QUALITY BY DESIGN (QBD) AS A TOOL FOR THE OPTIMIZATION OF INDOMETHACIN FREEZE-DRIED SUBLINGUAL TABLETS: *IN VITRO* AND *IN VIVO* EVALUATION

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ABSTRACT

Objective: This study aims to prepare and optimize indomethacin freeze-dried sublingual tablets (IND-FDST) by utilizing a quality by design (QbD) approach to achieve rapid drug dissolution and simultaneously bypassing the GIT for better patient tolerability.

Methods: A screening study was utilized to determine the most significant factors which the quality attributes, namely disintegration time and % friability. Then an optimization study was conducted using a full response surface design to determine the optimized formula by varying the amount of the matrix-forming polymer (gelatin) and super disintegrant (croscarmellose sodium (CCS)). The variables' effect on the % friability, disintegration time, wetting time, and amount of drug release after 10 min (%Q10) was studied. The optimized formula was tested for compatibility, morphology as well as stability studies under accelerated conditions in addition to the *in vivo* pharmacodynamics in rats. QbD was adopted by utilizing a screening study to identify the significant formulation factors followed by a response surface optimization study to determine the optimized IND-FDST formulation.

Results: Optimized IND-FDST comprised of gelatin/CCS combination in a ratio of 1:1 possessed adequate %friability (0.73±0.03%), disintegration time (25.40±1.21 seconds), wetting time (3.49±0.68 seconds), and % Q10 (100.99±5.29%) as well as good stability under accelerated conditions. IND-FDST also showed significant inhibition of edema, tumour necrosis factor-alpha, and interleukin-6 release *in vivo* compared to the oral market product by 70%, 42%, and 65%, respectively.

Conclusion: QbD presents a successful approach in the optimization of a successful IND-FDST formula that showed superior *in vivo* and *in vitro* characteristics.

Keywords: Quality by design, Indomethacin, Freeze-dried tablet, Sublingual tablet, Stability testing

INTRODUCTION

Oral drug delivery is the most customarily used route among all other drug administration routes [1] because of its recognizable advantages such as; its ease of application, ameliorate patient compliance, slightest sterility conditions needed, and high flexibility in developing the dosage form [2, 3]. Now a day's formulation research is breaking barriers of conventional methods to deliver drugs more conveniently with better performance and high bioavailability. Limitations associated with the oral tablets could be solved by using sublingual tablets as an alternative to the oral route [4]. The use of the sublingual route offers various advantages, such as ease of preparation and the ability to bypass the GIT harsh conditions [5]. In addition, the absorption of the drug by the veins located in the mouth floor, leading directly to the superior vena cava [6] combined with a small thickness of 100-200 μm. Allows faster distribution of the drug through the bloodstream compared to that of orally administered drugs [6–10]. Freeze-dried sublingual tablets have attracted much attention from researchers as it offers tablets of high porosity which allows better exposure of the drug to the saliva in the mouth resulting in immediate disintegration and dissolution of the tablet when placed under the tongue [11–16].

Non-steroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous drug group broadly prescribed for analgesic, fever-reducing, and anti-inflammatory effects [17, 18]. A model drug for NSAIDs in our study is Indomethacin (IND), a non-selective NSAID that is considered one of the most successful anti-rheumatic drugs and prime drug nominate for the management of pain and/or swelling in many conditions such as osteoarthritis, rheumatoid arthritis [19, 20], and joint pains [21], Despite, its high therapeutic efficacy, oral administration of IND causes several adverse effects, such as gastrointestinal ulceration especially upon the repetitive use

of the drug with high doses and in cases of self-medication [22, 23]. These effects are attributed to the cyclooxygenase (COX) inhibition which impairs the GIT protective barrier function as well as the prolonged contact of the drug with the GIT mucosa. The latter mechanism can be tackled via minimization/elimination of the IND contact with the GIT mucosa *via* sublingual route can be beneficial as an alternative route to overcome IND side-effects and to benefit from the advantages of this route [24].

Recently, Quality by design (QbD) has harnessed significant attention in the scientific and industrial community to shorten the time needed for the development of formulas with efficient resource allocation [25]. QbD is a regulatory, systematic, knowledge-based system with quality management thinking to ensure the quality of the predefined final target product [26]. QbD necessitates the determination of the quality target product profile (QTPP), and critical quality attributes (CQAs), which critically affect the quality properties of the final product [27]. Scientific knowledge from previously reported literature is the basic source for the selection of material attributes and critical process parameters (CPPs) which can significantly influence the QTPPs [26].

Thus, based on the above considerations, this study aims to develop IND freeze-dried sublingual tablets (IND-FDSTs) to allow a faster onset of action for IND and eliminate the direct contact of the drug with GIT mucosa. A QbD approach was utilized for screening of select factors, namely, matrix former type and amount as well as super disintegrant type and amount. The % friability and disintegration time were considered CQAs. An optimization formula was then conducted utilizing a response surface design for the determination of the optimized IND-FDST, which will be further tested for stability and compatibility. An additional aim of this study was to compare the anti-inflammatory effect of IND-FDSTs compared to free IND in rats.

MATERIALS AND METHODS

Materials

Indomethacin (IND) was kindly supplied by El Arabeya Co. for Pharmaceutical and Chemical Industries, Cairo (Egypt). Carrageenan and pectin extracted from citrus fruits were purchased from Sigma Aldrich Chemical Co., (St. Louis, USA). Sodium alginate and gelatin were purchased from AVI-CHEM Laboratories, (Mumbai, India). Methocel® E15 was purchased from Colorcon (Dartford, United Kingdom). Crospovidone was supplied by BASF Pharma, Isle of Lewis (UK). Sodium starch glycolate, croscarmellose sodium, and mannitol were obtained from Roquette Pharma (Zaventem, Belgium). Sucralose was kindly supplied from Marcyrl Pharmaceutical Industries (Cairo, Egypt). Glycine, disodium hydrogen phosphate, and potassium dihydrogen phosphate were obtained from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). Tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were purchased from NOVA (Beijing, China). Distilled water was used throughout the study. All other chemical reagents and solvents were of analytical grade and used as received. Forty adult male Wister albino rats (7-8-weeks old, weighing 130-150 g) were purchased from the National Research Centre animal house (Cairo, Egypt) to be used for the animal studies.

Preparation of indomethacin freeze-dried sublingual tablets (IND-FDSTs)

IND-FDSTs were prepared by combining matrix-forming polymers (either gelatin, pectin, or Na alginate) with a super disintegrant (either croscarmellose (CCS), crospovidone (CP), or sodium starch glycolate (SSG)) in distilled water and stirred with a magnetic stirrer till they completely dissolve. After that, glycine was added as a cryoprotectant and mannitol as a bulking agent and allowed to dissolve to give a clear solution. An accurately weighed amount of IND powder was then dispersed in the prepared aqueous solution using a magnetic stirrer. Then, an accurate volume of the resulted dispersion was transferred into the pocket of a PVC blister pack to give a dose of 25 mg of IND per tablet. The blister packs were freeze-dried for 24 h in a lyophilizer (Alpha 1-2 Idplus Christ) under a pressure of 0.02 mbar at-45 °C. The resultant IND-FDSTs were inspected for the integrity and smoothness of the surface as well as their adhesion to the blisters; the thickness and diameter were also measured using a vernier caliper (Mituyoto, Tokyo, Japan) and presented as mean value±standard deviation (SD) to ensure successful preparation and tablet properties.

Quality by design (QbD) and screening study

Based on literature reviews and preliminary studies, risk assessment was implemented to identify and prioritize all the high-risk material attributes and process parameters that may potentially affect IND-FDSTs. After identifying the critical quality attributes (CQAs), according to ICH8 [26], CQAs for oral tablets can be set as disintegration time and % drug released at 10 min (% Q10), % friability. Ishikawa (fishbone or cause-and-effect) diagram was set up as a graphical tool for highlighting all factors that can influence the CQA of IND-FDST. The diagrams were generated using Minitab® 17 software (Minitab Inc.; State College, PA, USA).

An extensive number of variables from many categories, i.e., materials, formulation, environment, homogenization process, methods, and measurements, *etc*., presented in the Cause and Effect (Ishikawa) diagram (fig. 1), were reported in the literature. Based on the results of the Ishikawa diagrams, the type and amount of matrix former and super disintegrant were determined to be critical factors for the preparation of IND-FDSTs, which in turn directly affect the disintegration and dissolution. The critical quality attributes (CQA) were set to the % friability and disintegration time.
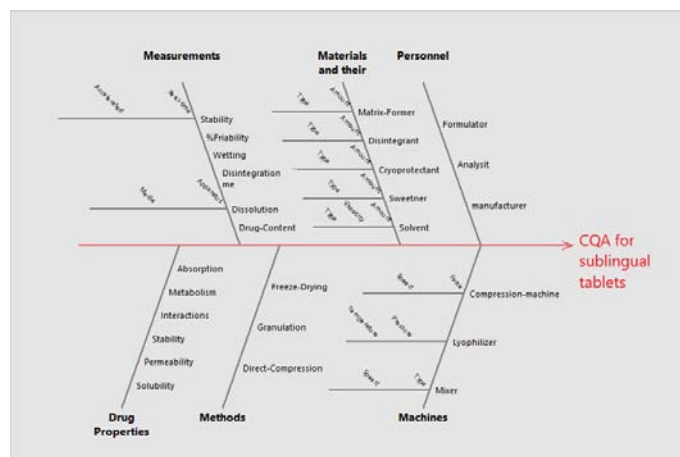

Fig. 1: Ishikawa diagram representing the formulation critical factors and CQAs for preparation of sublingual tablets based on historical data for QbD study

Herein, a screening study was conducted for the determination of the most significant formulation attributes to be included in the optimization study. IND-FDSTs were prepared according to the proposed screening study design using Minitab® 17 to determine the most critical attributes affecting the formulation for optimization of the preparation of IND-FDST. The screening design comprised two categorical variables: matrix former type (X1) in three levels (gelatin, pectin, and sodium alginate), super disintegrant type (X2) in three levels (CP, CCS, and SSG) as well as their amount (X3 and X4, respectively) in two levels (0, and 4 mg). The most promising formulas were prepared and their CQA's were determined, namely % friability (Y1) and disintegration time (Y2). All experiments were conducted in triplicates and the data were evaluated for the level of significance for the studied factors on the responses using one-way analysis of variation (ANOVA). The resultant 12 formulas (S1-S12) composition is listed in table 1. The optimized combination of the mentioned factor will be selected based on the desirability index (D) value with the most desirable formula with a value close to 1 to be included in the optimization study. The optimized formula will be defined as the formula with the least % friability and the least disintegration time with equal weights for both attributes.

Optimization study for IND-FDSTs

A response surface design was employed for the optimized matrix former/super disintegrant combination by varying their amounts (0, 2, 4, and 8 mg for super disintegrant and 0, 2, 4, 8 mg for matrix polymer) and employing the same preparation method previously described. The selected independent variables were % friability (Y1), disintegration time (Y2), wetting time (Y3), and % IND released at 10 min (% Q10) (Y4). All experiments were conducted in triplicates and the data were evaluated for the level of significance of

the studied factors on the responses using one-way analysis of variation (ANOVA). The resultant 9 formulas (O1-O9) composition is listed in table 2. The optimized formula was selected based on the D value with the most desirable formula has a value close to 1 to be employed for future studies.

All formulas were evaluated according to their thickness, diameter, weight variation, drug content, friability, disintegration time, and dissolution profile.

Evaluation of IND-FDSTs

Weight variation

From each formula, twenty tablets were weighed individually, and the average weight was calculated. The weight variation was calculated using Equation 1 [28].

$$\text{Weight Variation (\%)} = \frac{\text{Theoretical tablet weight} - \text{Average tablet weight}}{\text{Average tablet weight}} \times 100$$
.... (Equation 1)

Friability

Friability was determined using an Erweka friability tester (ERWEKA-GmbH, Germany) by introducing ten tablets of each formula after being de-dusted and weighed. The drum of the apparatus was rotated for 100 rotations for 1 minute after which the tablets were removed, brushed, and reweighed. The difference between the weight of the tablets before and after the test was calculated according to Equation 2.

$$\text{Friability (\%)} = \frac{\text{Initial tablet weight} - \text{Final tablet weight}}{\text{Initial tablet weight}} \times 100 \text{ .... (Equation 2)}$$

Drug content

Five tablets from each formula were powdered using mortar and pestle and an amount equivalent to one tablet was weighed and dissolved in methanol and phosphate buffer pH 7 in a ratio of 1:1 on a rotary shaker overnight so that IND content would constitute 25µg/100 ml. The solution was then centrifuged and the supernatant was collected and analysed for IND content using a UV-visible spectrophotometer (Shimadzu spectrophotometer 2401/PC, Japan) at λ_{max} 318 nm and the concentration of the drug was calculated from the constructed calibration curve (USP 41/NF 38)[29]. The measurements were repeated (n= 3) and the average drug content was calculated according to the following equation [30]:

$$\text{Drug content (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \text{ .... (Equation 3)}$$

Wetting time

A sample of 10 ml of methylene blue dye in distilled water was placed in a Petri dish of 10 cm diameter. One tablet was carefully placed in the center of the Petri dish and the time required for water to reach the upper surface of the tablet was measured and noted as the wetting time. The test results are presented as the mean value of three determinations±SD [31, 32].

Disintegration time

The disintegration times were determined *in vitro* by a USP disintegration tester (PTZ Auto 1 A, Pharma test, Germany) at 37 °C±2 °C (USP 41/NF 38) using distilled water as disintegration medium [29]. One tablet was introduced into each of the six tubes of the basket and the time required for complete tablet disintegration was recorded. The mean±SD of the six tablets was calculated.

Evaluation of the optimized IND-FDST

In vitro dissolution studies

The dissolution profiles of the optimized IND-FDST was compared to the pure drug (25 mg) and the current marketed product of IND (Indocin® capsules) of the same strength as well as formulas S1, S3, and S10 comprising Na alginate, gelatin, and pectin, respectively as matrix formers with the same ratios of all other excipients for comparison purposes. The experiments were carried out in triplets for each sample in a USP rotating paddle apparatus (Hanson SR8plus, USA). The dissolution media was 750 ml of phosphate buffer pH 7.4 and distilled water in ratio 1:4 (USP media) [29], the temperature was kept at 37±0.5 ° C and the paddle rotation speed was set to 100 rpm. Samples were withdrawn at 1, 2, 3, 5, 7, 10, 15, and 20 min. The sample volume was 5 ml of dissolution medium which was replaced by an equal volume of fresh buffer to maintain a constant total volume. Samples werefiltered using a syringe filter of 0.45 µm Millipore and the drug concentration was spectrophotometrically assayed (Shimadzu spectrophotometer 2401/PC, Japan) at 318 nm after appropriate dilution with dissolution media. The dissolution profiles were compared using similarity factor (f2) defined by Equation (4) [33]

$$f2 = 50\log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} Rt - Tt\right]^{-0.5}\right\}x100 \text{ .... (Equation 4)}$$

Where, n = the number of sampling time points, Rt = mean % dissolved of the reference at a given time point t, Tt = mean % dissolved of the test at the same time point t. The similarity factor fits the result between 0 and 100. For two dissolution profiles to be considered similar, f2 values should be higher than 50, whereas smaller values imply dissimilarity between dissolution profiles [34].

Differential scanning calorimetry (DSC)

DSC scanning was done for pure IND, mannitol, the physical mixture, and the optimized IND-FDST. Scanning was carried out using a differential scanning calorimeter (Perkin Elmer, Germany) by heating 5 mg of the tested sample in sealed aluminum pans from ambient temperature to 200 °C at a linear heating rate of 10 °C/min under atmospheric nitrogen. The resultant thermograms were compared.

Fourier-transform infrared spectroscopy (FT-IR)

IR spectra of the pure drug, physical mixture, the selected IND-FDST, and all the excipients individually were recorded on FTIR spectrophotometer (Perkin Elmer, Germany) at a scanning range from 500–4000 cm^{-1} and resolution of 4 cm^{-1} [35].

Powder X-ray diffraction (XRD)

Diffraction patterns for pure IND, the selected IND-FDST, and their corresponding physical mixtures were obtained using Scintag X-ray diffractometer (Massachusetts, USA) using Cu Kα radiation, and a voltage of 45 kV [36].

Scanning electron microscopy (SEM)

Samples were cut from the selected formula using a blade and observed by Jeol JSM-6400 scanning electron microscope (Jeol Ltd., United States of America) for surface and section view [37].

Accelerated stability study

The stability of the optimized IND-FDST formula was studied in an accelerated stability study. Tablets were stored in glass containers and kept in the stability chamber (Votsch, VP600, Germany) at 40 °C±2 °C, 75%±5% RH for six months according to the ICH guideline (Q1A (R2) section 2.1.7.1) [38, 39]. The physical appearance, drug content, disintegration time, and *in vitro* dissolution for IND-FDST tablets were chosen to be the stability determining attributes. Thus, the aforementioned factors were re-determined after storage and compared to the freshly prepared formulation for any significant changes.

In vivo pharmacodynamics

Animals

Forty adult male Wister albino rats (7-8-weeks old, weighing 130-150 g) were purchased from the National Research Centre animal house (Cairo, Egypt) and housed in standard cages (10 rats per cage), under specific pathogen-free conditions in facilities maintained at controlled room temperature (21-24 °C) with a relative humidity of 40-60% and under normal dark-light cycles. All animals had free access to a rat chow diet and water ad libitum. The rats were acclimatized for two weeks before the start of the study in the laboratory of the National Research Centre, Cairo, Egypt. All procedures followed the Helsinki Declaration as well as specific

national laws. This experiment was carried out in accordance with the Ethics Committee of the National Research Centre, Egypt, and followed the National Institutes of Health Guide Recommendations Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). The procedures were approved by the Ethical Committee of the October for Modern Sciences and Arts (MSA) University (approval number: Pt18/Ec18/2019 F).

Carrageenan induced paw Edema

Paw swelling was induced by the sub-plantar injection of 100 μl of 1% sterile lambda carrageenan suspended in saline into the right hind paw [40, 41]. The contralateral paw received an equal volume of saline as a control. The inflammation was quantified by measuring the hind footpad before carrageenan injection and one to four hours after carrageenan injection with a micrometer caliper [42]. Edema was expressed as a percentage of change from control (pre-drug) values. Animals were divided into four groups each of ten rats. The 1st group: Rats received orally saline (0.2 ml/rat) as control, 2nd group: Rats were injected with carrageenan, 3rd group: Rats received crushed Indocin® Cap (IND market product) (25 mg/kg) [43], and fourth group: Rats received selected IND-FDST, O4 (25 mg/kg).

Serum tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) determination

Serum levels of TNF-α and IL-6 were determined using Enzyme-Linked Immunosorbent Assay (ELISA) kit. The manufacturer's instructions of the NOVA kit (Beijing, China) were followed, for calculating the results. Standards and samples were pipetted into wells with immobilized antibodies specific for rat TNF-α and IL-6, then were incubated for 30 min at 37 °C. After incubation and washing, horseradish peroxidase-conjugated streptavidin was pipetted into the wells and incubated for another 30 min at 37 °C, which were washed once again. Tetramethyl benzidine (TMB) substrate solution was added to the wells and incubated for 15 min at 37 °C; colour developed proportionally to the amount of TNF-α, and IL-6 bound. Colour development was discontinued (using Stop Solution) and after 10 min colour intensity was measured at 450 nm.

Statistical analysis

All the values are presented as means±SD of the means. Comparisons between different groups were carried out using ANOVA followed by the least significant difference (LSD) test for multiple comparisons using Minitab 17® software. The difference is considered significant when p<0.05.

RESULTS

QbD and screening study

As shown in table 1, all tablets exhibited comparable thickness, diameter, and weight variation percentages with a smooth appearance. The tablets thickness and diameter ranged from 0.55±0.1 cm to 0.54±0.01 cm and 1.45±0.1 cm to 1.52±0.02 cm, respectively. Weight variation ranged from 0.11±0.04 to 0.95±0.12% (data not shown). The drug content of all the prepared tablets was adequate ranging from 97.31% to 99.14% thereby conforming to USP limits between 90 and 110%.

Table 1: Composition of IND-FDSTs during QbD screening study and the respective results of % friability (n=10), drug content (n=5), and disintegration time (n=6)*

Formula**	Matrix former	Super disintegrant	Matrix former (mg)	Super disintegrant (mg)	Drug content (%)	% Friability	Disintegration time (sec)
S1	Na alginate	CCS	4	4	99.14±4.87	0.13±0.01	101.23±2.21
S2	Na alginate	SSG	4	4	98.11±2.61	0.12±0.02	115.13±1.23
S3	Gelatin	CCS	4	4	99.23±2.01	0.73±0.03	25.40±1.21
S4	Gelatin	SSG	4	4	98.23±1.53	0.32±0.02	119.00±3.21
S5	Pectin	-	0	4	99.21±1.32	1.50±0.01	62.34±4.51
S6	-	-	0	0	99.65±2.33	1.75±0.23	75.32±9.28
S7	Gelatin	-	4	0	99.65±0.33	0.75±0.01	125.22±12.63
S8	Na alginate	CP	4	4	97.23±0.57	0.44±0.1	129.79±15.31
S9	Gelatin	CP	4	4	99.00±1.31	0.37±0.03	118.00±22.86
S10	Pectin	CCS	4	4	99.14±2.68	0.46±0.06	137.26±23.21
S11	Pectin	CP	4	4	99.25±3.25	0.25±0.02	171.21±27.53
S12	Pectin	SSG	4	4	98.72±1.72	0.16±0.05	150.11±33.21

*Results are represented as mean±SD, **Each formula contained 25 mg IND, 4 mg glycine, 5 mg sucralose, and mannitol was added so that the theoretical tablet weight is 200 mg.
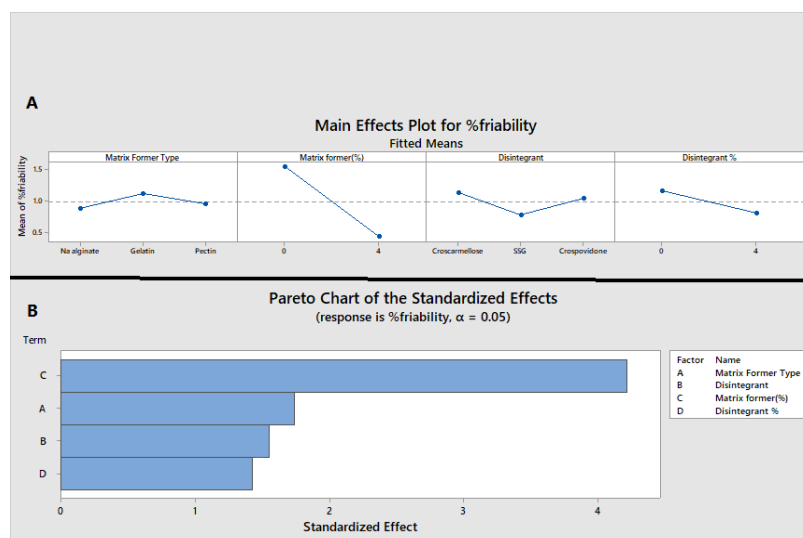
Fig. 2: Main effects plot (A) and Pareto Plot for (B) for % friability during the screening study for preparing IND-FDST

However, tablets prepared without matrix former were dusty and friable and showed pores on their surface indicative of the importance of the presence of a matrix former as a binder and to preserve tablet integrity. This result is also indicated in the Pareto plot (fig. 2B) which shows that the matrix former amount was the most significant factor affecting the % friability compared to the other factors in this study. This fact is also reflected in friability results as tablets prepared without matrix former displayed a high friability percentage which does not conform to USP limits that should be less than 1% [29]. In addition, the main effects plot in (fig. 2A), which displays the effects of matrix former amount on friability, showed a significant friability decrease ($p < 0.05$) upon the increasing amount of matrix former from 0 to 4 mg indicative of the binding effect of the matrix former on the FDST which in turn preserve the tablet integrity. However, the type of matrix formers present in this study did not have a significant effect on the % friability ($p > 0.05$). Nevertheless, sodium alginate showed the least % friability followed by pectin, and the highest % friability was shown by the gelatin. The super disintegrant type and disintegrant amount had an insignificant effect on the % friability ($p > 0.05$).

The importance of matrix former amount is also signified in disintegration time, which was also one of the most contributing factors as shown in Pareto Plot (fig. 3B) and main effects plot (fig. 3A). The plots showed a significant decrease in the disintegration time upon the addition of a matrix former ($p < 0.05$) regardless of its type. The type of the matrix former showed an overall insignificant effect ($p > 0.05$) on the disintegration time. The addition of a super disintegrant significantly decreased the disintegration time ($p < 0.05$) due to the increased wicking and subsequent decrease in breakage of the tablets. However, the types of the disintegrant had no significant effect on the disintegration time ($p > 0.05$).

Based on the above results of the statistical design, the matrix former amount and super disintegrant amount are the most significant factors and thus will be included in the optimization study in the QbD process. To determine the most suitable matrix former and super disintegrant to be included in the optimization study. The desirability index (D) value of the prepared formulas was calculated to determine the most desirable combination of matrix former and super disintegrant for the optimization study. The results revealed that the gelatin/CCS combination showed the most desirable attributes (low % friability and low disintegration time) and thus was included in the optimization study. As shown in fig. 4, gelatin-based FDSTs showed the highest *in vitro* dissolution profile, followed closely by Na alginate, and least was pectin-based FDSTs.
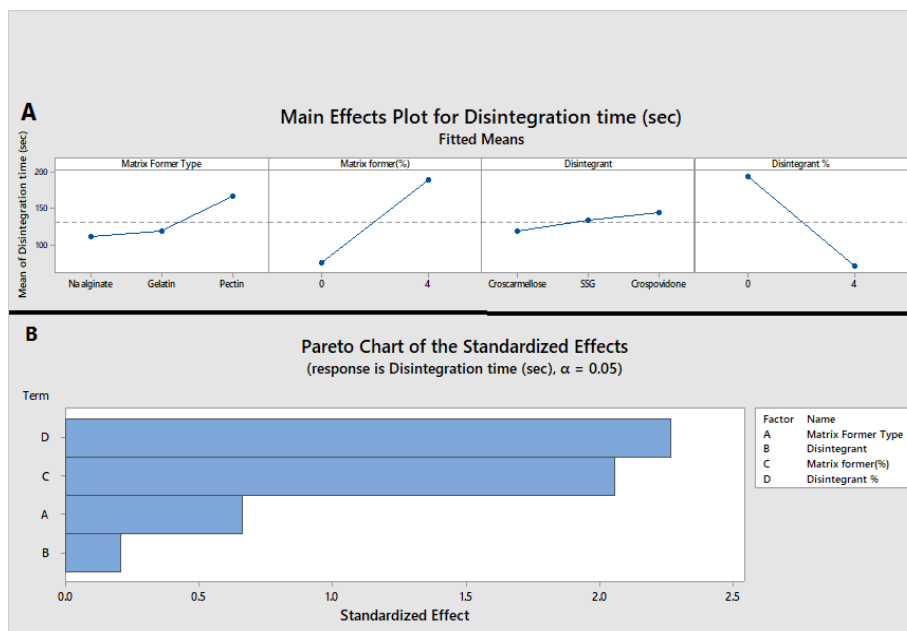

Fig. 3: Main effects plot (A) and Pareto Plot (B) for disintegration time during the screening study for preparing IND-FDST
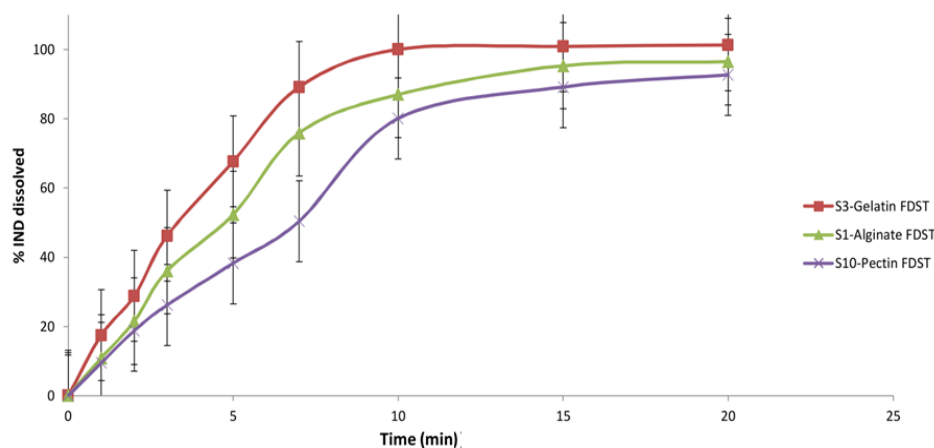

Fig. 4: Dissolution profiles of IND in USP media at 37±2 °C for formulas S3, S1, and S10 IND-FDST prepared during screening study (n=6)

Optimization study of IND-FDST

Table (2) summarizes the results for IND-FDST formulas O1-O9. As shown in the 3D plot for % friability (fig. 5A), friability was primarily affected by gelatin amount (p<0.05) contrary to the CCS amount (p>0.05) which did not significantly affect the friability. In addition, an indirect relationship was observed between the gelatin amount and friability which decreases as gelatin amount increases in FDST from 0 to 8 mg. It is also worth noting that tablets with a gelatin amount lower than 4 mg (O1 to O4) exhibited friability % higher than 1% which is non-conforming and out of specifications of the USP 39/NF 34 [29]. These results come in line with the screening study results and confirm the importance of matrix formers in FDST formulation.

Table 2: Composition of IND-FDSTs/tablet during response surface study and the results of %Friability (n=10), Drug content (n=5), Disintegration time (n=6), wetting time (n=3) and %Q10 (n=6)*

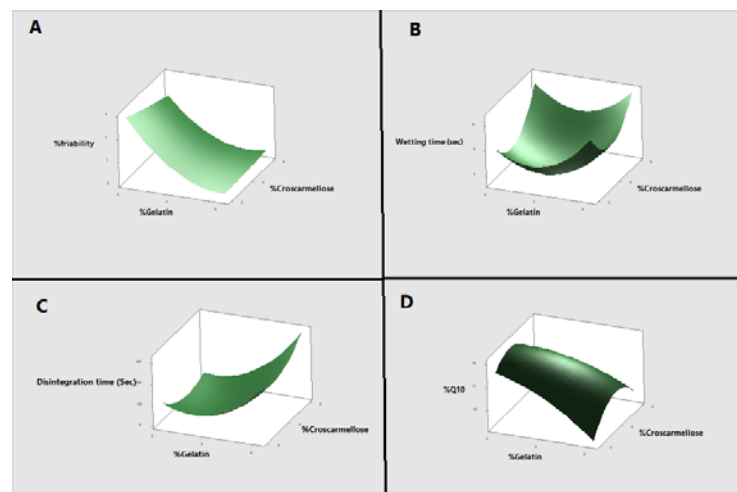| Formula*	Matrix former (mg)	Super disintegrant (mg)	Friability (%)	Disintegration time (sec)	Wetting time (sec)	%Q10
O1	0.00	2.00	2.35±0.76	62.34±5.31	5.31±0.04	83.12±1.21
O2	2.00	0.00	1.68±0.54	75.32±8.64	6.31±0.12	79.00±3.91
O3	2.00	4.00	1.42±0.61	20.32±1.91	3.21±0.26	72.00±1.09
O4	4.00	4.00	0.73±0.03	25.40±1.21	3.49±0.68	100.99±5.29
O5	4.00	0.00	0.75±0.12	123.45±22.76	4.21±0.09	65.21±2.01
O6	4.00	8.00	0.56±0.04	69.22±11,51	4.48±0.53	63.21±5.94
O7	8.00	0.00	0.30±0.03	251.12±32.98	10.01±0.29	55.61±1.26
O8	8.00	4.00	0.26±0.04	193.59±21.87	3.17±0.41	61.24±6.81
O9	8.00	8.00	0.34±0.02	332.12±52.75	12.02±0.15	57.22±2.73

*Results are represented as mean±SD.

Wetting time and disintegration time are predictably two closely related parameters that depend on the wetting properties of the polymers used in the formulation of FDSTs. This relationship is reflected in the main effects and contour plots of % gelatin and CCS and their effect on the wetting and disintegration times (fig. 5B, 5C). The same pattern of change in disintegration and wetting times upon changing gelatin and CCS amounts is noticed. Gelatin amount significantly (p<0.05) affected both disintegration and wetting times. As the gelatin amount increased from 0 to 2 mg, the wetting time decreased. However, as the gelatin amount increased from 4 to 8 mg an increase in the wetting and disintegration times of the tablets is noted.

Increasing the CCS amount has significantly decreased the disintegration and wetting time (p<0.05). Generally, disintegration time usually depends on the wetting properties of the super disintegrant causing its swelling and subsequent tablet disintegration [30]. However, the addition of 8 mg CCS resulted in a significant increase in disintegration time (p>0.05).

Dissolution profile represented by %Q10 also confirmed the significant effect of matrix former and super disintegrant amounts and ratios to each other on the dissolution of IND in FDSTs (p<0.05). As shown in the 3D plot (fig. 5D), the % Q10 significantly increased by decreasing the amount of gelatin. The same holds for the super disintegrant amount with the % Q10. These results come in agreement with the disintegration time curves that indicate that the optimum gelatin and CCS amounts are 4 mg with a ratio of 1:1, which is required to achieve the fastest disintegration time and highest % Q10.

Fig. 5: 3D plots for the effect of Matrix former type and % as well as super disintegrant type and % on the (A) %friability (n=3) (B) wetting time (n=3), (C) disintegration time (n=6), and (D) %Q10 (n=10) of the predicted IND-FDST during optimization study

Upon optimization of the response surface design, Formula O4 which contained 4 mg of gelatin and CCS was shown to be the most desirable formula with a D value of 0.718. Thus, it will be progressed to the accelerated and *in vivo* studies.

Worthy of note, formula O4 complies with USP [29] specifications for official sublingual tablets formulations with disintegration time less than 2 min (25.42 seconds) and % of drug released higher than 80 % in 20 min (% Q10 =100.99 %), % friability less than 1% (0.73±0.03 %). IND-FDST optimized formula O4 showed also comparable dimensions to those prepared during the screening study (0.55±0.05 cm thickness and 1.35±0.05 diameter). As well as a drug content of (99.23±2.01%) which falls within the USP [29] limits for acceptable drug content (90-110%) and acceptable weight variation limit less than 5% (0.95±0.12%) is also shown for formula O4.

Upon comparison of the dissolution profiles of O4 FDST and market product Indocin® capsules (fig. 6), it is evident that the FDST formula exhibited a faster dissolution profile than the market product confirmed by a similarity factor<50 (f2=33) indicating dissimilar dissolution profiles. This result can be explained by its faster disintegration which leads ultimately to faster dissolution.
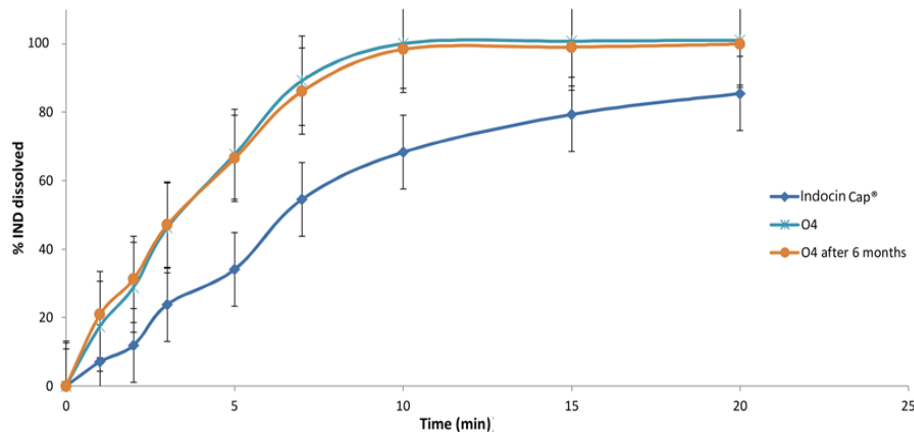

Fig. 6: Dissolution profiles of IND in USP media at 37±2 °C for Indocin Cap® market product, optimized IND-FDST formula O4 freshly prepared and after 6 mo (n=6)

Evaluation of optimized IND-FDST

Differential scanning calorimetry (DSC)

As shown in DSC thermograms, fig. 7, pure IND shows one sharp prominent endothermic peak observed at 159.99 °C indicating the melting transition point of the γ crystalline form of the drug [44]. The physical mixture shows a peak with low intensity at 159.5 °C for the drug and another peak at 166.5 °C which corresponds to the mannitol. O4 FDST showed no peaks at the drug melting transition temperature (159.5 °C), and another broader endothermic peak was observed at 163.5 °C.

Powder X-ray diffraction (XRD)

Fig. 8 shows the X-ray diffraction peaks for pure IND, FDST O4, and its corresponding physical mixture. XRD checks the crystallinity of the substance. IND is a crystalline drug where, the γ-crystalline form (most stable) diffraction pattern shows sharp peaks at 2θ 11.6°, 16.4°, 19.6°, 21.7°, 26.89°, and 29.3°. The diffraction pattern of the FDST (O4), shows a decrease in the intensity of the drug peaks at 2θ 21.7°, 26.89° as well as some changes in the position of the peaks are observed.

Fourier-transform infrared spectroscopy (FT-IR)

Fig. 9 shows the infrared spectroscopy of indomethacin, FDST O4 and its corresponding physical mixture, and all the components of the tablet. Generally, the FT-IR is used to determine the presence of any interaction between the drug and the tablet components at room temperature. The IR spectrum of indomethacin shows characteristic bands in the following wavenumbers: 1716 cm^{-1} for (C=O) in the COO group, 1625–1575 and 1479 cm $^{-1}$ for (C–C) stretching of the aromatic rings, 1692 cm^{-1} for amide group, 1261–1223 cm^{-1}, below 1012 and 737 cm^{-1} for (¼ C–O) of the ether group, (C–H) deformation, and (C–C) stretching modes, and 2927 and 2966 cm^{-1} for (CH$_2$) [36, 45, 46]. The bands present in the FTIR of the individual components superposed in the IND-FDST FTIR chart without the appearance of new peaks or disappearance of an existing peak of the drug significant peaks in FDST O4 and its corresponding physical mixture.

Scanning electron microscopy (SEM)

The SEM micrographs (fig. 10) illustrate the highly porous structure of FDST O4 which provides an excellent path for the water. In turn, this allows fast hydration of the formula to show rapid disintegration, enhanced IND released percentage, and dissolution rate.
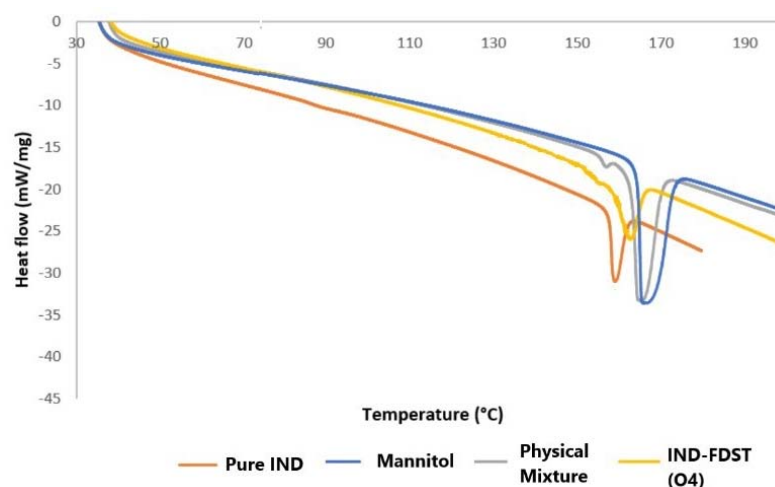

Fig. 7: DSC thermogram of Pure IND, IND-FDST optimized formula (O4), and physical mixture
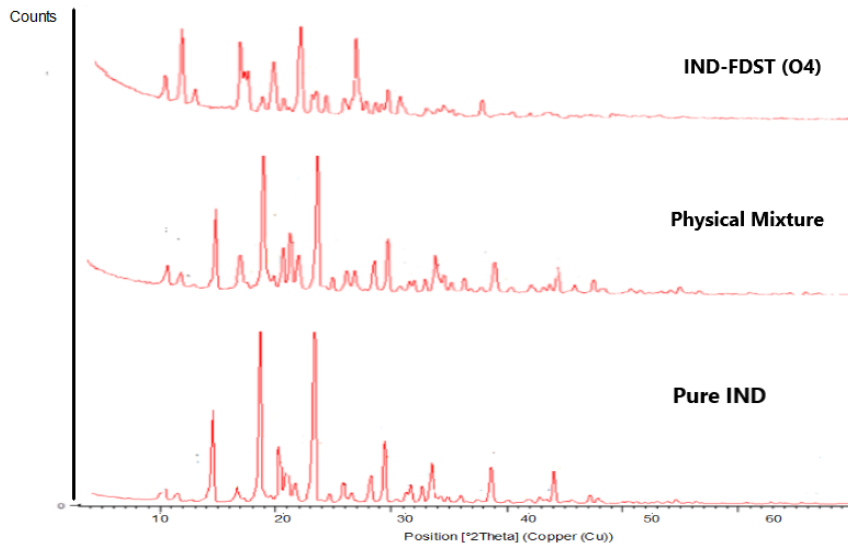
Fig. 8: XRD diffractogram of pure IND, IND-FDST optimized formula (O4), and physical mixture
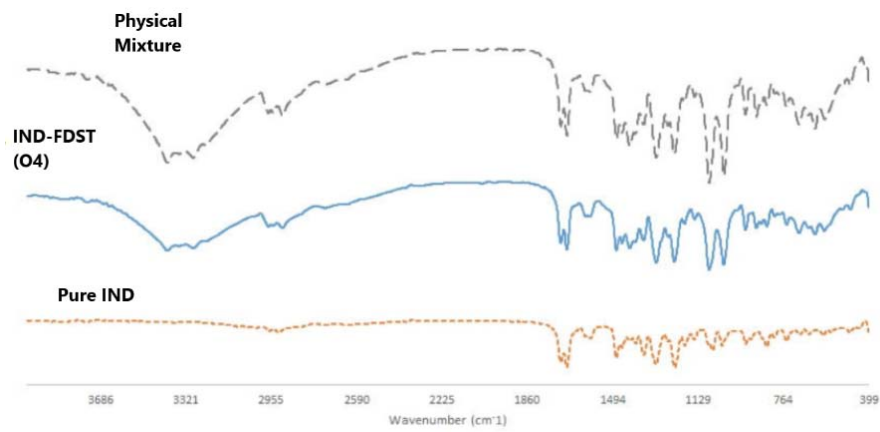

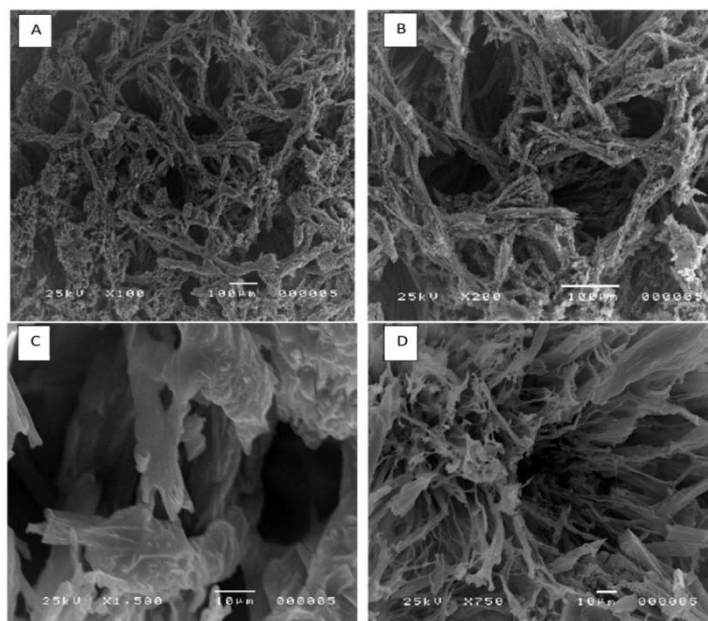**Fig. 9: FTIR diffractogram (C) of Pure IND, IND-FDST optimized formula (O4), and physical mixture**

Fig. 10: SEM micrographs of IND-FDST optimized formula (O4)

Accelerated stability study

Stability of FDST tablets O4 was done to assess the effect of storage on the stability of FDST tablets under accelerated stability conditions (40±2°C/75±5% RH) according to ICH guidelines [38]. IND-FDST (O4) has shown adequate stability and insignificant changes to the tested attributes and retained residual drug content of 97.21±3.15% from the fresh FDST. The change was within the 5 % limit specified by the ICH. Moreover, the tablet disintegration time and % friability insignificantly (p>0.05) changed from 25.40±1.21 seconds to 26.21±0.91 seconds and from 0.73±0.03% to 0.77±0.1%, respectively. These results confirm the ability of the freeze-dried dosage forms to preserve IND stability.

The dissolution profile (fig. 3) represented by % Q10 changed from 100.99±5.29%, for the fresh FDST to 99.83±3.17% for stored tablets with similarity factor f2>50 (f2=83). Thereby, the dissolution profile

of the stored tablets comes to confirm the stability of the tablet quality attributes and properties throughout the storage period.

***In vivo* anti-inflammatory effects**

Carrageenan induced paw edema

The sub-planter injection of 100 μl of 1% sterile carrageenan into the rat hind paw elicited an inflammation (swelling and erythema). A time-dependent increase in paw edema by 40%, 52%, 97%, and 94% in the 1st, 2nd, 3rd, and 4th hours, respectively, was observed as compared with pre-carrageenan control values. The application of IND oral suspension and FDST O4 showed significant inhibition of the edema formation at the 4th hour by 36% and 70%, respectively, as compared with the carrageenan control group at the same time. In addition, the application of FDST O4 showed significant inhibition of edema formation in the 4th hour by 54% compared with the free drug.

Table 3: Effects of IND-FDST optimized formula (O4) compared to Indocin® Cap market product, negative and positive control on TNF-α and IL-6 in rats (n=10)*

	Negative control	Positive control	Indocin® Cap	FDST (O4)
TNF-α (pg/ml)	525.00±7.50	978.75±11.25	816.25±1.25	563.75±3.75
IL-6 (pg/ml)	83.00±16.84	245.80±28.61	135.60±3.13	85.40±22.06

*Results are represented as mean±SD.

Effects of IND-FDST on tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)

As shown in table 3, carrageenan-induced an increase in the inflammatory cytokine release (positive control) namely, TNF-α by 86% and IL-6 by 2-fold as compared with the normal control group. The application of pure IND and FDST O4 showed significant inhibition of TNF-α release by 17% and 42%, and IL-6 release by 45% and 65%, respectively, as compared with the carrageenan control group, table 7. Therefore, the application of FDST O4 showed significant inhibition of TNF-α and IL-6 release by 31% and 37%, respectively, as compared with free drugs.

DISCUSSION

With the aim of preparing fast disintegrating sublingual tablets, Freeze-drying was chosen as a preparation technique. Tablets produced by freeze-drying are of high porosity which allows better exposure of the drug to the dissolution media (saliva in mouth). As a result, an immediate disintegration and dissolution of the tablet occur when placed under the tongue [11–16]. The tablet excipients were carefully selected to serve the aim of the research paper in providing a rapid and safe therapeutic action. The selected matrix-forming polymers are from natural sources and have a considerably good water solubility to allow rapid tablet dissolution. The other components of the tablets are held very well within the matrix of the polymer, which in turn affects the tablet's physical strength (% friability). The presence of a super disintegrant significantly decreased the disintegration time (p<0.05) due to the increased wicking and subsequent breakage of the tablets. Other additives were used to enhance the appearance, physical strength, and taste of the tablet. Glycine was added as it acts as a cryoprotectant to prevent the shrinkage of the tablet after freeze-drying. Sucralose was used as a sweetener to enhance the taste of the formula. Finally, mannitol was employed as a filler. In addition, mannitol possesses a well-established cryoprotectant ability as it crystallizes during freezing thereby protecting the tablet components from degradation while simultaneously offering an elegant appearance [16]. Besides, the high water solubility of the selected filler also improves the mouthfeel, texture, and taste of the formula [11].

Based on the literature review and preliminary studies, the selection of a good matrix-forming polymer and a compatible super disintegrant is the key to a successful fast disintegrating sublingual tablet (IND-FDST) formulation. As such, A QbD design was employed to screen the most significant formulation attributes for an optimization study namely lower % friability and disintegration time. The screening study focused on studying different variables namely (matrix former type

and amount, disintegrate type and amount) to determine the best matrix former/super disintegrant combination for the optimization study. Friability was chosen as a critical attribute to assess the success of tablet formulation in terms of its ability to withstand handling. In addition, disintegration time was chosen as a second critical attribute as rapid tablet disintegration ensures immediate dissolution and dispersibility in the oral cavity.

The gelatin and CCS were selected as the best matrix former and super disintegrant, respectively. The combination of the two polymers in the tablet provided a low % friability and rapid disintegration time from the screening study. This can be explained by the ability of the matrix former to hold the tablet components together to give enough physical strength to the tablet. In addition, the hydrophilic nature of the binder and CCS allow fast wetting of the tablet, and hence rapid disintegration is observed. Based on the results of the screening design, the gelatin and CCS amount were noted as the most significant factors and thus were included in the optimization study in the QbD process.

During the optimization study, wetting time and disintegration time were predictably found to be two closely related parameters that depend on the wetting properties of the polymers used in the formulation of FDSTs. It has been observed that the disintegration time usually depends on the wetting properties of the super disintegrant causing its swelling and subsequent tablet disintegration [30]. This relationship has been highlighted during the optimization study. Gelatin amount significantly (p<0.05) affected both disintegration and wetting times.

In the absence of gelatin (0 mg), the IND-FDST failed to properly bind and was very fragile, therefore it was rejected. At a low gelatin amount (2 mg), the wetting time decreased probably due to the impaired binding effect of the polymer on the tablet components, being used in a small amount, causing them to disintegrate rapidly [30]. In addition, this amount showed a high % friability above the acceptable values. The % Q10 was relatively low this can be explained by the high friability of the tablets which leads to the loss of an amount of the drug during handling, therefore the inability to fully release the drug though it has a fast disintegration time may be logical.

However, as the gelatin amount increased to 4 mg, more tablet binding and proper holding of the tablet components within the polymer matrix were observed. This is reflected by the acceptable % friability percentages with a very subtle increase in the wetting and disintegration time of the tablets, and a higher %Q10, which makes this amount of gelatin the optimum.

Further increase in the gelatin amount (8 mg) significantly increased the tablet wetting and disintegration time to be above the accepted values for the sublingual tablets. In addition to the low percentage of drug release. This is expected due to gelatin crosslinking that causes the formation of a swollen, water-insoluble pellicle which acts as a barrier and restricts the release of the drug [47]. Generally, in FDSTs the fast disintegration and dissolution of the drug are prioritized to allow the latter rapid absorption through the oral mucosa [6, 48].

Increasing CCS amount has significantly decreased the disintegration and wetting time (p<0.05) due to the wicking and super disintegrant action of CCS which causes faster disintegration of tablets. Surprisingly, the addition of 8 mg CCS resulted in a significant increase in disintegration time (p>0.05). However, this effect was previously reported and can be explained by the partial gelling that potentially could form a viscous barrier and delay the entry of water into the tablet thereby increasing the disintegration time [49].

Therefore, based on the previously discussed factors, the optimum amount for the gelatin and CCS in IND-FDST is 4 mg for each polymer present in formula O4, where this amount provides a low % friability (0.73±0.03%), adequate wetting time (3.49±0.68 seconds), disintegration time (25.40±1.21 seconds), and % Q10 (100.99±5.29%). The optimized formula IND-FDST O4 exhibited faster dissolution profiles compared to market product Indocin®capsules which are explained by its faster disintegration, leading to the faster dissolution of the IND. The faster disintegration can be attributed to the choice of the optimized ratio of gelatin/CCS as well as the freeze-drying technique for the tablet preparation, which is known for its ability to form porous, fast-dissolving cakes, making it a method of choice for the preparation of fast dissolving sublingual tablets. The high porosity of the O4 formula was confirmed by SEM micrographs (fig. 4D) [30, 50].

The physical-pharmaceutical tests performed as supportive evidence for the enhanced performance of the optimized FDST O4. The enhanced dissolution profile may be attributed to the probability of the presence of IND in an amorphous form which is known to have a faster dissolution rate than crystalline forms [51]. This probability was confirmed by DSC which suggests the presence of IND in a solubilized or amorphous form probably as a result of the freeze-drying process indicated by the disappearance of the IND endothermic peak in the O4 formula [30]. XRD confirmed the change of the crystalline structure of IND to a molecularly dispersed form in the tablet matrix explained by the decrease in the peak intensity and the slight shifting of the peaks [11, 52]. FTIR confirmed the absence of chemical interaction between the formula components at room temperature illustrated by the absence of any changes in IND characteristic peaks in the O4 IND-FDST FTIR diffractogram [28]. IND-FDST optimized formula (O4) also exhibited stability under accelerated conditions thereby enforcing the successful preparation of the optimized IND-FDST formula with characteristics suitable to be industrialized and marketed to the patients.

Inflammatory response induced by carrageenan stimulates the secretion of inflammatory cytokine [53]. Therefore, blood samples were withdrawn from the rats for the assessment of the level of inflammatory cytokines (TNF-α, and IL-6). The *in vivo* anti-inflammatory effects in rats confirmed the increased efficacy of the IND-FDST tablets compared to IND suspension. The better control of the formula FDST O4 on the paw edema in comparison to the oral suspension of the drug may be correlated to the rapid release and absorption of the drug. The rapid absorption is explained by the improved solubility of IND in the formula as illustrated by the increased *in vitro* dissolution of the IND-FDST. In addition, the sublingual route exhibits high vascularity as the drug is absorbed from the mouth floor veins leading directly to the superior vena cava combined with the thin epithelial cell lining of the mucosa. All these factors contribute to the faster absorption of IND and distribution of the drug to various body tissues leading to a faster onset of drug action [54]. Moreover, the better inhibitory effect of the formula FDST O4 on the inflammatory markers relative to the oral suspension may be correlated to the enhanced drug solubility and faster onset of action. Therefore, it may take the oral route a longer

duration to reach a similar inhibitory level of the markers. The results also suggest a higher bioavailability of IND when administered as FDST compared to the market product due to its superior pharmacodynamics implied by the increased anti-inflammatory effects in rats.

CONCLUSION

Based on the presented results, it can be concluded the QbD is a successful approach in optimization of a stable IND-FDST formula that surpasses the commercial oral IND tablets both *in vitro* and *in vivo* with the added advantage of reducing the unpleasant GIT side effects present in the oral tablets. IND-FDST prepared using gelatin/CCS combination with ratio 1:1 showed adequate tablet properties as well as better dissolution profile as well as good stability under accelerated conditions. IND-FDST tablets displayed significantly higher anti-inflammatory effects *in vivo* compared to the market product confirming its successful formulation. The authors recommend a further *in vivo* pharmacokinetic evaluation to assess the absorption, and bioavailability of IND-FDST compared to market product.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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