Supplementary material

A comprehensive understanding of lowly–hydrolyzed polyvinyl alcohol–based ternary solid dispersions with the use of a combined mixture–process design

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Design of experiment for HME

Design-Expert® software (version 11.0.2.0, Stat-Ease Inc., Minneapolis, MN, USA) was used to build a design of experiment (DoE) with 36 experiments (Supp. Table 1) involving a mixture design (three factors) combined with continuous factors (two factors) and applying the User-Defined Design mode (Combined Mixture-Process Design) which can evaluate mixture components and process factors simultaneously. The three component parameters were: indomethacin (10–30%), JR-05 (50–90%), and sorbitol (0–40%). The levels of IND and JR-05 were determined by considering the solubility of IND in JR-05 at a temperature close to melting point of IND, as determined in our previous report [1]. The level of sorbitol was designed based on a previous report [2] The process parameters were varied: the three extrusion temperatures (110–156°C) and screw speeds (20–100 rpm). In addition, the temperature was designed based on our previous report [1]. Moreover, considering an arrangement for uniform points from the range being studied of each factor, subtotal of nine points for mixture design was applied, resulting from the experimental options (selecting vertices, centers of edges and overall centroid). Similarly, subtotal of four points for process design was applied, resulting from the experimental option (selecting centers of edges). In total, 36 experiments were built by the Design-Expert® software. To reduce systematic errors, all experiments were randomly conducted based on the instructions from the Design-Expert® software. Furthermore, to evaluate the multiple factors and levels within a realistically possible number of experiment, DoE design was constructed with no replicates. Therefore, lack-of-fit, which represents how well the model fits the data, was unable to be obtained under the design. Instead, three validation studies were additionally performed to evaluate the predictability by using each prediction model obtained. Finally, coded values of mixture components and process factors used for regression are shown in Supp. Table 2.

Preparation of hot-melt extrusion

Binary and ternary mixtures were prepared using a TURBULA® mixer (Willy A. Bachofen AG, Basel, Switzerland) for 5 min. The 2.5 gram of physical mixtures (PMs) were manually charged into a HAAKE MiniCTW fully intermeshing conical mini twin screw extruder (Thermo Fisher Scientific K.K., Waltham, MA, USA). Temperature, the feed rate and the screw rate were controlled by an external operation system. Processing torques were recorded using an external operation system. After passing through the extruder with no circulation, melt extrudates were collected, allowed to cool at ambient temperature, pulverized using a laboratory micromill, and finally sieved through an open mesh size of 500 μm. After sieving, all samples were placed in 20 mL of amber glass bottles and stored with a desiccant at 4°C until measurement.

Characterization

Residual crystallinity

The degree of residual crystallinity of IND was evaluated using DSC measurement. The dried precipitates were analyzed using a Q2000 DSC (TA Instruments, Leatherhead, UK) equipped with a refrigerated cooling system. Dry nitrogen at a flow rate of 50 mL/min was used to purge the DSC cell. The instrument was calibrated at heating rates of 10°C/min using high purity indium to standardize the temperature and heat flow signals. The amounts of samples equivalent to 1 mg of IND were accurately weighted and loaded in non-hermetic aluminum Tzero pans, and measurement was performed in the temperature range of 25-200°C at a heating rate of

10°C/min. The results were analyzed using TA instruments Universal Analysis 2000 software. Furthermore, the residual crystallinity contained in the extrudates was determined according to Eq. (1):

$$\%_{\text{residual crystalline of IND}} = \frac{\Delta H_s}{\Delta H_c} \times 100 \quad (1)$$

Where ΔH_s is the melting enthalpy of IND within the extrudates and ΔH_c is the melting enthalpy of pure crystalline IND [3] Two different DSC profiles were observed in measurements. In the case of 1, which is the immiscible state, an endothermic peak derived from IND (range 140-160˚C) was separately observed from an endothermic peak derived from L-PVA (range 160-180˚C). In this case, ΔH_s of IND was calculated from the area under the curve (range 140-160˚C). In the case of 2, which is the partially miscible state, a broad single peak was observed in the range from 120 to 160˚C. In this case, ΔH_s of IND was calculated from the area under the curve (range 120-160˚C). Also, in order to reduce measurement variability, the DSC measurements were performed in triplicate.

Ultra performance liquid chromatography assay

Each extrudate was dissolved in 50% (v/v) acetonitrile aqueous solution using sonication. The drug concentrated in the filtrate was passed through a 0.45 μm PTFE membrane before being quantified by Ultra Performance Liquid Chromatography (UPLC, ACQUITY™, Waters, Milford, MA, USA). The measuremental conditions were as follows: ZORBAX SB-Phenyl column (1.8 μm, 50 mm x 2.1 mm) at 37°C; mobile phase, 50% (v/v) acetonitrile aqueous solution containing 0.2% phosphoric acid; detection by UV absorption at 237 nm; injection volume, 2.0 μL; and flow rate, 0.6 mL/min. The assay values obtained were defined as residual ratio in this study.

Dissolution study

Dissolution experiments were used to evaluate the *in vitro* drug release properties of the matrices. An equivalent amount of 50 mg IND in each extrudate after milling and sieving through an open mesh size of 250 μm were accurately measured on medicine wrapper and manually introduced into the dissolution vessels. These studies were carried out following the 17th edition of the Japan Pharmacopeia, using an automatic 6-series dissolution-testing device (Toyama Sangyo Co. Ltd., Osaka, Japan). Dissolution Test Method 2 (paddle method) was used, with a paddle speed of 100 rpm, to disperse the powder in 450 mL of distilled water at 37°C (n = 3). The samples were removed from the dissolution vessels at predetermined time intervals and assayed using a UV-Vis spectrophotometer at 320 nm (reference wavelength: 500 nm). The testing condition was followed the same method in our previous report so as to compare the supersaturation behavior [1, 4] The area under the dissolution curve (AUDC) from 0 to 4 h was used as the response factor for the DoE study.

Residual ratio

Thermal degradation of drug is the most important challenge in HME. Therefore, we evaluated the influence of each component and process factor on the residual ratio of IND. As shown in Supp. Table 3, several samples containing a large amount of sorbitol showed high residual ratio close to 110%, resulting from sublimation of sorbitol by applied temperature during the process. A statistical analysis of the residual ratio (R3) results was performed based on the data set. No transformation was performed, and a quadratic-linear model was applied because of best fitted to the data shown in Table 3. The Pred R^2 of 0.6103 obtained showed a low value caused by the data set, however, the difference between the Pred R^2 (0.6103) and the Adj R^2 (0.7762) represented less than 0.20, suggesting reasonable agreement. Therefore, an R^2 of 0.8530 indicates good predictive ability of the selected model [5]. Supp. Table 6 shows the result of the ANOVA. A model F-value of 11.12 and a very low probability value (Prob>F) less than 0.05 implies a significant model fit. In this case, the terms A, B, C, BC, CD, CE, ABD and BCD represent significant model terms. As shown in Supp. Figure 3(a), (b), and (c), the investigated components and process factors did not influence on fluctuations in the residual ratio of IND. Next, according to the result in the perturbation plot shown in Supp. Figure 3(d), no steep slopes were not detected in both factors, suggesting that factor D and E would have no impacts on residual ratio under the midpoint condition containing 16% sorbitol. The same tendency for the residual ratio was expected based on the results of the contour graph shown in Supp. Figure 3(e). As shown in Supp. Figure 4, no influence of investigated factors on the residual ratio of IND was suggested among the experimental design. These results suggest that the extent of residual ratio would be involved by multiple factors interaction such as BC, CD, CE, ABD and BCD which showed a significant difference (Supp. Table 6).

In our previous study, we reported that no degradation during HME was observed in the melt extrudate consisting of IND and JR-05 at 10 % (w/w) API prepared at 156°C [1]. In this study, we mainly focused on the influence of sorbitol as a plasticizer on the residual ratio of IND, and no degradation of IND in the thermal process was observed under the process conditions. However, the prediction model obtained consisted of numerous significant interactions, indicating that residual ratio in HME process might be governed by various factors. Therefore, to improve the predictability of residual ratio, water amount in each material should be taken account in the next model construction, leading more simplified prediction model. In either case, the results indicate that sorbitol is a suitable plasticizer for L-PVA because of its no influence on the residual ratio of IND in HME process.

Tables

Supp. Table 1. Formulation and process parameters for the design of experiment

Number	Formulation			Process	
	Factor A wt.% Indomethacin	Factor B wt.% JR-05	Factor C wt.% Sorbitol	Factor D Temperature (°C)	Factor E Screw speed (rpm)
1	10	90	0	156	60
2	30	70	0	133	100
3	30	50	20	156	60
4	20	50	30	133	20
5	20	65	15	133	100
6	30	60	10	110	60
7	10	70	20	110	60
8	20	50	30	156	60
9	30	70	0	110	60
10	10	50	40	133	20
11	20	50	30	133	100
12	20	65	15	133	20
13	30	70	0	133	20
14	10	70	20	156	60
15	20	65	15	156	60
16	20	80	0	156	60
17	10	50	40	133	100
18	20	50	30	110	60
19	10	90	0	133	20
20	30	50	20	110	60
21	20	80	0	133	20
22	30	50	20	133	20
23	30	70	0	156	60
24	30	60	10	133	100
25	10	70	20	133	20
26	10	90	0	133	100
27	20	65	15	110	60
28	10	90	0	110	60
29	10	50	40	110	60
30	30	50	20	133	100
31	30	60	10	133	20
32	20	80	0	110	60
33	30	60	10	156	60
34	20	80	0	133	100
35	10	70	20	133	100
36	10	50	40	156	60

Supp. Table 2. Coded values of (a) mixture components and (b) process factors for regression

(a) Mixture components

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	IND	%	Mixture	10	30	+0 ↔ 10	+0.5 ↔ 30	20.00	8.28
B	JR-05	%	Mixture	50	90	+0 ↔ 50	+1 ↔ 90	65.00	13.52
C	Sorbitol	%	Mixture	0	40	+0 ↔ 0	+1 ↔ 40	15.00	13.52
-	-	-	-	Total =	100.00	L_Pseudo Coding	-	-	-

(b) Process Factors

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
D	Temperature	℃	Numeric	110.00	156.00	-1 ↔ 110.00	+1 ↔ 156.00	133.00	16.49
E	Screw speed	rpm	Numeric	20.00	100.00	-1 ↔ 20.00	+1 ↔ 100.00	60.00	28.69

Supp. Table 3. DoE Results

Number	R1 Torque (Nm)	R2 Residual crystallinity [a] (%)	R3 Residual ratio [a] (%)	R4 AUDC [a] (μg hr/mL)
1	0.81	0.77±0.25	98.68±0.72	285.0±4.38
2	0.93	1.57±0.16	101.17±2.44	132.1±28.6
3	0.16	22.23±0.58	99.77±1.73	74.9±0.29
4	0.092	12.34±0.40	105.64±1.60	64.6±0.57
5	0.64	11.74±1.08	97.50±1.23	84.7±0.29
6	0.91	22.89±2.44	97.96±2.35	62.2±0.89
7	0.39	14.65±1.39	101.02±1.28	82.6±0.29
8	0.12	14.30±0.71	109.80±1.50	87.8±0.90
9	1.19	8.79±0.30	95.35±1.09	81.7±0.78
10	0.070	11.12±1.86	107.84±0.47	88.1±0.74
11	0.19	17.81±0.83	95.41±1.00	54.2±0.34
12	0.27	12.59±0.94	99.64±0.27	89.9±0.33
13	1.09	4.52±0.42	100.56±0.53	99.3±0.57
14	0.26	1.61±1.01	95.47±0.42	217.5±1.74
15	0.21	2.93±0.50	98.79±0.60	122.2±0.79
16	0.52	0.28±0.14	98.65±0.74	171.4±36.4
17	0.15	14.48±1.11	95.84±0.19	70.2±0.30
18	0.22	22.04±1.03	96.13±0.78	51.2±1.03
19	0.77	0.05±0.05	100.03±0.66	258.3±1.57
20	0.33	30.02±1.80	98.30±1.85	53.8±0.30
21	1.05	0.02±0.02	98.46±0.51	141.4±1.24
22	0.11	20.60±2.79	99.20±1.85	58.4±0.16
23	0.35	0.49±0.22	100.41±1.09	243.6±10.1
24	0.70	23.36±1.10	96.38±0.55	76.9±1.45
25	0.12	6.94±0.81	98.15±0.69	125.7±0.42
26	1.39	0.04±0.03	98.59±0.47	239.1±1.50
27	0.55	16.77±1.60	99.82±0.95	68.3±0.70
28	2.21	0.01±0.01	101.17±0.45	212.0±0.84
29	0.16	15.62±1.85	97.30±0.97	65.1±1.37
30	0.30	24.97±2.30	100.10±2.17	52.0±1.27
31	0.45	27.33±2.32	98.82±0.60	67.8±0.44
32	0.54	0.83±0.10	100.93±0.98	122.8±1.33
33	0.26	7.74±0.89	97.47±0.93	133.0±19.6
34	1.00	0.00±0.00	99.62±0.50	148.5±3.95
35	0.23	11.34±0.73	98.65±0.34	98.4±0.64
36	0.13	10.90±1.53	114.09±1.61	133.8±1.47

a Each value represents the mean ± S.E. of 3 experiments.

Supp. Table 4. Analysis of variance (ANOVA) table for the model for predicting average torque (R1) using mixture design combined with continuous factors (non-reduced responses)

Source/Equation	Sum of squares	df	Mean square	F value	P values (Prob $>F$)	
Model [1]	19.46	8	2.43	30.56	< 0.0001	significant
Linear Mixture [2]	15.32	2	7.66	96.24	< 0.0001	
AD	0.5104	1	0.5104	6.41	0.0175	
AE	0.0077	1	0.0077	0.0972	0.7576	
BD	0.0450	1	0.0450	0.5653	0.4586	
BE	0.0103	1	0.0103	0.1290	0.7223	
CD	0.1774	1	0.1774	2.23	0.1471	
CE	1.78	1	1.78	22.35	< 0.0001	
Residual	2.15	27	0.0796			
Cor Total	21.61	35				
Equation [3]	1/Sqrt (average torque) = 1.25367 × A + 0.999747 × B + 2.99882 × C + 0.641237 × AD + -0.0789479 × AE + 0.111607 × BD + -0.0533129 × BE + 0.221593 × CD + -0.701741 × CE					

1) A: IND, B: JR-05, C: Sorbitol, D: Temperature, E: Screw speed

2) Inference for linear mixtures uses Type I sums of squares.

3) Final equation in terms of L_Pseudo components and coded factors

Supp. Table 5. Analysis of variance (ANOVA) table for the reduced model for predicting average torque (R1) using mixture design combined with continuous factors

Source/Equation	Sum of squares	df	Mean square	F value	P values (Prob >*F*)	
Model [1]	19.20	4	4.80	61.98	< 0.0001	significant
Linear Mixture [2]	15.32	2	7.66	98.89	< 0.0001	
AD	1.43	1	1.43	18.51	0.0002	
CE	2.45	1	2.45	31.62	< 0.0001	
Residual	2.40	31	0.0775			
Cor Total	21.61	35				
Equation [3]	1/Sqrt (average torque) = 1.25367 × A + 0.999747 × B + 2.99882 × C + 0.874477 × AD + -0.735272 × CE					

1) A: IND, B: JR-05, C: Sorbitol, D: Temperature, E: Screw speed

2) Inference for linear mixtures uses Type I sums of squares.

3) Final equation in terms of L_Pseudo components and coded factors

Supp. Table 6. Analysis of variance (ANOVA) table for the reduced model for predicting residual ratio (R3) using mixture design combined with continuous factors

Source/Equation	Sum of squares	df	Mean square	F value	P values (Prob ＞*F*)	
Model [1]	464.05	12	38.67	11.12	< 0.0001	significant
Linear Mixture [2]	57.12	2	28.56	8.21	0.0020	
AB	6.99	1	6.99	2.01	0.1696	
AD	14.09	1	14.09	4.05	0.0560	
BC	43.12	1	43.12	12.40	0.0018	
BD	8.26	1	8.26	2.38	0.1369	
BE	0.0634	1	0.0634	0.0182	0.8938	
CD	199.69	1	199.69	57.41	< 0.0001	
CE	105.31	1	105.31	30.28	< 0.0001	
ABD	21.31	1	21.31	6.13	0.0211	
BCD	73.22	1	73.22	21.05	0.0001	
BCE	12.75	1	12.75	3.66	0.0681	
Residual	80.00	23	3.48			
Cor Total	544.05	35				
Equation [3]	Residual ratio = 94.5923 × A + 99.5557 × B + 103.896 × C + 7.47259 × AB + -5.17763 × AD + -13.6911 × BC + -1.92374 × BD + -0.131571 × BE + 8.90234 × CD + -5.3618 × CE + 18.4475 × ABD + -25.2325 × BCD + 10.4213 × BCE					

1) A: IND, B: JR-05, C: Sorbitol, D: Temperature, E: Screw speed

2) Inference for linear mixtures uses Type I sums of squares.

3) Final equation in terms of L_Pseudo components and coded factors

Supp. Table 7. Analysis of variance (ANOVA) table for the reduced model for predicting AUDC (R4) using mixture design combined with continuous factors

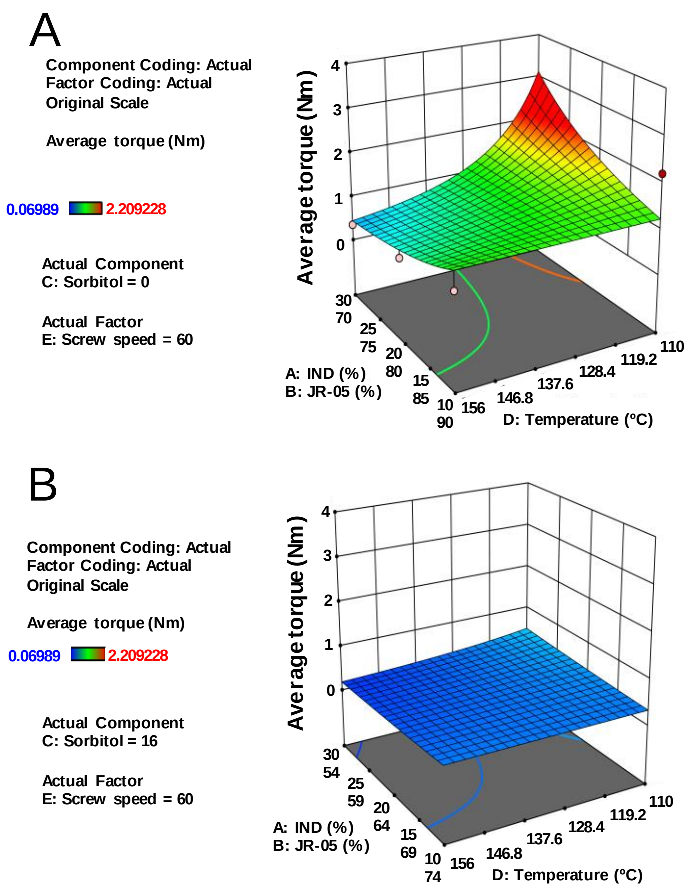
Source/Equation	Sum of squares	df	Mean square	F value	P values (Prob > F)	
Model [1)]	0.0008	11	0.0001	69.31	< 0.0001	significant
Linear Mixture [2)]	0.0005	2	0.0003	257.74	< 0.0001	
AC	0.0000	1	0.0000	43.21	< 0.0001	
AD	0.0000	1	0.0000	25.07	< 0.0001	
AE	3.530E-06	1	3.530E-06	3.42	0.0768	
BC	5.261E-07	1	5.261E-07	0.5095	0.4822	
BD	1.655E-07	1	1.655E-07	0.1602	0.6925	
CD	0.0000	1	0.0000	38.59	< 0.0001	
CE	0.0000	1	0.0000	14.19	0.0009	
ACD	5.052E-06	1	5.052E-06	4.89	0.0367	
BCD	4.115E-06	1	4.115E-06	3.99	0.0574	
Residual	0.0000	24	1.033E-06			
Cor Total	0.0008	35				
Equation [3)]	1/Sqrt (AUDC) = 0.012791 × A + 0.0043665 × B + 0.0122397 × C + 0.0188733 × AC + -0.00701768 × AD + -0.00153671 × AE + 0.00151239 × BC + -0.000256252 × BD + -0.00422416 × CD + 0.00201379 × CE + 0.00898141 × ACD + -0.00598176 × BCD					

1) A: IND, B: JR-05, C: Sorbitol, D: Temperature, E: Screw speed

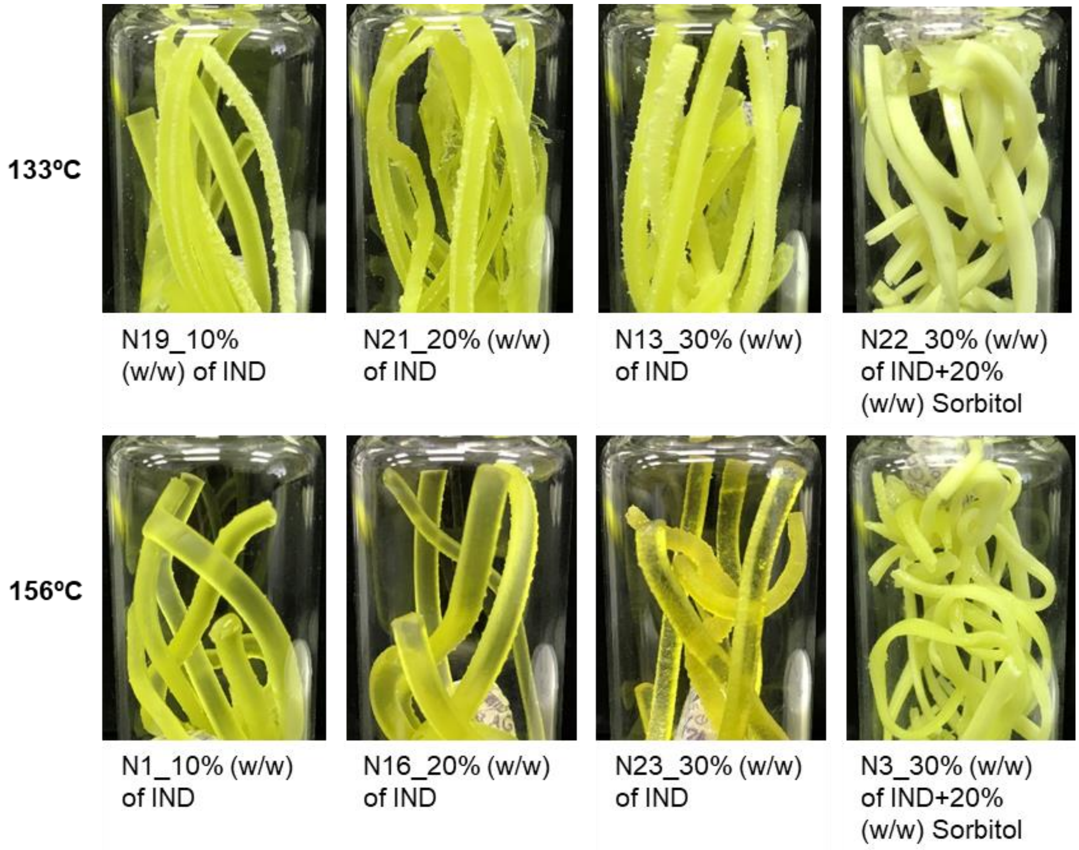
2) Inference for linear mixtures uses Type I sums of squares.

3) Final equation in terms of L_Pseudo components and coded factors

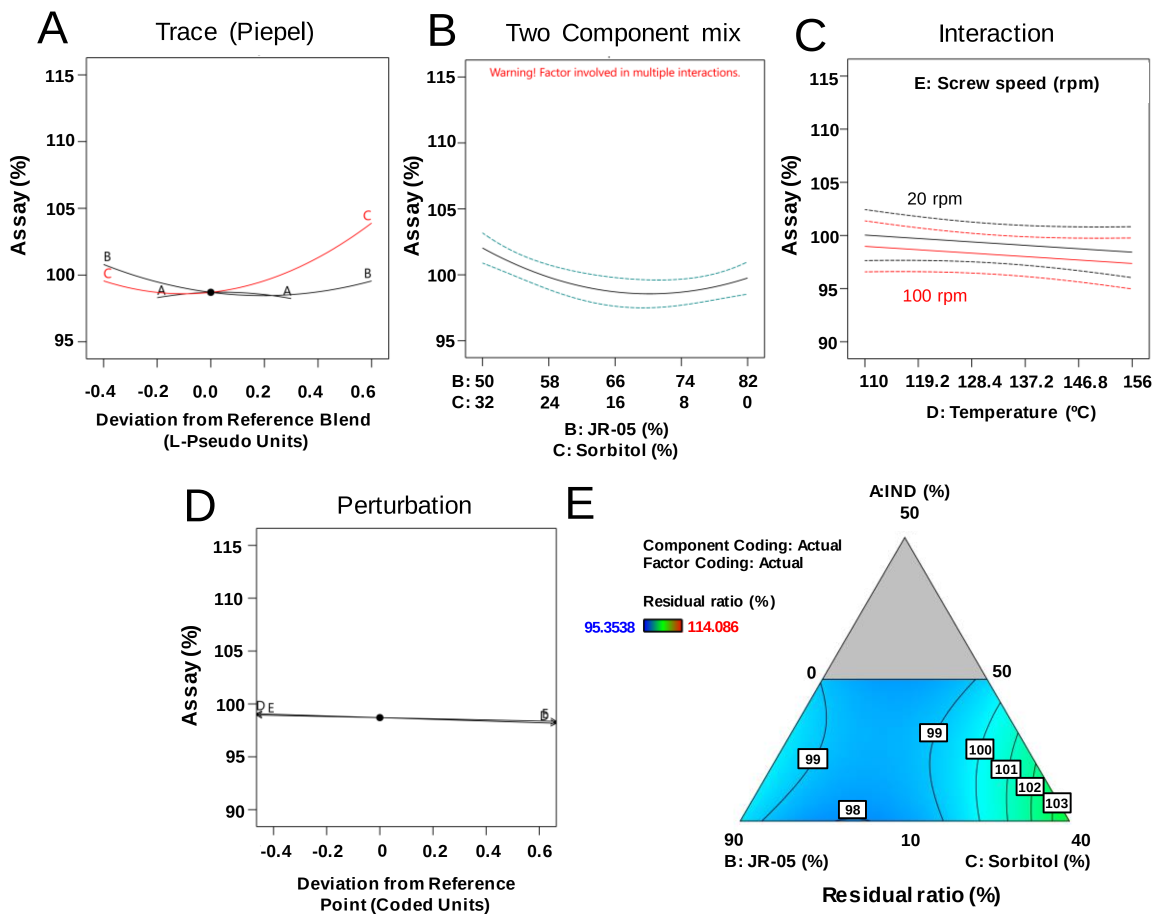
Figures



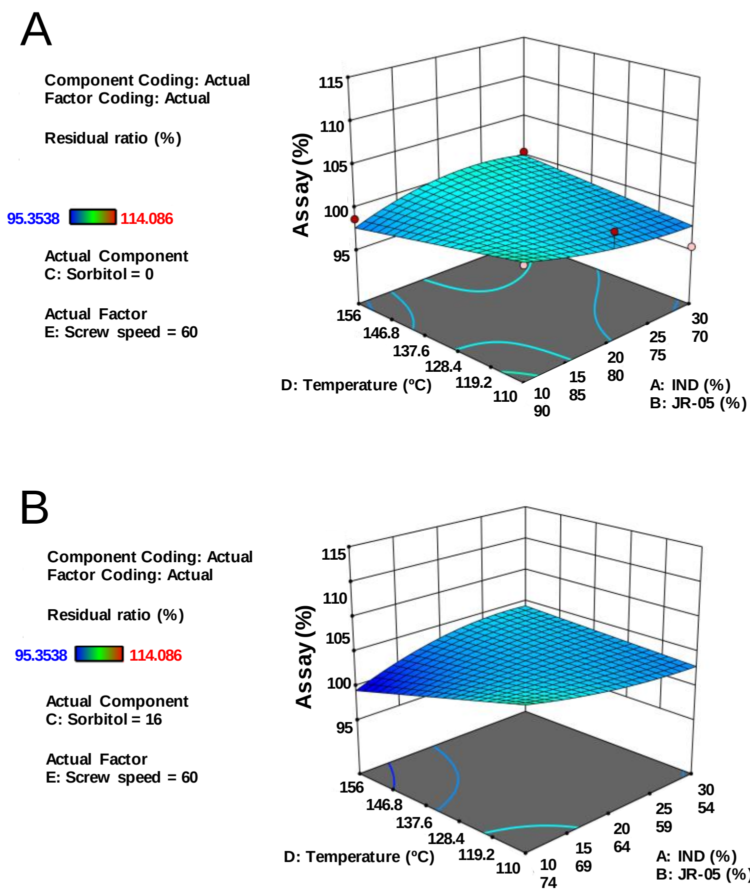
Supp. Figure 1. Influence of IND/JR-05 and temperature on average torque (Nm) in screw speed at 60 rpm: (A) Sorbitol 0 %: (B) Sorbitol 16% in three-dimensional Mix-process plots.



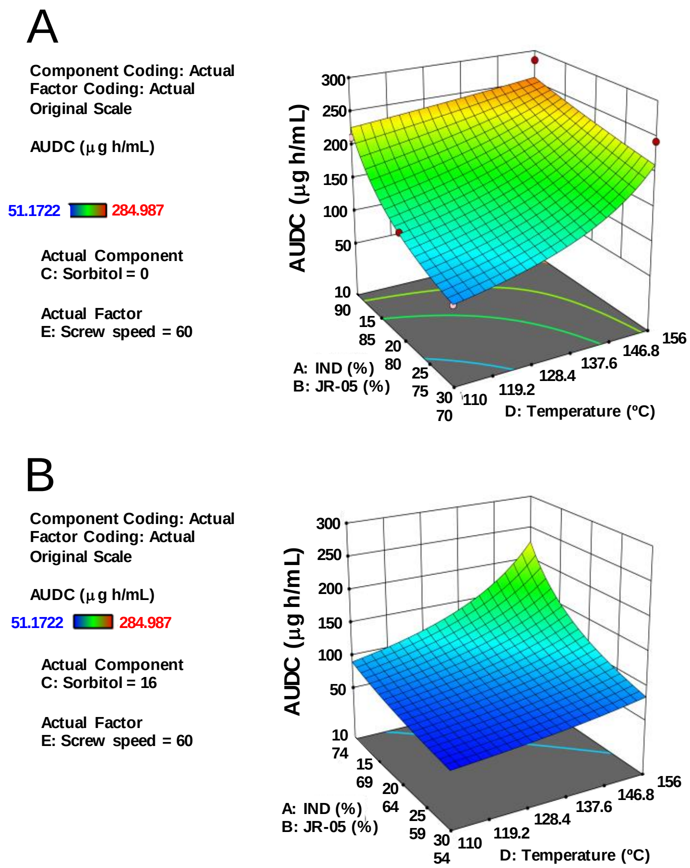
Supp. Figure 2. Examples for the physical appearance of melt extrudate consisted of IND (10% to 30%) and JR-05 with or without Sorbitol (20%) processed at 133ºC and 156ºC.



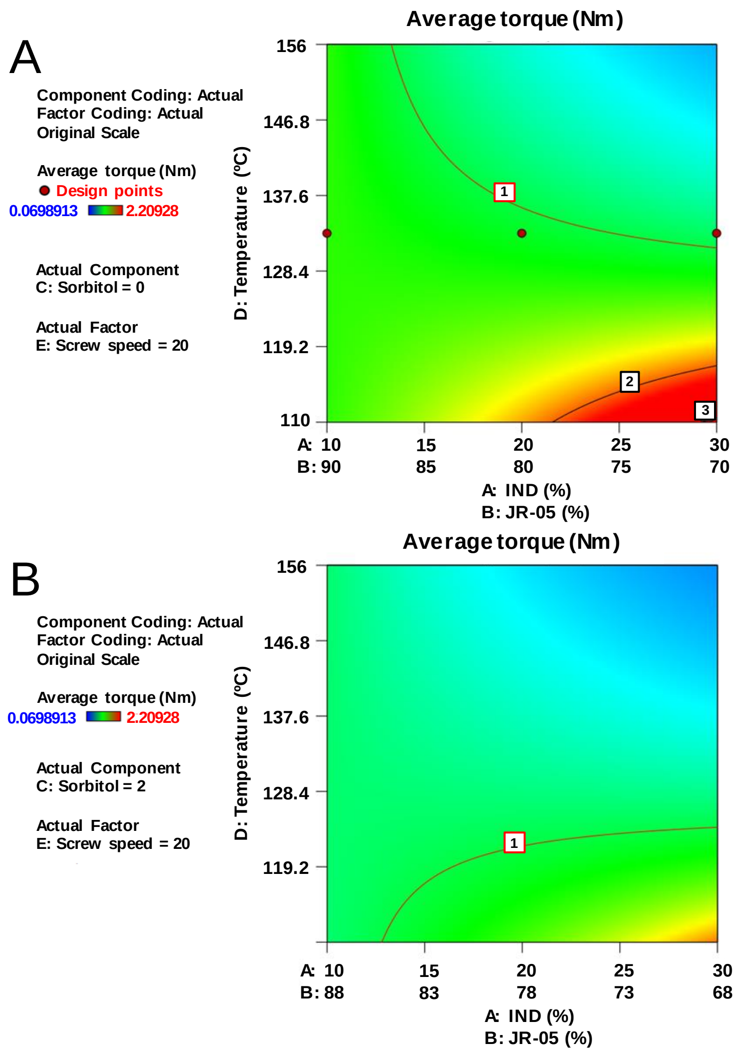
Supp. Figure 3. Influence of mixture components and process condition on residual ratio (%): Trace (Piepel) plot (A): Two component mix plot (B): Interaction graph (C): Perturbation plot (D) for IND content 18%, JR-05 66% and Sorbitol 16% in temperature at 133ºC and screw speed at 60 rpm. Dotted lines in two component mix plot (B) and interaction graph (C) represent 95% confidence intervals. Contour plot (E) represents the relationship between residual ratio (%) and mixture components in temperature at 133ºC and screw speed at 60 rpm.



Supp. Figure 4. Influence of IND/JR-05 and temperature on residual ratio (%) in screw speed at 60 rpm: Sorbitol 0 % (A): Sorbitol 16% (B) in three-dimensional mix-process plots.



Supp. Figure 5. Influence of IND/JR-05 and temperature on AUDC (µg hr/mL) in screw speed at 60 rpm: (A) Sorbitol 0 %: (B) Sorbitol 16% in three-dimensional Mix-process plots



Supp. Figure 6. Influence of IND/JR-05 and temperature on average torque (Nm) in screw speed at 20 rpm: (A) Sorbitol 0%: (B) Sorbitol 2% in Mix-process plot.

References

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