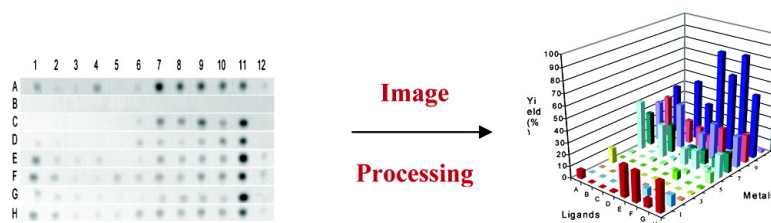Report

Image Analysis as a Quantitative Screening Test in Combinatorial Catalysis: Discovery of an Unexpected Ruthenium-Based Catalyst for the Sonogashira Reaction

Stefania Garbacia, Rachid Touzani, and Olivier Lavastre

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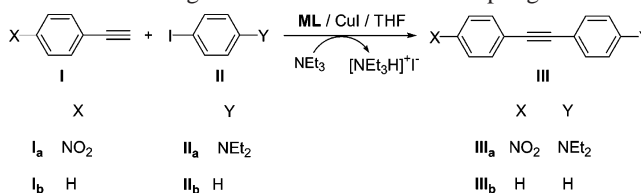Stefania Garbacia, Rachid Touzani, and Olivier Lavastre*

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Catalysis concerns a broad range of applications, including inorganic, enzymatic, or organic reactions. Evaluation and detection of new interesting candidates in short periods of time are crucial. In this respect, high-throughput analytical methods have a tremendous power and are of great interest in enabling the rapid screening of a large diversity of compounds.[1−4] This has boosted the design of efficient high throughput screening (HTS) tests for combinatorial catalysis. In addition to chromatographic methods,[5−7] elegant new concepts have been recently reported, such as infrared thermography,[8−9] colorimetric[10,11] or fluorescence-based[12−14] tests, mass spectrometry labeling methods,[15] or optical screening techniques, such as the resonance-enhanced multiphoton ionization, REMPI.[16] However, these methods are generally based on a HTS test specifically designed for a target, that is, the expected product. This prevents the analysis of the overall composition of the catalytic mixture. Reactions giving different and unexpected products will be not detected by a focused screening test, preventing the discovery of new and interesting results. Thus, the design of new screening tests, able to give both qualitative and quantitative evaluation of large libraries of samples without sample purification, has strong attraction. Herein, we report a fast and inexpensive screening method based on image analysis (IA) and its first application in the area of organometallic combinatorial catalysis screening.

If NMR spectroscopy and HPLC or GC chromatography are able to give a general overview of the composition of reaction mixtures by the number and relative position and integration of peaks, they are also highly demanding in terms of consumables when applied to large numbers of samples. In addition, and particularly for organometallic catalysis, a prepurification process is necessary in most cases to remove derivatives interfering with analysis, that is, paramagnetic metal species for NMR or salts and insolubles for HPLC or GC columns. This represents a time-consuming and potentially biasing process, and when very small volumes are involved, as in combinatorial approaches, significant errors can occur during filtration or extraction steps. Recently, capillary array electrophoresis (CAE)[17] was reported as an efficient screening test in combinatorial catalysis that is able to separate compounds from mixtures and to quantify them

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Scheme 1. Sonogashira Carbon−Carbon Coupling Reaction

by UV detection. Thin layer chromatograpgy (TLC), a much simpler and universal method, has also been applied in combinatorial chemistry as a qualitative screening tool[18−20] and for quantitative evaluation when coupled to mass spectrometers and surface sampling probes.[21] On the other hand, softwares for image analysis are broadly used in the life sciences area, particularly for the quantitative evaluation of gels after electrophoresis. The integration of different spots by image processing (size, intensity) is widely used as a quantitative method in the life sciences. Thus, the combination of TLC and image analysis software for the quick qualitative and inexpensive separation of compounds and quantification of corresponding spots represents a potential alternative to NMR spectroscopy, GC, and HPLC chromatographies or CAE/UV methods. Evaluation of a catalyst library by this method involves a three-step procedure: (a) thin layer chromatography for quick qualitative separation and visualization of the different compounds present in the catalytic mixture, (b) generation of digital pictures of TLC plates with an office scanner or a digital camera, and (c) quantification of the different spots by image analysis software. As a model, the catalytic Sonosgashira reaction[22] was used (Scheme 1). We report here the validation studies for this quantitative IA test and its first application as a high-throughput screening test for colored products generated by organometallic combinatorial catalysis and the generalization of this method for the quantitative screening of colorless compounds. The product IIIa is easily detected as an orange spot on TLC plate after elution. To check the reproducibility and accuracy, 12 identical aliquots of a solution of IIIa (from pure sample) were deposited on a TLC silica plate with a 12-channel pipet, and after elution, the image of the corresponding spots was analyzed. The image analysis software[23] transforms a two-dimensional spot into a three-dimensional volume, integrating both the area of the spot and its intensity, which is related to the amount of compound present. The impact of different parameters, such as the range of concentration of solution of IIIa, the volume of aliquots (1 μL versus 2 × 0.5 μL), distance of elution, elution power of solvent and eluent composition, were carefully investigated in order to find the optimum conditions. The key point is to have all the compound at the surface of the support by playing with the amount of product and the elution distance. The optimum conditions were obtained using the following criteria: (a) 0.001 M < concentration of IIIa < 0.01 M; (b) aliquots, 1 μL; (c) R_f, 0.6; (d) solvent, THF; eluent, heptane/ethyl acetate (v/v 7/3); TLC plates, silica gel 60 F254, aluminum sheets, Merck. It should be noted that this
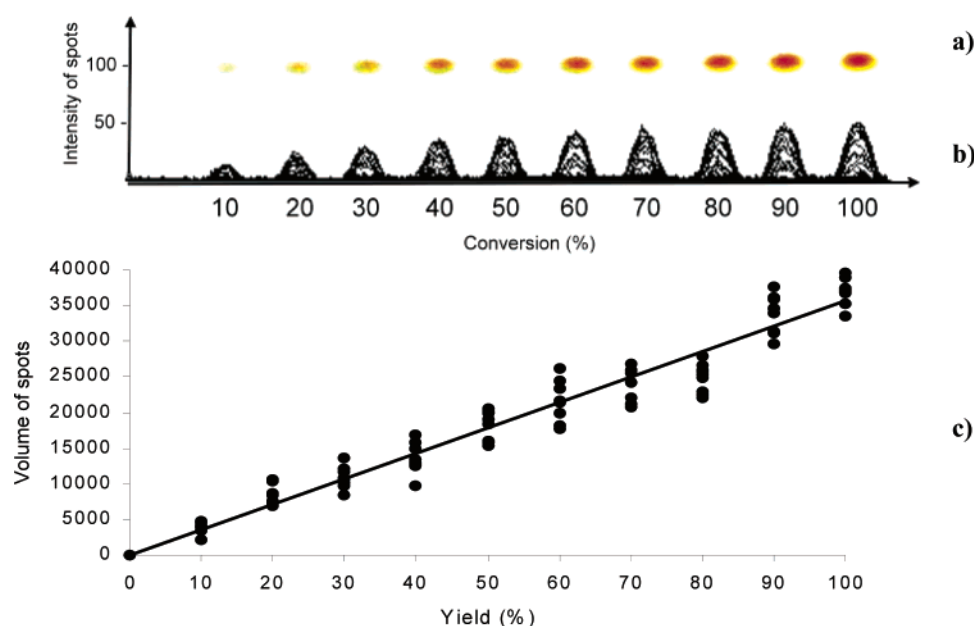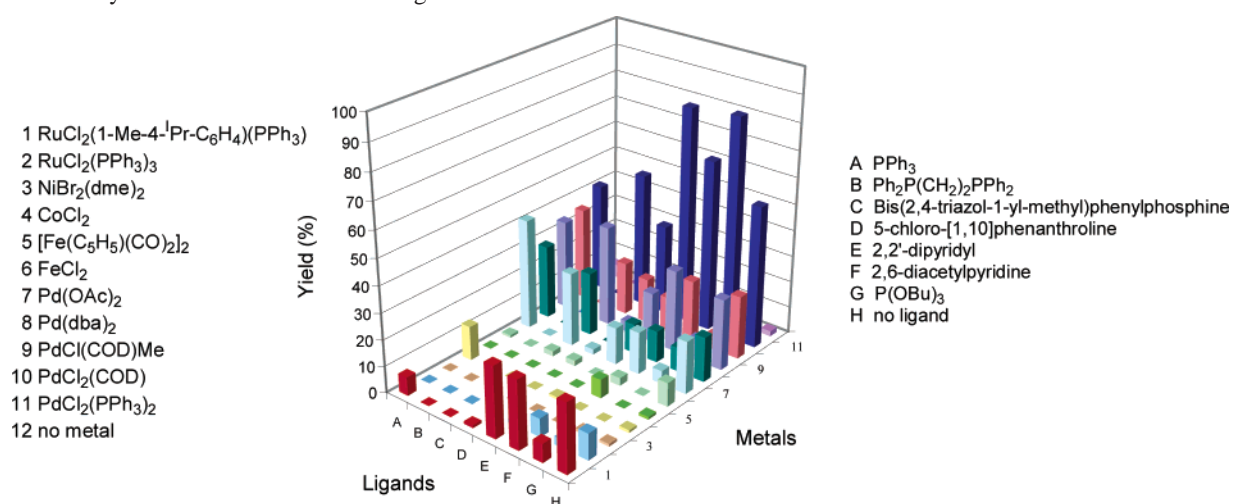
Figure 1. (a) 10 spots of IIIa at different concentrations, (b) corresponding image analysis, and (c) calibration curve.

Chart 1. Catalytic Activities of 96 Metal/Ligand Combinations

preliminary investigation to find the best condition to generate a good calibration curve is faster than classical optimization of HPLC or GC methods. Six independent identical measurements showed good reproducibility and accuracy with an average deviation below 10% (see annex 1, ref 24). For quantitative screening, 10 solutions of IIIa in THF were prepared at different concentrations to simulate yields of the reaction between 10 (0.001 M) and 100% (0.01 M). Figure 1 shows (a) the 10 spots after elution, (b) the corresponding analysis generated by the image analysis software, and (c) the resulting calibration curve.

Good correlation between yield and volume of each spot was observed from seven independent calibrations curves (see annex 2, ref 24). These results indicate that this IA test could be applied for the fast quantification of colored compound IIIa.

With this working quantitative assay developed, the catalytic reaction described in Scheme 1 was studied by an HTS approach. A diversity of potential catalysts was obtained from parallel dispatching of solutions of 11 metal salts, M,

and 7 ligands, L, into a 96-vial plate, each vial corresponding to a specific metal/ligand ML combination, as reported in Chart 1 (see Supporting Information Section for details). After 20 h at 50 °C, all reactions were quenched by fast cooling to room temperature, and 1 μL of each vial of the previous plate was immediately transferred to eight TLC silica plates (rows A to H). A representative example is shown in Figure 2 and shows the results corresponding to row F after elution (eluent: heptane/ethyl acetate, v/v 7/3).

A simple visual evaluation of the region of interest (ROI) corresponding to the orange product IIIa led to a general qualitative overview of the catalytic activity of the ML system in each vial, according to the presence or absence of the spot related to product IIIa. Starting products Ia and IIa are colorless and did not interfer with the colored spot of IIIa. It should be noted that a colorimetric evaluation with a UV microplate reader of the 96 crude solutions was not able to give a general overview of the exact composition of the catalytic mixture and was not applicable for this study because of the presence of red side products in several vials.
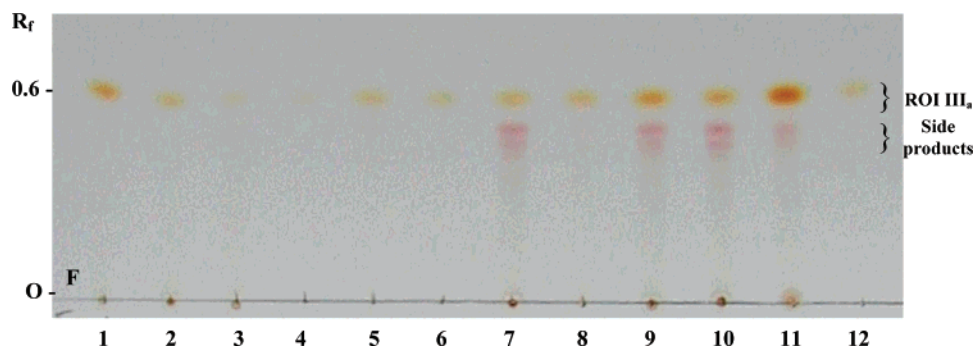
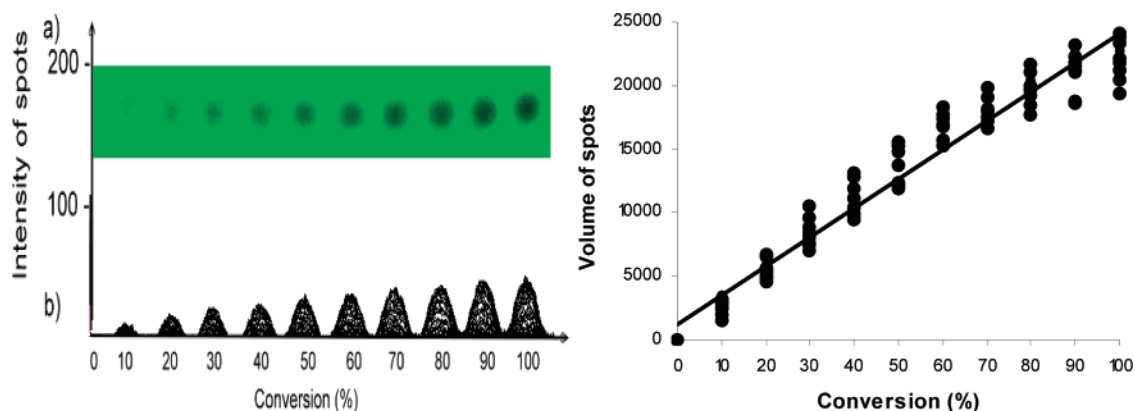**Figure 2.** Picture of the TLC plate corresponding to row F.

Figure 3. Quantification of TLC plate by image analysis and calibration curve for IIIb.

For quantitative screening, the previous library of 96 reactions was first quenched by cooling, then diluted by a factor of 10 in order to reach the optimum concentration range for quantitative evaluation. After elution, eight TLC plates A−H were scanned together, and the 96 reactions were evaluated by image analysis. By comparison with the calibration curve, the graph 1 was generated allowing a direct comparison of catalytic activities. Representative vials and corresponding reactions were reproduced independently in classical reactors at the 10-mL level and were analyzed by ^{1}H NMR spectroscopy. As expected for this kind of catalytic reaction,[22] palladium derivatives (columns 7−11) were effectively detected as active catalysts. However, this simple screening test also revealed a completely unexpected catalyst, $RuCl_2(1\text{-Me},4\text{-}^iPrC_6H_4)(PPh_3)$, clearly illustrating the impact of combinatorial approaches, because no reliable theories would have been able to predict this result.

This catalyst was active without any additional ligands (vial H1). The presence of other ligands induced deactivation. Additional experiments showed that there was no coordination between the ruthenium precursor and the bipyridine and diacetylpyridine ligands, explaining the values obtained for E1, F1, and H1. The activity at 50 °C was moderate (26%), but was improved at 80 °C (46%). Although the activity of this new catalyst is modest (46%) by comparison with the reference palladium complex (83%), it should be noted that it represents the first application of a ruthenium-based catalyst for the Sonogashira carbon−carbon coupling reaction. These results demonstrate the potential for the image analysis screening test in combinatorial catalysis. One could extrapolate this method to any library of "colored compounds" generated by combinatorial catalysis.

However, compounds can also be colorless. Thus, the detection and quantitative evaluation of the colorless compound IIIb was investigated as a model (Scheme 1). Six independent TLC plates, containing 12 spots generated from 1-μL aliquots of solution of pure IIIb (0.01 M in THF), and seven replications of TLC plates, obtained from 10 solutions of pure IIIb at different concentrations, were prepared and eluted (heptane/ethyl acetate, v/v 7/3). Images of TLC plates under UV irradiation (256 nm) were obtained (digital camera, Figure 3a) and analyzed by image analysis (Figure 3b), again showing good reproducibility and accuracy (see annexes 3 and 4, ref 24).

The low deviation measured for the calibration curve (<10%) (Figure 3) indicated that the method was also applicable for fast quantitative evaluation of libraries of colorless compounds by using UV visualization.

The combination of TLC for the quick separation of compounds and image analysis for quantification of the corresponding image is able to give direct, quantitative information from catalytic mixtures without a prior purification step. The application of this method as a high-throughput screening method in catalysis led to the discovery of a completely unexpected ruthenium catalyst and to the generation of a new quantitative database for the Sonogashira C−C coupling reaction in a rapid manner. Generally speaking, this new method could be applied to a large field of applications because practically all types of molecules could be detected directly in the case of colored products or under UV illumination. To circumvent the potential problem of co-eluting compounds, three different eluents with low, medium, and high eluting power could be used to choose the best conditions to separate efficiently the target product but also

to detect unexpected compounds.[20] Less than 1 h was necessary for the manual deposition of 96 aliquots, elution of eight TLC plates, and generation and analysis of the TLC images. If necessary, this could be easily improved with robotic liquid handling, parallel elution systems, and automatic integrated image generation and analysis (e.g., TLC scanner). However, the fact that no specific analytical equipment is necessary and inexpensive commercial image analysis softwares are readily available indicates that image analysis screening can be used as a general, fast, and inexpensive alternative to HPLC, GC, CAE, and NMR analytical methods in combinatorial catalysis screening and analysis.

Supporting Information Available. Experimental details are available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) Bio-profil Bio 1D (Vilber Lourmat) software.

(24) See Supporting Information: Experimental protocol for this IA test and corresponding pictures and annexes.