CHITOSAN BIOCOMPATIBLE HYDROGELS AS DRUG DELIVERY MATRICES FOR ANTICANCER APPLICATIONS

Anda-Mihaela Craciun,[1*] Gabriela Gavril,[1] Ioana Cristina Marinas,[2] Liliana Mititelu Tartau,[3] Luminita Marin[1]

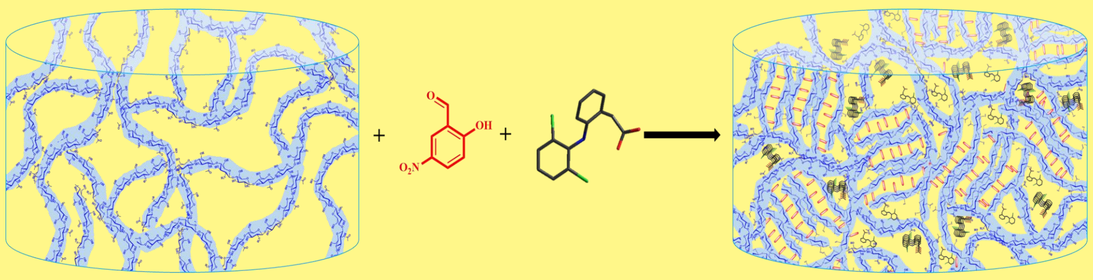
1 "Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy, Iasi, Romania
2 Sanimed Int Impex Srl, Ctr Serv & Res Adv Biotechnol, Bucharest, Romania
3 "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania
**olaru.anda@icmpp.ro*

Introduction

Chitosan is a biocompatible biopolymer with good biocompatibility, bioadhesivity and biodegradability in human body, by an enzymatic metabolism.[1] Based on these favorable properties, various forms of chitosan and its derivatives were developed for application as drug delivery systems. This research activity evidenced a new hydrogel based on chitosan and a monoaldehyde with suitable properties for local drug delivery in tumors: rapid hydrogelation in media of physiological pH and excellent *in vivo* biocompatibility.

Materials and methods

The hydrogels were obtained by acid condensation reaction between the amino groups of chitosan and a monoaldehyde, 2-hydroxy-5-nitrobenzaldehyde,[2] by varying the molar ratio between amine and aldehyde groups. In this way, a series of hydrogels with different crosslinking degree was obtained and noted **N1-N7**, the number corresponding to the molar ratio of the amine/formyl functional units. Also, a series of formulations were prepared by *in situ* encapsulation during hydrogelation of diclofenac sodium salt (**DCF**) as model drug (**N1D-N4D**).



Scheme 1. Schematic representation of the *in situ* hydrogelation in the presence of **DCF**

Structural characterization

The FTIR and ^{1}H-NMR spectroscopy demonstrated the formation of the imine linkage by the appearance of the characteristic stretching vibration band at 1613 cm^{-1} and specific chemical shift of imine proton at 8.7 ppm, respectively.

The encapsulation of **DCF** into the drug delivery systems was evidenced by the deconvolution of the 1620-1480 cm^{-1} FTIR spectral domain, which revealed the characteristic vibration band of the carboxyl group of **DCF**, at 1577 cm^{-1} (Figure 1).

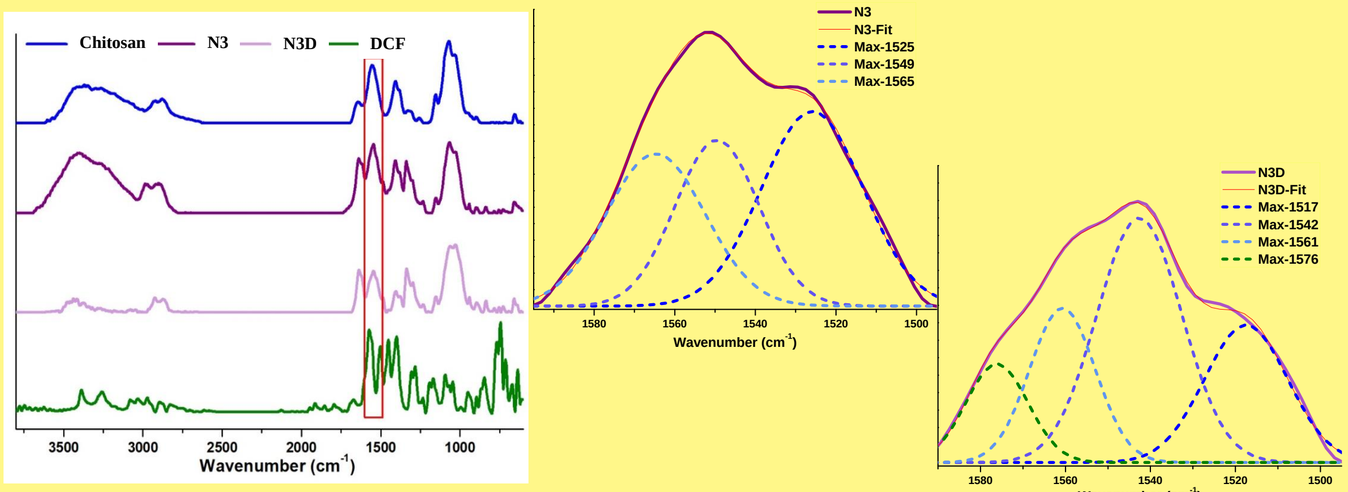
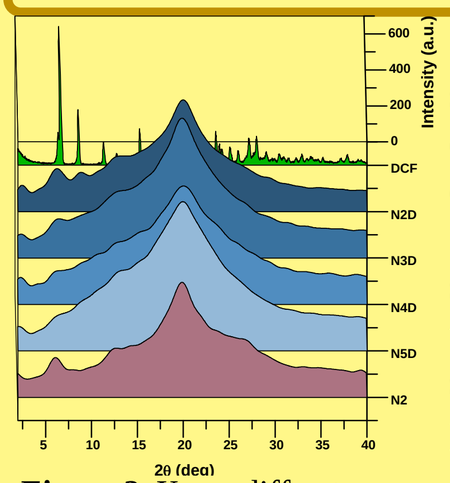


Figure 1. Comparative FTIR spectra and deconvolution of the 1620-1480 cm^{-1} spectral domain

Wide angle X-Ray diffraction

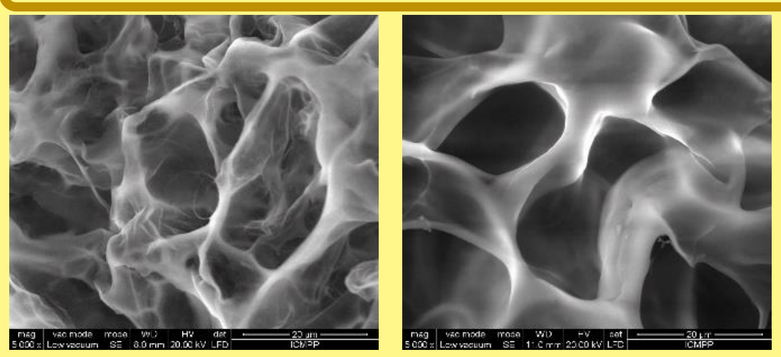


On the other hand, X-ray diffractograms proved the supramolecular ordering of the imine units by the occurrence of a sharp reflection band around 6°, characteristic to the formation of layered architectures.

The reflections of the delivery systems were slightly broader and shifted to wider angle compared to the reflections of the hydrogel references, indicating shorter intermolecular distances with a larger polydispersity (Figure 2).

Figure 2. X-ray diffractograms of the **N2D-N5D**, **DCF** and **N2**

Scanning electron microscopy



Comparative SEM images of both xerogels, with and without drug, suggested the encapsulation produced mainly into the xerogels' walls at submicrometric level (Figure 3).

Figure 3. SEM images of the **N3** reference and **N3D** drug delivery system

In vitro release of DCF

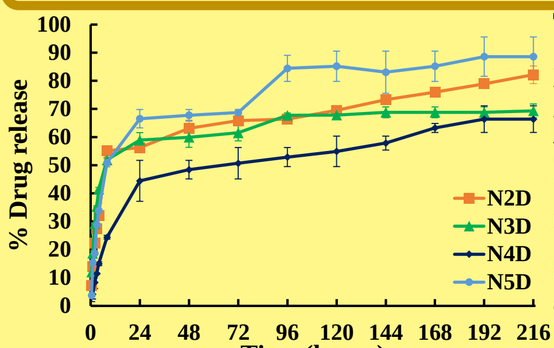


Figure 4. Percentage of **DCF** released during 8 days

The *in vitro* release study was realized in conditions mimicking the physiological environment (PBS, 37 °C). The drug release was more effective for the **N2D** and **N5D** samples, which released almost 90 % of drug after 8 days, while **N3D** and **N4D** ones released only 70 % (Figure 4).

In vitro enzymatic degradation

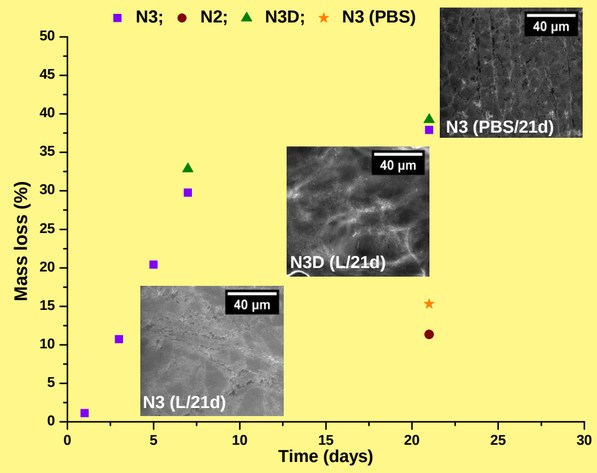


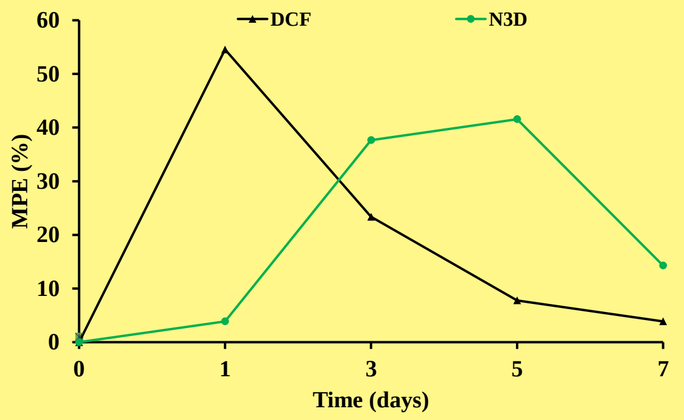
Figure 5. Graphical representation of the mass loss *versus* time of the formulations in the presence of lysozyme, and corresponding SEM images

The biodegradation rate was dependent on the crosslinking degree: increased as the crosslinking degree decreased, e.g. the sample **N3**, showed a mass loss of 37 % after 21 days, while **N2** biodegraded much slower, reaching a mass loss of only 11 % (Figure 5).

In vivo biocompatibility & drug release

In vivo investigation of biocompatibility and drug release were monitored on rats animal models, by determining the variation of the blood, biochemical and immune systems parameters, and the somatic antinociception, following the subcutaneous implantation of the samples.

Figure 6. The influence of the subcutaneous implantation of the formulations in rats, on the maximum possible effect (MPE%) in time



No statistic relevant toxic effects were recorded. The monitoring of the antinociception effect revealed a prolonged release of DCF during 7 days (**N3D**), compared to 24 hours in the case of the positive control (**DCF** pellet) (Figure 6).

Conclusions

- New drug delivery systems based on a chitosan hydrogel matrix were obtained by *in situ* encapsulation during hydrogelation.
- The hydrogelation took place by imination reaction followed by supramolecular layering of the imine units.
- The drug has been encapsulated into the hydrogel walls, at submicrometric level.
- The new formulations showed good biodegradability rate and *in vivo* biocompatibility.
- The *in vivo* release kinetics showed a prolonged drug release, with an improved bioavailability of the drug allowing a repetition dossing at every 5 days.

References

1. R.O. Beauchamp, M.B.G. St Clair, T.R. Fennell, D.O. Clarke, K.T. Morgan, Crit. Rev. Toxicol. 22, 143-174, 1992.
2. A.M. Olaru, L. Marin, S. Morariu, G. Pricope, M. Pinteala, L. Tartau-Mititelu, Carbohyd. Polym. 179, 59-70, 2018.

Acknowledgements

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PD 197/2020, within PNCDI III.