Alteration in Dissolution Characteristics of Gelatin-Containing Formulations

A Review of the Problem, Test Methods, and Solutions

Saranjit Singh,* K.V. Rama Rao, K. Venugopal, and R. Manikandan

The authors review the literature about the problem of the alteration in dissolution characteristics of gelatin-based formulations. This article also includes a brief introduction to gelatin as a pharmaceutical ingredient and describes examples of the altered dissolution profile; the established cause, mechanisms, influencing factors, and stress methods for study of the behavior; methods for determining the nature and extent of the change; and the reported solutions to the problem.

Saranjit Singh is a professor and department head, and K.V. Rama Rao is a postgraduate student, at the Department of Pharmaceutical Analysis of the National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar 160 062, India, tel. +91 172 214 682, fax +91 172 214 682, ssingh@niper.ac.in. K. Venugopal and R. Manikandan are postgraduate students from the same institute, presently working at Ranbaxy Research Laboratories (Gurgaon, India).

*To whom all correspondence should be addressed.

Manufacturers of pharmaceutical products have the responsibility — not only from marketing and ethical standpoints but also from a legal (regulatory) perspective — to ensure that their products meet dissolution specifications during storage conditions described on the label. This is necessary because dissolution per se is rate determining in terms of the absorption and bioavailability of a drug.

Unfortunately, a few dosage forms exist in which eventual change in the dissolution characteristics is a common problem. Formulations containing gelatin in the outer layer (i.e., hard and soft gelatin capsules) as well as sugar-coated tablets are typical examples. The problem has been ascribed to cross-linking of gelatin, which occurs with time. Because of this tendency, the very use of gelatin in pharmaceutical formulations has been put to question. Nevertheless, the material is used widely despite efforts to replace it with other substances.

This article reviews the literature describing the widespread problem of a drop in dissolution rates of gelatin-containing products and critical observations concerning their in vitro and in vivo behavior. The discussion includes a brief introduction to gelatin, some reported instances of altered dissolution profiles, the chemistry of change, responsible factors, suggested test methods, and reported solutions to the problem.

Gelatin — a brief introduction

Gelatin is a mixture of water-soluble proteins derived from collagen by hydrolysis (1). The protein fractions consist almost entirely of amino acids (see Table I). These amino acids are joined by amide linkage to form a linear polymer varying from 15,000 to 250,000 M_w.

Types of gelatins. The two types of gelatins are characterized by their mode of manufacture. Type A gelatin (pH 3.8–6.0; isoelectric point 6–8) is derived by acidic hydrolysis of pork skin and contributes plasticity and elasticity to the blend. Type B gelatin (pH 5.0–7.4; isoelectric point 4.7–5.3) is derived by basic hydrolysis of bones and animal skin and contributes high gel strength to the blend. Gelatin used in the pharmaceutical industries is a blend of these two types (2), although sometimes

only Type A or Type B is used. Various grades of gelatin with differing particle sizes and molecular weight are sold commercially in the form of translucent sheets, granules, or powders. Gelatin usually is graded according to jelly strength, expressed as *bloom strength,* which is the weight in grams that, when applied with a 12.7 mm–diameter plunger, will produce a depression exactly 4 mm deep in a matured jelly containing 6.66% w/w of gelatin in water.

Properties. Gelatin is practically odorless and tasteless. It is insoluble in acetone, chloroform, ethanol (95%), ether, and methanol. It is soluble in glycerin, acids, and alkalis, although strong acids or alkalis cause its precipitation. It swells and softens in water, gradually absorbing 5 to 10 times its own weight in water. It solubilizes in hot water. Upon cooling to 35–40 °C, it forms a jelly or gel. At temperatures >40 °C, the system exists as a sol. A gel of higher viscosity is formed in alkaline media as compared with acid media (3).

Because it is a protein, gelatin exhibits chemical properties characteristic of those materials (e.g., gelatin is hydrolyzed by most of the proteolytic systems to yield its amino components). Gelatin reacts with acids and bases, aldehydes and aldehydic sugars, anionic and cationic polymers, electrolytes, metal ions, plasticizers, preservatives, and surfactants.

Applications and uses. Gelatin is valuable to the pharmaceutical industry because it can be incorporated into a variety of formulations. It is the only ingredient used to form hard and soft elastic gelatin capsules (SEGCs). It is widely used in solutions, syrups, tablets, sugar-coated tablets, inhalants, and dental, vaginal, and topical preparations and injections. Its other uses include the preparation of pastes, pastilles, pessaries, and suppositories. In addition, it is used as a vehicle for parenteral formulations and as a tablet binder and coating agent. Low molecular weight gelatin has been investigated for its ability to enhance dissolution of orally ingested drugs.

Gelatin also is used for the microencapsulation of drugs, a process whereby the active drug is sealed inside a microsized capsule that then may be handled as a powder. Gelatin forms simple coacervates at temperatures >40 °C in the presence of dehydrating agents such as ethanol or 7% sodium sulfate solution. Peters et al. studied the properties of gelatin in complex coacervation processes (4). Gelatin capsules also can be coated for any application (5). Soft gelatin capsules can be given a film coat (6) and an enteric coat (7).

Cross-linking of gelatin before or after drying the capsules allows for sustained release of the drug (8). Formaldehyde exposure has been exploited to produce enteric hard and soft capsules (2,9,10). Drilling pores in formaldehyde cross-linked gelatin capsules to design a controlled-release dosage form also has been reported (11). A zero-order release of verapamil was observed with this approach. Several other reports describe the formation of gelatin microspheres and their cross-linking with glutaraldehyde with the objective of sustaining drug release (12–14). Cross-linked gelatin gels have been used as biomaterials in living tissues either as bioadhesives or as devices for sustained drug release (15). A novel system for gene delivery based on the use of DNA-gelatin nanoparticles (nanospheres) formed by salt-induced complex coacervation of gelatin and plasmid DNA has been developed. It consists of spherical particles in sizes ranging from 200 to 700 nm containing 25–30% (w/w) DNA. The particles are stabilized by the cross-linking of gelatin (16).

Table I: Amino acids and their content in gelatin.

Amino Acid	Percentage
Glycine	25.5
Proline	18.0
Hydroxyproline	14.1
Glutamic acid	11.4
Alanine	8.5
Arginine	8.5
Aspartic acid	6.6
Lysine	4.1
Leucine	3.2
Valine	2.5
Phenylalanine	2.2
Threonine	1.9
Isoleucine	1.4
Methionine	1.0
Histidine	0.8
Tyrosine	0.5
Serine	0.4
Cystine	0.1
Cysteine	0.1

Therapeutically, gelatin has been used as a plasma substitute and in the preparation of wound dressings. Soft capsules made of gelatin and containing a radiolabeled drug have been used in radioactive tracer studies (17). Gelatin also is widely used in food products and photographic emulsions.

In general, when it is used in an oral formulation, gelatin may be regarded as a nontoxic and nonirritant material. However, rare reports exist of gelatin capsules adhering to the esophageal lining (18–20), which may cause local irritation. Hypersensitivity reactions, including serious anaphylactoid reactions, have been reported following the use of gelatin in parenteral products (21).

Official status. Gelatin is included on the FDA list of inactive ingredients. In the United Kingdom, it appears on the list of licensed medicines. It also is described in most pharmacopeias.

The problem of gelatin cross-linking and change in dissolution profiles

A major problem with gelatin-based formulations is an apparent fall in dissolution upon aging, which is attributed to the cross-linking of stressed gelatin-containing products. The cross-linking causes the formation of a swollen, very thin, tough, rubbery, water-insoluble membrane, also known as a *pellicle.* The pellicle acts as a barrier and restricts the release of the drug. It is not disrupted easily by gentle agitation, and the dissolution values (Q values) drop often to the point of rejection (2,22).

The altered dissolution behavior of gelatin is reported in several studies in the literature. This section discusses examples for various categories of formulations.

Sugar-coated tablets (SCTs). In SCTs, gelatin forms part of the subcoat, where it is used mainly as a binder, film-forming agent, and coating agent. During the subcoating process, a coating solution and a dusting powder are applied alternately on the core, interspersed with relevant drying periods. This procedure results in the buildup of a laminated structure or a thick sandwich of alternate layers of binder and powder. These layers give the tablet a smooth profile by covering the core's original sharp edges and facilitate the application of the final color coating, which seals the tablet.

The problem of a reduced dissolution rate in SCTs was highlighted very early in a study by Khalil et al. (23). They developed a model subcoating system of SCT formulations consist-

ing mainly of cast gelatin films containing sucrose, dextrose, fructose, and calcium carbonate. The films were stored at 70, 80, 90, and 110 °C for varying time intervals, and a significant correlation was observed between an increase in the storage temperature and the rate at which these films dissolved. The disintegration and dissolution properties were adversely affected by an increase in temperature.

Subsequently, Barrett and Fell reported the influence of age on the dissolution of SCTs containing phenylbutazone (24). The formulations were stored at 20, 37, and 50 °C. The resulting progressive decrease in disintegration and also of dissolution was related to the adherence of the gelatin subcoat to the tablet core. The breakup pattern of older tablets (5 years old) was similar to that of the tablets stored at 50 °C. The disintegration time of the SCTs stored at 20 and 37 °C was not affected, except for that of the tablets stored for 14 weeks at 37 °C.

The retardation of dissolution in sugar-coated cyclothiazide and reserpine tablets when stored for 1 year at various temperatures is reported by Warren and Rowe (25). Very poor dissolution rates also were observed in SCTs of propantheline bromide (26). El-Fattah and Khalil examined the dissolution rates of 14 batches of sugar-coated chlorpromazine hydrochloride tablets and found that all the batches passed USP disintegration tests in 0.1N hydrochloric acid, yet none passed the dissolution limit of not less than 80% dissolved in 30 min (27). Ondari et al. observed retardation in the dissolution of the marketed products of sugar-coated chlorpromazine tablets stored at isothermal temperature (30 °C) and cyclic storage conditions (12 h at 30 °C, 12 h at room temperature, 12 h at 30 °C and 90% RH) for 4 weeks (28).

In another study, the dissolution behavior of eight commercially available brands of ibuprofen tablets was investigated after storage at 37 °C and 75% RH for 4 weeks (29). At the end of the storage period, a significant reduction in the dissolution rate of SCTs was noted, but film-coated tablets were unaffected. The SCTs were associated with a loss of clinical efficacy. In a study of SCTs of riboflavin, 10 batches of 2 brands were subjected to storage at temperatures ranging from 18 to 28 °C in a closed container and at 45 °C outside the container. The brand of tablets that contained gelatin in the subcoat exhibited poorer dissolution profiles than did the other brand (30). Dahl et al. found no change in the in vitro dissolution performance of gelatin-coated acetaminophen tablets that had been stored ⩽7 months at room temperature (31). However, when the tablets were stored in the presence of high humidity for 3.5 and 7 months, a significant reduction in both the amounts of drug released and standard deviation at each time point was observed.

Shah and Parsons compared the in vitro dissolution behavior of film-coated, sugar-coated, and plain tablets of valproic acid (VPA) after subjecting them to accelerated storage conditions (32). The plain and film-coated tablets were not affected adversely by the accelerated conditions of 40 °C and 75% RH for at least 3 months. The percent of VPA released from SCTs after 1 month also was not significantly different. However, after 2- and 3-month storage periods, the percent of VPA released during the first hour significantly decreased. The poor dissolution was ascribed to a disintegration problem in the sugar coating, but the fact that most of the core tablets still were dry at the end of the dissolution test indicated adherence of the seal coat or the subcoat to the tablet core.

In a recent study, Singh et al. studied the release rates of SCTs of chloroquine phosphate when exposed to various storage conditions such as 25 °C and 60% RH, 40 °C and 75% RH, 25 °C and 60% RH/light, and 40 °C and 75% RH/light (33). The storage of SCTs at 25 °C and 60% RH/light conditions for 16 days resulted in retardation of the dissolution rates. The storage of SCTs for 8 days in 40 °C and 75% RH/light conditions caused a drastic retardation in dissolution. Storage in these conditions for more than 8 days resulted in an even worse performance.

Hard gelatin capsules (HGCs). The shell of HGCs normally contains ~13–16% water, and HGCs can be safely stored at between 40 and 60% RH. Variations within the range of 12–18% moisture do not seriously impair the integrity of the shell. If an HGC shell contains <12% water, then it can become brittle and easily ruptured. If it contains >18% moisture, then the shell becomes moist, soft, and distorted and has a propensity to transmit moisture to the capsule contents if the contents are hygroscopic (34). The dissolution stability of HGCs is determined primarily by the moisture content of the shell, which in turn is related to the storage conditions. The moisture in the capsule gelatin shells will act as a plasticizer to impart flexibility to HGCs. Variations in the moisture content of a capsule shell as the storage conditions change may lead to undesired physical properties such as brittleness and stickiness. Moisture also can be transferred from the contents to the shell, potentially resulting in softening and stickiness problems. This problem is seen in efflorescent ingredients, for example. Conversely, moisture can move from the shell to the capsule contents during storage, especially for deliquescent and hygroscopic ingredients. Moisture transfer between shell and contents can be one of the reasons for a change in the properties of gelatin when stored at 40 °C, 50 °C, and 40 °C and 75% RH (35).

Langenbucher observed retardation of dissolution in capsule formulations containing lactose after 2–8 weeks of storage at 11–67% RH (36). Khalil et al. explored the effect of aging on the disintegration and dissolution of four brands of chloramphenicol HGCs (23). The products were stored in an open container at 25 °C in various humidity conditions for 32 weeks. No change was observed in the dissolution profile of samples stored at 49 and 66% RH. However, the capsules stored at 80% RH showed practically no drug release ⩽60 min. In another study, marketed preparations of tetracycline and chloramphenicol capsules stored at 30 °C and 75% RH for 1 month in an open container showed significantly slower in vitro release compared with the initial values. The shell failed to disintegrate in the test conditions (37). Mohamad et al. also observed partial insolubilization of the gelatin shell for tetracycline hydrochloride capsules stored for 48 months (38). Georgarakis et al. studied the influence of storage conditions on the performance of ampicillin trihydrate capsules stored in varying humidities (50–90% RH), and significant retardation of the dissolution rate of the capsule formulation was noted (39). This was attributed to the agglomeration and subsequent caking of the capsule contents as the result of moisture transfer from the shell.

Murthy et al. evaluated the effect of exaggerated storage conditions on the dissolution characteristics of hard-shell capsule preparations using three drugs as the model systems (40,41). In this study, the HGCs containing various dyes such as FD&C Red No. 3, Red No. 40, Yellow No. 5, Red No. 28, and Blue No. 1 were tested. The influence of light, humidity, and a combination of these two factors was assessed using capsules containing certified colorants in both the cap and the body as well as two-tone capsules containing dye only in the cap. The capsules containing FD&C Yellow No. 5, Red No. 3, and Blue No. 1 showed complete inhibition of drug release when stored for 2 weeks under fluorescent light at 80% RH. A marked decrease in the total amount of drug released was seen after the products were stored for 2 weeks under ambient light and 80% RH and for 2 days under UV light and 80% RH. When the same formulation was deposited into clear capsules and stored for 4 weeks under high-intensity fluorescent or ambient light at 80% RH, no effect on the dissolution and disintegration characteristics of the capsule was seen. Thus it was assumed that changes in the capsule shell were dye induced and were promoted by light and high humidity. The study underscored the need for avoiding high-humidity environments and supported the use of light-resistant containers during storage of capsule products containing FD&C-certified dyes in the capsule shell.

Dey et al. showed that etodolac capsules (200 or 300 mg), when stored at accelerated conditions (40 °C and 75% RH) unpacked or packed in poly(vinyl chloride) (PVC) or PVC–polychlorotrifluoroethylene blisters, failed to meet the dissolution specification of 80% Q in 30 min, but the capsules that were stored in high-density polyethylene (HDPE) bottles continued to conform to the dissolution specification (42). In another study, dissolution studies were conducted on ibuprofen HGCs stored in high temperature and high humidity, with and without light. The drug release was retarded when light was included with two other accelerated conditions (33).

Soft gelatin capsules (SGCs). Dissolution problems also are common knowledge in regard to this dosage form, although only a few reported studies exist. SGCs contain drugs either in a solution or a dispersion form. The shell contains a plasticizer, typically glycerine or sorbitol; polyethylene glycol (PEG); ethers of polyethylenated glycosides; and gelatin and water. High humidities cause the capsules to become soft, tacky, and bloated and create the likelihood of moisture migration from the shell into the fill material. Such a transfer potentially causes chemical and dissolution instability (43–45). The degree of pelliculation frequently differs between soft-shell capsules and hard-shell capsules because of the larger mass of gelatin in the soft-shell dosage form (46).

A typical reported case is the study by Chafetz et al., who found a significant decrease in the dissolution rate of gemifibrozil from SGCs (47). The capsules were stored at 37 °C, 37 °C and 80% RH, and 45 °C and were tested after 1, 2, and 3 months. All the capsules containing polysorbate 80 showed film formation after 1 month at 37 °C and 80% RH. A study by Bottom et al. showed that two batches of nifedipine SGCs failed during dissolution testing (48). In a recent study, dissolution testing was carried out on marketed nimesulide SGCs after their storage at 40 °C and 75% RH and light for various time periods (33). The change in dissolution behavior was drastic, and the drug was not released at all in all samples withdrawn on the eighth day and afterward.

Tablets containing gelatin as binder. Differing reports exist about formulations containing gelatin as a binder. In a study conducted by Asker et al., prednisone tablets containing gelatin as a binder were stored at 50 °C and 83% RH and 70 °C and 96% RH for 7 weeks (49). An increase in both disintegration and dissolution time was observed. However, the problem was not observed in a recent study of chloroquine tablets in which gelatin was used for the same purpose. All tablets stored at 40 °C and 75% RH under light ⩽3 weeks exhibited complete disintegration and little retardation in the dissolution rate, unlike other drug products containing gelatin in the outer layer (33).

Bioavailability–clinical behavior of cross-linked gelatin formulations and the role of gastrointestinal-tract (GIT) enzymes

Johnson et al. carried out bioavailability studies on soft-gel digoxin capsules stored for 10 months at 37 °C (50). Although the dissolution rate decreased, the extent of absorption was not reduced. Similarly, Chafetz et al. did not find a correlation between the results of in vitro and in vivo tests (47). Their study showed film-forming gemfibrozil capsules to be bioequivalent to the readily dissolving product. However, Martin et al. found that exposure to high humidities destroyed the clinical efficacy of phenytoin capsules (51). Later, Mohamad et al. also reported no change in the in vivo bioavailability of tetracycline capsules stored for 42 months, although in vitro dissolution decreased (38,52).

The role of GIT enzymes. Because the adverse effect on dissolution was not observed in vivo for the formulations that failed the in vitro tests, one could conclude that GIT enzymes were responsible for the digestion of denatured gelatin. It was perhaps for the same reason that before the 1960s, when simulated gastric and intestinal fluids were used as dissolution media to test the role of GIT enzymes, almost no reports were made about the failure of dissolution of gelatin-containing formulations during storage. Rather, an early report indicated that pepsin in the gastric fluid advanced the dissolution of slow-dissolving capsules (53). Hence, an extensive study was conducted by Murthy et al. to determine the influence of enzymes in the dissolution media on in vitro release characteristics of two formulations subjected to stressful storage conditions (40). Their study confirmed that the adverse effects on dissolution resulting from exaggerated conditions of humidity and light were virtually eliminated when the products were tested in dissolution media containing enzymes.

The same observation also was made by Dahl et al., who showed that gelatin-coated acetaminophen tablets, when stored in a humid chamber for 7 months and tested in 1% aqueous pancreatin solution, exhibited the same dissolution profile as the freshly prepared gelatin-coated tablets (31). Similarly, Dey et al. demonstrated that stressed HGCs (40 °C and 75% RH) containing 200 and 300 mg of etodolac that failed in vitro dissolution tests in phosphate buffer (pH 7.5) met dissolution specifications (not less than 80% drug release in 30 min) when

tested in phosphate buffer (pH 7.5) containing 1% w/v pancreatin (42). Furthermore, the rate and extent of absorption of the drug from the stressed 200- and 300-mg etodolac capsules in dogs were equivalent to capsules stored at room temperature that passed in vitro dissolution tests. Also, the bioavailability of etodolac from 300-mg stressed capsules that failed dissolution specifications was shown to be equivalent to that of control capsules (freshly packed) in a study of 24 adult male volunteers.

Recent studies. In one recent study, two batches of nifedipine SGCs were treated with 10–20 ppm formaldehyde and 80 ppm formaldehyde (48). Although the SGCs in the first batch were bioequivalent to fresh capsules after storage for 1.5 years, the SGCs in the second batch were not bioequivalent after storage for 1 year. In another study, the disintegration times of SGCs of vitamins were studied after storage at 40 °C and 75% RH for 6 months and at 25 °C and 60% RH for 1 year (54). The products failed in the disintegration media lacking enzymes but passed in media containing enzymes. Urine analysis showed that the disintegration rate was normal in both cases.

Brown et al. compared the in vivo disintegration behavior of unstressed and moderately stressed acetaminophen-containing HGCs (stressed with formaldehyde) using gamma scintigraphy (46). No difference was found in the disintegration properties of either group. A similar but more extensive study was conducted by Digenis et al., who used gamma scintigraphy for the study of in vivo capsule rupture and GI transit and also carried out a six-way single-dose bioequivalence study on amoxicillin from HGCs stressed with formaldehyde (55). The study showed a delay in the in vivo capsule rupture of severely cross-linked capsules, which then led to a delay in the onset of absorption and T_{max}. However, no effect was observed on $AUC_{(0-\alpha)}$ or C_{max}. In a more-recent study, an increase in C_{max} parallel to T_{max} was observed for acetaminophen SGCs and HGCs subjected to various degrees of cross-linking using formaldehyde (56). Although no difference was observed between the severely and moderately stressed formulations based on $AUC_{(0-\alpha)}$, bioequivalence did not exist on the basis of C_{max}.

The USP two-tier dissolution test involving enzymes

On the basis of the findings that satisfactory dissolution is obtained for bioavailable products by adding GIT enzymes (pepsin or pancreatin) to the dissolution medium, a call was made for the inclusion of the enzymes in the USP test medium for the specific evaluation of gelatin products (22). The recommendation was based on the logic that because enzymes would eliminate the impeding barrier that is exerted upon the drug molecule by a highly cross-linked gelatin capsule wall, a test of such kind could alleviate the time and cost of bioequivalence studies.

Accordingly, in July 1993 a USP subcommittee on dissolution and bioavailability (DBA) proposed to institute a second dissolution test using a medium containing an enzyme for aged capsules that failed to pass the first dissolution test. However, the proposal included the condition that there should be no evidence that the bioavailability of the capsules had changed adversely. The proposal was put forward in *Pharmacopeial Forum* in early 1994. At about the same time, FDA's Industry Gelatin Capsule Working Group was formed, in which USP also became a participant. DBA decided to defer formalization of its proposal until the working group completed its studies of the bioavailability–bioequivalence issue. Early in 1997, *Pharmacopeial Forum* introduced a two-tier test for HGCs for situations in which a formulation had failed the official dissolution test. It initially was proposed that the requirement for the second dissolution test would be included in the individual monographs. In the September–October 1997 issue of *Pharmacopeial Forum,* however, the second dissolution-test medium was included in the General Chapter ⟨711⟩ "Dissolution." This two-tier dissolution test eventually appeared in the 25th edition of *USP* (57).

The two-tier test. The two-tier test encompasses the initial dissolution study in the plain medium as specified in the individual monograph. The second dissolution is conducted in the medium that contains enzymes. Two types of enzymes are recommended on the basis of the pH of the dissolution medium. The recommendation is to use purified pepsin, resulting in an activity of $\leqslant$750,000 units/1000 mL for conditions in which water or a medium with $<$6.8 pH is specified in the monograph. For the medium with $\geqslant$6.8 pH, pancreatin is added at not more than 0.05 g/1000 mL (57).

Extending the test to insoluble drugs. Recent efforts are being directed toward extending the USP two-tier test to include formulations containing insoluble drugs. The use of nonionic surfactants combined with pepsin has been considered for that application (58).

The chemistry of the problem

An excellent review by Digenis et al. describes the mechanistic rationalizations that explain gelatin cross-linking in stress conditions relevant to pharmaceutical situations (2). Therefore, mechanisms are not discussed in detail in this article.

Postulated chemical events. The following chemical events are postulated to be involved in the cross-linking process:

- The reactivity of the gelatin arises from the trifunctional amino acid lysine. The lysine residues, which are proximal to each other, are oxidatively deaminated to yield terminal aldehyde groups. One of the aldehyde groups is attacked by a free ϵ-amino group of a neighboring lysine to yield an imine, which subsequently undergoes a series of aldol-type condensation reactions to produce a cross-linked product containing pyridinium ring(s).
- The lysyl ϵ-amino group reacts with aldehyde when it is present as an impurity. The reaction yields a hydroxymethylamino derivative, which loses water to form a cationic imine. The latter reacts with another hydroxymethylamino lysine residue to form dimethylene ether, which eventually rearranges to form a methylene link between two lysyl ϵ-amino groups, resulting in the development of a cross-link.
- The third type of gelatin cross-linking is the formation of aminal, the amine form of an acetal, which is produced by a reaction of a cationic imine intermediate (see previous bullet) with a free amino group. The pH of the environment plays an important role in this type of reaction.

- A similar type of reaction can occur with glucose or other aldose sugars that are commonly used in pharmaceutical formulations. The imine formed during the interaction of an aldehydic functional group of these saccharides reacts with the free amino group and produces ketose sugar upon rearrangement. The ketose sugar then reacts with another amine through its carbonyl functionality to form cross-linked gelatin.

In addition to lysine–lysine cross-linking, lysine–arginine and arginine–arginine cross-linking also are reported. In general, cross-linking of the gelatin polypeptides can occur in the following two ways:

- Bridging can take place within the same polypeptide strand (intrastrand, intramolecular cross-linking).
- Amino acid residues from two neighboring peptide strands can form a bridge (interstrand, intermolecular cross-linking), a process that increases the molecular weight of gelatin (39).

As a result of cross-linking, the interparticulate bonds formed in the original compact are removed and replaced by new bonds, culminating in a dosage form that has a different porosity and pore structure and therefore a different in vitro release pattern as compared with the original (34).

Table II: Chemical compounds reported to induce gelatin cross-linking.

Material	Reference
Aldehydes (furfural, acrolein, formaldehyde, glutaraldehyde, glyceryl aldehyde)	2, 47, 48, 52, 65, 81, 86
Imines	22
Ketones	22
Saccharides (glucose and aldose sugars)	2
Dyes (FD&C Red No. 3 or 40 and Blue No. 1)	2
Calcium carbonate	27, 68
Hydrogen peroxide	41, 86
Sulfonic acids and *p*-toluene sulfonic acid	41, 86
Carbodiimides (1-ethylene 3-(3-dimethylamino propyl) carbodiimide hydrochloride, guanidine hydrochloride)	81, 86
Benzene	86
Terephthaloyl chloride	8

Causative factors for cross-linking

The presence of some chemicals, high humidity, high temperature, and exposure to light has been found to play individual or synergistic roles in increasing the in vitro dissolution time of formulations containing gelatin in the outer layer. The reported examples are discussed in this section.

Chemicals. The chemicals that commonly are known to introduce modifications in gelatin are listed in Table II. Of all the reagents, formaldehyde has been studied most extensively (9). It is released in dosage forms from plasticizers and preservatives, fats, and polyethylenated compounds such as PEG, ethers of PEG and aliphatic alcohols or phenols, polyethylenated glycerides, nonionic surfactants (polysorbates, esters of unsaturated fatty acids), and corn starch containing hexamethylene tetramine as a stabilizer (9,38,47,52,59). Much work has been done to establish a correlation between the concentration of formaldehyde and the extent of reduction in dissolution of gelatin-containing preparations (60–64). Conversely, the cross-linking of gelatin with formaldehyde has been used advantageously to produce enteric hard and soft capsules (2,9–11,65).

Colorants, especially FD&C Red No. 3 and FD&C Red No. 40, also are known to play a crucial role in modifying the conformational properties of gelatin and rendering it insoluble (66,67). The dyes interact with gelatin by means of hydrophobic and hydrogen bonding (9,66). Murthy et al. (41) observed a significant decrease in the rate of dissolution in capsules containing FD&C Red No. 3 when they were stored in high humidity and light.

The interaction of gelatin with calcium carbonate and also with other water-insoluble compounds such as calcium sulphate and magnesium carbonate has been reported by Ray-Johnson and Jackson (68). No adverse effect, however, was found with the soluble salts (i.e., calcium chloride and magnesium chloride). Thus it was proposed that the change in the behavior of gelatin in the presence of insoluble calcium salts resulted from a physical change within the tablet subcoat and not the chemical reaction between cations and gelatin.

Two other studies reported a negative effect on the dissolution rate of gelatin capsules as a result of the rayon coiler that fills the headspace of HDPE bottles containing capsules. The rayon produces furfural, which when present in a saturated vapor phase rapidly insolubilizes gelatin capsules (65). Hartauer et al. (69) reported a significant decrease in the dissolution profile of rayon coiler–containing packaging for low count (10-count) capsules after 2 and 3 months of storage at accelerated environmental conditions.

Humidity. Humidity is another major factor that induces cross-linking in gelatin preparations. Examples highlighting its detrimental influence include studies of chloramphenicol (23), gemfibrozil (47), and phenytoin capsules (51) as well as gelatin-coated tablets of acetaminophen (31).

Humidity is proposed to act by

- indirect catalysis of imine formation, which is the first intermediate in all cross-linking reactions
- catalysis of excipient decomposition, yielding products that cause cross-linking of gelatin. For example, corn starch at times contains traces of the stabilizer hexamethyl tetramine, which decomposes in humid conditions to form ammonia and formaldehyde (38).
- influencing the rate of arginine–arginine cross-linking
- providing a vehicle for the denaturation of gelatin.

Temperature. Elevated temperatures increase the rate at which cross-linking occurs. Barrett and Fell found that storing SCTs at 20 °C and 37 °C did not affect the disintegration time, with the exception of those stored for 14 weeks at 37 °C. However, a significant change occurred at 50 °C (24). The breakup pattern of five-year-old tablets was similar to the pattern of those stored at 50 °C. Hakata et al. reported a significant decrease in the disintegration of SGCs upon storage at ⩾40 °C (70–72). In other studies, a model system was developed consisting

Table III: Reported examples of the use of formaldehyde for the induction of cross-linking in gelatin-containing formulations.

Dosage Form and Drug	Formaldehyde Concentration	Storage Condition and Time Period	Effect on Dissolution	Reference
Hard gelatin capsules:				
Amoxicillin	Lactose contaminated with 18 ppm	6 months at RT*, 40 °C and 75% RH	Significant decrease	55
Hard gelatin capsule shell	Lactose contaminated with 0, 20, 120 ppm	40 °C and 75% RH followed by 25 °C and 50% RH for 6 weeks	Significant decrease	60
Soft gelatin capsules:				
Acetaminophen and Nifedipine	0, 20, 80 ppm	25 °C and 60% RH, and 40 °C and 75% RH for 208 days	20 ppm or less were tolerable, but significant decrease with 80 ppm	48
Acetaminophen	Lactose contaminated with 20 ppm	1 day at 40 °C and 75% RH followed by 6 days at RT	Significant decrease	56

* RT = room temperature

mainly of cast gelatin films containing sucrose, dextrose, fructose, and calcium carbonate (30,73). These systems were stored at 70, 80, 90, and 110 °C for varying time intervals. During storage all the gelatin films showed a significant decrease in the disintegration rate.

Light. Light or UV–vis radiation also has been shown to influence the dissolution characteristics of formulations containing gelatin. Lengyel et al. observed that when gelatin had been exposed to ionization radiation and was used as a binding agent in tablets and capsules, changes occurred in the tablet stability and capsule characteristics (74). The influence of UV–vis radiation on gelatin cross-linking was observed by Murthy et al. during a study of hydrochlorothiazide and diphenhydramine hydrochloride capsules containing various dyes (41). They found that intense UV or visible irradiation promoted changes in the capsules, especially those containing FD&C Red No. 3. The result was decreased in vitro dissolution rates. They also observed that the adverse influence of light was enhanced when irradiation was combined with high humidity. Recently, Singh et al. (33) reported a further pronounced effect when the three environmental factors — temperature, humidity, and light — were combined. The increased adverse effect was seen in all types of preparations containing gelatin in the outer layer: SGCs, HGCs, and SCTs.

Stress methods for the induction of gelatin cross-linking to study polymerization behavior

To introduce cross-linking in gelatin preparations for the study of polymerization behavior, either formaldehyde or environmental conditions such as temperature, humidity, and light (alone or a combination thereof) have been used as stress methods. The conditions and the duration of tests are listed in Tables III and IV. The FDA Industry Gelatin Capsule Working Group mainly uses formaldehyde for stressing (46,48,56). Formaldehyde in various concentrations is added to the fill of HGCs and SGCs, and the formulations are exposed to accelerated conditions of temperature (40 °C) and humidity (75% RH) for periods of ≤6 months (see Table III). Table IV shows that a short or even an extended time period is used for stressing with humidity, temperature, and light either alone or in combination.

A major disadvantage of these test methods is the prolonged exposure period at accelerated conditions of temperature and humidity that is required before a sufficient adverse effect is noticed. A waiting period of several months is unreasonably slow, especially during formulation and packaging development when repeated trials must be carried out. Therefore, a better and more acceptable method is one whose duration is short and thereby provides opportunities for repeated studies.

A rapid test method. Recently, Singh et al. (33) proposed a test of much shorter duration that simultaneously uses all three environmental factors — temperature, humidity, and light — instead of only one or two factors at a time (Table IV). A mere 8 days' exposure of samples in a photostability chamber at 40 °C and 75% RH with a total illumination of 2 million lux h visible light and UV light of >200 Wh/m^2 causes pellicle formation in all formulations containing gelatin in the outer layer. Unlike the formaldehyde procedure used by the FDA–USP Industry Gelatin Capsule Working Group (46,48,56), this test method can simulate the conditions to which the product is exposed during its manufacture, transportation, distribution, and storage. The test uses the same conditions as those recommended in ICH guidelines for accelerated stability testing of pharmaceutical drug substances and products (75).

As described in an even more recent report, Venugopal and Singh (64) extended the test to include the evaluation of gelatin raw materials. The objective of the test was to predetermine which of the gelatin materials, of those available from various sources, would be least likely to create a decrease in dissolution when used in the manufacture of formulations. The films made from various raw materials were subjected to photostability-chamber exposure as well as to various doses of formaldehyde. The two stress methods revealed nearly the same results. On the basis of the results, it was possible to systematize the various raw materials according to their potential to undergo cross-linking. That is, each material was identified by the extent that it could show the problem of cross-linking when it was used in formulations.

Table IV: Literature reports about the influence of temperature, humidity, and light on the dissolution of gelatin-containing formulations.

Dosage Form and Drug	Storage conditions: Temp. (°C)*	%RH	UV and Vis Illumination	Time Period	Effect on Dissolution	Reference
Sugar-coated tablets:						
Phenylbutazone	20, 37, 50	—	—	2–14 weeks	Decrease in those stored at 50 °C for 14 weeks	24
Valproic acid	45 40	— 75	—	1, 2, and 3 months	Signficant decrease in 2- and 3-month samples	89
Ibuprofen	37	75	—	4 weeks	Significant decrease	29
Riboflavin	45	—	—	—	Significant decrease	30
Acetaminophen	RT	—	—	7 months	No change	31
	RT	High	—	3, 5, and 7 months	Significant decrease	
Hard gelatin capsules:						
Chloramphenicol	25	49 66 80	—	32 weeks	No change No change No release till 1 h	23
Nitrofurantoin	40	79		2 and 10 weeks	Significant decrease in 10-week samples	90
Gemfibrozil	37 37 45	— 80 —	— — —	1, 2, and 3 months	— Significant decrease at 1 month —	47
Hydrophobic drug in various colored capsules	—	80 80 80	Ambient light Fluorescent UV	2 weeks 2 weeks 2 days	Significant decrease Significant decrease Significant decrease	41
Hydrophophic drug in clear capsules	—	80	Ambient light Fluorescent	4 weeks 4 weeks	No change No change	41
Etodolac	40	75	—	8–20 weeks	Significant decrease in all	42
Triamterene/ hydrochlorothiazide	40	85	—	4 weeks	Significant decrease for both drugs	86
Poorly water-soluble drug	40	75	—	Ongoing stability study	Significant decrease	58
Acetaminophen	40 25	75 60	—	55 days 52 weeks	Significant decrease Significant decrease	56
Hard gelatin capsule shells	81	37	—	12–14 and 21 weeks	Significant decrease in all	60
Soft gelatin capsules:						
Digoxin	5, 25, and 37	—	—	1, 3, 6, and 10 weeks	Significant decrease in 10-week samples	50
Medium-chain triglycerides	⩾40	—	—	6 months	Significant decrease	71, 72
Acetaminophen and nifedipine	25 40	60 75	—	2–26 weeks	Significant decrease Significant decrease	48
Vitamins	40	75	—	6 and 24 months	Significant decrease in all	54
Acetaminophen	40 25	75 60		55 days 52 weeks	Significant decrease Significant decrease	56

*RT = room temperature.

Techniques used to determine the nature and extent of gelatin cross-linking

A few techniques have been described for the determination of the nature and extent of gelatin cross-linking occurring in films and formulations subjected to stress tests or marketed formulations during their shelf storage. The techniques are described in this section.

Carbon 13–nuclear magnetic resonance (NMR) spectroscopy. This technique can be used to study the mechanism and the site of development of cross-links (63,76–78). The technique de-

termines the involvement of amino groups in lysine–lysine, lysine–arginine, and arginine–arginine cross-links subsequent to reaction with formaldehyde. Gold et al. used Carbon 13–NMR to establish that pancreatin, a proteolytic enzyme present in the gastrointestinal tract, depolarized the cross-linked gelatin (76).

Fourier transform near-infrared (FT-NIR) spectroscopy. This technique is advantageous because it is rapid and nondestructive. Gold et al. reported how it is used to monitor the migration of formaldehyde from a PEG fill into the gelatin shell of SEGCs (61,62). The capsules were filled with various solutions of formaldehyde in PEG and stored at ambient conditions for 48 h. They then were emptied and scanned in a NIR spectrophotometer. A linear relationship was found between the NIR spectra and the amount of cross-linking induced by various concentrations of formaldehyde.

FT-IR spectroscopy. Salsa et al. reported the use of FT-IR spectroscopy for the determination of cross-linking of gelatin during a reaction with formaldehyde (79). The spectra were recorded in a potassium bromide pellet at various times during the reaction of an aqueous solution of formaldehyde with gelatin. The interpretation of the results involved principal component–regression analysis. It was established that the cross-linking reaction was initialized by the lysine–methylol formation, followed by the formation of an arginine–methylol link and the eventual origination of lysine–arginine cross-links.

UV and fluorescence spectrophotometry. Ofner et al. reported the use of a chemical assay method that uses 2,4,6-trinitrobenzenesulfonic acid (TNBS) reagent and resulted in the development of a UV chromophore that absorbs at 346 nm (14,60,80,81). The reaction of TNBS with the primary amino group of gelatin determines the un-cross-linked amino groups and hence helps reveal the loss of ϵ-amino groups, which participate in the cross-linking process. Another recent study reported about the use of intrinsic fluorescence determination for describing conformational changes in gels made as a result of the interaction of gelatin with glutaraldehyde (82).

Magnetic resonance imaging (MRI). The use of MRI for the study of gelatin cross-linking was explored recently by a group led by Professor J.H. Hornak at the Rochester Institute of Technology, Rochester, New York (www.cis.rit.edu/people/faculty/hornak/jph-part-2.htm). The group concluded that the technique can be used for the study of both the diffusion of ions into a gelatin–water matrix and the setting of gelatin as a result of cross-linking.

Monitoring of solubility and dissolution. Other methods used to determine gelatin cross-linking are the determination of the solubility of gelatin films or the dissolution of formulations (64,83). Although these procedures do not provide information about the mechanism or site of interaction and do not quantify the extent of cross-linking at the molecular level, they give a crude idea about the extent because the reduction in solubility or dissolution is linearly correlated to the extent of cross-linking (64). Some indirect methods to determine the amount of gelatin remaining in the undissolved state also have been used (e.g., gravimetric analysis, a protein assay method involving color reaction with bicinchoninic acid, and UV absorbance measurements at 214 nm) (60,83).

Inhibition of gelatin cross-linking

Much effort has been applied to identifying the means to protect gelatin-based formulations against changes in dissolution characteristics. Many approaches exist such as using additives and direct inhibitors (see Table V) and controlling humidity and photostabilization.

Using Type B gelatin. Type B gelatin is mentioned in the literature to be associated with less cross-linking than is type A gelatin, but unfortunately no details of the study are reported (84). Therefore, experiments can be conducted to evaluate the use of Type B gelatin for the development of more-stable pharmaceutical formulations (84).

Protecting against released aldehydes or preventing the formation of aldehydes. Compounds such as lysine, phenylamine, glutamine, hydroxylamine hydrochloride, *p*-amino benzoic acid, glycine, and others function as carbonyl scavengers, preventing the interaction of aldehydes with gelatin shells and thereby inhibiting cross-linking (85). It even is reported that if the formaldehyde initially present in the capsule fill is scavenged by the use of glycine, an amino acid, it prevents or reduces the further introduction of aldehyde (86). Another approach is to prevent the very formation of aldehydes, a process that can be accomplished by controlling the degradation of the capsule contents through manipulation of pH. Carboxylic acids such as benzoic acid, fumaric acid, maleic acid, and citric acid have been found to be effective for this purpose. Trials have shown that using a combination of an amino acid and a buffer significantly prevents pellicle formation. A typical example is the synergistic use of glycine and citric acid.

Use of direct inhibitors. Some compounds act as direct inhibitors, and they also have been found to help protect gelatin-based formulations against changes in dissolution characteristics. Examples include semicarbazide hydrochloride, hydroxylamine hydrochloride, piperazide hydrate, pyridine, piperidine, glycerine, and *p*-aminobenzoic acid (85,86).

Control of humidity. The dissolution characteristics of capsules become more seriously affected when they are stored in blister packaging made of PVC, which affords minimal protection against moisture. The best way to overcome this adverse effect is to use water-impermeable packaging systems. An alternate method is to add disintegrants to the HGC fill powder blend. Capsule formulations containing ⩾10% of disintegrant can withstand the stress of high-humidity storage conditions presumably because of the more-porous nature of the capsule fill (87).

Photostabilization. Thoma has reported photostabilization of gelatin capsules using two distinct approaches: coloring or pigmentation of the gelatin shell and core and manipulation of the thickness of the shell, size of granules or powder particles, and the size and height of the core (88). It has been shown that titanium oxide, iron oxide, and color pigments offer good protection against cross-linking introduced by light. A curcumin content of 0.4% in the capsule shell resulted in a threefold or higher increase in the half-life of the test compounds. Some dyes such as FD&C Yellow No. 5, Blue No. 1, and Red No. 3 also were able to protect dosage forms from light. The same was true of synthetic iron oxides, which are potent absorbers of wavelengths

<400 nm. However, an excess iron content (>15 ppm) should not be used because it causes discoloration in SGCs (45).

Conclusion

In the past few decades an all-encompassing understanding has developed about the problem of the decrease in the extent of dissolution in dosage forms containing gelatin in the outer layer. It is heartening that this problem is not so serious with respect to in vivo bioavailability unless the preparations are severely stressed or the drug has a narrow window of absorption (46).

The introduction of USP's two-tier dissolution test is a welcome development because it can save the manufacturer from difficulties associated with failed dissolution tests. Unfortunately the two-tier test is not yet official in other pharmacopeias. Therefore, gelatin products prepared according to compendia other than *USP* stand a chance of being recalled if they fail the standard dissolution tests. In this context, the remaining pharmacopeial organizations in the world must be cognizant of current developments concerning the problem and thus introduce necessary modifications in dissolution tests for gelatin preparations.

At their level, manufacturers can protect their interests by selecting gelatin raw materials that show minimum cross-linking behavior, a process that can be carried out easily with a recently proposed test (64). Alternately, the use of inhibitors (see Table V) and other approaches such as the use of aldehyde-free excipients can be explored to stabilize the preparations. Help also can be had from the recently reported rapid-test method for the study of the possibility of cross-linking in formulated products (33).

Table V: Agents that inhibit cross-linking of gelatin.

Inhibiting Agent	Preparation	Effect Observed	Reference
Semicarbazine hydrochloride, hydroxylamine, hydrochloride, piperazide hydrate, pyridine, piperidine, glycine, *p*-amino benzoic acid	Not specified	Cross-linking inhibiting agents	85
Sorbitol as a plasticizer	Gelatin films (Type B)	Showed increase dissolution	91
Semicarbazide HCl (1%)		Decreased cross-linking	
L-Histidine (1%)		Decreased cross-linking	
Citric acid (1%)		Decreased cross-linking	
Glycine (2.5% w/w)	Formulation	Showed some improvement in dissolution but did not prevent gelatin cross-linking	86
Citric acid (0.5% w/w),		Showed some improve ment in dissolution but did not prevent gelatin cross-linking	
Glycine (2.5% w/w), and Citric acid (0.5% w/w)		Cross-linking prevented	

References

1. *The Merck Index* (Merck & Co., Rahway, NJ, 11th ed., 1989), p. 685.
2. G.A. Digenis, T.B. Gold, and V.P. Shah, "Cross-Linking of Gelatin Capsules and Its Relevance to Their In Vitro–In Vivo Performance," *J. Pharm. Sci.* **83** (7), 915–921 (1994).
3. J.A.J. Robinson, I.W. Kellaway, and C. Marriott, "Effects of Blending on the Rheological Properties of Gelatin Gels," *J. Pharm. Pharmacol.* **27,** 188–824 (1975).
4. H.J. Peters, E.M.V. Bommel, and J.G. Fokkens, "Effect of Gelatin Properties in Complex Coacervation Processes," *Drug Dev. Ind. Pharm.* **18** (1), 123–134 (1992).
5. N.T. Naidoo, "Coating Trends of Solid Dosage Forms," *S. A. Pharm. J.* **59,** 86–87 (1992).
6. L.A. Felton et al., "Physical–Mechanical Properties of Film-Coated Soft Gelatin Capsules," *Int. J. Pharm.* **127** (17), 203–211 (1996).
7. L.A. Felton et al., "Physical and Enteric Properties of Soft Gelatin Capsules Coated with Eudragit L30 D-55," *Int. J. Pharm.* **113** (2), 17–24 (1995).
8. M. Guyot, F. Fawaz, and M. Maury, "In Vitro Release of Theophyllin from Cross-Linked Gelatin Capsules," *Int. J. Pharm.* **144,** 209–216 (1996).
9. B.E. Jones, in *Hard Capsules — Development and Technology,* K. Ridgway, Ed. (The Pharmaceutical Press, London, UK, 1987), pp. 39–48.
10. E.A. Swinyard and W. Lowenthal, "Oral Solid Dosage Forms," in *Remington: The Science and Practice of Pharmacy,* A.R. Gennaro, Ed. (Mack, Easton, PA, 1995), pp.1615–1649.
11. G.-L. Chen and W.-H. Hao, "Factors Affecting Zero-Order Release Kinetics of Porous Gelatin Capsules," *Drug Dev. Ind. Pharm.* **24** (6), 557–562 (1998).
12. Y. Tabata and Y. Ikada, "Synthesis of Gelatin Microspheres Containing Interferon," *Pharm. Res.* **6** (5), 422–427 (1989).
13. M.A. Vandelli et al., "Concentration of Cross-Linking Agent as a Tool for the Control of Release and Swelling Properties of Gelatin Microspheres," *J. Pharm. Bel.* **46,** 381–388 (1991).
14. M.A. Vandelli et al., "Gelatin Microspheres Cross-linked with D,L-Glyceraldehyde as a Potential Drug Delivery System: Preparation, Characterisation, In Vitro and In Vivo Studies," *Int. J. Pharm.* **215,** 175–184 (2001).
15. X. Lou and T.V. Chirila, "Swelling Behavior and Mechanical Properties of Chemically Cross-Linked Gelatin Gels for Biomedical Use," *J. Biomater. Appl.* **14** (2), 184–191 (1999).
16. V.L. Truong-Le, J.T. August, and K.W. Leong, "Controlled Gene Delivery by DNA–Gelatin Nanospheres," *Hum. Gene. Ther.* **9** (12), 1709–1717 (1998).
17. M.S. Tesconi, S.L. Bramer, and S.H. Yalkowsy, "The Preparation of Soft Gelatin Capsules for a Radioactive Tracer Study," *Pharm. Dev. Technol.* **4** (4), 507–513 (1999).
18. M. Weiner and L. Bernstein, *Adverse Drug Reaction to Drug Formulation Agents: A Handbook on Excipients;* Marcel Dekker, New York, NY, 1989.
19. H. Al-Dujaili, A.T. Florence, and E.G. Salole, "Adhesiveness of Proprietary Tablets and Capsules to Porcine Esophageal Tissue," *Int. J. Pharm.* **34,** 75–79 (1986).
20. D.A. Swisher, S.L. Sendelbeck, and J.W. Fara, "Adherence of Various Oral Dosage Forms to the Esophagus," *Int. J. Pharm.* **22,** 219–228 (1984).
21. Y. Blanloeil et al., "Severe Anaphylactoid Reactions after Infusion of Modified Gelatin Solution," *Therapie* **38,** 539–546 (1983).
22. J.T. Carstensen and C.T. Rhodes, "Pellicule Formation in Gelatin Capsules," *Drug Dev. Ind. Pharm.* **19** (20), 2709–2712 (1993).

23. S.A.H. Khalil, L.M.M. Ali, and A.M.M.A. Khalek, "Effects of Aging and Relative Humidity on Drug Release, Part 1: Chloramphenicol Capsules," *Pharmazie* **29** (1), 36–37 (1974).
24. D. Barrett and J.T. Fell, "Effect of Aging on Physical Properties of Phenylbutazone Tablets," *J. Pharm. Sci.* **64** (2), 335–337 (1975).
25. J.W. Warren and E.J. Rowe, "Influence of a Sugar Coating on Certain Physical Properties of Series of Core Tablets," *Can. J. Pharm. Sci.* **10** (2), 40–45 (1975).
26. S.R. Chapman et al., "Dissolution of Propantheline Bromide Tablets B.P.," *J. Pharm. Pharmacol.* **32,** 20P (1980).
27. S.A. El-Fattah and S.A.H. Khalil, "Variations in Dissolution Rates of Sugar-Coated Chlorpromazine Tablets," *Int. J. Pharm.* **18,** 225–234 (1984).
28. C.O. Ondari et al., "Effects of Short-Term Moderate Storage Stress on the Disintegration and Dissolution of Four Types of Compressed Tablets," *Pharm. Acta. Helv.* **59** (5–6), 149–153 (1984).
29. A.J. Romero, L.T. Grady, and C.T. Rhodes, "Dissolution Testing of Ibuprofen Tablets," *Drug Dev. Ind. Pharm.* **14** (11), 1549–1586 (1988).
30. S.A.H. Khalil, N.S. Barakat, and N.A. Boraie, "Effect of Aging on Dissolution Rates and Bioavailability of Riboflavin Sugar-Coated Tablets," *S. T. P. Pharm. Sci.* **1,** 189–194 (1991).
31. T.C. Dahl, I.L.T. Sue, and A. Yum, "The Effect of Pancreatin on the Dissolution Performance of Gelatin-Coated Tablets Exposed to High Humidity Conditions," *Pharm. Res.* **8** (3), 412–414 (1991).
32. I.G. Shah and D.L. Parsons, "Preparation and In Vitro Evaluation of Powder Solution Tablets of Valproic Acid," *Drug Dev. Ind. Pharm.* **19** (13), 1587–1602 (1993).
33. S. Singh, R. Manikandan, and S. Singh, "Stability Testing for Gelatin-Based Formulations: Rapidly Evaluating the Possibility of a Reduction in Dissolution Rates," *Pharm. Technol.* **24** (5), 58–72 (2000).
34. K.S. Murthy and I. Ghebre-Sellassie, "Current Perspectives on the Dissolution Stability of Solid Oral Dosage Forms," *J. Pharm. Sci.* **82** (2), 113–126 (1993).
35. R.K. Chang, K.S. Raghavan, and M.A. Hussain, "A Study on Gelatin Capsule Brittleness: Moisture Transfer between the Capsule Shell and Its Content," *J. Pharm. Sci.* **87** (5), 556–558 (1998).
36. F. Langenbucher, "Linearization of Dissolution Rate Curves by the Weibull Distribution," *J. Pharm. Pharmacol.* **24,** 979–981 (1972).
37. P. York, "The Shelf Life of Some Antibiotic Preparations Stored under Tropical Conditions," *Pharmazie* **32,** 101–104 (1977).
38. H. Mohamad et al., "Investigation of Drug Biopharmaceutical Stability: Application to Capsules of Tetracycline Hydrochloride, Part 2: In Vivo Study," *S.T.P. Pharma.* **2,** 630–635 (1986).
39. M. Georgarakis, P. Hatzipantou, and J.E. Kountourelis, "Effect of Particle Size, Content in Lubricant, Mixing Time, and Storage Relative Humidity on Drug Release from Hard Gelatin Capsules," *Drug Dev. Ind. Pharm.* **14** (7), 915–923 (1988).
40. K.S. Murthy, R.G.J. Reisch, and M.B. Fawzi, "Dissolution Stability of Hard-Shell Capsule Products, Part II: The Effect of Dissolution Test Conditions on In Vitro Drug Release," *Pharm. Technol.* **13** (6), 53–58 (1989).
41. K.S. Murthy, N.A. Enders, and M.B. Fawzi, "Dissolution Stability of Hard-Shell Capsule Products, Part I: The Effect of Exaggerated Storage Conditions," *Pharm. Technol.* **13** (3), 72–84 (1989).
42. M. Dey et al., "The Dissolution and Bioavailability of Etodolac from Capsules Exposed to Conditions of High Relative Humidity and Temperatures," *Pharm. Res.* **10** (9), 1295–1300 (1993).
43. J.P. Stanley, "Capsules: Soft Gelatin Capsules," in *Theory and Practice of Industrial Pharmacy,* L. Lachman, H.A. Lieberman, and J.L. Kanig, Eds. (Lea & Febiger, Philadelphia, PA, 1991), pp. 398–412.
44. H. Seager, "Soft Gelatin Capsules: Solution to Many Tableting Problems," *Pharm. Technol.* **9,** 84–104 (1985).
45. R.F. Jimerson, "Soft Gelatin Capsule Update," *Drug Dev. Ind. Pharm.* **12** (8, 9), 1133–1144 (1986).
46. J. Brown et al., "The Effects of Cross-Linking on the In Vivo Disintegration of Hard Gelatin Capsules," *Pharm. Res.* **15** (5), 1026–1030 (1998).
47. L. Chafetz et al., "Decrease in the Rate of Capsule Dissolution Due to Formaldehyde from Polysorbate 80 Autooxidation," *J. Pharm. Sci.* **73** (8), 1186–1187 (1984).
48. C.B. Bottom, M. Clark, and J.T. Carstensen, "Dissolution Testing of Soft-Shell Capsules — Acetaminophen and Nifedipine," *J. Pharm. Sci.* **86** (9), 1057–1061 (1997).
49. A.F. Asker, M.M. Abdel-Khalek, and I. Machloof, "Effect of Scaling Up and Formulation Factors on the Qualities of Prednisone Tablets," *Drug Dev. Ind. Pharm.* **7** (1), 79–111 (1981).
50. D.F. Johnson et al., "The Effects of Storage upon In Vitro and In Vivo Characteristics of Soft Gelatin Capsules Containing Digoxin," *J. Pharm. Pharmacol.* **29,** 576–578 (1977).
51. E.D. Martin, R.J.L. Fraser, and I. Camens, "Storage of Phenytoin Capsules," *Med. J. Aust.* **143,** 634–635 (1985).
52. H. Mohamad et al., "Investigation of Drug Biopharmaceutical Stability, Part 3: Study of Ampicillin Trihydrate Capsules (In Vitro–In Vivo Investigation)," *S.T.P. Pharma.* **2,** 912–917 (1986).
53. F.S. Hom, S.A. Veresh, and J.J. Miskel, "Soft Gelatin Capsules I: Factors Affecting Capsule-Shell Dissolution Rate," *J. Pharm. Sci.* **62,** 1001–1006 (1973).
54. K. Sukur, "Disintegration Test Media for Soft Gelatin Capsules — Need for Global Harmonization," paper presented at the Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), New Orleans, Louisiana, 14–18 November 1999.
55. G.A. Digenis et al., "Bioequivalence Study of Stressed and Unstressed Hard Gelatin Capsules using Amoxycillin as a Drug Marker and Gamma Scintigraphy to Confirm Time and GI Location of In Vivo Capsules Rupture," *Pharm. Res.* **17** (5), 572–582 (2000).
56. M.C. Meyer et al., "The Effect of Gelatin Cross-Linking on the Bioequivalence of Hard and Soft Gelatin Acetaminophen Capsules," *Pharm. Res.* **17** (8), 962–966 (2000).
57. USP, "Physical Tests and Determinations," *Pharm. Forum* **25,** 7516 (1999).
58. J.M. Shaw et al., "Studies Directed Toward the Development of Enzymatic Dissolution Test Methods for Cross-Linked Hard Gelatin Capsule Formulations Containing Poorly Water-Soluble Drug Substances," paper presented at the Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), New Orleans, Louisiana, 14–18 November 1999.
59. E. Doelker and A.C. Vial-Bernasconi, "Shell-Content Interactions in Gelatin Capsules and a Critical Evaluation of Their Effects on Drug Availability," *S.T.P. Pharma.* **4,** 298–306 (1988).
60. C.M. Ofner III et al., "Cross-Linking Studies in Gelatin Capsules Treated with Formaldehyde and Exposed to Elevated Temperature and Humidity," *J. Pharm. Sci.* **90** (1), 78–88 (2001).
61. T.B. Gold et al., "Determination of Extent of Formaldehyde-Induced Cross-Linking in Hard Gelatin Capsules by Near-Infrared Spectrophotometry," *Pharm. Res.* **14** (8), 1046–1050 (1997).
62. T.B. Gold et al., "Detection of Formaldehyde-Induced Cross-Linking in Soft Elastic Gelatin Capsules Using Near-Infrared Spectrometry," *Pharm. Dev. Technol.* **3** (2), 209–214 (1998).
63. S.K. Taylor, F. Davidson, and D.W. Ovenall, "Carbon 13–Nuclear Magnetic Resonance Studies on Gelatin Cross-Linking by Formaldehyde," *Photogr. Sci. Eng.* **22,** 134–138 (1978).
64. K. Venugopal and S. Singh, "Evaluation of Gelatins for Cross-Linking Potential," *Pharm. Technol. Drug Delivery,* Supplement, 32–37 (2001).
65. J.R. Schwier et al., "Rayon: Source of Furfural — A Reactive Aldehyde Capable of Insolublizing Gelatin Capsules," *Pharm. Technol.* **17,** 78–80 (1993).
66. J.J.W. Cooper, H.C. Ansel, and D.E. Cadwallader, "Liquid and Solid Interactions of Primary Certified Colorants with Pharmaceutical Gelatins," *J. Pharm. Sci.* **62** (7), 1156–1164 (1973).
67. B.E. Jones, "Hard Gelatin Capsules," *Manuf. Chem. Aerosol News* **40,** 22–24 (1969).
68. M.L. Ray-Johnson and I.M. Jackson, "Temperature-Related Incompatibility between Gelatin and Calcium Carbonate in Sugar-Coated Tablets," *J. Pharm. Pharmcol.* **28,** 309–310 (1976).

69. K.J. Hartauer et al., "Effect of Rayon Coiler on the Dissolution Stability of Hard-Shell Gelatin Capsules," *Pharm. Technol.* **17,** 76–83 (1993).
70. T. Hakata et al., "Effect of Storage Temperature on Disintegration Time of Soft Gelatin Capsules," *J. Pharm. Sci. Technol. Japan* **41,** 276–281 (1981).
71. T. Hakata et al., "Effect of Storage Temperature on the Physicochemical Properties of Soft Gelatin Capsule Shells," *Chem. Pharm. Bull.* **42,** 1496–1500 (1994).
72. T. Hakata et al., "Effect of Formaldehyde on the Physicochemical Properties of Soft Gelatin Capsule Shells," *Chem. Pharm. Bull.* **42,** 1138–1142 (1994).
73. S. Khalil, N. Barakat, and N. Boraie, "Insolubility of Gelatin Films in Systems Simulating the Subcoat of Sugar-Coated Tablets," *S.T.P. Pharm. Sci.* **1** (2), 145–150 (1991).
74. J. Lengyel, "Investigation and Evaluation of the Utilization of Gelatin Treated with Ionizing Radiation in the Pharmaceutical lndustry," *Gyogyszereszet* **20** (1), 5–7 (1976).
75. International Conference on Harmonization, "Stability Testing of New Drug Substances and Products," (International Conference on Harmonization, Geneva, Switzerland, November 2000).
76. T.B. Gold, S.L. Smith, and G.A. Digenis, "Studies on the Influence of pH and Pancreatin on 13C-Formaldehyde–Induced Gelatin Cross-Links Using Nuclear Magnetic Resonance," *Pharm. Dev. Technol.* **1** (1), 21–26 (1996).
77. K. Albert et al., "Cross-Linking of Gelatin with Formaldehyde: A ^{13}C NMR Study," *Z. Naturforsch* **41b,** 351–358 (1986).
78. K. Albert et al., "Investigation of the Hardening Reaction of Gelatin with ^{13}C-Labeled Formaldehyde by Solution and Solid-State ^{13}C NMR Spectroscopy," *Z. Naturforsch* **46b,** 385–389 (1991).
79. T. Salsa, M.E. Pina, and J.J.C. Teixeira-Dias, "Cross-Linking of Gelatin in the Reaction with Formaldehyde: An FTIR Spectroscopic Study," *App. Spectrosc* **50,** 1314 (1996).
80. W.A. Bubnis and C.M. Ofner III, "The Determination of ε-Amino Group in Soluble and Poorly Soluble Proteinaceous Materials by a Spectrophotometric Method Using Trinitrobenzenesulfonic Acid," *Anal. Biochem.* **207,** 129–133 (1992).
81. C.M. Ofner III and W.A. Bubnis, "Chemical and Swelling Evaluations of Amino Group Cross-Linking in Gelatin and Modified Gelatin Matrices," *Pharm. Res.* **13** (12), 1821–1827 (1996).
82. W.G. Liu et al., "Intrinsic Fluorescence Investigation on the Change in Conformation of Cross-Linked Gelatin Gel During Volume Phase Transition," *Polymer* **41,** 7589–7592 (2000).
83. M.M. Welz and C.M. Ofner III, "Examination of Self-Cross-Linked Gelatin as a Hydrogel for Controlled Release," *J. Pharm. Sci*. **81** (1), 85–90 (1992).
84. M.C. Levy and M.C. Andry, "Microencapsulation par Reticulation Interfaciale de Gelaitine," *S.T.P. Pharm. Sci.* **3,** 644–651 (1987).
85. C.A. Marks, D. Tourtellotte, and A. Andux, "A Phenomenon of Gelatin Insolubility," *Food Technol.* **22,** 1433–1436 (1968).
86. T.A. Adesunloye and P.E. Stach, "Effect of Glycine–Citric Acid on the Dissolution Stability of Hard Gelatin Capsules," *Drug Dev. Ind. Pharm.* **24** (6), 493–500 (1998).
87. T.C. Dahl, I.T. Sue, and A. Yum, "The Influence of Disintegrant Level and Capsule Size on Dissolution of Hard Gelatin Capsules Stored in High-Humidity Conditions," *Drug Dev. Ind. Pharm.* **17** (7), 1001–1016 (1991).
88. K. Thoma, "Photodecomposition and Stabilization of Compounds in Dosage Forms" in *The Photostability of Drugs and Drug Formulations,* H.H. Tonnesen, Ed. (Taylor & Francis, London, UK, 1996), pp.111–140.
89. V.P. Shah et al., "Phenytoin I: In Vitro–In Vivo Correlation for 100-mg Phenytoin Sodium Capsules," *J. Pharm. Sci.* **72** (3), 306–308 (1983).
90. H.W. Gouda, M.A. Moustafa, and H.I. Al-Shora, "Effect of Storage on Nitrofurantoin Solid Dosage Forms," *Int. J. Pharm.* **18,** 213–215 (1984).
91. F.S. Hom, "Soft Gelatin Capsules III: An Accelerated Method for Evaluating the Dissolution Stability of Various Gel Formulations," *Drug Dev. Ind. Pharm.* **10** (2), 275–287 (1984). **PT**