

# Determination of naproxen with solid substrate room temperature phosphorimetry based on an orthogonal array design

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## Abstract

A solid substrate room temperature phosphorimetric method (SSRTP) for the determination of naproxen in pharmaceutical products was developed. The experimental conditions were optimized by a  $L_{25}(5^6)$  orthogonal array design (OAD) with five factors at five levels using statistical analysis. The five factors contained pH value of the sample solution, drying time ( $t_d$ ) and drying temperature ( $T_d$ ) of solid substrate paper in the oven, concentration ( $c_{I^-}$ ) of heavy atom ( $I^-$ ), and exposure time ( $t_e$ ) of solid substrate paper after being dried. The pH value,  $t_d$ ,  $c_{I^-}$  and  $t_e$  had significant influences on the measurement of phosphorescence intensity. The optimization for sample preparation improved greatly the analytical performance of SSRTP. Under the optimal conditions, naproxen can be determined in a linear range from 10 to 400  $\text{ng ml}^{-1}$  with a detection limit of 2.7  $\text{ng ml}^{-1}$  at  $3\sigma$ . The method has been applied satisfactorily to the determination of naproxen in a commercial product. © 2003 Elsevier B.V. All rights reserved.

**Keywords:** Room temperature phosphorescence; Solid substrate; Naproxen; Orthogonal array design

## 1. Introduction

Naproxen, (+)-2-(6-methoxy-2-naphthyl)propionic acid, is extensively used in non-steroidal anti-inflammatory cures such as the treatment of rheumatoid arthritis, dysmenorrhea and acute gout [1]. Commercial formulation naproxen has been determined by UV spectrophotometry [2–4], high-performance liquid chromatography [5–7], fluorimetry [8] and mass fragmentography [9]. Recently, two groups have used room temperature phosphorimetry to determine naproxen in fluid solutions and achieved competitive simplicity and detection limit comparing to other methods [10–12].

Phosphorimetry is sensitive and selective for the determination of many kinds of compounds, but it was rarely used before 1970 because phosphorescence must be observed at a low temperature (77 K). Since the room temperature phosphorimetry (RTP) was proposed in 1970s, this technique has been widely applied both in fluid solutions and on solid substrates [13–15]. In fluid solutions, phosphorescent analytes are restricted to organized media, such as micelle and

cyclodextrin system, to decrease the collision between phosphors or phosphor and other molecules, which increases the phosphorescence intensity of analytes. When the analytes are absorbed on solid substrates to avoid the collision and increase their phosphorescence intensity, this technique is called as solid substrate room temperature phosphorimetry (SSRTP). SSRTP is a very attractive method because of its simplicity, speed and economical character. In SSRTP, several factors significantly influence the determination of phosphorescence intensity. These factors include the pH value of the sample solution, the concentration of heavy atom, the drying time ( $t_d$ ) and temperature ( $T_d$ ) of solid substrate paper and the exposure time ( $t_e$ ). The exposure time means the time that the substrate paper was exposed in atmosphere after it was dried and cooled in a sealed quartz tube.

In SSRTP, the ionization of analyte molecules is one of the essential conditions under which the analyte molecules show a strong adsorption on solid surface [16]. Thus, pH value of the sample solution plays an important role in the phosphorescence intensity determination of the analyte. Previous studies have already showed that the radiationless deactivation of the triplet state increases as the amount of heavy-atom salts increases, and the heavy atoms can increase the intensity of room temperature phosphorescence ( $I_{\text{RTP}}$ ) of the analytes [17]. To our knowledge, the salts containing heavy

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atoms such as I, Br, Tl, Ag and Pb have been used in SSRTP experiments [16–20]. Among these salts, KI and  $\text{TlNO}_3$  are frequently used because of their high efficiency to increase the radiationless deactivation of the triplet state [18,19]. To obtain intense and stable room temperature phosphorimetric signal, the concentration of heavy-atom salt should be sufficiently high. Moisture is another experimental factor affecting the RTP of a compound adsorbed on substrate paper [20]. The effect of moisture is attributed to the disruption of hydrogen bond formed between the analyte molecule and the substrate paper, which results in an additional detrimental effect and allows the transport of oxygen into the vicinity of the phosphor. In order to maintain the rigidity required for preventing collision and vibration quenching of triplet state, drying time, drying temperature and exposure time of the substrate paper must be optimized.

Orthogonal array design (OAD), also known as Taguchi design, is believed to incorporate the advantages of simplex method and factorial design [21–24]. It arranges different factors for effective optimization of experimental conditions. The results of the OAD experiment can be statistically treated by two ways: analysis of variance (ANOVA) and direct observation analysis [25,26]. In ANOVA, the effects of different factors on response functions can be evaluated by both significance ( $F$  ratio) and PC% (percentage contribution) value. In other words, the importance of a factor or the interaction among different factors can be estimated from the significance and PC% value. Direct observation analysis is also called range analysis. The fluctuation range and tendency of response functions versus the levels of different factors can be directly observed from a broken line plot. From ANOVA and direct observation analysis of experimental results, factors that significantly affect the output responses can be found and optimal parameters for an analytical procedure can be obtained. The use of OAD can simplify the experiment procedure without affecting the quality of results. Adopted as a chemometric method, OAD has been widely applied for the optimization of analytical procedures in recent years [21–24]. Here, for the first time, we use OAD in room temperature phosphorescence for the condition optimization of SSRTP and establish a novel method for the determination of naproxen.

In the present study, a  $L_{25}$  ( $5^6$ ) OAD procedure is performed with five factors at five levels that are important influence parameters in the phosphorescence intensity determination of SSRTP. This method has been used for naproxen determination in a commercial product with a satisfactory result.

## 2. Experimental

### 2.1. Reagents

Analytical grade reagents were used for the preparation of all solutions. Naproxen and potassium iodide were

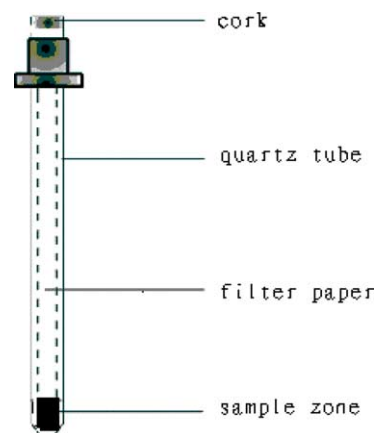


Fig. 1. Scheme of a quartz tube fitted with a strip of solid substrate paper.

purchased from Shanghai Chemical Plant (Shanghai, China). Whatman no. 3 substrate paper was from Merck Chemical Co. (Darmstadt, Germany). Freshly double-distilled water was used throughout experiments.

### 2.2. Instruments

SSRTP was performed on a Hitachi 650 spectrofluorimeter (Hitachi Ltd., Tokyo, Japan) with a low temperature phosphorescence accessory. For all phosphorescence measurements, the excitation monochromator slit width and emission monochromator slit width were set to 10 and 15 nm, respectively. The experimental data of the OAD were statistically analyzed with SAS 8.1 software.

### 2.3. Procedures

Whatman no. 3 substrate paper was exposed under an ultraviolet lamp for 8 h to lower its background signal [27]. The paper was cut to strips with an appropriate size (6.5 mm  $\times$  150 mm) to be fit in a quartz tube (Fig. 1). Stock solution of naproxen ( $1.0 \times 10^{-4}$  M) was prepared in 0.5 M KI ethanol/water 50:50 (v/v) solution. Working solutions were obtained by appropriate dilutions of the stock solution with a 0.5 M KI ethanol/water 50:50 (v/v) solution. The solution pH was adjusted to 9.5 using 0.1 M NaOH.

A strip of solid substrate paper was immersed in a beaker that contained a height of 1.0 cm sample solution for 1.0 min. The immersed paper strip was then dried in an oven for 3.0 min at 120 °C. After the resulting paper was swiftly put into a dried quartz tube and sealed up with a cork in the oven, it was cooled down to room temperature and then the phosphorescence measurement was carried out. The signal was detected at a  $\lambda_{\text{ex}}$  of 340 nm and a  $\lambda_{\text{em}}$  of 522 nm.

### 2.4. OAD

A  $L_{25}$  ( $5^6$ ) OAD was used in the present experimental procedure to search the optimal conditions for obtaining the

Table 1  
Attribution of factors to the levels in  $L_{25}$  ( $5^6$ ) OAD experiments

Level	pH	$t_d$ (min)	$c_{I^-}$ (M)	$T_d$ ( $^{\circ}$ C)	$t_e$ (min)
1	7.0	1	0	80	0
2	9.5	2	0.050	90	0.5
3	13	3	0.10	100	1
4	2.0	5	0.50	110	2
5	4.5	7	1.00	120	3

Table 2  
 $L_{25}$  ( $5^6$ ) OAD matrix with the experimental results

No.	pH	$t_d$ (min)	$c_{I^-}$ (M)	$T_d$ ( $^{\circ}$ C)	$t_e$ (min)	Dummy	Average $I_{\text{RTP}}$ ( $n = 3$ )
1	7.0	1	0	80	0	1	6.29
2	7.0	2	0.050	90	0.5	2	7.41
3	7.0	3	0.10	100	1	3	6.32
4	7.0	5	0.50	110	2	4	7.61
5	7.0	7	1.0	120	3	5	7.38
6	9.5	1	0.050	100	2	5	2.54
7	9.5	2	0.10	110	3	1	9.78
8	9.5	3	0.50	120	0	2	22.8
9	9.5	5	1.0	80	0.5	3	18.5
10	9.5	7	0	90	1	4	8.45
11	13	1	0.10	120	0.5	4	3.75
12	13	2	0.50	80	1	5	4.28
13	13	3	1.0	90	2	1	7.89
14	13	5	0	100	3	2	6.08
15	13	7	0.050	110	0	3	9.75
16	2.0	1	0.50	90	3	3	2.85
17	2.0	2	1.0	100	0	4	15.2
18	2.0	3	0	110	0.5	5	9.60
19	2.0	5	0.050	120	1	1	4.53
20	2.0	7	0.10	80	2	2	4.53
21	4.5	1	1.0	110	1	2	3.53
22	4.5	2	0	120	2	3	3.50
23	4.5	3	0.050	80	3	4	7.77
24	4.5	5	0.10	90	0	5	16.3
25	4.5	7	0.50	100	0.5	1	14.2

maximum phosphorescence intensity. The design involved five factors at five levels as shown in Table 1. These factors were pH value of the sample solution, drying time ( $t_d$ ) and drying temperature ( $T_d$ ) of solid substrate paper in the oven, concentration ( $c_{I^-}$ ) of heavy atom ( $I^-$ ), and exposure time ( $t_e$ ) of solid substrate paper after being dried. As shown in Table 2, the chosen  $L_{25}$  ( $5^6$ ) array has 25 rows (24 degrees of freedom) with six columns at five levels. Each of the 25 experiments was performed in triplicate, corresponding to a total of 75 tests. The sixth column is a dummy factor. Its levels can be called arbitrary levels of the dummy variance.

### 3. Results and discussions

#### 3.1. Spectral characteristic

Fig. 2 shows the phosphorescence spectrum of naproxen on a solid substrate filter paper, which was obtained at room

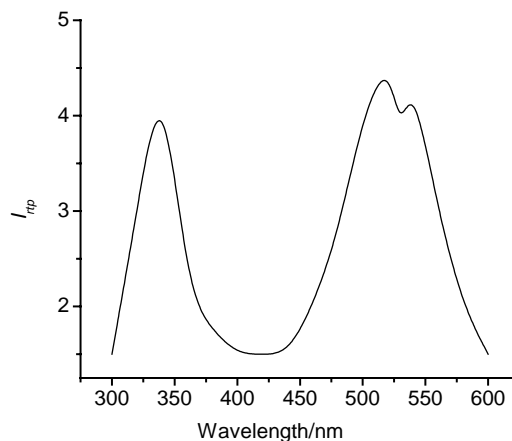


Fig. 2. Phosphorescence spectrum of naproxen on a substrate filter paper in presence of 0.5 M  $I^-$  at room temperature ( $25^{\circ}$ C) and  $\lambda_{\text{ex}}$  of 340 nm.

temperature ( $25^{\circ}$ C) in the presence of  $I^-$ . The RTP spectrum of naproxen displays strong phosphorescence at  $\lambda_{\text{ex}}$  of 340 nm and  $\lambda_{\text{em}}$  of 522 nm. Different instrumental parameters can seriously affect the phosphorescence response, this work sets the excitation monochromator slit width and emission monochromator slit width at 10 and 15 nm, respectively, the gate time at 1 ms and the chopping speed at “high” (rapid) position.

#### 3.2. ANOVA analysis of the OAD

The aim of the OAD was to select the optimal pH value,  $t_d$ ,  $c_{I^-}$ ,  $T_d$  and  $t_e$  for obtaining the maximum  $I_{\text{RTP}}$ . The factors and response function ( $I_{\text{RTP}}$ ) were given in Table 2. Table 3 shows the significance ( $F$  ratio) analysis, it can be seen that pH,  $t_d$ ,  $c_{I^-}$  and  $t_e$  are statistically significant at  $P < 0.01$ ,  $T_d$  is insignificant at  $P < 0.1$ . These results are further confirmed by the analysis of percentage contribution (PC%) contained in Table 3. The PC% is calculated by Eq. (1):

$$\text{PC\%} = \frac{SS'}{SS_{\text{total}}} \times 100 \quad (1)$$

Table 3  
ANOVA analysis and percent contribution for  $I_{\text{RTP}}$  in the  $L_{25}$  ( $5^6$ ) OAD matrix

Source of variation	SS	Degree of freedom	Mean square	$F^a$	SS'	PC%
pH	119	4	29.8	53.0	117	17.8
$t_d$ (min)	163	4	40.7	72.4	161	24.5
$c_{I^-}$ (M)	74.4	4	18.6	33.1	72.1	11.0
$T_d$ ( $^{\circ}$ C)	1.91	4	0.478	0.851	–	–
$t_e$ (min)	295	4	73.8	131	293	44.7
Error	2.25	4	0.562	–	13	1.98
Total	656	24	–	–	656	100

<sup>a</sup> The critical  $F$  value is 4.11 ( $P < 0.1$ ), 6.39 ( $P < 0.05$ ) and 16.0 ( $P < 0.01$ ).

Table 4  
The sum ( $K$ ) and average ( $k$ ) at each level of a factor and range of  $I_{\text{rtp}}$

	pH	$t_d$ (min)	$c_{\text{I}^-}$ (M)	$T_d$ ( $^{\circ}\text{C}$ )	$t_e$ (min)
$K_1$	35.0	19.0	33.9	41.4	70.3
$K_2$	62.1	40.2	32.0	42.9	53.5
$K_3$	31.8	54.4	40.7	44.3	27.1
$K_4$	36.7	53.0	51.7	40.3	26.1
$K_5$	45.3	44.3	52.5	42.0	33.9
$k_1$	7.00	3.79	6.78	8.27	14.1
$k_2$	12.4	8.03	6.40	8.58	10.7
$k_3$	6.35	10.9	8.14	8.87	5.42
$k_4$	7.34	10.6	10.4	8.05	5.21
$k_5$	9.06	8.86	10.5	8.39	6.77
Range	6.05	7.11	3.72	0.82	8.89

where  $SS'$  is the purified sum of squares and is given by  $SS - SS_{\text{error}}$ ,  $SS$  is the individual sum of squares,  $SS_{\text{error}}$  is the sum of squares of error and  $SS_{\text{total}}$  is the total sum of squares. It should be noted that the value of  $SS'_{\text{total}}$  is equal to  $SS_{\text{total}}$  in statistics, while  $SS'_{\text{error}}$  is obtained from the difference of  $SS_{\text{total}}$  and the sum of  $SS'$  of the considered factors. The results indicate  $t_e$  is the most important factor contributing to the  $I_{\text{rtp}}$  response (44.7%) and then followed by  $t_d$  (24.5%), pH (17.8%),  $c_{\text{I}^-}$  (11.0%) and  $T_d$  (0%). Obviously, the data analysis of PC% is in good agreement with the conclusion obtained from the significance analysis discussed above.

### 3.3. Direct observation analysis of the OAD

Except ANOVA, the results of the OAD experiment can also be statistically treated by direct observation analysis to evaluate the effect or the importance of a given factor. In direct observation analysis of the OAD, the sum ( $K$ ) and average ( $k$ ) of  $I_{\text{rtp}}$  at each level of a factor were calculated from the data listed in Table 2 and the results are listed in Table 4. Fig. 3 shows the dependence of  $k$  on the levels of investigated factors.

#### 3.3.1. Effect of pH on $I_{\text{rtp}}$

Fig. 3a indicates the effect of pH on  $k$  is very considerable. In this work, the influence of pH on the SS RTP intensity of naproxen was studied by adding different amounts of HCl and NaOH to adjust the solution pH. With a carboxyl group, naproxen can be ionized in weak alkaline solution and hydrogen bond with substrate filter paper can be formed, thus intense room temperature phosphorescence can be observed, while  $k$  decreases sharply in strong acidic solution due to the protonation of ketonic oxygen of naproxen. Contrarily, the strong alkaline can destroy the fiber construction of the substrate paper, which results in the decrease of  $k$ . The maximum  $k$  occurs at pH 9.5. Thus, pH 9.5 is selected as an optimal condition for following experiments.

#### 3.3.2. Dependence of $I_{\text{rtp}}$ on the concentration of heavy atom

Fig. 3b shows the relation between the  $k$  and  $\text{I}^-$  concentration. The  $k$  value increases with the increasing concentration of  $\text{I}^-$  and reaches a constant value at the  $\text{I}^-$  concentration of 0.5 M. Thus,  $\text{I}^-$  concentration is selected at 0.5 M for the determinations of naproxen.

#### 3.3.3. Effects of drying temperature, drying time and exposure time on $I_{\text{rtp}}$

Fig. 3c–e show the effects of  $t_d$ ,  $T_d$  and  $t_e$  of the substrate paper on  $k$ , respectively. The variation ranges of  $k$  with the changes of  $t_d$  and  $t_e$  are 7.11 and 8.89, respectively, which are much bigger than 0.82 resulted from the  $T_d$  change. In other words,  $t_d$  and  $t_e$  have significant influences on the  $k$ , while  $T_d$  has weaker influence. Although the influence of  $T_d$  on the  $k$  is insignificant in this work, it could not conclude that  $T_d$  does not affect the determination of  $I_{\text{rtp}}$  in every circumstance due to a limited span of  $T_d$  (80–120  $^{\circ}\text{C}$ ). From the direct observation analysis of these figures, following conclusions can be obtained. (1) The  $t_d$  must be sufficiently long for removing moisture from the substrate paper, but overlong  $t_d$  may affect the properties of the analyte compounds and destroy the fabric construction of the substrate paper.

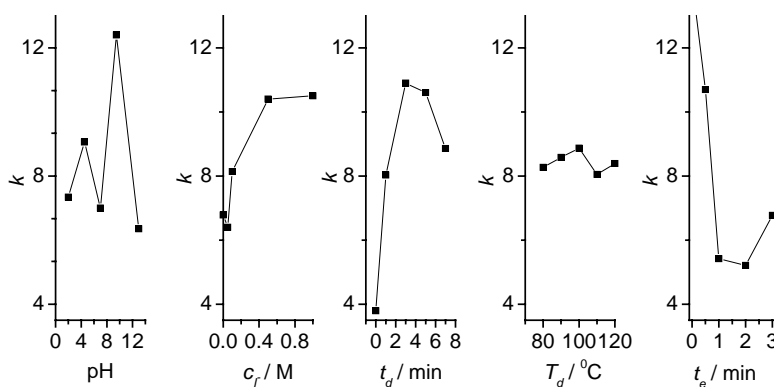


Fig. 3. Effect of factors on  $k$ : (a) effect of pH on  $k$ ; (b) dependence of  $k$  on  $\text{I}^-$  concentration; (c) plot of  $k$  vs. drying time; (d) relation between  $k$  and drying temperature; (e) effect of exposure time on  $k$ .

(2) The  $t_e$  of the substrate paper after being dried should be as short as possible for avoiding the adsorption of moisture on the paper from atmosphere. (3)  $T_d$  can be set at a high level, which accelerates the experimental procedure. Meanwhile, carefulness must be taken for avoiding detriment of the analyte compounds and paper construction at a higher temperature.

Above results show that the optimal conditions for the determination of naproxen by SSRTP are as follows: pH adjusted to 9.5,  $I^-$  concentration to 0.5 M,  $t_d$  to 3 min,  $T_d$  to 120 °C and  $t_e$  as short as possible.

### 3.4. Analytical application

$I_{\text{rtp}}$  of a series of standard solutions were determined under the optimal conditions. A regression line was then plotted for  $\log(I_{\text{rtp}} - I_{\text{rtp}}^0)$  versus  $\log c$ , where  $c$  is the concentration of naproxen in  $\text{ng ml}^{-1}$ ,  $I_{\text{rtp}}^0$  is the background signal. The linear range was found to be between 10 and 400  $\text{ng ml}^{-1}$  with a regression equation of  $\log(I_{\text{rtp}} - I_{\text{rtp}}^0) = 4.95 + 0.893 \log c$  and a correlation coefficient of 0.9991. The detection limit was 2.7  $\text{ng ml}^{-1}$  at  $3\sigma$ .

The method has been applied to the determination of naproxen in a commercial product, Antalgin 550 (Sintex Latino, Spain), with a nominal content of 673  $\text{mg g}^{-1}$ . In the assay procedure of naproxen in Antalgin 550, 10 capsules were taken, powdered and homogenized. The powder obtained was dissolved in water and aliquots of these solutions were treated according to the procedure shown in 2.3. The relative standard deviation of the results was 2.21%. The average recovery of the proposed method was 98.75%. These results were satisfactory. Thus, the method presented possessed good precision and accuracy.

## 4. Conclusions

This paper applies an orthogonal array design (OAD) to optimize the determination conditions of naproxen in pharmaceutical products with solid substrate room temperature phosphorimetric method. The pH value of the sample solution, drying time, concentration of  $I^-$  and exposure time of solid substrate paper after being dried have significant influences on the measurement of phosphorescence intensity based on the results of the OAD experiment statistically treated by ANOVA and direct observation analysis. Comparing to the previously proposed methods for the determination of naproxen [2–12], the new method is competitively rapid, simple and precise. It means that OAD can be a useful

tool for optimizing the determination conditions of room temperature phosphorimetry.

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