THE INFLUENCE OF CUTICULAR WAXES AND SELECTED CATIONS ON THE PERMEABILITY AND ELASTIC PROPERTIES OF TOMATO FRUIT CUTICULAR MEMBRANES

> Dissertation for the Degree of Ph. D. MICHIGAN STATE UNIVERSITY DOROTA HAMAN BURGESS 1983





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#### ABSTRACT

#### THE INFLUENCE OF CUTICULAR WAXES AND SELECTED CATIONS ON THE PERMEABILITY AND ELASTIC PROPERTIES OF TOMATO FRUIT CUTICULAR MEMBRANES

by

#### Dorota Haman Burgess

The permeability of the cuticular membrane of the tomato fruit influences the water potential of the fruit itself. Since water potential is related to fruit cracking, it is important to understand the influence of certain chemicals on the permeability of the cuticle surrounding the fruit. The strength of the cuticular membrane is another important factor in the cracking of tomatoes. The objectives of this investigation were

- to show the influence of cuticular waxes (lipids) on the permeability of the cuticular membrane of the tomato fruit,
- (2) to investigate the influence of selected cations (H\*, K\*, Ca\*\*, Al\*\*\*) on permeability,
- (3) to investigate the elastic properties of dewaxed and nondewaxed tomato cuticular membranes treated with these cations.

Using a modified technique for measuring water permeability, an increase in permeability due to the removal



of waxes (lipids) and due to certain treatments (Al<sup>+++</sup> for nondewaxed cuticular membranes and Ca<sup>++</sup> for both dewaxed and nondewaxed) was found. From the theory developed for a modified experimental tensile test, a decrease in the elastic constant Et, the product of the elastic modulus of the cuticular material and the thickness of the membrane, was found to occur in dewaxed cuticular membranes. Poisson's ratio was also calculated. More investigations on the influence of chemicals on material properties is suggested.



#### THE INFLUENCE OF CUTICULAR WAXES AND SELECTED CATIONS ON THE PERMEABILITY AND ELASTIC PROPERTIES OF TOMATO FRUIT CUTICULAR MEMBRANES

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Dorota Haman Burgess

A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Agricultural Engineering



To my parents: Zofia and Janusz - ----



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#### 1. INTRODUCTION AND OBJECTIVES

The outermost layer of a tomato fruit is the cuticular membrane. The elastic properties of this membrane contribute to the strength of the fruit, in particular, to its resistance to cracking. It is very likely that cracking is induced by a rapid change in water potential in the fruit and since the role of permeability of the tomato fruit cuticle to water vapor influences this water potential, permeability is another factor related to cracking of the tomato fruit.

There were two main objectives in this research:

- to investigate the permeability of cuticular membranes when treated with various cations, and
- to determine the change in the elastic properties of the membranes as a result of these treatments.

Since the application of certain chemicals to the tomato fruit are known to change the permeability of the cuticular membrane (Schonherr, 1976a);  $H^+$ ,  $K^+$ ,  $Ca^{++}$  and  $Al^{+++}$  ions were chosen for investigation in this research. Previous research indicates an increase in permeability with  $K^+$ treatment (see Chapter 2) and a change in cracking resistance with  $Ca^{++}$  treatment (Bengerth, 1973). These treatments may also influence the elastic properties of the cuticular



membrane and therefore must also be investigated for this effect. In this research, a modified tensile test was performed in order to analyze the elastic properties of chemically treated and untreated cuticular membranes. The influence of naturally occurring soluble waxes (lipids) on the elastic properties of the cuticle was also investigated.


2. LITERATURE REVIEW

#### 2.1 The Cuticle

The cuticle covers the aerial organs of terrestrial plants and serves as a barrier between the tomato fruit and its surrounding environment to limit water loss from the fruit. However, the cuticle is not impermeable to water and under extreme conditions, wilting may occur (Martin and Juniper, 1970).

The cuticle consists of a cutin polymer matrix of nonextractable esters of hydroxylated fatty acids. The main components of the cutin are two families of fatty acid monomers: a  $C_{16}$  and a  $C_{18}$  (Kolattakudy 1981). The cutin matrix is separated from the underlying epidermal cell wall by pectic substances. Extractable lipids (waxes) are deposited on the outside surfaces of cuticular membranes and embedded in the cutin matrix (Martin and Juniper, 1970).

The cuticular membrane can be viewed as a two component system; the extractable lipids (waxes), and the nonextractable polymer matrix (Norris and Bukovac, 1968). The permeability of the cuticle to water in an 'in-vitro' system is directly related to the amount of cuticular waxes (Skoss, 1955). Baker and Bukovac (1971) showed that the composition of surface waxes was important in the



permeability of the cuticle. Hydrocarbon and aldehyde fractions strongly impeded the passage of water while esters and fatty acids were found to be less restrictive. The composition of the tomato cuticle (cutin and waxes) was studied by Baker, Bukovac and Hunt (1982). For mature tomato fruit, the cutin was found to contain the following monomers:

16 Hydroxyhexadecanoic acid- 4.6%Hydroxyhexadecane-1, 16-doic acid- 4.1%10,16 - Dihydroxyhexadecanoic acid- 76.8%9,16 - Dihydroxyhexadecanoic acid- 6.4%8,16 - Dihydroxyhexadecanoic acid- 6.2%7,16 - Dihydroxyhexadecanoic acid- 2.2%

The constitutents of epicular wax fractions of the mature fruit were as follows:

Hydrocarbons	29%		
Fatty Acids	trace		
α-amyrin	6%		
β-amyrin	21%		
Naringenin	32%		
Chalconaringenin	11%		

The composition of cuticular wax varied from epicular wax. Cuticular wax was found to consist of:

Hydrocarbons	15%		
Fatty acids	43%		
$\alpha$ -amyrin	9%		
β-amyrin	32%		



Naringenin

0.8%

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Schonherr (1976a, 1976b) conducted extensive studies on the water permeability of isolated cuticular membranes. By treating the cuticular matrix and cuticular waxes as two resistances acting in series, Schonherr concluded that permeability to water was determined primary by the waxes and that permeability changes with different ionic forms of the membrane; permeability followed the order Li<sup>+</sup> < Na<sup>+</sup> < K<sup>+</sup> < Rb<sup>+</sup>. The dependence of permeability of cuticular transpiration on water activity was investigated (Schonherr and Schmidt, 1979). They found that the water potential across the membrane was the driving force behind cuticular transpiration.

Schonherr, Eckl and Gruler (1979) showed that the permeability coefficient for the cuticular membrane was temperature dependent. The original distribution of soluble cuticular lipids is irreversibly altered above 44°C and is accompanied by an increase in water permeability. Permeability of dewaxed cuticular membranes was shown to be strongly dependent on relative humidity due to the presence of polar functional groups in the polymer matrix. However, for non-dewaxed cuticular membranes the permeability coefficients were only slightly affected by relative humidity, showing that the movement of water was limited by a hydrophobic barrier that lacks dipoles (Schonherr and Merida, 1981).



The behavior of the cuticular membrane can also be greatly influenced by the nature and concentration of the fixed charges in the polymer. The fixed charge concentration of the polymer affects sorption and diffusion of water and electrolytes by affecting the water content (swelling) of the polymer and by imparting permselectivity. Schonherr and Bukovac (1973) found that at constant pH and salt concentration, the exchange capacity increased with increasing counter-ion valence and decreasing crystal radius.

Swelling of the matrix is a function of its chemical form. The cutin matrix in the Na<sup>+</sup> form swelled more than in the Ca<sup>++</sup> form (Schonherr, 1976a). Calcium ions associate more closely with fixed charges than sodium ions and reduce swelling because only one half the number of osmotically active particles are present (Schonherr and Bukovac, 1973).

An excellent review of the research and literature on water permeability in cuticular membranes was presented by Schonherr (1982) as a relationship between the cuticular membrane structure, membrane composition, and permeability.

Certain properties of the cuticle can be changed by treatment with different chemicals. The influence of calcium on plant membranes is widely discussed in the literature. Highly disorganized cell membranes can be restored by the addition of calcium (Bangerth, 1979). Ca in the cell walls and the middle lamella appears to play an important role in reducing cracking of fruits due to a



strengthening of the constituent of middle lamella. (Bangerth, 1973). Dickinson and McCollum (1964) also pointed out that calcium may be related to fruit crack resistance since it depends on cell wall strength. The distribution of calcium throughout the plant is closely correlated to the distribution of water along the xylem yessels. During the period of rapid growth, very little water enters the tomato fruit through the xylem since the water is supplied by mass flow through the phloem. This mass flow does not carry a significant amount of Ca. Because of this, a non uniform distribution of calcium may occur among different parts of the plant (Wiersum, 1966; Vangoor, 1968). Calcium deficiency in plants can be observed as the cells break down along with loss of turgor which causes the tissues to become water-soaked. Eventually, the tissue may become desiccated, yielding a dry area of necrosis (Simon, 1978).

Potassium has been known to enhance the drying of different plants (Dudman, 1962; Chambers and Possingham, 1963; Tullberg, 1978; Dunman and Grncarevic, 1962; Grncarevic, Radler and Possingham, 1968, Columbella (trans. 1945) described a method of making raisins in 60 A.D. by dipping grapes into a solution of boiled ashes of vines mixed with a little oil. This technique, which uses potassium carbonate and oil, has been proven to give good results under laboratory conditions (Dunman, 1962; Dunman and Grncarevic, 1962). It was suggested (Chambers and Possingham, 1963) that air spaces between wax platelets become filled with liquid



during dipping and the potassium carbonate in the solution changes the wax from hydrophobic to hydrophilic. However, washing the dipped grapes within two days reduced the increased transpiration to that of undipped grapes (Grncarevic, Radler and Possingham, 1968). One can conclude, therefore, that the potassium was not bound in the cuticular matrix since the  $K^+$  ions were removed during washing.

Potassium also enhanced the drying of alfalfa (Tullberg, 1978; Tullberg and Angus, 1972) and reduced tomato fruit cracking (Inverson, 1938). Inverson suggested that the decrease in tomato fruit cracking was due to the more fibrous type of root system resulting from an application of potassium permanganate to the soil.

### 2.2 Mechanical Properties of the Tomato Fruit

Mils, Friedley and Jorenzen (1969) suggested that the epidermis was the component of the fruit controlling mechanical strength which is related to cracking and puncture resistance. A number of tests have been developed and performed to describe the rheological properties of agricultural products. (Mohsenin, 1970; Sharma and Mohsenin, 1970; Morrow and Mohsenin 1965, Friedley et al., 1968). A widely accepted method of testing the strength of the tomato epidermis is the puncture test which is also used as an index in relating tomato epidermae strength to fruit cracking (Voisey and Lyall, 1965; Voisey and MacDonald, 1964; Voisey, Lyall and Kloek, 1970; Johannessen, 1949; Tennes, 1973). Miles, Friedley and Lorenzen (1969) tested



tomato fruits Using flat plate compression and internal pressure methods. Three different techniques to determine tomato fruit strength, tensile, puncture and bursting diaphram methods, were used by Voisey and Lyall (1965).

Altisent and Sierra (1979) applied quasi-static compression and impact tests to different varieties of processing tomatoes. They also studied the epidermis of processing tomatoes and concluded that epidermal strength depended on the shape of the epidermal cells and cuticle penetration. The correlation between the above properties of the epidermal layer and tomato cracking was also investigated by Conter, Burns and Leeper (1969); however, they could not relate the shape of the cells to tomato skin puncture resistance. They observed that tomato fruits resistant to concentric cracking possessed flattened epidermal cells.

Voisey, Lyall and Kloek (1970) suggested that crack resistance can be related to greater cutinization of the epidermal layer and underlying cells. However, they also concluded that the elongated shape of the epidermal cells did not change cracking resistance.

Failure and relaxation tests were used to evaluate tomato skin behavior (Hankinson and Rao, 1979). It was concluded that failure occurred in the middle lamella, between cells, and that the shape of the cells and the degree of deposition of cutin affected cracking. From the above, one can only conclude that there are contradicting

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opinions about the influence of cell shape on fruit cracking.

Since the change in water status of the fruit is thought to be the direct cause of tomato fruit cracking, Murase and Merva (1977) investigated the elastic modulus of the tomato epidermis as affected by water potential. They related mechanical properties with potential characteristics by applying relaxation tests to skin segments with different water potentials.

Only a few investigators have attempted to test tomato fruit strength by applying stresses similar to those which occur under natural conditions. Internal pressurization as used by Miles, Friedley and Lorenzen (1968) seems to be closer to the stress which tomatoes undergo in the field. In their experiments, force deformation characteristics were compared with those of an elastic sphere and a spherical membrane filled with water in order to gain insight into the structural composition of the fruit.

A preliminary theoretical development using stresses in thin shells was performed by Tennes (1973). The theory of shells has also been used by Considine and Brown (1981) to describe certain aspects of the physics of fruit growth. A theoretical analysis of the forces occurring during the growth period was related to cracking and splitting. The shape of the fruit was found to be a very important factor in determining the stress distribution and region of failure in the cuticular membrane.



3. MATERIALS AND METHODS

#### 3.1 Chemical Treatments

Cuticular membranes were enzymatically isolated from two sources (separate fields) of "Pik Red" tomatoes (Lycopersicon esculentum L.) and one source of "UC 82" processing tomatoes. Segments of tomato epidermis were taken from fruits free of visual defects and placed in a solution of 5% pectinase and 0.2% cellulase in 0.2M phosphate citrate buffer, pH 3.7. The pieces of epidermis were then incubated at 37°C and the solution was changed every three to four days. After about 10 days, when the cuticular membrane was free from the underlying cells, it was rinsed thoroughly with distilled water. This technique was developed by Orgell (1955) and modified by Yamada (1962). It is generally felt that the morphological and physiological features of the intact cuticular membrane are retained by this procedure (Norris and Bukovac, 1973).

The above method was compared by Hoch (1975) to isolation using the zinc chloride-HCl method and ammonium oxalote-oxalic acid reflux procedure for cuticular membrane isolation. It was found that the membrane isolated with pectinase and cellulase appeared most similar to the nonisolated cuticular membrane. Schmidt, Merida and Schonherr



(1981) compared fine structures of cuticular membranes and of dewaxed cuticular membranes to non-isolated membranes with a scanning electron microscope and found no harmful effects on the cuticular membrane due to above method of isolation.

The separated cuticular membranes from the three sources were divided into two groups. One group was dewaxed by extracting with chloroform for two hours followed by a methanol extraction for two hours. The extraction of waxes was done at 20°C using approximately 1000g of chloroform and 1000g of methanol for 50g of cuticular membrane. (Fig 3.1) The other group was not dewaxed. Both groups of the cuticular membranes were washed with 6 N hydrochloric acid (HCl) for three hours twice and rinsed with deionized water after each wash (see Fig 3.2). The procedure was done at 20°C with 1000g of acid for every 50g of cuticular membranes. Each of the two groups (dewaxed and nondewaxed) was divided into five subgroups which were subjected to the following chemical treatments (Fig 3.3): 1 N solutions of hydrochloric acid (HCl), potassium-chloride (KCl), calcium chloride (CaCl<sub>2</sub>), and aluminum chloride (AlCl<sub>3</sub>); the fifth subgroup in each group of cuticular membranes was left untreated and used as the control group. All of the treatments were done at 20°C with the cuticular membranes thoroughly submerged in the reagents using a weight proportion of 50g of cuticular membranes for every 1000g of reagent. The reagents were applied twice for 2 hours with a



deionized water rinse after each application.



Fig 3.1 Procedure for the extraction of waxes from cuticular membranes.

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ISOLATED DEWAXED AND NON-DEWAXED CUTICLES

(Repeated Twice)

Fig 3.2 Washing procedure for all groups of cuticles.





Fig 3.3 Procedure for application of ions to cuticular membranes.



The two sources of "Pik Red" tomatoes and one source of "UC 82" which were treated separately, yielded 24 sets of chemically treated samples and 6 sets of control samples (3 with and 3 without wax) see Fig 3.4. These samples of cuticular membranes were observed under a scanning electron microscope. There were significant differences between the dewaxed and nondewaxed surfaces of the cuticular membranes (Fig 3.5, 3.6). However, there was no way to distinguish between chemical treatments in the dewaxed and nondewaxed a subgroups.



## CUTICLES

FIELD A (PIK RED)		FIELD B (PIK RED)		FIELD C (UC-82)		
	н+	н+	н+	н+	н+	н+
	к+	к+	к+	к+	к+	К+
	Ca++	Ca <sup>++</sup>	Ca <sup>++</sup>	Ca <sup>++</sup>	Ca <sup>++</sup>	Ca <sup>++</sup>
	Al+++	Al+++	Al+++	Al+++	Al+++	Al+++
	CONTROL	CONTROL	CONTROL	CONTROL	CONTROL	CONTROL

Fig 3.4 Final thirty groups of cuticles obtained after chemical treatments.

17





(a)



(b)

Fig 3.5 (a) - outside surface of the nondewaxed cuticular membrane of the tomato fruit.

(b) - outside surface of the dewaxed cuticular membrane of the tomato fruit

magnification X600 15KV 65-70° tilt.





(a)



(b)

- Fig 3.6 (a) inside surface of the nondewaxed cuticular membrane of the tomato fruit.
  - (b) inside surface of the dewaxed cuticular membrane of the tomato fruit.



# 3.2 <u>A Procedure for Measuring Water Permeability Through</u> the Cuticular Membrane

The transpiration chambers were manufactured from 28.6 mm outside diameter aluminum rods. The chambers were 38.1 mm tall and were milled to contain a well in the chamber which was 9.5 mm in diameter and 31.8 mm deep, over which a circular membrane could be placed. A rubber o-ring 12.5 mm in diameter served as a seal between the membrane and the chamber. It was embedded in a 1.5 mm groove surrounding the center well. An aluminum cover with a center opening 8.3 mm in diameter was placed over the membrane and fastened with three screws. These chambers were very similar to the ones used by Schonherr and Lendzian (1981).

Schonherr and Merida (1981) showed that for the dewaxed membranes, permeability was strongly dependent on humidity due in their opinion to the presence of polar functional groups in the polymer matrix. For the nondewaxed cuticular membranes, changes in humidity did not influence permeability significantly. The permeability of the cuticular matrix is also a function of temperature and it changes drastically at about  $44^{\circ}C$  (Schonherr, Eckl and Gruler, 1979). For these reasons, the transpiration chambers were also stored in desiccators over blue silica gel. The desiccators were placed in a constant temperature water bath (25  $\pm$  .5°C). Under these conditions, the air in equilibrium with the silica gel contained only about 3 x 10<sup>-11</sup> kg-m<sup>-3</sup> water (Kolthoff et al., 1969), so for all




practical purposes, the activity of the water vapor at the surface of the silica gel was zero and the humidity and temperature in the desiccators were held constant. Therefore, the relative effects of humidity and temperature on permeability were eliminated.

At the beginning of each experiment, 1 cm<sup>3</sup> of distilled water was placed in every chamber. The membrane was then placed over the chamber opening followed by the aluminium cover which was secured by screws. The chambers were placed over silica gel upside down (membrane on the bottom) and a paper filter was inserted between the chambers and the silica gel. The upside down position is believed to be closest to the situation in vivo since the inner surface of the cuticular membrane on a fruit is in contact with liquid and the outer surface with dry air (Schonherr and Schmidt, 1979). The chambers were taken from the desiccators and weighed every two hours for dewaxed cuticular membranes and every 12 hours for non-dewaxed, after which, they were quickly returned to the desiccators.

From this, the transpirational flux across the membrane was obtained from the equation;

 $J_{tr} = R/A\rho \tag{3.1}$ 

where A is the area of the membrane exposed to water and air, and  $\rho$  is the density of water at 25°C (996.5 kg/m<sup>3</sup>). Note that J<sub>tr</sub> is expressed in (m/s). Since there was more than one sample for each group of cuticular membranes, the rate R (in kg/s) of water from the chamber in the presence



of the membrane was calculated by a modified least squares method which is presented in Appendix C. Then permeability coefficients (P) were obtained as the ratio of the flux  $J_{tr}$  per unit driving force. The driving force for the transpiration process is the water activity difference which was calculated assuming that the water activity inside the chambers was 1 (distilled water) and outside the chambers was zero by assumption (due to silica gel). The difference was therefore assumed to be unity.

Since permeability coefficients obtained using chambers without membranes are not infinite due to the distance of the water surface from the silica gel and due to the unstirred laver effect. permeability coefficients in the absence of cuticular membranes had to be determined. For this purpose, silica gel was placed in a screen basket above the water filled chambers. A paper filter was placed between the screen and the silica gel. The chambers were filled to the rim which was the position of the membrane during the experiment in the upside down position. The value of the permeability coefficient without the membrane was found to be  $1.07 \times 10^{-7}$  m/s (average of 10 chambers). The permeability coefficient for the membrane itself was calculated using the assumption that the membrane acts as resistance in series with the boundary layer resistance so that



1	1	1	
	=		(3.2)
Pmembrane	Ptot	Po	

#### where:

Pmembrane - permeability coefficient for the membrane

- Ptot permeability coefficient obtained from the experiment for the chamber in the presence of the membrane.
- P<sub>0</sub> permeability coefficient obtained from the experiment for the chamber in the absence of the membrane.

## 3.3 <u>A Procedure for Measuring Elastic Properties of the</u>

### Cuticular Membrane

The chemical treatments described earlier in of "Materials and Methods" yielded 30 different groups of cuticles. Since different ions in the polymer matrix could influence the elastic properties of the matrix, a tensile test was performed using the Instron tensile tester to determine the effect of chemical treatments on material properties. However, since the cuticle of the tomato is nearly spherical and therefore cannot be unfolded into a strip without introducing initial stresses, a modification of the tensile test was introduced and is presented in Figure 3.7. The edge of the cuticle was clamped between two plates with a opening of radius .79 cm. A force was applied to the surface of the membrane using a sphere with a radius of .48 cm. This arrangement did not introduce the initial stresses that would occur in using a thin straight strip of tomato skin and performing a classical tension test.



Two experiments were performed for each of the 30 groups of cuticles. In one experiment, it was made sure that there was friction between the sphere and the cuticular membrane. Several samples from a group were then selected and the tensile test was performed under these conditions.





Fig 3-7 The setup for the tensile test on the Instron device.



In the second experiment, the contact surfaces were made frictionless using a lubricant vegetable oil between the membrane and the sphere. The tensile test was again performed on the remainder of the samples in the group under the new conditions. The reason for this separation is explained in detail in the theory chapter. For now, it is sufficient to say that the calculation of the material properties, Et and v, is made easier by this separation into "slip" and "no-slip" groups.

From the experiment described above, curves of force versus displacement were obtained. Using the equations developed in the next chapter and the computer program QUICK shown in Appendix B, the elastic properties of the cuticles were calculated.



#### 4. THEORETICAL DEVELOPMENT

# 4.1 <u>Theoretical Solution for Elastic Properties of Tomato</u> <u>Cuticle</u>

A tomato fruit cracks due to the water status change in the fruit (Frazier (1934, 1947)). An increase in water potential of the fruit will cause an increase in pressure on the cuticular membrane surrounding the fruit. We can simulate this state in the laboratory by applying a pressure to the membrane using a smooth spherical indenter. The experimental procedure was described in the previous chapter and presented in Figure 3.7. Since the surface of the sphere can be made very smooth, nearly frictionless slip can be made to occur between the cuticular membrane and the spherical indenter. Then the cuticular membrane can be treated as a thin membrane under pressure.

Higdon et al. (1976) gives the following equation for a thin membrane under pressure;

 $(p/t) = (\sigma_1/\rho_1) + (\sigma_2/\rho_2) \tag{4.1}$ where p is the pressure applied to the membrane, t is the thickness of the membrane,  $\rho_1$  and  $\rho_2$  are the principal radii of curvature in two perpendicular directions, and  $\sigma_1$ and  $\sigma_2$  are the corresponding "in-plane" membrane stresses. (See Figure 4.1)





Fig 4.1 Rectangular section of membrane-forces  ${\rm T}^{}_1$  and  ${\rm T}^{}_2$  and internal pressure p.



Equation (4.1) can be rewritten in the following form,

 $p = (T_1/\rho_1) + (T_2/\rho_2)$ (4.2) where T\_1 and T\_2 are forces per unit length in the

same directions as  $\sigma_1$  and  $\sigma_2$  respectively. The complete state of tension (T<sub>1</sub>, T<sub>2</sub>) at any point on the membrane for this particular experiment will now be determined.

An examination of Figure 4.2 which describes the configuration of the membrane used in the experiment during deformation shows that the only parameter which is difficult to measure is the "contact angle",  $\theta_0$ . Fortunately,  $\theta_0$ can be expressed in terms of the other parameters R, h and a. In Appendix A-1 it is shown that  $\sin \theta_0 = (aR - (R - h) (h^2 + a^2 - 2Rh)^{1/2})/(R^2 + (R - h)^2)$ (4.3)

The state of stress in the upper portion of the membrane above the contact line can now be determined by "making a horizontal cut" z units from the top and drawing a free body diagram of the membrane and indenter below this cut as shown in Figure 4.3. With W equal to the downward force exerted on the spherical indenter and b equal to the radius of the horizontal circle formed by the cut, the balance of forces in the vertical direction requires that

 $\Sigma F_{vert} = 2\pi b \cdot T_1 \cos(90^\circ - \theta_0) - W = 0 \tag{4.4}$  From the geometry of Figure 4.2, it can be shown that

 $b \sin \theta_{0} = a \sin \theta_{0} - z \cos \theta_{0} \qquad (4.5)$ Substituting this into equation (4.4) gives

 $T_{1} = W/(2\pi \ (a \ sin \theta_{o} - z \ cos \theta_{o}), \quad 0 \le Z \le h-R+R \ cos \theta_{o}$ (4.6a)





Fig 4.2 Crossectional view of membrane being loaded by spherical indenter.





Fig 4.3 Perspective view of section of membrane under load W.



(for details see Appendix A-2(a)).

With  $T_1$  determined for any value of z above the contact line,  $T_2$  may be found using Equation (4.2). Since  $\rho_1$  =  $\infty$  and p = 0, Equation (4.2) reduces to

 $0 = (T_1/\infty) + (T_2/\rho_2)$ (4.6.b)

and, therefore,  $T_2 = 0$  for any  $z \le h - R + R \cos \theta_0$ 

Now that the tensions  $(T_1, T_2)$  at any point on the free surface of the membrane have been found, it remains only to calculate  $(T_1, T_2)$  for those points on the contact surface. The contact surface is defined to be that part of the membrane which is directly in contact with the sphere (below the contact line). Here, the situation is a little more difficult; the pressure, p, exerted by the sphere on the membrane varies from point to point on the contact surface as do  $T_1$  and  $T_2$ . Under the assumption of frictionless slip, Equation (4.2) still applies but it alone is not sufficient to determine all three unknowns, p,  $T_1$  and  $T_2$ . It will be necessary, therefore, to derive two additional equations.

For any point on the contact surface,  $\rho_1$  =  $\rho_2$  = R where R is the radius of the sphere so that Equation (4.2) can be written as

$$T_1 + T_2 = pR$$
 (4.7)

This equation relates the tensions  $T_1$  and  $T_2$  in two perpendicular directions to p. An equilibrium equation involving  $T_1$  and p alone can be obtained by making a horizontal cut through the membrane parallel to and just



below the contact line and drawing a free body diagram of the membrane below this cut. After having done this, in order to simplify matters, the entire free body diagram will be flipped upside down and a spherical coordinate system (R,  $\alpha$ ,  $\phi$ ) with its origin at C, the center of the sphere, will be used. See Figure 4.4.

Since all points on the membrane are equidistant from C and since axisymmetry of the stress field eliminates any dependence on the coordinate  $\alpha$ , only  $\phi$  will appear in the equilibrium equation. Note that in this free body diagram,  $\phi$ may assume values in the range,  $0 \leq \phi \leq \theta$ , where  $\theta$ marks the cut in the membrane. By summing forces in the vertical direction on Figure 4.5 we can obtain a second independent equilibrium equation

 $\Sigma F_{vert} = -T_1 \cos(90^\circ - \theta)(2\pi R \sin \theta) + \oint (p(\phi)\cos\phi) dA$  $0 \le \phi \le \theta \qquad (4.8)$ 

where  $dA = (2\pi R \sin \phi)(Rd\phi) =$  the element of area corresponding to the thin band shown in Figure 4.5. This equation can be rearranged to produce

 $(dT_1/d\theta) \tan\theta + 2T_1 = Rp(\theta)$  (4.9) (for details see Appendix A-3)

There are now two independent relations, (4.7) and (4.9), in three unknowns obtained from the two available equilibrium equations. The third equation must come from elasticity considerations. From Sokolnikoff (1956), in the three dimensional formulation of elasticity theory there are 3 equations of equilibrium, 6 stress-strain relations and 6





Fig 4.4  $\,$  Inverted perspective view of the contact surface and the coordinate system used.







strain-displacement relations. These equations are given in spherical coordinates in Appendix A-4.

All variables here are functions of 0 only since the assumption of axisymmetry immplies that  $\partial/\partial a = 0$  and  $u_{\alpha}=0$ . Axisymmetry also implies that there are no in-plane shear stresses. For this particular problem there are no body forces. These considerations are justified by the fact that one can obtain the same equilibrium Equations (4.7) and (4.9) by using them. (For details, see Appendix A-4). The advantage in taking the elasticity approach is that it yields the third equation in  $T_1$ ,  $T_2$  and p necessary for a solution; this equation comes in the form of a compatibility equation.

Applying the axisymmetry conditions to the straindisplacement equations gives only two nontrivial equations,

 $\varepsilon_{AA} = (1/R)(\partial u_A / \partial \theta) \qquad (4.10)$ 

 $\varepsilon_{\alpha\alpha} = (1/R) u_{\theta} \cot\theta$  (4.11)

Solving Equation (4.11) for  $u_{ extsf{ heta}}$  , differentiating and substituting into Equation (4.10) gives a compatibility equation

 $\epsilon_{\theta}\cos^2 = \epsilon_{\alpha\alpha} + (\partial \epsilon_{\alpha\alpha}/\partial \theta) \sin \theta \cos \theta$  (4.12) These strains can now be expressed in terms of the stresses which in turn can be expressed in terms of the tensions.

$$\sigma_{\theta\theta} = \sigma_1 = T_1/t$$
 (4.13.a)  
and

 $\sigma_{\alpha\dot{\alpha}} = \sigma_2 = T_2/t$  (4.13.b) The third stress,  $\sigma_{rr}$ , can be taken to be zero since it is



This is justified in the following way:

 $\sigma_{rr} = -p$  on the inside of the membrane

 $\sigma_{rr} = 0$  on the outside of the membrane (4.14) It is expected then that  $\sigma_{rr}$  is on the same order of magnitude as p everywhere along the thickness of the membrane. But

 $p = (T_1 + T_2)/R$  from Equation (4.7), so using Equation (4.2)

 $p = (\sigma_{\theta\theta} t + \sigma_{\alpha\alpha} t)/R = (t/R)(\sigma_{\theta\theta} + \sigma_{\alpha\alpha}).$   $r_r \text{ is therefore on the order of } t/R \text{ times } \sigma_{\theta\theta} \text{ or } \sigma_{\alpha\alpha}$ and since t/R << 1,  $\sigma_{rr} <<\sigma_{\theta\theta}$  and  $\sigma_{rr} <<\sigma_{\alpha\alpha}$ .
Therefore,  $r_r$  will be neglected (taken to be zero).
Using Equation (4.13) and Hook's law then, one obtains

 $\epsilon_{\theta\theta} = (1/Et)(T_1 - v T_2)$ 

 $\varepsilon_{nn} = (1/Et)(T_2 - vT_1)$  (4.15)

Substituting these into the compatibility Equation (4.12), the third independent equation is obtained.

 $(T_1 - \nu T_2)\cos^2\theta = (T_2 - \nu T_1) + \sin\theta \cos\theta (d/d\theta)(T_2 - \nu T_1)$  (4.16)

The two previous equations in the three unknowns p,  ${\rm T}_1$  and  ${\rm T}_2$  are rewritten here for reference,

 $T_1 + T_2 = pR$  (4.7)

 $(dT_1/d\theta)\tan\theta + 2T_1 = pR \tag{4.9}$ 

Combining these equations, (4.7), (4.9) and (4.16), and simplifying, a second order homogenous differential equation with non-constant coefficients is be obtained. From



Appendix A-5,  

$$(d^2T_1/d\theta^2) + (1 - v) T_1$$
sin $\theta \cos\theta + (dT_1/d\theta)(2 + \cos^2\theta)$   
= 0 (4.17)  
A series solution to this differential equation in the form

$$T_1(\theta) = \sum_{n=0}^{\infty} a_n \sin^n \theta$$

(4.18)

can be found. Appendix A-6 covers this in detail. The results are

$$T_{1}(\theta) = K \sum_{x=0}^{\infty} bn \sin^{n}\theta$$
(4.19)

where the  $b_n$  are pure numbers with  $b_0 = 1$ ,  $b_1 = 0$  and

$$n+2 = b_n(n^2 + n - 1 + v)/((n+2)(n+4))$$
 n=0,1,2...  
(4.20)

Note that all odd b<sub>n</sub>'s are zero. The constant k is found by evaluating Equation (4.19) at  $\theta = \theta_0$ 

$$k = T_1(\theta_0) / \sum_{n=0}^{\infty} b_n \sin^n \theta_0$$
(4.21)

and  $T_1(\theta_0)$  is obtained from the boundary condition that  $T_1(\theta)$  is continuous across the contact line. At  $\theta = \theta_0$ ,  $z = h - R + R \cos \theta_0$  and Equation (4.6a) gives  $T_1(\theta_0) = W/(2\pi R \sin^2 \theta_0)$  (4.22)

(See Appendix A-2(b))

Now that  $T_1$  is completely determined for any value of  $\theta$ in the range  $0 \le \theta \le \theta_0$  in terms of known parameters,  $T_2$  can be determined from equations (4.7) and (4.9),  $T_2 = pR - T_1 = (2T_1 + (dT_1/d\theta)tan\theta) - T_1$ 


$= T_1 + (dT_1/d\theta) \tan \theta$ (4.23) This can be rewritten as:

 $T_2 \cos\theta = T_1 \cos\theta + (dT_1/d\theta)\sin\theta. \qquad (4.24)$ Substituting (4.19) into (4.24) one obtains:  $T_2 \cos\theta = \cos\theta + \sum_{i=1}^{\infty} b_n \sin^n \theta$ 

+  $\sin\theta\cos\theta$  k  $\sum_{n=0}^{\infty}$  n b<sub>n</sub>  $\sin^{n-1}\theta$ 

Dividing through by  $\cos\theta$  which is never zero since  $0^{\circ} < \theta < 90^{\circ}$ ,

 $T_2 = k \sum_{n=0}^{\infty} (n+1) b_n \sin^n \theta$ (4.25) where  $b_n$  and k are the same as in Equations (4.20) and (4.21). It can be seen that  $T_1(0) = k = T_2(0)$  as expected since at  $\theta = 0$ ,  $T_1$  is physically indistinguishable from  $T_2$ .

An examination of the coefficients  $b_n$  in Equation (4.20) shows that  $b_0 = 1$  is the only positive one since  $b_2 = -(1-\nu)/8$ , and  $n^2 + n - 1 + \nu$  is positive for the remaining values of n = 2, 4, 6,...This means that the expressions for  $T_1$  and  $T_2$  in Equations (4.19) and (4.25) are monotonically decreasing functions of  $\theta$ . This renders both  $T_1$  and  $T_2$  maximized at  $\theta = 0$  with  $T_{1max} = T_{2max} = k$ . The strains  $\varepsilon_1$  and  $\varepsilon_2$  can be calculated using Equation (4.15),  $\varepsilon_1 = (1/Et)(T_1 - \nu T_2) = (k/Et) \sum_{n=0}^{\infty} (1-\nu-n\nu) b_n \sin^n \theta$ (4.26)

$$\begin{split} \varepsilon_2 &= (1/\text{Et})(T_2 - \nu T_1) = (k/\text{Et}) \sum_{\nu=0}^{\infty} (n+1-\nu) b_n \sin^n \theta \\ \text{Since } (n+1-\nu) > 0 \text{ for all } n, \text{ the sign considerations for the} \\ b_n \text{ mentioned above are unchanged so that } \varepsilon_2 \text{ is maximized at} \\ \theta &= 0 \text{ with } \varepsilon_{2\text{max}} = k(1-\nu)/\text{Et.} \text{ But since } (1-\nu-n\nu) \end{split}$$



changes sign for  $n > (1 - \nu)/\nu$ , so do the terms in the series and a determination of the point at which  $\varepsilon_1$ , is maximized and the value of that maximum cannot easily be made.

From the graph of load versus displacement obtained from the experiment described earlier (see Fig 3.7) in "Materials and Methods" it is possible to determine the material properties v and Et. This is done by comparing the actual displacement to the theoretical one for a given load. The total vertical displacement  $u_z$  is composed of two parts: the part below the contact line  $u_{zb}$  and the part above the contact line  $u_{za}$ . The first step is to relate the vertical displacement below the contact line to  $\theta_o$  and W. From the strain - displacement relation (Appendix A-4),

 $\varepsilon_{\alpha\alpha} = u_{\theta} (\cot \theta / R)$  (4.27) so,

$$u_{\theta} = R \tan \theta \epsilon_{\alpha \alpha}$$
 (4.28)  
From the stress-strain relations (Appendix A-4).

$$\varepsilon_{\alpha\alpha} = (1/E) \left( \sigma_{\alpha\alpha} - \nu \sigma_{\theta\theta} \right)$$
(4.29)

Using  $\sigma_{\alpha\alpha} = T_2/t$  and  $\sigma_{\theta\theta} = T_1/t$  we obtain

 $\varepsilon_{\alpha\alpha} = (1/Et)(T_2 - \nu T_1)$ so that

 $u_{\theta} = (R \tan \theta / Et)(T_2 - \nu T_1)$  (4.30) This correctly predicts that  $u_{\theta} = 0$  when  $\theta = 0$ .

The vertical component of the displacement below the contact line is the component of  $u_{\theta}$  evaluated at  $\theta = \theta_{\circ}$  in the vertical direction,



$$\begin{split} \mathbf{u}_{\text{Zb}} &= (\text{R tan } \boldsymbol{\theta}_{\text{O}}/\text{Et})(\text{T}_{2}(\boldsymbol{\theta}_{\text{O}})-\text{T}_{1}(\boldsymbol{\theta}_{\text{O}}))\sin\boldsymbol{\theta}_{\text{O}} \end{split} \tag{4.31} \\ \text{Now that } \mathbf{u}_{\text{Zb}} \text{ has been calculated in terms of known} \\ \text{parameters and the material properties, } \mathbf{u}_{\text{Za}} \text{ can be} \\ \text{determined.} \end{split}$$

For the free surface part,  $T_2 = 0$  and  $T_1 = W/(2\pi (a \sin \theta_0 - z \cos \theta_0))$  from Equation (4.6a).

From Hooke's law,

 $\sigma_1 = T_1/t = E\varepsilon_1$  so that  $\varepsilon_1 = T_1/Et$  (4.32) From Appendix A-7,

 $du_{za} = \varepsilon_1 dz$ 

After integration this gives the second part of the vertical displacement.

 $u_{za} = (W/2\pi Et) (ln(a/Rsin\theta_0)/cos\theta_0)$ (4.34) The total vertical displacement is  $u_z = u_{za} + u_{zb}$ which from (4.31) and (4.34) is

$$\mu_{Z} = (R\sin^{2}\theta_{0}(T_{2}-\nu T_{1})+(W/2\pi)\ln(a/R\sin\theta_{0}))/(Et \cos\theta_{0})$$

$$(4.35)$$

The term  $T_2 - \nu T_1$ , evaluated at  $\theta = \theta_0$  can easily be replaced in Equation (4.35) using Equations (4.21), (4.22) and (4.25).

$$T_{2} - \nu T_{1} = k \sum_{n=0}^{\infty} (n-1) b_{n} \sin^{n}\theta_{0} - \nu (W/2\pi R \sin^{2}\theta_{0})$$

$$T_{2} - \nu T_{1} = (W/2\pi R \sin^{2}\theta_{0}) ((\sum_{n=0}^{\infty} (n-1) b_{n} \sin^{0}\theta_{0}) / \sum_{n=0}^{\infty} b_{n} \sin^{n}\theta_{0}) - \nu)$$

$$(4.37)$$

Using this in equation (4.35) the total vertical displacement becomes

$$\begin{split} u_{z} = (W/2\pi Et \ \cos\theta_{O}) (( \ \tilde{\Sigma}_{o}(n+1-\nu) b_{n} \sin^{n}\theta_{O}) / ( \ \tilde{\Sigma}_{o} b_{n} \sin^{n}\theta_{O}) + \\ & \ln(a/R \ \sin\theta_{O})) \end{split} \tag{4.38}$$



Both sides can be divided by the load W,

 $u_{Z}/W = ((\sum_{r=0}^{\infty} (n+1-\nu)b_{n}\sin^{n}\theta_{O})/(\sum_{r=0}^{\infty} b_{n}\sin^{n}\theta_{O}) + \ln(a/R \sin^{2}\theta_{O}))/(2\pi Et \cos\theta_{O}).$ (4.39)

This is the theoretical "deflection over load" expression in terms of geometric parameters and material properties which should be compared to the actual values from the Instronexperiment for frictionless slip conditions.

If we look at the term two series in Equation(4.39), we see that the ratio of the two series can be approximated by  $(1-\nu)$  which uses only the first terms from each of the two series. The remaining terms ignored here turn out to be orders of magnitude smaller than the first one as can be seen from the rapidly decreasing values for the  $b_n$  (See Equation 4.20) and from the fact that  $\sin\theta_0 << 1$  in practice. Because of the above reasoning, program QUICK listed in Appendix B calculates Et and  $\nu$  using  $(1-\nu)$  as the ratio of the two series so that

 $u_{\tau}/W=((1-\nu)+\ln(a/R \sin\theta_{o}))/(2\pi Et \cos\theta_{o})$ (4.40)

As mentioned earlier in the methodology section, the experiments on the Instron were separated into two groups. In one of them, slip conditions were satisfied by applying a lubricant between the indenter surface and the cuticular membrane. In the second group, no-slip conditions were satisfied by covering the ball with the sticky material from masking tape. To make sure that the no-slip conditions were satisfied, the shape of the break in the membrane was checked after each experiment: in a "no slip" break, there



is no deformation below the contact line so that the break occurs at the contact line which can easily be checked.

Since there is no displacement below the contact line for no-slip conditions, the total vertical displacement is due to that above the contact line which is  $u_{ZB}$  (Equation 4.34). Since the first term in brackets in Equation (4.39) represents  $u_{ZD}$  which is zero for no-slip conditions the following form is obtained.

 $u_z/W=\ln(a/R \sin\theta_0)/(2\pi Et \cos\theta_0)$  (4.41) Equations (4.40) and (4.41) now represent the deflection over load for slip and no-slip conditions respectively. Since Equation (4.41) is independent of Poisson's ration,  $\nu$ , the only unknown is Et which can be easily calculated knowing  $u_z/W$  from the Instron experiment and the corresponding geometry terms, a, R,  $\theta_0$ . Now, since Et is a material property, in theory it does not change for a given group of cuticular membranes. The same Et can therefore be used in the "slip" experiment, and Poisson's ratio  $\nu$ , the only unknown left, can be directly calculated from Equation (4.40) and the Instron data for the "slip" experiment.

A step-by-step explanation of these calculations for Et and  $\nu$  is contained in Appendix A-8. The program performing the calculations, PROGRAM QUICK, is shown in Appendix B.



#### 5. RESULTS AND DISCUSSION

#### 5.1 Results of the Permeability Test

# 5.1.1 Comparison Between Dewaxed and Nondewaxed Cuticular Membranes

Permeability coefficients for all 30 groups of cuticles (see Figure 3-4) were determined using the procedure described in Chapter 3. The permeability coefficients were calculated using program DEWAX and NWAX shown in Appendix B. The results show that there was a significant change in permeability between the nondewaxed cuticular membranes and the dewaxed ones. These results are summarized in Tables 1, 2, 3. It can be concluded that soluble waxes are very important in determining the permeability of the cuticular membrane. It should be noted that the standard deviation is much smaller in groups of dewaxed membranes than in groups of nondewaxed membranes. This is very likely related to the nonuniform distribution of waxes in the nondewaxed cuticular membranes. Similar results were obtained by Schonherr (1976b).



The effect of soluble cuticular waxes on water permeability of isolated cuticular membranes for <u>Pik Red tomatoes (set 1)</u> using 4 different chemical treatments and a control group.

Chemical treatment	Number of samples in the group (dewaxed)	Permeability of dewaxed membrane P <sup>D</sup> [m/s]	Number of samples in the group (nondewaxed)	Permeability of nondewaxed membrane P <sup>ND</sup> [m/s]	P <sup>D</sup> P <sup>ND</sup>
HCl	8	2.33 x 10 <sup>-8</sup> (36)	10	2.08 x 10 <sup>-9</sup> (47)	11
ксі	5	1.87 x 10 <sup>-8</sup> (33)	10	2.50 x 10 <sup>-9</sup> (42)	7
CaCl2	5	2.44 x 10 <sup>-8</sup> (15)	10	3.12 x 10 <sup>-9</sup> (49)	8
AlCl <sub>3</sub>	5	1.42 x 10 <sup>-8</sup> (34)	10	2.50 x 10 <sup>-9</sup> (37)	6
Control	5	2.16 x 10 <sup>-8</sup> (16)	10	1.49 x 10 <sup>-9</sup> (51)	14

The coefficient of variation is given in parenthesis.



The effect of soluble cuticular waxes on water permeability of isolated cuticular membranes for <u>Pik Red tomatoes</u> (set 2) for 4 different chemical treatments and a control group.

Chemical treatment	Number of samples in the group (dewaxed)	Permeability of dewaxed membrane P <sup>D</sup> [m/s]	Number of samples in the group (nondewaxed)	Permeability of nondewaxed membrane P <sup>ND</sup> [m/s]	P <sup>D</sup> P <sup>ND</sup>
HCl	7	1.68 x 10 <sup>-8</sup> (31)	10	1.17 x 10 <sup>-9</sup> (30)	14
KCI	9	2.41 x 10 <sup>-8</sup> (38)	9	1.49 x 10 <sup>-9</sup> (52)	16
CaCl <sub>2</sub>	9	2.57 x 10 <sup>-8</sup> (21)	10	2.09 x 10 <sup>-9</sup> (54)	12
Alcl <sub>3</sub>	9	2.22 x 10 <sup>-8</sup> (18)	10	2.21 x 10 <sup>-9</sup> (64)	10
Control	10	2.54 x 10 <sup>-8</sup> (18)	9	1.95 x 10 <sup>-9</sup> (35)	13

The coefficient of variation is given in parenthesis.



The effect of soluble cuticular waxes on water permeability of isolated cuticular membranes for <u>"UC 82" processing tomatoes</u> for 4 different chemical treatments and a control group.

Chemical treatment	Number of samples in the group (dewaxed)	Permeability of dewaxed membrane P <sup>D</sup> [m/s]	Number of samples in the group (nondewaxed)	Permeability of nondewaxed membrane P <sup>ND</sup> [m/s]	P <sup>D</sup> P <sup>ND</sup>
HCl	7	1.97 x 10 <sup>-8</sup> (11)	10	1.66 x 10 <sup>-9</sup> (52)	12
ксі	5	2.16 x 10 <sup>-8</sup> (21)	9	1.27 x 10 <sup>-9</sup> (37)	17
CaCl <sub>2</sub>	4	1.87 x 10 <sup>-8</sup> (16)	9	1.05 x 10 <sup>-9</sup> (21)	18
AlC13	5	1.78 x 10 <sup>-8</sup> (18)	10	1.78 x 10 <sup>-9</sup> (40)	10
Control	4	$1.64 \times 10^{-8}$ (9)	9	1.43 x 10 <sup>-9</sup> (29)	11

The coefficient of variation is given in parenthesis.



5.1.2 Comparison Between Different Chemically Treated Cuticular Membranes and the Control Group

Differently treated cuticular membranes were compared to the control group for the three main groups of cuticles described earlier (Chapter 3) - two groups of "Pik Red" tomatoes and one of processing "UC 82." In each of the three sets were 4 differently treated groups (H+. K+. Ca<sup>++</sup>, Al<sup>+++</sup>) and a control group (see Fig 3.4). In order to determine if there was a significant difference in permeability between the chemical treatments and the control group, the Student's t test was performed for each of the three groups. The pooled standard deviation for each treatment and the control group, the degrees of freedom, and the "t" values were calculated in programs DEWAX and NWAX for dewaxed and nondewaxed subgroups respectively (see Appendix B). The levels of significance,  $\alpha$ , were found from standard tables (Oktaba, 1974) of critical t values for the Student's t test and are summarized in Table 5.4.



The results of the Student's t-test for the permeability comparison between the chemically treated cuticles and the control groups at a significance level of ( $\alpha$ ) = .05. (95% certainty in a difference between the sample and control).

Chemical Pik Red (set 1) Pik Red (set 2) Processing "UC-82" Treatment dewaxed nondewaxed dewaxed nondewaxed HCl - - + + - -

			(**)	(**)		
KCl	+	- (**)	+	+	-	+
CaCl <sub>2</sub>	-	(**)	-	-	-	(**)
AlC13	+	_ (**)	+	-	-	-

"-" means an increase in permeability

"+" means a decrease in permeability

(\*\*) means statistically significant ( $\alpha < .05$ )

Values of "t" and degrees of freedom are in Appendix B (Table

B.1).



5.1.3. Discussion of the Influence of Chemicals on Permeability Coefficients of Cuticular Membranes.

Table 5.4 shows that in most cases there were insignificant changes in permeability due to the application of chemicals. However, some trends are noticeable. Calcium ions (Ca<sup>++</sup>) increased permeability in all cases except for nondewaxed processing tomatoes "UC-82" where a significant decrease in permeability occurred. It would be interesting to check how many ions were absorbed by the samples, but this was not done in this investigation. An interesting effect connected with Al<sup>+++</sup> treated membranes can be seen. All nondewaxed samples showed an increase in permeability when treated with AlCl<sub>3</sub>, while almost all dewaxed samples, except for processing "UC-82" showed a decrease in permeability. It seems possible that there is some interaction between the waxes and the Al<sup>++++</sup> ions which should be investigated further.

In Pik Red (set 2), a significant decrease in permeability occurs when treated with HCl.

Potassium did not show a significant increase in permeability as would be expected from observations reported in the literature (Dudman 1962, Chambers and Possingham 1963, Tullberg 1978, Dudman and Grncarevic 1962, Grncarevic, Radler and Possingham 1968). If the potassium ion interacts with waxes as was suggested by Chambers and



Possingham (1963), an increase in permeability should be significant for non-dewaxed cuticular membranes in K<sup>+</sup> form. It is important to remember that the last procedure in the preparation of the cuticular membranes was to wash them in distilled water (see Figs 3.1. 3.2. 3.3 and 3.4). Recall from the literature review that the washing of grapes dipped in potassium carbonate solution within two days reduced the increased transpiration to that of undipped grapes (Grncarevic, Radler and Possingham 1968). There again, it would be interesting to check how much potassium was absorbed by the membranes. There was some potassium present in the cuticles since this was qualitatively checked in the laboratory. Quantitative tests were not performed. Since Ca++ and Al+++ appear to increase permeability, more research should be done with these cations.



#### 5.2. Results of the Instron Tensile Test

From the graph of load versus displacement obtained from the experiment described earlier (see Fig 3.7) and from the theoretical equations given in Chapter 4 (Eq. 4.40 & Eq. 4.41) the material property Et and Poisson's ratio  $\nu$  were found for all 30 samples of cuticles. The step-by-step procedure for these calculations is contained in Appendix A-8 and Appendix B (PROGRAM QUICK).

## 5.2.1 The Influence of Soluble Waxes (Lipids) on the Material Property Et.

Table 5.5 shows the influence of soluble waxes (lipids) on the material property Et of the cuticular membrane. In almost all cases, the cuticles without waxes had lower Et values than the cuticles with waxes in the same chemical subgroup. A Student's t test was performed on the results. The samples for which the difference was significant (above 95% confidence level) are indicated by "\*\*" in Table 5.5. It seems that the most significant difference among all the main groups is in the Ca\*+ treatment where the level of significance is consistently above 99%. The subgroup with the least significant difference is the control group, which shows almost no difference between dewaxed and nondewaxed cuticular membranes.

Ten samples of cuticular membranes from each of the 30 subgroups were measured to check if there was a noticeable change in thickness in dewaxed and nondewaxed cuticles. No



significant difference could be detected between cuticular membranes with waxes and without. This means that the change in Et was not due to the change in thickness.



Comparison between dewaxed and non-dewaxed cuticles in the same subgroups of chemical treatments. The influence of soluble waxes (lipids) on Et.

	Chemical		degrees of
group	Treatment	t - value	freedom
Pik Red (set 1)	HC1 KC1 CaCl2 AlCl3 Control	-2.179 ** 1.167 -3.583 ** .252 286	14 23 18 15 38
Pik Red (set 2)	HC1 KC1 CaC12 AlC13 Control	-2.661 ** -6.521 ** -5.672 ** .370 508	30 39 45 20 43
"UC-82" Processing	HCl KCl CaCl <sub>2</sub> AlCl <sub>3</sub> Control	-1.296 -2.585 ** -9.845 ** -3.577 ** 895	21 26 19 17 22

\*\* - satisfied 95% confidence level for a difference between samples.

Note: A negative sign indicates that Et for the nondewaxed cuticle is larger than Et for the dewaxed cuticle in the same chemical treatment.



In Table 5.6, the Student's t test was performed in order to determine the difference between chemically treated cuticular membranes and the control subgroups. The table contains the elastic property Et, the coefficient of variation c.v., the degrees of freedom d.f., and the t values for each of the subgroups.

In the majority of the cases, chemically treated cuticular membranes had lower Et values than the control subgroup. In set 1 of Pik Red tomatoes, there were two subgroups of cuticles, K<sup>+</sup> and Al<sup>+++</sup> (both dewaxed and nondewaxed) where Et was higher for chemically treated cuticular membranes than for control subgroups. In set 2 of Pik Red and in "UC-82", especially for dewaxed groups, Et decreased significantly with chemical treatments.

Since chemical treatments of cuticular membranes require some mechanical operations such as washing, mixing and stirring, there is some chance that minor mechanical damage took place. However, there were no noticeable differences between treated cuticular membranes and the control samples under the scanning electron microscope.



Comparison of Et values for chemical treated subgroups. Each subgroup is compared with the control subgroup for a given group of cuticles.

Group	Chemical	1	dev	vaxed	1		r	nondew	axed
	treatment	Et	c.v.	d.f	. t	Et	c.v.	d.f.	t
Pik Red (Set 1)	HCl KCl CaCl <sub>2</sub> AlCl <sub>3</sub>	239 440 194 434	44 30 24 14	20 22 21 18	-2.124** 2.603** -4.242** 2.576**	346 388 319 425	23 21 25 14	32 39 35 35	•557 2•608** <del>-</del> •581 4•704**
Pik Red (Set 2)	HCl KCl CaCl <sub>2</sub> AlCl <sub>3</sub>	240 207 204 270	13 14 16 30	28 42 40 29	-1.708 -4.750** -4.669** 387	300 284 270 259	21 16 16 21	45 40 48 34	.469 420 -1.436 -1.517
UC-82	HCl KCl CaCl <sub>2</sub> AlCl <sub>3</sub>	291 306 262 254	15 14 12 20	16 22 19 18	-6.998** -7.772** -10.854** -8.891**	319 354 382 467	11 14 6 33	27 26 22 21	-5.726** -4.051** -3.036** .047

For	control	groups:
-----	---------	---------

	dewaxed	non dewaxed		
Pik Red (Set 1)	Et = 327 c.v. = 22%	Et = 332 c.v. = 16%		
Pik Red (Set 2)	Et = 281 c.v. = 24%	Et = 291 c.v. = 23%		
"UC-82"	Et = 439 c.v. = 9%	Et = 465 c.v. = 19%		

c.v. - coefficient of variation (%)

d.f. - degrees of freedom

t - value for the Student t-test

\*\* - significance of 95% (confidence level that the samples are different)


The values of Et are in the range of values obtained previously by Murase and Merva (1977). They obtained a "Static Elastic Modulus" for epidermal tissue (not for the cuticular membrane only) in the range 6,000 - 11,000 (kPa) depending on the water potential of the tissue. The value obtained in this experiment for Pik Red (set 2) HCl of Et is from Table 5.6, 240 Nm. Since t for Pik Red (set 2) was on the average 2.54 x  $10^{-5}$ m, the value for E here is 9,450 kPa.

5.2.2 Discussion of the Results for Poisson's Ratio.

Table 5.7 shows the different values of Poisson's ratio calculated in PROGRAM QUICK for 30 different samples of cuticular membranes. The values of  $\nu$  are in the required range of 0 to .5. However, looking at the different groups of cuticular membranes (Pik Red (set 1), Pik Red (set 2) and ("UC-82")) one, notices extremely large variations in  $\nu$  even for the same chemical treatment. During calculation, it was observed that the value of  $\nu$  was very sensitive to the slope of the load versus displacement curve from the Instron experiment. Et in comparison was relatively insensitive. Since a difference of one degree in slope significantly changed the value of  $\nu$ , any oscillation in the values for  $\nu$  in Table 5.7 should be attributed to experimental error.

The load cell used in this experiment was the most sensitive available and had a full scale load range of 1 kg, which corresponded to 10 in. on the graph paper. The force exerted on the cuticular membrane was on the order of .98 -



Chemical treatment	Pik Red set 1		Pik Red set 2		Processing UC-82	
	dewaxed	non- dewaxed	dewaxed	non- dewaxed	dewaxed	non- dewaxed
нсі	•354	.224	•382	.161	.268	.480
KCl	•323	.274	.302	.172	.204	.128
CaCl <sub>2</sub>	•349	.151	•325	•432	.428	.168
AlC13	.118	•345	.449	.407	.249	.116
Control	.129	. 197	.081	•358	.198	.314

Table 5.7 - Values of Poisson's ratio  $\nu$  for different treatments.



2.94 N (which corresponds to .1 - .3 kg). So the displacement of the recording pen was 1 to 3 inches long. At the same time, the slope angle  $\beta$  (See Fig A8.1) was on the average about 75° - 85°. Since the slope of load versus displacement was approximated by a straight line, an error of one or two degrees in the angle measurement was very easily introduced. Adding to this mechanical errors in the equipment itself, a meaningful discussion of the influence of cheemicals on Poisson's ratio must be forfeited. However, since the values of are in the required range,  $0 < \nu < .5$ , and since the values of Et are in the range of values obtained previously (see discussion at the end of 5.2.1) the experiment was well designed even though more work should be done to obtain more precise data for Poisson's ratio.



6. CONCLUSIONS

Several conclusions can be drawn from this study.

- (1) The soluble waxes (lipids) in a cuticular membrane of the tomato fruit have a very significant influence on the permeability of the cuticular membranes. Removing the soluble waxes (lipids) increased permeability 12 times on the average.
- (2) Chemically treated cuticular membranes showed some significant differences in permeability over the control group. Increased permeability can be seen for the Ca<sup>++</sup> treatment. For the Al<sup>+++</sup> treatment, dewaxed cuticles showed some decrease in permeability while nondewaxed membranes showed an increase at the same time.
- (3) The soluble waxes (lipids) significantly influenced the elastic property Et (see Table 5.5). Dewaxing of the cuticular membrane of the tomato fruit significantly decreases Et.
- (4) The chemical treatments of cuticular membranes, especially for dewaxed ones, decreased the Et value of the cuticle in the majority of cases.
- (5) If we use an average thickness for the cuticular membrane, the theoretical values obtained for Et fall



in the same range of experimental values obtained by Murase and Merva in 1977.

(6) Poisson's ratio calculated from the Instron experiment was not correlated to chemical treatments. The large distribution of calculated values (.081 - .449) suggests a very large experimental error.



## 7. RECOMMENDATIONS

The following recommendations are suggested.

- Using analytical chemistry methods, the number of ions absorbed by each group of cuticular membranes should be investigated.
- (2) More research should be done with Al\*\*\* treated samples and the influence of aluminum on soluble waxes (lipids) should be investigated.
- (3) The permeability of potassium treated cuticles washed in distilled water should be compared to cuticular membranes which were not washed in distilled water but only treated with KC1.
- (4) More precise measuring equipment for obtaining the load versus displacement curve should be used in order to reduce experimental error.





APPENDICES



APPENDIX A-1

From Fig 4.2, 
$$a > R$$
 so  $0^{\circ} \langle \theta_{0} \langle 90^{\circ} \rangle$   
 $h = AB + BD = BP \tan \theta_{0} + BD = (a - Rsin \theta_{0}) \tan \theta_{0} + (R - Rcos \theta_{0})$   
 $hcos \theta_{0} = asin \theta_{0} - Rsin^{2} \theta_{0} + Rcos \theta_{0} - Rcos^{2} \theta_{0}$   
 $(h - R)cos \theta_{0} = asin \theta_{0} - R$   
 $(h - R)^{2} cos^{2} \theta_{0} = a^{2} sin^{2} \theta_{0} - 2aRsin \theta_{0} + R^{2}$   
 $(h - R)^{2} (1 - sin^{2} \theta_{0}) = a^{2} sin^{2} \theta_{0} - 2aRsin \theta_{0} + R^{2}$   
 $(a^{2} + (R-h)^{2})sin^{2} \theta_{0} - 2aR sin \theta_{0} + R^{2} - (R-h)^{2} = 0$   
 $sin \theta_{0} = (2aR^{\pm}(4a^{2}R^{2}-4(a^{2}R^{2}-a^{2}(R-h)^{2}+R^{2}(R-h)^{2}-(R-h)^{4}))^{1/2}/(2(a^{2}+(R-h)^{2}))$   
 $sin \theta_{0} = (aR^{\pm}((R-h))(a^{2}-R^{2}+(R-h)^{2})^{1/2})/(a^{2}+(R-h)^{2})$ .  
When  $a=R, \theta_{0} = 90^{\circ}$  provided  $h > 2R$ . Then,  
 $1 = (R^{2}(R-h)|R-h|/(R^{2}+(R-h)^{2})$ .  
Choose (-) since  $h > 2R$ .  
 $sin \theta_{0} = (aR-(R-h)(h^{2}+a^{2}-2Rh)^{1/2})/(R^{2}+(R-h)^{2})$   
Check; when  $h=0$ ,  $sin \theta_{0} = (aR-aR)/a^{2}+R^{2}) = 0$  which means  $\theta_{0} = 0$ .



(a). From Fig 4.3,  $\Sigma F_y = 2\pi b T_1 \cos(90^\circ - \theta_0) - W = 0.$ From Fig 2.4,  $x/z = \tan(90^\circ - \theta_0)$   $x = z \tan(90^\circ - \theta_0) = a - z \cot \theta_0$   $T_1 \cos(90^\circ - \theta_0) = a - z \cot \theta_0$   $T_1 (a \sin \theta_0 - z \cos \theta_0) - z \sin \theta_0 = W$   $T_1 (a \sin \theta_0 - z \cos \theta_0) = W/2\pi$  $T_1 = W/(2\pi (a \sin \theta_0 - z \cos \theta_0)) = 0 \le z \le h - R + R \cos \theta_0)$ 

(b). at  $\theta = \theta_0$  z=h-R+Rcos $\theta_0$ T<sub>1</sub> = W/(2 $\pi$ (asin $\theta_0$  - (h-R+Rcos $\theta_0$ )cos $\theta_0$ )) T<sub>1</sub> = W/(2 $\pi$ (asin $\theta_0$  - (h-R)cos $\theta_0$ -R(1-sin<sup>2</sup> $\theta_0$ ))). From Equation A.1 (Appendix A-1), asin $\theta_0$  - (h-R)cos $\theta_0$ =R Substituting, T<sub>1</sub> = W/(2 $\pi$ (R-R(1-sin<sup>2</sup> $\theta_0$ ))) = W/2 $\pi$ Rsin<sup>2</sup> $\theta_0$ .



Using spherical coordinates and summing forces in Fig 4.5 in the vertical direction,

$$\Sigma F_{y} = -T_{1}\cos(90^{\circ}-\theta)(2 \operatorname{Rsin}\theta) + \oint (p(\phi)\cos\phi) dA=0$$
$$0 \leq \phi \leq \theta$$

where

 $dA = 2\pi R \sin \phi R d\phi$ .

$$T_{1}\sin\theta 2\pi R\sin\theta = \int_{0}^{\theta} p(\phi)\cos\phi 2\pi R^{2}\sin\phi d\phi.$$

$$2\pi RT_{1}\sin^{2}\theta = 2\pi R^{2} \int_{0}^{\theta} p(\phi)\cos\phi \sin\phi d\phi.$$

$$T_{1}\sin^{2}\theta = R \int_{0}^{\theta} p(\phi)\cos\phi \sin\phi d\phi.$$
Taking the derivative with respect to 0 of both sides,
$$(dT_{1}/d\theta)\sin\theta + 2T\sin\theta\cos\theta = Rp(\theta)\cos\theta\sin\theta.$$

$$(dT_{1}/d\theta)\sin\theta + 2T_{1} = Rp(\theta).$$



APPENDIX A-4

In three dimensional elasticity using spherical coordinates (see Figure A. 4-1, A.4-2) there are 3 equations of equilibrium:

 $\frac{\partial \sigma_{rre}}{\partial r} + \frac{1}{r\sin\theta} \frac{\partial \sigma_{rx}}{\partial \alpha} + \frac{1}{r} \frac{\partial \sigma_{r\theta}}{\partial \theta} + \frac{2\sigma_{rre} - \sigma_{ax} - \sigma_{re} + \sigma_{re} cdt}{r} + F_r = 0$   $\frac{\partial \sigma_{rx}}{\partial r} + \frac{1}{r\sin\theta} \frac{\partial \sigma_{rxe}}{\partial \alpha} - \frac{1}{r} \frac{\partial \sigma_{r\theta}}{\partial \theta} - \frac{3\sigma_{re} + 2\sigma_{re} cdt}{r} + F_{\alpha} = 0$   $\frac{\partial \sigma_{rxe}}{\partial r} + \frac{1}{r_{elr,\theta}} \frac{\partial \sigma_{re}}{\partial \alpha} - \frac{1}{r} \frac{\partial \sigma_{r\theta}}{\partial \theta} - \frac{3\sigma_{re} + (\sigma_{\theta} - \sigma_{rxe}) cdt}{r} + F_{\theta} = 0$ 

and 6 stress-strain relations:

$$\begin{split} \epsilon_{rr} &= \left(\sigma_{rr} - \nu \left(\sigma_{se} + \sigma_{xx}\right)\right) / \in \\ \epsilon_{\theta\theta} &= \left(\sigma_{e\theta} - \nu \left(\sigma_{rr} + \sigma_{xx}\right)\right) / \in \\ \epsilon_{xx} &= \left(\sigma_{\sigma x} - \nu \left(\sigma_{rr} + \sigma_{e\theta}\right)\right) / \in \\ \gamma_{r\theta} &= \sigma_{r\theta} / G \\ \gamma_{re} &= \sigma_{xe} / G \\ \gamma_{rex} &= \sigma_{xx} / G \end{split}$$



and 6 strain-displacement relations:

$$\begin{aligned} \epsilon_{rr} &= \partial u_r / \partial r \\ \epsilon_{\theta\theta} &= (1/r) (\partial u_{\theta} / \partial \theta) + (u_r / r) \\ \epsilon_{\alpha\alpha} &= (1/r\sin\theta) (\partial u_{\alpha} / \partial \alpha) + (u_r / r) + (u_{\theta} \cot \theta / r) \\ \epsilon_{r\alpha} &= ((1/r\sin\theta) (\partial u_r / \partial \alpha) - (u_{\alpha} / r) + (\partial u_{\alpha} / \partial r)) / 2 \\ \epsilon_{r\theta} &= ((1/r) (\partial u_r / \partial \theta) - (u_{\theta} / r) + (\partial u_{\theta} / \partial r)) / 2 \\ \epsilon_{\alpha\theta} &= ((1/r) (\partial u_{\alpha} / \partial \theta) - (u_{\alpha} \cot \theta / r) + (1/r\sin\theta) (\partial u_{\theta} / \partial \alpha)) / 2 \end{aligned}$$

(see Sokolnikoff, 1956)

In order to relate the elasticity variables to p,  $T_1,$  and  $T_2,$  consider the definitions of  $T_1,$  and  $T_2$  in relation to Figure A-4.1.

 $\sigma_{\alpha\alpha}^{A} = T_{2}L$  $\sigma_{\theta\theta}^{A} = T_{1}L$ .

But A=L.t,

so

 $T_2 = \sigma_{\alpha\alpha} t + \sigma_{\alpha\alpha} = (1/t)T_2$  $T_1 = \sigma_{\theta\theta} t + \sigma_{\theta\theta} = (1/t)T_1.$ 

Now, if one assumes that both shear stresses and body forces are zero.

$$\sigma_{r_{\theta}} = \sigma_{r_{\alpha}} = \sigma_{\alpha \theta} = 0$$
  
$$F_r = F_{\theta} = F_{\alpha} = 0,$$

it can be shown that the three equilibrium equations of elasticity reduce to the two equilibrium equations corresponding to equations (7) and (9) in the text. For the



axisymmetric case ( $\partial/\partial \alpha = 0$ ) with no shear stresses or body forces, the equivalent equations of elasticity are ( $\partial \sigma_{rr}/\partial r$ ) + (1/r) ( $2\sigma_{rr} - \sigma_{\alpha\alpha} - \sigma_{\theta\theta}$ )=0 0=0 ( $\partial \sigma_{\theta\theta}/\partial \theta$ ) + ( $\sigma_{\theta\theta} - \sigma_{\alpha\alpha}$ )cot $\theta$ =0. The first equation can be rewritten as ( $r^{2}(\partial \sigma_{rr}/\partial r) + 2r\sigma_{rr}$ ) =  $r(\sigma_{\theta\theta} + \sigma_{\alpha\alpha})$ or ( $\partial/\partial r$ )( $r^{2}\sigma_{rr}$ ) =  $r\sigma_{aa} + r\sigma_{\alpha\alpha}$ .





Fig A4.1 Stresses on the element in spherical coordinates.





Fig A4.2 Displacement in spherical coordinates.



Integrating

$$\int_{\mathbb{R}}^{R+t} (\partial/\partial r)(r^2 \sigma_{rr}) dr = \int_{\mathbb{R}}^{R+t} r \sigma_{\theta\theta} dr + \int_{\mathbb{R}}^{R+t} r \sigma_{\alpha\alpha} dr$$
$$r^2 \sigma_{rr} |_{\mathcal{R}}^{R+t} = (RT_1(\theta) + RT_2(\theta))$$

 $(R+t)^2 \cdot 0 - R^2(-p) = RT_1 + RT_2,$ 

$$\begin{split} T_1 + T_2 &= pR & (\text{same as (7)}). \end{split}$$
 The third equilibrium equation of elasticity is (d/d0) (T\_1/t) + (T\_1/t - T\_2/t)cot0 = 0 (dT\_1/d0) + (T\_1 - (pR - T\_1))cot0 = 0 (dT\_1/d0) tan0 + 2T\_1 = pR (same as (9)). \end{split}

This means that the 'no shear stress, no body force' assumptions applied to 3-D elasticity in spherical coordinates are compatible with the membrane equations obtained by equilibrium considerations alone.

The advantage in taking the elasticity approach is that it yields the third equation in  $T_1$ ,  $T_2$  and p necessary for a solution; this equation comes in the form of a compatibility equation. Using the stress-strain relations, the no shear stress assumptions require that

 $\varepsilon_{r\alpha} = \varepsilon_{r\alpha} = \varepsilon_{\alpha \Theta} = 0.$ 

In view of the axisymmtry conditions, the straindisplacement equations for  $\epsilon_{r\alpha}$  and  $\epsilon_{\alpha\beta}$  are satisfied automatically. The remaining equation requires that

$$\label{eq:expectation} \begin{split} \epsilon_{\mathbf{r}\theta} = 0 = ((1/r)(\partial u_r/\partial \theta) - (u_\theta/r) + (\partial u_\theta/\partial r))/2. \end{split}$$
 It was assumed that the change in thickness during deformation is negligable. As a result  $u_r << u_\theta$ , and the above equation reduces to



 $(\partial u / \partial r) = (u_{\rho} / r)$ 

which has the general solution,

 $u_{\theta} = rf(\theta)$ 

where f is some arbitrary function. So,  $u_{a}$  increases

linearly with r as expected. The three remaining strain-displacement equations are (with  $u_{\rm r}{<\!\!<} u_{\rm a})$  ,

 $\varepsilon_{rr} = (\partial u_r / \partial r)$ 

$$\begin{split} & \varepsilon_{\theta\theta} = (1/r)(\partial u_{\theta}/\partial \theta) + (u_{r}/r) \simeq (1/r)(\partial u_{\theta}/\partial \theta) \\ & \varepsilon_{\alpha\alpha} = (1/r\sin\theta)(\partial u_{\alpha}/\partial \alpha) + (u_{r}/r) + (u_{\theta}\cot\theta/r) \simeq (u_{\theta}\cot\theta/r) \\ & \text{since } \varepsilon_{\theta\theta} \text{ and } \varepsilon_{\alpha\alpha} \text{ are both related to } u_{\theta}, \text{ they must be} \\ & \text{related to each other; hence, the compatibility condition.} \end{split}$$



## APPENDIX A-5

and the state of the

From Equation (4.7).  $T_{2} = pR - T_{1}$  $T_1 - vT_2 = T_1 - v(pR - T_1) = T_1 (1 + v) - vpR$ and from Equation (4.9).  $T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + (dT_1/d\theta)tan\theta) = (1 - v)T_1 - v T_2 = T_1 (1 + v) - v (2T_1 + v)T_2 = T_1 (1 + v) - v T_2 = T_1 (1 + v) - v (2T_1 + v) - v (2T_1 + v) = T_1 (1 + v) - v (2T_1 + v) - v (2T_1 + v) - v (2T_1 + v) = v (2T_1 + v) - v (2T_1 + v) = v (2T_1 + v) - v (2T_1 + v) - v (2T_1 + v) = v (2T_1 + v) - v (2T_1 + v) = v ($  $(dT_1/d\theta)) \tan\theta$ In similar manner,  $T_2 - vT_1 = pR - T_1 - vT_1 = (1 - v)T_1 + (dT_1/d\theta) \tan\theta$ Substituting into equation (4.16).  $((1 - v)T_1 - v(dT_1/d\theta)\tan\theta)\cos^2\theta = (1 - v)T_1 + (dT_1/d\theta)\tan\theta$ +  $\sin\theta\cos\theta (d/d\theta) ((1 - v)T_1 + (dT_1/d\theta)\tan\theta)$ . Or,  $(1-\nu)T_1\cos^2\theta-\nu T_1'\sin\theta\cos\theta=T_1(1-\nu)+T_1'\tan\theta+T_1'(1-\nu)$  $sin\theta cos\theta + T_1"sin^2\theta + T_1'sec^2\theta sin\theta cos\theta$ . Or,  $T_1"\sin^2\theta + T_1' (sin\theta\cos\theta + 2tan\theta + (1 - \nu)sin\theta\cos\theta) + T_1 (1 - \nu)$  $(1 - \cos^2 \theta) = 0$ . Or,  $T_1"sin^2\theta + T_1'(sin\thetacos\theta + 2tan\theta) + T_1(1 - v)sin^2\theta = 0.$ After one final rearrangement,  $(T_1'' + T_1(1 - v)) \sin\theta\cos\theta + T' (2 + \cos^2\theta) = 0.$ 


From Appendix A-5,  

$$(T_1" + (1 - \nu)T_1)\sin\theta\cos\theta + T_1'(2 + \cos^2\theta) = 0. \quad (4.17)$$

$$T_1 = \sum_{n=0}^{\infty} a_n \sin^n \theta$$

$$T_1' = \sum_{n=0}^{\infty} a_n (n^{-1}) \sin^{n-2}\theta \cos\theta = \cos\theta \sum_{n=0}^{\infty} a_n \sin^{n-1}$$

$$T_1" = \sum_{n=0}^{\infty} a_n ((n-1)) \sin^{n-2}\theta \cos^2\theta - \sin^n \theta) =$$

$$= \sum_{n=0}^{\infty} a_n ((n-1)) \sin^{n-2}\theta - n\sin^n \theta).$$
Substituting into (4.17),  
sin 0 \cos\theta \sum\_{n=0}^{\infty} (n\_n ((n-1)) \sin^{n-2}\theta - n\sin^n \theta) + (1 - \nu)a\_n \sin^n \theta) +
$$(2 + \cos^2 \theta) \cos\theta = and rearranging,$$

$$\sum_{n=0}^{\infty} (n_n ((n-1)) \sin^{n-1}\theta - n\sin^{n+1}\theta + (1 - \nu)a_n \sin^{n+1}\theta + (3 - \sin^2\theta)$$

$$na_n \sin^{n-1}\theta) = 0.$$

$$\sum_{n=0}^{\infty} (n^2 + 2n)a_n \sin^{n-1}\theta - \sum_{n=0}^{\infty} (n^2 + n - 1 + \nu)a_n \sin^{n+1}\theta = 0.$$

$$0 + 3a_1 + \sum_{n=0}^{\infty} (n+2)(n+4)a_{n+2} \sin^{n+1}\theta - \sum_{n=0}^{\infty} (n^2 + n - 1 + \nu)a_n \sin^{n+1}\theta = 0.$$
From the linear independence of the sine functions,  

$$a_1 + 0 = (n_2 + n - 1 + \nu)a_n / ((n+2)(n+4)).$$
Since  $a_1 = 0$ , all  $a_{odd} = 0$ .  
Let  $a_0 = k$ . Then,  
 $a_2 = -(1 - \nu)k/8$ 



 $a_{\perp} = -(1 - \nu)(5 + \nu)k/192$ 

 $a_6 = -(1 - \nu)(5 + \nu)(19 + \nu)k/9216$ 

In summary,

$$\begin{split} & T_1 \left( \Theta \right) = k \; \sum_{n=0}^{\infty} b_n \sin_n \Theta \quad \text{where} \\ & b_{n+2} = b_n (n_2 * n - 1 + \nu) / (n+2) (n+4) \; , \quad b_0 = 1 \\ & \text{and } k \; \text{is determined from the boundary condition that } T_1 \left( \Theta_0 \right) \\ & \text{is known (Equation (4.22)).} \\ & T_1 (\Theta_0) = k \; \sum_{n=0}^{\infty} b_n \sin_n \Theta_0 = W / 2 \pi \text{Rsin}_2 \Theta_0 \\ & \text{k} = (W / 2 \pi \text{Rsin}_2 \Theta_0) / \; \sum_{n=0}^{\infty} b_n \sin_n \Theta_0 = W / (2 \pi \text{R} \; \sum_{n=0}^{\infty} b_n \sin_{n+2} \Theta_0) \text{.} \end{split}$$



APPENDIX A-7

```
From Figure A7.1,
du1=E1ds
du_z = du_1 \sin \theta_0 = \epsilon_1 \sin \theta_0 ds
du, =E,dz
du_z = (T_1/Et)dz = (W/2\pi Et(asin\theta_o - zcos\theta_o))dz
\int_{2}^{u_{z}} du_{z} = W/2\pi Et \int_{2}^{h+R-R\cos\theta_{0}} dz/(a\sin\theta_{0} - z\cos\theta_{0} =
=(W/2\piEt) (ln¦asin\theta_{0} - zcos\theta_{0}|/(-cos\theta_{0}))
u_z = (W/2\pi Et)((ln|asin\theta_0 - hcos\theta_0 + Rcos\theta_0 - Rcos^2 e'/(-cos\theta_0)) -
(\ln|asin\theta_0|/(-\cos\theta_0))).
Using the relations from Appendix A-1, this can be simplified:
u_{\pi} = (W/2\pi Et)(\ln(Rsin^2\theta_0) - \ln(asin\theta_0))/(-\cos\theta_0)
u_{\pi} = (W/2\pi Et) \ln(Rsin^2\theta_0/asin\theta_0)/(-\cos\theta_0)
or
u_{\pi} = (W/2\pi Et)(\ln(a/Rsin\theta_0)/cos\theta_0).
Since a > R, \ln (a/Rsin\theta_0) > 0, so that u_7 > 0.
```





Fig A7.1 Displacement of the free surface part of the membrane.



## APPENDIX A-8

Equation (4.41) in the text represents the deflection over load result for no slip conditions. Solving (4.41) for Et,  $Et = (W/u_{\tau})(\ln(a/R\sin\theta_{0}))/2\pi\cos\theta_{0}$ (A8.1) Since W and  $u_{\tau}$  from the Instron experiment were read off of the graph in inches with a full scale load of 10 inches = 1 kg for W and with a 2 inch displacement on the paper corresponding to a 1 inch displacement of the indenter, W/u<sub>2</sub> = force/displacement = W/Wcotß =(W[in] \* 9.81[N]/10[in])/(Wcot8 [in]\*(1[in indenter]/2[in N/m chart]\* .0254[m]/1[in]) = 77.244 tang[N/m] where  $\beta$  is the angle from the graph shown on Figure A8.1. Using Equation (A8.2) in Equation (A8.1),  $Et=(12.294 \tan\beta / \cos\theta_{o})(\ln(a/Rsin\theta_{o}))$ (A8.3) for the no-slip part of the experiment. Let  $M_{NS}$  be the number of samples in each group of cuticular membranes which were tested under "no-slip" conditions and Ms the number under slip conditions. Since  $\beta$  and  $\theta_{0}$  change for each of the  $M_{NS}$  samples, from Equation (A8.3).  $Et_{avg} = (12.294 \sum_{n=1}^{M_{E}} tan\beta_{n} \ln (a/(Rsin\theta_{n}))/cos\theta_{n})/M_{NS} (A8.4)$ 

$$\begin{split} & \text{Et}_{\text{avg}} = (12.294 \sum_{n=1}^{\infty} \tan \beta_n \ln (a/(\text{Rsin}\theta_n))/\cos \theta_n)/M_{\text{NS}} \quad (A8.4) \\ & \text{In program QUICK, two vectors were generated,} \end{split}$$





where:

- H initial distance from the horizontal plane to the point of taut membrane (Figure 4.2)
- HF final distance H from the horizontal plane to the point of break
  - β "Instron angle"; ANG in program QUICK
- W load applied to the membrane

Figure A8.1 Example of graph from Instron experiment and graphical explanation of some variables required for PROGRAM QUICK (W and  $u_{\tau}$  were read in inches from the graph).







Using the above two vectors, (A8.4) can be written as

$$Et_{avg} = (12.294 \sum_{n=1}^{M_{av}} x_n y_n) / M_{NS}$$
 (A8.6)

The only unknown in (A8.6) is  $Et_{avg}$  since everything on the right hand side is known. For "slip" conditions, Equation (4.40) in the text should be used. Solving (4.40) for Et,  $Et=(W/(2\pi \cos\theta_{o}u_{z}))(1 - v + \ln(a/R\sin\theta_{o}))$  (A8.7) Since the same conversion factors used in Equation (A8.1) apply to Equation (A8.7) as well,  $Et=(12.294 \tan\beta/\cos\theta_{o})(1 - v + \ln(a/R\sin\theta_{o}))$  (A8.8) and the average Et for all "slip" samples in a given group of cuticles can be calculated as

$$Et_{avg} = 12.294 \left( (1-v) \sum_{n=1}^{M_{s}} (tan\beta_{n}/cos\theta_{n}) + \frac{M_{s}}{n=1} (tan\beta_{n}/cos\theta_{n}) \right)$$
(A8.9)

or in terms of the vectors in (A8.5)

$$Et_{avg} = 12.294 ((1-v) \sum_{n=M_{w_{t}}+M_{s}}^{M_{w_{t}}+M_{s}} \sum_{n=X_{w_{t}}+1}^{M_{w_{t}}+M_{s}} \sum_{n=M_{w_{t}}+1}^{M_{w_{t}}+M_{s}} (A8.10)$$

Now, since Equations (A8.6) and (A8.10) both give Et<sub>avg</sub>, the right hand sides can be equated and the resulting expression can be solved for the only remaining unknown, Poisson's ratio.



$$= (((M_{s}/M_{Ns})\sum_{n=1}^{M_{Ns}} x_{n}y_{n} - \sum_{n=M_{Ns}+1}^{M_{Ns}+M_{s}} x_{n}y_{n}) / \sum_{n=M_{Ns}+1}^{M_{Ns}+M_{s}} x_{n})$$
(A8.11)

Et and v for each group of cuticular membranes were calculated using Equations (A8.6) and (A8.11) and program QUICK listed in Appendix B. In the same program, the standard deviation for Et was calculated using the value of from Equation (A8.11) and generating an additional number (M<sub>S</sub>) of Et's from Equation (A8.8). In this way, the standard deviation applied to all samples in the group, "slip" and "no-slip", not just to the "no-slip" ones.

$$\sigma^2 = \sum_{n} (Et^{(n)} - Et_{avg})^2 / (M_{NS} + M_S)$$
(A8.12)

This can be expanded as

 $\sigma^{2} = \sum_{all} ((Et^{(n)})^{2} - 2Et^{(n)}Et_{avg} + Et_{avg}^{2})/(M_{NS} + M_{S}) \quad (A8.13)$ It will now be shown that  $\sum_{all} Et^{(n)} = (M_{NS} + M_{S}) Et_{avg}$  where  $Et_{avg}$  was calculated using only  $M_{NS}$  samples.

From Equation (A8.4)

$$\sum_{\substack{n=1\\n \neq i}}^{M_{MS}} Et^{(n)} - M_{NS}Et_{avg}$$
(A8.14)

From Equation (A8.10)

$$\prod_{n=1}^{N_{s}} \operatorname{Et}^{(n)} - \operatorname{M}_{S} \operatorname{Et}_{avg}$$
(A8.15)

But,

$$\sum_{\alpha \parallel} (Et^{(n)})^2 = \sum_{M_{NS}} (Et^{(n)})^2 + \sum_{M_S} (Et^{(n)})^2$$
(A8.16)

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Using (A8.14) and (A8.15) in (A8.16)

$$\begin{split} \sum_{all} (\text{Et}^{(n)})^2 &= (M_{NS} + M_S)(\text{Et}_{avg})^2 \quad (A8.17) \end{split}$$
  
Substituting the right hand side of (A8.17) into (A8.13)  
$$\sigma^2 &= (\sum_{all} (\text{Et}^{(n)})^2 - 2(M_{NS} + M_S)\text{Et}_{avg}^2 + (M_{NS} + M_S)\text{Et}_{avg}^2)/ (M_{NS} + M_S), \text{ or} \\ \sigma^2 &= (\sum_{all} (\text{Et}^{(n)})^2 - (M_{NS} + M_S) \text{Et}_{avg}^2)/(M_{NS} + M_S) \text{ or} \\ \sigma^2 &= (1/(M_{NS} + M_S)) (\sum_{all} (\text{Et}^{(n)})^2) - \text{Et}_{avg}^2 \quad (A8.18) \text{ Separating the sum into groups,} \\ \sigma^2 &= (1/M_{NS} + M_S)) (\sum_{M_{NS}} \text{Et}^{(n)})^2 + \sum_{M_S} \text{Et}^{(n)})^2) - \text{Et}_{avg}^2 \quad (A8.19) \\ \end{split}$$
  
Using the expressions (A8.3) and (A8.8) for the "no-slip" and (A8.8) and (A8.8) for the "no-slip" and (A8.8) for the "

"slip" parts respectively,

and the standard deviation is equal to the square root of (A8.20).

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PROGRAM DEWAX (INPUT,OUTPUT,TAPE1=INPUT,TAPE2=OUTPUT) DIMENSION N(20),SLOPE(20),W(20,3),AVG(5),STDDEV(5), \*,GERMC(20),STDPE5),CUIV(20) PROGRAM CALCULATES PERMEABILITY COEFFICIENTS OPT THE DEWAXED CUTIDULAR MEMBRANES AND GIVES TREATED MEMBRANES AND THE CONTROL GROUP PROUDE1-MCL,GROUP2-KCL GROUP3-CACL2 GROUP4-ALCL3 GPOUP5-CONTROL K-NUMBER OF GAMBERS FOR A GIVEN TREATMENT REAUC1,\*/CN(X),\*E1,5) CH-NEAUC1,\*/CN(X),\*E1,5) CH-NEAUC1,\*/CN(X),\*/CN(X),\*/CN(X),\*/CN(X) CH-NEAUC1,\*/CN(X),\*/C RAD (1+\*)((U([]),)=1+3),I=1+N(K)) U=UIDAT OF THE CHAMBER SUMMER OF CONSECUTIVE MEASURMENTS FOR THE SAME CHAMBER SUMMER OF CONSECUTIVE MEASURMENTS FOR THE SAME CHAMBER SUPE-OID I=1+N(K) DO 10 I=1+N(K) SLOPE-OID THE REGRESSION LINE FOR TALE MEMBRANE CUTJ-J=SUMPER PERMC(I)=C(UTJ(I)\*CH)/(CH-CUTJ(I)) PERMCAPLENELITY COEFFICIENT FOR THE MEMBRANE ITSELF NA CHAMBER AVG-AVEAGE SLOPE FOR N CHAMBERS SIDDEV(K)=SUM1/N(K))\*2 AVG-AVEAGE SLOPE FOR N CHAMBERS SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) N=CAMBERS SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) IN=DECRESOF FOR N CHAMBERS SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) IN=DECRESOF FOR N CHAMBERS SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) IN=DECRESOF FOR N CHAMBERS SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) N=DECRESOF FOR N CHAMBERS SIDDEV(K)=SORT(SUM2/N(K)-AVG(K)\*2) SIDDEV(K)=SORT(K)/N(K)=SORT(K)) SIDDEV(K)=SORT(K)/N(K)=SORT(K)) SIDDEV(K)=SORT(K)/N(K)=SORT(K)) SIDDEV(K)=SORT(K)/N(K)=SORT(K)) SIDDEV(K)=SORT(K)/N(K)=SORT(K) c C ř

## PROGRAM DEWAX



PROGRAM NWAX

PROGRAM NWAX (INPUT, OUTPUT, TAPEL = INPUT, TAPSBEOUTPUT) DIMENSION N(20) & SLOPE(20) & V(20,3) & AVG(3), STODEV(5), \* PERCK(20), STOP(5), & CUTV(20) PROGRAM CALCULATES PERMEABILITY COEFFICIENTS COMPARISON OF THE PERMEABILITY BETWEEN CHEMICALLY THEATLO MEMBRANES AND THE CONTROL GROUPS-CONTROL K-NUMBER OF CRANERS FOR A GIVEN TREATMENT RLADI, \*)(N(K) \*K=1,5) CH-AVSPAGE, PERMEABILITY COEFFICIENT FOR THE CHAMBERS WITHOUT THE FERMEABILITY COEFFICIENT FOR THE CHAMBERS WITHOUT THE FERMEABILITY COEFFICIENT FOR THE CHAMBERS UNTHOUT THE FERMEABILITY COEFFICIENT FOR THE SAME CHAMBER SUBJO (1) \*\*; (V(I)), J=1,3), I=1,N(K)) U-WITHOUT THE FERMEABILITY COEFFICIENT FOR THE SAME CHAMBER SUBJO (1) \*\*; (V(I)), J=1,3), I=1,N(K)) U-WITHOUT THE FERMEABILITY COEFFICIENT FOR THE SAME CHAMBER SUBJO (1) \*\*; (V(I)), J=1,3), I=1,N(K)) U-WITHOUT THE FERMEABILITY COEFFICIENT FOR THE SAME CHAMBER SUBJO (1) \*\*; (V(I)), J=1,3), I=1,N(K)) U-WITHOUT HE FERMEABILITY COEFFICIENT FOR THE MEMBRANE IN A CHAMBER OF CONSECUTIVE MEASURMENTS FOR THE SAME CHAMBER SUDJO (1) SUBJO (0) FINE KCGGESSON LINE FOR THE MEMBRANE IN A CHAMBER IN A CHAMBERS LICECOUNTION FOR THE MEMBRANE ITSELF SUDJO (1) SUBJO (1) //1/1/1/1/1/2) \*\*2\*W(I) //1/2) PREME-PERMEABILITY COEFFICIENT FOR THE AVERAGE SLOPE IN A CHAMBERS STODEV STANDARD DEVIATION FOR THE AVERAGE SLOPE FOR N CHAMBERS USED (1) //1/1/2) NOUVENT: STANDARD DEVIATION FOR THE AVERAGE SLOPE SUDJO STANDARD DEVIATION FOR CHE AVERAGE SLOPE STUDEV(S) \*\*5700EV(K)/AVG(K), STDDEV(K) \*\*STODEV(S) \*\*5700EV(K)/AVG(K), STDDEV(S) \*\*515.13,55X, \*\*STODEVCS) \*\*STOPEV(K)/AVG(S) STOP COEFFICIENT OF THE PERMEABILITY OF CHEMICALLY TREATED MEMBRANES VERSUS CONTROL GROUP DO SI I=1,4,4,(S) STUDENT T TEST FOR THE PERMEABILITY OF CHEMICALLY TREATED MEMBRANES VERSUS CONTROL GROUP DO SI I=1,4,4,(S) STUDENT T TEST FOR THE PERMEABILITY OF CHEMICALLY TREATED MEMBRANES VERSUS CONTROL GROUP DO SI I=1,4,4,(S) STUDENT T TEST FOR THE PERMEABILITY OF CHEMICALLY TREATED MEMBRANES VERSUS CONTROL GROUP DO SI I=1, 0000000000



PROGRAM OUICK(INPUT.BUTPUT) DIMENSION 4(50) +F(50) +NNG(3D) +X(3D) +Y(3D) DIMENSION 4(50) +F(3D) +NNG(3D) +X(3D) +Y(3D) C PF ELASTICITY AND THE STANDARD DEVIATION FOR THE READ.\* MNS.(W(1) +F(1) +ANG(1) +I=1+MNS) C MNS.\*MUHER OF SAMPLES UNDER SLIP CONDITIONS C MNS.\*MUHER OF SAMPLES UNDER SLIP CONDITIONS C MNS.\*MUHER OF SAMPLES UNDER SLIP CONDITIONS C MNS.\*MUSDITIONS C ANGLE OF THE SLOPE (SEE FIS.4B-1) C ARGE OF THE SLOPE (SEE APPENDING C ARGE OF THE SLOPE (SEE APPENDIX A-B) C TALCULATION OF THE VECTOR X (SEE APPENDIX A-B) C CALCULATION OF THE VECTOR Y (SEE APPENDIX A-B) SI S2525310. C CALCULATION OF THE VECTOR Y (SEE APPENDIX A-B) SI S2525310. C CALCULATION OF THE VECTOR Y (SEE APPENDIX A-B) SI S2525310. C CALCULATION OF THE VECTOR Y (SEE APPENDIX A-B) SI S2525310. C CALCULATION OF THE VECTOR Y (SEE APPENDIX A-B) SI S252547(1)\*Y(1) SI S252547(1)\*Y(1) SI S252547(1)\*Y(1) SI S253500, S RATIO VI. -(MS\*SI/MNS-S2)/S3 C CALCULATION OF ET FOR NON-SLIP POINTS EFIC.294+SI/MNS C CALCULATION OF ET FOR NON-SLIP POINTS EFIC.294+SI/MNS SI SU SSO I=MNS+I, MNS+MS SI SU SSO I=MNS+I END

PROGRAM QUICK



Table B.1

Additional information for Table 5.4

Group	chemical treatment	dewaxed		nondewaxed	
		t	d.f.	t	d.f.
	HCl	428	11	-1.391	18
Pik Red (Set 1)	HCl	•596	8	-2.262	18 (**)
	CaCl <sub>2</sub>	915	8	-2.770	18 (**)
	AlCl <sub>3</sub>	2.091	8	-2.463	18 (**)
Pik Red (Set 2)	HCl	2.667	15 (**)	2.976	17 (**)
	KCl	.127	17	1.220	16
	CaCl2	135	17	310	17
	AlC13	1.238	17	599	17
UC-82 (processing)	HCl	-1.131	9	681	17
	KCI	-1.224	7	.717	16
	CaCl <sub>2</sub>	564	6	2.273	17 (**)
	AlCl <sub>2</sub>	349	7	-1.195	16

d.f - degrees of freedom

(\*\*) - samples found to be significantly different from the control group at the uncertainty level  $\alpha = .05$  (5%)



Each of the ten chambers was weighed three times. It is easy to show that if the standard least square technique is used, the line fitted to three points has a slope

 $M = (W_3 - W_1)/4$ 

and y-intercept

 $b = (5W_1 - W_2 + 2W_3)/6$ 

where W  $_1$ , W  $_2$ , W  $_3$  are the consecutive weights of each chamber at 0, 2 and 4 hours for dewaxed samples.

This means that the slope of the line depends only on the first and last measurements and that the y-intercept is not  $W_1$ . This is unacceptable since at t = 0, the weight must be  $W_1$ . For this reason, a modified least square method which forces the line to pass through the first point is used. With this method, the slope is dependent on all three measurements; it takes into account the second measurement,  $W_2$ , as it should.

In general, if one wants to fit a straight line to N points while at the same time forcing it to pass through the first point  $(x_1,y_1)$ , an equation of the form

 $y = y_1 + M (x - x_1)$  is used. Here we want to minimize the variance

$$E^{2} = \sum_{k=1}^{N} (yk - (y_{1} + M (x_{k} - x_{1})))^{2}$$

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This requries that  $aF^2/aM = 0$  $-2\sum_{k=1}^{N} (y_k - (y_1 + M (x_k - x_1))) (x_k - x_1) = 0$ (C-1)Solving (C-1) for M,  $M = \sum_{k=2}^{N} (x_k - x_1)(y_k - y_1) / \sum_{k=2}^{N} (x_k - x_1)^2$ (C-2)For three points;  $(W_1,0)$ ,  $(W_2,2)$ ,  $(W_3,4)$  for dewaxed  $(W_1, 0)$ ,  $(W_2, 12)$ ,  $(W_3, 24)$  for nondewaxed and  $M_{dewaxed} = (-3W_1 + W_2 + 22W_3)/10$ (C-3) $M_{nondewaxed} = (-3W_1 - W_2 + 22 W_3)/60$ (C-4)The slope of the line represents the permeability rate R for a given chamber in (kg/h). The permeability rate was converted to (kg/s) and the flux J+r was calculated,  $J_{tr} = R/A\rho$ where  $J_{tr}$  - transpirational flux for the chamber (m/s) - rate of permeability for the chamber (kg/s) R - surface area of the exposed membrane  $(m^2)$ Α - density of water at 25<sup>0</sup>C (996.5 kg/m<sup>3</sup>) Then permeability coefficients for each chamber were obtained as the flux  $J_{tr}$  per unit driving force ( $\Delta a=1$ ), where  $\Delta a$  is the difference in water activity between the inside and outside of the membrane. The permeability coefficient for the membrane itself was calculated using Equation (3.2) in Chapter 3. Finally the average value for the permeability coefficient for each subgroup of membranes



was calculated together with a coefficient of variation (see PROGRAM DEWAX AND PROGRAM NWAX).



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