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Development of a rheometric technique to measure the mucoadhesive capacity of a dry powder formulation

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In the present study, the use of an *in vitro* technique applying oscillatory shear rheology to determine the interaction between a dry polymer powder and mucin is investigated. The polymers examined are Carbopol 974P, Noveon AA1 and a polymer mixture containing Carbopol 974P and Drum Dried Waxy Maize Starch. The degree of interaction is assessed by analysing the variation of the elastic and viscous modulus as a function of oscillation stress. Both moduli are used to calculate a mucoadhesive index at a selected single stress/frequency combination. The contribution of physical entanglements and secondary bond formation to the interaction can be derived from the mathematical analysis of the relation between the moduli and the oscillation frequency.

When considering the stress sweep data, the interaction between mucin and the various polymers investigated seems very similar. This similarity is also observed when calculating the mucoadhesive indexes. On the contrary, the mathematical analysis of the frequency sweep data clearly indicates that the secondary bond formation between the polymer powder particles and mucin can be obtained only in the case of Carbopol 974 P.

1. Introduction

Several bioadhesive drug delivery systems have been investigated to prolong the residence time of a dosage form at specific sites (such as the buccal, nasal and gastrointestinal tract) and enhance drug absorption [1]. ‘Bioadhesion’ is referred to as the attachment of natural or synthetic macromolecules to a biological substrate [2]. When the attachment occurs more specifically to a mucosal epithelium, this phenomenon is referred to as ‘mucoadhesion’ because the biological layer responsible for the adhesion is the mucus layer [3]. The mucoadhesive process has been described to begin with the establishment of an intimate contact between the macromolecules and the mucus gel present at the surface of the epithelium. The second stage involves the physical entanglements of both polymers to allow finally the formation of the secondary chemical bonds between the macromolecules and the mucus chains [4, 5]. The water transfer from mucus to an applied dosage form can also be a significant factor in adhesion, since the adhesive and cohesive nature of the mucus gel increases when the water content is decreased. Mucoadhesion of a dry or partially hydrated dispersion is probably due to mucus dehydration, while in case of fully hydrated polymers the mucoadhesion mechanism is more related to surface energy thermodynamics and/or interpenetration phenomena [6].

Dry powder formulations were proposed to deliver drugs to the nasal cavity [7–9], while Charrueau et al. investigated the application to the buccal cavity in order to decontaminate the oropharynx [10]. The aim of the present study was to investigate the formation of secondary bonds after mixing a dry powder formulation and a mucin dispersion. The technique developed to verify the secondary bond formation is based on controlled-stress rheology. After hydration of the polymer powder particles, which occurs rather fast because of the large contact surface area, interpenetration of the flexible polymer chains into the mucin network and formation of secondary bonds can be expected. The formulations evaluated to investigate the method developed are Carbopol® 974P NF (CP974), Noveon® AA1 (NOV) and a polymer mixture (MIX) containing 5% Carbopol® 974P.

2. Investigations and results

2.1. Characterisation of the interaction in a high molecular weight polymer solution

Two types of oscillatory measurements are performed. On the one hand a dynamic stress sweep is applied in which the oscillatory moduli are recorded at a constant frequency of 1 rad/s and a range of stress amplitudes (0.001 to 10 Pa). The linear viscoelastic region is determined by the maximum stress which can be applied without affecting G' and G''. Furthermore, the relative magnitude of the moduli is a qualitative indication for the structure in the sample. For a solution of a high molecular weight polymer, three different situations can be encountered: $G' \gg G''$ for a chemically crosslinked system, $G' > G''$ for a network consisting of secondary bonds and $G' \leq G''$ for a physically entangled polymer solution [11]. On the other hand, experiments have been performed in which the stress amplitude was kept constant and the frequency was varied. The structure of the system can be kept intact during the measurement by choosing the amplitude of the oscillations within the linear viscoelastic region. By performing such small stress oscillations at a whole range of frequencies (0.1–10 rad/s), the kind of network structure present in the sample can be revealed. Typical frequency sweeps from an entangled and covalently cross-linked network are shown in Fig. 1, which is a graphical representation of the theory of Ross-Murphy [12]. The main difference between a network of secondary bonds and one of physical entanglements is located in the low frequency range: in an entangled network the polymers can disentangle if the available time is long enough (low frequency); in a network with secondary bonds the bonds are fixed irrespective of the time scale. This structural behaviour results for an entangled solution in a limiting slope of 2 for G' and 1 for G'' at low frequency in a log-log plot of moduli versus frequency, while at intermediate frequency a plateau develops. For a network of secondary bonds an almost constant value of G' and G'' is observed over the whole frequency range, with the value of G' exceeding that of G''.

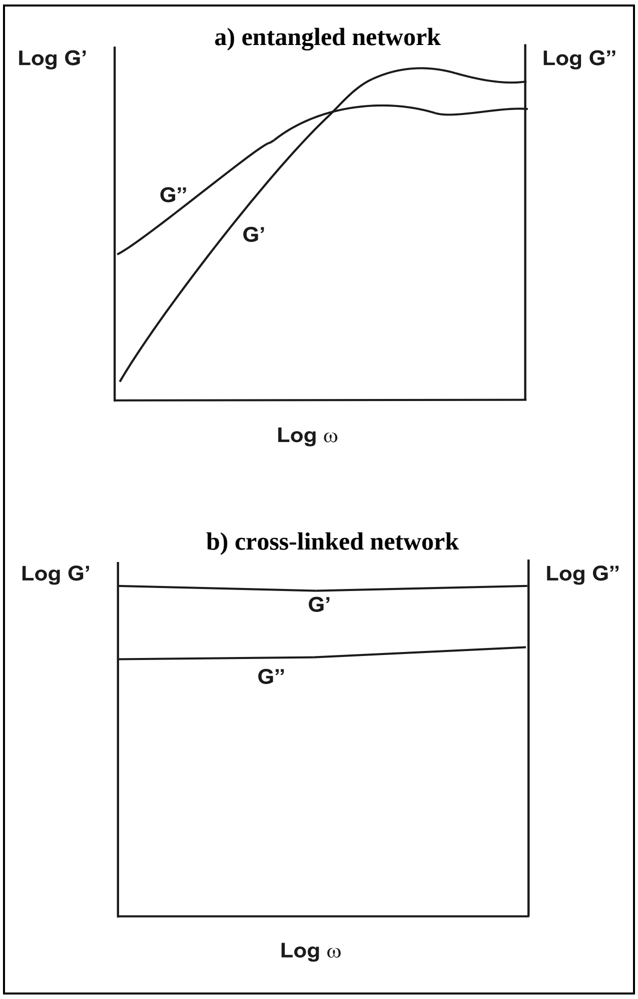

Fig. 1: Typical frequency sweeps from a) an entangled network and b) a covalently cross-linked network

2.2. Mixtures prepared to investigate the mucoadhesive interaction

An 8% (w/v) mucin dispersion (MUC) is prepared by dispersing the required amount of mucin powder in a salt solution (SALT) simulating the fluid in which the mucin is dispersed *in vivo*. To study the mucoadhesive interaction, the required amount of polymer powder is added to the mucin dispersion. An interaction between the polymer and mucin – either physical entanglements or secondary bonds – should be seen as a synergistic effect in the rheological properties [13]. Therefore it is essential to rheologically characterise the powder/MUC mixture as well as the single components. The first single-component (powder/SALT) consists of a dispersion of the polymer powder in SALT, having a concentration which is identical to the polymer concentration in powder/MUC. The rheological behaviour of this dispersion represents the particular interactions between the polymer investigated and the electrolytes in SALT. The second single-component consists of the mucin dispersion MUC to which no powder is added. The rheological behaviour of the MUC dispersion represents the interactions between the molecular chains of mucin.

2.3. Determination of the degree of interaction between the polymer and mucin

When taking the dynamic stress sweep results into consideration (Fig. 2), a clear rheological synergism can be observed: the oscillatory moduli of the powder/MUC mixture are higher compared to the oscillatory moduli of powder/SALT and MUC. This rheological synergism occurs independently of the kind of powder formulation under examination. Furthermore, the linear viscoelastic region of each powder/MUC mixture is characterised by an elastic modulus being higher compared to the viscous modulus, which points to mainly elastic behaviour of the powder/MUC mixture. The elastic modulus being higher compared to the viscous modulus is also observed for the powder/SALT mixture in the case of NOV and MIX, but only at very low stress values (2.64 mPa). The MUC dispersion has a linear region which is slightly smaller compared to the powder/MUC dispersion and is further characterised by G′ and G″ having similar values, pointing to a viscoelastic behaviour. The main conclusion concerning the stress ramps is that the addition of the polymer powder to the mucin dispersion, independently of the kind of polymer used, results in a clear increase of both G′ and G″. The oscillatory moduli of the powder/SALT mixture, being smaller compared to the moduli of the powder/

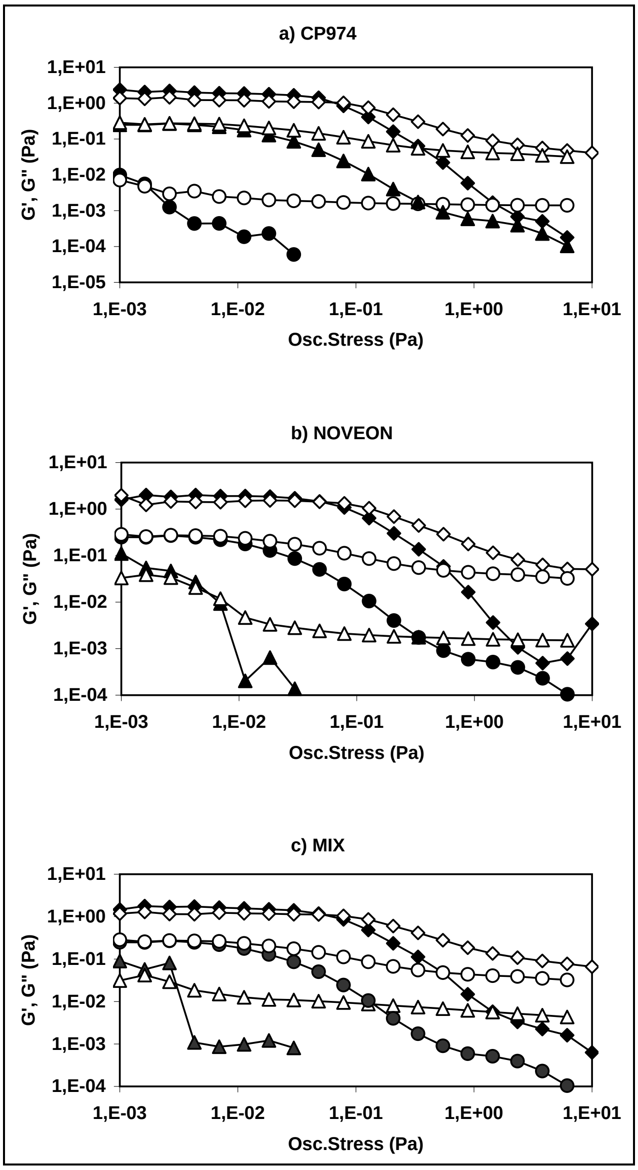

Fig. 2: Rheological investigation of the mucoadhesive interaction of a) CP974, b) NOV and c) MIX using the log G′(G″)/log stress relationship. Diamonds: powder/MUC; triangles: powder/SALT; circles: MUC. Filled symbols: G′; open symbols: G″

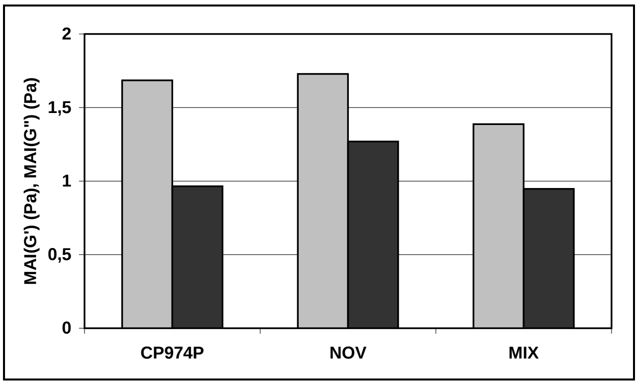

Fig. 3: Mucoadhesive indexes of the various polymers tested. MAI (G′): light grey; MAI(G″): dark grey

MUC mixture, indicate that the viscoelastic increase is not due to an interaction between the polymer and the salts present in the mucin dispersion.

The rheological synergism in the powder/MUC mixture can be asssessed by the calculation of the mucoadhesive index (MAI) based on the method of Rossi et al. (Fig. 3) [14]. The elastic and viscous modulus are measured at an oscillation stress situated within the linear region of the various mixtures and a frequency of 1 rad/sec.

$\mathrm{MAI}_{(G')} =$ Mucoadhesive index calculated with G'

$$\mathrm{MAI}_{(G')} = G'(\text{powder/MUC}) - [G'(\text{powder/SALT}) + G'(\text{MUC})] \quad (1)$$

$\mathrm{MAI}_{(G'')} =$ MucoAdhesive Index calculated with G''

$$\mathrm{MAI}_{(G'')} = G''(\text{powder/MUC}) - [G''(\text{powder/SALT}) + G''(\text{MUC})] \quad (2)$$

These indexes enable the quantification of the conclusion derived from the graphical analysis of the stress sweep curves. After mixing the polymers investigated and the mucin dispersion, a clear synergistic effect is observed for both the elastic and the viscous modulus. The difference between the effects of the different polymers is very small.

2.4. Determination of the kind of interaction between the polymer and mucin

The dynamic frequency sweeps (Fig. 4) are used to further explain the mechanism of interaction between the polymer powder and mucin. No frequency sweeps were performed with the powder/SALT mixtures since the viscoelastic region is often very small or even completely absent. Besides, stress sweep data clearly illustrate the minor importance of the powder/SALT mixture in the explanation of the interaction mechanism.

When considering the slopes of $\log G'/\log \omega$ and $\log G''/\log \omega$ for an entangled network ($\log G'/\log \omega$: slope 2; $\log G''/\log \omega$: slope 1) and a cross-linked network (both $\log G'/\log \omega$ and $\log G''/\log \omega$: slope ≈ 0) as presented in Fig. 1, it is clear that when additional secondary bonds are developed between the polymer and mucin, the slope of powder/MUC is smaller than the slope of powder/SALT and MUC. When only interpenetration and physical entanglements are responsible for the interaction, the slopes of the various mixtures are not different.

The CP974/MUC dispersion is characterised by oscillatory moduli which are less dependent on the frequency compared to the moduli of the MUC dispersion. Furthermore, the elastic modulus is clearly higher than the viscous modulus. On the contrary, the frequency dependency of the oscillatory moduli of the NOV/MUC and MIX/MUC dispersions is very similar to the frequency dependency of the MUC dispersion. The elastic and the viscous modulus curves of the latter mixtures cross each other at a frequency which is comparable to the cross-over frequency (ω_c) of the MUC dispersion. These results indicate that the additional formation of secondary bonds probably occurs only after mixing CP974 and mucin. The mucin dispersion itself is characterised by viscoelastic bonds having a relaxation time ($\tau = 1/\omega_c$) of 4.5 s, while after addition of the CP974 powder, ω_c is much smaller than 0.1 s^{-1}, which points to a relaxation time larger than 10 s. The increase of the relaxation time can be attributed to the formation of secondary bonds. Since the relaxation time of both the NOV/MUC and MIX/MUC mixture closely approaches the relaxation time of the MUC dispersion, the formation of additional secondary bonds can be excluded. Therefore, the rheological synergism observed in the dynamic stress sweeps is only due to interpenetration and physical entanglement of both components.

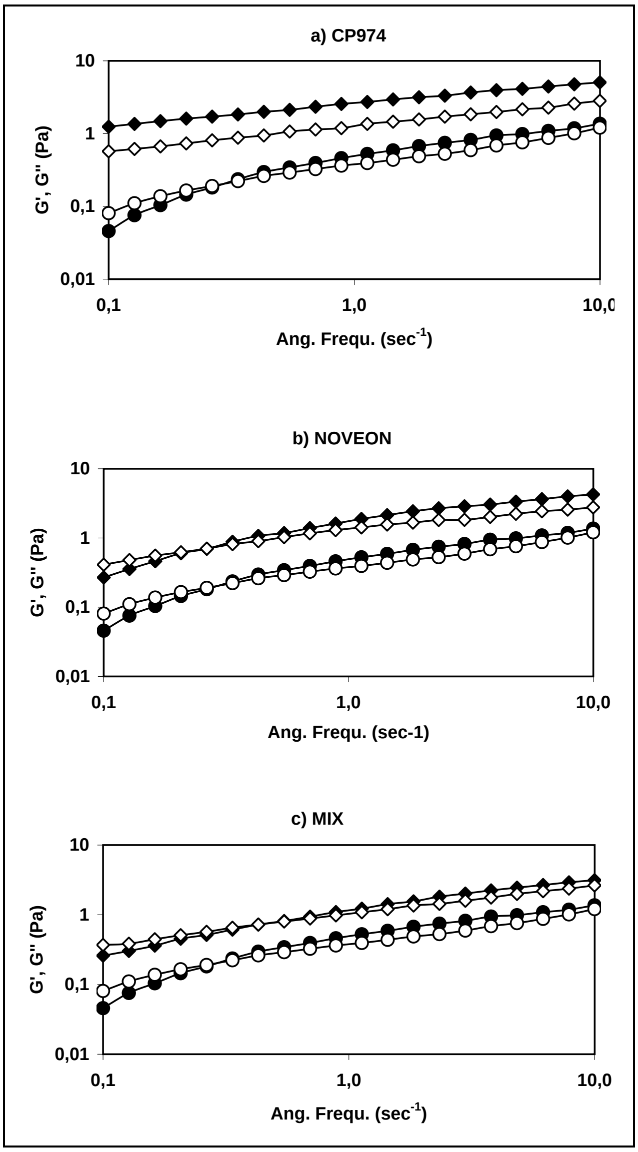

Fig. 4: Rheological investigation of the mucoadhesive interaction of a) CP974, b) NOV and c) MIX using the $\log G'(G'')/\log$ frequency relationship. Diamonds: powder/MUC; circles: MUC. Filled symbols: storage modulus; open symbols: loss modulus.

To quantify the results of the graphical interpretation of the dynamic frequency sweeps, the slopes of the curves

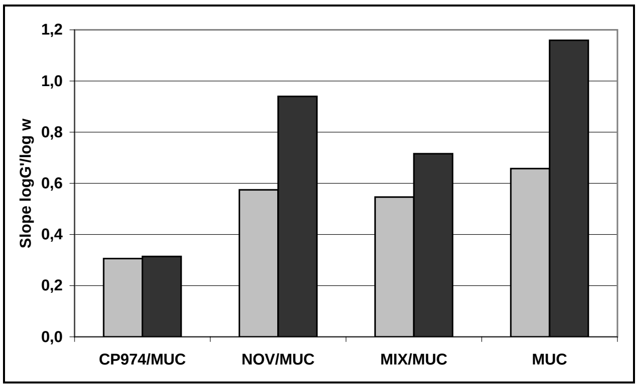

Fig. 5: Slope parameters obtained from the mathematical analysis of the dynamic frequency sweeps. Light grey: slope corresponding to the complete frequency range. Dark grey: slope corresponding to the low frequency range.

are calculated using the linear regression model (eq. 3, see Exp.). The values of the slope of the log G′/log ω and log G″/log ω relation in the low frequency region (Fig. 5) indicate the correlation with the interaction mechanism. Since the dimensional relationship between log G′ and log G″ is already discussed in the graphical interpretation of the frequency sweep curves, only the log G′/log ω relation is analysed mathematically. To demonstrate the importance of the choice of the frequency region selected to apply the linear regression model, two slopes corresponding with the complete curve (0.1–10 s^{-1}) on the one hand and the low frequency region (0.10–0.43 s^{-1}) on the other hand are calculated. When considering the complete frequency range, an additional secondary bond formation can be clearly demonstrated only for CP974, since only for this dry powder formulation the slope of the powder/MUC mixture is smaller than the slope of the MUC dispersion itself. For the other powders investigated, the difference is much less explicit. However, when considering only the low frequency range, the differences between the slope of the powder/MUC mixture and MUC are more pronounced. CP974 remains the polymer showing the highest secondary bond formation with mucin, but some additional secondary interactions can also be observed in the case of the mixture containing 5% CP974. The slope of the NOV/MUC mixture is also smaller than the slope of the MUC dispersion but the decrease is clearly more limited compared to the CP974P preparations. The correlation coefficients corresponding with the calculations of both the complete frequency curve and the low frequency region (Fig. 6) demonstrate that the conclusions based on the low frequency region data, showing the highest correlation coefficients, are most valuable. Especially the calculation of the slope of the MUC dispersion strongly depends on the frequency range applied. When performing the calculation with the complete set of data, the correlation coefficient is clearly smaller compared to the correlation coefficient obtained when considering only the low frequency data. Also the result of the NOV/MUC mixture depends on the frequency range applied, while the calculation for CP974 and MIX is not dependent on the frequency range.

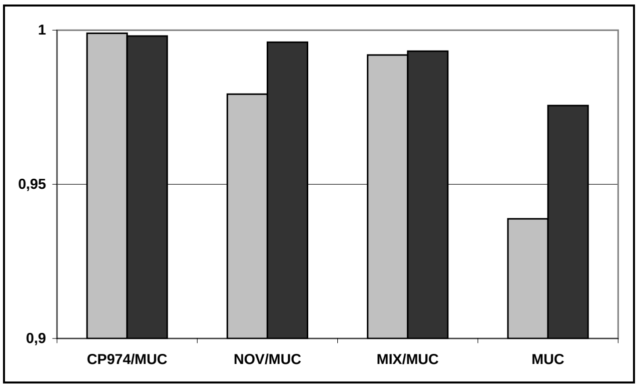

Fig. 6: Correlation coefficient obtained from the mathematical analysis of the dynamic frequency sweeps. Light grey: coefficient corresponding to the complete frequency range. Dark grey: coefficient corresponding to the low frequency range.

3. Discussion

Both the stress and frequency sweep data can provide useful information concerning the interaction between a dry powder formulation and a mucin dispersion, although the kind of information provided is clearly different. The graphical interpretation of the stress sweep curves and the calculation of the mucoadhesive index according to Rossi et al. [14] enable the determination of the degree of interaction, without giving an indication of the kind of interaction. The powders used in the present study show a comparable degree of mucoadhesive behaviour. To determine whether the interaction is due to interpenetration and physical entanglement only, or due to a combination of physical entanglement and secondary elastic bond formation, employing a frequency sweep procedure studying in detail (graphically and mathematically) the relaxation times of the powder/MUC network is necessary. Irrespectively of the frequency range considered, the difference between the mucoadhesive behaviour of the various powders is more explicit compared to the differences obtained with the former stress sweep procedure. However, the calculation of the correlation coefficient clearly indicates that the frequency range considered can have a major influence on the interpretation of the results. The frequency range to be selected can be derived from the graphical representation of the results. The combination of rheological procedures presented in this study enables the determination of the degree of the *in vitro* interaction between a dry powder formulation and mucin, as well as the identification of the bonds responsible for the interaction. However, future investigations have to be performed to assess the correlation between *in vitro* and *in vivo* results.

4. Experimental

4.1. Materials

Carbopol® 974P NF (CP974) and Noveon AA1 (NOV) were obtained as a gift from BF Goodrich (Brussels, Belgium). All other chemicals were purchased and used as received: sodium chloride (Federa, Brussels, Belgium); calcium chloride, magnesium chloride (Sigma Chemicals, Bornem, Belgium); potassium chloride, sodium hydrogen carbonate (Merck, Darmstadt, Germany); crude mucin from porcine stomach was purchased and used as received (Sigma Chemicals, Bornem, Belgium); the use of commercial mucins is justified by Rossi et al., who demonstrated the lower batch-to-batch variability shown by commercial samples with respect to those freshly prepared [15]. Purified water was used throughout the experiments.

4.2. Methods

4.2.1. Rheological measurements

Rheological analyses were carried out with a controlled stress rheometer (Carri-Med CSL^2 100, TA Instruments Ltd., Leatherhead, UK). The geometry applied is a standard size double concentric cylinder geometry, heated to 32 ± 0.1 °C by a warm water jacket. Each oscillatory procedure is performed two times on each sample. Mean values and standard deviations are calculated.

4.2.2. *Mucoadhesive investigation*

In the present study, 0.1% (w/v) is added to the mucin dispersion in the case of CP974 or NOV, while 1.5% (w/v) is added in the case of the powder mix containing 5% Carbopol 974P. These percentages are in accordance with the amount of polymer added in the case of a hydrated polymer preparation. The SALT solution is an electrolyte solution containing 1.7893 g/l KCl, 6.3118 g/l NaCl, 2.1842 g/l $NaHCO_3$, 44.4 mg/l $CaCl_2$ and 47.6 mg/l $MgCl_2$. Physiological pH (7.4 ± 0.1) was adjusted by adding the required amount of 0.1 N HCl.

4.2.3. *Mathematical analysis of the frequency sweep*

The mathematical model applied to calculate the slope is the linear regression model (eq. 3):

$$\log G = \log G_{(\omega = 0.1\,s-1)} + (\text{slope} \times \log \omega) \qquad (3)$$

$\log G = \log G'$ or $\log G''$

$\log G_{(\omega = 0.1\,s-1)} = \log G'$ or $\log G''$at a frequency of 0.1 s^{-1}

slope = slope of the log G/log frequency relation

$\log \omega$ = log frequency

The linear correlation r between log G and log ω is indicated by the correlation coefficient r (eq. 4):

$$r = \frac{n\,(\sum xy) - (\sum x)\,(\sum y)}{\sqrt{[n \sum x^2 - (\sum x)^2][n \sum y^2 - (\sum y)^2]}} \qquad (4)$$

$x = \log G'$ or $\log G''$

$y = \log \omega$

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