



Research Article

## Optimization of Controlled Release Matrix Formulations of the Chronobiotic Hormone Melatonin *via* Experimental Design

Marilena Vlachou\*, Angeliki Siamidi, Sofia Konstantinidou and Yannis Dotsikas

### Abstract

Melatonin (MT) is a chronobiotic hormone synthesized by the pineal gland and has a significant role in the regulation of the circadian biological clock. In order to be administered as a drug, the time of melatonin release from its formulation is of great significance. To this purpose, controlled release matrix tablets (200 mg) of MT were developed by using D-optimal experimental design, including 1 categorical and 2 numerical factors, aiming at effecting its sustained release using a USP XXIII dissolution apparatus II at pH 1.2 and 7.4 media. The excipients selected are polyvinyl pyrrolidone (M.W.: 10.000 and 55.000), hydroxylpropyl Methylcellulose K15M and lactose monohydrate. The *in vitro* release data were fitted to the Korsmeyer-Peppas empirical equation; the *n* exponent, which refers to release kinetics, was evaluated. The optimal composition was reached *via* the desirability function, as a compromise to the different responses (the time for 50% drug dissolution at pH=1.2 and the diffusional exponent (*n*) at pH values 1.2 and 7.4). As the differences in the *n* values amongst the formulations are closely related to variation in water penetration into the different excipients used, excipients with different physicochemical characteristics were used so that the desired formulation was produced. Overall, this procedure resulted in a suitable excipients' composition for the controlled release of melatonin with the minimal number of experiments.

### Keywords

Melatonin; Controlled release; Experimental design; Formulation; Matrix tablets

### Introduction

The pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine) is an important component in the regulation of seasonal and circadian rhythms [1]. Its action is believed to be mediated through a family of specific, high-affinity, G-protein-coupled cell membrane receptors. The secretion of the hormone is closely synchronized with the habitual hours of sleep in humans. Ingestion of melatonin affects sleep propensity, duration and quality of sleep. Human studies have

also indicated that increasing serum melatonin concentrations can trigger the onset of sleep [2]. However, the use of melatonin as a drug is hampered by its short biological half-life and poor bioavailability. As a result, dosage forms, which mimic the physiologically secreted melatonin concentration versus time model, are limited [3].

In our previous studies, matrix tablets of melatonin were tested with respect to their ability to fully release melatonin in a controlled manner within 8 h [4]. This time-frame is critical since melatonin's release in a quick initial pace is needed for treating sleep onset problems, while its release at a relatively slow initial pace, aims at improving sleep quality and/or sleep maintenance.

Until recently, scientists used to design new drug formulations by modifying the levels of one factor at a time (OFAT), while keeping all the rest constant. This strategy usually requires a large number of experimental runs and ignores any potential interactions among the factors. As a result it becomes quite costly and expensive, whilst in some cases it has been proven ineffective. Conversely, a more organized way of conducting experiments could be cost-effective and time saving and also reveal some critical interactions amongst the examined factors. The Design of Experiments (DoE), which has been applied to many fields, including tablets' formulation, has been well documented [5-10]. Performing a small number of preliminary experiments, DoE can provide detailed analysis of complex systems and resolve problems, which cannot be easily managed by the trial and error approach, with a minimal number of experiments.

Among the various types of designs, the D-optimal design has been established as a robust design strategy. It is an alternative to the classical factorial designs strategy, allowing the assessment of both numerical and categorical factors [11]. Furthermore, numerical factors are examined at many different levels (design matrix), which are created automatically by computer algorithms in order to satisfy the D-optimality criterion. D-optimal designs are constructed to minimize the generalized variance of the estimated regression coefficients without increasing the total number of experimental runs.

The aim of the current study was to produce improved melatonin controlled release formulations with the following characteristics: T50% (pH: 1.2)  $\leq$  150 min, so that an initial dose will be released to aid the sleep onset of patients, and *n* (pH:1.2)=0.89, in order to achieve zero order release kinetics and Case II diffusion, and *n* (pH:7.4)=0.80 for first order release kinetics and anomalous diffusion. To this purpose, a group of specific excipients were studied at ranges set by the preliminary experiments. In order to assess both numerical and categorical factors, in a limited number of experiments, the D-optimal design was selected in combination with Response Surface Methodology (RSM). The optimal settings for melatonin release, taking into account the aforementioned responses, were reached by utilizing the Derringer's desirability function.

### Experimental

#### Materials

*N*-acetyl-5-methoxytryptamine was purchased from Sigma Aldrich Co. (St. Louis, U.S.A.). As for excipients, polyvinylpyrrolidone (M.W.: 10.000-low and 55.000-high) and hydroxy propyl

\*Corresponding author: Marilena Vlachou, Department of Pharmacy, National and Kapodistrian, Department of Pharmacy, Division of Pharmaceutical Technology, Panepistimioupoli-Zografou, 84 Athens, Greece, Tel: +30 210 7274674; E-mail: vlachou@pharm.uoa.gr

Received: November 03, 2016 Accepted: December 02, 2016 Published: December 11, 2016

methylcellulose K15M were purchased from Sigma Aldrich Co. (St. Louis, U.S.A.), while lactose monohydrate and magnesium stearate were obtained from E. Merck (Darmstadt, Germany).

### Methods

**D-optimal experimental design:** A series of preliminary experiments took place, in order to define the significant factors in tablets composition. In the end, 1 categorical and 2 numerical factors were selected for optimization. The M.W. of polyvinylpyrrolidone (PVP) was chosen as a categorical factor (M.W.: 10.000-low and 55.000-high), since a significant differentiation was observed when a low M.W. PVP was replaced by a higher M.W. PVP. The two numerical factors selected were the mass (mg) of PVP and Hydroxypropyl Methylcellulose K15M (HPMC). As responses reflecting the best tablet characteristics criteria, the time for 50% drug dissolution (T50%) at pH=1.2 and the diffusional exponent (*n*) at pH values 1.2 and 7.4, were chosen. Table 1 presents the plan of 17 experiments suggested by the D-optimal Design along with the obtained responses (Table 1).

**Tablet preparation:** Each matrix tablet was comprised of melatonin (2 mg) and combinations of the following excipients: Hydroxypropyl Methylcellulose (HPMC) K15M, low viscosity sodium alginate, lactose monohydrate, polyvinylpyrrolidone (PVP) – M.W.: 10.000 or 55.000 and magnesium stearate as lubricant. All

the components (except the lubricant) were blended in a mixing apparatus (Wab Turbula Type T2F) for 10 min (32 rpm). Magnesium stearate was then added to the mixture and blended for 2 more min. The total weight of each tablet, irrespectively of their composition, was 200 mg. The flat tablets were produced using a 10 mm diameter die and a hydraulic press (Maassen type, MP 150). The weights (in mg) of sodium alginate and lactose were estimated for each experiment (Table 2).

**Tablet characterisation:** Tablet average weight, crushing strength and friability were determined on 10 samples for each batch using respectively an electronic balance, a hardness tester (Erweka, type TBH28) and a friabilator (Erweka, type TA3R) at 25 rpm for 4 min. The crushing strength is expressed in kp and values between 8-10 kp were considered acceptable. The friability is reported in terms of weight loss and has been calculated as percentage of the initial weight; according to Pharmacopeia specifications, friability under 1% was considered acceptable.

**Dissolution experiments:** The tablets were stirred at 50 rpm in a USP XXIII dissolution apparatus II (Pharmatest, Hainerp, Germany) containing 450 mL of either gastric (pH 1.2) or intestinal-like fluids (pH 7.4) at 37 ± 0.5°C. Samples (5 mL) were withdrawn at predetermined time intervals (every 30 min for the first 180 min and every 60 min thereafter), filtered and analyzed at λ max=278 nm using a Perkin–Elmer UV spectrophotometer (Norwalk, CT).

Table 1: Plan of experiments defined by D-optimal design and responses for each experiment.

| Exp. No | Factor A<br>PVP (mg) | Factor B<br>HPMC (mg) | Factor C<br>PVP M.W. | T50% (min)<br>pH=1.2 | n<br>pH=1.2 | n<br>pH=7.4 |
|---------|----------------------|-----------------------|----------------------|----------------------|-------------|-------------|
| 1       | 26.00                | 27.76                 | low                  | 155                  | 2.04        | 0.72        |
| 2       | 0.00                 | 16.33                 | high                 | 147                  | 1.12        | 0.70        |
| 3       | 0.00                 | 32.00                 | low                  | 144                  | 0.75        | 0.84        |
| 4       | 16.75                | 10.41                 | low                  | 143                  | 2.03        | 0.78        |
| 5       | 52.00                | 32.00                 | low                  | 135                  | 2.78        | 0.70        |
| 6       | 41.88                | 16.00                 | low                  | 172                  | 2.38        | 0.87        |
| 7       | 25.29                | 0.00                  | high                 | 142                  | 2.78        | 0.70        |
| 8       | 52.00                | 32.00                 | low                  | 135                  | 1.24        | 0.71        |
| 9       | 52.00                | 15.52                 | high                 | 136                  | 1.67        | 0.57        |
| 10      | 52.00                | 0.00                  | low                  | 144                  | 1.40        | 0.57        |
| 11      | 26.60                | 32.00                 | high                 | 147                  | 2.40        | 0.81        |
| 12      | 25.29                | 0.00                  | high                 | 142                  | 2.78        | 0.70        |
| 13      | 30.82                | 0.00                  | low                  | 152                  | 2.24        | 0.71        |
| 14      | 26.29                | 16.00                 | high                 | 145                  | 2.22        | 0.76        |
| 15      | 0.00                 | 0.00                  | low                  | 128                  | 0.73        | 0.81        |
| 16      | 52.00                | 15.52                 | high                 | 136                  | 1.65        | 0.57        |
| 17      | 0.81                 | 32.00                 | high                 | 147                  | 0.76        | 0.88        |

Table 2: Ingredients of melatonin matrices.

| Ingredients            | Quantity (%) |  | Amount (mg) |   |
|------------------------|--------------|--|-------------|---|
|                        | 1            | 2  | 1           | 2   |
| Melatonin              | 1            |  | 2           |   |
| PVP (low or high M.W.) | 0-26         | Boundaries:<br>Quantity <sub>PVP (low or high MW)</sub> + Quantity <sub>(Lactose monohydrate)</sub> = 26 | 0-52        | Therefore:<br>Amount <sub>PVP (low or high MW)</sub> + Amount <sub>Lactose monohydrate</sub> = 52 |
| Lactose monohydrate    | 0-26         |  | 0-52        |   |
| HPMC                   | 0-16         | Boundaries:<br>Quantity <sub>HPMC</sub> + Quantity <sub>Sodium Alginate</sub> = 72                       | 0-32        | Therefore:<br>Amount <sub>HPMC</sub> +<br>Amount <sub>Sodium Alginate</sub> = 144                 |
| Sodium Alginate        | 61-72        |  | 112-144     |   |
| Mg. Stearate           | 1            |  | 2           |   |
| Total                  | 100%         |  | 200         |   |

**Curve fitting release profile:** The *in vitro* release data were fitted to the Korsmeyer-Peppas Eq. (1),

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

Where,  $M_t$  and  $M_\infty$  express the absolute cumulative amount of drug released at time  $t$  and infinite time, respectively;  $k$  is the release rate constant and  $n$  is the diffusion coefficient. This equation is only valid for the first 60% of the fractional release [12]. The values assumed by the  $n$  exponent represent either Fickian or anomalous (non-Fickian) release kinetics. For the case of cylindrical tablets, in particular,  $n \leq 0.45$  corresponds to a Fickian diffusion release (case I diffusional),  $0.45 < n < 0.89$  to an anomalous transport, and  $n = 0.89$  to a zero-order (case II) release kinetics.

## Results and Discussion

The responses obtained from the experiments conducted, following the plan derived from the D-optimal design, are presented in Table 1. For all responses a quadratic model was suggested as the most adequate. The general form of the quadratic response model is given by the Eq. (2).

$$y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 \quad (2)$$

The calculated coefficients of the response model for coded factor levels are given in Table 3. All models had *Adj. R<sup>2</sup>* values > 0.75 and non-significant lack of fit ( $p > 0.05$  for 95% confidence level). The results of Analysis of Variance (ANOVA) show the statistical significance of the factors studied on the responses. For instance, as shown in Table 3, PVP M.W., (PVP mg)<sup>2</sup> and the 2-way interaction (PVP mg\*HPMC mg) are significantly affecting the T50% (pH:1.2) value. It is critical to mention that interactions evaluated as statistically significant ( $p < 0.05$ ) would have been ignored if the OFAT strategy was adopted. Three-dimensional response surface plots, depicted in Figures 1-3, as graphical representations of the obtained models, show each response T50% (pH:1.2)  $n$  (pH:1.2) and  $n$  (pH:7.4) as a function of the excipients (HPMC, PVP high and low MW).

In order to decipher the global optimal conditions, the multi objective optimization (MOOP) technique, via the Derringer's desirability function, was employed to optimize the three responses with different targets [13]. More specifically, the target for T50%  $n$  (pH:1.2) was the values maximization in the range 145-172 min, for  $n$  (pH:1.2) the value 0.89, while for  $n$  (pH:7.4) maximization of the

response. By adjusting the importance coefficients T50% (pH:1.2) was set as the most important response for consideration, whilst the rest 2 of equal importance), weights and range of responses, according to defined objectives, the optimal solution for tablet composition was produced (Figure 4). The global desirability was 0.907, which was considered as a very satisfactory value.

Based on the optimal solution suggested by the desirability function (PVP high M.W., PVP 0.36 mg and HPMC 26.09 mg) and taking into account the constant sums described in Table 2 (PVP + lactose and HPMC + sodium alginate) the optimal tablet composition (total weight: 200 mg) was as described in Table 4. This composition was tested (dissolution profile in Figure 4) and the obtained values for responses T50% (pH:1.2)=149 min,  $n$  (pH:1.2)=0.89 and  $n$  (pH:7.4)=0.80 were in accordance with the ones predicted (Figure 5). This outcome proves the quality of the design utilized in the current application and the significance of DoE for the development of novel drug formulations (Figures 1-5 and Table 4)

As previously mentioned, one of the problems related with sleep is its onset. This dysfunction is better treated by the use of melatonin formulations, like the one suggested above. This formulation could enable the initial dose to act in less than 150 min.

The value of the release exponent obtained by the experimental design,  $n$  (pH:1.2)= 0.89, indicates Case II transport, which is a process of moving boundaries and linear kinetics (zero order). Case II relaxation transport is controlled by polymer swelling. The use of HPMC is known to contribute to tablets' swelling, the extent of which depends on the pH value of the medium [14-16]. In our case (optimal formulation) this difference in the matrix swelling, caused mainly by HPMC, was not seriously affected by the pH of the medium, although a slight drop in swelling in the acidic environment could be deduced from Figure 2.

The obtained value of  $n$  (pH:7.4)=0.80 markedly exceeds the value of 0.45 for diffusion controlled systems and indicates significant contribution of erosion. The erosion and degradation of the polymer, lead to the breakdown of the chains of the polymer and the loss of material from the whole initial bulk of the polymer. During the anomalous transport, processes that also take place are polymer swelling and polymer and drug dissolution.

Through the use of DoE and mathematical models, the number of experiments needed to optimize the desired drug-release profile was

**Table 3:** Coefficients of quadratic response model for T50% at pH=1.2,  $n$  at pH values 1.2 and 7.4, along with  $p$ -values indicating the significance of the associated factors as shown in Equation 2.

|          | T50%<br>pH=1.2 | p-value | n<br>pH=1.2 | p-value             | n<br>pH=7.4 | p-value |
|----------|----------------|---------|-------------|---------------------|-------------|---------|
| $b_0$    | 155.44         | 0.0408* | 2.33        | 0.0197*             | 0.77        | 0.0420* |
| $b_1$    | -0.51          | 0.8489  | 0.40        | 0.0297 <sup>a</sup> | -0.083      | 0.0142* |
| $b_2$    | 1.82           | 0.4802  | -0.078      | 0.5987              | 0.052       | 0.0437* |
| $b_3$    | -3.50          | 0.0462* | 0.098       | 0.3988              | -0.024      | 0.2550  |
| $b_{12}$ | -7.29          | 0.0208* | 0.17        | 0.3997              | 0.016       | 0.6411  |
| $b_{13}$ | -4.91          | 0.0893  | -0.11       | 0.4741              | -0.011      | 0.6852  |
| $b_{23}$ | +0.26          | 0.9176  | -0.17       | 0.2677              | 0.019       | 0.4731  |
| $b_{11}$ | -11.14         | 0.0225* | -1.05       | 0.0019*             | -0.065      | 0.1480  |
| $b_{22}$ | -9.13          | 0.0577  | 0.057       | 0.8193              | 0.00027     | 0.9513  |

\*Significant model terms at 95% confidence level.

reduced. It was possible to use a semi-empirical approach to achieve zero-order release of a water-soluble drug through multi-step diffusion. The Korsmeyer-Peppas mathematical model indicated non-Fickian diffusion at both pH values and zero and first order drug-release kinetics. As previously stated [17,18], the differences in the  $n$  values between the formulations can be due to variation in water penetration into the different excipients used. In this investigation it has been demonstrated that when the dissolution medium penetrates into the swellable matrices, the polymer particles swell resulting to matrix volume and drug release kinetics changes, according to the characteristics of the incorporated excipients. From this investigation the optimal formulation technique was found to give a robust product with good modified released characteristics.

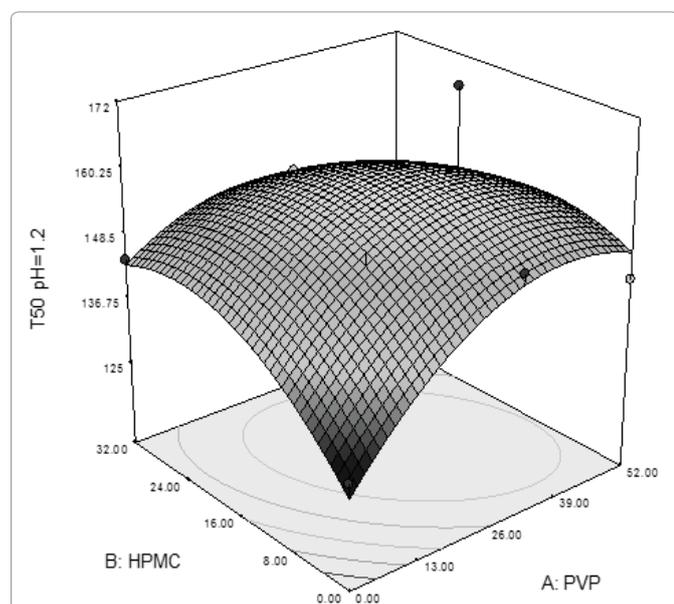


Figure 1: 3D graph  $T_{50}$  =  $f$ (HPMC content, PVP content) for high PVP M.W.

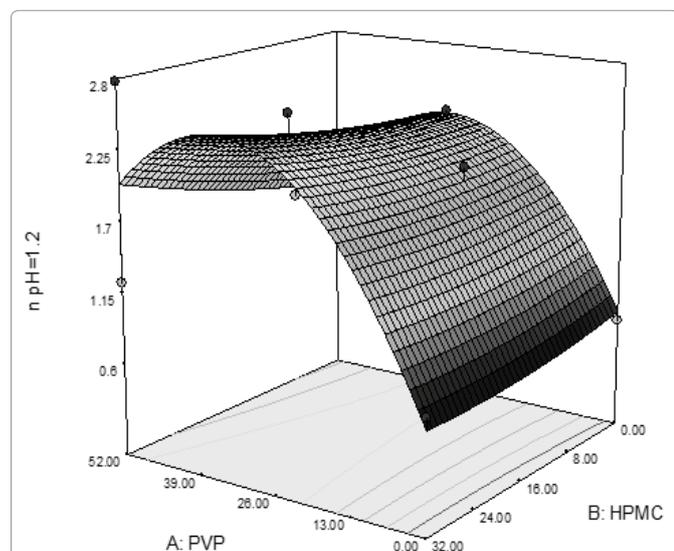


Figure 2: 3D graph  $n$  (pH=1.2) =  $f$ (HPMC content, PVP content) for low PVP M.W.

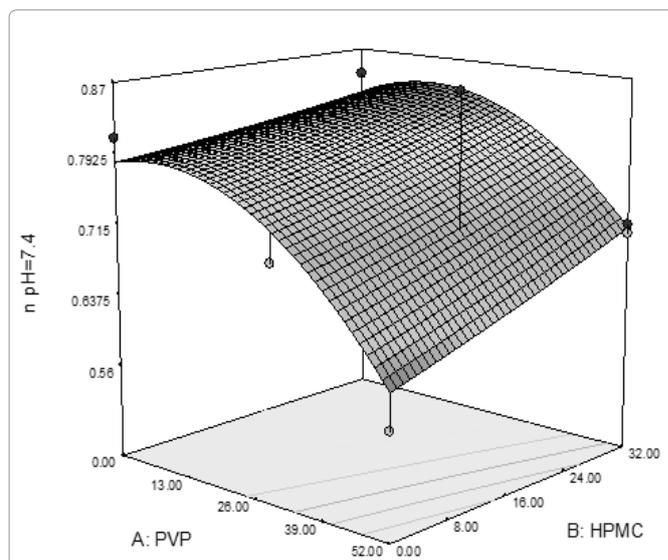


Figure 3: 3D graph  $n$  (pH=7.4) =  $f$ (HPMC content, PVP content) for high PVP M.W.

Table 4: The optimal solution for tablet composition (D=0.907).

| PVP (mg) | HPMC (mg) | PVP M.W. | Lactose (mg) | Sodium alginate (mg) | Magnesium stearate (mg) | Melatonin (mg) |
|----------|-----------|----------|--------------|----------------------|-------------------------|----------------|
| 0.36     | 26.09     | High     | 51.64        | 117.91               | 2                       | 2              |

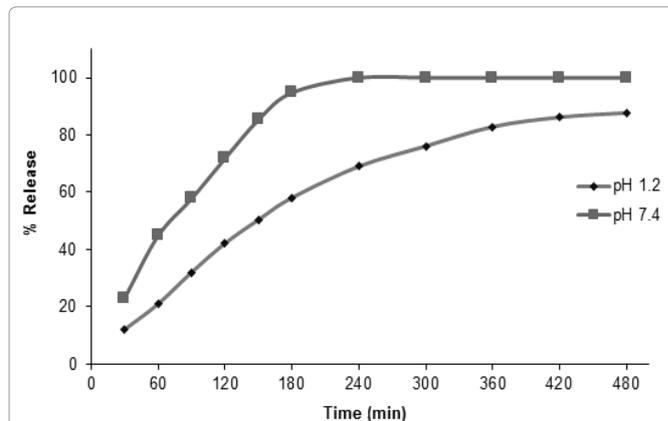


Figure 4: Drug release (%) vs. time - (pH 1.2 and 7.4).

## Conclusion

The current study confirms the usefulness of experimental design in optimizing tablets composition, in order to achieve suitable release characteristics of melatonin. Both categorical and numerical factors, related with excipients, were examined via the D-optimal design for the melatonin tablets. The optimal solution reached by the multi-objective optimization technique, via the Derringer's desirability function, resulted in tablets with the requisite properties in both acidic and basic media. This formulation is expected to be effective against sleep onset problems, as well as dysfunctions related to the duration of sleep. Information about the oral absorption profile of melatonin from the optimal formulation presented herein will be very useful for future *in vivo* release studies.

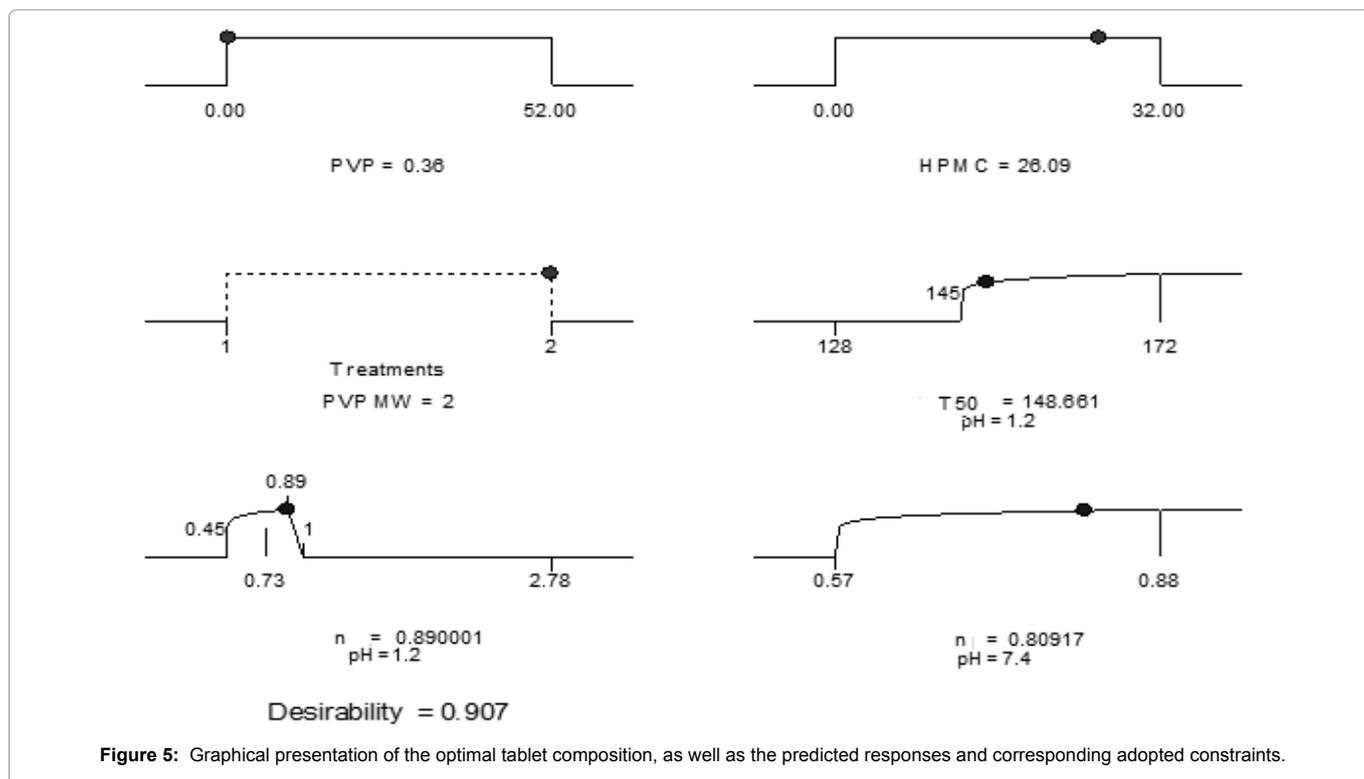


Figure 5: Graphical presentation of the optimal tablet composition, as well as the predicted responses and corresponding adopted constraints.

#### Declaration of Interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

#### References

- Zlotos DP (2005) Recent advances in melatonin receptor ligands. *Arch Pharm Chem Life Sci* 338: 229-247
- Turek FW, Gillette MU (2004) Melatonin, sleep and circadian rhythms: rationale for development of specific melatonin agonists. *Sleep Med* 5: 523-532
- Kumar A, Agarwal SP, Khanna R (2003) Modified release bi-layer tablet of melatonin using  $\beta$ -cyclodextrin. *Pharmazie* 58: 642-644.
- Vlachou M, Tsiakoulia A, Eikospentaki A (2007) Controlled release of the pineal hormone melatonin from hydroxypropylmethylcellulose/sodium alginate matrices in aqueous media containing dioctyl sulfosuccinate. *Curr Drug Discov Technol* 4: 31-38
- Vatsaraj N, Zia H, Needham T (2002) Formulation Optimization of a Sustained-Release Tablet of Ketorolac Tromethamine. *Drug Delivery* 9: 153-159
- Choi du H, Shin S, Khoa Viet Truong N, Jeong SH (2012) A new experimental design method to optimize formulations focusing on a lubricant for hydrophilic matrix tablets. *Drug Dev Ind Pharm* 38: 1117-1127.
- Furlanetto S, Cirri M, Maestrelli F, Corti G, Mura P (2006) Study of formulation variables influencing the drug release rate from matrix tablets by experimental design. *Eur J Pharm Biopharm* 62: 77-84.
- Martinello T, Kaneko TM, Velasco MV, Taqueda ME, Consiglieri VO (2006) Optimization of poorly compactable drug tablets manufactured by direct compression using the mixture experimental design. *Int J Pharm* 322: 87-95.
- Dey S, Mahanti B, Khila S, Mazumder B, Gupta SD (2012) Formulation development and optimization of bilayer tablets of aceclofenac. *Expert Opin Drug Deliv* 9:1041-1050.
- Vatsaraj N, Zia H, Needham T (2002) Formulation and Optimization of a Sustained-Release Tablet of Ketorolac Tromethamine. *Drug Deliv* 9:153-159.
- Vemić A, Rakić T, Malenović A, Medenica M (2015) Chaotropic salts in liquid

chromatographic method development for the determination of pramipexole and its impurities following quality-by-design principles. *J Pharm Biomed Anal* 102: 314-320.

- Korsmeyer RW, Doelker GFP, Peppas NA (1983) Mechanism of potassium chloride from compressed, hydrophilic, polymeric matrices: effect of entrapped air. *J Pharm Sci* 72:1189-1191.
- Malenović A, Dotsikas Y, Mašković M (2011) Desirability-based optimization and its sensitivity analysis for the perindopril and its impurities analysis in a microemulsion LC system. *Microchem J* 99: 454-460.
- Huang Y, Khanvilkar KH, Moore AD, Hilliard-Lott M (2003) Effects of Manufacturing Process Variables on In Vitro Dissolution Characteristics of Extended-Release Tablets Formulated with Hydroxypropyl Methylcellulose. *Drug Dev Ind Pharm* 29: 79-88.
- Vlachou M, Nassef H, Efentakis M, Tarantili P, Andreopoulos AG (2001) Swelling properties of various polymers used in controlled release systems. *J Biomater Appl*, 15, 4: 293-306.
- Akash MSH, Iqbal F, Raza M, Rehman K, Ahmed S, et al. (2013) Characterization of Ethylcellulose and Hydroxypropyl Methylcellulose Microspheres for Controlled Release of Flurbiprofen. *J Pharm Drug Deliv Res* 2:1
- Solinis MA, De la Cruz Y, Hernandez RM (2002) Release of ketoprofen enantiomers from HPMC K100M matrices diffusion studies. *Int J Pharm* 239: 61.
- Efentakis M, Vlachou M, Choulis NH (1997) Effects of excipients on swelling and drug release from compressed matrices. *Drug Dev Ind Pharm* 23: 107.

#### Author Affiliations

Top

Department of Pharmacy, Cipla Limited, National and Kapodistrian University of Athens, Athens, Greece