Date: 10.1. 2008 (English version)
Assessor: Juraj Kasala, Ph.D.
For: **Assessment of PhD thesis by Milan Navrátil: „Instrumentation and diagnostics of polymer composites."**

Originality and up-to-date of the topic:

The topic of this thesis is up-to date. Despite of the fact that the crosslinking reactions plays very important role in the collagen film/casing industry there is a lack of work done in solving problems regarding collagen crosslinking kinetics and description of the crosslinking reactions.

Relevance of the results, fulfilment of the objectives established in the thesis

With the exception of the objective „Processing of accessible information about collagen crosslinking" all of the given objectives were completed. The missing information above was replaced by description in details of present state of the related analytical methods.

Procedure of the problem solving, results of the thesis, actual contribution of the author

The significant contribution of this work is the simulating of the crosslinking reaction, which I have not observed before. It would be useful to compare obtained results to the practical observations.

The mathematical derivations in the theoretical part of the citied work is impressive and contributing part of the work. What is important, the mathematical simulations are presented in graphs. Noticeable is the difference among the plotted lines when the different rate constants are included in the simulation. Especially in case of k3 - the mentioned phenomenon is very often noticed in practice when the polymerisation of the glutaraldehyde is observed.

The graph simulating the continuously mixed reactor is not published, it would be interesting to compare it with the other types of simulated systems.

I see very important that the author used the unusual procedures and methods in process of the chemical reaction observation, such as dielectric spectroscopy, which is very novel method in field of collagen films production. I acknowledge that the author dealt with the repeatability of the measurement, so important for industrial practise. I would like to ask the author , what is the reason for variability of the dissipative factor D?

The other question is if the concentration of the said Hykol influences the factor Dn?

Value to practise :

The thesis is applicable and could have a wider value to practise. The use of dielectric spectroscopy is the novel approach for research in field of the collagen crosslinking reactions. Especially it is possible to measure kinetics of the crosslinking reaction also in case of non diluted solutions and mixtures.

The experiments with gas chromatography presented applicability for study of crosslinking reactions by means of hydroxyproline decrease. I would like to give a question: What was the procedure of the sample Hykolu-E preparation when determined by use of gas chromatography?

The optical method can be used in the industry, the limitation is the pollution of the used solutions.

Formal aspects

Excellent

I appreciate the use of English.

Bibliography

Pass without emendation

Recommendation:

Pass without emendation. The thesis is acceptable.

Report on Doctoral Thesis entitled 'Instrumentation and Diagnostics of Polymer Composites'

Doctoral Candidate: Milan Navratil

Supervisor: Prof Ing Karel Kolomaznik DrSc

Examiner: Professor Geoffrey Attenburrow, The University of Northampton

This thesis is concerned with an investigation of theoretical and experimental issues related to the characterization and measurement of the collagen crosslinking process. It is particularly aimed at better utilization of waste products from leather production and the use of collagen to prepare edible food casings.

There is an introduction chapter where there is general discussion about issues involved with processing leather and dealing with waste collagen arising from this and how it may be used in added value applications such as food casings. This is followed by a chapter on 'state of the art' which discusses the need to develop theoretical processing models and the requirement to be able to measure the development of crosslinking extent over time. In the latter case the possibility of taking some approaches developed for polymer physics is discussed. Both of these chapters are well written and make appropriate reference to existing literature in the field. Research objectives are also clearly stated.

The main bulk of the thesis comprises two substantial chapters one dealing with theory and the other dealing with experimental findings.

In the theory chapter there is a major section which deals with the development by the author of mathematical systems which can be used to model the changes in concentrations of components occurring during crosslinking of collagen in both a batch tank reactor and a continuous stirred tank reactor. In the latter case the progress reported is impressive given the complexity of the analysis required.

Following this there is a very interesting section in which the technique of dielectric spectroscopy is discussed in detail and proposed as a tool for evaluating the extent of crosslink development in a collagen. The technique has not been applied to such a system before. Various issues are considered from a theoretical and practical point of view. The theory of spectrophotometry is also reviewed.

The following chapter presents and discusses the experimental work which the candidate carried out. Materials used and instrumental techniques used are described. The results for Dielectric Spectroscopy are given in the form of standardized dissipation factor vs time in a range collagen hydrolisate – glutaraldehyde systems and were obtained continuously during reaction with the crosslinker. The novel results obtained are of considerable interest and demonstrate that the approach has significant promise for industrial practice.

Results are also reported for experiments in which spectrophotometry was used to continuously monitor the absorbance of a system during crosslinking and again the potential for industrial monitoring is apparent.

A further evaluation of the use of digital camera with optical sensor to continuously monitor crosslinking by measuring colour changes is reported.

Another procedure used was to measure free lysine/hydroxyproline ratio using gas chromatography. It is shown that this indicates crosslinking extent and correlates well with spectrophotometry data but that the technique is too expensive to use on an industrial scale.

The closing chapters review and further discuss the theoretical and experimental achievements of the research and discuss the wider implications of the results for industrial practice.

There is a comprehensive list of references as well as an impressive list of presentations and publications completed by the candidate and based on the work reported in the thesis.

In conclusion this reviewer finds that the thesis is well structured and presented and is written in a clear and unambiguous style. Novel findings concerning the mathematical modeling and experimental monitoring of collagen crosslinking reactions have been presented and discussed. These developments and advances in knowledge are clearly of significant importance to the collagen processing industry. The thesis demonstrates that the candidate has achieved a degree of research achievement worthy of the award of PhD.

G E Attenburrow

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QUÍMICAS Y AMBIENTALES
DE BARCELONA
"JOSEP PASCUAL VILA" (IIQAB)

REPORT ON DOCTORAL THESIS ENTITLED:
INSTRUMENTATION AND DIAGNOSIS OF POLYMER COMPOSITES .

DOCTORAL CANDIDATE : Milan Navrátil.

SUPERVISOR : Prof. Ing. Karel Kolomaznik Dr.Sc.

EXAMINER : Professor Jaume Cot, C.S.I.C. – Barcelona (Spain).

Leather Industry is one traditional industrial sector pollutant; therefore this Thesis contributes significantly to environment protection, thus moving towards a more sustainable developments. Leather manufacturing industries produce a considerable amount of waste which has a strong environmental impact. Main objective of this work is to minimise the amount of waste by recycling/processing them with the application of new technologies in order to obtain a new type of biopolymer that can be used as sausage casings. Specifically relevant are the chrome (III)-tanned wastes, which have a potential grade of toxicity by the oxidation of small amounts of chrome (III) to chrome (VI). Those wastes are treated by proteolitic enzymes; and the collagen hydrolysate obtained is submitted to a crosslinking reaction by glutaraldehyde.

This work is divided in two parts : a) theoretical and b) experimental.The former deals with modelling the micro and macro kinetic crossslinking reaction by means of glutaraldehyde. A complete study of the optimisation of the natural-based intermediates products was accomplished and several mathematical designs of non-linear differential equations were created for a better control of the kinetics speed of the crosslinking reaction with glutaraldehyde and also glyoxal.

Modelling is the first and usually the most complex step due to the high number of parameters that playing an important role in this reactions; such as : temperature, pressure, time, flow, concentration, etc. It is very well described the two parts of this mathematical design: a) empiric, which allows to obtain a second order surface, adjusted, therefore , optimum conditions can be found and b) functional, which searchs a function that could rule the whole process studied. Furthermore, a detailed description of a set of non lineal differential equations which are matrix transformed into lineal is given; in order to calculate the correspondent coefficients of this main equation. Different issues are considered and developed, such as : matter and heat balances, a detailed kinetic methods of analysis, first and second order reactions, model of batch tank reactor, model of continous stirred tank reactor, etc.

Another fundamental part of this Thesis is related to monitoring the course of the crosslinking reaction of the collagen hydrolysate with glutaraldehyde and glyoxal; although different methods of polymer physics were borrow and applied in this reaction. The following ones can be underlined :

a) Dielectric Spectroscopy : it is based on the dominant role that electrical charge play in the molecular interactions on the condensed biopolymers;in this way it is

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possible to study the mechanism of crosslinking which includes poly-electrolytic opening of the cyclic chemical structure (measurement of conductivity). A description of the real impedance produced around 10 KHz and caused by cables, connectors, etc were also introduced.

b) Spectrophotometry which is deviced for measuring light intensity in function of the wavelength of the light absorbed or transmitted.Every substance absorbs or transmit certain wavelength of radiant energy (UV/visible).This is a technique used to determine the amount of compounds that are coloured or that react to form coloured products.

c) RF. Reflectometer.

d) Optical fibre-based sensing system measuring the change of colour. This technique has proved to be reliable, efficient and non expensive.

Relationship of different analytical instruments tested were quite well selected; such as :

- Card NI-488.2
- LCR meter HP-4248 A
- Digital camera cannon Power A 70. It allows to make out a video from all measurements of RGB components during crosslinking process, thus showing the colour change in the various stages.
- Gas chromatograph HP 5859 Series II GC
- Spectrophotometer VARIAN carry 50 Bio.
- Different software : matlab, agilent VEE pro, etc
- Gas Chromatography – Mass Spectrosmetry.
  To evaluate the degree of crosslinking by using a coupled Gas Chromatography – Mass Spectrosmetry Unit has been set up. Although this analytical technique is extremely accurate, it is nevertheless, expensive and far away to become a common practice

This Thesis is very well structured dividing clearly the two main parts; theoretical and experimental, including an extense reference list. I would like to summarized the main target achieved by developing this work:

1.Important contribution to reduce the environmental impact of Leather Industry by minimizing the raw and chrome(III)-tanned wastes aiming therefore into a more sustainable environment.

2. Optimization by means of interesting mathematical modelling designs over the main reaction as it is the crosslinking of collagen hydrolysate with glutaraldehyde in order to isolate a new type of biodegradable casings, capsulates, medical supplies, etc.

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3. Amongst all polymer physical methods, accurate, efficient, but very expensive ,the author has developed a colorimetric method instrumentally simpler, easy to handle and by means of a digital camera system allows to measure the change of colour, monitoring the manufacturing process and controlling the production line; in addition is a non expensive technique.

4. This work can be continued in the direction of producing new type of ecological adhesive, protective colloids, capsulates, scaffolds, etc.

Finally I would like to express my satisfaction to Mr. Milan Navratil for this interesting work and underline that, to my point of view, can be considered as sufficient level to obtain the PhD Degree.

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