THE EFFECT OF ELECTRICAL PROCESSING ON MASS TRANSFER AND MECHANICAL PROPERTIES OF FOOD MATERIALS

by

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ABSTRACT

In this research work, the effect caused by electrical processing on mass transfer in food materials was studied by designing and performing experiments that allowed the visualisation of: the effect of moderate electrical fields (MEF ≤1000 $V\,cm^{-1}$) on mass transfer in cellular materials; the effect of MEF on mass transfer of solutes to polymer networks; and the effect of MEF and pulsed electrical fields (PEF) on mechanical properties of polymer networks.

MEF treatment was performed with continuous alternating current (50Hz frequency) at electrical fields up to 1400 $V\,m^{-1}$ using a jacket system processing cell to maintain constant temperatures. PEF treatment was performed with a pulse generator at Lund University, Sweden. Extraction of betanin from beetroot was monitored online and measured by spectrophotometry. Mass transfer of rhodamine6G into gel networks (alginate, albumin and gelatine) was measured by image analysis. Effective diffusion coefficients (D_{eff}) for mass transfer of betanin and rhodamine6G were estimated, assuming Fickian diffusion was valid. Mechanical properties of alginate and gellan gum treated with MEF and PEF were studied. Compression force of gel samples was measured with texture analysis.

Results showed that the application of MEF and thermal treatment had an enhancing effect on the extraction of betanin from beetroot. The orientation of the beetroot slab also appeared to have an enhancing effect on extraction when the slab was placed perpendicular to the electrical field. The application of MEF had a decreasing effect on mass transfer of rhodamine6G to gel networks set with ions. Mass transfer decreased as electrical field increased. This effect was influenced by electrical conductivities of the gel and rhodamine6G solution. No significant effect of MEF was observed on gelatin or albumin. MEF and PEF had an increasing effect on compression force of polymer networks.

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LIST OF ABBREVIATIONS

Abbreviation	**Stands for:**
3D	Three dimensional
AB	Pulses generator maker's name
AC	Alternating current
ANOVA	Analysis of variance statistical test
ß-GLUC	ß-glucosidase
β-lg	β-lactoglobulin
BVI	Brine vacuum impregnation
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
DC	Direct current
DMRBE	Leica Optical Microscope model
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
e.g.	'For example' [abbreviation for the Latin phrase *exempli gratia*]
E162	E-number for betanin dye
et al.	'And others' [abbreviation for the Latin phrase *et alii*]
EU	European Union
EW	Egg white
FAO	Food and agriculture organization
GEA	Gelatine maker's name
HEM	Human epidermal membrane
HHP	High hydrostatic pressure
HIPEF	High intensity pulsed electrical fields
i.e.	'That is' [abbreviation for the Latin phrase *id est*]
IR	Infrared
LOX	Lipoxygenase
MEF	Moderate electrical fields
min	Minute
NaCl	Sodium chloride

PEF	Pulsed electrical fields
PU	Pasteurisation unit
RFEF	Radio frequency electric fields
SEM	Electron microscopy
TA.XTPlus	Texture analyser model
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
VL	Visible light

NOMENCLATURE

Abs	Absorbance
A	Area [m^2]
A_{Mat}	Area of test material [m^2]
B	Normalised °Brix of sugar beet juice
C	Concentration [g ml^{-1}], [mol m^{-3}], [kg m^{-3}]
c,f,s	Terms for PDE in pdepe
C'	Concentration, for diffusion in gels [g ml^{-1}], [mol m^{-3}] or [kg m^{-3}]
C_0	Initial concentration of the solute [kg m^3]
C_1 and C_2	Relative average calculated concentrations in tissues type 1 and 2 [kg m^{-3}]
C_{esp}	Concentration of species [mol m^{-3}]
C_{ext}	Concentration in the solution surrounding red beet [kg m^{-3}]
c_p	Specific heat capacity [J $kg^{-1}K^{-1}$]
D	Diffusion coefficient [m^2s^{-1}]
d	Proportionality constant [$°C^{-1}$]
D'	Diffusion coefficient, for diffusion in gels [m^2s^{-1}]
D_0	Diffusion coefficient when no electrical field was applied [m^2s^{-1}]
D_E	Electrically enhanced diffusion coefficient [m^2s^{-1}]
D_{eff}	Effective diffusion coefficient [m^2s^{-1}]
$D_{eff,1}$ and $D_{eff,2}$	Diffusion coefficients for tissue type 1 and type 2, for bimodal diffusion [m^2s^{-1}]
E	Electrical field strength [V m^{-1}]
e_r	Activation energy [J mol^{-1}]
F	Faraday constant [C mol^{-1}]
$f(\varpi)$	Shape factor
$\vec{F}_E$	Electrophoretic force [N]
$\vec{F}_{El}$	Electric force [N]
$\vec{F}_R$	Relaxation force [N]
$\vec{F}_{Total}$	Total force [N]
G	Universal gas constant [J $kmol^{-1}K^{-1}$] or [J $mol^{-1}K^{-1}$]

H	Dimensionless time, $H=Dt/L^2$
h	Radius [m]
I	Current [A]
I_ρ	Electric current density [A]
I_i	Fraction of the current carried by the specie i [A]
J	Diffusion flux [mol m^2s^{-1}], species flux [kmol m^2s^{-1}]
J_0	Bessel function of the first kind of zero order
J_1	Bessel function of the first kind of first order
J_w	Water flux [mol m^2s^{-1}]
k'	Rate constant for Arrhenius equation
L	Length [m]
l	Semi thickness, length of infinite sheet of uniform material [m]
L_F	Distance from the centre in direction of the external electrical field [m]
L_{Gap}	Distance between electrodes [m] or [mm]
L_{Slab}	Thickness, length of the slab [m]
m	Coefficient for pdepe, depends on geometry of the sample: 0 for rectangular, 1 for cylindrical and 2 for spherical
M_∞	Amount of solute leaving the sheet after infinite time [kg]
M_t	Amount of solute leaving the sheet up to time t [kg]
p,q	Terms for boundary conditions in pdepe
PU	Pasteurisation unit [min]
Q	Heat generated by the passage of current [W]
q_ns	No-zero positive roots of $\tan q_n = -\alpha q_n$
r	Position, in cylindrical coordinates [m]
R	Resistance [Ω]
T	Temperature [K], [°C]
t	Time [s], [min]
T_{Mic10}	Change in temperature needed to change t_{Mic10} by a factor of 10 [°C]
t_{Mic10}	Time needed to reduce the amount of microbes by a factor of 10 [min]
T_{ref}	Reference temperature [°C]
u	Concentration, for solving pdepe [kg m^{-3}]
U	Integrated lethality [min]
V	Electric potential [V]
v	Valence of the species []

w/v	Weight per volume (grams of solute per 100ml of solution)
w_{Max}	Wavelength at which absorbance is the greatest [nm]
x	Position, in rectangular coordinates [m]
Z	Increase or decrease in temperature required to increase the decimal reduction time by one order of magnitude [°C]
z	Slope of the line of t_{Mic10} versus T_{Mic10} [°C]
Δc	Rate of solute enhancement or depletion at the interface []
ΔDif	Diffusion enhancement factor
ΔP	Applied pressure [bar]
$\Delta \varphi M$	Local membrane potential difference [V]
ΔX	Front depth [m]
ρ	Density [kg m^3]
τ	Dimensionless distance, $\tau = x/L_{Slab}$
ε	Electric resistivity (Eq. 7), $\varepsilon = \sigma^{-1}$ [m S^{-1}]
σ	Electrical conductivity [S m^{-1}]
σ_0	Electrical conductivity at reference temperature [S m^{-1}]
σ_{Bet}	Electrical conductivity of betanin (Chapter 4) [S m^{-1}]
σ_{Gel}	Electrical conductivity of gel cylinder (Chapter 5) [S m^{-1}]
σ_{NaCl}	Electrical conductivity of NaCl solution (Chapter 4) [S m^{-1}]
$\sigma_{Solution}$	Electrical conductivity of rhodamine6G solution (Chapter 5) [S m^{-1}]
σ_T	Electrical conductivity at a temperature T [S m^{-1}]
σ_{25}	Electrical conductivity of the material at 25°C [S m^{-1}]
ς	Fractional amount extracted corresponding to $D_{eff,1}$
Λ_0	Intensity of light passing through a blank
Λ	Intensity of light passing through the sample solution
ϑ	Maximum amount extracted for the individual samples [kg]
γ_r	Pre-exponential factor [min^{-1}]
α	Ratio of the volumes of solution and sheet
Y	Reflection coefficient
$\varpi_1, \varpi_2, \varpi_3$	Semi-axis of elliptical cells

Ψ	Solution for a long cylinder, by superposition principle
Φ	Solution for an infinite slab, by superposition principle
Θ	Temperature coefficient of resistivity
λ	Thermal conductivity [W $m^{-1}K^{-1}$]
Γ	Transmittance
$\Delta \iota$	Transport number difference across the interface
ι_i	Transport number of species i
ι_m	Transport number of membrane
ι_s	Transport number of adjacent solution
Π	Water permeability constant of the membrane

CHAPTER 1

1. INTRODUCTION

The general aim of this study was to build up an understanding of the effect produced on mass transfer when electrical fields are applied to food materials.

Electrical processing has been used in food and pharmaceutical industries mainly for pasteurisation and electrophoresis, respectively. Ohmic heating (also known as joule heating or electrical heating) provides a good way to sterilise or pasteurise foods due to its ability to provide volumetric heating in a rapid and uniform way (Uemura and Isobe, 2003; Zhang and Fryer, 1994). Pulsed electrical fields (PEF) treatment can be used to induce permeability of biological cells, e.g. to inactivate microorganisms (El Zakhem *et al.,* 2007; Gerlach *et al.*, 2008; Uchida *et al.*, 2008). However, there are many other potential applications for both processes: ohmic heating applied to meat patties reduces cooking time and makes the products safer and similar in moisture and fat content to the conventionally cooked ones (Özkan *et al.*, 2003); apples pre-treated ohmically show an increase in hot-air drying rate and juice yield over those pre-treated conventionally and with microwave heating (Lima and Sastry, 1999); a prototype ohmic thawing unit was shown to use less water and was more energy efficient than conventional methods (Roberts *et al.*, 1998).

Another potential application of electrical processing is its capacity to affect mass transfer. Mass transfer plays an important role in the food industry since most of the processes involve it, i.e. the purification of raw materials or the final separation of products and sub-products. Mass transfer in cellular materials under the application of electrical fields

(specially the extraction of betanin from beetroot) has been widely studied and it has been shown to be a process for the enhancement in extraction of cellular compounds (Lebovka *et al.*, 2006; Fincan *et al.*, 2004; Kulshrestha and Sastry, 2003; Schreier *et al.,* 1993).

Beta vulgaris is most widely known as beetroot. The colour of beetroot is due to a red-purple pigment betacyanin and a yellow pigment betaxanthin known collectively as betalains. The pigments are contained in cell vacuoles. Beetroot pigment is used commercially as a natural food dye (Stintzing, 2006; Hamilton, 2005).

Nevertheless, a smaller amount of research works have focused their attention to the effects of electrical processing on mass transfer in gel systems. These studies have reported that the application of electrical fields to gel networks causes either an enhancing effect in mass transfer, a decreasing effect or no effect at all.

For example, mass transfer was enhanced when moderate electrical fields (MEF) were applied to the infusion of synthetic colorants in potato, potato-alginate mix and agar gels (Kemp and Fryer, 2007); the adsorption behaviour of bovine serum albumin to Cibracron blue Sepharose in an electrochromatographic column increased when electrical fields were applied, making mass transfer of protein in the adsorbent particles more effective (Jia *et al.*, 2007). In another study, mass transfer of aminomethylcoumarin to polyacrylamide gels prepared with inclusions of silica nanoparticles and processed under direct current (DC) electrical fields was enhanced when the electrical current applied was +3mA (Matos *et al.*, 2006).

On the contrary, mass transfer decreased when the direction of the electrical current was reversed to -3mA (Matos *et al.*, 2006). Water vapour, oxygen and carbon dioxide

permeability coefficients in chitosan coating films showed a positive correlation, decreasing with the application of electrical fields at 100 $V\,cm^{-1}$ or higher (Souza *et al.*, 2009).

Diffusion of rhodamine6G and methylene blue into gels of agar and alginate was quantified when electrical fields from 0 to 667 $V\,m^{-1}$ were applied, showing that there was no effect of the electrical fields on diffusion (Samprovalaki, 2005).

The extensive use of polymer networks in the food industry as materials for the delivery of actives (effusion) and other applications like food coating, highlights the importance of studying the effect of electrical processing with MEF on mass transfer and mechanical properties of polymer systems. In this research work, mass transfer into gel networks and compression force of gel matrixes under electrical processing were studied.

Sodium alginate is one of the polymer networks used in this study. Sodium alginate is a polysaccharide isolated from seaweed (Vitz and Waldman, 1998). The interaction of alginate with divalent metal ions such as Ca^{2+} is an almost instantaneous process governed by the relative rate of diffusion of the ions and polymer molecules into the gelling zone (Blandino *et al.*, 1999). Sodium alginate is used to thicken, suspend, stabilise and gelatinise food products (FAO, 1997). It is also used in ointments, creams, and shampoos. In plant tissue, alginates are used to produce insoluble artificial seeds, in the pharmaceutical sector for immobilising enzymes by entrapment and encapsulation of actives. Another use is the encapsulation of biocatalysts within hydrogels (Blandino, 2003).

Gellan gum was also used in the experimental part of this thesis. Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by the bacterium *Pseudomonas elodea.* As for alginate, the introduction of divalent cations such as Ca^{2+} or Mg^{2+} promotes the gelation of aqueous solutions of gellan

gum (Moritaka *et al.*, 2003). Gellan gum is mainly used as thickening agent, gelling agent and stabiliser.

Entrapment in beads of gel is a widely used technique for immobilising living microbial and animal cells (Martinsen *et al.*, 1987; Mattiasson, 1983), controlled flavour release in mouth (Lian *et al.*, 2004), controlled drug release in stomach and intestine (Anal and Singh, 2007; Rastogi, 2006) and spherification for cooking (Adria *et al.*, 2006). Gel particles or gel capsules combine macroscopic structure formation, the ability to flow and an attractive soft solid texture (Burey *et al.*, 2008). Alginate beads are usually produced by spraying an aqueous solution of sodium alginate into a gelling agent, using a mechanical vibration nozzle or air jet. In order to change the mechanical and mass transfer properties of the matrixes produced, the concentrations of alginate and ionic solution are modified.

Nonetheless, electrical fields have been shown to be a potential method for modifying mechanical and textural properties of ionic polymer networks. Gel beads treated under a constant DC electrical field showed a general shrinkage effect that made them stronger and more rigid (Zvitov and Nussinovitch, 2003). pH gradients for ionic and non-ionic gels were experimentally detected when the gels were exposed to stationary electrical fields and found to affect the swelling ratio of the gels (Hirose *et al.*, 1991). Meanwhile, Souza *et al.* (2009) suggested that the presence of MEF during the preparation of chitosan coating solutions may influence their transport properties and proposed the use of electrical processing to tailor edible films and coatings for different applications such as increasing the shelf life of products.

The general aim of this study could be divided into the following specific aims for each of the experimental sets done:

- ✓ To visualise the effect of the application of MEF on mass transfer in cellular material at different conditions (temperature, electrical field strength and position of the slab respect to the electrical field).
- ✓ To study the effect of MEF on mass transfer in model foods like polymer gels due to their extensive use in food industry as materials for the delivery of actives and other applications where the infusion of substances is a non-desired process.
- ✓ To study the effect of the application of MEF during the formation of biopolymer gel particles in order to identify the changes in compression force of the particles.
- ✓ The application of PEF to alginate gel cylinders and slabs, in order to identify the effect of the treatment on the compression force of the gel sample.

The experimental system proposed in this study is based on the use of continuous alternating current at MEF strength (up to 1400 $V\,m^{-1}$) and constant frequency of 50Hz for the electrical processing; and a jacket system processing cell that can extract the heat generated during the application of the electrical fields.

1.1. THESIS OUTLINE

Chapter 1 covers an introduction to electrical processing to food materials, the aims of this research work and the relevance of it in the discipline.

Chapter 2 reports a review of the literature and previous studies on the structure of natural (cellular) food materials and gel networks, thermal and non-thermal food processing, theory of diffusion or mass transfer in food processing, different methods for modifying mass transfer and the effect electrical processing has on food material.

Chapter 3 includes a description of the materials, measurement and calibration techniques and experimental procedures used in the three experimental chapters performed (Chapters 4, 5 and 6).

Chapters 4, 5 and 6 start with a brief description of the work done followed by the results obtained from the experiments, a discussion of them and the comparison and reference to results reported in similar studies.

The first set of experiments performed is described in Chapter 4; it centres on mass transfer in cellular material under the application of MEF. The experimental procedure allows visualising the effect of the application of MEF on the extraction of betanin from beetroot. The differences in the amount of betanin extracted from beetroot at different temperatures, electrical field strength and position of the slab in respect to the electrical field were measured. It was not an objective of this work to optimise the system for maximal extraction of pigment from beet tissue, but to use this material and its properties (betanin colorant in specific) to demonstrate the ability of low to moderate AC electrical fields to modify the extraction. One of the advantages of this study, compared with previous similar studies in the extraction of betanin from beetroot, is that the release of betanin is measured in a continuous system using a simplified set-up and procedure, where the effect of electrical processing, even at moderate strength levels of the electrical field, can be measured. The experimental set-up and diffusion cell apparatus designed for the experiments with cellular material were used in the experiments with gel systems.

After the chapter covering experiments with cellular materials, Chapter 5 contains the experiments on mass transfer of solutes into gel networks. The objective of this part of the research was to investigate the effect of the application of alternating MEF on mass transfer of solutes into gel networks set thermally or with ions; continuing until some extent in the

research path of Kemp (2000) and having its work as a reference for the effect of MEF on mass transfer in gel systems. This study differs from Kemp (2000) in: the geometry used (a cylinder instead of a slab); the electrical processing cell; the use of gels set by different gelation methods; and, in experiments with polymer matrixes set ionically, varying the electrical conductivities of the gel network and the solution containing the solute to be infused. The variations enabled the identification of the effect of MEF on mass transfer. It was not intended to raise questions or try to invalidate previous similar research, but to extend the field of study.

Chapter 6 focuses on the experiments performed to study the changes in mechanical properties of gel systems under the application of MEF and PEF. This part of the study can be divided in two sections: the 1st one focuses on the application of MEF during the formation of biopolymer gel particles in order to identify the changes in mechanical properties of the particles; whilst the 2nd one covers the experiments where PEF were applied to alginate gel particles in order to identify the effect of the treatment on the mechanical properties of the gel sample, specifically force in compression. The 'spherical' particles (with an average 5mm diameter) were formed by dropping alginate solution in a solution of $CaCl_2$ under the application of electrical fields ranging from 0 to 1400 $V\,m^{-1}$. PEF were applied to alginate cylinders and slabs after their formation and the compression force of the gel particles was measured after the electrical treatment. The experiments corresponding to the application of PEF were carried out in Lund University, Sweden.

Chapter 7 summarises the general conclusions and makes suggestions for future work in the area of electrical processing of food material.

CHAPTER 2

2. LITERATURE REVIEW

2.1. INTRODUCTION

In Chapter 1, electrical processing was mentioned as an effective process for applications such as pasteurisation in the food industry; its potential as a treatment for modifying mass transfer was also presented. Chapter 2 includes an overview of previous research work in the topics: food material structure, food processing, diffusion in food material and electro-kinetic phenomena.

2.2. FOOD MATERIAL STRUCTURE

The food materials used in this study were beetroot as cellular material and calcium alginate, gellan gum, egg white albumen and gelatine as gel systems. A general review of their structure is essential in understanding the potential effect the application of electrical fields has on them.

2.2.1. Cellular material

The following description was principally taken from Kallem and Tauberer (1999):

Plants are eukaryotes, organisms whose cells have a nuclei and organelles enclosed by a membrane. The cell membrane and the cell wall cover the cell and the specialised structures inside it, including a central vacuole, plasmodesmata and chloroplasts (Fig. 2.1).

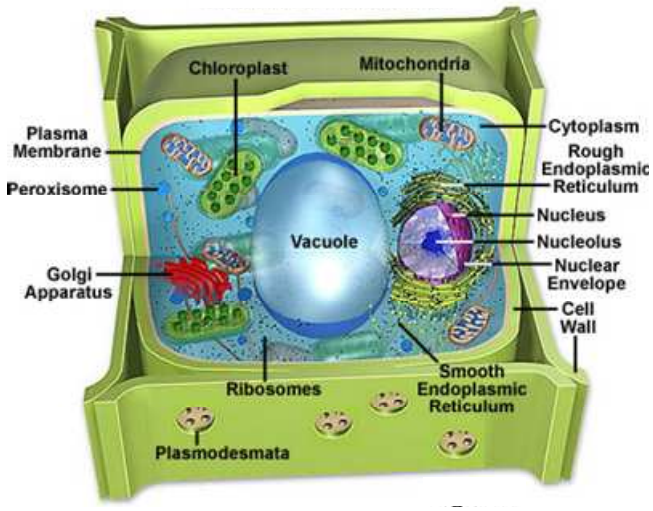


Fig. 2.1. Anatomy of a plant cell (Davidson, 2003).

The main functions of the cell membrane are to separate the cell from the external environment, to give physical protection and to regulate the transfer of materials into and out of the cell. The cell membrane is selectively permeable and consists mainly of molecules called phospholipids. Phospholipids have two parts: a polar phosphate "head" and two non-polar fatty acid "tails" (Fig. 2.2).

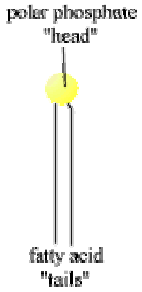


Fig. 2.2. Diagram of a phospholipid (Kallem and Tauberer, 1999):

The phospholipids are arranged in a double layer, the polar heads face the external and internal sides of the cell and the fatty acids form the inside of the membrane (Fig. 2.3). The phospholipids can move around exchanging places with other phospholipids, giving the cell membrane ability to stretch and change shape. Large protein molecules are also contained

in the cell membrane body extending either from one phospholipid to the other, or remaining on one side.

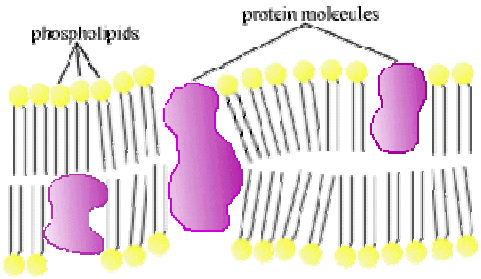


Fig. 2.3. "Double layer" representation of the cell membrane (Kallem and Tauberer, 1999).

The cell wall has three main parts: the primary cell wall, the middle lamella, and the secondary cell wall. The primary cell wall, made mostly of cellulose, is located closest to the inside of the cell and allows the wall to stretch as the cell grows. The middle lamella, is composed of polysaccharides called pectin while the secondary cell wall contains both cellulose and lignin. Lignin strengthens the wall and gives the cell a somewhat rectangular shape (Kallem and Tauberer, 1999).

Each plant cell has a large, single, central vacuole that stores compounds, helps in plant growth and plays an important structural role for the plant.

2.2.1.1. Beetroot and betanin

Beetroot (*Beta vulgaris L*) is a vegetable that has been chosen in many previous works where mass transfer was studied since it contains a soluble natural colorant betanin that is relatively simple to extract.

Beetroot cells contain a complex mixture of salts of betalamic acid called betalain pigments: red-purple betacyanins with betanin contributing to 75-95% of the red colour and yellow

betaxanthins with vulgaxanthin-I contributing to 95% of the yellow colour (Kujala *et al.*, 2001). The stability of betalains is affected by temperature, pH, oxygen, light, and water activity (Sapers and Hornstein, 1979).

Anthocyanins are also pigments present in plants but it is important to mention that these two groups of natural colorants have never been found together in the same plant (Socaciu, 2007).

Betanin is a pigment used in food industry as colorant for some desserts, gelatines, yogurt and cold dishes. Betanin resists acidic condition but it is damaged with heat, especially in presence of air (O_2), altering its colour from red to brown. E-number for betanin dye is E-162. The molecular weight of betanin is 564$g\ mol^{-1}$ (Nottingham, 2004).

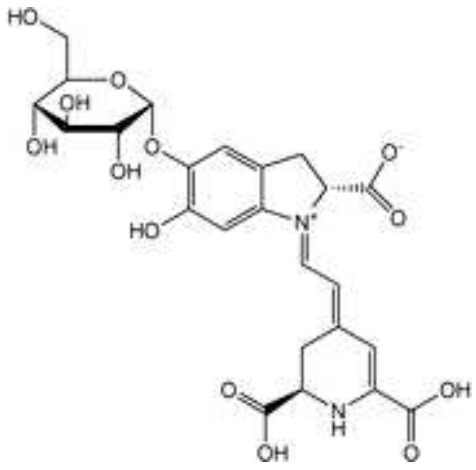


Fig. 2.4. Chemical structure of betanin (Wikipedia, 2010).

Betanin is found in the cell vacuole which also has a membrane helping to contain the betacyanins. Nevertheless, when the vacuole membrane or the cell membrane are affected by heat shock, by detergents or by solvents (e.g. ethanol or acidified methanol), the pore size increases and betanin molecules will acquire enough energy to move through the membrane by active transport and leak out of the cell.. Leakage of these betacyanins into the

external solution can be used as an indication of membrane permeability changes (White, 2003).

Fig. 2.5. Red beetroot (left) and a slab taken from the central part of it (right).

2.2.2. Model foods (Gels)

2.2.2.1. Alginate

Alginate and a mix of potato powder and alginate mix (model- experimental system) were chosen as the "ionically set" gels in Chapters 5 and 6 of this study. Their gelation is based on the affinity to calcium ions.

Sodium alginate is a polysaccharide isolated from seaweed composed of D-mannuronic acid and L-guluronic acid subunits (Vitz and Waldman, 1998). Upon interaction with divalent metal ions, such as Ca^{2+}, alginates form an ordered and irreversible gel structure. The gelation process involves binding of Ca^{2+} ions between aligned guluronic residues of two alginate chains, giving the gel a structure similar to an egg box (Grant *et al.*, 1973), Fig. 2.7. The gelation of calcium alginate is an almost instantaneous process, governed by the relative rate of diffusion of Calcium ions and polymer molecules into the gelling zone (Blandino *et al.*, 1999).

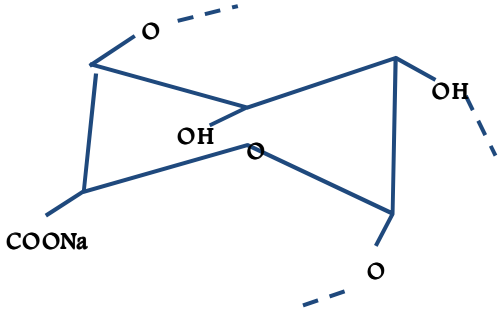


Fig. 2.6. Structure of sodium alginate.

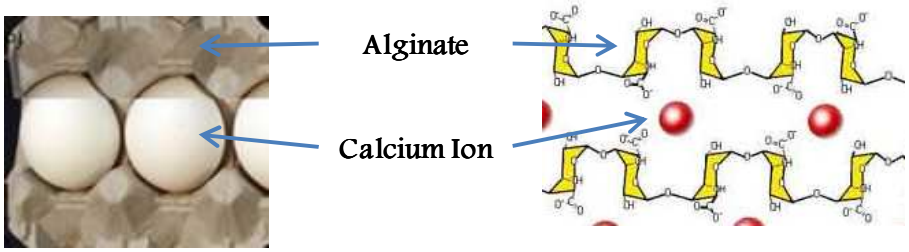


Fig. 2.7. Cardboard eggs-box (left) and Calcium alginate structure (right) (Calvo, 2003).

Sodium alginate is used to thicken, suspend, stabilise and gel foods and cosmetics (FAO, 1997). In the pharmaceutical sector, alginates are used for immobilising enzymes by entrapment and encapsulation of actives and to form highly absorbent and biodegradable medical dressings. Another use is the encapsulation of biocatalysts (Blandino, 2003).

The addition of dried potato to sodium alginate (Kemp, 2000) gave the mix a solid content similar to real potato but with the thermal resistance of alginate, and allowed diffusion coefficients of the same order of magnitude ($10^{-10} m^2 s^{-1}$).

2.2.2.2. Gellan gum

Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea* (McGee, 2004). The structure of gellan gum is presented in Fig. 2.8. As in sodium alginate, the introduction of divalent cations such as Ca^{2+} or Mg^{2+} in gellan gum promotes the gelation of aqueous solutions

forming a gel network (Moritaka *et al.*, 2003). Gellan gum is mainly used as thickening agent, gelling agent and stabiliser.

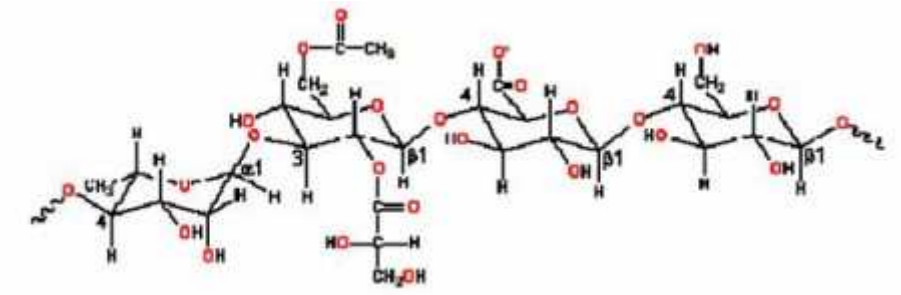


Fig. 2.8. Structure of gellan gum (Shah, 2007).

2.2.2.3. Albumin

Albumin is a globular (water soluble) protein. The most familiar examples of albumin can be found in egg whites (ovalbumin) and in human blood. The albumin used in this study is derived from chicken egg white albumen. Albumen helps holding the structure and binding of different materials in applications such as baking, purification and the treatment of poisoning (Smith, 2009).

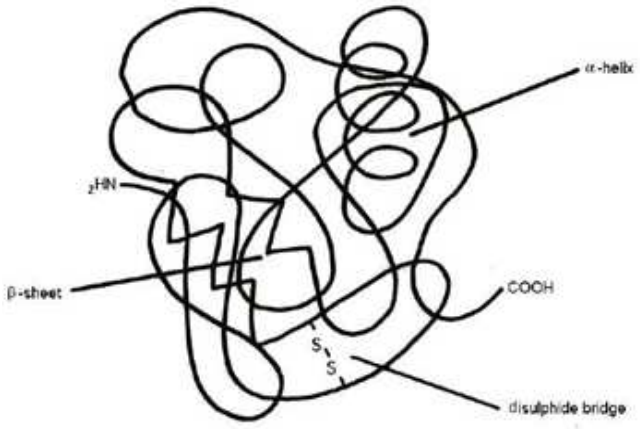


Fig. 2.9. Random coil of a globular protein (Faiers, 2007).

2.2.2.4. Gelatine

Gelatine is obtained from partial hydrolysis of collagen derived from skin, connective tissue and bones of animals. Pork skin gelatine was used in this study. Gelatine melts at 37°C and becomes firm when cool. Gelatine is used in the food industry as an ingredient for desserts, confectioneries, and as a binding and glazing agent in meat. In the pharmaceutical industry, it is used in hard and soft capsules for medicines and supplements (GEA Process Engineering Inc., 2010).

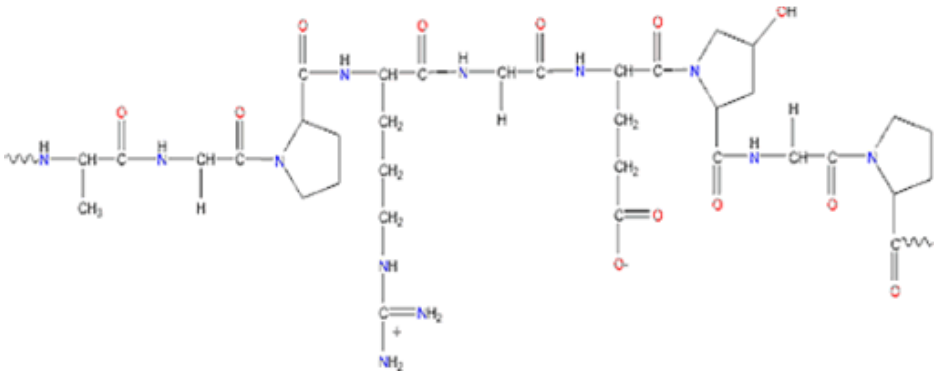


Fig. 2.10. Gelatine, molecular structure (GEA Process Engineering Inc., 2010)

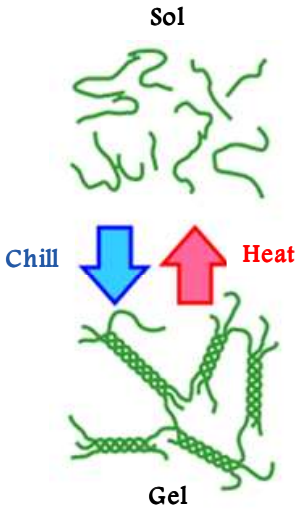


Fig. 2.11. Transition of gelatine from sol to gel (Nitta Gelatine India Limited, 2009).

Being proteins, both albumin and gelatine denaturate (unfold) and coagulate when heated up and during this process, protein loses its tridimensional structure and forms new bonds joining other proteins and forming a gel network.

2.2.3. Dye to be infused to gel networks.

Rhodamine6G ($C_{28}H_{30}N_2O_3$.HCl), is a synthetic fluorescence dye and chemical compound of 479.02$g\ mol^{-1}$ molecular mass and an ionic charge of +1 at pH 7 (Fluka catalogue UK, 2009). Rhodamine6G is widely used as a tracer within water to determine the rate and direction of flow and transport; its cost is relatively low. It was chosen in Kemp (2000) and Samprovalaki (2005) as one of the colorants to be infused in alginate, agar and carragenan gel matrixes, since infusion of the colorant to the gel was easy to visualise using optical light microscopy. Molecular structure of rhodamine6G is presented in Fig. 2.12.

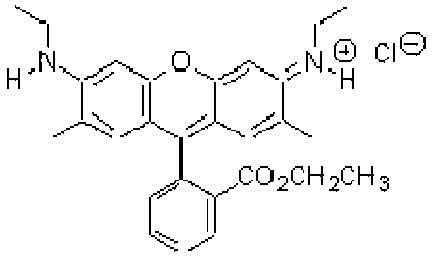


Fig. 2.12. Molecular structure of rhodamine6G (Prahl, 1998).

2.3. FOOD PROCESSING

Food processing involves the transformation of raw material or plant materials into consumer-ready products (Hogan *et al.*, 2005). Food producers intend to develop processes that help to retain or create the sensory and quality characteristics the consumer demands while reducing detrimental changes in the materials and products. The development of

alternative technologies for food processing helps to provide nutritive, safe and fresher-tasting food avoiding the use of heat or chemical preservatives (Hogan *et al.*, 2005).

2.3.1. Thermal processing of food material

Thermal processing involves heat transfer into the food from hot surroundings, with the difference in temperature being the driving force for the process. Thermal processing is applied in food industry to increase or reduce the rates of reactions in food, i.e., to concentrate and evaporate using heat; in sterilisation of foods heat is used to reduce to <12log pathogenic microorganisms in order to prevent decomposition and increase shelf life. Thermal processing is also necessary to obtain some particular sensory and textural attributes. For example, in the conversion of the dough from foam to sponge phase in bread baking, achieving a core temperature of about 92 to 96°C at the end of baking ensures that the product structure is fully set (Cauvain and Young, 2000).

Although in the mentioned cases thermal processing is necessary to ensure food safety and increase palatability, degradation of valuable components will also occur, i.e. alterations in taste, colour and aroma, causing some change in nutritional values (Kemp, 2000). Consumers desire long shelf life products which resemble those of a fresh product and also have high nutritive value.

The mechanisms that affect food material at high temperature in thermal processing include internal and dynamic changes:

- ✓ Internal changes: the structure of the food product is altered due to the rupture of cells membranes caused by denaturation of the proteins in the membrane or fractures in the lipid bilayer.

✓ Dynamic changes: there is an exchange of solutes between food solids and a surrounding solution.

Conventional cooking tends to be slow and the surface of the food always receives a more severe heating treatment than the rest of the food.

A number of novel non-thermal processing methods, such as high pressure, pulsed electric fields, UV, irradiation, microwave and radio frequency, are in active research and development in industry, academic and government sectors to overcome the problems that result from the use of thermal processing.

2.3.2. Thermal processing for microbial and enzyme inactivation

To provide the consumers with safe food, the materials have to be treated and the pathogens and microorganism killed. Examples of this are the use of thermal processing for the pasteurisation of milk and the sterilisation of canned food, in both processes it is necessary to achieve the required microbial inactivation with the lowest damage to the food structure as possible. The death of microorganisms can be expressed in terms of the temperature dependence of the rate constant, using the Arrhenius equation:

$$k' = \boldsymbol{\gamma_r} exp\left(-\frac{e_r}{GT}\right) \qquad \textbf{(2.1)}$$

Where k' is the rate constant, γ_r is a pre-exponential factor [min^{-1}], e_r is the activation energy [$J\,mol^{-1}$], G is the gas constant [$J\,mol^{-1}\,K^{-1}$] and T is the temperature [K].

In food industry, the sterilising effect of heat in a process is quantified by parameters such as integrated lethality. Integrated lethality is expressed as (Hallstrom *et al.*, 1988):

$$U = \int_0^t 10^{\frac{T-T_{ref}}{z}} dt \qquad \textbf{(2.2)}$$

Where U is the integrated lethality [min], T is the temperature [°C], T_{ref} is the reference temperature [°C] and z is the slope of the line of t_{Mic10} versus T_{Mic10}. t_{Mic10} is the time needed

for a reduction in the amount of microbes by a factor of 10 [min]. T_{Mic10} is the change in temperature needed to change D by a factor of 10 [°C].

In pasteurisation process, the pasteurisation unit (PU), a key standard for thermal load in relation to microorganisms can be obtained with (Shapton *et al.*, 1971):

$$PU = \int_0^t 10^{\frac{T-T_{ref}}{z}} dt \qquad (2.3)$$

where Z is the increase or decrease in temperature required to increase the decimal reduction time by one order of magnitude [°C]. Shapton *et al.* (1971) use a Z value of 10°C and a reference temperature of 80°C.

2.3.3. Alternative thermal processing

2.3.3.1. Microwave and radio-frequency processing.

Both microwave and radio-frequency processes cook or heat food by dielectric heating. The explanation for this phenomenon is that when an electromagnetic field is applied to material containing polar molecules, due to the presence of an electrical dipole moment those molecules will rotate and align themselves (Sumnu and Sahin, 2005). In an electromagnetic wave, where the field oscillates, the polar molecules will rotate continuously to align within the field. This is called dipole rotation.

Dielectric heating transfers energy directly to the product, targeting it and not the surrounding air. This is why in dielectric heating the product interior gets hot faster than the surface (Orsta and Raghavan, 2005).

A characteristic of radio-frequency process is that the electromagnetic energy has a longer wavelength than microwaves. In radio-frequency an electric field is developed between electrodes whilst in microwave a wave is propagated and reflected (Orsta and Raghavan, 2005).

Microwave and dielectric heating have been used in food industry and household appliances for reheating prepared foods, tempering of frozen foods, pre-cooking, pasteurisation of packaged foods and final drying of pasta products (Sumnu and Sahin, 2005). Previous works have shown the efficiency of these processes. In Huang *et al.* (2007) tea processed by microwave heating had significantly higher vitamin C and chlorophyll content and retained more chlorophyll during storage than oven heated samples, indicating a reduction in the decomposition of chlorophyll.

2.3.3.2. Dehydration using combined microwave-vacuum techniques

Fast low temperature dehydration is achieved when microwave and vacuum techniques are combined. The electromagnetic microwave energy penetrates the food where it is converted to thermal energy while vacuum reduces the boiling point of water keeping the product temperature low and creating a pressure gradient that enhances the drying rate. Drying can typically occur in minutes, compared to hours or days for air and freeze drying.

2.3.3.3. Ohmic heating

Ohmic heating consists of heating materials by the passage of alternating electric currents (Halden *et al.*, 1990); the food material heats as a result of its inherent electrical resistance (Fryer and Davies, 2001). The phenomenon was first explained by James Prescott Joule, this is why ohmic heating is also called Joule heating or electrical heating.

The equation governing ohmic heating is:

$$\frac{\partial}{\partial t}\left(\rho c_p T\right) = \nabla(\lambda \nabla T) + Q \qquad (2.4)$$

Where ρ is the density [$kg\ m^{-3}$], c_p is the specific heat capacity [$J\ kg^{-1}K^{-1}$], λ the thermal conductivity [$W\ m^{-1}K^{-1}$] and T is the temperature [K]. The term Q [W] is introduced to include the heat generated by the passage of current (de Alwis *et al.*, 1989).

$$Q = \sigma E^2 \quad \textbf{(2.5)}$$

Where E is the electrical field strength [$V\ m^{-1}$] and σ is the electrical conductivity [$S\ m^{-1}$].

The voltage distribution is given by:

$$\nabla(\sigma \nabla E) = 0 \quad \textbf{(2.6)}$$

Voltage distribution depends on both the distribution of electrical conductivity within the medium and the system geometry. In ohmic heating, the controlling factor is the electrical conductivity of the material being heated up. Food materials containing water in excess of 30% and dissolved ionic salts have been found to be suitable for ohmic heating, whilst non-ionised materials such as oils, fats, syrups and sugar do not conduct sufficiently the electric current applied (Halden *et al.*, 1990).

Electrical conductivity can be defined by using the equation (Rahman, 1999):

$$\sigma = \left(\frac{1}{R}\right)\left(\frac{L_{Gap}}{A_{Mat}}\right) \quad \textbf{(2.7)}$$

Where σ is the electrical conductivity [$S\ m^{-1}$], L_{Gap} is the distance between electrodes [m], A_{Mat} is the area of the test material in contact with the electrodes [m^2] and R is the resistance [Ω].

Conductivity of foods shows a linear increase with temperature at sufficiently high electric field strength (Biss *et al.*, 1989):

$$\sigma_T = \sigma_{25}[1 + d(T - 25)] \quad \textbf{(2.8)}$$

Where σ_T is the electrical conductivity [$S\ m^{-1}$] at a temperature T [°C], d is the proportionality constant [$°C^{-1}$] and σ_{25} is the reference electrical conductivity of the material at 25°C.

When food particles are large or have poor heat transfer properties, processing times for conventional heating are long and as a result, the quality of the product is reduced. The same problem occurs in the case of multiphase foods (either in cans or aseptic processing),

where the applicability of conventional thermal processing is limited due to the time needed to conduct heat to and from the centres of the particles.

Since in ohmic heating the food material heats from the inside to the outside (the interior of the food particle heats faster than the liquid surrounding it), it has the potential to heat faster and to overcome heat transfer problems in multiphase food and food particles. Electrical conductivity of the mixture and the amount of voltage applied significantly affects the heating rate of the solid-liquid mixtures during ohmic heating.

When ohmic heating is applied to a two phase mixture, if the liquid and solid have the same electrical conductivities, the two phases will generate heat at the same rate. If the phases have electrical conductivities significantly different from one another, it will result in "shadow regions" with large differences in temperature between the liquid and the solid (Davies *et al.*, 1999).

In general, different studies previously done to characterise food material according to its electrical conductivity have demonstrated an increase in electrical conductivity during heating. Halden *et al.* (1990) studied the effect of conventional and electrical heating on the electrical conductivities of cylinders of pork and different vegetables. It was found that the effects occurring in conventional heating, such as starch transition, melting of fats and cell structure changes, affect the electrical conductivity (Fig. 2.13).

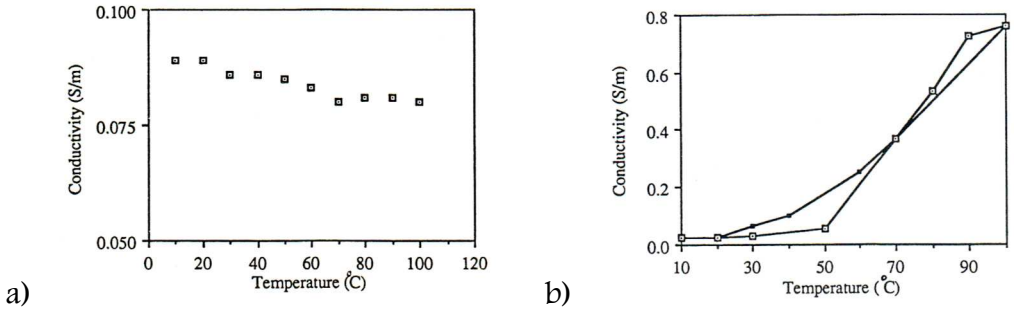


Fig. 2.13. Variation of electrical conductivity of a) pork meat with temperature during ohmic heating and b) beetroot with temperature during conduction (□) and ohmic heating (■) (Halden *et al.* 1990).

During the ohmic heating of corn and potato starch, Sastry and Wang (1997) observed that electrical conductivity increases with temperature but decreases with degree of gelatinisation. For carrot-starch mixtures the electrical conductivities increased linearly with temperature and decreased as the particle size and concentration of the samples increased (Zareifard *et al.*, 2002). Ohmic heating of hydrocolloids was found to be more efficient as the concentration of the hydrocolloids increased and was also uniform throughout the whole sample (Marcote *et al.*, 1998).

Ohmic heating has been used extensive and successfully in pasteurisation and sterilisation of food materials, resulting in products of an excellent quality. Eliot-Godéreaux and Goullieux (2001) applied ohmic heating to the sterilisation of cauliflower. Their results showed firm cauliflower florets stabilised at 25 and 37°C. In research done by Icier *et al.* (2007), ohmic heating was used to deactivate polyphenoloxidase from fresh grape juice. Critical deactivation temperatures for the enzyme were found to be around 60°C for electrical fields of 40 $V\ cm^{-1}$ and 70°C for 20 and 30 $V\ cm^{-1}$, regardless the processing temperature.

In similar studies, Ayadi *et al.* (2004) and Berthou *et al.* (2004) focused on the fouling of an ohmic heating unit with whey protein and β-lactoglobulin-xanthan gum mixture solutions,

to have an understanding of the fouling and consequently, cleaning of heat exchangers used in dairy processing. Their conclusions were that ohmic processing generates fouling on electrode surfaces causing an increase in electricity consumption and in the temperature of the electrode surfaces; the dissipation of the electrical energy into the deposit layers causes an increase in its temperature until boiling point is reached and boiling process and the removal of the fouling deposit starts. Ohmic heating processing has comparatively lesser heater cleaning requirements than those of traditional heat exchangers due to reduced product fouling on the food contact surface (Ramaswamy *et al.*, 2004).

The novel and effective use of ohmic heating for processes such as blanching, evaporation, dehydration, fermentation and cooking and extraction has been covered in previous works. Frozen blocks of shrimp were thawed ohmically (Roberts *et al.*, 1998) with the advantage of eliminating the use of water and being more energy efficient.

In comparison to microwave and no heating (Lakkakula *et al.* 2003), ohmic heating was probed to be an effective method for rice bran stabilisation with moisture addition, while increasing at the same time the total of lipids extracted from the rice bran to a maximum of 92%.

Beef muscle and ground beef were cooked conventional and ohmically (Icier *et al.*, 2009; Icier and Bozkurt, 2009). For both meats, ohmic cooking was faster, resulting in firmer meat with a fat retention to conventional cooking. The reduction in volume during cooking was also significantly smaller and the voltage gradient applied did not affect the quality of the cooked meat. Ohmic heating for assisting extraction and tissue damage will be considered in section 5 of this Chapter.

In general, ohmic process offers several advantages over other heating methods. Some of these advantages are: decrease of fouling of heat transfer surfaces, heating of particulates along with carrier fluid and gentle handling of particulates with no mechanical working (Biss *et al.*, 1989).

2.3.4. Non – thermal processing for food preservation

Although conventional thermal pasteurisation assures the safety of food products (Geveke, 2005) and can enhance their palatability, it also affects their sensorial and nutrient attributes, which in turn influences the quality of the products by destroying heat-sensitive nutrients and food product qualities such as flavour, colour and texture. New non-thermal methods for food preservation have been sought during the last years to overcome the disadvantages.

2.3.4.1. High pressure

In high pressure processing, the food product is placed in a pressure vessel capable of sustaining the required pressure and then submerged in a liquid, which acts as the pressure transmitting medium (Geveke, 2005). High pressure pasteurisation is able to preserve the nutritional substances in juices of fruits and vegetables. In a study done by Houška *et al.* (2006), high pressure pasteurisation process (500MPa for 10min) achieved a 5log reduction in viable microorganisms present originally in raw apple-broccoli juice and the final product was free of coli-form bacteria, yeast, moulds and salmonella during 30 days of storage at the chilled room temperature conditions (temperature up to 5°C).

2.3.4.2. Radio-frequency electric fields

Radio frequency electric fields (RFEF) have been shown to inactivate bacteria in apple juice at moderately low temperatures (Geveke, 2005). In RFEF, the power supply is continuous and high electric fields are produced by placing a liquid between two electrodes that are in

close proximity to each other and applying a high voltage to the electrodes. The population of *Escherichia coli* K12 in apple juice exposed to an electrical field of $20 kV\ cm^{-1}$ at 21Hz was reduced by 2.7log at 60°C (Geveke, 2005). In a previous study, Geveke *et al.* (2002) showed that microbial inactivation at low intensity radio frequency electric fields (less than $5 kV\ cm^{-1}$) was only due to heat but at higher intensities ($30 kV\ cm^{-1}$) suspensions of *Saccharomyces cerevisiae* in water were inactivated at non-thermal conditions, demonstrating the dependency of the inactivation on electric field strength at high intensity radio frequency electric fields.

2.3.4.3. High intensity pulsed light

High intensity pulsed light technology uses very high-power and very short-duration pulses of radiation (included in the spectra of ultraviolet UV, visible VL and infrared IR light) emitted by inert-gas flash lamps. In the food industry, pulsed light technology can be used to sterilise or sanitise foods, surfaces, plants and media involved in food processes. The electromagnetic radiations are emitted and propagated by means of waves which differ in wavelength, frequency and energy. The light radiation of energy hits the surface of a body, part of it is reflected by the surface, and part of it is absorbed by the body layers and transmitted to inner layers (Palmieri and Cacace, 2005).

The most accepted hypothesis for the mechanism of inactivation of microorganisms by pulsed light consists in the combination of a photochemical mechanism, which involves lethal effects on some constituents of the microbial cells, and photothermal mechanism, which involves a temperature increase produced by heat dissipation of the light pulse penetrating the material (Barbosa-Cánovas *et al.*, 2000).

A great inactivation effect of pulsed light was found by Dunn *et al.* (1995) with more than 7logs of *Aspergillus niger* spores inactivation by just a few light pulses in continuous UV light.

2.3.4.4. Ultrasound

The use of ultrasound for the inactivation of microorganisms was first observed by Harvey and Loomis (1929) when the reduction in light emission from a seawater suspension of rod-shaped *Bacillus fisheri* was examined. The microbial death was attributed to cell disruption by cavitations. The proposed mechanism of microbial killing by ultrasound is mainly due to thinning of cell membranes, localised heating and production of free radicals (Butz and Tauscher, 2002; Fellows, 2000).

Ultrasound as preservation technology has had a "revival" in the last 15 years (Mason *et al.*, 2005; Mason *et al.,* 2003). The use of ultrasound power in food processing covers a wide range of mechanical, chemical and biochemical effects. Used in combination with other non-thermal technologies like high pressure and magnetic fields, ultrasound reduced contamination by *E.coli* ATCC 11775 in model systems (San Martin *et al.*, 2002).

2.3.4.5. Irradiation

Irradiation is a physical treatment that consists of exposing food material to the direct action of electronic or electromagnetic rays (ionising radiation) that range from radio waves to high energy X-rays and gamma-rays (Lacroix, 2005). The irradiation sources that are permitted for use in food processing are gamma-rays produced from the radioisotopes cobalt-60 (1.17 and 1.33MeV) and caesium-137 (0.662MeV). The treatment applied is characterised by the irradiation dose, which is the amount of energy absorbed by the food when it is exposed to the irradiation field. The biological effects of irradiation are the damage in the genetic material and a lesion of the DNA, causing death of the cell; this is

why irradiation is principally used to inactivate pathogen organisms and improve shelf-life of food (Lacroix, 2005).

Foods like poultry, red meat, fish, egg products, spices and other dry food ingredients have been found to be good candidates for radiation decontamination (Farkas, 1998). Kampelmacher (1984) recommended treatment doses of 3 to 5kGy for frozen poultry and 1.5 to 2.5kGy for chilled poultry for the effective reduction of the most resistant serotype of *Salmonella* by about 3log-cycles, and *Campylobacter* by a still greater rate.

2.3.4.6. Pulsed electrical fields (PEF) and high intensity pulsed electrical fields (HIPEF)

In the area of non-thermal technologies, pulsed electrical fields (PEF) is one of the most advanced, mainly because even at low processing temperature and short residence time an effective inactivation of microorganisms is reached, but it is still undergoing scientific evaluation (Toepfl *et al.*, 2005).

PEF technology involves the use of an external electric field for a few microseconds to induce local structural changes and a rapid breakdown of the cell membrane (Toepfl *et al.*, 2005). Although many different pulse shapes are suitable for PEF processing, the ones commonly used are either exponential decay or square wave pulses (Toepfl *et al.*, 2005).

Fig. 2.14 is a schematic representation of the waveforms of pulses used for PEF treatment (Angersbach *et al.*, 2009).

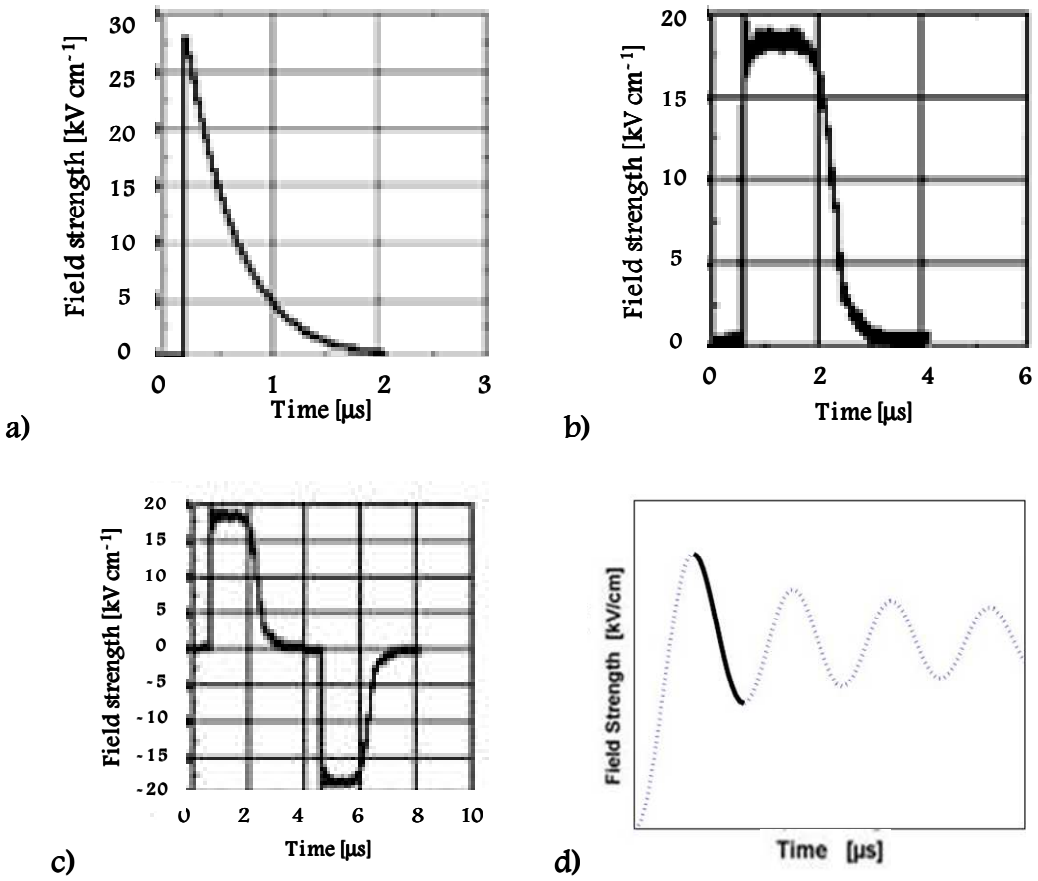


Fig. 2.14a) Exponential decay pulse, b) Rectangular pulse, c) Bipolar pulse and d) Oscillatory pulse (Angersbach *et al.*, 2009).

In order to affect the cell membrane, PEF should produce a potential difference. The potential difference (ΔφM) at the membrane of a biological cell with spherical shape and a radius h induced by the external electrical field E can be approximated by equation 2.9, derived from solving Maxwell's equations in ellipsoidal coordinates (Zimmermann *et al.*, 1974):

$$\Delta\varphi M = -f(\varpi)L_F E \qquad \textbf{(2.9)}$$

Where the local membrane potential difference ΔφM is given at the distance L_F from the centre in direction of the external electrical field (E). The shape factor f(ϖ) is a function of the three semi-axis ($\varpi_1, \varpi_2, \varpi_3$) of elliptical cells.

Zimmermann *et al.* (1974) reported that if the critical transmembrane potential exceeds a value of 1V, then a rapid electrical breakdown and local conformational changes of bilayer structures occur.

PEF have been shown to affect the integrity of the membrane of microbial and plant and animal cells in a short processing time; this advantage of PEF has been used for pasteurisation. Apple juice pasteurisation at high intensity pulsed electric fields (HIPEF) (electric field strength 50, 58 and 66$kV\ cm^{-1}$ and 2, 4, 8 and 16 pulses) combined with membrane ultrafiltration was proved to be a very efficient non-thermal preservation technique for microbial inactivation as well as in preserving quality attributes (Ortega-Rivas *et al.*, 1998). In another attempt to use PEF processing for apple juice pasteurisation, the results were also satisfactory, showing the potential of PEF treatment for liquid food decontamination (Heinz *et al.*, 2003).

The activity reduction of lipoxygenase (LOX) and ß-glucosidase (ß-GLUC) from strawberry juice in a continuous flow bench scale system for HIPEF treatment (35$kV\ cm^{-1}$) was effectively achieved at processing temperatures never higher than 40°C. The results also showed that monopolar rectangular pulses are more effective in reducing LOX activity than bipolar mode; and that the higher the pulse frequency and pulse width, the higher the ß-GLUC inactivation (Aguiló-Aguayo *et al.*, 2007).

If combined with heat treatment, PEF provides the possibility to develop a pasteurisation process at lower treatment temperatures compared to conventional heat treatment and extremely short residence times (Heinz *et al.*, 2003). HIPEF treatments have demonstrated a reduction in activity of enzymes that are involved in the formation of desirable flavour compounds, helping processors to obtain juices that keep their fresh flavour. (Aguiló-Aguayo *et al.*, 2007).

The mechanism of electro-permeabilisation is not completely understood yet. Parameters such as pulse shape, field strength, specific energy input per pulse and electrical conductivity of the cells affect the level of tissue and cell damage that could be achieved with PEF (Angersbach *et al.*, 2009).

2.3.4.7. Other applications of PEF processing for food material

Although PEF have been used mainly for microbial reduction, there are other applications where satisfactory results have been obtained. Gómez-Galindo *et al.* (2008) studied the metabolic responses of potato to PEF treatment at field strengths ranging from 30 to 500 $V\ cm^{-1}$ (single rectangular pulses). The metabolic response of potato tissue when it is affected by PEF involves oxygen consuming pathways; and is strongly dependent on pulsing conditions and independent on the total permeabilisation achieved by the pulse.

PEF was used to assist the clarification of synthetic and natural juices (Sarkar *et al.*, 2008). The electrical field was applied in both continuous and pulse mode at strengths ranging from 0 to 20 $V\ cm^{-1}$. The results obtained demonstrated that permeate flux increases with increase in electric field and pulse ratio. Pulsed electro-ultrafiltration was shown to be a promising alternative for clarification of citrus fruit juice and pectin containing solutions.

2.4. DIFFUSION

Molecular diffusion is mass transfer caused by concentration gradients, or more exactly, by gradients of the chemical potential. Diffusion occurs due to the attempt of the system to reach a position where the chemical potential is the lowest (equilibrium). In scientific terms, diffusion is the net movement of matter, attributed to the *random* movements of molecules, taking place from a region of high concentration to one of lower concentration (Agutter *et al.*, 2000).

Fig. 2.15 represents the diffusion of two different molecules in a container, separated by a barrier (a); when the membrane is removed (b) the pink molecules in the region of lower concentration go to the region with a high concentration of molecules in random movements (green line); until finally equilibrium in concentration is reached (c).

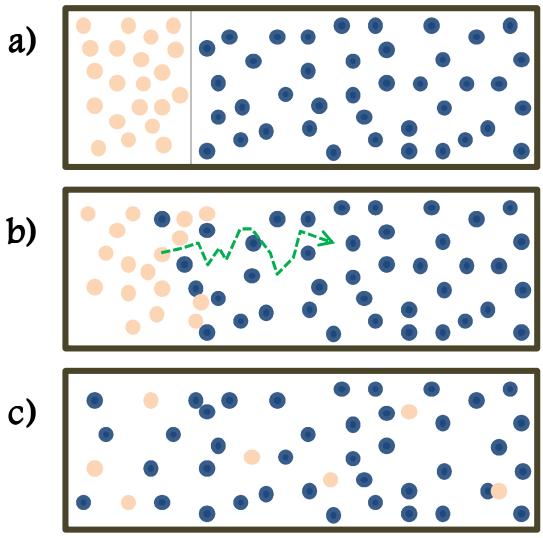


Fig. 2.15. Mixing of two substances, a representation of diffusion.

Two of the most used diffusion processes in food industry are leaching and infusion.

2.4.1. Leaching:

The term leaching can be defined as the extraction of certain materials from a carrier into a liquid (usually, but not always a solvent), also as the removal of materials by dissolving them away from solids (Wordnet, 2010). Depending on the process taking place, leaching could be either a beneficial or an unwanted step.

For many years leaching has been used to separate metals from their ores and to extract sugar from sugar beets (Bungay, 1997). The leaching of compounds from cellular material is limited by the degree of cell disintegration. For example, the extraction of betanin and sugars from beetroot and sugar beet for their numerous applications in food industry is

usually done using a multi-step method including extraction in a diffuser and pressing; the objective of the process is to damage either temporarily or permanently the cell membranes (Sugar Knowledge International, 2010).

2.4.2. Infusion:

Infusion is diffusion within a food solid (Wordnet, 2010). Infusion has been widely used for salting, pickling, addition of additives and smoking. In recent years, new and potential uses for infusion have attracted the attention of research and industry. These new processes include tenderisation of meats, citrus peel debittering, peach firming and the addition of nutrients in vegetables and fruits. Osmotic diffusion is the simplest method of infusion. The infusion of components is influenced by factors such as cell wall porosity and molecular size of the solute, and different process variables have to be set according to the type of food being infused and the active to be introduced.

The diffusion of a fluid (gas, liquid or solid) through a solid matrix or porous solid can be classified in two types (Geankoplis, 1972):

1. Diffusion is not a function of the actual solid structure.

It occurs when the fluid diffusing actually dissolves in the solid to form a homogeneous solution. i.e. in leaching the solid contains a large amount of water and the solute is diffusing through this solution. The solid is treated as a uniform material and the diffusion rate is predicted from Fick´s law.

2. Diffusion where the actual structure is important (porous solids).

Porous solids have interconnected voids or pores; as a result the diffusion rate is greatly affected by the type and size of the voids (Geankoplis, 1972). For homogeneous polymers the diffusion process can be considered as independent of the internal structure of the polymer.

There is an analogy between heat transfer by conduction and mass transfer by diffusion since both transfers are due to random molecular motions. Adolf Fick, in 1855, recognised this analogy and set the diffusion process on a quantitative basis with an equation derived from the equation proposed by Fourier, in 1822, for heat conduction.

The simplest mathematical expression for diffusion is known as Fick´s first law of diffusion (Crank, 1975):

$$J = -D\frac{dC}{dx} \qquad \textbf{(2.10)}$$

Where J is the diffusion flux [mol $m^{-2}s^{-1}$], D is the diffusion coefficient [m^2s^{-1}], C is the concentration [mol m^{-3}] and x is the position [m]. Fick´s first law of diffusion associates the molecular flux through a system to the concentration gradient in one dimension. The negative sign is introduced because diffusion occurs in the direction opposite to that of increasing concentration. A system is considered as Fickian when the diffusion coefficient is constant.

Mass transfer in food systems is described by Fick's second law of diffusion. Fick's second law predicts how diffusion causes the concentration field to change with time (t, [s]):

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \textbf{(2.11)}$$

In a few cases, Fick´s second law can be analytically solved if experimental data, as well as initial and boundary conditions are provided, in order to yield an effective mass transfer coefficient. Analytical solutions covering on varying specimen geometry are found in the book by Crank (1975). Numerical solutions are provided by computer programs which deal with the solution of the partial differential equations for momentum, heat and mass transfer, such as Navier-Stokes, Fourier and Fick.

The concentration profile curve method has often been used for the diffusivity of diverse substances, such as salt, small organic acids and aromas (Gross and Ruegg, 1987). The

method is based on the exact solution of Fick's equation for a semi-infinite medium using the method of "combination of variables". For the diffusion in a semi-infinite slab, the slab initially contains a uniform concentration of solute. At time zero, the concentration at the interface is suddenly increased. Diffusion occurs as the solute penetrates into the slab, producing the time-dependent concentration profile (Doulia *et al.*, 2000).

If the diffusion coefficient at constant temperature depends not only on the concentration, but also explicitly on time, the diffusion process is non-Fickian (Rehage *et al.*, 1970). Diffusion in polymers in rubbery state is generally Fickian because they respond rapidly to changes in their condition (volume); in glassy polymers diffusion is believed to be non-Fickian (Cussler, 1984).

2.4.3. Measurement of diffusion coefficient

Diffusivities for the infusion of rhodamine, methylene blue and fluorescein dyes in potato, calcium alginate gel and agar gel were estimated by Kemp (2000). Two types of diffusional process were considered: Fickian diffusion and Type 2 diffusion or displacement model. For describing the data in terms of Fickian diffusion, the diffusion coefficient D was estimated by using the following solution of Fick´s 2nd law:

$$\frac{C}{C_0} = 1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1} exp\left[-(2n+1)^2\pi^2\frac{H}{4}\right] cos\frac{(2n+1)\pi\tau}{2} \qquad \textbf{(2.12)}$$

Where H is dimensionless time $H=Dt/L_{Slab}^2$, L_{Slab} is the length of the slab into which the dye diffuses [m] and τ is the dimensionless distance $\tau=x/L_{Slab}$.

Since the D was found to depend on a number of variables, the experimental data was not represented by pure Fickian diffusion and a displacement model was used. For the displacement model, diffusion was modelled for a diffusion front moving into the material. The estimated D, for the case where no electrical field was applied, was used in equation

2.12 to fit the parameter ΔX, which is the front depth. The enhancement of diffusion was expressed with an enhancement factor ΔDif:

$$\Delta Dif = \frac{D_E}{D_0} \quad \textbf{(2.13)}$$

Where D_0 is the diffusion coefficient when no electrical field was applied [m^2s^{-1}] and D_E is the electrically enhanced diffusion coefficient [m^2s^{-1}]. For the displacement model used, the change in ΔX with electrical field strength is a measure of the enhancement in diffusion. For diffusion in alginate, both models fitted the experimental diffusion profiles at different process conditions.

Chalermchat *et al.* (2004) estimated the effective diffusion coefficient for the extraction of red pigments from thin slices of red beetroot by using an analytical solution of Fick´s 2nd law of diffusion and the superposition principle: the product of the infinite slab solution and the infinite cylinder solution for a finite cylindrical geometry found in Carslaw and Jaeger (1959) and Crank (1975). A fast (single) diffusivity described the process when high degree of permeabilisation of the red beet membrane was achieved, while at low levels of cell permeabilisation a bimodal diffusion model (with slow and fast diffusivities) was needed to describe the process effectively. As a consequence of the superposition principle, the concentration of the pigment at any point in the cylindrical sample can be determined by:

$$\frac{C}{C_0} = \Phi(x, l)\Psi(r, h) \quad \textbf{(2.14)}$$

Where C_0 is the initial concentration of the solute, 2l is the thickness of the slab.

Equation 2.14 is the product of the solution for an infinite slab of thickness 2l, **Φ**:

$$\Phi = \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{(2n+1)} \times exp\left(\frac{-D_{eff}(2n+1)^2\pi^2 t}{4l^2}\right) cos\frac{(2n+1)\pi x}{2l} \quad \textbf{(2.15)}$$

And the solution for a long cylinder with a diameter 2h, **Ψ**:

$$\Psi = \frac{2}{h}\sum_{n=1}^{\infty} exp\left(-D_{eff}\alpha_n^2 t\right) J_0(r\alpha_n)\alpha_n J_1(h\alpha_1) \quad \textbf{(2.16)}$$

The method of bimodal diffusion was proposed by Jason and Peters (1973) and Aguilera and Stanley (1990). In its simplest form the model is represented by the sum of two exponential terms containing diffusion coefficients in the exponents (Jason and Peters, 1973). For the extraction of red pigments from red beet, the equation for bimodal diffusion can be expressed as:

$$C_{ext} = \vartheta\{\varsigma(1 - C_1) + (1 - \varsigma)(1 - C_2)\} \qquad \textbf{(2.17)}$$

Where C_1 and C_2 are the relative average calculated concentrations in tissues type 1 and 2 corresponding to the diffusion coefficients $D_{eff,1}$ and $D_{eff,2}$, ς is fractional amount extracted corresponding to D_{eff1}, C_{ext} is the concentration in the solution surrounding the red beet and ϑ is the maximum amount extracted for the individual samples.

In another study, the effective sugar diffusion coefficient from a sugar beet was estimated by Lebovka *et al.* (2006) with a solution of Fick´s 2nd law taken from Crank (1975) for slabs of sugar beet of uniform thickness:

$$B = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}exp\left(-\frac{(2n+1)^2\pi^2 D_{eff}t}{4{L_{Slab}}^2}\right) \qquad \textbf{(2.18)}$$

Where B is the normalised °Brix of sugar beet juice, and L_{Slab} is the thickness of the slab. Only the first five leading terms of the summation were taken into account for D_{eff} estimation by least square fitting.

Doulia *et al.* (2000) remarked on the importance of the following matters taken into account for the general application of the Fickian approach for the study of diffusion in foods, and for the use of the effective diffusion coefficient (D_{eff}).

- ✓ The dependence of D_{eff} on concentration of the component being transferred. In this case the driving force for mass transfer is the difference in chemical potential and not the concentration difference (Gekas *et al.,* 1998).

✓ The dependence of D_{eff} on temperature. Application of an Arrhenius type relation in cases of sudden changes in the tissue structure is doubtful.

✓ The dependence of D_{eff} on volume changes taking place during dehydration (shrinkage) or re-hydration (swelling). In the majority of cases the influence of changes in volume is ignored. These effects are implicitly included in the value of D_{eff}.

✓ Evaluation of D_{eff} involves mass transfer occurs mainly by molecular diffusion mechanism. Coexistence of several other mechanisms of transport constitutes mechanical relaxation. The diagnosis of the exact mechanism is done experimentally with varying the thickness of a slice of food.

✓ In initial and boundary conditions the distribution coefficient between the two phases should be taken into account.

2.4.4. Diffusion in cellular material

As it has been already specified in this section, mass transfer in food systems can be described by Fick's 2nd law of diffusion, which in a few cases can be analytically solved if experimental data and initial and boundary conditions are given, in order to estimate an effective mass transfer coefficient (D_{eff}).

Diffusion coefficients of food material show variability due to factors such as season, weather conditions, variety difference and degree of maturity. The statistical analysis of diffusivity values of different types of foods shows that foods belonging to the same or relative category (i.e. meat, vegetables, fruits, food gels) have a typical value of effective diffusivity for a given temperature (25°C). For example untreated soft plant tissue at 25°C, with an average D_{eff} found to be $2x10^{-10} m^2 s^{-1}$ (Doulia *et al.*, 2000).

Table 2.1. Characteristic values of D_{eff}s of fruits and vegetables (Doulia *et al.*, 2000)

Category	Subcategory	Property or diffusant	Temperature [°C]	D_{eff} [$m^2 s^{-1}$]
Fruits	Apple raw	Moisture	30-70	0.9-28 x 10^{-10}
	Apple osm. process	Soluble solids	-	0.4-2.4 x 10^{-10}
	Bananas raw	Moisture	Warm air	0.3-2.7 x 10^{-9}
	Bananas osm. dried	Sucrose	-	1.3-4.3 x 10^{-10}
	Mango osm. dried	Moisture	50-70	6.0-10.2 x 10^{-10}
	Strawberries raw	Moisture	50	3.2-5.4 x 10^{-10}
Vegetables	Carrots diced	Moisture	15-100	5-24.1 x 10^{-10}
		NaCl	-	2.3-25.1 x 10^{-10}
	Carrots blanched	Sucrose	-	6-30 x 10^{-10}
	Onion	Moisture	25-65	0.2-2.3 x 10^{-10}
	Potatoes raw	Moisture	30-70	0.2-3.1 x 10^{-10}
		NaCl	5-120	2.5-44.5 x 10^{-10}
	Potatoes blanched	Reducting sugar	70-100	0.1-0.17 x 10^{-10}
	Soybeans	Moisture	50-78	0.5-3 x 10^{-10}
	Sugar beet diced	Moisture	40-84	0.4-1.3 x 10^{-10}
	Tomato diced	Moisture	60-80	6.6-23.6 x 10^{-10}

2.4.5. Diffusion in gel systems

A method to determine diffusion coefficients of solutes in extremely dilute solutions is to allow the solute to diffuse into or out of a gel. The method involves bathing the exposed surface of a gel in a cylindrical vessel with a constant concentration of solute. By measuring either the total amount of solute which penetrates the gel in a given time or by slicing the gel at the end of the experiment and measuring the concentration of solute as a function of distance from the interface, the diffusion coefficient can be determined (Lauffer, 1961).

Fick´s laws of diffusion can be applied to diffusion in gels. Fick's 2nd law of diffusion in a gel can be derived from Fick's 1st law by calculating the amounts moving into and out of an infinitesimal section. The equation obtained is of exactly the same form as Fick's 2nd law applied to diffusion of a solute in the absence of gel (Lauffer, 1961).

$$\frac{\partial C\prime}{\partial t} = D' \frac{\partial^2 C\prime}{\partial x^2} \qquad \textbf{(2.19)}$$

Essentially constant C_0' at x=0 can be attained experimentally by stirring continuously a volume of external solution large enough to contain an amount of solute much greater than the total which diffuses into the gel (Lauffer, 1961).

2.4.5.1. Diffusion in calcium alginate systems

Studies have found that the diffusion coefficient of glucose into calcium alginate gel is close to (Tanaka *et al.,* 1984) or smaller than (Hannoun and Stephanopoulos, 1986) the diffusion of glucose in water. For the diffusion of ethanol in calcium alginate gels the found values of diffusion coefficient were also smaller than for water (Merchant, 1987; Itamunoala, 1987; Axelsson and Persson, 1988).

In a study done by Hacimusalar and Mehmetoglu (1995), diffusion coefficients for ethanol and glucose were obtained by the moment analysis method. The effective diffusion coefficients of both glucose and ethanol are lower than the respective coefficients in water; these values decreased as gel concentration increased and increased with the rise in temperature (Table 2.2).

Table 2.2. D_{eff} values of glucose and ethanol at different gel concentrations (T=25°C, $C_{0glucose}$= 100$g\,l^{-1}$, $C_{0ethanol}$=10$g\,l^{-1}$) (Hacimusalar and Mehmetoglu, 1995)

Ca Alginate [%]	Solute	D_{eff} [$cm^2\,s^{-1}$] x10^{-6}
2	Glucose	5.29
3	Glucose	5.07
2	Ethanol	10.85
3	Ethanol	9.06
4	Ethanol	8.62

In another study about the diffusion of nitrate and glycerol through calcium-alginate gel beads, D_{eff}s for nitrate in gel beads of different alginate concentrations (calculated by the Mackie and Meares approach) were found to increase when the residence time of gel beads in calcium chloride solution was increased, and were found to decrease when alginate concentration was increased (Table 2.3). The experimental diffusion of glycerol (non-charged heavier molecule) occurred faster than the diffusion of nitrate. The different diffusion values were discussed to be caused by possible electrostatic interactions between

the negative charge of nitrate and calcium clusters around the polymer surface (Garbayo *et al.*, 2002).

Table 2.3. D_{eff}s for nitrate and glycerol in alginate gel beads (Garbayo *et al.*, 2002)

Ca Alginate [%]	Solute	D_{eff} [$cm^2\ s^{-1}$]
3	Nitrate	1.66×10^{-3}
4	Nitrate	1.64×10^{-3}
5	Nitrate	1.63×10^{-3}
6	Nitrate	1.63×10^{-3}
1	Glycerol	7.30×10^{-6}
3	Glycerol	6.72×10^{-6}
5	Glycerol	6.28×10^{-6}

The effective diffusivities for the diffusion of proteases (large molecules) within immobilised cells in calcium alginate beads were determined and compared with available values for small molecules (Table 2.4), showing that the simple correlation with the ~1/3 power could be useful. The study showed that the diffusion of some molecules can be limited by electrical or geometrical interactions between them (Longo *et al.*, 1992).

Table 2.4. D_{eff}s for proteases within immobilised cells in calcium alginate gel beads (Longo *et al.*, 1992)

Ca Alginate [%]	Solute	D_{eff} [$cm^2\ s^{-1}$] $\times 10^{-6}$	Reference
2	Oxygen	20.80-25.30	Kurosawa *et al.*, 1989
2	Xylose	6.40	Furusaki and Seki, 1985
2	Glucose	6.20-6.83	Tanaka *et al.*, 1984
2	Tryptophan	6.67	Tanaka *et al.*, 1984
2	α- Lactalbumin	1.02	Tanaka *et al.*, 1984
2	Bovine serumalbumin	None	Tanaka *et al.*, 1984
2	Bacilus total protease	1.04	Longo *et al.*, 1992
2	Bacilus metalloprotease	1.40	Longo *et al.*, 1992
2	Serratia total protease	0.89	Longo *et al.*, 1992

The D_{eff} of sucrose in calcium alginate gel containing *Saccharomyces cerevisiae* was investigated for two sizes of particle diameter involved (0.175cm and 0.3cm). The D_{eff}s were found to be similar for the two sizes of particle used. The average D_{eff} for sucrose in calcium alginate gel 5% w/v was found to be $4.05 \times 10^{-6} cm^2 s^{-1}$ (Mehmetoglu, 1990).

Table 2.5 includes a compilation of diffusion coefficients found by Samprovalaki (2005), for the diffusion of different solutes in alginate and gelatine gels.

Table 2.5. Values of D_{eff} in gels (Samprovalaki, 2005)

Product	Diffusant	Deff [$cm^2\ s^{-1}$] x 10^{-10}	Temp. [°C]	Reference
5.1% gelatine gel	Sucrose	2.8-4.1	5-20	Schwartzberg and Chao, 1982
Gelatine gels	Moisture	30-75	20	Boudrihoua, 2003
Gelatine gels	Moisture	10-50	10	Boudrihoua, 2003
Alginate 2%	A40926	28.6	-	Jovetic *et al.*, 2001
Alginate gum	Moisture	3	30	Doulia *et al.*, 2000
Alginate 1.2%	Sucrose	2.9	30	Handrikova *et al.*, 1996
Alginate gels	Pepsin	56.3		Amsden, 1998
Alginate gels	Ovalbumin	33.6		Amsden, 1998
Alginate gels	BSA	13.9		Amsden, 1998
Alginate gels	β-lactoglobulin	1.84		Amsden, 1998
Alginate membrane	Fructose	6		Venancio and Texeira, 1997
Alginate membrane	Glucose	6.7		Venancio and Texeira, 1997
Alginate membrane	Xylose	9.01		Venancio and Texeira, 1997
Alginate membrane	Lactose	4.66		Venancio and Texeira, 1997
Alginate membrane	Maltose	4.6		Venancio and Texeira, 1997
Alginate membrane	Sucrose	4.7		Venancio and Texeira, 1997

2.5. DIFFUSION ENHANCEMENT METHODS

2.5.1. High pressure and vacuum assisted diffusion.

High pressure treatment of food has resulted in enhanced mass transfer of solutes between food material and the liquid surrounding it. Corrales *et al.* (2008) made a comparison of 3 novel methods (ultrasound, high hydrostatic pressure [HHP] and pulsed electric fields) for the extraction of anthocyanins from grape to assist thermal extraction at 70°C. The total phenolic content of samples subjected to novel technologies was found to be 50% higher than in the control samples. Their explanation for the increase in extraction yields was the ability of HHP to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membranes which may lead to a higher permeability, similar to what Barbosa-Cánovas *et al.* (1998) proposed previously. It was also noticed that the decrease in the dielectric constant of water caused under HHP combined with thermal treatment leads to a decrease in the polarity of the media which may contribute to the higher levels of total phenolics and other antioxidants yielded (Corrales *et al.*, 2008).

The mechanism for diffusion enhancement in both high pressure and vacuum methods is not yet clear. Any enhancement is mainly believed to be due to the denaturation of bound proteins in the membrane of the cell (in the case of high pressure) and due to the compression of gas in products containing gas voids and the substitution of the gas with liquid (Dörnenburg and Knorr, 1993).

The use of brine vacuum impregnation (BVI) for salting process of meat, fish and cheese was studied by Chiralt *et al.* (2001). In general, a notable reduction of salting time, an increase in the process yields and greater values of the ratio of salt gain to water loss were observed. The reason proposed for the enhancement was that during BVI the samples lose natural gas or liquid phases entrapped in their structure and reach a flatter salt concentration profile than that obtained in conventional salting methods.

2.5.2. High intensity pulsed electrical fields (HIPEF).

HIPEF treatment has been widely used as a non-thermal process for food preservation due to its capacity to improve cell disintegration of cellular material. Electrical fields higher than $1 kV\,cm^{-1}$ will be considered in this study as high intensity electrical fields. The efficiency of HIPEF for cell disintegration depends on electrical field strength and number of pulses applied.

The mechanism proposed for the enhancement in mass transfer with the application of HIPEF is that the external electrical field induces an electric potential across the cell membrane; when this potential goes over a critical value E_k an electrostatic charge separation takes place in the cell membrane. It is known that high electric fields can cause either reversible or irreversible rupture of cell membranes; a phenomenon known as electroporation (Bryant and Wolfe, 1987).

Different studies have reported the enhancement of solutes extraction from cells when HIPEF are applied. Eshtiaghi and Knorr (2001) studied the use of HIPEF to cause cell disintegration of sugar beet. HIPEF at different field strength (1.2 to 2.5$kV\ cm^{-1}$) and pulse number (1 to 200) demonstrated to rapidly disintegrate sugar beet cell membranes in 20 seconds or less at ambient temperatures, reducing the energy and the time required for extraction. Field strength and pulse number had a key influence on the disintegration. The profiles for cell disintegration of sugar beets found by Eshtiaghi and Knorr (2001) are shown in Fig. 2.16.

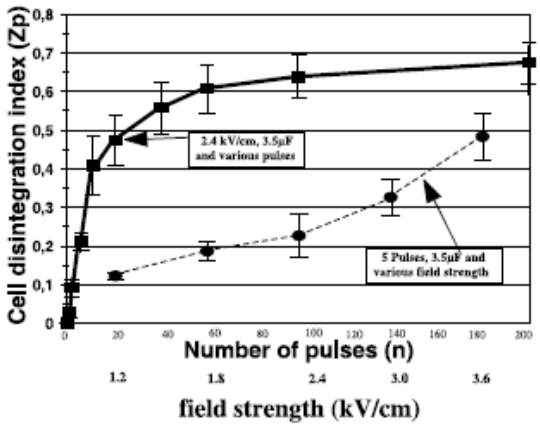


Fig. 2.16. Cell disintegration of sugar beets, influence of field strength and pulse number (Eshtiaghi and Knorr 2001).

Suga *et al.* (2006) found HIPEF useful for the production of homologous and heterogeneous proteins. In the study, electrical fields (typically of 5 to 50$kV\ cm^{-1}$ and microsecond duration) caused electroporation, damage of membranes, enhanced cytoplasmic ion leakage and the release of intracellular bio-products.

Another study, done by Yin and He (2008), showed that the extraction of dissoluble Calcium from bone was increased at a high pulse number and high electrical field intensity.

In a previous work made by Corrales *et al.* (2008), (see also in the section for high pressure and vacuum assisted diffusion) the enhancement in extraction of anthocyanins from grape during thermal processing assisted by HIPEF at electrical field strength of $3 kV\ cm^{-1}$ caused irreversible electroporation in cell membranes increasing the extractability of polyphenols and anthocyanins by their release into the solvent. HIPEF provided the possibility of inactivating degrading enzymes, which may explain the higher yields in antioxidant activity compared to the other methods for polyphenols and anthocyanins extraction (Corrales *et al.*, 2008).

HIPEF process has been satisfactorily used for structure modification of egg white (EW) proteins and β-lactoglobulin (β-lg) concentrate by long length pulses of high intensity electric fields ($12.5 kV\ cm^{-1}$, exponential decay shape). The results obtained indicated that pulses in the order of milliseconds partially modified the native structure of β-lg concentrate and EW. β-lg concentrate was more sensitive than EW to HIPEF treatment. HIPEF also caused differences in the gelation behaviour of both proteins, enhancing the gelation rate of β-lg and partially decreased that of EW (Pérez and Pilosof, 2004).

HIPEF used as pre-treatment in the vinification of Tempranillo grapes remarkably enhanced colour intensity, anthocyanins and index of total polyphenols. HIPEF treatment, at 5 and $10 kV\ cm^{-1}$ with 50 exponential decay pulses, contributed to reduce the duration of the maceration time during vinification of Tempranillo grapes. The wine obtained from the PEF treated grape presented a higher colour density, anthocyanins and phenolic content than the control samples but did not have a significant effect in the other physical–chemical properties of the wine (López *et al.*, 2008).

2.5.3. Ultrasound

Ultrasound can enhance mass transfer. This concept is used for delivering drugs into patients directly across the skin, but is used less extensively in mass transfer in food materials. If ultrasound is combined with another non-thermal process such as AC electrical fields, the diffusion may be increased further. In a study made by Corrales *et al.* (2008) (see also in the section for high pressure and vacuum assisted diffusion) where ultrasound, high pressure and pulsed electric fields were used in combination with thermal processing at 70°C for the extraction of anthocyanins from grape, ultrasound with thermal process improved the extraction yields compared to the control samples; nevertheless, the effect of ultrasound on antioxidant recovery was less pronounced than for PEF and HHP.

Ultrasonic extraction resulted in the disruption of biological cell walls to facilitate the release of sugar from sugar beet (Chendke and Fogler, 1975). Ultrasonically assisted extraction can also be applied to the production of medicinal compounds like helicid, berberine hydrochloride and bergenin from Chinese plants (Zhao *et al.,* 1991).

The main hypothesis of the mechanism for the mass transfer enhancement in ultrasound assisted extraction is the propagation of ultrasound pressure waves resulting in cavitation phenomena.

2.5.4. Electrical fields.

Electrical fields (ohmic heating, moderate electrical fields and mild pulsed electrical fields) as method for modifying mass transfer will be consider with more detail in the next section of this chapter since it is a main part of this study.

2.6. ELECTRICAL PROCESSING FOR MODIFYING MASS TRANSFER IN FOOD MATERIAL

The term "electrical processing of food material" refers to processes where an electrical field, either in a continuous or pulsed way or at a moderate or high electrical strength, is applied to a food material. In section 2 of this chapter, the necessity of applying thermal processing to food material in order to achieve a complete pathogen-free product was mentioned. It was also mentioned the novel use of ohmic heating, moderate electrical fields (with or without heating) and pulsed electrical fields for a wide range of applications in food processing, from the inactivation of microbial and enzymes to practical applications like meat cooking. This section focuses on electrical processing as a way to modify mass transfer in food.

2.6.1. Ohmic heating

In previous studies by Halden *et al.* (1990) and Schreier *et al.* (1993) it was demonstrated that betanin effusion from beetroot was enhanced by the application of an alternating electric field during ohmic heating, compared with conventional heating. The results also suggested that the enhancement follows the rules given for mass transfer coefficients and that the effect is a linear function of the applied electric field strength.

In Halden *et al.* (1990), cylinders of pork, pork-fat, potato, aubergine, mushroom and beetroot were heated ohmically from room temperature to 100°C. The presence of an electric field induced enhanced diffusion of cell fluids in the food, increasing the rate of change of conductivity with temperature above that found by conventional heating.

In Schreier *et al.* (1993) the effusion of betanin from beetroot and the mass transfer of rhodamineB solutions were studied. The samples were heated conventionally or ohmically (at 200V AC, electrical fields from 3 to 20 $V\ cm^{-1}$). The effusion of betanin was greater during electrical heating than during conventional heating (Fig. 2.17).

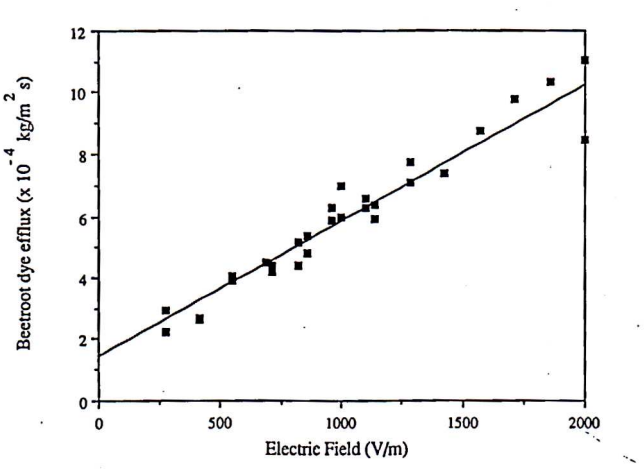


Fig. 2.17. Variation in betanin efflux from beetroot with the applied electrical field (Schreier *et al.*, 1993).

Another example of the modification of mass transfer with electrical processing is the study made on the drying rate and juice yield of apples, by Lima and Sastry (1999). Ohmic pre-treatment at 60 Hz sine wave (with 40, 60 and 70 $V\,cm^{-1}$ field strengths) and 4Hz saw tooth wave (with 20 and 40 $V\,cm^{-1}$ voltage gradients) was applied to the samples. The ohmic pre-treatment increased hot-air drying rate and shifted desorption isotherms, and increased as well juice yields over raw samples or those pre-treated with conventional or microwave heating. The highest drying rates and juice yields were obtained with the lower frequency and saw tooth waveform of the alternating current applied.

2.6.2. Pulsed electrical fields (PEF)

In this section the intensity of the pulsed electrical fields taken into account goes from 10 to 1000 $V\,cm^{-1}$, which are in the category of mild or moderate electrical fields. PEF have been principally used in the food industry as a non-thermal technology for pasteurisation of liquid and particulate foods (Calderón-Miranda *et al.*, 1999). Nevertheless, previous studies have shown the efficiency of using electrical fields as an electrically induced extraction technology, i.e. as treatment for selective extraction of different biomolecules from plant tissue. PEF is a less destructive method for plant tissues (Lebovka *et al.*, 2005).

Lebovka *et al.* (2001) studied cell breakage of apple tissue treated with PEF. Results suggested that the breakage of cellular material by PEF is governed by two key processes: resealing of cells and moisture transfer processes inside the cellular structure. For tissue damage of potato under PEF treatment with monopolar pulses of near-rectangular shape and electrical field below 500 $V\,cm^{-1}$, the results obtained by Lebovka *et al.* (2005) showed a pronounced decrease of the time needed to cause electrical damage, which was proportional to the increase of both temperature and electric field strength. It was found as well that the combination of PEF treatment with mild ohmic heating can give the opportunity to get high tissue disintegration degree at MEF strengths below 100 $V\,cm^{-1}$ without any noticeable losses of product quality.

The damage degree of alfalfa mash was researched by Gachovska *et al.* (2009). Under PEF treatment at field strengths of 1.25, 1.90 and 2.50 $kV\,cm^{-1}$ the maximum rate of change of damage degree was observed at the highest capacitance of 1.5μF for all the electric field strengths. A decrease in the capacitance led to a decrease in the rate of change of damage degree, while increases in the electric field led to a decrease in energy needed to obtain the maximum damage degree. It was suggested that fewer numbers of pulses and higher energy per pulse could be used in order to consume the least energy to obtain maximum damage degree of the alfalfa mash. Loghavi *et al.* (2009) observed permeabilisation of Lactobacillus acidophilus membrane under the presence of MEF of 2 $V\,cm^{-1}$ at five different values of frequency and 30°C constant processing temperature.

2.6.2.1. Extraction of red pigments from beetroot affected by PEF

A study made by Jemai and Vorobiev (2002) on the extraction of soluble substances from sugar beet by moderate PEF ranging from 160 to 780 $V\,cm^{-1}$ (50min of extraction) showed

that the transfer of soluble substances into the liquid can be significantly intensified with the electrical treatment. A minimal field strength of $150 V\ cm^{-1}$ was required to detect a significant enhancement of soluble transfer. At higher electrical field strength (500V cm^{-1}) a significantly large increase in the efficiency was obtained before reaching a stable value of soluble substances in the surrounding liquid.

Beets were also used in a work done by Fincan *et al.* (2004) on the extraction of betanin from red beetroot. PEF treatment at $1 kV\ cm^{-1}$ and monopolar rectangular pulses was compared with other extraction methods such as freezing and mechanical pressing. After 270 rectangular pulses of 10µs at $1 kV\ cm^{-1}$ field strength, with an energy consumption of $7 kJ\ kg^{-1}$, the samples released about 90% of total red pigment and ionic content following one hour of aqueous extraction. The results suggested that no differential permeabilisation of the intracellular components occurred.

The plasmolysis (or cell membrane damage) of sugar beet under PEF treatment at moderate strength ($100 V\ cm^{-1}$, bipolar pulses of near-rectangular shape) was compared with conventional heating (Lebovka *et al.*, 2006). PEF treatment noticeably accelerated the diffusion process, which required one hour with mild heating at 40°C, allowing to disintegrate efficiently the biological cells and noticeably decreasing the diffusion temperature (up to 40–50°C) without any loss of solution purity (Lebovka *et al.*, 2006).

2.6.3. Moderate electrical fields (MEF)

Not only PEF at a mild or high intensity, but also continuous MEF have been shown to alter the structure of food material. The expression "moderate electrical fields processing" does not yet have a formal definition. In this study, moderate electrical fields processing will mean:

"Processes that involve electrical fields less than or equal to 1000V cm^{-1}, they are too low to be in the range of high intensity pulsed electrical fields but have been shown to affect the permeability of cell membranes and to cause electroporation effects. MEF can be achieved with or without heating the material".

When MEF are applied, the heat that could be produced is removed from the system with water-baths or similar equipment and constant processing temperature is maintained.

2.6.3.1. Modification of mass transfer in cellular and gel systems with the application of MEF

Previous studies have demonstrated that the extraction of compounds from food material or the damage of cellular tissue can be accomplished or enhanced with the use of MEF. The diffusion of betanin from beet at MEF process using frequencies ranging from 0 (direct current) to 5000Hz, and field strengths ranging from 0 $V\ cm^{-1}$ (conventional heating) to 23.9 $V\ cm^{-1}$, for 3min at a constant 45°C temperature was found to increase with electric field strength and decrease with frequency (Kulshrestha and Sastry, 2003) (Fig. 2.18). The enhancement appeared to be significant when the product initially possessed an intact cell structure, since no enhancement was observed in samples where the cell structure was either absent or previously completely permeabilised. The mechanism of diffusion enhancement was attributed to pores formation in cell membranes when a threshold potential above which significant increases in permeabilisation occur is trespassed (Kulshrestha and Sastry, 2003).

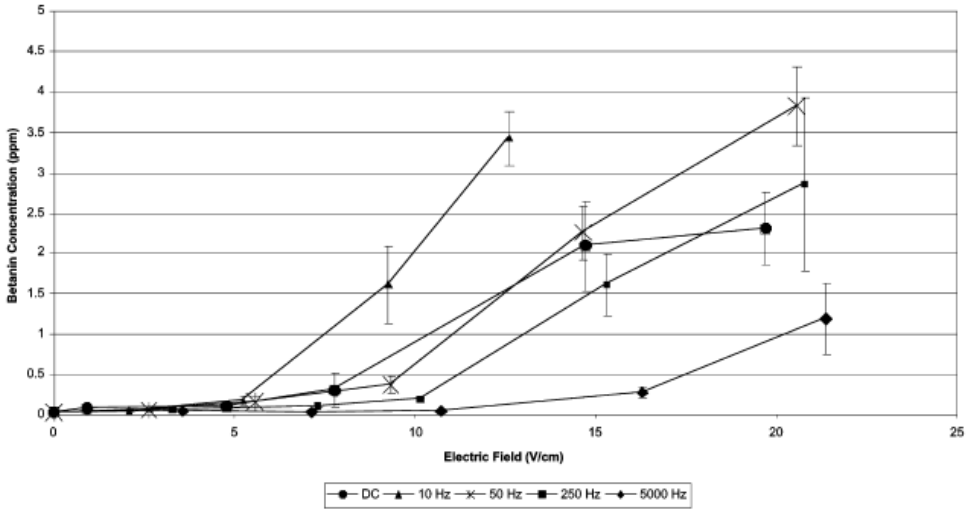


Fig. 2.18. Diffusion of betanin from fresh beetroot during MEF processing (Kulshrestha and Sastry, 2003).

The extraction by conventional heating and MEF treatment of solutes from fermented black tea leaves and fresh or dry mint leaves immersed in an aqueous fluid medium was reported in a work by Sensoy and Sastry (2004). Results obtained from control samples heated on a hot plate were compared with those MEF treated (0 to 125 $V\ cm^{-1}$); showing that MEF processing increased the extraction yield for fresh mint leaves (cellular material). The use of low frequency resulted in higher extraction rates for fresh mint leaves. Dried mint leaves and fermented black tea leaves (dried cellular materials) were not affected by the treatment type. It was proposed that the leaching of solutes is enhanced apparently because of membrane breakdown that could be caused by field-induced tension.

Red beet tissue samples were sandwiched between agarose, agar or alginate in a work done by Zvitov and Nussinovitch (2004) to study the effect of a DC voltage applied at electrical field strength of up to 40 $V\ cm^{-1}$, on the extraction of pigments and minerals from the red beet to the gels. It was observed that low DC electrification yielded extraction of pigments and minerals into the gels and that the stiffer the gel was, the lower the amount extracted by

the method proposed. Separation of different charged ions was detected in the different gel layers, i.e. cations in the gel near the cathode and anions in the gel near the anode.

The infusion of rhodamine, methylene blue and fluorescein dyes into potato, alginate and agar gels under the application of MEF at electrical field gradients of 0, 5.43, 8.15 and 10.87 $V\,cm^{-1}$ was shown to be enhanced. For rhodamine infusion into raw potato at 100°C the enhancement was around 70% in comparison with control samples (Kemp and Fryer, 2007).

MEF treatment was reported in Lebovka *et al.* (2007) to damage sugarbeet tissue. The tissue was subjected to thermal, MEF (AC at 50Hz, electrical fields from 20 $V\,cm^{-1}$) and PEF (monopolar pulses of near-rectangular shape) treatments. Results showed that electrically stimulated damage of a sugarbeet tissue occurs even at small electrical field strength (20 Vcm^{-1}) if treatment time is long enough (around one hour). MEF treatment with field strength under 100 $V\,cm^{-1}$ effectively disintegrated the sugarbeet tissue at temperatures of 50 to 60°C and treatment times below 100s. A dependence of damage efficiency on sample orientation with respect to the external electric field was observed.

Matos *et al.* (2006) studied mass transport of aminomethylcoumarin dye diffused in polyacrylamide gels doped with charged silica colloidal inclusions when an internal pumping on electro-osmotic flow was driven by an electric field applied across a gel. It was demonstrated that under the application of electrical fields the mass transport of aminomethylcoumarin is enhanced, mainly due to the generation of electro-osmotic flows around the charged inclusion surfaces. It was also found that by reversing the direction of the applied electrical current, the electro-osmotic flow can be used to hinder or suppress tracer mass transfer through the gel.

In a recent study by Machado *et al.* (2009), the inactivation of *Escherichia coli* under MEF (50, 160, 180, 220, and 280 $V\ cm^{-1}$) was reached at temperatures below 25°C. Electric fields above 220 $V\ cm^{-1}$ promoted death rates of 3log cycles of *E. coli* in less than 6min, and even higher rates at greater electric fields. MEF was assumed to overcome the thermal degradation caused by conventional high temperature treatments. SEM observations of *E. coli* cells after the exposure to the MEF treatment, revealed changes at the cell membrane level that could be a possible cause for the cell death rates (Machado *et al.*, 2009).

Although the mechanism of food heating by electrical fields is well understood, the mechanism for mass transfer enhancement is uncertain. The changes in structure observed under the presence of a MEF can be explained due to "temporary" permeabilisation of cell membrane. Electro-osmotic effects may produce enhanced diffusion; in addition, diffusion can be enhanced as a result of differences in transport number between a membrane surface (e.g. a food cell membrane) and the surrounding bulk solution. In this case the cell membrane suffers electroporation damage (electrical mechanism) (Vorobiev and Lebovka, 2009).

2.6.3.2. Modification of structure in gel systems

So far, this section has focused on the modification of mass transfer in cellular systems with the application of MEF. Nevertheless, different works have dealt with structure modification of gel systems under electrical fields. A review of the literature in this area is of importance because experimental Chapters 5 and 6 of this study are based on the application of moderate and pulsed electrical fields to gel matrixes.

In a work presented by Hirose *et al.* (1992), non-ionic and ionic (sodium acrylate or acrylic acid added) acrylamide gels were exposed to stationary electrical fields from 0.1 to 1.0 $V\ cm^{-1}$ at room temperature. The gels were found to develop pH gradients under the

application of the electrical field. This development appeared to be a relevant phenomenon in explaining the phase transitions and volume changes of ionic gels induced by an electric field. The results also showed that the occurrence of pH gradients was strongly affected by the type and concentration of the ions present in the gel.

The structure of gel beads produced from agar, agarose, alginate and gellan gum was modified by DC electrical fields (Zvitov and Nussinovitch, 2003). The results showed a general shrinkage and weight loss of beads as a result of the DC supply, accompanied by electrolysis. After shrinkage, all beads became stronger and more rigid. The most important effect of the electrical field seemed to be the migration and redistribution of counter and added ions within the electrified gel.

In the area of gel bead formation by electrical fields, Moghadam *et al.* (2008) suggested that (in their proposed alternative method of electro-spray for the formation of droplets from sodium alginate using constant DC voltage at a temperature of approximately 27°C) voltage had a pronounced effect on the size of beads as compared to flow rate, nozzle diameter and concentration of alginate liquid. Increasing the height from which the droplets fell improved the sphericity of the beads by increasing the time of flight for the droplets (Moghadam *et al.*, 2008).

MEF were also found to modify the physical properties of chitosan coatings from lobster (Souza *et al.*, 2009). Chitosan solutions were treated at different field strengths (from 50 to 200 $V\ cm^{-1}$) and an increase of temperature up to 60°C. Their results showed that the application of a moderate electric field to the film-forming solutions had significant effects on physical properties of the films such as solubility in water (Fig. 2.19), structure and water vapour, oxygen and carbon dioxide permeability coefficients. The surface of chitosan films was much more uniform when the electrical field was applied (Souza *et al.*, 2009).

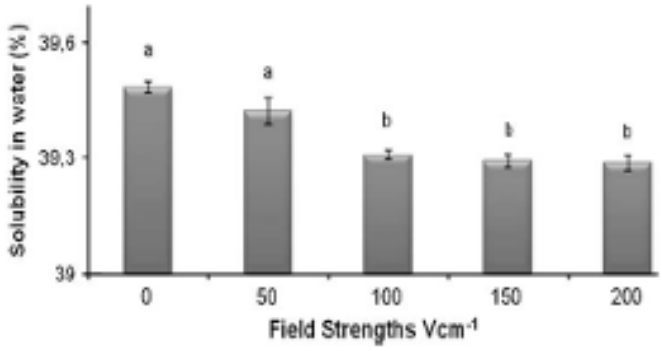


Fig. 2.19. Solubility in water of chitosan films treated at different electrical field strengths. Different letters in the same column correspond to statistically different samples ($p < 0.05$) (Souza *et al.*, 2009).

2.6.4. The use of AC instead of DC for the experiments performed in this study

A disadvantage of using direct current (DC) is that undesirable electrolysis reactions may occur (Qin *et al.*, 1994) and charged particles in the liquid may form a layer on the anode that would require periodic cleaning. The use of an alternating current (AC) overcomes these problems and the behaviour of ions in the solution may be similar to that of DC (as explained in section 2.7.1). This potentially simpler method of generating electrical fields may have lower capital and operating costs than those associated with PEF processing. When using AC electrical fields no net lateral motion might be assumed for an oscillating ion in solution.

2.7. ELECTROKINETIC PHENOMENA

2.7.1. Motion of an ion in solution under the application of an external electrical field

The "ionic cloud" describes the interaction between an ion and its environment (Brockris and Reddy, 1970). When the ion is in absence of a driving force, it is static, no direction in space from its central part (positive charge) is preferred (Fig. 2.20a). When around a stationary central ion, the ionic cloud is spherically symetrical, nevertheless, under the stimulus of a driving force, i.e. an electrical field, one direction in space becomes privileged,

displacing the reference ion in the x direction, giving the ionic cloud an egg-like shape (Fig. 2.20b).

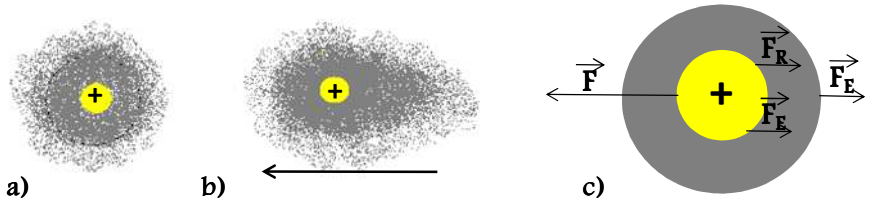


Fig. 2.20. Motion of an ion in solution under the application of an external field. a) The ion is static, b) under the external field, the reference ion is displaced in the x direction.

The spherical symmetry of the cloud can be restored if the ion and solvent molecules contained in it immediately readjust to the new position of the central ion. This readjustment involves ionic movements taking place in a finite time which is not available when the central ions keep moving on. In front of the central ion the cloud is being continually built up, but the part behind looks like it is being left standing (Brockris and Reddy, 1970).

The centre of charge of a central ion in motion does not coincide with the centre of charge of its ionic cloud, developing an electrical force between the ion and the cloud. The ion is subjected to an electrical field that receives the name of relaxation field, since it arises from the continual relaxation of the cloud behind the ion and the front part of the ion. An ion in motion has a "portable" field of force (Brockris and Reddy, 1970).

When an electrical field is applied, it acts on both the central ion and its charged cloud (with an opposite charge) making them move in opposite directions. If the ionic atmosphere is considered as a sphere (of radius κ^{-1}) (Fig. 2.21) and the ionic cloud to have a thickness of about 10nm, then the movement of the ionic atmosphere under the action of an electrical field corresponds to that of a fairly large particle. Electrophoresis is used to describe the

motion of particles of coloidal dimension, from 1 to 1000nm, in an electrical field. This is why it is appropiate to describe the migration of the ionic cloud as an electrophoretic effect. From Fig. 2.20c it can be seen that a force results from the ionic cloud moving and trying to move the central ion with it, the force is termed as electrophoretic force $\overrightarrow{F_E}$.

Fig. 2.21. Analogy between a central ion and surrounding cloud with a particle of colloidal dimensions (1 to 1000nm).

When studying the motion of ions under an external electrical field, not only the electric force $\overrightarrow{F_{El}}$ determines the drift velocity of the ion, but a total force $\overrightarrow{F_{Total}}$ that also involves the relaxation force $\overrightarrow{F_R}$ (resulting from the distortion of the cloud around a moving ion) and the electrophoretic force $\overrightarrow{F_E}$ (resulting from the ion sharing the electrophoretic motion of its cloud), both acting in opposite direction of $\overrightarrow{F_{El}}$.

$$\vec{F}_{total} = \vec{F}_{El} - \left(\vec{F}_E + \vec{F}_R\right) \qquad \textbf{(2.20)}$$

The motion of an ion due to diffusive and electrical forces can be determined using the Nernst-Planck electro-diffusion equation:

$$J = \frac{DC}{GT} VFE - D\frac{dC}{dx} \qquad \textbf{(2.21)}$$

Where J is the species flux [kmol $m^{-2}s^{-1}$], V is the valence of the species, C is the concentration of species [mol m^{-3}], G is the universal gas constant [J $kmol^{-1}K^{-1}$], T is the temperature [K], D is the diffusion coefficient [m^2s^{-1}], F is the Faraday constant [C mol^{-1}] and E is the electric field strength [V m^{-1}].

2.7.2. Ionic electric double layers

As explained previously in section 2.2.1, the cell membrane phospholipids form a double layer which in the presence of an electrical field acts as an ionic electrical double layer. Most double layers are relaxed, which means that they form spontaneously when absorbing charged species. Ionic double layers are formed by non-electrostatic forces. The formation of relaxed double layers depends on the non-electric affinity of charge-determining ions for a surface and their development depends on non electrostatic and electrostatic interaction. Induced pole moment, dielectric permittivity and conductivity are characteristics of double layers (Lyklema, 1995).

2.7.3. Electrokinetic phenomena

Electrokinetic phenomena are classified as second-order phenomena since forces create fluxes or flows (Lyklema, 1995). It is important to mention in this study some types of electrokinetic phenomena since it has been suggested that the electrokinetic mechanisms resulting from ionic motion when applying a direct current may be relevant to the case when an alternating current is used.

Electrokinetic phenomena could be defined as a group of different effects that occur in heterogeneous fluids (containing solid or liquid particles or gas bubbles) or in porous bodies filled with fluid. There is a common cause for the effects: the interfacial 'double layer' of charges. Under the influence of an external force, i.e. gravity, concentration gradient, electrical field or pressure gradient, a tangential movement on the diffuse layer of the fluid is generated with respect to a neighbouring charged surface. The phenomenon could also involve stationary particles with the liquid moving, i.e. in electro-osmosis. In addition, the moving phase might be either continuous fluid or dispersed phase. The electrokinetic effect is determined by the combination of driving force and moving phase (Hunter, 1989).

2.7.4. Most known electrokinetic phenomena:

2.7.4.1. Electrophoresis

The best known electrokinetic phenomenon is electrophoresis. This phenomenon was discovered by Reuss in 1809 when he saw that under the influence of an applied electrical field, clay particles dispersed in water migrate. Electrophoresis could be defined as the movement of dispersed particles relative to a fluid when an electrical field that is space uniform is applied. It is caused by the interfacial "double layer" of charges (Barfoot, 2010). Electrophoresis is used for molecular separation, its performance depends upon the charge distribution of the molecules being separated (Heidcamp, 2009).

2.7.4.2. Electroporation

Electroporation could be defined as the increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field (Lyklema, 1995). Small holes are formed in the cell wall, making it permeable for the diffusion of species. The damage of cells is also called plasmolysis. The mechanism of pore formation remains unknown; the hypotheses that have been suggested are presented in Fig. 2.22. The models taken into account are electro-mechanical, periodic lipid and viscoelastic. The electro-mechanical model proposes pore formation in response to extensive membrane compression and thinning (Zimmermann *et al.,* 1974), the viscoelastic model suggests pore formation with continuous borders (Dimitrov and Jain, 1984) and for the periodic lipid block model the lipids are rearranged into pores with discrete boundaries (Sugar and Neumann, 1984).

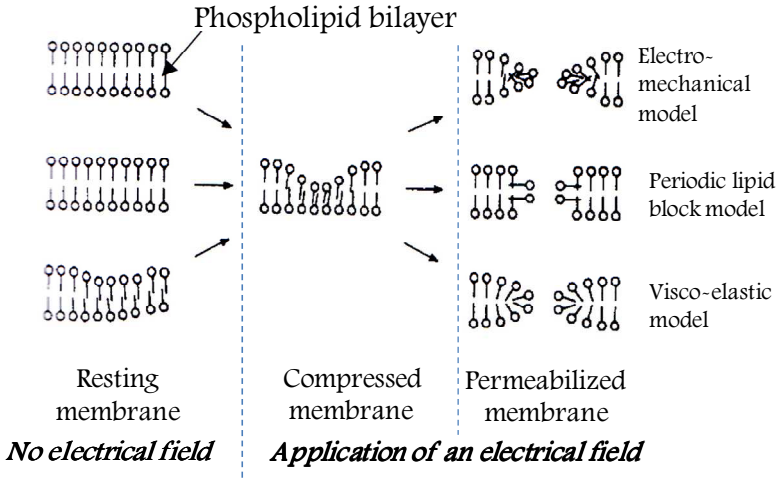


Fig. 2.22. Three models proposed for electropore formation; a schematic model (Joersbo and Brunstedt, 1991).

2.7.4.3. Electro-osmosis.

Osmosis is the net movement of water across a selectively permeable membrane driven by a difference in osmotic pressure across the membrane (Hunter, 1989). The permeable selective membrane allows passage of water, but rejects solute molecules or ions (Cath, 2006). The osmosis phenomenon has numerous applications, from water treatment and food processing to power generation and novel methods for controlled drug release.

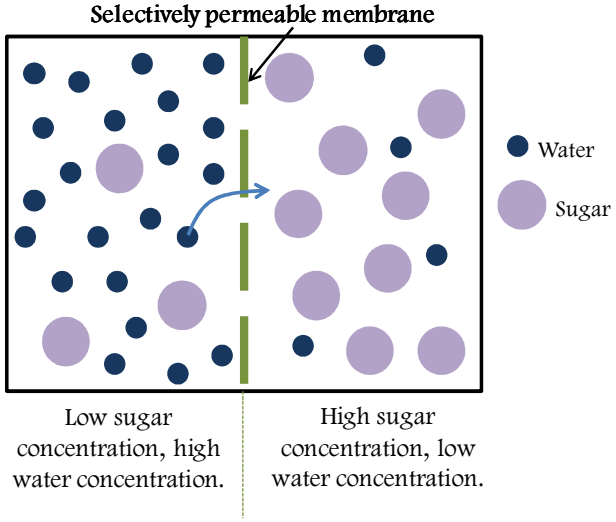


Fig. 2.23. Direct (forward) osmosis. Due to the selective permeable membrane and the concentration potential in the solutions, the molecules of water move across the membrane.

Osmosis direction can be reversed by applying pressure that acts as the driving force for mass transport through the membrane i.e., reversed osmosis uses hydraulic pressure for water treatment in order to oppose and exceed the osmotic pressure of an aqueous feed solution, and as a result the water is purified (Sourirajan, 1970).

In both forward and reversed osmosis the general equation describing water transport is:

$$J_w = \Pi(\mathrm{Y}\Delta\pi - \Delta P) \qquad \textbf{(2.22)}$$

Where J_w is the water flux, Π the water permeability constant of the membrane, Y the reflection coefficient, $\Delta\pi$ is the change in osmotic pressure [bar] and ΔP is the applied pressure (Cath, 2006).

The osmosis process in the presence of an electrical field is called electro-osmosis and can be defined as the motion of a polar liquid through an immobilised set of particles, porous plug, capillary or membrane as a consequence of an applied electrical field. Electro-osmosis is the result of the force exerted by the applied field on the countercharge in the liquid. The ions involved move and enter the liquid in which they are embedded. In some cases, the rate of ion movement is increased by the application of the electrical field compared with simple osmosis. Electro-osmosis is the opposite phenomenon to electrophoresis: in electrophoresis a solid particle moves through a stationary electrolyte while in electro-osmosis the electrolyte moves around a solid charged surface (Brockris and Reddy, 1970).

Electro-osmosis phenomenon also occurs in AC and is caused by molecular diffusion; the changes induced by the electrical field are only partially reversed during half cycle (Halden *et al.*, 1990). One of the applications of electro-osmosis is the removal of water from food suspensions. In electro-osmotic dewatering, the solid particles move to the anode, while the electro-osmotic flow causes the water to move in the other direction (Vijh, 2002). Electro-

osmotic dewatering is considered ideal for the dewatering of slurries with particle size in the ultrafine range, and possibly for heat-sensitive material which cannot be thermally dried (Vijh, 2002).

For the particular case of tomato paste, the water is removed by placing the paste between two electrodes. Al-Asheh (2004) found that electro-osmotic dewatering can remove significant amounts of water from tomato paste suspension with lower energy expenditure than the required to vaporise the water; the dewatering rate increased with the increase in the applied voltage (Fig. 2.24).

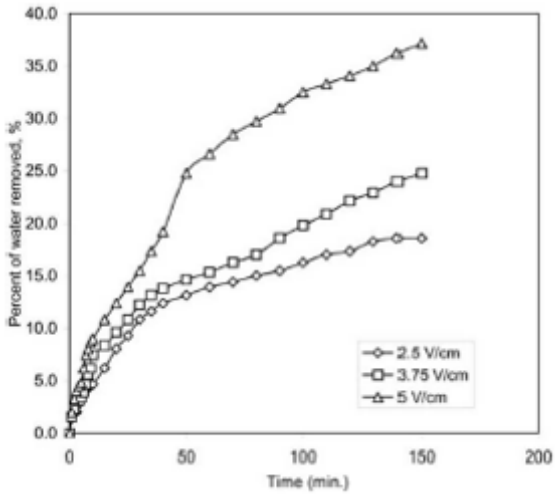


Fig. 2.24. Effect of electric field strength on the electro-osmosis dewatering of tomato paste suspension under constant DC voltage (Al-Asheh, 2004).

Another important application of electro-osmosis is the delivery of drugs, called "iontophoresis". Transdermal iontophoresis has traditionally been developed based upon the application of a constant direct current (DC) across the skin, expecting that the constant current would lead to constant permeant flux (Inada *et al.*, 1994).

Li *et al.* (1999) demonstrated that there is a direct proportionality between permeant flux and the electrical conductance of human epidermal membrane (HEM) during AC iontophoresis. It has also been demonstrated that during AC iontophoresis HEM electrical conductance can be enhanced and then maintained at the higher level by manually adjusting the applied AC voltage (Song *et al.*, 2002). These discoveries brought out the possibility of the use of AC voltage for iontophoresis. Zhu *et al.* (2002) developed a method for transdermal iontophoresis using AC voltage in order to reduce flux drift during DC iontophoresis and minimise skin-to-skin variability.

Iontophoretic flux variability for neutral permeant was significantly reduced by the method of constant conductance AC iontophoresis proposed. Constant conductance AC iontophoresis allowed to maintain a target constant resistance of the HEM and apparently permitted values of the membrane parameters (such as pore size, pore size distribution, and effective surface charge density) within a relatively narrow range (Zhu *et al.*, 2002).

2.7.4.4. Transport number effect.

Transport number effect could be defined as the fraction of the total current carried by a given ion in an electrolyte (McGraw-Hill Dictionary of Scientific and Technical Terms, 2003). For species i, the transport number ι_i is:

$$\iota_i = \frac{I_i}{I} \qquad \textbf{(2.23)}$$

Where I_i is the fraction of the current carried by the species i and I is the whole current.

It has been proposed by Barry and Hope (1969) that when a current is passed through a membrane system, the differences in transport numbers between the membrane (ι_m) and the adjacent solutions (ι_s) will result in depletion and enhancement of concentrations at the membrane-solution interfaces. This effect will be balanced by: diffusion back into the bulk

solution, diffusion of solute back across the membrane itself, and osmosis resulting from these local concentration gradients.

The transport number of a particular ion depends on whether it is situated in the membrane or in the contact solution. The transport number of a cation is greater in the membrane that in the electrolyte; when the direct field is applied the mobility of the cation within the membrane is enhanced and causes the levels of the cation to be diminished on the anodic side. This results in an osmotic potential across the membrane that drives an osmotic flux from the anodic to the cathodic half.

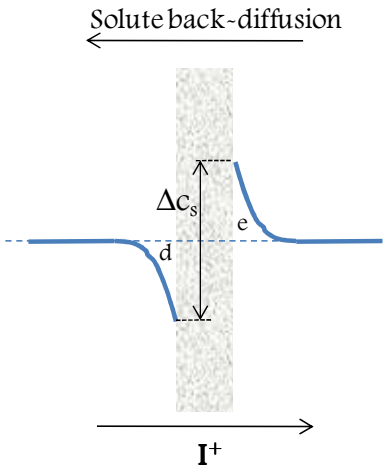


Fig. 2.25. Membrane segment under the application of a direct field. The arrows show the direction of the current I and solute back diffusion. 'e' refers to enhancement and 'd' to depletion.

The rate of solute enhancement (or depletion) Δc at the interface is given by:

$$\Delta c = \frac{\Delta \iota I}{F} \qquad \textbf{(2.24)}$$

Where I is the current [A], F is the Faraday constant [C mol^{-1}] and Δι the transport number difference across the interface.

CHAPTER 3

3. MATERIALS AND EXPERIMENTAL METHODS

3.1. INTRODUCTION

In Chapter 2, previous work in the area of electrical processing was presented and the relation between electrical processing, mass transfer and physical properties of food material was introduced. In this Chapter, the materials and experimental methods used in this research to study the effect of electrical processing on food material are described. The main aim of the experimental sets performed in this study was to visualise the effect of the application of moderate electrical fields (MEF) on food material, specifically on mass transfer in cellular material and gel networks, and the effect of electrical processing with MEF and pulsed electrical fields (PEF) on compression force for gel networks.

3.1.1. Mass transfer in cellular material under the application of MEF (summary)

In a first set of experiments, mass transfer in cellular material was investigated by measuring the differences in the amount of betanin extracted from beetroot. Experimental variables included temperature, electrical field strength and position of the sample in respect to the electrical field. Concentration of betanin was measured by spectrophotometry. The effect of the MEF was quantified by estimating effective diffusion coefficients (D_{eff}s).

It was not our intent to optimise the system for maximal extraction of pigment from beet tissue but to use this material and its properties (colorant) to demonstrate the ability of low-moderate AC electrical fields to modify the extraction.

3.1.2. Mass transfer in gel systems under the application of MEF (summary)

The effect of alternating MEF on mass transfer of a polar dye (rhodamine6G) on ionic (alginate, potato-alginate mix) and thermally set gels (albumen and gelatine) was studied by varying the strength of the applied electrical field and the electrical conductivities (σ) of the gel sample and the solution surrounding it. The concentration of rhodamine6G in the samples at the end of the process was measured using image analysis. The effective diffusion coefficients (D_{eff}s) for the infusion of rhodamine6G were estimated with a solution of Fick´s 2nd law of diffusion for cylindrical systems.

3.1.3. Mechanical properties of gel systems under the application of MEF and PEF (summary)

Mechanical properties of gel systems after the application of MEF and PEF were studied. MEF were applied during the formation of alginate and gellan gum particles and PEF to alginate samples after their formation. Mechanical properties were measured using a compression test.

3.2. MATERIALS

3.2.1. Beetroot

Raw beetroots were obtained from a local supplier (Sainsbury's, UK). The beetroots were stored in an airtight container in a fridge at temperatures from 4 to 6°C for no more than one week before use. A slab of 3x2x0.3cm (Fig. 3.1) was cut from the central part of the root the day of the experiment and soaked in distilled water at room temperature (20°C approximately) for 30 minutes before the experiment started. The dimensions of the slab were chosen so the ratio of the two shortest lengths of slab L/D was high enough that one-dimensional mass transfer could be assumed; for betanin slab L/D=2/0.3. The value of electrical conductivity (σ_{Bet}) for betanin at 20°C is $0.03 S\,m^{-1}$ (Schreier *et al.*, 1993).

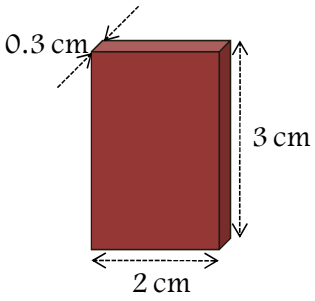


Fig. 3.1. Dimensions of the beetroot slab used in extraction experiments.

3.2.2. Gel systems, cylindrical samples

Gel networks were the chosen materials in Chapters 5 and 6. Four different gels were used for the experiments in Chapter 5, two of them set with calcium ions (alginate and potato powder-alginate gels) and two thermally set (chicken egg white albumen and pork skin gelatine). A summary of the gel used and the process conditions is presented in Table 3.1.

Table 3.1. Gel systems used in the study

Gel	σ_{Gel} [S m^{-1}] @25°C	$\sigma_{soaking\ CaCl2\ Solution}$ [S m^{-1}] @25°C	Concentration of $CaCl_2$ soaking solution [g l^{-1}]	Processing time [s]	Processing temperature [°C]	Processing electrical fields [V m^{-1}]
Calcium alginate 3% w/v	a) 2.7	0.0417	20	840	40	0 and 1000
	b) 0.0417		0.2			
	c) 0.01		0.05			
Potato- alginate mix	a) 2.7	0.0417	20	840	40	0 and 1000
	b) 0.0417		0.2			
	c) 0.01		0.05			
Chicken egg albumen 10% w/v	0.9			2340	40	0 and 1000
Pork skin gelatine 10% w/v	0.3			1140	20	0 and 1200

Cylindrical samples of 1.2cm diameter and 3cm long (Fig. 3.2) were obtained by pouring the gels in a cellulose dialysis membrane (1.2cm diameter) for the alginate and potato-alginate gels and by using a stainless steel cork borer (1.2cm internal diameter) for the albumen and gelatine gels. The dimensions of the cylindrical samples were defined by the

size of the cellulose dialysis membrane available at the laboratory and used by Sudera (2008), so comparison of results was possible.

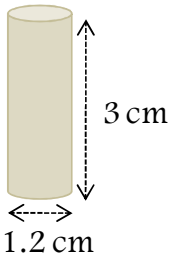


Fig. 3.2. Dimensions of the gel cylinders used in infusion experiments.

For Chapter 6, two polymer networks set ionically were used, calcium alginate to form particles, cylinders and slabs, and gellan gum to form sphere-like particles.

3.2.2.1. Calcium alginate gel samples

3.2.2.1.1. Cylindrical samples used in Chapter 5

Calcium alginate gel cylinders were made using sodium alginate (Sigma-Aldrich, UK). Alginate gels are stable at temperatures up to 140°C and it is possible to alter the electrical conductivity of the gel by modifying the electrical conductivity of the calcium chloride ($CaCl_2$) soaking solution (Kemp, 2000). A solution of sodium alginate 3% w/v was soaked for 24 hours in a $CaCl_2$ aqueous solution 2% w/v. Once the gel was already formed it was removed from the dialysis membrane and cut to be then soaked for 48 hours prior the experiments in another $CaCl_2$ solution which concentration depended on the electrical conductivity needed for the gel.

3.2.2.1.2. 'Spherical' samples used in Chapter 6

The particles were formed by dropping sodium alginate (Sigma-Aldrich, UK) 2% w/v in $CaCl_2$ 0.5% w/v. Concentrations of $CaCl_2$ above 0.5% w/v led to a significant increase in

temperature of the solution (approximately 50°C), that was not possible to control despite the use of a jacket system.

3.2.2.1.3. Slabs and cylinders used in Chapter 6

Calcium alginate gels were formed using a solution of sodium alginate 2% w/v (Sigma-Aldrich, UK) immersed in $CaCl_2$ 0.5% w/v for 48 hours. For $CaCl_2$ 0.5% w/v, the electrical conductivity at 25.5°C was 100.9$\mu S\ cm^{-1}$, value measured using a Seven Easy conductivity meter (Mettler Toledo, UK). The concentrations of alginate and $CaCl_2$ were the same to those for the formation of gel particles under the application of MEF. The samples where cut using parallel stainless steel razor blades to get the sample with geometry shown in Fig. 3.3.

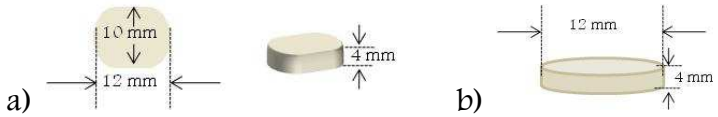


Fig. 3.3. Alginate gel sample used for PEF experiments in a) electrophoration cuvette and b) stainless steel electrodes.

3.2.2.2. Calcium potato-alginate mix gel samples

Calcium potato-alginate gels were formed using the method described by Kemp (2000). Potato powder was added to the alginate gel in order to obtain diffusion coefficients in the same order of magnitude to those of a real food material.

1. 20 g of "instant mash potato" (Sainsbury´s, UK), 7.2g of sodium alginate (Sigma-Aldrich, UK), 0.12g citric acid (Sigma-Aldrich, UK), 0.48g calcium sulphate (Sigma-Aldrich, UK) and 350ml of distilled water were blended for 5 minutes at room temperature until a paste was formed.
2. The mixture was allowed to de-gas for 10 minutes.
3. The mixture was poured into a cellulose dialysis membrane of 1.2cm diameter.

4. The dialysis membranes with the mixture inside were soaked for 24 hours in a $CaCl_2$ solution 2% w/v to get a full penetration of calcium ions.

5. The gel already formed was removed from the $CaCl_2$ and cut, to then be soaked for 48 hours prior to the experiments in calcium chloride solution to modify their electrical conductivity.

3.2.2.3. Albumen gel samples

Chicken egg white albumen (Sigma-Aldrich, UK) was dissolved at room temperature in distilled water at a ratio of 10% w/v. When the albumen was completely dissolved, it was placed in a water bath at 80°C temperature for approximately 40 minutes, until a gel was well formed. The sample was then left at room temperature for 30 minutes to cool down and stored in a fridge. The albumen gel used was never older than one week.

3.2.2.4. Gelatine samples

Gelatine from porcine skin (Fluka, Bio Chemika) was dissolved in distilled water at 30°C using a ratio of 10% w/v, then heated up to 60°C stirring continuously for 10 minutes. After 30 minutes at room temperature to cool down, the sample was placed in the fridge.

3.2.2.5. Gellan gum samples

Gellan gum particles were formed by dropping an aqueous solution of gellan gum at 1% w/v in $CaCl_2$ 0.5% w/v. Concentrations of $CaCl_2$ above 0.5% w/v led to a significant increase in temperature of the solution (approximately 50°C), that was not possible to control despite the use of a jacket system.

3.2.3. Rhodamine6G aqueous solution

An aqueous solution of rhodamine6G dye ($C_{28}H_{30}N_2O_3$.HCl) [molecular mass of rhodamine6G is 479.02$g\ mol^{-1}$, and ionic charge +1 at pH 7 (Fluka catalogue, 2009)], at

2.5mM ($1.2g\ l^{-1}$) concentration was prepared and infused to the different gel systems. Previous tests showed that a high concentration of rhodamine6G is necessary for a better visualisation of its infusion.

3.3. EQUIPMENT

3.3.1. Electrical field supply

In all MEF experiments an alternating current at a voltage up to 240V and 50Hz frequency was supplied through a voltage regulator (Fig. 3.4a) and either stainless steel or Nickel-Titanium plate electrodes (3cmx5.5cm, Fig. 3.4b and Fig. 3.4c) custom made at the University of Birmingham. Voltage and current readings were recorded using separate multimeters (Keithley 2000, RS components Ltd., UK).

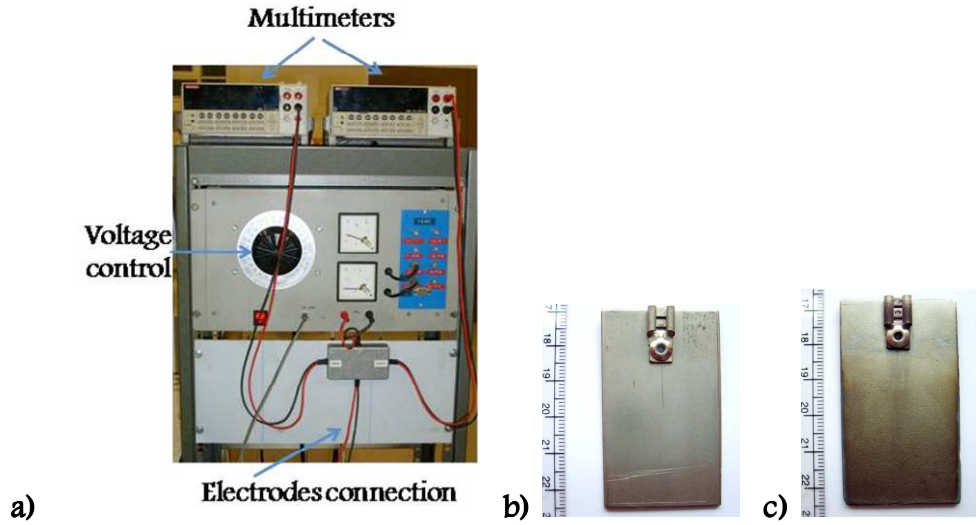


Fig. 3.4a) MEF supplier at the University of Birmingham. Plate electrodes for MEF application: b) stainless steel, c) Nickel-Titanium.

PEF were generated with an electrical pulses generator available at Lund University, Sweden (Cellect BioFusion AB, Sweden) (Fig. 3.5a). The shape of the applied pulse was rectangular monopolar throughout all the experiments, and the length of the pulse 1ms. The maximum number of pulses the trigger used sends is 9 pulses. The pulses were triggered manually for

the treatments with 18, 36, 45, 63 and 90 pulses. The first PEF experiments were made using an electrophoresis cuvette with Aluminium plate electrodes (the gap between electrodes was 4mm) (Fig. 3.5b). To discard possible effects of the Aluminium ions of the electrodes influencing the behaviour of the Calcium ions in the gel during the PEF treatment, stainless steel plate electrodes were also used (20mmx50mm. Fig. 3.5c), with a gap between electrodes of 4mm.

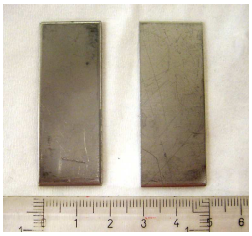
a)  b)  c) 

Fig. 3.5a) PEF generator at Lund University, Sweden. b) Electrophoration cuvette and c) stainless steel plate electrodes.

3.3.2. Temperature measurement

Temperature readings were performed using Type K Thermocouples (RS Components Ltd., UK) connected to the computer using a data logger (Pico Technology, UK). To minimise the effect of electrical processing on the performance of the thermocouples, the tip of the thermocouples were covered with capillary glass and shrinkable plastic.

3.3.3. Experimental chamber and temperature control system

The diffusion cell consisted of a 250ml glass jacket heating and cooling system connected to a water bath. A schematic of the jacket system is presented in Fig. 3.6.

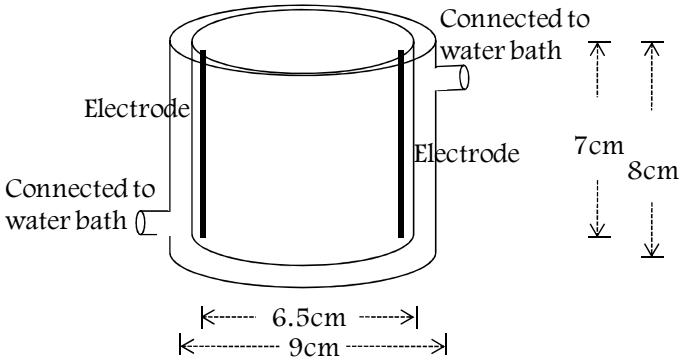


Fig. 3.6. Glass jacket system for experiments.

3.3.4. Flow through cuvette for spectrophotometer

A flow through cuvette (Hellma Analytics, UK) was used in order to have continuous measurements of the changes in concentration of betanin in the solution surrounding the beetroot slab. The specifications of the cuvette (shown in Fig. 3.7) are: SOG material, lightpath 10mm.

Fig. 3.7. Flow through cuvette.

3.4. MEASUREMENT TECHNIQUES

3.4.1. Spectrophotometry

Betanin concentration in the solution surrounding the beetroot slab was measured by spectrophotometry. UV/Visible Spectrophotometry is an analytical technique widely used in laboratories to measure the absorbance or the transmittance of different samples. The spectrophotometer works by measuring the amount of light absorbed by a solution when a beam of light is passed through it, and then the intensity of the light reaching a detector is

measured (Blauch, 2000). The features of the spectrophotometer used in Chapter 4, a Libra S32 (Biochrom Ltd, UK), are summarised in Table 3.2.

Table 3.2. Features and parameters of the Libra S32 spectrophotometer

			Instrument parameters			
Product	Lamp source	Optical system	Wavelength range [nm]	Absorbance range [A]	Bandwidth [nm]	Stray light at 340nm
Libra S32	Deuterium/ Tungsten press to read	Split beam, reference beam compensation	190 -1100	-0.3 - 3.000	<1.8	<0.025%T

The intensity of light (Λ_0) passing through a blank is measured (distilled water was used as blank solution), then the intensity of light (Λ) passing through the sample solution contained in the flow through cuvette is measured. The transmittance (Γ) and the absorbance (Abs) are estimated as follows:

$$\Gamma = \frac{\Lambda}{\Lambda_0} \qquad \textbf{(3.1)} \qquad\qquad Abs = - \log_{10} \Gamma \qquad \textbf{(3.2)}$$

Biological pigments (like betanin) absorb light at specific wavelengths, this reflection of light in the visible spectrum is characteristic of their colour (Kujala *et al.*, 2001). In order to identify the best wavelength at which betanin molecule absorbs light (w_{max}), a spectral scan was done. The scan for four solutions of betanin at different concentrations covered a range from 300 to 650nm. An absorption peak was found between 400 and 600nm. Fig. 3.8 shows the spectral scan for the betanin extracted from beetroot (detailed in section 3.5.4 of this chapter) and three decimal dilutions from it.

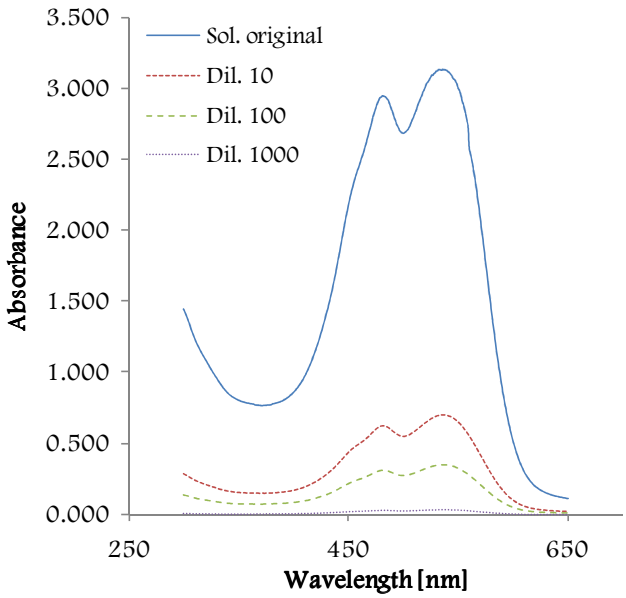


Fig. 3.8. Spectral scan in a range from 300 to 650nm for four solutions of betanin at different concentrations. The highest peak represents the wavelength of maximum absorbance.

For betanin values, the wavelength at which the absorbance is the greatest (w_{max}) was detected at 540nm.

3.4.1.1. Calibration curve for spectrophotometer

The colorant used for the calibration curve was Duranat Beetroot red food colour powder (Town End Leeds plc, UK), classification E162, obtained from red beet juice spray-dried on maltodextrin-free food lumps. Three solutions of beetroot red food colour at three different concentrations from $4x10^{-5}$ to $8x10^{-3} g\ l^{-1}$ and a blank solution (distilled water) were passed at $2 ml\ min^{-1}$ through the flow cuvette and the absorbance recorded at a wavelength of 540nm. Fig. 3.9 illustrates the calibration curve Absorbance-Concentration, as well as the linear equation that fitted the data.

$$C = 0.0039 * Abs \qquad (3.3)$$
$$R^2 = 0.9998$$

Where C is the concentration [g l^{-1}] and Abs is the absorbance.

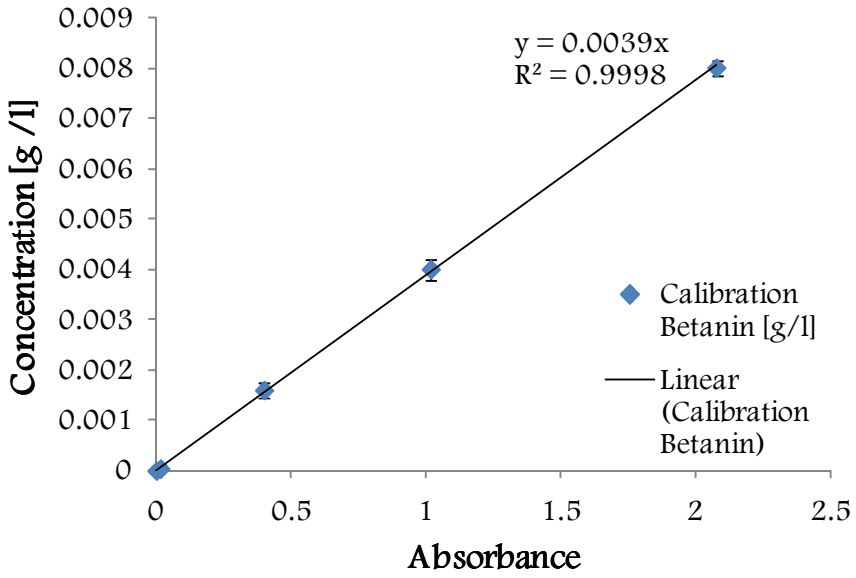


Fig. 3.9. Calibration curve for absorbance measurements of four solutions of betanin at different concentrations.

3.4.2. Image analysis

The infusion of rhodamine6G to gel systems was quantified by scanning discs of gel after the experiment (Hewlett Packard Scan Jet 5200C) and analysing the changes in mean grey values with the software Image J (Wayne Rasband, National Institutes of Health, USA.). Concentration was a function of radial distance, using the calibration curves to convert the grey values measured to the actual concentration of the dye.

3.4.2.1. Calibration curves for image analysis

Gel samples were equilibrated in rhodamine6G solutions having a range of concentrations from zero to 1.2g l. Five discs of the alginate, potato-alginate and albumen gels having the same dimensions than those cut from the gel cylinder after the experiment (1.2cm diameter and 0.3cm long) were soaked for 48 hours in the rhodamine6G solutions (Fig. 3.8b to 3.10b). The samples were then scanned and the average grey value was determined with image analysis to obtain the calibration curve. These calibration curves are presented in Fig. 3.10a to Fig. 3.12a.

The graph obtained by plotting the average grey value of the gel slices versus the average concentration value was fitted to an exponential model in order to get the calibration curve for each gel used. For all the gels used the relationship between intensity and concentration was non-linear, the best fitting was obtained with exponential curves of the form:

$$C = a * e^{-b(Grey\ value)} \quad \textbf{(3.4)}$$

Where C is the concentration in [g ml^{-1}], a and b were constants fitted using Microsoft Excel software.

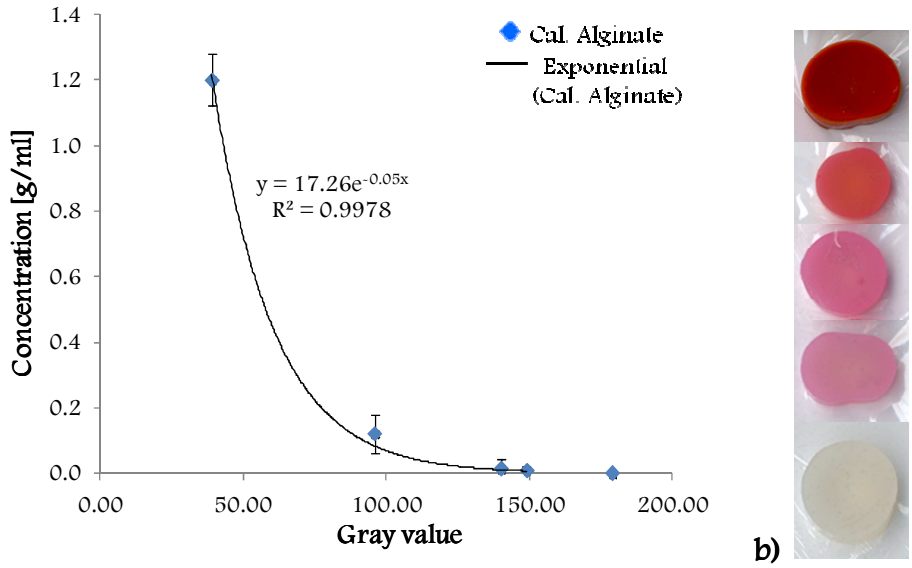


Fig. 3.10a) Calibration curve for the infusion of rhodamine6G in alginate; b) alginate discs equilibrated in rhodamine6G.

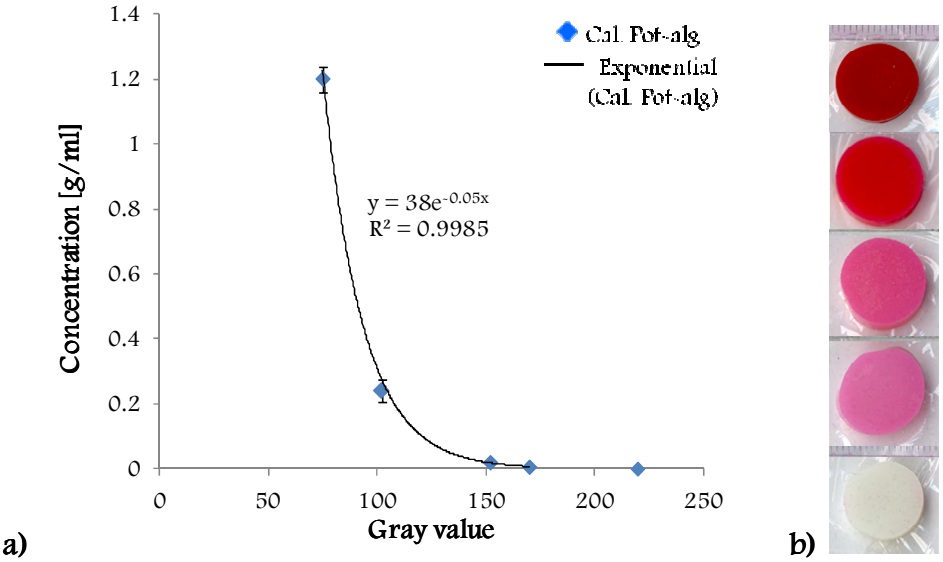


Fig. 3.11a) Calibration curve for the infusion of rhodamine6G in potato-alginate; b) discs of potato-alginate gel equilibrated in rhodamine6G.

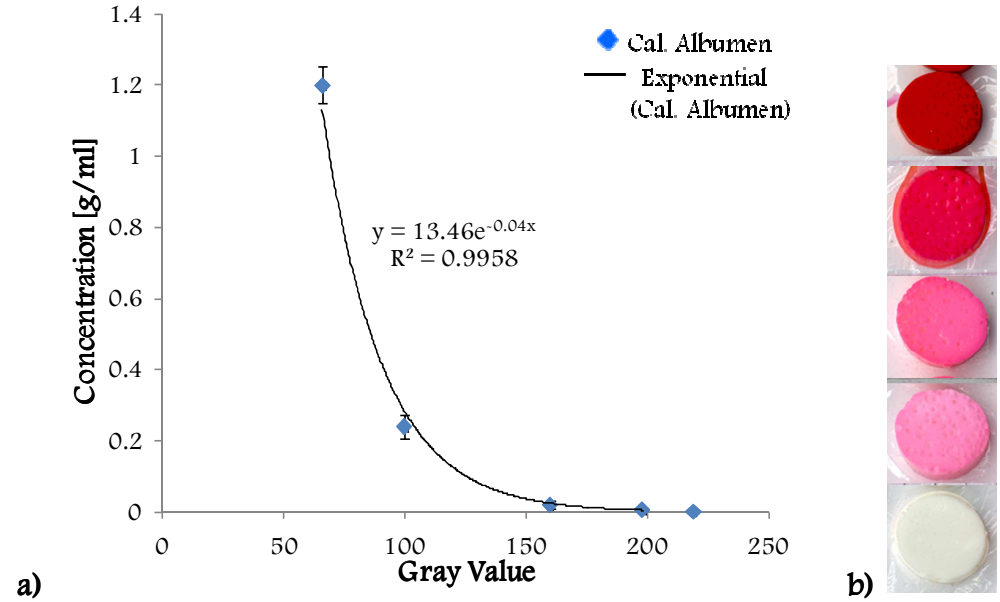


Fig. 3.12a) Calibration curve for the infusion of rhodamine6G in albumen gel; b) discs of albumen gel equilibrated in rhodamine6G.

3.4.3. Compression test

A compression test was used to study the mechanical properties of the gels.

For MEF experiments the compression test consisted in the determination of the strength of the sample subjected to compression with a 20mm diameter aluminium cylinder probe (which diameter was approximately four times bigger than the gel particle diameter). A schematic of sample and probe used can be seen in Fig. 3.13a. The speed used in the test was set at $0.2 mm\ s^{-1}$ and distance for compression at 1mm, as in Martinsen *et al.* (1989). The compression apparatus used was TA.XTPlus Texture Analyzer (Texture Technologies, Stable Microsystems), a photo of the equipment is presented in Fig. 3.13b.

For PEF experiments, samples were subjected after electrical processing to a uniaxial compression test in a 4400 Instron (Instron, EU) available at Lund University, Sweden; and the compression force necessary to compress 50% of the sample length (2mm) was

recorded. The particles were compressed from the top at 0.28$mm\ s^{-1}$ with a stainless steel compression platen of 30mm diameter, as is schematically shown in Fig. 3.14a.

Similar tests have been used in the past (Zvitov and Nussinovitch, 2003; Martinsen *et al.*, 1989).

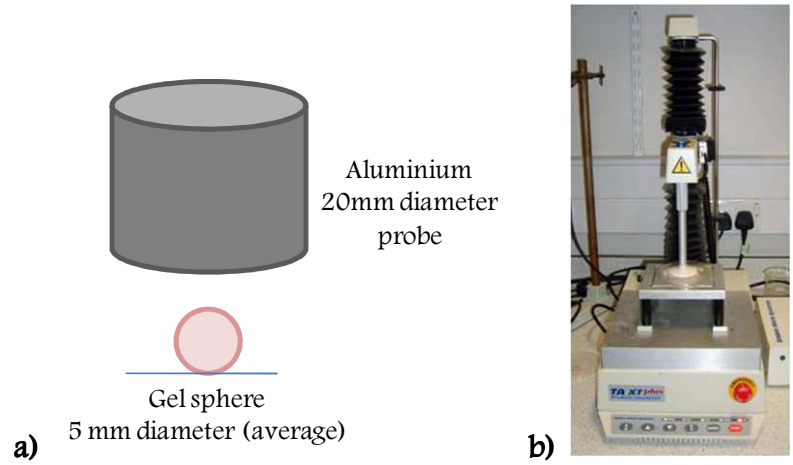


Fig. 3.13a) Schematic of the probe used for texture analysis of the gel particles, b) Texture analyser at the University of Birmingham.

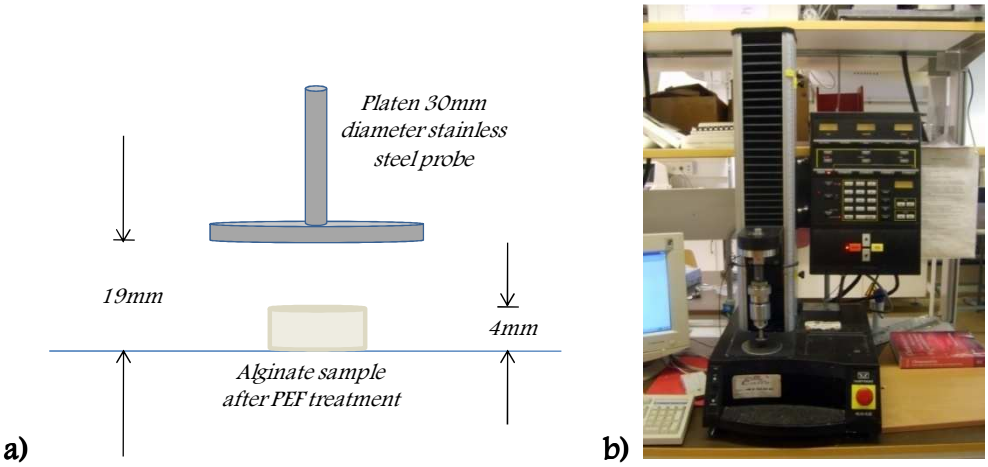


Fig. 3.14a) Diagram of probe and sample in compression test, b) Instron universal testing machine at Lund University.

3.5. EXPERIMENTAL PROCEDURES

3.5.1. For mass transfer in cellular material under the application of MEF

The diffusion cell was filled with 170ml of Sodium chloride (NaCl) solution 3.4mM ($\sigma_{NaCl\ solution}$=0.05$S\ m^{-1}$ at 25°C) and stirred with a magnetic follower. The water bath temperature was adjusted for each experiment to compensate the heat lost in the tubing and the beakers' walls. In order to make the measurement system continuous, NaCl solution was withdrawn from the diffusion cell using a peristaltic pump (Ismatec) at a rate of $15 ml\ min^{-1}$ and pumped to a flow-through cell. The absorbance value of the NaCl solution remained constant at 0.000. For the first 2 minutes, only the absorbance, temperature, voltage and current were recorded, after which the beetroot was immersed in the diffusion cell and electrically processed for 5 minutes, then at $0 V\ m^{-1}$ (no electrical processing) for a further 55 minutes. The diffusion experiments were made at steady temperatures of 40, 50 and 60°C, and electrical fields of 600 and 1000 $V\ m^{-1}$. Absorbance measurements were recorded each 2 seconds.

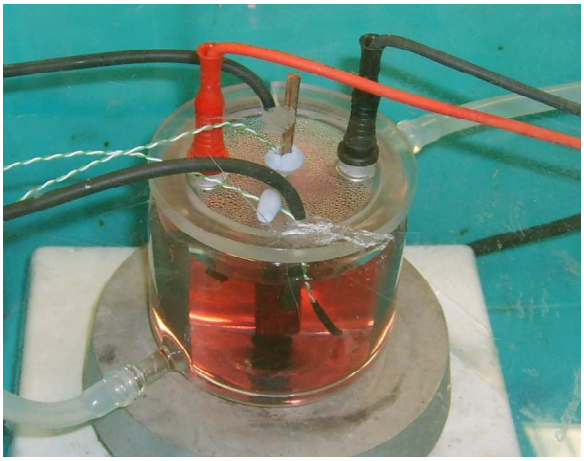


Fig. 3.15. System used for the diffusion experiments.

A photo of the system and a schematic of the experimental set-up for online monitoring of betanin diffusion from beetroot are presented in Fig. 3.15 and Fig. 3.16.

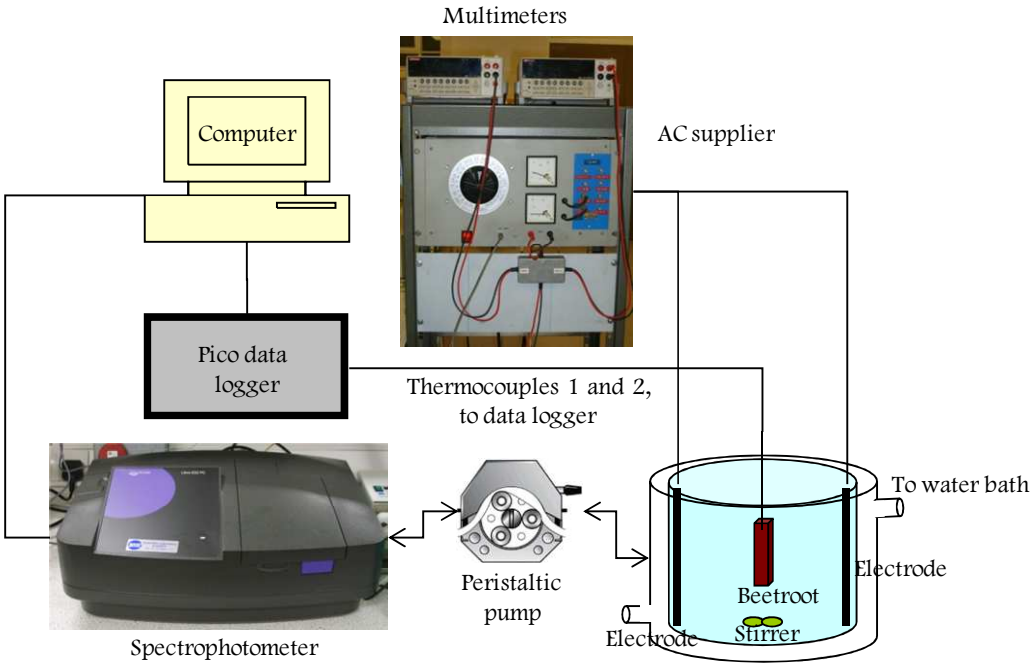


Fig. 3.16. Experimental set-up for the mass transfer experiments and online concentration monitoring.

Similar experiments were performed to investigate the effect of the orientation of the sample with respect to the electrical fields. A schematic of the experimental set-up is shown in Fig. 3.17.

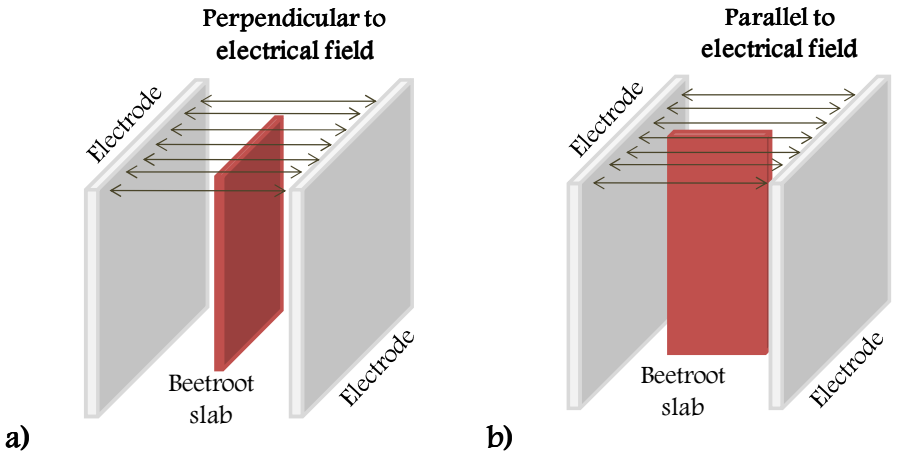


Fig. 3.17. Position of the slab respect to the electrical field: a) perpendicular and b) parallel.

3.5.2. For mass transfer in gel systems under the application of MEF

A cylindrical gel sample was soaked in a solution of rhodamine6G 1.2$g\ l^{-1}$ which was kept at a constant processing temperature. By adjusting the temperature of the water bath it was possible to remove the heat generated by the application of the electrical field. Gel samples were stored in a fridge (4 to 6°C) prior to the experiments; samples were then soaked in the same $CaCl_2$ solution at room temperature for 30 minutes. The diffusion cell was filled with 170ml of rhodamine6G solution and stirred with a magnetic follower (Magnetic stirrer Jenway 1000). One thermocouple was placed at the middle of the cylindrical gel sample and another one measured the temperature of the solution. The gel cylinder was immersed in the diffusion cell and electrically processed for 10 minutes at1000 or 1200$V\ m^{-1}$, then it was left in the processing cell at 0$V\ m^{-1}$ for a period of 5 minutes for alginate and potato-alginate samples, 30 minutes for albumen and 20 minutes for gelatine. After that time, the cylinder sample was weighted and its diameter measured, then it was cut in discs of 1.2cm diameter and 0.3cm long using parallel stainless-steel scalpels and taken to the flat scanner for the acquisition of images. The schematic of experimental set-up for rhodamine6G infusion experiments is shown in Fig. 3.18.

The experimental method used in this study allowed us to identify the effect on mass transfer of the electrical field applied and the differences in electrical conductivities on mass transfer. Three different levels of electrical conductivity of alginate and potato-alginate gels were set by soaking the samples in solutions of $CaCl_2$ at concentrations of 0.05$g\ l^{-1}$, 0.2$g\ l^{-1}$ and 20$g\ l^{-1}$. For thermally set gels (albumen and gelatine) the electrical conductivity was not modified and in both cases it was higher than the electrical conductivity of the rhodamine6G solution surrounding them. The concentration, and therefore the conductivity of rhodamine6G, solution was constant for all the experiments.

Three different cases were investigated by varying the electrical conductivity of the gel:

Case 1. Electrical conductivity of rhodamine6G solution was lower than the electrical conductivity of alginate and potato-alginate gels ($\sigma_{Gel}>\sigma_{Solution}$).

Case 2. Electrical conductivity of rhodamine6G solution was matched with that of alginate and potato-alginate gels ($\sigma_{Gel}=\sigma_{Solution}$).

Case 3. Electrical conductivity of rhodamine6G solution was higher than the electrical conductivity of alginate and potato-alginate gels ($\sigma_{Gel}<\sigma_{Solution}$).

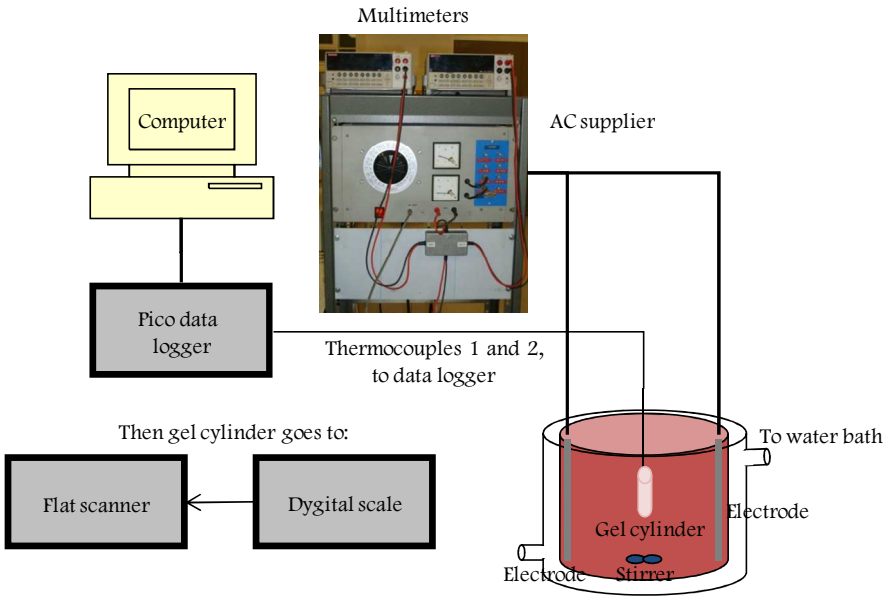


Fig. 3.18. Experimental set-up for the infusion of rhodamine6G in gel systems.

All alginate and potato-alginate samples were weighed before and after the experiment (removing first the excess of $CaCl_2$ and rhodamine6G solution with a napkin) and the diameter at the bottom end of the cylinder was measured with a digital calliper.

3.5.3. For compression force in gel systems under the application of MEF and PEF

3.5.3.1. MEF treatment

The experiments consisted in the formation of alginate and gellan gum particles at different MEF strengths (700, 1000 and 1400 $V\,m^{-1}$) and processing temperatures (20 and 40°C) by

dropping 5ml of gel solution with a plastic syringe (1.5mm diameter) in 100ml of $CaCl_2$ solution. The gel particles were kept in the $CaCl_2$ solution for 5 minutes after their formation. The average diameter of the particles formed was measured with a digital calliper.

The experimental set-up for MEF experiments is shown in Fig. 3.19 and Fig. 3.20.

Fig. 3.19. System used for the application of MEF during the formation of gel particles.

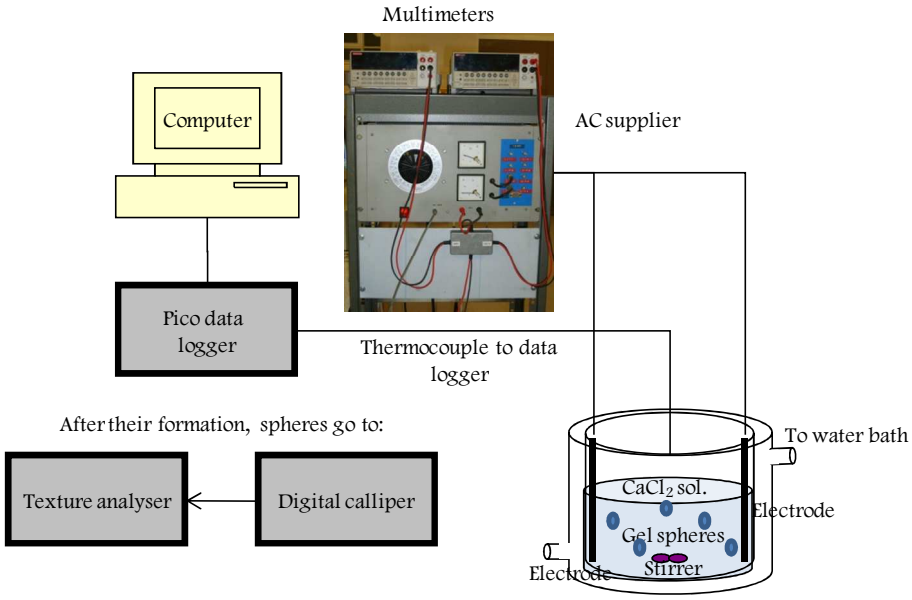


Fig. 3.20. Experimental set-up for gel particles' electrical treatment.

A Leica Optical microscope DMRBE (Leica Microsystems, Germany) was used to visualise the surface and structure of alginate gel particles. A photo of the microscope used is shown in Fig. 3.21.

Fig. 3.21. Leica Optical microscope used to visualise alginate gel particles.

3.5.3.2. PEF treatment

Calcium alginate samples were subjected to PEF treatment which included 1, 9, 18, 36, 45, 63 and 90 pulses of 1ms pulse length at 300V in the electrophoration cuvette and 54 and 90 pulses of 1ms at 300V with the stainless-steel electrodes. The electrical field in all cases was 750 $V\ cm^{-1}$. The PEF experiments for each treatment were repeated five times. All the experiments were done at room temperature (20°C). The experimental set up for the PEF experiments is presented in Fig. 3.22.

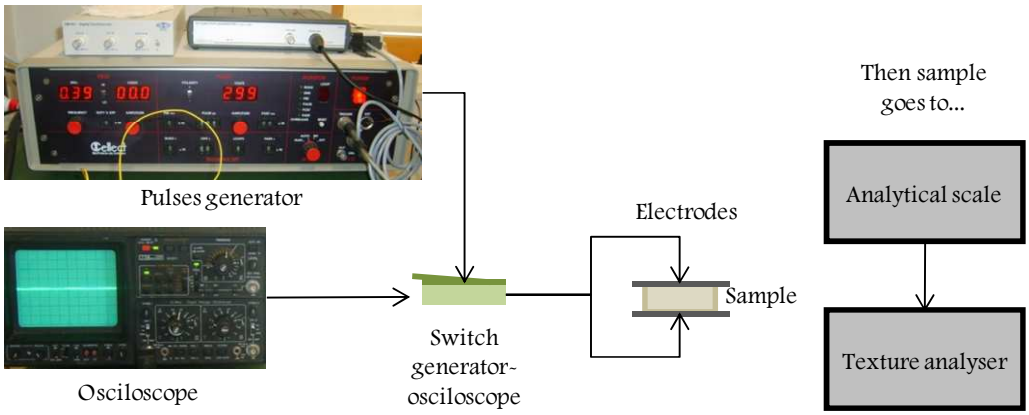


Fig. 3.22. Experimental set-up for PEF experiments.

The samples were weighted in an analytical scale before and after the PEF treatment and after the compression test in order to identify whether the application of the pulses was the reason for changes in weight and structure of the samples.

The experimental conditions used for MEF and PEF treatments are described in Table 3.3.

Table 3.3. Summary of experimental settings for MEF and PEF experiments

Parameter	*Moderate Electrical Fields (at the University of Birmingham)*	*Pulsed Electrical Fields (at Lund University)*
Gel formation	Drops of alginate 2% w/v in a $CaCl_2$ 0.5% w/v and left there for 5 minutes	Alginate 2% w/v inside a dialysis membrane and soaked in $CaCl_2$ 0.5% w/v for 48 hours
Sample geometry	Particles of 5mm average diameter	Slabs of 10x12x4mm and cylinders of 4mm long and 12mm diameter
Processing temperature	20 and 40°C	Room temperature, 20°C
Processing time	5 minutes	~~~~
% of sample compressed	20	50
Texture analyser	Texture analyser (TA XT plus Stable Micro systems)	Instron Single Column Testing System.
Electrical treatment	Electrical fields of 0, 7, 10 and 14 $V\ cm^{-1}$ during 5 minutes	0, 1, 9, 18, 36, 45, 63 and 90 pulses of 1ms at 300V, electrical field of 750 $V\ cm^{-1}$

3.5.4. Betanin extraction from fresh beetroots

The maximum amount of betanin (C_0) contained in beetroot was obtain as follows:

1. One beetroot was rinsed with tap water, peeled and then blended for one minute with 240ml of distilled water at a volume ratio 2:1.
2. The solution obtained was stirred at room temperature (approximately 25°C) for 30 minutes and a stirring velocity high enough to form a vortex. The pH of the solution was 6.22.
3. The solution was centrifuged for 10 minutes at 3000rpm.
4. The liquid obtained after the centrifugation was filtered.

5. 3ml of the resulting solution were filtered again with a syringe filter and kept in a fridge for calibration and characterisation matters.

Using spectrophotometry analysis it was calculated that there is $4.41x10^{-4}$g of betanin in 1g of beetroot. For a slab of dimensions 3x2x0.3cm ($1.8cm^3$ volume and 2g weight) the concentration of betanin is $8.82x10^{-4}$g of betanin per beetroot slab; agreeing with previous studies where the reported maximum amount of betanin extracted was:

* $1x10^{-3}$g per slab; up to 200mg of betanin in one beetroot of approximately 40g weight.

$2.6x10^{-4}$ to $1x10^{-3}$g per slab; an average 130mg of betanin per 100g fresh weight of beetroot, new red beetroot cultivars can yield up to 450 to 500mg per 100g fresh root (Nottingham, 2004).

* $6x10^{-4}$g to $1.2x10^{-3}$g per slab; 300 to 600 $mg\ kg^{-1}$ in a beetroot (Harmer, 1980).

3.5.5. Betanin charge according to pH

Betanin is highly susceptible to changes in both pH and temperature. w_{max} (best wavelength at which a molecule absorbs light), colour and ionic charge carried by betanin are influenced by the pH conditions. The charge of betanin becomes +1 at pH less than 1.5, -2 at pH between 2.5 and 7.5, and a charge of -3 at pH between 7.5 and 9.5. The charge of betanin becomes neutral (zero charge) and exhibits an isoelectric point at pH between 1.5 and 2.5 (Yang *et al.*, 2003).

pH value was measured for the experimental conditions used in the set of experiments for the extraction of betanin from beetroot, in a range of processing temperature from 20 to 60°C. Temperature vs. pH profile is shown in Fig. 3.2323. Ionic charge of betanin, for pH ranging from 5 to 6, is -2.

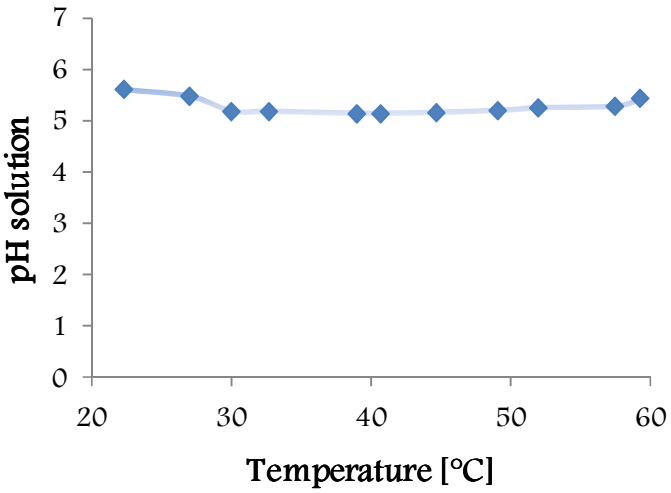


Fig. 3.23. Changes in pH of the solution where a slab of beetroot is immersed with increase in temperature.

3.5.6. Measurement of the changes in compression force when the gel is kept soaking in $CaCl_2$ solution

In order to identify if the compression force in gel samples varies in respect to the time it is left soaking in $CaCl_2$, dialysis membrane containing sodium alginate 2% w/v was left soaking in $CaCl_2$ solution 0.5% w/v for a week. Four cylinders of calcium alginate gel, having a 1.2cm diameter and a height of 4mm, were cut after 1, 2, 3, 7 and 8 days of soaking and the force necessary to compress 2mm of the cylinder was measured using a TA.XTPlus Texture Analyser (Texture Technologies, Stable Microsystems).

Fig. 3.24 represents compression force vs. time the material was left in $CaCl_2$ solution. The maximum compression force needed to compress 2mm of the cylinder reached a plateau after 2 days soaking in $CaCl_2$. Thus in the experiments performed the gels were used only after being soaked for 48 hours.

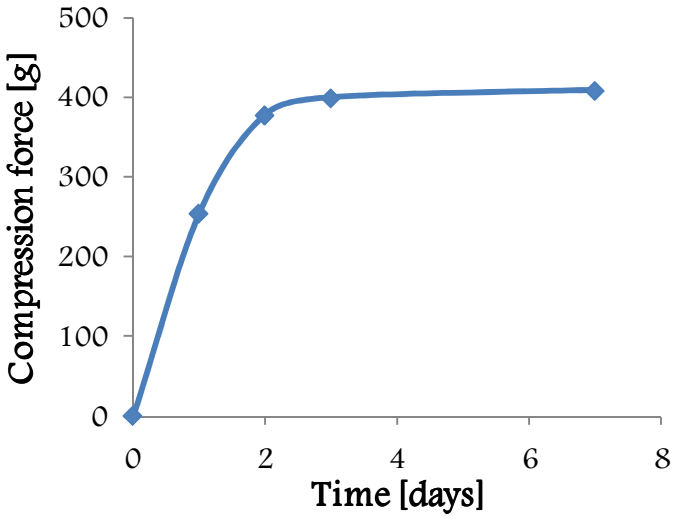


Fig. 3.24. Maximum compression force vs. time the alginate has been soaked in $CaCl_2$.

CHAPTER 4

MASS TRANSFER IN CELLULAR MATERIAL UNDER THE APPLICATION OF MODERATE ELECTRICAL FIELDS (MEF)

4.1. INTRODUCTION

The effect of the application of alternating MEF on the extraction of betanin from beetroot was studied by changing the processing temperature, the applied electrical field and the position of the beetroot slab. The concentration of betanin was monitored on-line and measured by spectrophotometry. The effect of the MEF was quantified by estimating the effective diffusion coefficients (D_{eff}s) with a solution assuming Fick´s 2nd law of diffusion is valid. The results obtained from the experiments are presented in this chapter. An enhancement in mass transfer was observed, it was proportional to the increase in processing temperature and electrical field strength.

4.2. RESULTS AND DISCUSSION

4.2.1. Temperature profiles for mass transfer experiments

Application of electrical fields creates a source of heat. Throughout the experiment, the temperature of the sample and the surrounding solution were kept constant by adjusting the temperature of the water bath connected to the diffusion cell. These adjustments to the temperature of the water bath also ensured similar temperature profiles were maintained for the experiments at different levels of electrical treatment. In Fig. 4.1, the temperature of the sample (a) and the surrounding solution (b) are shown at different times for a typical experiment. It can be seen that the profiles for beetroot slab (Fig. 4.1a) and NaCl solution (Fig. 4.1b) at 0 $V\,m^{-1}$ match the profiles at 600 and 1000 $V\,m^{-1}$.

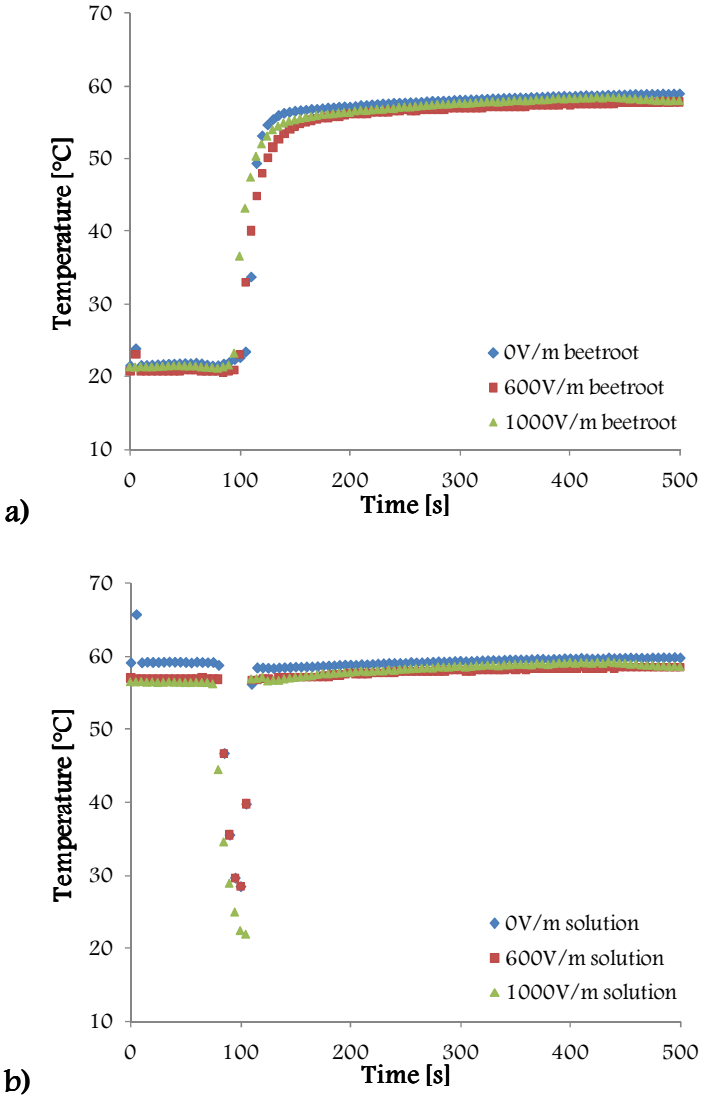


Fig. 4.1a) Temperature profiles at processing temperature of 60°C and applying MEF of 0, 600 and 1000 $V\,m^{-1}$, for a) beetroot slab and b) solution surrounding slab.

4.2.2. Effect of temperature and electrical field on the extraction of betanin

4.2.2.1. Preliminary 'Long' experiments

Preliminary diffusion experiments were carried out in order to achieve constant concentration of betanin in the NaCl solution at the three different processing temperatures used in this study. A beetroot slab was left soaking in the solution until constant concentration of betanin in the solution was achieved (Fig. 4.2). Using slabs from the same beetroot for the three experiments (to avoid possible differences in samples' structure), the

maximum amount of betanin extracted at 40°C was $3.29x10^{-4} kg_{Bet} kg_{Beet}^{-1}$ after 10 hours, $3.45x10^{-4} kg_{Bet} kg_{Beet}^{-1}$ after approximately two hours at 50°C and $4.39x10^{-4} kg_{Bet} kg_{Beet}^{-1}$ after one hour at 60°C. Extraction at both 40 and 50°C reached a similar steady concentration value but at different processing times, with extraction increasing relatively to temperature.

Concentration profile at 60°C, from Fig. 4.2, shows degradation of beetroot extract. The stability of betalains' colour and beet extract get affected principally by temperature (Chetana, 2007; Castellar, 2003). At high temperatures, the sugars present in the beet extract caramelise and cause fermentation; degradation of betanin occurs, changing its colour from red to brown.

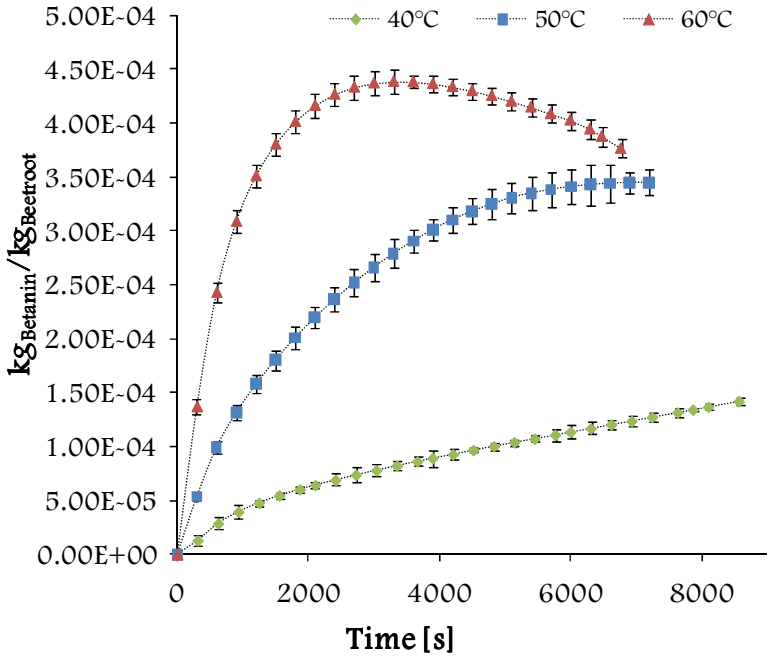


Fig. 4.2. Betanin extraction profiles for 'long experiments' at 40, 50 and 60°C. The period of time to the left of the vertical dashed line shows the duration of the electrical process.

4.2.2.2. Betanin degradation

From the 'long experiments' performed, presented in section 4.2.2.1, it was observed that betanin dye degradated at 60°C. The colour of betanin gets affected by heat, causing discolouration, going from red to light brown. As temperature increases, predominantly in the presence of oxygen (e.g. during storage or processing), irreversible betanin degradation is promoted. Thermal degradation of betanin has been shown to follow first-order kinetics over a pH range from 3.0 to 7.0 under aerobic conditions (Hendry, 1996).

Solutions of betanin dye at a concentration of 0.8$g\ l^{-1}$ were heated at different constant processing temperature ranging from 35 to 60°C, during 100 minutes. The colour of the solution gradually changed. The profiles for time vs. concentration of betanin are presented in Fig. 4.3.

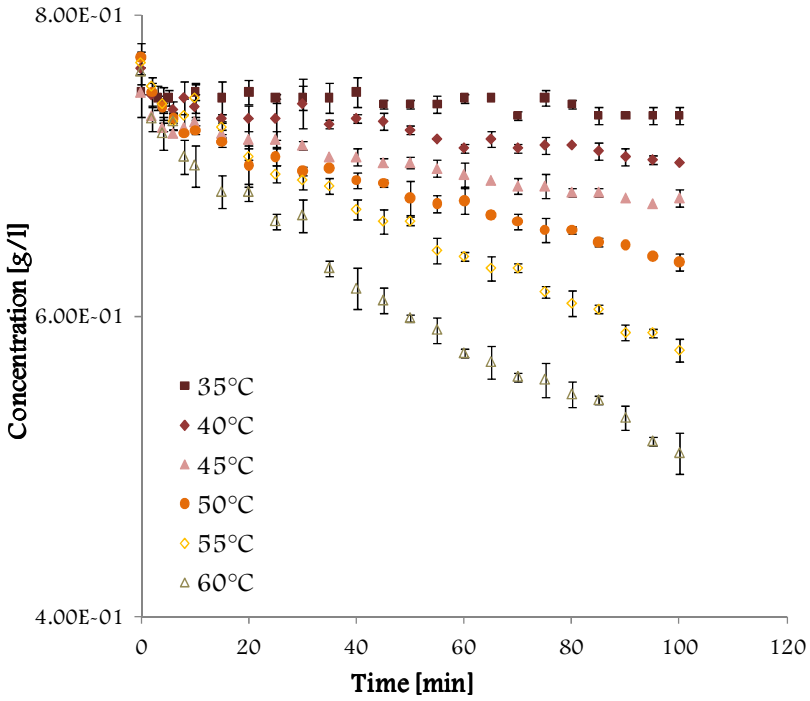


Fig. 4.3. Time vs. Concentration profiles for the degradation of betanin dye at different processing temperatures. Vertical error bars represent standard deviation for the duplicates done.

In the extraction experiments described in this experimental section of the study, the material undergoing processing temperatures of 50 and 60°C, and electrical processing at electrical fields up to $1000\,V\,m^{-1}$ was raw beetroot (thus the betanin present in it). In order to see whether degradation of betanin present in raw beetroot would occur in the extraction experiments and affect the results obtained (during both $0\,V\,m^{-1}$ and MEF application), experiments consisting in heating a slab of raw beetroot at constant processing temperatures of 52.5°C, 55°C and 57.5°C were made. Results are shown in Fig. 4.4, where it can be seen that thermal processing of raw beetroot at temperatures of 55°C or higher, accelerates extraction but degradation occurs for processing times around one hour.

For the experiments with powdered betanin, degradation of solution's colour was observed even at processing temperatures of 40°C, whilst in the experiments for betanin extraction from raw beetroot slabs degradation was observed only at temperatures of 55°C or higher. A possible explanation could be that when working with cellular material (raw beetroot) the vacuole membrane, cell membrane, fibres, polyphenols, antioxidants and other compounds present in beetroot protect the colorant from degradating.

Since the experimental set-up for the experiments for betanin extraction consists in processing temperatures of one hour, degradation of betanin was not considered to affect the differences in rate of extraction of betanin when beetroot was subjected to electrical processing. Nevertheless, the study of betanin colour degradation under the application of electrical fields could be a possible area for future work.

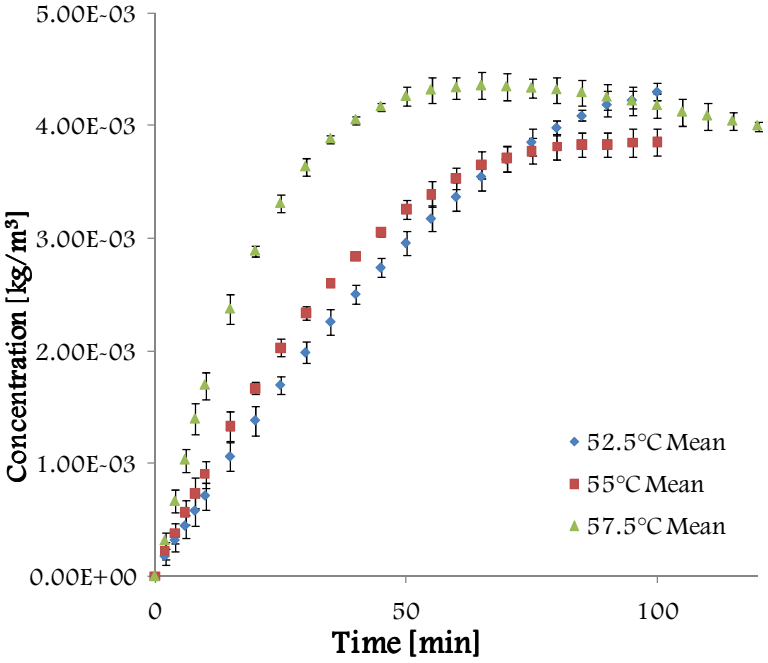


Fig. 4.4. Time vs. Concentration profiles for the degradation of betanin in raw beetroot at temperatures between 50 to 60°C. Vertical error bars represent standard deviation for the duplicates done.

4.2.2.3. Mass transfer experiments

A set of experiments to investigate the extraction of betanin at different temperatures and MEF strength was performed. Fig. 4.5 shows the profiles of the average amount of betanin extracted vs. time from the triplicates done for each temperature and electrical field combination. Both the application of MEF up to 1000 $V\,m^{-1}$ and the increase in processing temperature, enhanced the effusion of betanin from beetroot. The enhancement is proportional to the increase in temperature and electrical field. Temperature appears to have a more pronounced enhancing effect on extraction that the electrical field applied. At 60°C the enhancing effect of electrical processing was the highest compared with results at 40°C and 50°C.

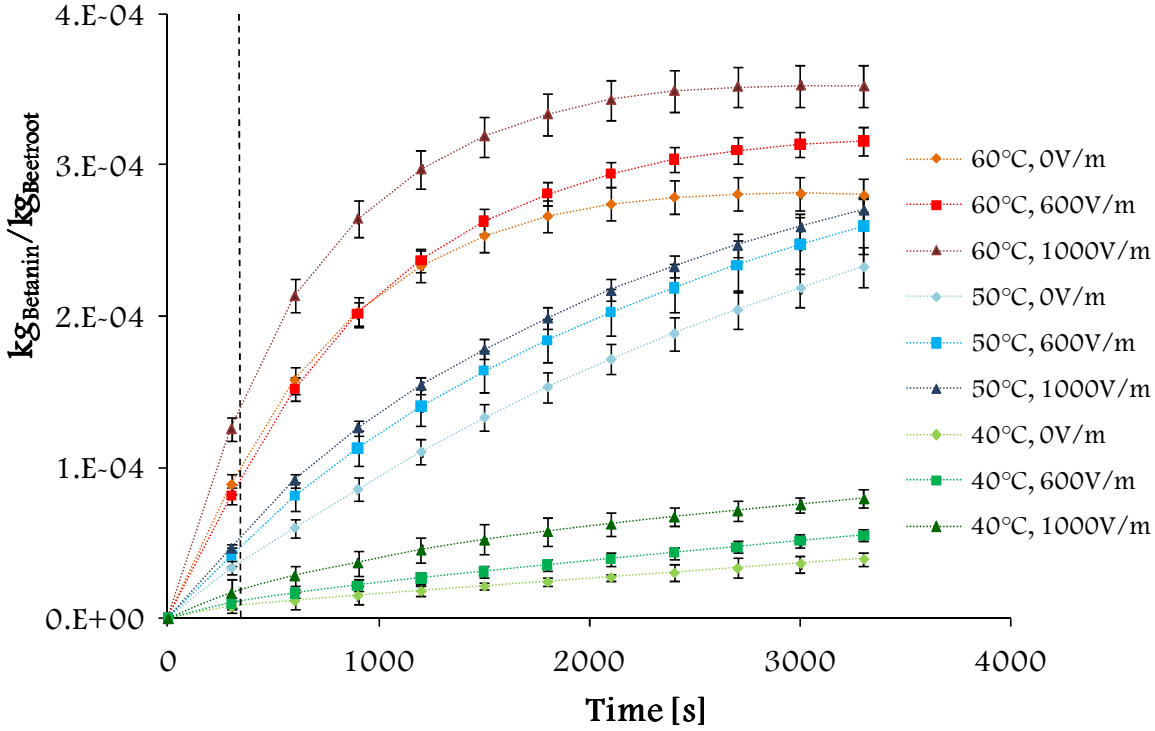


Fig. 4.5. Betanin extraction profiles at 40, 50 and 60°C and electrical fields of 0, 600 and 1000 $V\,m^{-1}$. Vertical error bars represent standard deviation of triplicates performed. The period of time to the left of the vertical dashed line shows the duration of the electrical process.

The same betanin extraction profiles are presented in Fig. 4.6 to Fig. 4.8 for each processing temperature. For 40°C (Fig. 4.6) at minute 55 in the experiments performed, the average amount of betanin extracted increased almost 40% from $3.95\text{x}10^{-5} kg_{Bet} kg_{Beet}^{-1}$ at $0\,V\,m^{-1}$ to $5.50\text{x}10^{-5} kg_{Bet} kg_{Beet}^{-1}$ at $600\,V\,m^{-1}$, it increased 100% with the amount of betanin extracted at $1000\,V\,m^{-1}$ of $7.94\text{x}10^{-5} kg_{Bet} kg_{Beet}^{-1}$.

The results from the experiments at 50°C (Fig. 4.7) showed an increase in the amount of betanin extracted at 55min, with the value of $2.33\text{x}10^{-4} kg_{Bet} kg_{Beet}^{-1}$ at $0\,V\,m^{-1}$ increasing 11% in the experiments at $600\,V\,m^{-1}$ where the amount of betanin released was $2.60\text{x}10^{-4} kg_{Bet} kg_{Beet}^{-1}$, and being 16% higher at $1000\,V\,m^{-1}$ with $2.70\text{x}10^{-4} kg_{Bet} kg_{Beet}^{-1}$ of betanin transferred to the NaCl solution.

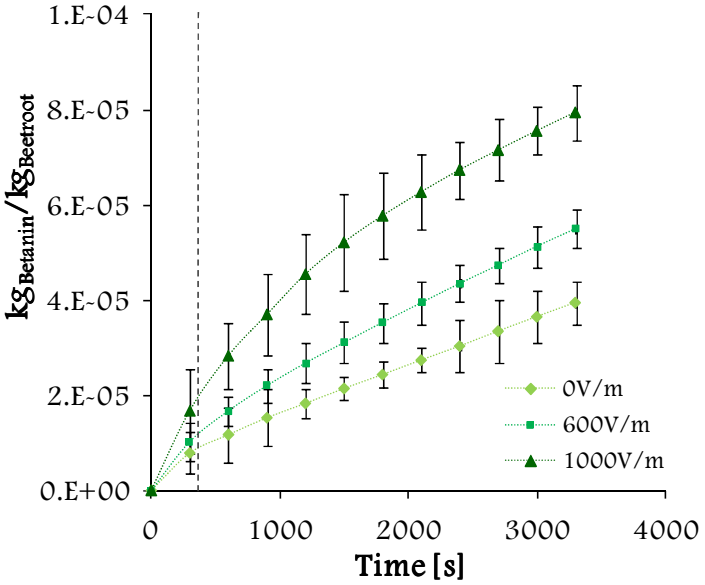


Fig. 4.6. Betanin extraction profiles for the experiments at 40°C and electrical fields of 0, 600 and 1000 $V\,m^{-1}$. Vertical error bars represent standard deviation of triplicates performed. Period to the left of dashed line shows duration of electrical processing.

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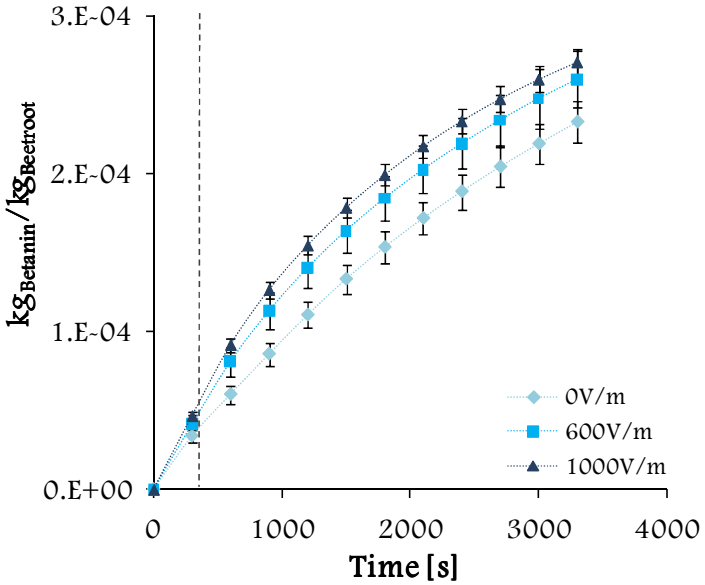

Fig. 4.7. Betanin extraction profiles for the experiments at 50°C and electrical fields of 0, 600 and 1000 $V\,m^{-1}$. Vertical error bars represent standard deviation of triplicates performed. Period to the left of dashed line shows duration of electrical processing.

At 60°C constant processing temperature (Fig. 4.8), the application of MEF resulted in an increase of 12% from 2.80x10^{-4} $kg_{Bet}kg_{Beet}^{-1}$ extracted at 0 $V\,m^{-1}$ to 3.16x10^{-4} $kg_{Bet}kg_{Beet}^{-1}$ at 600 $V\,m^{-1}$; and an even higher increase of 25% at 1000 $V\,m^{-1}$, reaching 3.52x10^{-4} $kg_{Bet}kg_{Beet}^{-1}$.

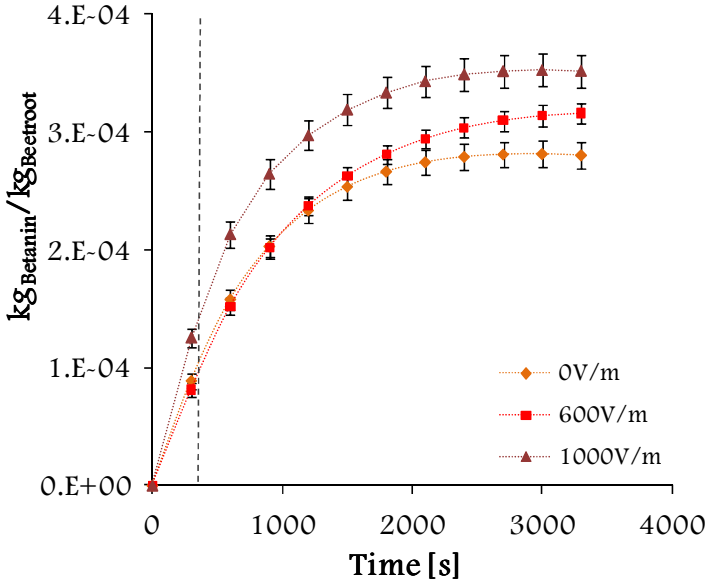

Fig. 4.8. Betanin extraction profiles for the experiments at 60°C and electrical fields of 0, 600 and 1000 $V\,m^{-1}$. Vertical error bars represent standard deviation of triplicates performed. Period to the left of dashed line shows duration of electrical processing.

Kruskal-Wallis statistical analysis of the results of all the different treatments showed significant differences between the treatments:

1. 40°C - 0 $V\,m^{-1}$ and 60°C - 0 $V\,m^{-1}$.
2. 40°C - 0 $V\,m^{-1}$ and 60°C - 600 $V\,m^{-1}$.
3. 40°C - 0 $V\,m^{-1}$ and 60°C - 1000 $V\,m^{-1}$.
4. 40°C - 600 $V\,m^{-1}$ and 60°C - 1000 $V\,m^{-1}$.

When performing the analysis for each temperature group (which includes treatments at 0, 600 and 1000 $V\,m^{-1}$), significant differences were found only between the treatments 40°C - 0 $V\,m^{-1}$ and 40°C - 1000 $V\,m^{-1}$.

An interpretation of the analysis is: MEF had a significant effect on mass transfer at the temperatures above 40°C used in this study (50 and 60°C) and at electrical field strength of 1000 $V\,m^{-1}$ or higher.

Nevertheless, the results indicate that MEF and thermal treatment cause an increase in the extraction of betanin and cell structure damage. The betanin that is outside the cell is removed by both thermal and electrical processes (mechanism referred as appoplastic transport by Marcote *et al.*, 1998), then the cell membrane suffers temporary damage, caused by thermal and electrical heating as well, allowing the betanin to be extracted from the vacuole (Symplastic transport [Marcote *et al.*, 1998]). Although the disruption in the cell wall of the beetroot is caused by both thermal and electrical damage, the effect of temperature to the cellular structure of the beetroot cells appeared to be more pronounced than the effect of MEF in the range of experimental conditions used for this study.

4.2.2.4. Micrographs of beetroot tissue

Optical light microscopy was used as an attempt to visualise the effect of processing on beetroot cells. Microscopy images (with a magnification of x80) of beetroot samples of 1mm thickness were taken before and after processing the samples. In Fig. 4.9 and Fig. 4.10, pictures of raw beetroot tissue and of processed samples at either 0 or 2000 $V\,m^{-1}$, for 10 minutes, are shown. Cellular structure appeared to collapse, resulting in an increase of the transfer of the components contained in the cells. In raw tissue, as well as in samples treated at 25°C and 2000 $V\,m^{-1}$, and tissue treated at 40, 50 and 60°C and no electrical processing, the cell structure can be seen; whereas the images of samples processed at 40, 50 and 60°C and 2000 $V\,m^{-1}$ showed a more disintegrated tissue.

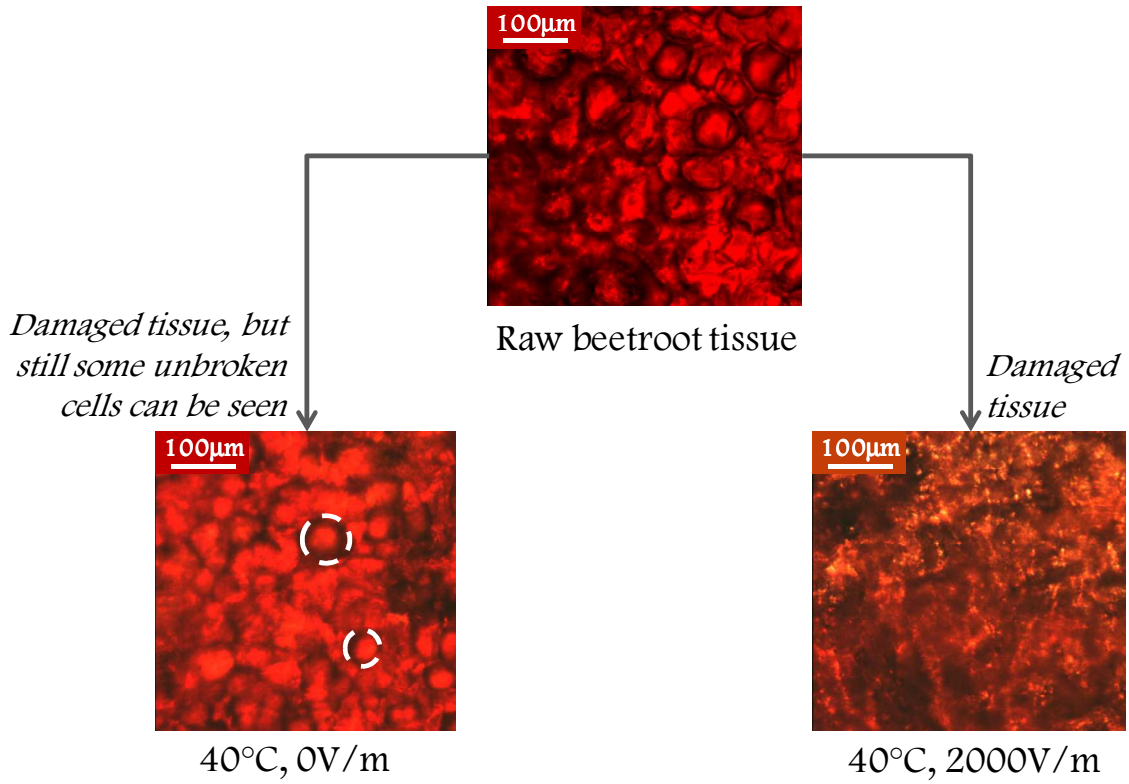

Fig. 4.9. Micrographs of raw beetroot tissue and treated one at 40°C and either 0 $V\,m^{-1}$ (left) and 2000 $V\,m^{-1}$ (right).

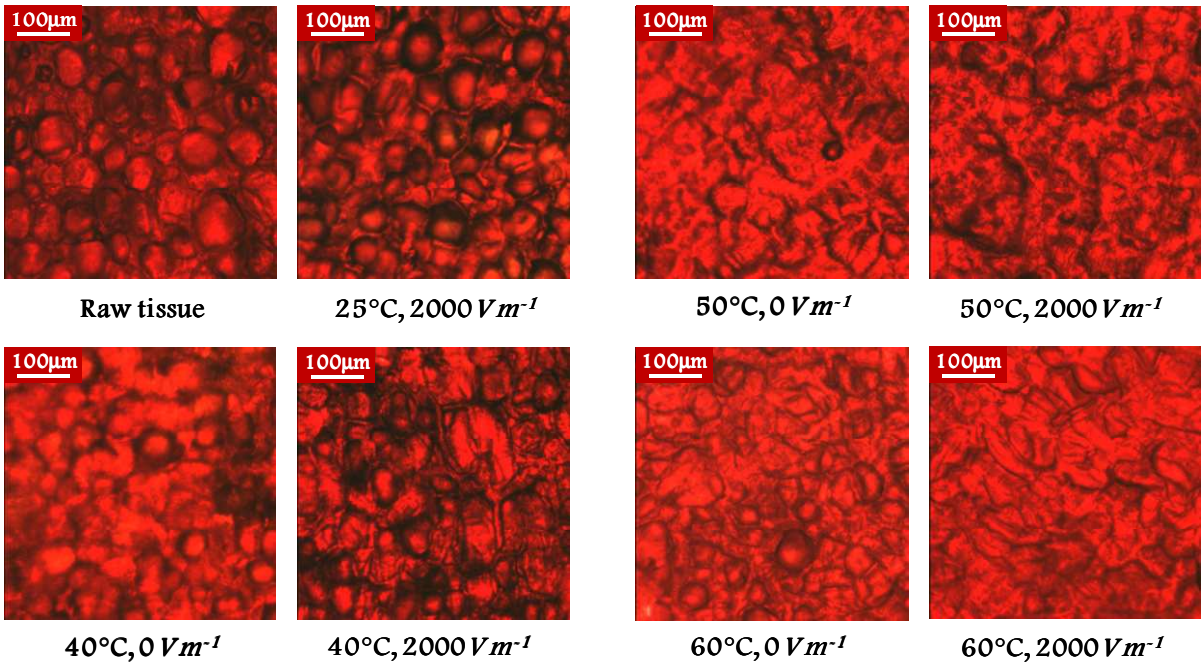

Fig. 4.10. Micrographs of beetroot tissue at different processing conditions.

The enhancing effect of MEF on mass transfer seen in this study has been reported in the literature. Kulshrestha and Sastry (2003), at a temperature of 45°C and electrical fields of $1000 V m^{-1}$ during 3 minutes, reported a betanin concentration of 0.6ppm approximately. Our results at a constant process temperature of 50°C and $1000 V m^{-1}$ during 3min, showed a concentration of betanin of $0.31 \times 10^{-6} g\ ml^{-1}$ = 0.31ppm. A process at a temperature of 60°C allows extraction of higher amounts of betanin in a shorter time, although the increased temperature leads to colour degradation of betanin. The degradation effect of high temperatures on betanin is shown graphically in Fig. 4.2 and Fig. 4.8, where the profile for the amount of betanin extracted decreases after one hour processing the beetroot at 60°C.

4.2.3. Effect of the position of beetroot slab in respect to the electrical field on betanin effusion

Remarkable effects have been seen when changing the position of particles treated ohmically. Davies *et al.* (1999) found that when ohmically heating a single rectangular particle of egg albumen submerged in salt water, the system is treated as an electrical circuit in parallel when the particle position is parallel to the electrical field. The same system is treated as an electrical circuit in series when the particle position is perpendicular to the electrical field. In their work the heating rate of the particle was affected by the position of the particle; when positioned in parallel circuit the egg albumen (with a higher electrical conductivity than salt water) heated faster than the salt water; while for the series circuit the egg albumen heated slower than salt water, at 100 seconds in "parallel-circuit" 130°C egg albumen and 50°C salt water, in "series-circuit" 30°C egg albumen and 60°C salt water. Lebovka *et al.* (2007) showed that the heating rate and electrically induced damage during MEF treatment was intensified when the sample had a perpendicular orientation in respect to the electrical field.

This part of the research work focussed on the enhancement of betanin extraction at 40, 50 and 60°C processing temperature and 1000 $V\ m^{-1}$ electrical field when modifying the beetroot slab´s position. Results of the experiments performed are shown in Fig. 4.11. An electrical field of 1000 $V\ m^{-1}$ was applied during 5 minutes at the beginning of the experiment. An enhancement in betanin extraction was observed when the slab was placed perpendicular to the electrical field in comparison to the extraction when it was placed in parallel.

At the end of the 55 minute experiment, the highest enhancement (approximately 50%) after varying the position was observed at 40°C. At 50°C a 17% increase was seen; whilst at 60°C the smallest effect of slab's position on betanin extraction was observed, with only approximately a 4% increase in extraction when the slab was placed perpendicular to the electrical field (difference of $8x10^{-6} kg_{Bet} kg_{Beet}^{-1}$).

The surface area of the slab that receives the effect of the electrical field is different in each case: $6.00x10^{-4}m^2$ when the slab is perpendicular to the field and $9.00x10^{-5}m^2$ when it is parallel to it. A bigger surface area allows more electricity to pass through the sample.

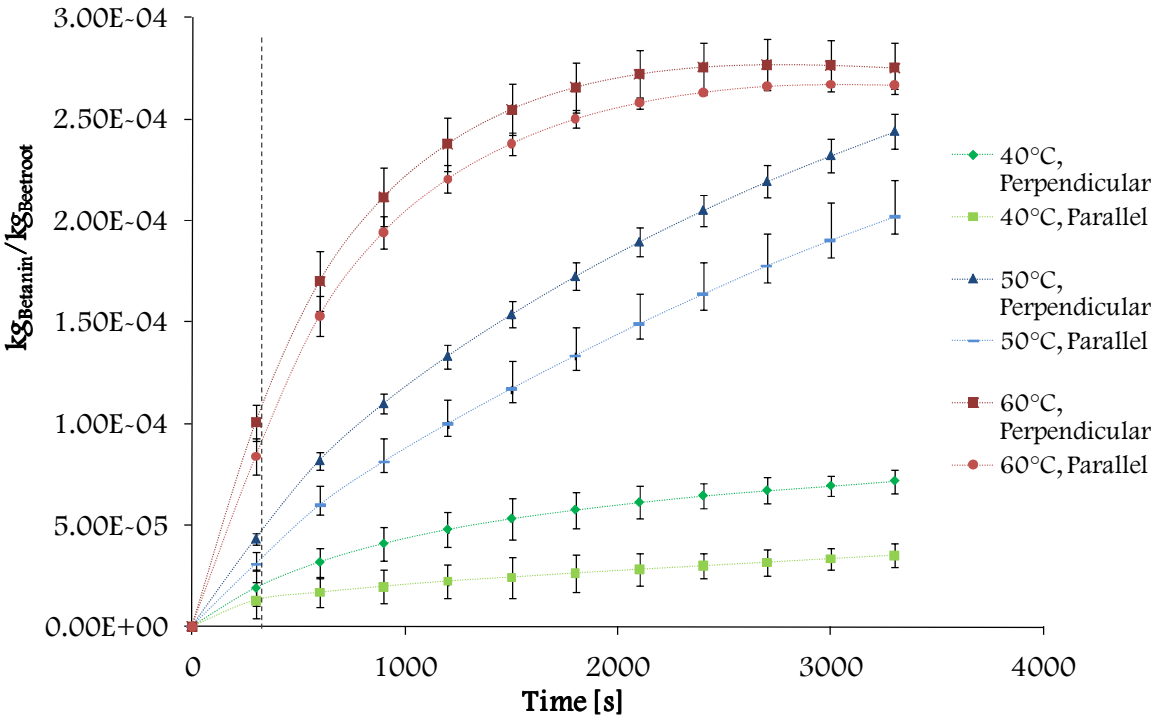

Fig. 4.11. Betanin extraction profiles for experiments to visualise the effect of slab position; at electrical field of 1000 $V\,m^{-1}$ and 40, 50 and 60°C. Vertical error bars represent standard deviation of triplicates performed. Period to the left of dashed line shows duration of electrical processing.

4.2.4. Estimation of D_{eff} for betanin from beetroot to the solution

As a 1st approximation to the D_{eff} of betanin in the solution; the following values were referred from previous works. Vukov (1977) reported that the D_{eff} for sucrose in sugar beet plasmolysed by mechanical shear stress and strong pressure was found to be in the range of $0.7x10^{-10}$ to $1.23x10^{-10} m^2s^{-1}$. In Chalermchat *et al.* (2004) the estimated D_{eff} values after treating beetroot with PEF of different durations (270, 54 and 27 total pulses) went from $1.6x10^{-10} m^2s^{-1}$ for 270 pulses to $4.89x10^{-10} m^2s^{-1}$ for 27 pulses (values of fast diffusion coefficient). Lebovka *et al.* (2006) reported estimated D_{eff} values for sugar diffusion from sugar beet at 60°C from $1.2x10^{-9} m^2s^{-1}$ when the beet was PEF treated to $5x10^{-10} m^2s^{-1}$ for the untreated tissue. At 50°C the values went from $1x10^{-9} m^2s^{-1}$ for samples treated with PEF to $2x10^{-10} m^2s^{-1}$ for the untreated one. Doulia (2000) mentioned the moisture diffusivity of diced sugar beet at temperatures of 40 to 84°C to be in a range from $0.4x10^{-10}$ m^2s^{-1} to $1.3x10^{-10} m^2s^{-1}$.

The estimated D_{eff} values (obtained as explained in Appendices 1 and 2) are summarised in Table 4.1.

The D_{eff}s presented in Table 4.1 are in the same order of magnitude ($10^{-10} m^2 s^{-1}$) as those reported in previous similar works (Lebovka *et al.,* 2006; Chalermchat *et al.*, 2004; Vukov, 1977). The variability in the diffusivities reported in the literature can be explained by different methods of plasmolysis leading to different values of D_{eff} (Vukov, 1977).

An increase in the values of D_{eff} as a result of electrical fields can be seen in Table 4.1. However, this increase is rather small when compared to the effect of temperature.

Table 4.1. Estimated diffusion coefficients (D_{eff}) for Betanin. Average ± Standard deviation

Temperature [°C]	Electrical Field [$V\,m^{-1}$]	D_{eff} [$m^2 s^{-1}$]
40	0	$2.14 \pm 0.50 \times 10^{-11}$
	600	$3.55 \pm 0.62 \times 10^{-11}$
	1000	$4.98 \pm 0.33 \times 10^{-11}$
50	0	$1.28 \pm 0.53 \times 10^{-10}$
	600	$1.70 \pm 0.70 \times 10^{-10}$
	1000	$2.80 \pm 0.28 \times 10^{-10}$
60	0	$9.31 \pm 1.12 \times 10^{-10}$
	600	$9.71 \pm 1.41 \times 10^{-10}$
	1000	$10.10 \pm 0.45 \times 10^{-10}$

Fig. 4.12 and Fig. 4.13 illustrate the curves for experimental data and the for the model used to estimate D_{eff}, for the experiments at 50 and 60°C, and electrical fields of 600 and 1000 $V\,m^{-1}$ strength. R squared values show a good fitting of the chosen model.

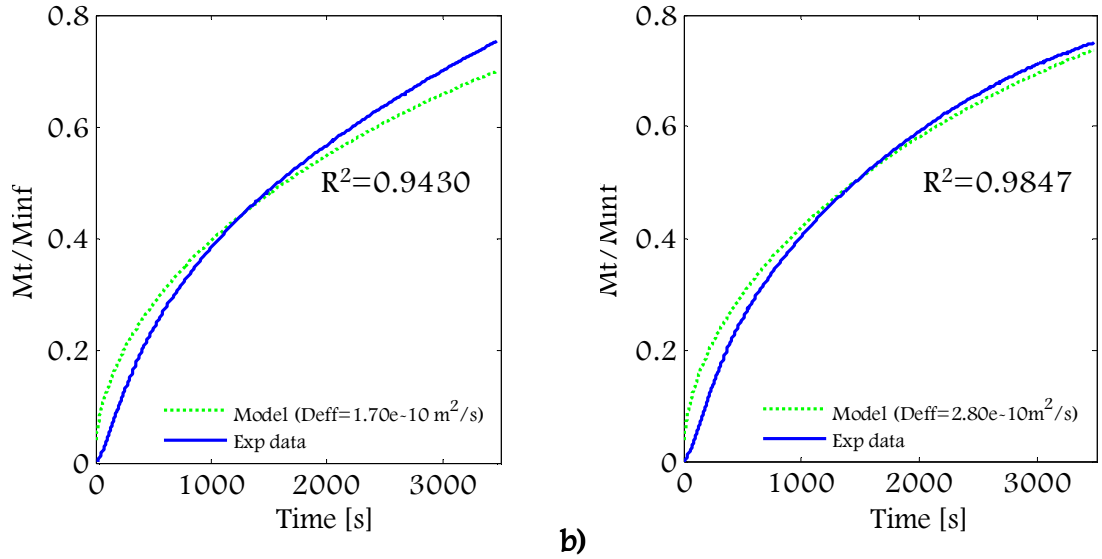

Fig. 4.12. Graphs showing curves for model and experimental data in experiments at 50°C, for a) 600 $V\,m^{-1}$ and b) 1000 $V\,m^{-1}$.

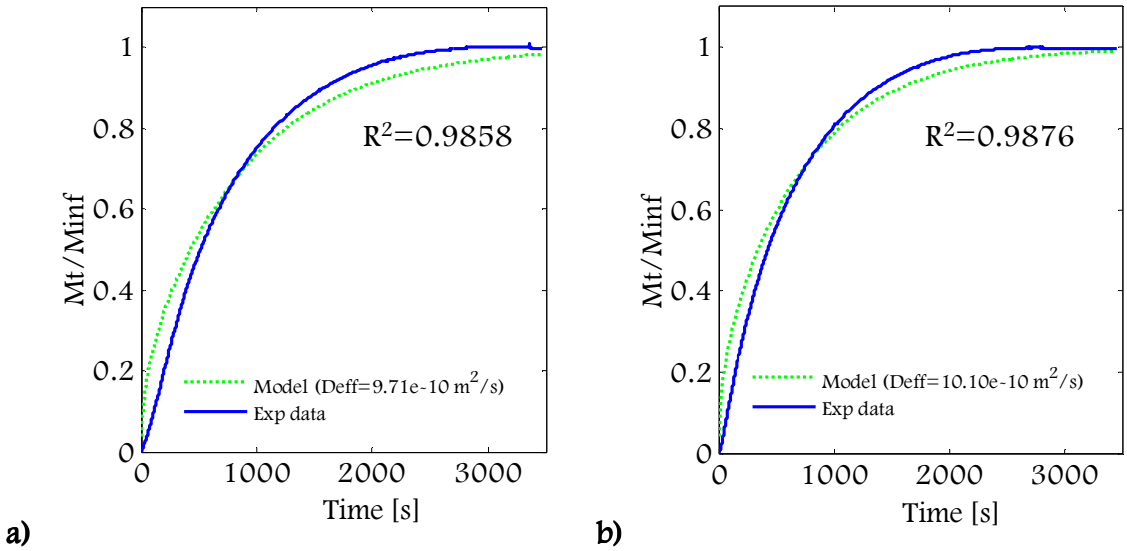

Fig. 4.13. Graphs showing curves for model and experimental data in experiments at 60°C, for a) 600 $V\,m^{-1}$ and b) 1000 $V\,m^{-1}$.

The Arrhenius plot of the estimated D_{eff}s presented in Fig. 4.14 shows a linear relation between temperature and the D_{eff} at the three different electrical fields applied, with values of R^2 (after linear fitting) of 0.9977 at 0 $V\,m^{-1}$, 0.999 at 600 $V\,m^{-1}$ and 0.9955 at 1000 $V\,m^{-1}$ demonstrating linear correlation.

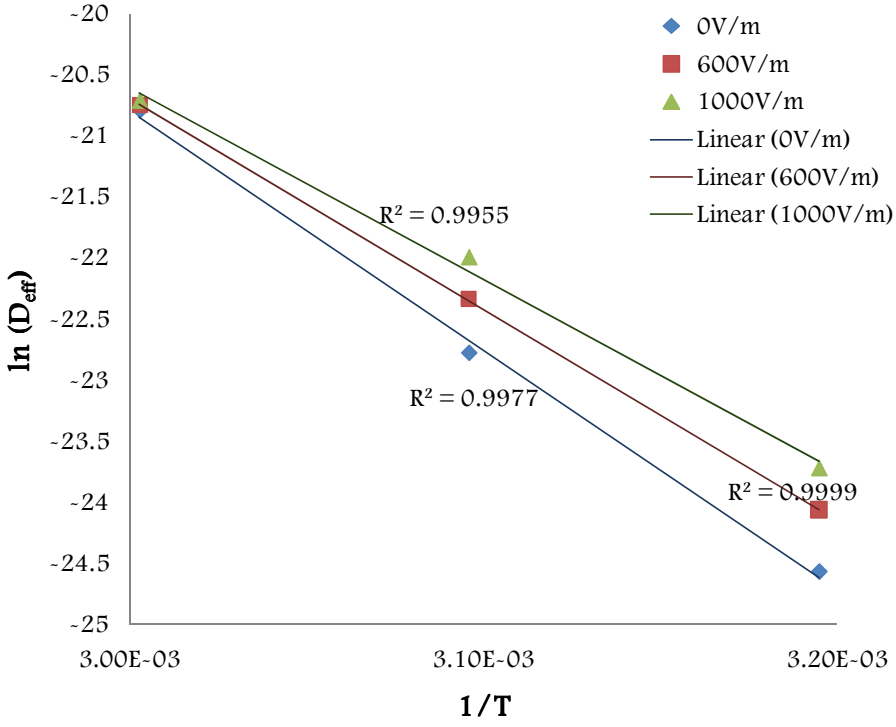

Fig. 4.14. Arrhenius plot for electrical field vs. estimated D_{eff}.

Activation energy for betanin extraction was estimated from the Arrhenius plot in Fig. 4.14. For the reaction at $0\,V\,m^{-1}$ the energy that must be overcome for the extraction of betanin to occur was $130.6 kJ\ mol^{-1}$, $143.3\ kJ\ mol^{-1}$ when the extraction happens at $600\,V\,m^{-1}$ electrical field and $163.4 kJ\ mol^{-1}$ when electrically processing at $1000\,V\,m^{-1}$. These values can be compared with data previously presented by Drdák and Vallová (1990) for activation energy for the extraction of betanin of $144.8 kJ\ mol^{-1}$.

4.3. CONCLUSIONS

In this experimental chapter, the results from the experiments to study the effect of the application of moderate electrical fields (MEF) on the extraction of betanin from beetroot were presented. Extraction experiments were performed at different treatment conditions by varying processing temperature, applied electrical field and position of the beetroot slab. Concentration of betanin was monitored online and measured by spectrophotometry. Results showed that the application of moderate electrical fields and thermal treatment

cause disruption in the beetroot cells' wall, producing an enhancing effect on the extraction of betanin. The enhancement in extraction was proportional to the increase in temperature and electrical field. The effect of temperature appears to be more pronounced than the effect of MEF in the range used for this study. At 60°C the concentration of extracted betanin at 55 minutes of experiment increased approximate 25% from experiments at 0V m^{-1} to those at 1000 $V\,m^{-1}$ were applied. The orientation of the beetroot slab in respect to the electrical current also appeared to have an effect on the amount of betanin extracted; being the highest enhancement of approximately 50% when the slab was placed perpendicular to the electrical field and processed at 1000 $V\,m^{-1}$ and 40°C, compared to the treatment at 0 $V\,m^{-1}$ and 40°C. The estimated diffusivities for the extraction of betanin from beetroot, which are in agreement with values reported previously in the literature, showed to be increased when extraction occurred under electrical fields of 1000V m^{-1}. Values of R^2, after fitting of experimental data and model, validate the assumption of Fickian diffusion for the extraction process.

CHAPTER 5

MASS TRANSFER IN GEL SYSTEMS UNDER THE APPLICATION OF MODERATE ELECTRICAL FIELDS (MEF)

5.1. INTRODUCTION

This experimental chapter covers the results obtained from the experiments where the effect of the application of alternating MEF on mass transfer of rhodamine6G into gel networks was studied. Rhodamine6G was diffused into networks set with ions (alginate, potato-alginate mix) or set thermally (albumen and gelatine), and the strength of the applied electrical field and the electrical conductivities (σ) of the gel sample and the colorant solution were varied. The concentration of rhodamine6G at the end of the process, measured by image analysis, showed that MEF only affects the gel matrixes set ionically and that the effect varies according to the values of σ in the process. The effective diffusion coefficients (D_{eff}s) for the infusion of rhodamine6G were estimated with a solution of Fick´s 2nd law of diffusion.

5.2. RESULTS AND DISCUSSION

5.2.1. Results for gels set with Calcium ions

Before performing the mass transfer experiments, preliminary simulation using Comsol software (Appendix 3) allowed estimation of the effects if direct current was applied to the system gel-solution and the electrical conductivities of the gels were modified.

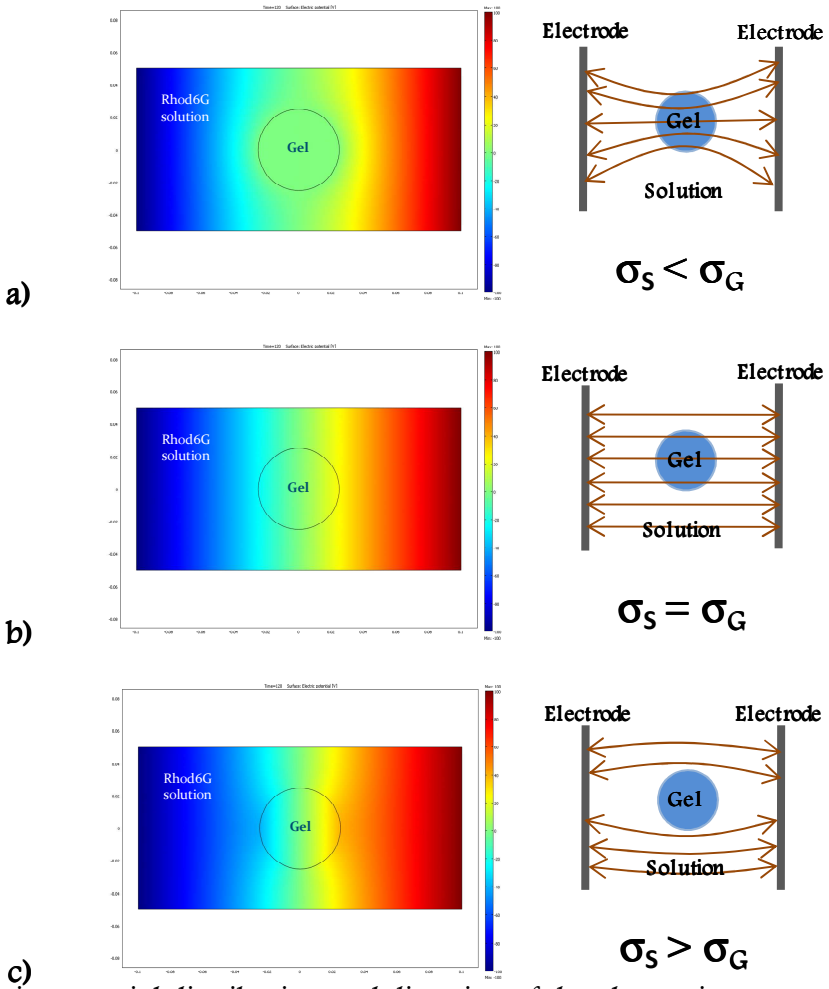

Fig. 5.1. Electric potential distribution and direction of the alternating current flow for the 3 cases of study: a) $\sigma_{Gel}>\sigma_{Solution}$, b) $\sigma_{Gel}=\sigma_{Solution}$ and c) $\sigma_{Gel}<\sigma_{Solution}$.

When DC is applied to a system where the electrical conductivity of the gel is higher than the one of the solution surrounding it ($\sigma_{Gel}>\sigma_{Solution}$), the electric potential is higher in the gel (Fig. 5.1a). In the case of AC, the current flowing from one electrode to the other and back, is attracted first by the gel, affecting its permeability in a way that the cross linking is enhanced, making the colorant molecules less likely to infuse. The Calcium ions and bonds present in the network get affected and as a consequence, the permeability of the gel.

If the electrical conductivities of gel and solution are matched ($\sigma_{Gel}=\sigma_{Solution}$) the distribution of the electric potential in the centre of the system (where the gel is located) is equally

distributed in the gel and the solution surrounding it (Fig. 5.1b). It is expected that when AC is applied it will affect ions present and structure in both gel and solution equally.

When the electrical conductivity of the gel is lower than the one of the solution ($\sigma_{Gel}<\sigma_{Solution}$), it is expected that the alternating current would affect mainly the rhodamine6G solution and not the permeability of the gel. Infusion of the colorant is expected to be the same (no significant differences) with or without the application of the electrical field (Fig. 5.1c).

5.2.1.1. Effect of electrical processing on mass transfer for alginate

A typical image of rhodamine6G transferred in gel samples is shown in Fig. 5.2. From those images, concentration values were obtained measuring the grey value with Image analysis. The graphs of concentration vs. radius for the infusion of rhodamine6G to calcium alginate cylinders are shown in Fig. 5.2 for the three electrical conductivity combinations and electrical field strength of the experiments performed. Error bars correspond to standard deviation of the triplicates done. Although alginate and potato-alginate solutions were degasified by leaving them resting for 30 minutes before soaking in $CaC1_2$, after the formation of the 3D network some air compartments could be seen when the gels were sliced for scanning. The red speckles visualised in the images of scanned slices are those air voids that got stained when in contact with the cutting blade.

The concentration of rhodamine6G at the end of the mass transfer process for both 0 and $1000 V\, m^{-1}$ experiments for alginate when $\sigma_{Gel}>\sigma_{Solution}$, started at $6.69\text{x}10^{-1} kg\, m^{-3}$ at the boundary of the gel-solution (maximum radius) and reached a steady point of $3.91\text{x}10^{-3} kg\, m^{-3}$ towards the centre of the sample. Fig. 5.2 also presents scanned images of two slices of alginate gel at the end of the mass transfer process, for the different experiments'

conditions. The probability obtained with a one way ANOVA analysis of the concentrations measured at radius 5mm was 3.5527×10^{-15}, which meant a significant difference between the values for the different treatments.

As can be seen in Fig. 5.2, the amount of rhodamine6G infused into alginate gels after 15 minutes' processing was lower in case 1 ($\sigma_{Gel} > \sigma_{Solution}$) than in cases 2 ($\sigma_{Gel} = \sigma_{Solution}$) and 3 ($\sigma_{Gel} < \sigma_{Solution}$). Concentration results were standardised by calculating C/C_0, to compare the effect of the strength of the electrical field applied. C_0 is the concentration of rhodamine6G at a radius of 6mm (boundary gel-rhodamine6G solution) which varies according to the characteristics of the experiment performed; going from $6.69 \times 10^{-1} kg\ m^{-3}$ for case 1, to $1.24 kg\ m^{-3}$ for cases 2 and 3.

The concentration of the colorant at the end of the process in case 2 was $1.2 kg\ m^{-3}$ at the maximum radius of 6mm and reached a point of $6 \times 10^{-3} kg\ m^{-3}$ towards the centre of the sample. For case 3 (Fig. 5.2c) the concentration of rhodamine6G in the cylinder goes from an initial value of $1.2 kg\ m^{-3}$ at the edge of the gel cylinder to a final value of $5 \times 10^{-3} kg\ m^{-3}$ observed at the centre of the scanned slice. These values are similar to those results from the experiments when $\sigma_{Gel} = \sigma_{Sol}$ (case 2) but still higher than the ones corresponding to $\sigma_{Gel} > \sigma_{Sol}$ (case 1).

The reason for these differences could be that in case 1 the gel matrix is "packed" with ions from the $CaCl_2$ solution in which it was soaked for 48 hours whilst in cases 2 and 3 there is more space in the network for the entrance of the rhodamine6G molecules.

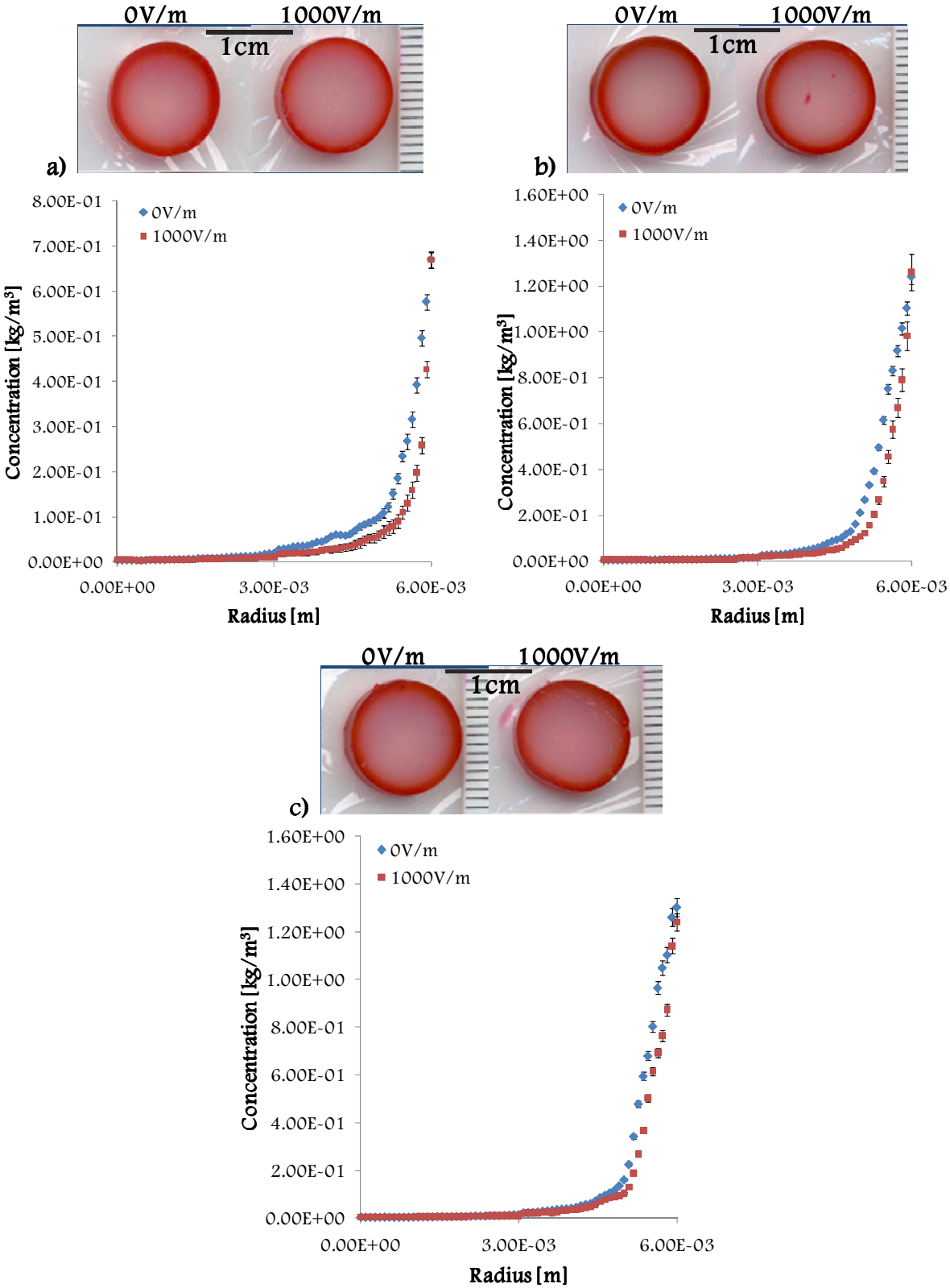

Fig. 5.2. Scanned images of the 3rd slice of the gel cylinder and radius vs. concentration profiles for the infusion of rhodamine6G into alginate after 15min processing time, when a) $\sigma_{Gel}>\sigma_{Solution}$, b) $\sigma_{Gel}=\sigma_{Solution}$ and c) $\sigma_{Gel}<\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

5.2.1.1.1. Case 1, $\sigma_{Gel}>\sigma_{Solution}$

Electrical conductivity of the gel is higher than the one of the solution surrounding it

The infusion of rhodamine6G to alginate networks was lower when an electrical field of $1000\,V\,m^{-1}$ was applied to the gel having a higher electrical conductivity than the solution in which it was processed, compared to the samples at $0\,V\,m^{-1}$. After a processing time of 15 minutes (10 minutes at either 0 or $1000\,V\,m^{-1}$ and 5 minutes at $0\,V\,m^{-1}$), the 3rd slice (cut from the bottom to the top of the gel cylinder) showed a difference of approximately 50% between the concentrations (at 5mm radius) of the samples at $0\,V\,m^{-1}$ and those at $1000\,V\,m^{-1}$ (Fig. 5.3). Error bars represent standard deviation of C/C_0 values for the triplicates done.

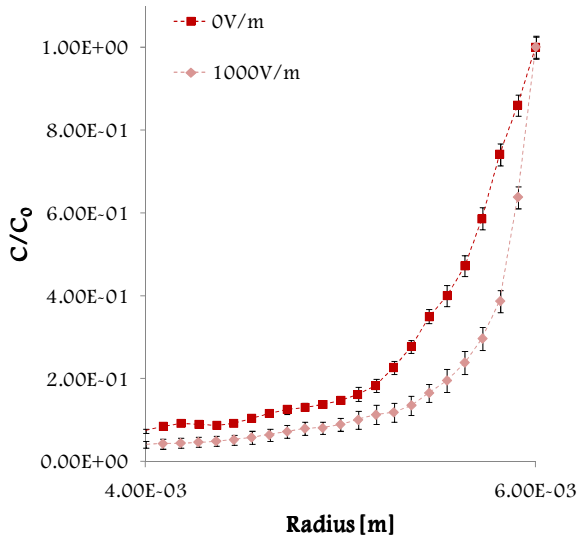

Fig. 5.3. Radius vs. C/C_0 profiles for the infusion of rhodamine6G into alginate cylinders after 15min processing time, when $\sigma_{Gel}>\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

From the shape of the radius vs. concentration profiles, Fickian diffusion was assumed. The estimation of the diffusion coefficients was done by fitting the experimental curve to the proposed model. As can be seen in Fig. 5.4, for the infusion of rhodamine6G to alginate networks when $\sigma_{Gel}>\sigma_{Solution}$, the experimental curve fits the model only at the beginning

and at the end of the curve, but from 5mm to 3.5mm approximately the fitting is not adequate, indicating that diffusion of rhodamine6G when $\sigma_{Gel}>\sigma_{Solution}$ is not Fickian.

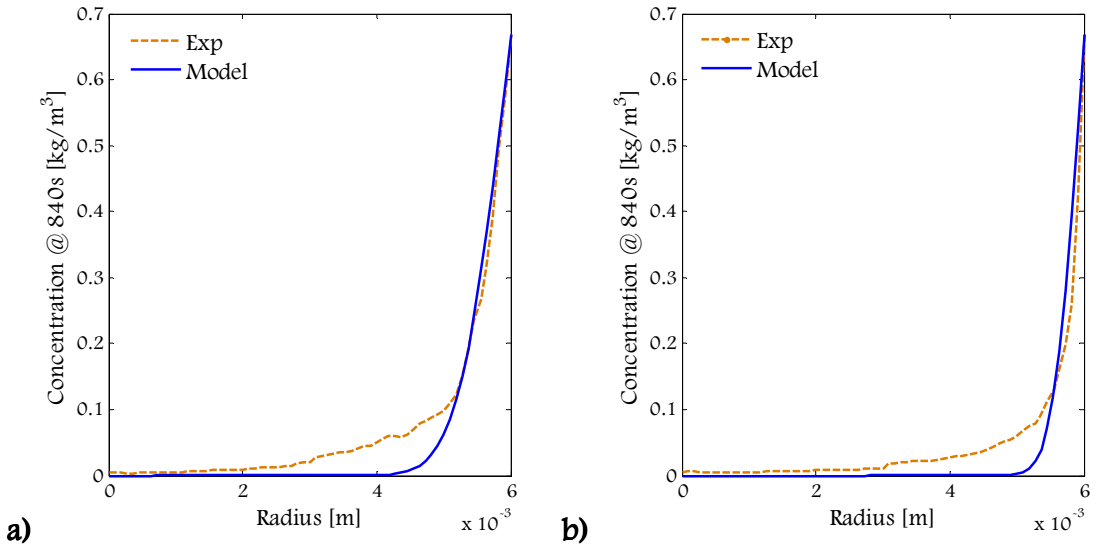

Fig. 5.4. Experimental data and model curve for the infusion of rhodamine6G into alginate when $\sigma_{Gel}>\sigma_{Sol}$ for the experiments at a) $0\,V\,m^{-1}$ and b) $1000\,V\,m^{-1}$.

Regardless the effect of the electrical field on mass transfer for the same process at either 0 or $1000\,V\,m^{-1}$, the difference in the electrical conductivities of gel and rhodamine6G solution influenced the infusion of the colorant molecule.

5.2.1.1.2. Case 2, $\sigma_{Gel}=\sigma_{Solution}$

Electrical conductivity of the gel and solution surrounding it are matched

When the electrical conductivities of the gel and the rhodamine6G solution surrounding it were matched, the results showed a decrease of approximately 40% in the amount of rhodamine6G infused into the alginate cylinder when the electrical field of $1000\,V\,m^{-1}$ was applied in respect to the amount measured at $0\,V\,m^{-1}$ (Fig. 5.5).

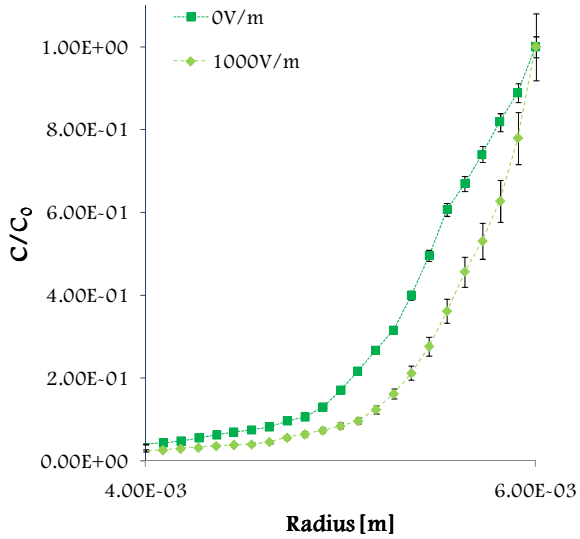

Fig. 5.5. Radius vs. C/C_0 profiles for the infusion of rhodamine6G into alginate cylinders after 15min processing time, when $\sigma_{Gel}=\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

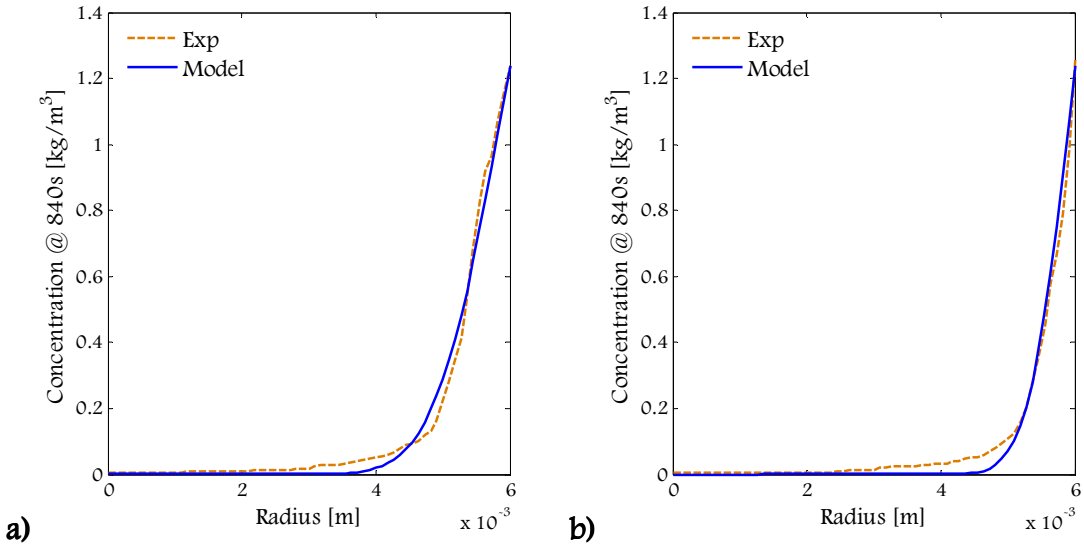

Fig. 5.6. Experimental data and model curve for the infusion of rhodamine6G into alginate when $\sigma_{Gel}=\sigma_{Sol}$ for the experiments at a) $0\,V\,m^{-1}$ and b) $1000\,V\,m^{-1}$.

Fig. 5.6 shows a comparison of the curves radius vs. concentration obtained experimentally and with the estimated D_{eff} for the infusion of rhodamine6G to calcium alginate, when $\sigma_{Gel}=\sigma_{Sol}$. At $0\,V\,m^{-1}$ the D_{eff} was $3.8\text{x}10^{-10} m^2 s^{-1}$ whilst at $1000\,V\,m^{-1}$ it decreased to $1.55\text{x}10^{-10} m^2 s^{-1}$. Both graphs show a good correlation between experimental and model curves.

5.2.1.1.3. Case 3, $\sigma_{Gel}<\sigma_{Solution}$

Electrical conductivity of the gel is lower than the one of the solution surrounding it

The results after performing the experiments showed a smaller decrease in the amount of rhodamine6G infused to the gel than the decrease seen in cases 1 and 2. Taking the concentration values at 5mm of radius as comparison point, the amount of rhodamine6G infused into the alginate was approximately 20% less when the electrical field of $1000\,V\,m^{-1}$ was applied, in respect to the amount measured at $0\,V\,m^{-1}$ (Fig. 5.2 and Fig. 5.7).

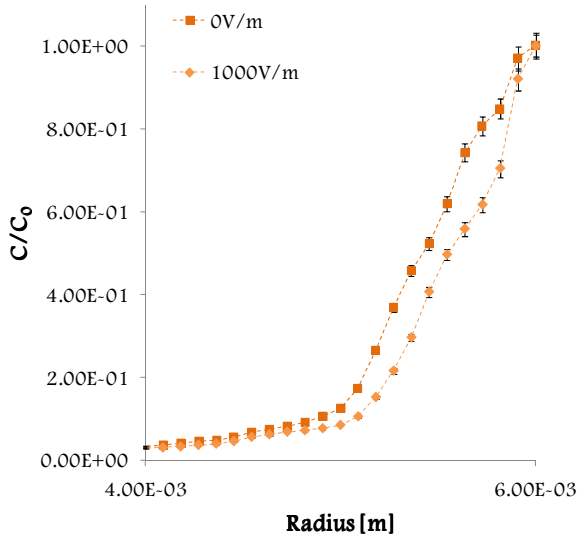

Fig. 5.7. Radius vs. C/C_0 profiles for the infusion of rhodamine6G into alginate cylinders after 15min processing time, when $\sigma_{Gel}<\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

The estimated diffusivities went from $3.50\text{x}10^{-10} m^2 s^{-1}$ to $2.50\text{x}10^{-10} m^2 s^{-1}$, these values of D_{eff} are in the same order of magnitude as the estimated D_{eff} for rhodamine6G into alginate for case 2 when $\sigma_{Gel}=\sigma_{Solution}$ ($\text{x}10^{-10} m^2 s^{-1}$). A comparison of the time vs. concentration curves of experimental data and model used for the infusion of rhodamine6G to alginate gel is presented in Fig. 5.8. The fitting of the curves is acceptable, indicating Fickian diffusion.

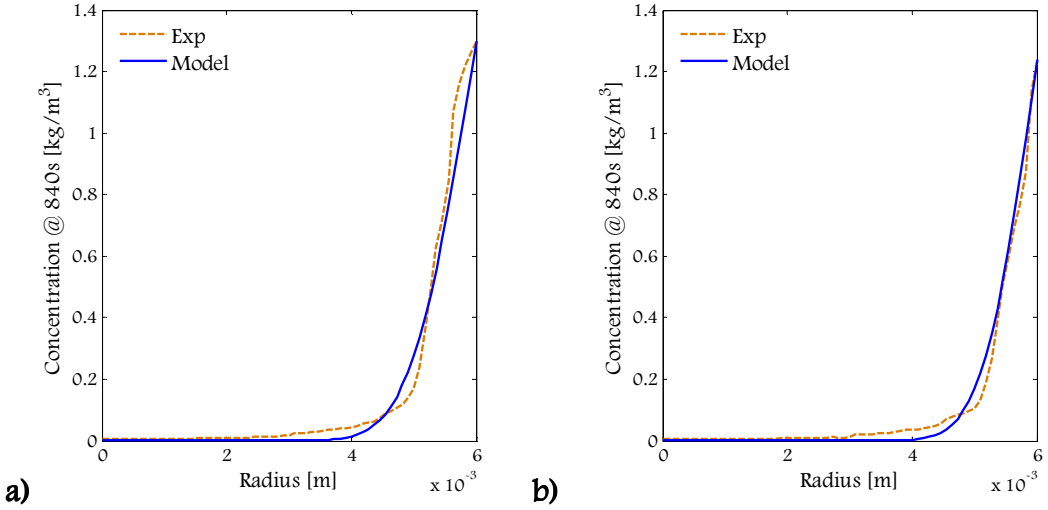

Fig. 5.8. Experimental data and model curve for the infusion of rhodamine6G into alginate when $\sigma_{Gel}<\sigma_{Sol}$ for the experiments at a) 0 $V\,m^{-1}$ and b) 1000 $V\,m^{-1}$.

Table 5.1 summarises the estimated D_{eff}s for mass transfer of rhodamine6G to alginate cylinders for the experiments performed.

Table 5.1. Estimated D_{eff}s for the infusion of rhodamine6G to calcium alginate samples at different electrical conductivity conditions and electrical field strength

E [$V\,m^{-1}$]	Case	D_{eff} [$m^2\,s^{-1}$]	R^2
0	$\sigma_{Sol} < \sigma_{Gel}$	0.5 x10^{-10}	0.8701
	$\sigma_{Sol} = \sigma_{Gel}$	3.8 x10^{-10}	0.9895
	$\sigma_{Sol} > \sigma_{Gel}$	3.5 x10^{-10}	0.9875
1000	$\sigma_{Sol} < \sigma_{Gel}$	0.1 x10^{-10}	0.8672
	$\sigma_{Sol} = \sigma_{Gel}$	1.55 x10^{-10}	0.9803
	$\sigma_{Sol} > \sigma_{Gel}$	2.5 x10^{-10}	0.9837

In general, the infusion in case 1 was slower than in cases 2 and 3, as it can be seen in the values for D_{eff} and in the scanned images of alginate. The estimated D_{eff}s for rhodamine6G into alginate gels are in the same order or magnitude as values reported by Doulia *et al.* (2000) for the diffusion of moisture into alginate gum at a processing temperature of 30°C with a diffusivity value of 3x10^{-10} $m^2\,s^{-1}$; and those found by Venancio and Texeira (1997) for the infusion of sugars like sucrose and fructose through alginate membranes, with D_{eff}s of 6x10^{-10} $m^2\,s^{-1}$ for sucrose and 4.7x10^{-10} $m^2\,s^{-1}$ for fructose.

5.2.1.2. Effect of electrical processing on mass transfer for potato-alginate

The images of rhodamine6G transferred to calcium potato-alginate samples, for the three electrical conductivity combinations and electrical field strength treatments preformed, are shown in Fig. 5.9. Concentration values were obtained measuring grey value with Image analysis. Concentration of rhodamine6G vs. radius graphs for the infusion of rhodamine6G to calcium potato-alginate cylinders are presented in Fig. 5.9. Error bars correspond to standard deviation of the triplicates done.

Concentration of the rhodamine6G at the end of the experiments when $\sigma_{Gel}>\sigma_{Solution}$ went from $3.23x10^{-1} kg\ m^{-3}$ at the boundary of the gel-solution (maximum radius of 6mm) to $5.00x10^{-3} kg\ m^{-3}$ at the centre of the sample for both treatments at 0 and $1000\ V\ m^{-1}$ electrical field strength. After 15 minutes processing the gel (10 minutes at either 0 or $1000\ V\ m^{-1}$ and 5 minutes at $0\ V\ m^{-1}$) the concentration values at a radius of 5mm, showed a difference of approximately 60% between the samples at $0\ V\ m^{-1}$ and those at $1000\ V\ m^{-1}$ (Fig. 5.9a).

When $\sigma_{Gel}=\sigma_{Solution}$ for potato-alginate gels, the concentration at the border of the cylinder was $1.1 kg\ m^{-3}$ for both 0 and $1000\ V\ m^{-1}$ decreasing along the radius to a constant concentration of $6x10^{-3} kg\ m^{-3}$ towards the centre of the sample. At a radius 5mm the concentration of rhodamine6G for the experiments for the experiments at $0\ V\ m^{-1}$ was nearly 50% higher than the values for the experiments at $1000\ V\ m^{-1}$ (Fig. 5.9b). The decreasing effect is lower than the observed when $\sigma_{Gel}>\sigma_{Solution}$.

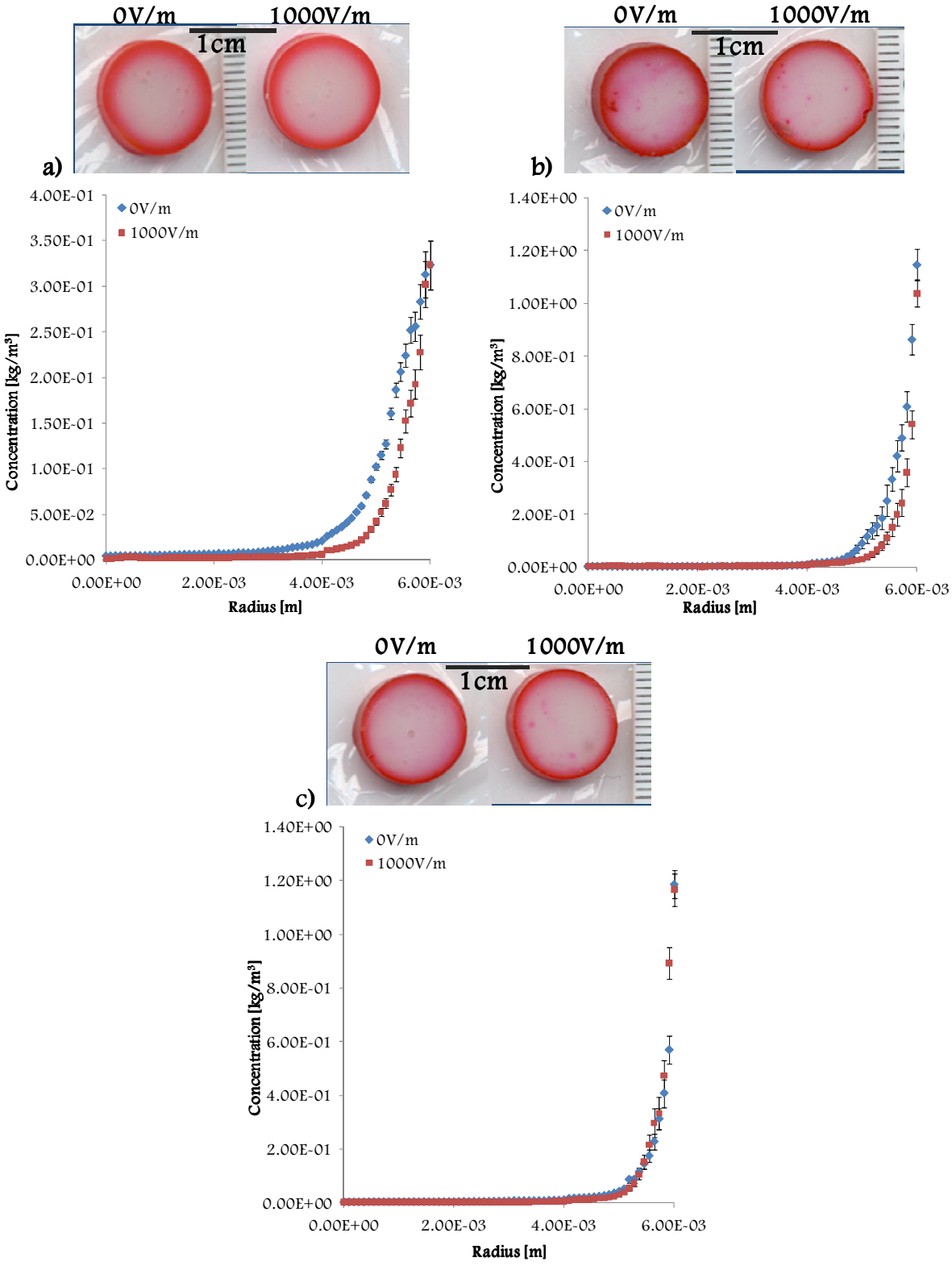

Fig. 5.9. Scanned images of the 3rd slice of the gel cylinder and radius vs. concentration profiles for the infusion of rhodamine6G into alginate after 15min processing time, when a) $\sigma_{Gel}>\sigma_{Solution}$, b) $\sigma_{Gel}=\sigma_{Solution}$ and c) $\sigma_{Gel}<\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

When $\sigma_{Gel}<\sigma_{Solution}$ at the end of the process the concentration of dye went from $1.2 kg\ m^{-3}$ at 6mm radius, to a concentration of rhodamine6G infused at the centre of the sample of $2.35x10^{-3} kg\ m^{-3}$. A decrease of around 10% was found for the amount of rhodamine6G infused when an electrical field of $1000 V\ m^{-1}$ was applied (Fig. 5.9c).

One way ANOVA analysis of the concentrations measured at radius 5mm gave a probability value of $2.4024x10^{-9}$, which represents significant differences between the concentration results for the different treatments.

Concentration results were standardised to compare the effect of the strength of the electrical field applied, by calculating C/C_0. C_0 is the concentration of rhodamine6G at a radius of 6mm (boundary gel-rhodamine6G solution); for potato-alginate gels varied from $3.23x10^{-1} kg\ m^{-3}$ for case 1 to $1.15 kg\ m^{-3}$ and $1.19 kg\ m^{-3}$ for cases 2 and 3. The standardised graphs for the infusion of rhodamine6G to potato-alginate gels are shown in Fig. 5.10, Fig. 5.12 and Fig. 5.14.

5.2.1.2.1. Case 1, $\sigma_{Gel}>\sigma_{Solution}$

Electrical conductivity of the gel is higher than the one of the solution surrounding it

The infusion of rhodamine6G to potato-alginate matrixes was lower when the electrical processing with $1000 V\ m^{-1}$ was applied to the gel having a higher electrical conductivity than the solution in which it was processed (Fig. 5.10).

The estimated D_{eff}s for the infusion of rhodamine6G to potato-alginate gel when $\sigma_{Gel}>\sigma_{Solution}$ were $9x10^{-12} m^2\ s^{-1}$ for the experiments at $0 V\ m^{-1}$ and $4.00x10^{-12} m^2\ s^{-1}$ when an electrical field of $1000 V\ m^{-1}$ was applied. Fig. 5.11 presents the experimental and fitted curves assuming Fickian diffusion.

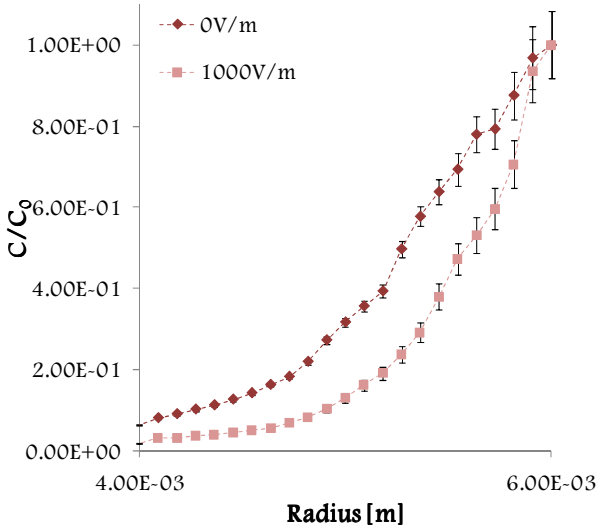

Fig. 5.10. Radius vs. C/C_0 profiles for the infusion of rhodamine6G into potato-alginate after 15min processing time, when $\sigma_{Gel}>\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

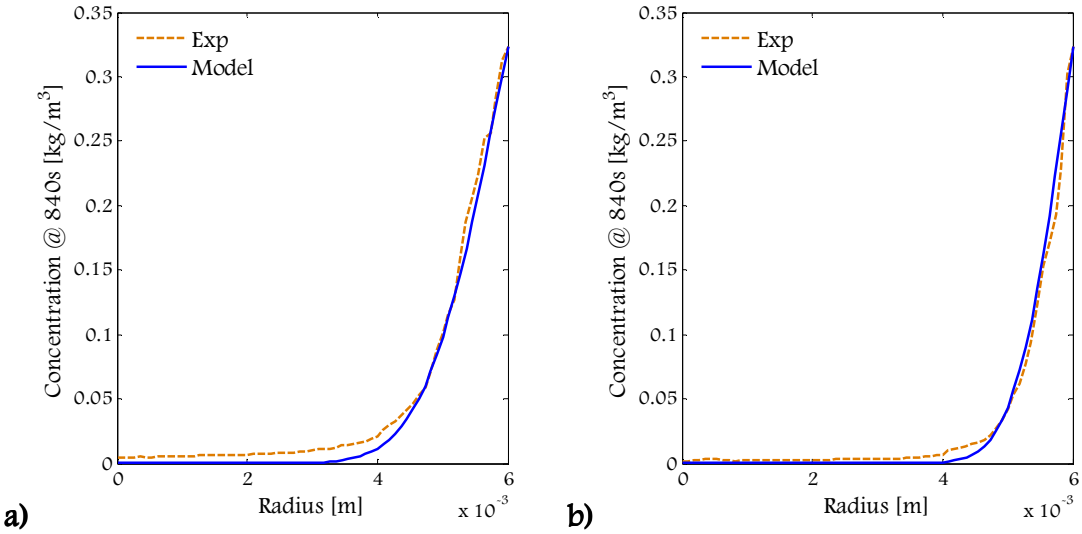

Fig. 5.11. Experimental data and model curve for the infusion of rhodamine6G into potato-alginate when $\sigma_{Gel}>\sigma_{Sol}$ for the experiments at a) $0\,V\,m^{-1}$ and b) $1000\,V\,m^{-1}$.

5.2.1.2.2. Case 2, $\sigma_{Gel}=\sigma_{Solution}$

Electrical conductivity of the gel and solution surrounding it are matched

In the experiments for potato-alginate when $\sigma_{Gel}=\sigma_{Solution}$, the same decreasing effect of electrical processing on mass transfer was found (Fig. 5.12).

In Fig. 5.13, the time vs. concentration of rhodamine6G curves for the experimental data and the model used for the estimation of D_{eff} are presented. Estimated D_{eff} for the infusion of rhodamine6G into alginate gels when $\sigma_{Sol}=\sigma_{Gel}$ at $0\,V\,m^{-1}$ was $7x10^{-11} m^2\,s^{-1}$, decreasing to $1.8x10^{-11} m^2\,s^{-1}$ for the process at $1000\,V\,m^{-1}$.

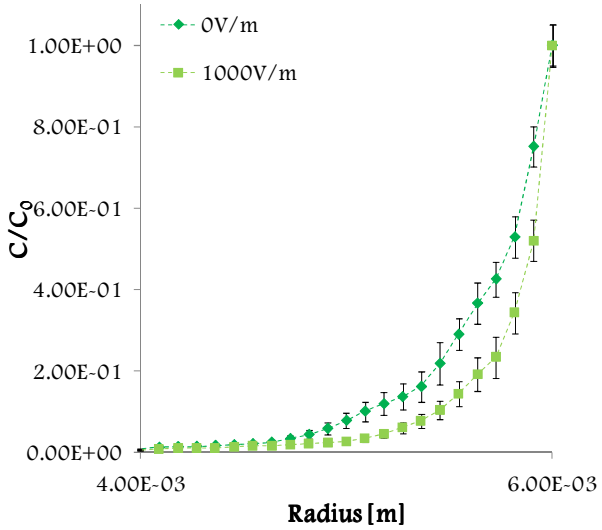

Fig. 5.12. Radius vs. C/C_0 profiles for the infusion of rhodamine6G into potato-alginate after 15min processing time, when $\sigma_{Gel}=\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

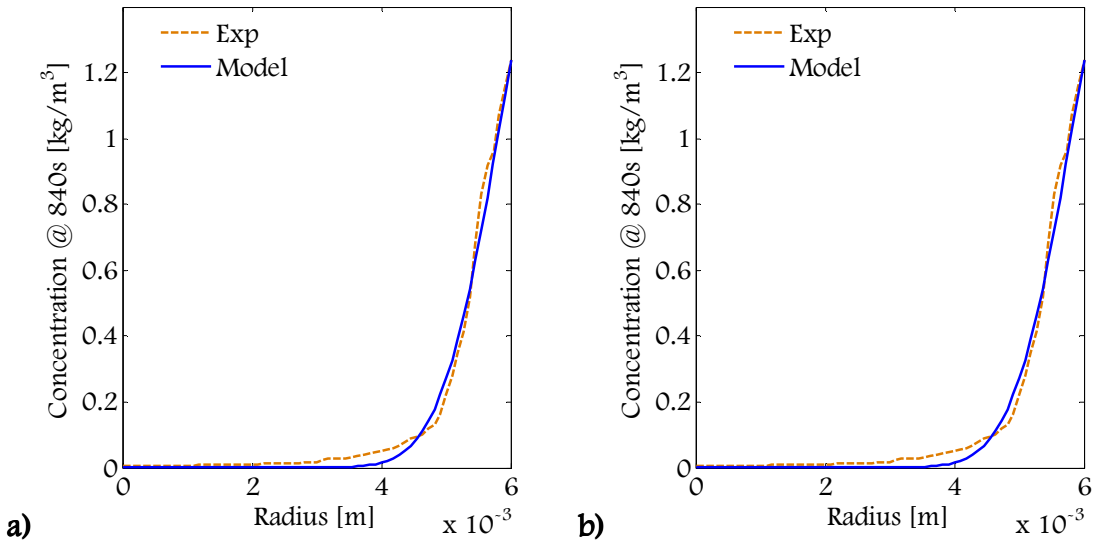

Fig. 5.13. Experimental data and model curve for the infusion of rhodamine6G into potato-alginate when $\sigma_{Gel}=\sigma_{Sol}$ for the experiments at a) $0\,V\,m^{-1}$ and b) $1000\,V\,m^{-1}$.

5.2.1.2.3. Case 3, $\sigma_{Gel}<\sigma_{Solution}$

Electrical conductivity of the gel is lower than the one of the solution surrounding it

The effect of the application of the MEF on the infusion of the rhodamine6G to potato-alginate when $\sigma_{Gel}<\sigma_{Solution}$ was even slower than for alginate.

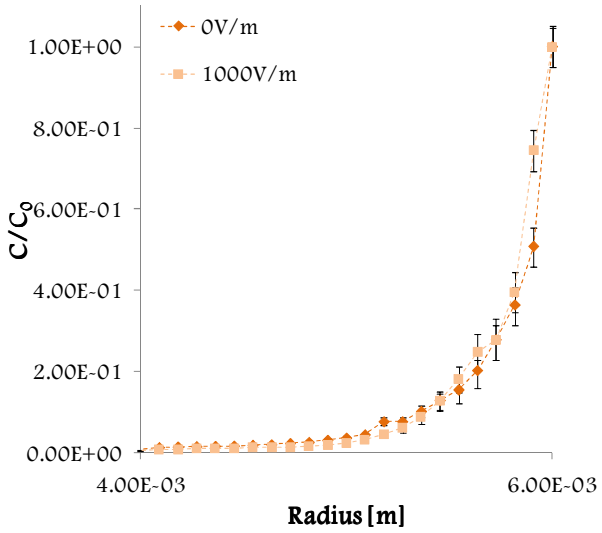

Fig. 5.14. Radius vs. C/C_0 profiles for the infusion of rhodamine6G to potato-alginate after 15min processing time, when $\sigma_{Gel}<\sigma_{Solution}$. Vertical error bars represent standard deviation of triplicates performed.

The estimated mass transfer coefficient for rhodamine6G into potato-alginate when $\sigma_{Gel}<\sigma_{Solution}$ decreased from $4x10^{-11} m^2 s^{-1}$ for the infusion at $0 V m^{-1}$ to $2.5x10^{-11} m^2 s^{-1}$ for the process at $1000 V m^{-1}$; as shown in Fig. 5.15 with the radius vs. concentration curves for the experimental data and the model.

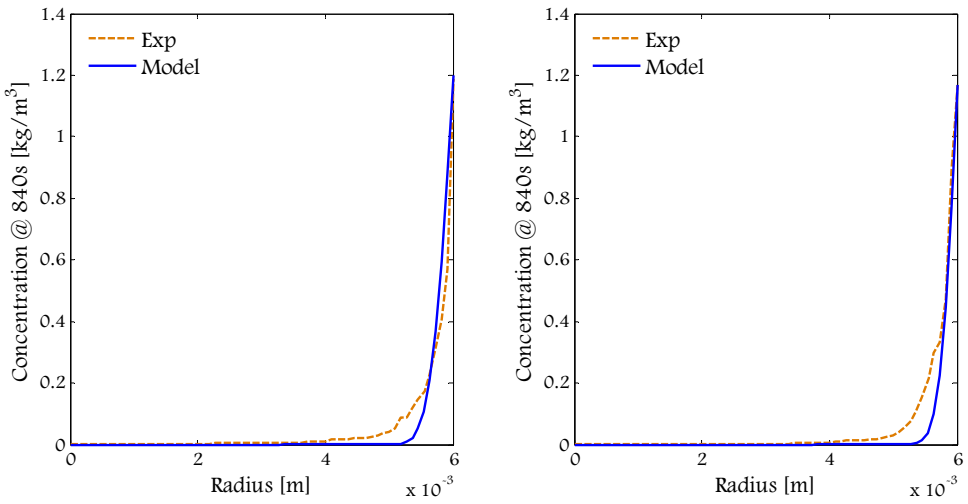

Fig. 5.15. Experimental data and model curve for the infusion of rhodamine6G into potato-alginate when $\sigma_{Gel}<\sigma_{Sol}$ for the experiments at a) $0 V m^{-1}$ and b) $1000 V m^{-1}$.

Table 5.2 presents the estimated D_{eff}s for mass transfer of rhodamine6G to potato-alginate.

Table 5.2. Estimated D_{eff}s for the infusion of rhodamine6G to calcium potato-alginate samples at different electrical conductivity conditions and electrical field strength

E [$V m^{-1}$]	Case	D_{eff} [$m^2 s^{-1}$]	R^2
0	$\sigma_{Sol} < \sigma_{Gel}$	$0.09 x10^{-10}$	0.9916
	$\sigma_{Sol} = \sigma_{Gel}$	$0.7 x10^{-10}$	0.9591
	$\sigma_{Sol} > \sigma_{Gel}$	$0.41 x10^{-10}$	0.9096
1000	$\sigma_{Sol} < \sigma_{Gel}$	$0.04 x10^{-10}$	0.9898
	$\sigma_{Sol} = \sigma_{Gel}$	$0.18 x10^{-10}$	0.9377
	$\sigma_{Sol} > \sigma_{Gel}$	$0.25 x10^{-10}$	0.9409

The results of this part of the study can be compared with the D_{eff} values obtained by Kemp (2000) for the diffusion of rhodamine6G to slabs of calcium potato-alginate gel. These ranged from $0.5x10^{-10} m^2 s^{-1}$ to $1.7x10^{-10} m^2 s^{-1}$ when the samples were processed with

electrical fields ranging from 0 to 940 $V\,m^{-1}$ and a constant processing temperature of 50°C, and from $0.2\text{x}10^{-10} m^2\,s^{-1}$ to $0.7\text{x}10^{-10} m^2\,s^{-1}$ after applying MEF ranging from 0 to 924 $V\,m^{-1}$ at a constant processing temperature of 30°C. The effect of electrical fields on mass transfer of colorants to gel networks found by Kemp (2000) was of enhancement, which differs from the decreasing effect presented in the results of this study. The discrepancy in resulting effect could have been caused by the differences in the experimental set-up.

D_{eff}s were estimated only in the experiments for the infusion of rhodamine6G into alginate and potato-alginate samples.

As it can be seen in the above Figures and Tables for the infusion of rhodamine6G into gel networks set with ions, a general decrease of the amount infused into the samples under the application of an electrical field of 1000 $V\,m^{-1}$ was found. The infusion of rhodamine6G to alginate was faster than in the potato-alginate samples as it can be seen when comparing the D_{eff}s from Tables 5.1 and 5.2.

As mentioned in the introduction of this chapter, Souza *et al.* (2009) found a similar decreasing effect of the application of electrical fields of higher strength (from 100 $V\,cm^{-1}$) than those applied in this study, when water vapour, oxygen and carbon dioxide permeability coefficients in chitosan coating films decreased from $0.3228\pm0.027 g\,m\,Pa^{-1}s^{-1}m^{-2}$ at 0 $V\,cm^{-1}$ to $0.2667\pm0.025 g\,m\,Pa^{-1}s^{-1}m^{-2}$ at 200 $V\,cm^{-1}$ for water vapour, from $6.98\pm0.030 g\,m\,Pa^{-1}s^{-1}m^{-2}$ at 0 $V\,cm^{-1}$ to $6.72\pm0.040 g\,m\,Pa^{-1}s^{-1}m^{-2}$ after the application of a 200 $V\,cm^{-1}$ electrical field for CO_2 permeability; and from 10.60 ± 0.420 to 9.62 ± 0.600 $g\,m\,Pa^{-1}s^{-1}m^{-2}$. Their results showed that the application of a MEF to the film-forming solutions has statistically significant effects on the physical properties of the film.

5.2.2. Results for thermally set gels

The interaction of the ions present in the gel (calcium ions in alginate 3D networks) with the electrical field applied was thought to be a cause for mass transfer modification. In order to try to probe that supposition, the same diffusion experiments were done with two gels set not by ions, but thermally: albumen from chicken egg and gelatine from pork skin.

5.2.2.1. ALBUMEN

The results for the experiments using albumen are presented in Fig. 5.16 with radius vs. concentration and radius vs. C/C_0 curves. Concentration profiles showed no modification in the infusion of rhodamine6G into albumen after MEF of 1000 $V\ m^{-1}$ was applied. The concentration of rhodamine6G at a radius of 6mm after 40 minutes of experiment was $4.7x10^{-1} kg\ m^{-3}$ for both 0 and 1000 $V\ cm^{-1}$.

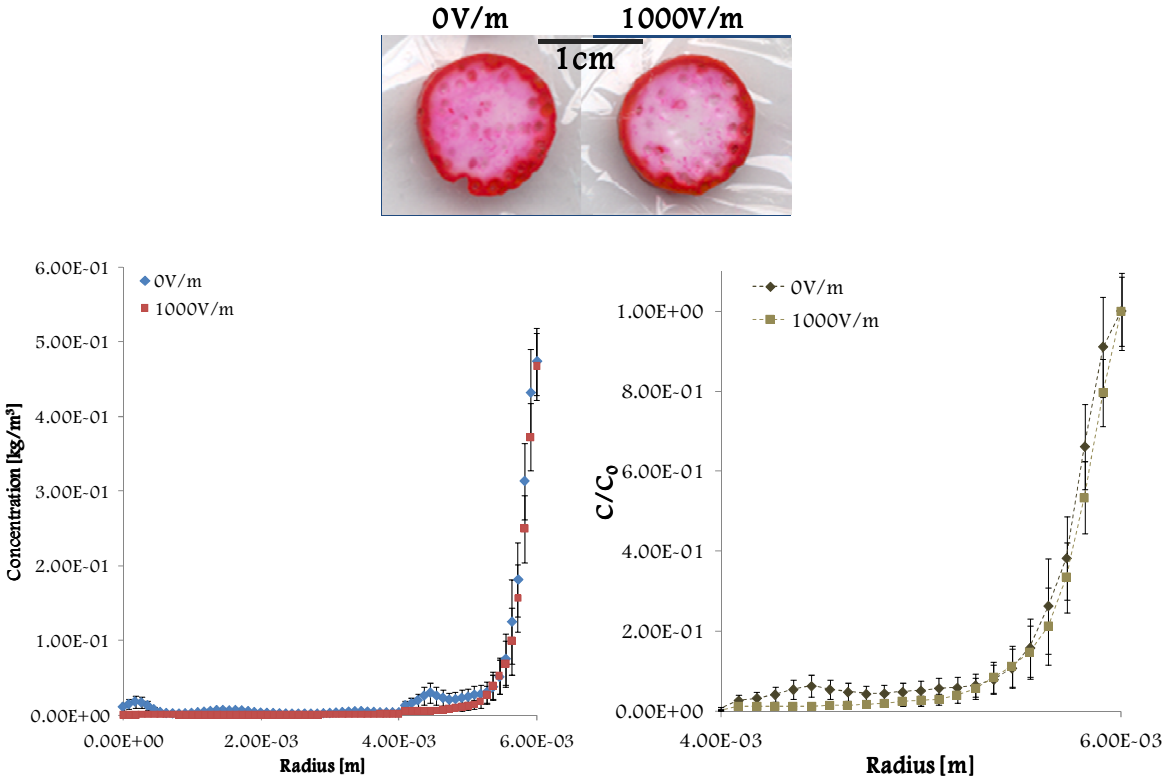

Fig. 5.16. Scanned images of the 3rd slice of the gel sample, radius vs. concentration and radius vs. C/C_0 profiles for the infusion of rhodamine6G to albumen after 40min processing time. Vertical error bars represent standard deviation of duplicates performed.

5.2.2.2. GELATINE

The effect (or lack of effect) of electrical processing on mass transfer of rhodamine6G into gelatine was similar to that for albumen. Radius vs. concentration and radius vs. C/C_0 curves with the results of the experiments using gelatine are presented in Fig. 5.17. It was observed that the application of $1200\,V\,m^{-1}$ electrical field did not have an effect on mass transfer, in spite of the electrical conductivity of gelatine being higher than the one of the solution of rhodamine6G. Scanned images of slices from a gelatine cylinder after 30 minutes soaking in rhodamine6G solution (with or without electrical processing) are also shown in Fig. 5.17.

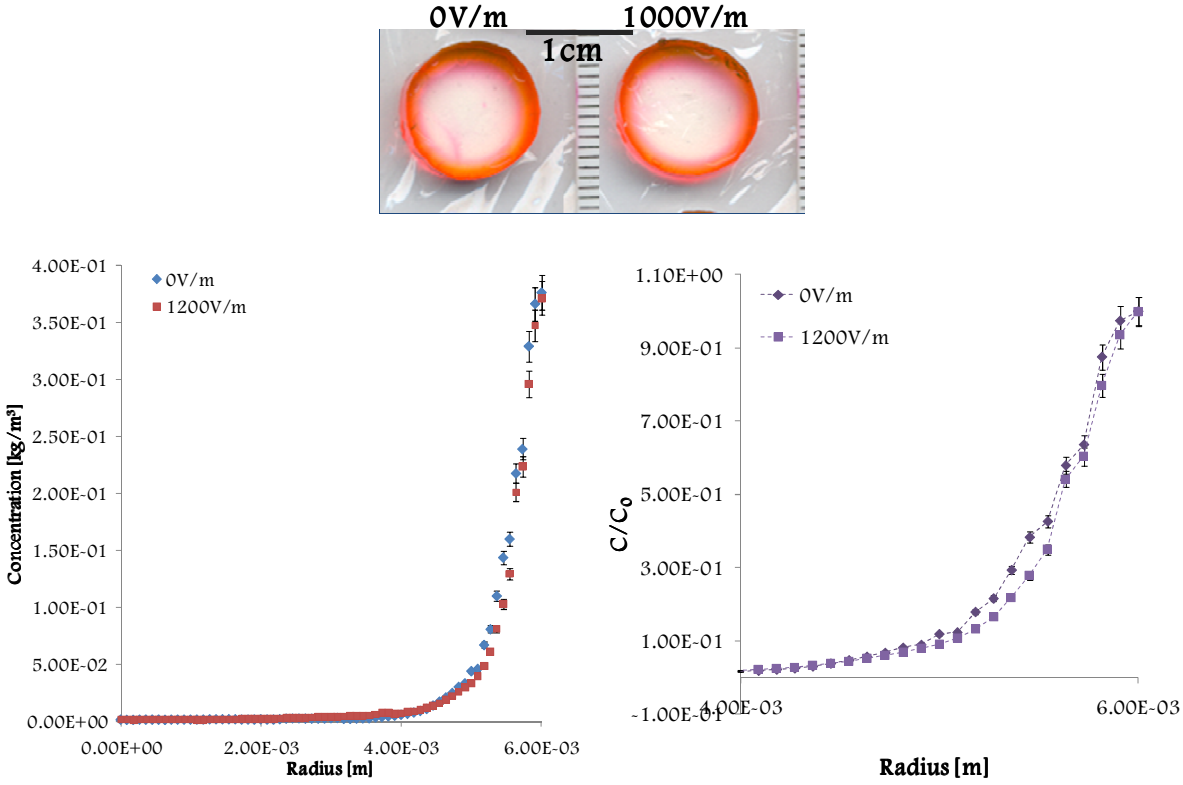

Fig. 5.17. Scanned images of the 3rd slice of the gel sample, radius vs. concentration and radius vs. C/C_0 profiles for the infusion of rhodamine6G into gelatine after 20min processing time. Vertical error bars represent standard deviation of duplicates performed.

5.2.3. Infusion of betanin to alginate

In order to repeat the infusion experiments done for rhodamine6G (which has a +1 ionic charge), but using a colorant with a negative ionic charge, natural colorant betanin with an ionic charge of -2 (at pH between 2.5 and 7.5) was chosen as the diffused colorant. Results in Fig. 5.18 showed that the concentration of betanin infused to alginate increased approximately 20% at MEF treatment of 1000 $V\,m^{-1}$ compared with the infusion at 0 $V\,m^{-1}$. Being a natural colorant with colour intensity lower than the intensity of rhodamine6G, both the calibration curve and the mass transfer profiles for betanin differed from those for rhodamine6G.

Due to time limitations it was not possible to extend this part of the study; however, it has been included in the Future work section in the Conclusions chapter.

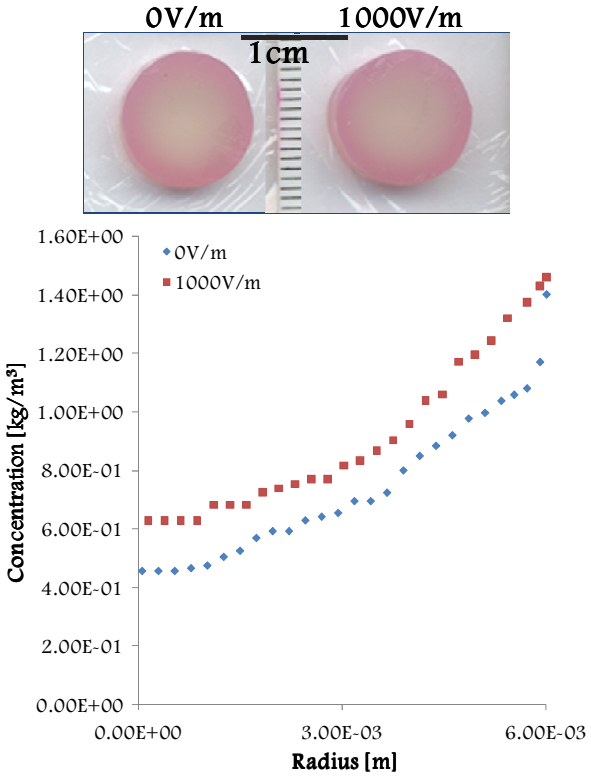

Fig. 5.18. Scanned images of the 3rd slice of the gel sample and radius vs. concentration profiles for the infusion of betanin to alginate after 30min processing time.

5.3. CONCLUSIONS

In chapter 5 mass transfer of rhodamine6G into gel networks under the application of alternating moderate electrical fields was studied. Polymer networks set either ionically (alginate, potato-alginate mix) or thermally (albumen and gelatine) were chosen as materials. Experimental conditions included changes in strength of the electrical field, the gel matrix used and the electrical conductivities (σ) of the gel sample and the colorant solution surrounding it. Results indicated that the application of MEF during colorant infusion process has a decreasing effect on mass transfer of rhodamine6G to gel systems set with ions. Mass transfer decreased as electrical field increased. This effect was influenced by the differences in the electrical conductivities of the gel and the rhodamine6G solution. In systems with electrical conductivities $\sigma_{Gel}>\sigma_{Solution}$, the current flowing through the electrodes is attracted first by the gel, affecting its permeability, enhancing cross-linking and decreasing the infusion of colorant. When the electrical conductivities in the gel-solution system are $\sigma_{Gel}=\sigma_{Solution}$, electric potential is distributed equally, affecting both the polymer sample and the solution containing the dye. In systems where electrical conductivities are $\sigma_{Gel}<\sigma_{Solution}$, infusion of the colorant did not present any significant differences with the application of the electrical field. No significant effect of MEF was observed in gelatine or albumen either.

CHAPTER 6

EFFECT OF MODERATE AND PULSED ELECTRICAL FIELDS (MEF AND PEF) ON COMPRESSION FORCE OF GEL NETWORKS

6.1. INTRODUCTION

In this experimental Chapter, the effect of the application of MEF and PEF on mechanical properties of polymers was studied. MEF were applied during the formation of alginate and gellan gum particles. Slabs and cylindrical samples of calcium alginate gel were treated with PEF after their formation. Compression force of gel samples was measured by texture analysis. Although the traditional approaches to control the mechanical properties of polymer networks include adjusting the composition of the monomers used, increasing or decreasing the cross-linking density and changing the conditions under which the polymer is formed, previous research works have found electrical processing as a potential way of changing the structure and mechanical properties of polymer networks (Moghadam *et al.*, 2008; Zvitov and Nussinovitch, 2003). Results obtained in this part of the study showed that both MEF and PEF had an effect on compression force of the gels, resulting in stronger networks after electrical treatment in comparison to control samples at 0 $V\,cm^{-1}$.

6.2. RESULTS AND DISCUSSION

6.2.1. MEF experiments

6.2.1.1. Alginate particles formation

Fig. 6.1 shows photos of alginate particles formed at 20°C for 0 and 10 $V\,cm^{-1}$. The particles formed at 10 $V\,cm^{-1}$ had a more spherical shape while those at 0 $V\,cm^{-1}$ had a "clearer" colour when the scanned images where analyzed with ImageJ, presenting a mean gray

value of 183.99 at 0 $V\,cm^{-1}$ which is whiter than the 167.13 value for the particles at 10 $V\,cm^{-1}$ (in a scale where 0 represents black and 255 white).

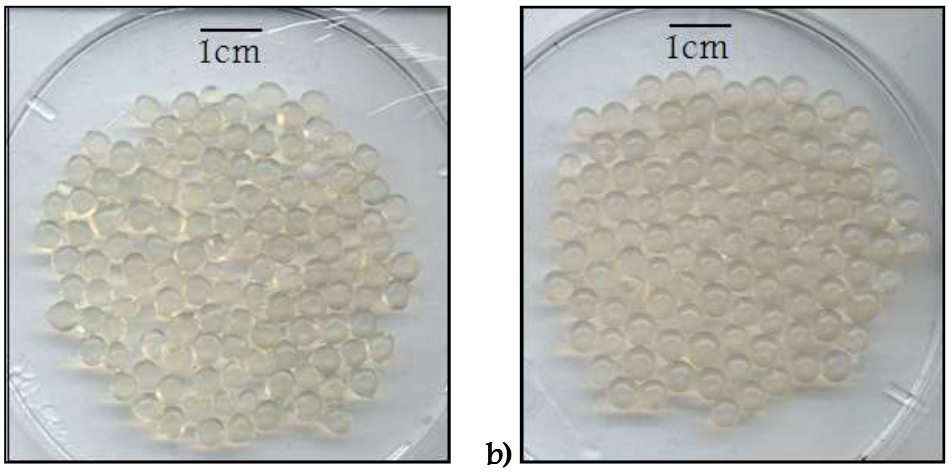

Fig. 6.1. Alginate particles formed by dropping sodium alginate solution 2% w/v in $CaCl_2$ solution 0.5% w/v at a) 0 $V\,cm^{-1}$ (no electrical field) and b) 10 $V\,cm^{-1}$.

The visualisation of the alginate particles under the optical microscope showed that processing temperature and electrical field influenced the shape and structure of the particles. Control samples (formed at 0 $V\,cm^{-1}$) presented a core with non-set alginate whilst those formed at 10 $V\,cm^{-1}$ were easier to cut with a scalpel and the core was as set as the rest of the particle. The colour of the particles formed at 0 $V\,cm^{-1}$ remained even after drying them in the stove at 40°C during three hours. Fig. 6.2 shows micrographs of particles formed at 0 and 10 $V\,cm^{-1}$.

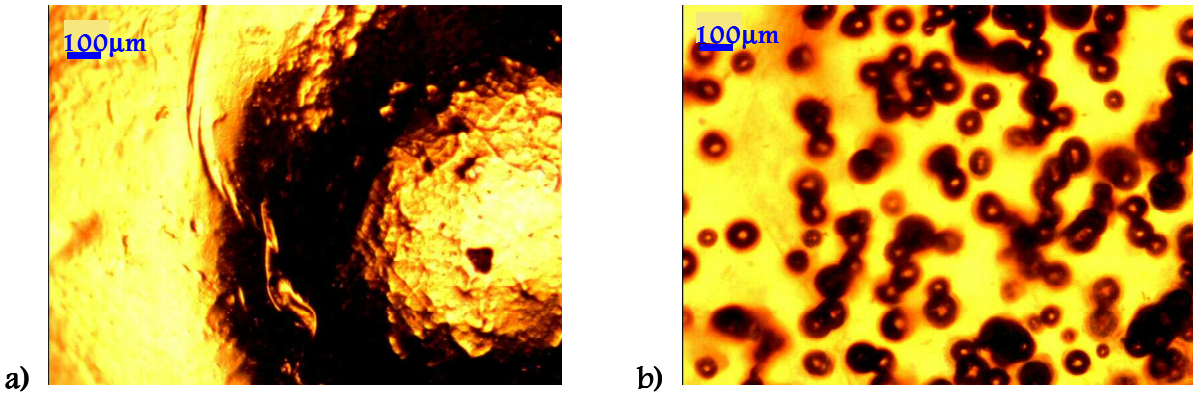

Fig. 6.2. Micrographs of alginate particles formed at a) 0 $V\,cm^{-1}$ and b) 10 $V\,cm^{-1}$.

Ten particles were selected from each batch and their diameter was measured with a digital calliper. The average diameters are shown in Table 6.1. In general, the diameter of the particles was approximately 3% smaller when formed at electrical fields of 10 $V\ cm^{-1}$ and processing temperature of 20°C, whilst at 40°C and 14 $V\ cm^{-1}$ the diameter of the particles was 9% smaller respect to the size of control samples. Kruskal-Wallis statistical test showed significant differences between the sizes of the beads formed at:

1. 20°C, 0 $V\ cm^{-1}$ and those at 40°C, 14 $V\ cm^{-1}$.
2. 40°C, 0 $V\ cm^{-1}$ and those at 40°C, 14 $V\ cm^{-1}$.

Table 6.1. Average size of alginate particles formed at different processing temperatures and electrical field strengths.

	20°C			40°C		
Electrical field	**6.3. 0 $V\ cm^{-1}$ (Control)**	**7 $V\ cm^{-1}$**	**10 $V\ cm^{-1}$**	**0 $V\ cm^{-1}$ (Control)**	**7 $V\ cm^{-1}$**	**14 $V\ cm^{-1}$**
Average diameter [mm]	4.954	4.854	4.814	4.962	4.74	4.52
Standard dev.	0.3501	0.3088	0.2516	0.2470	0.2119	0.1787

Calcium alginate is a hydrophilic, cross-linked and water-swollen polymers hydrogel (Anseth *et al.*, 1996). When in contact with $CaCl_2$ solution, the alginate solution swells and forms a network. The resulting lightly cross-linked polymer (calcium alginate) is highly swollen; its degree of swelling allows it to respond to external forces with a rapid rearrangement of the polymer. Swollen hydrogels are extremely weak materials that can exhibit poor mechanical strength; this fact could explain the relatively low compression force values presented in this study.

In order to analyse the mechanical properties of the calcium alginate particles, a uniaxial compression test was performed at low mechanical stress (as explained in Chapter 3). The

particles showed a complete recovery after removal of the deformation force immediately after the specified percentage of the particle size was compressed. Time dependence of the applied compression force respect to the polymer mechanical properties was not taken into account in this study. An example of a typical time vs. force profile given by the software Exponent (Texture Technologies, Stable Microsystems, UK) when performing the uniaxial compression test (previously described in Chapter 3) is presented in Fig. 6.3. Force value at the highest point in the curve is the force needed to compress 1mm of the sample's height and is the value reported as "maximum compression force" in this Chapter.

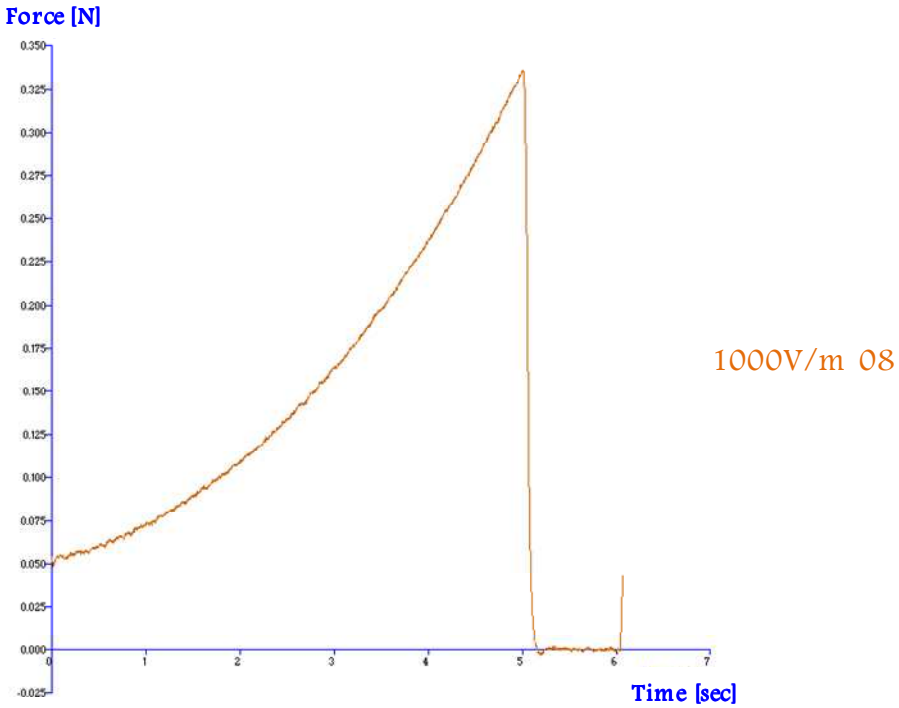

Fig. 6.3. Example of graph given by software Exponent (Texture Technologies, Stable Microsystems, UK) for the compression test performed.

Fig. 6.4 shows maximum force under compression as a function of the electrical field applied, for the alginate particles formed at 20 and 40°C. Particles formed at a constant processing temperature of 20°C presented an increase in compression force, from 20g at 0 $V\ cm^{-1}$ to 25.2g at 7 $V\ cm^{-1}$ (26% approximately) and to 27.78g at 10 $V\ cm^{-1}$ (39% approximately). The weakest alginate particles where those formed at 20°C and 0 $V\ cm^{-1}$.

For the alginate particles formed at 40°C, as well as in the process at 20°C, electrical processing resulted in an increase in the compression force; from 21.65g when the gel was formed at 0 $V\,cm^{-1}$, c compression force increased to 27.6g at when processing at 7 $V\,cm^{-1}$, which represents an increase of approximately 27%. An enhance of 46% in compression force was observed when forming the particles at 14 $V\,cm^{-1}$, with a difference in compression force of 9.95g. According to the Kruskal-Wallis statistical test performed, the only significant differences were found with particles formed at 0 $V\,cm^{-1}$, 20°C and those at 14 $V\,cm^{-1}$, 40°C; and also between particles formed at 0 $V\,cm^{-1}$, 40°C and those at 14 $V\,cm^{-1}$, 40°C.

From the results previously described, it could be deduced that in order to affect significantly the compression force of alginate particles during their formation in this study, it was necessary to electrically process at field strengths of 14 $V\,cm^{-1}$, regardless processing temperature. The alternating electrical fields used in these experiments and in the experiments described in Chapters 4 and 5, although in the category of moderate, are sometimes low to produce a significant effect on both cellular material and gel networks.

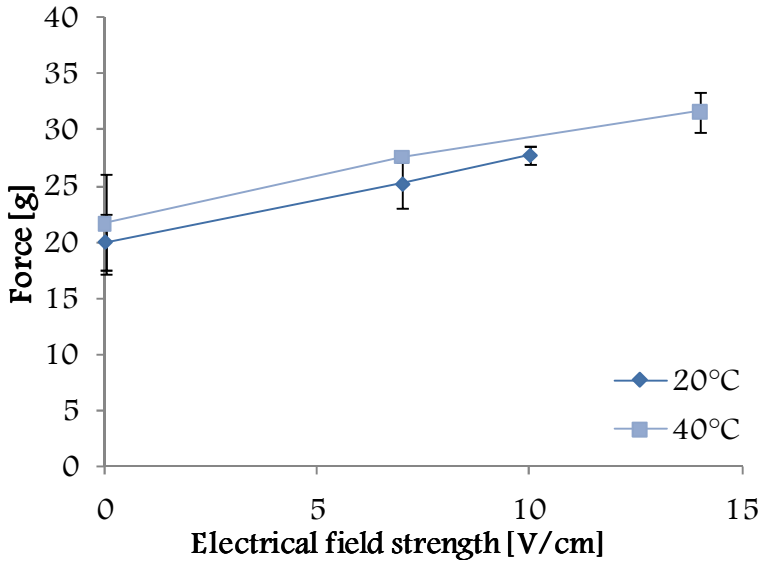

Fig. 6.4. Graph showing the force necessary to compress 1mm of alginate particles formed at 20 and 40°C and different electrical field strength. Vertical error bars represent standard deviation of triplicates performed.

6.3.1.1. Gellan gum particles formation

The experiments for the formation of alginate particles were also done using gellan gum as polymer. Although having characteristics similar to alginate (both gels form after the interaction with divalent ions), gellan gum forms clearer and more transparent gels which depending on the concentration used, go from brittle to elastic. Gellan gum´s use and consumption in the food and pharmaceutical industries are lower than alginate´s; nevertheless, the ability of gellan gum for being extremely effective in forming gels with low polymer levels and for forming thermo-reversible gels, are raising the importance of gellan gum for industrial applications (Shah, 2007). Gellan gum, which is a carbohydrate secreted by a bacterium, is an industrial discovery (McGee, 2004).

The average size of the gellan gum particles formed at various electrical field strengths and processing temperature is presented in table 6.2. The particles formed at 40°C did not have a spherical shape, which made the measurement of their "diameter" with the digital

calliper difficult; so the reported size corresponds to the thinnest length of the particle (shown graphically in Fig. 6.5). The shape of gellan gum particles formed at 40°C was spherical according to the increase in the electrical field applied.

Table 6.2. Average size of gellan gum particles formed at different processing temperatures and electrical field strengths

	20°C			40°C		
Electrical field	**6.4. 0 $V\ cm^{-1}$ (Control)**	**7 $V\ cm^{-1}$**	**10 $V\ cm^{-1}$**	**0 $V\ cm^{-1}$ (Control)**	**7 $V\ cm^{-1}$**	**14 $V\ cm^{-1}$**
Thinnest part [mm]	5.36	4.74	4.1	2.96	4.02	4.68
Standard dev.	0.2608	0.6768	0.4	0.5683	0.2168	0.4764

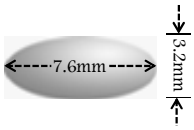

Fig. 6.5. Example of the shape of gellan gum particles formed at 40°C.

Compression test of formed gellan gum particles showed that regardless the processing temperature the effect produced by MEF is an enhancement in compression force according to the increase in the strength of the electrical field. Compression force values of the particles formed at 0, 7, 10 and 14 $V\ cm^{-1}$ can be compared in Fig. 6.6. It can be seen that in the experiments at 20°C the compression force was 7% higher for particles formed at 7 $V\ cm^{-1}$ (from 48.3g to 52.1g), and the force presented an increase of 15% in the particles formed under electrical fields of 10 $V\ cm^{-1}$ (from 48.3g to 56.5g).

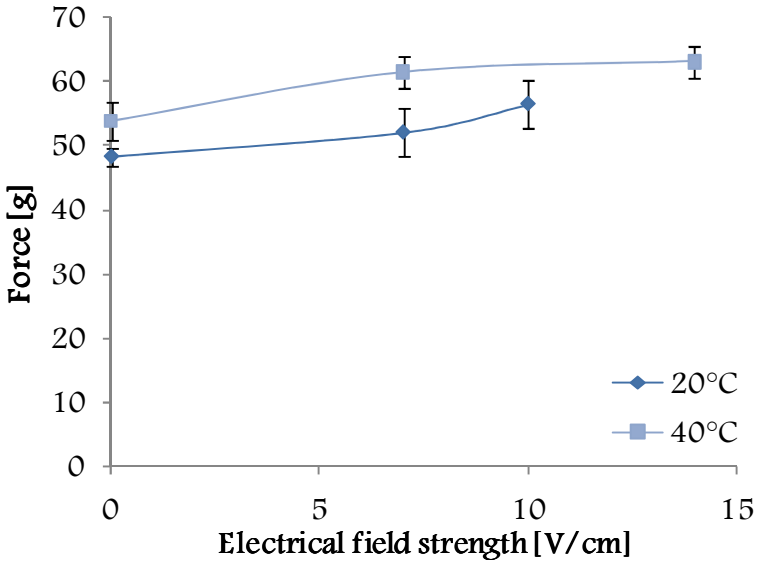

Fig. 6.6. Graph showing force necessary to compress 1mm of gellan gum beads formed at 20 and 40°C and different electrical field strengths. Vertical error bars represent standard deviation of triplicates performed.

At processing temperature of 40°C (Fig. 6.6), compression force of the particles increased when formation occurred under electrical processing, similarly to the results observed for alginate. In this case the force necessary to compress 1mm of the particle increased from 53.93g in control samples to 61.45g for particles formed under 7 $V\ cm^{-1}$, which corresponds to an increase of approximately 13%; and was 16% higher in the particles prepared at 14 $V\ cm^{-1}$. Statistical analysis (Kruskal-Wallis test) showed significant differences between the particles formed at:

1. 20°C, 0 $V\ cm^{-1}$ and those at 40°C, 0 $V\ cm^{-1}$.
2. 20°C, 0 $V\ cm^{-1}$ and those at 40°C, 14 $V\ cm^{-1}$.
3. 40°C, 0 $V\ cm^{-1}$ and those at 40°C, 14 $V\ cm^{-1}$.

Mechanical strength of gellan gum particles under electrical processing with MEF was enhanced when their formation occurred at electrical field strengths of 14 $V\ cm^{-1}$ in this study. Increase in thermal conditions also had an enhancing effect on compression force, being this raise proportional to the increase in compression force of the particles.

The observed enhancing effect in compression force after the application of MEF in this research work is in agreement with the results obtained by Zvitov and Nussinovitch (2003) and Moghadam *et al.* (2008). Agar, agarose, alginate and gellan gel beads treated under constant DC electrical fields ($30\,V\,cm^{-1}$) from 1 to 60 seconds presented a general shrinkage effect which made the beads stronger and more rigid (Zvitov and Nussinovitch, 2003). The degree of weight loss depended on time and intensity of the DC electrical field and on the composition of the bead and the solution in which it was immersed. At $64\,V\,cm^{-1}$, weight losses of 24% for alginate and 35% for gellan gum were observed.

In the work done by Moghadam *et al.* (2008) the effect of the application of the electrical field was attributed to the migration and redistribution of counter and added ions within the electrified gel. Alginate beads were produced by electro-spray using constant DC voltage. Voltage had a pronounced effect on the size of beads, reducing them from 2.07mm mean size for control samples (0V) to a minimum mean size of 0.63mm with a voltage applied of 9000V, and making possible to produce mono-sized and spherical beads.

6.4.1. PEF experiments

6.4.1.1. Temperature profile for PEF experiments

The temperature of an alginate slab was measured when 0, 1, 9, 45, 90 and 180 pulses were applied to it; the obtained increases in temperature are presented in Fig. 6.7. The maximum increase observed was 0.2°C which cannot be considered as a cause of the effect of PEF on compression force of gel networks.

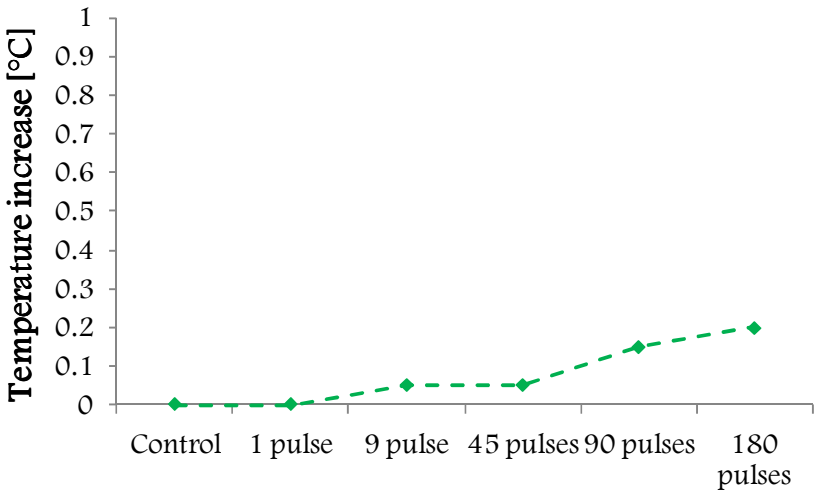

Fig. 6.7. Temperature increase profile when different amount of pulses were applied.

6.4.1.2. Experiments with Aluminium electrodes

6.4.1.2.1. Compression force results

The comparison of compression force of alginate samples after treatment with electrical pulses (applied with aluminium electrodes) is presented in Fig. 6.8. An increase of 0.303g was seen when comparing the compression force needed to compress control samples (0 pulses) and the compression force of the samples treated with 90 pulses. The increase represents approximately 50% in compression force; this percentage of increase is similar to the value obtained using MEF, the main difference was the processing time: from 5 minutes in MEF to milliseconds in PEF.

Kruskal-Wallis statistical test showed significant differences in compression force between:

1. Control samples (0 pulses) and samples treated with 45, 63 and 90 pulses.
2. Samples treated with one pulse and those treated with 63 and 90 pulses.
3. Alginate samples electrically processed with 9 pulses and the samples to which 90 pulses were applied.

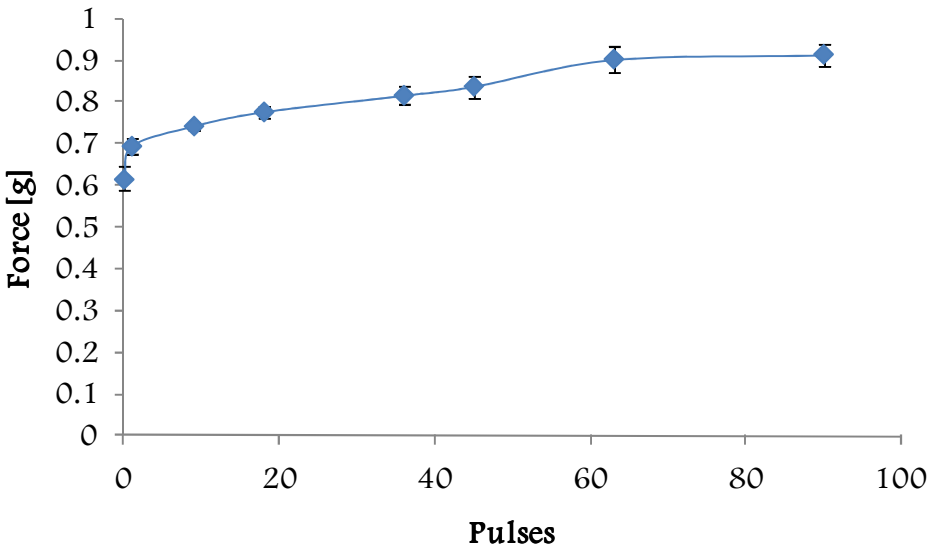

Fig. 6.8. Comparison of compression force of alginate samples at different PEF treatments when the electrodes were aluminium plates. Vertical error bars represent standard deviation of quintuplicates performed.

6.4.1.2.2. Weight reduction

Alginate slabs presented an increase in weight loss when treated with electrical pulses. In order to ensure consistency and clarity in the data collected, control samples were processed following the same experimental procedure and timings as those at 1, 9, 18, 36, 45, 63 and 90 pulses. Weight loss at different levels of treatment is shown in Fig. 6.9, where the profile number of applied electrical pulses vs. % of weight loss increases according to the increase in the pulses applied; with a highest difference in weight loss of 5% after comparison of control samples and samples treated with 90 pulses. Possible explanations for this phenomenon are water loss due to dehydration, and in samples electrically treated, the ability of PEF to affects cross-linking and the alginate network's strength.

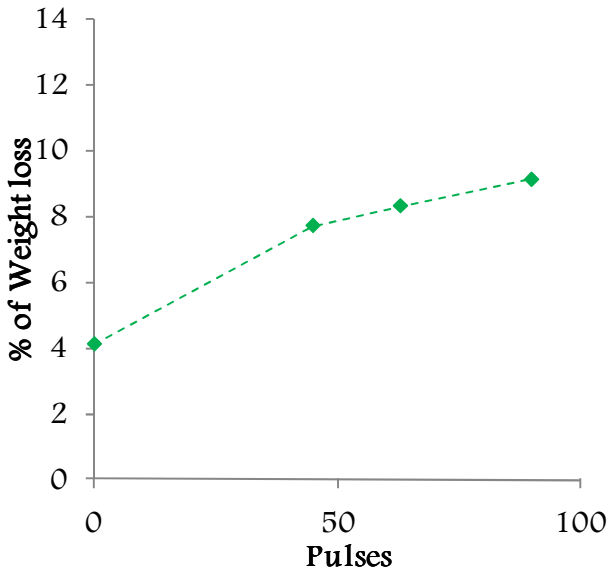

Fig. 6.9. Graph showing the % of weight loss profile at different PEF treatments, using the electrophoration cuvette.

6.4.1.3. Experiments with stainless steel electrodes

6.4.1.3.1. Compression force results

Results obtained after PEF treatment for alginate samples using stainless steel electrodes are shown in Fig. 6.10. Although in a smaller extent, the same increasing pattern in compression force was observed. Compression force was 14% higher when the samples received 90 electrical pulses, increasing from 0.97g when treated with 0 pulses to 1.1g with the application of 90 pulses. A statistical non-parametric test (Kruskal-Wallis test) of the results showed significant differences between control samples (0 pulses applied) and the samples that received 90 pulses.

6.4.1.3.2. Weight reduction, PEF with stainless steel electrodes

Alginate particles electrically treated (with PEF using stainless steel electrodes) presented an increase in weight reduction, which was proportional to the increase in applied pulses. The profile for number of pulses vs. % of weight loss is presented in Fig. 6.11. A difference of 2.5% can be observed between the percentages of weight loss for control samples and

samples treated with 90 pulses, showing that the weight of the samples is affected by the electrical treatment.

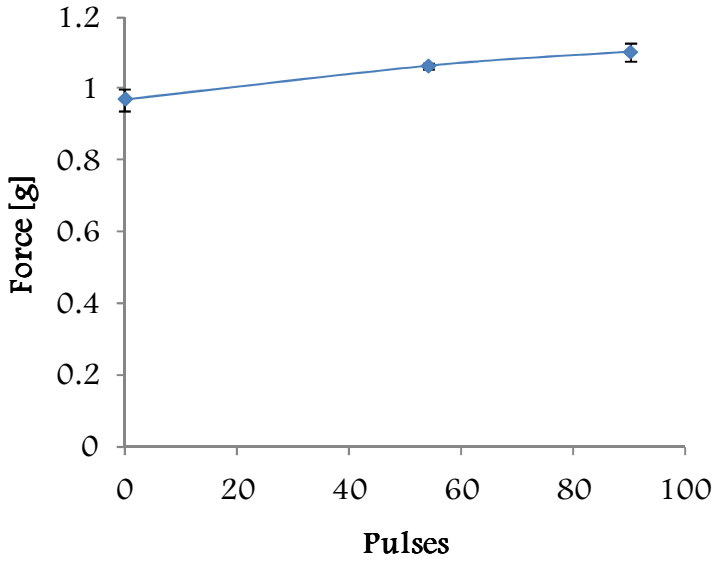

Fig. 6.10. Comparison of compression force of alginate samples at different PEF treatments when the electrodes were stainless steel plates. Vertical error bars represent standard deviation of quintuplicates performed.

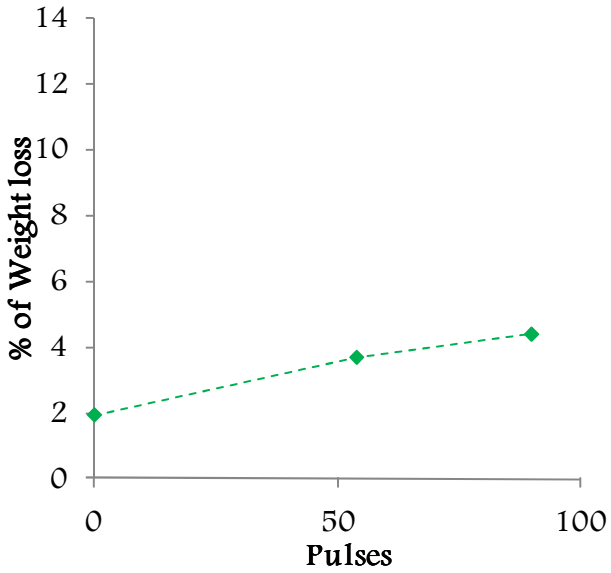

Fig. 6.11. Graph showing the % of weight loss profile at different PEF treatments, using the stainless steel plate electrodes.

A limited amount of research works have previously dealt with PEF applied to 'already formed' gel networks. As explained in the introduction of this Chapter, a reason for the lack of research done in this area could be attributed to the fact that the most common way of controlling mechanical properties of alginate, gellan gum and other polymer networks in the industry sector, is by adjusting the composition of the monomers and binding solution used.

6.5. CONCLUSIONS

The effect of MEF and PEF on mechanical properties of polymer networks was studied in this Chapter. Polymer particles were formed by dropping aqueous solutions of alginate and gellan gum in a solution of $CaCl_2$. Electrical fields ranging from 0 to 14 $V\,cm^{-1}$ were applied during the formation of the particles. Results showed that MEF have an increasing effect (of approximately 46%, the highest) on compression force of the gels. Alginate cylinders and slabs were treated with PEF (amount of pulses ranging from 1 to 90) at Lund University, Sweden. Compression force of the samples was higher (by approximately 50%) than the values for control samples. The increasing effect of electrical processing on mechanical properties (such as compression force) of gel systems could be used as a novel way of controlling the characteristics of polymer materials in the food and pharmaceutical industries.

CHAPTER 7

7. CONCLUSIONS AND FUTURE WORK

7.1. CONCLUSIONS

Electrical processing as an effective way of pasteurisation and for electrophoresis purposes is widely used in food and pharmaceutical industries. As it has been presented in the literature survey for this research work, electrical processing is an alternative process with the ability to affect mass transfer for food material. In cellular materials, electrical processing has been shown to enhance the extraction of cellular components, making electrical processing a novel process with many other potential applications in industry. In comparison with the amount of works that have focused on the effect of electrical processing for mass transfer in cellular material, a smaller amount of research has paid attention to the effects of electrical processing to mass transfer in gel systems. These studies have reported that the application of electrical fields to gel networks has either an enhancing effect in mass transfer, a decreasing effect or in some cases, no effect at all. The importance of studying the properties of polymer materials lies in the numerous applications they have in food and pharmaceutical industries (among other industrial areas); e.g. as materials for the delivery of actives. Electrical fields have also been proved to be a potential method for modifying mechanical and textural properties of ionic polymer networks.

This study has researched the effect of electrical processing on mass transfer for cellular materials and on mass transfer and mechanical properties of polymer networks, by the development and performing of three sets of experimental methods. The sets of

experimental methods allowed to measure the extraction of betanin from beetroot when thermal and electrical processing were applied, to visualise the infusion of rhodamine6G to four different types of gel networks and to electrically process alginate and gellan gum, with both MEF and PEF, to affect its compression force.

The first set of experiments was designed to allow measurement of the release of betanin from beetroot when the material was affected by thermal and/or electrical processing. Electrical processing consisted in MEF ranging from 0 to 1000 $V\,m^{-1}$ at constant processing temperatures in a range from 40 to 60°C. Results showed that the application of MEF has an enhancing effect on mass transfer of compounds from cellular materials, specifically for the extraction of betanin dye from beetroot, with the amount of betanin extracted from a beetroot slab immersed in a NaCl being higher when electrical fields of 600 and 1000 $V\,m^{-1}$ were applied in comparison to the results at 0 $V\,m^{-1}$. The enhancement was proportional to the increase in temperature and electrical field; although the effect of temperature appears to be more pronounced than the effect of MEF in the range of electrical field strength used for this study. The enhancing effect observed in this study could be compared with increase in the amount of betanin extracted from beetroot when treated with PEF of different durations, by Chalermchat *et al.* (2004) and with the resulting enhanced amount of material extracted when beetroot was treated at electrical fields of 1000 $V\,m^{-1}$, by Kulshrestha and Sastry (2003). The leaching of solutes (betanin) is believed to be caused by the disruption in the cell wall of beetroot due to both thermal and electrical damage. It could be seen that continuous electrical fields have an enhancing effect on the extraction of components from cellular material even at mild strengths, by damaging the membrane of the cell. In order to visualise the effect of the slab orientation respect to the electrodes and current, the slab was placed in parallel or perpendicular to the electrical field. The results showed that the amount of betanin extracted was higher when the slab was placed in a perpendicular position respect to the electrical field compared with the extraction when it

was placed in parallel. A possible cause for the difference in extraction is believed to be that a bigger surface area is affected by the flow of the current when the slab is situated perpendicularly to the current (or parallel to the electrodes). Effective diffusion coefficients (D_{eff})were estimated with a solution of Fick´s 2nd law of diffusion. The estimated values showed a linear relation between temperature and the D_{eff}, a good correlation model-experimental data and Fickian diffusion.

In the designed and performed experiments for electrical processing of MEF to gel networks, mass transfer coefficients for the infusion of rhodamine6G were estimated with a solution of Fick´s 2nd law of diffusion. The estimated diffusivities were comparable with values reported in previous works, for experimental conditions similar to those in this study. A general decreasing effect of electrical fields on mass transfer of rhodamine6G into polymer gels was observed in networks set with ions (alginate and potato-alginate mix), and no significant effect on gels set thermally (albumen and gelatine). A possible cause of the decreasing effect could be that the electrical field affects the ions and colorant particles present in the system, affecting the crosslinking of the gel polymer, strengthening the alginate gel bindings and making the infusion of the colorant molecule slower and more difficult. A similar decreasing effect of electrical processing on mass transfer was found in a previous work for the permeability of water vapour, oxygen and carbon dioxide in chitosan coating. For the mass transfer experiments performed on alginate and potato-alginate gels, the effect was also influenced by the differences in the electrical conductivities of the polymer and the rhodamine6G solution, being the highest decreasing effect, approx. 50%, when the electrical conductivity of the gel was higher than the electrical conductivity of the colorant solution surrounding it ($\sigma_{Gel}>\sigma_{Solution}$). In the experiments where the electrical conductivity of the gel was lower than the electrical conductivity of the rhodamine6G solution ($\sigma_{Gel}<\sigma_{Solution}$) and in those where the gel matrix was a gel set with temperature,

the infusion of the colorant was the same with or without the application of electrical fields, the results after the statistical analysis performed did not show significant differences.

The 3rd set of experiments was divided in two main sections, one to study the effect of MEF on polymer networks by forming alginate and gellan gum spheres under different electrical field and temperature conditions, to then measure the compression force for the particles formed. Alginate and gellan gum spheres formed under electrical processing at 7, 10 or 14 $V\ cm^{-1}$, presented a significant higher compression force than those formed at 0 $V\ cm^{-1}$, approximately 46% at 40°C and 14 $V\ cm^{-1}$. The differences in compression force were influenced by both thermal and electrical treatment, increasing with the raises of electrical field and temperature. Section number two of the chapter studied the effect of PEF on alginate slabs and cylinders that after their formation at 0 $V\ cm^{-1}$ were subjected to PEF at different number of electrical pulses ranging from 1 to 90; the effect of the electrical processing was measured in terms of the compression force of the gel samples. Results showed an increase in compression force with the increase in the amount of pulses applied. The increasing effect could be caused (as explained in the conclusions for the experiments on mass transfer of dyes to gel networks) by the rearrangement of the Calcium ions in ionic gels when the electrical field is applied, and by the water loss the swollen networks experience during the electrical process. In general, results from Chapter 6 evidenced that it is possible to influence the mechanical properties of gel spheres by electrical processing with MEF and PEF.

7.2. FUTURE WORK

In the results presented in chapter 4, for mass transfer of betanin under MEF at temperatures of 50 and 60°C, degradation of betanin was not considered to affect the differences in rate of betanin extraction. Nevertheless, the study of betanin colour

degradation under the application of electrical fields could be a possible area for future work.

The experiments for betanin dye mass transfer into gel systems on Chapter 5 could not be taken further due to time limitations. The two experiments performed showed an increase of mass transfer with the application of MEF, contrary to the effect found when using rhodamine6G as diffusing molecule. Future work could focus on the effect of electrical processing on polymer networks when the dyes used have ionic charges that are negative and/or positive.

A possibility that was not taken into account as a cause for the effect when electrical processing was applied is electrolysis happening near the electrodes. The reactions that could be taken place are likely to produce particles that react or affect the motion of Calcium ions, i.e. the oxygen produced in the bubbles near the electrodes. Future experiments, where an electrolyte solution (which electrical conductivity is the same than the solution surrounding the gel) flowing around the electrodes in order to remove the substances that might be produced near them, could be designed and performed. A membrane dividing the electrodes from the treatment chamber could be also included in the experiment set-up. Another variant to take into account for further experiments could be the use of a phosphate chloride solution surrounding the samples during the mass transfer experiments. Phosphate has an ion relative mobility that is higher than the one for Sodium or Calcium, so it would be expected that the phosphate ions would move prior to the movement of the Calcium ions in the alginate gel when exposing them to electrical processing.

A complete comparison of the effects of PEF and MEF to gel systems could not be done mainly because the 3rd set of experiments was done in different laboratory facilities at

different experimental conditions; and since the sample geometry was chosen to be most suitable one for the nature of the treatment, they had to be different from each other. Another limitation was that it was not possible to produce gel capsules in a microscopic size that were suitable for the compression test available at the University. The capsules formed consisted only in alginate and tended to dry out as soon as the light of the camera was switched on.

The experimental work of different researchers, many of them presented and referenced through the chapters of this work, cannot be totally compared with the results obtained in this study since the models used for the interpretation of the results depend strongly on the individual system and set-up used in each case. Nevertheless, in general the effect observed when the material was treated electrically, as well as the order of magnitude of the obtained results agree with those reported in previous similar works in the research areas of electrical processing and mass transfer.

Electrical processing is an area that is already being used in food industry, however, its importance is growing and potential applications are being widely researched, being the modification of mass transfer just one in the vast list. By using systems with only a few variables which experimental set-up allowed visualising and measuring diffusion, this research work has showed evidence of the ability of electrical processing to affect structure and mass transfer properties of different types of food material.

APPENDIX

1. Theoretical considerations for D_{eff} estimation, (for the diffusion of betanin from a beetroot slab) in Chapter 4.

A solution of Fick´s 2nd Law of diffusion (Crank, 1975) was used for the estimation of the effective diffusion coefficients (D_{eff}):

$$\frac{\partial C}{\partial t} = D_{eff} \frac{\partial^2 C}{\partial x^2} \quad \textbf{(1)}$$

for the correspondent initial and boundary conditions:

Initial conditions: $C = C_0,\ -l < x < l,\ t = 0$ **(2)**

Boundary conditions: $a \frac{\partial C}{\partial t} = \mp D_{eff} \frac{\partial C}{\partial x}$ **(3)**

assuming that an infinite sheet of uniform material of thickness 2l is placed in a solution and that the solute is allowed to diffuse from the sheet into the solution.

In a form expressing M_t, the amount of solute leaving the sheet up to time t as a fraction of M_∞, the corresponding quantity after infinite time, the solution is:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(\frac{-D_{eff} q_n^2 t}{l^2}\right) \quad \textbf{(4)}$$

Matlab (MathWorks Inc., USA) was used to evaluate the effective diffusion coefficients of experiments carried out by fitting the experimental data to the curve given by the model.

2. Matlab functions used for D_{eff} calculations in Chapter 4.

The diffusivities for the extraction of betanin from beetroot where estimated with two Matlab subroutines.

For the 1st subroutine it is necessary to get the values of α and q_s beforehand. These values differ depending on the maximum concentration of betanin extracted at varying experimental conditions. Function "my fun" calculates a curve of M_t values based on the solution of Fick´s law of diffusion and a random value of D_{eff} (which is obtained from the literature for the system used in the experiments).

```
function F=myfun(D,xdata)

alpha=3.6154;
l=1.5e-3;
t=xdata;
q=[1.7294    4.7703    7.8890   11.0207   14.1567  17.2948];
mt=zeros(size(t));

for tt=1:length(t)
    mt(tt)=1-
sum(2.*alpha.*(1+alpha)./(1+alpha+alpha.^2.*q.^2).*exp(-
D.*q.^2.*t(tt)/l.^2));
end

F = mt;
```

The model curve is then optimised and fitted to the experimental data with the functions:

```
xdata=t;
ydata=mexp;
D0=...D
options=optimset('TolFun',1e-14,'Tolx',1e-14);
[D,resnorm,residual,exitflag,output] =
lsqcurvefit(@myfun,D0,xdata,ydata,1e-11,1e-8,options)
```

In the 2nd subroutine, the solution is obtained by finding the value of D_{eff} where the error value is the lowest. The value obtained from both subroutines was the same after comparison of the results.

```
clear; close all; format short g;

%initial values
C0=9e-4;
Minf=7.36e-4;
L=1.5e-3;
%time vals
t=500:20:3400;
Lt=length(t);

alpha=fract(C0,Minf);

n=6;

qn=rootstan(alpha,n);

%select number of D values
dval=2000;
Dmax=2e-9;
Dmin=8e-10;

D=Dmin+(Dmax-Dmin)*rand(dval,1);
D=sort(D);
mt=zeros(dval,Lt);

%load experimental data
data=load('Mtmeas_data.txt');
tmeas=data(:,1); Mtmeas=data(:,2);
clear data

Mt_comp=interp1(tmeas,Mtmeas,t,'cubic');

for i=1:dval
    mt(i,:)=march2(D(i),t,alpha,L,qn);
    err(i)=sum((Mt_comp-mt(i,:)).^2);
end
[merr, place]=min(err);

mt_best=march2(D(place),t,alpha,L,qn);

disp('value of D at minmum error')
D(place)
```

```
subplot(3,1,1); plot(t,mt_best,tmeas,Mtmeas); xlabel('Time
(secs)'); ylabel('Total Diffused, Mt'); legend('model','meas')
subplot(3,1,2); plot(t,mt_best-Mt_comp); xlabel('Time');
ylabel('difference');
subplot(3,1,3); plot(D,err); xlabel('Diffusivity');
ylabel('error size'); legend('error')
```

3. Comsol programme for the simulation of the effects on electrical potential of the application of direct current to a gel-solution system when electrical conductivities of the gels are modified, Chapter 5.

The model for resistive heating in Comsol Multiphysics was used to visualise, previous to performing the experiments, if changes in the electrical conductivities of the gel in the system described for the set of experiments of Chapter 5, would have any influence of the effect caused by electrical processing on mass transfer of rhodamine6G.

The model assumes that the system is subjected to a current passing through it, as a result of an electric potential difference across two opposite sides. The current heats the system. The programme performs a transient analysis of the thermal-electric model (Comsol AB, 2009). Only the electric part of the model (Conductive Media DC application mode) was used to obtain Fig. 5.1a, 5.1b and 5.1c. Fig. 1 is a representation of the system gel-colorant solution in an electrical processing cell, seen from above.

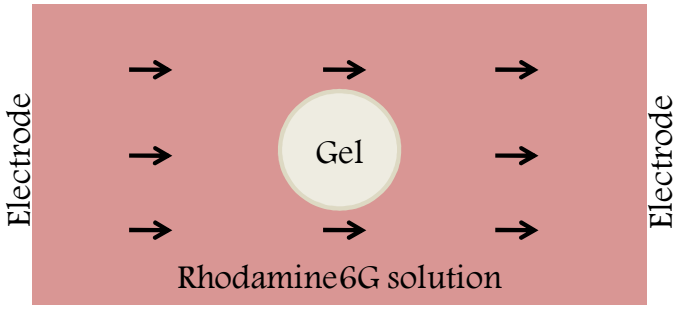

Fig. 1. System gel-colorant solution subjected to an electric potential difference across 2 opposite sides.

The electric potential, V, is the solution variable in the Conductive Media DC application mode. The electric current density in turn, I_ρ, is proportional to the electric field, which equals the negative of the gradient of the potential, V. The generated resistive heat, Q, is proportional to the square of the magnitude of the electric current density, I_ρ.

$$Q \propto \left|I_\rho\right|^2 \quad \textbf{(5)}$$

The coefficient of proportionality is the electric resistivity, $\varepsilon=\sigma^{-1}$, which is also the reciprocal of the temperature-dependent electric conductivity, $\sigma = \sigma(T)$. Combining these gives the fully coupled relation

$$Q = \frac{1}{\sigma}\left|I_\rho\right|^2 = \frac{1}{\sigma}|\sigma E|^2 = \sigma|\nabla V|^2 \quad \textbf{(6)}$$

Over a range of temperatures the electric conductivity σ is a function of temperature T according to

$$\sigma = \frac{\sigma_0}{1+\Theta(T-T_{ref})} \quad \textbf{(7)}$$

where σ_0 is the conductivity at the reference temperature T_{Ref}. Θ is the temperature coefficient of resistivity, which describes how the resistivity varies with temperature. In the Conductive Media DC application mode the electric conductivity for Joule heating in can be specified in terms of this equation (Comsol AB, 2009).

4. Theoretical considerations for D_{eff} estimation (for the infusion of rhodamine6G to a cylindrical polymer network) in Chapter 5.

Fick´s 2nd Law of diffusion was used to quantify effective diffusivities. Considering a long circular cylinder in which diffusion is everywhere radial. Concentration is a function of radius r and time t only. Fick´s diffusion equation becomes (Crank, 1975):

$$\frac{\partial C}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD_{eff}\frac{\partial C}{\partial r}\right) \quad \textbf{(8)}$$

For the following initial and boundary conditions:

$$\text{Initial conditions: } C = 0, \quad 0 < r < a, \quad t = 0, \quad \textbf{(9)}$$

$$\text{Boundary conditions: } C = C_0, \quad r = a, \quad \textbf{(10)}$$

Where C is the concentration [kg m^{-3}], t is time [s], D_{eff} is the effective diffusion coefficient [m^2 s^{-1}] and r is the radius [m]. Assuming that a long circular cylinder in which diffusion is everywhere radial is placed in a solution from where the solute is allowed to diffuse from into the cylinder.

For mass transfer in gel systems, Matlab's function "pdepe" gives a solution for the diffusion in a cylinder from a stirred solution of limited volume.

5. "pdepe" function (The Math Works, Inc., 2009).

pdepe solves PDEs of the form:

$$c\left(x,t,u,\frac{\partial u}{\partial x}\right)\frac{\partial u}{\partial t}=x^{-m}\frac{\partial}{\partial x}\left(x^{m}f\left(x,t,u,\frac{\partial u}{\partial x}\right)\right)+s\left(x,t,u,\frac{\partial u}{\partial x}\right) \quad \textbf{(11)}$$

For t = t_0 and all x, the solution components satisfy initial conditions of the form:

$$u(x,t_0)=u_0(x) \quad \textbf{(12)}$$

For all t and either $x=a$ or $x=b$, the solution components satisfy a boundary condition of the form:

$$p(x,t,u)+q(x,t)f\left(x,t,u,\frac{\partial u}{\partial x}\right)=0 \quad \textbf{(13)}$$

6. Matlab routine for D_{eff} estimation in Chapter 5.

In function 'alginate', the global coefficients D_{eff} and C_0 are defined at the beginning; these values are specific for each treatment. Then the subfunctions alginate_pde (for diffusivities), alginate_ic (for initial conditions) and alginate_bc (for boundary conditions) are used by pdepe in order to get a solution matrix, called u that contains the concentration values.

```
function alginate

global Deff
global C0

Deff = 6.17e-10;
C0 = 1.2e-01;
m=1;
x=linspace(3.73e-3,6.0e-3,11);
t=linspace(0,840,3);

sol=pdepe(m,@alginate_pde,@alginate_ic,@alginate_bc,x,t);
u=sol(:,:,1)

surf(x,t,u)

data = load('alsmeg0.txt');
xexp= data(1:11,1);
uexp= data(1:11,2);
umod= sol(end, 1:11);

hold off
plot(xexp, uexp, 'g')
xlabel('Radio [m]')
ylabel('Concentration @ 840s [kg/m^3]')
hold on
plot (x(1:11),umod,'b')

for i=1:11
    erro = sum((uexp-umod').^2);
end

format short eng
erro

function [c,f,s]=alginate_pde(x,t,u,DuDx)
global Deff
c=1;
f=Deff*DuDx;
s=0;
```

```
function u0=alginate_ic(x)
u0=0;

function [pl,ql,pr,qr]=alginate_bc(xl,ul,xr,ur,t)
global C0
pl=0;
ql=1;
pr=ur-C0;
qr=0;
```

Fitting of experimental and model curves was done by comparing the value of error given by Matlab when running the function 'alginate' with the error when another D_{eff} was set. The D_{eff} that gave the smallest error value represented the best fit for the model and the experimental curves, and was recorded as the D_{eff} for the process.

7. Kruskal – Wallis test non parametric statistic analysis.

The non-parametric equivalent to one-way ANOVA is the Kruskal-Wallis test. In the test, three or more independent groups of data are collected and the differences between those sets of data estimated. Kruskal-Wallis involves ranking each individual datum, and then the sums of the ranks within each treatment are used to calculate a test statistic which is used to examine the validity of the null hypothesis (Jones, 2002).

Matlab´s function p = kruskalwallis(X) performs a Kruskal-Wallis test to compare samples from two or more groups, and returns the p value for the null hypothesis that all samples are drawn from the same population (or equivalently, from different populations with the same distribution). If the p value is near zero, this casts suggests that at least one sample median is significantly different from the others (The Math Works, Inc., 2009).

kruskalwallis function displays two figures. The first figure is a standard ANOVA Table (Fig. 2); the second figure displays box plots of each column of X (Fig. 3).

Kruskal-Wallis ANOVA Table

Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Columns	967	5	193.4	19.35	0.0017
Error	182.5	18	10.139		
Total	1149.5	23			

Fig. 2. Example of ANOVA Table for Kruskal-Wallis test given by Matlab.

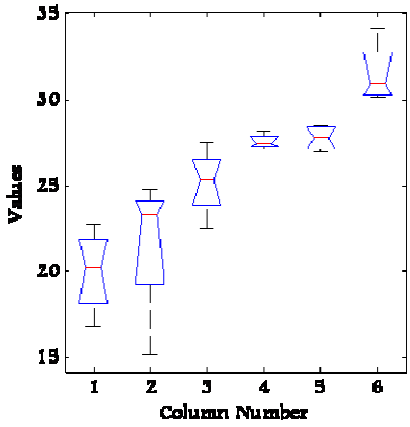

Fig. 3. Example of Box Plots for Kruskal-Wallis test, given by Matlab.

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