Supplementary Information.

Understanding the Metal Free Alginate Gelation Process.

Ornella Ursini [1*], Roberta Angelini[2], Silvia Franco[2] and Barbara Cortese[1]

[1] Nanotechnology Institute -National Research Council (CNR-NANOTEC), c/o Sapienza University of Rome, Piazzale A. Moro 2, 00185 Rome, Italy

[2] Institute for Complex Systems, National Research Council (CNR-ISC), c/o Sapienza University of Rome, Piazzale A. Moro 2, 00185 Rome, Italy

* Correspondence: ornella.ursini@cnr.it

2.3. Preparation of Formic acid solutions

Three different solutions of pure HCOOH containing exactly the same amount of formic acid present in the acidified Glutamine solutions were prepared, as described in the following Table 1.

Table 1. Experimental parameters of the formic acid solutions prepared as control samples (the assigned code to identify each sample is reported in the first column).

Control sample (sample code)	Volume (ml) of HCOOH [1M] put into 10 ml volumetric flask	Mole of HCOOH put into 10 ml volumetric flask	Molar Concentration of [HCOOH]	[H+] = rad (k_a*$Conc_a$)	pH
0.5 H+	0.883	8.830E-04	8.830E-02	3.953E-03	2.40
1.0 H+	1.72	1.720E-03	1.720E-01	5.518E-03	2.26
2.0H+	3.44	3.440E-03	3.440E-01	7.803E-03	2.11

The pH values were calculated taking into account the HCOOH K_a value: 1.77E^{-04}. The molar concentration of HCOOH was calculated from the final volume in a 10 ml graduate flask. Each volume of HCOOH solution [1.0] M was placed into three different volumetric flasks and deionized H_2O was added to achieve the final volume of 10 ml. These three different solutions allow the evaluation of the ability of formic acid as a gelling agent and the understanding of the gelling process as a function of the different molar concentrations.

2.5. Preparation of Glutamine solutions acidified with formic acid

We prepared three different solution of glutamine containing suitable volumes of HCOOH in order to obtain different molar ratios HCOOH / Glutamine as reported in Table 2.
Each amount of amino acid was dissolved with pure deionized water in a volumetric flask of 10 ml. Before bringing to the final volume, we added the measured quantities of HCOOH [1M] in order to have different formic acid / glutamine molar ratios.

Table 2. Experimental parameters of glutamine solutions acidified with suitable volumes of HCOOH in order to obtain different formic acid / glutamine molar ratios as described in the second last column.

sample code	Glutamine weight (g)	Glutamine (mole)	Glutamine concentration [M] into 10 ml volumetric flask	Volume (ml) of HCOOH [1M] added to Glutamine solution	Mole of HCOOH into 10 ml volumetric flask	Molar ratio HCOOH/ Glutamine	pH
Glu-0.5 H+	0.25839	1.768E-03	0.177	0.883	8.830E-04	0.5	3.51
Glu-1.0 H+	0.26106	1.786E-03	0.179	1.78	1.780E-03	1.0	3.21
Glu-2.0H+	0.25155	1.721E-03	0.172	3.44	3.440E-03	2.0	2.96

2.6. Preparation of Alginate Gels

2.6.1. Alginate Control Sample

Alginate blank proof was obtained using 1 ml of deionized pure water added to 1 ml of alginate 4 % w/v solution.

2.6.2. HCOOH - Alginate Gels

Table 3. Experimental conditions of the alginate gels obtained adding different concentrations of formic acid to 1 ml of aqueous alginate 4%w/v.

Alginate + Formic acid solutions. (sample code)	Mole of HCOOH into 10 ml volumetric flask	Mole of HCOOH contained into 1 ml and put into contact with 1 ml of alginate 4% solution	Molar concentration of [HCOOH] in 2 ml of solution (1ml Alg 4%+1 ml HCOOH)	[H+] = rad(k_a*$Conc_a$)	pH
Alg + 0.5 H+	8.830E-04	8.830E-05	4.415E-02	2.795E-03	2.55
Alg + 1.0 H+	1.720E-03	1.720E-04	8.600E-02	3.902E-03	2.41
Alg + 2.0H+	3.440E-03	3.440E-04	1.720E-01	5.518E-03	2.26

The gels were obtained taking 1 ml of the solution containing different concentrations of formic acid and put into contact with 1ml of aqueous alginate. (Table 3).
The molar concentration of HCOOH has been calculated considering the 2 ml as whole volume. (1ml of aqueous alginate 4% w/v + 1 ml of HCOOH solution).
The $[H^+]$ values were obtained considering the K_a value of HCOOH as 1,77E-04.
The value of pH has been calculated just considering the dissociation of formic acid.

2.6.3. {Glutamine - Alginate} Gels

Table 4. Experimental conditions of alginate gels obtained adding different Glutamine solutions to aqueous alginate solution.

Alginate + Glutamine solution (sample code)	**Starting Concentration of Glutamine [M]**	**Mole of Glutamine into 1 ml and put into contact with 1 ml of alginate 4% solution**	**Mole of HCOOH into 1 ml and put into contact with 1 ml of alginate 4% solution**	**Molar ratio HCOOH/ Glutamine**
Alg + Glu	0.175	1.751E-04		
Alg + Glu-0.5 H+	0.177	1.768E-04	8.830E-05	0.5
Alg+ Glu-1.0 H+	0.179	1.786E-04	1.780E-04	1.0
Alg+ Glu-2.0H+	0.172	1.721E-04	3.440E-04	2.0

Glutamine gels (Table 4) were obtained taking 1ml of aqueous alginate solution and adding 1 ml of each Glutamine solution (as pure Glutamine and acidified Glutamine mixtures).