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Rheological evaluation of dispersions, prepared with different non- and gamma-irradiated bioadhesive cospray dried powder mixtures

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Various polymer mixtures were used to prepare gels: native drum dried waxy maize starch®, Amioca® starch, Carbopol® 974P NF and powder mixtures obtained by cospray drying Amioca® starch and Carbopol® 974P NF, and by blending Carbopol® 974P NF with Amioca® starch or drum dried waxy maize starch®. Oscillatory rheology was employed to investigate the influence of gamma-irradiation, but also of the different compositions (i.e. Carbopol® 974P NF varied from 5 to 25% w/w in the powder mixtures tested) and the preparation procedures of the powder mixtures on the viscoelastic properties of the polymer dispersions. The rheological data showed that gamma-irradiation has a significant influence on the rheological behaviour of the polymers dispersed. More elastic properties were obtained when the concentration of Carbopol® 974P NF was increased. The dispersions containing at least an amount of 15% Carbopol® 974P NF (w/w) are mainly elastic. Contrary to cospray drying the powders, blending the powders leads to higher elastic and gel properties of the dispersed polymers.

1. Introduction

The application of eye drops used as conventional ophthalmic delivery systems results in a poor bioavailability and therapeutic response. This is due to reflex blinking, tear secretion and nasolacrimal drainage. Consequently, the drug is drained away from the precorneal area in a few minutes. As a result, frequent instillation of eye drops is needed to achieve the desired therapeutic effects (Lee and Robinson 1986). But, during lacrimal drainage the main part of the drug administered is transported via the naso-lacrimal duct where it can be absorbed, sometimes causing side effects (Salminen 1990). In order to increase its effectiveness, a dosage form should be chosen, which prolonges the residence time of the drug in the *cul-de-sac*. This may enhance the bioavailability, reduce the systemic absorption and the need for frequent administration, leading to an improved patient compliance. One possible strategy is to add polymers to ophthalmic solutions in order to increase the viscosity and thereby decrease the drainage rate (Swan 1945; Saettone 1982; Zaki 1986; Ludwig and Van Ooteghem 1992; Robinson and Mlynek 1995). In most cases, a substantial increase in viscosity leads only to a modest bioavailability enhancement. However, very viscous dosage forms are not always of practical value since they give rise to blurred vision and inaccurate instillation (Sintzel 1996). In recent years, bioadhesive polymers, usually consisting of macromolecular hydrocolloids with numerous hydrophilic (carboxyl-, hydroxyl- and sulfate-) functional groups, have been evaluated as adjuvants in ophthalmic drug delivery (Greaves and Wilson 1993; Robinson and Mlynek 1995; Kaur and Smitha 2002; Le Bourlais et al. 1998; Hornof et al. 2003, Bonferoni et al. 2004). Solutions or gels containing bioadhesive polymers are supposed to interact with the precorneal/conjuctival mucin layer via non-covalent bonds. Consequently, they rely on mucoadhesion as the primary mechanism for prolonging the residence time of ocular dosage forms in the *cul-de-sac* (Greaves and Wilson 1993; Kaur and Smitha 2002; Le Bourlais et al. 1998; Lee et al. 2000).

Recently, drum dried waxy maize starch® (DDMW), Amioca® (waxy corn) starch and Carbopol® 974P NF were used as bioadhesive polymers in the formulations of an ocular minitablet or in nasal drug delivery systems (Ceulemans et al. 2001; Weyenberg et al. 2003; Callens et al. 2003). Other mixtures and compositions based on Amioca® or DDWM with Carbopol® 974P NF were prepared in order to obtain a longer residence time in the *cul-de-sac* compared to the minitablets containing DDWM with 5% (w/w) Carbopol® 974P NF (Weyenberg et al. 2003).

In this study, cospray drying was used instead of freeze drying to obtain modified polymer mixtures (Ameye et al. 2002). The influence of this technique on the rheological behaviour of the polymers was examined and compared to the physical mixtures of the powders employed. The influence of varying amounts of Carbopol® (i.e. 5–25%, w/w) in combination with DDWM or Amioca starch® was also studied. As ocular dosage forms must be sterile, gamma irradiation (25 kGy) on the powders was performed and its influence on the rheological behaviour of the dispersions prepared was examined.

Table 1: Samples prepared for rheological characterisation

Type of powder formulation	Polymer ratio (%)	Concentration in PBS (w/w)	Code
Native polymer			
DDMW	100	6	DDWM
Amioca® starch	100	6	AS
Carbopol® 974 P	100	0.8	CAR08
		1.9	CAR19
Cospray dried polymer mixture			
(Amioca® starch : Carbopol® 974 P)	95 : 5	6	CS95
	90 : 10	6	CS90
	85 : 15	6	CS85
	75 : 25	6	CS75
Physical polymer mixture			
(DDMW : Carbopol® 974 P)	95 : 5	6	PD95
	90 : 10	6	PD90
	85 : 15	6	PD85
	75 : 25	6	PD75
Physical polymer mixture			
(Amioca® starch : Carbopol® 974 P)	95 : 5	6	PA95
	85 : 15	6	PA85

2. Investigations, results and discussion

Firstly dispersions of native DDWM, Amioca® starch and Carbopol® 974P NF were evaluated, secondly the preparations of cospray dried mixtures of Amioca® starch and Carbopol® 974P NF and finally dispersions containing the physical mixtures of Carbopol® 974P NF and DDWM or Carbopol® 974P NF and Amioca® starch (Table 1).

In the present study a controlled stress rheometer was employed to carry out two series of oscillatory measurements. Dynamic Stress Sweep (DSS) and Dynamic Stress Frequency (DFS) measurements were used for the evaluation of the elastic (G') and viscous (G'') parameters of the samples. Firstly, DSS measurements were performed to detect the linear viscoelastic region (LVER) of the dispersions, where the polymer network stays intact. The maximum stress in the LVER was determined by the stress value which can be applied without decreasing G' and G'' values. The relation between the strain and stress is only constant in this viscoelastic region. This relation is not more proportional after the LVER because of the destruction of the polymer network and consequently a larger deformation of the sample due to the stress used results in a decrease of the G' values. Thus, analyses of DSS measurements allow to characterise the polymers, the force of their intermolecular bonds and their resistance towards the stress applied. Three different situations can occur: G' (elastic) $\gg$ G'' (viscous) for a chemically crosslinked system, $G' > G''$ for a network consisting of secondary bonds and $G' < G''$ values for a physically entangled polymer solution (Ferry 1970). The DFS slope values were calculated when the logarithmic (log) of the elastic parameter (G') or viscous parameter (G'') were recorded as a function of the log value of the angular frequency (ω).

2.1. *Native polymer dispersions*

The dispersions of native non- and sterilised polymers in phosphate buffers solution were rheologically characterised in order to gain insight in their gel properties and to evaluate the influence of gamma-irradiation on the polymer network structure.

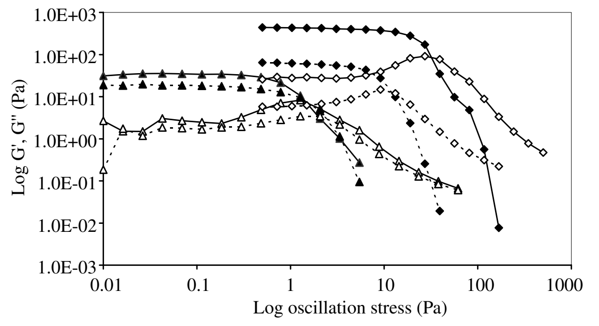

Fig. 1: Dynamic stress sweep curves of dispersions prepared with gamma (ST) and non-sterilised Carbopol® 974P NF formulations. —▲—, G'(CAR08); —△—, G''(CAR08); ···▲···, G'(CAR08)-ST; ···△···, G''(CAR08)-ST; —◆—, G'(CAR19); —◇—, G''(CAR19); ···◆···, G'(CAR19)-ST; ···◇···, G'' (CAR19)-ST

The results obtained from the Dynamic Stress Sweep measurements (DSS) indicated that increasing the concentration of Carbopol® 974P from 0.8% (w/w) to 1.9% (w/w) in the dispersion caused an increase of the G' and G'' values (Fig. 1). It is obvious that at higher polymer concentrations, more resistance occur against the increase of the oscillation stress applied. Lower G' and G'' values were measured after γ-irradiation of the carbomers used for the preparation of these dispersions. From the LVER, one can also conclude that γ-irradiation causes a decrease in resistance by the dispersed polymers to the increasing oscillation stress. The values of G' were higher than the values of G'' for both concentrations of the non- and the γ-irradiated Carbopol® 974P (Fig. 1), which suggests the presence of a network with secondary bonds (Ceulemans and Ludwig 2002a).

The Dynamic Frequency Sweep (DFS) slope values of $\log G'/\log \omega$ and $\log G''/\log \omega$ curves indicated that increasing the concentration of Carbopol® 974P from 0.8% (w/w) to 1.9% (w/w) in the dispersion caused a decrease of these slope values, which points to a moderate gel state for both dispersions. The DFS slope values of $\log G'/\log \omega$ and $\log G''/\log \omega$ curves of the dispersions prepared with non-sterilised powders were significantly lower than in the case of the sterilised Carbopol® 974P (p values are shown in Tables 2 and 3), thus demonstrating the existence of a weak gel structure. This observation is in accordance with the work of Deshpande and Shirolkar (1989) who observed also changes in the rheological properties of Carbopol® 940 gels after γ-sterilisation of the polymer used. When DFS slope values of $\log G'/\log \omega$ and $\log G''/\log \omega$ of Carbopol® 974P dispersions are compared to other preparations, it can be deduced that Carbopol® 974P dispersions have the strongest gel structure of all formulations tested.

In the DSS measurements, the viscosity (G'') was higher than the elasticity (G') of the dispersions prepared with the non- and sterilised starches Amioca® (AS) and drum dried waxy maize starch (DDWM), indicating the presence of a physically entangled system.

Furthermore, lower visco-elastic values were obtained after dispersing the sterilised powders (Fig. 2). Consequently, a decrease in elasticity (G') and viscosity (G'') was observed after γ-irradiation of the starches. DFS tests could not be performed on the samples prepared with sterilised powders since no linear region was present in the case of Amioca® and drum dried waxy maize starch.

Table 2: DFS slope values, p-values and regression coefficients of the log G'/log ω of dispersions prepared (mean ± S.D.)

Abbreviation	Slope		p-Values*	Regression	
	NST	ST		NST	ST
DDWM	0.824 ± 0.004	ND	–	0.999 ± 0.000	ND
AS	0.859 ± 0.004	ND	–	0.999 ± 0.000	ND
CAR08	0.064 ± 0.017	0.209 ± 0.018	0.004	0.987 ± 0.077	0.953 ± 0.029
CAR19	0.057 ± 0.026	0.101 ± 0.003	0.279	0.996 ± 0.081	0.935 ± 0.008
CS95	0.828 ± 0.083	ND	–	0.994 ± 0.005	ND
CS90	0.736 ± 0.057	0.767 ± 0.182	0.103	0.991 ± 0.004	0.985 ± 0.012
CS85	0.407 ± 0.024	0.724 ± 0.001	0.044	0.997 ± 0.003	0.993 ± 0.000
CS75	0.228 ± 0.014	0.254 ± 0.015	0.012	0.967 ± 0.015	0.996 ± 0.001
PD95	0.786 ± 0.008	0.867 ± 0.000	0.052	0.998 ± 0.001	0.974 ± 0.000
PD90	0.591 ± 0.067	0.792 ± 0.072	0.010	0.998 ± 0.002	0.984 ± 0.017
PD85	0.329 ± 0.028	0.555 ± 0.005	0.045	0.993 ± 0.004	0.993 ± 0.001
PD75	0.198 ± 0.002	0.201 ± 0.001	0.148	0.986 ± 0.003	0.983 ± 0.005
PA95	0.604 ± 0.002	0.877 ± 0.000	0.023	0.998 ± 0.001	0.944 ± 0.000
PA85	0.335 ± 0.014	0.537 ± 0.003	0.025	0.987 ± 0.009	0.993 ± 0.002

ND: not determined

* p-Values are obtained by comparing dispersions prepared with non-sterilised (NST) and gamma-irradiated (ST) powder mixtures

Table 3: DFS slope values, p-values and regression coefficients of the log G''/log ω of dispersions prepared (mean ± S.D.)

Abbreviation	Slope		p-Values*	Regression	
	NST	ST		NST	ST
DDWM	0.735 ± 0.049	ND	–	0.994 ± 0.001	ND
AS	0.796 ± 0.109	ND	–	0.971 ± 0.037	ND
CAR08	0.041 ± 0.003	0.045 ± 0.004	0.115	0.975 ± 0.000	0.982 ± 0.019
CAR19	0.033 ± 0.004	0.065 ± 0.002	0.085	0.986 ± 0.010	0.997 ± 0.000
CS95	ND	ND	–	ND	ND
CS90	0.348 ± 0.018	0.650 ± 0.138	0.223	0.940 ± 0.026	0.929 ± 0.073
CS85	0.103 ± 0.005	0.678 ± 0.022	0.020	0.998 ± 0.000	0.994 ± 0.001
CS75	0.052 ± 0.002	0.035 ± 0.002	0.098	0.997 ± 0.001	0.974 ± 0.002
PD95	0.862 ± 0.027	ND	–	0.970 ± 0.019	ND
PD90	0.639 ± 0.067	0.756 ± 0.092	0.488	0.995 ± 0.002	0.792 ± 0.096
PD85	0.167 ± 0.004	0.486 ± 0.003	0.009	0.996 ± 0.001	0.993 ± 0.005
PD75	0.074 ± 0.003	0.098 ± 0.005	0.138	0.995 ± 0.003	0.991 ± 0.000
PA95	0.602 ± 0.084	ND	–	0.986 ± 0.009	ND
PA85	0.167 ± 0.006	0.467 ± 0.028	0.032	0.996 ± 0.001	0.990 ± 0.004

ND: not determined

* p-Values are obtained by comparing dispersions prepared with non-sterilised (NST) and gamma-irradiated (ST) powder mixtures

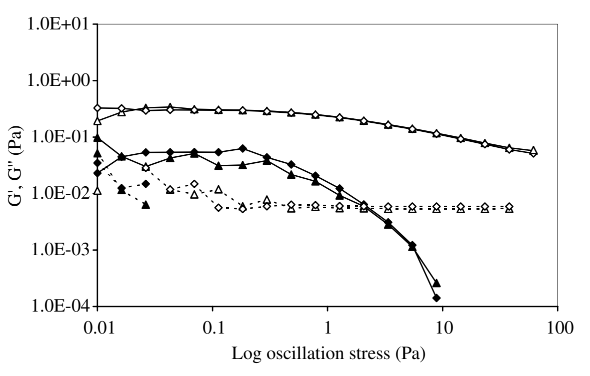

Fig. 2: Dynamic stress sweep curves of dispersions prepared with gamma (ST) and non-sterilised Amioca® starch and DDMW formulations. —▲—, G'(AS); —△—, G''(AS); ···▲···, G'(AS)-ST; ···△···, G''(AS)-ST; —◆—, G'(DDWM); —◇—, G''(DDWM); ···◆···, G'(DDWM)-ST; ···◇···, G'' (DDWM)-ST

2.2. *Cospray dried polymer mixture*

Furthermore, the rheological behaviour of the cospray dried mixtures were investigated by oscillation measurements.

Contrary to the dispersions prepared with the cospray dried powders CS75 and CS85, the G' values of CS90 and CS95 (composition, see Table 1) were lower than the G'' values due to the concentration effect of Carbopol® 974P in the polymer mixture (Fig. 3). In contrast to Amioca starch®, a high concentration of Carbopol® 974P in the cospray dried polymer mixture resulted in an elasticity increase (G'). For all mixtures prepared with γ-irradiated powders, lower G' and G'' values were recorded compared to the non-irradiated mixtures (Fig. 3). This is in agreement with the results observed for the native polymers.

Higher slope values of log G' or G'' versus log ω were observed for CS90 than for CS75 dispersions, made of non- and γ-irradiated powders. Thus, increasing the percentage of Carbopol® 974P in the cospray dried polymer mixture resulted in a stronger gel structure. The slope val-

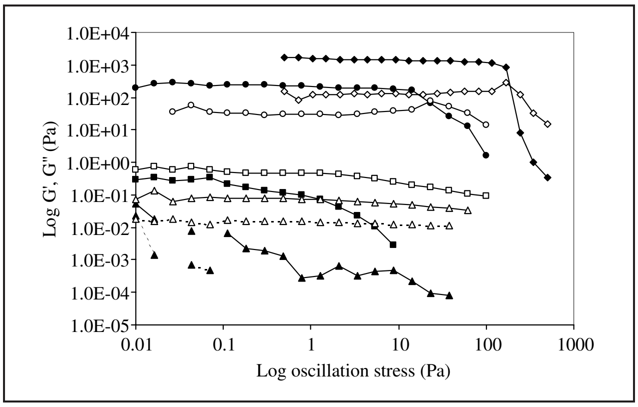

Fig. 3: Dynamic stress sweep curves of dispersions prepared with gamma (ST) and non-sterilised cospray dried polymer mixtures. —◆—, G′(CS75); —◇—, G″(CS75); —●—, G′(CS85); —○—, G″(CS85); —■—, G′(CS90); —□—, G″(CS90); —▲—, G′(CS95); —△—, G″(CS95); ···▲···, G′(CS95)-ST; ···△···, G″ (CS95)-ST

ues of log G′ or G″ versus log ω for all γ-irradiated powders, except for CS95 were significantly higher compared to the non-irradiated polymers, confirming the weak gel structure (p values are shown in Tables 2 and 3). In the case of CS95, log G′/log ω and log G″/log ω could not be determined, since no linear region (LVER) was present.

2.3. *Physical polymer mixtures*

The physical powder mixtures based on the both starches and Carbopol® 974P were evaluated in order to gain insight in the possible change of viscoelastic properties due to cospray drying the powder mixtures instead of mechanical blending.

The elasticity (G′) of the physical mixtures PD75 and PD85 (composition see Table 1) was higher than the viscosity (G″), while in the case of the PD90 and PD95 dispersions the opposite was measured. Thus an increase of the Carbopol® 974P concentration in the physical mixture leads to an increase of the elasticity, such as observed with the cospray dried mixtures. The values G′ and G″ were larger for all non-sterilised compared to sterilised powders (Fig. 4).

Higher slope values of log G′ or G″ versus log ω were measured for PD90 than for PD75, both before and after γ-irradiation of the powder mixtures. An increase of the amount of DDMW in the physical mixture results in a weak gel behaviour of the irradiated mixtures (Tables 2 and 3). Higher slope values of log G′/log ω and log G″/log ω for PD90, PD85 and PD75 prepared with γ-irradiated mixtures were recorded during the frequency measurements in comparison to those measured with the non-sterilised polymers. Thus, γ-irradiation induces a decrease of the gel strength of the samples (p values are shown in Tables 2 and 3). This can be explained by the fact that after γ-irradiation, a decrease in amylopectin fraction occurs, which is responsible for the swelling and rheologic properties of starches (Deschrieder 1960).

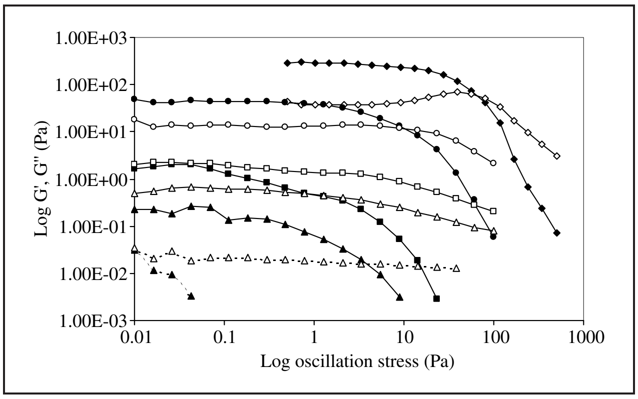

Fig. 4: Dynamic stress sweep curves of dispersions prepared with gamma (ST) and non-sterilised physical polymer mixtures based on Carbopol® 974P and DDMW. —◆— G′ (PD75); —◇—, G″(PD75); —●—, G′(PD85); —○—, G″(PD85); —■—, G′(PD90); —□—, G″(PD90); —▲—, G′(PD95); —△—, G″ (PD95); ···▲···, G′(PD95)-ST; ···△···, G″ (PD95)-ST

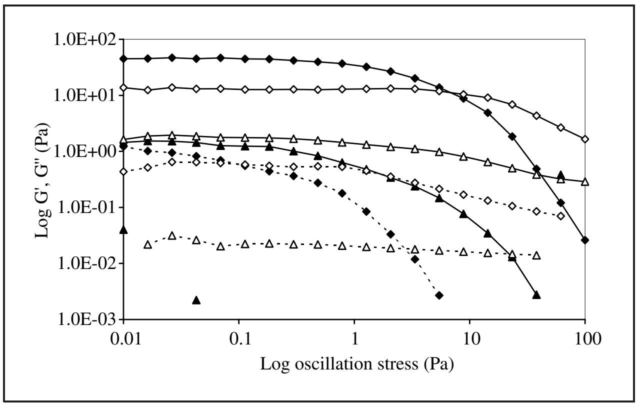

Fig. 5: Dynamic stress sweep curves of dispersions prepared with gamma (ST) and non-sterilised physical polymer mixtures based on Carbopol® 974P and Amioca® starch. —◆—, G′(PA85); —◇—, G″(PA85); ···◆···, G′(PA85)-ST; ···◇···, G″ (PA85)-ST; —▲—, G′(PA95); —△—, G″(PA95); ···▲···, G′(PA95)-ST; ···△···, G″(PA95)-ST

The DSS measurement results showed that the PA85 dispersion was more elastic than viscous, suggesting a network with secondary bonds. The G″ values of the PA95 dispersion were higher than the G′ values, indicating the presence of a physically entangled system (Fig. 5).

Higher slope values of log G′/log ω were recorded in the case of PA95 compared to PA85 dispersions prepared with the non- and γ-irradiated powders. Consequently an increase of the amount of Amioca® starch in the physical mixture results in a decrease of the gel strength of these mixtures (Table 2). The average slope value of log G″ versus log ω of the PA85 dispersion was lower when prepared with the non-sterilised than in the case of the sterilised powder mixture (Table 3). The gel behaviour of dispersions prepared with the γ-irradiated polymer mixtures PA85 and PA95 was weaker than those made with the non-sterilised powder mixtures. This can be derived from the higher slope values of log G′/log ω for the dispersions prepared with the mixtures sterilised (p values are shown in Table 2).

The advantage of cospray drying is that the mixtures obtained have less mucosal irritation properties than their corresponding physical blends (Adriaens et al. 2003). However, the elastic properties of the dispersions prepared with the cospray dried powder mixtures are lower in contrary to the physical blends. The powder mixtures with concentrations of Carbopol® 974P NF less than 10% (w/w) have smaller G′ values than G″ values. The most promising formulations, based on the highest G′ values or the lowest slopes from the log G′/log ω plots, are the preparations with only Carbopol® 974P NF (i.e. CAR19 and CAR08) followed by PD75, CS75, PA85, PD85 and CS85. Gamma-irradiation has a significantly negative influence on the rheological properties of the polymers examined. The dispersions prepared have less elastic and viscous properties in comparison to the samples made with the non-sterilised polymers. Gamma-irradiation sterilisation should be carefully evaluated and since it might cause changes in the rheological properties of polymeric formulations.

Further *in vitro* characterisation methods must be performed to select the most adequate formulation for a an *in vivo* evaluation in human volunteers.

3. Experimental

3.1. Materials

Purified water produced by Milli-Q (Millipore Co., USA) was used throughout the experiments. Carbopol® 974P NF and drum dried waxy maize starch® (DDMW) were supplied from Noveon (Cleveland, Ohio, USA) and Eridania Béghin-Say Cerestar (Vilvoorde, Belgium), respectively. Amioca® starch and the co-spray dried mixtures Amioca® starch with Carbopol® 974P NF (95/5, 90/10, 85/15 and 75/25) were received from National Starch and Chemical Company (Bridgewater, NJ, USA). Both starches are pre-gelatinized or pre-cooked: thus, if they are dispersed, then they will swell or thicken instantly even without cooking. They contain both a large amount of amylopectine (> 99%).

Sodium-dihydrogeno phosphate dihydrate ($NaH_2PO_4 \cdot 2\,H_2O$) and disodium-hydrogeno phosphate dihydrate ($Na_2HPO_4 \cdot 2\,H_2O$) were purchased from Merck (Darmstadt, Germany) and used to prepare an isotonic phosphate buffer solution (pH 7.4) or PBS.

3.2. Gamma-irradiation

The following powders were sterilised by γ-irradiation (25 kGy): native DDWM starch®, Amioca® starch and Carbopol® 974P NF; the physical mixtures prepared by blending Carbopol® 974P NF with DDWM or with Amioca®, and the cospray dried mixtures based on Amioca® starch and Carbopol® 974P NF. Gamma-irradiation sterilisation was performed using a ^{60}Co source (Gammir-I-Sulzer irradiator unicell, IBA-Mediris/Sterigenics, Fleurus, Belgium). The dose rate was set at 1.0 kGy/h and the irradiation dose applied was 25 kGy. All experiments were carried out at room temperature.

3.3. Polymer dispersions

The dispersions with native DDWM (6%, w/w), Amioca® starch (6%, w/w), Carbopol® 974P NF (0.8 and 1.9%, w/w) or the cospray dried mixture of Amioca® starch and Carbopol® 974P NF (CS95/5, CS90/10, CS85/15, CS75/25, 6%, w/w) were prepared by addition of irradiated or non-irradiated powder to PBS under continuous stirring until the polymers were completely dispersed (see Table 1). Similarly, the gels prepared with the physical powder mixtures were made by dispersing in PBS the required amount of irradiated or non-irradiated blends of Carbopol® 974P with DDWM or Amioca® starch. To ensure complete hydration all dispersions were stored at 6 °C for at least 12 h. The choice of the Carbopol® 974P concentrations 0.8 and 1.9% (w/w) is based on similarity with the Carbopol® 974P concentration present when dispersing 6% (w/w) of the cospray dried mixtures CS85/15 and CS75/25, respectively.

3.4. Rheological characterisation

The rheological analyses were carried out using a Carri-Med CSL^2 100 rheometer (TA Instruments Ltd., Brussels, Belgium) fitted with a 4 cm acrylic cone (1.59 degrees acrylic cone, truncation 57 μm). The samples were individually loaded on the Peltier plate after which a pre-shear stress was applied on the sample in order to homogenise them. All the rheological studies were conducted at 32 ± 0.1 °C, the temperature on the eye surface (Morgan et al. 1995). The samples were equilibrated during 10 min, allowing the polymers to recover from the destruction caused by the pre-shear procedure (Ceulemans and Ludwig 2002b).

In the oscillation measurements, the stress was increased logarithmically in the case of less viscous and more viscous samples from 0.01–100 and 0.5 to 500 Pa, respectively at a constant frequency of 1 rad/s to detect the LVER, during the dynamic stress ramp (DSS). A stress value (range: 0.05–5 Pa) derived from the LVER was chosen to perform a frequency ramp (DFS), during which the oscillation frequency was increased logarithmically from 0.1 to 10 rad/s (Ceulemans and Ludwig 2002b; Weyenberg and Ludwig 2002; Weyenberg et al. 2004). Three DSS and two DFS oscillation procedures of the various dispersions prepared were performed. Mean values and standard deviations were calculated.

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