

**RETROSPECTIVE STUDY ON THE ELECTROLYTES STATUS OF PUERPERAL WOMEN
WITH PERIPARTAL CARDIAC FAILURE IN NORTHERN NIGERIA**

By

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DECLARATION

I hereby declare that this thesis contains only the report of my research work and has not been previously presented for a higher degree. All information from other sources have been duly acknowledged by means of references.



Kasim Ibrahim Idrisa

10th DECEMBER, 2000
Date

APPROVAL

This thesis titled RETROSPECTIVE STUDY ON THE ELECTROLYTE STATUS OF PEURPERAL WOMEN WITH PERIPARTAL CARDIAC FAILURE (PPCF) IN NORTHERN NIGERIA by KASIM IBRAHIM IDRISA meets the requirement governing the award of the degree of Master of Science AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA and is approved for its contribution to knowledge and literary presentation.


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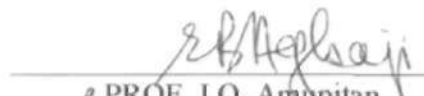
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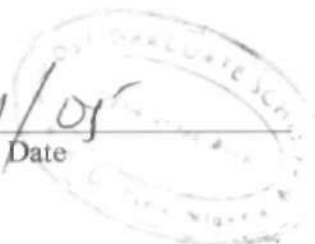
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DEDICATION

This work is dedicated to my parents,

Alh. Isah Magajin Mallam

and

Hajiya Ummul Salma I. Ibrahim.

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ABSTRACT

A detailed study protocol was produced and the protocol forms completed by the medical doctors who diagnosed and admitted the PPCF patients. The female wards of the Ahmadu Bello University Teaching Hospital, Zaria and Hajiya Gambo Sawaba General Hospital, Kofan Gayan, Zaria City, were used for sampling. A total of 14 patients from the two hospitals were used as source of blood samples. Also, 14 healthy puerperal women diagnosed to be PPCF free but of the same age group with the patients were employed to serve as the control subjects.

For each blood sample collected, the plasma was first separated from the red cells by centrifuging at 2500 rpm for 5 minutes. The plasma was then deproteinised using 2M nitric acid and the resulting mixture centrifuged at 3000 rpm for 10 minutes. The deproteinated plasma was then digested with nitric/sulphuric acids mixture before appropriate dilutions were made for onward quantification by AAS. A total of five electrolytes (sodium, potassium, calcium, sulphate and phosphate) were determined. Mutual interferences of the electrolytes of interest were also investigated and their correlations examined.

Sodium and sulphate were the only two electrolytes that proved statistically positive to play a vital role in the pathogenesis of PPCF in puerperal women in Northern Nigeria. Other electrolytes did not, although, a high level of calcium ion was observed in the blood plasma of the patients. Furthermore, the sodium heavy loading was found to be more acute in patients whose ages lie between 26 - 35 years. However, there was no bias on the basis of the consumption mode of the patients be it in terms of white "*kanwa*", red "*kanwa*" or those taking both white and red "*kanwa*" combined.

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ABBREVIATIONS AND FORMULAE

PPCF	-	Peripartal Cardiac Failure
Ppm	-	Parts per million
ml	-	Millilitre
mg/l	-	Milligram per litre
NS	-	Not significant
***	-	Very highly significant
**	-	Highly significant
LSD	-	Least square difference
DF	-	Degree of freedom
SS	-	Sum square
MS	-	Mean square
n	-	Number of patients in each column
r	-	Correlation coefficient
S/N	-	Serial number
ISE	-	Ion selective electrode
\bar{X}	-	Mean of a set of results
$t_{0.05}$	-	Tabulated T value at 95% confidence limit
CF	-	Correlation factor
X_T	-	Sum of readings in all the three strata
n_T	-	Number of subjects in all the three strata-42

$$LSD = t_{0.05} \sqrt{\frac{2 \text{ Error (ms)}}{n}}$$

$$r = \frac{n \sum xy - \sum x \sum y}{\sqrt{n \sum x^2 - (\sum x)^2 \times n \sum y^2 - (\sum y)^2}}$$

$$CF = \frac{(X_T)^2}{n_T}$$

CHAPTER ONE

INTRODUCTION

1.1 Medical Background of Peripartal Cardiac Failure (PPCF)

Traditionally, heart failure has been regarded as a disorder in which ventricles fail to pump adequate quantities of blood to meet the needs of peripheral organs. Therefore, for half a century, the physiological abnormalities of the disease have largely been described in haemodynamic terms (Parker, 1992). According to this model, heart failure follows an injury to the heart which impairs its ability to eject blood; renal blood flow is reduced and subsequent sodium retention leads to pulmonary and peripheral oedema (Parker, 1992). This focus on the haemodynamics led to widespread use of digitalis and diuretics in the treatment of the condition (Parker, 1992). However, heart failure is now thought of as a disorder of the circulation not merely a disease of the heart. Many patients have structural cardiac damage that adversely affects systolic or diastolic function but they do not have heart failure because compensatory mechanisms maintain cardiac output and peripheral perfusion (Parker, 1992). Since the response of the circulation to stress is governed by neurohormonal mechanisms in addition to haemodynamic factors, these compensatory processes cannot be understood in mechanical terms alone. Heart failure thus develops not when the heart is injured but when compensatory haemodynamic and neurohormonal mechanisms are overwhelmed or exhausted (Parker, 1992).

1.2 Background of the Study

All over the tropics there are patients whose cardiac failure cannot be ascribed to any obvious cause and some of these are women with symptoms

related to childbirth. These are the women described as having peripartur cardiac failure (PPCF)(Parry, 1994). The development of this type of heart failure after pregnancy in a previously healthy woman without any obvious cause is now a well-recognized, although rare, event, and it is generally accepted as a specific heart disease of the puerperium (Sanderson *et al.*, 1979).

Although occasional cases are also seen in temperate countries and some important series of cases have been reported in the USA almost exclusively among black women, the syndrome has been widely reported in the tropics (Davidson, 1979).

In northern Nigeria there is an elegant demonstration of the relationship between cultural practices and the disease, PPCF (Parry, 1994), and it is the intention of this research work to address the above assertion by looking at the concentration of certain electrolytes in the pathogenesis of the syndrome PPCF.

1.3 Postpartum Cardiac Failure and its Risk Factors

The definition of postpartum cardiac failure (PPCF) varies from place to place according to the interval between delivery and its onset. In this research we will adopt the following definition: it is a cardiac failure with symptoms beginning in pregnancy of up to six months duration, with no history of cardiac failure other than PPCF itself and with no discernible cause for cardiac failure other than anaemia or hypertension presumed to be acute (Brockington, 1971).

However, in some cases PPCF is limited to patients whose symptoms begin during the last months of pregnancy or in the first few months after delivery – the essential failure of PPCF is cardiac failure with no definable cause developing in a woman who is or was recently pregnant (Parry, 1994).

PPCF is a syndrome, which may conceal a number of conditions. Some risk factors may be important only in certain places while they are irrelevant in other places and the factors in northern Nigeria are apparently unique. The syndrome is primarily one of a dilated cardiomyopathy and therefore the factors in the pathogenesis of a dilated cardiomyopathy anywhere in the world is to be considered in PPCF (Parry, 1994). However, there are no data which link PPCF patients with a Particular genetic predisposition, which could affect the evolution of a viral infection, but there is unequivocal evidence of myocarditis, detected by endomyocardial biopsy in a high percentage of the cases reported in the USA (Parry, 1994). In Northern Nigeria there is an elegant demonstration of the relationship between cultural practices and the disease PPCF (Parry, 1994).

1.3.1 Cultural postpartum practice(s) in northern Nigeria

Heating: This commences immediately after a women deliver and continues for 40 to 120 days. The new mother takes two scalding hot baths each day, using a bundle of leaves to splash about 30 liters of very hot water on her body to keep out the cold (Davidson and Parry, 1979). Temperatures of 82⁰C have been measured immediately before the bathing is commenced and superficial burns are common. A young puerperal may well have to be forced to take her baths until she herself has accepted the importance. After taking the bath, the mother remain in a well- heated room with a fire or glowing embers underneath a specially constructed dried mud bed, which retains heat for several hours. Food is taken well cooked and highly peppered and all water is boiled before it is drunk. All these measures are attempts to prevent cold (sanyi) from entering the body (Davidson and Parry, 1979). Among Hausa women, cold is thought to be a common cause of illness and especially puerperal disease including swelling (Trevitt,

1973). Similar idea about cold and the need to roast the patient prevail in Malaysia (Wilson, 1973).

Drinking kununs kanwa: A special gruel or palp is prepared from guineacorn or millet with potash (kanwa) and pepper and is taken regularly as a medicine to increase the quantity and improve the quantity of breast milk. Potash is a misnomer for a dried lake- salt or natron which, according to Davidson et al., 1974 is largely hydrated sodium carbonate. However, it has a large sodium content but very little potassium and is also taken as medicine for all sorts of minor complaints in Northern Nigeria (Davidson et al., 1974). In the case of the puerperum, it is taken in very large quantities regularly for forty days or more (Buchanan and Pugh, 1969). The total amount taken varies greatly from women to women but is up to about 30g per day (Davidson et al., 1974).

The sodium loading caused by taking up to 30g of kanwa daily (equivalent to 450 mM of sodium) may well increase extracellular fluid and plasma volumes and thus lead to increased cardiac work (Davidson et al., 1974). The excessive heat loading from lying on a hot bed too is capable of increasing further the cardiac output and cardiac work (Parry, 1994). These two factors may together cause cardiac failure if the myocardium is for any reason vulnerable (Davidson et al., 1974). Therefore it is believed that the customs of Hausa women in Northern Nigeria are important in the pathogenesis of PPCF. Thus this present research shifts a little bit from the traditional way of looking at the syndrome and geared towards examining the role of electrolytes (Particularly Na^+ , K^+ , Ca^{2+} , So_4^{-2} and Po_4^{-3}) in the pathogenesis of PPCF rather than taking the aforementioned factors without substantial proof.

1.4 PPCF Mechanisms

1.4.1 Parry's hypothesis

It is established that the influencing factors of PPCF are postpartum state, hot season and hot beds and baths and ingestion of a sodium rich rock salt as stated earlier (Parry, 1994). The question now is how do these factors affect the function of the heart. According to (Parry, 1994), the heat causes vasodilatation so that the cardiac output rises in order to maintain flow and arterial pressure. The excessive sodium load, however, needs a high renal arterial pressure for its excretion (Sanderson et al., 1979). This renal arterial pressure can only be partially achieved by a further increase in cardiac output and blood pressure (Parry, 1994), oedema therefore, develops. The cycle is set for the PPCF to increase relentlessly, so that the syndrome with a high output state and vasodilatation, oedema and systemic and pulmonary venous congestion is established (Parry, 1994). Physiologically, PPCF is not a cardiac failure because the stroke volume can rise in response to a rise in filling pressure (Parry, 1994). In addition affected women respond quickly to diuretics although some with irreversibly dilated heart do not respond with diuretics immediately and have worse outcome (Parry, 1994).

1.4.2 Harrison's hypothesis

- (i) *Oedema in PPCF*: The consumption (postpartum) of large quantities of 'kanwa' by the puerperal women in Northern Nigeria could incite the oedema in PPCF which is due to the initial plasma volume contraction.
- (ii) *Geographical distribution of PPCF*: If the first hypothesis is correct, then geographical distribution of PPCF in Northern Nigeria is probably linked to the composition and quantity of *kanwa* consumed postpartum. The

incidence of the syndrome should therefore be higher in those localities where large quantities of *kanwa* with high soluble sulphate are consumed.

- (iii) *The parathyroid hormone hypothesis:* The relatively small but unknown proportion of women who are not able to manage the stress from the local postpartum practices noted in the first hypothesis are susceptible to the cardiomyopathy of PPCF perhaps because of moderate inefficiency in the parathyroid calcium metabolism.

1.5 Chemical Analyses and Pretreatment of Blood Samples

1.5.1 Deproteination

The direct determination of protein bound metals in blood samples is complicated by the high matrix effect of the proteins themselves. The nature of the interactions between blood and metals is the subject of much debate (Cernik, 1973) and numerous metals are present as complexes with blood components and to a lesser extent as the free ions. Release of the metals from such complexes requires either the use of chelating agents or destruction of the ligand. The usual procedure for destroying organic material in blood samples involves salting out of protein by alkaline earth metal salts and wet oxidation with mixtures of sulphuric and nitric acids (Cernik, 1973).

Carter (1980) described the use of low temperature ashing in an oxygen plasma although this procedure is time consuming. Various pressurized bomb digestion systems have been described but there is always an appreciable decomposition time and significant risk of contamination (Kaiser, 1978). Use of glassy carbon instead of traditional PTFE in the bomb material reduces contamination (Kolz, 1979).

Novel contamination free ultraviolet irradiation techniques were described by Battley and Farrar (1978) but these are even slower; the oxidation taking up to 12 hours. The use of acids is therefore preferred in metal analysis because of the availability of the acids in high purity grades. Ekanem *et al.* (1986) compared four acids (hydrochloric, nitric monochloroacetic and trichloroacetic acids) in terms of the viscosity (measured as nebulized uptake rate) and volume of the supernatant liquid produced and observed that nitric acid gave the largest supernatant liquid from blood serum/plasma. The nitric acid treatment gave a product with essentially the same uptake rate as the aqueous calibration standards whereas monochloroacetic and trichloroacetic acids gave products with remarkably high viscosity. It was also observed that the optimum concentration of nitric acid for sample preparation was 2.0M.

1.5.2 Analytical Methods – Principles

Atomic absorption can simply be defined as absorption of light by excited activated atoms. Such an absorption occurs over a very narrow spectral range, the so-called absorption line, the theoretical spectral width being of the order of 0.001 (Sobotka and Stewart, 1964). Absorption lines are entirely characteristic and specific for each element (Zettner and Seligson, 1964). If monochromatic light of a specific wavelength is provided, it will be absorbed only by atoms of that element for which that wavelength is characteristic and not by others (Zettner and Seligson, 1964). A field of atoms is opaque for monochromatic light when resonance line and source wavelength match, but for other wavelengths it is transparent. The degree of opacity is proportional to the total number of absorbing atoms. It follows then that with a beam of characteristic

Monochromatic light, the concentration of an element can be determined in a mixture of atomic species (Sobotka and Stewart, 1964).

Atomic absorption will take place only in a field of free, neutral, activated atoms. Atomic absorption cannot be brought about by ions, atoms bound in a compound or by a molecular gas (Zettner and Seligson, 1964). When metals are heated to their boiling points they vaporize as free atoms, provided that interaction with other species is prevented and it is for this reason that atomic absorption spectroscopy in its present form has found its most extensive applications in the analysis of the metallic elements (Sobotka and Stewart, 1964).

1.5.3 Methodology and principles of AAS

The technique of actually performing a measurement is simple and almost identical to that in flame emission photometry. A solution of the sample containing the test metal is aspirated into a flame. The degree of absorption, usually read out as % transmittance (T) but more conveniently as % absorption ($1-T$) is converted to absorbance or optical density ($2-\log T$) in the conventional way. Sample concentrations are obtained from interpolated readings of absorbance – concentration plots of working curves derived from appropriate standard solutions (Sobotka and Stewart, 1964). Even under the most ideal instrumental conditions such as low lamp current, homogenous flame, narrow slit width, optimal monochromatic efficiency, etc, analytical curves usually bend toward the concentration axis (Zettner and Seligson, 1964). The reasons for deviation from a straight line are complex and maximal correction of one contributing factor usually does not eliminate the curvature. The effects of Doppler broadening and self-reversal of the source line can be minimized running the lamp on a low current (Manzies, 1960). Flame inhomogeneities

Should be corrected wherever possible. These inhomogeneities cause variations in absorption in different parts of the elongated flame with regard to its horizontal as well as vertical extension (Manzies, 1960). This problem may be reduced by using fuel-rich flames in which the zone of maximum absorption is widened vertically and by focusing the light – beam onto the center of the flame (Zettner and Seligson, 1964).

While industrial or agricultural chemists, for the most part, have large samples available for analysis, the clinical chemist is faced with an ever increasing number of tests to be performed on a small single sample, for instance, a few millilitres of serum. The sample dilutions, aspiration rate and time of aspiration required to obtain one reading are some of the problems. These are in turn a function of sensitivity and instrumental stability. Since a few seconds (usually 10 – 30) of sample aspiration suffice for one reading, the total amount of samples sprayed usually is 1 – 5 ml (Zettner and Seligson, 1964). Due to their relatively high concentrations in biological samples, sodium, potassium and calcium require high dilutions and only small samples of the original material, the minimum size volumes rapidly and accurately (Zettner and Seligson, 1964). However iron, copper, zinc, manganese and other trace metals have to be determined on undiluted or even concentrated materials (Zettner and Seligson, 1964). In this case, removal of proteins, ashing or extraction of the analyte from the blood sample, will be part of the sample preparation for aspiration (Zettner and Seligson, 1964).

1.5.4 Interferences

Interferences can be defined as any physical or chemical agent capable of either increasing or decreasing the degree of absorption usually achieved with the test element in aqueous solution (Zettner and Seligson, 1964). All interferences in flame emission have one common effect, namely, changing the state or number of excited atoms. In atomic absorption, interferences can act in two ways: causing changes in the number of activated atoms in the ground state and attenuating the monitoring light beam by processes other than atomic absorption (Zettner and Seligson, 1964). Fortunately, the latter is rarely observed and it is actually the high transparency of flames over a wide spectral range that makes absorption measurement in flame photo feasible.

Spectral interference, a well known common difficulty in flame emission photometry, arises from excitation of other metals with emission lines or wavelength too close to be effectively separated from the line undergoing measurement (Manzies, 1960). A form of spectral interference encountered with hollow cathode tubes is the emission of a complex spectrum, from which it may be difficult to separate the resonance line (Manzies, 1960). This difficulty is usually circumvented by the use of high dispersion monochromators. Also light emitted from the flame itself is another form of interference. This is eliminated from measurement by the modulation of the resonance line source or by chopping of the light beam (Zettner and Seligson, 1964). The possible interference from atomic fluorescence should also be mentioned here. This effect, if significantly strong would be particularly difficult to exclude from measurement, if resonance fluorescence is involved. The latter not only possesses the same wavelength but also the modulation frequency of the hollow

cathode emission. However excitation interference appears to be entirely negligible in absorption, since the fraction of excited atoms of the total population in most flames is insignificantly small (Zettner and Seligson, 1964). Essentially, a significant reduction of the number of atoms in the ground state is possible through excessive ionization, as observed with more easily ionized metals (David, 1960a). The degree of ionization is exponentially dependent on the flame temperature (David, 1960b). Potassium, rubidium and calcium possess especially low ionization potentials and at the temperature of the commonly used air-acetylene flame, 30-70% of the total number of these atoms may be ionized (Dean, 1960). The degree of ionization of an alkali metal however, is reduced by the presence of other easily ionisable elements and consequently the admixture of such elements affords one means of controlling this type of interference (Foster, 1959).

Various interferences may arise from changes in the composition of the solution and flame matrices. The simplest matrix is encountered when an aqueous solution containing the analyte only is aspirated. In biological analysis this may very rarely be the case. Relatively large increases of the viscosity of the sample are necessary to lead to reduced vaporization, but the effect is usually combined with changes in the surface tension (Zettner and Seligson, 1964). Where discharged atomizers are used, the presence of acid and salts in the sample interferes with the evaporation of the aerosol. The mild depression of calcium absorption by sodium chloride in physiological concentrations may be related to this (Porter and Wyld, 1955). When solutions of high salt content (2% or higher) are aspirated, the salt particles formed from the aerosol are of sufficient size to pass through the flame without disintegrating. These particles

are capable of scattering the light from the hollow cathode tube which will slow up in the measurement erroneously (Willis, 1960a).

1.5.5 Interference control

There are several different possibilities to circumvent/compensate for or suppress interference.

Separation of the analyte from the solution matrix

This method has the attractive advantage that the test element can be isolated from all interferences at once. Although almost complete isolation is realized in the solvent extraction technique, it is often used only in those cases where complete isolation is unavoidable (Willis, 1960b). Separation by precipitation has been applied in sample preparations for atomic absorption spectroscopy (Zettner and Seligson, 1964), but the presence of an anion in the precipitate capable of binding the cation again poses the problem of anionic suppression (Willis, 1960b). It can be said that in general very accurate results are obtained with this method but where a great number of analyses are involved, it may prove too cumbersome.

Addition in excess

This method is extensively used in flame photometry and is based on the principle that interfering agents are added in equal amounts to both standards and samples. In practice, however, sensitivity is considerably curtailed and working curves appear to be less linear. However, small interferences like that of sodium on calcium absorption can easily be compensated for by this method (Zettner and Seligson, 1964).

Protective chelation

If a strong chelator is added to a sample containing the test element and an anionic depressor, the metal is preferentially bound by the former and thereby kept from interacting with the anions in solution or in the aerosol phase. This method was successfully applied in emission (West, 1960) and absorption (Willis, 1960b). It should be kept in mind however, that chelators themselves act as depressants.

There is considerable evidence that the concept of protective chelation may not be correct (West, 1960). For instance, greater concentrations of EDTA than unimolar between EDTA and calcium are needed for full protection (West, 1960). Furthermore, the action of EDTA is independent of the pH of the solution but dependent on its cation, the sodium salt being more active than the ammonium salt. Baker and Garton (1961) reasoned that the dry particles deriving from aerosol consist mainly of a matrix of EDTA in which calcium or other solution constituents are evenly dispersed. In the flame the organic matrix disintegrates rapidly, and when the metal is released the production of the metallic vapor is greatly accelerated by the high dispersal of the original particle. Thus, the effect of EDTA in abolishing phosphate depression would not be related to its chelating properties, but simply to the formation of a bulky matrix easily decomposed in the flame (Baker and Garton, 1961). This reasoning is supported by an earlier finding that with very fine aerosols the anionic depression is remarkably reduced (Alkemade, 1955).

Competitive cation technique

Mitchell and Robertson (1939) observed that the anionic depression of calcium emission in the flame could be abolished by the addition of strontium. Many other cations were also found to have similar effects. Lanthanum, neodymium, samarium, yttrium, magnesium, beryllium, barium, scandium, iron

and other cations are also capable of releasing calcium and other metals either completely or partially from the depressive effect of anions (Dean, 1960).

It is thought that the releasing or protecting mechanism depends on the competition between the cations for the anionic depressants during salt formation as the droplets of the aerosol evaporate (Alkemade, 1955). In order to suppress the anions completely and prevent them from interacting with the test element, the releasing cation has to be added in large excess over the concentration of both the metal to be determined and the interfering anion (Fukushima, 1960) since the depressive action of the anions does not originate from compound formation in the flame, but rather in the aerosol (Fukushima, 1960).

The protective action of competitive actions cannot be due to the refractoriness of their compounds in the flame, but must depend on other factors such as their concentration in the solution and the solubility of the salts they form with the depressant anions (Fukushima, 1960). For instance, if an excess of lanthanum chloride is used to control the depressive effect of phosphate on calcium, the drying salt particles forming from the aerosol will consist of lanthanum chloride, lanthanum phosphate and calcium chloride, the latter being readily dissociable in the flame. The true competitive nature of this phenomenon is discussed by Dean (1960).

The use of competitive cation for the control of anionic depressors appears to be the method of choice. No spectral interference arises in atomic absorption from the addition of another cation, an objection often raised in atomic emission. The concentrations of the added salt required for full anion control usually are less than 1%, a salt level below that at which light scattering is observed (Zettner and Seligson, 1964).

1.6 Aims and Objectives

We have seen in the introduction that there are basically two factors that can be primarily linked to the pathogenesis of PPCF. These include the excessive heat load from lying on hot bed and taking of hot baths and, the massive sodium loading by administering large quantities of 'kanwa'. However, this research work is centred on the role of electrolytes in the pathogenesis of PPCF with reference to specific electrolytes in the plasma. Thus, the objectives of this research include the following:

- (i) to ascertain the mean level of plasma ionized calcium, potassium, sodium; phosphate and, most importantly, the sulphate in the target population and compare that with the mean concentration in the healthy subjects (the controls);
- (ii) to further establish whether or not there is any association between plasma levels of the electrolytes in (i) above and the incidence of PPCF in the target population;
- (iii) to investigate whether or not the consumption of sulphate in the form of 'kunun kanwa' is a potential influencing factor in PPCF;
- (iv) to check the effect of age as a factor in PPCF;
- (v) to again investigate whether or not the consumption mode of 'kanwa' i.e. taking white only, red only or both white and red combined increases the risk of PPCF,
- (vi) to investigate the applicability of an indirect method of sulphate determination in body fluid (blood) using AAS;
- (vii) Finally, to check the interfering effects of selected ions in the determination of the electrolytes of interest using AAS.

CHAPTER TWO**LITERATURE REVIEW**

A very large volume of literature is available on the analyses of electrolytes in blood plasma. Most of the reports deal with the use of different analytical techniques. Prominent are the following: titrimetric, colometric/nephelometric, chromatographic (ion exchange) and electrochemical techniques. In recent times, ion selective electrode has become the method of choice. However, this review will be confined to essentially atomic absorption spectrophotometric analysis of calcium, potassium, sodium, phosphate and sulphate-ions.

2.1 Calcium

Calcium is one of the elements most frequently determined by atomic absorption spectrometry. The early work of David (1960) and Willis (1960 and 1961) used air-acetylene flame to determine calcium in soils, serum, urine and in plant samples respectively. The analysis can also be done in a nitrous oxide-acetylene flame with a characteristic concentration of 0.09 mg/l and has a detection limit of 0.001 mg/l. In the presence of high silicon and aluminium concentrations, no interference was observed in this flame whereas in the air-acetylene flame some interference was noted. The slight ionization observed was removed by the addition of small quantities of alkali metal. David (1960a) employed air-acetylene to determine calcium in plant materials. After wet-ashing of the samples, magnesium and sulphuric acid were added to overcome the effects of anions (such as phosphates, sulphate and silicates) and aluminium. The author further determined calcium in soils by

extraction with ammonium chloride but used strontium and lanthanum chloride to suppress the effect of phosphate, sulphate, silicate and aluminium.

Willis (1960b), studied the behaviour of calcium in oxygen-hydrogen and air-acetylene flames and found that the latter was the more suitable for absorption work. The author further investigated the effects of phosphate, sulphate and protein. Interference effects of these ions were suppressed by dilution of the sample with either strontium or lanthanum chloride or the disodium salt of EDTA. Although, the author concluded that good results could be obtained by all modes of sample preparation it was claimed that deproteination and dilution with strontium chloride gave the most accurate result.

Lethner (1964) conducted an extensive study of calcium interference deriving from serum constituents and other substances. In the air-acetylene flame, no effect was seen from excess concentration of the ions of potassium, ammonium, magnesium, chloride, bicarbonate and hydroxide. Phosphate, sulphate, oxalate and EDTA acted as strong anionic depressors. Sodium caused small but distinct depression of about 3%. The author also investigated the combined action of interfering agents. Mixtures of sulphates and phosphates showed an additive effect only at concentrations lower than those producing maximum depression, but the depression progressed with phosphate concentration.

Ramakrisma (1966) published a systematic study in which the effect of more than 50 ions on the absorption of calcium was investigated. Only two anions (bicarbonate and hydroxide) from the 20 investigated and 15 cations including magnesium from the 30 investigated were without any significant influence on the absorption of calcium. However, the author claimed that when

The work was carried out with a direct injection burner the interferences are much less. In a premix burner the interference of up to 50 mg/l silicon and 1000 mg/l aluminium or phosphorus was suppressed by the addition of 1% lanthanum chloride or disodium salt of EDTA.

Julio et al. (1978) described a method for the determination of total and unbound calcium concentration in volumes of about 20 to 30ml of physiological fluids. The technique involved the use of helium glow photometer (an original dialysis nanocell capable of processing volumes at nanolitre level). Lanthanum was used to displace calcium from its bound states. An equilibrium dialysis criterion were determined in cartilage fluid sample aspirated in vivo from the cartilage of the three different rats preparations as well as serum obtained from the correspondent animals.

Wisthorpe et al. (1978) measured plasma concentrations of calcium, proteins, phosphates and magnesium before and after cardiopulmonary bypass in 15 patients undergoing cardiac surgery. When calcium chloride was added to a pump priming solution which contained little or no blood, the concentrations of all calcium fractions were significantly greater after bypass than before bypass, with a mean ionized calcium concentration of 1.52 mmol/l plasma water, 30 minutes after completion of bypass. This iatrogenic hypercalcemia was increased significantly by the administration of more than 10 mg/kg calcium chloride in the first 30 minutes after bypass.

Fenukel and Earl Quarcov (1978) determined serum calcium, magnesium and inorganic phosphate concentrations in lactating Ghanaian women up to the 12th month after deliver. The mean serum calcium and magnesium were found to be

significantly lower than the corresponding means of the serum calcium and magnesium values for non-lactating women for whom these values were within the normal range. The changes in serum calcium concentrations were not accounted for by the levels of serum albumin. There was only a slightly significant reduction in the serum inorganic phosphate concentrations of the lactating women. Ion selective electrode (ISE) was the method the authors used.

Corrondo (1979) determined calcium in sewage sludge by dispensing a suspension in dilute nitric acid directly into the graphite furnace. The results were in good agreement with those from the flame technique and did not mention anything on interference.

In another separate development, Achilles and Cumme (1981) used ion exchange chromatography in conjunction with AAS to simultaneously determine free calcium and magnesium. In this method, calcium and magnesium were adsorbed from 1 ml samples of haemolysate, while blood or plasma was adsorbed on sulphuric acid activated polystyrenes by shaking the mixture for 15 to 20 minutes and diluting with 1 ml of 10 mM EDTA. 0.5 ml of the eluate was then diluted with 2 ml of water and calcium and magnesium were determined by AAS. Determination of 0.05 to 5.0 mmol calcium and magnesium were carried out in the presence of 100 to 200 mmol sodium and potassium ions and the coefficient of variation was 2 to 5%.

Demhordt (1981) worked on the continuous flow system for the determination of sodium, potassium and calcium ions in heparinized human blood by means of the ion selective electrode (ISE) method. Blood was withdrawn from peripheral vein at approximately 200 $\mu\text{l}/\text{min}$ and introduced immediately into the cell of the electrodes and the mixture was passed through the

Measurement cell, where contact was made with the three selective electrodes in succession. The emergent liquid made contact with the saturated calomel electrode (reference) electrode (SCE) and the respective readings were displaced on the digital voltmeters. This system also permits direct determination of changes in response to intravenous injections and during the course of operation. Excellent correlation ($r=0.988$) was obtained with flame photometric determinations.

Chopateau (1993) reported a new single-reagent colorimetric assay for the determination of calcium ion in serum. The assay was based on a novel chromogenic tetracarboxylic acid chelating agent. The results for 47 patients samples correlated well with the results obtained with an instrument laboratory AAS. The mean calcium values were 2.27, 0.06 mmol/l and 2.21, 0.05 mmol/l for the new and reference methods respectively. The on-system stability of the reagent was 1 week versus 8 hours for the o-cresol-phthalein complexone method.

Landt (1954) studied the effect of heparin anticoagulant preparation on ionized calcium in the whole blood by using ICA-1- analyzer (Radiometer – America). With lithium heparin, calcium ion concentrations were decreased by 0.7 mmol/l at 300 units/l of heparin and by greater amounts with increasing amounts of heparin. With zinc heparin the concentration of calcium ions was initially increased by 0.06 mmol levels of heparin. Use of heparin containing both lithium and zinc ions did not cause any interference in the determination of calcium ion or in the determination of total calcium.

2.2 Phosphate

Phosphate determination by atomic absorption spectrophotometry usually uses molybdenum hollow cathode lamp. David (1961) used the 312.92 nm line and obtained high sensitivity by the use of the said hollow cathode lamp, a long burner and the adjustment of air and acetylene pressures to ensure reducing conditions in the flame. However, molybdenum can be determined both in a fuel gas rich air-acetylene flame. At the 313 nm resonance line the characteristic concentration in air acetylene flame is about 1 mg/l, the detection limits are 0.1 mg/l and 0.3 mg/l respectively. Beside its high sensitivity, the nitrous oxide-acetylene flame is also largely free of interferences, so that it is used almost exclusively nowadays.

Roos (1969) found out that iron caused considerable interferences in the determination of phosphate using molybdenum lamp. However, Van Loon (1972) found that addition of 0.1 to 0.3% aluminum eliminated all interferences. In a related development, West (1973) accounted for this effect of aluminum as being due to an inhibition of the lateral diffusion of molybdenum leading to an increased atom concentration in the middle of the flame. Especially with organic solvents which gave considerable difficulties in the air acetylene flame, the hot nitrous oxide acetylene flames show its advantages.

Ram and Hamblg (1965) investigation showed that even under fuel gas rich conditions, the flame zone with the highest atom concentration was very limited, so that a large portion of the hollow cathode radiation passes through areas in which the formation of compound is possible.

In a detailed study on the determination of molybdenum in various flames Slurgeon (1967) found that the highest free atom concentration was always

located within a very narrow area in the flame. An adequate atom density was only found in strongly reducing flames; either a fuel gas rich air-acetylene flame or a nitrous oxide-acetylene flame. The author concluded that atomization was principally via the formation of MoO.

The possible interferences in the determination of molybdenum in fuel gas rich air-acetylene flames have been little investigated and matching of matrix is frequently necessary. A number of interferences occur in nitrous oxide-acetylene flame which can lead either to depression or enhancement of the signal.

Various complexing agents have been used by different authors for phosphate determination. Delaughter (1965) used toluene -3,4-diol as the complexing agent for extraction of phosphate into methyl isobutyl ketone (MIBK) before determination by AAS. However, Butler and Mathews (1966) used ammonium pyrrolidine dithiocarbonate of the complexing agent and also extracted into MIBK. In this technique, it was found necessary to mask iron with citric acid before the extraction. In a related development, Kirkbright (1966) complexed molybdenum with 8-hydroxyquinoline in the presence of fluoride and EDTA as masking agents and extracted it into butanol since butanol burns well in nitrous oxide-acetylene flame. On the other hand, Ure (1969) treated soil extracts with tri-N-Octylamine and 2% n-octanol in petrol and then back extracted the molybdenum with ammonia gas into the aqueous phase from which the determination was made with nitrous oxide acetylene flame.

Belle (1976) developed an automatic method for the determination of inorganic phosphate based on a reaction-complex formation with spectral shift between phosphomolybdate and the dye methyl green-oo. The advantages of the

method are its very high sensitivity when compared with the then existing methods, its extreme simplicity and very short reaction time (2.5 min) under relatively mild conditions. The hydrolysis of labile phosphates was found negligible. The reproducibility of the method is excellent (relative standard deviation 0.91%) as claimed by the author.

Peter *et al.* (1979) described a calorimetric assay for the determination of nanomole amounts of inorganic phosphate. The procedure combines a very high molar extinction with colour stability and insensitivity to newly released phosphate from labile organophosphate. This was achieved by introducing citrate into their mixture. The colour reaction was rendered insensitive to nascent phosphate, and hence became a stable and reliable measurement of extremely small amounts of inorganic phosphate in the sample.

Jakka and Reijo (1981) developed a new method for the determination of inorganic phosphate. Phosphomolybdate was measured colorimetrically without reduction to molybdenum blue, by dissolving the whole assay mixture in acetone, where phosphomolybdate is bright yellow. The hydrolysis of acid-labile phosphate (e.g. creatine phosphate) causes no problem, because the colour has been developed. Strong reductants and amino compounds interfere, if present in high concentrations. However, they can be eliminated by adding peroxide. Detergents, organic bases, protein and sucrose did not interfere. The assay was as sensitive as most modifications of the earlier method. In routine procedures, the useful range is 500 – 1500 nmol of inorganic phosphate. The application of the method to the assay of inorganic pyrophosphatase in the cells of *Escherichia coli* was also described.

Ignace (1983) developed a technique based on the formation of an insoluble rhodamine B-phosphomolybdate complex. After washing with 1M HCl, the precipitate was dissolved in acetone and rhodamine B was measured spectrophotometrically at 555 nm. In 1M HCl, the complex was composed of 3 molecules rhodamine B and 1 molecule phosphomolybdate. Due to the high molar absorbance of rhodamine B in acetone and the three fold amplification of dye concentration compared to inorganic phosphate concentration in the precipitated complex, a high molar extinction coefficient was obtained. This allows the determination of quantities as low as 1.5 nmol inorganic phosphate with good precision. However, the author did not specify the percentage precision.

Jefrey and David (1983) carried out a retrospective study of 120 patients with surgically proved primary hyperparathyroidism. This number included 71 patients surgically proved to be normotensive and 49 patients were hypertensive at the time of parathyroidectomy or had a history of hypertension. However, a review of this data obtained and related studies lead to a conclusion that the hypertension of hyperparathyroidism is heterogeneous in origin. The mean serum phosphate level in the hypertensive patients was significantly lower than that in the normotensive patients (2.2 ± 0.06 mg/dl versus 2.69 ± 0.05 mg/dl) which may be due to a decrease in renal tubular phosphate reabsorption secondary to hypertension.

Chiyo *et al.* (1987) proposed a method for the determination of trace amounts of phosphate and arsenate in water. Molybdophosphate and molybdoarsenate-malachite Green aggregates, formed by reaction of a reagent consisting of a mixed solution of ammonium molybdate and malachite Green, were selectively collected on a nitrocellulose membrane filter (pore size 3 μ m)

and dissolved in methylcellulosol together with the membrane filter. The absorbance ($\lambda=627\text{nm}$) denoted $A(P+As)$ was proportional to the sum of concentration of phosphate and arsenate with molar absorptivity $2.7 \times 10^5 \text{ mol}^{-1} \text{ cm}^{-1}$. Thiosulfate, a reducing agent for arsenate was added to the water samples, and the absorbance of the solution, $A(P)$, was measured as described above. The absorbance corresponds to the concentration of phosphate alone. The difference $A(P+As) - A(P)$ then corresponds to arsenate concentration. This method made it possible to determine phosphate and arsenate at levels ranging from 0.3 to 150 ppb which is an improvement of the earlier method of rhodamine B (Ignace, 1983).

2.3 Sodium and Potassium

Sodium and potassium are routinely determined in simple flame photometry with sufficient sensitivities and precision with a detection limit that is 10 times higher than that of AAS. Nevertheless, AAS still offers certain advantages for these elements. Furthermore, AAS appears to be free of interferences in the trace range in the presence of complex matrices which can be of great importance in practice. In AAS, there are a number of less sensitive lines which make the determination of higher sodium and potassium concentrations possible without excessive dilution.

Early work with AAS for sodium determination by David (1960a) employed an air – acetylene flame for the determination of sodium in soil extracts. The detection limit was 0.06 ppm. Phosphate, aluminum, sulfate and silicate exceeding the sodium level 100 – 200 folds apparently did not interfere.

Malmstadt and Chambers (1960) using an air-propane flame, claimed to obtain an accuracy, by their standard addition method, in the 1- 100 ppm range

with their null point instrument (a modified design of AAS). However, neither the percentage accuracy nor the design of the null point instrument were described by the authors. They observed a small decrease of sodium absorption, which was absent in the case of potassium determination. The depression was also observed with high concentrations of hydrochloric and sulphuric acids. Samples and standards contained 25% isopropyl alcohol.

On the other hand, Robinson (1960) used oxy-hydrogen flame (against air acetylene and air propane used by earlier authors) and found that interference appeared to be less than in other flames as no interference was observed from a 100 – 500 fold excess of potassium or lithium.

Willis (1960a) employed a sodium hollow cathode lamp to determine sodium in blood serum. With a 10 cm burner, sensitivity was so high as to make the dilution (500-fold) impractical and subject to contamination. The author reduced the sensitivity simply by shortening the absorption path and this was obtained by rotating the flame 90°. It was also shown that the use of another much weaker line at 330.2 Å reduced dilution requirements to only 10 folds. The author claimed that no interferences were encountered from any of the serum constituents.

Jorgen (1972) described an automated ion selective electrode method employing "Technicon Auto Analyser System" for the determination of sodium and potassium in serum. A simple 2 points calibration procedure was used. With the use of a high ionic strength buffer, the electrode was made to read in concentration units (instead of activity). Sodium and potassium in serum were determined at rates of up to 120 samples/ hour using 80ml of the sample. There

was excellent agreement between the electrode and the flame values which clearly indicated that the flame can be successfully replaced by the electrode.

Uddin *et al.* (1977) compared the analysis of sodium and potassium by conventional flame photometry (Corning H43, Corning Instruments Medfield MA 02052) method with the ion selective electrode (Orion SS 30, Orion Bio Medical Cambridge MA 02132) in aqueous standards, commercial control sera, serum specimens and whole blood specimens. Aqueous standards were prepared from sodium and potassium chloride reference materials. Within run, precision was 1.0%, 1.4%, 0.4% for sodium and 0.4%, 2.0%, 1.4% for potassium in 8 analyses of aqueous standards, control material and whole blood respectively.

Doherty *et al.* (1979) used the sodium ligand 1, 1, 1-tris (1, (2-oxa-4-oxo-5'-aza-5'-methyl) dodecanyl) propane dissolved in 3-nitro-o-xylene containing a small amount of the lipophilic anion tetrachlorophenol borate as a liquid ion exchanger in sodium selective microelectrodes. The microelectrodes gave rapid, stable responses that were linear functions of the logarithm of sodium activity. They were tested under conditions approximating those to be expected in the interior, and the results indicated that they can be used to measure *in vivo* sodium activity without significant interference from intracellular potassium. In contrast to ion selective electrode, Gabor (1981) developed a direct potentiometry method based on dilution of serum as done in flame photometry and the results were identical with those of ISE.

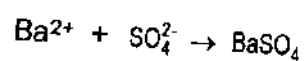
Cohen *et al.* (1982) measured sodium and potassium in serum, urine and red blood cells (RBC) in normotensive whites, normotensive blacks and severely hypertensive blacks with malignant hypertension. There were no important differences between the groups studied as regards the serum sodium, potassium and urinary sodium excretion values in contrast with earlier studies. However, the

urinary potassium excretion was significantly lower in normotensive and hypertensive blacks than in whites. The red blood sodium concentration showed no significant differences in the mean values across the range of degree of hypertension in blacks, although they tended to be higher in the more severely hypertensive group.

David *et al.* (1983) evaluated an accessory (no details of the accessory was given by the authors) that enabled the "Du Pont aca discrete analyser" to measure sodium and potassium by direct potentiometry. The authors claimed that sodium and potassium gave linear responses in both blood and urine modes with no carryover. No interfering species were identified in the blood mode. Intra-assay and inter-assay precision for sodium and potassium were more than adequate and comparable to those with flame photometry. The sodium and potassium values for plasma were comparable with those obtained by use of another direct potentiometric analyser (the Orion SS-30). Samples from patients with hyperlipemia and multiple myeloma gave clinically relevant values with the aca/ion selective electrode (ISE).

2.4 Sulphate

The entire principle of sulphate ion determination involves the precipitation of the sulphate as shown below:



Bertolacini (1957) worked on the colorimetric determination of sulphate using barium chloranilate. Solid barium chloranilate was reacted with sulphate at pH 4 in 50% ethyl alcohol solution to liberate a highly colored acid chloranilate ion. The absorption peak used was 530 m μ . The sensitivity of the method was 2

ppm of sulphate and the precision and accuracy were about 1%. Interfering cations were removed by ion exchange resins. Phosphate, oxalate, bicarbonate, chloride and nitrate did not interfere. The method was applied in the determination of sulphate in water and sulfur in petroleum products.

Bykova et al. (1966) worked on the complexometric determination of sulphate in coals. Sulphur was leached by water and subsequently precipitated with barium chloride. Excess barium was titrated with complexone III EDTA ($C_{10}H_{14}N_2Na_2O_2 \cdot 2H_2O$).

Toboleva and Kostikin (1967) improved this titrimetric procedure by using nitrosulphanazo III as indicator. The indicator which is bluish violet in colour in aqueous solutions turns greenish blue when the first excess of barium ion appears in the solution. The colour was quite distinct and the method was successfully used for titration of sulphate in industrial effluents and solutions. 0.5 mg of sulphate could be determined accurately by this method. The results obtained by this method and the usual gravimetric methods were identical.

In a related development, Lukin and Chermystsheva (1968) substituted the indicator nitrosulphanazo III with chlorophosphanazo III and the sulphate determined in 80% acetone at pH 1.3 with a solution of barium salt. Phosphate, arsenate, sodium, fluoride, ammonium, nitrate and sodium chloride did not interfere. The method was found most suitable for organic samples. Coada and Farmacia (1969) worked on the determination of sulphate in pharmaceuticals using thoron I (*o*-arsono - 1 - phenylazo - 2 - naphthol-3, 6- disulfonate disodium) as an indicator for the volumetric titration of the sulphate ion with an alcohol solution of barium chloride.

Hopin and Nancy (1970) used a turbidimetric method to determine soluble sulphate in water soluble colour additives. The color was adsorbed on activated carbon and the sulphate in the filtrate was precipitated as barium sulphate. The turbidity of the resulting suspension was measured spectrophotometrically at 440nm. The precision of this method was 0.10% in the range 0.1 – 0.25% sodium – sulphate.

On the other hand, Kadow et al. (1971) used AAS to determine sulphate in soil by the precipitation of the sulphate with barium ion and the subsequent determination of the excess barium in solution. An air – acetylene flame at 5536^oA barium line was used. 0.03M lanthanum was used to suppress the effects of aluminium and phosphate. Samples containing 6.26 – 304.7 mmol/l were recovered with a relative standard deviation of 36%.

Abdul- Waheed and Robert (1978) described a simple, rapid and sensitive procedure for the study of sulfatase kinetics using nonchromogenic substrates. The procedure utilized the dye potassium rhodizante which forms a pink coloured complex with barium ions indicates the quantity of sulphate. Some of the factors that could influence the pink colour were discussed and the optimum conditions for sulphate determination were also investigated. The assay procedure has good reproducibility and has a lower limit of detection of about 1 nmol of sulphate ion when 1.0m barium ion concentration was used.

Peter and Marjelyn (1983) described a method for the determination of inorganic sulphate, phosphate, nitrate and bromide in 100 ml serum. After a 10 fold dilution, the sample was directly injected into an ion chromatographic column without further pretreatment. The method the authors claimed was very accurate and reproducible over longer periods. The serum concentration of 4 of

the above mentioned anions was determined in 20 male and 20 female normal persons and found to be in agreement with results of earlier studies.

Pascoe et al. (1984) described the use of "Cabas bio centrifugal analyser" to carry out turbidimetry of inorganic sulphate, after precipitation with barium. Polyethylene glycol was used as the precipitate stabilizing agents. Reproducibility of precipitation was enhanced by the presence of barium sulphate particles which functioned as seed nuclei. There was no interference by normal or above normal concentration of phosphate, heparin, bilirubin, haemoglobin or erythrocyte contents or lipemia (triglyceride concentration up to 6.5 mmol/l).

Analytical recovery of added inorganic sulfate was found to be quantitative. Precision was similar to that for other methods for inorganic sulphate in plasma. The method is suitable for rapid, routine analysis of plasma inorganic sulphate and it is simple and less expensive to perform than alternative methods, but details of the analyser were not given.

Cole and Senver (1983) worked on the micro assay of inorganic sulphate in biological samples by continuous flow ion-exchange chromatography. In this method the sample of the blood serum and plasma were diluted with 1 mmol NaOH. Liver tissue was homogenized with 1 mmol NaOH, The mixture was then centrifuged at 2000 rpm for 10 minutes. The supernatant obtained was again centrifuged at 10,500 rpm for an hour and diluted 10 fold with 1 mmol NaOH. To separate the anions a pre-column (5 cm x 3mm) was connected-with a separator column (25 cm x 6 mm). The separator column was packed with Dionex anion ion-exchange resin while the pre-column had D-10 ion exchanger. The eluent (140 ml/h) was 2.4 mmol NaHCO₃ and conductance of the eluent was monitored. The recovery of added sulphate ion (0.1 to 0.5 mmol)

was 101.3% (n=9). Other anions such as succinate, malate, phosphate, and bromide did not interfere.

Panomareva (1974) described an indirect method of determining sulphate ion in natural water. The method involved the precipitation of sulphate with barium and the formation of a blue complex between unconsumed barium ion and nitrosulphanazo III indicator used. The colour developed instantaneously and remained stable for several days. The absorbance was universally proportional to sulphate contents in the range 0.1 to 2 mg in the 10 ml of the sample used. The sample was first passed through the cation exchanger before determination to remove interfering elements.

Parashar *et al.* (1994) described an indirect method for the determination of sulphate by AAS. The sulphate was precipitated with excess acidified barium chromate liberating an equivalent amount of chromic acid. The chromium was then determined by AAS after removing excess of barium chromate with lime. The method was highly selective and sensitive and was used in the determination of sulphate in high purity acids and antartic glacier samples. The sensitivity of the method was 0.1 ppm based on 1% absorption and Beer's law was obeyed up to 5 ppm of the sulphate concentration.

SAMPLING DESIGN, MATERIALS AND METHODS**3.1 Sampling Design****3.1.1 Target population**

The target population consisted of puerperal women who had been screened for a number of named medical conditions and lived in Zaria or its immediate environs. The sampling sites were two hospitals both located in different parts of Zaria. These were the Ahmadu Bello University Teaching Hospital (ABUTH) situated in Tudun Wada and the Kofan Gayan General hospital in Zaria city. Only the female wards were used in both hospitals. The sample consisted of 28 subjects divided into two basic strata with 14 subjects in each stratum.

3.1.2 The patient's stratum

These consisted of the patients which have been screened free of any heart failure except that due to PPCF. All the subjects in this stratum were also found to have some of the associated symptoms such as fever, cough and blue colouration of skin otherwise known as synosis. They also showed some of the following symptoms: palpitation, constipation, loss of appetite, nausea and indigestion.

3.1.3 The control stratum

This stratum consisted of healthy subjects whose ages are within child bearing and were actually nursing mothers. They were also screened free of any form of cardiac failure and related diseases of the heart including PPCF. They

were also found to be free of even the common diseases such as malaria and typhoid fever and were within the same social class with those patients in the first stratum.

3.2 Samples Collection

The patients and the control subjects whose blood samples were collected were all in a lying posture and must have fasted overnight or at least for 2 - 4 hours post prandial in the case of the control subjects before their blood samples were collected. The total volume of blood collected from each patient was 20 ml in 2 lots of 10 ml per lot. However for the control subjects only 10 ml of the blood sample was collected in a single lot.

For the patients, the first lot of 10 ml of the blood was collected at day 1 (pretreatment; that is when the patient had just been diagnosed and certified to have PPCF and admitted into the ward). The second lot of 10 ml of the blood was collected 72 hours (3 days) after the commencement of the treatment. Ordinary disposable 10 ml syringes with needles were used for the blood collection and the blood was usually collected by the medical doctor who diagnosed and subsequently admitted the patient.

The blood so collected was then carefully transferred into a special clinical tube otherwise known as vacutainer pretreated with lithium heparin to prevent the blood from coagulating (clotting). The vacutainer was gently shaken and then immediately placed in a vacuum flask containing a solution of sodium chloride at 4°C for onward delivery to the chemical pathology laboratory of ABUTH. On reaching the laboratory, the vacutainer was carefully removed from the flask and placed in a constant temperature water bath at 37°C for thirty minutes before the blood samples were spun in a centrifuge at 2500 rpm for 5 minutes to separate

the red cells from the plasma. The plasma so obtained was then carefully transferred into another clean vacutainer using a clinical pipette and then stored in a freezer in the same laboratory.

3.2.1 Sample Preparation

3.2.2 Deproteinisation of the plasma

All the plasma (samples) obtained from both patients and the control subjects were placed in a constant temperature water bath at 37°C for at least an hour to thaw. An equal volume of an already prepared and standardized 2 molar nitric acid was added on to the plasma (Ekanem *et al.*, 1986) to deproteinise the latter in the vacutainer. This precipitated the protein immediately, and the resulting mixture was spun at 3000 rpm for 10 minutes to separate the precipitate (protein) from the supernatant. The supernatant was then carefully collected using a clinical pipette and kept for further analysis. However, the resulting precipitates were discarded.

3.2.3 Test for protein in the deproteinised plasma

The supernatant collected was again mixed with a fresh portion of the nitric acid. Formation of another precipitate confirmed the existence of the protein in the supernatant. Thus, nitric acid was continuously added until a clear supernatant fluid was obtained.

3.2.4 Demineralisation of the deproteinised plasma

The 5 ml deproteinised samples obtained were then treated with a 10 ml volume of a mixture of nitric and sulphuric acids (in a volume ratio of 3:1 respectively) and gradually heated in a Kjeldahl flask using a heating mantle (Vogel, 1974). An average of not less than 8 hours was used to mineralize 5 ml of

the deproteinated plasma. The resultant demineralized samples obtained were properly labeled and stored in a refrigerator for further investigation. However, the demineralized samples could now be kept under ordinary laboratory condition.

3.3 Preparation of Standard Stock Solutions of the Elements

3.3.1 Sodium stock solution

About 3.500g of Analar grade sodium chloride was dried at 90°C for six hours and placed in desiccator to cool. From the dried-cooled sodium chloride, 2.520g was weighed and carefully transferred into a litre volume flask. The salt was dissolved with deionised water and made up to the mark. This prepared sodium chloride solution contained 1000 ppm sodium. From this stock solution a set of standard solutions containing 0.5 - 5.0 ppm of sodium was prepared by appropriate dilution. These were used to prepare the calibration curve. Prior to the determination, 1 ml of 0.2% lithium chloride was added to both the standards and the test solution.

3.3.2 Preparation of 0.2% lithium chloride

0.2000g of lithium chloride was weighed and dissolved with deionised water in 100 ml volumetric flask and the solution made up to the mark with more water.

3.4 Potassium Stock Solution

About 3.5000g of analytical grade potassium chloride was dried at 90°C for 6 hours and placed in a desiccator to cool. From this, 1.9090g was measured and dissolved with deionised water in a litre volumetric flask and the solution so obtained made up to the mark with more water. The stock solution contained 1000 ppm potassium. From the prepared stock solution, a set of standard

Solutions containing 0.5 - 5.0 ppm potassium were prepared by dilution. These were used to prepare the calibration curve which was used for the determination of potassium in the samples,

3.5 Calcium Determination

The determination of calcium using atomic absorption spectrophotometry is complicated because of the chemical interferences when air-acetylene flame is used. Therefore the use of releasing agents such as strontium chloride, lanthanum chloride or EDTA is necessary (Vogel, 1974). However, when the hotter nitrous oxide-acetylene flame is used the only significant interference arises from the ionization of calcium and under this condition an ionization buffer such as potassium chloride should be added to the test solutions. In the present work air-acetylene flame was used and strontium chloride was added to both the stock solutions and the samples.

3.5.1 Preparation of strontium chloride

3.8000g of analar grade strontium chloride was weighed and dissolved in a 500 ml volumetric flask with demineralised water and the solution made up to mark with the water. This solution had a concentration of 0.25%.

3.5.2 Calcium stock solution

About 3.500g of analar grade calcium carbonate was dried and allowed to cool in a desiccator. From this 2.4970g was accurately weighed and placed in a litre graduated flask. The salt was then dissolved with 50 ml of 1M hydrochloric acid. When the dissolution was completed with gradual heating, the resulting solution was diluted with deionised water to the 1000 cm³ mark. From this stock,

a set of standard solutions within the range of 0.5 - 5.0 ppm were obtained by appropriate dilution. These were used to prepare the calibration curve which was used for the determination of calcium in the samples.

Experimental parameters selected

Wavelength 422.7 nm; fuel-lean air-acetylene flame and calcium hollow cathode lamp. The measurement started with the blank then the standard solutions and the test solution coming last. All solutions contained 10 ml of the releasing agent solution.

3.6 Phosphate Determination

About 1.37250g potassium dihydrogen orthophosphate was accurately weighed, dissolved with deionised water and made up to the mark in a 1000 ml volumetric flask with more water. This solution contained 1,000 ppm phosphate. From this stock solution, a set of standard solutions were obtained by appropriate dilution. The working range of the standard solutions was within 0.5 - 5.0 ppm.

3.6.1 Preparation of ammonium molybdate solution

10.690g analar grade ammonium molybdate tetrahydrate was accurately weighed and transferred into a litre volumetric flask. It was then dissolved and made up to the mark with deionised water.

3.6.2 Procedure for phosphate determination

10 ml of each of the molybdate reagent and distilled water were transferred to a separatory funnel and sufficient hydrochloric acid added to make the solution 0.95M with respect to the hydrochloric acid. Then a standard phosphate solution was added to the mixture. After thorough mixing, it was allowed to stand for 5 minutes. 10

ml of isobutyl acetate solvent was added to the mixture in the separatory funnel. The mixture was shaken very well for a few minutes and allowed to stand so that the two phases separated properly. The lower aqueous layer was collected and discarded while the upper isobutyl acetate layer was aspirated into the air-acetylene flame for the molybdenum determination at 313.3 nm against a solvent blank of isobutyl acetate (Kirkbright, 1966). The procedure was repeated for all the standard and test solutions.

3.7 Sulphate Determination

3.7.1 Chromium stock

1.000g of a high purity chromium metal was weighed and placed in 1 litre volumetric flask. About 50 ml of concentrated hydrochloric acid and 2 ml of nitric acid were added on to the chromium metal and allowed to stand until the metal was completely dissolved. The solution contained, 1,000 ppm of chromium. From this stock a set of standard solutions within 0.5 - 5.0 ppm range of chromium were obtained by appropriate dilution. The readings were used to prepare the calibration curve.

3.7.2 Preparation of standard sulphate solution

0.6760g of analar grade anhydrous sodium sulphate was weighed and transferred into 1 litre volumetric flask. The salt was dissolved with deionised water and made up to the mark with more water. This stock solution contained 1,000 ppm sulphate. From the stock solution a set of standard solutions were obtained by appropriate dilution.

3.7.3 Standard barium chromate preparation

1.000g of analar grade barium chromate was dried at 110°C and 0.1267g was weighed and dissolved with 20 ml of hydrochloric acid 1M (1 mol dm⁻³) and diluted to the mark in a 1 litre volumetric flask with deionised water. The solution contained 1,000 ppm barium chromate.

Blank solution

This contained all the reagents except the sulphate solution.

Experimental parameters selected

- Wavelength = 357.87 nm; lamp current = 12 mA;
- slit width = 0.2 mm; air-acetylene flame; burner with an oxidant flow rate 3.51/min and the acetylene flow rate 1.01/min.

3.7.4 Procedure for sulphate determination

To 2.5 ml sulphate solution, about 10 ml of acidified barium chromate solution was added and the solution was allowed to stand for 15 minutes. Barium sulphate was then precipitated and equivalent amount of chromic acid was liberated. The excess barium chromate was removed by adding 0.2g of lime. The precipitate with the lime added was filtered using Whatman number 10 sponge filter paper and the supernatant collected and the volume made up to the mark with deionised water in a 50 ml volumetric flask. The supernatant solutions were then aspirated into the flame and the corresponding absorbance taken at the chromium wavelength. The concentration of sulphate in the blood plasma sample was measured using the earlier prepared calibration curve.

3.8 Preparation of Standard Ferric Chloride Solution

59.0% (w/v) ferric chloride solution was prepared by dissolving 59g FeCl₃ in water and diluting to 100 ml in a volumetric flask. This solution contained 59×10^4 ppm ferric chloride. 1.70 ml of this solution was diluted to 1 litre to give a solution containing 1000 ppm ferric chloride. From this stock solution subsequent dilutions were made to obtain the desired concentration of 0.5 ppm, 1.05 ppm, 52.5 ppm and 105 ppm in 100 ml.

3.8.1 Determination of potassium interference in sodium determination

1.5 ppm of sodium solution was obtained by appropriate dilution of the sodium stock solution. The sodium solution (1.5 ppm) so obtained was then doped with potassium solution of different concentrations (80 ppm; 160 ppm, 240 ppm, and 320 ppm). The corresponding absorbances of the different concentrations of the potassium containing 1.5 ppm sodium were measured and recorded in Table 9.

3.8.2 Determination of sodium interference in potassium determination

1.5 ppm of potassium solution was prepared by appropriate dilution of the potassium stock solution. The potassium solution so obtained was then doped with sodium solution of different concentrations: 62.5 ppm, 335 ppm and 650 ppm respectively. The corresponding absorbances of the resulting mixtures were measured and are included in Table 8.

3.8.3 Determination of phosphate interference in sulphate determination

1.5 ppm of sulphate solution was prepared by appropriate dilution of the sulphate stock solution. the sulphate solution obtained was then doped with phosphate solution of different concentrations: 15 ppm, 35 ppm, 175 ppm and 350

ppm respectively. The corresponding absorbances of the resulting mixtures were measured and recorded in Table 6.

3.8.4 Determination of iron interference in sulphate determination

1.5 ppm of sulphate solution was prepared by appropriate dilution of the sulphate stock. The sulphate solution obtained was then doped with the iron solution of different concentrations: 1.05 ppm, 52.5 ppm and 105 ppm respectively. The corresponding absorbances of the resulting mixtures were measured and recorded in Table 6.

3.8.5 Determination of calcium interference in sulphate determination

1.5 ppm sulphate solution prepared as described above was doped with 45 ppm; 98 ppm, 475 ppm and 1000 ppm Ca respectively. The corresponding absorbances of the resulting mixtures were measured and are included in Table 6.

3.8.6 Determination of sulphate interference in calcium determination

1.5 ppm of calcium solution was obtained by appropriate dilution of the calcium stock solution. The calcium solution so prepared was then doped with 9 ppm, 45 ppm, 90 ppm and 180 ppm sulphate respectively. The corresponding absorbances of the resulting mixtures were measured and the results are recorded in Table 7.

3.8.7 Determination of phosphate interference in calcium determination

1.5 ppm of calcium solution also obtained by appropriate dilution was doped separately with 15 ppm, 35 ppm, 175 ppm, and 350 ppm phosphate respectively. The corresponding absorbances of the resulting mixtures were measured and are included in Table 7.

3.8.8 Determination of calcium interference in phosphate determination

1.5 ppm phosphate solution was obtained by appropriate dilution of the phosphate stock solution. The phosphate solution prepared was then doped with 45 ppm, 98 ppm, 475 ppm, and 1000 ppm calcium respectively. The corresponding absorbances of the mixtures were then measured and recorded accordingly in Table 10.

3.8.9 Determination of sulphate interference in phosphate determination

The 1.5 ppm phosphate solution prepared was doped with 9 ppm, 45 ppm, and 180 ppm SO_4^{2-} respectively. The corresponding absorbances of the resulting mixtures were measured and are included in Table 10.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

Table 1: Plasma sodium concentration (ppm x 1.5 x 10⁴) in patients and control subjects.

S/No.	Before Therapy	After Therapy	Controls
1.	0.650	0.250	0.125
2.	0.650	0.325	0.200
3.	0.800	0.650	0.180
4.	0.575	0.525	0.200
5.	1.000	0.550	0.200
6.	0.750	0.575	0.125
7.	0.600	0.500	0.125
8.	0.775	0.125	0.200
9.	0.950	0.200	0.200
10.	0.875	0.275	0.200
11.	0.925	0.300	0.180
12.	1.200	0.280	0.200
13.	0.900	0.225	0.180
14.	1.200	0.250	0.200
Mean (\bar{x})	0.8464	0.359	0.1796

Table 2: Plasma sulphate concentration (ppm x 50) in patients and control subjects.

S/No.	Before Therapy	After Therapy	Controls
1.	2.9500	2.1750	2.0250
2.	2.7500	1.9750	2.1750
3.	2.7000	2.3500	2.1750
4.	2.6000	2.3500	2.2500
5.	2.6000	2.2500	2.1750
6.	2.6000	2.1750	2.1750
7.	2.5700	1.9750	2.1750
8.	2.9000	2.1750	2.1750
9.	2.7500	1.9250	2.1500
10.	2.9000	2.5500	2.1750
11.	3.3500	2.5250	2.1500
12.	3.1250	2.7500	2.1500
13.	3.3500	1.0000	2.2500
14.	2.9500	3.2500	1.7500
Mean (\bar{x})	2.8767	2.2446	2.1320

Table 3: Plasma calcium concentration (ppm x 10²) in patients and control subjects.

S/No.	Before Therapy	After Therapy	Controls
1.	0.8500	1.5000	0.8800
2.	1.6000	1.4900	1.0000
3.	2.0500	1.3000	0.4400
4.	1.3000	0.8000	0.4900
5.	1.5500	1.8000	0.5800
6.	1.2500	1.2500	0.4000
7.	1.1800	1.5000	0.5000
8.	2.9500	1.2000	0.1500
9.	2.9500	2.7500	0.4400
10.	1.2500	2.3000	0.3800
11.	1.4500	1.2000	0.3300
12.	2.7500	1.4500	0.4400
13.	1.6900	0.6000	0.4700
14.	2.2000	1.3500	0.3000
Mean (\bar{x})	1.7871	1.4636	0.4857

Table 4: Plasma potassium concentration (ppm $\times 5 \times 10^2$) in patients and control subjects.

S/No.	Before Therapy	After Therapy	Controls
1.	0.4900	0.3000	0.1500
2.	0.5250	0.2500	0.1000
3.	0.4500	0.4250	0.5000
4.	0.6250	0.3500	0.2500
5.	1.3500	0.4250	0.4500
6.	1.3500	0.4250	0.4500
7.	0.0750	0.3100	0.2500
8.	0.3750	0.2500	0.3700
9.	0.5500	0.5750	0.3000
10.	0.3500	0.5000	0.2500
11.	0.4250	0.3000	0.3000
12.	0.5500	0.3750	0.3500
13.	0.3250	0.3750	2.1000
14.	0.4750	0.2000	0.2500
Mean (\bar{x})	0.5011	0.3532	0.4268

Table 5: Plasma phosphate concentration (ppm x 10²) in patients and control subjects.

S/No.	Before Therapy	After Therapy	Controls
1.	0.7500	0.7000	0.7000
2.	0.7000	0.6500	0.7000
3.	0.7750	0.7000	0.7000
4.	0.7500	0.7000	0.7250
5.	0.700	0.7000	0.7000
6.	0.7500	0.7000	0.7000
7.	0.7750	0.7500	0.7500
8.	0.7000	0.7000	0.7000
9.	0.7500	0.7000	0.7500
10.	0.6800	0.7000	0.7250
11.	0.6500	0.6250	0.7000
12.	0.6800	0.6750	0.6750
13.	0.5000	0.4750	0.7250
14.	0.4750	0.4500	0.7500
Mean (\bar{x})	0.6882	0.6625	0.7143

Table 6: Absorbance of 1.5 ppm sulphate in the presence of the other analytes.

Concentration (ppm)	Absorbance	% Depression
1.5 SO ₄ ²⁻	1.5064	-
1.05 Fe ²⁺	1.175	78
52.5 Fe ²⁺	1.725	48
105.0 Fe ²⁺	0.258	17.2
45.0 Ca ²⁺	1.242	83
98.0 Ca ²⁺	1.217	81
475 Ca ²⁺	1.158	77
1000 Ca ²⁺	1.108	74
15 PO ₄ ³⁻	1.183	79
35 PO ₄ ³⁻	1.133	89
175 PO ₄ ³⁻	1.067	71
350 PO ₄ ³⁻	0.975	65

Table 7: Absorbance of 1.5 ppm calcium in the presence of the other analytes.

Concentration (ppm)	Absorbance	% Depression
1.5Ca ²⁺	1.506	-
9SO ₄ ²⁻	1.250	83
45 SO ₄ ²⁻	0.600	40
90 SO ₄ ²⁻	0.625	42
180 SO ₄ ²⁻	0.175	12
15PO ₄ ³⁻	1.025	68
35PO ₄ ³⁻	0.800	53
175PO ₄ ³⁻	0.558	37
350PO ₄ ³⁻	0.575	38

Table 8: Absorbance of 1.5 ppm potassium in the presence of the other analytes.

Concentration (ppm)	Absorbance	% Depression
1.5Na ⁺	1.5037	-
62.5Na ⁺	1.2330	82
325Na ⁺	1.2000	80
650Na ⁺	1.1500	77
1000Na ⁺	1.1500	77

Table 9: Absorbance of 1.5 ppm sodium in the presence of the other analytes.

Concentration (ppm)	Absorbance	% Depression
1.5Na ⁺	1.493	-
80K ⁺	0.433	29
160K ⁺	0.467	31
240K ⁺	0.525	35
320K ⁺	0.658	44

Table 10: Absorbance of 1.5 ppm phosphate in the presence of the other analytes.

Concentration (ppm)	Absorbance	% Depression
1.5 PO ₄ ³⁻	1.545	-
45Ca ²⁺	1.3750	89
98Ca ²⁺	1.2750	85
475Ca ²⁺	1.2500	83
1000Ca ²⁺	1.2500	83
9SO ₄ ²⁻	1.3250	88
45SO ₄ ²⁻	1.2500	83
90SO ₄ ²⁻	1.2500	83
180SO ₄ ²⁻	1.0500	70

Table 11: ANOVA table for sodium concentration (ppm) in the pretreated PPCF patients, treated PPCF patients and the control subjects.

Source	DF	SS	MS	F
Replication	13	0.2173	0.0167	0.6373NS
Strata	2	3.3329	1.6665	64.5930***
Error	26	0.6720	0.0258	
Total	41	4.2222		

LSD Strata = 0.1036.

Table 12: ANOVA table for sulphate concentration (ppm) in the pretreated patients, treated patients and their control subjects.

Source	DF	SS	MS	F
Replication	13	0.2644	0.0742	0.4469NS
Strata	2	4.4831	2.2416	13.5036***
Error	26	4.3152	0.1660	
Total	41			

LSD Strata = 0.2627.

Table 13: ANOVA table for calcium concentration in the pretreated PPCF patients, treated and the control subjects.

Source	DF	SS	MS	F
Replication	13	3.0104	0.2316	0.642NS
Strata	2	15.2370	7.6185	21,1331***
Error	26	9.3742	0.3605	
Total	41			

LSD Strata = 0.3872.

Table 14: ANOVA table for potassium concentration in the pretreated patients; treated and their control subjects.

Source	DF	SS	MS	F
Replication	13	1.0276	0.0790	0.7545NS
Strata	2	0.1531	0.0766	0.7316 NS
Error	26	2.7233	0.1047	
Total	41			

Table 15: ANOVA table for phosphate concentration in the pretreated patients, treated and their control subjects.

Source	DF	SS	MS	F
Replication	13	0.13193	0.01015	2.9420*
Strata	2	0.01878	0.00935	2.7101NS
Error	26	0.08960	0.00345	
Total	41			

LSD Replication = 0.0818.

Table 16: ANOVA table for the mean sodium based on whether the patients consumed only red *kunun kanwa* , white , or both white and red combined.

Source	DF	SS	MS	F
Replication	8	0.1226	0.0153	1.2541NS
Strata	2	0.2366	0.1183	9.696**
Error	16	0.1959	0.0122	
Total	26			

Table 17: ANOVA table for the mean sulphate concentration based on whether the patients consumed only red *kanwa*, white or both white and red.

Source	DF	SS	MS	F
Replication	8	0.0101	0.00125	0.0296NS
Strata	2	0.356	0.1780	4.218*
Error	16			
Total	26			

LSD Strata = 0.1691.

Table 18: ANOVA table for the mean calcium concentration based on whether the patients consumed only red *kanwa*, white or both white and red combined.

Source	DF	SS	MS	F
Replication	8	2.973	0.372	1.879NS
Strata	2	6.890	3.445	17.399***
Error	16	3.161	0.198	
Total	26			

LSD Strata = 0.3662.

Table 19: ANOVA table for the mean potassium concentration based on whether the patients consumed only red *kanwa*, white or both combined.

Source	DF	SS	MS	F
Replication	8	0.077	0.00963	0.629NS
Strata	2	0.285	0.1425	9.313***
Error	16	0.245	0.0153	
Total	26			

LSD Strata = 0.1018.

Table 20: ANOVA table for the mean phosphate concentration based on whether the patients consumed only red *kanwa*, white or both combined.

Source	DF	SS	MS	F
Replication	8	0.041	0.00513	1.125NS
Strata	2	0.0107	0.00535	1.173NS
Error	16	0.073	0.00456	
Total	26			

Table 21: ANOVA table for the mean sodium concentration of the pretreated patients based on their age groupings.

Source	DF	SS	MS	F
Replication	6	0.129	0.0215	1.693NS
Strata	2	0.196	0.0980	7.716***
Error	12	0.152	0.0127	
Total	20			

Table 22: ANOVA table for the mean sulphate concentration of the patients based on their age groupings.

Source	DF	SS	MS	F
Replication	6	0.097	0.0162	0.426NS
Strata	2	0.136	0.0680	1.789NS
Error	12	0.456	0.0380	
Total	20			

Table 23: ANOVA table for the mean calcium concentration of the pretreated patients based on their age groupings.

Source	DF	SS	MS	F
Replication	6	4.803	0.634	2.297Ns
Strata	2	0.154	0.077	0.278NS
Error	12	3.316	0.276	
Total	20			

Table 24: ANOVA table for the mean phosphate concentration of the patients based on their age groupings.

Source	DF	SS	MS	F
Replication	6	0.032	0.0053	0.109NS
Strata	2	0.052	0.0280	0.573NS
Error	12	0.586	0.0488	
Total	20			

Table 25: ANOVA table for the mean potassium concentration of the patients based on their age groupings.

Source	DF	SS	MS	F
Replication	6	0.157	0.0262	1.699NS
Strata	2	0.315	0.1575	1.021NS
Error	12			
Total	20			

Table 26: Table of electrolytes levels for normal Nigerian adults.

Electrolytes	Concentration (mmol/l)	Concentration (ppm)
Sodium	136 - 145	3125 - 3335
Potassium	3.6 - 5.0	140 - 195
Chloride	95 - 108	3325 - 3780
Bicarbonate	22 - 32	1342 - 1952
Calcium	2.25 - 2.75	90 - 110
Phosphate	0.8 - 1.9	76 - 1805

Table 27: Correlation coefficient (r) of different set of electrolytes

Electrolytes	Ca^{2+} / SO_4^{2-}	Ca^{2+} / PO_4^{3-}	Na^+ / SO_4^{2-}	Na^+ / PO_4^{3-}	K^+ / SO_4^{2-}	K^+ / PO_4^{3-}
Correlation coefficient (r)	-0.4656	0.0289	0.413	-0.603	-0.141	0.3418

4.2 Discussion of the Results

4.2.1 The Blood plasma concentration of the electrolytes in the patients and controls

Table 1 gives the plasma sodium concentration (ppm) in patients before therapy and after treatment and, their controls. The table reveals variation in the sodium concentration among the patients with the highest concentration being observed in the patients with serial numbers 12 and 14. The table also reveals that there are significant variations with respect to sodium concentration between the patients before therapy, after treatment and the control subjects – with the mean being highest in the first stratum and least in the controls stratum.

Table 2 shows the plasma sulphate concentration (ppm) in the patients before and after therapy and the controls. The table further shows that there are variations among the patients before therapy with the highest sulphate concentration (ppm) being observed in the patients with serial numbers 11 and 13. The table also reveals variations of sulphate concentration between the three strata with the mean level being highest in the pretreatment stratum.

Table 3 shows the plasma calcium concentration in the test patients and their controls. From the table there are variations among the patients with the highest plasma calcium concentration (ppm) found in the patients with serial numbers 8 and 9. The table also indicates variation in calcium concentration between the patients before therapy, after therapy and the controls with the average calcium concentration being highest in the patients before the commencement of treatment.

Table 4 gives the plasma potassium concentration (ppm) in all the three strata. The table reveals variation of potassium concentration among the patients within the same stratum with the highest level in patient with serial

number 5. Variations have also been noticed between the three strata with the average being highest in the first stratum (i.e. patients before therapy) and least in the patients after 72 hours of therapy.

Plasma phosphate concentration (ppm) are shown in Table 5. The table reveals variation of phosphate concentration among the patients with the highest concentration in patients with serial numbers 3 and 7. Variation was also observed between the three strata with the mean being highest in the patients before treatment and lowest in the patients after treatment.

4.2.2 Mutual interferences of the electrolytes of interest

Table 6 shows that iron causes slight interference during sulphate determination using the chromium lamp. This is because the percentage signal enhancement of sulphate was found to be less than what was expected in the absence of iron. The signal depression increased as the concentration of the iron increased. This observation has therefore confirmed that iron can cause a slight interference in sulphate determination using chromium cathode lamp. A similar observation was made by Plate (1965).

From Table 6 also, calcium was similarly observed to reduce the percentage signal enhancement of sulphate determination using the chromium cathode lamp. The table also reveals that the percentage signal depression of the sulphate concentration also decreases with increase in calcium concentration until when the concentration of calcium was 475 ppm in which the sulphate concentration appreciates again at 1000 ppm concentration of calcium.

Phosphate too has been observed to show this decreasing character of iron and calcium on sulphate determination as observed in Table 12. However,

contrary to what was observed for iron and calcium, phosphate did not obey the linear decreasing pattern but rather an erratic decrease.

Table 7 shows that during calcium determination using AAS both phosphate and sulphate anions have certain degrees of interfering effects with sulphate being the most serious of the two anions. Their interfering effects was also observed to increase with increasing concentration. A similar effect was observed by Ramakrishna (1966).

Table 8 shows that in the determination of potassium using AAS, the presence of sodium slightly reduces the signal enhancement as recorded in the literature. Again the literature has also shown that phosphate and other electrolytes considered in this work have noticeable interference on potassium determination using AAS (Lueke, 1975).

Sodium determination using AAS was found to be affected by the presence of potassium which has been observed as shown in Table 9 to reduce the absorbance signal in deviance to what was previously observed and reported in the literature. The table also reveals that this signal depression was observed to be increasing with the increase in potassium concentration. Thomson and Smith (1978) reported that heavy loading of other electrolytes such as calcium, magnesium, phosphate and sulphate do not affect the sodium absorption.

Table 10 indicates that in the AAS determination of phosphate using molybdenum cathode lamp, addition of calcium causes a slight signal depression with 1000 ppm of calcium producing up to 83% absorbance signal of the original phosphate concentration. The slight signal depression might be attributed to the interaction between calcium and phosphate prior to the phosphate determination. Sulphate also shows a similar behaviour of calcium ion with

phosphate; the reason for this slight depression may not be far from the fact that sulphate is in competition with the phosphate anion being SO_4^{2-} a divalent ion for the molybdate reagents added prior to the phosphate determination in AAS.

4.2.3 Significance of electrolytes levels in blood plasma of PPCF patients

Table 11 reveals that the mean sodium level in the blood plasma of the PPCF pretreated patients varies slightly significantly with respect to both treated patients and the control subjects. The table further reveals that the mean sodium level in the treated patients also varies significantly with respect to that of the control subjects. This observation has clearly shown that the 3 days duration for the collection of the second lot of the blood from the patients is enough time for the patients to recover from the effect of the increased sodium level. It could also be explained on the basis of the effectiveness of the drugs administered to the patients which are usually diuretics. The result has also agreed with the research hypothesis that the postpartum consumption of large quantities of 'kanwa' in the form of 'kunun kanwa' by the puerperal women in northern Nigeria could incite the oedema in PPCF. This is also in agreement with the result of 'kanwa' analysis carried out by Ekanem (1977) which showed that the single most important electrolyte in 'kanwa' is sodium sulphate. The latter salt is known to contract extracellular fluid volume (Sullivan, 1961). One of the immediate consequences of this contraction is the drop in cardiac output which would not have been observed in the reported cases from northern Nigeria based on the medical hypothesis of the syndrome. As expected, it is the response of the kidneys to the contraction of plasma volume that leads to salt and fluid retention and eventually oedema. Based on this observation, one can then draw conclusion that contrary

to the medical explanation that the syndrome, PPCF, is due to hot season, hot baths and beds (Parry, 1994) the result here has indicated that the ingestion of 'kanwa' leads to an initial plasma contraction preceding oedema and the compliance of the vascular system is assumed to have increased due to pregnancy and again that exposure to external heat can only be an aggravating factor. Therefore, sodium can then be said to play a vital role in the pathogenesis of the PPCF syndrome.

Table 12 clearly indicates that the mean sulphate anion concentration in the blood plasma of the pretreated patients varied significantly from that of both the treated patients and the control subjects. The table further reveals that the mean sulphate level in the plasma of the treated patients did not vary significantly from that of the control subjects. This result suggests that the three days follow up period for the collection of the second lot of the blood is not long enough for the patients to respond to the drugs administered. The first observation is in agreement with the research hypothesis on the postpartum consumption of large quantities of 'kanwa' as explained earlier for the case of sodium. This has only further confirmed the result of Ekanem (1977) on 'kanwa' analysis and agreed with the hypothesis that the consumption of 'kanwa' by the puerperal women in northern Nigeria is the genesis of the plasma volume contraction preceding oedema and not as suggested earlier medically or as explained by Parry *et al.* at different times. Consequently, this research can be said to suggest that sulphate ion can play a vital role in the pathogenesis of the syndrome, PPCF, in Northern Nigeria.

Table 13 clearly shows that the mean calcium level in the blood plasma of the patients before and after therapy did not vary significantly but both varied

significantly with respect to the control subjects. The two explanations that could be offered for these observations are whether the follow-up period of 3 days between the first lot of blood collection and the second lot is not long enough for the calcium level in plasma to fall or the diuretics administered were not effective enough to decrease the calcium level within the first three days of the commencement of the treatment. The first observation is in deviance from the earlier proposed hypothesis of the parathyroid hormone which suggested that the relatively small proportion of women who are not able to manage the stresses from the local postpartum practices noted earlier are susceptible to the cardiomyopathy of PPCF perhaps because of moderate inefficiency in the parathyroid/metabolite calcium metabolism. This assumption readily accounts for the association of PPCF with pregnancy and the postpartum periods during which plasma calcium normally falls. Fall in plasma ionized calcium could provoke hormocalcemic or hypocalcemic secondary hyperthyroidism (HPSH) (Partiff and Klecrekoper, 1980). The consumption of excess sulphate is another favourable condition for HPSH since it is expected to reduce ionized calcium level in plasma. This parathyroid hormone assumed did not imply the presentation of a clinical hormone deficiency. However, there could be the objection that at the clinical level, the hypocalcemia which the parathyroid anomalies may lead to, rarely has an effect on the heart muscle. This might indeed be the case but there are nevertheless documented cases of chronic hypocalcemia leading to heart failure. Based on this explanation and the results obtained (Table 3), one can then draw a conclusion that the high calcium level in the PPCF patients is not derived from 'kanwa' ingestion but could possibly be from other sources of the patients dietary which is in agreement with the initial 'kanwa' analysis reported

by Ekanem (1977). The explanation further indicates that the level of free ionized calcium cannot be used in the pathogenesis of the syndrome, PPCF, at least as far as the result of this investigation is concerned.

Table 14 shows that there is not a significant variation in the concentration of potassium in the plasma either between the patients before and after therapy and the control subjects and/or between the treated patients and the control subjects. As a matter of fact, the low level of potassium observed in the patients before therapy is in agreement with the '*kanwa*' analysis earlier reported which showed that the level of potassium was very low and consequently ingestion of '*kanwa*' could not really increase its level in the plasma. This has suggested clearly that the level of potassium did not play a vital role in the syndrome and hence can not be used in the pathogenesis of it.

Table 15 indicates that there is no significant variation of phosphate concentration between the patients before and after therapy and the control subjects. However, the table has clearly indicated that there is a significant variation among the patients before therapy. The observations are all in agreement with the research hypothesis which again agreed with the result of '*kanwa*' analysis. The result has further suggested that phosphate does not play a vital role in PPCF and therefore, can not play a role in the pathogenesis of the syndrome so long as the first hypothesis of this research is concerned and which attributed the syndrome to taking large quantities of '*kanwa*' which was found to very rarely contain phosphate.

4.2.4 *Kanwa* as an aggravating factor in PPCF

Table 16 clearly shows that the mean level of sodium in the plasma of the patients that consumed both white and red '*kanwa*' combined did not vary significantly compared to that of those who consumed the red '*kanwa*' alone but

varied significantly compared to that of those who consumed only the white 'kanwa'. The table has further shown that there was no significant variation between the patients that consumed only red 'kanwa' and those that consumed only the white 'kanwa'. These observations, derived from the table, clearly indicate that the mean level of sodium in the three categories of subjects cannot be used to draw a conclusion on which variety or mode of consumption can be a potential factor of PPCF incident in puerperal women in northern Nigeria. However, it signifies that the sodium loading is higher in the case of those taking both white and red 'kanwa' combined and least in those consuming only the white 'kanwa'. The result has further confirmed the hypothesis that heavy sodium loading is due to the ingestion of large quantities of 'kanwa'

Table 17 shows that the mean level of sulphate in the patient that consumed only red 'kanwa' varies significantly from that of those that consumed white 'kanwa' alone than those that consumed both white and red combined. This observation has shown that if sulphate is accepted as an influencing factor in PPCF as suggested and shown, then the effect is even more on the patients that consumed red 'kanwa' alone than in the other categories of the patients.

Table 18 indicates that the mean level of calcium in the blood plasma of the PPCF patients that consumed red 'kanwa' alone varies significantly from those that consumed only the white or both white and red 'kanwa' combined. This observation agreed with earlier report on 'kanwa' analysis which showed that it was red 'kanwa' that calcium tested positively significant which suggested that the calcium level be higher in those that consumed it (red 'kanwa') than in the remaining two categories.

Table 19 has shown that the mean level of potassium ion in the plasma of the patients that consumed only red 'kanwa' does not vary significantly from that of those that consumed only the white 'kanwa' but varied significantly with that of those that consumed both red and white combined. This has further buttressed the initial argument that potassium ion is not an influencing factor in PPCF syndrome.

Just as in the case of potassium ion, the phosphate ion too shows no bias against any mode of consumption. This observation agreed with the result shown in Table 17 and also with the result on 'kanwa' analysis which showed a very low concentration of phosphate (Ekanem, 1977). Consequently, one draws a conclusion that phosphate electrolyte is not playing a vital role in PPCF syndrome in agreement with our research hypothesis.

4.2.5 The role of age in PPCF syndrome

Table 20 shows that the mean level of sodium in blood plasma of the PPCF patients before therapy and whose ages lie in the range 26 - 35 years vary significantly with respect to that of both the patients whose ages lie between 15 - 25 years and those whose ages lie between 36 - 50 years. This observation suggested that the risk of sodium as an influencing factor in the PPCF syndrome is high in the patients whose ages lie between 26 - 35 years and which is the peak delivery age in women. This observation is in agreement with the result of the outcome of peripartal cardiac failure in Zaria, between 1969 - 1995 which suggested that there is less risk of PPCF in older women (Ford *et al.*, 1998).

However, the results of other electrolytes of interest including the sulphate ion as shown in Tables 11, 12, 13, 14 and 15 clearly indicate that there

has not been any prejudice against or for any age group as a most probable potential risk factor of PPCF patients in northern Nigeria.

4.2.6 Correlation among the electrolytes in PPCF patients before therapy

Table 27 shows that sodium and phosphate, potassium and sulphate, calcium and sulphate interactions have shown a negative but strong linear correlation among themselves whereas interactions among sodium and sulphate and vice versa and potassium and phosphate have positive and strong correlations. However, the interaction between calcium and phosphate has a positive but weak linear correlation.

CHAPTER FIVE**SUMMARY AND CONCLUSION**

Sodium and sulphate are the two electrolytes that proved statistically to play a vital role in the pathogenesis of postpartum cardiac failure (PPCF) in puerperal women in Northern Nigeria for all categories of the patients. Considering the sodium effect, the drug administered to the patients did really subdue the mean level of sodium in the patients within the first three days from the commencement of treatment and the effect of the sodium heavy loading is more acute in patients whose ages lie between 26 - 35 years than in younger or older ones. However, there was no clear bias on the consumption mode of the patients be it in the form of either red '*kanwa*' alone, white alone or white and red '*kanwa*' combined. The effect of plasma sulphate was also found to be more acute in patients that consumed red '*kanwa*' alone than in the other two categories of the patients that consumed either the white '*kanwa*' alone or both white and red '*kanwa*' combined. However, the effect of plasma sulphate does not show any prejudice with respect to age groupings as in the case of sodium.

The high level of calcium in the PPCF patients was observed to be due to other sources of calcium in the patients' diet and not due to '*kanwa*' consumption and consequently, was found not to influence the syndrome. Potassium and phosphate were also found not to influence the risk of the syndrome in all the categories of patients for all the modes of '*kanwa*' consumption.

5.1 Recommendations

More electrolytes should be looked into as potential factors in influencing the PPCF syndrome.

Analysis of the whole blood should be carried out as against the plasma. Better and on-the-spot method of analysis of the mean level of electrolytes such as the ion selective electrode method should be adopted and used.

The height, weight and parity should be focused as potential factors to see variation of the mean level of the electrolytes of interest.

The geographical coverage of the study should be expanded to include patients from other Teaching Hospitals in Northern Nigeria.

Comparative analysis of the patients in Northern Nigeria and their counterparts from the other parts of the country with respect to their postpartum cultural practices should be carried out.

The collection of the blood for onward analysis should begin before the physical manifestation of oedema in the PPCF patients.

The follow-up period for the collection of different lots of the blood should either be increased or the number of lots to be collected should be increased.

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APPENDIX A**STUDY PROTOCOL**

1. Serial No. _____
2. Age _____
3. Ethnic group _____
4. Occupation _____
5. Religion _____
6. Type of sleeping place:
 - (i) Traditional
 - (ii) Modern
7. Height in cm _____
8. Weight in kg _____
9. Obstetric History:
 - (i) How many pregnancies have you had? _____
 - (ii) Number of deliveries _____
 - (iii) Number of abortions _____
 - (iv) Any previous history of body swelling following delivery? _____
 - (v) If yes, was this during pregnancy? _____
 - (vi) If no to above, was it during the puerperum? _____
 - (vii) In addition to body swelling did you have any of the symptoms listed below?
 - (a) Breathlessness
 - (b) Palpitations
 - (c) Cough
 - (d) High blood pressure

10. Traditional practices

Which of the traditional habits listed below do you engage in after delivery?

- (a) Bath with boiling water
- (b) Lying on hot mud bed
- (c) Drinking *kunun kanwa*

If yes to (c) above is it:

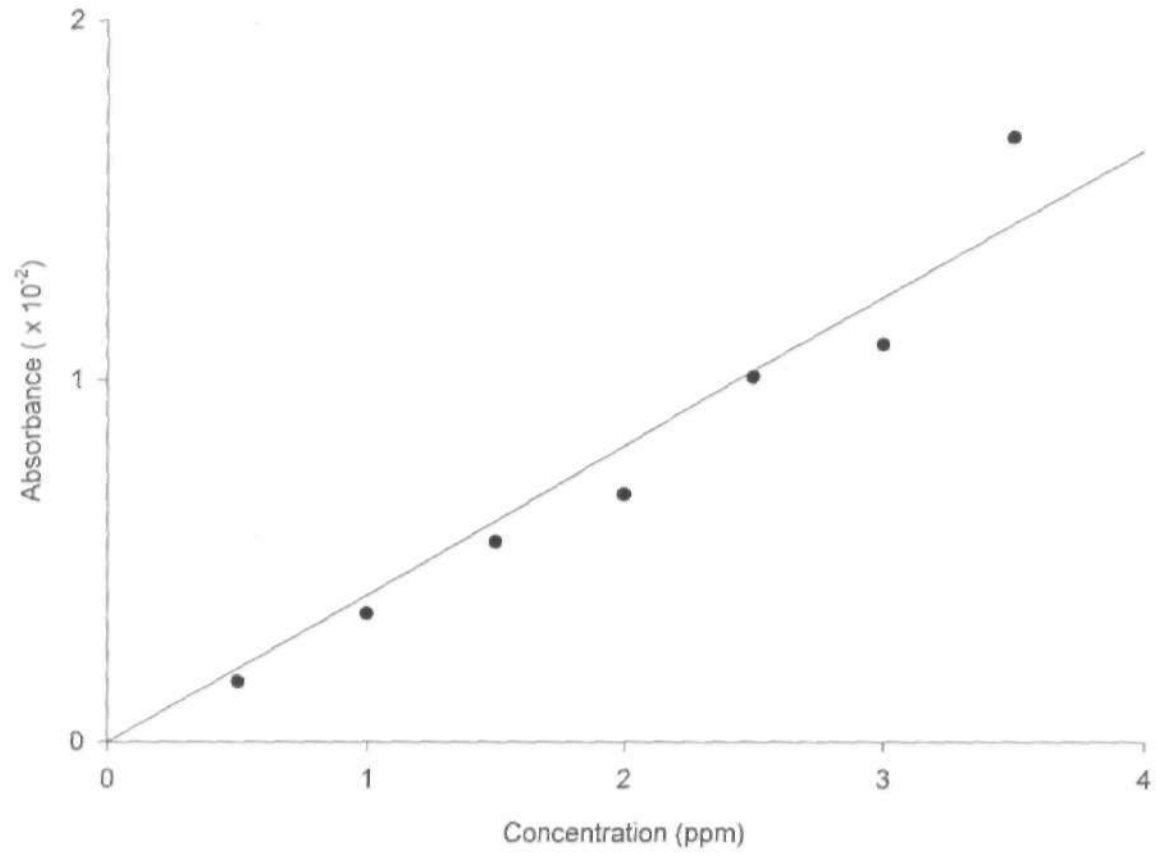
- (i) Red *kanwa*?
- (ii) White *kanwa*?
- (d) Other herbal drinks

11. Present pregnancy/delivery

- (i) How many weeks have you engaged in the traditional practices before you developed body swelling?
- (ii) Physical signs:
 - (a) Fever
 - (b) Body swelling
- (iii) Lab. Results:
 - (a) PR
 - (b) BP
- (iv) Heart failure

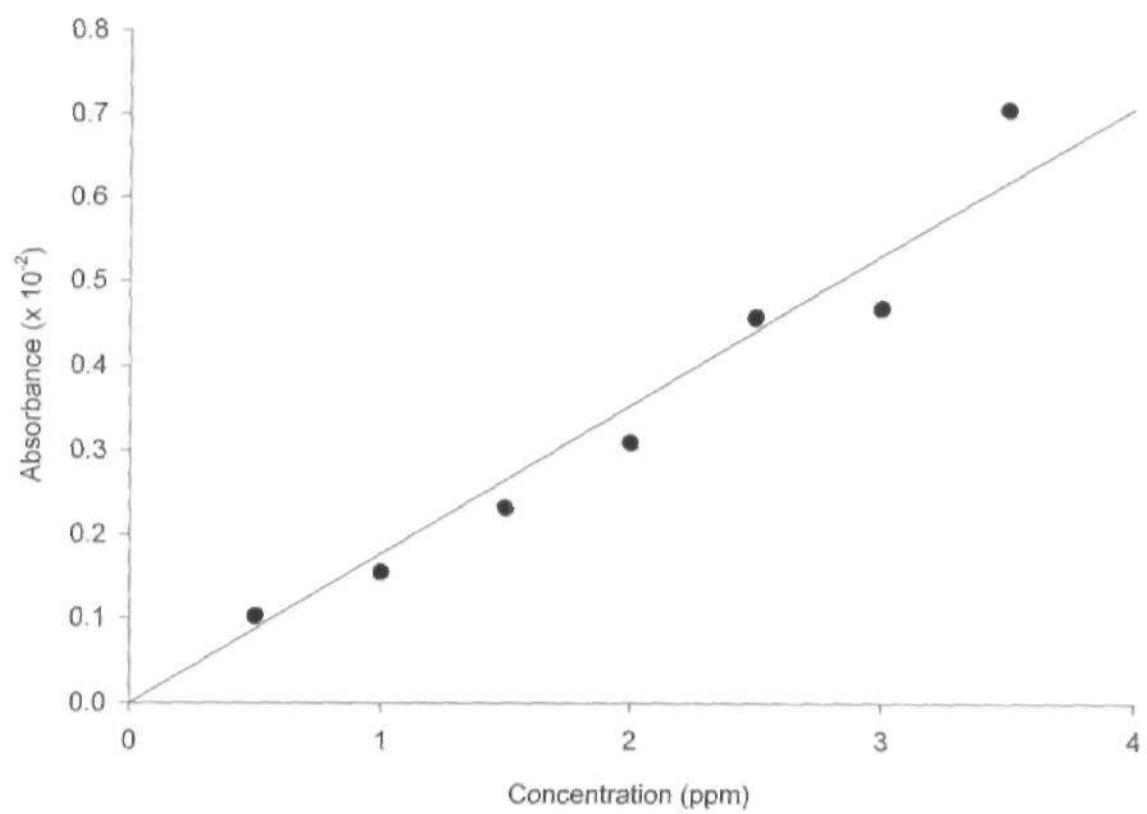
APPENDIX B

CALIBRATION GRAPH FOR SULPHATE DETERMINATION



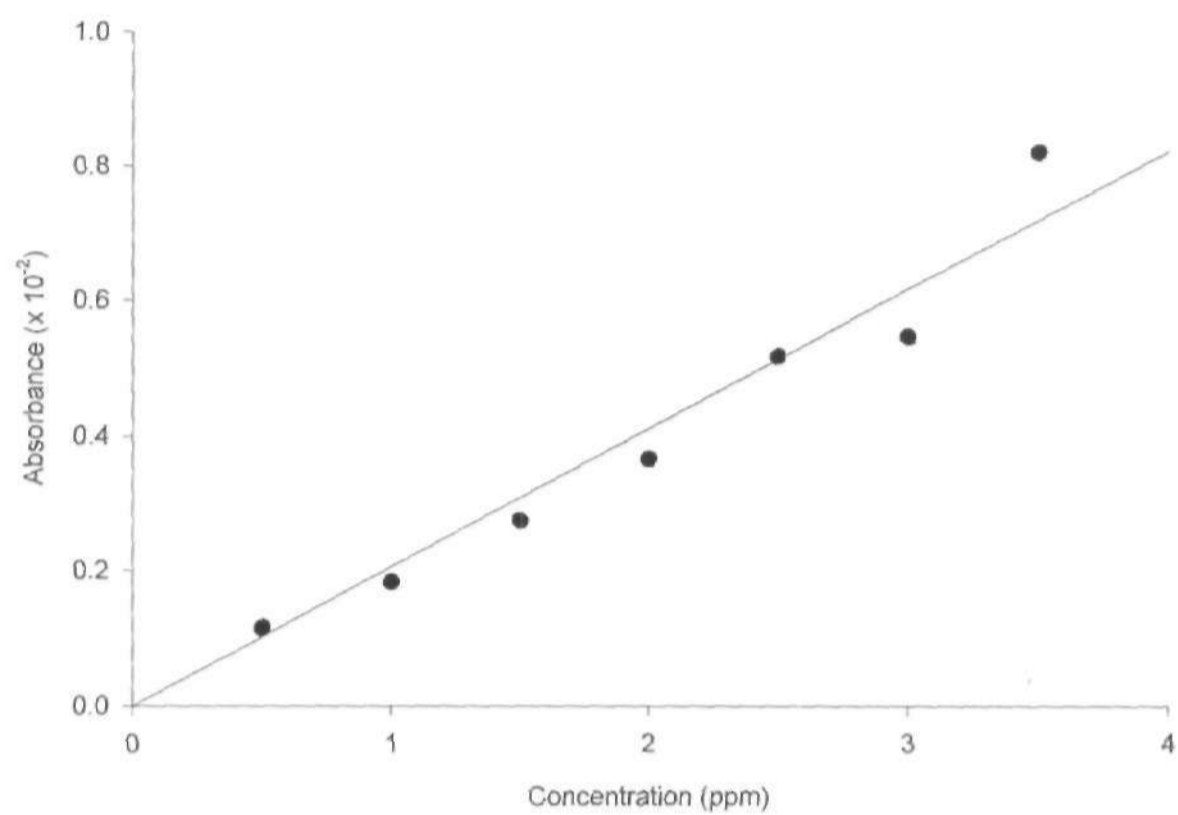
APPENDIC C

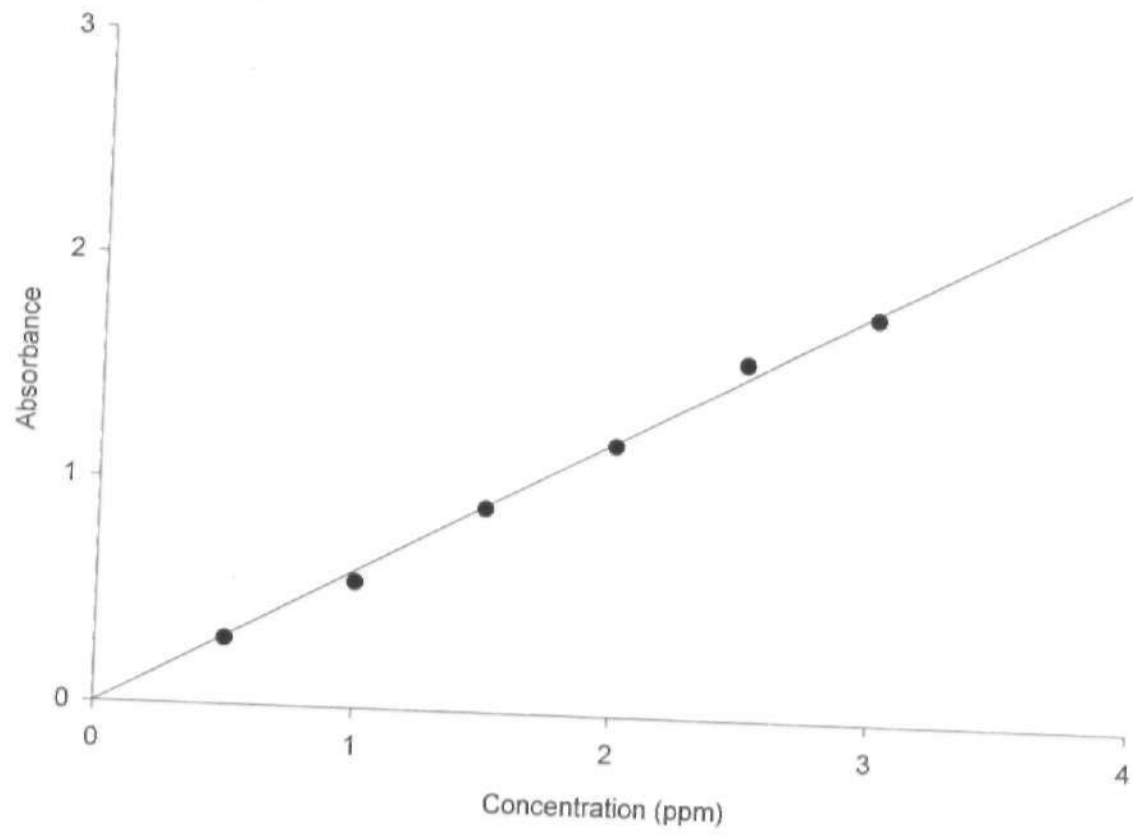
CALIBRATION GRAPH FOR PHOSPHATE DETERMINATION



APPENDIX D

CALIBRATION GRAPH FOR POTASSIUM DETERMINATION



APPENDIX E
CALIBRATION GRAPH FOR SODIUM DETERMINATION

APPENDIX F

CALIBRATION GRAPH FOR CALCIUM DETERMINATION

