Improved Texture Analysis for Hydrogel Characterization: Gel Cohesiveness, Adhesiveness, and Hardness

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ABSTRACT: The texture properties of formulation are an important parameter in optimization of topical formulations. These properties will affect applicability of the formulation at the administration site and therapy outcome. Our aim was to develop a fast and reliable method to characterize texture properties of hydrogels, namely cohesiveness, adhesiveness, and hardness. During the method development, we realized that the measurements setup needed to be adjusted for each hydrogel type, namely Carbopol, chitosan, and poloxamer hydrogels. The influence of the polymer concentration, pH, and incorporation of additives such as glycerol, drug solution, or liposomes on the texture properties, as determined by Texture Analyzer, was evaluated. In addition, the new method was applied to determine the changes during the accelerated stability testing. While Carbopol and poloxamer gels showed a linear relationship between the polymer concentration and texture properties, for low molecular weight chitosan gels the properties increased in exponential manner with increasing polymer concentration. The effect of incorporated liposomes on the gel properties was found to be dependent on the type of hydrogel. The hydrogel hardness was affected by the temperature as seen in accelerated stability testing. The method represents a valuable tool in pharmaceutical and cosmetics formulation development. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 180–188, 2012

Key words: hydrogels; hardness; biomaterials; vesicles

INTRODUCTION

The current aim in topical therapy of skin disorders, including wounds, is the development of advanced delivery systems, able to provide close and prolonged contact between the drug formulation and affected skin area.[1] An ideal wound dressing should provide good functional and aesthetic characteristics. It should be adhesive, elastic, durable, occlusive, and impermeable to bacteria.[2] Topical dosage forms destined for administration onto the skin need to posses optimal mechanical properties (such as spreadability), bioadhesion (prolonged contact time at administration site), acceptable viscosity and possibly, predictable release of active ingredients. Moreover, the wound dressing will be additionally subjected to flexing processes of the skin and its adhesiveness will directly affect the therapeutic outcome.[2,3]

Hydrophilic polymers as wound dressings are gaining more and more popularity. They are especially useful in the treatment of local wound infections providing the increased local concentrations of antibiotics while avoiding systemic effects. Moreover, biodegradable hydrogels can be easily washed off from the wound surface once they have exerted the desired therapeutic effects.[4] In addition, they possess low interfacial tension, high molecular and oxygen permeability, and good moisturizing and mechanical properties that resemble physiological soft tissue, all very important features considering improved wound therapy.[5] Due to their high water content and retention capacity, hydrogels can actually enhance wound healing.[6]

Among the most studied polymeric materials exhibiting potential in wound treatment, we focused on the Carbopols/Carbomers®, chitosan, and poloxamers.[7] Those three hydrogels were selected based on their specific properties, including mechanical differences and clear applicability as delivery systems/vehicles in wound therapy. In particular, physical hydrogels of chitosan were proposed as a very promising dressing for burned skin areas, even for the third degree burns.[8]

Carbomer polymers can be prepared as highly viscous gels at rather low concentrations and are known to exhibit bioadhesive, thermostable, and organoleptic properties which make these systems attractive both from pharmaceutical aspects and in respect to the patient acceptance.[9] Moreover, compatibility with many active ingredients and good bioadhesiveness are the additional advantages of Carbopol hydrogels.[10]

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Chitosan is widely used in different pharmaceutical applications due to its good biocompatibility, biodegradability, bacteriostatic as well as wound healing and hemostatic properties. It was shown that chitosan gels accelerate the reformation of connective tissues.[2,6,11]

A thermosensitive hydrogel was used as a third gel type in this study. Poloxamer 407 copolymer (ethylene oxide and propylene oxide blocks) shows thermoreversible properties. This type of hydrogel can be engineered in a way that the gelling temperature lays at body temperature. This can be of advantage for a facilitated administration of a topical formulation.[12]

In order to evaluate mechanical properties of hydrogels, we aimed at developing a fast and reproducible method, able to provide deeper insight on the properties of hydrogels as a first step in the evaluation of their potential in wound therapy. For this purpose, we have focused on mechanical properties and texture analysis. Texture profile analysis (TPA) was originally proposed as a suitable method to characterize semisolid drug dosage forms by Jones and his group.[3,13–15] The resulting mechanical parameters such as hardness, adhesiveness, and compressibility have been correlated to the therapeutic outcome of the drug formulation.[16,17]

However, we believe that the method could be modified to simplify the measurement procedure, allowing for a more straightforward characterization of hydrogel-based delivery systems targeting skin as administration site. Gel cohesiveness, adhesiveness, and hardness were characterized by the newly developed method. The method can serve as both an in-process and a quality control method in formulation development and pharmaceutical manufacturing. Moreover, as it is known that mechanical/rheological properties of polymeric gels can be manipulated by changes in the concentration of the polymer used, the pH of the formulation, and the presence of additives,[16] we used the newly developed method to evaluate the effect of those parameters on hydrogel cohesiveness, adhesiveness, and hardness.

EXPERIMENTAL

Materials

Carbopol Ultrez 10 NF was purchased from Noveon (Cleveland, USA). Low M_w chitosan (Brookfield viscosity 20.000 cps) and degree of deacetylation (DD, %) of 92, medium M_w chitosan (Brookfiled viscosity 200.000 cps) and DD of 82 and high M_w chitosan (Brookfield viscosity 800.000 cps) and DD of 77 were purchased from Sigma Aldrich Chemistry (St. Luis, USA). Poloxamer-407 was the products of Sigma-Aldrich Chemistry (St. Luis, USA). Lipoid S 100 was a generous gift from Lipoid GmBH (Ludwigshafen, Germany). Triethylamine was a product of Merck Schuchardt (Hohenbrunn, Germany). Glycerol was purchased from Merck KGaA (Darmstadt, Germany). Chloramphenicol was obtained from Sigma Aldrich (Steinheim, Germany). All other chemicals used in experiments were of analytical grade.

Preparation of liposomes

Liposomes were prepared by the conventional film method.[18] In brief, phospholipids (200 mg) were dissolved in methanol (approx. 20 mL) in a round bottom flask. The solvent was then completely removed on a rotary vacuum evaporator (Büchi R-124, Büchi Labortechnik, Flawil, Switzerland). The dry lipid film deposited on the flask wall was rehydrated by 10 mL of distilled water and hand-shaken for 20 min. The liposome dispersion was kept overnight at fridge temperature (4–8°C) prior to incorporation in hydrogels.

Preparation of hydrogels

Preparation of Carbopol Ultrez 10 hydrogels

Carbopol Ultrez 10 hydrogels were prepared according to the modified method by Fresno et al.[19] An appropriate amount of Carbopol resin was weighed and dispersed in distilled water and hydrogels of a polymer concentration ranging from 0.2 to 1.0% (w/w) were prepared. Triethylamine was used for neutralization purposes. The quantity of triethylamine was adjusted to achieve gel with desired pH (pH 5–11), respectively. The hydrogels were allowed to swell for 24 h at room temperature prior to the characterization.

Preparation of chitosan hydrogels

The preparation methods were based on Alsarra[2] and Cao et al.[20] Low (LMW), medium (MMW), or high (HMW) molecular weight chitosans were dispersed in appropriate volumes of acetic acid solution 2.5% (w/w). The hydrogels with chitosan content ranging from 1 to 6% (w/w) were investigated. The mixtures were stirred for 10 min manually and bath-sonicated for additional 30 min to remove entrapped air. The gels were allowed to swell in a sealed container for 48 h at room temperature.

Preparation of chitosan gels containing glycerol

A defined amount of glycerol (10%; w/w) was added and mixed with acetic acid (2.5%; w/w) and shaken manually until the blend was homogeneous. High molecular weight chitosan (2.5%; w/w) was dispersed in the prepared glycerol/acetic acid

TABLE I
Optimized Measurement Conditions

Conditions	Type of hydrogel		
	Chitosan	Carbopol	Poloxamer
Position of probe	Submerged below surface	Above gel surface	Above gel surface
Speed [mm/s]	4	1	1
Distance [mm]	10	15	15

mixture. The mixtures were stirred for 10 min manually and bath-sonicated for additional 30 min to remove entrapped air. The gels were allowed to swell in a sealed container for 48 h at room temperature.

Preparation of thermosensitive hydrogels

Poloxamer gels were prepared according to Park et al.[21] In brief, Poloxamer-407 (20%; w/w) was solubilized in distilled water and left at 4°C until a clear solution was obtained. Prior to investigation, the gels were kept in a 34°C water bath for 20 min until the gelling process was completed.

Incorporation of vesicles in hydrogels

Different amounts of liposomal dispersion were incorporated into Carbopol hydrogels to investigate the effect of vesicles incorporation on the texture properties of hydrogels. For this purpose up to 15% (w/w; liposomal dispersion/total weight) of dispersion was added to the gel and stirred carefully by hand until evenly distributed.[22]

Texture analysis

A Texture Analyser TA.XT Plus (Stable Micro Systems Ltd., Surrey, UK) was used to determine the texture properties of the hydrogels. Approximately 50 mL of the gel formulation was filled in a standard beaker (100 mL), thereby avoiding the introduction of air into the sample and assuring generation of a smooth upper surface. A 40-mm (diameter) disk was compressed into the gel and redrawn. The method settings, including speed rate and distance (depth of the insertion), were chosen according to hydrogel type (Table I). Five replicate analyses were performed at room temperature for each formulation, providing the same conditions for each measurement.

Gel parameters like hardness, cohesiveness, and adhesiveness were determined from the resultant force-time plot (Fig. 1). The maximum force does hereby present the hardness of the hydrogel formulation. Cohesiveness is defined as the work required to deform the hydrogel in the down movement of the probe. The second area shows the adhesiveness of the hydrogel to the probe.[3]

Stability testing

Stability testing was performed under the accelerated conditions. Gels were kept at 40°C for 4 weeks period in an airtight container. Texture analysis was used as a mean to compare the texture properties of hydrogels before and after the storage at 40°C.

RESULTS AND DISCUSSION

Hydrogels applied to the wounded area should provide a microgel network able to resist the physiological stress caused by the movement of skin (body), and at the same time provide closer and prolonged contact between gel and the skin area.[9] In designing the optimal topical formulation, particularly in respect to prolonged retention time at the site of administration for hydrogels destined for the treatment of wounds, a balance between gel adhesiveness and gel cohesiveness should be maintained. Texture analysis could provide a reliable overview of those properties. Gel hardness, which expresses the applicability of the gels to the skin, or adhesiveness, which can be an indicator for the retention time on the wound site, are directly correlated to the polymer concentration.[3,15] Gel viscosity, rheometry, and texture properties are known to depend on the composition of hydrogels. As a method to characterize the properties of gels, texture analysis offers a possibility to modify the measuring process in a way to achieve the desirable information in a reproducible and validated manner. Literature data provide information on different method options for hydrogel characterization, such as using the gel compressed in a tablet form,[23] or measuring hydrogel in original form as in the texture profile analysis developed by Jones et al.[13]

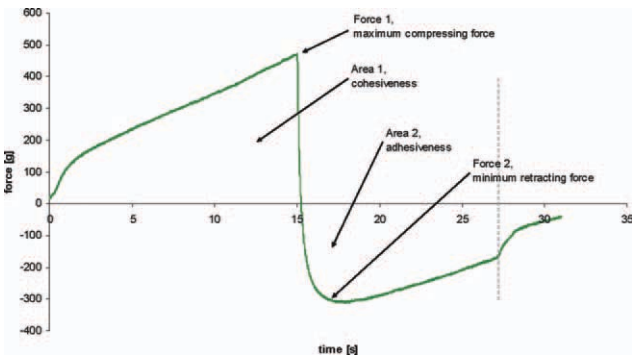

Figure 1 Typical force versus time plot of a backward extrusion measurement for Carbopol hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II
Texture Properties of Hydrogels Under the Optimized Measurement Conditions ($n = 1$)

Type of hydrogel and concentration (%; w/w)	Force 1 ± S.D. [g] (maximum compressing force; hardness)	Area 1 ± S.D. [g*s] (cohesiveness)	Force 2 ± S.D. [g] (minimum retracting force)	Area 2 ± S.D. [g*s] (adhesiveness)
Carbopol, 0.5	306.4 ± 9.7	3240.4 ± 82.0	−232.00 ± 5.9	−2676.00 ± 109.6
LMW chitosan, 5	44.6 ± 0.5	100.1 ± 0.5	−42.16 ± 0.5	-83.43 ± 0.7
Poloxamer, 22	753.2 ± 11.0	8571.6 ± 335.9	−662.25 ± 12.9	−5862.08 ± 471.5

Five replicate analyses were performed for each formulation, under the optimized conditions for that type of hydrogel.

However, in our opinion, the published methods have several limitations with respect to characterization of hydrogels destined for skin therapy. When the three selected hydrogels were characterized by the method based on the work of Jones and coworkers,[13] we could not obtain reproducible results (data not shown). Based on that fact and in order to gain more direct information on the cohesive and adhesive properties, and hardness of the hydrogels, we developed the new, improved, and simplified method using the Texture Analyzer.

Method development

We prepared all three types of hydrogels in distilled water rather than in buffer, as it is known that hydrogels, particularly Carbopol hydrogels, lose their viscosity in the presence of electrolytic components of the buffers.[18,24] However, one should consider that the viscosity of hydrogels applied to exudate-rich wounds for example, would be affected by the wound exudate.

It is well-established fact that the experimental conditions affect the measurements. At the same time, it is known to be rather difficult to directly correlate the mechanical properties of gels to their rheological properties. Jones et al. (2002) proposed correlation between mathematical interpretation of textural parameters and rheological measurements, providing possibility to interpret textural parameters as rheological evaluation.[16] Therefore, it is of great importance to establish and validate a simple, reproducible method, able to characterize both cohesive and adhesive properties of a gel, as well as gel hardness, without performing time consuming rheological characterization. The method should be easily applicable to various types of gels, such as the three model hydrogels selected in our experiments.

During the validation of the method, we realized that different types of hydrogel require different instrument settings, as the reproducibility of the results was rather problematic when the same measurement setup was applied for all three types of hydrogels (data not shown). Depending on the gel type, the different instruments setups such as starting point, speed and penetrating depth were finally adapted (Table I). Similarly, in the case of conventional texture profile analysis, an increase in the speed of measurement was reported to result in an increase in the hardness of the gel.[16]

Once the optimized measurement settings are determined for the particular hydrogel type, texture analysis can be easily applied for all types of characterization. The optimized measurements showed a very good reproducibility with a standard deviation being below 3% (for five consecutive measurements of the same gel) (Table II). Moreover, even a small change in texture properties could be reproducibly distinguished.

It was found that the optimal starting position of the disk probe for measuring Carbopol or poloxamer gels was exactly above the gel surface, whereas for the chitosan hydrogels a starting position below the gel surface was found to be more suitable. Due to its honey-like and sticky texture, chitosan hydrogels tend to stick to the container walls and thus affect the measured force, if the same method setup as for Carbopol and poloxamer gels was used for the chitosan gel measurement. In addition, the measuring speed had to be adjusted for each gel type. Chitosan hydrogels required a higher speed value for the compression than Carbopol and poloxamer gels, due to their rather low hardness, as compared to the other analyzed gels (Table II).

When analyzing the thermosensitive poloxamer hydrogels, the temperature had to be controlled before each measurement. In addition, a resting time had to be maintained to give the gel a chance for recovering into its original structure. Carbopol and chitosan gels were more robust regarding maintaining their structural strength after each measurement.

Each method setup was chosen after careful evaluation and based on the reproducibility of the measurements, with main criteria being SD below 3% within five measurements. Moreover, one should keep in mind that the texture analysis is a method particularly useful to compare gels of same origin, measured under the same measurement setup. In those conditions, the method is very valuable support both in formulation development and in-process control. It provides fast and reliable insight on mechanical gel properties, particularly pharmaceutically important

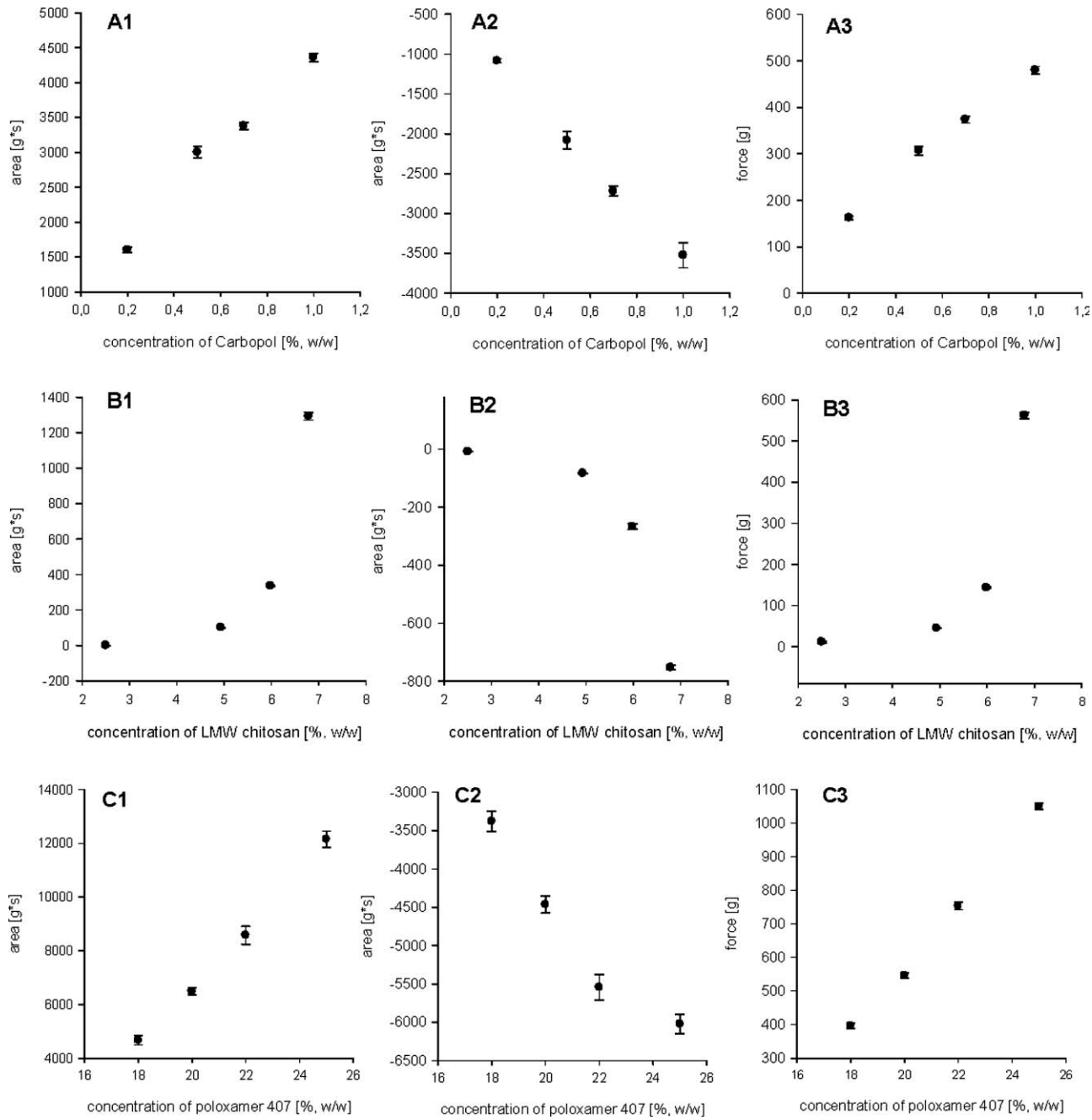

Figure 2 Influence of polymer concentration on the gel texture: A1, B1, and C1 indicate gel cohesiveness; A2, B2, and C2 indicate gel adhesiveness; A3, B3, and C3 indicate gel hardnesss ($n = 1$). Five replicate analyses were performed for each type of hydrogel.

parameters such as the effect of polymer concentration, stability of hydrogels, changes due to incompatibility, changes due to the incorporated vesicles, etc.

Influence of polymer concentration on gel cohesiveness, adhesiveness, and hardness

The amount of gelling agent in a formulation is of great importance with respect to its textural properties. It is expected that gel adhesiveness is correlated to gel bioadhesiveness, a parameter of great importance in wound therapy. The investigated hydrogels showed different correlation between the amount of gelling material and texture properties. Whereas cohesiveness, adhesiveness, and hardness of Carbopol and poloxamer hydrogels seem to increase in a linear fashion with increasing polymer concentration [Fig. 2(A,C)], chitosan hydrogel showed an exponential correlation between the texture properties and chitosan concentration [Fig. 2(B)]. However, it has to be mentioned that we examined only a rather narrow concentration range and that the changes in the

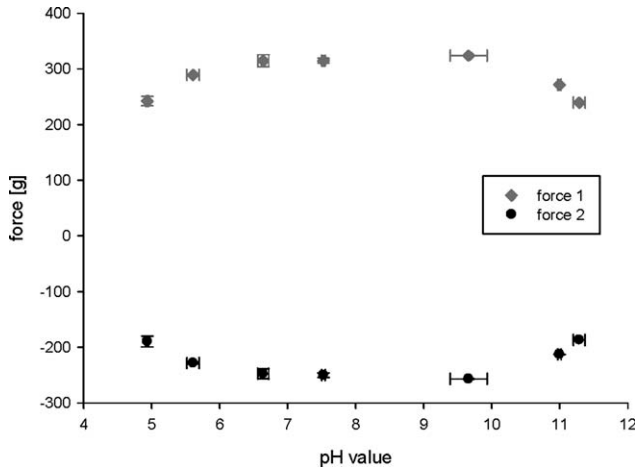

Figure 3 Effect of pH on the texture of Carbopol Ultrez hydrogels (0.5 %; w/w) ($n = 3$). Five replicate analyses were performed for three separate gel preparations.

polymer concentration outside the tested range might show different tendencies. It was previously reported that adhesiveness increases with an increase in polymer concentration when determined by texture profile analysis.[14,25] Tan et al.[26] reported the decrease in gel cohesiveness for Carbopol gels with increasing concentration of the polymers, and an increase in gel cohesiveness for polyvinyl–pyrrolidone gels. Sezer et al.[5] also reported an increase in gel adhesiveness with the increasing chitosan concentrations, but the cohesiveness was found to be negatively correlated. On the other hand, Karavana et al.[25] found an increase in gel cohesiveness with an increase in hydroxypropylmethylcellulose concentration and Cevher et al.[27] reported similar observations for Carbopol gels. The texture profile analysis (TPA) method used in their experiments varies from our newly developed method; therefore, the direct comparison of the findings is rather difficult. The main difference is the definition of gel cohesiveness. In TPA, the probe is compressed twice in the gel sample and cohesiveness is defined as the ratio of the area under the force-time curve produced on the second compression cycle to that produced on the first compression cycle with successive compressions being separated by a defined recovery period. In our case, a single compression into the probe was used and the cohesiveness was defined as the area under the curve for force 1 (Fig. 1), which we believe is the direct measure of gel cohesiveness.

Influence of pH on Carbopol gel texture

Carbopol gels are prepared by dispersing the polymer powder in water and neutralizing the obtained suspension by the addition of triethylamine. The amount of triethylamine is expected to affect the pH of the formed hydrogel. The texture analysis can determine the changes in gel properties in relation to the changes in pH of the gel formulation.

The Carbopol hydrogels showed rather stable texture properties in a pH range from 6 to 10, as can be seen in Figure 3. Outside that range, the measured forces (both force 1 and force 2) were found to be decreasing. At a low pH, the amount of triethylamine might not be sufficient for the deprotonation of the acidic environment and therefore for the successful gelling process. The decreased firmness at higher pH (over 10) is due to reduced electrostatic repulsion because of excess electrolytes. However, since the hydrogels are destined to be applied onto the skin with damaged barrier function, the pH close to neutral would be acceptable.

Texture properties of model formulations

Topical applications for wound healing are expected to contain several ingredients in addition to gelling polymer. In addition to the polymer and its dispersant, topical formulations can contain drugs and eventually their solvent, humectants such as glycerol, or drug carrier systems such as liposomes, providing sustained release of the drug. In order to evaluate the effect of various additives on gel properties, different model formulations were investigated by the texture analysis. Two types of hydrogels were evaluated, namely Carbopol and chitosan. Chloramphenicol was used as a model antibacterial drug.[28] Two different solvents (ethanol and propylene glycol) were used to dissolve chloramphenicol, which was then incorporated in a form of solution into the hydrogels. Figure 4 indicates the maximum compressing force (gel hardness) for selected formulations. Although we were able to determine the changes in original gel hardness, the changes were not significant. It appeared that the addition of drug in a form of solution, regardless of solvent used, did

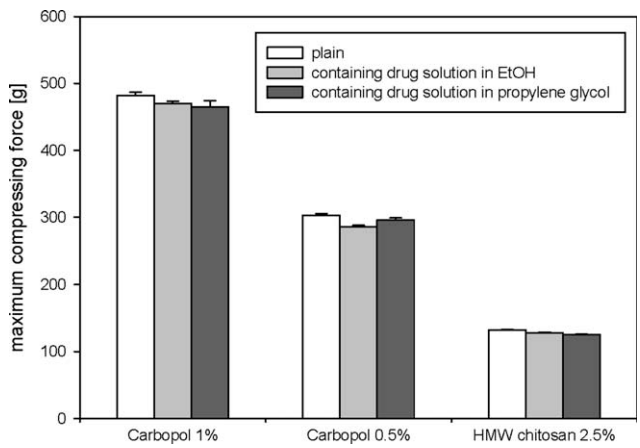

Figure 4 Changes in gel compressibility (force 1) in respect to gel composition ($n = 1$). Five replicate analyses were performed for each type of hydrogel.

TABLE III
The Effect of Glycerol on Texture Properties of HMW Chitosan Hydrogels (2.5%; w/w) (n = 1)

Chitosan hydrogel composition	Force 1 ± SD (g)	Area 1 ± SD (g*s)	Force 2 ± SD (g)	Area 2 ± SD (g*s)
Plain	205.8 ± 2.2	483.7 ± 6.4	−178.7 ± 2.1	−363.9 ± 4.0
Containing 10 % (w/w) glycerol	212.2 ± 0.9	460.7 ± 3.1	−165.4 ± 0.5	−349.3 ± 1.4
Containing 10 % (w/w) glycerol plus 10 % (w/w) of liposomal dispersion	250.7 ± 4.6	570.8 ± 1.9	−220.6 ± 1.9	−426.4 ± 1.5

HMW chitosan hydrogels of various compositions were analyzed by five replicate measurements.

not affect the compressibility of the hydrogels at the concentrations used in formulations.

Influence of incorporated glycerol on chitosan gel texture

Solvents like glycerine and propylene glycol are known to be able to modify the characteristics of hydrogen bonding between water, solvent, and polymer, affecting the swelling and properties of the polymer.[9] Chitosan hydrogels incorporating 10% glycerol (w/w) were examined by the texture analysis and the data are presented in Table III. Glycerol is expected to stabilize the chitosan gel structure, although a slight decrease in gel cohesiveness (Area 1) was observed. However, the adhesiveness of chitosan gel (Area 2) was found to be decreasing. Interestingly, the addition of liposomal dispersion to the chitosan/glycerol formulation results in more coherent hydrogels as both gel cohesiveness and adhesiveness increased (Table III). It appears that liposomes stabilize the chitosan network.

Influence of incorporated liposomes on gel properties

Although hydrogels exhibit good properties as wound dressings, solubility of a drug in a gel and control over the incorporated drug release remain to be the limiting factors for many of the drugs destined for wound therapy. Controlled delivery systems as dressings, such as liposomal hydrogels, can enable the delivery of drugs to the wound sites in a predictable and sustained manner. By using liposomes as a drug carrier system, the solubility of poorly water-soluble drugs and controlled release of an incorporated drug can be improved.[29,30] Up to now, no consensus on whether the addition of liposomes affects the rheological properties of Carbopol gels is achieved, as some reports suggest that liposomes affect the rheological properties,[18] whereas other suggests that the addition of cationic lipids significantly increased the viscosity of the gel.[24]

Table IV presents the effect of various amounts of liposomal dispersion incorporated in hydrogels on the texture properties of Carbopol and chitosan gels. Incorporated liposomes were multilamellar in structure with average size of around 1 μm (data not shown).

Carbopol hydrogel retained its original texture to a great extent. Even after the addition of liposome dispersion up to 15% (w/w), the resulting hardness (force 1) remained at 93% of the value for intact gel (Table IV). The polymeric chains of Carbopol in hydrogels can take up a rather large amount of additional liquid without significant change in their texture. This property is an advantage not only with regard to development of liposomal hydrogels but also with regard to possible intake of wound exudate.

However, chitosan hydrogels were found to be loosing their original texture properties, namely both maximum compressing force (force 1) and minimum retracting force (force 2) were reduced (Table IV), directly affecting the hydrogel hardness, so that the hydrogels with incorporated 15% (w/w) of liposomal dispersion retained only 40% of their original properties, as compared to the intact gels. This is an additional reason for using glycerol as stabilizing agent in chitosan-based hydrogels.

TABLE IV
The Effect of the Addition of Liposomal Dispersions on Gel Properties

Type of hydrogel and corresponding concentration (%; w/w)	Liposomal dispersion (%; w/w)	Force 1 ± SD (g)	Force 2 ± SD (g)
Carbopol, 0.5	0	306.4 ± 9.7	−232.0 ± 5.8
	5	293.1 ± 8.8	−229.3 ± 10.6
	10	294.4 ± 11.6	−230.8 ± 10.9
	15	286.0 ± 5.1	−221.4 ± 4.6
LMW chitosan, 6	0	170.3 ± 0.8	−123.1 ± 0.3
	5	123.0 ± 0.6	−89.1 ± 0.4
	10	97.0 ± 0.6	−75.2 ± 0.4
	15	74.7 ± 0.6	−61.8 ±0.2
MMW chitosan, 3.5	0	253.1 ± 1.1	−201.8 ± 0.6
	5	216.9 ± 0.6	−157.8 ± 0.7
	10	167.0 ± 0.7	−127.0 ± 0.5
	15	97.2 ± 0.2	−76.6 ± 0.2
HMW chitosan, 2.5	0	188.2 ± 1.0	−135.6 ± 0.6
	5	113.3 ± 1.8	−81.7 ± 0.5
	15	76.4 ± 0.7	−62.5 ± 0.1

Five replicate analyses were performed for each type of hydrogel.

TABLE V
The Effect of Temperature on Gel Properties (Stability Testing)

Hydrogel composition	Force 1 ± SD (g)		Force 2 ± SD (g)	
	Before	After	Before	After
HMW chitosan, 2.5 % (w/w) plain	205.8 ± 2.2	16.5 ± 0.3	−178.7 ± 2.1	−15.7 ± 0.2
HMW chitosan, 2.5 % (w/w) containing 10 % (w/w) glycerol	212.2 ± 0.9	120.6 ± 0.9	−165.3 ± 0.5	−108.9 ± 0.6
HMW chitosan, 2.5 % (w/w) containing 10 % (w/w) glycerol plus 10 % (w/w) liposomal dispersion	250.7 ± 4.6	199.4 ± 7.4	−220.5 ± 1.9	−136.4 ± 1.5
Carbopol, 0.5% (w/w) plain	306.4 ± 9.7	266.2 ± 6.5	−232.0 ± 5.8	−149.6 ± 3.0

Five replicate analyses were performed for each type of hydrogel before and after the storage for 4 weeks at 40 °C.

Stability

A hydrogel formulation destined for wound therapy should not only be easy to apply onto the skin but should also remain stable over a longer period of time. To evaluate the stability of the different hydrogel formulations, an accelerated stability test was performed and the texture properties determined prior and after the testing.

In this regard, maintaining the original gel hardness is an important parameter in determining gel stability. In Table V, force 1 and force 2 values before and after 4 weeks of storage of gels at 40°C are presented. Both forces show the same tendency of change. Carbopol hydrogel showed only a small loss in the original gel structure after the stability testing (85% of the original hardness was maintained). In addition, no change in visual appearance was visible. In contrast to that, plain HMW chitosan gel lost almost all of its original hardness, as it retained only 8% of its original hardness. In addition, a clear change in the color from almost colorless before the stability testing to light yellow after the testing was noticeable. As expected, liposomal chitosan gel, containing 10% glycerol, was found to be even more stable (Table V). It appears that embedding of liposomes within the chitosan hydrogel network increased its stability at increased temperatures (40°C).

Texture analysis as applied in this stability testing provided valuable information on changes in hydrogel properties due to increased temperature. The method can be easily applied in stability testing as quality control parameter.

CONCLUSIONS

In conclusion, the texture analysis as presented herewith can be used to characterize and optimize hydrogels destined for topical application to the wounded area. Rather simple and reproducible method provides direct information on gel cohesiveness, adhesiveness, and hardness, the properties of hydrogels directly affecting the outcome of wound therapy. Those properties of hydrogels will also affect the release of the incorporated drug from the delivery system; therefore, the next step in our research is to optimize the formulation in respect to drug release and formulation's bioadhesiveness. Moreover, to evaluate the efficacy of the optimized formulation, *in vivo* animal experiments remain to be the real prove of the concept.

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