High-throughput analytical techniques for reaction optimization

Miles S. Congreve and Craig Jamieson

This review describes recent developments in approaches to high-throughput reaction optimization, as well as the associated analytical techniques. The studies discussed include the use of UV-visible, IR-thermographic and mass spectrometric methods for application in catalyst development, process optimization and materials science. Other methods of potential use in a high-throughput format are also discussed.

Miles S. Congreve
Astex Technology
250 Cambridge Science Park
Milton Road
Cambridge
UK CB4 0WE
tel: +44 1223 226270
fax: +44 1223 226201
e-mail: m.congreve@astex-technology.com
Craig Jamieson
GlaxoSmithKline R&D
Medicines Research Centre
Gunnels Wood Road
Stevenage
UK SG1 2NY

▼ Combinatorial chemistry and associated techniques are now widely used for the synthesis of potentially biologically active molecules. Perhaps counter-intuitively, however, optimization of the individual reactions used in the synthesis of biological ligands is often performed using more traditional iterative methods, rather than by using a combinatorial approach. However, there are several other examples in which combinatorial methods have been used in optimization campaigns, particularly in the areas of asymmetric catalysis and materials science [1,2]. This review will introduce the topic of reaction scanning, discussing both the chemical methodologies and the high-throughput analytical methods used. Newly developed techniques with the potential for use in reaction scanning will also be described. Biocatalysis has been excluded and is reviewed in detail elsewhere [3].

Strategies and formats for high-throughput experimentation

There are several prominent, early examples of parallel reaction screening and optimization, which employed laboratory equipment or techniques already used for combinatorial library production (e.g. 96-well plates and solid-phase chemistry). In medicinal chemistry programmes, both Bray [4] and Warmus [5] have shown the value of parallel reaction scanning in the optimization of a particular process. In this way, numerous continuous (e.g. concentration, stoichiometry of reagent) and discrete (e.g. type of catalyst, solvent, base) variables were rapidly explored. In addition, there are several seminal reports on the parallel screening of modular catalyst systems for asymmetric processes, and selected examples are given in Fig. 1a–c [6–12].

An alternative to a modular optimization approach is one based on statistical design of experiments (Do E) methodology. Emiabata-Smith *et al.* [13] described the development of an automated workstation (SK233, Anachem, Luton, UK), which enabled solution-phase chemistries to be examined in parallel with online high-performance liquid chromatography (HPLC) analysis. Using the principles of DoE to examine process variables and predict optimum reaction conditions, the workstation enabled rapid process screening and optimization to be performed for a wide range of chemical applications. In an extension of this approach, amide formation using polymer-supported carbodiimide as a supported reagent was examined [14]. Stoichiometry of the resin, concentration, reaction time and solvent effects were varied and optimum conditions rapidly developed. These conditions were then found to be quite general in the synthesis of an 80-member amide array.

Reaction screening methodology

UV-visible methods

Crucial to the success of any reaction scanning campaign are the techniques employed for the high-throughput analysis and interpretation of the results. The remainder of this review will focus on current and potential methods used to interpret the results of reaction scanning experiments.

UV-visible spectroscopy is perhaps the most ubiquitous screening technique, with the examples mentioned previously using HPLC as

1359-6446/02/$ – see front matter PII:S1359-6446(02)02094-3

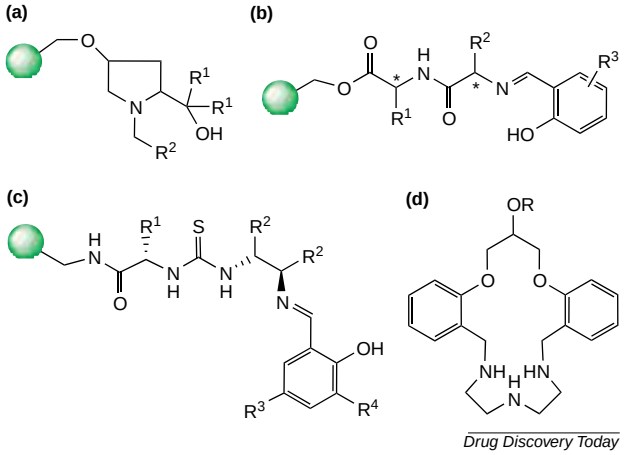

Figure 1. General structures of ligands used in reaction scanning campaigns. Supported ligands for **(a)** diethyl zinc addition to aldehydes [9], **(b)** Ti-catalyzed addition of TMSCN to *meso* epoxides [10] and **(c)** the Strecker reaction [11]. **(d)** Ligands for lanthanide-catalyzed phosphate ester hydrolysis [17]. The asterisks denote a mixture of isomers.

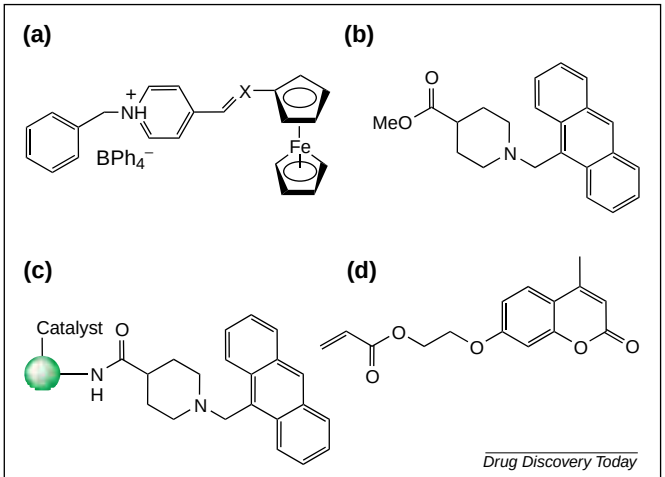

Figure 2. Sensor molecules used to assay reaction scanning experiments. **(a)** Dye substrates for catalystic hydrosilation reactions (X = CH or N) [22]. **(b)** Acetic acid fluorescence sensor [23]. **(c)** Acetic acid fluorescence sensor for single bead detection [23]. **(d)** Fluorescence substrate for the Heck reaction [24].

the method of choice. Many other researchers have used UV-visible detection methods in reaction scanning, and some examples are listed below. Burgess *et al.* [15] screened 13 phosphine oxazole ligands under a wide-range of reaction conditions as catalysts in the asymmetric allylation reaction of dimethylmalonate, assaying the results using chiral HPLC. Hoveyda and colleagues [16] recently studied asymmetric diethyl zinc addition to imines catalyzed with Zr-complexed chiral peptide-based Schiff bases by chiral HPLC. Using a kinetic enzyme-linked immunosorbent assay (ELISA) plate reader at 405 nm, Janda and colleagues [17] were able to assess the suitability of aza-crown ether ligand systems (Fig. 1d) as catalysts in the hydrolysis of *p*-nitrophenyl phosphate derivatives. Gao and Kagan [18] have assayed asymmetric reduction of small mixtures of prochiral ketones with a chiral catalyst, measuring the enantioselectivities of each component of the mixture by chiral HPLC. This strategy required careful pre-selection of the substrates used in the assay to avoid overlapping signals. A similar approach to HPLC, chiral gas chromatography (GC), has been applied to the analysis of catalytic diastereoselective aldol chemistry [19]. In this way, an array of 192 reactions was studied, identifying an effective chiral metal ligand.

Hartwig *et al.* [20] used a colourimetric assay to qualitatively assess the amount of unreacted aniline remaining after screening potential catalysts for the palladium-mediated hydroamination of 1,3-dienes. Using a 96-well plate format, the best palladium complex and phosphine ligand were readily identified. In a related study of the hydroamination of acrylates, the level of arylamine remaining after each reaction in a series was assessed using a spot test for amines [21].

Several approaches to assay reactions using sensor molecules have been reported. Dyes (Fig. 2a) were used as substrates for catalytic hydrosilation and the rates of conversion to saturated products assessed using a digital camera by measuring 'bleaching' of the colour of the reaction mixtures [22]. Copeland and Miller [23] have developed a fluorescent sensor for the detection of acetic acid, produced as a by-product from a model enantioselective acyl transfer reaction (Fig. 2b). A fluorescence plate reader was used to assay simultaneously rates of reaction in a 96-well-plate format studying the effects of a range of catalysts. It was also demonstrated that the sensor could be used on a solid support (Fig. 2c) to detect active catalysts on the same bead, making the approach suitable for single-bead–single-catalyst libraries.

Coumarin fluorophore (Fig. 2d) was used as a substrate for the Heck reaction with a resin-bound aromatic halide to assess qualitatively the extent of reactions with a range of ligands [24]. Incorporation of the substrate could be determined by the degree of fluorescence of the beads under a UV lamp, and this could then be correlated with the yield determined by GC after cleavage of the products from the resin.

In an extension of this approach, Hartwig *et al.* [25] studied the Heck reaction by tagging both coupling partners, one with a dansyl fluorophore, the other with an azodye quencher. The reactions were then assayed using fluorescence resonance energy transfer (FRET), in which any unreacted fluorophoric substrate (FRET donor) will emit

upon excitation, but the coupled product will not because of quenching of its emission by the azodye (FRET acceptor) present in the same molecule. Screening of the reactions with a range of ligands could then be done in a 96-well fluorescent plate reader.

Finally, using a split–mix-pool combinatorial strategy for the discovery of catalysts for the polymerization of olefins, Murphy and colleagues [26] used encoded resin to identify active catalysts. Pools of beads bearing potential catalysts were subjected to polymerization conditions and single beads viewed under a microscope to select visually those on which polymerization had occurred. Catalysts were then identified by HPLC with fluorescence detection by cleavage of the tertiary amine codes, followed by dansylation.

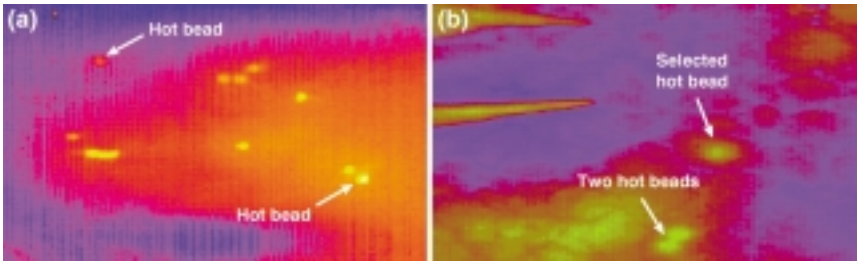

Figure 3. (a) Infra-red (IR) thermographic image of ~20 catalyst beads in the presence of ~3000 non-catalyst beads. Arrows indicate two of the 14 visible hot beads. **(b)** Close-up IR thermographic image of the trimeric catalyst library in the presence of acylation reagents, showing one hot bead being selected for decoding (tweezers in upper left). Reproduced, with permission, from Ref. [31].

Mass spectroscopy-based methods

Reetz and colleagues [27] prepared *pseudo*-enantiomeric and *pseudo-meso* pairs of substrate molecules, in which one was isotopically labelled and the other unlabelled, for use in screening of reactions by mass spectrometry (MS). A mixture of the compounds behaved chemically as a racemate or as a *meso* compound; however, the kinetic resolution of a racemate gave two products with different molecular weights, enabling the ratio to be determined. Using this technique, up to 1000 reactions can be assayed per day. Finn and colleagues [28] have independently reported a similar mass-tagging approach to the measurement of enantiomeric excess by MS.

Infra-red thermographic techniques

IR thermography, a technique in which microscopic changes in temperature, such as those occurring during a chemical reaction, can be measured using an IR camera, has been applied to reaction screening [29–31]. Reetz *et al.* [29] demonstrated that the method was suitable for the study of enantioselective reactions in 96-well-plate format. This work was later extended to illustrate that both exothermic and endothermic reactions could be studied [30]. Taylor and Morken [31] demonstrated that the technique could be successfully applied to study resin-bound catalysts. A 3150-member encoded split–mix-pool combinatorial library of potential acylation catalysts was prepared and the 'hottest' beads selected (Fig. 3). Three structurally similar catalysts were identified which, after resynthesis, were found to be active for the reaction under study. Other, less common screening techniques have been used for high-throughput screening of enantiomeric excess. These include capillary-array electrophoresis [32] and HPLC-circular dichroism (CD) [33].

Clearly, HPLC- and GC-based methods, despite being the most general and widely used, are subject to throughput limitations because of the length of time required for analysis. Attempts to enhance throughput, such as by use of parallel column systems, could help alleviate this bottleneck. In comparison, fluorescence-based techniques do offer enhanced throughput (screening of up to 1000 samples per hour is possible) [34], but they require specifically-labelled substrates. Other emerging techniques of potential use in reaction scanning are described next.

Future directions for reaction scanning

Single-bead Fourier transform infra-red (FTIR) and beam condenser FTIR have been found to be suitable for rapid analysis of resin-supported substrates and can provide qualitative information on resin-supported organic reactions [35]. Extension of this methodology to an automated format would significantly enhance throughput, making the method more attractive for scanning a large number of reactions in a parallel format. ReactIR (Mettler-Toledo, Leicester, UK) can also be used to follow the course of a solution-phase reaction in real-time [36], and again could be adapted to follow the course of multiple parallel reactions.

Analytical constructs, which facilitate analysis of resin-supported chemistries by enabling cleavage of the resin-bound products labelled with an analytical enhancer, have found application in scanning the reactivities of solid-phase linkers [37]. The approach has recently been extended to include a UV chromophore in the enhancer, enabling both the detection and quantitative analysis of resin-bound product mixtures with single bead sensitivity [38]. Clearly, analytical constructs have great potential for use

in reaction scanning, both in parallel synthesis and split–mix-pool combinatorial experiments.

Finally, microarrays have begun to find application for reaction optimization, although they are currently restricted to a small range of chemical processes. Gao *et al.* [39] have shown how conditions suitable for the removal of acid labile protection groups from nucleosides could be screened using a digital light-controlled microarray platform. Similarly, Senkan and Ozturk [40] determined the optimal composition of a heterogeneous metal catalyst system for the dehydrogenation of cyclohexane to benzene in 2.5 days using a microreactor assembly. Microarray and microfluidic technology can be expected to develop rapidly to expand the scope of chemistries for possible study.

References

1 Reetz, M.T. (2001) Combinatorial and evolution-based methods in the creation of enantioselective catalysts. *Angew. Chem., Int. Ed. Engl.* 40, 284–310

2 Jandeleit, B. *et al.* (1999) Combinatorial materials science and catalysis. *Angew. Chem., Int. Ed. Engl.* 38, 2494–2532

3 Reetz, M.T. and Jaeger, K-E. (2000) Enantioselective enzymes for organic synthesis created by directed evolution. *Chem. Eur. J.* 6, 407–412

4 Bray, A.M. *et al.* (1995) Rapid optimization of organic reactions on solid phase using the multipin approach: synthesis of 4-aminoproline analogues by reductive amination. *Tetrahedron Lett.* 36, 5081–5084

5 Warmus, J.S. *et al.* (1998) Rapid optimization of an ICE inhibitor synthesis using multiple reaction conditions in a parallel array. *Bioorg. Med. Chem. Lett.* 8, 2309–2314

6 Menger, F.M. *et al.* (1995) Phosphatase catalysis developed *via* combinatorial organic chemistry. *J. Org. Chem.* 60, 6666–6667

7 Burgess, K. *et al.* (1996) New catalysts and conditions for a C-H insertion reaction identified by high throughput catalyst screening. *Angew. Chem., Int. Ed. Engl.* 35, 220–222

8 Bromidge, S. *et al.* (1998) A parallel combinatorial approach to locating homochiral Lewis acid catalysts for the asymmetric aza-Diels–Alder reaction of an imino dienophile. *Tetrahedron Lett.* 39, 8905–8908

9 Liu, G. and Ellmann, J.A. (1995) A general solid-phase synthesis strategy for the preparation of 2-pyrrolidinemethanol ligands. *J. Org. Chem.* 60, 7712–7713

10 Cole, B.M. *et al.* (1996) Discovery of chiral catalysts through ligand diversity: Ti-catalyzed enantioselective addition of TMSCN to *meso* epoxides. *Angew. Chem., Int. Ed. Engl.* 35, 1668–1671

11 Sigman, M.S. and Jacobsen, E.N. (1998) Schiff base catalysts for the asymmetric Strecker reaction identified and optimized from parallel synthetic libraries. *J. Am. Chem. Soc.* 120, 4901–4902

12 Francis, M.B. and Jacobsen, E.N. (1999) Discovery of novel catalysts for alkene epoxidation from metal-binding combinatorial libraries. *Angew. Chem., Int. Ed. Engl.* 38, 937–941

13 Emiabata-Smith, D.F. *et al.* (1999) A practical approach to accelerated process screening and optimisation. *Org. Process R&D* 3, 281–288

14 Jamieson, C. *et al.* (2000) A rapid approach for the optimisation of polymer supported reagents in synthesis. *Synlett.* 1603–1607

15 Porte, A.M. *et al.* (1998) Design and optimization of new phosphine oxazoline ligands via high-throughput catalyst screening. *J. Am. Chem. Soc.* 120, 9180–9187

16 Porter, J.R. *et al.* (2001) Enantioselective synthesis of arylamines through Zr-catalyzed addition of dialkylzincs to imines. Reaction development by screening of parallel libraries. *J. Am. Chem. Soc.* 123, 984–985

17 Berg, T. *et al.* (1999) A combined parallel synthesis and screening of macrocyclic lanthanide complexes for the cleavage of phospho di- and triesters and double-stranded DNA. *J. Comb. Chem.* 1, 96–100

18 Gao, X. and Kagan, H.B. (1998) One-pot multi-substrate screening in asymmetric catalysis. *Chirality* 10, 120–124

19 Taylor, S.J. and Morken, J.P. (1999) Catalytic diastereoselective reductive Aldol reaction: optimization of interdependent reaction variables by arrayed catalyst evaluation. *J. Am. Chem. Soc.* 121, 12202–12203

20 Löber, O. *et al.* (2001) Palladium-catalyzed hydroamination of 1,3-dienes: a colorimetric assay and enantioselective additions. *J. Am. Chem. Soc.* 123, 4366–4367

21 Kawatsura, M. and Hartwig, J.F. (2001) Transition metal-catalyzed addition of amines to acrylic acid derivatives. A high-throughput method for evaluating hydroamination of primary and secondary alkylamines. *Organometallics* 20, 1960–1964

22 Cooper, A.C. *et al.* (1998) Reactive dyes as a method for rapid screening of homogeneous catalysts. *J. Am. Chem. Soc.* 120, 9971–9972

23 Copeland, G.T. and Miller, S. (1999) A chemosensor-based approach to catalyst discovery in solution and on solid support. *J. Am. Chem. Soc.* 121, 4306–4307

24 Shaughnessy, K.H. *et al.* (1999) A fluorescence-based assay for high throughput screening of coupling reactions. Application to Heck chemistry. *J. Am. Chem. Soc.* 121, 2123–2132

25 Stambuli, J.P. *et al.* (2001) Screening of homogeneous catalysts by fluorescence resonance energy transfer. Identification of catalysts for room-temperature Heck reactions. *J. Am. Chem. Soc.* 123, 2677–2678

26 Boussie, T.R. *et al.* (1998) Solid-phase synthesis and encoding strategies for olefin polymerization catalyst libraries. *Angew. Chem., Int. Ed. Engl.* 37, 3272–3275

27 Reetz, M.T. *et al.* (1999) A method for high-throughput screening of enantioselective catalysts. *Angew. Chem., Int. Ed. Engl.* 38, 1758–1761

28 Guo, J. *et al.* (1999) Measurement of enatiomeric excess by kinetic resolution and mass spectrometry. *Angew. Chem., Int. Ed. Engl.* 38, 1755–1758

29 Reetz, M.T. *et al.* (1998) Time-resolved IR-thermographic detection and screening of enantioselectivity in catalytic reactions. *Angew. Chem., Int. Ed. Engl.* 37, 2647–2650

30 Reetz, M.T. *et al.* (2000) IR-thermographic screening of thermoneutral or endothermic transformations: the ring-closing olefin metathesis reaction. *Angew. Chem., Int. Ed. Engl.* 39, 1236–1239

31 Taylor, S.J. and Morken, J.P. (1998) Thermographic selection of effective catalysts from an encoded polymer-bound library. *Science* 280, 267–270

32 Reetz, M.T. *et al.* (2000) Super-high-throughput screening of enantioselective catalysts by using capillary array electrophoresis. *Angew. Chem., Int. Ed. Engl.* 39, 3891–3893

33 Ding, K. *et al.* (1999) Super high throughput screening (SHTS) of chiral ligands and activators: asymmetric activation of chiral diol-zinc catalysts by chiral nitrogen activators for the enantioselective addition of diethylzinc to aldehydes. *Angew. Chem., Int. Ed. Engl.* 38, 497–501

34 Copeland, G.T. and Miller, S. (2001) Selection of enantioselective acyl transfer catalysts from a pooled peptide library through a fluorescence based activity assay. *J. Am. Chem. Soc.* 123, 6496–6502

35 Yan, B. *et al.* (1999) A comparison of various FTIR and FT Raman methods: applications in the reaction optimization stage of combinatorial chemistry. *J. Comb. Chem.* 1, 46–54

36 Pagenkopf, B.L. *et al.* (1998) Mechanistic insights into Cu-catalyzed asymmetric aldol reactions: chemical and spectroscopic evidence for a metalloenolate intermediate. *Angew. Chem., Int. Ed. Engl.* 37, 3124–3126

37 Murray, P.J. *et al.* (1999) Rapid reaction scanning of solid phase chemistry using resins incorporating analytical constructs. *Tetrahedron Lett.* 40, 5609–5612

38 Williams, G.M. *et al.* (2000) Analysis of solid-phase reactions: product identification and quantification by use of UV-chromophore-containing dual-linker analytical constructs. *Angew. Chem., Int. Ed. Engl.* 39, 3293–3296

39 LeProust, E. *et al.* (2000) Digital light-directed synthesis. A microarray platform that permits rapid reaction optimization on a combinatorial basis. *J. Comb. Chem.* 2, 349–354

40 Senkan, S.M. and Ozturk, S. (1999) Discovery and optimization of heterogeneous catalysts by using combinatorial chemistry. *Angew. Chem., Int. Ed. Engl.* 38, 791–794