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Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents

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Abstract

Carbopol is one of the most common thickening agent for water phases. It is used after neutralisation and its rheological properties in the aqueous medium are well known. The aim of this work was to investigate the gelation properties of Carbopol 971 e 974 polymeric systems in water-miscible cosolvents such as glycerine and PEG 400. Since in these cosolvents, carboxypolymethylene precipitates after neutralisation with a base, then the attention was pointed out of the gelation properties of the different systems at increasing temperature, in order to obtain Carbopols gels avoiding neutralisation and, at the same time, making possible the dissolution in these gels of insoluble or poorly soluble water drugs. Rheological properties of PEG 400 and glycerine samples were compared with similar systems in water by performing oscillatory analyses and measuring the main rheological parameters, G', G'' and δ. The results obtained showed that Carbopol 971 and 974 in PEG 400 gave rise after heating to gels that show a satisfactory rheological behaviour. The elastic modulus is greater than the viscous one showing a remarkable elastic character of these samples and the performed frequency sweeps show a typical spectrum of a "gel-like" structure. Being Carbopols well-known mucoadhesive polymers, gels adhesive properties were studied using the ex vivo method. Then, the possible cutaneous irritation were also tested using the in vivo method (Draize test). No signs of cutaneous irritation and good mucoadhesive properties were obtained for the PEG 400 and water gels of Carbopol 974 prepared by heating.

After rheological and mucoadhesive properties were set, paracetamol as a model drug was then inserted in the composition of the gels and the release characteristics were defined. Dissolution tests pointed out the greater release control properties of PEG 400-Carbopol 971 samples. These studies showed PEG 400-Carbopol systems as a first-rate alternative to traditional water gels.

Keywords: Gel; Rheology; Viscoelasticity; Mucoadhesion; Drug release

1. Introduction

Carbopols, which are very high molecular weight polymers of acrylic acid, have been used mainly in liquid or semi-solid pharmaceutical formulations, such as

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gels, suspensions and emulsions, as a thickening and viscosity agent, in order to modify the flow characteristics. Recently, they are also used for their mucoadhesive properties and a relevant amount of work has been done on the bioadhesive potential of Carbopol polymers. Formulations include ophthalmic, rectal, buccal, nasal, intestinal, vaginal and topical preparations. In particular, Carbomer grades with no residual benzene content, like Carbopol 971P e 974P chosen in this work, may be used in oral preparations, in suspensions and in tablets (Wade and Weller, 1994; BF Goodrich brochure) and certainly in topical preparations. Carbopol 974P NF and 971P NF are polymerised in ethyl acetate and it is for this reason that they are a toxicologically preferred alternative to Carbopol 934P NF resin. Carbopol 974P, like Carbopol 934P, is a highly cross-linked polymer, whilst Carbopol 971P is a lightly cross-linked polymer (BF Goodrich brochure).

In previous works (Tamburic and Craig, 1995a,b; Blanco-Fuente et al., 1996; Riley et al., 2001), the rheological and mucoadhesive properties of different kinds of Carbopols gels in water have been studied.

It is a fact that usually, Carbopol gels are prepared by the dispersing of polymer in water, in which it swells up to 1000 times the original volume (BF Goodrich handbook) and neutralises the system. It permits the ionisation of the carboxylic groups, and as a consequence of that, a strong gel then forms.

When a water insoluble drug has to be added to this gel it can only be dispersed, and a transparent aqueous gel cannot be obtained. However, in some cases, water insoluble drugs are soluble in hydrophilic water miscible cosolvents, for example in PEG 400 and glycerine.

The possibility then to obtain gels from these hydrophilic solvents, by using a polymer as thickening agent, should seriously be taken into account so in order to make possible the addition of these water insoluble drugs. Besides, a drug dissolved in the liquid phase of the gel under a non ionised form may potentially penetrate more a barrier like skin or a certain mucosa. Therefore, the aim of this work is the investigation of the gelation properties of Carbopol 974 and 971 in these cosolvents, so in order to create systems that are able to load and dissolve a large number of drugs. Previously (Chu et al., 1992), Carbopol 934P polymeric systems were studied in different mixtures of propylene glycol and glycerol, with the addition of a certain amount of water in order to make possible the Carbopol neutralization and to show that the addition of water to nonaqueous Carbopol samples increased strongly their elasticity.

In this paper simple dispersions of polymers in different pure cosolvents were compared with the same systems prepared by heating at 70 °C, so in order to verify changes in the polymer solvation after heating, and an improvement of the rheological properties giving rise to a gel structure. Samples were also compared with similar systems in water in order to verify the possibility of their use in topical or oral dosage forms instead of the water system. There were no cases in which PEG 400, glycerine and water were mixed in each other.

Despite the fact that polyethylene glycols are regarded as non-toxic and non-irritant materials and several dermatological formulations based on PEGs are on the market, some adverse effects have been reported (Fisher, 1978). The possible cutaneous irritation caused by the prepared gels were investigated.

Mucoadhesive power of the samples with better rheological properties were also tested. The bioadhesion can be defined as “any kind of adhesion phenomenon in which one or more of the involved phases, either substrate or adhesive, are part of a living organism” and this notion implies the presence of water (Waite, 1990). If the adhesion is on a mucosal surface then the phenomenon is called mucoadhesion. This occurs by a process of wetting and interpenetration of the mucoadhesive polymer with the mucus gel (Ponchel et al., 1987). The recent development of mucoadhesive dosage forms is due to the fact that a mucoadhesive drug formulation permits to localise a drug in a particular region, thereby increasing bioavailability and, at the same time, increasing the contact time between drug and mucosa.

Afterward, the examination of the ability of these gels to control the release of a drug and dissolution studies was carried out. Therefore, a model drug such as Paracetamol was chosen for these studies.

2. Materials and methods

Paracetamol USP (gift of ANGELINI Pharmaceuticals, Ancona, Italy), Carbopol 974 and Carbopol 971 (BF Goodrich, Cleveland, OH), glycerine Eur. Ph. (ACEF, Fiorenzuola d’Arda, Italy), Polyethylene Gly-

col 400 Eur. Ph. (ACEF, Fiorenzuola d'Arda, Italy), deionised water obtained from an ion-exchange system MF3 (San Salvatore di Cogorno, Genova, Italy).

2.1. Gel preparation

Two methods of gel preparation were used with both Carbopol C971 and C974, in order to verify if this could influence the rheological characteristics of the gel.

According to the first preparation method, a certain amount of Carbopol was dispersed in water, PEG 400 and glycerine, respectively. The dispersion was homogenised using an Ultraturrax T25 for 5 min at 12500 rpm, degassed under vacuum and then left at rest for one day before being analysed.

Since preliminary rheological studies of water or PEG 400 Carbopol gels revealed an increased consistency starting from 60 °C, in the second preparation method, after a complete dispersion with Ultraturrax T25 at the same conditions previously described, the sample was then heated at 70 °C and the system was stirred mechanically until a homogeneous and transparent dispersion was formed (30 min).

Carbopol concentration in all systems ranged between 0.5 and 4% (w/v).

For the release studies, the 0.5% (w/v) of Paracetamol was dissolved in the three different media at room temperature before the addition of the polymer. The final gels contained the 0.5% (w/v) of Paracetamol and the 4% (w/v) of Carbopol. Paracetamol was chosen as the model drug due to its intermediate water solubility (1:70) between an insoluble and a very soluble drug.

2.2. Rheological characterization

Rheological analyses were performed in triplicate using a stress control rheometer (Stress-Tech, Reologica) equipped with a cone-plate geometry (4/40) operating in the oscillation mode. The gap was 150 μm.

The following tests then were carried out:

- *Oscillation stress sweep*: The sample was exposed to increasing stress at a constant frequency; at 20 °C, 1 Hz frequency and different ranges of stresses (0.05–10 Pa, 0.05–100 Pa, 0.05–500 Pa). The G' values were plotted in logarithmic scale. This test allows the determination of the linear viscoelastic regime (LVR) of the sample, and therefore the consequent choice of the stress value to use in the other oscillation tests.
- *Temperature sweep*: The test was performed to outline sample behaviour at constant frequency and stress in a range of temperatures: 1 Hz frequency, 1 Pa stress, temperature range 10–70 °C and heating rate 0.5 °C/min were the experimental parameters. A cooling step followed the heating procedure at the same conditions in the temperature range 70–10 °C.
- *Time sweep*: The test was performed to see the changes in the samples with time at constant temperature (70 °C), stress (1 Pa) and frequency (1 Hz). Time range 0–90 min.
- *Frequency sweep*: The sample was exposed to a stepwise of increasing frequency at a constant stress (1 Pa); 0.05–50 Hz frequency range, in the field of linear viscoelasticity, at different temperatures between 10 and 70 °C. The frequency range and the G' values were plotted in logarithmic scale.
- *Creep-recovery*: The test was carried out at 20 °C at a stress of 1 Pa, which was maintained constant for 100 s. It was then instantly removed and the recovery was followed for 200 s. The Creep compliance J_C (defined as the ratio between the measured strain and the applied stress) is monitored against time. The test was also used to calculate the viscosity of the sample from the linear stress–strain region of the retardation curve.
- *Viscometry test*: Flow measurements were performed on the 0.5% (w/v) Carbopol gels at the 0.05–10 Pa range of stress at 20 °C. Viscosities of non newtonian systems were recovered from the beginning of the flow curves. The obtained data was analysed using the "Power Law":

$$\sigma = K\dot{\gamma}^n$$

where σ: shear stress, K: consistency index, $\dot{\gamma}$: shear rate, n: power law index.

The shearing behaviour of a fluid is represented by a straight line in a log–log shear rate/shear-stress plot, and it is possible to have a good approximation of the shearing properties of fluids. For example, for Newtonian samples the power law model gives $n = 1$ and $K = 1$, for a shear thinning (pseudo-plastic) fluid $n < 1$ and for a shear thickening (dilatant) fluid $n > 1$.

2.3. Cutaneous irritation

The 4% (w/v) glycerine and PEG 400 gels of Carbopol C974 prepared by heating were tested and the 4% (w/v) water C974 gel was used as a negative control.

Guinea pigs, weighing 300–400 g and 7–8 weeks old (Charles River, Calco, Lecco, Italy) were used for the modified Draize test protocol (Draize et al., 1944; Zisu, 1995). The animals were housed separately in stainless-steel cages and identified by tags attached to an ear. Water and food pellets were available ad libitum. The environment was controlled with a 12 h light–dark cycle, a room temperature of 22 °C and a relative humidity of 60%. Six animals were used in each test group, in all a total of three groups. The test gel was applied for 24 h by an occlusive patch of about 1 cm^2 on the flank of the animal that was carefully shaved the day before. Animals were observed for erythema and oedema in 0.5 and 24 h after gel removal according to the standard classification scores (Draize et al., 1944; Zisu, 1995).

2.4. Mucoadhesive tests

Mucoadhesive studies were performed on the 4% (w/v) gels, according to the ex-vivo method using a tensile tester Instron 5543 (Milan, Italy). This method determines the maximum force and work needed to separate two surfaces in intimate contact (Blanco-Fuente et al., 1996; Ponchel et al., 1987). The force and the work necessary to detach the gel from the surface of the mucous layer of the bovine oesophagus were measured recording force versus displacement curves. The oesophagus mucosa has been chosen for its smooth surface and thinness.

Bovine oesophageal mucosa were drawn immediately after the sacrifice of the animals at the slaughterhouse and then frozen at −20 °C (Lejoyeux, 1991). The mucosa were defrozen and cleaned before the tests, using an isotonic solution (NaCl 0.9%) at room temperature, cut into discs of 2 cm in diameter and then it was fixed on the lower support of the tensile tester by a cyanoacrylate glue (Duchêne et al., 1988). Very thin layers of the 4% Carbopol gels prepared by heating were applied in 1.5 cm disks of electrophoresis foils (CA251/0, Schleicher and Schuell, Dassel, Germany) and then glued on the upper metal probe. The tests were performed applying a pre-load of 10N for a time contact of 5 min and raising the upper probe at the constant speed of 5 mm/min. Ten replicates were performed for each type of gel and the average and standard deviations were then calculated.

2.5. Drug release studies

In vitro drug release tests were carried out on the 4% (w/v) Carbopol gels prepared according to the second method and the 0.5% (w/v) paracetamol was dissolved in the medium during the preparation step. The method used was the USP XXIV apparatus 2 with the use of the Enhancer Cell™ 4 cm^2 section (Rege et al., 1998), (VanKel, NJ). The height of the Enhancer Cells™ was set to an inner volume of 4 ml and gel samples were then placed into them. Therefore, only the upper surface of the gel disk was in contact with the dissolution medium. The dissolution media used were distilled water, phosphate buffer pH 6.8 and HCl 0.1N. Since Carbopol viscosity is sensitive to pH changes, then these three different media were chosen in order to verify if the drug release from the gels was influenced by the external conditions surrounding them. Tests were performed in triplicate for 480 min using an Erweka DT6.

The Enhancer Cells™ were settled at the bottom of the vessels containing 900 ml of the dissolution medium at 37 °C, and the distance between the gel surface and the stirring paddle (50 rpm) was found to be 1.5 cm. In 10 min intervals 3 ml of the dissolution medium were withdrawn, and then passed through a 0.45 μm membrane filter and assayed spectrophotometrically at 248 nm. The initial volume of the medium was maintained by adding 3 ml of dissolution medium after each sampling.

3. Results and discussion

3.1. Rheological characteristics

As previously described, the rheological characterisation pointed out an increasing sample elasticity at increasing temperature and on gelation properties of Carbopol 974 and Carbopol 971 in PEG 400, glycerine and water. Storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) were monitored. The elastic or storage modulus represents the elastic storage of energy and this is a measure of how well structured a material is. The loss or viscous modulus represents the

viscous energy dissipation and it will be large when the sample is predominantly viscous (Tamburic and Craig, 1995a). The loss tangent is the measure of the energy, lost to stored energy, in the cyclic deformation (tan $\delta = G''/G'$). A value of tan $\delta < 1$ means a prevalent elastic behaviour (Madsen et al., 1999).

The different parameters were used to define the rheological characteristics of samples so in order to verify if their structure corresponds to the rheological definition of gel. In the presence of a gel structure G' and G'' are frequency independent and the phase angle δ is small, whilst for concentrated solutions there is a frequency dependence of G' and G'' and the phase angle is variable (Carlfors et al., 1998).

A definition from Burchard and Ross-Murphy is that a gel shows a plateau in the real part of the complex modulus extending over an appreciable window of frequencies i.e. they are viscoelastic solids (Burchard and Ross-Murphy, 1990).

3.1.1. Carbopol 974 in PEG 400

The stress sweep of PEG 400 samples prepared without heating, shows a prevalent liquid behaviour (tan $\delta > 1$) with negligible variations of G' and G'' moduli and of the phase degree (δ) when polymer concentration is increased (Table 1).

The time sweep test (Fig. 1), performed at 70 °C, shows that in all cases (but particularly for the 4%, w/v gel) a remarkable variation of the G' modulus and of the phase degree, which decreases from 39.5 to 15. This means that at this temperature the system changes its structure gradually with time, raising its elastic character, through a probable increase of the polymer solvation (a transparent dispersion formed).

These data are confirmed by the temperature sweep test (Fig. 2) performed on the 4% (w/v) sample. On heating, the elastic modulus G' shows a temperature dependent behaviour increasing from 0.5 to 125 Pa. Of course, at the same time, the phase angle decreases from the value of 72 to the value of 13.15 during the heating cycle. These results confirm the change from a prevalent liquid status to a "gel-like" structure at increasing temperature. The "gel-like" structure is not reversible but gets even stronger as the temperature decreases during the cooling step of the test (Fig. 2).

Frequency sweep tests performed on the 4% (w/v) system (Fig. 3), show frequency dependence behaviour with crossover (when G' becomes equal to G'') value shifted towards the low frequencies when the temperature is increased. The reciprocal of the frequency at the crossover point can be regarded as the relaxation time of entangled network in the polymer solution (Kobayashi et al., 2002). For the 4% (w/w) system the crossover point shift from 3 Hz at 10 °C to 0.5 Hz at 60 °C showing longer relaxation time at increasing temperature. This crossover shift towards higher frequencies is a sign of more lasting elastic properties: thus it means that gel elasticity gets greater as the temperature increases, in agreement with the temperature sweep and the time sweep tests.

The creep-recovery test performed on the 4% (w/v) gels prepared without heating (Fig. 4) confirms the feature of a viscous liquid of this sample at room temperature, characterised by a low viscosity (0.190 Pa s). J_{0C}, which represents the total elastic component of a system and J_{0R} which is the total recovered elasticity of a system, both are 0.

Data collected from the viscometry test on the 0.5% w/v sample (Table 4) suggest that C974 gels prepared without heating respects the power law equation with a correlation coefficient of 1 and $n = 0.98$, showing Newtonian characteristics.

As expected, the corresponding gels prepared at 70 °C show an increase of the elastic character due to the temperature increase during the preparation step. In the stress sweep test, these systems (Table 1) show, particularly for the 2 and 4% (w/v) concentration, a greater

Table 1
Stress sweep results of Carbopol 974–Carbopol 971/PEG 400

	% (w/v)	G' (Pa)	G'' (Pa)
C 974	0.5	0.43 ± 0.020	0.89±0.007
	1	0.50 ± 0.007	0.77±0.050
	2	0.48 ± 0.007	0.94 ± 0.110
	4	0.47 ± 0.020	1.00 ± 0.080
	0.5 70 °C	0.40 ± 0.002	0.90 ± 0.060
	1 70 °C	0.31 ± 0.003	2.60 ± 0.007
	2 70 °C	44.0 ± 1.410	38.0 ± 0.640
	4 70 °C	960 ± 6.300	340 ± 10.50
C 971	0.5	0.43 ± 0.042	0.80 ± 0.060
	1	0.42 ± 0.030	0.94 ± 0.020
	2	0.44 ± 0.009	0.71 ± 0.080
	4	5.80 ± 0.470	18.1 ± 0.560
	0.5 70 °C	0.43 ± 0.007	1.05 ± 0.001
	1 70 °C	0.57 ± 0.006	5.80 ± 0.100
	2 70 °C	115 ± 5.520	74.2 ± 6.700
	4 70 °C	340 ± 4.240	155 ± 7.600

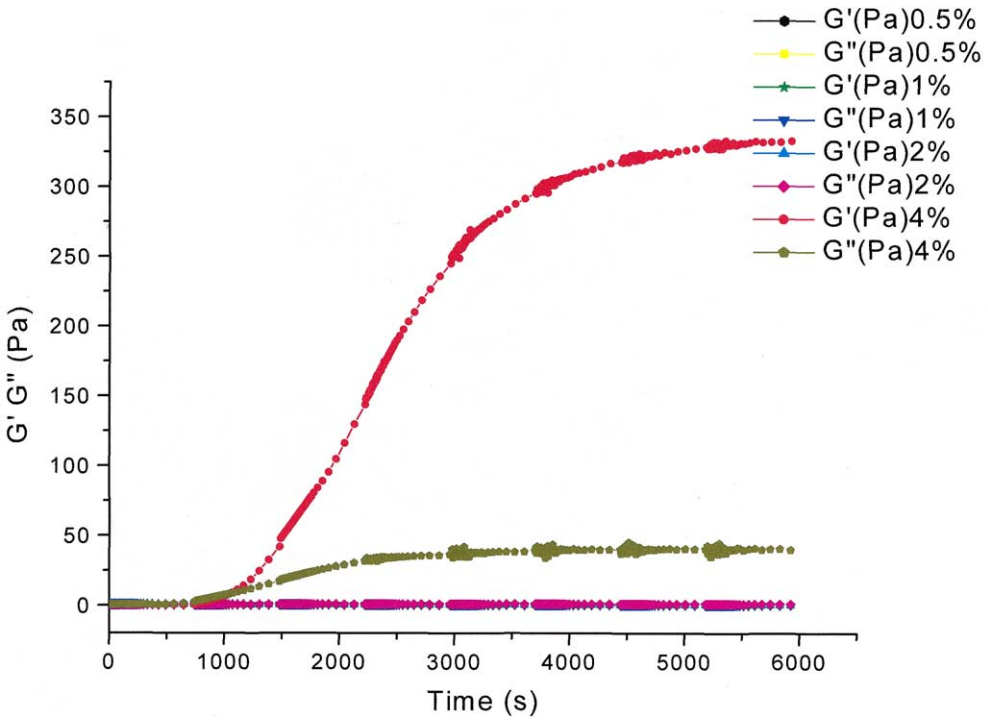

Fig. 1. Time sweep of Carbopol 974 in PEG 400.

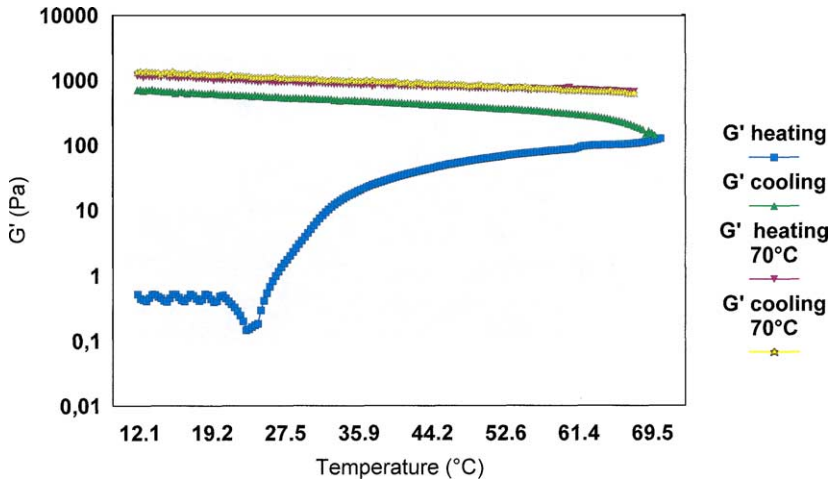

Fig. 2. Temperature sweep of Carbopol 974 in PEG 400.

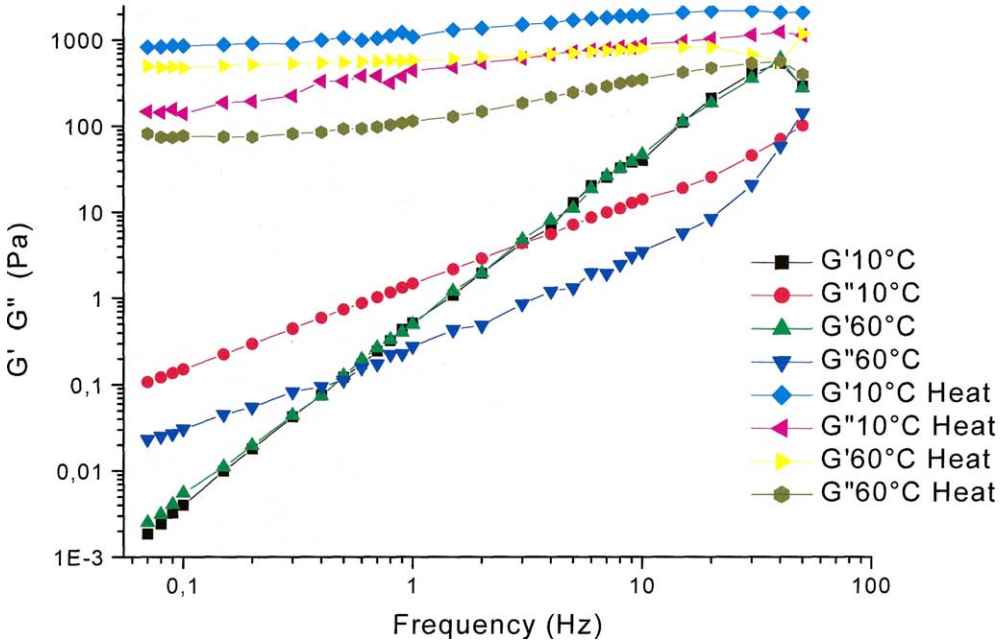

Fig. 3. Frequency sweep of Carbopol 974 gels in PEG 400 prepared at room temperature (I method) and at 70 °C (II method).

G' value than the sample prepared at room temperature, and a moduli crossover value that moves towards a higher stress. These results reflect the presence of a stronger and more resistant structure, thus confirming a remarkable change in the sample rheological properties. For example the 4% (w/v) gel has a G' of 960 Pa, a δ value of 20 and a crossover at 474 Pa.

The temperature sweep test (Fig. 2) shows a slight decrease in the moduli value during the heating cycle and a corresponding slight increase during the cooling

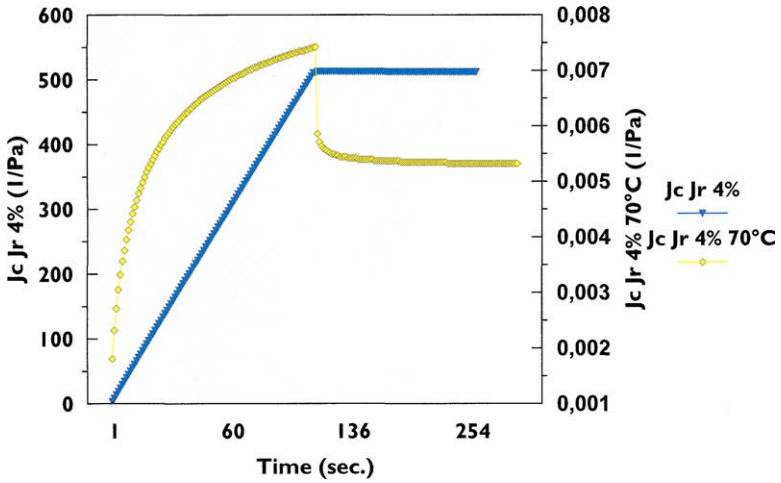

Fig. 4. Creep-recovery of Carbopol 974 in PEG 400.

cycle. Probably, at a further heating treatment (after the preparation step), the gel behaves like many other systems, with a consistency reduction as the temperature increases and a corresponding recovery as the temperature decreases. As expected the G' value at the end of the cooling cycle is higher than that of the corresponding gel prepared without heating at the end of the same step confirming that the greater elasticity is still maintained.

Fig. 3 represents the frequency behaviour of the 4% sample prepared at 70 °C compared to system obtained without heating. The heated sample exhibited a characteristic "gel-like" mechanical spectra with a G' modulus greater than G'' in the entire frequency range examined and both moduli almost frequency-independent. All the oscillatory results indicate that the heating procedure is able to transform PEG 400-C974 samples from low viscosity semi-dilute solution to a "gel-like" structure probably giving rise to interactions polymer-solvent. When a sufficient solvation is present then polymers chains are in a more extended form causing an increase in systems elastic behaviour (Chu et al., 1992).

The creep-recovery test, carried out on the 4% concentration sample (Fig. 4), gives a viscosity value of 422 kPa s, six order larger than that of the corresponding system prepared without heating, a J_{0C} of 2.3 10^{-3} 1/Pa and a J_{0R} of 1.9 $\times$ 10^{-3}. This time the curve is typical of a viscoelastic material, showing a considerable creep and recovered elastic component.

Regarding flow behaviour the 0.5% (w/v) system shows very similar characteristics to the sample obtained at room temperature (Table 4).

3.1.2. Carbopol 971 in PEG 400

Figures relative to these systems were spontaneously omitted and data simply described since in most cases their rheological properties were similar to those of C974 described above. In fact C974 and C971 have got a similar behaviour when dispersed in PEG 400. They show prevalent liquid properties when prepared at room temperature, and they are not proposable as semi-solid controlled drug delivery systems or even as an alternative to Carbopol water gels. Anyway, a temperature increase makes possible the complete solvation of the polymeric molecules with formation of "gel-like" structures.

The time sweep and temperature sweep, performed on the C971 samples prepared without heating, are similar to those obtained using C974. As already stated for the C974 gels, these results confirm the change from a prevalent liquid state to a "gel-like" structure at increasing temperature and time. Also the creep-recovery tests show, as already observed for the C974 samples, an increase in samples consistency and elasticity with a change in the viscosity value from 9.16 Pa s for the 4% sample prepared at room temperature to 6431 Pa s of the corresponding sample obtained by the heating procedure.

However, stress sweep and frequency sweep tests pointed out the lower elastic character of C971 systems compared with C974 samples. This result is in agreement with the cross-linking structure of the two Carbopols. In fact C974 possess a higher cross-links density.

Indeed, the stress sweep tests (Table 1) of samples obtained with both preparations methods show a greater dependence of G' and G'' on polymer concentration. Therefore, as for the C974 gels, it is possible to observe an increase in the elastic character after heating particularly for 2 and 4% (w/v) concentrations.

The frequency sweep test for the 4% (w/v) concentration, prepared at room temperature, outlines a certain frequency dependence of both moduli with $G'' > G'$: it's a typical mechanical spectrum of semi-dilute polymer solutions. But, at 60 °C, the storage modulus shows a slighter variation with frequency and the system has the typical behaviour of a polymer dispersion whose chains are becoming entangled.

The mechanical spectrum in the available frequency range of the 4% (w/v) gel prepared by heating confirms an increase in sample elasticity and a greater predominance of its elastic character. It shows a G' approximately 10 times bigger than G'' independently on the temperature of the test, but a certain dependence on frequency persists. For these reasons a real gel status is not present and the system can be considered as a "weak-gel" (Ikeda and Nishinari, 2001). Therefore, the C971 system shows an increasing elastic character at increasing temperature like the C974 sample but at the same time, a less remarkable solid-like behaviour.

The viscometry test shows for both preparation methods that there is not a modification of flow characteristics (Table 4) and it confirms similarity with C974 samples. We are in presence of Newtonian systems.

Table 2
Stress sweep results of Carbopol 974–Carbopol 971/H_2O

	% (w/v)	G' (Pa)	G'' (Pa)
C 974	0.5	0.31 ± 0.030	0.76v0.070
	1	0.40 ± 0.050	0.34 ± 0.030
	2	35.0 ± 2.830	7.40 ± 0.130
	4	360 ± 10.43	25.0 ± 4.070
	0.5 70 °C	0.4 ± 0.006	0.12 ± 0.050
	1 70 °C	0.31 ± 0.011	1.40 ± 0.040
	2 70 °C	100 ± 3.530	10.0 ± 0.700
	4 70 °C	480 ± 6.360	35.0 ± 3.650
C 971	0.5	0.27 ± 0.020	0.90 ± 0.040
	1	0.50 ± 0.060	2.40 ± 0.050
	2	4.00 ± 0.080	5.70 ± 0.070
	4	13.0 ± 1.300	8.50 ± 1.110
	0.5 70 °C	0.20 ± 0.006	1.10 ± 0.014
	1 70 °C	1.00 ± 0.101	2.50 ± 0.170
	2 70 °C	1.50 ± 0.020	4.40 ± 0.200
	4 70 °C	3.50 ± 0.130	7.00 ± 0.290

3.1.3. Carbopol 974 in H_2O

As demonstrated by the test described below, Carbopol 974 water based gels, prepared at room temperature, show after heating an increase in the elastic component. This behaviour is similar to that of the PEG 400 samples.

In the stress sweep test (Table 2), until the 2% (w/v) polymer concentration, it was very difficult to determine a linear viscoelastic regime for the prevailing liquid character of the samples prepared at room temperature. They present tan δ values bigger than 1 and depending upon the stress applied. The situation is different for the 4% (w/v) gel whose G' value is 360 Pa and δ value ranges quite low between 4 and 5. So, an increase in polymer concentration brings better rheological characteristics, until reaching a kind of gel-status.

While the time sweep test carried out at 70 °C (Fig. 5) shows for the 4% (w/v) gel, prepared without heating, a certain decrease of the storage modulus, the temperature sweep test (Fig. 6) shows an increase of G' modulus (from 318 to 815 Pa) and a decrease of δ (from 3.53 to 2.33) during the heating cycle, until reaching a temperature of 61 °C. A further temperature increase, above 61 °C, leads to a decrease of the storage modulus. This is in agreement with the time sweep test results and it confirms the slight decrease in elasticity observed at 70 °C. Then, during the cooling cycle the G' gradually returns to the value recorded at the beginning of the heating test (10 °C). The recovery of the sample structure demonstrates that the increase of the elastic properties at increasing temperature is not due to water loss, but to a temperature-depending behaviour. The gel status, seen in the stress sweep test, is confirmed by the frequency sweep test (Fig. 7). In the 4% (w/v) C974 gel, G' does not depend on the frequency, $G' > G''$ and the δ values are reduced from 8 to 3 as the frequency decreases, thus confirming the sample elasticity. The creep test for the 4% (w/v) gel prepared according to the first procedure confirms the presence of an elastic response in the gel with low values of J_{OC} and J_{OR} (2.8 $\times 10^{-3}$ 1/Pa).

So, concerning gels prepared without heating, despite the fact that at low Carbopol concentration water and PEG 400 systems possess similar characteristics, it can be concluded that, when the polymer concentration is increased, C974 water gels showed better rheological behaviour than the corresponding samples in PEG 400.

As expected, systems prepared at 70 °C possess a higher elasticity than the corresponding samples prepared at room temperature, and especially for the 2 and 4% (w/v) concentrations. The 4% gel shows, in the stress sweep (Table 2), a G' of 480 Pa and a low δ value. The increase in elasticity is confirmed by the G'–G'' crossover shift from 7.4 Pa for the sample prepared by the first method to 85 Pa for the one obtained by the heating procedure. As already described for the gel prepared without heating, the temperature sweep test performed on the same system prepared at 70 °C (Fig. 6) showed an increase of G' modulus and a decrease of δ during the heating cycle, until it reached a temperature of 60 °C. A further increase in temperature lead to a decrease in the storage modulus. Then, G' gradually returns to the initial value during the cooling cycle. It must be noted that G' values are higher for the gel prepared at 70 °C. The typical rheological behaviour of a gel structure is confirmed by the frequency sweep test (Fig. 7) performed on the 4% (w/v) gel prepared at 70 °C. The test points out the absence of frequency dependence, $G' > G''$ and very small phase angle (7) in the timescale of experiments.

The viscometry test (Table 4) reveals a soft shear thinning behaviour independently on the preparation method used.

3.1.4. Carbopol 971 in H_2O

In this case figures relative to these systems were spontaneously omitted and the data simply described.

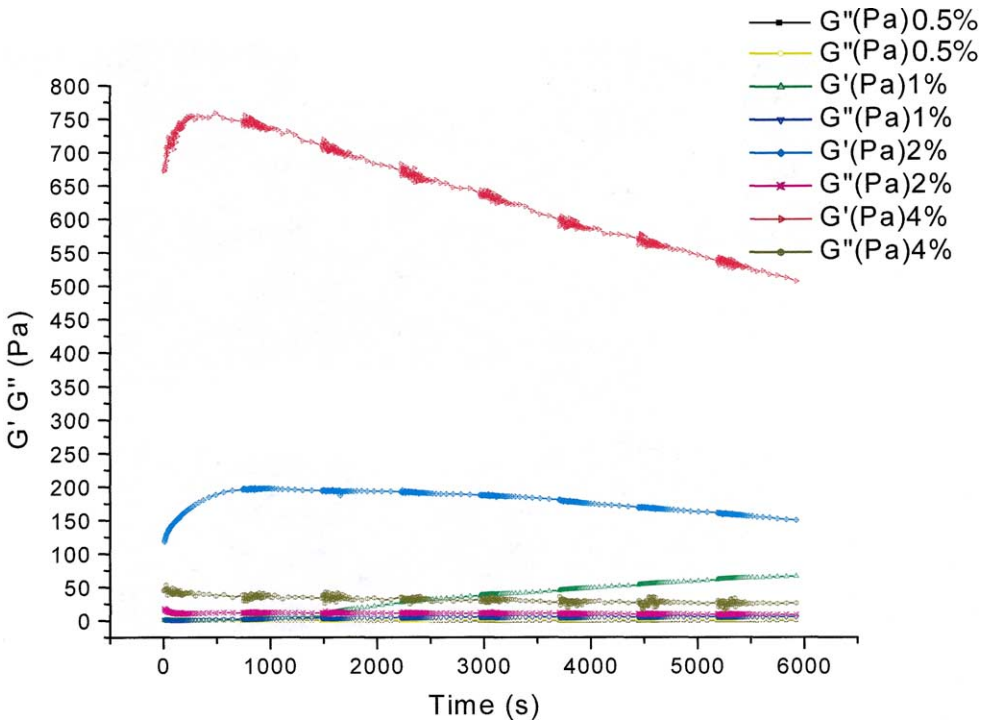

Fig. 5. Time sweep of Carbopol 974 in water.

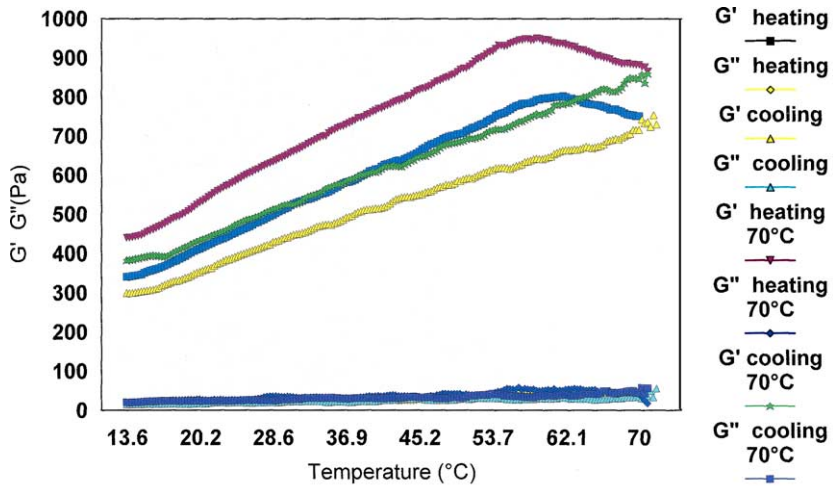

Fig. 6. Temperature sweep of Carbopol 974 in water.

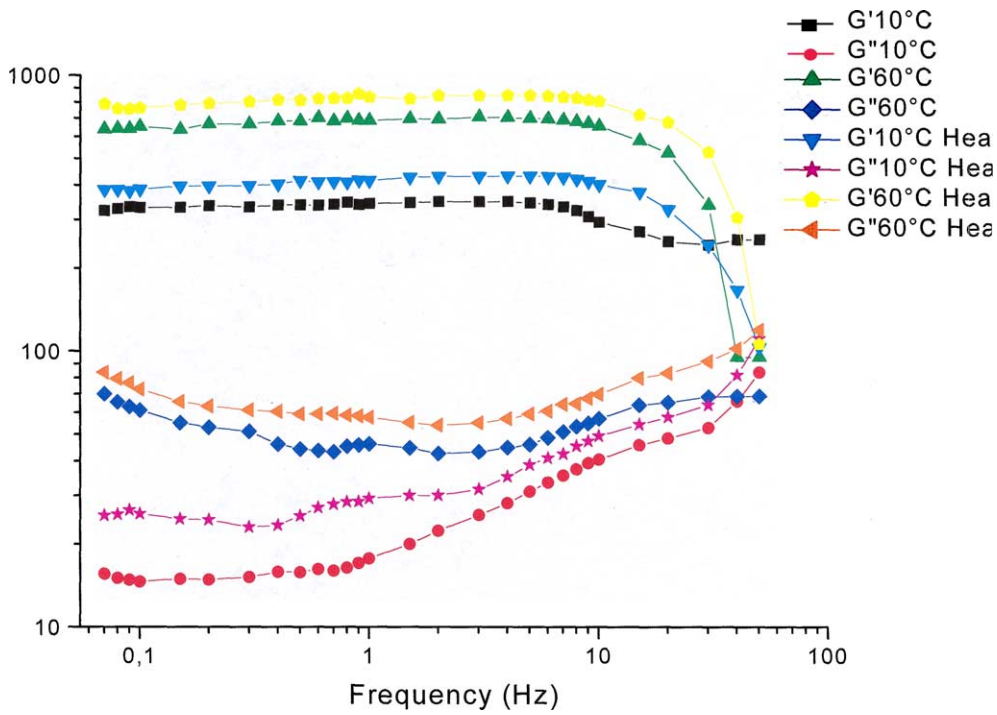

Fig. 7. Frequency sweep of Carbopol 974 gels in water prepared at room temperature (I method) and at 70 °C (II method).

In Carbopol 971-water based samples, it is possible to observe from the stress sweep test (Table 2), in particular for G', no sensible variation in the moduli value after the heating process. In fact, in the stress sweep of the sample prepared by both preparation procedures, the difficulty to outline a LVR persists, and there is not a positive variation of the G' modulus or the δ value.

Samples show tan $\delta > 1$ and δ quite variable depending on the stress applied.

This trend is confirmed by the time sweep test where it is possible to observe that both G' and G'' decreased with time at 70 °C, and from the temperature sweep where non sensible moduli variations is recorded.

The same gel prepared at 70 °C showed during the heating cycle of the temperature sweep test an increase in the moduli values, similar to that observed for Carbopol 974.

The frequency sweep shows no change in sample properties by changing the temperature of the preparation step. This behaviour confirms the stress sweep results. In fact, C971 gels show small frequency dependence and a G' value bigger than G'', but δ values change from 70 to 20. This variability of phase angle is typical of a non-real gel structure. This could rise from the less cross-linked polymer structure.

Also in the case of the creep test, similar results are obtained for both gel preparation methods. The analysis confirms the viscoelastic properties characterised by both a small recovery and a viscosity value of about 72 Pa s.From the viscosity test (Table 4) it is possible to observe a shear thinning behaviour.

3.1.5. Carbopol 974 and 971 in glycerine

Glycerine gels do not show a significant variation of the rheological parameters, independently of the gel preparation temperature, and the type of Carbopol used. In the stress sweep test (Table 3), for both polymers and both preparation methods, the storage modulus increases as the polymer concentration increases. The loss modulus is bigger than the storage modulus, except for the 4% (w/v) C974 gel prepared with and without heating, and for the 2% (w/v) sample obtained at 70 °C. Concerning the Carbopol 974 gels, in the time sweep, the G' value slightly increases while the C971

Table 3
Stress sweep results of Carbopol 974–Carbopol 971/glicerine

	% (w/v)	G' (Pa)	G'' (Pa)
	0.5	2.50 ± 0.160	23.0 ± 1.530
	1	8.70 ± 0.300	33.3 ± 2.050
	2	98.0 ± 1.130	158 ± 4.650
C 974	4	900 ± 6.720	620 ± 7.760
	0.5 70 °C	6.00 ± 0.230	27.0 ± 0.350
	1 70 °C	18.0 ± 0.850	56.0 ± 2.220
	2 70 °C	411 ± 5.310	290 ± 3.730
	4 70 °C	982 ± 8.480	600 ± 4.240
	0.5	8.00 ± 0.110	27.5 ± 0.850
	1	19.0 ± 0.640	48.5 ± 0.350
	2	81.0 ± 1.520	110 ± 3.020
C 971	4	230 ± 5.070	255 ± 2.120
	0.5 70 °C	8.50 ± 0.092	25.7 ± 0.700
	1 70 °C	27.5 ± 1.010	56.0 ± 0.560
	2 70 °C	85.0 ± 1.340	112 ± 1.410
	4 70 °C	202 ± 1.410	208 ± 1.710

gels show a loss of the elastic component if they are heated. Anyway, both systems do not show gelation properties at increasing temperatures.

Either Carbopol 974 or Carbopol 971 glycerine gels show a frequency dependence and $G'' > G'$. As already described, these characteristics are usually present in a concentrated dispersion rather than in an entangled system.

The creep-recovery test for the 4% gels give the typical curves of a viscoelastic material and the recovered viscosities were 6286 Pa s for C974 and 127 Pa s for the C971. No significant changes are detected in the samples prepared by heating. The viscometry tests show a shear thinning behaviour in both C974 and C971 systems.

So, both the polymers in glycerine show similar characteristics even after heating and do not possess a real gel structure.

3.2. Skin irritation

No sign of skin erythema or oedema was detected either after 0.5 or 24 h and 0 scores were recorded for both glycerine and PEG 400 gels and, of course, for the negative control gel (Table 4).

3.3. Mucoadhesion

Mucoadhesion studies point out a certain better mucoadhesiveness of Carbopol C974 in comparison with the C971 (Tables 5 and 6). The difference is particularly remarkable in PEG 400 and water based gels. This is probably due to the better rheological gels characteristics. In fact, these two gels are more mucoadhesive than glycerine gels.

Usually systems with higher elastic component possess a greater mucoadhesion. According to previous works (Tamburic and Craig, 1995a), δ smaller values correspond to higher detachment forces.

3.4. Dissolution tests

Mean values were used to construct the dissolution curves but standard deviation bars were omitted

Table 4
Viscometry results of Carbopol 974–Carbopol 971 0.5% (w/v) in the three different media

	Power law			Viscosity (Pa s)
	Behaviour	Correl. coeff.	n	
Peg400-C974	Newtonian	1	0.98 ± 0.014	0.14 ± 0.004
Peg400-C974 70 °C	Newtonian	1	0.98 ± 0.003	0.13 ± 0.005
Peg400-C971	Newtonian	1	0.98 ± 0.030	0.12 ± 0.014
Peg400-C971 70 °C	Newtonian	1	0.97 ± 0.010	0.16 ± 0.007
H_2O-C974	Shear thinning	1	0.91 ± 0.006	0.007 ± 0.0004
H_2O-C974 70 °C	Shear thinning	1	0.85 ± 0.003	0.013 ± 0.0003
H_2O-C971	Shear thinning	1	0.57 ± 0.064	0.17 ± 0.021
H_2O-C971 70 °C	Shear thinning	1	0.51 ± 0.070	0.77 ± 0.071
Glyc.-C974	Shear thinning	0.99	0.80 ± 0.070	4.76 ± 0.080
Glyc.-C974 70 °C	Shear thinning	0.99	0.72 ± 0.070	6.00 ± 0.540
Glyc.-C971	Shear thinning	1	0.85 ± 0.014	7.70 ± 0.210
Glyc.-C971 70 °C	Shear thinning	1	0.89 ± 0.099	7.30 ± 0.110

Table 5
Carbopol 974 mucoadhesion results

Carbopol 974	Load (N)	Work (mJ)
Peg 400 4% 70 °C	5.72 ± 1.46	2.12 ± 1.78
H_2O 4% 70 °C	6.21 ± 2.75	2.22 ± 1.44
Glycerine 4% 70 °C	2.77 ± 2.20	0.48 ± 0.46

Table 6
Carbopol 971 mucoadhesion results

Carbopol 971	Load (N)	Work (mJ)
Peg 400 4% 70 °C	1.36 ± 0.79	0.35 ± 0.28
H_2O 4% 70 °C	4.60 ± 1.71	0.62 ± 0.34
Glycerine 4% 70 °C	2.87 ± 2.40	0.47 ± 0.60

to avoid overlapping. The curves show that C974 gels in PEG 400 do not possess a good capability of controlling the drug release in the three tested media (Fig. 8). The full 100% of the drug is always released within 240 min.

Glycerine based C974 gels (Fig. 9) show a very poor release control, which is also influenced by the dissolution medium. Drug release slows down according to the following order: HCl 0.1N > distilled water > phosphate buffer pH 6.8. In this last medium 100% of the release is reached after 200 min.

The same ineffective drug release control was also found in water-based gels (Fig. 10), that is unless phosphate buffer (pH 6.8) is used as dissolution medium. In this case, 100% of the release is attained after 480 min.

When Carbopol C971 is dispersed in PEG 400, the gel has a good ability to control drug release. After 480 min, about 57% of the drug is released when the dissolution medium is water and 70% in phosphate buffer. The full 100% of drug release is reached after 390 min in HCl 0.1N. (Fig. 8). These results may appear in disagreement with the polymers structure and with rheological characterisation described above. Carbopol 974 is heavily cross-linked, whilst Carbopol 971 is lightly cross-linked, and this difference in the polymer structure is reflected in their rheological behaviour. In fact, C974 gives gels with a greater elastic character, particularly in PEG 400 and water. Usually, the release rate of a drug from a semi-solid matrix is inversely proportional to its solid character but previous studies (Lochhead et al., 1989) have already demonstrated that higher viscosity gels made by Carbopol 974 and Carbopol 934 showed a non-homogeneous viscosity with regions characterised by a very high macroviscosity and regions of water-thin micro viscosities. Due to these differences drugs behave differently in formulations containing different Carbopol grades. For example, Carbopol 974 in water shows the most rigid gel micro particles when fully hydrated and channels are present in the gel structure, while Carbopol 971 presents flexible micro particle at the same condition. The presence of these channels gives rise to a faster drug release rate for the Carbopol 974, while the more homogeneous structure of Carbopol 971 shows the slower release rate. This different gel microstructure does not change at different pH values.

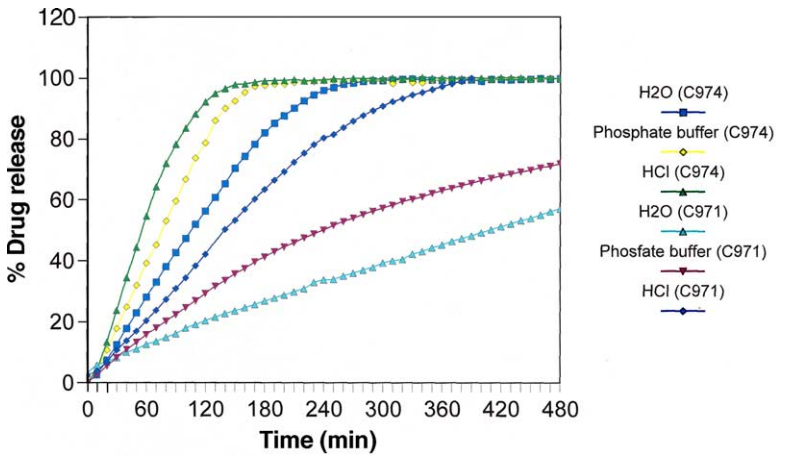

Fig. 8. Drug release from Carbopol 974 and Carbopol 971 in PEG 400 gels.

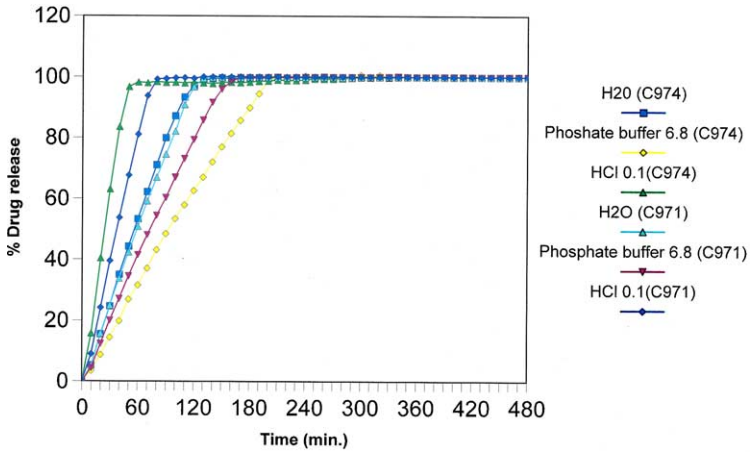

Fig. 9. Drug release from Carbopol 974 and Carbopol 971 in glycerine gels.

The different micro-viscous gel structure between C974 and 971 can explain the better ability of this last Carbopol grade to control drug release.

The glycerine based C971 gel possesses a very similar behaviour to the C974 glycerine gel, with a very poor release control in all of the three media. (Fig. 9).

Finally, the release control of the C971 water gel, also in this case, strongly depends on the dissolution medium (Fig. 10). Drug release is immediate in HCl 0.1N, more gradual in distilled water (100% after 280 min) and strongly controlled in phosphate buffer (only 85% released after 480 min).

So, a difference can be remarked between water and PEG gels. As expected, in water gels the rate of drug release is influenced by the pH value of the dissolution medium, since it determines the percentage of ionised Carbopol acidic groups at the interface medium gel, and may be also in the external layers of the gel matrix. In phosphate buffer these groups are completely dissociated and the gel becomes stiffer. As a consequence the drug release rate is lower. In HCl 0.1N the acidic groups are not dissociated and the gel is less viscous. In water they are partially dissociated and the release rate is in an intermediate position.

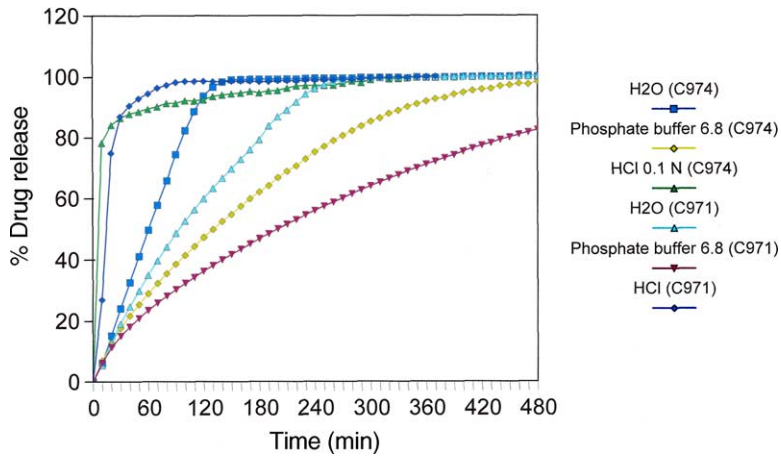

Fig. 10. Drug release from Carbopol 974 and Carbopol 971 in water gels.

In PEG 400 gels the drug release rate is slower in water than in phosphate buffer, and the above-explained difference in the release rate of drug between C971 and C974 gels is more remarkable. The slower release rate in water rather than in buffer can be explained with Carbopol desolvation and precipitation occurring in PEG 400 after salification of the carboxylic groups, with a consequent reduction of the gel consistency.

4. Conclusion

Carbopol C971 and C974 systems in the three different solvents show different rheological, mucoadhesive and release characteristics, which should be taken into consideration when formulating a dosage form. Carbopol–PEG 400 and Carbopol–water gels are more similar to each other than Carbopol–glycerine gel. PEG 400 gels, independently of the Carbopol used, and C974–water gels increase their thickening behaviour after a thermal treatment, and respond better to the rheological definition of gel, in particular when the systems are heated during the preparation step. So, heating may be used as an alternative procedure to neutralisation of Carbopol water dispersion, and as a real procedure for the preparation of PEG 400–Carbopol gels, particularly when C974 is used. In fact, generally, Carbopol C974 gives more elastic gels than C971. This may depend on the presence of a greater number of cross-links in C974 than in C971. The greater elasticity of C974 PEG 400 and water gels can also explain their good adhesive properties, which make them very interesting systems as semi-solid mucoadhesive formulations, prolonging drug residence time at application site.

Besides, the extremely effective drug release control of C971 gel in PEG 400 could for instance make possible the formulation of C971–PEG 400 gels, not only as topical formulations but also in gelatine capsules for oral controlled release systems.

In the final analysis, liquid PEGs can be successfully used as a medium to dissolve water insoluble drugs, since they are easily transformed in systems having great elasticity and "gel-like" behaviour, by using heat during the preparation step and Carbopol resins as thickening agent.

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