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RESEARCH PAPER

Effect of Glycine/Citric Acid on the Dissolution Stability of Hard Gelatin Capsules

T. A. Adesunloye* and P. E. Stach

Pharmaceutical Research and Development, Geneva Pharmaceuticals, Broomfield, Colorado 80038-0446

ABSTRACT

Gelatin capsule crosslinking is a well-known phenomenon that results in reduced dissolution of capsule products with the passage of time and/or under accelerated stability conditions. These studies describe one means of preventing capsule crosslinking by incorporating glycine and citric acid into a triamterene/hydrochlorothiazide 37.5/25 mg capsule formulation (triam/HCTZ). Triam/HCTZ without glycine and citric acid showed extensive capsule crosslinking and then failed the USP dissolution specification after a 4-week accelerated (40°C/85% relative humidity [RH]) stability study. Triam/HCTZ containing glycine alone showed some improvement in the dissolution stability but did not prevent gelatin crosslinking. This formulation also failed dissolution specifications after a 4-week accelerated stability study. The same results were obtained when only citric acid was incorporated into the triam/HCTZ. However when glycine and citric acid were incorporated together into the triam/HCTZ, crosslinking was completely prevented. Dissolution profiles remained the same throughout 12-week accelerated stability studies, with little or no drop in the dissolution values throughout the test period. The above results were confirmed with follow-up studies using gemfibrozil and piroxicam as model drugs. Disintegration times for gemfibrozil and piroxicam capsule formulations without glycine and citric acid increased dramatically with observed pellicle formation, but there was little or no change in the disintegration time of the model drugs formulated with glycine and citric acid. The results of these studies demonstrated that when glycine and citric acid are present in some gelatin capsule formulations, pellicle formation or crosslinking of the capsule gelatin is prevented.

*To whom correspondence should be addressed.

INTRODUCTION

Hard and soft gelatin capsule dosage forms are very popular in the pharmaceutical industry. Gelatin capsules provide an easy method of orally administering a drug. They are pharmaceutically elegant and disintegrate rapidly once the capsule enters the stomach, presenting the capsule contents to the gastric environment for the process of dissolution. However, there is the risk that the gelatin capsule may suffer from retardation of disintegration and dissolution with the passage of time and/or when placed under accelerated conditions (high temperature and humidity). One proposed mechanism is commonly referred to as crosslinking. Crosslinking is a carbonyl/gelatin phenomenon which results in insolubility of the capsule shell, and poor dissolution stability in some gelatin capsule products. Crosslinking is facilitated, and/or accelerated, when the formulation in the capsule either contains a carbonyl compound as an impurity or decomposes into a carbonyl compound or derivatives such as aldehydes, especially formaldehyde. With the passage of time and/or under the accelerated conditions, aldehydes react with the amino acid groups within the gelatin shell to generate a crosslinked structure. This is described as a very thin, tough, and water-insoluble film noted around the capsule contents during dissolution testing in USP dissolution media without enzyme. This film does not disrupt easily by gentle agitation and is usually referred to as a pellicle (1). As a result, water cannot enter between the polypeptide chains of the gelatin, and the gelatin becomes insoluble. The disruption of this film seems to be the dissolution rate limiting factor for the drug product.

The effect of accelerated storage conditions on the dissolution stability of hard shell capsule products was studied by Murthy et al. and a combination of high temperature and humidity were found to facilitate and/or accelerate gelatin capsule crosslinking (2). Other possible causes of gelatin capsule crosslinking include formaldehyde formed by the auto-oxidation of excipients such as polysorbate 80 in gemfibrozil capsules (3). This auto-oxidation results in denaturation of the inner surface of the capsule and a significant decrease in gemfibrozil capsule dissolution rate with the passage of time and exposure to humidity. The presence of colorants, especially FD&C red #3 and FD&C red # 40, is also known to play a crucial role in modifying the conformational properties of gelatin and rendering it insoluble (4,5). Rayon has been identified as a potential source of furfural, which is a reactive aldehyde capable of insolubilizing gelatin capsules. Digenis et al. (6) stress the importance of the interaction that occurs in the presence of certain chemical entities which may contribute to the occurrence of gelatin crosslinking in the gelatin shell of dosage forms. Thus, careful attention should be paid to the purity and chemical reactivity of all the excipients that are to be encapsulated in gelatin. Testing for contamination by formaldehyde as well as low molecular weight aldehydes should be a standard part of excipient evaluation procedures.

Materials which promote gelatin crosslinking include (7) glucose, formaldehyde, glutaraldehydes, glyceraldehyde, hydrogen peroxide, benzene, sulfonic acid, *p*-toluene sulfonic acid, and guanidine hydrochloride. Inhibitors of gelatin capsule crosslinking include (7) semicarbazide, hydrochloride, hydroxylamine hydrochloride, piperazide hydrate, pyridine, piperidine, glycerine, and *p*-aminobenzoic acid.

Current literature tends to indicate that crosslinking has a much greater impact on the result of in vitro dissolution testing than on the in vivo bioavailability of drugs formulated in gelatin capsules (6,8,9). However, since in vitro dissolution testing is commonly employed as a method of measuring the stability of drug products, it is important to utilize a capsule fill which minimizes crosslinking in the capsule shell and thus minimizes the impact of time and/or accelerated conditions on the dissolution profile of the filled gelatin capsule. To help compensate for this in vitro pellicle effect, the dissolution test of the capsule product in which pellicle formation is likely could employ media containing pepsin or pancreatin (10).

MATERIALS AND METHODS

Triamterene and piroxicam were obtained from Interchem/Moehs, Spain. Hydrochlorothiazide and gemfibrozil were obtained from Plantex, Israel, and SST/VIS, Italy, respectively. All excipients used were NF/USP grades.

Formulas A–D (Table 1) are triamterene/hydrochlorothiazide formulations (Triam/HCTZ). Formula A is the control formula with neither glycine nor citric acid. Formulas B and C contained glycine and citric acid, respectively, and formula D contained both glycine and citric acid. The above formulas were manufactured by a wet granulation method, similar to the method described below for the piroxicam and gemfibrozil formulations. Triam/HCTZ formulations were encapsulated in #4 gelatin capsules made with no colorant. Formulas E and F (Table 2) were gemfibrozil formulations and for-

Table 1

Triam/HCTZ Formulations

Ingredients	Formulas (%w/w) A	B	C	D
Triamterene	23.01	23.01	23.01	23.01
Hydrochlorothiazide	15.34	15.34	15.34	15.34
Glycine	–	2.50	–	2.50
Citric acid	–	–	0.50	0.50
Others	61.65	59.15	61.15	58.65

mulas G and H (Table 3) were piroxicam formulations. Glycine and citric acid were added to formulas F and H to study the effect of glycine/citric acid on gemfibrozil and piroxicam gelatin capsule disintegration stability and crosslinking.

Gemfibrozil and piroxicam formulations were manufactured by an aqueous wet granulation process. Active raw materials were placed into a Glatt 5 liter vertical mixer (Glatt Air Techniques Inc., Ramsey, NJ), along with lactose, corn starch, intragranular sodium starch glycolate, colloidal silicon dioxide, and povidone. These materials were mixed and granulated with polysorbate solution (polysorbate dissolved in purified water). Additional purified water was added until granules formed and no dry powder remained. Where applicable, glycine and citric acid were dissolved in the purified water. Wet granules were tray-dried in an oven at 110°F (Gruenberg, Williamsport, PA) until the loss on drying (LOD) was not more than 2.0%. Dried granules were milled with the extragranular sodium starch glycolate, using a Fitzpatrick comminutor, model D6 (Fitzpatrick Co., ELmhurst, IL), and blended and lubricated with screened magnesium stearate in a P-K twinshell blender (Patterson-Kelly Co., East Stroudsburg, PA). Gemfibrozil formulations (formulas E and F) were encapsulated in size 0 capsules which contained FD&C yellow #6, FD&C red #3, blue #1, and D&C red #33 as colorant. Piroxicam formulations (formulas G and H) were encapsulated in size 2 capsules which contain the following colorants, FD&C red #3, D&C yellow #10 lake, FD&C yellow #6 aluminum (al.) lake, and FD&C blue #1 al. lake. Capsules were packaged in 100 cm^3 high-density polyethylene (HDPE) plastic bottles and placed on accelerated stability stations (40°C/85% relative humidity [RH]) for 12-week stability studies.

The dissolution rate studies for the triam/HCTZ formulations (formulas A–D) were performed according to USP 23, supplement 3, test 1, dissolution method. Six

Table 2

Gemfibrozil Formulations

Formula	E (mg/capsule)	F (mg/capsule)
Gemfibrozil	100.000	100.000
Lactose, anhydrous	248.750	233.750
Corn starch	100.000	100.000
Sodium starch glycolate	25.000	25.000
Povidone	5.000	5.000
Polysorbate 80	15.000	15.000
Colloidal silicon dioxide	1.250	1.250
Glycine	–	12.500
Citric acid	–	2.500
Magnesium stearate	5.000	5.000
#0 Gelatin capsules	QS	QS
Total fill weight	500.000	500.000

QS: Quantity sufficient for manufacture.

Table 3

Piroxicam Formulations

Formula	G (mg/capsule)	H (mg/capsule)
Piroxicam	50.000	50.000
Lactose, anhydrous	124.375	116.875
Corn starch	50.000	50.000
Sodium starch glycolate	12.500	12.500
Povidone	2.500	2.500
Polysorbate 80	7.500	7.500
Colloidal silicon dioxide	0.625	0.625
Glycine	–	6.250
Citric acid	–	1.250
Magnesium stearate	2.500	2.500
#2 Gelatin capsules	QS	QS
Total fill weight	250.000	250.000

QS: Quantity sufficient for manufacture.

capsules were tested in a USP apparatus 1 (basket) at 100 rpm, in 0.1 N HCL solution at 37 ± 1°C. Physical disintegration of the gemfibrozil and piroxicam capsules carried out in a USP apparatus was 2 (paddle), with each of six capsules placed into 900 ml of purified water at 37 ± 1°C, at a paddle speed of 100 rpm.

RESULTS

As indicated in Tables 4 and 5, and Figs. 1 and 2, there was a significant drop in the dissolution profiles for formulas A, B, and C. For formula A only 13% of triam and 17% of HCTZ were released at the 45-min time point after 4 weeks at the accelerated condition. When glycine was added to the above formula (formula B), the dissolution profile improved slightly, but glycine alone did not completely prevent pellicle formation or the retardation of capsule dissolution in triam/HCTZ capsules. Only 50 and 54% of the triam and HCTZ, respectively, were released at 45 min after 4 weeks at the accelerated condition. Results from formula C with only citric acid also failed USP specifications at the 4-week time point. Only 27% of the triam and 31% of the HCTZ were released at 45 min after the 4-week time point. However, when glycine and citric acid were added to the formulation (formula D), the dissolution stability was greatly improved; the release rate for both triam and HCTZ was 96% in 45 min after a 12-week study at 40°C and 85% RH (Tables 4 and 5, Figs. 3 and 4).

Figure 5 shows the effect of glycine/citric acid on gemfibrozil 100 mg capsule disintegration stability. Gemfibrozil capsules without glycine/citric acid (formulation E), showed an increase in disintegration time and evidence of gelatin crosslinking was prevalent. Disintegration time went from 8 min initial to 22 min after a 4-week accelerated stability study, and further increased to 27 min at an 8-week time point and 45 min at a 12-week time point. Disintegration remained constant at about 8 min with no crosslinking or pellicle formation at all tested stability intervals for the gemfibrozil capsules with glycine/citric acid (formula F). Similar results were observed for the piroxicam experiments. Piroxicam capsules without glycine/citric acid (formula G) showed a dramatic increase in disintegration time throughout the 12-week accelerated studies (Fig. 6). Disintegration increased from 4 min initial to 12, 14, and 16 min after 4-, 8-, and 12-week accelerated studies, respectively. Pellicle formation was observed in all tested stability capsules. For piroxicam capsules with glycine/citric acid (formula H), disintegration remained

Table 4

Triam/HCTZ Capsules: Accelerated Stability at 40°C/85% RH, Triam Dissolution Profile at 15, 30, 45, and 60 min

Formula	Time (min)	Dissolution Profile			
		Initial (%)	4 Week (%)	8 Week (%)	12 Week (%)
A	15	78	5		
	30	88	10		
	45	93	13		
	60	95	16		
B	15	69	21		
	30	83	40		
	45	88	50		
	60	91	57		
C	15	92	4		
	30	104	14		
	45	107	27		
	60	109	38		
D	15	84	85	84	82
	30	94	95	94	93
	45	96	97	97	96
	60	97	98	98	97

Table 5

Triam/HCTZ Capsules: Accelerated Stability at 40°C/85% RH, HCTZ Dissolution Profile at 15, 30, 45, and 60 min

Formula	Time (min)	Dissolution Profile			
		Initial (%)	4 Week (%)	8 Week (%)	12 Week (%)
A	15	76	7		
	30	88	13		
	45	92	17		
	60	94	21		
B	15	69	24		
	30	82	42		
	45	87	54		
	60	90	61		
C	15	88	4		
	30	101	17		
	45	105	31		
	60	106	44		
D	15	84	83	81	82
	30	95	94	91	93
	45	95	96	94	96
	60	96	97	95	97

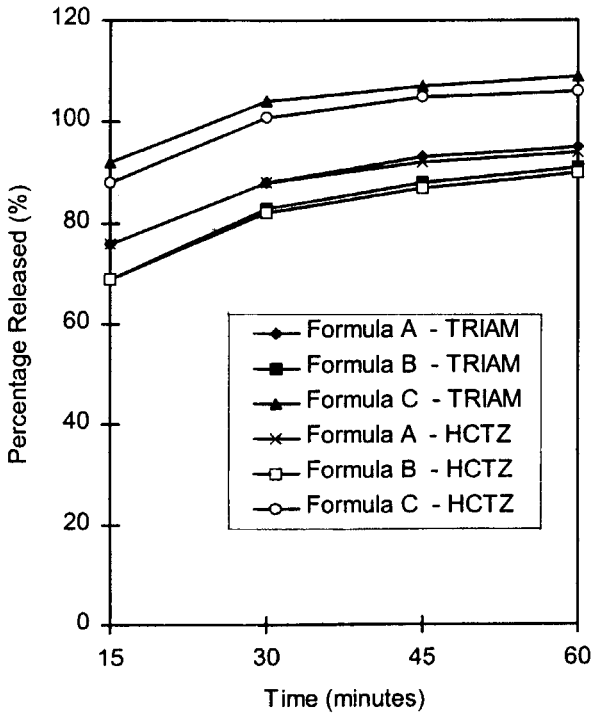

Figure 1. Triam/HCTZ capsules: accelerated stability studies at 40°C/85% RH, initial dissolution profile.

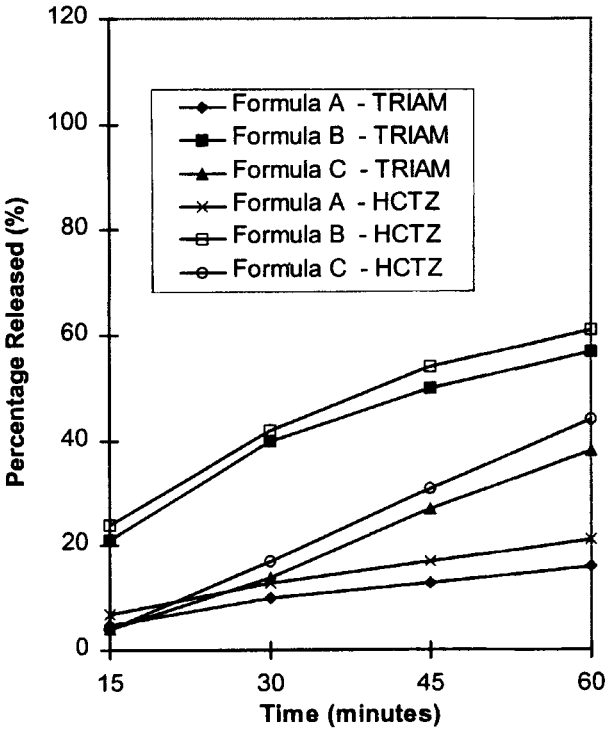

Figure 2. Triam/HCTZ capsules: accelerated stability studies at 40°C/85% RH, 4-week dissolution profile.

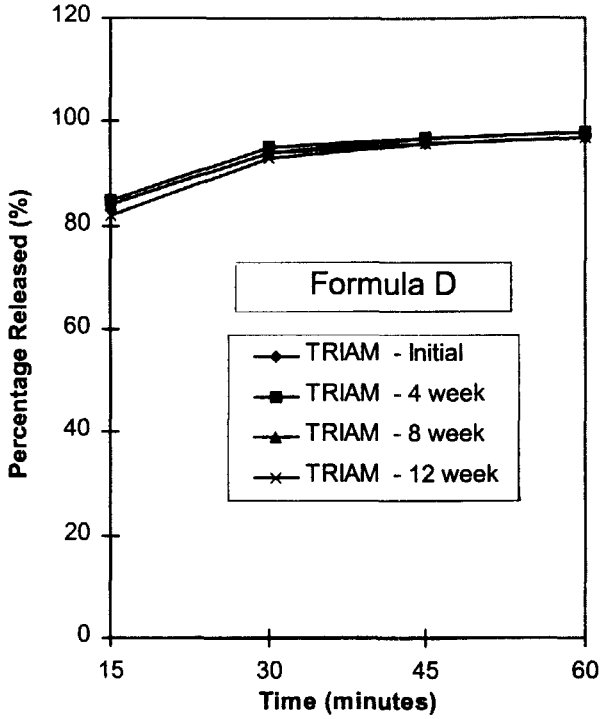

Figure 3. Triam/HCTZ capsules: accelerated stability studies at 40°C/85% RH, effect of glycine/citric acid on dissolution stability.

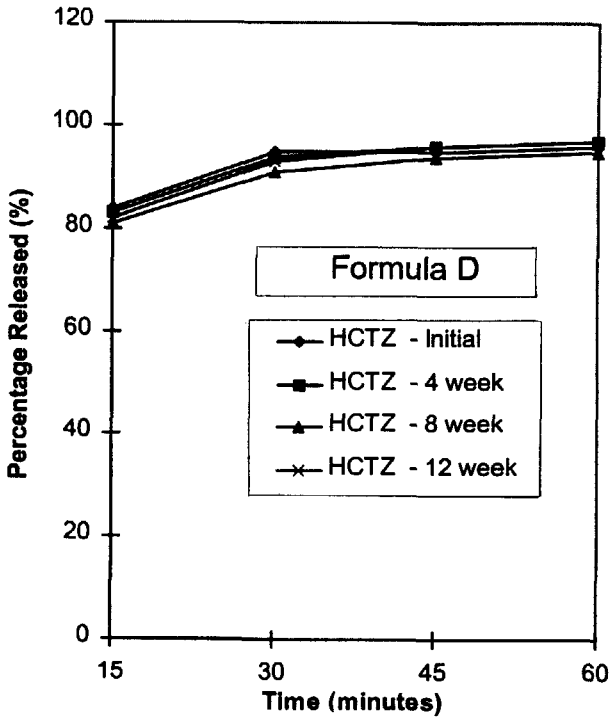

Figure 4. Triam/HCTZ capsules: accelerated stability studies at 40°C/85% RH, effect of glycine/citric acid on dissolution stability.

consistently between 4 and 6 min for all tested capsules at all tested stability time points. No visual evidence of gelatin crosslinking was observed.

DISCUSSION

It is well known that the presence of certain ingredients in the filling promotes crosslinking in the gelatin with the passage of time and/or under accelerated conditions such as high temperature and humidity. When crosslinking occurs, the gelatin shell becomes less soluble in aqueous media without enzyme, especially acidic aqueous media. Material that promotes crosslinking includes ingredients which contain a carbonyl compound as an impurity, or an ingredient which decomposes into a carbonyl compound or a derivative such as ketones or aldehydes, especially formaldehyde.

It has been suggested that amino acids such as lysine, phenylalanine, glutamine, hydroxylamine, hydrochloride, *p*-aminobenzoic acid, glycine, and others function as carbonyl scavengers in some gelatin capsule formulations, preventing the interaction of aldehyde with the gelatin shell, thereby preventing gelatin crosslinking. This knowledge was utilized by adding glycine to the triam/HCTZ formulas (formula B), but as indicated in Tables 4 and 5, and Figs. 1 and 2, glycine by itself did not prevent gelatin crosslinking. This may possibly be due to the relative amount of formaldehyde compared to glycine in the capsule fill. Another hypothesis could be that more formaldehyde may have been produced by the decomposition of the capsule contents with the passage of time and/or under accelerated conditions. One way to remedy this problem would have been to increase the amount of glycine in the formulation, but there is a limit to the amount of glycine that could be added to the formulation because of an observed adverse effect on triam/HCTZ capsule initial dissolution.

Hydrochlorothiazide is known to decompose through hydrolysis to produce formaldehyde and 6-chloro-2,4-disulfamoylaniline (11). According to literature, this degradation process is pH dependent. Between pH 2.5 and 11.5, the rate follows a bell-shaped curve with maximum degradation occurring at about pH 7.2. Below

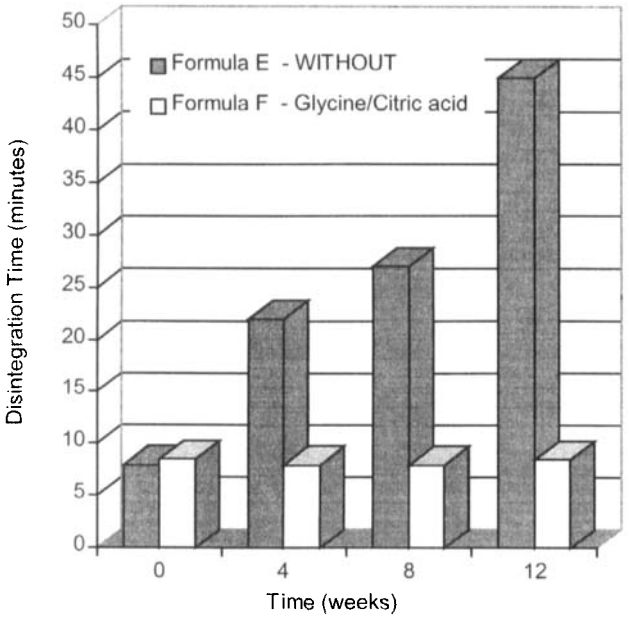

Figure 5. Gemfibrozil capsules: accelerated stability studies at 40°C/85% RH, effect of glycine/citric acid on disintegration stability.

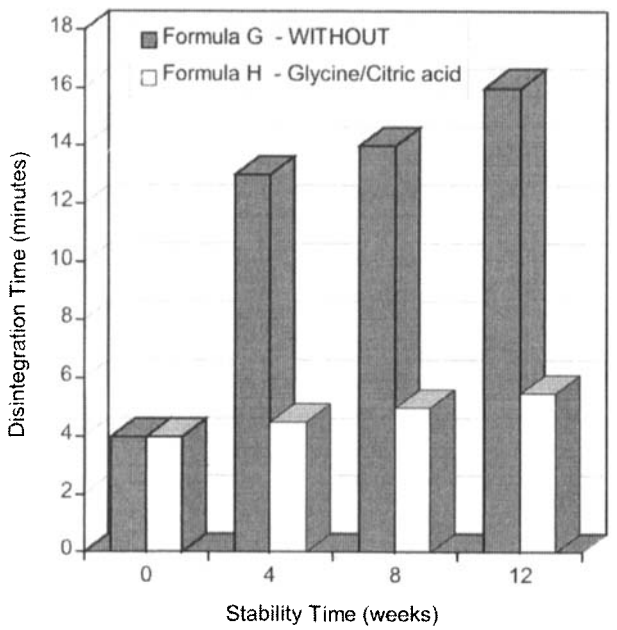

Figure 6. Piroxicam capsules: accelerated stability studies at 40°C/85% RH, effect of glycine/citric acid on disintegration stability.

pH 2.0 and above pH 12.0 the hydrolysis reaction rate increases rapidly. The optimum stable pH for hydrochlorothiazide was estimated to be 5.0.

With the above understanding of the chemistry of HCTZ decomposition, it was theorized that by adjusting the pH of the triam/HCTZ blend to approximately 5.0 with a carboxylic acid, hydrolysis of HCTZ and the formation of formaldehyde under accelerated conditions will be reduced, thereby reducing the possibility of gelatin crosslinking. Carboxylic acids, such as benzoic acid, fumaric acid, malic acid, citric acid, and others are commonly used in the pharmaceutical industry to control the pH of a formulation. Citric acid was added to the triam/HCTZ formulation (formula C) to study its effect on triam/HCTZ gelatin capsule crosslinking. Citric acid by itself did not prevent gelatin crosslinking in the triam/HCTZ capsules (Tables 4 and 5, Figs. 1 and 2). This may be because formaldehyde is already present in the capsule fill, therefore, reducing formaldehyde formation by minimizing the hydrolytic decomposition of capsule fill is not effective.

Formula D contained the combination of an amino acid (a formaldehyde scavenger) and a carboxylic acid (pH modifier) to control triam/HCTZ capsule crosslinking. If the amino acid is able to scavenge the initial formaldehyde present in the capsule fill, and further introduction of formaldehyde into the capsule fill is reduced or prevented by controlling the degradation of capsule fill, capsule crosslinking may be reduced or eliminated. This theory proved to be correct when glycine was combined with citric acid in a triam/HCTZ formulation. Pellicle formation and the subsequent retardation of triam/HCTZ capsules dissolution was prevented. Dissolution remained unchanged from the initial values up to 12 weeks at elevated temperature and humidity when tested in a dissolution medium without enzyme (Tables 4 and 5, Figs. 3 and 4).

The results of these tests clearly demonstrated that when glycine and citric acid are present in a triam/HCTZ gelatin capsule formulation, pellicle formation or crosslinking of the gelatin capsules is prevented. Another proposed theory evolved from observations made during manufacturing. It appeared that citric acid facilitated solubilization of glycine in the granulation solvent, which may have resulted in glycine being more evenly distributed throughout the granulation.

Further experiments were undertaken to study if this method of preventing crosslinking was applicable to other capsule formulations. Gemfibrozil and prioxicam were selected as model drugs because of their long his-

tory with crosslinking problems. Excipients such as lactose, polysorbate 80, and corn starch and its derivatives were selected because they had been identified in the literature as promoting crosslinking in gelatin capsules. Gelatin capsule shells with colorants such as FD&C red #3, which had also been identified as promoting gelatin capsule crosslinking, were used in this work to simulate "worst-case" formulations. There was a significant difference between model drugs with the glycine/citric acid combination and capsules without glycine/citric acid in the formula. Disintegration time remained relatively constant in the capsules with the glycine/citric acid throughout the test period. Disintegration increased dramatically throughout the test period for the capsules without the glycine and citric acid. This established that capsules other than triam/HCTZ can be protected from crosslinking and dissolution stability problems by incorporating an amino acid and a carboxylic acid into the formula.

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