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Functionality testing of gellan gum, a polymeric excipient material for ophthalmic dosage forms

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Abstract

Low acetyl gellan gum, originally a food ingredient, has been used to devise novel ophthalmic formulations, significantly improving drug ocular bioavailability. This increase in drug bioavailability results from the unique gelling property of gellan gum in the presence of tear fluid cations. The aim of this study was to develop a functionality test ensuring the consistency of the dosage form's gelling property and a reproducible pharmacological effect. The rupture strength of the gel was shown to be a reliable indicator of the ocular drug bioavailability in the albino rabbit. The test parameters susceptible to influence the test results were identified, evaluated and optimized. The influence of the raw material characteristics and of the processing parameters on the final product gel strength were determined and optimized, and finished product specifications established. © 1997 Elsevier Science B.V.

Keywords: Functionality test; Gel strength; Ophthalmics; Gellan gum; Gelrite; Ocular bioavailability

1. Introduction

Excipients have long been considered as inert components of a drug formulation. This can no longer be considered true in view of the development of new dosage forms with excipients added to the formulation that drastically modify the drug release. The pharmaceutical community is becoming acutely aware of the necessity to characterize and control these excipient materials: a key issue of the Second Joint Pharmacopoeia Open Conference on Harmonization of Excipients Standards held in Florida in 1994, was the functionality testing of such excipients.

Timoptic XE®[1] (Timoptol LP® or Timoptol LA® in Europe) is a novel ophthalmic dosage form which enhances the ocular bioavailability of the antiglaucoma drug timolol in the albino rabbit

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[1] Timoptic XE®, Timoptol LP®, Timoptol LA®, Timoptic® and Timoptol® are trademarks of Merck and Co. Inc., (Whitehouse Station, N.J., USA).

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by three- to four-fold, when compared to a plain timolol solution (Rozier et al., 1989a). In man, studies have shown that Timoptic XE® given once a day was equivalent to regular Timoptic® (Timoptol® in Europe) administered twice a day (Shedden, 1994). This amelioration was obtained by the addition to the formula of a polymer excipient, Gelrite®[2]. Gelrite® (low acetyl gellan gum), originally developed as a food ingredient, has a unique property, forming viscous solutions in deionized water which gel in the presence of cations. The ophthalmic formulations containing gellan gum are instilled as liquids and gel upon contact with the tear fluid cations (Mazuel and Friteyre, 1985). Once gelled, the formulations resist the natural elimination process of drainage from the precorneal area; their residence time at the site of drug absorption is prolonged (Greaves et al., 1990), and subsequently, the amount of drug absorbed is increased. The importance of the gelation mechanism was demonstrated by the comparison of the intraocular drug levels obtained with Timoptic XE® and a non-gelling polymeric solution of comparable viscosity (Rozier et al., 1989b). The former are significantly higher, indicating that gelation is indeed the key parameter. It thus appeared early during the drug development program that a functionality test characterizing this parameter was mandatory to obtain a dosage form with a reproducible pharmacological effect.

The test had to be rapid, simple and ultimately usable as a routine test, while not necessitating overly sophisticated equipment. It had to allow the testing of both the raw material and the finished dosage form. In addition, as it was intended to be used as a product stability indicator, and because the capacity of an ophthalmic vial is relatively small (2.5 ml per vial in the case of Timoptic XE®), the amount of product necessary to run the test had to be as small as possible. Finally, the test had to be accurate, reproducible and validated.

Available methods to evaluate gel characteristics include fundamental tests such as low shear oscillatory rheology. While this kind of technique was used during product development, it requires sophisticated equipment that is not adapted to routine analysis in a control laboratory. Empirical tests include Texture Profile Analysis (TPA) and gel strength measurement. TPA is widely used in the food industry (Szczesniak et al., 1963) and consists of the analysis of the force/deformation curve generated by compressing a gel sample using an Instron Universal Testing Machine. Gel strength measurements can be obtained using a variety of instruments which do not necessarily measure the same textural parameter. A gel strength test is described in the USP (US Pharmacopeia XXIII, 1995) for gelatin (commonly called the Bloom test), which measures gel firmness at small strain levels, but which does not measure rupture strength. The USP test requires too large a sample size (> 100 g) for routine examination of ophthalmic samples. It also measures the combined reaction forces of the gel itself and of the 'skin' formed at the air-gel interface. As such a 'skin' would not form in the eye, the test was not thought to be representative. Another inconvenience is that the sample is not unmolded before testing and interferences from the container walls can be expected. TPA provides a comprehensive understanding of gel texture, but requires expensive equipment and is particularly indicated when comparison of two different gelling agents is required (Sanderson et al., 1988). Since the ultimate goal was to compare identical formulations of the same gelling agent, attention was therefore directed towards a simple gel strength test adapted to small size samples and allowing the determination of the gel rupture strength. The test sample preparation was designed to ensure sample conservation and to avoid the interferences mentioned above. The strain was applied to the total sample surface area and the force required to break the gel was determined.

2. Materials and methods

2.1. Apparatus

A Stevens[3] LFRA 1000 texture analyzer equipped with a 38 mm cylindrical plunger and a

[2] Gelrite® is a trademark of Kelco, unit of Monsanto Co., St. Louis, Missouri, USA.

[3] Stevens and Son Ltd., 2–8 Dolphin Yard, Holywell Hill, St. Albans, Hertfordshire AL1 1EX, England.

Table 1
Gelling reagent formulation

Cation	Added as	Concentration (mg/ml)
Sodium	Sodium chloride	24
Calcium	Calcium chloride, dihydrate	0.225

one-way recorder was chosen to perform the measurements. The gel strength is evaluated by compressing the sample up to rupture point with a mobile plunger moving downward at a defined rate (0.2 mm/s), for a preset distance (5.0 mm). A sensor is connected to the plunger and records the sample reaction forces to compression and in particular, the force necessary to break the gel or gel strength. Gel strength is reported in grams. It is proportional to the test sample surface area and for convenience can be reported as a mass/surface ratio in g/cm^2.The sensor is calibrated using standard masses suspended from the plunger. The weights of two masses bracketing the working range (100 and 1000 g) are checked against specifications ($\pm$ 1%).

The gel strength measurement calibration is carried out using a 'dummy bloom strip' available from the manufacturer. It consists of a spring steel strip of adjustable length. It is provided with a certificate of calibration which lists the reaction forces obtained in a standard deflection test, for a series of different strip positions. The same standard test conditions are used to measure the reaction forces with the texturometer to be calibrated. These should agree within 1% with the values of the certificate of calibration.

2.2. Test sample preparation

The test sample is molded from a casting solution prepared by boiling a known amount of the gellan formulation or raw material with the gelling reagent. The gelling reagent is a solution in deionized water of the tear fluid gelling cations in physiological proportion (see Table 1). After boiling, the casting solution weight is adjusted so as to obtain in the test sample a final gellan concentration of 0.4% and 25% of gelling reagent. The samples are cast in molds consisting of a Plexiglass ring sitting on a Plexiglass plate. 2.1 ml of the casting solution is needed for the 14.6 mm internal diameter mold. Another plate is positioned on the ring to avoid the entrapment of air bubbles between the sample and the plate. The gel is allowed to set without disturbance for a given time and unmolded just before measurement.

Table 2
Influence of boiling time

Gel strength (g/cm^2 $\pm$ R.S.D.; number of single determinations)					
14.6 mm molds			19.3 mm molds		
1 min	3 min	5 min	1 min	3 min	5 min
ND	68 $\pm$ 3 (6)	62 $\pm$ 2 (7)	ND	67 $\pm$ 4 (8)	68 $\pm$ 3 (8)
ND	67 $\pm$ 3 (7)	66 $\pm$ 3 (7)	ND	ND	ND
65 $\pm$ 4 (8)	65 $\pm$ 4 (7)	ND	65 $\pm$ 3 (5)	65 $\pm$ 2 (7)	ND
ND	63 $\pm$ 4 (8)	64 $\pm$ 2 (7)	ND	64 $\pm$ 3 (8)	64 $\pm$ 2 (8)
Avg. = 65	Avg. = 66	Avg. = 64	Avg. = 65	Avg. = 65	Avg. = 66

R.S.D., relative standard deviation.
ND, not determined.

Table 3
Influence of setting time

Sample	Gel strength ($g/cm^2 \pm$ R.S.D.; number of single determinations)				
	14.6 mm molds			19.3 mm molds	
	2 h	4 h	6 h	2 h	6 h
Gellan solution (autoclaved)	65 ± 1 (6)	ND	66 ± 1 (6)	68 ± 2 (6)	65 ± 3 (8)
Gellan solution (non-autoclaved)	169 ± 5 (3)	160 (2)	159 ± 2 (3)	ND	ND
Raw material					
Lot 1	182 ± 15 (3)	179 (2)	184 (2)	ND	ND
Lot 2	95 ± 8 (3)	96 (2)	102 (2)	ND	ND
Lot 3	183 ± 14 (3)	186 ± 23 (3)	174 ± 17 (3)	ND	ND

R.S.D., relative standard deviation.
ND, not determined.

2.3. Method validation

The influence of boiling time (1 to 5 min), setting time (2 to 6 h), casting temperature range (84–88 or 62–69°C) and weighing variations were evaluated and the operator-to-operator reproducibility checked.

2.4. Ocular bioavailability evaluation

Ocular bioavailability was evaluated in albino rabbits. Bilateral instillations, 50 μl of 0.25% Timoptic XE® solutions with different gel strengths, were made into the conjunctival sac of the albino rabbits. Cornea, aqueous humor and iris + ciliary body were subsequently sampled at 10 minutes, 0.5, 1, 2 and 4 h post-treatment for quantitation of their timolol content by HPLC and UV detection at 294 nm.

Table 4
Influence of casting temperature (determination with 14.6 mm molds)

Gel strength ($g/cm^2 \pm$ R.S.D.; number of single determinations)	
Casting temperature: 84–88°C	Casting temperature: 62–69°C
66 ± 1 (5)	63 ± 2 (7)
68 ± 4 (6)	60 ± 1 (8)

2.5. Gellan gum molecular weight evaluation

The differences in the raw material molecular weights were evaluated using intrinsic viscosity data extrapolated from the viscosities of dilute aqueous gellan gum solutions at various concentrations and measured using a Contraves Low Shear 30 viscometer. To ensure complete polymer dissolution and to perform the measurements above the conformational transition in the random coil conformation (Robinson et al., 1991), measurements were carried out on 45°C solutions containing 25 mM Na^+.

3. Results and discussion

Each of the test parameters susceptible to influence the final sample gel strength was evaluated and optimized, when deemed necessary, to insure method reproducibility. The correlation between gel strength results and ocular bioavailability was established and limits set. Finally, the influence of various factors such as raw material batch and processing parameters on the final product gel strength, was evaluated.

3.1. Method validation

3.1.1. Influence of boiling time

Because of potential polymer degradation during boiling of the casting solution, the influence of

Table 5
Influence of gellan concentration, reagent concentration and precision of final q.s. on gel strength

Gel strength ($g/cm^2 \pm$ R.S.D.; number of single determinations)							
Gelling reagent added:	−5% 57 ± 4 (12)	−2% 61 ± 3 (21)	−1% 65 ± 3 (15)	Nominal 62 ± 3 (32)	+1% 64 ± 3 (13)	+2% 65 ± 2 (20)	+5% 65 ± 3 (9)
Water added for q.s.:	−5% 71 ± 2 (11)	−2.5% 71 ± 3 (11)	−1.25% 63 ± 2 (15)	Nominal 62 ± 3 (27)	+1.25% 61 ± 2 (13)	+2.5% 62 ± 3 (12)	+5% 54 ± 2 (12)
Gellan formulation added:		−10% 62 ± 4 (6)	−5% 67 ± 6 (6)	Nominal 72 ± 5 (9)	+5% 73 ± 4 (3)	+10% 86 ± 5 (6)	

R.S.D., relative standard deviation.

boiling time on gel strength was studied. Gel samples prepared with 14.6 and 19.3 mm diameter molds were allowed to stand for 4 h before gel strength determination. Results shown in Table 2 indicate no difference between 1 and 5 min of boiling time. For practical reasons, a time window of 3 to 5 min was chosen. The data show no difference in gel strength between 14.6 and 19.3 mm molds.

3.1.2. Influence of setting time

After the samples have been molded, some time is required for the gel to set. In order to determine the influence of setting time on gel strength results, various gellan samples (raw materials, non-autoclaved and autoclaved solutions) were tested. Results given in Table 3 indicate that a setting time between 2 and 6 h does not affect gel strength results.

3.1.3. Influence of casting temperature

The gel strength of the sample will be influenced if the casting temperature approaches the gel setting temperature (around 40°C). The casting temperature may therefore be critical. It is a function of the time that elapses between the end of boiling and the actual casting of the solution, which includes the final q.s. adjustment by weight. Its influence was evaluated by allowing the casting solution to cool down to different temperatures before molding the test sample. Results shown in Table 4 reveal a small effect on gel strength values. However, instead of setting molding temperature limits which proved to be impractical, the time elapsed between the end of boiling and the casting of the solution was set to 5 min, which resulted in a solution temperature within the range 84–88°C.

3.1.4. Influence of weighing variations

Sample preparation involves several weighing operations: test formulation, reagent and water for q.s. adjustment, which are not all done under analytical conditions (the q.s., for example, needs to be done with the hot solution). Therefore, the effect of variation in the amounts of gelling reagent, gellan formulation and water was evaluated in several exploratory experiments. Results in

Table 6
Reproducibility study

Sample	Average gel strength ($g/cm^2 \pm$ R.S.D., $N = 12$)	95% Confidence interval (g/cm^2)
Raw material lot 1	103 ± 3.2	101–105
Raw material lot 2	189 ± 5.0	183–195
0.5% Timoptic XE lot 1	157 ± 5.1	152–162
0.5% Timoptic XE lot 2	51 ± 4.6	50–52
0.25% Timoptic XE lot 1	92 ± 7.7	88–97
0.25% Timoptic XE lot 2	63 ± 7.3	60–66

Averages are obtained from the results obtained by three analysts on two different days, each day in duplicate.
R.S.D., relative standard deviation.

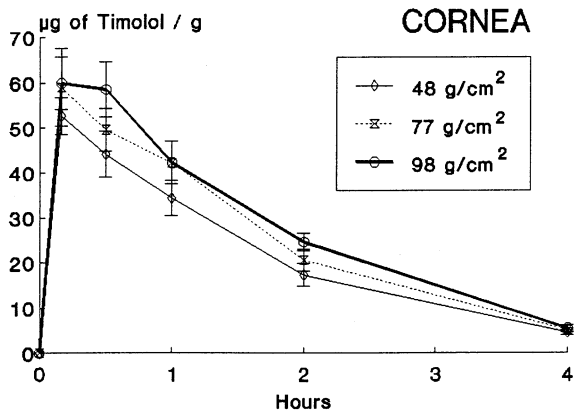

Fig. 1. Corneal concentration/time profile for different gel strengths.

Table 5 show that all these parameters impact on the final gel strength and especially those influencing the gum concentration (formulation weight and final q.s.). For this reason, it was decided to set a limit of ± 0.05 g for all these variables, which corresponds to $\pm 1.7\%$ for the gelling reagent, $\pm 0.4\%$ for the water added for q.s. and $\pm 0.6\%$ for the gellan formulation.

3.1.5. Reproducibility

In order to evaluate the reproducibility of the gel strength test, measurements were made on raw materials and formulations with gel strength values bracketing the range normally encountered. Samples were assayed by three analysts on two different days and each day in duplicate (i.e. 12 measurements per sample) (see Table 6).

3.2. In vivo performances/gel strength correlation

In the albino rabbit, the pharmacokinetic parameters C_{max}, T_{max} and $T_{0.5}$ of timolol in the eye were similar for the three different gel strength formulations (See, for example, the concentration/time profiles for cornea in Fig. 1.) In addition, increasing gel strength produced a gradual concomitant increase in ocular bioavailability, as assessed by the measurement of the area under the concentration versus time curve, $AUC_{(0-4\,h)}$, at the three ocular sites (Fig. 2). An analysis of variance (Student–Newman–Keuls) showed only borderline statistical differences among treatments ($P = 0.05$) (see Table 7).

Gel samples are obtained under conditions (heating the ophthalmic solution with the gelling cations to boiling point, then cooling the mixture to room temperature) that are different from the physiological ones. Gelation occurs in the eye (at 33°C; Adler's Physiology of the Eye, 1981) by diffusion of the gelling cations, mostly sodium and calcium, into the instilled formulation. Nevertheless, these data confirm that ocular bioavailability is influenced by gel strength as measured by the method described above. When gel strength is within set limits, a consistent pharmacological effect can be expected. This has been confirmed in man by clinical observations made with Timoptic XE® using gel strengths within this range.

3.3. Raw material characterization

Gel strength results obtained from different gellan raw material batches show a significant batch-to-batch variation. Since gellan gum is produced by a fermentation process, it contains residual cations in variable amounts. Its intrinsic viscosity, which is a function of the polymer molecular weight was also found to vary. The amounts of monovalent and divalent cations in the test gel sample were a hundred-fold and a ten-fold, respectively, that of the raw material content, therefore no correlation was found between gel strength and raw material residual cations (data not reported). On the other hand, gel strength was found to be proportional to gellan gum intrinsic viscosity (Fig. 3). Although intrinsic viscosity is commonly used to assess relative polymer molecular weight, the gel strength method was found more practical for routine testing. Specifications were set for the raw material gel strength, as this was found to influence the final product gel strength.

3.4. Influence of the manufacturing process on finished product gel strength

Since the route of administration for Timoptic XE® is ocular, a major concern was to assure

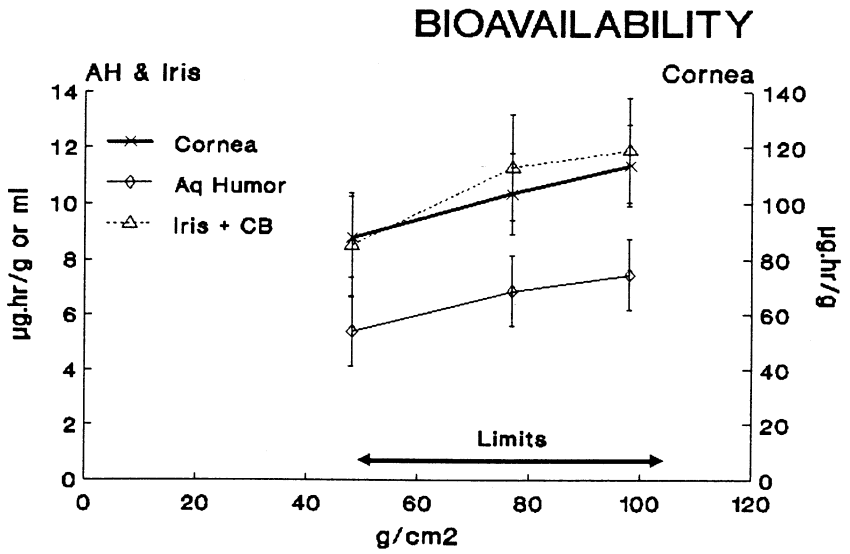

Fig. 2. $AUC_{(0\text{-}4h)}$ by tissue and gel strength.

Table 7
Influence of gel strength on ocular bioavailability as assessed by $AUC_{(0-4\,h)}$ measurements at three ocular sites

Gel strength (g/cm^2)	Cornea ($\mu g \cdot h/g$)	Aqueous humor ($\mu g \cdot h/ml$)	Iris+ciliary body ($\mu g \cdot h/g$)
48	87.9	5.39	8.52[b]
77	103.7	6.86	11.30
98	113.5[a]	7.45	11.90
Standard error	7.1	0.62	0.91

[a] Statistically ($P<0.05$) greater than 48 g/cm^2 gel strength.
[b] Statistically ($P<0.05$) lower than 77 or 98 g/cm^2 gel strength.

sterility of the finished product. Steam sterilization was proven the only viable option. Autoclaving gellan solutions leads to a significant reduction in the finished product gel strength (Fig. 3). This reduction was found to be proportional to the autoclaving time. During manufacturing process development and scale-up, the gel loss during sterilization was used as an indicator to determine optimal sterilization conditions.

4. Conclusion

The development of a functionality test, based on gelation properties, has been described above for gellan gum (Gelrite®). This test satisfies the requirements of simplicity and rapidity for small sample volumes encountered in ophthalmics. The influence of experimental parameters has been investigated and conditions defined to obtain a reproducible and robust test for both the raw material and the finished product. The functionality test has proven to be an invaluable tool at each stage of the development of the dosage form and has enabled the establishment of finished product specifications ensuring a consistent pharmacological effect. In addition, the control of the raw material based on gel strength measurements was shown to be possible. Finally, the test allowed the establishment of a manufacturing process that retained the funda-

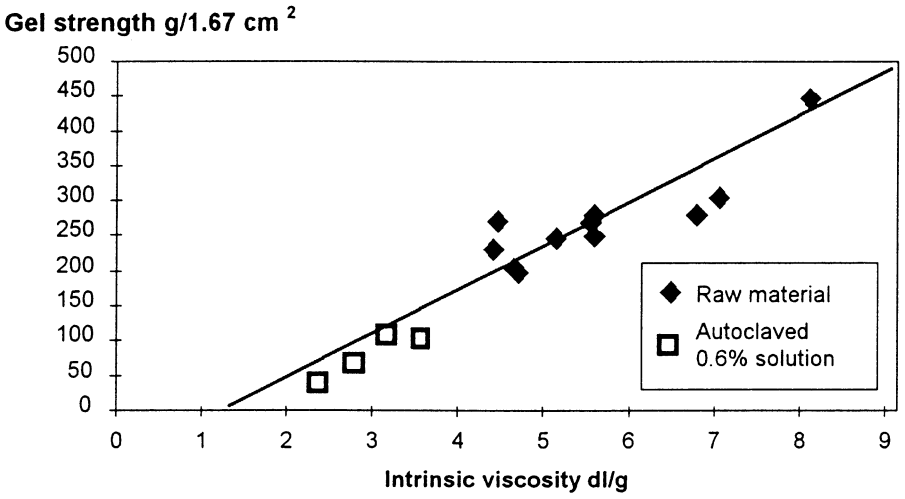

Fig. 3. Intrinsic viscosity vs. gel strength.

mental gelling properties of the excipient for exploitation in ophthalmic drug delivery.

The present test for a polymeric excipient is perhaps too specific to find a place in Pharmacopoeias. Nevertheless, this work highlights how much this specificity should be the basic requirement for a functionality test and how fundamental a well-focused test can be during development. Taking the reasoning one step further, it can also be reflected that if a functionality test is general and is included in a monograph, it may not be relevant to all applications. This highlights the difficulties encountered by the Pharmaceutical Industry to agree on a definition of functionality tests. It is especially true for polymer excipients that are incorporated into numerous different formulations, where they do not necessarily fulfill the same function.

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