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# Modelling of HPMC thermogelation as a function of polymer concentration, pH and ionic strength

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## ABSTRACT

Structure and texture are crucial aspects for new non-conventional foods interesting for people having specific dietary preferences or restrictions. In developing these products, in fact, as some basic ingredients are substituted, the performances of traditional foods are hard to be achieved. The lack of conventional structuring ingredients (e.g. gluten proteins, sucrose, fats) may be overcome with the use of hydrocolloids, such as pectins, alginates, agar-agar, carrageenans, cellulose and derivatives, that are usually added to control or minimize possible defects. Despite the large number of factors that can influence hydrocolloids properties, only a limited basic research has been performed on this topic. The aim of this work was to methodically study the thermo-reversible gelation process of hydroxy-propyl-methyl-cellulose (HPMC) in aqueous systems, as a function of three experimental factors: polymer concentration (1-3%), pH (4-10) and ionic strength (50-150 mM) of the dispersing medium. To simultaneously evaluate the effects of the different factors and of their interactions, a Box Behnken Design of Experiment was applied and replicated twice. The thermogelation process was evaluated by means of fundamental rheological methods. In particular, strain sweep and frequency sweep tests were carried out both at 20°C and 80°C; temperature sweep test was performed from 20°C to 80°C. Significant response surface models able to describe the HPMC structuring properties as a function of the three considered experimental factors were calculated. At lower temperatures, the most significant factor ( $P < 0.001$ ) was the HPMC concentration, whereas at higher temperatures, also the pH and the ionic strength had a significant influence ( $P < 0.001$ ) on the rheological properties of the gels. In particular, both main ( $P < 0.001$ ) and interaction ( $P < 0.01$ ) effects of these two last factors were observed for the gelation temperature. The models obtained represent a first step for the identification of the optimal structuring conditions of HPMC in aqueous systems. As future perspectives, these results could make easier the modulation of the structural and textural features of food products as a function of recipes, technological processes, and consumer requirements.

## 1 Introduction

Knowledge of specific interactions and synergistic effects amongst different food components is of high relevance not only for the optimisation of conventional food formulations, but also for creating new formulations and for enabling ingredient substitution. Food industries, in fact, are continuously searching for new non-conventional products, interesting for people with specific dietary preferences, needs or restrictions (vegans, vegetarians, people with high cholesterol levels, or intolerant/allergic, etc.). Generally, structure and texture are the greatest problems in developing a non-conventional food. However, the fundamentals of the networks in such non-conventional foods still have to be completely understood.

Proteins and polysaccharides are the main determinants of the texture and overall sensory properties of food products (deJong et al., 2009). Amongst polysaccharides, hydrocolloids (non-starch polysaccharides) are widely available, have a relatively low cost, and provide a wide range of functionalities, largely responsible for the textural and other physico-chemical properties of food products. The properties of polysaccharides are mainly determined by the shape of their molecules, and these shapes are complex, because polysaccharide molecules themselves are polymolecular and/or polydisperse, and in solutions and gels they are in a constant state of dynamic structural interconversion. However, also the nature of the aqueous environment surrounding polysaccharide molecules affects their conformations, properties and functionalities. Many studies (mainly applied research) investigated hydrocolloids properties as a function of single variables, but very few investigated those variables acting in combination with one another.

The aim of this work was to methodically study the thermo-reversible gelation process of hydroxy-propyl-methyl-cellulose (HPMC) in aqueous systems, as a function of three experimental factors: polymer

concentration, pH and ionic strength of the dispersing medium. To simultaneously evaluate the effects of the different factors, and of their interactions, a Box Behnken Design of Experiment was applied.

## 2 Material and Methods

### 2.1 HPMC dispersions

The HPMC powder used (Sigma-Aldrich, St. Louis, MO) had a molecular weight of 86000Da and a viscosity, in 2% aqueous solution, of 4000mPa·s. The powder was dispersed, at various concentrations, in buffer solutions having different pH and ionic strength values (as reported later on, § 2.2), coming from suitable proportions of  $K_2HPO_4$  and  $KH_2PO_4$  (Sigma-Aldrich, St. Louis, MO). Dispersions were obtained following a highly standardized procedure, specifically set up in the lab at this purpose.

### 2.2 Box-Behnken experimental design

A Box-Behnken experimental design (3 factors, 3 levels) was applied, both to carry out the study in a systematic way, and to model HPMC rheological behaviour not only as a function of the three single factors taken into account but also of their interactions. The three factors (independent variables) were: HPMC concentration (1, 2, 3%w/v), dispersion pH (4, 7, 10) and ionic strength (IS; 50, 100, 150mM). Ranges of factors were selected on the basis of preliminary tests. As a result of the Box-Behnken experimental design (DoE), 15 different HPMC dispersions were produced (Table 1) and characterized in a random sequence, to avoid systematic bias and to minimize the effects of unexpected variability. Three replicates of the combination of the mid-level of the three factors (HPMC=2%w/v, pH=7, IS=100mM) were included in order to estimate the experimental error and to anchor the quadratic model. The whole DoE was replicated twice, to improve the significance of the experimental results.

### 2.3 Consistency as determined by Bostwick consistometer

The consistency of the various HPMC dispersions, maintained at 25°C in a thermostatic chamber, was determined by means of a Bostwick Consistometer LS100 (Labo-Scientifica s.r.l., PR, Italy). It consists of a slope divided into two sections by a gate: the smaller section serves as a reservoir for the dispersion before starting the measurement; the larger section is graded every 0.5cm to allow the measurement of the flow distance. The gate spring is held by a release mechanism, permitting the instant flow of the sample once the gate is opened. The consistency was measured as the distance (cm) the fluid flew after a fixed period of time (10s). Results (expressed as 'distance 10s') are the average of three measurements performed for each sample.

### 2.4 Gelling properties as determined by penetration test

The gelling properties were evaluated on each HPMC dispersion, pouring about 30g of samples in 16 PET cylindrical containers provided with caps, which were placed at 80°C, in a thermostatic chamber. After 30, 60, 90 and 180min at 80°C, 4 containers were taken from the chamber, opened, and a penetration test was carried out on the forming gel. The test was performed by means of an Instron Universal Testing Machine 3365 (Instron Corp., Norwood, MA, USA), controlled by a dedicated software (BlueHill V2.9). The following test conditions were adopted: load cell, 100N; probe diameter, 27mm; crosshead test speed, 50mm/min; maximum strain, 25% of the original height of the sample. Results are expressed as load (N) and energy (mJ) at 15% strain, and they represent the average of 4 determinations for each sample at each testing time. Eight response variables were thus obtained, for each sample, from this test.

### 2.5 Fundamental rheological properties

The fundamental rheological behaviour of the various HPMC dispersions was determined by means of dynamic oscillatory tests performed using a Physica MCR300 rheometer (Anton Paar GmbH, Graz, Austria), supported by the software Rheoplus/32 V3.00 (Anton Paar Germany GmbH, Ostfildern, Germany) and equipped with a 50 mm parallel plate geometry (PP50), with a gap of 2 mm.

In particular, strain sweep tests (SS; both at 20°C and 80°C), frequency sweep tests (FS; both at 20°C and 80°C) and temperature sweep tests (TS; from 20°C to 80°C back to 20°C) were carried out ( $n \geq 2$ ) on the samples. SS were performed to determine the linear viscoelastic region of the different samples, and they were run at a frequency of 1Hz, varying the strain from 0.01% to 100%. When the SS were carried out at 80°C, the heating phase was performed adopting a temperature rate of 3°C/min, a strain of 0.1% and a frequency of 1Hz. Mechanical spectra of HPMC dispersions were evaluated by means of FS performed over the range 0.1–10 Hz, at a constant strain of 0.1% (chosen from the linear viscoelastic region of the samples, both at 20°C and 80°C).

When the FS were carried out at 80°C, the heating phase was performed adopting the same conditions of SS. Finally, TS were made in order to determine both the gelation temperature of the different samples and to evaluate their rheological behavior during heating and cooling, having HPMC a thermo-reversible behavior. TS were performed at a constant heating rate of 3°C/min, a strain of 0.1% and a frequency of 1Hz. The temperature was regulated by a circulating bath and a controlled Peltier system. The presence of a moisturizing external chamber avoid samples dehydration during the test. Before starting each tests, the samples, after loading, were allowed to rest for 5 min to relax stresses.  $G'$  (elastic modulus, Pa),  $G''$  (viscous modulus, Pa),  $\tan\delta$  (damping factor,  $G''/G'$ ),  $G^*$  (complex modulus, Pa), and  $\eta^*$  (complex viscosity, Pa·s) were obtained both from SS at 20°C and 80°C, considering a strain of 0.1%, and from FS at 20°C and 80°C, at a frequency of 1Hz, while only the gelation temperature (GT) was here considered for the TS. In total, 21 response variables were obtained to describe the fundamental rheological behavior of each HPMC dispersion.

## 2.6 Statistical analysis

To discriminate amongst the means, the analysis of variance (ANOVA) with Fisher's least significance difference (LSD) test was firstly performed on the analytical results (Statgraphics Centurion XVI, Statistical Graphics Corp., Herndon, VA, USA). The DoE was created and analyzed by means of a dedicated software (Design-Expert V 8.0.7.1 Trial). In total, results related to 30 experimental samples and 30 response variables were analysed.

## 3 Results

A preliminary data analysis was performed by means of ANOVA and LSD test. Only the results related to the empirical rheological behaviour of the HPMC dispersions and to their gelling temperature (GT) are here reported. Thereafter, data evaluation concerned the statistical elaboration of each DoE and of the two DoE joined together. In the last case, response variable values represent the average of data obtained from each DoE, and only data related to this solution are here presented.

### 3.1 Consistencies, gelling properties and gelation temperatures of HPMC dispersions

Results related to the empirical rheological properties (consistency expressed as 'distance at 10s' and gelling features expressed as 'load' at fixed testing times) and to the gelation temperature of the different HPMC dispersions are reported in Table 1. For their discussion, see § 4.1.

**Table 1.** Results of the ANOVA performed on the empirical rheological variables and on the gelation temperature (GT)

Sample	Independent Factors			Variables					
	HPMC (%w/v)	pH	IS (mM)	Distance 10s (cm)	Load 30' (N)	Load 60' (N)	Load 90' (N)	Load 180' (N)	GT (°C)
Cube-001	1.0	4	100	20.71 <sup>g</sup>	0.034 <sup>a</sup>	0.044 <sup>a</sup>	0.054 <sup>abc</sup>	0.056 <sup>a</sup>	50.60 <sup>gh</sup>
Cube-002	3.0	4	100	9.33 <sup>bc</sup>	0.046 <sup>abcd</sup>	0.196 <sup>de</sup>	0.249 <sup>ef</sup>	0.379 <sup>de</sup>	52.23 <sup>hi</sup>
Cube-003	1.0	10	100	22.04 <sup>g</sup>	0.035 <sup>a</sup>	0.040 <sup>a</sup>	0.048 <sup>abc</sup>	0.057 <sup>a</sup>	43.80 <sup>bc</sup>
Cube-004	3.0	10	100	6.33 <sup>a</sup>	0.055 <sup>d</sup>	0.172 <sup>cde</sup>	0.299 <sup>f</sup>	0.348 <sup>cde</sup>	45.95 <sup>cd</sup>
Cube-005	1.0	7	50	24.08 <sup>h</sup>	0.035 <sup>a</sup>	0.040 <sup>a</sup>	0.043 <sup>ab</sup>	0.050 <sup>a</sup>	52.30 <sup>hi</sup>
Cube-006	3.0	7	50	8.04 <sup>b</sup>	0.050 <sup>bcd</sup>	0.202 <sup>e</sup>	0.332 <sup>f</sup>	0.347 <sup>cde</sup>	51.90 <sup>hi</sup>
Cube-007	1.0	7	150	25.00 <sup>h</sup>	0.036 <sup>ab</sup>	0.041 <sup>a</sup>	0.040 <sup>a</sup>	0.099 <sup>ab</sup>	40.40 <sup>a</sup>
Cube-008	3.0	7	150	8.88 <sup>bc</sup>	0.070 <sup>e</sup>	0.215 <sup>e</sup>	0.474 <sup>g</sup>	0.516 <sup>e</sup>	42.68 <sup>b</sup>
Cube-009	2.0	4	50	12.75 <sup>f</sup>	0.039 <sup>abc</sup>	0.077 <sup>ab</sup>	0.127 <sup>abcde</sup>	0.163 <sup>abc</sup>	53.90 <sup>i</sup>
Cube-010	2.0	10	50	12.75 <sup>f</sup>	0.041 <sup>abcd</sup>	0.098 <sup>abc</sup>	0.113 <sup>abcd</sup>	0.132 <sup>ab</sup>	50.53 <sup>gh</sup>
Cube-011	2.0	4	150	12.83 <sup>f</sup>	0.039 <sup>abc</sup>	0.163 <sup>cde</sup>	0.209 <sup>def</sup>	0.265 <sup>bcd</sup>	48.83 <sup>fg</sup>
Cube-012	2.0	10	150	10.00 <sup>cd</sup>	0.051 <sup>cd</sup>	0.141 <sup>bcd</sup>	0.168 <sup>bcd</sup>	0.217 <sup>abcd</sup>	39.15 <sup>a</sup>
Cent-a	2.0	7	100	10.96 <sup>de</sup>	0.038 <sup>abc</sup>	0.106 <sup>abc</sup>	0.168 <sup>bcd</sup>	0.228 <sup>abcd</sup>	48.73 <sup>efg</sup>
Cent-b	2.0	7	100	12.46 <sup>ef</sup>	0.039 <sup>abc</sup>	0.117 <sup>abcd</sup>	0.172 <sup>cde</sup>	0.256 <sup>bcd</sup>	47.68 <sup>def</sup>
Cent-c	2.0	7	100	11.08 <sup>de</sup>	0.040 <sup>abc</sup>	0.174 <sup>cde</sup>	0.222 <sup>def</sup>	0.264 <sup>bcd</sup>	46.63 <sup>de</sup>

Note: for the same variable, values followed by different letters are significantly ( $P < 0.05$ ) different

### 3.2 DoE elaboration and models building

All data were firstly analyzed in terms of Gaussian distribution and transformed, when necessary. Data transformations, in fact, are often a very effective way to deal with the problem of non-normal response and the associated inequality of variance (Montgomery, 2001). As far as concern the investigated response variables, a  $\log_{10}$  transformation was applied to all the variables obtained from the fundamental rheological tests, with the

only exception of GT. No data transformation was performed on the other parameters.

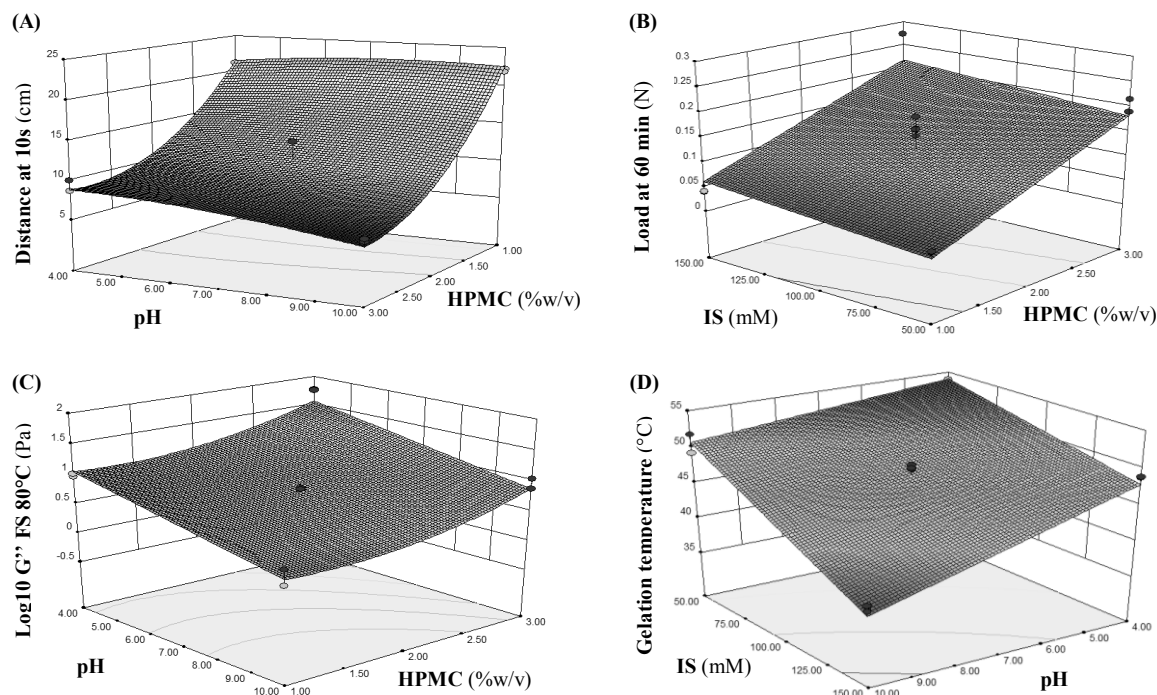
The data matrix obtained after this pre-treatment was elaborated by means of the response surface methodology (RSM). A response surface is the geometric representation obtained when a response variable is plotted as a function of one or more quantitative factors. Data from Box–Behnken designs can be efficiently used to fit a response surface model; in particular they can be used to fit a full quadratic response surface model. The full quadratic response surface model, applied to this study, is described by Eq. (1):

$$(1) Y = \beta_0 + \beta_1 * C + \beta_2 * pH + \beta_3 * IS + \beta_{12} * (C * pH) + \beta_{13} * (C * IS) + \beta_{23} * (pH * IS) + \beta_{11} (C)^2 + \beta_{22} (pH)^2 + \beta_{33} (IS)^2 + \varepsilon$$

where Y is the value of the considered response variable; C, pH and IS are the values of the three independent factors;  $\beta_0$  is the constant value (intercept);  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are the linear coefficients;  $\beta_{12}$ ,  $\beta_{13}$ , and  $\beta_{23}$  are the interaction coefficients;  $\beta_{11}$ ,  $\beta_{22}$ , and  $\beta_{33}$  are the quadratic coefficients;  $\varepsilon$  is the random error. As 30 responses have been measured, a separate analysis has been performed on each one.

First, ANOVA was carried out, in order to determine the significance of each model and of each coefficient within the model. For various response variables, some coefficients came out to be not significant, and the model described by Eq. (1) was consequently simplified considering only the significant factors. In particular, the following response variables were better described by a linear model: load at 30, 60, 90 and 180min; energy at 60 and 90min;  $\tan\delta$  SS 0.1% 20°C;  $\tan\delta$  FS 1Hz 20°C. Otherwise, the following response variables were better described by a 2-factors linear interaction model:  $\tan\delta$  SS 0.1% 80°C;  $\tan\delta$  FS 1Hz 80°C; GT.

For each model describing each response variable, the determination coefficient ( $R^2$ ), the coefficient values (with their own significance), as well as the lack of fit (LOF), were determined (*data not showed*). The LOF tests the error in response prediction: if the P-value for LOF is smaller than 0.05, the model does not describe the true shape of the response surface. As an evidence of the fairness of the selected 30 models, LOF was always non significant ( $P > 0.05$ ), with the only exception of two specific cases (load at 30min;  $\tan\delta$  FS 1Hz 80°C). Generally, all the models were characterized by a high significance ( $P < 0.001$ ). The response surfaces of some of the variables investigated in this research have been reported in Fig. 1; specifically: distance at 10s (as determined by the Bostwick consistometer), load at 60min (from the penetration test),  $G''$  (from the FS performed at 80°C), and GT (from the TS).



**Figure 1.** Response surfaces of some of the parameters used to characterize the various HMPC dispersions.

## 4 Discussion

### 4.1 Consistencies, gelling properties and gelation temperatures of HPMC dispersions

Data reported in Table 1 refer to some of the empirical rheological properties measured and to the gelation temperature of the different HPMC dispersions. With regard to their consistency, the significant ( $P < 0.05$ ) differences evidenced amongst the samples were mainly related to HPMC concentration: dispersions containing 1% HPMC, in fact, exhibited the fastest flows, while those containing 3% HPMC presented a reduced flow rate. HPMC concentration thus seemed to influence the consistency of the samples at 25°C at the most, while pH and IS did not appear as significant factors.

In terms of gelation kinetics, none of the independent factors seemed to influence gel load at the beginning of the 80°C storage period. This was probably due to the fact that the systems were in evolution and no definite property could be outlined yet. Later on, HPMC concentration, again, came out to be the main influencing factor, being those gels containing 3% HPMC the most consistent. When the focus was on the same sample at the different testing times, it was possible to highlight how the majority of the systems became stable only after 180min of storage at 80°C.

Apparently, none of the three independent factors seemed to play a key role on GT. However, looking at samples Cube-005 and Cube-006 (different HPMC concentrations; same pH; same IS), it was assumed that pH and IS, joined together, probably influenced the gelation temperature of the various HPMC systems to a larger extent than HPMC concentration by itself.

### 4.2 Modelling of HPMC thermogelation

Generally, response variables related to the empirical rheological properties of the various HPMC systems were better described by linear models, with a highly significant effect ( $P < 0.001$ ) of the hydrocolloid concentration. On the contrary, response variables related to the fundamental rheological properties of HPMC dispersions were better described by quadratic models, with a significantly high ( $P < 0.001$ ) predominance of HPMC concentration in all the range of temperatures investigated. However, at higher temperatures (80°C), an increase in the influence of pH and IS on the system features was observed. In particular, pH and IS (both as single factor and as interactive factors) significantly influenced ( $P < 0.01$ ) GT.

For some of the 30 response variables taken into account, more details can be noticed in Fig. 1. The variation of consistency (determined by the Bostwick consistometer), as a function of pH and HPMC concentration, was reported in Fig. 1A. The curvature is an indication of the quadratic model found to describe its evolution. The most significant influencing factor was HPMC concentration. Its negative  $\beta$  coefficient (-19.920) signified an inverse correlation: the highest the concentration the lowest the consistency of the hydrocolloid dispersion. The HPMC concentration was the most significant factor ( $P < 0.001$ ) also for gel hardness (load at 60min; Fig.1B): in this case, however, the model was linear and the positive  $\beta$  coefficient (0.078) indicated a direct correlation between hydrocolloid concentration and gel firmness. An example of the response surfaces obtained for the fundamental rheological variables investigated was reported in Fig. 1C: the pH of the environment in which the hydrocolloid was dispersed became highly influent ( $P < 0.001$ ) at higher temperatures (80°C). In particular, all the three independent factors significantly ( $P < 0.001$ ) influenced the specific response variable ( $\log_{10}$  of  $G''$  from FS at 80°C; quadratic model). The response surface related to GT (2-factor linear interaction model) was reported in Fig. 1D: pH and IS came out as the most significant ( $P < 0.001$ ) factors affecting the gelation temperature of the different HPMC systems. Both the  $\beta$  coefficients associated to pH and IS were negative (-0.039 and -0.020, respectively), indicating that an increase in the pH or in the IS of the environment in which the hydrocolloid was dispersed determined a decrease of HPMC gelation temperature. The maximum GT value recorded during the experiments, in fact, was obtained when both the pH and the IS of the system were at their minimum.

## 5 Conclusions

The extreme complexity of the HPMC thermogelation was highlighted by this study, as this process was significantly influenced by all the three independent factors investigated (polymer concentration, pH and ionic strength of the dispersing medium) as well as by their interactions. In particular, the effect of HPMC concentration on the properties of the systems was predominant at the lower temperatures. However, during the systems settling due to temperature increase, pH and ionic strength assumed a significant influence, too. Despite the complexity of the process, this study allowed to calculate some mathematical models able to describe, in a proper way, HPMC thermogelation as a function of polymer concentration, pH and ionic strength,

as well as of their interactions, thus highlighting the importance of fundamental systematic studies in this sector. The models obtained represent a first step for the identification of the optimal structuring conditions of HPMC in aqueous systems, as well as a first step towards a more conscious selection of the proper hydrocolloids to be used in a specific food preparation. As future perspectives, these results could make easier the modulation of the structural and textural features of food products as a function of recipes, technological processes, and consumer requirements.

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