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# Evaluation of the effect of physical variables on *in vitro* release of diclofenac pellets using Box-Behnken design

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ABSTRACT

***Objective(s):*** A Box-Behnken design was used for evaluation of Eudragit coated diclofenac pellets. The purpose of this work was to optimize diclofenac pellets to improve the physicochemical properties using experimental design.

***Materials and Methods:*** Diclofenac was loaded onto the non-pareil beads using conventional coating pan. Film coating of pellets was done at the same pan. The effect of plasticizer level, curing temperature and curing time was determined on the release of diclofenac from pellets coated with polymethacrylates.

***Results:*** Increasing the plasticizer in the coating formula led to decrease in drug release and increasing the curing temperature and time resulted in higher drug release. The optimization process generated an optimum of 35% drug release at 3 hr. The level of plasticizer concentration, curing temperature and time were 20% w/w, 55 ˚C and 24 hr, respectively.

***Conclusion:*** This study showed that by controllinig the physical variables optimum drug release were obtained.

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## Introduction

Most recently, much emphasis is being laid on the progression of multiparticulate dosage forms. Among them, oral sustained release pellets have attracted much attention (1-3). Pellets could be prepared by size expansion process which is called pelletization (1, 3).

Eudragit RL30D and Eudragit RS30D are aqueous dispersions of copolymers of acrylic acid and methacrylic acid esters. They are prevalently used for the preparation of controlled-release oral pharmaceutical dosage forms (4, 5).

Plasticizers are added in coating formulations to improve the mechanical and film-forming properties of the polymers (6, 7).

Thermal annealing or treatment plays an important role in controlling characteristics of polymer films. In this process a polymer is heated to a specified temperature, for a certain time period. Annealing of amorphous polymers generally obliges the heating of polymer to temperatures above Tg, in which the stress relaxation and orientation are the most rapid. Curing can result in extensive structural changes within a polymeric film coating by inducing coalescence of the polymer particles. This coalescence will lead to the formation of tougher and stronger films (8, 9).

The Box-Behnken design (BBD) is an experimental design employed for the optimization procedure.

The principal objective of the present investigation was to employ a simple, suitable and applicable method to prepare pellets and to evaluate the effect of some intensive factors on cumulative percent of drug release, to statistically determine the levels of these factors and to optimize the product applying mathematical equations and response surface plots.

## Materials and Methods

Diclofenac sodium (Dipharma, Italy) polyvinyl pyrrolidone (PVP K25) and triethyl citrate (TEC) was purchased from merck, Germany. Eudragit® RS30D and Eudragit® RL30D (Evonik, Germany) was donated by Akbariye Pharm. Co, Iran and Non-pareil beads with the size of 25 - 30 mesh was supplied by Soha pharm. Co., Iran. Other excipients used to prepare pellets were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

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**Table 1.** Factors and their levels for Box-Behnken design and responses

| Factors | Levels | | | Responses |
|---|---|---|---|---|
| | -1 | 0 | +1 | |
| Plasticizer concentration (%, $X_1$) | 10 | 20 | 30 | Cumulative% drug released in 3 hr ($Y_1$) |
| Curing temperature (°C, $X_2$) | 40 | 50 | 60 | Cumulative% drug released in 4 hr ($Y_2$) |
| Curing time (h, $X_3$) | 24 | 96 | 168 | Cumulative% drug released in 6 hr ($Y_3$) |

A Box-Behnken design with three factors was used for optimization procedure. It is suitable for investigating the quadratic response surfaces and for constructing a second-order polynomial model, thus enabling optimization of a process with a small number of experimental runs (15 runs). Response surface modeling was performed with SPSS software (version 17).

The primary studies provided a setting of the levels for formulation variables. The studied factors were plasticizer concentration ($X_1$), curing temperature ($X_2$) and curing time ($X_3$). Table 1 summarizes the factors and their levels (independent variables). The chosen dependent variables were cumulative percentage values of diclofenac sodium dissolved in a determined time (after 3, 4 and 6 hr). It has been shown that these dependent variables have great effectiveness on drug release (7, 8).

### *HPLC analysis*

A HPLC method was applied for determination of diclofenac in pellets. A liquid chromatograph (Smart line; Knauer, Berlin, Germany) equipped with an ultraviolet detector (Wellchrom, K-2600; Knauer) and C18 (Nucleosil H.P. 25 cm × 0.46 cm internal diameter, pore size 5 µm; Knauer) column was used. The mobile phase consisted of a mixture of methanol and pH 2.5 phosphate buffer (700:300) and was delivered at a flow rate of 1.00 ml /min. Aliquots of 20 µl from samples prepared in methanol were injected to the system. The column effluent was detected at 254 nm.

### *Preparation of drug loaded pellets*

Pellets were prepared by powder layering of diclofenac sodium on nonpareils in a 35-cm diameter, conventional coating pan (Erweka, Germany). At first the required amount of the poly vinyl pyrrolidone (Kollidon 25) was dissolved in distilled water to prepare binding solution. Then, the desired size (25/30 mesh) of non-pareil seed (NPS) was loaded into rotating pan (160 rpm). The required amounts of diclofenac sodium powder, lactose, and maize starch was mixed properly by cubical blender. The powder blend was loaded manually on NPS with simultaneous spraying of binding solution. Diclofenac layering condition were as follows; inlet temperature 50–55 °C, product temperature 37–40 °C, outlet temperature 35–38 °C, nozzle diameter 1.0 (mm), atomisation pressure 2.0 (bar) and spray rate 25-35 (g $min^{-1}$).

After completion of each cycle of wetting and powder layering the sphere bed was dried completely. This was continued until all the drug powder was applied to NPS, and then sieved through 18 mesh and 20 mesh, to get the desired size (18-20 mesh). The layered pellets were dried in the oven for 12 hr at 35 °C.

### *Drug loading determination*

A known amount of samples was dissolved in a mixture of methanol and water (70:30 (v/v)) as diluent solution. Then the solution was filtered into a vial, and was analyzed by HPLC.

### *Coating of drug loaded pellets*

Briefly, Eudragit RS (30% w/w) and Eudragit RL (30% w/w) were mixed (3:1) in water. The polymer content of the mixture was then adjusted to 20% w/w (related to the dried polymer) by dilution with water. With gentle stirring, suspension of talc was added to the prepared acrylic dispersion. At the end, aqueous polymer dispersion was plasticized with TEC (10, 20 and 30%, based on the experimental design in Table 2).

The amount of 80 g of dried pellets containing 30% drug loading transferred to the same conventional coating pan with speed of 160 rpm. The coating dispersion was sprayed on the pellets with the following condition; inlet temperature 35–40 °C,

**Table 2.** Coating formulation for diclofenac sodium sustained release pellets

| Ingredients (g) | Amount (g) | Amount (g) | Amount (g) |
|---|---|---|---|
| Eudragit RS30D | 50 | 50 | 50 |
| Eudragit RL30D | 16.6 | 16.6 | 16.6 |
| Purified talc | 4 | 4 | 4 |
| Tri-ethyl citrate | 2 (10% plasticizer) | 4 (20% plasticizer) | 6 (30% plasticizer) |
| Water | 33.3 | 33.3 | 33.3 |

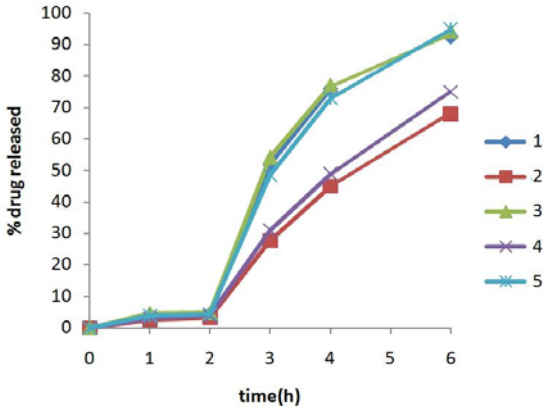


**Figure 1a.** Dissolution profiles of diclofenac sustained release pellets according to the Box-Behnken design (runs 1–5)

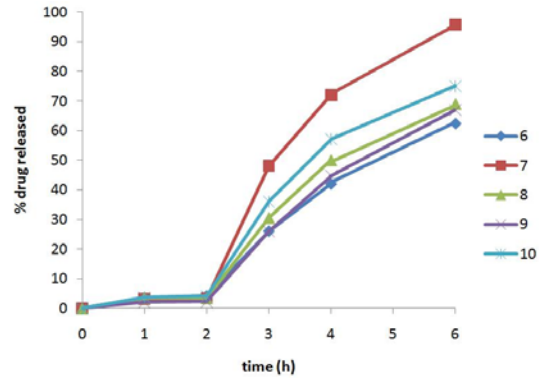


**Figure 1b.** Dissolution profiles of diclofenac sustained release pellets according to the Box-Behnken design (runs 6–10)

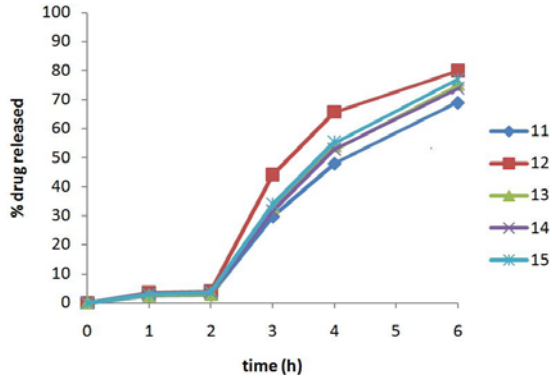


**Figure 1c.** Dissolution profiles of diclofenac sustained release pellets according to the Box-Behnken design (runs 11–15)

outlet temperature 30–32 ˚C, the nozzle diameter of 1 mm, atomization pressure 2 bar and spray rate 15 – 20 g.min$^{-1}$. This process was continued till all the coating dispersion (100 ml) was used.

Then pellets were sieved and the fraction of mesh 16-18 was separated (95% product yield).

The prepared formulations (containing 10, 20 and 30% plasticizer, respectively) was divided to three parts and each part was taken in an oven with adjusted temperature (40, 50 and 60 ˚C). The weight increase of pellets after polymer coating was about 4-6 g.

### *In vitro drug release*

*In vitro* drug release studies were carried out based on USP39 dissolution apparatus I. In the first stage, accurately weighed samples containing the equivalent of about 100 mg of diclofenac sodium were introduced in the dissolution medium (1000 ml 0.1 N HCl solution) at 37 ˚C at a basket speed of 100 rpm. After 1 and 2 hr, 5 ml samples were taken from the vessels and fresh medium was replaced each time, samples passed through a filter, then assayed by HPLC. In the second stage, acidic medium was immediately replaced with the phosphate buffer (pH 6.8), then the dissolution testing was continued. Additional samples were taken in the same way as before at 3, 4 and 6 hr and analyzed by HPLC.

### *Scanning electron microscopy (SEM)*

The optimized formulation of the pellets was taken for the surface characteristic studies. The pellets were scanned using SEM (EM 3200, KYKY, montage china).

## Results

Dissolution profiles of all 15 formulations are shown in Figures 1a, 1b and 1c. All formulations demonstrated a release of less than 10 percent in acidic medium while in buffer phosphate medium more than 60 percent of drug released.

The resulted equations of all responses represented the quantitative effect of the formulation variables on the three responses $Y_1$–$Y_3$, respectively.

The observed, predicted and residual values for the dependent variable indicatad the mean effect for each factor in the model. The obtained results were complied with our expectations and previous studies as regards the effect of $X_1$, $X_2$ and $X_3$ on the drug release after 3 hr (data not shown).

It can be also deduced that by increasing TEC concentration from 10 to 20%, diclofenac release is drastically decreased. Only slight changes in the response can be observed upon increasing its concentration to 30%.

The surface morphology of pellets cured at 40 °C for 24 hr ($S_1$) as shown in Figure 2a is smooth and uniform. Surface morphology of the pellets cured at 60 ˚C for 168 hr ($S_2$) as shown in Figure 2b, is rough and small pores and voids could be observed.

After generating polynomial equations for representing the effect of formulation variables, the process was optimized for response $Y_1$. In order to find the optimized conditions different experiments was performed. Based on previous study, optimization was done with limitation of the drug release, i.e. cumulative percentage released in 3 hr of dissolution of 35%. Some batches were made and

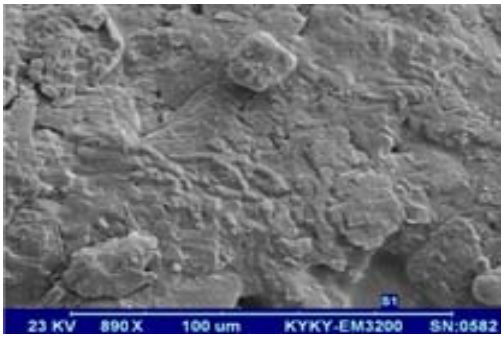


**Figure 2a.** SEM of pellets containing 20% plasticizer, cured at 40 ˚C for 24 hr with a magnification 890 × ($S_1$)

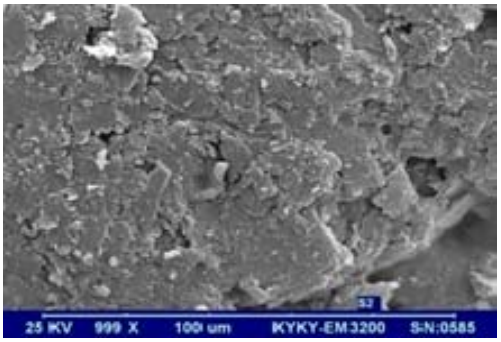


**Figure 2b.** SEM of pellets containing 20% plasticizer, cured at 60 ˚C for 168 rh with a magnification 999 × ($S_2$)

tested (9). Therefore, the optimized conditions for 35% drug released in 3 hr were attained at the level of plasticizer concentration 20% w/w, curing temperature 55 °C and curing 24 hr time.

To check the validity of the optimization procedure, a new batch of diclofenac pellets coated with the predicted levels of formulation factors was prepared. The obtaned results showed that the optimized formulation prepared according to computer-determined levels ensured the release profile which was close to the predicted values.

## Discussion

The dependent variables were cumulative percent released of drug within 3, 4 and 6 hr based on previous studies (9, 10). The limits for the responses were based on the Box-Behnken design, the interaction between factor caused different diclofenac release rates.

Previous studies showed that the dissolution profiles were very low and independent of composition and thickness of coating in acidic medium. In this medium, the main limiting factor was very poor solubility of diclofenac sodium (9). These are correlated with the results in this study.

On the other hand, in phosphate buffer dissolution rates were much higher and very sensitive to any changes in composition of the polymer film and curing condition. This is correlated with the previous studies in the literatures (10-13).

The results showed that predicted values were compared with the observed values and were found to be in good agreement. The results indicated that $X_1$ has negative effect but $X_2$ and $X_3$ have positive effect on the response $Y_1$ (data not shown). In other words, by increasing the amount of plasticizer in the coating polymer, we obtained a remarkable effect in delaying the release of diclofenac. On the contrary, increasing the curing temperature and time, cause more drug release from the pellets.

The interaction between plasticizer concentration and curing time in model (X1X3) indicated that increasing the amount of plasticizer and curing time led to increase in cumulative percentage values of diclofenac sodium dissolved in a determined time.

The possible explanation is that increasing the curing time causes more plasticizer evaporation. Therefore the drug release increases by losing the quality of the mechanical properties of the polymer film. More plasticizer concentration results in more curing time influence on drug release rate.

The previous studies showed that the loss of plasticizer TEC with further curing cause losing the quality of some physical and mechanical properties including the weight and thickness of polymethacrylic film (10-13). From the results, it was suggested that pellets cured for longer times would have imbibed less water after immersion than pellets cured for shorter times (13).

Plasticizer loss could also lead to the formation of molecular pores and voids within the polymeric film coatings that could act as an alternate path for the passage of drug from film-coated pellets. This could result in the higher rate of drug release that was observed for the Eudragit® RS coated pellets cured for longer times at higher curing temperatures (13).

Further curing resulted in extensive loss of plasticizer (TEC), leading to an increase in the glass transition temperature (Tg) (14, 15). The increase in Tg rendered the film samples brittle and weak (15).

The model can also further explain the relationship between the dependent and independent variables when their values are shifted from lower to higher level.

The possible explanation for this behavior is that there is an incomplete formation of the polymer film with poor mechanical properties at low plasticizer concentration, which results in faster drug release. The second possible reason may be found in TEC influence on hydrophobicity of the films. Surface morphology confirmed that the polymer particles has coalesced and inter-diffused, indicates that it has cured over T*g*.

## Conclusion

The results of this study allow us to conclude that this formulation of diclofenac may be useful for future pharmaceutical application.

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