Robotic Catalysis: A High-throughput Method for Miniature Screening of Mesoporous Metal Oxides

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Graphical Abstract
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Keywords
Mesoporous metal oxides; high-throughput catalysis; Echo liquid handler; Opentrons; 3D printing; oxidation of morin.

Abstract
The conventional method for heterogeneous catalyst discovery involves the synthesis and evaluation of a single catalyst at a time. This method is time-consuming and only a few catalysts can be prepared and evaluated successfully. We have developed a high-throughput system to synthesise and explore up to 96 heterogeneous catalysts at the same time. The system was developed using a standard glass plate and a customised 3D printed bottomless 96-well plate. Nano-droplets of mesoporous metal oxide (MMO) formulations and/or polyvinyl pyrrolidone (PVP) nanoparticle formulations were transferred to the glass plate using an acoustic liquid handler. The glass plate containing up to 96 droplets of formulae was subjected to different heat treatments (ageing at 80 °C, drying at 150 °C and calcination at 400 °C) to form the miniature mesoporous metal oxide catalysts. A customised 3D printed bottomless 96-well plate was fixed to the glass plate, to give 96 individual
wells, each containing a catalyst. This was achieved by using a glue dispensing robot and a slow curing epoxy glue. The catalysts were evaluated for the oxidation of morin dye, using hydrogen peroxide (H₂O₂) as an oxidant. Four catalyst plates were prepared (Co₃O₄, Au/Co₃O₄, Pd/Co₃O₄ and Co/Mn-MMO) to determine if different catalyst systems could be screened using this method. The observed reaction rates (k_{obs}) for each catalyst were calculated to identify the most active catalyst. The general method described herein requires microscopic amounts of catalysts with high degrees variability of the catalyst’s composition.

1. Introduction

The use of heterogeneous catalysis in industry has become popular in recent years. Heterogeneous catalysis has numerous advantages over homogeneous catalysis. In comparison with homogeneous catalysis, the heterogeneous system is a two-phase system (the catalyst being solid), making the catalyst recyclable and reusable, which is impossible with a homogeneous system since catalyst, substrate and products remain in the same phase [1]. This generates unnecessary waste increasing the environmental footprint. It is known that heterogeneous catalysts are often less efficient than a homogeneous catalyst. This problem is often overcome by supporting a homogeneous catalyst onto a solid support. This enables the catalyst to be as efficient as a homogeneous catalyst that can be recovered and recycled after each reaction [1]–[3]. Catalysts that can be supported include transition metal complexes, mono-and bimetallic nanoparticles [1], [3]. However, it should be mentioned that leaching of these catalysts from the support, even though small, may occur. This might lead to the deactivation of the catalysts, leading to a reduced turnover [4], [5].

Silica [2], carbon [6], clay, zeolites and metal oxides [7]–[9] are mostly used as heterogeneous catalysts or supports. Among these solid supports, mesoporous materials (with pores sizes ranging from 2 – 50 nm) are particularly interesting: increased surface area and uniformity of the pore network create spaces where the catalysts and reactants can interact [1], [10], [11]. The most general definition of porous materials (with pore sizes ranging between 1 and 100 nm) is the continuous and solid networks filled through voids, which can be filled with something such as a gas, liquid or sometimes even with solids [12].

These materials can be used for many applications which include catalysis, sorption, battery technology as well as for gas sensing and optics purposes [13]–[15]. Moreover, it has also been used for organic synthesis reactions such as Heck and Suzuki cross-coupling reactions [16]–[18], the reduction of p-nitrophenol [7], [19], [20], oxidation reactions such as the oxidation of CO [14], [21],
and hydroformylation of 1-octene [22]. Mesoporous metal oxide materials discussed here are used as oxidation catalysts. Mesoporous metal oxide materials including Fe, Co and Mn have mostly been used as oxidation catalysts for their stability at multiple oxidation states [14]. Not only have Co mesoporous metal oxide been used for oxidation reactions, but also as a support material [15] for noble metals as such Pd and Au nanoparticles.

Mesoporous metal oxides can be used as oxidation catalysts for the oxidation of pollutants (especially dyes) into a more environmentally friendly species. This is mainly important for aquatic ecosystems. Dyes are mostly used in the industry for textiles, paper, rubber, plastics, and cosmetics as a colouring agent. However, the pollution of the environment from these dyes is of great concern, since it affects the aquatic systems and food chain, even in low concentrations [23]. Efforts to remove polluting dyes from the environment includes process such as flocculation-precipitation, adsorption, solvent extraction [24] as well as oxidation of the dyes [15], [25]. Morin is a dye that is used in industry as a textile dye and the waste is released into the water, polluting the environment. It is therefore important to find a catalyst to efficiently degrade morin and its metabolites. The oxidation of morin can be evaluated using UV/Vis at the wavelength of $\lambda$ 410 nm. Morin is oxidised to morin oxide using the catalysts in the presence of hydrogen peroxide.

In this paper, cobalt oxide ($\text{Co}_3\text{O}_4$) and derivatives thereof, including a multicomponent mesoporous metal oxide derivative (Co/Mn) in catalytic amount were screened for the oxidation of morin. Subsequently, the activity of stabilised gold and palladium nanoparticles were assessed when immobilised within cobalt mesoporous materials ($\text{Au/Co}_3\text{O}_4$-MMO and $\text{Pd/Co}_3\text{O}_4$-MMO)

Conventional catalyst development and discovery is a tedious process and often based on trial-and-error [26]. Using a high-throughput and/or combinatorial approach, one can synthesise and evaluate multiple catalysts simultaneously, increasing the efficiency of the process [27], [28]. With this approach, a large number of new materials can be discovered. This is done by using different combinations of specific building blocks of atoms and/or molecules [29]. These methods were first established for early-stage drug discovery in the pharmaceutical industry, where libraries of compounds were screened against a target to identify a potential drug treatment [30]. These methods (parallel synthesis and screening) are being implemented to reduce the time and cost of compound optimisation from a hit with relatively low affinity with a protein, to a lead that can progress to a future clinical stage of the drug development. In recent years, these methods have also been adapted for the discovery and optimisation of catalysts [31]. The terms high-throughput and/or combinatorial
approach may also refer to the automated parallel or rapid sequential synthesis or evaluation of materials [32]. One of the drawbacks of combinatorial methods is the analysis and characterisation of the properties of the materials. This is due to the amount of each sample that is generated. The samples are miniaturised to evaluate more targets at once. This makes it extremely difficult to characterise and analyse the samples [30]. High-throughput screening methods have been applied in the fields of luminescence [33], [34], magnetoresistivity [35], ferroelectric/dielectric materials [36], and also solid-state catalysis [26].

The assessment of catalytic activity using a high-throughput method can be very problematic since it requires the unambiguous detection of a specific product above a small catalyst site. And using multiple catalysts in a library can make it even more difficult [26]. The first screening approach was reported by Moates et al., in 1996 [37]. They used a reactor capable of holding 16 catalyst pellets on a platform. The system was used to test the activity of metal catalysts for the oxidation of H₂. The activity of the catalysts was then analysed using infrared thermography. The technique used an IR camera for analysing the heat generated from the pellets once the reaction occurred. An active catalyst would produce an exothermic reaction which was captured and recorded by the IR camera [37], [38]. The drawback of this technique is that no information on the reaction products is given. Other techniques involve resonance-enhanced multiphoton ionization from the conversion of cyclohexane to benzene [26]. However, this study could not state the conversion, hence no quantitative data was given [38]. Another technique used for high-throughput screening of catalysts is microprobe sampling mass spectrometry [39].

Some of the initial high-throughput combinatorial catalysis involved the synthesis of crystalline compounds in libraries that can be identified using X-ray micro-diffraction. The reaction volume is in microliters which makes it possible to miniaturise the samples to obtain amounts of 50 – 150 μg [30]. These small volumes were pipetted manually or by a robot. Using this technique, more catalysts (100) can be tested simultaneously compared to the previous technique mentioned where only 16 catalysts are evaluated at a time. Recently, inkjet printing has become a popular technique to produce large numbers of materials in parallel. It has the potential to be used for combinatorial and high-throughput screening of different materials or catalysts [40]–[42].

Inkjet printing can be used for the synthesis of a large amount of material or material libraries in parallel. This technique has been used for the synthesis of many oxides including and indium-zinc tin oxide, Al₂O₃ [43], TiO₂ [44]–[47], graphene oxide sheets [48]. Nanoparticles (such as GaPd₂) have
been used as inks to print catalysts for the selective hydrogenation of acetylene [49]. Metal oxides and mesoporous metal oxide have also recently been synthesised using inkjet printing, for the high-throughput discovery of catalysts [48]. Multicomponent metal oxides have been printed for their catalytic activity potential, and also used for the “ultra-fast” catalyst discovery [40]–[42], [50], [51]. The main advantage of using inkjet printing is that a large number of materials or catalysts can be produced simultaneously. This makes it possible to construct libraries of catalysts to be used for combinatorial high-throughput screening purposes.

Inkjet printing can be used in parallel with a multidimensional group testing method. This was shown by Liu et al. in 2012, whereby they synthesised a multicomponent mesoporous metal oxide photocatalytic library, which was screened for hydrogen evolution activity [40]. Inkjet printing assisted synthesis of mesoporous oxide materials was used to synthesis a large library of multicomponent mesoporous metal oxides (eg. \( \text{Mg}_{0.1}\text{Ni}_{0.1}\text{Mn}_{0.1}\text{Cu}_{0.1}\text{Fe}_{0.1}\text{Co}_{0.1}\text{Ti}_{1.0}\text{O}_x \)). This technique can be used to synthesis up to 1 000 000 formulations in one hour using up to eight-component compositions.

In this paper, we demonstrate the design and use of a custom high-throughput setup for the parallel synthesis and screening of 96 mesoporous metal oxide oxidation catalysts. This proof of concept shows that a liquid handler designed for biological serial dilutions can also be customised for the high-throughput synthesis and screening of heterogeneous catalysts. More importantly, with acoustic dispensing (ECHO liquid handler), nano-amounts (as small as 2.5 nL droplets) of metallic species could be transferred to a glass surface in a 96-matrix format, suitable for plate type automated reactions. Finally, modification of the method highlighted here to 384 or even 1536 plate format, for which the instrument is compatible, it is conceivable to think that more than 1000 catalysts could be routinely screened. The ECHO acoustic liquid handler is mostly used in biological applications such as drug discovery [52], compound management, genomics research, synthetic biology, proteomics, and functional screening. Recently, this technique was used for the preparation of boronic acid libraries using an acoustic liquid handler [53]. This paper demonstrates that biological application and chemistry can be bridged. To the best of our knowledge, acoustic transfer has yet to be exploited for the synthesis of mesoporous metal oxide catalysts.
2. Experimental

2.1. Chemicals and materials:
Cobalt (II) nitrate hexahydrate (99%), Manganese (II) nitrate hexahydrate (99%), Pluronic surfactant F127, ethanol (absolute), ethylene glycol, K$_2$PdCl$_2$ (99%), AuCl$_3$ (99%) and polyvinylpyrrolidone (ave mol weight 10 000) were all purchased from Merck (formerly known as Sigma-Aldrich). Dimethyldichlorosilane and hydrogen peroxide (30%) were purchased from Fischer Scientific. All the chemicals were used as received without any further purification.

Lipidic cubic phase (LCP) sandwich glass plate set was purchased from Marienfeld. Ultem 3D printing filament was purchased from Stratasys. The slow-curing epoxy glue, manufacturer 3M, DP490 epoxy AD BK 50 mL CAR 12/CV, was purchased from RS Components (UK), RS stock no 189-3159, MFr part no DP-490 50ML 1.

2.2. Synthesis of mesoporous metal oxide (MMO) formulations:

The synthesis of the mesoporous metal oxides was adapted from Lui, et al. [40].

2.2.1. Synthesis of mesoporous cobalt oxide formulation:
The surfactant, F127 (1.6 g) was dissolved in ethanol (15 mL). Cobalt (II) nitrate hexahydrate (2.9 g, 10 mmol) was added to the mixture. The mixture was stirred until all the solids had dissolved. Ethylene glycol (15 mL) was added to the mixture and it was further stirred for 2 hrs.

2.2.2. Synthesis of mesoporous manganese oxide formulation:
The surfactant, F127 (1.6 g) was dissolved in ethanol (15 mL). Manganese (II) nitrate tetrahydrate (2.5 g, 10 mmol) was added to the mixture. The mixture was stirred until all the solids had dissolved. Ethylene glycol (15 mL) was added to the mixture and it was further stirred for 2 hrs.

2.3. Synthesis of polyvinyl pyrrolidone (PVP) stabilised nanoparticles (NPs):

2.3.1. Pd-PVP NPs:
The Pd-PVP NPs were synthesised in ethylene glycol serving the dual role of solvent and reducing agent [54]. The appropriate amount of K$_2$PdCl$_4$ (0.979 g, 0.003 mol) was weighed out to give a 0.3 M solution. It was dissolved in 10 mL ethylene glycol. PVP (0.28 g, average Mw = 10 000) was added to the mixture. The mixture was heated to 120 °C for 2 hours. During the heating process and
favoured by the presence of ethylene glycol, palladium species are formally reduced from Pd(II) to Pd(0), which can be observed by a change of colour of the solution from dark orange to a brown [54]. The synthesised particles formulations were used without further purification.

2.3.2. Au-PVP NPs:
The synthesis of the Au-PVP NPs was modified from the method used by State, et al [55]. A solution of PVP (0.51 g, average Mw = 10 000) in ethylene glycol (5 mL) was prepared. The mixture was stirred for 2 hours at 80 °C, to dissolve all the PVP. A solution of AuCl₃ (0.16 g) was prepared in water (2.5 mL). The Au solution was added to the PVP solution and the mixture was stirred for 15 min. The pH of the solution was adjusted to ~9 by the addition of NaOH (1 M, 2.5 mL). The solution was then heated to 100 °C for 2 hrs after which the nanoparticles formed, evidenced by the change of colour of the reaction mixture, from dark brown to burgundy.

2.4. Customised catalyst plate/s:
2.4.1. Dispensing of the catalyst formulation onto the glass plate:
Catalysts droplets were transferred from a source plate (containing the catalyst formulation and/or PVP-NPs, 384PP Labcyte) onto the glass plate (or destination plate) using a Labcyte ECHO 550 acoustic liquid handler following a 96-well plate template. Thereafter, the glass plate was subjected to different heat treatments to form the mesoporous metal oxide materials.

2.4.2. Heat treatment of the glass plate to form the catalyst plate:
After catalyst formulation transfer to the glass plate/s, the glass plate was placed into an oven for heat treatments of the catalysts. The catalyst plates were aged between 50 – 80 °C overnight. Then dried between 100 – 150 °C for 6 hours. The catalysts were finally formed after calcination of the plates at 400 °C for 4 hrs (using a ramped temperature of 1 – 2 °C/min).

2.4.3. Custom 3D printed catalyst plate:
A 3D model of a bottomless 96-well plate was designed using the Onshape software (academic license). The model was printed on a Stratasys Fortus 450 (F-450) 3D printer using Ultem (polyetherimide) printing material. The 3D print was fixed to the glass plate using a slow curing (4 – 6 hrs) epoxy glue (3M Scotch-Weld DP490). The glue was dispensed onto the 3D print using a 2D pattern with a Nordson EFD Pro 4 glue dispensing robot. The 3D print was fixed to the plate containing the calcined catalysts and was slightly pressed down to ensure an even distribution of the
glue around each well was achieved. The glued plate was left overnight to complete the curing process.

2.5. Characterisation of bulk and miniature catalysts:

2.5.1. The surface area analysis of the bulk Co$_3$O$_4$-MMO, Au/Co$_3$O$_4$-MMO, Pd/Co$_3$O$_4$-MMO and Co/Mn- MMO catalysts were determined using nitrogen sorption:

The surface area of the bulk samples was determined by the Brunauer-Emmett-Teller (BET) analysis. Before the analysis of the samples (approximately 20 mg) were weighed out and degassed under nitrogen at 200 °C overnight. The BET surface area, pore volume and pore size (diameter) analyses were carried out at a temperature of -196 °C using N$_2$ gas. The Brunauer-Emmett-Teller (BET) method was used to calculate the surface area. The pore size and volume were calculated using the adsorption-desorption curves using the Barrett-Joyner-Halenda (BJH) model (results are shown in the SI).

2.5.2. Transmission electron microscopy (TEM) imaging:

The mesoporous metal oxide and/or PVP-NP formulations (1000 μL x 96) were transferred to the glass plates. The catalysts were formed by subjection to the heat treatments and thereafter the catalysts were scraped off the plate and collected. The catalysts were then dispersed in deionised H$_2$O using a sonicator and deposited onto a copper TEM grid. The images were analysed using Transmission Electron Microscopy (TEM) JEOL JEM-2100.

2.6. Catalytic evaluation of the catalyst:

After the formation of the catalysts on the glass plate, the catalysts were evaluated for their potential oxidation activity. To each well, water (100 μL), aqueous morin dye (50 μL, 150 μM) and hydrogen peroxide (50 μL, 7.5 mM) were added using an Opentrons OT2 pipetting robot. The catalyst plates were evaluated for the activity in the oxidation of morin. The decrease in absorbance was measured at $\lambda$ 410 nm over time using a SpectraMax M2e microplate reader.

For the catalytic evaluation of the bulk Co$_3$O$_4$-MMO catalyst, the bulk sample was dispersed in deionised water (1 mg/mL) and a concentration of 0.05 mg/mL was used in a round bottom flask. A concentration of 150 μM and 10 mM was used for the morin and hydrogen peroxide, respectively. The reaction was evaluated for 1 hour by sampling (150 μL) every 10 min. The reaction was followed on a microplate reader (SpectraMax M2e) at a wavelength of $\lambda$ 410 nm.
2.7. **Data fitting:**
The catalytic data were fitted and processed using Kinetic Studio and Origin Pro 8.5 graphing and modelling software.

3. **Results**
To set up an efficient workflow for high-throughput catalyst evaluation, the synthesis of the catalysts should be done in a fast way and using small amounts. While difficult with conventional methods, a state-of-the-art acoustic liquid handler is capable of accurately and precisely dispensing multiple nano-droplets (2.5 – 5000 nL) of different formulations from a source plate (containing the catalyst formulations) into or onto destination plates of various format and material. For this study, a glass plate was used. After catalyst synthesis, a customised 3D printed bottomless 96-well plate was adhered over the catalysts, to subject each catalyst to an individual reaction. For this study, the catalysts were evaluated for the oxidation of morin.

3.1. **Morphology comparison between the miniature and bulk catalyst:**
Since the catalysts were synthesised using small volumes of the formulation/s (2.5 – 5000 nL), a morphology study was included to compare the miniature catalysts and the bulk catalyst. It had to be confirmed that the mesoporous metal oxides could be formed when small volumes of the formulation were used. Transmission electron microscopy (TEM) characterisation of bulk and miniature samples (Co$_3$O$_4$-MMO) was used to compare the morphology. From Fig. 1.Error! Reference source not found. and Fig. 2, it can be seen that the morphology of the bulk samples and miniature samples are comparable. This confirms that nano-droplets can be used to synthesise miniature mesoporous metal oxide catalysts. The same pore network can be seen from both the miniature and bulk samples. The images for the Au/Co$_3$O$_4$-MMO, Pd/Co$_3$O$_4$-MMO and the multicomponent Co/Mn-MMO miniature samples, are comparable to the bulk catalyst. This is illustrated in Fig. 3.
3.1.1. TEM imaging:

![TEM images](image1)

**Fig. 1.** Transmission electron microscopy (TEM) images to compare the bulk and miniature Co$_3$O$_4$-MMO catalysts. TEM images of the bulk Co$_3$O$_4$-MMO sample (A), and for the miniature Co$_3$O$_4$-MMO sample (B). Comparing the images of both samples it can be seen that the particles size is uniform, approximately 20 nm.

![TEM images](image2)

**Fig. 2.** Transmission electron microscopy (TEM) images indicating the pore networks for the bulk and miniature Co$_3$O$_4$-MMO catalysts. These images are from the bulk Co$_3$O$_4$-MMO sample (A), and for the miniature Co$_3$O$_4$-MMO sample (B). From these images, the pore network structures can be seen for both the bulk and the miniature samples.
Fig. 3. Transmission electron microscopy (TEM) images of the Co$_3$O$_4$-MMO derivatives. A comparison of the Au/Co$_3$O$_4$-MMO (A), Pd/Co$_3$O$_4$-MMO (B) and Co/Mn-MMO (C) miniature catalysts under the microscope. The particles sizes are comparable to the bulk sample, around 20 nm. The mesoporous channels can be seen in all three samples.

3.2. High-throughput catalyst screening system:

3.2.1. Nano-droplet transfers to the glass plate

For high-throughput catalyst screening, the system requires the parallel synthesis of the catalysts on a small scale. Small amounts of catalyst formulation/s were transferred onto a glass plate (treated with a silane solution) which was then aged, dried and calcined to form the catalysts in small amounts (Fig. 4). Up to 96 different catalysts can be synthesised in this way, however, for the initial study, we used one catalyst formulation (Co$_3$O$_4$) which was increased in volume on the glass plate, giving an increase in the concentration of the catalyst. This was done to show proof of principle, to show that up to 96 catalysts can be synthesised and evaluated for an oxidation reaction simultaneously. Thereafter, other plates including Au/Co$_3$O$_4$-MMO, Pd/Co$_3$O$_4$-MMO and Co/Mn-MMO, with increasing concentration of the variable, were synthesised.
3.2.2. The customised catalyst plate:
To analyse each catalyst on the glass plate, the catalysts should be separated into individual reactions. A custom bottomless 96-well plate was designed and 3D printed (using a chemically inert material) to be placed on top of the glass plate, over the already calcined catalysts, to separate the catalysts into different wells (Fig. 5). The bottomless 96-well plate was adhered to the glass plate using a slow curing epoxy glue. This glue was dispensed onto the well plate, in a specific pattern, using a Nordson EFD Pro4 glue dispensing robot. This robot ensured that the glue was dispensed accurately in between each well to secure the well plate onto the glass plate without leakages occurring between neighbouring wells.
3.3. Evaluation of the catalyst plates for the oxidation of morin:

To evaluate the activity of the glass supported Co$_3$O$_4$ mesoporous metal oxide species, a 96-matrix of an increasing amount of metal oxide transferred was prepared. After ageing, calcining and addition of the bottomless chemical resistant plastic well-plate, the morin reaction was set up using the conditioned mentioned in the experimental section. A decrease in the absorbance of morin was seen with the Co$_3$O$_4$-MMO plate indicating that this system could potentially be used to screen the activity of catalysts. Therefore, plates containing different derivates of Co$_3$O$_4$-MMO catalysts (Au and Pd immobilised nanoparticles, and a Co$_3$O$_4$/MnO$_x$-MMO) were subsequently synthesised and evaluated for activity in the oxidation of morin.

These plates include the addition of PVP nanoparticle in the MMO catalysts, Au/Co$_3$O$_4$-MMO and Pd/Co$_3$O$_4$-MMO and also a multicomponent MMO catalyst plate (Co/Mn-MM0). As with the Co$_3$O$_4$-MMO plate, the concentration of the variables (Au, Pd or Mn) was varied over 80 catalysts. The other 16 wells on each plate were used for the inclusion of a negative (the absence of a catalyst) and positive (0.025 mg/mL bulk Co$_3$O$_4$-MMO) control. Analysis of the rate of reaction (Fig. 6) highlights the following: (i) for all systems, the reaction rate was increased compared to the negative control, for which no catalyst was added. (ii) the rate when the reaction uses Co$_3$O$_4$-MMO synthesised on the glass plate is decreased compared to the positive control, 0.025 mg/mL bulk
Co$_3$O$_4$-MMO (synthesised traditionally) dispersed in deionised water by sonication before the addition into the custom catalyst plate. Moreover, the rate is also decreased (ca 2 folds) compared to the rate of bulk Co$_3$O$_4$ catalyst when determined using the conventional method (CM) in a round bottom flask. Even though the rate of the miniature Co3O4-MMO catalyst is decreased compared to the bulk, it should be mentioned that the concentration is much lower. This is both expected and interesting. Expected because the catalyst synthesised on the glass plate hasn’t been dispersed properly in the reaction mixture, hence fewer active sites are available. Interesting because it means that the activity of a screened catalyst will almost certainly be higher when scaled-up and/or properly dispersed by sonication. (iii) Cobalt and Pd/Co$_3$O$_4$ are not significantly increasing the reaction rate (ca 3-fold). (iv) Both Au/Co$_3$O$_4$-MMO and Mn/Co-MMO species increase the rate by more than 18-folds (18- and 20-fold, respectively).

![Figure 6](image.png)

**Fig. 6. Identification of the best catalyst from the rates.** The observed rates ($k_{obs}$) obtained for the best catalyst from each catalyst plate. Negative and positive controls were included for all the catalyst plates, and the average rate was calculated for each control and represent in the figure. The rate of reaction for the bulk Co$_3$O$_4$ catalyst determined using the conventional method (CM) in a round bottom flask (RBF) for 1 hour. A negative control (NC) was included to show that the reaction takes place slowly in the absence of a catalyst, and a positive control (PC, 0.025 mg/mL bulk Co$_3$O$_4$ dispersed in deionised water and added to empty well in the custom catalyst plate prior to the analysis in the plate reader) to show that Co$_3$O$_4$ is active in the oxidation of morin.
4. Discussion

4.1. High-throughput catalyst discovery

The mesoporous metal oxide catalysts were subsequently formed after a few heat treatments. Using a customised 96-well plate over the calcined catalysts, these catalysts were screened for the oxidation of morin. Generally, ethanol is used as a solvent for the mesoporous metal oxide formulations. However, droplets generated with the liquid handler was not stable on the glass plate. The formulations were, therefore, stabilised with ethylene glycol. The addition of the ethylene glycol to the formulation and the synthesis on a small scale (using nL droplets of the formulations) are factors that might influence the formation of the mesopores. Morphological and surface area (found in the SI) analyses were performed to determine the influence of these factors on the catalysts.

4.2. Morphological studies:

4.2.1. TEM imaging:

The morphology of the bulk and the miniature Co₃O₄-MMO catalysts were compared using TEM imaging in Fig. 1 and Fig. 2. From the images, it can be seen that morphology is comparable. The particle sizes for both catalysts are consistent around 20 nm. The porous networks can also be seen in both samples. This indicates that the catalysts can be formed on a small scale, by using nano-droplets generated by Echo acoustic liquid handlers. Moreover, the addition of the ethylene glycol also does not influence the formation of the mesoporous metal oxide catalysts.

The morphology of the other catalysts, Au/Co₃O₄-MMO, Pd/Co₃O₄-MMO and the Co/Mn-MMO, are also comparable to the bulk Co₃O₄-MMO catalyst. This can be seen in Fig. 3. The Au and Pd-PVP nanoparticles can be seen as the black spots on the MMO catalysts. Due to the high concentration of Au and Pd-PVP NPs that had to be used to achieve relatively low percentages (1% - 13% for Pd, and 0.3% - 4% for Au) of the Au and Pd supported onto the catalyst, some agglomeration can also be observed.

4.3. High-throughput catalyst screening system:

4.3.1. Glass as catalyst support

For biological application, high-throughput screening methods are generally achieved using commercially available microtiter well plates which are made of plastic. To synthesise the mesoporous metal oxide materials, plastic microtiter well plates cannot be used since the catalysts...
are formed at a high temperature (400°C). It was, therefore, necessary to use an alternative system to synthesise the catalysts in parallel. Moreover, the catalysts were screened for the oxidation of morin. This reaction was evaluated using UV/Vis spectroscopy. The decrease in absorbance of the morin is followed as oxidation takes place. Therefore, it was important to deposit the catalysts onto a material that was suitable for high temperatures and which was compatible to be used in a UV/Vis plate reader. The UV/Vis plate reader measures the absorbance of a particular solution in a microtiter plate. Moreover, this reading takes place from the bottom of the plate reader. Hence, to evaluate the catalyst of the oxidation of morin, a clear substrate was needed for the catalyst deposition, to enable the UV/Vis plate reader to analyse each well.

A glass substrate was the only appropriate material for this use since it can withstand a temperature of 400 °C, and it is a transparent material/substrate, which allows the UV/Vis light to penetrate and evaluate the reaction. An LCP sandwich set glass plate (Fig. S.12) was used as a substrate or support for the synthesis and evaluation of each catalyst activity. These LCP sandwich set glass plates are usually used for the screening of membrane proteins in lipidic mesophase.

4.3.2. 3D printed bottomless 96-well plate
A 96-well plate pattern was used for the deposition of the catalyst formulation on to the glass plate. A droplet was placed in the same position on the glass plate, as it would have been in a 96-well plate. Therefore, a custom bottomless 96-well plate was designed and 3D printed to be placed over the catalyst on top of the glass plate, to ensure that each catalyst was contained in an individual well (Fig. 5). A chemically resistant 3D printing filament (Ultem, polyetherimide) was used to print the 96-well plate. The custom 96-well plate was glued to the glass plate after catalyst calcination. A slow curing epoxy glue was dispensed onto the 96-well plate using a Nordson EFD Pro4 glue dispensing robot. This robot ensured that the glue was evenly dispensed in between each well. The plate was then fixed to the glass plate and left overnight for curing. Thereafter, the catalyst was evaluated for the oxidation of morin.

4.4. Evaluation of catalysts plates
Four catalysts plates were prepared for the evaluation of oxidation of morin. The morin dye is oxidised to only one product (A) during the first few minutes of the reaction, however upon over-oxidation two products are formed (B and C) (Fig. 7).
Fig. 7. Oxidation of morin. The figure shows the oxidation and over-oxidation of morin.

This was observed by Bingwa et al. [56] by the formation of one absorption band at λ 310 nm (with isosbestic points) which indicate the presence of only one product. However, over time it was seen that the isosbestic points disappeared indicating the formation of more than one product due to over oxidation. For this study, the oxidation of morin was evaluated over 2 hours which might lead to the over oxidation of the morin resulting in 2 oxidised products and not one.

Initially, a Co₃O₄-MMO plate with increasing volumes (ultimately increasing amount of catalyst formed, hence, increasing the concentration of the catalysts in the final reaction) of the formulation was used on the glass plate. This was used as a proof of concept, to show that miniature catalysts can be synthesised in such a way, and still be active in the oxidation of morin. Three other plates were prepared to determine if other variables can also be screened in this way, the concentration of each variable was increased over the plate. Nanoparticles (NPs) supported onto the Co₃O₄ and a multicomponent MMO was investigated. The plates included an Au/Co₃O₄-MMO and Pd/Co₃O₄-MMO for the NPs supported catalysts, and Co/Mn-MMO for the multicomponent MMOs. Negative and positive controls were included with each plate. The negative control was included to show that the reaction occurs slowly in the absence of a catalyst. The positive control showed that the Co₃O₄-MMO bulk catalyst can be used as a catalyst for the oxidation of morin.

The reagents for the oxidation reactions were added to each well using an Opentrons OT2 pipetting robot. The robot can accurately dispense the appropriate volume of water, peroxide (H₂O₂) and aqueous morin solution into each well before the analysis. Thereafter, the decrease in absorbance of the morin at λ 410 nm was followed using a UV/Vis plate reader. The reaction was followed for two hours with a reading interval of 2 min. The rate of reactions (kₐₒₛₜ) was determined from the decrease in absorbance over time. The best catalyst from each plate was identified by highest kₒₛₜ value (Fig. 6).
As expected, the negative control gave the lowest \( k_{\text{obs}} \) (0.01185 s\(^{-1}\)) value. This shows that the reaction takes place in the absence of a catalyst, however, slowly. The \( k_{\text{obs}} \) (0.09412 s\(^{-1}\)) value for the positive control is much higher (8 folds) than the negative control, indicating that the Co\(_3\)O\(_4\)-MMO bulk catalyst is active towards the oxidation of morin. The \( k_{\text{obs}} \) for Co\(_3\)O\(_4\)-MMO plate is lower than the positive control. Moreover, the rate was also decreased (2 folds) compared to the rate of the Co\(_3\)O\(_4\) bulk catalyst determined in a round bottom flask. Even though the rate for the miniature Co\(_3\)O\(_4\)-MMO catalyst is lower compared to the positive control and for the reaction in the round bottom flask, the concentration is also lower (25 and 50 μg/μL for the PC and CM, compared to 4.1 μg/μL for the miniature catalyst). This may indicate that the rate for the miniature catalysts might increase when the amount is upscaled or if the catalyst is properly dispersed in the reaction solution.

The positive control, as well as the catalyst in the round bottom flask reaction, were prepared by the dispersion of the bulk Co\(_3\)O\(_4\)-MMO using a sonicator bath. For the positive control, the dispersed catalyst was subsequently added to empty wells of each catalyst plate under investigation. Sonication ensures that all the particles are properly dispersed, making the entire surface area available for adsorption of reagents. When the particles are not sonicated and dispersed, it decreases the available active sites on the catalyst. The Co\(_3\)O\(_4\)-MMO plate was not sonicated. The particles were therefore agglomerated, which decreased the surface area, explaining why the \( k_{\text{obs}} \) might have been lower than expected. All plates were subsequently sonicated.

For the plate containing an incremental ratio of Au-PVP NPs (0.05 M, 0.4 to 4%) in a fixed (2500 nL) amount of Co\(_3\)O\(_4\) formulation the \( k_{\text{obs}} \) for the best identified Au/Co\(_3\)O\(_4\)-MMO catalyst (3.2%) is higher than the Co\(_3\)O\(_4\)-MMO and positive control. This indicates the Au supported onto the Co\(_3\)O\(_4\) catalyst shows a synergistic effect, increasing the rate [57]. The Au-PVP NPs increase the surface to volume ratio of the catalyst hence increasing the activity of the catalyst. This was previously shown by Ilunga et al., [57]. They showed a synergistic effect between Au/Pd supported nanoparticles on Co\(_3\)O\(_4\) in the oxidation of morin. Similarly, with the Pd/Co\(_3\)O\(_4\)-MMO plate, fixed amounts of the Co\(_3\)O\(_4\)-MMO (2500 nL) catalyst was used with an increasing amount of Pd-PVP NPs (0.3 M). The Pd loading was approximately 1 – 10%. The \( k_{\text{obs}} \) of the best-identified catalyst (11.8%) is slightly higher than the Co\(_3\)O\(_4\)-MMO catalyst but lower than the Au/Co\(_3\)O\(_4\)-MMO catalyst. This indicates that Pd-PVP NPs also increases the activity of the catalyst, however, not as much as the Au-PVP NPs.
A multicomponent MMO plate was also investigated and it contained Co/Mn-MMO catalysts. A fixed amount for the Co$_3$O$_4$ formulation was used and the Mn formulation was increased over the plate. From Fig. 6, it can be seen that the Co/Mn-MMO with (22% Mn) catalyst gave the highest $k_{obs}$. This is ascribed to a synergistic effect between the Co and Mn in the catalyst. Both catalysts are active, however, in combination, the $k_{obs}$ for the catalyst is increased considerably by approximately 20-fold.

The success in the parallel synthesis of the MMO catalyst on the glass plate, and the screening thereof implies that this high-throughput screening system can be used to identify the most active catalyst on the glass plate. It can also be developed further as a combinatorial high-throughput system, where multicomponent MMO catalysts can be synthesised and screened. The established workflow can be further standardised and accommodated to other reactions which will be reported in due course.

**5. Conclusion**

High-throughput miniaturisation of catalysis can be achieved by using microscopic amounts of mesoporous metal oxide formulations, for the miniature synthesis of heterogeneous mesoporous metal oxide catalysts. In this study, a workflow was set up for the rapid screening of miniature multi-metallic species of mesoporous metal oxides catalyst for their activity towards the oxidation of morin. This was conducted by using a combination of state-of-the-art acoustic liquid handling (Echo acoustic handler) as well as affordable (Opentrons and 3D printing) technologies. In this study, a customised 96-well plate was created by using a 3D printed bottomless plate fixed to a glass plate containing the catalysts. Catalysts such as Co$_3$O$_4$-MMO as well other derivatives (immobilisation of Mn, Au-PVP NPs and Pd-PVP NPs on the Co$_3$O$_4$-MMO) could also be synthesised and screened in this manner. The most active catalyst could be identified from the highest $k_{obs}$ value obtained. It can, therefore, be concluded that this high-throughput technique can be used for the simultaneous synthesis and evaluation of numerous catalysts, ultimately for catalyst screening for a specific reaction. This technique can be further developed for the screening of multicomponent mesoporous metal oxides using a combination of different metals. Moreover, as well as for the high-throughput synthesis of 96 different molecules State-of-the-art equipment such as the Echo acoustic liquid handler might not be available in many research groups, however, the same study can be conducted using an affordable liquid handling robot such as the Opentrons for the liquid handling for catalyst formulations.
6. References


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