

Sensitivity of Gelatin Raw Materials to Cross-Linking

The Influence of Bloom Strength, Type, and Source

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The authors discuss the results of a study that was conducted to determine how sensitivity of cross-linked

gelatin materials varied with respect to their bloom strength, type, and source. Twelve gelatin samples were studied: four of type A and eight of type B, all with bloom strengths varying between 100–275. Gelatin rings prepared from these materials were tested separately by the formaldehyde test and the photostability chamber test. Although cross-linking behavior was found to relate to bloom strength, no correlation was found regarding gelatin type or source.

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Gelatin is used extensively in the pharmaceutical industry despite its tendency to become cross-linked as a result of interaction with chemicals such as aldehydes and exposure to humidity, temperature, and light (1,2). Cross-linking can result in an altered dissolution profile for formulations that use gelatin in the outer layer (3). Consequently, marketed preparations are at risk of failing dissolution tests. Although cross-linking has been found to have little relevance to in vivo bioavailability because of digestion by GIT enzymes, a Fail/Fail dissolution in both the absence and presence of gastric enzymes indicates that the drug would not be released at all from the formulation. Therefore, cross-linking within gelatin formulations must be controlled.

One way to resolve the problem is to use a gelatin raw material that is most resistant to cross-linking. In a recent publication, the authors proposed a combination of formaldehyde and photostability chamber tests to evaluate the cross-linking resistance capability of various gelatin raw materials (4). In the study presented in this article, the proposed tests were used to evaluate how the cross-linking potential of various gelatin raw materials differed with respect to the gelatin's bloom strength, type, and source.

Experimental

Materials. Gelatins were supplied by Kind & Knox (Sioux City, Iowa, USA) and Rama Industries (Dappar, Punjab, India). Kind & Knox supplied two types of gelatins—Type A NF pork skin gelatin and Type B NF bone gelatin—each in bloom strengths of 100, 150, 200, and 275. Rama Industries supplied Type B gelatin in bloom strengths of 100, 150, 200, and 250. Table I lists the exact bloom strength, viscosity, pH, and percent moisture values of each sample used.

Glycerol and pepsin (1:3000 LR) were purchased from LOBA Chemie Pvt. Ltd. (Mumbai, India). Methyl paraben, propyl paraben, and formaldehyde solution (37–41% w/v LR) were purchased from s. d. fine-chem Ltd. (Boisar, India).

Equipment. A rotary film evaporator (B-480, Buchi Labortechnik AG, Flawil, Switzerland) and a water bath equipped with a precision controller (MV, Julabo Labortechnik GmbH, Seelbach, Germany) were used for the preparation and maturation of the gelatin mass, respectively. The films were prepared using a laboratory coating device (SV-M-101301, Mathis, Oberhasli/Zurich, Switzerland). Film thickness was determined using a digital screw gauge (Mitutoyo Corporation, Kanagawa, Japan). The rings were exposed in a photostability chamber equipped with an interior light bank (KBF 240, WTF Binder, Tuttlingen, Germany). Dissolution studies were conducted using a magnetic heating and stirring device (RCT basic, IKA Labortechnik, Staufen, Germany) that included a digital controller and platinum probe (Ikatronik, IKA Labortechnik).

Preparation of gelatin films and rings. The films were prepared using the procedures described in a previously published article (4), except the film casting was performed mechanically by a laboratory coating device rather than by manual spreading. The spread film was refrigerated for 12 h to harden it. Thereafter, it was removed with a spatula and stored in ambient conditions. Two batches of films were prepared for each sample of each type of gelatin.

Rings measuring 11 mm i.d. × 14 mm o.d. and weighing 35 ± 2 mg were cut out of the prepared films and subjected to further studies.

Exposure of rings to formaldehyde and photostability tests. The rings were exposed separately to both formaldehyde vapors and to the combined temperature–humidity–light test in a photostability chamber (3,4). The experimental setup for formaldehyde exposure was the same as the setup used in a previously published study (4). The rings were exposed to 78.0 and 97.5 μg formaldehyde in squat-type weighing bottles and dried in ambient conditions for 12 h. The exposure conditions in the photostability chamber were 40 °C, 75% RH, and light, according to the ICH recommendation for UV and visible illumination, option 2 (5). The total visible illumination exposure was 1.34 million lx-h, and the UV energy exposure was 232 Wh/m², both above the minimum ICH requirements of 1.2 million lx-h and 200 Wh/m² (5). Before dissolution, the rings were withdrawn from the chamber and dried for 12 h in ambient conditions.

Dissolution studies. The dissolution studies on the rings were performed both in the presence and the absence of enzymes, as previously reported (4). The complete disappearance of the ring as a result of dissolution was considered the end point. Cases in which dissolution completion exceeded 60 min were given recorded times of 60 min.

Results and discussion

Dissolution results and general observations. Table II lists the dissolution results for rings made from different gelatin materials and exposed to various test conditions. Data are presented for two batches of each material, both in the presence and the

Table I: Gelatin materials used in this study.

Material Type/Code	Source	Bloom (g)	Viscosity (6.67%, Mps)	pH	Moisture (%)
A 100 KK	Kind & Knox	118	20.10	5.13	11.30
A 150 KK	Kind & Knox	157	25.80	5.39	11.10
A 200 KK	Kind & Knox	212	33.40	5.24	11.00
A 275 KK	Kind & Knox	282	47.00	5.44	10.90
B 100 KK	Kind & Knox	118	26.50	5.49	10.80
B 150 KK	Kind & Knox	157	36.70	5.54	11.10
B 200 KK	Kind & Knox	209	48.40	5.59	11.60
B 275 KK	Kind & Knox	285	46.40	5.70	11.60
B 100 R	Rama	101	30.96	5.41	9.58
B 150 R	Rama	161	28.77	5.59	8.79
B 200 R	Rama	196	43.00	5.59	11.15
B 250 R	Rama	245	49.43	5.48	10.56

absence of enzymes. A “Pass” case is one in which the rings dissolved within 60 min, and a “Fail” case is one in which the dissolution time exceeded 60 min.

The data in Table II indicate that when dissolution studies are carried out without enzymes, a Fail result is observed in all three types of exposures (i.e., photostability chamber and two concentrations of formaldehyde). However, the presence of enzymes indicates a high rate of Pass results. Furthermore, the dissolution times are about the same for formaldehyde (78 μg) and photostability chamber exposures, which is similar to the results found in a previous study (4). As expected, dissolution times overall are higher in the case of exposure to 97.5 mg of formaldehyde.

Influence of bloom strength. The data in Table II show that gelatins of bloom strengths 100 (A 100 KK, B 100 KK, and B 100 R) and 150 (A 150 KK, B 150 KK, and B 150 R) show Fail/Pass behavior in all three types of exposures. Rings made of gelatins with bloom strength 200 (A 200 KK, B 200 KK, and B 200 R) show Fail/Pass behavior after exposure to 78 μg of formaldehyde and in a stability chamber; a Fail/Fail result is observed after exposure to 97.5 mg of formaldehyde. Gelatins with bloom strengths 250 (B 250 R) or 275 (A 275 KK and B 275 KK) show Fail/Fail behavior after all three exposures.

The two batches of rings made of gelatin A 100 KK dissolved in the presence of enzymes within 30 ± 2 min after exposure to 78 mg of formaldehyde and in a stability chamber. The corresponding values for materials A 150 KK, A 200 KK, and A 275 K are 35 ± 2 min, 49 ± 1 min, and ≥ 60 min, respectively. A similar behavior of increasing the dissolution period with bloom strength is observed even for the gelatin groups B 100–275 KK and B 100–250 R. The increase in dissolution time with an increase in bloom strength also is evident from the data obtained after exposure to 97.5 mg formaldehyde. Cross-linking clearly increases with an increase in the bloom strength of gelatin raw materials.

Influence of the type of gelatin. The influence of each gelatin type in this cross-linking study can be seen by comparing the dissolution times resulting from the three exposure conditions of the material groups A 100–275 KK and B 100–275 KK (both were received from the same manufacturer). The dissolution

times of both groups are comparable, suggesting the absence of the influence of gelatin types A and B on the degree of cross-linking. This finding contradicts previous information in literature that type B gelatin is more resistant to cross-linking than type A (6).

Comparison between gelatin materials from two suppliers. The comparison of data for gelatin groups B 100–275 KK and B 100–250 R provides clear evidence that the dissolution times are not significantly different between the two groups after exposure to all three types of test conditions. Thus, one can conclude that no difference exists in the cross-linking behavior of materials obtained from two separate suppliers.

Conclusions

Under the three conditions specified in this article, the dissolution time of gelatin rings is influenced only by bloom strength, and no influence whatsoever is exerted by type A and B raw gelatin materials or their source. The dissolution time increases with a rise in bloom strength, and materials with bloom strength ≥ 200 have a pronounced potential to cross-link. Hence, one must critically evaluate the formulations whenever gelatin raw materials with bloom strengths >200 are used in the outer layer. In such cases, the possibility exists that a Fail/Fail situation might develop during storage as a result of exposure either to chemical catalysts (such as aldehydes) or adverse environmental conditions.

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Table II: Comparison of mean dissolution time of various gelatin raw materials.

Material Type/Code	Batch Number	Photostability Chamber		Formaldehyde (78.0 mg)		Formaldehyde (97.5 mg)	
		Without Enzyme	With Enzyme	Without Enzyme	With Enzyme	Without Enzyme	With Enzyme
A100 KK	1	F*	P** (31.00)†	F	P (32.00)	F	P (43.66)
	2	F	P (29.66)	F	P (30.00)	F	P (44.00)
A 150 KK	1	F	P (35.00)	F	P (36.66)	F	P (45.33)
	2	F	P (34.33)	F	P (35.66)	F	P (46.33)
A 200 KK	1	F	P (48.66)	F	P (49.00)	F	F
	2	F	P (48.66)	F	P (50.33)	F	F
A 275 KK	1	F	F	F	F	F	F
	2	F	F	F	F	F	F
B 100 KK	1	F	P (28.00)	F	P (27.33)	F	P (41.66)
	2	F	P (29.66)	F	P (30.00)	F	P (43.00)
B 150 KK	1	F	P (37.00)	F	P (36.00)	F	P (45.33)
	2	F	P (37.66)	F	P (34.66)	F	P (46.00)
B 200 KK	1	F	P (44.66)	F	P (45.00)	F	F
	2	F	P (44.33)	F	P (44.00)	F	F
B 275 KK	1	F	F	F	F	F	F
	2	F	F	F	F	F	F
B 100 R	1	F	P (26.66)	F	P (26.00)	F	P (39.66)
	2	F	P (25.66)	F	P (24.00)	F	P (38.66)
B 150 R	1	F	P (39.66)	F	P (35.66)	F	P (44.66)
	2	F	P (39.66)	F	P (34.66)	F	P (44.66)
B 200 R	1	F	P (40.00)	F	P (41.66)	F	F
	2	F	P (40.00)	F	P (42.00)	F	F
B 250 R	1	F	F	F	F	F	F
	2	F	F	F	F	F	F

* Fail
 ** Pass
 † Mean of results of three films

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