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Evaluation of FD&C Red No. 40 dye concentration and type of gelatin in the dissolution of gelatin sheets

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Abstract

Objective: To study the relationship between the type of gelatin and dye concentration in relation to crosslinking in soft capsules.

Materials and methods: Two types of gelatin, bovine RXL Type B 130 Bloom and pig NF Type B 150 Bloom, and three concentrations of FD&C Red 40 Dye were used in six pre-formulations in six replicates, each for a total of thirty-six trials. Each pre-formulation was analyzed by a sample dissolution test under conditions of natural stability and of 21 days.

Results: Using the logistic regression model, it was found that the type of gelatin and dye concentration were statistically significant, while the interactions between them showed no significance.

Conclusion: The use of the RXL Type B 130 Bloom gelatin in pre-formulation of soft gelatin capsules increases the probability of success with non-crosslinking.

Keywords: factorial design, logistic regression, crosslinking, gelatin, dye

INTRODUCTION

Pre-formulation development of soft gelatin capsules often present quality problems in relation to the physicochemical and biopharmaceutical properties of the active ingredient alone or when combined with excipients. Variation in the quality of the finished product is one of the most acknowledged problems affecting the disintegration and dissolution of the capsules. Exposure to high humidity, high temperatures, or interaction with aldehydes can cause crosslinking, making it resistant to dissolution in water[1]. These problems may occur due to environmental factors, combination with some chemicals or interaction with the excipients, in most cases affecting the bioavailability of the medicine. Oral bioavailability is one of the most important properties in the design and development of drugs. High oral bioavailability reduces the amount of drug necessary to achieve the desired pharmacological effect, which reduces the risk of side effects and toxicity. Poor oral bioavailability can result in low efficacy, which leads to an unpredictable response to a medication[2].

There are studies that have evaluated the bioavailability of drugs in soft gelatin in comparison to other pharmaceutical forms. According to L. M. Fucella[3], the bioavailability of Temazepam in soft capsules is significantly faster and it causes higher and earlier maximum plasma levels. G. Bende[4] compared the bioavailability of Diclofenac Potassium in soft capsules against oral solutions and tablets, demonstrating that the area under the curve (AUC) is similar among different pharmaceutical presentations that were compared. The maximum plasma concentration of Diclofenac in the soft capsule was 67% higher than that in the existing tablet formulation, and 15% lower than that in the oral solution formulation. The review by Benza[5] discussed the progress and challenges in improving the solubility of soft gelatin capsule formulations, and the absorption-enhancing techniques developed by the manufacturing industry. The benefits that can be obtained with the use of pharmaceutical forms of soft gelatin capsules are many. The improvement in bioavailability of melatonin has been described by S. Proietti[6], where the bioavailability of melatonin as classic commercially available powder and as soft gelatin capsules was compared. The study concluded that the soft gelatin capsules showed improved bioavailability even with a low dose of melatonin (1 mg), which represents a clinical advantage for the treatment of various physiological and pathological disorders in which melatonin supplementation is recommended.

Among the major excipients in the soft capsule pre-formulations is gelatin, which is the cover that protects the active substance and is responsible for its release for subsequent absorption. Gelatin is a purified protein that is obtained from collagen of animals (including fish and poultry) by partial alkaline hydrolysis and/ or acid hydrolysis, enzymatic hydrolysis, or thermal hydrolysis[7]. Gelatin is a mixture of water-soluble proteins, derived from collagen by hydrolysis. The Pharmacopoeia of the United States of America[8] describes the meaning of gelatin as: sheets, scales or fragments, or coarse or fine dust. It has a slightly yellow amber color; the intensity of the color varies according to the size of the particles. It is stable in the presence of air when dry but is subject to microbial decomposition when wet or in solution. The adequate consistency of gelatin is determined by its bloom value, which is measured in a gelometer[8]. The jelly 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[9]. In water, it swells and softens, gradually absorbing 5 to 10 times more than its own weight in water. It is soluble in water above 40°C forming a colloidal solution, which gels when cooled to 35-40 ° C.

Gelatin has been considered a unique material with a wide range of applications in the industry, especially for the preparation of pharmaceutical products, such as capsules (soft and hard) or microspheres. In the biomedical field, it is used as a wound dressing and provide support for tissue regeneration, and in the preparation of packaging[10]. Since it is an abundant material produced worldwide at low cost and has excellent film-forming properties, gelatin is still used in film-forming studios[11]. Type A gelatin is obtained by an acid hydrolysis process while type B gelatin is obtained by a basic hydrolysis process; the essential differences between these two types of gelatin lie in the amino acid composition. In general, type B gelatins have a higher hydroxyproline content and lower tyrosine content than type A. The most likely cause of this difference in the composition of type A and B gelatins is that alkaline-processed raw materials lose poor peptides as in hydroxyproline and tyrosine-rich[12] type B gelatin; this difference in amino acid composition provides differences in reactivity with different chemicals.

The formulation of each film depends directly on the application that it is required for, which also determines the desirable mechanical and barrier properties. In many cases, it is necessary to add a plasticizer so that the film is not brittle and can be easily handled[13]. The nature of the excipients whose purpose is to provide stability, compatibility, and good performance from manufacturing to obtaining the finished product[14], must be taken into account. The soft capsules are manufactured using a mixture of gelatin, a plasticizer, such as glycerin and/ or sorbitol, and water[15]; in addition, color is incorporated to improve its appearance and in other cases help with the stability of the encapsulated asset. There are different types of synthetic dyes used in pharmaceutical formulations that are approved by FDA; for example, FD&C Blue No. 1, D&C Blue No. 2, D&C Green No. 5, D&C Red No. 22, FD&C Red 40, and FD&C Yellow No. 5, among others. Red FD&C 40 dye is an azo-derivative of the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid (Figure 1) that is widely used in the food, pharmaceutical, and cosmetic industry.

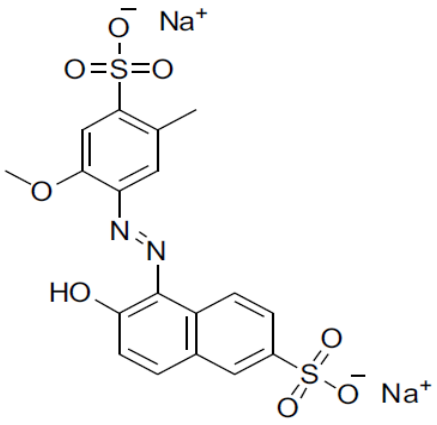

Figure 1. FD&C Red No40

The dissolution test is used at the beginning of the formulation and in subsequent phases because it allows the study of the release mechanisms of active principle. The dissolution test is a physicochemical test, which determines the percentage of drug that can dissolve per unit of time under standardized conditions of the liquid/ solid interface, temperature, and solvent composition. It is an essential test for compliance with the dissolution requirements in the manufacturing processes of oral dosage forms (tablets, capsules) as specified by the Pharmacopoeia of the United States of America[8]. This trial seeks to establish the dissolution behavior of the active ingredients contained in an oral dosage form, setting the criteria for evaluating the physical and biopharmaceutical properties of the drug. The test must be carried out on an equipment that meets the dissolution requirements established by the Pharmacopoeia of the United States of America[8] as described in chapter 711 on 'Dissolution', and on which a performance verification test can be performed when there is one available.

The non-dissolution of the gelatin shell in soft capsule formulations is due to the reactions of the aldehyde, amine, or ketone groups present in the gelatin. These groups are not only naturally present in the raw material, such as the skin of the capsule, but also in the drugs contained in the capsules, or can be added during the manufacturing process of the capsules. In general, crosslinking within the gelatin polypeptide can occur by intramolecular reaction within the same polypeptide chain and intermolecularly[16]. Crosslinking of soft gelatin capsules is a frequent problem, which directly alters the dissolution characteristics in formulations containing gelatin, and thus the bioavailability of the drug, as exposed by S. Singh[17].

In the study conducted by M.C. Meyer[1], the authors demonstrated the effects of crosslinking on *in vitro* dissolution rate of hard and soft gelatin capsules of Acetaminophen. Bigi[18] has shown the influence of glutaraldehyde concentration on the mechanical, thermal, swelling, and release properties of gelatin films. On the other hand, T. Toru[19] showed that the effect of formaldehyde on the physicochemical properties of the shell (gelatin husks) of soft gelatin capsules, which leads to an alteration in the dissolution rate of the capsules. Ofnerll[20] studied the effect of formaldehyde in soft gelatin capsules exposed to high temperature and humidity conditions to simulate crosslinking, and its negative effects on the dissolution rates of soft capsules. Botton[21] studied the effect of aging and crosslinking on two soft gel products containing Acetaminophen and Nifedipine. In this study, formaldehyde was used to create controlled crosslinking in the products, and crosslinking in the capsules was then evaluated by two United States Pharmacopeia (USP) dissolution methods to determine which method most accurately detects crosslinking.

This study aimed to evaluate the effects of the varied concentrations of FD&C RedNo 40 dye (Figure 1) and type of gelatin in the dissolution of gelatin sheets. Important problems in gelatin-based formulations result in dissolution failures due to its interaction with the formula

excipients and excipients aging. Crosslinking can be attributed to the formation of a swollen membrane, very thin, resistant, and insoluble in water, which acts as a barrier preventing the release of assets.

MATERIALS AND METHODS

A factorial design studies the effect of factors at certain levels and all of their possible interactions; if there are k factors at 2 levels, the whole factor has combinations of factor levels. The factorial experiment allows us to observe the effect that each independent variable has on the dependent variable, and the effect that the interaction between these variables have. The factors and levels of the experiment must be defined. One factor is any influence that may affect the response variable and is controlled by the experimenter. The levels are the categories or intensities that each previously established factor has[22]. In this study, a factorial experiment design 2^2 with a central point for the variables of gelatin type and dye concentration for the preparation of gelatin sheets is proposed (Table 1).

There were two types of gelatin, gelatin 1 (RXL Type B 130 Bloom) and gelatin 2 (NF Type B 150 Bloom), and three concentration values of FD & C Red No. 40 dye: 0.5%, 1%, and 1.5%. The different pre-formulations were prepared in the order and as specified in the design matrix (see table 1), using the type of gelatin and the indicated dye concentration; the amount of dough prepared is 500 g. For each pre-formulation, with the prepared gelatin mass, gelatin sheets were obtained by adding a portion of the melted gelatin mass on a glass plate with a thickness of approximately 0.04 ± 0.01 inches; the hardened sheets of the plate were removed from the glass, gelatin sheets with an area of 4 cm^2 were cut, and allowed to dry for 48 h at room temperature, subjected to natural stability tests (temperature 25 ± 2 ° C, humidity 60 ± 5%), and accelerated (temperature 40 ± 2 ° C, humidity 75 ± 5% 40 ± 2 ° C) for 21 days in stability cab.

The gelatin sheets of each natural and accelerated stability pre-formulation were evaluated at day 21 by the dissolution test. A Distek Evolution 6100 Apparatus II dissolver was used, a gelatin sheet was added to each vessel of the dissolving equipment (apparatus II), using 500 ml of dissolution medium. The dissolution medium is Type I water degassed at 37.0 ± 0.5°C, with a stirring speed of 50 rpm for 30 min. It was observed and recorded that there was no total dissolution of the gelatin sheets, relating these cases to the presence of crosslinking. In such a case in the dissolution tests where crosslinking identification yields binary responses if crosslinking is formed or crosslinking is not formed, it complicates the analysis of the DoE by conventional methods. Therefore, the logistic regression model was applied, which is suitable for situations where we want to explain the probability P of occurrence of an event of interest by means of the values of certain explanatory variables. If a dichotomous variable is associated with the event of interest, then this is a Bernoulli variable with conditional hope p[23].

The interaction between a quantitative variable X and a qualitative A is introduced into the model as the products of the quantitative variable multiplied by all design variables associated with variable A, which is equivalent to considering the quantitative variable in each of the levels of the qualitative variable[24]. The different statistical procedures were performed with the statistical program RStudio IDE[25].

RESULTS

For the analysis of the problem, some notations described below were used. Y = 0 indicates a presence of crosslinking and Y = 1 indicates no presence. Table 2 shows the results of the solutions made for the different pre-formulations of gelatin sheets detailed in Table 1.

Table 1. Matrix of Factorial design 2^2 of the experiment, where the factors are: Concentration of Red FD&C dye No. 40 expressed in% (0.5; 1; 1.5) and Jelly type (1: RXL Type B 130 Bloom; 2: NF Type B 150 Bloom)

Experiment	Order	FD&C Red 40	Gelatin type
1	4	1.5	2
2	1	0.5	1
3	3	0.5	2
4	5	1	1
5	6	1	2
6	2	1.5	1

Table 2. Results natural dissolutions and accelerated stability of gelatin sheets.

Experiment	Natural stability												Accelerated stability											
	15 minutes replicates						30 minutes replicates						15 minutes replicates						30 minutes replicates					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 (G2-C1.5)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
2 (G1-C0.5)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3 (G2-C0.5)	1	0	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0
4 (G1-C1)	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1
5 (G2-C1)	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0
6 (G1-C1.5)	1	0	1	0	1	0	1	0	1	1	1	0	1	1	0	1	0	0	1	1	1	1	0	0

Table 3. Percentages of crosslinking

Experiment	Natural stability		Accelerated stability	
	15 minutes	30 minutes	15 minutes	30 minutes
	%	%	%	%
1 (NF Tipo B 150 Bloom - C1.5)	100	83	100	83
2 (RXL Tipo B 130 Bloom - C0.5)	0	0	0	0
3 (NF Tipo B 150 Bloom - C0.5)	67	50	83	67
4 (RXL Tipo B 130 Bloom - C1)	33	17	33	17
5 (NF Tipo B 150 Bloom - C1)	83	67	100	67
6 (RXL Tipo B 130 Bloom - C1.5)	50	33	50	33

Table 4. Frequency distribution for presence = 0 and absence = 1 of crosslinking, gelaltin type (RXL Type B 130 Bloom and NF Type B 150 Bloom) and percentage of FD&C Red 40 dye (C0.5, C1 and C1.5) in formulations of gelatin sheets.

Crosslinking	FD&C Res 40	RXL Type B 130 Bloom	NF Type B 150 Bloom	Total
0	C0.5	0	16	73
	C1	6	19	
	C1.5	10	22	
1	C0.5	24	8	71
	C1	18	5	
	C1.5	14	2	
Total		72	72	144

Table 2 shows the results of the dissolution analysis in natural stability and accelerated stability of 21 days. For natural and accelerated stability in time periods of 15 and 30 min, the percentages of crosslinking obtained from different experiments are shown in Table 3.

Table 4 shows the frequency of distribution for the presence and absence of crosslinking, taking into account the type of gelatin and the percentage of dyes.

Figure 2 shows 51% crosslinking percentage versus a 49% non-formation for a sample size of 144. The percentages corresponding to the type of gelatin and percentage of FD&C Red 40 dye are uniform.

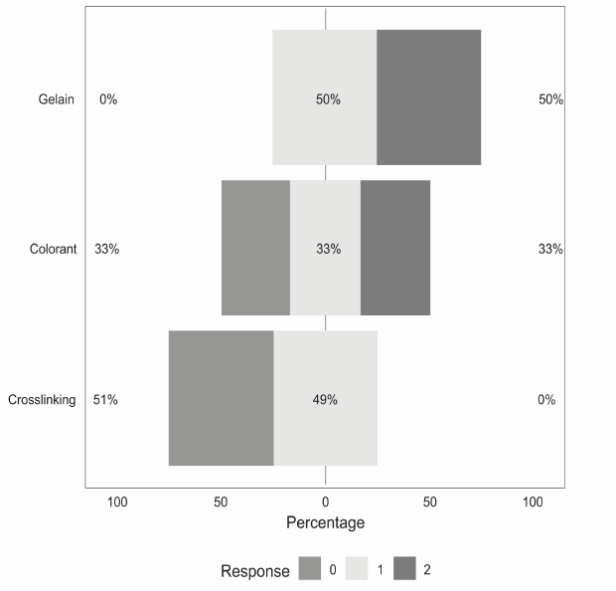

Figure 2. Percentage distribution of presence levels = 0 and absence = 1 of crosslinking, type of gelaltin (RXL Type B 130 Bloom = 1 and NF Type B 150 Bloom = 2) and percentage of FD&C Red 40 dye (C0.5 = 0, C1 = 1 and C1.5 = 2), in formulations of gelatin sheets

Figure 3 shows that the percentage of crosslinking in RXL Type B 130 Bloom jelly is 22% as compared to 78% of non-crosslinking. In addition, it was observed that in the NF Type B 150 Bloom gelatin, the percentages are reverted.

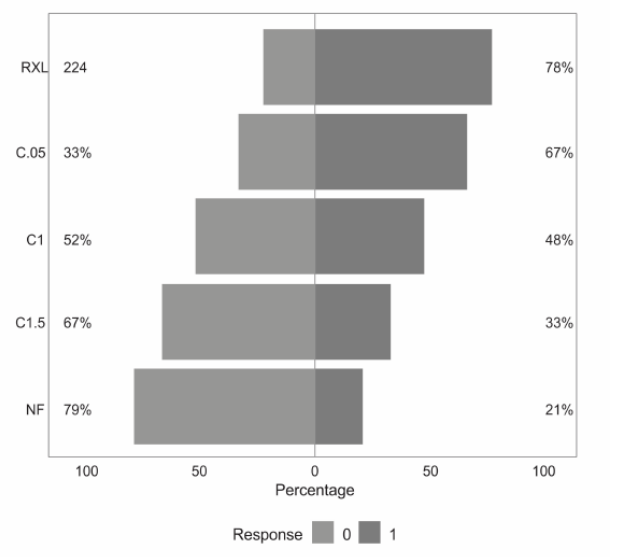

Figure 3. Percentage distribution of presence levels = 0 and absence = 1 of crosslinking, type of gelaltin (RXL Type B 130 Bloom = 1 and NF Type B 150 Bloom = 2) and percentage of FD&C Red 40 dye (C0.5 = 0, C1 = 1 and C1.5 = 2), in formulations of gelatin sheets

In case of dyes, it was observed that as the concentration increased, crosslinking also increased (33% for 0.5, 52% for 1, and 67% for 1.5); similarly, the percentage of no crosslinking decreased with increasing dye concentration (33% for 1.5, 48% for 1, and 67% for 0.5). The logistic regression model was applied, and estimates of the logistic model coefficients are shown in Table 5.

Table 5. Parameter estimates of the logistic model for the design of factorial experiment 2^2 (With interaction)

Variables	Coefficients	Standard error	p Value
Intercept	4.569	1.208	<0.001
FD&C Red 40	-2.939	0.939	0.002
RXL Type B 130 Bloom	-4.405	1.413	0.002
FD&C Red 40 * RXL Type B 130 Bloom	1.313	1.227	0.284

Table 6. Parameter estimates of the logistic model for the design of factorial experiment 2^2 (without interaction)

Variables	Coefficients	Standard error	P value
Intercept	3.711	4.778	<0.001
FD&C Red 40	-2.234	0.600	<0.001
RXL Type B 130 Bloom	-3.045	0.492	<0.001

Table 7. Predicted probabilities of non-crosslinking, for each type of gelatin and for some percentages of dyes.

Gelatine	% FD&C Red 40	Probability	Gelatine	% FD&C Red 40	Probability
RXL Type B 130 Bloom	0.000	0.980	NF Type B 150 Bloom	0.000	0.660
	0.010	0.980		0.010	0.660
	0.100	0.970		0.100	0.610
	0.150	0.970		0.150	0.580
	0.200	0.960		0.200	0.550
	0.250	0.960		0.250	0.530
	0.300	0.950		0.300	0.500
	0.350	0.950		0.350	0.470
	0.400	0.940		0.400	0.440
	0.450	0.940		0.450	0.420
	0.500	0.930		0.500	0.390
	0.550	0.920		0.550	0.360
	0.600	0.910		0.600	0.340
	0.650	0.910		0.650	0.310
	0.700	0.900		0.700	0.290
	0.750	0.880		0.750	0.270
	0.800	0.870		0.800	0.250
	0.850	0.860		0.850	0.230
	0.900	0.850		0.900	0.210
	0.950	0.830		0.950	0.190
	1.000	0.810		1.000	0.170
	1.050	0.800		1.050	0.160
	1.100	0.780		1.100	0.140
	1.150	0.760		1.150	0.130
	1.200	0.740		1.200	0.120
	1.250	0.710		1.250	0.110
	1.300	0.690		1.300	0.100
	1.350	0.670		1.350	0.090
	1.400	0.640		1.400	0.080
	1.450	0.620		1.450	0.070
	1.500	0.590		1.500	0.060

This table shows that the interaction between the gelatin types and the percentage of FD&C Red 40 dye is not significant in the model (P-value = 0.284> 0.05); therefore, it was eliminated from the logit model. This same result can be seen graphically in Figure 4. Almost parallel lines indicate that there is no interaction between these two variables.

A new model is adjusted without considering the interaction. The values of the parameter estimation are shown in Table 6.

We see that all the variables (type of jelly and percentage of FD&C Red 40) are statistically significant in the model (P-value <0.05). Based on the estimates of the logistic parameters, for each type of gelatin, some probabilities of non-crosslinking have been calculated in formulations of varying composition of percentages of the FD&C Red 40 dye. The results found are shown in Table 7.

The table shows that for each type of gelatin, the probability of non-crosslinking increases as the percentage of dye decreases, obtaining the best results when using

RXL Type B 130 Bloom gelatin with low concentrations of FD&C Red 40 dye from 0.700 % up to 0.010%. These same results can be seen more clearly in a graphic representation (as shown in Figure 5).

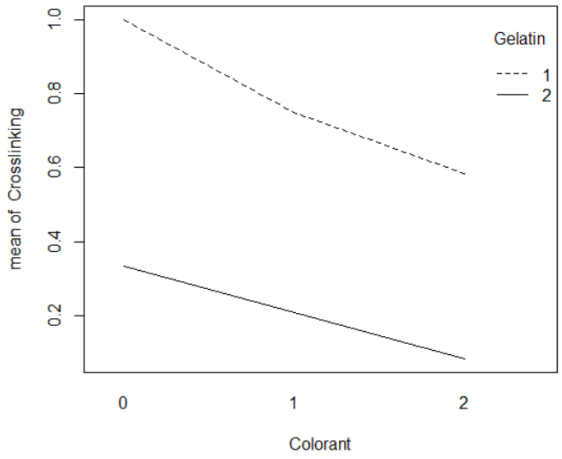

Figure 4. Interaction between the type of gelatin and the percentage of FD&C Red 40 dye in gelatin sheets

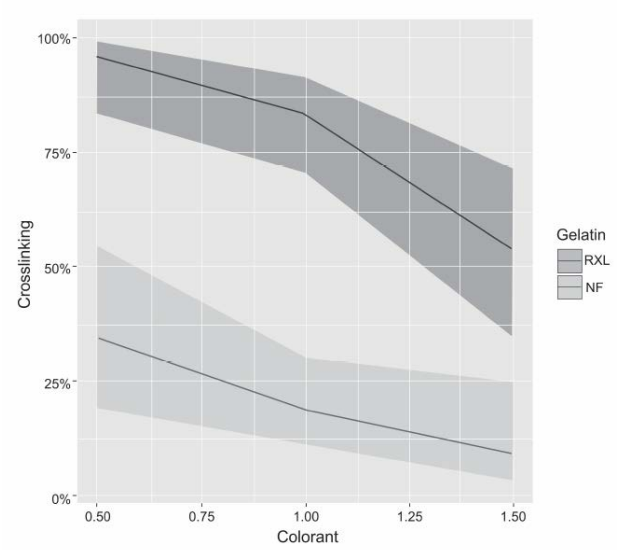

Figure 5. Predicted probabilities for non-crosslinking for each type of jelly and for some percentages of FD&C Red 40 dyes

DISCUSSION

Among the many problems of pre-formulation of pharmaceutical forms of soft gelatin capsules, the resistance of the capsules to dissolve constitutes a main factor causing rejection of the product and low effectiveness in pharmacological therapy.

The main objective of this investigation was to determine the contribution of crosslinking formation using two types of gelatin, RXL Type B 130 Bloom and NF Type B 150 Bloom, at different concentrations of FD&C Red 40 dye, which was evaluated by the dissolution test of Jelly sheets. Out of the 6 pre-formulations that were prepared and evaluated, it was observed that the pre-formulation containing NF Type B 150 Bloom jelly is where the highest percentage of crosslinking occurred. The percentage increased as the concentration of FD&C Red 40 dye increased. On the other hand, pre-formulations containing RXL Type B 130 Bloom jelly had a lower percentage of crosslinking. Likewise, with this jelly the presence of crosslinking is seen when the concentration of FD&C Red 40 dye is increased. Due to the nature of these two types of gelatin, it could be assumed that the proportion of amino acids in their gelatin chains and the chemical nature of the dye could explain the high percentage of crosslinking when using a specific type of gelatin and how the increase affects the percentage of dye. In this study, it was found that the main effects of the variables under study (type of gelatin and dye concentration) were significant; the intercept or their interactions did not show statistical significance. The variable that presents the greatest weight on the response is the type of jelly, for the logit models generated; the value of the intercept is not taken into account because it was not significant. It is evident that the relationship between gelatin type 1 (RXL Type B 130 Bloom) with the concentration of dye (FD&C Red 40) is the one that offers the highest probability of non-crosslinking, as the coefficient of the variable dye concentration is negative, indicating that the lower dye concentration may have greater chance of success.

It has been found that the effects of gelatin types and dye concentration on crosslinking are statistically significant. The use of the RXL Type B 130 Bloom jelly type in pre-formulations for soft gelatin capsules increases the likelihood of non-crosslinking. Gelatins obtained by alkaline hydrolysis process are more likely to undergo crosslinking when combined with FD&C Red 40. The higher proportion of hydroxyproline in the gelatin chains increases the probability of crosslinking when combined with FD&C Red 40. Increasing the concentration of the dye Red FD&C 40 in gelatin pre-formulations for soft capsule processing increases the probability of crosslinking. The higher the concentration of FD&C Red 40 dye (Figure 1), the greater the number of free sulfonate groups that can react with amino acid residues present in the gelatin chains and lead to crosslinking. In conclusion, the ideal relationship of the variables under study for the preparation of pre-formulations provides a greater probability that crosslinking does not occur is RXL Type B 130 Bloom jelly with low concentration of FD&C Red 40 dye.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author: A. Espinosa, e-mail: cespinosaa@uninorte.edu.co. The data are not publicly available due to restrictions, e.g. their containing information that could compromise the privacy of research participants.

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