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International Journal of Pharmaceutics 307 (2006) 129–140
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Rheological, adhesive and release characterisation of semisolid Carbopol/tetraglycol systems

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Received 9 March 2005; received in revised form 30 August 2005; accepted 12 September 2005
Available online 16 November 2005

Abstract

Gels dosage forms are successfully used as drug delivery systems considering their ability to control drug release and to protect medicaments from an hostile environment. This study deals with the gelation properties of Carbopol 971 e 974 polymeric systems in tetraglycol, a water-miscible cosolvent. In this paper, the attention was noted of the thickening properties of the different Carbopol in tetraglycol solvent at increasing temperatures, in order to obtain gels avoiding neutralisation and, at the same time, to make possible the dissolution in these gels of insoluble or poorly soluble water drugs. Samples were prepared by simply dispersing different Carbopols amount (0.5–4%) were added to tetraglycol and different systems were prepared at room temperature and by heating at 70 °C. All these systems were then characterised rheologically. Frequency sweep, creep-recovery, temperature sweep and time sweep analyses outlined that Carbopol 971 and 974 in tetraglycol gave rise after heating to gels with satisfactory rheological behaviour: the elastic modulus was greater than the viscous one and a remarkable elastic character was found to be present.

Systems obtained by heating procedure were examined also from a mechanical point of view using a texture profile analysis. Besides, being Carbopols well known mucoadhesive polymers, gels adhesive properties were also studied using the ex vivo method. Texture and adhesion characterisation confirmed rheological results pointing out a certain greater elasticity and adhesiveness of Carbopol 974 systems. Then, the possible cutaneous irritation was also tested using the in vivo method (Draize test). No signs of cutaneous irritation were obtained for all the samples that were analysed.

After rheological and mucoadhesive properties were set, paracetamol as a model drug was inserted in the composition of the gels and the release characteristics were defined. Dissolution tests pointed out the greater release control properties of tetraglycol/Carbopol 971 samples. These studies showed tetraglycol/Carbopol systems as a first-rate alternative to traditional water gels when low water-soluble drugs have to be added.

Keywords: Hydrogels; Viscoelasticity; Viscosity; Mechanical properties; Mucoadhesion; Controlled release

1. Introduction

Different strategies have been proposed to achieve efficient drug delivery systems and in the last few years hydrogels and gels in general have been considered as good candidates for oral, rectal, ocular, cutaneous and subcutaneous administration.

The term "gel" is not easy to define and to understand, giving rise often to different opinions. According to the "Encyclopaedia of Polymer Science and Engineering": a gel is a cross-linked polymer network swollen in a liquid medium. Its properties depend strongly on the interaction of these two components" (Tanaka, 1987).

According to its phenomenological characteristics a gel is better defined as a soft, solid or solid-like material consisting of two or more components, one of which is a liquid, present in substantial quantity (Almdal et al., 1993).

Hydrogels in particular have been widely utilised in the medical and pharmaceutical fields for their biocompatibility and their similarity to a natural tissue (Ratner and Hoffman, 1976; Peppas et al., 2000).

Moreover, in some cases, when a water insoluble drug has to be added to a hydrogel, it can only be dispersed, and a transparent aqueous gel cannot be obtained. On the contrary, water insoluble drugs are often soluble in hydrophilic water miscible cosolvents, such as PEG 400 and tetraglycol (glycofurol 75).

Tetraglycol, in particular, can be also used in parenteral products for intravenous or intramuscular injection in concentration up to 50% (v/v). The amount administered parenterally should

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0378-5173/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2005.09.034

not exceed 0.07 ml/kg body weight daily (Wade and Weller, 1994) and it is an irritant if injected undiluted (Spiegel and Noseworthy, 1963).

Besides, different studies have been done on tetraglycol ability as an absorption enhancer. In a nasal formulation containing insulin it is able to enhance the uptake of the drug and to significantly lower blood glucose level in rabbits (Bechgaard et al., 1996). Also butemide was better absorbed after intranasal administration when the vehicle was formed by glycofurol/coconut oil 60:40 (v/v) or pure glycofurol (Nielsen et al., 2000).

In this paper tetraglycol is studied as a medium to obtain gels, with the help of a polymer as a thickening agent, in order to make possible the dissolution of water insoluble drugs. One of the most used thickeners is Carbopol, which is a very high molecular weight polymer of acrylic acid. 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 (Noveon bulletin) and certainly in topical formulations. Carbopol 974P NF and 971P NF are polymerised in ethyl acetate and for this reason 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 (Noveon bulletin).

Recently, Carbopols have also been used for their mucoadhesive properties and a relevant amount of work has been done on their rheological and mucoadhesive properties (Tamburic and Craig, 1995a; Tamburic and Craig, 1995b; Blanco-Fuente et al., 1996; Riley et al., 2001).

Usually Carbopol gels are prepared by dispersing the polymer in water, in which it swells up to 1000 times the original volume (Noveon bulletin), and by neutralising the systems. This permits the ionisation of the carboxylic groups, and as a consequence of that, a strong gel forms.

In this work the gelation properties of Carbopol 974 and 971 in tetraglycol are investigated, so in order to create systems that are able to load and dissolve a large number of water insoluble drugs, for the formulation of semi-solid systems which could be potentially administered in different ways including ophthalmic, rectal, buccal, nasal, intestinal, vaginal and topical routes. So, the issue is not to demonstrate that Carbopol/tetraglycol systems are better than Carbopol/water gels but just to describe an alternative method that could be used with water insoluble drugs.

Previously, 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 non-aqueous Carbopol samples increased strongly their elasticity (Chu et al., 1992). Also Carbopol/PEG 400 pure systems were already investigated showing the possibility of obtaining samples characterised by good rheological properties typical of a gelified structure (Bonacucina et al., 2004).

In this paper simple dispersions of polymers in tetraglycol were compared with the same systems prepared by heating at 70 °C, so in order to verity changes in the polymer solvation after heating, and an improvement of the rheological properties giving rise to a gel structure avoiding neutralisation. Studying the rheology of the systems is important in order to predict their performance giving a physical sample characterization. Mechanical or texture properties can be related with pre-formulation and formulation studies of semisolid dosage form and with sensory parameters in-vivo such as the product removal from the container and good spreadability on the skin.

Besides, a good bioadhesion should be desirable since a mucoadhesive drug formulation permits to localise a drug in a particular region, thereby increasing the contact time between drug and mucosa and consequently bioavailability.

So a rheological, mechanical and mucoadhesive characterization of the obtained samples has been performed to evaluate Carbopol/tetraglycol gels as possible basis for oral, topical and vaginal delivery systems.

Despite the fact that tetraglycol is regarded as a non-toxic and a non-irritant material the possible cutaneous irritation caused by the prepared gels was investigated.

Finally the ability of these systems to control the release of a drug was investigated by performing dissolution studies after having loaded Paracetamol in the gels. This model drug was chosen as a tracer for its intermediate solubility in water (1:70).

2. Materials and methods

Paracetamol USP (gift of ANGELINI Pharmaceuticals, Ancona, Italy), Carbopol 974 and Carbopol 971 (BF Goodrich, Cleveland, OH), Tetraglycol Eur. Ph. (Sigma Chemical Co, St. Louis), 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, as reported in a previous work for other systems (Bonacucina et al., 2004).

According to the first preparation method, a certain amount of Carbopol was dispersed in tetraglycol. The dispersion was homogenised using an Ultraturrax T25 for 5 min at 12,500 rpm, degassed under vacuum and then stored at room temperature for 1 day before being analysed.

Since preliminary rheological studies of tetraglycol Carbopol gels revealed an increased consistency after heating at 70 °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 left under mechanical stirring until a homogeneous and transparent dispersion was formed (30 min). Samples were then stored at room temperature for 1 day before being analysed. 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 medium 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.

2.2. Rheological characterization

Rheological analyses were performed using a stress control rheometer (Stress-Tech, Reologica) equipped with a cone-plate geometry (4/40) with a 150 μm gap operating in the oscillation mode. Samples were equilibrated for 5 min before starting the rheological analyses, except for the time sweep test. In fact, this test is usually carried out to follow sample changes (even sudden changes) at a chosen temperature with time. The equilibration of the sample could hide some changes in the sample and compromise the aim of the test. The following tests were performed in triplicate:

- *Oscillation stress sweep*: The sample was exposed at 20 °C to increasing stress at a constant frequency: 1 Hz frequency and different ranges of stresses (0.05–10, 0.05–100, and 0.05–500 Pa). 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 (1 Hz, 1 Pa) in a range of temperatures. The temperature was increased from 10 to 70 °C at a rate of 0.5 °C/min. 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 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. Slope values of $\log G'$ and $\log G''$ versus log frequency have been calculated in order to investigate the frequency dependence of dynamic moduli (Rosalina and Bhattacharya, 2002). Afterwards a deeper analysis of samples mechanical spectra and behaviour on frequency was carried out by fitting the obtained curves with a one unit Maxwell model (Jiménez-Regalado et al., 2004) and by studying the power law ($G' \sim \omega^n$, $G'' \sim \omega^n$) dependence (Desbrieres, 2004).
- *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 test was used to calculate the viscosity of the sample from the linear stress/strain region of the retardation curve.

2.3. Texture profile analysis

A texture profile of 4% systems prepared by heating at 70 °C was performed using a Tensile Tester Instron 5543 in compression mode. Samples were transferred into 10 ml beaker (20 mm internal diameter) and packed to a fixed height, avoiding the introduction of air bubbles. The analytical probe (10 mm diameter) was compressed twice into each sample to a depth of 15 mm at a rate of 2 mm/s. A delay period of 20 s was chosen between the end of the first and the beginning of the second compression.

The textural properties were calculated from the resultant force–time curve. The calculated parameters were: hardness (force to attain a given deformation), compressibility (force required to deform the samples during the first compression cycle), recovery (the rate at which the deformed sample returns to its initial state after the removal of the deforming force) and adhesiveness (work necessary to overcome the attractive forces between samples and metallic probe). All the tests were performed in five replicates at room temperature (Jones et al., 1996, 1997).

2.4. Cutaneous irritation

The 4% (w/v) tetraglycol/Carbopol C974 samples 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/12 h 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).

2.5. 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 (Lejoyeux, 1991), cleaned before the tests using an isotonic solution (NaCl 0.9%) at room temperature, cut into discs of 2 cm in diameter and then 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 & Schuell, Dassel, Germany) and then glued on the upper metal probe. The tests were performed applying a pre-load of 10 N 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.

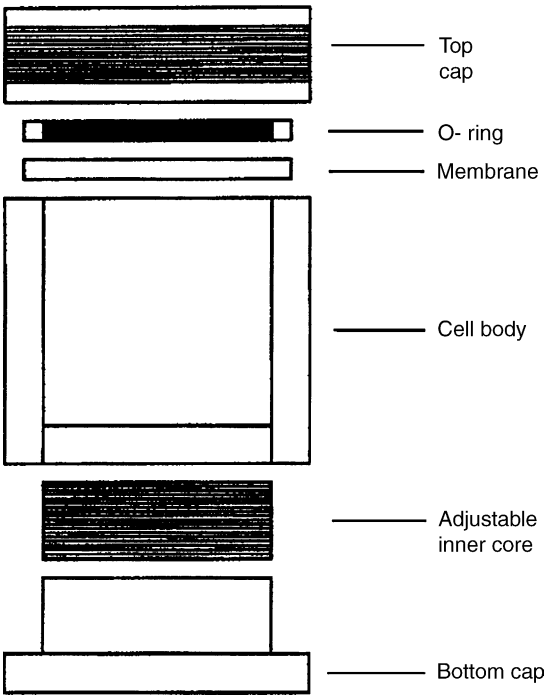

Fig. 1. The Enhancer Cell™.

2.6. Drug release studies

In vitro drug release tests were carried out on the 4% (w/v) Carbopol gel prepared according to the second procedure and loaded with 0.5% (w/v) paracetamol. The USP XXIV apparatus 2 was used with the aid of the Enhancer Cell™ 4 cm^2 section (VanKel, NJ). The enhancer cell is a device made in Teflon (inert and not reactive material), which can be used to study the release profile of semisolid formulations. It consists of a cap, an o-ring, a washer and a drug reservoir. If necessary a membrane may be placed on the top of the reservoir (Fig. 1). A previous work (Rege et al., 1998) demonstrated advantages and reliability on utilising this method in comparison with other diffusion cells as screening procedure in preformulation studies. Only one of the four surfaces is in contact with the dissolution medium. So the contact area remains rather constant for the duration of the test, even when matrix erosion occurs.

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 were distilled water, phosphate buffer pH 6.8 and HCl 0.1N. Since Carbopol viscosity is sensitive to pH changes, then these 3 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 dissolution medium at 37 °C, and the distance between the gel surface and the stirring paddle (50 rpm) was set to 1.5 cm. In 10 min intervals, 3 ml of the dissolution medium was 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.

The mean release profiles were fitted according to the power law equation in order to describe the drug release mechanism from the polymeric systems examined:

$$\frac{M_t}{M_\infty} = Kt^n$$

where M_t and M_∞ are the absolute amount of drug released at time t and infinite time respectively; K is a constant reflecting structural and geometric characteristic of the device, and n is the release exponent characterizing the difrusional mechanism.

When $n = 0.5$ the fraction of drug released is proportional to the square root of time (Higuchi equation) and the drug release is pure diffusion controlled, when $n = 1$ drug release is swelling controlled (zero order release kinetics or case-II transport). Values of n between 0.5 and 1 indicate anomalous transport and a superposition of both phenomena (Rinaki et al., 2003; Siepmann and Peppas, 2001).

3. Results and discussion

3.1. Oscillatory rheological tests

Non-destructive oscillatory measurements, performed in this study, allow obtaining rheological main parameters as the storage or elastic modulus (G'), the loss or viscous modulus (G'') and the loss tangent ($\tan\delta$). These parameters were monitored as function of time, frequency and temperature pointing out the thickening properties of Carbopol 974 and Carbopol 971 in tetraglycol solvent and the increase of sample elasticity occurring when the temperature is raised.

The different parameters were used to define the rheological characteristics of samples 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). Graphical data are reported as the mean of the three curves obtained from the repetition of the same test, but standard deviation bars were omitted to avoid overlapping since S.D. values were very low.

3.1.1. Systems prepared at room temperature

Rheological results outline a certain influence of temperature on the thickening properties of C974/C971-tetraglycol systems as already seen previously for PEG 400/Carbopols samples (Bonacucina et al., 2004). They show prevalent liquid properties when prepared at room temperature but a temperature increase makes possible the complete solvation of the polymeric molecules with the formation of gel-like structures.

Stress sweep tests show a typical liquid behaviour particularly in the concentration range 0.5–2% (w/v) with a consequent difficulty in defining the linear viscoelastic region and the rheological parameters G' G'' and δ depend on polymer concentration.

Besides, definite lower values of elastic modulus are measured for C971 samples with respect to the correspond-

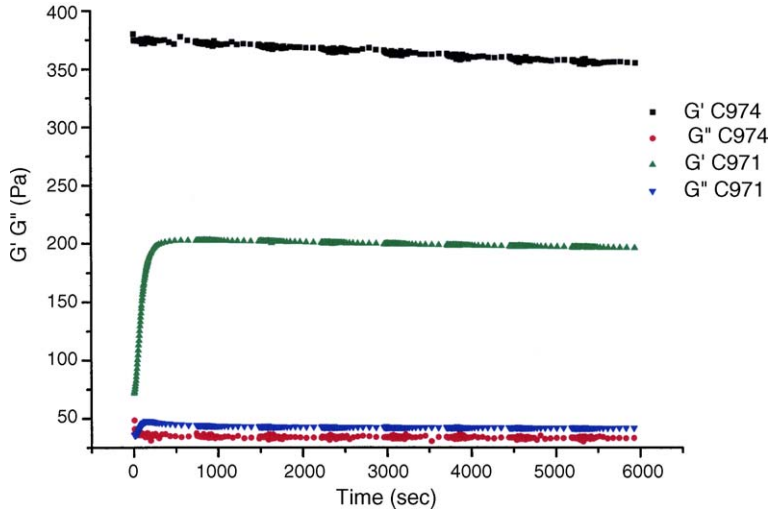

Fig. 2. Time sweep of Carbopol 974 and Carbopol 971 4% (w/v) samples at 70 °C (1 Pa, 1 Hz).

ing systems obtained using C974. Carbopol 974 is heavily crosslinked whilst Carbopol 971 is lightly crosslinked and this difference is obviously reflected in their rheological behaviour.

In the time sweep analysis (Fig. 2) a sudden increase in elasticity is observed when samples are placed on the lower plate of the rheometer at the temperature of 70 °C especially for the 4% (w/v) system. C971 sample needs only 50 s to reach the plateau region and the same process occurs immediately for the C974 system with a three-fold increase in G' modulus values if compared to those measured in the stress sweep test (from 110 to 380 Pa), showing an important influence of the temperature on sample consistency.

Temperature sweep analysis confirms the time sweep results. On heating to 70 °C, the elastic modulus of the C974 4% concentration system (Fig. 3) increases rapidly from 35 °C until reaching a plateau region between 50 and 70 °C. On the contrary, G'' modulus remains virtually constant giving rise to a lowering of the phase angle (from 22.5 to 5.3°). Even the C971 samples show a progressive increase in both elastic and viscous moduli until reaching a plateau at 60 °C (Fig. 4). The increase in G' was greater than the increase in G'' (10 times for G' and four times for G'') giving rise also in this case to a four-fold decrease of the phase angle. In both cases, the cooling step shows a further increase in sample consistency. For example, sample elasticity of the C974 system increases until reaching at 20 °C a G' value six times higher than that observed at the same temperature during the heating step.

So, both polymers form gel-like structures in tetraglycol. Very probably a heat treatment gives rise to a considerable solvation of the polymer chains. In fact, visually the systems changed from a milk-like dispersion to a transparent semisolid. So, heat is very important in the preparation of Carbopol/tetraglycol systems since the elasticity increase is not reversible. Once polymer chains are solvated, a temperature decrease reduces the energy of the system and the mobility of these chains and consequently the viscosity increases. Figs. 3 and 4 only report G' values in order to make the figures more comprehensive.

Figs. 5 and 6 show the frequency dependence of elastic and viscous modulus of 4% (w/v) samples at different temperatures.

For the C974 sample (Fig. 5) in all cases G' is larger than G'' in the entire frequency range examined but a certain frequency dependence is present particularly at 20 °C. In fact, sample are characterised by a low consistency at this temperature.

Besides, the presence of a temperature dependent behaviour is confirmed from the mechanical spectra obtained at 60 °C. As it is possible to observe, elastic modulus presents only a slight

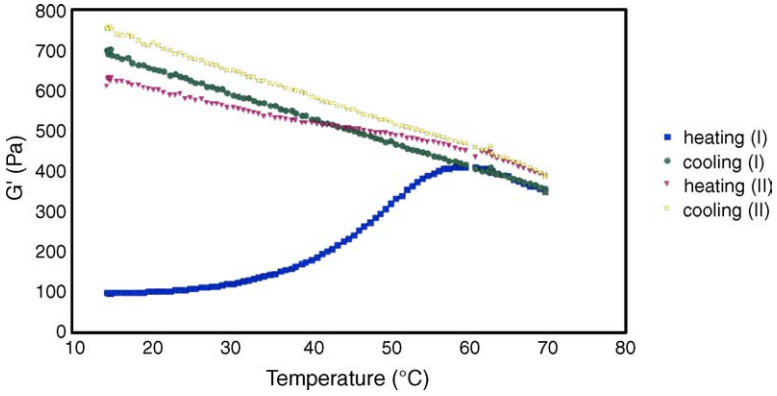

Fig. 3. Temperature sweep of C974 4% (w/v) samples obtained at room temperature (I) and at 70 °C (II).

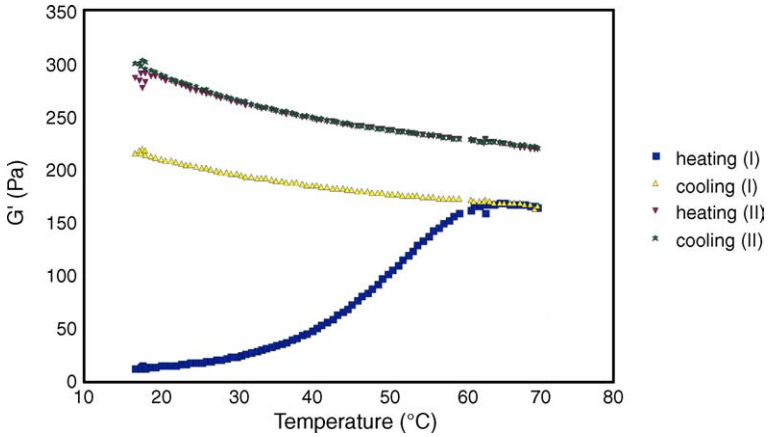

Fig. 4. Temperature sweep of C971 4% (w/v) samples obtained at room temperature (I) and at 70 °C (II).

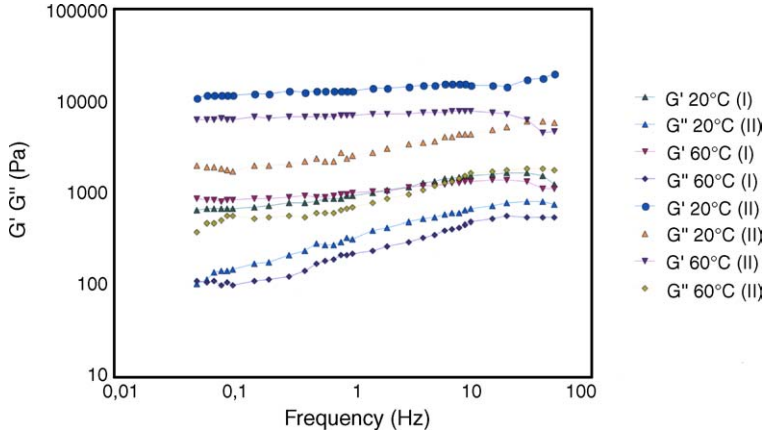

Fig. 5. Frequency sweep of Carbopol 974 4% samples prepared at room temperature (I) and at 70 °C (II) and analysed at 20 and 60 °C.

dependence in the low frequency region confirming the presence of a gel-like structure at this temperature. A tan δ value approximately of 0.1° and a G'' value ten times lower than that of G' are typical features of "weak gel-type "(Ikeda and Nishinari, 2001) In Table 1 values of G', G'' and phase angle δ of 0.5–4% samples at 1 Hz frequencies and at 20° and 60 °C are reported. It is possible to observe an increase in the elastic modulus particularly for the 2 and 4% samples, giving rise to a lowering of the phase angle and to a greater systems consistency.

The mechanical spectra for the C971 4% concentration sample (Fig. 6) are similar to those of a dilute solution for which G'' is bigger than G' and the cross-over between the two moduli is found in the high frequencies region as usually observed for low consistency samples. When the sample is analysed at 20 °C the

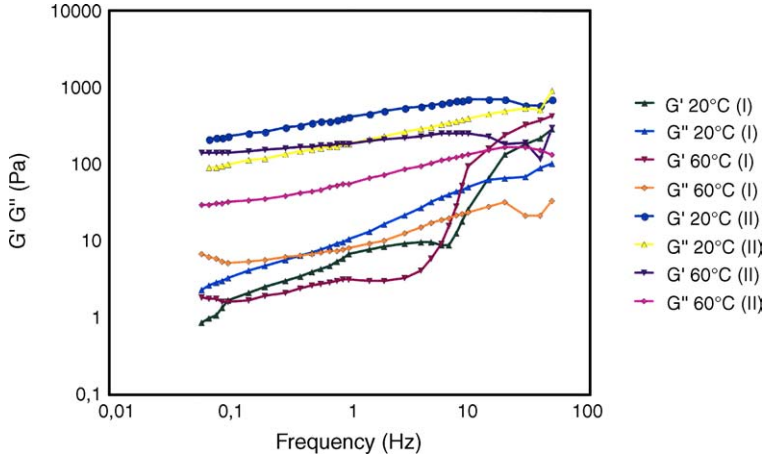

Fig. 6. Frequency sweep of Carbopol 971 4% samples prepared at room temperature (I) and at 70 °C (II) and analysed at 20 and 60 °C.

Table 1
Dynamic rheological parameters determined at 1 Hz (mean and standard deviation were calculated for $n = 3$) for C974 samples prepared at room temperature (I) and at 70 °C (II)

Concentration (%p/v)	T (°C)	G' (Pa)		G'' (Pa)		δ (°)	
		I	II	I	II	I	II
0.5	20	0.48 ± 0.02	0.46 ± 0.04	0.16 ± 0.02	0.33 ± 0.03	18.0 ± 2.17	27.1 ± 3.28
	60	0.51 ± 0.02	0.48 ± 0.01	0.08 ± 0.01	0.12 ± 0.01	9.07 ± 1.86	13.5 ± 1.40
1	20	0.44 ± 0.02	0.19 ± 0.02	0.21 ± 0.06	1.55 ± 0.06	25.3 ± 6.77	82.9 ± 0.84
	60	0.51 ± 0.01	0.48 ± 0.03	0.16 ± 0.04	0.33 ± 0.05	17.9 ± 4.33	34.1 ± 4.75
2	20	0.05 ± 0.03	27.1 ± 5.45	1.98 ± 0.15	18.3 ± 1.94	88.5 ± 0.53	34.2 ± 2.62
	60	0.73 ± 0.25	1.54 ± 0.05	3.17 ± 0.45	4.34 ± 0.07	77.3 ± 2.52	70.4 ± 0.28
4	20	79.0 ± 8.66	5.23 ± 46.3	32.3 ± 2.47	103 ± 8.40	22.3 ± 0.73	11.2 ± 0.60
	60	308 ± 18.7	370 ± 38.9	44.0 ± 1.94	48.0 ± 3.80	8.16 ± 0.14	7.42 ± 0.33

cross over occurs at 10 Hz whilst when the selected temperature is 60 °C, G' is higher than G'' until 8–9 Hz of frequency where the two moduli finally converged towards a common value: this means a longer relaxation time at increasing temperature. 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). The crossover shift towards higher frequencies is a sign of more lasting elastic properties. So, gel elasticity gets greater as the temperature increases, in agreement with the temperature sweep and the time sweep tests but the presence of a certain frequency dependence makes difficult the exact quantification of the effect of temperature on the rheological parameters. In fact, as shown in Table 2, a difference in G' or phase angles is not present between 20 and 60 °C at the frequency value of 1 Hz. This means that the sample cannot be considered a true gel even at 60 °C.

The analysis of $\log G' - \log G''$ versus log frequency plots confirmed that similar considerations can be done for the C974 and C971 samples from the analysis of the slopes of the different curves.

In fact, for the C974 system different slope values of $\log G'$ and $\log G''$ versus log frequency confirm that both viscous and elastic moduli are slightly dependent on frequency (G' slope = 0.17, G'' slope = 0.33) at 20 °C while at 60 °C the slope becomes definitely lower for the elastic modulus (0.09) even if viscous modulus still remains little dependent (slope = 0.30). The results outline a slight frequency dependence ($0.28 < \text{slopes} < 0.59$) even for C971 samples. This type of spectrum is usually associated with a weak gel behaviour (Rosalina and Bhattacharya, 2002). In fact, weak gel possesses intermediate rheological properties between solution and strong gels showing a progressive breakdown of the three dimensional network as deformation increases.

Then, relaxation times (T_R) and plateau modulus (G_0) are determined by fitting with one unit Maxwell model. The fitting of the C974 curve shows a good accordance with Maxwell equation at low frequency. G_0 moduli obtained from the fitting of G' curve at 20 and 60 °C (values are 1594.16 and 1368.70 Pa, respectively) are higher than the corresponding G_0 values calculated from the analysis of G'' spectra (639.43 and 493.60 Pa, respectively) confirming a predominance of the elastic component.

Anyway, the analysis of relaxation times shows, at both temperatures, a slower relaxation for G'' modulus (T_R at 20 = 3.969 s, T_R at 60° = 3.600 s) in comparison with the elastic modulus (T_R at 20° = 3.257 s, T_R at 60° = 1.483 s). This means that the energy stored by the systems relaxes quicker than the dissipated part confirming that samples cannot be considered true gels. Moreover, cross-linking systems possess a dynamic mechanical behaviour at gel point usually characterised by a scaling relation between dynamic moduli and frequency ($G'(\omega) \sim G''(\omega) \sim \omega^n$), i.e. power law dependence is observed.

Table 2
Dynamic rheological parameters determined at 1 Hz (mean and standard deviation were calculated for $n = 3$) for C971 samples prepared at room temperature (I) and at 70 °C (II)

Concentration (%p/v)	T (°C)	G' (Pa)		G'' (Pa)		δ (°)	
		I	II	I	II	I	II
0.5	20	0.53 ± 0.02	0.01 ± 0.03	0.24 ± 0.04	1.67 ± 0.03	24.5 ± 4.38	88.3 ± 1.76
	60	0.52 ± 0.01	0.45 ± 0.04	0.10 ± 0.02	0.34 ± 0.07	10.6 ± 1.67	38.8 ± 1.40
1	20	0.19 ± 0.14	4.80 ± 0.18	1.56 ± 0.32	7.71 ± 0.16	82.3 ± 6.11	58.1 ± 0.46
	60	0.26 ± 0.05	0.72 ± 0.23	1.03 ± 0.15	3.00 ± 0.38	75.4 ± 4.65	76.8 ± 2.40
2	20	0.13 ± 0.09	54.4 ± 1.25	1.97 ± 0.24	30.0 ± 0.64	86.1 ± 3.00	28.9 ± 0.22
	60	0.50 ± 0.41	20.5 ± 0.85	2.56 ± 0.97	13.4 ± 0.35	80.5 ± 5.87	33.2 ± 0.40
4	20	6.27 ± 0.35	229 ± 25.4	10.5 ± 0.51	95.7 ± 9.18	53.3 ± 0.86	22.7 ± 0.40
	60	6.09 ± 1.33	189 ± 11.0	7.70 ± 1.35	56.8 ± 3.02	54.3 ± 3.43	16.7 ± 0.20

On the contrary, in this case all the systems present different n values for G' and G'' in agreement with the assertion that samples behaviour is not typical of a true gels and a gel point is not present. However, n values calculated from the fitting of the elastic modulus are in general lower (n at 20 °C = 0.186, n at 60 °C = 0.099) than the corresponding n related to the viscous modulus (n at 20 °C = 0.321, n at 60 °C = 0.331) showing once again a less dependence on frequency for the G' modulus.

The fitting with the one unit Maxwell model at low frequency and with the power law confirms the presence of a lower elasticity for C971 samples compared to the corresponding C974 systems with a predominance of the viscous component either at 20 and 60 °C. For example, at 20 °C the G_0 plateau modulus related to the fitting of the G' curve was 9.38 Pa while the G_0 obtained from the G'' was 63.41 Pa. Besides, the analysis of relaxation times outline a quicker relaxation process for the G' modulus ($0.97\,s < T_R < 1.21\,s$) compared to the G'' modulus ($7.59\,s < T_R < 23.11\,s$) confirming the progressive breakdown of the structure at increasing force and deformation, characteristic of a weak gel. On the contrary, the power low fitting shows very similar results to those observed for the C974 system since the n exponent values do not respect the power law dependence $G'(\omega) \sim G''(\omega) \sim \omega^n$.

3.1.2. Systems prepared by heating

The oscillation stress sweep shows a concrete change in the rheological behaviour of the systems when samples are prepared at 70 °C. The increased elastic character is demonstrated by the presence of a defined linear region for all concentrations tested. In particular the storage modulus of 2 and 4% systems, are considerably higher than those of the corresponding samples obtained at room temperature. They also show low phase angles, indicating a greater predominance of the elastic behaviour (tan δ lower than 1).

As an example, the G' modulus is five times bigger for the 4% concentration sample containing C974 in comparison with the corresponding systems prepared at room temperature (G' increases from 110 ± 2.12 to 568 ± 36.1 Pa).

The temperature sweep tests carried out on samples prepared at 70 °C show a slight decrease in the moduli value during the heating cycle, and a corresponding slight increase during the cooling cycle (as shown in Figs. 3 and 4 where the behaviour of the 4% (w/v) samples are reported). Probably, at further heating treatment (after the preparation step), the gels behave like many other systems, with a consistency reduction as the temperature increases and a corresponding recovery as the temperature decreases. Sample consistency at the beginning of the heating cycle and at the end of the cooling step is always higher than that of the corresponding samples prepared at room temperature and tested under the same conditions. The frequency spectra (Fig. 5) of the C974 samples show small values of the phase angles at all temperatures tested and G' is bigger than G'' in the entire frequencies range but a slight dependence on frequency still persists. The overall response approaches that of a solid material but the system behaviour is more similar to a "weak-gel" response. In fact, the analysis of the slopes of log G' and log G'' curves versus log frequency shows a similar trend to that observed for the corresponding samples prepared at room temperature even if results obtained for G' outlines a definitely lower dependence on frequency: both G' slopes at 20 and 60 °C are 0.009 while G'' shows a slight dependence. Slopes in this case are between 0.176 and 0.229. This behaviour is still typical of a weak gel response. G_0 plateau moduli obtained from the fitting with one unit Maxwell model are, as expected, in any case higher that those calculated for the corresponding samples prepared at room temperatures. However, G' relaxation times (1.4 s at 20 °C and 1.9 s at 60 °C) are definitely shorter than G'' relaxation times (3.6 s at 20 °C and 8.16 s at 60 °C) showing more lasting viscous properties and a quicker dissipation of the energy stored. So, on the basis of these results, it is not possible to consider the studied system "gels" from a rheological point of view. As observed for the samples prepared at room temperature, the above results are confirmed by the analysis of the power law dependence of dynamic moduli versus frequency. Even if n exponents outline an increased elasticity and a lower dependence on frequency in particular for G' modulus, values are still different for the two moduli. So, on the basis of their mechanical spectra they can be considered as weak gels, despite the heat treatment during the preparation step.

Table 1 shows a certain influence of the temperature on the elastic modulus for all concentrations, as observed in the temperature sweep, but the overall samples response, as demonstrated by phase angles values, is not compromised. In fact, the G' decrease is followed by a corresponding decrease of the viscous modulus.

Similar considerations may be done for the C971 systems. As can be observed in Fig. 6 a slight frequency dependence is still present for the 4% sample but, in comparison with the same system prepared at room temperature, this time, G' is higher than G'' in all the examined frequency range showing a predominance of the elastic over the viscous behaviour (tan δ lower than 1). Also the analysis of the slopes of log G' and log G'' curves versus log frequency shows a definitely lower dependence than that of the corresponding samples prepared at room temperature but the behaviour is still typical of a weak gel ($0.13 < G'$ slopes < 0.29, $0.25 < G''$ slopes < 0.31). In Table 2 the expected elasticity increases in comparison to the sample prepared at 20 °C is clearly visible from the reported moduli values at both the analysed temperatures. Besides, a general decrease of G' and G'' can be observed when samples are tested at 60 °C. G_0 plateau moduli obtained from the fitting with one unit Maxwell model are, as expected, higher than those calculated for the corresponding samples prepared at room temperatures. For example, the value obtained from the fitting of G' curve at 20 °C is 680.11 Pa. However, G′ relaxation times (2.08 s at 20 °C and 1.99 sat 60 °C) remained shorter than G″ relaxation times (3.28 s at 20 °C and 4.59 s at 60 °C) showing less lasting elastic properties and a quicker dissipation of the energy stored. This is another affirmation of the weak gel character of these systems. Furthermore, the power law behaviour is not that usually observed for a "real" gel being n exponents still different for the two moduli. Anyway a slight frequency dependence is present for both moduli at all temperatures tested ($0.13 < n < 0.35$).

Table 3
The mechanical properties of tetraglycol 4% 70 °C gels determined using texture profile analysis (mean and standard deviation were calculated for $n = 5$)

	Compressibility (N mm)	Adhesiveness (N mm)	Hardness (N)	Recovery
C974	1.240 ± 0.46	0.754 ± 0.14	0.335 ± 0.12	0.979 ± 0.06
C971	0.800 ± 0.19	0.520 ± 0.12	0.200 ± 0.03	1.050 ± 0.07

Table 4
Load and work of adhesion for C974-C971/tetraglycol samples of 4% (w/v) concentration prepared at 70 °C (mean and standard deviation were calculated for $n = 10$)

	Load (N)	Work (mJ)
C974	3.95 ± 1.50	1.49 ± 0.92
C971	2.77 ± 0.71	0.71 ± 0.18

3.2. Creep test

Creep-recovery analysis confirms the temperature dependent behaviour of such systems outlining the higher viscosity value and the lower compliance for samples prepared by the heating procedure. Comparing the two 4% C974 Carbopol concentration samples, prepared at room temperature and at 70 °C, this last one shows a higher viscosity value (171,270 Pa s) than that of the system obtained at room temperature (viscosity = 68,000 Pa s) confirming the temperature influence on system elastic properties.

Same considerations for the C971 4% sample: heating significantly increases viscosity of the from 5.26 ± 0.39 to $12{,}120 \pm 170$ Pa s.

All these rheological characteristics are similar to those encountered for the Carbopol/PEG 400 systems previously described (Bonacucina et al., 2004): samples elasticity is strongly dependent on temperature and preparation procedure and also on the type of Carbopol used. In fact, with both solvents, C974 gives systems characterised by higher elastic behaviour than the corresponding samples containing C971 as thickening agent.

3.3. Texture profile analysis

The mechanical properties of C974 and C974 tetraglycol gels are presented in Table 3. This study permits to evaluate the textural properties of the different formulations in order to obtain information about physical gels structure and to predict samples behaviour under the physiological conditions as, for example, the application of a stress during sample administration. In fact, examples of textural properties are the ease of sample removal from the container or its spreadability on mucosal or non-mucosal surface, the problems encountered during product filling and the "feel" of the formulation.

In agreement with rheological and mucoadhesive studies, C974 system presents the greatest compressibility, hardness and adhesiveness confirming the role of sample elasticity in these textural parameters.

Compressibility and hardness quantify sample deformation under compression and shear and they are related to sample "consistency". Since a remarkable viscosity of the tested systems produces a greater resistive force to its deformation, an increased force per unit time is necessary for compression and consequently an increased hardness is measured for C974 gels. As already observed in the rheological analysis, the more entangled structure of C974 influences polymer gels characteristics giving rise to more elastic and thick systems.

Adhesiveness is the work required to remove the probe from the sample and it is related to the breaking of cohesive bonds. Obviously it is dependent on system viscosity but it is also considered a measure of the affinity for non-mucous surface. This makes the C974 sample a good candidate for topical delivery systems (Jones et al., 1996; Jones et al., 1997).

The recovery parameter gives an indication about sample ability to "recover" its initial position and it is related to temporal and spatial structural reformation after compression. Lower numerical results (C974 samples) mean greater elasticity being more difficult for higher viscous systems to return to the initial position in a short time.

3.4. Skin irritation

No sign of skin erythema or oedema is detected either after 0.5 or 24 h and 0 scores are recorded for tetraglycol gels and, of course, for the negative control gel.

3.5. Mucoadhesion

Carbopols are well-known adhesive polymers when formulated in tablets or water neutralized gels.

In this case adhesive properties have been tested on tetraglycol/Carbopol semisolid systems and the obtained results show a good correlation with polymers characteristics.

Mucoadhesion studies point out a certain better mucoadhesiveness of Carbopol C974 in comparison with the C971 (Table 4) in agreement with data already reported in the literature (Bonacucina et al., 2004; Tamburic and Craig, 1997).

In fact, as already explained C974 and C971 possess similar chemical structures, but the C971 is considerably less cross-linked. Therefore C974 gives rise to more elastic gels as it is shown by the rheological analysis. Usually systems with a higher elastic component possess a greater mucoadhesion. According to a previous work (Tamburic and Craig, 1995), δ smaller values correspond to higher detachment forces.

3.6. Dissolution studies

When Carbopol C971 is dispersed in tetraglycol, the gel has a good ability to control drug release. After 480 min, about 80% of the drug is released when the dissolution medium is water while in HCl 0.1N the full 100% of drug release is reached after 300 min and after 200 min in phosphate buffer (Fig. 7).

Carbopol 974 samples do not present a control of drug release in any of the considered dissolution media being the 100% of drug released in a maximum of 150 min. These results may

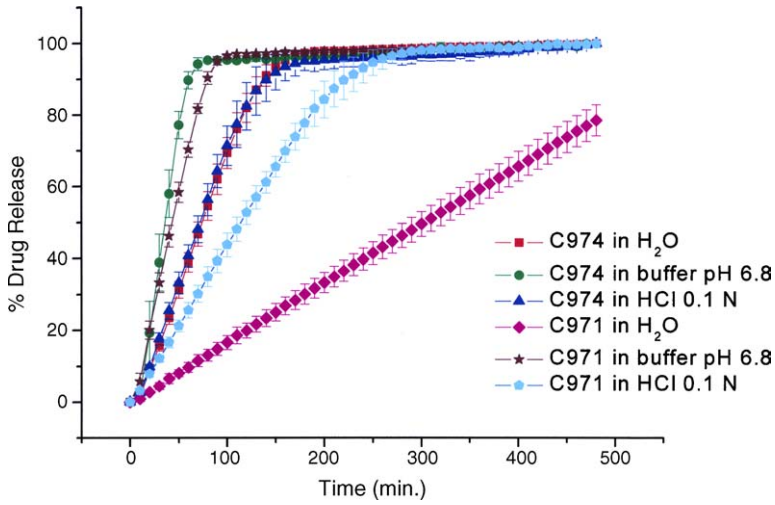

Fig. 7. Release profile (mean curves ± S.D., $n = 3$) obtained from tetraglycol/Carbopol 974 and Carbopol 971 systems containing Paracetamol.

appear in disagreement with the polymers structure and with rheological characterisation described above. In fact, C974 gives gels with a greater elastic character. Usually, the release rate of a drug from a semisolid matrix is inversely proportional to its solid character, however 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 microviscosities. Due to these differences drugs behave differently in formulations containing different Carbopol grades. For example, Carbopol 974 in water shows the most rigid gel microparticles when fully hydrated and channels are present in the gel structure, while Carbopol 971 presents flexible microparticles at the same condition (Lochhead et al., 1989). 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 (Lochhead et al., 1989).

The different micro-viscous gel structure between C974 and 971 can explain the better ability of this last Carbopol grade to control drug release. Besides, the release control of the C971/tetraglycol gel strongly depends on the dissolution medium. Drug release is nearly immediate in phosphate buffer, more gradual in HCl 0.1N and strongly controlled in distilled water.

Therefore 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.

The slower release rate in water rather than in buffer can be explained with Carbopol desolvation and precipitation occurring in tetraglycol after salification of the carboxylic groups, with a consequent reduction of the gel consistency.

In HCl 0.1N the acidic groups are not dissociated and the gel is less viscous but the polymer does not precipitates. This can explain why the release rate is intermediate between water and phosphate buffer.

These dependence of release kinetics on the dissolution medium used was already observed for Carbopol/PEG 400 systems (Bonacucina et al., 2004) even if in that case samples showed a definitely higher control of drug release.

The fitting of the release profiles according to the Power law equation ($y = kt^n$) is reported in Table 5. As expected the exponents n, related to drug release kinetics, are in all cases different from the ideal value of 0.5 indicating a Fickian diffusion mechanism. In fact, results show values of n approaching a zero order kinetics. This means drug release is independent of time and a swelling-controlled mechanism followed by erosion for both Carbopols samples in all dissolution media is present. A swelling controlled kinetics implies a polymer relaxation process and a significant change in system volume. Slight differences observed in n values and more pronounced changes in K values, K being a constant incorporating structural and geometric characteristic of the device and related to the drug/polymer system studied, could be explained with the difference in the swelling behaviour depending on the polymer and on the dissolution medium studied. In fact, K values reflect the release kinetics obtained. Systems with highest values of K are C971 and C974 tested in phosphate buffer which show the highest

Table 5
Results of the regression for the dissolution data generated by the power law equation (mean and standard deviation were calculated from the three dissolution curves)

	K	n	R^2
C974 H_2O	0.67 ± 0.01	0.99 ± 0.00	0.99
C974 buffer pH 6.8	1.07 ± 0.04	1.07 ± 0.01	0.96
C974HCl 0.1N	0.69 ± 0.01	1.00 ± 0.00	0.99
C971H_2O	0.18 ± 0.00	0.99 ± 0.00	1
C971 buffer pH6.8	0.90 ± 0.18	1.00 ± 0.04	0.99
C971HCl 0.1N	0.51 ± 0.04	0.97 ± 0.01	1

release rate while the C971 system in water present the lowest K and the better ability of controlling drug release.

The fitting was quite good considering the correlation coefficients values (R^2) all approaching to 1.

4. Conclusion

Carbopol C971 and C974 in Tetraglycol solvent show similar rheological behaviour while mucoadhesive and release characteristics are different. This should be taken into consideration when formulating a dosage form.

Tetraglycol systems, independently of the Carbopol used, increase their thickening behaviour after a thermal treatment and when systems are heated during the preparation step. Therefore, heating may be used as a real procedure for the preparation of tetraglycol/Carbopol gels, and its effect is more remarkable when C974 is used. In fact, Carbopol C974 gives more elastic gels than C971. However, as shown by the fitting of the frequency sweep curve, even after thermal treatment, systems are still weak gels, characterised by a lacking of strong bonds among the polymeric chains and by a predominance of polymer–solvent interactions. Thus, samples may be considered more entangled systems than true gels. In fact, the rheological behaviour let suppose the existence of topological interactions among polimeric chains rather than the presence of a real solid network or of a crosslinking structure.

The texture characterisation is completely in agreement with the rheological results confirming the greater mechanical properties of C974 samples in terms of hardness, compressibility and adhesiveness and also showing a consequent slower recovery of systems structure after compression. The greater elasticity of C974 gels can also explain their good adhesive properties, which make them interesting systems as semisolid mucoadhesive formulations, prolonging drug residence time at application site.

However, because of the different micro-viscous gel structure, Carbopol C971 shows a better ability to control drug release particularly when water is the dissolution medium even if the fitting of the dissolution curves with the power law equation outlines that drug release is for both Carbopols independent of time. So, in general, a swelling-controlled mechanism followed by erosion is present in all dissolution media.

Independently on the Carbopol used, systems appear sound alternatives for the formulation of semi-solid systems, which could be potentially administered in different ways including rectal, buccal, nasal, intestinal, vaginal and topical routes.

Besides, the effective drug release control of C971 gel could, for instance, make possible the formulation of C971/tetraglycol gels, not only as topical formulations but also for hard gelatine capsules filling as oral controlled release systems.

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

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