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From Lab to Upscale—Boosting Formulation Performance through In Vitro Technologies †

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Abstract: Pre-stability studies carried out throughout the development of a diclofenac emulgel formulation have shown a clear decrease on drug release rate. In order to address the root-cause associated with this phenomena, product historical data was retrieved and analyzed following a retrospective quality by design (rQbD) approach. Product quality target product profile (QTPP) was established and risk assessment tools were used to identify the most relevant parameters affecting formulation performance. These consisted in (i) mixing time, (ii) sodium hydroxide content and (iii) carbopol grade. Following a 2^3 full factorial design, the pH, viscosity, in vitro release rate and cumulative amount of drug released in the end of the release experiment were selected as responses to statistically model the available data. It was observed that higher sodium hydroxide concentrations induce a decrease in viscosity, consequently resulting in a superior pharmaceutical performance. Moreover, as a secondary effect, a lower carbopol viscosity yields lower release outputs. The estimated models were used to define a feasible working region, which was further confirmed at an industrial scale. This work highlights the use of rQbD principles to achieve a greater product understanding. By doing so, specific strategies can be applied to product manufacture in order to consistently meet QTPP requirements.

Keywords: IVRT; Topical drug delivery; DoE; scale-up

1. Introduction

The release profile of a topical semisolid dosage form, extracted from in vitro release testing (IVRT), typically carried out through Franz diffusion cells, enables the determination of the in vitro release rate (IVRR). This kinetic parameter provides important information on the microstructure characteristics of the product, such as particle size and rheological behavior. For this reason, it is considered a product critical quality attribute [1–6]. In this context, the determination of the in vitro release profile is a valuable tool during product development and optimization [7].

The present work aimed at providing the assumptions to assist a sustainable improvement of the pharmaceutical performance of an anti-inflammatory semisolid dosage form. The qualitative/quantitative composition and the production process were already well-established, however, there were some parameters that lacked optimization, since in pre-stability studies, a marked decrease on drug release outcomes was observed. To address this constraint, the historical data of the product was thoughtfully analyzed following Quality by Design (QbD) principles. This is

referred to as "retrospective QbD" (rQbD), since it focuses on product historical data and not on classic QbD approaches, which are mainly directed towards new product development [8].

A cause-and-effect diagram and a risk estimation matrix were constructed to identify potential CPPs (Critical Process Parameters) and CMAs (Critical Material Attributes) that could impact the formulation CQAs (Critical Quality Attributes). From this analysis (data not shown), three main factors were identified as critical: (i) mixing time; (ii) sodium hydroxide content and, finally, (iii) carbopol viscosity. Efforts were then made to rationalize, predict and ultimately maximize the effects of these parameters on the product pharmaceutical quality. For that, a 2^3 full factorial design was employed to assess the impact of the above mentioned variables, on the pH, viscosity, IVRR and cumulative amount released at the end of the IVRT study. During this optimization phase, all manufactured batches were produced at a laboratory scale. To confirm these assumptions, the formulations were then translated from lab to industrial scale, envisioning the validation of the working conditions in line with the predefined quality target product profile (QTPP).

2. Materials and Methods

2.1. Materials

All formulation components (medium chain triglycerides, hydroxiethylcellulose, carbopol 980, propylene glycol, propylparabene, methylparabene, sodium diclofenac and sodium hydroxide) were kindly provided by Laboratórios Basi Indústria Farmacêutica S.A. (Mortágua, Portugal). The commercial name of the raw materials is not disclosed for confidential purposes. For IVRT tests, propylene glycol was acquired from Merck and phosphate buffered saline was purchased from Sigma. Water was purified (Millipore®) and filtered through a 0.22 μm nylon filter before use. All other chemicals were of analytical grade or equivalent.

2.2. Methods

2.2.1. Diclofenac Emulgel Production

Emulgels regard pharmaceutical dosage forms gathering emulsion and gel properties, which enables their use as a controlled topical delivery system [9]. Their production firstly involves the preparation of an emulsion by the hot emulsification method. Briefly, water, medium chain triglycerides, hydroxiethylcellulose and the carbopol) were mixed with propylene glycol, propylparabene and methylparabene), which had been previously heated to 40 °C to enable the complete solubilization of both preservatives. Please note that different carbopol viscosities were used, as this was one of the critical material attributes (CMAs) retrieved from the risk assessment analysis. Both phases were homogenized by an Ultra-Turrax (T50B IKA) for a specified rotation, time and temperature. Afterwards, the drug was dissolved in water at 70 °C and blended into the previously prepared mixture by using a mechanical stirrer (Heidolph AZA 2051). The formulation was then cooled down to 25 °C and a 10%(w/V) sodium hydroxide solution was slowly added following fixed intervals of time according to the design of experiments (DoE). The formulation was then filled into suitable lined collapsible aluminum tubes (100 g). 1 Kg batches were considered for laboratory scale studies, whilst 600 kg were considered for industrial scale batches.

2.2.2. Quality Target Product Profile (QTPP) Definition

The establishment of a QTPP is regarded as the basis of formulation development, as it refers to a prospective summary of the quality characteristics intended for the product [10]. Therefore, the QTPP was established envisioning the emulgel quality features intended to reach, considering the drug product efficacy and safety aspects.

2.2.3. Rectrospective Quality by Design Applied to Diclofenac Formulation Optimization

Since the qualitative/quantitative composition and the production process were already well-disclosed, it was possible, based on prior knowledge, to retrospectively identify several production settings/critical material attributes which might have a direct repercussion on the formulation. The (i) neutralizer addition (final hydroxide concentration) and (ii) thickener grade (carbopol viscosity) were considered as CMAs. On the other hand, as CPPs, the mixing time (40 vs. 80 min) during production was selected. As critical quality attributes (CQAs), due to their overall importance in semisolid microstructure, the following parameters were considered: viscosity, pH and IVRT outputs – IVRR and cumulative amount of drug released in the end of the study (Qf).

A 2^3 full factorial design was performed for the optimization of the diclofenac emulgel formulation. This design envisions an in-depth analysis of the impact and interactions between the previously referred CMAs and CPP, in the formulation CQAs. Coded (−1, +1) levels were used for each independent variable, X_1, X_2, and X_3 (mixing time, NaOH final product concentration and carbopol viscosity), in which the −1 level corresponds to the lower value of each variable and +1 to the upper one. Table 1 describes the settings used for each formulation. The experimental design and the polynomial models were solved resorting to JMP Pro software. These models were used to describe the influence of each factor and to check for potential synergisms between them.

Table 1. Process and formulation experimental settings according to a 2^3 full factorial design.

Formulation	Mixing Time	Sodium Hydroxide Concentration	Carbopol Viscosity
F1	40 min (−1)	0.26% (−1)	44400 cPs (−1)
F2	80 min (+1)	0.3% (+1)	48800 cPs (+1)
F3	40 min (−1)	0.3% (+1)	48800 cPs (+1)
F4	80 min (+1)	0.26% (−1)	44400 cPs (−1)
F5	40 min (−1)	0.3% (+1)	44400 cPs (−1)
F6	80 min (+1)	0.26% (−1)	48800 cPs (+1)
F7	40 min (−1)	0.26% (−1)	48800 cPs (+1)
F8	80 min (+1)	0.3% (+1)	44400 cPs (−1)

Equation (1) defines the polynomial equation used to describe the behavior of each selected independent variable.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

where, Y refers to the response in the absence of effects, β_1, β_2 and β_3 the linear coefficients of the independent variables, β_{12}, β_{13} and β_{23} the interaction coefficients between the factors. Analysis of variance (ANOVA) and Student's t-test were applied to test pair-wise multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

2.2.4. pH Measurement

Topical products should be manufactured with an appropriate pH range in order to assure an adequate drug solubility, stability, and ultimately product biocompatibility. pH values were determined at room temperature (25 °C), in triplicate, using a digital pH meter pH/ION seven compact—Metler Toledo, previously calibrated using standard buffer solutions (pH of 4.00, 7.00 and 10.00). About 1 g of each sample was weighed and dispersed in 10 times the volume of distilled water. Afterwards, the respective pH value was recorded. The determination was performed 24 h after batch manufacturing.

2.2.5. Viscosity Measurement

Formulation viscosity was evaluated 24 h after production at 25 °C using a rotational viscometer (Brookfield Viscosimeter®, RV DV-II, USA) with a spindle T-A.

2.2.6. In Vitro Release Testing and HPLC Analysis

The IVRT method was conducted using static vertical Franz diffusion cells (PermeGear, Inc., PA, USA) with a diffusion area of 0.636 cm^2 and a receptor compartment of 5 mL. 300 mg of the formulation were applied in the donor compartment, separated from the receptor compartment by a polysulfone membrane, previously soaked in distilled water for 30 min. The receptor media comprised a phosphate buffered saline (PBS): proplylene glycol mixture (80:20, V/V), continuously stirred at 600 rpm and maintained at a temperature of 37 °C. Samples of the receptor phase were withdrawn at 15, 30, 60, 90, 120, 150, 180 min. After each collection, the same volume of medium was replaced with receptor solution. A n = 4 was performed in the same conditions. The concentration of diclofenac in IVRT samples was determined though HPLC, following the experimental procedures previously described [7,11].

3. Results

3.1. QTPP Definition

To follow a rQbD-based development approach, it is essential to define the desired product performance profile, also known as quality target product profile (QTPP). This refers to a prospective summary of quality characteristics to be achieved for a pharmaceutical product [12]. Taking into account the defined QTPP, presented in Table 2, as well as the historical data gathered during the initial development studies, it was possible to identify CPP, CMA and CQA pertaining to the diclofenac emulgel formulation. This information was then integrated within quality risk management principles and with DoE, to effectively apply QbD principles [8].

Table 2. Quality target product profile (QTPP) specifications of a diclofenac emulgel.

QTPP Element	Target	Scientific Rationale
Dosage form	Emulgel	Emulgels combine emulsion and gel characteristics. This dosage form can be regarded as a controlled topical delivery system. This technological feature is most useful in musculoskeletal disorders management [13].
Administration route	Topical	Local administration avoids systemic side effects. Moreover, this route is non-invasive, convenient and painless and, therefore, promotes high patient compliance [14].
Dosage strength	1% w/w	A 1% w/w diclofenac emulgel ensures formulation efficacy.
Assay	90–110%	A correct dosing is required to ensure therapeutic efficacy. Nevertheless, this parameter was not considered as a formulation CQA within this rQbD approach, since compliance with this parameter had been consistently documented during pre-development studies.
Physical attributes		
Organoleptic characteristics	White smooth emulgel	Even though this parameter is not directly related with safety, it is considered relevant to ensure patient compliance and acceptance. Moreover, inadequate homogenization conditions may lead to phase separation which ultimately impact the product efficacy profile.
pH	6–7.5	Topical products should be designed within a suitable pH range to assure an adequate drug solubility, stability and skin compatibility.
Viscosity	255,000–270,000 cPs	The viscosity profile of a semisolid dosage form is highly linked with the product sensorial properties, such as consistency, spreadability and feel, which strongly impact patient compliance [11,15].

		From a quality perspective, viscosity measurements can be regarded as not solely a monitoring tool during process development and stability assessment, but also as a performance indicating tool, since these attributes correlate with drug release and diffusion rate [14].
		Product performance
IVRR	>690 µg/cm²	A compliant API release assures an adequate product pharmaceutical performance [16].
Qf	>490 µg/cm²/√h	

3.2. Quality by Design Outputs

As presented in Figure 1, there are distinct release behaviours among the formulations. Three principal groups can be observed: (i) High release (F2, F3, and F5); (ii) Moderate release (F6, F7 and F8), and (iii) Low release: F4, and F1.

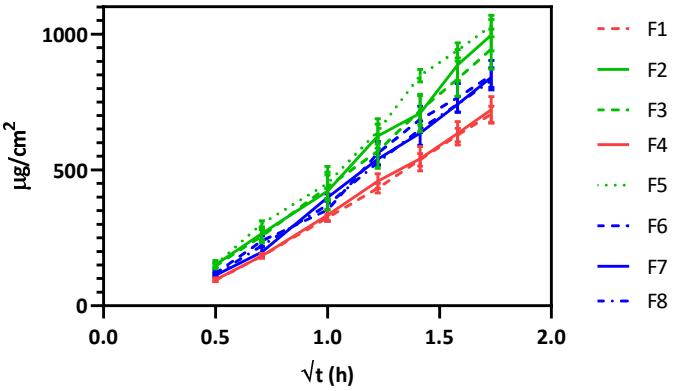

Figure 1. IVRT profiles of the formulations prepared according to the DoE.

Tables 2 and 3 gather the values of the coefficients obtained from the experimental design, as well as the corresponding statistical significance.

Table 2. Parameters of the response surface for viscosity, IVRR and Qf obtained from the 2^3 full factorial design, respective t ratio and Prob > |t|.

		β_0	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}
Viscosity	**Coefficients**	317113	6863	−11188	−12713	−2038	−263	6288
	t ratio	29.19	0.63	−1.03	−1.17	−0.19	−0.02	0.58
	Prob>|t|	0.0218	0.6413	0.4906	0.4501	0.8820	0.9846	0.6660
IVRR	**Coefficients**	604	−14.2	50.3	33.0	−18.9	26.2	−17.4
	t ratio	23.5	−0.55	1.96	1.28	−0.73	1.02	−0.68
	Prob>|t|	0.0271	0.6779	0.3008	0.4215	0.5969	0.4941	0.6216
Qf	**Coefficients**	858	−22.1	79.4	49.3	−27.9	37.0	−16.2
	t ratio	22.1	−0.57	2.05	1.27	−0.72	0.95	−0.42
	Prob>|t|	0.0288	0.6704	0.2891	0.4240	0.6032	0.5149	0.7481

Table 2. Summary of least squares fit for each response.

Responses	Summary of Fit		ANOVA
	RSquare	Rsquare Adj	Prob > F
Viscosity	0.762	−0.665	0.78
IVRR	0.88	0.205	1.30
Qf	0.89	0.199	1.29

3. Discussion

A higher positive coefficient indicates that an increase of that specific CMA/CPP promotes an increase in the response, whilst a negative coefficient bears the opposite system response, meaning that with its increase, the system response decreases. The higher the magnitude of the coefficients, the higher is the influence of that variable on the system, either positively or negatively [17].

As can be seen from Table 2, higher coefficient magnitudes are attained for sodium hydroxide content (β_2) and carbopol viscosity (β_3). These are the main CMA that affect formulation CQA, meaning that formulation parameters prevail over the CPP. The sodium hydroxide content, negatively affects the final product viscosity, resulting in a better product performance in terms of release behavior. Similarly, carbopol viscosity also negatively impacts formulation viscosity, i.e., higher carbopol viscosity values prompt a decrease in final product viscosity, which in turn is correlated with higher IVRT outputs. Another important analysis relies on the interaction terms, which indicate how the variation of one factor may modulate the response of another one, and consequently influence the selected response. Regarding possible synergistic effects between the studied CMA/CPP, solely the coefficient β_{13} registered higher magnitudes.

It should be remarked that despite pH had been selected as a product CQA, this individual parameter showed no significant effect (p-value > 0.05, data not shown).

As topical semisolid microstructure is highly dependent on batch size, these assumptions needed to be further validated at an industrial scale. In order to draw plausible conclusions two opposite formulations were produced: one with lower sodium hydroxide content and higher carbopol viscosity, and a second one with superior percentage of sodium hydroxide and lower carbopol viscosity. The results, presented in Figure 2, sustain DoE estimates.

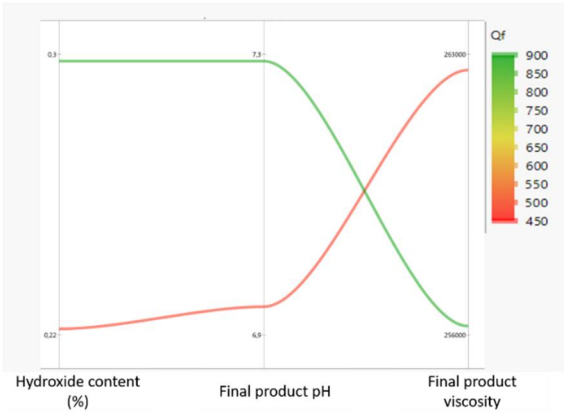

Figure 2. Parallel plot portraying diclofenac 10 mg/g industrial batches.

5. Conclusions

Based on DoE results, the following assumptions may be retrieved: (i) The principal effect seems to be correlated with sodium hydroxide concentration. In other words, to yield a superior formulation performance, the concentration of hydroxide should be set to the higher level. This will enable a decrease in final product viscosity which in turn promotes a superior IVRT performance. (ii) A secondary effect regards carbopol viscosity, with lower values yielding lower IVRT outputs. These results were further confirmed at an industrial scale.

Such findings consubstantiate the relevance of the application of rQbD principles in the redesign of pharmaceutical drug products.

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Abbreviations

The following abbreviations are used in this manuscript:

CMA	critical material attributes;
CPP	critical process parameters;
CQA	critical quality attributes;
DoE	design of experiments;
IVRR	in vitro release rate;
IVRT	in vitro release testing;
QbD	Quality by Design;
Qf	Cumulative amount of drug released in the end of the IVRT study;
QTPP	quality target product profile.

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