Original Article

Using factorial design to improve the solubility and *in-vitro* dissolution of nimesulide hydrophilic polymer binary systems

Ibrahim S. Khattab*, Saleh M. Al-Saidan, Aly H. Nada, Abdel-Azim A. Zaghloul

Department of Pharmaceutics, Faculty of Pharmacy, Kuwait University, Kuwait.

ABSTRACT: The aim of the present study was to use factorial design to enhance the dissolution rate of nimesulide using solid binary systems with the hydrophilic carriers D-mannitol and polyethylene glycol (PEG 4000). Two-factor full factorial design was employed to investigate the effects of the drug/carrier ratio (X_1, 10 and 20%) and the method of preparation (X_2, physical or co-melted mixture) on the percent drug release after 60 min (Y_1). Drug-carrier co-melted mixtures were prepared by melting the carriers D-mannitol or PEG with the drug. For physical mixtures, the drug and carrier were mixed thoroughly in a mortar until a homogeneous mixture was obtained. Drug-carrier interactions were investigated by differential scanning calorimetry (DSC). All prepared mixtures were filled in hard gelatin capsules, size 0, and then their dissolution rate was tested. The results showed an increase in the solubility of the drug with increasing polymer concentrations. Thermal analysis revealed no notable differences regarding thermal events of nimesulide, D-mannitol, PEG 4000, and their physical or co-melted mixtures. The percent drug released after 60 min was 29.5% for nimesulide alone, 37.14 and 32.0% for a PEG/Physical mixture with a 10 or 20% drug/polymer ratio, and 69.7 and 53.1% for a PEG/Co-melted mixture with the same ratios. For nimesulide/D-mannitol, this percent drug released was 33.57 and 29.6% for a physical mixture and 63.13 and 48.04% for a co-melted mixture. Formulations with PEG showed an increase in solubility as well as dissolution in comparison to those prepared with D-mannitol. Factorial design was successfully used to optimize the dissolution rate of nimesulide. The chosen polymers caused a notable increase in drug solubility and co-melted formulations generally showed a higher dissolution than those prepared with physical mixtures.

Keywords: Nimesulide, Binary systems, Factorial design

**Correspondence to:* Dr. Ibrahim S. Khattab, Pharmaceutics Faculty of Pharmacy, Kuwait University, Kuwait;
e-mail: Khattab@hsc.edu.kw

1. Introduction

Nimesulide is a non-steroidal anti-inflammatory agent that differs from many similar compounds in that it is acidic by virtue of a sulfonanilide rather than a carboxyl group. It is an inhibitor of cyclo-oxygenase 2, hence it inhibits the synthesis of destructive prostaglandins and spares cytoprotective prostaglandins. Nimesulide is practically insoluble in water (0.01 mg/mL). The poor aqueous solubility and wettability of the drug give rise to difficulties in the pharmaceutical formulation of oral preparations or solutions and may lead to its varying bioavailability. Increasing the aqueous solubility of nimesulide is an important way to overcome these drawbacks (*1*).

The improvement of both solubility and dissolution rate by inserting a drug into a solid dispersion has been widely discussed and reported in a number of articles (*2-6*).

The selection of the carrier has ultimate influence on dissolution characteristics. Research has shown that water soluble carriers result in a fast release of the drug from the matrix. Poorly soluble carriers, however, lead to slow release of the drug from the matrix (*7*). To date, several methods have been used to increase the water solubility of poorly soluble drugs such as physical modification of the drug and use of co-solvents, nanoparticles, a film coating, a complexation approach, and solid dispersion technology (*8-13*). Polyethylene glycols have been used extensively as water-soluble carriers and stabilizers for pharmaceutical dosage forms because of their favorable solution properties and low toxicity and cost (*14-16*). D-Mannitol is primarily used in pharmaceutical preparations as a diluent. It has been also used to improve the dissolution and bioavailability of thiazolidinedione and triamterene (*17-19*).

The purpose of the present study was to employ an experimental design to develop an optimization strategy to increase the water dissolution of nimesulide by using carriers such as D-mannitol and polyethylene glycol at various drug/polymer concentrations. A two-factor, two-level factorial experimental design was employed to determine whether a particular treatment or combination of treatments was satisfactorily significant

in influencing system response. Mathematical elaboration of experimental data was carried out by the computer program Design Expert®.

2. Materials and Methods

2.1. *Materials*

Nimesulide (NS) was from Sigma (Italy), and Polyethylene Glycol 4000 (PEG 4000) and D-mannitol (DM) were from Fluka (Germany). Empty capsules were generously supplied by KSPICO (Kuwait). All other materials and solvents were of analytical grade and used as received.

2.2. *Preparation of physical mixtures and binary systems*

2.2.1. *Preparation of physical mixtures*

Physical mixtures were prepared according to the design; NS content was at a concentration of 10 and 20% (w/w) with a carrier of DM or PEG 4000. The drug and carrier were accurately weighed, pulverized, and then mixed thoroughly by titration in a glass mortar until a homogeneous mixture was obtained. The systems were designated NS/DM10, NS/DM20, NS/PEG10, and NS/PEG20 for the drug D-mannitol and the drug PEG, respectively.

2.2.2. *Melting carrier method*

Solid dispersions of NS with carriers were prepared by the melting method at ratios of 1:9 and 2:8 (NS: PEG or DM). The required amount of each carrier was melted over a thermostatically controlled magnetic stirrer at its respective melting point, *i.e*. 50-60°C for PEG and 165°C for DM. When the carrier appeared to have completely melted, the required amount of NS was incorporated into the molten carrier mass. The blend was heated at the corresponding temperature for 5 min with constant mixing. The resultant melted mixture was transferred to a glass mortar and mixed thoroughly for another 5 min and then left to cool at room temperature. After solidification, the solid obtained was ground and passed through a no. 60 sieve, and the fraction between 50-200 μm fractions was selected. All prepared solid dispersions or physical mixtures were filled into hard gelatine capsules (size 0) such that each capsule contained 10 or 20% drug in different carriers.

2.3. *Phase solubility studies*

The apparent solubility of NS pure drug and binary mixtures in water was obtained at 37°C. Various quantities (1, 2, 3, 10 and 20%, w/v) of aqueous solutions of PEG and DM were prepared, and 50 mL of these solutions were placed in conical flasks with screw caps. An excess amount of NS was added to the flasks. The flasks containing NS-mixtures were shaken mechanically for 12 h at 37 ± 2°C in a mechanical shaker, rpm 175 (Elico Pvt. Ltd, Mumbai, India). These solutions were allowed to equilibrate for the next 24 h and then centrifuged for 5 min at 2,000 rpm. The supernatant of each flask was filtered through Whatman filter paper no. 1; the filtrate was diluted and analyzed spectrophotometrically at 393 nm. Solubility studies were performed in triplicate ($n = 3$).

2.4. *Differential scanning calorimetry*

Thermal analysis was carried out by a Setaram instrument (model 41 L, Caluire, France WB/M00) under a dry nitrogen purging gas flux. Thermoregulation of the instrument head was guaranteed by connection to a cooling system. About 5 mg of samples were weighed in a standard open aluminum pan. An empty pan of the same type was utilized as a reference. Heating and cooling rates of 5°C/min in the temperature range of 25-360°C were used. Calibration of temperature and heat flow was performed with indium.

2.5. *In vitro dissolution test*

A paddle dissolution apparatus, USP23/NF (model DT80, Erweka, Germany), was used. Dissolution fluid was 900 mL of a pH 7.4 phosphate buffer. Temperature was thermostated at 37 ± 0.5°C and the buffer was stirred at 50 rpm. Sample solution (2.5 mL) was withdrawn at predetermined time intervals, and the fluid was filtered (0.45 nm membrane) prior to entering the working cell and analyzed spectrophotometrically at 393 nm; each test was performed in triplicate ($n = 3$). An equal amount of fresh dissolution medium was replaced after withdrawal of the test sample. The considered parameters were: the initial dissolution rate and the percent NS released after 1 h of dissolution.

2.6. *Experimental design*

To optimize the *in-vitro* dissolution of NS from different carriers, a two-factor full factorial design was employed to investigate the effects of the drug/carrier ratio (X_1, 10-20%), represented by –1 and +1 and analogous to the respective low and high values, and the method of preparation (X_2, physical or co-melted mixture) on the percent drug release after 1 h (Y), with X_1 and X_2 serving as independent variables. The polynomial equations that completely describe the system were also calculated. The general expression for these equations is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2$$

Mathematical elaboration of experimental data was carried out by the computer program DESIGN EXPERT®. Analysis of variance (ANOVA) was also performed on the resulting data.

3. Results

3.1. *Phase solubility studies*

The solubility of NS increased as the PEG concentration increased, while with DM a slight change occurred in comparison to solubility with pure NS, as indicated by the solubility data given in Table 1 and shown in Figure 1. The enhancement of solubility with PEG as directly relates to the increase in the polymer content may be attributed to the hydrophilic nature of the carrier.

3.2. *DSC studies*

Differential scanning calorimetry (DSC) analysis is widely used to investigate the structure of solid dispersions and to demonstrate possible drug/matrix interactions through the shape of the peaks, the melting temperatures, and the specific melting heats offered by the thermograms.

The thermograms were carried out separately with the drug and excipient. NS shows quite a narrow melting peak and a melting point at 146°C. The melting point for PEG is at 58°C, with a wide and asymmetric peak due to its chemical composition, which is a mixture of macromolecules with a molecular mass around 4,000. The thermograms of DM gave rise to two sharp melting endotherms at 170.15°C and 345.17°C (Figure 2).

Thermal analysis revealed no notable differences regarding thermal events of NS, PEG, DM, and their physical or co-melted mixtures, even though some NS influence on thermal crystallization of PEG can be appreciated. The main reason for this is that the situation inside the system is altered as far as scanning proceeds.

Table 1. Summary of NS/polymer solubility studies

Polymer conc (%)	NS/PEG (x 10^{-5} M)	St/So*	NS/DM (x 10^{-5} M)	St/So*
1	2.36	1.00	2.00	0.85
2	2.40	1.17	2.20	0.93
5	3.24	1.37	2.46	1.04
10	4.45	1.89	2.87	1.22
20	7.37	3.12	1.78	0.75

* St/So, solubility change; So, solubility of nimesulide in water (2.36 x 10^{-5} M).

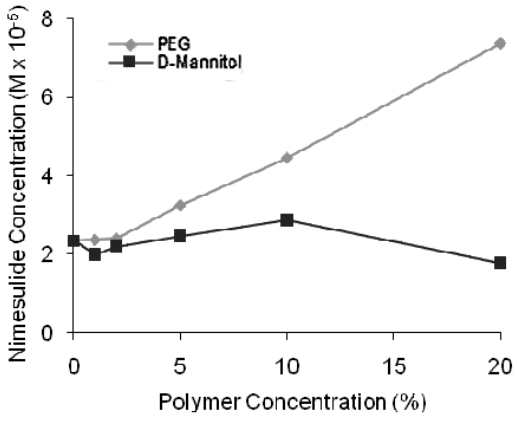

Figure 1. Phase solubility diagram of NS with (PEG/ Mannitol) solutions.

3.3. *Experimental design*

A full factorial experimental design was adopted to investigate the effect of the drug/carrier ratio (X_1) and method of preparation (X_2) on the percent drug release (Y) as illustrated in Table 2. In a full factorial design, the two variables polymer ratio and method of preparation at two levels are represented by –1 and +1, analogous to low and high values. Response surface methodology was used to generate contour plots to visually illustrate the impact of the tested variables on dissolution. Response surfaces were generated by employing one of the following mathematical equations.

Linear model: $Y = b_0 + b_1X_1 + b_2X_2$

Interaction model: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2$

Quadratic model: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + X_1^2 + X_2^2$

where Y represents the dependent variables, X_1 and X_2 are the independent variables (X_1 represents the polymer ratio and X_2 is the method of preparation),

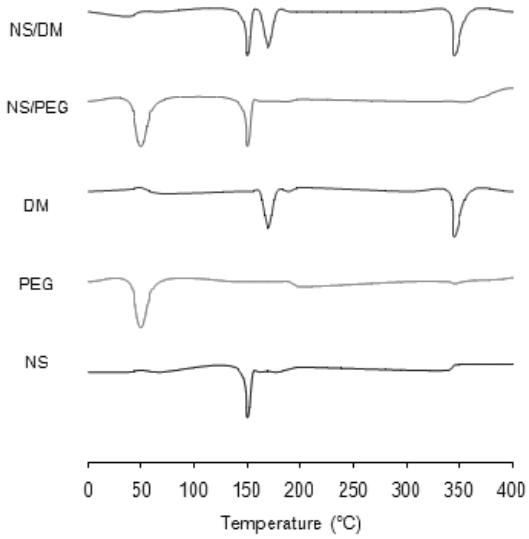

Figure 2. DSC thermogram of nimesulide, PEG 4000, and D-mannitol and NS/PEG and NS/DM (2:8) binary systems.

Table 2. Two-factor, two-level full factorial design

Run No.	Controlled factor	
	X_1	X_2
1	–1	–1
2	1	–1
3	–1	1
4	1	1
5	–1	–1
6	1	–1
7	–1	1
8	1	1

and the coefficients b_0, b_1, b_2 and b_3 are the least square regression coefficients.

The results indicate an increase in the solubility of the drug with increasing polymer concentrations. Dissolution of NS from its physical mixtures was slightly higher than that for the pure drug, but maximum improvement in the dissolution rate was observed with co-melted mixtures. The percent drug released after 60 min (R_{60}) was 29.5% for NS alone, 37.14 and 32.06% for PEG/PM with a 10 or 20% drug/polymer ratio, and 69.7 and 53.1% for PEG/CM with the same ratios. For NS/DM, this percent drug released was 33.57 and 29.5% for PM and 63.13 and 48.04% for CM. Formulations with PEG showed a notable increase in solubility as well as dissolution in comparison to those prepared with D-mannitol (Figures 3 and 4).

To optimize the percent NS release, mathematical relationships were generated between the dependent and independent variables; the resulting polynomial equations for dissolution after 60 min in terms of coded factors are as follows:

$$R_{60}\ (NS/DM) = 43.64 - 4.96X_1 + 11.87X_2 - 2.7X_1X_2$$
$$R_{60}\ (NS/PEG) = 47.88 - 5.32X_1 + 13.05X_2 - 3.02X_1X_2$$

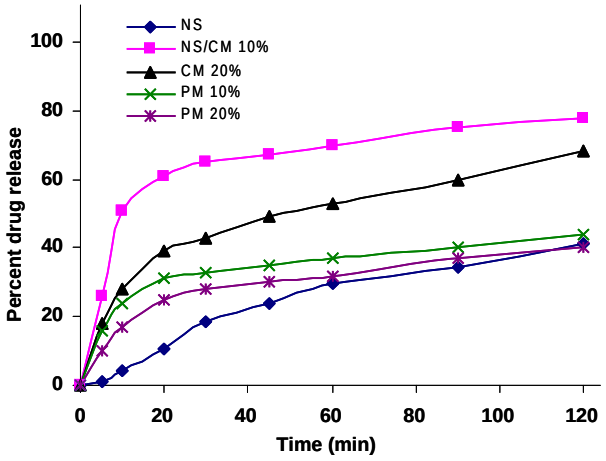

Figure 3. Dissolution profiles of NS alone and with PEG 4000 from different mixtures ratios (10% or 20%) prepared by different methods (PM, Physical mixture; CM, Co-melted mixture).

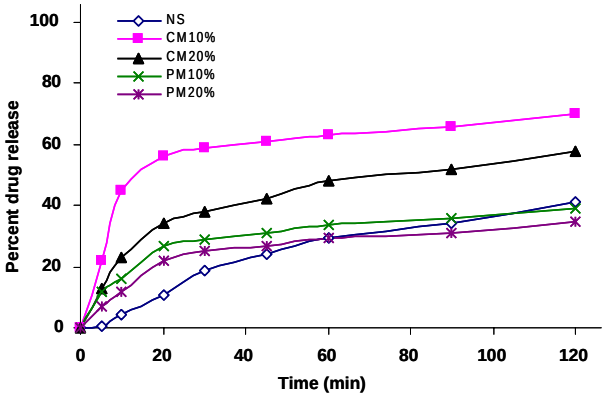

Figure 4. Dissolution profiles of NS alone and with D-mannitol from different mixtures ratios (10% or 20%) prepared by different methods (PM, Physical mixture; CM, Co-melted).

Generating the respective model equations allows selection not only of the best formulation experimentally prepared but also of the best formulation within the experimental range.

The observed, predicted, and residual values are shown in Tables 3 and 4. The "Predicted R-Squared" of 0.9965 is in reasonable agreement with the "Adjusted R-Squared" of 0.9985 for NS/PEG after time of 60 min. The same finding applied to NS/DM, which had a "Predicted R-Squared" of 0.9996 and "Adjusted R-Squared" of 0.9993. As the "Adequate Precision" measures the signal-to-noise ratio, a ratio greater than 4 is desirable; the resulting ratios of 86.68 for NS/PEG and 131.388 for NS/DM indicate an adequate signal.

Figures 5 and 6 represent response surface plots showing the influence of the percent NS released from capsules containing D-mannitol or PEG 4000, which explains the relationships between the dependent and independent variables. The figures show the effect of drug concentration and method of preparation on the response in terms of percent NS release. The results indicate that the effect of the increase in polymer concentration was more significant with co-melted mixtures than with physical ones.

Using the least square regression coefficients b_0, b_1, b_2 and b_3 in the model, one can find which X(s) produces the maximum drug release value (optimization). An experiment was performed under optimal conditions corresponding to 10% PEG 4000 using the co-melted method; the results conformed to the predicted values, indicating that the response surface

Table 3. Actual, predicted, and residual values for percent of nimesulide released after 60 min from capsules containing a NS/DM (X_1: 10 and 20%) physical mixture or co-melted mixture (X_2)

Standard order	X_1	X_2	Actual value	Predicted value	Residual value
1	–1	–1	33.57	33.49	0.08
2	1	–1	29.50	30.12	–0.62
3	–1	1	63.13	63.42	–0.29
4	1	1	48.04	48.19	–0.15
5	–1	–1	33.02	32.88	0.14
6	1	–1	30.51	30.80	0.29
7	–1	1	64.42	63.80	0.62
8	1	1	47.87	47.95	–0.08

Table 4. Actual, predicted, and residual values for percent of nimesulide released after 60 min from capsules containing a NS/PEG (X_1: 10 and 20%) physical mixture or co-melted mixture (X_2)

Standard order	X_1	X_2	Actual value	Predicted value	Residual value
1	–1	–1	37.14	36.84	0.30
2	1	–1	32.00	32.15	–0.09
3	–1	1	69.72	70.03	–0.31
4	1	1	53.08	53.17	0.09
5	–1	–1	40.27	40.10	0.17
6	1	–1	32.47	32.65	0.18
7	–1	1	71.75	71.93	–0.18
8	1	1	52.11	52.27	–0.16

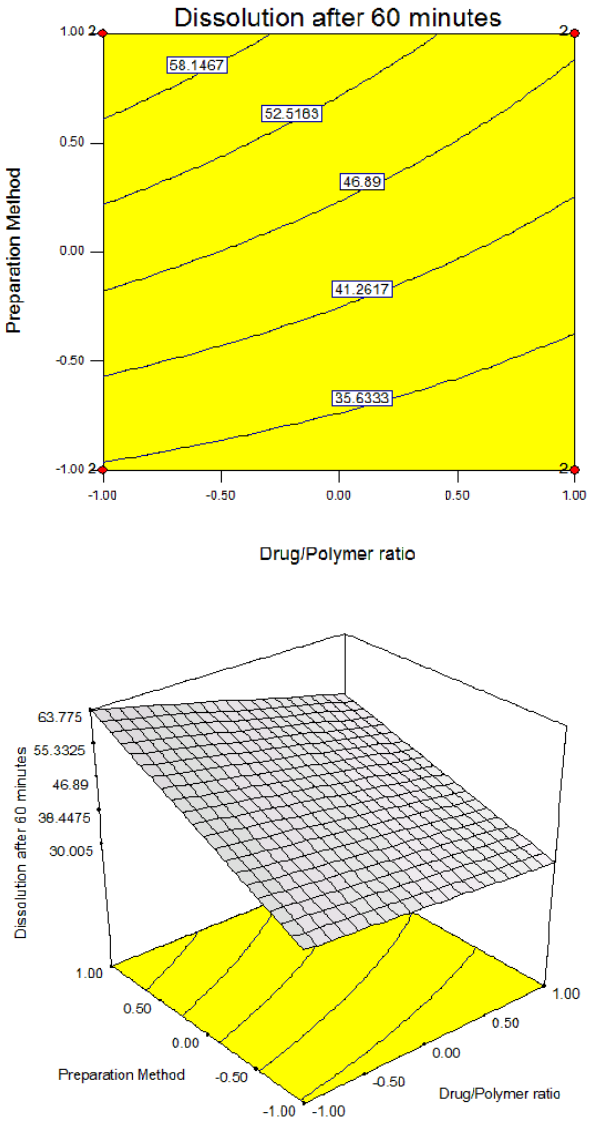

Figure 5. Response surface plot showing the influence of the NS/DM ratio (X_1) and method of preparation (X_2, Physical mixture or Co-melted mixture) on the nimesulide dissolution rate after 60 min.

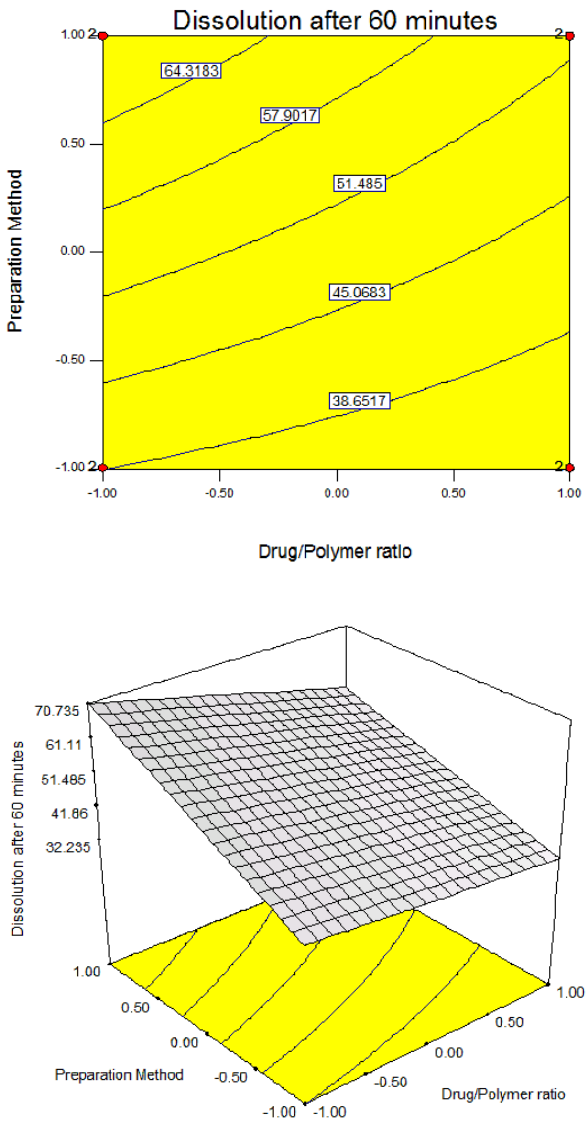

Figure 6. Response surface plot showing the influence of the NS/PEG ratio (X_1) and method of preparation (X_2, Physical mixture or Co-melted mixture) on the nimesulide dissolution rate after 60 min.

methodology optimization technique was quite useful in optimizing *in-vitro* dissolution of nimesulide.

4. Conclusions

Thermal analysis revealed no notable differences regarding thermal events of NS, PEG, DM, and their physical or co-melted mixtures. A co-melted binary system showed higher solubility as well as dissolution in comparison to NS alone or a physical mixture. Capsules containing PEG show a higher dissolution compared to those containing DM. Binary systems of NS/PEG 4000, formulated as solid dispersions, produce satisfactory drug release from capsules after 1 h. A factorial experimental design is an excellent way to optimize both formulation and process factors as well as to save time by reducing number of runs.

Acknowledgment

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