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Use of highly efficient Draper–Lin small composite designs in the formal optimisation of both operational and chemical crucial variables affecting a FIA-chemiluminescence detection system

Laura Gámiz-Gracia *, Luis Cuadros-Rodríguez, Eva Almansa-López, Jorge J. Soto-Chinchilla, Ana M. García-Campaña

Department of Analytical Chemistry, School of Qualimetrics, University of Granada, E-18071 Granada, Spain

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Abstract

A new formal strategy in the multidimensional optimisation of the experimental variables affecting the chemiluminescence (CL) detection in flow injection analysis (FIA) is proposed here. The strategy implies several steps, being the most significant: selection of the variables to be studied and their experimental domain; use of a screening design to detect significant variables and interactions into the experimental region; study of the main effect of variables and second-order interactions; and finally application of a Draper–Lin small composite design (orthogonal) to obtain the optimum values of the significant variables. The methodology is applied to the determination of methylamine by FIA based on the use of the peroxyoxalate CL (PO-CL) reaction. Considering the high number of experiments required due to the different chemical and instrumental variables to be taken account and their adequate compatibility to obtain maximum sensitivity, the methodology offers a rigorous study of the main effects and interactions, achieving a reduction of experimental work.

Keywords: Optimisation; Draper–Lin small composite designs; Peroxyoxalate chemiluminescence; Flow injection analysis

1. Introduction

Chemiluminescence (CL) is a high sensitive analytical technique that permits kinetic measurements, since CL emission is not constant but varies with time, as the light flash is composed of a signal which increases after reagent mixing, passing through a maximum, then declining to the baseline. Thus, the analytical signal can be obtained from the measurement of the CL emission at a strictly defined period from the moment of reagent mixing [1]. Flow injection analysis (FIA) is an

* Corresponding author. Tel.: +34-958-248-593; fax: +34-958-249-510.
E-mail address: lgamiz@ugr.es (L. Gámiz-Gracia).

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advantageous methodology in the application of kinetic techniques, as it allows us the mixing of analyte and reagents in a constant flow-rate, controlling the measurement time in a very reproducible way [2]. Due to its dynamic characteristics, analytical signals obtained from a FIA-manifold are transitory, as a result of the short resident-time of the analyte in front of the detection cell. Thus, FIA signals are peak-shaped and can be quantified in terms of both height and peak area, like in chromatographic analysis.

The optimisation of two types of variables is mandatory in an analytical FIA-method: (i) those variables inherent to the FIA-manifold, such as flow rate of the different reagents, mixing reactor length and sample injection volume; and (ii) chemical variables involved in the reaction, such as pH, ionic strength, composition of the carrier and concentration of the different reagents. The traditional one-at-time univariate strategy has been usually employed in the optimisation of those FIA variables, being a time-consuming approach (as a high number of experiments are required) that can not assure accurate conclusions, as possible interaction between the different variables, both FIA and chemical ones, are not taken into account [3]. By contrast, the proper use of formal optimisation techniques based on experimental designs to model and predict the analytical signal can avoid these drawbacks. However, very few examples of this methodology in the optimisation of FIA systems have been found in the literature [4–10].

In the coupling of FIA with CL detection it is also necessary to combine the kinetic requirements of the CL response, which depend on factors such as concentration and nature of reagents, pH, temperature, composition and ionic strength of the carrier, with the dynamic requirements of the FIA system. Optimum sensitivity is achieved by controlling flow rates, mixing/reaction and detection point distance, and characteristics of the detection cell, with the aim of obtaining the observed portion of emission profile at the maximum of the CL intensity-versus-time profile (see Fig. 1). For this reason, a great number of experimental factors should be simultaneously optimised, considering their possible interactions

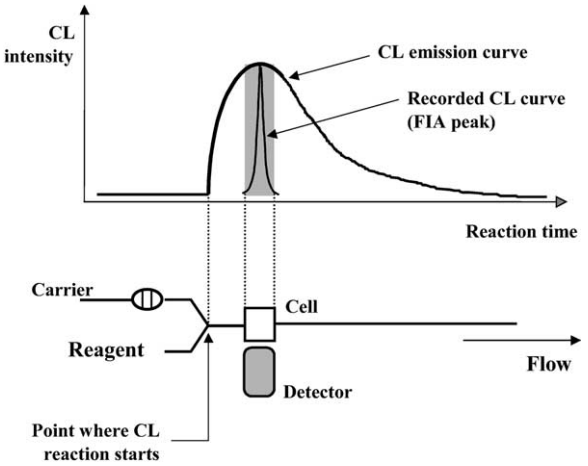


Fig. 1. Observed portion of the emission profile in a CL–FIA system.

and effects [11]. However, the use of experimental design in the optimisation of FIA-CL systems has not been commonly reported [12,13].

In this paper, we propose a methodology for the simultaneous optimisation of both operational and chemical variables involved in the determination of methylamine (MA) using the peroxyoxalate CL (PO-CL) reaction, based on the previous formation of a fluorescent derivative with *ortho*-phthalaldehyde (OPA) and mercaptoethanol (MEt) in alkaline medium [14]. Bis(2,4,6-trichlorophenyl) oxalate (TCPO) is oxidised by hydrogen peroxide in the presence of imidazole (IMZ) as a catalyst and a high energy intermediate, 1,2-dioxetane-3,4-dione, forms a charge transfer complex with the fluorophore, donating one electron to the intermediate, which is transferred back to the fluorophore raising it to an excited state and liberating an emission typical for the nature of this fluorescent derivative [15]. The formal application of the proposed methodology in this system comprises the following steps: (i) selection of the different influent factors and delimitation of the experimental domain; (ii) application of a two level design (fractional saturated or Plackett–Burman design) which allows us the study of the selected experimental region, as a previous screening of

the significant factors of the CL detection; (iii) establishment of some univariate experiments for the differentiation of the main effects of significant variables from the effect of the second order interactions confounded with them; (iv) application of a three level design (Draper–Lin small composite design) to model the CL response as a function of the significant factors and interactions selected in the previous step; (v) location of the optimum values predicted by the model; and (vi) application of a sequential strategy of design contractions for the verification of the optimum experimental values, if necessary. This proposed methodology could be easily applied in the optimisation of different FIA systems, following the different steps and selecting the proper variables, which will depend on each particular problem. In this sense, this work is included in a research project about the chemiluminescent determination of carbamates, which under certain conditions can generate methylamine by hydrolysis, being the aim of further experiments to implement this detection system in CE and HPLC for the determination of these compounds.

2. Draper–Lin small composite designs

Only one reference has been found in relation to the application of small composite designs for optimisation purposes in Analytical Chemistry [16]. In this sense, an introduction about the basical aspects and the application of this design is presented in this part. Further information is included in the original papers from Draper and Lin [17,18].

For the establishment of a quadratic model that describes a multivariate system, it is necessary to carry out experiments where the different variables are studied at three different levels ($l \geq 3$ where l is the number of levels), although a higher number of levels would be more reliable. Box, Wilson and Hunter [19,20] developed the use of composite designs obtained by adding extra star-points ($2k$ points, where k is the number of variables) and central-points (c represents the number of central points and its value is decided by the user [21]) to two-level full (2^k) or fractionated (2^{k-f}) factorial designs (f, fraction; usually $f=0$ if $k<5$; $f=1$ if $5 \leq f \leq 7$, $f=2$ if $k>7$). These five level designs ($l=5$) are able to be fitted to quadratic polynomial models from a reduced number of experiments, N ($N=2^{k-f}+2k+c$) (see Table 1).

The efficiency of an experiment, ϕ, is a parameter which measures the needed 'experimental work' in relation to the achieved 'mathematical aim', and it can be calculated from the quotient between the number of coefficients of the model to be fitted, p, and the minimum number of different required experiments to complete the design, $N_{\min}$, that is, $\phi = p/N_{\min}$, where $p = 1/2(k+1)(k+2)$ for quadratic polynomial equations. The value of ϕ must be equal to 1 ($p = N_{\min}$, maximum efficiency) or higher than 1 ($p > N$), but lower efficiency will be obtained as ϕ value is more different to 1. In addition, and independently on the design efficiency, it is convenient to include some replicate experiments (1–3) with the aim of evaluating statistically the quality of the fit.

Composite designs are extensively used because of their high efficiency, although it decreases as the number of variables increases, even using some fractions from the factorial design (see Table 1). With the purpose of increasing their efficacy, several strategies have been carried out to reduce the number of points of the factorial design, which constitutes the 'design skeleton' (named 'cube-portion' in this article), obtaining by this way the so-called 'small composite designs'. Among the different strategies, Draper and Lin [17,18], have presented an attractive proposal to find the needed points of the 'cube-portion' based on the removal of columns of two-level Plackett–Burman designs [22,23]. These designs show efficiency close to 1 (Table 1), and can be easily increased by addition of central points in relation to the degrees of freedom required for the evaluation of the model and/or the need of establishing an orthogonal design.

The Draper and Lin approach is based on the following steps: (i) calculate the minimum number of points, m, required for the cube-portion, given by $m = p - 2k$; (ii) start from a two-level Plackett–Burman design with a number of experiments equal to or higher than m; (iii) select k columns

Table 1
Efficiency of some composite designs excluding central points

	Number of variables, k						
	2	3	4	5	6	7	8
Number of coefficients of the quadratic polynomial model, p	6	10	15	21	28	36	45
Number of runs in B–W designs[a] (efficiency, ϕ_{B-W})	8 (1.33)	14 (1.40)	24 (1.60)	42 (2.00)	78 (2.79)	142 (3.94)	272 (6.04)
Number of runs in B–H designs[b] (efficiency, ϕ_{B-H})	–	–	–	26 (1.24)	44 (1.57)	78 (2.17)	80 (1.77)
Number of runs in D–L designs[c] (efficiency, ϕ_{D-L})	–	10 (1.00)	16 (1.07)	21 (1.00)	28 (1.00)	36 (1.00)	46 (1.02)

[a] Box–Wilson complete composite designs.
[b] Box–Hunter fractional composite designs.
[c] Draper–Lin small composite designs.

of the original Plackett–Burman design and remove the rest; (iv) in case of duplicate rows, remove one for each duplication; (v) establish the cube-portion with the rest of rows; and (vi) add the corresponding experiments to the selected star and centred points, obtaining the definitive Draper–Lin small composite design (see Table 2).

In this paper, we propose the use of a Draper–Lin small composite design for the optimisation of four variables (Fig. 2) that influence the CL emission using the PO-CL system for the detection of methylamine after derivatisation with OPA. The quadratic equation for four variables includes 15 coefficients (an independent term, four quadratic terms, four linear terms and six interaction terms). Considering that the number of star-points needed is 8 (twice than the number of variables), at least seven points are required for the cube-portion of the design.

From a two-level Plackett–Burman design with eight experiments (for seven variables), the columns 1, 2, 3 and 6, are selected, removing the three others (columns 4, 5 and 7), and obtaining a two-level design for four variables. This design does not show any replicated row and so, the cube-portion is constructed with the eight experiments. The corresponding star-points are adding to this design (with $\alpha = 1.414$), which is completed with two central points (in order to maintain the orthogonal condition).

Considering that these designs are currently implemented in some statistical software [24], they could be easily applied for optimisation purposes in Analytical Chemistry.

3. Experimental

3.1. Apparatus

CL measurements were carried out on a Jasco CL 1525 detector (Jasco Corporation), equipped with a PTFE spiral detection cell, data control and acquisition programme. Two Gilson Minipulse-3 (Gilson) peristaltic pumps, two Rheodyne 5020 manual injection valves (Rheodyne, L.P.), and Omnifit tubing and connections were used for constructing the FIA manifold in Fig. 3 [25].

3.2. Chemicals

A 500 mg l^{-1} OPA solution (Sigma-Aldrich) was prepared weekly by adding 0.05 g of OPA, 1 ml methanol (Panreac), 5 ml borate buffer 0.1 M, pH 9.0 (Sigma-Aldrich) and 0.1 ml of 2-mercaptoethanol (Sigma-Aldrich Química S.A.) to a 100 ml volumetric flask, diluting to the mark with deionised water [26]. A 2 M stock solution of imidazole (Sigma-Aldrich) was prepared weekly in water and proper working solutions were prepared daily in sodium dihydrogen phosphate buffer

Table 2
Construction of Draper–Lin small composite designs (excluding central points)

	Number of variables, k							
	2	3	4	5	6	7	8	
Number of coefficients of the quadratic polynomial model (p)	6	10	15	21	28	36	45	
Number of star points ($s=2k$)	4	6	8	10	12	14	16	
Minimal number of need points in the cube portion ($p-k$)	2	4	7	11	16	22	29	
Number of runs in the P–B design[a] (N_{P-B})	–	4	8	12	16	24	36	
Number of columns (factors) in the P–B design (k_{P-B})	–	3	7	11	15	23	35	
Selected columns from the P–B design (k)	–	All	1, 2, 3, 6	1, 2, 3, 9, 11	1, 2, 3, 4, 5, 14	1, 2, 5, 6, 7, 9, 10	1, 3, 4, 6, 8, 10, 16, 17	
Number of duplicated runs (d)	–	0	0	1	0	2	6	
Minimal number of runs in the D–L design[b] ($N_{D-L}=s+N_{P-B}-d$)	–	10	16	21	28	36	46	

[a] Plackett–Burman two-level designs.
[b] Draper–Lin small composite designs.

(Panreac) 0.1 M and used as catalyst. A working solution of proper concentration of hydrogen peroxide (from 30% p/v solution, Panreac) was prepared daily in 0.1 M sodium dihydrogen phosphate buffer and used as oxidant. A working solution of TCPO (Wako Pure Chemical Industries) was prepared daily in acetonitrile (Panreac). Methylamine (MA) hydrochloride (Sigma-Aldrich) was heated at 110 °C for 30 min and then placed in a calcium chloride desicator until room temperature. Then, a standard solution of 1 g l^{-1} was prepared in methanol (Panreac). Methylamine (MA) hydrochloride working solution of 5 mg l^{-1} was prepared daily by dilution with methanol, which corresponds to a concentration of 2.3 mg l^{-1} of methylamine.

All the reagents or solvents were of analytical reagent or HPLC grade. Deionised water (Milli-Q Plus 185, Millipore) was used for the experimental work.

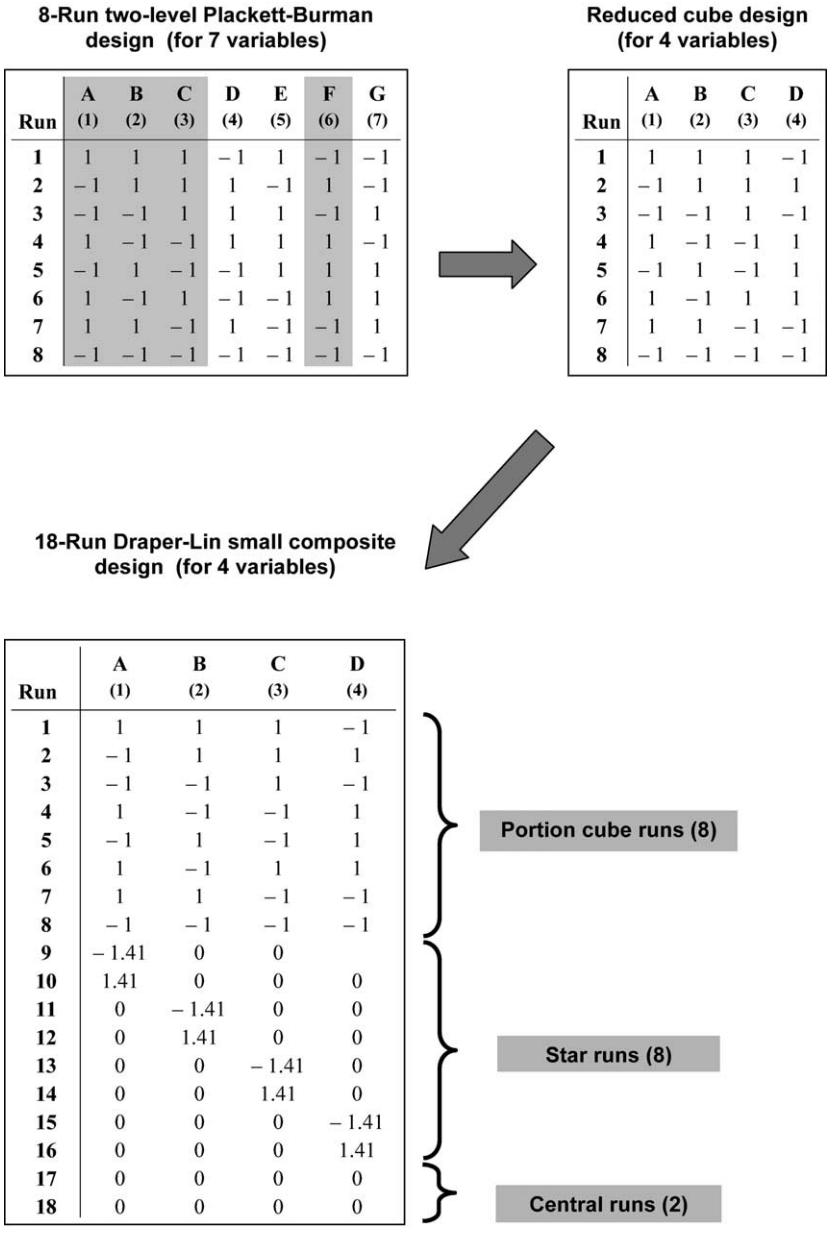
3.3. Procedure

3.3.1. Derivatisation reaction

The labelling reaction of MA was developed off-line. A volume of 4 ml of the MA hydrochloride working solution was mixed with 4 ml of OPA solution and placed in an ultrasound bath for 1 min. The resulting solution was injected into the carrier in the FIA system. The blank was prepared and measured in the same way, substituting methanol for the MA solution.

3.3.2. Measurement procedure

TCPO solution of proper concentration was injected manually using valve 1 (500 μl loop) in the FIA manifold (see Fig. 3) and the labelled analyte (or blank) was injected using valve 2 (100 μl loop). Both valves were then turned to the 'inject' position, beginning with valve 1 and with a difference of 5 s between both injections. After incorporating into the buffer carrier stream, the TCPO and the labelled analyte were mixed in a reaction coil (50 cm length, 0.5 mm i.d.), being subsequently merged with the imidazole and hydrogen peroxide streams, achieving the production of the chemiluminescent



Run	A (1)	B (2)	C (3)	D (4)	E (5)	F (6)	G (7)
1	1	1	1	−1	1	−1	−1
2	−1	1	1	1	−1	1	−1
3	−1	−1	1	1	1	−1	1
4	1	−1	−1	1	1	1	−1
5	−1	1	−1	−1	1	1	1
6	1	−1	1	−1	−1	1	1
7	1	1	−1	1	−1	−1	1
8	−1	−1	−1	−1	−1	−1	−1

Run	A (1)	B (2)	C (3)	D (4)
1	1	1	1	−1
2	−1	1	1	1
3	−1	−1	1	−1
4	1	−1	−1	1
5	−1	1	−1	1
6	1	−1	1	1
7	1	1	−1	−1
8	−1	−1	−1	−1

Run	A (1)	B (2)	C (3)	D (4)
1	1	1	1	−1
2	−1	1	1	1
3	−1	−1	1	−1
4	1	−1	−1	1
5	−1	1	−1	1
6	1	−1	1	1
7	1	1	−1	−1
8	−1	−1	−1	−1
9	−1.41	0	0	
10	1.41	0	0	0
11	0	−1.41	0	0
12	0	1.41	0	0
13	0	0	−1.41	0
14	0	0	1.41	0
15	0	0	0	−1.41
16	0	0	0	1.41
17	0	0	0	0
18	0	0	0	0

Fig. 2. Construction of an orthogonal 18-run (four-variables) Draper–Lin small composite designs (used in the experimental optimisation study of this paper) from an eight-run (seven-variables) two-level Plackett–Burman design.

emission in the detection cell just in front of the photomultiplier. All the testing solutions (blank and solutions containing the derivatives of MA with OPA) were injected by triplicate. The net signal was then calculated as the difference between the average height from the signals corresponding to the MA solution and those corresponding to the blank.

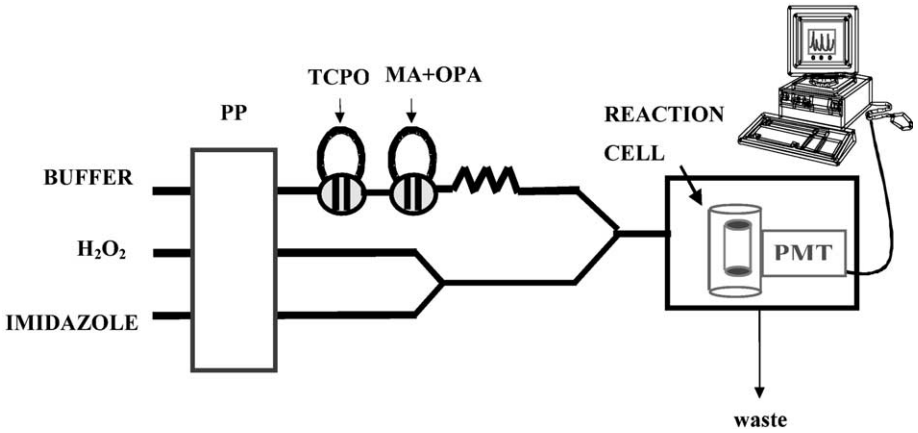


Fig. 3. Proposed manifold. PMT, photomultiplier tube; PP, peristaltic pump; TCPO, bis(2,4,6-trichlorophenyl) oxalate.

4. Results and discussion

4.1. Optimisation

The optimisation of the overall method was developed in four steps, namely: (i) selection of variables and delimitation of the experimental region; (ii) selection of the carrier flow rate; (iii) screening of the significant variables concerning the FIA system and estimation of their effects; (iv) optimisation of the significant variables.

4.1.1. Selection of variables and delimitation of the experimental region

Those variables affecting the FIA system (i.e. concentration of the reagents and flow rates) were optimised following a formal strategy based on sequential experimental designs. The different experimental regions were selected taking into account the chemical requirements for the reaction and including those values usually found in the bibliography for each variable. The selected variables and values are showed in Table 3.

Table 3
Experimental values for variables in the 2^{7-4} fractional factorial screening design

Variables	Levels		
	−1	0	+1
(A) log[peroxide] (M)	−2	−1	0
(B) log[Imidazole] (M)	−3	−2	−1
(C) [TCPO] (M)	5×10^{-4}	1.5×10^{-3}	2.5×10^{-3}
(D) pH	5	7	9
(E) Imidazole flow rate (ml min^{-1})	0.25	2	3.75
(F) Peroxide flow rate (ml min^{-1})	0.25	2	3.75
(G) Dummy	–	–	–

4.1.2. Selection of the carrier flow rate

As a previous step, the flow rate of the carrier (sodium dihydrogen phosphate buffer 0.1 M) was optimised separately, as this variable is the only one affecting the time between the turnings of both injection valves. This flow rate was studied in the range between 1 and 4 ml min^{-1}. A flow rate of 3 ml min^{-1}, with an interval of 5 s between both injections, was finally selected because of the best signals, in terms of both sensitivity (signal height) and precision (relative standard deviation) were obtained at these conditions.

The optimisation of the rest of variables affecting the FIA system was developed by applying a formal strategy based on sequential experimental designs.

4.1.3. Screening of the significant variables affecting the FIA system

First of all, a 2^{7-4} fractional factorial screening design was carried out, selecting the variables and levels showed in Table 3. A 'dummy' variable was necessary to complete the design. Once the design was carried out, the total effects of the different

Table 4
Estimated effects for the variables affecting the CL signal (from a 2^{7-4} fractional factorial screening design)

Variable	Total effect[a]	Significant
(A) log[peroxide]+BD+CE	52	Yes
(B) log[imidazole]+AD+CF	−103	Yes
(C) [TCPO]+AE+BF	−19	Non
(D) pH+AB+EF	−49	Yes
(E) Imidazole flow rate+AC+DF	−35	Non
(F) Peroxide flow rate+BC+DE	−738	Yes
(G) Dummy+AF+BE+CD	37	Yes

Standard deviations, S.D., are based on pure error with 2 degrees of freedom. (Effects and S.D. are expressed in arbitrary units of the CL signal).

[a] Standard deviation = ±12.8.

variables as well as their second order interactions were evaluated considering the corresponding total effect estimated, shown in Table 4.

4.1.3.1. Effect of main variables. All those variables (and confounded second order interactions) whose total effect estimated was lower than 5% of the absolute value of the highest effect (which corresponds to the total effect of peroxide flow rate, that is $737.51 \times 0.05 = 36.88$) were considered as non-significant. Thus, TCPO concentration, imidazole flow rate and second order interactions, which are confounded with them (see Table 4) were considered as non significant variables and the values corresponding to their '0 level' were selected for subsequent studies, except in the case of TCPO concentration, where a concentration of 1×10^{-3} M was selected, as higher concentrations produced precipitation.

4.1.3.2. Second order interaction effects. In the previous screening study, the total effect of each variable is confounded with second order interactions of the rest of the variables. In the case of the peroxide flow rate (F), the effect is confounded with the second order interactions, log[imidazole]–[TCPO] (BC) and pH-imidazole flow rate (DE). As the variables C and E were considered as non-significant, their second order interactions were also considered non significant, so the total effect estimated was due to the effect of the main variable, that is the peroxide flow rate. For the purpose of elucidating if the total effect of the other variables considered as significant was due to the variable itself or to second order interactions, a deeper study of the effects was carried out. In this sense, an univariate study of the significant variables (namely: log[imidazole], log[peroxide] and pH) was carried out, which consisted of measuring the CL signal at three different levels for each variable (see Table 3), keeping the rest of the variables constant at the '0 level'. The main sided effects were then calculated as:

Sided-up effects: variation in the response when the value of the studied variable is changed from the '0' to the '+1' level:

$$E(+) = y(+1) - y(0).$$

Sided-down effects: variation in the response when the value of the studied variable is changed from the '0' to the '−1' level:

$$E(-) = y(-1) - y(0).$$

where $y(+1)$, $y(-1)$ and $y(0)$ are the response when the variable is in the +1, −1 and 0 level, respectively. Thus, the main total effects were calculated as:

$$E = E(+1) + E(-1)$$

The estimated effects are shown in Table 5. Those sided effects are both deviations that are caused by logarithmic changes of the unit from the zero value (that is, a factor of ten in concentration up and down). These differences in the concentration could explain why the sided-up and sided-down effects are so different.

Table 5
Estimated effects for significant variables confounded with second order interactions (univariate study)

Variable	Positive effect	Negative effect	Total effect
(A) log[peroxide]	−587	106	−481
(B) log[imidazole]	−241	119	−122
(D) pH	−149	363	214

Effects and S.D. are expressed in arbitrary units of the CL signal.

Once the main total effect of the significant variables has been estimated, the effects of the second order interactions are estimated too, as the difference between the total effect obtained from the screening design and that one obtained from the single study of each significant variable. At this point it must be remembered that the second order interactions AE, BF, AC and DF were considered as non-significant in the screening design. Also, the significance of the 'dummy' variable indicates that at least, one of the second order interactions confounded with this variable (AF+BE+CD) is significant. The obtained results for the remaining second order interactions are shown in Table 6.

Following the same criteria than in the case of the main studied variables, those second order interactions whose estimated total effect is lower than 5% of the absolute value of the total effect of peroxide flow rate, are considered as non-significant. In this sense, the variables and interactions finally considered as significant were: log[peroxide], log[imidazole], pH, peroxide flow rate, and the second order interactions log[peroxide]–log[imidazole] (AB), log[imidazole]–pH (BD) and log[peroxide]–peroxide flow rate (AF). These variables were considered in the next optimisation step.

4.1.4. Optimisation of the significant variables

The next step was the optimisation of the significant variables, namely: log[peroxide], log[imidazole], pH and peroxide flow rate. With this purpose, a Draper–Lin small composite design (orthogonal), which permits the optimisation of the variables with a minimum number of experiments, was selected (see Fig. 2). The selected experimental region is shown in Table 7. Once the response was obtained and the data were analysed by means of the ANOVA, those quadratic coefficients whose P-value was lower than 5% were not considered in the model. These coefficients were: AB, CD and DD. The ANOVA was performed again and the final P-values are shown in Table 8.

The equation of the fitted response surface was:

$$\begin{aligned}\text{CL signal} = {} & 502.4 + 105.8 \times A - 173.1 \times B \\ & - 150.0 \times C - 87.0 \times D - 126.4 \times A^2 \\ & - 100.5 \times AC - 261.5 \times AD - 88.6 \\ & \times B^2 - 179.2 \times BC + 78.0 \times BD \\ & - 52.7 \times C^2\end{aligned}$$

The approximated optimum scores were obtained from this equation. For log[Imidazole] and peroxide flow rate these values were 0.093 and −1.34, respectively, which are included in the selected experimental region (see Fig. 4). These codified values correspond to real values of 1.2×10^{-2} M and 0.66 ml min^{-1}, respectively. On the other hand, the optimum values for log[peroxide] and pH were 1.41 and −1.41, respectively, which are in the limit of the experimental region, and correspond to real values of 0.54 and 5 M, respectively.

4.1.5. Verification of the first optimum

In order to verify these optimum coordinates, a further optimisation design was carried out in a more limited experimental region. In this sense, a narrow Draper–Lin small composite design (face-centred) around the predicted optimum was constructed. The new selected experimental region is shown in Table 9. Once the response was obtained and the data were analysed by means of the ANOVA, those quadratic coefficients whose P-value was lower than 5% were removed of the

Table 6
Estimated effects for confounded second order interactions

Effect			
(A) log[peroxide]+BD+CE = 51.59	(A) log[peroxide] = −481	BD = 532	CE = NS*
(B) log[imidazole]+AD+CF = −102.91	(B) log[imidazole] = −122	AD = 20 (NS*)	CF = NS*
(D) pH+AB+EF = −48.84	(D) pH = 214	AB = −263	EF = NS*

Effects and S.D. are expressed in arbitrary units of the CL signal.
* NS, non-significant.

Table 7
Experimental values for variables in the first Draper–Lin small composite design (orthogonal)

Variable	Level				
	−1.41	−1	0	+1	+1.41
log[peroxide] (M)	−1.735	−1.5	−1	−0.5	−0.265
log[imidazole] (M)	−3	−2.71	−2	−1.29	−1
pH	5	5.64	7	8.36	9
Peroxide flow rate (ml min^{-1})	0.53	1	2	3	3.47

model. These coefficients were: A (log[peroxide]), B (log[imidazole]), AA, AB, BD and DD. A new ANOVA was performed on the reduced model and the final *P*-values are shown in Table 10.

The equation of the final fitted response surface was:

$$\text{CL signal} = 858.3 - 112.3 \times C - 30.6 \times D - 16.0 \times AC - 114.7 \times AD - 113.6 \times B^2 - 101.6 \times BC - 185.7 \times C^2$$

The final optimum values were obtained from this equation. For log[Imidazole] and pH these values were 0.29 and −0.10, respectively, which correspond to real values of 1.4×10^{-2} M and 5.7, respectively. On the other hand, the optimum values for log[peroxide] and peroxide flow rate were 1 and −1, respectively, which are in the limit of the experimental region, and correspond to real values of 0.56 M and 0.5 ml min^{-1}, respectively. Those values are close to those reported by the previous Draper–Lin small composite design. First and final optimum values for all optimised variables are summarised in Table 11.

4.1.6. Influence of the methylamine concentration on the CL signal

Once the crucial experimental variables had been optimised and in order to check the dependence of the methylamine concentration on CL signal at these final optimum values, a study was performed varying the concentration of MA hydrochloride from 0.5 to 10 mg l^{-1}, which corresponds to values of MA in the range from 0.23 to 4.6 mg l^{-1}. The obtained response is shown in Fig. 4. It can be stated that a linear response can be expected up to a concentration of approximately 1.4 mg l^{-1} of MA.

Table 8
Analysis of variance (ANOVA) for CL signals obtained from the first Draper–Lin small composite design (orthogonal) without the non-significant coefficients

Source	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	*P*-value
(A) log[peroxide]	44 748.4	1	44 748.4	467.91	0.0294
(B) log[imidazole]	119 883.0	1	119 883.0	1253.56	0.0180
(C) pH	269 864.0	1	269 864.0	2821.83	0.0120
(D) Peroxide flow rate	90 883.4	1	90 883.4	950.32	0.0206
AA	127 762.0	1	127 762.0	1335.95	0.0174
AC	80 866.9	1	80 866.9	845.58	0.0219
AD	182 288.0	1	182 288.0	1906.09	0.0146
BB	62 776.4	1	62 776.4	656.42	0.0248
BC	256 922.0	1	256 922.0	2686.50	0.0123
BD	16 232.1	1	16 232.1	169.73	0.0488
CC	22 225.5	1	22 225.5	232.40	0.0417
Lack of fit	63 816.6	5	12 763.3	133.46	0.0647
Pure error	95.6344	1	95.6344		

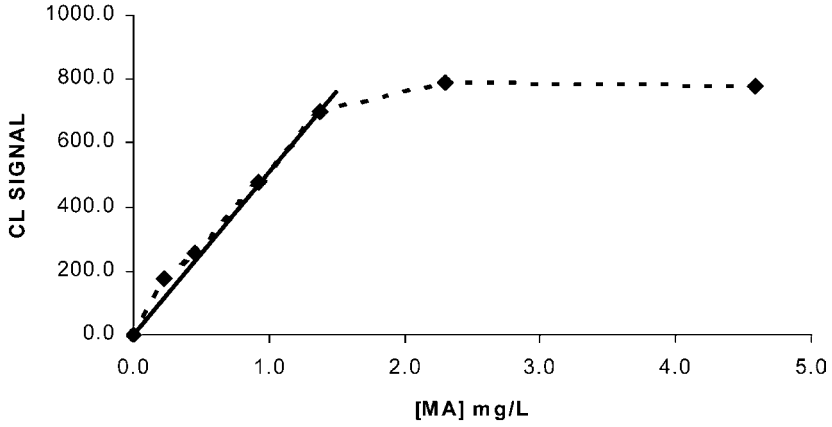


Fig. 4. Plot for CL-signal vs. methylamine concentration (CL signal is expressed in arbitrary units).

Table 9
Experimental values for variables in the second Draper–Lin small composite design (face-centred)

Variable	Level		
	−1	0	+1
log[peroxide] (M)	−0.75	−0.5	−0.25
log[imidazole] (M)	−2.5	−2.0	−1.5
pH	5.0	5.75	6.5
Peroxide flow rate (ml min^{-1})	0.5	1.0	1.5

Table 11
First and final optimum values for optimised variables involved in the PO-CL–FIA system

Variable	First optimum	Final optimum
Carrier flow rate (ml min^{-1})	3	3
[Peroxide] (M)	0.54	0.56
[Imidazole] (M)	1.2×10^{-2}	1.4×10^{-2}
[TCPO] (M)	1.0×10^{-3}	1.0×10^{-3}
pH	5.0	5.7
Peroxide flow rate (ml min^{-1})	0.66	0.5
Imidazole flow rate (ml min^{-1})	2	2

5. Conclusions

The use of the PO-CL reaction coupled to a FIA manifold as an alternative detection system for methylamine has been proposed. The method implies: (i) formation of a MA-OPA derivative (fluorophore) in presence of 2-mercaptoethanol; (ii) oxidation of TCPO by H_2O_2 using imidazol as catalyst, in presence of the fluorophore, whose CL emission is proportional to the methylamine con-

Table 10
Analysis of variance (ANOVA) for CL signals obtained from the second Draper–Lin small composite design (face-centred) without the non-significant coefficients

Source	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	*P*-value
(C) pH	126 065.0	1	126 065.0	122.13	0.0001
(D) Peroxide flow rate	9374.01	1	9374.01	9.08	0.0296
AC	2059.86	1	2059.86	2.00	0.2169
AD	105 251.0	1	105 251.0	101.97	0.0002
BB	40 746.3	1	40 746.3	39.48	0.0015
BC	82 513.4	1	82 513.4	79.94	0.0003
CC	108 795.0	1	108 795.0	105.40	0.0002
CD	24 537.8	1	24 537.8	23.77	0.0046
Lack of fit	36 284.5	5	7256.91	7.03	0.0259
Pure error	5160.89	5	1032.18		

centration. A formal strategy has been carried out for the multidimensional optimisation of the experimental variables of the PO-CL–FIA system with the aim to determine methylamine derivatives. Due to the interdependence of the chemical and operational variables, a formal strategy based on the use of experimental designs has been proposed for optimisation purpose. This implies the consecution of several steps, including the use of Draper–Lin small composite designs, scarcely used in the optimisation of analytical methods. This strategy offers interesting possibilities in the optimisation of analytical signals from other analytical techniques. In this sense, further research is being orientated to the determination of carbamates by employing the PO-CL system, using the new experimented strategy proposed in this paper.

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