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Influence of the concentrations of liposomes and a submicron emulsion on the rheological properties of a topical gel

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Received December 2, 2005, accepted March 3, 2006

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Pharmazie 61: 938–942 (2006)

Two different kinds of liposomes and a submicron emulsion were added to a topical hydrogel consisting of alginates. The rheological characteristics of the gels after various dilutions were investigated. The various preparations studied exhibited a similar rheological behaviour. No important interactions were observed by mixing the hydrogel with liposomes or a submicron emulsion. The viscoelastic properties and gel state were preserved, only a decrease in consistency was measured.

1. Introduction

Liposomes and emulsions have been widely used as drug carriers for dermatological purposes, aiming at better penetration or prolonged action in the skin of a variety of drugs (Cevc 2004). In order to enhance the accumulation of drug at the site of application and to improve the application of topical liposomes, the use of viscous, but refreshing vehicles is appreciated by patients. Pavelic et al. (2001, 2005) demonstrated the compatibility of liposomes with crosslinked poly(acrylic acid) gels such as Carbopol®.

The aim of present study was to evaluate the influence of liposomes and submicron emulsions based on the same phospholipids on the rheological properties of a topical gel consisting of alginates. The influence of concentration of the carrier system in the hydrogel, and the presence of lecithin in the liposomes were investigated.

2. Investigations, results and discussion

The physical characteristics of both kinds of liposomes and submicron emulsion were measured 1 day after preparation and before mixing with the hydrogel. The data obtained are given in Table 1.

The particle size of the liposomes and the emulsion droplets were quite similar. The various carrier systems were negatively charged.

Table 1: Physical characteristics of liposomes and submicron emulsion

Preparations	Particle size (nm) (± SD)	Zetapotential (mV) (± SD)
Liposomes S75	477 (3.6)	−34.4 (1.1)
Liposomes S75 + Chol	463 (26.4)	−47.9 (0.6)
Submicron emulsion	326 (1.1)	−31.7 (0.7)

The influence of different dispersions of submicron emulsions and liposomes on the rheological properties of the hydrogel was examined. Concentrations of 10%, 20% and 30% (w/w) carrier systems in Kelset® gel were compared to the characteristics of the hydrogel diluted in the same manner with the aqueous vehicle (mannitol 5.07% w/v). Flow measurements were used in order to study the relation between the stress (related to the force applied) and the shear rate on the samples, and to determine the viscosity and the flow characteristics. The rheological parameters derived from the flow measurements are summarized in Table 2.

The rheograms of all gels diluted with mannitol solutions, submicron emulsions or containing both kinds of liposomes exhibited a pseudoplastic behaviour. The rate index values ranged from 0.32 to 0.51. The yield stress values were about zero for all preparations examined. The best curve fitting was obtained with the Herschel-Bulkley model. The submicron emulsion and the liposome preparations had a Newtonian behaviour with a rate index of 1.0 and a viscosity of 1.06 Pa · s.

The addition of various amounts of liposomes or submicron emulsion to Kelset® did not change the rheological characteristics of the hydrogel. The rate indeces lower than 0.51 indicated the pseudopastic behaviour was preserved but depended of the dilution. Mixing of the hydrogel with the submicron emulsion had less influence on the rate index than addition of liposomes.

Of course, compared to the pure gel (K100) a decrease in consistency was recorded after mixing as a function of the dilution. The consistency of the hydrogels diluted with liposomes was higher than the hydrogel preparations mixed with submicron emulsions.

The statistical analysis of the consistency values of the preparations was performed (Scheffé test, significance level $p = 0.005$). Comparing K80E20 with K80S20 and K90E10 with K90S10, p-values of 0.004 and 0.0002 were calculated respectively. This means that significant effects

Table 2: Rheological parameters of flow measurements

Samples	Code	Consistency (Pa.s) (SD)	Rate index (SD)	Regression (SD)
Kelset gel	K100	158.2 (7.7)	0.318 (0.005)	0.971 (0.002)
Gel-emulsion 90 : 10	K90E10	73.6 (6.7)	0.362 (0.007)	0.984 (0.014)
Gel-emulsion 80 : 20	K80E20	44.8 (6.4)	0.346 (0.003)	0.979 (0.003)
Gel-emulsion 70 : 30	K70E30	41.1 (5.1)	0.360 (0.019)	0.973 (0.006)
Gel-liposomes S75 90 : 10	K90S10	128.2 (15.5)	0.351 (0.075)	0.986 (0.017)
Gel-liposomes S75 80 : 20	K80S20	83.8 (9.1)	0.434 (0.016)	0.991 (0.008)
Gel-liposomes S75 70 : 30	K70S30	62.3 (5.9)	0.505 (0.039)	0.990 (0.007)
Gel-liposomes S75 + CHOL 90 : 10	K90SC10	101.4 (14.1)	0.408 (0.020)	0.995 (0.001)
Gel-liposomes S75 + CHOL 80 : 20	K80SC20	91.5 (2.9)	0.387 (0.012)	0.994 (0.003)
Gel-liposomes S75 + CHOL 70 : 30	K70SC30	60.5 (10.8)	0.411 (0.034)	0.995 (0.001)
Gel-mannitol sol 90 : 10	K90M10	84.0 (8.2)	0.383 (0.006)	0.991 (0.003)
Gel-mannitol sol 80 : 20	K80M20	47.8 (2.4)	0.424 (0.004)	0.988 (0.002)
Gel-mannitol sol 70 : 30	K70M30	36.5 (4.3)	0.443 (0.008)	0.988 (0.004)

on the rheological behaviour of the hydrogel were observed when the gel was combined in a ratio 90/10 or 80/20 with an emulsion on the one hand and with liposomes on the other hand. The differences were, however, not significant in the case of a 70/30 ratio. Moreover, no significant differences were noted, when the hydrogels diluted with the vehicle mannitol were compared with the gels mixed with submicron emulsion.

Two series of oscillation measurements were carried out with a controlled stress rheometer in order to investigate the elastic (G') and viscous (G'') parameters of the gels. During DSS (dynamic stress sweep) measurements the oscillation stress was increased logarithmically from 0.3 to 3000 Pa in order to detect the linear viscoelastic region (LVER), which is the stress region characterised by a directly proportional relation between the stress applied on the sample and the strain of the sample. The polymer network stays intact, while applying an oscillation stress in this linear viscoelastic region. This relation is not proportinal any more after the LVER because of the destruction of the polymer network: a larger deformation of the sample is obtained due the stress used resulting in a decrease of the elastic value (G'). Consequently, analyses of DSS measurements allow the characterisation of polymeric dispersions, the force of their intermolecular bonds and their resistance towards the stress applied (Ceulemans and Ludwig 2002a). 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 $G' < G''$ for a physically entangled polymer solution (Ferry 1970).

The viscoelastic behaviour of the pure hydrogel is shown in Fig. 1. The Kelset® gel exhibited elastic properties at low oscillation stress values. The values of G' were higher than the values of G'', suggesting the presence of a network with secondary bonds. The linear viscoelastic region reached 9 Pa. At higher values a sharp decline of the elastic properties was observed. From Fig. 1 one could also deduce that when the stress values increased the decrease of the elastic properties (G') was more important than the decrease of the viscous properties (G''). The cross-over stress was around 20 Pa. A possible explanation of this phenomenon is that by applying an oscillation stress secondary bonds between hydrated polymers were broken down, but the free polymer chains then participated in physical entanglement ($G'' > G'$). The same phenomenon was observed in Carbopol® gels (Bozdag et al. 2005).

The DSS (Dynamic Stress Sweep) results of the various diluted gels are summarised in Figs. 2–5.

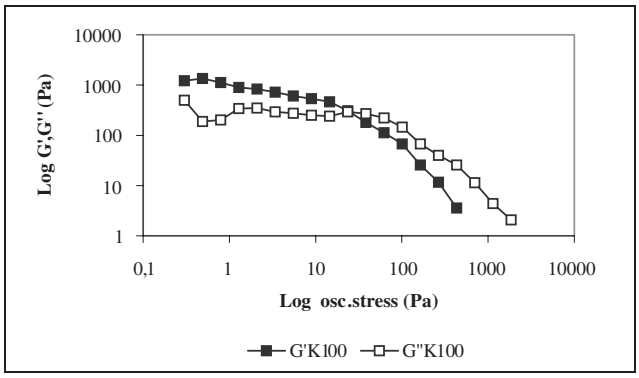

Fig. 1: Rheological characteristics of Kelset® gel

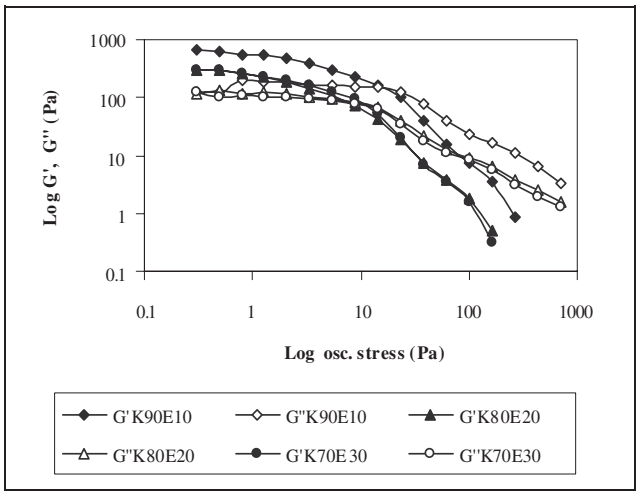

Fig. 2: DSS graphs of hydrogel diluted with submicron emulsion. (composition see Table 2.)

The preparation K90E10 containing the lowest amount of emulsion, possessed the highest viscoelastic properties (highest G' values), indicating that emulsion droplets did not improve the elastic behaviour of the hydrogel. The values of G' being higher than G'' for all preparations examined suggested that the network with secondary bonds was preserved. The LVER of K90E10 is 5.0 Pa and is significantly longer than both other preparations (2–3 Pa). From the LVER one could conclude that the higher dilution of the gel caused a decrease in resistance of the dispersed polymers to the increased oscillation stress.

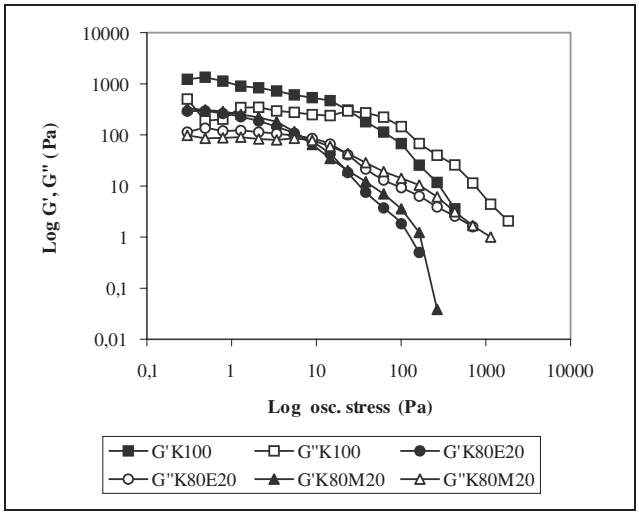

Fig. 3: DSS curves of K100, K80E20 and K80M20 preparations. (composition see Table 2)

One should also point out that the elastic moduli (G′) of K80E20 and K70E30 were quite similar. The cross-over stress of K90E10 is significantly higher than that of K80E20 and K70E30, which indicates a closer interaction between the hydrated polymer chains in this preparation, due to the lower oil concentration present.

The DSS curves of the pure gel K100, the gels K80E20 and K80M20 are presented in Fig. 3. The elastic properties of these 3 gels were more pronounced compared to the viscous moduli (G′ > G″). The LVER of K100 is 9.0 Pa in contrast to both other preparations with a LVER of 3.0 Pa. The cross-over stress is also decreased after mixing from 20 Pa to less than 10 Pa. Thus, structure change from a network with secondary bonds to physical entanglement occurred at lower oscillation stress compared to K100. Furthermore, lower viscoelastic values were observed after mixing the hydrogel with 20% submicron emulsion or mannitol solution. However, the viscoelastic properties of K80E20 and K80M20 were not significantly different.

The DSS measuremets of the gels containing both kinds of liposomes are given in Figs. 4 and 5. Both graphs show the elastic behaviour of these gels mixed with liposomes without and with cholesterol. A decrease of G′ values and G″ values was registered as a function of dilution. But at low oscillation stress the elastic moduli were higher than the viscous moduli, which means that the addition of liposomes did not change the structure of the network. The

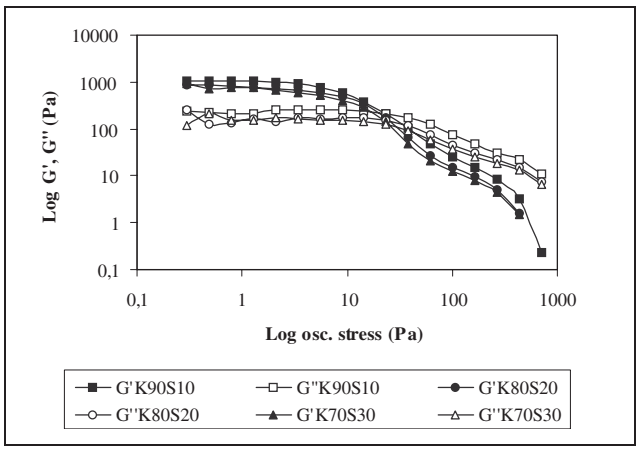

Fig. 4: DSS curves of hydrogels mixed with liposomes. (composition see Table 2)

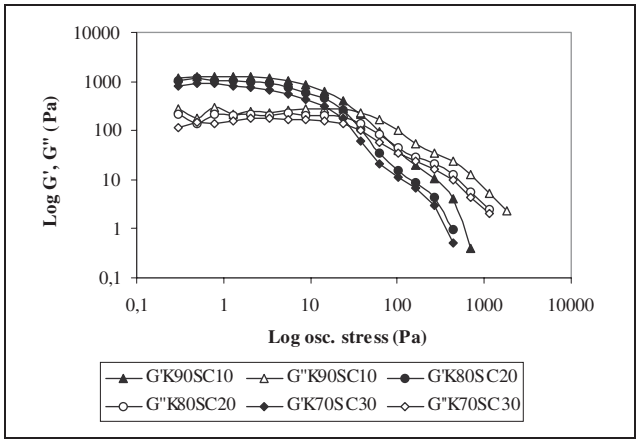

Fig. 5: DSS curves of hydrogels mixed with liposomes containing cholesterol. (composition see Table 2)

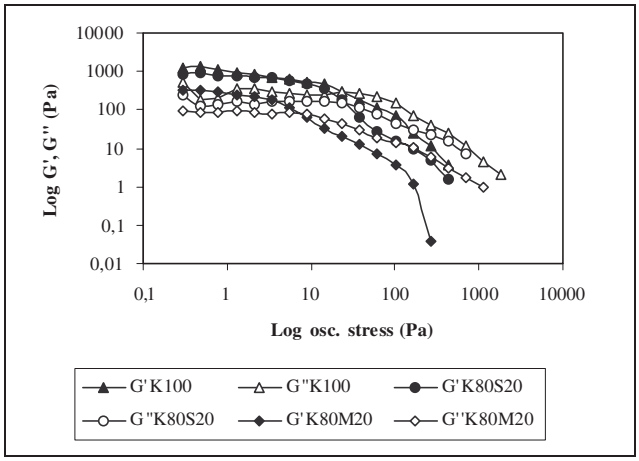

Fig. 6: DSS curves of K100, K80S20 and K80M20. (composition see Table 2)

LVER and the cross-over stress were influenced slightly by the concentration of the liposomes incorporated into the gel. The LVER is 8.0 Pa in the case of K90S10 and K90SC10 but 5–6 Pa for K70S30 and K70SC30.

From Figs. 4 and 5 one could deduce that cholesterol present in the bilayers of the liposomes had no influence on the rheological properties of the various samples.

In Fig. 6 a comparison is made between the pure hydrogel K100 and the diluted gels K80M20 and K80S20. The stress sweep curves of K100 and K80S20 were almost similar. The decrease of G′ values in comparison with K100 is more important in the case of K80M20 than for K80S20.

When analysing the DSS results recorded after addition of liposomes and submicron emulsion, following conclusions can be drawn. The elastic moduli of samples with liposomes are higher than the G′ values of corresponding mixtures with microemulsions. Also G″ values are for all samples measured always higher in the case of liposomes compared to similar addition of emulsions. The LVER and cross-over stresses are greater in hydrogels mixed with liposomes than after addition of submicron emulsion.

Dynamic frequency sweep (DFS) measurements were performed in order to gain insight in the gel or sol state of the preparations and to evaluate the possible change in hydrogel structure after mixing with submicron emulsion or liposomes. The oscillation stress is maintained at a low constant value deduced from the DSS results in order to keep the structure of the network intact, meanwhile the oscillation frequency is increased. In an entangled network

the polymer chains can disentangle only if the time is long enough (low frequency), while the bonds are fixed irrespective of the angular frequency in a network with secondary bonds. This structural behaviour results in the case of an entangled solution in a limiting slope of 2 for G′ versus frequency in a log-log plot. In the case of network with secondary bonds, a constant value of zero is observed for G′ over the whole frequency range (Ferry 1970; Ceulemans et al. 2002b).

Results of the DFS (dynamic frequency sweep) measurements are represented in Fig. 7. The G′ and G″ moduli of K100 and K80S20 were almost similar. The rheological behaviour of the pure hydrogel and the gel containing liposomes were comparable over the whole frequency range studied, while for K80E20 lower moduli values were recorded.

The DFS measurements were carried at a constant oscillation stress of 1.5 Pa. These measurements allowed to investigate which kind of polymer network was present in the samples. The DFS slope values of the log G′/log ω and log G″/log ω curves are given in Table 3.

The values of the slopes calculated demonstrated that all samples tested were gels and no sols, because the values were very low approaching zero. If mixing of the hydrogel with the carrier systems should have broken down the network to a dispersion with only physical entanglements of the polymer chains, a slope value of about 2 should have been calculated.

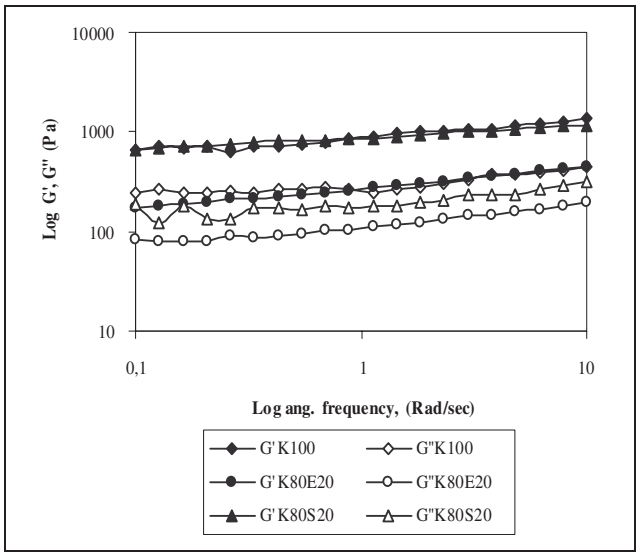

Fig. 7: DFS curves of hydrogels mixed with liposomes and submicron emulsion. (composition see Table 2)

Table 3: DFS slope values of the log G′/log ω and log G″/log ω curves (mean ± SD)

Sample	Slope log G′/log ω	Slope log G″/log ω
K100	0.18 (0.01)	0.24 (0.02)
K90E10	0.16 (0.01)	0.26 (0.04)
K80E20	0.19 (0.03)	0.26 (0.08)
K70E30	0.28 (0.04)	0.24 (0.02)
K90S10	0.11 (0.01)	0.23 (0.25)
K80S20	0.12 (0.03)	0.24 (0.10)
K70S30	0.10 (0.01)	0.29 (0.08)
K90SC10	0.11 (0.01)	0.20 (0.08)
K80SC20	0.09 (0.01)	0.19 (0.10)
K70SC30	0.12 (0.01)	0.18 (0.06)

The higher slope value of log G′ versus log ω of K70E30, confirmed the weaker gel structure and lower consistency measured during flow measurements (Table 2).

Statistical analysis was performed comparing the slope data of K90S10 with K90SC10, K80S20 with K80SC20 and K70S30 with K70SC30. No significant differences were observed, indicating that cholesterol in the bilayers had no influence.

From the rheological measurements performed one can conclude that the pure hydrogel and the gels containing both kinds of liposomes exhibit a similar rheological behaviour.

No important interactions and change in the network structure were observed by mixing the alginate hydrogel with liposomes or a submicron emulsion.

3. Experimental

3.1. Materials

The oil phase of the submicron emulsions consisted of medium chain triglycerides (Akomed E from Karlhamns, Karlshamn, S). The soybean lecithin S75 was kindly donated by Lipoid GmbH (Ludwigshafen, D) and used as received.

Mannitol, macrogol 400, and cholesterol were of pharmaceutical grade and were purchased from VWR-International (Leuven, B). Chloroform p.a. was supplied by Sigma Aldrich Chemie (Bornem,B). Kelset® was purchased from ISP (San Diego, USA), and alginic acid from ISP (Ayrahine, UK) .

Purified water produced by Milli-Q (Millipore Co., USA) was used throughout the experiments.

3.2. Preparation of liposomes

Liposomes were prepared by the film method. Lecithin S75 (100mg/ml) without or with cholestrol (2mg/ml) (ratio 1/1) was dissolved in chloroform evaporated to dry at 60 °C using a rotovapor apparatus (Buchi R205-V805, Flawil, CH). The film was hydrated by a aqueous mannitol solution (5,07% w/w). The preparation was held at 5 °C till mixing with the hydrogel.

3.3. Preparation of submicron emulsions

Microemulsions were prepared at 70 °C by dispersing the oil phase (20 g) into the water phase (80 g) containing the required amount of tonicity agent (mannitol 5,07% w/w). As emulsifier lecithin S75 (2 g) was used.

The primary emulsion obtained by mechanical mixing during 5 min was further homogenized with an ultrasonic probe at 50 W (Branson Digital sonifier 450-D, Danbury, USA) during 10 min.

3.4. Preparation of the hydrogel

The aqueous hydrogels were prepared with 2.5% (w/w) Kelset®, 1% (w/w) alginic acid and 25% macrogol 400 in water.

The submicron emulsion and the liposomes were carefully mixed manually until complete homogeneous preparations were obtained.

3.5. Physical characterisation

Measurement of droplet size and zetapotential were performed using the zetasizer 3000 (Malvern Instruments, Malvern, UK). The mean droplet size was measured by photon correlation spectroscopy after appropriate dilution with the vehicle. Each sample was measured three times. Zetapotential values were obtained using laser doppler anemometry. Each sample was diluted with vehicle, resulting in an optimum signal intensity, and measured 10 times.

The measurements were performed in triplicate and mean values were calculated.

3.6. Rheological evaluation

Rheological measurements were performed with a controlled stress rheometer (Carri-Med CSL^2 100, TA Instruments, Brussels, B) equipped with a 2 cm cone (2.00°, truncation 60 microns). The experiments were carried out at room temperature (25 °C). A pre-shear procedure was used to homogenise the samples. The test samples were equilibrated during 10 min allowing the hydrogel to recover from the destruction caused by the manipulations of sampling.

During the flow procedure the shear rate was increased from 0 to 15 (1/s) in 3 min (flow step/linear ramp mode).

The Herschel-Bulkley model expressed by Eq. (1) was employed as mathematical model to fit the data obtained.

$$\sigma = \sigma_y + K \cdot \gamma^n \quad (1)$$

σ = shear stress (Pa), σ_y = yield stress (Pa), K = consistency (Pa · s), γ = shear rate (1/s), n = shear rate index (n = 1, newtonian behaviour; n < 1, pseudopastic behaviour).

In the oscillation measurements the stress was increased logarithmically from 0.3 to 3000 Pa at a constant frequency of 1 rad/s to detect the linear viscoelastic region (LVER), during a dynamic stress ramp (Dynamic Stress Sweep (DSS)). A stress value in the LVER was chosen to perform a frequency ramp (Dynamic Frequency Sweep (DFS)) during which the oscillation frequency was increased logarithmically from 0.1 to 10 rad/s (Weyenberg and Ludwig 2002). Three flow measurements, three DSS and two DFS oscillation procedures were carried out in triplicate. Mean values and standard deviations were calculated.

3.7. Statistical analysis

The software Statistica® version 5.5 (Statsoft Inc. Tulsa, OK, USA) was used to perform statistical analyses of data.

Acknowledgements: The authors wish to thank the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT) for the financial support provided.

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