ADVANCES IN CHITIN SCIENCE

Volume XII

Proceedings of the 5th Iberoamerican Chitin Symposium

06 – 09 June 2010
Santiago do Chile
– Extended Abstracts –

Organized by

Dr. Telma T. Franco

Professor of Chemical Engineering

V SIAQ Chair and President of SIAQ

State University of Campinas, Brazil

Dr. Martin G. Peter

Emeritus Professor of Natural Products

University of Potsdam, Germany

and

Visiting Professor

State University of Campinas, Brazil

Optimization of the *N,N,N*-trimethyl chitosan (TMC) synthesis by factorial design

Douglas de Britto[1*], Fernando R. Frederico[2], Odilio B.G. de Assis[1]

[1] Embrapa - CNPDIA, R. XV de Novembro, 1452, CP 741, 13560-970, São Carlos, SP – Brazil
[2] Faculdade de Engenharia Química, UNICAMP, Campinas, SP – Brazil
*E-mail: britto@cnpdia.embrapa.br; odilio@cnpdia.embrapa.br, Tel: + 55 (16) 2107-2800, Fax + 55 (16) 2107-2902.

Abstract
The factorial design was applied to optimizes the synthesis of the N,N,N-trimethyl chitosan via dimethylsulfate synthetic route. The responses analyzed were weight yield, solubility and degree of quaternization. It was verified that the temperature was the most important variable, causing the main variation in the response, as X(T) = -54.52; -9.50 and -7.25 respectively for weight yield, solubility and degree of quaternization. Further analyses, including viscosimetry, contact angle and gel permeation chromatography gave additional information for some representative samples.

Keywords: optimization, factorial design, *N,N,N*-trimethyl chitosan, GPC, molecular weight

INTRODUCTION

Talking about chemical processes, words as chemiometry and optimization methods have become very common nowadays owing to the usefulness of the statistical tools in planning experiments.[1] One of these methods is the Factorial Design Analysis (FDA) largely used in synthesis processes optimization. For chitosan, FDA has been applied to study models of cupper ions-polymer adsorption,[2] to optimize film's mechanical properties[3] and in deacetylation processes.[4] Surprisingly, applications of factorial design on the synthesis of chitosan derivatives were found to be absent from published works.

To optimizes chitosan derivatives, as polysaccharides derivatives in general, is a challenger task due the complexity of the reaction as well as the many roles of the reagents. But it is indispensable mainly for new synthetic routes, as that the synthesis of the N,N,N-trimethyl chitosan (TMC) by means of dimethylsulfate (DMF) as methylant agent.[5]

Such chitosan quaternary salts are a special class of polyelectrolyte with permanent positive charges[6-9] and enhanced hydro solubility in a wide pH range. These derivatives have found several applications nowadays as gene delivery tool;[10,11] absorption enhancer for hydrophilic drugs across intestinal epithelia;[12,13] improved antibacterial activity[14,15] and nanoparticles for vaccine;[16] and controlled drug release.[17] Furthermore, properties such as biodegrability, non toxicity, bactericidal and fungicidal activities[18,19] and easy film forming ability[20] make these derivatives potential materials for uses on natural products.[21] Considering its potential applications, this work applies the FDA in an attempt to optimize the synthesis of the TMC via DMF.

EXPERIMENTAL

The reaction sequence comprised[5,7,21,22] a suspension of 1.0 g of chitosan in 16 mL of DMF and 4 mL of deionized water. Following, 1.2 g of NaOH and NaCl were added and the solution mixed over the desired times under magnetic stirrer. The factorial design was arranged for α=3 (temperature, time and NaCl addition) and b=2 (25/50°C; 3/6 hours and 0/0.44g), resulting in 2^3 = 8 independent experiments, each done in duplicate. Finally, the

TMC was submitted to dialysis in a cellophane membrane (cut-off ~13000 g/mol), precipitated with acetone and dried. The three analyzed responses were weight yield, solubility and degree of substitution (DQ) with help of the software FATORIAL®, developed by Barros Neto *et al.*[1,23] Representative samples were further characterized by viscosimetric, contact angle and Gel Permeation Chromatography (GPC) techniques, as following:

i) **Solubility** was estimated by static method from an aqueous solution at 2.0 mg/mL at 25°C. After the dissolution for 24 h, it was centrifuged (10000 rpm) and supernatant aliquots were taken dried and weighted. From the difference between the initial and final weights the percentage of soluble was calculated. The experiment was realized in triplicate.

ii) **Viscosity** and **hydrophilic character** measurements were carried as described before.[5,24]

iii) The **GPC** analysis was carried out by using dextran and poly(ethylene oxide) standards from American Polymer Standard Corp.® for calibration and check the detectors. The system was composed by pump model 515 from Waters®, degasser Viscotek model VE7510, injector Rheodyne model 7715i and detectors (refractometer, viscosimeter, light scattering 90° and 7°) ViscoteK model TDA302. It was used two columns (Ultrahydrogel Linear, 7.8 x 300 mm from Waters®, the gel is a cross-linked hydroxylated polymer and contains some residual carboxyl functionality) set at 40°C and eluent flow rate of 0.8 mL/min. Eluent and solvent for solutions preparation (1 mg/mL) was a buffer acetic acid-sodium acetate solution (pH=4,5). Analyses were conducted in duplicate and the data handled through the software Viscotek OmniSEC v. 4.1.0.224.

RESULTS AND DISCUSSION

Based on suitable statistical equations[25] and imputing the averages response, the FATORIAL® software returned the values summarized in Table 1. The FDA showed that synthesis realized at high temperature, extensive reaction time and in the presence of NaCl resulted in samples with the lowest weight yield, taking in account the negative value for the global average (Table 1). Indeed, the temperature was the most critical factor to weight yield once X(T) showed high magnitude value compared with the other main effects (Table 1). The negative sign indicates that the weight yield decreases as the reaction temperature increases.

Table 1. Calculated effects for the factorial design for weight yield, solubility and $\overline{DQ}$ responses.

Responses	Weight yield	Solubility	$\overline{DQ}$
Global Aver.	-21.05	71	29.38
Main effects:			
X(T)	-54.52	-9.5	-7.25
X(t)	-9.84	2.0	4.75
X(S)	4.07	1.0	-1.75
2nd effects:			
X(Tt)	-13.46	-12.5	1.75
X(tS)	11.72	-4.0	-0.75
X(TS)	-6.23	-20.5	1.25
3rd effect:			
X(TtS)	7.79	11.5	9.25

Following, FDA showed that the reaction time, X(t), has also a negative influence on the weight yield (extensive reaction time decreases the weight yield) but not so intensively as that

seen for temperature factor. However, this feature must be analyzed taking in account the strong correlation between the variables, especially X(Tt). The value found for this correlation is expressive (second effects, in Table 1) and its negative sign can be due the highly negative influence of the X(T). This conclusion is supported by the fact that X(TS) has also a negative sign but X(tS), in with the temperature is absent, has a positive sign. The cause of the high reaction temperatures give samples with low weight yields in the quaternization synthesis is due to the formation of H^+ as byproduct in the reaction medium.[5] The acid medium provokes the chitosan chain depolymerization that becomes more expressive at high temperature. Finally, the fraction of small chains is inevitable washed out during the dialysis process. The salt addition does not influence so much in the weight yield, according to X(S) that is not high in magnitude.

The Fig. 1 shows the normal probability plot for the estimative effects. In such a plot, the low magnitude effects or not significant, must show normal distribution data close to zero and a constant variance. This behavior is followed for all effects, where the points fit a nearly linear scattering, excepting the X(T). On the other hand, significant effect presents normal distribution far from zero and is seen to be distant from that linear pattern, as the case of X(T). In fact, the temperature is a very significant factor X(T) for the weight yielding of the quaternization reaction.

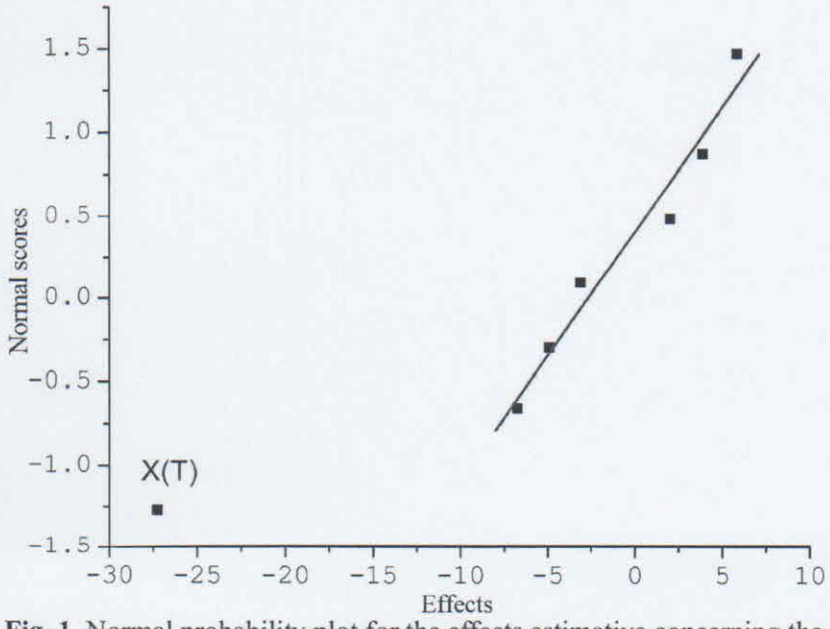

Fig. 1. Normal probability plot for the effects estimative concerning the reaction weight yield.

A very similar result is attained for the influence of these three factors on the solubility and $\overline{DQ}$ (Table 1). The main effects followed the same trend as found above, showing, however, lower magnitude values in comparison with that one. The $\overline{DQ}$ is the main concern in working with polysaccharide derivatives once its small variation may cause great changes in the derivative property. Considering this, if a high substituted TMC is desired the time must be increased, keeping the temperature near the lower level.

Further characteristics of the TMC

Degradations which took place during the reaction can be assessed by measuring the intrinsic viscosity that is related to the average molar mass by the Mark-Houwink equation, $[\eta] =$

K $\overline{M_v}$ [a]. The samples $TMCy_3$ and $TMCy_7$ presented [η] reduction[24] in comparison with the parent chitosan (Table 2). However, for the sample $TMCy_7$, reacted at high temperature, the [η] reduction was more expressive then for $TMCy_3$, reacted at low temperature. From the Mark-Houwink equation and the empiric values for K and a for chitosan reported in the literature,[5] was calculated $\overline{M_v} \cong 154$ kg/mol.

Table 2. Values of the intrinsic viscosity according to Huggins, $[\eta]_{Hug}$, and Kraemer, $[\eta]_{Kra}$, for chitosan and TMC in buffer aqueous solution (0.3/0.2 M CH_3COOH/CH_3COONa).

Samples	$[\eta]_{Hug}$	r	$[\eta]_{Kra}$	r
Chitosan	637	0.9978	646	-0.9819
TMC y_3	160	0.9773	162	-
TMC y_7	50	0.9980	50	0.9962

Contact Angle is a useful tool to evaluate the hydrophilic character expressing it in terms of wettability. For TMC a sharp decrease in the contact angle values was found when compared with the parent sample (Fig. 2), mainly for $TMCy_3$. As matter of fact, the introduction of permanent positive charges into the chitosan chains must increase its hydrophilic character. On the other hand, the methyl group is apolar and contributes to decrease of the hydrophilic character. Consequently the hydrophilic character will depend on a balance of these phenomena.[5] For $TMCy_7$ sample, the huge methylation imparts a very apolar characteristic, overtaking any hydrophilic feature. Evidently, this condition is not good for a desirable aqueous soluble derivative.

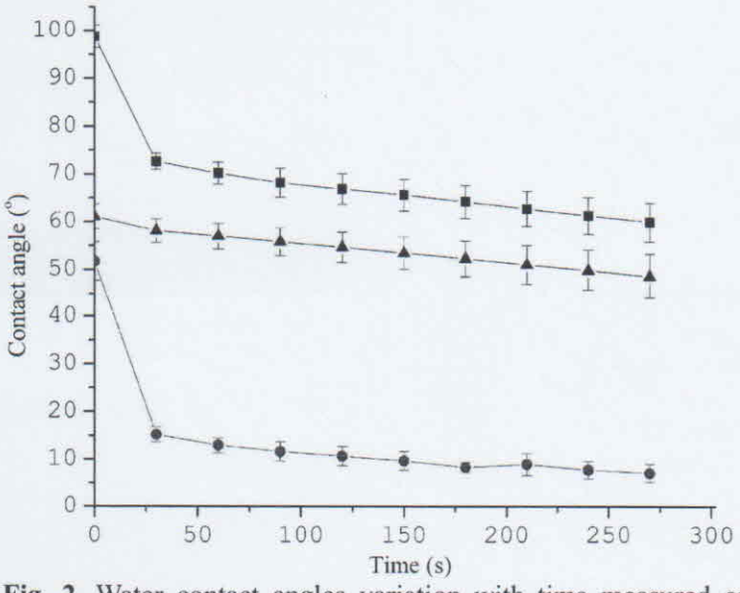

Fig. 2. Water contact angles variation with time measured on polymeric film surface for chitosan and TMC, in which: (■) chitosan; (●)TMC y_3 and (▲)TMC y_7.

The GPC data confirms the molecular weight of chitosan is altered after the quaternization reaction (Table 3). The weight-average molecular weight $\overline{M_w}$ and the number-average

molecular weight $\overline{M_n}$ decrease for the derivatives.[26] In confirmation, the sample $TMCy_7$ was the most degradated one with lowest $\overline{M_w}$ and $\overline{M_n}$. For the experiment conducted at room temperature ($TMCy_3$), the preservation of the polymeric chain is notable great. Other important feature is that the TMC salts show low polydispersity ($\overline{M_w}/\overline{M_n}$), what can be attributed to the purification dialyzes process that washes out small chain. This fact trends to become more accentuated as the degradation increases, as experienced for $TMCy_7$. For chitosan, the viscosimetric Mark-Houwink constant **a**, given by GPC analysis (Table 3) is in agreement with the literature,[27] while log **K** differ considerably. However, these parameters are empiric and dependent on the conditions, *e.g.*, temperature and solvent, and adopted methodology.

Table 3. Molecular weight and viscosimetric constants by GPC.

Sample	$\overline{M_n}$	$\overline{M_w}$	$\overline{M_w}/\overline{M_n}$	a	log K
Chitosan	60,146	218,718	3.636	0.583	-2.061
TMC y_3	56,779	116,813	2.059	0.744	-3.137
TMC y_7	12,593	19,364	1.538	0.836	-3.433

CONCLUSIONS

The use of FDA for evaluating the quaternization process of chitosan allowed concluding that the temperature is the most critical factor influencing the weight yield, solubility and degree of substitution of the derivative. Also, according to Contact Angle measurements, viscosimetric and GPC analyses, effective chain preservation is attained at room temperature and by extensive reaction time. The addition of NaCl did not show any positive influence on the response and can be definitely suppressed from the reaction.

ACKNOWLEDGMENTS

The authors are grateful to EMBRAPA, FAPESP and CNPq for financial support. Also to: Prof. Dr. José Fabian Schneider (IFSC-USP) and Prof. Dr. Antonio Gilberto Ferreira (DQ-UFSCar), solid state NMR; Prof. Dr. Paulo Sérgio Campana Filho (IQSC-USP), viscosimetry; and Prof. Dra. Telma Teixeira Franco (FEQ-UNICAMP), GPC; that kindly made available their equipments to data acquiring.

REFERENCES

1. Barros-Neto B, Scarmínio IS and Bruns RE, *Planejamento e otimização de experimentos*. Editora da Unicamp, Campinas (1995).
2. Lima IS and Airoldi C, *Colloids Surf A* **229:** 129-136 (2003).
3. Tao YG, Pan J, Yan SL, Tang B and Zhu LB, *Mater Sci Eng B* **138:** 84-89 (2007).
4. Weska RF, Moura JM, Batista LM, Rizzi J and Pinto LAA, *J Food Eng* **80:** 749-753 (2007).
5. de Britto D and Assis OBG, *Carbohydr Polym* **69:** 305-310 (2007).
6. de Britto D and Campana-Filho SP, *Polym Degrad Stab* **84:** 353-361 (2004).
7. Curti E, de Britto D and Campana-Filho SP, *Macromol Biosci* **3:** 571-376 (2003).
8. Dung P, Milas M, Rinaudo M and Desbrières J, *Carbohydr Polym* **24:** 209-214 (1994).
9. Sieval AB, Tahanou M, Kotzé AF, Verhoef JC, Brusse J and Junginger HE, *Carbohydr Polym* **36:** 157-165 (1998).
10. Germershaus O, Mao SR, Sitterberg J, Bakowsky U and Kissel T, *J. Controlled Release* **125:** 145-154 (2008).

11. Sajomsang W, Ruktanonchai U, Gonil P, Mayen V and Opanasopit P, *Carbohydr Polym* **78:** 743-752 (2009).
12. Mourya VK and Inamdar NN, *J Mater Sci: Mater Med* **20:** 1057-1079 (2009).
13. Sahni JK, Chopra S, Ahmad FJ and Khar RK, *J Pharm Pharmacol* **60:** 1111-1119 (2008).
14. Kim CH, Choi JW, Chun HJ and Choi KS, *Polym Bull* **38:** 387-393 (1997).
15. Jia Z, Shen D, Xu W, *Carbohydr Res* **333:** 1-6 (2001).
16. Amidi M, Romeijn SG, Verhoef JC, Junginger HE, Bungener L, Huckriede A, Crommelin DJA and Jiskoot W, *Vaccine* **25:** 144-153 (2007).
17. Cafaggi S, Russo E, Stefani R, Leardi R, Caviglioli G, Parodi B, Bignardi G, De Totero D, Aiello C and Viale M, *J. Controlled Release* **121:** 110-123 (2007).
18. Bautista-Baños S, Hernández-Lauzardo AN, Velázquez-del Valle MG, Hernández-López M, Barka EA, Bosquez-Molina E and Wilson CL, *Crop Protection* **25:** 108-118 (2006).
19. Rabea EI, Badawy MET, Stevens, CV, Smagghe, G and Steurbaut W, *Biomacromolecules* **4:** 1457-1465 (2003).
20. Hsu SH, Whu SW, Tsai CL, Wu YH, Chen HW and Hsieh KH, *J. Polym Res* **11:** 141-7 (2004).
21. de Britto D and Assis OBG, *Int J Biol Macromol* **41:** 198-203 (2007).
22. de Britto D, Forato LA and Assis OBG, *Carbohydr Polym* **74:** 86-91 (2008).
23. http://www.chemkeys.com (accessed February 2010).
24. Curti E and Campana Filho SP, *J Macromol Sci, Part A: Pure Appl Chem* **43:** 555-572 (2006).
25. Box GPG, Hunter WG and Hunter JS, *Statistics for Experimenter: An Introduction to Design, Data Analysis and Model Building*. John Wiley & Sons, New York (1978).
26. Jayakumar R, Reis RL and Mano JF, *J Macromol Sci, Part A: Pure Appl Chem* **44:** 271-5 (2007).
27. Yomota C, Miyazaki T and Okada S, *Colloid Polym Sci* **271:** 76-82 (1993).