Tomas Bata University in Zlín
Faculty of Applied Informatics
Department of Electrotechnics and Measurements

Ing. Milan Navrátil

Doctoral Thesis

INSTRUMENTATION AND DIAGNOSTICS OF POLYMER COMPOSITES

Study branch: Technical Cybernetics

Supervisor: Prof. Ing. Karel Kolomazník, DrSc.

Consultant: Doc. RNDr. Vojtěch Křesálek, CSc.

Zlín, Czech Republic, 2007

ACKNOWLEDGEMENTS

I wish to thank to all the people who contributed to the completion of this work. First, I would like to express my deepest gratitude to Professor Karel Kolomazník, my advisor, for help, guiding and encouraging me to complete this dissertation. I also owe a great deal to my consultant Vojtěch Křesálek, for his support, smart ideas, guidance and most importantly his perseverance. Without their numerous suggestions and immense knowledge, this work would have never been completed.

My special thanks belong to Michaela Uhlířová, who helped me with technical terms not only in chemistry and revised my English in this work.

At last but not least I appreciate great help by Santiago Clara and top-class laboratory access offered by British Leather Technology Centre in Northampton.

Finally, I want to thank to my family, my girlfriend and friends for their unfailing support.

SUMMARY

One of the most often methods of intermediate product stabilization is their cross-linking. Aldehydes are commonly used as cross-linking chemicals, from which glutaraldehyde and formaldehyde have the greatest importance. Final quality and optimal behaviour of the end product depend on various conditions during the cross-linking reaction. This thesis deals with mathematical description of cross-linking process represented by the nonlinear vector differential equations. Linearization of the equations is given to determine a transfer matrix.

In the experimental part, the physical methods exploiting changes of electrical and optical properties of investigated polymer are used. These changes allowed accomplishing not only experimental identification of the cross-linking system but also partial clarification of the first-step mechanism when poly-electrolytic circle of the cross-linking protein is opening. Kinetic curves acquired by the above-mentioned physical measurements are presented. In addition to these curves, relevant dependencies on concentration were also measured. They can be further used not only as calibration curves but also as possible methods how to get some information during the cross-linking process. At the end of this work, the results that can be applied in practice during natural polymer treatment are discussed.

RESUMÉ

Jedním z nejčastějších metod stabilizace proteinových meziproduktů je jejich síťování. Jako síťovací chemikálie se běžně používají aldehydy, z nichž největší význam mají glutaraldehyd a formaldehyd. Výsledné vlastnosti konečného produktu jsou určeny řadou podmínek síťovací reakce. Předkládaná dizertační práce se zabývá matematickým popisem dynamiky síťovacího procesu, který je reprezentován nelineární vektorovou diferenciální rovnicí, je provedena také její linearizace s cílem stanovení přenosové matice. V experimentální části jsou použity fyzikální metody využívající změn především elektrických a optických vlastností zkoumaného biopolymeru, které umožnily nejen experimentální identifikaci síťovacího systému, ale i částečně vysvětlit mechanismus prvního kroku síťování, kdy se otevírá polyelektrolytický kruh síťované bílkoviny. Jsou prezentovány kinetické křivky s využitím uvedených fyzikálních metod. Vedle kinetických křivek byly také naměřeny příslušné koncentrační závislosti nejen jako kalibrační křivky, ale i jako možné metody získáni informací během síťovacího procesu. Na závěr jsou uvedeny výsledky, které mohou byt přímo aplikované pro praktické zpracování přírodních polymerů.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

A	$[-]$	absorbance
A_λ	$[-]$	absorbance at wavelength λ
Φ_A	$[W]$	absorbed radiant flux
c	$[mol.m^{-3}]$	concentration
E_q	$[J]$	energy base level
ΔE	$[J]$	energy difference
E_p	$[J]$	energy excited level
Φ_I	$[W]$	input radiant flux
Φ_O	$[W]$	output radiant flux
c_L	$[m.s^{-1}]$	velocity of light
λ	$[m]$	wavelength
$\vec{E}_0$	$[V.m^{-1}]$	amplitude of electric field strength
ω	$[rad.s^{-1}]$	angular frequency
S	$[m^2]$	area of electrodes
$\vec{J}^*$	$[A.m^{-2}]$	complex current density
ε^*	$[F.m^{-1}]$	complex dielectric function
$\vec{E}^*$	$[V.m^{-1}]$	complex electric field strength
Z^*	$[\Omega]$	complex impedance
$\vec{J}$	$[A.m^{-2}]$	current density
ρ_e	$[C.m^{-3}]$	density of charge
$\vec{D}$	$[A.s.m^{-2}]$	dielectric displacement
ε_0	$[F.m^{-1}]$	dielectric permittivity of vacuum
D	$[-]$	dissipation factor

l	$[m]$	distance between electrodes
$\vec{E}$	$[V.m^{-1}]$	electric field strength
ρ	$[\Omega.m]$	electric resistivity
C_P	$[F]$	equivalent parallel capacity
R_P	$[\Omega]$	equivalent parallel resistance
e	$[-]$	Euler's number, e= 2.71828 18284 59045 23536...
f	$[Hz]$	frequency
$\varepsilon"$	$[F.m^{-1}]$	imaginary part of the complex dielectric function
i	$[-]$	imaginary unit
Z	$[\Omega]$	impedance
δ	$[-]$	loss angle
$\vec{H}$	$[A.m^{-1}]$	magnetic field strength
$\vec{B}$	$[A.s.m^{-2}]$	magnetic induction
ε_λ	$[m^2 mol^{-1}]$	molar absorption coefficient at wavelength λ
N	$[-]$	number of periods
U_j''	$[V]$	orthogonal component of the harmonic base wave
ε	$[-]$	permittivity of surrounding
U_j'	$[V]$	phase component of the harmonic base wave
h	$[J.s]$	Planck constant $h = 6,626176.10^{-34}\, J.s$
ε'	$[F.m^{-1}]$	real part of the complex dielectric function
I_S	$[A]$	sample current
Z_S	$[\Omega]$	sample impedance
U_S	$[V]$	sample voltage
σ	$[S.m^{-1}]$	specific conductance
b	$[m]$	thickness of layer
t	$[s]$	time

T_P	$[s]$	time of one period
C_0	$[F]$	vacuum capacitance
U	$[V]$	voltage
$f_1 \dots f_5$		five ordinary differential equations
V	$[m^3]$	volume of reactor
c_A	$[mol.m^{-3}]$	voluminal concentration of component A
c_B	$[mol.m^{-3}]$	voluminal concentration of component B
c_C	$[mol.m^{-3}]$	voluminal concentration of component C
c_D	$[mol.m^{-3}]$	voluminal concentration of component D
c_E	$[mol.m^{-3}]$	voluminal concentration of component E
k_1	$[mol^{-1}s^{-1}]$	velocity constant
k_2	$[s^{-1}]$	velocity constant
k_3	$[mol^{-1}s^{-1}]$	velocity constant
$r_1 \dots r_7$		auxiliary variables
A		system matrix
B		excitation matrix
G		transfer matrix
Δ**U**		vector of input parameters
Δ**X**		vector of state values
Δ**Y**		vector of output parameters
q_A	$[m^3s^{-1}]$	flow of component A
q_B	$[m^3s^{-1}]$	flow of component B
r_A	$[mol.m^{-3}s^{-1}]$	formation rate of component A
r_B	$[mol.m^{-3}s^{-1}]$	formation rate of component B
r_C	$[mol.m^{-3}s^{-1}]$	formation rate of component C
r_D	$[mol.m^{-3}s^{-1}]$	formation rate of component D

r_E	$[mol.m^{-3}s^{-1}]$	formation rate of component E
$a_{11}...a_{55}$		individual elements of matrix **A**
$b_{11}...b_{54}$		individual elements of matrix **B**
c_{1A}	$[mol.m^{-3}]$	initial voluminal concentration of component A
c_{1B}	$[mol.m^{-3}]$	initial voluminal concentration of component B

LIST OF ABBREVIATIONS

AC		alternate current
DC		direct current
GC-MS		gas chromatography - mass spectrometry
GP		gelation point
VAPG		variable amplitude-phase generator

1 INTRODUCTION

At the present time, there are still industrial branches which can be called typical polluters in spite of the effort to reduce their negative impact on environment. Leather industry is one of them and as a whole; it can not be covered with any list of steps that should contribute to environment protection. Leather industry has various manufacturing sectors with specific impacts on surrounding. It is necessary to assert an individual approach during judgment of these impacts and to minimize negative influences in accord with the national legislation. Moreover, the development of environmental friendly products is main objective of sustainable development, which is the fundamental of the European Union program.

Leather manufacturing produces considerable amount of wastes which means unfavourable impacts on elementary components of environment, especially soil, water and air. There are two ways how this problem can be solved. The former is preventive way and leads to limitation of waste production by force of clear technology installation or recycling methods. Recycling procedure allows utilizing wastes as a source of secondary raw material without any reference to place or time of waste formation. The latter way removes consequences of industrial production which disturbs balance of nature or has negative impacts on environment.

New conception of manufacturing from utilizing products and waste formation point of view brings many steps which prospectively results in installation of wasteless technologies. It means that the amount of wastes can be decreased by force of a suitable change of the original manufacturing process. It is spoken about high degree of material use and significant decrease of processing waste. These technologies can be considered as a specific case of recycling when no time shifts either spatial shift arises between waste formation and their utilization. Amount of energy which is consumed for reutilization of waste should be minimal and demonstrates how the wasteless technology is effective.

Wasteless technologies are based on conceptual solution of whole cycle: raw material – manufacturing – consumption – recycling of waste. Principle of solution is product with desiderative parameters which is produced with minimal material and energy usage. From practical point of view, the realization of wasteless technology is inaccessible. In practice, every technology of this type produces wastes in minimal amount, but they always have specific impact on environment. There is no problem to meet the term “environmentally wasteless technology”, which has not at least negative consequences for nature.

Let us concentrate to leather industry which processes hides from slaughter cattle, sheep, goats, buffalo etc. The hides are changed through physical, chemical and mechanical processes from raw hide into the leather. There are many production steps in the process which determine properties and behaviour of final product. Moreover, they have appreciable impact on amount of waste. Subsequent processing of waste chrome shavings by enzymatic hydrolysis can help with decreasing of wastes during hide processing because it can be further processed and utilized. More concrete procedure of processing chrome shavings is described below in chapter 6.1.

Collagen hydrolyzate, obtained from originally minor waste product, is further utilized for production of synthetic sausage casings in nutritional industry and glutaraldehyde is usually used as a cross-linking agent. Quality of these casings is sensorial assessed by committee. Final product must have specific qualitative requirements such as visual aspect, consistence, colour, elasticity etc. Production of these casings is realised in batch so it is not exception that intestine has length in hundreds meters. Product with inconvenient properties is not usable and energy and material losses arise in this case. In order to reach desired parameters of the final product, processes must be well managed and controlled. It includes knowledge about reactions and their mechanism especially cross-linking process with glutaraldehyde.

This work is divided into two main parts – theoretical and practical. The theoretical part deals with modelling of processes and kinetic methods of analysis which are concentrated on cross-linking reaction of collagen with glutaraldehyde. Creation of the mathematical model and determination of transfer function matrix for control purposes are described as well. Principles of used measurement techniques such as dielectric spectroscopy method and spectrophotometry method are characterized. These methods are used for diagnostics of the system represented by experimental identification. The practical part contains description of materials, instruments and other technical equipment used during experiments. Experimental data acquired by mentioned methods are presented and at the end the obtained results are discussed. Suggestions for further research are also given.

2 STATE OF ART

Increasing prices of chemicals, water and energy as well as serious problems related to environmental protection and permanent leather manufacturing as a topic of growing importance, require optimization and rationalization of measurement processes in the leather industry (Hüffer, et al., 2004), (Fond, et al., 2005). In 1972 Corning published an extensive article summarising his own long-standing experience of chemical engineering work in the tanning industry (Corning, 1972). He dealt with the potential application of theoretical tools of chemical engineering for rationalising the treatment process of nature polymers. He came to the conclusion that mathematical simulation of chemical processes, as the chief tool of chemical engineering, was not used in treatment of polymers at all. This fact was very sharply criticised and proposals were suggested. Stages of treatment process using mathematical simulation can be fundamentally used.

More and more waste production steadily occurs due to industrial expansion. Those are mostly stock-pilled without any further conception of their use. In general, material which can not be further used is considered as waste. One of the solutions to the problem consists in wasteless technology (Kupec, et al., 2000). This technology stands closed technological cycles in which waste is recycled and gone back into the production. Moreover, leather manufacturing ranks a specific position. It processes leather of fatstock and game, which are wastes of meat-processing industry. From this point of view, the leather industry is the first branch, the main raw material of which is waste from other industrial productions. However, in addition to valued product it produces considerable amount of liquid and solid wastes. By way of physical and chemical processes the leather is gradually transmuted into the hide. Production of 250 kg of leather requires 1000 kg of hide (Kupec, et al., 2000). Recovery factor stands 25%, which is quite low value. The rest comprises secondary products from which the hide trimmings are the most valued (Langmaier, 1974). These are very important raw material for pharmaceutical, nutritional, cosmetics and stock-feeding industry (Mládek, 1971). Another utilization of secondary products is production of

proteinaceous casings for nutritional industry; in particular, it is biodegradable casings used in butcher production. In practice, this process is realised discontinuously. Hide trimmings are non-homogenous collagenous material and require specific technological methods during its processing. These methods are substantially different from common procedures which are used in leather technology. Initial and very important procedure is a long-run liming which results among others in homogenization of the material. In addition, lipids and soluble proteins are removed; cross atomic bonds of collagen are cracked. Ripe fleshings are partially got rid of lime by force of elutriation. A residual content of strongly bonded calcium hydroxide is removed by hide pickling which is process when calcium hydroxide is neutralised and calcium sulphate originates. It is not bonded with collagen. Mechanical disintegration follows to separate collagen fibres. Prepared material is extruded into infinite tube (synthetic sausage skin) with thickness from 50 to 150 μm. Extruded material passes through baths of specific composition when curing agent and other ingredients diffuse from the solution into the collagenous material. According to temperature and type of curing bath the diffusion is finished within drying process which follows.

There are many steps and procedures during hide transformation into the leather, respectively chrome shavings into nutritional casing. The most important and key operation is chemical reaction of collagen with any curing agent. Course of cross-linking reaction has cardinal influence on properties of final product. For example, digestibility, biodegradability, moisture and fume permeability, tenacity, wholesomeness or bulging resistance to higher temperatures. Moreover, there is another interesting relation between biodegradation of cross-linked collagen under anaerobic conditions and the cross-linking grade (Kupec, et al., 2003). To fulfil all mentioned preconditions signifies thorough knowledge of used procedures, especially course of cross-linking reaction (Heidemann, 1993), (Wong, 1991), (Lundblad, et al., 1983). From engineering point of view, the course of that reaction can not be determined or predicted without knowledge of its mechanism, which is currently intensively studied (Covington, 2001). Some studies, in which the

theoretical tools of process engineering are used, can be exceptionally found (Kasala, 2005). These tools are applied as a basis for stage of production called “research of production line”. Complexity of reaction is given by origin of collagen material. It is a biological substance, especially heredity and environment (in which livestock be situated) have significant influence on its quality and structure. Texture and chemical composition knowledge is one of necessary preconditions for understanding of cross-linking problems. Nowadays, there are nineteen types of collagen. Their individual functions and structural composition are described in detail (Kupec, et al., 2000). Valuable information can be also found in (Brinckmann, et al., 2005).

It is evident, that the most complicated problem is optimizing the cross-linking reaction itself. Nowadays, a fully automatic control of cross-linking process is practically the only possibility. Key problem is a selection of any suitable measurable quantity. This sensory system informs actuator about reaction state or kinetics conditions. As a result it allows execution of actions in individual technological steps.

One possible way how to monitor course of the cross-linking reaction, respectively to measure some quantity, can be methods of polymer physics, which deal with an investigation of structure and physical properties of polymers. Empirical relations and phenomenological theories are the result of polymer physics deducing from experimental observations. Polymer physics foundations were established in the early 40s of previous century. In the 50-70s, this field was concurrently with rapid growing of production and due to using of synthetics polymers intensively expanded. Theoretical background was deepened and number of experimental works increased. In the late 50s, a comprehensive monograph was published, which contained entire field of polymer physics of that time (Stuart, 1956). Subsequent publications deal with only part of problems from polymer physics (Hedwig, 1977), (Kausch, 1978), (Rabek, 1980), (Ferry, 2004).

Polymer physics disposes of broad palette of measuring methods and techniques (Doi, et

al., 1996). Only some of these techniques are suitable and usable in practise during production of mentioned collagen casings. One of suitable method is the dielectric spectroscopy which proceeds from the dominant role which electrical charges play in the molecular interactions of condensed matter. It utilizes electrical charge distributions as naturally present molecular marks in order to monitor the short-range liquid order and its fast variations with time. Dielectric spectroscopy applications are broad and diverse and cover presently the enormous frequency range of 19 decades, ranging from about 10^{-6} Hz to almost 10^{13} Hz. Owing to this broad frequency range of measurements relaxation phenomena with characteristic time constants between about 10 fs and some days are accessible to experimental investigations (Kremer, et al., 2003).

Study of chemical reactions course by means of material dielectric properties was employed more then seventy years ago. In 1934, this method for examination of poly-esterifical reaction was applied (Kienle, et al., 1934). Recently, that method is commonly used not only for study of process which is related to plastic curing (Maistros, et al., 1994), (Lairez, et al., 1991), (Radhakrishnan, et al., 1993), but also as a new method for testing various pharmaceutical systems with water content. In this field the electrical properties of liposome suspensions, gels, creams, proteins and bio-molecules were studied. Also region of vitreous transition and rate of cross-linking reaction were detected (Craig, 1995). In the same way the polyvinyl-acetate plastic wraps and polyvinyl-acetate filled with gelatine were investigated (Abo-Ellil, et al., 2000). Another study demonstrating the use of a dielectric spectroscopy technique was published (Shah, et al., 1997) where the measurement of dielectric constants and dielectric losses in the frequency domain help to quantify the physical-chemical changes in the bulk due to high energy irradiation. However, a monitoring of cross-linking reaction of collagen with aldehydes by dielectric spectroscopy is not described in available literature.

Another possibility lies in study of interaction of electromagnetic radiation with the matter which is called spectrophotometry. Spectrophotometry involves the use of a spectrophotometer, which is a device for measuring light intensity that can measure intensity as a function of the wavelength of light. Spectrophotometry methods are often

preferred especially in analytical chemistry as they include inexpensive instrument and provide high sensitivity (Němcová, et al., 1996), (Thomas, et al., 2007). There are lot of articles dealing with spectrophotometric measurement of cross-linking processes, particularly in pharmaceutics (Gold, et al., 1997), (Yoshioka, et al., 2007). In food industry, quality of chicken, sausages and pastry products during their cooking processes using are monitoring by an optical fibre-based sensing system. The sensor monitors the food colour online as the food cooks by examining the reflected light from both the surface and the core of the product (Sheridan, et al., 2006). From practical point of view, use of spectrophotometer in industrial surrounding or manufacturing is unnecessarily complicated. There are also colorimetric methods which describe colours in numbers, or provide a physical colour match using a variety of measurement instruments. Theoretical background with focusing on the principles and observations is described in (Shevell, 2003). Colorimetry is used in chemistry and in industries such as colour printing, textile manufacturing, paint manufacturing and in the food industry (Movshovich, et al., 1985).

3 STATEMENT OF RESEARCH OBJECTIVES

- Processing of accessible information about collagen cross-linking
- Description of dynamic system which represents cross-linking reaction, application of mathematical simulation for optimizing collagen casings production
- Determination of transfer matrix of cross-linking reactor
- Selection of physical quantities suitable for measurement of cross-linking process to identify the system and to determine its kinetic characteristics
- Apparatus arrangement with connection to PC, implementation of sensory system for monitoring, software creation for automatic data collection
- Acquiring of experimental data
- Suggestions for further research in this field

4 FORMULATION OF MEASUREMENTS METHODS

Dielectric spectroscopy is non-selective method which integrates contributions of all components of the cross-linking reaction to total complex permittivity. To the contrary, optical methods are selective because they can be tuned for unique chemical component. During the study of essential reactions of pure polymer components it is suitable to use the optical spectrophotometry method as a standard method. This advantage disappears during industrial production of novel materials. Filling agents are often used in order to improve mechanical, electrical and also economical properties. These agents make rational use of optical methods impossible due to light scattering and its absorption. This is the reason why acoustic methods are more and more often applied, especially then dielectric spectroscopy methods. Another advantage of dielectric spectroscopy method is shorter time of measuring and data evaluation. Due to automation of experiment execution, embedded systems and digital signal processors the time decreases down to seconds. This time is substantially shorter then time constants of the systems and therefore this methodology becomes regenerated nowadays. Moreover, electrical signals are very well processed and represent great advantage at introduction of automation systems. From mentioned reasons, the dielectric spectroscopy is suitable method and in this study I have mainly concentrated on this method.

5 THEORETICAL PART

5.1 Modelling of systems

State of the process is characterized by quantities which are called state functions. Relations between these quantities represent a mathematical model of the process. A project must be created before new technological process is designed. This project is the basis for implementation of desired appliance. There is a need to obtain experience from a model because exact mechanism is unknown for many processes. There are two types of modelling – direct and indirect. The former consists in accomplishment of laboratory experiment so that it is possible to know the behaviour of the technological process without any calculations, mathematical, chemical or physical analysis.

The latter is focused on acquisition of mathematical models which allows description of examined process behaviour on the basis of elementary processes, for example, kinetics of chemical reactions, sorptive, physical-chemical or transport processes. This method comes out from high-abstracted data which is independent on used experimental device and its regime. In fact, the model can differ from real device. The stress is put on universality of data and independence between experimental and real device.

Nowadays, direct modelling is very difficult and complicated because it is economically and time demanding at contemporary ascending production. Nevertheless, indirect modelling can not represent guaranteed success. In some cases, it is not possible to dispense with experiment, because information about chemical reaction can be obtained only from laboratory measurement.
Purpose of modelling should be execution of experiments in that way in order that it could be carried out their exact evaluation and then elaborated the project for a specific device.

From methodical point of view, there are two ways how to get the mathematical model. Black box method is based on systematic monitoring of time behaviour of the studied

process on the change of their parameters. Model is obtained by experimental determination of relations between input and output for whole interval of conditions. Final result is described with suitable mathematical relation. Advantage of this method lies in monitoring only input and output quantities. There is no need to watch the processes inside the object. However, if the mechanism of process inside object is unknown, it is not possible to transfer the model into another device.

Another method, based on conception of mechanism of the process, is quantitative formulation of physical or chemical reactions, which are the substance of process. Mutual interactions of the considered reactions are also taken into account. The examined process is decomposed into the system of elementary processes, for which it is supposable to define what laws they are followed. In this model, quantities have specific significance. If the model is correctly arranged, there is no problem to predict behaviour of the system when some parameters are changed. Main advantage of this method is that any direct experiment can not be executed. In contrast to black box method, it puts great emphasis on amount of information, which is necessary to obtain. Decision, which method to choose, depends on circumstances.

From mathematical point of view, models can be further divided into deterministic and stochastic. Deterministic models consider all influences which has impact on the system. These models describe system state. On the other hand, stochastic models calculate with unknown and random influences and describe probability that a given state of the system occurs in a specific point.

Certain simplification is that examined object is taken as a system. System is a collection of elements, which are in mutual interactions. The element is a part of the system characterized by properties, which are typical only for that element. Interactions describe mutual activity of elements and they are common property of element collection.

During assembling of useful mathematical model, it is always necessary to decide to what extent the decomposition of the system is purposeful. Despite the system is possible to arbitrarily divide into elements, it is suitable to prefer such partitions, which are convenient for acquisition and processing of experimental data.
It is a question, if mathematical model describing element behaviour is the same for all devices, or it differs for individual devices. According to that question, elements can be divided into two groups:

1) Micro-kinetic elements
   Laws describing elements behaviour are the same for all objects. Mathematical model is common for all devices.
2) Macro-kinetic elements
   Laws describing elements behaviour are specific for individual object. Mathematical model is specific for individual device.

Mathematical model of micro-kinetic element can be used for other object whereas model of macro-kinetic element can not.

The purpose of modelling is a reliable description of system behaviour and for its success there is a need:

- to accomplish an experimental measurement on the object
- to complete an analysis of the measured experiments including division into micro-kinetic and macro-kinetic elements
- to create a mathematical models of the micro-kinetic elements
- to determine properties of the macro-kinetic elements for target object
- to make synthesis: mathematical models of the micro-kinetic elements are unified with models of the macro-kinetic elements of target object due to definition of interactions and balancing relations

Modelling is the first, usually the most complicated step during suggestion of a suitable

control strategy. Process is usually described with various quantities (temperature, pressure, flow, concentration etc.). These quantities are called state. Mathematical relations between them then characterize mathematical model of the process. Mathematical model is very often set of the ordinary or partial equations, but also set of linear and nonlinear equations.

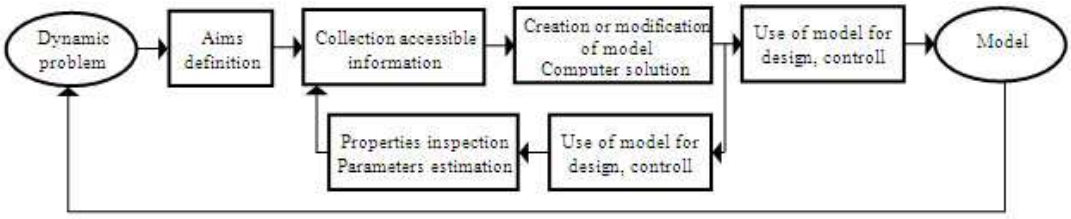


Figure 5.1 Algorithm of model formation

Procedure of model formation is graphically shown in Figure 5.1. These five steps can be described as follows:

a) Exact definition of the problem and aims of modelling
b) Collection of all accessible information including practical experiences with similar problems and then also formulation of the physical model
c) Mathematical definition of the problem and its solution by computer simulation
d) Appropriateness of nominated model is compared with known information and experiments on real process and then used for unknown parameters estimation. Steps a) up to d) are usually repeated in specific intervals.
e) Model can be used for regulator design and then for control purposes

Balances

Balances constitute application of laws about conservation for the system and they are very often used during mathematical model formation. Only extensive quantities can be balanced. In practice, law of conservation of matter and energy conversation law are often used. Balanced system is an area, which has defined boundaries with surrounding.

Surrounding is what rounds the system. Balancing interval is a time span during which the quantities are watched. Basic balancing equation is given by

$$\text{Input} + \text{Source} = \text{Output} + \text{Accumulation} \qquad (5.1.1)$$

To accomplish a control action, the dynamic model of the system must be known. It is given by vector state differential equations which represent system with lumped parameters. State differential equations are derived from balance relations. State equations comprise ordinary derivative of state quantities according time. In our case, they represent concentration of reaction components (concentration of collagen hydrolyzate and glutaraldehyde). To propose mentioned model, there is a need to deal with reaction kinetics, which is further presented in chapter “Kinetics of cross-linking reaction”.

Essential step during model creation is formulation of material and heat balances which include relevant kinetics equations for rate of chemical reactions, transmissions of heat and equations which represent changes of properties, for example. Sum of these reactions gives mentioned mathematical model, which can be from simple case up to model considerably complex with many equations. In this case, it is important to introduce some simplification, which decreases complexity. The aim of this simplification is to create such a model that is as simple as it can and it describes the most important properties of the process.

5.2 Kinetic methods of analysis

Most quantitative methods rely on the use of relative fast chemical reactions and equilibrium systems. Not all reactions will proceed rapidly. It is important to point out that as a reaction proceeds, it will have an initial kinetic region where concentrations change with time. Kinetic methods rely on using this region, see Figure 5.2.

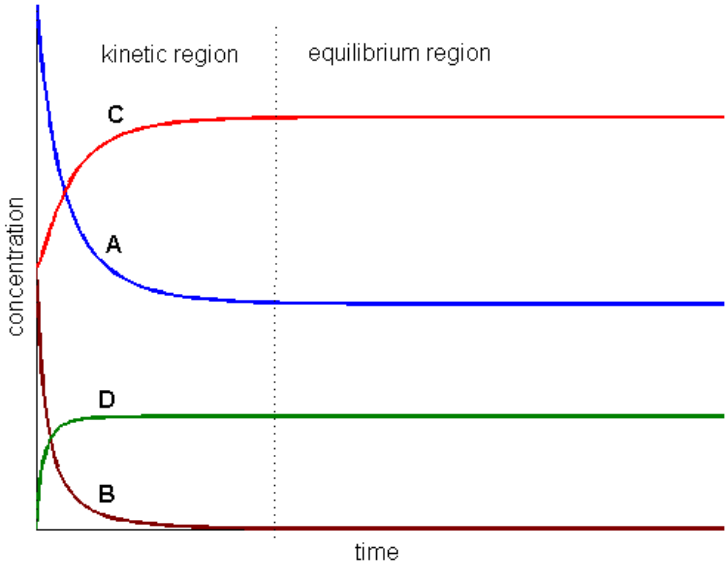


Figure 5.2 Kinetic and equilibrium regions

Order of reaction

Order of reaction represents the relationship between concentration and reaction rates. The general reaction can be expressed as follows:

$$aA + bB \xrightarrow{k} C \qquad (5.2.1)$$

Where

$$rate = kc_A{}^a c_B{}^b \qquad (5.2.2)$$

Reaction order is equal to *a* plus *b*. It should be noted that the order of reaction is not

necessarily related to the stoichiometry of the reactants.

First-order reaction

For this class of reaction the rate directly depends on the concentration of a single species. For the reaction:

$$A \xrightarrow{k} B \tag{5.2.3}$$

The rate is equal to kc_A. The mathematical expression of the rate is based on the decrease of component *A* with respect to time as follows:

$$-\frac{dc_A}{dt} = kt \tag{5.2.4}$$

Where *k* is the rate constant expressed in units of time^{-1} (s^{-1}, min^{-1},…)

The first-order reaction depends only on the value of *k* and c_A. Constant $-k$ can be determined as the slope of the line, see Figure 5.3.

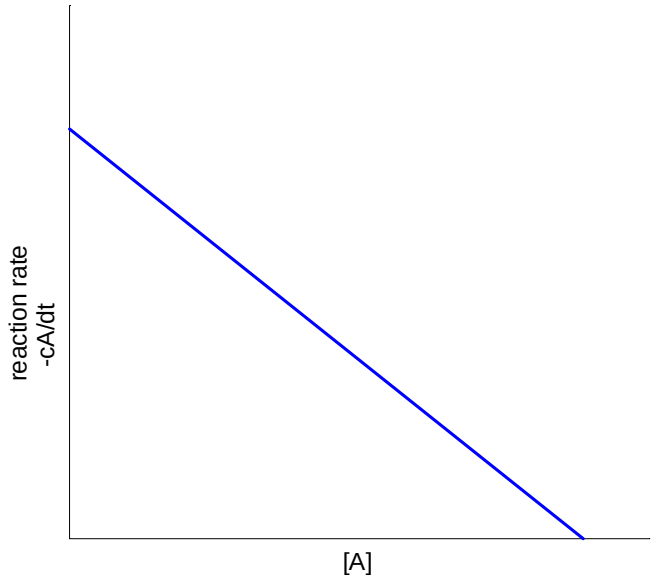


Figure 5.3 Reaction rate

Second-order reactions

For this type of reactions, the order typically depends on the concentration of two species:

$$aA + bB \xrightarrow{k} C \tag{5.2.5}$$

It should be noted that the order and the stoichiometry need not be the same. The differential form of the expression is given by:

$$-\frac{dc_A}{dt} = -\frac{dc_B}{dt} = kc_A c_B \tag{5.2.6}$$

The rate is equal to kc_Ac_B.

5.3 Kinetics of cross-linking reaction

Schema of reaction

Reaction of collagen hydrolyzate with glutaraldehyde can be schematically described as follows:

$$A + B \xrightarrow{k_1} C \tag{5.3.1}$$

$$C \xrightarrow{k_2} D \tag{5.3.2}$$

$$2A \xrightarrow{k_3} E \tag{5.3.3}$$

Protein *B* reacts with the cross-linking agent *A* resulting in the intermediate product *C*. Product *C* then reacts with itself due to its two reactive bonds and the final product *D* (raw material for production of biodegradable casings) arises. Simultaneously, glutaraldehyde reacts with itself (aldol synthesis) resulting in aldol resins *E*. These resins have typical coloration and this reaction is accompanied with colour change.

From the chemical point of view, the hydrolyzate is a product of skin collagen and contains seventeen amino acids. The most important and mostly represented of them are

glycine, proline and hydroxyproline. For this reason, for mathematical simulation the collagen hydrolyzate is considered a copolymer of the above-mentioned amino acids (Heidemann, 1993).

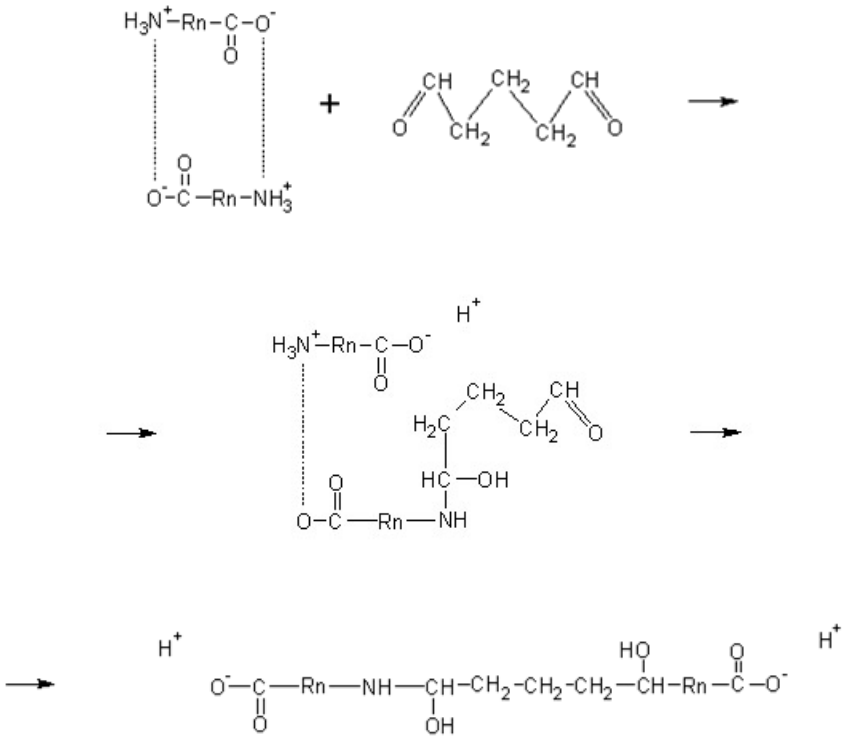
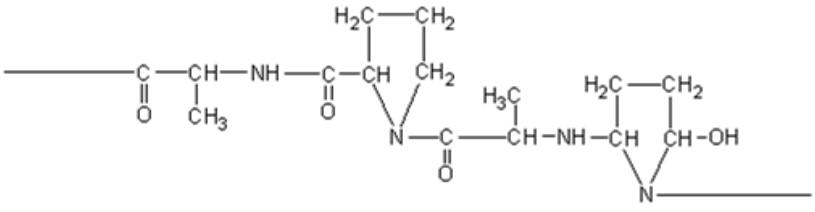


Figure 5.4 Intermediate product

where R is



and n=100.

Model of batch tank reactor

Let us consider batch reactor, where components *A* and *B* react to each other. As a result this reaction, components *C*, *D* and *E* originate.

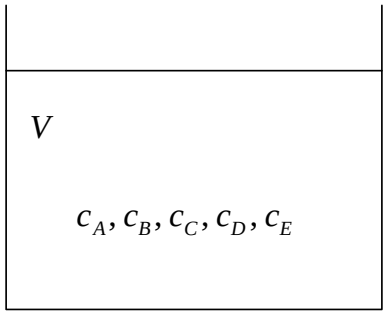


Figure 5.5 Batch tank reactor

Mentioned reaction system is possible to quantitatively express by mathematical notation:

$$-\frac{dc_A}{dt} = k_1 c_A c_B + k_3 \left(c_A\right)^2 \tag{5.3.4}$$

$$-\frac{dc_B}{dt} = k_1 c_A c_B \tag{5.3.5}$$

$$\frac{dc_C}{dt} = k_1 c_A c_B - k_2 c_C \tag{5.3.6}$$

$$\frac{dc_D}{dt} = k_2 c_C \tag{5.3.7}$$

$$\frac{dc_E}{dt} = k_3 \left(c_A\right)^2 \tag{5.3.8}$$

Simulative calculations based on mentioned equations were carried out for different values of kinetics constants k_1, k_2 and k_3, graphical results are depicted in following pictures:

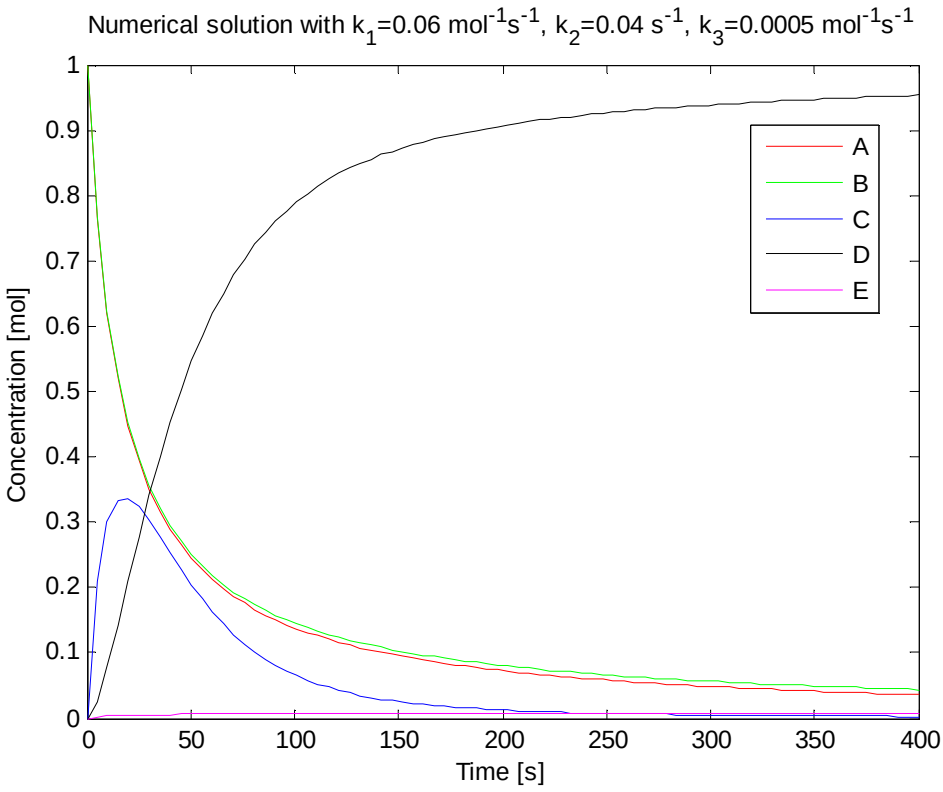


Figure 5.6 Simulative calculations with velocity constant $k_3=5.10^{-4}$ $mol^{-1}.s^{-1}$

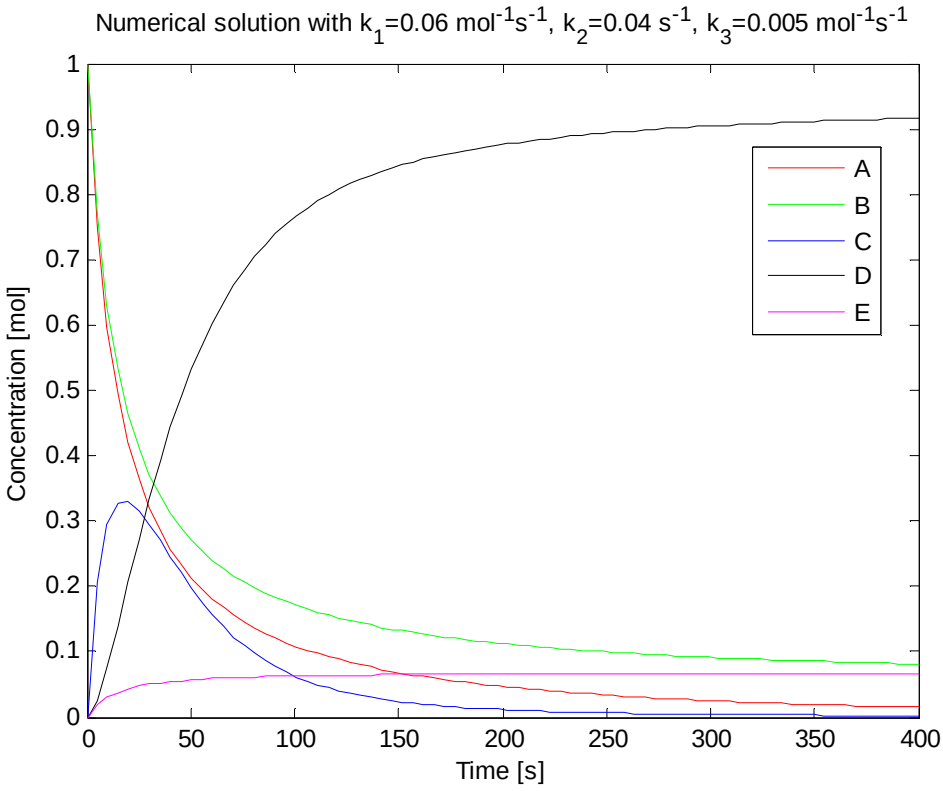


Figure 5.7 Simulative calculations with velocity constant $k_3=5.10^{-3}$ $mol^{-1}.s^{-1}$

Model of continuous stirred tank reactor

We assume that the reactants inside the tank are perfectly mixed and volume contraction of the reactants is negligible during the reaction.

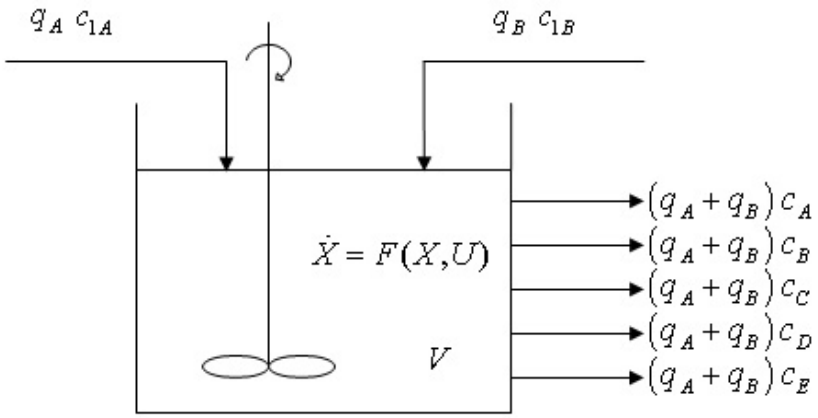


Figure 5.8 Continuous stirred tank reactor

The mathematical model of the system is then derived from the material balances of the reactor:

$$q_A\, c_{1A} = (q_A + q_B)\, c_A + V\, r_A + V \frac{dc_A}{dt} \tag{5.3.9}$$

$$q_B\, c_{1B} = (q_A + q_B)\ c_B + V\, r_B + V \frac{dc_B}{dt} \tag{5.3.10}$$

$$0 = (q_A + q_B)\ c_C + V\, r_C + V \frac{dc_C}{dt} \tag{5.3.11}$$

$$0 = (q_A + q_B)\ c_D + V\, r_D + V \frac{dc_D}{dt} \tag{5.3.12}$$

$$0 = (q_A + q_B)\ c_E + V\, r_E + V \frac{dc_E}{dt} \tag{5.3.13}$$

Let us mark each individual ordinary differential equation with letter f_x, where $x=1,2,..5$:

$$f_1: \quad \frac{dc_A}{dt} = \frac{q_A}{V} c_{A1} - \frac{(q_A + q_B)}{V} c_A - k_1 c_A c_B - k_3 \left(c_A\right)^2 \tag{5.3.14}$$

$$f_2: \quad \frac{dc_B}{dt} = \frac{q_B}{V} c_{B1} - \frac{(q_A + q_B)}{V} c_B - k_1 c_A c_B \tag{5.3.15}$$

$$f_3: \quad \frac{dc_C}{dt} = -\frac{(q_A + q_B)}{V} c_C + k_1 c_A c_B - k_2 c_C \tag{5.3.16}$$

$$f_4: \quad \frac{dc_D}{dt} = -\frac{(q_A + q_B)}{V} c_D + k_2 c_C \tag{5.3.17}$$

$$f_5: \quad \frac{dc_E}{dt} = -\frac{(q_A + q_B)}{V} c_E + k_3 \left(c_A\right)^2 \tag{5.3.18}$$

Now we have complete set of ordinary differential equations, which are nonlinear. For automatic control purposes, it is suitable to linearize them. One of possible methods is using of the Taylor's series. Let us consider linear, time invariant state system with four inputs and five state variables and five outputs.

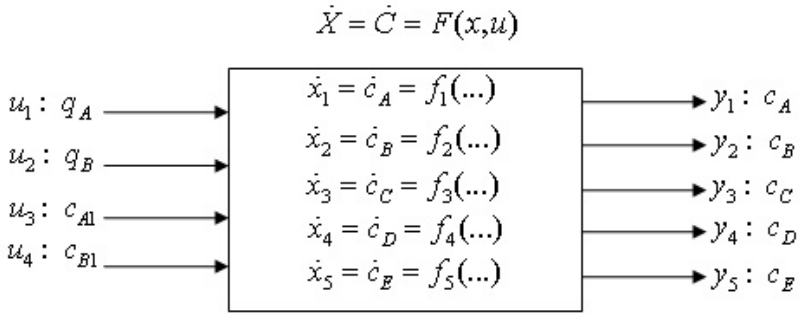


Figure 5.9 Scheme of the state model

Matrix notation of such linearized model is given as follows:

$$\Delta\dot{\mathbf{X}}_{5x1} = \mathbf{A}_{5x5}\Delta\mathbf{X}_{5x1} + \mathbf{B}_{5x4}\Delta\mathbf{U}_{4x1} \tag{5.3.19}$$

$$\Delta\mathbf{Y}_{5x1} = \Delta\mathbf{X}_{5x1} \tag{5.3.20}$$

where $\Delta\mathbf{X}$ is vector of state values, $\mathbf{A}$ is system matrix, $\mathbf{B}$ is excitation matrix, $\Delta\mathbf{U}$ is vector of input parameters, $\Delta\mathbf{Y}$ is vector of output parameters.

$$\mathbf{A} = \begin{pmatrix} a_{11} & a_{12} & a_{13} & a_{14} & a_{15} \\ a_{21} & a_{22} & a_{23} & a_{24} & a_{25} \\ a_{31} & a_{32} & a_{33} & a_{34} & a_{35} \\ a_{41} & a_{42} & a_{43} & a_{44} & a_{45} \\ a_{51} & a_{52} & a_{53} & a_{54} & a_{55} \end{pmatrix} \tag{5.3.21}$$

$$\mathbf{B} = \begin{pmatrix} b_{11} & b_{12} & b_{13} & b_{14} \\ b_{21} & b_{22} & b_{23} & b_{24} \\ b_{31} & b_{32} & b_{33} & b_{34} \\ b_{41} & b_{42} & b_{43} & b_{44} \\ b_{51} & b_{52} & b_{53} & b_{54} \end{pmatrix} \tag{5.3.22}$$

$$\Delta\mathbf{X} = \begin{pmatrix} \Delta c_A \\ \Delta c_B \\ \Delta c_C \\ \Delta c_D \\ \Delta c_E \end{pmatrix} \tag{5.3.23}$$

$$\Delta\mathbf{U} = \begin{pmatrix} \Delta q_A \\ \Delta q_B \\ \Delta c_{A1} \\ \Delta c_{B1} \end{pmatrix} \tag{5.3.24}$$

Individual elements of matrix **A** are derived from the Tailor's series with the neglected members of higher order as follows:

$$a_{11} = \frac{\partial f_1^{\,0}}{\partial c_A} = -\frac{(q_A + q_B)}{V} - k_1 c_B^0 - 2k_3 c_A^0 \tag{5.3.25}$$

$$a_{12} = \frac{\partial f_1^{\,0}}{\partial c_B} = -k_1 c_A^0 \tag{5.3.26}$$

$$a_{13} = \frac{\partial f_1^{\,0}}{\partial c_C} = 0 \tag{5.3.27}$$

$$a_{14} = \frac{\partial f_1^{\,0}}{\partial c_D} = 0 \tag{5.3.28}$$

$$a_{15} = \frac{\partial f_1^{\,0}}{\partial c_E} = 0 \tag{5.3.29}$$

$$a_{21} = \frac{\partial f_2^{\,0}}{\partial c_A} = -k_1 c_B^0 \tag{5.3.30}$$

$$a_{22} = \frac{\partial f_2^{\,0}}{\partial c_B} = -\frac{(q_A + q_B)}{V} - k_1 c_A^0 \tag{5.3.31}$$

$$a_{23} = \frac{\partial f_2^{\,0}}{\partial c_C} = 0 \tag{5.3.32}$$

$$a_{24} = \frac{\partial f_2^{\,0}}{\partial c_D} = 0 \tag{5.3.33}$$

$$a_{25} = \frac{\partial f_1^{\,0}}{\partial c_E} = 0 \tag{5.3.34}$$

$$a_{31} = \frac{\partial f_3^0}{\partial c_A} = k_1 c_B^0 \tag{5.3.35}$$

$$a_{32} = \frac{\partial f_3^0}{\partial c_B} = k_1 c_A^0 \tag{5.3.36}$$

$$a_{33} = \frac{\partial f_3^0}{\partial c_C} = -\frac{(q_A + q_B)}{V} - k_2 \tag{5.3.37}$$

$$a_{34} = \frac{\partial f_3^0}{\partial c_D} = 0 \tag{5.3.38}$$

$$a_{35} = \frac{\partial f_3^0}{\partial c_E} = 0 \tag{5.3.39}$$

$$a_{41} = \frac{\partial f_4^0}{\partial c_A} = 0 \tag{5.3.40}$$

$$a_{42} = \frac{\partial f_4^0}{\partial c_B} = 0 \tag{5.3.41}$$

$$a_{43} = \frac{\partial f_4^0}{\partial c_C} = k_2 \tag{5.3.42}$$

$$a_{44} = \frac{\partial f_4^0}{\partial c_D} = -\frac{(q_A + q_B)}{V} \tag{5.3.43}$$

$$a_{45} = \frac{\partial f_4^0}{\partial c_E} = 0 \tag{5.3.44}$$

$$a_{51} = \frac{\partial f_5^0}{\partial c_A} = 2k_3 c_A \tag{5.3.45}$$

$$a_{52} = \frac{\partial f_5^0}{\partial c_B} = 0 \tag{5.3.46}$$

$$a_{53} = \frac{\partial f_5^0}{\partial c_C} = 0 \tag{5.3.47}$$

$$a_{54} = \frac{\partial f_5^0}{\partial c_D} = 0 \tag{5.3.48}$$

$$a_{55} = \frac{\partial f_5^0}{\partial c_E} = -\frac{(q_A + q_B)}{V} \tag{5.3.49}$$

Individual elements of matrix **B** are defined as follows:

$$b_{11} = \frac{\partial f_1^0}{\partial q_A} = \frac{c_{A1}^0}{V} - \frac{c_A}{V} \tag{5.3.50}$$

$$b_{12} = \frac{\partial f_1^0}{\partial q_B} = -\frac{c_A}{V} \tag{5.3.51}$$

$$b_{13} = \frac{\partial f_1^0}{\partial c_{A1}} = \frac{q_A^0}{V} \tag{5.3.52}$$

$$b_{14} = \frac{\partial f_1^0}{\partial c_{B1}} = 0 \tag{5.3.53}$$

$$b_{21} = \frac{\partial f_2^0}{\partial \dot{V}_A} = -\frac{c_B^0}{V} \tag{5.3.54}$$

$$b_{22} = \frac{\partial f_2^0}{\partial \dot{V}_B} = \frac{c_{B1}^0}{V} - \frac{c_B^0}{V} \tag{5.3.55}$$

$$b_{23} = \frac{\partial f_2^0}{\partial c_{A1}} = 0 \tag{5.3.56}$$

$$b_{24} = \frac{\partial f_2^0}{\partial c_{B1}} = \frac{q_B^0}{V} \tag{5.3.57}$$

$$b_{31} = \frac{\partial f_3^0}{\partial q_A} = -\frac{c_C^0}{V} \tag{5.3.58}$$

$$b_{32} = \frac{\partial f_3^0}{\partial q_B} = -\frac{c_C^0}{V} \tag{5.3.59}$$

$$b_{33} = \frac{\partial f_3^0}{\partial c_{A1}} = 0 \tag{5.3.60}$$

$$b_{34} = \frac{\partial f_3^0}{\partial c_{B1}} = 0 \tag{5.3.61}$$

$$b_{41} = \frac{\partial f_4^0}{\partial q_A} = -\frac{c_D^0}{V} \tag{5.3.62}$$

$$b_{42} = \frac{\partial f_4^0}{\partial q_B} = -\frac{c_D^0}{V} \tag{5.3.63}$$

$$b_{43} = \frac{\partial f_4^0}{\partial c_{A1}} = 0 \tag{5.3.64}$$

$$b_{44} = \frac{\partial f_4^0}{\partial c_{B1}} = 0 \tag{5.3.65}$$

$$b_{51} = \frac{\partial f_5^0}{\partial q_A} = -\frac{c_E^0}{V} \tag{5.3.66}$$

$$b_{52} = \frac{\partial f_5^0}{\partial q_B} = -\frac{c_E^0}{V} \tag{5.3.67}$$

$$b_{53} = \frac{\partial f_5^0}{\partial c_{A1}} = 0 \tag{5.3.68}$$

$$b_{54} = \frac{\partial f_5^0}{\partial c_{B1}} = 0 \tag{5.3.69}$$

Now we have linearized set of differential equations in matrix notation:

$$\begin{pmatrix} \dot{c}_A \\ \dot{c}_B \\ \dot{c}_C \\ \dot{c}_D \\ \dot{c}_E \end{pmatrix} = \begin{pmatrix} a_{11} & \cdots & a_{15} \\ \vdots & \ddots & \vdots \\ a_{51} & \cdots & a_{55} \end{pmatrix} \begin{pmatrix} \Delta c_A \\ \Delta c_B \\ \Delta c_C \\ \Delta c_D \\ \Delta c_E \end{pmatrix} + \begin{pmatrix} b_{11} & \cdots & b_{14} \\ \vdots & \ddots & \vdots \\ b_{51} & \cdots & b_{54} \end{pmatrix} \begin{pmatrix} \Delta q_A \\ \Delta q_B \\ \Delta c_{A1} \\ \Delta c_{B1} \end{pmatrix} \tag{5.3.70}$$

in expanded notation:

$$\begin{aligned} \frac{dc_A}{dt} = {} & a_{11}\Delta c_A + a_{12}\Delta c_B + a_{13}\Delta c_C + a_{14}\Delta c_D + a_{15}\Delta c_E + \\ & + b_{11}\Delta q_A + b_{12}\Delta q_B + b_{13}\Delta c_{A1} + b_{14}\Delta c_{B1} \end{aligned} \tag{5.3.71}$$

$$\begin{aligned} \frac{dc_B}{dt} = {} & a_{21}\Delta c_A + a_{22}\Delta c_B + a_{23}\Delta c_C + a_{24}\Delta c_D + a_{25}\Delta c_E + \\ & + b_{21}\Delta q_A + b_{22}\Delta q_B + b_{23}\Delta c_{A1} + b_{24}\Delta c_{B1} \end{aligned} \tag{5.3.72}$$

$$\frac{dc_C}{dt} = a_{31}\Delta c_A + a_{32}\Delta c_B + a_{33}\Delta c_C + a_{34}\Delta c_D + a_{35}\Delta c_E + \\ + b_{31}\Delta q_A + b_{32}\Delta q_B + b_{33}\Delta c_{A1} + b_{34}\Delta c_{B1} \tag{5.3.73}$$

$$\frac{dc_D}{dt} = a_{41}\Delta c_A + a_{42}\Delta c_B + a_{43}\Delta c_C + a_{44}\Delta c_D + a_{45}\Delta c_E + \\ + b_{41}\Delta q_A + b_{42}\Delta q_B + b_{43}\Delta c_{A1} + b_{44}\Delta c_{B1} \tag{5.3.74}$$

$$\frac{dc_E}{dt} = a_{51}\Delta c_A + a_{52}\Delta c_B + a_{53}\Delta c_C + a_{54}\Delta c_D + a_{55}\Delta c_E + \\ + b_{51}\Delta q_A + b_{52}\Delta q_B + b_{53}\Delta c_{A1} + b_{54}\Delta c_{B1} \tag{5.3.75}$$

For control purposes there is a need to transform mentioned linearized differential equation system into dimensionless shape. Let us assume:

$$\Delta c_A = \Delta c_A^* \; c_A^0 \tag{5.3.76}$$

$$\Delta c_B = \Delta c_B^* \; c_B^0 \tag{5.3.77}$$

$$\Delta c_C = \Delta c_C^* \; c_C^0 \tag{5.3.78}$$

$$\Delta c_D = \Delta c_D^* \; c_D^0 \tag{5.3.79}$$

$$\Delta c_E = \Delta c_E^* \; c_E^0 \tag{5.3.80}$$

$$\Delta q_A = \Delta q_A^* \; q_A^0 \tag{5.3.81}$$

$$\Delta q_B = \Delta q_B^* \; q_B^0 \tag{5.3.82}$$

$$\Delta c_{A1} = \Delta c_{A1}^* \; c_{A1}^0 \tag{5.3.83}$$

$$\Delta c_{B1} = \Delta c_{B1}^* \; c_{B1}^0 \tag{5.3.84}$$

Then we can have equations as follows:

$$\frac{d\left(c_A^* c_A^0\right)}{dt} = \left(-\frac{(q_A+q_B)}{V} - k_1 c_B^0 - 2k_3 c_A^0\right)\Delta c_A^* c_A^0 - k_1 c_A^0 \Delta c_B^* c_B^0 + \left(\frac{c_{A1}^0}{V} - \frac{c_A^0}{V}\right)\Delta q_A^*\, q_A^0 + \left(-\frac{c_A^0}{V}\right)\Delta q_B^*\, q_B^0 + \frac{q_A^0}{V}\Delta c_{A1}^* c_{A1}^0 \tag{5.3.85}$$

$$\frac{d\left(c_B^* c_B^0\right)}{dt} = -k_1 c_B^0 \Delta c_A^*\, c_A^0 + \left(-\frac{(q_A+q_B)}{V} - k_1 c_A^0\right)\Delta c_B^*\, c_B^0 + -\frac{c_B^0}{V}\Delta q_A^*\, q_A^0 + \left(\frac{c_{B1}^0}{V} - \frac{c_B^0}{V}\right)\Delta q_B^*\, q_B^0 + \frac{q_B^0}{V}\Delta c_{B1}^*\, c_{B1}^0 \tag{5.3.86}$$

$$\frac{d\left(c_C^* c_C^0\right)}{dt} = k_1 c_B^0 \Delta c_A^*\, c_A^0 + k_1 c_A^0 \Delta c_B^*\, c_B^0 + -\left(\frac{(q_A+q_B)}{V} + k_2\right)\Delta c_C^*\, c_C^0 - \frac{c_C^0}{V}\Delta q_A^*\, q_A^0 + -\frac{c_C^0}{V}\Delta q_B^*\, q_B^0 \tag{5.3.87}$$

$$\frac{d\left(c_D^* c_D^0\right)}{dt} = k_2 \Delta c_C^* c_C^0 - \frac{(q_A+q_B)}{V}\Delta c_D^* c_D^0 - \frac{c_D^0}{V}\Delta q_A^*\, q_A^0 + -\frac{c_D^0}{V}\Delta q_B^*\, q_B^0 \tag{5.3.88}$$

$$\frac{d\left(c_E^* c_E^0\right)}{dt} = 2k_3 c_A^0 \Delta c_A^* c_A^0 - \frac{(q_A + q_B)}{V} \Delta c_E^* c_E^0 - \frac{c_E^0}{V} \Delta q_A^* \, q_A^0 + \\ - \frac{c_E^0}{V} \Delta q_B^* \, q_B^0 \tag{5.3.89}$$

After mathematical modification we have the set of linearized differential equations in dimensionless shape:

$$\frac{dc_A^*}{dt^*} = \left(-\frac{q_A^0}{q_B^0} - 1 - \frac{k_1 V c_B^0}{q_B^0} - 2\frac{k_3 V c_A^0}{q_B^0} \right) \Delta c_A^* - \frac{k_1 V c_B^0}{q_B^0} \Delta c_B^* + \\ \left(\frac{q_A^0 c_{A1}^0}{q_B^0 c_A^0} - \frac{q_A^0}{q_B^0} \right) \Delta q_A^* - \Delta q_B^* + \frac{q_A^0 c_{A1}^0}{q_B^0 c_A^0} \Delta c_{A1}^* \tag{5.3.90}$$

$$\frac{dc_B^*}{dt^*} = -\frac{k_1 V}{q_B^0} \Delta c_A^* + \left(-\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} - \frac{k_1 V}{q_B^0} \right) \Delta c_B^* - \frac{q_A^0}{c_A^0 q_B^0} \Delta q_A^* + \\ + \left(\frac{c_{B1}^0}{c_A^0 c_B^0} - \frac{1}{c_A^0} \right) \Delta q_B^* + \frac{c_{B1}^0}{c_A^0 c_B^0} \Delta c_{B1}^* \tag{5.3.91}$$

$$\frac{dc_C^*}{dt^*} = \frac{k_1 V c_B^0}{c_C^0 q_B^0} \Delta c_A^* + \frac{k_1 V c_B^0}{c_C^0 q_B^0} \Delta c_B^* + \left(-\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} - \frac{k_2 V}{c_A^0 q_B^0} \right) \Delta c_C^* + \\ - \frac{q_A^0}{c_A^0 q_B^0} \Delta q_A^* - \frac{1}{c_A^0} \Delta q_B^* \tag{5.3.92}$$

$$\frac{dc_D^*}{dt^*} = \frac{k_2 V c_C^0}{c_A^0 c_D^0 q_B^0}\Delta c_C^* + \left(-\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} \right)\Delta c_D^* + \\ -\frac{q_A^0}{c_A^0 q_B^0}\Delta q_A^* - \frac{1}{c_A^0}\Delta q_B^* \tag{5.3.93}$$

$$\frac{dc_E^*}{dt^*} = 2\frac{k_3 V c_A^0}{c_E^0 q_B^0}\Delta c_A^* + \left(-\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} \right)\Delta c_E^* + \\ -\frac{q_A^0}{c_A^0 q_B^0}\Delta q_A^* - \frac{1}{c_A^0}\Delta q_B^* \tag{5.3.94}$$

Individual elements of dimensionless matrix **A** are following:

$$a_{11}^* = \left(-\frac{q_A^0}{q_B^0} - 1 - \frac{k_1 V c_B^0}{q_B^0} - 2\frac{k_3 V c_A^0}{q_B^0} \right) \tag{5.3.95}$$

$$a_{12}^* = -\frac{k_1 V c_B^0}{q_B^0} \tag{5.3.96}$$

$$a_{13}^* = 0 \tag{5.3.97}$$

$$a_{14}^* = 0 \tag{5.3.98}$$

$$a_{15}^* = 0 \tag{5.3.99}$$

$$a_{21}^* = -\frac{k_1 V}{q_B^0} \tag{5.3.100}$$

$$a_{22}^* = -\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} - \frac{k_1 V}{q_B^0} \tag{5.3.101}$$

$$a_{23}^* = 0 \tag{5.3.102}$$

$$a_{24}^{*} = 0 \tag{5.3.103}$$

$$a_{25}^{*} = 0 \tag{5.3.104}$$

$$a_{31}^{*} = \frac{k_1 V c_B^0}{c_C^0 q_B^0} \tag{5.3.105}$$

$$a_{32}^{*} = \frac{k_1 V c_B^0}{c_C^0 q_B^0} \tag{5.3.106}$$

$$a_{33}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} - \frac{k_2 V}{c_A^0 q_B^0} \tag{5.3.107}$$

$$a_{34}^{*} = 0 \tag{5.3.108}$$

$$a_{35}^{*} = 0 \tag{5.3.109}$$

$$a_{41}^{*} = 0 \tag{5.3.110}$$

$$a_{42}^{*} = 0 \tag{5.3.111}$$

$$a_{43}^{*} = \frac{k_2 V c_C^0}{c_A^0 c_D^0 q_B^0} \tag{5.3.112}$$

$$a_{44}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} \tag{5.3.113}$$

$$a_{45}^{*} = 0 \tag{5.3.114}$$

$$a_{51}^{*} = 2\frac{k_3 V c_A^0}{c_E^0 q_B^0} \tag{5.3.115}$$

$$a_{52}^{*} = 0 \tag{5.3.116}$$

$$a_{53}^{*} = 0 \tag{5.3.117}$$

$$a_{54}^{*} = 0 \tag{5.3.118}$$

$$a_{55}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} - \frac{1}{c_A^0} \tag{5.3.119}$$

Individual elements of dimensionless matrix **B** are following:

$$b_{11}^{*} = \left(\frac{q_A^0 c_{A1}^0}{q_B^0 c_A^0} - \frac{q_A^0}{q_B^0} \right) \tag{5.3.120}$$

$$b_{12}^{*} = -1 \tag{5.3.121}$$

$$b_{13}^{*} = \frac{q_A^0 c_{A1}^0}{q_B^0 c_A^0} \tag{5.3.122}$$

$$b_{14}^{*} = 0 \tag{5.3.123}$$

$$b_{21}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} \tag{5.3.124}$$

$$b_{22}^{*} = \frac{c_{B1}^0}{c_A^0 c_B^0} - \frac{1}{c_A^0} \tag{5.3.125}$$

$$b_{23}^{*} = 0 \tag{5.3.126}$$

$$b_{24}^{*} = \frac{c_{B1}^0}{c_A^0 c_B^0} \tag{5.3.127}$$

$$b_{31}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} \tag{5.3.128}$$

$$b_{32}^{*} = -\frac{1}{c_A^0} \tag{5.3.129}$$

$$b_{33}^{*} = 0 \tag{5.3.130}$$

$$b_{34}^{*} = 0 \tag{5.3.131}$$

$$b_{41}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} \tag{5.3.132}$$

$$b_{42}^{*} = -\frac{1}{c_A^0} \tag{5.3.133}$$

$$b_{43}^{*} = 0 \tag{5.3.134}$$

$$b_{44}^{*} = 0 \tag{5.3.135}$$

$$b_{51}^{*} = -\frac{q_A^0}{c_A^0 q_B^0} \tag{5.3.136}$$

$$b_{52}^{*} = -\frac{1}{c_A^0} \tag{5.3.137}$$

$$b_{53}^{*} = 0 \tag{5.3.138}$$

$$b_{54}^{*} = 0 \tag{5.3.139}$$

For the determination of transfer matrix we use Laplace transformation in the following procedure. Linearized model of the reaction system in matrix form is given:

$$\Delta\dot{\mathbf{X}} = \mathbf{A}\Delta\mathbf{X} + \mathbf{B}\Delta\mathbf{U} \tag{5.3.140}$$

$$\Delta\mathbf{Y} = \Delta\mathbf{X} \tag{5.3.141}$$

Applying the Laplace transformation (index *L*) we can obtain:

$$s\Delta\mathbf{X}_L = \mathbf{A}\Delta\mathbf{X}_L + \mathbf{B}\Delta\mathbf{U}_L \tag{5.3.142}$$

$$\Delta\mathbf{Y}_L = \Delta\mathbf{X}_L \tag{5.3.143}$$

Solving equations (5.3.142) and (5.3.143) results in transfer matrix *G(s):*

$$\mathbf{G}(s) = \frac{\Delta\mathbf{Y}_L}{\Delta\mathbf{U}_L} = (s\mathbf{I} - \mathbf{A})^{-1}\mathbf{B} \tag{5.3.144}$$

For calculation members of invertible matrix $(s\boldsymbol{I}\text{-}\boldsymbol{A})^{-1}$ computer algorithm based on Gauss-Jordan elimination was used (Anderson, et al., 1999).

Individual elements of transfer function matrix **G** are following:

$$g_{11}(s) = \frac{s(-b_{11}) - a_{12}b_{21} + b_{11}a_{22}}{-s^2 + s(a_{11} + a_{22}) + a_{21}a_{12} - a_{11}a_{22}} \tag{5.3.145}$$

$$g_{12}(s) = \frac{s - a_{22} - a_{12}b_{22}}{-s^2 + s(a_{11} + a_{22}) + a_{21}a_{12} - a_{11}a_{22}} \tag{5.3.146}$$

$$g_{13}(s) = \frac{s(-b_{13}) + b_{13}a_{22}}{-s^2 + s(a_{11} + a_{22}) + a_{21}a_{12} - a_{11}a_{22}} \tag{5.3.147}$$

$$g_{14}(s)=\frac{-b_{24}a_{12}}{-s^2+s(a_{11}+a_{22})+a_{21}a_{12}-a_{11}a_{22}} \tag{5.3.148}$$

$$g_{21}(s)=\frac{s(-b_{21})-a_{21}b_{11}+b_{21}a_{11}}{-s^2+s(a_{11}+a_{22})+a_{21}a_{12}-a_{11}a_{22}} \tag{5.3.149}$$

$$g_{22}(s)=\frac{s(-b_{22})+a_{21}+b_{22}a_{11}}{-s^2+s(a_{11}+a_{22})+a_{21}a_{12}-a_{11}a_{22}} \tag{5.3.150}$$

$$g_{23}(s)=\frac{b_{13}a_{21}}{s^2-s(a_{11}+a_{22})-a_{21}a_{12}+a_{11}a_{22}} \tag{5.3.151}$$

$$g_{24}(s)=\frac{s(-b_{24})+b_{24}a_{11}}{-s^2+s(a_{11}+a_{22})+a_{21}a_{12}-a_{11}a_{22}} \tag{5.3.152}$$

$$g_{31}(s)=\frac{s^2b_{31}+s(b_{11}a_{31}-b_{31}a_{11}+b_{21}a_{32}-b_{31}a_{22})+r_1}{s^3+s^2(-a_{33}-a_{11}-a_{22})+sr_2+r_3} \tag{5.3.153}$$

where

$$r_1=b_{11}a_{21}a_{32}-b_{31}a_{11}a_{22}+b_{21}a_{11}a_{32}+b_{11}a_{31}a_{22}-b_{21}a_{12}a_{31}+b_{31}a_{21}a_{12}$$

$$r_2=a_{11}a_{22}-a_{21}a_{12}+a_{22}a_{33}+a_{11}a_{33}$$

$$r_3=-a_{11}a_{22}a_{33}+a_{21}a_{12}a_{33}$$

$$g_{32}(s)=\frac{s^2b_{23}+s(b_{22}a_{32}+b_{32}a_{11}+b_{32}a_{22}+a_{31})-r_1}{s^3+s^2(-a_{11}-a_{22}-a_{33})+sr_2+r_3} \tag{5.3.154}$$

where

$r_1=a_{21}a_{32}+b_{22}a_{11}a_{32}-a_{31}a_{22}+b_{32}a_{21}a_{12}-b_{32}a_{11}a_{22}-b_{22}a_{12}a_{31}$

$r_2=a_{11}a_{22}+a_{11}a_{33}-a_{21}a_{12}+a_{22}a_{33}$

$r_3=a_{21}a_{12}a_{33}-a_{11}a_{22}a_{33}$

$$g_{33}(s)=\frac{s(a_{31}b_{13})+b_{13}a_{21}a_{32}-b_{13}a_{31}a_{22}}{s^3+s^2(-a_{33}-a_{11}-a_{22})-sr_1+r_2} \tag{5.3.155}$$

where

$r_1=a_{21}a_{12}+a_{11}a_{22}+a_{22}a_{33}+a_{33}a_{11}$

$r_2=a_{21}a_{12}a_{33}-a_{11}a_{22}a_{33}$

$$g_{34}(s)=\frac{s(b_{24}a_{32})+b_{24}a_{12}a_{31}-b_{24}a_{11}a_{32}}{s^3+s^2(-a_{33}-a_{11}-a_{22})-sr_1+r_2} \tag{5.3.156}$$

where

$r_1=a_{21}a_{12}+a_{11}a_{22}+a_{22}a_{33}+a_{33}a_{11}$

$r_2=a_{21}a_{12}a_{33}-a_{11}a_{22}a_{33}$

$$g_{41}(s)=\frac{s^3b_{41}+s^2r_1+sr_2+r_3}{s^4+s^3r_4+s^2r_5-sr_6+r_7} \tag{5.3.157}$$

where

$$r_1=-b_{41}a_{11}-b_{41}a_{22}-b_{41}a_{33}+a_{43}b_{31}$$

$$r_2=b_{41}a_{22}a_{33}-a_{43}b_{31}a_{11}+b_{41}a_{11}a_{22}-b_{41}a_{21}a_{12}+b_{41}a_{11}a_{33}+a_{43}b_{11}a_{31}-a_{43}b_{31}a_{22}+a_{43}b_{21}a_{32}$$

$$r_3=a_{43}b_{21}a_{12}a_{31}-a_{43}b_{21}a_{11}a_{32}+a_{43}b_{31}a_{11}a_{22}+a_{43}b_{11}a_{21}a_{32}-b_{41}a_{11}a_{22}a_{33}+$$
$$-a_{43}b_{31}a_{12}a_{21}-a_{43}b_{11}a_{31}a_{22}+b_{41}a_{12}a_{21}a_{33}$$

$$r_4=-a_{11}-a_{33}-a_{22}-a_{44}$$

$$r_5=a_{11}a_{33}+a_{11}a_{44}+a_{33}a_{44}+a_{11}a_{22}+a_{22}a_{44}+a_{22}a_{33}-a_{21}a_{12}$$

$$r_6=a_{11}a_{22}a_{33}-a_{22}a_{44}a_{33}+a_{21}a_{12}a_{33}-a_{11}a_{44}a_{33}+a_{21}a_{12}a_{44}-a_{11}a_{22}a_{44}$$

$$r_7=a_{11}a_{22}a_{44}a_{33}-a_{21}a_{12}a_{33}a_{44}$$

$$g_{42}(s)=\frac{s^3b_{42}+s^2(-b_{42}a_{33}-b_{42}a_{11}-b_{42}a_{22}+a_{43}b_{32})+sr_1+r_2}{s^4+s^3(-a_{44}-a_{11}-a_{22}-a_{33})+s^2r_3+sr_4+r_5} \tag{5.3.158}$$

where

$$r_1=b_{42}a_{11}a_{33}+b_{42}a_{22}a_{33}-a_{43}b_{32}a_{11}+b_{42}a_{11}a_{22}-a_{43}b_{32}a_{22}-a_{43}a_{31}-b_{42}a_{21}a_{12}+a_{43}b_{22}a_{32}$$

$$r_2=-a_{43}b_{22}a_{11}a_{32}+a_{43}b_{32}a_{11}a_{22}-b_{42}a_{11}a_{22}a_{33}-a_{43}b_{32}a_{12}a_{21}+a_{43}a_{31}a_{22}-a_{21}a_{32}a_{43}+$$
$$+a_{43}b_{22}a_{12}a_{31}+b_{42}a_{12}a_{21}a_{33}$$

$$r_3=a_{11}a_{22}+a_{22}a_{33}+a_{11}a_{33}-a_{21}a_{12}+a_{33}a_{44}+a_{11}a_{44}+a_{22}a_{44}$$

$$r_4=-a_{11}a_{22}a_{44}-a_{11}a_{44}a_{33}+a_{21}a_{12}a_{44}-a_{11}a_{22}a_{33}-a_{22}a_{44}a_{33}+a_{21}a_{12}a_{33}$$

$$r_5=a_{11}a_{22}a_{44}a_{33}-a_{21}a_{12}a_{33}a_{44}$$

$$g_{43}(s)=\frac{s(a_{43}b_{13}a_{31})-a_{43}b_{13}a_{31}a_{22}-a_{43}b_{13}a_{21}a_{32}}{s^4+s^3r_1+s^2r_2+sr_3+r_4} \tag{5.3.159}$$

where

$$r_1=-a_{11}-a_{44}-a_{33}-a_{22}-a_{22}a_{12}$$

$$r_2=a_{11}a_{22}+a_{11}a_{33}+a_{33}a_{44}+a_{22}a_{33}+a_{11}a_{44}+a_{22}a_{44}$$

$$r_3=-a_{22}a_{44}a_{33}-a_{11}a_{44}a_{33}-a_{11}a_{22}a_{44}+a_{21}a_{12}a_{33}-a_{11}a_{22}a_{33}+a_{21}a_{12}a_{44}$$

$$r_4=a_{11}a_{22}a_{44}a_{33}-a_{21}a_{12}a_{33}a_{44}$$

$$g_{44}(s)=\frac{s(a_{43}b_{24}a_{32})-a_{43}b_{24}a_{12}a_{31}-a_{43}b_{24}a_{11}a_{32}}{s^4+s^3r_1+s^2r_2+sr_3+r_4} \tag{5.3.160}$$

where

$$r_1=-a_{44}-a_{11}-a_{22}-a_{33}$$

$$r_2=a_{11}a_{22}+a_{33}a_{44}+a_{11}a_{33}+a_{22}a_{33}+a_{11}a_{44}-a_{21}a_{12}+a_{22}a_{44}$$

$$r_3=-a_{11}a_{22}a_{44}-a_{11}a_{22}a_{33}-a_{11}a_{44}a_{33}-a_{22}a_{44}a_{33}+a_{21}a_{12}a_{44}+a_{21}a_{12}a_{33}$$

$$r_4=a_{11}a_{22}a_{44}a_{33}-a_{21}a_{12}a_{33}a_{44}$$

$$g_{51}(s)=\frac{s^2b_{51}+s(-b_{51}a_{22}-b_{51}a_{11}+a_{51}b_{11})+r_1}{s^3+s^2(-a_{11}-a_{22}-a_{55})+sr_2+r_3} \tag{5.3.161}$$

where

$$r_1=-b_{51}a_{21}a_{12}+b_{51}a_{11}a_{22}+a_{12}a_{51}b_{21}-a_{51}b_{11}a_{22}$$

$$r_2=a_{22}a_{55}-a_{21}a_{12}+a_{11}a_{22}+a_{11}a_{55}$$

$$r_3 = -a_{11}a_{22}a_{55} + a_{21}a_{12}a_{55}$$

$$g_{52}(s) = \frac{s^2 b_{52} + s(-b_{52}a_{11} - a_{51} - b_{52}a_{22}) + r_1}{s^3 + s^2(-a_{11} - a_{55} - a_{22}) + sr_2 + r_3} \tag{5.3.162}$$

where

$$r_1 = -b_{52}a_{21}a_{12} + a_{51}a_{22} + b_{52}a_{11}a_{22} + a_{12}a_{51}b_{22}$$

$$r_2 = -a_{21}a_{12} + a_{11}a_{22} + a_{22}a_{55} + a_{11}a_{55}$$

$$r_3 = a_{21}a_{12}a_{55} - a_{11}a_{22}a_{55}$$

$$g_{53}(s) = \frac{s(a_{51}b_{13}a_{22}) - a_{22}a_{51}b_{13}}{s^3 + s^2 r_1 + sr_2 + r_3} \tag{5.3.163}$$

where

$$r_1 = -a_{55} - a_{11} - a_{22}$$

$$r_2 = a_{11}a_{22} + a_{22}a_{55} - a_{21}a_{12} + a_{11}a_{55}$$

$$r_3 = -a_{11}a_{22}a_{55} + a_{21}a_{12}a_{55}$$

$$g_{54}(s) = \frac{-b_{24}a_{12}a_{51}}{-s^3 + s^2 r_1 + sr_2 + r_3} \tag{5.3.164}$$

where

$$r_1 = a_{55} + a_{22} + a_{11}$$

$$r_2 = -a_{11}a_{55} + a_{21}a_{12} - a_{11}a_{22} - a_{22}a_{55}$$

$$r_3 = +a_{11}a_{22}a_{55} - a_{21}a_{12}a_{55}$$

5.4 Dielectric spectroscopy

The interaction of electromagnetic fields with matter is described by Maxwell's equations (5.4.1) - (5.4.4)

$$rot \quad \vec{E} = -\frac{\partial}{\partial t}\vec{B} \tag{5.4.1}$$

$$rot \quad \vec{H} = \vec{J} + \frac{\partial}{\partial t}\vec{D} \tag{5.4.2}$$

$$div \quad \vec{D} = \rho_e \tag{5.4.3}$$

$$div \quad \vec{B} = 0 \tag{5.4.4}$$

In this set of equations $\vec{E}$ $[Vm^{-1}]$ and $\vec{H}$ $[Am^{-1}]$ describe the electric and magnetic field, $\vec{D}$ $[Asm^{-2}]$ the dielectric displacement, $\vec{B}$ $[Asm^{-2}]$ the magnetic induction, $\vec{J}$ $[Am^{-2}]$ the current density and ρ_e $[Cm^{-3}]$ the density of charge. For small electric field strengths $\vec{D}$ can be expressed by

$$\vec{D} = \varepsilon^* \varepsilon_0 \vec{E} \tag{5.4.5}$$

where ε_0 is the dielectric permittivity of vacuum ($\varepsilon_0 = 8.854 \cdot 10^{-12}$ $[Fm^{-1}]$). ε^* is the complex dielectric function or dielectric permittivity. According to Maxwell's equations (5.4.1) - (5.4.4), ε^* is time (or frequency) dependent if time dependent processes take place within the sample. There can be different reasons for this. Resonance phenomena are due to atomic or molecular vibrations and can be analysed by optical spectroscopy. Relaxation phenomena are related to molecular fluctuations of dipoles due to molecules or parts of them in a potential landscape. Moreover, drift motion of mobile charge carriers (electrons, ions or charged defects) causes conductive contributions to the dielectric

response. In general, time dependent processes within a material lead to a difference of the time dependencies of the outer electrical field $\vec{E}(t)$ and the resulting dielectric displacement $\vec{D}(t)$. For a periodic electrical field $\vec{E}(t) = \vec{E}_0 e^{-i\omega t}$ the complex dielectric function ε^* is defined by

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega) \tag{5.4.6}$$

Where $\varepsilon'(\omega)$ is the real part and $\varepsilon''(\omega)$ is the imaginary part of the complex dielectric function.

The complex dielectric function (5.4.6) can be measured in the extraordinary broad frequency regime from 10^{-6} Hz up to 10^{12} Hz (in wavelength 3.10^{16} cm - 0.03 cm). To span this dynamic range, different measurement systems based on different measurement principles have to be combined. From 10^{-6} do 10^{7} Hz lumped circuit methods are used in which the sample is treated as a parallel or serial circuit of an ideal capacitor and an ohmic resistor. Effects of the spatial extent of the sample on the electric field distribution are neglected. With increasing frequency the geometrical dimensions of the sample capacitor become more and more important limiting this approach to about 10 MHz. In addition parasitic impedances caused by cables, connectors, etc. become important at frequencies 100 kHz.

Using distributed circuit methods (10^{7} Hz - 10^{11} Hz) the complex dielectric function is deduced by measuring the complex propagation factor (in reflection or transmission). Both, waveguide as well as cavity techniques can be applied.

In the dynamic range between 10^{-6} - 10^{10} Hz the complex dielectric function $\varepsilon^*(\omega)$ can also be deduced from a measurement of the time dependent dielectric function $\varepsilon(t)$. The latter is related to $\varepsilon^*(\omega)$ via a Fourier transformation

$$\varepsilon^*(\omega) - \varepsilon_\infty = \int_{-\infty}^{+\infty} \dot{\varepsilon}(t) e^{-i\omega t}\, dt \tag{5.4.7}$$

Where $\varepsilon_\infty = \varepsilon'(\nu \approx 10^{11}\,Hz)$.

For frequencies between 10^{10} - 10^{12} Hz quasi-optical set-ups as polarizing Mach-Zehnder interferometers or oversized cavity resonators are employed. The latter has the advantage that it is suitable for very low loss materials and for measurements at low temperatures.

The main experimental difficulty in this frequency range arises from the large coherence length of the mm- and sub-mm-wave sources (backward wave oscillators, klystrons, gun oscillators, etc.) resulting in multiple standing wave patterns. Above 10^{11} Hz Fourier-Transform spectrometers are used. Due to the throughput and the multiplex advantages they are superior to arrangements using monochromators.

Measurements in the frequency domain from 10^{-6} - 10^{11} Hz

For a capacitor C^* filled with a material under study the complex dielectric function is defined as

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega) = \frac{C^*(\omega)}{C_0} \tag{5.4.8}$$

where C_0 is the vacuum capacitance of the arrangement. ω is the angular frequency with $\omega = 2\pi\nu = 2\pi T_P^{-1}$ with T_P as time for one period. $\varepsilon'(\omega)$ and $\varepsilon''(\omega)$ de-scribe the real and imaginary part of the complex dielectric function. Using a sinusoidal electric field $\vec{E}^*(\omega) = \vec{E}_0 e^{i\omega t}$ with the angular frequency ω and at field strengths within linear response (for most materials $\vec{E}_0 \leq 10^6\,Vcm^{-1}$) the dielectric function can be derived by measuring the complex impedance $Z^*(\omega)$ of the sample

$$\varepsilon^*(\omega) = \frac{\vec{J}^*(\omega)}{i\omega\varepsilon_0\vec{E}^*(\omega)} = \frac{1}{i\omega Z^*(\omega)C_0} \tag{5.4.9}$$

Where $\vec{J}^*$ is the complex current density and ε_0 the permittivity of free space. To cover the frequency domain from 10^{-6} up to 10^{11} Hz, four different systems based on different

measurement techniques are employed: Fourier correlation analysis in combination with dielectric converters (10^{-6} - 10^{7} Hz), impedance analysis (10^{1} - 10^{7} Hz), RF-reflectometry (10^{6} - 10^{9} Hz) and network analysis (10^{7} - 10^{11} Hz).

Fourier correlation analysis

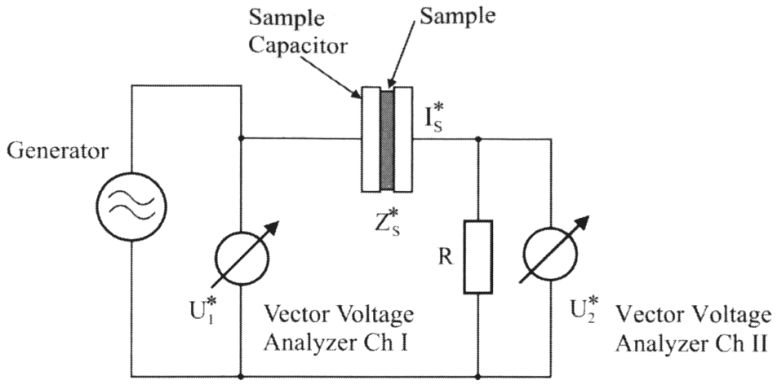


Figure 5.10 Scheme of a Fourier correlation analyzer

The basic principle is shown in Figure 5.10. Typically a sine wave voltage $U_1(t)$ with frequency $\omega/2\pi$ is applied to the sample by a generator, covering the frequency range from 10^{-6} - 10^{7} Hz. The resistor R converts the sample current $I_S(t)$ into a voltage $U_2(t)$. $U_1(t)$ and $U_2(t)$ are analyzed with respect to the amplitudes and phases of their harmonic Fourier base waves $U_1^*(\omega)$ and $U_2^*(\omega)$ by two phase sensitive sine wave correlators. The complex sample impedance Z_S is calculated

$$Z_S^*(\omega) = \frac{U_S^*(\omega)}{I_S^*(\omega)} = R\left(\frac{U_1^*(\omega)}{U_2^*(\omega)} - 1\right) \qquad (5.4.10)$$

where $U_S^*(\omega)$ and $I_S^*(\omega)$ are the sample voltage and current. The response of the correlator to the signal $U_i(t)$ are the two components

$$U'_j(\omega) = \frac{1}{NT}\int_0^{NT} U_j(t)\sin(\omega t)dt \qquad (5.4.11)$$

$$U''_j(\omega) = \frac{1}{NT}\int_0^{NT} U_j(t)\cos(\omega t)dt \qquad (5.4.12)$$

where j=1, 2. $U'_j(\omega)$ is the phase component of the harmonic base wave and $U''_j(\omega)$ the orthogonal (90° shifted or quadrature) component of the harmonic base wave. N is the number of periods with duration $T=2\pi/\omega$ measured by the correlator. For technical implementation of Fourier correlation analysis one has to realize mentioned equations. This is done in conventional systems by analogue components. From the main oscillator, the reference sin and cos signals are created by a 90°. Modern systems digitize the test signal and approximate numerically by a signal processor avoiding by that the drift and the non-linearity of analogue components. Fourier correlation analyzers are commercially available as frequency response analyzers or lock-in amplifiers from Agilent Technologies, EG & G, Novocontrol, Solartron and Stanford Research Systems.

Impedance analysis

Two kinds of techniques have to be distinguished. The first is the I-V method referring to the direct phase sensitive measurement of the sample current and voltage. It is similar to the dielectric converter techniques described above. Instead of an electrometer amplifier, a broadband current to voltage converter is used without reference technique.

An alternative technique is the AC impedance bridge shown in Figure 5.11; which consists of the sample capacitance $Z_S^*(\omega)$ and the adjustable compensation impedance $Z_C^*(\omega)$. On the left hand side of the bridge, the generator drives the sample with the fixed and known a.c. voltage $U_S^*(\omega)$ which causes the current $I_C^*(\omega)$ to flow into P_1. On the right hand side of the bridge, the variable amplitude-phase generator (VAPG) feeds the current $I_C^*(\omega)$ through the compensation impedance $Z_C^*(\omega)$ into P_1. The bridge will be balanced, if $I_S^*(\omega)$ equals $-I_C^*(\omega)$ which corresponds to $I_0=0$. Any deviation is detected by the zero voltage

detector which changes the amplitude and phase of the variable amplitude phase generator (VAPG) as long as $I_0 \neq 0$. In the balanced state, the sample impedance is calculated as

$$Z_S^*(\omega) = \frac{U_S^*(\omega)}{I_S^*(\omega)} = -\frac{U_S^*(\omega)}{U_C^*(\omega)} Z_C^*(\omega) \qquad (5.4.13)$$

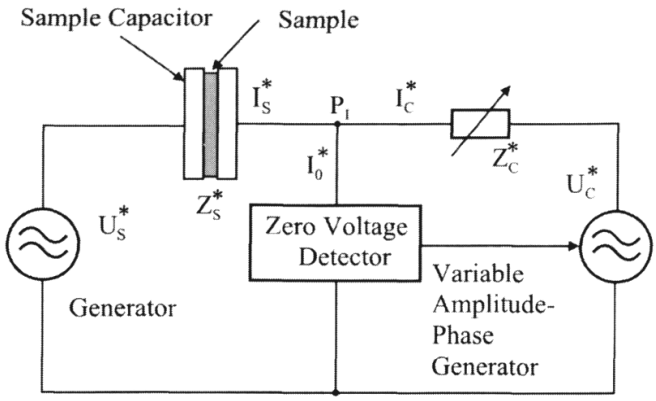


Figure 5.11 Scheme of an impedance bridge

Impedance bridges or analyzers which are not specially designed for dielectric measurements typically work from 10 Hz to 10 MHz. For dielectric samples, they suffer from the inverse problem of the dielectric converter systems as optimized for low (typically 10 mΩ) to medium impedance with a limit of about 100 MΩ. This limit is reached for typical dielectric samples (100 pF, tan δ=0.01) at about 1 kHz. The typical accuracy in tan δ is less then 10^{-3}. Here, AC impedance analyzers or bridges offer a suitable and inexpensive solution for applications which do not require a large frequency and a high impedance range. Due to the fact that the measurement time is lower compared with systems using reference techniques, AC-impedance bridges are particularly suited for measurements on materials with time-dependent dielectric properties (monitoring of chemical reactions, characterization of phase transitions, etc.). Impedance analyzers or bridges are commercially available from several manufactures like, e.g., Agilent Technologies, Novocontrol, Solartron, QuadTech, and Wayne Kerr.

RF reflectometry

Coaxial line reflectometry can be employed at frequencies from 1 MHz to 10 GHz. In contrast to the low frequency techniques already described, above 1 MHz the measurement cables significantly contribute to the sample impedance. Above approximately 30 MHz standing waves arise in the line and a direct measurement of the sample impedance completely fails. This can be avoided by application of microwave techniques taking the measurement line as the main part of the measured impedance into account. Therefore, precision lines and sample cells with defined propagation constants are required.

5.5 Spectrophotometry

Interaction of electromagnetic radiation with matter is an important and versatile tool. In fact, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample. A principle of spectrophotometry is that every substance absorbs or transmits certain wavelengths of radiant energy but not other wavelengths. The light energy absorbed or transmitted must match exactly the energy required to cause an electronic transition (a movement of an electron from one quantum level to another) in the substance under consideration. Only certain wavelength photons satisfy this energy condition.

Molecules have an ability to absorb electromagnetic radiation of specific wavelengths and can exist in definite quantum states which differ in different energy content. If molecule can go over from state with smaller energy E_q into state with higher energy E_q, then molecule absorbs radiation with frequency f, which corresponds to energy difference ΔE between energy levels E_p and E_q of both quantic states according Planck condition:

$$\Delta E = E_P - E_q = hf = h\frac{c_L}{\lambda} \tag{5.5.1}$$

where h is Planck constant, f is frequency, c_L is velocity of light and λ is wavelength.

Absorption of ultra violet light (190 - 400 nm) and visible light (400 - 800 nm) leads to transitions between electron energy levels which are the most energetically demanding.
Part of radiation from input radiant flux (Φ_I) is absorbed by sample during absorption measurement (Φ_A) and in ideal case, the rest of radiant flux passes through (Φ_O).

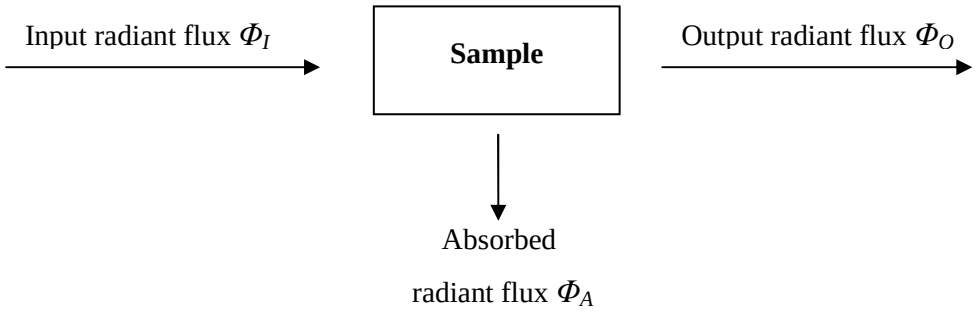


Figure 5.12 Scheme of radiant flux through the sample

Quotient of output and input radiant flux is defined as transmittance:

$$\tau = \frac{\Phi_O}{\Phi_I} \tag{5.5.2}$$

Absorbance is defined as the negative logarithm of the transmittance:

$$A = -\log(\tau) = \frac{\Phi_I}{\Phi_O} \tag{5.5.3}$$

Dependence of absorbance on wavelength (or frequency) results in absorption spectrum.
It is used as qualitative identification of compounds, especially organic with groups which cause colouration of molecules, for example *C=O*, *N=N*, *N=O*. In such grouping,

numerous energetic transitions happen and then molecules containing those groups absorb in visible spectrum. Absorbance at specific wavelength depends on concentration and thickness of layer according Lambert-Beer law:

$$A_\lambda = \varepsilon_\lambda bc \qquad (5.5.4)$$

where A_λ is absorbance at wavelength λ, ε_λ is molar absorption coefficient at wavelength λ, *b* is thickness of layer and *c* is concentration.

Mentioned absorbance dependence on concentration is valid for monochromatic radiation at low concentration (less then 10^{-2} mol.l^{-1}). Lambert-Beer law also is not valid if other interactions between matter and surrounding occur, for example, fluorescence or phosphorescence. However, in most cases, mentioned law is valid and it is used for ascertainment of matter concentration in solution.

In recent years spectrophotometry methods have become the most frequently used and important methods of quantitative analysis. They are applicable to many industrial and clinical problems involving the quantitative determination of compounds that are coloured or that react to form a coloured product.

6 EXPERIMENTAL

6.1 Materials

Hydrolyzate of chrome shavings – Hykol-E

It was industrially produced from waste chrome shavings by enzymatic hydrolysis. Shavings were mixed with fivefold amount of water. Heterogenous mixture was alkalinized by using organic basis (cyklohexylamin, izopropylamin) together with magnesium hydroxide. After reaching uniform alkalinity of chromium shavings, the reaction blend is warmed to 70°C. After 1 hour, approximately 0.02 % of proteolytic enzyme is added, and the reaction is then kept running at 70°C another 2 or 4 hours. After termination of the reaction time, the hot heterogeneous blend is filtered by a vacuum filter and the filtrate is vacuum evaporated to the concentration of 30-40% of the dry matter. Detailed description of the procedure is mentioned in (Kolomazník, et al., 2000). Technical specification is described in Table 6.1.

Table 6.1 Chemical composition of Hykol-E.

Nitrogen in dry matter	5 -17%
Ash in dry matter	3 - 7%
Chromium content	max 0,05%
Free primary amino groups	0,06 - 0,2 mol/g
Molecular mass determined by osmometry	5000 - 8000
pH 5% of aqueous solution	7,5 - 8,5

Cross-linking agents

Glutaraldehyde

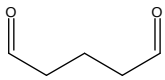


Figure 6.1 Structural formula

Table 6.2 Some properties of glutaraldehyde (Večeřa, 1975).

Summation formula	$C_5H_8O_2$
Mass density	1,06 g/cm^3
Molar mass	100,117 g/mol
Boiling point	101 °C
Melting point	-6 °C
Length of molecule	0,616 nm

Glyoxal

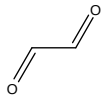


Figure 6.2 Structural formula

Table 6.3 Some properties of glyoxal (Večeřa, 1975).

Summation formula	$C_5H_8O_2$
Mass density	1,14 g/cm^3
Molar mass	58,0366 g/mol
Boiling point	51 °C
Melting point	15 °C
Length of molecule	0,154 nm

Used instruments

Technological card NI-488.2

IEEE-488 is a short-range, digital communications bus specification. It was originally created for use with automated test equipment, and is still in wide use for that purpose. IEEE-488 is also commonly known as HP-IB (Hewlett-Packard Instrument Bus) and GPIB (General Purpose Interface Bus). It allows up to 15 devices to share a single 8-bit parallel electrical bus by daisy chaining connections. The slowest device participates in control and data transfer handshakes to determine the speed of the transaction. The maximum data rate is about one Mbyte/sec in the original standard, and about 8 Mbyte/sec with IEEE-488. The IEEE-488 bus employs 16 signal lines — eight bi-directional used for data transfer, three for handshake, and five for bus management — plus eight ground return lines.

LCR meter HP 4284A

The Agilent 4284A precision LCR meter is a cost-effective solution for component and material measurement. The wide test frequency range (20 Hz to 1 MHz) and superior test-signal performance allow testing components to the most commonly-used test standards, such as IEC/MIL standards, and under conditions that simulate the intended application. Whether in research and development, production, quality assurance, or incoming inspection, the Agilent 4284A will meet all of LCR meter test and measurement requirements.

Figure 6.3 Used LCR meter HP 4284A

Digital camera Cannon PowerShot A70

Power Shot A70 is a 3MPx digital camera which among others allows acquisition of compressed video in AVI format. This instrument was used for measurement of RGB components during the cross-linking process.

Figure 6.4 Digital camera Cannon PowerShot A70

Gas chromatograph HP 5890 Series II GC

This chromatograph is equipped with Hewlett Packard - 5890 GC-FID-FID System Including: 5890 Series II GC, Dual Flame Ionization Detectors, Dual Split-Splitless Injection Ports with Manual Pneumatics, HPIB Communication, 7673B Autosampler Including: Controller, Injector, 100 Position Tray and Mounting Bracket

Spectrophotometer VARIAN Carry 50 Bio

It is very precise spectrophotometer equipped with xenon flash lamp and the highly focussed and very intense light beam. The fibre optic dip probe was used for measurement.

Figure 6.5 The fibre optic dip probe

6.2 Used software

Automated system of data acquisition for dielectric measurement was realized in Agilent VEE Pro (Angus, et al., 2004). Three own user applications were created in this graphical programmable environment. They allow us various settings of parameters, such as frequency, sampling period, delays etc. These applications were tested, debugged and then used for all measurements. Illustration pictures of one of them are shown in Figure 6.6 and Figure 6.7. Own user m-files in Matlab software were created and used for data evaluation and data post-processing.

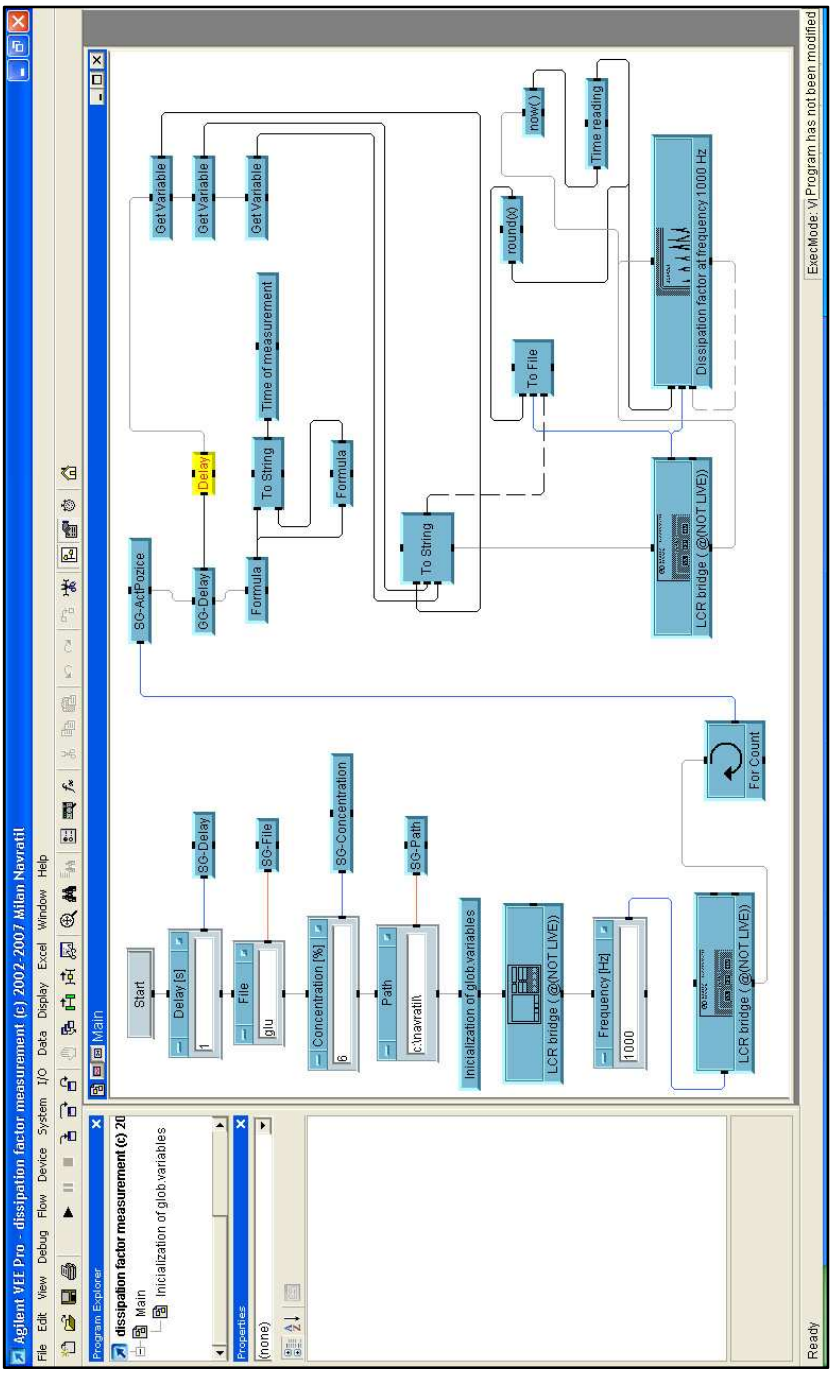


Figure 6.6 Source code of the user application created in Agilent VEE Pro software

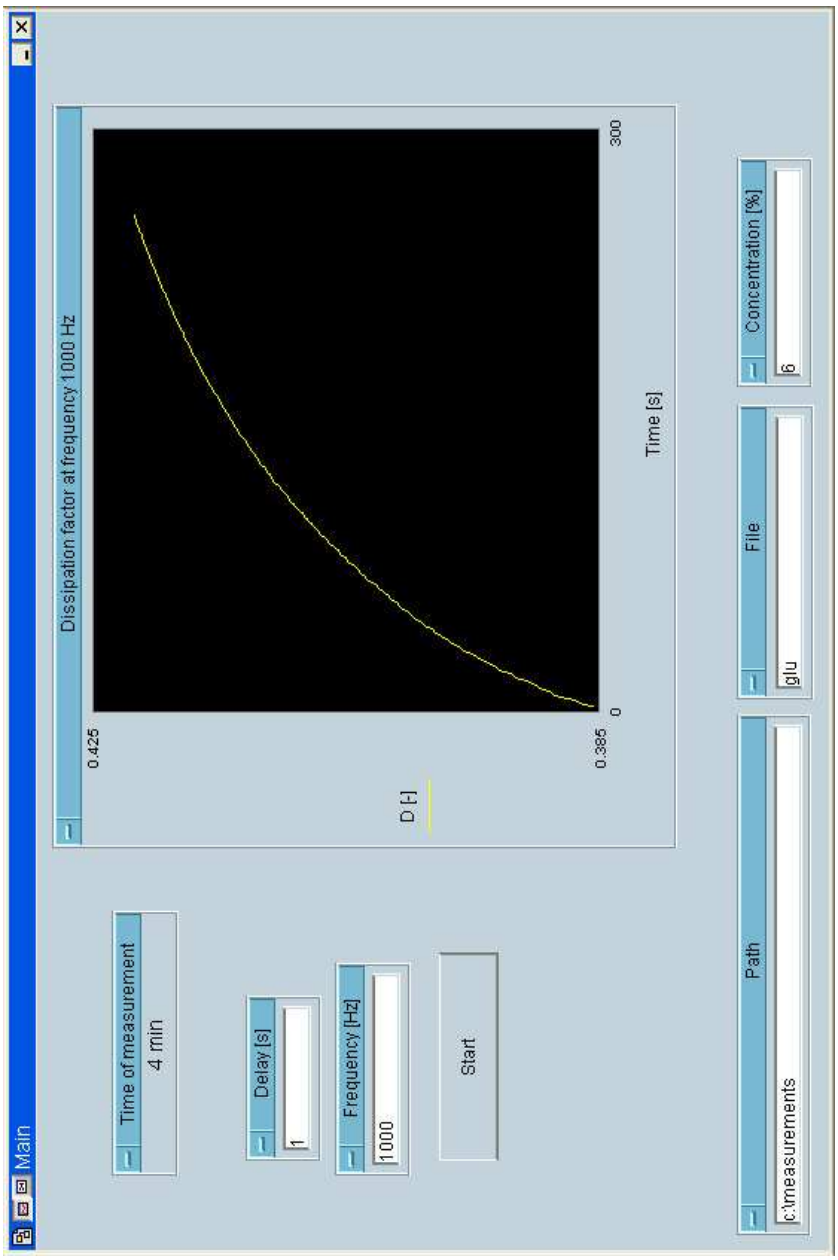


Figure 6.7 The appearance of the user application screen used for measurement of the dissipation factor created in Agilent VEE Pro.

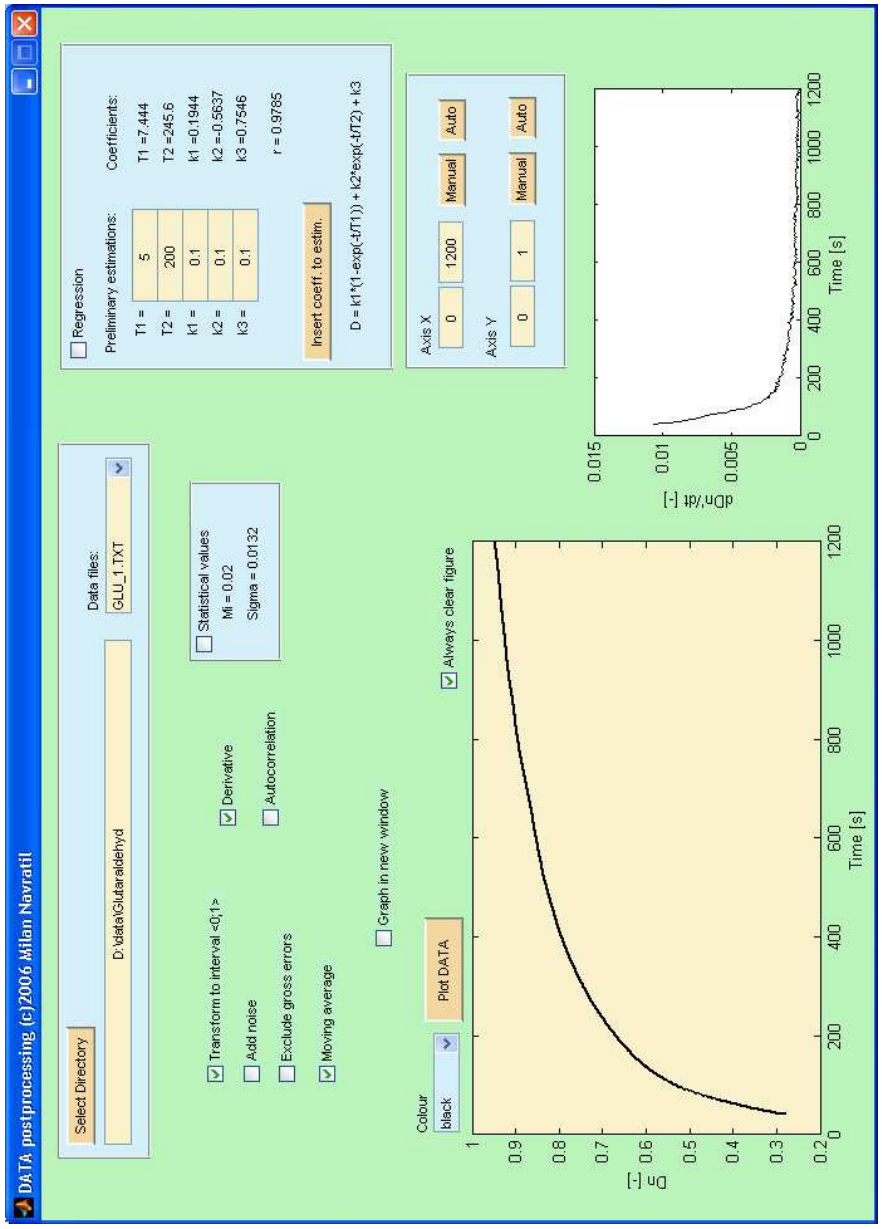


Figure 6.8 The appearance of the user application screen used for data post-processing and evaluation created in Matlab

6.3 Measuring methods

Dielectric spectroscopy method

Personal computer together with technological card NI-488.2 and LCR precise meter HP4284A including two-electrode measuring system was used for data acquisition. Instruments were connected via GPIB interface; scheme is shown in Figure 6.9. Practical realization and application procedure are shown in Figure 6.10.

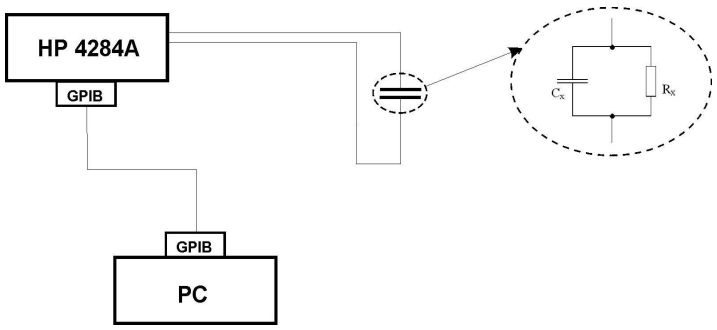


Figure 6.9 Scheme of measuring apparatus

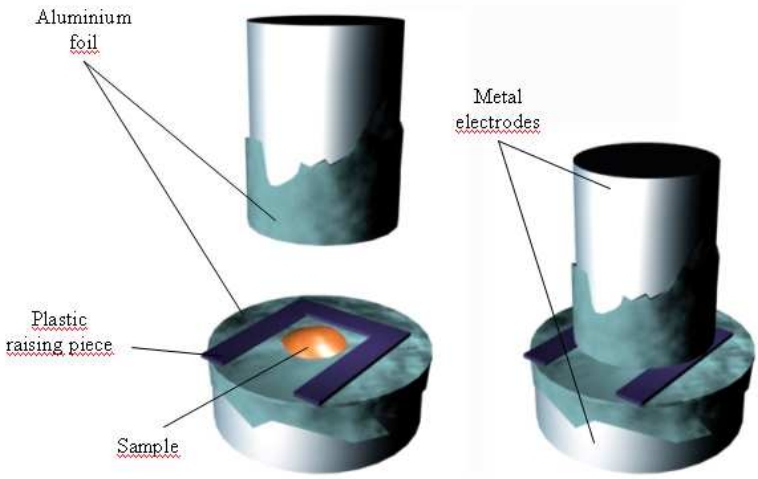


Figure 6.10 System of electrodes before (left) and during measurement (right)

In all measurements, 25% aqueous solution of glutaraldehyde and 40% aqueous solution of glyoxal were used as cross-linking agents. Hykol-E in powder was dissolved in water so that spare 56% aqueous solution of Hykol-E was prepared. Concentration of Hykol-E was experimentally chosen according to sample viscosity. Sample preparation was realised in the following way: spare solution of Hykol-E was weighed (0,500 ± 0,005)g on aluminium foil which was placed on an electrode and a certain amount of the cross-linking agent added from micropipette (1 up to 60 μl ± 0.1 μl). Then the sample was stirred by plastic stick (the procedure is evident from Figure 6.10). During all measurements, the laboratory temperature was (23 ± 1) °C.

Dissipation factor was chosen as a measured quantity, because it is not dependent on geometric parameters of sample. It is the ratio of the power loss in a dielectric material to the total power transmitted through the dielectric, the imperfection of the dielectric. It is also equal to the tangent of the loss angle:

$$D = \frac{\varepsilon''}{\varepsilon'} = \tan(\delta) = \frac{1}{\omega R_P C_P} \tag{6.1.1}$$

$$R_P = \rho \frac{l}{S} \tag{6.1.2}$$

$$C_P = \varepsilon \frac{S}{l} \tag{6.1.3}$$

$$\sigma = \frac{1}{\rho} \tag{6.1.4}$$

where δ is loss angle, ω is angular frequency, R_P is equivalent parallel resistance, C_P is equivalent parallel capacity, ρ is electric resistivity, ε is permittivity of surrounding, S is area of electrodes, l is distance between electrodes, σ is specific conductance, so after elementary mathematical modification we can obtain:

$$D = \frac{1}{\omega R_P C_P} = \frac{1}{\omega \rho \frac{l}{S} \varepsilon \frac{S}{l}} = \frac{1}{\omega \rho \varepsilon} = \frac{\sigma}{\omega \varepsilon} \tag{6.1.5}$$

From above mentioned is evident that dissipation factor is dependent only on angular

frequency, permittivity and conductivity of the material. Frequency 1 kHz was chosen as a suitable frequency for dissipation factor measurement because we assume that molecules in creating gel have relaxation time in milliseconds. All measurements then were carried out at frequency 1 kHz. From practice that the most suitable sampling period is about one second because time of duration of cross-linking reaction is in minutes.

It is also known from practice that necessary amount of cross-linking agent (glutaraldehyde) is from 1 % up to 3 % b.w. for successful cross-linking process with collagen. At first, four samples were measured in the same way (1 % b.w. of the glutaraldehyde).

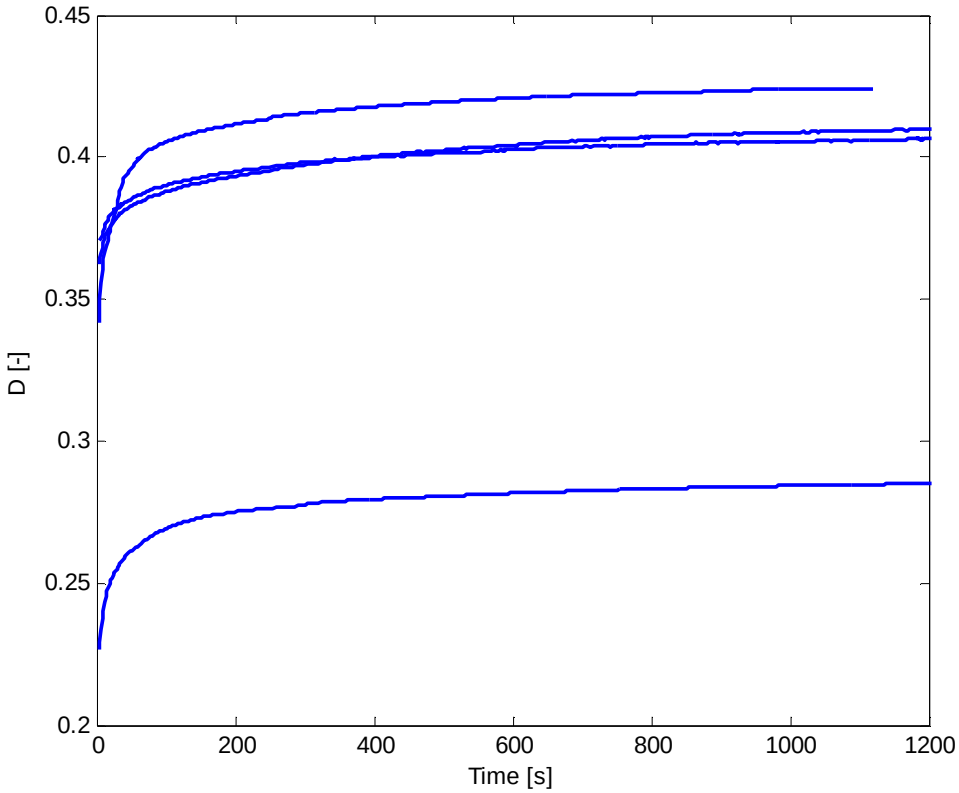


Figure 6.11 Sample (Hykol-E with 1% b.w. of glutaraldehyde), four same measurements

From the Figure 6.11 it is evident that if we repeat the measurement with the same sample, dissipation factor values have different quantity but the trend is the very similar to each other. From aspect of the technological purposes, we are interested in time courses of measured data, especially rather time constants then specific values of dissipation factor.
For that reason, we transform measured data of dissipation factor into standardized dissipation factor:

$$D_n = \frac{D - \min(D)}{\max(D) - \min(D)} \tag{6.1.6}$$

Where D is vector of dissipation factor values and D_n is vector of standardized values.

At first, the effect of the cross-linking agent concentration on the reaction was studied. Six samples were prepared and measured: Hykol-E with 1 %, 2 % up to 6 % b.w. of cross-linking agent – glutaraldehyde. The same experiment was carried out with glyoxal. Measured data is shown in Figure 6.12 and Figure 6.13. We obtain modified data by application of standardized dissipation factor which is illustrated in Figure 6.14 and Figure 6.15.

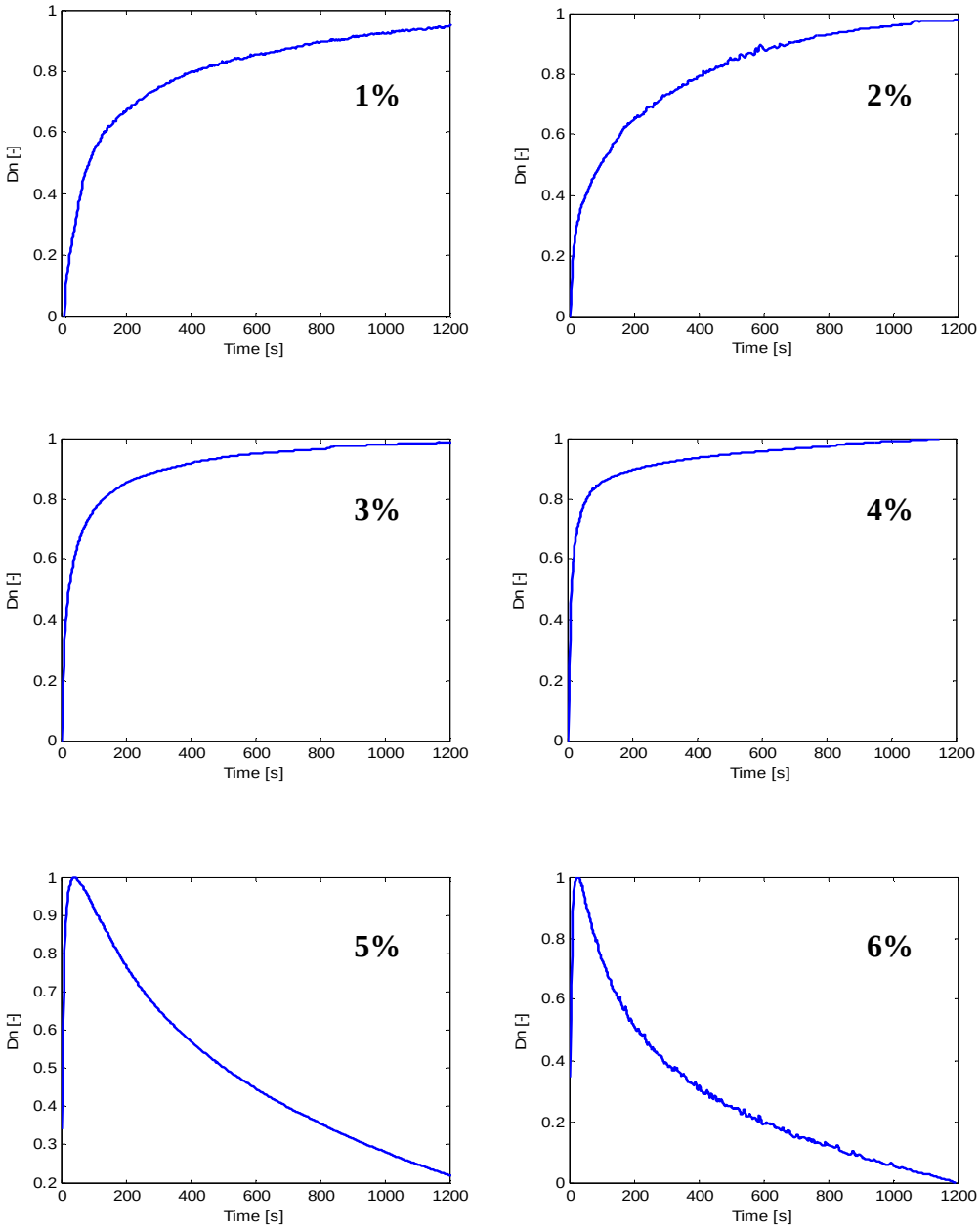


Figure 6.12 Time dependence of standardized dissipation factor – reaction of Hykol-E with 1 % - 6 % b.w. of glutaraldehyde.

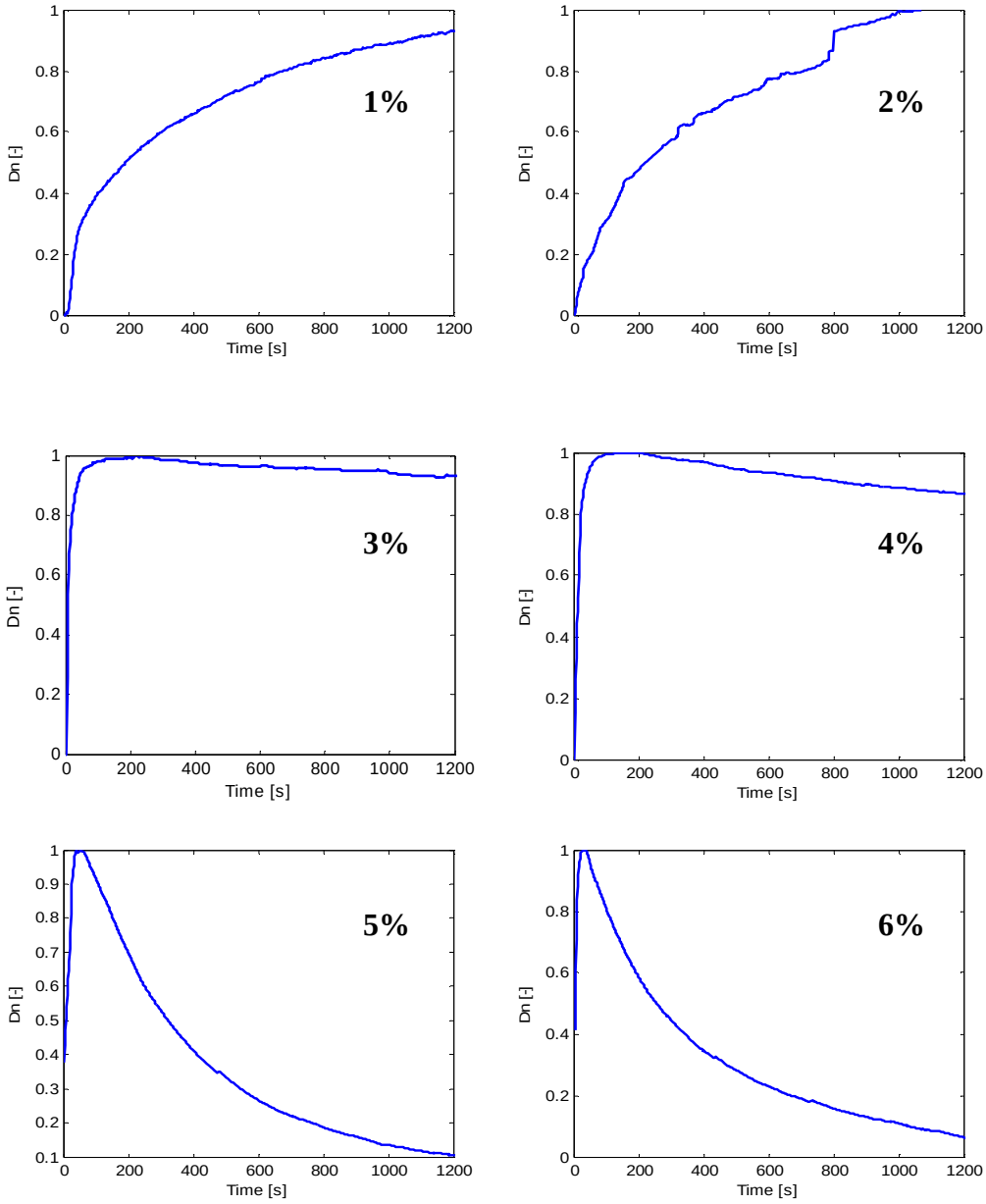


Figure 6.13 Time dependence of standardized dissipation factor – reaction of Hykol-E with 1 % - 6 % b.w. of glyoxal.

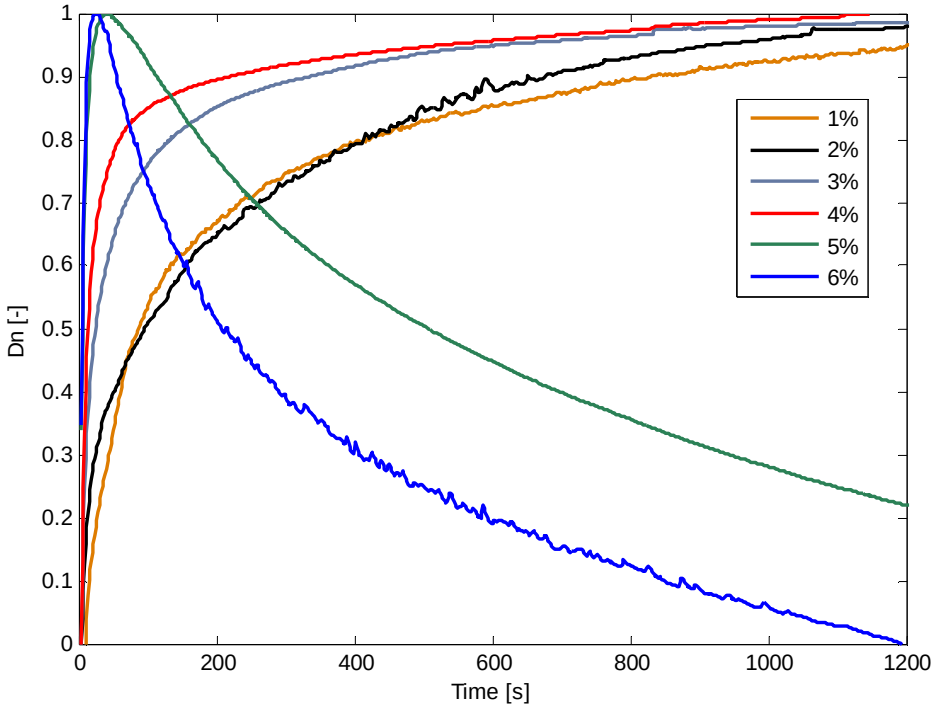


Figure 6.14 Comparison of time dependence of standardized dissipation factor – reaction of Hykol-E with 1 % - 6 % b.w. of glutaraldehyde.

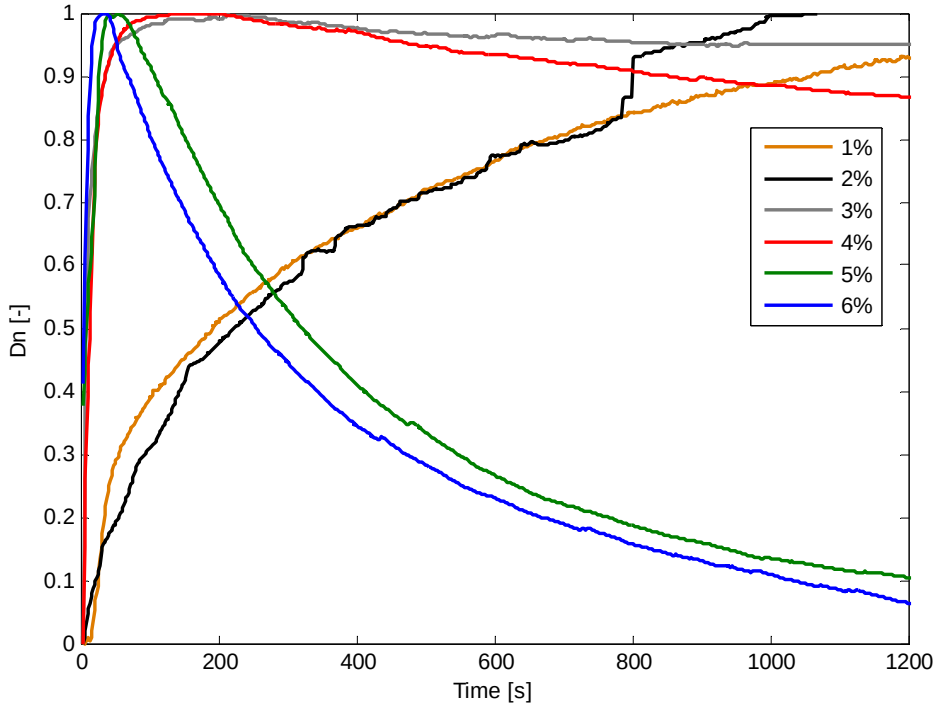


Figure 6.15 Comparison of time dependence of standardized dissipation factor – reaction of Hykol-E with 1 % - 6 % b.w. of glyoxal.

In Figure 6.14, there is an obvious change of curve shapes for glutaraldehyde concentration between 4 % and 5 %. Glutaraldehyde concentrations up to 4 % b.w. result in increasing shape of curves only. Concentrations above 5 % b.w. result in curve shapes which are at first rapidly increasing and then exponentially decreasing.

The curves differ in the rate of growing and they are directly proportional to the amount of the cross-linking agent. The changes of curve shapes in the further text are referred to as

“gelation point” (GP). In this point, we assume that the gel is created in the whole volume of the sample. GP is reached when enough amount of cross-linking agent is added. Enough means such number of reactive groups of agent that corresponds to equivalent number of reactive groups of collagen.

We verified the above-mentioned assumption in the same experiment, where glyoxal was used instead of glutaraldehyde. Both agents are dialdehydes and have the same number of reactive groups. They differ in molecule length and molar weight as it is described in chapter (6.1). Molecules of glutaraldehyde are approximately four times longer and have 1.7 times greater weight then molecules of glyoxal. Therefore, we expect that GP appears at 1.7 times lower concentration of glyoxal (from 2.3 % b.w. up to 2.9 % b.w). In fact, similar behavior can be seen in Figure 6.15, where GP appears between 2 % and 3 % of glyoxal.

Although excess of aldehydes would guarantee reaching GP, it is unsuitable from health and economic point of view as it was discussed above. The ideal case would be the addition of minimal amount of cross-linking agent needed for successful reaction.

Moreover, the cross-linking reaction was subjected to an experimental study which was carried out to prove whether the reaction releases or absorbs energy in the form of heat. The change in temperature during the cross-linking process was measured for Hykol-E with 6 % b.w. of glutaraldehyde and a two-component Epoxy resin (see Figure 6.16).

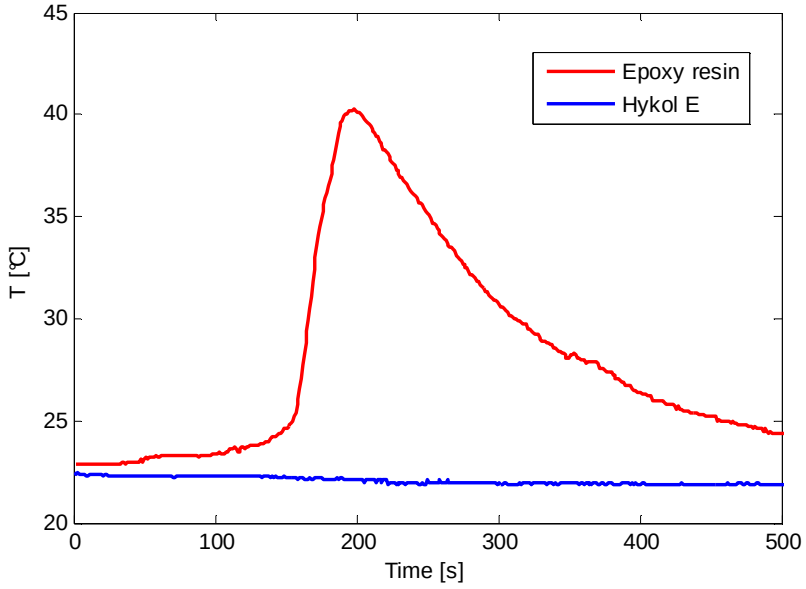


Figure 6.16 Change in temperature of Hykol-E during cross-linking reaction with 6 % b.w. of glutaraldehyde at laboratory temperature 22.7°C.

The cross-linking reaction temperature of Hykol-E with glutaraldehyde is approximately the same during the entire cross-linking process. In industrial practice, during technological process we suppose approximately constant temperature which is normally about 20°C. Investigation of temperature influence can be the object of further research. In general, we can say that higher temperature accelerates the reaction according to Arhenius law.

Evaluation of the degree of cross-linking using Gas Chromatography – Mass Spectrometry method with lysine as a model system

Four aqueous based lysine solutions (0.5 % b.w. of lysine) were prepared. Two of them were subjected to cross-linking with excess of glutaraldehyde (9 mol of glutaraldehyde / 1 mol of lysine). In addition, we added hydrochloric acid to two samples, as it is shown in Table 6.4. The cross-linking degree of the glutaraldehyde mediated cross-linking with lysine in comparison with acid hydrolysis was assessed.

Table 6.4 Four prepared samples

Lysine
Lysine + Acid
Lysine + Glutaraldehyde
Lysine + Glutaraldehyde + Acid

These samples were appropriately diluted and the free lysine content was measured by Gas Chromatography with Mass Selective Detection (GC-MS).

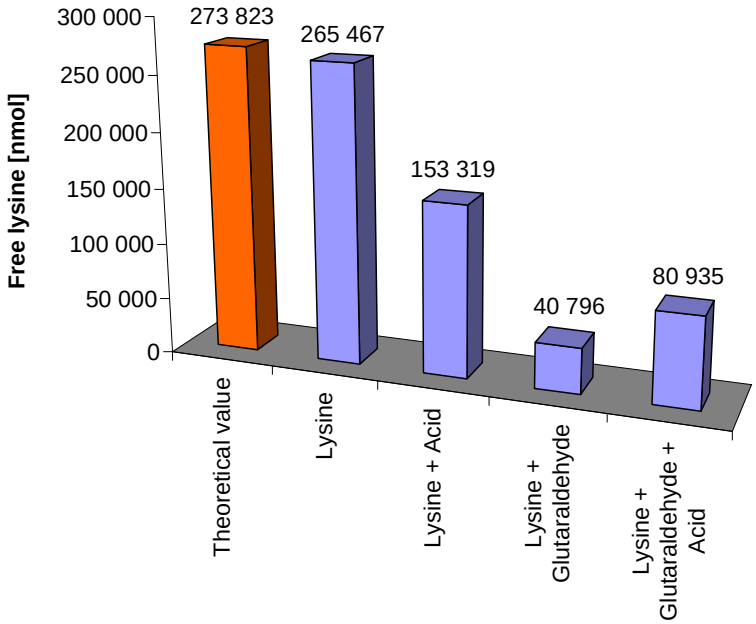


Figure 6.17 Concentration of free lysine in analysed four samples

From the results displayed in Figure 6.17 it is clear that the analytical technique is useful for the detection of free lysine. The analysis of a sample of known concentration resulted in a value close to the theoretical value. Treating lysine with the acid resulted in a decrease in the levels of detected lysine, due to hydrolysis. Cross-linking of lysine with glutaraldehyde resulted in an almost seven times reduction in the levels of free lysine. This indicates that cross-linking reaction proceeds, however the reaction has not gone to completion. If cross-linking is complete, there will be no detectable free lysine.

The cross-linked sample with acid contained increased levels of free lysine compared with the fully cross-linked sample. This indicates that the glutaraldehyde-lysine adduct is not stable to acid hydrolysis. This experiment showed that indication of the cross-linking process degree can be obtained using free lysine concentration as a model system by GCMS method.

Further analysis of collagen hydrolyzate, the results of which are illustrated in Figure 6.18 indicates that it is possible to detect two substances, lysine and hydroxyproline, in this sample.

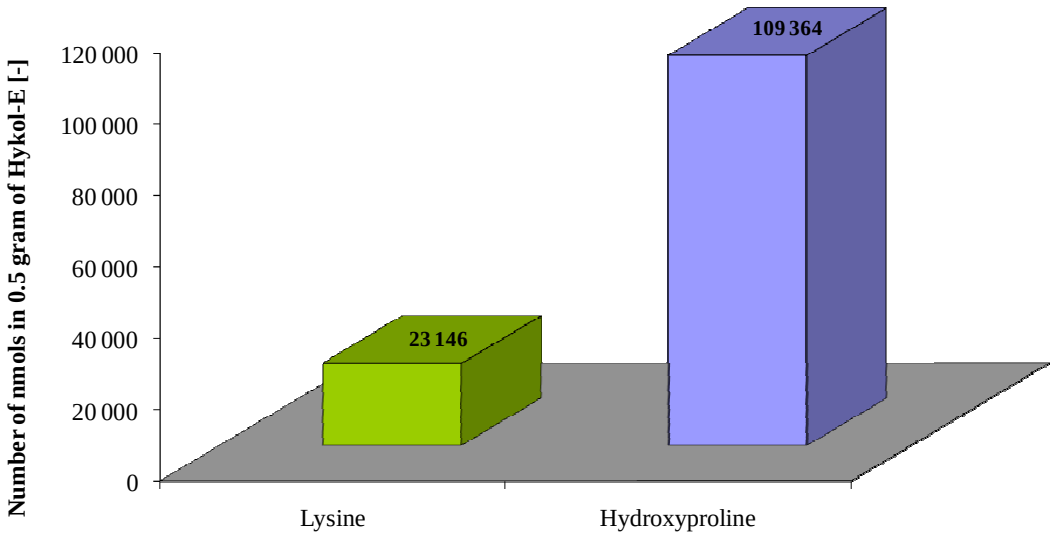


Figure 6.18 Content of free lysine and hydroxyproline in the measured sample of 0.5 g of Hykol-E

Gas chromatography method is a very accurate laboratory technique and can be used as a reference measurement, but it is expensive and due to complexity of fine chemical methodology it is not suitable for common practice. It was used as an alternative to physical methods and for comparison with methods which are instrumentally simpler and usable in practice.

Optical method

Spectrophotometric measurement of absorbance

This measurement was carried out to evaluate the potential of spectrophotometric analysis for the monitoring of cross-linking reactions. Samples containing collagen hydrolyzate and 6 % b.w. of glutaraldehyde were prepared and allowed to react for varying lengths of time (0, 2, 5, 10 and 20 minutes) before addition of acid to prevent further reaction. Samples containing collagen hydrolyzate and distilled water for blank test were also evaluated. The UV/Visible spectrum of the solution was obtained for each sample, which is shown in Figure 4.

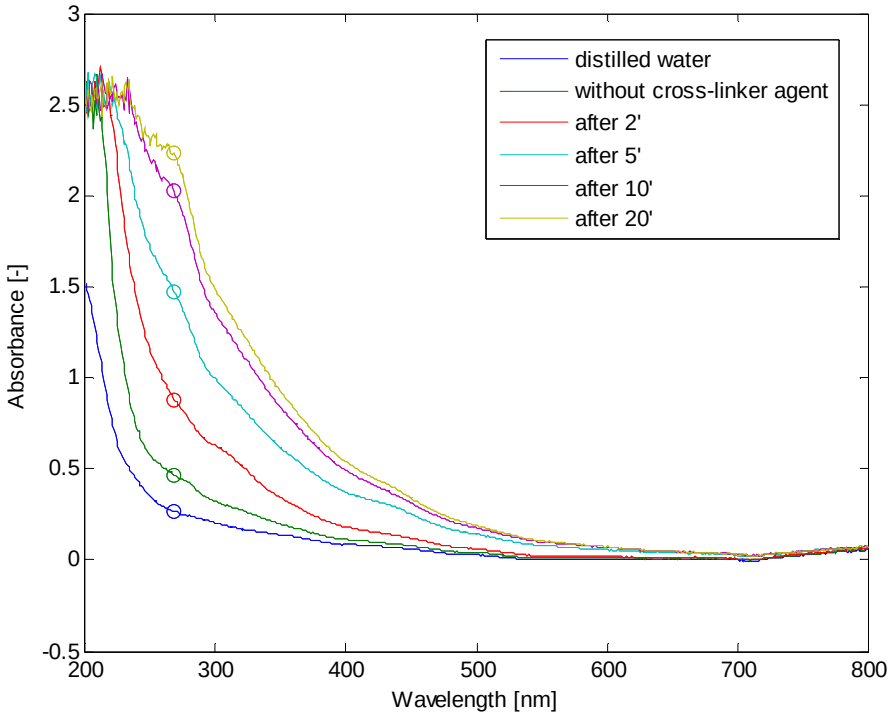


Figure 6.19 UV/Visible Spectrum of time-variously cross-linked Hykol-E with 6 % b.w. of glutaraldehyde and distilled water

The aim of comparing the UV/Visible spectrum was to determine the optimum point on the spectrum for monitoring differences in the reaction rates. From Figure 6.19, the largest difference in absorbance can be seen at about 268 nm (the points are highlighted by a circle). For practical applications, however, the visible range between approximately 400 nm and 750 nm is the most suitable.

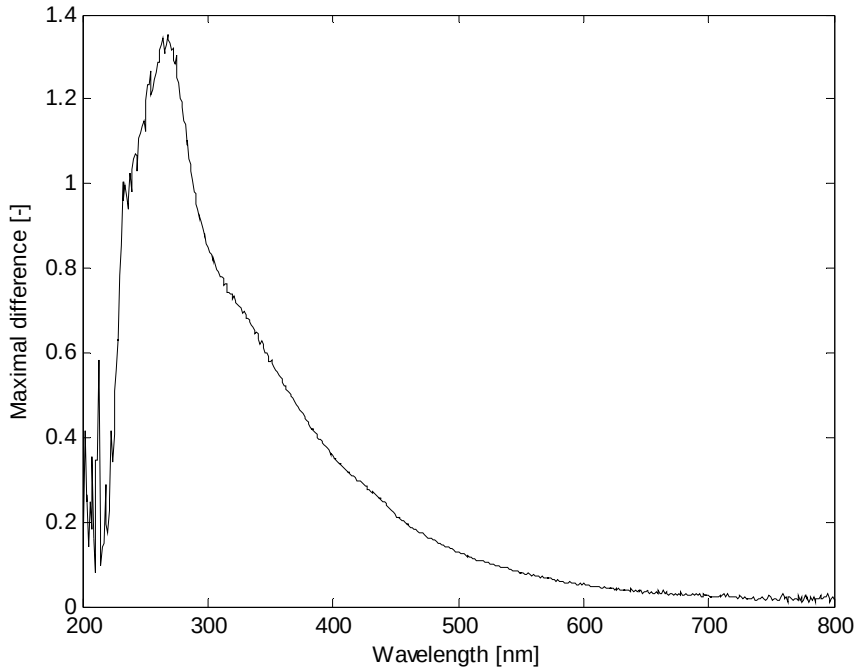


Figure 6.20. Maximal difference in absorbance with respect to wavelength

Within the visible range it can be determined (see Figure 6.20) that the biggest differences in absorbance occur at about 400 nm. For practical reasons, absorbance of the experimental samples was measured in visible spectrum at 400 nm and the results are displayed in Figure 6.21.

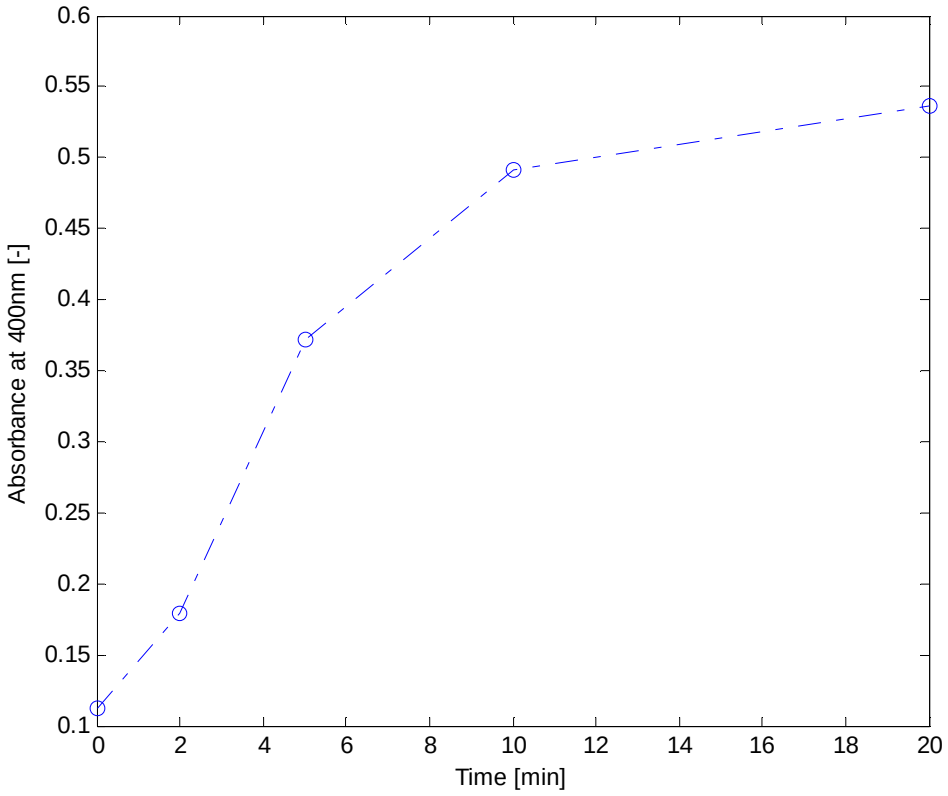


Figure 6.21 Absorbance at 400 nm during reaction of collagen hydrolyzate with glutaraldehyde

As this technique proved to be successful (illustrated in Figure 6.21), absorbance of the cross-linking reaction was monitored continuously. The results are shown in Figure 6.22. From the automatic control point of view, this seems to be a valuable tool for the on-line monitoring of the cross-linking reaction involving glutaraldehyde.

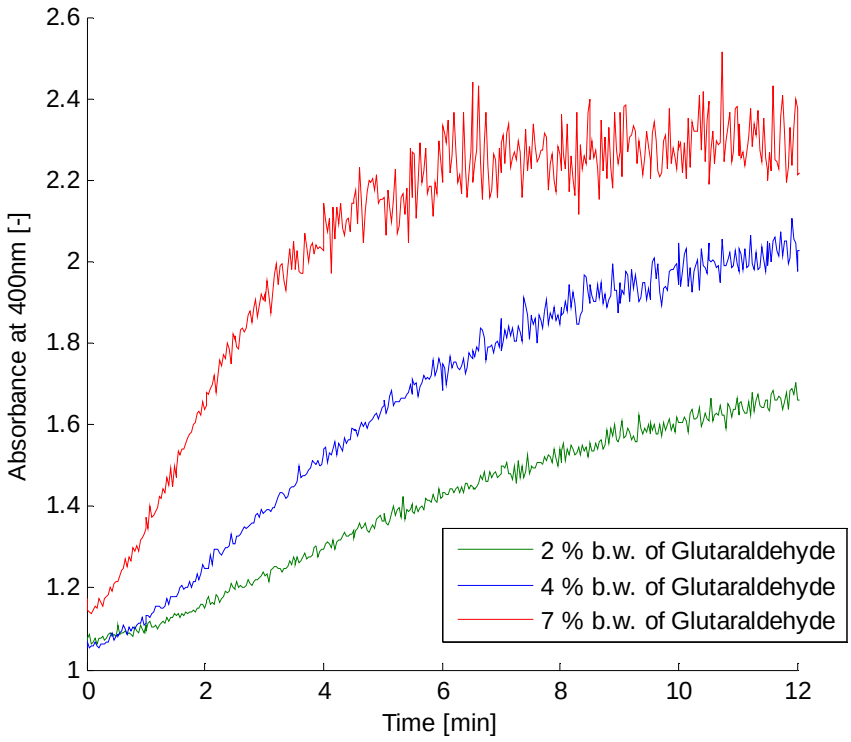


Figure 6.22 Time dependence of absorbance during the cross-linking reaction

This research illustrates the potential for developing measurement techniques of the cross-linking degree that occurs between proteins and cross-linking agents. These trials have shown that this method can be utilized in industrial practice. The method of monitoring the absorbance changes of the solutions is promising as an online technique. It is obvious that a quick and reliable method can be developed and may have implications in many industrial areas including the productions of gelatines or sausage casings.

Colorimetric measurement of RGB components

Using a digital camera containing optical sensor represents simple and relatively cheap way for automatic monitoring of sample colour changes. In this experiment, Canon PowerShot A70 containing CCD sensor was used for colorimetric measurement of the cross-linking process of Hykol-E with 6 % b.w. of glutaraldehyde. Five pictures were obtained, each of them at specific time during the cross-linking reaction. The obtained pictures were pre-processed in Adobe Photoshop CS2 and appropriately trimmed and properly saved. The resulting images were then processed using own user function realised in MATLAB and shown in Figure 6.23. It was possible to determine the red, green and blue colour components for each individual pixel in defined area of the picture. All selected pixels in the image were averaged for each component.

Figure 6.23 Change of solution colour during the cross-linking reaction, from left: without glutaraldehyde, after 2, 5, 10 and 20 minutes.

Individual components were drawn with respect to time and can be seen in Figure 6.24.

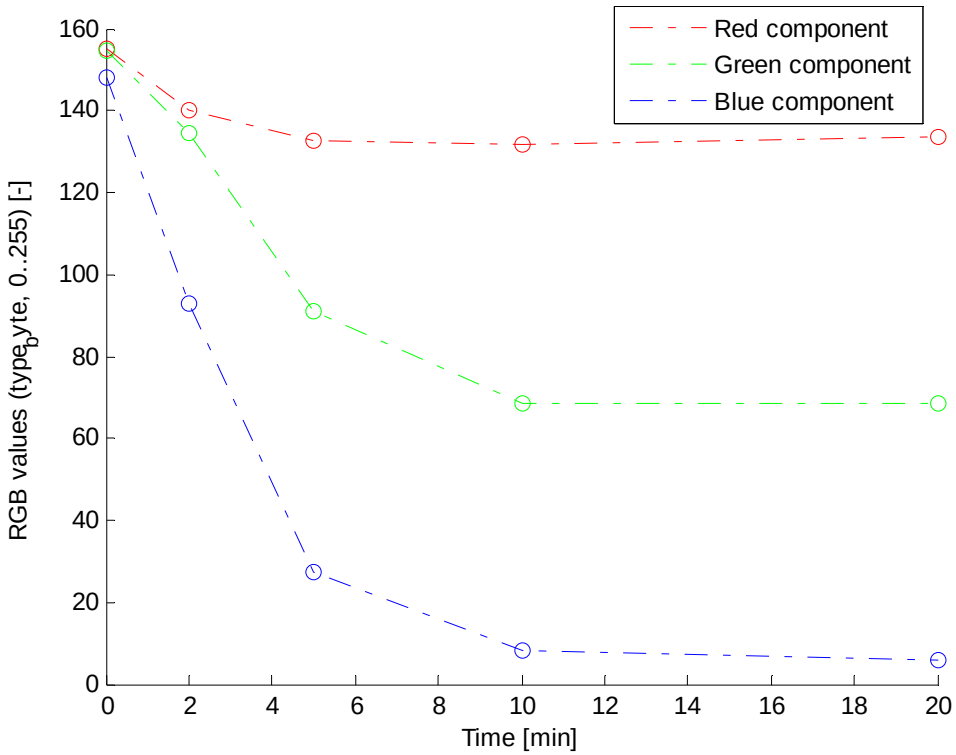


Figure 6.24 Change of RGB components during the cross-linking reaction of Hykol-E with 6 % b.w. of glutaraldehyde measured discontinuously

Moreover, four samples containing Hykol-E with 2%, 3%, 5% and 6 % b.w. of glutaraldehyde were prepared. The cross-linking reaction was continuously monitored by web-camera with CMOS sensor. Stored data in form of the video file *(*.avi*) was processed via own user function in Matlab and as a result, picture obtained from every key frame were further processed. The suitable part of the picture was evaluated as the change of RGB component. The results are shown in Figure 6.25, Figure 6.26, Figure 6.27 and Figure 6.28.

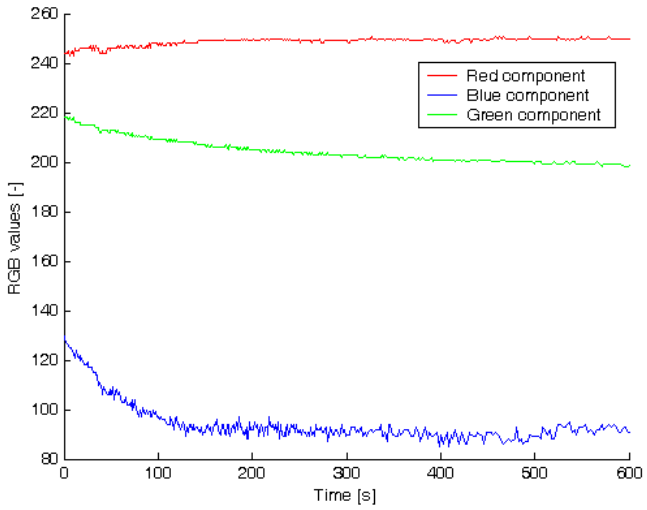


Figure 6.25 Change of RGB components during the cross-linking reaction of Hykol-E with 2 % b.w. of glutaraldehyde

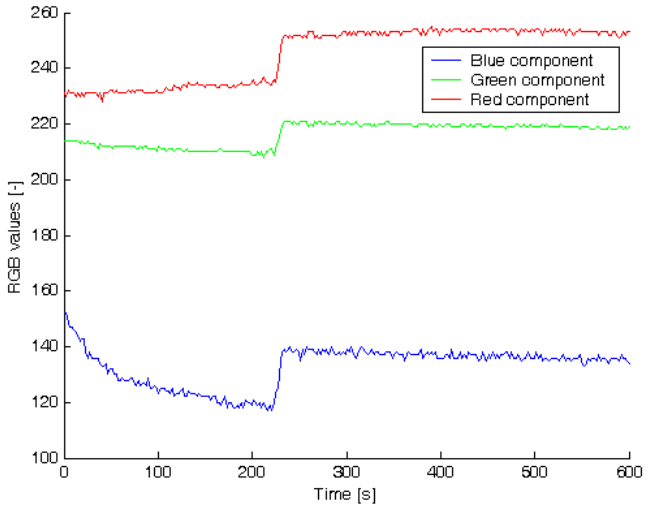


Figure 6.26 Change of RGB components during the cross-linking reaction of Hykol-E with 3 % b.w. of glutaraldehyde

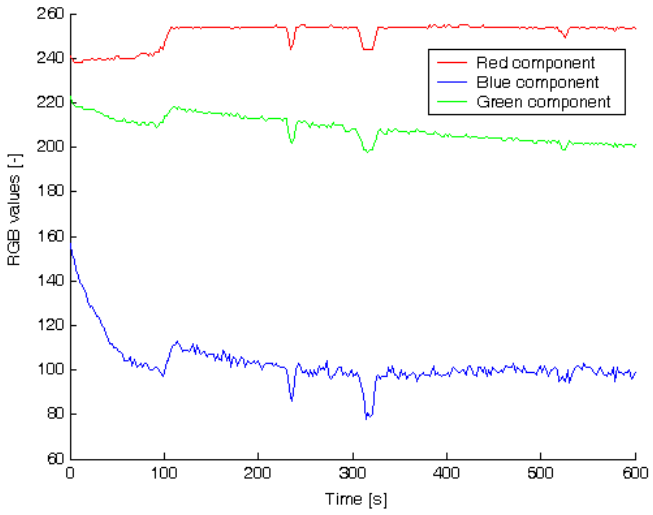


Figure 6.27 Change of RGB components during the cross-linking reaction of Hykol-E with 5 % b.w. of glutaraldehyde

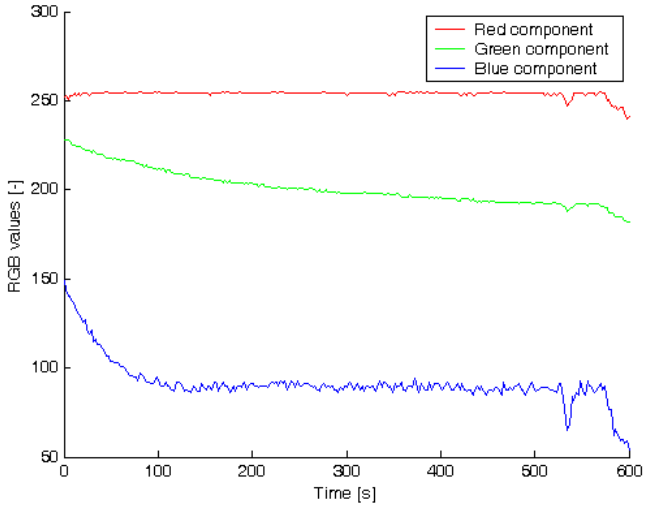


Figure 6.28 Change of RGB components during the cross-linking reaction of Hykol-E with 6 % b.w. of glutaraldehyde

As can be seen in above-mentioned pictures, every single curve is similar to each other. Some accidental disturbances occurred during the reaction. For example, in Figure 6.26, there is a step at approximately 220 second. The same problem is in Figure 6.27 and Figure 6.28, there is fluctuation of RGB values as well. Although an artificial lighting was used, some disturbances occurred due to different light conditions during the measurement. The main changes are in blue colour and these results prove absorbance measurement described above.

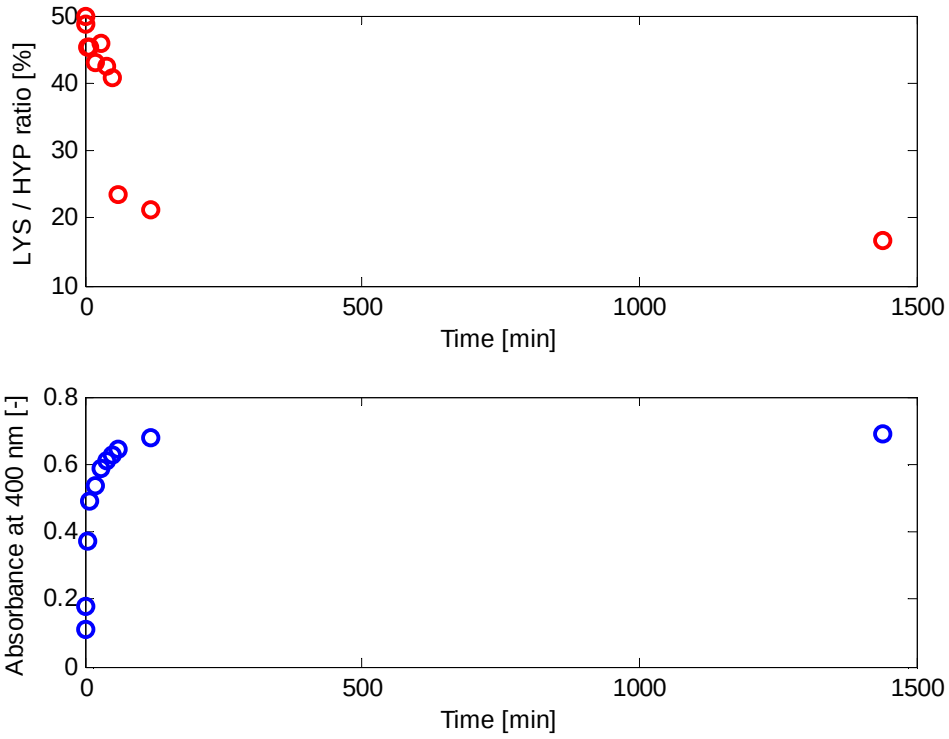


Figure 6.29 Comparison of LYS/HYP ratio measurement by gas chromatography and absorbance measurement at 400 nm by spectrophotometry.

Relationship between absorbance measurement and Lysine/Hydroxyprolyne ratio during the cross-linking process can be observed and this fact could be utilized in manufacturing practice.

In general, we can say that optical methods reflect changing values of absorbance which also means concentration of relevant aldol resin and therefore we can utilize it and control the reaction process in desired way by proper control actions. It is clear that these colour parameter measurements represent a valuable tool for the monitoring of the cross-link formation. The colorimetric methods are instrumentally simpler and allow using camera systems not only for measurement of change in colour but also for partial monitoring of manufacturing process and supervision of the production line or work safety, for example.

7 DISCUSSION OF THE RESULTS

Increasing prices of chemicals, water and energy as well as serious problems related to environmental protection and permanent leather manufacturing as a topic of growing importance, require optimization and rationalization of measurement processes in the leather industry.

In this thesis, cross-linking reaction of collagen material was chosen as tangible case of natural polymer treatment. This reaction has practical meaning especially in production of biodegradable casings, capsulates, medical supplies, in health service (singe treatment) etc. Another utilization of collagen hydrolyzate can be production of clearly natural bio-composite material. Suitably cross-linked hydrolyzate collagen together with vegetal fibres such as linen or hemp comprise matrix. These composites can be used as an adhesive material in wood industry. It could be also used instead of plastic bags for packaging.

This thesis is focused on the problems with production of artificial casings. The first step of mathematical modelling was description of the dynamic system consisted of kinetics of consecutive and lateral reactions. With respect to the fact that this is the first approach to quantitative description, we assumed that diffusion retarding influence of reactants is negligible and the reaction rate is determined by kinetics of cross-linking reaction. Quantitative description of dynamic system is represented by nonlinear vector differential equations and due to the character of the work, it was necessary to linearize these equations. Development in Taylor's series was applied with neglect of the higher order members. This procedure resulted in determination of transfer matrix, which reflects the real cross-linking system. Before control design, simulative calculations of time dependencies of individual components were carried out. Time and concentration profiles were dependent on the velocity constants, the values of which differ according to acidobasic medium of cross-linking process. In addition to the main cross-linking reaction,

another undesirable lateral reactions represented by aldol processes arise. The result of these parasitic reactions is not only the coloration of the end product, but also inconvenient sensorial quality. Cardinal advantage of mathematical simulation lies in the fact that we can estimate desirable conditions of reaction by proper change of acidobasic concentration and control the reaction in desired way.

From the reaction mechanism investigation point of view, a very valued result is the experimental measurement based on conductivity during the cross-linking process. Aldehydes were used as cross-linking agents, in our case glutaraldehyde and glyoxal, both of them being non-conducting chemicals. Conductivity of Hykol-E is practically insignificant. Molar weight of collagen hydrolyzate used for our measurements was approximately 30 kD; therefore it is considered to be polyelectrolyte, the terminal groups of which comprise closed cycle. Movement of these cycles is very small and for that reason their small conductivity can be explained. Effect of cross-linking process in the first step causes opening the cycle and simultaneously hydrogen ion is released. This activity decreases pH value of the reaction system, *i.e.* affects the velocity constants and among others, makes the system conductible as a consequence of small size. Mentioned mechanism has not been described in available literature yet.

Finding successful application of sensory methods, which can monitor cross-linking process, represents another important result. However, with regard to complexity and mutual interaction between individual reactionary steps and chosen sensory measurements, the exact principle was not sufficiently explained. Nevertheless, these methods can be useful during experimental identification of the system and for control via stochastic models. A brief overview of used measurement techniques, their advantages and disadvantages is given in the following lines.

Dielectric measurements have advantages as follows:

- quick measurement
- cheap
- historically well researched
- high sensitivity

Disadvantages:

- contact measurement
- measurement of electrical quantities carry some interferences (noise)
- problem can be high water content in sample

Spectrophotometry methods have advantages as follows:

- good reproducibility
- very accurate

Disadvantages:

- more expensive
- instrumentation is more complex
- prevention necessity of parasitic light sources

Colorimetry measurements have advantages as follows:

- nowadays very cheap
- versatile

Disadvantages:

- scene lightning influence on measured quantities

Gas Chromatography Mass Spectrometry method has advantages as follows:

- accurate reference method

Disadvantages:

- very expensive
- suitable for laboratory, not for practice and industry

- not applicable to online measurement

Suggestions for further research in this field:

- to apply mentioned methods during treatment of recyclable composites and bio-composites
- to engage the deterministic model in other cross-linking systems, for example, another aldehydes (glyoxal, acetaldehyde), enzymatic cross-linking (trans-glutaminase) or other physical procedures (ionizing radiation)

At the end I would like to remark that many measurements and trials were realised, but only some of them are published in this work. Moreover, most presented data in Appendix A - B was proportionally reduced to fixed length of 35 values over the measured interval. If I would put all measurements in this thesis, it would have mammoth size. All measured data was saved and can be available either in electronic form or in my laboratory note book.

8 OUTPUTS FOR MANUFACTURING PRACTICE

One of the most important results was the proof of serviceability of process engineering theoretical tools for rationalization, optimization and application of automated systems during natural polymer treatment. The results of mathematical simulation can determine optimal conditions of manufacturing process not only from the energy saving or environment protection point of view, but they also optimize desired mechanical and sensorial quality of the end products. The results are absolutely general and can be extrapolated; it means that verified mathematical models can be used for other processes described by the same chemical-physical mechanism. For example, kinetic equations of artificial casing cross-linking are valid for production of ecological adhesive, protective polymer colloids, capsulates, medication etc.

Another practical output is the fact that experimental methods can be directly used for control of devices producing collagen foils by blow moulding, extruding, casting or impregnation.

Treatment of natural material represented by complex and often variable input parameters produces considerable amount of waste, the economic utilization of which is still being searched for. Results of this work, both in theoretical and practical respect, can minimize formation of minor products and waste during natural polymer treatment.

REFERENCES

Abo-Ellil M. S. [et al.] Effect of fast neutrons on dielectric properties of pure and gelatin doped-poly (vinyl alcohol) films [Article] // Journal of Polymer Research. - [s.l.] : Springer Netherlands, 2000. - Volume 3 : Vol. 7. - pp. 149-153. - ISSN 1022-9760.

Anderson E. [et al.] LAPACK User's Guide, third edition [Online]. - SIAM, 1999. - http://www.netlib.org/lapack/lug/lapack_lug.html.

Angus R. B. and Hulbert T. E. VEE Pro: Practical Graphical Programming [Book]. - [s.l.] : Springer, 2004. - First Edition. - ISBN 978-1852338701.

Blažej A. [et al.] Technologie kůže a kožešin [Book]. - Praha : SNTL, 1984.

Brinckmann J., Notbohm H. and Müller P. K. Collagen: Primer in Structure, Processing and Assembly [Book]. - [s.l.] : Springer, 2005. - 1st : pp. 207-230. - ISBN 3-5402-3272-9.

Corning D. R. [Article] // Society of Leather Technologists and Chemists. - 1972. - Volume 56. - p. 95.

Covington A. D. Chrome Tanning Exploding the Perceived Myths, Preconceptions and Received Wisdom [Article] // The Journal of the American Leather Chemists Association. - [s.l.] : American Leather Chemists Association, Cincinnati, OH, ETATS-UNIS, 2001. - 12 : Vol. 96. - pp. 467-480. - ISSN 0002-9726.

Craig D. M. Dielectric spectroscopy as a novel analytical technique within the pharmaceutical sciences [Article] // STP pharma sciences. - [s.l.] : Editions de santé, Paris, 1995. - 6 : Vol. 5. - pp. 421-428. - ISSN 1157-1489.

Doi M. and See H. Introduction to Polymer Physics [Book]. - [s.l.] : Oxford University Press, 1996. - ISBN 0-19-851789-0.

Ferry J. D. Viscoelastic Properties of Polymers [Book]. - [s.l.] : Wiley, 2004. - 3rd. - ISBN 0-4710-4894-1.

Fond D. and Redwood M. Managing technology in complex networks [Article] // American Leather Chemists Association. - 2005. - Volume 100. - pp. 93-101.

Fond David and Redwood Michael Managing technology in complex networks [Article] // American Leather Chemists Association. - 2005. - Volume 100. - pp. 93-101.

Gold T. B. [et al.] Determination Of Extent Of Formaldehyde-Induced Crosslinking in Hard Gelatin Capsules By Near-Infrared Spectrophotometry [Article] // Pharmaceutical Research. - 1997. - Volume 14. - pp. 1046-1049.

Hägerström H., Edsman K. and Stromme M. Low-frequency dielectric spectroscopy as a tool for studying the compatibility between pharmaceutical gels and mucous tissue [Article] // Journal of Pharmaceutical Sciences. - [s.l.] : Wiley-Liss, Inc., 2003. - 9 : Vol. 92. - pp. 1869 - 1881.

Hedwig P. Dielectric Spectroscopy of Polymers [Book]. - Bristol : Adam Hilger Ltd., 1977.

Heidemann E. Fundamentals of leather manufacturing [Book]. - Darmstadt : Eduard Roether KG, 1993. - ISBN 3-7929-0206-0.

Hüffer S. and Taeger T. Sustainable leather manufacturing – a topic with growing importance [Article] // American Leather Chemists Association. - 2004. - Volume 99. - p. 424.

Ingham J. [et al.] Chemical Engineering Dynamics: An Introduction to Modelling and Computer Simulation [Book]. - Weinheim : VCH Verlagsgesellshaft, 2000. - ISBN 978-3-527-31678-6.

Kanagaraj J [et al.] A new approach to less-salt preservation of raw skin/hide [Article]. - 2000. - Volume 95. - p. 368.

Kanagaraj J. [et al.] A new approach to less-salt preservation of raw skin/hide [Article] // American Leather Chemists Association. - 2000. - Volume 95. - p. 368.

Kasala J. The collagen crosslinking [Book]. - Zlín : UTB, 2005.

Kausch H. H. Polymer fracture [Book]. - New York : Springer-verlag, 1978.

Kienle R. H. and Race H. H. The electrical, chemical and physical properties of alkyd resins [Article] // Transactions of the Electrochemical Society. - 1934. - Vol. 65.

Kolomazník K. [et al.] Experience in Industrial Practice of Enzymatic Dechromation of Chrome Shavings [Article] // The Journal of the American Leather Chemists Association. -

2000. - 2 : Vol. 95. - pp. 55-63.

Kremer F. and Schönhals A. Broadband dielectris spectroscopy [Book]. - [s.l.] : Springer, 2003. - 3-540-43407-0.

Křesálek V. Studie využití svazku urychlených elektronů pro síťování materiálů na bázi kolagenu // Engeneering report. - Slavičín : [autor neznámý], 1998.

Kupec J. [et al.] Effect of Cross Linking Waste Protein with Dialdehydes on Its Biodegradation under Anaerobic Conditions [Article] // Journal of Polymers and the Environment. - [s.l.] : Springer Netherlands, July 2003. - pp. 93-100. - ISSN 1566 – 2543.

Kupec J. and Ludvík J. Ekologické aspekty kožedělné výroby [Book]. - Brno : VUT, 2000.

Lairez D. [et al.] Real time dielectric measurements of network formation in a crosslinked epoxy resin system [Article] // Plastics rubber and composites processing and applications. - [s.l.] : Institute of Materials, London, 1991. - 4 : Vol. 16. - pp. 231-238. - ISSN 0959-8111.

Langmaier F. [et al.] Calorimetry of the Reactions of Hydrolysates of Chromed Shavings with Aldehydes [Article] // Journal of Thermal Analysis and Calorimetry. - [s.l.] : Akadémiai Kiadó, co-published with Springer Science+Business Media B.V., Formerly Kluwer Academic Publishers B.V., 2004. - 3 : Vol. 67. - pp. 659-666. - ISSN 1388-6150.

Langmaier F. Pomocné přípravky kožedělného průmyslu [Book]. - Brno : VUT, 1974.

Lundblad R. L. and Strauss S. Chemical Reagents for Protein Modification [Book]. - [s.l.] : CRC Press, 1983. - 3rd : pp. 285-301. - ISBN 0-8493-1983-8.

Maistros G. M. and Bucknall C. D. Modelling the Dielectric Behavior of Epoxy Resin Blends During Curing [Article] // Polymer Engineering and Science. - 1994. - 34 : Vol. 20. - pp. 1517-1528. - ISSN 0032-3888.

Marek M., Opatová H. and Voldřich M. Odpady a druhotné suroviny v zemědělsko-potravinářském komplexu [Book]. - Ostrava : Technická universita, 1996.

Mládek M. Zpracování odpadů kožedělného průmyslu [Book]. - Praha : SNTL, 1971.

Movshovich I. M. [et al.] Objective-colorimetry methods in instrumental estimation of dye quality [Article] // Measurement Techniques. - [s.l.] : Springer New York, 1985. -

Volume 28. - pp. 501-504. - ISSN 0543-1972.

Němcová I., Čermáková L. and Gasparič J. Spectrophotometric reactions [Book]. - [s.l.] : Marcel Dekker, Inc., 1996. - ISBN 0-8247-9451-6.

Nimni M. E. Collagen: Biochemistry [Book]. - Boca Raton : CRC Press Inc., 1988. - ISBN 0849346010.

Pethrick R. A. and Hayward D. Real time dielectric relaxation studies of dynamic polymeric systems [Article] // Progress in polymer science. - [s.l.] : Elsevier Science, Oxford, 2002. - 9 : Vol. 27. - pp. 1983-2017. - ISSN 0079-6700.

Phenomenex Phenomenex website [Online] // Phenomenex 2004-2005 Catalog, Introduction to Peptide and Protein HPLC: a user's guide, GC Users Guide, Zebron GC Column Selection Guide brochure, EZ:faast amino acid sample testing kit users guide & brochure. - 28 September 2004. - http://www.phenomenex.com.

Rabek J. F. Experimental Methods in Polymer Chemistry [Book]. - New York : John Wiley and Sons Ltd, 1980. - ISBN 0-4712-7604-9.

Radhakrishnan S. and Saini D. R. Dielectric monitoring of the curing process in cyanoacrylate resin [Article] // Polymer engineering and science. - [s.l.] : Society of Plastics Engineers, Brookfield, 1993. - 3 : Vol. 33. - pp. 125-131. - ISSN 0032-3888.

Shah C. S., Patni M. J. and Pandya M. V. Dielectric spectroscopy — an analytical tool for monitoring the deterioration due to high energy radiation in ethylene propylene diene terpolymer (EPDM) vulcanizates [Article] // Journal of Materials Science. - [s.l.] : Springer Netherlands, 1997. - Volume 32. - pp. 6119-6126. - ISSN 0022-2461.

Sheridan C. [et al.] Monitoring food quality using an optical fibre based sensor system—a comparison of Kohonen and back-propagation neural network classification techniques [Article] // Measurenent science and technology. - 2006. - 17. - pp. 229-234.

Shevell S. K. The Science of Color [Book]. - [s.l.] : Elsevier Science, 2003. - Second Edition. - ISBN 978-0444512512.

Stuart H. A. Die Physik der Hochpolymeren. IV. Theorie und molekulare Deutung technologischer Eigenschaften von hochpolymeren Werkstoffen [Book]. - Berlin : Springer, 1956.

Thomas O. and Burgess C. UV-visible Spectrophotometry of Water and Wastewate [Book]. - [s.l.] : Elsevier, 2007. - First Edition : Vol. Volume 27. - ISBN 0-444-53092-4.

Večeřa M. Chemické tabulky organických sloučenin [Book]. - Praha : SNTL, 1975.

Wong S. S. Chemistry of Protein Conjugation and Cross-Linking [Book]. - [s.l.] : CRC Press, 1991. - pp. 75-133. - ISBN 0-8493-5886-8.

Yoshioka S. A. and Goissis G. Thermal and spectrophotometric studies of new crosslinking method for collagen matrix with glutaraldehyde acetals [Article] // Journal of Materials Science: Materials in Medicine. - [s.l.] : Springer Netherlands, 2007. - ISSN 0957-4530.

PUBLICATION

Conference papers

NAVRÁTIL, M., KŘESÁLEK, V. (2003): *Dielectric Spectroscopy Method of Collagen Crosslinking by Glyoxal*. In: Proceedings International Carpathian Control Conference, High Tatras, Slovak Republic, p. 160 – 163.

NAVRÁTIL, M., KŘESÁLEK, V. (2003): *Measurement of Collagen Crosslinking by Dielectric Spectroscopy*. In: Proceedings of 14th International Conference on Process Control, Štrbské Pleso, Slovak Republic, Paper No. 048.

NAVRÁTIL, M., KŘESÁLEK, V. (2003): *The Bases of the Sensor Construction for Continuous Production of the Composite with Collagen*. In: Proceedings of 14th DAAAM International Symposium, Sarajevo, Bosnia and Herzegovina, p. 317 – 318.

NAVRÁTIL, M., KŘESÁLEK, V. (2004): C*ross-linking reaction of collagen hydrolyzate as a nonlinear system with time-variable parameters.* In: Proceedings of International Carpathian Control Conference, Zakopane, Poland, p. 183 – 188.

NAVRÁTIL, M., KŘESÁLEK, V. (2005): *Monitoring of cross-linking reaction of collagen hydrolyzate with glutaraldehyde by optical method*. In Proceedings of the 44th Microsymposium of P.M.M. Programme Booklet, Vol. K83, pp. 149. ISBN 80-85009-50-1. Institute of Macromolecular Chemistry of the Academy of Sciences of the Czech Republic. Prag, July 10-14.

NAVRÁTIL, M., KŘESÁLEK, V. (2005): *Síťovací reakce hydrolyzovaného kolagenu s glutardialdehydem sledovaná metodou dielektrické spektroskopie*. In Proceedings of the International expert seminar SEKEL 2005, pp. 120 – 124- ISBN 80-73-18-346-3. Rusava, Czech Republic, September 14-16.

NAVRÁTIL, M., KŘESÁLEK, V. (2005): *Development of optical sensing method for continuous production of cross-linked collagen hydrolyzate*. In Proceedings of the 9th International Research/Expert Konference „"Trends in the Development of Machinery and Associated Technology" TMT 2005, pp. 1073-1076. ISBN 9958-617-28-5. Antalya, Turkey, September 26-30.

NAVRÁTIL, M., KŘESÁLEK, V. (2005): *New measuring methods for monitoring of collagen cross–linking reaction with glutaraldehyde.* In Proceedings of the DAAAM International Symposium „"Intelligent Manufacturing & Automation", pp. 265-266. ISBN 3-901509-46-1, Opatija, Croatia, October 19-22.

NAVRÁTIL, M., KŘESÁLEK V. (2005): *Matematické modelování vícevrstvých systémů pro úpravu reflektance kovových povrchů v mikrovlnné oblasti spektra pro ochranu vojenských objektů,* In. Proc. Force protection: Significance in contemporary warfare, Brno, p. 71-77, ISBN: 80-210-3692-3

NAVRÁTIL, M., KŘESÁLEK V. (2006): *Using of microwave microscope for surface scanning*. In Proc. 7th International Scientific - Technical Conference Process Control, Kouty nad Desnou, Paper No. R219, ISBN 80-7194-860-8, June 13-16.

KŘESÁLEK V., NAVRÁTIL M. (2006): *Surface scanning with microwave microscope*. In: Proceedings of the 10th International Research/Expert Conference „Trends in the Development of Machinery and Associated Technology" TMT 2006, pp. 1267-1270. ISBN 9958-617-30-7. LLoret de Mar, Spain, September 11-15.

NAVRÁTIL, M., KŘESÁLEK, V. (2007): *Condensing reaction of collagen hydrolyzate with glutaraldehyde – mathematical modelling.* In Proceedings of the 34th International conference of SSCHE, pp. 299, ISBN 978-80-227-2640-5, Tatranské Matliare, Slovakia, May 21-25.

NAVRÁTIL, M., KOLOMAZNÍK, K., KŘESÁLEK, V. (2007): *Approach to mathematical model of cross-linking reaction of polymer composite.* In: Proceedings of 16th International Conference on Process Control, Štrbské Pleso, Slovak Republic, Paper No. 009, ISBN 978-80-227-2677-1, June 11-14

NAVRÁTIL, M., KOLOMAZNÍK, K., KŘESÁLEK, V. (2007): *Transfer matrix determination of the system of collagen cross-linking with aldehydes described by mathematical model of continuous stirred tank reactor*. In: Proceedings of the 11th International Research/Expert Conference „Trends in the Development of Machinery and Associated Technology" TMT 2007, pp. 1419-1422. ISBN 9958-617-34-X. Hammamet, Tunisia, September 05-09.

Contributions to the technical journals

KUPEC, J., CHARVÁTOVÁ, K., NAVRÁTIL, M., KŘESÁLEK, V., KŘESÁLKOVÁ M. (2003): *Effect of Cross-Linking Waste Protein with Dialdehydes on Its Biodegradation under Anaerobic Conditions*. Journal of Polymers and the Environment, Vol. 11, 3, p. 93 – 100. ISSN 1566 – 2543.

NAVRÁTIL, M., KŘESÁLEK, V. (2004): *Digital Camera Application for Monitoring of Cross-linking Reaction of Biopolymers*, Fine Mechanics and Optics, p. 78 – 80, Institute of Physics of Academy of Sciences of the Czech Republic, ISSN 0447 – 6441

NAVRÁTIL, M. (2005): *Counting cross-links.* BLC Journal, Vol. 53, p. 16 – 18.

KŘESÁLEK, V., NAVRÁTIL, M. (2005): *Surface scanning with microwave microscope*, Fine Mechanics and Optics, p. 319 – 321, Institute of Physics of Academy of Sciences of the Czech Republic, ISSN 0447 – 6441

NAVRÁTIL, M., KŘESÁLEK, V., KOLOMAZNÍK, K. (2007): *Approach to mathematical model of the cross-linking reaction of polymer composite*, AT&P Journal, (accepted and published soon)

CURRICULUM VITAE

Personal information:

Name: Ing. Milan Navrátil

Born: June 4th 1979 in Kroměříž

Address: Česká 4757, 760 05 Zlín

Phone: +420 605 832 656

E-mail: navratil@fai.utb.cz

Education:

1993 – 1997: Dairy high school in Kroměříž, branch: Milk processing, finished with A levels in1997

1997 – 2002: Tomas Bata University in Zlín, Faculty of Technology, Master's of degree (M.Sc.), branch: Automation and control technology in consumer goods industry, finished

2002 – present: Tomas Bata University in Zlín, Faculty of Applied informatics, Department of Electrotechnics and Measurements, (Ph.D.), branch: Technical cybernetics

Language knowledge:

Czech – mother tongue

English – active (writing, reading, speaking, listening)

German – passive

Study stays:

September – November 2004: Study and research at BLC Leather Technology Centre Ltd, United Kingdom

Pedagogical activities:

2002 – present: Teaching of seminars and laboratory courses: Basics of Computer

Technology and Informatics, Signal processing, Instrumentation and measurement
2003 – present: Supervising of master (3 defended) and bachelor (7 defended) thesis at Tomas Bata University in Zlín, Faculty of Applied Informatics

Work experience:

2005 – 2006: Lecturer at Tomas Bata University in Zlín, Faculty of Applied Informatics.

2006 – present: Assistant at Tomas Bata University in Zlín, Faculty of Applied Informatics
Member of the Academic Senate of Faculty of Applied Informatics at Tomas Bata University in Zlín

Grant participations:

2003: Grant project FRVŠ under No. G1/1404/2004 – main-participant

2005 – present: Grant project of Czech Ministry of Education, Youth and Sports No. MSM 7088352102 "Modelling and Control of Processing Procedures of Natural and Synthetic Polymers", co-participant

Other related activities:

2002: Attendance at SVOČ with Diploma thesis (price for 1st place)

APPENDIX A

Measurement 1		Measurement 2		Measurement 3		Measurement 4	
Time [s]	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*
22	0,3767	41	0,3841	36	0,2575	32	0,3864
43	0,3815	78	0,3886	70	0,2646	62	0,3998
63	0,3843	115	0,3912	103	0,2694	92	0,4044
83	0,3864	168	0,3937	141	0,2726	123	0,4073
105	0,3881	217	0,3958	222	0,2757	153	0,4093
127	0,3897	274	0,3974	307	0,2782	183	0,4108
147	0,3908	311	0,3980	356	0,2790	213	0,4121
179	0,3923	362	0,3991	395	0,2795	243	0,4132
235	0,3947	408	0,4001	449	0,2801	273	0,4148
295	0,3971	463	0,4010	500	0,2807	303	0,4156
354	0,3987	543	0,4014	537	0,2810	333	0,4163
400	0,4004	600	0,4025	574	0,2814	363	0,4170
440	0,4012	650	0,4033	611	0,2818	392	0,4176
480	0,4021	692	0,4037	648	0,2821	423	0,4182
520	0,4030	747	0,4041	690	0,2824	453	0,4187
561	0,4034	794	0,4046	726	0,2827	483	0,4192
601	0,4043	837	0,4047	761	0,2830	514	0,4196
635	0,4048	889	0,4052	797	0,2832	544	0,4200
685	0,4057	934	0,4051	832	0,2834	574	0,4204
723	0,4063	972	0,4054	867	0,2836	604	0,4208
765	0,4069	1019	0,4055	903	0,2838	634	0,4213
799	0,4070	1071	0,4059	938	0,2840	664	0,4215
840	0,4074	1132	0,4060	973	0,2841	694	0,4218
892	0,4080	1176	0,4065	1009	0,2843	724	0,4220
926	0,4082	1212	0,4065	1044	0,2845	754	0,4222
963	0,4087	1249	0,4067	1080	0,2846	784	0,4224
990	0,4087	1285	0,4063	1116	0,2847	814	0,4226
1027	0,4088	1323	0,4067	1151	0,2849	844	0,4228
1054	0,4092	1360	0,4069	1187	0,2850	874	0,4233
1091	0,4093	1397	0,4072	1224	0,2851	905	0,4235
1139	0,4094	1435	0,4070	1260	0,2853	934	0,4236
1176	0,4097	1473	0,4073	1296	0,2854	965	0,4237
1201	0,4099	1518	0,4075	1331	0,2855	996	0,4238
1229	0,4099	1561	0,4073	1367	0,2856	1036	0,4240
1271	0,4100	1600	0,4073	1403	0,2857	1074	0,4241

Table A.1 Sample (Hykol-E with 1% b.w. of glutaraldehyde), four same measurements of dissipation factor with respect to time.

Measurement 1		Measurement 2		Measurement 3		Measurement 4	
Time [s]	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*
22	0,2990	41	0,3593	36	0,5193	32	0,5421
43	0,4007	78	0,4803	70	0,6400	62	0,7044
63	0,4592	115	0,5502	103	0,7218	92	0,7609
83	0,5034	168	0,6184	141	0,7748	123	0,7958
105	0,5397	217	0,6739	222	0,8274	153	0,8195
127	0,5733	274	0,7181	307	0,8701	183	0,8377
147	0,5949	311	0,7340	356	0,8839	213	0,8537
179	0,6274	362	0,7632	395	0,8924	243	0,8673
235	0,6772	408	0,7901	449	0,9030	273	0,8858
295	0,7269	463	0,8155	500	0,9126	303	0,8960
354	0,7617	543	0,8256	537	0,9189	333	0,9045
400	0,7969	600	0,8545	574	0,9252	363	0,9128
440	0,8151	650	0,8779	611	0,9310	392	0,9203
480	0,8334	692	0,8875	648	0,9365	423	0,9270
520	0,8530	747	0,8974	690	0,9419	453	0,9335
561	0,8600	794	0,9118	726	0,9471	483	0,9395
601	0,8802	837	0,9144	761	0,9525	514	0,9450
635	0,8910	889	0,9278	797	0,9560	544	0,9500
685	0,9084	934	0,9241	832	0,9593	574	0,9545
723	0,9214	972	0,9333	867	0,9627	604	0,9590
765	0,9332	1019	0,9352	903	0,9657	634	0,9647
799	0,9365	1071	0,9462	938	0,9685	664	0,9679
840	0,9443	1132	0,9498	973	0,9715	694	0,9712
892	0,9568	1176	0,9626	1009	0,9742	724	0,9737
926	0,9615	1212	0,9634	1044	0,9768	754	0,9762
963	0,9719	1249	0,9695	1080	0,9792	784	0,9786
990	0,9719	1285	0,9575	1116	0,9817	814	0,9809
1027	0,9746	1323	0,9672	1151	0,9840	844	0,9835
1054	0,9821	1360	0,9743	1187	0,9862	874	0,9893
1091	0,9845	1397	0,9823	1224	0,9885	905	0,9913
1139	0,9877	1435	0,9774	1260	0,9912	934	0,9933
1176	0,9936	1473	0,9844	1296	0,9933	965	0,9947
1201	0,9968	1518	0,9909	1331	0,9953	996	0,9960
1229	0,9969	1561	0,9836	1367	0,9968	1036	0,9976
1271	0,9987	1600	0,9853	1403	0,9983	1074	0,9989

Table A.2 Sample (Hykol-E with 1% b.w. of glutaraldehyde), four same measurements of standardized dissipation factor with respect to time.

1 % b.w. of glutaraldehyde		2 % b.w. of glutaraldehyde		3 % b.w. of glutaraldehyde		4 % b.w. of glutaraldehyde		5 % b.w. of glutaraldehyde		6 % b.w. of glutaraldehyde	
Time [s]	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*
64	0,2950	48	0,2526	61	0,3180	33	0,3000	61	0,3533	35	0,3052
118	0,3047	94	0,2547	121	0,3246	64	0,3065	120	0,3490	68	0,2998
173	0,3096	140	0,2563	180	0,3277	96	0,3090	180	0,3449	101	0,2952
228	0,3125	187	0,2575	240	0,3296	127	0,3103	239	0,3413	134	0,2915
282	0,3151	233	0,2583	299	0,3309	159	0,3112	299	0,3385	167	0,2887
337	0,3170	279	0,2590	359	0,3318	190	0,3120	358	0,3361	200	0,2865
392	0,3188	326	0,2597	418	0,3327	222	0,3126	418	0,3340	233	0,2852
446	0,3199	372	0,2604	478	0,3334	253	0,3131	478	0,3322	266	0,2833
501	0,3211	418	0,2609	545	0,3341	284	0,3135	537	0,3305	299	0,2819
555	0,3219	464	0,2613	604	0,3344	316	0,3139	597	0,3291	332	0,2811
610	0,3228	511	0,2618	664	0,3347	347	0,3143	656	0,3277	366	0,2795
665	0,3234	557	0,2622	724	0,3350	379	0,3146	716	0,3264	399	0,2783
719	0,3243	604	0,2623	783	0,3353	410	0,3149	775	0,3253	432	0,2777
774	0,3250	650	0,2627	843	0,3359	442	0,3152	835	0,3242	465	0,2769
828	0,3256	696	0,2629	902	0,3360	473	0,3154	894	0,3231	498	0,2763
883	0,3260	742	0,2631	962	0,3362	504	0,3157	954	0,3221	531	0,2753
938	0,3268	789	0,2633	1022	0,3363	536	0,3159	1013	0,3212	564	0,2747
992	0,3270	835	0,2635	1081	0,3364	567	0,3161	1073	0,3203	597	0,2740
1047	0,3276	881	0,2637	1141	0,3365	599	0,3163	1132	0,3195	630	0,2738
1101	0,3280	928	0,2638	1201	0,3366	630	0,3165	1192	0,3187	663	0,2732
1156	0,3281	974	0,2639	1260	0,3367	661	0,3167	1251	0,3182	696	0,2723
1211	0,3289	1021	0,2640	1319	0,3368	693	0,3169	1311	0,3174	729	0,2722
1265	0,3289	1067	0,2643	1380	0,3369	724	0,3170	1371	0,3166	762	0,2715
1320	0,3292	1113	0,2643	1441	0,3369	755	0,3172	1431	0,3158	795	0,2714
1376	0,3294	1160	0,2643	1503	0,3370	787	0,3173	1493	0,3151	829	0,2711
1433	0,3297	1206	0,2644	1565	0,3371	818	0,3175	1555	0,3144	862	0,2701
1489	0,3300	1252	0,2645	1626	0,3372	850	0,3179	1617	0,3138	895	0,2701
1546	0,3302	1299	0,2645	1688	0,3372	881	0,3180	1678	0,3131	928	0,2693
1603	0,3305	1346	0,2646	1750	0,3372	913	0,3182	1740	0,3125	961	0,2690
1659	0,3308	1393	0,2646	1812	0,3373	944	0,3183	1802	0,3119	994	0,2689
1716	0,3311	1440	0,2647	1874	0,3373	976	0,3185	1863	0,3112	1027	0,2684
1772	0,3313	1488	0,2647	1935	0,3374	1007	0,3185	1925	0,3107	1061	0,2679
1829	0,3315	1536	0,2647	1997	0,3374	1038	0,3186	1987	0,3100	1094	0,2675
1885	0,3318	1584	0,2648	2059	0,3374	1070	0,3187	2048	0,3095	1127	0,2672
1942	0,3320	1632	0,2648	2121	0,3375	1101	0,3188	2110	0,3089	1160	0,2668

Table A.3 Sample (Hykol-E with 1% - 6 % b.w. of glutaraldehyde), measurements of dissipation factor with respect to time.

1 % b.w. of glutaraldehyde		2 % b.w. of glutaraldehyde		3 % b.w. of glutaraldehyde		4 % b.w. of glutaraldehyde		5 % b.w. of glutaraldehyde		6 % b.w. of glutaraldehyde	
Time [s]	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*
64	0,4161	48	0,3905	56	0,6646	33	0,7112	61	0,9815	35	0,9825
118	0,5690	94	0,4970	111	0,7766	64	0,8087	120	0,8868	68	0,8462
173	0,6459	140	0,5731	165	0,8287	96	0,8459	180	0,7969	101	0,7296
228	0,6918	187	0,6373	220	0,8631	127	0,8661	239	0,7188	134	0,6371
282	0,7325	233	0,6732	275	0,8843	159	0,8805	299	0,6557	167	0,5668
337	0,7637	279	0,7100	329	0,9000	190	0,8915	358	0,6028	200	0,5109
392	0,7911	326	0,7436	384	0,9141	222	0,9006	418	0,5580	233	0,4783
446	0,8092	372	0,7811	438	0,9269	253	0,9081	478	0,5180	266	0,4282
501	0,8272	418	0,8060	493	0,9370	284	0,9149	537	0,4819	299	0,3948
555	0,8405	464	0,8254	555	0,9456	316	0,9209	597	0,4496	332	0,3738
610	0,8554	511	0,8504	609	0,9511	347	0,9263	656	0,4193	366	0,3316
665	0,8637	557	0,8707	664	0,9556	379	0,9313	716	0,3913	399	0,3021
719	0,8792	604	0,8767	719	0,9597	410	0,9359	775	0,3668	432	0,2866
774	0,8889	650	0,8969	773	0,9640	442	0,9399	835	0,3417	465	0,2680
828	0,8990	696	0,9060	828	0,9679	473	0,9438	894	0,3181	498	0,2529
883	0,9056	742	0,9164	882	0,9773	504	0,9472	954	0,2966	531	0,2255
938	0,9184	789	0,9270	937	0,9786	536	0,9506	1013	0,2767	564	0,2116
992	0,9218	835	0,9362	992	0,9810	567	0,9536	1073	0,2572	597	0,1925
1047	0,9301	881	0,9436	1047	0,9830	599	0,9566	1132	0,2395	630	0,1892
1101	0,9368	928	0,9506	1101	0,9848	630	0,9593	1192	0,2223	663	0,1735
1156	0,9391	974	0,9556	1156	0,9863	661	0,9619	1251	0,2108	696	0,1495
1211	0,9509	1021	0,9619	1210	0,9878	693	0,9650	1311	0,1925	729	0,1486
1265	0,9513	1067	0,9754	1265	0,9890	724	0,9674	1371	0,1754	762	0,1301
1320	0,9557	1113	0,9745	1319	0,9900	755	0,9697	1431	0,1593	795	0,1280
1376	0,9585	1160	0,9748	1375	0,9913	787	0,9719	1493	0,1443	829	0,1208
1433	0,9641	1206	0,9785	1431	0,9925	818	0,9742	1555	0,1278	862	0,0940
1489	0,9677	1252	0,9837	1487	0,9933	850	0,9805	1617	0,1139	895	0,0943
1546	0,9724	1299	0,9864	1544	0,9943	881	0,9826	1678	0,1004	928	0,0754
1603	0,9767	1346	0,9893	1601	0,9949	913	0,9845	1740	0,0858	961	0,0681
1659	0,9813	1393	0,9908	1658	0,9965	944	0,9869	1802	0,0726	994	0,0650
1716	0,9858	1440	0,9929	1714	0,9968	976	0,9890	1863	0,0584	1027	0,0513
1772	0,9897	1488	0,9948	1771	0,9970	1007	0,9902	1925	0,0459	1061	0,0381
1829	0,9929	1536	0,9968	1828	0,9982	1038	0,9915	1987	0,0320	1094	0,0299
1885	0,9966	1584	0,9982	1884	0,9993	1070	0,9929	2048	0,0199	1127	0,0222
1942	1,0000	1632	0,9983	1941	0,9996	1101	0,9944	2110	0,0075	1160	0,0114

Table A.4 Sample (Hykol-E with 1% - 6 % b.w. of glutaraldehyde), measurements of standardized dissipation factor with respect to time.

1 % b.w. of glyoxal		2 % b.w. of glyoxal		3 % b.w. of glyoxal		4 % b.w. of glyoxal		5 % b.w. of glyoxal		6 % b.w. of glyoxal	
Time [s]	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*	*Time [s]*	*D [-]*
35	0,2136	31	0,1563	36	0,2582	36	0,2834	37	0,3870	37	0,2842
68	0,2214	61	0,1569	71	0,2603	71	0,2865	72	0,3867	70	0,2824
101	0,2253	91	0,1575	106	0,2610	106	0,2870	107	0,3837	103	0,2807
134	0,2279	121	0,1580	141	0,2612	141	0,2872	141	0,3808	136	0,2792
167	0,2302	151	0,1586	176	0,2612	176	0,2872	176	0,3776	169	0,2779
201	0,2328	181	0,1589	211	0,2614	211	0,2871	211	0,3749	202	0,2768
236	0,2349	211	0,1591	245	0,2614	246	0,2868	245	0,3722	235	0,2758
272	0,2369	241	0,1594	280	0,2612	281	0,2866	280	0,3702	269	0,2750
305	0,2386	271	0,1596	315	0,2610	316	0,2864	315	0,3684	302	0,2743
338	0,2399	301	0,1599	349	0,2610	351	0,2863	349	0,3668	335	0,2737
372	0,2410	331	0,1603	384	0,2608	386	0,2861	384	0,3653	368	0,2731
405	0,2422	361	0,1603	419	0,2606	421	0,2858	419	0,3640	401	0,2726
438	0,2434	391	0,1606	454	0,2606	455	0,2855	453	0,3628	434	0,2722
471	0,2446	421	0,1606	489	0,2605	490	0,2852	488	0,3621	467	0,2718
505	0,2460	451	0,1608	523	0,2604	525	0,2850	523	0,3610	500	0,2715
538	0,2468	481	0,1610	558	0,2603	560	0,2847	557	0,3601	533	0,2711
571	0,2479	511	0,1611	593	0,2603	595	0,2846	592	0,3593	566	0,2708
605	0,2488	541	0,1612	628	0,2603	630	0,2845	627	0,3585	599	0,2705
638	0,2501	571	0,1613	662	0,2602	665	0,2843	661	0,3578	633	0,2703
672	0,2509	601	0,1615	697	0,2601	700	0,2841	696	0,3573	665	0,2700
705	0,2514	631	0,1616	732	0,2601	735	0,2839	730	0,3569	699	0,2698
738	0,2522	661	0,1617	766	0,2601	770	0,2837	765	0,3565	732	0,2697
771	0,2531	691	0,1617	801	0,2600	805	0,2835	800	0,3559	765	0,2695
805	0,2535	721	0,1618	836	0,2599	840	0,2833	834	0,3555	798	0,2692
838	0,2542	751	0,1619	871	0,2599	875	0,2831	869	0,3552	831	0,2691
871	0,2547	781	0,1620	906	0,2598	910	0,2831	904	0,3549	864	0,2689
905	0,2552	811	0,1629	940	0,2598	945	0,2829	939	0,3545	897	0,2688
938	0,2558	841	0,1629	975	0,2599	980	0,2827	974	0,3541	930	0,2686
972	0,2564	871	0,1630	1011	0,2595	1015	0,2826	1010	0,3539	963	0,2685
1005	0,2567	901	0,1631	1045	0,2593	1050	0,2824	1046	0,3537	996	0,2684
1039	0,2569	931	0,1631	1080	0,2592	1085	0,2823	1081	0,3533	1029	0,2682
1072	0,2576	961	0,1632	1115	0,2592	1120	0,2822	1117	0,3531	1062	0,2681
1105	0,2581	991	0,1633	1150	0,2591	1155	0,2821	1153	0,3531	1095	0,2680
1138	0,2586	1021	0,1634	1184	0,2592	1189	0,2819	1189	0,3528	1128	0,2679
1172	0,2588	1051	0,1634	1219	0,2591	1224	0,2819	1224	0,3525	1161	0,2678

Table A.5 Sample (Hykol-E with 1% - 6 % b.w. of glyoxal), measurements of dissipation factor with respect to time.

1 % b.w. of glyoxal		2 % b.w. of glyoxal		3 % b.w. of glyoxal		4 % b.w. of glyoxal		5 % b.w. of glyoxal		6 % b.w. of glyoxal	
Time [s]	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*	*Time [s]*	*D' [-]*
35	0,2195	31	0,1540	36	0,9019	36	0,9042	37	0,9868	37	1,0000
68	0,3515	61	0,2151	71	0,9622	71	0,9804	72	0,9771	70	0,8887
101	0,4183	91	0,2967	106	0,9821	106	0,9936	107	0,8915	103	0,7878
134	0,4630	121	0,3477	141	0,9899	141	0,9995	141	0,8077	136	0,7002
167	0,5031	151	0,4179	176	0,9878	176	0,9986	176	0,7183	169	0,6223
201	0,5468	181	0,4554	211	0,9942	211	0,9973	211	0,6412	202	0,5537
236	0,5827	211	0,4881	245	0,9945	246	0,9901	245	0,5639	235	0,4952
272	0,6174	241	0,5175	280	0,9897	281	0,9830	280	0,5068	269	0,4472
305	0,6461	271	0,5479	315	0,9836	316	0,9782	315	0,4556	302	0,4072
338	0,6687	301	0,5727	349	0,9827	351	0,9752	349	0,4077	335	0,3699
372	0,6880	331	0,6206	384	0,9776	386	0,9722	384	0,3666	368	0,3324
405	0,7080	361	0,6233	419	0,9716	421	0,9644	419	0,3275	401	0,3020
438	0,7294	391	0,6602	454	0,9709	455	0,9557	453	0,2940	434	0,2785
471	0,7500	421	0,6661	489	0,9674	490	0,9485	488	0,2747	467	0,2570
505	0,7739	451	0,6886	523	0,9659	525	0,9443	523	0,2431	500	0,2349
538	0,7871	481	0,7036	558	0,9624	560	0,9365	557	0,2160	533	0,2136
571	0,8051	511	0,7183	593	0,9629	595	0,9342	592	0,1928	566	0,1936
605	0,8213	541	0,7278	628	0,9634	630	0,9305	627	0,1719	599	0,1798
638	0,8433	571	0,7394	662	0,9598	665	0,9265	661	0,1521	633	0,1632
672	0,8567	601	0,7729	697	0,9570	700	0,9205	696	0,1374	665	0,1470
705	0,8662	631	0,7767	732	0,9581	735	0,9176	730	0,1245	699	0,1368
738	0,8789	661	0,7934	766	0,9560	770	0,9122	765	0,1131	732	0,1262
771	0,8953	691	0,7946	801	0,9535	805	0,9070	800	0,0984	765	0,1157
805	0,9023	721	0,8039	836	0,9528	840	0,9018	834	0,0864	798	0,1021
838	0,9143	751	0,8161	871	0,9511	875	0,8961	869	0,0761	831	0,0915
871	0,9219	781	0,8331	906	0,9489	910	0,8961	904	0,0672	864	0,0834
905	0,9312	811	0,9305	940	0,9489	945	0,8901	939	0,0564	897	0,0748
938	0,9400	841	0,9396	975	0,9502	980	0,8862	974	0,0444	930	0,0645
972	0,9511	871	0,9488	1011	0,9401	1015	0,8848	1010	0,0389	963	0,0563
1005	0,9569	901	0,9539	1045	0,9357	1050	0,8799	1046	0,0327	996	0,0521
1039	0,9596	931	0,9623	1080	0,9326	1085	0,8758	1081	0,0240	1029	0,0418
1072	0,9712	961	0,9737	1115	0,9306	1120	0,8725	1117	0,0177	1062	0,0314
1105	0,9806	991	0,9846	1150	0,9290	1155	0,8700	1153	0,0157	1095	0,0263
1138	0,9880	1021	0,9954	1184	0,9329	1189	0,8673	1189	0,0091	1128	0,0234
1172	0,9919	1051	0,9981	1219	0,9284	1224	0,8653	1224	0,0011	1161	0,0128

Table A.6 Sample (Hykol-E with 1% - 6 % b.w. of glyoxal), measurements of standardized dissipation factor with respect to time.

	Epoxy resin	Hykol-E
Time [s]	*Temperature[°C]*	
13	22,9	22,4
27	22,9	22,3
41	23,0	22,3
55	23,2	22,3
69	23,3	22,3
83	23,3	22,3
97	23,3	22,3
111	23,6	22,3
125	23,8	22,3
139	24,1	22,2
153	24,8	22,2
167	30,5	22,2
181	36,8	22,1
195	40,2	22,1
209	39,5	22,1
223	37,9	22,0
237	36,4	22,0
251	35,1	22,0
265	33,6	22,0
279	32,3	22,0
293	31,2	22,0
307	30,3	21,9
321	29,5	22,0
335	28,8	22,0
349	28,1	22,0
363	27,9	22,0
377	27,4	21,9
391	26,7	22,0
405	26,2	21,9
419	25,8	21,9
433	25,5	21,9
447	25,2	21,9
461	25,0	21,9
475	24,8	22,0
489	24,6	21,9

Table A.7 Temperature changes of Hykol-E during cross-linking reaction with 6 % b.w. of glutaraldehyde at laboratory temperature 22.7°C.

APPENDIX B

	Sample					
	distilled water	without coss-linking agent	after 2'	after 5'	after 10'	after 20'
Wavelength [nm]	Absorbance [-]					
206	1,3209	2,3666	2,5172	2,6267	2,5450	2,4881
223	0,6914	1,4152	2,2841	2,4994	2,6089	2,5186
240	0,4317	0,7072	1,4068	1,9367	2,3509	2,4410
257	0,3041	0,5262	1,0298	1,6047	2,1018	2,2780
274	0,2521	0,4449	0,8264	1,3812	1,9017	2,1107
291	0,2171	0,3479	0,6675	1,0753	1,4775	1,6210
308	0,1897	0,2998	0,5922	0,9278	1,2602	1,3835
325	0,1650	0,2592	0,4768	0,7978	1,0824	1,1857
342	0,1455	0,2128	0,3727	0,6654	0,9082	0,9942
359	0,1301	0,1787	0,3049	0,5639	0,7620	0,8424
376	0,1077	0,1458	0,2399	0,4683	0,6332	0,6972
393	0,0878	0,1173	0,1926	0,3941	0,5231	0,5743
410	0,0824	0,1055	0,1680	0,3480	0,4492	0,4901
427	0,0748	0,0961	0,1475	0,3149	0,3925	0,4307
444	0,0643	0,0833	0,1272	0,2724	0,3335	0,3635
461	0,0524	0,0676	0,1026	0,2174	0,2688	0,2928
478	0,0336	0,0507	0,0757	0,1720	0,2167	0,2361
495	0,0295	0,0446	0,0646	0,1485	0,1849	0,1997
512	0,0220	0,0337	0,0523	0,1242	0,1552	0,1657
529	0,0089	0,0192	0,0346	0,0974	0,1239	0,1335
546	0,0022	0,0110	0,0254	0,0808	0,1011	0,1094
563	0,0029	0,0137	0,0238	0,0739	0,0920	0,0964
580	0,0054	0,0129	0,0210	0,0640	0,0797	0,0840
597	0,0040	0,0122	0,0213	0,0588	0,0704	0,0751
614	0,0032	0,0084	0,0171	0,0509	0,0612	0,0612
631	0,0035	0,0082	0,0124	0,0450	0,0507	0,0520
648	0,0060	0,0077	0,0143	0,0434	0,0503	0,0495
665	0,0056	0,0107	0,0150	0,0394	0,0448	0,0425
682	0,0041	0,0076	0,0124	0,0349	0,0404	0,0371
699	-0,0008	0,0029	0,0057	0,0271	0,0289	0,0294
716	-0,0015	0,0002	0,0048	0,0232	0,0237	0,0269
733	0,0140	0,0178	0,0201	0,0362	0,0390	0,0394
750	0,0257	0,0300	0,0307	0,0508	0,0493	0,0469
767	0,0361	0,0396	0,0457	0,0556	0,0536	0,0573
784	0,0397	0,0525	0,0504	0,0659	0,0631	0,0674

Table B.1 UV/Visible spectrum for variously cross-linked Hykol-E with 6 % b.w. of glutaraldehyde

	Sample		
	2 % b.w. of glutaraldehyde	4 % b.w. of glutaraldehyde	7 % b.w. of glutaraldehyde
Time [s]	Absorbance [-]		
18	1,0696	1,0665	1,1774
38	1,0911	1,0874	1,2545
58	1,0912	1,1329	1,3438
77	1,1169	1,1789	1,4931
97	1,1363	1,2035	1,5832
117	1,1589	1,2566	1,6964
137	1,1752	1,3094	1,8076
157	1,2052	1,3763	1,9055
176	1,2311	1,4095	1,9928
196	1,2490	1,4618	2,0287
216	1,2617	1,4984	2,0354
236	1,2879	1,5437	2,1449
256	1,3183	1,6067	2,1497
275	1,3472	1,6480	2,1562
295	1,3748	1,6665	2,1174
315	1,3928	1,7135	2,2759
335	1,4026	1,6975	2,2912
355	1,4108	1,7767	2,2168
374	1,4424	1,7904	2,1565
394	1,4452	1,8173	2,2320
414	1,4842	1,8662	2,2486
434	1,4997	1,8898	2,2009
454	1,4946	1,8957	2,1635
473	1,5422	1,9014	2,2725
493	1,5415	1,8761	2,2411
513	1,5347	1,9018	2,2967
533	1,5746	1,9269	2,2097
553	1,5615	1,9399	2,2933
572	1,6039	1,9882	2,4174
592	1,5890	1,9902	2,2444
612	1,6287	2,0302	2,2354
632	1,6153	2,0485	2,2234
652	1,6429	1,9969	2,2306
671	1,6550	2,0859	2,3273
691	1,6404	2,0716	2,1902

Table B.2 Time dependence of absorbance during the cross-linking reaction of Hykol-E with various amount of glutaraldehyde

APPENDIX C

		IS	Lys	Ratio	Area	N[nmol]	Navg[nmol]
Standards	**Standard 20a**	684 640	187 556	0,2739	0,0000	0	
	Standard 20b	1 717 100	506 584	0,2950	0,2845	4	
	Standard 40a	1 516 361	1 295 410	0,8543			
	Standard 40b	1 460 309	1 061 057	0,7266	0,7904	8	
Sample 1	**Sample 1-1a**	1 018 854	676 523	0,6640			
	Sample 1-1b	229 779	186 121	0,8100	0,7370	7,833	
	Sample 1-2a	1 882 399	1 510 342	0,8023			**7,96**
	Sample 1-2b	1 362 777	983 785	0,7219	0,7621	8,087	
Sample 2	**Sample 2-1a**	2 348 097	10 503 032	4,4730			
	Sample 2-1b	374 178	2 530 905	6,7639	5,6185	57,240	
	Sample 2-2a	3 166 652	11 455 416	3,6175			**51,80**
	Sample 2-2b	2 109 142	11 534 242	5,4687	4,5431	46,356	

Table C.1 Amino-acid analysis: Measurements by gas chromatography method used for free lysine determination in Sample 2 (50 mg of LYSINE + 10,25 ml of 0,1M $NaHCO_3$) and in the Sample 1, which is the same solution plus excess of glutaraldehyde (0,25 ml). Samples were further diluted as follows: 1 ml of previous samples + 9 ml of water then 2 ml of previous samples + 8 ml of 0,1M $NaHCO_3$ and then 0,1 ml of previous samples were used for amino-acid analysis.

		IS	Lys	Ratio	Area	N[nmol]	Navg[nmol]
Standards	**Standard 20a**	2 317 384	714 749	0,308429	0	0	
	Standard 20b	1 403 948	394 396	0,280919	0,294674241	4	
	Standard 40a	1 943 159	1 259 158	0,647995			
	Standard 40b	3 966 756	2 027 826	0,511205	0,579600239	8	
Sample 1	**Sample 1-1a**	1 154 075	1 392 901	1,206941			
	Sample 1-1b	1 198 099	967 147	0,807235	0,930572781	12,813	
	Sample 1-2a	1 173 135	912 162	0,777542			**13,61**
	Sample 1-2b	762 257	1 002 944	1,315756	1,046648968	14,414	

Table C.2 Amino-acid analysis: Measurements by gas chromatography method used for free lysine determination in Sample 1 (50 mg of lysine + 10 ml of 0,1M NaHCO3 + 0,25 ml of glutaraldehyde). Samples were further diluted as follows: 1 ml of previous sample + 4 ml of water + 5 ml of 6M HCl then 2 ml of previous sample + 9,6 ml of 0,68M NaOH and then 0,1 ml of previous sample was used for amino-acid analysis.

		IS	Lys	Ratio	Area	N[nmol]	Navg[nmol]
Standards	Standard 20a	1 302 327	219 240	0,168345	0	0	
	Standard 20b	2 151 791	287 754	0,133728	0,151036244	4	
	Standard 40a	2 271 699	604 655	0,266169			
	Standard 40b	2 577 196	635 618	0,246632	0,256400118	8	
Sample 2	Sample 2a	2 505 177	1 560 595	0,622948			
	Sample 2b	1 266 754	1 155 125	0,911878	0,767412959	23,670	
	Sample 2c	741 621	1 110 226	1,497026			**29,92**
	Sample 2d	1 034 012	868 319	0,839757	1,168391644	36,162	

Table C.3 Amino-acid analysis: Measurements by gas chromatography method used for free lysine determination in Sample 2 (50 mg of lysine + 10,25 ml of 0,1M NaHCO3). Samples were further diluted as follows: 1 ml of previous sample + 4 ml of water + 5 ml of 6M HCl then 0,1 ml of previous sample + 9,9 ml of 0,1M NaHCO3 and then 2 ml of previous sample was used for amino-acid analysis.

Sample	Free LYSINE content in diluted sample [nmol]	Free LYSINE content in original sample [nmol]
Theoretical value	**534**	**273 823**
Lysine	518	265 467
Lysine + Acid	299	153 319
Lysine + Glutaraldehyde	80	40 796
Lysine + Glutaraldehyde + Acid	136	80 935

Table C.4 Determination of free lysine content in original samples recalculated from diluted samples.

			IS	Lysine	Hydroxyproline	Lys Ratio	Hyp Ratio	Area Lys	Area Hyp	N [nmol]		
Measurement 1	Standards	**Standard 20a**	2 304 790	352 985	583 986	0,1532	0,2534	0,0000	0,0000	0		
		Standard 20b	2 376 347	273 034	396 847	0,1149	0,1670	0,1340	0,2102	4		
		Standard 40a	2 502 540	772 822	716 722	0,3088	0,2864					
		Standard 40b	2 990 976	1 080 702	1 369 416	0,3613	0,4578	0,3351	0,3721	8		
	Hykol-E	**Sample 1**	1 164 166	1 032 958	5 242 096	0,8873	4,5029	~~21,443781~~	~~96,66402~~			Navg [nmol]
		Sample 2	3 260 569	2 129 761	9 547 382	0,6532	2,9281	15,8565	62,7986		LYS	HYP
		Sample 3	2 384 363	1 481 443	7 519 763	0,6213	3,1538	15,0958	67,6512		30 952	130 450
Measurement 2	Standards	**Standard 20a**	4 286 127	2 028 707	2 386 558	0,4733	0,5568	0,0000	0,0000	0		
		Standard 20b	6 124 840	2 895 823	3 795 740	0,4728	0,6197	0,4731	0,5883	8		
		Standard 40a	7 112 166	6 798 738	6 672 275	0,9559	0,9381					
		Standard 40b	7 387 596	5 591 840	5 191 367	0,7569	0,7027	0,8564	0,8204	12		
	Hykol-E	**Sample 1**	9 255 015	5 237 700	24 999 899	0,5659	2,7012	8,3993	38,8075			Navg [nmol]
		Sample 2	11 683 364	5 850 305	24 757 148	0,5007	2,1190	~~7,4857564~~	~~30,49433~~		LYS	HYP
		Sample 3	10 051 744	6 655 541	28 375 548	0,6621	2,8229	9,7565	40,4814		18 156	79 289
Measurement 3	Standards	**Standard 20a**	7 333 293	1 108 648	2 312 518	0,1512	0,3153	0,0000	0,0000	0		
		Standard 20b	5 851 768	816 593	1 314 482	0,1395	0,2246	0,1454	0,2700	8		
		Standard 40a	7 033 024	3 055 109	3 000 762	0,4344	0,4267					
		Standard 40b	7 770 883	3 880 536	4 289 986	0,4994	0,5521	0,4669	0,4894	12		
	Hykol-E	**Sample 1**	8 137 797	2 585 430	20 951 183	0,3177	2,5746	9,8209	64,8745			Navg [nmol]
		Sample 2	8 893 474	3 492 226	22 672 086	0,3927	2,5493	11,8017	63,6816		LYS	HYP
		Sample 3	12 326 060	3 522 990	24 086 906	0,2858	1,9541	8,8722	48,9722		20 330	118 352

Total average:	**LYS**	**HYP**
	23 146	**109 364**

Table C.5 AminoAcid analysis: Measurements by gas chromatography method for detection of free lysine and hydroxyproline in analyzed sample of Hykol-E.

Time [min]	Ratio Lys/Hyp [-]	Absorbance at 400 nm [-]
0	49,9	0,112
2	48,8	0,180
5	45,4	0,372
10	45,2	0,491
20	43,1	0,536
30	46,0	0,586
40	42,6	0,612
50	40,8	0,630
60	23,5	0,645
120	21,1	0,680
1440	16,7	0,691

Table C.6 Comparison of results obtained by gas chromatography and spectroscopy methods during cross-linking process of Hykol-E with glutaraldehyde.