PREPARATION OF REAGENTS FOR DETERMINATION OF SODIUM, POTASSIUM, CREATININE AND CHLORIDE ON ASTRA-8

THESIS

Submitted in Partial Fulfilment of
Master Degree of Clinical
and Chemical Pathology

By

MONA SALAH ELDIN ABD EL BASET
M.B.B.Ch.
Clinical Pathology Department
Faculty of Medicine, Ain Shams University

SUPERVISORS

Prof. Dr. SAWSAN HOUSNY HAMZA
Prof. of Clinical Pathology Dept.
Faculty of Medicine
Ain Shams University

Prof. Dr. MAHMOUD SABRY SALLAM
Prof. of Clinical Pathology Dept.
Faculty of Medicine
Ain Shams University

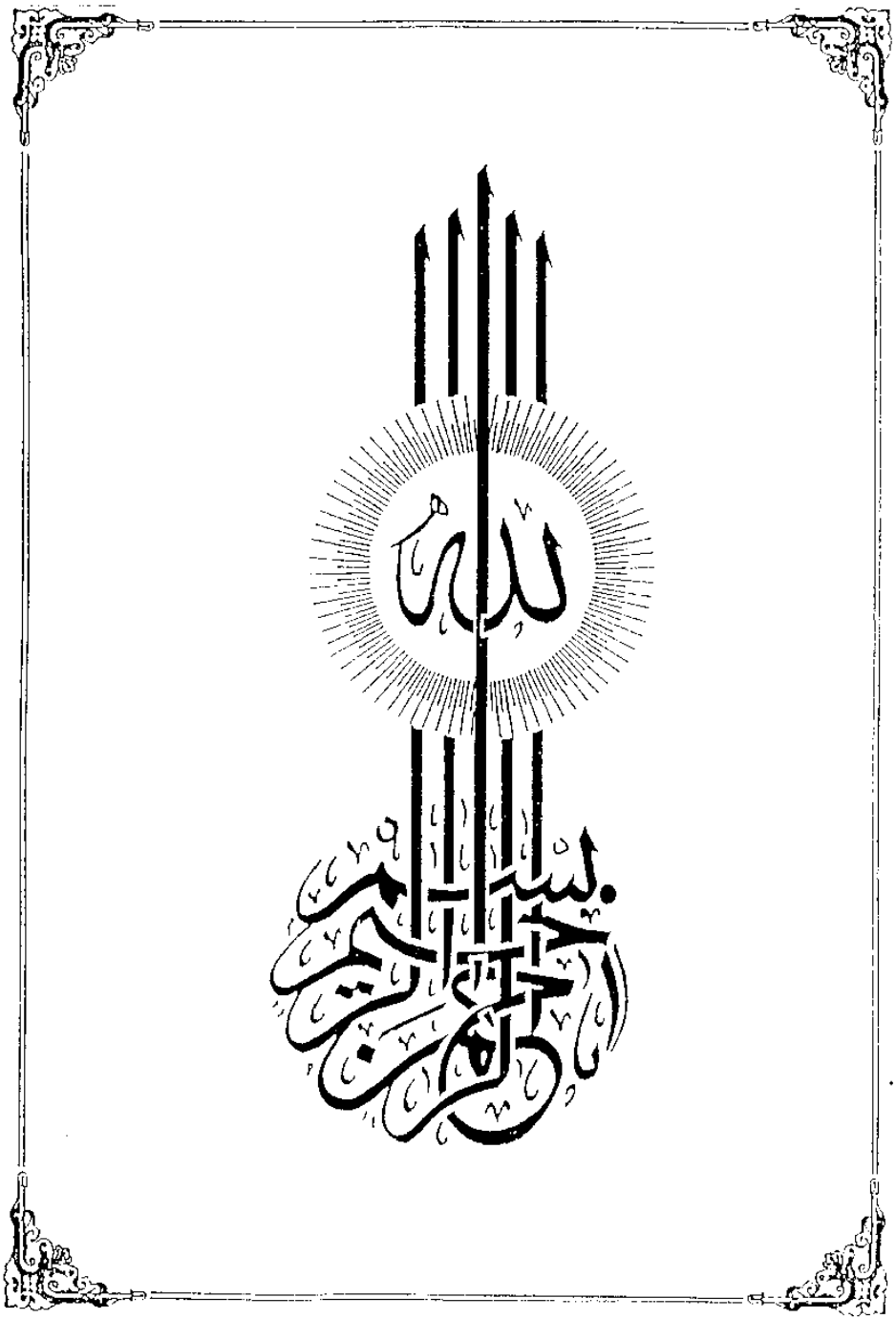
Faculty of Medicine
Ain Shams University

(1987)

بسم الله الرحمن الرحيم

"وقل رب زدني علما"

صدق الله العظيم



TO MY PARENTS

HUSBAND

AND

BROTHER

ACKNOWLEDGEMENT

I would like to express my deepest thanks and gratitude to Professor ***SAWSAN HOUSNY HAMZA*** *for her valuable suggestions and faithful guidance that contributed to success of the present work.*

My sincere appriciation to Professor ***MOHAMOUD SABRY SALAM*** *who offered me a great help through his continuous advice, support and encouragement.*

I am also deeply indebted to Dr. ***GEEHAN KAMAL HASSAN*** *for her generous help. I will always remember her unforgetable sincere encouragement and kindness.*

Finally my deep thanks to Dr. ***NASER REZK*** *for his good help.*

Mona Salah El Din

1987

CONTENTS

Introduction
&
AIM OF WORK

A) INTRODUCTION

Automation is necessary in any modern clinical hospital laboratory. Any medium-sized hospital (50-20 beds), or large-sized hospital (more than 200 beds), its laboratory should have a form of automation. Automation provides a mean by which increased work load can be processed rapidly and reproducibly.

Finely et al., 1978 have evaluated the performance of *Astra-8 with Beckman's reagents and found that it has an excellent precision and accuracy compared to values obtained by other methods.

Also the Astra-8 proved to be suitable for both routine and stat purposes (Rezk, 1986) where it constitutes the main work station in clinical chemistry section of clinical pathology department in Ain Shams Faculty of Medicine.

However being a developing country, the Beckman reagents are quite expensive compared to the funds available. Also transportation of refregirated reagents with the probability of stucking in the custom departments may affect the validity of these reagents. Besides they have to be imported in hard currency from abroad.

---

* See manufacturer's list.

B) AIM OF THE WORK

To prepare our own reagents and check its performance characteristics compared to that of the original company in an attempt to replace eventually the latter if they prove well.

The material of this study wil include the different chemicals used in the preparation of reagents for sodium, potassium, creatinine and chloride determination.

The following will be covered:

1. Preparation of some reagents of Astra-8 according to local availability of their constituting chemicals.
2. Evaluation of performance of these reagents as regards their accuracy, Precision, Linearity, stability, sensitivity and specificity.
3. Comparative statistical study between Beckman's reagents and local reagents.

Review of Literature

REVIEW OF LITERATURE

(A) Principles of electrolyte and creatinine measurement on Astra-8.

1. Measurement of Sodium and Potassium, by ion selective electrode:

Although ion selective electrodes have existed for sometime for determining Sodium and Potassium ions in biological fluids (Cremer, 1906 and Haber et al., 1909) their use in clinical routine practice was heralded by the widespread publicity of other techniques such as flame photometry (Annino, 1967). With the development of newer and better electrodes and better electronic circuity, the ion selective method became rapidly one of the routinely used techniques. Recently considerable progress has been made in the development of Sodium Selective glass. The thin layered lithium aluminium silicate glass electrode is particularly excellent at measuring sodium in biological fluids. This electrode is insensitive to hydrogen ion in the pH range 6-10 (Freiser, 1980).

One of the most important developments for measurement of potassium by electrode is the valinomycin electrode described by Proda and Simon in 1970. This electrode is insensitive to hydrogen ions in solutions buffered from pH 3-9. (Lustgarten et al., 1974).

The development of sodium and potassium selective electrodes opened the possibility of potentiometric determination which offers advantages over the flame photometry traditionally applied for measurement of sodium and potassium in plasma or serum. (Levy, 1981).

Advantages of the use ion-selective electrodes include rapid determinations, as there is no need for warm up time, which is of great clinical importance for electrolyte studies in acutely ill patients. Electrodes are stable and durable, even the potassium electrode which has an organic sensor, Valinomycin. No requirements for external fuel, air or water supply. There is no observable effect of hyperbilirubinemia, hyperglycemia or uremia on the ion selective electrodes. (Lustgarten et al., 1974).

There are two general types of ion selective elctrode measurements on clinical samples. "Direct potentiometric systems, measure the ion activity in an undiluted sample. It involves aspirating an undiluted biological sample, typically serum, plasma or whole blood directly into a vessel in which the electrodes are housed. "Indirect" ion selective electrode systems measure the ion activity in a prediluted sample. It involves the aspiration of a finite volume of biological sample and the appropriate

volume of the diluent to produce a dilution of the sample suitable for the given sensitivity of the individual instrument. (Miller, 1984). Direct reading systems use a type of unit described as ionized electrolyte/mmol/L of plasma water. Plasma water is measurable by osmolality and differs from sample to sample. Indirect reading systems use mmol/L of serum, plasma or urine units for reporting (Flores and Chittenden, 1981).

Because ion selective electrode measurements determine the activity of an ion in the water volume fraction in which it is dissolved, that is $\frac{\text{water volume}}{\text{Total serum volume}}$; "direct" measurements are unaffected in conditions such as hyperproteinemia or hyperlipidemia, which alter the volume fraction of water in serum. However, "indirect" methods are usually sensitive to this physiological effect because the dilution step itself is based on total volumes, and after dilution the volume occupied by soluble serum molecules becomes insignificant with respect to the total diluent volume. This physiological interference in the indirect methods is responsible for the pseudohyponatremia observed in cases of hyperproteinemia or hyperlipidemia with reduced plasma water volume fraction. This volume fraction error, when present, does not usually create a problem of clinical interpretation for potassium, because the normal range is fairly large with respect to the magnitude of concentration of potassium

in serum (Miller, 1984).

The shelf-life of electrodes is high in the indirect potentiometric systems. It may have indifinite life span, but it is low in the direct potentiometric systems & need to be changed every 2-3 months.

The Beckman Sodium-Potassium chemistry module is a subsystem operating in conjunction with Beckman Astra automated stat routine analyzer system. (Figure I)

Principle: depends on the indirect potentiometric determination of ions by ion selective electrodes.

Reagents: The module is designed to use reaction buffer solution, electrode conditioning solution and Astra system calibration standards in the quantitative determination of sodium and potassium in serum, plasma and urine samples.

Method: The module operation begins with the reaction cup filled with electrode conditioner reagent which acts as a stabilizing reagent and is used whenever sample measurements are not being taken. The conditioning solution brings the electrodes to an equilibrium potential between measurements and during stand by (Flores and Chittenden, 1981). On work, this reagent is replaced with buffer solution of high molar strength which serves to set a constant activity coefficient for the electrode.

With constant activity established, the electrode system is calibrated to concentration values using the Astra system calibration standards. A 50 ul sample is then injected into the buffer reagent by Astra system sample probes. The reaction cup contents are thoroughly mixed with a magnitically- driven stirrer located in the bottom of the reaction cup. When the mixture of the sample in fixed ionic strength buffer contacts the sodium electrode, sodium ions undergo an ion exchange in the hydrated outer layer of the glass electrode.

As the ion exchange takes place, a change in potential (voltage) is developed at the electrode. This change is measured by voltage-sensitive circuit within the Astra system allowing the calculation of sodium ion concentration in the injected sample when compared to the reference electrode.

The potassium electrode consists of a membrane system rather than glass. It has an organic sensor: the antibiotic valinomycin dissolved in a suitable solvent. Valinomycin is a neutral carrier that binds potassium ions in the center of a ring of oxygen atoms. (Freiser, 1980). The physical structure of the valinomycin membrane is such that, the ion exchange cavities in the membrane nearly equals the diameter of potassium ions allowing selective permeability for these ions. (Similar basis of glomerular membrane).