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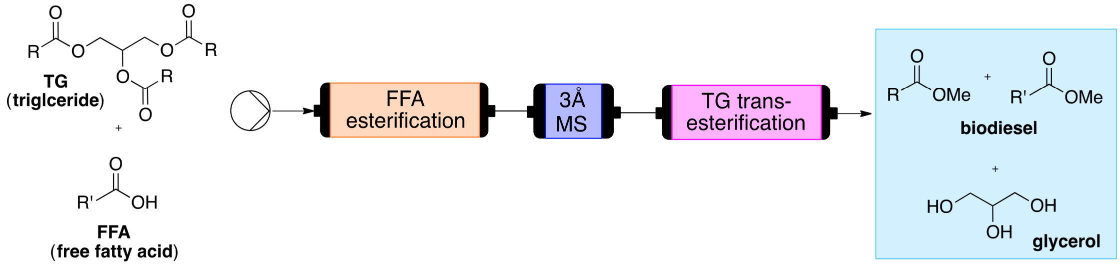

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ABSTRACT

The use of biodiesel derived from oils and fats is making an increased contribution as a renewable energy sources. The synthesis of biodiesel involves transesterification of triglycerides to the monoalkyl esters, a process often made challenging by the presence of free fatty acids (FFA). Herein an integrated flow system for the synthesis of biodiesel, employing a solid supported acid catalyst for esterification of FFAs, followed by transesterification using polymer supported *N*-heterocycle carbene (NHC) catalyst is reported. This system delivers biodiesel in high yields from a variety of fat and oils, using robust and recyclable catalysts.

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1. Introduction

As energy consumption shifts from fossil fuels, the use of biofuels is making an increased contribution to energy production. The EU has targeted the use of 10% biofuels in the transportation sector by 2020,[1] while US production of biodiesel is expected to exceed 6.4 million tonnes in 2016. Biodiesel is a fuel consisting of long chain monoalkyl esters derived from vegetable oils or animal fats. Biodiesel is an attractive replacement for petroleum based diesel, as the two fuels can be blended in any proportion and biodiesel can be used in existing diesel engines without modification. The use of biodiesel reduces emissions of particulate matter,[2] carbon monoxide and hydrocarbons by a significant amount (45–70%), although NO_x emissions are introduced slightly.[3,4] The lifecycle CO_2 emission of biodiesel are dependent on the feedstock material and land usage.[5,6] However, the use of third generation feedstock oils derived from algae have shown a 68% reduction in CO_2 emissions compared to traditional diesel fuel.[7]

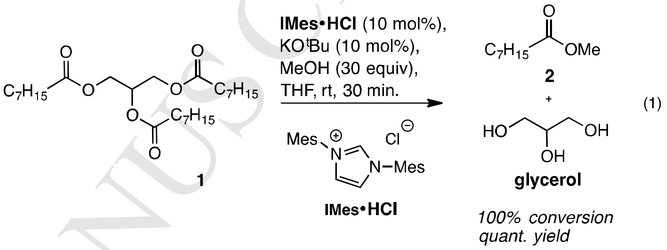
Although the first diesel engines demonstrated by Rudolf Diesel were powered by peanut oil the high viscosity of triglycerides means that they not ideal fuels, and are more commonly converted to the monoalkyl esters before combustion. This is typically achieved by transesterification to the methyl ester in the presence of a catalyst.[6] The most common method for transesterification employs a homogeneous Brønstead base catalyst, such as alkali metal alkoxides or hydroxides. Heterogeneous base catalysts such as metal oxides, zeolites, and hydrotalcites have also been extensively used, which can improve separation and recycling of catalyst from reaction mixtures.[8] Li and coworkers recently reported the synthesis of biodiesel from soybean oil, using an N-heterocyclic carbene (NHC) catalyst generated *in situ* by thermolysis of a NHC-CO_2 adduct.[9] This method employs low reaction temperatures and provides high yields of biodiesel when using oils and fats with low water, and free fatty acid (FFA), content (<1%). Unfortunately, many inexpensive feedstocks have significantly higher levels of FFAs, which neutralize the catalyst and lead to soap formation that can inhibit separation of the biodiesel from glycerol. In addition to base catalysts, homo- and heterogeneous acid catalysts have been used for the production of biodiesel.[6] These catalysts, while less sensitive to FFA, are typically less efficient, requiring higher temperatures and corrosion-resistant reaction vessels. In practice, many processes employ a two-stage procedure, where the fat or oil is first treated with an alcohol under acidic conditions to esterify the FFAs. This mixture of monoalkyl esters and triglycerides is then treated with a basic transesterification catalyst, producing a separable mixture of glycerol and biodiesel.

Continuous flow processes potentially offer a number of advantages in the production of biodiesel, allowing integration of multiple steps and ease of scale-up.[10] A number of continuous flow approaches to biodiesel production have been reported employing microwave heating,[11,12] ultrasonic reactors[13] or recirculating membrane reactors.[14] Herein, a new integrated continuous flow process for biodiesel production using fixed-bed catalytic reactors is reported. This process integrates esterification of FFAs over a solid acid catalyst with transesterification of triglycerides over a polymer-supported NHC catalyst.

2. Results and Discussion

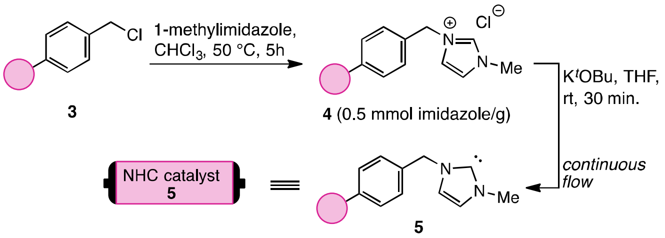
*2.1. Transesterification of triglyceride **1***

Studies began by demonstrating the NHC catalysed transesterification of triglyceride **1** with methanol. While the intension was to exploit an immobilized NHC catalyst studies commenced by examining batch transesterification of triglycerides. Concurrent reports by Hedrick/Waymouth[15] and Nolan[16] demonstrated that IMes is a highly efficient transesterification catalyst. Although subsequent studies were unlikely to exploit immobilized IMes (*vide infra*) it was decided to proceed with this catalyst as a model due to its use in the studies mentioned. Furthermore, these studies found that alkyl imidazole derived NHCs behaved similarly in the transesterification reaction. Pleasingly, with moderate modification to the reported conditions it was possible to convert triglyceride **1** to methyl ester **2** at room temperature in quantitative yield in less than an hour (Scheme 1).



Scheme 1 – IMes catalyzed transesterification of **1**

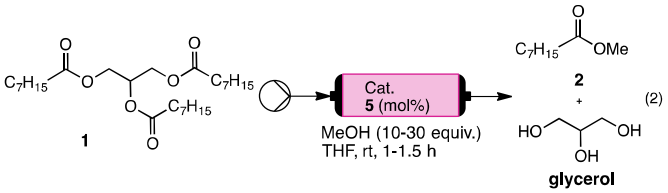
The solid-supported NHC catalyst **5** was prepared in two steps. Thus, commercially available Merrifield resin **3** was treated with 1-methylimidazole in $CHCl_3$ to yield polymer supported imidazolium chloride **4**[17] with around 0.5 mmol/g of imidazolium as determined by elemental analysis. Activation of **4** was achieved by passing a solution of KO^{t}Bu across the catalyst under continuous flow, followed by washing with THF (Scheme 2). To allow good solvent flow the catalyst was diluted with ground 3Å molecular sieves in glass Omnifit columns. All experiments were performed using a Vapourtec E-series equipped with temperature control and pressure monitoring.



Scheme 2 – Preparation of polymer supported NHC **5**

Optimization of the continuous flow transesterification began by examining the impact of catalyst loading on the reaction (Table 1). Increasing the catalyst loading from 5 mol% (Table 1, entry 1) to 10 mol% increased the yield while 20 mol% resulted in only marginal additional increase (Table 1, entries 2 and 3). Similarly increasing residence time from 30 min to 60 min gave a small increase in yield (Table 1, entry 4), while a further increase to 90 min showed no further improvement (Table 1, entry 5). Finally, increasing methanol content from 20 or 30 equivalents with 10 mol% catalyst led to quantitative conversion, and a 98% isolated yield of monoalkyl ester **2** (Table 1, entry 6).

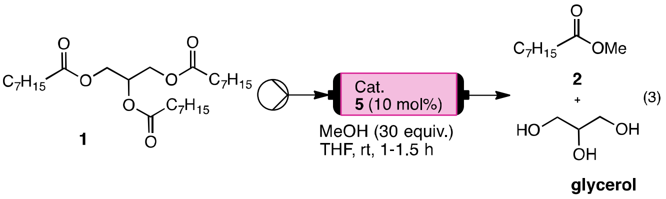
Table 1 – Optimisation of NHC catalysed transesterification



Entry	Cat. **5** (mol %)	Residence time (min)	Methanol (equiv.)	Conversion (%)^a	Yield (%)^b
1	5	30	10	84	80
2	10	"	"	90	87
3	20	"	"	91	88
4	10	60	"	92	87
5	"	90	"	92	88
6	**"**	**60**	**20**	**100**	**98**
7	"	"	30	100	98

^{a}Determined by GC analysis, ^{b}Isolated yield of **2** following column chromatography.

Having established conditions for the continiuous flow transesterification of triglyceride **1**, the stability of the polymer-supported NHC catalyst was examined. A column of catalyst **5** was subjected to five consecutive runs for the conversion of **1** to the monoalkyl ester **2** (Scheme 3, runs 1–5). Over five runs, a drop of 5–6% was observed in the conversion and yield, indicating that the solid catalyst **5** was largely stable to the reaction conditions. It was speculated that formation of channels through the catalyst bed might reduce the effective contact of the solution with the catalyst, and cause the small reduction in yield. To test this hypothesis, the NHC catalyst was removed under a nitrogen atmosphere then repacked into the reactor column. This reloaded catalyst was subjected to the same reaction conditions and the reaction repeated (Scheme 3, run 6), unfortunately its performance was not improved. Next the possibility the NHC catalyst was being deactivated by protonation was examined. Thus, the catalyst was reactivated with KO^{t}Bu (1.5 equiv) followed by washing with THF. These washings were performed until no spectrospocpic evidence for KOtBu was observed. This is necesssary as in control experiments it has been demonstrated that homogeneous transesterification can be achieved using KO^{t}Bu. After this treatment the transesterification was carried out 3 more times, displaying improved reactivity similar to the initial activity of the catalyst (Scheme 3, runs 7–9).



run	conversion (%)	yield (%)	run	conversion (%)	yield (%)	
1	100	98	6 (repacked)	97	94	K^{t}OBu, THF
2	100	98	7	100	98	
3	98	97	8	99	96	
4	97	95	9	97	96	
5	95	93				

Scheme 3 – Recyclability of NHC catalyst **5**

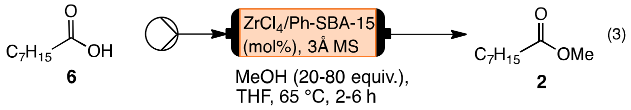
These results demonstrate good reactivity and reusability of NHC catalyst **5** for continuous flow transesterification. However, as expected, when triglycerides contaminated with FFA were examined with NHC catalysts **5** only trace amounts of the product were observed. To address this limitation our attention turned to the preparation of solid acid catalysts for the esterification of FFAs.

2.2. Solid acid catalysis for the esterification of FFAs

In order to achieve the esterification of free fatty acids in flow, a $ZrCl_4$ based catalyst was selected.[18] The activity of zirconium Lewis acids can be increased by deposition over a porous solid support, such as mesoporous silica. Hence, the active catalyst was prepared according to known procedures, by treating SBA–15 mesostructured silica with $ZrCl_4$ and $PhSi(OCH_3)_3$ in refluxing toluene.[19] Analysis of the resulting solid $ZrCl_4$/Ph–SBA–15 catalyst by inductively coupled plasma atomic emission spectroscopy (ICP–AES) confirmed the presence of 1.34 mmol of Zr per gram.

Esterification of FFAs was optimized by passing a solution of octanoic acid (**6**) and methanol in THF over a fixed-bed column of $ZrCl_4$/Ph–SBA–15 catalyst and ground 3Å molecular sieves at 65 °C. Varying the catalyst loading from 5 to 40 mol% showed an optimal loading of 20 mol% (Table 2, entries 1–5), while incrementally increasing the residence time from 2 to 6 hours showed no additional improvement beyond 4 hours (Table 2, entries 5-7). Finally, the amount of methanol was increased from 10 equivalents to 30 equivalents giving a 93% isolated yield of **2** (Table 2, entry 8). This level of conversion for pure octanoic acid was considered highly satisfactory, as the FFA content of vegetable oils is typically lower than 1%,[6] while high FFA feedstocks such as animal fats vary from 0.5% to 35%. The recyclability of the $ZrCl_4$/Ph–SBA–15 catalyst was tested by subjecting the catalyst column to 6 consecutive runs with octanoic acid (Table 3, entries 12-16). Although a reduction in yield was seen after each run, it should be noted that each of these runs would correspond to approximately 100 cycles using a typical feedstock with 1% FFA content.

Table 2 –$ZrCl_4$/Ph-SBA-15 catalysed esterification



6

2

(3)

Entry	$ZrCl_4$/Ph–SBA–15 (mol %)	Residence time (hours)	Methanol (equiv.)	Conversion (%)[a]	Yield (%)[b]
1	5	2	20	61	57
2	10	"	"	70	69
3	20	"	"	75	71
4	30	"	"	75	73
5	40	"	"	74	72
6	20	4	"	86	81
7	"	6	"	83	82
8	**"**	**4**	**30**	**96**	**93**
9	"	"	40	96	93
10	"	"	80	95	93
Recyclability					
12[c]	**"**	**4**	**30**	**95**	**90**
13[c]	"	"	"	93	91
14[c]	"	"	"	91	88
15[c]	"	"	"	81	77
16[c]	"	"	"	72	68

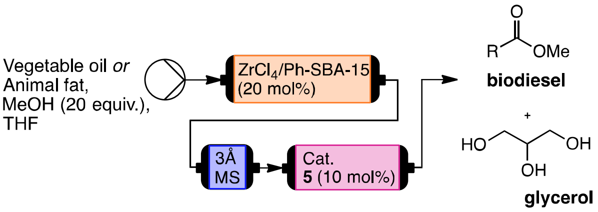
[a]Determined by GC analysis, [b]Isolated yield of **2** following column chromatography. [c]Repeat of entry 8

2.3. Integrated esterification/transesterification flow system

Having established methods for the esterification of FFAs over solid supported acid catalyst, and transesterification of triglycerides over polymer bound NHC catalyst **5**, an integrated system to convert oils and fats into biodiesel was developed. The $ZrCl_4$/Ph–SBA–15 column, and the NHC catalyst **5** were connected in series with an intermediate drying column containing ground 3Å molecular sieves. The synthesis of biodiesel in continuous flow was tested using a variety of feedstocks from vegetable oils and animal fats (Table 3). While a systematic examination of the impact of FFA on performance was not undertaken this study allowed materials with as little as 0.4% FFA (Entry 1) through to 1-2% FFA (Entries 8,9) to be examined. In all cases, complete conversion of the feedstock to the monoalkyl ester was observed and products were obtained in excellent yields (Table 3, entries 1-9), thus the system was robust across a range of application specific conditions.

The stability and recyclability of the catalysts was demonstrated by the use of a single batch of $ZrCl_4$/Ph–SBA–15 catalyst for all feedstocks tested (Table 3, entries 1–9). The NHC catalyst column was regenerated in-line every three runs by pumping a solution of KO^{t}Bu (2 equivalents) over 30 minutes. In addition, the reaction with beef fat was run on 30 g (35.5 mmol) scale using freshly prepared catalysts, resulting in a 96% isolated yield of biodiesel.

Table 3 – The synthesis of biodiesel in continuous flow



Entry	Feedstock	Conversion (%)	**FFA Content (%)**	Yield (%)
1	Rice Bran oil	100	0.4[20]	96
2	Beef fat	"	0.3-0.6[21]	99
3	Pork fat	"	0.5[21]	99
4	Olive oil	"	<0.8[22]	97
5	Canola oil	"	0.65-0.8[21]	100
6	Sunflower oil	"	0.8[23]	100
7	Peanut oil	"	0.9[21]	97
8	Sesame oil	"	1.3-1.8[24]	94
9	Grapeseed oil	"	0.5-2.6[25]	95
10	Beef fat (30 g scale)	98	0.3-0.6[21]	96

[a]Determined by GC analysis, [b]Isolated yield of **2** following column chromatography.

These results demonstrate an efficient method for the synthesis of biodiesel using integrated solid supported zirconium catalyst and polymer immobilised NHC catalyst **5** in continuous flow. Both catalysts were shown to be robust, and delivered biodiesel in high yields from a wide variety of feedstock. Further studies in this area are focused on improvements in the ease of preparation and efficiency of both the acid and base catalysts to improve the potential applicability of this system.

3. Experimental section

3.1. General Procedures for immobilized catalyst characterization and biodiesel quantification

The nitrogen content of the supported NHC catalyst was determined by elemental analysis according to AS1038.6.4, using a LECO Truspec CHN analyser. Bulk zirconium contents of the synthesized materials were determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Typically, 200 mg of sample were dissolved in aqueous aqua regia (HNO_3 + 3HCl, v/v) and heated at reflux temperature overnight. After dissolution and filtration, the sample was transferred to a 250 ml calibrated flask and diluted with water. An absorption standard solution of metal (zirconium or palladium, 1000 mg/L in water) was used to calibrate the equipment. X-ray powder diffraction patterns (XRD) were collected on a Philips X'pert diffractometer using the CuKα line. The XRD data were recorded in the 2θ range from 0.6° to 5.0° with a step size of 0.02° for low angle analysis and in the 2θ range from 5.0° to 50° using a step size of 0.04° for high angle analysis. Transmission electron micrographs (TEM) and elemental analysis by EDS (spot beam analysis) were collected in a Philips FEI Tecnai G2 T20 electron microscope operating a 200 kV. Fatty Acids Methyl Esters (FAME) were characterized using gas chromatography (GC) with flame ionization detection (FID) using a polyethylene glycol phase (Agilent Technologies, BP20; 25 m x 0.32 mm x 0.25 μm). Helium was used as the carrier gas and the gas line was equipped with an oxygen scrubber. The following temperature program was employed: 190 °C for 1 min and ramping at 10 °C min^{-1} and held at 260 °C for 4 min.

*3.2. Synthesis of supported NHC catalyst **5**.*

To a suspension of the Merrifield's peptide resin (3.0 g,~ 4.0 mmol Cl-/g) in dried $CHCl_3$ (30 mL) under a N_2 atmosphere 1-methylimidazole 98% (1.45 mL,18 mmol) was added at room temperature. The reaction temperature was increased to 50 °C and after 5 h the mixture cooled to ambient temperature. The resin beads (i.e. **4**) were filtered and washed with $CHCl_3$ (3 x 50 mL), ethyl acetate (3 x 50 mL), then dried under high vacuum. The transformation to NHC **5** was carried out in the flow system by passing KO^{t}Bu (2 equivalents in THF) over imidazolium **4** over 30 min under a N_2 atmosphere. During this process the colour of the column immediately changed from pale yellow to red.

3.3. Transesterification optimization (Table 1)

A reaction mixture containing glyceride trioctanoate **1** (2 mmol), methanol (1.28 g, 40 mmol, 20 equivalents) and THF (10 mL) was pumped through the NHC catalyst bed (0.50 cm^3, 400 mg, 0.2 mmol NHC, 10 mol% NHC). The NHC catalyst was diluted with the same volume of ground molecular sieve 3Å in order to decrease the back pressure. The flow rate of the flow system was set at 17

μL/min to deliver a residence time of 60 min. After removal of the solvent and methanol under reduced pressure the residue was washed through a very short silica gel plug using ethyl acetate/hexanes (1:99 v/v) to give, after evaporation pure biodiesel for GC analysis.

3.4. General procedure for synthesis of $ZrCl_4$/Ph–SBA–15

SBA–15 was synthesised following literature procedures[18,19] in which pluronic acid P123 (4.0 g) was dissolved in water (30 mL) and HCl (120 g of a 2M aqueous solution) with stirring at 35 °C for 2 h. Then tetraethylorthosilicate (TEOS) (8.0 g) was added to the solution with stirring at 35 °C for 20 h. The mixture was aged at 80 °C overnight without stirring. The solid product was recovered, washed and air-dried at room temperature for several hours. The white powder was calcined by slowly increasing temperature (1 °C /min) from room temperature to 500 °C in 8 h and heating at 500 °C for 6 h.

To prepare $ZrCl_4$/Ph-SBA-15 a suspension of SBA-15 (1.25 g) in toluene (50 mL) was treated with $ZrCl_4$ (910 mg, 3.90 mmol) then heated at reflux. After 2 h the reaction mixture was cooled to ambient temperature and then 0.51 ml (2.77 mmol) of $PhSi(OCH_3)_3$ was added and the resultant mixture refluxed. After 2 h, toluene was removed in vacuo and the residue was washed with toluene (3 x 50 mL) and then dried using high vacuum at 80 °C for 2 h to give the final product $ZrCl_4$/Ph-SBA-15 as a pale orange solid.

3.5. FFA esterification optimization (Table 2)

A reaction mixture containing octanoic acid **6** (4 mmol), methanol (3.84 g, 120 mmol, 30 equivalents) and THF (10 mL) was pumped through the $ZrCl_4$/Ph-SBA-15 catalyst bed (0.86 cm^3, 600 mg, 0.80 mmol Zr, 20 mol% Zr). The $ZrCl_4$/Ph-SBA-15 catalyst was diluted with 1.54 cm^3 of ground molecular sieve 3Å. The flow rate of the flow system was set 10 μL/min to serve residence time of 4h. After removal of the solvent and methanol under reduced pressure the residue was washed through a very short silica gel plug using ethyl acetate/hexanes (1:99 v/v) to give methyl octanoate for GC analysis.

3.6. General procedure for reactor testing of Biodiesel integrated flow system

Catalytic reaction runs were driven in a range of glass columns (Omnifit) fitted in a flow device (Vapourtec, E-series), equipped with temperature controller and pressure monitor. All vegetable feedstocks were purchased from the local super market. In the case of vegetable oils we evaporated them using rotary evaporator (equipped with oil bath) at 110 °C under reduced pressure to remove water content. To have a clear and dried feed from animal sources, animal tallow was purchased from local butchers and heated at 110 °C in a silicon oil bath for 1h. Then the solid portion was removed via filtration and the filtrate extracted using hexanes/ethyl acetate (95/7, v/v). The organic phase was dried over $MgSO_4$ followed by a second filtration and then evaporated using rotary evaporator at 110 °C under reduced pressure to remove water content. In a typical example, olive oil (5 g), methanol (5.5 g, 172 mmol) and THF (10 mL) was pumped through two catalysts beds with flow rate 11 μL/min and residence time 4 h. The first catalyst bed was containing $ZrCl_4$/Ph-SBA-15 catalyst (1.23 mL, 858 mg, 20 mol % of Zr + 1.22 mL of grinded molecular sieve 3Å) at 65 °C. After inline removal of the produced water in an intermediate molecular sieve 3Å column at room temperature, the reaction mixture was passed through the second column containing the supported NHC catalyst **46** (1.63 mL, 1.24 g, 10 mol% of NHC + 0.82 mL of ground molecular sieve 3Å) at room temperature. After removal of the solvents under reduced pressure the residue was washed through a very short silica gel column using ethyl acetate/hexanes (1:99, v/v) to give pure biodiesel for GC analysis. In experiments with larger amount of feed oil, the light phase of biodiesel was separated, washed with hot distilled water (60-65 °C), heated at 100 °C for 30 min then dried with anhydrous $MgSO_4$ before filtration.

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