Application of Arrhenius Equation and Plackett-Burman Design to Ascorbic Acid Syrup Development

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Received 19 April 2004; accepted 29 June 2004

Abstract

Stability testing and drug-excipients compatibility of pharmaceutical dosage forms are necessary to perform during the early stages of product development. The aim of this study was to apply Arrhenius equation and Plackett-Burman design for stability testing and drug-excipients compatibility of formulations, respectively. The stability of ascorbic acid in syrup was examined using an accelerated stability testing according to the Arrhenius equation. The formulations were stored at room temperature and over the temperature range of 40-70°C. A linear regression line was obtained from Arrhenius plot of the reaction rate (k) against reciprocal of degree kelvin. The heat of activation was found to be 17.96 kcal/mole. Plackett-Burman experimental design was used to investigate the compatibility of ascorbic acid with various syrup excipients. It was found that glycerin (5%v/v) exerted a significant stabilizing effect on ascorbic acid, whereas sugar cane syrup (33.3%v/v) had destabilizing effect. The other excipients appeared to be no significant effect on ascorbic acid stability. The study demonstrated that Arrhenius equation would be useful for the prediction of product stability and the stabilizing or destabilizing effects of excipients could be identified by Plackett-Burman design.

Keywords: Arrhenius equation, Plackett-Burman design, Ascorbic acid syrup

Introduction

Stability testing of pharmaceutical dosage forms usually begins during the early stages of product development, the main purpose is to establish a product shelf life. According to the long duration of room-temperature shelf lives (may range up to several years), stability tests are often performed under stressed conditions (e.g. elevated temperatures) to accelerate the degradation process (Ertel and Carstensen, 1990). Garrett (Garrett, 1962) has introduced the principles of chemical kinetic to evaluate drug stability at higher temperature. The room-temperature stability or any lower temperature stability could be extrapolated from accelerated data by using Arrhenius relation. The Arrhenius equation is expressed mathematically as:

$$k = Ae^{-E_a/RT} \quad (1)$$

$$\ln k = \ln A - E_a/RT \quad (2)$$

where k is the reaction rate constant of any order, R denotes the gas constant (1.987 calories degree^{-1} mole^{-1}), A is the frequency factor, E_a is the activation energy and T is the absolute temperature.

Besides the stability testing, the evaluation of drug-excipient compatibility is an essential aspect of any preformulation study. The formulation of a stable and effective dosage form requires careful selection of excipients. One of an alternative approach to excipient compatibility testing involves the application of factorial experimental design to isothermal stress testing. Plackett and Burmann (Plackett and Burman, 1946) have developed saturated fractional factorial design that allow the researcher to investigate accurately many factors simultaneously without having to investigate all the possible combinations of factors (Durig and Fassihi, 1993). This design allows determination of the effect of variables with a minimum number of experiments. The disadvantage of this design is that it does not yield estimates of the extent or type of interaction between variables (Motola and Agharkar, 1992). However, within the bounds of these limitations, the use of these screening procedures invariably results in a well designed, efficient experiment, the outcome of which can be supported with statistical significance. Thus, Plackett-Burman design has been recommended for preformulation compatibility studies. In this study, ascorbic acid syrup was developed and its stability was predicted by Arrhenius equation. A Plackett-Burman experimental design was used to investigate the compatibility of ascorbic acid with various syrup excipients.

Materials and Methods

Materials

L-ascorbic acid (O.V. Chemical & Supply Ltd., Chiang Mai, Thailand) was used as received. The following materials were obtained from the indicated sources: ethylenediaminetetraacetic acid (EDTA), disodium salt, chelating agent (Riedel-DeHaen, Seelze, Germany); sodium metabisulfite, antioxidant (Vidhyasom Co., Ltd., Bangkok, Thailand); propylene glycol and glycerin (K.H. Co., Ltd., Bangkok, Thailand); methyl paraben and propyl paraben, preservative (Srichand United Dispensary Co., Ltd., Bangkok, Thailand); tartrazine (Sigma, St. Louis, MO, USA); lemon oil (Vidhyasom Co., Ltd., Bangkok, Thailand); saccharin sodium (Srichand United Dispensary Co., Ltd., Bangkok, Thailand); sorbitol (O.V. Chemical & Supply Ltd., Chiangmai, Thailand); and sugar cane (Pure refined sugar, Mitrphol, Mid Siam Sugar Co., Ltd., Bangkok, Thailand).

Methods

Preparation of ascorbic acid syrup

The compositions of the formulations are shown in Table 1. Ascorbic acid syrups were prepared by dissolving the drug in distilled water, adding EDTA (chelating agent), sodium metabisulfite (antioxidant) and saccharin sodium (sweetening agent), and then stirring until the clear solution was obtained. Vehicles (such as propylene glycol, glycerin, sorbitol, 85%w/v sugar cane syrup), preservative (paraben concentrate), and/or color, flavor were then added and mixed. The final volume

was adjusted to 60 ml with distilled water.

Evaluation of taste acceptability of ascorbic acid syrup

Since ascorbic acid has a sharp, acidic taste (Kibbe, 2000), the taste acceptability of the formulations could be improved by using suitable excipients in the formulations such as sweetening agents. The taste acceptability of the formulations was evaluated by using flavor panel (Behl et al., 1976). The panel consisted of volunteers from among the faculty, students at Faculty of Pharmaceutical Sciences, Naresuan University. Each formulation was evaluated by 25 volunteers. The panel members were given a verbal explanation of the nature and purpose of the work. They were given a brief outline of instruction on evaluation of the product as followings:

1. Take the test formulation half teaspoonful for each volunteer.
2. Record score of rating of the formulation on the evaluation sheet.
3. Allow at least 10 minutes before testing the next formulation.

The rating was between 1 to 10. Rating 1 indicated dislike extremely while rating 10 indicated the most favorable formulation. The highest rated formulation was chosen for further study.

Table 1 Ascorbic acid syrup formulations designed for taste acceptability evaluation

Excipient	Formulation					
	1	2	3	4	5	6
Ascorbic acid (g)	3	3	3	3	3	3
10%w/v EDTA (ml)	0.06	0.06	0.06	0.06	0.06	0.06
10%w/v sodium metabisulfite (ml)	0.6	0.6	0.6	0.6	0.6	0.6
10%w/v saccharin sodium (ml)	0.6	0.6	0.6	0.6	0.6	0.6
Propylene glycol (ml)	3	3	3	3	-	-
Paraben concentrate* (ml)	0.6	0.6	0.6	0.6	0.6	0.6
Glycerin (ml)	3	3	3	-	3	3
70%w/v sorbitol (ml)	15	10	5	15	10	5
85%w/v sugar cane syrup	15	20	25	15	20	25
Distilled water	20.64	20.64	20.64	23.64	23.64	23.64

Note: *Paraben concentrate consists of 20%w/v methyl paraben and 2%w/v propyl paraben in propylene glycol

Kinetic study on the stability of ascorbic acid

The highest rated formulation was prepared and filled in amber glass bottles. The bottles were then stored at room temperature and in thermostatically controlled ovens (Termaks KBS, Bergen, Norway) at 40, 60, 70, and 80°C. Samples were taken after predetermined time intervals and were diluted by distilled water (1:2500). The amount of ascorbic acid remaining was assayed by UV spectrophotometer (Model DU 605i, Beckman Instrument, Fullerton, CA, USA) at a wavelength of 258 nm (n = 3). Percentage of ascorbic acid remaining was calculated as a relative percentage of initial amount of ascorbic acid at time zero to amount of ascorbic acid at time t. These data were used to predict the stability of ascorbic acid syrup using Arrhenius relation.

Identification of stabilizing and destabilizing effect of excipients on ascorbic acid syrup using Plackett-Burman design

Nine excipients (variables) of the highest rated formulation were investigated at two levels of magnitude. A (+) sign represents the presence of a variable and (-) sign represents the absence of a variable with a constant amount of ascorbic acid (5%w/v) (Table 2). The composition of 20 mixtures prepared for this study is shown in Table 3. These mixtures were filled in 8 ml amber glass bottles and then incubated at 80°C in thermostatically controlled oven. After 7 days storage, the amount of ascorbic acid remaining in each bottle was determined by UV spectrophotometer.

Table 2 (+) and (-) levels for the assigned variables used in the twenty-run Plackett-Burman design to study the effect of excipients on stability of ascorbic acid

Variable	(+)	(-)
(A) 85%w/v sugar cane syrup	33.3%	0
(B) Glycerin	5.0%	0
(C) 70%w/v sorbitol	16.7%	0
(D) EDTA, disodium salt	0.1%	0
(E) Saccharin sodium	1.0%	0
(F) Sodium metabisulfite	1.0%	0
(G) Paraben concentrate	1.0%	0
(H) Color (tartrazine)	0.0005%	0
(I) Flavor (lemon oil)	0.01%	0

Table 3 Twenty-run Plackett-Burman design to study the effect of excipients on stability of ascorbic acid

Trial	Assigned variables (A-I)									Unassigned variables (J-S)									
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-
2	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+
3	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+
4	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-
5	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-
6	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+
7	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+
8	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+
9	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+
10	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-
11	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+
12	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-
13	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+
14	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-
15	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-
16	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-
17	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-
18	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+
19	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Statistical analysis

The best fitted straight line was determined by the least squares method. The correlation coefficient of the curves was calculated.

Results and Discussion

Evaluation of taste acceptability of ascorbic acid syrup

Ascorbic acid possesses a sharp acidic taste. The taste of formulations was improved by varying ratios of the excipients in formulation such as sweetening agents. A total of 12 formulations were evaluated by the investigator (acting as an initial screening taste panel) for taste acceptability. The 6 highest rated formulations from this section level were selected for evaluation by 25 volunteers using taste panel. The numerical evaluations of the results are reported in Table 4. The highest rated formulation (formulation 5) was then selected for further study.

Table 4 Rating of six most preferred ascorbic acid syrup formulations

Formulation	Mean rating (SD)
1	6.84 (1.31)
2	7.60 (1.44)
3	6.88 (1.42)
4	6.92 (1.44)
5	7.94 (1.33)
6	6.62 (1.63)

Kinetics studies on the stability of ascorbic acid

Ascorbic acid is an unsaturated lactone (cyclic ester). It is stable in dry state but unstable in solution, especially in alkaline solution. It readily undergoes oxidation on exposure to air (Kibbe, 2000; Touitou et al., 1992). In accordance with previous studies (Blasco et al., 2004; Esteve et al., 1998), it is assumed that ascorbic acid follows first-order degradation kinetics as the following equations:

$$\ln C = \ln C_0 - kt \qquad (3)$$

where C_0 and C are the percentages of ascorbic acid at the beginning of reaction and after time t incubating at a given temperature, respectively, k is the first-order rate constant (day^{-1}), and t is the time (day).

The stability of ascorbic acid in formulation 5 was determined by accelerated stability testing method at 40, 60, 70, 80°C and compared to the results obtained at usual room temperature (32 + 2°C). The plots of natural logarithm of the percentage of ascorbic acid remaining against time are shown in Figure 1. All plots were found to be linear, indicating first-order reaction with respect to ascorbic acid concentration. From the slopes of the curves obtained by fitting by least squares it is possible to calculate k, which indicates the rate constants of ascorbic acid degradation.

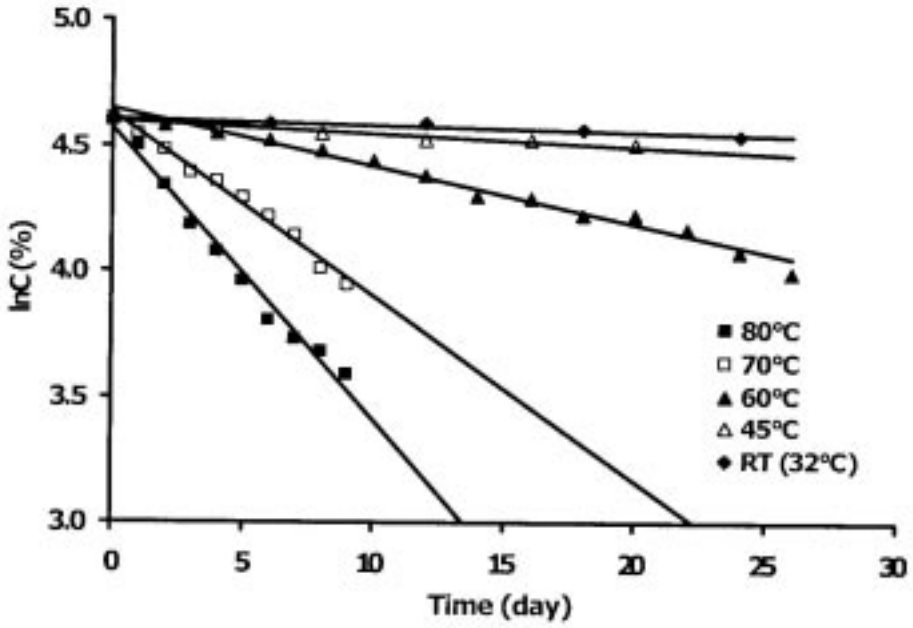


Figure 1 First-order plot for the degradation of ascorbic acid in syrup at various temperatures

Table 5 shows the observed rate constants at each storage temperature. It can be seen that the ascorbic acid degradation rate increases with increasing temperature.

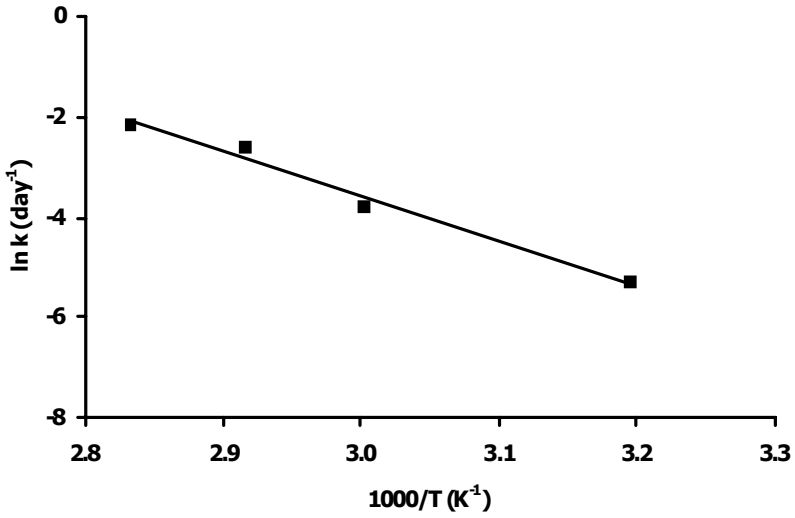
Table 5 Observed rate constants (k) for ascorbic acid degradation in syrup during storage at various temperatures

Temperature (°C)	k x 10^3 (day^{-1})(SD)	R^2
40	4.90 (0.09)	0.9306
60	22.85 (0.98)	0.9807
70	73.04 (0.27)	0.9893
80	116.65 (0.34)	0.9841
Room temperature (32 ± 2)	2.58 (0.66)	0.9657

In this study, the natural logarithm of observed rate constants over the temperature range of 40-70 °C were plotted versus reciprocal of temperature according to the Arrhenius equation (Connors et al., 1986; Carstensen, 2000). The plot exhibits linearity ($R^2 = 0.9874$) as shown in Figure 2. The equation of the curve corresponding to equation (2) is:

$$\ln k = 23.53 - 9.0 \times 1000/T \qquad (4)$$

Figure 2 Arrhenius plot for the first-order rate constant of ascorbic acid degradation over the temperature range of 40-70 °C



The heat of activation, E_a, was calculated from the slope of the straight line obtained by least squares. The E_a value for ascorbic acid degradation in syrup was 17.96 kcal/mol. This value lies within the range of the values in the previous reports (Connors et al., 1986; Blaug and Hajratwala, 1972; Finholt et al., 1965). The linear nature of Arrhenius plot is excellent verification of the method's utility in prediction of rates of degradation under a different range of temperatures (Bibart, 1979; Garrett and Carper, 1955). The predicted rate of degradation (k) can be used to determine shelf-life (t_{90}) of the formulations from the following equation:

$$t_{90} = 0.105/k \qquad (5)$$

where k is the first-order rate constant.

Table 6 shows the predicted and actual values of rate constants and shelf life at room temperature (32 + 2°C) and 4°C (in refrigerator). The predicted value of rate constant at room temperature was slightly lower than that obtained from actual storage. This resulted in the slightly longer shelf-life of the predicted value. It may be due to the variation of room temperature during day time and night time. However, both rate constant and shelf-life (at room temperature) lied in the range of the actual values. The predicted rate constant and shelf-life of ascorbic acid syrup at 4°C was also calculated and the values are 0.11×10^{-3} day^{-1} and 945.35 days, respectively. It indicated that we could prolong the product's shelf-life by storing in refrigerator or at lower temperature.

Table 6 Predicted and/or observed degradation rate constant (k) and shelf-life (t_{90}) of ascorbic acid in syrup at room temperature (32 + 2 °C) and 4 °C (in refrigerator)

	Temperature			
	Room temperature (32°C)		Refrigerator (4°C)	
	k x 10^3 (day^{-1})(SD)	t_{90} (days) (SD)	k x 10^3 (day^{-1})(SD)	t_{90} (days) (SD)
Predicted value	2.23	47.11	0.11	945.35
Observed value	2.58 (0.66)	40.70 (7.63)	N/A	N/A

Identification of stabilizing and destabilizing effect of excipients on ascorbic acid syrup using Plackett-Burman Design

The effect of the excipients in the highest rated formulation (formulation 5) on stability of ascorbic acid was determined by using Plackett-Burman design. The design showing the percentage of ascorbic acid recovered from each excipient combination is shown in Table 7. From the percentage of ascorbic acid recovered, the average effect was determined for each assigned variable and unassigned variable (Motola and Agharkar, 1992). Using the average effects of the unassigned variables, the standard deviation of the factor effect, S_{FE}, was calculated. This S_{FE} was then used to calculate a minimum significant factor effect, E_{ms} (E_{ms} = t x S_{FE}), at the 90% level of confidence. As t has degree of freedom equals to the number of unassigned variables (10 in this study), the t value is 1.812 at 90% confidence level in this study. Any average effect showed absolute value greater than the E_{ms} was considered to be statistically significant on the stability of ascorbic acid.

Table 8 compares the average effects of the assigned variables with the E_{ms} at 90% level of confidence. It appears that 85%w/v syrup is the dominant destabilizing factors. Since syrup USP (85%w/v syrup) was one of the most widely used vehicles in vitamin preparation (Lachman et al., 1986), it was surprised that 85%w/v syrup destabilized ascorbic acid. A possible explanation is that some impurities in commercial cane sugar might be responsible for this enhancing effect (Kibbe, 2000). The positive effect was observed for glycerin. This stabilizing effect was significant at the 90% level of confidence. Glycerin is an organic polyhydric alcohol that widely used in the formulation. It is misible with water and might reduce dissolved oxygen thereby producing a stabilizing effect upon the oxidation of ascorbic acid in the preparations. This result was in agreement with the earlier findings (Bandelin and Tuschhoff, 1954; Bandelin and Tuschhoff, 1955; Bartilucci and Foss, 1954; Giral, 1947). The other components (sorbitol, EDTA, saccharin sodium, sodium metabisulfite, paraben concentrate, tartrazine, lemon oil) did not significantly affect ascorbic acid stability.

Table 7 Plackett-Burman design showing %ascorbic acid recovered per trial (excipient combination)

Trial	Assigned variables (A-I)									Unassigned variables (J-S)									% REC
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	47.94
2	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	47.22
3	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	54.37
4	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	54.78
5	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	51.66
6	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	54.19
7	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	48.37
8	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	41.92
9	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	59.00
10	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	49.91
11	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	58.07
12	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	46.19
13	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	47.24
14	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	48.27
15	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	52.19
16	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	57.97
17	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	48.35
18	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	45.51
19	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	58.38
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50.07

Note: 85%w/v sugar cane syrup; B-glycerin; C-70%w/v sorbitol; D-EDTA, disodium salt; E-saccharin sodium; F-sodium metabisulfite; G-paraben concentrate; H-color (tartrazine); I-flavor (lemon oil); J to S - unassigned variables. %REC-percentage of ascorbic acid recovered or remaining.

Table 8 Summary of the compatibility test using a Plackett-Burman factorial design

Variable	Average effect	Significance ($P<0.1$)* ($E_{ms} = 2.322$)
85%w/v sugar cane syrup	-5.910	yes
Glycerin	5.584	yes
70%w/v sorbitol	2.016	no
EDTA, disodium salt	-0.220	no
Saccharin sodium	-1.710	no
Sodium metabisulfite	0.148	no
Paraben concentrate	0.387	no
Color (tartrazine)	-0.220	no
Flavor (lemon oil)	0.751	no

Note: * 90% level of confidence.

Conclusions

Stability testing is usually performed to determine the product shelf-life during the early stages of product development. The results obtained in our study demonstrated that Arrhenius equation could be applied for the accelerated stability testing to calculate and predict the rate constant and shelf-life of ascorbic acid syrup. Plackett-Burman design showed the ability to identify both destabilizing and stabilizing factors. Thus, it was recommended for preformulation compatibility study. These methods provide a helpful tool for pharmaceutical scientists to develop formulations.

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