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Characterization and evaluation of β -glucan formulations as injectable implants for protein and peptide delivery

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Abstract

Context: Injectable implants are biodegradable, syringeable formulations that are injected as liquids, but form a gel inside the body due to a change in pH, ions or temperature.

Objective: To investigate the effect of polymer concentration, pH, ions and temperature on the gel formation of β -glucan, a natural cell-wall polysaccharide derived from barley, with particular emphasis on two-phase system formation after addition of dextran or PEG.

Materials and methods: Oscillation viscometry was used to evaluate the gel character by measuring flow index (N), storage (G') and loss (G'') moduli. Two-phase systems were further characterised for hardness and syringeability using a texture analyzer. Finally, *in vitro* release characteristics were determined using Franz diffusion cells.

Results: Oscillation viscometry revealed that only addition of dextran or PEG resulted in distinct gel formation. This was seen by a decrease in N after polymer addition. Moreover,

hardness (in g) of the gels increased significantly ($P < 0.001$) from 3.65 ± 0.43 to 34.30 ± 8.90 (dextran) and 805.80 ± 5.30 (PEG) 24 h after polymer addition. *In vitro* release profiles showed significantly ($P < 0.05$) reduced AUC_{0-8h} , k and % of drug released from two-phase systems compared to β -glucan dispersions, with the PEG system resulting in the lowest amount released over 8 h ($15.1 \pm 1.6\%$).

Discussion: The unfavorable mixing enthalpy and higher water affinity of PEG resulted in the formation of a dense β -glucan gel.

Conclusion: 1.5% (w/w) β -glucan combined with PEG at a ratio of 1:3 seemed to be the most promising injectable formulation with respect to fastest gel formation, increased hardness and sustained release.

Introduction

(1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- β -glucan is a cell-wall polysaccharide occurring in bacteria, fungi, yeasts, algae and the endosperm and aleurone cell walls of cereals such as barley, oats, rye and wheat^{1,2}. The US Food and Drug Administration (FDA) has acknowledged β -glucan soluble fibre from whole oats as part of a diet low in saturated fat and cholesterol, which may reduce the risk of coronary heart disease¹. Barley β -glucan results in highly viscous solutions which can cause problems in the brewing process¹. Curdlan (β -1,3-glucan) tablets containing theophylline were evaluated with regards to their *in vitro* release properties and showed sustained release³. Schizophyllan, an extracellular polysaccharide produced by a fungus, was investigated in terms of its ability to serve as carrier for nucleotide delivery⁴. Nucleotides as well as proteins and peptides such as human growth hormones, insulin or monoclonal antibodies play an important role in the defense of diseases⁵. Recent progress in genetic engineering has led to an increase in therapeutic proteins and peptides; however, there are a number of challenges with delivering such molecules to the target site⁵⁻⁷. As a consequence of their susceptibility to enzymatic degradation and fast clearance from the bloodstream, injections of therapeutic proteins and peptides have to be applied frequently which leads to high costs and poor patient compliance⁵. Thus, the importance of developing parenteral systems for sustained release of proteins and peptides is evident. Several injectable formulations including emulsions, liposomes, micelles and microspheres have already been evaluated^{5,8}. Considering the drawbacks of some parental formulations the need for a system which provides ease and convenience of application as well as reliability is apparent⁹. Implants can be divided into solid and injectable implants⁹. While solid implants are fabricated *ex vivo*, injectable implants are formed *in vivo*. Due to well controllable manufacturing processes solid implants normally provide very reproducible release kinetics⁹. However, surgery is required to insert and remove the implant from the body which causes discomfort for the patient and may lead to infections^{10,11}. Injectable implants are biodegradable, syringeable formulations, which are injected subcutaneously or intramuscular as liquids and subsequently form a gel inside the body due to various mechanisms^{8,10}. Important properties of a polymer suitable for an injectable implant include biodegradability, fast gelling, biocompatibility, low viscosity of the polymer dispersion to be injected and short setting time to avoid excessive progression of the implant^{12,13}. The main advantage of biodegradable injectable implants is that removal is

unnecessary thus helping to reduce the risk of infections¹¹. Like other sustained parental delivery systems injectable implants provide sustained release over a prolonged period of time. They may therefore increase patient compliance, reduce the overall costs and contribute to drug safety due to more constant blood drug levels^{9,14}. Mechanisms that can lead to sol-to-gel phase transition of a polymer include a change in temperature, pH, ions or solvent⁸ as well as the formation of two-phase systems due to phase separation. In ternary systems containing two different water soluble polymers such as dextran and polyethylene glycol (PEG) and water, two-phase separation is observed in certain ratios¹⁵ and has been found universal for most water-soluble polymer-polymer systems¹⁶. Two-phase formation occurs when limiting concentrations of the polymers are exceeded, which depends on the type as well as the molecular weight of the polymers and ionic strength of the formulation¹⁶. The thermodynamic condition for the formation of a two-phase system has previously been described by Stenekes *et al.*¹⁵. So far, two-phase systems have been used extensively as green reaction media for liquid-liquid partitioning of proteins with PEG/dextran as the most studied system¹⁶. Furthermore, two-phase systems have been used for the preparation of dextran microspheres while using aqueous PEG/dextran phase separation¹⁵. However, the use of two-phase systems as injectable implants has not been evaluated so far. The objectives of this study were to investigate the effect of polymer concentration, pH, ions and temperature on the gel formation of β -glucan, with particular emphasis on two-phase system formation after addition of dextran or PEG.

Materials and methods

Materials

Glucagel (crude aqueous extract, 80-90% pure) was purchased from GraceLinc (Canterbury, New Zealand). Dextran 40,000 and PEG 20,000 were purchased from ThermoFisher (Auckland, New Zealand). NaCl (ECP Ltd, Auckland, New Zealand), CaCl₂ (Scharlau Chemie, Barcelona, Spain), AlCl₃ (Ajax Finechem, Auckland, New Zealand), Na₂CO₃ (Scharlau Chemie, Barcelona, Spain) and sodium fluorescein (Sigma Aldrich, USA) were of analytical grade. PBS tablets were purchased from Oxoid Ltd. (Hampshire, England). Water used throughout all the experiments was purified by ion exchange (Millipore, Bedford, USA) to a resistance of 18.2 M Ω cm at 25 °C.

Sample preparation

The appropriate amount of β -glucan was added to water of 90 °C under constant stirring for 1 h to ensure complete hydration and dispersions were left to equilibrate at room temperature for 24 h. To evaluate the influence of pH, sodium hydroxide (0.1 M) or hydrochloric acid (0.1 M) were added to the β -glucan dispersions until the desired pH value was achieved (pH meter, Mettler Toledo, Schwerzenbach, Switzerland). For the evaluation of the influence of ions, weighed amounts of NaCl, MgCl₂ or AlCl₃ were added to the dispersions and left to equilibrate at room temperature overnight. Formulations were evaluated visually by tilting to an angle of 90°, classifying them as solid gels, gels with syneresis, partial gels or solutions respectively. Gels with syneresis were defined as systems consisting of a well formed gel

with a water layer on top, while partial gels consisted of gel lumps in suspension. For preparation of the two-phase systems, weighed amounts of dextran or PEG were gradually added to the β -glucan dispersions under constant stirring. In addition to the visual classification, the time until phase separation occurred and the volume of the two separate phases formed was measured.

Rheological characterization

All samples were prepared as described above and were left to equilibrate at room temperature overnight prior to rheological measurements. Stress, frequency and temperature sweeps were performed on a controlled stress rheometer (AR G2, TA instruments, USA) equipped with a 40 mm diameter parallel plate. The gap was set to 1 mm and samples were allowed to equilibrate to 37 ± 1 °C for 2 min prior to measurement. Initially, oscillatory stress sweeps (from 0.001 to 1 Pa) were performed to find the linear viscoelastic region. Frequency sweeps from 0.1 to 10 Hz were used to obtain the storage (G') and loss (G'') moduli, complex viscosity (η^*) and $\tan \delta$. In the case of two-phase system formation, the individual phases were separated and only the gel phase was measured. Temperature sweeps were performed from 6 to 75 °C at a rate of 1 °C min⁻¹. The flow index (N) was determined from the slope of the power law, by plotting the shear rate against the shear stress on a logarithmic scale. All measurements were performed in triplicate.

Mechanical properties and syringeability

Mechanical properties (hardness, adhesiveness and cohesiveness) were measured using a TA.XT plus texture analyzer (Stable Micro Systems, Godalming, England) with a load cell capacity of 5 kg. A 10 mm probe was compressed twice into each sample to a depth of 5 mm at a rate of 1 mm s⁻¹ allowing a relaxation period of 30 s between compression cycles. The trigger force was set to 0.1 g. All measurements were performed in triplicate. Hardness, adhesiveness and cohesiveness were determined from the force *versus* time plots as described by Jones *et al.*^{17,18} and compared using a one-way ANOVA followed by Tukey's pairwise comparison at a 99% confidence interval (MinitabTM Student Release 12.1, Minitab Inc., State College, PA, USA).

For syringeability, the maximum force required to squeeze the sample through a 38 mm long 22 gauge needle attached to a 3 ml syringe was measured at predetermined time points (0, 5, 10, 20, 30 and 60 min). A probe of 10 mm diameter was used to compress the plunger 20 mm into the barrel of the syringe at a rate of 1 mm s⁻¹. The trigger force was set at 0.5 g.

***In vitro* release profiles**

Standard Franz diffusion cells with a 15 mm diameter orifice (providing a diffusion area of 1.77 cm²), thermostated at 37 ± 1 °C by means of a water jacket connected to a VTC-220 heat circulator, were mounted to a FDC-6 diffusion cell drive console (Logan Instrument Corp., Somerset, NJ, USA). Receptor chambers (12 ml volume) were filled with PBS buffer (pH

7.4) and were constantly stirred at 600 rpm by small magnetic bars. Donor and receptor chambers were separated by a cellulose membrane (MWCO 8000 Da), soaked in receptor medium overnight prior to the experiment. A volume of 1 ml of test formulation (1.5 and 2.4% β -glucan dispersions with and without the addition of PEG or dextran at a ratio of 1:3) containing 50 μ g/ml of sodium fluorescein as model drug was loaded into the donor compartment. Samples of 1 ml were removed from the receptor chamber at predetermined time points (0, 0.5, 1, 2, 3, 4, 6 and 8 h) and replaced with fresh receptor medium to maintain sink conditions. Triplicates were performed for each formulation and samples were analyzed using a fluorescence spectrophotometer (Fluorometer Synergy™ 2, BioTek, Singapore) with an excitation and emission wavelength of 485 and 528 nm respectively. Cumulative amounts of sodium fluorescein released were plotted against time and the area under the curve (AUC_{0-8h}) was calculated according to the trapezoidal method. Formulations were compared in terms of the AUC_{0-8h}, the percentage of drug released after 8 h and the release rate constant (k) using a one-way ANOVA followed by Tukey's pairwise comparison at a 95% confidence interval (Minitab™ Student Release 12.1, Minitab Inc., State College, PA, USA). Release data was also fitted to various kinetic release models including zero and first order, Higuchi¹⁹, Hixon-Crowell cube root law²⁰ and Baker-Lonsdale model as previously described^{21,22}.

Results

Influence of β -glucan concentration, pH, ions and temperature on gel formation

A change in pH or addition of ions did not result in any pronounced gel formation of 2.4% β -glucan as seen by relative constant values for G' and G'' (data not shown). An increase in temperature over 45°C resulted in thinning of 2.4% β -glucan, which was evidenced by a 100-fold decrease in G' values and a 10-fold decrease in G'' values. However, once the temperature rose over 60°C, an increase in G' and G'' exceeding values at room temperature was observed. The temperature dependency decrease in G' and G'' did not occur for 1.5% β -glucan dispersions.

Of all the tested parameters, β -glucan concentration had the most pronounced effect on gel formation. While concentrations ranging from 1 to 2.4% were characterized as dispersions, 2.6 and 3% were classified as partial gels, 4% as a gel with syneresis and 5% as a solid gel.

Dispersions of 1, 2 and 3% β -glucan were easily syringeable, while 4 and 5% required much greater force to expel the material. This resulted in breakdown of the gel structure and therefore only excess water being expelled, while the remaining gel was prone to block the needle.

Oscillatory stress sweeps revealed the linear viscoelastic region and 0.3 Pa was chosen for subsequent frequency sweeps. Inconsistent G' and tan δ values were obtained for β -glucan concentrations of less than 2.4%. The higher the β -glucan concentration the more evident the gap between G' and G'' after 48 h. Thus, for 2.4% β -glucan G' values were only slightly higher than G'' values whereas G' values for 4% β -glucan were approximately 30-fold higher than G'' values. Moreover, the higher the β -glucan concentration the higher the η^* and the lower the N values obtained. Complex viscosity η^* versus angular frequency plots after 24 h

illustrated a negative slope of -0.3122 for 2.6% β -glucan while lower concentrations displayed positive values. The gel character of all formulations seemed to increase over time as seen by further decrease in the slope to -0.9027 for 2.6% β -glucan after 48 h.

Influence of dextran and PEG addition on formation of two-phase systems

Visual observations

β -glucan dispersions ranging from 1.2 to 2.4% were investigated with various ratios of dextran or PEG. In general, the higher the β -glucan concentration and the higher the ratio, the more pronounced the phase separation and therefore the gel formation observed. A concentration of 2.4% β -glucan combined with dextran at a ratio of 1:3 (Glu2.4/Dex) and 1.5% β -glucan combined with PEG at a ratio of 1:3 (Glu1.5/PEG) demonstrated the best phase separation as visually observed in measuring cylinders and were therefore chosen for further. In Glu2.4/Dex the gel phase formed at the top, while the gel phase was situated at the bottom in the Glu1.5/PEG system. Additionally, gels obtained with PEG were more compact in comparison to gels attained with dextran. With respect to the time required for gel formation, PEG addition to 1.5% β -glucan resulted in formation of a solid gel after 30 sec, while 3 min were required after addition of dextran to 2.4% β -glucan.

Rheological characterization

An increase in the dextran or PEG to β -glucan ratio resulted in higher storage and loss moduli with a more pronounced gap between the two parameters. In general, higher G' and G'' values were observed for PEG compared to dextran systems despite the lower β -glucan concentration used. To determine which polymer concentration and ratio resulted in the best gel formation, 1.5% and 2.4% β -glucan dispersions were selected, and after addition of various ratios of dextran and PEG, flow indices were determined directly and 15 min after mixing (Table 1). The lowest N -value and therefore highest pseudoplasticity for dextran addition was observed at a ratio of 1:3 to 2.4% β -glucan (0.720 ± 0.247), while PEG addition at a ratio of 1:3 to 1.5% and 2.4% β -glucan led to N -values below 0.3 (0.096 ± 0.103 and 0.240 ± 0.363 respectively) and therefore revealed much stronger gel formation.

Mechanical properties and syringeability

The syringeability of both Glu2.4/Dex and Glu1.5/PEG systems was similar within the first 10 min after mixing with a maximum force below 1000 g. After 10 min the PEG systems exhibited a more pronounced increase in the force required to expel the formulation with values being at least two-fold that of the dextran system (2148 g and 866 g at 30 min respectively). After 30 min formulations could not be expelled anymore due to blockage of the needle by the formed gels.

Mechanical properties (hardness, adhesiveness and cohesiveness) were derived from the force *versus* time plots as described by Jones *et al.*^{17,18} and are listed in Table 2. Statistical

analysis revealed that 24 h after addition of dextran and PEG values for hardness and adhesiveness for Glu2.4/Dex and Glu1.5/PEG were significantly ($P<0.001$) increased compared to 2.4% and 1.5% β -glucan dispersions respectively. The formed gel in the PEG system displayed a significant ($P<0.001$) 24-fold higher hardness after 24 h compared to the dextran system.

***In vitro* release**

Cumulative amounts of sodium fluorescein released from the various systems are shown in Figure 1. As can be seen the 1.5% β -glucan dispersion showed the fastest release (23.5% over 8h), while only about half the amount (12.3% over 8h) was released from the Glu1.5/PEG system.

In order to objectively quantify this phenomenon and compare the different systems to each other, the area under curve (AUC_{0-8h}) as well as the percentage of drug released after 8 h were determined (Table 3). Fitting the release profiles to various models, the best fit was found for the Hixon-Crowell cube root law, which was then used to calculate the release rate constants (k). Statistical analysis revealed that the AUC_{0-8h} , percentage of drug released after 8 h and k were significantly lower ($P<0.05$) for the Glu1.5/PEG systems when compared to a 1.5% β -glucan dispersion, while the Glu2.4/Dex systems only exhibited significantly lower values with respect to k (Table 4).

Discussion

Visual observations were supported by rheological results with important parameters being N , G' , G'' and $\tan \delta$. The flow index N is derived from the power law and is a measure for the pseudoplasticity of the sample²³. G' (storage modulus) characterizes the energy stored per sequence within the sample and is also referred to as the solid response of the formulation²⁴. G'' , the loss modulus, represents the energy dissipated per series within the sample and consequently gives information about the liquid response²⁴. $\tan \delta$ is the ratio of G'' and G' ; a predominance of elastic over viscous nature is therefore shown by a $\tan \delta$ value of less than 1. η^* is defined as complex modulus divided by angular frequency, in which the complex modulus is the overall resistance to deformation of a material. A gel is generally characterized by G' being nearly independent of the angular frequency, G'' being at least one decade below G' and a complex viscosity η^* with a slope of -1 on a double logarithmic plot against angular frequency²⁵. Therefore, visual observations characterizing β -glucan concentrations from 1 to 2.4% as dispersions were supported by rheological results which indicated no gel formation (negative G' and $\tan \delta$ values, positive slopes for complex viscosity η^* versus angular frequency plots). Formulations of 2.6 to 3% β -glucan were visually classified as partial gels, which was confirmed by G' values being independent of the frequency as well as a negative slope of the complex viscosity η^* versus angular frequency plots. Visual characterization of 4% as a gel with syneresis and 5% as a solid gel was supported by a wider gap between G' and G'' . Therefore, the higher the β -glucan concentration, the stronger the gel character which consequently resulted in reduced syringeability. Therefore high concentration β -glucan dispersions without gel character should be used as starting material, as they would be easily

syringeable, but would allow sufficient gel formation after addition of dextran or PEG. This was found to be the case for a 2.4% β -glucan dispersion, which was the highest concentration without any gel character and was therefore selected for subsequent experiments.

A change in pH or the addition of ions was not observed to trigger gel formation of β -glucan dispersions and was to be expected as β -glucan is non-ionic possessing no ionizable groups. An increase in temperature resulted in less viscous gels, which is in agreement with previous rheological studies performed on β -glucan dispersions²⁵ and can be explained by the disentanglement and increased solvation of polymer chains at higher temperatures. No changes in dynamic moduli were observed at low temperatures; thus, storing formulations in the fridge for stability of the incorporated protein and peptide drugs would not affect the gel structure. The detected decrease in viscosity of 2.4% β -glucan dispersions over 45 °C is at high enough temperatures to allow handling and storage without any structural breakdown, although the temperature independence of the 1.5% β -glucan dispersion would be even more favorable. The increase in G' and G'' values for both 1.5 and 2.4% dispersions over 60 °C is likely to be an aberration of the analytical technique due to partial evaporation of water at high temperatures and consequently an increase in gel viscosity.

Formation of two-phase systems was found to be the most promising approach to obtain a firm gel rapidly with the additional advantage of polymers such as PEG being able to stabilize the native structure of added proteins²⁶. The thermodynamic conditions for the formation of a two-phase system has previously been described by Stenekes *et al.*¹⁵ and include a positive free energy of mixing ΔG_{mix} as described in Equation 1, where ΔG_{mix} is the Gibbs free energy, ΔH_{mix} the enthalpy of mixing, T the absolute temperature and ΔS_{mix} the entropy of mixing.

Equation 1

If mixing is performed, the disorder increases and consequently the product of temperature and entropy also increase. The enthalpy of mixing is influenced by various factors such as the polymer concentration, polymer interactions, temperature and addition of ions¹⁶. If the gain in entropy of mixing is insufficient to compensate the repulsive interaction from the enthalpy, mixing of the two polymers is unfavorable from a thermodynamic point of view and therefore phase separation occurs¹⁵.

In general, the higher the β -glucan concentration and the ratio of the added polymer the more rapid the phase separation and the more viscous the gel formed. This is comparable with previous studies of PEG/dextran systems reporting increased density of the dextran-rich phase with increasing polymer concentration¹⁶ and can be explained by the unfavorable thermodynamics as described above. PEG addition led to faster and more compact gel formation at lower β -glucan concentration and lower ratios compared to dextran. This may be explained by the fact that dextran is structurally very similar to β -glucan and therefore has a comparable water affinity, while PEG is structurally vastly different to β -glucan and seems to have a higher water affinity, therefore taking more water away from β -glucan resulting in faster formation of denser β -glucan gels. According to previous studies on two-phase systems consisting of PEG, dextran and water, the more hydrophobic lighter phase is enriched in PEG while the more hydrophilic heavier phase is enriched in dextran¹⁶. This may explain the fact

that the liquid PEG phase was situated at the top in the Glu1.5/PEG systems, while the dextran phase was located at the bottom with regards to the Glu2.4/Dex formulation.

In general, PEG addition led to quicker and denser gel formation than dextran which can also be seen by the N-values listed in Table 2. Again, higher ratios and longer time intervals resulted in more pseudoplastic gels (lower N values).

With respect to syringeability, the maximum force of 1000 g was exceeded about 10 min after mixing; thus, formulations should ideally be injected within 10 min after the addition of PEG or dextran. Mechanical properties revealed significantly increased values for hardness 24 h after mixing, especially for the PEG systems, which can again be related to the higher water affinity of this polymer. This makes the Glu1.5/PEG more favorable as injectable implant as it would form a rather solid implant once injected into the body cavity.

In vitro release curves were found to best fit the Hixson-Crowell cube root law, which describes release of a drug from systems where the release process involves a change in surface area and diameter of the matrix²⁰. This is in agreement with visual observations showing a decrease in gel phase volume over time leading to a more compact gel with reduced surface area. However, also the reference formulations (1.5 and 2.4% β -glucan dispersions) seemed to best fit the Hixson-Crowell model. Since all release models have considerable overlap, solely relying on release data is not sufficient to explain the overlap or identify the correct release mechanism. Therefore, further studies using NMR and Raman spectroscopy could be used in future to determine the interactions between the polymers and the drug. Moreover, physical examination of the formed depot for zones of depletion could be performed. Nevertheless, the sustained release of the formed two-phase systems as opposed to the β -glucan dispersions is apparent with Glu1.5/PEG showing the lowest amount released over 8 h, which is in accordance with previous results obtained for rheological and mechanical properties for this system.

Conclusion

Development of biodegradable, sustained release injectable implants to deliver protein and peptide drugs such as insulin could considerably improve patient convenience and compliance. This study evaluated the use of a natural occurring polymer, β -glucan, for its suitability in injectable implant formulations. It was found that the concentration of β -glucan had an effect on gel formation, while a change in pH or ion concentration could not be used to achieve adequate gelling. In addition, the formation of two-phase system with the addition of other water soluble polymers such as PEG or dextran was found to be the most promising approach to obtain rapid and solid gel formation. This was shown by a dramatic increase in viscosity and hardness of the formed gel over time, as well as the sustained release characteristics. In practice, two-phase system formation could be achieved by using a two chamber syringe with mixing of the additional polymer with the β -glucan dispersion occurring shortly before or during the injection. However, injection would need to be completed within 10 min of mixing to avoid gelation within the needle, as shown by the syringeability tests performed. 1.5% β -glucan combined with PEG at a ratio of 1:3 therefore seemed to be the most promising formulation with respect to fastest gel formation, increased

hardness and sustained release and should therefore be further evaluated for its suitability as an injectable implant.

Declaration of Interest

The authors report no declarations of interest.

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Tables

Table 1. Flow indices (N) for 1.5% and 2.4% β -glucan dispersions with various ratios of dextran and PEG directly and 15 min after polymer addition; data points represent mean values $\pm$ SD, n = 3.

	N (1.5% β -glucan)		N (2.4% β -glucan)	
	0 min	15 min	0 min	15 min
β -glucan (reference)	0.990 $\pm$ 0.078	0.990 $\pm$ 0.078	0.997 $\pm$ 0.019	0.997 $\pm$ 0.019
β -glucan:PEG (1:1)	0.970 $\pm$ 0.392	0.748 $\pm$ 0.331	0.647 $\pm$ 0.146	0.573 $\pm$ 0.058
β -glucan:PEG (1:2)	0.627 $\pm$ 0.131	0.276 $\pm$ 0.408	0.633 $\pm$ 0.051	0.190 $\pm$ 0.220
β -glucan:PEG (1:3)	0.619 $\pm$ 0.626	0.240 $\pm$ 0.363	0.624 $\pm$ 0.070	0.096 $\pm$ 0.103
β -glucan:dextran (1:1)	0.905 $\pm$ 0.019	1.070 $\pm$ 0.201	0.981 $\pm$ 0.041	0.997 $\pm$ 0.014
β -glucan:dextran (1:2)	1.044 $\pm$ 0.015	1.010 $\pm$ 0.009	0.954 $\pm$ 0.028	0.828 $\pm$ 0.025
β -glucan:dextran (1:3)	1.006 $\pm$ 0.036	1.125 $\pm$ 0.020	0.862 $\pm$ 0.186	0.720 $\pm$ 0.247

Table 2. Mechanical properties of Glu2.4/Dex and Glu1.5/PEG over time; data points represent mean values $\pm$ SD, n = 3.

Time	Glu1.5/PEG			Glu2.4/Dex		
	0 min	15 min	24 h	0 min	15 min	24 h
Hardness (g)	3.37 $\pm$ 0.15	3.53 $\pm$ 0.31	805.80 $\pm$ 5.30	3.83 $\pm$ 0.25	4.50 $\pm$ 0.46	34.30 $\pm$ 8.90
Adhesiveness (g s ⁻¹)	0	0	27.45 $\pm$ 4.43	0.27 $\pm$ 0.08	0.41 $\pm$ 0.13	8.44 $\pm$ 1.31
Cohesiveness	0.88 $\pm$ 0.02	0.88 $\pm$ 0.01	0.71 $\pm$ 0.06	0.84 $\pm$ 0.02	0.81 $\pm$ 0.02	0.81 $\pm$ 0.02

Table 3. Areas under curve (AUC_{0-8h}), percentages of drug released after 8 h, release rates constants (k) and correlation coefficients (R^2) according to the Hixon-Crowell cube root law for the release of sodium fluorescein from the different formulations; data points represent mean values $\pm$ SD, $n = 3$.

	AUC_{0-8h} (% h cm^{-2})	Drug released after 8 h (%)	k (% $cm^{-2} h^{-0.5}$)	R^2 (Hixson – Crowell)
1.5% β-glucan	106.8 $\pm$ 20.7	23.5 $\pm$ 3.1	9.8 $\pm$ 1.1	0.987 $\pm$ 0.010
2.4% β-glucan	86.8 $\pm$ 7.9	19.9 $\pm$ 2.0	8.4 $\pm$ 0.8	0.994 $\pm$ 0.002
Glu1.5/PEG	55.9 $\pm$ 0.9	12.3 $\pm$ 0.2	5.2 $\pm$ 0.1	0.986 $\pm$ 0.001
Glu2.4/Dex	56.9 $\pm$ 20.7	15.1 $\pm$ 1.6	6.3 $\pm$ 0.7	0.996 $\pm$ 0.001

Table 4. Tukey's pairwise comparison of the AUC_{0-8h} , percentage of drug released after 8 h and *release rate constant (k)*; S = significant ($P \leq 0.05$), NS = not significant ($P > 0.05$).

	1.5% β-glucan	2.4% β-glucan	Glu1.5/PEG
2.4% β-glucan	NS; <u>NS</u> ; NS	-	-
Glu1.5/PEG	S; <u>S</u> ; S	S; <u>S</u> ; S	-
Glu2.4/Dex	S; <u>S</u> ; S	NS; <u>NS</u> ; S	NS; <u>NS</u> ; NS

Figure Captions

Figure 1. Cumulative amounts of fluorescein released versus time; data points represent mean values $\pm$ SD, $n = 3$.