

Microwave enhanced reaction of carbohydrates with amino-derivatised labels and glass surfaces

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The reaction between carbohydrates and amino-derivatised labels has been improved through microwave heating. We show it proceeds principally *via* solvent mediated heating rather than either direct microwave heating of the reagents or microwave influenced changes in the rates of mutarotation in the sugars beyond those obtained by conventional thermal heating. The method is applied to the attachment of sugars to an aminosilane-derivatised glass surface suitable for the construction of carbohydrate microarrays.

The condensation reaction between the aldehyde group of reducing sugars and an amine to form an imine (or Schiff's base), Fig. 1, is well-known¹ and has been exploited widely for the labeling of sugars and saccharide sequencing.² Its use for the attachment of sugars to amino-derivatised glass surfaces for the construction of microarrays has become an attractive prospect, particularly in light of the many biological activities and potential medical applications of carbohydrates.³ Such a development would allow rapid screening of large numbers of structures, while consuming small amounts of material. However, the reaction is slow, typically requiring 36 h with conventional heating and considerably longer without heating.

The rate of labeling with an amine containing fluorophore, 7-aminonaphthalene-1,3-disulfonic acid (Fluka), of a number of model compounds (reducing mono- and di-saccharides) was investigated in formamide, which affords good solubility of sugar and label. The reaction rates were measured for hexoses, pentoses, aminosugars and disaccharides, Table 1. Typically, 0.05 mmol of sugar were dissolved in 3 ml of formamide in a standard quartz reaction vessel, to standardize heating, and 3 equivalents of 7-aminonaphthalene-1,3-disulfonic acid added. Microwave heating, which is potentially quick and convenient, was applied for 90 s at 20% power (in an 850 W oven). Standard reaction conditions (rate and yield defined as 1) were also performed (without microwave heating, 24 h, 25 °C) with the above quantities of reagents. Rates were quantified by measurement of fluorescent intensity ($\lambda_{\text{exc}} = 315 \text{ nm}$, $\lambda_{\text{emit}} = 450 \text{ nm}$) of the product following separation by electrophoresis

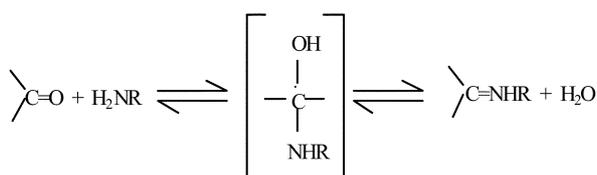


Fig. 1 Formation of imine.

and calibrated with reference to an authentic standard. The microwave heated reactions resulted in significantly improved reaction rates for all of the reducing sugars studied, suggesting that the method is widely applicable. Acid promotes imine formation, giving higher yields, but cannot be applied to many biologically active carbohydrates because of their sensitivity. These include N-sulfated sugars (*e.g.* glycosaminoglycans), which undergo de-sulfation, and sialic acid derivatives (*e.g.* N-linked oligosaccharides, bacterial lipo-oligosaccharides and capsular polysaccharides), which can be cleaved or undergo lactonisation. Acid catalysis was therefore not pursued further.

The use of microwave radiation to drive specific amination reactions in a solvent-free environment, in which the reactants (*e.g.* ammonium formate) were microwave absorbing has been reported.⁴ The possibility that this could explain the observed improvements in the reaction rate was eliminated by measurement of the dielectric properties (the real ϵ' and imaginary ϵ'' components) over the range 0.13 to 12 GHz by means of a HP8722A network analyzer employing a specially designed open-ended coaxial probe.⁵ Neither carbohydrate nor label showed any appreciable imaginary dielectric component, nor loss tangent (ϵ''/ϵ') at 2.45 GHz, which are a good measure of the ability of a system to heat under microwave irradiation.⁶

It has been suggested that the improved reaction of

Table 1 Rate improvements for amination of model sugars with 7-aminonaphthalene-1,3-disulfonic acid in formamide

Sugar	Rate ^a
D-glu	10 ³
D-glcNAc	10 ³
D-glcNS	10 ³
D-man	10 ³
D-gal	10 ³
D-xyl	10 ³
L-ara	10 ³
Lactose	10 ³
Maltose	10 ³
Sucrose ^b	0
Maltose ^c	10 ²
Maltose ^d	10 ²

^aDetermined as the development of fluorescent intensity of the separated product over time. The yield for this reaction is low, typically a few %, although variations occur as a result of subtle structural features influencing the rate of mutarotation and reactivity.

^bSucrose, a non-reducing sugar, was included as a control. No reaction was expected. ^cThermal heating in an oil bath at 90 °C for 90 s. ^dMicrowave equivalent to thermal heating in *c*, in a CEM MARS 5 temperature control microwave oven.

carbohydrates can be explained by an increased rate of mutarotation (the process of equilibration between unreactive closed ring structures that proceeds *via* the reactive open chain intermediate⁷) above that achieved by equivalent thermal heating. Alternatively, the reaction may be accelerated *via* solvent heating which increases the rate of mutarotation. This was investigated by several experiments. First, reaction between model sugars and label with microwave assistance was compared to one under equivalent thermal heating (last two entries in Table 1). In these experiments the rates were comparable. Second, the real (ϵ') and imaginary (ϵ'') components of the dielectric constant were measured for formamide and mixtures of formamide and water, Fig. 2, which also includes loss tangent data calculated from Maxwell Garnett equations.⁸ Both of these solvents exhibited varying absorbance of microwaves from 0.13 to 12 GHz but are strong absorbers at 2.45 GHz, the operating frequency of commercial microwave ovens. The correspondence between yield and solvent composition (*i.e.* varying loss tangent) at 2.45 GHz is shown in Fig. 3 for mixtures of water and formamide. These results confirm that the reaction is improved primarily by solvent driven heating and implies that processes proceeding by this reaction can also be improved where a suitable microwave absorbing solvent can be employed.

One application of this process is the rapid and facile attachment of saccharides (in formamide, 1 nl per spot) of the heparan sulfate family, a glycosaminoglycan with many important biological and pharmacological properties,⁹ to glass slides derivatised with γ -aminopropylsilane (GAPSII,

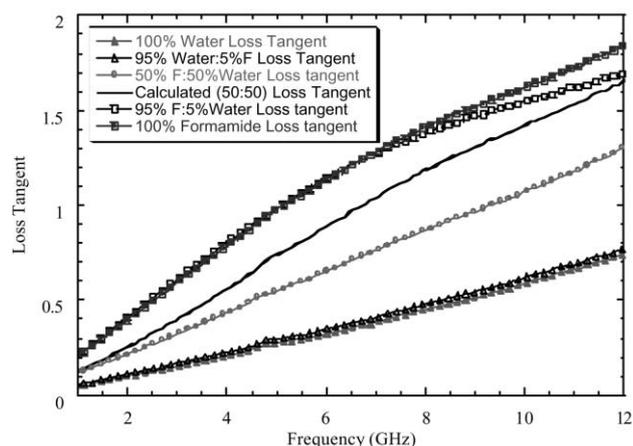


Fig. 2 Loss tangent (ϵ''/ϵ') for mixtures of formamide and water in the microwave frequency range (1–12 GHz).

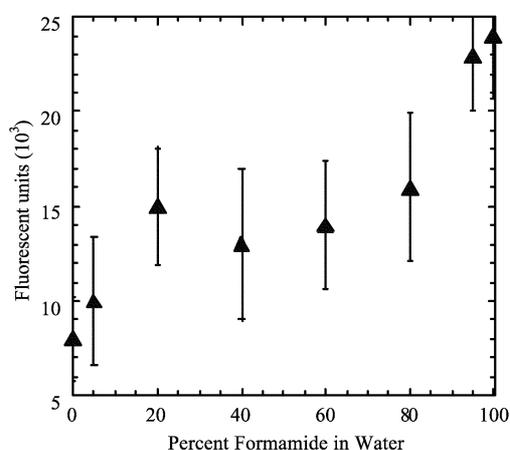


Fig. 3 Reaction yield *versus* percentage formamide in water at 2.45 GHz.

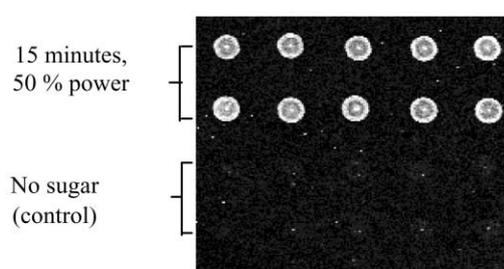


Fig. 4 Rapid attachment of biologically active sugars to amino-derivatised glass slides.

Corning B.V., Amsterdam, Netherlands). Attachment was achieved after microwave heating at 2.45 GHz (15 min at 50% power in an 850 W oven), Fig. 4, a considerable rate improvement (*ca.* 140-fold) compared to conventional conditions (36 h, 80 °C). The rate of heating of the glass slide alone was two orders of magnitude less than that observed in the presence of formamide. Consequently the temperature of the system is considered to be approximated by that of the solvent. The sugars were detected by an anti-heparan sulfate primary antibody followed by secondary, followed by an Alexafluor labeled (546 nm) antibody (Molecular Probes, Portland, OR, USA), then observed under standard microarray conditions. Similar experiments, in which the carbonyl group is blocked, indicate the attachment is predominantly covalent.

In conclusion, we report significant improvements in reaction rates between sugars and amine-containing labels and surfaces.[†] We show that this is principally due to solvent mediated heating rather than either direct heating of reactants or microwave influenced rates of mutarotation of the sugar beyond those observed in equivalent thermal heating. The method has been applied to the attachment of bioactive and medically important carbohydrates to amino-derivatised glass slides and should aid the construction of functional carbohydrate array systems.

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Notes and references

[†]Imine formation: FTIR; 1680 cm^{-1} , C=N stretch. A control surface (as in Fig. 4), sugars reacted (15 min) in the absence of microwave heating (*i.e.* at 25 °C) showed no attachment. Formamide is less volatile (b.p. 210 °C) than water, allowing longer reaction time for small volumes. Formamide allows sugars to mutarotate effectively owing to its highly polar, ampholytic nature, at a rate comparable to that in water. The imaginary component (loss, ϵ'') of the dielectric constant for formamide has its maximum value at 4.43 GHz,¹⁰ and at 2.45 GHz its value is about 80% of the maximum. At 2.45 GHz, the penetration depth in formamide and water is in the cm size regime.

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