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Chemistry

X-ray CT Analysis

The Unique Australian Flora

Highlights

Green Corrosion Inhibitor

Converter Slag Fine Aggregate

Discovering Thoughts, Inventing Future

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X-Ray Ct Analysis of FNS Mortars Mixed with Converter Slag Fine Aggregate

By Won Jung Cho & Ji Seok Kim

Hanyang University

Introduction- Aggregate is an indispensable component in concrete occupying nearly 65-80% of the total volume, whose effects on concrete performance are rarely studied [1]. Due to their inert and impervious characteristics, the durability of concrete is also influenced by the quality of aggregates. To meet the global demand for concrete in the future, it is becoming a more challenging task to find suitable alternatives to natural aggregate for preparing concrete [2]. Hence, the use of alternative sources for natural aggregates is becoming increasingly important.

Keywords: ferronickel slag (FNS), converter slag (BOF), X-ray CT analysis, supplementary cementitious materials (SCMs).

GJSFR-B Classification: FOR Code: 090799

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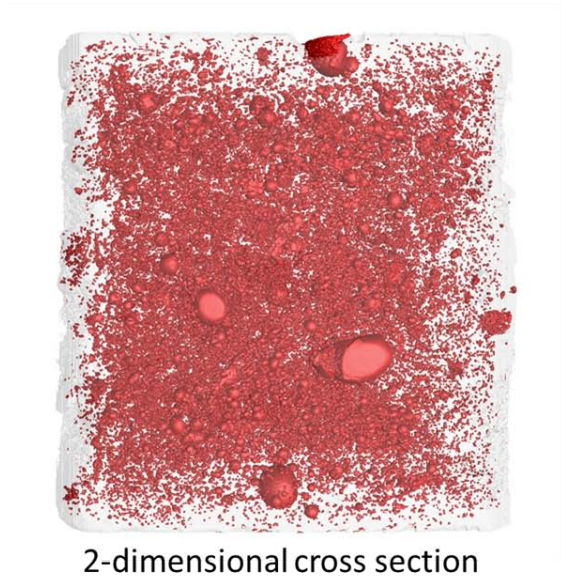
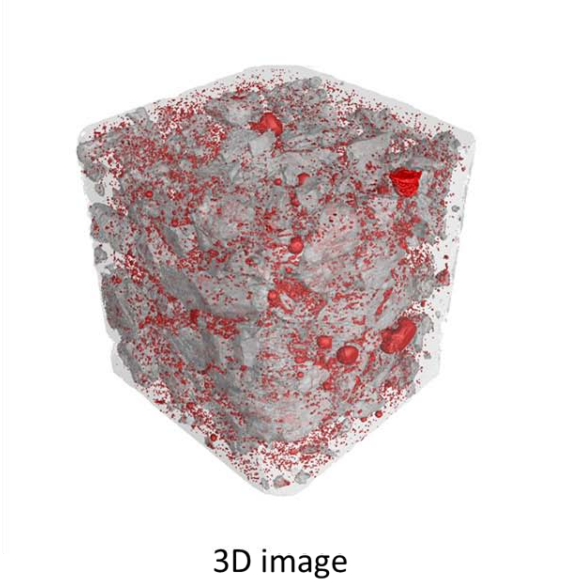
X-Ray Ct Analysis of FNS Mortars Mixed with Converter Slag Fine Aggregate

Won Jung Cho [α] & Ji Seok Kim [σ]

Graphical Abstract-



2-dimensional cross section



3D image

Keywords: ferronickel slag (FNS), converter slag (BOF), X-ray CT analysis, supplementary cementitious materials (SCMs).

I. Introduction

Aggregate is an indispensable component in concrete occupying nearly 65-80% of the total volume, whose effects on concrete performance are rarely studied [1]. Due to their inert and impervious characteristics, the durability of concrete is also influenced by the quality of aggregates. To meet the global demand for concrete in the future, it is becoming a more challenging task to find suitable alternatives to natural aggregate for preparing concrete [2]. Hence, the use of alternative sources for natural aggregates is becoming increasingly important.

Steel slag is a vast majority of solid wastes produced as a by-product during the steelmaking process. It is reported that 2-4 tons of wastes are being generated per one ton of steel production [3]. In currently, the annual emission of steel slag in China is 101 million tons [4] while 43.43 million tons in Korea but its utilization is very low due to the soundness effect; 1) poor grindability, 2) lower reactivity, 3) bad stability of volume [5]. Especially, converter slag (BOF) has been suggested a high potential of causing expansion as suggested in Eq (1) when utilized with ordinary Portland cement (OPC), hence its usability as a construction material is very low. Accordingly, the produced BOF is buried or stacked, however, the problems such as storage yard with a width that can be stacked and environmental pollution by leachate generated during the storage process have generated.

$$\text{Free CaO} + H_2O = Ca(OH)_2 \cdots \text{Eq} \quad (1)$$

Ferronickel slag (FNS) is an industrial by-product obtained by melting nickel ore and bituminous coal at high temperatures (between 1500℃ and 1600℃) and thereafter separating from ferronickel [6,7]. The main clinker of FNS is Forsterite (Mg_2SiO_4), and it also contains Fe_2O_3, CaO, and Al_2O_3 as an oxide composition. The production of FNS is estimated at about 2 million tons in Korea [6], 30 million tons in China [8] and approximately 3 million tons in Japan annually [9]. For decades, there was an effort to use FNS as a construction material. Due to the depletion of natural aggregate, the use of FNS as a replacement of aggregates has been extensively conducted [7,9-12]. Recently, as the reactivity of FNS was confirmed, the utilization of FNS as a binder was also investigated [8,10]. When mixed with ordinary Portland cement

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(OPC), FNS clinker reacts with $Ca(OH)_2$ to create secondary C-S-H gel, thereby contributing to the long-term strength development [13]. However, it has been still discussed whether FNS can provide a similar performance compared to conventional binders. Huang et al. [8] reported a reduction of compressive strength and chloride penetration resistance of FNS concrete than conventional OPC concrete. On the other hand, some studies showed that the compressive strength of FNS incorporated mortars was similar to the pulverized fly ash (PFA) [6,14]. Recently, economical or environmental advantages have been also reported through comparative analysis with conventional cementitious materials.

The purpose of this study is to investigate the pore structure of mortar which is mixed with FNS binder and fine aggregate replaced by using BOF. In order to characterize the expansion behavior, the pore structure analysis was examined by using X-ray CT analysis.

II. Experiment Work

a) Raw materials

i. The particle size distribution of OPC and FNS

The materials used in this study were OPC and electric arc furnace FNS powder produced by Korean company "P." The particle size distribution is shown in Figure 1 (modified from [15].

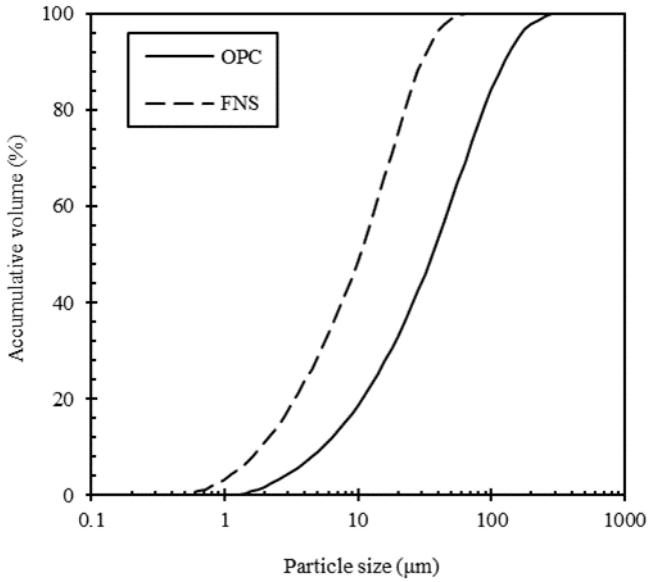

Fig. 1: The particle size distribution of OPC and FNS

ii. X-ray fluorescence (XRF)

Chemical analysis was carried out with X-ray fluorescence (XRF) and shown in Table 1 together with physical properties. The main chemical components of the FNS powders are SiO2, MgO, and Fe2O3. The CaO contents of FNS were counted for only 6.28% which is lower than OPC [15]. BOF fine aggregate has mainly consisted of CaO, SiO2 and Fe2O3, thus BOF can be classified as high calcium and ferrous materials.

Table 1: Chemical and physical properties of raw materials

	Oxides (%)							Physics		Loss on ignition (%)
	CaO	SiO_2	Al_2O_3	MgO	Fe_2O_3	SO_3	TiO_2	Density (g/cm^3)	Fineness (cm^2/g)	
OPC	66.98	17.43	3.97	1.60	4.16	3.41	0.27	3.14	3.539	0.40
FNS	6.28	48.23	3.59	23.01	15.76	0.50	0.11	3.14	3.400	0.02
BOF	44.95	11.60	6.50	2.19	28.12	0.18	-	3.27	-	-

iii. X-ray diffraction (XRD)

Figure 2 presented the X-ray diffraction (XRD) curves of the OPC, FNS and BOF, respectively. In the case of OPC, alite (C3S, 3CaO·SiO2) and belite (C2S, 2CaO·SiO2) are the main clinkers while other clinkers such as gypsum, periclase, brownmillerite (4CaO·Al2O3·Fe2O3) accounted for lower quantities. For FNS, the composition mainly accounted for forsterite (Mg2SiO4) and fayalite (Fe2SiO4) which have crystalline nature. Most of the MgO peaks detected from XRF

analysis indicated forsterite and it originally shows very late hydration which takes about 2 years for the complete reaction [16].The BOF sand is also mainly composed of crystalline clinker such as wuestite (FeO), srebrodolskite (Ca2(Fe+3)2O5, mayenite (Ca12Al-14O33), and larnite (Ca2SiO4).

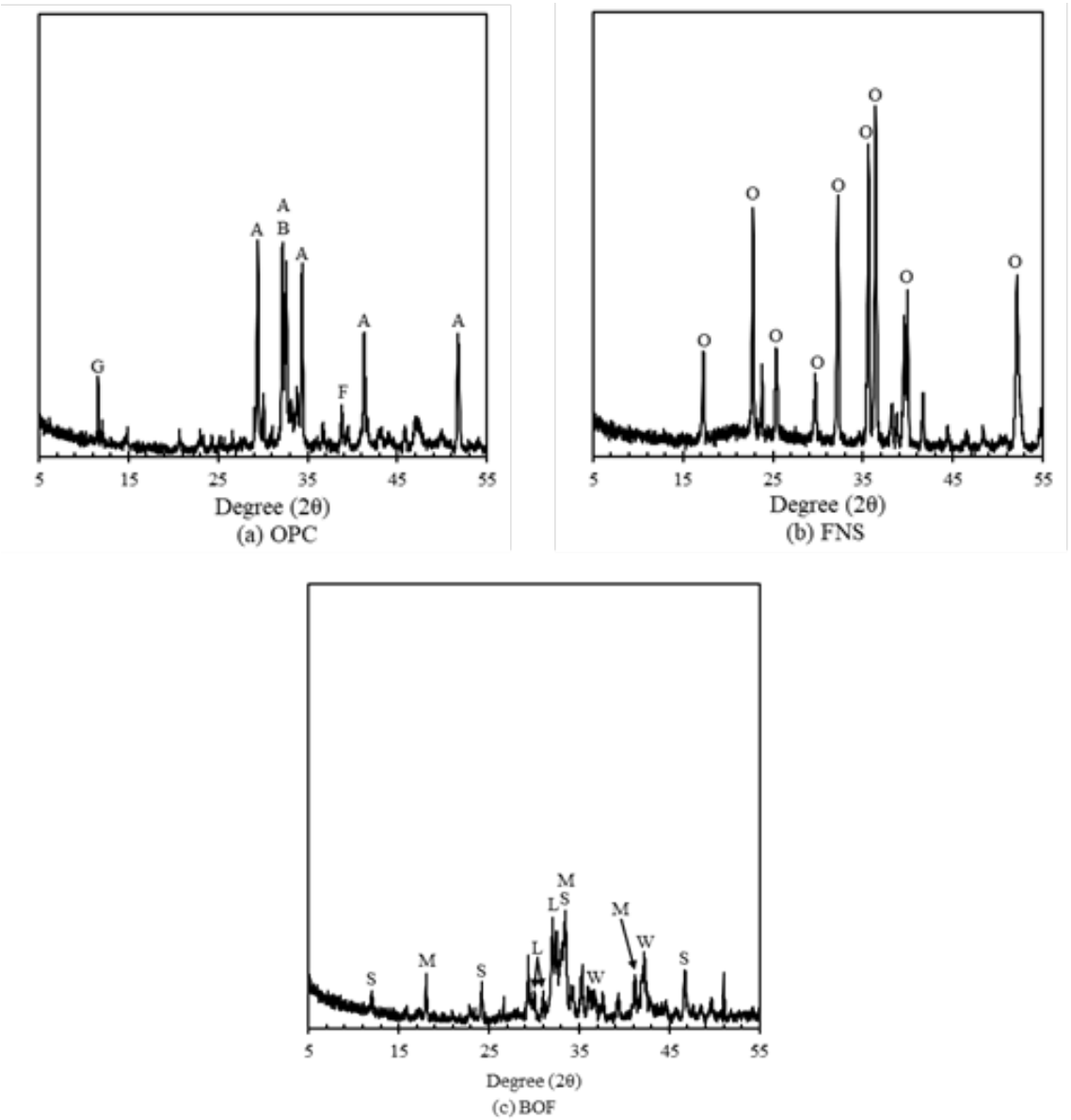

Figure 2: XRD curve of raw materials; (a) OPC, (b) FNS. A: Alite (C3S), B: Belite (C2S), F: Ferrite (C4AF), G: Gypsum (CaSO4), O: Olivine (Mg, Fe)2SiO4, W: Wuestite (FeO), S: Srebrodolskite (Ca2(Fe+3)2O5, M: Mayenite (Ca12Al14O33), L: Larnite (Ca2SiO4)

b) Mix proportion

The mix proportion of the specimen is shown in Table 2. The replacement ratio of 30% (by mass) FNS was chosen for the test specimen. For fine aggregates, washed aggregate with a specific gravity of 2.60 g/cm3 and fineness modulus of 2.73 was used. In the case of BOF fine aggregate, a specific gravity of 3.12 g/cm3 and a maximum size of 4.75 mm was employed.

To examine the hydration products, the mortar specimen was mixed with 0.45 of a total water-binder ratio (W/B). After mixing of the dry powder and fine aggregate with distilled water for 5 min., the mortar was placed into a cubic mold (50 × 50 × 50 mm), which was in turn cured at room conditions (20 ± 2 °C, RH 65 ± 5%) for 24 h. Then, the specimen was demolded and stored in a water bath (20 ± 2 °C) for specified periods. After it, the hardened mortar specimen was fragmented or ground off for its application to each microscopic examination, i.e. XRD analysis.

Table 2: Mix design of mortar (Kg/m3)

	OPC	FNS	BOF	Sand	Water
B20F30	381	163	267	1306	272

c) X-ray CT imaging

After curing for 90 days, the mortar specimens (50 × 50 × 50 mm) were kept in ambient temperature for 1 h stabilization of mass. Then, the sample was placed in CT scanning. The details of equipment and operation are given in Table 3 and the directional target was applied in this study. CT imaging process of mortar is suggested in Figure 3.

Table 3: X-ray Source

	Transmission Target	Directional Target	High Power Target
Voltage	30~120kV	30~225 kV	20~320kV
Forcal Spot size	0.4μm	6 μm	400 μm

For X-ray Detector specification is as below;
Type: Digital flat panel detector
Radiation energy: 40 ~ 320 kV
Active area: 400 mm (h) x 400 mm (v)
Pixel matrix: 1,024 (h) x 1,024 (v)
Pixel pitch: 200 μm
Resolution: 2.5 lp•mm@15 FPS(1×1), 1.25 lp•mm@30 FPS(2×2)

For X-ray Detector specification is as below;
Max. size of 3-D CT scanning: Ø 300 mm × 900 mm (h)
Repetition accuracy: 0.004 °

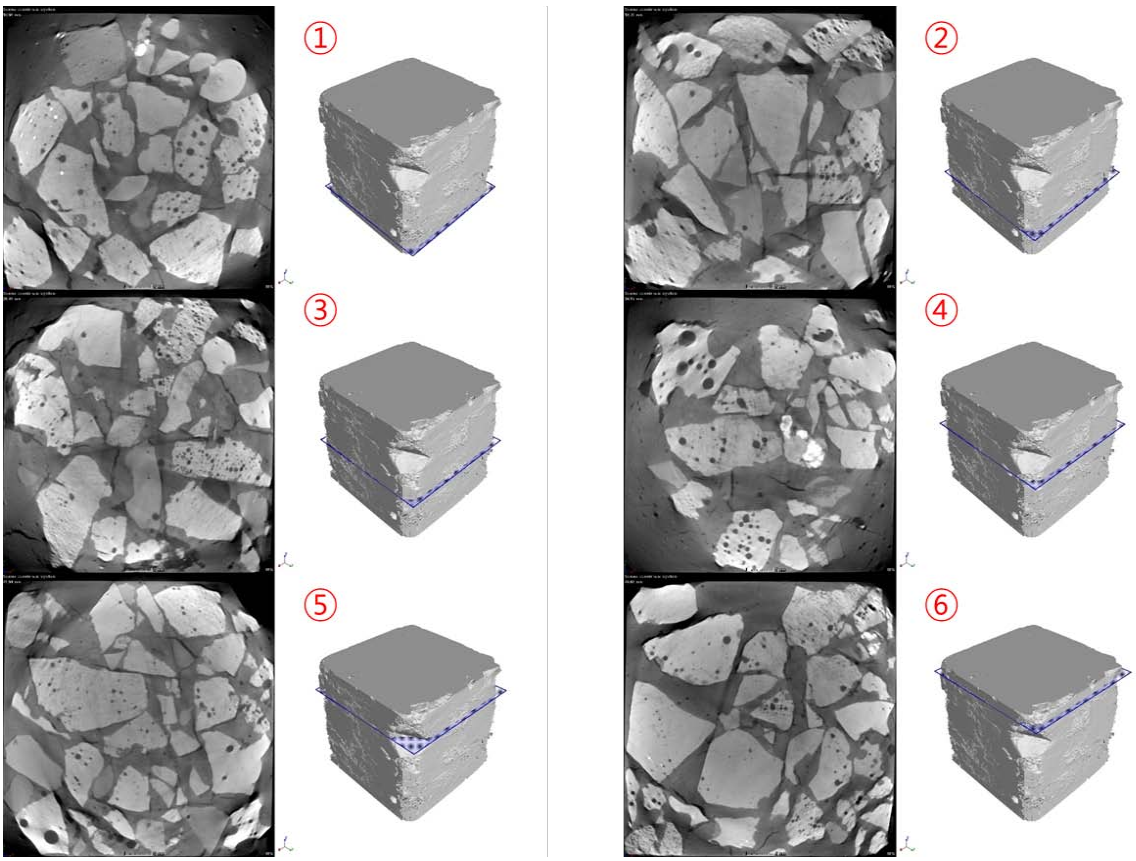

Figure 3: CT scanning process of mortar

After scanning, the obtained images are filtered to remove the noises and segment the pores using OTSU (otsu algorithm) [17]. Then, reconstruct the obtained images into a 3D image in Avizo software [18]. Detailed scan conditions are described in Table 4.

Table 4: Scan condition [15]

Diameter (mm)	Voltage (kVp)	Current (mA)	Transmission Time (sec)	Source-Object Distance (mm)	Pixel Pitch (mm)
100	200	0.8	1	316	0.106488

III. Test Results

a) XRD test results

To figure out the hydraulic reactivity of B20F30 blended mortar containing OPC, FNS, and BOF fine aggregate, the XRD examination was performed for sieved mortars at 90 days, as given in Figure 4. Hydration products for B20F30 mainly include portlandite (Ca(OH)2) and ettringite (Ca6Al2(SO4)3 32H2O), calcite (CaCO3), and anhydrate olivine crystalline ((Mg, Fe)2·SiO4), presumably being originated from raw FNS powder. These results indicated that FNS has low hydration reactivity. In fact, it is notable that there was no further formation of hydration products in B20F30 blended mortar. A formation of calcite may be attributed to the long-term curing age.

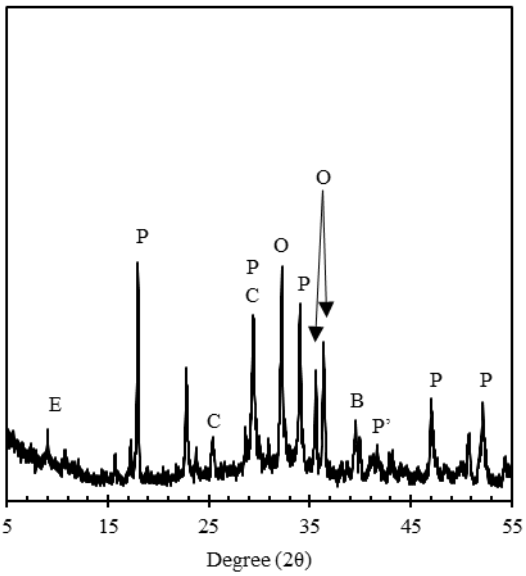

Figure 4: XRD curve of B20F30 mortar after 90 days of curing; E: Ettringite Ca6Al2(OH)12(SO4)326H2O, P: Portlandite (Ca(OH)2), C: Calcite (CaCO3), B: Brucite (Mg(OH)2), P': Periclase (MgO), O: Olivine (Mg, Fe)2SiO4

b) X-ray Tomography

Figure 4 represents the 3D pore size distribution in mortar distinguished by red color. As shown in figure 4 (b), for B20F30 mortar, the average porosity was 2.268%. However, despite the low average porosity, large pores appearing dark red voids were detected in figure 4 (a), on the 3D image. In general, when performing the fraction in the 3D image process, voids were classified and indicated depending on their size and shape. For the case of dark red voids in figure 4 (a), those were seen as a BOF aggregate that was not excluded from the fractioning process due to physical characteristics of BOF which has an irregular shape.

As shown in Figure 4 (a) and (b), no expansion due to the incorporation of BOF aggregate was found. This may be ascribed to the reduction of cement clinker by FNS substitution. From the XRD test result in figure 2, free CaO which can contribute to the reaction of causing expansion was seen in figure 2 (a) OPC, whereas BOF in figure 2 (c) has consisted of crystalline phases except for Wustetite. Besides, it can be seen that the BOF aggregate has similar oxide composition as shown in table 1 and figure 2, which contributed to the hydration reaction in the cement matrix which cement clinker has been reduced due to the replacement by FNS incorporation. This is identical with the XRD test results in figure 3, both BOF aggregate clinker and new hydrates were not observed. Furthermore, the low reactivity of FNS was incorporated as seen in figure 4, B20F30 mortar shows a low porosity. This is because

low reactivity FNS contributed to the filler effect especially extra space; as the filler does not produce hydrates, at the same water to solids ratio, the water to clinker ratio is higher and there is more space for the hydration products of the clinker phases [19]. Thus, it can be said that there would be no adverse effect in using FNS and BOF aggregate for cementitious materials, presumably due to the no significant change of the hydration products and porosity.

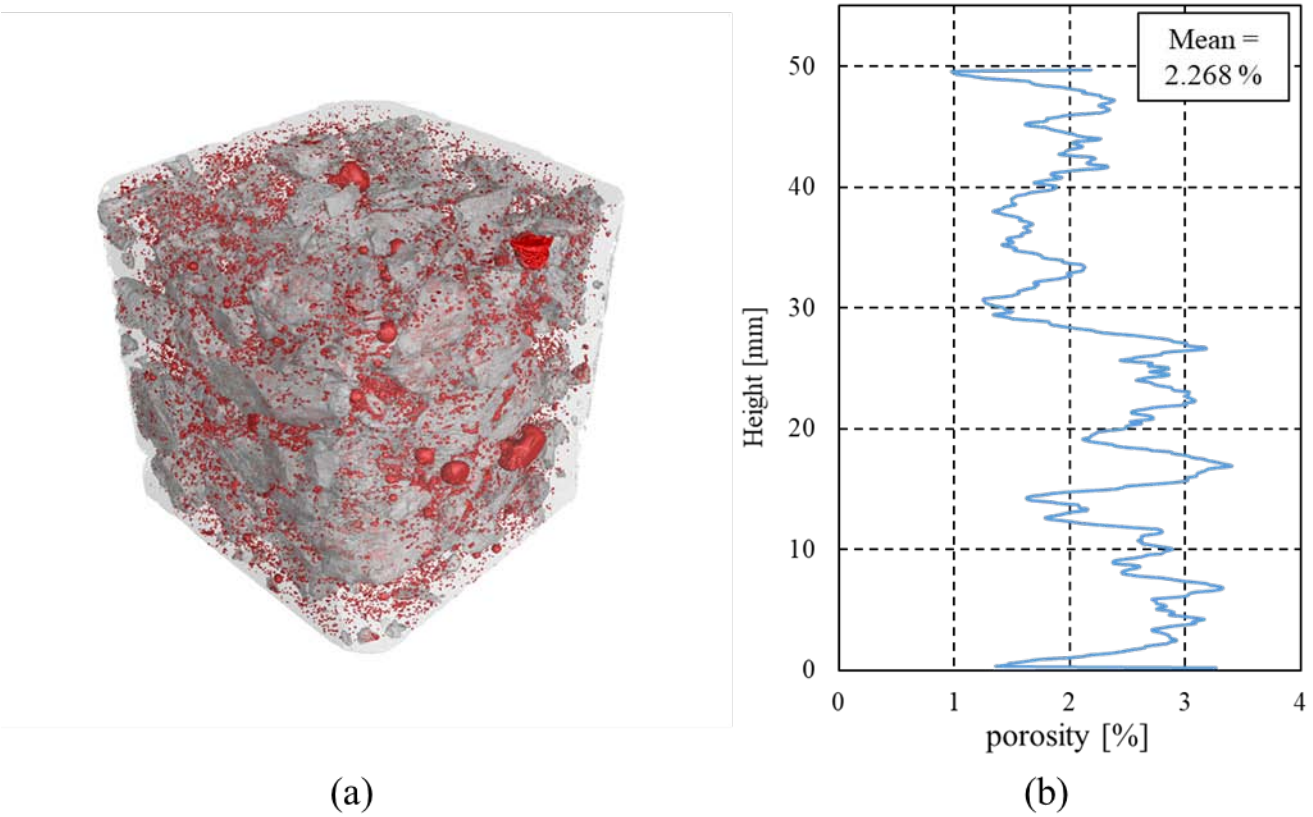

Figure 4: X-ray tomography of B20F30 mortar; (a) 3D image of mortar, (b) porosity with scan height

IV. Conclusion

In this study, the FNS cement with BOF fine aggregate mortar was investigated by the chemical microscopic analysis (X-ray fluorescence, X-ray diffraction) and pores distribution including an examination of X-ray CT analysis. Detailed experimental results and conclusion derived from the study are given as follows:

(1) The BOF aggregate has consisted of similar clinker of cement in XRD test results. For FNS, the composition mainly accounted for forsterite (Mg_2SiO_4) and fayalite (Fe_2SiO_4) which have crystalline nature. The low reactivity FNS contributed to the filler effect in the cement matrix while BOF aggregate clinker participated in the formation of hydration products. Moreover, there may be some reaction of BOF aggregate and cement matrix, due to similar clinker composition of BOF aggregate compared to OPC, there were no new hydrates found in B20F30 mortar.

(2) The apparent pore distribution and porosity were obtained by x-ray CT analysis at each height. The irregular shape of BOF aggregate resulted in large voids image in the 3D CT image. The large pores were detected in the 3D CT image while porosity with each height was 2.268%.

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Electrochemical Study by using Candesartan Drug as Green Corrosion Inhibitor of Aluminum Alloy in 1M HCL Medium

By Adel H. Ali

Taiz University

Abstract- The candesartan as a pharmaceutical drug compound was used as a corrosion inhibitor for Aluminum. Many techniques are utilized for the corrosion of the Aluminumin 1 M HCl such as weight loss (WL), open circuit (E_{OCP}), potentiodynamic polarization (PP) techniques, electrochemical impedance spectroscopy (EIS), electrochemical frequency modulation (EFM), and surface examination (SEM, EDX, and AFM) that used to analyze the surface of the metal before and after adsorption processes. WL is investigated at various temperatures between (25 – 45°C) but all electrochemical studies at room temperature. The inhibition efficiency (% IE) increased with increasing concentration of the Candesartan drug. The activation and adsorption parameters were calculated and illustrate the nature of the adsorption of candesartan drug on the metal surface.

Keywords: *corrosion inhibition; adsorption; electro-chemical techniques; SEM; EDX; AFM.*

GJSFR-B Classification: *FOR Code: 030604*

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Adel H. Ali

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Keywords: corrosion inhibition; adsorption; electro-chemical techniques; SEM; EDX; AFM.

I. Introduction

Aluminum is widely used as a material in automobiles, aviation, household appliances, containers, and electronic devices [1]. The resistance of Aluminum against corrosion in aqueous media is attributed to the rapid formation of oxide films on the surface. However, Aluminum gets easily corroded in the presence of corrosive acids [2]. Studies of the corrosion behavior of Aluminumin different aggressive environments have continued to attract attention because of its important applications. Hydrochloric acid is one of the most widely used agents in the industrial sector and it corrodes metals such as Aluminum. There is a need to use inhibitors for retardation of the metal dissolution process [3].

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Among several techniques used in mitigating corrosion problems, the use of chemical inhibitors remains the most cost-effective and practical method [4]. The development of Aluminum corrosion inhibitors based on organic compounds is of growing interest in corrosion chemistry [5].

The reason for this is that even though inorganic substances like phosphates, chromates, dichromate, and arsenates were found to be effective as metal corrosion inhibitors, the major disadvantage is their toxicity. Such as their use has come under severe criticism [6]. Research has shown that organic inhibitors are viable, and highly beneficial because they are efficient, environmentally benign, and comparatively cheap and are more effective than inorganic compounds [7-11]. Numerous authors, for the most part, concur that medications are inhibitors that can compete favorably with green inhibition of corrosion and that most medications are synthesized from natural products. Selection of some medication as corrosion inhibitors due to the followings: (1) drug molecules contain oxygen, sulfur, and nitrogen as active sites, (2) it is environmentally friendly furthermore vital in organic responses, and (3) drugs are produced easily, and purified (4) nontoxic compering organic inhibitors [12].

The scope of this article is to use Candesartan drug as saving corrosion inhibitor for Aluminum in the acid medium by various chemical, and electrochemical methods to elucidate the mechanism of corrosion inhibition.

II. Experimental Techniques

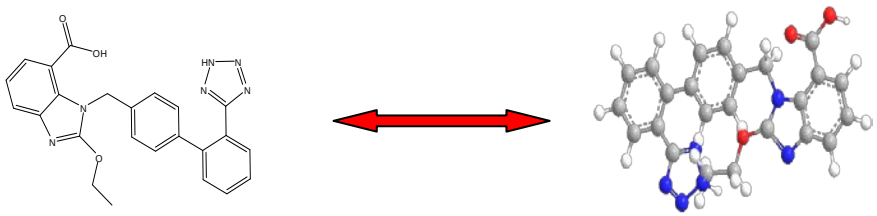
a) Chemical materials

i. Metal sample Aluminum

The Aluminum alloy used has the chemical composition (% weight) 0.30 Si; 0.60 Fe; 0.10 Cu; 1.40 Mn; 0.05 Mg; 0.05 Cr; 0.05 Ti and the rest Aluminum alloy.

ii. Inhibitor

Candesartan drug is an organic compound, which has the chemical formula $C_{24}H_{20}N_6O_3$ and purchased from Sandozinc and Pfizer in c companies.



2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] benzimidazole-4-carboxylic acid

iii. *Corrosive medium*

The corrosive medium is 1M HCl, which is prepared by diluted the (%37) HCl with distilled water, and the different concentrations of inhibitor (50, 100, 150, 200, 250, and 300 ppm) were prepared by dilution.

III. Methods

a) Weight loss technique (WL)

For WL estimations, coins have the area surface (2 cm x 2 cm) x 2 that presented to the destructive solution that was utilized. The coins were scraped with SiC polisher sheet coarseness sizes (400, 800, and 1200), and clean with (CH3)2CO. At that point, clean a few times with bi-distilled water, lastly dried by soft tissue. The WL estimations were done in a 100 ml glass-measuring beaker put in an indoor regulator thermostat or water path. The coins were then quickly dipped in the test medium in nonexistence and existence various concentration of the investigate compound.

All aggressive corrosive medium were opened to air. After three hour, the coins were taken out, washed, dried, and weighted correctly per thirty minutes. The average WL for seven square Aluminum specimens will be obtained .

The % IE and surface coverage (θ) of Candesartan drug for the corrosion of Aluminum were determinate from the following equation[13]:

$$IE\% = \theta x100 = [1-(W/W^{o})] \times 100 \quad (1)$$

Where W^{o} and W are the WLs in the nonexistence and existence of adding the various concentrations of investigating drugs, respectively.

ii. *Potentiodynamic polarization technique*

Polarization tests were done in a traditional three-electrode cell with a Pt counter electrode terminal and an immersed calomel electrode (SCE) as the reference electrode. The working electrode consists of a square sheet from Aluminum settled in epoxy resin like polytetrafluoroethylene, which does not affect by acid and covered the coin sample until the level surface that has 1.0 cm^2 area. The working terminal was polished by SiC polisher papers. Before estimation, the electrode was submerged in the corrosive medium at the normal potential for 30 min. until the point when an enduring or steady-state was occurring. The potential of the open circuit (E_{ocp}) started in the blank -533 mV, and the presence of different concentrations of Candesartan drug started from -515.7 to -490.5 mV. All tests were done in newly arranged or preparing corrosive medium at room temperature. The data were constantly rehashed no less than three times to check the accuracy or valid results. Determination of % IE, and the θ are calculated by the following equations[14]:

$$\%\, IE = \theta \times 100 = \left[1 - \frac{i_{corr\,(inh)}}{i_{corr\,(free)}}\right] \times 100 \quad (2)$$

Where $i_{corr(free)}$ and $i_{corr(inh)}$ are the corrosion current densities in the nonexistence, and existence of Candesartan drug as an inhibitor, respectively.

iii. *Electrochemical impedance spectroscopy technique (EIS)*

All EIS measuring data were performed at open circuit potential E_{ocp} at 25±1°C more than a broad frequency range of ($1x10^5$ Hz to x 0.1 Hz). The perturbation potential was ten mV in abundance, peak to peak. The obtain diameters of the capacitive loops raise in the occurrence of Candesartan drug. They are indicated of the capacity of the extent of inhibition of oxidation progression, contrary to the reducing of the capacitance of double layers (C_{dl}), which is determined by[15]:

$$C_{dl} = \frac{1}{(2\pi f_{max}\, R_p)} \quad (3)$$

Where f_{max} is the highest frequency.

The % IE, and the (θ) acquired the impedance estimations were characterized by the accompanying connection:

$$\%\, IE = \theta \times 100 = \left[1 - \frac{R_p^o}{R_p}\right] \times 100 \quad (4)$$

Where, R^{o}_{p} and R_p are resistance of the charge move in the nonattendance and nearness of Candesartan, separately.

iv. *Electrochemical frequency modulation technique*

From the large peaks were utilized for the determination of the corrosion current density (i_{corr}), the Tafel slopes (β_c and β_a), and the causality factors CF_2, and CF_3 [16].

The % IE_{EFM} was calculated as follows:

$$\% IE_{EFM} = \left[1 - \frac{i_{corr}}{i^{o}_{corr}}\right] \times 100 \quad (5)$$

Where i^{o}_{corr} and i_{corr} are the corrosion current densities in the nonexistence, and existence of Candesartan drug.

All potentiodynamic, open circuit potential, EIS, and EFM as electrochemical analysis were investigated using Gamry tool PCI300/4.

v. *Surface examinations*

The Aluminum specimens utilized for analysis of the surface morphology were prepared in 1M HCl acid (blank), and add 300 ppm of Candesartan at room temperature for one day after abraded automatically utilizing various emery sheets up to 1200 gravel size. Then, the coins were dipped in the corrosive medium at even time; the coins were cleaned quietly with distill water, charily dried, and mount into the performed specimens examined by using SEM, EDX, and AFM.

IV. Result and Discussion

a) Weight loss measurement

The WL of Aluminum relative to the surface area at different periods in the nonexistence, and existence of various doses (50, 100, 150, 200, 250, and 300 ppm) of the Candesartan. The curves acquired within sight of various drug doses fall essentially underneath that of the free corrosive medium is appeared. The % IE is recorded in Table 1. In all cases, the %IE of the drug increment with increasing concentration of the drug, but the rate of corrosion was decreased. These results indicated that the Candesartan under study is the good substance that prevents corrosion of Aluminumin corrosive medium Fig.1.

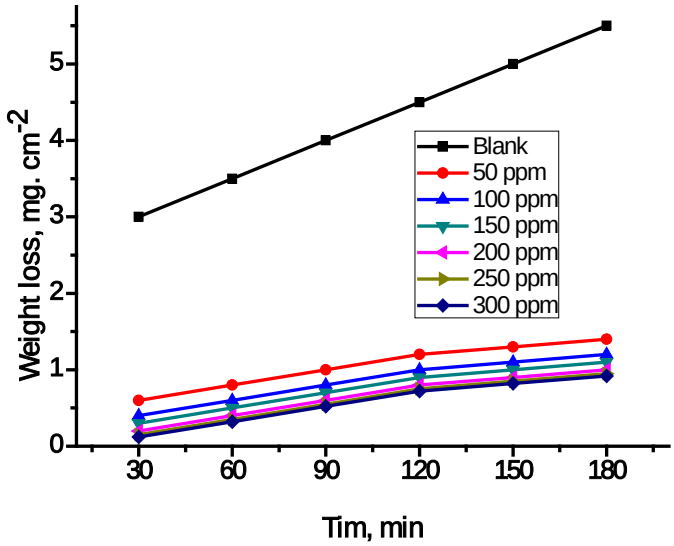


Fig. 1: W L-time bending curves for the oxidation of Aluminumin the nonexistence, and existence of various concentrations of Candesartan drug

Table 1: Variation of % IE of Candesartan with various concentrations from WL testing at 120 min. dipping in 1 M hydrochloric acid

Compound	Conc. ppm	WL(mg/cm^2)	C. R. x 10^{-3}(WL/min.)	θ	% IE
Blank	Blank	4.5	37.5		
Candesartan	50	1.20	10.0	0.733	73.3
	100	1.00	8.3	0.778	77.8
	150	0.90	7.5	0.800	80.0
	200	0.80	6.7	0.822	82.2
	250	0.75	6.3	0.833	83.3
	300	0.72	6.0	0.840	84.0

i. *Temperature effect*

Study the effect of temperature by applied Arrhenius equation and the rate constant of corrosion ($k_{corr.}$) can be determined:

$$\log k_{corr} = A - \left[\frac{E_a}{2.303\ RT}\right] \quad (6)$$

Where E_a^* is the activation of inhibition for the oxidation of Aluminum in the corrosive medium in nonexistence and existence of Candesartan inhibitor, R is universal gases constant, T is the absolute temperature and A is the Arrhenius pre-exponential consistent relies upon the metal sort and electrolyte. Plots of log k_{corr} versus (1/T) for Aluminumin 1 M hydrochloric acid in the nonexistence and existence of various doses of Candesartan drug is shown graphically in Fig. 2. Gives straight lines and the estimation values of E_a^* from the slope value that equal ($-E^*_a$ /2.303R), and recorded in Table 2. These outcomes propose that the drug is comparative in the system of activity. It is obvious that the E^*_a increases with increasing various doses of Candesartan drug indicating that, the energy barrier for the corrosion reaction increased. After additionally shown that the entire procedure is controlled by the relative of surface corroded occurs, since the activation energy of the consumption oxidation process is more than 24.9 kJ mol^{-1}[17].

The (ΔS^*, ΔH^*) of activation are determinate from the theory of transition state by applying the following relation (10)[18]:

$$k_{corr} = \left[\frac{RT}{Nh}\right] \exp\left(\frac{\Delta S^*}{R}\right) \exp\left(\frac{-\Delta H^*}{RT}\right) \quad (7)$$

Where N is Avogadro's number, h is Planck's constant. A plot of log (k_{corr}/T) versus (1/T) likewise gave straight lines as appeared in Fig. 3, for Aluminum dissolution in 1M of HCl in the absence and presence of various concentrations of Candesartan. The slopes of these lines equal ($-\Delta H^*$/2.303R) and the intercept value equal [log (R/Nh) + (ΔS^*/2.303R)], that the estimation of ΔH^* and ΔS^* were determined, and recorded in Table 2. These outcomes demonstrate that the used compound acts as an inhibitor. The estimations of ΔH^* are reflected the strength of the adsorption of this drug on Aluminum surface. The estimations of ΔS^* without and with using Candesartan is large and negative; this demonstrates that the rate-determination step is an association on the surface of the Aluminum rather than dissolution[19].

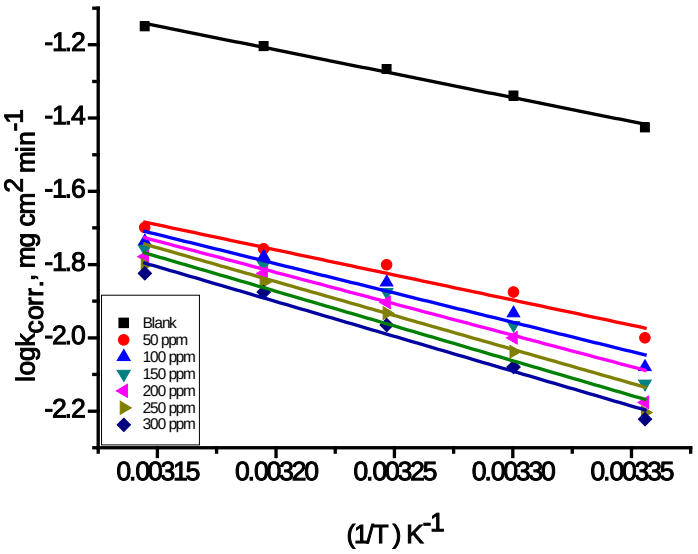


Fig. 2: Diagram (log k_{corr} vs. 1/ T) for oxidation of Aluminumin HCl acid in the nonexistence and existence of various doses of Candesartan

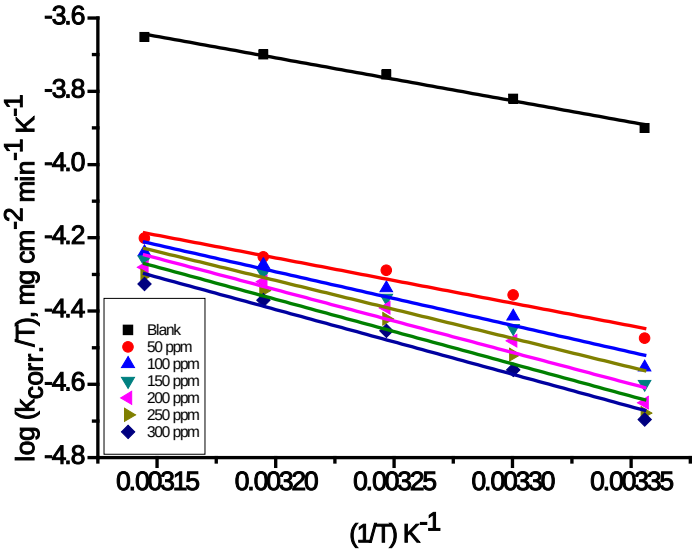


Fig. 3: Diagram (log k_{corr} / T) vs. (1/ T) for oxidation of Aluminumin 1 M HCl in the nonexistence and existence of variant doses of Candesartan

Table 2: E^*_a, ΔH^* and ΔS^*variables for the oxidation of Aluminumin 1 M hydrochloric acid in the nonexistence and existence of variant doses of tested drug

Conc. Ppm	Activation parameters		
	E^*_a kJ mol^{-1}	ΔH^* kJ mol^{-1}	$-\Delta S^*$ J mol^{-1} K^{-1}
Blank	24.9	22.4	196.9
50	26.23	23.7	203.3
100	30.6	28.0	190.1
150	32.8	30.3	183.4
200	35.4	32.9	175.5
250	36.3	33.7	173.2
300	36.4	33.8	173.4

ii. *Adsorption isotherms*

Candesartan adsorbed on the metal surface, and the values of (θ) for various doses of the drug in 1M HCl was determined from WL data utilizing the follows relation:

$$\theta = \left[\frac{weight\ loss_{(pure)} - weight\ loss_{(inh)}}{weight\ loss_{(pure)}}\right] \quad (8)$$

From the Θ values, it is obvious that the increment with raising the doses of Candesartan. By utilizing these values, and for applying various adsorption isotherms, Langmuir adsorption was found the best one and to follow the next relation[20]:

$$C/\theta = 1/K_{ads} + C \quad (9)$$

Where K_{ads} is the equilibrium constant of adsorption. Plotting (C/Θ) against (C) of Candesartan at various temperatures is shown in Fig. 4. The linear relationship is given with intercept equal to ($1/K_{asd}$), and slope approach the unity. The ΔG°_{ads} values were determined by the equation (13):

$$\Delta G^{o}_{ads} = -RTln\,(55.5\,K_{ads}) \quad (10)$$

Where R is the general gas constant, T is the absolute temperature and 55.5 is the concentration of water in the solution in M/L. The ΔH°_{ads}) was determined according to the Van't Hoff equation (14)[21]:

$$\log k_{ads} = \left(\frac{-\Delta H^{\circ}_{ads}}{2.303RT}\right) + constant \quad (11)$$

Plotting (log K_{ads}) against (1/T) give straight lines as shown in Fig. 5, the straight lines gives slope equal ($-\Delta H^{\circ}_{ads}/2.303R$), from this slope, the ΔH°_{ads} values were calculated, and are listed in Table 3. Then by applying the following equation:

$$\Delta G^{\circ}_{ads} = \Delta H^{\circ}_{ads.} - T\Delta S^{\circ}_{ads.} \quad (12)$$

From introducing the values of ΔG°_{ads}, ΔH°_{ads}, and ΔS°_{ads} were calculated by applied the above equations (13, 14 and 15) spontaneously. From all thermodynamic adsorption parameters for Candesartan

inhibitor on Aluminum from 1M HCl medium can be concluded that:

1. The correlation coefficients (0.99) reflected that the experimental data give good curves fitting for the adsorption isotherm, and K_{ads} values increase with increasing temperatures from 25 to 45°C
2. The negative values of ΔG°_{ads} reflect that the adsorption of Candesartan on Aluminum surface in 1 M HCl solution is a spontaneous process. The ΔG°_{ads} values are more negative than -20.8 kJ mol^{-1} lead to the Ven Der Waal's forces or electrostatic attraction between the positive charge of the metal surface and the negative charge of Candesartan molecules in the bulk of the medium and formation thin film adsorbed on the metal surface. i.e. physical adsorption.
3. The positive sign of ΔH°_{ads} and less than 20Kj/molrefer to the adsorption of inhibitor molecules is an endothermic process, indicating that the adsorption is physical adsorption. The unshared pairs electron from the investigate molecule may attractive with a positive center on the surface of Aluminumby electrostatic attraction to provide a protective film prevent corrosion process[22].
4. The ΔS°_{ads} values, in the existence of the investigate drug are positive and large and decreases with increasing temperatures tend to more order tend to chemisorption.

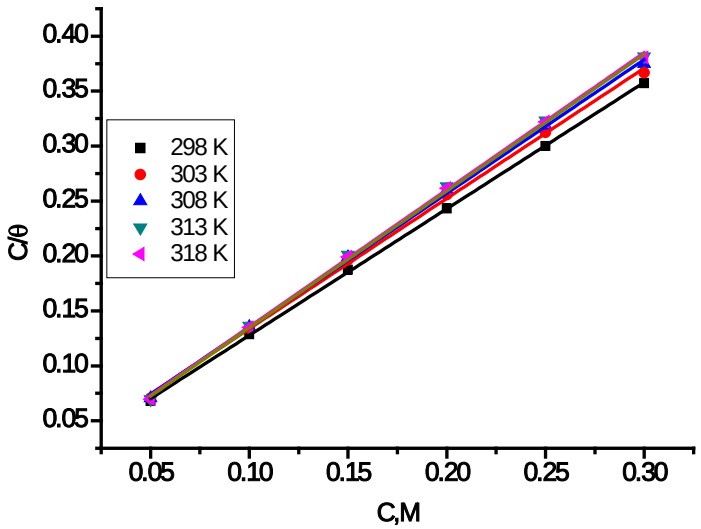


Fig. 4: Diagram illustrates the Langmuir adsorption that plotted (C) against (C/θ) of the Candesartan drug for corrosion of Aluminumin 1 M HCl from WL technique at 25°C

Table 3: K_{ads} and adsorption free energy (ΔG°_{ads}) for the adsorption of Candesartan drug on Aluminumin 1 M hydrochloric acid from WL method at 25°C

Temp.' °C m	K_{ads} M^{-1}	$-\Delta G^{\circ}_{ads}$ kJ mol^{-1}	ΔH°_{ads} kJ mol^{-1}	ΔS°_{ads} J $mol^{-1}K^{-1}$
25	79.2	20.8	11.9	109.7
30	65.9	20.7		107.5
35	75.9	21.4		108.0
40	92.3	22.2		109.0
45	98.2	22.7		108.9

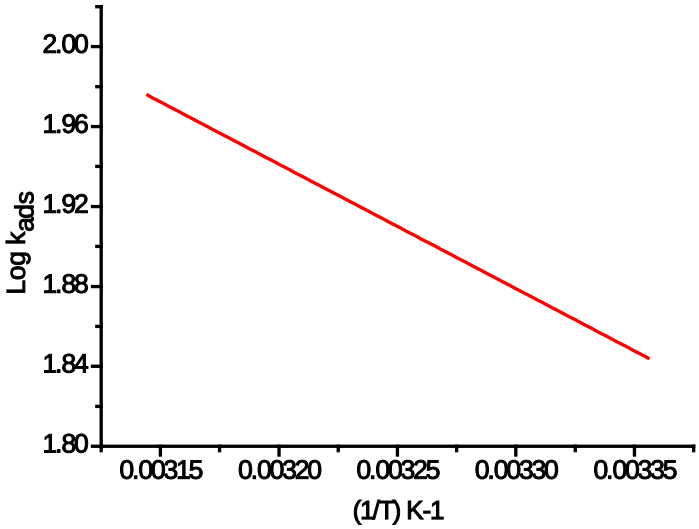


Fig. 5: Plotting (log K_{ads}) vs. (1/T) for the corrosion of Aluminumin 1M HCl in the existence of Candesartan at various temperatures

b) Open circuit potential (E_{ocp})

From the Fig.6 is shown several interesting points:

1. The E_{OPC} in the blank solution is beginning from -535 mV, then shifted anodically, and reached a steady state after 300 seconds, indicating that the initial dissolution process, and formation of oxide film on the metal surface.
2. The E_{OCP} is started in the existence of Candesartan drug, at less negatively potential(515.7, 507.4, 503.9, 500.8, 495.3 and 490.5) compared with that in the nonexistence of the drug, and shifted anodically, due to the increasing of concentrations of the drug, that shown in Table 4. The steady-state is attained rapidly, with increasing the doses of the drug comparing with the blank. The shift in the potential of E_{OCP} increment in the positive direction position and the drug might certain act as an anodic inhibitor [23]. However, from Fig.6, the shift in E_{OCP} on the addition Candesartan drug is about 57.5 mV revealing that the present drug acts as anodic inhibitor.

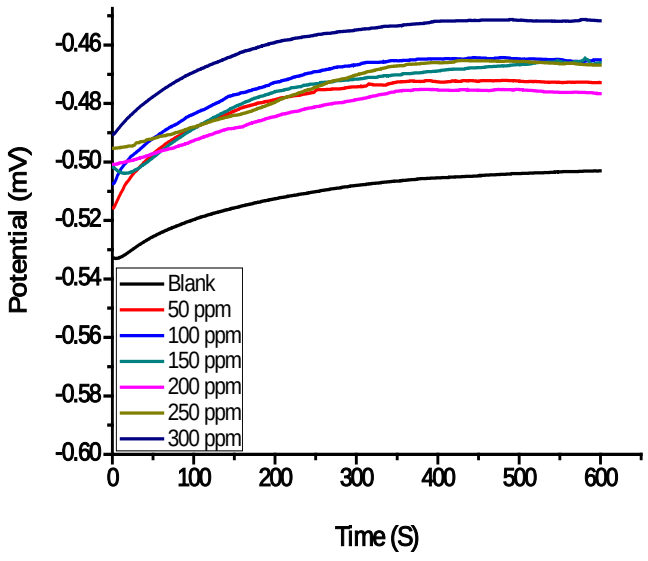


Fig. 6: Open circuit potential, E_{OCP} vs. time relations for Aluminum immersed in 1M HCl in the nonexistence and, the existence of Candesartan drug

Table 4: Open circuit potential of the Aluminumin nonexistence and in the existence of Candesartan drug

Conc. Ppm	$-E_{Min}$ (mV)	$-E_{Max}$ (mV)
Blank	535.0	505.0
50	519.7	472.1
100	503.4	466.3
150	500.9	464.5
200	500.8	463.2
250	495.3	460.2
300	490.5	451.3

c) Potentiodynamic Polarization technique(PP)

Polarization technique is carrying out in 1 M HCl acid mediumin the nonexistence and existence of different concentrations of Candesartan drug at 25°C. The results are collectedin Table 5, and drowning in Fig. 7, respectively. These outcomes information showing that the cathodic, and anodic curve lines obtained by Tafel-type behavior. The type of the curved lines is the same, which shows the dissolution of Aluminum and hydrogen evolution, obviously, stay without changing. The corrosion current densityre ducing with increasing the concentration of the drug, but the small change of the E_{corr} revealed that the drug acts as mixed type inhibitor[24]. The information data additionally demonstrate that the anodic and cathodic Tafel slope (β_a & β_c) were slightly changed with increasing of the concentration of the drug. This demonstrates that there is no change of mechanism, but % IE increases with increasing the concentration of Candesartan drug. The way of the approximations of β_c are somewhat higher than the approximations of β_a. This is attributed to the cathodic activity of the drug. The steadiness and the cathodic slope obtained from the electrochemical estimations demonstrate that the hydrogen evolution reaction was activation controlled [25].

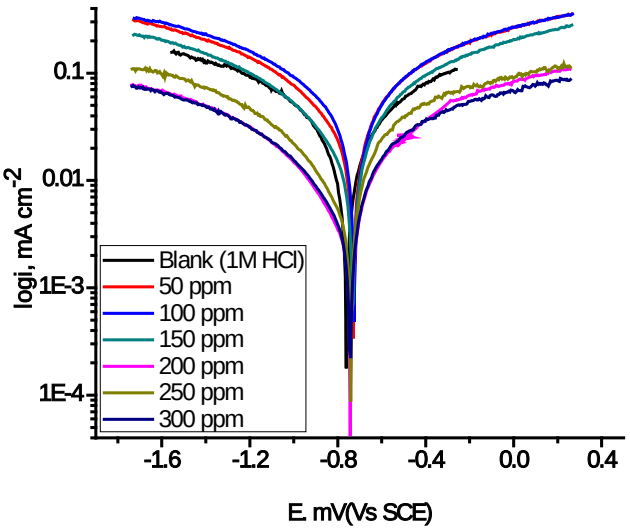


Fig. 7: The PP curves for the oxidation of Aluminumin 1M HCl in the nonexistence and existence of varied doses of Candesartan

Table 5: The effect of doses of Candesartan on the E_{corr}, i_{corr}, Tafel slopes (β_a& β_c), % IE, and Θ for the oxidation of Aluminumin 1M HCl

Conc. Ppm	$I_{corr.}$ mA/cm^{-2}	$-E_{corr.}$ mV(SCE)	β_ax10^{-3} mV dec^{-1}	β_cx10^{-3} mV dec^{-1}	C. R. mpy	θ	% IE
0	145	502	242	995	60	----	----
50	63.2	484	122	276	26	0.564	56.4
100	58	447	83	191	24	0.6	60
150	52	450	79	188	22	0.641	64.1
200	47	455	78	184	19.5	0.676	67.6
250	41	439	75	170	17	0.717	71.7
300	38	442	71.6	164	16	0.738	73.8

d) Electrochemical impedance spectroscopy technique(EIS)

The (EIS) charts (Nyquist and Bode) and data at various frequencies range between 0.1 Hz to 10^5 Hz with ten mV plenitude position at OCP for Aluminumin 1M HCl acid medium in the nonexistence and existence of varied measurements of Candesartan drug concentration are required. The identical circuit that describes for Aluminum and electrolytes are found in Fig. 8: EIS parameters, and % IE were determined, and listed in Table 6. The obtained Nyquist, and Bode plotting in curves for Candesartan drug are shown in Fig. 9 a, b. Nyquist spectra are described by a semicircle loop. These demonstrate that a charge transfer processrefer to the oxidation of Aluminummetal [26]. The diameter (R_p) of the capacitive circle loop increments, with increasing the Candesartan drug concentration, these means the expanding inhibition efficiency, and reducing the consumption oxidation process [27]. More details, the increase of R_p values improve the increase of the %IE because of the progressive replacement of water molecules by the adsorption of the medication particles on the metal surface by an adherence thin film formed on the metal surface. The formation film on the metal surface reduced the double layer thickness. Also, the decreasing of capacitance double layer (C_{dl}) with rises the drug doses as a result from reduce in local dielectric constant which indicating that, the drug was adsorbed on anodic sites, and covered the cathodic sites on the surface area of the metal[28].

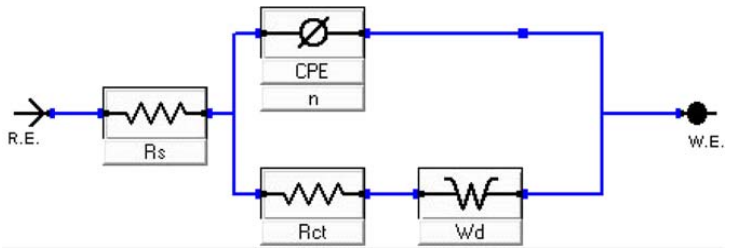
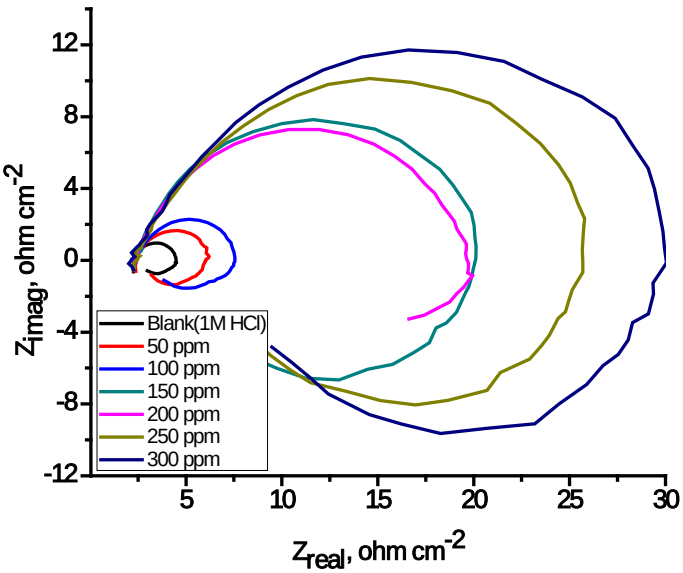
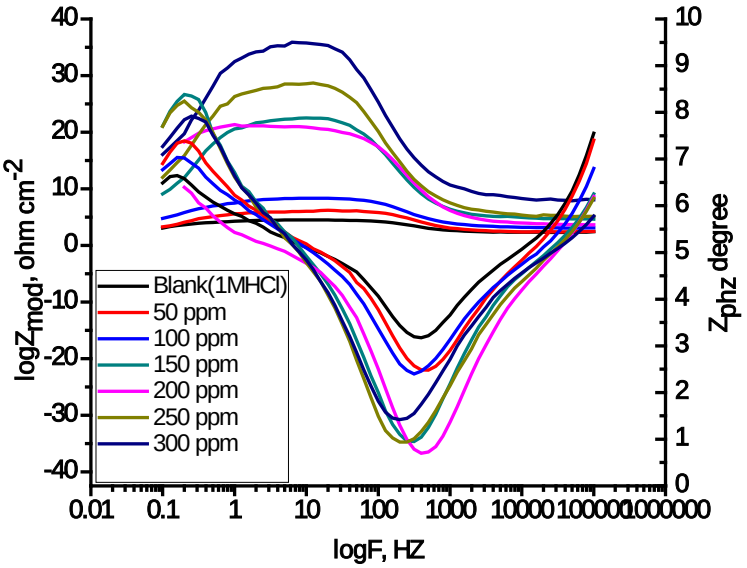


Fig. 8: Circuit model used to fit the experimental data, R_s refer to solution resistance and R_p or R_{ct} charge transfer resistance, respectively



(a)



(b)

Fig. 9: The Nyquist (a) and Bode (b) plots for oxidation of Aluminumin one 1M HCl in the nonexistence and existence of various doses of Candesartan

Table 6: Electrochemical kinetic variables occur by EIS technique for oxidation of Aluminumin 1M HCl nonexistence and existence various doses of Candesartan

Conc. ppm	R_p Ω cm²	C_{dl} μF cm²	θ	% IE
0	1.8	22	---	---
50	2.8	5.5	0.357	35.7
100	3.1	4.2	0.419	41.9
150	4	3	0.55	55
200	6.1	2.8	0.705	70.5
250	7.2	2.4	0.75	75
300	11	2.1	0.836	83.6

e) Electrochemical frequency modulation technique (EFM)

EFM is regarded as a very good technique to determine corrosion information directly and quickly because EFM is a nondestructive technique to determine corrosion [29]. The measurements data of EFM become valid data when the practical causality factors (CF2 and CF3) are equals or near the hypothetical values (2 and 3), which determination from the frequency spectrum of the current reaction. Fig. 10, illustrated the EFM inter-modulation spectrum of Aluminumin 1 M HCl in nonexistence and existence deferent concentrations of Candesartan drug. It is clear that the treatment EFM data utilizing two various models: (1) the activation model by solved three nonlinear equations, and assuming no change of the corrosion potential due to the polarization of the working electrode (2) cathodic reaction controlled by complete diffusion[30]. The corrosion currentdensity (i_{corr}), the (β_a and β_c), and (CF2 and CF3) are calculated from the two large peaks of inter-modulation spectrum, and then listed in Table 7. It is obvious, that the addition of tested Candesartan drug at given concentrations to the corrosive medium reducing the (i_{corr}), indicating that the Candesartan drug inhibits the corrosion of Aluminum by the adsorption process. The (CF2 and CF3) are equal, or near the hypothetical values (2 and 3) indicative of that. The estimation information data are valid and good value [31]. The % IE $_{EFM}$ values are increments by expanding the concentrations of Candesartan drug, which determined and recorded in Table 7.

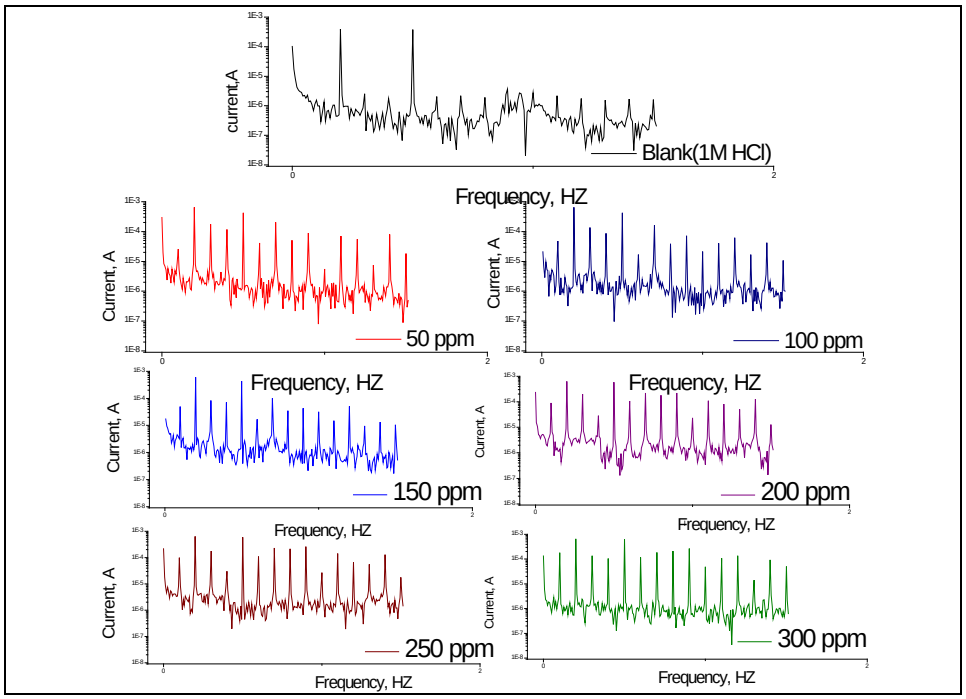


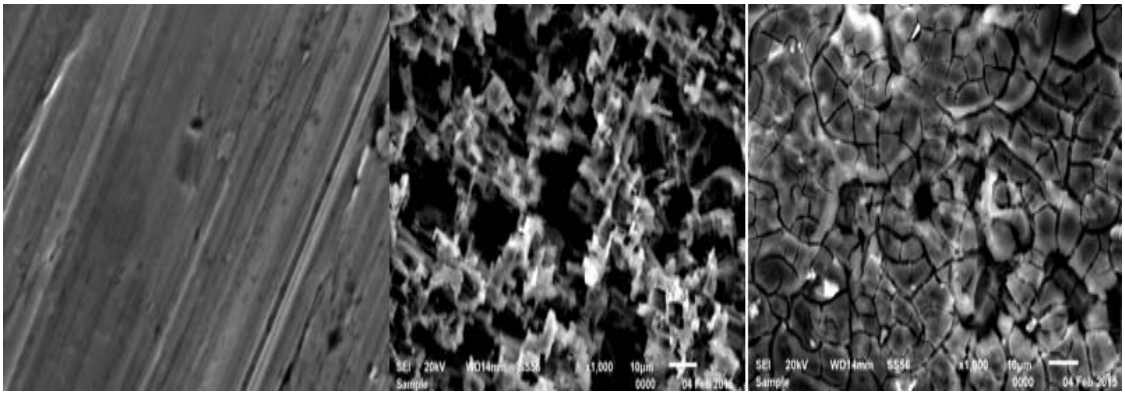
Fig. 10: EFM for Aluminumin 1M HCl with and without various doses of the used Candesartan

Table 7: Electrochemical kinetic variables occur by EFM method for Aluminumin 1 M HCl nonexistence and existence different doses of Candesartan

Comp.	Conc. Ppm	$i_{corr.}$ μAcm^{-2}	$\beta_a \times 10^{-3}$ $mVdec^{-1}$	$\beta_c \times 10^{-3}$ $mVdec^{-1}$	CF (2)	CF (3)	CR Mpy	Θ	% IE
Blank	0.0	140	182	195	1.9	3.3	61.1	----	----
Candesartan	50	80	32	102	1.8	4	37.7	0.429	42.9
	100	70.2	31	66	1.8	3.7	31.9	0.499	49.9
	150	60.3	34	55	2	2.9	30.5	0.569	56.9
	200	49.8	24	36	1.7	3.9	26.1	0.644	64.4
	250	40	19	33	2.1	4	24.2	0.714	71.4
	300	32	18	25	1.8	2.7	14.4	0.771	77.1

f) Scanning electron microscopy analysis (SEM)

The micrograph or the topography obtains on the Aluminum coins in existence and in nonexistence of 300 ppm of Candesartan inhibitor after contact for one day submersion. The coin morphology of Aluminum surface is smooth before immersion in the corrosive medium and in corrosive medium with 300 ppm of Candesartan inhibitor. It is obvious that inter-granular corrosion occurs due to the coin immersion in a corrosive medium for only one day. After that, the coin immersion in the corrosive medium after adding 300 ppm of inhibitor, that adsorbed on the surface of metal, and formation thin passive film that prevent corrosion processes, due to the development film that aspersion on the whole surface of the Aluminum, Fig. 11, This means, the inhibitor particles association with dynamic locales on the Aluminum surface, that is reducing the contact between Aluminum surface, and the destructive medium [32, 33].



(a) Free sample (b) Blank (in 1M HCl only) (c)In 1M HCl with 300 ppm of Candesartan

Fig. 11 a, b and c: SEM micrographs for Aluminumin the nonexistence and existence of 300 ppm of Candesartan after immersion for one day

g) Energy Dispersive X-ray analysis (EDX)

Determination the existence elements that adsorbed on Aluminum surface by EDX spectrum after one day immersion in 1M HCl with optimum concentration of Candesartan inhibitor.

Fig. 12, gives the EDX examination of Aluminumin 1M HCl and the presence 300ppm of Candesartan inhibitor. The spectrum demonstrates extra lines, showing C, N, and O (inferable from the chemical structure of Candesartan drug). This information demonstrates that the C, N, and O atoms secured the coins surface.

The elements carbon, nitrogen and oxygen werethe determination by EDX analysis and shown that protective film contained the chemical formula of Candesartan inhibitor adsorbed on the surface of Aluminum[34].

It is seen that the percent weight of adsorbing elements C, N, and O were present in the spectra and recorded in Table 8.

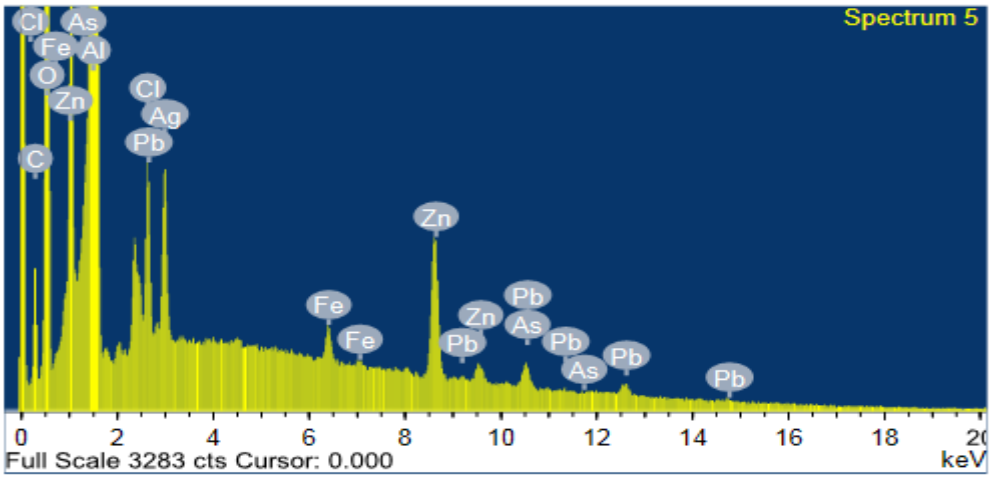


Fig. 12: EDX examination on Aluminumin the existence and nonexistence of Candesartan drug for one day submersion

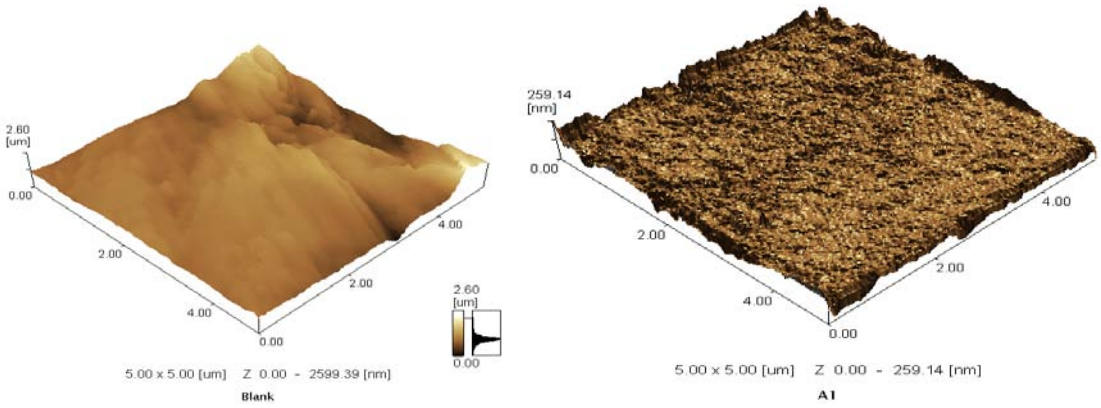
Table 8: Surface composition (% wt) of Aluminum after one day of submersion in 1M HCl nonexistence and existence the 300 ppm of Candesartan

(% Wt)	AL	C	O	N	Cl
Candesartan	55.12	8.5	32.1	2.5	0.56

h) Atomic force microscopy analysis (AFM)

From AFM analysis, it can be gained regarding the roughness on the surface. The roughness profile values play an important role in identifying and report the efficiency of the inhibitor under study. Among the roughness, take a role in explanation about the nature of the adsorbed film on the surface [35, 36]. Fig. 13, shows the 3D images as well as elevation profiles of polished of Aluminumin absence and presence Candesartan as an inhibitor. It observed that the surface of Aluminum specimen (a) exposed to corroded solution affected vales structure with large and deep crack but the

surface (b) reveal that is covering thin film adsorbed on the metal surface. The conclusion that the adsorption film can protected the surface of the metal from corrosion processes [37]. Analysis of the values indicating that the higher values of roughness parameter in corrosive medium than in the presence, 300 ppm of inhibitor drug which becomes less roughness values and according to the Z value of the image that be found (2.60 μm) for the blank in acid solution which placed in 1M HCl one day and analyzed but (259.14 nm)that observed of the image of the metal surface which immersion in 1M HCl in presence of 300 ppm of Candesartan as an inhibitor that becomes less roughness. The diminish in the roughness value reflected the adsorption of Candesartan molecule as a thin film on the metal surface and prevent the corrosion processes, in other hand reducing the rate of corrosion and increasing the inhibition efficiency.



(a) (b)

Fig. 13: The 3D of optical images of AFM in nonexistence (a) and existence (b) of Candesartan

i) Mechanism of inhibition

To illustrate the mechanism of inhibition of corrosion on the Aluminum surface in acid medium by using Candesartan compound as an inhibitor, it is must be know the nature of metal surface and the nature of the component of inhibitor structure.

All previous results prove that the Candesartan inhibitor compound under study was actually inhibiting the corrosion of Aluminumin 1M HCl acid solution as a corrosive medium. The corrosion inhibition is due to their physical and chemical adsorption that formation of protection thin film adsorbed on the metal surface. The effect of Candesartan inhibitor compound under study may be corresponding to the accumulation of the inhibitor molecules on the metal surface, which prevent the direction contact of the metal surface with corrosive environment. The surface of the Aluminum sample is positively charge in aqueous acid solution [38, 39]. The partial negative charge that presence in function group (O and N) and electronic density of benzene ring in Candesartan molecules under study, which adsorbed on the positively charge of the metal surface. Like electrostatic attraction between the opposite charge, in the form of neutral molecules, that involving displacement of water molecules from the metal surface and sharing electrons between electron density of π bonding, oxygen and nitrogen to vacant orbitals on the metal surface on anodic side and the skeleton of inhibitor compound cover the cathodic sites this action form thin layer film adsorbed on the metal surface that prevent the corrosion processes [40], Fig. 14. The present of benzene ring, this has electrons density and π-bonding that enhancement the adsorption process and gives the very good inhibition efficiency.

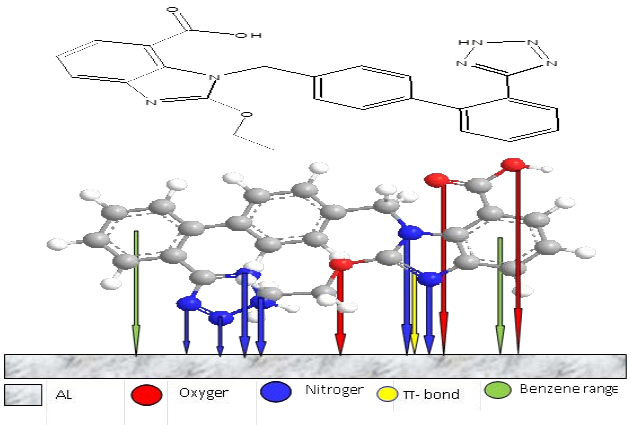


Fig. 14: Schema model illustrated the mechanism of the adsorption of Candesartan structure on the Aluminum surface

This meaning, the Candesartan molecule attached with anodic site and covered somewhat of cathodic area, so that the corrosion rate in presence of Candesartan is anodic-cathodic control.

V. Conclusion

Inhibition of the corrosion of Aluminumin 1M HCl solution by Candesartan is determine by weight loss, potentiodynamic polarization measurements, electrochemical impedance spectroscopy (EIS) and the electrochemical frequency modulation method (EFM). The surface of Aluminumexamination by Scanning Electron Microscopy (SEM), energy Dispersive X-ray (EDX) and atomic force microscopy (AFM). It was found that the inhibition efficiency depends on concentration, nature of metal, the mode of adsorption of the inhibitor and surface conditions. The observed corrosion data in presence Candesartan inhibitor, namely:

1) The tested Candesartan inhibitor establish a very good inhibition for Aluminum corrosion in 1M HCl solution
2) Candesartan inhibit the Aluminumfor the corrosion by adsorption on its surface and make thin film layer.
3) The inhibition efficiencies of the tested compound increase with increasing of their concentrations.
4) Double layer capacitances decrease with increasing concentration of inhibitor. This fact may explained by adsorption of the inhibitor molecule on the Aluminumsurface.
5) The adsorption of Candesartan inhibitor on the Aluminumsurface in1M HCl solution applied by Langmuir adsorption isotherm.
6) The values of inhibition efficiencies obtained from the different independent techniques used, showed the validity of the obtained results.
7) The Candesartan molecule attached with anodic site and covered somewhat of cathodic area, so that the corrosion rate in presence of Candesartan is anodic-cathodic control.

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Review Article: Applications and Methods of Preparation of Iron Oxide Fe_3O_4 Nanoparticle

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Abstract- Metal Oxide Nanoparticles are of interest in modern research and applications. Fe_3O_4 nanoparticles have interesting properties that can be manipulated in a various applications,some of these applications represent an advanced technology to biological and medical sciences [1-5], drugs and gene mapping [6-9], Detection of proteins moieties [10],Tumour removal [11,12] bioanalysis of pathogens [13], Tissue analysis [14], monitoring of DNA structure[15,16],and Magnetic Resonance Imaging resolution improvement [18-23] chromatographic separation and purification of molecules and biological cells [24].The purpose of this review is to summarize the different methods of preparations of Fe_3O_4 nano particles and describes these methods of preparation allowing control of the size, morphology, and surface treatment. These nanoparticles can be prepared by different methods such as Gas, liquid, two-phase methods, Sol-gel methods, High pressure hydrothermal methods and Co-precipitation Method.

GJSFR-B Classification: *FOR Code: 259999*

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Review Article: Applications and Methods of Preparation of Iron Oxide Fe_3O_4 Nanoparticle

Marwa Yousef Freshik [α] & Fathi Mohammed Asseid [σ]

Abstract- Metal Oxide Nanoparticles are of interest in modern research and applications. Fe_3O_4 nanoparticles have interesting properties that can be manipulated in a various applications,some of these applications represent an advanced technology to biological and medical sciences [1-5], drugs and gene mapping [6-9], Detection of proteins moieties [10],Tumour removal [11,12] bioanalysis of pathogens [13], Tissue analysis [14],monitoring of DNA structure[15,16],and Magnetic Resonance Imaging resolution improvement [18-23] chromatographic separation and purification of molecules and biological cells [24].The purpose of this review is to summarize the different methods of preparations of Fe_3O_4 nano particles and describes these methods of preparation allowing control of the size, morphology, and surface treatment. These nanoparticles can be prepared by different methods such as Gas, liquid, two-phase methods, Sol-gel methods, High pressure hydrothermal methods and Co-precipitation Method.

I. Introduction

Iron(III)oxide Fe_3O_4a ferromagnetic metal oxide is one of several iron oxides and a member of the spinel structural group (of inverse type)[25,26]. The systematic name is iron (II, III) oxide. Also known as magnetite mineral whose chemical formula can be written as $FeO \bullet Fe_2O_3$, a composition of wustite (FeO) and hematite (Fe_2O_3). This formular eflects the different oxidation states of the iron in the spinel inverse type structure.

The dimensions of iron oxide nanoparticles are generally described by its diameters that are between 1 and 1000 nanometers. The forms magnetite (Fe_3O_4) and its oxidized form Maghemite (Fe_2O_3) are synthesized , well characterized, and attracted a number of research groups due to their magnetic properties and potential for use in many technical applied sciences. These mineral do not pose any toxicity threats and environment friendly type of oxides with respect to other transition metal oxides, which are highly magnetic materials, known to be toxic and easily oxidized.

Row quality Fe_3O_4, usually called synthetic magnetite, can be prepared using by processing industrial wastes, and scrap iron or solutions containing iron salts (by-products of industrial processes such as the acid treatment of steel, through the following steps:

1. Oxidation of Fe metal by the Lucas process. Treatment of nitrobenzene with iron metal in presence of iron chloride, $FeCl_2$ as a catalystto form in Fe_3O_4

$$C_6H_5NO_2 + 9Fe + 2H_2O \rightarrow C_6H_5NH_2 + Fe_3O_4$$

2. Oxidation of Fe^{2+} compounds, to precipitate iron(II) salts as hydroxides followed by oxidation by aeration and the oxide produced determined by careful control of the pH.
3. Reduction of Fe_2O_3 with hydrogen [28]:

$$3Fe_2O_3 + H_2 \rightarrow 2Fe_3O_4 + H_2O$$

4. Reduction of Fe_2O_3 with CO:

$$3Fe_2O_3 + CO \rightarrow 2Fe_3O_4 + CO_2$$

Production of nano-particles can be done chemically by mixing salts of Fe^{II} and Fe^{III} and followed by addition of alkali to precipitate Fe_3O_4incolloidal form. Control of the conditions of the reaction are important to the determination of the particle size desired.

Magnetite exhibits a cubic inverse spinel structure in which oxygen forming a face centered cubic closed packing structure (Fig 2.6). With, the tetrahedral sites are occupied by Fe^{3+} and octahedral sites are occupied by both Fe^{3+} and Fe^{2+} ions. Maghemite and Magnetite minerals are different structurally from each other. In Magnetite mineral most of the iron ions are trivalent (Fe^{3+}) accompanied by the presence of cationic vacancies within the octahedral sites. Maghemite mineral exhibits a cubic unit cell, each cell contains 32 O ions, 21 1/3 Fe^{3+} ions and 2 1/3 vacancies. The iron ions are distributed randomly over the 8 tetrahedral and 16 octahedral sites.

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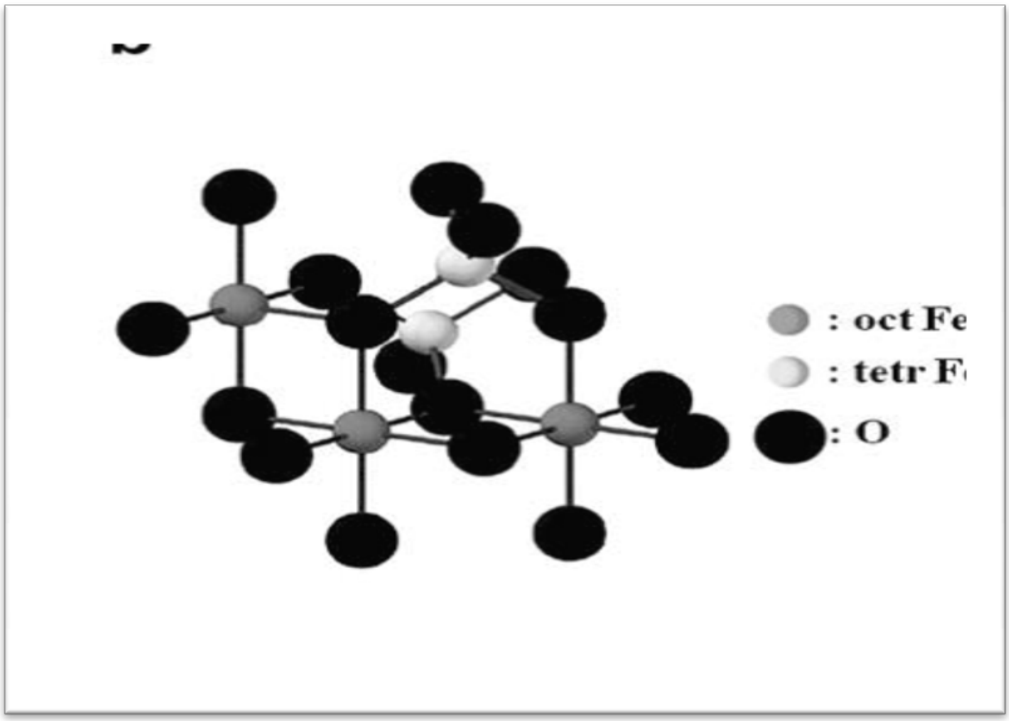


Fig. 2.6: Crystal structures of magnetite, Adapted from ref [27]

Fe_3O_4 is ferromagnetic with a phase transition at 120K, the so-called Verwey transition, in which there is a discontinuity in the structure, conductivity and magnetic properties. This effect has been extensively investigated with a number of explanations were proposed, however it is not fully clear. Fe_3O_4exhibits electrical conducting property with conductivity significantly higher (x 10^6) than that of Fe_2O_3, this is due to electron exchange between the Fe^{II} and Fe^{III} centers. Physical and magnetic properties for Magnetite are summarized in Table 2.3.

Table 2.3: Physical and magnetic properties of iron oxides

Property	Oxide (Magnetite)
Molecular formula	Fe_3O_4
Density(g/cm3)	5.18
Melting point (°C)	1583
Hardness	5.5
Type of magnetism	Ferromagnetic
Curie temperature (K	850
MS at 300 K (A-m2/kg)	92-100
Standard free	_1012.6
Crystallographic	Cubic
Structural type	Inverse spinel
Space group	Fd3m
Lattice parameter(nm)	a=0.8396

It is well known fact that iron atom has a strong magnetic moment as a result of four unpaired electrons in its 3*d*orbitals. Formation of crystals of iron atomsis due to different magnetic states as shown in Fig. 2.7.The paramagnetic state, usually arise paired electons in its 3d orbitals of the individual atomic magnetic moments randomly aligned with respect to each other, so the crystal has a zero net magnetic moment. However, if this crystal is exposed to an external magnetic field, some of these moments align, and the crystal will attain a small net magnetic moment. In the case of ferromagnetic crystal, all the individual moments are aligned without an external field effect. The ferromagnetic crystal, however has a net magnetic moment result from two types of atoms with moments of different magnetic strengths arranged anti parallely (Fig. 2.7). When anti parallel magnetic moments are similar in magnitude, the crystal is known as anti ferromagnetic and possesses net magnetic moment of zero.

Bulky ferromagnetic material, in which the magnetization *M* is the vector sum of all the magnetic moments of all the atoms per unit volume of the material. The magnitude of *M* is generally less than its value when all atomic moments are totally aligned, because the bulk material consists of domains, with each domain is having its own magnetization vector resulting from the alignment of atomic magnetic moments within the domain (Fig. 2.8). The magnetization vectors of all the domains in the material may not be aligned, and this leads to a decrease in the overall magnetization (Fig. 2.8). During the length scale of the material becomes small, the number of domains decrease until there is a single domain at which the characteristic size of the material is below some critical size d_C [27].

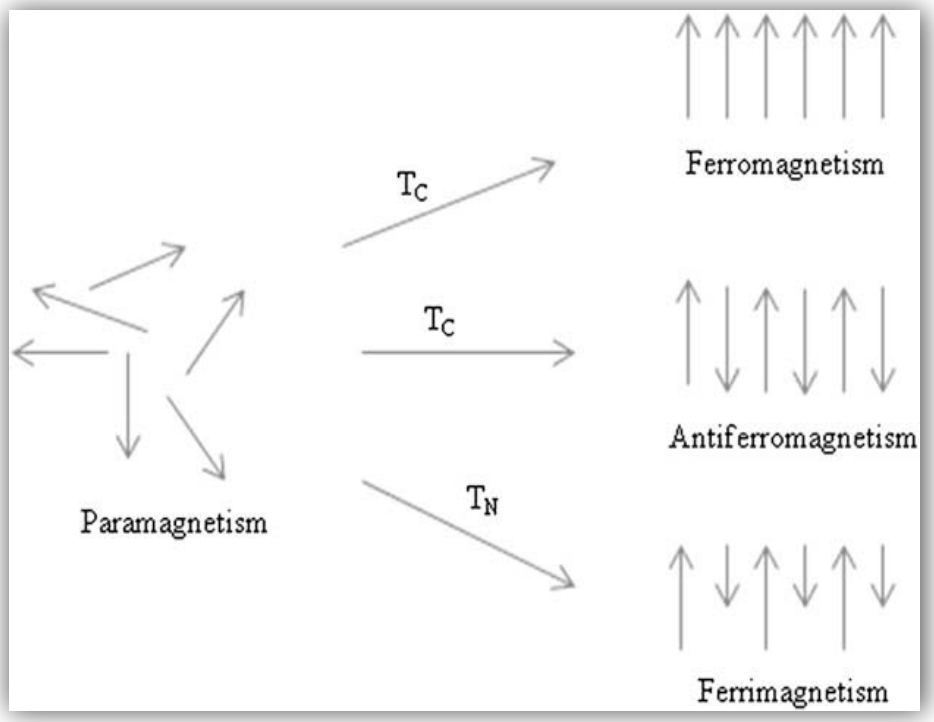


Fig. 2.7: Alignment of individual atomic magnetic moments in different types of materials. Adapted from ref [27]

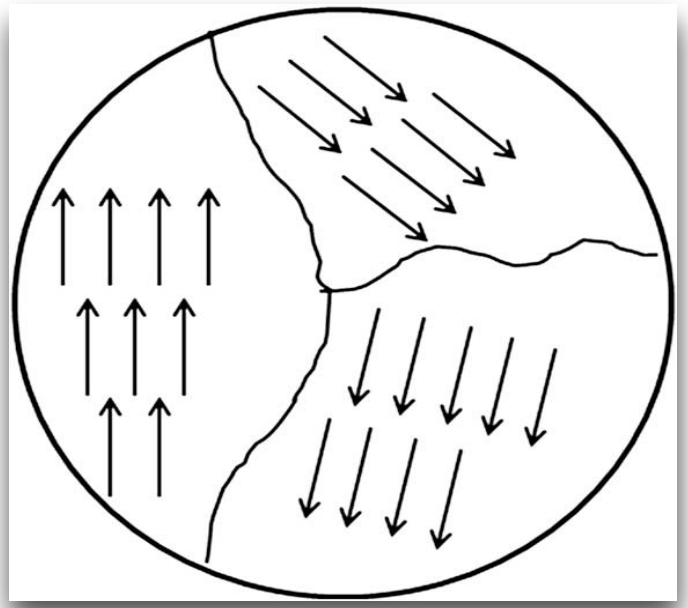


Fig. 2.8: Magnetic domains in a bulk material

Whenever an external applied magnetic field of H is introduced to a ferromagnetic materials of magnetic strength *M*, the magnetization curve obtained, Fig. 2.9showing that *M* increases with *H* until a saturation value M_S is reached. The magnetization curve displays a hysteresis loop, because all domains do not stabilize to the original state of orientations when *H* is decreased after the saturation magnetization value is attained. Consequently, when *H* returns back to zero, there is a remnant magnetization M_R which can only be removed by having a coercive field H_C applied in the opposite direction to the initially applied field. A single domain magnetic material does not show similar loop and is said to be super paramagnetic. Iron oxide nanoparticles of size smaller than about 20 nm often display super paramagnetic behavior at room temperature a character usually demanded in nanoparticle materials.

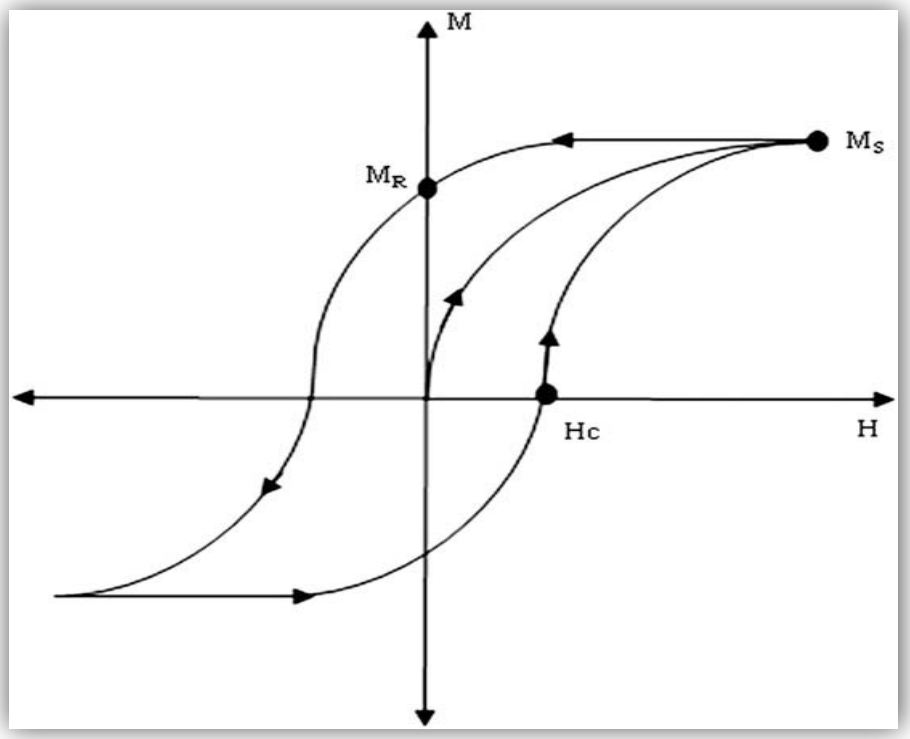


Fig. 2.9: Magnetization M as a function of an applied magnetic field *H*

Ordered arrangement of magnetic moments decrease with increasing temperature due to thermal fluctuations of the individual moments. Beyond the Neel or Curie temperature, the material in this case becomes disoriented and loses its net magnetization. The transition temperature is known as the Curie temperature T_C for ferromagnetic substances, and the Neel temperature T_N for anti ferromagnetic substances. Super paramagnetic particles are usually ordered below a blocking temperature, T_B. Magnetite is ferrimagnetic at room temperature and has a Curie temperature of 850 K. Magnetite particles smaller in size than 6 nm are super paramagnetic at room temperature, although their magnetic properties depend strongly on the methods of preparation used in their synthesis.

Tartaj and co-workers., reported that the magnetic properties of Nano sized magnetite depending strongly on changes in the crystal morphology[29]. The crystal morphology has coercivity factor in the order of: spheres < cubes <octahedra with increasing the number of magnetic axes along this series of shapes. Magnetite particles with coercivities ranging from2.4 to 20 kA/m have been produced by controlling their synthetic conditions.

Surface effects influence the magnetic properties of iron oxide nanoparticles. As a result, iron oxide nanoparticles net magnetization decreases at a fast rate with increasing temperature higher than that of the corresponding bulk material because a large number of the atoms near the surface where the exchange field is low. Modifications of the surface by chemical treatments enhances the coercivity of oxide nanoparticles. This is due to the dependence on size and surface treatment, and nano structuring of magnetic materials through the method of preparation can be used to improve magnetic properties, this will be discussed below.

II. Applications of Fe_3O_4

The magnetic properties of iron oxides have been used in a wide range of applications including, catalysts, magnetic seals and inks, magnetic recording

media, and ferro-fluids, in addition to contrast agents for magnetic resonance imaging (MRI) and therapeutic agents for cancer treatment. These applications require nanomaterials of specific sizes, shapes, surface characteristics, and magnetic properties.

Usage in data storage applications, the particles must have a stable, switchable magnetic state that is unaffected by temperature variations. For high performance in recording, the particles should exhibit both high coercivity and high remanence, and they should be uniformly small, and resistant to corrosion, friction, and temperature changes [27].

Magnetite used in ferro-fluids was proposed to optimize performance seals in space technology applications. Ferro-fluids is composed super paramagnetic nanoparticles dispersed in aqueous or organic solvents. This type of fluid has net magnetic moment when subjected applied field. An external field is therefore able to trap the fluid in a specific location to become a seal. Ferro-fluids exhibits properties such as magnetic field dependent optical anisotropy proved to be useful in optical switches, and tunable diffraction gratings. They are usually used in sealing computer disk units, and in vibrating environments in place of regular seals. Ferro-fluids are also use in NMR probes for receiving and transmitting coils, they have also been suggested for use in eye surgery to repair damaged retinas. Ferro-fluids are shown to have a high degree of colloidal stability in a magnetic field gradient. One of the keys to improving their performance in these applications is to make the particles smaller and more uniform, which requires an optimum method of preparation.

The application of magnetite nanoparticles received significant interest in last decade specially in magnetic resonance imaging contrast agents, and targeted drug delivery vehicles, as well as in magnetic hyperthermia. These applications demand particles that exhibit super paramagnetic property at room temperature. Magnetization is controlled to desired levels as excess magnetization sometimes lead to agglomeration of these nanoparticles, and this could lead to blockage of blood vessels. In addition, applications in biology and medical diagnosis require stable magnetic nanoparticles in water at neutral pH and physiological conditions. The colloidal stability of magnetic fluids depends on the size of particles, which should be sufficiently small to minimize precipitation due to gravitation forces, and on the charge and surface chemistry. Magnetite is the most commonly employed materials for biomedical applications[30].

Focused attention on the use of Magnetite in biomedical applications due to their biocompatibility and lower toxic effect in the human body. A major area of applications has been in the field of bio-assays where the magnetic properties have been exploited in vitro to manipulate magnetite nanoparticles with an external magnetic field. Wang andco-workers have developed extremely sensitive magnetic microarrays using ferromagnetic sensors to detect binding of target DNA and proteins [31].

Magnetic nanoparticles have been used in vivo as magnetic resonance imaging (MRI)contrast agents for molecular and cell imaging. Super paramagnetic magnetite is used as the core in these agents which are used to differentiate between healthy and unhealthy tissues and cells. The superparamagnetic nanoparticles are coated with a polysaccharidic layer for colloidal stability and better sensitivity. In vivo MRI cell tracking has been successfully performed by Song and co-workers[32]. Magnetic nanoparticles with a polymer coating have been used in cell separation, protein purification, environment and food analyses, organic and biochemical syntheses, industrial water treatment and biosciences. Encapsulation of magnetic nanoparticles with organic polymers is used to enhance their chemical stability, dispersability and functionality [33].

Another important application of magnetic nanoparticles is hyperthermia in cancer therapy. Super-paramagnetic magnetic nanoparticles when exposed to an alternating magnetic field can be used to heat tumor cells to 41-45^{o}C, where tissue damage of normal tissue is reversible while the tumor cells are irreversibly damaged, a process constitutes a giant step in irradiating cancerous cells.

Magnetite is used industrially as catalysts for a number important reactions, including the synthesis of NH_3 (the Haber process), the high temperature water gas shift reaction, and the desulfurization of natural gas[34]. Other reactions include the dehydrogenation of ethyl benzene to styrene, the Fisher-Tropsch synthesis for hydrocarbons, the oxidation of alcohols, and the large scale manufacture of butadiene.

Magnetite is semiconductors and can catalyze oxidation/reduction reactions. Iron oxides can be used as acid/base catalysts and to catalyze the degradation of acrylnitrile-butadiene-styrene copolymer into fuel oil.

Magnetic iron oxide is commonly used in synthetic pigments in paints, ceramics, and porcelain. Pigments made from magnetite are also used in magnetic ink character recognition devices, and super paramagnetic magnetite particles are used in metallography for detecting flaws in engines[35].

As noted above, many of the useful attributes of iron oxides depend on the preparation method for the nanomaterials. The preparation method plays a key role in determining the particle size and shape, size distribution, surface chemistry and therefore the applications of the material. In addition, the preparation method also determines the degree of structural defects or impurities present in the particles, and the distribution of such defects. Many synthetic routes have been developed to achieve proper control of particle size,

polydispersity, shape, crystallinity, and the magnetic properties. Some of these methods are described in the following sections.

III. Methods of Preparation

a) *Gas phase method of preparation of metal oxide nanoparticles*

These methods of preparations of nanomaterials depend on thermal decomposition (pyrolysis), reduction, hydrolysis, disproportionation reactions, oxidation/reduction, and other reactions to precipitate solidnano materials from the gas phase form. Chemical Vapor Deposition (CVD) process, allows for a carrier gas stream with precursors to be delivered continuously by a gas delivery system to a reaction chamber under vacuum at high temperature, above 900°C. This type of reaction takes place in the heated reaction chamber and the precursors combine to form nanoparticles. Growth and agglomeration of the nanoparticles are mitigated through rapid expansion of the two-phase gas stream at the outlet of the reaction chamber (Fig.2.10). A follow-up heat treatment of the synthesized nano powders in various high-purity gas streams allows for compositional and structural modifications, including nanoparticle crystallization, and transformation to a desirable size, composition, and morphology [36].This process employed to deposit iron oxide through the reaction of iron halide, such as iron trichloride, with water, heated to 800-1000°C. The success of this method depends on control of concentrations of precursor in the carrier gas, as well as rapid expansion and quenching of the nucleated clusters or nanoparticles as they exit from the reactor [37]. The use of organometallics as precursors in the MOCVD process, allows reactions to take place at lower temperature range 300-800°C and at pressure varying from less than 1torr to ambient conditions. Iron oxide thin films have been producedvia reaction of thermal decomposition of acetylacetonate at 400-500°C, and iron trifluoro-acetylacetonate at 300°C in oxygen(Singh et al .2008) [38a]. Precursors include tris(2,2,6,6-tetramethyl-3,5-heptadionato) Fe(III) and tris-t-butyl-3-oxo-butanoato Fe(III) were also used. Recently, Park et al. [38b] deposited magnetite thin films using Fe(II) dihydride complexes $H_2Fe[P(CH_3)_3]_4$ at 300°C in oxygen by direct growth of magnetite has been achieved by a low-pressure CVD using metal-organic ferric dipivaloyl-methanateas a precursor. Upon oxidation, these films were converted to maghemite. Another MOCVD reaction uses microwave plasma to decompose either iron cyclopentadienyl in an oxygen atmosphere at 300-500°C and 1-20 Tor pressure.

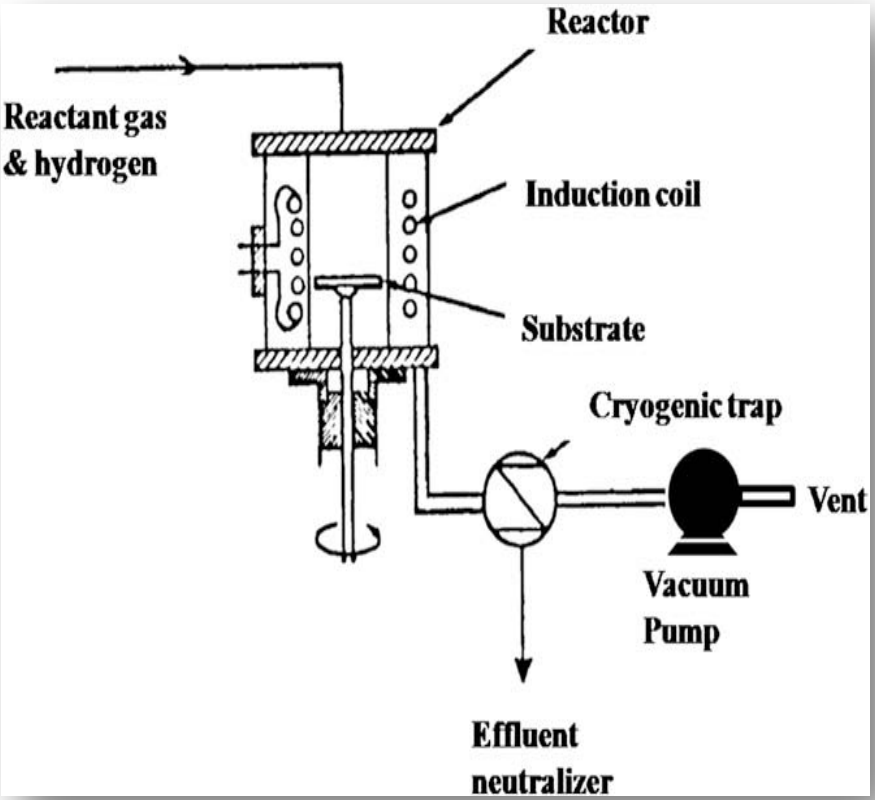


Fig. 2.10: Schematic diagram of a CVD apparatus (Adapted from ref [39]).

Laser pyrolysis of organometallic precursors based on resonant interaction between laser photons and gaseous species, reactant or sensitizer. Either of these energy transfer agents tha is excited by absorption of CO_2 laser radiation and transfers the absorbed energy to the reactants by collision [40]. The method involves heating a flowing mixture of gases with a continuous wave CO_2 laser to initiate and sustain a chemical reaction until a desired concentration of nuclei is reached in the reaction, which leads to homogeneous nucleation of particles. A schematic representation of a CO_2 laser pyrolysis device is shown in Fig.2.11. The nucleated particles formed during the reaction are driven by the gas stream and collected at the exit [29].

Well-crystallized and uniform iron oxide nanoparticles, including nanoparticles of hematite and maghemite, were obtained through one step using laser pyrolysis. Iron pentacarbonyl is usually used as a precursor in this method and ethylene is used as the carrier gas to transport the carbonyl vapor to the reaction chamber, this is because ethylene does not absorb radiation at the laser wavelength. The iron penta carbonyl breaks to iron and carbon monoxide molecules, followed by oxidation using air, then introduced into the system with the iron pentacarbonyl vapor or mixed with argon. Iron particles with 14 nm mean diameter and about 4 nm oxide shell thickness have been produced by this procedure of iron pentacarbonyl and ethylene mixtures followed by a controlled step-by-step passivation process [41].

The effect of process conditions on the structural and magnetic properties of maghemitenano particles produced by laser pyrolys is have been studied by Verdaguer et al.(2002) [42]. They reported that the particle size depends on the oxygen content of the gas phase and is independent of the laser conditions, and suggested that the amount of oxygen be used to optimize the size and crystallinity of the particles.

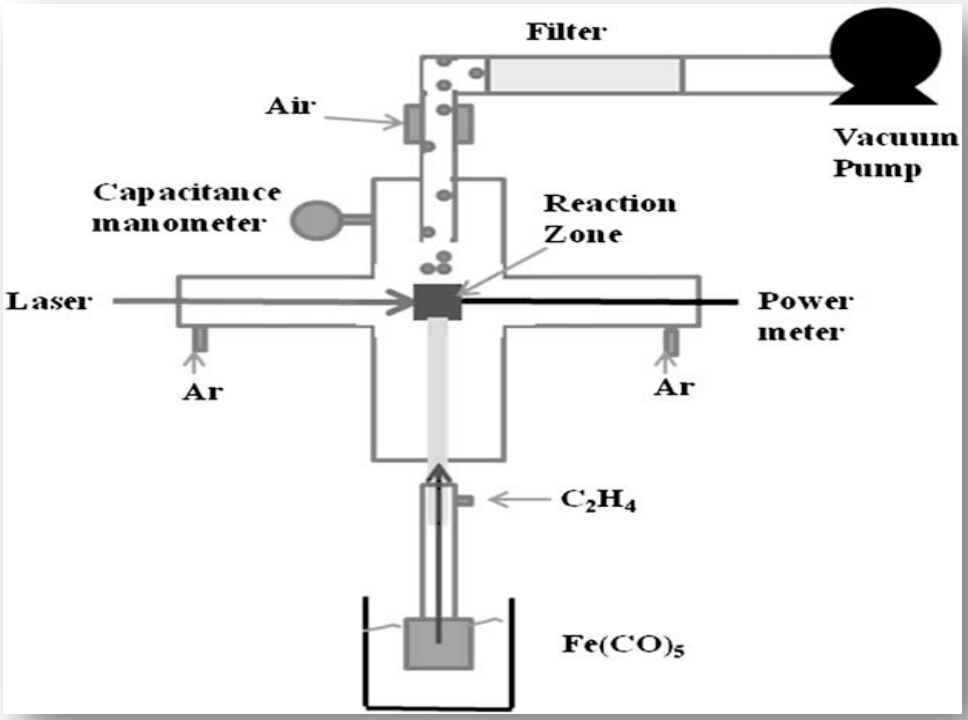


Fig. 2.11: Schematic diagram of a CVD apparatus (Adapted from ref[29])

Although gas phase methods are able to deliver high quality products, the yields are usually low and scale-up can be maintained. Variables such as oxygen concentration, gas phase impurities, and the heating time must be controlled precisely to obtain pure products.

b) *Liquid phase methods of preparation of metal oxide nanoparticles*

Liquid phase methods are more practical, generally affordable, and offer better yields of products as well asease of surface modifications. Most nanoparticles prepared by such methods have been via coprecipitation from aqueous solutions, although other organic solvents can also be used. It has been shown that magnetite particles with mean diameters ranging from 30 to100 nm can be achieved by the reaction of a Fe (II) salt, a base and a mild oxidant (nitrate ions)in aqueous solutions. Stoichiometric amounts of ferrous and ferric hydroxides can also be reacted in aqueous media to yield homogeneous spherical particles of either magnetite ormaghemite. The phase and size of

the particles depend on contents of cations, the counter ions present, and the pH of the solution. However, it is possible to control the mean size of the particles by adjusting the pH and the ionic content.

Due to the large surface-area to volume ratio, nanoparticles formed by liquid phase coprecipitation tend to aggregate in solution in order to reduce their surface energy. The suspension of nanoparticles can be stabilized by adding anionic surfactants as dispersing agents. The nature of the counterions, pH, and ionic strength can then be used to stabilize the charged particles via interactions between electrical double layers. Increasing the concentration of inert electrolyte in the system compresses the double layer and promotes coagulation. However, suspensions of iron oxide that are stabilized entirely by electrostatic repulsion are too sensitive to external conditions such as pH and ionic strength to offer any flexibility in engineering the surface properties of the particles.

Stabilization can also be achieved by coating the particle surfaces with proteins, starches, non-ionic detergents, or polyelectrolytes. Adsorption of such substances stabilizes the particles at electrolyte concentrations that would otherwise be high enough for coagulation to occur. Charles [43] prepared water-based magnetic fluids by dispersing magnetite particles in water containing oleic acid. Khalafalla & Reimers (1980) [44] also produced stable aqueous magnetic fluids using dodecanoic acid as a dispersing agent. Usage of saturated and unsaturated fatty acids to stabilize magnetic fluids has been intensively studied.

Better quality monodisperse and monocrystalline iron oxide nanoparticles can be produced by the thermal decomposition of organo metallic precursors in organic solvents containing stabilizing surfactants such as oleylamine, oleic acid, and steric acid. Precursors that have been investigated include iron acetylacetonate, iron carboxylate, iron cupferronates and iron carbonyls. High quality magnetite nanoparticles with diameters ranging from 3 to 20 nm were synthesized via thermal decomposition of Fe(III) acetylacetonate in phenyl/benzyl ether and 2-pyrrolidone.

Recently, Novak et al.[45] investigated the synthesis of maghemite by thermal decomposition of some complex combinations of Fe(III) with carboxylate type ligands obtained from the redox reaction between polyols and ferric nitrate. Maghemite was obtained at 250-300°C and hematite at 400-500°C.

Sun and co-workers [64a] have reported the preparation of mono disperse iron oxide nanoparticles by the thermal decomposition of iron acetylacetonate. They also proposed a simple method to transform hydrophobic nanoparticles into hydrophilic ones by adding bipolar surfactants and produced 4 nm magnetite nanoparticles by decomposition of Fe(III) acetylacetonate in a mixture of phenyl ether, 1,2-hexadecanediol, oleic acid, and oleylamine . It has also been reported that 6-7 nm maghemite nanoparticles can be produced via the reaction of iron cupferronates with trioctylamine at 300°C. Maghemite nanoparticles with sizes ranging from 4 to 16 nm have been produced by decomposition of iron pentacarbonyl in octylether and oleic acid or lauric acid. These results show the effectiveness of the thermal decomposition method for the synthesis of iron oxide nanoparticles. However, the presence of residual surfactants may hamper the efficiency of subsequent surface modification of the synthesized nanoparticles. In addition, the use of toxic solvents and surfactants may be detrimental to the biocompatibility of the product [46b].

c) *Two-phase methods of preparation of metal oxide nanoparticles*

Water-in-oil microemulsions consisting of nanosized water droplets dispersed in an oil phase and stabilized by surfactant molecules at the water/oil a method interface usually used to obtain iron oxide nanoparticles. The surfactant-covered water pools show a unique microenvironment for the production of nanoparticles and limiting their growth. The size of the microemulsion droplets is determined by the water to surfactant ratio, although the overall size of the nanoparticles may also be influenced by factors such as concentration of reactants, and flexibility of the surfactant film [47].

Pialli and co-workers [48] studied a number of ways to manipulate microemulsions to synthesize nanoparticles. They reacted A and B by dissolving in the aqueous phases of two identical water-in-oil microemulsions and formed AB precipitate upon mixing (see Fig. 2.12a). The precipitate from droplets, hence limiting the size and shape of the particle formed as targeted. Alternatively, nanoparticles are produced by the addition of a reducing agent to a microemulsion containing the primary reactant in the aqueous phase (Fig. 2.12b). Fig. 8c outlines the formation of oxide, hydroxide or carbonate products by passing gases like O_2, NH_3, or CO_2 through a microemulsion containing salts of the cations [48].

Water-in-oil microemulsions have been also used to produce iron oxide, metallic iron nanoparticles, magnetic polymeric iron oxide nanoparticles, and silica-coated iron oxide nanoparticles. Various surfactants have been used when preparing these materials, including bis(2-ethylhexyl) sulfosuccinate, sodium-dodecyl sulfate, cetyltrimethylammonium bromide, polyvinylpyrrolidone, diethyl sulfosuccinate[49].

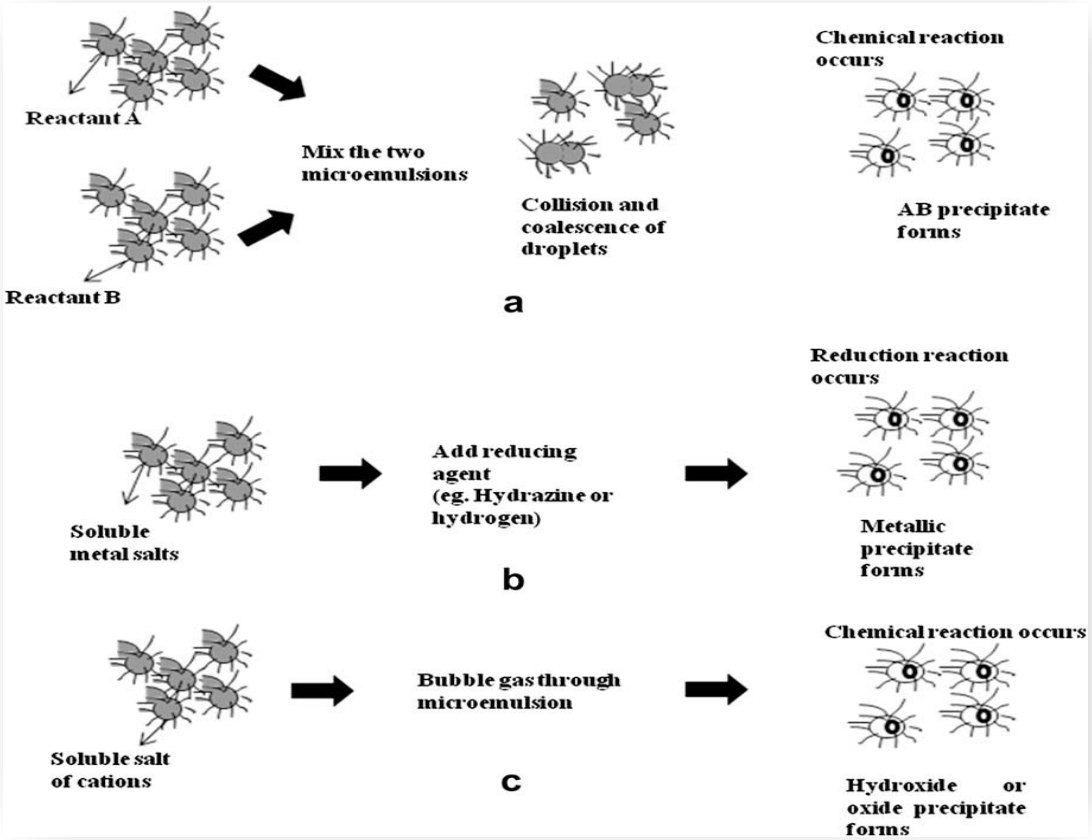


Fig. 2.12: Nanoparticle synthesis in microemulsions (a) by mixing two microemulsions, (b) using a reducing agent, and (c) by passing gas through the microemulsion [48]

d) Sol-gel method of preparation of metal oxide nanoparticles

Sol-gel methods, well developed methods for preparation of metal oxides nanoparticles, it refer to the hydrolysis and condensation of metal alkoxides or alkoxide precursors, resulting in dispersions of oxide particles in a sol. The sol is then dried or gelled by means of solvent removal or by chemical hydrolysis. The precursors can also be hydrolyzed by an acid or base media. Basic catalysis leads to the formation of a colloidal gel, whereas acid catalysis produces a polymeric gel. The rates of hydrolysis and condensation are the parameters that control the properties of the end products. Usually smaller particle sizes are obtained at lower controlled hydrolysisrates. The particle size also depends on the solution mixtures, pH value, and temperature. Magnetism of the sol-gel system relies on the resulting phases formed and the particle volume fraction, and is sensitive to the size distribution and dispersion of the naonparticles. Nanocomposites extracted from gels, structural parametersm and material porosity are determined by how fast the hydrolysis and condensation gel precursors, and by other oxidation-reduction side reactions that occur during the gelling and subsequent heat treatment [36].

Iron oxidesilica aerogels have been prepared by the sol-gel methods mentioned above, and found to be more reactive than conventional iron oxide. The increase in reactivity most likelydut to the large surface area of iron oxide nanoparticles supported on the silica Aerogel. Precursors, TEOS and Fe(III) solutions are dissolved in an alcoholic aqueous media, and the gels produced after a few days calcined to produce the final desired materials. Ferric nitrate, ferric acetylacetonate and ferricchloride are the candidate metal oxide precursors, even though the use of the metalliccomplex FeNa(EDTA) and a mixture of this metallic complex with ferric nitrate has also been reported. Experimental work with pure metallic complex resulted in iron oxide nanoparticles in the size range 20-160 nm. Low solubility of EDTA salt in the solvent prevented the synthesis of high iron content aerogels when this complex is used as a precursor [50]. Aerogels generated from both pure ferric nitrate and its mixture with the metallic complex show low saturation magnetization values of 14 and 8.5 emu/g, respectively. This compares with bulk maghemite values of 74-76 emu/g. The low magnetization values suggest that the aerogels may not be suitable for magnetic applications, although they may still be useful in catalysis and other applications.

Generally, methods of preparing iron oxide-silica supported reagents, iron oxide precursors were mixed with silica precursors in a solvent to form a ''sol''. Recently, Popovici and co-workers [51] produced iron oxidesilica supported reagents using a synthetic procedure that implements impregnating silica gels with anhydrous Fe(II) precursors followed by drying of the gels using ethanol. Maghemite dispersed nanoparticles doped in silica aerogels were yielded in a single-phase process. The nanocomposites showed a high saturation magnetization value and were superparamagnetic at ambient temperature. The success of his process is due to the impregnations of precursors after gelation, the exchange of water with ethanol before impregnation, and finally use of the anhydrous ferric salt.

Deng et al., reported that sol-gel method has also been used to synthesize magnetite and maghemite thin films, transparentiron-doped titanium oxide thin films, ferroelectromagnetic bismuth iron oxide films, mixed iron oxides, and iron oxide-alumina nanocomposites[52].

Disadvantages of the method of sol-gel approach, the contamination targetd product by byproducts of reactions, and the need for post-treatment of the products.

e) High pressure hydrothermal methods of preparation

These methods depend on the ability of water at elevated pressures and temperatures to hydrolyze and dehydrate metal salts, and the low solubility of the produced metal oxides in water at these conditions to generate required super saturation [39]. The higher temperatures favor higher dehydration rates, and so does the high diffusivity of reactants in water at these conditions. High super saturations is achieved by this process because of the low solubility of metal hydroxides and oxides, to obtain the desired fine crystals. Variables such as pressure, temperature, reaction time, and the precursor product system can be adjusted for high nucleation rates and to control growth. The process is environmentally favored and versatile, since it does not demand organic solvents or post-treatments such as calcination. Consequently, high pressure hydrothermal processes have been widely investigated for the synthesis of metal oxides as powders, nanoparticles and single crystals [53].

Xu and Teja reported that the hydrothermal synthesis of iron oxides can also be performed in situ within porous structures. They also have successfully deposited hematite in the pores of activated carbon using supercritical water. The hematite nanoparticles were 16-36 nm in diameter and were uniformly distributed throughout the carbon pellets (see Fig. 2.13). The resulting activated carbone iron oxide nanoparticles were used as catalysts in the oxidation of propanal at rates that were an order of magnitude higher than those for activated carboncatalysts solely[54].

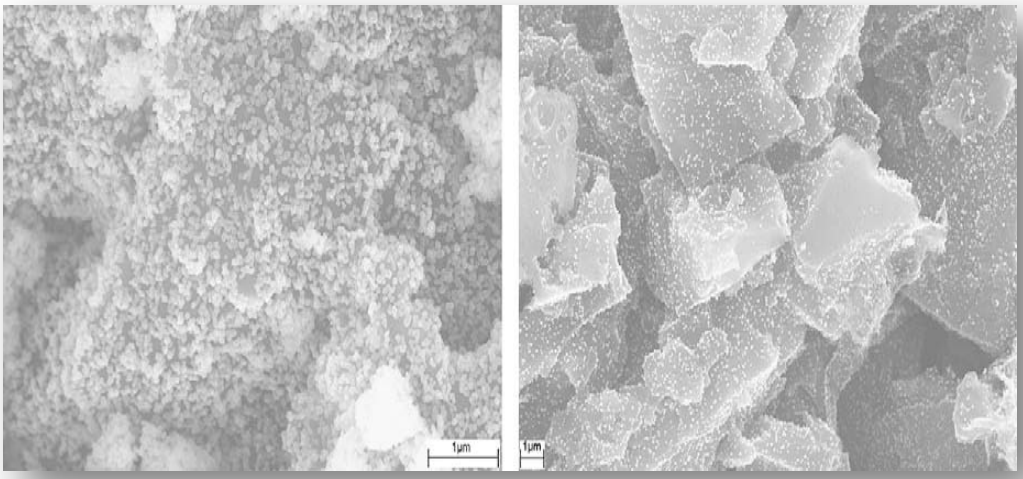


Fig. 2.13: Distribution of hematite nanoparticles deposited (a) on the surface and (b) in the interior of activated carbonpellets by supercritical water[54].

Darab and Matson [55] succeeded inusing the hydrothermal method to synthesize fine iron oxidenanoparticles in a continuous flow reactor. Their method involves fast heating of flowing solutions by contact with supercritical water at residence times of 5 to 30s in the reactor to control growth. A schematic roadmap of their experimental procedures is shown in Fig. 2.14. Hematite nanoparticles less than 10 nm in size were obtained from ferric nitrate and ferric ammonium sulfate solutions using this method. Magnetitenano particles were also synthesized using ferrous sulfate and urea reaction. It was observed that the particle size and

morphology were dependent on operating temperature and processing time. The particle size increased dramatically as the operating temperature increased and different forms of iron oxide were produced from the same precursor by elevating the temperature. For instance, mixtures of 6-line ferrihydrite and hematite were formed at 250-350°C, while pure hematite was obtained attemperatures higher than 350°C [55].

Adschiri and coworkers [56a] also introduced another flow technique, it used rapid and thorough mixing of the water stream with the precursor solution in a T-mixer. High super saturation rates were resulted in the T-mixer due to the low solubility of metalhydroxides in supercritical water. Hematite, magnetite, and other oxides were achieved in a size range of 1 nm to 1onm at temperature of 400-490°C and pressure of 30-40 MPa. The residence time of the precursor solutions was nearly 2 min in the experiments accomplished.

Cabanas and Poliak off, also studied the process of hydrolysis of metal acetates in supercritical water. They produced a number of oxides, including magnetite, in the size range 3-105 nm at 200-400°C and 25 MPa [56b]. Teja and Koh also produced a Uniform particles of hematite using two variations of the continuous hydrothermal technique [57]. Hawa and co-workers [58], successfully synthesized 50 nm magnetite nanoparticles without the addition of a strong base using the continuous technique. The mixture of ferric ammonium sulfate, ferric nitrate, ferric sulfate, and ferrous chloride were used for the synthesis of hematite, while ferric ammonium citrate was used as a precursor for magnetite. In the magnetite experiments, Fe(III) reduced to Fe(II)by carbon monoxide. In this experiment CO was produced via the thermal decomposition of ammonium citrate. Since CO gas is miscible in supercritical water, it provided a uniform reducing atmosphere throughout the reaction. This implies that the product form can be managed by the control of an oxidizing or reducing gas during continuous hydrothermal process using above mentioned conditions.

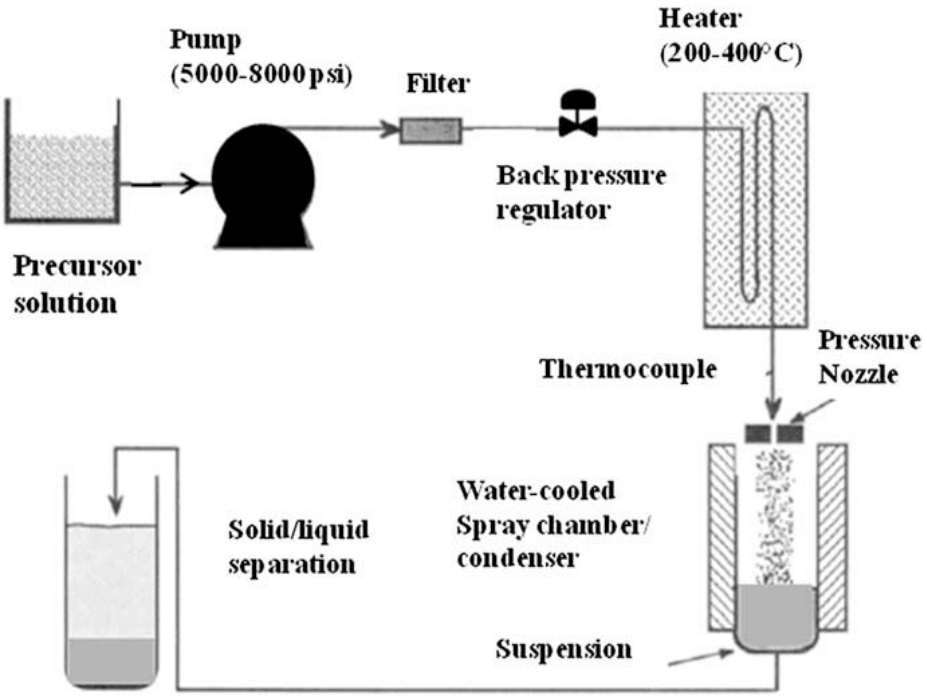


Fig. 2.14: Schematic diagram of the apparatus of Darab et al. 1998

Lester and his group[59]reported a design that takes advantage of differences in the densities of supercritical water and precursor solutions by using a nozzle mixer to improve mixing inside the reactor. The reactor diagram is shown in Fig. 2.15. The diagram shows supercritical water is introduced into the reactor from the top and the precursor metal salt stream from the bottom, upon mixing of the two streams allows for making use of buoyancy induced eddies to produce ''ideal'' mixing conditions. This leads to short residence times and limits subsequent particle growth. Lester and his group synthesized a variety of metal oxides, for which size range from 6 to 64 nm, there by demonstrating the effectiveness of separation of nucleation and growth steps in continuous hydrothermal process. The continuous hydrothermal process offers various opportunities for controlling particle size and morphology, and this done by maintaining residence times low and mixing processes worthwhile. However, deign of particle surfaces cannot be accheived in situ and requires additional post-processing steps.

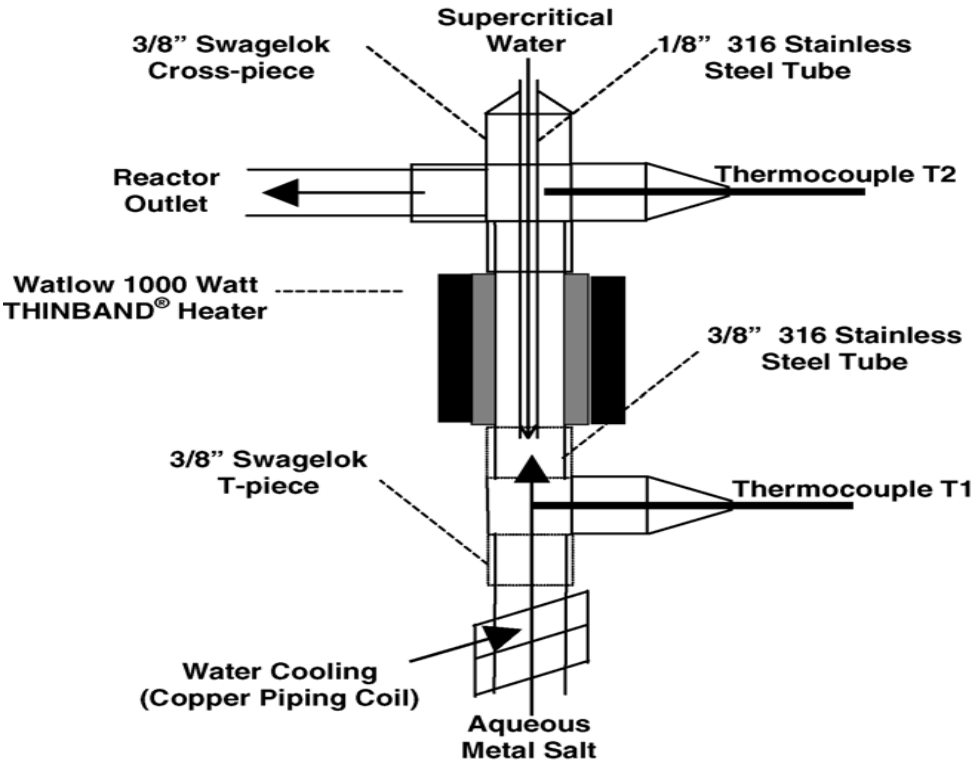


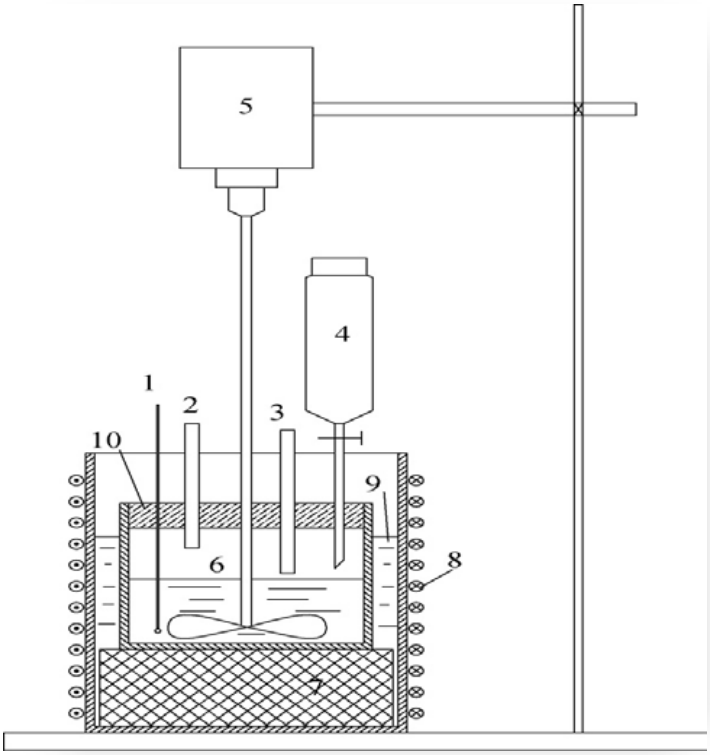
Fig. 2.15: Schematic of the nozzle mixer [59]

f) Co-precipitation Method of preparation of metal oxide nanoparticles

Co-precipitation method is a well-developed method and widely used in the preparations of nanoparticles metal oxides. The method takes advantage of the low solubility of an element in the soil solution than predicted by the solubility product, in addition to the solubility of an ion is lower in mixed ionic solution than in pure ionic solutions. The reason for the reduced solubility is due to co-precipitation. Co-precipitation process is as a result of incorporation of trace element into mineral structure during solid solution formation and recrystallization of minerals. This process will reduce the mobility of the trace elements that are incorporated into the mineral.

Minerals will only incorporate isomorphs elements into their structure through replacement of elements, these elements must have similar ionic radii for changing of the composition of the mineral. For example, during the formation of calcite, Mn^{2+}, Cd^{2+}, and Fe^{2+} can possibly be incorporated into the mineral structure. In the formation of Fe and Al-oxides, Cr^{3+}, Mn^{3+}, and V^{3+} may be incorporated into the structure. Mn and Fe-oxides have more possibility for co-precipitation than Al-oxides and aluminosilicate minerals such as clay and zeolites. Co-precipitation reactions are also controlled by the rate of soil mineral dissolution.

Wei and Viadero concluded that in order to achieve the precipitation of magnetite, the molar ratio of $[Fe_3]$ / $[Fe_2]$ should be 2:1 at high pH [60]. Synthesis system was used for this study at molar ratio of 1:2:8 for the Fe (II): Fe (III): OH and also de-oxygenated. This is to achieve phase purity of magnetite without the presence of impurities such as goethite and iron oxyhydroxide [61]. In this regard the green precipitate was observed when the Fe_2 ion initially added to the system followed by a brown precipitate was also resulted with the addition of Fe_3 ions in 1:2 ratio, as discussed earlier. When the alkaline NaOH was added into the mixed precursor of Fe_2 and Fe_3 ions, a thick black precipitate was observed and this was subsequently shown the resulting product is magnetite, as discussed by Schwertmann and Cornell [63].



Schematic diagram of synthesis system with solenoid . 1, Thermometer. 2,Condenser port. 3, N2 gas. 4, Dropper. 5, Stirrer. 6, Reactor. 7 Cushion. 8, Electromagnetic solenoid. 9, Hot water bath. 10, Sealed rubber[62].

IV. Conclusion

This review article describes a substantial progress has been made in methods and applications of iron oxides nanoparticles, with emphsis on advances in nanotechnology and biotechnology. Several methods have been reported to show developements that implement control over physical properties, such as size, size distribution, shape, crystal structure, defect distribution and surface structure.Among the methods reviewed, Continuous Supercritical Co-precipitation method technique assures process control and scalability. Furthermore, this method is environmentally suitable, andthe surface treatment of nanoparticles, however, requires additional steps. The method has been found to be very simple ,easy to carry on, low cost, and higher yield and without the use of organic reagents.

The challenge for all methods is the design of magnetic nanoparticles with effective surface coatings that provide optimum performance in vitro and in vivo biological applications. Additional challenges include scale-up, toxicity, and safety of large-scale particle production processes. Numerous progress were made in the synthesis Fe_3O_4 Nanoparticles.

The aim in various industrial applications and technologies remain in the optimization of the processes leading to desirable nanoparticles, nevertheless technical complexity does not prevent access to chemicals with high quality applications and specifications. The physical and chemical characteristics associated with these materials are often determined by the type and nature of the method used in the preparation, There is a disparity between the methods used depending on the economic cost and production capacity of each method. The differences in the use and application of prepared nanomaterials are also an important basis for different methods of preparation.

The increasing demand and advances for these materials since its inception has led to a wide range of methods for the production of high quality and quantitative materials, especially in the industrial fields (electrons and telecommuni-cations), medical fields (different treatments and the manufacture of human alternatives). Or a huge economic cost. One of the most common features of all methods is to deal with the atomic scale (atom towards another atom) for the purpose of reaching a design that is considered in advance to obtain desired results The difference in the scale of the mass of a single substance leads to different chemical effectiveness. The smaller the scale, the greater the chemical effectiveness due to the increased chemical impact of this substance.

On this basis, nanoscience and nanoscale preparation techniques are improving in accordance with the requirements of the global technological advances and expansion in various sectors. This allows

for great global competition to continue to arrive at the best results for better safe technology and quality of life.

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Chemical Evolution of Solutions from Beans Soaking and Cooking Processes: Case Study of Phaseolus Vulgaris L.

By J. C. Fopoussi Tuebue & I. N. Tchinda

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Keywords: *waste water, sanitation, recycling, fertilization, agriculture, nutrition, temperature.*

GJSFR-B Classification: *FOR Code: 039999*

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J. C. Fopoussi Tuebue [α] & I. N. Tchinda [σ]

Abstract- The present paper aims to highlight the physico-chemical evolution of solutions from soaking and beans cooking processes. For that purpose, solutions from soaking were produced by putting in contact 2kg of sorted and quickly washed beans seed with 8kg of water with known physico-chemical characteristics. Concerning the solutions from cooked beans, they were produced by putting on fire the pot containing the mixture of the water from soaking and bean seeds. The beans were a variety of *Phaseolus vulgaris L.*, known as "Meringue". The cooking process was done without salts. The samples of solutions were collected as follow: 30 and 60 minutes respectively after the beginning of the soaking, 30, 60, and 90 minutes after the beginning of the cooking process of the beans soaked during 60 minutes. After each sampling, the equal volume of the solution collected was replaced with the water used for the cooking process. Solutions obtained from beans soaking and beans cooking gradually enriched in mineral salts, particularly major macro elements (N and K), minor macro elements (Ca, S, Mg), and oligoelements compared to the situation noticed in the water used for the cooking process. Concerning the third major macro element, notably the phosphorous, it is present in low amounts. The pH and the electric conductivity (EC) of the solutions increase with the duration of soaking and cooking processes. The amounts and values start their weak increasing thirty minutes after the beginning of the soaking, and continue their shy increasing up to the end of the sixtieth minute of the soaking. With the beginning of the cooking process, the increasing become abrupt. The correlation between all the parameters followed up in the present study are globally positive. But some facts reveal the independence between some of the parameters taken into account here. Also, the study of the clouds of dots reveal the impact of the temperature as the major responsible of the behavior of some of those elements despite the positive correlations established. The Pearson index in the correlations including sodium are the lowest.

Keywords: waste water, sanitation, recycling, fertilization, agriculture, nutrition, temperature.

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I. Introduction

From the onset of the nineteenth century, like swarms of bees, springing from fountain pens of thinkers, disturbing facts with increasing intensities aggregated. These are facts, as different as day and night and numerous like grains of sand on a beach, which reflect the occurrence of the multifaceted links that exist between the food consumed and the state of health of the consumer. In this sense, the better the quality and composition of the food, the better the health of the consumer, and vice versa [1]. The quality of a food is commonly seen as being the consequence of a more or less adequate state of conservation of the given material [2]. Regarding its composition, it is linked either to the nature of the food [3] or to the modification that the food has undergone during handling [4]. Therefore, the limit between the causes of malnutrition and good nutrition becomes a little more difficult to define because we are in the presence of a multifactorial scourge [5]. Contributing to better nutrition among a people then comes down to carefully scrutinizing all the practices linked to this biological function. When we talk about nutrition, we are referring to the set of processes by which a living being transforms food to ensure its survival [6]. Regarding humans, the great variety of food resources has logically led to highly varied diets [7]. Thus, while some people will adopt simple diets, others will adopt rather complex diets [8]. Regarding the type of food consumed by humans, we will distinguish some that are eaten raw and others that necessarily require the cooking phase [9]. From an essentially dietary perspective, the specialists in the field increasingly recommend the consumption of at least one fruit per day. It is indeed the undisputed source of vitamins for the body, the vast majority of these first-degree functional nutrients being heat labile [10]. Such a prescription, carried out daily with insistence and authority, is very quickly found on the front line as generating many questions in the minds of the informed man. One might therefore be tempted to ask the question whether the vitamin concentration in cooked foods is the only one to change during the cooking process. It is undoubtedly in this sense that [11] undertook to test, fortunately for scientific research, with success, the water at the end of cooking beans on

strongly desaturated and very strongly acid soils as a potential way towards soils fertility recovery. Observations from these trials subsequently led [12] to also successfully test the mixture of end-of-cooking solutions from beans and human urine on plant growth. If in the first case there were significant improvements in the chemical fertility parameters of the soils involved, the characterization of the end-of-cooking solution of the beans remained only embryonic, thus helping to keep a heavy veil on the deeper knowledge of this fluid. In the context of this study, it is therefore a question of returning to the shortcomings of the work of [11] and [12] in order to try to shed light on the real composition of solutions at the end of cooking and soaking the beans, pledge of a strong advance in Science in the management of what one might be tempted to call "kitchen waste". This will be done in reference to the chemical changes undergone by the food during the processes mentioned. It will be in detail a question of specifying and quantifying the elements lost when food products enter the cooking phase on the one hand and the soaking phase on the other hand. In accordance with the principles of the inferential approach as recommended by [13], these losses will be assessed by progressive monitoring of the composition of the cooking water and of the soaking respectively. For this, the beans, because of its compulsory passage through the cooking phase and optional through the soaking phase was retained. The choice of this plant species is linked to the fact that it is one of the foods not only abundantly produced, but also appreciated in Cameroon [14]. From this large group, "Meringue", one of the main representatives of the *Phaseolus vulgaris L.* species, was chosen.

II. Materials and Methods

a) Materials

i. Bean seeds

Most of the African population is engaged in agriculture ([15], [16], [17]). Among the main cultivars, we have the bean [18]. This food is very popular throughout Cameroon because of its flavor and its dietary potential ([19], [20], [21]). For 100g of this food, there are organic compounds such as proteins (9.06g), carbohydrates (27.91g), lipids (0.49g), fibers (5.3g); water (61.2g); varieties of vitamins including vitamins B1 or thiamine (0.257mg), B2 or riboflavin (0.063mg), B3 / PP or niacin (0.57mg), B5 or pantothenic acid (0.299mg), B6 (0.175mg), E or tocopherol (0.98mg), K (3.7µg); total folate (168µg); many mineral elements including potassium (508mg), sulfur (225mg), phosphorus (165mg), magnesium (65mg), calcium (52mg), iron (2.3mg), sodium (2mg), zinc (0.96mg), manganese (0.548mg), copper (0.271mg), selenium (1.4µg) ([22], [23], [24], [25]). The total nitrogen content (1450mg) was deduced from the protein content by applying the Jones factor [26] according to which protein content = total nitrogen * 6.25.

About 85% of dry beans are consumed in some of the countries where they are grown. The remaining 15% are marketed ([27]). In the case of Cameroon, importation is almost absent. Large quantities are produced, but the bulk is for export ([28]).

The flatulent effect of beans is universally known and has, undoubtedly, been a source of discomfort throughout history [29].

ii. Solution from cooked beans

Solution from cooked beans is aheterogeneous mixture, and particularly a proteicglobular suspension, with considerable amounts of carbohydrates within. It has a pH value of 6.4. At rest, that fluid divides itself into two superimposed domains: a flaky superficial domain and a liquid lower domain. The flaky domain is the organic part and theliquid domain is the water and the mineral salts provider. The density of the flaky domain is 0.964and that of the liquid domain is 1.011. The average speed of the growth of *Aspergillus L.* at the surface of the water from cooked beans is3, 17 cm^2/H; they cover in five days a surface of379.74 cm^2.The physic-chemical characteristics of the flaky domain floating on the liquid domain make it an adequate area for the development of molds (*Aspergillus L.*). Solution from cooked beans Seems then to contain all the nutrients required for an optimal development of those beings (*Aspergillus L.*), and in the same way for anoptimal fertilization of soils; this include water, organic matters and mineral salts among which nitrogen, potassium, phosphorus, sulfur and calcium can be named. It is then a complete liquid organic fertilizer. That solution positions itself also as a high grade activator for soils microflora. It represents finally a way for a sustainable improvement of agriculture in developing countries and a way for a sustainable development of soils micro flora, required for the reach of the food self-sufficiency. Solution from cooked beans is finally not only a source of nutrients for plants, but it also positions itself as a high grade activator for soils micro flora [13].

b) Methods

To achieve the fixed objectives, the work was carried out in the field and in the laboratory.

i. In the Field

In the field, it was a question of obtaining the beans necessary for the production of the end-of-cooking solution for this food. The variety purchased is "Meringue" (photograph 1), one of the representatives of the species *Phaseolus vulgaris L.* It was also a question of getting closer to the families to question them in relation to the actions they take during the preparation of the bean-based meal. In total, between September 2019 and December 2019, 500 families were interviewed, i.e. 50 families in each of the 10 regions of Cameroon.

Photograph 1: « Meringue »

ii. *In the laboratory*

a. Handling of bean samples and preparation of water samples

In the laboratory, the bean was sorted in order to remove the stones. Then followed the weighing phase at the end of which 2,000 g of beans were taken for testing. The sample was then quickly washed to remove dust and all forms of impurities, then soaked for 1 hour in 8 liters of water, or 1 mass of bean for 4 mass of water. In detail, we started on the basis that 1 liter of water weighs 1 kg according to [30]. During the soaking phase, two samples of the same amount of water were taken. The first setting was made thirty minutes after the start of the soak and the second setting 60 minutes after; after each intake, the amounts of water removed were replaced by water drawn for cooking. Just before placing the pot containing the soaked water-bean mixture on the fire, a gauge line was marked on the rim of the pot in order to keep the water level intact during cooking by regular addition of water provided for cooking. Three water samples of the same quantities were taken during the cooking phase: the first 30 minutes after the start of cooking, the second 60 minutes after the start of cooking, and the third 90 minutes after the start of cooking. After each setting, the cooking water was brought back to the mark by adding water provided for cooking. The collected waters were cooled, bottled and the bottles labeled. These samples were supplemented by a sample of water used for cooking as control. Has followed the analytical phase. It focused on pH, electrical conductivity (EC), Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, HPO_4^{2-}, SO_4^{2-}, NH_4^{+}, Cl^{-}, and HCO_3^{-}.

The IonPac CS12A and IonPac AS12A analytical columns were respectively used for the separation of ammonium ($NH4^{+}$), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}) as far as cations were concerned, and chloride (Cl^{-}) and sulfate ($SO4^{2-}$) in the case of anions. The flow rate was set to 1.0 mL/min for both cations and anions optimization. The different cations were isocratically separated with a 20mM Methanesulfonic acid solution within 15 minutes. Concerning the ions studied here, isocratic elution with 4mM and 20mM Sodium hydroxide solution were employed for their separation, respectively for chloride and sulfate, during 15 minutes. All eluents were degassed and pressurized under high purity nitrogen to prevent dissolution of carbon dioxide and subsequent production of carbonate.

Concerning bicarbonate ion ($HCO3^{2-}$), its amount was measured by using titrimetric method. The pH was determined with a pH-meter having a glass electrode. Electric conductivity for its own was measured using a conductivity meter fitted with a calibrated measuring cell; the results were expressed in mS/cm.

b. Determination of bean seeds mass loss

In other to determine the mass loss by bean seeds during soaking and cooking processes, 1kg of beans was taken. From there, six batches of 100g beans were made. The first batch served as a control (mt or control mass = 100g). The other five batches were respectively washed quickly. The first batch of the five was soaked for 30 minutes, then dried (mht30cr = raw bean mass soaked for 30 minutes). The second batch was soaked for 60 minutes then dried (mht60cr = raw bean mass soaked for 60 minutes). The third batch was soaked for 60 minutes then cooked for 30 minutes and dried (mht60cu30 = bean mass soaked for 60 minutes and cooked for 30 minutes). The fourth batch was soaked for 60 minutes then cooked for 60 minutes and dried (mht60cu60 = bean mass soaked for 60 minutes and cooked for 60 minutes). The fifth batch was soaked for 60 minutes then cooked for 90 minutes and dried (mht60cu90 = bean mass soaked for 60 minutes and cooked for 90 minutes). In all five samples, drying was carried out in the open air until complete desiccation. To evaluate the mass losses (pm), we set respectively pm = mt- (mhtc)i. The percentage of material lost at each stage (pmpce) was obtained by setting pmpce = (pm / mt) * 100.

III. Results and Discussion

a) Results

i. *Field facts*

In Cameroon, beans are consumed in more than one form: boiled then fried, boiled then pounded, in the form of donuts, in the form of a cake, in the form of stew, boiled and then fried in mixture with boiled corn. Etc. By far, the most popular are the forms of cooking that integrate boiling in their cooking phases. The volume of water from cooking this cultivar is large, and increases with the amount of beans boiled.

To cook the beans, the populations, in general, sort, float, wash, soak, and boil. Regarding the trends in the use of end-of-cooking solution for beans (figure 1), 465 (93% of all) families (F1) throw end-of-cooking solution far from the habitat due to the subsequent odor induced. 15 (3% of all) families (F2) continue cooking with this water. 20 (4% of the whole) families (F3) cool this water and give it to their pigs to drink ...).

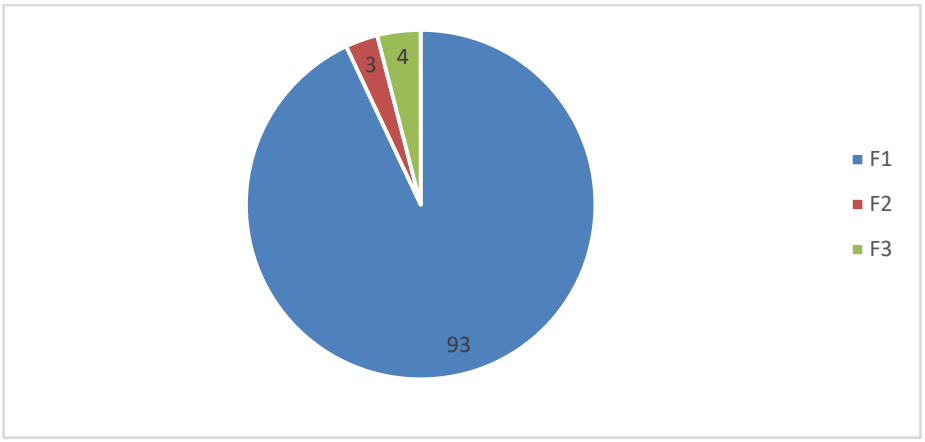


Figure 1: Managing the bean end-of-cooking water in Cameroon

Pig farmers who feed their animals with spent grain mixed with bean end-cooking solution justify their actions by the extraordinary results obtained. The families who continue to cook with this solutoin justify this act by an inheritance from their parents, who have regularly claimed that in doing so, we are better nourished. They do it as part of the preparation of the culinary dish called "pounded". For this, they use the solution from the boiling of the beans to cook what they will pound the beans with. The families who throw out the bean after cooking water justify their act by the fact that it is dirt. Indeed, they are based on the fact that when one comes to pour the water at the end of cooking around the house, shortly after, one notices the emanation of a strong odor which can only disappear after two or even three days depending on the amount of bean solution poured. It shows that the majority of the population at the end of the cooking process rejects as waste water the water from the end of cooking of the bean (Figure 1).

b) Analytical facts

All the parameters monitored within the framework of this study are on the rise despite local variations.

i. *Cations*

Among the cations, the decreasing order of concentration of the elements in the different solutions is as follows: $NH_4^+>>>>K>>>Mg>Ca>>>>>Na$.

a. *Earth alkali cations*

There is a strong similarity in the behavior of calcium and magnesium when the seeds are either soaked or boiled. In fact, the calcium contents (Figure 2A and Table 1) are as follows: 0.0045g / l in the water used for cooking the seeds (E1), 0.046g / l after 30 minutes of soaking (E2) , 0.103 after 60 minutes of soaking (E3), 0.183g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.321g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.403g / l after 60 minutes of soaking and 90 minutes of cooking (E6). Regarding magnesium (Figure 2B and Table 1), its contents change as follows: 0.0034g / l in the water used for cooking the seeds (E1), 0.062g / l after 30 minutes of soaking (E2), 0.138 after 60 minutes of soaking (E3), 0.244g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.429g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.538g / l after 60 minutes of soaking and 90 minutes of cooking (E6). With the involvement of the temperature, the calcium and magnesium contents gradually and significantly increase during cooking to reach their maximum values at the end of the phenomenon. It can also be noted that between the thirtieth minutes and the sixtieth minutes of cooking, the respective content of these two elements tend to double before gradually increasing until the end of the cooking process.

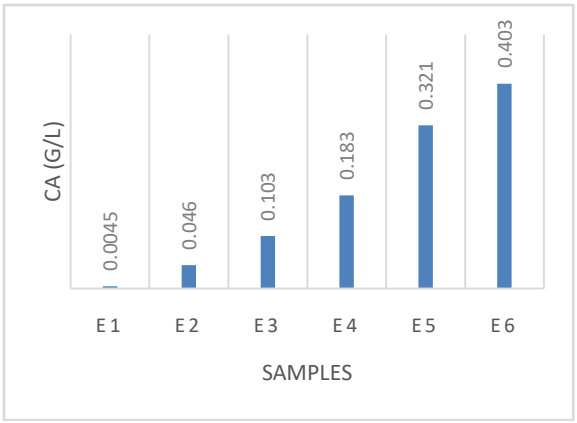


Figure 2A: Evolution of the amounts of Ca

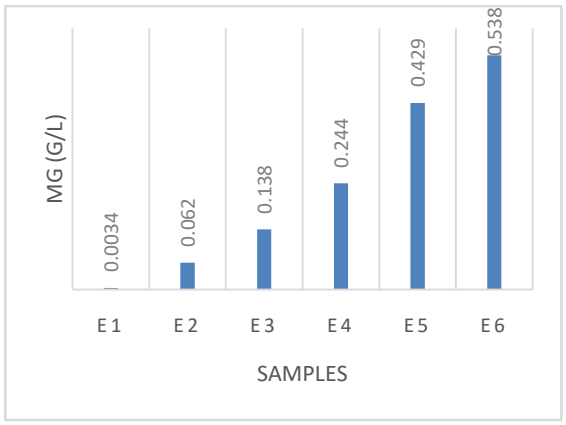


Figure 2B: Evolution of the amounts of Mg

Figure 2: Evolution of the amounts of Calcium and magnesium in response to the contact of bean seeds with water

b. *Alkali cations*

The potassium contents (Figure 3A and Table 1) behave as follows: 0.0095g / l in the water used for cooking the seeds (E1), 1.255g / l after 30 minutes of soaking (E2), 1.294g / l after 60 minutes of soaking (E3), 2.358g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 2.519g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 2.68g / l after 60 minutes of soaking and 90 minutes of cooking (E6). Concerning sodium (Figure 3B and Table 1), its contents change as follows: 0.0154g / l in the water used for cooking the seeds (E1), 0.008g / l after 30 minutes of soaking (E2), 0.019 after 60 minutes of soaking (E3), 0.034g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.0596g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.07487g / l after 60 minutes of soaking and 90 minutes of cooking (E6). The intervention of temperature contributes to greatly increase the potassium and sodium contents in solutions even if the sodium concentrations remain low. Unlike potassium, the increase in sodium content occurs in conjunction with the duration of cooking. On the other hand, for potassium, the sudden increase is felt from the start of cooking; thereafter and until the end of the process, it increases rather slightly.

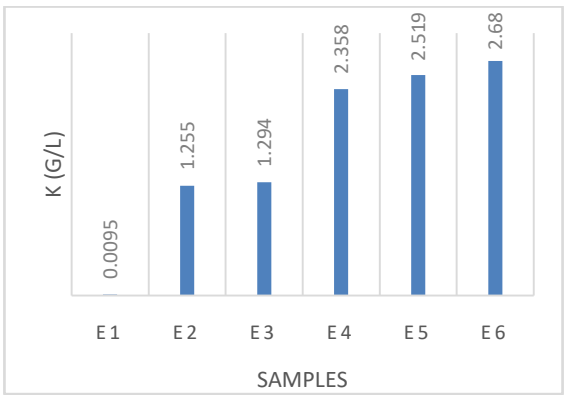


Figure 3A: Evolution of the amounts of K

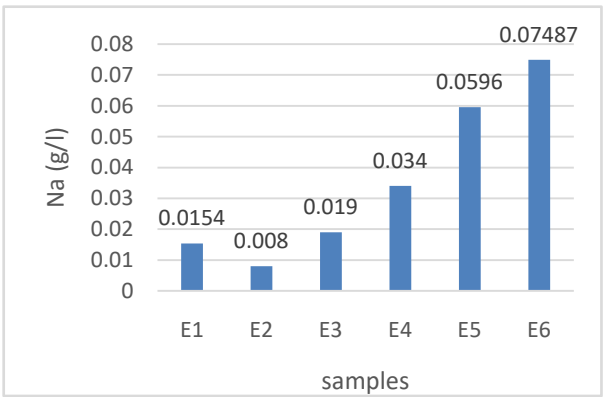


Figure 3B: Evolution of the amounts of Na

Figure 3: Evolution of the amounts of potassium and sodium in response to the contact of bean seeds with water

c. *Ammonium*

Ammonium is the most concentrated cation. Its contents (Figure 4 and Table 1) change as follows: 0.009g / l in the water used for cooking the seeds (E1), 0.31g / l after 30 minutes of soaking (E2), 0.32g / l after 60 minutes of soaking (E3), 4,851g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 5.183g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 5.41g / l after 60 minutes of soaking and 90 minutes of cooking (E6). We can then note that for this element, the intervention of the temperature causes a strong increase in its concentration in the solutions.

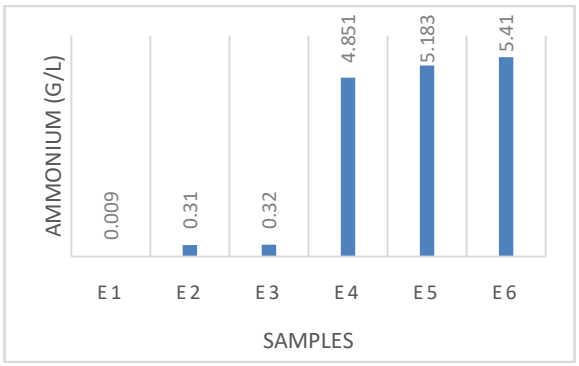


Figure 4: Evolution of the amounts of NH_4^+ in response to the contact of bean seeds with water

d.*Anions*

Among the anions, the decreasing order of concentration of the elements in the different solutions is as follows: S>>>HCO3>>P>Cl.

The sulfate contents (Figure 5A and Table 1) change as follows: 0.0085g / l in the water used for cooking the seeds (E1), 0.1236g / l after 30 minutes of soaking (E2), 0.1237g / l after 60 minutes of soaking (E3), 0.134g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.2633g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.29g / l after 60 minutes of soaking and 90 minutes of cooking (E6). Concerning bicarbonate (Figure 5B and Table 1), its contents change as follows: 0.0013g / l in the water used for cooking the seeds (E1), 0.0012g / l after 30 minutes of soaking (E2), 0.0011 after 60 minutes of soaking (E3), 0.0026g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.0182g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.095g / l after 60 minutes of soaking and 90 minutes of cooking (E6). These two most concentrated anions are also influenced by temperature. This physical magnitude allows them respectively to reach their respective highest content at the end of cooking. However, in the particular case of sulfates, its content increases suddenly and then remains relatively constant in the solutions from the start of soaking until the end of the thirtieth minute of cooking. At the end of the 60th minute of cooking, this content doubles before gradually increasing until the end of cooking. Regarding bicarbonates, their contents remain very low until the end of the thirtieth minute of cooking before being multiplied by 6 at the end of the sixteenth hour of cooking; at the end of the 90th hour of cooking, there is a more than sudden increase in these quantities, or they are multiplied by about 8.

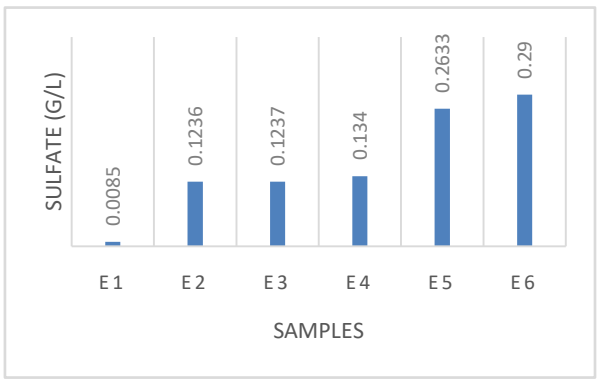


Figure 5A: Evolution of the amounts of SO_4^{2-}

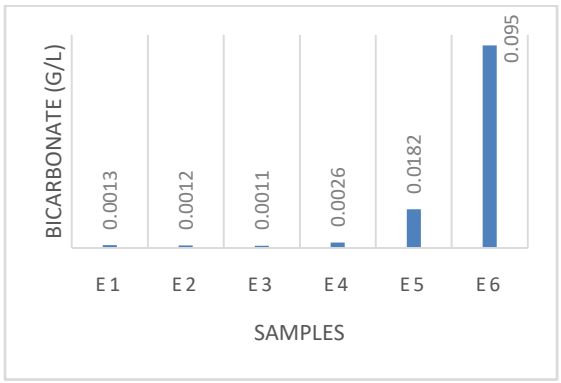


Figure 5B: Evolution of the amounts of HCO_3^-

Figure 5: Evolution of the amounts of sulfates and bicarbonates in response to the contact of bean seeds with water

Regarding the levels of phosphates (figure 6A and Table 1), they are 0.00146g / l in the water used for cooking the seeds (E1), 0.001826g / l after 30 minutes of soaking (E2), 0.00246g / l after 60 minutes of soaking (E3), 0.0298g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.0319g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.0333g / l after 60 minutes of soaking and 90 minutes of cooking (E6). For the chloride contents (Figure 6B and Table 1), we have 0.0016g / l in the water used for cooking the seeds (E1), 0.0034g / l after 30 minutes of soaking (E2), 0.0098 after 60 minutes of soaking (E3), 0.00476g / l after 60 minutes of soaking and 30 minutes of cooking (E4), 0.0171g / l after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.029g / l after 60 minutes soaking and 90 minutes of cooking (E6). Despite the low levels of these two anions, they respond favorably to seed contact with water, and especially with the involvement of temperature. In the particular case of phosphates, its contents first increase very slightly until the sixtieth minute of soaking. From the start of cooking, this content is multiplied by 10 at the end of the thirtieth minute before continuing to increase gradually until the end of cooking. For chlorides, despite an unexplained drop at the end of the thirtieth minute of cooking, the tendency is upward, with the end of cooking as the time of reaching their maximum concentration.

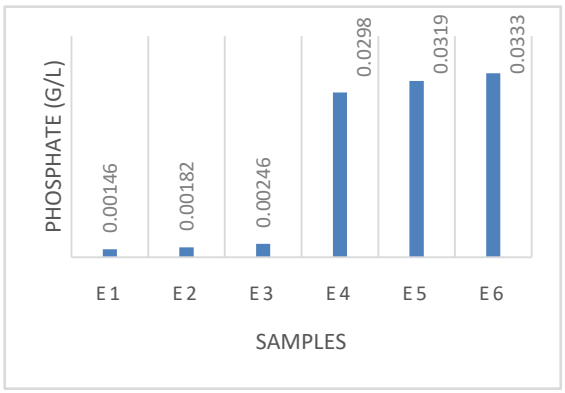


Figure 6A: Evolution of the amounts of HPO_4^{2-}

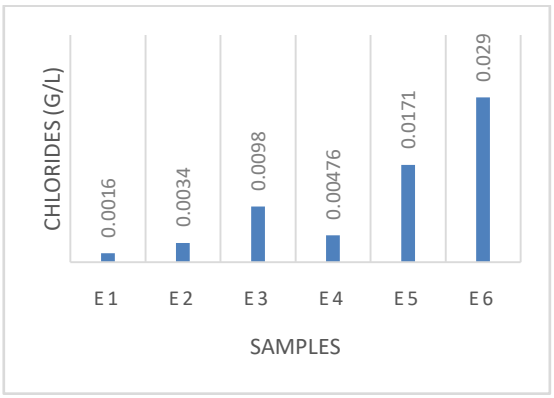


Figure 6B: Evolution of the amounts of chlorides

Figure 6: Evolution of the amounts of phosphates and chlorides in response to the contact of bean seeds with water

e. *EC et pH*

According to Figure 7A and Table 1, the values of electrical conductivity remain low. However, they increase gradually from the end of the thirtieth minute of soaking until the end of the cooking where the value is maximum. In detail, we have in turn 0.029 in the water used for cooking the seeds (E1), 0.071 after 30 minutes of soaking (E2), 0.169 after 60 minutes of soaking (E3), 0.303 after 60 minutes of soaking and 30 minutes of cooking (E4), 0.532 after 60 minutes of soaking and 60 minutes of cooking (E5), and 0.668 after 60 minutes of treping and 90 minutes of cooking (E6).

As for the pH, its value increases by about one unit at the end of the thirtieth minute of soaking (from 5.1 to 6.02). From this point on, there is a slight but constant increase until the end of cooking. We then have respectively 6.02 in E2, 6.1 in E3, 6.3 in E4, 6.41 in E5, and 6.45 in E6 (Figure 7B and Table 1).

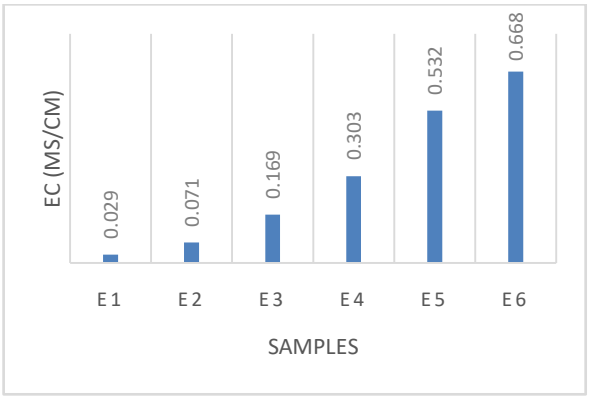


Figure 7A: Evolution of EC values

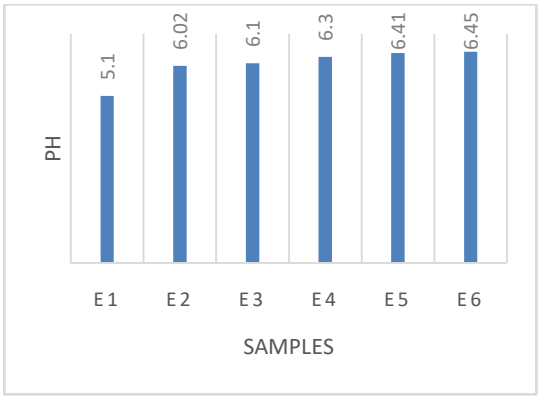


Figure 7B: Evolution of the pH values

Figure 7: Evolution of the EC and pH in response to the contact of bean seeds with water

From the above analysis, it appears that in the different solutions, ammonium followed by potassium are the two most concentrated main elements. They are followed from afar by magnesium, calcium, and sulfates, then very far by bicarbonates, sodium, phosphates and chlorides respectively (figure 8).

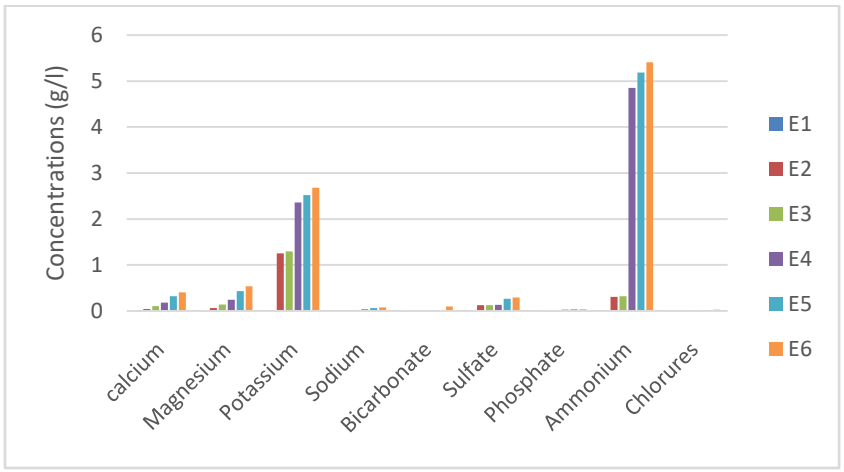


Figure 8: Global distribution of ions in solutions from the contact of bean seeds with water

c) Correlation studies

The correlation is positive and perfect linear between magnesium and calcium; the cloud seems to regroup in three sets (the two red points for the first set, the three blue points for the second set, and the yellow point almost halfway for the third set); the correlation coefficient here is 1 (Figures 9 and 63). That between calcium and potassium is just as positive with nonlinear positive monotonicity; the correlation coefficient is 0.8299 (Figures 10 and 63).Dots seem to constitute three groups: the three red dots for the first group, the middle yellow dot for the second group, and the two blue dots for the third group.

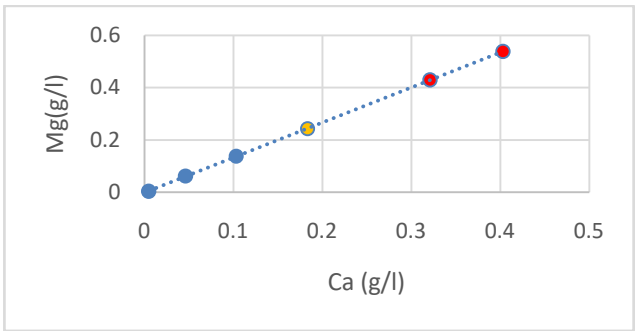


Figure 9: Ca^{2+} and Mg^{2+}correlation

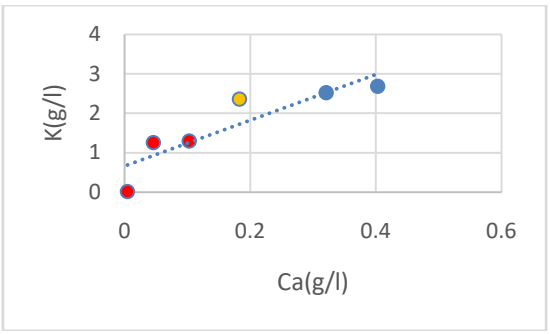


Figure 10: Ca^{2+} and K^{+} correlation

The correlation between sodium and calcium is positive with 0.9813 as correlation coefficient. The major trend is linear. However, we note a distribution of the points in three groups: the two yellow points for the first group, the almost median red point for the second group, the three blue points for the third group (Figures 11 and 63). The bond between bicarbonate and calcium appears to be positive, with a correlation coefficient of 0.7956. But, the grouping of points is very telling. Indeed, the first four points in blue, forming the first group, are arranged horizontally; red (second group) and yellow (third group) dots deviate from the first four respectively (Figures 12 and 63).

Table 1: Characterization of fluids during the process of soaking and beans cooking (mg/l)

	Masse totale (g)	pH	Ca2+	Mg2+	K+	Na+	CO_3^{2-}	$SO4^{2-}$	$HPO4^{2-}$	$NH4^{+}$	Cl-	E.C. (mS/cm)
Eau simple (E1)	88.9	5.1	0.0045	0.0034	0.0095	0.0154	0.0013	0.0085	0.00146	0.009	0.0016	0.0290
Eau de trempage durant 30 minutes (E2)	91.3	6.45	0.046	0.062	1.255	0.008	0.0012	0.1236	0.00182	0.310	0.0034	0.071
Eau de trempage durant 60 minutes (E3)	94.2		0.103	0.138	1.294	0.019	0.0011	0.1237	0.00246	0.320	0.0038	0.169
Eau de cuisson durant 30minutes apres 60 minutes de trempage (E4)	98.3	6.46-6.47	0.183	0.244	2.358	0.034	0.0026	0.134	0.0298	4.851	0.00476	0.303
Eau de cuisson durant 60minutes apres 60 minutes de trempage (E5)	105	6.30	0.321	0.429	2.519	0.0596	0.0182	0.2633	0.0319	5.183	0.0171	0.532
Eau de cuisson durant 90minutes apres 60 minutes de trempage (E6)	109.6	6.31	0.403	0.538	2.63	0.07487	0.095	0.29	0.0333	5.41	0.029	0.668

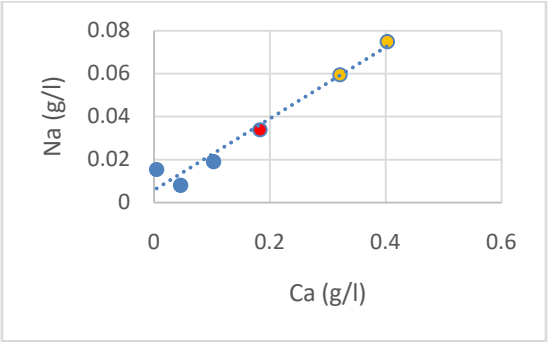


Figure 11: Ca^{2+} and Na^{+} correlation

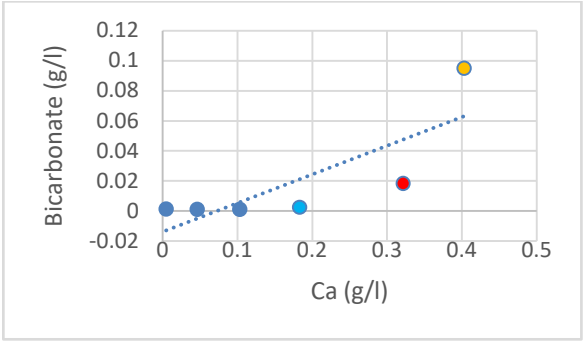


Figure 12: HCO_3^{2-} and Ca^{2+} correlation

Figure 13 shows a positive nonlinear monotonic correlation between sulfate and calcium, with Pearson's coefficient 0.9537 (Figure 63). But in detail, we note the grouping of points in three sets: the yellow point for the first set, the three blue median points for the second set, and the two red points for the third set. Regarding Figure 14, it reveals equally a monotonic positive nonlinear correlation between phosphate and calcium, withPearson'sindex 0.9051 (Figure 63). The clustering of points is just as notable here. Thus, we have the first group represented by the three blue dots, the second group represented by the red dot, and the third group represented by the two upper yellow dots.

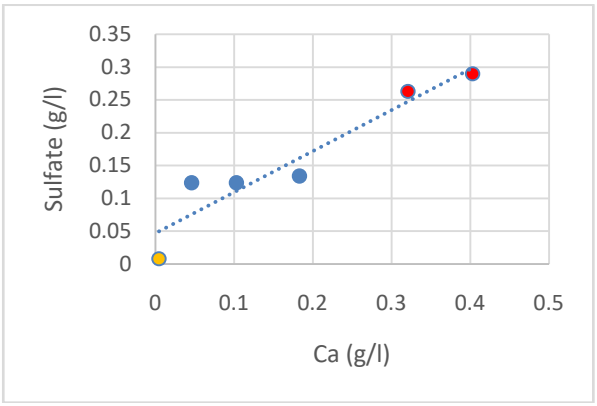


Figure 13: SO_4^{2-} and Ca^{2+} correlation

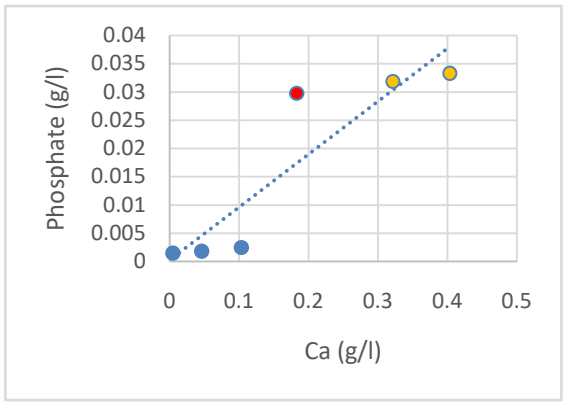


Figure 14: HPO_4^{2-} and Ca^{2+} correlation

For Figure 15, it reveals an equally monotonic positive nonlinear correlation between ammonium and calcium, with Pearson's coefficient 0.9061 (Figure 63). The regrouping of the points here brings out a first group (the three blue points), a second group (the yellow point), and a third group (the two red points). Figure 16 for its one shows a globally monotonic positive nonlinear binding between chlorides and calcium, with 0.923 as correlation coefficient (Figure 63). The distribution of points around the middle line shows a grouping into five groups: the two blue points (group 1), the red point (group 2), the yellow point (group 3), the green point (group 4), and the purple point (group 5).

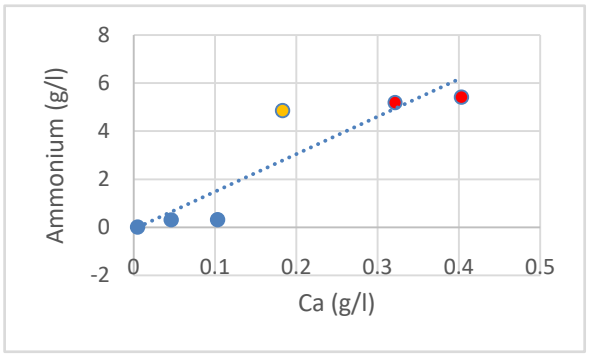


Figure 15: NH_4^{+} and Ca^{2+}correlation

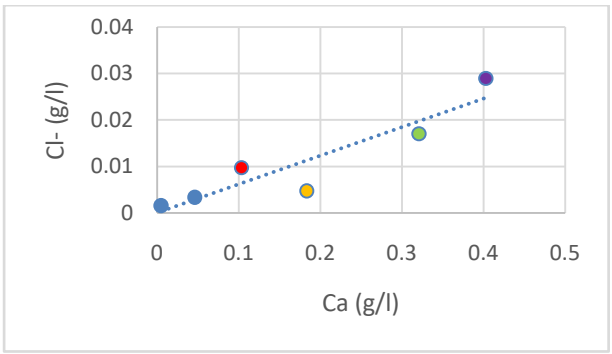


Figure 16: Cl^{-} and Ca^{2+} correlation

The correlation is almost linearly positive between electrical conductivity (EC) and calcium; the cloud seems to be grouped into three sets (the two blue points for the first set, the median red point for the second set, and the three yellow points for the third set); the correlation coefficient is 0.9994 (Figures 17 and 63). That between calcium and pH is non-linear monotonic positive, with the correlation coefficient 0.9081 (Figures 18 and 63); the points, located very high, seem to constitute three groups (the three blue points for the first group, the median red point for the second group, and the two yellow points for the third group). The steepest slope along the trendline is delimited between the first two blue points (from left to right); the rest of the points, almost perfectly aligned, generate a slight slope according to the trendline.

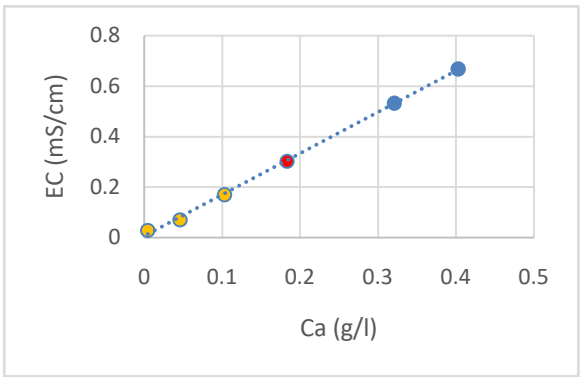


Figure 17: EC and Ca^{2+}correlation

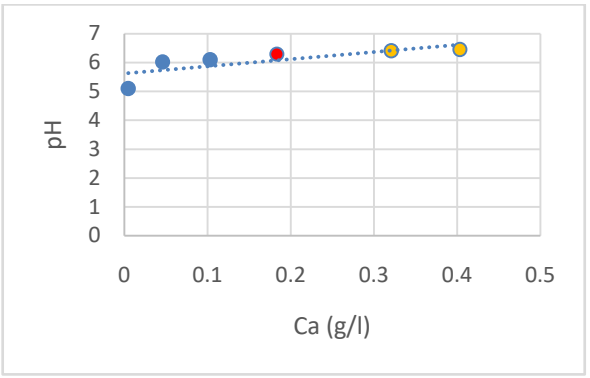


Figure 18: pH and Ca^{2+} correlation

Figure 19 shows a globally monotonic positive non-linear bond between potassium and magnesium, with the correlation coefficient 0.8942 (Figure 63). The distribution of points around the middle line shows a grouping into four groups: the two brown points (group 1), the red point (group 2), the two blue points (group 3), and the yellow point (group 4). The correlation between sodium and magnesium is positive with the correlation coefficient 0.16601. The major trend is linear. However, we note a distribution of the points in three groups: the two blue points for the first group, the almost median yellow point for the second group, the three red points for the third group (figure 20 and 63).

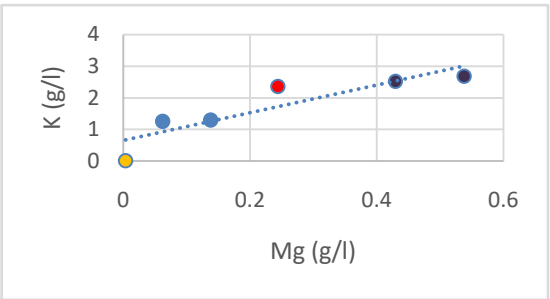


Figure 19: K^{+} and Mg^{2+}correlation

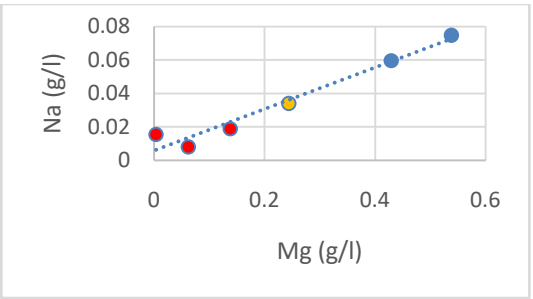


Figure 20: Na^{+} and Mg^{2+} correlation

The bond between bicarbonate and magnesium seems positive, with correlation coefficient 0.802. But, the grouping of points is very telling. Indeed, the first four points in blue, forming the first group, are arranged horizontally; the red (second group) and yellow (third group) dots respectively deviate from the first four (figures 21 and 63). The correlation between sulfate and magnesium is monotonic positive nonlinear, with the correlation coefficient 0.9515. We note a distribution of the points in three groups: the two yellow points for the first group, the three blue points for the second group, and the red point for the third group (figures 22 and 63).

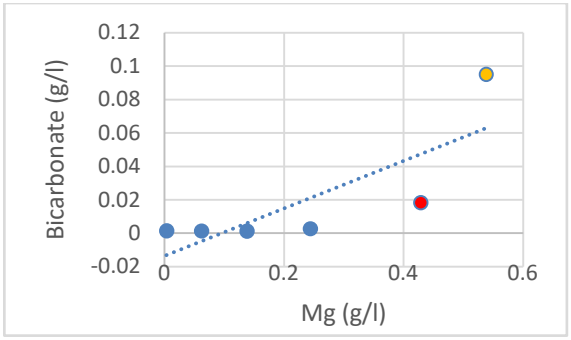


Figure 21: HCO_3^- and Mg^{2+} c orrelation

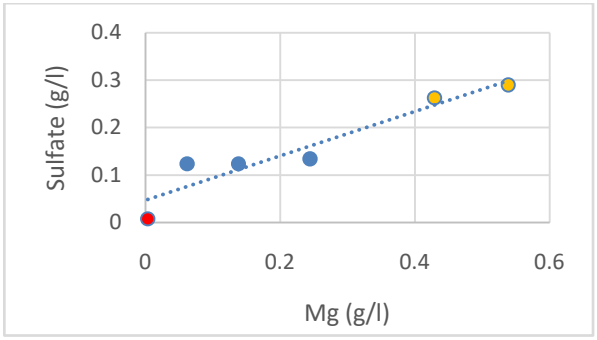


Figure 22: SO_4^{2-} and Mg^{2+} correlation

Figures 23 and 24 show a positive nonlinear monotonic correlation between phosphate and magnesium and between ammonium and magnesium, respectively. The correlation coefficient is 0.9044 in the first case and 0.9055 in the second case (Figure 63). The regrouping of the points here brings out a first group (the three blue points), a second group (the yellow point), and a third group (the two red points).

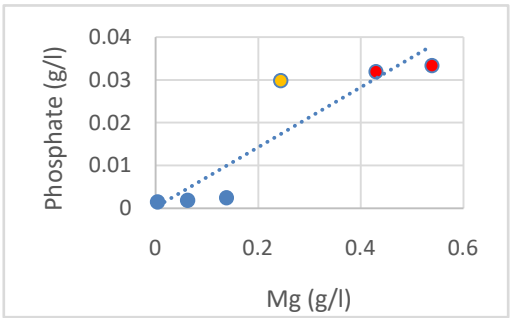


Figure 23: HPO_4^{2-} and Mg^{2+}correlation

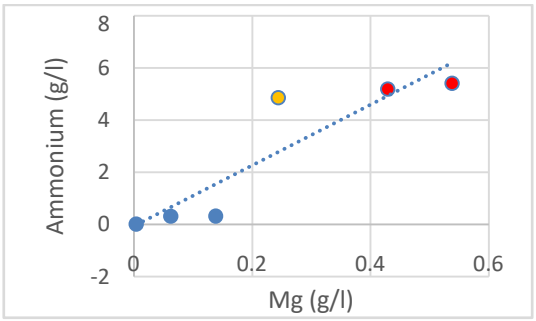


Figure 24: NH_4^+ and Mg^{2+} correlation

The correlation is globally positive nonlinear monotonic between chlorides and magnesium (Figure 25); their correlation coefficient is 0.9228 (Figure 63). The distribution of points around the middle line shows a grouping into five sets: the two blue points (group 1), the red point (group 2), the yellow point (group 3), the green point (group 4), the brown point (group 5). The correlation is quite positive linear between electrical conductivity (EC) and magnesium; the cloud seems to be grouped into three sets (the three blue points for the first set, the median red point for the second set, and the two brown points for the third set); the correlation coefficient here is 0.9989 (Figures 26 and 63).

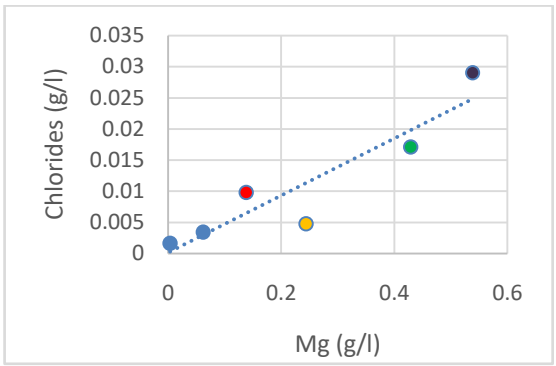


Figure 25: Cl^- and Mg^{2+} correlation

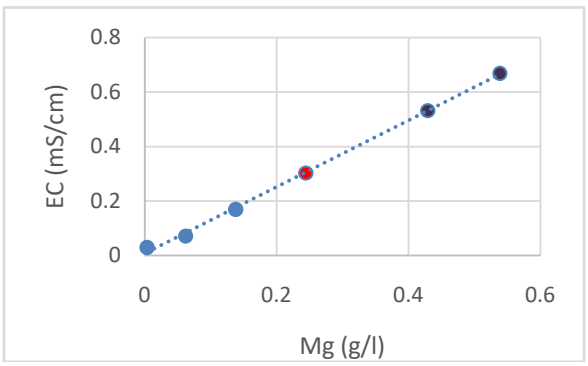


Figure 26: EC and Mg^{2+} correlation

The bond between magnesium and pH is monotonic positive nonlinear, with coefficient of correlation 0.9236 (figures 27 and 63); the points, located very high, seem to constitute three groups (the three red points for the first group, the median yellow point for the second group, and the two blue points for the third group). The steepest slope along the trendline is delimited between the first two red dots (from left to right); the rest of the points, almost perfectly aligned, generate a slight slope according to the trendline.

Regarding the correlation between sodium and potassium, it seems positive with regard to the trend curve (Figure 28), with the correlation coefficient 0.8031 (Figure 63). The scatter plot here reveals four groups: the blue point for the first group, the two red points for the second group, the yellow point for the third group, and the two purple points for the fourth group.

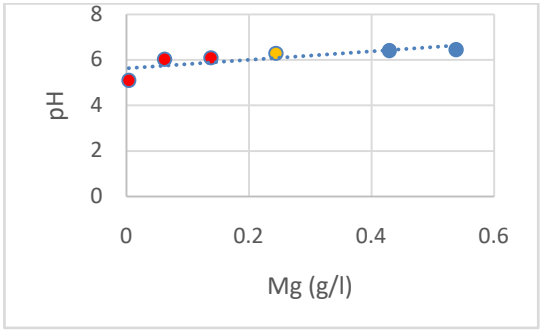


Figure 27: pH and Mg^{2+} correlation

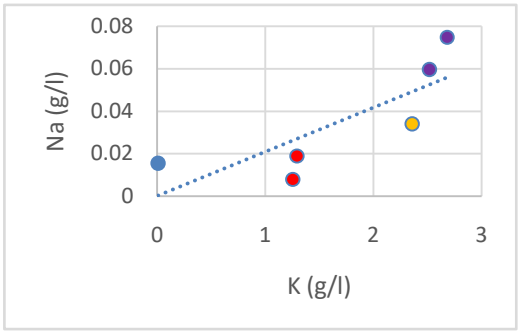


Figure 28: Na^{+} and K^{+} correlation

According to the trend curve in Figure 29, the correlation is positive between bicarbonate and potassium, with the correlation coefficient 0.6175 (Figure 63). But, the distribution of points is ambiguous, suggesting four groups: the red point for group 1, the two blue points for group 2, the two yellow points for group three, and the purple point for group 4. Also, we see that the points of group 1 and group 2 are completely aligned horizontally. From the analysis of Figure 30, there is also a positive correlation between sulfate and potassium, with Pearson's coefficient 0.8577 (Figure 63). As before, the distribution of the points suggests four groups: the red point for the first group, the two blue points for the second group, the yellow point for the third group, and the two purple points for the fourth group. However, the points of the first and second group are well aligned along the trendline.

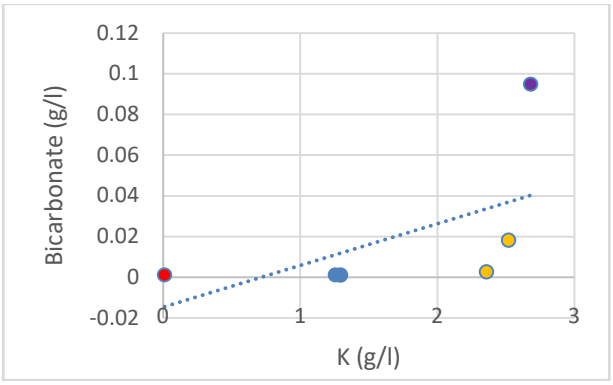


Figure 29: HCO_3^{-} and K^{+} correlation

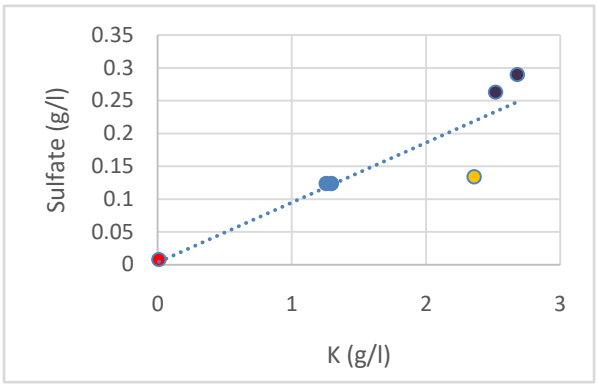


Figure 30: SO_4^{2-} and K^{+} correlation

Figures 31 and 32 respectively highlight the positive correlation that exists between phosphate and potassium on the one hand, and between ammonium and potassium on the other. The correlation coefficients are 0.8995 and 0.9101 respectively (Figure 63). In both cases, the cloud generated brings out three groups: the red point for the first group, the two blue points for the second group, the three yellow for the third group.

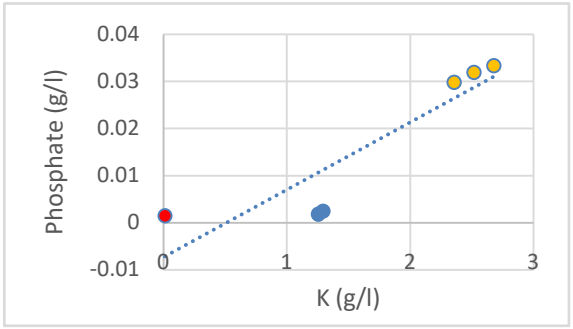


Figure 31: HPO_4^{2-} and K^{+} correlation

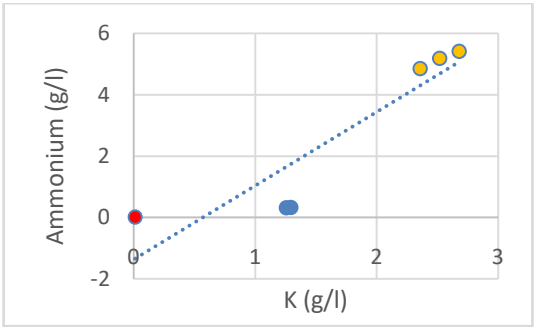


Figure 32: NH_4^{+} and K^{+} correlation

The correlation between chlorides and potassium is positive with regard to the trend curve (Figure 33); the correlation coefficient is 0.7109 (Figure 63). But, we note a strong dispersion between the points of the cloud, bringing out five groups in total: the red point for group 1, the two blue points for group 2, the yellow point for group 3, the green point for group four, and the purple point for group 5. Concerning figure 34, it shows for its part a positive correlation between electrical conductivity (EC) and potassium, with the correlation coefficient 0.8797 (Figure 63). The dispersion of the dots brings out four groups: the red point for group 1, the two blue dots for group 2, the yellow point for group 3, and the two purple dots for group 4.

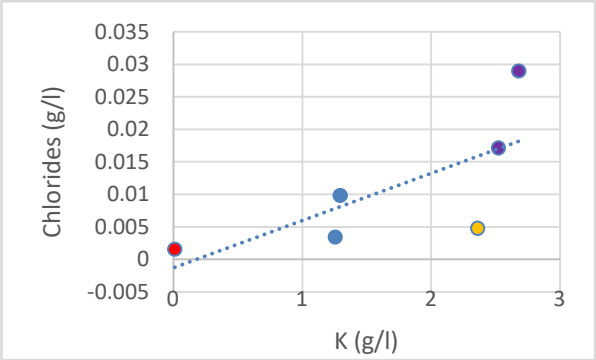


Figure 33: Cl^- and K^+ correlation

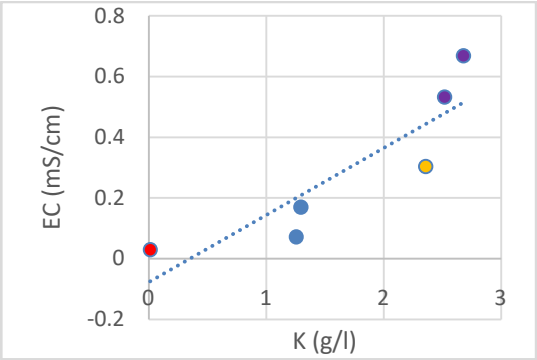


Figure 34: EC and K^+ correlation

The correlation is positive between pH and potassium (Figure 35), with Pearson's coefficient 0.9511 (Figure 63). The cloud of points, located very high, is organized into three groups: the red point for group 1, the two blue points for group 2, and the three yellow points for group 3. Overall, despite the dispersion points, they are all very close to the trendline. The correlation is also positive between sodium and bicarbonate by referring to the trend curve (Figure 36); their correlation coefficient is 0.1405 (Figure 63). The cloud inducted individualizes four groups: the three blue points for the first group, the red point for the second group, the yellow point for the third group, and the purple point for the fourth group. But, the points of the first and second group line up horizontally along the abscissa axis.

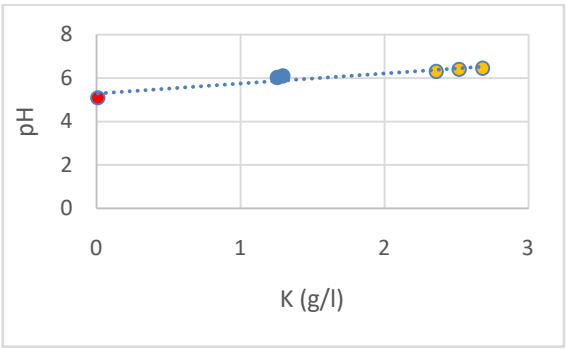


Figure 35: pH and K^+ correlation

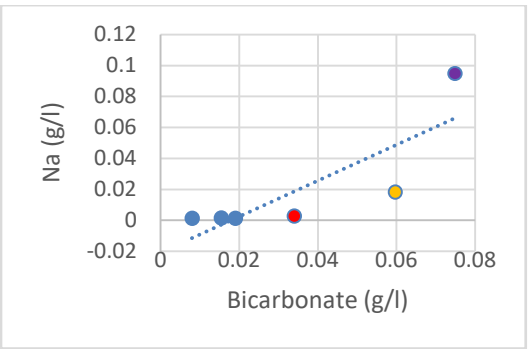


Figure 36: HCO_3^- and Na^+ correlation

Figure 37 shows a positive correlation between sulfate and sodium, with the correlation coefficient 0.1503 (Figure 63). The distribution of points individualizes three groups: the red point for the first, the three blue points for the second, the two yellow points for the third. Figure 38 for its part also shows a positive correlation between phosphate and sodium with regard to the trend curve. The correlation coefficient is 0.1515 (Figure 63). The points of the cloud are individualized into three groups: the three blue points for the first, the red point for the second, and the two yellow points for the third.

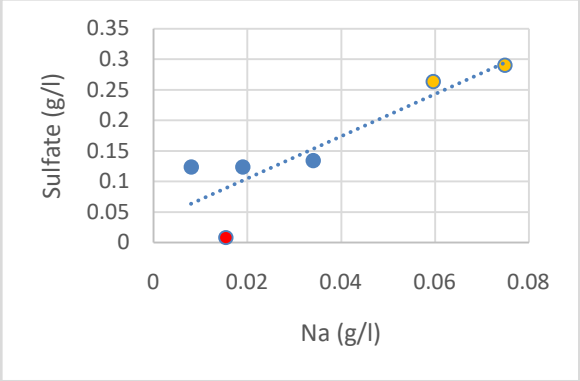


Figure 37: SO_4^{2-} and Na^+ correlation

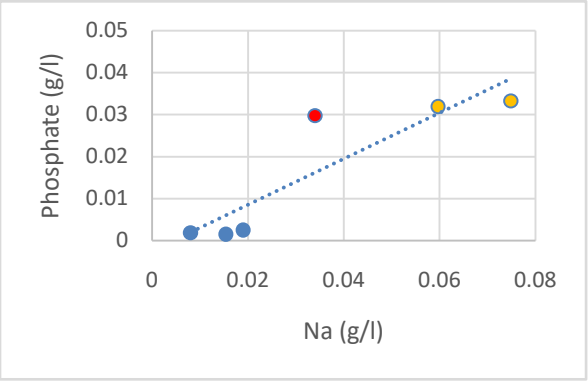


Figure 38: HPO_4^{2-} and Na^+ correlation

The trend line in Figure 39 shows a positive correlation between chlorides and sodium. The correlation coefficient is 0.1549 (Figure 63). The dispersion of the points of the cloud individualizes four groups: the three blue points for the first, the red point for the second, the yellow point for the third, and the purple point for the fourth. Regarding figure 40, the distribution of points highlights a positive correlation between electrical conductivity (EC) and sodium, with the Pearson's index of 0.1672 (Figure 63). The distribution of the points of the cloud reveals three groups: the three blue points for the first, the red point for the second, and the two yellow points for the third. The vast majority of points are almost carried by the trendline.

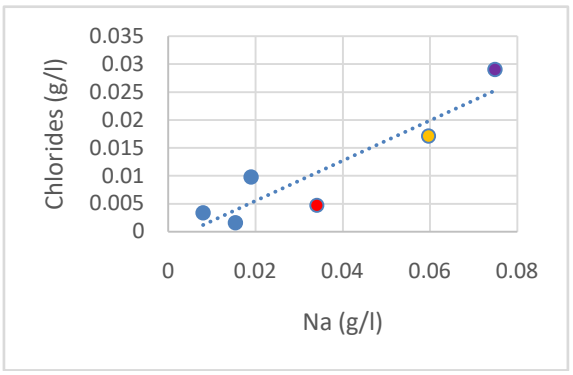


Figure 39: Cl- and Na^+ correlation

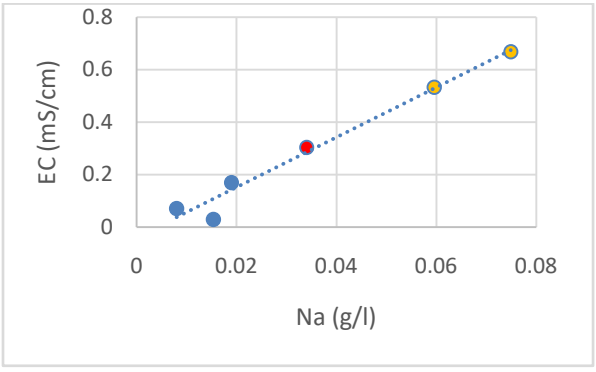


Figure 40: EC and Na^+ correlation

The correlation is positive between pH and sodium (Figure 41), with 0.1312 as the correlation coefficient (Figure 63). Located high, the scatter plot for the most part clusters very close to the trendline. Also, this cloud individualizes three groups: the three blue points for the first, the red for the second, and the two yellow points for the third. The trend curve in Figure 42 shows a positive correlation between sulfate and bicarbonate, with the correlation coefficient 0.7332 (Figure 63). The distribution of points reveals four groups: the red point for the first, the two blue points for the second, the yellow point for the third, the purple point for the fourth. We note in detail that the fourth group is very eccentric with respect to the other three groups.

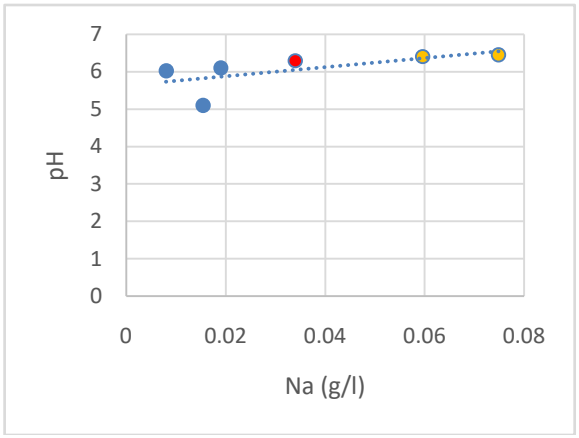


Figure 41: pH and Na+ correlation

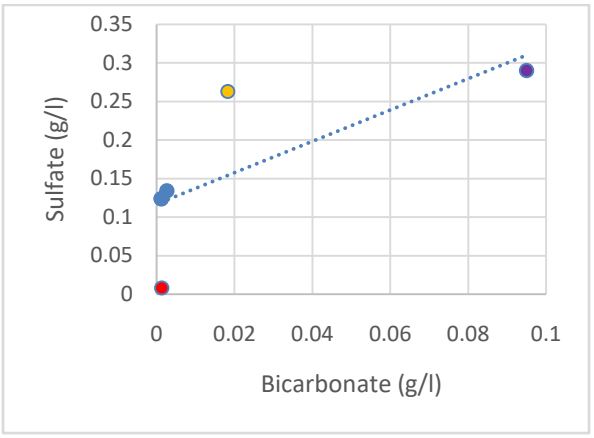


Figure 42: SO_4^{2-} and HCO_3^{2-} correlation

In Figures 43 and 44, there is a positive non-linear monotonic correlation between phosphate and sulfate on the one hand and between ammonium and bicarbonate on the other hand. The correlation coefficients are respectively 0.5972 and 0.5949 (Figure 63). The distribution of points shows four groups in the first case and three groups in the second case. In the first case we have: the two blue points for the first group, the red point for the second group, the two yellow points for the third group, and the purple point for the fourth group. In the second case, we have: the three blue points for the first group, the two yellow points for the second group, and the red point for the third group. In both cases, if the first groups tend to approach, the last group is completely eccentric.

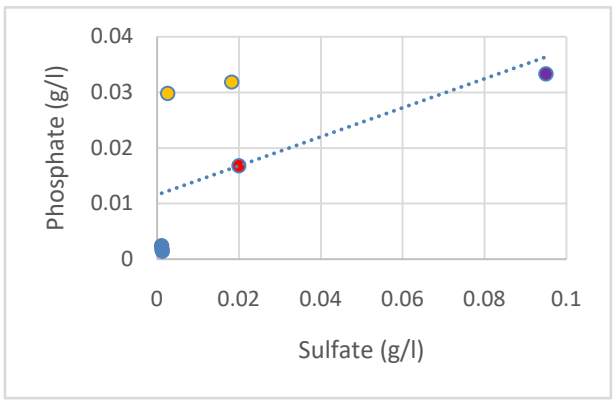


Figure 43: HPO_4^{2-} and HCO_3^- correlation

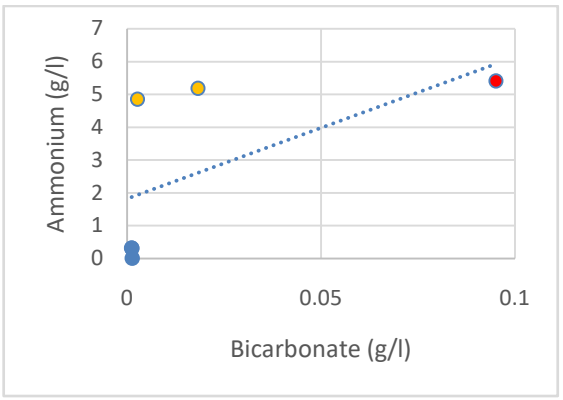


Figure 44: NH_4^+ and HCO_3^- correlation

Figures 45 and 46 show a monotonic positive non-linear correlation between chlorides and bicarbonate on the one hand and between electrical conductivity (EC) and bicarbonate on the other. The correlation coefficients are respectively 0.9138 and 0.8088 (Figure 63). The distribution of points highlights three groups in both cases: the three blue points for the first group, the two yellow points for the second group, and the red point for the third group. In both cases, if the first two groups tend to come closer, the last group is completely eccentric. Also, the points of the first group and one of the points of the second group are almost vertically arranged along the y-axis.

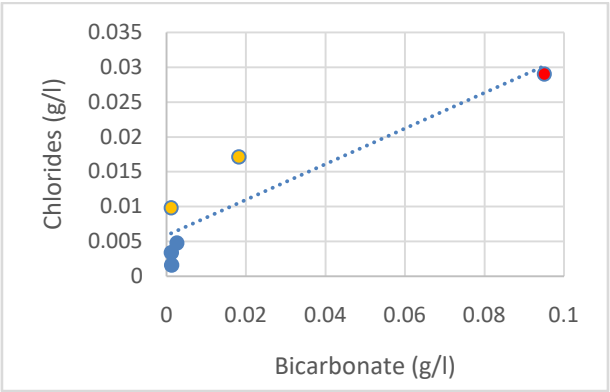


Figure 45: Cl- and HCO_3^- correlation

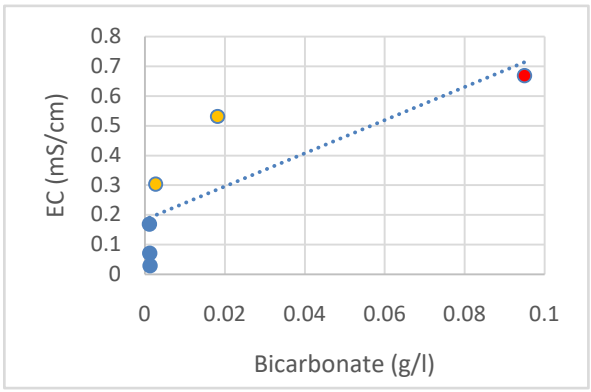


Figure 46: EC and HCO_3^- correlation

The correlation is positive nonlinear monotonous between the pH and the bicarbonate (Figure 47), with the correlation coefficient 0.4522 (Figure 63). The point cloud is high. Most of these points are concentrated towards the left end of the trendline. This cloud individualizes three groups: the four blue points for the first, the red point for the second group, and the yellow point for the third group. The correlation between phosphate and sulfate (Figure 48) on the one hand and between ammonium and sulfate (Figure 49) on the other hand is monotonic positive nonlinear, with correlation coefficient 0.8003 and 0.8060 respectively (Figure 63). The point cloud in both cases is organized into four groups: the brown point for the first, the two blue points for the second, the yellow point for the third, the two red points for the fourth. We note in detail that these groups are highly distant from each other.

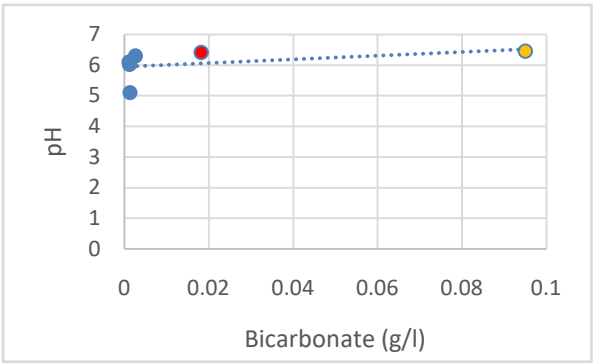


Figure 47: pH and HCO_3^- correlation

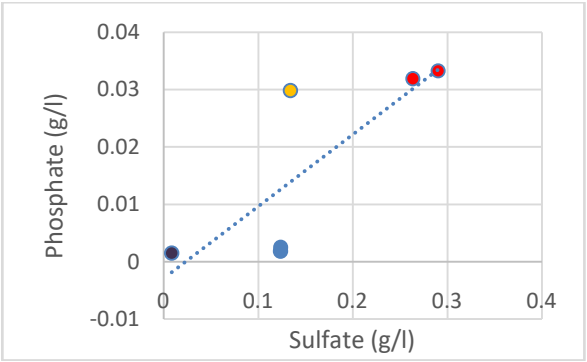
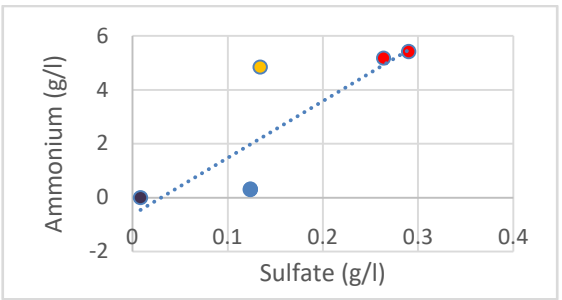


Figure 48: HPO_4^{2-} and SO_4^{2-} correlation

Regarding the correlation between chlorides and sulfates (Figure 50), the correlation between electrical conductivity (EC) and sulfate (Figure 51), and the correlation between pH and sulfate (Figure 52) respectively, the trend curve shows that it is globally linear positive, with the respective correlation coefficient 0.8987, 0.3662, 0.8696 (Figure 63). The point cloud of the pH-sulfate correlation has the particularity of being very high. Overall, this cloud makes it possible to visualize three groups of points, relatively distant from each other: the red point for the first group, the three blue points for the second group, and the two yellow points for the third group.



Fugure 49: NH_4^+ and SO_4^{2-} correlation

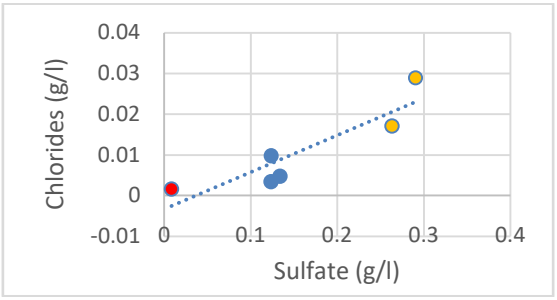


Figure 50: Cl^- and SO_4^{2-} correlation

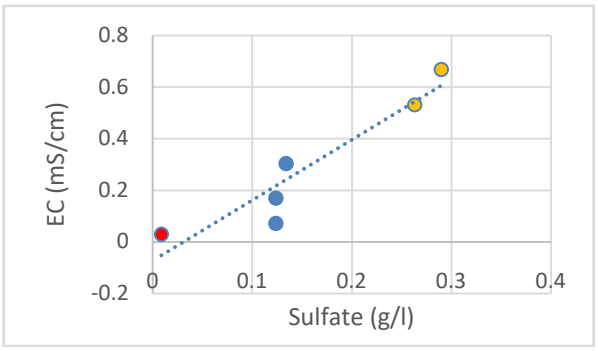


Figure 51: EC and SO_4^{2-} correlation

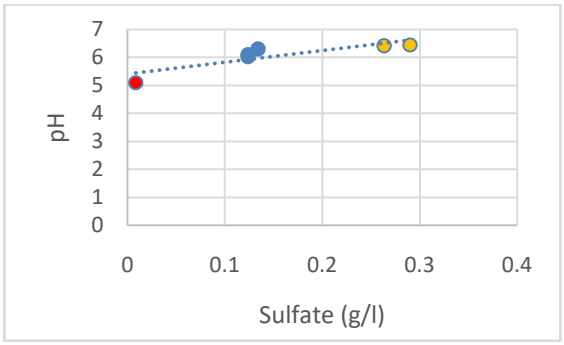


Figure 52: pH and SO_4^{2-} correlation

The trendline of the correlation between ammonium and phosphate (Figure 53), pH and ammonium (Figure 54), and pH and phosphate (Figure 55) shows a positive relationship. The correlation coefficient is 0.7964 in the first case, 0.7395 in the second case, and 0.7275 in the third case (Figure 63).

The point cloud initiated by the correlation pH-NH_4^+ and pH-$HPO_4$2- is positioned higher (Figures 54 and 55). But, in all three cases, this cloud individualizes two main groups, distant from each other: the three blue dots for the first group and the three red dots for the second group.

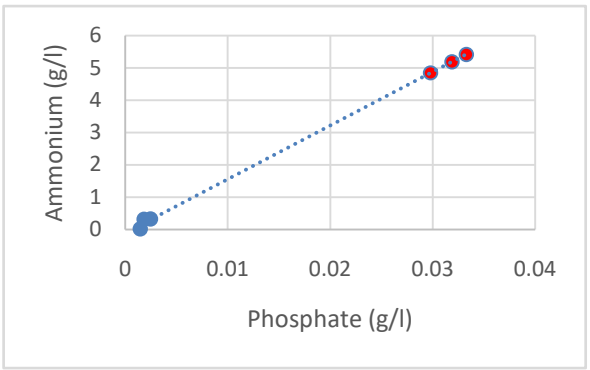


Figure 53: NH_4^+ and HPO_4^{2-} correlation

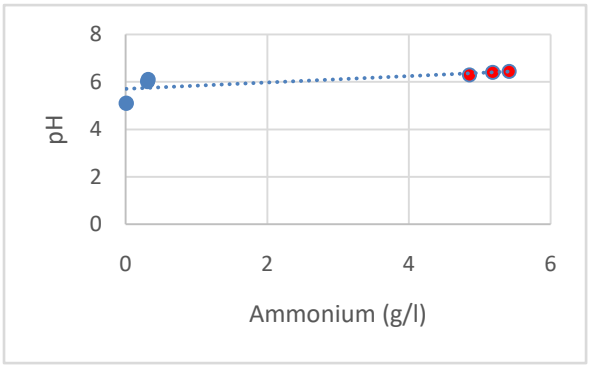


Figure 54: pH and NH_4^+ correlation

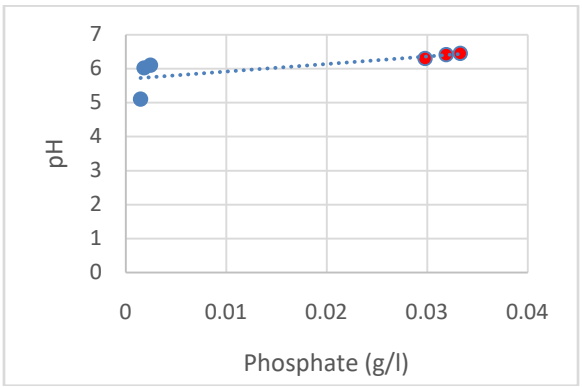


Figure 55: pH and HPO_4^{2-} correlation

Regarding the correlation between electrical conductivity (EC) on the one hand (Figure 56) and the correlation between electrical conductivity and phosphate on the other hand (Figure 57), the trend line shows a positive correlation. The respective correlation coefficients are 0.9065 and 0.9064 (Figure 63). The cloud of points individualizes three groups: the three blue points for the first group, the red point for the second group, and the two yellow points for the third group.

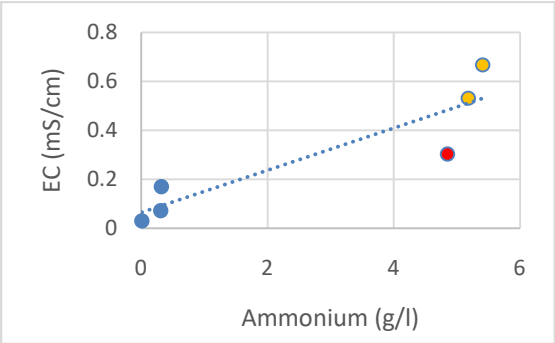


Figure 56: EC and NH_4^+ correlation

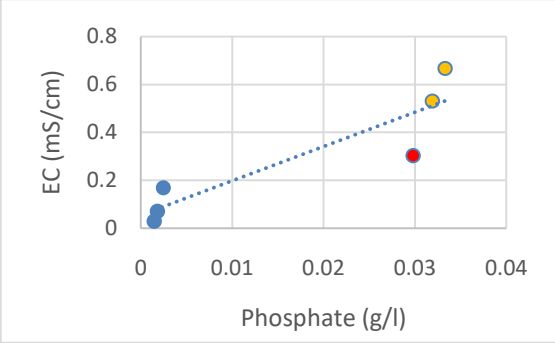


Figure 57: EC and HPO_4^{2-} correlation

In the context of the correlation between chlorides and ammonium (Figure 58) on the one hand and between phosphates and chlorides (Figure 59) on the other hand, the trend curve shows a positive relationship. The correlation coefficients are 0.6826 and 0.6823 respectively (Figure 63). The scatter plot shows four groups: the three blue points for the first group, the red point for the second group, the purple point for the third group, and the yellow point for the fourth group.

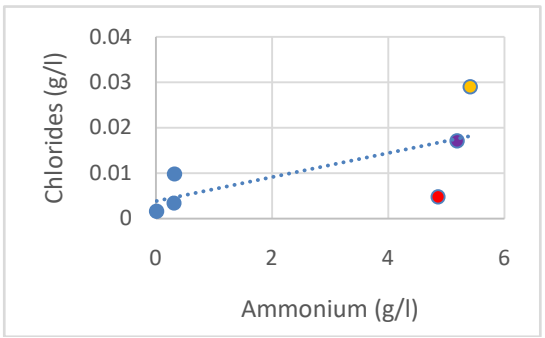


Figure 58: Cl^- and NH_4^+ correlation

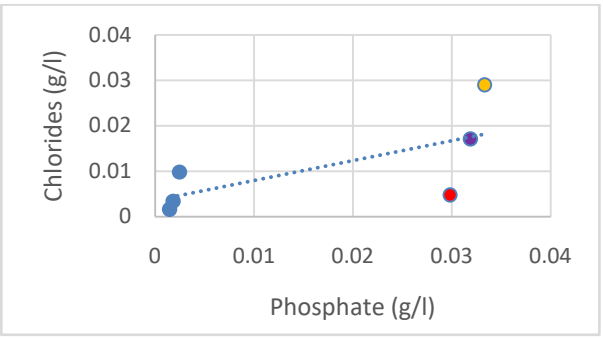


Figure 59: HPO_4^{2-} and Cl^-correlation

The trend curve for the correlation between pH and chlorides (Figure 60) on the one hand and between pH and electrical conductivity (EC) on the other hand (Figure 61) shows a positive relationship in both cases, with for respective correlation coefficient 0.6456 and 0.7576 (Figure 63). The distribution of points here brings out three groups for each of these cases. Regarding the correlation between pH and chlorides, the groups are: the four blue points for the first group, the red point for the second group, and the yellow point for the third group. Regarding the second case, the groups are: the three blue dots for the first group, the red dot for the second group, and the two yellow dots for the third group. In both cases, the third group is distant from the first two groups.

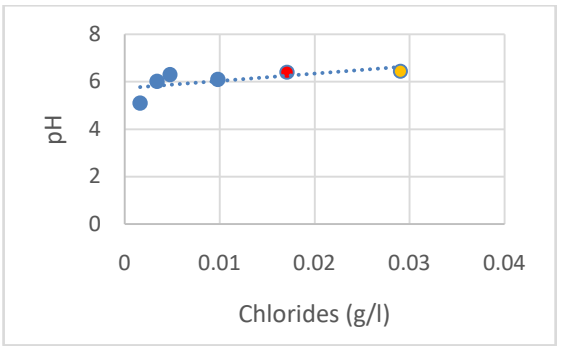


Figure 60: pH and Cl^- correlation

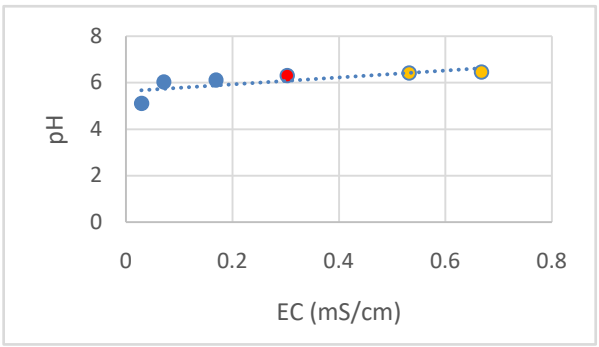


Figure 61: pH and EC correlation

Figure 62 highlights a generally linear positive correlation along the trendline. The correlation coefficient is 0.9235 (Figure 63). The cloud of points makes it possible to visualize three groups: the four blue points for the first group, the yellow point for the second group, and the red point for the third group. In detail, these three groups are significantly distant from each other.

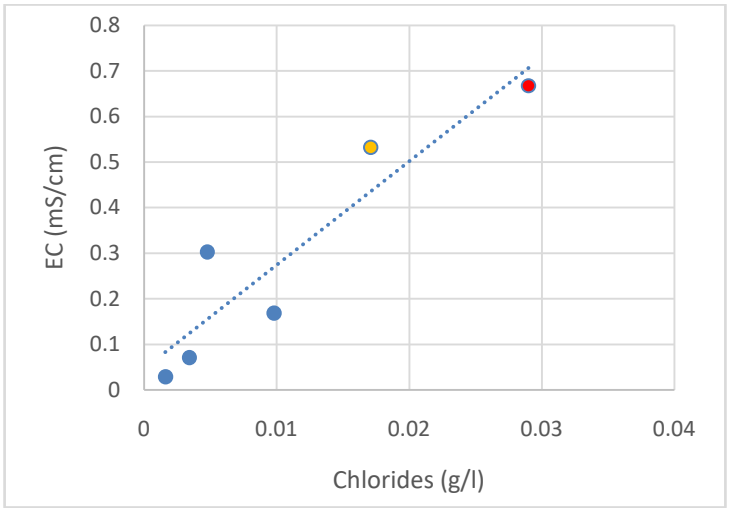


Figure 62: EC and Cl^- correlation

	Ca^{2+}	Mg^{2+}	K^+	Na^+	HCO_3^-	NH_4^+	SO_4^{2-}	HPO_4^{2-}	Cl^-	EC	pH
Ca^{2+}	1										
Mg^{2+}	1	1									
K^+	0.8299	0.8942	1								
Na^+	0.9813	0.16601	0.8031	1							
HCO_3^-	0.7956	0.802	0.6175	0.1405	1						
NH_4^+	0.9061	0.9055	0.9101	0.1509	0.5949	1					
SO_4^{2-}	0.9537	0.9515	0.8577	0.1503	0.7332	0.8061	1				
HPO_4^{2-}	0.9051	0.9044	0.8995	0.1515	0.5972	07964	0.8003	1			
Cl^-	0.923	0.9228	0.7109	0.1549	0.9138	0.6826	0.8987	0.6823	1		
EC	0.9994	0.9989	0.8797	0.1672	0.8088	0.9065	0.3662	0.9064	0.9235	1	
pH	0.9081	0.9236	0.9511	0.1312	0.4522	0.7395	0.8696	0.7275	0.6456	0.7576	1

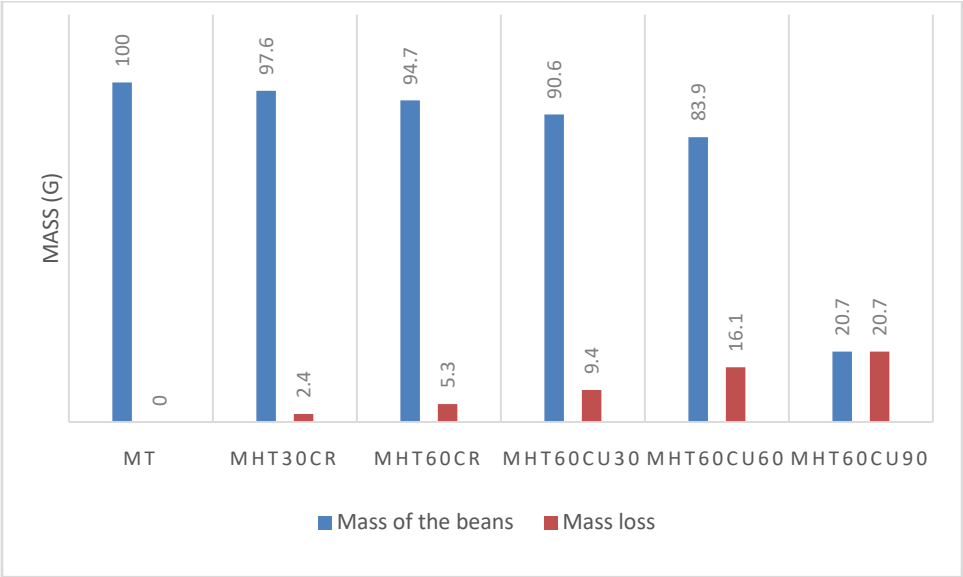
Figure 63: Matrix of correlation

d) Mass variations of the beans during soaking and cooking processes

During soaking and cooking the beans, one can denote the mass loss at the different stages by bean seeds (Figure 64 and Table 2). However, the mass loss is lower during the soaking of the beans compared to the situation observed during the cooking process. In detail, from 100g at the beginning (mt), the mass reaches 97.6g after 30 minutes of soaking (mht30cr), 94.7g after 60 minutes of soaking (mht60), 90.6g after 60 minutes of soaking and 30 minutes of cooking (mht60cu30), 83.9g after 60 minutes of soaking and 60 minutes of cooking (mht60cu60), and 79.3g after 60 minutes of soaking and 90 minutes of cooking (mht60cu90). Then, compared to the initial situation (mt), the loss is as follow: 2.4g, 5.3g, 9.4, 16.1g, 20.7g respectively in mht30cr, mht60cr, mht60cu30, mht60cu60, mht60cu90 stages (Figure 64).

Table 2: Mass variations of the beans during soaking and cooking processes

	mt	mht30cr	mht60cr	mht60cu30	mht60cu60	mht60cu90
M(g)	100	97.6	94.7	90.6	83.9	79.3
Perte de masse (g)	0	2.4	5.3	9.4	16.1	20.7
Pourcentage de matière perdue (%)	0	2.4	5.3	9.4	16.1	20.7



IV. Discussion

When the seeds are soaked, the amounts of the various target ions in the scope of this study increase significantly. During boiling, the stage after the soaking phase, this increase is greater. Overall, this increase is due to the hydrolysis of reserves contained in the cotyledons according to [31] and [32], the main seed storage organs as proved by [33]. The products resulting from this hydrolysis then pass through the teguments by diffusion to end up in the soaking or cooking solutions. Rachidi (2012) explains this integument crossing by relying on the permeable or semi-permeable character of biological membranes. This postulate corroborates the observations made by [35]. The sudden increase in the quantities of ions in solution during cooking is justified by the fact that the temperature, high or low, greatly influences the lysis of compounds (Davis, 1983). The addition of the amounts of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, SO_4^{2-}, HPO_3^{2-}, Cl^{-}, NH_4^{+}, Cl^{-}, and HCO_3^{-} in the soaking or cooking solutions is in accordance with the composition of bean seeds as highlighted by [24] have shown this. In addition, [35] completes these observations by specifying the amounts of sulfur in bean seeds. NH_4^{+} and HCO_3^{-}come from the hydrolysis of organic compounds present in the cotyledons, in particular proteins for the first ion, and carbohydrates and lipids for the second ion. This is in agreement with the results of [36].Likewise, the increase in pH and electrical conductivity (EC) is noted. The increase in pH is the consequence of the increase in the amounts of exchangeable basic cations in soaking or cooking solutions. This observation is commonly made in soil physiology by pedologists like [37], [35], etc. Regarding electrical conductivity (EC), this is a quantity that is related to the presence of electrolytes in solution. Its behavior is consistent with the increase in ion quantities in the solutions followed here. However, according to [38], it is weak. This is the consequence of the low sodium contents in the solutions. The main ions contained in the solutions are NH_4^{+} and K^{+}respectively, followed by Mg^{2+}, Ca^{2+}, $SO_4^{2-}$for the most abundant. This is in accordance with the order of decreasing concentration of bean seeds in these different elements. However, one can also relate the enrichment of solutions resulting from cooking bean seeds in Ca^{2+}, Mg^{2+}, K^{+}, and $SO_4^{2-}$to the fact that these elements are highly influenced by temperature. In this sense, [39] and [40] shows through his two series of crystallization of minerals in a magmatic environment that the Ferromagnesians (forsterite type olivines) crystallize parallel to the calcium feldspars (anorthites) at high temperature. Lower, that is, at a lower temperature, crystallize potassium and sodium silicates, followed by other minerals. Moreover, it has been observed that during volcanic eruptions, many gases including sulfur

dioxide are released [41]. This ease of sulfur to express in the presence of heat is consistent with the increase in sulfates in bean seed cooking solutions.

Further on, all the correlations are positive between the parameters followed in this present study. This is in agreement with the joint increase of these different parameters. One would therefore be tempted to think that the different parameters influence each other in solutions when bean seeds are soaked or boiled. However, a detailed observation shows that the point clouds organize themselves into groups, and that the points of several of these groups are arranged either horizontally as is the case in the correlation between sodium and bicarbonate, potassium and bicarbonate, bicarbonate and magnesium, and between bicarbonate and calcium, either vertically, as is the case in the correlation between electrical conductivity and bicarbonate, chlorides and bicarbonates, phosphates and sulfates, ammonium and bicarbonate, sulfate and bicarbonate, pH and bicarbonate, electrical conductivity and sulfate, and between electrical conductivity and ammonium. This demonstrates independence in the behavior of the parameters concerned according to the work of [42]. This is the reason why in a magmatic environment, some minerals will form first before others depending on the environmental conditions. Also, the points of the clouds organize themselves in groups which are sometimes very distant. This is the consequence of the presence of another underlying parameter which controls the behavior of the elements involved in the correlation. This is in agreement with the observations made by [42]. On a completely different level, we can see that in the organization of point clouds, many points are completely off-center, imposing a final trend that does not correspond to reality. This is the case in the correlation between pH and bicarbonate, electrical conductivity and bicarbonate, sulfate and bicarbonate, phosphate and sulfate, ammonium and bicarbonate, sulfate and bicarbonate, bicarbonate and potassium, bicarbonate and magnesium, and between bicarbonate and calcium. This corroborates the notable independence in the behavior of certain parameters taken in pairs according to [42]. In the same vein, the trendline of the correlations between some of these elements taken into account are linear positive as is the case for the correlation between calcium and magnesium, or even non-linear monotonic positive as it is the case for the correlation between pH and chlorides, pH and electrical conductivity, pH and phosphate, pH and ammonium, pH and bicarbonate, chlorides and bicarbonate, electrical conductivity and bicarbonate, ammonium and bicarbonate, phosphate and sulfate, and between magnesium and pH. [42] explains this fact by specifying that in this case, the value of one of the elements concerned in the correlation can, depending on the environmental conditions, strongly or weakly influence that of the other. This is in accordance with the temperature variation brought into play in the present study through the simple soaking and cooking of bean seeds channel. The correlations involving sodium have the lowest coefficients. This reflects a low degree of enrichment of sodium in solutions. This can be justified by the starting composition of bean seeds, which have low concentrations of this element, in particular about 2 mg per 100 g of bean seeds according to [24]. Such a cause and effect phenomenon has also been observed in the magmatic environment, where the composition of the final rock is closely related to that of the starting magma as shown by [40]. Still in the analysis of correlations, we see that between magnesium and calcium, the correlation is positive and perfect linear, with correlation coefficient 1. By drawing a parallel with the magmatic environment once again, we will notice that in [39]'s crystallization series, forsterite-type Ferromagnesians (Magnesian olivine) and anorthite-type calcium feldspars (calcium plagioclase) are the first minerals to form in the respective series, in particular the discontinuous series (black minerals) and continuous series (white minerals).

In the various solutions resulting from the contact between bean seeds and water, and especially in those whose production has undergone the intervention of temperature, the three main nutrients are, and in order of importance, nitrogen, potassium, and magnesium. According to [43], this are two of the three major macroelements (N and K), and one of the minor macroelements (Mg). After these three elements, we have the other minor macroelements (Ca and S) analyzed within the framework of the present study, and the trace elements here represented by the chlorides. Concerning the third major macroelement, in particular phosphorus, its contents are rather low. These contents in solutions, compared with those of calcium and magnesium, do not agree with the contents in seeds as showed by [24]; in fact, the phosphorus contents of bean seeds are at least twice the calcium and magnesium contents respectively. The phosphorus contents in the solutions could therefore have been at least comparable to those of calcium and magnesium. However, [44] clarifies the mystery by declaring at the end of his work that phosphorus is rather an element sensitive to the composition of the environment. In this sense, this author shows that in the presence of calcium among other things, the phosphate reacts with it to precipitate in the form of calcium phosphate as showed by [45], thus becoming not very concentrated in the solution. This is in agreement with the observations of [11].Also, by referring to the correlations established between phosphorus and calcium, between phosphorus and magnesium, between phosphorus and potassium, and between phosphorus and sodium, we see in the cloud of points the formation of groups of points the

arrangement of which is reminiscent of staircases, that is to say sequentially linear, thus revealing not only the influence that these elements can have on each other taken two by two, but also revealing the impact of temperature on their behavior according to [42]. Further on, we will establish such a theory by referring to the magmatic medium or in the same rocks, where we will find mineral associations highlighting the presence of magnesian (forsterite), calcium (anorthite), phosphate (apatite), sodium (albite) as shown by [41]. Apart from the nutrients observed here, notably the major macroelements, minor macroelements, and trace elements, the bean end-of-cooking solution has a very high water content as showed by [46]. In addition, [13] showed that the bean end-of-cooking solution, at rest, settles, depositing organic matter. It is therefore a complete fertilizer. [13] has showed this quality by testing this solution as a substrate for heterotrophic beings, whose food needs are summed up in water, mineral salts, and organic matter according to [47]. The parallelism drawn by [46] between this solution and human urine supports the observations made by [12].

During the processes of soaking and cooking the beans, one can denote the differential mass loss at the different stages by bean seeds.This situation is due to the more or less extended duration of the contact between the bean seeds and the water. In that vein, [48] demonstrated the lysis capacity of water when it gets in contact with matters. According to [49], the products from the bean lysis traversed the seeds membrane and reach the solutions drowning the beans. This is consistent with the increase of the concentrations of the different ions followed up in the case of the present study. However, the mass loss is lower during the soaking of the beans compared to the situation observed during the cooking process. After [50], the involvement of temperature often increases the intensity of chemical reactions. This is in accordance with the amount of mass loss at different stages. In that point of view, compared to the initial situation, the loss of matter is 2.4g after 30 minutes of soaking. After 60 minutes of soaking, the loss is a little more than the double (5.3g) of the amount previously observed. After 60 minutes of soaking and 30 minutes of cooking the beans, the lost is quite the double (9.4g) of the former amount. After 60 minutes of soaking and 60 minutes of cooking the beans, the loss is equally quite the double (16.1g) of that observed before. After 60 minutes of cooking and 90 minutes of cooking the beans, the loss is about 1.3 time (20.7g) that of the earlier situation. The reduction of the increasing in losing the matter can be due to the progressive reach of the saturation point of the solutions as demonstrated by [51].

V. Conclusion

Solutions obtained from beans soaking and beans cooking are gradually enriched in mineral salts, particularly major macro elements (N and K), minor macro elements (Ca, S, Mg), and oligoelements compared to the situation noticed in the water used for the cooking process. Concerning the third major macro element, notably the phosphorous, it is present in low amounts. The pH and the electric conductivity (EC) of the solutions increase with the duration of the soaking and cooking processes. The amounts and values start their weak increasing thirty minutes after the beginning of the soaking, and continue their shy increasing up to the end of the sixtieth minute of the soaking. With the beginning of the cooking process, the increasing become abrupt. The correlation between all the parameters followed up in the present study are globally positive. But some facts reveal the independence between some of the parameters taken into account here. Also, the study of the clouds of dots reveal the impact of the temperature as the major responsible of the behavior of some of those elements despite the positive correlations established. The Pearson index in the correlations including sodium are the lowest.

VI. Recommandation

Consumers would benefit from recycling the bean end-of-cooking water. This recycling can be done by reintroducing this water in the rest of the bean cooking processes, either as inputs in pig feed, or then as fertilizer. To keep the water at the end of cooking the beans, it is advisable to put it in a cool place if it is stored for gastronomic purposes, or directly in the ground if it is stored for agrarian purposes.The use of solutions from cooked beans as fertilizers must be directly followed by earthing up in other to avoid the loose of nitrogen and sulfur through gas emanation. It use does not require any dilution.

Competing Interests

Authors have declared that no competing interests exist.

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Measuring the Effect of using two Carbon Sources (Sodium Bicarbonate with Rice Washing Water) on the Chemical Composition, Fatty Acids and Amino Acids of Marine Microalgae *Nannochlorpsis Oceanica*

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Abstract- Microalgae breeding media must be cost-effective, enable high growth, meet exact requirements and be readily available. The effect of different levels of sodium bicarbonate and rice wash water [25, 50, and 75%] in the growth medium on the biochemical constituents (protein, carbohydrates, lipids, fatty acids, and amino acids) of the *Nannochloropsis oceanica* was assessed compared to the F/2 Guillard standard medium. The obtained results revealed that the chemical constituents of *Nannochloropsis oceanica* affected by the level of urea used and the rice washing water. The highest protein, carbohydrate contents, and highest EAA (51.39%) obtained using ME1. medium (25% RWW and 75% SB) as compared to the control (100% F/2). The highest total lipid content was achieved by using the ME3 medium (75% RWW & 25% SB) producing (42.71 %), were the obtained the of the highest biomass productivity and lipid productivity in ME3 medium. In accordance, the highest total saturated fatty acids percentage (TSFA) of *Nannochlorpsis oceanica* recorded by ME3 medium.

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Keywords: amino acids, fatty acids, nannochloropsis oceanica, proximate composition.

I. Introduction

Micro-organisms with extremely high growth rates in various cultural conditions, such as microalgae, have major chemical diversity applications in many fields, including biotechnology, food science, and aquaculture this is due to their nature (Templeton & Leuins, 2015). As microalgae placed as a staple food for humans in the future. Microalgae are a source of many essential elements not only in biomedicine and balanced nutrients but also in technology.

In addition to natural use in aquaculture, microalgae are used directly in feed for larvae and juveniles (Sarker *et al*., 2016), provide a supply of n-3 LC-PUFA for fish farmed in marine hatcheries, and *Nannochloropsis* is considered the species. The main component of algae is of great importance in aquaculture (Bundioli *et al*., 2012). More aspects are requirement in to increase aquaculture production to create new high-quality microalgae species and to use microalgae species as feed sources (Hemaiswarya *et al*., 2011).

The amino acid requirements of prawns were analyzed to determine protein quality in dinoflagellates (Lim *et al*., 2018). Microalgae help improve the traditional nutritional value of foods and promote the growth and development of targeted and main products (Tokuşoglu & Ünal, 2003). The chemical profile of microalgae varies with cultural conditions and age (Carvalho *et al*., 2009). Diverse cultures influence a large number of microalgae species that have been studied for understanding organ function and generating a group culture (Grobbelaar, 2010). El-Muhsnawi *et al*. (2020) show the distinct diets of *Chlorella Vulgaris* have improved the productivity of omega-3 and omega 9 fatty acids, resulting in higher food quality for humans and aquaculture . Suain *et al*. (1987) to Alonso *et al*. (2000) suggested that nitrogen reduction was to transfer microalgal metabolism to lipid production. Chisti (2007) and (Abugrara *et al*. 2019). For a novel cellular lipid storage synthesis, an adequate supply of inorganic carbon dissolved in the medium of the environment is require for carbon fixation. Sodium bicarbonate was used as a carbon source to examine the growth and biochemical composition of microalgae (Yeh *et al*., 2010). Most of the inorganic form of dissolved carbon in seawater is find in the form of bicarbonate (HCO_3^-), and the conversion speed from HCO_3^- to CO_2 is low (Skirrow, 1975). The intensive production of commercial microalgae can be supported by addition bicarbonate salt as sources of carbon (Chi *et al*., 2011). The use of SB from external media can vary from one species to another (Dason *et al*., 2004). Species of *Nanochloropsis* take bicarbonate ions from extras through media to the

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cytosol via the plasma membrane and extract CO_2 from HCO_3^- through the action of carbonic anhydrase (Li *et al*., 2018). Several microalgae were examined for the effect of adding sodium bicarbonate and the results indicated that fatty acids such as triglycerides and n3 fatty acids accumulate rapidly (Guihéneuf & Stingle, 2013). It was observed in a previous study of seaweed *Dunaliella salina* that the addition of sodium bicarbonate significantly enhanced the lipid and fatty acid content while increasing the activities of carbonic anhydrase enzymes and reducing the oxidative stress induced by ROS (Srinivasan *et al*., 2018). Despite this, the animal lacks the enzymes required to synthesize PUFA, it must be obtained from food and, therefore, it is often known to be biotic (Milledge, 2011), so deficiency in PUFAs appears to be the main reason behind low larval survival rates (Patel). *et al*., 2005). As a result, microalgae have been used as a food source for aquatic organisms, with fatty acid contents being the central factor in selecting the microalgae species (Huerlimann *et al*., 2010). The full use of algal biomass may involve a combination of different techniques (Wiley *et al*. 2011).

is increasing and can be saturated by lipid biosynthesis using appropriate nutrients as well as by optimizing harvesting strategies that lead to cell / biomass recovery. Various physico-chemical conditions such as production cost. However, the question is, did N. oceanicacultured at different levels of SD concentrations achieve biochemical composition (protein, carbohydrates and fats) and fatty acids and amino acids such as those temperature, stress, light intensity, culture grown on F / 2 Guillard medium? time, organic carbon and inorganic nutrients including iron (Fe), phosphorous (P), nitrogen

II. Materials and Methods

On the industrial production scale of marine hatcheries, optimizing an effective media for cultivating microalgae species for nutritional cultivation is very necessary. The microalgae nutrient media should prepare quickly, economically, hit high growth, and fulfill the quality and quantity of all microalgae. Although the medium of F/2 Guillard is regarded as the most popular medium of *Nanochloropsis* cultivation in marine hatcheries, F/2 medium has some drawbacks, such as difficulties in preparation and preparation of outdoor and costly mass culture. Among the different nutritional factors in particular, nitrogen is one of the most important nutrients for growth, as it is a component of all structural and functional proteins such as peptides, enzymes, chlorophyll, energy transport molecules and genetic material in algal cells (Cai *et al*., 2013). The nitrogen concentration in the middle of the culture affects the rate of cell growth and the biochemical compositions of the microalgae (Wang *et al*. 2013), and several studies have shown that when nitrogen is limited in the middle of the culture, the microalgae slow down the rate of cell growth and increase their fat or carbohydrate content, Which reduces protein synthesis (Ho *et al*., 2014).The demand for algae-based fats (N), manganese (Mn), zinc (Zn), sulfur. (S), cobalt (Co), and others, affect and regulate the growth and lipid accumulation of many types .of microalgae (Bajpai *et al*., 2014) *Nannochloropsis sp*. has been widely accepted as a productive strain of microalgae due to its high growth rate, high lipid content, and strong resistance to biological contamination (Biondi *et.al*., 2013). Therefore, commercial agricultural fertilizers (CAGF) should be used more commonly in place of F / 2 cultivation medium (Lopez-Elias *et al*., 2005). As aquatic organisms, microalgae need water, salts, and carbon dioxide to thrive. The main primary nutrients are nitrogen (N), phosphorous (P) and silicon (Si, for diatoms only). Certain vitamins and micronutrients are also required for algae growth (such as magnesium, sulfur, iron, etc.). Of all the nutrients, nitrogen and phosphorous are the main nutrients that limit growth, lipid content and microalgae productivity (Bajpai *et al*., 2014). At the level of industrial production of marine hatcheries, the improvement of the effective culture medium for microalgae species for food culture is absolutely necessary. Nutrient media should quickly prepare microalgae, hit high growth and meet the quality and quantity of all microalgae.

This study was designed to assess the effects of adding different levels of $NaHCO_3$ and (RWW) on the biochemical composition of *N. oceanica* and the rate of lipid and amino acid production. Therefore, different media were prepared using different levels of SB and (RWW) (25, 50 and 75%) to cultivate *N. oceanica* to replace F / 2 medium to reduce Cultivation and growth conditions *Nannochloropsis oceanica* strain was obtained from an algal unit of the marine hatchery presented in the National Institute of Oceanography and Fisheries, NIOF Aquaculture Division, Alexandria, Egypt. *N. oceanica* was maintained under controlled conditions of illumination (55 μmol photons $m-2$ $s-1$), salinity (35 ± 2ppt), and temperature (25 ± 2°C) using F/2 medium (Guillard & Rhyter, 1962), with continuous aeration and 16:8 h light to dark cycle in three replicates. Cultures were incubated for homogenous mixing on a shaker at 80rpm. The cellular dry weight (CDW) and biochemical composition of algal cells were monitored in the late exponential growth phase (after 10 days culturing). The cellular dry weight (CDW) was determined, according to (Abomohra *et al*.,2013).

III. Experimental Design

The F/2 medium contained (mg. L^{-1}) $NaNO_3$, 75; $NaH_2PO_4.H_20$, 5; Na_2 EDTA. H_20, 4.16; $FeC1_3$. $6H_20$, 3.15; $CuSO_4.5H_20$, 0.01; $ZnSO_4.7H_20$, 0.022; $COC1_2.6H_20$, 0.01;

$MnC1_2.4H_20$, 0.18; Na_2MoO_4. 2H20, 0.006;

Vitamin B12, 0.0005; Vitamin B1, 0.1; and Bi tin, 0.0005 (Guillard & Rhyter, 1962). Carboys loaded with 5 liters of filtered disinfected saline water (35±2ppt), then enriched by sodium bicarbonate (SB) as a percentage against F/2 medium (control) as shown in Table 1. The seawater used in the study was obtained from Alexandria Beach (Egypt). SB stock solution was prepared by dissolving 10 g of NaHCO3 (SB) in 100 ml of distilled water.

Use liquid plastic bottles of 1.5 liters and 1 liter of sterile saline water (35 ± 2ppt) and 1kilo of rice was washed (RWW) with 1.5 liters of water in a first wash. 50 ml of water was taken and filtered using filter paper, and water was used as a medium, rice washing water was used without fermentation (Table 1).

The cultures were inoculated by the experimented alga for the last harvesting after 10 days

a) Estimation of the biochemical constituents of N. oceanica

Total protein and carbohydrate content .The extraction of protein content was carried out by the procedure described by Lowry *et al*. (1951) using Bovine Serum Albumin (BSA) as standard. Dubois *et al*. (1956) were followed for extraction and estimation of total carbohydrates "phenol-sulfuric acid" by using D-glucose µg/ml as standard.

$$\text{Biomass productivity (mg } L^{-1} \text{ day}^{-1}) = (CDWL - CDWE) \text{ x } (tL - tE)^{-1}$$

With CDW_E representing the CDW (mg L^{-1}) at the days of early exponential phase (t_E) and CDW_L at the days of late exponential phase (t_L). (Abomohra , *et al.,* 2016).

b) Total lipid content and fatty acid profile

Total lipid and fatty acids were extracted as described by Folch *et al*. (1957) and Bligh & Dyer (1959). Preparation of fatty acids methyl ester from total lipids was performed according to the procedure of (Radwan, 1978). All analyses for identification of fatty acids fractions were performed on GS-MS, model HP (Hewlett Packard) 7890GC equipped with a flame ionization detector. GC Conditions: Device Model: HP (Hewlett Packard) 6890GC, Column: HP-INNOWax (Polyethylene glycol), 60m, 0.25mm ID, 0.2*µ*m film thickness.

Detector: FID (Flame Ionization Detector). Detector temperature: 250°C. Injector temperature: 220°C, injection volume 3*µ*l, split ratio 50:1.

$$\text{Lipid productivity (mg } L^{-1} \text{ day}^{-1}) = (LC_L - LC_E) \text{ x } (t_L - t_E)^{-1}$$

with LC_E representing the lipid content (mg L^{-1}) at the days of early exponential phase (t_E) and LC_L at the days of late exponential phase (t_L) . (Abomohra , *et al.,* 2013).

c) Amino acids determination

Amino acids of N. oceanica were analyzed by hydrolysis in 6N HCL for 22hrs at 110°C; after hydrolysis, the acid was evaporated in a vacuum oven. The residue of the algal sample was dissolved in 1 ml of sample dilution (diluting buffer) (0.2M, pH 2.2) to complete the sample dissolving. Automatic amino acid analyzer was used for amino acid determination (Dionex ICS3000) (Block, 1948).

IV. Statistical Analysis

Table 1: The experimental design used in the cultivation of *Nannochloropsis oceanica*

	CO	ME1	ME2	ME3
F/2	100	---	---	--
Sodium bicarbonate (SB(	----	0.75	0.50	0.25
Rice wash water (RWW)	----- -	0.25	0.50	0.75

V. Results

Samples were harvested for analysis of *Nannochloropsis oceanica* was cultured under biochemical composition after late stationary different concentrations of sodium phase (10 days). The cellular dry weight and bicarbonate and rice washing water (25%, 50% biochemical compositions of the isolated and75%) in the early stationary phase, where species were examined. Moreover, the

The statistics have been conducted using the General Linear Univariate Model (ANOVA) analysis. The differences between means were assigned as significant at P< 0.05 with the use of the least significant difference LSD for multiple ranges of post hoc comparisons to resolve the differences between the replication means by using SPSS (2007). characteristics of biodiesel were examined by international standards and compared to the previous studies. The presented results indicated that there is no significant difference in the cellular dry weight (CDW) between the media contained different levels of SB, RWW and the control. The obtained data (Table 2) showed significant variations in the biochemical composition of *N. oceanica* between different treatments. The highest total protein and carbohydrate percentages of dry weight (18.76%± 0.02 and 24.66%± 0.02, respectively) were achieved by ME1 medium (75% SB and 25% RWW) in comparison with control and other treatments. The highest total lipid content (42.71%± 0.02) was exhibited by ME3 medium (25% SB and 75% RWW) relative to the control and other treatments.

a) Biomass productivity and lipid productivity

The obtained data (Table 2) showed significant variations in the biomass productivity of *N. oceanica* between day^{-1}) was exhibited by ME3 and ME2 medium relative to the control and other treatments.

b) The fatty acids analysis

The fatty acids profile of *N. oceanica* was presented in Table 3. The data revealed that there is no change in the fatty acids profile between the different treatments. In contrast, there is a noticeable change in the content of each individual fatty acid between the different treatments. The most abundant saturated fatty acid was the palmitic acid (C16:0), which recorded its highest value (27.14%) with ME3 medium (25% SB and 75%RWW) than the other media. Following the palmitic acid, is the stearic acid (C18:0), which has nearly the same percentage values in all used media. In addition Oleic acid (C18:1) was remarkable the most prevalent monosaturated fatty acid in all treatments, however its value reduced to reach its minimum amount (22.71%) with ME3 medium, which means that the Oleic acid content decreased with the addition of RWW to the culture medium. Also, palitoleic acid (C16:1) showed an highest value (5.91%) with ME3 (25%SB and 75% RWW) medium, while it recorded the lowest value with F/2 medium. Moreover, linoleic acid (C18:2) was the most common polyunsaturated fatty acid with all treatments, where the data revealed that the highest value of this fatty acid (15.85%) was recorded with ME3 media relative to the control medium (13.64%). Ecosa-pentaenoic acid (EPA) was the second polyunsaturated fatty acid, where its maximum percentage value (9.54%) was recorded with ME3 medium. Similarly, dcosa-hexaenoic acid (DHA) was the third polyunsaturated fatty acid which recorded its highest value (3.67%) with the same medium. Low percentage values of linolenic acid (C18:3) were detected by control and ME1 media. However the highest value of linolenic acid was achieved by ME3 medium (1.48%). The results revealed that the highest percentage of total saturated fatty acids TSFA (41.69%) was achieved by ME3 medium (25% SB and75% RWW), which was higher than TSFA percentage (35.75%) recorded by the control medium (CO) (100% F/2). The present study explained that the highest rate of the total unsaturated fatty acids USFA (65.80%) was detected by ME1 medium (25% RWW and 75% SB), where this percentage is mainly consisting of 33.66% MUFA and 32.14% PUFA. On the other hand, the highest ratio (0.66) between SFA/USFA was achieved by control medium (25% SB and 75%%RWWME3), In addition, the highest ratios between n-3/n-6 and DHA/EPA were 1.08% and 0.52 % respectively, which exhibited by ME1 medium (75% SB and 25% RWW), (Table 3).

c) Amino acids analysis

Amino acid profiles of different culture media of *N. oceanica* diets were presented in (Table 5). The present study revealed that there is no change in the amino acid profile between the different media. In contrast, there is a clear variation in the content of each individual amino acid between the different treatments.

The results showed that *N. oceanica* recorded the highest percentage of essential amino acid EAA (51.39%) by ME1 medium (75% SB and 25% RWW), while the lowest value was achieved by ME3 medium (25% SB and 75% RWW). (Table 5). The non-essential amino acids (NEAA), where the highest percentage of nonessential amino acids NEAA (53.61%) was detected by ME3 medium (25% SB and 75% RWW), while the lowest value of NEAA was achieved by ME1 medium.

Table 2: The Average biochemical composition (in % dry basis) mg/g DW of *N. oceanica* at different levels of sodium bicarbonate (SB) and rice wash water (RWW) medium harvested after 10 days incubation period

Medium	CDW (g L^{-1})	Protein (%CDW)	Carbohydrate (%CDW)	Lipid (%CDW)	Biomass productivity (mg L^{-1} day^{-1})	Lipid productivity (mg L^{-1} day^{-1})
CO	0.74 ± 0.02^{d}	21.64 ± 0.03^{a}	17.29 ± 0.02^{d}	36.55 ± 0.03^{d}	69.76 ± 0.03^{d}	27.13 ± 0.02^{d}
ME1	0.84 ± 0.00^{c}	18.74 ± 0.02^{b}	24.66 ± 0.02^{c}	38.43 ± 0.02^{c}	91.64 ± 0.03^{c}	29.01 ± 0.02^{c}
ME2	0.87 ± 0.00^{b}	16.86 ± 0.02^{c}	22.74 ± 0.02^{b}	41.64 ± 0.03^{b}	102.05 ± 0.03^{b}	32.23 ± 0.02^{b}
ME3	0.97 ± 0.00^{a}	14.97 ± 0.01^{d}	20.26 ± 0.02^{a}	42.71 ± 0.02^{a}	104.14 ± 0.03^{a}	33.27 ± 0.03^{a}

Data are statistically analyzed using ONE-WAY ANOVA. Significant result is obtained at P= 0.05

Table 3: Total fatty acids profiles and their individual (%) of *N. oceanica* at different levels of sodium bicarbonate (SB) and rice wash water (RWW) medium harvested after 10 days incubation period

Fatty acid	CO	ME1	ME2	ME3
C14:0 (Myristic acid)	3.49 ± 0.03^{d}	4.37 ± 0.02^{c}	4.78 ± 0.03^{b}	4.92 ± 0.03^{a}
C15:0 (Pentadecylic acid)	0.63 ± 0.02^{d}	0.75 ± 0.02^{c}	0.85 ± 0.02^{b}	0.96 ± 0.02^{a}
C16:0 (Palmitic acid)	23.27 ± 0.02^{d}	22.45 ± 0.03^{c}	24.62 ± 0.03^{b}	27.14 ± 0.02^{a}
C17:0 (Margaric acid)	0.29 ± 0.02^{d}	0.37 ± 0.02^{c}	0.62 ± 0.02^{b}	0.67 ± 0.02^{a}
C18:0 (Stearic acid)	4.41 ± 0.02^{d}	4.63 ± 0.02^{c}	4.75 ± 0.02^{b}	4.86 ± 0.02^{a}
C21:0(Heneicosanoic acid)	1.93 ± 0.02^{d}	1.15 ± 0.03^{c}	1.17 ± 0.02^{b}	1.22 ± 0.02^{a}
C24:0 (Lignoceric acid)	1.73 ± 0.02^{b}	1.78 ± 0.02^{d}	1.86 ± 0.03^{c}	1.92 ± 0.02^{a}
∑Saturated (SFA)	53.53	53.33	56.13	96.14

C14:1 (Myristoleic acid)	0.12±0.02^{c}	0.18±0.02^{a}	0.23±0.02^{b}	0.28±0.02^{cc}
C15:1 (cis-10-pentadecenoic acid)	0.06±0.02^{a}	0.08±0.02^{b}	0.06±0.02^{bb}	0.09±0.02^{c}
C16:1 (Palitoleic acid)	5.76±0.02^{d}	5.84±0.02^{c}	5.75±0.02^{b}	5.91±0.02^{a}
C17:1(cis-10-Heptadecenoic acid)	0.50±0.02^{a}	0.40±0.02^{c}	0.33±0.02^{d}	0.42±0.02^{b}
C18:1n9 (Oleic acid)	25.84±0.02^{d}	24.83±0.02^{c}	23.62±0.02^{b}	22.71±0.02^{a}
C20:1 (Paullinic acid)	2.84±0.02^{a}	1.67±0.02^{d}	1.79±0.02^{c}	1.67±0.01^{b}
C22:1 (Erucic acid methyl)	0.63±0.02^{b}	0.66±0.02^{d}	0.59±0.02^{c}	0.67±0.02^{a}
∑Monosaturated (MUFA)	53.53	55.11	53.55	56.53
C18:2n6 (Linoleic acid)	13.64±0.02^{d}	14.72±0.03^{b}	15.75±0.02^{c}	15.85±0.02^{a}
C18:3n6 (y-Linoleic acid)	0.20±0.01^{a}	0.12±0.01^{d}	0.13±0.02^{c}	0.14±0.02^{b}
C18:3n3 (α- Linolenic acid)	1.41±0.02^{d}	1.65±0.02^{c}	1.36±0.02^{b}	1.48±0.02^{a}
C20:2n6 (Eicosadienoic acid)	0.87±0.02^{a}	0.64±0.02^{c}	0.75±0.02^{d}	0.63±0.02^{b}
C20:5n-3 (Ecosapentaenoic acid)	8.15±0.02^{d}	9.87±0.02^{c}	9.65±0.02^{b}	9.54±0.02^{a}
C22:6n-3 (Docosahexaenoic acid)	3.81±0.02^{d}	5.14±0.02^{b}	3.74±0.02^{c}	3.67±0.02^{a}
∑Polyunsaturated (PUFA)	36.36	53.69	56.56	56.56
∑Usaturated	15.65	13.63	15.53	15.31
SFA/MSFA	6.33	6.66	6.35	6.56
SFA/PSFA	6.35	6.66	6.35	6.55
SFA/USFA	3.31	3.39	3.16	3.11
∑n 3	65.51	61.11	69.53	69.14
∑n6	69.56	63.96	61.15	61.13
∑n3/n6	3.46	6.36	3.64	3.66
DHA/EPA	3.95	3.33	3.54	3.54

Table 4: Amino acids profile (%) in *N. oceanica* at different levels of sodium bicarbonate (SB) and rice wash water (RWW) medium harvested after 10 days incubation period

	Medium			
Amino acid (AA)%	CO	ME1	ME2	ME3
Essential amino acids (*EAA)*				
Arginine	4.13±0.02^{d}	6.16±0.02^{c}	6.32±0.02^{a}	6.20±0.02^{b}
Histidine (HIS)	1.68±0.02d	3.62±0.02^{c}	3.75±0.02^{b}	3.85±0.02^{a}
Isoleucine (ILE)	5.09±0.02^{a}	3.25±0.02^{b}	3.16±0.02^{d}	3.22±0.02^{c}
Leucine (LEU)	9.22±0.02^{a}	7.12±0.02^{b}	6.42±0.01^{c}	6.16±0.02^{d}
Lysine (LYS)	3.11±0.02^{d}	7.41±0.02^{a}	5.45±0.02^{c}	5.66±0.02^{b}
Methionine (MET)	1.56±0.02^{b}	4.16±0.02^{d}	4.63±0.02^{a}	4.21±0.02^{c}
Phenylalanine (PHE)	4.89±0.03^{a}	5.47±0.02^{b}	5.14±0.02^{d}	5.31±0.02^{c}
Threonine (THR)	4.58±0.02^{a}	4.62±0.02^{b}	4.11±0.02^{c}	4.07±0.02^{d}
Tryptophan (TRP)	14.48±0.02^{d}	4.24±0.02^{a}	3.61±0.03^{b}	3.12±0.01^{c}
Valine (VAL)	6.09±0.02^{a}	5.34±0.02^{b}	4.69±0.02^{c}	4.59±0.03^{d}
Total EAA	39.6	36.54	95.34	91.54
Non-essential amino acids (*NEAA*)				
Alanine (ALA)	5.59±0.02^{a}	4.55±0.02^{d}	4.76±0.02^{c}	4.92±0.03^{b}
Aspartate (ASP)	9.68±0.01^{a}	9.44±0.02^{b}	9.23±0.02^{c}	9.46±0.02^{d}
Cystine (C-C)	3.31±0.02^{c}	4.34±0.02^{d}	6.47±0.02^{a}	5.86±0.02^{b}
Glutamine (GLU)	11.55±0.02^{a}	11.23±0.02^{b}	9.62±0.03^{d}	10.13±0.02^{c}
Glycine (GLY)	4.39±0.02^{d}	5.65±0.02^{b}	5.76±0.02^{a}	5.20±0.02^{c}
Proline (PRO)	4.34±0.02^{d}	5.83±0.02^{c}	7.77±0.02^{a}	7.34±0.02^{b}
Serine (SER)	4.22±0.02^{d}	5.41±0.03^{c}	6.55±0.02^{b}	8.37±0.02^{a}
Tyrosine (TYR)	2.12±0.01^{d}	2.16±0.02^{c}	2.55±0.02^{a}	2.34±0.02^{b}
Total NEAA	93.3	96.16	33.56	35.16

VI. Discussions

The improvement of culture conditions is essential to raise efficiency and economic value for microalgae productivity in the future. New methods of extraction, production, and cultivation can be efficiently established to improve productivity and reduce costs. For more than 50 years, Guillard F/2 medium has been popular for marine aquaculture in the cultivation of microalgae, currently, because of the different use of microalgae in various biotechnological domains; the F/2 Guillard medium has many drawbacks. Our results investigated that some sodium bicarbonate and rice

washing water levels achieved significant biochemical constituents higher than F/2 medium (control).

The present study showed that low addition of SB and RWW to ME1 medium (25% RWW and 75% SB) could improve protein, carbohydrate, PUFA and EAA contents of *N. oceanica*, which may be exhibited by increasing inorganic dissolved carbon concentration as additional sources of energy.

Similar findings have been found with *Chlorella pyrenoidosa* and *Scenedesmus obliquus* exposed to increased CO_2 (Yang & Gao, 2003; Srinivasan *et al*., 2018). (Pancha *et al*. 2015) recorded that bicarbonate addition raises the protein content of freshwater alga *Scenedesmus sp*. (Jegan *et al*. 2013) recorded that the protein and carbohydrate contents of *Desmococcus sp*., *Chlorococcum sp*., and *Chlorella sp*. strains elevated when they were cultivated in media provided with bicarbonate Microalgae protein content can be explained by intake of nitrogen internally, possibly due to the high level of nitrate intake. The decrease in the nitrogen level in the ME1 medium (25% RWW and 75% SB) than that in F/2 medium (control) caused an increase in the carbohydrate content in ME1 medium due to nitrogen limitation. This result is following (Millán-Oropeza *et al*. 2015), who revealed that nitrogen starvation caused the accumulation of carbohydrate in *Chlorella sp*. In this research, the replacement of all nutrient salts from the culture by 25% SB and 75% RWW (ME3 medium) resulted in a significant decrease in protein content of *N. oceanica*. Similarly, (Pancha *et al*. 2015) showed a decrease in protein content under nutrient-starved conditions. The present study also revealed that ME3 medium (25% RWW and 25%SB) significantly decreases the carbohydrate content; this finding in contrast to(Pancha *et al*., 2015).

As for the lipid, it was higher than (Abugrara, *et al*., 2020) as 100% sodium bicarbonate was used on *N.oculata*, the percentage was higher than (Abugrara, *et al.,* 2019) in using starch by 75% on the same algae, higher than (Ashour, *et al*., 2019's results when using Medium F/2, and higher than (Ashour M. and Abd ElWahab K., 2017) in its use of 50-50% nitrogen and phosphorous, and higher than (Zhang, *et al.,* 2016), which used Different nitrogen levels and the carbohydrates were less than what (Abugrara, *et al.,* 2020) reached when different levels of sodium bicarbonate were used on *N.oculata*, and it was higher than (Ashour, *et al*., 2019) by using medium F / 2 on the same algae, and less than (Ashour M. and Abd ElWahab K., 2017) when using N - P by 50 - 50%, and less than (Chun W. *et al*.,2012) when it used me to medium F/2 on the same algae.

The biomass productivity was higher than (Ashour, *et al*., 2019) using Medium F/2 on the same alga, higher than (Mata, et al., 2010) reported on *N.oculata*.

The lipid productivity was higher than (Ashour *et al.,* 2019) results for its use of Medium F / 2 on the same algae It was similar to (Aarón Millán *et.al*., 2015) results that used nitrate and carbon dioxide on the alga *N.oculata* and higher than (Chun Wan, *et al.,* 2013) results on the same algae with different sources of nitrogen used Below is what (Mata, *et al*., 2010) has found on *N.oculata*.

The present work demonstrated an increase in polyunsaturated fatty acids (PUFAs) yield as eicosapentaenoic fatty acid (EPA) and docosa-hexaenoic fatty acid (DHA) with the addition of different sodium bicarbonate levels in relative to the control. This is in agreement with Ma et al. (2016), who detected that EPA is the dominant PUFA in *Nannochloropsis*, which makes it a possible partial replacement of fish oil for fish foods (Sørensen et al., 2017).

In the present study, the highest percentage of essential amino acids EAA was detected by ME1 medium (75% SB and 25% RWW), which recorded the highest percentage of protein content. In contrast, the highest percentage of non-essential amino acids NEAA revealed by ME3 medium (25% SB and 75% RWW), which recorded the lowest percentage of protein content. The data are supported by those published by Barkia et al. (2019), who mentioned that amino acids differ with growth conditions as do other bioactive compounds synthesized by the microalgae. The most abundant EAA in the profile of N. oceanica cultured on ME1 medium is Arginine. The percentage of Arginine (6.16%), is higher than that of *Tetraselmis spp*. (Brown, 1991), *Chlorella sp*. (Brown & Jeffrey, 1992), and dinoflagellates as represented by Lim et al. (2018). Leucine as the second largest EAA (7.12%) is higher than that of *Heterocapsa rotundata* (7.5 %) as recorded by Lim et al. (2018). Therefore, *N. oceanica* cultured on ME1 medium can be used as arginine and leucine-rich mixed algal diets in aquaculture. Our results showed an increase in the lipid content of *N. oceanica* especially at ME3 culture medium (25%SB and 75% RWW). Pancha et al. (2015) recorded an increase in the cellular storage lipids (namely triacylglycerides or TAGs) of microalgae with the addition of bicarbonate to the nutrient deficiency medium. The present data revealed that the highest lipid content and percentage of total saturated fatty acids TSFA were obtained by culturing *N. oceanica* on ME3 medium (25%SB and 75% RWW). Therefore, these results in accordance with other authors who showed that increasing concentration of bicarbonate in algal cultures will increase the number of fatty acids (Xia & Gao 2005; Chiu et al., 2009). El-Sheekh et al. (2013), indicated that sodium bicarbonate addition in *Scenedesmus obliquus* culturing medium has a negative effect on the production of the fatty acid. Similarly, (White et al. (2013) showed that there is no effect of sodium bicarbonate addition on the composition of fatty acids in *Tetraselmis suecica*

cultures, while a marked effect was observed on *N. salina* cultures. Our data revealed that Palmitic acid (C16:0) was the main saturated fatty acid and this result was in agreement with Abugrara et al. (2019), who declared that palmitic acid is the common component in the crude lipids of the *N. oceanica*. The data explained that SFA was predominantly in *N. oceanica* cultured on ME3 medium (25% SB and 75% RWW) than MUFA and PUFA recorded at the same medium. This finding was recommended by Guihéneuf & Stengel (2013).

VII. Conclusion

In summary, this research has the potential to promote the development of industrial using sodium bicarbonate as a useful inorganic source of carbon to enhance the growth of biomass and lipid production. Our findings suggested that sodium bicarbonate and rice wash water addition in low percentage (25%SB and RWW) to the culture medium had significant effects on the production of cellular compounds including protein, carbohydrates, polyunsaturated fatty acids and essential amino acids (especially arginine and leucine), where these valuable substances are used for feeding in aquaculture. The present study nominates *N. oceanica* as a promising candidate for lipid production by cultivation on level of sodium bicarbonate and rice wash water (25%SB and 75% RWW) in the culture medium.

As C16 - C18 (palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid) is the most widely obtained fatty acid ester in biodiesel, and is considered the most widespread component of biodiesel, the use of *N. oceanica* has shown Clearly, the supply of sodium bicarbonate as well as rice wash water has a positive effect on FAME content and therefore it can be used in the production of biofuels.

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The Unique Australian Flora, A Veritable Pandora's Pharmacopeia of Compounds with Therapeutic Biomedical Potential: Are the Chalcones the Geni in The Box?

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Abstract- The aim of this review was to highlight the unique biodiversity of the flowering plants and shrubs of Australia and their component chemicals that evolved during the separation of the Australian continent from Gondwanaland. The chemicals produced by these flowering plants provided protection ensuring the survival of the Australian flora which had to contend with often harsh Australian climatic conditions. The diversity of plant phytochemicals produced by these flowering plants reflects the unique diversity of the Australian Flora and these represent a Pharmacological goldmine. It was beyond the scope of this review to cover the full spectrum of these chemical compounds present in Australian plants instead we focused on the chalcones in this review. This compound has a special status in medicinal chemistry as a base intermediate for the synthesis of a large repertoire of polycyclic compounds that display anti-bacterial, anti-fungal, anti-viral and anti-tumour properties and these are thus of considerable interest in Biomedicine.

Keywords: phytochemicals; chalcones; polyphenolics; flavonoids; biomedicine; australian native plants; anti-viral; anti-bacterial; anti-cancer; SARS-CoV-2; COVID 19.

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Keywords: phytochemicals; chalcones; polyphenolics; flavonoids; biomedicine; australian native plants; anti-viral; anti-bacterial; anti-cancer; SARS-CoV-2; COVID 19.

I. Introduction

a) Geographical isolation of the Australian continent

Separation of Gondwanaland from the Pangea super-continent during the mid- Jurassic period resulted in the Australian land-mass becoming isolated in the Southern Ocean. This geographic isolation is reflected in the present day by the biodiversity and uniqueness of Australia's flowering plants, shrubs and trees. Many of these are unique to Australia although occasional related species have also been recorded in S. Africa, S. America and Hawaii. Remnant vegetation resembling that seen in Gondwanaland remains in pockets of rainforest in the Blue Mountains, New South Wales and Tasmanian wilderness.

In the last few million years the Australian continent and its variable climate and geological activity moulded the continent into climatic regions that are arid and dry in its central regions, high-rainfall in tropical regions of the north and sub-tropical temperate easterly and southern regions[1].This Australian climatic diversity is reflected in the incredible range of its native flora.

The demands of the extreme climatic conditions that prevailed in Australia over this evolutionary period was an important selection determinant resulting in the unique spread of flowering plants evident today and these evolved a bio diverse range of chemical compounds through natural selection processes that equipped them for survival in the harsh climatic conditions that prevailed and these continue to be important functional entities of plants in present day Australia. Australia is one of 17 mega diverse countries in the world and has a wealth of unique plant species[2].

b) The biodiversity of the Australian flora

It has been estimated that the Australian native flora accounts for ~10% of the world's total plant species with the total number of vascularised plant species estimated to be 25,000, and is greater in number than all plant species in the countries of Europe combined[3]. After World War II a systematic survey of the Australian flora for plants of chemical and pharmaceutical interest uncovered a large number of plants and compounds of interest. Large numbers of pharmaceutical compounds including 500 alkaloids were discovered in Australian plants from 1949 to 1969, 40% of these were identified as new compounds[4].The majority of these have yet to be examined for their chemical composition or potential biological activities[5].It is quite clear from the diverse unique morphologies displayed by Australian native plants that they contain a veritable Pandora's box of genetic information which will undoubtedly be useful to plant breeders in the future as reference genetic information that may be useful in the development of new plant traits in prospective plant breeding programs.

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c) *Flora of Australia (http://www.environment.gov.au/science/abrs)*

The *Flora of Australia* is an authoritative comprehensive collection of data on Australia's native and naturalised vascular plant biodiversity and integrates a wide range of botanical information from many sources, such as nomenclature, distribution maps, images, biodiversity data, and identification keys. This interactive, online platform was developed through a collaboration between the Australian Biological Resources Study (ABRS), Council of Heads of Australasian Herbaria (CHAH, www.chah.gov.au) and the Atlas of Living Australia (ALA, www.ala.org.au).This platform includes a comprehensive linked Australian Plant Image Index; and taxonomy and nomenclature information from the National Species List.The Australian Biological Resources Study [abrs@awe.gov.au] (ABRS, http://www.environment.gov.au/science/abrs), is a unit within the Department of the Environment and Energy, Australian government and manages the production of the "Flora of Australia", with content provided by botanists.

d) *The chemical biodiversity present in native Australian plants*

A similar chemical platform to Flora of Australia [http://www.ausflora.org.au] to systematically identify and characterise the pharmacological compounds present in individual Australian native plant species would be an invaluable resource and would greatly simplify the characterisation of compounds of potential pharmaceutical application in Biomedicine. It should be noted that historical evidence shows approximately 25% of all current prescription drugs and 75% of all anti-cancer drugs were originally developed from plant compounds.From the early 1940s until the late 1970s CSIRO (Commonwealth Scientific and Industrial Organisation) was involved in a major screening program of Australian native plants, searching for medically active chemicals. A major target was alkaloids, but triterpenes, diterpenes, phytoecdy-steroids, flavanoids and phenolics were also identified.

II. Plant Phytochemicals

Plants contain a biodiverse range of chemical compounds (phytochemicals) that convey important survival properties. The unique bioactive properties of these compounds may be useful in alternative strategies to conventional medications and may represent a platform for new drug discovery. Australia's geographic isolation, and harsh environmental climatic conditions led to the evolution of a large range of unique and distinct flora with adaptations not seen elsewhere in the world. In response to the unique environmental conditions that native Australian plants had to contend with they evolved some unique survival adaptations and unique phytochemicals that ensured the survival of the plants that inhabited Terra Australis. The Amazon region in S. America is often lauded as an important source of plant and genetic biodiversity of global significance however the unique collection of native plant species in Australia should also be recognized as a resource of equal importance.

The knowledge of Aboriginal First Nation communities gleaned over40,000 years of observation, clinical interpretation, experimentation and trial and error in the medicinal use of Australian Native Plants that led to anecdotal evidence of numerous antiseptics, analgesics, sedatives, astringents, antipyretics, hypnotics, expectorants, muscle relaxants and mood-altering drugs has come full circle. Current day analytical chemical investigations and pre-clinical studies have led to the confirmation of the wealth of health promoting properties present in Australian native plants. Current medicinal practice however dictates that the active molecules should be isolated and fully characterized in order to gain medical certification in order that they be used in the clinic. This task is daunting given the diversity of compounds present in Australian native plants but based on pre-clinical investigations is warranted.

a) *Genomics provide clues as to the existence of chalcones in plants*

The colour of a flower is predetermined by the plants genome which encodes for the production of certain chemicals. Red, purple, blue and pink colors are produced by anthocyanin pigments which are members of the flavonoid chemical family[6]. Yellow or white coloured flower regions are due to the presence of chalcone an intermediate chemical in the biosynthesis of the anthocyanin pigment family. The dominant green colour of plantstems is due to chlorophyll which has light absorbing properties essential for the conversion of atmospheric CO_2 to plant carbohydrate and O_2 essential for planet Earths survival. The vivid coloration of many unique native Australian flowers is evidence of their anthocyanin pigmentation and their unique structural features (Fig 3, 5). However, many Australian flowers lacksuch pigmentation and occur as white flower heads and inflorescences due to the prominence of chalcone and absence of anthocyanin pigments (Fig 4). Chalcone synthasecatalyzes the first committed and key regulatory step in flavonoid biosynthesis. Thus genetic silencing of the chalcone synthase gene results in an absence of colour in flower heads. Fig 4 shows this effect is widespread in Australian native flowers. While formal analyses reported to identify chalcone levels in native Australian plants is limited the presence of chalcone can nevertheless be deduced from the presence of these non-coloured flower varieties.

Post-transcriptional gene silencing (PTGS) is a mechanism whereby gene silencing is mediated and may be open to epigenetic regulation[7]. PTGS exploits

cellular mechanisms using transcripts that have sequence similarity to cellular double-stranded RNA (dsRNA) that undergo degradation in-situ. PTGS is a process that is closely related to RNA-mediated viral resistance and is a protective anti-viral feature that evolved in plants. Gene silencing and the cellular machinery that undertakes this process is a natural anti-viral protective mechanism in plants. In PTGS, small interfering RNA (siRNA) of 21-23 nucleotide residues in size act as homolous guides to trigger the systemic degradation of transcripts that are homologous to the si RNAs. PTGS was first discovered in transgenic petunias harbouring chalcone synthase genes. This process has been exploited to target degradation of specific gene transcripts to develop desirable features in crop plants. This has been achieved by the introduction of DNA constructs encoding dsRNA, anti-sense RNA, or by using co-suppression constructs producing si RNAs directed against the transcript of interest. A greater understanding of this gene silencing mechanism has led to the development of strategies for the silencing of deleterious genes and the induction of desirable plant traits in a precise and controlled way ushering in a new era in plant genetic manipulation. Such an approach has been utilised by Japanese plant breeders in the production of pure white chrysanthemum flower heads.

b) Plant breeding to prepare pure-white flower varieties

Pure white flowers hold special significance in the Japanese Psyche. Plant breeders have attempted to produce pure white colored Dahliahs in Japan for over 100 years with over 50,000 hybrids so far produced. However none of these cultivars had a pure-white coloration with the closest to white being an ivory coloration (Fig 1). Japanese flower breeders have striven for over 100 years to produce pure white flower cultivars. A white flower holds special aesthetic significance in Japanese society and invokes powerful emotions. White flowers are used in symbolic ceremonial events of high importance and are described as "Hanakotoba". White Chrysanthemums hold special significance in Japan and have appeared on the Japanese Imperial Family's crest for generations. White chrysanthemums depict purity, grief, and truth, and are traditionally used for funerals. White plum blossoms are considered to depict elegance and loyalty. The Sakura (cherry blossom), is the Japanese national flower, and has a brief flowering season. Sakura viewing parties (hanami) are popular in the spring time. White cherry blossoms symbolise "accomplishment" and "beauty of heart". The white rose is an aesthetic of innocence, devotion and silence while white Dahlia symbolises pureness, innocence and focussed behavior. The presence of a diverse range of pure white Australian native flowers encompassing the entire flower head is significant considering that flower breeders have found this very difficult to replicate.

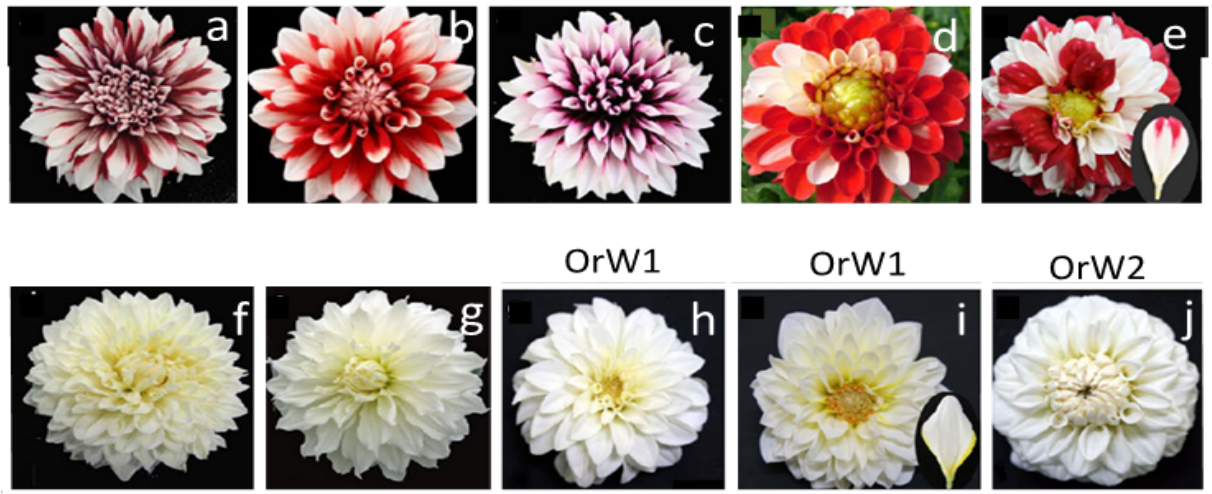

Figure 1: The stunning features of Star type Dahlia flower cultivars with variegated colour combinations (a-e) and a few near white cultivars (f-j). Notice the Orihime, *OriW1* cultivar (h) with a pure white *OriW1* inflorescence that spontaneously produces yellow petals (i) and *OriW2* with pure white inflorescence (j). Orihime is a labile cultivar which rarely produces red and white flowers in the same flower head despite the fact its parents are red-white bicolor plants. A careful analysis of the pigments in pure-white petals from OriW1 and OriW2 cultivars demonstrated a complete absence of flavonoid derivatives and an absence of *Chalcone-1* and *2* gene expression but small interfering RNAs (siRNAs) from the *Chalcone-1* and *2* genes were found. The pure white pigmentation was thus due to epigenetic gene silencing of *Chalcone-1* and *2* and the variable distribution of the pure-white colouration was due to differential inactivation of these genes. The fact that most Dahlia cultivars do not occur as entirely pure-white flower heads indicates that this gene silencing process rarely occurs with 100% efficiency [8]. However there are many pure-white Australian native flowers.

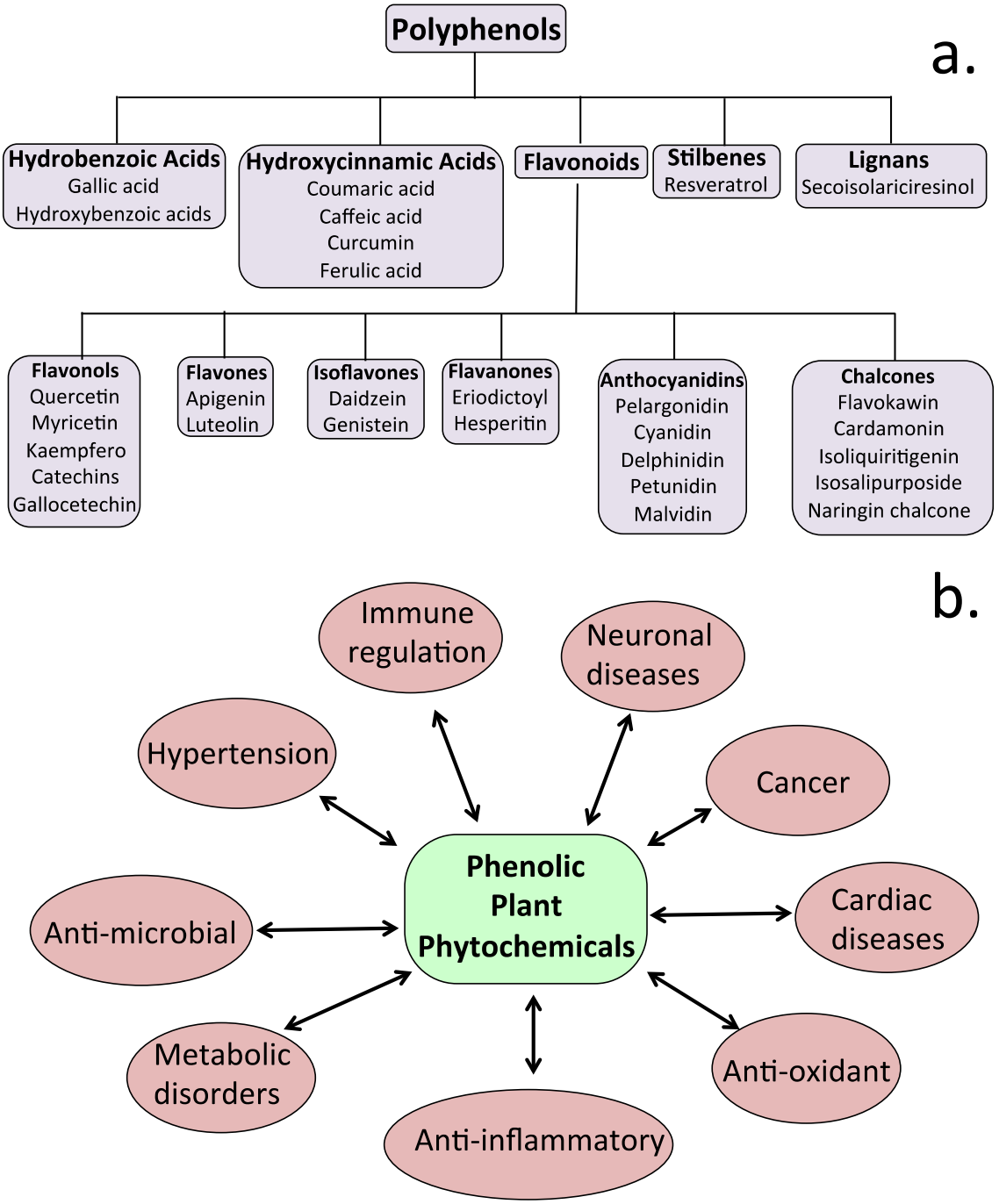

Figure 2: The biodiversity of plant phytochemicals (a) and their areas of biomedical application (b).

c) Medicinal uses for Australian native plants

Many of Australia's plants are unique and were used by its First Nation peoples for many generations not only as a food resource but also for medicinal purposes. The Aboriginal communities of Australia are one of the oldest civilisations recorded, it is estimated they have lived in Australia for at least 60,000 years. Unfortunately the Traditional Aboriginal peoples of Australia have no tradition of recording medicinal plant use information in a written form but such informationis passed down the generations in myths, traditional symbolic paintings and legendary story telling by elders. Thus the information available now on Australian native plants is incomplete and their medicinal properties are often known only from anecdotal observations. Moreover, information that has been recorded in print is somewhat fragmented and not readily accessible being recorded in government agency, statutory authority and institutional reports and writings put together by aboriginal communities. Some publications have made in-roads into this knowledge gap [3, 9-29] and some recent publications and on-line

platforms [The *Flora of Australia (http://www.environment.gov.au/science/abrs)]* have made significant inroads into the coverage of Australian native plant polydisperity. The wealth of medicinal compounds in native Australian plants is indisputable and an incredible resource which needs to be preserved and better understood for future generations. The development of analytical techniques to characterize pharmaceutical compounds from native plants will also aid in the plants taxonomic identification and in the elucidation of the functional properties of these compounds. Based on accumulated anecdotal evidence many of these will undoubtablyprove to be of application in Biomedicine [30-32]. Such studies need to be conducted on pure phytochemical components isolated from Australian native plants in order to establish their credentials in biomedicine on a firm scientific basis and to obtain certification from safety agencies that will allow their use in the clinic.

III. The Ancient Practice of the use of Plants for Medicinal Purposes

Ancient Chinese herbal remedies are undergoing a resurgence with several research groups striving to demystify the properties of Chinese herbal compounds and Traditional Chinese health practices [33-36]. This is important in order to avoid deaths from complications when traditional medicines are consumed along with conventional Western medicines. Cases have already been recorded of inadvertent deaths in chronic low back pain patients co-administered traditional Chinese medications in conjunction with conventional Western medications. The current epidemic of over-use of opiate medications in the USA indicates a cautious approach is advisable in the co-administration of traditional Chinese medications with conventional Western medicines

a) *Modern evidence of the mechanism of therapeutic acupuncture and traditional chinese medicines*

In a historical study on "The Meridians" by Li Shizhen in the Ming dynasty, the internal organs were considered to be regulated by meridians which represented a "life-threatening, cure-all disease pivot point" as proposed by the inner canon of Yellow Emperor Lingshu[37, 38]. Acupuncture points in the human body are distributed along the fourteen meridians. It has been proposed that acupuncture points are regions of communication with resident pluripotent stem cell niches[39, 40]. Acupuncture and traditional Chinese medicines have been proposed to activate these stem cell niches[41]and it is the subsequent release of programmed pluripotent stem cells that migrate to sites of tissue damage that provides healing properties [42]. A recent study suggests that some fluorescent dyes can visualize the pericardium meridian [43].

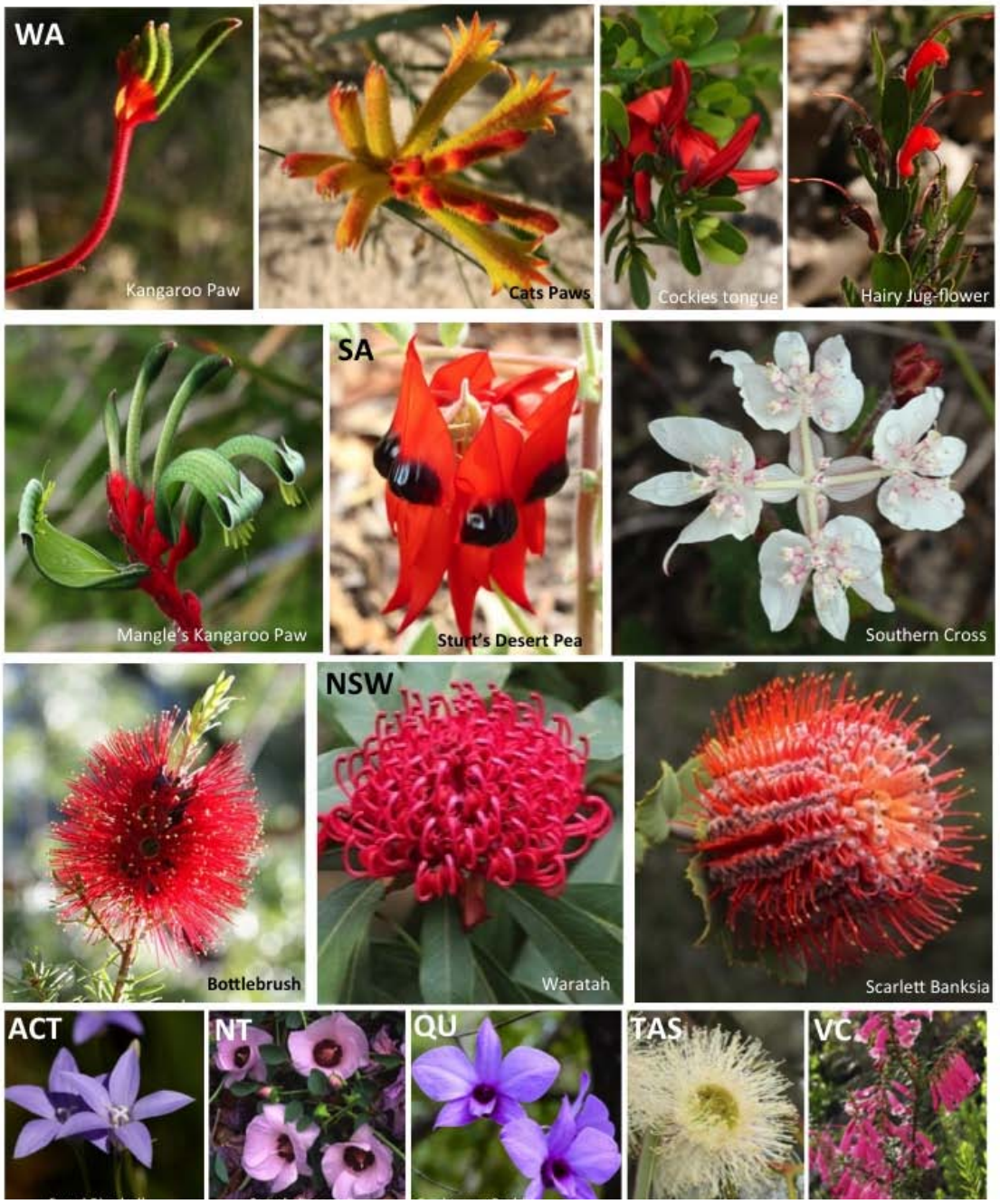

Figure 3: A few examples of the unique, stunning iconic Australian native wild flowers and flowering shrubs. Many of the flowers shown are Australian National State Emblems, state abbreviations are indicated in the top left hand side of these images. These include the red and green Kangaroo Paw, Anigozanthos manglesii (Western Australia); Sturt's desert pea, Swainsona Formosa (South Australia); Waratah, Telopea speciosissima (New South Wales); Royal Bluebell, Wahlenbergia gloriosa (Australian Capital Territory); Sturt's Desert Rose, Gossypium sturtianum, (Northern Territory); Cooktown Orchid, Dendrobium phalaenopsis, (Queensland); Tasmanian Bluegum, Eucalyptus globolus Labill (Tasmania); Common Heath, Epacris impressa (Victoria). Flower images supplied courtesy of Mr Philip Bouchard ©Bouchard 2010.

Figure 4: The biodiversity of the flowerheads and inflorescences displayed by Australian Native "white" coloured wild-flowers. Flower images supplied courtesy of Mr Philip Bouchard ©Bouchard 2010-2014.

IV. Historical Perspectives on Australian Native Plants

When Europeans first settled in Australia from 1788 onwards local First Nation Communities aided them with knowledge of many native Australian food plants and also aided in their medicinal applications. This was essential for the survival of the settler communities since farming practicesat this time were primitive and not properly adapted to Australian conditions thus yields were low. Furthermore, the uncertainty of re-supply of food and other essential commodities by the First Fleet was also an important consideration. The medicinal plants that were available in these early days of the settlement of Australia were recorded by the curator of The Technological Museum of New South Wales Mr JH Maiden in the form of a manuscript entitled *The Useful Native Plants of Australia* in 1889[25]. This 696 page document extensively documented all known native Australian plants and their uses and also included a 30 page listing of their common and botanical names. While information on individual native plants was often sketchy, this document is nevertheless an extremely important and invaluable historical document which is one of the earliest English records of the biodiversity and usefulness of Australian Native Plants. While a number of publications have appeared sincethen, on Australian Native Plants these mainly document anecdotal evidence of the usefulness of these plants with little in depth scientific analysis [3, 9-11, 14, 16, 17, 19, 21, 23-26, 29]. Two major key presentations should also be noted since they represent significant contributions to the fabric of Australian Ethnobotanical Biomedical History. In 1988 Dr Ella Stack, the first and former lady Mayor of Darwin delivered the third Eric Johnston lecture entitled *Aboriginal Pharmacopoeia*[44]. This prestigious occasional lecture series was named after Commodore Eric Johnston, The Northern Territories ex administrator and was an initiative of The Northern Territory Government. This lecture was published by The Northern Territory Library Service and was also televised by the Australian Broadcasting Corporation, and video tapes of this occasion are held in their historical archive. In 2004, Major General John Pearn former Surgeon General, Australian Defence Force, Prof of Paediatrics and Child Health, Royal Childrens Hospital, Brisbane was invited to address the Linnean Society in Piccadilly, London, UK and he delivered a presentation entitled *Medical Ethnobotany of Australia Past and Present*[27]. In this presentation he showed how 40,000 years of observation, clinical interpretation, experimentation and trial and error in the use of Australian Native Plants by the Aboriginal peoples had allowed the identification of

numerous antiseptics, analgesics, sedatives, astringents, antipyretics, hypnotics, expectorants, muscle relaxants, carminative anti-flatulent and mood-alteringdrugs. Aboriginal peoples used drugs and medicinal compounds obtained from leaves, bark, roots, and the flowers of various native plants in the form of infusions, decoctions and maceration poltices or in emulsifications with kangaroo or emu fat for topical applications as ointments or creams. Incidentally, Emu oil itself has powerful anti-inflammatory and anti-oxidant compounds which undoubtably contributed to the efficacy of such preparations so it is not simply an inert carrier for the bioactive plant components being administered. Emu oil is high in omega-3, omega 6, and omega-9 fatty acids which help reduce inflammation and it has useful trans-dermal transfer properties for bioactive substances this is a particularly beneficial trait in the treatment of conditions such as swollen joints or the repair of cutaneous wounds. Emu oil also has known pain relief properties and accelerates healing responses of wounds[45, 46].Furthermore, Emu oil also contains a protein related to cyclosporine-A, a cyclic 11 amino acid fungal peptide used as an immunosuppressant medication in heart transplant patients. Unfortunately long-term use of cyclosporine-A leads to inevitable tumour development and the demise of the patient[47, 48]. The emu-oil equivalent to cyclosporine-A is a magnitude more active raising the possibility that if used in heart recipients this may not lead to the inevitable development of tumours.

Due to a declining interest in the 1980's by young Aboriginals in their traditional medical practices Government sources considered that there was a danger that knowledge of these compounds might disappear. A jointly funded inititiative by the Federal and Northern Territory Governments was therefore begun in 1984 as part of a Bicentennial Commemorative Program to record botanically all known medicinal native Australian plants in Central Australia and the Northern Territory, how they were harvested and how they should best be used medicinally. Vouchers for all documented plants are held in the Northern Territory Herbarium at Alice Springs and at the Darwin Herbarium. An initiative of the Northern Territory Government in 2003 "Desert Knowledge Australia, thriving Desert Knowledge Economics" www.desertknowledge.com.au was also made. Desert Knowledge Australia (DKA) is a statutory authority of the Northern Territory Government established in 2003 by an Act of Parliament to "encourage and facilitate learning, research and sustainable economic and social development relating to deserts and arid lands". DKA is located in Alice Springs and promotes all aspects of desert culture and knowledge generation and dissemination. A similar institution, the Australian Institute of Aboriginal and Torres Straight Islander Studies (AIATSIS) [http://aiatsis.gov.au/publications/australian-aboriginal-studies-journal]islocated in Canberra, Australian Capital Territory and holds extensive information on indigenous Australian plants and their beneficial uses as food items and in medical applications in their collections catalogue which can be consulted on site. Despite these initiatives Australian native plants remain relatively poorly studied and despite their massive potential it is surprising that more research is not being undertaken.

Traditional Australian Aboriginal knowledge of plants as therapeutics is disappearing as the Aboriginal culture merges into main-stream society in Australia and the passing of oral traditions between each generation diminishes [22]. Given the diverse and unique nature of the Australian flora and inadequate maintenance of the traditional knowledge base on these plants, there is a very real need to document the traditional usage of Australian native and indigenous plants before this knowledge is permanently lost. Australian native plants have a long history of being used for a wide variety of uses including food, clothing, shelter, tools, weapons, and as medicines. Before modern medicine, ancient civilizations afflicted with illnesses and diseases were reliant on a wealth of therapeutic agents which were discovered in the plant kingdom. Knowledge of these medicinal preparations and any dangerous side effects they may have was passed down through the generations by oral tradition and was sometimes recorded in herbal literature. This information generated by the First Nation peoples in Australia certainly rivals other historical medical information databases written down in more conventional texts and tablets. The earliest recorded plant medications can be traced back 6000 years to Sumerian clay tablets (4000 BC) which detail 1000 medicinal plants and plant remedies [49]. These cuneiform clay tablets also provide evidence Sumerian physicians were aware of the symptoms of Stroke (chronic apoplexy) and document procedures for its treatment [50]. Greco-Roman physicians became aware of this condition centuries later however debated whether the source of the condition was the brain or the heart. The Greek physician Galen considered the brain to be the source of this condition [51]. Neurotrophic properties known in some Aboriginal medications would probably have been of benefit in the treatment of stroke. The Pun-tsao, a Chinese record of thousands of herbal cures assembled by Shen-nung, a legendary Chinese emperor dates to 2500 BC. The Hippocratic Corpus (a collection of medical texts of herbal remedies) by Greek physician Hippocrates was recorded in the late fifth century BC and the Roman De Materia Medica by Dioscorides, identifies 600+ medicinal plant species [51]. It is estimated that Aboriginal peoples in Australia were aware of many thousands of plants which they used for medicinal purposes for at least 30,000 years, with knowledge of these passed down orally rather than being recorded in a written text.

V. Plant Phytochemicals Represent a Diverse Functional Group of Molecules

Plant phenolic phytochemicals are widely distributed in all plant parts and many of these have beneficial anti-oxidant, anti-inflammatory and anti-microbial profiles that combat infection when used in man and also convey protective properties to plants preventing them from being consumed by plant pests. Figure 5 shows how plant phenolics have been categorized and the widespread areas in Biomedicine where these compounds have found application. The plant phytochemicals have been sub-classified into hydrobenzoic acids, hydroxycinnamic acids, stilbenes, lignans and flavonoids. This latter group has been subcategorized into flavonoids, flavones, isoflavones, flavanones, anthocyanidins and chalcones (Fig 2a). Chalcones are a particularly versatile group of phytochemicals. Polyphenolic phytochemicals have been utilized in a very diverse range of biomedical applications (Fig 2b).

a) Australian native plants contain bioactive compounds of interest in biomedicine

Some particularly outstanding examples of Australian native plants and the uses of chemical compounds they contain are noteworthy. Aboriginal peoples used the kangaroo apple *Solanumlaciniatum* and *Solanum-aviculare* in poltices that were applied to joint swellings. This plant contains the steroid solasodine which is a precursor for the production of other steroids such as those used in the contraceptive pill. Solasodine displays diuretic, anticancer, antifungal, cardiotonic, antispermatogenic, antiandrogenic, immunomodulatory, antipyretic and various effects on the central nervous system. While this plant is native to Australasia it has been widely exported to Russia and Eastern Europe where plantations were established and used for steroid production and represented a cost effective alternative to the synthesis of steroid intermediates[44].

b) Australian plants contain a diverse range of alkaloids of medicinal potential

After World War II a systematic survey of the Australian flora for plants of chemical and pharmaceutical interest uncovered a large number of compounds of interest. A total of 500 alkaloids were identified and 200 of these were novel compounds. In 1963 The Australian Institute of Aboriginal Studies was set up to promote the recording of the medicinal uses of native Australian plants and several texts on these have appeared[2, 10, 16, 21, 52-66].

Australia is one of 17 megadiverse countries in the world and has a wealth of unique plant species [2]. Although a systematic survey of Australian native plants for chalcone compounds has yet to be undertaken, the presence of chalcones in Australian native plants was noted in a recent review of the PubMed, SciFinder, Web of Knowledge, Scopus, and Science Direct databases for medicinal compounds in Australian plants and shrubs [67]. A number of compounds of potential medicinal interest were identified that are unique to Australian plants. One class of these compounds are the terpenes which are fragrant highly aromatic unsaturated hydrocarbons found in essential plant oils. These have found application in fragrances, aromatherapy, cosmetics and house-hold cleaning products. Terpenes are of the general formula $(C_5H_8)_n$ and are classified by the number of carbon atoms they have into monoterpenes (C_{10}), sesquiterpenes (C_{15}) and diterpenes (C_{20}). The monoterpene Terpinen-4-ol, the main bioactive component of tea-tree oil has a range of beneficial biological properties [68]. Terpinen-4-ol is useful in the treatment of a range of dermatological disorders [69-72] and has also found application in cosmeceuticals[73]. Terpinen-4-ol also induces a significant dose-dependent growth inhibition of colorectal, pancreatic, prostate and gastric cancer cells in-vitro [74],has anti-microbial activity [75, 76]and has been used to treat protozoan and helminthic infections [77]and oralcandidosis in cancer patients [77].

c) Some Australian condiment herbs have medicinal properties

Tasmanian pepper leaf (*Tasmannialanceolata*, *Winteracea*), anise myrtle (*Syzygiumanisatum*, *Myrtaceae*) and lemon myrtle (*Backhousia*, *Myrtaceae*) are unique polyphenolic Australian native herbal condiments that display cytoprotective, anti-oxidant and anti-cancer properties significantly reducing the growth of colon, stomach, bladder and liver cancer cells in-vitro [78]. Extracts of these herbs induced apoptosis in promyelocytic leukaemia (HL-60) and colon adenocarcinoma (HT-29) cells indicating they had beneficial therapeutic properties in the treatment of these tumours.

Figure 5: Examples of some of the unique highly coloured native flowering plants and shrubs present in Australia. Flower images supplied courtesy of Mr Philip Bouchard ©Bouchard 2010-2014.

d) Specific chemical compounds of interest in Australian native plants

Figure 6 shows examples of a few bioactive compounds that have been identified in some native Australian plants. The leaves and fruits of the kangaroo apple (*Solanum laciniatum)* contain the steroid alkaloid solasodine which binds to oestrogen receptors and has contraceptive properties. Solasodine is a chemical precursor that can be converted to progesterone which suppresses ovulation by inhibiting production of follicle stimulating hormone and luteinizing hormone.The Kangaroo Apple was used by native Australian Aboriginal people for centuries, as a food item, it is rich in vitamin C, and medicinally as an antibacterial and anti-fungal agent and a source of anti-inflammatory and antioxidants that were applied in the treatment of swollen joints. This plant was exported to Russia and Eastern block countries in the 1960s and 1970s where it

was grown in plantations and the processed plant material used for the production of steroids, alkaloids, and contraceptive compounds for biomedical applications. Solasodine also has anti-cancer properties[79-81].

The corkwood tree *Duboisiamyoporoides* contains the psycho-active drugs hyoscyamine (daturine) and hyascine (scopolamine)[82, 83]. Aborigines traditionally used corkwood infusions to aid in fish capture by drugging waterholes to paralyse the fish to simplify their collection. A thriving industry was established in Northern Queensland based on the corkwood tree during World War II[84]. Scopolamine was widely used to counter sea-sickness during the D-day landings of World War II. Hyoscyamine provides symptomatic relief of lower abdominal muscle spasms, bladder disorders, peptic ulcers, irritable bowel syndrome, bladder disorders, diverticulitis, pancreatitis, colic, and interstitial cystitis and is also used in the control of neuropathic pain and chronic intractable pain in resistant, untreatable, incurable diseases[85-87].

*Lactucavirosa*is, a wild "lettuce" found in Australia that contains two noteworthy bio-active compounds, lactucopicrin and lactucin. These are sesquiterpene lactones that bind to opioid receptors and produce pain relief during painful menstruation, joint and muscle pain, and they also act as anti-malarials[88]and have anti-tumor properties [88, 89] and promote neuritogenesis and neurotrophic effects in the CNS/PNS [90].

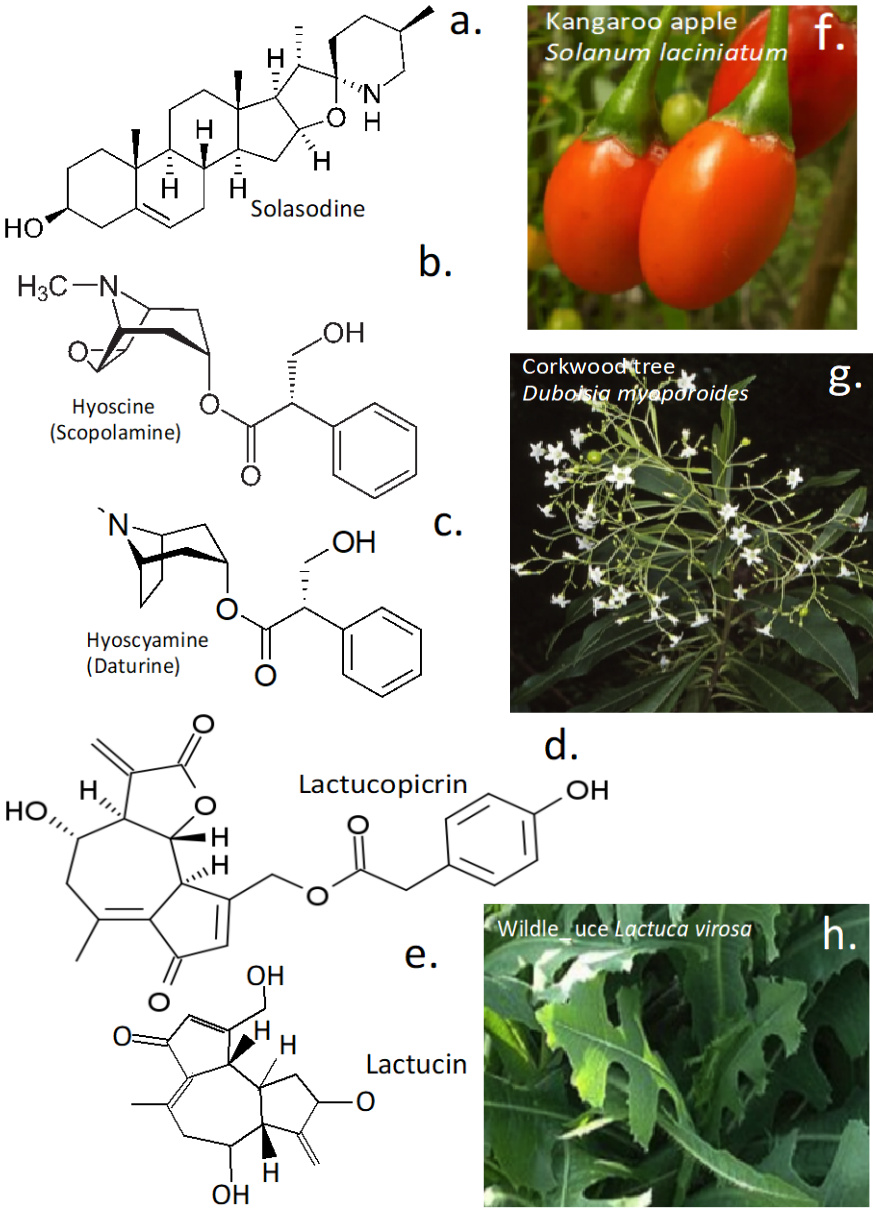

Figure 6: A few examples of bioactive compounds present in Australian native plants with important biomedical applications. Images reproduced under Open Access from Wikipedia

Table 1 lists a number of selected bioactive compounds which have been identified in Australian native plants. Clearly these are of vast potential in prospective therapeutic medical applications. However it should be noted that these entries only represent a relatively small selection of all compounds that are present in Australian native plants. The definitive list of all bioactive compounds in Australian native plants and their scientific characterization has yet to be undertaken although several studies have documented examples of a range of bioactive compounds [12, 13, 18, 28, 91]. There is a clear need to undertake this systematically, it should be noted that approximately 25% of all current prescription drugs and 75% of all anti-cancer drugs were developed from plant compounds, Australian Native Plants offer a vast potential [29].

Table 1: Examples of Bioactive Compounds Detected in Australian Native Plants and Shrubs

Compound	Application and Comments
Aesculetin	Removal of venous congestion, treatment of varicose veins
Allantoin	Moisturising properties, Cosmetics applications
Anthocyanin	Anti-inflammatory, anti-oxidant, anti-obesity properties
Atropine	Reverses pesticide induced nerve dysfunction, produced by corkwood native shrub *Duboisiamyoporoides*
Catechin	Vasodilatory, blood pressure regulation. Found in lemon myrtle, wild lime, kakadu plum.
Meteloidine	Cocaine-like alkaloid of *Erythroxylumaustrale* (cocaine bush) N. Australia
Chalcone	Intermediate in anthocyanine and isoflavone production, derivatives have anti-bacterial, anti-cancer, anti-inflammatory, anti-diabetic, anti-oxidant, anti-microbial, anti-viral properties. Targets Microtubule, NFkB, RTK and EGFR signaling
Colchicine	Alternative non-steroidal anti-inflammatory drug for the treatment of gout
Curcumin	anti helminthic properties
Digitalin, Digitoxin Digoxin	Treatment of cardiac arythmias and regulation of blood pressure. Produced by native Australian foxglove
Emetine	An anti-protozoal drug that induces vomiting
Ephedrine	cough expectorant stimulant, produced by *Ephedracea* gymnosperm shrubs
Glycyrrhizin	sucrose/glucose substitute obtained from native liquorice *Glycyrrhiza glabra*
Hydrastine	haemostatic drug intermediate
Kainic acid	CNS stimulant, induces seizures and epilepsy in experimental animals
Lactucopicrin and Lactucin	Sesquiterpene lactones that bind to opioid receptors and produce pain relief during painful menstruation, joint and muscle pain, and they also act as anti-malarials. Found in *Lactucavirosa*, a wild Australian lettuce
Lovostatin	Cholesterol lowering drug, Member of statin family
Quinine	Anti-malarial of Quinine Tree (Native Quince/Bitterbark)*Alstonia-constricta*
Picrotoxin	Interacts with the inhibitory neurotransmitter GABA, acts as a stimulant and convulsant impacting the CNS, causing seizures and respiratory paralysis if used in high enough doses.
Pseudoephedrene	Amphetamine stimulant, decongestant. Ephedra desert shrub contains pseudoephedrine
Physostigmine	Highly toxic reversible inhibitor of cholinesterase, glaucoma treatment
Resveratrol	polyphenolic anti-oxidant, a phytoalexin – antibacterial, anti-fungal drug
Salicin	anti-inflammatory related to aspirin found in *Salix*sp trees and shrubs
Scopolamine	treats sea-sickness, pain-killing anaesthetic, soothes post operative neausea
Solasodine	Present in *Solanumlaciniatum* and *Solanumaviculare*. Solasidinehas diuretic, anticancer, antifungal, cardiotonic, contraceptive, antispermatogenic, immunomodulatory, antipyretic properties and various effects on the CNS
Stevioside	Sucrose/glucose substitute sweetener suitable for diabetics
Strychnine	Asphyxiant poison
Tetrahydropalmatine	analgesic, suggested as less addictive alternative to benzodiazepine/opiates.
Theophylline	treatment of COPD and asthma, phosphodiesterase inhibiting drug
Xanthotoxin	treatment of acne, vitilago, and psoriasis. Produced by wild parsnip, angelica seeds, bullwort, parsley

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; GABA, γ-amino butyric acid; CNS, central nervous system; RTK, receptor tyrosine kinase; EGFR, epidermal growth factor receptor.

e) *The chalcones are widely distributed in many native Australian plants*

We can deduce from the widespread occurrence of pure-white native Australian flower forms that chalcones are also present in Australian Native Plants where they evolved to provide protection from the adverse, harsh environmental Australian climatic conditions. Chalcones also act as attractant molecules for pollinators and have strong fungitoxic, antimicrobial and anti-viral properties that protect plants from pathogenic organisms [92-98]. Although chalcones and flavonoids play prominent functional roles in flowers they also have a widespread distribution in fruits, vegetables and many different plant tissues. Chalcones, and flavonoids have important roles to play in plant physiology and biochemistry in pollination processes [99, 100], UV light protection [101-104] and the protection of plants from insect and plant pathogens [105]. Chalcones and flavonoids are members of the polyphenol superfamily of plant phytochemicals. Over 10,000 plant flavonoids have been detected and categorized into several subclasses, including flavonols, anthocyanins, flavanones, flavones, isoflavones and chalcones (Fig 1a). Chalcones in particular represent a privileged structure in medicinal chemistry and a versatile template that is widely used in drug discovery investigations. Chalcone is a common simple intermediate scaffold structure found in many naturally occurring plant compounds and biodiversechalcone derivatives have also been synthesized due to their ease of preparation. The chalcone family of compounds are widely distributed in the plant kingdom where they are both intermediates and end products in flavonoid biosynthesis.The flexible versatile structure of chalcones and its derivatives have provided a wide array of properties regulating many biological activities and the ability to target several cellular targets in particular regulatory pathways.

f) *Chalcones counter the damaging features of oxidants during inflammatory conditions*

Antioxidants prevent or slow damage to cells due to the generation of free radicals in tissues which damage cellular structure and function. Oxidative stress is a common feature of chronic diseases such as heart disease, stroke, cancer, arthritis, respiratory syndromes, and neurological disorders such as Parkinson's and Alzheimer's disease, and is a standard feature of inflammatory disorders. Chalcones have many anti-inflammatory and anti-oxidative properties which aid in tissue protection. The molecular target molecules the chalcones are interactive with are indicated in Table 2. Such interactions either inhibit the action of key molecules that lead to tissue degradation or downregulate their expression at the gene level. Some beneficial tissue promoting proteins are also up-regulated depending on tissue context and disease process.

VI. Chalcone Derivatives have Multifunctional Properties Useful in Medicinal Applications

Chalcones or analogues or derivatives of[E]1,3-diphenyl-2-propene-1-one , and represent an extremely diverse family of compounds. Chalcones have Anti-inflammatory, Anti-bacterial, Anti-tuberculosis, Anti-diabetic, Anti-oxidant, Anti-microbial, Anti-malarial, Anti-viral properties and combat tumour development. A number of chalcones are licenced for clinical use [106].

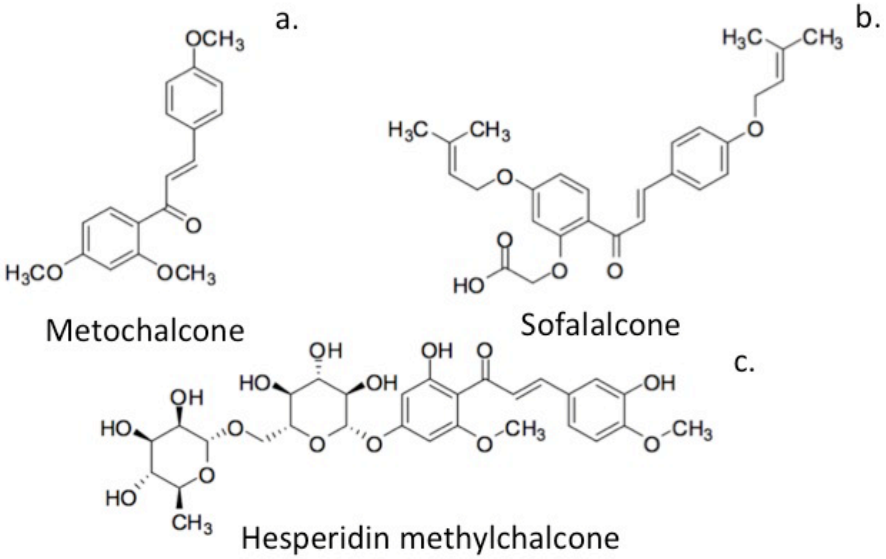

Figure 7: Chalcone drugs that are licenced for medicinal applications[107-110]. Figure reproduced with permission © 2020 Tekale S, Mashele S, Pooe O, Thore S, Kendrekar P, Pawar R. under CC BY 3.0 license. Available from: http://dx.doi.org/10.5772/intechopen.91626

Chalcone has a widespread distribution in fruits and vegetables and in flowering plants. Chalcone is an intermediate compound in the biosynthesis of members of the anthocyanin family and has been proposed as a promising base molecule for the development of new molecules with specific biological therapeutic activities [111, 112]. Chalcone has therefore been derivatised into many anti-cancer agents [91, 113-144] which act in many different ways on tumor cells in a number of cancer types. Chalcone derivatives inhibit microtubule formation or destabilize these structures in tumor cells [125, 126, 133, 142], promote apoptosis [113, 117, 120, 130, 134], inhibiting proliferation of tumour cells [115, 116, 123, 126, 131, 133, 134, 136, 139, 142-144].

a) Anti-viral properties of plant phytochemicals against SARS-CoV-2 viral functions

A number of flavonoids have become of interest as anti-viral agents in the treatment of the infective phase or the replication of SARS-CoV-2. Advanced computational molecular docking methods have been developed to assess the molecular fit of prospective anti-viral agents and the measurement of free energy values which drive binding to defined regions of SARS-CoV-2. Thus potential anti-viral interactions with RNA dependent RNA polymerase, main protease and Spike glycoprotein have been evaluated with a view to determining the ability of these compounds to suppress COVID 19 infections. Several flavonoids and related indolechalcones are capable of combatting SARS-CoV-2 viral functions through interaction with RNA dependent RNA polymerase (rdrp), main protease (M^{pro}) and Spike (S) protein impacting on the function of SARS-CoV-2 activity and by implication may provide disease suppression. Out of 23 natural flavonoids and 25 synthetic indolechalcones, 30 compounds were capable of M^{pro} deactivation potentially lowering the efficiency of M^{pro} function. Furthermore, cyanidin displayed inhibitory activity against RNA polymerase and quercetin blocked interaction sites on the viral spike protein. Cyanidin is a pigmented anthocyanidin intermediate compound found in many red berries grapes, bilberry, blackberry, blueberry, cherry, cranberry, elderberry, hawthorn, loganberry, and raspberry. Quercetin is a polyphenolic pigment flavonoid found in fruit and vegetables which displays neuroprotective anti-oxidant activity [99, 145, 146].

Further studies are thus warranted with these plant phytochemicals to fully determine their potential as SARS-CoV-2 anti-viral agents [147]. Flavonoid glycosides and their putative human metabolites can play a key role as inhibitors of the SARS-CoV-2 3CLpro and RNA-dependent RNA polymerase RdRp enzymes impeding viral replication [148]. Certain flavonoids exhibit angiotensin-converting enzyme (ACE2) inhibitory activity and have crucial roles to play in the regulation of arterial blood pressure. Interaction of these flavonoids with ACE2 may also impede the binding of SARS-CoV-2 spike protein and is in line with the anti-viral properties of these compounds [149].The interaction of flavonoids with Mpro, Spike receptor binding domain (Spike-RBD), RNA - dependent RNA polymerase (RdRp or Nsp12), non-structural protein 15 (Nsp15) and the host ACE-2 spike-RBD binding domain have been examined using advanced computation molecular docking techniques. These en-silicoSARS-CoV-2 binding studies indicated that tribuloside, legalon and isosilybin had anti-viral anti-infective properties, warranting further evaluation to determine how effective they are in the inhibition of SARS-CoV-2 infectivity [150].A range of chalcone derivatives have been evaluated using molecular docking methods to assess the effectiveness of these re-purposed compounds on the prevention of the infectivity and replication of SARS-CoV-2 [151-155].

VII. Concluding Remarks

Unique plant lineages in Australia can be traced back to their origins in the super-continent of Gondwanaland[156], these represent part of the ancient evolutionary records of the pre-history of the Australian continent[157]. The origins of some key plant species have even been traced back to the Gondwanaland super-massif prior to its break-up and some of these have been identified in the present day Australian flora. The rice genus (*Oryza*) originated about 130 million years ago in Gondwanaland withsub-species subsequently becoming established in the land-masses that arose from Gondwanaland's break-up. This plant was the basis of all rice varieties, a staple food of a significant proportion of the worlds population and as such is an invaluable source of original genetic information when creating back-crosses in the production of new rice cultivars [158]. This is but one example of the importance of the gene pool resident in native Australian plants.The plants of the Amazon basin are often extolled as an important source of genetic biodiversity of global significance for good reason. However, the genetic code resident in Australian flora should also be recognized for its unique qualities and be recognized to be of global significance. Australian native plants are a significant genetic resource that needs to be preserved, this also emphasizes the sustainable exploitation of plant biodiversity in the development of new medicinal products.The chemical compounds which evolved in Australian plants to facilitate plant survival under the often harsh climatic conditions that prevailed at the time led to an impressive collection of chemical compounds of significant chemical diversity. Many of these compounds have yet to be identified and characterized but nevertheless represent an invaluable Pharmacologic resource in need of full categorization and characterization. It should be remembered that many medicinal compounds have

been identified from plant materials. These compounds in Australian plants are a considerable resource with many of these compoundsliable to have beneficial properties of application in Biomedicine. This is built on anecdotal evidence accumulated on the medicinal uses of Australian native plants overmany generations of First Nation Aboriginal Communities. Their contributions gleaned from 40,000 years of the use of native medicinal plants should be duly acknowledged and the Aboriginal communities should continue to be involved in the development of native plant species as a biomedical resource.

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Conflicts

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Author contributions

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

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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