Sudan University of Science and Technology

Post College of Graduate Studies

Characterization of Gum From

Anogeissus leiocarpus

توصيف صمغ شجرة الصهب

A Thesis Submitted in Fulfillment of the Requirement

of the Degree of Ph.D. in Chemistry

By

Muzamil Hassaballa Abd El-Mula

(B.Sc., M.Sc., Chemistry)

Supervisor

Professor: Mohammed El-Mubarak Osman

Co-Supervisor

Professor: Elfatih Ahamed Hassan

Oct., 2018

Approval Page

(To be completed after the college council approval)

Name of Candidate: Muzmil | Hassabu | Abdelmula | Mail Omer

Thesis title: .....

.....

.....

.....

Degree Examined for: .....

.....

Approved by:

1. External Examiner

Name: Mohamed Ali Hussein

Signature: ..... Date: 15.11.2018

2. Internal Examiner

Name: Elmugdad Ahmed Ali

Signature: ..... Date: 15/11/2018

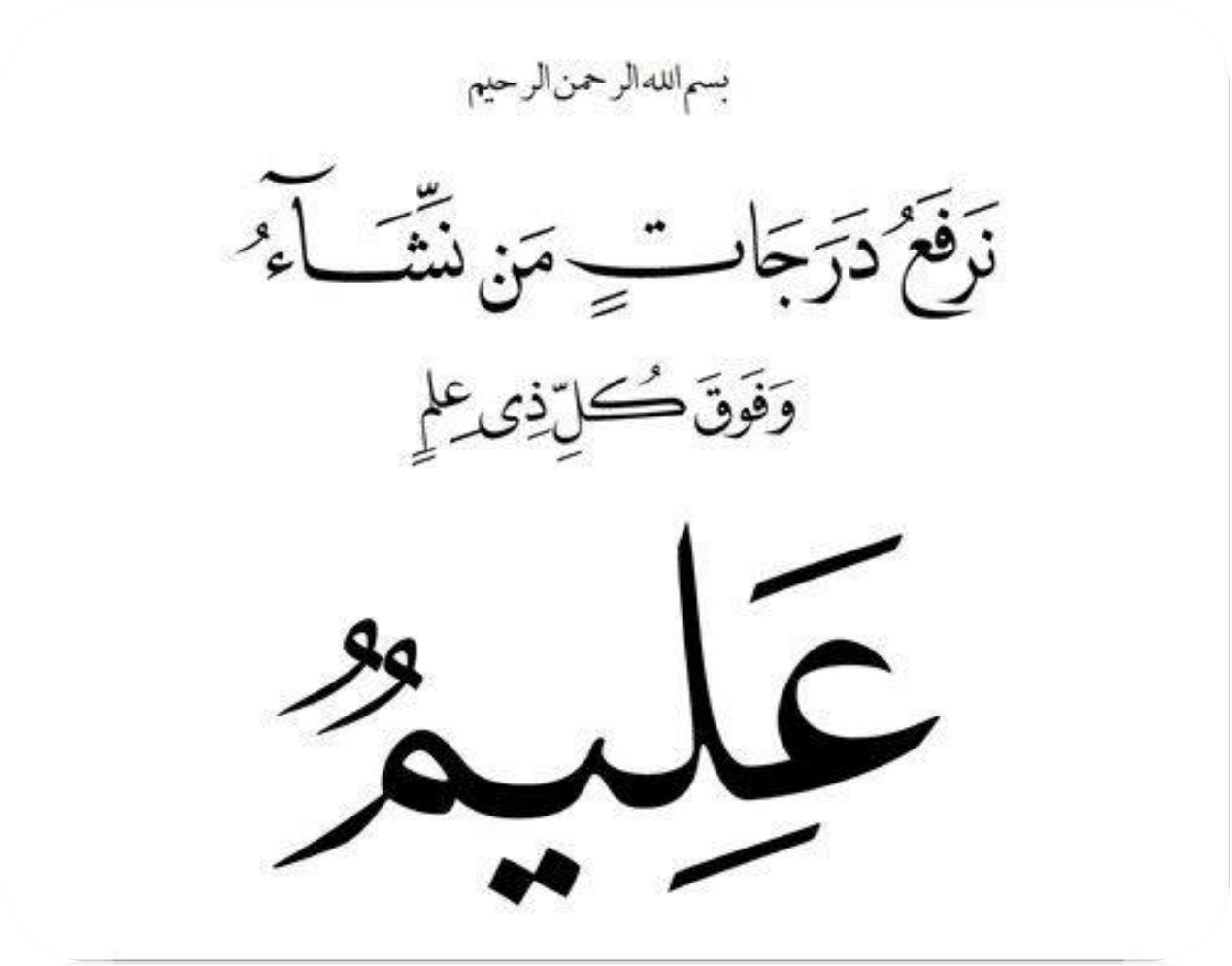
3. Supervisor

Name: Mohammed Elmubark Osman

Signature: ..... Date: 15.11.2018


بسم الله الرحمن الرحيم

الأستهلال



سورة: يوسف

الآية: 76

DEDICATION

To Souls of my parents, who inspired and encouraged me, through hard times, and to my brothers and sisters.

ACKNOWLEDGEMENTS

First and foremost, I would like to praise ALLAH who made all things possible...

I cannot express gratitude to supervisors professor, Mohammed El-Mubark Osman, and; professor. El-Fatih Ahmed Hassan for their continued support and encouragement. I offer my sincere appreciation for the learning opportunities provided by them.

I would like to thank my classmates, Dr. Mai Makki, Dr. Amira Abd Elaziz; and my children, Doaa, Waed , Shahed, Ahed, Daed and Bassam for moral support. Thanks are due to Mr. Alrasheed Awad Mohammed for technical support, University of Khartoum.

To my caring, loving, and supportive wife, Nadia Bushra: my deepest gratitude. Your encouragement when the times got rough was much appreciated and duly noted. It was a great comfort and relief to know that you were willing to provide management of our household activities while I completed my work. Special thanks to my Azal Pharma colleagues for their undivided support and interest who inspired and encouraged me to go my own way. Last but not the least I want to thank my friends who appreciated my work and motivated me.

ABSTRACT

Samples *of Anogeissus leiocarpus* gum were randomly collected as natural exudates from different locations of the Sudan gum belt.

The physicochemical properties of composite samples studied were moisture content, ash, nitrogen, protein, specific rotation, relative viscosity, refractive index, density, also UV electromagnetic radiation, metals ions, *p*H, uronic acid, reducing sugars and infrared spectra. Analysis of variant of results showed insignificant differences ($p < 0.05$) in all properties studied. The mean value of all parameters studied were; moisture content 7.82%, ash content 2.02%, nitrogen content 0.14%, protein content 0.87%, specific optical rotation -35.5°, relative viscosity 0.914*kg/(m s)*, refractive index 1.589, density 1.066g/ml. UV absorption spectra gave a maximum absorption ($\lambda_{max)}$ at 277nm, the cationic composition was in the order; the calcium (Ca^{++}) 0.45325 ppm > potassium (K^{+}) 0.35200 ppm > magnesium (Mg^{++}) 0.10465 ppm > sodium (Na^{+}) 0.06000 ppm > iron (Fe^{++}) 0.05625 ppm > lead (Pb^{++}) 0.02580 ppm > zinc (Zn^{++}) 0.01973 ppm and trace amount of manganese (Mn^{++}) 0.00745 ppm, chromium (Cr^{++}) 0.00045ppm, and there is no cadmium (Cd^{++}). The pH was found to be 4.52, and glucuronic acid 0.14%.

The composition chemically, consisted mainly of high-molecular, rhamnose 9.288%, arabinose 34.00%, and galactose 27.50%.

Anogeissus leiocarpus gum was used as suspending agent for cefixime suspension powder, compared with the reference sample, the pH were measured, density and sedimentation volume, the results of the pH for seven days for samples between 3.77 in day one , 3.68 in day seven and reference sample between 3.82 in day one and 3.71 in day seven, the relative density for sample was 1.45g/ml in day one and

1.40 g/ml in day seven, and for reference sample was 1.40 g/ml in day one and 1.36 g/ml in day seven and the sedimentation volume for four weeks as follows: the first week 0.83, second week 0.80, third week 0.77, and the fourth week 0.71. The reference sample as follows: the first week 0.83, second week 0.80, third week 0.77, and the fourth week 0.71. Also, kinetic chemical reaction rate for cefixime dry suspension powder were studied in 45^{o} C and relative humidity 75% for six months, the assay in initial time were 100.05%, the first month were 87.22%, second month 80.01%, third month 63.25% and sixth month, 55.15%.

Also, cytotoxicity of *Anogeissus leiocarpus* gum was investigated after cell growth. The concentration at which the growth of cells was inhibited to 50% of the control (IC_{50}) was 370.5μg/mL ± 15.68. It presented an accentuated inhibition of cell growth at 125 μg/mL, 250μg/mL and total inhibition at 500 μg/mL was 27.5 ± 1.5, 49.0 ± 1.42 and 49.2 ± 1.4 respectively. The limit of the toxicity is IC_{50} < 30μg/mL and the non-toxic IC_{50} > 100μg/mL, shows the effect on cell growth. Practically non-toxic and the value of IC_{50} result indicate that the *Anogeissus leiocarpus* gum is non-toxic and cytotoxicity assays did not interfere in the results because all reagents and chemicals used are non-toxic. The *in vitro* cytotoxicity assays are used as screening tests for evaluation *Anogeissus leiocarpus* gum biocompatibility.

المُستخلص

تم جمع عينات من صمغ شجرة الصهب *(Anogeissus leiocarpus)* عشوائيا من مناطق حزام الصمغ العربى فى السودان وتمت دراسة الخصائص الفيزيوكيمائية لعينات الصمغ (الرطوبة والرماد والنيتروجين و البروتين، الدوران النوعى، اللزوجة، معامل الانكسار، الكثافة ، وتم تحديد أطياف امتصاص الأشعة فوق البنفسجية وتحديد الطول الموجى لأعلى امتصاصية، وأيونات المعادن، ودرجة الحموضة، وحامض اليورانيك، وكمية السكريات المختزلة، كما تم تحديد أطياف امتصاص الأشعة تحت الحمراء) . وكانت النتائج فى الدراسة بحساب قيمة المتوسط فى جميع العينات على النحو التالي: الرطوبة 7.82% والرماد بنسبة 2.2%، و النيتروجين 0.14%، والبروتين 0.021%، والدوران النوعى ^{O}34.5-، و اللزوجة النسبية 0.914 كيلوجرام. متر$^{-1}$. ثانية $^{-1}$، ومعامل الانكسار1.589، والكثافة فى الملتر 1.066 وتم تحديد أطياف امتصاص الأشعة فوق البنفسجية وتحديد الطول الموجى لاعلى امتصاصية وكانت تساوى 277 نانوميتر وأظهرت النتائج ان أعلى معدل لأيونات المعادن كانت لأيون الكالسيوم (Ca^{++}) 4532.5 جزء من المليون ثم يلية أيون البوتاسيوم (K^{+}) 3520.0 جزء من المليون ثم أيون المغنيسيوم (Mg^{++}) 1046.5 جزء من المليون، ثم أيون الصوديوم (Na^{+}) 600.0 جزء من المليون ثم الحديد (Fe^{++}) 562.5 جزء من المليون، والرصاص (Pb^{++}) 258.0 جزء من المليون، والزنك (Zn^{++}) 197.25 جزء من المليون و كمية ضئيلة من المنغنيز (Mn^{++}) 74.5 جزء من المليون والكروم (Cr^{++}) 4.5 جزء من المليون ولم تظهر النتائج وجود أيون الكادميوم (Cd^{++}).

وفى هذه الدراسة تم تحديد درجة الحموضة 4.52، وحامض اليورانيك 0.14% وكمية السكريات المختزلة الرامنوز 9.288% والرابينوز 34.00% والجلاكتوز 27.50% .

كما تم فى هذه الدراسة أستخدام صمغ شجرة الصهب *(Anogeissus leiocarpus)* كعامل تعليق فى شراب السيفيكسيم Cefixime for oral suspension powder مقارنة مع عينة مرجعية تجارية وتم قياس درجة الحموضة pH والكثافة النوعية density وحجم التركيد sedimentation volume وفحص النقاوة Assay وكانت النتائج للحموضة pH للعينة 3.77 وللعينة المرجعية 3.82 والكثافة النوعية للعينة هى 1.45جم/مل وللعينة المرجعية هى 1.40جم/مل وحجم التركيد لمدة اربع أسابيع للعينة وكانت كالاتى : الاسبوع الاول 0.83 الاسبوع الثانى 0.69 الاسبوع الثالث 0.66 الاسبوع الرابع 0.63 اما العينة المرجعية

التجارية كانت كالاتى : الاسبوع الاول 0.83 الاسبوع الثانى 0.8 الاسبوع الثالث 0.77 الاسبوع الرابع 0.71.

كما تمت دراسة حركية التفاعل الكيميائى عند وضع بدرة من عينات شراب السيفيكسيم فى درجة حرارة 45 درجة مئوية و رطوبة نسبية 75% لمدة ستة أشهر وكانت نتيجة فحص النقاوة Assay فى بداية الفترة 100.05% وفى الشهر الاول 87.22% وفى الشهر الثانى 80.01% وفى الشهر الثالث 63.25% وفى الشهر السادس 55.15%.

كما تمت دراسة السمية لصمغ شجرة الصهب بعد نمو الخلية، وكان اقل تركيز يثبط نمو الخلية للمستخلص يساوى 50% ومن العينة المرجعية يساوى 370.5 ± 15.68 ميكروجرام/مل وتم دراسة التثبيط لنمو الخلايا في التراكيز الاتية: 125 ميكروجرام/مل، 250 ميكروجرام/مل وللتثبيط الكلي على 500ميكروجرام/مل، وكانت نتائج التثبيط على النحو التالى: 27.5 ± 1.5 ، 49 ± 1.42 و49.2 ± 1.4 .وحدود السمية هى: (IC_{50} < 30) ميكروجرام/مل شديد السمية، و (IC_{50} >100) ميكروجرام /مل. غير سام. و نتائج هذه الدراسة تشير الى عدم سمية صمغ شجرة الصهب وكذلك قيمة نتيجة IC_{50} والجدير بالذكر أن كل الكواشف والمواد الكيميائية المستخدمة فى المختبرغير سامة .

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page No.**

APPENDIX

Appendix **Page No.**

Appendix **Page No.**

LIST OF ABBREVIATIONS

Da :	Dalton.	2
EPS :	Extracellular Polymeric Substances.	6
TFA :	Trifluoroacetic acid.	11
FAO :	Food and Agriculture Organization of the United Nations.	15
cps :	Centipoise.	22
IUPAC:	International Union of Pure and Applied Chemistry.	29
SEC :	Size Exclusion Chromatography.	32
MW :	Molecular Weight.	32
APIs:	Active pharmaceutical ingredients.	50
PSD :	Particle size distribution.	50
PS :	Particle size.	51
FPP :	Finished pharmaceutical product.	64
FDCs :	Fixed Dose Combination.	65
RH :	Relative Humidity.	65

LDH :	Lactate dehydrogenase.	71
MTT :	3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide.	71
ATP :	Adenosine triphosphate.	72
SRB :	Sulforhodamine B.	72
ECIS :	Electric Cell-Substrate Impedance Sensing.	72
ADCC :	*Antibody-dependent cell-mediated cytotoxicity*.	72
CDC :	*complement-dependent cytotoxicity*.	72
NCF :	Nitrogen conversion factor.	79
HPLC :	High Pressure Liquid Chromatography.	80
U.S.P :	United State Pharmacopeia.	84
B.P. :	British Pharmacopeia.	84

Chapter one

1. Introduction and literature review

Chapter One

1. Introduction and literature review

1.1 Natural Gums

"Polysaccharide" is a general term given to high molecular weight substances which contain a large number of structural units called monomers or glycoses (monosaccharides) joined by O-glycosidic linkage (Yalpani,1988). The monomers are joined by linkages often forming long repeating units of polymer chains.The polysaccharides are also known as carbohydrate polymers. They have been described as the most abundant,versatile and diverse class of organic compounds occurring in nature (Yalpani, 1988; Izydorczyk, 2005). Polysaccharides or their derivatives which, when dispersed in hot or cold water at low dry substances content, swell to produce gels, highly viscous dispersions or solutions, have been classified as gums (Glicksman and Schachat, 1959; Wallis, 1967). These include the water-insoluble or water-soluble derivatives of cellulose and the derivatives and modifications of other polysaccharides which in their natural forms are insoluble. They also include polysaccharides and their derivatives which are slimy or mucilaginous.

1.2 Source of Natural Gums

Gums are complex neutral or slightly acidic polysaccharides produced by trees, and shrubs, either spontaneously or after mechanical injury, and also produced from micro-organisms. Each polysaccharide gums contains three or more constituent sugars including D-glucuroflic acid (or its 4-methyl ether) and/or D-galacturonic acid.

Highly branched structures are invariably encountered and since each constituent sugar may be present in furanose or pyranose ring forms may have different

configurations at its glycosidic linkages, and may be involved in a variety of types of linkage, the elucidation of the detailed molecular structure of exudate gums provides one of the most challenging problems in organic chemistry. There are three types of polymer heterogeneity that have been recognized amongst the polysaccharide constituents of exudate gums (Aspinall and Baillie, 1963).

Firstly, there are those gums, such as gum tragacanth (Aspinall and Baillie, 1963) and *Khaya senegalensis* gum (Aspinall, *et al*;. 1960;. Aspinall, Johnston, and Young,1965), in which two polysaccharide components of entirely different structural type are present.

Secondly, at the other end of the scale, there is the kind of micro-heterogeneity, found in Combretum leonense gum (Aspinall and Bhavanandan,1965) in which polysaccharide subfractions show small differences in composition but no differences in the nature of the structural units or in the linkages between them.

Thirdly, heterogeneity of an intermediate type has been encountered recently in *Anogeissus leiocarpus* ʻformerly A. schimperiʼ gum (Aspinall and Christensen, 1961). This gum contains two discretepolysaccharide components which are sufficiently different to permit fractionation on a preparative scale.

Gums, a branched polysaccharide consisting of more than 90% arabinogalactan having a molecular weight around 250,000 Da is the oldest and best known of all natural gums (Nishi, 2007).

Gums are an organic adhesive substance, produced from a tree called *Acacia Senegal var*. it is a natural gum that exudes from the exterior of Acacia trees in the form of dry, hard nodules, with over 1,100 Acacia species worldwide, it produces a natural gum made of hardened sap mostly taken from three species of the *acacia*tree. In Sudan, the major Acacia utilized for commercial Gum Arabic production are *Acacia senegal* and *Acacia seyal* (Nishi, 2007).

The produced materials are used as a thickener, suspender, emulsifier, stabilizer, flavor carrier, binder and encapsulating material. It is therefore needed in industries such as the food, pharmaceutical, beverage, dairy and ice cream, cosmetic, confectionary and textile, thereby making the market for it quite robust. The gum has binding or adhesive properties. In food products, it serves as a stabilizer, emulsifier, and binding and gelling in jams. In pharmaceuticals, the gum is a suspending agent, a binder in lozenges, tablets, pills, throat pastilles and cough drops. In textile industry, it is used for fabric stiffening and as a binder in textile printing gums. In miscellaneous industries it is used in producing ink, water colors, paints, carbon papers, pottery glacing; it is also used in the plastic industry. In confectionery industry it is used for hard gums, soft gum and gum pastilles (Edward, 1992).

1.2.1 Exudate gums

A large number of plants can produce the complex polysaccharides commercially known as 'plant-based gums'. Several studies on various plant-based gums (mainly plant gum exudates and seed sums) have resulted in the identification of valuable natural sources of complex carbohydrate polymers that promote the desired quality, and functionality (Francisco, 2013).

Plant exudates include saps, gums, latex, resin, and sometimes nectar is considered an exudates (Power and Michael, 2010).

Plant roots exude a variety of molecules into the rhizosphere, including acids, sugars, polysaccharides and ectoenzymes; this can account for 40% of root carbon (Marschner, Horst, 1995). Exudation of these compounds has various benefits to the plant and to the microorganisms of the rhizosphere (Walker, *et.al*., 2003).

1.2.2 Mucilage gums

Some gums are not exuded from the plant upon incision but rather obtained by extraction from various plant parts such as the seeds or soft stems of the plant. Such plant gums are described as intracellular or mucilage gum (Izydorczyk *et. al*., 2005). A naturally occurring, high-molecular-weight (ranging 200,000 and up), organic plant product of unknown detailed structure. The term is loosely used, often interchangeably with the term gum. Chemically, mucilage is closely related to gums and pectins but differs in certain physical properties.

Although gums swell in water to form sticky, colloidal dispersions and pectins gelatinize in water, mucilages form slippery, aqueous colloidal dispersions which are optically active and can be hydrolyzed and fermented. Mucilages are formed in normal plant growth within the plant by mucilage-secreting hairs, sacs, and canals, but they are not found on the surface as exudates as a result of bacterial or fungal action after mechanical injury, as are gums. Mucilages occur in nearly all classes of plants in various parts of the plant, usually in relatively small percentages, and are not infrequently associated with other substances, such as tannins. The most common sources are the root, bark, and seed, but they are also found in the flower, leaf, and cell wall. Their biological functions within the plant are unknown, but they may be considered an aid in water storage, decrease diffusion in aquatic plants, aid in seed dispersal and germination, and act as a membrane thickener and food reserve. Mucilages are commonly identified by physical properties, most recently by infrared spectroscopy (Elbert, 2010).

1.2.3 Seaweed polysaccharides

Many species of seaweed (marine macroalgae) are used as food and they have also found use in traditional medicine because of their perceived health benefits. Seaweeds are rich sources of sulphated polysaccharides, including some that have become valuable additives in the food industry because of their rheological properties as gelling and thickening agents (e.g., alginates, agar, and carrageenan). Sulphated polysaccharides are recognized to possess a number of biological activities including anti-coagulant, anti-viral, anti-tumor, anti-inflammatory, andimmunestimulating activities that might find relevance in nutraceutical functional food, cosmetic, and pharmaceutical applications (Pereira, 2011). Some seaweeds produce hydrocolloids, associated with the cell wall and intercellular spaces. Members of the red algae (Rhodophyta) produce galactans (e.g., carrageenans and agars) and the brown algae (Heterokontophyta, Phaeophyceae) produce uronates (alginates) and other sulphated polysaccharides (fucoidan and laminaran) (Pereira, 2011).

1.2.4 Microbial polysaccharides

Polysaccharides are either extracted from biomass resources like algae and higher order plants or recovered from the fermentation broth of bacterial or fungal cultures. For sustainable and economical production of bioactive polysaccharides at industrial scale, rather than plants and algae, microbial sources are preferred since they enable fast and high yielding production processes under fully controlled fermentation conditions. Microbial production is achieved within days and weeks as opposed to plants where production takes 3–6 months and, highly, suffers from geographical or seasonal variations anever, increasing concerns about the sustainable use of agricultural lands. Moreover, production is not only

independent of solar energy which is indispensible for production from microalgae but also suitable for utilizing different organic resources as fermentation substrates (Donot, *et.al.*, 2012).

The microorganisms used as industrial or technical producers of Extracellular Polymeric Substances(EPS) are chiefly the bacteria. Species of *Xanthomonas*, *Leuconostoc*, *Sphingomonas*, and *alcaligenes* which produce *Xanthan*, *dextran*, *gellan*, and *curdlan* are the best known and most industrially used (Kenyon, 2002, Sarwat, 2008, Palaniraj, 2011).

1.2.5 Animal polysaccharides

Polysaccharides are an important class of biological polymers. Their function in living organisms is usually either structure-or storage-related. Starch(a polymer of glucose) is used as a storage polysaccharide in plants, being found in the form of both amylose and the branched amylopectin. In animals, the structurally similar glucose polymer is the more densely branched glycogen, sometimes called 'animal starch'. Glycogen's properties allow it to be metabolized more quickly, which suits the active lives of moving animals.

Cellulose and chitin are examples of structural polysaccharides. Cellulose is used in the cell walls of plants and other organisms, and is said to be the most abundant organic molecule on earth. It has many uses such as a significant role in the paper and textile industries, and is used as a feedstock for the production of rayon (via the viscose process), cellulose acetate, celluloid, and nitrocellulose. Chitin has a similar structure, but has nitrogen-containing side branches, increasing its strength. It is found in arthropodexo skeletons and in the cell walls of some fungi.

It also has multiple uses, including surgical threads. Polysaccharides also include callose orlaminarin, chrysolaminarin, xylan, arabinoxylan, mannan, fucoidan and galactomannan (Campbell, 1996).

1.2.6 Less known source of polysaccharides

Fruit gum is obtained from *Dyspyros cardifolia* (Fam. Ebenceae), a tree grown in tropical countries like India (Parija, *et.al*., 2001), pectins are obtained from plant cell walls, and scleroglucan, a natural exocelluar polysaccharide, is secreted by fungi of the genus *Sclerotium rolfsii* (Bhardwaj, *et.al*., 2000). Mesona blumes gum is extracted from the leaves of Liangfen Cao, also called Hsian tsao. Liangfen cao a herb found growing in south China, Indonesia, Vietnam and Burma (Feng, *et.al*., 2004). Mesquite gum from *Prosopis julifora* (Fam. Leguminosae) and gums from *Cissus populnea* of the family ampelidaceae are also intracellular gums from the stem bark of the plants (Feng, *et.al*., 2004).

1.3 Classification of natural gums

Natural gums have been classified in various ways based on source, chemical composition and structure, physical properties or whether they are acidic or neutral (Whistler and Smart, 1953; Smith and Montgomery, 1959; Izydorczk, 2005).

Classification of plant gums according to their chemical composition and structure is based on the hydrolytic nature of the product. Those gums hydrolyzing to only a single monosaccharide type are classified as homoglycans while those hydrolyzing into two monosaccharide species are classified asdi-hetroglycans. The antigenic polysaccharide produced by *Eubacterium saburreum*, strain T17, is a homoglycan composed of *d-glycero-d-galacto* heptose residues (Nazakawa, 1985).

Plant gums containing three, four and five different monosaccharide residue are classified as tri-, tetra-, and penta-hetroglycans respectively (Smith and Montgomery, 1959; Izydorczyk, 2005).

Polysaccharides have also been classified based on the arrangement of the different monomers present. They may be linear, branched or cyclic. This classification is related to the physical properties of the gum. Linear polysaccharides do not dissolve in water but swell to form thick gels and give translucent viscous solutions. The linear polysaccharides are closely packed and form many intermolecular secondary attachments which make the structure strong, rigid and insoluble. They are therefore associated with structural material in those plants in which they occur (Berger, 1962; Mithal and Khasgiwal, 1972). Examples include cellulose, xylan and chitin. Branched polysaccharides on the other hand, generally dissolve in water to give clear solutions with adhesive properties (Smith and Montgomery, 1959). The commonly branched polysaccharides are those which serve as food reserve such as glycogen and dextran. Pullulan is a branched polysaccharide obtained from the fungus *Aureobasidium pullulans.* They are easily soluble in water and have immense thickening properties (Whistler and Smart, 1953). The cyclic polysaccharides are also known as cycoamyloses.

Polysaccharides have been classified based on the number of the different monomers present as either homopolysaccharides (examples include pullulan, starch, cellulose and glycogen) or hetropolysaccharides for examples: include xanthan gum which consists of trisaccharide chain, guar gum made of mannose and galactose and acacia gum (Aspinall, 1972). While the homopoly-saccharides consist of only one type of monosaccharide, the hetropolysaccharides consist of two or more types of monosaccharide units joined by O-glycosidic linkages.

(Izydorczyk, 2005). The homopolysaccharides are further classified based on the type of glycosidic linkages which join the monosaccharide units.

A classification of gums which accommodates all the natural gums is that based upon whether the gum is acidic, neutral or basic. Acidic polysaccharide gums contain sugar acids in their structure and carry negative charges.Examples include *Xanthan* gum, *Acacia* gum, and *Tragacanth* gum. They are said to be anionic polysaccharides and carry a negative charge (Izydorczyk, 2005). Polysaccharide gums which consist of sugar units only are known as neutral polysaccharides. Most seed gums containing galactose and mannose (galactomannan) are neutral gums (Cunha et al., 2009). Chitosan obtained by modification of the neutral animal polysaccharide chitin is the only example of a cationic polysaccharide (Izydorczyk, 2005) and is an example of a polysaccharide with basic groups (Whistler and Smart, 1953; Smith and Montgomery, 1959).

1.4 Composition of natural gums

Natural gums are chain macromolecules which are highly complex polymers usually composed of different monosaccharide residues joined together by glycoside bonds. The monosaccharide residues may be hexose, pentose, and/or sugar acid units. The most common hexoses found in natural gums are glucose, galactose, rhamnose and mannose. The pentoses include arabinose and xylose, while glucuronic, galacturonic and mannuronic acids constitute the sugar acids frequently isolated from plant gums (Smith and Montgomery, 1959; Berger, 1962; Kharbade and Joshi, 1995, Bhardwaj *et.al*.2000, Parija *et.al.*,2001). (Figure 1.1) shows molecular structure of some monosaccharides and sugar acids. Lipids, lignin, salts, colouring matter, and other substances may associated with natural gums, the composi-

tion of natural gums may be achieved by degradation of the polysaccharide to the constituent monosaccharide units before analysis.

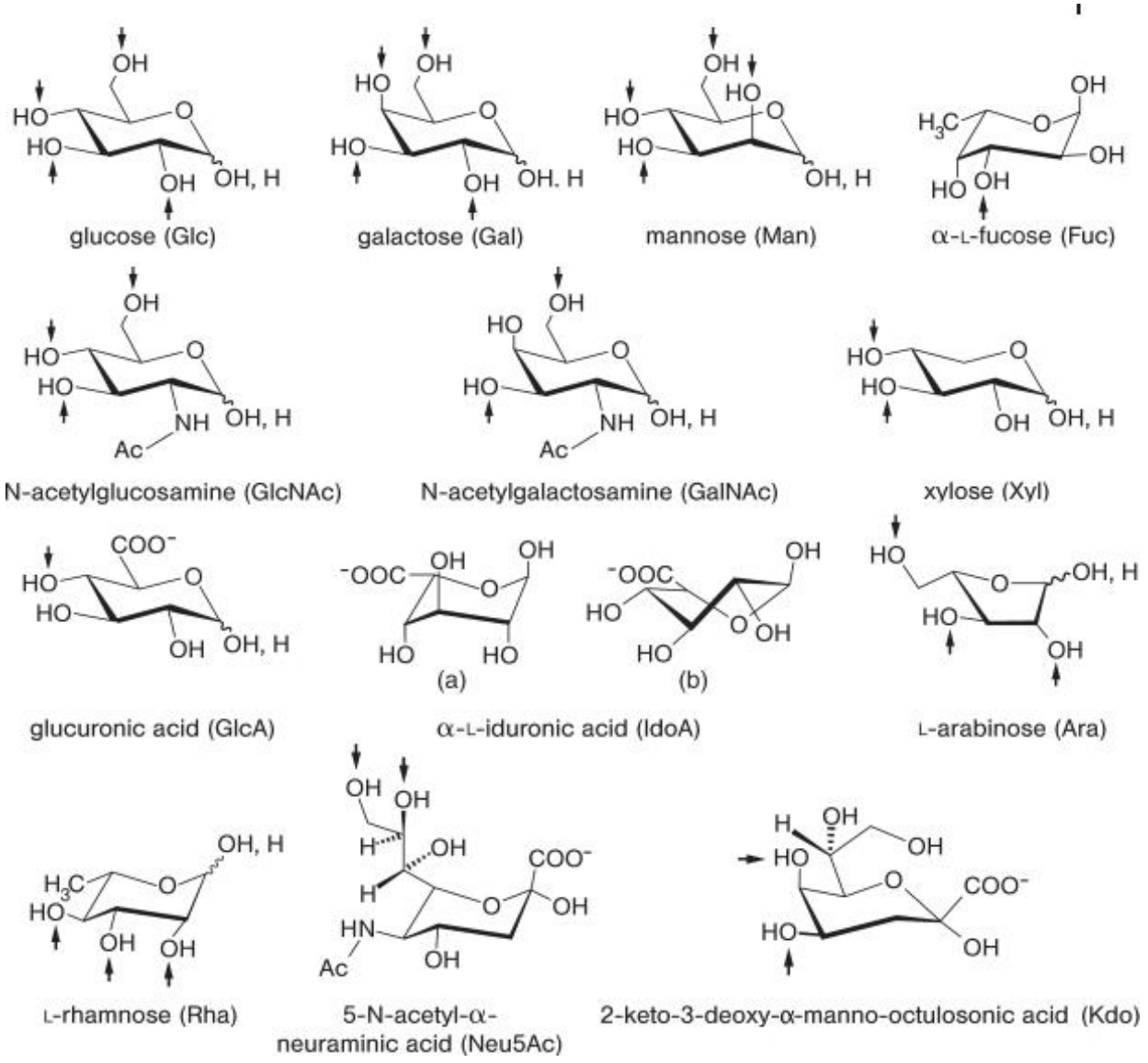


Figure 1.1: Molecular structure of some monosaccharides and sugar acids.

This degradation process has been achieved in a number of ways: controlled acid hydrolysis of the gum in the mineral acid (such as HCl or H_2SO_4) or in organic acid such as trifluoroacetic acid (TFA). Methanolysis, and also by acetolysis of polysaccharide under the influence of heat (Cui, 2005).

Polysaccharides with sugar acids present some difficulty in hydrolysis because the glycosidic linkages of uronic acids are highly resistant to hydrolysis. In such instances the stronger mineral acids are the choice for hydrolysis. The components of the hydrolysate can be separated and detected by chromatographic techniques (Cui,2005).

1.5 Degradation of Polysaccharides

The determination of the monosaccharide composition of polysaccharide polymers requires first of all that the polysaccharide be broken down into its constituent monosaccharide units.

The hydrolysis and specific or partial degradation of the polysaccharide prior to analysis of the monosaccharide or oligosaccharide components can be achieved in a number of ways.

The monosaccharide components of polysaccharides are linked by glycosidic bonds. In order to free the constituent monosaccharides, the glycosidic bonds or linkages must be cleaved. This can be achieved by the use of a mineral or organic acid in the presence of heat. However the glycosidic bonds do not all cleave at the same rate. Notably, the glycosidic bonds linking uronic acid units are difficult to cleave. This indicates that the hydrolysis of acidic polysaccharides presents greater difficulty as compared to neutral polysaccharides (Brummer and Cui, 2005). For this reason sufficient time must be allowed for complete hydrolysis of all glycosidic linkages in a sample.

It must however be borne in mined that while sufficient time is required to allow for complete hydrolysis of the sample, the length of time must not be too lengthy as to lead to degradation of the sample.

Sulphuric acid has been reported to be more efficient than trifluoroacetic acid (TFA) in the hydrolysis of fibrous substrates such as microcrystalline cellulose, wheat bran, apple and straw (*Garleb et.al*., 1989). The draw-back with sulphuric acid however, is the difficulty to remove it from sample after hydrolysis. This is not the case with TFA which is volatile and relatively easily removed prior to analysis.

Several hydrolysis procedures have been reported for natural polysaccharide gums using TFA (Brummer and Cui, 2005). When working with a new polysaccharide sample, it is worthwhile to monitor the hydrolysis protocol. The quantity of each sugar present in the solution is monitored at time intervals to ensure that the protocol is not resulting in excessive decomposition and bonds are cleaved quantitatively. The progress of hydrolysis is indicated by an initial increase in the quantity of each sugar to an optimum.

Thereafter when further hydrolysis results in degradation, this will be evident as a decrease in monosaccharide concentration as hydrolysis time increases (Brummer and Cui, 2005).

1.5.1 Methanolysis

Methyl glycosides are formed when the glycosidic bonds of polysaccharides are broken down and a methyl group introduced. Methanolysis is usually done on permethylated polysaccharides. Acid catalysed methanolysis of permethylated polysaccharidea results in the formation of methyl glycosides (Cui, 2005).

1.5.2 Acetolysis

When polysaccharides are heated in a mixture of acetic anhydride, acetic acid, and sulphuric acid in the ratio 10:10:1(Lindberge *et. al*., 1975), there is peracetylation and cleavage of selected glycosidic bonds. This process is termed acetolysis. It has been reported that glycosidic linkages in α-configuration are more susceptible to acetolysis than those in β-configration (Pazur, 1986; Harvey, 2001).

1.5.3 Smith degradation

Smith degradation is a combination of three procedures periodate oxidation, reduction, and mild acid hydrolysis. The procedure results in monosaccharide units and/or oligosaccharides (Cui, 2005).

1.5.4 Enzyme hydrolysis

Enzymes have been used to cleave acid resistant polysaccharide structures to give high yields of oligosaccharides. B-mannase is an example of a purified and specific enzyme that has been used in the enzymatic analysis of galactomannans (McCleary, 1994).

1.6 Purification of natural gums

The source of a gum, its chemical composition and nature of contaminants or impurities determine, to a large extent, the technique employed for the extraction and purification of the gum. Usually, the extraction process involves milling of the crude dried part of the plant. This is followed by dispersion in aqueous medium to form mucilage which is filtrated to remove insoluble impurities and the gum recovered by precipitation with alcohol. Repeated precipitation from acidified aqueous solution with ethanol serves to remove inorganic ions and proteinaceous impurities (Yang *et.al*., 2006), electro-dialysis may also be employed for the

elimination of inorganic ions by passing an aqueous solution of the material through a cation exchange resin (Smith and Montgomery, 1959).

Some natural gums can be purified by precipitation from aqueous or acidified aqueous solution with acetic acid while others containing a large proportion of uronic acid are best precipitated from aqueous solution with dilute mineral acids. When further purification of the gum is required, this may be achieved by fraction precipitation from aqueous solution with dilute mineral acid. When further purification of the gum is required, this may be achieved by fractional precipitation from aqueous solution with ethanol. Levels of pH that do not adversely affect the polysaccharides are maintained during fraction. Low pH values can cause hydrolysis while high pH values results in alkaline degradation (Smith and Montgomery, 1959).

Separation of natural gums may also be partially achieved by successive or repeated extraction with solvents of increasing efficacy. Successive extraction with cold or hot water, cold dilute alkaline or hot dilute alkaline can give fraction of different compositions and properties (Smith and Montgomery,1959). Natural gums that have undergone purification are usually dried by solvent exchange or by freeze-drying. Such procedures produce light amorphous powders that are reactive. When gums containing moisture are dried by methods other than this, they form hard, horny masses that are difficult to pulverize and study (Smith and Montgomery,1959). Other methods for purification of plant polysaccharides include ultra filtration. Gel filtration, column chromatography and electrophoresis (Smith and Montgomery,1959; Flindt *et.al*., 2005; Morthlagh *et.al*., 2006).

1.7 Physical properties of natural gums

The physical properties and appearance of natural gums are of high significance in determining their marketability and end use. These differ with different botanical sources. There is a considerable dissimilarity in gums from the same species collected from plants grown under different climatic conditions or even from the same plant in different seasons. Physical properties are also affected by the exudates and treatment of the gum after collection by, for example, washing, drying, sun-bleaching and storage temperatures (Glicksman and Schachat, 1959).

1.7.1 Color

Color is a perceptual phenomenon that depends on the observer and on the conditions under which it is observed. It is a characteristic of light, which is measurable in terms of intensity and wavelength. A material's color only becomes visible when light from a luminous object or source illuminates or strikes the surface (Sahin and Sumnu, 2006). Color is of great importance in thecommercial valuation of gums, with-colored gum being preferred. More than 80 years ago, the claim was made that there are no completely colorless gums, but this is still open to question (Wiesner, 1927): for example, the color of the finest gum Arabic in the Sudan has been described as colorless (Blunt, 1926). Commercial grades of *A. Senegal* from Sudan include the best grade i.e. the hand- picked, selected, cleans and largest pieces, with the lightest color, the second-best grade includes that which remains after the hand-picked material and siftings have been removed. This grade comprises whole and broken lumps with a pale to dark amber color. The standard grade has a light to dark amber color (Islam., 1997) another example of higher gum grades can be found with commercial gum *karaya* which contains less foreign matter and has a lighter color than the non-commercial gum (FAO, 1995). Gum colors depend on the plant species, climate and soil.

In its solid state, gum colors vary from almost transparent while through various shades of yellow, amber and orange to dark brown. Certain gums possess a pink, red or greenish hue. Color is primarily due to the presence of impurities: it often only appears as the gum ages on the tree and may be due to substances that have washed onto the gum (Howes, 1949). There is no doubt that old trees give off a dark gum. In addition, scorching from bush or grass fires darkens gums, and tannin from the sap or tissues of the parent plant is also believed to account for some of the very dark gums yielded by certain trees (Howes, 1949).

1.7.2 Size and shape

As seen or collected under natural conditions, gums are represented by a variety of shape and forms (Howes, 1949). The fragments are generally irregularly round, drop- or pear-shaped, as is well-illustrated in the variety of commercial grade of gum Arabic. Some gums are characterized by stalactitic shapes. Following collection and possible fraction and possible fracturing,irregular rod-shaped shaped segments can appear, as demonstrated by cashew gum (Howes, 1949). Gum tragacanth s̓ name is derived from the two Greek words *tragos* (goat) and *akantha* (horn) and probably refers to the curved shape of the ribbons in the best grade of commercial gum (Verbeken *et.al*., 2003). The surface of most gums is, perfectly, smooth when fresh, but may, quickly, become rough or covered with minute cracks due to weathering (Howes, 1949). These fissures are not restricted to the surface, and therefore in some gums might serve to break a tear into smaller fragments.

After collection, the gum is cleaned and graded. This is traditionally done by manual sorting according to the size of lumps (FAO, 1995). The gum can be further processed into kibbled and powdered forms size distribution,to facilitates dissolution of the gum in water. Better solubility can be obtained with powdered gum, which is usually produced by dissolving the gum in water, removing

impurities and spray- drying (Verbeken *et.al*., 2003). It is possible to specify the size of regular gum lumps, but for irregular pieces, the size must be arbitrarily specified (McCabe *et.al*., 1993).

Size can be determined using the projected-area method: the longest dimension of the maximum project area, the minimum diameter of the maximum projected area diameter and the shortest dimension of the minimum projected area defined (Sahin and Sumnu, 2006). Shape can be expressed in terms of sphericity and aspect ratio. Sphericity is the volumetric ratio of the solid to a sphere that has diameter equal to the major diameter of the object such that it can circumscribe the solid sample (Mohsenin, 1970). Sphericity was first defined 70 years ago (Wadell, 1935). Ψ, of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of particle:

$\Psi = \pi^{1/3} (6 V_p^{2/3})/A_p$----------► (Equation 1.1)

where V_p is the particle volume and $A_p$is its surface area. Shericity has also been defined differently by other researchers (McCabe *et.al*., 1993; Bayram, 2005). The aspect ratio is calculated using the length and width of a sample. It may be applied to two characteristic dimensions of a three dimensional shape, such as the ratio of the longest to short axis, or for symmetrical objects that are described by just two measurements, such as the length and diameter of a rod (Maduako and Faborode, 1990). Such definitions should be entered into the shape field of the classification of collected exudates in order to render it less descriptive, and more scientific.

1.7.3 Taste and smell

True gums are generally odorless, or nearly so (Howes, 1949). In contrast, resins and oleoresins have distinctive odors. For example, the gum resin opoponax is obtained by wounding the roots of *opopamax chironium* (L.) W.D.J. Koch (*syn. Pastinaca opoponax*), a native plant of the countries surrounding the levant.

It occurs in lumps that are reddish-yellow on the surface, but white within. It has a bitter and acrid taste, and a peculiar smell. Due to its exotic aroma, opoponax has long been used in perfumery. Its aroma has also been described as smooth and sweet, like a bit of toffee mixed with subtle myrrh (http://www.somaluna .com/prod/opoponax.asp). Another example is myrrh, which forms as reddish yellow tears. It has a peculiar smell, an aromatic bitter taste. In water, it forms a yellow opaque solution. It becomes opaque in an alcoholic solution when water is added, but there is no precipitate (Turcotte, 2006).

In order to experience plant odors, a courtyard of senses was created in 1999 in montreal Botanical Garden, designed to give its visitors a whole new way of "seeing" the plant world. In the last area of the garden, one finds the common gums cistus, which is covered in a resinous secretion that sticks to the fingers, perovskia, which has a penetrating fragrance that takes the visitor by surprise if the leaves are rubbed, and the blue gum tree, which has a strong medicinal camphor smell (Turcotte, 2006). Gum resins denote a class of vegetable substances which are regarded by chemists as consisting of gum and resin. They are generally contained in the vessels of the plant, i.e. the root, stem, branch, leaves, flowers, or fruit. Gum resins, in their solid state, some are brittle, and they generally some have a strong smell, which is sometimes bitter and nauseating (Howes, 1949). Gums may be tasteless or devoid of any characteristic taste. Nevertheless there is evidence that some gums are slightly sweet or bitter according to their botanical origins. Some gums have, a distinctly, bitter, lingering after taste, and this should of course be considered when gums are used as food additives (Howes, 1949).

1.7.4 Hardness and Density

Hardness characterizes the resistant to scratching of various minerals based on the ability of a harder material to scratch a softer one. Attempts to classify gum hardness as with minerals in order to use hardness as diagnostic character for gum identification have not proven at all satisfactory (Howes,1949).

Among many other things, hardness in gums depends on moisture content, which in general ranges between 12% and 16% (Howes,1949). For example, the moisture content, of the gum obtained from *A. Senegal var*. *Senegal* ranges from 12.5% to 16.0% (Idris *et al*. 1998). Analytical data for exudates from the Turkish *Astragalus* species demonstrate a loss of dryingbetween 12.7% and 9.9% weight (Anderson and Bridgeman, 1985). Density also proves unpredictable, even in the same gum, as it depends in part on the quantity of air that may have been trapped during its formation.

1.7.5 Specific optical rotation

Optical rotation or activity defines the rotation of linearity polarized light as it passes through the solution of certain materials: these can be solutions of chiral molecules, e.g. sucrose (sugar), solids with rotated crystal planes such as quartz, and spin-polarized gases of atoms or molecules, polarization is used to measure syrup concentration, in optics to manipulate polarization, in chemistry to characterize substances in solution, and it is being developed as a method to measure blood sugar concentration in diabetics (http://en.wikipedia.org/ wiki/Optical _rotation). In aqueous solutions, gums are either levorotatory or dextrorotatory. For example, eight gum specimens from *Pereskia guamacho* (Cactaceae) are dextrorotatory acidic arabinogalactans and give very clear solutions of moderate viscosity (De Pinto *et.al*.,1994). The gum from *Cyamopsis tetragonolobus* (guar gum) (Omaira *et.al*., 2007).

Samanea samana and *Pithecellobium mangense*exude clear yellow gums, both dextrorotatory (De Pinto *et.al*., 1995). Venezuelan gum exudates from nine specimens of *Parkinsonia* (Leguminosea) were examined. The samples, which were highly soluble in water and levo-rotatory, have viscosities comparable to that of gum Arabic from *A. Senegal var. Senegal* (De Pinto *et.al*., 1993).

The sugar composition and amino acid content of a gum solution influences whether gums are levorotatory or dextrorotatory. In *acacia seyal* and *A. Senegal var. senegal* gums, the sugar composition and amino acids are identical but are present in different proportions, which is the main reason why *A. SeyalVar. Seyal*is dextro-rotatory and *A. SenegalVar. Senegal*is levorotatory (Flindt *et.al*., 2005).

1.7.6 Solubility

Gum s' solubility may be influenced by age and the length of time that it has been attached to the tree. Most gums yield a certain amount of insoluble residue when mixed with water and in general, there is more of this residue with dark-colored gums with the pale or light-colored gums (Howes, 1949). For example, native gum *karaya* is insoluble and only swells in water, due to the presence of acetyl groups (Imeson, 1992). Based on their solubility in water, three fractions were distinguished in gum *Karaya* (Le Cerf *et.al*., 1990).

Only 10% of the native gum solubilizes in cold water, but this fraction increases to 30% in hot water. After deaceylation, 90% of the native gum dissolves in water. Only low molecular weight molecules are able to dissolve in cold water. While deacetylation leads to the solubilization of higher- molecule weight material (Le Cer *et.al*., 1990). Better solubility can be achieved by breaking large lumps into small more uniform granules, and solubility can be further enhanced using spray-dried powder (Verbeken *et.al*., 2003).

Many macromolecules are made up of a hydrophobic portion that creates an "inside", consisting of segments not in contact with the solvent, "outside" consisting of the more hydrophilic groups that are in contact with water (Whistler, 1973). Some electrolytes are able to stabilize macromolecular conformations in aqueous solutions (Whister, 1973).

The free energies of the macromolecule states are the sum of the individual group solvent and group-group interactions. A change of a kilocalorie or less per mole in one of stabilizing interactions engaged in maintaining the delicate balance is sufficient to generate cooperative transition to a different conformation (Whistler, 1973).

1.7.7 Viscosity

"Viscosity" (or "absolute viscosity") is frequently referred to as dynamic viscosity. It is the internal friction of a liquid or its tendency to resist flow (Bourne, 1982). In colloidal suspensions, viscosity is increased by the thickening of the liquid phase due to liquid absorption and resultant swelling of the dispersed colloid (Glicksman, 1969). This in turn, is accountable for functional properties such as suspension of solid particles, emulsification of oil and water phases, stabilization of liquid-solid-gas phases, and related phenomena (Glicksman, 1969).

Selection of the proper hydrocolloid is crucial for an operation's success and for the food system's shell stability. The viscosity of the hydrocolloid system depends on 10 factors: concentration, temperature, degree of dispersion, solvation, electrical charge, previous thermal treatment, previous mechanical treatment, presence or absence of other lyophilic colloids, age of lyophilic sol and presence of both electrolytes and non-electrolytes (Ostwald, 1922).

These factors are considered “the ten commandments of food preparation” (Lowe, 1955). Gum exudates are usually ground to a powder, with particle size varying according to the desired use. Although gum Arabic is highly branched, it has compact structure (Nussinovitch, 1997).

Gum Arabic solutions are distinguished by their low viscosity, enabling the use of high gum concentrations in various applications (Dziezak, 1991; Imeson, 1992). Solutions display Newtonian behavior at concentrations of up to 40% and become pseudo plastic at higher concentrations. Above~ 30%, the hydrated molecules effectively overlap and steric interactions results in much higher solution viscosities and increasing pseudo plastic behavior (Nussinovitch, 1997).The *p*H of the solution is usually around 4.5 to 5.5, but maximal viscosity is found at a *p*H 6.0 (extension of the molecule). At still higher *p*H, ionic strength of the solution increases until the repulsive electrostatic charges are masked, yielding a compact conformation with lower viscosity (Anderson *et.al*., 1990; Williams *et.al*., 1990a; Imeson, 1992). Due to gum Arabic’s high water solubility, low viscosity, and emulsification properties, it is used in soups and dessert mixes (Glicksman and Farkas, 1975). The high quality gum tragacanth has high viscosity, good solution color and low microbial count (Nussinovitch, 1997). Storage of the dry gum results in loss of viscosity (Dziezak, 1991).

The viscosity of a 1% solution of gum *Karaya* at normal *p*H is approximately 3,300 cps (Table 1.1). Maximal viscosity is achieved at *p*H 8.5. Boiling of the dispersion results in a permanently reduced viscosity (Whistler, 1973).

Heating predominantly via pressure, gives smooth, uniform, semi-transparent, colloidal dispersions, with concentrations as high as 20 to 25% being achievable in this method relative to 3 or 4% maximal concentration obtained by non-heated water hydration (Glicksman, 1969). The viscosity of a 1% solution of the highest grade of gum tragacanth is approximatly 3,400 cps. In cold preparations, the maximum viscosity is usually reached after 24 hours, but this can be obtained in about two hours by raising the temperature of the solution to about 50^{o}C (Glicksman, 1969).

Viscosity of food products is not necessarily correlated with their mouthfeel, for example, beverages of identical viscosity can be either slimy and coat the mouth or smooth and pleasant (Glicksman, 1969). A correlation between the organoleptic characteristics of hydrocolloid solutions and rheological behavior has been established (Szczesniak and Farkas, 1962). Measurement of solution viscosities at various rates of shear showed a relationship between the shape of the curve and the degree of sliminess. This was conformed in a work on gum-thickened sucrose solutions (Stone and Oliver, 1966). Various hydrocolloid solutions were grouped into two categories: slimy (pectin, methylene cellulose, carboxymethyl cellulose, sodium alginate, locust bean gum), slightly slimy (starch). This method makes it possible to select the best gums for a desired mouthfeel or texture.

1.7.8 Intrinsic Viscosity

When a polymer is in solution, its viscosity is always directly related to the relative size and shape of the polymer molecules (Wang and Cui, 2005). Dilute solution viscometry is one of the methods most widely used for the characterization of the molecular weight of polysaccharides. The instrumentation is minimal and data interpretation simple and straight forward.

In dilute solution viscosity, relative viscosity$[\eta_r]$ is measured for a series of dilute solutions of the polymer or polysaccharide, which allow for calculation of interinstic viscosity $[\eta]$. Molecular weight can then be calculated using the Mark-Houwink equation (Idris *et.al*., 1998):

$[\eta] = KM^a$----------► (Equation 1.2)

Where, M is the viscosity average molecular weight, a and k are constants. This requires that the constants *k* and *a* be known. These are determined by use of absolute methods such as sedimentation and osmometry. Dilute solution viscosity does not give absolute molecular weight values but only a relative measure of the polymer's molecular weight (Wang and Cui, 2005).

The determination of intrinsic viscosity $[\eta]$, is theoretically based on the Huggins (Huggins, 1942) and Kraemer (Amalvy, 1997) equations:

$$\eta_{sp} = [\eta]c + k\,[\eta]^2c^2 \text{---------►} \quad \text{(Equation 1.3)}$$

$$In(\eta_{rel}) = [\eta]c + (k' - 0.5)[\eta]^2c^2 \text{---------►} \quad \text{(Equation 1.4)}$$

Where k' is called the Huggins coefficient and *C* is the concentration of the polymer. Determination of $[\eta]$ requires that the viscosity of several dilute solutions are determined and plotted in the forms of η_{sp}/c or $In(\eta_{rel})/c$ *vs. c.* Ideally, both lines usually extrapolate to the same point at zero concentration to give the intrinsic viscosity. The average of the two intercepts gives the intrinsic viscosity where the two intercepts are not identical (Vinod *et.al*., 2008).

The intrinsic viscosity of the polymer solution is an index of the inherent ability of a polymer to increase solution viscosity (Xiaodong and Marek, 2007) and is directly related to its molecular weight. Intrinsic viscosity is measure of the dynamic volume occupied by the isolated polymer chains in a given solvent. Unlike reduced or specific viscosity, intrinsic viscosity does not depend on

polymer concentration. It depends on three factors: molecular structure, molecular weight and solvent quality.

Table 1.1: **Variation of viscosity with concentration of various Gums.**

Exudate %	Gum Arabic	Gum *Ghatti*	Gum *Karaya*	Gum*Tragacanth*
0.5		-	400	-
1.0	-	2	3.300	54
1.5	-	-	-	-
2.0	-	35	-	906
2.5	-	-	-	-
3.0	-	-	-	10.605
3.5	-	-	-	-
4.0	-	-	-	44.275
5.0	7.3	288	-	111.000
6.0	-	-	-	183.500
7.5	-	1.012	-	-
10.0	16.5	2.444	-	-
20.0	40.5	-	-	-
30.0	200.0	-	-	-
35.0	423.8	-	-	-
40.0	936.3	-	-	-
50.0	4162.5	-	-	-

1.7.9 Newtonian fluids

When the viscosity of a material does not change irrespective of the shear rate at a constant temperature, in accordance with Newton ś law, such a material is said to exhibit Newtonian flow and obey the relationship:

$$\eta = \frac{\tau}{d\gamma/d\tau} = \frac{\tau}{\gamma} \longrightarrow \text{(Equation 1.5)}$$

Where, the viscosity (η)is defined as ratio of applied shearing stress (τ) to rate of shear strain ($d\gamma/d\tau$) or simply shear rate (γ). Examples of fluids that exhibit New-

tonian flow are water and thin motor oils. The viscosity of a Newtonian fluid will remain the same at constant temperature (www.brookfieldengineering.com/education/what-is-viscosity-asp).

1.7.10 Non-Newtonian fluids

The relationship $d\gamma/d\mathcal{T}$ is not a constant in non-Newtonian fluids. Such polymer solutions or dispersions do not obey equation 1.1. In these fluids the viscosity changes as the share rate is varied and the model of viscometer, spindle number and speed (in the case of the Brookfield viscometer) all affect the measured viscosity. The measured viscosity is called the apparent viscosity of the fluid and is accurate only when the explicit experimental parameters are adhered to (Cui and wang, 2005) ; (www.brookfieldengineering.com/education/what-is-viscosity-asp). In these types of fluid when shear rate increase apparent viscosity, the polymer solution or dispersion is said to exhibit a shear thinning decrease behavior.

This phenomenon is also called pseudoplastic flow behavior. Examples of pseudoplastic fluids are paints, emulsions and different types of dispersed systems.

Conversely, when a polymer solution or dispersion shows an increase in viscosity with an increase in shear rate, it is said to exhibit a shear thickening behavior also called dilatants flow behavior.

Dilatant flow is very often displayed by highly deflocculated systems such as clay slurries, candy compounds, corn starch in water. However, under static conditions, certain fluids behave as solids and force must be applied in order to induce flow. This force that must be applied in order to induce flow is called the yield value. In such systems the polymer solution or dispersion only starts to flow when the yield value is exceeded usually by vigorously shaking. Such fluids are said to exhibit plastic fluids will exhibit Newtonian, pseudo plastic or dilatants flow characteristics (www.brookfieldengineering.com/education/what-is-viscosity-asp).

Under conditions of constant shearing, some fluids show a change in viscosity over time. Two categories are identifiable. If when shearing is constant over a period of time a fluid shows a decrease in viscosity, it is said to exhibit thixotropy.

When a fluid's viscosity increases as a function of time under continuous shear at constant rate, it is said to exhibit rheopexy.

The knowledge of a material's rheological characteristics is valuable in predicting pumpability and pourability performance in a dipping or coating operation, or the ease with which it may be handled, processed or used. It is also of value predicting redispersibility of dispersed systems during storage.

1.7.11 Solubility characteristics

A wide range of solubility is displayed by plant polysaccharides. Some dissolve readily in cold water while some are only soluble in hot water, and some insoluble ones will not dissolve even in boiling water. For example *Xanthan*gum dissolves in cold water, *Khaya grandifolia* (Fam. *Meliaceae*) gum dissolves only in hot water (Aslam *et.al*., 2006) while *karaya* gum will not dissolve even in boiling water, this behaviour is determined primarily by two factors: molecular structure and molecular weight of the plant gum or polysaccharide (Wang and Cui, 2005). Natural polysaccharides are not as the charged polysaccharides while linear polysaccharides with high regularity in structure are essentially insoluble in aqueous media. Polysaccharides with regular conformation that can form crystalline or partial crystalline structures are usually insoluble in water. The irregularity of molecular structure allow many polysaccharides to readily hydrate and dissolve in water. Generally, solubility increases with reduced regularity in molecular structure. Polysaccharides with high levels of branching or substitution of polysaccharide chains tend to show an increase in solubility due to the reduced possibility of intermolecular association.

According to Wang and Cui (2005), any structures that contain especially flexible units such as (1- 6) glycosidic linkages are more soluble because of large favourable entropy of the solution.

The dissolution rate for polysaccharide samples of similar particle sizes decreases with increasing molecular weight. This is because disentanglement from the particle surface and subsequent diffusion to bulk solution of large molecules takes a longer time compared to small molecules (Wang and Cui, 2005).

The process of dissolution of plant gums is sometimes complex and occurs in stages. When the polymer is introduced into solvent, no obvious interaction may take place initially. Later, the polymer becomes swollen as the solvent molecules diffuse into it.

Swelling is usually rapid for finely divided polymers and when the solvent is a small, compact molecule, which the rate determining step in the dissolution process. The volume of the polymer phase increases as the solvent is imbibed, but few polymer molecules enter the solvent phase because of their low diffusion rates (Collins *et.al*., 1973). Finally, the swollen polymer particles disintegrate, and the individual polymer molecules diffuse until a true solution is formed.

Agitation is helpful in speeding this final process. The higher the molecular weight of the polymer, the slower is this final stage in the dissolution process.

Up to 37% w/v of *AcaciaSenegal* var. *Senegal* gum dissolves in water at 25^{o}C to form a clear solution whereas tragacanth gum forms a thick, viscous and mucilaginous semi-gel in water (Smith and Montgomery, 1959; Whistler and Smart, 1953). Some plant gums swell in water to form viscous dispersions while others are either completely water-soluble or water-insoluble. Upon addition of mono-valent, divalent or trivalent metals, solutions of most plant gums form or produce precipitates.

The stability of these polymer solutions is also affected by *p*H changes. The polymers are precipitated out of solution by high concentrations of organic solvents. Gum polymers are precipitated out of solution by high concentrations of organic solvents such as ethanol or acetone.

Swelling is an integral property requirement for efficient and effective disintegration action and water penetration or swelling is now the most widely accepted mechanism for tablet disintegration (Hahm, 2000; Zhao and Augsburger, 2005).

The ability of plant gums or polysaccharides to absorb water and swell is suggestive of good disintegrant action which depends upon the concentration of the gum used. Swelling has been described as either intrinsic or bulk swelling, both of which have been shown to be dependent on pH, temperature and concentration of the polymer in question (Shangraw *et. al*.,1980; Chen *et.al*., 1997).

1.7.12 Molecular Weight

Natural gums or polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water (Varki A; 2008).

When all monosaccharides in a polysaccharide are the same , the polysaccharide is called a homopolysaccharide or homoglycan, but when more than one type of monosaccharide is present they are called heteropolysaccharides or hetero-glycans (Varki A; 2008).

Natural saccharides are, generally simple carbohydrates called monosaccharides with general formula $(CH_2O)_n$ where n is three or more. Examples of monosaccharides are glucose, fructose, and glyceraldehyde (IUPAC; 1997).

Polysaccharides, meanwhile, have a general formula of $C_x(H_2O)_y$ where x is usually a large number between 200 and 2500. Considering that the repeating units in the polymer backbone are often six-carbon monosaccharides, the general formula can also be represented as $(C_6H_{10}O_5)n$ where $40 \leq n \leq 3000$ (Figure 1.2).

Natural gums or polysaccharides contain more than ten monosaccharide units. Polysaccharides are an important class of biological polymers. Their function in living organisms is usually either structure- or storage-related. Starch (a polymer of glucose) is used as a storage polysaccharide in plants, being found in the form of both amylose and the branched amylopectin. In animals, the structurally similar glucose polymer is the more, densely, branched glycogen, sometimes called 'animal starch'. Glycogen's properties allow it to be metabolized more quickly, which suits the active lives of moving animals.

Cellulose and chitin are examples of structural polysaccharides (Figure 1.3). Cellulose is building unit of the plants cell walls, such as organisms, and is said to be the most abundant organic molecule on earth (Mathew S.; 2008). It has many uses such as its significant role in the paper and textile industries, and is used as a feedstock for the production of rayon (via the viscose process), cellulose acetate, celluloid, and nitrocellulose. Chitin has a similar structure, but has nitrogen containing side branches, increasing its strength. It is found in arthropodexo skeletons and in the cell walls of some fungi. It also has multiple uses, including surgical threads. Polysaccharides also include callose or laminarin, chrysolaminarin, xylan, arabinoxylan, mannan, fucoidan and galactomannan (Campbell; 1996).

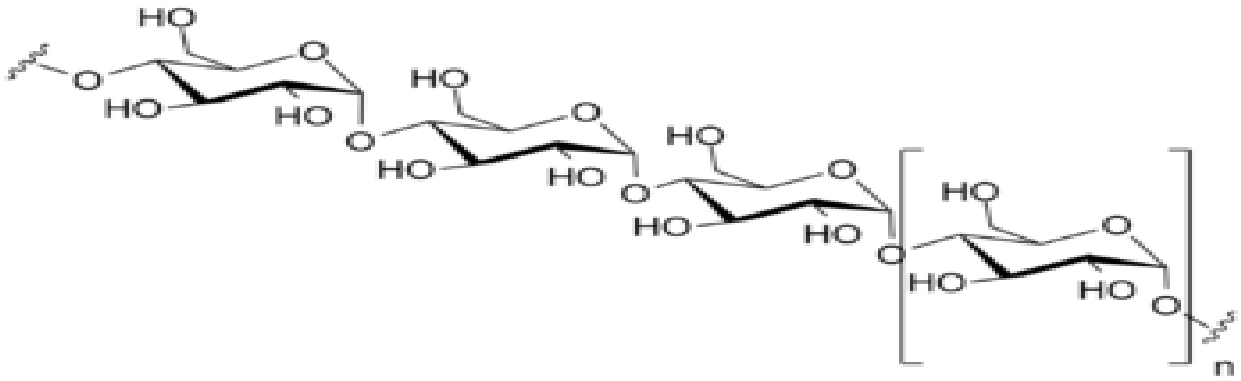


Figure 1.2: Amylose is a linear polymer of glucose mainly linked with α(1→4) bonds. It can be made of several thousands of glucose units. It is one of the two components of starch, the other being amylopectin.

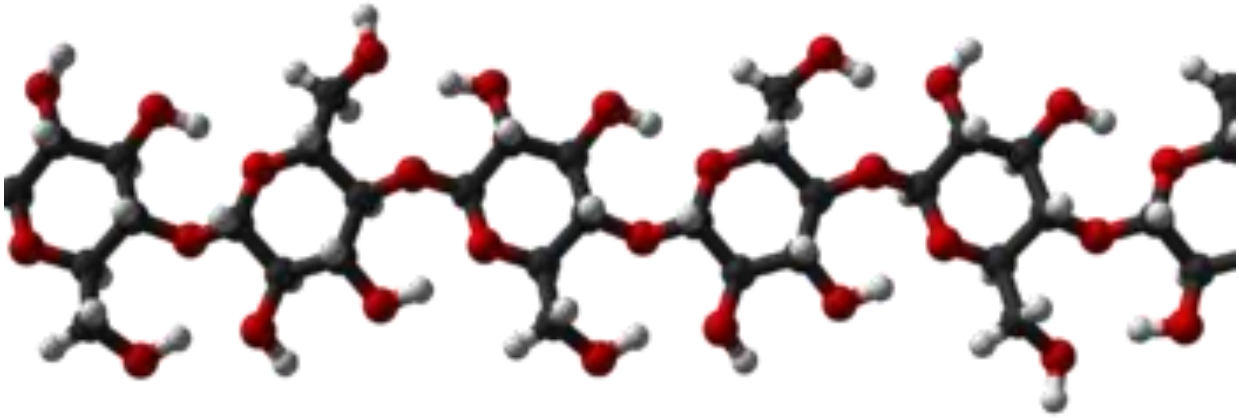


Figure 1.3: 3D structure of cellulose, a beta-glucan polysaccharide.

1.7.13 Size Exclusion Chromatography (SEC)

Size exclusion chromatography (SEC) is also known as gel permeation chromatography or gel filtration chromatography. Separation is by molecular size rather than by chemical properties. The polymer solution is pushed through the SEC column causing polymer chains to separate according to differences in hydrodynamic volume. Column construction allows access of smaller molecules and excludes the larger ones. Thus the retention volume (or time) of fraction provides a measure of the molecular size. The resulting chromatogram represent a molecular size the resulting chromatogram represents a molecular size distribution. SEC does not yield absolute molecular weight rather it requires calibration using standards of known molecular weight by converting the retention volume to a molecular weight for a given column set. Several approaches have been used for calibration. The peak position method is that approach in which narrow fraction standards (e.g. pullulan and dextran) are used for calibration curve of log molecular weight (*MW*) against retention volume. The calibration curve of log used for determination of unknown molecular weight.

Under or over-estimation of the molecular weight of test polymer can occur if there is a difference in structure between the calibration standard and the test polymer. Values are therefore reported as pullulan or dextran equivalent molecular weight as appropriate (Wang and Cui, 2005).

Inconsistency caused by use of improper standard as already described, can be overcome by using the universal calibration approach which is based on the observation that [η] x *MW* is directly proportional to hydrodynamic volume of a polymer (Benoit *et.al*., 1966).

In this case, a plot of log [η] *MW* against retention volume emerges to a common line (the so-called universal calibration curve). Narrow fraction standards are also used. Provided the intrinsic viscosity is known, the peak *MW* is read from the calibration curve.

1.7.14 Polysaccharide gels

Polysaccharides have the ability to form gels and often used as gelling agents in both Food and Pharmaceutical industries. Gellation by a polysaccharide is induced in the first instance by dissolution or dispersion of polysaccharide in solution. This forces the disruption of the hydrogen bonds, in the solid state resulting in the formation of sols. In order for sols to transform to gel, subsequent treatment by addition of cations or co-solutes, temperature and/or pH change is required (Wang and Cui, 2005).

These treatments can either decrease intramolecular interactions or increase intra-molecular interactions. Intermolecular attractions contributing to the gelation process include hydrogen bonding, ionic or ion dipole bonding, *Van der waals* attractions, and hydrophobic interactions.

The gelation of polysaccharides can be affected by several factors. These factors include structural features of the polysaccharide, polymer concentration, molecular weight, ionic strength, *p*H, and the presence of co-solutes. The formation of polysaccharide gels requires that polysaccharide chains or segments must assume ordered structures which cross-link with each other to form a stable three dimensional network. Gels with different properties and gelation mechanisms can result for the same polysaccharide if there are variations in the fine structure of the polymer chains.

The formation of gels only occurs when the polymer concentration exceeds a critical concentration and there is a minimum critical chain length necessary for the cooperative nature of the interaction. This is in the range of 15 to 20 residues (Whistler,1973). Co-solutes such as sugars can enhance the gelation of poly-saccharides. Altering the pH or the type and amount of counter-ions can also considerably affect the properties of polysaccharide gel (Wang and Cui, 2005).

1.7.15 Surface activity

The property of some polysaccharides that has made them useful as emulsifiers and emulsion stabilizer is the ability to reduce the surface tension of water. They form protective layers through adsorption at oil-water interfaces (Dickinson, 2003). The general trend by which the surface tension is reduced has been documented (Wang and Cui, 2005): the initial addition of polysaccharides dramatically reduces the surface tension; further increase in polysaccharide concentration results in continued decrease of the surface tension of water until a saturated concentration is reached; above which, no further reduction of surface tension should be observed forming protective layers. A polymer must possess a number of characteristic in order to be effective in stabilizing dispersed particles or emulsion droplets (Dickin-son, 2003).

(i) The polymer must be able to adsorb ,strongly, at the oil-water interface. This means that the polysaccharide should possess a good degree of hydrophobic as well as hydrophilic character (amphiphilic). This property will ensure that the polysaccharide remains permanently anchored at the interface.

(ii) The polysaccharide must have , complete, surface coverage of both oil and water phases. This requires good solubility of the polysaccharide in water so that sufficient polymer chain is present in the continuous phase that will allow for full coverage of the surface of the oil droplets.

(iii) The ability to form a thick steric protecting layer and / or charged protecting layer will help to stabilize the emulsion systems.

Polysaccharides such as methylcellulose and hydroxypropyl methylcellulose have been converted to amphiphilic polymers by the introduction of hydrophobic groups to the sugar units (Gaonkar, 1991).

1.8 Uses of gums

Gum is used primarily in the food industry as a stabilizer. It is edible and has E number E414. Gum is a key ingredient in traditional lithography and is used in printing, paint production, glue, cosmetics and various industrial applications, including viscosity control in inks and in textile industries, although less expensive materials compete with it for many of these roles (http://en.wikipedia.org/wiki/Gum_arabic).

Gum is a mixture of polysaccharides and glycoproteins gives it the properties of a glue and binder which is edible by humans. Other substances have replaced it in situations where their toxicity is not an issue, as the proportions of the various chemicals in gum vary widely and make it unpredictable. Still, it remains an important ingredient in soft drink syrups, "hard" gummy candies such asgumdrops, marshmallows, M&M's chocolate candies, and edible glitter, a very popular modern cake-decorating staple. For artists, it is the traditional binder used in water color paint, in photography for gum printing, and it is used as a binder in pyrotechnic compositions. Pharmaceutical drugs and cosmetics also use the gum as a binder, emulsifying agent and a suspending or viscosity increasing agent (Smolinske, 1992). Gum has been used in the past as a wine fining agent (Vivas N., 2001).

It is an important ingredient in shoe polish, and can be used in making homemade incense cones. It is also used as a lickable adhesive, for example on postage stamps, envelopes, and cigarette papers. Lithographic printers employ it to keep the non-image areas of the plate receptive to water (*Printing Process Explained*., 2012) . This treatment also helps to stop oxidation of aluminium printing plates in the interval between processing of the plate and its use on a printing press.

1.8.1 Food

Gum is also used as an emulsifier and a thickening agent in icing, fillings, chewing gum and other confectionery treats (Laura Rinsky., 2009).

1.8.2 Painting and art

Powdered gum for artists, one part gum is dissolved in four parts distilled water to make a liquid suitable for adding to pigments.

Gum is used as a binder for water color painting because it dissolves easily in water. Pigment of any color is suspended in varying amounts, resulting in water color paint (Laura Rinsky., 2009).

Water acts as a vehicle or a diluent to thin the watercolor paint and helps to transfer the paint to a surface such as paper. When all moisture evaporates, the acacia gum binds the pigment to the paper surface. After the water evaporates, the acacia gum in the paint film increases luminosity and helps prevent the colors from lightening. Gum allows more precise control over washes, because it prevents them from flowing or bleeding beyond the brush stroke. In addition, *Acacia Senegal* gum *Var.Sensegal* slows evaporation of water, giving slightly longer working time (Laura Rinsky., 2009).

1.8.3 Photography

The historical photography process of gum bichromate photography uses gum mixed with ammonium or potassium dichromate and pigment to create a coloured photographic emulsion that becomes relatively insoluble in water upon exposure to ultraviolet light. In the final print, the *Acacia Senegal* gum *Var.Sensegal* permanently binds the pigments onto the paper (Laura Rinsky., 2009).

1.8.4 Printmaking

Gum is also used to protect and etch an image in lithographic processes, both from traditional stones and aluminum plates. In lithography, gum by itself may be used to etch very light tones, such as those made with a number five crayon. Phosphoric, nitric or tannic acid is added in varying concentrations to the *Acacia Senegal* gum*Var.Sensegal* to etch the darker tones up to dark blacks. The etching process creates a gum adsorbing layer within the matrix which attracts water, ensuring that the oil based ink will not stick to those areas. Gum is also essential to what is sometimes called paper lithography, printing from an image created by a laser printer or photocopier (Renard, D., 2006).

1.8.5 Pyrotechnics

Gum is also used as a water-soluble binder in fireworks composition (http://en. wikipedia.org/wiki/Gum_arabic).

1.9 Production of gums

The gum exported came from the band of *Acacia* trees which once covered much of theSahel region: the southern littoral of the Sahara Desert running from the Atlantic to the Red Sea. Today, the main populations of gum-producing *Acacia* species are harvested from the African gum belt countries at a broad band, situated at a latitude of between 12° and 16° North, (Figure 1.4) :Mauritania, Senegal, Mali, Burkina Faso, Niger, Nigeria, Chad, Cameroon, Sudan, Eritrea, Somalia, Ethiopia, Kenya and Tanzania. *Acacia SenegalVar. Senegal*is tapped for gum by cutting holes in the bark, from which a product called *kordofan* or *Senegal* gum is exuded (Navarro Alain., 2008). *Seyal* gum, from *Acacia seyal Var. Seyal*, the species more prevalent in East Africa, is collected from naturally occurring extrusions on the bark. Traditionally harvested by semi-nomadic desert pastoralists in the course of their transhumance cycle, *Acacia* gum remains a main export of several African nations, including Mauritania, Niger, Chad, and Sudan. The hardened extrusions are tapped after the rainy season mid October, and collection start in December, after that exported at the dry season (Navarro Alain., 2008).

Total world gum arabic exports are today (2008) estimated at 60,000 tonnes, having recovered from 1987–1989 and 2003–2005 crises caused by the destruction of trees by the desert locust. Sudan, Chad, and Nigeria, which in 2007 together produced 95% of world exports (Navarro Alain., 2008).

1.9.1 Political aspects

Gum grows across the 'African gum belt' stretching across the Africa continent, from Senegal and Mauritania in the west, to Ethiopia, Somalia and Kenya in the east. Traditionally gum has been associated with Sudan, which remains an important supplier to the industry.

However, over the past 15 - 20 years there has been a substantial diversification on the part of processors across the sub-Saharan belt, so the principle harvesting countries today would be Chad, Nigeria and Sudan, with insignificant volumes coming fromcountries like Mali and Kenya (John Corcoran, and Karen Manheimer, 2010).

1.9.1.1 Gums Arabic production

In 1445, Prince Henry the Navigator set up a trading post on Arguin Island (off the coast of modern Mauritania), which acquired *Acacia* gum and slaves for Portugal. With the merger of the Portuguese and Spanish crowns in 1580, the Spaniards became the dominant influence along the coast. In 1638, however, they were replaced by the Dutch, who were the first to begin exploiting the *Acacia* gum trade. Produced by the *Acacia* trees of Trarza and Brakna, and used in textile pattern printing, this *Acacia* gum was considered superior to that previously obtained in Arabia (ARGUIM, 1911). By 1678, the French had driven out the Dutch and established a permanent settlement at Saint Louis at the mouth of the Senegal River, where the French Company of the Senegal River (Compagnie Française du Sénégal) had been trading for more than fifty years (ARGUIM, 1911). Moorish tribes meet to trade gum arabic at Bakel on the Senegal river, 1890 (Webb, James., 2009). For much of the 19th century, gum arabic was the major export from French and British trading colonies in modern Senegal and Mauritania. France in particular first came into conflict with inland African states over the supply of the commodity, providing an early spur for the conquest of French West Africa (Webb, James., 2009). Gum Arabic continued to be exported in large quantities from the Sahel are as of French West Africa (modern Senegal, Mauritania, Mali, Burkina Faso, and Niger) and French Equatorial Africa (modern Chad) until these nations gained their independence in 1959–61(Webb, James., 2009).

Figure 1.4: The African Gum Belt

1.9.1.2 Gum Arabic production in the Sudan

Although from the 1950s to the early 1990s, Sudan accounted for roughly 80% of gum Arabic production, today, that figure is under 50% (Policy Note., 2007).

Hundreds of thousands of Sudanese are dependent on gum Arabic for their lively-hoods in gum belt area (Figure 1.5). It is, however, still the world's largest single producer (Gerstenzang, James and Sanders, 2007).

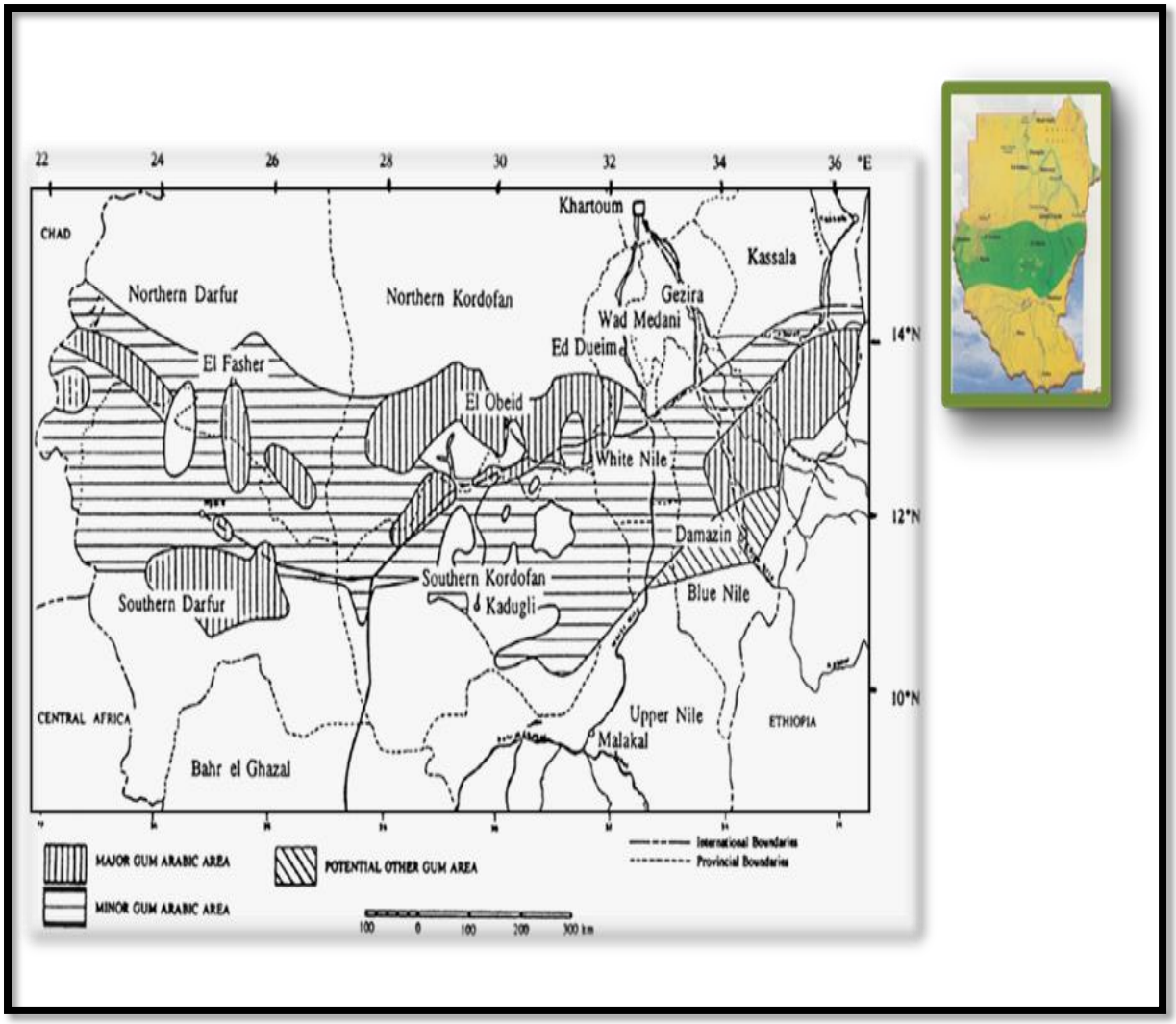


Figure 1.5: Gum belt in Sudan

1.9 Gum Tapping and Collection

Gums are collected after tapping, which involves removing sections of the bark with an axe taking care not to damage the tree. Tapping begins when the trees are just starting to shed their leaves, around the end of October or the beginning of November in Sudan. In order to reach this stage, trees have to grow for a period of 3 to 7 years depending on the method of establishment. Again in the Sudan, there are two tapping seasons, an earlier one before the onset of the colder weather which is between the months of December and March and a later one in the dry spell after the month of March. After tapping, exudation occurs gradually forming a hard, but slightly elastic,nodule (Coppen, 1995). As more gum exudes the outer skin expands or cracks and the nodule grows to about 15–30 mm in diameter. When the outer casing becomes so hard that the liquid cannot force it to expand any further, the nodule is ready for picking. The time taken to reach this stage is from 3-6 weeks and as soon as the nodules are picked, new ones start to form and within 10-15 days a second picking is possible. Several branches are treated in this manner at one tapping (Coppen, 1995).

In the following years, other branches or the reverse side of the same treated branches are tapped. An average of four pickings is common, up to seven. The nodules are picked by hand sorted according to colour and size. Some typical grades of Sudanese gum available are listed in (Table 1.2).

The production and trade of gum Arabic has been dominated by Sudan followed by Chad and Nigeria (Islam *et al*., 1997).

Table 1.2: Grades of Sudanese gum (Islam *et al*., 1997).

Grade	Description
Hand-picked selected	The most expensive grade. Cleanest, lightest colour and in the form of large whole nodule, Ø >20 mm**.**
Cleaned and sifted	The material that remains after hand-picked selected and siftings are removed. Comprises whole or broken lumps varying in colour from pale to dark amber.
Cleaned	The standard grade varying from light to dark amber. Contains siftings but dust removed.Whole nodules plus fragments , 10 < Ø <20 mm**.**
Siftings	Fine particles remaining following sorting of the choicer grades. Contains some sand, bark and dirt.Fragments and siftings, 2.5<Ø < 10 mm.
Dust	Very fine particles collected after the cleaning process. Contains sand and dirt.Ø<2.5 mm**.**
Red	Dark red particles - Rejects – local use only**.**

1.9.1 *Anogeissus leiocarpus*

(Bambara: *ngálăma*) is a tall ever green tree native to savannas of Tropical Africa and Southeast Asia (Steentoft, Margaret,1988; Edward,1992). It is the sole West African species of the genus *Anogeissus*, in Sudan it is found in southern Kordofan (Nuba mountain) and southern Darfour (Hamza;1990). *A. leiocarpa* germinates in the new soils produced by seasonal wetlands and grows at the edges of the rain forest, although not in the rainforest, in the savanna, and along river banks forming gallery forests. The tree flowers in the rainy season, from June to October. The seeds, winged samaras, are dispersed by ants (Margaret; 1988).

1.9.2 Botanical classification

Kingdom : *Plantae*

(Unranked): *Angiosperms*

(Unranked): *Eudicots*

(Unranked): *Rosids*

Order : *Myrtales*

Family : *Combretaceae*

Genus : *Anogeissus*

Species : *A. Leiocarpa*

Arabic Name: *Sahab.*

Figures 1.6 to 1.9 show a part of *Anogeissus leiocarpa.*

Figure 1.6: Part of *Anogeissus leiocarpus* tree

Figure 1.7: *Anogeissus leiocarpus* Leaves

Figure 1.8: *Anogeissus leiocarpus* flowers

Figure 1.9: *Anogeissus leiocarpus* gum

1.9.3 Uses of *Anogeissus leiocarpus*

The wood is used, mainly, in transmission and building poles, fences posts, forked poles and as beam of local building construction. It is also used for firewood and charcoal. The leaves and barks contain tannin materials (Hamza,1990). In northern Nigeria and Burkina Faso the ashes are used for preparation of goatskin. In Mali all parts of *Anogeissus leiocarpus* tree are used as medicine mixed with other plants in traditional system. Also *Anogeissus leiocarpus* gum was used as food additives mixed with gum Arabic or as a substitute for it (Arbonnier, Michel,2004).

It is one of the plants used to make bògòlanfini, a traditional Malian mud cloth. Small branches with leaves are crushed to make one of the yellow dyes (Arbonnier, Michel,2004). The inner bark of the tree is used as human and livestock anthelmintic for treating worms, and for treatment of a couple of protozoan diseases in animals, nagana an animal trypanosomiasis, and Babesiosis (Bizimana, Nsekuye, 1994). The inner bark is used as a chewing stick in Nigeria and extracts of the bark show antibacterial properties (Mann, *et.al*, 2008). The stem barks contains castalagin (Shuaibu, *et.al* 2008) and flavogallonic acid dilactone (Tanaka, T.; Kouno, I., 2008).

1.9.4 Pharmaceutical Applications of Natural Gums

Natural polymers are often preferred to synthetic materials because of their non-toxicity, low cost and, relative, availability. They can however exhibit uncontrolled rates of hydration and thickening, decrease in viscosity on storage and microbial contamination which may make modification necessary (Durso, 1980). Irrespective of these, natural plant gums have several applications in pharmacy and medicine. In pharmaceutical formulations, they have been used for film coating of tablets and some in drug delivery systems to control the release of medications, some have been used as plasma expanders, bulk laxatives or demulcents (Durso, 1980). When

finely divided, insoluble solid (drug) particles are dispersed uni-formly throughout a fluid, they are known as suspensions (Billany, 2007). The solid particles are also called the dispersed phase while the liquid or fluid in which they are dispersed is known as the dispersion medium or continuous phase. Pharmaceutical oral suspensions are coarse dispersions (usually aqueous) having a dispersed phase with mean particle diameter of greater than 1.0 μm while colloidal suspensions have a dispersed phase of mean particle diameter that is less than 1.0 μm (Kulshreshtha, 2010).

Aqueous suspensions are useful for administration of insoluble or poorly water soluble drugs. The dispersed drug provides a large surface area that ensures and enhances dissolution and hence absorption of the drug. They however come with the challenge of physical stability (Kulshreshtha, 2010).There are a number of feature desirable in pharmaceutical oral suspensions. These include: the suspended material should not settle too rapidly; the particles which do settle to the bottom of the container must not form a hard mass but should be readily dispersed into a uniform mixture when the container is shaken; and the suspension must not be too viscous to pour freely from the orifice of the bottle (Billany 2007).

A reduction in the zeta potential to a point where attractive forces pre-dominate produces, primarily, coarse masses or coagulates which is difficult to readily re-disperse.

Conversely, the particles may form a loosely bonded structure or aggregate called a floc or flocculate. And the process of its formation flocculation. These flocs separate out and sediment fairly rapidly but because they are loosely packed, high volume sediments which retain their structure, they are easily redispersible. The supernatant liquid is clear because the colloidal particles are trapped within the flocs and sediment with them. This later state is desirable for a pharmaceutical

suspension. Figure1.10 shows a schematic representation of stability dynamics in a coarse or colloidal system (Billany, 2007).

One parameter for assessing a pharmaceutical suspension is the sedimentation volume ratio, *F*, defined as the ratio of the final volume V_u to the original volume V_o

$F = V_u/V_o$ ---------► (Equation 1.6)

The ratio *F* is a measure of the aggregated-deflocculated state of a suspension. The ratio *F* may be plotted together with the measured zeta potential, against concentration of additive to enable assessment of the state of the dispersion. Also the appearance of the supernatant liquid should be noted and the redispersibility of the suspension evaluated (Billany, 2007).

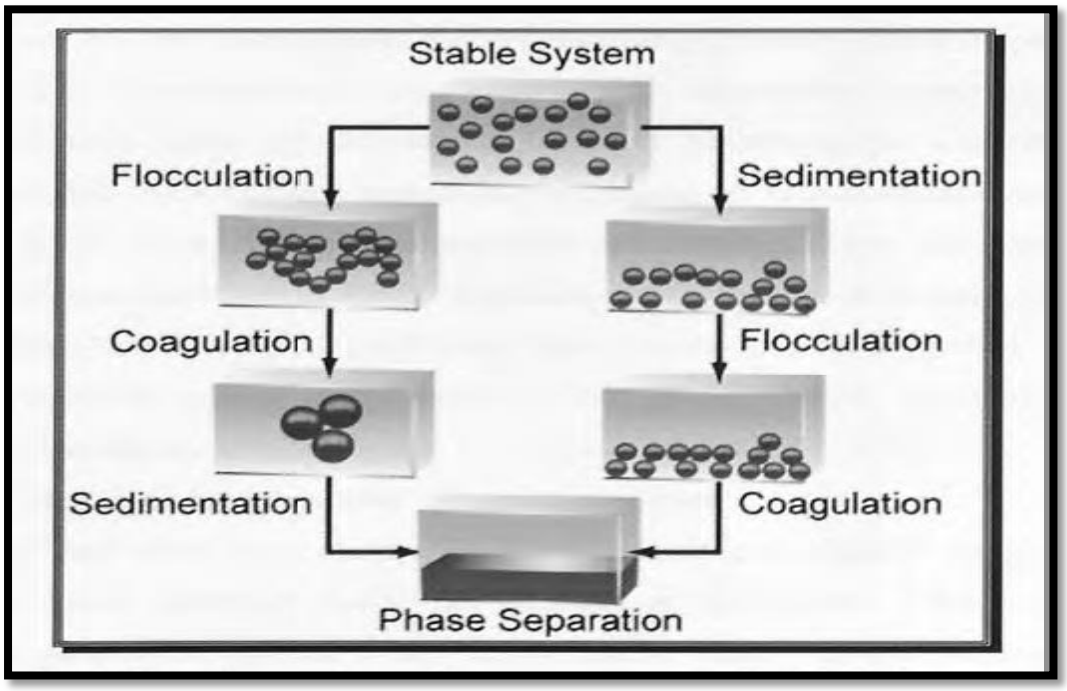


Figure 1.10: Stability dynamics of a coarse or colloidal dispersed system.

Adapted from www.malvern.com/LabEng/industry/colloids_stability.htm

1.9.4.1 Stabilization of Suspensions

Physical stability can refer to any number of attributes ranging from appearance to color to viscosity. Many stability parameters are at least partially, if not completely, a function of suspension stability – the ability of the suspension to remain in its original state. This is critical for pharmaceutical formulations since any change could negatively impact its performance. Suspension stability is the result of various physical and electrochemical forces over which formulators often feel that they have little control. However, an understanding of the fundamental forces involved will, in fact, empower the formulator to create more effective and stable formulations (Niazi, Sarfaraz, 2009).

The preparation of oil-in-water (O/W) and water-in-oil (W/O) emulsions requires emulsifiers and other tensides (Becher; 1985). Similarly, the preparation of suspensions of powders in liquids usually necessitates the use of chemical dispersing aids, including surfactants (Parfitt; 1981). These materials, dispersants and emulsifiers, while they can also provide some degree of stability to the final suspension or emulsion are not true stabilizing aids. It is, therefore, often advantageous to consider stabilization as a separate issue. This is, particularly, so when systems are to be challenged by extreme process conditions such as freeze/thaw cycling and high temperature / long term storage. Thus, when stability is an issue it is important to consider, more closely, the mechanics of the stabilization process (Parfitt; 1981).

A suspension comprises, in the simplest case, a mixture of two phases. The phases are described by the terms dispersed phase (for the material forming any particles) and the "dispersion medium or vehicle" (for the material in which particles are distributed) (Parfitt; 1981).

The dispersed (internal) phase can be solid particles, as in nail polish, liquid droplets, such as in an emulsion or air/vapor/gas droplets in a foam. For this discussion, the term "particle" will be used for the dispersed phase be it solid, liquid or gaseous. The (external) liquid phase can be aqueous or non-aqueous; examples are all around us from car polish to salad dressing. The overall properties of the suspension are influenced by the chemical and physical characteristics of both the dispersed phase and the dispersion medium/vehicle and the interactions between these two individual materials when they are mixed together (Parfitt; 1981). The physical condition of the dispersed phase can vary from round, such as oil droplets in emulsions to needle-or plate-like crystals of clays and oxides. A very important physical property that influences the performance of a suspension is the particle size distribution (PSD) (Figure 1.11). PSD, directly affects important characteristics such as the bioavailability of Active Pharmaceutical Ingredients APIs and the tone and hue of colored pigments, such as the iron oxides used in daily-wear skincare formulations. In addition, the PSD determines settling properties and affects rheological behavior (flow and deformation) as can be clearly demonstrated by varying the droplet size in a given emulsion (Parfitt; 1981). All suspensions, including coarse emulsions, are, inherently, thermo-dynamically unstable. They will, through random motion of the particles over time, aggregate because of the natural and dominant tendency to decrease the large specific surface area and excess surface energy (Ronald E, 2005).

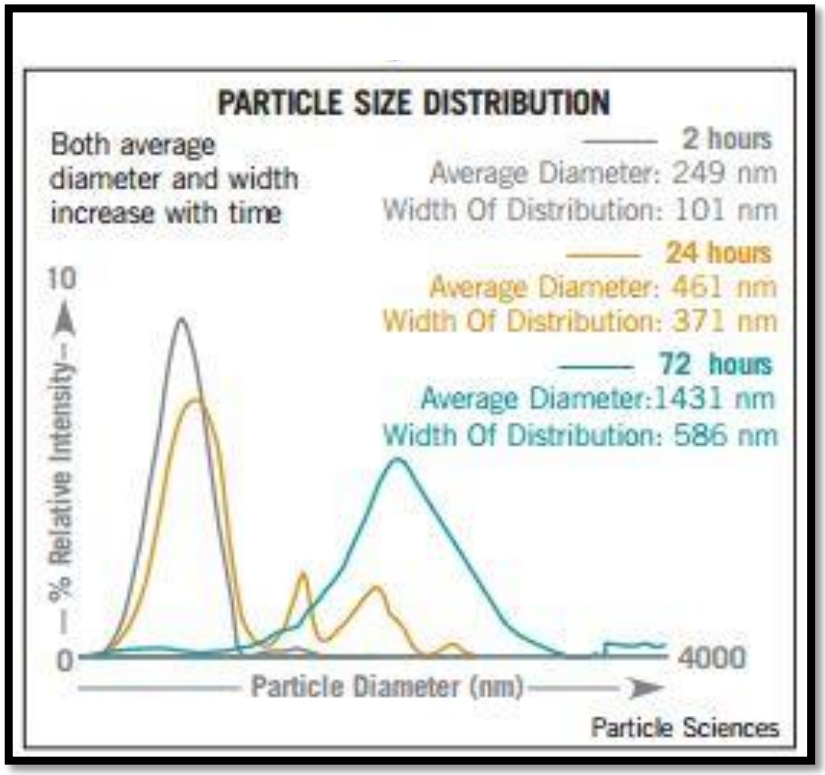


Figure 1.11: Particle size distribution of formulated suspensions

This tendency becomes increasingly important the smaller the initial PS, and is especially significant for colloidal-sized particles (less than 1 micron). Hence attention must be paid to any process of attrition or comminution to reduce PS because of the potential for creation of a population of submicron particles (Ronald, 2005).

The PS will always tend to increase with time unless some barrier is present to keep them from coalescing or sticking when they get close to each other. This is true for both liquid/liquid and solid / liquid suspensions. This will also result in a change in shape and width of the PSD (Krister, 2002). This is illustrated in Figure1.11, when an emulsion is destabilized by dilution in a solution of an ionic surfactant of opposite charge.

The frequency of particle - particle collision depends on PSD, particle concentration, dispersion medium viscosity and temperature.The suspension stability thus refers to the resistance to change in the dispersed state of the system. This is somewhat of a moving target that each formulator must define on a case - by - case basis because it depends upon the time-scale of the observations – how long it takes for the system to remain "stable"? For most commercial, industrial, and pharmaceutical products this is usually measured in years. However, suspensions used as an intermediate during manufacturing may only need to survive for hours or days. It should be noted that the process of settling is not necessarily an indication of instability. Many particulates, especially oxides, have a high density and will simply settle over time under gravity. If the particles are not well-stabilized they will eventually aggregate. However, a well-stabilized, but settled sytem will re-disperse quite readily with agitation – think of creaming in whole milk (Krister, 2002).

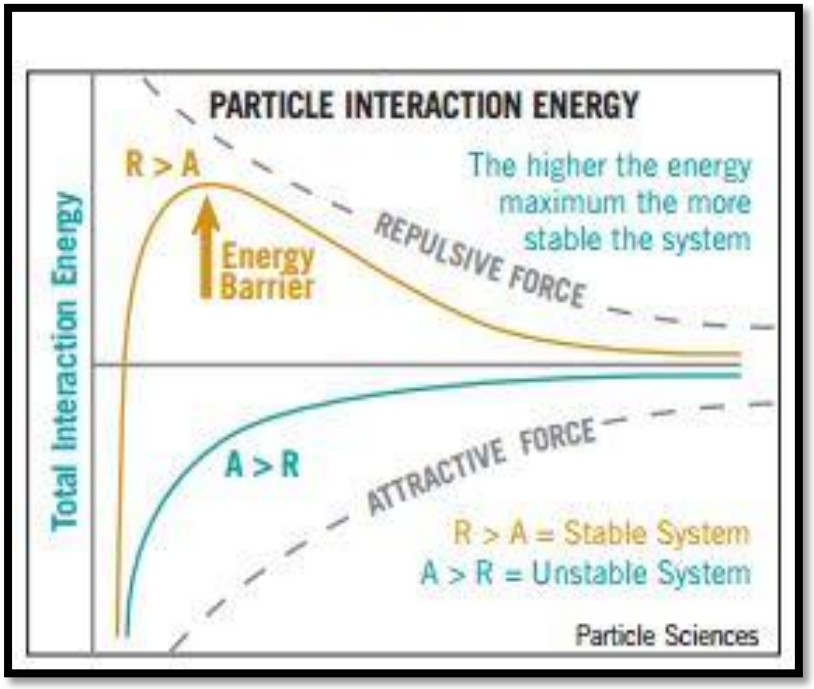


Figure 1.12: Particle interaction energy

There are two fundamental types of interaction between particles – attraction and repulsion (Himenz; 1986, Everett; 1988). Whether two approaching particles will combine, or not, depends on the potential energy barrier between them. This potential energy barrier arises as a consequence of the difference in magnitude of repulsive forces in comparison with attractive electrostatic forces. When the attractive forces are dominant, the particles will adhere; if the repulsive forces are stronger then the particles will remain suspended (Figure 1.12). The forces of attraction (van der Waals forces) are always present pulling particles together even in completely nonpolar systems; these forces of attraction act over, surprisingly, large distances of separation. The magnitude of the attractive forces increases as the polarity of the medium/vehicle decreases. This is very important in, for example W/O emulsions where the presence of small amounts of less polar impurities in the oil-phase can promote flocculation or even coalescence. In order to maintain the highest degree of suspension stability the forces of attraction must be kept as low as possible and there must be sufficient repulsive force to counteract them. There are basically two ways to attain this – electrostatically and sterically – as shown schematically in Figure 1.13. Either method can be used but it can be seen from this simple picture that each will result in completely different rheological (flow and deformation) behavior of the stabilized suspension (Himenz; 1986, Everett; 1988).

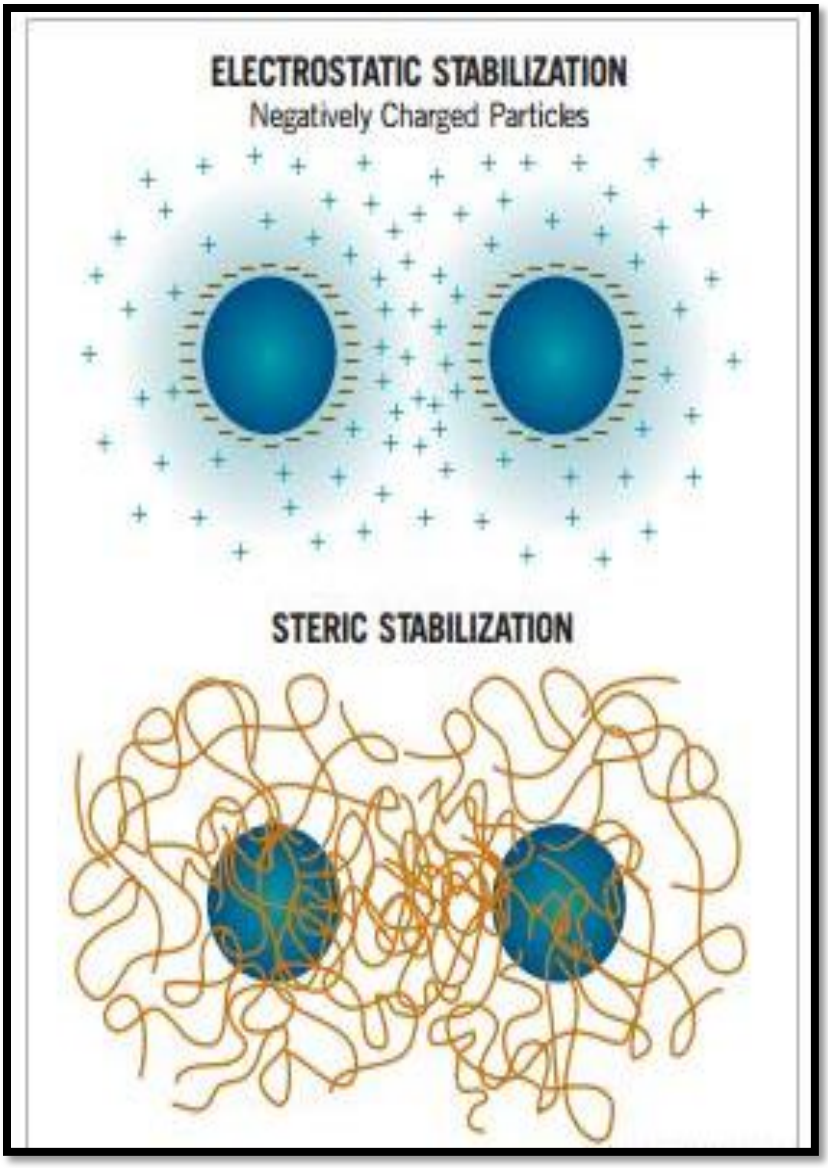


Figure 1.13: Electrostatic stabilization

The electrostatic method places an ionic charge at the surface of particles that results in mutual repulsion. For maximum stability the electrostatic force of repulsion should be as large as possible, i.e., the particle surface charge should be as large as possible. The force of repulsion becomes smaller as the ionic strength of the medium increases. Thus, the lowest electrolyte concentration possible should be used. This is problematic for many pharmaceutical formulations that tend to be isotonic with blood, i.e., they have a very high ionic strength (Krister, 2002).

Any method of making the particle(s) in the suspension display a net charge will work for electrostatic stabilization and there is much room for creativity. One common process is dissociation of ionogenic surface hydroxyl or carboxyl groups. This process is pH dependent (in water); oxides and clays can readily be stabilized by this method. A second is through physical adsorption of ionized surfactants and polyelectrolytes. Indeed, physical adsorption is the foundation of particle dispersion technology and has been, successfully, employed for decades. However, the inherent shortcoming is that any physically adsorbed species can, and often, will, be displaced during subsequent processing because of the often severe thermal and mechanical agitation employed (Krister, 2002).

The sign and magnitude of the surface charge is an important factor not only in the choice of dispersing and processing aids but also when preparing co-suspensions containing different dispersed materials. A reliable and well-established method to determine the surface charge of suspensions is zeta potential (Hunter; 1981).

The steric method of repulsion produces a structure-mechanical barrier of non-ionic long chain molecules around interacting particles to prevent collision, rather like the bumper on a car.

The use of macromolecules to provide steric stabilization is almost as much an art as a science and various conditions must be met if stable dispersions are to be successfully prepared. The dispersion medium or vehicle must be a good "solvent" for the adsorbed macromolecule to allow for the polymer chains to extend as far as possible into bulk solution. The surface coverage must be complete and the adsorbed moiety must be firmly attached to the surface but not so strongly that it will collapse because the "depth" or "thickness" of the steric barrier has to be large (Amit, 2009).

Figure 1.14 illustrates schematically just a few difficulties in achieving these conditions simultaneously. The characteristic feature of any macromolecule in solution is its high degree of conformational freedom. The shape and dimensions of a macromolecule in solution are critically affected by the macromolecule concentration, the nature of the "solvent", and the temperature. This, in turn, will affect how the moiety adsorbs onto a surface (Fleer *et.al*; 1993).

The conditions are such that the macromolecule is well solvated and adopts the conformation of an extended chain. For complete surface coverage the macromolecule must adsorb flat-on. Although there may be firm attachment, it produces only a thin layer and, hence, virtually no steric barrier (Fleer *et.al*; 1993).

The conditions are such that the macromolecule is, a tightly bunched random coil. Although adsorption onto the surface results in a thick layer, there are only a few contact points. Displacement can readily occur, particularly under shear conditions. Also, surface coverage is not optimum.

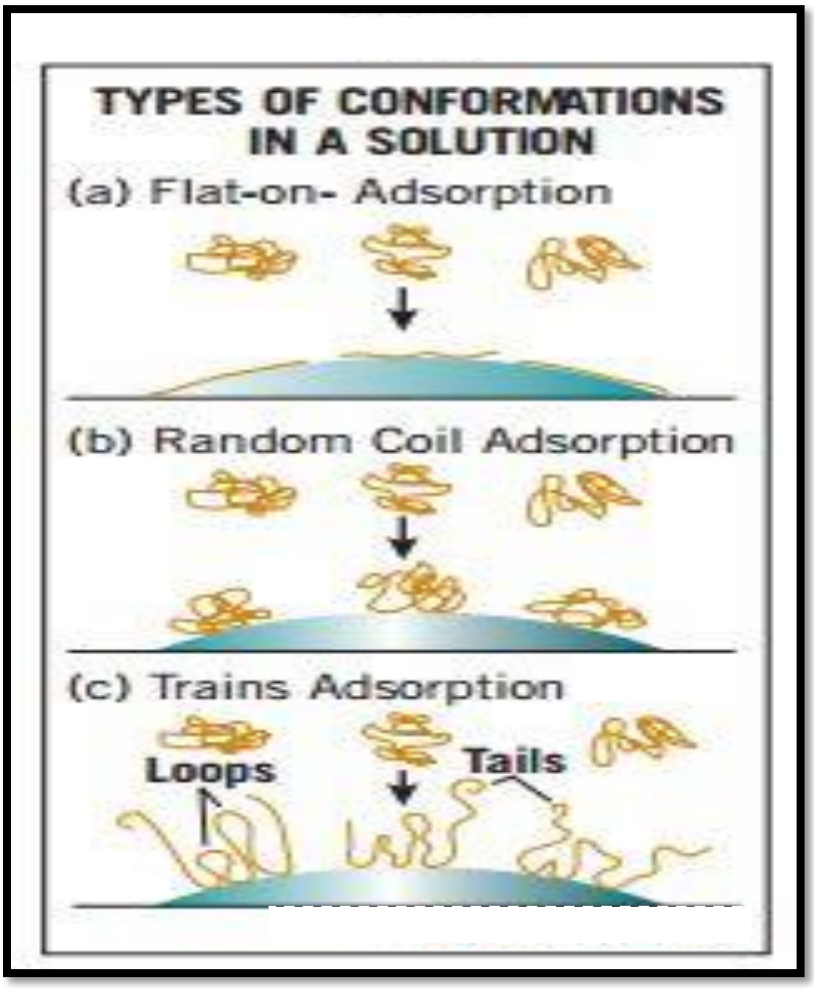


Figure 1.14: Types of conformations in a solution.

Good steric stabilization is achieved when the solution conditions are such that the macromolecule adsorbs onto the surface by many contacts. The open structure of the "loop" segments also provides a solvation layer that, itself, supplies additional repulsive force; there is resistance to squeezing out this layer because it entails removal (or displacement) of solvent. The "tails" provide additional depth to the barrier (Fleer *et.al*; 1993). Thus, in practice, the most useful steric stabilizers are block and graft copolymers. They are generally composed of two parts; one of which is insoluble in the dispersion medium and which, firmly, anchors the stabilizing moiety, and a second part which is soluble in the dispersion medium,

providing the steric repulsion. Naturally occurring polymers such as gelatin, casein gum Arabic and even vegetable oils have been used. Sterically stabilized systems are much less sensitive to electrolytes and they provide tolerance at very high ionic strengths.Thus, they are very useful in formulating pharmaceutical suspensions (Napper, 1983).

They exhibit more progressive rheological properties and generally display better freeze/thaw behavior. Sterics also allow higher solids loadings and they work in non-aqueous media. The disadvantage is that a suspension can be readily destabilized if there is poor coverage or if there is excess steric stabilizer in solution (Napper, 1983).

1.9.5 Rheological properties of suspensions

Rheology is the study of the flow of matter, primarily in the liquid state, but also as 'soft solids' or solids under conditions in which they respond with plastic flow rather than deforming, elastically, in response to an applied force (Schowalter, 1978). It applies to substances which have a complex microstructure, such as muds, sludges, suspensions, polymers and other glass formers (*e.g.*, silicates), as well as many foods and additives, bodily fluids (*e.g.*, blood) and other biological materials or other materials which belong to the class of soft matter (Schowalter, 1978).

Newtonian fluids can be characterized by a single coefficient of viscosity for a specific temperature. Although viscosity will change with temperature, it does not change with the strain rate. Only a small group of fluids exhibit such constant viscosity, and they are known as Newtonian fluids.

But for a large class of fluids,the viscosity changes with the strain rate (or relative velocity of flow) are called non-Newtonian fluids.

Rheology is principally concerned with extending continuum mechanics to characterize flow of materials, that exhibits a combination of elastic, viscous and plastic behaviour by properly combining elasticity and (Newtonian) fluid mechanics. It is also concerned with establishing predictions for mechanical behaviour (on the continuum mechanical scale) based on the micro or nano-structure of the material, e.g. the molecular size and architecture of polymers in solution or the particle size distribution in a solid suspension. Materials with the characteristics of a fluid will flow when subjected to a stress which is defined as the force per area.

There are different sorts of stress (e.g. shear, torsional, etc.) and materials can respond differently for different stresses. Much of theoretical rheology is concerned with associating external forces and torques with internal stresses and internal strain gradients and velocities (Bird, 1960; Schowalter, 1978 and Byrin Bird, 1989; Morrison, 2001).

1.9.6 Electrokinetic properties of suspensions

The surfaces of particles in a suspension or emulsion, usually, acquire an electric charge when brought into contact with an aqueous medium. The charge is more, often, negative and it may arise in number of ways: the surface of the particles may contain chemical groups which can ionize to produce charged surfaces; the particle surface may adsorb ions of one sign of charge in preference to charges of opposite sign; deliberate addition of chemical compounds that, preferentially, adsorb on the particle surface to generate a charge.

The amount of charge on the particle surface is an important particle characteristic and it determines the properties of the suspension (Billany, 2007).

Although particles are referred to as being electrically charged, the suspension overall is neutral because the charge on the surface of each particle is counter-balanced by charges (ions) of opposite sign in surrounding solution. This distribution of ions is in turn affected by thermal agitation which tends to redisperse the ions in solution and results in the formation of an electric double layer made up of charged surface and a neutralizing excess of counter-ions over co-ions distributed in a diffused manner in the aqueous medium is a schematic representation of the electric double layer. The liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and outer (diffuse) region where they are less firmly associated. Within this diffuse layer is a national boundary known as the slipping plane, within which the particle acts as a single entity. This surface separating the bound charge from the diffuse charge around the particle, marks where the solution and the particle move in opposite directions when an external field is applied. It is called the surface of shear or the slip surface. The electrostatic potential on slip surface or slipping plane is called the zeta potential and it is that potential which is measured, when the velocity of the particles in a direct current electric field is determined (Billany, 2007).

The potential stability of a dispersed system may be indicated by the magnitude of the zeta potential. Dispersed systems with high negative or positive zeta potential show no tendency to coagulate because the particles tend to repel one another. However, low zeta potential means that the particles can easily come togather and coagulate.

The general dividing line between stable and unstable suspensions is generally taken at either + 30 mV or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than 30mV are considered stable (www.nbtc.cornell.edu/facilities/downloads/Zetasizer%20chapter%2016.pdf)

1.9.7 Theory of Sedimentation

Sedimentation means settling of particle (or) floccules occur under gravitational force in liquid dosage form.

1.9.8 Force due to gravity F_G

The force acting on any body at the earth's surface due to the action the earth's gravity described by equation (1.7) (Donald E, 2002).

$$F_G = mg \quad \text{--------►(Equation 1.7)}$$

Where:-

m The mass of the particle (expressed in kg)

g The acceleration due to gravity (approximately 9.81 m.s^{-2}).

The above equation is not very helpful for small particles such as pollen grains as it would be quite a challenge to find scales sensitive enough to determine the mass of a single pollen grain (http://www.hettichag.ch/en/zentrifugen/kleinzentrifugen/eba_20_2002.htm).

Hence a change is made, using the concept of density. Density is defined as the mass per unit volume, see equation (1.8).

$$\rho = m/V \quad \text{--------►(Equation 1.8)}$$

Where:-

ρ density of particle (expressed in kg.m^{-3})

m Mass (kg)

V Volume (m^3)

If we assume the particle is a perfect sphere (typically not too far from the truth for many pollen grains) then we can calculate its volume from its radius.

$$V=¼r^3 \text{---------►} \text{(Equation 1.9)}$$

Where:-

r: Radius of a sphere (m)

¼ ratio of circle circumference to diameter (3.14159265 . . .)

V: Volume (m^3)

From combining above equations (1.7), (1.8) and (1.9) gives the required result, equation(1.10).

$$F_G=¼r^3\rho_p g \text{ --------►} \text{(Equation 1.10)}$$

Where:-

ρ_p density of a particle particle (expressed in kg.m^{-3}).

1.9.9 Controlled Release of Medicaments

In the design of sustained-release dosage forms, a single dose unit is expected to provide a prompt or immediate release of an amount of drug that would produce the desired therapeutic plasma concentration followed by a gradual and continuous release of smaller amounts of the drug to maintain the attained therapeutic plasma level. Several formulation methods have been used to achieve sustained-release of drug substances (Leon Shargel, 2004).

1.9.10 Coated granules / tablets and similar multilayered systems

Multi-layered systems are designed in such a way that the drug has to cross barrier(s) on its way from the device to the physiological environment. The release process is controlled by the nature and number of barriers (Zahirul, 1995).

To design appropriate barriers, formulation scientists have relied on polymers from the very beginning of the emergence of controlled-release technology. This is, perhaps, attributed to the fact that these substances can be fabricated according to the needs of the system and most of them are inert and biocompatible (Zahirul, 1995).

In their simplest form, coated tablets comprise a core containing either the drug alone or loaded into an inert material which may be hydrophilic or hydrophobic, or a mixture of both in which the ratio is optimized according to the needs of the system (Zahirul, 1995).

Several, differently, coated groups of granules may have to be combined to produce the final desired availability rate. This may produce more uniform absorption from the gastrointestinal tract. The coated granules may be encapsulated or tableted or even formulated into suspension (Rawlins, 1980; Zahirul, 1995). In order to extend the release profile drugs from coated tablets, more complex systems have introduced. The common approach is to use multiple coatings with polymers of different physicochemical properties or polymers in combination with other hydrophobic materials (Rawlins, 1980).

Other attempts to extend the release of the drug from coated tablets include modification of the drug with a carrier in the matrix which results in prolonged drug release. Multi-layered systems having the drug in both outer layers and within the core have also been described in the literature. The rationale behind this type of formulation is that a rapid release of the drug followed by a controlled-release asrequired for prolonged action can be achieved using using these devices (Zahirul, 1995).

1.9.11 Ion exchange products

Ion-exchange resins are water-insoluble cross-linked polymers containing salt-forming groups in repeating positions on the polymer chain (Rawlins, 1980). The design of ion-exchange preparations involves an insoluble resin capable of reacting with either an anionic or cationic drug to form an insoluble Resin-Drug complex. Upon exposure to the ions in the gut (K^+, Na^+,Cl^-), the drug is displaced from the resin releasing the drug which is then absorbed freely.

The charging of drug ions to an ion-exchange resin may be accomplished in two ways: highly concentrated drug solution is percolated through a column or bed of resin particles until equilibrium is reached. Alternatively, resin particles are stirred with a large volume of concentrated drug solution. The charged resin particles are then washed with deionised water to remove unassociated drug and other ions, and the resin particles sub-sequently dried (Rawlins, 1980; Zahirul, 1995). The particles may then be tableted or encapsulated.

1.9.12 Drug Particle-Size Increase

This is usually aimed at increasing the surface to volume ratio which allows the slow rate of drug release. The particle-size increase is limited to poorly soluble drugs. Good grinding techniques and/or addition of filler with larger or smaller particles provide means of getting desired particle-size range (Zahirul, 1995).

1.9.13 Stability Testing Of Finished Pharmaceutical Products

The purpose of stability testing is to provide evidence of how the quality of an Finished Pharmaceutical Product FPP varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The stability programme also includes the study of product-related factors that

influence its quality, for example, interaction of Active Pharmaceutical Ingredients API with excipients, container closure systems and packaging materials (Kinjal N., 2012). In Fixed Dose Combination (FDCs) Finished Pharmaceutical Products FPPs the interaction between two or more APIs also has to be considered. As a result of stability testing a re-test period for the API (in exceptional cases, e.g. for unstable APIs, a shelf-life is given) or a shelf-life for the FPP can be established and storage conditions can be recommended. Various analyses have been done to identify suitable testing conditions for WHO Member States based on climatic data and are published inthe literature, on the basis of which each Member State can make its decision on long-term (real-time) stability testing conditions. Those Member States that have notified WHO of the long-term stability testing conditions they require when requesting a marketing authorization (Kinjal N., 2012).

1.9.14 Stress testing

Stress testing of the API can help to identify the likely degradation products,which, in turn, can help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual API and the type of FPP involved.

For an API the following approaches may be used:

— When available, it is acceptable to provide the relevant data published in the scientific literature to support the identified degradation product sand pathways;

— When no data are available, stress testing should be performed.

Stress testing may be carried out on a single batch of the API. It should include the effect of temperature (in 10 °C increments (e.g. 50 °C, 60 °C,etc.) above the temperature used for accelerated testing), humidity (e.g. 75% relative humidity (RH) or greater) and, where appropriate, oxidation and photolysis on the API

(Kinjal N., 2012). The testing should also evaluate the susceptibility of the API to hydrolysis across range of pH values when in solution or suspension.Assessing the necessity for photostability testing should be an integral part ofa stress testing strategy (Kinjal N., 2012).

1.9.15 Chemical kinetics

Chemical kinetics deals with the experimental determination of reaction rates from which rate laws and rate constants are derived. Relatively simple rate laws exist for zero-order reactions (for which reaction rates are independent of concentration), first-order reactions, and second-order reactions, and can be derived for others. In consecutive reactions, the rate-determining step often determines the kinetics. In consecutive first-order reactions, a steady state approximation can simplify the rate law. The activation energy for a reaction is experimentally determined through the Arrhenius equation and the Eyring equation (Wikipedia, 2012; Upper Saddle, 2004). The main factors that influence the reaction rate include: the physical state of the reactants, the concentrations of the reactants, the temperature at which the reaction occurs, and whether or not any catalysts are present in the reaction (Wikipedia, 2012; Upper Saddle, 2004).

1.9.16 First-order reactions

A first-order reaction depends on the concentration of only one reactant (a unimolecular reaction). Other reactants can be present, but each will be zero-order. The rate law for a reaction that is first order with respect to a reactant A is

$$\frac{-d[A]}{dt} \equiv r = k[A] \longrightarrow \quad \text{(Equation 1.11)}$$

k is the first order rate constant, which has units of 1/s.

The integrated first-order rate law is

$$\ln [A] = -kt + \ln [A]_0 \quad \longrightarrow \text{(Equation 1.12)}$$

A plot of $\ln [A]$ vs. time *t* gives a straight line with a slope of $-k$.

The half-life of a first-order reaction is independent of the starting concentration and is given by $t_{\frac{1}{2}} = \frac{\ln (2)}{k}$ ——————► (Equation 1.13)

Examples of reactions that are first-order with respect to the reactant:

$$\mathrm{H_2O_2}(l) \rightarrow \mathrm{H_2O}(l) + \frac{1}{2}\mathrm{O_2}(g) \quad \longrightarrow \text{(Equation 1.14)}$$

$$\mathrm{SO_2Cl_2}(l) \rightarrow \mathrm{SO_2}(g) + \mathrm{Cl_2}(g) \quad \longrightarrow \text{(Equation 1.15)}$$

$$2\mathrm{N_2O_5}(g) \rightarrow 4\mathrm{NO_2}(g) + \mathrm{O_2}(g) \quad \longrightarrow \text{(Equation 1.16)}$$

1.9.17 Further properties of first-order reaction kinetics

The integrated first-order rate law

$$\ln [A] = -kt + \ln [A]_0 \quad \longrightarrow \text{(Equation 1.17)}$$

is usually written in the form of the exponential decay equation

$$A = A_0 e^{-kt} \quad \longrightarrow \text{(Equation 1.18)}$$

A different (but equivalent) way of considering first order kinetics is as follows: The exponential decay equation can be rewritten as:

$$A = A_0 \left(e^{-k\Delta t_p}\right)^n \longrightarrow \text{(Equation 1.19)}$$

Where Δt_p corresponds to a specific time period and $n$is an integer corresponding to the number of time periods (Szallasi, 2006), (Iglesias, 2010). At the end of each time period, the fraction of the reactant population remaining relative to the amount present at the start of the time period, f_{RP}, will be:

$$\frac{A_n}{A_{n-1}} = f_{RP} = e^{-k\Delta t_p} \longrightarrow \text{(Equation 1.20)}$$

Such that after $n$time periods, the fraction of the original reactant population will be:

$$\frac{A}{A_0} \equiv \frac{A_n}{A_0} = \left(e^{-k\Delta t_p}\right)^n = (f_{RP})^n = (1 - f_{BP})^n \longrightarrow \text{(Equation 1.21)}$$

Where: f_{BP}corresponds to the fraction of the reactant population that will break down in each time period. This equation indicates that the fraction of the total amount of reactant population that will break down in each time period is independent of the initial amount present. When the chosen time period corresponds to $\Delta t_p = \frac{\ln(2)}{k}$, the fraction of the population that will break down in each time period will be exactly ½ the amount present at the start of the time

period (i.e. the time period corresponds to the half-life of the first-order reaction) (Szallasi, 2006), (Iglesias, 2010).

The average rate of the reaction for the n^{th} time period is given by:

$$r_{avg,n} = -\frac{\Delta A}{\Delta t_p} = \frac{A_{n-1} - A_n}{\Delta t_p} \longrightarrow \text{(Equation 1.22)}$$

Therefore, the amount remaining at the end of each time period will be related to the average rate of that time period and the reactant population at the start of the time period by:

$$A_n = A_{n-1} - r_{avg,n}\Delta t_p \longrightarrow \text{(Equation 1.23)}$$

Since the fraction of the reactant population that will break down in each time period can be expressed as:

$$f_{BP} = 1 - \frac{A_n}{A_{n-1}} \longrightarrow \text{(Equation 1.24)}$$

The amount of reactant that will break down in each time period can be related to the average rate over that time period by:

$$f_{BP} = \frac{r_{avg,n}\Delta t_p}{A_{n-1}} \longrightarrow \text{(Equation 1.25)}$$

Such that the amount that remains at the end of each time period will be related to the amount present at the start of the time period according to:

$$A_n = A_{n-1}\left(1 - \frac{r_{avg,n}\Delta t_p}{A_{n-1}}\right) \longrightarrow \text{(Equation 1.26)}$$

This equation is a recursion allowing for the calculation of the amount present after any number of time periods, without need of the rate constant, provided that the average rate for each time period is known (Walsh R, 2010).

1.10 Cytotoxicity

Is the measure of how materialis being toxic to cells. Examples of toxic agents are an immune cell or some types of venom, e.g. from the puff adder (*Bitis arietans*) or brown recluse spider (*Loxosceles reclusa*).

1.10.1 Cell physiology

Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis).

Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, shut down metabolism and release their contents into the environment. Cells that undergo rapid necrosis in vitro do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers (Promega Corporation, 2006). Apoptosis is characterized by well defined cytological and molecular events including a change in the refractive index of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments (Promega Corporation, 2006). Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. They will shut down metabolism, lose membrane integrity and lyse (Promega Corporation, 2006 and Riss, 2004).

1.10.2 Measuring cytotoxicity

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high through put drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical (Riss, 2004).

Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components (Riss, 2004). Alternatively, membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside. One molecule, lactate dehydrogenase (LDH), is commonly measured using LDH assay (Decker,1988). Protease biomarkers have been identified that allow researchers to measure relative numbers of live and dead cells within the same cell population. The live-cell protease is only active in cells that have a healthy cell membrane, and loses activity once the cell is compromised and the protease is exposed to the external environment. The dead-cell protease cannot cross the cell membrane, and can only be measured in culture media after cells have lost their membrane integrity (Niles, 2007).

Cytotoxicity can also be monitored using the 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) or MTS assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTS reagent to a colored formazan product.

A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In addition to using dyes to indicate the redox potential of cells in order to monitor their viability, researchers have developed assays that useadenosine triphosphate ATP content as a marker of viability (Riss, 2004).

Such ATP-based assays include bioluminescent assays in which (ATP) is the limiting reagent for the luciferase reaction (Fan, 2007).

Cytotoxicity can also be measured by the sulforhodamine B (SRB) assay, WST assay and clonogenic assay.

A label-free approach to follow the cytotoxic response of adherent animal cells in real-time is based on electric impedance measurements when the cells are grown on gold-film electrodes. This technology is referred to as electric cell-substrate impedance sensing (ECIS). Label-free real-time techniques provide the kinetics of the cytotoxic response rather than just a snap shot like many colorimetric end point assays (Fan, 2007).

1.10.3 Immune system cytotoxicity

Antibody-dependent cell-mediated cytotoxicity (*ADCC*) describes the cell-killing ability of certain lymphocytes, which requires the target cell being marked by an antibody. *Lymphocyte-mediated cytotoxicity*, on the other hand, does not have to be mediated by antibodies; nor does *complement-dependent cytotoxicity* (*CDC*), which is mediated by the complement system.

Chapter Two

2. Materials and Methods

Chapter Two

1. Materials and Methods

All of the experimental techniques used in this thesis is present in this chapter.

2.1 Materials

Samples of *Anogeissus leiocarpus* gum were obtained from different location in Sudan, the gum samples were collected from gum pelt namely from Abojebiha and Elfula.

2.1.1 Sample Preparation

The gum samples were cleaned by hands to remove foreign particles, the samples were then grind using mortar and pistle, sieved through sieve no. 250 µm, after that grouped into three composite samples and kept in plastic container for further analysis without any purification technique.

2.2 Methods

2.2.1 Bulk And Tapped Densities

Using bulk and tapped density instrument (Pharma test Model:PT-TD1) a quantity of *Anogeissus leiocarpus* gum was powdered and passed through a sieve with apertures equal to 1.0 mm, to break up agglomerates. Into a dry, graduated, 250 mL cylinder (readable to 2 mL), gently introduced, without compacting, approximately 10.0 gm of the gum powder carefully weighed, the powder leveled without compacting, and the unsettled apparent volume (V_0) read to the nearest graduated unit.

The bulk density in grams per millilitre calculated using the formula;

$$m/V_0. \text{ ——————————►} \quad \text{(Equation 2.1)}$$

the cylinder in the support. A 10, 500 and 1250 taps Carried out on the same powder sample and the corresponding volumes was read V_{10}, V_{500} and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than 2 mL,

V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments of, for example, 1250 taps, until the difference between successive measurements is less than 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density in grams per millilitre using the formula m/V_f (where V_f is the final tapped volume). Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results (WHO; 1998. BP 2013).

2.2.2 Solubility

This test is visual comparative tests; was carried out by using colourless, transparent, neutral glass tubes with a flat base. The volume of liquid prescribed are for use with tubes 16 mm in internal diameter; tubes with a larger internal diameter, the volume of 1.0 gram. *Anogeissus leiocarpus* gum powder with purified water increased, until sample dissolved completely with shaking. The solution was examined down the vertical axis of the tube against a white back-ground (WHO; 1998. BP 2013).

2.2.3 Density

The *weight per millilitre* was determined by dividing the weight in air, expressed in gram, of the quantity of the solution of 10.0 gram of the gum dissolved in 100 ml purified water that filled a pycnometer at the 20^{o} C temperature by the capacity, expressed in mL, of the pycnometer at the same temperature (BP 2013). The capacity of the pycnometer was ascertained from the weight in air, expressed in g, of the quantity of *water* required to fill the pycnometer at that temperature. The weight of a litre of water at specified temperatures when weighed against brass weights in air of density 0.0012 gram per mL was given in the following table **(Table 2.1)**. Ordinary deviations in the density of air from the above value, here taken as the mean:

Table 2.1: Density of water at different temperature

Temperature ^{o}C	Weight of a litre of water (g)
20	997.18
25	996.02
30	994.62

The density, ρ_{20}, of 10.0 g of gum dissolved in 100 ml purified water was the ratio of its mass to its volume at 20°. It is expressed in kg m^{-3}.

The density was determined by dividing the weight in air of the quantity of the solution of gum being examined that fills a pycnometer (25 ml) at 20°C by the weight in air of water required filling the pycnometer after making allowance for the thrust of the air.

The density was calculated from the expression:

$$\rho_{20} = \frac{998.2\,(M_1+A)}{M_2+A} \longrightarrow \text{(Equation 2.2)}$$

Where:

M_1 = weight in air (apparent mass) in grams of the substance being examined, M_2 = weight in air (apparent mass) in grams of *water*,

A = the correction factor for the thrust of the air, $0.0012M_2$.

998.2 = the density of water at 20° in kg m^{-3}.

In most cases, the correction for the thrust of the air may be disregarded.

2.2.4 Loss On Dry

A 1.0 gram of the powder gum was placed in an oven (RADWAG, Model:MAX 50/NH) at temperature 105°C ± 2 °C , in a weighing bottle previously dried under the 105^{O} C, the powder gum dried to constant mass (FAO Corporate Document Repository, 1997. BP, 2013).

The losses calculated by the following equation:

$$\text{Loss On Drying} = \frac{W1-W2}{W1} \longrightarrow \text{(Equation 2.3)}$$

Where:

Weight of sample before drying = W1

Weight of sample after drying = W2

2.2.5 Total ash

A silica crucible ignited at 600 ±50 °C for 30 minutes, and cooled in a desiccator over silica gel, and a 1.0 gram of powder gum was weighed and placed in the crucible and weighed. Heated gently at as low a temperature as practicable until the sample is thoroughly charred. After cooling, ignite at 550 ± 50 °C on muffle (Wisetherm-wised) until the residue is completely incinerated. Allowed the crucible to cool in a desiccator over silica gel, weighed it again and the percentage of residue calculate (FAO Corporate Document Repository, 1997. BP 2013).

$$\text{Total ash} = \frac{W4-W3}{W4} \longrightarrow \text{(Equation 2.4)}$$

Where:

Weight of sample before ignition = W4

Weight of sample after ignition = W3

2.2.6 ρH of Dispersion Of *Anogeissus leiocarpus* gum solution

The pH-meter (Sartorius, Model: Professional meter PP-20) was calibrated with the buffer solution of potassium hydrogen phthalate (primary standard) and one other buffer solution of different pH. The pH of a third buffer solution of intermediate pH read off on the scale (not differ by more than 0.05 pH unit from the value corresponding to this solution).

The electrodes were immersed in the 10.0 grams of the gum, dissolved in 100 ml carbon dioxide-free water, and the reading taken in the same conditions as for the buffer solutions (BP 2013).

2.2.7 Specific Optical Rotation.

The polarimeter (Uni pol L Model: SCHMIDT+HAENSCH) was set to zero using purified water and the angle of rotation of polarised light at the wavelength of the D-line of sodium (λ= 589.3 nm) at 20 ± 0.5 °C. 10.0 gm. of the gum was dissolved in 100 ml carbon dioxide-free water, a 20 cm tube was filled with the solution to determined specific optical rotation (FAO Corporate Document Repository, 1997 and BP, 2013).

The specific optical rotation calculated by using the following formula.

$$\{\alpha\}^{20}_{D}=\frac{1000\ \alpha}{l.C} \longrightarrow \text{(Equation 2.5)}$$

Where:

C is the concentration of the solution in grams per litre.

λ = angle of rotation in degrees read at 20 ± 0.5°C,

l= length in decimetres of the polarimeter tube.

2.2.8 Refractive Index

The refractive index (Instrument:Krus Model; A.Kruss Optrionic) of a medium with reference to air is equal to the ratio of the sine of the angle of incidence of a beam of light in air to the sine of the angle of refraction of the refracted beam in the given medium.

The refractive index was measured at 20 ± 0.5 °C, with wavelength of the D-line of sodium (λ= 589.3 nm); and one drop of the liquid (zimtöl solution) was put on the measuring prism and lightly press the smooth surface of the sample of gum onto it, reflecting mirror was opened and direct light by way of the mirror onto the prism,

the measurements occur in reflected light, the light/dark fields one exchanged and contrast becomes less, the symbol is then n_D^{20} (Karamalla *et.al*, 1998).

2.2.9 Intrinsic Viscosity

1.0 gram of gum sample were dissolved in 100 ml of 1M NaCl, and another 1.0 gram were dissolved in 100 ml of purified water to give solution with concentrations of it. The solutions was filtered through $3\mu m$ Millipore filter into clean containers and the viscosity determined using a cannon ubbelohde ($\mu 130$) semi micro viscometer size 75. The viscometer was cleaned by washing with distilled water and dried in acetone. Exactly 2 ml samples were pipettes into the reservoir and the viscometer was placed into the holder and interested into a constant temperature water bath set at 25^0C.

The initial relative viscosity was determined; three subsequent readings for the flow time were taken.

Further dilutions of the samples were made inset by adding appropriate amount of the solvent and the flow time for each concentration was determined as described previously (López Martínez *et.al*, 2003).

2.2.10 Nitrogen and Protein Content

Kjeldal method was used to determination the total nitrogen in *Anogeissus leiocarpus* gum samples. The procedure used (Bradstreet, 1965) is two stages, process in which the gum samples are digested in hot concentrated sulphuric acid and ammonia released using sodium hydroxide is neutralized using standard acid.

The reactions involved in these steps can be shown as follows:

Sample + H_2SO_4 (conc.) catalyst + Heat $\rightarrow (NH_4)_2.SO_4 \text{---►}$ (Equation 2.6)

$(NH_4)_2.SO_4 + 2NaOH \rightarrow 2NH_3 + NaSO_4 + 2H_2O \text{ ------►}$ (Equation 2.7)

$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$ ——————————► (Equation 2.8)

$H_2BO_3^- + HCl \rightarrow H_3BO_3 + Cl^-$ ——————————► (Equation 2.9)

0.5 gram of each sample (in triple) was weighed and transferred to kjeldahl digestion flasks and kjeldahl tablet (copper sulphate-potassium sulphate catalyst) was added to each 10.0 cm^3 of concentrated, nitrogen free, sulphuric acid was added. The tube was then mounted in the digestion heating system which was previously set to 240^o C and capped with an aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish-green color was observed which indicates the completion of the digestion. The tubes were then allowed to attain room temperature.

Their contents were quantitatively transferred to two separate kjedahl distillation apparatus followed by addition of distilled water and 30% (w/v) sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 25 cm^3 of 2% boric acid. Back titration of the generated borate was then carried versus, 0.02M, hydrochloric acid using methyl red as an indicator. Back titration was carried in the same way.

$$N = \frac{14.01 \times M \times (\text{volume of titrant} - \text{volume of blank}) \times 100}{\text{Weight of sample}} \text{-►(Equation 2.10)}$$

Which M is the molarity of hydrochloric acid.

Protein content was calculated by using nitrogen conversion factor (NCF) of 6.25 (Anderson, 1986) as follows:

%protein = %N X 6.25——————————► (Equation 2.11)

2.2.11 Sugar Composition by HPLC

The samples were hydrolyzed to liberate the sugar residues (Randall, R.C.,1989). Sample was weighed out (200.0 mg, accurately weighed out – including allowing for moisture content) and added to 10 cm^3 of H_2SO_4 and incubated at 100^oC for 6 hours. Following this, 1.0 gram of $BaCO_3$ were added to the solution and left over night (minimum 12 hours to neutralize the solution, after $BaCO_3$ treatment, universal indicator strips were used to ensure that the samples are neutral before proceeding to the next stage, the solution was centrifuged at 2500 rpm for 10 minutes to allow the Barium Sulphate (formed from neutralizing the H_2SO_4) to settle. The supernatant solution was removed and filtered through a 0.45 µm Whatman nylon filter and then diluted 1:1 with 70:30 Acetonitrile : buffer. This constituted the final solution of which 1.0 ml was analyzed using HPLC (Shimadzu Model: DGU – $20A_3$ Prominence), in the central laboratory at University of Khartoum.

2.2.12 Ultraviolet Absorption Spectrophotometry

U.V Spectrophotometer Shimadzu U.V model 1800s was used for measuring in the ultraviolet range of the spectrum consist of an optical system capable of producing monochromatic radiation in the range of 190 - 400 nm and measuring the absorbance.

0.5gram of gum powder dissolved in 100ml purified water, measure the absorbance at the prescribed wavelength using a path length of 1 cm, the measurements are carried out with reference to the same solvent or the same mixture of solvents. The absorbance of the solvent measured against air and at the prescribed wavelength shall not exceed 0.4 and is preferably less than 0.2. Plot the absorption spectrum with absorbance or function of absorbance as ordinate against wavelength or function of wavelength as abscissa (G.O.Phillips, 2009).

2.2.13 Equivalent Weight and Uronic Acid

Acid equivalent weight was determined according to the method reported in (encyclopedia of chemical technology, 1966) with some modification.

A cation exchange column packed with Amberlite -120 resin (acid form) was thoroughly washed with 2 M Sulphuric acid. Leave to stand for 10 minutes then continuous washing with about 150 cm^3, followed by distilled water until free of sulphate. Check the presence of sulphate by testing with Barium Chloride solution (Saturated solution). 50 $cm^3$of 3% (W/V) of gum sample were introduced on top of the column and allowed to elute under gravity action. Adjust the elution rate by one drop per 2 second. Collect the elute and wash with about 150 Cm^3 distilled water, the elution rate does not have to be adjusted. The eluent and washing were collected and titrated against standard 0.1 M solution of sodium hydroxide; by using phenolphathalein as indicator (2 drops were added), phenolphathalein was prepared as follows: 2% in ethanol.

Calculations:

$$\text{Acid equivalent weight} = \frac{\text{weight of sample X50.000}}{\text{Volume of titrant X molarity of alkali}} \quad \text{(Equation 2.12)}$$

$$\text{Uronic acid \%} = \frac{\text{molar mass of uronic acid unhydride X100}}{\text{Acid equivalent weight.}} \quad \text{(Equation 2.13)}$$

2.2.14 Cationic Composition

Using an atomic emission spectrometer Shimadzu model AA-6800 in accordance with the manufacturer's instructions at the prescribed wavelength. The experimental optimise conditions (flame temperature, burner adjustment, by using of an ionic buffer, concentration of solutions in plastic labware) for the specific element to be analysed and in respect of the sample matrix.

A blank solution was introduced into the atomic generator and the instrument adjusted to reading zero or to its blank value. A reference solution was introduced in the most concentrated and sensitivity was adjusted to obtain a suitable reading (Buffo *et.al*., 2001).

The calibration plots curved and then applied with appropriate calibration software. The cationic composition determined by made comparison with reference solutions with known concentrations of the element to be determined.

Samples prepared by using dry ashing method, two grams of *Anogeissus Leiocarpus* gum sample were placed in a well-glazed porcelain dish. Start in a cold furnace, and then heated to 550 ^{o}C, maintain temperature for four hours. Cool the sample and add 10.0 ml of 3N HCl. Cover with watch glass, and boil sample gently for 10 minutes. Cool, filter into a 100 cm^3 volumetric flask, and dilute to volume with deionized water.

Atomic absorption spectrometerwas used to determination seven elements: Potassium, Sodium, Magnesium, Calcium, Iron, Zinc, Chromium, and lead (Buffo *et.al*., 2001).

2.2.15 Infrared Spectra

By using Disc technique, a 2 mg of the *Anogeissus leiocarpus* gum powder treated with 300-400 mg, of finely powdered and dried *potassium bromide R*. These quantities are usually sufficient to give a disc of 10 - 15 mm diameter and a spectrum of suitable intensity. The mixture was grind carefully, and spreaded uniformly in a suitable die, and submitted it to a pressure of about 800 MPa (8 $t \cdot cm^{-2}$). Under normal atmospheric conditions, the disc was pressed *in vacuo*. The disc was examined at transmittance 400 cm^{-1} - 4000 cm^{-1}, by using FTIR spectrophotometer Shimadzu, Model IR Affinity–1(B.P 2013).

2.3 Preparation of Cefixime Oral Suspension

Cefixime trihydrate oral suspension was prepared by dry direct mixing method. The calculated amount of active ingredient was weighed and manually sieved through 18 mesh screen (1mm) and taken in a polybag. All other excipients were sieved through 18 mesh screen (1mm) and taken in previous polybag. Mixture was manually blended for 5 minutes. Then the blend was sieved through 18 mesh screen (1mm). Finally the powder was filled manually in 75 ml amber bottles for fit prescription and closed tightly. In the cefixime for oral suspension formula, sodium benzoate was used as preservative agent, areosil was used a glidant to improved flowability, *Anogeissus leiocarpus* gum was used as suspending agent. To impart palatability, sucrose was used as a sweeting agent and strawberry flavor as flavouring agent, the representative formula for the preparation of dry mixture are presented in (Table 2.2). Dry suspension required addition of water at the time of dispensing or testing.

Table 2.2: Representative Formula of Cefixime trihydrate 100 mg/5 ml for Oral Suspension.

No.	Ingredients	Weight per unit (gm)	Purpose
1	Cefixime trihydrate	0.600	Active
2	Sodium benzoate	0.060	Preservative agent
3	Strawberry flavor	0.150	Flavouring agent
4	Sucrose	13.920	Sweeting agent/Diluent
5	Areosil	0.150	Glidant
6	*Anogeissus Leiocarpus* gum	0.120	Suspending agent
Total		15.000	

2.3.1 Assay for Cefixime for Oral Suspension

Solution A: 0.4 M tetrabutyl ammonium hydroxide solution and water (1:39). Adjust with 1.5 M phosphoric acid to a pH of 6.5.

Solution B: 13.6 mg/mL of monobasic potassium phosphate

Solution C: 14.2 mg/mL of anhydrous dibasic sodium phosphate. Adjust a volume of this solution with a sufficient volume of Solution B to a pH of 7.0.

Mobile phase: Acetonitrile and Solution A (1:3)

System suitability solution: 1 mg/mL of USP Cefixime RS. [Note— Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly].

Standard solution: 0.2 mg/mL of USP Cefixime RS in Solution C. [Note— Use this solution promptly].

Sample solution: Constitute Cefixime for Oral Suspension as directed in the labeling. Quantitatively dilute a suitable aliquot of the suspension, freshly mixed and free from air bubbles, with Solution C to obtain a solution having a nominal concentration of 0.2 mg of cefixime/mL (BP, 2013).

Chromatographic system

Mode: HPLC Shimadzu Model: DGU – $20A_3$ Prominence

Detector: UV 254 nm

Column: 4.6-mm × 12.5-cm; 4-µm packing L1

Temperature: 40°C.

Flow rate: Adjust flow rate so that the retention time of cefexime is about 10 min.

Injection size: 10 µL

System suitability

Samples: System suitability solution and Standard solution

[Note — The relative retention times for cefixime (E) - isomer and cefixime are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between cefixime and cefixime (E) isomer, System suitability solution

Column efficiency: NLT 4000 theoretical plates, Standard solution. Use the following formula to calculate column efficiency:

Result = 5.545(t/Wh/2)2

Tailing factor: NLT 0.9 and NMT 2.0 for the analyte peak, Standard solution. Use the following formula to calculate tailing factor:

Result = W0.1/2f

W0.1 = peak width at 10% peak height

Relative standard deviation: NMT 2.0%, Standard solution (BP, 2013).

Analysis:

Samples: Standard solution and Sample solution.

Calculate the percentage of $C_{16}H_{15}N_5O_7S_2$ in the constituted suspension prepared from the Cefixime for Oral Suspension (BP,2013):

Result = $(r_U / r_S) \times (C_S / C_U) \times 100$ ——————————► (Equation 2.14)

r_U = peak response of cefixime from the Sample solution

r_S = peak response of cefixime from the Standard solution

C_S = concentration of USP Cefixime RS in the Standard solution (mg/mL)

C_U = nominal concentration of cefixime in the Sample solution (mg/mL)

Acceptance criteria: 90.0% –120.0% (BP, 2013).

2.3.2 Rheological Method

It provides information about settling behaviour. The arrangement of the vehicle and the particle structural features.

Brookfield viscometer is used to study the viscosity of the suspension. It is mounted on heli path stand and using T- bar spindle. T- bar spindle is made to descend slowly into the suspension and the dial reading on the viscometer is then a measure of the resistance the spindle meets at various levels (Prentice, 1983).

This technique also indicates at which level of the suspension the structure is greater owing to particle agglomeration. The dial reading is plotted against the number of turns of the spindle. The better suspension show a lesser rate of increase of dial reading with spindle turns, i.e. the curve is horizontal for long period.

2.3.3 Sedimentation Method.

The suspension formulation (30mL) was poured separately into100m measuring cylinders and sedimentation volume was read after 1, 2, 3 and 7 days and there after at weekly intervals for 12 weeks. Triplicate results were obtained for each formulation. Sedimentation volume was calculated according tothe equation:

$F = V_u/V_o$ ——————————► (Equation 2.15)

Where, F = sedimentation volume, V_u= ultimate height of sediment and V_o= initial height of total suspension (A. Khusnoor, 2003).

2.4 Cytotoxicity Screening

2.4.1 Principle of Cytotoxicity Screening

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrical-

ly. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

2.4.2 Preparation of Extracts, Solutions

Using a sensitive balance 5 mg of each extracts were weighed and put in eppendorf tubes. 50 µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

2.4.3 Cell Line and Culturing Medium

Vero (Normal, African green monkey kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37^{o}C. The cells were subculture twice a week.

2.4.4 Cell line used

Vero cells (Normal, African green monkey kidney).

2.4.5 Cell counting

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted (Patel S, 2009). The following formula was used for calculating cells:

$$(\text{Cells/ml})\ N = \frac{\text{Number of cells counted X dilution factor X } 10^4}{4} \longrightarrow \quad \text{(Equation 2.16)}$$

Procedure:

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant solution that flicked out (Patel S, 2009). 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37 ^{o}C for 96 hours (Patel S, 2009).

On the fourth day, the supernatant solution was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96 - well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37^{o}C. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well.

The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader (Patel S, 2009). The percentage growth inhibition was calculated using the formula below:

% cell inhibition = 100-{(Ac-At)/Ac} × 100−−−−−−−−−−► (Equation 2.17)

Where, At = Absorbance value of test compound;

Ac = Absorbance value of control.

Chapter Three

3. Results and Discussions

Chapter Three

3. Results and Discussion

Unless otherwise stated, all chemicals were of analytical grade and procured from Romil (England). The results from all studies are expressed as mean of at least three values, unless otherwise indicated.

3.1 Statistical Analysis

The main statistical test employed for analysis of results was the one- way analysis of variance (ANOVA), carried out using the software (Exell) at 95% confidence interval.

Table 3.1. **Values for some physical and chemical parameters of the *Anogeissus leiocarpus* gum.**

Tests	Results
Bulk density gm/ml	0.82
Tapped Density gm/ml	0.97
Density gm/ml	1.07
Loss on drying % w/w	7.82
Residue on ignition % w/w	2.02
ρH	4.52
Refractive index	1.59
Nitrogen % w/w	0.14
Protein % w/w	0.88
Specific optical rotation	-34.51^{o}
Equivalent weight	1384.33
Uronic Acid% w/w	14.03

3.2 Determination of Bulk Density

The bulk density of *Anogeissus leiocarpus* gum powder is the ratio of the mass of an untapped *Anogeissus leiocarpus* gum powder sample to its volume, including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of *Anogeissus leiocarpus* gum powder particles and the spatial arrangement of particles in the *Anogeissus leiocarpus* gum powder bed. The bulk density is expressed in grams per millilitre despite the International Unit being kilogram per cubic metre (1 g/mL = 1000 kg/m^3), because the measurements are made using cylinders. It may also be expressed in grams per cubic centimetre.

The bulking properties of *Anogeissus leiocarpus* gum powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it has been handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of *Anogeissus leiocarpus* gum powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of *Anogeissus leiocarpus* gum powder is determined by measuring the volume of a known mass of powder sample, which may have been passed through a sieve, in a graduated cylinder, the bulk density determine and the results shown in the table (3.1).

3.3 Determination of Tapped Density

The tapped density is an increased bulk density attained after mechanically tapping a receptacle containing *Anogeissus leiocarpus* gum powder sample. The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder is mechanically tapped, and volume or mass readings

are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass, a specified distance as described in table 3.1. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down, the result shown as below:

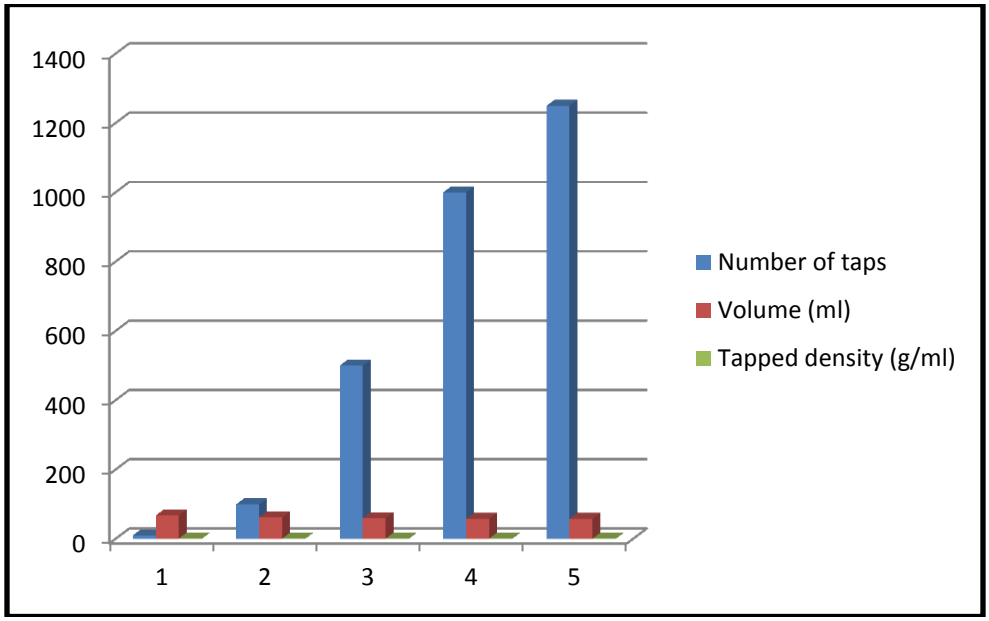


Figure 3.1: Tapped density of *Anogeissus leiocarpus* gum powder.

3.4 Determination Solubility

Solubility is often said to be one of the "characteristic properties of *Anogeissus leiocarpus* powder gum", which means that solubility is commonly used to describe the gum powder, to indicate a gum powder's polarity, to help to distinguish it from other gums, and as a guide to applications of the gum.

Statements on solubility given under the side-heading Characteristics are intended as information on the approximate solubility at a temperature between 15° and 25°, and are considered as official requirements.

1.0 gm of *Anogeissus leiocarpus* gum powder was dissolved completely in 7.0 ml of purified water that means the *Anogeissus leiocarpus* gum is freely soluble in purified water.

Table 3.2: **Approximate solubility grading.**

Descriptive term	**Approximate volume of solvent in millilitres per gram of solute**
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10000
Practically insoluble	More than 10 000

3.5 Determination of Density

The specific gravity of *Anogeissus leiocarpus* gum were 1.066± 0.026 (Table 3.1) since specific gravity is a constant that varies slightly for any given kind of carbohydrates. It is of little value in determining the purity of the sample and it can only be used in the characterization of a given sample. Specific gravity of the solution is nearly equal to 1 which is the density of water that controls the distribution of particles in the solution (Kuntz 1999). Thus, addition of other substances to the gum solution can be accommodated and may alter the characteristic by improving the texture of added material.

3.6 Determination of Loss On Drying

The loss on drying test is designed to measure the amount of water and volatile matters in a sample of *Anogeissus leiocarpus* gum powder when the sample is dried under conditions specified in table 3.1, the results shown as below:

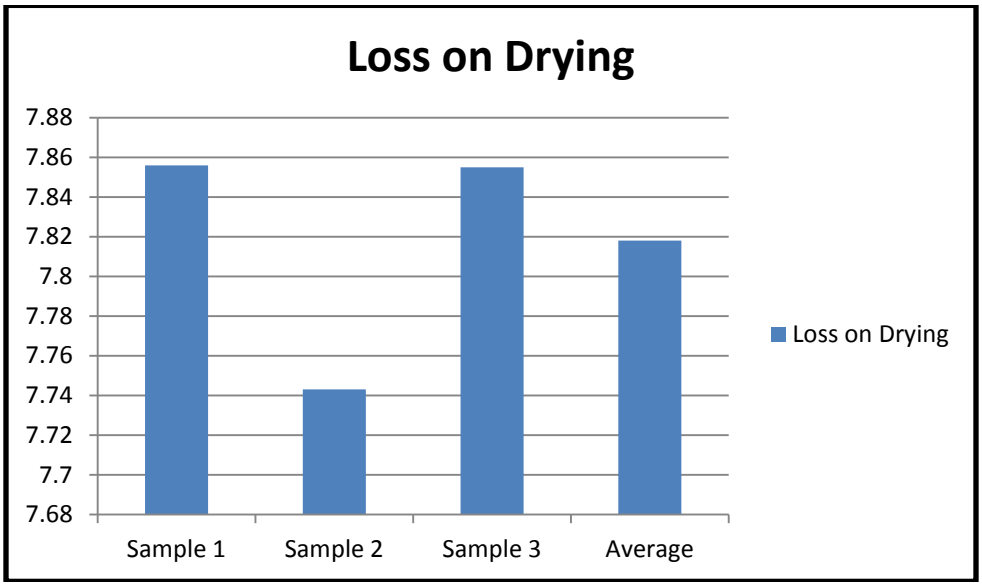


Figure3.2: Loss On Drying of *Anogeissus leiocarpus* gum powder

3.7 Determination of Total ash

The Residue on ignition test utilizes a procedure to measure the amount of residual substances on *Anogeissus leiocarpus* gum not volatilized from sample when the sample is ignited according the procedure descripe in 2.2.5. This test is usually used for determining the content of inorganic impurities in an organic substance.

3.8 Determination of ρH value

Anogeissus leiocarpus gum is easily and quickly soluble in cold water. Highly concentrated solutions with relatively low viscosity can be produced. At concentrations of less than approx. 40%, solutions react like authentic Newtonian fluids; at higher concentrations they show pseudo-plastic behaviour.

In aqueous solutions, *Anogeissus leiocarpus* gum shows slightly acid character at temperature between 25 ^{o}C to 27 ^{o}C (pH 4 to 5) as shown below.

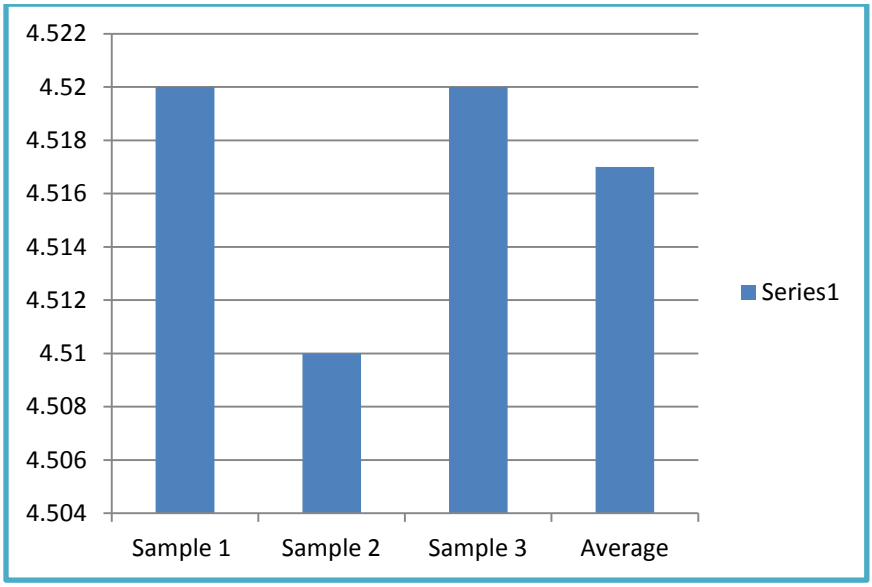


Figure 3.3: ρH of *Anogeissus leiocarpus* gum powder

3.9 Determination of Specific Optical Rotation

The Optical rotation for the *Anogeissus leiocarpus* gum samples were analysed found to be -34.513 (Table 3.1). This is approximately similar to the range of the international standards of the FAO, (1995) for Acacia gums which is (-26) to (-34).

3.10 Determination of Refractive Index

The refractive index which is a fundamental physical property can be used to identify the substance, confirm its purity and to measure its concentration. From the results of refractive index (Table 3.1), the the average of refrective index of *Anogeissus leiocarpus* gum samples was 1.5895, if the samples of *Anogeissus leiocarpus* gum compared to the standard (gum acacia) with refractive index, 1.334, the observed that the *Anogeissus leiocarpus* gum samples was almost pure.

3.11 Determination of Intrinsic Viscosity

Anogeissus leiocarpus gum has high water solubility and a relatively low viscosity compared with other gums. Most gums cannot dissolve in water in concentrations above 5% due to their high viscosity. Instead, *Anogeissus leiocarpus* gum can get dissolved in water in a concentration of 40% w/v, forming a fluid solution with acidic properties (pH =4.517). The highly branched structure of the *Anogeissus leiocarpus* gum molecules may leads to compact relatively small hydrodynamic volume and, consequently *Anogeissus leiocarpus* gum will only become a viscous solution at high concentrations. Solutions containing less than 10% of *Anogeissus leiocarpus* gum have a low viscosity and respond to Newtonian behavior. However, steric interactions of the hydrated molecules increase viscosity in those solutions containing more than 30% of *Anogeissus leiocarpus* gum resulting in an increasingly pseudoplastic behavior.

Table 3.3: **The intrinsic viscosity of *Anogeissus leiocarpus* gum using NaCl as Solvent.**

Conc.%	T_i. sec	T_i/T_o	$T_i/T_o=\eta_i/\eta_o$	$(\eta_i-\eta_o)/\eta_o=\eta_{sp}$	η_{sp}/C	$\ln((\eta_o/\eta i)/C)*C$
0.01	1019	1.676	1019	0.676	67.599	-0.392
0.005	844	1.388	844	0.388	77.632	-0.946
0.0025	745	1.225	755	0.225	90.132	-1.490
0.0013	690	1.135	670	0.135	103.745	-2.003
0.0006	658	1.082	658	0.082	137.061	-2.498

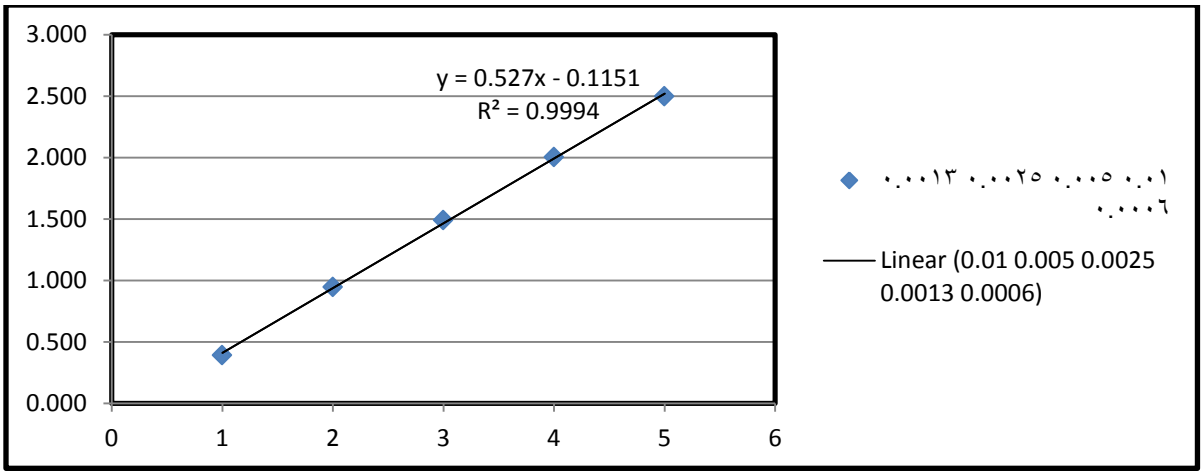


Figure 3.4. Logarithmic plot of the intrinsic viscosity of the gum as a function of concentration in NaCl. ([η], cm^3/g) in function of 1/T of viscosity (Arrhenius plot).

Table 3.4: **The intrinsic viscosity of *Anogeissus leiocarpus* gum using water as solvent.**

Conc.%	T_i. sec	T_i/T_o	$T_i/T_o=\eta_i/\eta_o$	$(\eta_i-\eta_o)/\eta_o=\eta_{sp}$	η_{sp}/C	$\ln((\eta_o/\eta_i)/C)*C$
0.0100	1503	2.472	2.472	1.472	147.204	0.387
0.0050	1149	1.890	1.890	0.890	177.961	-0.117
0.0025	919	1.512	1.444	0.512	204.605	-0.670
0.0013	790	1.299	1.286	0.299	230.263	-1.206
0.0006	720	1.184	1.184	0.184	307.018	-1.692

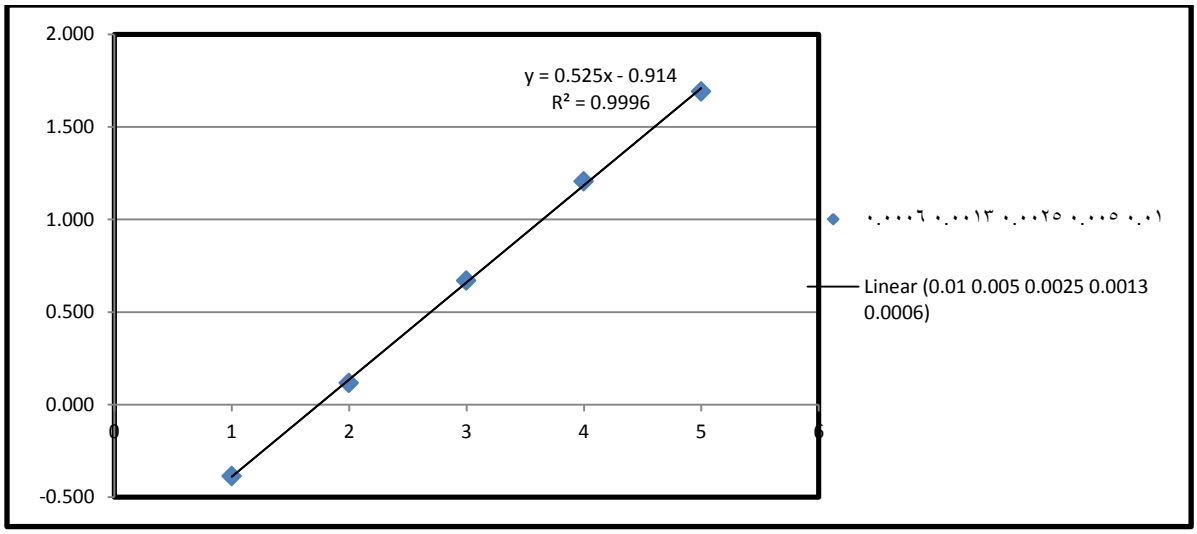


Figure 3.5: Logarithmic plot of the intrinsic viscosity of the gum as function of concentration in water. ([η], cm^3/g) in function of 1/T of viscosity (Arrhenius plot).

The intrinsic viscosity was determined by taking specific viscosity at various concentrations and extrapolating the concentration to c = 0. The concentration dependence is also expressed by Huggins equation.

The value of Huggins constant for sodium chloride was 0.1151 and in water was 0.9142, it was observed that the Huggins constant was much greater in water than sodium chloride media that indicating the poor incompatibility of solvent with solute. Increasing in intrinsic viscosity can be attributed to the change in water structure affecting the interaction between water and *Anogeissus leiocarpus* gum molecules. The weak protonation of the hydroxyl groups in *Anogeissus leiocarpus* gum and water molecules caused by high H^+ may reduce the inter and intra molecular hydrogen bonding. It is possible that *Anogeissus leiocarpus* gum molecules are less expanded in sodium chloride media and the hydrogen bonding between sodium chloride and *Anogeissus leiocarpus* gum is less prevalent.

3.12 Determination of Nitrogen Content and Protein

The mean nitrogen content of the gum was found to be relatively low 0.14%, Table 3.1 compared to the international standard of 0.26–0.39 (FAO, 1995), with corresponding low protein content of 0.88% using the 6.25 standard nitrogen conversion factor (NCF). This is result considering the significance of nitrogen in the application properties of gum arabic. It is important to study the nitrogen content of gum because it forms an important component which absorb unto the surface of oil droplet for the purpose of emulsion stability (Dickinson, 1992). reported that the protein moiety of gum arabic affect its emulsification ability and that the best emulsion stability is found in gums with highest amount of nitrogen.

3.13 Determination of Sugar composition by HPLC.

The polysaccharides isolated from *Anogeissus leiocarpus* gum by HPLC contained galactose, arabinose and rhamnose, the content of arabinose was found 34.00% is predominating in the in the *Anogeissus leiocarpus* gum, galactose was found 27.50% and rhamnose the less values was found 9.288% in the gum. Table 3.5 showed the sugar content for Rhamnose, Arabinose, and Galactose, a higher arabinose content in comparison with values found for the in situ gum and for gums from Prosopis species (Anderson & Farquhar, 1982; Anderson & Weiping, 1989). The sugar composition of the in situ gum is very close to that reported by Anderson and Farquhar (1982) for P. laevigata.

Table 3.5: The sugar content for Rhamnose, Arabinose, and Galactose.

Sugar	Retention time	Area	Sugar Concentration%
Rhamnose	4.808	42309	9.288
Arabinose	5.775	429239	34.000
Galactose	7.317	4806	27.500

3.14 Ultraviolet Absorptionof *Anogeissus leiocarpus* Gum.

The ultraviolet (UV)-visible spectra from 190 to 400 nm were measured for sample of *Anogeissus leiocarpus*. **Appendix 3.29, 3.30, 3.31** shows λ_{max} of *Anogeissus leiocarpus* gum solution was distinctive in having higher relative absorbance of the light above 190 nm, also *Anogeissus leiocarpus* gum sample had peaks in the 194.8 to 276.6 nm range.

3.15 Determination of the equivalent weight and uronic acid

Table 3.1, showed that the equivalent weight of samples ranged between 1372and 1396,and the mean value of the three samples was 1384.33.This value fall within that of*Acacia Senegal* gum *Var. Senygal* (1136 - 1875),also the presence of uronic acids in all samples of *Anogeissus leiocarpus* gum were ranged between 13.91% to 14.15%, and the mean value of the three samples was 14.02%. which is within the range *of Senegal* gum*Var. Senygal* (10.34%- 23.32 %) (Siddig 1996).

3.16 Determination of Cationic Composition

A calibration curve was prepared from the mean of the readings obtained with the reference solutions by plotting the means as a function of concentration. Not fewer than three reference solutions of the element to be determined applied. The concentration of the element in the test solution was determined from the curve obtained.

Table 3.6: shows that calcium; magnesium, potassium, sodium, iron, and copper are the most abundant elements in all gum samples. The mean values in the table show that the major elements in *Anogeissus leiocarpus* samples are, in the decreasing order Ca^{++} was 4532.5 ppm, K^{+} was 3520.0 ppm, Mg^{++} was 1046.5 ppm, Na^{+} was 600.0ppm, Fe^{++} was 562.5 ppm, Pb^{++} was 258.0 ppm, Zn^{++} was 197.25 ppm, Mn^{++} was 74.5 ppm, Cr^{++} was 4.5 ppm and Cd^{++} was not detected. It could be observed that Calcium (Ca^{++}), Potassium (K^{+}) and Magnesium (Mg^{++}) has

the higher value in the *Anogeissus leiocarpus* gum may be a source of metals and indicates the nutritive values of this gum. Also the transition metals which form colored complexes, therefore they can be considered to be the main reason for coloration.

Table 3.6: Cationic Composition of *Anogeissus leiocarpus* Gum.

Metals	Actual conc.(ppm)
Ca^{++}	4532.5
K^{+}	3520.0
Mg^{++}	1046.5
Na^{+}	600.0
Fe^{++}	562.5
Pb^{++}	258.0
Zn^{++}	197.3
Mn^{++}	74.5
Cr^{+++}	4.5
Cd^{++}	n.d.

3.17 IR Spectra

The FTIR spectrum of *Anogeissus leiocarpus* gum powder was obtained by using KBr disc, the FTIR spectrum of *Anogeissus leiocarpus* gum powder is obtained between 400 and 4000 cm^{-1}. The FTIR spectra of *Anogeissus leiocarpus* gum the spectrum was very clear and strong features assigned to the polysaccharide molecules in *Anogeissus leiocarpus* gum. The major and strongest vibrational modes in the *Anogeissus leiocarpus* gum spectrum are those located at 1066, 1430, 1624, 2309, 2930, and a broad absorption band at 3000 - 3600 cm^{-1}. In addition to these major peaks, an additional peak appears at low frequency with low intensity at 650 cm^{-1}. The strong vibrational mode located at 3000-3600 cm^{-1} is assigned to the stretching vibrations of the O-H bond, the other strong vibrational mode located at 1624 cm^{-1} is assigned to the stretching vibrations of the C=O bond of carboxylate group associated with the *Anogeissus leiocarpus* gum molecules, the two vibrational modes located at 1066 and 1430 cm^{-1}, with relatively low intensity, are assigned to the stretching vibrations of the C-O bond, and the weak vibrational mode located at 2930 cm^{-1} is assigned to the stretching vibrations of the C-H bond (Robert *et.al*, 1981). The absorption band located at 2309 cm^{-1}, with relatively low intensity, is usually assigned to the CO_2 vibration (Robert *et.al*, 1981). It is worth mentioning that *Anogeissus leiocarpus* gum is made up of large amount of polysaccharide and very less amount of glycoprotein (Robert *et.al*, 1981). However, according to the FTIR spectra shown in Appendix 3.32, 3.33 and 3.34, no significant absorption bands have been observed for glycoprotein molecules. This result is attributed to the presence of broad absorption band at 3000–3600 cm^{-1} due to the O–H stretch of polysaccharide which might cover the characteristic bands of glycoprotein molecules.

3.18 Formulation of Cefixime trihydrate Oral Suspension using *Anogeissus Leiocarpus* gum

Cefixime trihydrate 100 mg oral suspension was prepared using *Anogeissus Leiocarpus* gum as suspending agent, and its in-vitro quality evaluation. Dry direct mixing method was adopted for preparation of suspensions using different excipients namely; sodium benzoate, strawberry flavour, sucrose, areosil and *Anogeissus Leiocarpus* gum. The dry direct mixing method for suspensions were evaluated for pH, density, sedimentation, rheological assessment, also suspensions were subjected to assay in vitro studies. The formulate was satisfactory as per cefixime trihydrate 100 mg oral suspension properties, and complied with the USP pharmacopoeia standard requirements when compared with marketed products.

3.19 Rheological Volume for Cefixime for Oral Suspension

The rheological properties of suspensions were investigated in order to clarify the relationship between the viscosity and suspendability using a rotation viscometer. As shown in Figure 3.6, rheological properties of excipients are important criteria for deciding their commercial use, (Pritam, 2014) the greater viscosity of suspensions offers the advantage of slower sedimentation; however, it may compromise other desirable properties for oral suspensions, and ease of administration for oral suspensions. The property of shear thinning is highly desirable so that the suspension is highly viscous during storage when minimal shear is present so that the sedimentation is slow and has low viscosity for oral suspension (high shear) facilitate ease of pourability from the bottle (Alok K., 2010).

They are added with the objective to increase apparent viscosity of the continuous, phase thus preventing rapid sedimentation of the dispersed particles.

The selection of the type and concentration of a suspending agent depends on sedimentation rate of dispersed particles, pourability and spreadibility.

The ideal suspending agent should have a high viscosity at negligible shear i.e., during shelf storage and it should have a low viscosity at high shearing rates i.e., it should be free flowing during agitation, pouring and spreadibility. A suspending agent that is thixotropic as well as pseudoplastic should prove to be useful as it forms a gel on standing and becomes fluid when shaken. They include natural polysaccharides (Gum *Acacia*, Gum *Tragacanth*, *Guar* Gum, Sodiun Alginate, *Xanthan* Gum and *Carrageenan*), Semi-synthetic polysaccharides (Sodium Carboxymethylcellulose, Methyl Cellulose, Hydroxyethyl Cellulose, Hydroxypropyl Cellulose, Hydroxypropyl Methyl Cellulose and Microcrystalline Cellulose), Clays (Aluminium Magnesium Silicate, Bentonite and Hectorite) and synthetic agents (Carbomer, Colloidal silicon dioxide). Pseudoplastic substances like tragacanth, sodium alginate and sodium carboxymethyl cellulose show these desirable qualities.

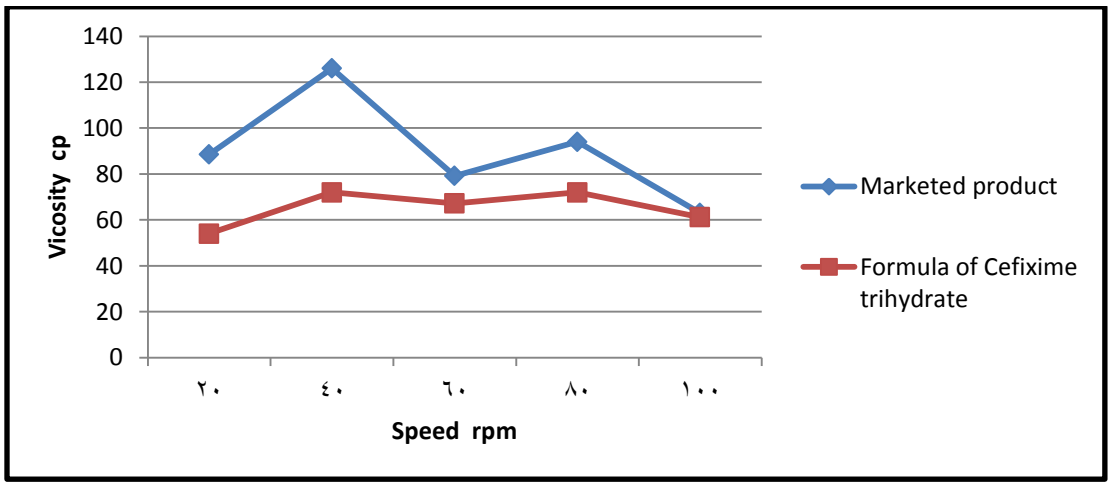


Figure 3.6: Effect of speed of rotation on the viscosity of Cefixime oral suspension formulated with *Anogeissus leiocarpus* gum.

3.20 SedimentationVolume for Cefixime For Oral Suspension

It is observed that the sedimentation volume is between 0.8-0.7 at the end of 28 days of the formula of cefixime trihydrate, and between 0.83 – 0.71 of the marketed product. The shape of the curve shows good stability of formulated of Cefixime trihydrate suspension,and this was shown in the Figure 3.7.

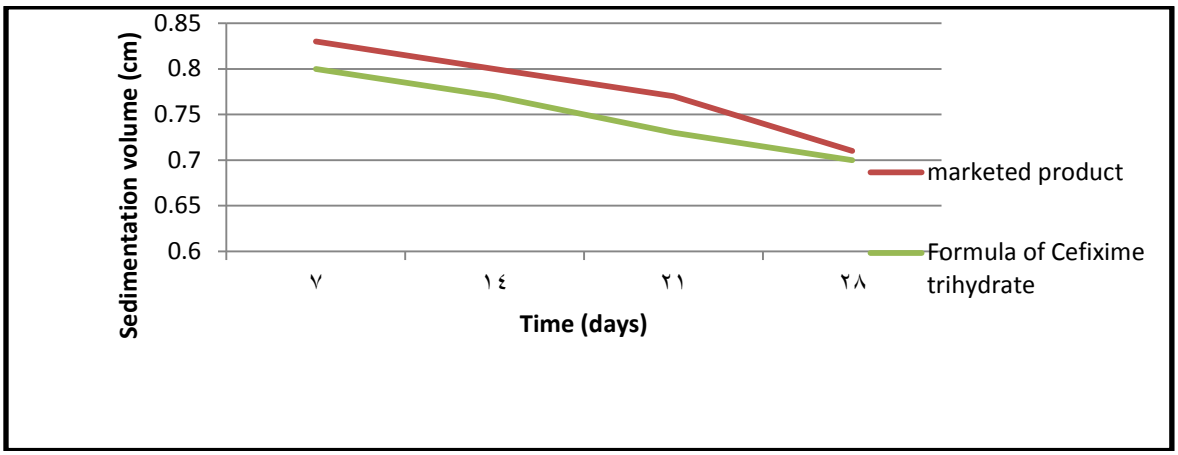
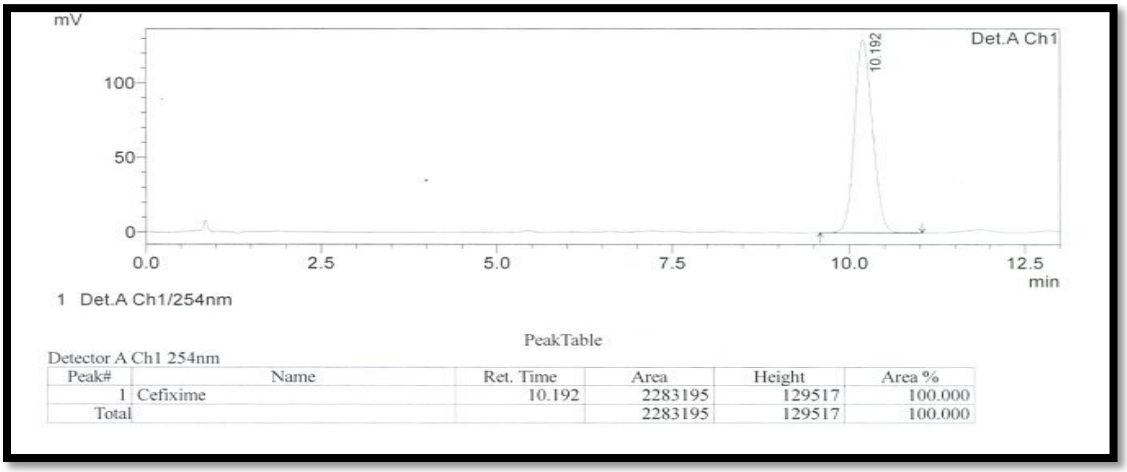


Figure 3.7: Sedimentation of formula of Cefixime trihydrate oral suspension using *Anogeissus leiocarpus* gum as suspending agent in comparison with marketed product.

3.21Assay for Cefixime for Oral Suspension

The assay for constituents formula of cefixime trihydrate for oral suspension and constituents of marketed product was carried out as per USP 2014, the assay was 100.05% – 92.41% (Figure 3.8) and for formula of cefixime trihydrate, and 110.6% – 103.30% for the marketed product respectively (Figure 3.9), it was in good agreement with the label claim during the 5 days at home storage conditions. The assay test in the constituents' marketed product the high percentage may be due to overage.

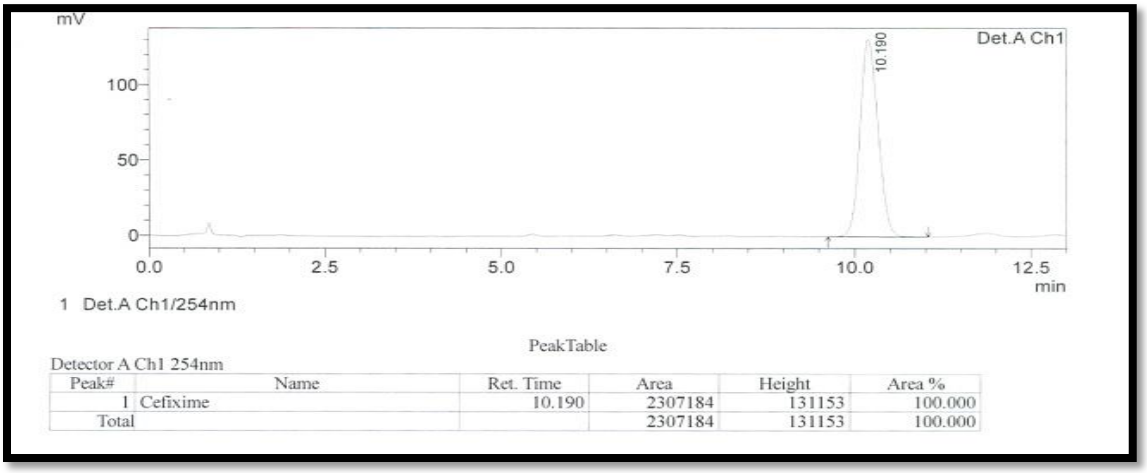


PeakTable

Detector A Ch1 254nm

Peak#	Name	Ret. Time	Area	Height	Area %
1	Cefixime	10.192	2283195	129517	100.000
Total			2283195	129517	100.000

Figure 3.8: Chromatogram of Cefixime Trihydrate formula



PeakTable

Detector A Ch1 254nm

Peak#	Name	Ret. Time	Area	Height	Area %
1	Cefixime	10.190	2307184	131153	100.000
Total			2307184	131153	100.000

Figure 3.9 Chromatogram of marketed product.

3.22 FT - IR Studies

When IR of Cefixime trihydrate (Figure 3.10) was correlated with cefixime trihydrate and excipients (Figure 3.11) the region of 3400 – 3500 cm^{-1} was found due to the N-H (aromatic) stretching. However other peaks related to C-H, C-O and carbonyl stretching remain unchanged. This indicates that overall symmetry of the molecule might not be significantly changed; therefore the FTIR study revealed that there is no interactions' taking place between Cefixime trihydrate and excepient in formulated product.

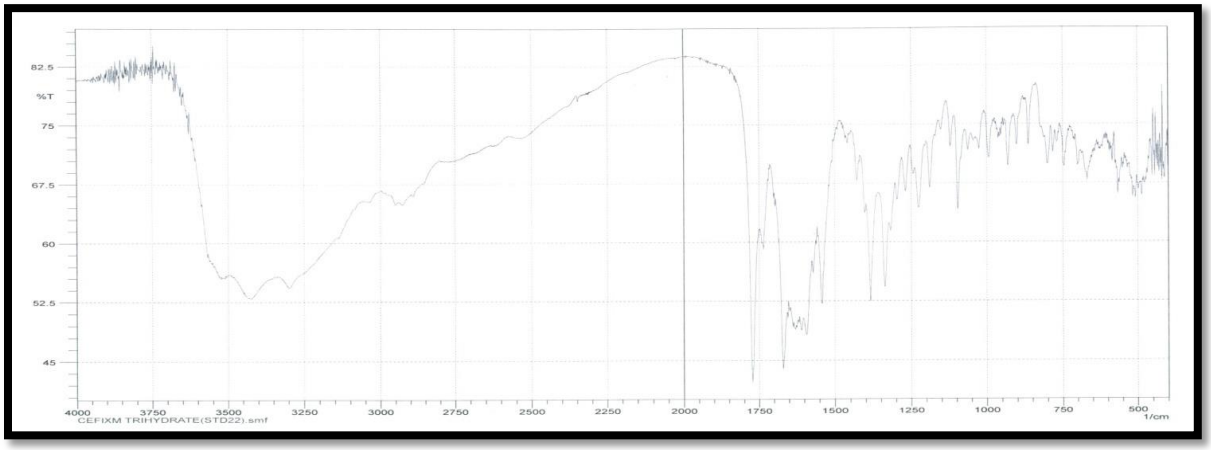


Figure 3.10: FTIR spectra of the pure Cefixime trihydrate.

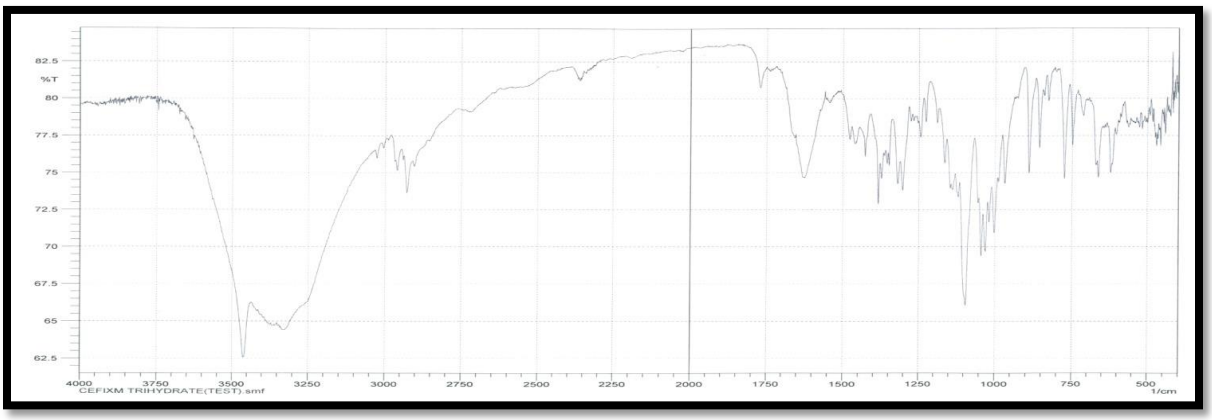
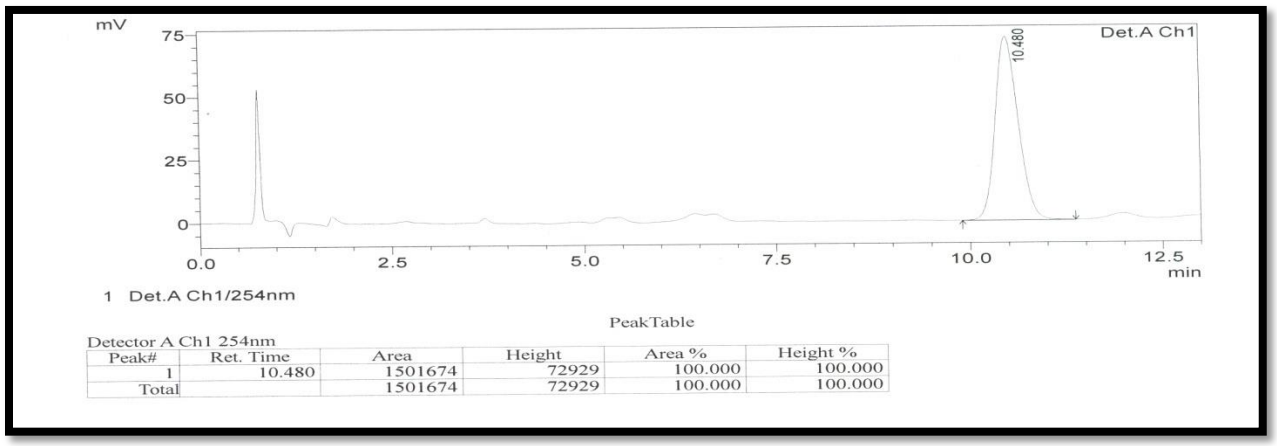


Figure 3.11: FTIR spectra of the Cefixime trihydrate in marketed formula.

3.23 Degradation of Cefixime trihydrateOral Suspension under stress conditions

Degradation behavior of cefixime trihydrate oral suspension (CEF-$3H_2O$) which was investigated under stress conditions, temperature 75^o C and humidity 75 RH, and some pharmaceutical excipients using HPLC. Stability - indicating methods HPLC were applied as per USP 2014 that could separate the drug from its degradation products formed under these stress conditions. As shown in (Figure 3.12) in comparison with standard (Figure 3.13) degradation was occurred under stress conditions, temperature 75^o C and humidity 75 RH.

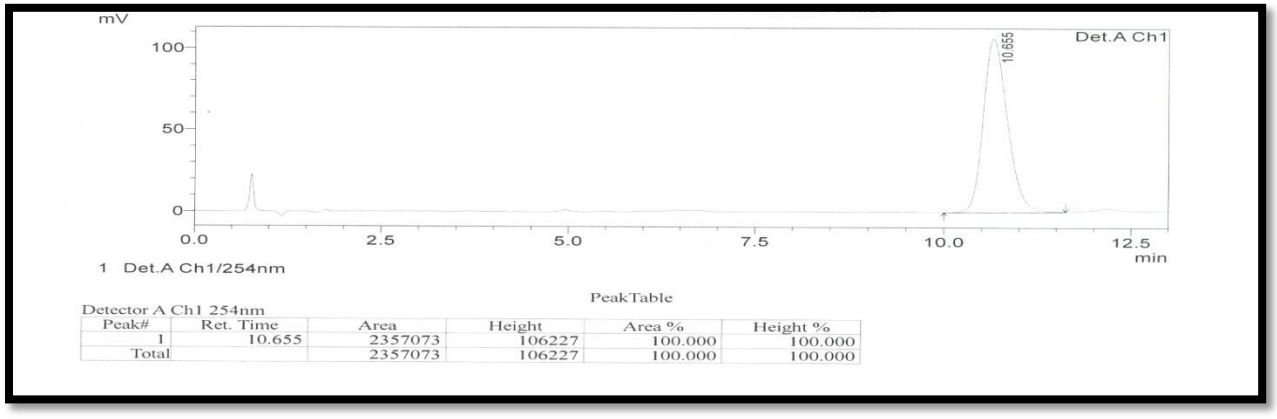


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	10.480	1501674	72929	100.000	100.000
Total		1501674	72929	100.000	100.000

Figure 3.12: Degradation of cefixime trihydrate oral suspension.



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	10.655	2357073	106227	100.000	100.000
Total		2357073	106227	100.000	100.000

Figure 3.13: Standard of cefixime trihydrate.

The assay of the stability studies as per ICH guidance (Figure 3.14) was in initial time were 100.05%, the first month were 87.22%, second month 80.01%, third month 63.25% and sixth month, 55.15%.

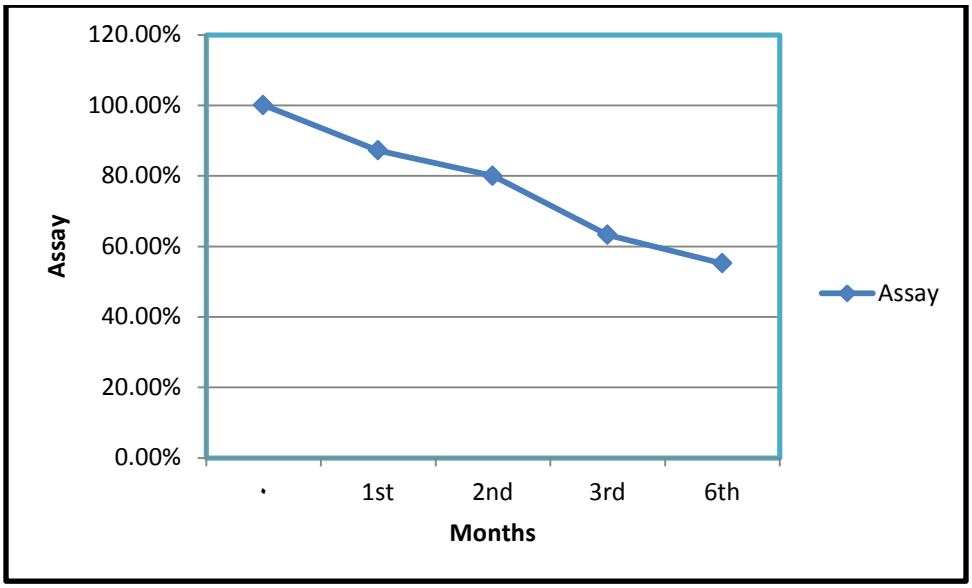


Figure 3.14: The assay of stability studies for 6 months

3.24 Cytotoxicity Screening

The cytotoxicity of *Anogeissus leiocarpus* gum was investigated after cell growth, the concentration at which the growth of cells was inhibited to 50% of the control (IC_{50}) was 370.5 µg/mL ±15.68. It presented a accentuated inhibition of cell growth at 125 µg/mL, 250 µg/mL and total inhibition at 500 µg/mL was 27.5 ± 1.5, 49.0 ± 1.42 and 49.2 ±1.4 respectively. The limit of the toxic is IC_{50}< 30µg/mL, and the non toxicIC_{50} > 100µg/mL. Table 3.7 shows the effect on cell growth, practically non toxic and the value of IC_{50} result indicates that the *Anogeissus leiocarpus* gum non toxic, and cytotoxicity assays did not interfere in the results because all reagents and chemicals used non toxic. The *in vitro* cyto-toxicity assays are used as screening tests for evaluation *Anogeissus leiocarpus* gum biocompatibility.

Table 3.7: The results of MMT assay of *Anogeissus leiocarpus* gum.

Name of sample	Concentration (µg/ml) Inhabitation (µg/ml) ± SD			IC_{50} (µg/ml)	The degree of toxicity
	500	25	125		
Anogeissus leiocarpus gum	49.2±1.4	49.0±1.42	27.5±1.5	> 100	Non toxic
Control	95.3			< 30	High toxic

Key: IC_{50} < 30µg/ml) : high toxic.

Control: Triton X 100 was used as the control positive at 0.2 µg/ml.

The maximum concentration used was 500 µg/ml, when this concentration produced less than 50% inhabitation, the IC_{50} cannot be calculated, IC_{50} < 30µg/ml) : high toxic.

3.25 Conclusion

The aim of this research was to evaluate and characterize of the *Anogeissus leiocarpus* gum with particular focus on physicochemical properties, also the potential application of this gum as pharmaceutical excipient. The objectives included:

1. Physicochemical characterization of the processed gum. Properties such as moisture content, ash, nitrogen, protein, specific rotation, relative viscosity, refractive index, density, also UV electromagnetic radiation, metals ions, *p*H, uronic acid, reducing sugars and infrared spectra.
2. The present study indicates that *Anogeissus leiocarpus* gum may be a poly-saccharide gum. A techniques of HPLC showed the gum is composed of galactose, rhamnose, and arabinose as natural sugars with uronic acid as sugar acid.
3. Comparative evaluation of the suspending ability of *Anogeissus leiocarpus* gum will be compared with standard suspending agent as market product. The evaluation parameters include viscosity and rheology, sedimentation volume and density.
4. The cellular toxicity effects of the *Anogeissus leiocarpus* gum were evaluated by MTT-formazan viability assay, and the results within the defined cytotoxicity range, therefore the use of these gum in food and medicine is possible.

3.26 Recommendations:

1. Data obtained from this study can be used for propose specification of this gum.
2. More toxicological study should be done on the gum to conform its safety for used in food and pharmaceutical industries.
3. Further details of structural study should be carried out on the gum to obtain the final structure of the gum.

References

A. Khusnoor, Physical Pharmacy, (2003). Tara Publishers, New Delhi,1st Edition, pp 61-69.

Afaf H. Rahim; (2010).Competition in the gum arabic market:a game theoretic modelling approach, Philipps-Universität Marburg, Germany, Quarterly Journal of *International Agriculture* **49**.

Alok K. Kulshreshtha, Onkar N. Singh, G. Michael Wall; (2010). *Pharmaceutical Suspensions*, Springer, Pp 103 - 127.

Amalvy, J. I. (1997). Dilute solution viscometry of carboxylated acrylic lactices. *Pigment and Resin Technology* **26**, 363-369.

Amit Bhattacharya, James W. Rawlins, Paramita (2009). *Polymer grafting and crosslinking*. Hoboken, N.J.: John Wiley.Pp 145-177.

Anderson, D. M. W.; Brown Douglas, D. M.; Morrison, N. A. & Weiping, W. (1990). Specifications for gum arabic (*Acacia Senegal*); analytical data for samplescollected between 1904 and 1989. *Food Additives and Contaminants*. Journal of Taylor and Francis Online**7**.

Anderson, D. M. W., and M. M. E. Bridgeman, (1985). The composition of the proteinaceous polysaccharides exuded by Astragalus microcephalus, A. gummifer and A. Kurdicus- the source of Turkish gum tragacanth. Phytochemistry **24**:2301-4

Anderson, D.M.W, (1986). *Food Addit. Contam.*, **3**: 225.

Arbonnier, Michel, (2004). *Trees, Shrubs and Lianas of West African Dry Zones*. Publishers GMBH. Pp 27:133.

Arguin, (1911). Encyclopædia Britannica **2**, 23:54

Aslam, M., Pass, G. and Phillips, G.O., (2006). Properties of *Khaya granification* gum. *Journal of the Science of Food and Agriculture* **29**, 563-568.

Aspinall, G.O. (1972). *The polysaccharides*. New York: Academic Press.**1**:86-97.

Aswathanarayana SC. et al.,(1997). Seed viability and microflora of forest tree species. *Indian Journal of Forestry*, **19** (4): 326-329.

Bayram, M., (2005). *Determination of the sphericity of granular food materials*. J. Food Eng. **68**:385-90.

Bencher, p., (1985) Ed. *Encyclopedia of emulsion technology*, Vol. 2 Marcel Dekker: New York, Pp 1-28.

Benoit, H., Grusbisic, Z., Rempp, P., Decker, D., and Zillox, J.G. (1966). Liquid phase chromatographic study of branched and linear polystyrenes of known structure. *Journal of Chemical Physics* **63**, 1507 – 1514.

Berger, L.D. (1962). Water soluble polymers. In Davidson, R. L. and Sittig. M. (eds.), Water-soluble resins, New York: Reinhold Publishing Corporation, pp 1-20.

Bhardwaj, T.R., Kanwar, M., Lal, R. and Gupta, A. (2000). Natural gums and modified natural gums as sustained- release carriers. *Drug Development and Industrial Pharmacy* **26**, 1025-1038.

Billany, M.R., (2007) Suspension and emulsions. In Aulton, M.F. (ed) *Pharmaceutical: the design and manufacture of medicines* (Third Ed.) Philadelphia: Churchhill Livingstone, Pp. 383-405.

Bizimana,Nsekuye., (1994). *Traditional Veterinary Practice in Africa*. German Technical Cooperation.

Blunt, H. S., (1926). *Gum Arabic, with special reference to its production in the Sudan*. London: Oxford University Press. pp 45.

Bourne, M. C., (1982). *Food and viscosity*. New York: Academic Press.**10**, pp. 483-487

Bradstreet, RB.,(1965). *The Kjeldahl Method for Organic Nitrogen*. Academic Press,.Catfish. New York Science Journal. 2010; 3(**6**)
British Pharmacopoeia, (2013) Volume I & II.

Brummer, Y. and Cui, S.W. (2005). Understanding carbohydrate analysis. In Cui, S. W. (ed.), Food Carbohydrates: *Chemistry, Physical Properties*, and Applications. Boca Raton, Florida: Taylor and Francis, pp. 68-99.

Buffo, R.A., G.A. Reineccius and G.W. Oehlert, (2001)Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions. *Food Hydrocolloids*, **15**: 53-66.

"Cemotherapy Principles" (PDF). American Cancer Society. Retrieved 20 August 2014.

Chemical Kinetics, (2012).From Wikipedia, the free encyclopedia.

Chen, C.R., Lin, Y.H., Cho, S.L Yen, S. Y. and Wu, (1997). Investigation of the dissolution difference between acidic and neutral media of the acetaminophen tablets containing a super disintegrant and a soluble excipient, *Chemical and Pharmaceutical Bulletin* **45**, 509-512.

Chen, L. and Wang, R. and Li, C. and Aihara, K. (2010). Modeling biomolecular networks in cells: *structures and dynamics*. Springer.

Collins, E. A., Bares J. and Billmeyer, F.W.(1973). Molecular Weight and its Distribution. *In Experiments in polymer Science*, New York: John Wiley and Sons, Pp.147-153.

Cui, S. W. (2005). "*Structural analysis of polysaccharides*," In: Food Carbohdrates: Chemistry, Physical Properties, and Applications, Cui, S. W. (ed.), Taylor and Francis Group: Florida, Pp 105-157.

Cunha. P. L. R. Viera, I. G. P., Arriaga, A.M.C., de paula, R.C.M. and Feitosa, J.P.A. (2009). *Isolation and characterization of galactomannan from Dimorphandra gardneriana Tul*. Seeds as a potential guar gum substitute. Food hydrocolloids **23**, 880-885.

D. F. Durso.(1980) *Handbook of Water Soluble Gums and Resins*. New York, NY: McGraw Hill, Kingsport Press; pp 12.

De Pinto, G. L., Demoncada, N. P., Martinez, M., Degotera, O. G., Rivas, C., and E. Ocando. (1994). Composition of Pereskia-Guamacho gum exudates. Biochem. Systematics Ecol. **22**:291-5.

De Pinto, G., Martinez, M., De Gutierrez, G. O., Vera, A., Rivas, C., and E. Ocando., (1995). Comparison of two Pithecellobium gum exudates. Biochem. Systematics Ecol. **23**:849-53.

De Pinto, G., Rodriguez, O., Martinez, M., and C. Rivas., (1993). Composition of Cercidium-Pracos gum exudates. Biochem. Systematics Ecol. **21**:297-300.

Decker T, Lohmann-Matthes ML; Lohmann-Matthes (November 1988). "A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity". *J. Immunol. Methods* **115** (1).

DH Everett,(1988). Basic Principles of Colloid Science, RSC Publications, London, pp 30-53.

DH Napper.,(1983). Polymeric Stabilization of Colloidal Dispersions, Academic Press, New York, British Polymer Journal **18** (4):278.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food hydrocolloids **17**, 25-39.

Dickinson, E., (1992). An introduction to food colloids. Oxford University Press, Oxford.pp 10-12.

Donald E. Aylor. (Nov. 2002) *Journal of Aerosol Science*,. **33**, pp. 1601-1607.

Donot F, Fontana A, Baccou J C, Schorr-Galindo S., (2012) Microbial exopoly-saccharides: main examples of synthesis, excretion, genetics and extraction. Carbohyd Polym **87** :951–962

Dziezak, J. D., (1991). *Afocus on gums. Food Technol*. **45**:116-32.

Edward Barbier., (1992). Rehabilitating Gum Arabic systems in Sudan: economic and environmental implications. Environmental and resources economics, **4**, pp 341-358.

Elbert H. Hadley., (2010). Formerly, Department of Chemistry and Biochemistry, Southern Illinois University, McGraw-Hill Global Education Holdings.

Encyclopedia of Chemical Technology, (1966). *Executive Encyclopedia of Chemical Technology*, Executive Editor Anthony Stander, Inter Science Publishers John Wiely and Sons Inc. London.

Faith A. Morrison, (2001). Understanding Rheology, Oxford University Press, Pp 127-131.

FAO. (1995).*Gums, resins and latexes of plant origin*. Non wood forest products series, Rome. pp.152.

FAO. (1997) Corporate Document Repository Prepared at the 49th JECFA superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Addendum 3 (1995).

Fan F, Wood KV; Wood (February 2007). "Bioluminescent assays for high-throughput screening". *Assay Drug Dev Technol* **5** (1): 127–36.

Feng. T., Gu, Z.B. and Jin, Z.Y. (2004). Evaluation of the suspending properties of *Mesona blumes gum. Food Science and Technology International* **33**, 55-61.

Flindt, C., Al-Assaf, S., Philips, G. O. and Williams , P. A. (2005). Studies on acacia exudates gums. Part V: structural features of *Acacia seyal*. Food hydro-colloids **19**, 687-701.

Francisco Jimenez- Colmenero, 2013. Potential application of multiple emulsions in the development of healthy and fuctional foods. *Food Research International*, Volume 52, Issue 1, June 2013, Page 64-74.

G. O. Aspinall and T. B. Christensen (1961). *Anogeissus schimperi* gum. J. *Chem. Soc*. 3461.

G. O. Aspinall and V. P. Bhavanandan. I. (1965). Gum *ghatti* (Indian gum). Part V. Degradation of the periodate-oxidised gum. J. *Chem. Soc*. 2685, 2693.

G. 0. Aspinall and J. Baillie. (1963). Gum tragacanth. Part I. fractionation of the gum and structure of tragacanthic acid. *J. Chem*. Sec. 1702-14.

G. 0. Aspinall, M. J. Johnston, and A. M. Stephen. (1960). Plant Gums of the Genus Khaya. Part II. The major Major Component of *khaya* senegalensis Gum. J. *Chem. Soc*. 4918.

G. 0. Aspinall, M. J. Johnston, and R. Young. (1965). Plant Gums of the Genus Khaya. Part II. The major Major Component of *khaya* senegalensis Gum. *J. Chem.* Soc. 2701.

G. Jiao, G. Yu, J. Zhang, and H. S. Ewart,(2011) "Chemical structures and bio-activities of sulfated polysaccharides from marine algae," Marine Drugs, **9**, pp. 196–233.

G.O. Phillips and P.A. Williams,(2009). *Handbook of hydrocolloids*, Woodhead publishing limited Oxford Cambridge New Delhi, Pp 8-28.

Gaonkar, A.G. (1991). Surface and interfacial activities and emulsion characteristics of some hydrocolloids. *Food hydrocolloids* **5**, Pp 329-337.

Garleb, K. A, Bourquin, L. D, and Fahey, G. C. Jr.(1989). Neutral monosaccharide composition of various substances: A comparison of hydrolytic processes and use of anion-exchange high performance liquid chromatography with pulsed amperometric detection of monosaccharides. *Journal of Agricultural and food Chemistry* **37**, pp 1287-1293

GD Parfitt (Ed), (1981). Dispersion of Powders in Liquids, Third Edition, Applied Science, London Pp 332- 334.

Gerstenzang, James and Sanders, Edmund (30 May 2007). "Impact of Bush's Sudan sanctions doubted". Los Angeles Times. Retrieved 1 June 2007.

GH Fleer et al, (1993), *Polymers at Interfaces*, Chapman & Hall, New York, pp 133-162.

Glicksman, M. (1969). *Gum technology in the food industry*. New York: Academic Press.

Glicksman, M. and Schachat, R.E. (1959). Some lesser known seawed extracts. In : whistler, R.L. (ed) *Industrial Gums*, New York: Academic press, pp 213-298.

Glicksman, M., and E.H Farkas. (1975) Method of preventing gelation in canned gravy-based per foods. US patent Application **3**.881.031.

Hahm, H. A. (2000). *Effect of Sorbed water on the efficiency of super disintegrants: Physical and mechanistic considerations*. Baltimore, MD: University of Maryland press, pp.6.

Hamza, M.E/Amin, (1990): *Trees and Shrubs of the Sudan*, IthacPress, Exeter,UK.
Hans, J.P., (1990). Trees and Shrubs of the Sahel: *Their characteristic and uses*, Print by Typo-druck.

Harvey, D. J. (2001). Review: Identification of Protein-bound carbohydrates by mass spectrometry. *Proteomics* **1**, 311-328.

Heinrich, R. and Schuster, S. (1996). The regulation of cellular systems. Chapman & Hall, New York.

Howes, E. N. (1949). *Vegetable gums and resins*. Waltham, MA: Chronica Botanica Company.

Huggins, M. L. (1942). The viscosity of dilute solutions of long chain molecules, IV: Dependence on the concentration. Journal of American Chemical Society **64**, 2716-2718.

Idris, O. H. M., Williams, P. A., and G. O. Phillips.(1998), Characterization of gum from Acacia Senegal trees of different age and location using multi detection gel permeation chromatography. Food Hydrocolloids **12**:379-88.

Iglesias, P.A. and Ingalls, B.P. (2010) Control theory and systems biology. MIT Press Cambridge.

Imeson, A. (1992). Exudae gums. In thickening and gelling agents for food, ed. Imeson, London: Chapman and Hall. Pp 66-97.

International Journal of *Pharmaceutical Sciences Review and Research*. Volume 11, Issue 1, November – December 2011; Article-012

Islam, A, M ., Phillips, G. O., Sljivo, M. J.,and P.A. Williams. (1997). A review of recent developments on the regulatory, structural and functional aspects of gum Arabic. Food Hydrocolloids **11**:493-505.

IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (1997). Online corrected version: (2006) "homopolysaccharide (homoglycan)".

IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (1997). Online corrected version: (2006) "heteropolysaccharide (heteroglycan)".

IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997).

Izydorezyk, M (2005) Understanding the chemistry of food carbohydrates. In Cui, S.W. (ed.). Food Carbohydrates; Chemical Physical Properties, and Applications. Boca Raton, Florida: Taylor and Francis, pp. 1- 64

J.J.W. Coppen,(1995). non-wood forest products; *Gums, resins and latexesof plant origin*; FAO, Rome,ISBN 92-5-103757 - 4. p 142 .

John Corcoran, Karen Manheimer, (2010): *North African and Mediterranean Essential Oils and Aromas: Tales and Realities of our Industry – a new decade of challenges and opportunities*'in the printed Conference Proceedings.p 217-226

Journal of *Ethnopharmacology*, (1990) **30**:233-264

Journal of Pharmaceutics (2014), Pritam Dinesh Choudhary and Harshal Ashok Pawar, *Recently Investigated Natural Gums and Mucilages as Pharmaceutical Excipients*: An Overview Dr. L.H. Hiranandani College of Pharmacy, Smt. CHM Campus, Opp. Ulhasnagar Railway Station, Ulhasnagar, Maharashtra 421003, India, Volume 2014, Article ID 204849, p 9.

Marschner, Horst (1995). Mineral Nutrition of Higher Plants. 3rd edition, Publisher, Academic press. Pp 125-366. .

Karamalla, K.A., N.E. Siddig and M. E. Osman (1998). Analytical data for *Acacia senegal* gum samples collected between 1993 and 1995 from Sudan. *Food Hydrocolloids*, **9**: 1- 6.

Kenneth A. Connors *Chemical Kinetics*,(1991). *The study of reaction rates in solution*, VCH Publishers, pp 200-203.

Kenyon WJ, Buller CS, (2002). Structural analysis of the curdlan-like exopoly-saccharide produced by Cellulomonas flavigena KU. J. Ind. Microbiol Biotechnol **29**:200–203

Kharbade, B. V and Joshi, G. P.(1995). Thin layer chromatographic and hydro-lysis methods for the identification of plant gums in art objects. Studies in Conser-vation **40**, 93-102.

Kinjal N. Desai, Rina H. Gokani,(2012). *International Journal of Advances in Pharmaceutical Analysis;* Stability Study: Regulatory Requirenment, IJAPA Vol. **2**(3) pp 62-67.

Krister Holmberg, (2002), *Handbook Of Applied Surface and Colloid Chemistry*; John Wiley & Sons, Ltd.

Kuntz LA. 1999. Special Effects with Gum. A report on application. Food Product Design. N orthbrook : Virgo Publishing, pp10.

L. Pereira, (2011)"A Review of the nutrient composition of selected edible seaweeds," in Seaweed: Ecology, Nutrient Composition and Medicinal Uses, V. H. Pomin, Ed, Nova Science, New York, NY, USA, pp. 15–47.

Laura Halpin Rinsky; Glenn Rinsky (2009). *The Pastry Chef's Companion: A Comprehensive Resource Guide for the Baking and Pastry Professional*. Chichester: John Wiley & Sons. pp. 1-134.

Le Cerf, D., Irine, F., and G. Muller,(1990). Solution properties of gum exudates from Sterculia urens (*Karaya* gum). Carbohydr. Polym. **13**:375-86.

Leon Shargel, Susanna WU- Pong, Andrew B.C. Yu,(2004).*Applied Biopharma-ceutics & Pharmacokinetics*, 5th Edition; Leon Shargel, Susanna Wu-Pong, An-drew B.C. Yu.

Lindberge, B. L., Lonngren, J., and Svensson, S(1975). Specific degradation of polysaccharides. *Advances in Carbohydrate Chemistry and Biochemistry* **31**, 185-240.

López Martínez, M. C., Díaz Baños, F. G., Ortega Retuerta, A., García de la Torre, J., (2003) Multiple Linear Least-Squares Fits with a Common Intercept: Determination of the Intrinsic Viscosity of Macromolecules in Solution. Journal of Chemical Education, **80** (9), 1036-1038.

Lowe, B. 1955. Expermental cookery from the chemical and physical standpoint. New York: Wiley. **4**,16-18.

Maduako, J. N. , and M. O. Faborode. 1990. *Some physical properties of cocoa pods in relation to primary processing*, IFE J. Technol **2**, Pp 1-7.

Mann, A.; Y. Yahaya, A. Banso and G. O. Ajayi. (March 2008) "*Phytochemical and antibacterial screening of Anogeissus leiocarpus against some micro-organisms associated with infectious wounds*". Academic Journals.

Marschner, Horst (1995). Mineral Nutrition of Higher Plants, new York:Academic press, second addition, **149**, 441-456.

Mathew, S.; Abraham, T. E (2008). Characterization of ferulic acid incorporated starch-chitosan blend films. *Food Hydrocolloids* **22**, 826-835.

Matthews, C. E.; K. E. Van Holde; K. G. Ahern (1999). *Biochemistry*. 3rd edition. Benjamin Cummings.

McCabe, W. L., Smith, J. C., and P. Harriot. (1993). Unit operations of chemical engineering, 5th ed. Singapore: McGraw-Hill.

McCleary, B. V. (1994). Enzymatic analysis of the fine structure of galactomannans. Methods in *Carbohydrate Chemistry* **10**, 175-182.

Mithal, B.M. and Khasgiwal, P.C. (1972). Fraction of polysaccharides. *International Journal of Pharmaceutics* **34**, 51-57.

Mohsenin, N, N. (1970). *Physical properties of plant and animals*, Volume I. London: Gordon and Breach Science publisher.

Morthlagh, S., Ravines, P., Karamallah, K.A. and Ma, Q. (2006). The analysis of acacia gums using electrophoresis. *Food hydrocolloids* **20**, 848-854.

N.A.Campbell (1996), Biology (4th edition). Benjamin Cummings, NY. p.23

Navarro, Alain (10 July 2008) Sudan's manna from heaven and strategic weapon, AFP. Archived 28 May, 2013 at the Wayback Machine.

Nazakawa, F. (1985). Structural studies of the antigenic polysaccharide of Eubacterium saburreum, strain T17. *Carbohydrate Research* **143**, 185-190.

Niazi, Sarfaraz., (2009). *Handbook of pharmaceutical manufacturing formulations*, Library of Congress Cataloging.

Nichols, E. L.; Franklin, W. S., (1898). *The Elements of Physics*. Vol. 1. Macmillan, **1**, p. 101.

Niles AL, Moravec RA, Eric Hesselberth P, Scurria MA, Daily WJ, Riss TL; Moravec; Eric Hesselberth; Scurria; Daily; Riss (July 2007). "A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers". *Anal. Biochem.* **366** (2): 197–206.

Nishi, K. K.; Antony, M.; Mohanan, P. V.; Anilkumar, T. V.; Loiseau, P. M.; Jayakrishnan, A., (2007) (Pharmaceutical Research). **24** (5), 971-980.

Nussinovitch, A., (1997*). Hydro colloid applications: gum technology in the food and other industries*, London: Blackie Academic & Professional.pp125-139.

Omaira, A., Gladys, L, D., Maritza, M., Omaira, G., and S. Lilian., (2007). Structural features of a xylogalactan isolated from *Hymenaea courbarial* gum. Food hydrocolloids **21** (8), p. 1302-1309,

Ostwald, W.,(1922). Theoretical and applied colloid chemistry (translated by M. H. Fisher). New York: Wiley.

P. Becher (Ed),(1985, 1987 and 1988). Encyclopedia of Emulsion Technology, Vol 1 Basic Theory, Vol 2 Practical Applications, Vol 3, Basic Theory Measurement, Applications, Marcel Dekker, New York.

Palaniraj A, Jayaraman V (2011) Production, recovery and applications of xanthan gum by Xanthomonas campestris. J Food Eng **106**:1–12.

Parija, S., Misra, M. and Monhanty, A.K. (2001). Studies of natural gum adhesive extracts – an overview. *Polymer Reviews* **4,** 175-197.

Patel S, Gheewala N, Suthar A, Shah A**.,** (2009). In-Vitro Cytotoxicity Activity of Solanum Nigrum Extract Against *Hela* Cell Line And*Vero* Cell Line. *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. **1**, Suppl 1, Nov.- Dec, 38-46.

Pazur, J. H.(1986). Neutral polysaccharides. In : Chaplin, M. Z. and Kennedy, J. F. (Eds), Carbohydrate analysis, a practical approach. IRL Press, Oxford, Pp. 55-96.

PC Hiemenz, (1986). Principles of Colloid and Surface Chemistry, Marcel Dekker, New York, pp 681 – 703.

Policy Note, Export Marketing of acacia gum from Sudan. (March 2007)World Bank, p. 4.

Power, Michael L. (2010). Anne M. Burrows, Leanne T. Nash, ed. The Evolution of Exudativory in Primates / Nutritional and Digestive Challenges to Being a Gum feeding Primate. Springer. p. 28. Retrieved 2 October 2012.

Prentice, J. H., (1983). Precision and accuracy on viscometric measurement on foods. Journal of Rheology, **27**, 621–635.

Price, M.L., V.S. Scoyoc and L.G. Butler., (1978). A criticalevaluation of the vanillin reaction as an assay fortannin in sorghum grain. J. Agric. Food Chem., **26**:1214-1218.

Printing Process Explained". *dynodan.com*. Retrieved 29 August 2012.

Promega Corporation (2006) Protocols and Applications Guide. Cell Viability.

Promega Corporation (2007) Protocols and Applications Guide. Apoptosis.

R. B. Bird, W. E. Stewart, E. N., (1960). Light foot Transport Phenomena, John Wiley & Sons, pp 455.

R. Byrin Bird, CharlesF. Curtiss, Robert C. Armstrong., (1989). Dynamics of Polymeric Liquids, Vol 1 &2 , Wiley Interscience.

Randall, R.C., Phillips, G.O. and Williams, P.A. (1989). Fractionation and characterization of gum from acacia senegal. Food Hydrocolloids **3**, 65–75.

Rawlins E.A., (1980). Bentley's textbook of pharmaceutics (8th edition). ELBS and Tindall Bailliere, London.

Renard, D; Lavenant-Gourgeon, L; Ralet, MC; Sanchez, C(2006). "Acacia senegal gum: Continuum of molecular species differing by their protein to sugar ratio, molecular weight, and charges" *Biomacromolecules.*

Riss TL, Moravec RA; Moravec (February 2004). "Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays". *Assay Drug Dev Technol* **2** (1): 51–62.

RJ Hunter,(1981). Zeta Potential in Colloid Science, Academic Press, New York, p 109.

Ronald E, (2005) *Hand book of food analytical chemistry*. Published by John Wiley & Sons, Inc., Hoboken, New Jersey.

Robert M. Silverstein, Francis X. Webster' David J. Kiemle. (1981). SPECTROMETRIC IDENTIFICATION OF ORGANIC COMPOUNDS, John wiley and Sons Inc., New Jersey.pp 78 – 84.

S. Peat, W. J. Whelan, and H. G. Lawley, (1958). "The structure of laminarin. Part I. The main polymeric linkage," *Journal of the Chemical Society*, Pp. 724–728.

Sahin, S., and S. G. Sumnu. (2006). Physical properties of foods. New York: Springer, Pp 166-173.

Sarwat F, Ul Qader SA, Aman A, Ahmed N., (2008). Production and characterization of a unique dextran from an indigenous Leuconostoc mesenteroides CMG713. *Int. J. Biol Sci*. **4:**379–386

Shangraw, R.F., Mitrevej A. and Shah M., (1980). A new era of tablet disintegrants. *Pharmaceutical Technology* **4**, 49-57.

Shuaibu, M. N.; Pandey, K.; Wuyep, P. A.; Yanagi, T.; Hirayama, K.; Ichinose, A.; Tanaka, T.; Kouno, I., (2008) "Castalagin from *Anogeissus leiocarpus* mediates the killing of Leishmania in vitro". *Parasitology Research*, **103** (6): 1333–1338 .

Shuaibu, M. N.; Wuyep, P. A.; Yanagi, T.; Hirayama, K.; Tanaka, T.; Kouno, I. (2008)."The use of microfluorometric method for activity-guided isolation of antiplasmodial compound from plant extracts". *Parasitology Research*,**102** (6): 1119–1127 .

Siddig, N. E., (1996). Nitrogen and specific rotation as qualifying indices for gum Arabic derived from Acacia Senegal (L) wild. M.Sc.Thesis, faculty of Agriculture. University of Khartoum.

Smith, F. and Montgomery, R., (1959). Occurrence and isolation of some of the more important gums and mucilages. In : The chemistry of plant gums and mucilages. New York: Reinhold Publishing Corporation, Pp. 14-39.

Smolinske, Susan C. (1992). *Handbook of Food, Drug, and Cosmetic Excipients*. Publishing CRC Press, Taylor and Francis Group, p. 7.

(Steentoft, Margaret. (1988). Flowering plants in west Africa. Cambride University Press. Pp 24-123.

Stone, H., and S. Oliver., (1966). Effect of viscosity on the detection of relative sweetness intensity of sucrose solutions. *J. Food Sci*. 31:129-34.

Szallasi, Z. and Stelling, J. and Periwal, V., (2006). System modeling in cell biology: from concepts to nuts and bolts. MIT Press Cambridge, Pp 97 - 124.

Szczesniak, A. S., and E. H. Farkas, (1962). Objective characterization of the mouthfeel of gum solutions. *J. Food Sci* ., 27:381-5.

Turcotte, D. 2006. A Garden for visually impaired visitors. The nature of success: Success for nature, 1-3. Montreal Botanical Garden, Montreal, Canada, www. Bgci.org/educationcongress/proceedings/

Upper Saddle River, (2004). Preparing for the Chemistry AP Exam. New Jersey: Pearson Education, pp 131-134.

Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, Etzler M., (2008). "Essentials of glycobiology". Essentials of Glycobiology (Cold Spring Harbor Laboratory Press; 2nd edition).

Verbeken, D., Dierckx, S., and K. Dewettinck., (2003). Exudate gums: occurrence, production, and applications. Applied Microbiology and Biotechnology **63**:10-21.

Vinod, V.T.P., Sashidhar, R.B., Suresh, K.I., Rao, B. R., Saradhi, U.V.R. and Rao, T.P. (2008). Morphological, Physico-chemical and structural characterization of gum kondagogu (Cochlospermim gossypium): Atree gum from India. *Food hydro-colloids* **22**,899-915.

Vivas N, Vivas de Gaulejac N, Nonier M.F and Nedjma M (2001). "Effect of gum arabic on wine astringency and colloidal stability". *Progres Agricole et Viticole* (in French) **118** (8): 175–176.

W. R. Schowalter (1978), Mechanics of Non-Newtonian Fluids Pergamon published Cambridge University Press, Pp 660-672.

Wadell, H., (1935). *Volume, shape and roundness of quartz particles*. J. Geology. **43**:250-80.

Walker, T. S.; Bais, H. P.; Grotewold, E.; Vivanco, J. M. (2003). Root exudation and rhizosphere biology. Plant Physiol. **132**, 44-51.

Wallis, O.L. (1967). *Chemistry of plant gums and mucilages*. Reinhold Publishing Corporation, New York: pp. 501

Walsh R, Martin E, Darvesh S., (2010) A method to describe enzyme-catalyzed reactions by combining steady state and time course enzyme kinetic parameters... Biochim Biophys Acta, 117: 307 - 310.

Wang, Q. and Cui, S.W. (2005). Understanding the physical properties of food polysaccharides. In : Cui, S.W. (ed.), Food Carbohydrates: Chemistry, physical properties, and Applications. Boca Raton, Florida: Taylor and Francis, Pp 162-214.

Webb, James L. A. "The Trade in Gum Arabic: Prelude to French Conquest in *Senegal*"(2009). *The Journal of African History* **26** (2–3): 149.

Whistler, R. L.,(1973). Industrial gums, 2nd edition. New York: Academic Press, pp 567.

Whistler, R.L.and Smart, C.L. (1953). Polysaccharide Chemistry, New York: Academic Press. pp. 1-25.

WHO Library Cataloguing-in-Publication Data; Quality control methods for herbal materials. Updated edition of *Quality control methods for medicinal plant materials*, (1998).

Wiesner, J. V.,(1927). Die Rohstoffe des Pflanzenreichs (Leipzing, Engelmann). In Vegetable gums and resins, ed. F. N. Howes, 6. Waltham, MA: Chronica Botanica Company.

Williams, P. A., Phillips, G. O., and R. C. Randall. (1990a.) Structure function relationships of gum Arabic. In gums and stabilizers for the food industry 5,ed. G. O. Phillips, D. J. Wedlock, and P. A. Williams,Oxford: IRL press at Oxford University Press. pp 25-36.

Williams, P. A.; Phillips, G. O. & Stephen, A. M.(December 1990).Spectroscopic and molecularcomparisons ofthree fractions from Acacia senegal gum. *Food Hydrocolloids*, **4** (4): 305-311.

Xiaodong M. and Marek P, (2007). Intrinsic viscosities and Huggins constants of guar gum in alkali metal chloride solutions. *Carbohydrate Polymers* **70**: 15-24.

Yalpani, M. (1988) *Polysaccharide: synthesis, modification andstructure/property relationships*. Amsterdam; Elsevier, pp. 42

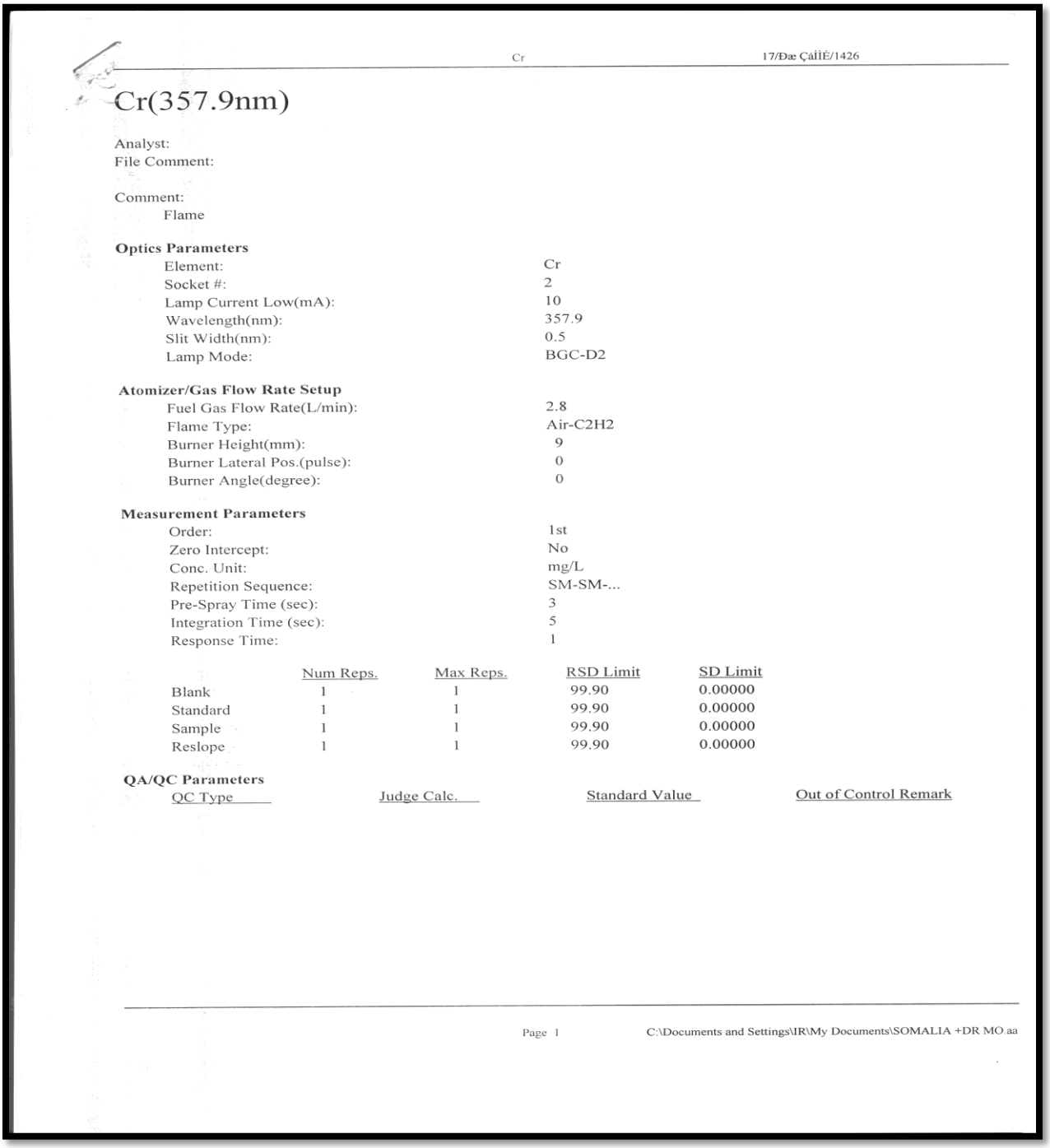
Yang. B., Wang, J.S., Zhao, M.M., Liu, Wang, W. and Jiang, Y. M., (2006). Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis Sonn.*) fruit in relation to their antioxidant activities, *Carbohydrate research***341**: 634-638.

Young, A.,(1989). Agroforesty for soil conservation, Oxon, UK: CAB and ICRAF, **58**: 501-508.

Zahirul Khan, M. (1995). Recent rends and progress in sustained or controlled oral delivery of some water-soluble drugs: morphine salts, diltiazerm and captopril. *Drug Development and Industrial Pharmacy*, **16**(8): 1249-1277.

Zhao, N., and Augsburger, L. L. (2005). The influence of swelling capacity of super disintegrants in different pH media on the dissolution of hydrochlorothiazide from directly compressed tablets. *AAPS pharm.Sci.Tech.*, **6**(01), 120-126.

Appendix



Cr 17/Ðæ ÇáÌÉ/1426

Cr(357.9nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Cr
Socket #:	2
Lamp Current Low(mA):	10
Wavelength(nm):	357.9
Slit Width(nm):	0.5
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.8
Flame Type:	Air-C2H2
Burner Height(mm):	9
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-SM-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

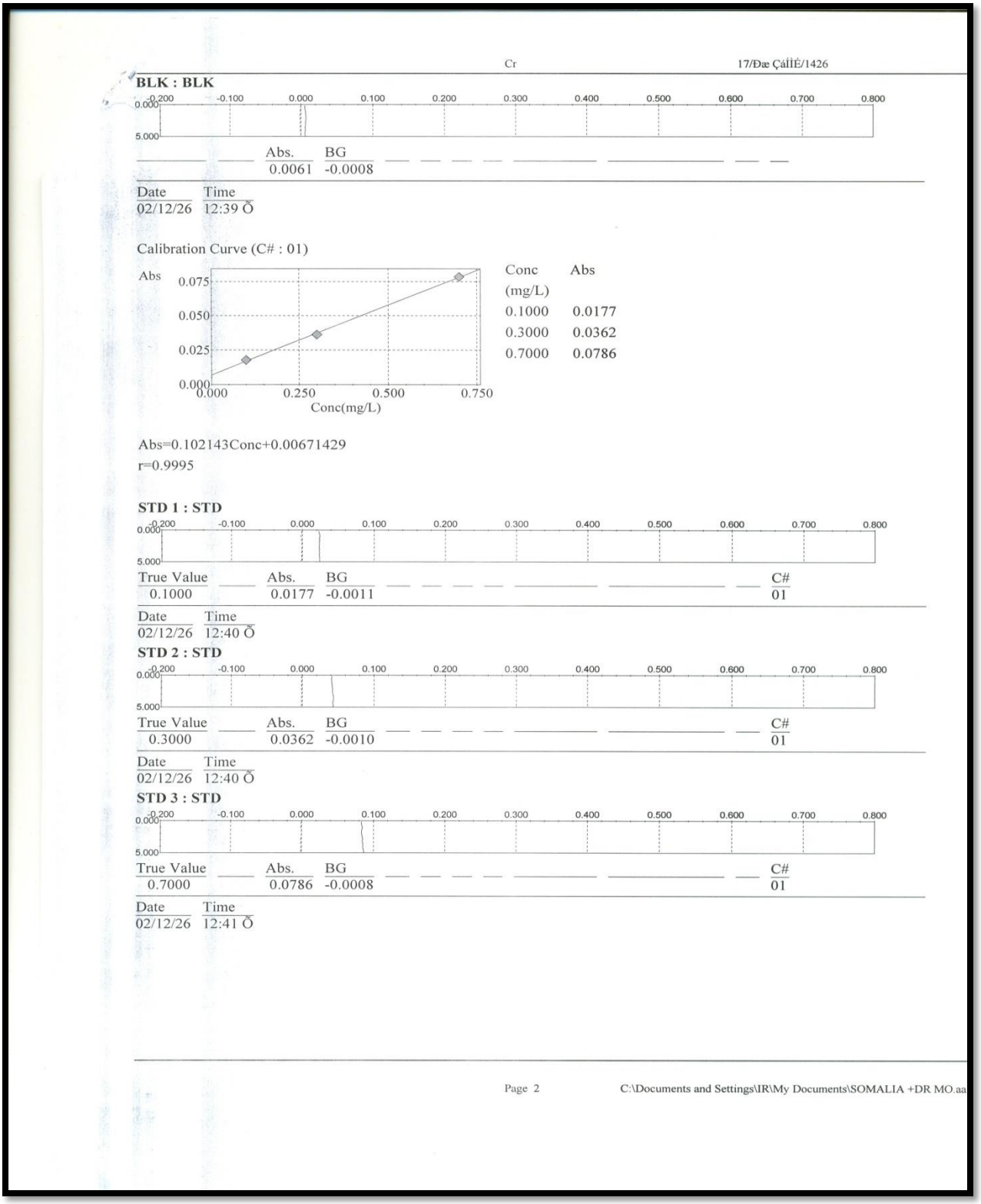
	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	99.90	0.00000
Standard	1	1	99.90	0.00000
Sample	1	1	99.90	0.00000
Reslope	1	1	99.90	0.00000

QA/QC Parameters

QC Type	Judge Calc.	Standard Value	Out of Control Remark

Page 1 C:\Documents and Settings\IR\My Documents\SOMALIA +DR MO.aa

Appendix 3.1: Atomic absorption condition for determination Cr^{++}



Cr 17/Đæ ÇáÍÍÉ/1426

BLK : BLK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

Abs.	BG
0.0061	-0.0008

Date	Time
02/12/26	12:39 Õ

Calibration Curve (C# : 01)

Abs

0.075

0.050

0.025

0.000

0.000 0.250 0.500 0.750

Conc(mg/L)

Conc (mg/L)	Abs
0.1000	0.0177
0.3000	0.0362
0.7000	0.0786

Abs=0.102143Conc+0.00671429

r=0.9995

STD 1 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

True Value	Abs.	BG	C#
0.1000	0.0177	-0.0011	01

Date	Time
02/12/26	12:40 Õ

STD 2 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

True Value	Abs.	BG	C#
0.3000	0.0362	-0.0010	01

Date	Time
02/12/26	12:40 Õ

STD 3 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

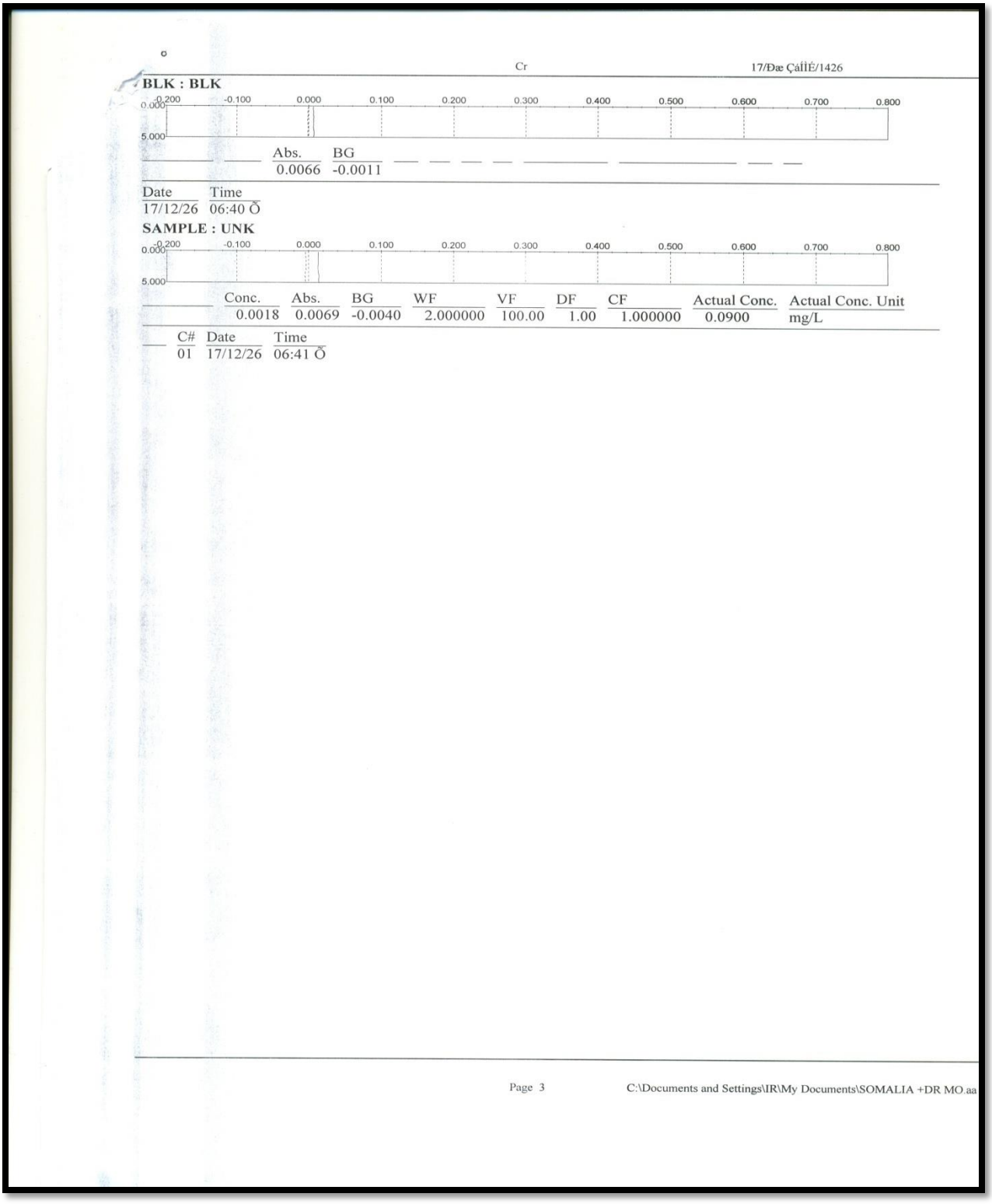
5.000

True Value	Abs.	BG	C#
0.7000	0.0786	-0.0008	01

Date	Time
02/12/26	12:41 Õ

Page 2 C:\Documents and Settings\IR\My Documents\SOMALIA +DR MO.aa

Appendix 3.2: Calibration curve for determination Cr^{++}



Cr 17/Ðæ ÇáÍÌÉ/1426

BLK : BLK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

	Abs.	BG
	0.0066	-0.0011

Date	Time
17/12/26	06:40 Õ

SAMPLE : UNK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

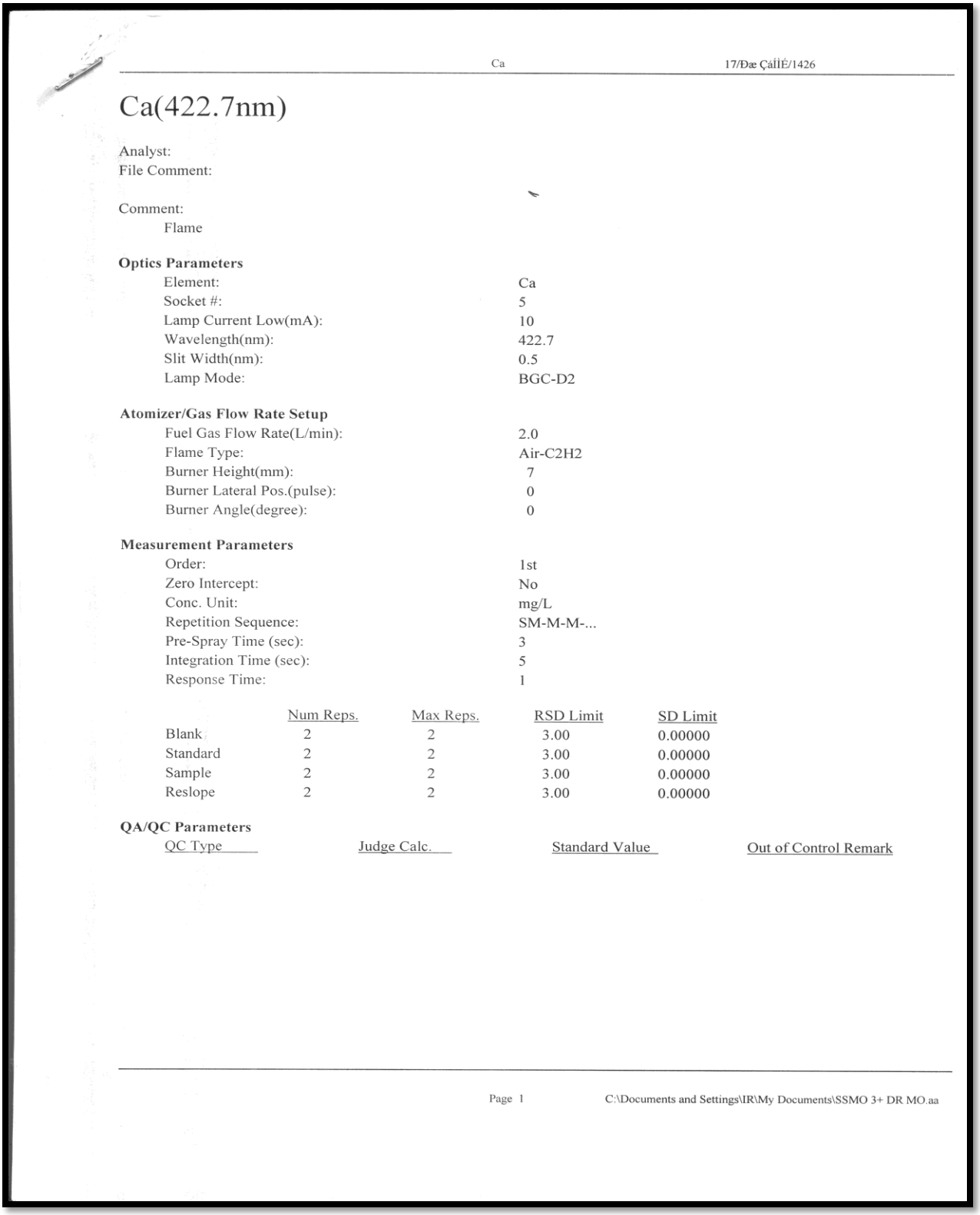
5.000

	Conc.	Abs.	BG	WF	VF	DF	CF	Actual Conc.	Actual Conc. Unit
	0.0018	0.0069	-0.0040	2.000000	100.00	1.00	1.000000	0.0900	mg/L

	C#	Date	Time
	01	17/12/26	06:41 Õ

Page 3 C:\Documents and Settings\IR\My Documents\SOMALIA +DR MO.aa

Appendix3.3:Results for determination Cr^{++}



Ca(422.7nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Ca
Socket #:	5
Lamp Current Low(mA):	10
Wavelength(nm):	422.7
Slit Width(nm):	0.5
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.0
Flame Type:	Air-C2H2
Burner Height(mm):	7
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

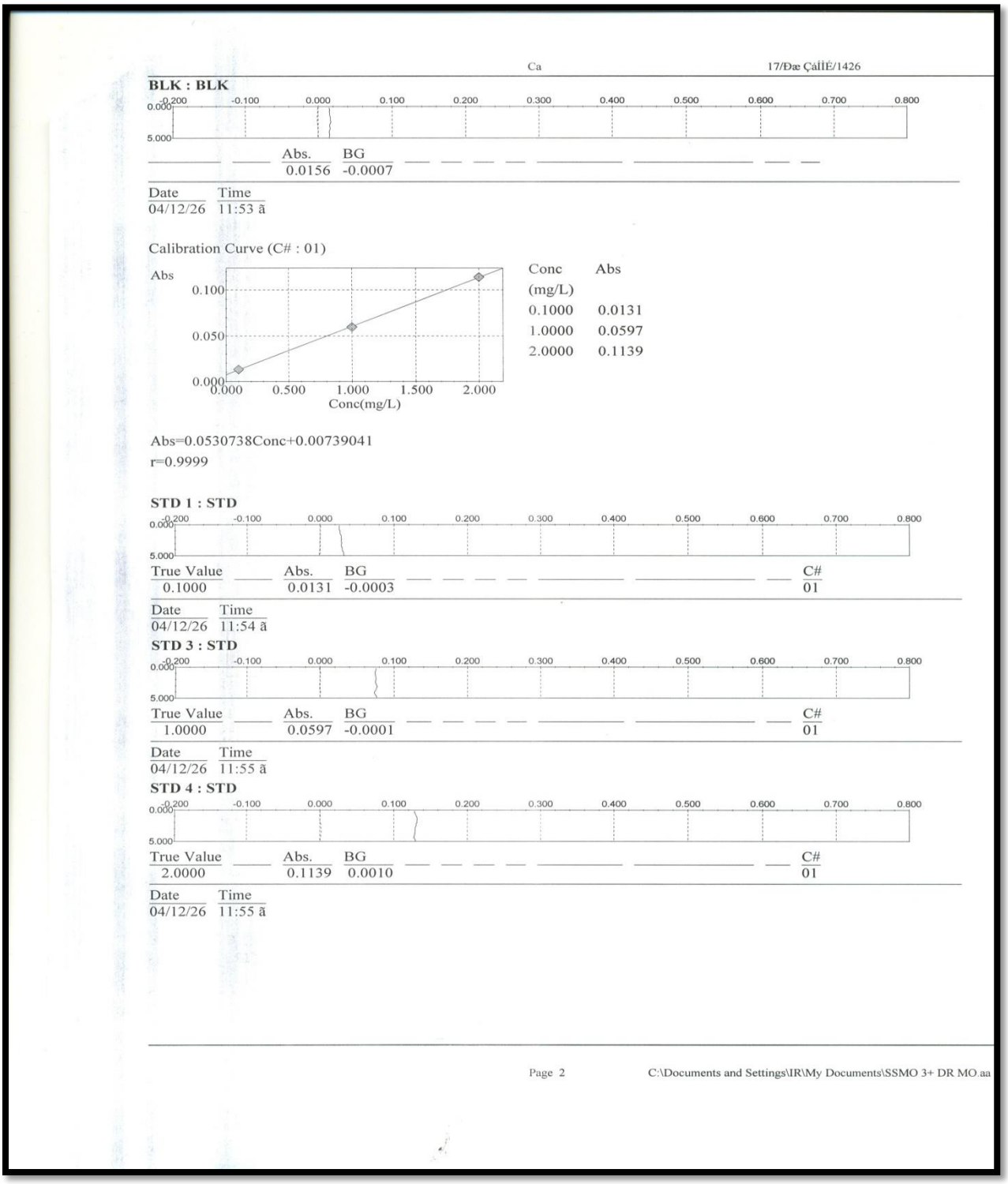
Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-M-M-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	2	2	3.00	0.00000
Standard	2	2	3.00	0.00000
Sample	2	2	3.00	0.00000
Reslope	2	2	3.00	0.00000

QA/QC Parameters

QC Type	Judge Calc.	Standard Value	Out of Control Remark

Appendix 3.4: Atomic absorption condition for determination Ca^{++}



Ca 17/Đæ ÇáÎÉ/1426

BLK : BLK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000
5.000

Abs.	BG
0.0156	-0.0007

Date	Time
04/12/26	11:53 ã

Calibration Curve (C# : 01)

Abs

0.100
0.050
0.000
0.000 0.500 1.000 1.500 2.000
Conc(mg/L)

Conc (mg/L)	Abs
0.1000	0.0131
1.0000	0.0597
2.0000	0.1139

Abs=0.0530738Conc+0.00739041

r=0.9999

STD 1 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000
5.000

True Value	Abs.	BG	C#
0.1000	0.0131	-0.0003	01

Date	Time
04/12/26	11:54 ã

STD 3 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000
5.000

True Value	Abs.	BG	C#
1.0000	0.0597	-0.0001	01

Date	Time
04/12/26	11:55 ã

STD 4 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

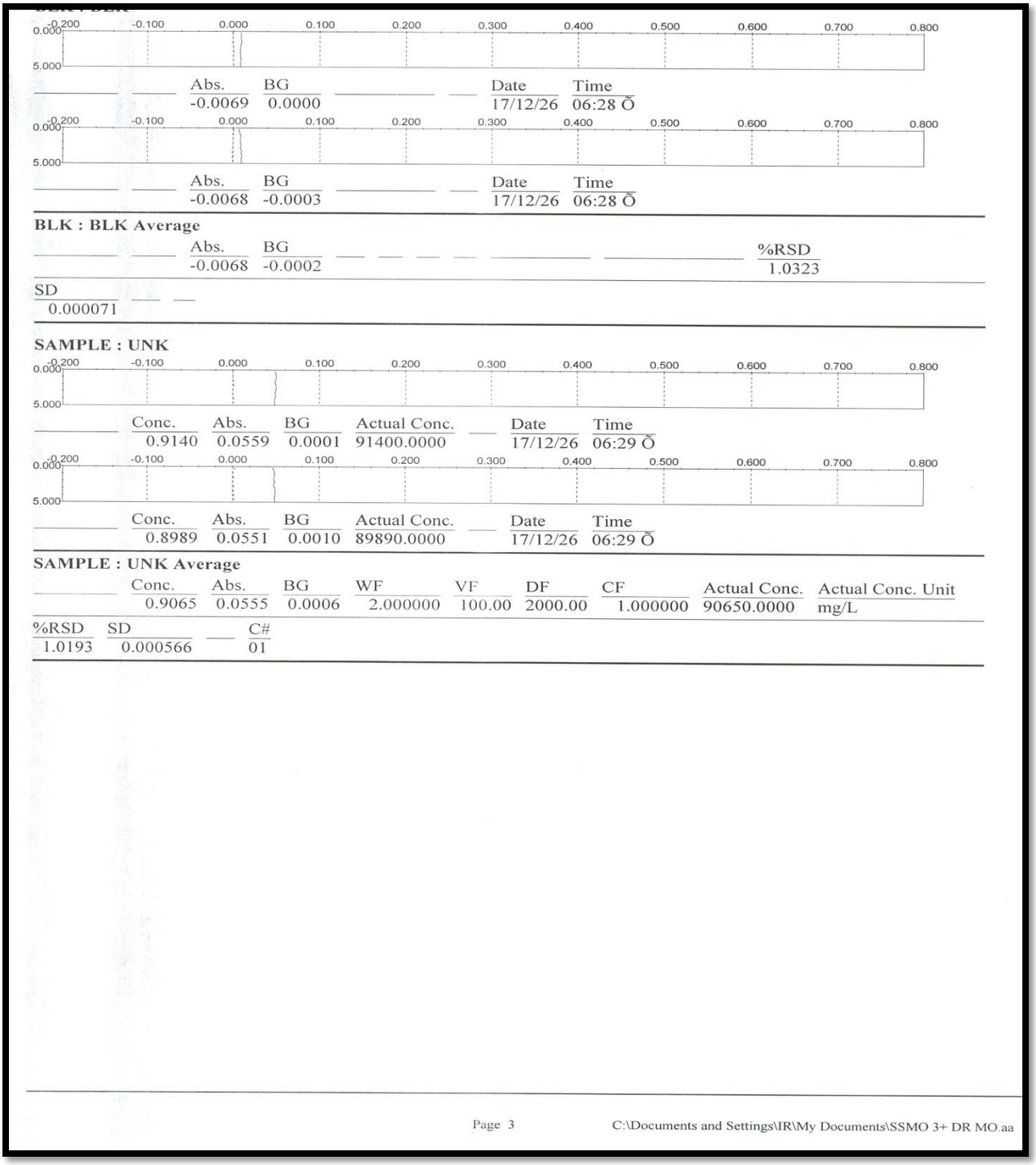
0.000
5.000

True Value	Abs.	BG	C#
2.0000	0.1139	0.0010	01

Date	Time
04/12/26	11:55 ã

Page 2 C:\Documents and Settings\IR\My Documents\SSMO 3+ DR MO.aa

Appendix 3.5:Calibration curve for determination Ca^{++}



	Abs.	BG		Date	Time
	-0.0069	0.0000		17/12/26	06:28 Õ

	Abs.	BG		Date	Time
	-0.0068	-0.0003		17/12/26	06:28 Õ

BLK : BLK Average

	Abs.	BG	%RSD
	-0.0068	-0.0002	1.0323

SD		
0.000071		

SAMPLE : UNK

	Conc.	Abs.	BG	Actual Conc.		Date	Time
	0.9140	0.0559	0.0001	91400.0000		17/12/26	06:29 Õ

	Conc.	Abs.	BG	Actual Conc.		Date	Time
	0.8989	0.0551	0.0010	89890.0000		17/12/26	06:29 Õ

SAMPLE : UNK Average

	Conc.	Abs.	BG	WF	VF	DF	CF	Actual Conc.	Actual Conc. Unit
	0.9065	0.0555	0.0006	2.000000	100.00	2000.00	1.000000	90650.0000	mg/L

%RSD	SD		C#
1.0193	0.000566		01

Appendix 3.6: Results for determination Ca^{++}

Zn(213.9nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Zn
Socket #:	6
Lamp Current Low(mA):	8
Wavelength(nm):	213.9
Slit Width(nm):	0.5
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.0
Flame Type:	Air-C2H2
Burner Height(mm):	7
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-M-M-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	3.00	0.00000
Standard	1	1	3.00	0.00000
Sample	1	1	3.00	0.00000
Reslope	1	1	3.00	0.00000

QA/QC Parameters

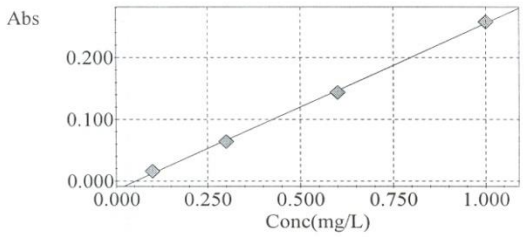
QC Type	Judge Calc.	Standard Value	Out of Control Remark

Appendix 3.7: Atomic absorption condition for determination Zn^{++}

	Abs.	BG
	-0.0061	0.0000

Date	Time
08/08/34	10:25 Õ

Calibration Curve (C# : 01)

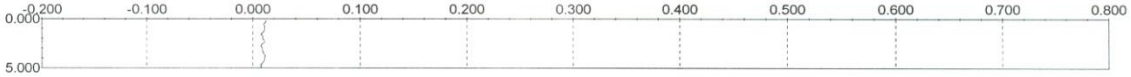


Conc (mg/L)	Abs
0.1000	0.0158
0.3000	0.0642
0.6000	0.1438
1.0000	0.2572

Abs=0.269174Conc+-0.014337

r=0.9995

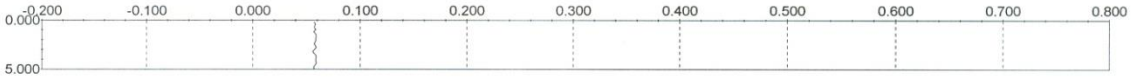
STD 1 : STD



True Value	Abs.	BG	C#
0.1000	0.0158	0.0000	01

Date	Time
08/08/34	10:25 Õ

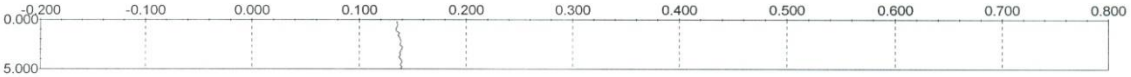
STD 2 : STD



True Value	Abs.	BG	C#
0.3000	0.0642	0.0000	01

Date	Time
08/08/34	10:26 Õ

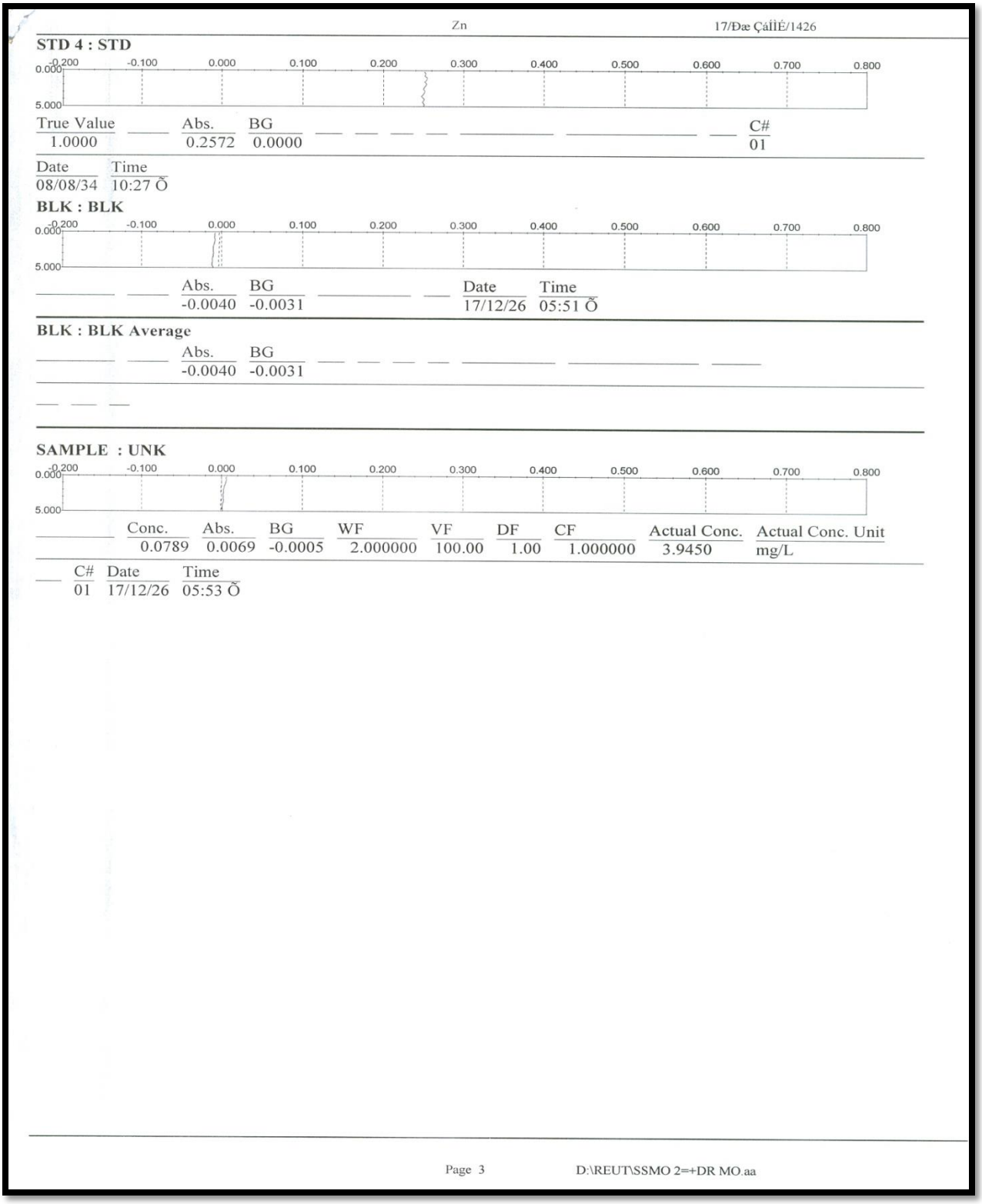
STD 3 : STD



True Value	Abs.	BG	C#
0.6000	0.1438	0.0000	01

Date	Time
08/08/34	10:26 Õ

Appendix3.8: Calibration Curve for determination Zn^{++}



Zn 17/Đæ ÇáÌÉ/1426

STD 4 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

True Value		Abs.	BG								C#
1.0000		0.2572	0.0000								01

Date	Time
08/08/34	10:27 Õ

BLK : BLK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

		Abs.	BG			Date	Time
		-0.0040	-0.0031			17/12/26	05:51 Õ

BLK : BLK Average

		Abs.	BG								
		-0.0040	-0.0031								

SAMPLE : UNK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

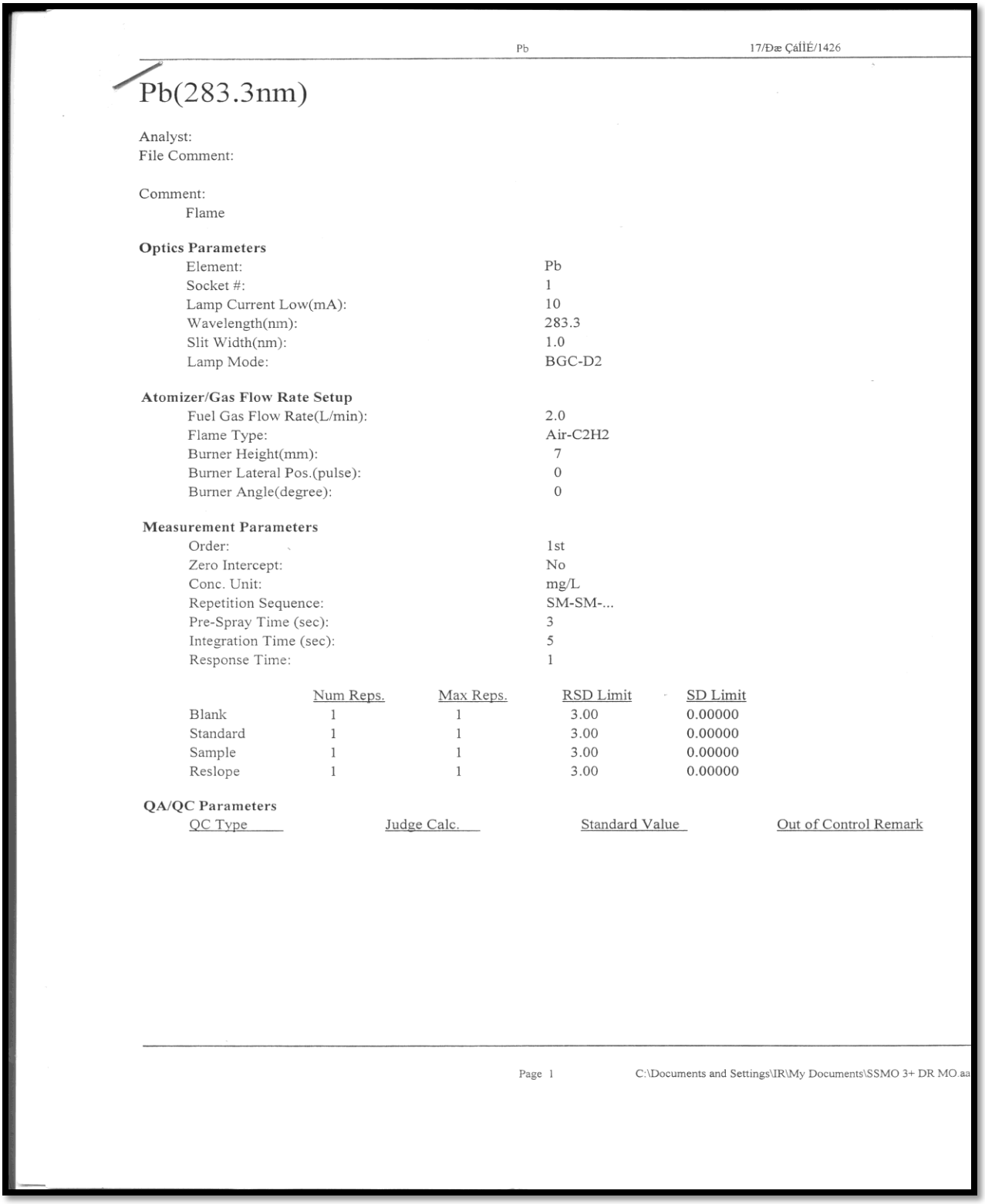
5.000

	Conc.	Abs.	BG	WF	VF	DF	CF	Actual Conc.	Actual Conc. Unit
	0.0789	0.0069	-0.0005	2.000000	100.00	1.00	1.000000	3.9450	mg/L

C#	Date	Time
01	17/12/26	05:53 Õ

Page 3 D:\REUT\SSMO 2=+DR MO.aa

Appendix 3.9: Results for determination Zn^{++}



Pb 17/Đæ ÇáÌÉ/1426

Pb(283.3nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Pb
Socket #:	1
Lamp Current Low(mA):	10
Wavelength(nm):	283.3
Slit Width(nm):	1.0
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.0
Flame Type:	Air-C2H2
Burner Height(mm):	7
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-SM-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	3.00	0.00000
Standard	1	1	3.00	0.00000
Sample	1	1	3.00	0.00000
Reslope	1	1	3.00	0.00000

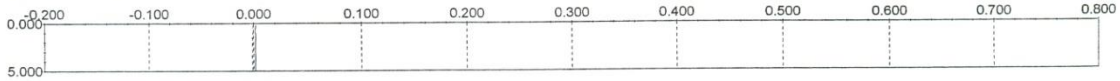
QA/QC Parameters

QC Type	Judge Calc.	Standard Value	Out of Control Remark

Page 1 C:\Documents and Settings\IR\My Documents\SSMO 3+ DR MO.aa

Appendix 3.10: Atomic absorption condition for determination Pb^{++}

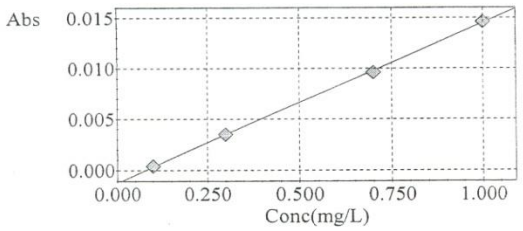
BLK : BLK



Abs.	BG
0.0016	-0.0010

Date	Time
04/12/26	06:17 Õ

Calibration Curve (C# : 01)

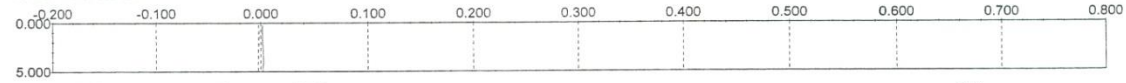


Conc (mg/L)	Abs
0.1000	0.0004
0.3000	0.0035
0.7000	0.0096
1.0000	0.0146

Abs=0.0157077Conc+-0.00122154

r=0.9998

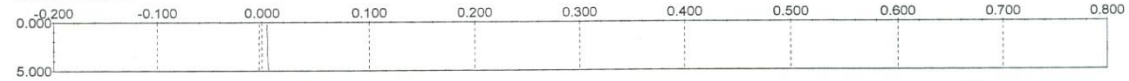
STD 1 : STD



True Value	Abs.	BG	C#
0.1000	0.0004	-0.0023	01

Date	Time
04/12/26	06:18 Õ

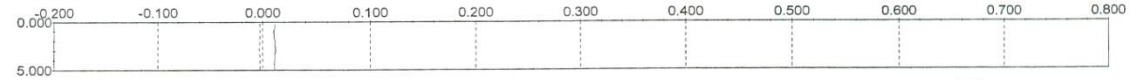
STD 2 : STD



True Value	Abs.	BG	C#
0.3000	0.0035	-0.0027	01

Date	Time
04/12/26	06:18 Õ

STD 3 : STD



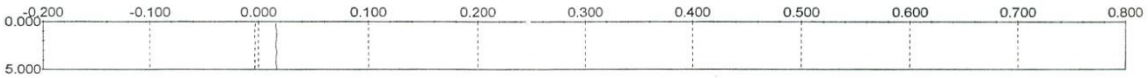
True Value	Abs.	BG	C#
0.7000	0.0096	-0.0027	01

Date	Time
04/12/26	06:19 Õ

Appendix 3.11: Calibration Curve for determination Pb^{++}

Pb 17/Ðæ ÇáÏÈÉ/1426

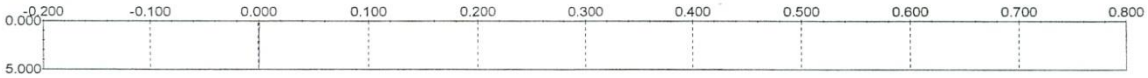
D 4 : STD



True Value	Abs.	BG	C#
1.0000	0.0146	-0.0031	01

Date	Time
04/12/26	06:19 Õ

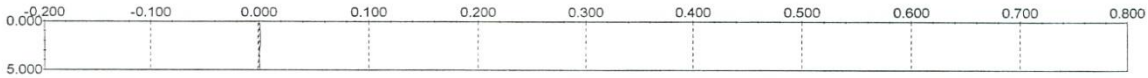
BLK : BLK



Abs.	BG
0.0003	-0.0006

Date	Time
17/12/26	06:08 Õ

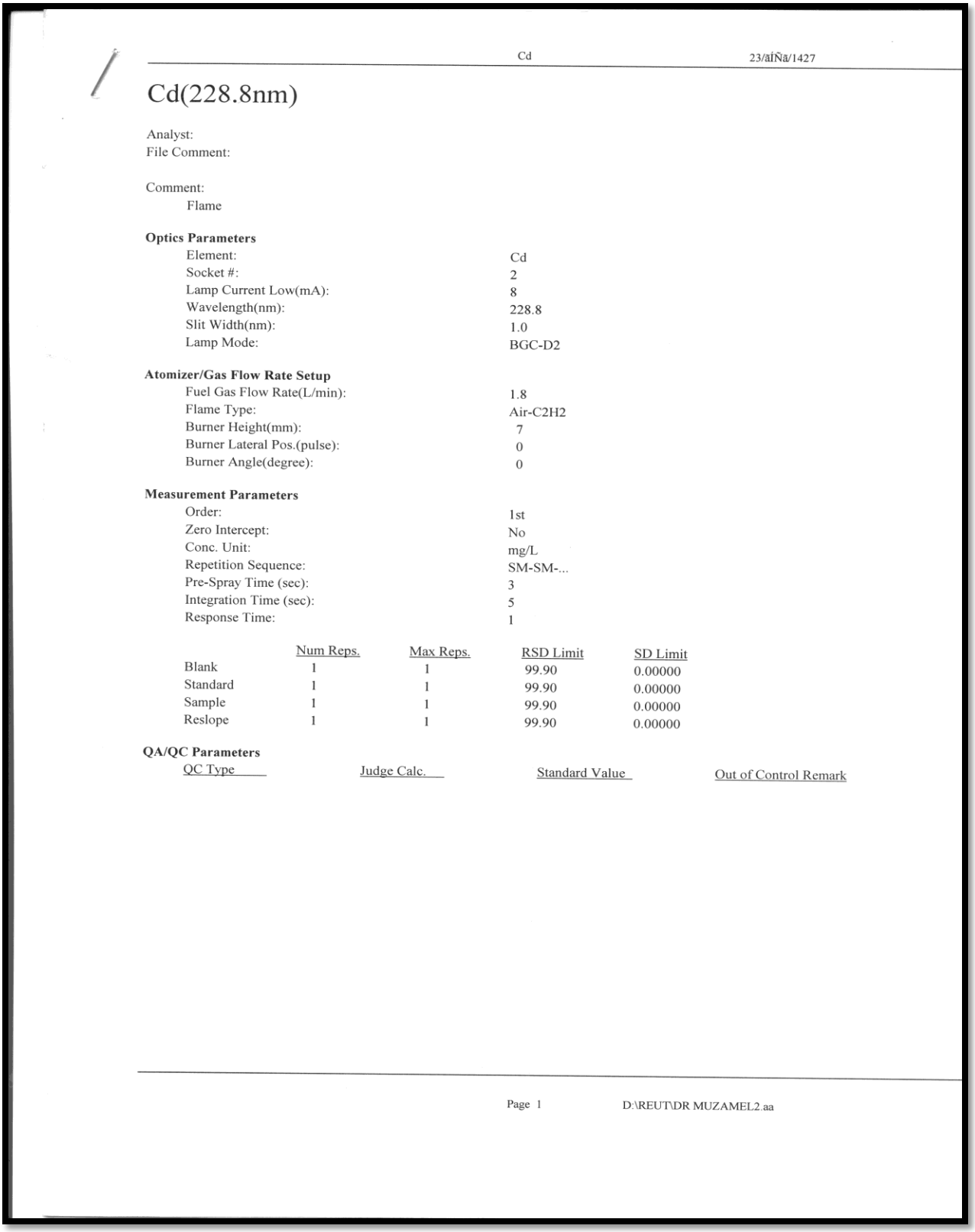
SAMPLE : UNK



Conc.	Abs.	BG	WF	VF	DF	CF	Actual Conc.	Actual Conc. Unit
0.1032	0.0004	-0.0009	2.000000	100.00	1.00	1.000000	5.1600	mg/L

C#	Date	Time
01	17/12/26	06:08 Õ

Appendix 3.12: Results for determination Pb^{++}



Cd 23/aíÑa/1427

Cd(228.8nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Cd
Socket #:	2
Lamp Current Low(mA):	8
Wavelength(nm):	228.8
Slit Width(nm):	1.0
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	1.8
Flame Type:	Air-C2H2
Burner Height(mm):	7
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-SM-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	99.90	0.00000
Standard	1	1	99.90	0.00000
Sample	1	1	99.90	0.00000
Reslope	1	1	99.90	0.00000

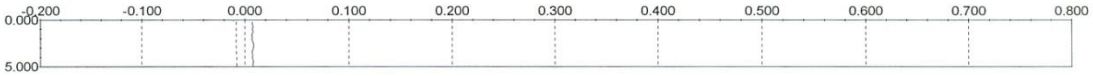
QA/QC Parameters

QC Type	Judge Calc.	Standard Value	Out of Control Remark

Page 1 D:\REUT\DR MUZAMEL2.aa

Appendix 3.13: Atomic absorption condition for determination Cd^{++}

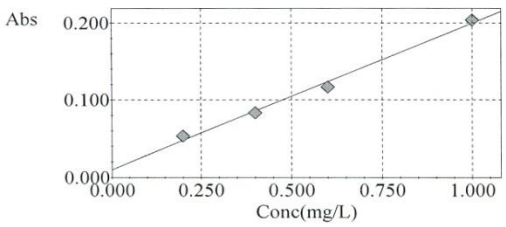
BLK : BLK



Abs.	BG
0.0075	-0.0081

Date	Time
11/01/27	12:00 Õ

Calibration Curve (C# : 01)

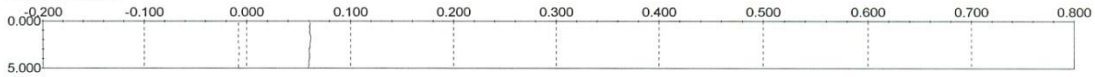


Conc (mg/L)	Abs
0.2000	0.0532
0.4000	0.0833
0.6000	0.1166
1.0000	0.2040

Abs=0.190043Conc+0.00975143

r=0.9959

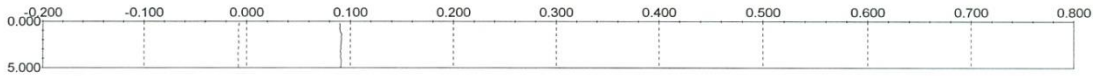
STD 1 : STD



True Value	Abs.	BG	C#
0.2000	0.0532	-0.0080	01

Date	Time
11/01/27	12:00 Õ

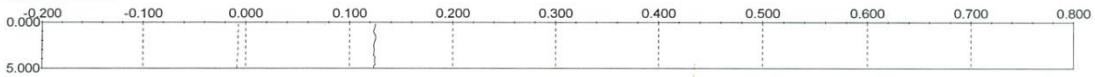
STD 2 : STD



True Value	Abs.	BG	C#
0.4000	0.0833	-0.0079	01

Date	Time
11/01/27	12:01 Õ

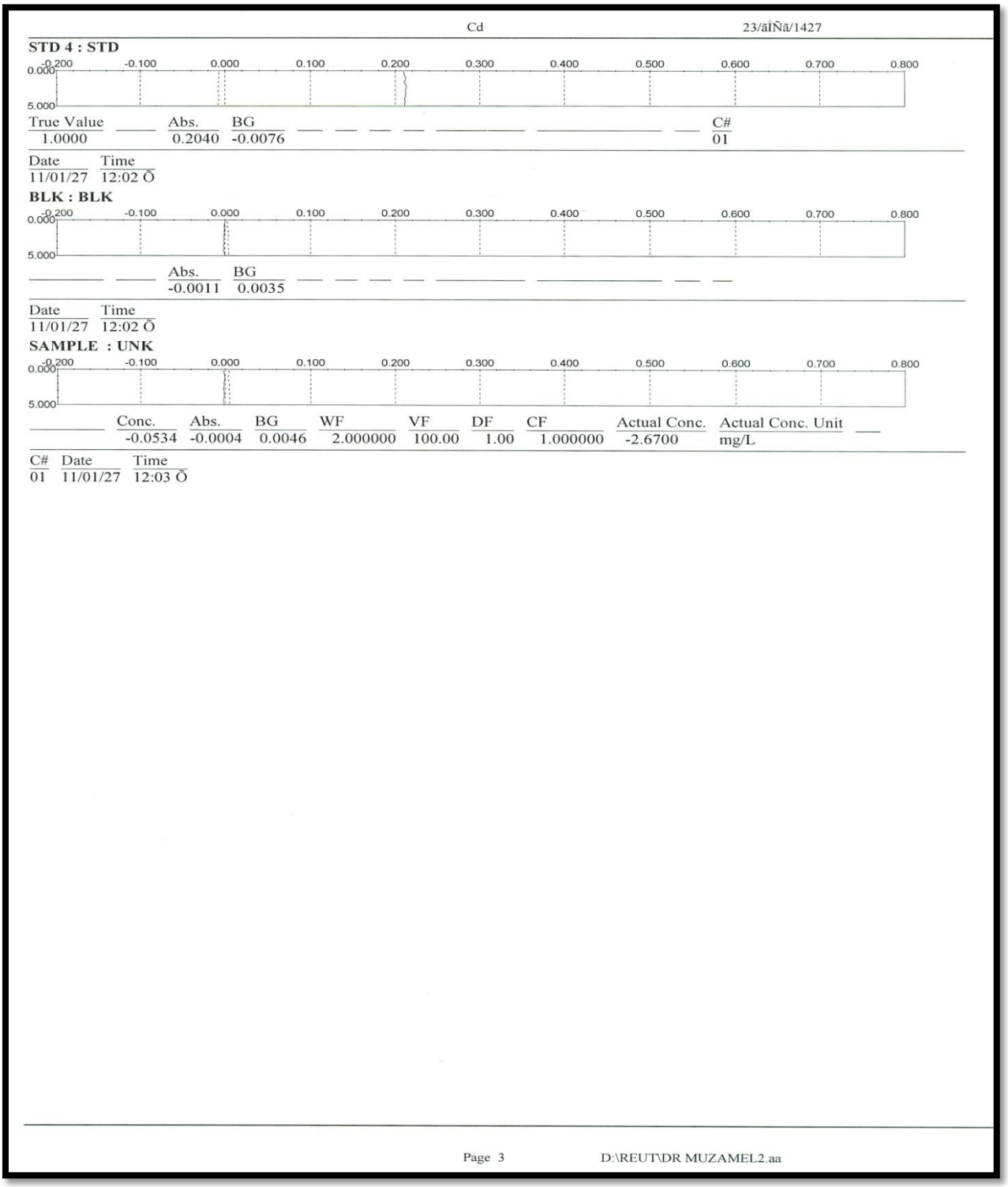
STD 3 : STD



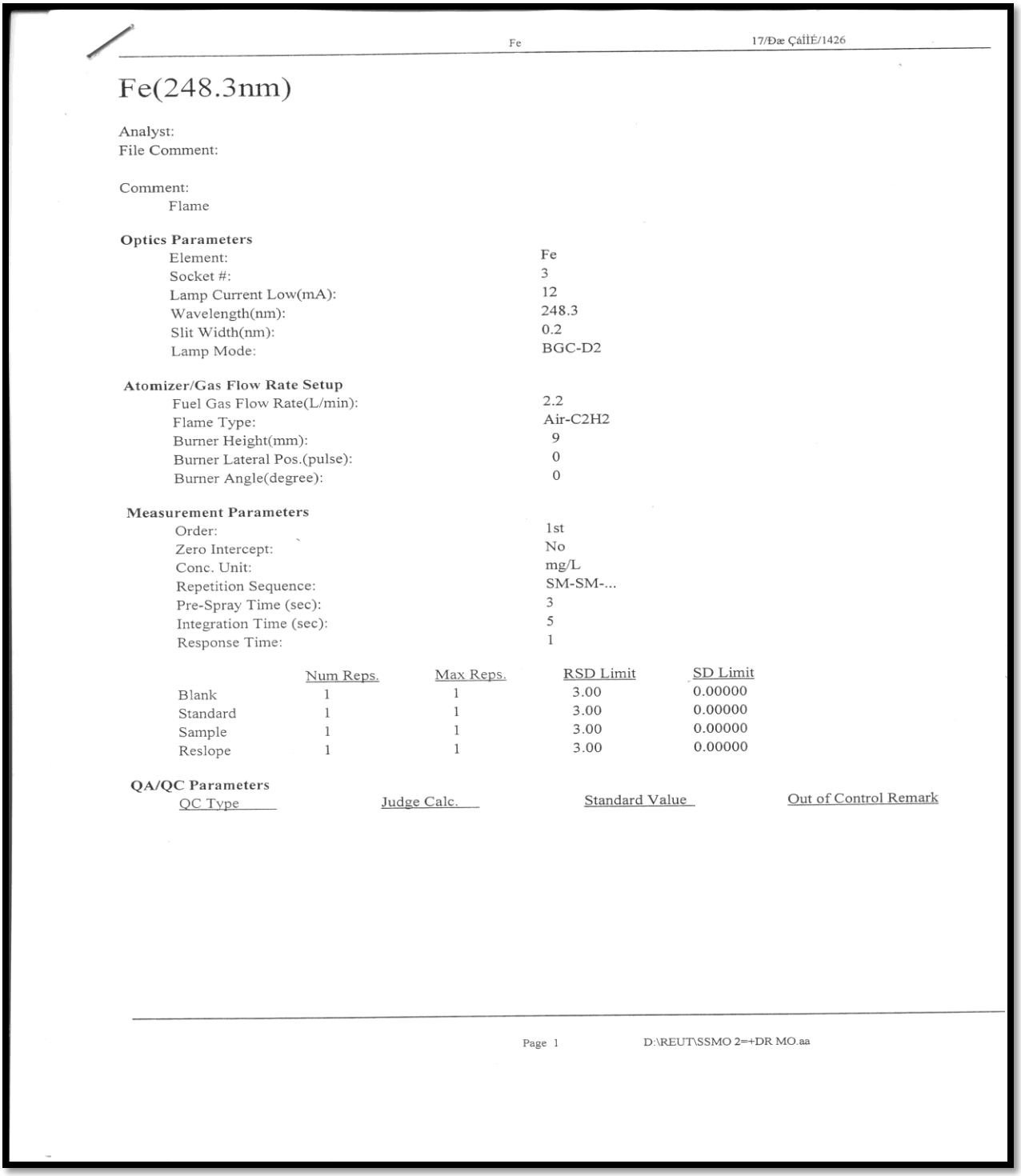
True Value	Abs.	BG	C#
0.6000	0.1166	-0.0078	01

Date	Time
11/01/27	12:01 Õ

Appendix3.14: Calibration curve for determination Cd^{++}



Appendix 3.15: Results for determination Cd^{++}



Fe 17/Ðæ ÇáÎÉ/1426

Fe(248.3nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Fe
Socket #:	3
Lamp Current Low(mA):	12
Wavelength(nm):	248.3
Slit Width(nm):	0.2
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.2
Flame Type:	Air-C2H2
Burner Height(mm):	9
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-SM-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	3.00	0.00000
Standard	1	1	3.00	0.00000
Sample	1	1	3.00	0.00000
Reslope	1	1	3.00	0.00000

QA/QC Parameters

QC Type	Judge Calc.	Standard Value	Out of Control Remark

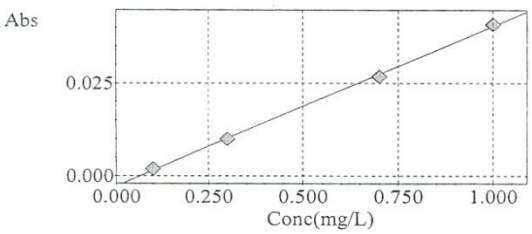
Page 1 D:\REUT\SSMO 2=+DR MO.aa

Appendix 3.16: Atomic absorption condition for determination Fe^{++}

Abs.	BG
0.0003	0.0000

Date	Time
08/08/34	11:31 Õ

Calibration Curve (C# : 01)

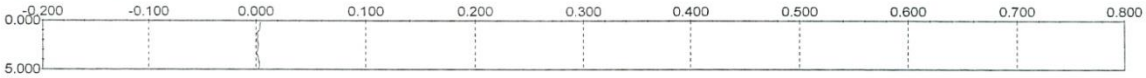


Conc (mg/L)	Abs
0.1000	0.0019
0.3000	0.0099
0.7000	0.0268
1.0000	0.0409

Abs=0.0432462Conc+-0.00282923

r=0.9995

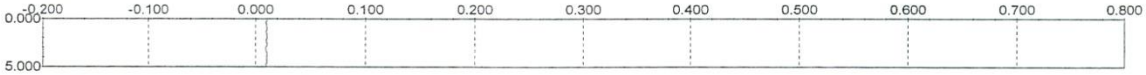
STD 1 : STD



True Value	Abs.	BG	C#
0.1000	0.0019	0.0000	01

Date	Time
08/08/34	11:32 Õ

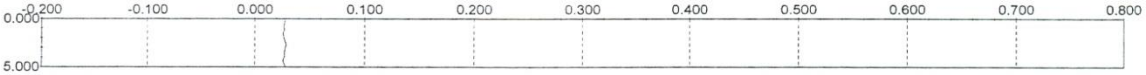
STD 2 : STD



True Value	Abs.	BG	C#
0.3000	0.0099	0.0000	01

Date	Time
08/08/34	11:32 Õ

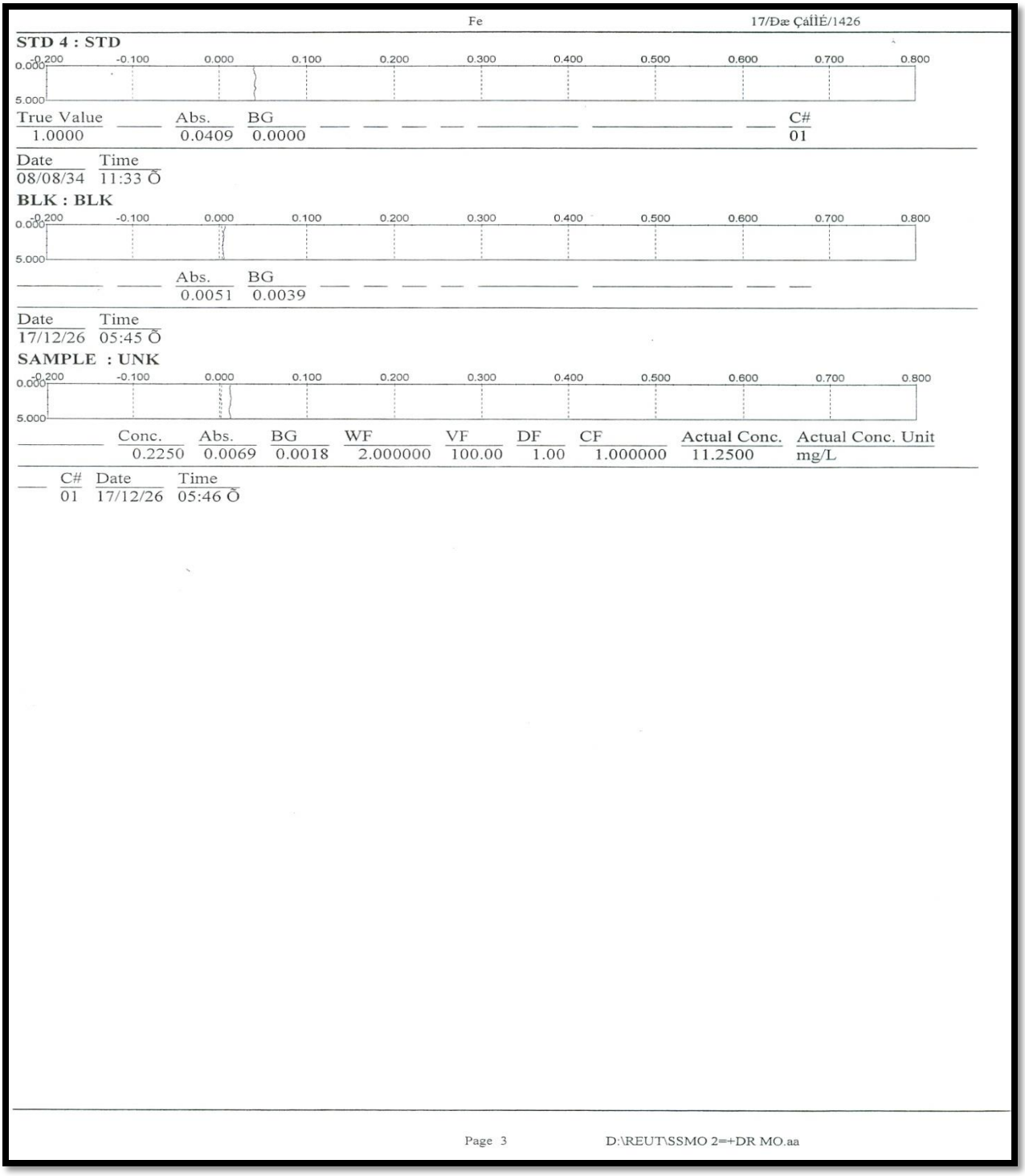
STD 3 : STD



True Value	Abs.	BG	C#
0.7000	0.0268	0.0000	01

Date	Time
08/08/34	11:33 Õ

Appendix 3.17: Calibration curve for determination Fe^{++}



Fe 17/Đæ ÇáÍÉ/1426

STD 4 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

True Value		Abs.	BG		C#
1.0000		0.0409	0.0000		01

Date 08/08/34 Time 11:33 Õ

BLK : BLK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

		Abs.	BG
		0.0051	0.0039

Date 17/12/26 Time 05:45 Õ

SAMPLE : UNK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

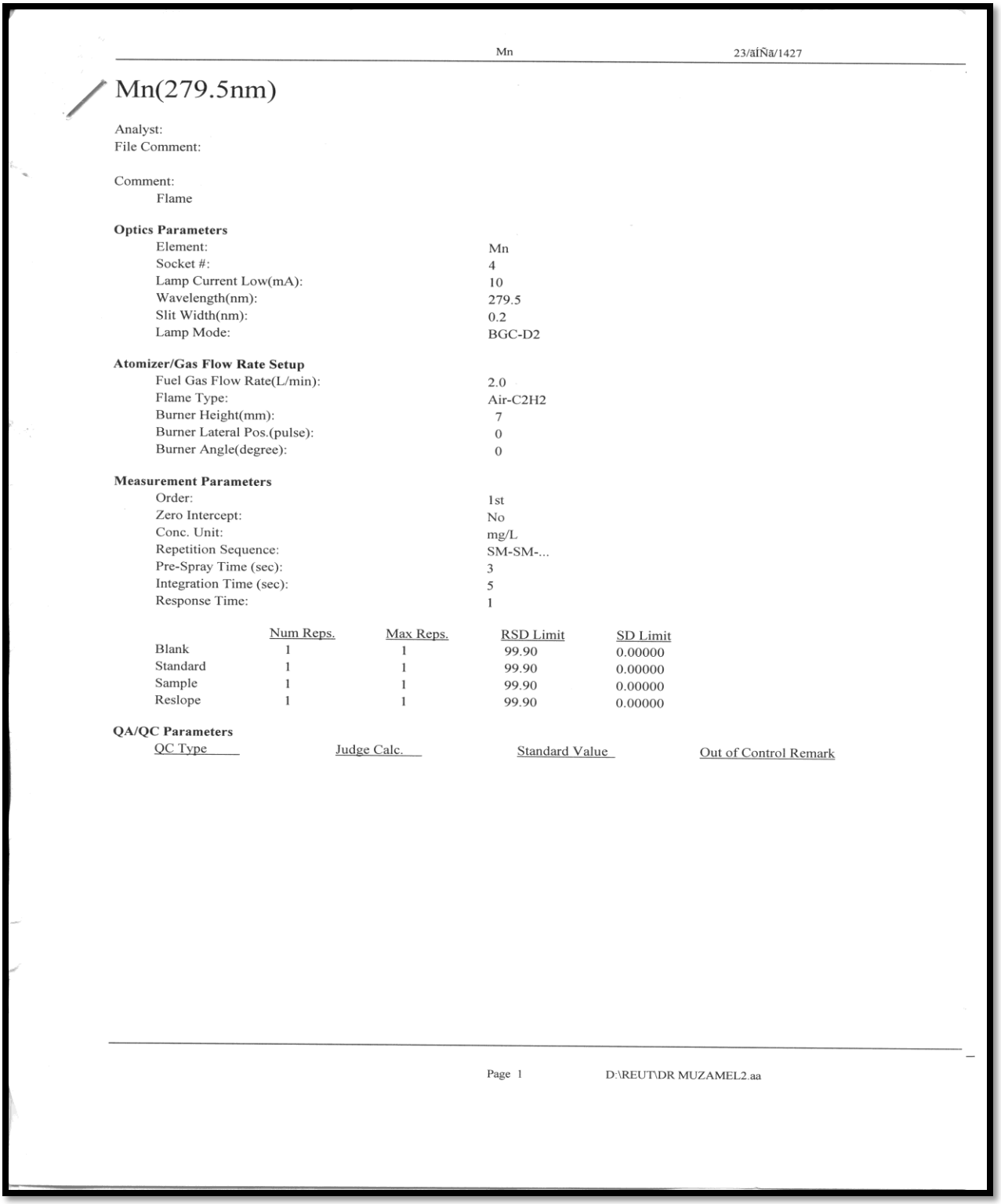
5.000

	Conc.	Abs.	BG	WF	VF	DF	CF	Actual Conc.	Actual Conc. Unit
	0.2250	0.0069	0.0018	2.000000	100.00	1.00	1.000000	11.2500	mg/L

	C#	Date	Time
	01	17/12/26	05:46 Õ

Page 3 D:\REUT\SSMO 2=+DR MO.aa

Appendix 3.18: Result for determination Fe^{++}



Mn(279.5nm)

Analyst:
File Comment:

Comment:
Flame

Optics Parameters

Element:	Mn
Socket #:	4
Lamp Current Low(mA):	10
Wavelength(nm):	279.5
Slit Width(nm):	0.2
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.0
Flame Type:	Air-C2H2
Burner Height(mm):	7
Burner Lateral Pos.(pulse):	0
Burner Angle(degree):	0

Measurement Parameters

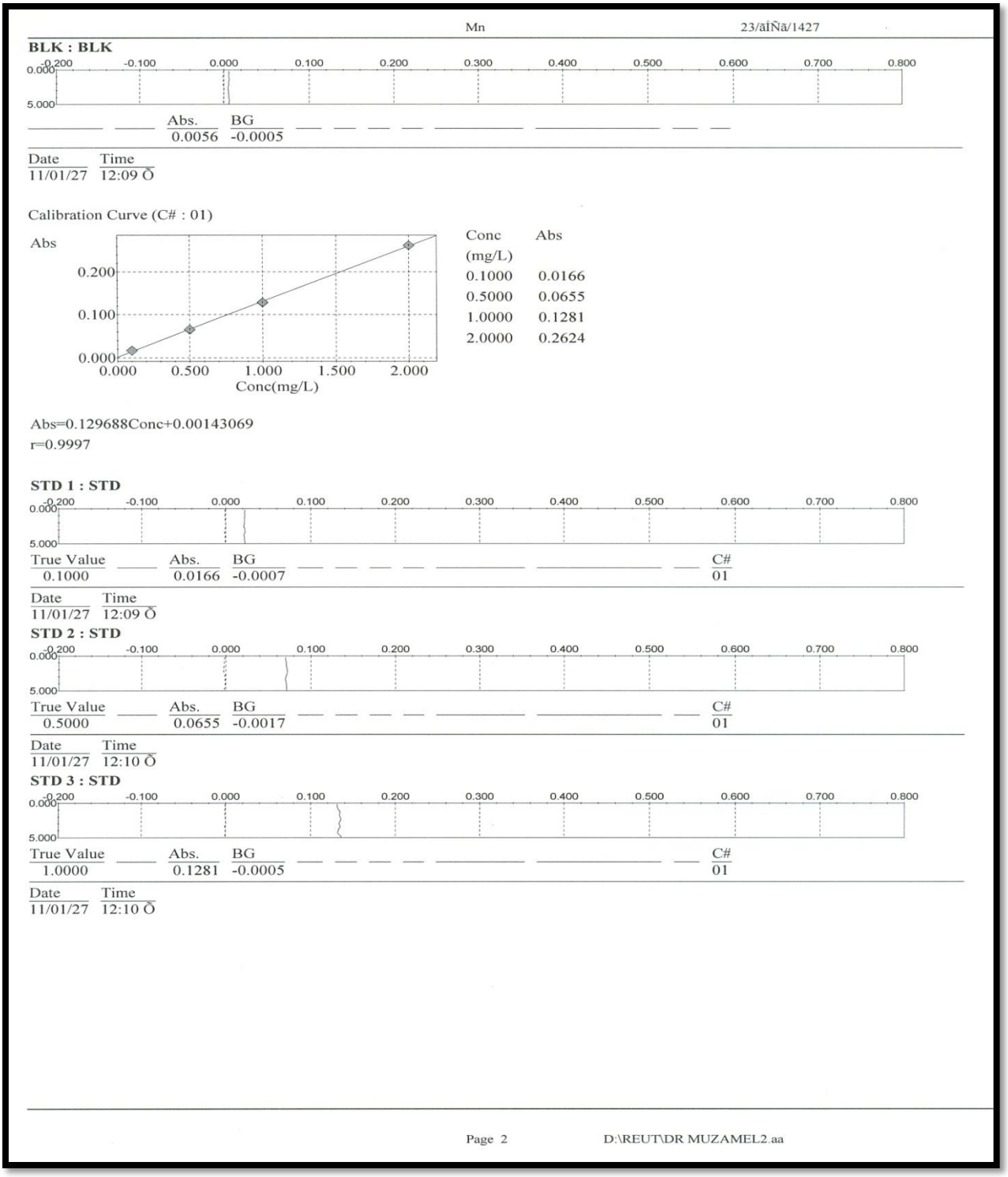
Order:	1st
Zero Intercept:	No
Conc. Unit:	mg/L
Repetition Sequence:	SM-SM-...
Pre-Spray Time (sec):	3
Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	99.90	0.00000
Standard	1	1	99.90	0.00000
Sample	1	1	99.90	0.00000
Reslope	1	1	99.90	0.00000

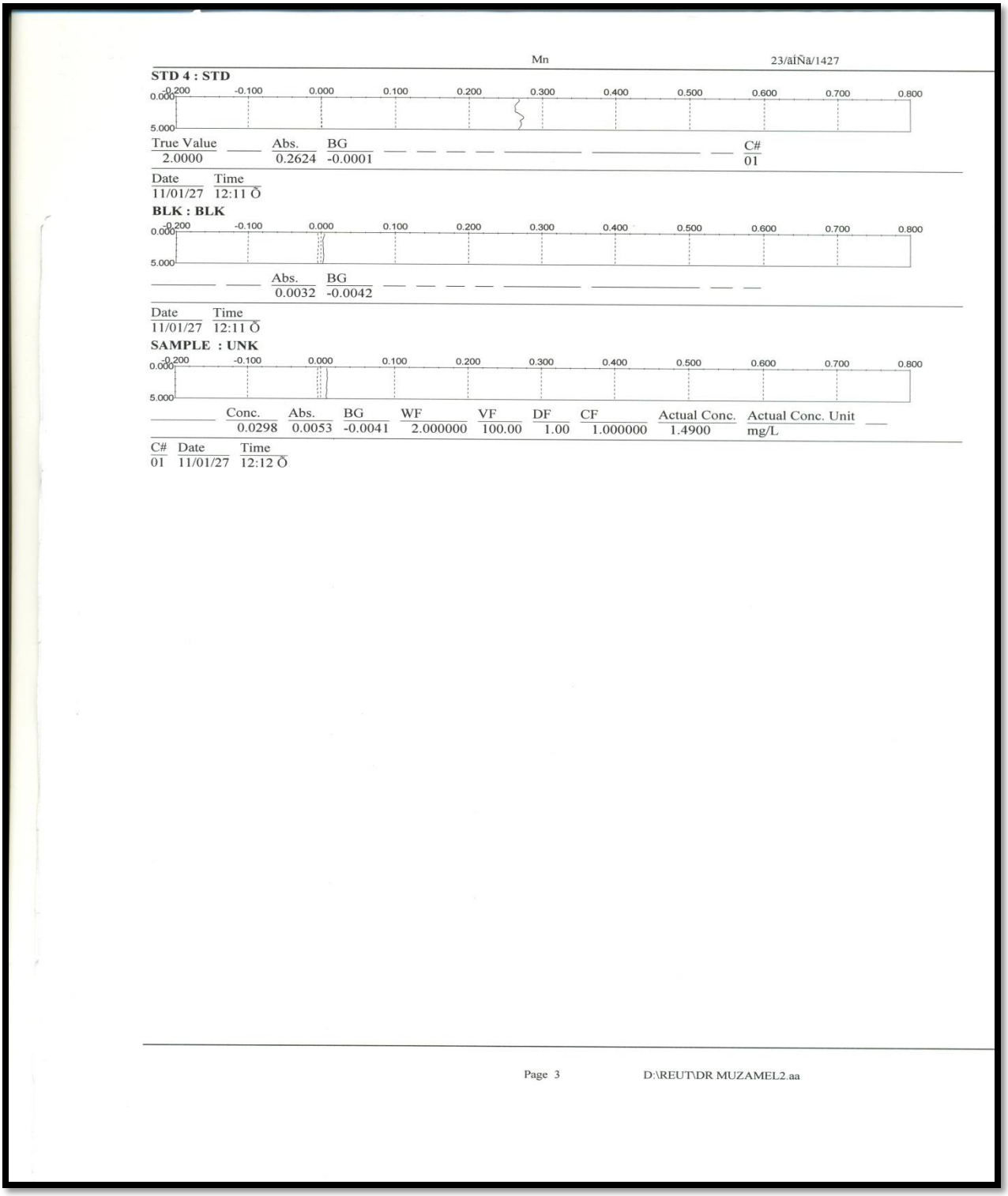
QA/QC Parameters

QC Type	Judge Calc.	Standard Value	Out of Control Remark

Appendix 3.19: Atomic absorption condition for determination Mn^{++}



Appendix 3.20: Calibration curve for determination Mn^{++}



Mn 23/äíÑä/1427

STD 4 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

True Value		Abs.	BG		C#
2.0000		0.2624	-0.0001		01

Date	Time
11/01/27	12:11 Õ

BLK : BLK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

5.000

		Abs.	BG
		0.0032	-0.0042

Date	Time
11/01/27	12:11 Õ

SAMPLE : UNK

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

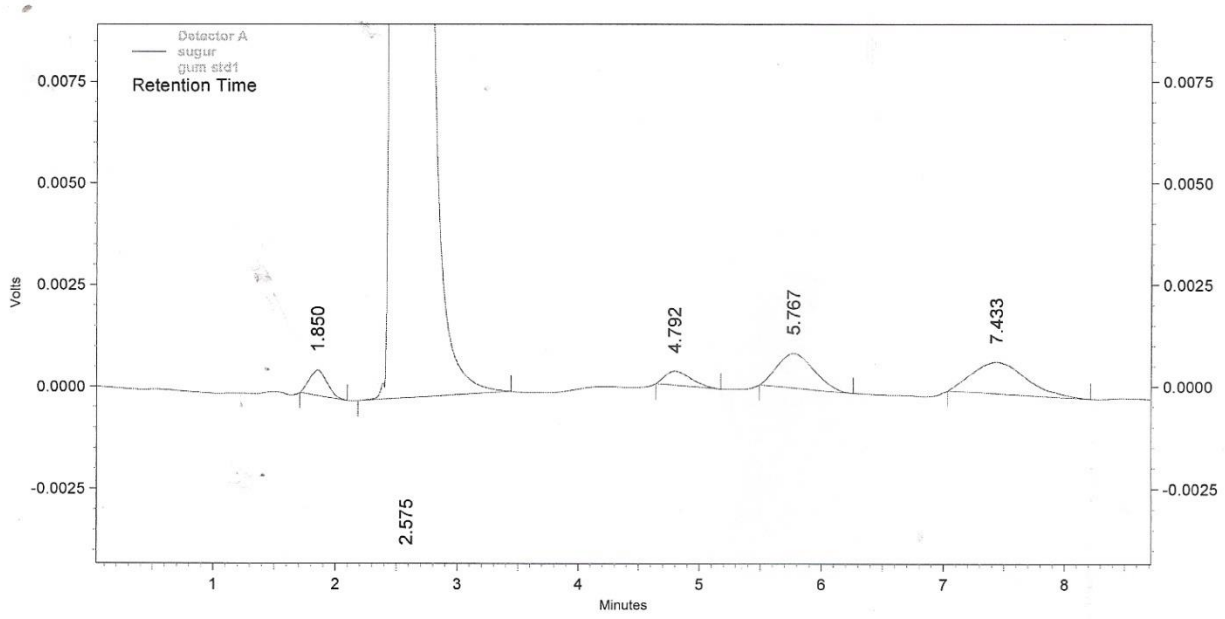
5.000

	Conc.	Abs.	BG	WF	VF	DF	CF	Actual Conc.	Actual Conc. Unit	
	0.0298	0.0053	-0.0041	2.000000	100.00	1.00	1.000000	1.4900	mg/L	

C#	Date	Time
01	11/01/27	12:12 Õ

Page 3 D:\REUT\DR MUZAMEL2.aa

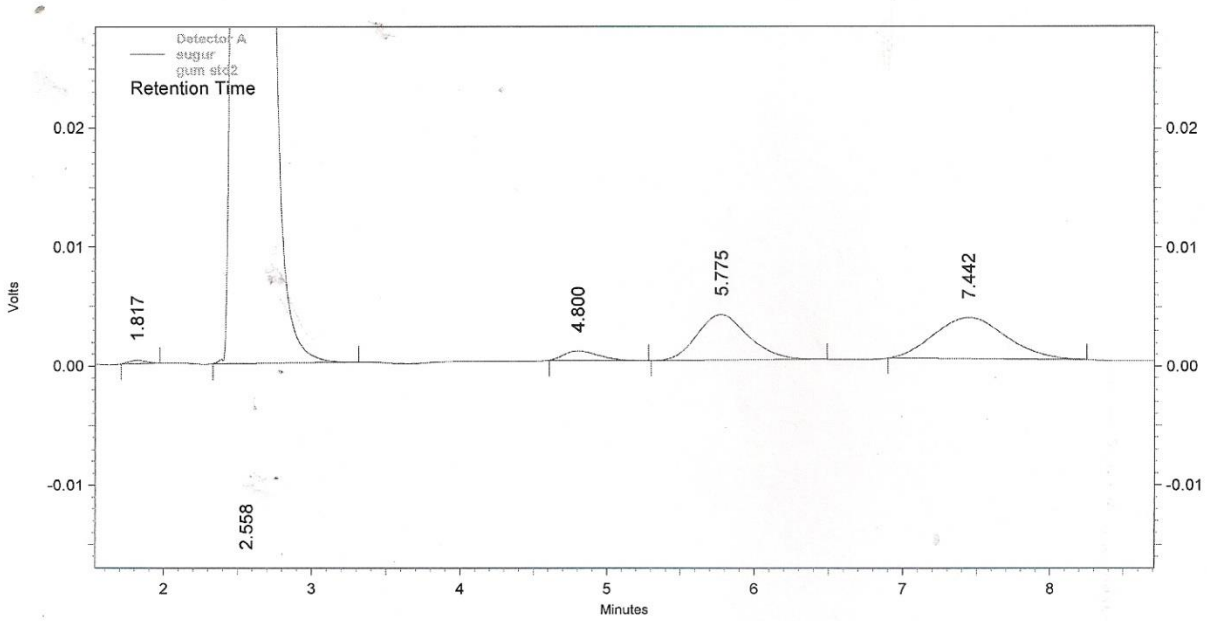
Appendix 3.21: Results for determination Mn^{++}



Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Units
3	Rhamnose	4.792	5161	0.200 CAL	**mg/ml**
4	Arebanose	5.767	17914	1.000 CAL	**mg/ml**
5	Galactose	7.433	24354	1.000 CAL	**mg/ml**

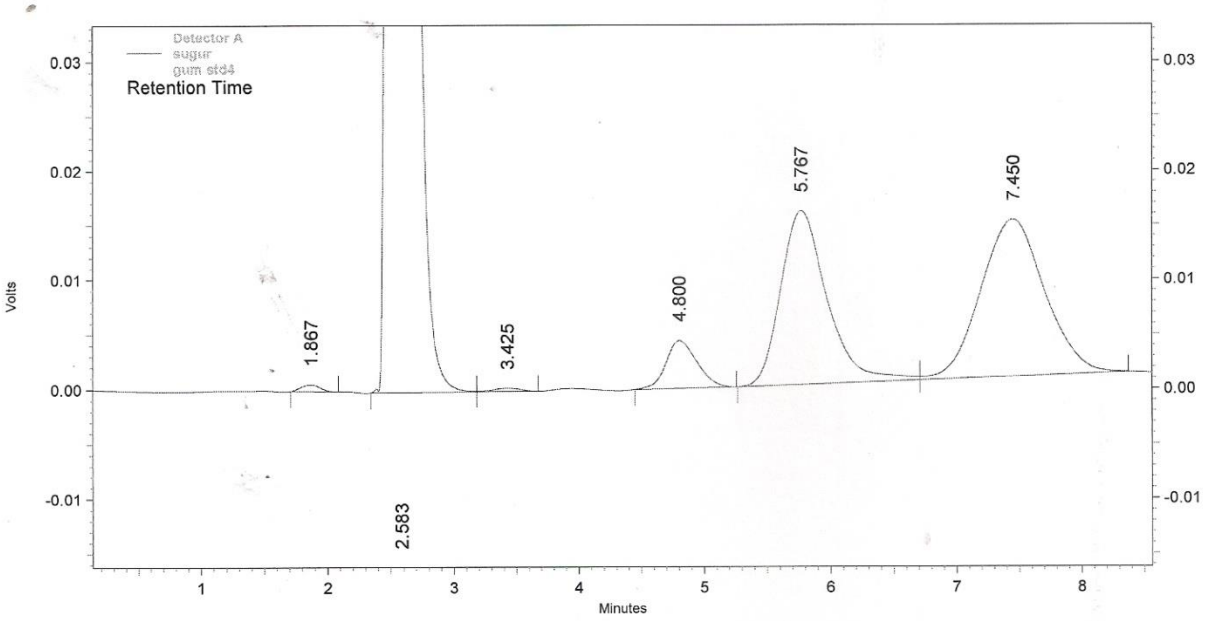
Appendix 3.22: Chromatogram (1) calibration curve for sugars



Detector A

Pk #	Name	Retention Time	Area	ESTD concentration	Units
3	Rhamnose	4.800	12345	0.400 CAL	**mg/ml**
4	Arebanose	5.775	91185	4.000 CAL	**mg/ml**
5	Galactose	7.442	117763	4.000 CAL	**mg/ml**

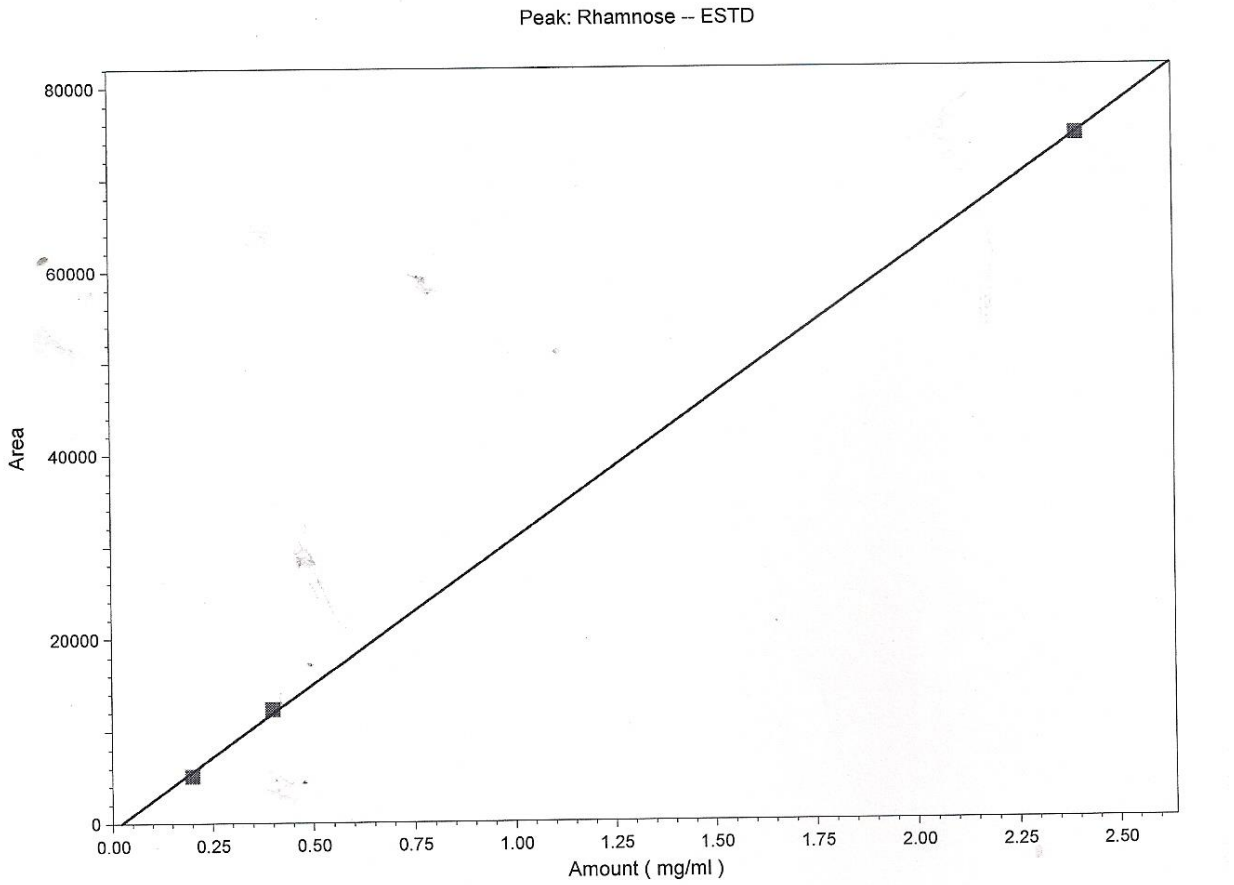
Appendix 3.23: Chromatogram (2) calibration curve for sugars



Detector A

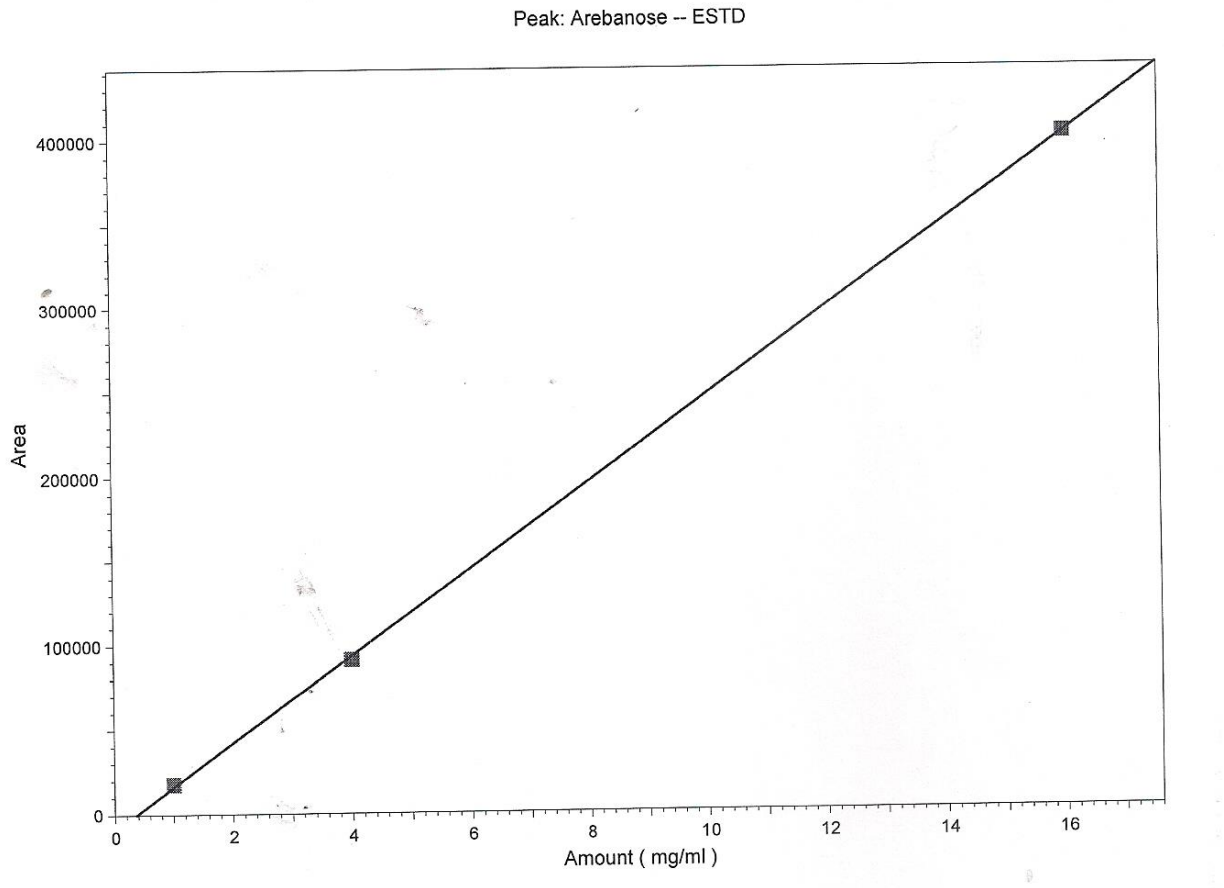
Pk #	Name	Retention Time	Area	ESTD concentration	Units
4	Rhamnose	4.800	74582	2.400 CAL	**mg/ml**
5	Arebanose	5.767	402171	16.000 CAL	**mg/ml**
6	Galactose	7.450	507518	16.000 CAL	**mg/ml**

Appendix 3.24: Chromatogram (3) calibration curve for sugars



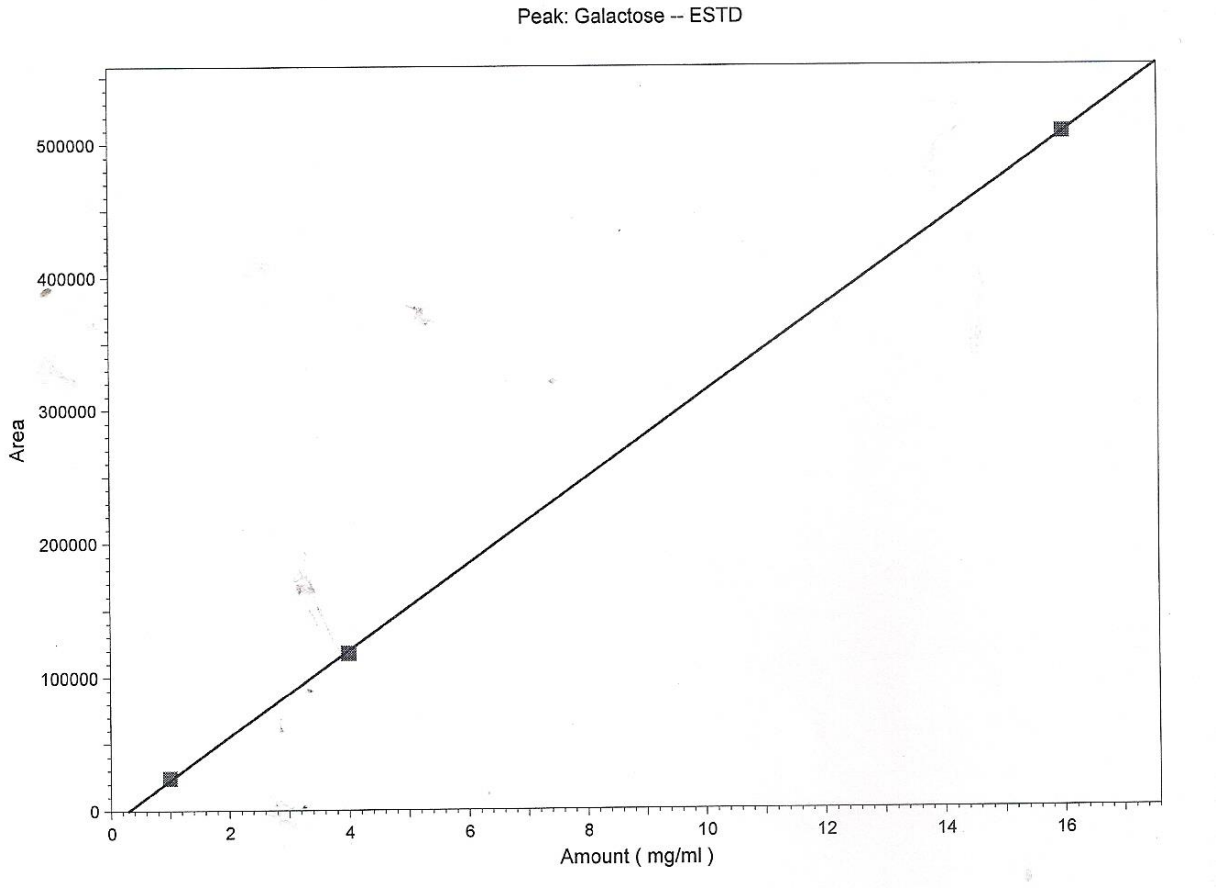
S#	Retention time	Area	Standard con-centration	Concentration unit
Standard1	4.792	5161	0.200	mg/ml
Standard2	4.800	12345	0.400	mg/ml
Standard3	4.800	74582	2.400	mg/ml

Appendix 3.25: Calibration curve for Rhamnose



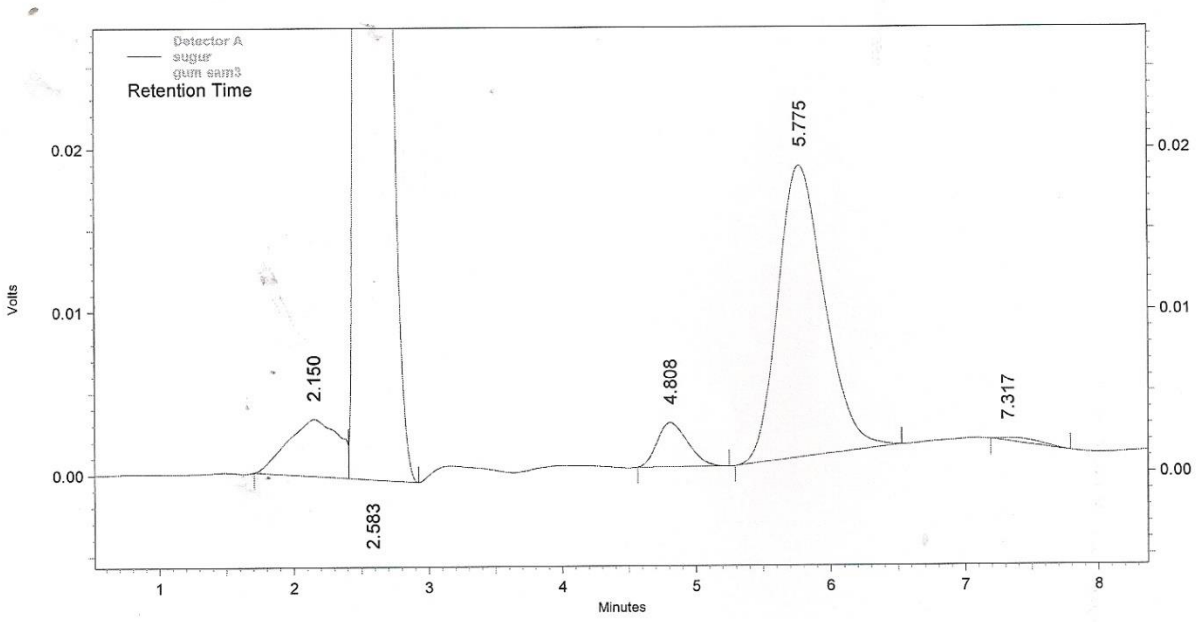
S#	Retention time	Area	Standard con-centration	Concentration unit
Standard1	5.767	17914	1.000	mg/ml
Standard2	5.775	91185	4.000	mg/ml
Standard3	5.767	402171	16.000	mg/ml

Appendix 3.26: Calibration curve for Arabinose



S#	Retention time	Area	Standard con-centration	Concentration unit
Standard1	7.433	24354	1.000	mg/ml
Standard2	7.442	117763	4.000	mg/ml
Standard3	7.450	507518	16.000	mg/ml

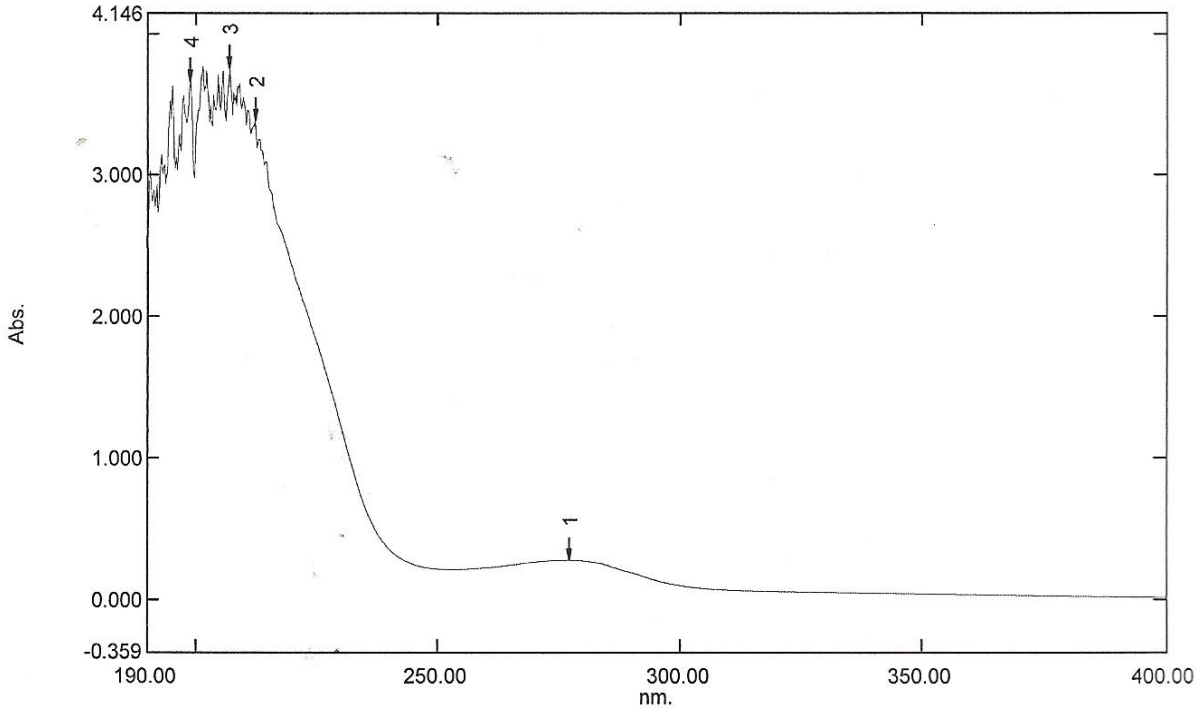
Appendix 3.27: Calibration curve for Galactose



Detector A

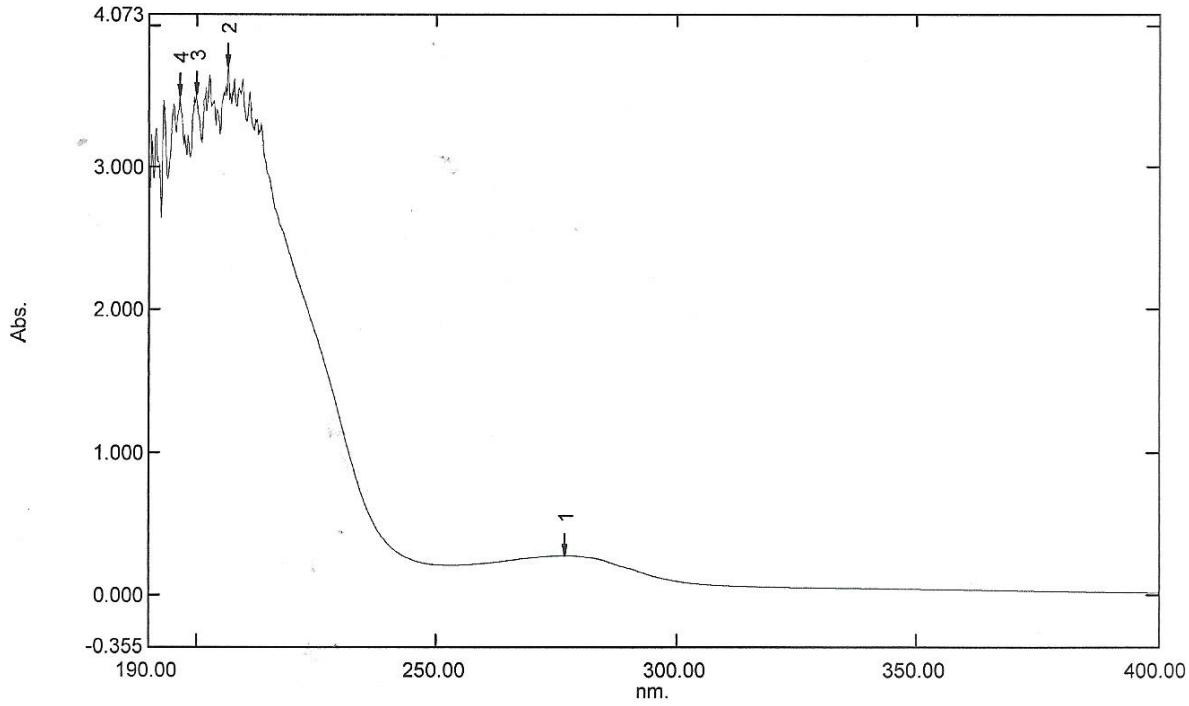
Pk #	Name	Retention Time	Area	ESTD concentration	Units
3	Rhamnose	4.808	42309	1.370	**mg/ml**
4	Arebanose	5.775	429239	17.070	**mg/ml**
5	Galactose	7.317	4806	0.442	**mg/ml**

Appendix 3.28: Chromatogram for sugar content for *Anogeissus leiocarpus* gum.



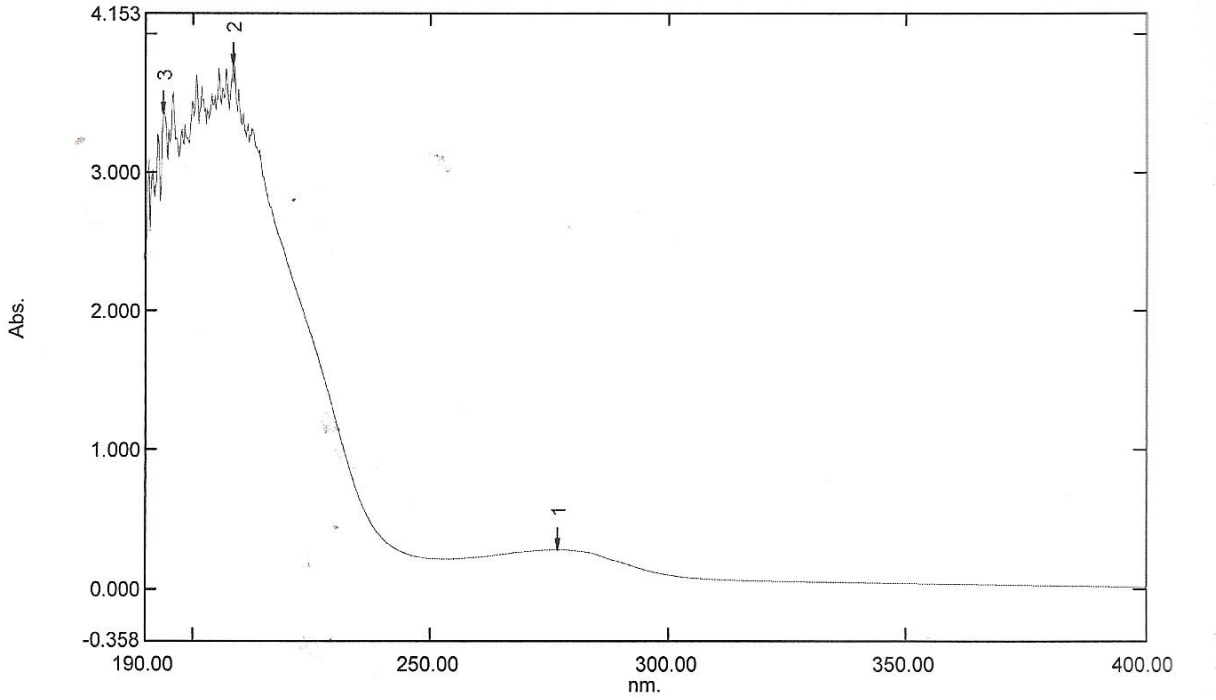
No.	P/V	Wavelength	Abs.	Description
1		277.00	0.281	
2		212.40	3.384	
3		207.20	3.761	
4		199.00	3.665	
5		253.20	0.215	
6		211.60	3.296	
7		199.80	2.979	

Appendix 3.29: The λ_{max} of *Anogeissus leiocarpus* gum solution (1).



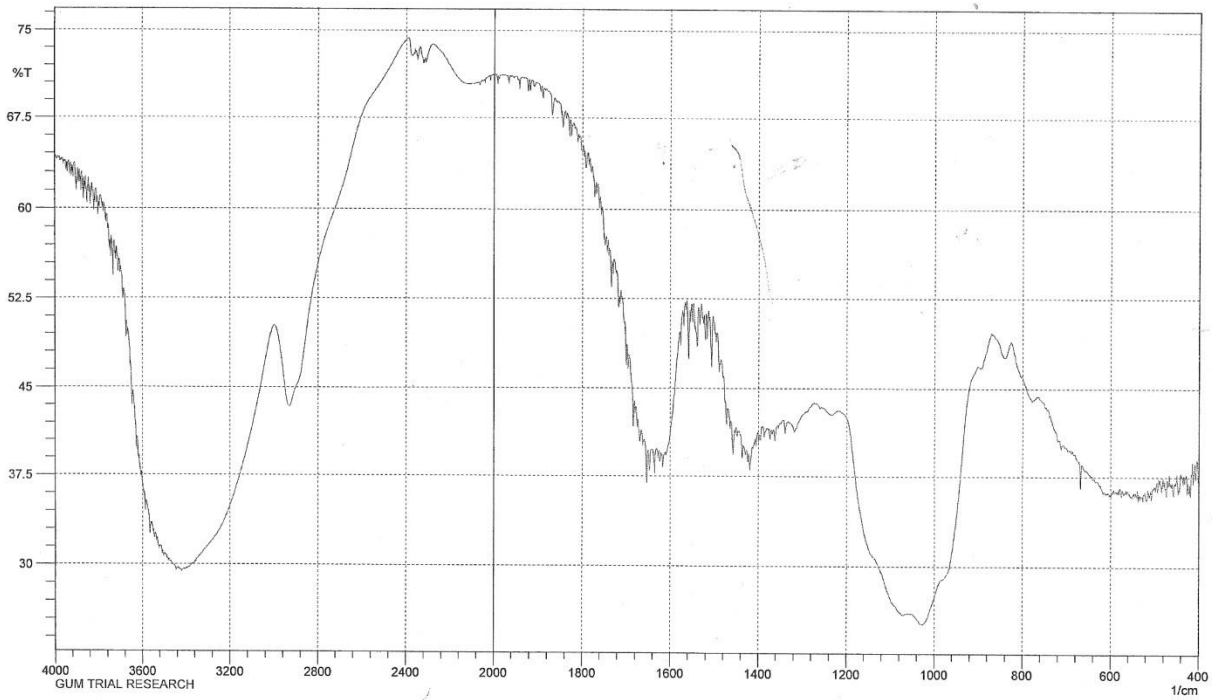
No.	P/V	Wavelength	Abs.	Description
1	↑	276.80	0.276	
2	↑	206.80	3.704	
3	↑	200.00	3.506	
4	↑	196.60	3.501	
5	↓	253.20	0.209	
6	↓	201.20	3.171	
7	↓	198.80	3.070	
8	↓	194.00	2.917	

Appendix 3.30: The λ_{max} of *Anogeissus leiocarpus* gum solution (2)

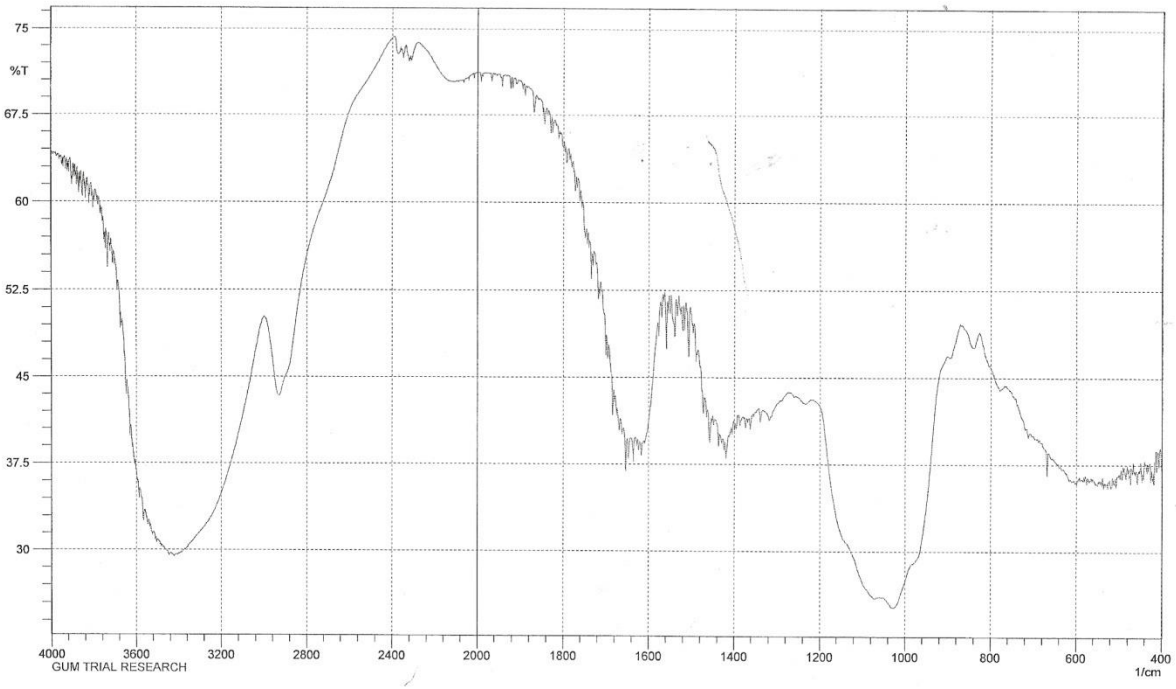


No.	P/V	Wavelength	Abs.	Description
1		276.60	0.285	
2		208.60	3.777	
3		194.00	3.438	
4		253.20	0.219	
5		194.80	3.098	

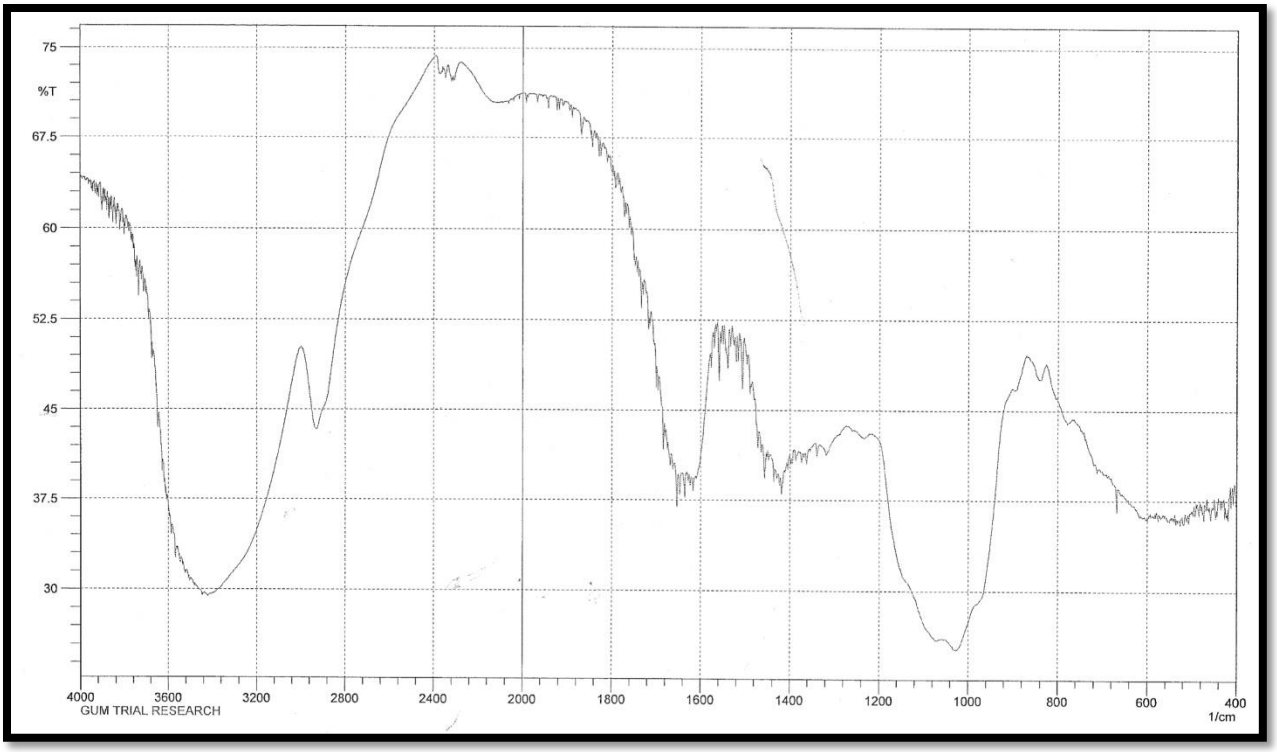
Appendix 3.31: The λ_{max} of *Anogeissus leiocarpus* gum solution (3)



Appendix 3.32: The I.R Spectra of *Anogeissus leiocarpus* gum (1)



Appendix 3.33: The I.R Spectra of *Anogeissus leiocarpus* gum (2).



Appendix 3.34: The I.R Spectra of *Anogeissus leiocarpus* gum (3).