Aloe Extracts

213-0

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Current Status of Quality Control of Aloe barbadensis Extracts

Summary

For over 11 years now the International Aloe Science Council (IASC) has been striving to ensure that Aloe products are undiluted, unadulterated, and of good quality. For the last three years, scientists, supported by the Aloe Research Foundation (ARF), have been at work perfecting tests to make aloe certification and quality control possible *(2–5)*. In this article, a summary of a paper at the recent European Conference on Aloe, we will examine the developments in analytical chemisty that are bringing us closer to the IASC's goal of benchmark tests for Aloe gel identity and quality; **1.** measurement of ions *(5)*, **2.** high pressure liquid chromatography or HPLC *(2, 3)* and **3.** measurement of total polysaccharides.

Background – Certification and Quality Control of Aloe

Ultimately, analytical chemistry serves two functions for the Aloe Industry: **1.** establishing that a material contains Aloe (Certification) and **2.** determining the quality of the Aloe (Quality Control). Certification **(Table I)** is a process developed by the International Aloe Science Council (IASC), a trade organization composed of approximately 150 corporate members engagend in various aspects of growing, processing, and marketing Aloe products. The IASC certification seal **(Figure 1)** indicates that the company has undergone an inspection and audit to verify **1.** that operations are conducted under sanitary conditions and products are bacteriologically clean **2.** that plant capacity matches product output **3.** that consumption of raw materials equals product output and **4.** conducts chemical tests of Aloe products to determine if the product's alleged content of Aloe is consistent with what is currently accepted as the chemical composition of bona fide Aloe. At present, for liquid gel extracts, the IASC Standards consist of four test parameters **(Table II)**. The certification process is overseen by a Science and Technology Subcommittee comprised of quality control personnel (TAW) from some of the larger and more technologically advanced companies. University-based scientists are not part of the IASC. However, for the last 11 years, the IASC

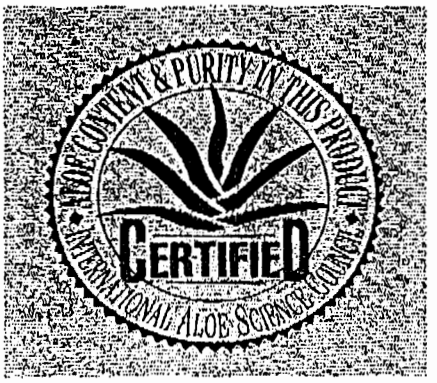

Fig. 1 IASC certification seal

On Site Activities

A. Aloe Facility Inspection
1. Assembly Line/Processing Line
2. Laboratory
3. Storage – Cold
4. Storage – Room Temperature
5. Raw Materials Records
6. Equipment – Production

B. Aloe Raw Materials Audit

C. Aloe Finished Product Audit (for each product)
1. Percent of Aloe in Final Product Based on Label Claim
2. Percent of Aloe in Final Product Based on Formulation Data

D. Manufacturing Audit (for each product)
1. Total Amount of Aloe Purchased
2. Products Manufactured
3. Starting and Finishing Inventory

Off Site Activities

A. Laboratory Tests
1. Solids Content
2. Calcium Content
3. Magnesium Content
4. HPLC Profile
5. Microbiology (as part of Onsite Inspection A.2)

Table I IASC – ARF Quality Assurance Program

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Test	Average	Range
Solids (105°C Nonvolatiles)	0.92 g per dl	0.75 to 1.50
Calcium	33 mg per dl	23.3 to 52.3
Magnesium	3.9 mg per dl	3.2 to 4.7
HPLC Profile*	67% of freshly prepared leaf	51 to 110%

* As originally stated, the peaks to be examined in this test were unspecified as was the standard sample of Aloe to which the analyte was compared. For certifications conducted in 1991–1992, the peak analysed was »E Peak« and the standard sample was Aloe Research Foundation Standard Sample ARF'91A Process A (Crude Gel), Frozen.

Table II Chemical Laboratory Tests for IASC Certification of Aloe barbadensis Gel (1982–83 Database)

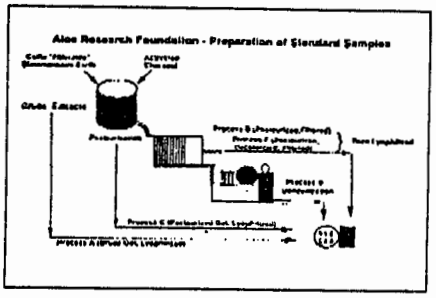

Fig. 2. Preparation of Aloe Research Foundation (ARF) Standard Samples.

sponsors an annual Scientific Seminar at which academicians summarize recent scientific studies of Aloe materials. By and large the IASC does not sponsor scientific work although academic scientists (RPP) have collaborated in the certification process *(4)* and have been supplied (YTW) with fresh Aloe barbadensis leaves for the purpose of establishing a analyte data base *(5)*.

The Aloe Research Foundation (ARF), chartered three years ago, is funded by several of the largest companies in the Aloe industry and is charged with sponsoring and supervising research on basic and applied aspects of Aloe materials. The Scientific Advisory Board of the ARF, composed of industrial and academic scientists with established credentials in Aloe research, reviews grant applications, supervises the progress on funded projects, and acts as a pool of experts to advise the ARF and IASC. Two of us (RPP & TAW) are members of the Scientific Advisory Board of the ARF. The ARF also sponsors a yearly scientific symposium on Aloe research and the biennial International Congress of Phytotherapy. The ARF is in the process of establishing a professional service library to make available scientific and technical documents on Aloe materials.

Since 1991, the ARF has sponsored and supervised the preparation of Standard Samples of Aloe barbadensis extracts **(Figure 2, Table III)**. These carefully prepared extracts are made on a semi-industrial scale starting with metric ton amounts of leaves and producing up to several kg of freeze dried extracts. The ARF Standard Samples represent the best approximation of authentic Aloe materials available and are dispensed in lyophilized from to scientists and industrial quality control laboratories regardless of whether or not the scientist is funded by the ARF or whether the company is a contributer to the ARF. The only proviso is that the scientists be of academic repute and that the companies use the Standard Samples for their own internal quality control purposes. To date, 7 Standard Samples of gel extracts have been made (ARF'91A through ARF'93A). The major thrust of these programs of the ARF is elucidating the biological properties of Aloe materials and developing quality control tests for Aloe.

Field Operations

Highest degree of sanitization obtained
Gel filleted from leaves within 1 hour of leaf harvest
Pulp removed by passage through 250μm mesh
Chilled 4°C within minutes of depulping

Plant Operations

Crude Gel (Process A)
Lyophilized, within 2 hours of filleting

Pasteurized (Process C)
65°C, 15 min then lyophilized

Filtered – (Process D)
After Pasteurization, absorbed with Celite »Filteraid« Diatomaceous Earth Silica Gel and passed through 20μm Cellulose Filter

Decolorized – (Process E)
Pasteurized, absorbed with Norite Darcoal activated charcoal, »Filteraid« Diatomaceous Earth, and filtered

Concentrated – (Process B)
Any of the above materials concentrated by rising/falling thin film evaporation under reduced pressure prior to freeze drying

Storage

Dessicated, 4°C or -20°C

Table III Aloe Research Foundation Standard Samples

Many of the tools that industrial Aloe quality control labs use are those that are also used for IASC Certification. These are record keeping, bacteriology, solids content, Ion determinations, and HPLC analysis. Over the last two years these tests have increased in their power because of a statistical database developed at Texas A & M University, Weslaco Agricultural Experiment Station *(5)*.

Quality control labs also employ tests such as pH and color that are primarily for specification purposes and other tests (such as anthraquinone content) that are primarily for health and regulatory purposes. Some of these tests are not yet completely chemically or statistically validated. Examples of these are assays for polysaccharide or anthraquinones. Hopefully, future scientific development will bring these tests up to the standards that the IASC Certification Tests are now approaching.

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The Chemistry of Aloe

All the chemical tests are based upon our knowledge of the chemical composition of Aloe and its relationship to the biological activities of Aloe. Aloe barbadensis gel extracts are 99.5% water – all of the biological activities specific to Aloe thus reside in the 0.5% non aqueous molecules that comprise the solids content of Aloe. In general *(6,7)*, Aloe is composed of molecules such as the quinones aloin and aloe-emodin *(8)*, polysaccharides of various chemical linkages and varying molecular weight *(9–13)*, reducing sugars, a mixture of proteins *(15–17)*, and molecules of lower molecular weight such as salts, fats, amino acids and other molecules *(7)*.

The solids of Aloe gel extracts can be chemically thought of as containing two crude classes of molecules – large molecules and small molecules. The macromolecules make up approximately 20% (range in ARF Standard Samples, 10 to 25%) of the solids content of Aloe gel and are overwhelming polysaccharide. Studies at the University of Texas have confirmed earlier published studies *(9–13)* indicating that Aloe gel extracts contain a mixture of polysaccharides including a linear highly acetylated β1–4 mannan, branched dextrans, pectic acids and other polysaccharides with negative charge. In contrast to the claims of some commercial concerns *(14)*, we do not find that only one highly linear polysaccharide (the acetylated β1–4 mannan) is essentially the only polysaccharide of Aloe species gel extracts. Polysaccharide hexose makes up approximately 10% of the total mass of the solids of Aloe. Associated closely with these polysaccharides are small molecules, primarily positively charged ions. Proteins, on the other hand, make up only approximately 1% of the solids content of Aloe barbadensis extracts. The predominant proteins in gel extract are of low molecular weight (11,000 and 14,000 kd respectively for the two polypeptide chains). They are probably associated with the Aloe lectin *(15, 16)* and may account for the mitogen activity described for Aloe *(17)*. Caution must be exercised in interpreting the protein content of Aloe because the β1–4 mannan appears to react in some of the biochemical assays commonly used to determine protein content (the Lowry assay and the Coumassie Blue dye binding assay).

The small molecules of Aloe extracts fall into four chemical groups **1.** cations **2.** anions, **3.** sugars, and **4.** neutral or amphipathic small molecules. Of these, the cations and anions comprise approximately 50% of the mass of the small molecules. Later, we will devote an entire section of this paper to the cations and their measurement. The anions consist of chloride and the organic anions, chiefly organic acids. The organic acids are notable mostly for their tendency to form insoluble precipitates with calcium when Aloe extracts are concentrated. The free, reducing sugars, consisting primarily of glucose and mannose, make up about 20% of the small molecules but vary greatly in content from extract to extract *(5)*. Therefore they are of little utility for purposes of certification and quality control although their presence or absence may be useful in detecting cases of adulteration. The remaining small molecules consist of a mix of at least 100 compounds including sterols, terpenes, amino acids, neutral lipids and of course anthraquinones.

A great deal of the published literature on Aloe species is devoted to the anthraquinones. The commercial importance of the anthraquinones lies in the area of quality control. Anthraquinones are undesirable in consumer products because of concerns over their long term toxicity *(21–23)*. Furthermore, anthraquinones are susceptible to oxidation and therefore tend to be associated with product color change problems subsequent to processing and compounding. Although fascinating to the analytical biochemist, anthraquinones are generally removed during the processing of Aloe extracts by absorbtion with activated charcoal. This removal makes them unsuitable markers for certification.

Biologically, Aloe latex was traditionally associated with the cathartic activity of anthraquinones *(8)*. Gel extracts of Aloe barbadensis **(Fig. 3)** are associated with emolliency (see the numerous patents on the subject), acceleration of wound healing *(18, 19)* and reduction of inflammation *(18, 20)*. Aside from the laxative activity of the Aloe anthraquinones (which are not present in significant concentration in most commercial gel extracts) we do not know for certain which molecules are associated with which biological activities.

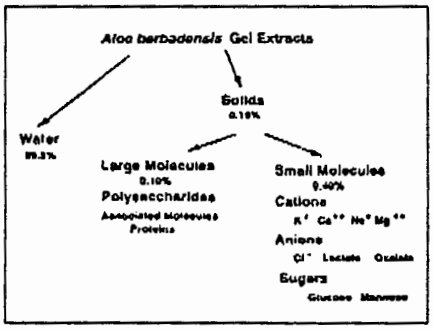

Fig. 3. The chemical composition of Aloe barbadensis gel extracts based on *(6)* and our own unpublished results. For details of preparation see **Table II.**

Aloe Chemical Data Bases

Given this state of knowledge, between 1982–1984 the IASC somewhat arbitrarily selected a set of analytes **(Table II)** to serve the purposes of quality control *(1)*. This was based upon the **1.** presence of these molecules in Aloe, **2.** their stability and persistance during processing into commercial products and **3.** our ability to quantitate them. The IASC and ARF commissioned one of us (YTW) to determine the concentration of these 4 analytes in samples consisting of 3 Aloe leaves (yeilding approximately 500 g of gel extract). Leaves were raised on 4 different plantations and were harvested at weekly intervals throughout a two year cycle. Recently, we completed the sample acquisation phase of the study and have presented the data on solids content and Ca^{++} and Mg^{++} *(5)*. We have also studied the content of Na^{+}, K^{+} and reducing sugars. It is hoped that the complete database will soon be made available to the entire industry and serve as the statistical basis for future Certification Programs. Analyte values for the ARF Standard Samples closely approximate the mean values for the analytes in the Texas A & M study.

Determination of Ions

The determinations of solids, K^{+}, Ca^{++}, Na^{+} and Mg^{++} are straightfoward. Confer Table IV for the values of these analytes to date in the Texas A & M study. Solids are measured by taking an aliquot, evaporating at about 65° C and drying overnight at 105° C. Salts are best determined by atomic absorbance spectroscopy because the salts are partially tied up with the organic acids and charged polysaccharides. For example,

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the calculated ionic strength for the Texas A & M distribution of the four major cations is 3.70 ± 1.38 mMHO (mean ± Standard Deviation) based on a solution of the four cations, with Cl^- as the anion. Direct determination of Aloe barbadensis gel extract ionic strength yeilds a value of 1.7 ± 0.49 mMHO. This indicates that only 50% of all ions exist in solution in the freely ionized form. Therefore determination of individual ion concentrations by a method such as ion selective electrodes is fraught with danger. Ion selective electrodes (or determination of ionic strength) can be very useful in a relative sense when appropriate standards (such as ARF Standard Samples) are employed. However, this was the reason that a primary reference method (atomic absorbance) was employed in the Texas A & M study.

Figure 4 illustrates the distribution of the 4 cations in the four plantations sampled. There are two significant sources of variability in the distributions. First there is sample to sample variability over time at a single farm (for example, at Farm 1, Ca^{++} versus Mg^{++} versus K^+ and Na^+). The second source of variability is farm to farm variability (for example Farm 1 versus Farm 4). Realizing that there are multiple sources of variability in the data base, we can examine the distribution of ion content values (**Figure 5**). With the exception of Na^+ (skewed by the low values of Farm 4), the values are normally distributed. We can state with high confidence that there is a threshold of combined cation content that essentially all Aloe barbadensis gel extracts contain. If we take twice the standard deviation as our confidence limit (**Figure 5, arrows**), the probability of falsely rejecting an Aloe sample is very low. This is because when the values for one analyte are low (for example, **Figure 4**; Farm 4, Na^+; Farm 3, K^+), the value for other analytes compensate.

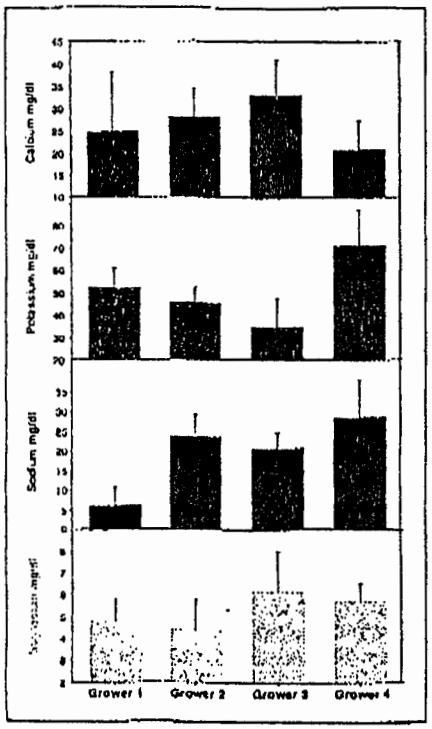

Fig. 4. Variation in Content of Calcium, Potassium, Sodium, and Magnesium in Fresh Aloe barbadensis Gel Extracts Among the Four Participating Growers. Data are from that portion of the Texas A & M population that was analysed as of June, 1992 (**Table IV**). Values expressed are the mean ± one standard deviation (1 σ).

Atomic absorbtion analysis has serious deficiencies as a routine quality control tests. The equipment is expensive, difficult to maintain, and requires skill to operate. If conducted commercially, the cost of analysing a single sample of Aloe for all four cations generally exceeds $ 100. In this case ionic strength (determined by conductivity) can be used as a screening test because the equipment is cheap and the test is easily and quickly performed. **Figure 6** illustrates the results of conductivity testing on the 7 ARF Standard Samples reconstituted to a solids content of 0.59 g/dl (the average solids content of the Texas A & M distribution). The mean ionic strength of the sample was 1.74 mMHO with a standard deviation of 0.49 mMHO and a coefficient of variance of 28%. Maltodextrin, a common adulterant, has negligible conductivity (0.026 mMHO). The average coefficient of variance of the Texas A & M cation distribution is somewhat larger (37.5%) than that for the ARF Standard Sample sample (28%). Adopting the Texas A & M distribution, the lower two standard deviation limit for conductivity is 0.44 mMHO. Using this as the lower confidence limit for Aloe Ion content allows us to quickly and cheaply screen samples and perform atomic absorbance spectroscopy

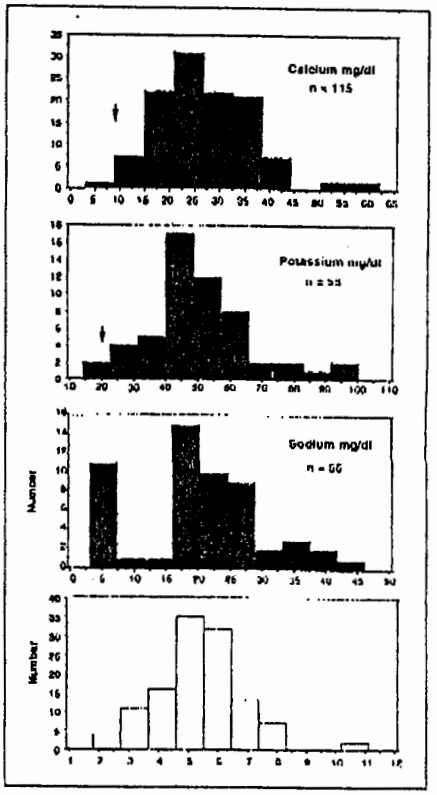

Fig. 5. Frequency Distribution Analysis of Calcium, Potassium, Sodium, and Magnesium Content in Fresh Aloe barbadensis Gel Extracts. The arrows indicate two standard deviations (2 σ) below the mean for the samples. These data are derived from the same samples as described in **Figure 4** and **Table IV.**

Test	Mean	Standard Deviation	Coefficient of Variance	n
Solids (105°C Nonvolatiles)	0.59 g per dl	0.11	18%	201
Calcium	27 mg per dl	10	37%	115
Magnesium	5 mg per dl	2	28%	120
Sodium	20 mg per dl	10	50%	55
Potassium	51 mg per dl	17	34%	55
HPLC Profile*	»E Peak« Present In All Cases			200

*The standard sample of Aloe to which the analyte was compared Aloe Research Foundation Standard Sample ARF'91A Process A (Crude Gel), Frozen.

Table IV Texas A & M Study of Aloe barbadensis Gel 1991–93 Database (Analyses to Date)

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for the four cations only on those materials with conductivity below 0.44 mMHO at a solids content of 0.59 g/dl. This sort of screening results in a great savings of money and time.
It is theoretically possible to adulterate Aloe by addition of salts and organic solids with the aim of disguising dilution.

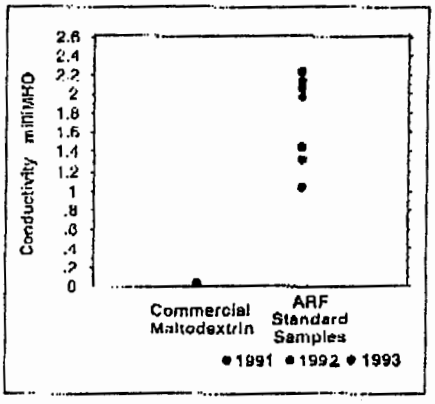

Fig. 6. Conductivity of Aloe Research Foundation (ARF) Standard Samples, Process A. The seven Standard Samples of Aloe barbadensis gel extract, Process A (Crude gel, lyophilized directly from 1:1 gel without processing) were suspended at a concentration of 0.59 g/100 ml in deionized water and conductivity determined with a Radiometer Conductivity Meter (Copenhagen, Denmark). Values expressed are mean conductivity in reciprical OHMs per cm.

We have not observed this to date in our analysis of commercial Aloe powders (which are usually adulterated with low ionic strength maltodextrin) although it may occur with liquid gels. Further efforts are underway to chemically analyse Aloe in order to discover molecules in Aloe – particularly those chemicals mediating the biological effects of Aloe that can serve as rigorous certification standards. Recently studies have focused on the use of HPLC because of the ability of this technique to separate components without prior extraction or derivitization. Efforts have been made using this technique to find a molecule that serve as a diagnostic marker for high quality Aloe.

What is HPLC

Chemical tests tend to fall into two categories: **1.** tests where the molecule we attempt to measure (the analyte) is detected by a highly specific chemical reaction and **2.** tests which first separate analytes one from another and then detect analytes by methods which rely on general physical-chemical properties of broad classes of compounds. Classic examples of the first type of test are the analysis of sugar and protein in blood and urine. HPLC, thin layer chromatography and gas chromatography are the best known examples of the second type of test. **Figure 7** illustrates in skematic fashion an HPLC for Aloe barbadensis gel extracts and **Table V** lists the conditions for its use.

The IASC Diagnostic Molecule – E Peak

Approximately 10 years ago, Dr. Fleming found emperically that cation exchange matrices (bonded amine HPLC columns) yeilded a pattern for Aloe that could be interpreted after some degree of experience. At Aloecorp, it was noted that in commercial preparations of Aloe, there was a late eluting peak that was consistently present in fresh Aloe (**Figure 8**, upper Panel, peak eluting at approximately 12.5 minutes). This peak appeared to correlate with a commercially acceptable product. In many commercial preparations this was the fifth peak, in order, to elute. Since peaks are usually named with letters in the order that they elute (e.g. A, B, C, D, E, etc.) this diagnostically useful material was termed »E peak«. Unfortunately, the chemical identity of this material was unknown at that time. Furthermore, no chemically pure reference standards were available for calibration of quantitation and no standard samples were available for standardization of retention time. Even so, the needs for quality control and quality assurance were pressing enough so that the HPLC method was adopted as part of the certification process. A standard proceedure has been suggested by the IASC for this proceedure (**Table V**).
In January of 1991 the Aloe Research Foundation decided to adopt this system as one of criteria for the analysis of Aloe and systematic efforts were made to include this analysis in our explorations of the biological activities of purified Aloe materials. The first stage in our studies was to produce a set of reference samples of Aloe barbadensis representative of the native plant or of commercial materials prepared under carefully defined conditions – the ARF Standard Samples. In the Texas A & M study, »E Peak« was present in all samples tested. By comparison with this population we are confident that the HPLC profiles of the ARF Standard Samples are representative of Aloe barbadensis commercially grown in the lower Rio Grande Valley.

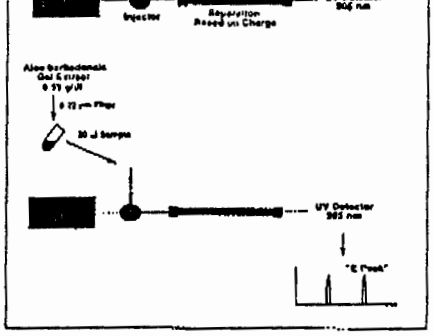

Fig. 7. Skematic of HPLC

Properties of »E Peak«

Preliminary studies of the IASC diagnostic molecule »E Peak« has yeilded the following preliminary findings **Table VI.** »E Peak« is a highly acidic molecule based on its extractive properties and late elution from the cationic amino column. It is heat stable (105° C) even in the presence of sodium hydroxide (although it is labile at pH 1). It is reasonably stable in sterile solutions (several weeks at 4° C). **Figure 9** demonstrates that »E Peak« is not removed during usual industrial processing which includes Pasteurization, activated charcoal absorbtion and filtration in the presence of Celite diatomaceous earth (On this particular HPLC column, »E Peak« elutes at approximately 10 minutes. The variability of elution time with aging of the column emphasizes the need for the use of ARF Standard Samples for calibration of the technique). »E peak« has relatively little UV absorbance suggesting the absence of aromatic rings (thus ruling out an anthraquinone or chromone structure) or conjugated dienes. Studies that we will present later demonstrate that this material is readily assimilated by bacteria that are capable of growing in Aloe. Gel fil-

tration of commercial Aloe materials results in concentration of this material in fractions of molecular weight less than 500 but it is larger than the inorganic anions and cations abundant in Aloe. Studies of these gel filtration fractions by Dr. *W. D. Winters* at the University of Texas Health Sciences Center in San Antonio suggest that this material is neither an in vitro growth factor nor is it particularly toxic to cells in culture.

Over the last year we have been purifying »E peak« from a number of ARF Standard Samples by classical organic chemical means. The lower panel of **Figure 8** illustrates the purity of the material we have isolated. Currently, we and others are engaged in determining the structure of »E peak«. The techniques we are using include measuring the absorbtion of light in the ultraviolet and infrared regions (which tells us about the bonds connecting the atoms), mass spectroscopy (which measures the size of the molecule and tell us how it breaks apart) and nuclear magnetic resonance spectroscopy (which measures the vibration of the atoms in a magnetic field and tells us which atoms are hooked to each other). These studies will allow us to draw a picture of the chemical structure of »E Peak«.

Bonded Amino Column (Alltech, 4 mm × 250 mm)	
Mobile Phase:	70% Acetonitrile 0.05M dihydrogen phosphate (pH 5.6)
Flow rate:	1 ml per minute
Eluates monitored at 205nm.	
Samples prepared by passage through 0.22 µm filter	
Sample volume:	20 µl

Table V IASC Standard Procedure for HPLC Analysis of Aloe barbadensis Extracts

Present in Highest Concentration in Fresh Leaf Extracts
Unaffected by Commercial Processing
Presumably Acidic with Significant Nonpolar Component
Stable to Heat and Base
Not an Anthraquinone
Assimilable by Aloe Associated Bacteria
Low Molecular Weight
Neither Growth Inhibitor nor Growth Factor

Table VI Characteristics of the IASC Diagnostic Molecule (»E Peak«)

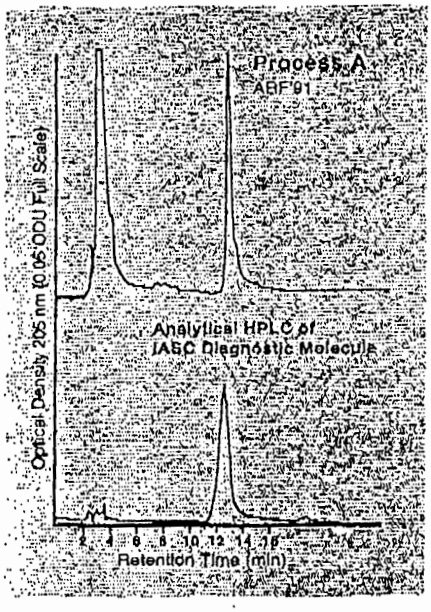

Fig. 8. Upper panel: HPLC Analysis of ARF Standard Samples of Aloe barbadensis Gel Extract # 1 for 1991 (ARF '91A). This material was Process A (Crude gel, frozen 1:1 gel, without processing) This material was thawed, passed through a 0.2 µm filter and 20µl was injected. Samples were chromatographed under IASC conditions (**Table V**). »E Peak« is considered to be the peak eluting at 12.5 minutes. Note that in the lower panel, »E Peak« is considered to be the peak eluting at 12.3 minutes. This degree of variation is considered to be usual with isocratic elution from Amino columns and appears to be a partial function of column loading as exemplified by the relative size of the upfield peaks. As the column »ages« there is a tendency for the peak to elute at earlier retention times which is only partially reversed by treatment of the column with strong organic solvents. Extremes of pH are to be avoided in attempts to »regenerate« the column as these may »strip« the amino groups from the matrix.

Lower Panel: HPLC Analysis of Purified »E Peak« IASC Diagnostic Material. Material was isolated by two cycles of solvent extraction followed by preparative HPLC on a 26 mm diameter Spherisorb NH_2 column in IASC buffer. Pooled material was concentrated by rotary evaporation to remove acetonitrile and the aqueous solution was lyophilized. The amount of phosphate in the sample was calculated from the composition of the Buffer. Approximately 5 µg of purified »E Peak« was injected. Conditions of HPLC are IASC Standard conditions except for scale (0.05 ODU full scale in this figure).

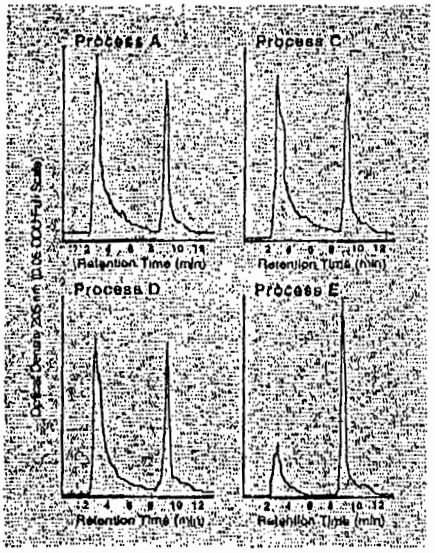

Fig. 9. Effect of »Commercial Processing« of Aloe barbadensis Gel Extracts upon Content of »E Peak«. The materials were from Aloe Research Foundation Standard Sample # 4 for 1991 (ARF '91D). Samples consisted of the following: Process A (Crude Gel, lyophilized directly from 1:1 gel without processing), Upper Left Panel; Process C (Gel Pasteurized for 15 minutes at 65° C and then frozen and lyophilized without

further processing), Upper Right Panel; Process D (Pasteurized Gel, incubated with Celite diatomaceous earth »Filteraid« and then passed through a cellulose filter), Lower Left Panel; and Process E (Pasteurized Gel, incubated with Norite activated charcoal »Darcoal« and diatomaceous earth and then filtered), Lower Right Panel. These materials were stored frozen and after thawing filtered through 0.2 µm filters before 20 µl were injected. Columns were run under IASC Standard conditions.

HPLC and the Bacteriology of Aloe

Studies, at the University of Texas, in collaboration with Dr. *J. Heggers*, and in the laboratory of Dr. *L. Sheffield* at the University of Wisconsin, have shown that Aloe barbadensis extracts are bacteriostatic for a wide variety of organisms. However, there are some bacteria, particularly those organisms associated with the rind of Aloe, that are capable of growing in Aloe gel. We have isolated a wide variety of bacteria from ARF preparations and have maintained these organisms as pure cultures on artificial media. Under certain circumstances bacteria can cause commercial problems with Aloe extracts.

Some of the organisms we have identified are, indeed, the same as organisms causing commercial problems. Earlier, studies at Aloecorp had noted that »E Peak« was decreased or absent in preparations of Aloe contaminated with large numbers of bacteria. An emloyee of Nam Yang Aloe Company, *B. S. Kang* recently spent some time in the University of Texas laboratory examining this problem systematically. Aloe extracts can be absolutely sterilized – either by membrane filtration or by high (105° C) heat without the loss of »E. Peak«. This sterile Aloe can then serve as a growth media to explore, in controlled fashin, the growth of defined organisms **(Figure 10)**. Some bacteria, such as members of the genus Bacillus **(Figure 10, Panel A)**, are incapable of growing in liquid Aloe-containing medium. Other bacteria, such as the Gram– rod Cedecea (Panel B) or the Gram+ coccus Enterococcus **(Panel C)**, are capable of multiplying in Aloe 9 fold in 24 hours at room temperature. Aloe extracts in which bacteria have not grown have a normal »E Peak« content **(Figure 10, Panel A)**. However, the growth of certain bacteria such as Cedecea or Enterococcus causes the disappearance of »E Peak« from the Aloe – presumably because the bacteria eat it. Additionally new peaks appear in the chromatogram (e.g. Panel B, at 5.5 and 12 minutes; Panel C, 5.5 minutes) that were not present in fresh Aloe. Interestingly enough, by these experiments we can produce a chromatograph that looks very much like the HPLC profile (Panel D) we observe in ARF Crude Gel that has been allowed to sit at 4° C for three weeks without Pasteurization or any preservatives.

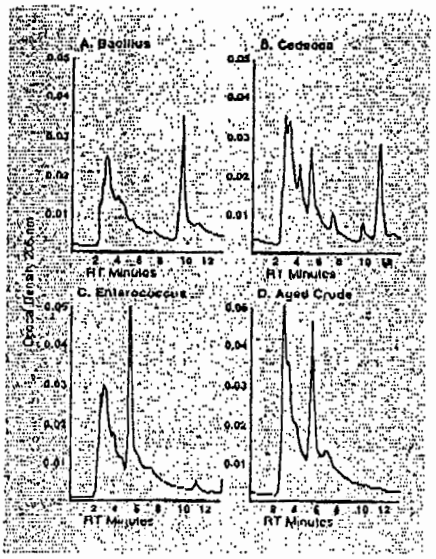

Fig. 10. Effect of growth of bacteria in Aloe barbadensis Gel Extracts upon Content of »E Peak«. The culture media consisted of Aloe Research Foundation Standard Sample # 4 for 1991 (ARF '91D) Process D (Pasteurized Gel, incubated with Celite diatomaceous earth »Filteraid« and then passed through a cellulose filter). The Aloe liquid was sterilized by sequential passage through membrane filters terminating in a 0.2 µm filter. Organisms were isolated by at least two cycles of picking and streaking isolated colonies. Taxonomy was determined by morphology on Gram stain and biochemical typing (Autobac). Bacteria were innoculated into sterile Aloe at a concentration of approximately 30,000 CFU/ml. Bacterial numbers were determined by dilution and spread plating on respectively Yeast Extract Nutrient Agar (Bacillus), Levine Agar (Gram negative organisms), and Columbia CNA Agar (Gram positive cocci). Cultures were incubated at 23° C and organisms enumerated and samples taken for HPLC analysis at 8 hour intervals. By 36 hours, the cultures innoculated with the Gram negative rods and the Gram positive cocci had attained stationary phase (> 100,000,000 CFU). Samples were filtered through 0.2 µm filters prior to HPLC analysis. Aged Aloe consisted of ARF '91D Process A material that was never lyophilized or frozen. Rather it was stored, without preservatives, at approximately 23° C until analysis.

Caveats About HPLC

Experiments such as these suggest that HLC cannot only be used to tell if fresh Aloe is present, but can also tell us something about how carefully the Aloe was handled and processed. HPLC promises to add a whole new dimension to Aloe quality control. However, it is obvious that the Interpretation of HPLC profiles is complicated and we should be cautious when we read HPLC chromatograms. Commercial materials frequently contain preservatives (anti-oxidants and anti-microbials). These compounds and their breakdown products introduce novel peaks to the chromatograms. During the production of beverages other additives are added that can further complicate interpretation. Since the final elucidation of the structure of »E Peak« remains incomplete, we must at present rely on the use of ARF Standard Samples for calibration of the HPLC technique. All of the authors strongly recommend against over interpretating HPLC »fingerprints« and over reliance on a single technique for quality control of Aloe.

New Techniques Under Development

Currently we are developing an assay to measure the polysaccharides that comprise the bulk of the large molecules in Aloe. The purpose of these tests is not based solely because of their content in Aloe but because maltodextrin apparently is commonly being used to adulterate Aloe powders. The technique, using freeze-dried or spray-dried powder **(Figure 11)** is based upon the older publications isolating and characterizing the different subclasses of polysaccharides found in different Aloe species *(9–13)*. The polysaccharides are precipitated by classical means using alcohol. In authentic Aloe only 10–25 % of the material precipitated is polysaccharide.

Aloe Extracts

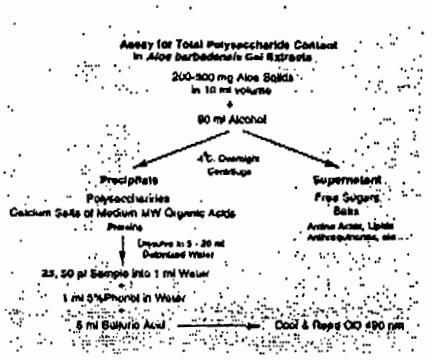

Fig. 11. Assay for Total Polysaccharides.

The rest of the mass consists of poorly alcohol soluble complexes of organic acids and divalent cations. The polysaccharide in the precipitate is then measured with the Dubois assay for total hexose *(25)*. As **Figure 12** illustrates, free sugars such as mannose have minimal activity in this test while approximately one half of the mass of commercial maltodextrin is alcohol precipitable hexose. ARF Standard Sample Aloe barbadensis gel extract (ARF'93A, Process E) contains about 6 % polysaccharide as alcohol precipitable hexose -- a value in keeping with those in the scientific literature. Commercial »Aloe« powders containing amounts of alcohol precipitable hexose similar to maltodextrin are likely, in fact, to be maltodextrin. Confirmation that this is so requires hydrolysis of the polysaccharide, sugar analysis, and analysis of the likages involved. Such confirmatory studies are obviously more the task of academic laboratories than industrial quality control laboratories. However, the test for total polysaccharides outlined above offers a quick, cheap and easy method for initial screening of powders.

Obiously, our tests for polysaccharides must undergo the same sorts of analytical development, data base accumulation, and industrial confirmation as the tests for ions and HPLC have undergone. However, this is the first assay that tests the macromolecules of Aloe. Hopefully with time assays for Aloe macromolecules will, probably in some modified form, become accepted by the IASC.

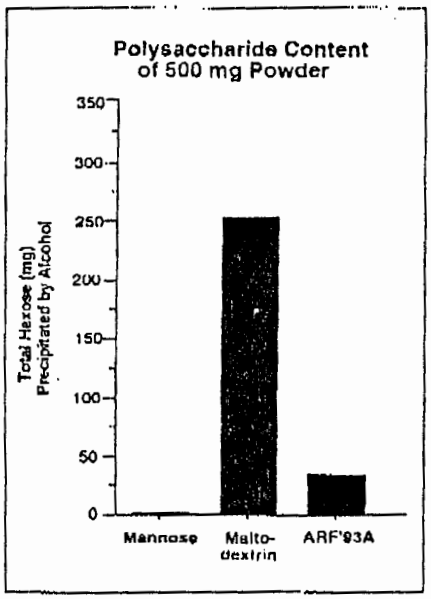

Fig. 12. Polysaccharide content of 500 mg powder.

Conclusions

What we have achieved to date is this. There are at present several tests for determining the quality of Aloe feedstocks. The authors do not regard any single test as definitive but we believe that they are best run in ordered sequence. The best strategy is to buy a IASC Certified feedstock. In the case of materials such as Aloe powders where no IASC Certified feedstocks are available, it is best to buy material from an IASC Certified producer. Common sense dictates that producers who have something to hide, avoid certification. If tests are needed, the first test to be run can be ionic strength using ARF Standard Samples as controls. If ionic strength is more than 75 % lower than ARF Standard Sample then the individual ions should be measured by atomic absorbtion spectroscopy. If HPLC equipment is available, a profile should be generated using a Spherisorb Amino packing under IASC conditions. The fingerprint should be compared to ARF Standard Sample Aloe. The degree of testing employed should be a function of the capabilities of the quality control lab and the suspection with which the feedstock is viewed.

We are continuously adding new analytes to the QC armamentarium. In the future, the availability of purified »E peak« will mean that we can quantitate the freshness of Aloe with a precision and reproducibility we never before had. Whether or not »E peak« will be truely useful in identifing a product as Aloe will depend upon how widely distributed »E peak« is in nature, its structural identity and its biological activity. Next, will be the deployment of assays for polysaccharides. In the far future, as the ARF identifies the active ingredients in Aloe, we will be able to develope chemical tests based on biological activity.

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Copies of articles pertaining to Aloe not commonly accessible in general public or academic libraries may be obtained from the Aloe Research Foundation library service. These are often from industrial publications such as references 1–5 above or from somewhat obscure scientific journals (such as references 6, 11, 18 & 19 above). Contact Dr. *T. Tolbert,* Executive Director, Suite 800, 910 Houston St., Fort Worth, Texas 76102-6627.

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