Active transport: How rheological modifiers and preservatives impact release

Polowyj Rachael, Stevic C Milica and Tamburic Slobodanka

Cosmetic Science Research Group, London College of Fashion, University of the Arts London, UK

Summary

This study explores two formulation parameters that could influence the diffusion of caffeine through the hydrogel system: the type of gelling agent and the presence of a preservative system. The aim was to assess whether and to which extent the changes in rheological properties exerted by the above two parameters affect the *in vitro* release of caffeine from the hydrogels.

A range of 12 gelling agents, belonging to five chemical categories, have been used, as follows: cellulose derivatives (sodium carboxymethyl cellulose, hydroxyethyl cellulose), clays (hectorite, magnesium aluminium silicate), natural polymers (xanthan gum, carrageenan, gellan gum), polyacrylic acid polymers (carbomer, acrylates C_{10-30}/alkyl acrylate crosspolymer, sodium polyacrylate) and silica-based thickeners (hydrated silica and silica). A simple hydrogel formulation, with and without preservative (a mixture of methyl and ethyl paraben in phenoxyethanol, at 1% w/w) was prepared in each case.

Continuous flow and dynamic (oscillation) tests were used in conjunction to produce complete rheological profiles of the test samples. Release studies were performed on the Franz cell vertical diffusion system (Copley Scientific, UK), using the hydrophilic polysulphone membrane during the period of four hours.

The results have shown a variety of release patterns, which were both gelling agent- and preservative-dependent. All hydrogels have released 100% of caffeine within four hours, with the exception of xanthan gum (a maximum of 80% release). The addition of preservative has, in most cases, strengthened the hydrogel internal structure, evidenced by increased viscosity, complex modulus (rigidity) and yield stress. It has generally produced either identical or higher release rates than the sample without preservative.

In conclusion, this study has shown that both the rheological parameters (defined by the type of gelling agent) and the presence of preservative in the gel formulations affect the rate of release of a hydrophilic active caffeine.

Introduction

It is known that diffusion and partition are the two most important phenomena in the complex process of skin penetration (e.g. Wiechers, *et al*., 2004). A diffusing permeant must undergo a series of consecutive steps to penetrate the skin. Firstly, the molecule must diffuse through the formulation to the skin surface and partition into the skin, before diffusing through the SC via

one of the three delivery routes (intercellular, intracellular or via skin appendages). It must then partition into the viable epidermis and diffuse through this structure before partitioning into the dermis, if applicable. These processes are dependent on the properties of both active ingredient and the topical formulation used for its delivery. Generally, a topical active should have the following characteristics in order to penetrate skin efficiently: octanol-water partition coefficient of about 100 (Log $P_{o/w}$=2), good solubility in both lipophilic and hydrophilic media and relatively small M_W (Lane *et al.*, 2012), usually below 500 Da (Bos and Meinardi, 2000).

To start this process, however, the active ingredient has to be released, i.e. it has to diffuse through the formulation and reach the stratum corneum in sufficient quantity. For the given active ingredient, the diffusion is known to be dependent on the structural properties of the three-dimensional network of the vehicle.

In this study, we have used caffeine, a methylxanthine derivative with molecular weight of 194.2 Da and Log $P_{o/w}$ of -0.07, as a model hydrophilic cosmetic active ingredient. Caffeine is increasingly used as a hydrophilic model substance for topical *in vitro* testing, due to its ability to penetrate the skin barrier (Luo and Lane, 2015) and the ability to exert cosmetic effects (e.g. anti-cellulite, reduction of periorbital puffiness). There is also evidence that caffeine possesses anti-oxidant properties, which may protect cells against the effects of UV radiation (León-Carmona and Galano, 2011; Koo *et al*., 2007).

Two formulation parameters that could influence the diffusion of a hydrophilic ingredient caffeine through the hydrogel system were explored in this study: the type of gelling agent and the presence of a preservative system. The aim was to assess whether and to which extent the changes in rheological properties exerted by the above two parameters affect the *in vitro* release of caffeine from the hydrogel formulations.

Materials and Methods

A simple hydrogel formulation, presented in Table 1, was used in the study. Given the fact that gelling agents could have very different gelling potential, it was decided that they would be used in different concentrations, sufficient to achieve a similar value of apparent viscosity. The target viscosity and pH, established by measuring a suitable commercial benchmark, were 44,000 mPa.s at 20^0C (Brookfield DV-E, Brookfield Ametek, UK, measured at 6 rpm with T-bar S93 and a helipath) and a pH of 5.93, respectively. A tolerance limit of ±20% from the above values has been applied.

Table 1. Generic hydrogel formulation

	INCI Name	**% w/w**
Phase A	Aqua	Up to 100.0
	Caffeine	2.0
Phase B	Gelling agent	As required to achieve target viscosity
Phase C	Methylparaben 15%, ethylparaben 10% and phenoxyethanol 75%	1.0 (if used)
Phase D	Citric Acid	As required to achieve target pH
	Sodium Hydroxide	As required to achieve target pH

To form the hydrogel vehicle for the topical delivery of caffeine, 12 gelling agents, belonging to 5 chemical categories, were used, as follows: **cellulose derivatives** (sodium carboxymethyl cellulose, hydroxyethyl cellulose), **clays** (hectorite, magnesium aluminium silicate), **natural polymers** (xanthan gum, carrageenan, gellan gum), **polyacrylic acid polymers** (carbomer, acrylates C_{10-30}/alkyl acrylate crosspolymer, sodium polyacrylate) and **silica-based thickeners** (hydrated silica and silica). The preparation of samples has followed a generic process, consisting of: dissolution of caffeine in water at 45^0C with stirring, dispersion and mixing of the gelling agent and addition of the preservative and/or pH adjuster, when required. This method was modified when the gelling agent had specific requirements in terms of the higher temperature or the pH of water used for dispersion.

Table 2. Concentration, pH and Brookfield (at 20^0C and 6 rpm, T-bar S93 with helipath) viscosity values of the series of hydrogels, with and without the addition of the preservative system

INCI Name	Concentration (%w/w)	pH without preserv.	pH with preserv.	Viscosity without preserv. (mPa.s)	Viscosity with preserv. (mPa.s)
Sodium carboxymethylcellulose	3.75	6.60	6.95	45,500	45,700
Hydroxyethylcellulose	2.20	6.31	6.32	48,700	45,800
Xanthan gum	6.00	7.08	6.69	40,000	42,700
Carrageenan	2.75	6.48	5.78	41,200	42,300
Gellan gum	1.75	5.80	5.56	42,500	37,200
Hectorite	3.50	6.71	6.90	36,200	42,200
Magnesium aluminium silicate	2.50	6.69	6.49	41,200	40,000
Carbomer	0.35	6.22	5.52	36,700	40,000
Acrylates/C_{10-30} alkyl acrylate crosspolymer	0.35	6.02	6.23	50,200	44,300
Sodium polyacrylate	1.00	5.94	5.92	50,000	48,000
Hydrated silica	13.50	6.29	6.23	50,200	38,700
Silica	6.00	5.33	5.94	41,300	52,800

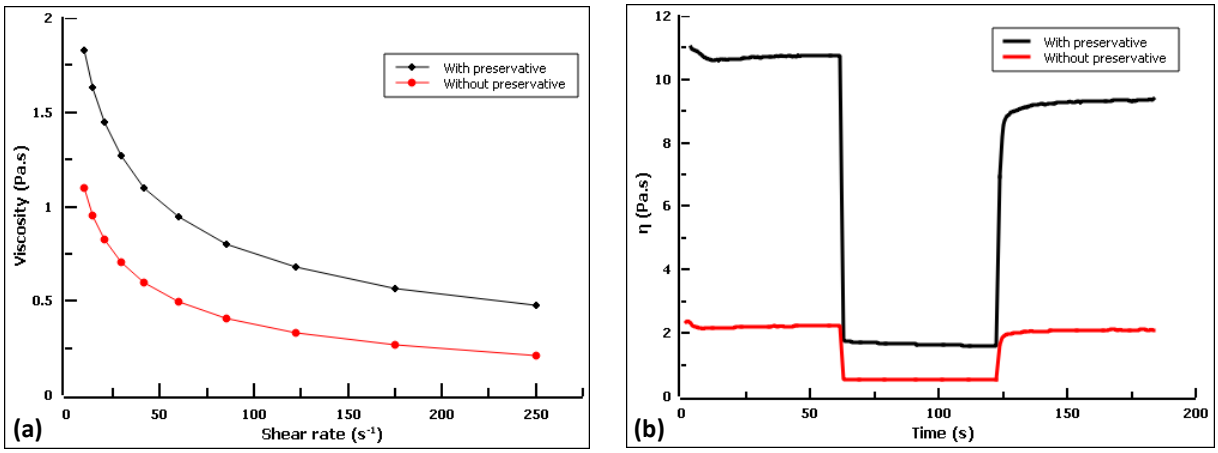
Rheological measurements were carried out on the RheoStress RS75 Rheometer (Haake, Germany), using a 35-mm serrated parallel plate and the gap of 1 mm. Continuous flow and dynamic (oscillation) tests were used in conjunction to produce complete rheological profiles of the test samples. Two types of flow measurements were employed: the shear rate sweep (from $250s^{-1}$ to $10s^{-1}$ during the period of 100s) and 3-step thixotropy method (at the constant shear rate of $10s^{-1}$, followed by $250s^{-1}$ and again $10s^{-1}$, each step taking 60s). Oscillatory stress sweep was conducted to establish the viscoelastic properties of the samples, measured by complex modulus G* and phase angle δ. The test was carried out at the constant frequency of 1Hz and

the oscillatory stress range of 0.5-500 Pa. The method was also used to establish the yield stress of each hydrogel, expressed as the stress values at which the complex modulus declines by 10%.

In vitro release testing of caffeine was performed in a Vertical diffusion cell test system, HDT 1000 (Copley Scientific, UK) consisting of 10 diffusion cells. The testing was carried out at the temperature of 32 ± 1^0C and the stirring rate of 600 rpm, through a hydrophilic Tuffryn membrane (Pall Corporation, Sigma Aldrich, UK). The membrane was composed of polysulfone, with a diameter of 25.0 mm, pore size of 0.45 μm and thickness of 300 μm. A release profile of caffeine for each hydrogel was determined by taking 20 μL-samples from each of 10 diffusion cells and analysing their caffeine content spectrophotometrically. The testing was conducted over a four-hour period and the sampling was done after 30, 60, 120, 180 and 240 min, followed by prompt replacement of the receptor medium (phosphate buffered saline, pH 7.4) in order to maintain the constant volume in the cell. The concentration of caffeine was quantified immediately after sampling, using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA) at 275 nm and a standard curve for caffeine. Statistical analysis of the release data were performed using repeated measures one-way ANOVA, with p=0.05 as a significance threshold.

Results and Discussion

An example of a complete data set obtained for the gelling agent (in this case sodium carboxymethylcellulose) was given in Fig. 1. It presents the graphs obtained from the three rheological tests (shear rate sweep, 3-step thixotropy and oscillatory stress sweep), alongside the *in vitro* caffeine release profile, for the samples with and without the chosen preservative system.



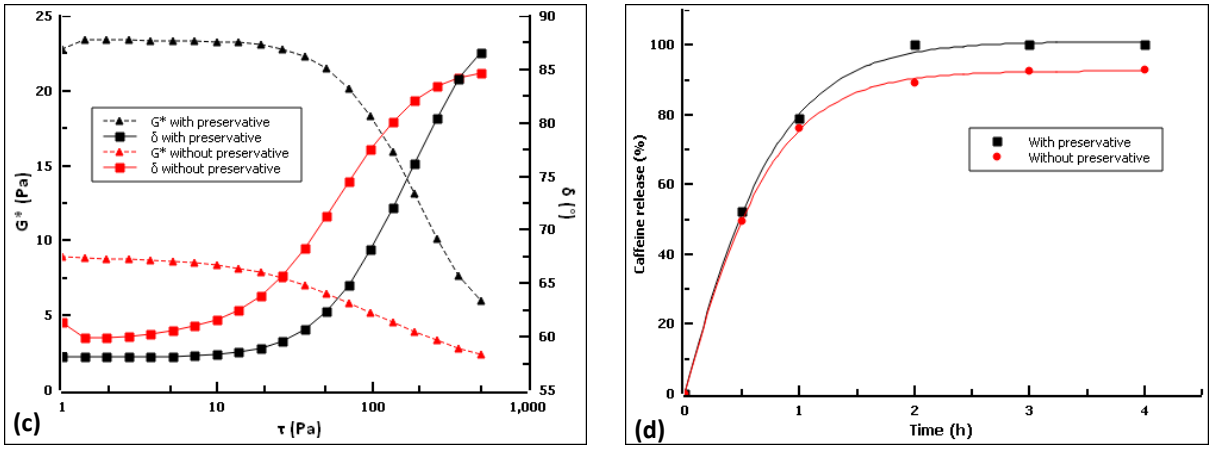


Fig. 1. Results of the rheological characterisation of sodium carboxymethylcellulose hydrogel, with and without preservative, using shear rate sweep (a), 3-step thixotropy test (b), oscillatory stress sweep (c) and the caffeine release profiles (d)

In common with all tested hydrogels, the sodium carboxymethylcellulose sample has shown a shear-thinning rheological behaviour, with distinct yield stress (i.e. the value of shear stress at which the material starts flowing), the behaviour known as plastic flow (Fig.1a, Table 3). The sample with preservative has shown higher viscosity within the whole shear rate range, the finding that was mirrored in the 3-step thixotropy test (Fig.1b). This thixotropy test uses two shear rates (in this case 10 s^{-1} and 250 s^{-1}) to assess not only the effect of shear, but also of time, on the loss and the subsequent recovery of the sample structure, measured by the changes in viscosity (Tamburic et al., 2017). In addition to the graphs, the method produces a quantitative measure in the form of percentage of viscosity recovery (Table 3), calculated from the end parts of the viscosity curves obtained in the first and third step. It is evident from Table 3 that the % recovery has generally increased in the presence of preservative, with small exceptions in the cases of sodium carboxymethylcellulose and carrageenan. The structure of clay samples could not withstand the high shear of the second step, hence no data were obtained for those hydrogels.

Table 3. Rheological parameters obtained from the 3-step thixotropy test (percentage of structural recovery, measured by viscosity) and from the oscillatory stress sweep (the value of yield stress) of the series of hydrogels, with and without the addition of preservative system. In some cases, it was not possible to derive data under the conditions of the test (-).

INCI Name	% recovery without preserv.	% recovery with preserv.	Yield stress without preserv. (Pa)	Yield stress with preserv. (Pa)
Sodium carboxymethylcellulose	93.86	87.13	19.62	69.26
Hydroxyethylcellulose	86.57	100	92.01	111.00
Xanthan gum	88.79	91.83	83.66	101.70
Carrageenan	93.34	76.22	-	80.82
Gellan gum	32.57	95.60	13.72	26.33
Hectorite	-	-	259.90	312.50

Magnesium aluminium silicate	-	-	135.10	200.00
Carbomer	95.56	96.55	34.87	28.02
Acrylates/C_{10-30} alkyl acrylate crosspolymer	93.97	95.36	37.58	42.60
Sodium polyacrylate	92.37	98.26	85.11	83.18
Hydrated silica	0.03	83.46	18.57	39.80
Silica	4.45	71.35	45.76	196.00

As a semisolid system, each hydrogel belongs to the group of viscoelastic materials, having both liquid-like (viscous) and solid-like (elastic) characteristics (Miner, 1993). Dynamic (oscillatory) rheology is a standard method used to assess viscoelasticity, whereby an oscillating shear stress is applied to the sample and the resulting strain measured as its response (Brummer, 2006). Dynamic tests are performed at very low shear stresses, normally below the yield point, allowing an insight into the internal structure of a semisolid without destroying it (Craig et al., 1994). The oscillatory stress sweep method, shown in the recent study to be the most reliable for the detection of shear stress (Tamburic et al., 2017) was used to detect this parameter in all samples.

The graph in Fig. 1c shows the behaviour of the two relevant parameters in this test – the complex modulus G* (known as 'rigidity') and the phase angle δ (known as 'the lag phase'). It is clear that the presence of preservative has strengthened the internal structure of the hydrogel, evidenced by an increased complex modulus G* and decreased phase angle δ (the lower the phase angle, the higher the elasticity of the material). Yield stress was detected as the point where rigidity of the sample starts decreasing. Since the yield stress is not a point, but a region, the same approach was used to detect the yield value as previously published (Tamburic et al., 2017), i.e. the value of stress causing the rigidity to fall by 10% (Table 3). It could be concluded from Table 3 that the addition of preservative has considerably increased the yield value for each hydrogel, except in the case of three polyacrylic acid polymers, where it was almost unchanged.

Despite the changes in the internal structure, captured by rheological measurements, the release profile of caffeine from the sodium carboxymethylcellulose sample stayed almost the same after the addition of preservative (Fig.1d), with no significant changes at either 30 min or 4 hours (Table 4).

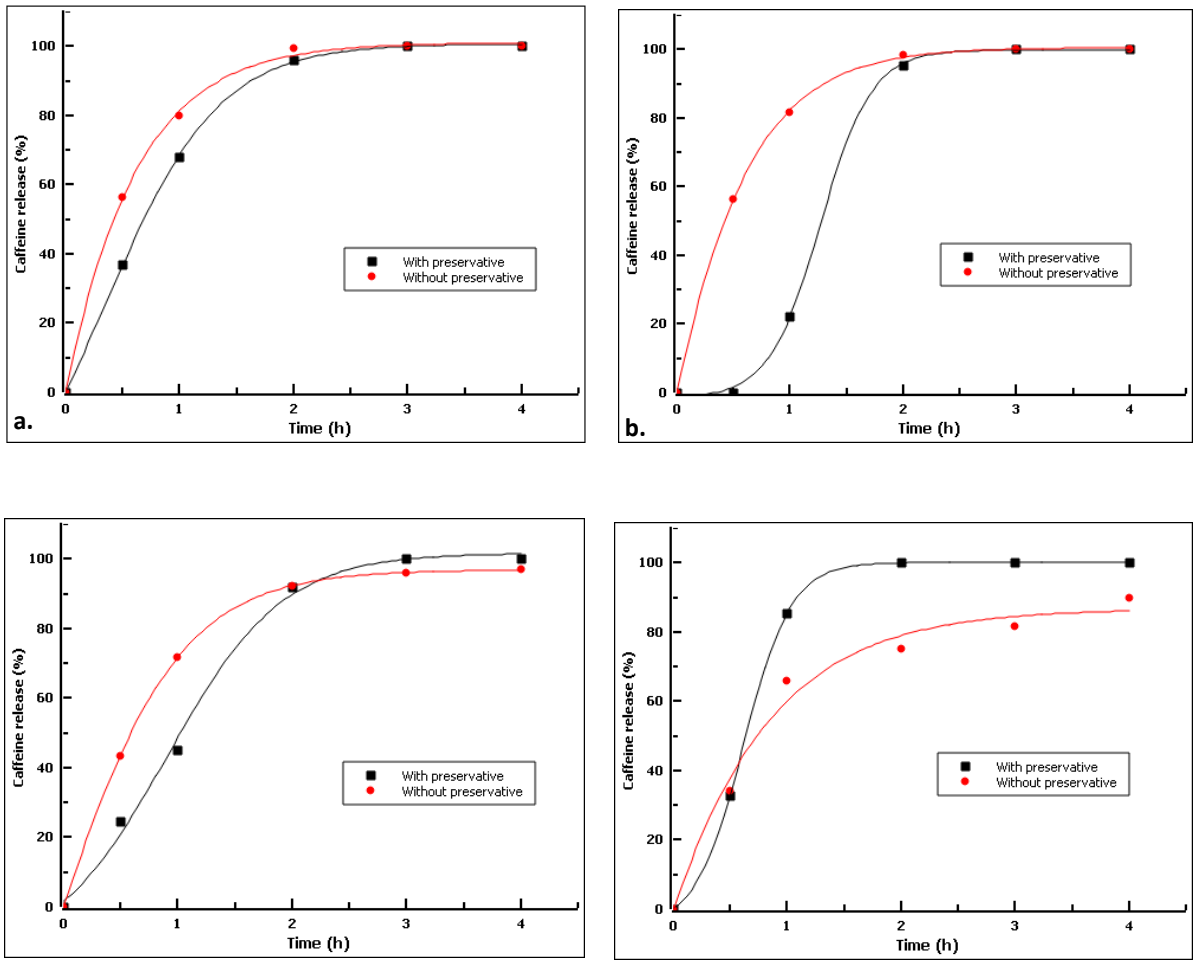


Fig.2. Results of the *in vitro* release of caffeine from carrageenan (a), gellan gum (b), hydrated silica (c) and silica (d) hydrogels

The overall release results, however, have shown a variety of patterns, which were both gelling agent- and preservative-dependent. All hydrogels have released 100% of caffeine within the four hour-test, most of them between the first and second hour, with the exception of xanthan gum (a maximum of 80% release, Table 4). The addition of preservative has generally produced either identical or higher release rate than the sample without preservative (e.g. Fig.2d), but the difference was not always significant. The exceptions were hydroxyethyl cellulose, hydrated silica and all natural gums, whereby a lower release rate was detected during the first 3 hours, with the tendency to equalise later (e.g. Fig. 2a, b and c).

A deviation from the above observations was detected in the case of xanthan gum, which has shown the lowest overall release. The set of results obtained for xanthan gum with and without preservative is shown in Fig 3. Rheological results have revealed very little change in the internal hydrogel structure, with almost identical viscosity, rigidity and elasticity (Fig. 3a and c) and a small increase in the percentage of thixotropic recovery and yield stress (Table 3). However, the *in vitro* caffeine release profile with preservative was consistently, although not significantly, lower (Fig. 3d), which indicates that the rheological effect was not the only parameter affecting the diffusion coefficient of caffeine in xanthan gum.

The fact that this hydrogel has shown the lowest release rate for caffeine is congruent with the earlier observation by Talukdar and Kinget (1997). They measured the diffusivity of three drugs,

including caffeine, from the hydrated polymeric matrices of xanthan gum and hydroxypropyl-methyl cellulose and found that it was lower in the case of xanthan gum. They concluded that the slow diffusion through the xanthan gum hydrogel was the controlling factor in the retarded release of caffeine from the relevant tablets. This finding did not apply to the hydrophobic actives tested in their experiments. In terms of the present study, it would be useful to observe the release profile of caffeine during a longer period (e.g. 8 hours) in order to establish whether and at which time point a complete release of caffeine occurs.

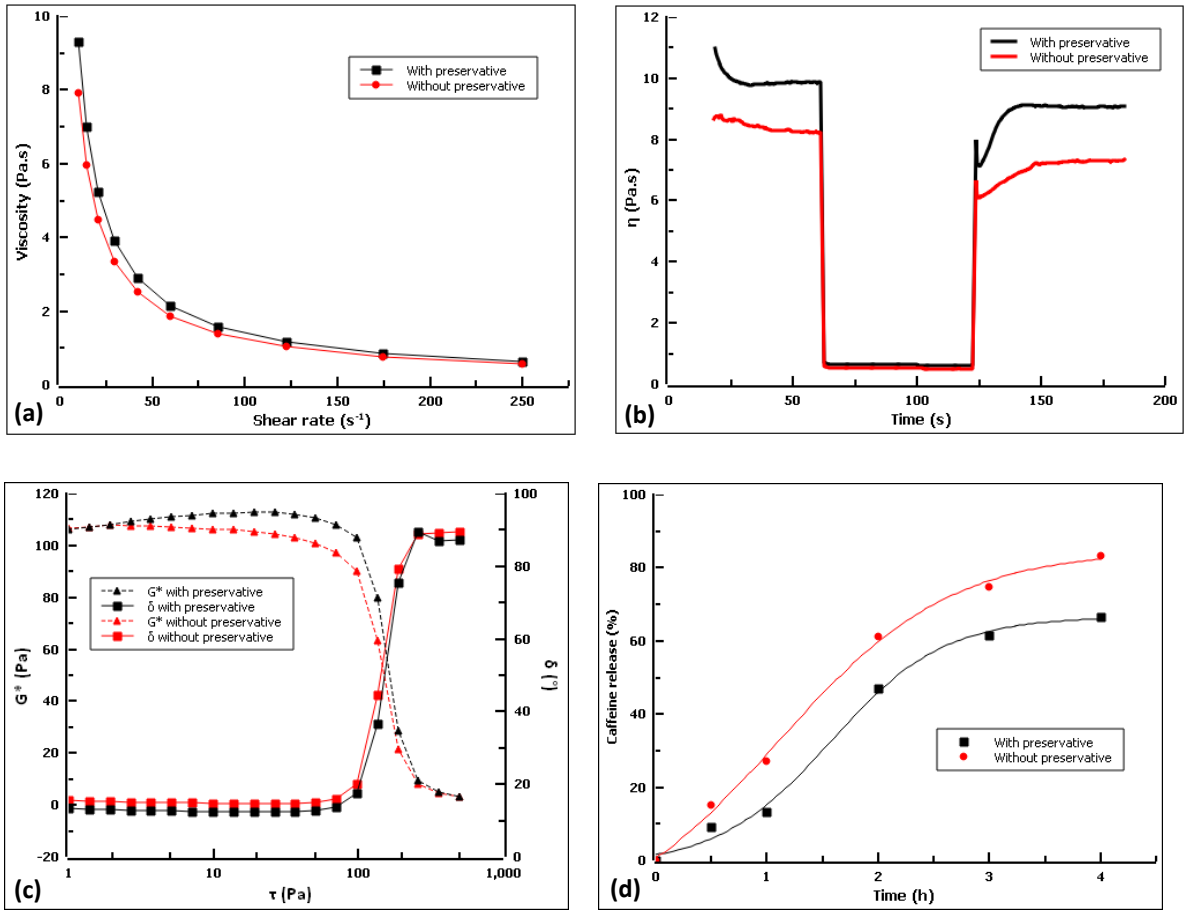


Fig. 3. Results of the rheological characterisation of xanthan gum hydrogel, with and without preservative, using shear rate sweep (a), 3-step thixotropy test (b), oscillatory stress sweep (c) and the caffeine release profiles (d)

The hydrogel based on acrylates/C_{10-30} alkyl acrylate crosspolymer (Fig. 4) presents an example where the addition of preservative has made very small alterations to its internal structure. The viscosity, thixotropy level and rigidity have shown small differences, while the phase angle δ, expressing sample's elastic properties, was unaltered. In line with the theory, the release profile of caffeine from the two variations of this formulation did not differ either (Fig. 4d).

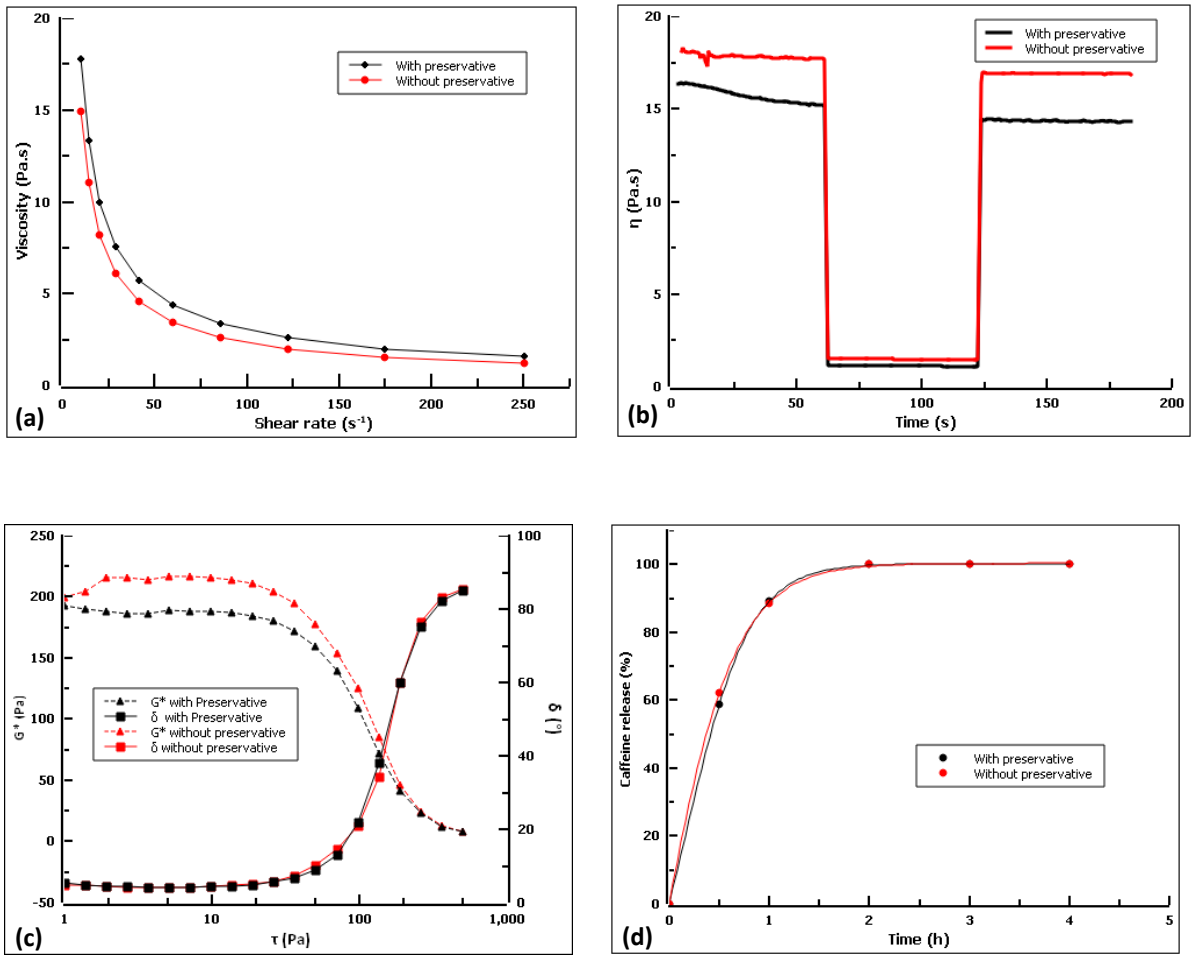


Fig. 4. Results of the rheological characterisation of acrylates/C_{10-30} alkyl acrylate crosspolymer hydrogel, with and without preservative, using shear rate sweep (a), 3-step thixotropy test (b), oscillatory stress sweep (c) and the caffeine release profiles (d)

Table 4 presents the results of the caffeine release after 30 minutes and four hours from all test samples, with their statistical analysis (derived from a repeated measures one-way ANOVA test, followed by Tukey HSD test). After 30 minutes, three samples have shown significant difference in caffeine release, with gellan gum shown in Fig. 2b. These differences have disappeared in all three samples and new differences were showing after four hours (Table 4). It should be noted that carbomer has presented an anomaly in terms of showing a decrease in the % caffeine released between the first and the fourth hour, which could be an instrumental error. This leaves the two clays (hectorite and magnesium aluminium silicate) as the only hydrogels in which the presence of preservative has significantly increased the release of caffeine after four hours. Since clays have very specific, ion-dependent, mechanism of gel formation, it is reasonable to assume that the diffusion of caffeine molecules was made easier due to the rearrangement of platelets caused by the presence of preservative.

Interestingly, the expected pattern of decreased diffusion rate with increased viscosity was not consistently observed. This reveals the influence of additional factors that affect diffusion rate of caffeine through the hydrogel system. It is known that the diffusion through polymeric networks takes place through the liquid-filled pores, and that it mainly depends on the pore size, tortuosity and partition coefficient for the large pores (Karlsson *et al*., 2001). For the small pores, however,

it is also dependent on the steric hindrance and the sliding friction. It is possible that some of these parameters have been changed by the addition of preservative, but not detected through rheological measurements, and vice versa. Due to this complexity, it is not possible to accurately predict the *in vitro* release pattern of caffeine through the tested hydrogels, apart from the fact that most of them release 100% of caffeine during the first two hours.

Table 4. Results of the *in vitro* release of caffeine from the series of hydrogels, with and without the addition of preservative system, and their statistical analysis (* denotes significant difference at 5% level)

INCI Name	% released after 30 min without preserv.	% released after 30 min with preserv.	Significance (p) after 30 min	% released after 4 h without preserv.	% released after 4 h with preserv.	Significance (p) after 4 hours
Sodium carboxymethyl cellulose	49.45	52.27	1.000	93	100	0.970
Hydroxyethyl cellulose	63.70	16.63	**0.000***	100	100	0.994
Xanthan gum	14.99	9.03	1.000	83.19	66.69	0.996
Carrageenan	56.47	36.95	0.946	100	100	1.000
Gellan gum	56.22	0	**0.000***	100	100	1.000
Hectorite	34.08	51.51	0.984	58.09	98.18	**0.039***
Magnesium aluminium silicate	23.93	42.30	1.000	50.23	100	**0.001***
Carbomer	47.90	70.64	0.807	99.90	53.13	**0.004***
Acrylates/C_{10-30} alkyl acrylate crosspolymer	62.13	58.70	1.000	100	100	1.000
Sodium polyacrylate	4.56	82.69	**0.000***	85.29	100	0.571
Hydrated silica	43.50	24.53	0.957	96.99	100	1.000
Silica	34.27	32.89	1.000	89.94	100	0.915

Conclusion

This study has shown that the presence of preservative, in addition to the type of gelling agent, could strongly affect the rheological properties of the hydrogel vehicles used for the topical delivery of caffeine. For the majority of hydrogels evaluated in this study, the change in rheological properties affects the rate of release of caffeine from the formulation, hence this effect could be used to control the initial stage in the complex topical delivery process.

Note: This work was presented at the 30th IFSCC congress in September 2018 in Munich, Germany

Acknowledgements
The authors wish to thank London College of Fashion for supporting this study and the Society of Cosmetic Scientists and Azelis UK Ltd for supporting the attendance of Rachael Polowyj at the congress.

References

Bos, J. D. and Meinardi, M. M. H. M. (2000) 'The 500 Dalton rule for the skin penetration of chemical compounds and drugs', Experimental Dermatology, **9** (3), pp. 165-169.

Brummer, R. (2006) *Rheology essentials for cosmetic and food emulsions*, Hamburg: Springer

Craig, D.Q., Tamburic, S., Buckton, G. and Newton, J.M. (1994) 'An investigation into the structure and properties of Carbomer 934 gels using dielectric spectroscopy and oscillatory rheometry', *J. Control. Rel.*, **30**, pp. 213-223.

Lane, M.E. *et al*. (2012) 'Rational formulation design', *International Journal of Cosmetic Science*, **34** (6), pp. 496-501.

León-Carmona, J.R., Galano, A. (2011) 'Is Caffeine a Good Scavenger of Oxygenated Free Radicals?', *J. Phys. Chem. B* **115**, pp. 4538–4546.

Luo, L., Lane, M.E. (2015) 'Topical and transdermal delivery of caffeine', *Int. J. Pharm.* **490**, 155–164.

Karlsson, J., Stubbs, J.M., Karlsson, L.E. and Sundberg, D.C. (2001) 'Estimating diffusion coefficients for small molecules in polymers and polymer solutions', *Polymer*, **42** (11), pp. 4915-4923.

Koo, S.W., Hirakawa, S., Fujii, S., Kawasumi, M., Nghiem, P. (2007) 'Protection from photodamage by topical application of caffeine after ultraviolet irradiation', *Br J Dermatol.* **156**, pp. 957– 964.

Miner, P.E. (1993) *Emulsions rheology: creams and lotions*, edited by Laba, D. *Rheological properties of cosmetics and toiletries*, New York: Marcel Dekker, pp. 131-369

Talukdar, M.M. and Kinget, R. (1997) 'Comparative study on xanthan gum and hydroxypropylmethyl cellulose as matrices for controlled-release drug delivery. II. Drug diffusion in hydrated matrices', *International Journal of Pharmaceutics*, **151** (1), pp. 99-107.

Tamburic, S., Sisson, H., Cunningham, N. and Stevic, M.C. (2017) 'Rheological and texture analysis methods for quantifying yield value and level of thixotropy', *SOFW Journal*, **143** (6), pp. 24-30.

Wiechers, J.W. *et al*. (2004) 'Formulating for efficacy', *International Journal of Cosmetic Science*, **26** (4), pp. 173-182.