Journal of Colloid and Interface Science 504 (2017) 721–730

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

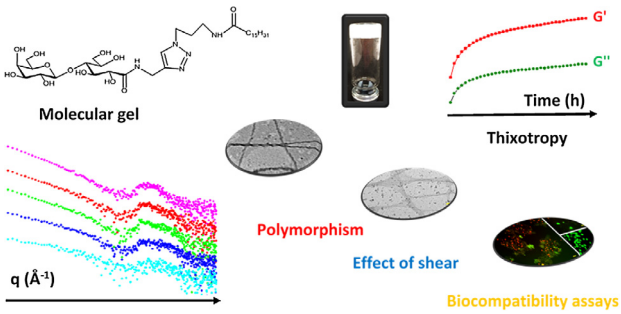
Regular Article

A shear-induced network of aligned wormlike micelles in a sugar-based molecular gel. From gelation to biocompatibility assays

Juliette Fitremann [a,*], Barbara Lonetti [a], Emiliano Fratini [b], Isabelle Fabing [c], Bruno Payré [d], Christelle Boulé [e], Isabelle Loubinoux [f], Laurence Vaysse [f], Luis Oriol [g]

[a] CNRS – Université de Toulouse III Paul Sabatier, Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique (IMRCP, UMR 5623), Bat 2R1, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France
[b] Department of Chemistry "Ugo Schiff" and CSGI, University of Florence, via della Lastruccia 3-Sesto Fiorentino, I-50019 Florence, Italy
[c] CNRS UMR 5068, LSPCMIB, Université de Toulouse, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse cedex 9, France
[d] Centre de Microscopie Electronique Appliquée à la Biologie (CMEAB), Faculté de Médecine Rangueil, Université de Toulouse III Paul Sabatier, Bâtiment A5, R.D.C., 133 Route de Narbonne, 31400 Toulouse, France
[e] Université Claude Bernard UCBL Lyon1, Service de Prestations CTμ EZUS, Bâtiment Darwin B, 5 rue Raphaël Dubois, 69622 Villeurbanne Cedex, France
[f] TONIC, Toulouse NeuroImaging Center, Université de Toulouse, Inserm, UPS, France
[g] Instituto de Ciencia de Materiales de Aragon (ICMA),Universidad de Zaragoza-CSIC, Dpto. Quimica Organica, Facultad de Ciencias, Pedro Cerbuna 12, 50009 Zaragoza, Spain

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 April 2017
Revised 6 June 2017
Accepted 6 June 2017
Available online 9 June 2017

Keywords:
Supramolecular
Gel
Self-assembly
Carbohydrate amphiphile
Saccharide
Triazole
Fiber
Fibre
Cylindrical micelle
Shear

ABSTRACT

A new low molecular weight hydrogelator with a saccharide (lactobionic) polar head linked by azide-alkyne click chemistry was prepared in three steps. It was obtained in high purity without chromatography, by phase separation and ultrafiltration of the aqueous gel. Gelation was not obtained reproducibly by conventional heating-cooling cycles and instead was obtained by shearing the aqueous solutions, from 2 wt% to 0.25 wt%. This method of preparation favored the formation of a quite unusual network of interconnected large but thin 2D-sheets (7 nm-thick) formed by the association side-by-side of long and aligned 7 nm diameter wormlike micelles. It was responsible for the reproducible gelation at the macroscopic scale. A second network made of helical fibres with a 10–13 nm diameter, more or less intertwined was also formed but was scarcely able to sustain a macroscopic gel on its own. The gels were analysed by TEM (Transmission Electronic Microscopy), cryo-TEM and SAXS (Small Angle X-ray Scattering). Molecular modelling was also used to highlight the possible conformations the hydrogelator can take. The gels displayed a weak and reversible transition near 20 °C, close to room temperature, ascribed to the wormlike micelles 2D-sheets network. Heating over 30 °C led to the loss of the gel macroscopic integrity, but gel fragments were still observed in suspension. A second transition near 50 °C, ascribed to the network of helical fibres, finally dissolved completely these fragments. The gels showed thixotropic behaviour,

* Corresponding author.
E-mail address: fitremann@chimie.ups-tlse.fr (J. Fitremann).

http://dx.doi.org/10.1016/j.jcis.2017.06.021

Cell culture
Biomaterial
LMWG
Low molecular weight gelator

recovering slowly their initial elastic modulus, in few hours, after injection through a needle. Stable gels were tested as scaffold for neural cell line culture, showing a reduced biocompatibility. This new gelator is a clear illustration of how controlling the pathway was critical for gel formation and how a new kind of self-assembly was obtained by shearing.

1. Introduction

Low molecular weight (LMW) hydrogelators provide an alternative family of gelling agents compared with polymers, leading to soft materials with possible applications in the field of wet materials, switchable gels, controlled release or uptake, cell culture. LMW gelators belong to different structural families (peptides, cholesteryl, nucleobase or sugar amphiphiles, etc...) and the now quite large amount of work done on these self-assembling molecules has been well reported in several reviews [1–21]. We are more especially interested in sugar-derived molecular gelators (see the recent review on this family of gelators and the following references for the most recent works [22–50]). Sugar gelators provide generally a neutral hydrophilic polar head, with a low sensitivity to temperature changes on the contrary to PEG amphiphiles. In the context of biological applications, carbohydrate derived hydrogels will interact differently with biomolecules or cells compared with PEG or peptide derived gelators, notably by mimicking to some extent the saccharidic components of the glycocalix, composed of glycoproteins and glycolipids [22].

From a practical point of view, simple, rapid and cheap syntheses are essential when considering sugar-based LMW hydrogelators applications. In former results, a family of hydrogelators based on a disaccharide head has been described. It has been shown that the presence of a triazole linker enhanced gelation, but the synthetic pathway consisted of six steps [23–26]. Other amphiphilic molecules with close structures (namely, a sugar head, triazole linkers, and a fatty chain) have been described as well, including hydrogelators [27–29], organogelators [30] and micelles [31–33], all of them involving also protection-deprotection multistep synthesis and purification by chromatography. In this work, a new gelator inspired from these structures has been prepared with a simpler synthetic route with only three steps starting from lactobionic acid as the polar head (Scheme 1). Another important aspect in the field of LMW hydrogelators is to control in a precise manner the supramolecular structure sustaining the gel. In the case of very flexible molecules many conformations are possible. It can give rise to polymorphism. Polymorphism is the main cause for the lack of reproducible gelation [34–37]. Accordingly, the importance of controlling the conditions of the self-assembly in order to reach a reproducible final state, with the related macroscopic properties, has been well pointed out in several papers. But it still remains underestimated, quite unexplored and not well controlled [38–42]. Compound **3** is a typical example of such a situation. Its gelation behaviour appeared more complex than expected and gave the opportunity to explore the effect of different pathways for the gel preparation. The self-assembled structures have been elucidated by electronic microscopy and Small Angle X-ray Scattering (SAXS). The rheology and thermal transitions of the gel have also been studied. Finally, the results related to the use of these gels for cell culture are briefly discussed.

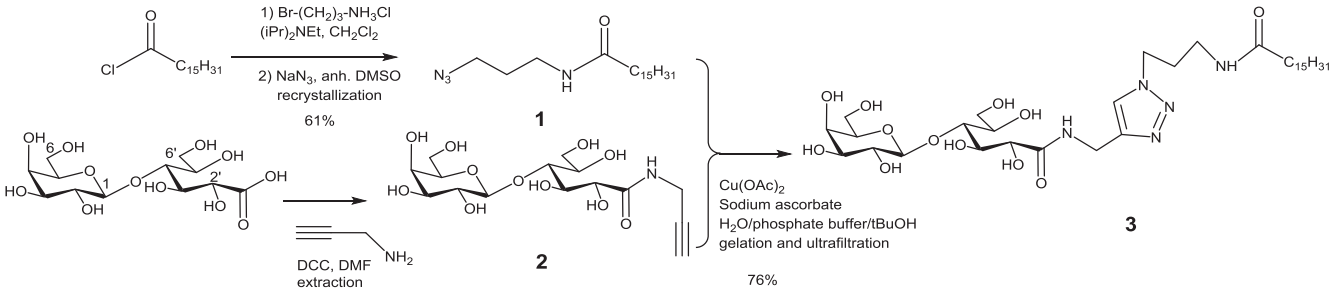
2. Results and discussion

2.1. Preparation of the gelator

An easy access to the sugar based gelator **3** is represented in Scheme 1. The gelator was obtained in three synthetic steps and was purified without chromatography. The fatty chain **1** was purified by recrystallization (yield 60%) while the product **2** was obtained nearly pure by extraction and was used without further purification in the next step. After the azide-alkyne "click chemistry" step, leading to the compound **3**, the unreacted and sparingly soluble azide was discarded by centrifugation. The cleared and concentrated reaction medium was diluted in water and allowed to rest for several hours, until a gel was formed. The gel was purified by ultrafiltration or dialysis, removing water soluble by-products. Analysis of the filtrate showed that about 5% of the gelator went out through the membrane after four volumes and 24 h of ultrafiltration, evidencing a low proportion of free molecules. A last step of filtration in methanol enabled to get rid of traces of the azide remaining after ultrafiltration. After this sequence, the gelator was pure (yield 76%, freeze-dried), according to NMR, HPLC-MS chromatograms (see Fig. SI-11–13). Residual copper was analysed and it did not exceed 300 ppm (0.3 μg/mg) when ultrafiltration was performed in the presence of EDTA.

2.2. Gelation

Gelation of the gelator **3** was at first quite puzzling. The crude product coming directly from the reaction mixture and diluted in water (after solvent removal) provided directly the hydrogel within 24 h of standing. Conversely, the purified and dried samples gave a non-reproducible gelation behaviour. The usual method consisting in heating the solution until solubilisation followed by



Scheme 1. Synthetic scheme for the preparation of the gelator **3.**