

# Reaction optimization by parallel kinetic studies with the FLUOstar Omega

James E. Redman  
Cardiff University, School of Chemistry, Main Building, Park Place, Cardiff, CF10 3AT, United Kingdom

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- Kinetic studies on the metallation of porphyrin
- Changes in the absorbance spectra monitored with the FLUOstar Omega
- Relative reaction rate in twelve different solvents determined in parallel

## Introduction

The yields of chemical reactions may vary considerably with parameters such as solvent, catalyst, temperature and reaction time. A challenge for the chemist is to optimize these parameters to obtain a satisfactory yield at acceptable time and cost. To aid optimization, a number of devices are available that increase the number of reactions that can conveniently be performed in parallel by an individual chemist. A method of reaction monitoring and analysis is then required, with thin layer chromatography (TLC) or LCMS being common choices. TLC in its simplest implementation is not quantitative, and highly polar non-volatile solvents may cause interferences. Although LCMS coupled with UV-visible detection is quantitative, an initial reaction quench and sample clean-up may be necessary to avoid damage to instrumentation and columns.

In situations where reactants and products differ in their molar extinction coefficients the reaction progress can be followed directly by UV-visible spectroscopy. Rather than determining the concentrations of species by physical separation and individual quantitation, a time-series of spectra in which these species vary in their relative proportions can be analysed mathematically accompanied by derivation of kinetic rate constants by least squares fitting.

Devices such as the Radleys carousel reactor allow 12 reactions to be performed simultaneously on one standard hot-plate stirrer. Reaction aliquots sampled as a function of time are therefore readily accommodated in the 12 columns of a 96-well microplate. Monitoring is ideally suited to the BMG LABTECH FLUOstar Omega microplate reader due to its ability to acquire a full UV-visible spectrum of each well in under one second.

In this example we have monitored the metallation of a porphyrin with  $Zn^{2+}$  (Figure 1) as a function of solvent. The porphyrin starting material and product are both highly coloured and the reaction proceeds with a perceptible but subtle colour change. The absorption spectra of the two species overlap significantly making a global least squares analysis of spectra desirable for quantitative rate comparisons.

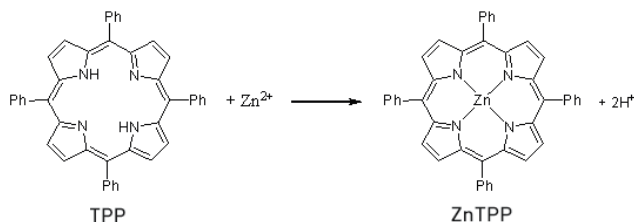


Fig. 1: Metallation of tetraphenylporphyrin (TPP) with zinc.

## Materials and Methods

- BMG LABTECH FLUOstar Omega microplate reader (Figure 2)
- Corning Costar flat bottom polypropylene 96-well microplates (cat. #3364)
- Radleys Carousel 12 Reaction Station, with magnetic stirrer
- Specfit/32 software (Spectrum Software Associates)



Fig. 2: BMG LABTECH's FLUOstar Omega

5,10,15,20-Tetraphenylporphyrin (TPP) was prepared by the method of Adler et al. and recrystallized from chloroform layered with methanol.<sup>1</sup> Solvents (*N,N*-dimethylformamide (DMF), toluene, chloroform, pyridine, isopropanol, acetone, *N*-methyl-2-pyrrolidone (NMP), dichloromethane, acetonitrile, tetrahydrofuran, ethylacetate and dimethylsulfoxide) were obtained from Acros or Aldrich.

Background scans of all wells of a microplate were made on the FLUOstar Omega using the UV/Vis absorbance spectrometer (20 flashes per well, 450 – 680 nm with 1 nm resolution).

TPP (2 mg) was placed with a stirrer bar in each of the 12 tubes of the carousel reactor. Solvent (10 mL) was added to each tube, which was stirred for ~15 minutes to allow dissolution of the porphyrin. Aliquots (50  $\mu$ L) were removed from each tube and diluted with DMF (200  $\mu$ L) in columns 1-12 of row A of the microplate, which was immediately scanned.

$Zn(OAc)_2 \cdot 2H_2O$  (obtained from Avocado) portions were weighed (12  $\times$  7 mg), then one portion added in quick succession to each carousel tube to initiate the reaction. After total times of 10, 30, 60, 120, 240, 480 and 1440 minutes, aliquots (50  $\mu$ L) were withdrawn from each tube, diluted with DMF (200  $\mu$ L) in a microplate row and scanned immediately. The microplate was removed from the reader between measurements.

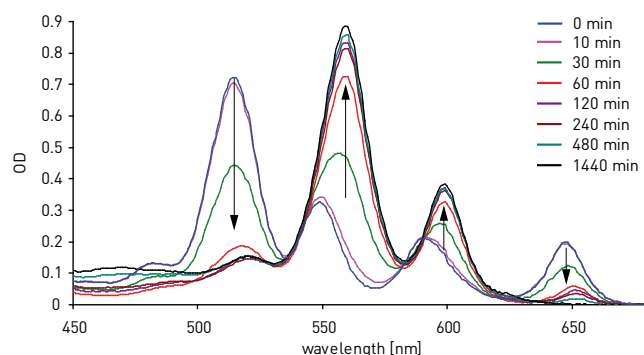
Spectral scans were exported as Microsoft Excel spreadsheets, the empty plate background subtracted and the absorbance at 680 nm set to zero for each scan by subtracting a constant offset at all wavelengths. The data were reformatted in Excel to create a separate table for each solvent with columns of absorbance values as a function of time at each wavelength. These data were subjected to singular value decomposition using the Specfit/32 software, followed by kinetic modelling according to the scheme  $TPP \rightarrow ZnTPP$  where both are coloured species with first order kinetics with respect to TPP. The  $Zn^{2+}$  concentration remains approximately constant due to the ten fold excess used, and no significant difference in the relative rates or quality of fit was observed if the reaction was modelled as  $TPP + Zn^{2+} \rightarrow ZnTPP$ , with colourless  $Zn^{2+}$  and first order with respect to both TPP and  $Zn^{2+}$ .

## Results and Discussion

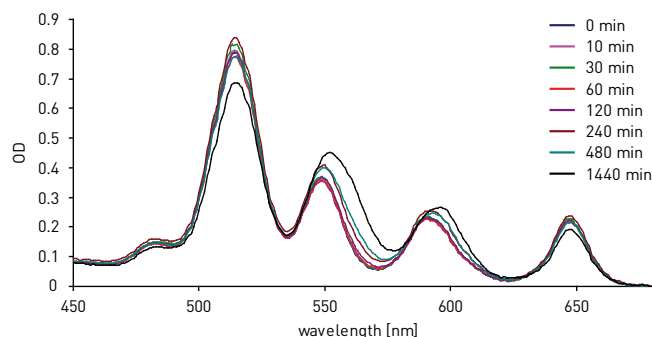
We anticipated that the rate of the metallation reaction of tetraphenylporphyrin (TPP) with  $Zn^{2+}$  (Figure 1) might show solvent dependence, due to differing degrees of solvation of the porphyrin and zinc salt and the possibility for bases to assist in porphyrin deprotonation. In preliminary experiments polystyrene microplates were found to be attacked too rapidly by most organic solvents, so we investigated the possibility of using flat bottom polypropylene storage plates as an alternative to costly and fragile quartz. At the visible wavelengths of interest, these plates proved to be sufficiently transparent, although it was found necessary to collect a background scan of each well of the empty plate due to well-to-well variation.

Twelve common laboratory solvents spanning a range of polarity and chemical properties were chosen for comparison. To combat the problem of high volatility of some of these solvents leading to rapid evaporation of small volumes and precipitation of porphyrin the reaction aliquots were diluted in relatively non-volatile DMF prior to measurement of visible spectra. Starting material and product were both found to dissolve adequately in DMF.

The reaction progress could be qualitatively assessed by visual inspection of spectra (Figure 3 and 4) using the MARS evaluation software, which revealed most rapid reaction in the halogenated solvents dichloromethane and chloroform and little change in *N*-methyl-2-pyrrolidone (NMP). Isopropanol and acetonitrile reactions gave lower maximum OD values than the other samples which can be attributed to poor solubility of the TPP starting material in these solvents. For DMSO a black precipitate was apparent in the sampled reaction aliquot, possible evidence of a side reaction.



**Fig. 3:** Changes in visible spectrum accompanying zinc metallation of TPP in chloroform. Arrows indicate the evolution of the absorption bands with time.



**Fig. 4:** Changes in visible spectrum accompanying zinc metallation of TPP in NMP.

To quantify the relative reaction rates in the different solvents, spectra were analysed using the Specfit/32 software. Spectra were subjected to singular value decomposition and globally fitted to a kinetic model in which the only coloured species were TPP and ZnTPP. The reaction was assumed to be first order with respect to TPP. There was no evidence from the factor analysis of more than two coloured species, and we were unable to observe the presence of an intermediate that had been suggested in the literature.<sup>2</sup> Acceptable fits to the model were obtained for all solvents (estimated errors <20%), with the exceptions of acetonitrile, isopropanol and DMSO. The relative rates deduced from this analysis are given in Table 1. Acetonitrile and isopropanol are excluded due to insolubility of the TPP starting material, and the value for DMSO should only be taken as approximate.

**Table 1:** Rates of reaction of tetraphenylporphyrin with  $Zn(OAc)_2 \cdot 2H_2O$  in different solvents, relative to the rate in *N*-methyl-2-pyrrolidone (NMP). Data are omitted for acetonitrile and isopropanol due to poor solubility of the TPP starting material. \*A black precipitate formed during this reaction.

Solvent	Relative initial rate	Solvent	Relative initial rate
DMF	8	NMP	1
Toluene	10	Dichloromethane	300
Chloroform	100	Tetrahydrofuran	30
Pyridine	2	Ethylacetate	30
Acetone	50	Dimethylsulfoxide <sup>a</sup>	~1

The metallation reaction is fastest in the two halogenated solvents, dichloromethane and chloroform. The rates in the highly polar solvents NMP, DMF and DMSO are amongst the slowest. The basic and aromatic solvent pyridine, which has been reported to catalyse  $Mg^{2+}$  metallation of porphyrins,<sup>3</sup> was less effective than non-basic toluene and two orders of magnitude slower than dichloromethane.

## Conclusion

A carousel reactor could be used in conjunction with the FLUOstar Omega to conveniently monitor the kinetics of twelve reactions in parallel. Polypropylene microplates were found to be compatible with measurement of visible spectra of organic solutions, and sampling and scanning of the twelve reactions could be achieved in three minutes. The ability of the FLUOstar to collect complete spectra enabled the use of singular value decomposition and global least squares fitting to analyse reaction kinetics. From a range of common laboratory solvents, the rate of metallation of TPP with  $Zn(OAc)_2 \cdot 2H_2O$  was found to be fastest in dichloromethane. The same principles will be applicable to optimization of other reactions which are accompanied by a change in UV-visible absorbance.

## References

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- 2) Fleischer, E. B.; Wang, J. H., (1960), *J. Am. Chem. Soc.*, **82**, 3498-3502.
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Germany: **BMG LABTECH GmbH** Tel: **+49 781 96968-0**

Australia: **BMG LABTECH Pty. Ltd.** Tel: **+61 3 59734744**  
France: **BMG LABTECH SARL** Tel: **+33 1 48 86 20 20**  
Japan: **BMG LABTECH JAPAN Ltd.** Tel: **+81 48 647 7217**  
UK: **BMG LABTECH Ltd.** Tel: **+44 1296 336650**  
USA: **BMG LABTECH Inc.** Tel: **+1 919 806 1735**

Internet: **www.bmglabtech.com** info@bmglabtech.com