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Influence of rheological properties and microstructure of microemulsions on the kinetic release of bioactive compounds

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In memory of Dr. Alicia Califano (1951–2019), Lead Scientist (CIDCA-CONICET, Argentina) and a highly respected colleague who will be greatly missed.

Abstract

Microemulsions (ME) are one of the most effective ways to incorporate lipophilic active compounds into water-based food matrices. Great stability, colloidal domain droplets, and optical isotropy are some of the ME advantages. The present work analyzes the influence of rheological behavior and microstructural characteristics of food microemulsions on the release rate of lipophilic vitamins. A fluid ME was compared to a ME with higher dispersed phase content and a bicontinuous structure (gel-ME). Additionally, carboxymethyl cellulose (CMC) was added to fluid ME (3.5 and 10 g/100g), obtaining systems with different viscosity and microstructure. All ME were characterized using FTIR, rheological analysis, and TEM microscopy. Those systems with similar zero-shear viscosity showed significantly different viscoelastic behavior; gel-ME behaved like a concentrated suspension of macromolecules but with the largest plateau modulus. Microemulsions with CMC exhibited a viscoelastic solid type with G' > G''. Mechanical spectra were satisfactorily fitted with Generalized Maxwell model and the relaxation time spectra were determined. Kinetic release of vitamins E and D was studied and modeled at 37 °C. Fluid and gel-ME reached higher percentages of release in a very fast manner, while CMC systems showed a matrix-driven nature of the release, in agreement with the relaxation time behavior determined from rheological experiments.

Keywords: Microemulsions; viscoelasticity; bioactive compound release; relaxation time sprectrum; carboxymethyl cellulose; vitamins

Author's Contribution

N.M.C. participated in: Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing

S.S.M. contributed to: Methodology, Investigation, Writing - Review & Editing

M.E.R. worked mainly in: Conceptualization, Investigation, Writing - Review & Editing, Supervision,

A.C. was a critical part in: Conceptualization, Project administration, Funding acquisition

G.L. did a general contribution in: Conceptualization, Investigation, Writing - Review & Editing, Project administration, Supervision, Funding acquisition.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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1. INTRODUCTION

In recent years, consumers' demand for functional foods has increased as a result of the increased knowledge of functional ingredients and their impact on human health and physiological functions [1, 2]. There are a wide variety of foods where lipophilic active compounds (such as bioactive lipids, flavors, antimicrobials, antioxidants, and nutraceuticals) must be incorporated into aqueous media, to make them suitable for oral consumption [3, 4]. Particularly, vitamins are essential for human growth and development, but most of them are not produced in the human body and need to be supplied through foods or food supplements [1]. Adequate intake of vitamin D increases the intestinal absorption of calcium and promotes normal bone formation and mineralization [5, 6]. Vitamin E, on the other hand, provides health benefits to humans such as the prevention of cardiovascular diseases and cancer [7, 8]. Furthermore, vitamin E plays a fundamental role in the treatment of Alzheimer and other neurological diseases [9].

Material and microstructures that can enable the controlled release of active ingredients are increasingly used in both the foods and pharmaceutical industries [10]. Colloidal delivery systems such as microemulsions, nanoemulsions, and emulsions are some of the most effective ways to encapsulate and incorporate lipophilic active compounds into water-based matrices. These vehicle systems should be capable of releasing the active compound after consumption at a particular site within the human body, for example, bioactive compounds in the gastrointestinal tract and flavors in the mouth [11]. Therefore, controlled release systems were developed to maintain the desired concentration of the active compounds, exerting control on the release rate and duration.

Microemulsions have been attracted the attention of the cosmetic and food industries since they are optically isotropic, thermodynamically stable, and can improve the bioavailability of bioactive compounds. Some studies indicate that the bioavailability of bioactive compounds may be increased when delivered as an o/w microemulsion because the interfacial layer of the nanodroplets can enhance transmembrane passage across the digestive tract [12]. Additionally, most microemulsions, mainly those with nonionic emulsifiers, presented great stability in a wide range of pH, temperature, and ionic strength [13-15]. Furthermore, the transparency of microemulsions allows the incorporation of lipophilic bioactive compounds in wide variety of food matrices that must be transparent or have a slightly

cloudy appearance (i.e., flavored waters, herbal drinks, isotonic drinks, jellies, fruit gummies, etc.).

From a structural point of view, microemulsions contain oil and water microdomains separated by surfactant layers like lyotropic liquid crystals, which gives a wide range of flow behaviors depending on the concentration of the components [16]. Thus, a fluid water-like microemulsion with tiny oil droplets could be obtained as well as a gel-type bicontinuous structure altering layers of oil and water, depending on the disperse phase content.

This great variety of structures and behavior makes microemulsions very attractive systems for food applications as bioactive compounds delivery. However, the interaction between components and the rheological behavior could probably affect the way of bioactive compounds are released from a food matrix. Therefore, the hypothesis of this work is that the kinetic release of bioactive compounds from the internal lipid phases depends on both the viscosity of the continuous phase and the microemulsions structure.

The objective of the present work was to analyze the influence of rheological behavior and microstructural characteristics of food microemulsions on the release rate of lipophilic vitamins.

2. MATERIALS AND METHODS

2.1 Materials

Macrogolglycerol hydroxystearate (Kolliphor RH40) was kindly donated by BASF (Argentina S.A.). Commercial sunflower oil was purchased from Molinos Cañuelas (SACIFIA, Argentina). Analytical grade ethanol > 99.5% was used as cosurfactant (Soria, Argentina). Vitamin E acetate and vitamin D were provided by Laboratorios Bagó (Argentina S.A.). Butylated hydroxytoluene (BHT) was used as antioxidant (Sigma Aldrich, Argentina). Carboxymethyl cellulose (CMC) was employed as thickener (Saporiti SACIFIA, Argentina). Sodium azide was used as antimicrobial agent (Anedra, Argentina). Distilled water was added to all continuous phases and microemulsions.

2.2 Formation of continuous phases

In order to formulate o/w microemulsions with thickener with a target viscosity, the flow behavior of aqueous continuous phases with CMC was previously determined.

158 Dispersions with 3, 3.5, 4, and 5 g CMC/100 g water were evaluated. Samples were
159 prepared by slowly adding the hydrocolloid in a vessel with distilled water at 40°C and
160 with constant stirring. They were allowed to stand for 24 h before use.
161
162 **2.3 Formation of microemulsion**
163 Microemulsions were prepared following the procedure previously described with
164 slight modifications [14]. First, vitamins E and D (1:1) together with BHT (0.01 g/100 g
165 lipid phase) were dissolved in sunflower oil. Then, disperse phase was prepared by
166 mixing appropriate amounts of surfactant (Kolliphor RH40), sunflower oil and absolute
167 ethanol (cosurfactant). Microemulsions were prepared by slowly adding water over the
168 lipid phase with constant stirring. Depending on the water content, two microemulsions
169 were obtained: gel-ME (with a bicontinuous structure) and o/w-ME (fluid).
170 Additionally, two CMC concentrations were added to the o/w-ME, to obtain the low
171 CMC and high CMC microemulsions (Table 1).
172
173 **Table 1**. Composition of the studied microemulsions. Contents are given in g/100 g of
174 final microemulsion.

	o/w-ME	gel-ME	ME-low CMC	ME-high CMC
Water	80	40	76.5	70
Sunflower oil	0.67	3.34	0.67	0.67
Surfactant	18	54	18	18
Cosurfactant	0.67	2	0.67	0.67
Vit. E & D (1:1)	0.66	0.66	0.66	0.66
CMC	-	-	3.5	10

175
176
177 **2.4 Rheological measurements**
178 Rheological tests were carried out for the different formulations using a Haake
179 RS600 controlled stress rheometer (Haake, Germany). Parallel plates of 60 mm
180 diameter were used for the most viscous samples and double gap concentric cylinders
181 for the most fluid. All measurements were performed at least in duplicate and
182 maintaining a constant temperature of 37°C to simulate the thermal condition of the
183 release study. The data was processed using the IRIS Rheo-hub 2012 program to
184 perform the flow behavior and spectra calculations [17]. The standardized protocols

used were previously described by Mori Cortés et al. [14]. Briefly, steady state flow measurements were applied to determine the viscosity dependence on the shear rate. Additionally, stress sweeps were performed at a frequency of 1 Hz (6.28 rad/s) to determine the linear viscoelastic range (LVR). Afterwards, within the LVR, frequency sweeps were carried out to obtain the variation of the elastic (G') and viscous (G'') moduli. Lastly, changes in the rheological behavior were monitored as a function of time and over a temperature sweep (8°C-80°C), using a fixed stress within the LVR.

2.5 Fourier-transform infrared spectroscopy (FTIR)

Samples were analyzed using a Thermo Nicolet iS10 spectrometer (Thermo Scientific, MA, USA) with an attenuated total reflectance (ATR) accessory. FTIR spectra were recorded as a result of 32 consecutive scans and a resolution of 4 cm^{-1}. Scanning was conducted from 4000 to 600 cm^{-1}. Before each determination a background without sample was determined. At least four replicates of samples were recorded. Spectrum analysis was performed using the software Omnic 9.0 (Thermo Scientific, Madison, USA).

2.6 Transmission electron microscopy (TEM)

The microemulsions were observed with a transmission electron microscope (JEM 1200EX II, Jeol, USA) by using a negative staining method. A small aliquot of microemulsion was deposited on a collodion coated grid and stained with one drop of aqueous solution of phosphotungstic acid. The excess droplet was removed before TEM observation.

2.7 Release studies

The release profiles of vitamins were determined using dissolution equipment (USP Apparatus I, basket). Phosphate buffer (250 ml) at pH 6.8 was used as dissolution medium. The temperature was set at 37°C and the dissolution medium was continuously stirred at 50 rpm. The formulations were previously placed in rigid gelatin capsules (two capsules per glass). At predetermined time intervals (0.5, 1, 2, 4, 6, 10 and 24 hours), 1 ml samples were taken and replaced by the same volume of fresh release medium to maintain sink conditions at all times. The percentage of vitamins released was determined in a Dionex Ultimate 3000 UHPLC (Thermo Scientific, Sunnyvale, CA, USA) equipped with a dual gradient tertiary pump (DGP-3000) and a DAD-3000

diode array detector. A C18 column (Phenomenex, USA), packed with 5 μm particles was utilized for chromatographic separation. Two wavelengths were selected for the determination of vitamins in order to obtain greater sensitivity for the detection of each one: 265 nm (vitamin D) and 284 nm (vitamin E).

2.8 Statistical analysis

All statistical procedures were computed using the SYSTAT software (SYSTAT Inc., Evanston, IL, USA). Analyses of variance were conducted separately on the dependent variables analyzed. Tukey's test was chosen for simultaneous pairwise comparisons. Differences in means and F-tests were considered statistically significant when $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Flow behavior of the microemulsions

Fig.1a shows the shear rate dependence of the viscosity for carboxymethyl cellulose dispersions at different concentrations (3, 3.5, 4 and 5 g CMC/100 g), at a temperature of 37°C. As it was previously reported, this rise in viscosity could be attributed to the increase in the intermolecular interaction between CMC molecules [18]. All suspensions exhibited a marked non-newtonian behavior, typical in concentrated hydrocolloid dispersions. Although an overall shear-thinning behavior is observed for all samples, it is noteworthy that at low shear rates, CMC suspensions showed a shear thickening behavior. At large shear rates, macromolecule chains are disentangled and aligned in the flow direction, which is the general consensus of the shear-thinning behavior of biopolymeric suspensions. However, shear-thickening was not deeply discussed and it is not fully elucidated. Benchabane and Bekkour [19] found the same shear-thickening behavior in CMC suspensions and related with a stiffer inner structure caused by the formation of entanglements between polymer coils and the increase in the intermolecular interactions, as the shear rate rises.

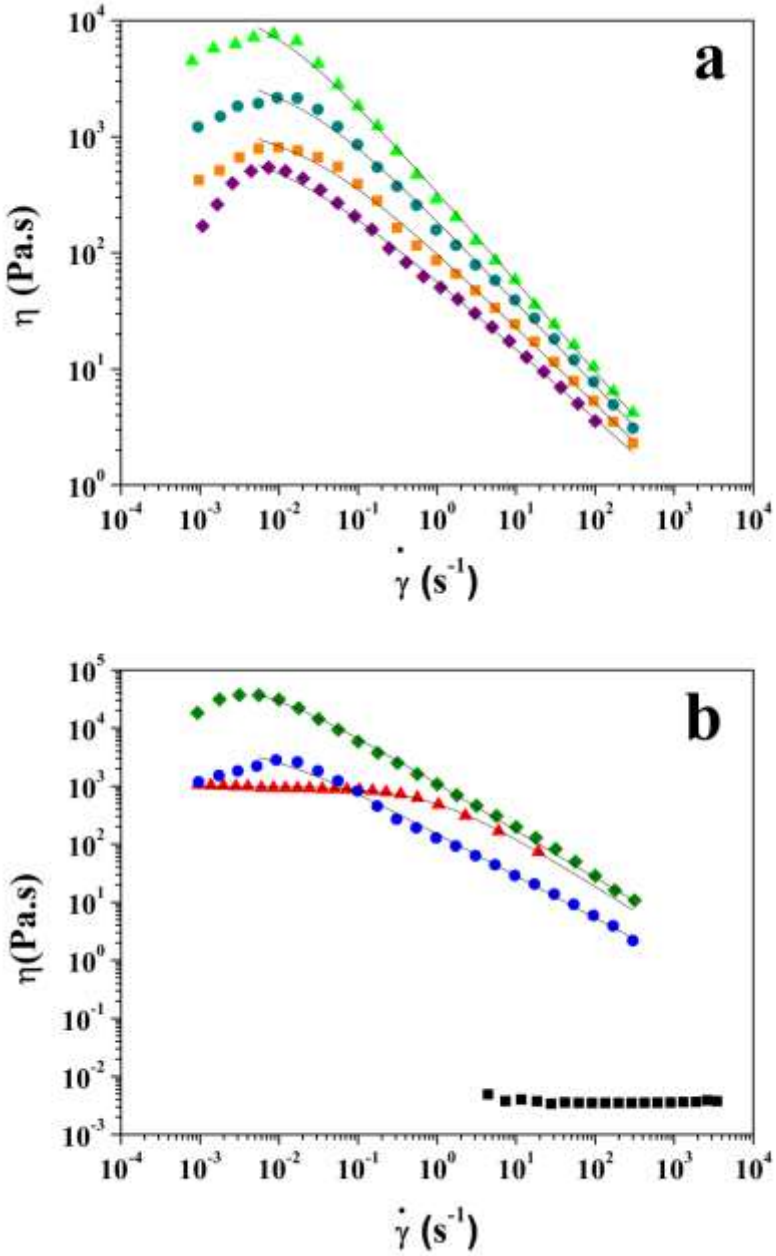


Fig. 1 a) Flow curves of aqueous suspensions with different concentrations of carboxymethyl cellulose: (◆) 3, (■) 3.5, (●) 4 y (▲) 5 g CMC/100 g distilled water. b) Flow curves for microemulsions: (■) o/w-ME, (▲) gel-ME, (●) ME-low CMC and (◆) ME-high CMC according to Table 1 codes. Continuous line: Cross model

To interpret the shear thinning dependence, the Cross model was used [20]:

$$\eta = \frac{\eta_0}{(1+(\tau\dot{\gamma})^n)} \qquad (1)$$

where η is the viscosity corresponding to a shear rate $\dot{\gamma}$; η_0 is the asymptotic value at zero shear rate; τ is a transition time that represents the reciprocal of the shear rate required to halve the viscosity, and n a measure of the pseudoplastic characteristics.

It was observed that by increasing the concentration of thickener from 3 to 5 g/100 g, the value of η_0 increased from $9.7x10^2$ to $1.6x10^4$ Pa.s. This is because the CMC generates a structure formed by a three-dimensional network of interconnected CMC chains. Then, increasing their concentration increases the interactions between the chains causing an increase in the strength of the network. As shown in Fig. 1b, the ME-low CMC also exhibited a qualitatively similar behavior to the corresponding continuous phase but with a marked increase in viscosity values. This suggests that the oil droplets are trapped in the network formed by the polymer chains of CMC thus reinforcing the structure.

Fig. 1b shows that the gel-ME containing 60 g dispersed phase/100 g emulsion also exhibited a marked pseudoplastic behavior. However, its behavior was qualitatively different from ME-low CMC. Although both values of η_0 could be considered within the same range ($2.3x10^3$ Pa.s and $9.9x10^2$ Pa.s for the microemulsion with thickener and gel, respectively), the values of τ were significantly different. The ME-low CMC had a characteristic transition time of 30.2 s while for the gel-ME it was only 1.02 s. This value corresponds to the inverse of the shear rate that is necessary for the structure of the material to begin to flow. Therefore, gel-ME presented a more stable structure and a greater range of deformation rates without modifying its viscosity. On the other hand, microemulsion containing 10 g CMC/100 g (ME-high CMC) exhibited pseudoplastic behavior with a value of $\eta_0 = 7.6x10^4$ Pa.s. This microemulsion presented a transition time ($\tau = 58.7$ s) longer than ME-low CMC. At rest, the microemulsion with higher thickener content has a stronger structure given by the greater number of interactions between the CMC polymer chains. However, by increasing the shear stress these interactions are easily broken causing weakening of the structure.

The decrease in the dispersed phase content at 20 g disperse phase/100 g emulsion generated changes in the flow characteristics of the microemulsion. The o/w-ME exhibited a Newtonian behavior. This behavior is characteristic of microemulsions where oil drops are freely dispersed in a large volume of continuous phase.

3.2 Viscoelastic behavior of microemulsions

Stress sweeps of the different microemulsions were carried out to determine their linear viscoelastic range. Fig. 2 shows the complex modulus ($G^*=((G')^2 + (G'')^2)^{1/2}$) as a function of the stress amplitude. The fluid o/w-ME was invariant throughout the entire range of measured stresses. While the gelled microemulsions either by the addition of thickener or by the increase of disperse phase showed a decrease of G* to approximately 300 Pa.

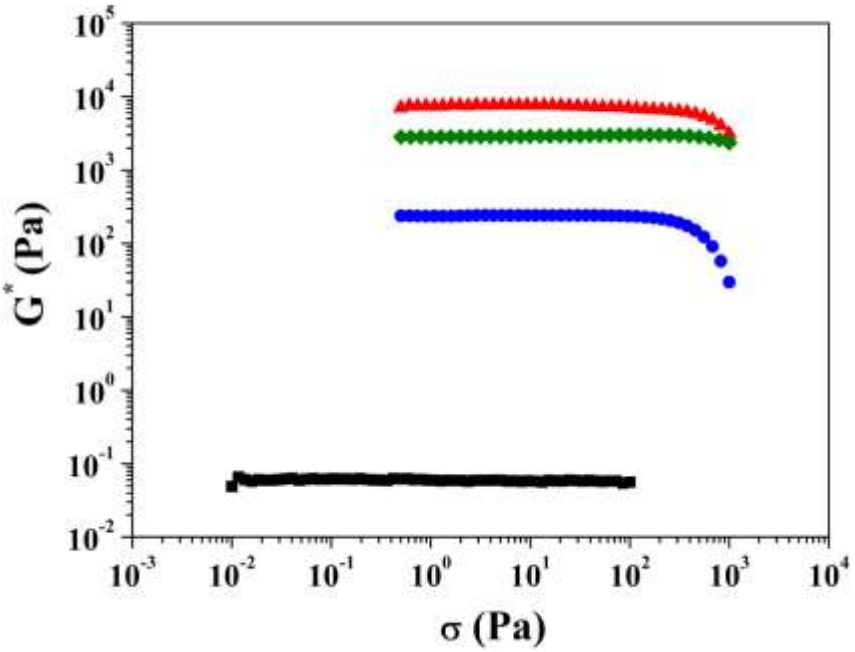


Fig. 2 Stress sweeps for microemulsions: (■) o/w-ME, (▲) gel-ME, (●) ME-low CMC and (◆) ME-high CMC according to Table 1

Fig. 3 shows the elastic modulus (G') and the viscous modulus (G'') as a function of the oscillatory frequency (ω) at 37°C. It can be seen that although the ME-low CMC and the gel-ME had similar zero-shear viscosities, they exhibited a markedly different viscoelastic behavior. The gel-ME showed characteristics of a concentrated solution of macromolecules with G''>G' and a crossover of both moduli at ω~25 rad/s. On the contrary, both o/w microemulsions containing thickener exhibited a viscoelastic solid type behavior with G'>G'' throughout the studied frequency range and with a low dependence of both moduli with the frequency. Although the ME-low CMC exhibited a weak gel-like behavior, its mechanical spectrum was below the gel-ME spectrum. As the thickener content increased, there was an increase in the elastic characteristics of the system. Thus, the ME-high CMC showed a significant increase in both moduli,

exceeding the mechanical spectra of the gel-ME. Larger differences between G' and G'' and a smaller variation with oscillation frequency were observed, which is a typical behavior of a more elastic matrix. On the other hand, the o/w-ME exhibited a markedly viscous behavior, typical of a Newtonian fluid. Very low values of G' and G'' and a marked linear dependence of these parameters were observed with frequency throughout the studied range ($G' \propto \omega^2$ and $G'' \propto \omega$).

The results obtained were adjusted satisfactorily using the generalized Maxwell model, where the values of G' and G'' at each frequency are given by the sum of N contributions from N Maxwell elements in parallel, defined by the elastic response of a spring (G_i) and the relaxation time λ_i [21].

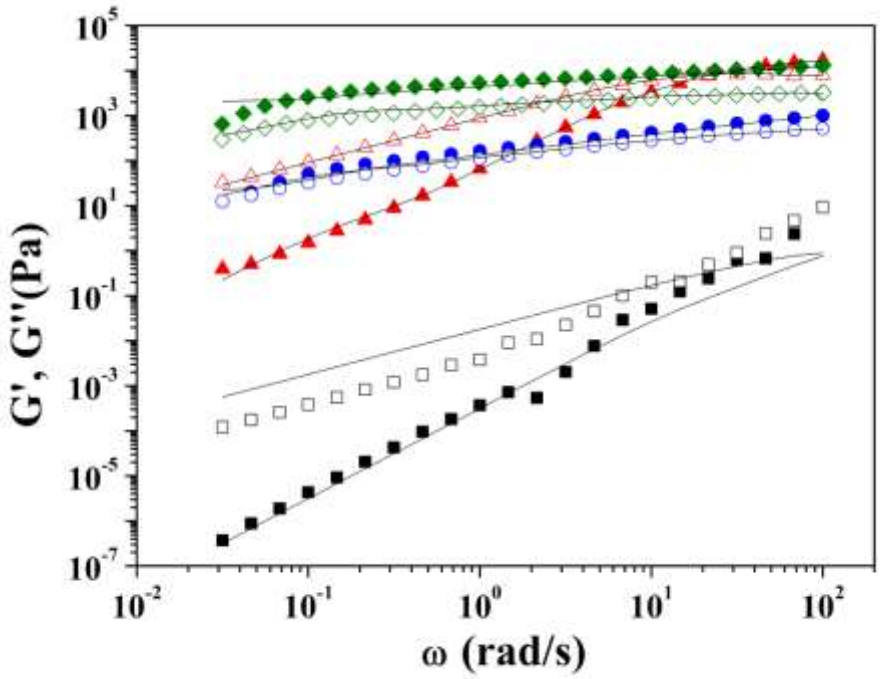


Fig. 3 Frequency sweeps for microemulsions: (■,□) o/w-ME, (▲,△) gel-ME, (●○) ME-low CMC and (◆,◇) ME-high CMC, according to Table 1. Full symbols: G'; empty symbols: G''. Continuous lines: Maxwell model

3.3 Thermo-rheological analysis

Fig. 4 shows G' and G'' vs temperature for the gel-ME and ME-low CMC. The microemulsions showed markedly different behaviors with temperature. At low temperature, the gel-ME constituted a stronger gel than the ME-low CMC. However,

when the temperature increased, an abrupt fall of both moduli and a crossover at 36.5ºC were observed. This behavior is associated with the thermal properties of the emulsifier that were previously reported in other works, where an abrupt fall in the complex viscosity η* of the Kolliphor RH40 was observed at a similar temperature, related to its complete fusion [14]. Conversely, the ME-low CMC showed a slight variation of the moduli with the temperature and a crossover of G' and G'' at around 60°C. This behavior is similar to that previously reported in CMC suspensions, which shows that the rheology of the hydrocolloid governs the mechanical properties of microemulsions [22]. Both the o/w microemulsion with thickener and gelled completely recovered their structure after the cooling process (supplementary material S1).

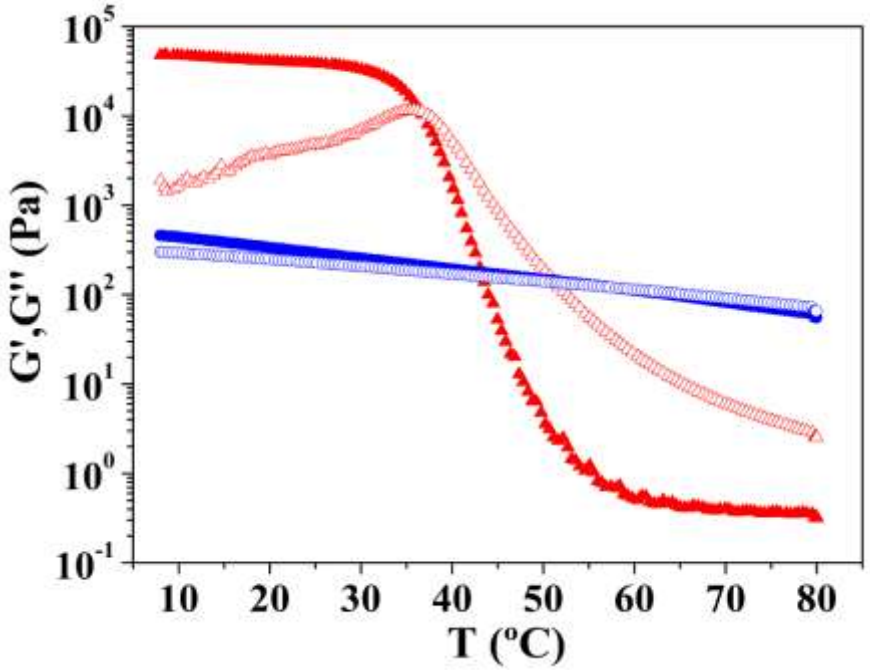


Fig. 4 Thermo-rheological analysis: (▲,△) gel-ME and (●,○) ME-low CMC according to Table 1. Full symbols: G'; empty symbols: G''

3.4. FTIR Analysis

To evaluate the interactions involved in the formation of the microemulsion, ATR-FTIR spectra was determined (Fig. 5) on CMC (A), hydrogels of CMC (B), CMC+Kolliphor RH40 (C), and CMC+Kolliphor RH40+ethanol (D), o/w-ME (E), ME-low CMC (F), ME-high CMC (G) and gel-ME (H). The FTIR spectrum of CMC (A) showed a broad band at 3245 cm^{-1} due to the –COO stretching, which overlaps with the –OH stretching region at 3400- 3300 cm^{-1} [23]. The band at 2880 cm^{-1} arises from the

C–H stretching. The peak at 1585 cm^{-1} was attributed to asymmetric stretching of –COO group. The bands around 1412 and 1321 cm^{-1} were assigned to–CH_2 scissoring and C–OH bending vibration, respectively [24]. The band at 1019 cm^{-1} was associated with CH_2–O–CH_2 stretching. FTIR of the hydrogels (B, C, and D) and microemulsions (E, F, G, and H) presented a peak at 1635 cm^{-1}, assigned to the scissoring vibration of water and a broad band around 3650-3000 cm^{-1} corresponding to the asymmetric stretching and hydrogen bonds of the –OH group. The band in the 3650-3000 cm^{-1} region can be divided into a lower frequencies band (3300-3000 cm^{-1}) corresponding to strong hydrogen bonds arranged in a tetrahedral structure, and a higher frequencies band (3650-3300 cm^{-1}), related to weak hydrogen bonds (non-tetrahedral ordering) of water molecules. Compared to pure water, the spectra of microemulsions showed a decrease in the relative area of the band around 3250 cm^{-1}, which is a measure of the fraction of water molecules that vibrates within a tetrahedral structure [25]. Additionally, a shift of the vibration frequency was observed from 3313 cm^{-1} in pure water to 3382 cm^{-1} in gel-ME that could be attributed to hydrogen bond weakening. Similar results were reported by Moayedzadeh, Gunasekaran, and Madadlou [26] when studying gelled emulsions with gellan gum. On the other hand, by subtracting the spectral contribution of water to the spectrum of the hydrogels and the microemulsions, some of the characteristic peaks of CMC and surfactant could be observed (supplementary material S2). All the microemulsions presented a band with two peaks at approximately 2924 and 2859 cm^{-1} that were attributed to asymmetric and symmetric –CH_2 stretching vibrations, respectively. While the peak at 1733 cm^{-1} was assigned to the C=O stretching [27]. Furthermore, the microemulsion showed an intense band at 1085 cm^{-1} due to C-O-C stretching. These peaks were assigned to the surfactant since they were not found in the CMC or in CMC hydrogel.

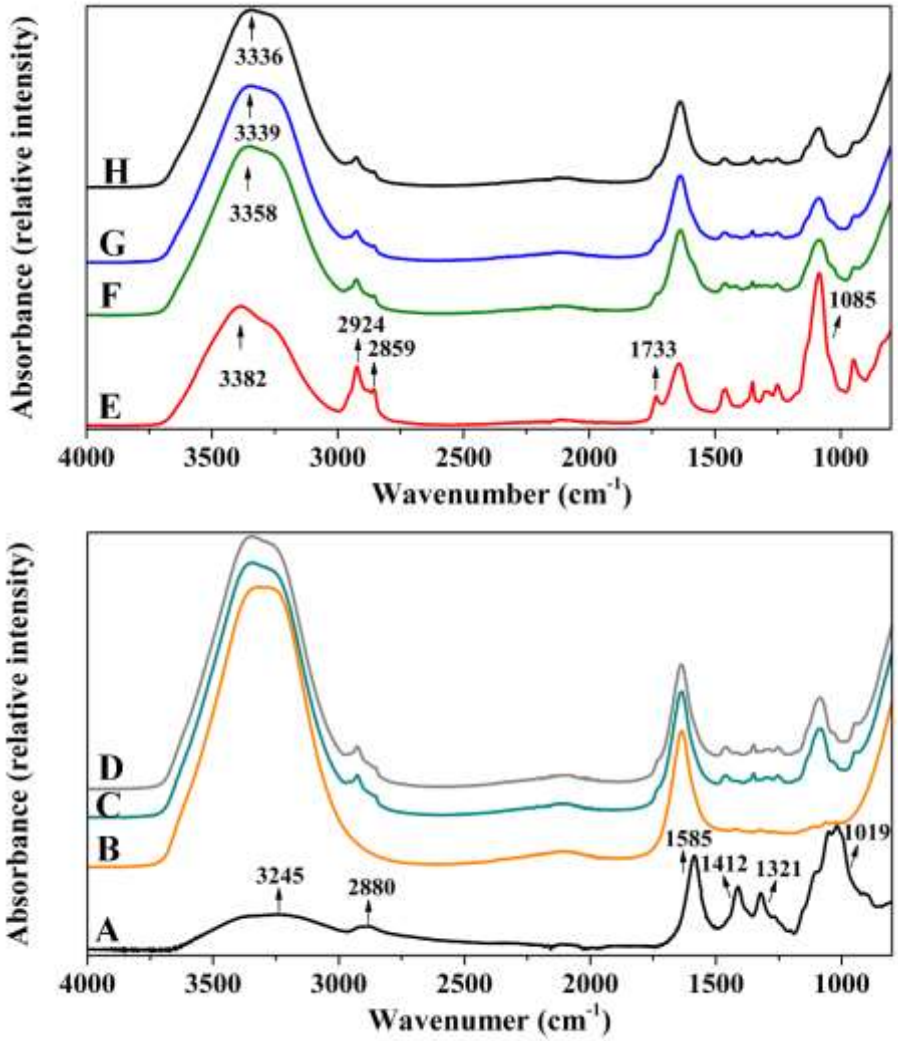
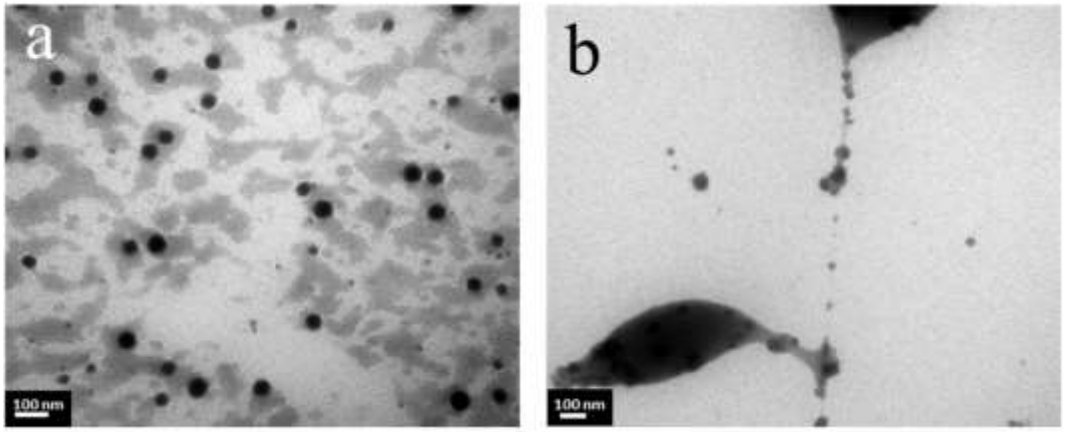


Fig. 5 ATR-FTIR spectra of CMC (A), hydrogels of CMC (B), CMC+Kolliphor RH40 (C), CMC+Kolliphor RH40+ ethanol (D), o/w-ME (E), ME-low CMC (F), ME-high CMC (G) and gel-ME (H), according to Table 1

3.5 Transmission electron microscopy

In Fig. 6 the images obtained by TEM can be observed. The o/w-ME presented small spherical droplets homogeneously dispersed without presence of aggregates. For TEM observations it was necessary to eliminate the thickener from the continuous phase since it interfered with the observation of the drops. In the gel-ME, filamentous aggregates that interconnect irregular clusters of droplets were observed. Guo, Zhang, Wang, Liu, and Gu [28] observed a similar transition in microstructural characteristics when increasing the disperse phase content. The extended network of interconnected

390 particles and emulsifier micelles could be the responsible for the significant increase in
391 viscosity and elastic characteristics reported earlier.
392



393
394 **Fig. 6** Microscopic images obtained by TEM for: (a) o/w-ME and (b) gel-ME according
395 to Table 1
396
397 **3.6 Release studies**
398 Before the release assays, each component of the sample was analyzed separately
399 by HPLC (surfactant, sunflower oil, BHT and CMC) to detect possible interference
400 (peak overlap) with the bioactives. The BHT showed a chromatographic peak at
401 retention times markedly different from the retention times corresponding to the
402 vitamins, so no interference was observed. On the other hand, surfactant, sunflower oil
403 and CMC could not be detected under the chromatographic conditions tested. This
404 demonstrates that the usual concentrations of alpha-tocopherol from sunflower oil have
405 no detectable absorption peaks, which can overlap and interfere with the detection of
406 vitamin E incorporated in the microemulsion.
407 Fig. 7 shows the release profiles of vitamins E and D as a function of time for the
408 studied formulations. Vitamins E and D showed similar release rate regardless of the
409 type of structure or viscosity of the microemulsion. Initially there was a marked
410 difference in the release profile of the fluid o/w-ME and gel-ME (bicontinuous
411 structure). After 30 minutes, the o/w-ME presented more than 90% of the vitamins
412 release while the gel-ME showed a release ~45% for all bioactive components (Fig. 7a).
413 However, after 1 hour of testing, no significant differences were observed between the
414 release profiles of both microemulsions. Feng, Wang, Zhang, Wang, and Liu [29] found
415 similar results of the amount released when studying the rate of release of vitamin E in

o/w microemulsions produced with a non-ionic emulsifier. The release rate of bioactives in o/w-ME was mainly affected by the gradient concentration. In these systems, the oil droplets containing the bioactives were dispersed in an aqueous medium with low viscosity whereby they could easily diffuse into the release medium. On the other hand, the gel-ME having a higher viscosity had a lower diffusion coefficient and consequently a lower release rate.

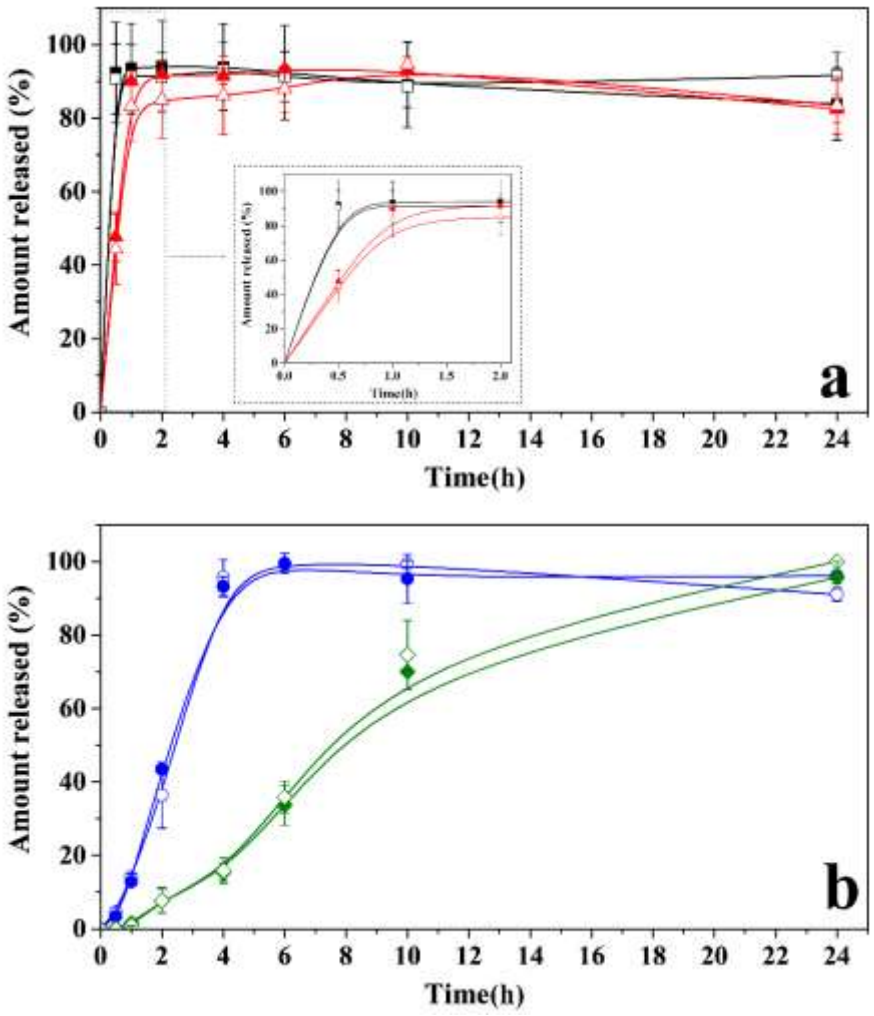


Fig. 7 Vitamins release percentage as a function of time for (a) o/w-ME (■,□),gel-ME (▲,△), (b) ME-low CMC (●○) and ME-high CMC (◆,◇), according to Table 1. Full symbols: vitamin D; empty symbols: vitamin E. The inset in (a) corresponded to a zoom in the first 2h of release

However, the present study showed that changes in viscosity were not the only variable that affected the kinetic release. Gel-ME (bicontinuous structure) and ME-low CMC were formulated with similar zero-shear viscosity at the test temperature (η ~

1x10^{3}Pa.s for $\dot{\gamma}$<0.01 s^{-1}) and exhibited notable differences in their release profiles (Fig. 7b). This suggests that the formation of a bicontinuous structure, where there are bilayers of surfactant molecules containing alternate layers of oil and water, could allow a greater availability of bioactives to diffuse into the release medium. Conversely, in the microemulsion with CMC, the oil droplets with bioactives are immobilized in a network formed by the entanglements of the polymeric chains of the thickener. The addition of CMC to the fluid o/w-ME led to a marked decrease in the release rate of the bioactives. The ME-low CMC presented a release below 5% after 30 minutes and only after 4 hours could release more than 90% of vitamins. The rate reduction effect was enhanced by increasing the hydrocolloid concentration up to 10 g CMC/100 g since after 30 minutes the release percentage was less than 0.3% and only about 75% of vitamins were released after 10 hours of testing. Feng, Xiong, Wang, and Yang [29] have reported that the addition of a gelling agent to the continuous phase of a w/o microemulsion decreased the release rate of the bioactives contained in the dispersed phase.

3.7 Mathematical model of release profiles

According to the shape of the release profiles obtained with all the assayed systems, it was decided to adjust the simplest expression of the Korsmeyer-Peppas model, also called *Power Law* [30]. This simple model has the advantage of a high degree of flexibility: it can be adjusted to different release mechanisms according to the value of the exponent (Equation 2).

$$f_t = \frac{M_t}{M_\infty} = k \cdot t^n \quad (2)$$

It is a semi-empirical model initially developed to describe the release of a drug from a polymeric matrix, such as a hydrogel [31]. In the equation, M_t and M_∞ are the cumulative amounts of the compound released at time t and infinite time, respectively; k contains structural and geometric information about the matrix or device, and n is indicative of the drug release mechanism. To determine the exponent n, it is recommended to use the first portion of the release curves, approximately until $M_t/M_\infty \leq 0.60$ [32]. Thus, only the slow release systems were modelled, since the fluid o/w-ME and the gel-ME reached that percentage of release in a very fast manner (less than 1 h in both cases).

462 Model fitting was made first for each system (ME-low CMC and ME-high CMC) and
463 for each vitamin (D and E) separately. However, it was observed that there were no
464 significant differences between the estimates of the parameters by drug, and therefore it
465 was decided to group the data into two unique models (ME-low CMC and ME-high
466 CMC). The fact that a single model fits both vitamins means that the release profiles do
467 not depend on the encapsulated compound, but only on the matrix. At the same time,
468 grouping the data allows the estimation of the parameters with greater precision.
469 The results of the modelling analysis are shown in Table 2, along with some goodness
470 of fit measures.
471
472 **Table 2.** Modelling analysis of the vitamins' release profiles

ME-low CMC **(N=20)***			
Parameter	Estimate	Std. Error	p-value
k	15.0604	1.0172	<0.0001
n	1.3280	0.0521	<0.0001
R: 0.9913; Adj-R: 0.9908; Error MS: 11.93; Regression p-value <0.0001			
ME-high CMC **(N=28)***			
Parameter	Estimate	Std. Error	p-value
k	2.1419	0.3037	<0.0001
n	1.5300	0.0649	<0.0001
R: 0.9859; Adj-R: 0.9853; Error MS: 9.38; Regression p-value <0.0001			

** For the fitting, time points until a minimum of 60% of release were used (i.e., until 4 h for ME-low CMC, and 10 h for ME-high CMC)*

473
474
475 As it can be seen in Table 2, both models exhibit a value of n>1, corresponding to the
476 so-called Super Case II transport, and extreme form of transport representing purely
477 relaxational behavior [33].
478 This matrix-driven nature of the release is in accordance to the fact that both vitamins
479 fitted into the same model.
480 It is worth mentioning that the Korsmeyer-Peppas model that considers a lag time (t_{lag}),
481 fitted slightly better in all cases, with estimated t_{lag} between 10 and 15 min (data not
482 shown). However, we decided to use the model without t_{lag} (Eq. 2) because the observed

lag time corresponded to the delay imposed by the rigid gelatin capsule used during the release test, so it was not justified to incorporate one more parameter to the model.

Resuming the analysis, the addition of CMC to the o/w-ME generated a polymeric matrix where the oil droplets with bioactives were homogeneously dispersed. When the system contacted the dissolution medium, an interface buffer/polymer matrix was generated, with the corresponding concentration gradient. This could induce a buffer flow into the microemulsion, causing a matrix swelling and, therefore, an increase in the dimensions of the system. Consequently, this gain in the aqueous medium could increase the mobility of the polymer chains, allowing the droplets of the microemulsion to come into contact with the surrounding environment and diffuse out of the expanded matrix. In these types of polymeric matrices, with strong interaction with the release medium, its expansion will depend on the physicochemical properties, such as viscosity [34]. As the concentration of CMC increases, a more closed structure was generated. Thus, the steric hindrance of the polymer chains within the crosslinked networks increased and consequently, the tortuosity of the diffusion path to the release medium. Based on the model results, the release rate of the bioactives from microemulsions depended not only on the dissolution rate of the bioactive but mainly on the entanglements between hydrocolloids molecules that affect the relaxation of the microstructure. Chain entanglement is probably best regarded as a special type of intermolecular interaction, affecting mainly the large-scale motions of the chains, and through them, the long time end of the viscoelastic relaxation time spectrum [35]. The mechanical properties of microemulsions obtained with rheological analysis could be useful to explain the differences observed in the release rate. Using the Maxwell elements previously determined (G_i, λ_i), the plateau modulus and the characteristic relaxation time were calculated as it was expressed by several authors [36, 37]:

$$G_0^N = \sum_{i=1}^{N} G_i \quad (3)$$

$$\lambda_{char} = \frac{\sum_{i=1}^{N} G_i \lambda_i^2}{\sum_{i=1}^{N} G_i \lambda_i} \quad (4)$$

The plateau modulus showed an increase in the elastic properties of the matrix in the order: fluid o/w-ME<ME-low CMC<ME-high CMC<gel-ME with values of 1.89 Pa, 1.58×10^3 Pa, 1.49×10^4 Pa, 2.70×10^4 Pa, respectively. In the quiescent state, gel-ME

exhibited higher elastic behavior than those containing large amount of thickener in the continuous phase. Conversely, λ_{char} showed a different behavior, increasing in the order: fluid o/w-ME<gel-ME<ME-low CMC<ME-high CMC, with values of $1.75x10^{-2}$ s, $2.55x10^{-1}$ s, $4.74x10^{2}$ s, $1.11x10^{3}$ s, respectively. This behavior is in agreement with the release profile obtained. The rise in the longest relaxation time of the matrices with the increase in CMC concentration could be related to the delay in the large-scale chain motions associated with the loosening of the interconnected structure and the consequent droplet release.

Several authors have investigated the processes of hydrogel swelling and polymer relaxation concerning controlled-release drug delivery systems [38, 39]. They relate it to both the penetrant transport mechanism and the concurrent drug release mechanism assuming the relaxation time for the hydrogels as a characteristic time for diffusion. Particularly, with physical gels like those containing CMC, not only the relaxation behavior of the hydrated macromolecules influences on bioactive release rate and water penetration but also the structure of the gel layer [40]. Hence, in the quiescent state, the higher zero-shear viscosity concurred with the lower kinetic release and with the longer relaxation times.

On the other hand, the significantly different microstructure of gel-ME showed a rapid drug release that could be related to the almost instantaneous dilution of the lipophilic phase in the aqueous medium without the additional resistance of the polymeric matrix swelling.

4. Conclusion

The present study showed that different strategies to obtain gel microemulsion could be useful in controlling the release rate of bioactive compounds from the lipophilic phase, which is in agreement with the hypothesis stated in this work. Increasing the dispersed phase content to a fluid o/w-ME generated a gel-ME (with bicontinuous structure), with a similar zero-shear viscosity (η_0) to those obtained using CMC in the continuous phase. However, a markedly different viscoelastic behavior was observed. The thickener addition generated systems with weak gels characteristics (G'>G'') and a slight variation of both moduli with temperature. This greater structure stability was reflected in the decrease in the release rate of vitamins. In other words, CMC not only controls the mechanical properties of the microemulsion but also, the release rate of the bioactive compound, by controlling the relaxation time of the polymeric entanglements.

Conversely, microemulsions with a bicontinuous structure resulted in a highly viscoelastic matrix, which is useful to incorporate in different food systems without significantly altering the bioavailability of bioactive compounds of a fluid microemulsion. Therefore, by changing the viscoelasticity of the microemulsion, it is possible to control the kinetic release of lipophilic components with different chemical structure and behavior incorporated in gelled microemulsions.

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