ENVIRONMENTAL

A Multicommutated Flow System Based on an Opened-Loop with Micropump Propulsion

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Abstract: A multicommutated flow system using an opened-loop configuration and incorporating micropump propulsion was developed and applied in the determination of highly concentrated species in wastewater samples.

The developed flow system enabled a suitable dilution level to be attained and continuously monitoring of the analytical process. A synergetic effect was also obtained with the continuous removal of the dispersed front and trailing zones of the sample plug.

The developed strategy was evaluated on the determination of iron in high concentrated wastewater samples without any pre-treatment. The continuous sample recirculation permitted an increase in linear range up to 10 g \( \cdot \) l\(^{-1}\).

Keywords: Multicommutation, micropump, open-loop, flow analysis, total iron

INTRODUCTION

The utilization of closed-loops in flow analysis was initially proposed for flow recirculation in a loop where the sample was injected and was initially applied for species regeneration (Eswara Dutt and Mottola, 1975) and enzymatic assays (Wolff and Mottola, 1978; Iob and Mottola, 1980). Nevertheless, with slight differences in the closed-loop configuration, those flow systems
were employed in other analytical purposes, like multiple-peak recording of one injected sample with only one detector (Ríos et al. 1985; Valcárcel et al. 1989), regeneration of reagents (Ríos et al. 1987), determination of conventional kinetics parameters (Ríos et al. 1985), simultaneous determination of species by kinetics methods (Ríos et al. 1986), solvent extraction (sample preconcentration) (Atallah et al. 1987), sample dilutions (Ríos et al. 1985), and amplification of the determination range (Valcárcel et al. 1989).

The multicommutation concept in flow analysis (Rocha et al. 2002; Icardo et al. 2002; Cerdà and Pons, 2006) enabled the development of new flow assemblages such as new loop configurations. This improvement was obtained due to distributed commutation and to simple control of the commutation devices, usually three-way solenoids valves. In this sense, the achievement of a loop in an open circular configuration was possibly (Lapa et al. 1998), with advantages relatively to the closed-loops, respectively: the adaptation of the dilution level to the sample concentration without any previous sample dilution and an amplification of the analytical range.

When high dilution levels are necessary, several strategies can be considered for its performance on-line, such as decrease sample volume, increase the radius or length of the reactors, use of merging zones technique, employ dilution chambers (Růžička and Hansen, 1988), split the sample volume into two portions—one analyzed, and the other discarded (Clark et al. 1989), make use of dialysis units (Lima et al. 1991) or use the zone sampling technique (Reis et al. 1981). In cases where high degrees of dispersion are necessary, is more efficient and reproducible the use of zone sampling process (Reis et al. 1981).

More recently, the use of solenoid micropumps for solutions insertion and propulsion in flow systems was proposed (Lapa et al. 2002). The micropumps produce a pulsed flowing stream by the successively repetition of an abrupt diaphragm movement (pulse) followed by a period of immobilization. The pulsed flow generated contributes to a faster and more efficient mixing of the solutions (Lima et al. 2004).

In this work, a flow system with an opened-loop configuration associated with micropump propulsion was developed and evaluated. The main aims were to develop a flow system that allowed high levels of sample dilution based on the recirculation in an opened-loop, which enabled the frame of the analytical signal in the zone of analytical meaning and assessed its behaviour in the recirculation process. Multicommutation conjugated with micropump propulsion was tested and showed adequate operational characteristics which enabled the development of a reduced dimensional flow system and an increase in mixture efficiency in the flow line where the micropump was located, due to the pulsed nature of the stream (Lima et al. 2004). In addition, significant advantages relative to cost, easy installation and facility of operation were achieved. During the sample recirculation process, continuous zone sampling occurs, allowing a faster sample dilution, and the achievement of variable dilution degrees. With this strategy, the dilution level required can be obtained, without any manifold reconfiguration. The
only requirement was to control the number of sample recirculations in the opened-loop, according to the dilution level claimed. The proposed flow system was applied in the determination of total iron in manufacturing effluents, in order to assess the opened-loop applicability.

EXPERIMENTAL

Reagents

All chemicals were of analytical reagent grade and double deionised water (conductivity <0.1 μS cm⁻¹) was used throughout.

The acetate buffer solution (pH 4.0) was prepared using approximately 90 ml of sodium acetate 0.5 mol · l⁻¹ solution and 410 ml of acetic acid 0.5 mol · l⁻¹ solution, being the resulting solution diluted to 1000 ml.

Chromogenic reagent solution was prepared by dissolving 1.250 g of 1,10-phenanthroline in 400 ml of water, previously heated to 80°C. After cooling to room temperature, the volume was completed to 500 ml.

Carrier solution was prepared by mixing one third of the chromogenic reagent solution with two thirds of the acetate buffer solution.

The stock iron (III) solution (12.5 g · l⁻¹) was prepared by dissolving 4.525 g of Fe(NO₃)₂ · H₂O in 50 ml of HNO₃ 1 mol · l⁻¹, with the working standards being prepared by appropriate dilution of the previous solution with HNO₃ 1 mol · l⁻¹.

Ascorbic acid solution 0.284 mol · l⁻¹ was prepared daily by dissolving 0.500 g in 10 ml of water.

Apparatus

The flow system was designed with six three-way solenoid valves (161 T031, NREsearch) and flow lines of 0.8 mm i.d. PTFE tubing. Home-made end-fittings and confluence points were also used.

The solenoid micropump used for propelling the solutions, operated at a fixed displacement diaphragm (BIOCHEM Valve Inc.) which dispensed a fixed volume of 8 μl per stroke (Ref. 090SP).

A lab-made power drive based on an ULN 2003 integrated circuit was used for activating the valves and micropump. For data acquisition and control of the analytical system, a Pentium based microcomputer equipped with an Advantech PCL-711B interface card was used. All software was developed in Visual Basic 6.0 (Microsoft) with the low level dynamic libraries being supplied by Advantech.

The absorbance measurements were made at 510 nm with a Jenway 6300 spectrophotometer equipped with a 30 μl inner volume flow cell and the analytical signals were recorded on a Kipp and Zonen model BD 11E recorder.
Flow manifold

The flow manifold design (Fig. 1) comprised as active components, a set of three-way valves and a micropump. Solution insertions were made by aspirating from the selected channels, controlling the frequency of the micropump (consequently the flow rate), being the pathway established through the solenoid valves actuation, accordingly with the control algorithm (Table 1). The developed strategy permitted control of solution insertions and recirculation in the opened-loop, where the detector was placed and used for assessing the evolution of the analytical signal.

The working characteristics of the developed analytical system were assessed regarding the main variables and its performance was optimized. In this sense, the performance of the micropump was tested to estimate the efficiency of propulsion and its influence on the dispersion.

The inserted solution volumes were calculated based on the number of pulses, with the flow rate being established by the frequency of actuation and by the pulse volume of the micropump.

Reference Method

To assess the accuracy of the results obtained by the developed system, the results were compared with those furnished by the reference method. The reference method was carried out by spectrophotometric determination according to the Standard Methods for the Examination of Water and

![Figure 1. Schematic representation of the developed flow system: C—carrier solution; RA—reducing agent solution; S—standard/sample solutions; V1 to V6—three-way solenoid valves: solid lines inside the valves correspond to the inactive position and dashed lines to the active position; X1 e X2—confluences; R1 to R4—reactors; MP—micropump; D—detector; W—waste.](image)
Wastewater (Condike et al. 1998). Samples were treated with 1,10-phenanthroline at pH 3.2 to 3.3, after reducing the iron to the ferrous state by boiling with acid and hydroxylamine. Spectrophotometric determination was carried out at 510 nm with a light path of 1 cm.

RESULTS AND DISCUSSION

The continuous sample recirculation in the opened-loop enabled a selection of the dilution level required and, in the case of the detector be placed in the opened-loop, the monitoring of the reaction evolution. The control algorithm was implemented based on the evaluation of the analytical signals.

Flow Procedure

The developed flow methodology was based on 4 solenoid valves assembled in a circular configuration (Fig. 1). Two of them (V₃ and V₅) were connected to a confluence point (X₁) used for insertion of the solutions. The other two
solenoid valves (V4 and V6) were connected to the other confluence point (X2) and with the waste. Carrier solution could be introduced through valve V3 or V5, and two ways out were possible, through valve V4 or V6.

Different possibilities were initially considered concerning the positioning of the micropump (or micropumps) in the analytical system. The possibilities considered were: using a micropump for each solution propulsion; placing one micropump in the output (waste) of the system manifold, or; placing one micropump in the middle of the opened-loop. The evaluation of the different assemblies revealed that the last option enabled a faster and more efficient mixture of the solutions and was consequently adopted.

In the flow system proposed (Fig. 1), the detector was placed in the opened-loop, more properly, in reactor R4. With the detector in this position, it was possible to study the analytical signal during the recirculation process because an analytical signal was obtained each time the mixture (sample and carrier) passed in the detector. Therefore, for a sample determination, the number of signals obtained corresponded to the number of times the mixture passed in the detector. A multidetection signal was obtained (Fig. 3) which enabled the evolution of the analytical signal to be followed.

In implementing the control algorithm, one of the most important aspects was synchronizing the micropump with the commutation time of the solenoid valves. It was necessary to ensure the exact number of micropump pulses executed in each step of the table control enabling a good reproducibility of the recirculation process.

For a more efficient mixture with ascorbic acid solution, the sample and the ascorbic acid solution were inserted using binary sample technique (Reis et al. 1994). Then, the carrier solution (chromogenic reagent and buffer solution) was introduced as represented in Fig. 2A over the time required to transport the mixture sample/ascorbic acid to reactor R3 (step N = 11 of

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**Figure 2.** Schematic representation of the functioning system mode: the sample and the reducing agent were inserted using the binary sampling approach, through solenoid valve V2; A—the obtained mixture was transported to reactor R3 by the carrier solution; B—thereafter, the valves’ commutation was changed and the sample plug that remained in reactor R3 was transported to reactor R1 by the carrier solution inserted through solenoid valve V5. By repeating steps A and B, the sample was recirculated in the opened-loop.
Table 1), at the same time that the reaction started to take place. Thereafter, the commutation of the valves was changed, which permitted the carrier solution to be introduced in the opened-loop through valve V₅ (step N = 12 of Table 1) and enabled the mixture in the reactor R₃ to circulate in the opened-loop (Fig. 2B) and reach reactor R₁. Then the valves were once more commutated and the carrier solution introduced in the opened-loop from valve V₃ (step N = 13 of Table 1) (Fig. 2A) and the mixture was transported from reactor R₁ to reactor R₃. Thereafter, the commutation of the valves was changed once more. By intercalating the two last steps described (steps N = 12 and N = 13 of Table 1), it was possible to continually recirculate the sample in the opened-loop (steps N = 14 to N = 20 of Table 1). During this process, dilution of the sample occurred rapidly because the sample/carrier interface was constantly renewed due to the entrance of new carrier solution and the zone sample process that was occurring in reactors R₁ and R₃.

Time control modifications could enable a faster or slower sample dilution. For example, if had been chosen to recirculate the middle plug, it would have taken more time (requiring more recirculations) to obtain the same level of dilution that had been obtained with the recirculation of the

Figure 3. Typical detection signals obtained with a $2.50 \times 10^3$ mg·l⁻¹ standard solution.
head or tail of the sample plug. The plug that was recirculated corresponded to
the part that remained in coil R3, when the valve commutation was changed
between steps 11 and 12 (Table 1). Therefore, the opened-loop system
worked as a selective zone sampling device in which the sample plug
depended on the coil length and synchronization time. Recirculation of the
central part of the plug was chosen.

During sample recirculation, one signal was obtained each time the
mixture passed through the detector. The initial signals were in the non-
linear zone of the calibration curve. After a few recirculations the analytical
signals began to be framed within the linear response interval.

The flow system proposed enabled the determination of one sample con-
centration up to its maximum concentration. The difference between the
analysis of more concentrated and more diluted samples was in the number
of times required to recirculate the sample in the opened-loop. This avoided
any previous sample dilution step and any manifold reconfiguration.

Manifold Optimization

Analytical system control was based on the data obtained from studying the
working characteristics of the valves and micropump and the pathways
(reactors R1, R2, R3, and R4). The time was used as independent variable in
the opened-loop control and the micropump frequency was directly related
to the flow rate. During this stage, the time for each control step was
optimized with a view to yielding a high signal with good repeatability.

The recorded signals obtained in the sample recirculation (Fig. 3) rep-
resented an evolution, corresponding to two phases of the process: initially
the sample concentration was too high and no relationship between sample
concentration and analytical signal can be established; in a second phase,
the analytical signals were framed in the linear zone of the analytical curve.
Since, several analytical signals were obtained for each analytical process
and considering the concentration level of the samples, the first determination
in which the analytical signals were in the linear zone of the calibration curve,
was used for evaluation purposes.

The optimization of the manifold parameters was made with the main
goal of obtaining a system capable of carrying out sample analysis without
the need for any additional pre-treatment and with good reproducibility.
With these objectives, evaluation of the micropump frequency, sample
volume and length of the opened-loop reactors were studied. For these
studies, a standard 2.5 g·l⁻¹ iron solution was used.

The micropump frequency was a parameter that required evaluation since
it determined the flow rate and high micropump frequencies could cause a
decrease in repeatability due to a lack of coordination between the
micropump frequency and the solenoid valves’ commutation in the opened-
loop. Therefore, a frequency interval of the micropump between 1 and 4 Hz
was established. The relative standard deviation (RSD) obtained was lower for a frequency of 3 Hz (Fig. 4) and this frequency value was chosen.

As expected, the sample volume showed to have significant influence on sample dispersion, which affected the zone sampling process, particularly in terms of repeatability. Increased sample volumes gave rise to higher analytical signals and more time was needed to recirculate the samples in the system. Therefore, it was necessary to establish the sample volume that allowed a good repeatability without a large increase in sampling rate. Thereafter, sample volumes between 8 and 40 μl were studied (Fig. 5). A compromise between sample rate and repeatability was established, which led to the choice of a sample volume of 32 μl. The sample volume (32 μl) was inserted by 4 intercalating cycles of 8 μl of sample with 8 μl ascorbic acid solution (binary sampling).

**Figure 4.** Standard deviation (in percentage) obtained in the fifth detection, with a 2.50 × 10^3 mg · l^{-1} iron solution, as a function of the micropump frequency.

**Figure 5.** Standard deviation (in percentage) obtained in the fifth detection, with a 2.50 × 10^3 mg · l^{-1} iron solution, as a function of the sample volume.
The zone sampling process occurred in the reactors R\textsubscript{1} and R\textsubscript{3}, so the length of those reactors was directly related with the plug that was recirculated. In this sense, for one recirculation in the opened-loop, the dilution level increased with a decreased in the length of reactors R\textsubscript{1} and R\textsubscript{3}, because the plug recirculated was smaller. Different lengths, between 6 and 100 cm, were tested for those two reactors, and a length of 21 and 11 cm was chose for R\textsubscript{1} and R\textsubscript{3}, respectively. For those reactors length, a good compromise was established between sampling rate and the dilution level obtained in one recirculation in the opened-loop. The length of the reactors R\textsubscript{2} and R\textsubscript{4} was as short as possible (5 and 57 cm, respectively) corresponding to the minimum length necessary to established the connection between the valves (V\textsubscript{4} and V\textsubscript{5}) and (the valve V\textsubscript{3}, the detector and the valve V\textsubscript{6}), respectively.

Analytical determinations were performed using the analytical signals framed in the linear response zone of the analytical curve. The calibration model was previously established with standard solutions between $5.00 \times 10^2$ and $1.00 \times 10^4 \text{ mg} \cdot \text{ l}^{-1}$ of Fe(III).

The analytical frequency obtained with the proposed system depended on the iron concentration in the samples and can range between 8 and 20 determinations per hour, in case of 5 and 1 recirculations in the opened-loop, respectively.

**Determination of Iron in Wastewaters**

The figures of merit obtained with the proposed flow system and the reference method are summarized in Table 2. In the fifth determination was possible to obtain a high amplification of the linear range of the calibration curve (up to $1.00 \times 10^5 \text{ mg} \cdot \text{ l}^{-1}$) and, at the same time, determine directly the samples concentration. Accordingly to these results, this detection was chosen for the samples determination. The flow system was calibrated with standard solutions ranging between $5.00 \times 10^2$ and $1.00 \times 10^4 \text{ mg} \cdot \text{ l}^{-1}$ of Fe(III). A linear regression for the fifth detection was obtained between iron concentration and peak height, which was represented by the equation $y = (5.95 \times 10^{-4} \pm 2 \times 10^{-6}) \times x + (0.00 \pm 0.01)$, with a correlation coefficient ($R^2$) of 0.9999 and $n = 6$, where $y$ corresponds to peak height and $x$ represents concentration expressed in mg \cdot l\textsuperscript{-1}.

Wastewater samples furnished by metallurgic factories were analyzed by the proposed method and the results compared with those obtained by the reference method (Table 3). A good correlation was obtained between the results of the two methods with a correlation coefficient ($R^2$) of 0.994 and a relative deviation expressed as a percentage of the proposed method relative to the reference procedure from $-1.68$ to $1.75\%$. The results obtained show a good agreement, in spite of the difference in the sample pre-treatment that was need to do in the reference method namely, dilution of 2000 times of samples and boil with acid and hydroxylamine, compared to the developed system where the samples were introduced directly in the flow system.
### Table 2. Analytical data for spectrophotometric determination of total iron by employing the opened-loop system proposed and the reference method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Opened-loop&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear regression</td>
<td>$y = (5.95 \times 10^{-4} \pm 2 \times 10^{-6})x + (0.00 \pm 0.01)$</td>
<td>$y = (0.190 \pm 0.001)x + (0.009 \pm 0.002)$</td>
</tr>
<tr>
<td>Correlation coefficient ($R^2$)</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
<tr>
<td>Dynamic range (mol·l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>8.95 $\times$ 10&lt;sup&gt;-3&lt;/sup&gt; to 0.179 (5.00 $\times$ 10&lt;sup&gt;2&lt;/sup&gt; to 1.00 $\times$ 10&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>1.79 $\times$ 10&lt;sup&gt;-6&lt;/sup&gt; to 5.37 $\times$ 10&lt;sup&gt;-5&lt;/sup&gt; (0.10 to 3.00)</td>
</tr>
<tr>
<td>RSD (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Detection limit (mol·l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>4 $\times$ 10&lt;sup&gt;-3&lt;/sup&gt; (2 $\times$ 10&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>4 $\times$ 10&lt;sup&gt;-7&lt;/sup&gt; (2 $\times$ 10&lt;sup&gt;-2&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Sample consumption (µl)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>1,10-phenanthroline consumption (mol)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.6 $\times$ 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>2.0 $\times$ 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Buffer solution consumption (mol of CH&lt;sub&gt;3&lt;/sub&gt;COO&lt;sup&gt;-&lt;/sup&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.8 $\times$ 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.18</td>
</tr>
<tr>
<td>Ascorbic acid consumption (mol)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.1 $\times$ 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Hydroxylamine consumption (mol)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>—</td>
<td>1.4 $\times$ 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hydrochloric acid consumption (mol)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>—</td>
<td>2.4 $\times$ 10&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waste (ml)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>Throughput (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>8 to 20</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Analytical data corresponding to the fifth detection.

<sup>b</sup> RSD (%) is the relative standard deviation corresponding to 10 independent measurements of solutions containing 4.48 $\times$ 10<sup>-2</sup> mol·l<sup>-1</sup> (2.5 g·l<sup>-1</sup>) and 1.79 $\times$ 10<sup>-5</sup> mol·l<sup>-1</sup> (1.0 mg·l<sup>-1</sup>) for the opened-loop and reference methods, respectively.

<sup>c</sup> Detection limit was calculated using the regression line (with $y_b = a$ and $s_y = s_y/x$) (Miller and Miller, 2000).

<sup>d</sup> Sample and reagent consumption in one determination.

<sup>e</sup> Waste generated by one determination.
The quality of the results provided by the flow system was also confirmed by Student $t$-test of paired values ($t_{\text{calc}} = 0.00 < t_{\text{crit}} = 2.78$, $n_1 = n_2 = 5$ and $P = 0.05$) with no significant statistical difference having been verified between the two methods.

The detection limit calculated by using the regression equation (with $y_b = a$ and $s_b = s_{y/x}$) (Miller and Miller, 2000) was $4 \times 10^{-3} \text{mol} \cdot \text{l}^{-1}$ ($2 \times 10^2 \text{mg} \cdot \text{l}^{-1}$) of iron, for the fifth detection.

### CONCLUSIONS

The use of an opened-loop configuration gave rise to a versatile flow system enabling the determination of one species concentration without any previous dilution. Another characteristic of the developed flow system was the possibility of carrying out different dilution levels without the need for any physical change in the manifold. For samples with lower concentration the initial detection signals would be used and for more concentrated ones, it would be required to further recirculate the sample in the opened-loop in order to obtain more analytical signals. Nevertheless, in this work, only the analytical signal for the fifth detection was taken into account and a large analytical range was obtained.

The application of the developed flow system in the determination of iron allowed the opened-loop characteristics to be studied while also providing a foresight into its application in other analytical situations such as kinetic based determinations.

In a general way, the developed system based on the use of an opened-loop system using micropump propulsion represents a simple strategy to develop analytical systems based on continuous flow, combining the advantages of multicommutation with pulsed stream. This combination allows analytical systems to be obtained with a wide analytical application, high

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**Table 3.** Determination of total iron in wastewaters

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reference procedure$^a$</th>
<th>Developed methodology$^a$</th>
<th>Relative deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.63 ± 0.02</td>
<td>2.6 ± 0.1</td>
<td>−1.05</td>
</tr>
<tr>
<td>2</td>
<td>1.96 ± 0.01</td>
<td>2.0 ± 0.2</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>1.52 ± 0.01</td>
<td>1.49 ± 0.03</td>
<td>−1.68</td>
</tr>
<tr>
<td>4</td>
<td>2.19 ± 0.01</td>
<td>2.2 ± 0.1</td>
<td>1.75</td>
</tr>
<tr>
<td>5</td>
<td>2.30 ± 0.01</td>
<td>2.29 ± 0.09</td>
<td>−0.49</td>
</tr>
</tbody>
</table>

$^a$Average ± standard deviation of 3 independent analysis of each sample.
degree of automation and autonomy together with a low cost of installation and reduced dimensions.

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