A STUDY OF THE ELEMENTS IN *ALOE VERA* POWDER BY NEUTRON ACTIVATION ANALYSIS

CHAIDAN PACHANON

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A STUDY OF THE ELEMENTS IN *ALOE VERA* POWDER BY NEUTRON ACTIVATION ANALYSIS

C. Pachanon

Mr. Chaidan Pachanon

Candidate

M. Tuntawiroon

Assoc.Prof. Malulee Tuntawiroon

M.Sc.(Med.Phys.),M.S.(Nucl. Med)

Co-Advisor

N. Sritongkul

Assoc. Prof. Nopamon Sritongkul

M.Sc.(Biochem)

Major-Advisor

Nitaya Suparit

Mrs.Nitaya Suparit

M. Eng.(Nucl. Tech.)

Co-Advisor

W. Dharmvanij

Lect. Wanchai Dharmvanij

M.Eng.(Nucl. Tech.)

Co-Advisor

Salee Kiewkarnka

Asst. Prof. Salee Kiewkarnka, Ph.D

Acting Dean

Faculty of Graduate Studies

M. Tuntawiroon

Assoc. Prof. Malulee Tuntawiroon

M.Sc.(Med. Phys.), M.S. (Nucl. Med)

Chair

Master of Science Programme in

Radiological Science

Faculty of Medicine, Siriraj Hospital

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on

28 September, 2005

C. Pachanon

Mr. Chaidan Pachanon
Candidate

S. Khaweerat

Mrs. Sasiphan Khaweerat,
M.Eng.(Nucl. Tech.)
Member

N. Sritongkul

Assoc. Prof. Nopamon Sritongkul,
M.Sc.(Biochem)
Chair

Nitaya Suparit

Mrs. Nitaya Suparit,
M.Eng.(Nucl. Tech.)
Member

W. Dharmvanij

Lect. Wanchai Dharmvanij,
M.Eng.(Nucl. Tech.)
Member

Salee Kiewkarnka

Asst. Prof. Salee Kiewkarnka, Ph.D
Acting Dean
Faculty of Graduate Studies
Mahidol University

P. Sakolsatayadorn

Clin.Prof. Piyasakol Sakolsatayadorn,
M.D., FRCST
Dean
Faculty of Medicine, Siriraj Hospital
Mahidol University

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Chaidan Pachanon

A STUDY OF THE ELEMENTS IN *ALOE VERA* POWDER BY NEUTRON ACTIVATION ANALYSIS

CHAIDAN PACHANON 4336289 SIRS/M

M.Sc.(RADIOLOGICAL SCIENCE)

THESIS ADVISORS : NOPAMON SRITONGKUL, M.Sc.(BIOCHEM), MALULEE TUNTAWIROON, M.Sc.(MED.PHYS.), M.S.(NUCL.MED.), WANCHAI DHARMVANIJ, M.ENG.(NUCL.TECH.), NITAYA SUPARIT, M.ENG. (NUCL. TECH.)

ABSTRACT

Aloe vera is a medicinal plant that has been used for a long time in traditional medicine in several tropical countries. *Aloe vera* gel has several beneficial biological and physiological properties, such as healing skin burns, inhibitory action against some bacteria and fungi, and anti-ulcer activity. The gel is also used for manufacturing cosmetics. The objective of this study was to find out the optimum formula for preparing *Aloe vera* powder and to evaluate the elements in the *Aloe vera* powder by neutron activation analysis (NAA) and by inductively coupled plasma atomic-emission spectrometry (ICP-AES).

In this study *Aloe vera* plants were collected from 10 different areas of a herbal garden in Rayong province. The gel extraction was prepared in both freeze-dried and spray-dried forms. Five elements, Na, Ca, Mg, Mn and K were found by both methods. The NAA method showed high accuracy and precision.

The content of elements in the freeze and spray-dried samples as determined by NAA were not different statistically at 0.05 level. These results indicate that the method of extracted gel preparation has no significant effect on the measuring of elemental content.

In conclusion, NAA gives us another tool for simultaneously determining a number of elements. The method is sensitive, non destructive and suitable for evaluating composition of *Aloe vera* powder or other natural products for industrial, pharmaceutical or medicinal uses.

KEY WORDS : *ALOE VERA* (Linn.)/ NAA / SPRAY DRY/ FREEZE DRY

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การศึกษาแร่ธาตุในผงวุ้นว่านหางจระเข้โดยวิธีการอาบนิวตรอน
(A STUDY OF THE ELEMENTS IN *ALOE VERA* POWDER BY NEUTRON ACYIVATION ANALYSIS)

ชายแดน ภาชนนท์ 4336289 SIRS/M

วท.ม.(วิทยาศาสตร์รังสี)
คณะกรรมการควบคุมวิทยานิพนธ์ : นภมน ศรีตงกุล, M.Sc.(Biochem),มลุลี ตัณฑวิรุฬห์, M.Sc.(Med.Phys.), M.S.(Nucl.Med.), วันชัย ธรรมวานิช, M.Eng.(Nucl.Tech.), นิตยา ศุภฤทธิ์, M.Eng.(Nucl.Tech.)

บทคัดย่อ

ว่างหางจระเข้ เป็นพืชสมุนไพรที่ใช้กันมานานในยาแผนโบราณของประเทศในแถบโซนร้อน วุ้นจากว่านหางจระเข้มีผลทางชีววิทยาและทางสรีระวิทยา เช่นการนำมารักษาแผลไฟไหม้ มีฤทธิ์ยับยั้งเชื้อแบคทีเรีย เชื้อรา และรักษาแผลในกระเพาะอาหาร นอกจากนี้ยังนำมาใช้เพื่อผลิตเครื่องสำอาง ในงานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาหาสูตรตำรับที่เหมาะสมสำหรับการเตรียมผงว่านหางจระเข้ และเพื่อศึกษาหาปริมาณธาตุในผงว่านหางจระเข้โดยวิธีการอาบรังสีนิวตรอนและการวิเคราะห์สเปกตรัมที่เปล่งออกมาจากการใช้พลาสมาเป็นต้นกำเนิดแสง

ในการศึกษาได้ใช้ใบว่านหางจระเข้จากแปลงปลูก 10 แปลง ที่อยู่ภายในสวนสมุนไพร จังหวัดระยอง โดยการใช้สูตรตำรับที่ศึกษาได้ นำมาเตรียมเป็นผงแห้ง ทั้งแบบแช่แข็งและแบบพ่นฝอย ผลการวิเคราะห์ธาตุในผงว่านหางจระเข้ พบธาตุ 5 ชนิด คือ โซเดียม แคลเซียม แมกนีเซียม แมงกานีส และโปตัสเซียม จากการวิเคราะห์ทั้งสองวิธี และพบว่าเทคนิคการวิเคราะห์ธาตุด้วยการอาบรังสีนิวตรอนให้ค่าความถูกต้องแม่นยำสูง

การวิเคราะห์หาปริมาณธาตุในผงว่านหางจระเข้ที่ถูกทำแห้งแบบแช่แข็งและแบบพ่นฝอยไม่มีความแตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่น 0.05 ผลการวิเคราะห์จึงชี้ให้เห็นว่าวิธีการเตรียมผงแห้งไม่มีผลต่อปริมาณธาตุ

สรุปว่าเทคนิคการอาบรังสีนิวตรอนเป็นเครื่องมือที่สามารถวิเคราะห์ธาตุได้หลายชนิดพร้อมกัน มีความไวของการวิเคราะห์ ไม่ทำลายสารตัวอย่าง ดังนั้นจึงสามารถที่จะนำมาวิเคราะห์ผงว่านหางจระเข้หรือผลิตภัณฑ์สมุนไพรอื่นๆที่จะใช้ในอุตสาหกรรม เภสัชวิทยา ตลอดจนทางการแพทย์ได้

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LIST OF ABBREVIATIONS

ABBREVIATIONS	Term
NAA	Neutron activation analysis
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ϕ	Neutron flux
N*(t)	The number of radionuclides of any time, t
$t_{1/2}$	Half life of the radionuclide
N_o	Avogadro′s number
w	Weight of element
σ	Cross section
HPGe	Hyperpure (or intrinsic) germanium detector
t_i	Irradiation time
θ	Isotopic abundance
NIST	The Nation Institute of Standard and Technology
SRM	Standard reference material
ND	Not detected
ppm	Part per million
t_d	Decay time
λ	Decay constant
SD.	Standard of deviation

CHAPTER I
INTRODUCTION

Aloe vera (Linn.) is a tropical plant, its′leaves contain a particular tissue that can retain big quantities of water. It has a short-stemmed succulent, large and long leaves with sharp spine around the rim. When it was young, it had a spreadly white spot and disappeared when it grew up. Flowers are in the middle between the leaves. There are two major products derived from the leaves. One is the yellow, bitter juice present in specialized cells beneath the thick epidermis which is called drug aloe, contain high concentrations of an anthraquinones. Another part, *Aloe vera* gel or the mucilage is found in the center (the pulp) of the leaves and contain glucomannans or Aloctin A which is a polysaccharides compound. The other constituents are protein, lipid, vitamins and minerals. Because of the varieties of components in the gel, it has been used in medicine, cosmetic and food. In medicine, *Aloe vera* gel has been recognized as a good medicine for the treatment of thermal burn and antiinflammatory. In cosmetic, it is used as an active ingredient for nourishing the skin, UV-ray protection and treatment of hair. In food, it is used as an health food ingredients in the manufacturing of beverage, candies, yogurt and several other. Fresh gel cannot be kept for a longtime even at low temperature and all active ingredients are easily destroyed. Freeze dry and spray dry methods are the two processes which have been provided to prolong the effective life of the *Aloe vera* gel.[1,2,3] Because of the popularity of *Aloe vera* products has dramatically increased, a need has risen to provide a good quality and stability of *Aloe vera* powder for industry. The one of the purpose in this study was to find out the optimum formula for preparing the *Aloe vera* powder by freeze dry and spray dry method to get more yield and longer stability.

Since mineral constituents of *Aloe vera* in Thailand have not been evaluated whereas A. barbadensis juice have been examined by neutron activation analysis.[4] The Inductively Coupled Plasma Atomic Emission Spectrometry have been used for multielement determination of major-to-ultratrace elements in plant reference

materials.[5] Thus another purpose of this study is to examine the element contents in the *Aloe vera* powder cultivated in Thailand by NAA and ICP-AES. The results of this study may be useful not only for our local industry in ensuring the method of producing *Aloe vera* powder instead of importing from other countries but also to have a data on element contents of *Aloe vera* in Thailand.

CHAPTER II
LITERATURE REVIEW

1 *Aloe vera* (Linn.)

1.1 Nomenclature

A. vera has been known under various names ;

Scientific names: *Aloe vera* (L.) Burm.f.
Aloe barbadensis Miller
A. perfoliata var. vera L.
A. vera Tournefort ex Linne
A. vulgaris Lamarck

Common names: Barbados aloe
Curacao aloe
Medicinal aloe
Unguentine aloe

Local name: *Aloe vera*

At present, the official name is *Aloe vera* (L.) Burm.f. or *Aloe barbadensis* Mill..

1.2 Botanical Description

Aloe barbadensis Miller or *Aloe vera* (Linn.) Burm. f. is a tropical plant of the genus Aloe and a subspecies of the Liliaceae family. It is a short-stemmed succulent, herb. The succulent leaves are crowded on the top of their stems, spreading, grayish green and glaucous; spotted when young, 20-50 cm. long, 3-5 cm. wide at the base, tapering gradually to the point tip, 1-2.5 cm. thicked; having edges spiny, and bitter latex inside. Flowers are on the upper part of a slender stalk, 50-100 cm. height. Each flower is composed of six-petaled yellow color, about 2-5 cm. long, with 6 protruding stamens and one 3-celled ovary in long style. Sizes of leaves and colors of flowers are vary according to the species.

The epidermis of the leaves has a thick cuticle and beneath is a zone of chlorenchyma. The central bulk of the leaf contains the colorless mucilaginous pulp,

consisting of large thin-walled mesophyll cells containing the aloe gel. Along the junction between the pulp and the chlorenchyma, the numerous vascular bundles are found with accompanying inner bundle cell. The bundle sheath cells at the phloem poles are thin-walled and axially elongated, and contain the bitter yellow sap which exudes from the leaves when they are cut. [6]

1.3 Properties of *Aloe vera* (Linn.) Constituents [7]

There is a wide range of research from all over the world based upon different species of Aloe. Table 2.1 shows the constituents found in *Aloe Barbadensis* Miller (*Aloe vera*) in which listed in an alphabetical order along with their properties and activities. All constituents are derived from the leaf which consists of three primary sections: the rind (photosynthesis) with sap contained in the pericyclic transport tubules (xylem and phloem), the mucilage (container) layer and the parenchyma or gel fillet (storage) layer. The relative quantities of the aloe constituents is quite small (the total solid fraction varies from 0.5 to 1.5%), but most researchers claim that the effects that are observed may be due to the synergistic actions of the 75 (total about 200 different ingredients) known ingredients, including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid.

Table 2.1 Properties of *Aloe vera* (Linn.) constituents

Constituents	Number & Identification	Properties & Activity	Comment
Amino Acids	Provides 20 of the 22 human required amino acids & 7 of the 8 essential ones	Provides the basic building blocks of proteins in the production of muscle tissue etc	The 8 essential amino acids are those the human body cannot manufacture
Anthraquinones	Provides 12 anthraquinones: Aloe emodin, Aloetic Acid, Aloin, Anthracine, Antranol, Barbaloin, Chrysophanic Acid, Emodin, Ethereal Oil, Ester of Cinnamonic Acid, Isobarbaloin, Resistannol.	In relatively small concentrations together with the Gel fraction they provide Analgesic, Antibacterial, Antifungal & Antiviral activity. In high concentration on their own they can be toxic.	Traditionally known as laxatives.The anthraquinones are found in the sap. The anthraquinone derivatives (anthrones & chromones) comprise the phenolic fraction of the sap. The primary sap component is Aloin/Barbaloin anthrone derivative
Enzymes	Provides 8 enzymes: Aliiase, Alkaline Phosphatase, Amylase, Carboxypeptidase, Catalase, Cellulase, Lipase, Peroxidase	Helps breakdown of food sugars and fats aiding digestion & enhancing nutrient absorption	
Hormones	Auxins & Gibberellins	Wound Healing & Anti-inflammatory	
Lignin	Cellulose based substance	Thought to provide penetrating power in *Aloe vera* skin preparations and may act as a carrier for other components	
Minerals	Provides 9 minerals: Calcium, Chromium, Copper, Iron, Magnesium, Manganese, Potassium, Sodium, Zinc	Essential for good health and is known to work in certain combimation with each other, vitamins and other trace elements	
Salicylic Acid	Aspirin like compound	Analgesic	
Saponins	Glycosides	Soapy substance both cleansing and antiseptic	
Sterols	Provides 4 main plant steroids: Cholesterol, Campesterol, Lupeol, ß Sitosterol	Anti-inflammatory agents. Lupeol also possesses antiseptic and analgesic properties	
Sugars	Monosaccharides: glucose & fructose Polysaccarides: gluco-mannans / polymannose	Anti-inflammatory action Anti-viral, immune modulating activity of Acemannan	The long chain gluco-mannans are absorbed intact by the pinocytotic process of certain cells lining the digestive tract.
Vitamins	A, C, E, B, Choline, B12, Folic Acid	Antioxidant(A,C,E): neutralises free radicals	B's & Choline involved in amino acid metabolism, B12 required for production of red blood cells, Folic Acid in the development of blood cells

The water content of homogenized aloe vera gel was determined by conventional Cryodesiccation techniques. In raw leaf mucilage,it is about 99.5 percent water and has a pH of 4-5. The remaining solid materials are described in more detail below.

1.3.1 Vitamins

An important antioxidant, vitamins A, C and E, are found in the aloe vera constituents. Vitamins B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_5 (niacin or nicotinic acid), choline and folic acid are also present. Some authorities suggest that there is also a trace of vitamin B_{12}.

1.3.2 Enzymes

At least six enzymes have been claimed to be present: cellulase, carboxy-peptidase, bredykinase, catalase, anylase and an oxidase.When taken orally, several of these biochemical catalysts, such as amylase and lipase, can aid digestion by breaking down fats and sugars. One important enzyme, a carboxypeptidase, inactivates bradykinins and produces an antiinflammatory effect.[8-9] During the inflammatory process, bradykinin produces pain associated with vasodilation and, therefore, its hydrolysis reduces these two components and produces an analgesic effect. [10]

1.3.3 Minerals

Sodium, potassium, calcium, magnesium, manganese, copper, zinc, chromium and iron are all found in the aloe plant. Magnesium lactate inhibits histidine decarboxylase and prevents the formation of histamine from the amino acid.[9,11]Histamine is released in many allergic reactions and causes intense itching and pain. The prevention of its formation may explain the antipuritic effect of *Aloe vera*.

1.3.4 Sugars

Sugars are derived from the mucilage layer of the plant under the rind, surrounding the inner parenchyma or gel. They are approximately 25 percent of the solid fraction and comprise both mono- and polysaccharides. Two monosaccharides, D-glucose and D-mannose, were identified by Thin Layer Chromatography (TLC) and Gas Liquid Chromatography(GLC.) Trace amounts of xylose, rhamnose, galactose and

either arabinose or fucose were also found. The most important are the long chain polysaccharides, which one linear polymers contain 1-4 glycosidic linkage of glucose and mannose, known as the gluco-mannans (Beta - (1, 4) - linked acetylated mannan). When taken orally, some of these bind to receptor sites that line the gut and form a barrier, possibly helping to prevent 'leaky gut syndrome'. Others are ingested whole by a method of cellular absorption known as pinocytosis. Unlike other sugars which are broken down prior to absorption, these polysaccharides are absorbed complete and appear in the blood stream unchanged. Here, they act as immuno-modulators capable of enhancing and retarding the immune response. (12-15)

1.3.5 Anthraquinones

These phenolic compounds are found in the sap. They consist of free anthraquinones and their derivatives:

- Barbaloin
- Isobarbaloin
- Anthrone-C-glycosides and chromones.

These compounds in large amounts exert a powerful purgative effect, but when smaller they appear to aid absorption from the gut, they are not only potent antimicrobial agents but also possess powerful analgesic effects. Topically, they can absorb ultraviolet light, inhibit tyrosinase activity, reduce the formation of melanin and any tendency to hyper-pigmentation. (16-17)

1.3.6 Lignin

This woody substance, inert in itself, endows topical aloe preparations with their singular penetrative ability to carry other active ingredients deep into the skin to nourish the dermis.

1.3.7 Saponins

These soapy substances form 3 per cent of the gel and are general cleansers, having antiseptic properties.(18)

1.3.8 Salicylic acid

This is an aspirin-like compound possessing antiinflammatory and antibacterial properties. Topically, it has a kerolytic effect which helps to debride a wound of necrotic tissue.[19-20]

1.3.9 Amino acids

These are the building blocks of proteins. *Aloe vera* gel provides 20 of the 22 necessary amino acids required by the human body and seven of the eight essential amino acids which the body cannot synthesize and must be ingested in food.

1.4 Medicinal components of the aloe leaf

The *Aloe vera* leaf contains two different parts for medicinal use, the gel and the latex.

1.4.1 The *Aloe vera* gel

Aloe gel is defined by the third edition of CTFA Ingredient Dictionary as "the mucilage obtained as the juice expressed from the leaves of *Aloe vera*". This transparent slippery mucilage is produced by the thin-walled tubular cells found in the inner central zone (parenchyma) of the leaf. Hair-like connective matrices may be observed in the raw gel which are somewhat reminiscent of colorless gelatin in appearance.[21-22] This gelatinous substance has an original characteristic: an anti-inflammatory effect that soothes and calms tissues, bringing about reductions in pain and damage.

Fresh *Aloe vera* gel is unstable. The mucilagenous (gel like) properties of the fresh parenchyma "pulp" are slowly lost after exposure to air and light with subsequent discoloration which progressed from pinkish to purplish and finally to brown. Anthraquinones has been suggested as the cause of discoloration.[23] But normally the gel does not contain anthraquinones or anthroglycoside. It may be found in trace amount by contamination. Enzymatic degradation and micro-biological decomposition are probable causative of the instability of the fresh *Aloe.* Hence, after the leaves are cut, they should be stored in a darkly chilled place to maintain their aerobic respiration to allow the continuation of the carbohydrate and the organic acid

metabolism inside the leaf and also the continuation of forming the protective coating which hinders microbial attack.[21]

The raw unpreserved gel contains approximately 0.5% solids and 99.5% water with pH of 4 to 5. The solids are composed mainly of carbohydrates and proteins.[21] The carbohydrate constituents can be categorized as monosaccharides (e.g.glucose, mannose, arabinose, galactose and xylose), polysaccharides (e.g. glucomannan, cellulose, hemicellulose, dextrin, starch and pentose) and glycosides which are sugar that are condensed with non sugar material. Wound healing is one of the specific effects of the aloe gel and scientific research asserts that mannose increases the macrophage activity and help healing.

A large number of amino acids, pro-vitamins, vitamins, triterpenoid, steroids and enzymes are present in *Aloe vera* gel.[24]

At least four different partially acetylated glucomannans are thought to be responsible for producing the thick, stringy mucilage-like properties that are characteristic of the raw *Aloe vera* gel. When these glucomannans are hydrolyzed, viscosity of the gel is reduced.[25]

About nine minerals are found in the *Aloe vera* gel. They are quantitatively detected (ppm); Al (below 5), B (52), Ca (35400), Fe (4), Mg (7200), Mn (67), Na (1720), P (3210) and Sr (86). It is showed that *Aloe vera* belongs to a group of calcium-rich vegetables, which contain a little more magnesium, phosphorus and sodium.[26]

Fatty acids such as lauric acid, myristic acid, pentadecanoic acid, palmitoleic acid, palmitic acid, stearic acid, arachidonic acid and also dehydroabietic acid derivatives are identified in the acetone extract of the *Aloe vera* gel.[26]

The *Aloe vera* gel also contains glycoprotein, Aloctin A and Aloctin B.[27-29] Aloctin A and Aloctin B are peptides having molecular weight of 18000 and 24000, respectively. Aloctin A composes of two peptide subunits of 10500 and 7500. Aloctin B composes of two subunits each of 12000. Aloctin A has been reported to have several bioactivities including immunomodulation[14,30], mitogenic for lymphocyte and binding human alpha 2 macroglobulin[31], antiinflammatory activity, antitumor activity, antiulcer, and inhibition of gastric secretion and gastric lesion.

1.4.2 The *Aloe vera* latex

The *Aloe vera* latex (Aloe sap or Aloe leaf exudate) is the bitter yellow liquid contained in the pericyclic cells of the rind, a principal constituent is aloin.

The compounds found in the *Aloe vera* latex can be divided into 3 major groups which are the anthracene derivatives, phenolic compounds (which comprise chromone derivatives of 6-phenylpyran –2-one) and derivatives of 2-naphthoic acid.[32]

The anthracene derivatives can be divided into anthraquinones, anthranols and anthrones. A number of anthraquinones have been recognized both in the free state and as glycosides. Free anthraquinones seem to be present at low levels more prominent are in the bound forms. It was estimated that the total aloe-emodin released from leaf eluates only about 10-14% was present as the free anthraquinones and the glycosylated anthraquinones structure with C-glycoside bonds have been found much more than those with O-glycosidic bonds. Barbaloin is the main anthraquinone-derived constituent of commercial bitter aloes and its structure has been shown to be that of a C-glycoside of aloe-emodin anthrone.[32] It has been used as active ingredients in laxative preparations.

1.5 Pharmacological activities of *Aloe vera* (Linn.)

The *Aloe vera* possesses a variety of biological effects. It is difficult to ascertain when Aloe started to be used therapeutically. It has been important in China for several centuries and still a household remedy today. Several diseases are effectively treated with Aloe today. The pharmacological activities of *Aloe vera* gel and latex can be described as follow.

1.5.1 Healing effect

Aloe vera has been recognized as a good medicine for the treatment of thermal and radiation burn.[33] There are many factors in the gel involves in the healing activity. The watery composition of aloe has been shown to increase the migration of epithelial cell for the improvement of wound healing.[34] The healing effect with tissue regeneration may be by dilating capillaries to increase blood flow to injured area[35]

providing the more oxygen available for collagen formation by fibroblasts.[36] Natural substances contained in *Aloe vera* such as enzymes, amino acids, vitamin C, vitamin E and Zinc are the important ingredients necessary for wound healing.[37] Acemannan, an acetylated β-(1,4)-linked mannan derived from *Aloe vera* gel, is believed to exert its effects on the wound healing by two mechanism.[38] First, it is a potent macrophage-activation agent and may therefore stimulate the release of fibrogenic cytokines. Alternatively, growth factors may bind directly to acemannan, promoting their stability and possibly prolonging their stimulating effects on granulation tissue formation.

Aloe has also been shown to inhibit thromboxane A_2 (a potent vasoconstrictor) synthesis at the injury tissue and maintain a homeostasis within the vascular endothelium as well as the surrounding tissue.[39-41]

1.5.2 Antiinflammatory effect

Aloe extract and *Aloe gel* exhibit anti-inflammatory activity.[42] The active principles include aloctin A and bradykinase.[43] Aloctin A has been demonstrated the anti-inflammatory effect by inhibiting the synthesis of prostaglandin. *Aloe gel* also contains bradykinase[44], the enzyme needed for breaking down bradykinin, an inflammatory agent causing vasodilatation, inflammation and pain. Since this enzyme is carboxypeptidase[45], it can convert the angiotensin I to angiotensin II, the vasopressor. Therefore, the decreasing of bradykinin and the increasing of angiotensin II can inhibit the inflammation.

Recently, a new anti-inflammatory agent from aloe extract has been identified as a C-glucosyl chromone.[46] The anti-inflammatory was assessed by the measurement of ear swelling induced by castor oil. The C-glucosly chromone at a dose of 200 μg/mouse ear exhibits topical anti-inflammatory activity equivalent to 200 μg/mouse ear hydrocortisone.[47]

1.5.3 Antiulcer activity

It is known that the gel of *Aloe vera* shows anti-ulcer activity. The active principle has been clarified as Aloctin A.[29] Which plays a role in the inhibition of gastric secretion and gastric lesion.

1.5.4 Hypoglycemic activity

The hypoglycemic activity of aloe was first discovered in 1958. The active principles have been reported to be polysaccharides and anthraquinones. The hypoglycemic effect has been believed to be caused by the stimulation of insulin secretion from residual pancreatic islets beta cells (β-cells), but the exact mechanism is still unknown.[48]

In streptozotocin-induced diabetic rats, the single dose of fresh or preserved aloe gel exhibits no antidiabetic effect but daily feeding of fresh or preserved aloe gel shows a significant antihyperglycemic effect.[49] The antihyperglycemic effect starts at the second week of treatment. The proposed mechanism is the acceleration of blood glucose clearance.

The antidiabetic activity in patients has been reported. Oral administration of *Aloe vera* juice significantly decreases blood sugar levels in new cases of diabetes mellitus.[50] Furthermore, the combination of *Aloe vera* juice and glybenclamide has been studied in new cases of diabetes mellitus whom glybenclamide alone had no effect on blood sugar level. The combination of *Aloe vera* juice and glybenclamide exhibits antihyperglycemic activity.[51]

1.5.5 Antibacterial activity

The antibacterial activity of aloe is due to the latex containing anthraquinones.[52-53] *Aloe vera* gel has been shown to exhibit antibacterial activity only at a concentration of 80 %.[54]

1.5.6 Antifungal activity

Antifungal activity of aloe is due to anthraquinones fraction.[54-56] It has been demonstrated to inhibit the growth of *Trichophyton mentagophytes* and *Aspergillus niger.* [52-54]

1.5.7 Antiviral activity

The extract of *Aloe barbadensis* Mill. has the virucidal effect against herpes simplex virus type 1.[57] The active components are identified as anthraquinones. Aloe emodin has been found to inactivate herpes simplex virus type 1 and type 2, Varicella-Zoster virus, pseudorabies virus and influenza virus by partially disruption of the virus envelopes.[57]

1.5.8 Analgesic activity

Inhibition of prostaglandin synthesis is attributed to analgesic activity of *Aloe vera* (Linn.) Burm. f.. The pain relieving activity of aloe was demonstrated by many experiments including aloe gel that has been used to apply for treatment of jellyfish poison.[58]

1.5.9 Antihistaminic activity

The antihistaminic compounds of aloe are aloe ulcin and aloin, which isolated from aloe latex. They have an inhibitory effect on aromatic amino acid decarboxylase and histidine decarboxylase, which are the histamine synthesizing enzyme.[59]

1.5.10 Cathartic activity

Cathartic action of aloe has been recognized for centuries with aloin, barbaloin, anthraquinone C-glycoside which are the main ingredients in aloe latex.[60] Aloin has been demonstrated to increase peristaltic contraction of intestine.[61] During passage through the intestine, progressive hydrolysis of anthraquinone glycoside has occurred. The greatest absorption of the hydrolyzed products occurs in the large intestine and they are excreted fairly rapidly by kidneys.[60] The laxative activity of aloe is primarily produced by free anthraquinones. The glycosides become effective only after cleavage to a glycones by contact with the mucosa as well as by absorption and reexcretion in the colon.[59] It is found that the activity requires activation by intestinal flora. Besides stimulation of the large intestinal movement, the carthatic

activity may be due to increasing the water content of large intestine.[62] It is gently laxative if taken in small quantity, overdosage causes abdominal pain, bloody diarrhea, hemorrhagic gastritis and sometimes nephritis.

1.5.11 Miscellaneous

The *Aloe vera* plant has other biological activities such as immune modulating activity, cardiovascular effect, allergic, mutagenic and carcinogenic activity. [63-64] The role of stimulating the immune system makes *Aloe vera* a valid detoxifier. Today several diseases are effectively treated with *Aloe vera*: dermatitis, peptic and duodenal ulcers, burns and herpes goster inflammations. Nowadays *Aloe vera* has not only available commercially in cosmetic formulations but also in health food production.

2. Drying Technology

The aim of drying technology is to improve shelf life of material by making them to be an dehydrated product. There are four technology of drying methods to cover a different style of dried product. That are hot air dry, spray dry, freeze dry and drum dry. The different of each drying is the change of physical water state. The hot air dry is in the state of evaporation but the freeze dry is in the state of sublimation. The spray dry is most rapid drying for liquid to be power within second. The drum dry use a hot drum surface to dry a thin film product in a minute.

In this study, the technology of spray dry and freeze dry are used for the preparation of *Aloe vera* powder.

2.1 Spray Dry[66-67]

Spray dry is the process involving particle formation and drying. The production of particle or dry solid is from liquid feed stocks as emulsion or suspension. The method can be divided into 4 steps.

2.1.1 Atomization

The atomization of a liquid feedstock into a spray of droplets are produced by either rotary or nozzle atomizers. The particle size of powder can be operated by adjust the atomizer speed or change of nozzle.

2.1.2 Airflow

The process air is heated indirectly by electrical heater or steam prior to entering the drying chamber. The intensive contact between the hot process air and the mist liquid droplet ensure fast and gentle drying. There are three modes of concept:

1. Co-current

Drying air and particles move through the drying chamber in the same direction. Product temperatures on discharge from the dryer are lower than the exhaust air temperature, and hence this is an ideal mode for drying heat sensitive products. When operating with rotary atomizer, the air disperse creates a high degree of air rotation, giving uniform temperatures though out the drying chamber. However, an

alternative non-rotating airflow is often used in tower or filter mat-type spray dryers using nozzle atomizers with equal success.

2. Counter-current

Drying air and particles move through the drying chamber in opposite directions. This mode is suitable for products which require a degree of heat treatment during drying. The temperature of the powder leaving the dryer is usually higher than the exhaust air temperature.

3. Mixed flow

Particle movement through the drying chamber experiences both co-current and counter-current phases. The mode is suitable products where coarse powder requirements necessitate the use of nozzle atomizers, spraying upwards into an incoming airflow, or for heat sensitive products where the atomizer sprays droplets downward towards an integrated fluid bed. The air inlet and outlet are located at the drying chamber.

2.1.3 Evaporation

Evaporation of moisture from the droplets and formation of dry particles proceed under controlled temperature and airflow conditions.

2.1.4 Collection

Powder is discharged from the drying chamber. It will come into the separation cyclone and let separate the product powder into a container.

The essential elements of the spray dryer: atomizer, air disperse, drying chamber, inlet and exhaust air handing are combined into a system that meets individual operational safety, environmental protection, and powder handing requirements.

Spray dry is the most widely used in industrial process because the operating condition and dryer design can be selected according to the characteristic of the product and powder specification.

Apart from its uniform powder size product, the less nutrition loss because of a short time contact to the hot air and easily dissolve in water are the advantage of spray drying method

2.2 Freeze Dry [68-70]

Freeze dry or lyophilization is the method of drying a frozen product. It is the best preserved its essential quality, retain fresh flavor and nutrition value. The freeze dry process is performed by rapidly cooling the moist samples to -40 °C and dry under vacuum to remove water by a combination of sublimation and desorption, yielding a finished product that is stable and provides an acceptable activity. Final product is a dry product that has a long shelf life and that can be disolved again easily.

The process can be divided into 3 steps

1. **Freezing**

This step is the removal of heat from the original product.

2. **Primary drying (Sublimation)**

It is the removal of water vapor in the product by pumping off the condensable water vapor and the non-condensable passes.

3. **Secondary drying (Desorption)**

It is the removal of adsorbed water from the inner surfaces by overcoming the capillary strength. This process is not only adjusting a strong pressure gradient between the dry product and its environment but also adjusting the shelf temperature in accordance with the temperature resistance of the dry product.

Low temperature condenser, high vacuum and controlled heat to product are the three important equipment for freeze drying method. This method has an advantage that the dry product can be storage in a room temperature without deterioration because two primary cause; moisture and oxygen are removal by freeze dry processing steps.

3. Neutron Activation Analysis (NAA)[71-73]

Neutron activation analysis is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analysis of major, minor and trace elements in sample. The basic principle of NAA is that a stable isotope, when irradiated by neutrons, can undergo a nuclear transformation to produce a radioactive nuclide. After the irradiation, the characteristic gamma rays from radionuclides are measured by suitable radiation detector, the specific radionuclide will be identified from the gamma-ray energies and the concentration from their intensity. The applications for NAA are practically limitless for solid, liquid or gaseous samples, e.g. environmental, semiconductor materials and pharmaceutical materials analysis etc..

3.1 Nuclear reactions[73-74]

Stable isotopes can undergo a variety of nuclear transformations. A reaction used extensively in activation analysis is the captured reaction or (n,γ). The common notation of this type of reaction is ${}^{A}_{Z}X(n,\gamma){}^{A+1}_{Z}X$, e.g ${}^{75}_{33}As(n,\gamma){}^{76}_{33}As$.

The (n,γ) reaction is primarily a thermal-neutron reaction, although it can occur with neutrons of other energies. The neutron is captured by a target atom, and one or more gamma rays are emitted immediately. Since no change of the atomic number occurs, the radioelement retains the chemical identity of the target material.

Although the (n,γ) reaction is the most widely applied nuclear reaction for activation analysis, a number of other reactions are important. In the (n, p) reaction, which requires high energies, a neutron enters a target atom with sufficient energy to cause a proton to be released. The common notation of this type of radiation is ${}^{A}_{Z}X(n,p){}^{A}_{Z\text{-}1}Y$, e.g ${}^{32}_{16}S(n,p){}^{32}_{15}P$.

The (n,α) reaction , like the (n,p) reaction, usually requires high-energy neutrons. In the (n, α) process, a neutron enters a target atom and causes an α particle to be emitted. The common notation of this type of radiation is ${}^{A}_{Z}X(n,\alpha){}^{A\text{-}4}_{Z\text{-}2}Y$, e.g ${}^{27}_{13}Al(n,\alpha){}^{24}_{11}Na$.

3.2 Nuclear reactor[75]

A nuclear reactor is a device in which nuclear fission reaction are initiated, controlled, and sustained at a steady rate to produce neutron. Most of the reactors used for neutron activation analysis are based on the fission of ^{235}U, where the reactor core is made up of fuel elements of uranium enriched in ^{235}U. ^{235}U will only undergo fission with thermal neutrons and so the core is surrounded with a moderator to slow the neutrons down. The moderator is usually in the form of light water, heavy water, graphite or beryllium. Most reactors contain within their cores sizable magnitudes of three fairly distinct neutron-flux components (1) fast-neutron component, (2) epithermal or resonance neutron component, and (3) thermal-neutron component.

The typical neutron energy spectrum of most nuclear reactors are shown in Fig. 2.1. The curve indicates qualitatively the neutron-flux energy distribution and not the precise values of neutron flux. In any particular case, the energy distribution of one or more of the flux components may be altered slightly due to effects of moderation, neutron escape, neutron resonance capture, etc.

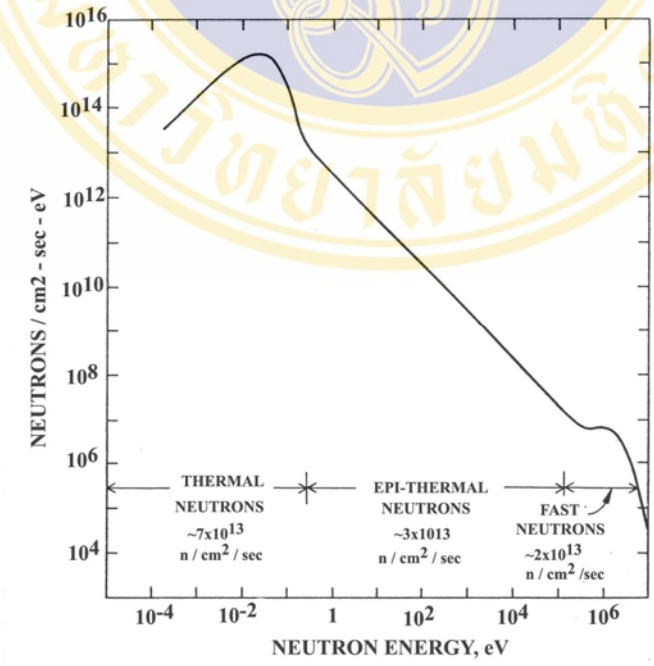

Fig. 2.1 General features of a reactor neutron spectrum

3.3 The Theory of Neutron Activation Analysis[73,76]

When neutron interact with the target nucleus the rate of formation of any radionuclides (P) can be determined from the thermal neutron flux (ϕ), the neutron capture cross section of the target nuclei (σ) and numbers of target nuclide (N) as illustrated in Eq. 2.1.

$$P = N\sigma\phi \tag{2.1}$$

If the activation product is radioactive and decays with its characteristic half-life. The rate of change of radionuclide is

$$\frac{dN^*(t)}{dt} = \sigma\phi N - \lambda N^*(t) \tag{2.2}$$

where $N^*(t)$ = the number of radionuclides at anytime, t.

λ = the decay constant = $\frac{0.693}{t_{1/2}}$

$t_{1/2}$ = half life of the radionuclides

For irradiation time (t_i), the solution of Eq. 2.2 is given by

$$N^*(t_i) = \frac{P}{\lambda}(1 - e^{-\lambda t_i}) + N^*(0)e^{-\lambda t_i}$$

Since at $t_i = 0$, $N^*(0) = 0$ hence

$$N^*(t_i) = \frac{P}{\lambda}(1 - e^{-\lambda t_i})$$

and the activity at the end of irradiation is

$$A(t_i) = \lambda N^*(t) = N\sigma\phi(1 - e^{-\lambda t_i}) \tag{2.3}$$

After irradiation, the activity at any decay time (t_d) can be determined from

$$A(t_d) = N\sigma\phi(1 - e^{-\lambda t_i})e^{-\lambda t_d} \quad (2.4)$$

Eq. 2.4 may also be expressed in terms of the count rate (R(t)) and weight of element, since

$$R(t) = A(t)\varepsilon \quad (2.5)$$

where ε = Absolute photopeak efficiency

and the number of target nuclides (N) in the sample can be calculated from

$$N = \frac{N_0 w\theta}{M} \quad (2.6)$$

where N_0 = Avogadro's number (6.022×10^{23} atoms/gram-atom)

w = The weight of element present in the sample

θ = The isotopic abundance of target

M = The atomic weight of the element

Thus, Eq. (2.4) can be written as

$$R(t_d) = \frac{N_0 w\theta}{M}\sigma\phi(1 - e^{-\lambda t_i})e^{-\lambda t_d} \quad (2.7)$$

3.4 Qualitative analysis[76]

Qualitative analysis is based on the fact that most radionuclides exhibit characteristic beta and gamma-ray energies and half-lives. It is only necessary to characterize the radiations from an irradiated sample, using the appropriate detection apparatus compared to result (decay data) and relate these characteristics to sample composition.

3.5 Quantitative analysis

From Eq.2.7, the weight of element can be calculated from

$$w = \frac{R(t_d) \times M}{N_0 \theta \sigma \phi (1 - e^{-\lambda t_i}) e^{-\lambda t_d}} \qquad (2.8)$$

But due to the uncertainty in the value of nuclear data and reactor parameter, these affect the accuracy of the result. Thus, the comparative method is used to solve this problem. In this method, a standard containing the sought element and the unknown sample are irradiated simultaneously for the same time in the same flux and count under identical conditions. The weight of element can be determined by

$$w_x = \frac{w_s R_x (td_x) e^{-\lambda td_x}}{R_s (td_s) e^{-\lambda td_s}} \qquad (2.9)$$

where subscript x and s refer to sample and standard respectively.

3.6 Advantages of Neutron Activation Analysis.[76-77]

3.6.1 It is very sensitive for many elements.

3.6.2 The problem of sample contamination by impurities in chemical reagents used in the analysis is eliminated, since normally no reagent are added until after sample activation.

3.6.3 The sample can be analyzed nondestructively, by simple irradiation with neutron and then examining the activated sample by means of gamma-ray spectrometry. The analyses are very rapid and require small quantity of sample.

3.6.4 A number of elements can be determined simultaneously.

3.6.5 It can be used in both qualitative and quantitative analysis, because the nuclear properties of radionuclide, such as half-life, type of radiation, and gamma energy are uniquely characteristic of that isotope.

3.7 Disadvantages of Neutron Activation Analysis.[76-77]

3.7.1 It only measures the total weight of an element and takes no account of different states of combination.

3.7.2 Special permission is required for organic and liquid sample.

3.7.3 It can not determine all the elements in periodic table, especially the elements with atomic number less than 10, such as H, C, etc.

3.7.4 Working with radioactive sample implies safety precautions. It requires well trained personnel.

3.7.5 Instrument used, e.g. neutron source, counting equipment, etc. are expensive.

3.8 Errors in Neutron Activation Analysis.[76-77]

3.8.1 Flux variation during irradiation

Flux Variation can be divided into three groups: variations in time, variations in positions and flux depression inside the sample. Flux variations with time and with position are caused by the reactor operation and by the differences in the distance to the reactor-core, respectively. These types of flux variations can be eliminated by using flux monitors.

3.8.2 Preparation of Standard and Samples.

The error may arise due to the sample and standard having different composition, concentration and type of containers. If standard material is hygroscopic, it will cause the error in weighting.

The contamination and the losses of some volatile elements from the samples during freeze drying and irradiation are the main sources of error during the sample preparation. These errors are eliminated by preparing the sample in a “clean room” where no chemicals and reagents are stored, and by using clean containers and tools.

3.8.3 Interference by nuclear reactions

Interference in NAA are of two types as follows:

3.8.3.1 Interference from other radionuclides, e.g. the 280 KeV peak

of Hg-203 has interference from the 279 KeV γ-energy of Se-75 . This interference can be eliminated by specific radiochemical separations in which the interfering radionuclides are removed from the element of interest.

3.8.3.2 Interference from two or more different nuclear reactions producing the same radionuclide, e.g.

$^{27}Al(n, \gamma)^{28}Al$ when n is the thermal neutron

$^{28}Si(n, p)^{28}Al$ when n is the fast neutron

$^{31}P(n, \alpha)^{28}Al$ when n is the thermal neutron

This interference is usually produced by fast neutron reactions. Thus, it can be eliminated by irradiating samples in thermal column or wrapping samples with Cd-sheet.

3.8.4 Counting error

The origins of counting errors are as follows:

3.8.4.1 Self absorption in the sample being counted. In simple terms this means that all the radiation produced in the sample do not manage to escape from it. The proportion which is lost in this way depends on the energy of the radiation (often occurs with low-energy γ-rays) and on sample thickness (the more thickness, the more self absorption). To minimize this error, samples and standards must be thin and have same thickness.

3.8.4.2 The counting errors of back-scattering is easily eliminated: the former by counting all samples on containers of uniform thickness and material: the latter by controlling sample size.

3.8.4.3 Variation in geometrical efficiency and errors due to scattering by air and shielding materials: They are normally eliminated by counting all samples at a fixed distance from the counter.

4. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)[78-80]

Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) is one of the most common techniques for elemental analysis. Its high specificity, multi-element capability and good detection limits result in the use of the technique in a large variety of applications. All kinds of dissolved samples can be analyzed, varying from solutions containing high salt concentrations to diluted acids. A plasma source is used to dissociate the sample into its constituent atoms or ions, exciting them to a higher energy level. They return to their ground state by emitting photons of a characteristic wavelength depending on the element present. This light is recorded by an optical spectrometer. When calibrated against standards the technique provides a quantitative analysis of the original sample.

4.1 Principle of ICP-AES

In ICP-AES, the sample is usually transported into the instrument as a stream of liquid sample. Inside the instrument, the liquid is converted into an aerosol trough a process known as nebulization. The sample aerosol is then transported to the plasma where it is desovated, vaporized, atomized, and excited and/ or ionized by the plasma. The excited atoms and ions emit their characteristic radiation which is collected by a device that sorts the radiation by wavelength. The radiation is detected and turned into electronic signals that are converted into concentration information for the analyst. The major components and the layout ICP-AES instrument is shown in Fig. 2.2.[80]

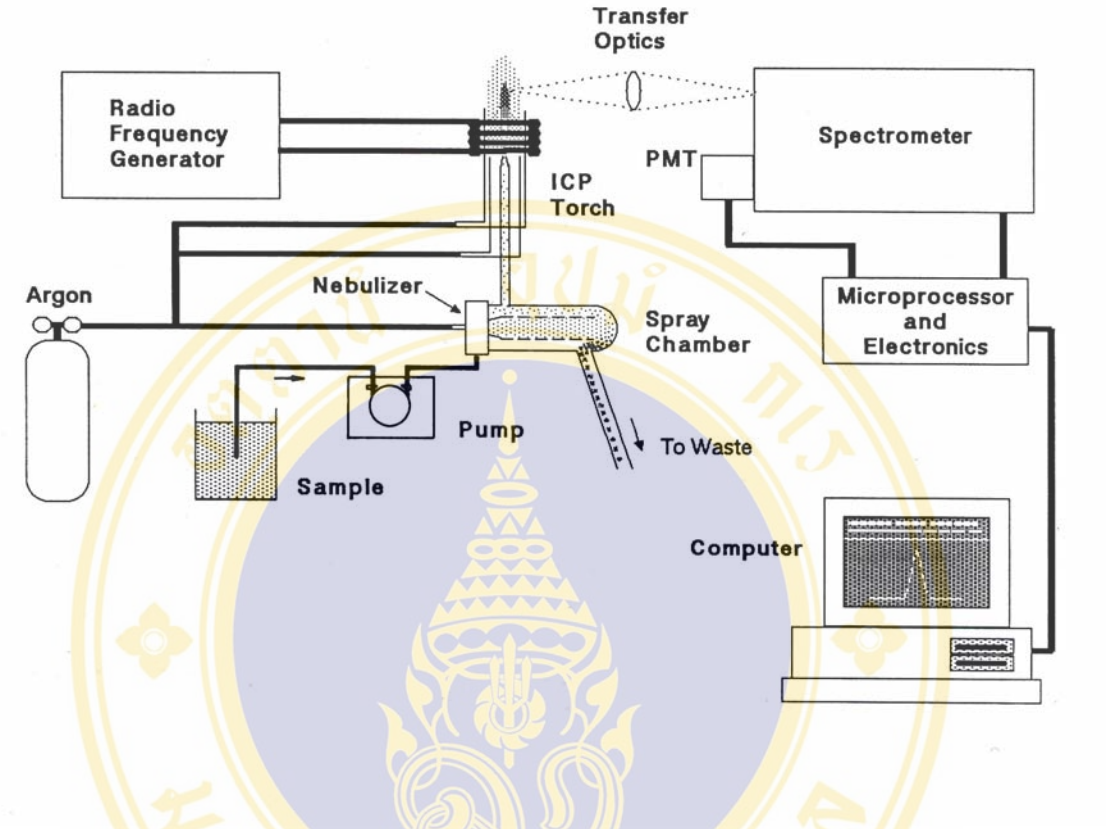

Fig. 2.2 Major components and layout of a typical ICP-AES instrument

4.1.1 Sample introduction

The sample solution was prepared from the solid sample and solubilized with diluted acid solution. The sample solution is transformed into an aerosol by a so-called nebulizer. The large droplets are separated from the smallest in a specially spray chamber. The smallest droplets (about 10 μm or smaller) are transferred to the plasma by an argon flow.

4.1.2 Production of emission

The gas flow that carries the sample aerosol is injected into the plasma though the central tube, or injector. Due to the small diameter at the end of the injector, the gas velocity is such that even the 1L/min. It is often called the sample flow or nebulizer flow which is the part of ICP torch.[80]

The ICP torch design is the one-piece torch shown in Fig. 2.3. It generally provide good plasma stability and are easy to use. At present, the most popular torches are of the demountable type such as the on shown in Fig. 2.4. The main advantage of the demountable torch is the lower costs and the ability to use a variety of injector tubes.

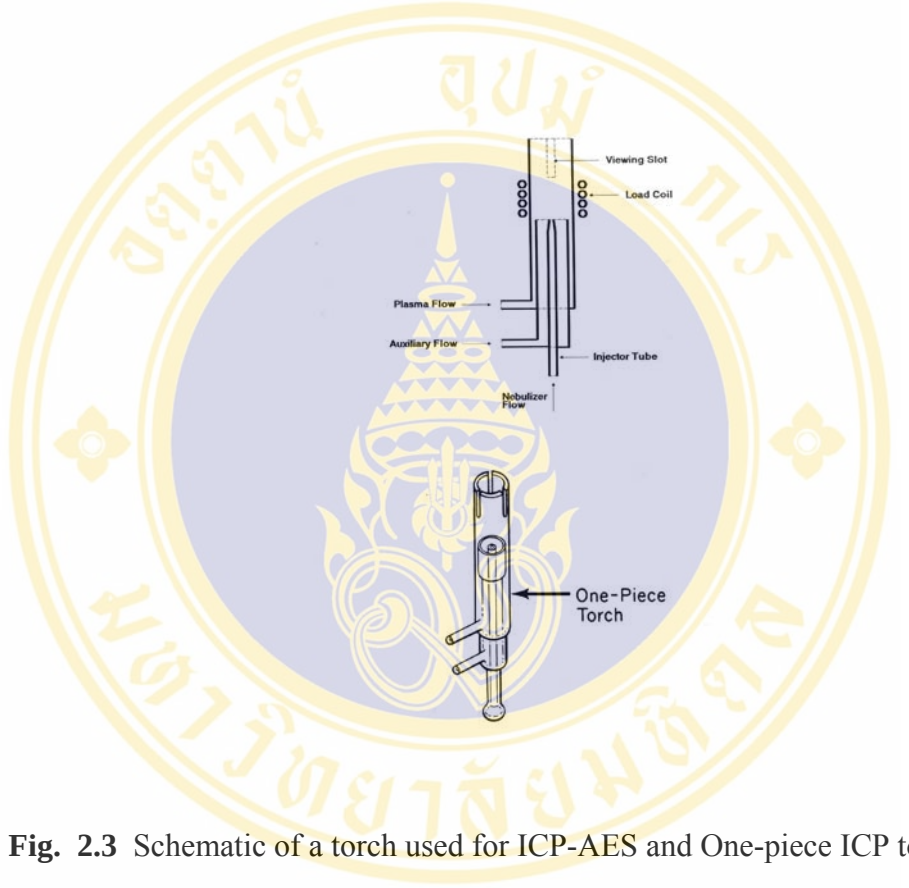

Fig. 2.3 Schematic of a torch used for ICP-AES and One-piece ICP torch

The radio frequency (RF) generator is the device that provides the power for the ICP torch and sustainment of the plasma discharge. This power, typically ranging from about 600-1800 watts, is transferred to the plasma gas through a load coil surrounding the top of the torch. The load coil, which acts as an antenna to transfer the RF power to the plasma, is usually made from copper tubing and is cooled by water or gas during operation.

Most RF generators used for ICP-AES operate at a frequency between 27 and 56 MH_z. An increasing number of instruments now operate at 40 MH_z due to improvements in coupling efficiency and reductions in background emission intensity

realized at this frequency. Frequencies greater than 40 MH_z also have been used but have not been as successful commercially.[80]

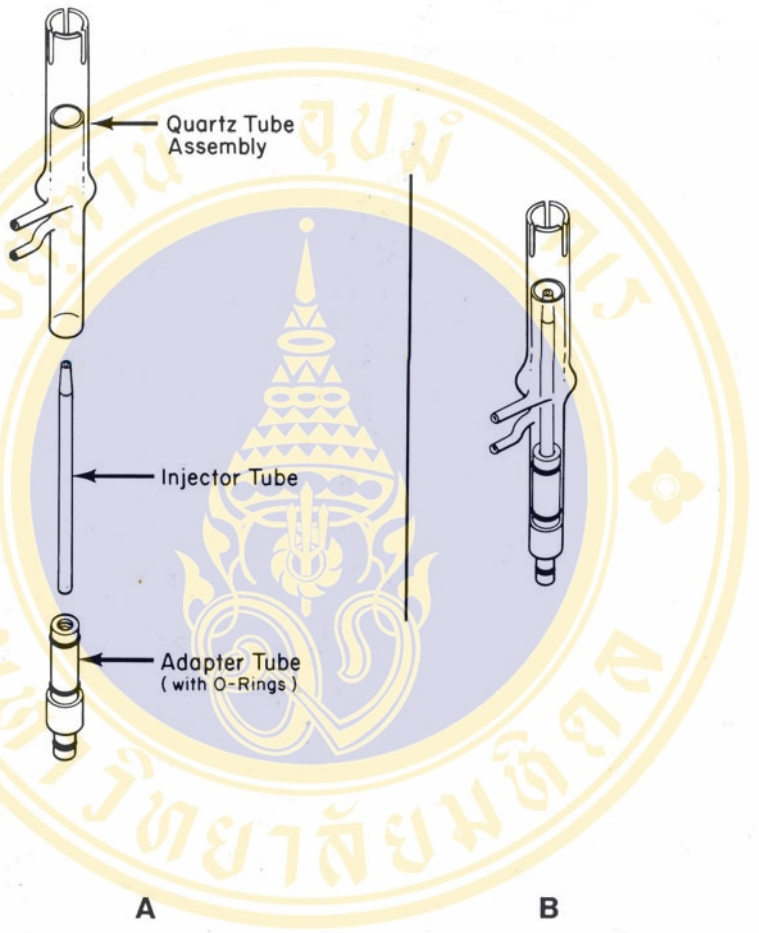

Fig. 2.4 Demountable ICP torch, A-expanded view, B-assembled view.

4.1.3 Spectrometer and Detector

A spectrometer consists of three main parts: an emission source which produces the spectrum, an optical system which scatters the spectrum and the device to measure the proper emitted lines which processes the result. Common spectrometer

optical systems consists of a polychromator or a monochromator which scatters the spectrum and isolates the analytical lines of the elements to be analyzed.

Nearly all recently manufactured emission spectrometer systems include a microprocessor or a microcomputer system. The degree of computer involvement in the experiment varies from model to model. In some cases the computer merely acquires the emission data, computes calibration data from standards, calculates the concentrations of unknowns from stored calibration equations, and outputs the data to a printer or video display. Some programs allow emission data to be corrected for matrix effects by including various empirical correction factors. Others allow background correction to be implemented under program control. Many instruments also provide an automatic sampler and in some cases the computer can control the sampler.

Parameters such as gas flow rates, nebulizer flow rates, observation windows, and many others are amenable to control by the computer. Placing as many parameters as possible under computer control allows self-optimization routines to be used to develop optimum analysis conditions.

CHAPTER III
MATERIALS AND METHODS

1. Apparatus and materials for preparation of *Aloe vera* (Linn.) powder

1.1 Apparatus

1.1.1 Lyophillizer, Shell Freezer (Labconco, USA.)

1.1.2 Minispray dryer SD-06 (LabPlant, England)

1.1.3 Stirrer (KIKA-Laboretechnik, Thailand)

1.1.4 Balance 4 position scale (precisa U&V holding Ltd.(Thailand); Switzerland)

1.1.5 Blender (Severin, Thailand)

1.1.6 Filter bag

1.1.7 pH meter (744 metrohm, Switzerland)

1.1.8 Viscometer (Anton paar, DV-3p, Austria)

1.1.9 Moisture analyzer (Sartorius MA 50, Germany)

1.2 Chemical

1.2.1 Maltodextrin (A.E. Staley Mfg., Co. Starch & Specialty Group; USA.)

1.2.2 Sodium benzoate (BDH Limited Poole., England)

1.2.3 Sodium metabisulfite (BDH Limited Poole., England)

1.2.4 Ethylenediaminetetraacetic acid (EDTA) (Merck, Germany)

2. Apparatus and materials for determination of elements in *Aloe vera* powder

2.1 Apparatus

2.1.1 Nuclear reactor: Thai Research Reactor-1/Modification-1 (TRR-1

/M1); which is a swimming pool type designed to operate at a maximum power of 2 MW but routinely operate at 1.2 MW and has been operated for 10 hours a day, four days a week, operated by the Office of Atoms for Peace (OAP), Bangkok, Thailand,

2.1.2 High resolution gamma ray spectrometer consists of HPGe detector model GCW 1522 from Canberra, and MCA card model S-100 with GENIE 2000 software.

2.1.3 Polyethylene irradiation container (rabbit) and vials.

2.1.4 Scissors

2.1.5 Other accessories such as tracing paper, paper towel, spoon, non-tooth forceps, magic ink and stainless steel tray.

2.1.6 Balance 4 position scale (Mettler Toledo, Switzerland)

2.1.7 Filter paper Whatman No. 42.

2.1.8 Volumetric flasks

2.1.9 Pipettes

2.1.10 Sonicating bath (Bransonic model B2200 E-3)

2.1.11 Inductively Coupled Plasmas - Atomic Emission Spectrometer (Perkin Elmer model 1000,USA.)

2.2 Chemical

2.2.1 65 % Nitric acid (A.R. Grade, Lab-Scan Analytical Science; Ireland)

2.2.2 Deionized water

2.2.3 SRM 1573a “Tomato leaves” and SRM 1547 “Peach leaves” from the National Institute of Standards &Technology, USA.

3. Sampling and Sample preparation

3.1 Sampling and storage

The *Aloe vera* (Linn.) samples were collected from ten areas of Herbal garden of the Department of Medical Science in Rayong province. Full size mature leaves were cut and wrapped with newspaper and stored in refrigerator at 2-4 °C.

3.2 Sample preparations

3.2.1 Preparation of *Aloe vera* gel and *Aloe vera* powder

Aloe vera (Linn.) leaves in herbal garden were first washed three times with water. After washing, they were air-dried on a clean aluminium tray at room temperature. Each dried aloe sample was weighed and cut to small pieces. The gel was separated by scratching with a spoon, soaked with deionized water and weighed again. Deionized water was added to *Aloe vera* gel by the ratio of 1:1 and then homogenized in blender. The homogenized gel was filtered through filter bag. The filtrate were equally divided into 10 parts for investigation of the optimum condition in pretreated *Aloe vera* gel by adding various amount of anticaking agent (Maltodextrin), preservative (Sodium benzoate), antioxidant (Sodium metabisulfite)and chelating agent (Ethylenediaminetetraacetic acid) as shown in the Table 3.1.

Table 3.1 The formulation of the *Aloe vera* gel

Formula No. / Diluents	1	2	3	4	5	6	7	8	9	10
- Anti-caking agent (%w/v)	-	-	3.50	4.00	1.00	1.50	4.00	2.00	2.50	3.00
- Preservative (%w/v)	-	0.10	0.10	0.10	0.20	0.20	0.20	0.20	0.20	0.20
- Chelating agent (%w/v)	-	-	0.10	-	0.10	0.10	0.10	0.10	0.10	0.10
- Antioxidant (%w/v)	-	0.05	-	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Each mixture solution was divided into two portions. One portion was freeze dried by Lyophilizer while the other portion was spray dried by Minispray dryer. All *Aloe vera* powder samples were determined for chemical and physical properties. The scheme of the powder preparation was shown in Fig 3.1.

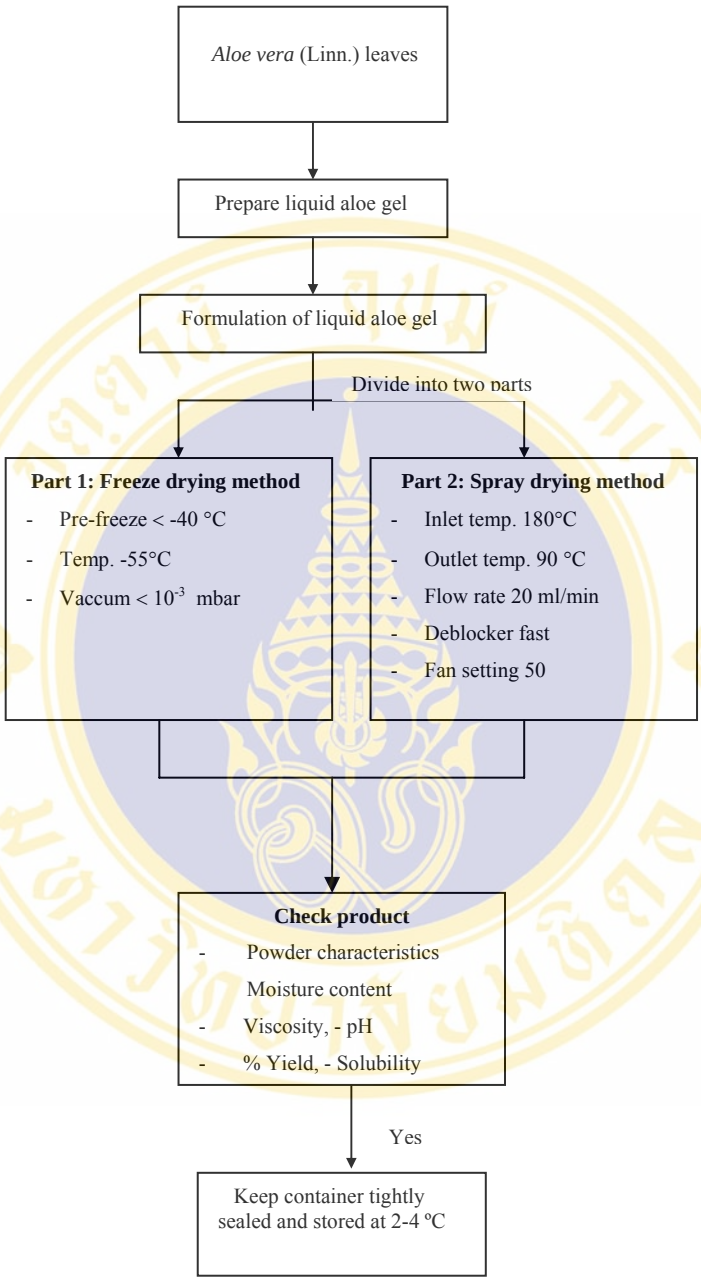

Fig. 3.1 Flow chart of the preparation of *Aloe vera* powder

4. Determination of chemical and physical properties of *Aloe vera* powder

4.1 Appearance and color

Appearance and color were observed the eye.

4.2 Solubility in water

0.2 mg of *Aloe vera* powder was dissolved with deionized water and the solubility was determined from the following criteria.

Table 3.2 Solubility test

Water volume	Solubility
≤ 1	Very soluble
1-2	Soluble
≥ 2	Insoluble

4.3 pH value

An *Aloe vera* gel was by dissolved 2.0 g with 100 ml of deionized water and the pH was determined by pH meter.

4.4 Viscosity

The viscosity of *Aloe vera* gel prepared in 4.3 was determined by Viscometer.

4.5 Moisture contents

Aloe vera powder was weighted 1.0 g into a heating tray and determine for moisture content at 150 ºC for 15 min by Moisture analyzer.

4.6 The process yield or % yield

The process yield in % was calculated from

$$\text{yield}\,(\%) = \frac{X}{Y} \times 100$$

Where X = weight of dry powder

Y = weight of *Aloe vera* gel

After comparison of chemical and physical of *Aloe vera* powder sample. The sample with suitable properties will be used for elemental analysis.

5. Coding System

The coding system of the sample for NAA analysis is as follows:

- AVF for freeze dry samples
- AVS for spray dry samples
- AVS-USA for spray dry samples purchased from USA

6. Neutron Activation Analysis (NAA)

6.1 Qualitative analysis

About 50 mg of sample was weighted in polyethylene vial and sealed. The sample was irradiated in the irradiation facility A-1 of TRR-1/M1 research reactor and counted by gamma-ray spectrometer. The experiments were performed by varying irradiation time and decay time.

6.2 Method validation for neutron activation analysis

The SRM 1547 was treated as unknown and was analyzed by comparing with SRM 1573a. The experimental condition was selected from 6.1 and the distance between sample and detector is two centimeters. The concentration of interested elements were calculated from Eq. 2.9.

6.3 Quantitative analysis

For quantitative analysis SRM 1547 was used as a standard. About 50 mg of standard was weight in polyethylene vial and sealed. Two samples and one standard were packed in the rabbit and irradiated in irradiation facility A-1. Triplicate analyses were performed for each sample using the same procedure as in 6.2.

7. Determination of elements by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

7.1 Preparation of standard solutions

The standard reference material of SRM 1573a and SRM 1547 were accurately weighed into a beaker, dissolved with 2 % nitric acid in sonicating bath at 45 °C for 15 minute. The insoluble residue was then filtered with filter paper Whatman No.42 and the filtrate was transferred into a volumetric flask and diluted to the volume with deionized water. The conditions of standard preparation were shown in Table 3.3. All standard solutions were prepared in the range of 1-6 ppm for Mn, Na and for K, Mg and Ca in the range of 8-50 ppm which were shown in Table 3.4. The 2 % nitric acid was used as blank for calibration curve.

Table 3.3 Preparation of standard stock solutions for ICP-AES method

Elements	Standard preparation
Mn	SRM:1573a (Tomato leaves) 0.4001 g/25 ml
Ca	SRM:1573a (Tomato leaves) 0.4002 g/25 ml, Then diluted 20 times
Na	SRM:1573a (Tomato leaves) 1.0001 g/25 ml
K	SRM:1573a (Tomato leaves) 0.4004 g/25 ml, Then diluted 10 times
Mg	SRM:1547 (Peach leaves) 0.4003 g/25 ml, Then diluted 2 times

Table 3.4 The concentration of elements in standards reference material were analized by ICP-AES

Element	Concentration (μg/ml)			Mean of emission		
	1	2	3	1	2	3
Mn	0.9859	1.9718	3.9436	11869.10	23823.30	46848.70
Ca	10.1000	20.2000	40.4000	37304.45	74617.86	149254.80
Na	1.3600	2.7200	5.4400	584.21	1168.74	2337.75
K	10.8000	21.6000	43.2000	281.20	562.52	1124.92
Mg	8.6400	17.2500	34.5600	752.23	5495.46	11011.02

7.2 Preparation of samples

The samples: AVF-5, AVS-7 were prepared as same as the standard solution and then they were diluted or pre-concentrated to the working range of the standard solution as shown in Table 3.5.

Table 3.5 Preparation of sample for ICP-AES

Elements	Sample No.	Sample preparation
Mn	AVF-5	2.0002 g/50 ml
	AVS-7	2.0001 g/50 ml, Then diluted 5 times
Ca	AVF-5	2.0002 g/50 ml, Then diluted 10 times
	AVS-7	2.0003 g/50 ml, Then diluted 10 times
Na	AVF-5	0.2005 g/50 ml, Then diluted 10 times
	AVS-7	0.2004 g/50 ml, Then diluted 10 times
K	AVF-5	2.0001 g/ 50 ml, Then diluted 10 times
	AVS-7	2.0003 g/ 50 ml, Then diluted 10 times
Mg	AVF-5	2.0007 g/ 50 ml, Then diluted 10 times
	AVS-7	2.0002 g/ 50 ml, Then diluted 10 times

7.3 ICP measurements

All ICP measurements were performed on a sequential ICP plasma spectrometer Perkin-Elmer model plasma 1000 which was operated in conditions as shown in Table 3.6. Table 3.7 lists the analytical wavelengths of the elements of interest.

Sample and standard solutions were introduced to the nebulizer with the flow rate of 1 ml/min. Argon flow velocity, about 1 L/min, was used to transport the sample aerosol to the plasma where it was desolvated, vaporized, atomized, and excited and/or ionized by the plasma. The excited atoms or ions emitted their characteristic radiation which was detected and turned into electronic signal. The radiation intensities were then converted into element concentration information when they were calibrated against standards.

Table 3.6 ICP-AES operating parameters

RF power	1.00 kW
Plasma Ar flow	15.00 L/min
Auxiliary Ar flow	1.00 L/min
Sample uptake rate	1.00 mL/min
Viewing Height	15 mm above load coil

Table 3.7 The analytical wavelengths for the interesting elements

Element	**Wavelength (nm)**
Na	589.592
Ca	393.366
K	766.490
Mg	279.550
Mn	267.716

CHAPTER IV
RESULTS

1. The chemical and physical properties of *Aloe vera* powder

The properties of spray dried and freeze dried *Aloe vera* powder were shown in Table 4.1 and Table 4.2 respectively.

Table 4.1 The properties of *Aloe vera* powders by spray drying method

Formula No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Description										
- Color	White	White	White	White	White	White	White	White	White	White
- Appearance	Lump Powder	Lump Powder	Lump Powder	Lump Powder	Fine Powder	Fine Powder	Fine Powder	Fine Powder	Fine Powder	Fine Powder
- Moisture Contents (%)	6.05	5.93	5.30	5.00	4.50	5.29	5.26	5.20	5.35	5.05
- Viscosity (cps.)	10.41	10.73	10.93	11.03	11.24	13.41	12.73	11.93	11.75	11.62
- pH	5.03	5.10	5.07	5.45	5.30	5.03	5.10	5.07	5.45	5.30
- % Yield	0.02	0.04	0.06	0.08	0.12	2.50	2.00	1.80	1.60	1.50
Solubility in water	Soluble in water	Soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Soluble in water	Soluble in water	Very soluble in water	Very soluble in water	Very soluble in water

Table 4.2 The properties of *Aloe vera* powders by freeze drying method

Formula No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Description										
- Color	White with yellow	White with yellow	White with yellow	White	White	White	White	White	White	White
- Appearance	Lump powder	Lump powder	Lump powder	Fine powder	Fine Powder	Fine powder	Fine powder	Fine powder	Fine powder	Fine powder
- Moisture Contents (%)	6.75	5.01	4.60	3.95	6.30	6.15	6.29	6.00	5.58	4.81
- Viscosity (cps.)	11.41	11.73	13.93	14.03	11.34	11.41	10.73	11.33	11.75	12.62
- pH	5.16	5.45	5.80	5.30	4.77	4.82	4.68	4.80	4.85	4.75
- % Yield	0.50	1.58	2.81	3.40	0.88	0.92	0.80	1.00	1.20	2.15
Solubility in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water	Very soluble in water

2. Qualitative analysis

The qualitative analysis of *Aloe vera* powder sample was shown in Table 4.3.

Table 4.3 Result of qualitative analysis

Condition	Sample	Isotope found				
		Mn	Mg	Na	K	Ca
T_i = 1 min	AVS-1	+	+	+	+	+
T_d = 5 min	AVF-1	+	+	+	+	+
T_c = 300 sec	AVS-USA	+	+	+	+	+
T_i = 1 min	AVS-1	+	+	+	+	+
T_d = 10 min	AVF-1	+	+	+	+	+
T_c = 300 sec	AVS-USA	+	+	+	+	+
T_i = 5 min	AVS-1	+	+	+	+	+
T_d = 5 min	AVF-1	+	+	+	+	+
T_c = 300 sec	AVS-USA	+	+	+	+	+
T_i = 5 min	AVS-1	+	ND	+	+	ND
T_d = 2 hr	AVF-1	+	ND	+	+	ND
T_c = 300 sec	AVS-USA	+	ND	+	+	ND
T_i = 1 hr	AVS-1	ND	ND	+	+	ND
T_d = 24 hr	AVF-1	ND	ND	+	+	ND
T_c = 300 sec	AVS-USA	ND	ND	+	+	ND
T_i = 6 hr	AVS-1	ND	ND	+	+	ND
T_d = 24 hr	AVF-1	ND	ND	+	+	ND
T_c = 300 sec	AVS-USA	ND	ND	+	+	ND

+ = Isotope found

ND = not detectable

From Table 4.3, the selected condition for further experiment is T_i = 5min, T_d = 5 min and T_c = 300 sec.

3. The method validation for Neutron Activation Analysis

The SRM 1547 (Peach leaves) were used to validate the procedure used in this study. The result was shown in Table 4.4. Where error is determined by

$$\text{Error}\ (\%) = \frac{\text{Certified value - Analyte value}}{\text{Certified value}} \times 100$$

Table 4.4 The analytical elemental contents of SRM 1547 (Peach leaves) and their certified values[81-82]

Elements	Analysis number	Concentration ; % wt.
Na	1	24.9
	2	23.3
	3	24.0
Nuclide: Na-24	Mean ± SD.	24.1 ± 0.8
	Certified value	24 ± 2 *
	Error (%)	0.33
Ca	1	1.555
	2	1.525
	3	1.557
Nuclide: Ca-49	Mean ± SD.	1.546± 0.018
	Certified value	1.56 ± 0.02
	Error (%)	0.92
Mn	1	101.8
	2	100.0
	3	98.1
Nuclide: Mn-56	Mean ± SD.	100.9± 1.39
	Certified value	98± 3*
	Error (%)	1.67
K	1	2.467
	2	2.444
	3	2.434
Nuclide: K-42	Mean ± SD.	2.448± 0.017
	Certified value	2.43 ± 0.03
	Error (%)	0.75
Mg	1	0.431
	2	0.435
	3	0.429
Nuclide: Mg-27	Mean ± SD.	0.432± 0.003
	Certified value	0.43 ± 0.08
	Error (%)	0.39

*** = ppm**

4. The quantitative analysis of *Aloe vera* powders by Neutron Activation Analysis

The concentration of Na, Ca, Mg, Mn and K was determined from the peak energy 1368, 3084, 1014, 846 and 1524 keV respectively. The elemental concentration of freeze dried sample and spray dried sample were shown in Table 4.5 and 4.6 respectively.

Table 4.5 The elemental concentration of freeze dried samples

Sample	Concentration				
	Na (%wt.)	Ca (%wt.)	Mg (%wt.)	Mn (ppm)	K (%wt.)
AVF -1	0.66±0.01	0.88±0.02	0.23±0.01	61.3±1.1	1.02±0.02
AVF -2	0.84±0.02	1.09±0.01	0.23±0.01	55.5±2.0	1.11±0.06
AVF -3	0.82±0.03	1.00±0.01	0.24±0.01	60.9±2.2	1.09±0.06
AVF -4	0.63±0.03	0.92±0.06	0.27±0.01	61.1±2.4	0.81±0.01
AVF -5	0.70±0.02	1.19±0.14	0.40±0.01	59.7±3.2	1.03±0.01
AVF -6	0.80±0.01	1.23±0.05	0.34±0.01	267.3±7.0	0.97±0.01
AVF -7	0.81±0.01	0.94±0.04	0.27±0.01	273.7±1.2	1.00±0.03
AVF -8	0.77±0.02	0.99±0.01	0.23±0.02	254.7±7.4	1.04±0.02
AVF -9	0.78±0.01	1.15±0.01	0.29±0.01	273.6±3.7	0.93±0.02
AVF -10	0.69±0.01	1.09±0.01	0.25±0.01	286.8±5.7	0.99±0.01
Mean ± SD.	0.75±0.07	1.05±0.12	0.28±0.06	165.5±111.8	0.99±0.09

Table 4.6 The elemental concentration of spray dried samples

Sample	Concentration				
	Na (%wt.)	**Ca (%wt.)**	**Mg (%wt.)**	**Mn (ppm)**	**K (%wt.)**
AVS -1	0.74±0.01	0.80±0.02	0.25±0.01	57.6±0.7	0.94±0.01
AVS -2	0.78±0.02	0.99±0.01	0.26±0.01	53.0±0.8	1.02±0.01
AVS -3	0.76±0.02	0.93±0.01	0.25±0.02	56.4±0.6	1.00±0.05
AVS -4	0.64±0.01	0.88±0.03	0.29±0.01	53.2±0.4	0.91±0.01
AVS -5	0.65±0.03	1.13±0.07	0.39±0.02	63.2±3.4	0.82±0.02
AVS- 6	0.71±0.02	1.11±0.02	0.36±0.01	271.3±12.9	1.07±0.01
AVS -7	0.81±0.02	0.90±0.01	0.31±0.02	265.4±11.6	0.96±0.01
AVS -8	0.71±0.01	0.95±0.03	0.28±0.01	289.7±4.2	0.93±0.01
AVS -9	0.82±0.03	1.05±0.05	0.36±0.01	265.1±5.6	0.82±0.01
AVS-10	0.63±0.03	0.96±0.01	0.30±0.01	263.0±2.8	0.84±0.03
Mean ± SD.	0.73±0.07	0.97±0.10	0.28±0.06	163.8±113.2	0.93±0.09
AVS-USA-1	0.09±0.01	0.21±0.01	0.05±0.01	10.4±1.3	0.18±0.00
AVS-USA-2	0.10±0.01	0.17±0.02	0.04±0.00	9.6±5.0	0.16±0.01
Mean ± SD.	0.09±0.01	0.19±0.02	0.045±0.01	10.2±0.8	0.172±0.01

5. The quantitative analysis of *Aloe vera* powders by Inductively Coupled Plasma Atomic Emission Spectrometry

All ICP measurements were performed on a sequential ICP plasma spectrometer Perkin-Elmer model plasma 1000. Freeze dried powder AVF-5 and spray dried powder AVS-7 were analyzed by ICP-AES. The elemental concentration was shown in Table 4.7.

Table 4.7 The elemental concentration of *Aloe vera* powders by ICP-AES

Sample	Elemental concentration				
	Na (%wt.)	**Ca (%wt.)**	**Mg (%wt.)**	**Mn (ppm)**	**K (%wt.)**
AVF -5	0.67±0.02	1.07±0.03	0.42±0.02	55.5±1.6	0.95±0.02
AVS -7	0.74±0.05	0.76±0.04	0.28±0.01	247.7±1.4	0.84±0.01

6. Comparison of elemental concentration between NAA and ICP-AES

The comparison of elemental concentration between NAA and ICP-AES for freeze dried sample (AVF-5) and spray dried sample (AVS-7) was shown in Table 4.8. The difference of the result was shown in Table 4.9. Where difference is determined by

$$\text{Difference (\%)} = \frac{\text{Mean}_{\text{NAA}} - \text{Mean}_{\text{ICP-AES}}}{\text{Mean}_{\text{NAA}}} \times 100$$

Table 4.8 Comparison of elemental concentration between NAA and ICP-AES

Sample	Elemental concentration									
	Na (% wt.)		Ca (% wt.)		Mg (% wt.)		Mn (ppm)		K (% wt.)	
	NAA	ICP-AES	NAA	ICP-AES	NAA	ICP-AES	NAA	ICP-AES	NAA	ICP-AES
AVF-5	0.70± 0.02	0.67± 0.02	1.19± 0.18	1.07± 0.03	0.40± 0.01	0.42± 0.02	59.7± 3.9	55.5± 1.6	1.03± 0.01	0.95± 0.02
AVS-7	0.81± 0.02	0.74± 0.05	0.90± 0.01	0.76± 0.04	0.31± 0.01	0.28± 0.01	265.4± 14.2	247.7± 1.4	0.96± 0.01	0.84± 0.01

Table 4.9 Percentage difference of elemental concentration between NAA and ICP-AES

Sample	Difference (%)				
	Na	Ca	Mg	Mn	K
AVF-5	4.29	10.08	-5.00	7.09	8.82
AVS-7	8.64	15.56	9.68	6.69	12.50

CHAPTER V
DISCUSSION

In this study, *Aloe vera* powder was prepared in the laboratory by spray drying and freeze drying methods. The advantage and disadvantage between this two methods was shown in the table below.

Spray dry method	Freeze dry method
Advantage	**Advantage**
1. Low cost units	1. Preserve of physical properties and active ingredient
2. Easy to operate	2. No contamination
Disadvantage	**Disadvantage**
1. Loss of sample	1. High cost units
2. Deterioration of the active ingredient	2. Required specific technical
3. Contamination	3. Complicate to operate

According to the advantage in preserving of physical properties and active ingredient with no contamination of freeze dry method, it might be recommended for preparing preserved the natural products. In case of no need in essential active ingredient, negligible of some contamination or loss of sample, the low cost unit and easy to operate, spray dry method was recommended.

The quantitative analysis of NAA under the condition of 5 minute irradiation time, 5 minute decay time and 300 second counting time, 5 elements (Na, K, Mn, Mg ,Ca) which have short half-life were found. Except Mn, other elements content in the *Aloe vera* samples expressed a very low standard deviation. The quantitation of Mn , sample No. 6 to 10 show higher concentration than sample No. 1 to 5. This may be from the variation of Mn composition in soil or from the difference fertilization.

A comparison between NAA and ICP-AES revealed some differences in the elemental contents, 4 to 10 % for freeze dried samples and 6 to 15 % for spray dried samples. The ICP-AES results showed lower amount than NAA, this might be from the complicated procedure which composed of many steps in ICP-AES that bring to the error. The NAA method showed not only high accuracy and high precision but also uncomplicated procedure.

CHAPTER VI
CONCLUSION

Various diluents were formulated to prepare *Aloe vera* powder by freeze dry and spray dry. The suitable formula (No. 10) was chosen which composed of an anti-caking agent 3 % w/v, preservative 0.2 % w/v, chelating agent 0.1 % w/v and antioxidant 0.05 % w/v. By considering on the basis of chemical and physical properties; the yield, solubility in water, pH, moisture content etc. formula No. 10 was the most optimum condition for preparation of *Aloe vera* powder.

The *Aloe vera* powder samples were qualitatively analyzed for elemental contents by NAA after optimizing experimental condition at 5 minute irradiation time, 5 minute decay time and 300 second counting by gamma spectrometer. The Na, K, Mn, Mg and Ca were found. For the determination of the accuracy of this method, Standard Reference Materials (SRM 1547) was analyzed. The results obtained are in good agreement with the certified values with relative error less than 2 %. No significant differences at 0.05 level were observed between the quantitative analysis of freeze dried and spray dried samples (Appendix A). The precision of the results obtained from spray dry and freeze dry samples were satisfactory within the limits expected of less than 10 % CV (Appendix B). A comparison between NAA and other analytical method, ICP-AES in AVF-5 and AVS-7 revealed that elemental contents by ICP-AES is less than NAA.

The results in this study indicate that methods of sample preparation had no effect on the elemental contents in *Aloe vera* powder. However, the freeze drying method and the NAA might be recommended as promising method for determining elemental components in natural products.

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APPENDIX

APPENDIX A

Comparison of concentration between spray dried and freeze dried sample

The elemental concentration in spray dried sample and freeze dried sample was test by unpaired t-test at 95% confidence level with the following hypothesis

H_0: $\mu = 0$

H_1: $\mu \neq 0$

The test result is shown in Table A.

Table A Comparison of elemental concentration between spray dried and freeze dried samples by NAA

Sample No.	Elemental Concentration									
	Na (%wt.)		Ca (%wt.)		Mg (%wt.)		Mn (ppm)		K (%wt.)	
	AVF	AVS	AVF	AVS	AVF	AVS	AVF	AVS	AVF	AVS
1	0.66	0.74	0.88	0.80	0.23	0.25	61.31	57.57	1.02	0.94
2	0.84	0.78	1.09	0.99	0.23	0.26	55.53	53.00	1.11	1.02
3	0.82	0.76	1.00	0.93	0.24	0.25	60.87	56.35	1.09	1.00
4	0.63	0.64	0.92	0.88	0.27	0.29	61.07	53.23	0.81	0.91
5	0.70	0.65	1.19	1.13	0.40	0.39	59.73	63.17	1.03	0.82
6	0.80	0.71	1.23	1.11	0.34	0.36	267.33	271.30	0.97	1.07
7	0.81	0.81	0.94	0.90	0.27	0.31	273.73	265.43	1.00	0.96
8	0.77	0.71	0.99	0.95	0.23	0.28	254.70	289.67	1.04	0.93
9	0.78	0.82	1.15	1.05	0.29	0.36	273.63	265.07	0.93	0.82
10	0.69	0.63	1.09	0.96	0.25	0.30	286.80	263.03	0.99	0.84
Mean ±SD	0.75± 0.07	0.73± 0.07	1.05± 0.12	0.97± 0.10	0.28± 0.06	0.31± 0.05	165.47± 111.77	163.79± 113.18	0.99± 0.09	0.93± 0.09
P- value	0.445		0.137		0.221		0.966		0.092	

The result shown that there is no statistically significant differences in the elemental contents between freeze dried and spray dried powder at 0.05 level ($P>0.05$).

APPENDIX B

Determination of precision

The precision was determined from the coefficient of variation (CV) and the results for spray dried sample and freeze dried sample were shown in Table B.1 and Table B.2 respectively.

Table B.1 The elemental concentration of freeze dried samples

Sample No.	Mean ± % CV				
	Na (%wt.)	Ca (%wt.)	Mg (%wt.)	Mn (ppm)	K (%wt.)
AVF -1	0.66±0.71	0.88±1.86	0.23±5.35	61.3±1.8	1.02±1.66
AVF -2	0.84±2.03	1.09±1.14	0.23±2.78	55.5±3.6	1.11±5.29
AVF -3	0.82±3.23	1.00±1.25	0.24±3.88	60.9±3.6	1.09±5.25
AVF -4	0.63±4.19	0.92±6.93	0.27±3.45	61.1±3.9	0.81±0.59
AVF -5	0.70±2.42	1.19±12.02	0.40±2.41	59.7±5.3	1.03±0.46
AVF -6	0.80±1.77	1.23±3.82	0.34±3.71	267.3±2.6	0.97±0.49
AVF -7	0.81±0.58	0.94±3.79	0.27±4.68	273.7±7.7	1.00±2.61
AVF -8	0.77±2.22	0.99±1.43	0.23±7.10	254.7±2.9	1.04±2.08
AVF -9	0.78±1.61	1.15±0.41	0.29±1.64	273.6±1.3	0.93±1.76
AVF -10	0.69±1.18	1.09±0.43	0.25±3.27	286.8±2.0	0.99±1.26

Table B.2 The elemental concentration of spray dried samples

Sample No.	Mean ± % CV				
	Na (%wt.)	Ca (%wt.)	Mg (%wt.)	Mn (ppm)	K (%wt.)
AVS -1	0.74±1.69	0.80±3.06	0.25±3.27	57.6±1.3	0.94±1.66
AVS -2	0.78±2.77	0.99±0.48	0.26±3.14	53.0±1.4	1.02±5.30
AVS -3	0.76±3.22	0.93±1.34	0.25±8.11	56.4±1.1	1.00±5.25
AVS -4	0.64±0.74	0.88±3.25	0.29±2.82	53.2±2.6	0.91±0.59
AVS -5	0.65±4.06	1.13±6.50	0.39±4.19	63.2±5.3	0.82±0.46
AVS- 6	0.71±2.38	1.11±1.47	0.36±1.30	271.3±4.8	1.07±0.49
AVS -7	0.81±2.02	0.90±0.52	0.31±6.56	265.4±4.4	0.96±2.62
AVS -8	0.71±0.67	0.95±3.49	0.28±2.92	289.7±4.9	0.93±2.08
AVS -9	0.82±3.23	1.05±4.73	0.36±1.32	265.1±2.1	0.82±1.76
AVS-10	0.63±4.19	0.96±0.98	0.30±2.36	263.0±1.1	0.84±1.26

BIOGRAPHY

NAME Mr. Chaidan Pachanon

DATE OF BIRTH 19 March 1977

PLACE OF BIRTH Phayao, Thailand

INSTITUTIONS ATTENDED Narasuan University, 1996-1999:
Bachelor of Science (Chemistry)
Mahidol University, 2000-2002:
Master of Science (Radiological Science)

POSITION & OFFICE 2000-2002 Department of Medical Science
Position: Medical Scientist
Tel 0-2589-9850-8 Ext. 9083
E-mail: chaidan_p@hotmail.com

HOME ADDRESS 183/21429 Tungsrigun 2 R.
Tungsrigun Donmueang
Bangkok Thailand 10210
Tel. 0-2929-2918