that the factory effluents contains large amount of dissolved solids, this salinity behavior of water sample due to the dumping of chemicals both organic and inorganic matter in to the river water. (WHO and BIS limit: 500 ppm -2000 ppm; palatability decrease and may cause gastro intestinal irritation).

h) Dissolved Oxygen (DO)

Oxygen is a regulator of metabolic processes of plants and animals. The depletion of oxygen level is due to high amount of organic wastes and industrial wastes. In the present investigation water samples at the industrial effluent discharge point their DO values found to be in between 1.8 ppm to 3.8 ppm during the monitoring months. While the upstream water showed the DO values in between 6.0 to 6.9 ppm and the downstream water showed the DO values in between 4.3 ppm to 6.6 ppm this indicates the river water gets natural purification. The lower value of DO at the effluent discharge point water may be due to inclusion of high inorganic and organic load in the water which leads to oxygen depletion.

i) Total Hardness (TH)

The total hardness may be due to the presence of divalent cations like Ca, Mg found abundant in water. The total hardness is sum of the calcium and

Magnesium concentrations, both expressed as Calcium carbonates in ppm values. Water is conventionally classified as hard or soft from the following classification 50-100 ppm (Soft), 100-250 ppm (Moderately hard), 250 – 350 ppm (Hard). Hardness is the property of water which prevents the later formation with soap and increases the boiling points of water14.

In the present investigation the TH of the upstream water samples ranged in between 45 ppm to 68 ppm. Similarly the TH values of downstream water found in between 65 ppm to 178 ppm during the monitoring periods. Whereas the water samples at industrial effluent point showed TH range was from 194 ppm to 330 ppm. Thus the water samples were found to be moderately hard and thus need to be treated before using it for the domestic uses.

j) *Calcium and Magnesium Hardness*

The water samples collected at the industrial effluent discharge point contain the calcium hardness from 87 ppm to 172 ppm these values were close resembling and were within the permissible limit. Magnesium hardness values from 18 ppm 54 ppm and were within the permissible limit of 30 to 150 ppm (WHO). Jain et al15 and other reported that the high concentration of hardness (150 to 300 ppm) may cause heart disease and kidney problems.

k) *Alkalinity*

Alkalinity of water is acid neutralizing capacity of the water to predestinated pH. Alkalinity in water is mainly due to CO3'' HCO3' and OH- content. Borates, phosphates, silicates or other bases if present also contribute for alkalinity. (WHO and BIS limit: 200 to 600 ppm; taste become unpleasant). In the present study the water samples collected at disposal point the total methyl orange alkalinity was found in between the range was within the prescribed limits.

l) *Biological Oxygen Demand(BOD)*

Biological oxygen demands are a measure of the quantity of oxygen used by microorganisms (aerobic bacteria) in the oxidation of organic matter. In the present work the BOD values of water samples during the monitoring periods at the industrial effluent disposal point was found in between 74 ppm to 112 ppm, this indicates the effluent runoff carries chemical wastes during the polyfibre synthesis. This is due to the discharge of untreated or partially treated industrial effluent directly in to the river. There are makeable variation in the BOD content along the downstream from the discharge point during the study period which was ranged in between 20.1 ppm to 36 ppm. The upstream water showed low BOD values 3.1 ppm to 6.4 ppm. The decrease in BOD and increase in DO from effluent discharge point was due to natural self purification.

m) *Chemical Oxygen Demand (COD)*

COD determines the oxygen required for the chemical oxidation of organic matter. COD values conveyed the amount of dissolved oxidizable organic matter including non-biodegradable matter present in it. The COD value in the sample effluent was found to be 122 ppm to 179 ppm; the high COD value determines the organic load. The COD values found within the 10 ppm to 24 ppm found within the permissible limit.

n) *Chloride (Cl)*

The chloride concentration serves as an indicator of pollution by sewage, industrial effluents. Chloride occurs in all ground waters widely in varying concentration. Excessive chloride in potable water is not particularly harmful. Chloride in excess (more than 250 ppm) imparts a salty taste to water. People accustomed to higher chloride in water are subjected to laxative effects16. In the present investigation the chloride values ranged from 26.5 ppm to 36.8 ppm from the upstream water samples, and chloride found in between 31.08 ppm to 54.5 ppm along the downstream water. Whereas the chloride content of the water samples collected at the effluent discharge point ranged in between 123 ppm to 276 ppm, slight more than the permissible legal range. The tolerance of chloride by human being varies with climate, exertion and loss through perspiration. (WHO and BIS limit: 250 to 1000 ppm decreases potability, salty taste and corrosion).

o) *Sodium (Na)*

Sodium levels in ground waters vary widely; depends upon geological formation. In surface water generally the sodium concentration ranges in between 1 and 300 ppm depending upon the geographical area. Excessive intake of sodium chloride causes vomiting. (Guideline values: 200 ppm; based on taste threshold and health consideration). In the present investigation the sodium concentration of the water samples collected from upstream water,at the industrial effluent disposal point and water samples of downstream were found in between the ranges of 20 ppm to 34 ppm, 59 ppm to 110 ppm and 26 ppm to 54 ppm respectively, this indicates the more concentration of sodium in the industrial effluent point due to the chemical combination of compounds leads to change in the quality of water.

p) *Potassium(K)*

The quality of water in the natural process most commonly found the concentrations of both sodium and potassium. The concentration of potassium is quite lower than sodium. It remains most common in solution without undergoing any precipitation. The potassium concentration of upstream river water sample found to be very low i.e., less than the prescribed limit. Whereas the potassium concentration in water sample collected at discharge point found in between 26 ppm to 30 ppm also less than legal range. The concentration of potassium falls with the downstream due to the natural purification.

q) *Acidity*

Acidity is a measure of the effects of combination of compounds and conditions in water. It is the power of water to neutralize OH- and is expressed in terms of CaCO3. Water attains acidity from industrial effluents, municipal waste (drainage) and from humic acid. No phenolphthalein acidity and methyl orange acidity was found in the studied water samples.

r) *Pathogens*

The most common danger associated with drinking water is contamination by sewage, by other waste or by human or animal excreta. In general this is determined by conducting test for total coli form counts, fecal coli form counts. The pathogen observed was salmonella species which is gram negative and short rod shape. (BIS limit: 10 CFU/ 100 ml and absent; causes illness and fatal for life).

s) *Sulphate (SO4)*

In the present investigation sulphate concentration of upstream water sample was recorded and ranged in between 8 ppm to 17 ppm. In downstream water was ranged in between 94 ppm to 280 ppm. The SO4 content in the water sample collected at the discharge point found in between 194 ppm to 330 ppm. All the values found within the permissible limits. It is known that the sulphate concentration in water samples around 1000 ppm; it has Laxative effect and causes gastrointestinal irritation17. Excess Na2SO4 in water causes cathartic action. High concentration of Na and MgSO4 is associated with respiratory illness.

t) *Iron (Fe)*

Iron is an important essential element to human body which is present in hemoglobin and myoglobin. When iron concentration exceeds permissible limit in drinking water it gives stringent taste to water. According to WHO standards, limit of Fe concentration in drinking water is 0.3 mg/L, exceeding which causes toxicity. In the present investigation the iron content of the water samples analyzed was found to be more than the permissible limits. These values vary from 1.56 ppm to 2.08 ppm and indicate the effluent consists with more iron content because of using chemical compounds in industrial process.

u) *Copper (Cu)*

Copper is essential element of human body, but excessive large doses may lead to mucosal irritation, corrosion, wide spread capillary damage, hepatic and renal damage and also to central nervous system. Copper below 20 µ gram/L may found in drinking water. Due to the industrial effluent concentration of copper in water may increase. The concentration of copper found in natural waters is not known to have adverse effects on humans. Though copper in excess of 1.0 mg/L may impart some taste to water18. Toxicity of copper to aquatic life is dependent on the alkalinity of water. At lower alkalinity, copper is more toxic to aquatic life. In the present investigation the concentration of copper recorded a maximum 0.09 ppm at industrial effluent disposal point.

v) *Zinc (Zn)*

Zinc is an essential element and it is necessary for the functioning of various enzyme systems, deficiency and low intake of Zinc of which results in retardation of growth, immaturity and anemia, condition known as “Zinc deficiency syndrome”. Symptoms of Zn toxicity in humans include vomiting, dehydration, abdominal pain, dizziness and lack of muscular coordination. Zinc imparts undesirable bitter astringent taste to water at levels above 5.0 m gram/L. zinc deficiency in human body may result in infantilism, impaired wound healing and several other diseases. Pollution from industrial sources to a great extent is responsible for high concentration of zinc in water. The concentration of zinc in the study area (at discharge point) ranges from 1.50 ppm to 4.95 ppm which below the prescribed limits.

w) *Manganese (Mn)*

Excess Manganese in a diet prevents the use of Iron in the regeneration of blood hemoglobin. Large doses of Manganese cause apathy, irritability, headaches, insomnia, and weakness of the legs.

Psychological symptoms many also develop including in impulsive acts, absent-mindedness, hallucinations, aggressiveness and unaccountable laugher. Finally a condition similar to Parkinson’s disease may develop19. Manganese Pollution from HPF industrial effluent discharging of chemical waste at disposal site was analyzed, the concentration of Manganese in the study area ranged from 0.21 to 0.42 mg/L [BIS Limits: 0.30 mg/L]. Hence, Manganese-values exceed this limit at some monitory period. Thus, the quality of water is suspect able for drinking and other domestic Purposes. The other elements such as Cobalt and Nickel are also important and essential to human body.

III. CONCLUSION

- Data reveals that the river water polluted to some extent at the industrial effluent discharge point, as indicated by a very high value of COD and other activities. The data also indicates that the water bodies total hardness were sound to be beyond the permissible limits as prescribed by WHO and other standards.
- Qualitatively the water in the studied area is not potable, hard and chemical mixed at the discharge

point. Hence, the water at this area is may be used for irrigation but not for domestic and drinking purposes.

- From the analysis it may be concluded that proper environment management plan may be adopted to control the release of effluent. Hence, it is suggested to exercise all the necessary precaution before the water is used for domestic and irrigation. Otherwise it may lead to much adverse health effect.
- To improve or to keep the quality of water at the effluent discharge point, the industrial waste should be treated properly before disposal into the river stream. Hence, there should be continuous monitoring of the pollution level.
- The polluted water due to the disposal of industrial effluent, used for irrigation which would not improve the soil fertility but also reduces the enrichment of nutrients present in the soils.
- The Figures 1 to 3 indicate the comparative studies in the variation of the physicochemical parameters and presence of trace elements in the water samples at various selecting points. The result implies the water sample at industrial effluent discharge point found more polluted rather than upstream, the water in the downstream itself gets natural purification.

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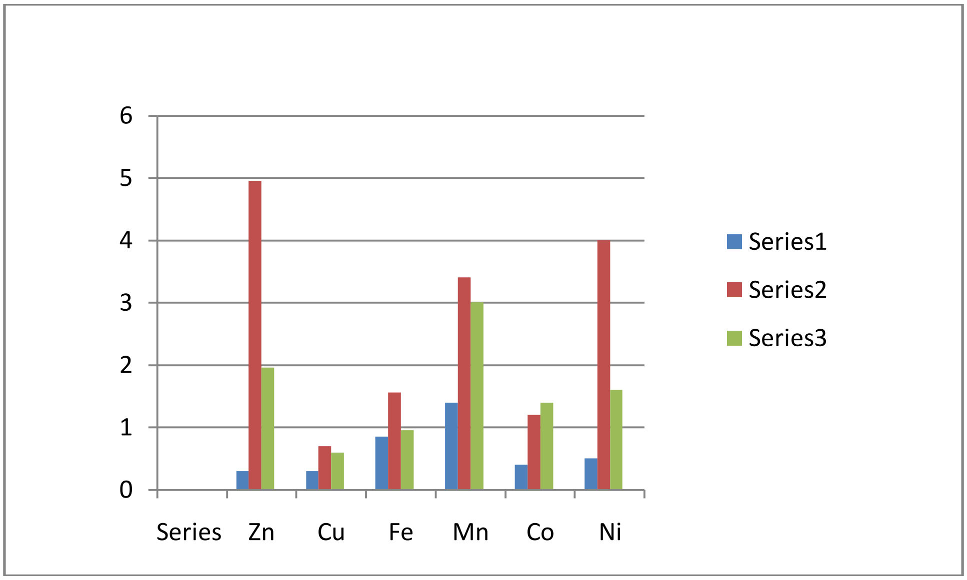

Figure 1 : Comparative study of physicochemical parameters at Upstream (Series 1), Discharge point (Series 2) and Downstream (Series 3) water samples

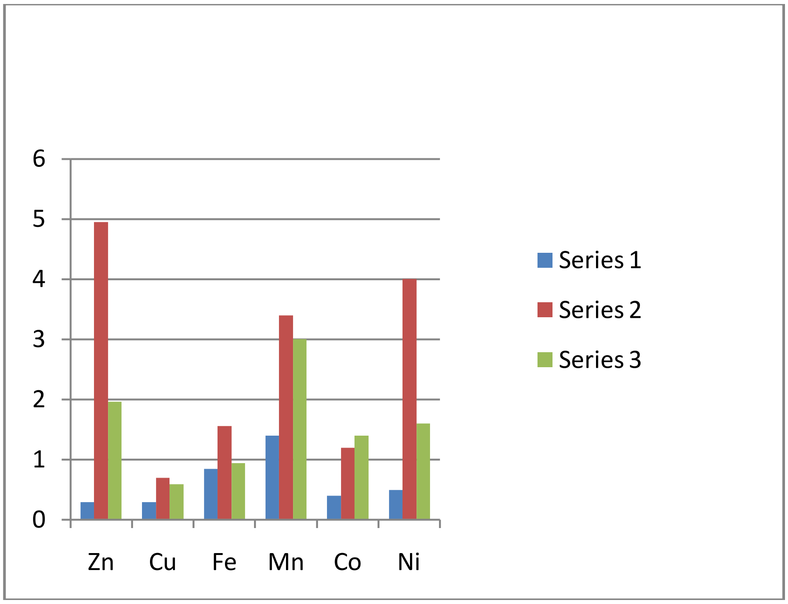

Figure 2 : Comparative study of physicochemical parameters at Upstream (Series 1), Discharge point (Series 2) and Downstream (Series 3) water samples

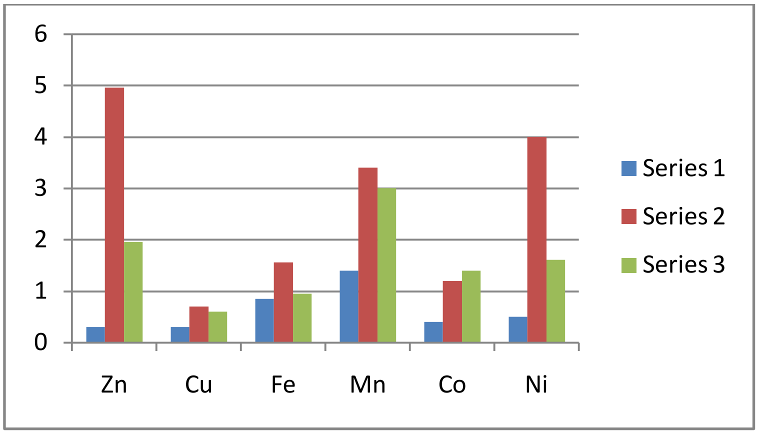

Figure 3 : Comparative study of Elements at Upstream (Series 1), Discharge point (Series 2) and Downstream (Series 3) water samples

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Synthesis, Characterization and Antimicrobial Activity of Azetidin-2-One based Phenyl Sulfonyl Pyrazoline Derivatives

By Shailesh H. Shah, Pankaj S. Patel, Mitesh Prajapati, Bharat Vaghari & Jayesh Parmar

Patel Jdkd Science College, India

Abstract- A new series of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substitutedphenyl)-1-(phenylsulfonyl)-4,5-dihydro-pyrazol-3- yl]phenyl}azetidin-2-one are synthesized by reacting 3-chloro-1-{4-[5-(Substitutedphenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Chlorophenyl) azetidin-2-one with Benzene sulfonyl chloride in presence of pyridine. All these compounds were characterized by means of their IR, 1H NMR, Spectroscopic data and were tested for their antibacterial and antifungal activities by broth dilution method.

Keywords: *chalcones, phenyl sulfonyl-pyrazolines, azetidin-2-one, antimicrobial activity.*

GJSFR-B Classification : *FOR Code: 030306p*

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Abstract- A new series of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-(phenylsulfonyl) -4,5-dihydro-pyrazol-3-yl]phenyl}azetidin-2-one are synthesized by reacting 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Chlorophenyl)azetidin-2-one with Benzene sulfonyl chloride in presence of pyridine. All these compounds were characterized by means of their IR, 1H NMR, Spectroscopic data and were tested for their antibacterial and antifungal activities by broth dilution method.

Keywords: chalcones, phenyl sulfonyl-pyrazolines, azetidin-2-one, antimicrobial activity.

I. INTRODUCTION

Heterocyclic compounds are the well-known class of compounds for its Biological applications. Keeping in view the importance of these Biological activities, it was considered of interest to synthesize some new phenyl sulfonyl derivatives of pyrazoles.

Pyrazolines are heterocyclic compounds which posses wide range of interesting biological activities such as anti-inflammatory [1], insecticidal [2], anti-tubercular [3], antitumor [4], tranquilizing [5], immunosuppressive [6], diuretic [7], anticonvulsant [8], antifungal [9], antidepressant activities [10-11], antibacterial activities [12], molluscidal [13]. In the present communication, we report the reaction of 3-chloro-1-{4-[5-(Substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one with Benzene sulfonyl chloride in presence of pyridine to form phenyl sulfonyl Pyrazoline (5a-j). The structures of the various synthesized compounds were assigned on the basis of IR, 1H-NMR spectral data and elemental analysis. These compounds were also screened for their antimicrobial activity.

II. EXPERIMENTAL

The IR spectra were recorded on IR affinity-1, DRS-8000A, Shimadzu, Ptc. Ltd., Japan spectrophotometer. The 1H-NMR was recorded in DMSO on Bruker Advance II 400 MHz spectrometer using TMS as an internal standard. Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC-using Silica gel-G (Merck). Column chromatography was performed on silica gel. All the compounds were tested for their antibacterial and antifungal activities by broth dilution method

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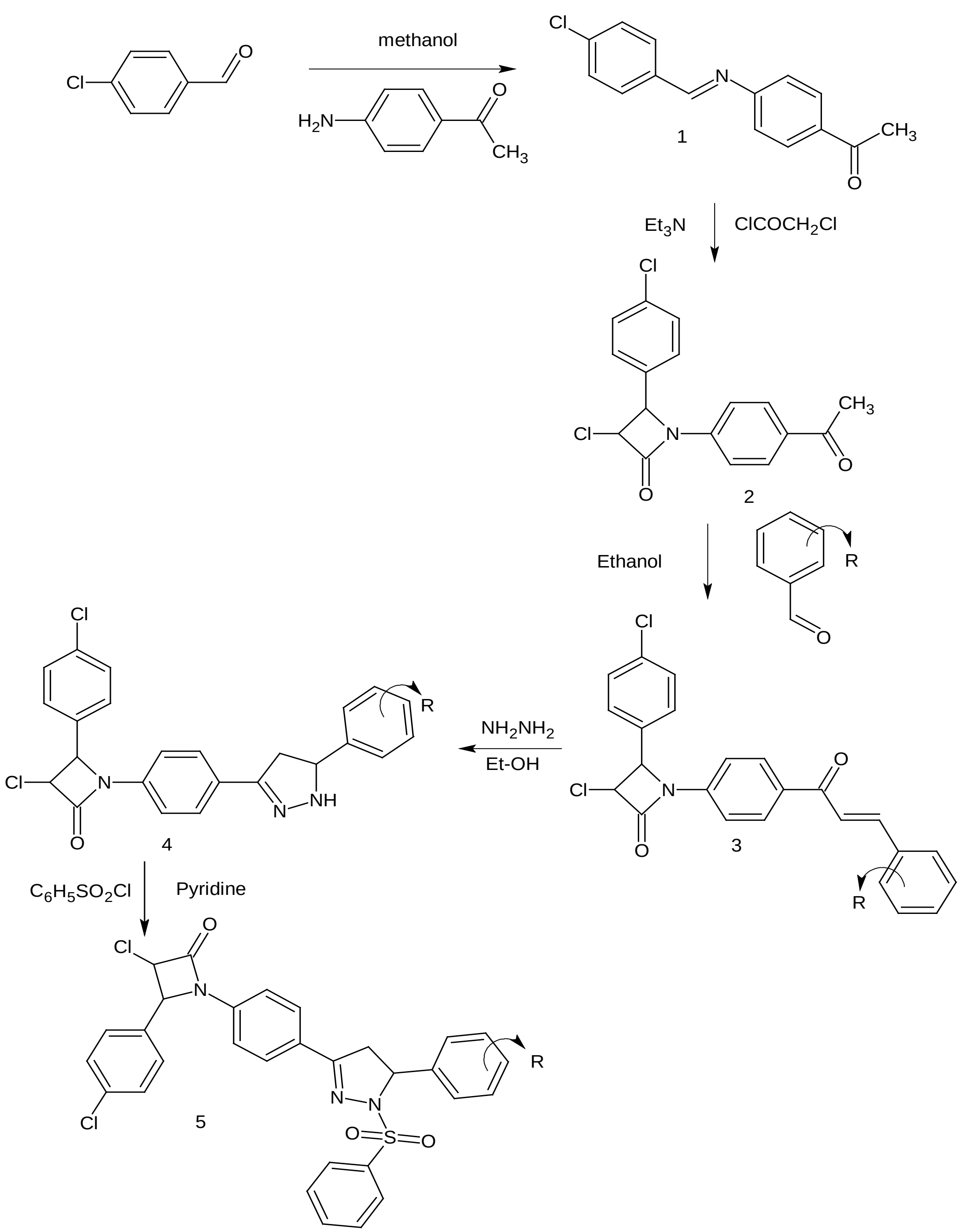
*Author α: Department of Chemistry, Patel JBR Arts, Patel AMR Commerce & Patel JDKD Science College, Borsad.
e-mail: shailchem@yahoo.com*

Author σ: Department of Chemistry, Sheth L.H. Science College, Mansa, Gujarat. e-mail: pspatel_mansa@yahoo.co.in

Authors ρ ω ¥: Students of Patel JBR Arts, Patel AMR Commerce & Patel JDKD Science College, Borsad, Gujarat-India.

Reaction Scheme



a) Preparation of 1-(4-{[(4-Chlorophenyl) methylene] amino} phenyl) ethanone (1)

A mixture of 4-Chloro Benzaldehyde (0.01M), 1-(4-aminophenyl) ethanone (0.01M) and methanol (30ml) was heated for about 5 min. in a beaker (250 ml) to get a clear solution. The solution was kept overnight at room temperature to get the respective crude solid which was recrystallized from ethanol to obtain the pure crystals of 1-(4-{[(4-chlorophenyl) methylene] amino} phenyl) ethanone respectively. The yield of the product was 75% and the product melts at 1200C. Found: C(69.88%) H(4.65%) N(5.41%) , Calcd. for C15H12ClNO: C(69.91%) H(4.69%) N(5.43%). IR, cm-1: 3084(=C-H), 2922(-C-H), 1678(>C=O), 1628(>C=N-), 1595 (>C=C<), 1408(-CH3, bend), 1301(-C-N<), 1240(-C-CO-C-), 738(-C-Cl). 1H-NMR (DMSO, d, ppm): 2.5785 (3H, s, COCH3), 6.5144-7.7992 (8H, m, Ar-H), 8.803 (1H, s, -CH=N-).

b) Preparation of 1-(4-acetylphenyl)-3-chloro-4-(4-Chlorophenyl) azetidin-2-one (2)

In a 100ml Round bottom flask 1-(4-{[(4-Chloro phenyl) methylene] amino} phenyl) ethanone (0.01M) in

70ml benzene was taken. Chloro acetyl chloride (0.01M) was added at room temperature with constant stirring and triethylamine 1ml was added and the reaction mixture was refluxed for 7 hours. After the completion of reaction, solvent was removed by vacuum distillation. The solid was filtered, dried and recrystallized from toluene. The yield of the product was 60% and the product melts at 1080C. Found: C(61.07%) H(3.88%) N(4.17%), Calcd. for C17H13Cl2NO2: C(61.10%) H(3.92%) N(4.19%). IR, cm-1: 3041(=C-H), 2921(-C-H), 1712(>C=O), 1548(>C=C<), 1365(-CH3, bend), 1292(-C-N<), 1197(-C-CO-C-), 642(-C-Cl). 1H-NMR (DMSO, d, ppm): 2.5550 (3H, s, COCH3), 4.8102 (1H, d, >CH-Ar), 5.4594 (1H, d, >CH-Cl), 7.3170-8.0618 (8H, m, Ar-H).

c) Preparation of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (3a-j)

To the solution of 1-(4-acetylphenyl)-3-chloro-4-(4-Chloro phenyl) azetidin-2-one (0.01M) in absolute ethanol (50 ml), substituted Benzaldehyde (0.01M) and 2% NaOH were added and refluxed for 10 hours. After refluxing the reaction mixture was concentrated, cooled, filtered and neutralized with dil. HCl. The solid residue thus obtained was crystallized by absolute ethanol. IR(3d), cm-1: 3043(=C-H), 1722(>C=O), 1624 (>C=C<), 1451(-N=O), 1286(-C-N<), 1232 (-C-O-), 684(-C-Cl). 1H-NMR (3g-DMSO, d, ppm): 4.8757 (1H, d, >CH-Ar), 5.4224 (1H, d, >CH-Cl), 6.3621-8.5674 (12H, m, Ar-H), 7.9978 (2H, d, -CH=CH-), 9.9660 (1H, s, Ar-OH).

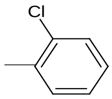
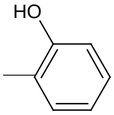
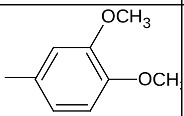
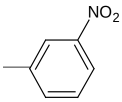
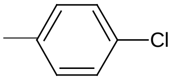
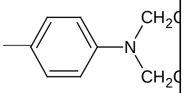
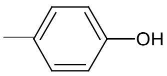
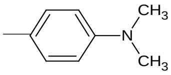
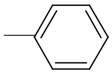
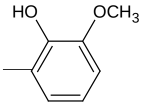
d) Preparation3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Chlorophenyl)azetidin-2-one.(4a-j)

A mixture of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (0.01M) and 99% hydrazine hydrate (0.015M) in ethanol (50ml) refluxed gently for 3 hours. Then the mixture was concentrated and allowed to cool. The resulting solid was filtered, washed with ethanol and recrystallized from ethanol to give a pale brown solid. IR(4e), cm-1: 3041 (=C-H), 2931(-C-H), 1728(>C=O), 1643(>C=N-), 1552 (>C=C<), 1452(-CH2, bend), 1313(-C-N<),1290 (-N-N), 663 (-C-Cl-),. 1H-NMR (4b-DMSO, δ, ppm): 3.61 (2H, d, CH2-of Pyrazol), 4.33 (1H, t, >CH-Ar of Pyrazol), 4.80 (1H, d, >CH-Ar of Azetidine), 5.32 (1H, d, >CH-Cl of Azetidine), 6.56-7.92 (13H, m, Ar-H), 9.65 (1H, s, Ar-OH).

e) Preparation of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-(phenyl sulfonyl)-4, 5 -dihydro-pyrazol-3-yl] phenyl} azetidin-2-one (5a-j)

A solution of 3-chloro-1-{4-[5-(Substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (0.001M) in dry pyridine (25ml) cooled in an ice-bath and to it benzene sulfonyl chloride (0.0011M) was added. The mixture was stirred for 1 hour at room temperature and was then treated with cold dilute HCl (2N). The resulting solid was filtered, washed with water, and recrystallized from absolute ethanol IR(5f), cm-1: 3040(=C-H), 1730(>C=O), 1631(>C=N-), 1529 (>C=C<), 1452(-CH2,bend), 1375 (CH3,bend), 1313 (-C-N-), 1230(-N-N), 696 (-C-Cl-), 1133 (>S=O). 1H-NMR (5e-DMSO, δ, ppm): 3.9 (2H, d, CH2- of Pyrazol), 4.3 (1H, t, >CH-Ar of Pyrazol), 4.8 (1H, d, >CH-Ar of Azetidine), 5.6 (1H, d, >CH-Cl of Azetidine), 6.9-8.0 (17H, m, Ar-H).

Table 1 : Physical constant of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-(phenyl sulfonyl)-4, 5 -dihydro-pyrazol-3-yl] phenyl} azetidin-2- one

Compd	R	M.F.	Yield %	M.P. °C	Elemental Analysis		
					% C Found (Calcd)	% N Found (Calcd)	% H Found (Calcd)
5a		$C_{30}H_{22}Cl_3N_3O_3S$	66	65	58.93 (58.98)	6.84 (6.88)	3.61 (3.63)
5b		$C_{30}H_{23}Cl_2N_3O_4S$	65	82	60.76 (60.81)	7.03 (7.09)	3.88 (3.91)
5c		$C_{32}H_{27}Cl_2N_3O_5S$	63	79	60.33 (60.38)	6.56 (6.60)	4.22 (4.28)
5d		$C_{30}H_{22}Cl_2N_4O_5S$	67	100	57.93 (57.98)	8.98 (9.01)	3.54 (3.57)
5e		$C_{30}H_{22}Cl_3N_3O_3S$	62	78	58.92 (58.98)	6.84 (6.88)	3.59 (3.63)
5f		$C_{34}H_{32}Cl_2N_4O_3S$	68	80	63.02 (63.06)	8.61 (8.65)	4.92 (4.98)
5g		$C_{30}H_{23}Cl_2N_3O_4S$	70	108	60.78 (60.81)	7.04 (7.09)	3.88 (3.91)
5h		$C_{32}H_{28}Cl_2N_4O_3S$	64	145	62.00 (62.03)	9.01 (9.04)	4.51 (4.56)
5i		$C_{30}H_{23}Cl_2N_3O_3S$	71	138	62.46 (62.50)	7.25 (7.29)	3.98 (4.02)
5j		$C_{31}H_{25}Cl_2N_3O_5S$	69	75	59.78 (59.81)	6.71 (6.75)	4.01 (4.05)

III. RESULTS AND DISCUSSION

a) *Antimicrobial activity*

The MICs of synthesized compounds were carried out by broth micro dilution method as described by Rattan (2000). It is one of the non automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms.

The invitro antimicrobial activity of test compounds were assessed against 24 hr cultures of several selected bacteria and fungi. The bacteria used were E. coli, S. aureus, P. aeruginosa, and S. pyogenus; the fungi used were C. albicans, A. Niger, and A. clavatus.

The antimicrobial activity was performed by broth dilution method in DMSO. Gentamycin, Ampicilin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Greseofulvin were used as standard for the evaluation of antibacterial and antifungal activities respectively. The activity was reported by Minimal Inhibition Concentration. The results are summarized in Table-2

Table 2 : Antimicrobial activity 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-(phenyl sulfonyl)-4, 5 -dihydro-pyrazol-3-yl] phenyl} azetidin-2-one

SR. NO.	COMP. NO.	R	ANTIBACTERIAL ACTIVITY MINIMAL INHIBITION CONCENTRATION				ANTIFUNGAL ACTIVITY MINIMAL INHIBITION CONCENTRATION		
			E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS	C.ALBICANS	A.NIGER	A.CLAVATUS
			MTCC 443	MTCC 1688	MTCC 96	MTCC 442	MTCC 227	MTCC 282	MTCC 1323
1	5a	-2-Cl	200	250	200	62.5	1000	>1000	>1000
2	5b	-2-OH	200	250	125	250	1000	1000	1000
3	5c	-3-OCH_3, -4-OCH_3	175	225	200	125	800	800	800
4	5d	-3-NO_2	200	200	225	200	1000	1000	**>1000**
5	5e	-4-Cl	100	125	100	250	>1000	>1000	>1000
6	5f	-4-$N(C_2H_5)_2$	125	200	62.5	100	1000	1000	1000
7	5g	-4-OH	200	200	175	250	800	1000	800
8	5h	-4-$N(CH_3)_2$	225	225	150	200	>1000	800	700
9	5i	-H	175	225	200	150	1000	1000	1000
10	5j	-3-OCH_3, -4-OH	100	125	100	250	500	>1000	1000

Table 3 : Antibacterial Activity: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS
-	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
(MICROGRAMME/ML)				
GENTAMYCIN	0.05	1	0.25	0.5
AMPICILLIN	100	--	250	100
CHLORAMPHENICOL	50	50	50	50
CIPROFLOXACIN	25	25	50	50
NORFLOXACIN	10	10	10	10

Table 4 : Antifungal Activity: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	C.ALBICANS	A.NIGER	A.CLAVATUS
-	MTCC 227	MTCC 282	MTCC 1323
(MICROGRAMME/ML)			
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100

Biological screening result of activities 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-(phenyl sulfonyl)-4, 5 -dihydro-pyrazol-3-yl] phenyl} azetidin-2-one based derivatives shows that compound 5e & 5j have shown better activity against E. coli. Compound 5f show good to very good activity against S. pyogenus, while rest of all compound possessed good activity against S.aureus in the range of 62.5-225 µg/ml. Compound 5j is found to be good antifungal activity against C. albicans, against standard drugs Greseofulvin. While rest of all derivatives are poor against A. Niger, and A.clavatus.

IV. CONCLUSION

The Main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized phenyl sulfonyl Pyrazoline derivatives, structures of synthesized compounds were confirmed and characterized with the help of analytical data's such as IR and 1H-NMR. In summary, we have described the synthesis and antimicrobial activity of some new 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-(phenyl sulfonyl) -4, 5 -dihydro-pyrazol-3-yl] phenyl} azetidin-2-one MIC values revealed that amongst newly synthesized compound having Methoxy-Hydroxide type linkage has shown good activity against the bacterial strains.

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Elimination of Methylene Blue Dye with Natural Adsorbent « Banana Peels Powder »

By Faisal Moubarak, Rachid Atmani, Ibtissam Maghri, M’hamed Elkouali, Mohammed Talbi, Mona Latifa Bouamrani, Mohammed Salouhi & Abdelkbir Kenz
University Hassan II, Morocco

Abstract- The investigation focused on the adsorption of the blue methylene on banana peels powder.

The studies led to determine contacting time (40 minutes for 90% elimination of substrate methylene blue). However, this contacting time can vary with dye concentration. The optimization of parameter such as the temperature, agitation and initial concentration of dye has showed a sensitive improvement of the capacity and rate adsorption. Moreover, the adsorption of this compound is well described by both Langmuir and Freundlich isotherm models.

Keywords: adsorption, blue methylene, dye, isotherm models, banana peels.

GJSFR-B Classification : FOR Code: 259999

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Keywords: adsorption, blue methylene, dye, isotherm models, banana peels.

I. INTRODUCTION

Intensive use of colors in everyday life has created a lot of problems both in the environment and in food [1]. It is important to mention that the water pollution is due to discharges of textile industries, and in feed, the toxicity is due to incorporation of several synthetic dyes [2] .

To address this situation, several decontamination methods have been developed, we cite as examples the adsorption [3,4], ion exchange [5,6], coagulation - flocculation [7] etc. In this work, we present the results for the adsorption blue methylene in banana peels powder. We determined successively contact time and the influence of parameters such as particle size, the initial concentration of substrate, the mass of the adsorbent and the temperature. These studies are needed to better understand the mechanisms governing the adsorption process.

II. THEORETICAL AND EXPERIMENTAL PART

The adsorbent used in this work, banana peels powder was first washed with distilled water to remove impurities and then dried in air for 15 days , then ground in mortar and analyzed by size in order to obtain two different types of fractions, the first fraction is characterized by a diameter less than 80 microns (d < 80 microns), and the second fraction has a diameter of between 80 microns and 2 mm (80 microns < d < 2mm) .

The measurements and spectral evolution of the optical density of the solutions of methylene blue at different reaction times were followed by UV/visible spectrometry (ShimadzuUV mini - 1240)

The experiments were conducted in "batch method" (100 ml Erlenmeyer flask) at an ambient temperature (22°C). It should be noted that the temperature control was made using a digital thermometer.

Moreover, to ensure good dispersion of solid particles Banana peels powder, we adopted the value of 1g/l (0.1g/100 ml) for solid/liquid ratio.

To investigate the adsorption power of our support, we applied models Langmuir and Freundlich. Due to their simplicity, the kinetic models the most commonly used are those of Langmuir and Freundlich.

a) Adsorption Isotherms

The adsorption data from experiments were fitted with:

i. *Langmuir isotherm [8]*

Langmuir adsorption isotherm sheds no light on the mechanistic aspects of adsorption. It provides information on uptake capabilities and also reflects the usual equilibrium process behaviour The Langmuir non linear equation is:

$$q_e = q_m \cdot \frac{b.C_e}{1+b.C_e} \quad (1)$$

Where q_e is the amount of methylene blue sorbed per unit weight of biomass at equilibrium, C_e is the residual equilibrium Dye, q_m is the maximum possible amount of dye adsorbed per unit weight of biomass and b is the equilibrium constant related to the affinity of the binding sites for the methylene blue, lower is b more is the affinity of methylene blue to biomass.

Low values of parameter b indicate that banana peel powder have high affinity for methylene blue. The table 1 presents linear equations of Langmuir model.

The essential characteristic of a Langmuir isotherm can be expressed as a dimensionless constant, defined as the separation factor:

Authors α σ ρ Ѡ ¥ § ν: Faculty of Sciences Ben M'Sik, Avenue Cdt Driss Elharti, BP: 20702 , Casablanca, Morocco.
e-mails: moubarak46@hotmail.fr, atmanirachid12@gmail.com, maghri.ibtissam@gmail.com, m.elkouali@gmail.com, maarifcentre@yahoo.fr, a.kenz@voila.fr, mlbouamrani@gmail.com
Author χ: Laboratory of bio systemes science and ingenierie, EMI, Rabat Agdal, Morocco. e-mail: m.salouhi@gmail.com

$$R_L = \frac{1}{1+b.C_0}(2)$$

b) Freundlich isotherm [9]

The Freundlich non linear equation is:

$$q_e = K_F C^n{}_e (3)$$

Where, K_F and n are constants indicating adsorption capacity and adsorption intensity, respectively. The constants were obtained from the plots of the linearized equations:

$$\log q_e = \log K_F + n.\log C_e (4)$$

Another use of the results is to plot the variation of the distribution coefficient K_d as a function of q_e in logarithmic scale:

$$\log K_d = (\frac{1}{n}).\log K_F.+[\frac{(n-1)}{n})].(\log q_e)(5)$$

III. RESULTS AND DISCUSSIONS

a) Optimization of methylene blue adsorption

i. Determination of equilibrium time

The study of the adsorption of methylene blue on banana peels powder (PBP), clearly implies the determination of contact time, time which corresponds to the adsorption equilibrium or a state of saturation of the support the substrate . In this case, the experimental procedure is simple " batch method" which involves 10 mg / l of methylene blue with 1g / l of powdered banana peels . Analysis by UV/visible allows us to determine the residual concentrations of the substrate at different reaction times.

Thus, the determination of the equilibrium time, allowed the establishment of adsorption isotherms that are essential for the calculation of the maximum adsorption capacity and to identify the type of adsorption to occur either in mono or multilayers. The results obtained at the end of these experiments showed that the contact time required for the elimination of 90 % of methylene blue is 40 minutes. Furthermore, the extension of the time to (90 minutes) does not lead to a significant improvement of the percentage removal. This justifies the inclusion of this contact time for other adsorption experiments (Figure 1).This result was obtained by the ratio :

$$q = V.\frac{C_0 - C_t}{m}(6)$$

q: Adsorption capacity of the support (mg/g).
C_0: Initial concentration of dye (mg/l).
C_t: Concentration of dye (mg/l) at time t of the adsorption process .
V: Volume of solution (substrate).
m: Mass of support (g).

Other factors such as the particle size, the initial concentration of the substrate, the mass of the adsorbent and the temperature may affect the adsorption capacity [10]. It finds its application in various adsorption models used.

b) Effect of particle size

To study the influence of the particle size of the adsorbent We choose two types of fraction, the first fraction having a particle size less than 80 microns, and the second fraction is between 80 microns and 2 mm, results is shown in fig 2, it is found that the adsorption is fast and relatively large for the fine particles (d <80 microns), this could be explained by the fact that the extent of adsorption depends on the external surfaces of the particles, More the particle size is small over the exchange surfaces are provided important promoting high speed transfer of dye to the adsorbent. On considering this, we will continue work with fine particle (d <80 microns).

c) Effect of mass of adsorbent

The influence of the mass of the adsorbent was studied in the range 0.1- 0.3 g. The figure 3 shows the variation of the adsorption amount depending on the time chosen for different masses ; 0.1 g, 0.2 g and 0.3 g, the mass 0.1g provides removal rate which can reach 96%. This effect, we chose the mass 0.1 g, as an optimal weight for the rest of our work.

d) Effect of initial concentration

Figure 4 shows the influence of the initial concentration of methylene blue on the dye adsorption capacity (the mass of the support being fixed), it shows that the adsorption capacity is better for the high initial concentration of methylene blue.

e) Effect of temperature

Figure 5 shows the influence of temperature on the adsorption of the dye. When we use different temperatures to 40 minutes of stirring, the adsorption capacity of powdered banana peels over time increases, the values obtained were Q = 8.8991 mg / g at 22°C.

The experimental results show that this parameter does not affect the adsorption retention of methylene blue on the support of the study process.

f) Adsorption Isotherm

Adsorption isotherms play an important role in determining the maximum capacity and in determining the type of adsorption front is produire.Elles are obtained first by the knowledge of the time and then the graphical presentation of Qe = f (Ce) and Qe These are respectively the amount of adsorbed dye per g of adsorbent and the equilibrium concentration of this colorant.les experimental results obtained show that the S-type is isothermal, which classification coorspond

Gilles[11]. This indicates the adsorption of growth when the concentration of the adsorbate augmante (FIG. 6)

The shape obtained shows that the adsorption of the dye process could occur in a monolayer.

g) *Langmuir isotherm*

The values of equilibrium relation parameter, RL were calculated. As shown in Table 1, RL values lie between 0 and 1 which indicate favourable sorption isotherm for methylene blue. Low values of parameter b indicate that banana peels powder have high affinity for methylene blue.

h) *Freundlich isotherm*

The values of equilibrium relation parameter, n were calculated for two Freundlich model's, the results is shown in table 1, n values is high than 1 so the methylene blue adsorption was not well fitted with Freundlich model and The low values of maximum adsorption capacities obtained from the Freundlich model, confirm that the molecule of Methylene Blue is not strongly adsorbed inside the pores because of its size.

The main parameters characterizing each model are summarized in Table 1. The results shown in Figures 7 and 8 show that the adsorption of Methylene blue on banana peels powder (PBP) follow the two linear models Freundlich and Langmuir.

Table1 : Valeur des paramétres d'adsorption Qm,K et Kf du BM sur les Pelures de Bananes en Poudre

Models	Equation	Q_m (mg/g)	K (l.mg^{-1})	K_f(mg/g)	R
Langmuir	$Q_e = \frac{Q_m\ KC_e}{1+KC_e}$ $\frac{1}{Q_e} = \frac{1+KC_e}{Q_m KC_e} = \frac{1}{Q_m KC_e} + \frac{1}{Q_m}$	111,111	0,310	-	0,972
Freundlich	$Q_e\ = KC_e^n$ $\ln(Q_e) = \ln K_f + n\ln(C_e)$	-	-	25,559	0,943

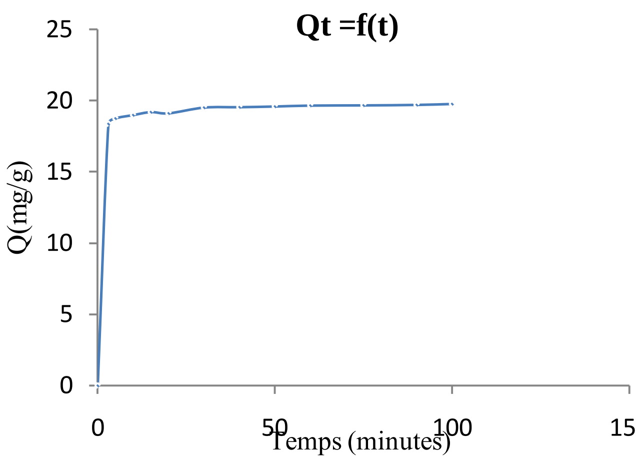

Fig. 1 : Adsorption kinetic of BM on banana peel powder, [BM]=20 mg/l ; m =1g/l ; T=22°C and d<80 μm

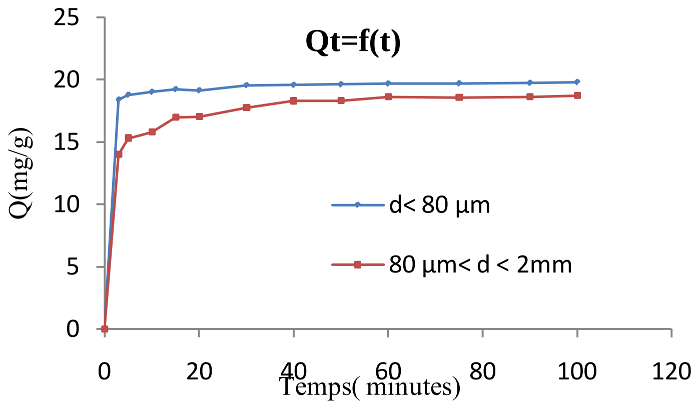

Fig. 2 : Effect of Granulometry banana peel powder, m=1g/l and T=22°C

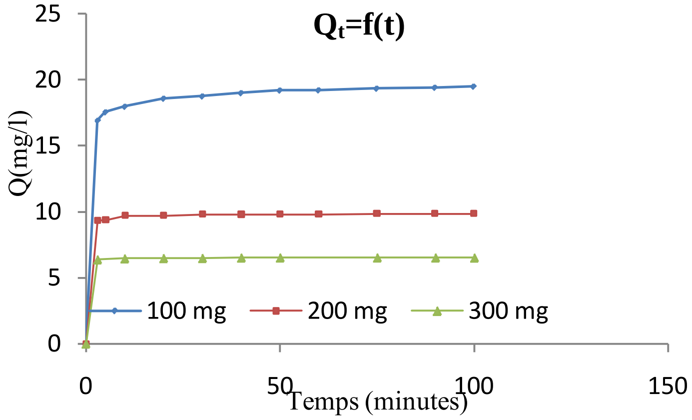

Fig. 3 : Effect of banana peel powder mass. [BM]=20 mg/l ; T=22°C and d<80 µm

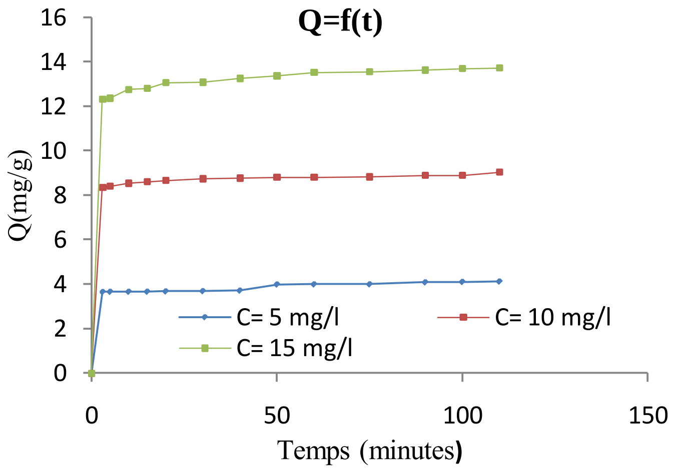

Fig. 4 : Effect of methylene blue initial Concentration, m =1g/l; T=22°C and d< 80µm

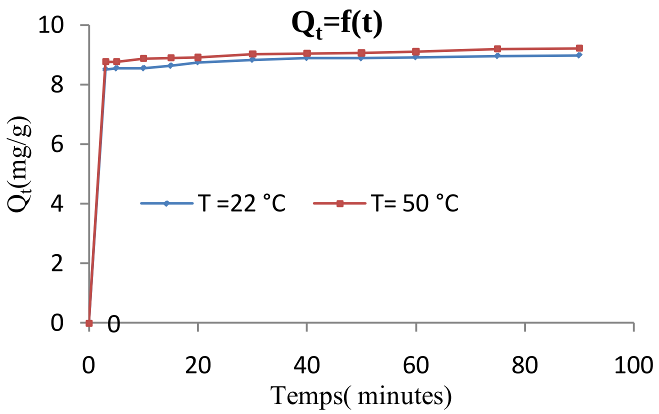

Fig. 5 : Effect of Temperature, [BM]= 20 mg/l. m 1g/l and< 80µm

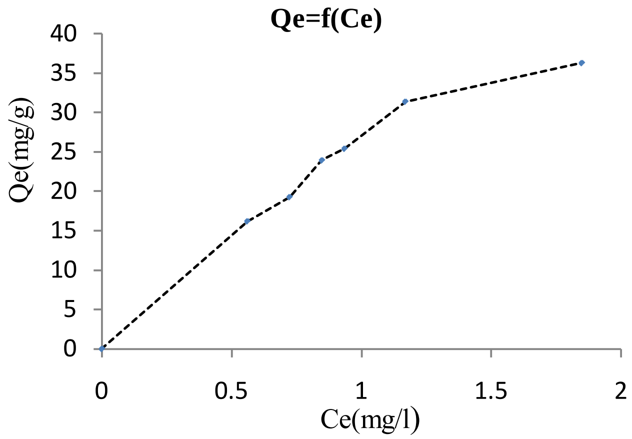

Fig. 6 : Isotherm of methylene blue adsorption. C0=20 mg/l; m =1g/l; T=22 °C and d <80 µm

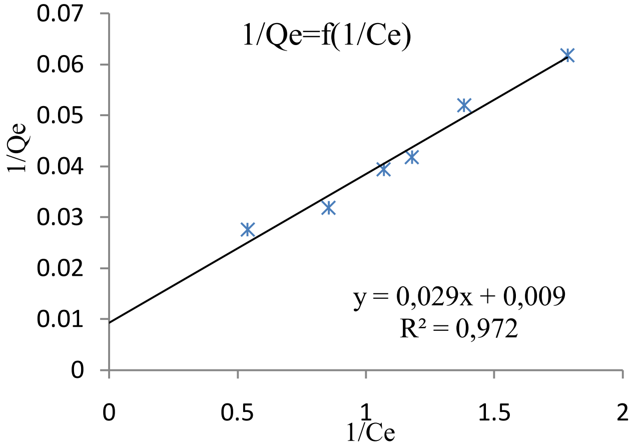

Fig. 7 : Langmuir linear Model of Methylene blue adsorption,T=22°C; d<80 µm

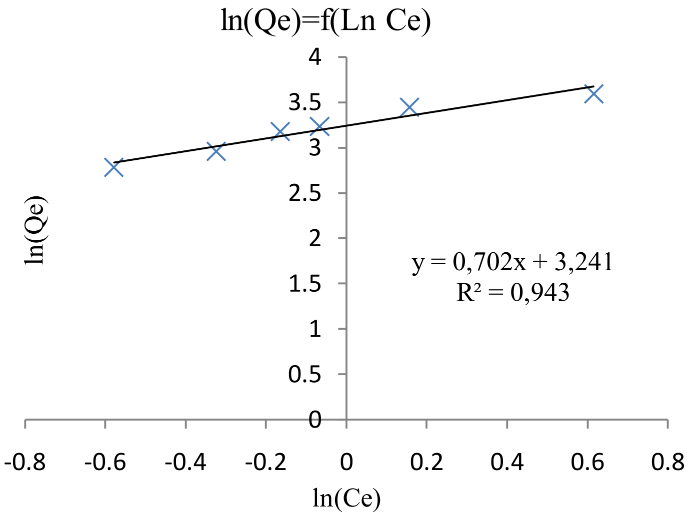

Fig. 8 : Freundlich Model of Methylene blue adsorption, T=22°C; d<80μm

IV. CONCLUSION

The results obtained in this work have to mount that :

- Methylene blue (20 mg/l) is adsorbed relatively well on this medium with a contact time of 40 minutes which corresponds to a removal rate of 90 %. Giving it a relatively large affinity with respect to peels Banana Powder (PBP).
- The effect of particle size, the mass of the adsorbent and the adsorbate concentration had a positive influence on the capacity and kinetics of retention Methylene Blue on our support study. Models of Langmuir - Freundlich and correctly describes the adsorption process.

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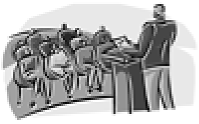

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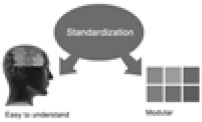

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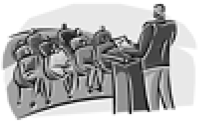

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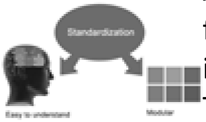

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- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
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Approach:

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- If use of a definite type of tools.
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- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

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- Skip all descriptive information and surroundings - save it for the argument.
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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
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- If you desire, you may place your figures and tables properly within the text of your results part.

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- Give details all of your remarks as much as possible, focus on mechanisms.
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- Try to present substitute explanations if sensible alternatives be present.
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Approach:

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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