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DEVELOPMENT OF THERMOGELLING HYDROGELS BASED ON A POLOXAMER 407 / CHITOSAN MIXTURE FOR SKIN APPLICATION

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ABSTRACT

Aim: The objective of this study was to develop Poloxamer 407/Chitosan mixtures. Thiolated or not for the treatment of certain skin ulcers like Buruli Ulcer after having synthesized 2-Iminothiolane or Thiobutylamidine (TBA), intermediate for the synthesis of thiolated chitosan. **Material and méthods:** Synthesis of TBA was done in two steps: synthesis of 4 (acetylthio)-butyronitrile by using thioacetic acid and 4-bromobutyronitrile; synthesis of 2-Iminothiolane HCl by using 4 (acetylthio)-butyronitrile. Modification of chitosan has been made by using 2 Iminithiolane and some chitosans. The poloxamer solutions were prepared in 0,1M acetic acid, and demineralised water. **Results:** The preparations had a good homogeneity and the gelling temperature of the F127/Chitosan mixtures is essentially controled by poloxamer. **Conclusion:** This study has shown that the gelation process is favoured by an increase in the concentration of F127 and is significantly disadvantaged by 0,1M acetic acid. The gelling temperature of the F127 / Chitosan mixtures is essentially controlled by the poloxamer.

KEYWORDS: Hydrogels, Poloxamer, Chitosan.

INTRODUCTION

The Poloxamers, better known by the BASF trade name Pluronic®, they are triblock copolymers consisting of a central hydrophobic block of poly (oxypropylene) (POP) flanked by two hydrophilic blocks of the same length of poly (oxyethylene) (POE). These polymers are not very toxic and their aqueous solutions can change reversibly from the solid state to the gel state at a temperature closer to the human body temperature (**Gilbert and al, 1986**) which

is an advantage for their use as a delivery system for active principle. Chitosan, a biopolymer with glucosamine and N-acetylglucosamine, is a product of N-deacetylation of chitin, one of the most abundant polysaccharides in nature. Chitosan has many advantages like they are biocompatible, biodegradable, non-toxic and especially mucoadhesive (**Kim and al, 2007**). These mucoadhesive properties can be improved through thiolation of chitosan (**Langoth et al, 2006**).

Poloxamer / Chitosan mixtures are under the controlled release systems. It is, therefore, appropriate to study these systems in order to be able to explain their behaviour and also to improve on them in order to obtain satisfactory materials for drug therapy in the area of controlled release. Poloxamer can gel at human body temperature. The gels obtained could be bioadhesive by the presence of Chitosan.

The objective of this study was to develop Poloxamer 407 / Chitosan mixtures. Thiolated or not for the treatment of certain skin ulcers like Buruli Ulcer after having synthesized 2-Iminothiolane or Thiobutylamidine (TBA), intermediate for the synthesis of thiolated chitosan

MATERIALS AND METHODS

Materials

Material for the synthesis of the Thiobutylamidine

Potassium carbonate: Lot 93H0360 (SIGMA), Demineralized water: (RIOS 5 and Synergy 185 (Millipore®), Thioacetic acid: Lot 446769/1 (FLUKA), Ethanol 96%: Code n ° 414008 (CARLO EBRA), 4 -bromobutyronitril: Lot 01005DE-296 (ALDRICH), Magnesium sulphate: Lot 23H02982 (SIGMA), ethyl acetate 99, 5%: Lot A019815601 (ACROS ORGANICS), Commercial solution of 5-6N hydrochloric acid in 2- Propanol: Lot A0230413 (ACROS ORGANICS), commercial chitosan 400000 and 145000 (SIGMA).

Material for the formulation of gels

Lutrol® F127 (Poloxamer 407) from BASF Corporation, Ledgewood, New Jersey and Reference Art: 51632903, Lot: WPHB615B (67056 Ludwigshafen, Germany).

A commercial chitosan (CC 400000) batch reference: 433278/1122606245 [9012-76.4] from Fluka Biochemika, and a degree of deacetylation of 71% (result obtained by NMR) with a molar mass of 400000g/mol.

A non-thiolated chitosan (CNT) obtained by depolymerization of the commercial chitosan and molar mass 145000g/mol.

Two thiolated chitosans (CT) obtained by thiolation of the preceding sample (a previously thiolated chitosan CT1 and a synthesized thiolated chitosan CT2).

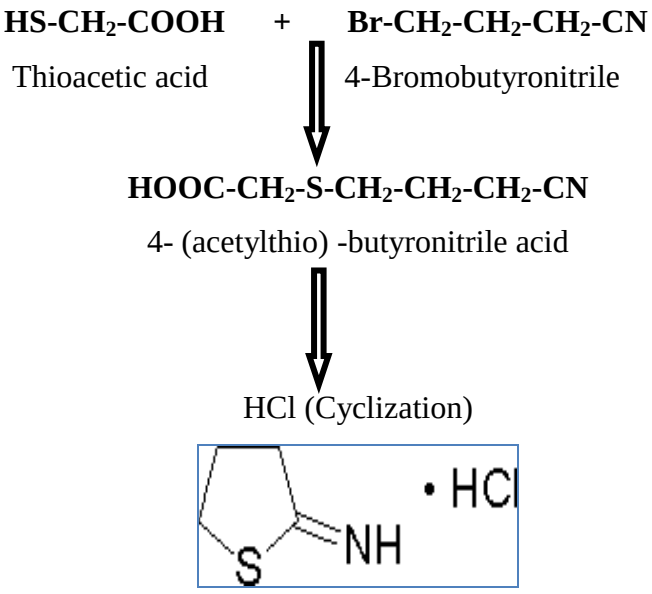
99-100% RECTAPUR ™ acetic acid with a molecular mass of 60,05g/mol and a density of d=1, 05 code 103.295 Lot L212 was used for the preparation of a 0,1M aqueous solution which served as the dissolution solvent.

For all preparations, we have used ultrapure water purified by RiOs ^{TM} 5 water purification system and Synergy 185 (Millipore®) by a reverse osmosis system.

METHODS

TBA synthesis Procedure

The procedure for the synthesis was done in two steps: The synthesis of 4 (acetylthio) -butyronitrile and the synthesis of 2-Iminothiolane-HCl from 4 (acetylthio) -butyronitrile.(**Fig1**).



2-Iminothiolane.

Figure 1: Diagram of 2-Iminothiolane synthesis.

- **Synthesis of 4 (acetyl thio)-butyronitrile**

In a 10ml flask containing water, add 6,8g of potassium carbonate, and stir with a mechanical stirrer (exothermic reaction). Still stirring, add 3,55mL of thioacetic acid, add 20ml of

ethanol, 5ml of 4-bromobutyronitrile and 0,76g of thioacetic acid. Stir it at room temperature for 12 hours. Filter the solution to remove the precipitates and wash once with ethanol 96%. Extract the precipitate with ethyl acetate. Evaporate the solvents using a Rotavapor and concentrate. Add magnesium sulphate and filter.

- **Synthesis of 2-Iminothiolane-HCl**

In a 50ml flask, mix 4 (acetyl thio) butyronitrile and a 5-6N HCl commercial solution in propanol (11mL). Stirring the solution for more than 24hours. Add ethanol. Filter and wash with ethanol. Dry at least 24 hours in the oven and store in the refrigerator.

Methods of Chitosan modification

The modification of chitosan was carried out on a single batch of chitosan in this case, the chitosan with molar mass of 145000g/mol (**Fig 2**).

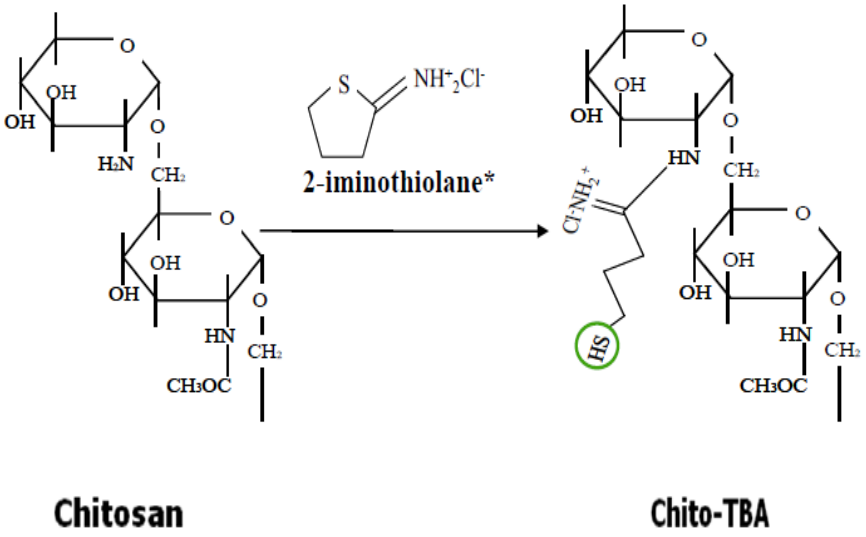


Figure 2: Diagram of synthesis of thiolated chitosan.

The mixture of Thiol groups in chitosan was carried out following the method developed by **Bernkop-Schnürch and al. (2003):** 1g of chitosan 145000 was dissolved in 100ml of acetic acid solution (1% w/v). The pH of the solution was adjusted to 6, 5 with a solution of NaOH (1N).

Traut's reagent (2-Iminothiolane) was added to chitosan in a mass ratio of 5: 2. Left for 24 hours under a magnetic stirring at room temperature and dialyzed against different aqueous medium (Spectra / Por®3 membrane MWCO 3500):

- 8h against 5L of 5mM HCl.
- 8h against 5L of 5mM HCl with 1% NaCl) x 2.
- 8h against 5L of 5mM HCl.
- 8h against 5L of 1mM HCl.

(40h in total).

The dialysate was frozen at least 24 hours before lyophilization. The product of the dialysis was lyophilized (Christ Alpha 1-4 Freeze-dryer, Bioblock Scientific, Illkirch, France) 48h and refrozen until use.

Preparation of studied systems

The poloxamer solutions were prepared in 0,1M acetic acid and in demineralized water, while those of chitosan and Poloxamer / chitosan mixtures were prepared in 0,1M aqueous acetic acid solution as chitosan is only soluble in pH below 6,5.

Preparation of the stock solution of F127

The Poloxamer solution was prepared in cold 0.1M acetic acid or deionized water. Two 32% and 35% (g / g) stock solutions were prepared.

A bottle containing the required amount of 0,1M acetic acid or deionized water was magnetically stirred in a crystallizer filled with ice and water. Then the F127 powder was added slowly to the solution while still stirring. After all the F127 has been poured into 0,1M acetic acid, the mixture was left stirring for 5h in ice until complete dissolution of F127. The solution was then refrigerated overnight to remove foam and air bubbles. 16% and 17, 5% solutions were prepared by dilution of this stock solution either in 0,1M acetic acid or in demineralized water.

Preparation of stock solutions of chitosan

Chitosan solutions at 1%, 2%, 3% and 4% (g/g) were prepared in 0,1M acetic acid. The chitosan was poured into a pillbox containing a solution of 0,1M acetic acid and this system were left stirring for 4 hours. Then the solution was refrigerated overnight until use.

Preparation of F127/Chitosan mixtures

The solutions of chitosan 1%, 2%, 3%, 4% in acetic acid and F127 (35%) were mixed in equal proportion in a 25ml pillbox and left stirring in the ice. Each mixture was then put in the refrigerator.

Several samples have been prepared:

- F127 at 16% in demineralised water.

- F127 at 16% in 0.1M acetic acid.

- F127 at 17, 5% in demineralised water.

- F127 at 17, 5% in acetic acid.

- Non-thiolated chitosan (CNT 145000) at 1%; 2%; 3% and 4% in acetic acid Thiolated chitosan (CT_1 and CT_2) (145000) at 1%; 2%; 3% and 4% in acetic acid.

- F127/CT_2 Mixture (145000) 17,5/0,5; 17,5/1; 17,5/1,5 and 17,5/2.

- F127 / CNT Mixture 17,5/0,5; 17,5/1; 17,5/1,5 and 17,5/2

RESULTS AND DISCUSSION

Systems's Formulation

The different systems prepared and their macroscopic appearance are summarized in **Table I**.

Table I: Appearance of the different solutions prepared at 4, 5°C.

Systems	Appearance
F127 at 32% in demineralized water	Viscous, transparent
F127 at 32% in acetic acid 0,1M	Viscous, transparent
F127 at 35% in demineralized water	Very Viscous, transparent
F127 à 35% in acetic acid 0,1M	Very Viscous, transparent
Non-thiolated chitosan (145000) at 1% in acetic acid	Slightly viscous, Light yellow (+)
Non-thiolated chitosan (145000) at 2% in acetic acid	Slightly viscous, Light yellow (++)
Non-thiolated chitosan (145000) at 3% in acetic acid	Slightly viscous, Light yellow (+++)
Non-thiolated chitosan (145000) at 4% in acetic acid	Viscous, Light yellow (++++)
Thiolated Chitosan (145000) at 1% in acetic acid	Slightly viscous, transparent
Thiolated Chitosan (145000) at 2% in acetic acid	Slightly viscous, Light yellow (+)
Thiolated Chitosan (145000) at 3% in acetic acid	Viscous, Light yellow (++)
Thiolated Chitosan (145000) at 4% in acetic acid	Viscous, Light Yellow(+++)
F127/ Demineralized water 16/0	Homogeneous-No separation
F127/Acetic acid 0,1M 16/0	Homogeneous-No separation
F127/ Demineralized water 17,5/0	Homogeneous-No separation
F127/Acetic acide 0,1M 17,5/0	Homogeneous-No separation
F127/ Non-thiolated chitosan (145000) 17,5/0,5	Homogeneous-No separation
F127/ Non-thiolated chitosan (145000) 17,5/1	Homogeneous-No separation
F127/ Non-thiolated chitosan (145000) 17,5/1,5	Homogeneous-No separation
F127/ Non-thiolated chitosan (145000) 17,5/2	Homogeneous-No separation
F127/ Thiolated Chitosan (145000) 17,5/0,5 CT_2	Homogeneous-No separation
F127/ Thiolated Chitosan (145000) 17,5/1 CT_1	Presence of microgels
F127/ Thiolated Chitosan (145000) 17,5/1 CT_2	Homogeneous-No separation
F127/ Thiolated Chitosan (145000) 17,5/1,5 CT_2	Homogeneous-No separation
F127/ Thiolated Chitosan (145000) 17,5/2 CT_2	Homogeneous-No separation

Color intensity

Comments

The homogeneity of this phase is a very good point compared to Poloxamer/HPMC mixtures which present separation appearance in the mixed phase (**Koffi, 2006**).

Chitosan and its counterions have a greater degree of freedom when the mixtures are homogeneous. The entropy of these counter ions would, therefore, be favourable to the compatibility of the two polymers.

Summary of gelling temperatures

Table II: below gives a summary of the gelling temperatures of our different systems.

Table II: Summary of Gelling Temperatures.

Solutions	Gelling temperature (T_{gel})
F127 at 16% in demineralised water	27,4°C ± 0,1°C
F127 at 16% in 0,1M acetic acid	Difficult to determine
F127 at 17,5% in demineralised water	25,3°C ± 0,1°C
F127 at 17,5% in 0,1M acetic acid	29,3°C ± 0,1°C
F127/CNT 145 17,5/0,5	28,4°C ± 0,1°C
F127/CNT 145 17,5/1	27,3°C ± 0,1°C
F127/CNT 145 17,5/1,5	27,3°C ± 0,1°C
F127/CNT 145 17,5/2	27,4°C ± 0,1°C
F127/CT_2 145 17,5/0,5	29,3°C ± 0°C
F127/CT_2 145 17,5/1	28,3°C ± 0°C
F127/CT_2 145 17,5/1,5	28,3°C ± 0,1°C
F127/CT_2 145 17,5/2	28,3°C ± 0,1°C

Comments

When the concentration of poloxamer increases, the micelles formed are closer and therefore interact and crystallize at lower temperatures, hence the drop in the gelling temperature observed for the concentration of F127 at 17.5% (Table II).). This effect has already been reported by **Zhou and al., (1988)**, **Alexandris and al., (1994)**.

CONCLUSION

In order to develop a topical treatment for Buruli ulcer, we are interested in thermogelling and bioadhesive systems based on poloxamer F127 and chitosan of different molar masses thiolated or not.

This study has shown that the gelation process is favoured by an increase in the concentration of F127 and is significantly disadvantaged by 0.1M acetic acid. The gelling temperature of

the F127 / Chitosan mixtures is essentially controlled by the poloxamer. As for the presence of chitosan, it essentially varies the viscosity of the solution before gelling and very little influence on the gelling temperature.

At 25 ° C, our systems behave like an elastic liquid, whereas at 37 ° C the behaviour is rather elastic and this is influenced by the poloxamer.

REFERENCES

1. Alexandris P., Holtzwarth J.F., Hatton T.A. ``Micellization of Poly (ethylene oxide)- Poly (propyle oxide) triblock copolymers in aqueous solutions: Thermodynamic of copolymer association.´´Macrom, 1994; 27: 2414-2425.
2. Bernkop-Schnürch A., Kast C.E., Guggi D. Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: thiomer/GSH systems. J. Control. Release, 2003; 93: 95-103.
3. Brugnerotto J., Lizardi J., Goycoolea F.M., Argüelles-Monal W., Desbrières J., et Rinaudo M. An infrared investigation in relation with chitin and chitosan characterization. Polymer, 2001; 42: 3569-3580.
4. Cafagi S., Leardi R., Parodi B., Caviglioli G., Russ E., Bignardi G. ``Preparation and evaluation of a chitosan-salt poloxamer 407 based matrix for buccal drug delivery.´´J. of Contr. Rel, 2005; 102: 159-169.
5. Gilbert J. C., Hadgraft J., Bye A., Brookes L.G. ``Drug release from Pluronic F-127 gels.´´Int. J. of Pharma, 1996; 32: 223-228.
6. Kim I.Y., Yoo M. K., Seo J. H., Park S. S., Na H. S., Lee H. C., Kim S. K., Cho C. S. ``Evaluation of semi-interpenetrating polymer networks composed of chitosan and poloxamer for wound dressing application.´´ Int. J. of Pharma 2007; 341: 35-43.
7. Koffi A. A, F. Agnely, G. Ponchel, J. L. Grossiord. Modulation of the rheological and mucoadhesive properties of thermosensitive poloxamer-based hydrogels intended for the rectal administration of quinine. European Journal of Pharmaceutical Sciences, 2006; 27(4): 328-335.
8. Langoth, N., Kahlbacher, H., Schömann, G., Schmerold, I., Schuh, M., Franz, S., Kurba, P., Bernkop-Schnürrch, A.``Thioled chitosans: Design in vivo evaluation of mucoadhesive buccal peptide drug delivery system.´´ Pharma. Res, 2006; 23(3): 573-579.
9. Martinez L. Application du procédé de prilling pour la préparation de micro réseaux à base de chitosane. Caractérisations physico-chimiques et utilisation dans la formulation

de principes actifs pour la voie orale. Doctorat de l'Université de Paris XI-Pharmacotechnie et Physico-chimie-Université de Paris XI, 285p.

10. Westerink M. A. J., Smithson S. L., Srivastava N., Blonder J., Coeshoot C., Rosenthal G.J. ''ProJuvant™ (Pluronic F127®/Chitosan) enhances the immune response to intranasaly administered tetanus toxoid''. Vaccine, 2002; 20: 711-723.
11. Zhou Z., Chu B. ``Light scattering study on the association behavior of triblock polymers of ethylene oxide and propylene oxide in aqueous solution.´´ J. of Coll. and Interf. Sci, 1988; 126(1): 171-180.