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Bioavailability of Acemanan: An Active Compound Found in Aloe Gel

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Abstract:- Acemannan is said to be the biologically active substance in aloe vera (Aloe barbadensis). Many producers of aloe products utilize inadequate production and extraction methods, resulting in aloe products that contain little or no acemannan. This article outlines a systematic procedure for extracting the bioactive polysaccharide compound from the aloe plant. This paper also provides a description of the physical distinctive features of acemannan. The study also emphasized the determination of physical properties, such as the pKa and Log P values, of acemannan. The physical characteristics were used to evaluate the bioavailability and hydrophilicity of this chemical. The primary approach used to acquire these physical characteristics involves the extraction of acemannan from aloe vera, the creation of phosphate buffer with varying pH levels, the separation of acemannan between chloroform and buffer using the shake flask technique, and the utilization of spectrophotometric analysis. Chloroform was used as a representation of the lipid membrane in the experiment, whereas phosphate buffer was utilized to symbolize the blood. A buffer solution was used to maintain a steady pH at a desired value. The acemannan compound had a pKa value of 4.82 at a pH of 3.45, indicating its acidity. Additionally, the Log P value (chloroform/buffer) was determined to be -3.282, indicating its hydrophobicity. Thus, it was deduced that acemannan exhibited hydrophilic properties throughout the gastrointestinal system.

I. INTRODUCTION

Aloe, belonging to the lily family, has been used both externally and internally for several millennia. The active component of the aloe plant is located in the gel of the inner leaf and is deemed to be safe and efficacious when appropriately handled.

A. Plant Taxonomy

Classification: Plantae, Classification: Angiosperms Classification: Monocots, Classification: Asparagales Family: Xanthorrhoeaceae Subfamily: Asphodeloideae. The genus is Aloe. Species: Aloe vera, The binomial name for Aloe vera is Aloe vera. Aloe vera is often referred to as the real aloe (Ombrello, 2008).

The term "aloe" is derived from the Greek word "alsos" and specifically denotes the acrid sap extracted from the leaves of these botanical specimens. It likely originates from the preceding Arabic term "alloeh" or the Hebrew term "allal," both denoting bitterness. Aloe extract is one of the most ancient forms of medicinal substances. The many forms of aloe plant extract include: fresh entire leaf, fresh gel (pulp), juice (sap), juice extract, and dried gel. Of these, the first three were limited in their use due to the need to protect the plant, but the latter two were commercially accessible. The crude has been obtained by the process of evaporation.

Boiling the juice separates the water and results in the formation of a black, solid, and glossy substance. This substance was used as both food and medicine. When produced by freeze drying, it often appears as a yellow or brown powder (Lee et al., 2001).

The health advantages of Aloe vera are mostly due to acemannan, the primary polysaccharide found in the gel of the plant's leaves. These biological actions include the stimulation of wound healing, the ability to combat fungal infections, the capacity to lower blood sugar levels, and the ability to protect the stomach from damage (Eric et al., 2009).

Although the existing biological activities of A. vera are briefly mentioned, the objective of this effort is to emphasize the newly found effects and uses of the leaf gel and its bioavailability. These effects include the capacity of A. vera's whole leaf or inner leaf gel liquid solutions to increase the absorption and availability of co-administered chemicals in the intestines, as well as improve the permeation of substances via the skin. Aloe vera has bioactive compounds that have anti-parasitic properties, as well as a rich concentration of essential minerals, vitamins, and amino acids. Furthermore, the plant also has elevated concentrations of a polysaccharide called acemannan. (Sinha et al., 2001)

Acemannan is a complex molecule made up of beta-(1, 4)-linked acetylated mannan. It has been shown to have antiviral activities and has been demonstrated in AIDS papers to limit the replication of HIV in laboratory tests. Additionally, it has been seen to excite macrophages, enhance the antiviral effects, and lower their toxicities (McDaniel et al., 1987).

Many makers of aloe products utilize improper production procedures, resulting in Aloe products that contain very little or no Acemannan, as was the case in ancient times. At present, the majority of manufacturers do not test for the presence of Acemannan in their finished goods.

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In order to constantly assure the effectiveness of the commercial aloe product and the associated health advantages, it is necessary to regularly use approved assays to measure the Acemannan content.

The active component of Aloe, Acemannan, is partly or completely eliminated by manufacturers during the manufacturing stage. Several of the preservatives used in Aloe products to control microorganisms may be harmful to skin fibroblast cells, which cancels out the advantages and prevents consumers from experiencing the potential benefits of Acemannan. Stabilized Aloe goods are now defined as products that include sufficient preservatives to regulate the development of microorganisms. On the contrary, a stabilized aloe product should ensure that the appropriate quantity and dimensions of Acemannan are maintained during the product's storage period.

During the processing step, it is possible to use appropriate extraction procedures to ensure that the correct amount of acemannan is retained in aloe products. The mass ratio of acemannan to aloe vera is very small, mostly due to the volatility of acemannan, resulting in a decrease in weight or mass following extraction. To avoid its loss, this highly volatile substance must be held in nitrogen gas.

Acemannan has diverse health ramifications when administered both internally and topically. Therefore, it is crucial to perform an experiment to determine its bioavailability.

Bioavailability refers to the percentage of a medicine in a certain dose form that is accessible to the body. By using the shake flask technique, we can determine the partition coefficient, which will enable us to make inferences on its bioavailability to the body.The shake-flask approach is applicable just to compounds that are very pure and soluble in both water and n-octanol. Surface active materials are exempt from this use

A computed value or estimate based on the individual n-octanol and water solubilities should be supplied.

The partition coefficient (P) is the ratio of the equilibrium concentrations of a dissolved material in a two-phase system composed of two solvents that are mostly unable to mix with each other. An example of a substance that may be used is n-octanol, which can be mixed with water or a buffer solution.

The partition coefficient (P) is defined as the ratio of two concentrations and is often expressed as the logarithm to the base 10 (log P).

When the logarithm of P is equal to zero, it indicates that acemannan is evenly distributed between the blood and the lipid membrane, resulting in a 50% bioavailability.

If the logarithm of P is greater than 0, it indicates a higher degree of lipophilicity, resulting in a greater amount of the substance remaining in the lipid membrane.

At a logarithmic value of P equal to 1, there exists a ratio of 10:1 between the organic and aqueous layers. It indicates that a significant amount of acemannan is distributed throughout the lipid membrane. If the logarithm of P is less than 0, it indicates that the substance is very hydrophilic. The Log P (Log10 partition coefficient) is used to comprehend the behavior of a medicine molecule inside the body (Sanjivanjit, 2007).

B. Objectives

- Determining the pKa and log P of acemannan in aloe plant
- The main objective of this experiment is to assess the behavior of Acemannan in the body and its bioavailability

C. Project Justification

Acemannan, the primary active component in the aloe plant, is often lost during the processing phase, resulting in a decrease in the effectiveness of most aloe products. Therefore, it is necessary to explore appropriate extraction procedures to ensure the retention of acemannan throughout processing.

Acemannan has diverse health consequences when administered both internally and topically. Hence, it is crucial to perform an experiment to determine its bioavailability. This information is useful for determining the amount of a substance that enters the bloodstream.

D. Chemical Assays

The composition of the substance is mostly made up of carbohydrates, which account for 0.3% of the total, and water, which makes up 98.5%. Gas liquid chromatography was used to analyze the composition of polysaccharides, as described by Hansel et al. in 1994.

II. LITERATURE REVIEW

Initial investigations suggest the potential efficacy of aloe as a supplementary treatment to conventional AIDS treatments, including AZT. Research done by H.R. McDaniel revealed that HIV-1 infected individuals who consumed 500-800 mg of acemannan daily had an increase in CD8 levels and maintained CD4 levels The study conducted by Sheets et al. in 1991 found that there was a significant increase in circulating monocytes/macrophages and an improvement in phagocytic activity.

The ability to enhance the immune system Acemannan has been shown to stimulate macrophages to produce interferon (INF), tumor necrosis factor-α (TNF-α), and interleukins (IL-This may aid in the prevention or elimination of viral infections. These three cytokines are recognized for their ability to induce inflammation, whereas interferon is secreted as a reaction to viral infections. Laboratory experiments have shown the ability to hinder the reproduction of HIV; nevertheless, experiments conducted on living organisms have yielded uncertain results (Harris et al., 1991). Acemannan is being used for the treatment and therapeutic care of fibrosarcoma in dogs and cats. Research has indicated that the administration of acemannan leads to an increase in

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tumor necrosis or extended life. Additionally, the mice treated with acemannan have displayed lymphoid infiltration and encapsulation, as reported by Harris et al. in 1991.

➢ *Adverse Reactions*

The use of topical aloe vera does not have any notable adverse effects. A 2-year research conducted by the National Toxicology Program (NTP) investigated the effects of consuming non-decolorized whole leaf extract of aloe vera. The study revealed conclusive evidence of cancer-causing activity in both male and female rats, namely in the large intestine where tumors were seen. Based on current knowledge, the NTP has found no evidence to suggest that these results are irrelevant to people. Gastrointestinal discomfort, including abdominal cramps and diarrhea, has been documented as potential side effects of oral use of aloe vera. Individuals with diabetes who are using glucose-lowering medication should exercise caution while consuming aloe orally due to early research indicating that aloe may have the potential to reduce blood glucose levels. Several case studies have documented instances of severe hepatitis resulting from the oral use of aloe vera. Nevertheless, the evidence lacks conclusiveness (Wang et al., 2004).

In addition, it is advisable for pregnant women to refrain from using laxatives derived from aloe during pregnancy. This is due to the potential adverse effects of consuming excessive amounts of oral aloe juice products, such as nausea, vomiting, and diarrhea (Yakugaku, 2003).2.2.0 Chemical tests The composition of the substance is mostly made up of carbohydrates, accounting for 0.3% of the total, while water makes up the majority at 98.5%. Gas liquid chromatography was used to analyze the composition of polysaccharides, as described by Hansel et al. in 1994.

➢ *Chemical Structure, Bioavailability, and Properties of Acemannan*

The physiochemical parameters used to determine the bioavailability of pharmaceuticals are the ionization constant (pKa) and the actual partition coefficient (Papp).

➢ *The Ionization Constant (pKa) of Pharmaceuticals*

The pKa refers to the pH value at which drug molecules undergo deprotonation or protonation in a solution. Acidic medications remain neutral when the pH is lower than their pKa, but they release hydrogen ions and become negatively charged when the pH is higher than their pKa. Fundamental medications have contrasting behavior. Uncharged molecules have a higher affinity for lipids and lower solubility, while charged species or molecules have greater solubility in an aqueous medium and lower affinity for lipids (Ràfols et al., 2012).

The pKa values give essential data on the interaction between ionizable drugs and charged biological membranes and receptor sites. They also provide information about the potential absorption sites of the drug in the digestive system (Sarfaraz, 2007). Understanding the pKa value allows scientists to determine the necessary pH adjustment to achieve complete ionization or non-ionization of a chemical.

For analytical and other reasons, such as formulation, solubility, and stability. Formulators need knowledge of the specific site of drug absorption inside the gastrointestinal system and its alignment with the most favorable area for absorption, particularly when developing an orally administered dosage form. Additionally, the significance of pKa lies in the fact that the majority of medications undergo ionization when dissolved in a solution. Furthermore, features such as lipophilicity, solubility, and permeability are contingent upon the pKa value.

Since most medications are classified as weak acids or bases, knowing the dissociation constant for each drug is crucial in determining the ionic form the molecule will assume at different pH levels. In physiological systems, the ionization state plays a crucial role in determining the rate at which a molecule may diffuse across membranes. The pKa of pharmaceuticals has a direct impact on their lipophilicity, solubility, protein binding, and permeability. These factors, in turn, have a direct effect on the pharmacokinetic features of the medications, including absorption, distribution, metabolism, and excretion (Manallack, 2007).

➢ *The Partition Coefficient (log P) and Lipophilicity of Medicines*

Lipophilicity is often quantified using the partition coefficient P (log p), which represents the ratio of a compound's (drug's) concentration in equilibrium between octanol and water (Lucas et al., 2010).

The logarithm of the partition coefficient (log P) serves as an indicator of whether compounds have the potential to be absorbed by humans or other living tissues, or whether they are more likely to be transported or spread by water. The lipophilicity of pharmaceuticals refers to their inclination to choose a lipid or oil-based environment over an aqueous one. The main physiochemical parameter that connects membrane permeability (drug absorption and distribution) with the clearance or elimination pathway is known as the key physiochemical parameter (Ania de la Nuez et al., 2008). Partitioning refers to the capacity of a medicine to disperse itself across two systems that are unable to mix with one other. When a medication is introduced into an immiscible system.

The medication is distributed in both octanol and water and finally finds equilibrium.The distribution coefficient determines the ratio of the medicine in each phase (AAPS NEWMAGAZINE, 2007).

The lipid/water partition characteristics have a significant impact on the fundamental mechanisms involved in medication action. For ionizable medications, the pharmacokinetic phase of drug action (absorption, distribution, and excretion) is influenced by the drug's dissociation in the watery parts of a living system that are separated by a lipid membrane. Thus, it is important to ascertain the lipophilicity (hydrophobicity) parameter and ionization constants of potential therapeutic candidates during the first phases of drug development (Kalisz et al., 2007).

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➢ *Properties and Bioavailability*

As noted from its structure, Acemannan is a mucopolysaccharide with mannoacetate as the monomer linked by β-1, 4- glycosidic. This polymer is hydrophilic: 50 hydrogen bond acceptors, and 19 hydrogen bond donors. Therefore, it is permeable and hence can be absorbed into the blood stream. The bioavailability of acemannan can be determined after finding its physical parameters log P and pK_a. It has 540 nm maximum wave length absorbance. (Eberendu, 1996)

III. MATERIALS AND METHODS EMPLOYED

A. Introduction

Materials included, aloe vera leaves (raw material), chemicals and reagents for analyses

B. Sample Collection

Raw material (Aloe vera) for analysis was collected at Bolgatanga and Kumasi located in northern and southern part of the Ghana respectively. The species of the aloe was identified as *Aloe barbadensis* by a botanist.

➢ *Equipments and Apparatus*

- pH meter
- Analytical balance
- Separating funnel (50 mL)
- UV-Vis spectrophotometer
- Laboratory glassware
- Blender
- Retort stand

➢ *Chemicals and Reagents for the Experiment*

- Sodium hydroxide
- Phosphate buffer [KH_2PO_4]
- HCl

C. Methods

➢ *Methods that Were Employed in the Analysis Included the Following*

- Extraction of acemannan from aloe
- Preparation of phosphate buffer with variable pH values of phosphate buffer solution
- Partitioning of acemannan between chloroform and buffer by the shake flask method
- Spectrophotometric analysis

➢ *Extraction of Acemannan from Aloe Vera using Ethanol*

- *Aloe barbadensis* leaves were cut gently from its plant
- *Aloe barbadensis* leaves were weighed and recorded as $\mathbf{m_1}$
- The leaves were then washed thoroughly with bacterialcidal solution (ethanol) to get rid of microbes (bacterial) from the leaves
- The lower end portion of the leaves were cut using a sharp knife to drain the yellow sap in the leaves
- The remaining portions of the leaves were cross cut into segments and placed upright in an aqueous solution to drain all remaining yellow sap for about twenty (20) to thirty (30) minutes.
- Using sharp knife, the rind (outer cover) of the leaf was removed to expose internal gel matrix portion (fillet).
- The fillets were washed under running water to remove any yellow sap and rinds that might have adhered to the fillet.
- The internal gel matrix was drained for an hour by placing the fillet on a mesh to obtain a gel matrix strip.
- The weight of the drained gel was recorded as $\mathbf{m_2}$.
- The gel matrix strips was transferred into a blender and blended at high speed for two minutes.
- The blended fillets were cooled in a refrigerator to allow the foam formed during blending to settle.
- The cooled blended fillets were then transferred into a Pyrex measuring cylinder and the volume was measured and recorded as $\mathbf{v_1}$.
- Ethanol solution of 95% was gently added to the measured volume in the ratio 3:1 while stirring.
- The flocculent precipitate (fiber) floating on the ethanol was removed using a stirring bar.
- The mixture was allowed to settle for four hours under room temperature.
- The pH of the precipitate was measured and adjusted with a 10 mL volume of HCl
- The ethanol together with other residue floating on the ethanol was decanted without disturbing the precipitate at the bottom of the mixture.
- The precipitates were transferred into test tubes and centrifuge at 5000 rpm for two minutes.
- The supernatant was decanted and the precipitates were transferred into a VWR brand test tube.

➢ *Isolation of Acemannan*

- Propylene glycol, 20 mL (v/v) was added to the precipitate in the VWR brand test tube and shacked vigorously.
- The mixture was centrifuged at 5000 rpm for two minutes.
- The thick smooth gel forming the supernatant was collected from the sediment.
- The viscous sample was diluted using distilled water to obtain a 30% (v/v) propylene glycol.
- The solution was then kept in a -30 ^oC freezer for 24 hours.
- The frozen solution was freeze dried for 24 hours

➢ *Physical Characterisation of Sample*

- A 20 mL 80% propylene glycol was prepared by:
- Measuring 16 mL of propylene glycol using graduated Pyrex.
- Distilled water was then added till the solution reached the 20 mL mark of the cylinder.

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- The mixture was then shaken vigorously for the two components to mix.
- The 20 mL 80% propylene glycol was then added to the precipitate and the mixture vigorously shaken.
- The mixture was then centrifuged at 5000 rpm.
- Supernatant was then transferred into a different test tube and the inference noted.

✓ Alkali Test

- A 50 mg gel was weighed in a test tube using a beam balance.
- One drop of concentrated NaOH was added.
- The mixture was then mixed by beating the tip of the test tube.
- One drop of distilled water was then added and the inference observed.

✓ pH Test

- A 0.2 (w/v%) solution of the gel was prepared by:
- Weighing 100 mg of the gel in test tube.
- 50 mL of distilled water was then added.
- The pH meter was then used to measure the pH at 27 ^{o}C.

➢ *Preparation of Buffer Solution*

Phosphate buffer solutions at different pH were prepared by adding specific volumes of 0.2 M NaOH to potassium dihydrogen phosphate of 0.2 M as prescribed by British pharmacopoeia using pH meter

➢ *Spectrophotometric Analysis*

- Different concentrations of acemannan (0.01, 0.015, 0.02, 0.05, 0.071, 0.17) in (w/v%) was prepared with 15 mL buffer solution of variable pH
- The solution was shaken vigorously and absorbancies were then recorded.
- The above process was repeated using chloroform as the solvent
- Two separate standard curves were then prepared

➢ *Partitioning of Drug Sample between Chloroform and Buffer Solution*

- A known mass (0.0034, 0.0062, 0.0114, 0.024) g of the sample was weighed and placed in the mixtures of the two solvents of equal volumes in a separating funnel.
- The solution was shaken vigorously then allowed equilibrium to establish between the two solutions
- The two phases were then separated and their respective absorbance recorded.
- The various concentration was determined using the standard curves

➢ *Determination of pKa and log P from a Linear Graph*

- Knowing the concentration in both phases P_{app} was calculated as

$$P_{app} = \frac{C_o}{C_w}$$

- **pKa** and **log P** was then calculated from a linear graph using the relation below:

$$\frac{1}{\Pi_{\alpha\pi}} = \frac{1}{\pi}\left(1 + \frac{K\alpha}{[H^+]}\right) + \frac{1}{\Pi}$$

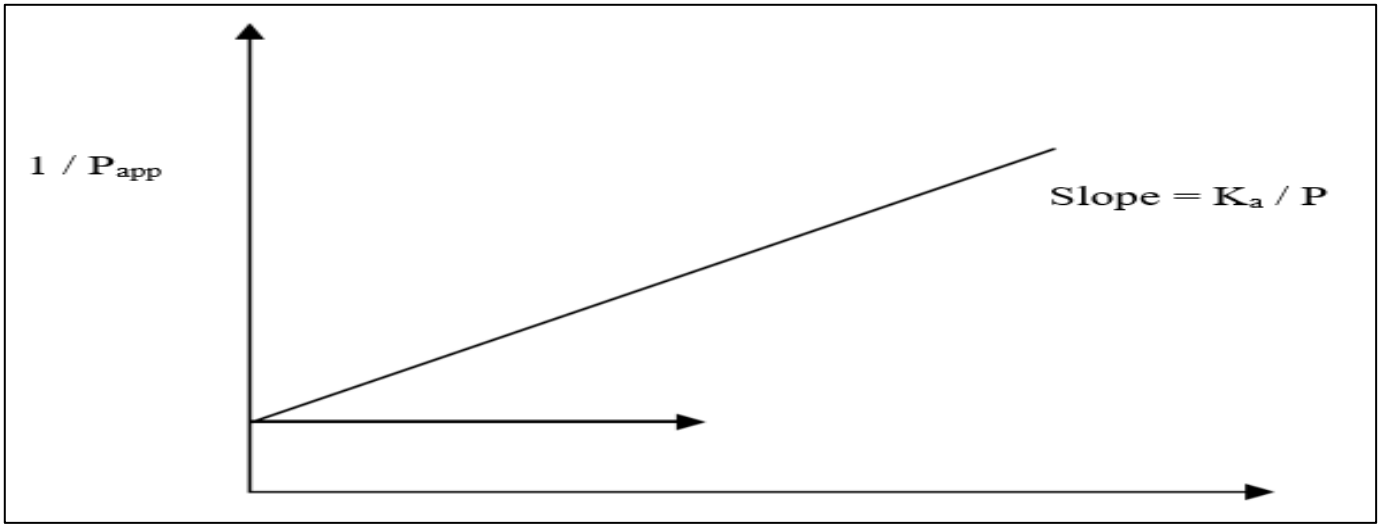

Fig 1: Graph

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IV. RESULTS AND DISCUSSION

The physiochemical characteristics, such as the dissociation constant (pKa) and partition coefficient (logP), are being considered.

Critical values are primarily taken into account while making judgments about medication composition. These physiochemical parameters provide insight into the dissolution and interaction of the manufactured medicine with biological membranes, as well as its absorption in the digestive tract (GIT).

A. Results Yielded from the Extraction of Acemannan from Aloe Vera ***(Aloe barbadensis)***

Mass of aloe vera plant (m_1) = 8.8 kg

Mass of aloe juice yielded from the plant = 600 g Weight of the leaves of *Aloe barbadensis* (m_1) = 8.8 kg.

Weight of gel matrix strip (m_2) = 600 g

Percentage of aloe juice yielded from the plant,

$$= \frac{\text{weight of gel}}{\text{weight of plant}} \times 100$$

$$\frac{0.06\ kg}{8.8\ kg} \times 100\%$$

$$0.06818182 \times 100\%$$

6.82%

Weight loss = Weight of outer leaves + water (m_3) = m_1-m_2

8.80 kg – 0.60 kg = 8.20 kg

$$\frac{8.20\ kg}{8.8\ kg} \times 100$$

Percentage weight loss = 93.12

Volume of aloe juice yielded from the plant = 500 mL

Initial pH of aloe juice = 4.6 at a temperature of 10.4 ^{0}C
Volume of HCl added = 5 mL

Final pH of aloe juice = 3.1 at a temperature of 10.4 ^{0}C
Volume of ethanol added = 1500 mL 4.1.2

➢ *Isolation*

Initial volume of propylene glycol and distilled water used = 20 mL

Volume propylene glycol added = 16 mL

Volume of distilled water added = 4 mL

(80% propylene glycol and 20% water)

Volume of propylene glycol and distilled water retrieved after centrifugation = 15 mL

volume of propyleneglycol in 15 mL = $\frac{80}{100} \times 15\ mL$

$0.80 \times 15\ mL$

$12mL$

Volume of distilled water in 15 mL = (15 – 12) mL = 3 mL

Volume of distilled water added during freeze drying = 28 mL
Total volume of distilled water used = (28 + 4) mL

= 32 mL

Volume of thick gel (isolate) after freeze drying = 3 mL

Yield of isolate after freeze drying = 1.50 g

B. Results Yielded from the Physical Characterization of Sample

After adding the 20 mL 80% propylene glycol and shaking vigorously, a thick smooth gel was formed indicating the presence of acemannan.

➢ *Alkali Test*

The isolate was unable to form gel after adding a drop of NaOH. So there is the presence of acemannan in which the acetal bonds have been cleaved.

➢ *pH Determination*

pH of the 0.2 (wt/v%) prepared at 27 ^{o}C was 6.60

C. Preparation of Buffer Solution

PREPARATION OF 0.2 M POTASSIUM DIHYDROGEN PHOSPHATE (KH_2PO_4) Weight of potassium dihydrogen phosphate (KH_2PO_4) = 6.8045 g

Molar mass of potassium dihydrogen phosphate (KH_2PO_4) = 136.09g/mol

$$\text{Mole of KH2PO4} = \frac{6.8045\ g}{136.09\ g/mol}$$

$= 0.05\ mol$

Volume of distilled water added = 250 mL

$$\text{Concentration of KH2PO4} = \frac{0.05 mol}{250\ mL}$$

$0.2\ M$

D. Preparation of 0.2 M Sodium Hydroxide (NaOH)

Weight of sodium hydroxide (NaOH) = 2 g

Molar mass of sodium hydroxide (NaOH) = 40 g/mol

$$\text{Mole of sodium hydroxide (NaOH)} = \frac{2g}{40g/mol}$$

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= 0.05 mol

Volume of distilled water added = 250 mL

Concentration of (NaOH) = $\frac{0.05\ mol}{250\ Ml}$

= 0.2 *M*

Specific volumes of 0.2 M NaOH was added to 0.2 M 50 mL KH_2PO_4 to yield the respective pH values

Table 1: pH Values of Phosphate Buffer Solution and Volume of NaOH

pH	**3.39**	**3.45**	**3.62**	**3.8**
Volume (mL) of 0.2 M NaOH	25.8	19.2	12.6	5.8

E. Spectrophotometric Results Concentration of Acemannan and Absorbance in Buffer Solution.

Table 2: Results of Concentration of Acemannan Absorbance in Buffer Solution

Concentration of Acemannan in buffer (wt/v %)	**Absorbance (540 nm)**
0.01	0.0021
0.017	0.015
0.02	0.00483
0.05	0.0091
0.12	0.025

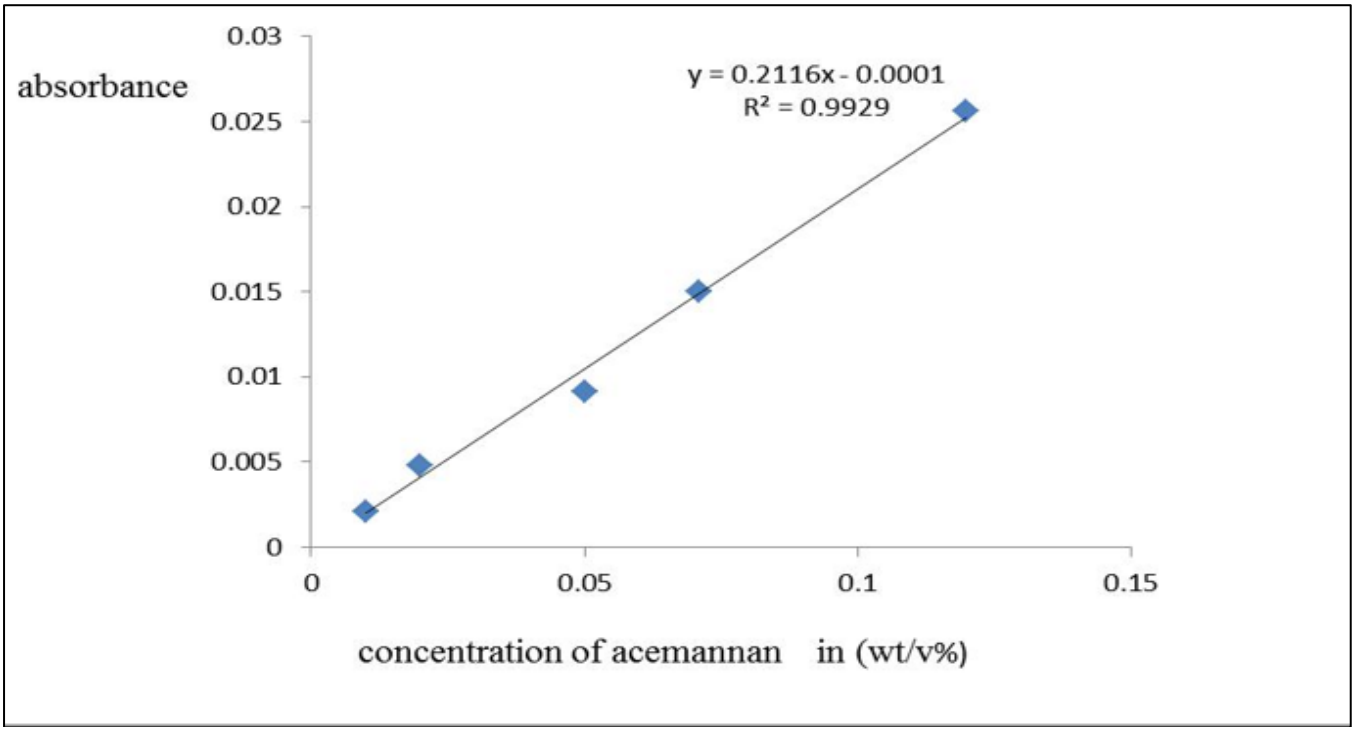

Fig 2: Standard Curve of Absorbance Against Concentration of Acemannan in Buffer Solution

F. Concentration of Carrisyn (Acemannan) and Absobance in Chloroform

Table 3: Results of Concentration of Acemannan Absorbance in Chloroform

Concentration of Acemannan in Chloroform (wt/v %)	**Absorbance (540 nm)**
0.01	0.0011
0.017	0.0019
0.02	0.0021
0.05	0.0054
0.12	0.0132

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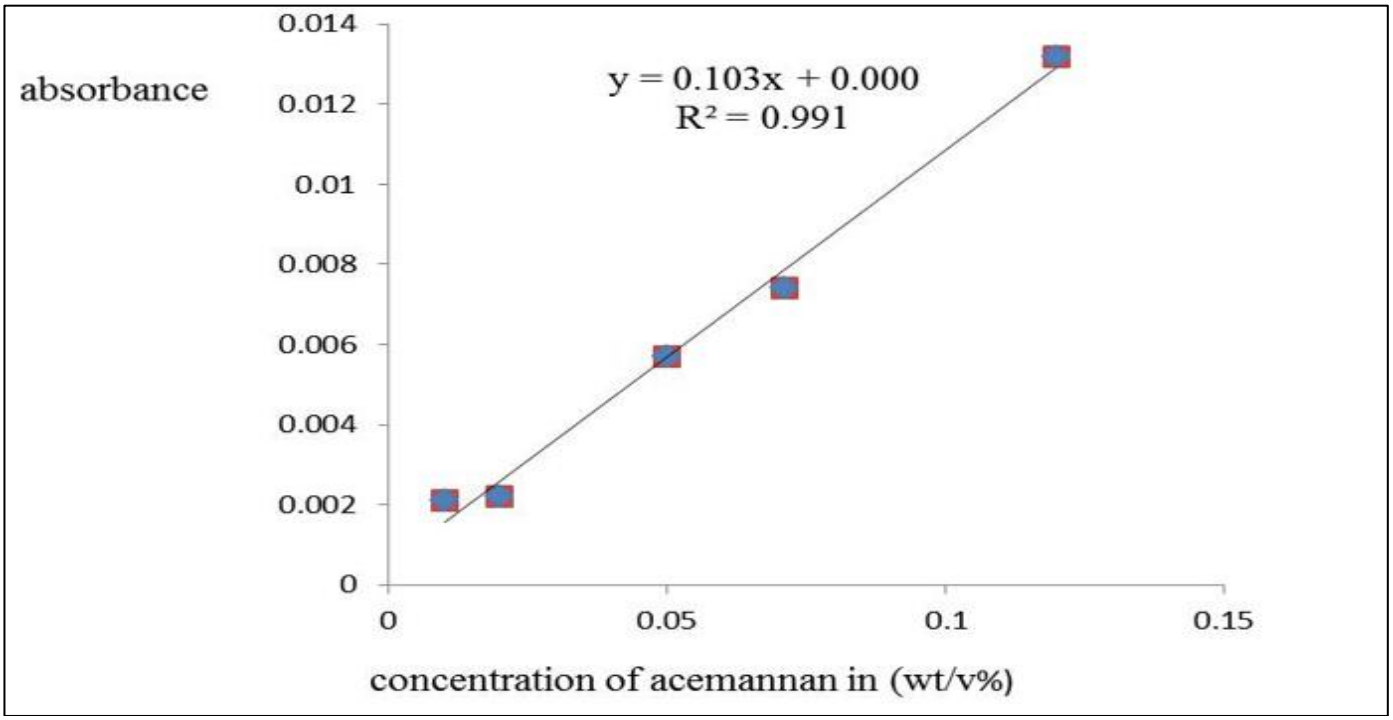

Fig 3: Standard Curve of Absorbance against Concentration of Acemannan in Chloroform

G. Concentration of Acemannan in Buffer Extrapolated from Standard Curve of Buffer Solution

Table 4: Results of Concentration of Acemannan Extrapolated from Standard Curve of Buffer Solution

Absorbance (540nm)	pH of buffer	$[H^{\square}]\ \square 10^{pH}$	Concentration of Acemannan in Buffer Solution
0.0034	3.39	$4.0738x10^{-4}$	0.0168
0.0068	3.45	$3.548x\ 10^{-4}$	0.0325
0.012	3.62	$2.39x\ 10^{-4}$	0.0571
0.024	3.80	$1.585x\ 10^{-4}$	0.115

H. Concentration of Acemnnan in Chloroform Extrapolated from Standard Curve of Chloroform

Table 5: Results of Concentration of Acemannan Extrapolated from Standard Curve of Chloroform.

Absorbance (540nm)	Concentration of Acemannan in Chloroform (wt /v %)
0.01	8.480×10^{-6}
0.018	1.632×10^{-5}
0.031	2.84×10^{-5}
0.060	5.48×10^{-5}

I. Concentration of Acemnnan in Chloroform (C_o), Buffer (C_w) and Apparent Partition Coefficient (P_{app})

Table 6: Results of Concentration of Acemannan in Chloroform, Buffer and Apparent Partition Coefficient

Concentration of acemannan in chloroform (wt/v %)	Concentration of acemannan in buffer C_w (wt/v %)	$P = C_o\ app\ C_w$
8.480×10^{-6}	0.0168	5.047×10^{-4}
1.632×10^{-5}	0.0325	5.02×10^{-4}
2.84×10^{-5}	0.0571	4.92×10^{-4}
5.48×10^{-5}	0.115	4.767×10^{-4}

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J. Hydrogen Ion Concentration [H^+] and Apparent Partition Coefficient (P_{app}) with their Respective Inverses

Table 7: Results of Hydrogen ion Concentration and Apparent Partition Coefficient and their Inverses

[H^+]	$\frac{1}{[H^+]}$	$P_{app} = \frac{C_o}{C_w}$	$\frac{1}{P_{app}}$
1.66x	2454.71	5.047×10^{-4}	1981.66
4.07x	2824.86	5.02×10^{-4}	1992.81
2.39x 10^{-3} 10^{-4}	41684.75	4.92×10^{-4}	2033.32
1.95x 10^{-4} 10^{-4}	6309.15	4.767×10^{-4}	2097.83

K. Determination of pKa and log P Ofacemannan

The physiochemical parameters pKa and log P were determined using the relation below:

$$\frac{1}{P_{app}} = \frac{1}{P}\left(1 + \frac{Ka}{[H^+]}\right)$$

Plotting $1/P_{app}$ against $1/[H^+]$ yielded the value of physiochemical parameters pKa and log P as shown below:

P_{app} = Apparent partition coefficient of drug; P = true partition coefficient

$[H^+]$ = hydrogen ion concentration of buffer solution; K_a = acid dissociation constant RESULTS OF $1/P_{app}$ AND $1/[H^+]$

Table 8: Plotted values of inverse of apparent coefficient ($1/P_{app}$) and inverse hydrogen ion concentration ($1/[H^+]$)

$1/[H^+]$	$1/P_{app}$
2454.71	1981.66
2824.86	1992.81
41684.75	2033.32
6309.15	2097.83

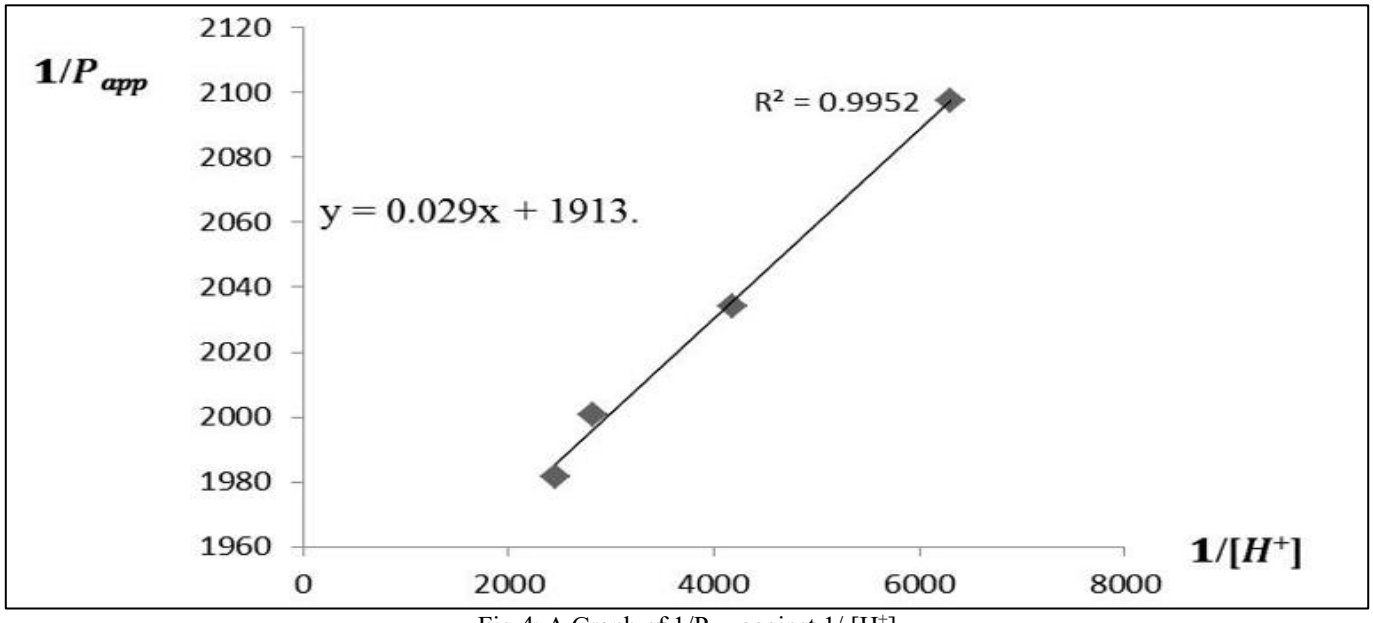

Fig 4: A Graph of $1/P_{app}$ against $1/[H^+]$

Comparing the equation of the line to the general equation

Slope $= K_P a$

Log P of acemannan was found to be -3.282
Also, slope=0.0291

$\frac{K_a}{5.2274}$

$P = 5.2274\times10^{-4}$

$\Rightarrow 10^{-4} = 0.0291$

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$K_\alpha = 1.51329 \times 10^{-4}$

$\pi K_\alpha = -\lambda o\gamma\, K_\alpha$

$= -\lambda o\gamma(1.51329 \times 10^{-4})$

$= 4.82$

Hence, $pK_a = 4.82$

Therefore the, pK_a of acemannan is 4.82. The partitioning coefficient log P (chloroform/buffer = -3.282)

Using Henderson-Hasselbach equation ionized portion and unionized portion of the drug can be calculated at pH = 3.45

$$\pi H = \pi K + \lambda o\gamma \frac{[A^-]}{[HA]},$$

$[A^-]$ represent ionized drug

[HA] represent unionized drug

$$3.45 = 4.82 + \lambda o\gamma \frac{[A^-]}{[HA]}$$

$$-1.37 = \lambda o\gamma \frac{[A^-]}{[HA]}$$

$$\frac{0.0427}{1} = \frac{[A^-]}{[HA]} \quad or \quad \frac{1}{23.4423} = \frac{[A^-]}{[HA]}$$

L. Percentage of Acemannan Ionized and Unionized at Ph = 3.45

$$\frac{I}{U+I} \times 100\% = \%\ \text{Ionised} \qquad \left(\text{b} \frac{U}{I+U} \times 100\% = \%\ \text{unionized}\right.$$

$$\frac{1}{23.4423+1} \times 100\% = 0.0427\ \% \qquad \frac{23.4423}{1+23.4423} \times 100\% = 95.73\ \%$$

0.0427 % ionized of drug 95.73% unionized of drug

V. DISCUSSION

Experimental findings have determined that allowing the mixture of ethanol and aloe gel to settle for four hours results in the highest yield of the active chemical substance (Mcanalley, 1993). The ratio of ethanol to aloe gel used in the experiment is 3:1, which produces a greater yield of the active compound compared to a 2:1 ratio (Mcanalley, 1993). Additionally, the amount of acemannan obtained from the plant is 1.5 g in 8.8 kg, confirming the literature's specified percentages of carbohydrates (0.3%) and water (98.5%). The carbohydrate's polymannan content is 60% (Hansel et al., 1994). Experiments have revealed that when ethanol is added, a significant amount of inorganic acids, specifically calcium oxalates, co-precipitate with the active compound. This is due to the high insolubility of calcium oxalates in both ethanol and water (Mcanalley, 1993). However, the addition of a mineral acid converts the insoluble oxalates into soluble ones.

$$2HCl + CaC2O4 + \longrightarrow CaCl2 + H2C2O4$$

Oxalic acid formed is soluble in ethanol whiles the salt, calcium chloride, is also soluble in water. This action reduces the amount of calcium oxalates that co-precipitate with the active compound when ethanol is added. This also allows the retrieving of high yield of the active compound. The pH range between 3.00 – 3.50 has shown to give much yield of the active compound (Mcanalley, 1993).Which helped me to adjust the pH of my sample within that range with HCl.

Acemannan, the potent pharmacological component in the aloe vera, has found to be soluble in 80% (v/v) propylene glycol and 20% water. It dissolves to give a thick smooth gel which 33 remains stable indefinitely. This property was observed during the isolation process when 16 mL propylene glycol and 4 mL distilled water was added to the sample. The freezing point of propylene glycol was found to be -55 ^{0}C so the sample had to be diluted with 28 mL distilled water. This made the freezing point of propylene glycol to be reduced and

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frozen at a desirable temperature of -30 ºC. Similarly, alkali treatment of acemannan destroys its gel forming property, indicating that the o-acetylation of acemannan influences its viscosity. Also, a 0.2% (w/v) acemannan has shown to have a pH of 6.31 ± 0.33 (Mcanalley, 1993).

➢ *Ionization of the Drug in Gastrointestinal Tract (GIT)*

pH of phosphate buffer used for the standard curve was 3.45 which correspond to the pH of the stomach and small intestine of GIT; therefore, by the use of Henderson-Hasselbach equation the ionized and unionized form of was determined. Also, the percentage ionize and unionize form of acemannan was also determined. From the calculation, unionized form of drug in a region of pH of 3.45 dominates the ionized. This is because for acidic drugs to fully ionize, the pKa of the drug should be below the pH of the surrounding fluid according to (Sarfaraz, 2007). Comparing the value of log P which is -3.282 to that of the standard set by literature which is -3.27 we can fairly conclude that acemannan is highly hydrophilic.

The primary objective in drug design and formulation is to make the drug interact with biological system and to produce a positive biological response. Drug brings a positive biological response when the drug is able to interact with main target such as lipids, carbohydrate, nucleic acid and proteins. Drugs ability to interact with macromolecules in the biological system stems from the parameters such as pK_a and log P. The pK_a and the log P enables drug designers to know the medium in which the drug would be absorbed, thus whether in aqueous medium (blood) or lipid medium (lipid membrane).

VI. CONCLUSION

Employing appropriate extraction processes and techniques may result in a greater yield of acemannan from aloe vera. The value of Log P, which is -3.282, suggests that acemannan is hydrophilic and does not have an affinity for lipids. Highly hydrophilic medicines have low absorption due to their difficulty to traverse lipid-rich cell membranes (Greg M. Peal, 2001). In order for a medicine to be easily absorbed, it has to be soluble in both aqueous and lipid solutions. Highly lipophilic drugs are carried in the aqueous solutions of the body by carrier proteins, such as albumin. Acemannan exhibits hydrophilic features, but also has lipophilic characteristics that enable its absorption into the bloodstream.

VII. SUGGESTION

Acemannan, the primary active component found in the aloe vera plant, is responsible for its beneficial effects. Unfortunately, many aloe products have become less effective because they have undergone processing that results in the loss of acemannan. Hence, I suggest the use of appropriate extraction techniques throughout the processing stage.

Studying the pKa and log P of medications is essential for comprehending their behavior in the biological system. Additionally, it is advisable to do additional study on the subject matter of the assignment.

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