

Cite this: *Green Chem.*, 2012, **14**, 1480

www.rsc.org/greenchem

PAPER

# Hydrolysis of chitosan to yield levulinic acid and 5-hydroxymethylfurfural in water under microwave irradiation†

Khaled W. Omari, Jessica E. Besaw and Francesca M. Kerton\*

Received 11th January 2012, Accepted 9th March 2012

DOI: 10.1039/c2gc35048c

A novel route has been developed that yields levulinic acid (4-oxopentanoic acid, LA) and 5-hydroxymethylfurfural (5-HMF) from chitosan. Hydrolysis of chitosan was performed in the presence of a range of Lewis acids with  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  providing the best results. All reactions were performed in sealed vessels under microwave irradiation at 200 °C for 30 min. Typical pressures achieved were 17 to 19 bar. 23.9 wt% LA was produced from 100 mg chitosan using 0.24 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  and 4 mL water. Under more dilute conditions, 10.0 wt% 5-HMF was obtained using 0.12 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  and 15 mL water. We propose that under more concentrated reaction conditions the 5-HMF formed reacts further to produce LA. When chitin is treated similarly, no 5-HMF is produced but up to 12.7 wt% LA can be obtained. For comparison, 32.0 wt% LA was produced from 100 mg glucosamine hydrochloride using 0.26 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  and 20 mL water. This corresponds to a yield of 59.4%. The  $\text{SnCl}_4$  forms  $\text{SnO}_2$  and HCl in solution and under similar conditions using  $\text{SnO}_2$  and HCl, chitosan formed 27.4 wt% LA.

## Introduction

Chitin is an important biopolymer that can be sourced from the ocean and is the second most abundant biopolymer on Earth after cellulose, Fig. 1.<sup>1,2</sup> The estimated annual production of chitin worldwide is about  $1.5 \times 10^5$  tons.<sup>2</sup> It is mainly available from crustaceans' shells such as crab, lobster and shrimp, and as

such it is an industrial waste material of fisheries and a renewable feedstock with much potential. Three steps are used to purify chitin from crustacean waste. These are (i) deproteinization using a strong base such as NaOH, (ii) demineralization using an acid such as HCl, and (iii) decolouration using a bleaching agent such as  $\text{H}_2\text{O}_2$ .<sup>2</sup> New methods are being developed that use green chemistry techniques in this field, for example, the use of ionic liquids.<sup>3</sup>

Chitin is deacetylated under alkaline conditions to yield chitosan. 100% deacetylation cannot be achieved and therefore, chitosan is a copolymer of glucosamine and *n*-acetylglucosamine.<sup>4</sup> It is readily available in a range of molecular weights and degrees of deacetylation,  $5 \times 10^4$  to  $2 \times 10^6$  Da and 40 to 98%, respectively.

In recent years, many useful chemicals have been produced from renewable feedstocks.<sup>5,6</sup> For example, catalytic conversions of cellulose,<sup>7–10</sup> fructose<sup>11–15</sup> and glucose<sup>16–21</sup> into 5-HMF and LA have been reported but amino-sugars and carbohydrates have been overlooked. The chemistry of chitin and chitosan should be investigated in concert with current studies on cellulose in order to achieve maximum benefits from the most abundant bio-feedstocks available. The processing and usage of these *N*-containing polysaccharides has been somewhat restricted over the years because they contain many hydroxyl groups that are able to form strong intra- and inter-molecular hydrogen bonds. Chitosan is insoluble in basic media and water.<sup>22,23</sup> Likewise, chitin is insoluble in water, most organic solvents, and dilute acidic or basic solutions.<sup>3</sup>

Despite the currently limited industrial applications of these biopolymers, their hydrolysis has been quite widely studied. They can be hydrolyzed using enzymes such as cellulase, hemicellulase, lysozyme, papain, pectinases and lipases to

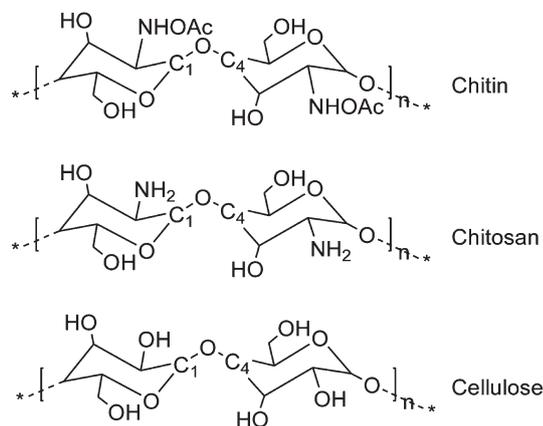


Fig. 1 Comparison of chitin, chitosan and cellulose formulae.

Centre for Green Chemistry and Catalysis, Department of Chemistry, Memorial University of Newfoundland, St. John's, NL, Canada, A1B 3X7. E-mail: [fkerton@mun.ca](mailto:fkerton@mun.ca)

† Electronic supplementary information (ESI) available: Graphs concerning typical temperature, pressure and power conditions for microwave-assisted reactions. Typical GC traces and mass spectra. SEMs. See DOI: 10.1039/c2gc35048c

produce glucosamine, *n*-acetylglucosamine and oligomers.<sup>24,25</sup> Chitinases depolymerize chitin to produce chito-oligosaccharides consisting of one to six *N*-acetylglucosamine (GlcNAc) subunits.<sup>25</sup> High yields of diacetylchitobiose (GlcNAc)<sub>2</sub> can be obtained through hydrolysis of colloidal chitin in dimethylsulfoxide and lithium chloride using *Vibrio furnissii* chitinase.<sup>26</sup> Chito-oligosaccharides can also be produced by chitin and chitosan cleavage using mineral acids. Depolymerization of chitosan in nitrous acid produces chito-oligosaccharides and 2,5-anhydro-D-mannose (M).<sup>25</sup> Cleavage of chitin in sulfuric acid in the presence of acetic anhydride produces *N*-acetylchito-oligosaccharide peracetates.<sup>25,27</sup>

However, aside from the production of simple sugars and oligosaccharides, there have been few reports on the production of chemicals from chitosan or chitin. One published example is the conversion of chitin to 5-(chloromethyl)furfural and LA using aqueous HCl and large amounts of 1,2-dichloroethylene.<sup>1</sup> The trimer (GlcNAc)<sub>2</sub>-M has been prepared by treating chitosan (fraction of *N*-acetylated units = 0.59) in HNO<sub>2</sub>.<sup>28</sup> This trimer was then reported to produce 1% 5-HMF upon further exposure to concentrated HNO<sub>2</sub>. Conversion of glucosamine (GlcN) to 5-HMF has been briefly reported by other researchers using organic acids in the presence of DMSO.<sup>29</sup> The current main use of glucosamine is as a dietary supplement and therefore some studies concerning its stability have been performed. Pyrolysis of glucosamine at 200 °C in the solid-state yields a mixture of furans, pyridines, pyrroles and pyrazines. The most abundant products 2-acetylfuran and 2-(2-furyl)-6-methylpyrazine were present at levels of only 0.063 mg per g of glucosamine pyrolyzed.<sup>30</sup> In aqueous solution, when glucosamine was heated to 100 °C, a similar mixture of products formed and around 20 mg of furan products were produced from 1 g of the sugar.<sup>31</sup> These results show that glucosamine has the potential to be a renewable feedstock for furans and *N*-containing heterocycles and that clearly, there is a need to further investigate the conversion of chitin and chitosan into useful renewable chemical building blocks.

We present here our attempts to hydrolyze chitosan in the absence of concentrated acids by generating superheated water under microwave conditions and enhancing the hydrolysis processes that occur by using a Lewis acid. Water is a clean, non-corrosive, non-flammable, renewable, readily available, cheap and environmentally friendly solvent.<sup>32,33</sup> Some of the advantages of using microwave heating are that it reduces reaction times and can increase product yields compared with conventional heating methods.<sup>34–36</sup> Microwave heating is particularly efficient for chemical transformations in water as it can be superheated in sealed vessels.<sup>35</sup> In our study, different parameters were varied, such as temperature, concentration, the Lewis acid used and reaction time. Two compounds were identified as the primary products from the hydrolysis of chitosan described herein: levulinic acid (LA) and 5-hydroxymethylfurfural (5-HMF). In addition, chitin under similar conditions produced LA. LA can be used to produce many compounds such as ethyl levulinate and 2-methyltetrahydrofuran that can be used as miscible diesel biofuel additives,  $\delta$ -aminolevulinic acid a herbicide and  $\beta$ -acetylacrylic acid, diphenolic acid and 1,4-pentanediol, which are polymer building blocks.<sup>37</sup> 5-HMF can yield other renewable building blocks such as 2,5-furandicarboxylic acid (FDCA), 2,5-

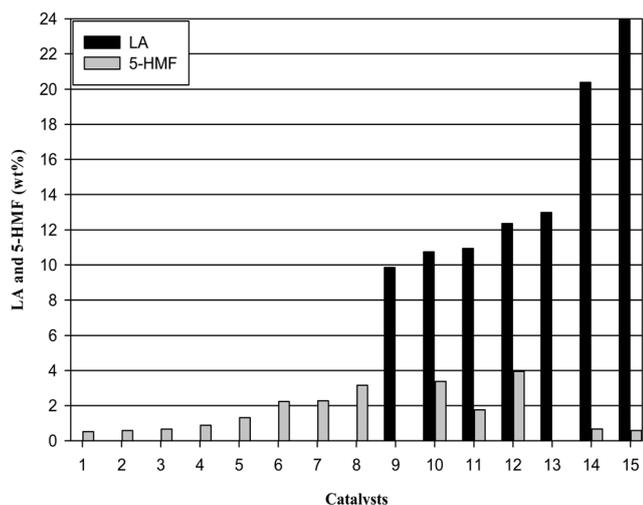
dihydroxymethylfuran, 2,5-bis(hydroxymethyl)tetrahydrofuran and 2,5-dimethylfuran. The latter is a promising liquid transportation fuel.<sup>17,38</sup> FDCA can be used in polyester production in the place of terephthalic acid.<sup>39</sup>

## Results and discussion

### Catalyst screening

Two series of reactions were performed initially to identify whether a catalyst was required to hydrolyze chitosan in superheated water under microwave conditions and whether an acidic or basic catalyst would give superior results. For each set of reactions, a control reaction (no catalyst) was performed and 21 catalysts were screened and the amounts of LA and 5-HMF produced from medium molecular weight chitosan were determined. The amount of chitosan processed under the two conditions was fixed at 100 mg. The first condition was 0.24 mmol catalyst and 4 mL deionized water. The second was 0.12 mmol catalyst and 20 mL deionized water. The potential catalysts that were assessed were anhydrous lanthanum trifluoromethanesulfonate La(CF<sub>3</sub>SO<sub>3</sub>)<sub>3</sub> (**1**), gadolinium trifluoromethanesulfonate hydrate Gd(CF<sub>3</sub>SO<sub>3</sub>)<sub>3</sub>·xH<sub>2</sub>O (**2**), ytterbium trifluoromethanesulfonate hydrate Yb(CF<sub>3</sub>SO<sub>3</sub>)<sub>3</sub>·xH<sub>2</sub>O (**3**), zinc perchlorate hexahydrate Zn(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (**4**), amberlyst 15 hydrogen form (**5**) which is an acidic resin, anhydrous indium(III) chloride InCl<sub>3</sub> (**6**), hydrochloric acid HCl (**7**), iron perchlorate hydrate Fe(ClO<sub>4</sub>)<sub>3</sub>·xH<sub>2</sub>O (**8**), nickel perchlorate hexahydrate Ni(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (**9**), zirconyl chloride octahydrate ZrOCl<sub>2</sub>·8H<sub>2</sub>O (**10**), copper perchlorate hexahydrate Cu(ClO<sub>4</sub>)<sub>3</sub>·6H<sub>2</sub>O (**11**), bismuth chloride BiCl<sub>3</sub> (**12**), chromium perchlorate hexahydrate Cr(ClO<sub>4</sub>)<sub>3</sub>·6H<sub>2</sub>O (**13**), zirconium tetrachloride ZrCl<sub>4</sub> (**14**), tin chloride pentahydrate SnCl<sub>4</sub>·5H<sub>2</sub>O (**15**), manganese perchlorate hydrate Mn(ClO<sub>4</sub>)<sub>2</sub>·xH<sub>2</sub>O (**16**), yttrium trifluoromethanesulfonate Y(CF<sub>3</sub>SO<sub>3</sub>)<sub>3</sub> (**17**), acetic acid CH<sub>3</sub>COOH (**18**), ammonia NH<sub>3</sub> (**19**), sodium hydroxide NaOH (**20**), and basic alumina Al<sub>2</sub>O<sub>3</sub> (**21**). These were chosen because the metal complexes are generally stable in water<sup>40</sup> and some of the other species, *e.g.*, amberlyst, have given good results for glucose/cellulose transformations.<sup>18</sup> HCl was studied because it is known to yield LA from chitin.<sup>1</sup> SnCl<sub>4</sub>·5H<sub>2</sub>O has been reported to yield 5-HMF from glucose in an ionic liquid.<sup>17</sup> Both the concentrated and dilute reaction mixtures were heated under microwave irradiation at 200 °C for 30 min. In the control reactions and in the presence of **16** to **21** neither LA nor 5-HMF were produced (details concerning the limit of detection and limit of quantitation levels for GC-MS determination of LA and 5-HMF are provided in ESI†). For the concentrated reaction mixtures (0.24 mmol catalyst, 4 mL water), Fig. 2 shows the weight percentages of LA and 5-HMF produced using catalysts **1** to **15**. 25 wt% LA corresponds to a mol% yield of 33.8 for 80% deacetylated chitosan.

Generally, the metal-containing catalysts gave superior conversions than the simple acids and bases. Acetic acid and the bases studied, **18–21**, yield no products, and HCl and amberlyst produced only a small amount of 5-HMF (<2.3%) and no LA. The reason for the poor performance of amberlyst may be insufficient swelling of the resin in aqueous solvents and also deposition of biopolymer on the surface of the beads, which was evident through SEM studies (Fig. S1 in the ESI†).

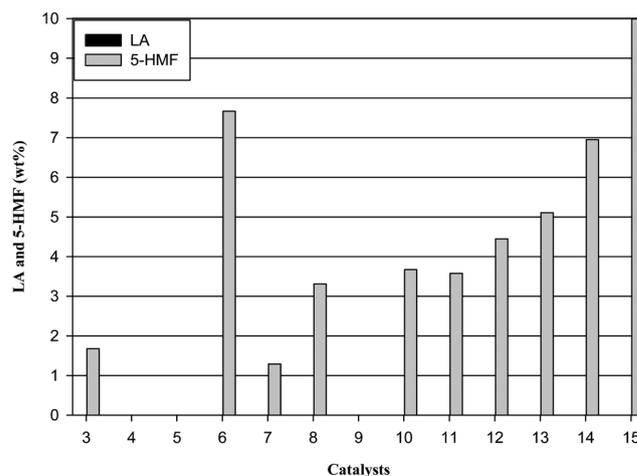


**Fig. 2** Comparison of weight percentages LA and 5-HMF produced under concentrated reaction conditions for a range of catalysts.

Of the metal salts, the triflate catalysts were also ineffective for this process, **1** to **3** produced small amounts of 5-HMF (<0.7%) and **17** was completely inactive. This may be due to the non-coordinating nature of these anions compared with chloride. The metal perchlorate Lewis acid catalysts were generally a little more effective and in most cases produced LA and/or 5-HMF, however, the Mn(II) salt (**16**) produced neither LA nor 5-HMF. The Fe(III) salt (**8**) produced only 5-HMF (3.16 wt%), whereas the Ni(II) salt (**9**) and the Cr(III) salt (**13**) produced only LA (9.9 wt% and 13.0 wt%, respectively). The Cu(II) salt (**11**) was the only metal perchlorate that produced LA and 5-HMF simultaneously (10.9 wt% and 1.8 wt%, respectively). These results indicate that the choice of metal ion is important in determining the selectivity of the hydrolysis reaction. However, in general, the overall yields of LA were strongly dependent on the anion and follow the reactivity trend:  $\text{CF}_3\text{SO}_3^- < \text{ClO}_4^- < \text{Cl}^-$ .

Under concentrated conditions, the metal chloride Lewis acid catalysts generally produced the largest amounts of LA and 5-HMF.  $\text{InCl}_3$  (**6**) produced a small amount of 5-HMF (2.2 wt%) but no LA was produced.  $\text{BiCl}_3$  (**12**) produced LA (12.4 wt%) and the largest amount of 5-HMF (4.0 wt%) under these conditions amongst the catalysts screened.  $\text{ZrCl}_4$  (**14**) and  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  (**15**) produced the largest amounts of LA (20.4 wt% and 23.9 wt%, respectively).  $\text{ZrCl}_4$  is known to react with water to produce  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  (**10**) and HCl (**7**).<sup>41</sup> As a result, we examined catalyst **10**, which produced a moderate amount of LA and 5-HMF (10.8 wt% and 3.4 wt%, respectively). However, neither  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  nor HCl gave the largest amounts of LA and 5-HMF under the conditions studied. Thus indicating that this process is catalyzed more effectively by a suitable Lewis acid rather than a Brønsted acid. The largest amounts of LA (23.9 wt%) and 5-HMF (3.95 wt%) produced in this set of reactions came from those using  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  (**15**) and  $\text{BiCl}_3$  (**12**), respectively.

For the more dilute reaction conditions screened (0.12 mmol catalyst, 20 mL water), Fig. 3 summarizes the weight percentages of LA and 5-HMF produced using the catalysts screened. Under



**Fig. 3** Comparison of weight percentages LA and 5-HMF produced under dilute reaction conditions for a range of catalysts.

these conditions, a smaller number of catalysts were effective, namely, **3**, **6–8**, and **10** to **15**.

Interestingly, under these dilute conditions, no catalyst was ever able to produce LA indicating that LA production is strongly dependent on reaction concentration. Catalysts **1**, **2**, **4**, **5** and **9** although able to hydrolyze chitosan to 5-HMF or LA (in the case of **9**) under concentrated conditions, did not yield any 5-HMF under these more dilute conditions. HCl (**7**) was the only conventional (Brønsted) acid or base that produced 5-HMF (1.3 wt%) but this was a significantly smaller amount than that generated by some of the Lewis acids studied. Also,  $\text{Yb}(\text{CF}_3\text{SO}_3)_3 \cdot x\text{H}_2\text{O}$  (**3**) was the only metal triflate that produced 5-HMF (1.7 wt%), whereas under more concentrated conditions  $\text{La}(\text{CF}_3\text{SO}_3)_3$  (**1**) and  $\text{Gd}(\text{CF}_3\text{SO}_3)_3$  (**2**) were also active. Fe(III) (**8**), Cu(II) (**11**) and Cr(III) (**13**) perchlorates produced 5-HMF (3.3 wt%, 3.6 wt% and 5.1 wt%, respectively) in similar quantities to those afforded under concentrated conditions. However, no LA was produced. Metal chloride Lewis acid catalysts yielded larger amounts of 5-HMF under dilute conditions compared with the more concentrated reaction mixtures. It is worth noting that  $\text{InCl}_3$  (**6**), in particular, was a much more effective catalyst under dilute reaction conditions, yielding 7.7 wt% 5-HMF compared with 2.2 wt% under concentrated conditions. Under both concentrated and dilute reaction conditions  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  (**15**) afforded the highest conversions, to 23.9 wt% LA (concentrated conditions) and 10.0 wt% 5-HMF (dilute conditions). At this point it is worth noting that  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  is a relatively cheap and easily handled reagent compared to some of the other species studied.

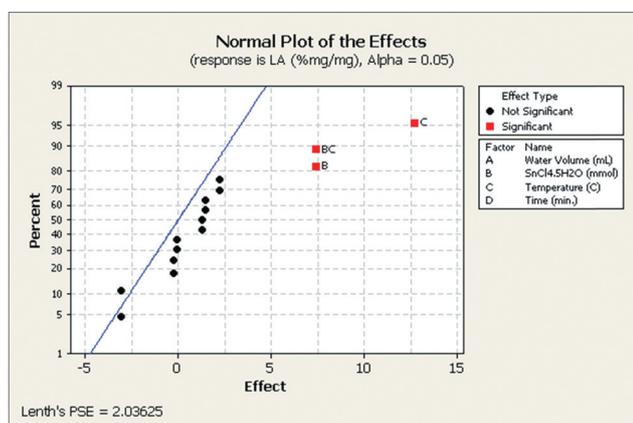
#### 2<sup>4</sup> Full factorial designs

In general, several factors are varied in order to optimize experimental conditions. Chemists can use factorial design (FD) to understand the interactions between factors and thereby obtain a full picture of information and optimize a chemical reaction. If each factor is studied at two different levels (high and low), FD is expressed as  $2^k$ , where  $k$  is the number of factors.<sup>42</sup> FD has been practiced in many catalytic research applications.<sup>43–46</sup>

**Table 1** Weight percentage of LA and 5-HMF produced from 100 mg chitosan in the presence of  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  (**15**) using  $2^4$  factorial designs

Water volume (mL)	$\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ (mmol)	Temperature (150 °C)		Temperature (200 °C)	
		Time (15 min)	Time (30 min)	Time (15 min)	Time (30 min)
Yield LA (wt%) <sup>a</sup>					
4	0.12	ND	ND	10.2 (6.2)	10.9 (4.9)
	0.24	ND	ND	18.0 (1.0)	23.9 (0.6)
20	0.12	ND	ND	0 (8.8)	0 (10.0)
	0.24	ND	ND	16.8 (3.3)	21.6 (3.6)

<sup>a</sup> ND = not detected. Value in parentheses is the wt% 5-HMF produced concomitantly.

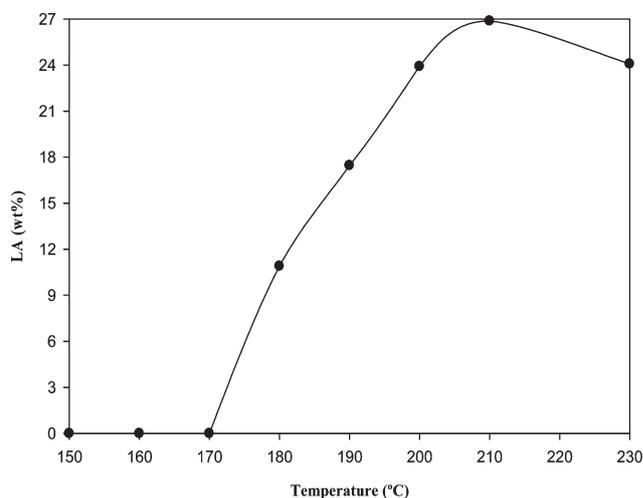
**Fig. 4** Normal plot of the effects on the weight percentage of LA.

In order to optimize the reaction conditions using  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  (**15**), we applied  $2^4$  FD by varying the following four factors: volume of water, catalyst loading, temperature and reaction time to study all effects and their interplay at once. Table 1 shows weight percentages of LA and 5-HMF produced at different levels for each variable. The low and high levels of each factor were determined from preliminary experiments.  $2^4$  FDs were studied using Minitab software. We used Lenth's analysis to analyze our FD (an alternative procedure to a normal plot). We studied 15 effects. In order to determine the significant effects out of the 15 effects, pseudo standard error (PSE) should be calculated to determine the margin of error (ME). After calculating the median ( $m$ ) of the absolute values of the effects, let  $s = 1.5 \times m$ . The median ( $m_o$ ) of absolute values that are less than  $2.5 \times s$  were calculated. PSE is then equal to  $1.5 \times m_o$ . ME is when an effect lies within 95% confidence interval. Therefore, ME is given by  $t_{(1-\alpha/2), df} \times \text{PSE}$ . Where  $t$  is  $t$ -distribution,  $\alpha = 1-95/100$  and  $df$  is the degree of freedom that is equal to number of effects/3.<sup>47</sup>

### Effects on LA production

The significant effects on LA production as shown in Fig. 4, when  $\alpha = 0.05$  and  $\text{ME} = 5.23$ , are the catalyst amount (B) and the temperature (C) factors.

Based on the data generated by the  $2^4$  FD experiment, we changed the significant factors keeping the water volume constant at 4 mL and the time constant at 30 min. Temperature was

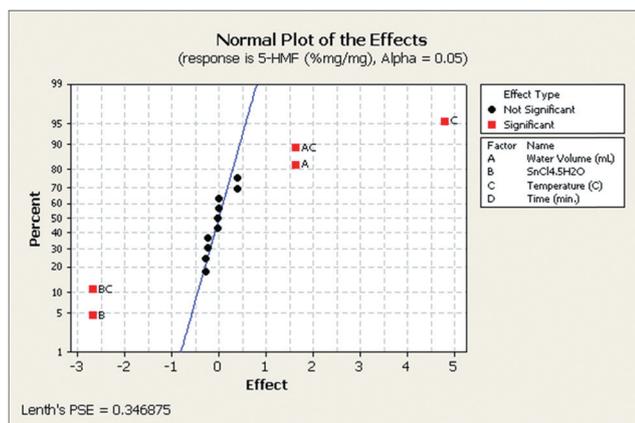
**Fig. 5** Effect of temperature on the weight percent LA produced from 100 mg chitosan in 4 mL water with 0.24 mmol of  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ .

varied between 150 and 230 °C while the catalyst loading was kept constant at 0.24 mmol. Fig. 5 shows that no LA was produced at lower temperatures (150–170 °C). However, the amount produced increased almost linearly from 170 °C to 210 °C and then levelled out.

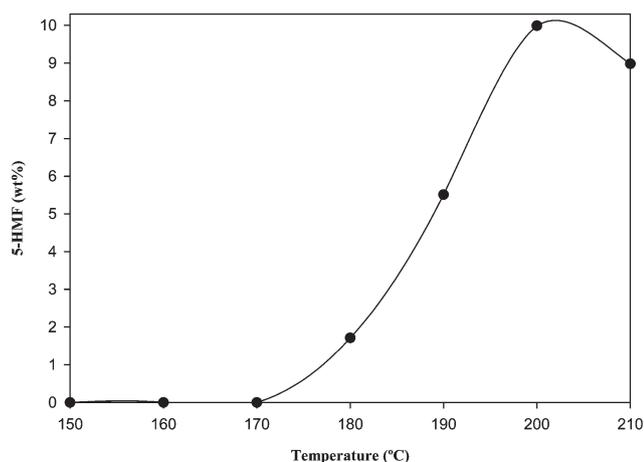
Additional experiments were then performed in order to determine if any of the other variables would have a significant effect on the amount of LA obtained. Doubling the amount of catalyst to 0.48 mmol and performing the reaction at 200 °C yielded 26.6 wt% LA compared with 23.9 wt% at tin levels of 0.24 mmol. Performing the hydrolysis reaction at 210 °C at high tin levels (0.48 mmol), yielded 28.6 wt% LA. Actually, increasing the time did not have a positive effect on the wt% of LA. For example, the optimum condition at 30 min reaction time produced 23.9 wt% LA, which increased to reach 26.0 wt% at 60 min. Therefore, although close to 30 wt% LA could be obtained by increasing reaction time and the amount of tin used, the effects of these changes are not significant when compared with the effect of temperature as shown in Fig. 5.

### Effects on 5-HMF production

A similar factorial design experiment was performed in order to optimize the reactions under dilute conditions and the production of 5-HMF from chitosan. The significant effects, when  $\alpha =$



**Fig. 6** Normal plot of the effects of factors on the weight percentage of 5-HMF produced during microwave-assisted tin-catalyzed hydrolysis of chitosan.



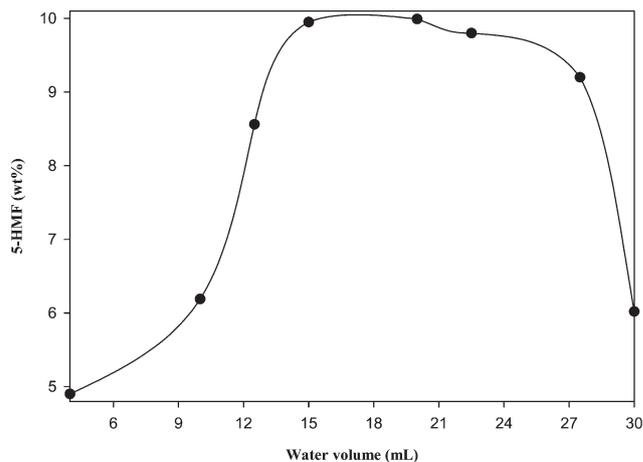
**Fig. 7** Effect of temperature on weight percent 5-HMF produced under dilute reaction conditions (100 mg chitosan, 20 mL water and 0.12 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ ).

0.05 and  $\text{ME} = 0.89$ , are volume of water (A), catalyst amount (B) and temperature (C) factors as shown in Fig. 6.

Based on the data from the factorial design experiment, we changed the significant factors keeping the time constant at 30 min. The effect of temperature on the production of 5-HMF was investigated between 150 and 210 °C while the volume of water was kept constant at 20 mL and the catalyst amount maintained at 0.12 mmol. Fig. 7 shows that no 5-HMF was detected for reactions conducted at 170 °C or lower. Between 170 and 200 °C, 5-HMF production increased linearly but decreased above this temperature. This decrease is likely due to more rehydration reactions occurring at higher temperatures to yield LA from the 5-HMF initially generated.

Catalyst loading was studied at 0.06 and 0.24 mmol of Sn in 20 mL water at 200 °C and the percentages of 5-HMF were 6.3 and 3.6, respectively. This shows that the highest 5-HMF production was at 0.12 mmol, which yielded 10 wt% 5-HMF.

The effect of dilution on production of 5-HMF was studied by performing reactions in 4.0 to 30.0 mL water at a constant temperature of 200 °C and a catalyst loading of 0.12 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ . Fig. 8 shows that the weight percent of 5-HMF



**Fig. 8** Effect of dilution on weight percent 5-HMF produced (100 mg chitosan, 0.12 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ ).

produced increases from 4 mL until 15.0 mL as conditions become more dilute and then levels out. When the volume of water used was 15.0 mL or 20.0 mL, the amount of 5-HMF produced was similar. Further dilution of reactions mixtures, *e.g.* 30 mL water, was detrimental in terms of wt% 5-HMF obtained.

In order to examine the significant effects proposed from FD analysis for 5-HMF production (*i.e.* AC, volume and temperature, and BC, [Sn] and temperature, Fig. 6), we compared the wt% 5-HMF produced from simultaneously varying two factors. Increasing the volume of water and temperature, whilst keeping [Sn] constant at 0.12 mmol, produced moderately less 5-HMF: 15 mL water at 200 °C yielded 10.0 wt% 5-HMF, Fig. 8 and 20 mL water at 210 °C yielded 9.0 wt% 5-HMF, Fig. 7. In contrast, simultaneously increasing the temperature from 200 to 210 °C and decreasing the catalyst loading from 0.12 to 0.06 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  (in a constant volume of 15 mL  $\text{H}_2\text{O}$ ) caused a significant decrease in the amount of 5-HMF observed (4.6 wt%) in line with changes predicted by FD.

In summary, the optimum conditions for 5-HMF production (9.9 wt%) were microwave irradiation of 100 mg chitosan in 15 mL water with 0.12 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  at 200 °C for 30 min.

### Conventional heating compared with microwave irradiation

As microwave heating, at present, is less widely used than conventional heating methods, efforts were made to study the reactions using conventional heating (stirrer-hotplate) with the same optimised catalyst loadings and volumes of water as described above. The reaction times and temperatures studied were chosen using the Biotage Prediction Chart and Time Converter in reverse,<sup>48</sup> and as such similar yields of products would be predicted. The two mixtures (concentrated and dilute) were heated at 100 °C in a round-bottomed flask equipped with a reflux condenser. This conventional heating process produced 12.08 wt% LA after 9 days from 100 mg chitosan, 4 mL water, 0.24 mmol  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ . This amount is almost half of that obtained *via* microwave irradiation. In attempts to yield 5-HMF using the optimum conditions from the microwave experiments, no product was obtained after 9 days of conventional heating. Reactions of chitosan at atmospheric pressure using microwave

heating under both concentrated and dilute conditions were performed (100 °C, 2 h, CEM Discover SP system). Unfortunately, LA and 5-HMF were not produced. These results suggest a critical role for pressure in the reactions and they may be occurring under near-critical water conditions.

### Studies using chitin as the feedstock

In our studies, we have found that chitin can also be used to produce LA. Different sources of chitosan and chitin were tested under the optimum conditions of LA production that were determined using medium molecular weight chitosan. Table 2 summarizes these results. Chitin produced an average of 12.3 wt% LA and no differences were seen between chitin from crab or from shrimp. Similarly, the amount of LA produced from different sources and molecular weights of chitosan resemble each other (average 24.2 wt%). This was somewhat surprising given the tough appearance of high molecular weight chitosan. Generally, from our studies it can be seen that chitosan produces two times more LA than chitin.

As shown in Table 2, 5-HMF was produced in similar amounts from chitosan of different sources and molecular

**Table 2** Summary of results for different types of chitin and chitosan treated using the optimized procedures for generation of LA and 5-HMF<sup>a</sup>

Biopolymer	LA (wt%)	5-HMF (wt%)
Low M.Wt chitosan	24.7	10.3
Medium M.Wt chitosan	23.9	10.0
High M.Wt chitosan	25.1	10.3
Chitosan, without catalyst	ND	ND
S-189	24.1	9.3
S-190	23.0	8.8
Chitin	11.5	ND
T-187	12.7	ND
T-188	12.6	ND

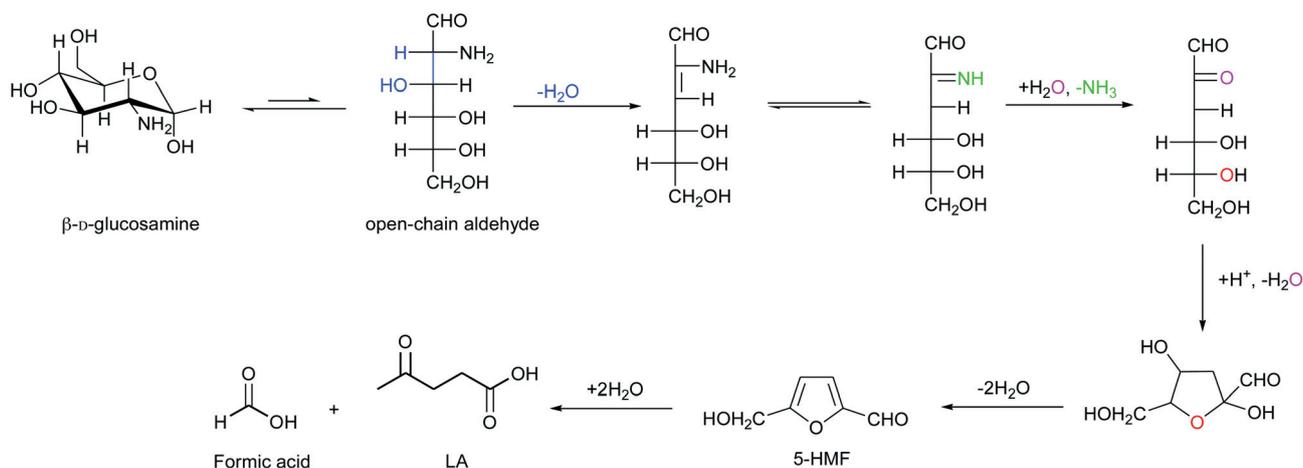
<sup>a</sup> ND = not detected. Chitosan and chitin purchased from Aldrich and used as received. S-189 = shrimp chitosan, S-190 = crab chitosan, T-187 = crab chitin and T-188 = shrimp chitin from Newfoundland fishery waste provided by ChitinWorks America LLC.

weights (average 9.8 wt%). In contrast, chitin failed to produce 5-HMF, or in fact LA or any small EtOAc soluble organic compounds under the conditions studied (Table 2). In the absence of the catalyst, under the optimized conditions, chitosan produced neither LA nor 5-HMF, Table 2. Also, reduced catalyst loadings failed to yield the desired products under the conditions studied.

### Proposed mechanism

Our proposed mechanism for this process is shown in Fig. 9. In the first step, chitosan is hydrolysed to yield glucosamine *via* cleavage of the glycosidic bond (C<sub>1</sub> → C<sub>4</sub> linkage in the polymer) in the presence of SnCl<sub>4</sub>·5H<sub>2</sub>O and microwave irradiation. Microwave-assisted acid-catalysed biopolymer hydrolysis has previously been performed on starch to yield glucose.<sup>49</sup> This process occurs in a stepwise fashion and oligomers are formed initially. Evidence for the formation of chito-oligosaccharides has been obtained through mass spectrometric studies, see below. We assume that as we see no product in the absence of catalysts (Brønsted or Lewis acids) that coordination of the amine functional group to either a proton or a metal centre facilitates this bond-breaking process by weakening the proximal C<sub>1</sub>–O bond and making it more amenable to hydrolysis. Furthermore by performing the reactions in water, both hydronium and hydroxide ions are readily available to react at this site. However, it is well known that an aqueous solution of SnCl<sub>4</sub> is mostly hydrolyzed and exists in equilibrium with colloidal tin (iv) oxide and HCl(aq).<sup>50</sup> Therefore, reaction mixtures prepared for this process are acidic. If colloidal tin oxide (Alfa Aesar) and HCl(aq) were used together rather than SnCl<sub>4</sub>·5H<sub>2</sub>O, 100 mg of chitosan yielded 27.4 wt% LA under the optimized conditions described above. This is slightly greater than the yield under the same conditions using SnCl<sub>4</sub>·5H<sub>2</sub>O, and is significantly greater than the yields obtained using either HCl(aq) or tin oxide alone.

For comparison and to provide some evidence for the last steps in the proposed mechanism, the reaction of glucosamine was studied under similar conditions. 32.0 wt% LA was produced from 100 mg glucosamine hydrochloride using 0.26 mmol SnCl<sub>4</sub>·5H<sub>2</sub>O and 20 mL water heated to 200 °C for



**Fig. 9** Proposed mechanism for 5-HMF and LA production from glucosamine. The glucosamine forms *in situ* *via* acid-catalysed hydrolysis of the biopolymer.

30 min. This corresponds to a yield of 59.4%. Unfortunately, attempts to date to detect the ammonia or any reducing sugars in the aqueous phase after extraction of the products from the reaction mixtures have been unsuccessful. Therefore, we cannot provide unequivocal evidence for all steps in the proposed mechanism.

In recent literature, glucose is known to yield 5-HMF,<sup>17–21</sup> and the subsequent conversion of 5-HMF to give LA is also known.<sup>18,21</sup> In such processes, glucose dehydrates to yield 5-HMF through loss of three water molecules – this process is also facilitated by the presence of a Lewis acid. Furthermore, in the presence of a high concentration of the Lewis acidic catalyst, as in the optimum conditions for generation of LA from chitosan, 5-HMF is rehydrated into LA and formic acid through addition of two water molecules. In order to test the last step in the mechanism, namely rehydration of 5-HMF into LA, 5-HMF (26.0 mg) was processed under the optimum conditions for LA production from chitosan (*i.e.*, 4 mL water, 0.24 mmol SnCl<sub>4</sub>·5H<sub>2</sub>O, *T* = 200 °C and *t* = 30 min). The weight percent yield of LA was 85.4 (or 92.7 mol%) thus providing evidence for this last step. In the absence of the Lewis acid, no LA was detected thus indicating the essential role of a metal centre in this last step.

It should be noted that two volatile compounds (furan and 2-methylfuran) were also identified *via* head-space analysis of the gas phase inside the sealed reaction vessel. These have previously been observed when sugars are roasted.<sup>51</sup> These compounds have a lower degree of solubility in aqueous solution than LA and 5-HMF and therefore, they were not found upon extraction of organics from the aqueous reaction mixture. Other hydrophilic or otherwise insoluble compounds would also be overlooked using the GC-MS method employed. MALDI-TOF mass spectra were obtained from aliquots of reaction mixtures in an attempt to observe intermediate depolymerization products and any compounds remaining in the aqueous phase. 2,5-dihydroxybenzoic acid (DHB) was used as the matrix, as it had been used previously in the study of chitooligosaccharides.<sup>52</sup> For aliquots taken 15 min into the reaction, for both dilute and concentrated reaction mixtures, some interesting peaks were observed in the mass spectra. For concentrated reaction samples (4 mL water), peaks were observed at *m/z* 1188 and 543. These could be assigned to the oligosaccharides (glucosamine)<sub>6</sub>-*n*-acetylglucosamine and (glucosamine)<sub>2</sub>-*n*-acetylglucosamine, respectively. These peaks were not present in spectra from aliquots taken after 30 min reaction time. This suggests that the oligosaccharides are true intermediates, as they are formed at an early stage in the reaction and then consumed. For dilute reaction samples (15 mL water), a peak was observed at *m/z* 501. This could be assigned to the trisaccharide, (glucosamine)<sub>3</sub>. This peak was also present in aliquots analyzed after 30 min reaction time. This indicates that incomplete degradation/hydrolysis of the biopolymer occurs under the dilute conditions studied.

## Conclusions

This study demonstrates that chitosan can be used to produce LA and 5-HMF in water under microwave conditions. This work is a proof-of-principle that *N*-containing biopolymers can be

degraded using green chemistry principles to give useful chemical building blocks in a similar way to ongoing research in the area of cellulosic feedstocks. The volume of water used and the loading of SnCl<sub>4</sub>·5H<sub>2</sub>O can be varied to produce either LA or 5-HMF with good selectivity. Factorial design was successfully employed to optimize the reaction conditions for this process. Microwave irradiation proved to be a more effective heating method for the generation of these small molecules compared with conventional heating, as 5-HMF could not be generated effectively under the conditions studied using conventional heating. A mechanism for this process has been proposed based on the known chemistry of cellulose and glucose, and some studies using glucosamine. Furthermore, evidence for oligosaccharide intermediates has been obtained using MALDI-TOF mass spectrometry. We were also able to perform the last step in the mechanism under microwave irradiation to convert 5-HMF to LA in good yields in water. The method developed for chitosan could be extended to the more robust parent carbohydrate chitin from which LA can also be obtained, albeit in smaller quantities. The results of this study will open the possibility of chemical and thermochemical transformations of the non-toxic and cheap biopolymers, chitosan and chitin, to yield useful, sustainable chemicals with possible industrial applications. Studies in our group are ongoing using chitosan, chitin and amino-sugars as feedstocks in different solvents *e.g.* ionic liquids (ILs) and employing a wide range of catalysts.

## Acknowledgements

We thank Memorial University, NSERC of Canada (Discovery and RTI Grants–FMK, Undergraduate Student Research Award–JEB), the Provincial Government of Newfoundland and Labrador (IRIF grant–FMK), RDC-Newfoundland (Ocean Industries Research Award – KWO) and Canada Foundation for Innovation (Leaders Opportunity Fund Award–FMK) for generous funding. Thanks also to ChitinWorks America LLC for provision of chitosan and chitin samples sourced from the Newfoundland fishery.

## Notes and references

- M. Mascall and E. B. Nikitin, *ChemSusChem*, 2009, **2**, 859–861.
- G. A. F. Roberts, *Chitin Chemistry*, Macmillan Press Ltd., London, UK, 1992.
- Y. Qin, X. Lu, N. Sun and R. D. Rogers, *Green Chem.*, 2010, **12**, 968–971.
- M. F. A. Goosen, *Applications of Chitin and Chitosan*, Technomic Publishing Company, Pennsylvania, USA, 1997.
- D. M. Alonso, J. Q. Bond and J. A. Dumesic, *Green Chem.*, 2010, **12**, 1493–1513.
- J. J. Bozell and G. R. Petersen, *Green Chem.*, 2010, **12**, 539–554.
- J. H. Clark and F. E. I. Deswarte, *Introduction to Chemicals from Biomass*, John Wiley & Sons, Ltd, West Sussex, UK, 2008.
- R. Palkovits, K. Tajvidi, J. Procelewaska, R. Rinaldi and A. Ruppert, *Green Chem.*, 2010, **12**, 972–978.
- M. Stocker, *Angew. Chem., Int. Ed.*, 2008, **47**, 9200–9211.
- J. N. Chheda, G. W. Huber and J. A. Dumesic, *Angew. Chem., Int. Ed.*, 2007, **46**, 7164–7183.
- S. Hu, Z. Zhang, Y. Zhou, B. Han, H. Fan, W. Li, J. Song and Y. Xie, *Green Chem.*, 2008, **10**, 1280–1283.
- X. Qi, M. Watanabe, T. M. Aida and R. L. Smith Jr, *ChemSusChem*, 2009, **2**, 944–946.
- J. Y. G. Chan and Y. Zhang, *ChemSusChem*, 2009, **2**, 731–734.

- 14 X. Qi, M. Watanabe, T. M. Aida and R. L. Smith Jr, *Green Chem.*, 2009, **11**, 1327–1331.
- 15 Y. Roman-Leshkov, J. N. Chheda and J. A. Dumesic, *Science*, 2006, **312**, 1933–1937.
- 16 M. Chidambaram and A. T. Bell, *Green Chem.*, 2010, **12**, 1253–1262.
- 17 S. Hu, Z. Zhang, J. Song, Y. Zhou and B. Han, *Green Chem.*, 2009, **11**, 1746–1749.
- 18 A. Takagaki, M. Ohara, S. Nishimura and K. Ebitani, *Chem. Commun.*, 2009, 6276–6278.
- 19 T. Staahlberg, M. G. Sorensen and A. Riisager, *Green Chem.*, 2010, **12**, 321–325.
- 20 G. Yong, Y. Zhang and J. Ying, *Angew. Chem., Int. Ed.*, 2008, **47**, 9345–9348.
- 21 H. Zhao, J. E. Holladay, H. Brown and Z. C. Zhang, *Science*, 2007, **316**, 1597–1600.
- 22 G. Ma, D. Yang, D. Su, X. Mu, J. F. Kennedy and J. Nie, *Polym. Adv. Technol.*, 2010, **21**, 189–195.
- 23 B. Krajewska, *Sep. Purif. Technol.*, 2005, **41**, 305–312.
- 24 L. Ramirez-Coutino, M. d. C. Marin-Cervantes, S. Huerta, S. Revah and K. Shirai, *Process Biochem.*, 2006, **41**, 1106–1110.
- 25 R. A. A. Muzzarelli and M. G. Peter, *Chitin Handbook*, European Chitin Society, Grottammare, Italy, 1997.
- 26 J. H. Yoon, *Enzyme Microb. Technol.*, 2005, **37**, 663–668.
- 27 K. Kurita, K. Tomita, S. Ishii, S. Nishimura and K. Shimoda, *J. Polym. Sci., Part A: Polym. Chem.*, 1993, **31**, 2393–2395.
- 28 K. Tømmeraas, K. M. Varum, B. E. Christensen and O. Smidsrød, *Carbohydr. Res.*, 2001, **333**, 137–144.
- 29 T. Wu and S. Zivanovic, Abstracts of Papers, 234th ACS National Meeting, Boston, MA, United States, August 19–23, 2007, IEC-015.
- 30 J. Chen and C.-T. Ho, *J. Agric. Food Chem.*, 1998, **46**, 1971–1974.
- 31 M. Jun, Y. Shao, C.-T. Ho, U. Koetter and S. Lech, *J. Agric. Food Chem.*, 2003, **51**, 6340–6346.
- 32 F. M. Kerton, *Alternative Solvents for Green Chemistry*, Royal Society of Chemistry Publishing, Cambridge, UK, 2009.
- 33 U. M. Lindstrom, *Organic Reactions in Water: Principles, Strategies and Applications*, Blackwell Publishing, Oxford, UK, 2007.
- 34 C. O. Kappe, D. Dallinger and S. S. Murphree, *Practical Microwave Synthesis for Organic Chemistry: Strategies, Instruments, and Protocols*, WILEY-VCH, Weinheim, Germany, 2009.
- 35 D. Dallinger and C. O. Kappe, *Chem. Rev.*, 2007, **107**, 2563–2591.
- 36 J. D. Moseley and C. O. Kappe, *Green Chem.*, 2011, **13**, 794–806.
- 37 M. Mascal and E. B. Nikitin, *Green Chem.*, 2010, **12**, 370–373.
- 38 F. Ilgen, D. Ott, D. Kralisch, C. Reil, A. Palmberger and B. Koenig, *Green Chem.*, 2009, **11**, 1948–1954.
- 39 J. N. Chheda, Y. Roman-Leshkov and J. A. Dumesic, *Green Chem.*, 2007, **9**, 342–350.
- 40 S. Kobayashi, S. Nagayama and T. Busujima, *J. Am. Chem. Soc.*, 1998, **120**, 8287–8288.
- 41 V. V. Guzeev and A. N. D'yachenko, *Glass Ceram.*, 2002, **59**, 49–52.
- 42 R. D. Moen, T. W. Nolan and L. P. Provost, *Quality Improvement Through Planned Experimentation*, 2nd edn, McGraw-Hill, New York, USA, 1999.
- 43 G. Epane, J. C. Laguerre, A. Wadouachi and D. Marek, *Green Chem.*, 2010, **12**, 502–506.
- 44 L. Veum, S. R. M. Pereira, J. C. van der Waal and U. Hanefeld, *Eur. J. Org. Chem.*, 2006, 1664–1671.
- 45 F. Stazi, G. Palmisano, M. Turconi and M. Santagostino, *Tetrahedron Lett.*, 2005, **46**, 1815–1818.
- 46 J. R. Ochoa-Gomez, O. Gomez-Jimenez-Aberasturi, B. Maestro-Madurga, A. Pesquera-Rodriguez, C. Ramirez-Lopez, L. Lorenzo-Ibarreta, J. Torrecilla-Soria and M. C. Villaran-Velasco, *Appl. Catal., A*, 2009, **366**, 315–324.
- 47 G. E. P. Box, J. S. Hunter and W. G. Hunter, *Statistics for Experimenters: Design, Innovation, and Discovery*, John Wiley & Sons, New Jersey, USA, 2005.
- 48 Biotage Pathfinder, Prediction chart and time converter, <http://www.biotagepathfinder.com/util.jsp>, accessed March 9th 2012.
- 49 H.-M. Yu, S.-T. Chen, P. Suree, R. Nuansri and K.-T. Wang, *J. Org. Chem.*, 1996, **61**, 9608–9609.
- 50 N. Wiberg, A. F. Holleman and E. Wiberg, *Holleman-Wiberg Inorganic Chemistry*, 34th edn (101st printing), Academic Press, San Diego, 2001, p. 908.
- 51 A. Limacher, J. Kerler, T. Davidek, F. Schmalzried and I. Blank, *J. Agric. Food Chem.*, 2008, **56**, 3639–3647.
- 52 S. Trombotto, C. Ladavière, F. Delolme and A. Domard, *Biomacromolecules*, 2008, **9**, 1731–1738.