Application of a Multilevel Factorial Design to the Formulation of Indomethacin-loaded Nanoparticles

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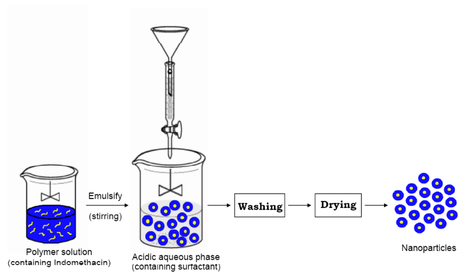
Introduction:

Indomethacin, a potent nonsteroidal anti-inflammatory drug (NSAID), is typically used for chronic inflammatory arthritis. However, patients receiving Indomethacin often experience gastrointestinal side effects. Thus, the design of targeted delivery formulations to reduce side effects of NSAIDs continues to be a focus of current research. In this regard, enteric nanoparticles have been recognized as a potential system to reduce gastrointestinal irritations. The aim of this study was to apply a two-level factorial design with three factors to the formulation of indomethacin-loaded nanoparticles.

Materials & Methods:

Figure 1. Schematic representation of nanoencapsulation procedure.



- *Eudragit® L100 was supplied by Evonik Degussa (Brazil).*
- *Indomethacin, Polyethylene glycol and Tween® 80: purchased from Sigma-Aldrich® (Brazil).*
- *Homogenization -Ultra Turrax homogenizer (Ultra Turrax T 25, IKA, Germany).*
- *Qualitative X-ray diffraction studies - PANalytical X-ray diffractometer ,Netherlands).*
- *Particle size - transmission electron microscope (TEM) (JEOL-JEM 2000EX).*
- *DLS measurements - Zetasizer Nano-ZS®, Malvern Instruments Ltd, Malvern, UK.*
- *Dissolution tests were performed under simulated GI conditions (two stages: acid and buffer). Dissolution apparatus 2 (Nova Ética, São Paulo, Brazil).*
- *The multilevel factorial design, consisting of 24 runs (to be run in 3 blocks) was analyzed using Statgraphics (Version 5.1 Plus). Variance analysis results for encapsulation efficiency were used to test the statistical significance of the estimated effects. P-values lower than 0.05 (P<0.05) were considered statistically significant.*

Table 1. Experimental design: factors and response.

Factors (Independent variables)		Levels -1	Levels 1	Response (Dependent Variable)
***X1*:** Surfactant (Tween® 80)		30	40	***Y*:** Encapsulation Efficiency
***X2*:** Hydrophilic polymer (PEG 2000)		0	40	
***X3*:** Enteric polymer (Eudragit® L100)		140	200	

Table 2. Experimental design matrix, EE and PY values.

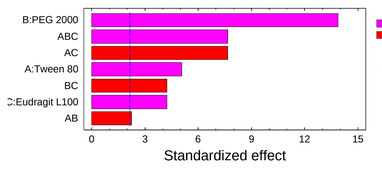
Formulation	Factors X1	X2	X3	Encapsulation Efficiency EE (%) ± SD	Process Yield PY (%) ± SD
I 1	30	0	140	56 ± 1	91 ± 8
I 2	40	0	140	88 ± 4	92 ± 13
I 3	30	40	140	96 ± 5	71 ± 15
I 4	40	40	140	100 ± 0	75 ± 15
I 5	30	0	200	90 ± 4	94 ± 3
I 6	40	0	200	79 ± 5	94 ± 6
I 7	30	40	200	96 ± 2	87 ± 12
I 8	40	40	200	100 ± 0	89 ± 10

Results & Discussions:

*As can be seen in **Table 2**, all PY values are quite respectable result considering that polyethylene glycol is highly soluble in water (min 71%; max 94%). Moreover, all the EE values are greater than 50% (min 56%; max 100%). The variance analysis results for EE is showed below:*

Statistical analysis

Figure 2. Standardized pareto chart for encapsulation efficiency.



Any bar extending beyond the blue line correspond to an statistically significant effect (95% confidence level). Then, in this study, there are 7 statistically significant effects.

Table 3. Summary of estimated effects for encapsulation efficiency.

Effects	Value	Standard Errors
Average	88.1667	0.709033
A:Tween® 80	7.16667	1.41807
B:PEG 2000	19.6667	1.41807
C:Eudragit® L100	6.0	1.41807
AB	-3.16667	1.41807
AC	-10.8333	1.41807
BC	-6.0	1.41807
ABC	10.8333	1.41807
block	1.91667	2.00545
block	-1.08333	2.00545

Table 4. ANOVA for encapsulation efficiency.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Tween® 80	308.167	1	308.167	25.54	0.0002
B:PEG 2000	2320.67	1	2320.67	192.34	0.0000
C:Eudragit® L100	216.0	1	216.0	17.90	0.0008
AB	60.1667	1	60.1667	4.99	0.0424
AC	704.167	1	704.167	58.36	0.0000
BC	216.0	1	216.0	17.90	0.0008
ABC	704.167	1	704.167	58.36	0.0000
blocks	11.0833	2	5.54167	0.46	0.6409
Total error	168.917	14	12.0655		
Total (corr.)	4709.33	23			

X-ray diffraction

Figure 3. Qualitative X-ray diffractograms of Indomethacin and pure polymers.

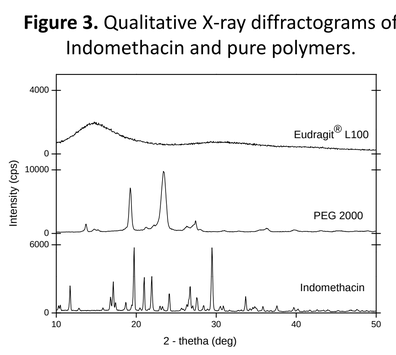
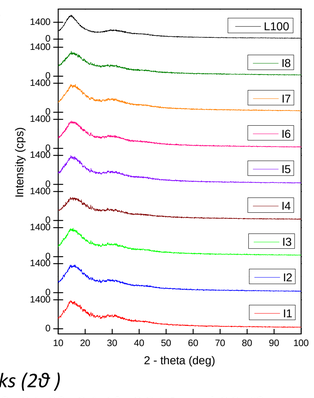


Figure 4. Comparison of the qualitative x-ray diffractograms of nanoparticles and Eudragit® L100.



Diffraction peaks (2ϑ)

Indomethacin: *11.7°; 17.1°; 19.7°; 20.9°; 21.9°; 24.1°, 26.7º and 29.4°*

PEG: *19.3° and 23.4°*

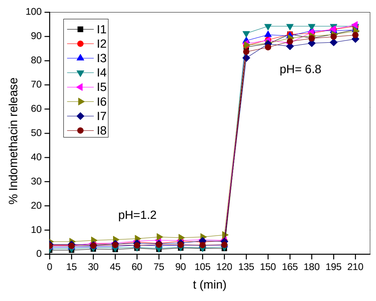
Eudragit® L100: *two amorphous halos between 10º and 37º (2ϑ)*

Formulations: *Equal to Eudragit® L100*

✓ *Reduced degree of crystallinity of the drug in the formulations.*

Dissolution tests

Figure 5. *In Vitro* dissolution profiles of Indomethacin loaded in nanoformulations.



Particle Size and Morphology

Figure 6. SEM micrographs of sample I5.
A) x 4 000, B) x 4 000, C) x 16 000, D) x 33 000.

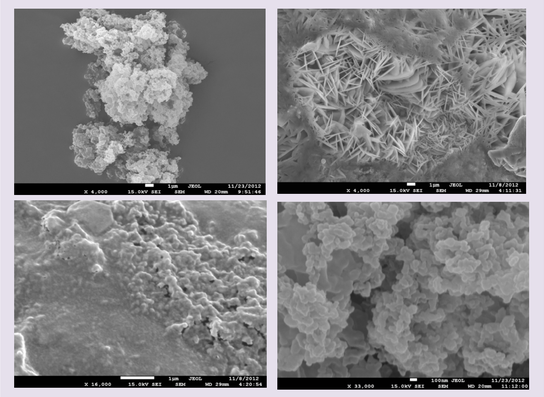
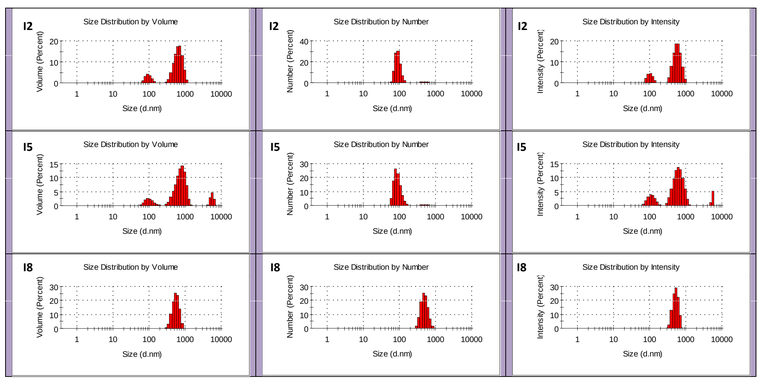


Figure 7. Particle size distributions (Volume, Number, Intensity).
From top to down: Samples I2, I5 and I8.



In the intensity and volume distributions, for Sample I5, can be seen three size distributions classes around 100 nm, 1 µm and 6 µm. However, on a number basis, it becomes more obvious that the 100 nm individual particles are the most significant size.

Conclusions:

✓ Statistical analysis of multilevel factorial design has shown 7 significance effects on encapsulation efficiency (95% confidence level), while PEG had shown the greater influence due to its co-surfactant behavior.

✓ Yield values and encapsulation efficiency are higher than 70% and 50%, respectively.

✓ X-ray diffraction (XRD) did not show evidence of strong interaction between the polymers and Indomethacin.

✓ From DLS measurement and Morphological analysis, the particle sizes and agglomerates sizes are around 100 nm and 1000 nm, respectively. These results indicate that nanoparticles aggregates are governed by electrostatic effects.

✓ All formulations have shown a delayed release, demonstrating great potential as Indomethacin carrier for oral administration.

References:

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