Original Article

Stability studies of the effect of crosslinking on hydrochlorothiazide release

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ABSTRACT: The aim of this study was to determine the changes in the *in vitro* drug release from cross-linked hard gelatin capsules containing a water-insoluble drug. An immediate release hydrochlorothiazide (HCTZ) capsule formulations containing drug, lactose, starch 1500 were prepared and exposed to accelerated stability study (40°C/ ambient RH (relative humidity), 40°C/60% RH, 40°C/ 75% RH, and 40°C/90% RH) in closed dark bottles for 4 weeks. Notable decrease in drug dissolution was observed after 4 weeks in all humidity conditions as compared with freshly prepared capsules. In an attempt to overcome capsule cross-linking, glycine alone, citric acid alone and both glycine and citric acid were added to the prepared formulations. In all humidity conditions, addition of glycine alone or citric acid alone did not affect the decrease in dissolution profile. On the other hand, addition of both glycine and citric acid together was found to prevent capsule cross-linking completely. Fourier transfer infra-red (FTIR) spectroscopy and differential scanning calorimetry (DSC) were performed on blank capsules (with no glycine or citric acid) and after storage for 4 weeks to identify the physicochemical changes in drug and other capsule components hence its effect on dissolution.

Keywords: Hard gelatin capsule, cross-linking, hydrochlorothiazide, relative humidity, starch

1. Introduction

Exposure of a dosage form to high temperature and relative humidity is an attempt to assess its long-term stability in a relatively short period of time (*1*). While accelerated conditions at temperatures greater than 30°C and humidities outside the range of 40-60% are not recommended by gelatin capsule shell manufactures (*2*), storage conditions more stressful than these are routinely required by governmental agencies as evidence of the long-term stability of the dosage form and the drug entity itself (*3*). Gelatin capsule cross-linking is a well known phenomenon that results in reduced dissolution of capsule products by time and/or under accelerated conditions. Cross-linking is facilitated when the formulation in the capsule either contains a carbonyl compound as an impurity or decomposes into a carbonyl compound or derivative such as formaldehyde (*4,5*). Combination products of various antihypertensive drugs with hydrochlorothiazide (HCTZ) are routinely formulated to augment their pharmacological effects or to provide step-up therapy (*6*). HCTZ when incorporated in hard gelatin capsules can undergo hydrolysis with the formation of formaldehyde and 4-amino-6-chloro-l,3-benzenedisulfonamide (free amine) (*7,8*). The degradation of HCTZ in a dosage form is undesirable since the US Pharmacopeia (USP) sets a tight limit for the free amine content of not more than 1% of the HCTZ potency due to toxicological reasons. On the other hand, by time and/or under the accelerated conditions, formaldehyde reacts with the amino acid groups within the gelatin shell to generate a cross-linked structure. This leads to the formation of a very thin, tough, and water insoluble film noted around the capsule contents during dissolution testing, this water-insoluble thin film acts as a barrier, restricting drug release (*9*). The purpose of the present study is to evaluate the effect of storage conditions on the disintegration and dissolution of HCTZ from hard gelatin capsule. In addition, the study aimed to indicate that cross-linking has a great impact on the results of the *in vitro* dissolution testing which is commonly employed as a method to assess the stability of drug products (*10*). Besides, the study aims to overcome this capsule cross-linking problem throughout all humidity conditions.

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2. Materials and Methods

2.1. *Materials*

Hydrochlorothiazide was a kind gift from Hikma-Egypt Pharmaceuticals, 6th October City, Egypt. Lactose monohydrate powder was purchased from Cooper (Melun, France), pre-gelatinized starch (Starch® 1500) from Colorcon (Shizuoka, Japan), glycine from Winlab Laboratory Chemicals (Leicestershire, UK), citric acid and hydrochloric acid (34%) from Adwic (Cairo, Egypt). Magnesium stearate was supplied by SEDICO Pharmaceutical Co., 6th October City, Egypt. Capsules (size 1) were Coni-Snap® type (Capsugel, Colmar, France). All chemicals were of commercial analytical grade.

2.2. *Preparation of capsules*

The composition of the HCTZ capsules is shown in Table 1 as follows: Formulation (A) is the blank formula with neither glycine nor citric acid. Formulation (B) contains citric acid alone. Formulation (C) contains glycine alone, while formulation (D) contains both glycine and citric acid. All ingredients were passed through sieve #40, properly mixed together, and carefully filled into gelatin capsule.

2.3. *In vitro dissolution study*

Dissolution was performed using USP Apparatus 2 (Vankel Industries, Cary, NC, USA) at 100 ± 1 rpm in 900 mL of SGF (pH 1.2) at 37 ± 0.5°C. To avoid floating, the capsules were ballasted by using a wire. Samples were filtered through a 0.45 µm pore size membrane filter (Millipore Co., Bedford, MA, USA) and analyzed spectrophotometrically (Shimadzu, model-UV-1601 PC, Kyoto, Japan) at 271 nm. The dissolution medium was replenished with fresh SGF (pH 7.4) maintained at 37°C. A cumulative correction factor was exploited to compensate for the withdrawn samples. Data were computed with reference to a standard calibration curve of the drug (r = 0.999) and the values obtained were the mean of three determinations. For dissolution stability evaluation, the capsules were packed in amber-colored glass containers and were exposed to 40°C/ambient RH, 40°C/ 60% RH, 40°C/75% RH, and 40°C/90% RH in a stability cabinet (Climacell, Medcenter, Einrichtungen GmbH, MMM group, Germany) for 4 weeks after which dissolution of all stored formulations was performed under the same conditions previously mentioned.

2.4. *Fourier transfer infra-red spectroscopy (FTIR)*

Samples (2-3 mg) of the fresh and stored capsules were mixed each with about 100 mg of dry potassium bromide, and were compressed into discs under pressure of 10-15 pounds/inch^2. The FTIR spectra were recorded.

2.5. *Differential scanning calorimetry (DSC)*

The instrument was calibrated using indium. Samples (3.49-5.8 mg) of the fresh and stored capsules were weighed directly into platinum pans and scanned between 80-140°C at a rate of 10°C/min. Dry nitrogen was used as a carrier gas with a flow rate of 30 mL/min.

2.6. *Data analysis*

A two factors three variables factorial is used which requires 9 experiments. The two factors X_1, humidity percent and X_2, addition of citric/glycine are represented by –1, 0, and +1, analogous to the low, middle and high values respectively (Table 2).

The following quadratic model was built to describe the response:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{12}X_2^2 + b_{13}X_1X_2$$

where y is the response, x the factors, and b the coefficients of each term calculated by multiple regression analysis. The responses studied for the drugs were the disintegration time (Y_1), amount dissolved or dissolution after 10 min (Y_2) and after 20 min (Y_3).

3. Results and Discussion

3.1. *In vitro dissolution study*

Complete drug release was observed from the prepared HCTZ capsules after 20 min as shown in Figure 1. This

Table 1. Composition of different HCTZ formulations

Ingredients	Formulations (%w/w)			
	A	B	C	D
Hydrochlorothiazide	50	50	50	50
Lactose	244	242.2	235.25	233.5
Citric acid	–	1.75	–	1.75
Glycine	–	–	8.75	8.75
Starch 1500	52.5	52.5	52.5	52.5
Magnesium stearate	3.5	3.5	3.5	3.5

Table 2. Experimental domains and coding of the variables

Variables	Levels		
	–1	0	+1
Citric/glycine (X_1)	Citric	Glycine	Both
Humidity (X_2)	60	75	90
Responses			
Y_1 disintegration time of capsule			
Y_2 dissolution after 10 min			
Y_3 dissolution after 20 min			

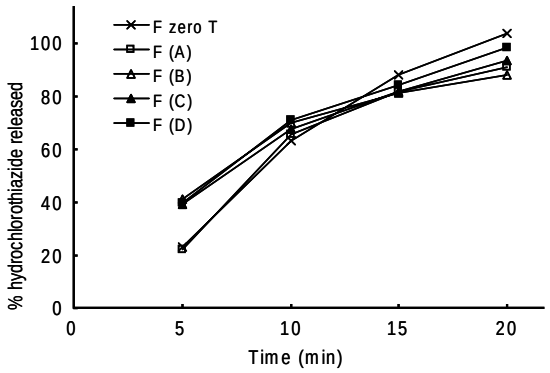

Figure 1. Percent HCTZ released (F zero time) and after 4 weeks at 40°C/ambient humidity.

is due to the presence of the super-disintegrant starch 1500 which acts by absorbing water, swelling, and producing the fast disintegration effect. After 4 weeks, there was a slight drop in the dissolution profiles for formulations A, B, and C at 40°C/ambient humidity, while formulation (D) showed no significant change in drug release ($p > 0.05$) (Figure 1). After 4 weeks, storage at 40°C/60, 75, or 90% RH, there was a significant drop in the dissolution profiles for formulations A, B, and C ($p < 0.05$), while formulation (D) exhibited no change in drug release. This is illustrated in Figure 2 and Table 3 in which formulation (D) gave maximum DP_5% (percent drug dissolved in 5 min), maximum DP_{20}% (percent drug dissolved in 20 min), and maximum DE_{20} (Dissolution efficiency at $t = 20$ min). At all accelerated conditions, the remarkable decrease in dissolution was attributed to the formation of trace amounts of formaldehyde due to hydrolysis of HCTZ in humid environment, which interact with the amino acid groups within gelatin shell to generate a cross-linked structure and it becomes less soluble in aqueous media and thus decreasing the drug release from the capsule (*11*). In addition, formaldehyde which is a highly reactive substance, reacts with starch 1500 leading to the loss of its swelling capacity (*12*), hence retarding its disintegration effect. Addition of citric acid (formulation B) was found to improve the dissolution slightly. This is due to the fact that the hydrolysis process of HCTZ is a pH dependent, so through manipulation of pH by adjusting the pH of the capsule content with citric acid to nearly 5.0 (optimums pH for HCTZ), hydrolysis of drug and subsequent formaldehyde formation will be reduced, thereby reducing gelatin crosslinking (*13*). Addition of glycine (formulation C) also improves the dissolution profiles as it functions as a carbonyl scavenger in gelatin capsule formulations, preventing the interaction of the aldehyde with the gelatin shell, thereby preventing gelatin cross-linking (*14*). It is even reported that if the formaldehyde, initially present in the capsule fill, is scavenged by the use of glycine, it prevents or reduces the further introduction of aldehyde. Formulations containing both citric acid and glycine exhibited no decrease in dissolution profiles. It appeared that citric acid

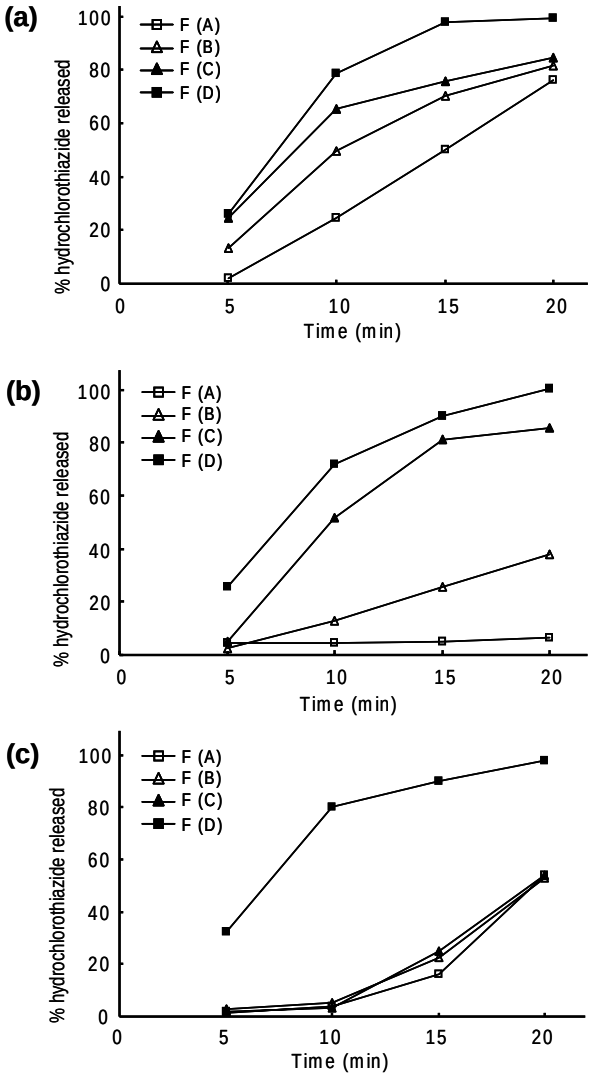

Figure 2. Percent HCTZ released after 4 weeks at (a) 40°C/60% RH, (b) 40°C/75% RH, and (c) 40°C/90% RH.

Table 3. Dissolution parameters of HCTZ formulations

Formulation	DP_5 (%)	DP_{20} (%)	DE_{20} (%)
F zero T	35.9	100	82.1
F (A)i	1.8	76.1	47.7
F (B)i	13.3	81.7	64.2
F (C)i	24.7	84.6	73.2
F (D)i	25.9	99	87.7
F (A)ii	3.8	5.9	5.4
F (B)ii	2.1	37.4	23.8
F (C)ii	4.6	85	65.9
F (D)ii	25.1	100	83.9
F (A)iii	0.91	53.4	24.9
F (B)iii	2.5	52.5	27.1
F (C)iii	1.6	53.7	27.5
F (D)iii	31.8	97.3	86.8

DP_5: Percent drug dissolved in 5 min. DP_{20}: Percent drug dissolved in 20 min. DE_{20}: Dissolution efficiency at $t = 20$ min (calculated from the area under the dissolution curve at $t = 20$ min and expressed as % of the area of the rectangle described by 100% dissolution in the same time). Each value is the average of three determinations. (i): 40°C/60 RH, (ii): 40°C/75 RH, and (iii): 40°C/90 RH.

facilitated solubilization of glycine in humid conditions, thus becoming more evenly distributed throughout the capsule content and preventing cross-linking. FTIR and

DSC spectrum were carried out on all stored capsules in different conditions but only three formulations were chosen in this study to show the effect of storage on them. These formulations were F_1 which contains citric acid only, F_2 with glycine only, and F_5 with both glycine and citric acid, all stored at 40°C/90% RH and were compared with blank formulation F_{blank} (containing no glycine and citric acid).

3.2. *Fourier transfer infra-red spectroscopy*

The FTIR spectrum of plain HCTZ (Figure 3) illustrates peaks at 3362, 3267, and 3170 cm^{-1} assigned to NH and NH_2 stretching. It also shows peaks at 1602 and 1520 cm^{-1} corresponding to the heterocyclic ring system, and peaks at 2361 and 2339 cm^{-1} assigned to C-H stretching of the thiazide ring. In addition, it shows a peak at 1321 cm^{-1} corresponding to SO_2 asymmetric stretching and at 1174 and 1152 cm^{-1} corresponding to SO_2 symmetric stretching.

All fresh and stored formulations at different relative humidities showed a peak at 3526 cm^{-1} which characterized the stretching vibrations of O-H bonds of lactose alcohol group (corresponding to lactose used as filler), which could be free or bonded (*15*).

By comparing FTIR spectra of formulations F_{blank}, F_1, and F_2 stored at 40°C/90% RH for 4 weeks, the peak at 3170 cm^{-1} assigned to NH stretching of secondary amine of the intact drug disappeared, which might indicate the cleavage of the heterocyclic ring in the above formulations, while it was still present in formula F_5 (Figure 3).

The intensity of the peaks at 1165, 1143, 1068, and 1030 cm^{-1} corresponding to C-H stretching of formaldehyde was increased in spectra of formulae F_1, F_2, and F_{blank} indicating the presence of formaldehyde in large amount. On the other hand, the same peaks were found in the spectrum of formula F_5, but with much less intensity indicating the presence of trace amounts of formaldehyde (*15*).

By examining the FTIR spectra of the formulations, the peak at 2300 cm^{-1} assigned to the thiazide ring of the drug was absent in the spectrum of blank formulation which may be attributed to the absence of both glycine and citric acid. Conversely, this peak was found in the spectra of F_1, F_2, and F_5 indicating the presence of the thiazide ring intact in these formulations (*16*). This result was correlated with that obtained from the dissolution studies where the blank formulation showed the least $DP_{5\%}$, $DP_{20\%}$, and DE_{20}.

3.3. *Differential scanning calorimetry*

The DSC of plain HCTZ (Figure 4) shows an endothermic peak corresponding to its melting point at 271.13°C with an apparent heat of fusion of -123.41 mJ which agrees with the melting point reported in Analytical Profiles (*13*). The disappearance of the peak of the drug in the thermograms of formulation F_5 proved that the drug was completely miscible in the excipients used. On the other hand, the same peak of the drug appeared in the thermograms of F_{blank}, F_1, and F_2 with relatively small intensity. This may be due to the absence of glycine in F_1, absence of citric acid in F_2 and absence of both in F_{blank} which caused the drug to be less soluble in the mentioned formulations resulting in the appearance of its peak.

3.4. *Data analysis*

The causal factor and response variables were related using polynomial equation with statistical analysis through Design-Expert® software (*17*).

The contour plots illustrating the simultaneous effect of the causal factors on individual and combined response variable are represented in Figures 5-7. This expression gives an insight into the effect of the different independent variables (response). A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response (Tables 4-7).

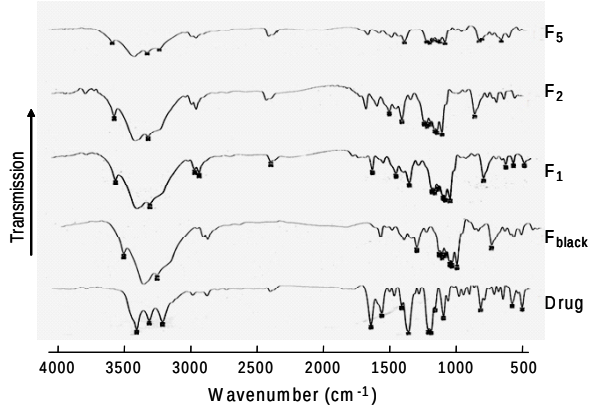

Figure 3. FTIR spectra of drug, F_{blank}, F_1, F_2, and F_5 stored at 40°C/90% RH for 4 weeks.

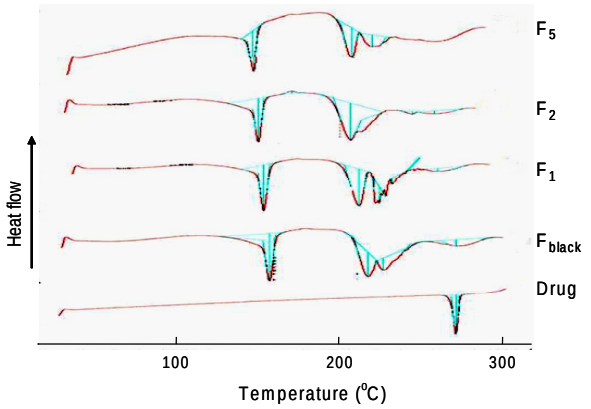

Figure 4. DSC spectra of drug, F_{blank}, F_1, F_2, and F_5 stored at 40°C/90% RH for 4 weeks.

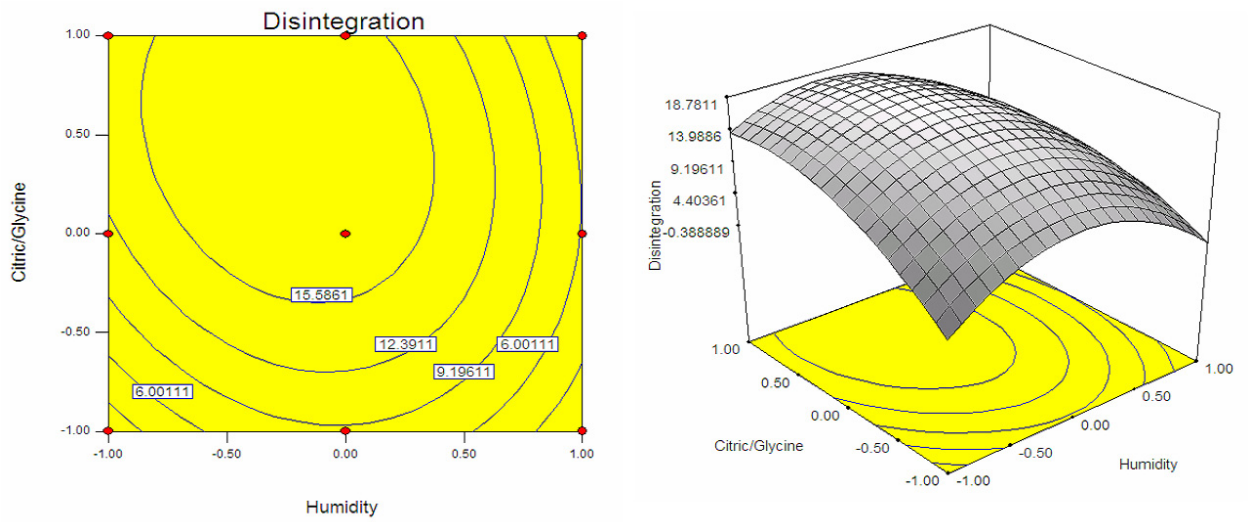

Figure 5. Contours of disintegration time (Y_1) as a function of humidity % (X_1) and addition of citric/glycine (X_2).

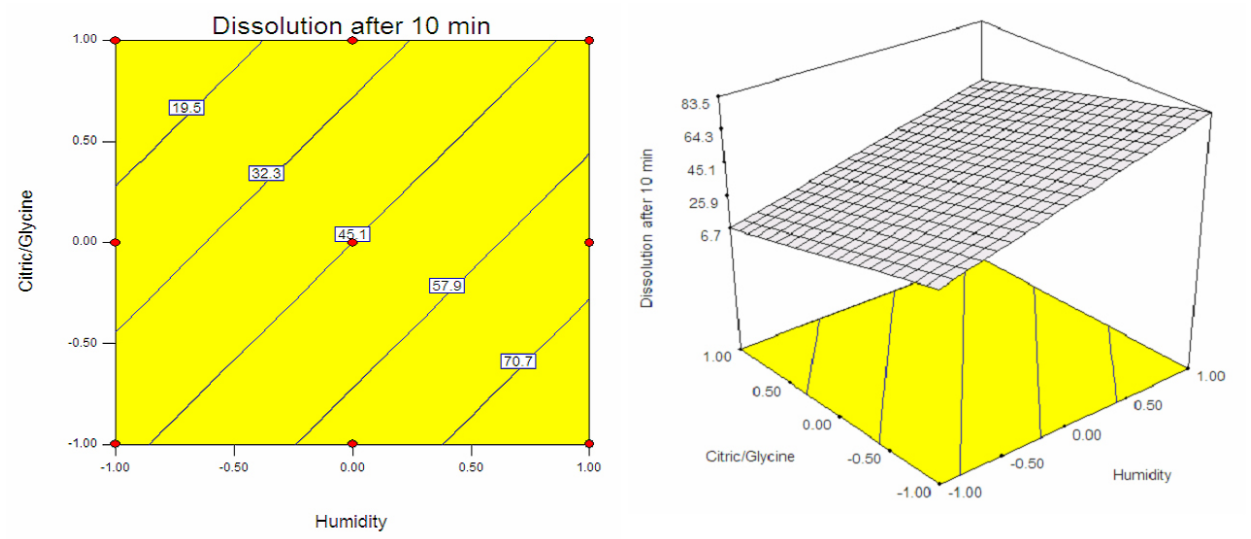

Figure 6. Contours of dissolution after 10 min (Y_2) as a function of humidity % (X_1) and addition of citric/glycine (X_2).

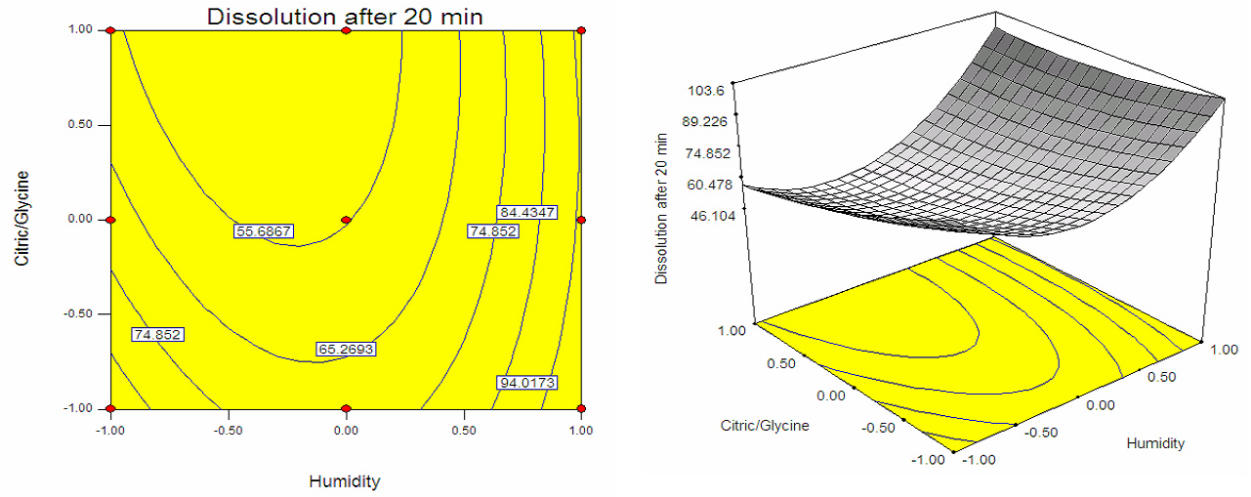

Figure 7. Contours of dissolution after 20 min (Y_3) as a function of humidity % (X_1) and addition of citric/glycine (X_2).

Table 4. Actual, predicted, residual values for disintegration time of capsules in minutes as a function of humidity % (X_1) and addition of citric/glycine (X_2)

Standard order	X_1	X_2	Actual value	Predicted value	Residual	Run order
1	–1	1	3.00	5.78	–2.78	7
2	0	0	2.00	–0.39	2.39	8
3	0	–1	3.00	8.78	–5.78	3
4	–1	0	7.00	11.78	–4.78	4
5	1	1	25.0	17.44	7.56	2
6	–1	–1	3.00	2.61	0.39	5
7	1	0	4.00	0.61	3.39	6
8	1	–1	15.0	16.78	–1.78	9
9	0	1	15.0	13.61	1.39	1

Table 5. Actual, predicted, residual values for percent drug released from capsules after 10 min as a function of humidity % (X_1) and addition of citric/glycine (X_2)

Standard order	X_1	X_2	Actual value	Predicted value	Residual	Run order
1	–1	1	71.00	72.83	–1.83	7
2	0	0	78.70	84.50	–5.80	8
3	0	–1	65.50	43.27	22.23	3
4	–1	0	51.00	31.57	19.43	4
5	1	1	2.50	20.10	–17.60	2
6	–1	–1	79.60	71.97	7.63	5
7	1	0	49.80	66.23	–16.43	6
8	1	–1	3.10	7.73	–4.63	9
9	0	1	4.70	7.70	–3.00	1

Table 6. Actual, predicted, residual values for percent drug released from capsules after 20 min as a function of humidity % (X_1) and addition of citric/glycine (X_2)

Standard order	X_1	X_2	Actual value	Predicted value	Residual	Run order
1	–1	1	99.00	95.53	3.47	7
2	0	0	99.30	103.6	–4.30	8
3	0	–1	84.60	70.27	14.33	3
4	–1	0	85.00	70.07	14.93	4
5	1	1	37.00	55.40	–18.40	2
6	–1	–1	97.30	96.47	0.83	5
7	1	0	81.70	91.73	–10.03	6
8	1	–1	53.60	49.53	4.07	9
9	0	1	52.50	57.40	–4.90	1

Table 7. Optimal regression equation for each response variable a function of humidity % (X_1) and addition of citric/glycine (X_2)

Model	Coefficient	Y_1	Y_2	Y_3
	B_0	79.67	91.11	19.67
	$b_1(X_1)$	14	0.5	–9
	$b_2(X_2)$	–27	–11	13
	$b_{11}(X_1^2)$	–14.5	–10.67	0.5
	$b_{12}(X_2^2)$	–11.5	–1.17	13.5
	$b_{13}(X_1X_2)$	3.75	3.75	–11.75
Quadratic	CV	80.82	50.85	23.08
	R^2	0.7200	0.8181	0.7807
	Adjusted R^2	0.2532	0.5150	0.4152
	PRESS	1,138	1,5919	7,832

4. Conclusion

In the environment of high humidity, the decrease in the dissolution of HCTZ capsules was attributed to the formation of formaldehyde due to the hydrolysis of HCTZ in the presence of moisture and excipients. The liberated formaldehyde reacted with the gelatin in the capsule shell and starch 1500 in the formulation to form an insoluble compound that led to a decrease in the dissolution profile and a decrease in the capsule disintegration capacity. This capsule cross-linking was overcome by using a combination of an amino acid (glycine) and a buffer (citric acid) which prevent the formaldehyde formation inside the capsule and thus attain its dissolution profile.

References

1. Mike D, Robin E, Mike K, David G, Doug S, Richard W. The dissolution and bioavailability of etodolac from capsules exposed to conditions of high relative humidity and temperature. Pharm Res. 1993; 10:1295-1300.
2. Elanco Qualicaps. Technical Service Reference Manual, 1991.
3. Guideline for submitting Documentation for the Stability of Human Drugs and Biologics, Centre for Drugs and Biologics, Food and Drug Administration, 1987; pp.

11-43.
4. Adesunloye TA, Stach PE. Effect of glycine/citric acid on the dissolution of hard gelatin capsules. Drug Dev Ind Pharm. 1998; 24:493-500.
5. Ofner CM, Zhang YE, Jobeck VC, Bowman BJ. Cross-linking studies in gelatin capsules treated with formaldehyde and in capsules exposed to elevated temperature and humidity. J Pharm Sci. 2001; 90:79-88.
6. Desai DS, Rubitski BA, Varia SA, Jain NB. Povidone- and poloxamer-mediated degradation of hydrochlorothiazide in an antihypertensive combination tablet product. Int J Pharm. 1996; 142:61-66.
7. Mollica JA, Rehm CR, Smith JB. Hydrolysis of hydrochlorothiazide. J Pharm Sci. 1969; 58:635-636.
8. Deventer K, Pozo O, Van Eenoo P, Delbeke F. Detection of urinary markers for thiazide diuretics after oral administration of hydrochlorothiazide and altizide-relevance to doping control analysis. J Chromat A. 2009; 1216:2466-2473.
9. Carstensen JT, Rhode CT. Pellicle formation in gelatin capsule. Drug Dev Ind Pharm. 1993; 19:2709-2712.
10. Digenis GA, Gold TB, Shah VB. Cross-linking of gelatin capsules and its relevance to their i*n-vitro in-vivo* performance. J Pharm Sci. 1994; 83:915-921.
11. Singh S, Rao KVR Venugopal K, Manikandan R. Alteration in dissolution characteristics of gelatin-containing formulations. A review of the problem, test methods, and solutions. Pharm Technol. 2002; 26:36-58.
12. Desai DS, Rubitski BA, Bergum JS, Varia SA. Effect of different types of lactose and disintegrant on dissolution stability of hydrochlorothiazide capsule formulations. Int J Pharm. 1994; 110:257-265.
13. Deppeler H. Hydrochlorothiazide. In: Analytical Profiles of Drug Substances, Vol. 10 (Florey K, ed.). Academic Press, New York, NY, USA, 1981; pp. 405-441.
14. Murthy KS, Enders NA, Fawzi MB. Dissolution stability of hard-shell capsule products. Part 1: The effect of exaggerated storage conditions. Pharm Technol. 1989; 13:72-85.
15. Spectrometric Identification of Organic Compounds, seventh edition (Silverstein RM, Webster FX, Kiemle DJ, eds.). John Wiley & Sons, NJ, USA, 2005; pp. 72-126.
16. Juan M, Aceves-Hernández J, Agacino E, Paz M, Hinojosa J. Experimental and theoretical study of the conformational analysis of hydrochlorothiazide. J Mol Struct. 2006; 786:1-8.
17. Vaughn N, Polnaszek C, Smith B, Helseth T. Program DESIGN-EXPERT, Stat-Ease Inc., 2000.

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