# A Film Approach for the Stabilization of Gelatin Preparations Against Cross-Linking

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**The authors present an approach for reducing or eliminating pellicle formation in gelatin preparations. The methodology involves an effective cross-linking–reducing combination of glycine and citric acid during the preparation of the film mass or solution, as opposed to the traditional method of incorporating these substances in the fill of hard-gel capsules. The optimized concentrations of the stabilizer combination needed to generate stable soft- and hard-gelatin films are reported.**

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Gelatin is used extensively in the food and pharmaceutical industries to prepare films, sheets, ribbons, and soft- and hard-gelatin capsules and as a seal coat for sugar-coated tablets. However, a major disadvantage of gelatin is that it cross-links over a period of time upon exposure to excessive temperature, humidity, and light conditions or in the presence of chemicals such as aldehydes (1). This cross-linking effect retards the rate and extent of dissolution of gelatin, which is a concern for pharmaceutical manufacturers that are bound by ethical, moral, legal, and economical reasons to ensure that the dissolution profile remains either unchanged or within specifications during their products' shelf lives.

A few solutions to this problem have been published (1). One method especially appropriate for soft- or hard-gelatin capsules is to use the fill, which is devoid of the detrimental cross-linking influence on the film. A second method is to use stabilizers, such as a combination of glycine and citric acid, in the capsule fill to minimize the effect of environmental conditions or chemical catalysts (2). However, the fill-stabilization approach has several disadvantages. For example, although the method is applicable mainly to the stabilization of hard-gelatin capsules, the cross-linking problem also exists with soft gels and sugar-coated tablets (3). In soft gels, the fill approach is expected to have limited application because stabilizers such as glycine and citric acid are insoluble in nonaqueous solvents and oils, which commonly are used in soft gels to dispense the drug. Moreover, the fill method cannot be used to stabilize other presentations of gelatin where there is no encapsulation and no fill (e.g., films, sheets, and ribbons). Furthermore, the concentration of stabilizers to be added in the fill can vary with the type of drug and the ingredients used. For example, drugs containing keto or aldehydic groups themselves can act as catalysts toward cross-linking. This effect may occur with other ingredients that form part of the capsule fill. In another situation, a substance such as glycine (owing to the presence of a primary amino group) may be neutralized or become less effective in the presence of drug or additives, thereby requiring larger quantities of stabilizer combination in the fill. For example, lactose typically is

**Table I: Standard formulae for the preparation of soft- and hard-gelatin films.**

| | % w/w | |
|---|---|---|
| **Ingredient** | **Soft-gelatin film** | **Hard-gelatin film** |
| Gelatin powder | 45.00 | 45.00 |
| Glycerol | 15.00 | 4.00 |
| Propyl paraben | 0.15 | 0.15 |
| Methyl paraben | 0.03 | 0.03 |
| Water | 39.82 | 50.82 |

used as a diluent and as a granulating agent, but it can interact with glycine, thereby reducing its anti–cross-linking potency.

Taking these points into consideration, the authors envisaged that stabilization approaches targeted to gelatin film or to gelatin raw material could prove more useful than the fill approach by overcoming the disadvantages associated with the latter. The film approach was expected to stabilize all preparations or formulations made out of gelatin film or mass. Moreover, the intervention to pellicle formation or cross-linking was anticipated to occur in a more efficient manner because of the

**Table II: Dissolution of standard soft- and hard-gelatin films.**

| | Without exposure | | Photostability chamber | | Formaldehyde (78.0 μg) | | Formaldehyde (97.5 μg) | |
|---|---|---|---|---|---|---|---|---|
| **Batch number** | **Without enzyme** | **With enzyme** | **Without enzyme** | **With enzyme** | **Without enzyme** | **With enzyme** | **Without enzyme** | **With enzyme** |
| Soft-gelatin films | | | | | | | | |
| 1 | P[a] (6.60)[b] | P (5.60) | F[c] | P (46.66) | F | P (46.33) | F | F |
| 2 | P (5.36) | P (6.00) | F | P (46.66) | F | P (46.33) | F | F |
| 3 | P (5.33) | P (5.00) | F | P (43.33) | F | P (44.33) | F | F |
| Hard-gelatin films | | | | | | | | |
| 1 | P (6.33) | P (5.33) | F | F | F | F | F | F |
| 2 | P (6.66) | P (5.33) | F | F | F | F | F | F |
| 3 | P (6.33) | P (5.00) | F | F | F | F | F | F |

[a]Pass
[b]Time in minutes. Mean result of three films
[c]Fail

**Table III: Influence of glycine and citric acid alone on the dissolution of gelatin films.**

| | | Photostability chamber | | Formaldehyde (78.0 μg) | | Formaldehyde (97.5 μg) | |
|---|---|---|---|---|---|---|---|
| **Stabilizer** | **Batch number** | **Without enzyme** | **With enzyme** | **Without enzyme** | **With enzyme** | **Without enzyme** | **With enzyme** |
| Glycine, % | | | | | | | |
| 0.1 | 1 | F[a] | P[b] (45.66)[c] | F | P (46.00) | F | F |
| | 2 | F | P (46.00) | F | P (44.00) | F | F |
| 0.5 | 1 | F | P (45.33) | F | P (44.66) | F | F |
| | 2 | F | P (45.66) | F | P (43.33) | F | F |
| 1.0 | 1 | F | P (43.66) | F | P (43.33) | F | F |
| | 2 | F | P (45.00) | F | P (43.33) | F | F |
| 1.5 | 1 | F | P (43.33) | F | P (44.66) | F | F |
| | 2 | F | P (44.00) | F | P (45.33) | F | F |
| 2.0 | 1 | F | P (45.66) | F | P (43.33) | F | F |
| | 2 | F | P (43.33) | F | P (44.66) | F | F |
| 2.5 | 1 | F | P (38.00) | F | P (36.00) | F | F |
| | 2 | F | P (35.33) | F | P (36.33) | F | F |
| Citric acid, % | | | | | | | |
| 0.1 | 1 | F | P (46.00) | F | P (45.33) | F | F |
| | 2 | F | P (44.00) | F | P (45.66) | F | F |
| 0.5 | 1 | F | P (42.00) | F | P (40.00) | F | F |
| | 2 | F | P (43.00) | F | P (39.00) | F | F |

[a]Fail
[b]Pass
[c]Time in min. Mean of results of three films

**Table IV: Effect of the combination of glycine and citric acid on the dissolution of soft-gelatin films.**

| Glycine:citric acid % | Batch number | Photostability chamber | | Formaldehyde (78.0 μg) | | Formaldehyde (97.5 μg) | |
|---|---|---|---|---|---|---|---|
| | | Without enzyme | With enzyme | Without enzyme | With enzyme | Without enzyme | With enzyme |
| 0.1:0.1 | 1 | F[a] | P[b] (45.33)[c] | F | P (42.33) | F | F |
| | 2 | F | P (44.00) | F | P (43.33) | F | F |
| 0.1: 0.5 | 1 | P (35.33) | P (12.66) | P (29.33) | P (7.66) | F | P (26.00) |
| | 2 | P (32.66) | P (15.33) | P (31.33) | P (9.66) | F | P (24.00) |
| 0.5: 0.5 | 1 | P (7.66) | P (7.33) | P (11.00) | P (8.33) | F | P (23.33) |
| | 2 | P (10.88) | P (10.50) | P (10.50) | P (7.00) | F | P (20.00) |
| 1.0: 0.5 | 1 | P (13.00) | P (6.66) | P (15.33) | P (7.50) | F | P (22.50) |
| | 2 | P (7.66) | P (7.33) | P (11.00) | P (8.33) | F | P (23.33) |
| 2.0: 0.5 | 1 | P (6.33) | P (8.00) | P (6.00) | P (8.00) | P (7.33) | P (8.00) |
| | 2 | P (7.33) | P (6.33) | P (6.00) | P (8.50) | P (6.33) | P (8.33) |
| 2.5: 0.5 | 1 | P (7.00) | P (8.33) | P (8.00) | P (9.00) | P (7.66) | P (5.00) |
| | 2 | P (6.33) | P (8.00) | P (6.66) | P (5.66) | P (8.66) | P (7.00) |

[a]Fail
[b]Pass
[c]Time in min. Mean of results of three films

addition of stabilizer directly into the gelatin layer. It also was expected that incorporation of a stabilizer in the gelatin layer would overcome all possible catalytic challenges, whether from the fill or from external exposure to chemical catalysts. It was further anticipated that the addition of stabilizers in the gelatin film would neutralize very effectively the pellicle formation and cross-linking caused by exposure to accelerated conditions of temperature, humidity, and/or light, because the effect of environmental factors is an external-surface phenomenon, and the same could be better tackled if the stabilizer combination was present in the film than in the fill. The chances of stabilizers interacting with formulation fill also were expected to be minimal, especially in solid dosage forms, because of the small chance of their leaching from the gelatin film.

Accordingly, the authors decided to explore the film approach for the stabilization of soft- and hard-gelatin capsules. Initial studies were carried out on a previously established and patented stabilizer combination of glycine and citric acid (4) to determine its potential in preventing cross-linking when added to films prepared according to the standard formulae for the manufacture of soft- and hard-gelatin capsules. This article presents the results of the study.

**Table V: Influence of accelerated storage at 40 °C and 75% RH on the dissolution of solft-gelatin films containing 2.5:0.5 ratio of glycine:citric acid.**

| Period (months) | Batch number | Films with stabilizer | |
|---|---|---|---|
| | | Without enzyme | With enzyme |
| 0 | 1 | P[a] (4.66)[b] | P (4.66) |
| | 2 | P (6.33) | P (5.33) |
| 1 | 1 | P (6.00) | P (5.00) |
| | 2 | P (5.33) | P (5.50) |
| 3 | 1 | P (5.00) | P (4.50) |
| | 2 | P (6.00) | P (4.50) |

[a]Pass
[b]Time in min. Mean of results of three films.

## Experiment

**Materials.** Gelatins of bloom strength 100 for soft-gelatin films and 200 for hard-gelatin films were provided by Kind and Knox (Sioux City, IA). Glycerol, methyl paraben, and pepsin 1:3000 LR were purchased from LOBA Chemie Pvt. Ltd. (Mumbai, Maharashtra, India). Propyl paraben and formaldehyde solution (37–41% w/v LR) were purchased from s.d. Fine-Chem Ltd. (Boisar, Maharashtra, India).

**Equipment.** Gelatin mass was prepared on a rotary film evaporator (model B-480, Buchi Labortechnik AG, Flawil, Switzerland) and matured on a water bath equipped with precision controller (MV, Julabo Labortechnik GmbH, Seelbach, Germany). The films were prepared using a laboratory coating device (model SV-M-101301, Mathis, Switzerland). Thickness of the films was measured using a digital screw gauge (Mitutoyo Corp., Kanagawa, Japan). The rings were exposed in a photostability chamber equipped with a light bank on the inside top (KBF 240, WTF Binder, Tuttlingen, Germany). Dissolution studies were carried out using a magnetic heating and stirring device (RCT basic, IKA Labortechnik, Staufen, Germany) equipped with a digital controller and platinum probe (Ikatronic) obtained from the same supplier.

**Preparation of gelatin rings and strips without and with stabilizer.** Table I lists the formulae for the preparation of soft- and hard-gelatin films. The procedure for the preparation of soft-gelatin films was the same as described in a previous article (5). The film casting was done mechanically using a laboratory coating device and kept in refrigerator for 12 h for hardening. Thereafter, it was

**Table VI: Effect of the combination of glycine and citric acid on the dissolution of hard-gelatin films.**

| Glycine: citric acid % | Batch number | Photostability chamber[a] Without enzyme | Photostability chamber[a] With enzyme | Formaldehyde (78.0 μg) Without enzyme | Formaldehyde (78.0 μg) With enzyme | Formaldehyde (97.5 μg) Without enzyme | Formaldehyde (97.5 μg) With enzyme |
|---|---|---|---|---|---|---|---|
| 0.1:0.1 | 1 | - | - | F[c] | F | F | F |
| | 2 | - | - | F | F | F | F |
| 0.5:0.5 | 1 | - | - | F | F | F | F |
| | 2 | - | - | F | F | F | F |
| 1.5:0.5 | 1 | - | - | F | F | F | F |
| | 2 | - | - | F | F | F | F |
| 2.0:0.5 | 1 | - | - | F | P (49.00) | F | F |
| | 2 | - | - | F | P (40.00) | F | F |
| 2.5:0.5 | 1 | - | - | F | P (28.33) | F | P (47.00) |
| | 2 | - | - | F | P (25.66) | F | P (52.66) |
| 2.5:1.0 | 1 | - | - | P (44.00)[d] | P (25.00) | P (60.00) | P (37.00) |
| | 2 | - | - | P (42.66) | P (25.33) | P (40.00) | P (39.00) |
| 2.5:1.5 | 1 | - | - | P (29.66) | P (13.66) | P (38.33) | P (18.00) |
| | 2 | - | - | P (25.00) | P (14.00) | P (40.00) | P (20.66) |
| 2.5:1.8 | 1 | - | - | P (16.66) | P (10.66) | P (25.66) | P (17.00) |
| | 2 | - | - | P (20.66) | P (11.33) | P (27.66) | P (17.66) |
| 2.5:2.0 | 1 | P[b] (4.00) | P (3.50) | P (7.66) | P (5.66) | P (9.33) | P (5.66) |
| | 2 | P (3.50) | P (3.00) | P (6.66) | P (6.00) | P (6.66) | P (6.00) |

[a]Data for photostability chamber studies are only for citric acid:glycine concentration of 2.5:2.0, which yielded the most acceptable results in the formaldehyde exposure test
[b] Pass
[c]Fail
[d]Time in min. Mean of results of three films.

removed with a spatula and stored under ambient conditions. The hard-gelatin films were prepared in a similar manner (5) but with slight differences in the processing steps, described as follows.

**Chilling phase.** All materials were weighed according to the formulae and transferred to a 100-mL beaker that was kept in a freezer for half an hour for chilling. Chilling of gelatin mass facilitated its swelling and also removed air bubbles entrapped in the gelatin granules.

**Melting phase.** In this phase, the temperature was maintained constant at 60 °C for approximately 2 h.

**Maturation phase.** During this phase, air bubbles in the gelatin mass slowly rose to the surface and were removed with a spatula.

**Casting.** Finally, the film was cast on a glass plate of specified dimensions using a spreader.

**Storage.** The film was stored at 4–6 °C for 7 h, after which it was kept under ambient conditions.

Films with stabilizers were prepared by adding glycine and citric acid either alone or together to the standard formula. Two 100-g batches of films were prepared in all cases. In the case of soft films, rings of 35 ± 2 mg and 11-mm i.d. × 14-mm o.d. were cut out. Because the rings of hard gelatin films were difficult to cut out, strips of size 0.4 × 2.5 cm and weight of 23 ± 3 mg were prepared.

**Exposure of the rings and strips to formaldehyde and photostability tests.** The rings and strips were exposed separately to both formaldehyde vapours and to a combined temperature–humidity–light test in a photostability chamber. The experimental setup for formaldehyde exposure was reported in a previous article (5). The rings and strips were exposed to 78.0 μg and 97.5 μg formaldehyde in squat-type weighing bottles. After the exposure, the materials were dried under ambient conditions for 12 h. Exposure conditions in the photostability chamber were 40 °C, 75% relative humidity (RH), and light according to ICH-recommended UV and visible illumination, option 2 (6). The samples were exposed for the time until total visible illumination exposure was >1.2 M lx-h, and the UV energy exposure was >200 Wh/m$^2$, thus meeting the minimum requirements of ICH (6). The rings and strips were withdrawn from the chamber and dried for 12 h under ambient conditions before dissolution.

**Dissolution studies.** The dissolution studies on the rings and strips were carried out both in the absence of and in the presence of enzymes, as per the details described previously (5).

## Results and discussion

**Dissolution behavior of standard films.** Table II lists the mean dissolution times of both soft- and hard-gelatin standard films without stabilizers. Both types of films without exposure show pass/pass dissolution behavior by taking only 6 ± 1 min to dissolve. The soft films show fail/pass behavior after exposure in the photostability chamber and 78 μg of formaldehyde and fail/fail behavior after exposure to 97.5 μg of formaldehyde. However, the hard films show fail/fail behavior after all three exposure conditions.

**Effect of glycine and citric acid on the dissolution of soft-gelatin films.** The concentration of glycine and/or citric acid required for stabilization was determined initially for soft-gelatin films.

First, glycine and citric acid were tried alone. The glycine concentration was varied between 0.1 and 2.5%, and that of citric acid varied between 0.1 and 0.5%. Results showed that the films mostly failed the dissolution tests (see Table III). Even in the case of films that passed the dissolution test in the presence of enzyme, the dissolution time was >40 min, except films with glycine concentration of 2.5%, where dissolution was ~36 min. These results demonstrated that glycine or citric acid alone cannot protect against pellicle formation, an observation that also was made by Adesunloye and Stach (2).

Table IV lists the dissolution results of soft-gelatin films prepared with the combination of glycine and citric acid in various ratios. At the combined ratio of 0.1:0.1, the results are similar to those shown by individual stabilizers (see Table III). As the concentration of either glycine or citric acid, especially the former, is increased in the combination, the situation improves to show pass/pass results, with only films exposed to high concentration of formaldehyde (97.5 µg) showing fail/pass results. At glycine:citric acid ratios of 2.0:0.5 and 2.5:0.5, the dissolution results for films under all exposure conditions evidently become similar to those shown by unexposed standard soft-gelatin films (see Table II), indicating that good stabilization is achieved.

**Accelerated stability testing of control and stabilized soft-gelatin films.** The soft-gelatin films with a combination of glycine and citric acid in the ratio of 2.5:0.5 were subjected to accelerated stability testing at 40 °C and 75% RH for three months to confirm dissolution stability during shelf storage. The films were withdrawn periodically and subjected to dissolution studies in the absence and in the presence of enzyme (see Table V). The dissolution time shows no change, meaning that the stabilization property of the glycine–citric acid combination is not lost with time in storage.

**Influence of glycine and citric acid together on the dissolution of hard-gelatin films.** On the basis of the successful combination of glycine–citric acid to stabilize soft-gelatin films, the authors conducted studies to optimize the percentages of stabilizers required for hard-gelatin films prepared according to the formulae listed in Table I (see Table VI). The dissolution data show that a combination of glycine and citric acid in the ratio of 2.5:2.0 can provide the required stabilization equivalent to that shown by the standard films (see Table II).

## Conclusion

This study indicates that the fall in dissolution rate for gelatin preparations as a result of cross-linking can be solved by the addition of a combination of glycine and citric acid at the time when the gelatin films are prepared (7). A significant advantage of this "film" approach is that it can be easily exploited by both soft-gelatin and hard-gelatin capsule manufacturers. Capsule manufacturers produce billions of units per year and when the addition of stabilizers in the film formula does not influence

*Continued on page 84*

the properties of final capsules, it is likely that the problem of cross-linking can be eliminated to a large extent.

The authors are presently working to search out other types of efficient stabilizers that can be added into gelatin films.


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