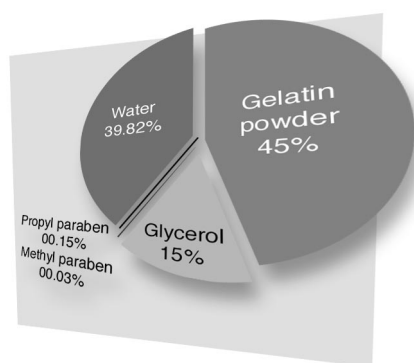


Evaluation of Gelatins for Cross-Linking Potential

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This article describes an approach to evaluate various gelatin raw materials for their resistance to cross-linking. The methodology, which can be used as a control test, encompasses dissolution testing of films made of various materials after they have been exposed to ICH-recommended accelerated conditions of temperature, humidity, and light in a photostability chamber and separately to different strengths of formaldehyde. The authors show that the combined approach of exposing gelatin films to environmental factors and formaldehyde can result in the selection of the most suitable material.

The cross-linking of gelatin and subsequent changes in dissolution behavior of gelatin-based products are well recognized. The problem is ascribed either to the interaction of gelatin with aldehydes (generated in situ in formulations because of drug–drug or drug–excipient interaction) or to environmental factors, including temperature, humidity, and light (1). One can control the former by proper selection of formulation ingredients, and the influence of environmental factors can be controlled to some extent by proper packaging. Still the problem is seen in marketed preparations; therefore, the best solution lies in the selection of the most appropriate type of gelatin, which shows minimum cross-linking behavior, so that there is no influence per se of any type of adverse causative factor.

Unfortunately, no method to date has been defined to evaluate gelatin raw materials with respect to their stability and resistance to cross-linking. Therefore, our endeavor was to develop a control test method, which can be used to find the best gelatin material. Because maximum cross-linking behavior among various types of gelatin preparations is seen in soft gelatin capsules, the study was performed on

gelatin films prepared in the same manner as is done commercially during the manufacture of soft gelatin capsules.

Experimental

Materials. Gelatin used as standard in the studies was purchased from s. d. fine-chem Ltd. (Mumbai, India). Various grades of raw gelatin were supplied by Panacea Biotec Ltd. (Lalru, India). The authors purchased glycerol from LOBA Chemie Pvt. Ltd. (Mumbai, India); methyl and propyl parabens from s. d. fine-chem Ltd. (Boisar, India); formaldehyde solution (37–41% w/v LR) from s. d. fine-chem Ltd. (Poicha, India); and pepsin 1:3000 LR from s.d. fine-chem Ltd. (Boisar, India) and LOBA Chemie Pvt. Ltd.

Equipment. A photostability chamber (KBWF 240, WTF Binder, Germany) was used for the stability studies. Dissolution studies on gelatin rings were carried out using a magnetic heating and stirring device (RCT basic, IKA Labortechnik, Germany) equipped with a digital controller and platinum probe (IKATRONIC), obtained from the same source. The gelatin mass was prepared on a rotary film evaporator (B-480, Buchi Labortechnik AG, Switzerland) and matured on a water bath

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equipped with a precision controller (MV, Julabo Labortechnik GmbH, Germany). The thickness of the films was measured using a digital screw gauge (Mitutoyo Corporation, Japan).

Preparation of gelatin films. Table I shows the optimized formula for the preparation of soft gelatin films. Figure 1 gives the flow chart of the steps involved. The authors weighed all the materials according to the given formula and transferred them to a 1000-mL, round-bottom flask, which was connected to a rotary film evaporator, with its bath filled with ice. The flask was stirred at a low speed under mild vacuum for 1 h. Chilling facilitated the swelling of the gelatin mass and removal of air bubbles. Subsequently, the ice was removed and the bath was filled with fresh water. The temperature was increased to 75 °C and the mass was maintained under vacuum for 4 h with constant stirring to facilitate appropriate mixing of the ingredients. The mass thus prepared was transferred to a beaker and kept in a water bath at 60 °C for 4 h to allow maturation and transfer of entrapped air to the surface. The film was finally cast using a 20 × 20 cm thin-layer chromatography plate onto which glass slides had been fixed on four sides (some margin was left on two sides) to give an internal area of 12 × 12 cm with a 1.2-mm thickness. The hot mass was dropped onto one side of the internal area and was spread with the aid of a 20 × 5 cm glass plate. After spreading the mass, the authors kept the plate in the refrigerator for 12 h to allow hardening of the film. They then removed the hardened film from the plate with the help of a spatula and stored it under ambient conditions.

Preparation of films of various grades of gelatin. The authors prepared films of 10 different grades of gelatin (coded G1–G10) using the method described above. They prepared two 100-g batches of film for each grade of gelatin.

Exposure to formaldehyde. A 5-mL beaker was placed in an inverted position in a 20-mL, squat-form weighing bottle (60 mm o.d. × 30 mm height). Two films at a time were rested on the top of the beaker (see Figure 2 for experimental setup). The required quantity of formaldehyde solution was dropped along the wall of the weighing bottle with the aid of a micropipette.

The films were exposed for 12 h. Several studies were performed using various volumes of formaldehyde stock solution (3.9 mg/mL) to keep the total formaldehyde in the weighing bottle in the range of 39–195 µg. The exposed films were dried for 12 h under ambient conditions and subjected to dissolution.

Exposure in photostability chamber. The films were simultaneously exposed to a temperature/humidity/light test described earlier by the authors (2). The exposure conditions were 40 °C, 75% RH, and light according to ICH-recommended UV and visible illumination, option 2 (3). The samples were exposed for a total of 10 days. The overall illumination at the point of placement was 6000 lx, and the near-UV energy (UV-A) at the same place was 2 W/m². These were determined, respectively, using calibrated lux (model 545, Testo GmbH, Germany) and UV (Dr. K. Honle GmbH, Germany) meters. Thus the total visible illumination exposure was 1.44 Mlx-h and the UV energy exposure was 480 Wh/m², both above ICH minimum requirements (3). The authors withdrew the films from the chamber for 12 h of drying under ambient conditions before subjecting them to dissolution.

Dissolution studies. The dissolution studies on the films were carried out in an open circular glass dish (10 cm diameter and 4 cm height). With the use of a stainless steel sieve, the authors created three compartments in this dish. In each of the three compartments, they placed two films at a time, one that was unexposed and the other that was exposed to formaldehyde or the photostability chamber. The authors tested three film replicates at the same time. They performed the dissolution studies separately, in 200 mL of water and in 200 mL of dissolution medium that contained pepsin, prepared according to the Tier-II test prescribed in *USP/NF* (4). The temperature was set at 37 ± 2 °C and constant stirring was maintained with help of a magnetic stirrer. The end point was the time taken for complete disappearance of the film as a result of dissolution. It was possible to record the end point within ± 1 min in repetitive analysis of unexposed samples and within ± 5 min for the exposed samples. In cases in which the time taken for dissolution was 60 min or more, the time was recorded as 60 min.

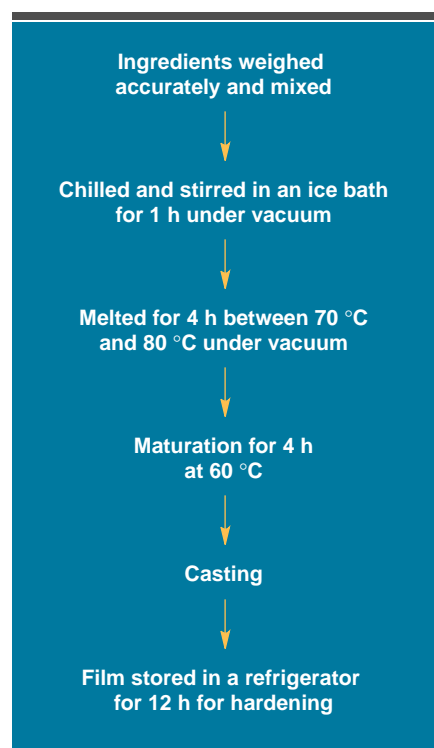


Figure 1: Flow diagram showing the method used for the preparation of soft gelatin films on a laboratory scale.

Table I: Optimized formula for preparing soft gelatin films on a laboratory scale.

Ingredient	% w/w
Gelatin powder	45.00
Glycerol	15.00
Propyl paraben	00.15
Methyl paraben	00.03
Water	39.82

Results and Discussion

Observations on gelatin mass preparations and formation of films. At the stage of mass preparation, none of the various films exhibited any problems. A general observation, however, was that a less viscous mass resulted when the gelatin was in a fine powder form. Films of gelatin with code G5 did not harden even after refrigerated storage for 18 h. This gelatin therefore was not studied further. The mass and films prepared with material G3 contained black particles. The films of this material were also very soft and melted after exposure for three days in a photostability chamber. The films formed with gelatin G4 were sticky and could not be removed intact from the glass plate.

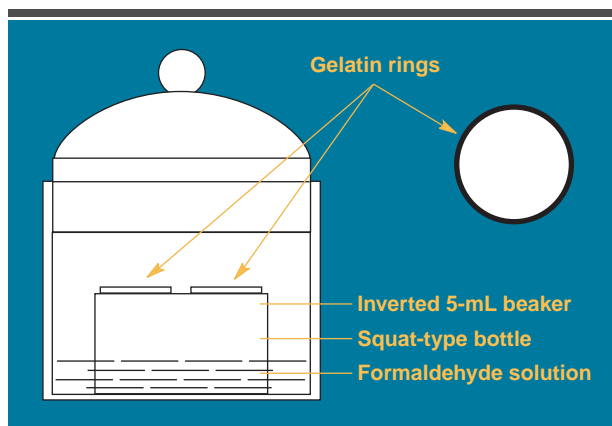


Figure 2: The set-up for formaldehyde exposure of gelatin rings.

The mass of gelatin with code G2 was viscous and turbid.

Optimization of the formaldehyde exposure method. Because the objective for the present study was to test the suitability of various gelatin materials for forming soft gels, the method used by the Gelatin Capsule Working Group was inappropriate. That method involves spiking lactose with formaldehyde and placing it into hard gelatin capsules before exposing them to high temperature and humidity (5). Nor did the authors follow the method used by Bottom et al., which involves the filling of formaldehyde-spiked polyethylene glycol into soft gels, because of a lack of availability of prototype machines for manufacturing soft gelatin capsules (6). Even the method involving the dipping of gelatin film into formaldehyde solution, as used by Hakata et al., was not considered suitable because of the chances of wetting and swelling of gelatin in the first stage (7).

Therefore, the authors decided to expose the films to formaldehyde vapors by

using formaldehyde solution in a closed chamber with the films kept away from the solution. For this purpose, squat-type weighing bottles were used, in which a beaker was kept in an inverted state, and the films were placed over the inverted beaker (see Figure 2). Initial studies were carried out on the films made from standard gelatin, and the influence of the following parameters was evaluated: diameter, weight, and thickness of the film; amount of formaldehyde (either mixed in lactose or directly as a solution); and time of exposure. The key observations were that the films cut as a circular disk were not fully cross-linked by formaldehyde vapors, and in almost all studies a core that dissolved faster than the boundaries was formed. Therefore, the authors removed the fast-dissolving core by punching out the central part to make hollow gelatin rings.

The use of formaldehyde-spiked lactose (6) gave erratic results, perhaps because of a loss of formaldehyde during mixing and its addition into the container as the lid was opened for subsequent placement of the inverted beaker and the films. Much better, more precise results were obtained when a solution of formaldehyde was used directly because it could be dropped from the side of the bottle, with the inverted beaker and the films already inside the chamber, and the cover could be closed immediately. In this situation, the expo-

sure period of 12 h resulted in complete and reproducible cross-linking. Trials were then made with rings of various sizes and weights. Best results were obtained with rings that weighed 35 mg and were 11 mm i.d. \times 14 mm o.d. in size. Table II shows the data for exposure of these rings to various amounts of formaldehyde in the range of 39 to 195 μ g. Figure 3 illustrates the corresponding dissolution profiles for the exposed films. It shows an increasing dependence on formaldehyde amounts between 39 and 117 μ g, after which a plateau is reached, with dissolution time \geq 60 min for all subsequent amounts of formaldehyde. Based on these observations, two amounts of formaldehyde (78 and 97.5 μ g) were selected with the expectation that they would generate maximum differentiating behavior for various gelatin films. Lower amounts were expected to produce a pass/pass result for all materials. A fail/fail outcome was expected with amounts \geq 117 μ g in all cases.

Photostability chamber method. A photostability test method developed earlier by the authors (2) for rapidly evaluating the possibility of a reduction in dissolution rates of gelatin-based formulations was applied to the gelatin rings to compare the results with those of the formaldehyde exposure method. The method involves exposure of gelatin formulations to accelerated temperature and humidity conditions of 40 $^{\circ}$ C and 75% RH along with ICH recommended light exposure according to option 2 (3).

Dissolution of rings. Table III lists the dissolution results for rings made from various grades of gelatins. The data are presented for two batches of each material. The dissolution results are given both in the absence and presence of enzyme. In the table, P indicates *pass*, meaning disappearance of gelatin film in less than <60 min and F means *fail*, in which case dissolution time was >60 min.

As is evident from the table, the results were the same for both the photostability chamber and formaldehyde exposure (78 μ g) methods, except in one case, i.e., the dissolution test of film G6 without enzyme. The exposure to 97.5 μ g formaldehyde further helped differentiate the films into two types: those that would either show or not show problems at higher concentrations of formaldehyde.

Table II: Dissolution data for films exposed to various amounts of formaldehyde.

Formaldehyde (μ g)	Dissolution time (min) \pm SEM			
	Unexposed		Exposed	
	Without enzyme	With enzyme	Without enzyme	With enzyme
39.0	7.67 \pm 0.33	6.33 \pm 0.33	11.67 \pm 0.33	9.33 \pm 0.33
58.5	8.00 \pm 0.00	7.00 \pm 0.00	31.00 \pm 1.00	18.33 \pm 0.33
78.0	7.67 \pm 0.33	6.67 \pm 0.33	44.00 \pm 1.15	30.67 \pm 1.20
97.5	7.67 \pm 0.33	7.00 \pm 0.00	60.00*	55.00 \pm 0.58
117.0	8.00 \pm 0.00	6.67 \pm 0.33	60.00*	60.00*
136.5	7.33 \pm 0.67	6.33 \pm 0.33	60.00*	60.00*
156.0	8.00 \pm 0.00	7.33 \pm 0.33	60.00*	60.00*
195.0	8.00 \pm 0.00	6.67 \pm 0.33	60.00*	60.00*

*Films did not dissolve even after 60 min.

The films of gelatins G1, G4, and G8 showed the same fail/pass results under all exposure conditions. The gelatins G2, G9, and G10 also showed fail/pass results upon exposure to the photostability chamber and 78 µg of formaldehyde, but showed a fail/fail situation upon exposure to 97.5 µg of formaldehyde. The gelatin G3, which melted during the chamber test method, showed pass/pass behavior upon exposure to both 78 µg and 97.5 µg of formaldehyde. The exposure of gelatin G6 film produced pass/pass in the photostability chamber and fail/pass on exposure to both 78 µg and 97.5 µg of formaldehyde. The film of gelatin G7 showed pass/pass when exposed to the chamber method and 78 µg of formalde-

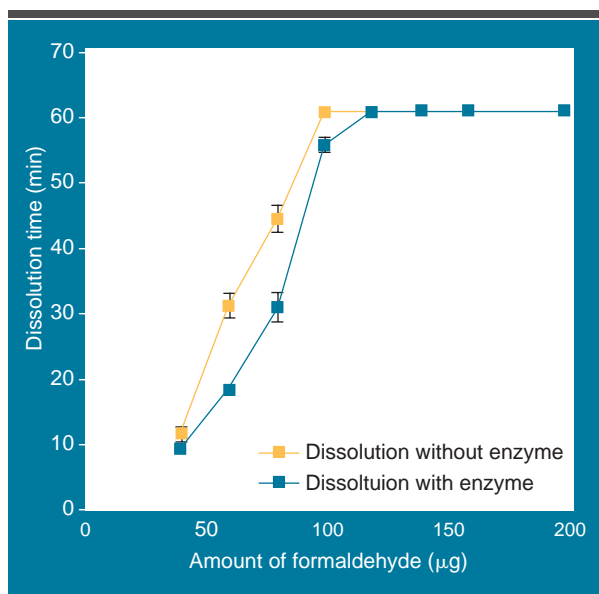


Figure 3: Dissolution profiles of formaldehyde-exposed films without and with enzyme.

hyde, but was fail/pass when exposed to 97.5 µg of formaldehyde.

Ranking of gelatins. On the basis of the results, different gelatin raw materials can be ranked with respect to their resistance to cross-linking. Foremost, G3 is the only

gelatin that showed pass/pass behavior after exposure to both concentrations of formaldehyde. But this gelatin resulted in very soft films, which even melted on exposure to 40 °C and 75% RH conditions; therefore, it cannot be considered suitable for commercial exploitation. Soft gelatin capsules made of this film are likely to exhibit physical stability problems during storage. Similarly, gelatins G4 and G5 can be taken out of the ranking because they also are considered commercially unsuitable for the reasons provided in the footnotes of Table III. The first ranking, therefore, is given to film G7, which shows pass/pass behavior under exposure to both chamber and 78-µg formaldehyde conditions and fail/pass upon exposure to 98 µg of formaldehyde. Second rank can be assigned to gelatin G6; it showed pass/pass under chamber exposure and fail/pass under the two formaldehyde conditions. On a similar basis, gelatins G1 and G8 can be ranked third, and gelatins G2, G9, and G10 fall to fourth place.

Table III: Comparison of different grades of gelatin.

Material code	Batch number	Photostability chamber		Formaldehyde (78.0 µg)		Formaldehyde (97.5 µg)	
		Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme
G1	1	F*	P* (30.33)**	F	P (30.00)	F	P (44.00)
	2	F	P (31.33)	F	P (31.00)	F	P (46.33)
G2	1	F	P (53.33)	F	P (35.00)	F	F
	2	F	P (52.67)	F	P (35.00)	F	F
G3	1	—***	—***	P (3.33)	P (3.00)	P (3.00)	P (3.00)
	2	—***	—***	P (3.00)	P (4.00)	P (3.33)	P (4.00)
G4†	1	F	P (43.33)	F	P (30.33)	F	P (45.33)
	2	F	P (42.67)	F	P (31.33)	F	P (46.67)
G5††		—	—	—	—	—	—
G6	1	P (10.00)	P (9.00)	F	P (30.33)	F	P (34.67)
	2	P (12.33)	P (9.00)	F	P (29.67)	F	P (33.33)
G7	1	P (10.00)	P (6.33)	P (26.67)	P (14.33)	F	P (31.33)
	2	P (11.33)	P (7.00)	P (20.67)	P (15.00)	F	P (33.00)
G8	1	F	P (23.67)	F	P (33.33)	F	P (43.00)
	2	F	P (23.00)	F	P (32.33)	F	P (45.00)
G9	1	F	P (22.33)	F	P (39.00)	F	F
	2	F	P (22.33)	F	P (40.33)	F	F
G10	1	F	P (45.00)	F	P (44.67)	F	F
	2	F	P (45.00)	F	P (45.00)	F	F

*F: fail; P: pass

**Mean of results of three films

***Melted in the photostability chamber

†Film was too sticky to the spreader plate. It could be scraped only in pieces. The studies were hence done on pieces, of uniform weight as of rings.

††Film was not formed

Verification from the formulation manufacturer. For verification of the ranking and validation of the approach, the authors contacted the soft gel manufacturer that provided the raw materials for the study. The manufacturer confirmed that gelatin coded G7 had proved best among all raw materials during the actual manufacture of soft gels and study of the marketed and aged samples.

Photostability versus formaldehyde. The study shows that both the photostability chamber and the formaldehyde (especially 78 μg) methods give largely the same results. The question then arises regarding whether only one type can be used instead of both types. The answer to this question is yes and no. The answer is yes if a photostability chamber is unavailable. In that case, the study can be conducted using the two strengths of formaldehyde. The answer is no if a photostability chamber is available because in that way two different catalytic factors (aldehydes and environmental factors) are evaluated simultaneously. That approach may be safer because the mechanism for cross-linking differs on exposure to formaldehyde and to environmental factors (1), despite the fact that the net influence might be the same with the two factors (as shown in this study). Another study in the literature also has reported that both types of catalytic influences can at times produce the same magnitude of cross-linking (7,8), though different chemistries are involved (1).

Scope of the control test. Although the test approach described in this study was established using films prepared according to the methodology prescribed for the preparation of soft gel formulations, there is a strong possibility that the final selected material would be equally suitable for use in the preparation of hard gelatin capsules and for use in sugar coating. The problem of cross-linking is worst in soft gels (2), and if a material shows resistance for this dosage form, it is more likely to prove a good material for hard gelatin capsules and as a film former during sugar coating. Thus it is assumed that the control test as suggested in this study can be safely extended to selecting the material resistant to cross-linking for all types of dosage forms for which gelatin is used in the outer layer.

One should, however, expect that rare situations might exist in which the test conditions would need readjusting, such as when dissolution limits are <45 min. A typical example is nifedipine capsules, for which *USP* prescribes a requirement of 80% drug release in 20 min (9). Table III shows that the maximum dissolution times for films made of first-ranked gelatin G7 in the presence of enzymes — on testing in the photostability chamber and upon exposure to 78 and 97.5 μg of formaldehyde — are 7, 15, and 33 min, respectively. So determining whether this material would be suitable for drug release within 20 min requires a more intensive study, especially when actual formulations are involved. In the latter case, bursting and disintegration of the gelatin shell or layer is a phenomenon that may help in dissolution but for which prediction cannot be made based on the study of films. Therefore, additional studies are suggested in demanding and specific situations.

Conclusions

Gelatin is one of the raw materials used in the pharmaceutical industry that has a natural origin and in which a lot of variation is seen among the supplies of different vendors. Moreover, gelatin carries a disadvantage in that it gets cross-linked after exposure to chemicals (like aldehydes) in very small quantities and on exposure to environmental factors. The cross-linking creates changed dissolution behavior in formulations in which it is used in the outer layer, and because of this there is always a risk of marketed preparations failing in dissolution tests.

As of today no quality control test can predict and differentiate various raw materials in terms of their resistance to cross-linking. The approach suggested in this study can be used to select the best raw material. The authors suggest that before a gelatin raw material lot is used in bulk, some experimentation should be conducted at the laboratory scale to identify whether a particular gelatin will be suitable for making the film and whether it will be resistant to cross-linking. Initially the mass can be prepared in small quantities and the spreading properties can be observed critically. Those raw materials, which form either a very soft (e.g., G3) or a very hard film (e.g., G4), or which have

any other type of problem (e.g., film not formed in G5 and black particles seen in material G3), can be rejected. The materials that yield satisfactory film can be subjected to further testing as was performed in this study. The substance that shows the best resistance to cross-linking can eventually be used in manufacturing the formulations.

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