THE ROLE OF ION EXCHANGE IN THE MOVEMENT OF CHEMICALS THROUGH PERIDERM AND CUTICULAR MEMBRANES

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Dedicated

to the memory of my late grandfather

and

also my grandmother

DECLARATION

I hereby declare that the following thesis is based on the results of investigations conducted by myself, and that it is of my own composition. This thesis has not, in whole or in part, been previously presented for a higher degree or qualification. Work other than my own is clearly indicated in the text by reference to the relevant researchers or their publications.

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SUMMARY

This thesis is principally an investigation on the factors contributing to the role of ion exchange in the movement of chemicals through the potato periderm and pear fruit cuticular membranes. This was investigated by monitoring the penetration of alkali cations, neutral amino acids and some pesticides through the potato periderm and pear fruit cuticular membranes in terms of physicochemical parameters such as permeability, diffusion, selectivity and adsorption by using the infinite-dose system, finite-dose and sorption process.

Isolated cuticles or periderms, which represent the prime barrier to penetration, provide a physical system by which transport studies can be conducted under well defined and highly controlled conditions. Most studies with isolated cuticles have focused on sorption, desorption and infinite-dose cuticular transport of chemical compounds in aqueous systems. Transport systems, using isolated cuticles or periderms, may be used to quantify the effects of permeability, sorption, environmental pollutants, foliar absorption and spray additives on pesticide penetration.

The work can be subdivided as follows:

A brief discussion was made on the background chemistry, structure and ion exchange properties of potato periderm and pear fruit cuticles with regard to the use of chemicals on isolated membrane samples. The mechanisms of penetration of chemicals, the nature and chemistry of the potato periderm and pear fruit cuticles and some of the interacting factors contributing to variability were considered. The focus was on the use of potato periderm and pear fruit cuticles as model systems.

The ion exchange properties and counter ion selectivity of the potato periderm and pear fruit cuticles towards alkali cations were studied. The important

role of ion exchange, pretreatment and counter ion selectivity were demonstrated by results on the capacity and selectivity of alkali ions, which generally followed the order; $Li^+ > Na^+ > K^+ > Cs^+$. The ion exchange capacity and selectivity of the potato periderm and pear fruit cuticles depended on the different ionic forms, increasing with increasing pH and counter ion valence and decreasing with decreasing crystal radius.

The titration curves obtained with NaOH in the presence of NaCl depend markedly on pretreatment. Three separate groups can be distinguished over the pH ranges studied. Within these ranges, the first was between pH 3-5.5, the second between pH 5.5-9 and, the endpoint of the third group was estimated between pH 9-11.5. The isolated potato periderm and pear fruit cuticles exhibited a behaviour typical of highly cross-linked, high capacity ion exchange resins of the weak acid cation exchanger type. The adsorption equations obtained from plotting the experimental data gave a fair agreement with the Freundlich equation.

Permeability and diffusion coefficients can be calculated from cuticular transport studies. These transport parameters provide a better understanding of the mechanisms of cuticular penetration phenomena. Further, they are useful when comparing both penetration characteristics of selected compounds and permeability studies.

The details of penetration rates and the mechanisms of alkali cation uptake through the isolated potato periderm and pear fruit cuticles were determined. and the permeability and diffusion coefficients and, also from these parameters, relative values of permeability and diffusion coefficients were calculated. The general order observed was Cs> K> Na> Li. This is the order of increasing hydration radii. The penetration rates of alkali cations were markedly dependent on pH and the nature of counter ions used. The penetration rates increased almost 3 fold from pH 3 to pH 8.

The possibility that K^+ ion could be transported its concentration gradient through the isolated membranes was investigated using different pH gradients but this was not found. A tentative transport mechanism due to fixed charges was proposed. The studies were then extended to examine the selective transport of alkali cations by using K-Na and K-Li binary systems with various pH gradients.

An investigation on amino acid transport and selectivity studies were carried out. The penetration rates of the amino acids on the isolated potato periderm and pear fruit cuticles were observed and followed the order of increasing molecular weights and the extension of the hydrophobic $-CH_2-$ groups. Amino acid-ammonium exchanges were identified by the ion exchange isotherms which resembled a characteristic Freundlich isotherm type.

The factors affecting the penetration of pesticides with particular emphasis on the influence of commercial formulations were investigated. The % penetration and % penetration rate were given. The result showed that the penetration of commercial formulations of pesticides depends on water solubility and pH. The transcuticular penetration of pesticides was compared using infinite dose and finite-dose systems. A finite dose system may be useful in studying spray droplet/deposit interaction with the cuticle and, transcuticular penetration. These systems may prove useful in optimising spray formulations or parameters, leading to pesticide development and application in fields studies.

Some conclusions were drawn as to the outcome and relevance of the main findings. The implications of the above studies were discussed and some suggestions for further investigation were also highlighted.

CONTENTS

ABBREVIATIONS

A	: Planar membrane area (cm^2)
A°	: Angstrom
A^+	: Monovalent amino acid
A^{2+}	: Divalent amino acid
AAS	: Atomic absorption spectrophotometry
C	: Concentration
C_H	: Equilibrium concentration of H
cm	: Centimetre
CM	: Cuticular membrane
C_M	: Equilibrium concentration of alkali cations
$C_{A^{+n}}$	: Amino acid in solution phase
$C_{NH_4^+}$	: Ammonium ion in solution phase
C_{M_0}	: Initial concentration of alkali cations
C_{H_0}	: Initial concentration of H ion
ΔC	: Concentration difference
°C	: Degrees centigrade
D	: Diffusion coefficient ($cm^2\ s^{-1}$)
D/D_{Li^+}	: Relative diffusion coefficients of cations as a Li ion.
DM	: Dialysis membrane
e	: Electronic charge
e.t.c.	: Etcetera
E_{io}	: Electric potential from inside to outside of cuticles
et al.	: and others (authors)
F	: Faraday constant
f_i	: Activity coefficient of i ion species
FMOC-Cl	: 9-Fluorenylmethyl chloroformate
Fr	: Flow rate (mol s^{-1}) eqn (4.3)
F_r	: Gibbs free energy
$\Delta F_{hydration}$	: Free energy of hydration
$\Delta F_{ion/site}$	: Free energy of ion
$\Delta F_{transfer}$	: Free energy of transfer
g	: Gram
h	: Hour

HLB	: Hydrophobic-lipophilic balance
HPLC	: High performance liquid chromatography
i.d.	: Internal diameter
i.e.	: That is
J_i	: Flow of species i (mol cm^2 s^{-1})
J_m	: Flow of chemical process
K	: Partition coefficient
K_d	: Distribution coefficient
K'^{B}_{A}	: Corrected selectivity coefficient eqn. (3.3)
K^{B}_{A}	: Selectivity coefficient for B ion over A ion eqn. (3.2)
K^{M}_{H}	: Selectivity coefficients of alkali cations
$K^{A}_{NH_4}$	: Selectivity coefficients of amino acid eqn. (6.2)
$[K^+]_0$	: Initial concentration of potassium ion
$[K^+]_{i,0}$	: Initial concentration in inner surface of membrane
$[K^+]_{max}$	: Concentration of potassium at time t maximum
$[K^+]_{o,t}$	: Concentration in outer surface of membrane at time t
L	: Litre
l	: Membrane thickness (cm)
L_i	: Phenomenological coefficients
m	: Metre
M	: Molarity
M^+	: Alkali cation
meq	: Milliequivalent
mg	: Milligram (10^{-3} gram)
min	: Minute
ml	: Millilitre (10^{-3} litre)
mmol	: millimol
M_S	: Amount diffused
nm	: Nano metre
P	: Permeability (cm s^{-1})
p	: Statistical probability
P/P_{Li^+}	: Relative permeability of cations as a Li ion.
P_{Cl}	: Permeability of chloride
PFCM	: Pear fruit cuticular membrane

P_K : Permeability of potassium

PM : Potato periderm membrane

P_M :Permeabilities of alkali cations

pp. : Pages

ppm : Part per million

Q : Ion exchange capacity eqn. (6.3)

R : Gas constant

r_i : Activity coefficients of cation species p.

R_{ir} : Coupling of species i against electrochemical potential gradient

$R_{A^{+n}}$: Amino acid in exchanger phase

$R_{NH_4^+}$: Ammonium ion in exchanger phase

s : Second

SD : Standard deviation

T : Temperature

t : Time (min or sec)

T : Titre (p.)

t_e : Time-lag for diffusion (s) eqn. (4.4).

U.V. : Ultraviolet detector

V : Volume

V_i : Partial molar volume of i ionic species

v : Versus

w : Weight

x : Space coordinate

X_i : Conjugate forces

X_M : Equivalent ionic fraction of alkali cations in the solution phase

$\overline{X}_M$: Equivalent ionic fraction of alkali cations in the exchanger phase

$\overline{X}_i$: Equivalent ionic fraction of i ion species in the exchanger phase

z : Valence

α^i : Ionic activity

ϕ_j^{io} : Fluxes of ion j from inside to outside of membrane eqn. (5.11)

ϕ_j^{oi} : Fluxes of ion j from outside to inside of membrane eqn. (5.11)

μg : Microgram (10^{-6} gram)

μ_i : Thermodynamic potential of component i

μl : Microlitre (10^{-6} litre)

μm : Micrometer

ν_i	: Positive for products, negative for reactants of reaction
%	: Percentage
<	: Less than
>	: Greater than
>>	: Much greater than
$\geq$	: Greater than or equal to

CHAPTER 1

Introduction

1.1 Background

Increased industrialisation and human population have led to a progressive degeneration of the existing environment. Chemicals are constantly being released into rivers, lakes, soils and atmosphere, and their harmful effect to human, animals and nature are main causes to be concerned. Pollutants in the physical and biotic environment are subject to redistribution, deterioration, and transformation.

Plants may accumulate pollutants by uptake from soil and water or acid rains through the roots. This is the preferential route for relatively polar materials. Those materials which are soluble in water either inorganic or organic, their absorption take place through the surface.

The mechanism of foliar penetration is necessary to find out what the pathways are through which penetrating solutions pass from the surface to the protoplasts of epidermal cells and vice versa. These pathways are at three sites: in the cuticle, in the cellulose wall and in the plasma membrane (Franke, 1967).

The plant cuticle is a noncellular, nonliving lipophilic membrane that forms the interface between the plant and its environment (Martin and Juniper, 1970). The covering on the epidermal cells on the outer surface, is commonly named the cuticle. The cuticle is a barrier, but it is not impermeable, and serves an important structural and physiological roles since all aerial plants organs are covered with a cuticular membrane (Martin and Juniper, 1970). The cuticle prevents the uncontrolled loss of water from the plant body and protect against environmental and biotic factors, and also serves as the prime barrier to penetration of agrochemicals (Ramirez et al., 1992).

Kolattukudy (1980) described that the cutin and suberin as biopolyester membranes of plants and such polyesters not only constitute the major protective barrier between the plant and its environments but also function as a rather permanent biological barrier within a variety of organs so that diffusion of molecules can be controlled. Schonherr and Huber (1977) reported that cuticles are bipolar polyelectrolytes.

Plant cuticle are potential sorption compartments for lipophilic compounds (Riederer and Schonherr, 1984). Throughout this study the emphasis is towards quantitative analytical and physicochemical procedures for chemicals penetrated and adsorbed by periderms and cuticles. The need to know the effect of chemical substances in plant materials, i.e., their transport, sorption, accumulation and desorption in cuticles was pointed out over the last three decade, but idea was not pursued, at least from a quantitative, physical and thermodynamical viewpoint. This kind of approach has been studied last 10 years. (Riederer and Schonherr, 1984, 1986, 1988; Riederer and Schneider, 1990; Schonherr and Riederer, 1989; Schreiber and Schonherr, 1990)

The physicochemical properties of the aerial surfaces of all higher plants are governed mainly by the cuticle, or cuticular membranes. Fundamental knowledge of the morphology and properties of plant cuticles is crucial to the design formulations for agrochemicals, especially in explaining their behaviour and performance following spray application to foliage (Holloway, 1993). This information is also relevant to any sorption and penetration studies with pesticides and adjuvants which might be undertaken in vitro using isolated cuticle preparations (Holloway, 1993).

Cutin is the structural component of the plant cuticle, which is attached to the outside of the epidermal cell wall. Suberin is deposited at an extracellular location, on the plasma membrane side of the cell wall. Cutin is found on the aerial parts, and suberin in the underground parts and wound surfaces of plants (Kolattukudy, 1980).

1.2 Cuticular membrane

It is generally agreed that the cuticular is a lipid membrane which covers aerial organs of terrestrial plants. The structure and physico-chemical nature of plant cuticle have been comprehensively reviewed (Franke, 1967; Hull, 1970; Martin and Juniper, 1970). The mechanism of foliar uptake and cuticular penetration through the cuticle have also been well discussed by various workers (Franke, 1967; Hull, 1970; Martin and Juniper, 1970; Schonherr and Riederer, 1989). The cuticle is a continuous, protective membrane (McPhail, 1984), which seems mostly to be uniform (Franke, 1967).

Most chemicals are applied as foliar sprays and many (i.e., herbicides, pesticides, nutrients and growth regulator) must penetrate the cuticle in order to be effective. Cuticle thickness and composition can differ substantially between plant species (Martin and Juniper, 1970). Orgell (1955) has stated that the cuticle may be characterised by an imbricate arrangement of lipoid platelets cemented together by hydrophilic pectinaeous materials.

The early works is mostly descriptive. In most cases, it is not possible to calculate permeability and diffusion coefficients as pointed out by Hartley and Graham-Bryce (1980). Davis et al (1979) pointed out that results from different sources, species and compound cannot be compared.

The chemical constituents of cutin have been discussed in some details (Baker, 1982; Holloway, 1972, 1982b, 1993; and Kolattukudy, 1980). A Comprehensive review of chemistry, structure and function of cuticles and periderms is found in Martin and Juniper (1970). Penetration of cuticles by solutes applied to the foliage has been studied extensively and was reviewed by Hull (1970). The chemical structure of cutin is assumed to be a polymerisation and a cross-linked condensation polymer of C_{16} and C_{18}-hydroxy fatty acids combined by ester bonds and peroxide (also ether) bridges (Franke, 1967). It shows, like lignin, the amorphous, stereochemically mixed

structure of inanimate polymers rather than the unique regularity of cellulose or proteins (Franke, 1967). This is a three-dimensional cross-linked polymeric structure of lipophilic nature, consisting mainly of a complex mixture of esterified, long chain fatty acids, predominantly of C_{16} and C_{18} chain length (McPhail, 1984). The main substituent of monomers being hydroxyl groups, though aldehyde, ketone, epoxide, unsaturated groups and additional carboxyl groups also occur (McPhail, 1984).

Embedded within this biopolymer matrix will be cuticular wax, possibly highly orientated or lamelear in form (mainly associated with the cuticle proper), and then a gradation into the incorporation of more hydrophilic compounds, polysaccarides such as pectin, hemicellulose and cellulose microfibrils, and possibly proteins and tannins (McPhail, 1984). Pectin and the hemisubstances of the matrix possess free carboxyl groups which contribute to polarity (Franke, 1967).

Cutin is largely a polyester comprising mainly hydroxy and epoxy fatty acids. The insoluble fraction of cuticles is never pure cutin, it also contains various nonlipid components such as cellulose, polyuronic acids, proteins and phenolic compounds (Hunt and Baker 1980; Kolattukudy, 1980; Martin and Juniper, 1970; Schonherr and Huber, 1977). In isolated cuticles the cutin ranges from 20 to 1.030 $\mu g\ cm^{-2}$ or from 20% to 84% by weight (Holloway, 1972). The soluble cuticular lipids are very complex mixture, comprising 6 to 380 $\mu g\ cm^{-2}$ or from 2% to 30% by weight (Schonherr, 1982).

Cuticles carry weakly acidic and weakly basic group, the pH of the external solution has an effect on water content of the membrane via swelling. Cuticles carry fixed negative charges above pH 3 (Schonherr and Huber 1977). This leads to Donnan exclusion of anions, to varying degrees, depending on pH, ionic strength and valency of anions (Schonherr, 1976). Cutin has the attributes of a weak acid cation exchanger. Cellulose, located more deeply in the epidermal layer, is also a weak acid cation exchanger.

The equivalent weight of the residual cutin was estimated to be about 2600 corresponding to 1 in 10 of C_{16} hydroxyacids having a free carboxyl group and the ionic content makes the cutin significantly swellable in water (Hartley and Graham-Bryce, 1980).

Cutin consists of a C_{16} and C_{18} family of monomers. Chemical examination of the nature and the quantity of the hydroxyl groups that are free in the polymer by the two methods suggested that in the polymers which contain mainly the C_{16} family of monomers, about half of the monomers contain a free midchain hydroxyl group (Kolattukudy, 1977). The proposed model for the structure of cutin is shown in Fig. 1.1.

Information about the internal structure and constituent by histochemical methods was observed with light or transmission electron microscopes (Holloway, 1982). Monomers with primary hydroxyl groups can form only a linear structure, whereas cross-linking of the aliphatic chains is possible with those possessing secondary hydroxyl groups; most cutins show an excess of hydroxyl over carboxyl groups on depolymerisarion (Holloway, 1993).

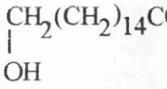
1.3 Periderm membranes

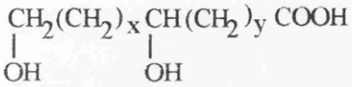
Periderm membranes are cellular membranes (Schonherr, 1982). Secondary walls of the individual cells are free of cellulose and consist of suberin, polyphenols, and soluble lipids (Kolattukudy 1975, 1980, 1984). Cutin and suberin are closely related chemical compounds, both are polymers with a high proportion of fatty acids. Martin and Juniper (1970) noticed that suberin and cutin from different sources have the same basic polyester structure, but differ in the relative proportions of constituent acids. Brieskorn and Boss (1964) draw attention to the occurrence of pairs of related w-hydroxy and dicarboxylic acids in cutin and suberin. Martin and Juniper (1970) pointed out that suberin differs from cutin by possessing a significant proportion of acids of C_{22} chain length.

Cutin
Major monomers

C_{16}-Family

$CH_3(CH_2)_{14}COOH$

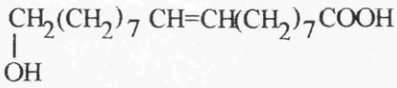


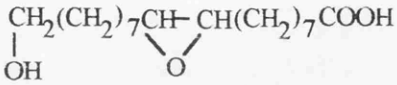


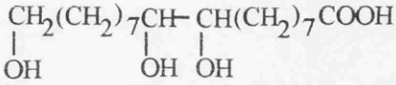
(y = 8, 7, 6 or 5 x+y = 13)

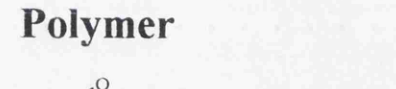
C_{18}-Family

$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$









Polymer

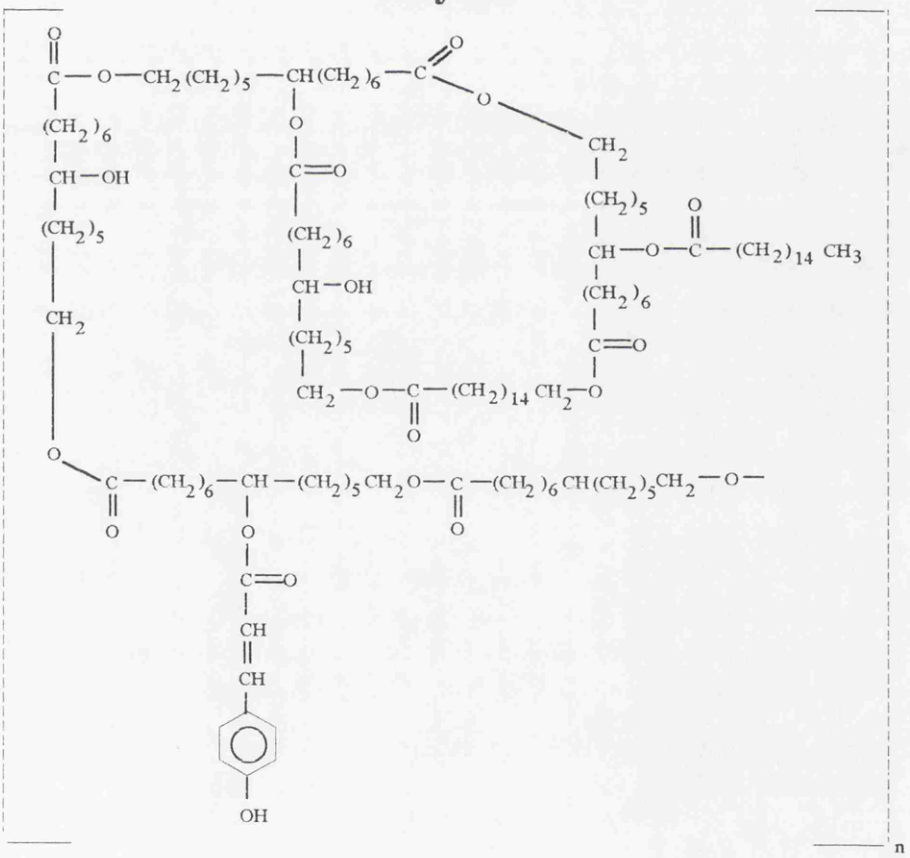


Fig. 1.1. Structure of the monomers of cutin and proposed model of the polymer. (Kolattukudy, 1980).

Suberin contains mainly polyester domains (Kolattukudy and Dean, 1974), and suberized layers show a lamellaer structure (Kolattukudy, 1980, 1981). The fine structure of isolated and non-isolated potato periderm layer has been investigated (Schonherr and Schmidt, 1982), the structure of suberin has been extensively studied (Kolattukudy, 1980, 1981, 1984; Kolattukudy and Agrawal, 1974; Agullo and Seoane, 1981; and Sukumaran et al. 1990), and information concerning the chemistry of suberin is also available (Hollaway, 1972a, 1972b; Martin and Juniper, 1970; Kolattukudy, 1978).

Vogt et al. (1983) investigated the fine structure and water permeability of potato tuber periderms which are composed of, six layers of phellem cells. These cells consist of cellulosic primary and tertiary walls and suberized secondary walls which are lamellated. Middle lamellae and primary walls contain lignin. In *Betula* periderm middle lamellae and primary walls are very polar (Schonherr, 1982).

Suberin contains a phenolic matrix that might be structurally somewhat similar to lignin; to this matrix are attached aliphatic domains that might have some resemblance to cutin. The composition of the aliphatic monomers suggests that hydroxyl groups are not in excess of the number of carboxylic groups present and that the aliphatic components themselves can hardly from an extensive polymer (Kolattukudy, 1980). The proposed model for the structure of suberin was shown in Fig. 1.2.

Suberin is virtually insoluble in all solvents and it is depolymerised by hydrolysis into monomers, which are mainly w-hyrdoxy-acids. These result prove that all the primary hydroxy groups in the w-hydroxy-acids are not free in suberin (Kolattukudy, 1980, 1984). The only free hydroxyl groups of suberin are the secondary groups of phloionic and phloionolic acids. (Agullo' and Seoane, 1981). The most common aliphatic components are fatty acids, fatty alcohols, ω-hydroxyfatty acids, and dicarboxylic acids. Treatments of potato suberin with aqueous dioxane or

Suberin
Major monomers

$CH_3(CH_2)_mCOOH$

$CH_3(CH_2)_mCH_2OH$

$CH_2(CH_2)_nCOOH$
|
OH

$HOOC(CH_2)_nCOOH$

Phenolics

(m=18-30; n=14-20)

Polymer

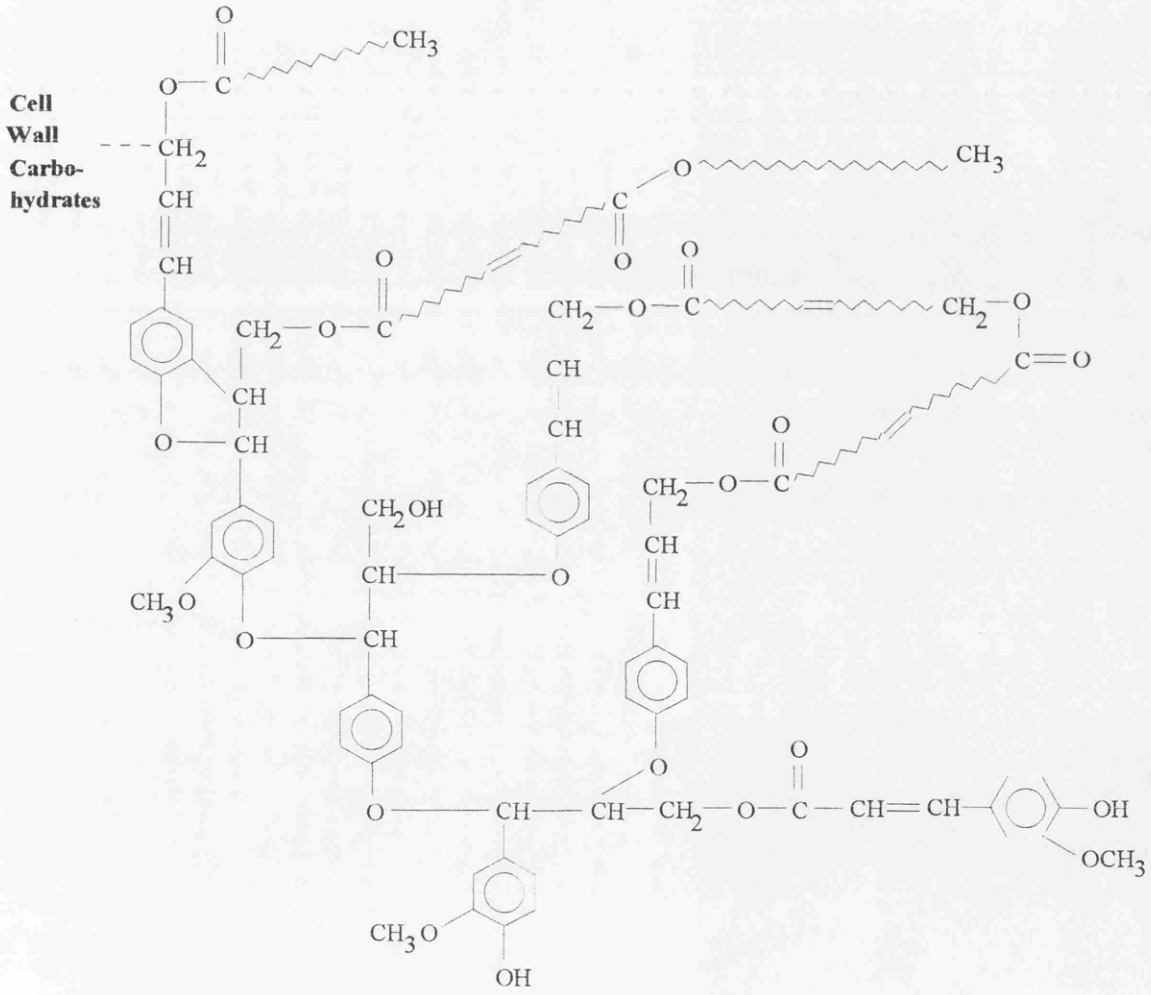


Fig. 1.2 Structure of the monomers of suberin and proposed model of the polymer. (Kolattukudy, 1980)

dioxane-HCl, released soluble phenolic materials in a manner somewhat similar to that observed with lignin (Kolattukudy, 1981).

Suberin from many sources has been examined for the composition of the aliphatic domains which are held together by ester bonds. Among these major components, monosaturated C_{18} monomers appear to be a dominant component, and C_{16} and C_{22} monomer appear to be the next (Kolattukudy, 1981; 1984).

1.4 Ion exchange medium

In a homogenous solution the transfer of materials can occur by processes such as diffusion and ionic migration. In living cells, transfer by combination of these processes is restricted or modified by the presence of porous materials which occur within the cells or constitute their walls (Martin and Juniper, 1970).

The uptake of mineral nutrients by roots is associated with an exchange of ions between the root surface and the soil components. Reichenberg and Sutcliffe (1954) proposed a mechanism of uptake of ions based on ion exchange by the root membrane and showed that the energetics of transport bi ion exchange account readily for the fact that ions may be absorbed against a concentration gradient. An expenditure of free energy is required.

An ion exchanger consists of a polymeric skeleton, insoluble in water and organic solvents, which is held together by crosslinkages from one chain to the next. Ion exchange group carried on the skeleton exchange readily with other ions in a surrounding solution without any change occurring in the material. When immersed in water, the exchanger takes up water which fills the pores of the network and the polymer swells.

There is evidence that not all of the carboxylic groups of the cutin acids are esterified (Martin and Juniper, 1970), those not involved in the linkages are likely to be ionised, with the hydrogens ions exchangeable with cations. Cations which at first are bound to negative charges may later dissociate and be displaced by other cations.

Indirect evidence suggest that the cuticular membrane can be considered to possess some properties of weak acid cation exchange membranes, as has been pointed out by various workers (Schonherr and Bukovac 1973; Cook and Duncan 1983). Three dissociable groups have been attributed in the pH ranges: at pH 3-6 to -COOH carboxyl groups of pectic materials and protein embedded in the membrane; at pH 6-9 to non-esterified -COOH carboxyl group of the cutin polymer, and at pH 9-12 to -OH phenolic groups in the membrane and amino groups $-NH^+$ proteins (Schonherr and Bukovac 1973). The cuticular membrane exhibits a behaviour typical of highly cross-linked, high capacity ion exchange resins of weak acid type (Schonherr and Bukovac, 1973). The inner surface of cuticular membrane are more highly charged, due to the pectin materials and this has been noted to have effect on the penetration and binding of cation through isolated cuticle membranes (Yamada et al. 1964a, 1964b). A mechanism of a cation exchange through the cuticle would give a preferential adsorption of cation over anions (Yamada et al., 1965). Yamada et al. (1965) examined the penetration of organic and inorganic substances through the enzymatically isolated astomatous cuticles and suggest that the more rapid penetration of cations than anions may be partially explained by the greater binding of the cations on the negatively charged cuticular surfaces. Middleton and Sanderson (1965) examined the uptake of ions by barley leaves and evidence was obtained that an ion exchange mechanism, in addition to metabolic processes, operated in the uptake. Crafts and Foy (1962) obtained that in a gradient of polarity from the exterior of the cuticle, which is more or less apolar, to the interior of the cuticular layers and of the cellulose wall, which exhibits high polarity.

1.5 The penetration of substances

The cuticle appears principally to be penetrable via intermolecular spaces. In order to study the penetrability and the mechanism of cuticular penetration, investigations have been performed with isolated cuticles (Darlington and Cirulis, 1963; Horrocks, 1964; Schonherr, 1976a, 1976b, 1978, 1982; Schonherr and

Bukovac, 1973; Silva Fernandes, 1965; Yamada et al., 1964a, 1964b, 1965). This aspect has also been well reviewed (Franke (1967; Hartley and Graham-Bryce, 1980; Hull (1970; Martin and Juniper, 1970; Schonherr and Riederer, 1989).

Martin and Juniper (1970) showed that there are pores in the cuticle which exist in specialised areas and are detectable under light and electron microscope. Crafts (1961) suggest that the cuticle is perforated by micropores which are more or less filled with an aqueous phase depending on the environmental conditions. Through these continuous aqueous phases are thought to pass polar substances moving parallel to non-polar substances which are believed to move through permanent lipoidal pathways. The cuticle lying immediately above the anticlinal walls of the epidermal cells is also believed to be easily penetrated by substances in aqueous solution (Currier and Dybing, 1959). Crafts and Foy (1962) noticed that the penetration of substances in the cuticle consisted of sorption in and diffusion across the cuticle followed by desorption at the cuticle/cell wall boundary. They introduced the concept of two parallel pathways in the cuticle, which are a lipoidal pathway for penetration of lipophilic molecules, and a polar pathway for transport of polar molecules.

Martin and Juniper (1970) noted that many complete and undamaged plant cuticles are freely penetrated by a wide range of both polar and non-polar substances. Some workers supposed that intermolecular channels exist through which relatively large molecules can penetrate, because of the distance between the hydroxy fatty acid chains within the cutin units, as well as between macromolecules themselves, intermolecular spaces exist as in the structure of cellulose. Presumably these spaces are large enough for passage of small molecules (Kamimura and Goodman ,1964). Dyestuffs, streptomycin and other antibiotics can penetrate quite readily (Franke, 1967).

Penetration is affected not only by intermolecular spaces but also by intermolecular forces and charges of polar groups within the macromolecules of cutin.

Cutin contains both hydrophilic (-OH and -COOH) and lipophilic groups (-CH_2- and -CH_3). Because of the presence of hydrophilic groups, hydration and imbibition of the cuticle proper and of the cuticular layers become explicable (Franke, 1967). Consequently the penetration of water and water-soluble substances through these layers to the outer or inner surface of the cuticle is facilitated.

The compound may penetrate is likely to be greatly influenced by its properties, in particular its water- or lipoid solubility and by the relative proportion of cutin and wax in the cuticle concerned. The extent of dissociation of polar compounds is an important factor governing their penetration.

Comparison of the permeability and diffusion coefficients indicates that the penetration of ions through isolated cuticles took place by diffusion and was impeded by charge interactions between the solute and charge sites in the penetration pathway. An increase in pH was accompanied by an increase in the penetration of ions (Schonherr, 1976b, 1978). Sorption is the first phase in the cuticular penetration process. Initially it is rapid, and is influenced by pH, polarity of solvent and solute, charge on the penetrating particles and additive particles, and concentration.

Crowdy (1959) points out that cutin is polar and will absorb water and swell. The permeability of enzymatically isolated cuticular membranes increased almost 5 fold between pH 3 and 11 and approximately 3 fold between pH 3 and 9 (Schonherr, 1976b). He obtained that water permeability increased with increasing salt concentration at pH 9, and in the order $Li^+ < Na^+ < K^+ < Rb^+ = NH_4^+$. McFarlane and Berry (1974) point out that transcuticular penetration of K^+ as a function of solution pH, coefficient values of K^+ above pH were about five times greater than below pH 5. They studied also the anion effect of K^+ penetration that the permeability of K^+ ions was essentially the same for the anions Cl^-, I^-, and NO_3^-, when the anions was SO_4^{2-} it was significantly lower. From these data, it appears that although

various anions affect penetration of alkali cations, it is their effect on ionic activity which is most significant.

The penetration of substances through cuticular membranes is easier from inside to outside than in the reverse direction. This is consistent with the hypothesis of Crafts and Foy (1962) according to which a gradient of polarity exists with more polar groups in the inner side of the cuticle than in the outer. Tyree et al. (1990a) measured diffusion potentials of K^+ ion at various concentration across the cuticles and they tested the polar pore model. They explained asymmetric property of cuticles in terms of a charged-pore model of transport through cuticles. High concentrations of mobile cations accumulate adjacent to the fixed anions in the pore; mobile anions are excluded from this region. The thickness of the double layer is a function of fixed-charge density; the greater the charge, the thicker the double layer. They also suggest that the cuticle is more densely charged on the inner than on the outer surface. Structurally the inner surface blends in with cell wall that has an abundance of charged groups such as proteins, polyuronic acids, etc., whereas the outer surface is predominantly uncharged waxes.

Although, according to the structure described, one could visualise that penetration should occur through all the interspaces of the cuticle and of the cellulose wall. From the kinetics of the appearance of penetrated substances in the pure water chamber it appears that the movement of ions and organic compounds occurs by diffusion (Darlington and Cirulis, 1963). Penetration was directly proportional to concentration, and increased with increasing temperature and with the higher lipophilic character of the applied compounds (Franke, 1967).

It may be stated that isolated cuticular membranes are permeable to both organic and inorganic ions and undissociated molecules. The penetration of ions is determined by the kind of charge, adsorbability, and ion radius. The mechanism of penetration is a physical process of diffusion. Urea seems to penetrate by a process of

facilitated diffusion, while lipophilic substances may penetrate by a process of solution. Their penetration is determined by the solubility, partition, and molecular size (Schonherr, 1976b).

The fact that the cuticle is negatively charged as a whole has the effect that these charges are at first neutralised by cations (Franke, 1967). Therefore the additional application of cations, which bring about dehydration, may increase the entry of larger cationic molecules such as streptomycin. Cations such as Ca^{++} and Mn^{++} are able to penetrate more quickly because of their smaller ion radius, thus facilitating the passage of larger cationic molecules (Franke, 1967).

The water permeability of periderm membranes has been studied (Muir, 1990, Soliday et al., 1979, Vogt et al., 1983). Evidence is presented that two pathways for water movement exist in parallel. Pathway 1 is represented by middle lamellae and primary walls extending in radial direction across the membranes. This pathway has a relatively high specific permeability and all relatively polar, as they consist of polyuronic acids, cellulose and lignin (Brieskorn and Binnemann, 1972, 1974, 1975). Pathway 2 is represented by a polyaminated structure made up of tangential walls of phellem cells which are orientated normal to the direction of water flow. This pathway has a low specific permeability because of the properties of secondary walls encrusted with soluble lipids. The presence of lignin is probably responsible for the fact that cellulase and pectinase did not cause disintegration of PM (Vogt et al., 1983).

The water permeability of plant cuticles is completely determined by the soluble lipids associated with cutin (Schonherr, 1976a, 1976b). Soluble lipids play a major role in the water permeability of periderm membranes (Soliday et al. 1979). The relationship between potato tuber membrane permeability and membrane lipid composition was examined by Spychalla and Desborough (1990).

1.6 Thesis Objectives

The object of this thesis is to investigate the role of ion exchange in the movement of chemicals through the isolated potato periderm and pear fruit cuticular membranes. Because of the importance of the periderm/cuticle as a barrier to penetration of a wide variety of compounds, its permeability and diffusion characteristics were studied. This was with a view to studying transport of some chemical compounds through the periderm/cuticle membranes and discussing their possible relationship to the structure and ion exchange properties of the membranes and ionic and other physical properties of the transporter species. The focus was on the use of isolated periderm/cuticles as model system. In addition to the data obtained here, a review of pertinent studies will be undertaken and their relevance related to foliar penetration of herbicides.

CHAPTER 2

Ion Exchange Properties

2.1 Introduction

Transport properties of membranes depend strongly on the nature and density of charges fixed to the membrane matrix (Helfferich, 1962; Lakshminarayanulah, 1969). Ion exchangers such as charged membranes can be described by their capacity. Ion exchange capacity is defined by the total amount of materials bound to ion exchange matrix. It is characterised as the number of counter ion equivalents in a specified amount of matrix.

It is important to characterise the ion exchange membranes before use in terms of capacity. Ion exchange capacities of the isolated periderm membranes (PM) and pear fruit cuticular membranes (PFCM) were determined in different ionic H^+, Li^+, Na^+, K^+ and Cs^+ forms and whose exchange capacities were also compared in different ionic Li^+, Na^+, K^+ and Cs^+ forms. Their physical parameters, such as membrane weight, diameter and thickness have been determined for alkali metal chloride solutions at various concentrations.

Cation exchange resins are high molecular weight polyacids which are virtually insoluble in aqueous and nonaqueous media. The acid or acids which constitute the exchange groups are usually of the sulphonic, carboxylic or phenolic type, and are substituents in the resin structure. Their exchange properties can be described as being entirely due to the exchange of various cations for the dissociable hydrogen ion.

It was seen that the titration curves of the isolated PM and PFCM exhibited a behaviour typical of carboxylic acid resins of the weak acid type. The fact that exchange properties of the isolated PM and PFCM in the presence of 0.1 N NaCl depend on acid pretreatment was examined.

2.2 Materials and methods

2.2.1 Materials

The salts were analytical grade, NaCl, KCl standard NaOH and HCl solutions from BDH, LiCl from Hopkin and Williams, CsCl form Formachem. Cellulose from Sigma, Pectinase from ICN Biomedicals, sodium acetate and acetic acid from May and Baker.

Salt solutions were prepared using deionized water without further purification by dissolving a weighed quantity of Analar salts. Standard solutions were prepared using standard concentrated solutions. The solutions were prepared under carbon dioxide free conditions. The indicator used was phenolphthalein.

2.2.2 Methods

2.2.2.1 Isolation of periderms and cuticles

Periderms were isolated from potatoes cultivar (cv.) Record by Dr. Boyd. Pears were purchased at the local market. Sampled potatoes and pears were washed carefully by hand before several cores of 17 mm and 22 mm in diameter were taken from each potato or pear. Isolation was carried out using a modification of the method of Schonherr (1976b) and Vogt et al. (1983). Most of the flesh below the skin of the cores was removed using a knife taking care to leave a layer of 2-3 mm of flesh attached to the skin. The cores from each tuber were placed in a suspension of 0.1% cellulase and 2% pectinase buffered at pH 3.8 in a 150 cm^3 conical flask. The buffer used was 0.2 M sodium acetate/0.2 M acetic acid. The flasks were stored at 25 ^{o}C for 14 days before isolating the periderms and cuticles from the remaining attached flesh.

At the end of the incubation period the cores were removed from the flasks and placed on a 100 mesh stainless steel sieve. Deionized water was sprayed onto the inside surface of the periderms to separate any cellular material still loosely adhering to the periderms. The isolated periderms were then rinsed and placed between single

Whatman No.1 filter papers (Whatman, UK.) and air dried at room temperature. The dried periderms and cuticles were stored between layers of tissue paper until required for determination of ion exchange capacity, properties and selectivity, permeability and transport measurements.

2.2.2.2 Weights

Isolated air dried periderms were weighed (±0.0005 g) using an Oertling LA 164 electronic balance. 10 periderms and 10 cuticles isolated from potatoes and pear respectively were weighed and subsequently used for all the ion exchange capacity and selectivity, permeability and transport measurements.

2.2.2.3 Thickness

The membrane thickness was calculated, as a mean thickness by measuring different points of each membrane by a micrometer accurate to ± 10 μm. The hydrated membrane was clamped between two microscope cover slips and measured at four different places on each membrane. The thickness of the cover slips was subtracted. The average of ten such determinations was taken as the thickness of the membrane and was reproducible to ± 0.1%. The result was 88±10.5 μm for periderm and 4.75±2.17 μm for pear fruit cuticular membranes, respectively.

2.2.2.4 Diameter

The membrane was removed from its equilibrating solution and placed, still wet, between two optically flat glass plates. It was then placed under the 10X-A lens of Nikon Profile Projector (Model 6C) and its diameter measured using the micrometer stage of this microscope. The average of ten determinations was taken as the diameter.

2.2.2.5 Appearance

In the isolated periderms and cuticles it was easy to distinguish the outer surface and inner surface by eye. The membrane samples were checked by

microscopy to observe stomata in pear fruit cuticular membranes and lenticels in periderm membranes. Stomata were detected in pear cuticles but no lenticels in the periderms used.

2.2.2.6 Integrity

Before use all membranes were inspected microscopically (Nikon 10 X). Membranes with imperfections were discarded and those remaining were tested for defects with an experimental diffusion diaphragm cell. The half cell containing the receiver solution was equilibrated for 30 min. After this time leaky membranes were detected by passing receiver solution across the opposite side of the cell.

2.2.2.7 Ion exchange capacity and properties

The ion exchange capacity of the isolated periderm and cuticular membranes was determined by potentiometry. The ion exchange properties of the isolated periderm and cuticular membranes were investigated using various salt concentrations.

The ion exchange capacity was determined by potentiometric titration after converting the membranes to the H^+ form by successive equilibrations with hydrochloric acid (1.0 M). They were then removed from the acid and thoroughly washed with deionized water to remove sorbed HCl, i.e. washed until the water remained free of Cl^-. After blotting between filter papers, the membrane was equilibrated with 1.0 M NaCl (20 ml) for several hours. In most cases equilibrium was achieved within 1 day. The equilibrium was checked by pH meter, and was taken as being when the pH of supernatant no longer decreased. Exchange capacity was calculated for a given pH from the amount of base added minus the amount of base remaining at equilibrium as determined by back titration of the supernatant. The following exchange;

$$RH + NaCl \longrightarrow RNa + H^+ + Cl^-$$

takes place. The sodium ions are exchanged for hydrogen ions of the membrane. Here, exchange was equated to the amount of H^+ released, which was titrated with standard sodium hydroxide solutions and a glass electrode assembly and titration was recorded by potentiometric recorder. An Orion model 720 pH/mV meter was used. The amount of H^+ released by the membrane is stoichiometrically equivalent to the amount of NaOH added at the end point.

The ion exchange properties and titration curves of the isolated cuticular membranes were obtained by back titration with NaOH in the presence of NaCl. Membranes were pretreated with either 1 M or 2 M HCl. This was repeated more than three times. In a separate experiment various NaCl concentrations (1.0 M, 0.1 M and 0.05 M), were used.

In this experiment the progressive back titration method was used. The membranes were weighed into the conical flask. The salt solution and standard base were added into the conical flask, such that salt solution concentration was constant and only amount of base varied. The flask was maintained at constant temperature in the incubator (25 ^oC). After equilibration had taken place the solution was titrated with standard base.

This system was used to determine the exchange capacities of various salt concentrations the same piece of cuticle. Between solute changes the cuticle was rinsed thoroughly with dilute nitric acid (10% V/V) (McFarlane and Berry, 1974) and then washed with deionized water to remove sorbed cations.

2.3 Results

2.3.1 Effect of Pretreatment

The titration curves of the periderm and pear fruit cuticular membranes obtained with NaOH in the presence of 0.1 N NaCl were dependent on pretreatment (Fig. 2.1). The isolated periderm and pear fruit cuticular membranes pretreated with 1 M HCl gave the following ion exchange capacities 0.415 meq g^{-1} for periderm; 0.25

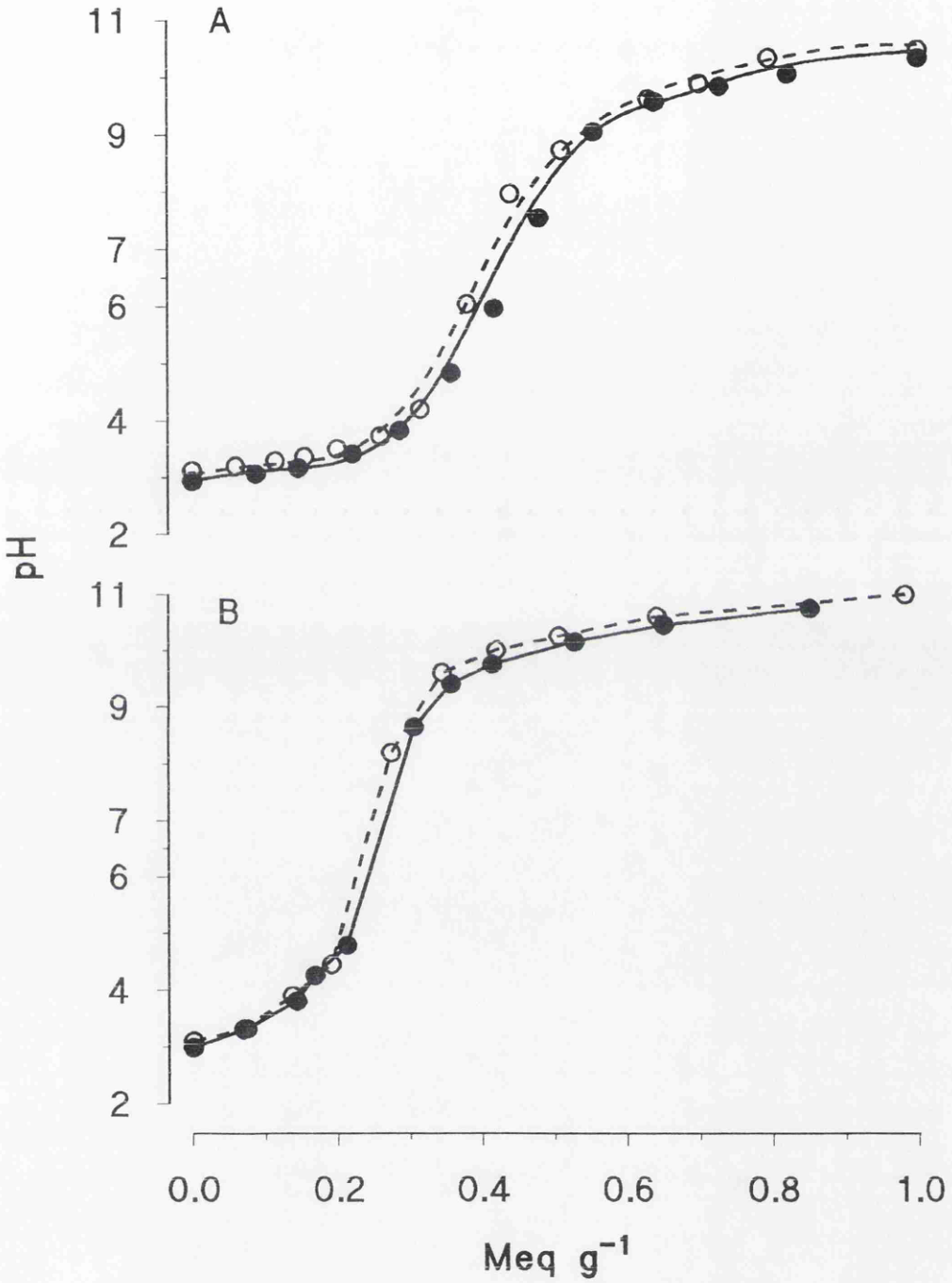


Fig. 2.1 Titration of PM (A) and PFCM (B) with NaOH in the presence of 0.1 N NaCl as affected by pretreatment; (●) pretreatment with 2.0 M HCl, (○) pretreatment with 1.0 M HCl.

meq g^{-1} for pear fruit cuticular membrane, respectively. When pretreatment was with 2 M HCl, the ion exchange capacities were increased to the following values 0.435 meq g^{-1} for periderm; 0.265 meq g^{-1} for pear fruit cuticular membrane, respectively. Repeating the 2 M HCl pretreatment had no further effect. Pretreatment with a stronger acid concentration was not attempted due to the possibility of damaging the cuticle samples.

2.3.2 Effect of neutral salt

Three separate groups can be distinguished in the pH ranges, the first point between pH; 3-5.5 and the second between pH 5.5-9, and third between pH 9-11.5. The end point of the third group was not observed clearly in some experiments, but it can be estimated from some titration curves that the endpoint group can be assumed around 12.

The exchange capacity of the periderm membranes and pear fruit cuticular membranes exhibited the characteristic properties of a weak acid cation exchanger whose exchange capacity is dependent on neutral salt concentration. At constant pH of 7, the exchange capacity increased with increasing concentration of neutral salt (Figs. 2.2 and 2.3). At pH 7, the exchange capacities of PM and PFCM are shown Table 2.1.

2.3.3 Effect of counter ion

The ion exchange capacities of PM and PFCM were also dependent upon the nature of the counter ion. At constant neutral salt concentration and constant pH 7, the order of decreasing capacity is $Li^{+} > Na^{+} > K^{+} > Cs^{+}$, (Fig. 2.4 and Table 2.1). This sequence was apparent over the pH ranges 3-9. This order follows the sequence of hydration energies, which is also the sequence of hydrated radii.

Table 2.1. Exchange capacity of the periderm and pear fruit cuticular membranes at pH 7.0 as a function of nature and external solution of counter ions.

Counter Ion	Exchange Capacity (meq g^{-1})					
	Periderm			Pear		
	1.0 M	0.1 M	0.05 M	1.0 M	0.1 M	0.05 M
Li	0.433	0.417	0.408	0.255	0.238	0.235
Na	0.410	0.393	0.387	0.238	0.225	0.223
K	0.398	0.380	0.377	0.215	0.197	0.191
Cs	0.370	0.350	0.345	0.200	0.183	0.180

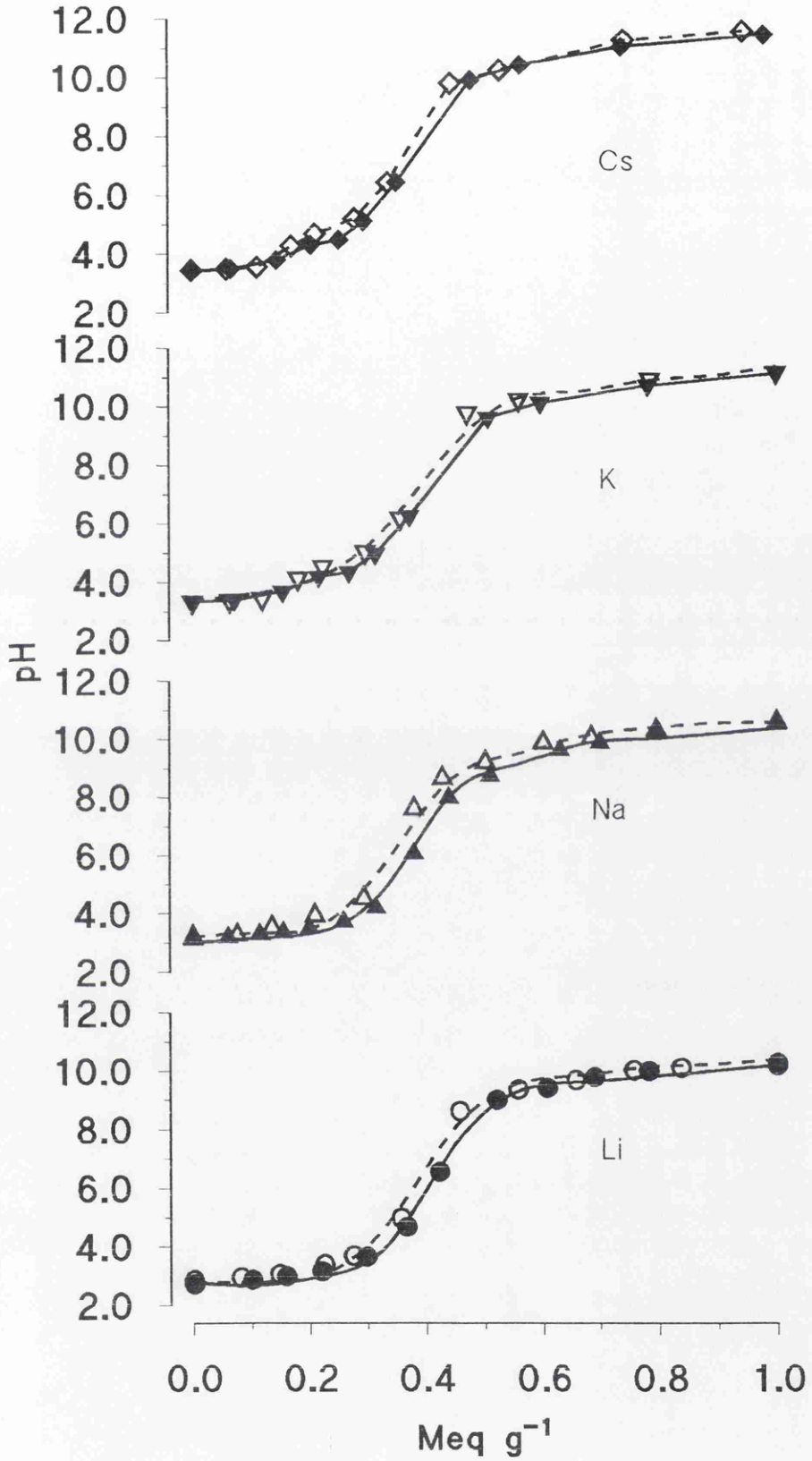


Fig. 2.2 Exchange capacity of PM as a function of pH and salt concentration, symbols indicates; solid 1.0 M, open 0.1 M salt concentration.

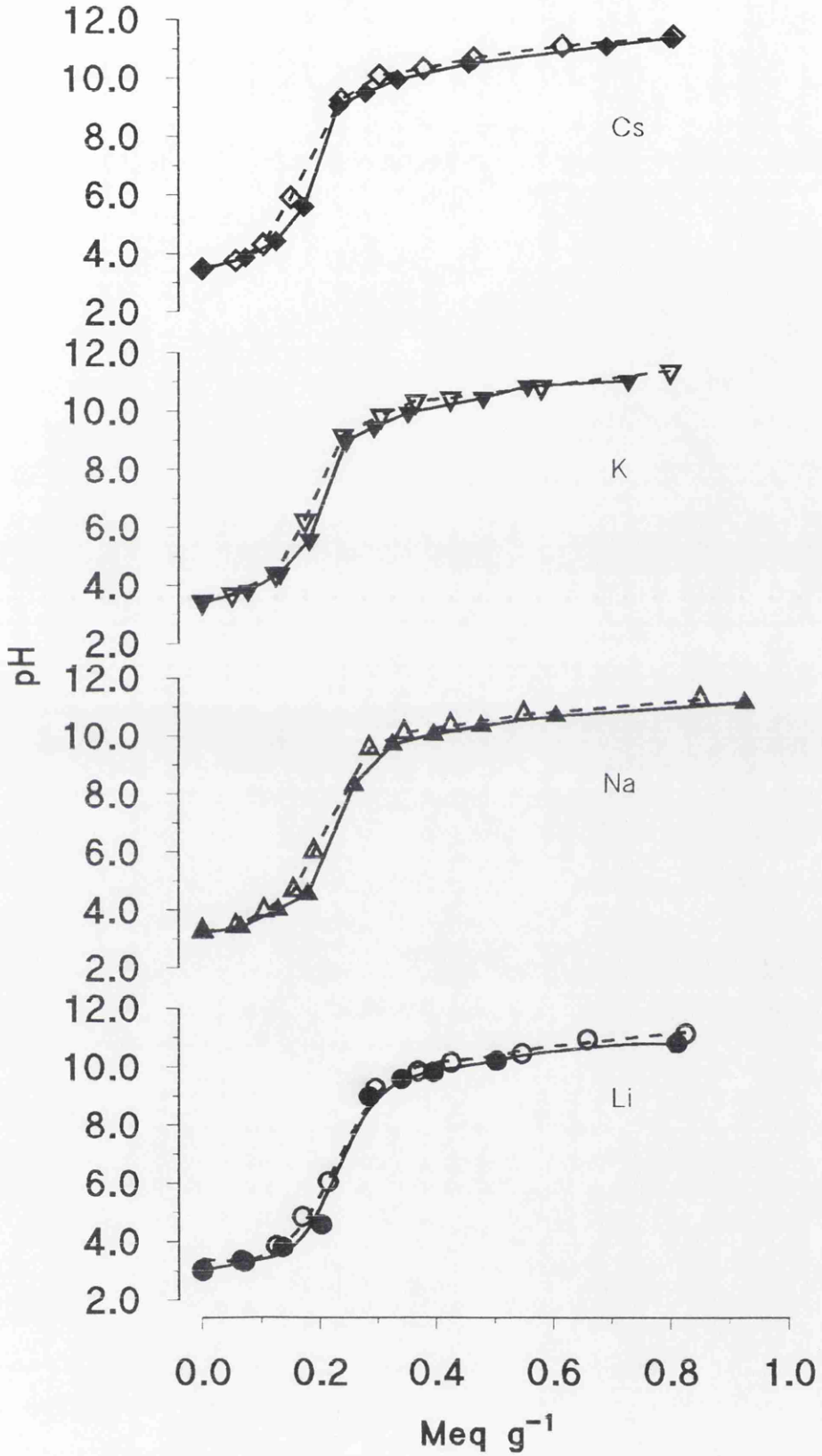


Fig. 2.3. Exchange capacity of PFCM as a function of pH and salt concentration, symbols indicates; solid 1.0 M, open 0.1 M salt concentration.

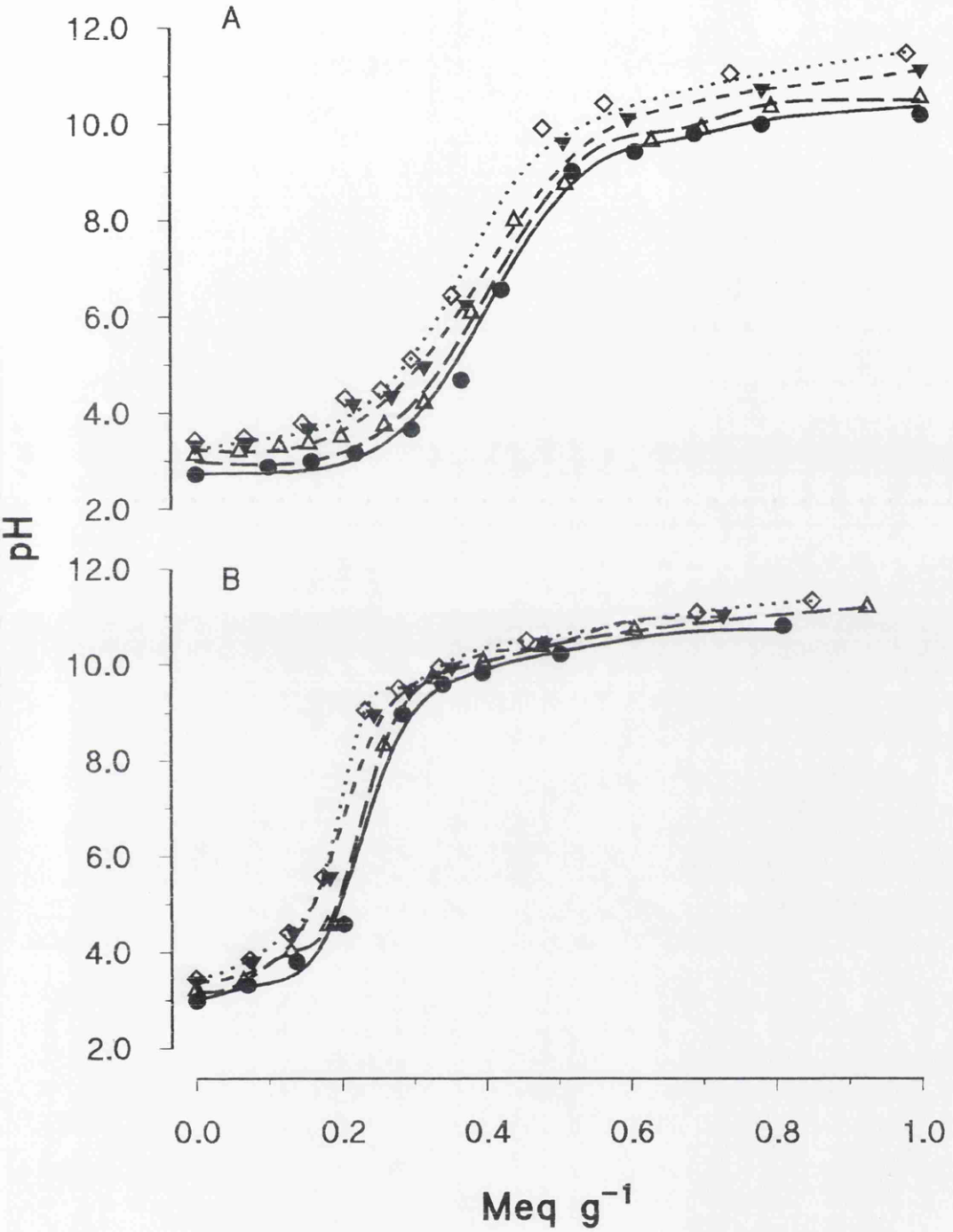


Fig. 2.4 Exchange capacity of PM (A) and PFCM (B) as a function of pH and nature of counter ion; (●) Li, (△) Na, (▼) K and (◇) Cs.

2.4 Discussion

The ion exchange capacity is dependent on different ionic Li^+, Na^+, K^+ and Cs^+ forms. The findings at constant pH 7, as a function of nature and concentration of counter ions at three different external solution concentrations are listed in Table 2.1 (1.0 M, 0,1 M and 0.05 M).

The titration curves of the isolated periderm and cuticular membranes exhibited a behaviour typical of weak acid resins. The initial pH of the aqueous phase is slightly higher. The titration curves dependent on different alkali metal cations are shown in Fig. 2.4, for PM and PFCM. The ion exchange capacity increased with increasing pH and neutral salt concentrations. The ion exchange capacities of PM and PFCM toward alkali metal cations were observed in sequence as $Li^+ > Na^+ > K^+ > Cs^+$, and were shown Fig. 2.4. At constant pH and salt concentration, the exchange capacity increased with concentration of neutral salt and decreased with decreasing crystal radius. Three separate groups can be distinguished in the pH ranges, the first between pH 3-5.5 and the second between pH 5.5-9. The endpoint of the third group was not observed obviously in some experiments, but it may be estimated from some titration curves and was the pH range between 9-11.5 or 12. Schonherr and Bukovac (1973) observed three dissociable groups in the pH ranges 3-6 (0.2 meq g^{-1}), 6-9 (0.3 meq g^{-1}) and 9-12 (0.55 meq g^{-1}), for the tomato fruit cuticular membranes. These investigators reported that the first group was tentatively assigned to -COOH group of pectic material and protein embedded in the membrane, the second to nonesterified -COOH groups of the cutin polymer and the third to phenolic -OH groups, such as nonexractable flavenoids present in the membrane, and to a small amount of $-NH_3^+$ groups of proteins. They have also obtained ion exchange capacity in isolated tomato fruit cuticular membrane sharring the selectivity sequence [tris(ethylenediamine) $Co]^+3 > Ca^+2 > Ba^+2 > Li^+ > Na^+ > Rb^+ > N(CH_3)_4^+$.

The results show that the exchange capacity of the isolated PM and PFCM indicated the characteristic dependence on neutral salt concentration observed with

weak acid type ion exchangers. At constant pH (7.0) the exchange capacity increased with increasing concentration of neutral salt solution.

It has been found that the exchange capacity of PM is higher than PFCM. Kolattukudy (1980) reported that the major aliphatic components of suberin are ω-hydroxy fatty acids and dicarboxylic acids. He proposed a model for cutin and suberin; the composition of the monomers in cutin shows that hydroxyl groups are in excess of the number of carboxyl groups, Whereas, the composition of the monomers in suberin suggest that hydroxyl groups are not in excess of the number of carboxyl groups. It can be estimated from this proposed model that the carboxyl groups of PM are in excess over that of PFCM, therefore the exchange capacity of PM is higher than PFCM.

For weak acid resins e.g., of carboxylic, phosphoric and phosphonic types, due consideration must be given to the fact that the degree of dissociation of the functional groups varies with the composition of the external solution and especially with the pH (Samuelson, 1963). Compared with sulfonic acid resins, the selectivity scale for alkali metal ions is reversed for both carboxylic and phosphonic resins. (Bregman, 1953). Thus, lithium is preferred to potassium ions, whereas sodium takes an intermediate position.

The selectivity of a cation exchange resin for alkali cations is affected by the nature, effect of neutral salt and acidity of the exchange group. The apparent acid strength increased with increasing concentration of neutral salt and decreasing crystal radius of counter ions (Table 2.1, Fig. 2.4). This behaviour is typical for polyelectrolytes of the weak acid type (Gregor et all., 1955, 1956). The main reason for this behaviour of polyelectrolytes is the electrostatic free energy arising from the mutual repulsion of neighbouring fixed charges (Schonherr and Bukovac, 1973, Katchalsky, 1954). Schonherr and Bukovac have observed that as the electrostatic potential increases during titration, the tendency to form more negative groups will

diminish and the apparent acid strength therefore decreases as the degree of ionisation and exchange capacity increases.

Generally, smaller ions have larger heats of hydration and a small ion contains a more concentrated charge, leading to a greater electrostatic interaction between the ion and polar water molecules. The hydration of ions in aqueous solutions is frequently represented as the strong binding of nearby molecules. Therefore this type of hydration is characterised by the number of molecules bound of water, the so called hydration number of ion. The concept of a more or less strong bond among molecules of water and ions is justified only in the case of strongly hydrated ions. The hydration number is directly proportional to the charge and inversely proportional to the size of the ion.

According to electrostatic theory, the electric field at the surface of a charged sphere of radius, r is proportional to ze/r^2, where z is the number of charges, e is the electronic charge. The ion-dipole interaction between dissolved ions and water molecules can effect a number of bulk properties of water, hence small ions such as Li^+ and Na^+ are called structure-making ions (Raymond, 1981). The high electric fields exerted by these ions can polarise the water molecules, producing additional order beyond the first hydration layer. This interaction leads to an increase in the solution's viscosity. And large monovalent ions such as K^+ and Cs^+ are structure-breaking ions. As a result of their diffuse surface charges and hence weak electric fields, these ions are unable to polarise water molecules beyond the first layer of hydration.

The electrostatic free energy can be reduced by association between fixed charges and counter ions, and by screening of neighbouring fixed charges (Gregor et al., 1955). The smaller the crystal radius of a counter ion the greater the interaction with the fixed charge, the lower the electrostatic free energy and the greater the apparent acid strength (Schonherr and Bukovac, 1973). The decreasing order of

crystal radii of counter ions used in this study is $Li^+ < Na^+ < K^+ < Cs^+$, which is also the order of decreasing acid strength observed.

Ions are hydrated in solution and their effective radii can be appreciably greater than their crystal or ionic radii. The radii of the hydrated Li^+, Na^+, K^+ and Cs^+ ions are 0.30 nm, 0.22 nm, 0.15 nm and 0.13 nm respectively, although the ionic radius is in the reverse order Cs^+, K^+, $Na^+$and Li^+. The mobility of an ion is inversely proportional to its hydrated radius. The ionic hydration plays a role in the exchange capacity. The ion exchange capacity of PM and PFCM has been found to follow the sequence of hydration radii of alkali cations.

Samoilov (1972) reviewed the ionic hydration of electrolytes in aqueous solutions, and developed a method of evaluating hydration for ions. He observed that Li^+ and Na^+ are hydrated positively, and K^+ and Cs^+ are hydrated negatively.

The selectivity of ion exchangers is also affected by interaction in the external solution. Schonherr and Bukovac have described that increasing the concentration of neutral salt increases the apparent acid strength because; a) the sorbed electrolytes tends to shield neighbouring fixed charges and thus decreases electrostatic free energy, and b) reduces the Donnan potential, i.e., reduces the pH difference between external solution and polymer.

The "field strength" or polarising ability of the unhydrated alkali cations decreases with increasing ionic size if the negatively ionised group is more polarizable than water, then the polarisation energy and columbic energy are additive, and the order of affinity for the negative group is $Li^+ > Na^+ > K^+$ (Bregman, 1953)

Gregor et al. have observed for insolubility of dicarboxylic acids the following sequence $Li^+ > Na^+ > K^+$. In this work the observed sequence at pH 7 is $Li^+ > Na^+ > K^+ > Cs^+$. These sequences are the same as the hydration energies, $Li^+ > Na^+ > K^+ > Cs^+$.

CHAPTER 3

Ion Exchange Selectivity

3.1 Introduction

Ion exchangers can distinguish different counter ions. Ion exchangers possesses positive or negative charges which are compensated by ions of opposite charge called counter ions. The counter ions are the ions responsible for ion exchange and can be replaced by other ions of the same charge preserving the electroneutrality of the exchanger. When an ion exchanger is brought into contact with an electrolyte solution, exchange of ions will take place until equilibrium is attained. Thus counter ions are exchanged. The ion exchanger usually takes up certain counter ions in preference to others. This selectivity can arise from one or several of the following physical causes. The ion exchanger tends to prefer; counter ions exhibiting the following properties; hydrated ionic radius, with smaller (solvated) equivalent volume, greater polarizability, interacts more strongly with the fixed ionic groups, steric factors, entropy effect, electrostatic effect and higher valence.

While the information available at present on the selectivity and interaction of alkali metal ions with sulphonic, inorganic and other type of exchangers is abundant, nothing is known about the selectivity and interaction of alkali metal ions with periderm and cuticular membranes. Therefore, in these studies Li^+/H^+, Na^+/H^+, K^+/H^+ and Cs^+/H^+ selectivities were examined. These ions were chosen because they are ions which have different tendencies in the exchanger.

3.2 Membrane Isotherm-Selectivity Coefficient

Ion exchange equilibrium can be characterised by ion exchange isotherms. The mole fraction, or equivalent fraction, of one of the competing ions in the exchanger is plotted against those of the same ions in a solution. This isotherm is a graphical representation which, in principle, covers all possible experimental conditions at a given temperature. In a simple ion exchanger and its equilibrium with two monovalent

counter ions which are both present in the exchanger and in the solution in contact with it. their rates define the selectivity coefficient. The ion exchange isotherm shows the ionic composition of the ion exchanger as a function of the experimental conditions.

It is known that when an ion exchange reaction occurs, selectivity is shown in nearly all instances. This is demonstrated by the fact that for a reaction such as;

$$\overline{A}^{+} + B \rightarrow \overline{B}^{+} + A \qquad (3.1)$$

where, the bars indicating the exchanger phase. For exchanges of ions of equal ionic charges the selectivity coefficient is defined by the following expression;

$$K_{B/A} = \frac{\overline{X}_B}{\overline{X}_A} \frac{X_A}{X_B} \qquad (3.2)$$

where $\overline{X}_A$ and $\overline{X}_B$ represent the equivalent ionic fractions of the counter ions in the membrane phase and X_A and X_B corresponding equivalent fraction of these ions in the solution phase. $K_{B/A}$ is defined in a quantitative way as being the relative ionic composition of the membrane and solution phase. Any value of $K_{B/A}$ that is different from unity measures the relative difference in preference of the solution and membrane phases for the two competing ions. The preference for one ion over another in the particular phase is a measure of the relative effect of the two components on the thermodynamic properties of the two phases. These effect are defined in the term activity which is a measure of the escaping tendency. Thus the preference of the solution phase for the two counter ions is the inverse of the ration of their activities α^{B}/α^{A}

The quantity $K_{B/A}$ represents the net resultant of all interactions in both membrane and solution phases that give rise to selectivity. It is also defined by another quantity $K'_{B/A}$ which is called "corrected selectivity coefficient" and represents the preference of the exchanger phase only, relative to the same standard

and is obtained from $K_{B/A}$ after "correcting" the latter for preferences of the solution phase. In this case;

$$K'_{B/A} = K_{B/A}\left(\frac{f_A}{f_B}\right) \tag{3.3}$$

The combination of Eq. (3.2) with (3.3), gives;

$$K'_{B/A} = \frac{\overline{X}_B}{\overline{X}_A}\frac{X_A}{X_B}\frac{f_A}{f_B} \tag{3.4}$$

or

$$K'_{B/A} = \frac{\overline{X}_B}{\overline{X}_A}\frac{\alpha_A}{\alpha_B} \tag{3.5}$$

where f_A and f_B are the activity coefficients of the individual ions in the solution phase. Together with counter ions there is present in the solution an equivalent amounts of coions to maintain electroneutrality of the solution. In the experiments carried out here there is only one type of coion present in the solution therefore the f_A and f_B may be written f_A^+ and f_B^+, respectively.

$$\frac{f_A^+}{f_B^+}\frac{f_A^+ f_{Cl}^-}{f_B^+ f_{Cl}^-} = \frac{(f_{\pm ACl})^2}{(f_{\pm BCl})^2} \tag{3.6}$$

where $f_{\pm ACl}$ and $f_{\pm BCl}$ are the mean ion-activity coefficients of the salts ACl and BCl, respectively, in the solution phase.

Ion exchange selectivity is determined in systems in which the concentration of ions in the solution phase is sufficiently low (0.02 M or less) (Reichenberg, 1966).

Dilute solutions $K'_{B/A} = K_{B/A}$ (Reichenberg, 1966; Helfferich, 1962; Abe, 1993), and,

$$K'_{B/A} = \frac{\overline{X}_B}{\overline{X}_A}\frac{X_A}{X_B} \tag{3.7}$$

It seems that by introducing the factor f_A / f_B it is "corrected" $K_{B/A}$ for that part of the selectivity due to interactions in the solution phase and thus, while converting the quantity $K_{B/A}$ into the quantity $K'_{B/A}$ is eliminated all selectivity due to interactions in the solution phase. It is done by introducing a factor f_B^- / f_A^-, f_A^- and f_B^- being activity coefficients in the exchanger phase. It would be eliminate all selectivity, therefore the quantity on the left side of equation would be unity;

$$1 = \frac{\bar{X}_B}{\bar{X}_A} \frac{X_A}{X_B} \frac{\bar{f}_B}{\bar{f}_A} \frac{f_A}{f_B} \tag{3.8}$$

that is,

$$\frac{\bar{f}_A}{\bar{f}_B} = \frac{\bar{X}_B}{\bar{X}_A} \frac{X_A}{X_B} \frac{f_A}{f_B} = K'_{B/A} \tag{3.9}$$

or

$$K'_{B/A} = \frac{\bar{f}_A'}{\bar{f}_B'} \tag{3.10}$$

The corrected selectivity coefficients $K'_{B/A}$ is nothing more than the ratio of the activity coefficients of the ions in the exchanger, provided that these activity coefficients in the exchanger phase are defined so as to be analogous to those in the solution phase. This way of expressing $K'_{B/A}$ was first suggested by Bauman and Eichorn (1947).

$K_{B/A}$ has been defined as a constant $K'_{B/A}$ which is known to vary with ionic composition of the exchanger. It is clear that all the variation of $K'_{B/A}$ with ionic composition is contained within the factor f_A / f_B (Boyd et al. 1954) and without an additional convention or assumption there is no means of evaluating f_A, f_B and $K_{B/A}$ separately or even of evaluating f_A / f_B and $K_{B/A}$ separately. Some authors (Ekahdahl et al 1950, Argersinger et al. 1952, Gaines and Thomas, 1953) adopted the convention that when the exchanger is entirely in the A form, f_A is unity, and when

the exchanger is entirely in the B form, f_B is unity. Adopting these conventions, these authors showed that application of the Gibbs-Duhem equation leads to the results.

$$\ln K_{B/A} = \int_0^1 \ln K'_{B/A} d\overline{X}_B \qquad (3.11)$$

From the above equations, it is seen that ln $K_{B/A}$ is effectively, a kind of average of the values of ln $K'_{B/A}$ over all ionic compositions in the exchanger phase. It is often very useful to express the selectivity of a given exchanger for a given pair of ions by such as a single meaningful value. The exact evaluation of $K_{B/A}$ is often rather tedious, since it requires that measurements be made of $K'_{B/A}$ for a large number of values of $\overline{X}_B$ ranging from nearly zero to nearly unity. However, for many purposes it is sufficiently accurate to measure $K'_{B/A}$ for only a few values of $\overline{X}_B$, plot a graph of log $K'_{B/A}$ (or $K'_{B/A}$) against X_B, determine by interpolation the value of log $K'_{B/A}$ (or $K'_{B/A}$) at X_B = 0.5 and take this as the value of log $K_{B/A}$ (or $K_{B/A}$). Therefore Reichenberg applied the approximation equation as below;

$$K_{B/A} = \left[K'_{B/A}\right]_{\overline{x}} = 0.5 \qquad (3.12)$$

This procedure is acceptable wherever log $K'_{B/A}$ is a linear function of $\overline{X}_B$.

Selectivity coefficients, $K_{B/A}$ are not constant and they are changed with membrane composition. It is noteworthy that in the exchange of ions of equal valence the selectivity coefficient is independent of the units used to measure the concentrations in the two phases. If $K_{B/A}$ >1 the membrane takes up B ion more strongly than A ion. If $K_{B/A}$ is less than unity, the affinity for A ion is greater than for B ion.

The distribution coefficient is defined as the ratio of the concentrations of the solute in the sorbent and in the solution. The distribution coefficient $K_{B/A}$ is expressed as;

$$K_d = \frac{\overline{X}_B}{X_B} = K \frac{\overline{X}_A \alpha_B \bar{f}_A}{\alpha_A \bar{f}_B} \frac{1}{X_A} \qquad (3.13)$$

where, α_A and α_B are the ionic activity coefficients of X_A and X_B in solution, and $\overline{\alpha}_A$ and $\overline{\alpha}_B$ are the ionic activity coefficients of X_A and X_B in the exchanger phase respectively, $\bar{f}_A$ and $\bar{f}_B$ are the activity coefficient of X_A and X_B in the exchanger phase, respectively. If the exchange reactions were carried out using micro quantities of the metal ions, then $X_A \gg X_B$ and $\overline{X}_A \gg \overline{X}_B$. Under these conditions, eq. (3.13) reduces to,

$$\text{Log}\, K_d = \text{const.} - n \log[X_A] \qquad (3.14)$$

If an ideal reversible exchange reaction takes place, a plot of log K_d versus log $[X_A]$ should be a linear relationship with slope of -n of the valency of the exchanged metal ions. For a discussion of the assumption in this equation see Abe, 1993. If the ion exchanger shows selectivity, hence K_d is not constant and depends upon the equivalent fraction. For any given conditions, the distribution coefficient can be calculated from the selectivity coefficient.

3.3 Materials and Methods

3.3.1 Materials

Materials and preparation of solutions are given in section 2.2.1.

3.3.2 Methods

Each membrane in M^+ or H^+-form was usually equilibrated with a mixed HCl+MCl (M: Li, Na, K and Cs) solution of total composition 0.05 M. After equilibrium (generally 24 hours) the equilibrated membrane was removed from solution. The change in composition of the equilibrated solution was determined by titration of H^+ ions. The membrane composition was also determined by conversion to the M^+-form and titration of the released H^+ ion. The concentration of metal cations in the solution and in the membrane phase was determined by Atomic

Absorption Spectrophotometer (Perkin Elmer 1100 B). In all cases mass balance was confirmed.

Consider that it beginning the membrane was in the M^+-form. To determine the ionic composition in the solution.

C_{M_0} = Initial concentration

C_{H_0} = Initial concentration

The concentration of HCl, after equilibrium is;

$$C_H = \frac{MT}{V}$$

where M= the molarity of NaOH, T= titre and V= the total volume of the solution. The concentration of M^+ ion;

$$C_M = C_{M_0} + C_{H_0} - C_H$$

Therefore, the ionic fraction of H^+ and M^+ ions in the solution after equilibrium are;

$$X_H = \frac{C_H}{C_{M_0} + C_{H_0}} \quad \text{and} \quad X_M = \frac{C_M}{C_{M_0} + C_{H_0}}$$

The ionic composition in the exchanger membrane was determined as follows;

The number of moles of metal, M in the membrane is equal to the capacity of the membrane, X meq g^{-1}, while in the solution, C_{M_0} became C_M and after equilibrium gain in metal ion M^+ by the solution is equal to $(C_{M_0} - C_M)V$ moles which is equivalent to the loss of H^+ by the solution.

$$\overline{X}_M = \frac{\overline{X} - (C_M - C_{M_0})V}{\overline{X}}$$

$$\overline{X}_H = \frac{(C_M - C_{M_0})V}{\overline{X}}$$

Selectivity coefficient;

$$K_H^M = \frac{\overline{X}_M}{\overline{X}_H} \frac{C_H}{C_M}$$

3.4 Results

The counter ion selectivity was studied by titrating PM and PFCM in solutions of various M^+/H^+ ratios. Alkali metals-hydrogen exchanges on the isolated PM and PFCM are characterised by the ion exchange isotherms (Fig. 3.1). Selectivity coefficient isotherms for alkali metals-hydrogen ion exchange of the isolated PM and PFCM at 25±1 °C is shown as a function of equivalent fraction of alkali metal ions in solution of HCl+MCl of 0.05 M (Fig. 3.3). The results of selectivity measurements were made to fit the Gibbs-Duhem equation (3.11). The ionic composition of alkali cations in the exchanger phase dependence of ln $K_{M/H}$ for PM and PFCM is shown in Fig. 3.3.

The experimental results were also fitted to adsorption isotherms either Langmuir or Freundlich type. The ion exchange selectivity of alkali cation-hydrogen exchange on the isolated PM and PFCM is shown as a characteristic feature of Freundlich isotherm type, Figs. 3.4.

The distribution coefficients of alkali cations for PM and PFCM versus hydrogen concentration in the solution phase (a plot of log K_d vs log $[H^+]$) are shown in Fig. 3.5.

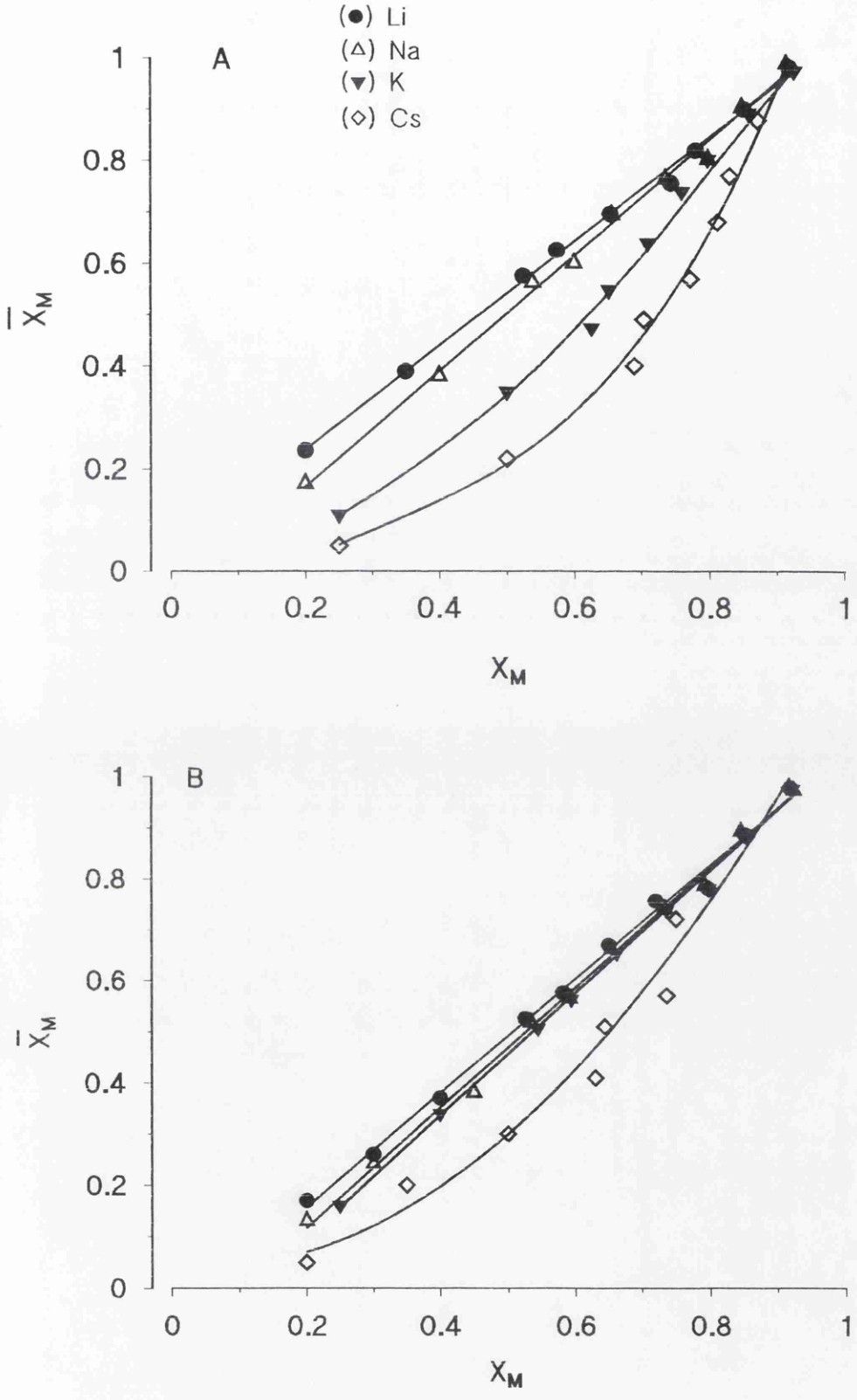


Fig. 3.1 M^+/H^+ exchange isotherms for isolated PM (A) and PFCM (B) membranes equilibrated with mixed alkali metal chloride and hydrochloric acid solutions of total concentration 0.05 M.

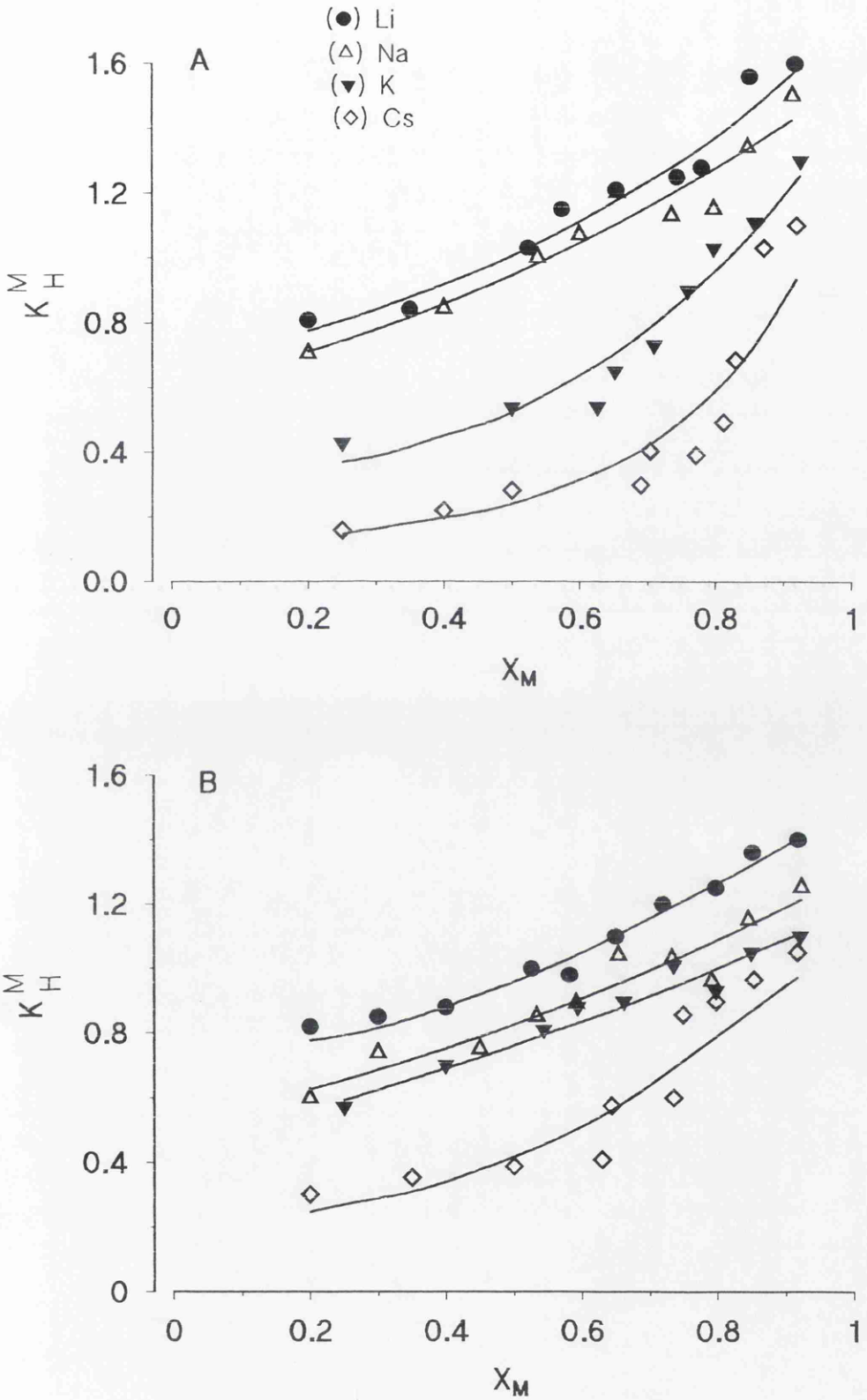


Fig. 3.2 Selectivity coefficients, $K_{M/H}$ for isolated PM (A) and PFCM (B) membranes as a function of the equivalent fraction of alkali cations in solution of (MCl+HCl) of total concentration 0.05 M.

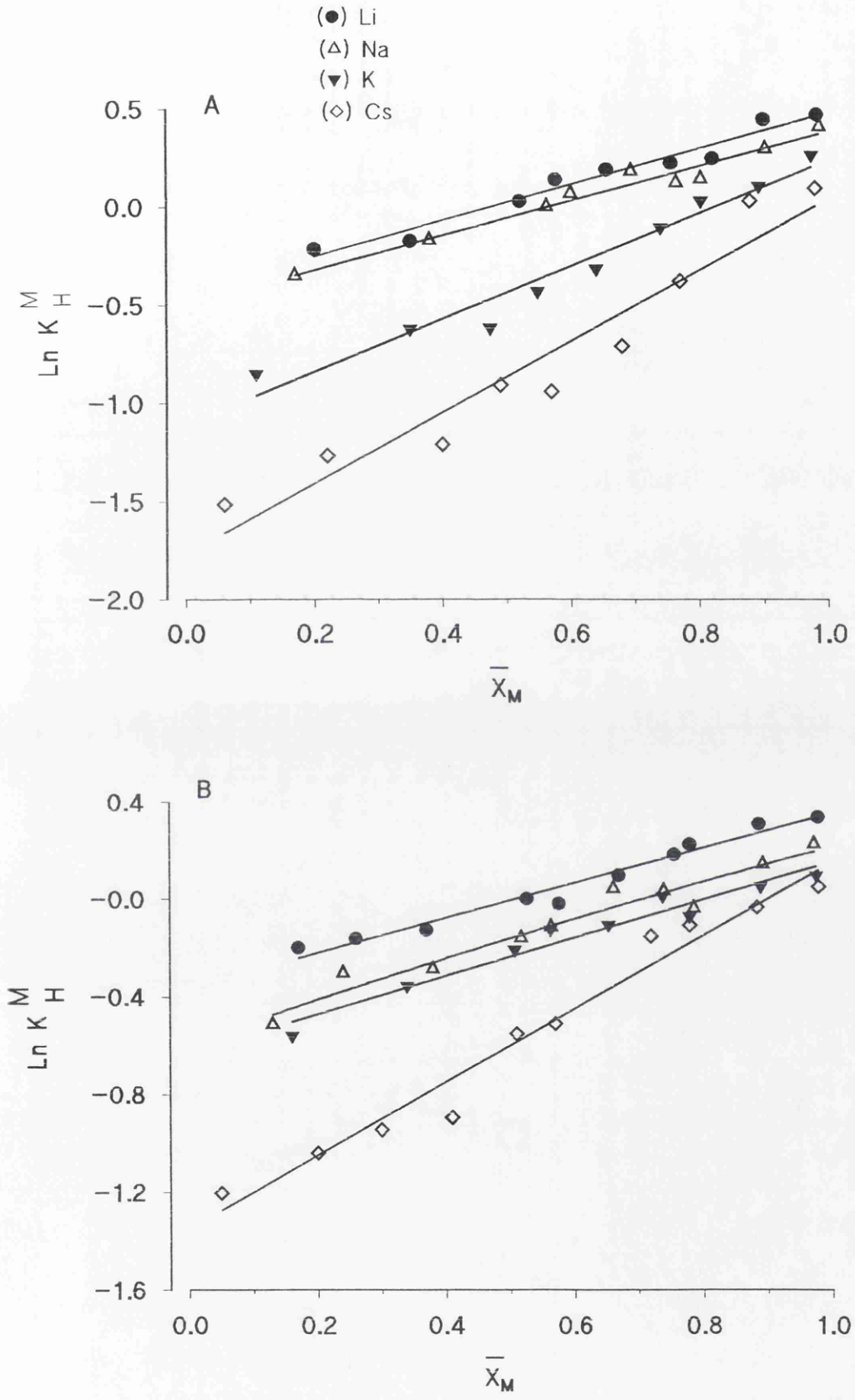


Fig. 3.3 $\bar{X}_M$ dependence of ln $K_{M/H}$ for isolated PM (A) and PFCM (B) cuticular membranes.

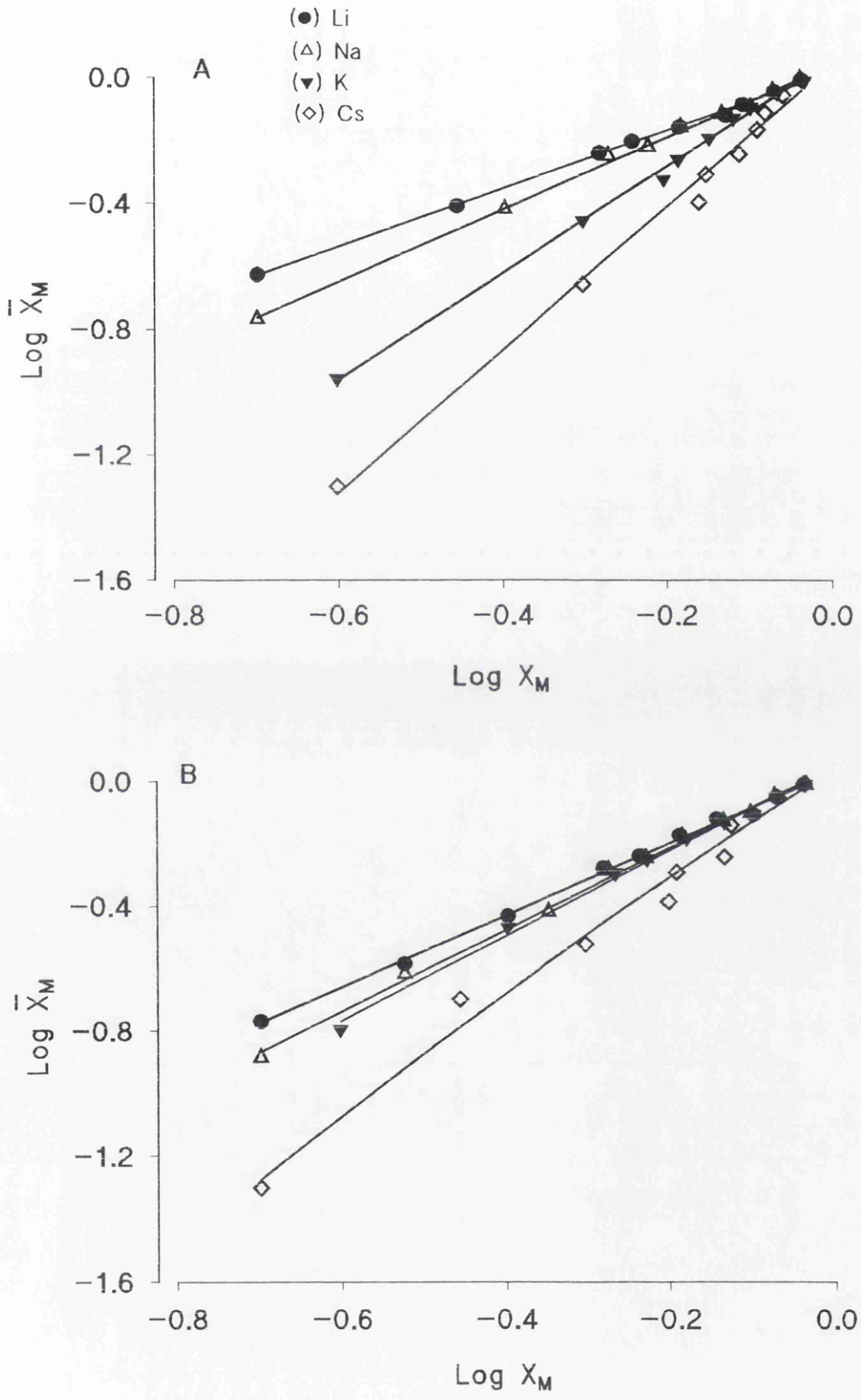


Fig. 3.4 The Freundlich isotherms of M^+/H^+ exchange systems for PM (A) and PFCM (B).

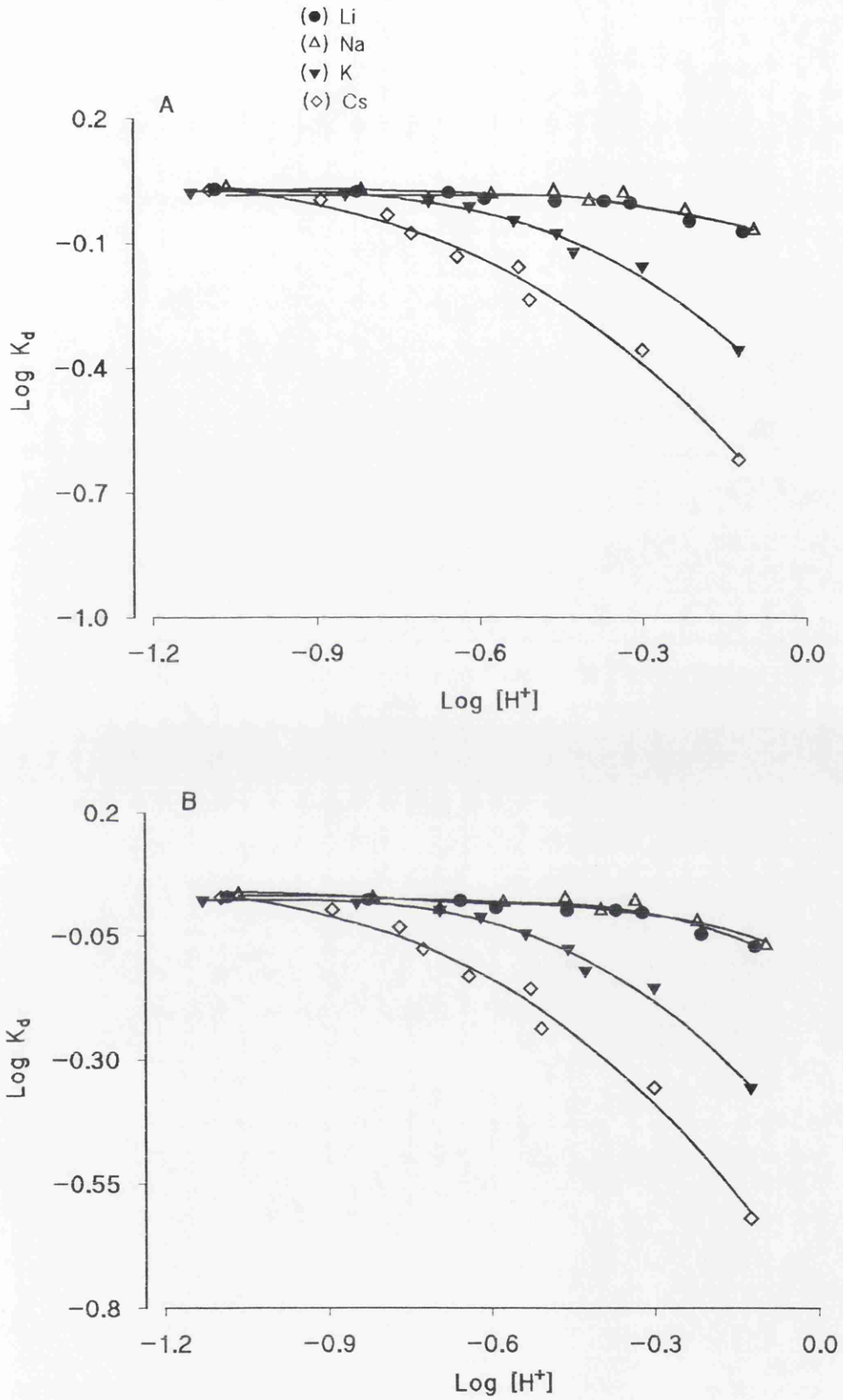


Fig. 3.5 A plot of Log K_d for M^+/H^+ as a function of Log [H^+] of solution phase for PM (A) and PFCM (B).

3.5 Discussion

Selectivity is usually expressed in terms of the selectivity term (K) derived from the Mass Action Law. The selectivity coefficient integrated over all ionic composition is that value obtained at ionic fraction = 0.5. (Yeager and Steck, 1979). These values for PM and PFCM, obtained by interpolation of the data shown in Fig. 3.2, are Li^+: 1.03; Na^+: 0.95; K^+: 0.62 and Cs^+: 0.28 for periderms, and Li^+: 0.99; Na^+: 0.83; K^+: 0.76 and Cs^+: 0.46 for pear fruit cuticular membranes, respectively.

For exchanges of two ions of equal ionic charge the selectivity coefficient defines the relative affinity of the two counter ions. It was seen from Figs. 3.2 and 3.3 that the selective uptake of alkali metal ions in the isolated PM and PFCM, follows the general order of preference $Li^+ > Na^+ > K^+ > Cs^+$.

The $K_{M/H}$ for K^+ and Cs^+ are lower than unity when the ionic fraction of the membrane phase is less than 0.8 and the membrane shows preference for hydrogen over potassium and caesium. The membrane demonstrated a reversed preference for potassium and caesium over hydrogen when the ionic fraction is greater than 0.8.

In most theoretical studies of ion exchange equilibria, attempts are made to fit empirical equations to experimental results. The equations used are modifications of either the mass-action law or adsorption isotherms of the Langmuir or Freundlich type (Helfferich, 1962). For ion exchange equilibria, either empirical and semi-empirical or adsorption isotherm equations derived from them are used by many workers (Kielland, 1935; Kunin and Myers, 1949; Walton, 1943; Hogfeldt, 1955).

Ion exchangers have a very large external surface. The ion exchange takes place to a great extent at this external surface, and exchangeable cations are held in part as a diffuse double layer, shading off gradually into the solution (Nachod, 1949). These experimental data were made to fit either Langmuir or Freundlich isotherm equation. The Freundlich isotherm is one of the first equation proposed to relate the amount of material adsorbed to the concentration of the material in the solution. The

PM and PFCM has exhibited a behaviour characteristic of Freundlich isotherm type Fig. 3.4.

There is no assurance that the derivation of the Freundlich equation is unique; consequently, if data fit to the equation, it is only likely, but not proven, that the surface is heterogeneous (Adamson, 1990). It is obviously seen from Fig. 3.4 that, the periderm membranes and pear fruit cuticular membranes are heterogeneous. Various plots were made of the data utilising the Freundlich and Langmuir mass action. A fair agreement was obtained with the Freundlich equation.

The selectivity coefficient for lithium and sodium ions is higher than that of potassium and caesium ions (Figs. 3.2 and 3.3) The isolated PM and PFCM show a low order or equal of preference for lithium over hydrogen at ionic fraction 0.5. The preference of sodium ions is reversed in the case of lithium ions in that both PM and PFCM exhibited very low order of preference for hydrogen over sodium ions at ionic fraction 0.5.

With carboxylic acid resins, the hydrogen ion exhibits the highest exchanging power (Kunin, 1958). The hydrogen ion is unique in its ion exchanging powers, because it forms covalent compounds, as it is a weak acid exchanger (Nachod, 1949). This ion exchanger prefers the counter ion which forms the stronger ion pairs or bonds with the fixed ionic groups. The preference of weak acid resins for H^+ ions, is associated with the weak acid fixed groups. If the counter ions are available, the polymer preferentially exchanges that ion due to results in the minimum free energy, therefore, the polymer prefers the counter ion that associates more closely with the fixed charges of the polymer (minimising the electrostatic free energy) and which results in the smallest polymer volume (minimising the free energy of stretching and maximising the configurational entropy of the polymer chains) (Gregor et al., 1956; Schonherr and Bukovac, 1973). PM and PFCM showed the pronounced selectivity order of carboxylic type ion exchangers.

According to electrostatic theory, generally the smaller ions have larger heats of hydration. A small ion contains a more concentrated charge, leading to a greater electrostatic interaction between ion and the polar water molecules. These ions are hydrated in water molecules, the radii of hydrated ionic sequence follows the order $Li^+ > Na^+ > K^+ > Cs^+$. Carboxylic acid groups are proton acceptor, thus these fixed groups prefer the larger, and more strongly hydrated. There is an agreement between these findings obtained and the affinity sequence of the hydrated ionic radius. The hydrated ionic values and crystal ionic radius of alkali cations are given, and the interaction between fixed groups of polymer and the counter ions in terms of hydrated ionic radius and crystal ionic radius is discussed with details in Chapter 2.

Gregor and et al. have measured the selective uptake of Li^+, Na^+ and K^+ by a series of methacrylic acid cation exchange resins of various divinylbenzene contents. They have observed the general order of preference as $Li^+ > Na^+ > K^+$. This preference became more marked as the degree of cross-linking increased, or as the degree of neutralisation of any given resin increased. They also revealed the order of activity coefficient for alkali salts, alkali hydroxides and alkali acetates. The general order for chlorides, bromides and iodides is $Li^+ > Na^+ > K^+$, for the acetates and hydroxides is $K^+ > Na^+ > Li^+$ (Gregor et al., 1956). Gregor's theory postulates a model that is relevant with hydrated volumes of the counterions. If it is assumed that the ions in the exchanger are "hydrated" and that when appropriate values for the volumes of the hydrated ions are taken other kinds of interactions are neglected.

The selectivity of a cation exchange resin for alkali cations is affected by the nature and acidity of the exchange groups. Some authors have reported that the weak acid resins show inverse selectivities to those of the sulphonic resins. Under alkaline conditions the adsorption sequence $Li^+ > Na^+ > K^+$ has been observed by Bregman (1953). The carboxylic and phenolic resins, are weak acid cation exchangers and require high pH values for their efficient utilisation.

The selectivity coefficient isotherms for alkali metal-hydrogen ion exchange in the isolated cuticular membranes show preference for hydrogen over alkali metal ions when the membrane phase is rich in hydrogen. With alkali metal ions rich membranes this preference is reversed and the membranes prefer alkali metal ions over hydrogen to a marked degree.

Bregman (1953) has shown that an ion exchange resin containing carboxylate groupings can prefer sodium to potassium. Gregor et al, (1956) criticised this and said that this is true only if all or nearly all the carboxylate groupings are ionised. When less than half of these groupings are ionised potassium is preferred to sodium. Reichenberg (1966) has confirmed and extended Bregman's observation. By working with a number of resins containing carboxylate groups of various specific capacities and degrees of cross-linking and with three different types of polymer matrix, and three different affinity sequences he observed the following;

$Li^+ > Cs^+ > Na^+ > K^+$

$Li^+ > Na^+ > Cs^+ > K^+$

$Li^+ > Na^+ > K^+ > Cs^+$

Williams (1990) noted that weak acid resins have the following affinity order $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. The result obtained here are in agreement with those findings of Williams (1990). The selectivity also increases with increasing hydrated ionic radii and decreasing crystal ionic radius of alkali cations. Muzarelli et al. (1974) studied the interaction of metal ions with alginic acid, polygalacturanic acid, carboxymethylcellulose and cutin. They found that the cutin behaves like carboxymethylcellulose.

It is well known (Robinson and Stokes, 1959) that the mobilities of the alkali-metal cations in aqueous solutions increase in the order $Li^+ < Na^+ < K^+ < Cs^+$. The most familiar interpretation of this is in terms of Stokes' law. Qualitatively, therefore, the conclusion is drawn that, in water, these ions exist in such a form that Cs^+

behaves as if it were the smallest and Li^+ the largest. The most natural way of accounting for this to suppose that the Li^+ ion has many more water molecules "stuck" to it than the Cs^+ ion; in fact, when unhydrated, the Li^+ ion is the smallest of the series, when hydrated it is the largest. This approach has been strongly criticised by Gurney (1953).

There are a number of other lines of experimental evidence; activity coefficients (Glueckauf, 1953) and diffusion (Robinson and Stokes, 1959) which may be interpreted to give the same qualitative conclusion that the term hydrated ionic radius has a valid meaning corresponding to real phenomena and that this radius decreases in the order $Li^+ > Na^+ > K^+ > Cs^+$.

The attracting interactions between the metal ion and the water molecules are primarily electrostatic in character. Most theories of ion exchange selectivity attach key importance to ionic solvation phenomena. The earlier theories employed quantities such as solvation number, solvated ionic radius, and solvated ionic volume. Reports by Link (1960) deal with the heat, entropy and free energy of solvation. Selectivity in the "normal" order is governed by the free energies of hydration of the counterion.

Most theoretical treatments of ion exchange selectivity assume, explicitly or implicitly, that all the fixed grouping within a given exchanger behave in an identical manner with respect to their selectivity properties. Glueckauf and Watts (1962) showed that electrolyte uptake from dilute solutions by ion exchange membranes did not obey predictions based on a simple Donnan equilibrium.

According to the theory of Eisenman (Reichenberg, 1966); if a mono valent counterion is taken from the bulk solution and brought into contact with a fixed group, two types of interaction energy are involved. 1. The electrostatic interaction between the fixed grouping and the ion. 2. The free energies required to remove from the fixed grouping and the counterion as many water molecules as are necessary to

permit the contact of the fixed grouping and counterion. Such free energies would be closely related to the standard free energies of hydration of the fixed group and counterion. In the case of a fixed grouping of high field strength, the exchanger will now give preference to the ion of smaller radius. This is exactly what is found with carboxylate resins, which is regarded as a grouping of high field strength, owing to its small size.

Many studies have been carried out for the determination of K_d values. A plot of log K_d versus log $[H^+]$ for isolated PM and PFCM is given Fig. 3.5. Using the experimental results for isolated PM and PFCM either reversible exchange or irreversible exchange reaction between alkali cations and hydrogen ion were tested according to equation (3.14). If an ideal reversible exchange reaction takes place, there should be a linear relationship with slope of -n of valency of the exchanged metal ions. (Abe, 1993). It can be seen from Figs. 3.5, the exchange reaction between some alkali metal cations and hydrogen ion is incomplete and is presumably due to the preference for more hydrated ions of the fixed groups of polymer matrix. Exchange of ions does not go to completion although the entering alkali cations are initially preferred and the degree of exchange gives a value lower than unity. It can also be seen from ion exchange isotherms curves (Fig. 3.1) that the distribution coefficients of Li^+ and Na^+ ions are very close to unity in both membranes, whereas K^+ and Cs^+ is lower than unity, because, $\overline{X}_M$ dependence of ln $K_{M/H}$ is not constant with concentration (Fig. 3.3).

CHAPTER 4

Penetration of Alkali Cations

4.1 Introduction

Diffusion through membranes is fundamentally no different from diffusion in liquid systems. The mechanism involves transfer of a molecule to a vacancy or "hole" in the medium. Energy of activation is required for formation of such a hole whether in a liquid or in a membrane. Transfer through membranes is by diffusion, a process of mass transfer which occurs as a movement of individual molecules. This movement may be induced by an electric field, a concentration, thermal or pressure gradient, or by other means.

A membrane is a solid or liquid film or layer with a thickness. It is described in simple terms as a phase, acting as a barrier to the flow of molecular and ionic species present in the liquids contacting the two surfaces.

Membranes can be classified into natural and artificial. Natural membranes existing in biological systems (biomembranes) are considered to have a fundamental unit membrane structure which is a bimolecular leaflet of lipid with their polar groups oriented toward the two aqueous, the extracellular and the introcellular, phases of the cell.

Ion exchange membranes combine the ability to act as a barrier between two solutions with the chemical and electrochemical properties of ion exchangers. The most important of these are the pronounced difference in permeability for counter ions, co-ions and neutral molecules, and their high electric conductivity.

The permeability of an ion exchanger membrane for counter ions, co-ions and non-electrolytes is quite different. The differences are most strongly pronounced when the concentration of fixed ionic groups is high and the solutions are dilute. When in contact with electrolyte solutions, the membrane contains a large number of counter

ions but relatively few co-ions (Donnan exclusion). Counter ions are admitted to the membrane and thus have little difficulty in passing through from one solution to the other. Co-ions, on the other hand, are rather efficiently excluded from the membrane and thus find it difficulty to pass through. The permselectivity is reflected not only in differences in permeability, but also in the electrical potential difference which arises between the two solutions.

In weak acid ion exchanger membranes the ionic concentration as well as swelling and diffusion coefficient depend strongly on the pH within the ion exchanger. The pH inside is a function of the equivalent ionic fraction of the H^+ ion in the solution. In weak acid ion exchangers there is association of H^+ with fixed ionic groups if equivalent ionic fraction of H^+ ion in solution is high. This reduces the counter ion concentration in the membrane, the efficiency of Donnan exclusion of the co-ion, and swelling and diffusion coefficient.

The mechanism of the interaction between simple ions and a charged group of a polymer chain is an interesting subject in ion transport phenomena through charged membranes as well as in polyelectrolyte solutions. It is known that transport rates of ions and water in membranes are affected by the physical and chemical properties of ions, solvents and membranes.

Diffusion and permeation of simple salts in polymers are closely related to the ionic transport phenomena in various systems. Many experiments on the diffusion of simple salts in ion exchangers have been reported, and the subject has been reviewed comprehensively (Helfferich, 1962; Lakshminarayanulah, 1969), and extensively studied.

So far permeability information in periderms is scarce and analysis of periderm permeability on the basis of chemistry is still lacking. Relatively little information is known about the mechanism of alkali ion permeation through cuticle membranes, and

nothing is known about penetration and diffusion of alkali cations in periderm membranes.

In gas diffusion studies the time lag method is commonly used to determine both the diffusion coefficient and the permeability of gases in polymer membranes. The diffusion of gases has most frequently been studied by permeation through the polymer membrane from one vessel to another. Gas permeability in the plant cuticles has been studied by Lendzian (1982). Provided measurements are made in both the transient state that follows a change in the gas phases outside the membrane and in the steady state ultimately reached, the data can be analysed to give the diffusion and sorption coefficients separately. The gas under study is introduced to one side of the cell and its pressure and temperature held constant thereafter. Gas which permeates the membrane collects in the other side of the cell which may then be measured by monitoring the pressure increase in that half with time. The steady state flow is obtained after a sufficient length of time. The relation between diffusion coefficient and permeability was first given by Daynes, 1920 (Al-Zubaidi, 1986).

Permeation of liquids or vapours across membranes is usually described and analysed in terms of Fick's law. The flux (J) of any species, is proportional to D and the driving force dC/dx where C is the concentration and x is the space coordinate in direction of flow. In steady state,

$$J = \frac{-D\Delta C}{l} \qquad (4.1)$$

If a planar membrane, area A (cm^2), thickness l, is exposed to a concentration gradient, the quantity of that solute which will have permeated in a certain time, t, is measured as a function of the magnitude of the driving force.

$$\Delta C = C_{don} - C_{rec} \qquad (4.2)$$

where ΔC is the concentration difference between the solutions of donor and receiver. If the amount diffused of a substance (M_S) is plotted against time, it will be linear at

large times (when the system is in steady state) and intercept on the time axis, whereby a curve is obtained. Thus, from a simple steady state experiment the flow rate (F_r) is obtained from the intercept of extrapolated steady state flow with the time axis hold-up time and with A and ΔC known, After a time lag, the flow rate $\Delta M_s/\Delta t$ becomes independent of time.

$$P = \frac{\Delta M_s}{A \Delta t \Delta C} = \frac{F_r}{A \Delta C} \qquad (4.3)$$

P can be used to calculate the flow of a penetrated solute across a membrane as a function of driving force. The corresponding diffusion coefficient can be calculated from the time required reach the steady state described by equation (4.1). This is conventionally done using the hold-up time equation (4.4) (Helfferich, 1962).

$$t_e = \frac{l^2}{6D} \qquad (4.4)$$

From this permeability , P is obtained from equation (4.3) and the corresponding diffusion coefficient, from the hold-up time, (equation 4.4). The method is convenient, rapid and relatively accurate.

Cuticles have a major influence on the absorption and ultimate impact of foliarly applied materials. Cation penetration in cuticles has been investigated by some workers(Yamada et al., 1964a, 1964b; McFarlane and Berry, 1974; Tyree et al., 1990a, 1990b) Cuticular permeability has been studied in the past and thoroughly reviewed (Currier and Dybing, 1959; Franke, 1967; Hull, 1970; Martin and Juniper, 1970 and Schonherr and Riederer 1989). Water permeability of periderm has been investigated (Muir, 1990, Vogt et al., 1983, Schonherr and Ziegler, 1980).

In this study, alkali cation penetration from K^+, Cs^+, Li^+ and Na^+ forms in isolated potato periderm and pear cuticle membranes were investigated and the rates of penetration determined. The steady state rates were measured by using a

diaphragm diffusion cell. The diffusion experiments were repeated using dialysing membrane.

It was the purpose of this research to determine the rates and mechanism of alkali cation penetration through isolated potato periderm and pear fruit cuticular membranes. The effect of external concentration and pH were also investigated in order to obtain data pertinent to the mechanism involved.

4.2 Materials and Methods

4.2.1 Materials

The isolation of periderm and cuticular membranes were given in section 2.2.2.1. Spectrapor Dialysis membrane was used to compare with PM and PFCM (obtained from Spectrum Medical Industries Inc., wet cellulose dialysis tubing in 1% sodium azide) which was composed of cotton cellulose, 0.05 mm thick, with a nominal mol. wt. cutoff of 3,500.

0.01 M Citric acid (May & Baker) - Na_2HPO_4 (Analar, BDH), for the penetration of Cs, Li, K ions and 0.05 M KH_2PO_4 (Analar, BDH) - KOH (Analar, BDH) for the penetration of Na ion, buffers were used in the pH range of 4 to 8. The experiments were carried out with aqueous hydrogen chloride solution (pH $\cong$ 3), and deionized water.

4.2 Methods

The isolated PM , PFCM and Dialysis membranes were converted to the H+ form by treatment with 1 M HCl, followed by rinsing with deionized water and air drying at room temperature. Dry membranes were stored for further use.

The apparatus used in the study of penetration of materials through membrane samples is illustrated which consisted of two borosilicate half cells as depicted in Fig. 4.1. Transport studies were made in the systems, aqueous solution (=donor) /membrane/ aqueous solution (=receiver).

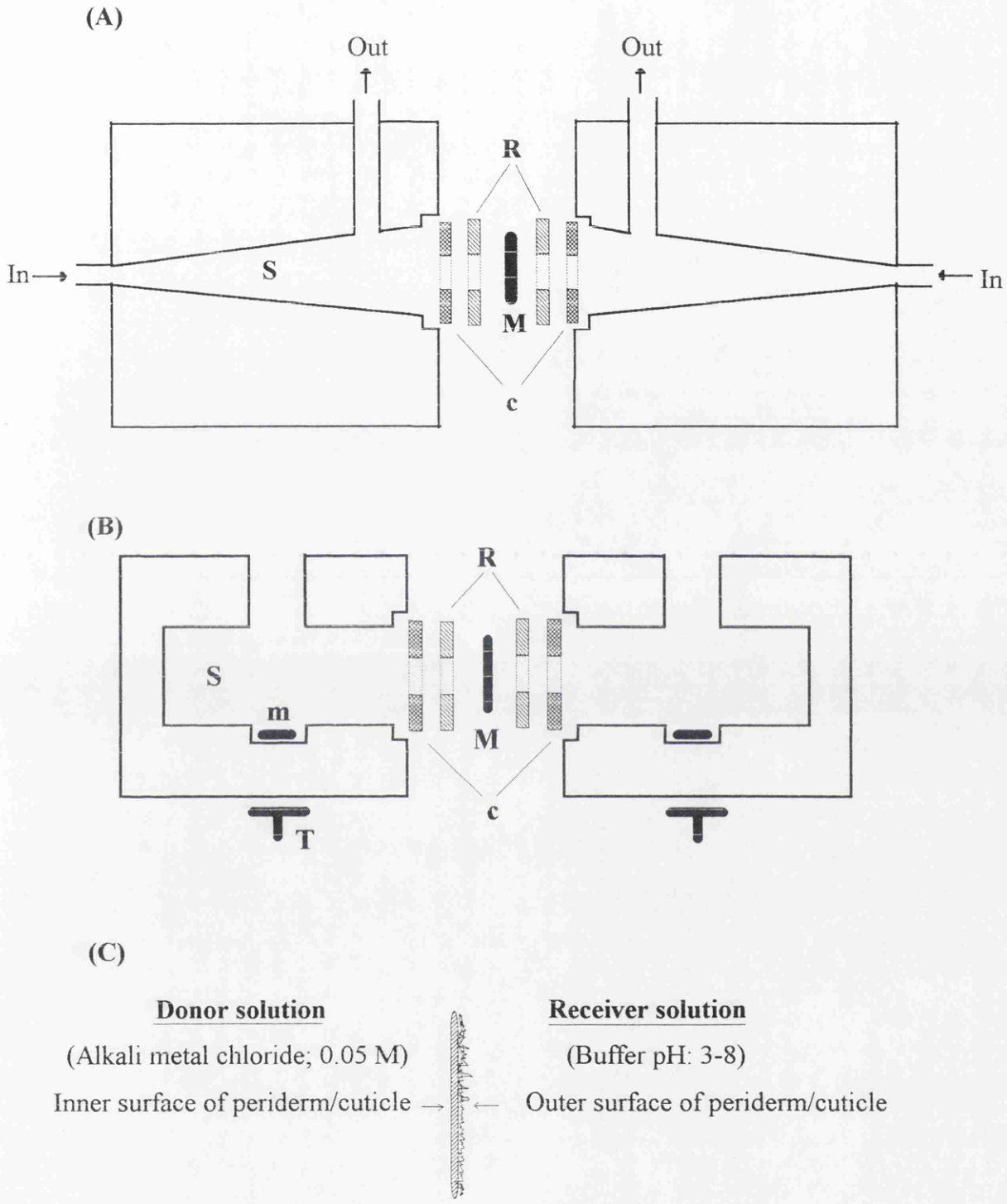


Fig. 4.1 Transport apparatus (A and B) (Infinite dose system). S: solution chamber; M: membrane; R: silicon rubber gaskets; c: clamps; m: stirring magnet; T: stirrer, (C) solutions on either side of the membranes.

The membrane samples were mounted between the silicon rubber gaskets and clamped between the two half cells in which the outer surface of the membrane was

receiver solution and the inner surface of the membrane (solution facing the morphological inside of the periderms/cuticular membranes), donor solution. The appropriate solutions were added into each compartment, and the unit was submerged, in a water bath (for experiment apparatus see Fig. 4.1 A) and in an incubator (for apparatus see Fig. 4.1 B), and maintained at 25±1 °C. The donor solutions were salt chloride solutions and the receiver solutions were the buffer solutions applied at different pH ranges. (pH ranges; pH 3 aqueous hydrogen chloride, pH 4-8 Na_2HPO_4-Citric acid and KH_2PO_4-KOH buffer, and Deionized water). Alkali cations were allowed to penetrate from the inner surface to the outer surface of the membrane (Fig. 4.1C).

The receiver and donor solutions were prepared in two 20 ml flasks and after being stirred, the solutions were fed into the transport apparatus (both sides of the cuticle simultaneously) by a peristaltic pump. For experiments using apparatus described in Fig. 4.1 B the solutions were poured into the two half cells (13 ml) and then stirred. The solution containing the ion, whose penetration rate was being measured, flowed over the inside surface of the cuticle and, at the same flow rate, a solution of receiver flowed over the outer surface to serve as a collection medium. At zero time and at time intervals a 1 ml aliquot was withdrawn from the receiver solution. One ml of "cold" collection solution was added to the receiver solution immediately after withdrawing the sample to maintain a constant volume in the receiver. At least 12 samples were taken during the experiment. When the penetration of alkali cations was being determined, the pH 3.0-8.0 range of solutions as above mentioned, were used as the collection medium. The concentrations of metal cations were determined by Perkin-Elmer 1100B Atomic Absorption Spectrophotometer. The effective membrane areas in the cell for potato periderm membranes were 2.05 and 1.33 cm^2, and for pear fruit cuticular membranes 1.33, 0.78 and 0.5 cm^2 and dialysis was 0.78 and 0.5 cm^2. The pear fruit cuticular membranes were very fragile hence a small diameter was used.

This system was used to determine the penetration rates of many different solutes through the same piece of cuticle. Before starting a new experiment the membranes were converted into the H^+ form by a treatment with 1 M HCl for 45 to 60 min. followed by rinsing with deionized water to remove all Cl^- ions. Determinations were repeated more than three times for each set of experimental conditions.

4.3 Results

Steady-state is defined by a time-independent transport rate and a constant concentration gradient between donor and receiver solution. Steady-state conditions verified many times and obtained within 10 min and maintained for a long time due to the low permeability of the PM and PFCM. Steady-state conditions were also obtained within 2 minutes for dialysing membrane. The flow rate F_r (mol s^{-1}) was determined from the linear slope of amount penetrated versus time by the least squares method. The permeability P (cm s^{-1}) was calculated from the equation (4.3). From the intercept of extrapolated steady-state flow with the time axis the so-called hold-up time was obtained from equation (4.4) (Helfferich,. 1962). Diffusion coefficient was calculated from the hold-up time.

The time course of penetration of alkali cations across PM, PFCM and dialysis membranes as a function of pH are shown in Figs. 4.2-4.4. The penetration rates of the alkali cations in the potato and pear isolated cuticular membranes were observed in the order $Cs^+ > K^+ > Na^+ > Li^+$. This is the order of increasing crystal radii, and the order of decreasing exchange selectivity observed for selectivity experiments. A linear increase of the outer solution with time was observed for Cs^+ and K^+ cations. The experimental points gave a straight line for Cs^+ and K^+ ions, but after an initial hold-up time, penetration rates reach a maximum and then level off for Na^+ and Li^+ ions. None of the slopes of amount penetrated versus time reach a constant value i.e. the flow rates are not independent of time.

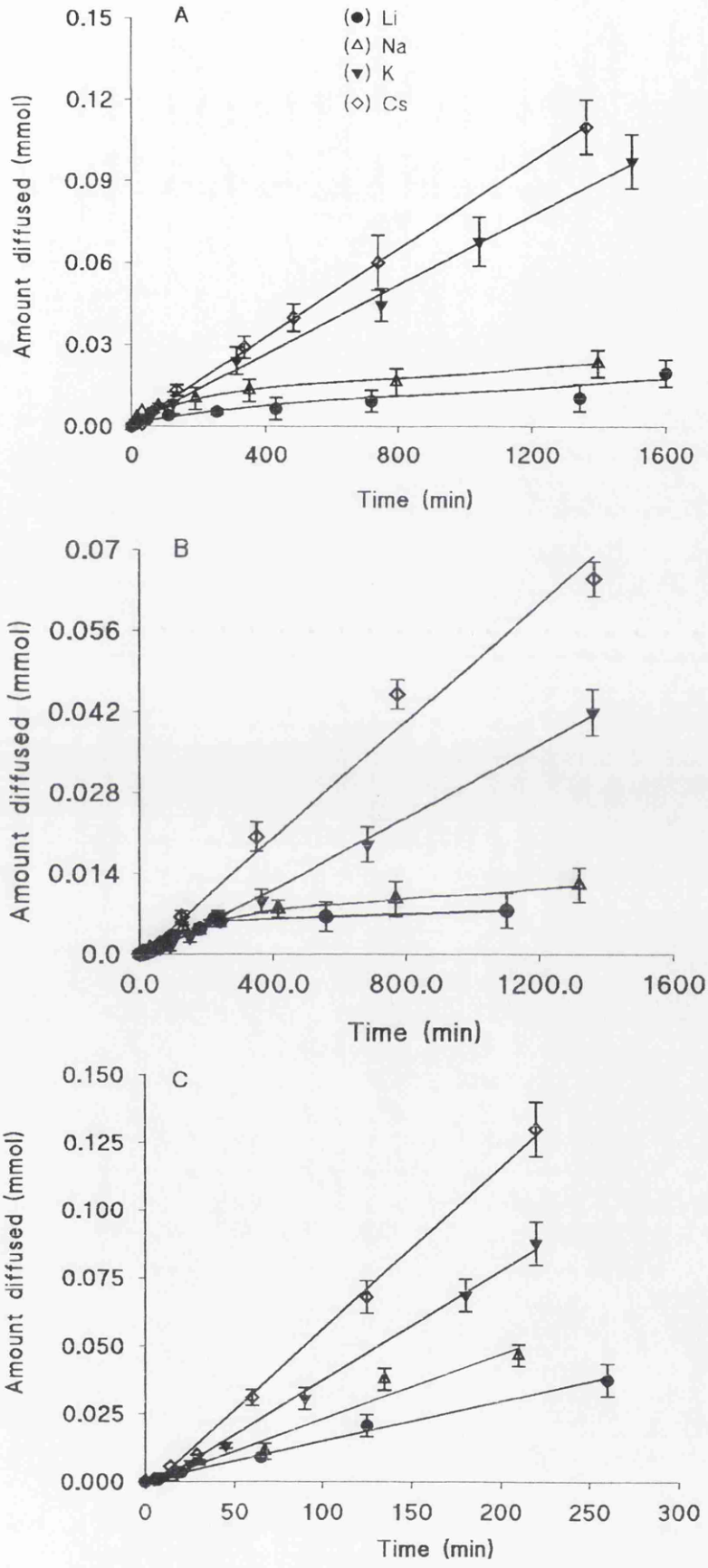


Fig. 4.2 Time course of penetration of alkali cations across (A) PM, (B) PFCM and (C) DM, as a function of pH 3.0.

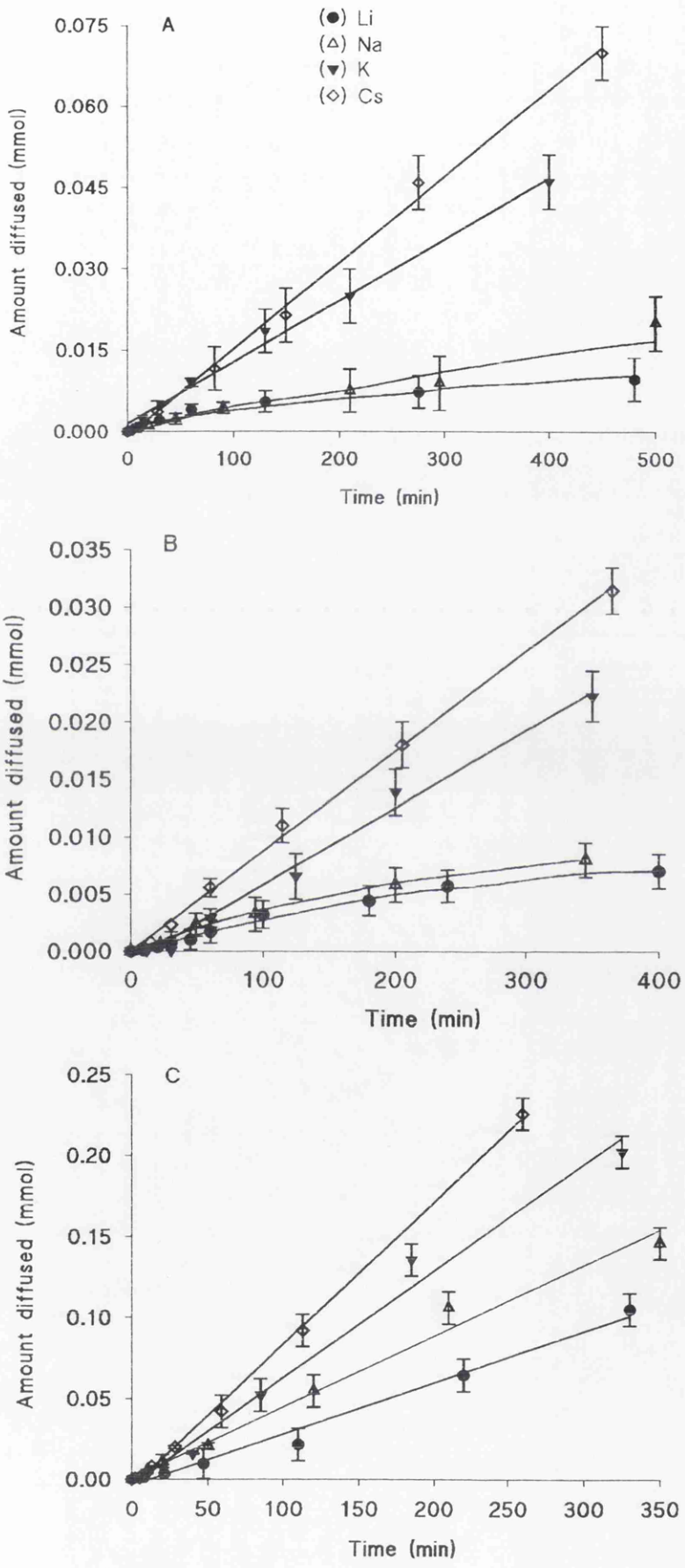


Fig. 4.3 Time course of penetration of alkali cations across (A) PM, (B) PFCM and (C) DM, as a function of pH 6.0.

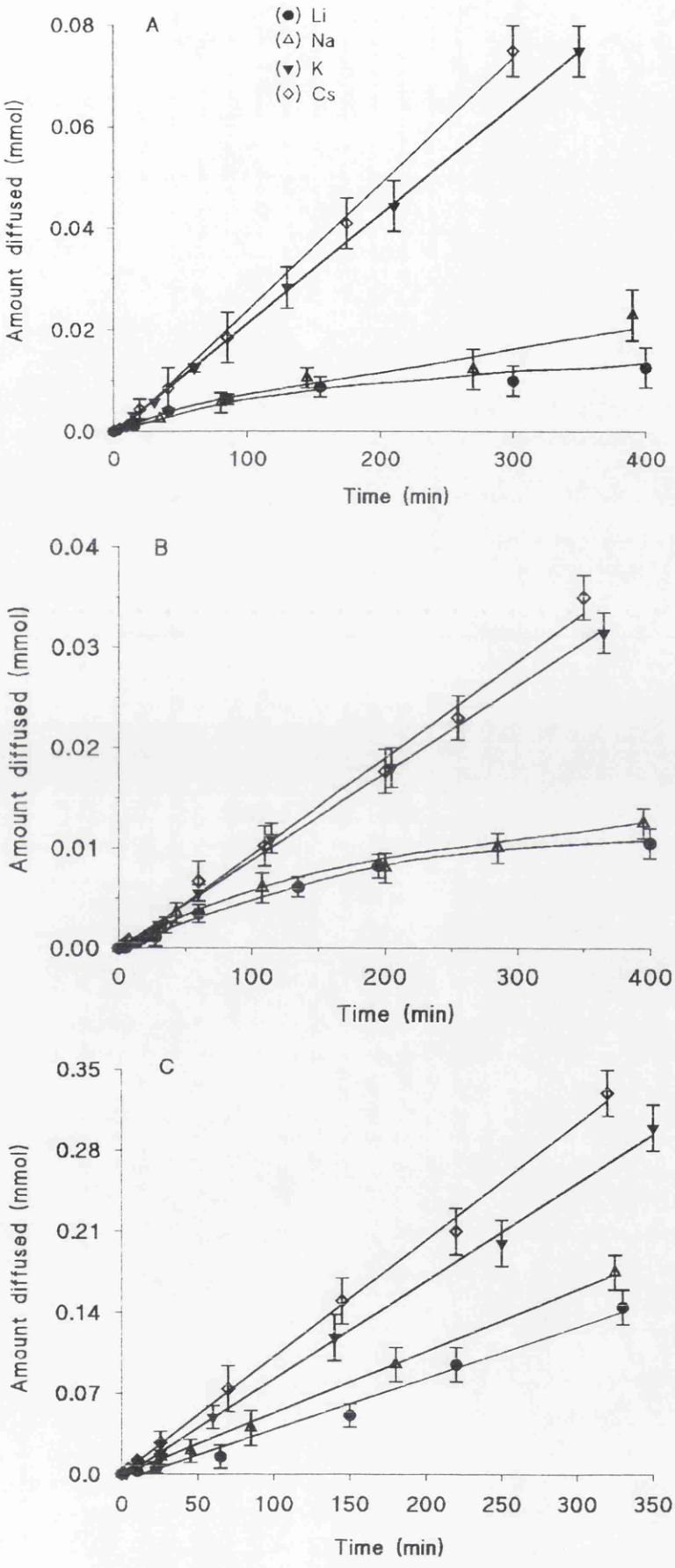


Fig. 4.4 Time course of penetration of alkali cations across (A) PM, (B) PFCM and (C) DM, as a function of pH 8.0.

The effects of the pH of the external solution on the alkali cation penetration and diffusion across PM, PFCM and dialysis membranes are given in Figs. 4.5 and 4.6. The penetration of alkali cations was studied between pH 3-8 and over this pH range two dissociable groups were identified as two carboxylic groups between pH 3 to 6 and 6 to 8. The carboxylic group dissociating between pH 6 and 9 discriminated between alkali metal ions according to their ionic radius.

The diffusion coefficients of all counter ions increased as the external concentration was increased. The permeability and diffusion coefficients are found to increase in a sequence of CsCl> KCl> NaCl> LiCl. This suggests that the diffusion coefficients and permeabilities of alkali cations in membranes decrease also with increasing Stokes radius of the cations.

The permeability of enzymatically isolated PM and PFCM increased almost 3 fold between pH 3 and 8. Higher pH values were avoided due to the risk of ester hydrolysis. Dialysis membranes gave very similar results. The diffusion of alkali cations was dependent on the pH. There is little affect observed between pH 3 and 4, but the permeability of alkali cations was increased above pH 5.

Relative permeability and diffusion coefficients as a function of hydrated ionic radius of alkali cations are shown in Figs. 4.7 and 4.8, here Li was arbitrarily chosen as a reference ion. P/P_{Li} and D/D_{Li} indicate the relative magnitude of any cation through the membrane samples. The relative diffusion coefficients and permeability of alkali cations as a function of hydrated values and pH are given in Figs. 4.9 and 4.10. The experimental points fit a curve, and the regression coefficient was between 0.94-0.98.

Comparison of data from various experiments was made by expressing coefficient values relative to values of another ion through the same cuticle. The penetration rate of Li^+ was less than for other ions, therefore Li^+ was arbitrarily chosen as a reference ion and the ratio $P/P_{Li}{}^+$ indicates the relative magnitude of

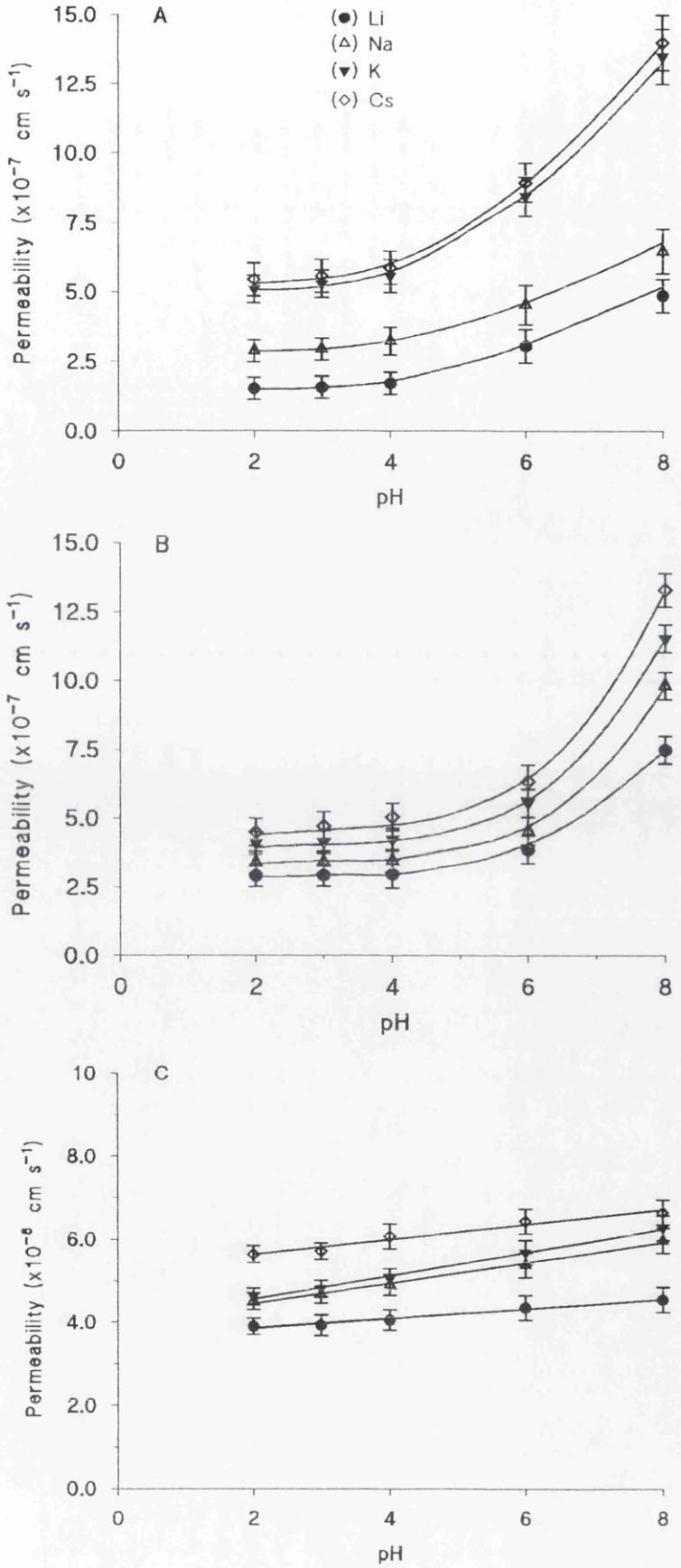


Fig. 4.5 The effect of the pH of the external solutions on alkali cation permeabilities across (A) PM, (B) PFCM and (C) DM, each point represents the mean of determinations.

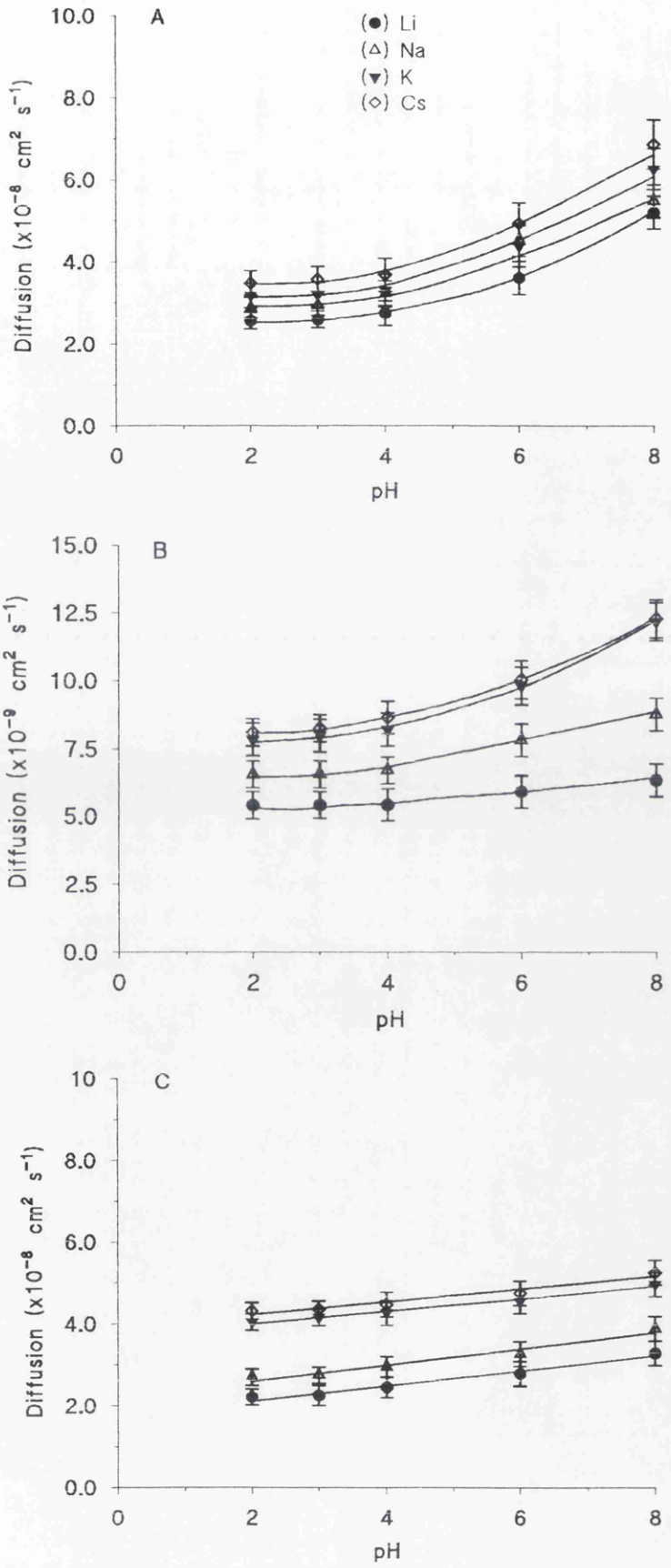


Fig. 4.6 The effect of the pH of the external solutions on alkali cations diffusion across (A) PM, (B) PFCM and (C) DM, each point represents the mean of determinations.

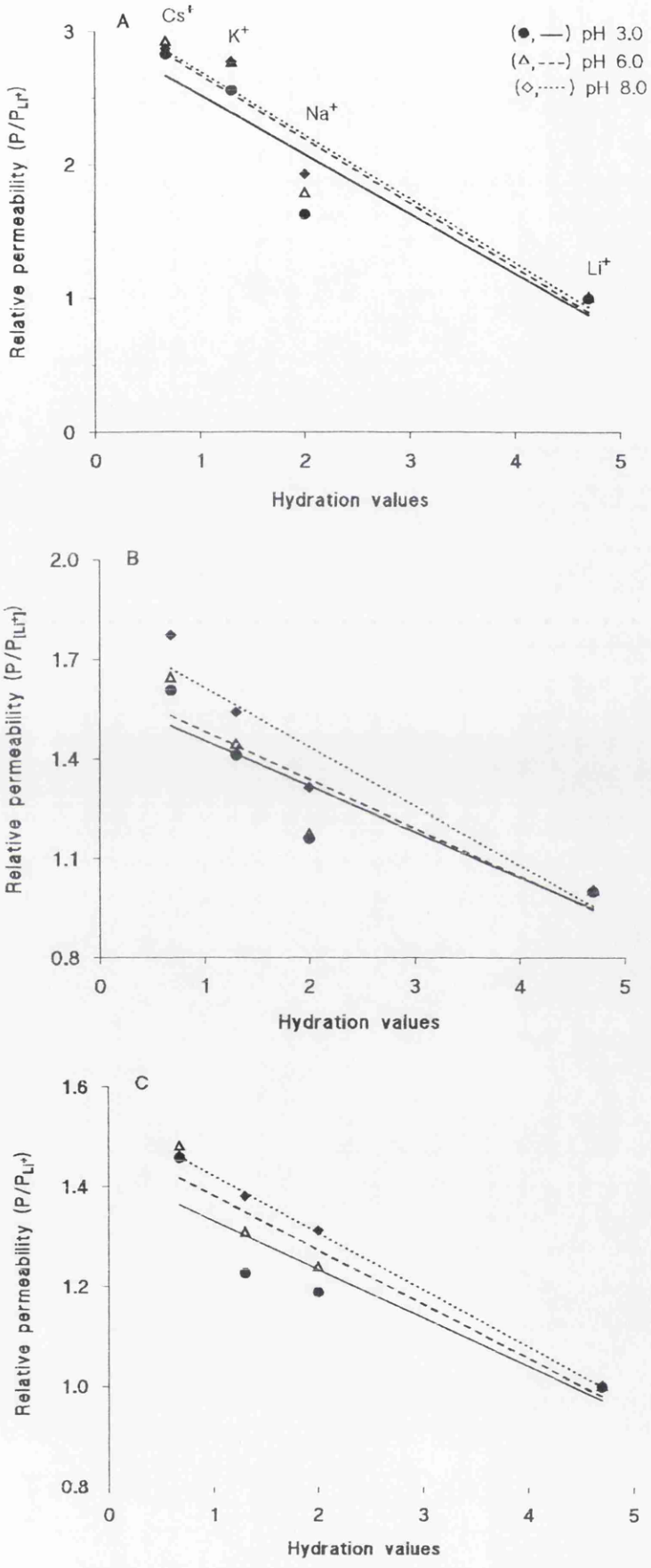


Fig. 4.7 Relative permeabilities of (A) PM, (B) PFCM and (C) DM, as a function of hydrated values and effect of pH of external solutions.

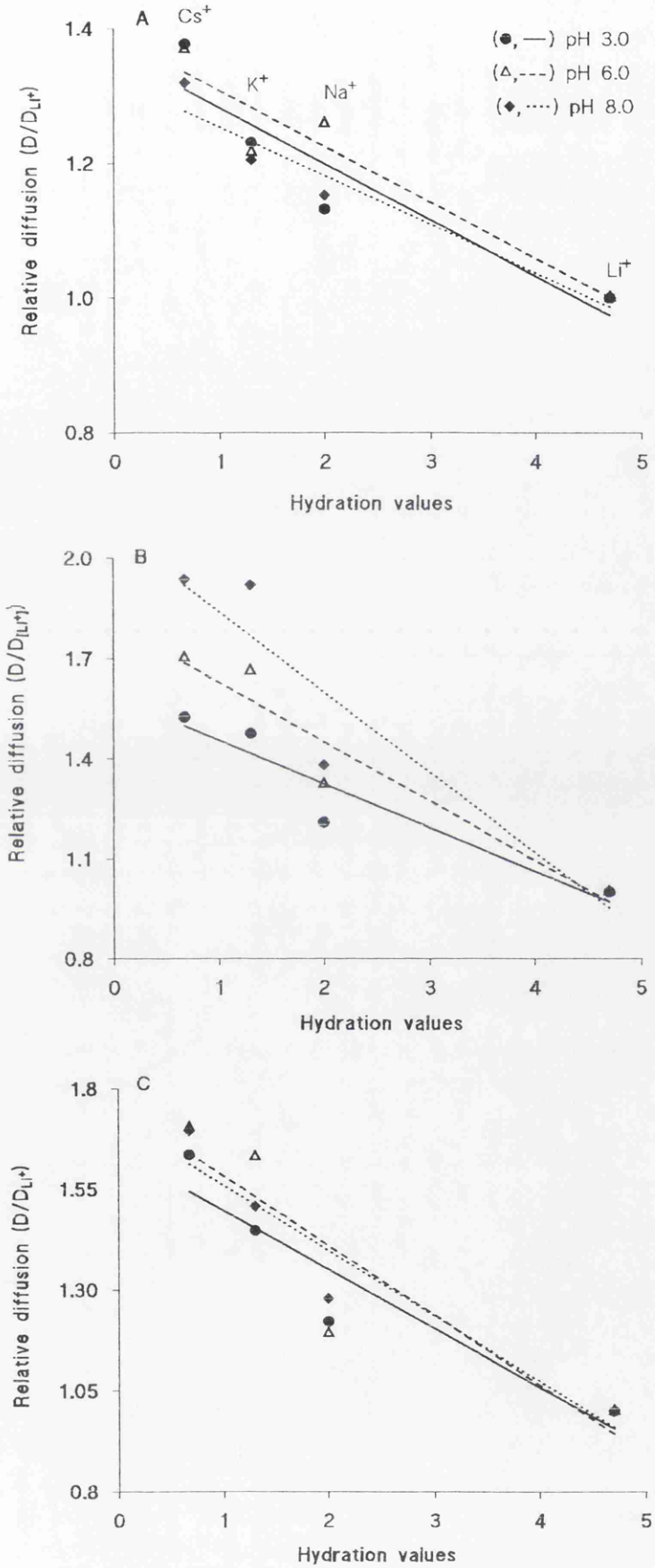


Fig. 4.8 Relative diffusion coefficients of (A) PM, (B) PFCM and (C) DM, as a function of hydrated values and effect of pH of external solutions.

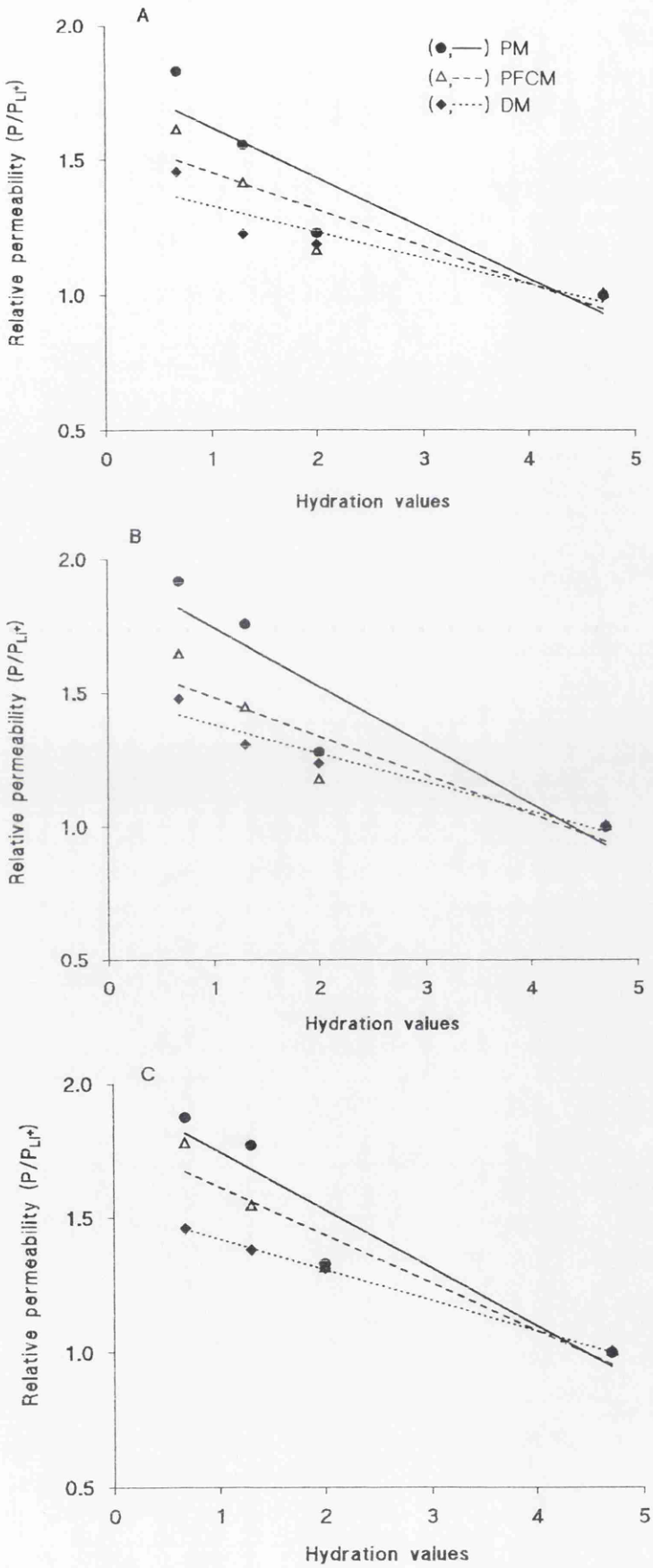


Fig. 4.9 Relative permeabilities of alkali cations as a function of hydration values and pH (A; 3.0, B; 6.0 and C; 8.0) in the PM, PFCM and DM.

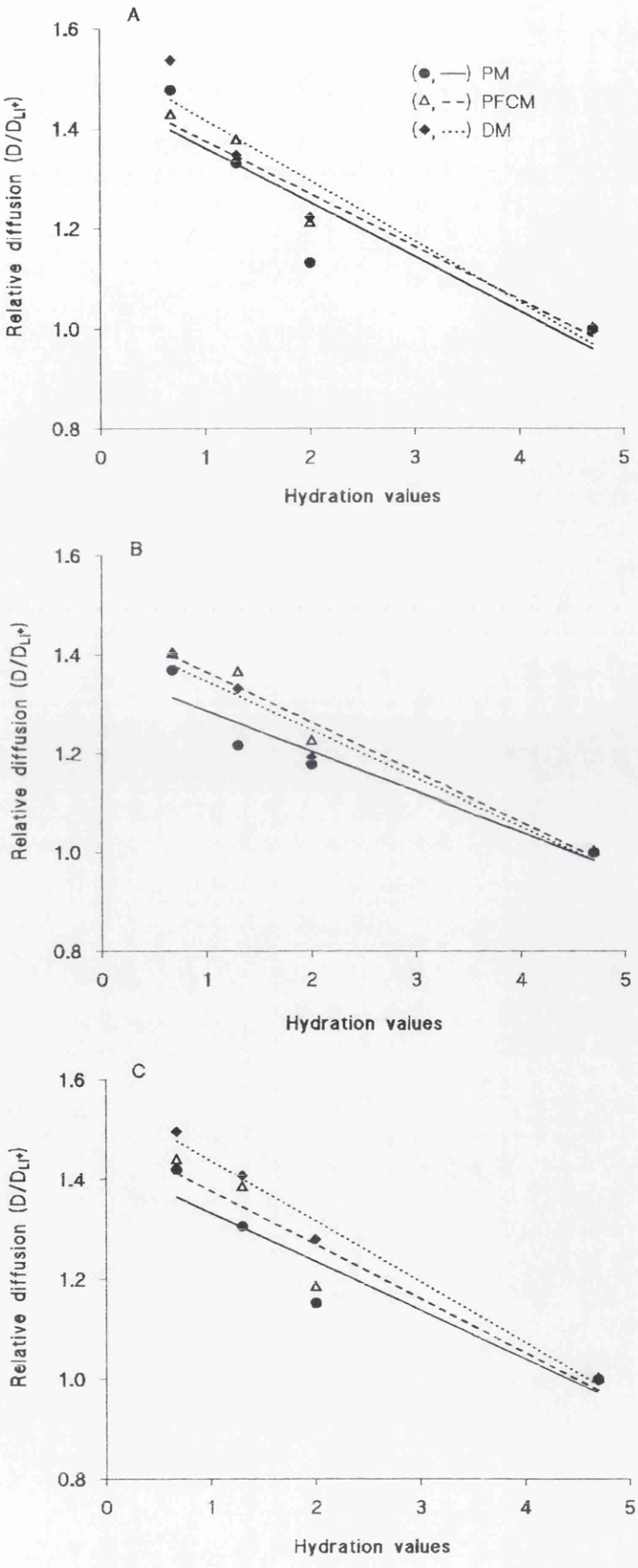


Fig. 4.10 Relative diffusion coefficients of alkali cations as a function of hydration values and pH (A; 3.0, B; 6.0 and C; 8.0) in the PM, PFCM and DM.

movement of any cation through the cuticle. The relative order of permeabilities for alkali cation penetration was $Cs^+ > K^+ > Na^+ > Li^+$.

4.4 Discussion

The permeability is a parameter that is characteristic for a given type of cuticle and a given solute (or solvent) and temperature. Permeability may depend on concentration of the solute. The process taking place also depends on the nature of the membrane or cuticles.

The penetration rates of the alkali cations in the potato and pear isolated cuticular membranes were observed in the following order $Cs^+ > K^+ > Na^+ > Li^+$ and this is the order of increasing crystal radii, and is in agreement with result from tomato fruit cuticular membranes (Schonherr and Bukovac 1973), and carboxylic acid type ion exchangers in general (Gregor et all, 1956).

A cation exchanger prefers the cation which results in the smallest volume. The ionic content makes the cutin significantly swellable in water. Schonherr and Bukovac (1973) estimated that the water content is between 10% and 20%. Some workers assumed the presence in cutin of hydrophilic ionic groups, without any attempt at measurement, that cutin will be "swollen" by water and you can neglect its probably greater swelling in less polar solvents. The swollen volumes and water contents of -COOH type ion exchangers increase in the order $Li^+ < Na^+ < K^+ < Rb^+$ (Gregor et al., 1956) which is the order of the alkali cation permeabilities observed in these experiments. The polymer matrix of cuticles was shown to be a porous polyelectrolyte, its water content and water permeability depending on the pH and the nature of counterions (Schonherr, 1976b).

It has been found that cuticular transpiration was markedly dependent on pH and the nature of buffer ions. Some workers found that the water permeability in cuticles and periderms was strongly dependent on the pH value and the cations of the buffer solutions, and the shape of the plot, water diffusion versus pH, suggests the

presence of 3 different dissociable groups fixed to the membrane matrix (Schonherr, 1976b, Vogt et al., 1983, Schonherr and Schmidt, 1979). They are tentatively identified as, two carboxylic groups dissociating between pH 3 to 6 and 6 to 9, respectively, and phenolic hydroxyl groups dissociating above pH 9. The carboxyl group dissociating between pH 6 and 9 discriminated between alkali metal cations according to their ionic radius. Water permeability was lowest in the Li^+ form and increased in the order $Li^+ < Na^+ < K^+ < Rb^+$ (Schonherr, 1976b). These results were taken to indicate an effect of pH and ions on swelling and pore size of the cuticle. The increase in permeability with increasing pH is definite evidence for the existence of fixed dissociable groups of the weak acid type in the cuticular membranes. Since sulphur and phosphorus are generally not present in cuticles (Martin and Juniper, 1970) these groups are most likely to be carboxyl below pH 9 and phenolic hydroxyl groups above pH 9. Therefore, the permeability of alkali cations has been studied below pH (9), to compare with carboxylic type ion exchangers. The presence of some amino groups together with -COOH groups can not be precluded. The existence of weakly basic groups has been assumed for the apricot leaf cuticle (McFarlane and Berry, 1974).

This pH-dependent increase in permeability is the consequence of the higher water content which accompanies dissociation and neutralisation of fixed charge (Katchalsky, 1954; Gregor et al., 1955, 1956) Due to ion pair formation -COOH groups are little ionised and little hydrated. When neutralised with monovalent alkali metal ions association between $-COO^-$ and M^+ is less close than with H^+ and the polymer swells (Schonherr, 1976a, 1976b). These swelling forces are counteracted by the cross-links of the polymer and an equilibrium is established between these forces. The position of the equilibrium depends on the concentration and the degree of dissociation of fixed charges, which in turn depends on the pH, the nature of counter ions and the concentration of the external solution (Schonherr, 1976b). The degree of

dissociation of fixed -COOH groups at constant pH is increased by addition of neutral salt (Katchalsky 1954, Gregor et all 1955, 1956, Schonherr and Bukovac, 1973).

Tyree et al. (1990a) have studied diffusion potentials of monovalent cations across leaf cuticles of both *Citrus* and *Acer*, and the order of permeabilities observed was $K^+ \geq Cs^+ > Na^+ > Li^+$. McFarlane and Berry (1974)). determined that the relative permeabilities of monovalent cation penetration of apricot leaf cuticles was in the order $Cs^+ \geq Rb^+ > K^+ > Na^+ > Li^+$. The ranking order close to the results, obtained here followed the order of decreasing ionic radius and increasing hydrated ionic radius for alkali cations. When this study was initiated it was unclear whether the order obtained by McFarlane and Berry or by Tyree et al. would result. The results here followed the same order as that of McFarlane and Berry.

Cuticles are chemically inert (Martin and Juniper 1970) except at high alkaline pH values, which were avoided (above pH 10.0) in the experiments due to the risk of ester hydrolysis. The enzymatic isolation was carried out under very mild conditions (pH 3.8) and was not likely to alter cutin or the other possible components of cuticles (Schonherr and Bukovac, 1973).

The permeability and diffusion coefficient in Figs. 4.5 and 4.6 shows that the order of cation penetration was $Cs^+ > K^+ > Na^+ > Li^+$. This sequence is the same as the sequence of mobilities in free solution, which is also the sequence of ionic radii. The sequence differs from the sequence of hydration energies, $Li^+ > Na^+ > K^+ > Cs^+$, which is also the sequence of hydrated radii. Yet the alkali cations are very similar to each other physically and chemically and differ mainly respect to size, the Ladd ionic radii (1968).

Comparison of quantitative values for diffusion coefficient and permeability is not possible but since all cations in these experiments penetrated the same area of the same cuticles, it is assumed that pathways were identical. Comparison of trends in diffusion coefficients and permeabilities between replicates are therefore valid.

Because permeability for each cuticle was different the same cuticles were replicated several times.

Selectivity isotherms can be used to predict empirically the permeability for any ion in a given individual situation at a given pH, once the permeability of any other ion under the same conditions has been measured. In all such cases, the difference between the free energy of ion ($\Delta F_{ion/site}$) and the free energy of hydration ($\Delta F_{hydration}$) is the equilibrium free energy change ($\Delta F_{transfer}$) to transfer the ion from water to the carrier, to the ion exchanger or membrane.

$$\Delta F_{transfer} = \Delta F_{ion/site} - \Delta F_{hydration}$$

Equilibrium constant depends on the difference between ion/site forces and hydration energies, thus, permeability depends both on equilibrium and non-equilibrium factors. The selectivity sequences could be related to differences among cations in values $\Delta F_{transfer}$, the free energy change in transferring the cation from water to the membrane sites that control permeation. For relatively nondeformable, nonpolarizable ions (alkali ions) and sites, $\Delta F_{ion/site}$ is likely to be dominated by the electrostatic attractive forces implicit in Coulomb's law (Blaustein and Goldman, 1968).

Tyree et al. (1990b) proposed charge pore model in which pore walls bear negative charges and the electric double layer near these charges attracts cations and excludes anions. They measured diffusion potential of alkali cations across leaf cuticles and E_{io} (electric potential from inside to outside of cuticles) was always positive, indicating that the permeability of K^+ was always greater than that of Cl^-. The magnitude of E_{io} in cuticles and artificial membranes also was dependent on ionic strength, decreasing as ionic strength increased. These observations are explained by combining classical transport equations with equations that describe the equilibrium ion distribution between ionic double layers in the cuticle or membranes and the bathing solutions. Most people assume that the driving force on ion movement across

cuticular membranes is determined only by the concentration difference. Tyree et al. have believed that both electrical and concentration factors determine the force on ion migration across membrane.

Govach et al. (1985) investigated selective ion transfer of NaCl and KCl in the carboxylic ion exchange membrane, and observed the amount of observed KCl was bigger than NaCl. They proposed that the parameter determining co-ion exclusion in these membranes remains the fixed charge density and this depends here on the degree of protonation of the carboxylic groups, and it is interesting and useful, to know what pH range corresponds exactly to the optimum co-ion exclusion.

The diffusion coefficients of all counter ions increased as the external concentration was increased. This suggests that the diffusion coefficients and permeability of alkali cations in membranes decrease also with increasing Stokes radius of the cation of the salts. From the cation permeability data it can be concluded that in isolated cuticular membranes the strength of association between -COOH groups and cations decreases in the order $Li^+ > Na^+ > K^+ > Cs^+$. Schonherr (1976b) reported that at pH values above 2, the carboxyl groups in the membrane will begin to dissociate and no more water will enter the membrane because of;

a) The higher dipole moment of dissociated carboxyl group compared to the non-dissociated one,

b) The additional water making up the hydration shell of the counter ion,

c) Electrostatic repulsion of the neighbouring charges of equal sign,

d) The osmotic pressure difference between the external solution and interstitial fluid.

McHardy et al (1969) found the diffusion coefficients order Cs> Na> Sr. In every case it can be seen that the diffusion coefficients increased with increasing concentration over the whole range. The counterions are attracted by the fixed charges and as a result experience considerable frictional interaction with polymer matrix.

The membrane model generally adopted was a porous membrane with the pore size depending mainly upon swelling in the presence of water. Schonherr (1978) proposed transport models, which are porous membrane and solubility membrane models. Polymer matrix behaves as a porous membrane, the soluble cuticular lipids as solubility membranes. Depending on the properties of solute molecules, cuticular membranes behave either as a porous, as solubility membrane or as a mixture of both (Schonherr, 1978). According to these models; firstly, if membrane is traversed by continuous solute filled polar pores, transfer of solvent and solute takes place via these pores. The mechanism of transport depends on the pore size and type of driving force. Secondly, if membrane does not contain solute filled pores penetration of this membrane takes place by dissolution and random motion of molecules in the membrane matrix and there is no continuity across the membrane.

Cutin matrix contains polar pores and ion transport caused by a chemical potential gradient is both by diffusion and by viscous flow. The porous nature of the membranes confirm the fact that they are permselective according to size of the permeating molecule. The polymer matrix of plant cuticles is a polyelectrolyte carrying both positive and negative charges fixed to the membrane matrix. Positive charges originate from amino acid residues of proteins embedded in the matrix, negative charges from -COOH groups of cutin, polyuronic acids and protein embedded in the cutin (Schonherr, 1976a, 1978).

It was concluded from earlier works that the pH effect on permeability was due to the dissociation of -COOH groups fixed to the membrane matrix. Schonherr (1976b) noted that these groups are not randomly distributed throughout the cuticular membranes, but rather that groups of similar acid strength are clustered together. These clusters must be continuous from one side of the membrane to the other in order to develop into continuous pores when the -COOH groups dissociate. At low pH values only those pores lined with relatively strongly acidic groups conduct water

and small solutes. With increasing pH value new pores lined with -COOH groups of lower acid strength come into being.

CHAPTER 5

Transport of K^+ Ion and Selective Transport of Alkali Cations

5.1 Introduction

The transport of metal ions against their concentration gradient has been studied [Teorell, 1935, 1937, 1953; Shimadzu et al., 1981a, 1981b; Uragami et al., 1983a, 1983b, 1987; Nonaka et al., 1987). Transport of anions, (Ogata et al., 1980) and organic cations (Uragami et al., 1982) against their concentration gradient through synthetic polymeric membranes with a fixed specific carrier has been also carried out. Transport of alkali metal ions against their concentration gradient through the cation exchange membranes containing carboxyl groups has been investigated (Uragami et al., 1982 and 1983b).

Active transport may be defined as flow dependent on the activity or energy change of another system (Conway, (1954), and criteria of active transport have been developed with the aid of which the coupling between transport and metabolism could be demonstrated (Ussing & Zerahn, 1951; Zerahn, 1956). Early investigators tried to relate transport of solutes to specific metabolic processes. From time to time various workers have put forward a number of criteria for active transport. Bowling (1976) concluded that accumulation against a concentration gradient is satisfactory only for uptake of non-electrolytes. For electrolytes where there is an electrical potential gradient it is possible to have accumulation of an ion against the concentration gradient without active transport.

The first definition of active transport was put forward in 1949 by Ussing. He defined active transport as a process by which an ion is moved against an electrochemical potential gradient. An ion will move spontaneously down a gradient of electrochemical potential and movement against such a gradient is dependent on a decrease in free energy of some metabolic process. Another definition of active

transport in terms of irreversible thermodynamics was proposed by Kedem (1961) who defined it as an entrainment between a transport flux and a metabolic reaction, or in irreversible thermodynamics it is characterised by a non-zero coupling coefficient between the flow and the metabolic reaction.

In recent years, liquid membranes have been used widely in studies of ion transport against concentration gradient. Baker et al. (1977) analysed the coupled ion transport of M^+-H^+ in a liquid membrane system. They assumed stationary-state bulk phase concentrations, and their experimental results for the system of Cu^{2+}-H^+ are in fair agreement with their prediction. Higa et al., (1988) studied ion concentration changes in a multi-ionic system across a strong and weak-acid membrane,simulated as a function of time using Donnan equilibrium and the Nernst-Planck equation.

The active and selective transport of ions in biomembranes play important roles for the function of life. The transport of specific ions is a common function of a biomembrane and has been developed for synthetic membranes recently. It was observed in the active transport of ions through the cell wall, the proton transport in oxidative phosphorylation, and the selective transport of K^+ and Na^+ through the protoplasmic membrane. To develop cuticular membranes that can transport ions against a concentration gradient is an important objective in pesticide and environmental chemistry, that does not need to involve active transport.

It is the aim of this study to investigate the transport of ions against their concentration difference between two solutions across both PM and PFCM separating these solutions under the simultaneous influence of a concentration gradient and a potential gradient within the membrane. The objective was to demonstrate that the ions can be transported against their concentration difference between the outside solutions, but move in the direction of their membrane potential difference.

The first quantitative experimental studies of an ion transport against a concentration gradient across microporous membranes was carried out by Teorell

(1935). Again, Teorell used cation exchange membranes with vanishing small mechanical permeabilities (1953). Neihof and Sollner (1957) studied transitional accumulations of electrolytes in one of the two outside solutions of ion exchange membranes. These investigations were directed toward the contribution of convection to the ion transport in non isobaric systems. In connection with the studies of the influence of different driving forces and different pH gradient on the transport of K^+ ion across PM and PFCM in isothermal-isobaric systems, the results were compared with dialysis tube membranes.

5.2 Description of Transport Mechanism:

An isothermal-isobaric system is considered in which two electrolyte solutions are separated by a weak acid cation exchange membrane. The interest here was in the transport of potassium ion against its concentration difference in the outside solutions. The values chosen were 1.0 mM KCl, pH 3-8 on one side and the other side 0.5 mM KOH. It was intended that K^+ transport would be driven by the membrane potential created by the pH difference.

Ion exchangers usually exhibit a preference for the counterion species with the smaller volume in the hydrated state. As a consequence of this distribution of K^+ and other ions at the membrane and both solution interfaces, concentration gradients for ion species are formed inside the membrane and an interdiffusion of these ions takes place. The concentration gradient of the potassium ions is directed from left to right (Fig. 5.7).

Teorell (1935 and 1953) employed a steady supply of HCl to one side, and discussed ion transport in terms of the steady state of the diffusion system. Woermann (1968) investigated the transport of ions against their concentration gradient across cation-exchange membranes with very small mechanical permeabilities, and driving forces of the ion transport are a concentration gradient and membrane potential gradient within the membrane separating these solutions, and passive transport is not

influenced by chemical reaction. He considers an isothermal-isobaric system and described a transport mechanism of ions transport against a concentration gradient. The electrolyte concentrations in the outer compartments, and the concentration, potential, and pressure profiles play a role, and at least at phase boundaries (membrane-solution), there exist jumps of concentrations of the mobile ions, jumps of the electrical potential, and jumps of the hydrostatic pressure caused by the electric charged groups of the membrane matrix. He also discussed his findings in terms of the equation of ion transport across membranes with narrow pores. Schwahn and Woermann (1986) investigated the transport of mono- and bivalent cations across cation exchange membranes. In their system also, a steady supply of HCl to one side of the cell was used. They assumed that the volume flow of HCl was negligibly small, and that the molar flux of ion species to be transported against their concentration gradient across the membrane vanished in the stationary phase. They measured the membrane potential of the system and calculated the ion concentration ratio between the two sides at equilibrium state.

Mass transfer through synthetic membranes is explainable by the difference of pressure, concentration, and potential, etc. across the membrane. Many complex phenomena which cannot be understood by physicochemical mechanisms are found in biomembranes (Uragami et al., 1983a, 1983b; Shimadzu et al., 1981a, 1981b). The phenomena involved are the active transport of ions against the osmotic pressure, concentration, and potential gradients and the selective transport of K^+ and Na^+ ions in the cell membrane (Uragami et al., 1982, 1983a). Uragami et al. (1983a, 1983b) studied selective and what they called "active" transport of metal ions and anions through ion exchange membranes. They reported that it was possible to transport "actively" and selectively metal ions through the polymer membranes with a fixed specific functional group fixed to the membrane. The function of specific carriers such

as lactone rings or carboxyl groups, were shown by reversible and rapid changes of chemical structure of their carriers with pH change.

5.2.1 The Equations of Flow

It is considered to be a system in which two electrolyte solutions are separated by a membrane. The solvent and a number of ions or solutes can pass from one solution into the other and a chemical reaction takes place in the membrane. It has been found that the flow of substances in a direct and linear manner depends not only on the conjugated force as current flow is conjugated, but also non-conjugate forces as well as temperature. To cover all possibilities, the flows J_1 and J_2 of two substances, 1 and 2, whose conjugate forces are X_1 and X_2 are considered

$$J_1 = L_{11} X_1 + L_{12} X_2 \qquad (5.1)$$

$$J_2 = L_{21} X_2 + L_{22} X_2 \qquad (5.2)$$

where L_{11}, L_{12}, L_{21} and L_{22} are phenomenological coefficients and in general are non-zero. For n substances;

$$J_i = \sum_{k=1}^{n} L_{ik} X_k \qquad (5.3)$$

Independent coefficients are reduced by Onsager's law, applicable under some conditions which states:

$$L_{ik} = L_{ki} \qquad (5.4)$$

Equations (5.1) and (5.2) can be transformed into,

$$X_1 = R_{11} J_1 + R_{12} J_2 \qquad (5.5)$$

$$X_2 = R_{21} J_1 + R_{22} J_2 \qquad (5.6)$$

or in the other case,

$$X_i = \sum_{k=1}^{n} R_{ik} J_k \qquad (5.7)$$

Ussing and Teorell developed independently in 1949 an expression which can be used in non equilibrium conditions provided the fluxes of the ions across the membrane are known. This equation relates the fluxes to the chemical activities of an ion as follows;

$$\frac{\phi_j^{oi}}{\phi_j^{io}} = \frac{C_j^{o}}{C_j^{i}\exp\left(z_j \frac{FE_M}{RT}\right)} = \frac{\bar{\mu}_j^{o}}{\bar{\mu}_j^{i}} \qquad (5.8)$$

ϕ_j^{oi} = The fluxes of the ion j from the outside to the inside of the membrane.

ϕ_j^{io} = The fluxes of the ion j from the inside to the outside of the membrane.

5.3 Materials and Methods

5.3.1 The transport of K^+ ion across the membranes

The isolated cuticular membranes were converted to the H^+ form by treatment with 1 M HCl, followed by rinsing with deionized water and air drying at room temperature. The apparatus used in the study of penetration of materials through isolated cuticular membranes consisted of two borosilicate half cells as depicted in Fig. 4.1. One half-cell contained 0.5 mM KOH. The other half-cell contained 1 mM KCl at pH 3-8, see Fig. 5.1.

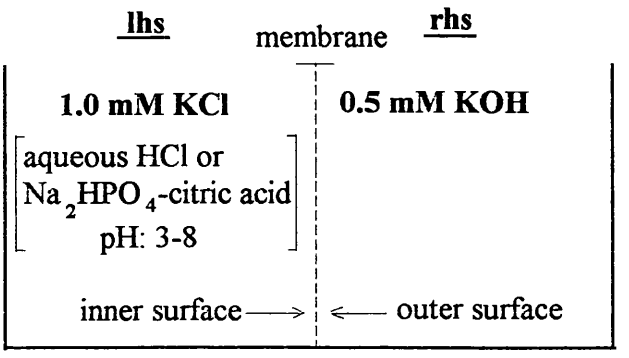


Fig. 5.1 Transport arrangement.

The isolated membrane samples were mounted between the silicon rubber gaskets and clamped between the two half cells in which the outer surface of the membrane was the alkaline side and the inner surface of the membrane the acidic side (pH range in this side was 3.0-8.0). The appropriate solutions were added into each compartment, and the unit was submerged in a water bath maintained at 25±1 °C.

The driving force for the transport of the K^+ ion is generated by a concentration difference and pH difference between the two solutions as the electrolytes were chosen to obtain different pH ranges. (pH ranges 3.0 aqueous HCl, 4.0-8.0 Na_2HPO_4-citric acid buffer or only 1 mM KCl solution) During the experiment the outer side solutions and the concentration of the KOH were kept constant, in order to avoid a high pH value.

At zero time and at time intervals a 1 ml aliquot was withdrawn from both side of the membrane solutions. One ml of "cold" collection solution was added to both sides immediately after withdrawing the sample, to maintain a constant volume in the solutions. The concentration of K^+ ions was determined by Perkin-Elmer 1100B Atomic Absorption Spectrophotometer. The effective membrane area in the cell was 1.33 and 2.05 cm^2 for PM and PFCM, and was 0.78 cm^2 for dialysing membranes.

This system was used in an attempt to determine the transport of K^+ ions against its concentration gradient through the same piece of cuticle. Determinations were repeated more than two times for each set of experimental conditions.

5.3.2 Selective transport of alkali cations

Selective transport experiments were carried out at 25±1 °C under magnetic stirring, using the same diaphragm type cells. The donor solution was salt chloride solutions such as KCl-NaCl, and KCl-LiCl binary systems and receiver solutions were the buffer solutions applied at different pH ranges. (pH ranges; pH 3 aqueous hydrogen chloride, pH 4-8 Na_2HPO_4-Citric acid and KH_2PO_4-KOH buffers.

Selective transport of alkali cations was measured from the inner surface to the outer surface of the membrane. The initial concentration of alkali cations in the inner surface of the membrane was kept constant at 0.05 M and that of receiver solutions in the outer surface of the membrane were applied at different pH ranges and various buffers. Selective transport experiment tests were made with the 0.05 M chloride solutions.

5.4 Results

5.4.1 The transport of K^+ ion across the membranes

The transport of K^+ ion through the isolated PM, PFCM and dialysis membrane with different pH gradient and various buffer contents was carried out using the system having 1.0 mM KCl-aqueous HCl or various buffers, at the inner surface of the cuticular membrane and 0.5 mM KOH at the outer surface of the membrane samples.

The transport of K^+ ion through the membranes with time-transport curves is shown in Figs. 5.2-5.4. In all systems, the concentration of K^+ ion in the outer side was kept constant in order to avoid a high pH value to avoid damaging the structure and ester hydrolysis of isolated PM and PFCM.

Effect of the initial concentration in the inner surface of the cuticular membrane side on mean transport rate (mM/L.min) and transport fraction (%) of K^+ ion, can be defined (Uragami et al., 1983) as equations (5.9) and (5.10);

$$\text{Mean transport rate} = \frac{[K^+]_{max} - [K^+]_0}{t_{max}} \qquad (5.9)$$

$$\text{Transport fraction} = \frac{[K^+]_{max} - [K^+]_0}{[K^+]_0} \qquad (5.10)$$

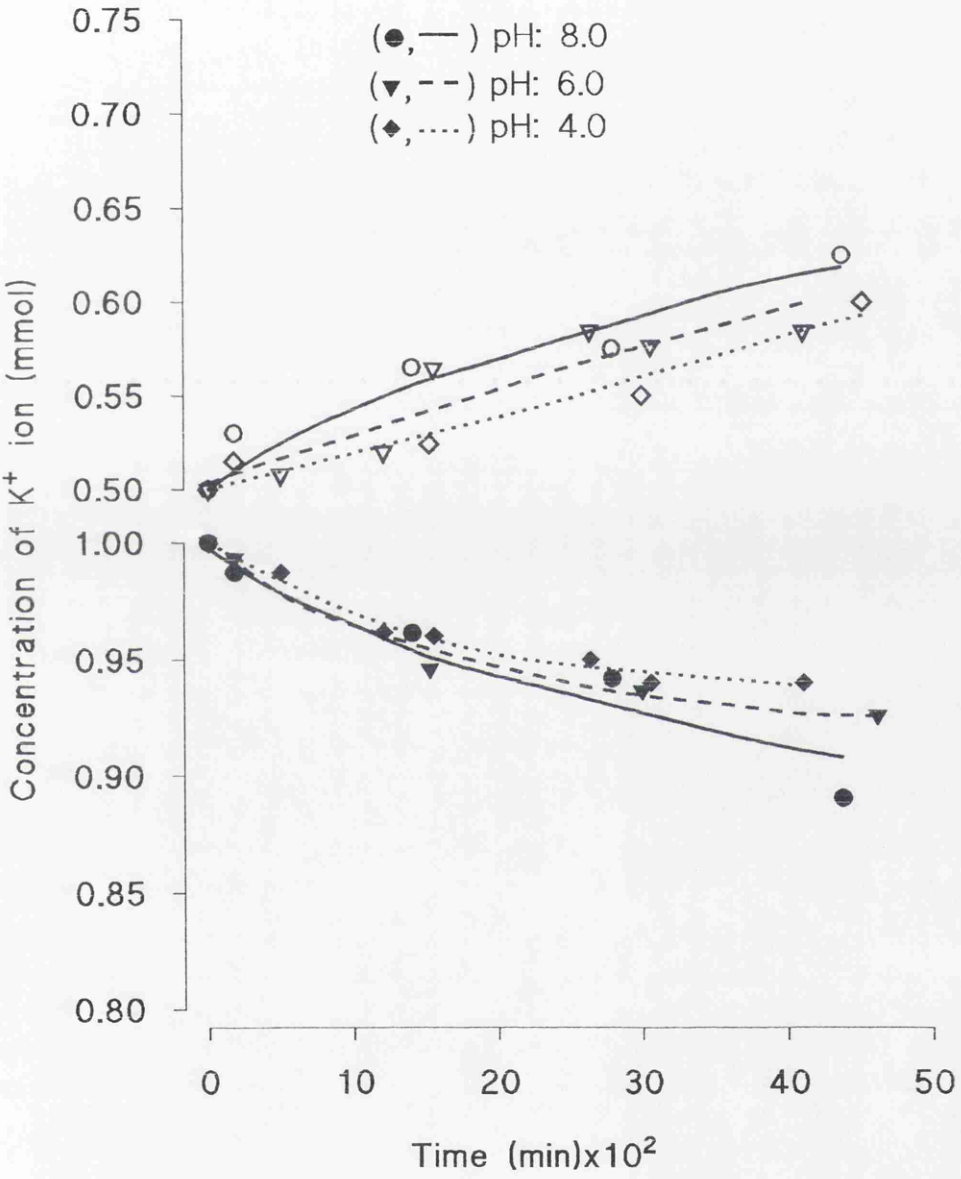


Fig. 5.2 Changes of K^+ ion concentration in both sides with time through PM (Solid forms, lhs; open froms, rhs).

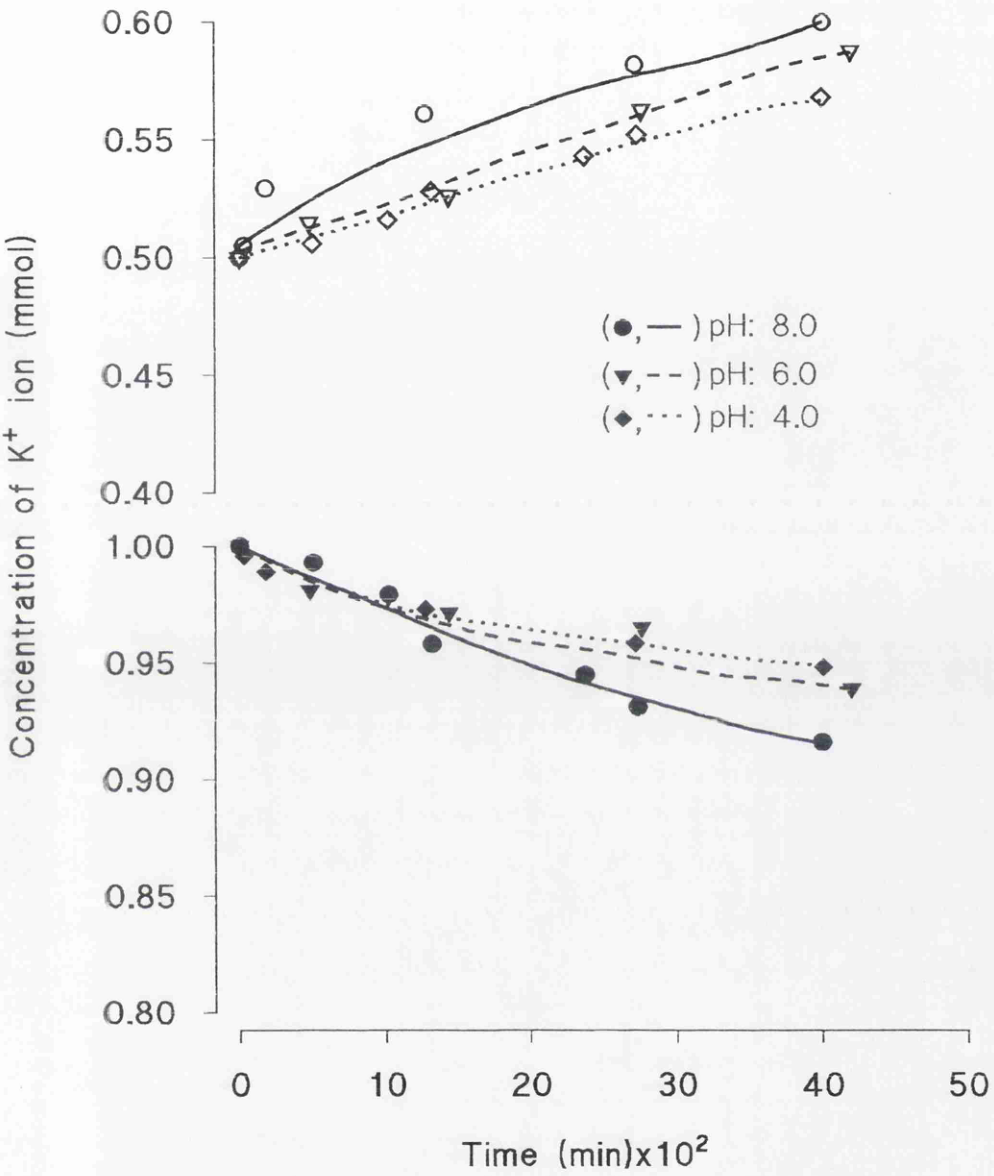


Fig. 5.3 Changes of K^+ ion concentration in both sides with time through PFCM (solid forms, lhs; open forms, rhs).

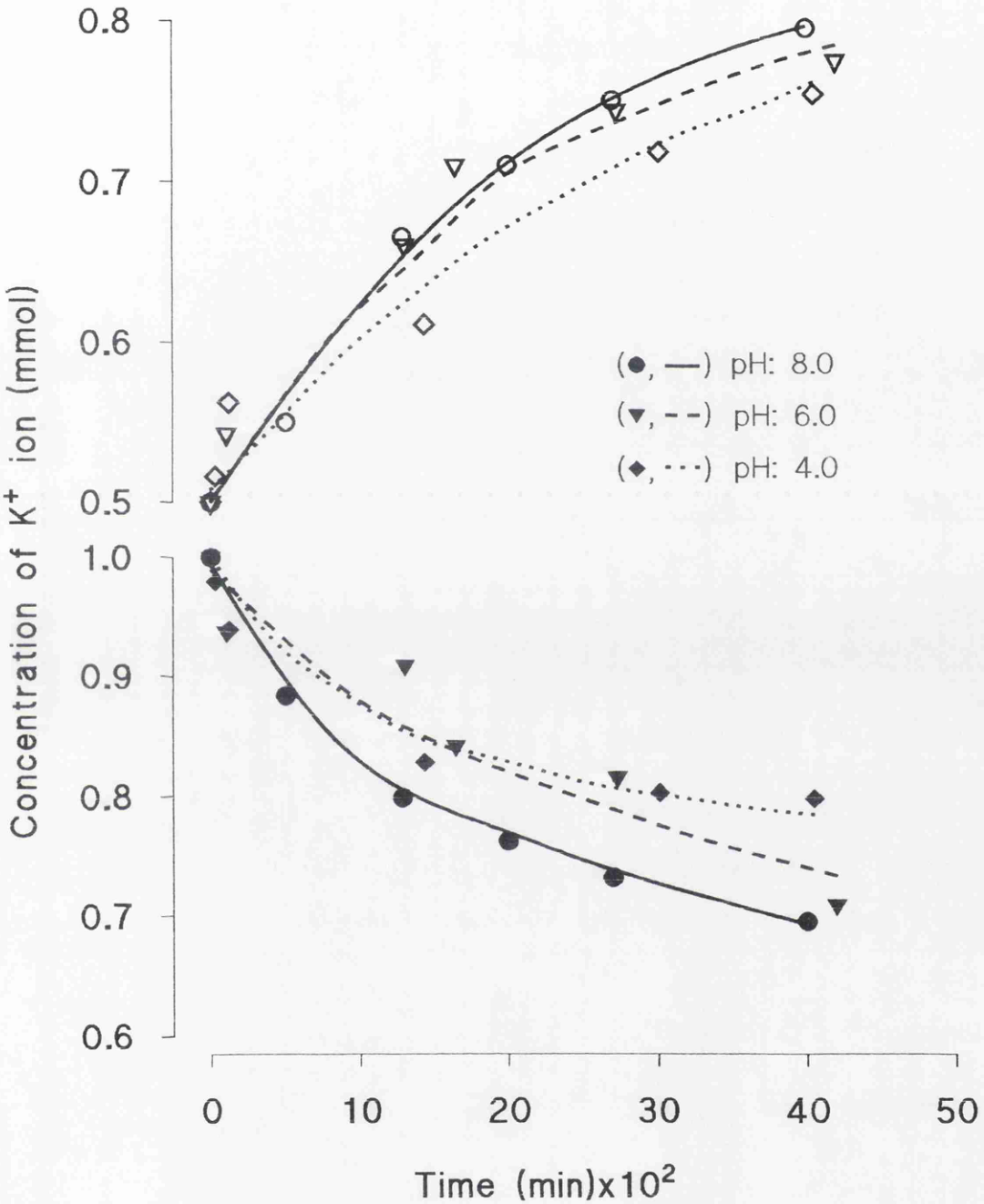


Fig. 5.4 Changes of K^+ ion concentration in both sides with time through DM (solid forms, lhs; open forms, rhs).

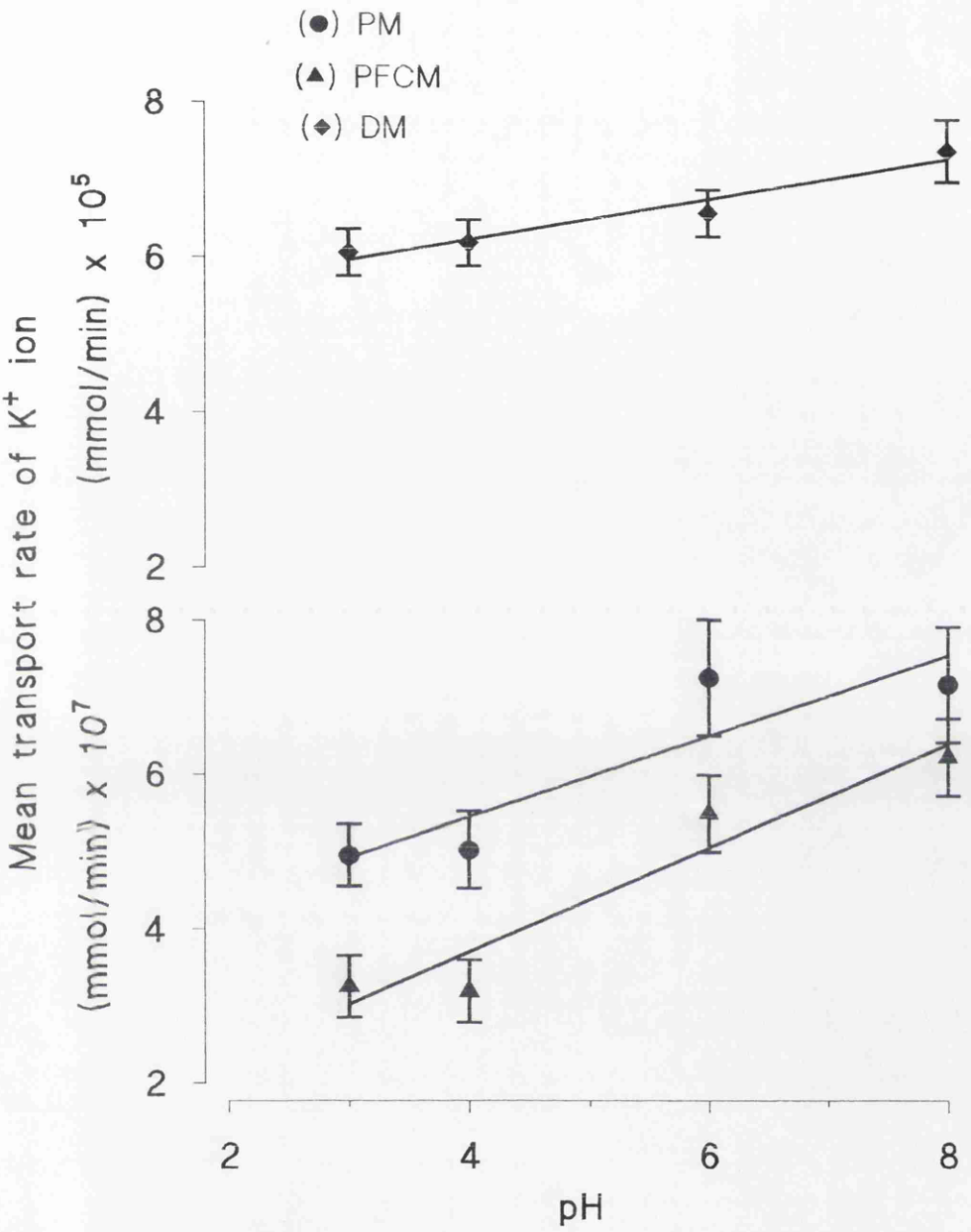


Fig. 5.5 Effect of the initial pH in the lhs on the mean transport rate of K^+ ion.

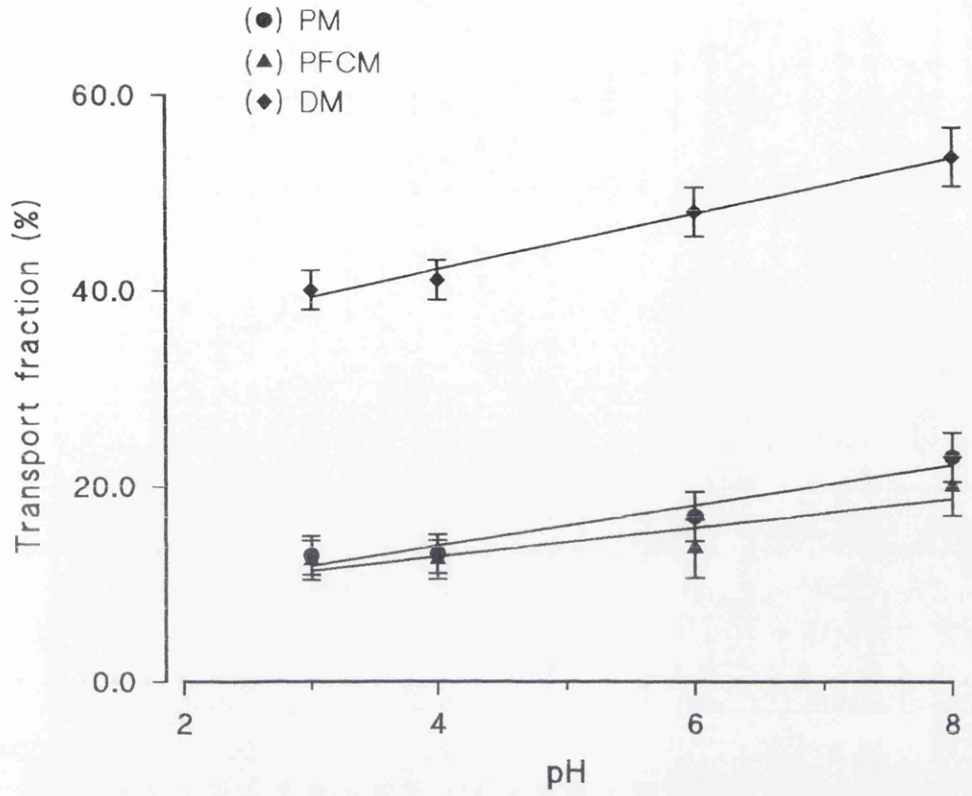


Fig. 5.6 Effect of the initial pH in the rhs on the transport fraction of K^+ ion.

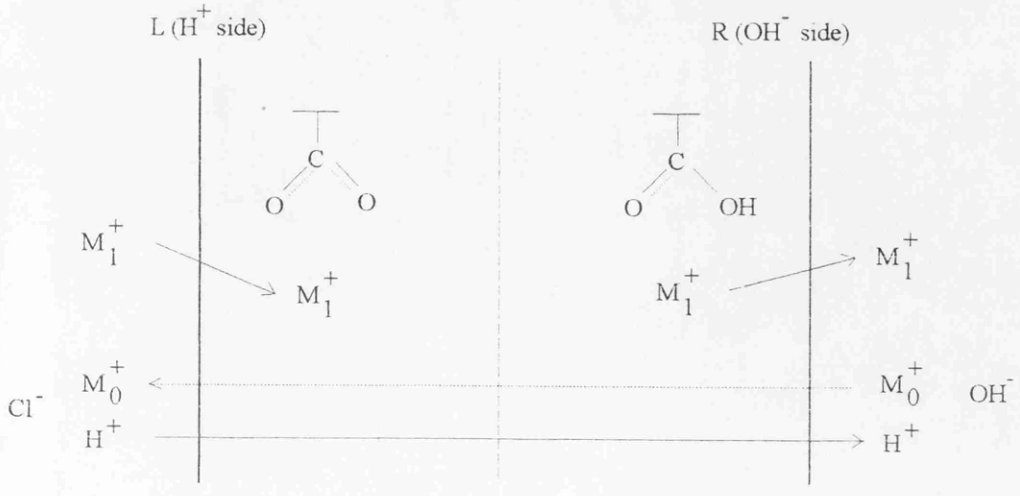


Fig. 5.7 Tentative mechanism of transport of K^+ ion through the membranes Having the carboxyl group.

where $[K^+]_{max}$ is the concentration in the outer surface of the PM and PFCM at time t maximum and $[K^+]_0$ is the initial concentration in the inner surface of the membrane samples. In these experiments, the $[K^+]_{max}$ value has not been reached, therefore $[K^+]_t$ at time t for transport rate of K^+ ion against its concentration gradient through isolated PM and PFCM was used. The mean transport rate and transport fraction of K^+ ion from the inner surface to the outer surface of the membrane during the transport of K^+ ions, against the pH are given in Figs. 5.5 and 5.6, respectively.

5.4.2 Selective transport of alkali cations

The initial concentrations of KCl-NaCl and KCl-LiCl in the inner surface membrane were kept constant at 0.05 M and that of pH and buffer solutions in the receiver phase were changed. The ratios of transported metal ions from the inner surface to the outer surface through isolated cuticular membranes were calculated from the following equation (Uragami et al., 1983);

$$\text{Selectivity} = \frac{[K^+]_{o,t} / [K^+]_{i,o}}{[Na^+]_{o,t} / [Na^+]_{i,o}} \qquad (5.11)$$

where o,t is the concentration in the outer surface of the membrane at time t and, i,o is the initial concentration in the inner surface of the membrane. The selectivity transport of K-Na and K-Li binary systems against various pH gradients through the membranes are shown in Fig. 5.8.

5.5 Discussion

5.5.1 The transport of K^+ ion across the membranes

Examples of the concentration changes of K^+ ions in both sides with time through isolated PM and PFCM and dialysis membranes are shown in Figs. 5.2-5.4. The effect of the initial pH in the outer side on the mean transport rate and transport fraction of K^+ ion are illustrated in Figs. 5.5 and 5.6. In all systems, the concentration of K^+ ion increased the outer side and decreased on the inner side with time, while pH

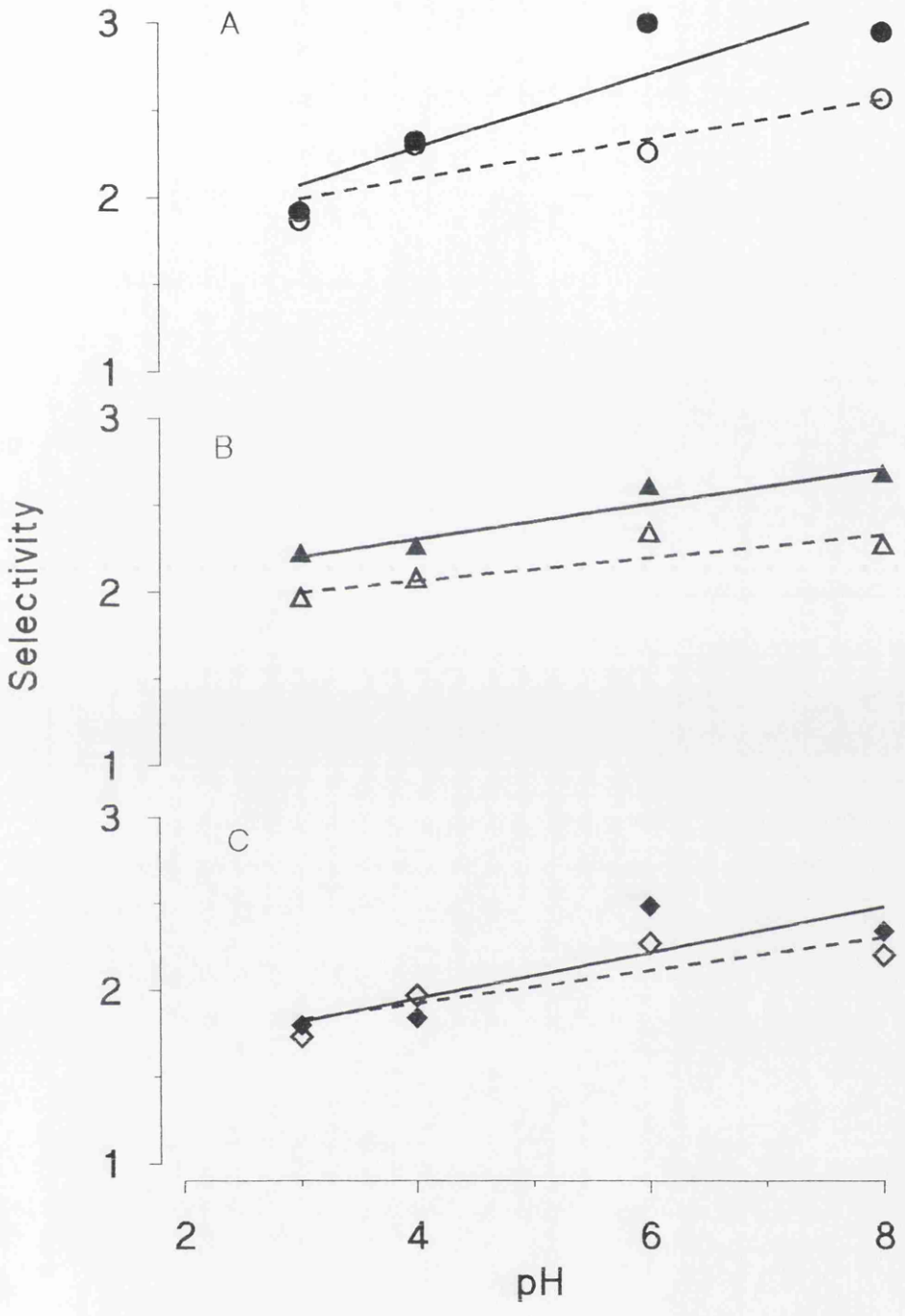


Fig. 5.8 The effect of the initial pH in the receiver phase on the selectivities of transport of alkali cations across (A) PM, (B) PFCM and (C) DM: Solid forms, K–Li; open forms K–Na binary system.

increased on the inner side. In these experiments transport of K^+ ion against its concentration gradient was not observed, as might be expected due to the nonliving character of the membranes. If a membrane potential was established by the pH gradient it was too small to cause K^+ transport, perhaps due to the ion-pair formation within the membrane resulting from co-ion exclusion.

The mean transport rate and the transport fraction had maximum values at the initial pH 8.0 in the inner side. These results are due to the fact that under these conditions the outer side remained alkaline and inner side neutral, at around pH 6-8, which corresponds to the ionisation of non-esterified -COOH groups of the suberin and cutin polymers. The transport of K^+ ions through membranes depended on a proportion of carboxyl groups in the membrane matrix, as observed in the previous chapter, and took place by diffusion with an exchange interaction between solute and charge sites in the penetration pathway.

The mean transport rate and the transport fraction of K^+ ion from the inner side to the outer side increased with an increase of pH. The increase of transport fraction had a maximum value at pH 6-8 which may be caused by the non-esterified -COOH groups.

5.5.2 Selective transport of alkali cations;

The selectivity, K^+/Na^+ and K^+/Li^+ binary systems are given in Figs. 5.8., where the initial concentrations of potassium chloride and sodium chloride or lithium chloride in the inner side were kept constant at 0.0 5 M and that of aqueous HCl and buffers in the different pH ranges, in the outer side were changed. The permeation ratios of K^+ ion to both Na^+ and Li^+ ions changed with initial pH in the outer side. In the lower initial pH, the selectivity K^+/Na^+ and K^+/Li^+ is close to unity, but it was increased with increasing pH in the outer side. In the former region both K^+ and Na^+ or Li^+ ions diffused simply without physical interaction with the membrane such as a permeation resistance in the membrane; but in the latter region a frictional resistance

between each ion and the membrane occurred because the membrane became denser, and consequently the increase of selectivity was due to the difference of hydrated size of each ion.

K^+ ion permeated preferentially toNa^+ and Li^+ ions. A decrease of selectivity in the treatments with higher initial pH in the outer side was caused by the fact that hydrated Na^+ and Li^+ ions are larger than that of K^+ ion. The relationship between the initial pH in the outer side and the selectivity of the transport of K^+/Na^+ and K^+/Li^+ ions from the inner side to the outer side, was calculated from equation 5.11. This result is attributable to the fact that the permeation of K^+ ion was not hindered by the hydrated Na^+ and Li^+ ions which were the most bulky, because the difference between the hydrated ion sizes of K^+ and Na^+ is greater than that between Na^+ and Li^+.

From the above results, the selective transportability for the alkali metal ions from the inner side to the outer side across the membrane is in the following series:

$$K^+ > Na^+ > Li^+$$

The ionic radius for these metal ions was in the order $K^+ > Na^+ > Li^+$. This order agreed with the above selective transportability but was not a measure for the selectivity of the metal ions because, in aqueous solution, the alkali metal ions were certainly hydrated. Therefore, the selective transportability had to be compared with the hydrated ionic radius for the alkali metal ions, whose order is $K^+ < Na^+ < Li^+$. It can be suggested that the selective transportability for the alkali metal ions was governed by the hydrated size of these metal ions. Also a contribution of hydrated ionic size to the selective transportability implies that these the physical factors were partly responsible for the selectivity of the metal ions.

In these systems, the metal ions were transported from the inner side to the outer side of the membrane by both diffusive flow based on the concentration gradient

and the transport caused by the pH difference between both sides. The transport of metal ions tended to decrease with the decrease in the initial pH in the outer side of the membrane. This decrease was caused by the frictional resistance between metal ions and the isolated PM and PFCM, because the isolated cuticle became denser with decrease in pH. This is due to the fact that the non-esterified -COOH groups in the suberin and cutin polymer dissociate very little at lower pH. This is caused by the higher mobility of the potassium ions within the membrane. The membrane has a distinct selectivity for potassium ions with respect to lithium ions. This is an agreement with the common rule that a cation exchange resin takes up preferably that cation species with the smaller volume in the hydrated state.

Govach et al. (1985) studied the selectivity of ion transfer in carboxylic membranes and predicted that with an apparent pK_a value of 4.1 for the carboxylic groups, the membrane behaves, as stated, as a cationic exchange substance in neutral media, but as an uncharged diaphragm at lower pH. At lower pH, where the carboxylic groups are not dissociated, the membrane acts as an uncharged diaphragm. As the pH of the aqueous solution increases the carboxylic groups become deprotonated and, in view of Donnan's exclusion of co-ion, the transport number of K^+ increases while that for Cl^- decreases and Cl^- ion does not participate to any extent in the electrical transport in such a solution. They interpreted this as the formation of ion pairs between Na^+ and Cl^- ions, therefore the membrane is permeable to the Na^+ ions but not at all to the Cl^- ions. This behaviour is not simply a result of the co-ion exclusion, but also a consequence of ion-pair formation within the membrane, which appears to have a very large association constant.

The transport and selectivity of metal ions through the isolated PM and PFCM were investigated under various conditions, and compared with dialysis membrane. The isolated PM and PFCM exhibited selective transport of metal ions. The tentative mechanism for the transport of K^+ ion was proposed and discussed in some detail. In

the selective transport of metal ions, the selectivity depended on both the hydrated ionic size and the interaction between the carboxyl groups in the membrane and the metal ions.

CHAPTER 6

Amino Acid Transport and Selectivity

6.1 Introduction

Amino acids are now manufactured in most cases by the fermentation method. Ion exchange is used for the recovery, separation and purification of amino acids from the fermentation broth. Amino acids are very important compounds because they participate in a great variety of metabolic processes; their permeation through biological membranes depends on their predominantly hydrophilic character, so that coupling with carrier systems is assumed for their transport (Ring, 1970).

The ecotoxicological importance of plant cuticles as a lipophilic sorption compartment has been pointed out by Riederr and Schonherr (1984). The need to know the effect of chemical substances in plant materials, i.e., transport, sorption, and desorption especially in cuticles was pointed out over the three decades, but the idea was not pursued, at least from the quantitative, physical and thermodynamical viewpoint. However, there is very little general information available concerning the relationship between permeabilities of periderms. Rates of uptake tend to increase with increasing lipophilicity of compounds (Kerler and Schonherr, 1988). This behaviour was predicted by transport theory (Crank, 1975) and it is generally observed with biological and synthetic membranes.

The interaction of materials with plant cuticles are described quantitatively using permeability, and diffusion coefficients. Foliar penetration, accumulation and transport of organic chemicals in plant cuticles has been studied comprehensively (Schonherr, 1976a, 1976b, 1978; Schonherr and Riederer, 1986; Riederer and Schonherr, 1984, 1985, 1986, 1988, 1989; Kerler et al., 1984, Kerler and Schonherr, 1988a, 1988b).

Transport phenomena through membranes in biological systems, as well as in many industrial processes, are important because of their potential use in various

separation processes. Charge plays also an important role in the sorption and the transport of simple electrolytes in both synthetic and biological membrane systems. In such systems, the mobility of ions is strongly affected by the fixed charge of the membranes.

In homogeneous membranes, transport and sorption are related to the permeability coefficient (p_C), the diffusion (D) and partition (K) coefficients (Crank, 1975). To date, however these coefficients have only been determined for some pesticides. In addition, the relationship between these coefficients and the physicochemical properties of chemicals is still obscure.

All nonvolatile compounds reaching the plant surface from the atmosphere must penetrate the cuticle before entering the plant (Hull, 1970). Since plant cuticles are mainly of lipid material, they are likely to sorb and accumulate lipophilic compounds (Riederer and Schonherr, 1984). Therefore, a program was initiated to quantitatively measure permeability and selectivities of amino acids on the ammonium form of periderm and cuticles. The objective is to establish an empirical relationship between periderm/cuticle and amino acids and to estimate the relative importance of selectivity coefficients and permeability of PM and PFCM. Kawalita et al., (1990) studied selectivity coefficients of amino acids for the ammonium ion on a strong cation exchange resin. They obtained a good relationship between selectivity coefficients and physicochemical parameters and expressed the selectivity coefficients with a regression equation for each amino acid as a function of the physicochemical parameter, viz partition coefficient, hydration number and partial molar volume. They pointed out that the selectivity coefficient of amino acids is extensively affected by the hydrophilic interaction, together with the molecular size.

There is information available at present on the permeation of nonelectrolytes. On the basis of permeation studies and thermodynamic arguments, Schonherr (1976b, 1979) concluded that small nonelectrolytes permeate through cuticular pores of up to

0.9 nm in diameter, the hydrated diameter of many ions is less than 0.8 nm (Tyree et al. 1990a). The permeabilities of PM and PFCM were measured using amino acids and whether or not permeability of periderm/cuticle took place and if so the selectivity coefficient of the compounds on the NH_4-form were predicted.

Selectivity coefficients of amino acids for the ammonium form of PM and PFCM were determined, using the mass action law. In this study, the transport and selectivity coefficients of some amino acids through the PM, PFCM and dialysis membranes were determined. The quantitative relationship between selectivity coefficients and the physical properties of amino acids were analyzed to develop a method for predicting the selectivity coefficients for amino acids. It was the objective of the study to provide a general description of amino acid transport of PM and PFCM in terms of permeability.

6.2 Description of selectivity

The ion exchange isotherm shows the ionic composition of the ion exchanger as a function of the experimental conditions. The ion exchange reaction between a ion exchanger and counter ions in solution is expressed as in eqn. 6.1;

$$C_{A^{+n}} + n R_{NH_4^+} \rightarrow R_{A^{+n}} + n C_{NH_4^+} \qquad (6.1)$$

The mass action law is applied to eqn. 6.1 and the selectivity coefficient, $K^A_{NH_4}$, is defined in equilibrium as follows:

$$K^A_{NH_4} = \left(\frac{C_{NH_4}}{R_{NH_4}}\right)^n \left(\frac{R_A}{C_A}\right) \qquad (6.2)$$

where R_A and R_{NH_4} represent the equivalent ionic fractions of the counter ions in the membrane phase and C_A and C_{NH_4} the corresponding equivalent fraction of these ions in the solution phase. The description of selectivity is discussed in details in Chapter 3.

As an amino acid is an amphoteric electrolyte, the cationic ratio varies, depending on the pH of the solution. The selectivity coefficients of amino acids were determined using eqn. 6.2. The relationship of the electrical neutrality for cations adsorbed on the ammonium form of a cation exchange resin in equilibrium with the solution is defined as in eqn. 6.3;

$$Q = R_{NH_4^+} + R_{A^+} + 2R_{A^{2+}} + R_{H^+} \qquad (6.3)$$

In the neutral pH, the ionic form of a basic amino acid is monovalent, and both A^{2+} and H^+ can be neglected and the following relation obtained (Kawalika et al., 1990);

$$\frac{Q}{R_{NH_4}} = 1 + K^{A}_{NH_4} \frac{C_A}{C_{NH_4}} \qquad (6.4)$$

Based on the slope of the relation of Q/R_{NH_4} with C_A/C_{NH_4}, the selectivity coefficient of A^+ for the ammonium form of the ion exchanger could be determined.

6.3 Materials and methods

6.3.1 Materials

The amino acids were analytical grade. Glycine and potassium dihydrogen phosphate were obtained, from BDH, NH_4Cl from Hopkin and Williams, DL-Methionine, D-Alanine, D-Valine, DL-Cysteine and 9-Fluorenylmethyl chloroformate from Sigma, L-Alanine, DL-Leucine from Biochemical, Sodium tetraborate from May&Baker, Acetone and Acetonitrile from Merck.

Amino acid solutions were prepared using deionized water without further purification. 35% (W/V) HCl, and ammonium chloride of analytical grade were used as reagents. Each amino acid was dissolved in deionized water to prepare a solution of about 0.1 mol/l, and then this solution was adjusted to a pH around 3.5-4 with dilute HCl, followed by final adjustment of its concentration from 0.01 to 0.05 mol/l with deionized water.

Phosphate buffer was made up on each occasion, at 0.1 M. Before making up to solution, the potassium dihydrogen phosphate being used was oven dried for at least two days so as to remove all the water. The salt was dissolved thoroughly in deionized water and then made up to the desired volume. The newly made phosphate solution was then adjusted to the appropriate pH by adding orthophosphoric acid, and recording pH with pH meter.

The 9-fluorenylmethyl chloroformate (FMOC-Cl) reagent was dissolved in acetone and had a concentration 0.01 M.

6.3.2 Methods

6.3.2.1 Transport

The ion exchange capacities of the isolated PM and PFCM were determined by measuring ammonia released. Membranes were converted to NH_4^+ form by treatment with 0.1 M NH_4Cl then followed, by washing with deionized water to remove Cl^- ions. This was followed by washing with deionized water, then the membranes were treated with 0.1 M HCl to remove adsorbed NH_4^+ ions into solution.

For transport experiments, citric acid-Na_2HPO_4 buffer in the pH ranges 6-8 and deionized water were used as the receiver solution in contact with the outer surface of the membrane. Amino acid transport experiments were made with 0.05 M solutions. The methods followed and apparatus details are given in Chapter 4.

6.3.2.2 Selectivity

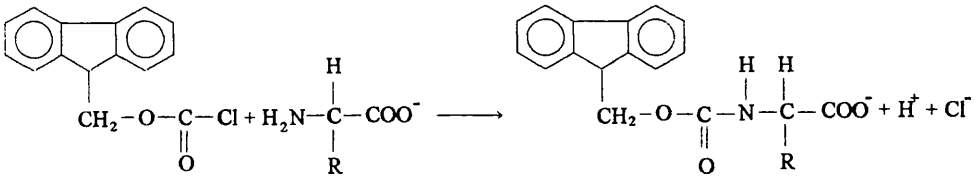
Each membrane in A^+ or NH_4^+-form was usually equilibrated with a mixed amino acid+NH_4Cl solution of total composition 0.05 M. After equilibrium (generally 24 hours) the equilibrated membrane was removed from solution. The selectivity coefficients of amino acids were measured by the batch equilibrium method at the temperature of 25±1 °C, with various ratios of amino acid cations to ammonium ions.

In all cases mass balance was confirmed. Selectivity coefficients were calculated from the results of these experiments using the equation 6.2.

6.3.2.3 Derivatisation

The majority of amino acids contain an amino functional group and the fundamental problem is the detection of the an amino group which is difficult by U.V. detector, so a fluorescence detector was used. This require derivatisation of amino acids. Pre-column derivatisation involves reacting the mixture of amino acids with an appropriate reagent before the amino acids are separated. Derivatisation of samples are carried out by the following steps;

To 0.1 ml of sample were added 0.9 ml sodium tetraborate buffer (0.025 M), 0.9 ml acetone and 0.1 ml of the FMOC-Cl reagent and left for 20 minutes. FMOC-Cl has been shown to be a suitable reagent for the determination of primary and secondary amino acids, giving products that are stable and highly fluorescent (Einarsson et al., 1983 and Einarsson, 1985). The reaction of FMOC-Cl with the amino acids proceeds as follows;



The borax buffer ensures alkaline conditions which are necessary for the derivatisation of the amino acids by FMOC-Cl to take place. Acetone makes the reagents miscible, facilitating reaction between them. After 20 min. 3 ml of diethyl ether was added and stirred, and caused 2 phases to form-derivatised and nonderivatised. This partitioned off the underivatised amino acids. This step was repeated 3 times, the top phase was carefully pipetted off avoiding turbulence. The bottom phase was the derivatised sample, excess diethyl ether was degassed by Nitrogen gas and the samples were now ready for injection.

6.3.2.4 Determination of amino acid

The determination of amino acid were carried out by high performance liquid chromatography (HPLC). The chromatograph consisted of gradient Perkin Elmer Series 400 solvent delivery pumps. The samples were introduced to the column by Perkin Elmer ISS-100 autosampler. For detection Shimadzu Fluorescence HPLC Monitor RF-530 detector was used. The emission light was monitored at 315 nm, with an excitation wavelength of 270 nm. The output was recorded by a Perkin Elmer CCI-100 Integrator. Column (25 cm x 4 mm) packed with APS-3u Hypersil (Shandon) was used for the separations.

The separation was achieved by gradient elution. Degassing of the solvents for the removal of dissolved oxygen is necessary to prevent bubble formation in the detector cell, therefore, helium gas was introduced into the solvents to prevent air dissolving. The eluent was acetonitrile-phosphate buffer (30/70, V/V). A flow rate of 1 ml/min was used throughout the separation. The amino acids were quantified by peak area measurements.

6.3.2.5 Determination of ammonium

Ammonium is determined by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hydroxides in the reagent system. Reagents; Alkaline phenol, complexing reagent (sodium nitroprusside), sodiumhypochloride, ammonium nitrogen standard stock solution. These reagents was used for the determination of ammonium.

The technicon Autoanalyzer II was used in this study for the analyses of ammonium ion. The system comprised a sampler, pump, a water bath with constant temperature and a spectrophotometer. Results from the samples were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used for the measurement of peak heights and calculation of results.

The exchanger and solution phase were analyzed using the Technicon Autoanalyzer II including standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour and the colour development was carried out in the water bath at 37 °C. The colour intensity was measured at 650 nm.

6.4 Results

6.4.1 Transport

The ion exchange capacities of PM and PFCM in the ammonium form were determined 0.381±0.043 meq g^{-1} for PM, 0.205±0.03 meq g^{-1} for PFCM, respectively. The time course of penetration of amino acids across PM, PFCM and dialysis membranes are shown in Fig. 6.1. The penetration rates of the amino acids in the PM and PFCM were observed in the order Gly> L-Ala≥ D-Ala> D-Val> DL-Leu> DL-Cys> DL-Met. This is the order of increasing molecular weights of amino acids, and the extension of the hydrophobic -CH_2- group. Steady state conditions of permeability of amino acids were found to be very variable due to each membrane showing different permeability characteristics. These experiments were studied between pH 6-8. The permeability of amino acids across PM, PFCM and Dialysis membranes as a function of molecular weight and partial volume is given Fig. 6.2.

6.4.2 Selectivity

The amino acid selectivity was studied in solutions of various amino acid/ammonia ratios. Amino acid-ammonium exchanges on the isolated PM and PFCM are characterized by the ion exchange isotherms (Fig. 6.3). Selectivity coefficient isotherms for amino acids-ammonium ion exchange of the isolated PM and PFCM at 25±1 °C are shown as a function of the equivalent fraction of amino acids in solution of A^++ NH_4^+ at 0.05 M (Fig. 6.4). The selectivity coefficient integrated over all ionic composition at ionic fraction = 0.5.

The experimental results were also assessed in terms of either the Langmuir or Freundlich adsorption isotherm. The selectivities of amino acid-ammonium exchange

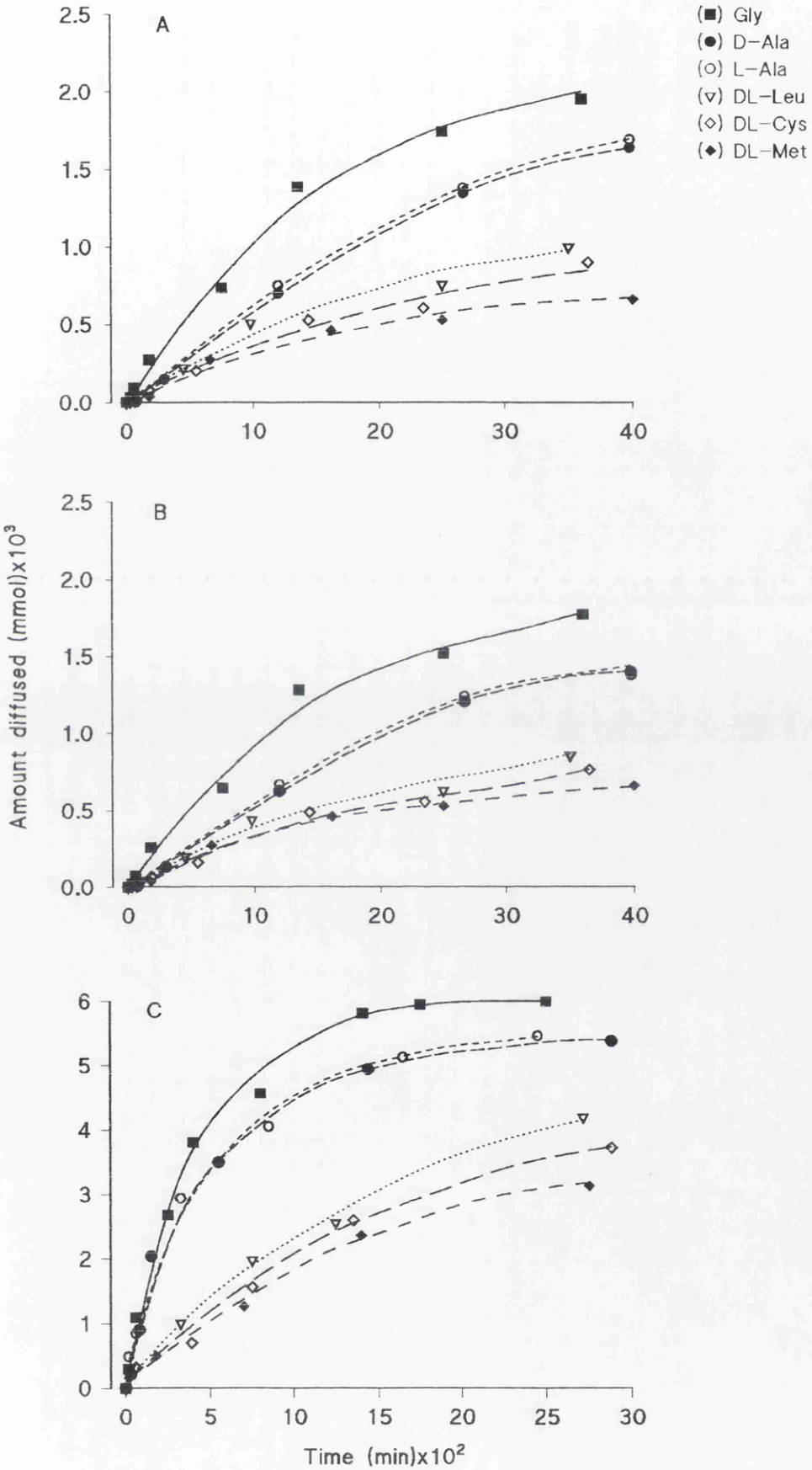


Fig. 6.1 Time course of transport of amino acids across (A) PM, (B) PFCM and (C) Dialysis membrane.

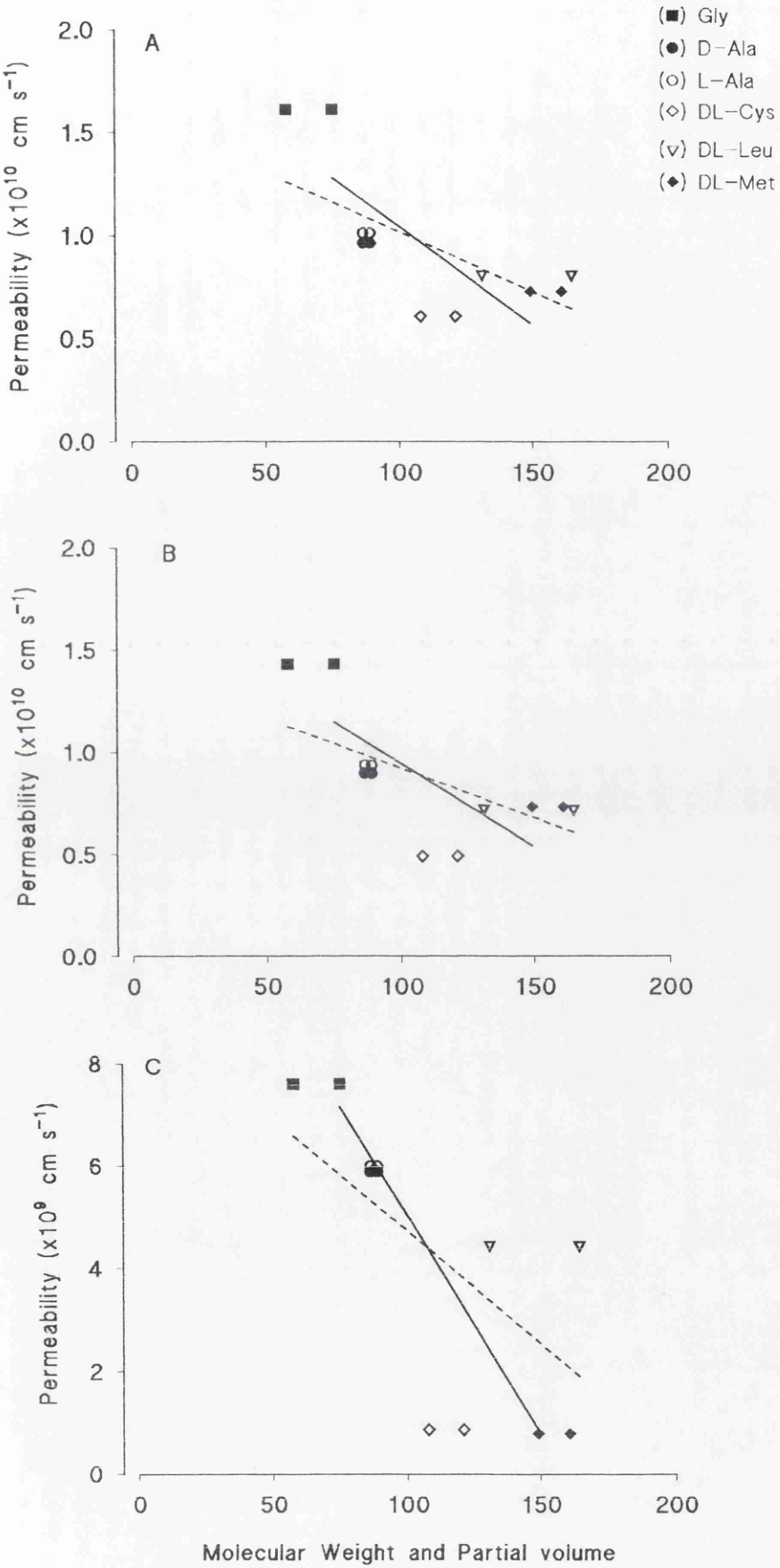


Fig 6.2 Relation between permeability, and the molecular weights (——) and partial volume (---) of amino acids, (A) PM; (B) PFCM and (C) Dialysis membrane

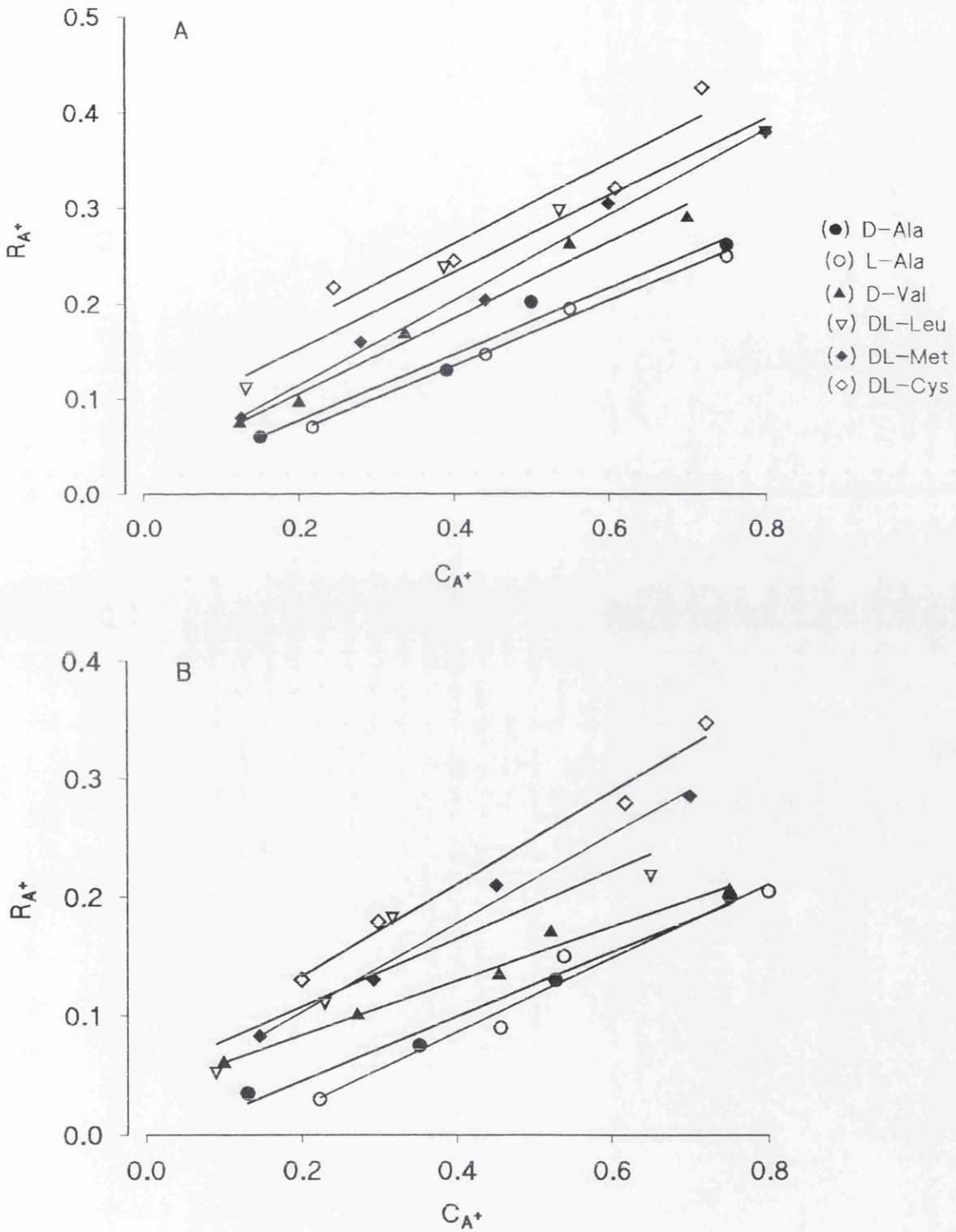


Fig. 6.3 A^+/NH_4^+ exchange isotherms for amino acids on the (A) PM and (B) PFCM, total molarity 0.05 M.

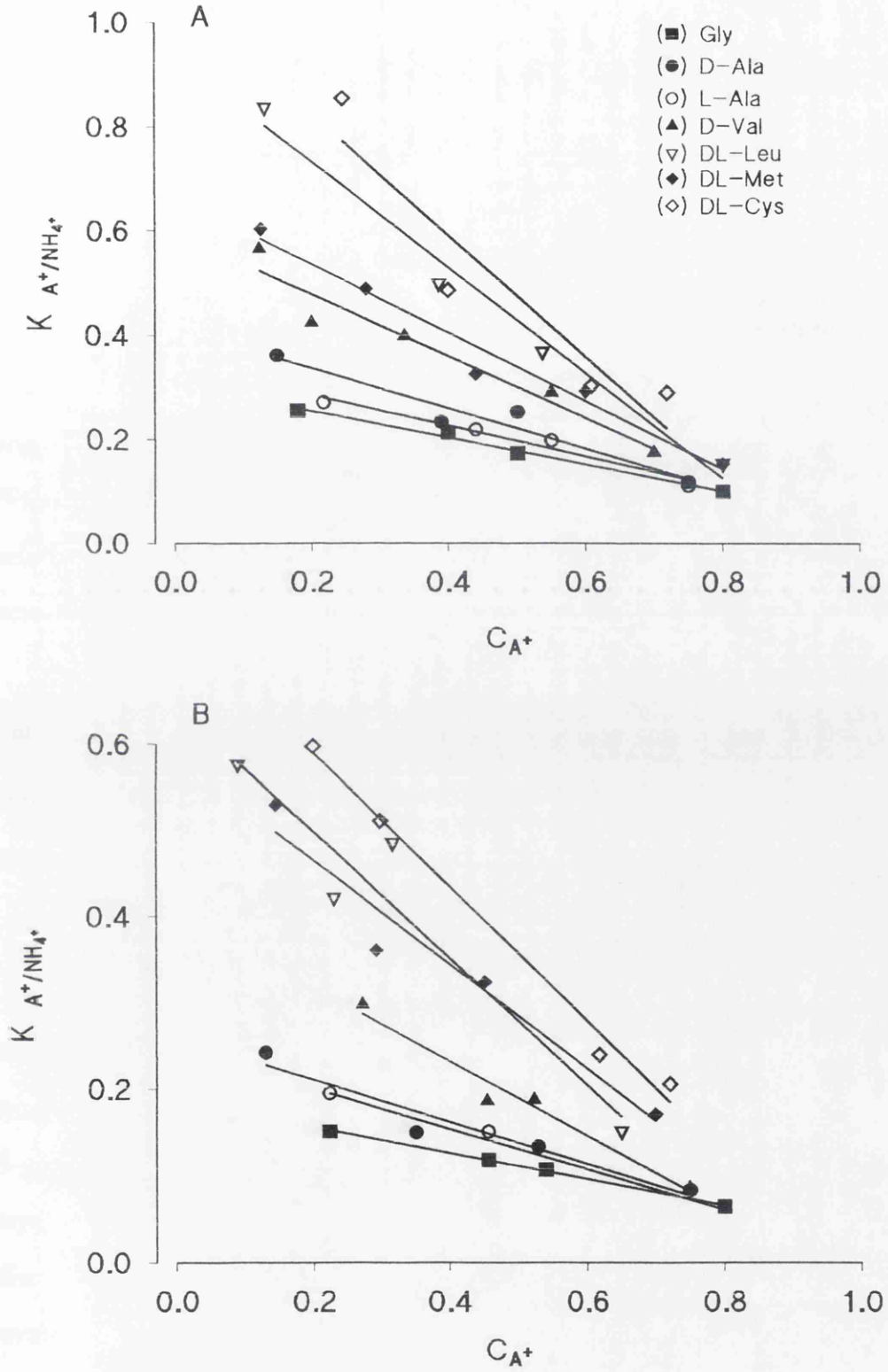


Fig. 6.4 The selectivity coefficients, for (A) PM and (B) PFCM, as a function of the equivalent fraction of amino acids in solution phase of total concentration 0.05 M.

on the isolated PM and PFCM resembled a characteristic Freundlich isotherm type, (Fig. 6.5). Sorption of amino acids in isolated PM and PFCM obeys the Freundlich isotherm equation.

In the case of amino acids, Q/R_{NH_4} was plotted against C_A/C_{NH_4} in the solution, as shown in Fig. 6.6. In order to clarify the characteristics of the interaction between the exchanger and amino acids, correlation between the selectivity coefficients of amino acids and molecular weights and partial volume of the respective amino acids were examined. The molecular weights and partial volume of the amino acids are plotted against $K^A_{NH_4}$, as shown in Fig. 6.7. These results show that the selectivity coefficient value increases with an increase in molecular size of the amino acids through extension of the hydrophobic-CH_2-group.

In Fig 6.6 shows the isotherms of ion exchange for amino acids. The ratio of ion exchange capacity, Q, to adsorbed ammonium $R_{NH_4^+}$ is plotted against the equivalent fraction of amino acids in solution. A method for measurement of amino acid transport through enzymatically isolated PM and PFCM is presented.

6.5 Discussion

The transport rate of amino acid in the PM and PFCM depend on the molecular size of amino acid and decrease with an increase in molecular size through extension of the hydrophobic-CH_2- group. The permeability also decreased through S-group and the slopes of the graphs are smaller than unity. The transport-limiting layer in the CM studied, acts both as a mobility and solubility barrier. The preceding discussion shows that the permeability of CM quantitatively cannot be explained in terms of properties of cuticles and compounds. This is due to insufficient information concerning the structural aspects of cutin, soluble lipids and the cutin/soluble lipid complex (Shafer and Schonherr, 1985). There was no direct correlation in permeability of cuticles and periderms for the amino acids studied.

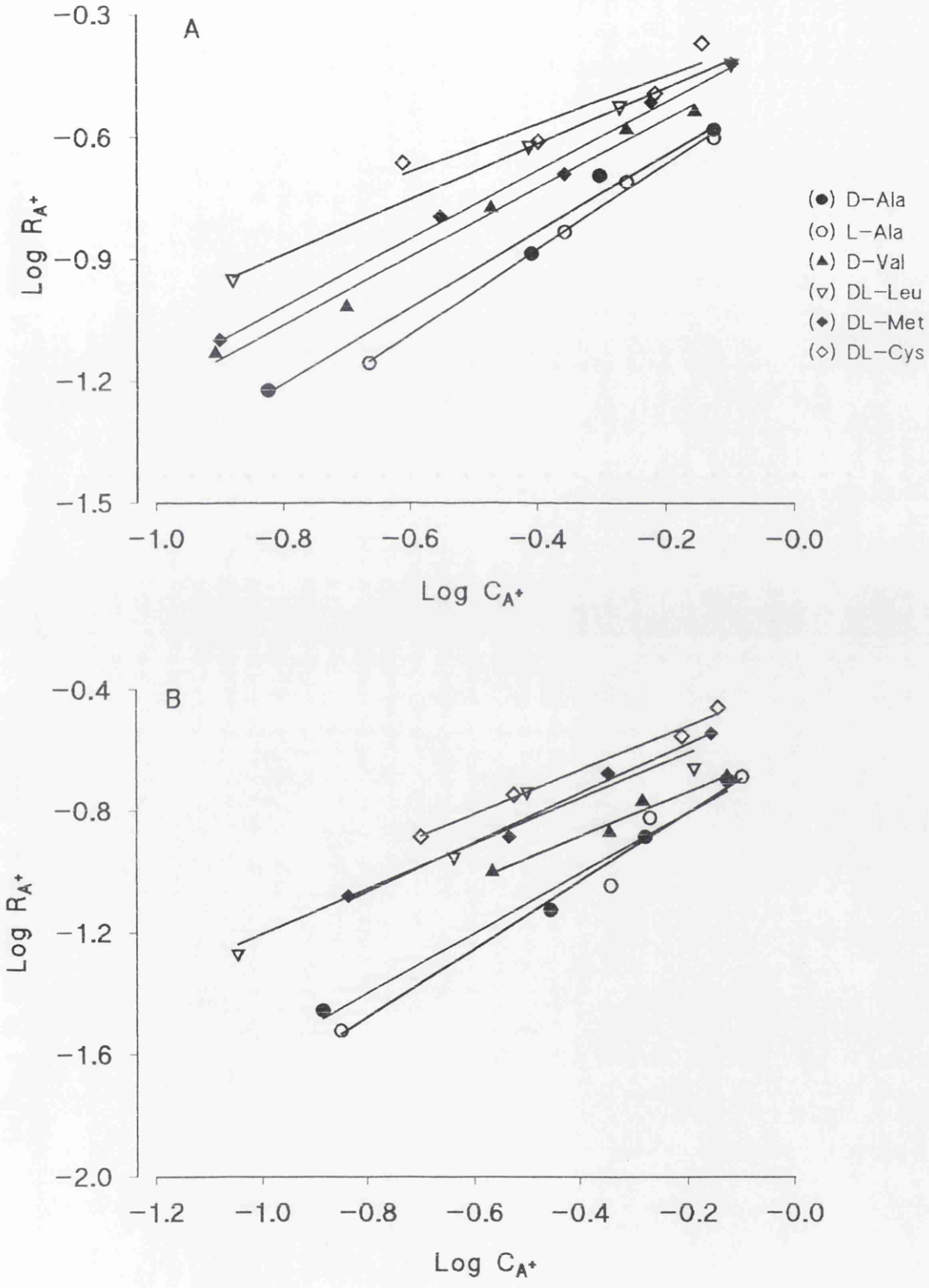


Fig. 6.5 The Freundlich exchange isotherms of amino acids on the (A) PM and (B) PFCM.

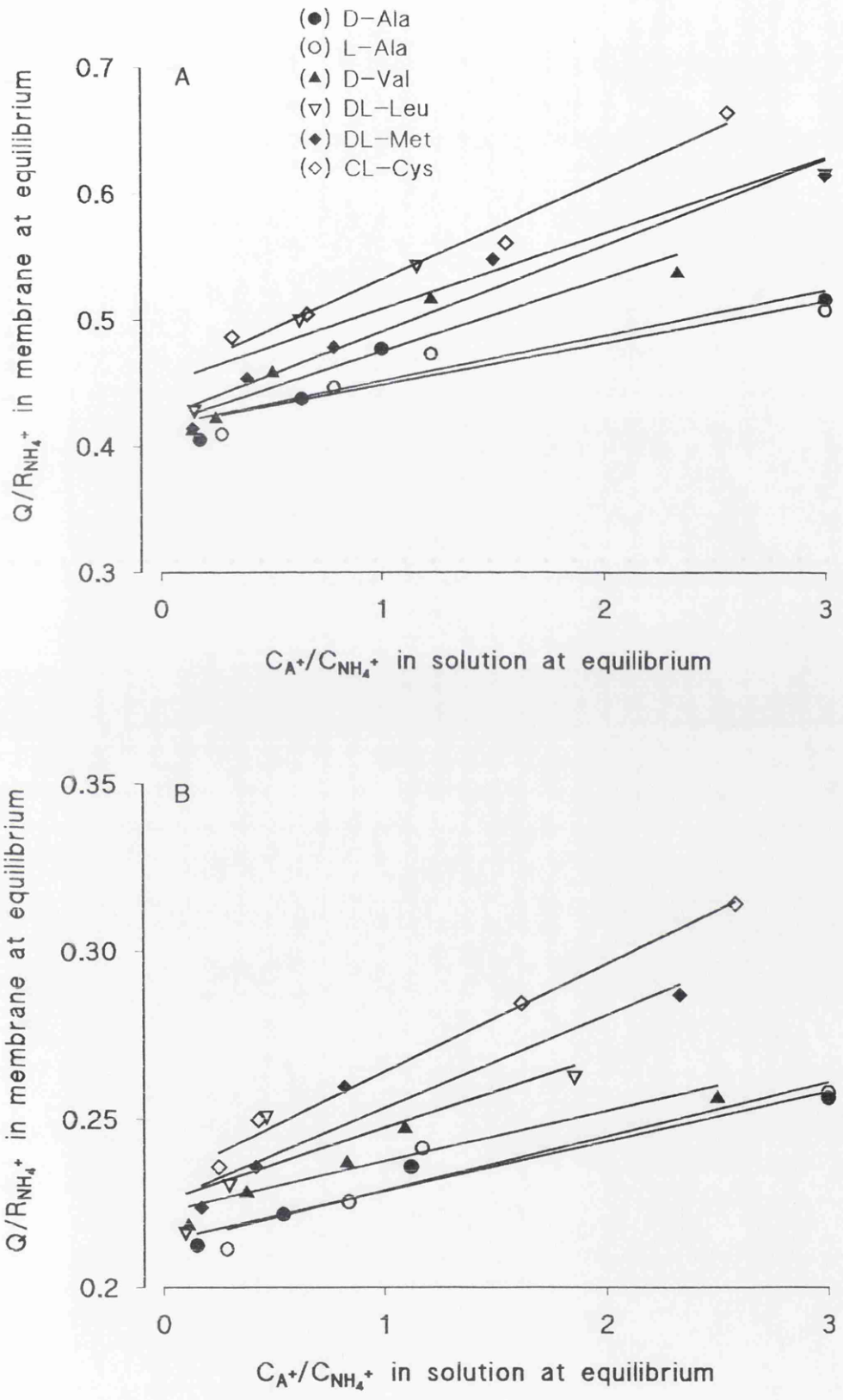


Fig. 6.6 Relationship of ion exchange capacity/$R_{NH_4^+}$ to the ratio, A^+/NH_4^+, at equilibrium in solution, (A) PM and (B) PFCM

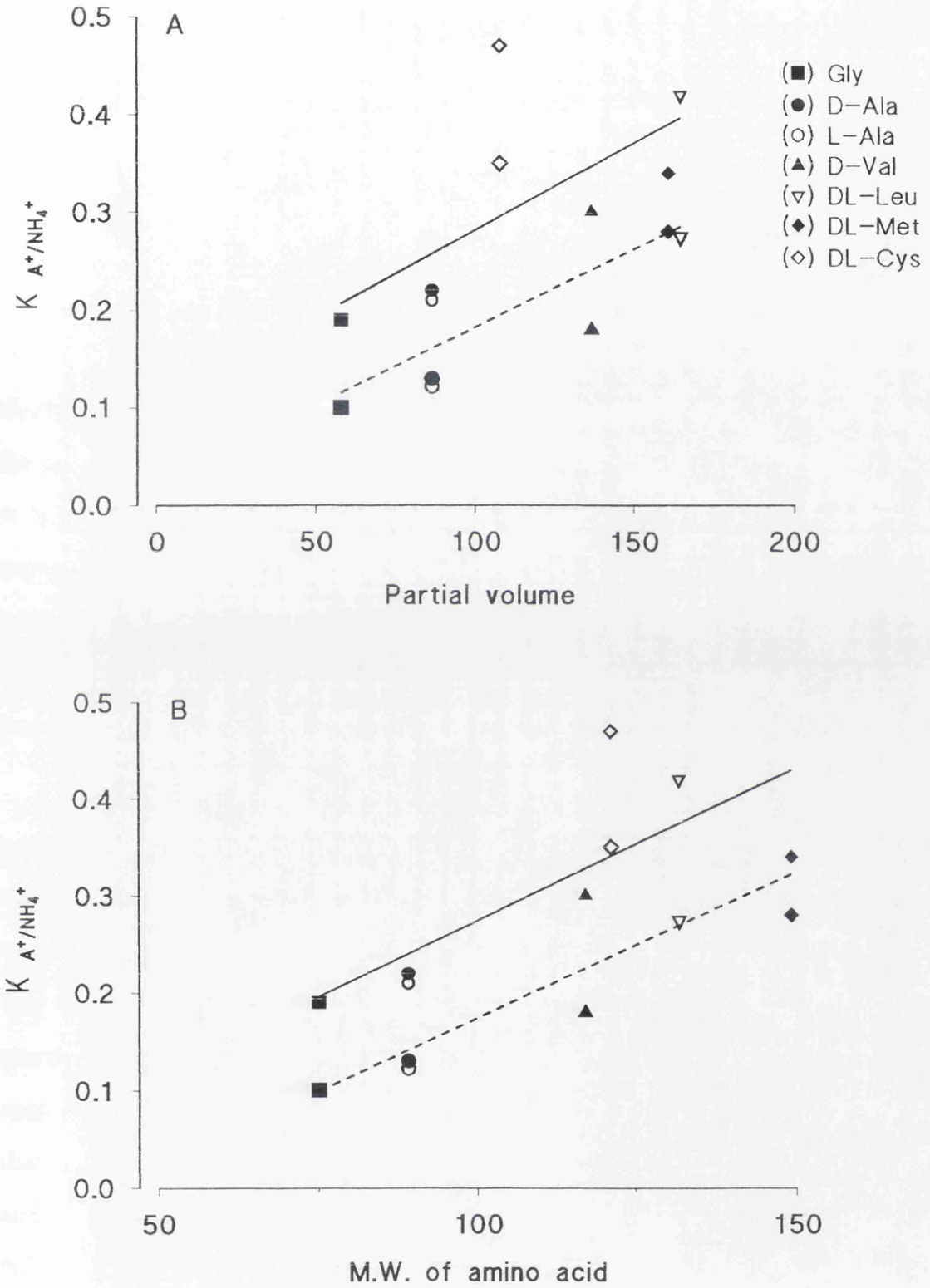


Fig. 6.7 Relation between selectivity and the partial volume (A) and molecular weights (B) of amino acids, (——) PM and (---) PFCM.

Many investigations have attempted to correlate cuticular penetration to physicochemical properties of both penetrant and cuticle (Hull, 1970). Permeability of cuticles observed so far range from about 10^{-6} to 10^{-10} m/sec and is quite large (Kerler and Schonherr, 1988; Riederer and Schonherr, 1989). Davis et al. (1979) tried to find a correlation between water permeability of cuticles and their permeabilities to organic solutes differing widely in lipid solubility. Riederer and Schonherr (1989) tried to find a relationship between water and pesticide permeability and plotted log P (ion) versus log P (pesticide) and the result showed a linear dependence. In the case of non-electrolyte permeability, a correlation has been suggested between lipid solubility of the penetrant and its penetration rate, as observed with cytoplasmic membrane (Kerler et al., 1984). Since the cuticle is a lipophilic membrane such a relationship can be expected, but has been confirmed only qualitatively within a series of chemically homologous compounds (Darlington and Cirulis, 1963). Most solutes are larger than urea and even larger than the pores of the polymer and they will move mainly or exclusively by diffusion (Riederer and Schonherr, 1989).

The penetration of amino acids were found to be very different between both periderm and cuticles and the fluxes observed also vary extremely among periderm discs. The variation was large and complicated by the fact that almost half of the total membranes were shown to exhibit a permeability characteristic to the amino acids. The failure to adequately describe permeability of PM and PFCM was due to membrane heterogenity. It was the purpose of these experiments to find the appropriate conditions for transport of amino acids through PM and PFCM, rather than to obtain permeability and diffusion coefficients. The time transport curves of amino acids is given in Fig. 6.1. The plots of amount v time are similar to sigmoidal. After an initial hold-up time, penetration rates reach a maximum and then level off. None of the slopes of amount penetrated v time reached a constant value.

Studies on membrane permeability should be made under steady-state conditions with constant, well-defined surface concentrations on each membrane

surface (Crank and Park 1968). Under such conditions simple relations hold between the flux of a compound under a defined driving force and the parameters of diffusibility and solubility of the compound within the membrane. Another disadvantage is that the diffusion coefficient cannot be given quantitatively due to the extreme variability realised at steady state.

The penetration of molecules through plant surfaces is of immense practical importance, particularly as regards pesticide deposition, accumulation and degradation. Several workers have attempted to simplify the study of chemical penetration into plant tissues by isolating the cuticle and following the movement of chemicals through them (Darlington and Cirulis, 1963; Martin and Juniper, 1970; Schonherr, 1976, 1979, 1982; Schonherr and Bukovac, 1973; McFarlane and Berry, 1974; Tyree et al. 1990; Yamada et. al, 1965). However, there are many problems inheret in this approach that are sometimes not obvious from the literature, such as extreme variability in the data and the permeants used, and differences in experimental design. Most reports on cuticular penetration omit calculations of a single parameter that can be used to compare values. The permeability coefficient, often expressed in cm s^{-1}, depends only on membrane thickness and characteristics and the properties of the permeant in the ideal case, and comparisons can be made regardless of area, compartment volume or substrate concentration (Davis et al., 1979). A student t-test analysis was run on the data and the variation was large and a t-test indicated ($p > 0.05$) no statistical differences in permeation of amino acids through PM and PFCM. The variation found between periderm/cuticle discs were also quite large, no statistical differences were observed, so that interpretation of the data was difficult. Plant cuticles are not homogeneous (Schonherr and Riederer, 1988; Tyree et al., 1990a) which means that gradients of mobilities and solubilities exist across cuticles (Bauer and Schonherr, 1992).

There is a significant correlation between permeability of PM and PFCM and the penetrant molecular weights and molar volumes and the correlation coefficients

are; r = -0.78 and r = -0.75 (for molecular weights for PM and PFCM), and r = -0.71 and r = -0.66 (for partial volume for PM and PFCM), respectively. The coefficients of determinations mean that molecular size and partial volume are the major determinants for the amino acids tested. A somewhat better result can be obtained, when log P is plotted v log of the physical parameter.

In these experiments, it can be shown that permeability and selectivity coefficients of amino acids can be accounted for using these parameters which are molecular size, molar volume and hydrophobic groups through extention groups. Unfortunately, the practical value of the data is limited by the fact, that permeabilities through both periderm and cuticles are extremely variable and also variability between these species is high. If this is in fact the case, one would expect that the differences in permeability of amino acids between periderm and cuticle should decrease with decreasing polarity of the compounds and increasing molecular size of amino acids.

The selectivity coefficient value increases with an increase in molecular size and partial volume of the amino acids through extension of the hydrophobic-CH_2-group. The study of solute sorption by a solid as a function of the solute concentration can elucidate some of the structural features of the materials on a molecular level (Riederer and Schonher, 1986). Sorption of solutes in cuticles, a reversible process, and desorption can be very rapid (Riederer and Schonherr, 1989). Sorption isotherms can be interpreted in terms of sorbent-substrate interactions and the microstructure of the solid phase (Giles et al. 1974). For a large number of liquid/liquid systems it has been shown, that the partition coefficient of a chemical species dramatically decreases upon ionization because the polarity of the molecule is greatly increased (Leo et al., 1971). More specifically, this data is discussed in terms of the mechanism of the transfer of amino acids into the periderm/cuticle and different sorptive properties.

Diamond (1966) reported that a change in ion-water interactions in the exchanger phase might be responsible for the selectivity of this ion exchange reaction. Theoretical treatment based on the Gibbs-Donnan model (Kawakita et al., 1989) leads to the following Eq. (6.5) for the selectivity coefficients of ion exchange for monovalent ions;

$$\ln K_{A/B} = \ln(r_B/r_A) + \pi(V_B - V_A)RT \qquad (6.5)$$

where $K_{A/B}$ denotes the selectivity coefficient of A to B, r_B and r_A the activity coefficients of cation species in the exchanger phase and the solution phase, respectively, and V_B and V_A the partial molar volume of ionic species.

Most theories of ion exchange selectivity attach key importance to ionic solvation phenomena. Selectivity in the "normal" order is governed by the free energies of hydration of the counterion. Gregor (1948, 1951) and, Gregor and Bregman (1951) proposed the model for predicting the selectivity coefficients from the solvation effect of the exchange ions. The hydration is directly proportional to the charge and inversely proportional to the size of the ion. The electrostatic free energy can be reduced by association between fixed charges and counter ions, and by screening of neighbouring fixed charges (Gregor et al., 1955). It would be assumed that the ion exchange system behaves ideally except for the hydration effect. The activity coefficient terms of eqn. 6.5 would be determined by the hydration effect and could be rewritten by including the hydration effect in the partial molar volume term as follows;

$$\ln K_{A/B} = \pi(V_B - V_A)RT \qquad (6.6)$$

It was proved that Gregor's model, which is not thermodynamically rigorous, is a very useful approximation for the system in which the hydration effect of ions is a predominant factor (Kawakita et al., 1989).

Riederer and Schonherr (1984) studied sorption of plant cuticles and demonstrated that sorption isotherm curves followed a straight line. Sorptive capacity

of plant cuticles for lipophilic chemicals is high (Riederer and Schonherr, 1985). The cuticle acts not only as a transport barrier but also as an ion exchange capacity. The extrapolated hold-up time gives the time needed to fill one-sixth of the total sorption capacity of the membrane (Riederer and Schonherr, 1985) The linearity of the sorption isotherm was demonstrated over the whole range of amino acids.

Taking periderm and cuticles as the solid phase their behaviour can be expressed by the Freundlich isotherm equation (Fig. 6.5). Isotherms obeying this relationship could be established for the sorption of amino acids in PM and PFCM. Freundlich isotherms are frequently encountered when solutes interact with heterogeneous substrates (Adamson, 1990).

Non-polar molecules are attracted to the aliphatic regions of cutin by London forces, while compounds having additional active substituents like hydroxyl, carbonyl or amino groups can also form hydrogen bonds with appropriate functional groups of the polymer (Riederer and Schonherr, 1986). In terms of sorptive properties, the cuticle behaves like an amorphous solid phase where both apolar and polar sorption sites are present with the aliphatic ones predominating. The interaction of a solute molecule with the molecular structure of a polymer arrests the molecule for a certain time at a given place and thus increases order, at the same time, it produces a large heat of sorption (Riederer and Schonherr, 1989).

The concentration dependence of the partitioning process is best described by sorption isotherms (Adamson, 1990). These are plots of the molal equilibrium concentration of solute in the membrane versus the molal concentration in the aqueous solution at a given experimental condition. Straight lines over the whole concentration range would be obtained if the two phases behaved as ideal solutions (Riederer and Schonherr, 1989). In these experiments, straight lines were obtained over the whole concentration range for both PM and PFCM. Feitelson (1961, 1963) discussed thermodynamically the correlation between steric properties of amino acids

and the configuration of the resin using selectivity coefficient data. Riederer and Schonherr (1986) calculated the partition coefficients in the system cuticle/aqueous solution as a function of solute concentration using sorption isotherms, and they predicted that at a low concentration range from 10^{-4} to about 10^{-1} moles per kg cuticle, the partition coefficient slightly varied with the logarithm of internal solute concentration but drastically decreased at higher concentration. The selectivity coefficients decreased with increase of amino acid concentration (Fig. 6.4).

A significant correlation was found between selectivity for PM and PFCM and their molecular weights and molar volumes and the correlation coefficients are; r = -0.77 and r = -0.83 (for molecular weights for PM and PFCM), and r = -0.67 (for partial volume for PM and PFCM), respectively. A somewhat better result can be obtained, when log $K^{A}_{NH_4}$ versus log of the physical parameter is plotted. The selectivity coefficients of amino acid can be obtained using molecular weights, partial molar volume and hydrophobic groups through extention groups.

The result of this study may be looked at from different viewpoints. Knowledge about the dependence of transport, concentration, selectivity, and type of periderm and cuticle is essential for the understanding and analyses of the uptake of amino acids, pesticides or chemical substances and environmental pollutants into leaves or fruits.

CHAPTER 7

Penetration of Herbicides

7.1 Introduction

The cuticle is an effective barrier to transport into and out of the plant leaf surface, retaining water and high concentration of solutes within the tissue and inhibiting penetration. It is not a complete barrier, however solutes and water are able to cross the cuticle (Tukey, 1970) by cuticular transpiration and pesticides can enter the plant after application to the surface. Because of the widespread use of pesticide sprays for crop production, as well as the interest in improving their performance, the cuticle has been the subject of studies in relation to structure and composition (Bukovac and Petracek, 1993; Schonherr and Bukovac, 1973; Baker et al., 1982 and Holloway, 1982a) and also spray retention and penetration of spray constituents (Price, 1982). Poor performance of foliar-applied systemic pesticides has been attributed to limited penetration, and has focused attention on the formulation of active ingredients to improve spray solution characteristics and cuticular penetration (Bukovac and Petracek, 1993).

Most studies using isolated cuticles have focused on sorption and transport across the membrane (Riederer and Schonherr, 1989). Diffusion is a much more likely mechanism for the transport of pesticides and other solutes across the cuticle and is a continuous event. For study and analysis cuticular penetration may be viewed as consisting of a three component process, namely sorption into, diffusion through and desorption from the cuticle (Bukovac and Petracek, 1993). Diffusion processes exist under field conditions for only a short time depending on spray volume, additives and environmental factors affecting drying time, but the greatest amount of penetration may take place during this time period (Wiitwer and Teubner, 1959; Sargent, 1965). Penetration from spray droplets on plant surfaces is a complex process not well understood (Bukovac and Petracek, 1993). Therefore, the isolated periderm and

cuticular membranes are used to gain an understanding of the mechanisms of cuticular penetration and to identify and quantify factors that affect the penetration process.

There is a simplified model of the cuticle, with carbohydrate fibres extending from the underlying wall and middle lamella into cuticle (Norris & Bukovac, 1968). Such fibres provide possible hydrophilic pathways extending from the aqueous apoplast to close proximity to the external surface of the cuticle (Hoch, 1979). Price (1978) mentioned that partition of a solute between the lipid and aqueous components of the cuticle is an equilibrium process which implies that no one route will be exclusive.

The rate by which foliar-applied pesticides move to the cuticle is determined by a series of complex interactions commencing with the adhesion and spreading of the impinging droplet (Baker and Chamel, 1990) and continuing via a sequence of sorption-desorption processes, both passive and active (Kirkwood, 1983, 1987). The penetration into leaves of a wide range of agrochemicals (Baker and Hunt, 1988) and surfactants (Silcox and Holloway, 1989) has been shown to be facilitated transport.

Foliar penetration of herbicides is influenced by plant factors, environmental factors (Ashton and Crafts, 1973; Currier and Dybing, 1959), composition of spray solution and methods of application (Babiker, 1976; Kirkwood and Fletcher, 1983). There are two systems by which herbicide molecules may move rapidly in plants; the phloem and the xylem (Babiker, 1976).

It is not known, whether or not sorption capacity of periderms and cuticles is the same for all compounds. The polymeric lipid membrane has a high sorption capacity for lipophilic compounds (Riederer and Schonherr, 1984, 1985, Shafer and Schonherr, 1985). The permeabilities of a given type of cuticle depends on the properties of chemicals and very large differences have been observed in the past (Darlington and Cirulis 1963, Schonherr 1976, Shafer and Schonherr, 1985). However, some quantitative data on permeabilities of cuticles are available up to now,

and nothing in the way of quantitative data on permeabilities of periderms to pesticides have appeared so far. Reason for differences in permeabilities are not well understood.

Most studies on transcuticular penetration of pesticides have been performed using infinite (Yamada et al., 1964; Norris and Bukovac, 1969; Kerler and Schonherr, 1984) (Fig 4.1) or finite dose systems (Darlington and Cirulis, 1963; Baker and Chamel, 1990; Kirkwood et al., 1982; Davis et al., 1979; Baker, 1987). In the infinite dose system (classical permeability) the diffusion of a pesticide is followed from an aqueous donor of essentially constant concentration, across the cuticle to an aqueous receiver. In finite dose systems (Fig. 7.1) a given dose of pesticide is supplied in an agar block or as an aqueous droplet(s) that is placed directly onto isolated cuticle which in turn is positioned on an agar block or in contact with an aqueous solution serving as a receiver.

This section is principally an investigation of the factors affecting the penetration of pesticides with particular emphasis on the influence of commercial formulations of three herbicides.

Herbicides are commonly formulated as water soluble powders and liquids, emulsifiable concentrates, flowable suspension concentrates, flowable emulsions, wettable powder concentrates, dust concentrates, field strength dusts, granular products and fumigants (Cook, 1979). Any formulation normally contains the toxicants and one or more of the following: solvents, emulsifiers and carriers (Cook, 1979). An additive is a substance or formulation of substances which when added to a herbicide formulation increases its effectiveness (Cook, 1979). Surfactants may act secondarily as humectants (Currier and Dybing, 1959) while many have considerable penetrating properties (Price, 1976).

Glyphosate, amitrole and asulam are translocated herbicides along with other herbicides introduced earlier such as 2,4-D (Yusuf, 1988). The commercial

formulations of these three herbicides have been specifically chosen, because they have been regularly used in the environment and on crops. These herbicides were selected for the study because of their general importance as herbicides, their considerable use on bracken in the past and their known sensitivity to environmental variables.

Amitrole is a non-selective herbicide adsorbed by roots and leaves and tronslocated, inhibiting chlorophyll formation and regrowth from buds. It is used around established fruit trees between harvest and the following summer (Worthing, 1979). It can penetrate foliage reasonable well.

Asulam is a foliar applied systemic herbicide for bracken control (Babiker, 1976), and interferes with plant processes. Babiker (1976) showed that asulam may possess auxin-type properties and it is a sulphonamide derivative. Asulam is absorbed by leaves and roots causing, in suspectible plants, a slow chlorosis (Worthing, 1979). It is used for pre- and post emergent control of bracken in agricultural situations.

The glyphosate sold commercially as Round-up, is a post-emergence non-selective herbicide and translocatable, claimed to be a new wonder environmental green herbicide, introduced in 1971. Glyphosate is a popular choice as it exhibits many unique biological properties, has been applied to crops and cereals, and is different from the other two herbicides and would not cause any surface damage. The properties that make this compound effective include its relatively high water solubility, rapid foliar absorption and translocation by plants and a low degree of *in vivo* metabolism and degradation (Guinivan et al., 1982). It is commonly recognised that glyphosate and its ionic forms are biologically equivalent. Glyphosate possesses distinctive properties compared to most other pesticides especially when it is dealt with at low residue levels. This compound is closely related to the exceptionally polar ones in which its main characteristic is its relatively high solubility in water but insolublity in organic solvents. (Yusuf, 1988).

7.2 Materials and methods

7.2.1 Materials

Glyphosate (commercial formulation, 97%) was obtained from Chem Service, Asulam (technical, 98%) was purchased from the Greyhound Chromatography & Allied Chemicals. Amitrole was purchased from Koch-Light Laboratories.

7.2.2 Methods

The rates of penetration of the pesticides through isolated PM and PFCM were examined in two separate experiment sets. Treatment periods were made by using agar discs (finite dose) (Fig. 7.1) and classical permeability measurements (infinite dose) (Fig. 4.1).

The cuticle/periderm was converted to H^+ form with 1 M HCl, followed by washing with deionized water to remove Cl^- ions. The membrane samples were placed on an agar disc (31 mm diameter x5 mm thick) of oxoid No:3 agar (2% W/V) held in a Petri dish (Fig. 7.1). For penetration studies, cuticles were supported on a layer of aluminium foil which was perforated at the centre by a hole 12 mm in diameter. The aluminium layer acted as a support to attach the cuticle to the agar disc. Prior to droplet application, cuticles and supports were stored in Petri dishes containing a thin strip of moistened tissue paper.

The pesticides 50 µl (1 mg/ml) were applied using the automatic pipette to the central area (12 mm diameter) of an isolated cuticle/periderm mounted on an aluminium support (Baker, 1987). The outer section of the membrane sample was shielded from droplets by placing a Whatman No: 1 grade filter paper(1.0 cm diameter), perforated by a hole (12 mm diameter), above the central section of the membrane sample. Following droplet application, the mounted membrane was placed on an agar disc positioned in a covered Petri dish which rested in an incubator maintained at 25±0.5 °C. Membrane samples were transferred at various intervals to fresh agar discs. Three replicates per treatment were used in all time course

experiments. At the end of the treatment period the membrane samples were removed from the final agar disc. Surface residues of pesticides were recovered from the membrane by washing with water. Residues of pesticides were recovered from the agar by washing with acetone- water (2/3, V/V).

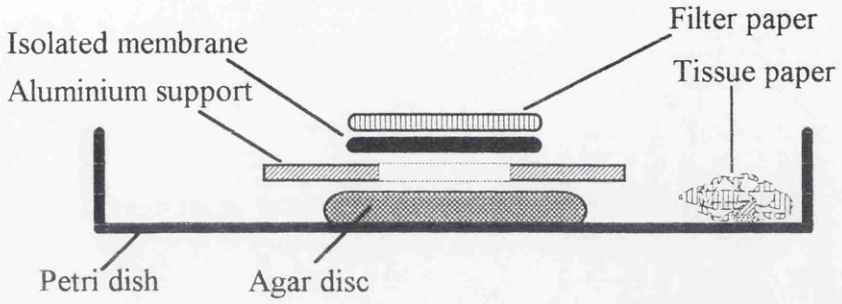


Fig. 7.1 Model (finite-dose) system used in penetration studies.

For classical permeability measurements, citric acid-Na_2HPO_4 buffer in the pH ranges 6-8 and deionized water were used. Pesticide classical permeability experiments were made with 1000 μg ml^{-1} solutions. The methods and apparatus details are given in Chapter 4.

Glyphosate contains 3 polar functional groups, a carboxylic acid group, a phosphoric acid group and a secondary amine group, and so the fundamental problem is the detection of the glyphosate, as detection can be difficult by U.V., a fluorescence detector was used. This requires derivatisation of glyphosate. Pre-column derivatisation involves reacting the mixture of glyphosate with an appropriate reagent before the glyphosate is determined. Glyphosate carries a secondary amino group which can react with reagents. 9-FMOCL reacts via an Sn^2 mechanism with amino nitrogen of both primary and secondary amines producing a carbamate having a fluorenyl group as the fluorophor (Yusuf, 1988). The derivatisation and separation procedures are given Chapter 6. The determination of glyphosate was carried out by high performance liquid chromatography (HPLC).

Amitrole (3-amino-1,2,4-triazole) was determined using colour development by colorimetric method (Storherr and Burke, 1961).

Asulam concentrations were determined by the Bratton-Marshall reaction as described by Brocklesby and Muggleton (1973).

7.3 Results

Changes of penetration of three herbicides through isolated potato periderm and pear fruit cuticular membranes were examined in two separate experiments involving infinite and finite dose systems. The time course study of herbicides and % penetration (finite dose systems used) are given in Table 7.1. Time course of penetration of herbicides (infinite dose system used), penetration total (% amount applied) and penetration rate (% amount applied h^{-1}) are shown in Fig. 7.2 and 7.3, respectively.

The penetration rates of these herbicides, particularly asulam and glyphosate decreased substantially after 1 h droplet application, whereas, amitrole diffused steadily into agar receiver discs throughout the whole of the 68 h treatment period. Consequently all herbicides penetration rates examined decreased after 1 h droplet application.

7.4 Discussion

The time course study of herbicides and % penetration are given in Table 7.1 and Fig. 7.2 for finite dose and infinite dose systems, respectively. The permeability of three herbicides was found to follow the order of their solubility. The water solubility order is Amitrole> Glyphosate> Asulam. The result can suggest that the penetration rates of these herbicides depends on the solubility. The mode of action is different for all three herbicides. Amitrole and glyphosate are both reasonably water soluble in the formulation. The point should be stressed that only three herbicides were tested in the present investigation. The epicuticular wax layer constituted a major barrier (Baker and Chamel, 1990) to the passage of these water soluble herbicides.

Table 7.1 The penetration of herbicides through PM (A) and PFCM (B)

(A)

Herbicide	Time(h)	Penetration (μg) Mean and SD (±)	% Penetration
Amitrole	0-1	1.89 (0.48)	3.78
	1-4	2.71 (1.43)	5.42
	4-24	5.01 (2.03)	10.01
	24-48	6.95 (2.53)	13.90
	48-68	8.03 (3.75)	16.06
Glyphosate	0-1	2.63 (1.98)	5.26
	1-4	3.40 (2.05)	6.81
	4-24	4.53 (2.34)	9.07
	24-48	5.94 (3.08)	11.88
	48-68	2.14 (1.85)	4.28
Asulam	0-1	3.96 (1.91)	7.92
	1-4	4.58 (2.08)	9.16
	4-24	3.07 (1.82)	6.14
	24-48	4.54 (2.66)	9.08
	48-68	3.75 (2.62)	7.51

(B)

Herbicide	Time(h)	Penetration (μg) Mean and SD (±)	% Penetration
Amitrole	0-1	2.05 (0.77)	4.10
	1-4	2.78 (1.32)	5.57
	4-24	5.08 (2.56)	10.15
	24-48	7.15 (2.21)	14.30
	48-68	8.36 (4.43)	16.73
Glyphosate	0-1	1.82 (1.24)	3.63
	1-4	3.54 (2.64)	7.08
	4-24	4.53 (3.08)	9.06
	24-48	4.79 (3.02)	9.58
	48-68	2.49 (1.76)	4.98
Asulam	0-1	3.28 (2.10)	6.56
	1-4	4.04 (2.04)	8.07
	4-24	2.95 (2.41)	5.89
	24-48	4.42 (2.82)	8.86
	48-68	3.79 (2.33)	7.57

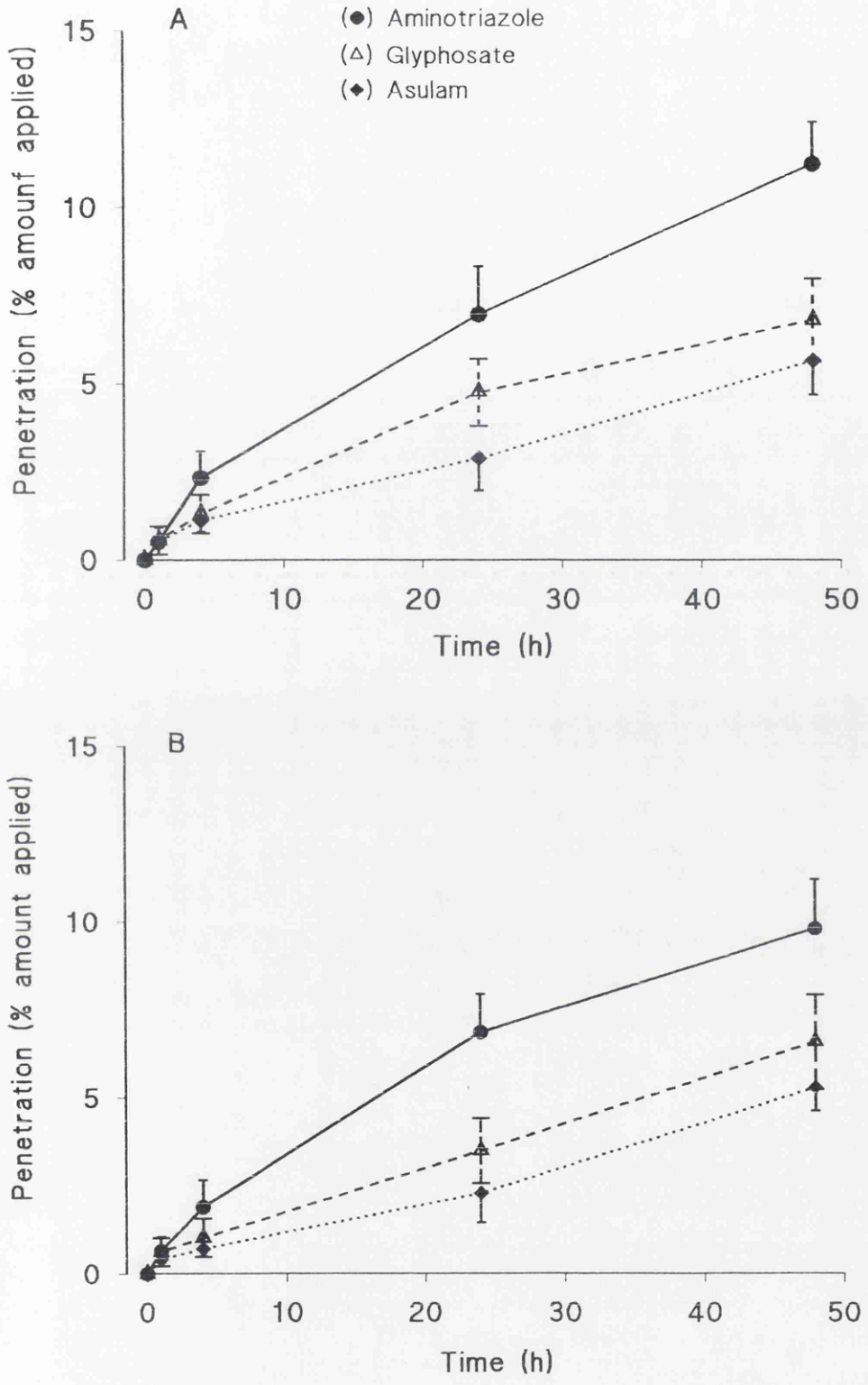


Fig. 7.2 Time course of penetration of herbicides across (A) PM and (B) PFCM. These data was obtained from infinit dose system.

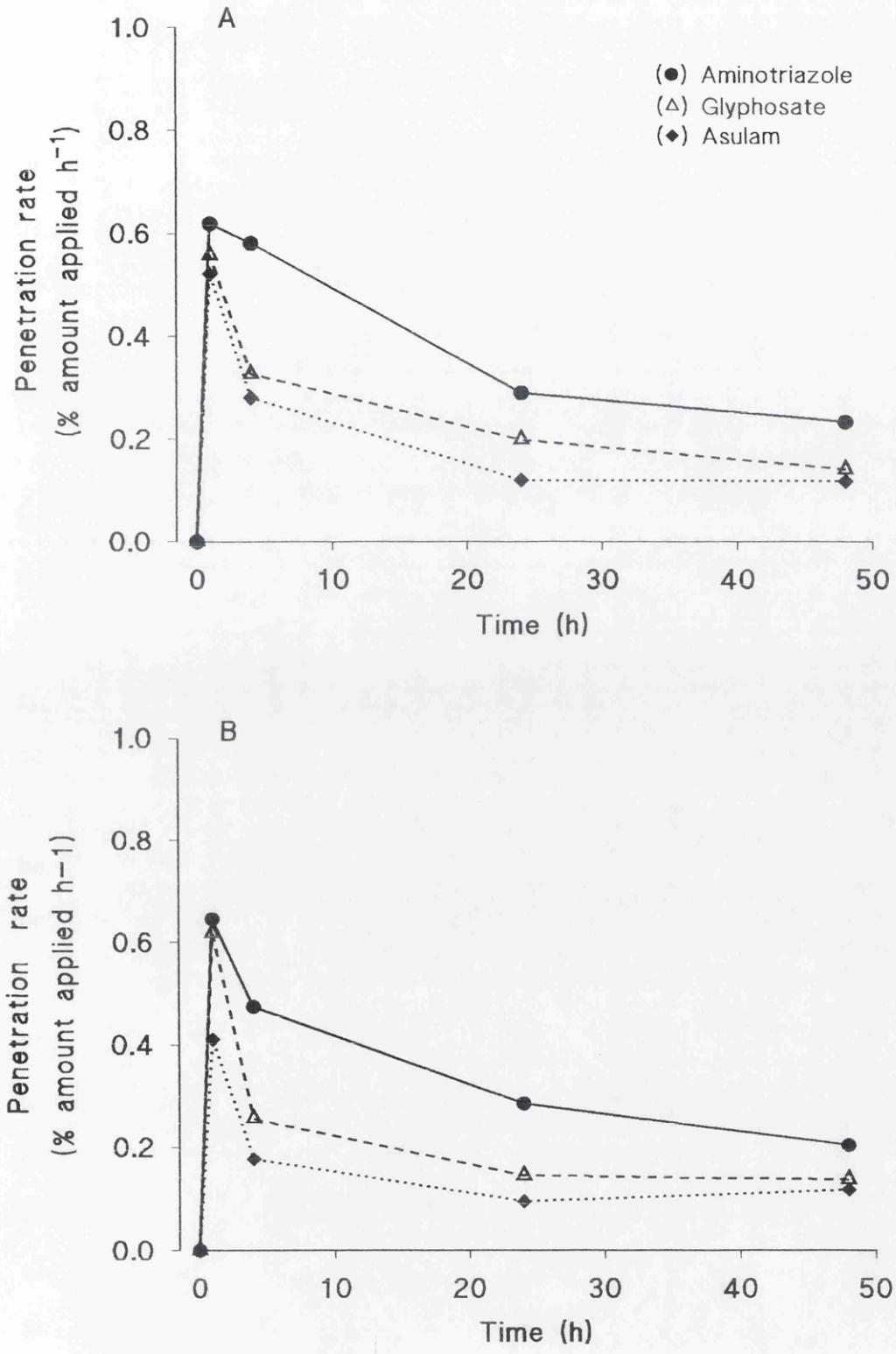


Fig. 7.3 Time course of penetration rates of herbicides across (A) PM and (B) PFCM.

This study also showed that rates of movement of formulation forms of applied herbicides vary through the isolated periderms/cuticles. The result obtained using the infinite and finite dose systems are in general agreement with the whole data, all three herbicides penetrated rapidly through the PM and PFCM during the initial 1 h treatment period then decreased, whereas amitrole maintained a steady rate of diffusion throughout the experimental period. A student t-test analysis was run on both set of data and the variation was large and t-test indicated ($p > 0.05$) no statistical differences in permeation of three herbicides through PM and PFCM.

Both techniques, generally gave similar results for the three herbicides. Further investigations are required to determine rates of herbicide penetration with and without surfactants, humectants and wetting agents, e.t.c.

It is difficult to relate penetration data obtained using isolated PM and PFCM systems to foliar uptake profiles, due to the problem of variability of data and determining the precise location of chemicals applied to periderms/cuticles. Because following foliar application, chemical recovered from within the treatment zone may be retained in the surface in the periderm/cuticular membrane or in the epidermal cell or may be interacting with the solute within the membrane.

This is a penetration study in which herbicides have been applied to isolated PM and PFCM using two sorts of experimental systems. These experiments could be used to test the practicability of carrying out model penetration studies with spray droplets and classical permeability systems applied to isolated periderms/cuticles. The finite dose technique would permit the rapid screening of a range of herbicides with widely differing physicochemical properties formulated with different carrier solvents and adjuvants.

Schonherr et al., (1984) also reported that cuticular lipids were the major barrier to the movement of aniline, p-nitrophenol, 2,4-D and 2,4,5-T through isolated *Citrus aurantifolia* cuticles. These authors found difficulty in defining the nature of

the barrier because the lack of correlation between partition coefficient from the permeability coefficient indicated that solutes do not dissolve in the soluble cuticular lipids. They postulated that diffusion might proceed through defect structures between wax crystallites which arise from interference by the hydroxyl groups of the cutin polymer with the crystallisation of the soluble cuticular lipids. It should be noted that no increase in penetration of glyphosate and asulam into periderms/cuticles took place. These findings may suggest that solute movement through the cuticle is controlled by the waxes embedded in the cuticular membrane.

The influence of pH on penetration of herbicides has been examined through the periderms/cuticles. Overall, cuticular penetration of the three herbicides was significantly affected by pH, and as cuticular penetration generally was recorded above pH 4.2, the penetration of herbicides was studied between pH 6-8 range. The data obtained were variable and variability was also noted among periderms/cuticles due to the fact each membrane exhibited different permeability characteristics. Kirkwood et al., (1987) examined the influence of pH in relation to the effect of surfactants on penetration of herbicides such as ^{14}C-MCPA and ^{14}C-MCPB. They reported that the cuticular retention of both herbicides was significantly affected by pH, and greatest penetration occurred at higher pH levels. The pH, can affect the permeability of the membrane directly, and has an effect on the driving force via electrical potentials, and can change the properties of the solutes by dissociation.

Further model studies using herbicides with formulation forms from different chemical classes, combined with a range of hydrophilic and lipophilic carrier solvents and additives could provide valuable information concerning the mechanisms of pesticide penetration. The presence of hydrophilic groups, hydration and imbibition of the cuticle proper and of the cuticular layers become explainable. Consequently the penetration of substances through the cuticle is facilitated.

Kerler and Schonherr (1988b) analysed the permeability of cuticular membranes for solutes differing widely in lipid solubility. Permeance increases with increasing lipid solubility. With regard to the penetration of lipophilic substances through the cuticular membranes, a process of solution by means of lipophilic components of cutin and waxes may take place. Penetration was directly proportional to concentration, and increased with increasing temperature and with the higher lipophilic character of the applied compounds (Darlington and Cirulis, 1963). The equilibrium depends on the dissociation of fixed charges which depends on the pH and concentration of the external solution. There are major differences in the permeability of different plants, but in general there is a tendency for uptake to be more closely related to minimum molecular radius than to maximum molecular radius or molecular weights. (Price, 1980)

Commercial formulations contains wetting agents, humectants and surfactants, which allow chemicals to remain in the solution. The formulation forms have been designed to assist their penetration into plants.

Surfactant-enhanced penetration is most probably related not only to improved wetting and modification of the physical nature of the deposit, but also to direct effects on the permeability of the cuticular membrane (Bukovac and Petracek, 1993). Knoche and Bukovac (1993), using the infinite-dose system, and Schonherr (1993) using a desorption system, presented evidence that surfactants increase the mobility (diffusion) of herbicides in the cuticle (Bukovac and Petracek, 1993). Surfactants definitely increase the chances of the component going across the barrier in penetration. Spray retention may improve by incorporating surfactants in the spray solution.

Penetration of herbicides (atrazine, diclofob-methyl, dinoseb and glyphosate-mono(isopropylammonium) through isolated pear leaf cuticles into agar and across intact cuticles was studied by Baker and Chamel (1990). They found that penetration

into pea leaves ranged for glyphosate-mono(isopropylammonium) 5%, atrazine 14%, dinoseb 91% and diclofob-methyl 82% (of amount applied). Penetration of glyphosate-mono(isopropylammonium) decreased after 4 h.

The amount, chemical composition and the physical configuration of the waxes on the leaf surface influence greatly the wettability of surfaces (Silva Fernandes, 1965). Cutinised surface of the cuticle provides a distinct barrier to optimum herbicide penetration (Martin and Juniper, 1970; Storherr and Burke, 1961).

The effect of humidity on amitrole penetration was quite apparent (Cook, 1979) and its penetration is greatly influenced by changes in atmospheric humidity (Babiker and Duncan, 1975). The lipid character of the cuticular waxes and the negative charge in the cuticle make it a unique barrier for the penetration of hydrophilic compounds (Babiker, 1976; Franke, 1968). Many workers consider that the obstacle caused by the cuticle is so great that uptake of hydrophilic compounds could only be envisaged as stomatal (Franke, 1967).

Amitrole is taken up by the leaf via an aqueous route (Crafts, 1961). A water continuum is essential for efficient penetration of the leaf surface barrier (Babiker, 1976). Presumably surfactants help to increase penetration by establishing or maintaining this water continuum, and bring about increased contact between chemical and leaf surface and thereby encourage penetration by diffusion (Martin and Juniper, 1970; Babiker, 1976). Cook (1979) studied factors affecting foliar uptake and translocation in particular the influence of additives on the herbicide amitrole. He found that penetration of bean leaves was greatly enhanced under high humidity conditions and amitrole penetration in the presence of additives and adjuvants was increased.

In general, the surfactant molecule consists of two more or less distinct moieties, one of which is hydrophilic, the other hydrophobic. Because of the hydrophilic-lipophilic nature of the molecule, it possesses only limited solubility in

both water and organic solvents (Cook, 1979; Behrens, 1964). In the field the chemical categories of importance are nonionic, cationic and anionic depending on the nature of active species. Numerous studies have demonstrated that surfactants increase the activity of herbicides on plants (Babiker, 1976; Behrens, 1964; Crafts and Foy, 1962; Mcwhorter 1963a, 1963b; Smith and Foy, 1967; Smith et al., 1967;).

The presence of additives (i.e. surfactants, humectants and wetting agents) within the commercial formulations of herbicides may facilitate penetration, although, the presence of salts may retard penetration (Babiker, 1976). Salts may compete with the herbicide for adsorption and ion exchange sites. Such reactions are known to take place when sprayed droplets are drying out on the leaf surface (Babiker, 1976). Possibly, the penetration of herbicides without the addition of surfactants, could be slow. However, the possibility that there is a specific herbicide-plant-surface interaction (Foy and Bingham, 1970) including the hydrophilic-lipophilic-balance of the system could be a factor (Babiker, 1976).

Asulam could be considered as an aniline-based herbicide (Babiker, 1976) as a substantial proportion of this herbicide contains unsubstituted or variously substituted anilines. Among the attractive features of this type of herbicide are their effectiveness, selectivity and biodegradability (Babiker, 1976; Kirkwood et al., 1982). However, in common with many foliar-applied herbicides the erratic behaviour of asulam noted and attributed to climatic factors, could be caused by slow penetration (Currier and Dybing, 1959). Babiker (1976) found that the penetration of asulam without surfactants is slow. He also reported that the percentage penetration is unaffected by concentration. Similar findings were observed by Cook (1979).

Of the surfactants which bring about a significant increase in penetration of asulam (Babiker, 1976), variations have been observed with other herbicides, which have been attributed to a specific interaction between the surfactant and the herbicide and the plant surface (Freed and Montgomery, 1958).

Takeno and Foy (1974b) have demonstrated that surfactants may solubilise surface waxes while it has also been proposed that surfactants may induce swelling of the cutin or dissolve certain cuticular components (Jansen, 1964a, 1964b). Swelling of the cutin could increase the hydrophilic and lipophilic channels thereby promoting uptake (Jansen, 1964a, 1964b). Jansen (1964a) has also proposed that cuticle hydration due to water from a droplet containing a surfactant being more available to the cuticle, may be a primary function. Surfactants have also been shown to have direct effects on translocation (Cook, 1979).

Humectants are much less widely used in herbicide formulation than are surfactants. Generally they are low molecular weight (Babiker and Duncan 1975a). They prevent drying of the spray solution on the surface thus increasing the penetration time, and they become sorbed by the cuticle so increasing its polar nature and compatibility with water soluble chemicals (Crafts, et al., 1958). Cook (1979) concluded that the penetration is not greatly influenced by the addition of humectants to spray solutions of amitrole, but additional inclusion of a surfactant brought about substantial increases in penetration over both the aqueous and humectant controls.

Experiments with isolated cuticles or periderms, possibly using surfactant concentration gradients comparable to the droplet drying situation, should provide more specific and quantitative data to support whole plant studies. Data from the agar or aqueous droplet systems are difficult to interpret due to experimental conditions and penetration from spray droplets on plant surfaces being a complex process. A critical evaluation of this system is needed to determine its usefulness as a model system. If meaningful transport parameters can be confirmed, this system offers the advantage of quantifying and relating the effects of spray application components, common to field conditions, on deposit formation to transcuticular penetration.

CHAPTER 8

General Discussion

The role of this chapter is to draw together the main findings and conclusions of the studies. Since there was not enough time to continue the study of many of the aspects covered, some suggestions for further investigations are also made.

The work concentrated on the role of ion exchange in the movement of chemicals through the isolated potato periderm and pear fruit cuticular membranes. The data obtained were discussed from a quantitative and physicochemical viewpoint, backed up by knowledge on the dependence of transport, concentration, selectivity, and type of periderm or cuticle, which is essential for a physicochemical understanding and analysis of the uptake of chemical substances and environmental pollutants into periderms or cuticles.

The exchange capacities, selectivities and interaction of alkali cations in the isolated potato periderm and pear fruit cuticular membranes were characterized. Both isolated membranes exhibited a behaviour typical of ion exchange resins of the weak acid type. It was found that the exchange capacity of periderm membranes is higher than that of cuticular membrane. This was thought to be due to the monomer composition of the suberin polymer in the periderm ,which has a higher carboxylic:hydroxyl ratio than the cuticular membranes. The isolated periderm and cuticular membranes also exhibited cation binding behaviour characteristic of the Freundlich isotherm type.

The penetration rates and diffusion coefficients increased as the external concentration and pH were increased. Transport properties of isolated membranes depended on the nature and density of charges fixed to the membrane matrix because their permeabilities increased 3 fold between pH 3 and 8 due to the carboxylic groups dissociating. The penetration of ions through isolated membranes took place by

diffusion and was impeded by charge interaction between solute and charge sites in the penetration pathway.

The transport of K^+ ion through the isolated periderm and cuticular membranes with different pH and various concentration gradients was demonstrated using two separated solutions by the membrane. A tentative transport mechanism model could be proposed. Experimental data suggested that this system is characterised by asymmetry of binding on both sides of the membrane for the formation and dissocation of carrier groups in the membrane matrix. The transport of ions against their concentration gradient was not observed. In the selective transport of metal ions, the selectivity depended on both the hydrated ionic size and the interaction between the carboxyl groups in the membrane and the metal ions.

The selectivity and permeability of the ammonium form of periderm and pear fruit cuticular membranes for some amino acids were determined, using the ion exchange concepts established above, and were found to be correlated with various physicochemical parameters such as the partial volume and molecular weight. The results showed that the selectivity coefficient increased with an increase in molecular weight and partial volume of the amino acids through extension of the hydrophobic - hydrocarbon- group. Permeability was in the reverse direction.

The penetration of three pesticides depended on their water solubility, and also was significantly affected by pH. All three herbicides penetrated rapidly through the isolated membranes during the initial 1 h treatment period then more slowly. Thus transport of herbicides was not explained by ion exchange theory except the effect of pH. This work could be used in practice to model penetration with spray droplets and compare with classical permeability systems.

The kinetics of diffusion across the cuticle were readily determined in the infinite-dose system and useful transport parameters could be calculated. Data from the agar or aqueous droplet systems were difficult to interpret due to experimental

conditions and penetration from spray droplets on plant surfaces is a complex process not well understood. A critical evaluation of the infinite-dose system is needed to determine its usefulness as a model system. If meaningful transport parameters can be confirmed, this system offers the advantage of quantifying and relating the effects of spray application components, common to field conditions, on deposit formation through transcuticular penetration. Further model studies using herbicides and formulations of different chemical classes, combined with a range of hydrophilic and lipophilic carrier solvents and additives could provide valuable information concerning the mechanisms of pesticide penetration.

In conclusion, the theory of ion exchange and ion transport can contribute to understanding the properties of periderms or cuticles in plants. Future models predicting leaching of nutrients and agrochemicals from plants during acid rain or water pollution events can incorporate transport parameters to account for the probable influence of diffusion and permeability characteristics on ion migration. Initial rate, maximum rate of penetration and lag time etc. are all potentially useful parameters that should be evaluated as essential background to environmental pollution issues.

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