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Inaugural Lecture Series 151

EQUILIBRIUM DYNAMICS IN LIFE AND COTERMINOUS PROCESSES

BY

OLASOJI A. AMIRE
Professor of Chemistry

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Life is the state or quality that distinguishes a living being or organism from a dead one and from inorganic matter[*] *The Holy Bible* is more specific. In Leviticus, chapter seventeen verse eleven, the Bible says "for the life of the flesh is in the blood..." and in Job, chapter seven verse seven, it says further "O remember that my life is wind..." Furthermore, in Genesis, chapter two verse seven, the Bible says "And the Lord God formed man of the dust of the ground, and breathed into his nostrils the breadth of life; and man became a living soul".

Modern science shows us that the wind is, in fact, heterogeneous. The component of interest in the wind is now well known as oxygen. Hope-Seyler was the first person to use the term haemoglobin to describe the pigments of blood; today, the term is used to include the respiratory proteins of all vertebrates. Available data suggests that there is a phytogenetic relationship between haemoglobins, myglobins, and even erythrocruorins. They carry out the job of transport and storage of oxygen. This lecture is partly about how they perform these functions, that is, the equilibrium dynamics in life processes.

There are many variants of haemoglobin molecules. Those from normal human hosts are called human haemoglobin A and those from hosts that suffer from sickle cell disease are known as

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[*] Collins Concise Dictionary gives the definition as "the state and quality that distinguishes living beings or organisms from dead ones and from inorganic matter".

haemoglobin S. Sickle cell disease has a genetic origin, that is, it is passed from parents to children. The prevalence of the disease is about ten percent in Africans. Red blood cells carry oxygen from the lungs and discharge it in the arteries where they diffuse into the muscle and other centres where they are used for other life processes. The red blood cell, after the discharge of its oxygen load, returns to the lungs to absorb oxygen, which it carries around the body again. The cycles of oxygenation and deoxygenation are repeated many times in one minute and continue endlessly in living human beings. When normal haemoglobin is fully oxygenated, its cell is morphologically biconcave and remains so even after deoxygenation. But when haemoglobin S is deoxygenated, its cell forms tactoids with sickle shapes; and it polymerizes within the cell. Haemoglobin S is irreversibly sickled, the sickled cell clogs the blood vessels and makes the job of pumping blood round the body difficult. The sickle cell crisis occurs from the blockage and anaemia from high rate of waste of the haemoglobin S arising from the irreversibility of the sickling phenomenon.

Studies on sickle-cell anaemia and the discovery of haemoglobin S (Pauling *et al*, 1949) gave a strong impetus to the investigations carried out on many haemoglobin variants by the Ibadan group of Professors D.H. Irvine, J.G. Beetlestone and L. Luzzato in the late sixties and later, A.C. Anusiem, G.B. Ogunmola, and others in the early seventies, when I became a graduate student in the Biophysical Research Laboratory at the Department of Chemistry, University of Ibadan. The observation

that the abnormality of this haemoglobin is caused by the exchange of a single amino acid, acidic glutamic acid in the normal beta-chain for neutral valine in the abnormal (Ingram, 1958), opened one of the most fascinating and challenging areas of human genetics. Today, the primary, secondary, tertiary and quaternary structures of haemoglobin are well known. Haemoglobin remains one of the most widely studied molecules in human history. However, the exact mechanism relating structure to function in sickle cell disease remains unknown.

A haemoglobin molecule consists of four chains: two alpha-subunits, each with one hundred and forty-one amino acid residues, and two beta-subunits each with one hundred and forty-six amino acid residues (Perutz, 1970). The four subunits are tetrahedrally arranged to produce an ellipsoid. The haem groups, which bind gaseous ligands (O_2, CO_2, CO, etc.), are placed at the corners of the tetrahedron. The reversible binding of gaseous molecules at the haems (called homotropic interactions) are modulated by the reversible binding of non-haem ligands to specific sites on the globin moiety (Amire *et al*, 1978; Ogunmola *et al*, 1978). This is known as heterotopic interactions.

Many methods of analysis of haem ligand binding are known and have been variously described for the oxygenation equilibria of many types of haemoglobin (Imai, 1981). These include, to mention a few: Hill plot, Scatchard plot, Watari-Isogai plot, Least squares method and Imai-Adair method, etc. References and detailed

discussions of these methods are given in the cited publication. The question that may be asked is what new contribution can one make to this endless list? At first, when I joined the Biophysical Research group in 1972, this question looked formidable and one to which no answer was ever forthcoming. Later in my academic career, I found an answer, or so I thought. At another time, the answer appeared trite. Whatever it is, I now have to say something and leave the judgement to you and the future generation of scientists that may pass my way. Honestly, I would have preferred to be left alone.

I will try to discuss a theory (Amire, 1994), which appears to be the basis of most multiple equilibria experiments (including, of course, multiple equilibria in haemoglobin) that I have come across in the last thirty years or so. If this has not been part of my contribution to knowledge, I would have spared you the agony of going through it for I know that many of my students hate mathematics. I promise the process of going through will be as painless as it is lucid. But before the presentation of this portion of my lecture, I will quickly go through some important developments in the field of multiple equilibria. Thereafter, I will endeavour to describe, in summary, some experimental results that highlight the applications of the principles of the theory.

Now the review. Consider a general macromolecule, M which has n identical binding sites and reacts with ligand, L one molecule of which can become attached to each of the sites at a time. Haemoglobin falls in this category and has four identical

binding sites and its ligand is usually the oxygen molecule. For the sake of simplicity I will present my arguments in terms of haemoglobin as the macromolecule because of its small number of binding sites. The results will thereafter be generalized. In this regard, consider the stepwise association of oxygen to each of the four haem groups in the haemoglobin molecule according to the following scheme:

$$(S1)$$
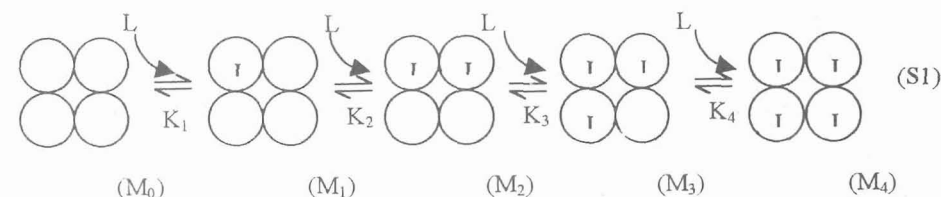
Suppose M_o, M_1, M_2,..., M_4 represent macromolecules carrying 0, 1, 2, ..., 4 molecules of the ligand, respectively. If the coefficients that measure the amounts of the macromolecules present as M_1, M_2, etc. at equilibrium, called the step equilibrium constants, are K_1, K_2, etc., respectively, then the law of mass action requires that $[M_1] = K_1[M_o][L]$, $[M_2] = K_2[M_1][L]$, ..., $[M_4] = K_4[M_3][L]$, where the square brackets represent the concentration of the species enclosed in number of moles per litre of the solution in which the species exist. The concentration of bound ligand, $[L_b]$ is equal to $[M_1] + 2[M_2] + 3[M_3] + 4[M_4]$ and the total concentration of the macromolecule of all forms, C_M is also equal to $[M_o] + [M_1] + [M_2] + [M_3] + [M_4]$. From the total concentration of

ligand bound and total concentration of macromolecules, we may obtain the average number of molecules of ligand bound per macromolecule, $\bar{\nu}$ which is equal to $[L_b]/C_M$. In terms of the step equilibrium constants and free ligand concentrations:

$$\bar{\nu} = \frac{K_1[L] + 2K_1K_2[L]^2 + 3K_1K_2K_3[L]^3 + 4K_1K_2K_3K_4[L]^4}{1 + K_1[L] + K_1K_2[L]^2 + K_1K_2K_3[L]^3 + K_1K_2K_3K_4[L]^4} \quad (1)$$

This equation is known as Adair equation after the British Biophysical Chemist, Gilbert Smithson Adair, who first derived it in 1925 (Adair, 1925). However, this equation is not useful as it stands, since in the days of Adair, it was impossible to easily evaluate the four constants K_1, K_2, K_3 and K_4 with any degree of accuracy from an experimental curve. To analyse an experimental result, where there was about ten binding sites was formidable and unimaginable. Therefore, some special cases needed to be considered.

Suppose the binding of a ligand at one site influences the other in such a way that they fill up in rapid succession, that is, the sites are extremely cooperative, in other words, K_4 is much larger than K_1, K_2 and K_3. The Adair equation reduces to,

$$\bar{\nu} = \frac{4K_1K_2K_3K_4[L]^4}{1 + K_1K_2K_3K_4[L]^4} = \frac{4K[L]^4}{1 + K[L]^4} \quad (2)$$

The product of the step equilibrium constants, $(K_1\ K_2\ K_3\ K_4)$ is designated the overall equilibrium constant, K for the equilibrium

reaction, $M + 4L = M_4$. For the general case of n identical highly cooperative binding sites,

$$\bar{\nu} = \frac{nK[L]^n}{1 + K[L]^n} \quad (3)$$

and the fraction of the binding sites occupied, $\bar{Y} = \bar{\nu}/n$ is given by,

$$\bar{Y} = \frac{K[L]^n}{1 + K[L]^n} \quad (4)$$

This last equation is called the Hill equation (Hill, 1910), which has been used extensively in the analysis of haemoglobin reactions with oxygen and is named in honour of the British Physiologist, Archibald Vivian Hill, who first used the equation in 1910 for oxygenation experiments. We conclude this section by saying that where the sites are extremely cooperative, *Adair equation reduces to Hill equation*.

Suppose on the other hand, that we have sites which are identical but independent (that is, non-cooperative) and k (small k) is identified as the intrinsic equilibrium constant for the binding of ligand, L to a site in a macromolecule, M. Since there are four ways for L to become attached to a haemoglobin and one way for it to come off, K_1 is equal to 4k. After one molecule of ligand is attached, there are three ways for a new one to go unto the macromolecule and two ways to come off and K_2 is equal to $(3/2)$k, and so on. Following this line of consideration, we have that K_1, K_2,

K_3 and K_4 are equal to $4k$, $(3/2)k$, $(2/3)k$ and $(¼)k$, respectively. The coefficients of the intrinsic constant, k are called statistical factors. If we use these values in the Adair equation and factorize the resulting expression we obtain a new equation for the fraction of sites occupied on the macromolecule, $\overline{Y}$, as:

$$\overline{Y} = \frac{k[L]}{1 + k[L]} \tag{5}$$

Equation 5 is known as Langmuir isotherm (Langmuir, 1918) and has been employed in various forms to analyze multiple equilibria in many systems.

I observed very early in my career that the statistical factors were no more than a comfortable way of stating what appears to be a breach of a basic logic of reaction kinetics. The fact that four sites are open in a macromolecule, such as haemoglobin, does not necessarily mean that the four sites can be filled simultaneously as this would mean that five entities must collide at the same instant in the reaction medium. This is what physical chemists call a reaction of order five. We know that the probability that five entities collide at a given point at the same instant in a reaction vessel to form a product or products is vanishingly small and we may in fact conclude that it does not exist; which is the only condition that the equilibrium constant for the first step can be equal to four times the intrinsic constant. An article, which took account of this inadequacy, was published by a Japanese team led by Tachiya

(1978). We followed in 1985 (Amire and Burrows, 1985) with an article on multiple equilibria in micelles and microemulsion. Later (Amire, 1994) the principle was used to develop a binding isotherm for the spontaneous self-assembly of surfactants unto a solid substrate. Permit me, Mr. Vice-Chancellor, at the risk of boring my audience, to consider the arguments that form the basis of the Tachiya equation.

(S2)
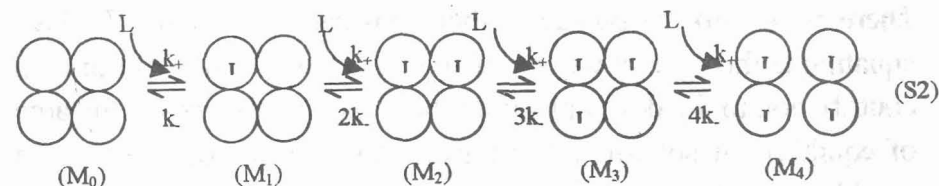
The notations in scheme S2 have the same definitions and significance as those used in scheme S1. The new notations k_+ and k_- represent the second-order rate constant for the combination of ligand, L to the macromolecule, M_o and the first-order rate constant for the dissociation of L from complex, M_1, respectively. The distinguishing feature of this model is that only one ligand can enter at a time and as many ligands as are already on the macromolecule can leave together at once; and the ratio of the forward and reverse processes in step one of S2 is equal to K_1, the step equilibrium constant for the first step in the multiple equilibria. The other step equilibrium constants K_2, K_3 and K_4 are related to the intrinsic constants by $k^2/1.2$, $k^3/1.2.3$ and $k^4/1.2.3.4$, respectively. No

physical chemistry law has been breached. From the relationships between the intrinsic and step equilibrium constants, expressions for the concentrations of the various forms of the macromolecule and ligand present in the solution were obtained using the law of mass action. The average number of ligands bound per macromolecule was found to be given by the Equation (6):

$$\bar{v} = k[L] \tag{6}$$

There is no time to pursue further considerations of the Tachiya equation at this time; it will be more instructive to consider our own contribution to the development of these binding theories in the area of equilibria at solid-liquid interface. An example of this type of equilibria is observed when molecules or ions deposit on a solid support (substrate). Suppose that we allow adsorption to take place and produce the aggregates shown in the Figure below:
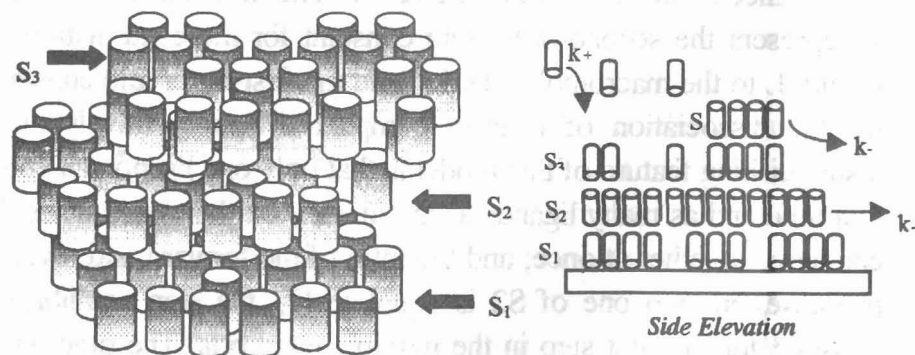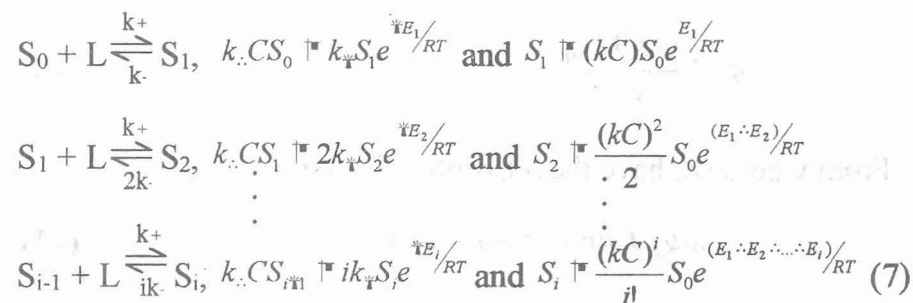

Side Elevation

Fig. 1

Let S_0, S_1, S_2, ..., S_i, ... represent the surface areas covered by only, 0, 1, 2, ..., i, ... layers of adsorbed amphiphiles. The addition of a single amphiphilic molecule will change the size of any one of the surfaces according to the equilibria in Equation (7). In these equilibria, E_i is the heat of adsorption of an adsorbent to the ith surface. It is necessary to modify our law of mass action as shown below because the adsorbent is entering a new phase and there is always a heat of adsorption due to change of phase.

$$S_0 + L \underset{k-}{\overset{k+}{\rightleftharpoons}} S_1, \quad k_- C S_0 = k_+ S_1 e^{E_1/RT} \text{ and } S_1 = (kC) S_0 e^{E_1/RT}$$

$$S_1 + L \underset{2k-}{\overset{k+}{\rightleftharpoons}} S_2, \quad k_- C S_1 = 2k_+ S_2 e^{E_2/RT} \text{ and } S_2 = \frac{(kC)^2}{2} S_0 e^{(E_1 + E_2)/RT}$$

$$S_{i-1} + L \underset{ik-}{\overset{k+}{\rightleftharpoons}} S_i, \quad k_- C S_{i-1} = ik_+ S_i e^{E_i/RT} \text{ and } S_i = \frac{(kC)^i}{i!} S_0 e^{(E_1 + E_2 + \cdots + E_i)/RT} \tag{7}$$

This is to be compared with crystallization: as a molecule enters into the crystal lattice it loses its kinetic energy as heat of fusion, E_f. From the above equations, we obtained an expression for the surface area covered by a section of the film containing i layers, as given in Equation (8):

$$S_i = bS_0 \frac{(k_0 C)^i}{i!} \tag{8}$$

where k_o is the observed equilibrium constant given by

$k_0 \, \vdash \, k \exp[E_f/RT]$, $\quad k \, \vdash \, \dfrac{k_{\cdot\cdot}}{k\text{\TeX}}$ is an intrinsic constant and $\beta \, \vdash \, \exp[(E_1 \, \text{\TeX} E_f)/RT]$. The total adsorbent surface area, A is given by the summation expression in Equation (9):

$$A \, \vdash \, \overrightarrow{\bigstar}_{i \vdash 0} S_i \, \vdash \, bS_0 e^{k_0 C} \tag{9}$$

The fraction of the surface covered by m layers, q_m is given by:

$$q_m \, \vdash \, \frac{(k_o C)^m / m!}{e^{k_o C}} \tag{10}$$

From where we have the adsorption isotherm:

$$\ln q_m \, \text{\TeX} \, m \ln C \, \vdash \, \ln K_m \, \text{\TeX} k_0 C \tag{11}$$

where the step equilibrium constant for the formation of the mth layer, $K_m \, \vdash \, (k_0)^m / m!$. We have found that Equation (11) is the adsorption isotherm for the formation of multi-layer organic films on a solid substrate. The equation has also, in our latest manuscript, been shown to be applicable to the analysis of oxygenation equilibria in haemoglobin (Amire *et al*, 1999). This is why this presentation is titled "the equilibrium dynamics in life and conterminous processes". In the rest of this lecture, I will now attempt to show the experimental results obtained from careful application of these principles.

In the application to haemoglobin-oxygen equilibria, m was identified with the class of binding sites on the haemoglobin molecule and q_m with fractional saturation, $\overline{Y}$ to which it was rigorously shown to be proportional. A plot of the left-hand-side of Equation (11) against oxygen concentration is compared with the Hill's plot below.
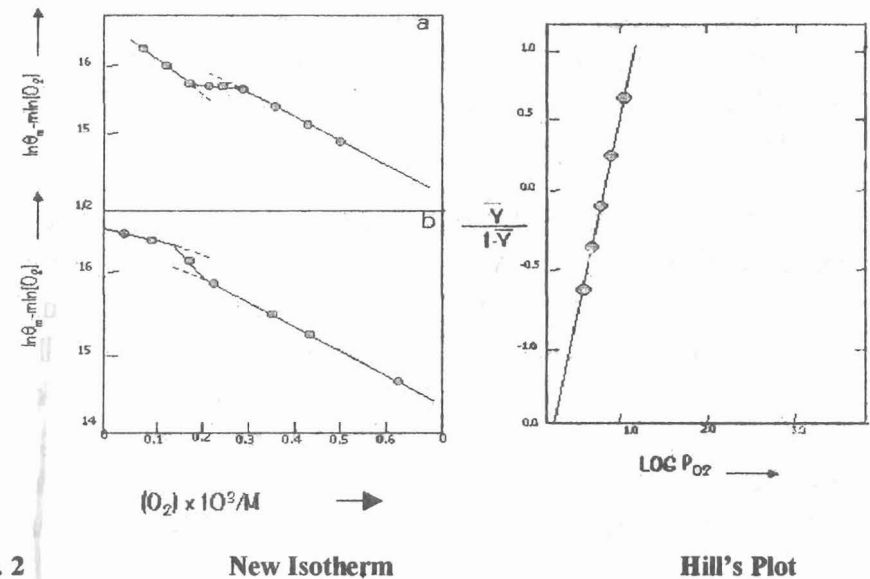

Fig. 2   New Isotherm   Hill's Plot

Hill's plot gives an average equilibrium constant, and consequently, the average affinity of the haems for oxygen, and the cooperativity of the haem groups. The new equation provides information about the number of class of binding sites. The high and

low oxygen affinity forms of haemoglobin have been known for a long time. As shown above, the high affinity form is obtained after the release of the first three oxygen molecules, that is, at about thirty percent oxygenation. In combination with calorimetric measurements, the method of analysis provides information on the heat of allosteric transition. From the heat of allosteric transition the heat of formation of what has hitherto been called salt-bridges was calculated and found to be about $\mp 32.4\,kJmol^{-1}$, a value which is comparable with hydrogen bond energy of between ∓ 33 and $\mp 40\,kJmol^{-1}$; suggesting that salt-bridges may actually be hydrogen bonds.

In their efforts to understand how a single change in the amino acid residue in each of the beta sub-units of the normal haemoglobin molecule could produce the sickle cell haemoglobin molecule whose oxygenation properties differ so drastically from that of the normal human haemoglobin, scientists looked for explanation in the heterotrophic interaction between haemoglobin S and the cell wall. In pursuance of this logic, we sought for mimetic cell membrane that could be used to test the hypothesis. In that

search, we stumbled on what is known as microemulsion. In apolar solutions, surfactant or surface active molecules may aggregate according to the following scheme:
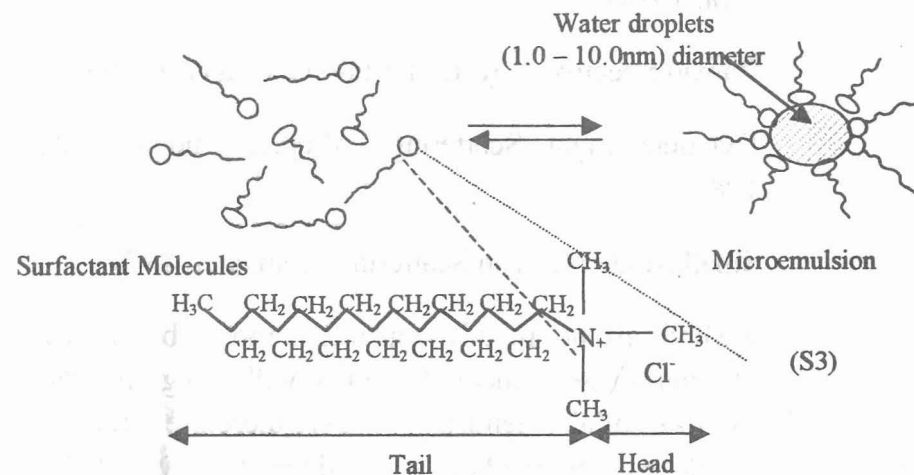
Microemulsion is prepared by dissolving dry surfactant into a given volume of dry apolar solvent, shaking to dissolve and introducing specified amount of polar solvent. The amount of polar solvent introduced determines what is known as water-to-surfactant molar ratio (R) which characterizes the microemulsion. The characteristics include the aggregation number, $\bar{n}$, the average number of surfactant molecules in each microemulsion aggregate, and the critical micelle concentration (CMC). The methods known

to have been used to determine the aggregation parameters include the following:

- ☐ Nuclear Magnetic Resonance Spectroscopy (Wong *et al*, 1978).

- ☐ Raman Spectroscopy (Amorin-da-Costa *et al*, 1981).

- ☐ Dynamic Light Scattering (Almgren and Loforth, 1981).

- ☐ Small Angle Neutron Scattering (Sein *et al*, 1979).

These methods are not readily available in most laboratories. We intended to introduce a method, which will bring micellar studies to the doorstep of all scientists. We were therefore forced to think of and introduce a new method, which depends only on U/V-visible spectrophotometer. In this method, microemulsion was made and a coloured solubilizate injected into it. Using various combinations of surfactant and probe concentrations, the principles outlined earlier in this lecture, and Lamber-Beer law, useful expressions were derived which gave the following results (Amire *et al*, 1992).
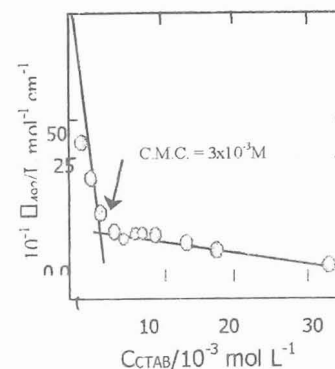
Fig 3. Plot of the apparent absorption coefficient of acridine orange against Total concentration of CTAB
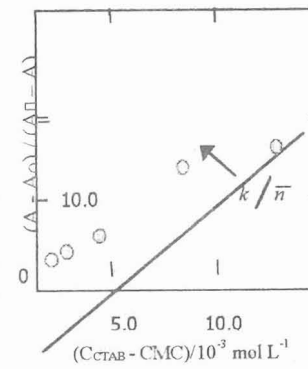
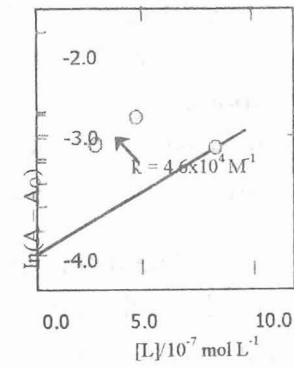Fig 4. Graph of (A - Ao) / (A□ – A) against (Cs – CMC). Temperature = 25.0 ± 0.1°C; the solvent is benzene and Chloroform (1:1)

Fig 5. Plot of ln(A – Ao) versus (L) . Temperature = 25.0 ± 0.1°C; solvent is benzene and Chloroform (1:1)

From these graphs, we obtained the CMC, $\bar{n}$ and the binding constant of probe to the microemulsion.

Adequate interpretations of many experimental results obtained in microemulsion systems, whether catalysis (Fendler and Fendler, 1975), solubilization (Shinoda *et al*, 1963) or even stepwise association reactions in general (Amire and Burrows, 1985), had been hindered by lack of accurate information about hydrogen ion concentration (pH) in the water micro-droplet. The question of pH measurements in the water pools of reverse micelles (microemulsion) has been addressed using several techniques. Menger and Yamada (1979) used a glass electrode to measure hydrogen ion activity. The main criticism of this method is that only

a small fraction of the solution (< 1%) is available to the electrode. This criticism is strengthened by the substantial variation in the pH reported for the same buffer solution under varying water-to-surfactant molar concentration ratios. Many other methods have similarly proved inadequate.

The main objective of the work to be described was to find a simple and direct method of determining the hydroxonium ion concentration in any part of the microemulsion water pool (Amire, 1988). The method of "corresponding solutions" first reported by Bjerrum (1944) appeared to us as the most suitable. The principle of this method involves finding solutions which without being identical in total concentrations, still have the same molar extinction coefficient at all wavelengths. Two solutions which meet this requirements are called "corresponding solutions". This principle was combined with that of multiple equilibria already discussed and Lambert-Beer law to obtain a final expression from which the fractional saturation of bromocresol green (BCG) with hydroxonium ion and free hydroxonium ion concentrations could be obtained. A Hill's plot of these parameters is shown in the next figure:
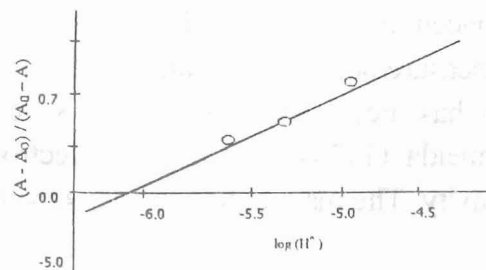

Fig 6. Graph of $(A - A_0) / (A_{\infty} - A)$ Against (C_a - CMC) Temperature = 25.0 ± 0.1°C; the solvent is benzene and Chloroform (1:1)

These set of experiments were performed for varying water-to-surfactant molar ratios, R and the data in Table 1 was obtained. The data suggests that as the water-to-surfactant molar ratios (R) increases (and consequently the dielectric constant of the core water increased) bromocresol green becomes more basic or less acidic, that is, the droplet size has large effects on the pK_a.

Table 1

The pK$_a$ Values of Bromocresol Green at Some Water Surfactant Molar Ratios in CTAB-Stabilized Without Microemulsion and Aqueous Medium (I=0.05 mol dm^{-3}, Temperature = 21.2 ± 0.1EC)

R (=[H$_2$O]/[CTAB])	pK_a^b	ΔpK_a^b
□	4.63	0.00
15.12	5.42	-0.79
12.06	6.35	-1.72
9.05	7.22	-2.59

[a] $\Delta pK_a^b = (pK_a$ in water $- pK_a$ in the micelle)

[b] Error in pK_a is □ ±0.05 pK_a units. $K_a = \dfrac{1}{K}$ (see definition of K in the text).

This, unlike other published methods, does not rest on any assumption. For the first time, the pH (hydrogen ion concentration) was measured for the interior of the microemulsion water droplet whose diameter is about 10nm.

Reverse micelles generally and microemulsion in particular, is one of the many ways surfactant molecules can aggregate in solution. Amphiphilic molecular aggregates in such systems, that is, micelles and microemulsions form lyotropic liquid crystals because their molecular order has been reduced by the solvent. There are some single-component aggregates called liquid crystals, liquids which have partial characteristics of crystals such as positional and orientational order. Liquids, apart from short range ordering, are positionally and orientationally disordered. Thus, liquid crystals are in between the two extremes of crystalline order and liquid disorder. The liquid crystalline state may be attained either by melting surfactant crystals and allowing them to gradually go into the molten liquid state and arrest the intermediate form between the two extremes or have a solid surface onto which surfactant molecules spontaneously assemble from solution; the resulting state which is generally referred to as thin liquid film is a form of lyotropic liquid crystalline state.

In microelectronics, conductivity is mainly by substances called semiconductors; which are substances such as silicon and germanium which have completely filled valence band and would be expected to be insulators. They do, however, conduct under certain conditions. Intrinsic semiconductors such as silicon or germanium conduct as the temperature is increased (Smart and Moore, 1992).
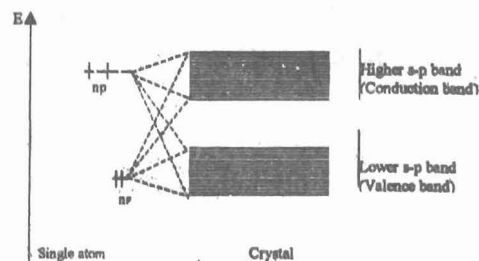
The current in semiconductors will depend on the number of electrons free to transport charge. This is the number of electrons promoted to the conduction band (the higher s/p band) plus the number in the valence band (lower s/p band) that have been freed to move by this promotion. As the temperature increases, the number of electrons promoted increases and so the current increases and this, of course, depends on the band-gap. Electrons can also be promoted by light. If the photon energy ($h\nu$) of light shining on a semi-conductor is greater than the energy of the band gap, then the valence electrons will be promoted to the conduction band and conductivity will increase; semiconductors with band gap energies corresponding to photons of visible light are called photoconductors, being essentially non-conducting in the dark but conducting electricity in the light.

The conductivity of semiconductors can also be increased by introducing a very low concentration of impurity known as dope to form extrinsic semiconductors: silicon doped with boron, an element one valency electron less than silicon, is called a p-type semiconductor. When silicon is doped with phosphorus, an element with one valency electron more than silicon, an n-type semiconductor is produced. It is electron rich. Silicon doped with boron is electron deficient because of the positive holes created by the boron atoms in the lattice. Thus, in the region of crystal where n- and p-type semiconductors meet, electron flow from the n-type to p-type semiconductors leaving positive holes behind in the n-type. At equilibrium, the concentration of electrons varies smoothly

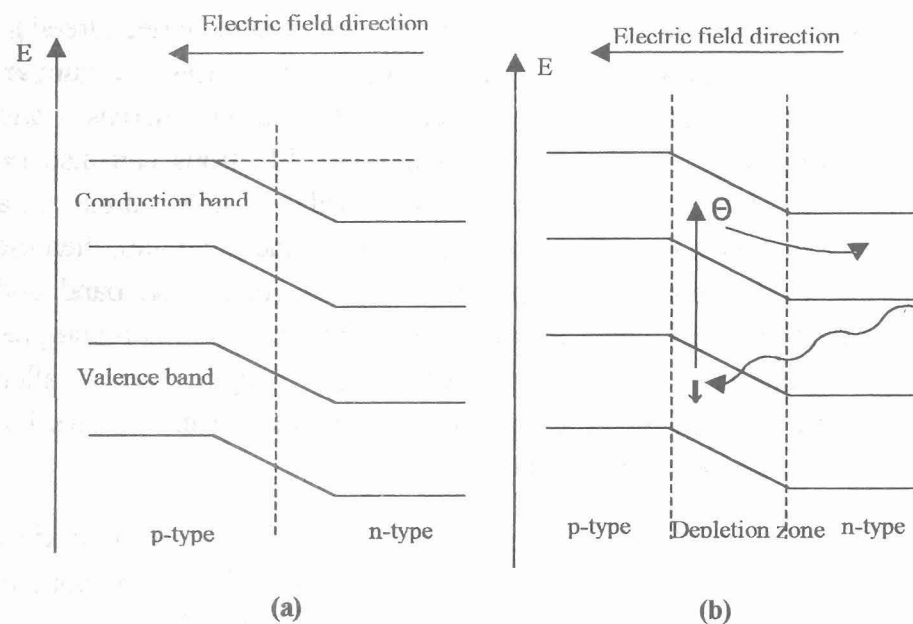across the junction in the dark. The use of p-n junction as a solar cell depends on its response to light.

Fig. 8(a) Bending of Energy Levels across a p-n junction and (b) the effect of light on a p-n junction

If the wavelength is short enough for the photon energy to be greater than the band gap energy, then electrons are promoted across the band gap to the conduction band. The promoted electron and the hole left behind are now separated in space and so the conduction electron cannot simply return to its hole in the valency band by emission of light, it travels through the n-type semiconductor to an external circuit to do some work and returns

through its valence band to its hole in the depletion zone. Thus, the illuminated p-n junction is acting as a battery with limited efficiency. This is the basis of many solar cells. The next set of experiments were prompted by the drive to find organic molecular equivalent(s) of the p-n type solar cell, and hence the study of thin organic liquid films on quartz slides. The structure of the donor-acceptor molecule, D(10)A, investigated at the Catholic University of Leuven, Belgium where this study was carried out is shown in the Figure below:
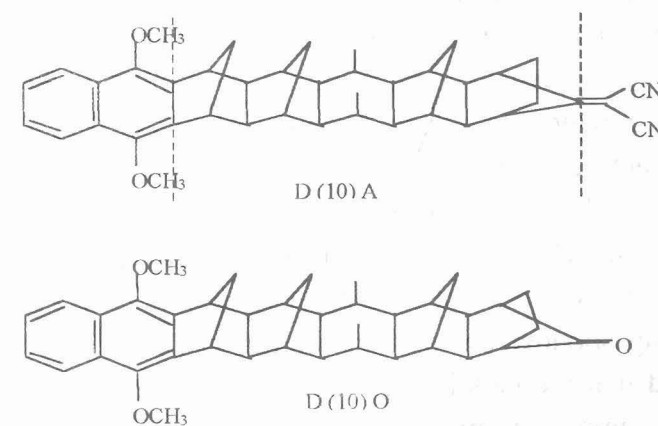

Fig. 9. Structures of donor-acceptor bridged molecule, D(10)A, and a reference compound D(10)O

Multi-layers of D(10)A and D(10)O analogue mixed with arachidic acid were compressed (with a circular trough of the

Fromherz type based on the Blodgett method) and deposited on quartz slides. The electronic absorption, static and time-resolved florescence spectra were recorded. The results showed that there were charge-transfer emissions from the electron-rich naphthalene moiety to the electron deficient dicyano group and there was none observed for the D(10)O films where oxygen replaced the dicyano group (Amire, 1996). This suggests that this type of molecules can be used in the same way as p-n junctions of semiconductors are used in solar cells.

The films of D(10)A molecules reported above were made in Belgium with an experimental setup called Langmuir-Blodgett trough. The potential application of the scientific finding in the Equatorial region to which Nigeria belongs makes this problem very attractive. Langmuir-Blodgett trough was unavailable in this University. This fact posed a new set of problems. Intensive literature search revealed that organic films could be made by spontaneous self adsorption method. Bigelow *et al* (1946) in their pioneering spontaneous self adsorption study from apolar solvents observed that monomolecular films of cholesterol were formed on platinum dippers from cyclohexyl solvent. Cleaned quartz slides were carefully lowered into a solution of 6-alkyl-2-naphthol (about 20ml) kept in a desiccator at a constant temperature for various periods of time (Amire *et al*, 1992). After equilibration, the slides were withdrawn with stainless steel tweezers and kept in empty weighing bottles with caps and stored in a desiccator for later use.

Limiting contact angles were measured on the films; these were recorded as advancing contact angles.

Table 2
Average[a] advancing contact angles for drops of triply-distilled water on 6-alkyl-2-naphthol films as a function of equilibration time

6-Tetradecyl-2-naphthol films		6-Hexadecyl-2-naphthol films	
Time/h	Average[a] contact angle, θ ($\pm$ 2)/$^\circ$	Time/h	Average[a] contact angle, θ ($\pm$ 2)/$^\circ$
16	61.0	8	58.1
24	61.1	16	58.0
48	61.3	24	58.7
62	61.8	48	58.1
110	61.7	66	59.1

Table 3
The thickness and the number of layers or 6-alkyl-2-naphthol films obtained after adsorption for 66h[a]

Compound	Film thickness/nm	Number of layers in film[b]
6-Tetradecyl-2-naphthol	143.2	52
6-Hexadecyl-2-naphthol	144.8	48

[a]Temperature $25 \pm 1^\circ$C

[b]Number of layers were calculated by assuming the structure shown in Figure 1 and using standard bond lengths and they were rounded off to the lowest whole numbers.

The results of the contact angle measurements suggest that there were films in which the surfactants are in a head-to-head tail-to-tail arrangements. The electronic and steady state spectra indicate that the multi-layer films contain close-packed platelets of 2-naphthols in sandwich arrangements. Ellipsometric measurements show that up to fifty-two layers of surfactant molecules were formed during a period of about one hundred and ten hours. We have, by the success of this experiment, established an alternative method of making well-organized multi-layer amphiphilic films on solid substrates. All we now need is to work on more samples and work out the mechanisms of the adsorption method.

One main impediment to the study of liquid thin films is that the process(es) involved in multi-layer adsorption is not well understood as theory lags behind experimentation. Langmuir adsorption isotherm developed in 1918 (Langmuir, 1918) for monolayer gas adsorption continued to be used in experimental analysis of multi-layer adsorption from solution. Benko *et al* (1979) used modified forms of Langmuir Equation.

In the earliest theory of multi-layer adsorption, de Boer and Zwikker (1929) proposed that adsorption of non-polar molecules unto ionic adsorbents is due to induced dipoles. The uppermost layer of the adsorbent induces dipoles in the first layer of adsorbed molecules which in turn induce dipoles in the next layer and so on until several layers are built-up. Brunauer, Emmett and Teller (BET) (1939) believed that the polarization of the second layer of

adsorbed gas by the first was too small to constitute the major portion of the binding energy between the adsorbed layers; particularly where such molecules do not possess permanent dipoles. It was proposed (Amire, 1994) that free-energy considerations, which account in large part for micellization and flocculation are also responsible for aggregation at solid-liquid interfaces. The expression developed from the consideration of the dynamics of multi-layer spontaneous self-adsorption of organic amphiphiles unto a solid adsorbent surface has been presented in the theoretical part of this lecture as Equation (11).
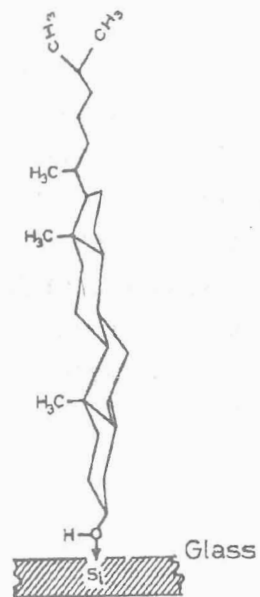
Fig. 10. Model Structure on which the unimolecular layer thickness of cholesterol was based. Bond-lengths are taken from various sources. The model structure is based on published Electron Micrographs of cholesterol in Langmuir-Blodgett films

Fig. 11. Plots of $(\ln q_i - i \ln C_{eq})$ versus C_{eq}. Aerosil substrate in, heptane: $C_{eq} = (0–3)\times 10^{-3}$ mol dm^{-3}: □-□-□-□ and, benzene: $C_{eq} = (1–3)\times 10^{-2}$ mol dm^{-3}: +-+-+-+. Graphitized furnace carbon black in heptane: $C_{eq} = (0–3)\times 10^{-3}$ mol dm^{-3}: o-o-o-o, and $(1–3)\times 10^{-2}$ mol dm^{-3}: □-□-□-□. Temperature = 293K. Alumina substrate in benzene: $(0–2)\times 10^{-3}$ mol dm^{-3}: □-□-□-□. Temperature = 293K. The lines were fitted with least-square method with correlation coefficients better than 0.967.

The equation was tested with the adsorption of cholesterol onto quartz slide (Figures 10 and 11) and various other mobile pieces, such as aerosol, graphiti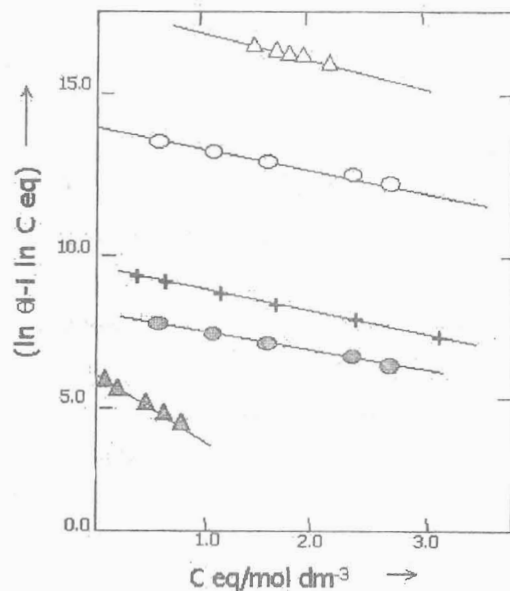zed furnace carbon black, and alumina; and steric acid on alumina. The plots in the Figure show beautiful linear fits that confirm the wide applicability of the new isotherm.

☐ Mr. Vice Chancellor, as you can see, I have developed a versatile adsorption equation, which seems to apply to all sorts of interfacial adsorption. The equations that were known before it were the gas monolayer adsorption equation by Langmuir and the BET theory of multi-layer gas adsorption, which do not represent adsorption from solution adequately.

☐ Also, we have developed spectrophotometric methods of determination of the aggregation parameters of reverse micelles and microemulsions. The main advantage of this method is that every scientist interested in this area of scientific endeavour can have access to it.

☐ I have developed a spectrophotometric method for determining the hydrogen ion activity or pH in microemulsion water droplets of the size of 100Å diameter (1Å is 10^{-8} cm).

☐ I have also worked on a molecule which can be used in making solar cells just as the p-n semiconductors, although we do not have the technical expertise to directly convert this to use now; this may become available in the near future.

☐ We have also developed the method of making multi-layer thin liquid films by spontaneous self-assembly method. This method allows large body application of thin liquid films.

☐ Work is continuing in our search for molecular explanation of the sickle-cell phenomenon. If we can identify the trigger substance, in these days of genetic engineering, hope is not lost for the more than 10% of the black race that suffer from this deadly disease, and such discovery will put us on the global map of eminent scientists. It is my hope that some day, and very soon, we will be there.

This, Mr. Vice-Chancellor, in summary, is my adventure so far into macromolecular chemistry. It has been challenging and frustrating as it has been exciting. It may be noted sir, that out of such frustration usually comes discoveries and advancement. As nature installs her barriers in the way of genuine discovery, a tenacious worker tries doggedly to remove them and as he does, he breaks new grounds and confirms the statement that at the end of a dark alley comes bright light.

In order to highlight the relevance and significance of our modest contributions at Ife in reference to global efforts, I refer to a recent exploratory paper published by James B. Beal (1996), an Electromagnetic Fields Interface consultant, he proposed "that the origins of terrestrial life may require the presence of electromagnetic field flux of different wave forms and intensities;

and the prevalence and interaction within the living cell, of a variety of biological liquid crystal forms which provide fundamental support for life and detection mechanisms to explain their quantum-level sensitivity. The lipid molecules of biological membranes exist in a liquid crystalline state and provide the matrix for membrane proteins to perform their function. Not only lipids but other major classes of compounds such as proteins, carbohydrates and nucleic acids exists in liquid crystalline phases under well defined conditions". Beal said that "it is therefore important to know the properties of liquid crystal materials in order to better understand biological processes". Beal noted further that "Natural electromagnetic fields associated with and transmitted from biosystems also provide survival factors in defense, attracting food and mates, and establishing territory". The author further said that "intriguing and controversial anomalous human electrical and electronic equipment interactions, if well understood, will lead to improved medical diagnostic devices, therapies for specific and whole body applications and monitoring of healing processes as influenced by mind and medicine". A good start the author claimed had been made "in two-dimensional brain electrical activity mapping of electroencephalographic signals and the more complex three dimensional brain magnetic activity mapping of magneto-encephalographic signals".

I have quoted extensively from this exploratory paper as it clearly defines the frontiers of knowledge and may be seen against our little contributions at Ife in the field of thin liquid films and

microemulsions. In the past thirty years or so, I have been led by my own intuition rather than the drive to excel in certain areas; it would appear that this is the best approach to research: if you like, call it spirit or nature led. This is why I would like to call on the authorities of our University, that is, the University Research Committee (URC) not to put too much emphasis on applied research as it amounts to putting ourselves in straight jackets. We should allow for maximum entropy in our research efforts as this amounts to allowing nature to move freely within us. Mr. Vice-Chancellor, already we have enough information in scientific literature to cater for the mundane needs of our country.

To demonstrate this, I want to show this audience a bottle of 96% alcohol, which I made from a still by the side of my house in the staff quarters. Surely, the information for making alcohol is available in our libraries, all we need to do is settle down and do something about our situation. The literatures are not covered by intellectual property rights because they are more than 50 years old. What seems to be holding the project is availability of clean Winchester bottles, at least on a small scale, the issue of expansion to industrial size is a future problem.

Research Institutes are the appropriate places for the conversion of knowledge into industrial outputs; and it is recommended that our government at the state and national levels should address national problems at these centres or create new ones for goal oriented research work as it is done in Germany. The

Institutes should be given projects of national significance and be mandated to build machines and plants to make specific substances of national need with specified time schedule. Subsequently, the plants so built can be sold to Nigerians interested in them. Government must identify national needs and rally scientists around them. Members of the academic communities who have knowledge of problems arising from these needs are made associate members of the Institutes. Thus, high quality research will continue in the Universities and national problems are solved at the same time in appropriate Institutes.

Mr. Chairman, I crave your indulgence to use the rest of this lecture to talk on urgent national issues; it is not for want of something to say, it is because unless the issues I want to raise are attended to our research efforts may be meaningless. Each of the selected papers summarized above is enough to engage this audience for one hour.

Within the last thirty years, extensive structural changes have occurred in our Universities and it is an act of patriotism to raise alarms at the debilitating state of our Universities in Nigeria today. Earlier this year, specifically on April 30 and May 1, 2001, I raised this alarm in *The Guardian Newspapers* for the second time, first internally as a submission to the visitation panel arising from the students' crisis that claimed some lives in this University in 1999 and the Guardian publication under reference was the second time. I will like to use this forum as the third time of raising the alarm

about the sorry state of our educational system, in general and the Universities in particular. I consider it necessary to repeat myself again in this forum to underline the importance that I attach to the University system as the fastest way forward for any nation. The title of the article is "Bantu education in Nigeria?"

What is the meaning of Bantu? Bantu is a Zulu word originally meaning, "people"; in South African vocabulary, it means "black people". Bantu Education means a separate system of education designed for the Black, Coloured and Indians under the apartheid regime of South Africa. Under the apartheid regime, there was no mixing of races in schools, but the apartheid government was concerned with more than physical separation. The Bantu Education Acts (of 1953 and 1954) were designed to bring all schools for Africans under the control and administration of a South African central government department where they could be starved of funding until they could eventually be abolished. Schools in private hands or churches had to register and the minister could, at any time, cancel the registration. Penalties were prescribed for running unregistered schools or permitting children to attend them.

The fundamental purpose of the Acts was to adapt Bantu Education to the role, which Bantu could be expected to play in a white country or world. Another purpose was to prevent Africans from becoming "black Englishmen". There would be less English and more Afrikaans, in the syllabus, social studies would be adapted accordingly, and there would be more emphasis on

practical subjects. The teachers would be under effective discipline, would be forbidden to comment adversely on government policies, and if dismissed would find no other employment, as there would be only one employer in their profession – government! All these features and more have been observed in Nigeria under various education ministers.

In 1976, the Federal Government of Nigeria took over the then University of Ife, ostensibly to allow the Western Region benefit from Federal funds as their counterparts in the other regions, namely, Ahmadu Bello University, Zaria, in the North and the University of Nigeria Nzukka, in the East. A decree was subsequently promulgated to back up the take over. The gigantic edifice built from the toil of the Western Nigeria farmers was now firmly in the kitty of the Federal Government with its assets and liabilities. My understanding was that all the investments and fixed assets of the University of Ife (OAU) in Britain were subsequently taken over by the National Universities Commission (NUC), a new organ of the Federal Government charged with the administration of Universities. All money budgeted for capital and recurrent expenditure in the Universities pass through the NUC, which paid subventions to the Universities according to its own guidelines, one of which is student population. Facilities in the Universities were consequently stretched beyond their elastic limits to accommodate more students and attract larger Federal funds from the NUC. The National Universities Commission (NUC) also issued guidelines on the Universities syllabi, which was called minimum standards. This

is currently in use in all the Federal and State Universities. Thus the autonomy of the University was totally wiped out.

Universities were no longer to conduct admission examinations individually, admission examinations are now to be centralized and conducted by a new institution, the Joint Admissions and Matriculations Board (JAMB), which prescribed the admission conditions and quotas and also ratified the admissions. No University can vary the admission policy; Ife's College of Health Sciences that hitherto conducted interviews for prospective students was forced to stop the practice by the JAMB.

New Universities began to spring up from all corners of Nigeria. Within a space of less than two decades Universities grew phenomenologically in number from five to about thirty-five (a factor of 7). In 1977, shortly after the takeover, the same Federal Government began to complain that there was too much waste in the Universities, started to cut subventions to them and stopped all physical developments. Construction of new hostel accommodations must stop. Students must henceforth be encouraged to live in off-campus accommodations "so that the students may live among those with whom they will eventually work", thereby denying them the civilization and culture that go with the University education. These measures went down to the states where the state governments manned by the military administrators took over all secondary and primary schools from their proprietors, the churches, without any compensation. The

governments at the state and federal levels proclaimed that all schools were free at all levels of education.

Some lecturers at Ibadan and Lagos were actually dismissed by a minister of education. One of those dismissed was Comrade Ola Oni, who died recently in Ibadan and another, Mr. Simeon Adu of the University of Lagos, father of the singer, Shade Adu, he died shortly after his dismissal because of shock.

As if Nigerians have not had enough, the ill-conceived 6-3-3-4 educational policy, a creation of Aliyu Babs Fafunwa, a professor of Education, was foisted on the Nigerian child. Every Nigerian child was thus made to believe that it is part of the education plan for it to attend a University and obtain a University degree. There is no civilized country in the World where more than ten percent of the population possess University degrees. Since the publication, the United States of America has announced that twenty four percent of her citizens possess "college" degrees, a rare feat indeed. Nevertheless, the target of many nations is ten percent. This is because education is about developing and maximizing the human potentials or put in another way, developing and maximizing our God given talents.

The introduction of a four-year University programme increased student population astronomically and more than doubled the recurrent expenditure in the University as the cost of running practical courses in the first year alone far outstrips what is needed

for the remaining three years. Because of the size of the classes and lack of corresponding physical expansion, the number of experiments conducted in these classes dropped from about 28 – 30 to 8 – 10 per session. Lately, the number of students in a group has risen from two in the nineties to six; and so many students stand by and watch. Thus, the practical content of our curriculum has decreased by a factor of about ten. Student accommodation problem was further compounded; rooms meant for two students now house between ten and twelve students. The social costs of these things are enormous.

Three important points emerge from our description of Bantu Education: over-centralization, starvation with funds and discipline of teachers. All these have happened in Nigeria; do we really intend a Bantu Education for our country? If not, we must look at the history of education in our nation and see how we can make amendments, stop experimenting with the lives of our children and make positive advancement.

In the early history of education in this country, students fit for University education were identified by their school certificate performance. Applicants with at least Division II in the West African School Certificate Examination were selected for University admission examinations. Usually everywhere in the world (except the communist states of the East, which is now of historical interest) there is a level of performance below which a person does not need to seek admission into a University. The minimum level of performance required in Great Britain and Ghana are the same.

In all these countries, there is no structural problem in their educational system. We should think seriously of reverting to this old system that worked well in the sixties and the early seventies. There is no Nigerian University. University is expected to have universal appeal. We must know that we can never expect the benefits of the University education such as technological advancement, if we fail to uphold the rules that govern Universities in technologically advanced communities around the world.

My suggestions are that:

☐ Privately owned Primary and Secondary Schools taken over by Government across the nation should be returned to their original owners. Government should give grants in aid where appropriate. Federal Government colleges nation-wide should now be strengthened and made to have Higher School Certificate sections. This will both be remedial and re-organizational. We must re-introduce classification into the school certificate results, that is, Division 1, 2, 3 and so on. We should revert to the University admission requirements of the sixties, that is, Division 1 or 2 at one sitting and a good pass at the concessional entrance examination, for admission to a four-year concessional University Degree programme for special students only. This

may be considered as a concessional parallel option to the normal three-year degree programme. The requirement for this is two or three Advanced Level subjects at a single sitting. This system is still practiced in Ghana. I know of many Nigerians that send their children to Ghana for education.

☐ The Government must change the thinking that the output of the secondary schools must go to the University, the so-called 6-3-3-4-education system. The Federal Government should realize that they must create jobs for school leavers or provide other low level avenues for technical, trade or other forms of training for Nigerians at an intermediate level. The education policy of the old Western Region under the leadership of Chief Obafemi Awolowo may be instructive in this regard.
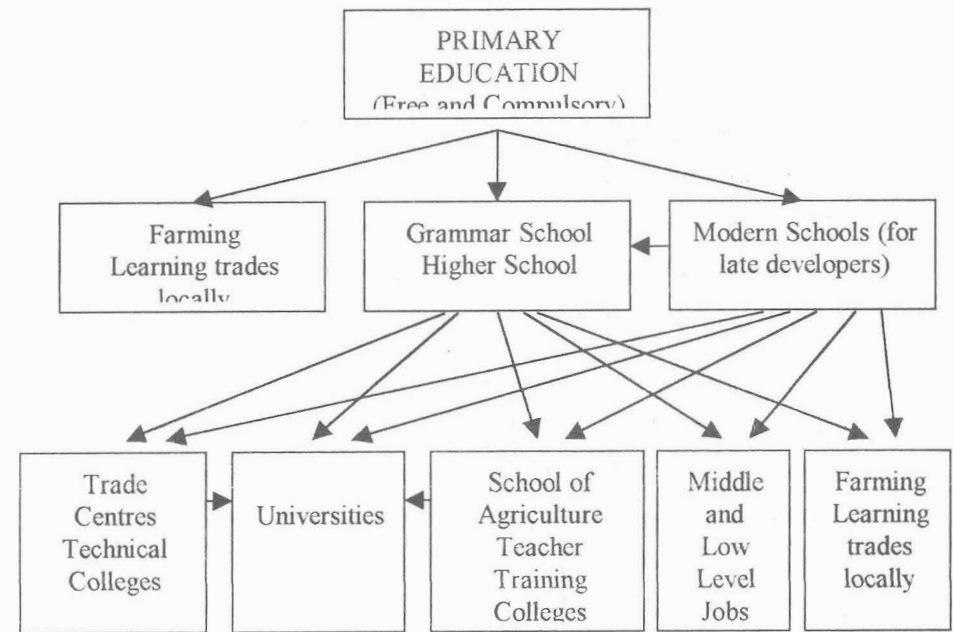

Fig. 12. The flow diagram shows the salient aspects of the Western Nigerian Educational Policy of 1955. Selection into these units was by keen competitive examinations. This is to allow for sorting into talent domains or what is called the natural attrition.

☐ A very important area to look into is the salary structure in the public service that discriminates against teachers. In 1981 when I was in Oregon State, USA, I discovered that the salary of a graduate teacher was 15,000 USD per annum while the salary of those in other jobs was 12,000 USD. That certainly makes teaching jobs attractive to the best calibre of

graduates. The converse is the case in Nigeria. The salary structure immediately after independence may have to be used as a frame of reference to adjust the newly introduced salary scales. Job evaluation and remuneration have become skewed in favour of those at the corridors of power since Independence. This can be seen from the salaries of political office holders and those in regular jobs as recently canvassed by various trade unions and in particular, Academic Staff Union of Universities (ASUU).

☐ The Federal Government has to make definite pronouncement on the level of Education that must be free and compulsory for all Nigerians. In doing this, age fifteen appears to be a good time to change carrier without trouble, and this should be taken into consideration. The current position of free education at all levels may be described, with due respect, as aimless and hypocritical, more so when the parents of the beneficiaries have been thrown out of their jobs. Of course, scholarships must be made available to needy students and possibly loans to not too bright students as is done in many third world nations. **Nobody who merits a university studentship must be denied it for lack of funds**.

☐ There are three basic necessities of life; they are food, shelter and clothing. These basic needs distinguish us from lower forms of animal. Of these three things none is guaranteed our

University undergraduates. Many of the students come to class in the morning without breakfast and sometimes they are not sure of what they will eat after the class. This is totally unacceptable. To compound the problems further, they do not know where they will sleep at night. Many of the students carry their backpacks and sleep wherever they find themselves! I have seen many students that sleep in lecture theatres! Clothing, though important, is minor and it is the least of the average student's problems. It is my humble opinion that government should pay attention to this accommodation problem urgently.

☐ We must dissolve those new structures such as NUC and JAMB and instead of NUC create a department of higher education in the presidency. The Minister of education should be made to take charge of Universal Basic Education and Federal Secondary Schools. This is what we have in many advanced countries of the world. **If these structures are to remain, their functions and objectives must be drastically reviewed to take account of the above observations**.

☐ The structural changes imposed on the School system have generated what social scientists call **systemic problems**, that is, disproportional allocation of human resources to one area of activity to the detriment of other areas. If there are teachers, there must be engineers, farmers, builders, etc. No

area of human endeavour must be allowed to suffer for want of gifted personnel. When I was putting this paper together, I read in the newspapers that Nigeria imports food to the tune of $2 billion a year! If we revert to those structures that produced first-rate scholars of today we will realize the University of our dream and wipe off systemic problems.

Mr. Vice-Chancellor, let me make one final comment; and it is about what is generally titled "intellectual property". The intellectual property of a people is the knowledge or knowledgeable people that belong to their society. From the beginning of our history the black race has never appreciated what is called intellectual property. Our history is replete with stories of how, during the slave trade, tribal chiefs sold out their brave young men and women for bottles of wine. I have carefully thought about how wasteful of intellectual property we have been in history. I think this same thought engaged the attention of our intellectual giant, Professor Wole Soyinka, when he said, "we are a wasted generation". We need to protect our intellectual properties. One economic adviser once said "Nigerians who travel abroad will earn the dollar and bring it home and so enrich our country monetarily"; forgetting that emigration of intellectuals permanently impoverishes a nation.

When Chief Obafemi Awolowo was alive he was styled an "unforgiving leader". Eventually, when he died he was described as "the best president Nigeria never had". Someone far away in

Britain said, "He could have been a good prime minister of Great Britain". All the same, he never won the favour of the British as a Nigerian leader, alive. This is how, through primitivity, we waste our intellectual property. An index of primitivity is selfishness and so a primitive society is where there are too many selfish people. We are so inundated with our selfish evil designs and forget to notice that the destiny of a nation is always tied to that of the individuals; the destiny of the Yoruba race to that of Moremi. Or how can a nation in a hurry to develop close the door of her House of Assembly against the best brains the world can be proud of? Yes, Nigerian university people are *primus inter peres*. Most American presidents are first-class brains.

☐ The House of Assembly in making a new electoral law should revert to the Independence Constitution, which allows University men and women who are interested in politics contest elections without resigning their appointments, and if they win, are allowed on leave of absence to serve the nation.

☐ **The Government should take the pain to catalogue Nigeria's intellectual properties and protect them, in and out of Nigeria, because such men and women are usually the targets of the rulers of the world of any age**. Examples are replete in history but an outstanding one is Marcus Mosiah Garvey, Jr. of Jamaica, a third-world country like Nigeria.

Finally, Mr. Vice Chancellor, distinguished ladies and gentlemen, I thank you for your attention.

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