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Title: Visualising substrate-fingermark interactions: solid-state NMR spectroscopy of amino acid reagent development on cellulose substrates

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Abstract

Most spectroscopic studies of the reaction products formed by ninhydrin, 1,2-indanedione-zinc (Ind-Zn) and 1,8-diazafluoren-9-one (DFO) when reacted with amino acids or latent fingermarks on paper substrates are focused on visible absorption or luminescence spectroscopy. In addition, structural elucidation studies are typically limited to solution-based mass spectrometry or liquid nuclear magnetic resonance (NMR) spectroscopy, which does not provide an accurate representation of the fingermark development process on common paper substrates. The research presented in this article demonstrates that solid-state carbon-13 magic angle spinning NMR (^{13}C-MAS-NMR) is a technique that can not only be utilised for structural studies of fingermark enhancement reagents, but is a promising technique for characterising the effect of paper chemistry on fingermark deposition and enhancement. The latter opens up a research area that has been under-explored to date but has the potential to improve our understanding of how fingermark secretions and enhancement reagents interact with paper substrates.

Keywords: latent fingermark; paper; indanedione-zinc; DFO; ninhydrin; NMR

1. Introduction

Structural studies of Joullié's pink (JP), Ruhemann's purple (RP) and the 1,8-diazafluoren-9-one (DFO) product *N*-(9*H*-pyrido[3',2':3,4]cyclopenta[1,2-*b*]pyridine-9-yl)-9*H*-pyrido[3',2':3,4]cyclopenta[1,2-*b*]pyridine-9-imine (DFO ylide; DFOY) – formed, respectively, by the reaction of the fingermark reagents indanedione-zinc (Ind-Zn), ninhydrin and DFO with amino acid deposits in latent fingermarks on paper substrates (Figure 1) – are typically limited to visible absorption and luminescence microspectrophotometry *in situ* on cellulose substrates [1-3]. Further structural information is often revealed using traditional solution-based analytical techniques such as electrospray ionisation mass spectrometry, liquid nuclear magnetic resonance (NMR) spectroscopy or Fourier transform infrared spectroscopy (FTIR) [4-10]. Single crystal X-ray diffraction crystallography of transition metal complexes of DFOY [11-12] and Ruhemann's purple [13-14] has also been used for structural elucidation. However, this approach has not yet been successfully implemented for Joullié's pink due to poor coordination complex formation and difficulty in producing suitable crystals. Furthermore, these techniques do not take into account the influence of the cellulose substrate on product conformation.

Figure 1: *The reaction schemes for (a) DFO, (b) 1,2-indanedione, (c) ninhydrin and (d) isatin (hypothesised) with amino acids.*

Previous spectroscopic studies directed at RP, DFOY and JP have demonstrated the effects of solvent polarity and hydrogen bonding interactions on characteristic features observed in UV-visible and NMR spectra [4,5,7,15]. Lower polarity and/or aprotic solvents such as dimethylformamide (DMF), pyridine and dimethylsulfoxide (DMSO) typically resulted in highly resolved ^{1}H-NMR spectra due to the suppression of keto-enol tautomerisation [7,15]. However, ^{1}H-NMR spectra collected in highly polar and protic solvents resulted in unresolved aromatic regions and the loss of functional group signals due to intra- and intermolecular hydrogen bonding and the rapid rate of keto-enol tautomerisation [7,15]. Proton migration in DFOY caused by residual methanol was also identified by Wilkinson to cause significant broadening across all regions of the ^{1}H-NMR spectrum in deuterated chloroform [4]. As yet, no published studies exist on the structural elucidation of the reaction product of isatin (3-[(2-oxo-1,2-dihydro-3*H*-indol-3-ylidine)amino]-1,3-dihydro-2*H*-indol-2-one; isatin ylide) with primary α-amino acids in latent fingermark residue, with previous biochemical research focusing on the reaction with proline or proteinaceous tyrosine residues [16-19].

Although solution-based techniques such as liquid NMR spectroscopy can provide important information on chemical structure, the study of fingermark reagents in solution does not provide an accurate account of the interactions between the paper substrate and fingermark reagents such as indanedione-zinc [10]. Consequently, an approach that permits the study of these reagents and their reaction products *in situ* is required to advance our understanding of fingermark detection on these complex matrices.

Recent developments in the NMR field include the use of high resolution and fast magic angle spinning (MAS) solid-state NMR to study insoluble materials in their natural state and to determine the unit cell arrangement of crystalline materials, a route of investigation typically reserved for X-ray diffraction crystallography [20]. Spinning sidebands and other anisotropic effects observed in the solid-state NMR spectra of highly ordered materials can also be used to determine bond length and internuclear distance between bonded and non-bonded moieties up to 10 Å apart [21-22]. Of particular interest to fingermark enhancement research is the ability to exploit the chemical shift anisotropy effects to characterise the intermolecular interactions between fingermark components, fingermark reagents and the

substrate. This approach is routinely used for structural elucidation of active protein conformations [23], and has been utilised for other matrices with tertiary structures, such as cellulose [24-28].

The results of our previous study indicated that ^{13}C-MAS-NMR was a suitable method for characterising the formation of JP (extracted under controlled conditions) with four common amino acids [10]. The aim of the research presented in this paper was to investigate whether ^{13}C-MAS-NMR could provide a simple and effective method for studying solvent-sensitive amino acid reaction products (resulting from the application of fingermark detection reagents) *in situ* on cellulose substrates. While the study focused on indanedione, ninhydrin and DFO, isatin [16] (Figure 1) was also included to determine whether small changes in the functionality of fingermark enhancement reagents affect the interaction with paper substrates.

2. Material and Methods

2.1 Preparation of reaction products

Alanine was chosen as the universal model for amino acids [4,10]. In order to assess the relative reaction rates of four amino acid reagents, no zinc chloride catalyst was added to the indanedione reaction [10]. This had the additional benefit of removing problems with paramagnetic materials in the sample, previously observed to partially quench the ^{13}C signal [10].

All solvents (AR or HPLC grade) used for these experiments were obtained from ChemSupply (Adelaide, Australia). 1,2-Indanedione was obtained from the Casali Institute (Jerusalem, Israel), 1,8-diazafluoren-9-one was obtained from Lumichem (Belfast, Northern Ireland), and ninhydrin and isatin were obtained from Sigma-Aldrich. All fingermark enhancement reagents were used as supplied without further purification.

Cellulose reaction media were prepared using a similar method to that previously published [10]. L-Alanine (Fluka, 98% w/w; 0.1000 g; 1.12 mmol) was dissolved in Milli-Q water (18.2 mΩ, 5 mL) and the solution mixed thoroughly with chromatography grade cellulose (SigmaCell type 101; 1.00 g). The cellulose mixture was dried at room temperature overnight and ground into a homogenous powder. Two sets of alanine–cellulose media were prepared for each reagent tested, one set for the extracted product studies and one set for the *in situ* studies as set out in Table 1. An equimolar quantity of 1,2-indanedione (0.1639 g; 1.12

mmol) dissolved in absolute ethanol (2 mL) was mixed with the alanine–cellulose powder and reacted in a Memmert laboratory oven at 160° C for 10 seconds. Ruhemann's purple was prepared by reacting the alanine–cellulose media with ninhydrin (0.1995 g; 1.12 mmol) under the same conditions as indanedione. DFOY (0.2040 g; 1.12 mmol DFO) and isatin ylide (0.1648 g; 1.12 mmol isatin) were prepared at 180° C for 10 and 30 seconds, respectively. A second sample set was prepared and reacted at 22° C and 65% relative humidity in darkness for 18 hours to monitor the effect of heat on the reaction. The reaction conditions highlighted in Table 1 correspond to the conditions typically employed when these reagents are applied to fingermark detection on paper substrates [29-31].

Table 1: *The reaction conditions used for each reagent set for the ^{13}C-NMR analysis of JP, RP, DFOY and IY extracted from and adsorbed to cellulose. Bolded conditions correspond to typical fingermark detection protocols.*

Set 1 – extracted products		***Set 2 – in situ products***	
Indanedione, 22° C	**Indanedione, 160° C**	Indanedione, 22° C	**Indanedione, 160° C**
Ninhydrin, 22° C	Ninhydrin, 160° C	**Ninhydrin, 22° C**	Ninhydrin, 160° C
DFO, 22° C	**DFO, 180° C**	DFO, 22° C	**DFO, 180° C**
Isatin, 22° C	**Isatin, 180° C**	Isatin, 22° C	**Isatin, 180° C**

One set of reaction products were extracted in cold absolute ethanol (25 mL), immediately chilled in liquid nitrogen, and freeze dried at -85° C using a Christ Alpha 2-4 LD plus freeze-drier. The second set of reaction media was freeze dried in the same manner with the products remaining *in situ* on the cellulose substrate.

2.2 ^{13}C-MAS NMR analysis

The isolated and *in situ* reaction products were ground into homogenous powders and firmly packed into an 18 mm zirconia MAS rotor (internal diameter 4 mm). Samples of insufficient volume to fill the rotor were diluted with spectroscopy grade potassium bromide (Scharlau, Germany) and manually ground to form a homogenous powder. A ^{13}C-NMR Hartmann-Hahn cross polarisation spectrum was obtained using a Bruker Spectrospin 300 MHz NMR fitted with a 4 mm ^{13}C-^{1}H MAS probe and MAS remote control unit. A contact pulse of 1.0 ms, 90° high pulse of 6.0 μs, relaxation delay of 4.0 s and time domain of 1024 were utilised for all samples. Extracted products and cellulose-bound samples were analysed at a spin rate of

6000 Hz, and copy paper samples (Section 2.3) at a spin rate of 4000 Hz. A 5 mm DUL ^{13}C-^{1}H liquid probe was used to collect comparative solution-based NMR spectra.

2.3 Correlating cellulose features in copy paper to fingermark development

Three brands of white copy paper available on the Australian market were purchased from a local stationary supplier (Table 2). Three sheets of paper were selected from random locations within each ream. Samples for ^{13}C-MAS NMR analysis were prepared by finely shredding and combining 1 cm strips of paper from each sheet (to reduce intra-ream variation), with the analyses conducted as per Section 2.2. Complementary FTIR spectroscopic studies of blank paper samples were performed on a Nicolet Magna-IR 760 FTIR with a germanium attenuated total reflectance FTIR microscope attachment (μATR-FTIR) (256 scans, 4000-650 cm^{-1}, 4 cm^{-1} resolution, averaged over 3 sheets).

Natural fingermarks (no grooming procedures) [32] were collected from 1 male and 1 female donor, stored on an open benchtop at 22^{o} C (65% RH) for 24 hours and then developed with indanedione-zinc [29-30] prior to absorption microspectrophotometry (Craic PV20/20, 36x magnification; 250-800 nm, 343 ms (male) and 700 ms (female) integration time; 25 scans; resolution factor = 5). Cross-sectional fluorescence microscopy of developed fingermarks was performed using a Leica DMLM microscope (20x objective; VG9 excitation filter, N2.1 590 nm longpass filter cube). Surface fluorescence micrographs were captured with a Leica FSM macroscope at 4-16x magnification (green Foster+Freeman Crime-Lite excitation; Schott OG570 longpass filter).

Table 2: *Fibre type and country of manufacture of the copy papers selected for this study (as specified by ream packaging). All papers had a measured pH c.a. 9 when wetted.*

Brand	Country of manufacture	Virgin fibre content (%)	Recycled fibre content (%)
Reflex Ultrawhite	Australia	100	0
Corporate Express EXP 800	Australia	100	0
Evolve Everyday	France	0	100

3. Results and Discussion

3.1 Relative reaction rates

Each of the isolated reaction products resulted in CP-MAS NMR spectra containing similar functional groups – with methylene, secondary amine, imine, ketone and aromatic signals observed in all samples – confirming that this class of reagents all react in a similar manner with amino acids to produce highly coloured and/or luminescent products. A comparison of samples reacted at room temperature against those heated in an oven indicated that heating the indanedione, DFO and isatin reaction samples improved the quality and resolution of the ^{13}C-MAS NMR spectra, along with an increase in the intensity of the imine and co-crystallised acetaldehyde peaks at approximately 152 and 207 ppm, respectively. The complementary reduction of alanine signal intensity was observed for these samples. Broad signals comprised of partially resolved peaks were observed across the aliphatic and aromatic regions, possibly due to the formation of multiple product resonance structures and orientations with subtly unique magnetic environments.

Isatin exhibited the slowest reaction rate of the four amino acid reagents, followed by DFO, although increasing the temperature and reaction time significantly improved the product and byproduct yields (Figure 2). Ninhydrin and 1,2-indanedione reacted with alanine at a comparable rate at room temperature, with 1,2-indanedione producing higher yields at 160^{o} C than at 22^{o} C, even without the addition of the zinc catalyst. The addition of the zinc chloride catalyst, as observed in previous experiments [10], would result in near complete conversion to JP, which is consistent with anecdotal and published reports that the application of ninhydrin is far less effective after 1,2-indanedione-zinc treatment than after 1,2-indanedione or DFO [29].

Peaks consistent with the chemical shifts for the imine, enolate and carbonyl functional groups were weak in most of the spectra obtained compared to those in the aliphatic and aromatic regions. This was due to the distribution of torsion angles and rotational conformations between adjacent indanone ring structures, with low sample concentration and the necessity for long-range transfer of magnetisation between these groups and neighbouring protons during the cross-polarisation experiments further contributing to low signal intensity. Highly conjugated molecules and functional groups with no directly coupled hydrogen atoms

typically require long contact pulse and relaxation times [33]; however, in this study, shorter contact pulse and relaxation delay times were utilised to maintain a balance between acquisition time and overall spectral intensity.

3.2 In situ ^{13}C-MAS NMR spectroscopy of reaction products

Each of the reaction products for DFO, ninhydrin, 1,2-indanedione and isatin were identified from the solid ^{13}C-NMR spectra, although the signals were substantially weaker than those from the cellulose itself, due to the low concentration of alanine able to be adsorbed to the substrate (1.12 mmol per gram of cellulose). Unreacted alanine also contributed to the carbonyl and aliphatic signals observed in each spectrum, suggesting that the hypothesised 2:1 stoichiometry of the reaction is realistic (this was confirmed by liquid ^{1}H- and ^{13}C-NMR of JP after liquid-liquid extraction).

Spectral broadening of the product peaks, particularly in the aromatic region, was generally more pronounced for the spectra collected *in situ* (Figure 2) although the observed effect varied for each reagent. The entire aromatic region of the DFOY-cellulose spectrum exhibited a 6 ppm broadening, while the RP aromatic region produced both general broadening and a decrease in neighbouring peak resolution due to increased anisotropy. Signal broadening was more subtle in the IY and JP *in situ* spectra (1-3 ppm), and one JP aromatic signal at 129 ppm became more resolved upon adsorption to cellulose. This suggests that the reaction products interact with the surface of the helical cellulose fibres, with a few particular orientations being more populated due to steric effects and hydrogen bonding.

At least two structures of cellulose were observed in the ^{13}C-MAS spectra as evidenced by multiple inequivalent magnetic environments for C4 (dark blue) and C6 (red), and broad peaks for the remaining carbon moieties. The broadness of the spectra, coupled with the presence of a strong upfield C4 peak at 82 ppm, indicated that the samples were largely amorphous, with approximately 10% crystalline I_β cellulose [28]. The unresolved, broad triplet at 62 ppm also suggests the presence of a third cellulose allomorph, cellulose II, formed by alkaline pulping methods [24]. Each of the spectra obtained from the cellulose-bound reaction products demonstrated partial resolution between the C2 (green) and C3/5 (yellow) cellulose carbons (Figure 2) – not observed in the pure cellulose samples – as well as a distinct separation of the doublet C6 and partial separation of the C4 I_β signals, suggesting an increase in rigidity or order of the cellulose molecule upon hydrogen bonding

with amino acids and fingermark enhancement reagent products. A similar effect was observed by Park *et al*. upon the addition of up to 70% w/w water to cellulose samples [27].

Figure 2: *The ^{13}C-MAS NMR spectra of chromatography cellulose (green), extracted reagent products (blue) and in situ reagent products (on cellulose; red) for (a) Jouillé's pink (Ind-Zn), (b) Ruhemann's purple (ninhydrin), (c) DFOY (DFO), and (d) isatin ylide (isatin).*

Although there was an observed change in the chemical shift of each cellulosic carbon, the most distinct downfield shifts were observed for the C1 (pale blue), C4 (dark blue) and C6 (red) carbons in the JP and RP samples. Similar deshielding effects of C4' and C6' were observed in the isatin and DFO samples, although, in both cases, very weak interactions between the reaction products and other cellulosic groups were observed. In particular, the C4' and C6' carbons from the second I_β cellulose chain exhibited the greatest downfield shifts coupled with a dramatic increase in relative intensity, due to localised electron withdrawal and assisted dipolar coupling (hence improved cross-polarisation signal intensity) of these carbon atoms [34-35]. Each of these downfield migrations corresponded to previously reported chemical shift values for cellulose I_β [25,27-28]. This suggests that intermolecular hydrogen bonding largely occurs between the C6 alcohol group and the C4 glycoside linkage of the I_β' cellulose chain and the products formed by the amino acid-targeting fingermark reagents.

The products of DFO, ninhydrin and isatin development exhibited small upfield peak shifts when bound to cellulose compared to the spectra of the freeze-dried extracts, suggesting that the interaction with cellulose chains results in a slight shielding effect on these compounds. This effect is most noticeable in the CH-NH peaks of the RP-cellulose and isatin ylide-cellulose spectrum, both producing 7 ppm upfield shifts. Chemical shift changes in DFOY were subtle, but outside the observed intra- (0.06 ppm) and inter-sample (0.10 ppm) variability. However, all functional groups across the JP molecule (including the aromatic region) demonstrated small downfield shifts in the cellulose-bound samples; the largest shift observed was with the indole CH_2 carbon of JP. While these deshielding effects were typically less pronounced than the upfield shifts observed in RP and the isatin product, as with DFO these fell outside the expected variability.

Further experiments (including ^{1}H-MAS NMR) are required to characterise the nature and extent of hydrogen-bonding networks between cellulose chains and the amino acid reagent

reaction products – and amino acid residues prior to development – but the trends observed in these preliminary studies indicate that the influence of cellulose (by extension, the paper substrate) differs for the three commonly used amino acid reagents. From these results, our current hypothesis is that the 1,2-indanedione product JP forms in a sheet arrangement between cellulose chains, interacting primarily with the C6 CH_2-OH group, but being spatially proximate to other electronegative groups in the cellulose. The DFO and ninhydrin reaction products appear to interact only with specific functions of the cellulose due to steric effects, with the pyridyl N of DFO primarily contributing to intermolecular H-bonding with the C2 (green) and C3 (yellow) hydroxyl groups and RP interacting with C6 and the glycoside linkages.

The preferential hydrogen bonding to I_β cellulose may be due to the spatial orientation of the C6 hydroxy chain on the accessible chains of each fibre and the packing of individual cellulose chains within the fibres. The ordered parallel chains and sheet packing of cellulose I_β, in comparison to the densely-packed helical structure of cellulose I_α, results in a large number of surface primary OH groups capable of intra- and intermolecular hydrogen bonding [26,36]. This spatial preference becomes important for understanding the effect of commercial paper substrates on amino acid-targeting fingermark enhancement reagents.

3.3 Correlating cellulose features in copy paper to fingermark development

The ^{13}C-MAS-NMR results indicated that all three copy papers were predominantly cellulose I, with I_β, I_α, cellulose II and hemicelluloses contributing to the spectrum of each paper (Figure 3). The order of crystallinity in the refined wood pulp papers is far more pronounced than that of the chromatography cellulose, but was still difficult to observe for most of the carbon peaks; C4 was the only signal that showed good separation between allomorphs. While the two virgin fibre copy papers (Reflex and EXP 800) produced similar spectra, the 100% reclaimed fibre paper Evolve contained a greater cellulose II composition than the virgin fibre papers. The presence of hemicelluloses was fairly uniform across all substrate types. Interestingly, no contribution from $CaCO_3$ filler (identified in the ATR-FTIR) or top layer finishing treatments was observed.

These results agree with previously published ^{13}C-NMR studies of mixed and pure wood bleached and unbleached pulps produced by the Kraft and sulfite processes – the two most commonly used pulping techniques for large scale paper production [24-25,37]. Although

Liitiä *et al*. observed a slight decrease in the crystallinity of refined fibres compared to raw materials, this was hypothesised to be partially due to the migration of crystalline hemicelluloses to the external fibre surfaces, rather than the loss of I_β chains [24,38]. The overall change to the cellulose structure was not substantial, although the presence of hemicelluloses and processing techniques such as alkaline deinking of recycled pulp – which may increase cellulose II formation – could affect the hydrogen bonding of amino acids and fingermark reagents to these paper types [24,37,39-40].

Figure 3: *The ^{13}C-MAS NMR spectra obtained from three white copy papers compared to chromatography cellulose. Peak annotations indicate cellulose I and II allomorphs. Grey boxes denote hemicelluloses.*

As with the NMR results, the μATR-FTIR spectra indicated that the papers contained predominantly cellulose I, with observable differences in the spectral shape and features (Figure 4). Notably, the COH bend at ~1428 cm^{-1} was distinct for each paper, illustrating different H-bonding environments and interactions between cellulose chains. Each paper showed indications of high order or crystallinity, with distinct peaks for I (I_α and I_β) and II observed [41]. Evolve recycled paper appeared to have more spectroscopic features characteristic of cellulose II and I_α than the virgin fibre papers, complementing the NMR data. The peak at 873 cm^{-1} – greatest in Evolve, followed by EXP 800 and Reflex – was consistent with calcite, a common filler material for white copy papers. None of the papers contained lignin, as expected for archival quality products.

Figure 4: *The μATR-FTIR spectra of the three white copy papers. Peak annotations indicate cellulose I and II allomorphs, and calcium carbonate filler.*

Only two fingermark donors across three substrates were used, and these experiments should be considered as a pilot study requiring further examination with a larger donor and substrate set. However, some interesting preliminary trends were observed between paper morphology, paper chemistry and fingermark development with Ind-Zn. Fingermark enhancement with amino acid reagents appears to be more complex than simple cellulose-reagent interactions; the macromolecular and physical cellulose structure along with finishings and the physical properties of the paper appear to play a role in multiple facets of the deposition and development processes.

As illustrated in Figure 5, both donors produced good quality development with sharply defined maxima and minimal residual indanedione (as evidenced by a small shoulder at 470 nm) [1,10] present on Reflex Ultrawhite, correlating with clear, highly luminescent ridge detail. The male donor also produced high quality fingermarks on EXP 800 paper, with similar macroscopic and spectroscopic trends to those observed for the same donor on Reflex paper. Conversely, the female sample exhibited broader peaks and baseline than the Reflex and male samples (twin peak at 520 nm). A high degree of inter-sample variation was observed in the UV region of the spectra, but further investigation is required to determine whether this is due to variations in paper morphology or donor secretions.

Figure 5: *Normalised visible spectra of fingermarks on the three copy papers developed with* 1,2-*indanedione-zinc and representative images of fingermark development from the male (M) and female (F) donors captured using the Rofin Poliview IV/PL500 (excitation 530 nm, 590 nm bandpass barrier filter).*

Interestingly, the reclaimed fibre Evolve paper demonstrated inverse behaviour to EXP 800, with the female fingermarks producing better defined spectra than the male. The male sample resulted in broad maxima at 550 and 520 nm, and the presence of a strong unidentified peak at 410 nm. The female sample baseline was greater than that of the male sample and the maxima were broader than that observed for Reflex with the same donor, with a greater contribution from λ_{max2} at 520 nm. The latent fingermarks developed on Evolve not only exhibited the poorest colour and luminescence, but produced non-uniform ridges with localised areas of high intensity flaring and broadening most noticeable in male marks, potentially due to the initial interaction between substrate and deposit.

Longitudinal cross-sections of each fingermark visualised by fluorescence microscopy suggested that fingermark secretions on Reflex only penetrated the superficial layers of the paper, while Evolve and EXP 800 had ridge development deeper within the paper matrix. Furthermore, the cross-sections illustrated distinct differences between the copy papers; Reflex paper having more closely packed layers, less feathering of the edge fibres and a highly reflective cut surface compared to EXP 800 and Evolve, which had the loosest fibre packing. There was no substantial difference in the depth of ridge penetration between EXP 800 and Evolve, supporting the inference that the quality of the enhanced fingermark is not simply a function of paper porosity or density [42].

Surface incident light and fluorescence microscopy indicated that the calcium carbonate filler was largely inert for Ind-Zn developed fingermarks, with luminescent ridge detail confined to the cellulose fibres. Thus, it may be inferred that fingermark secretion deposits are confined to the fibres due to strong intermolecular interactions between amino acid residues and cellulose. The difference in ridge continuity and paper morphology is clearly demonstrated by the micrographs in Figure 6. Reflex Ultrawhite was observed to have a high density of short fibres distributed across the surface, while EXP 800 had a less even distribution and a slightly higher proportion of long fibres. Evolve Everyday paper had the poorest distribution of fibres, with large gaps present between fine fibres. Consequently, fibre distribution, density and length appear to be an influencing factor on fingermark quality.

This preliminary study on white copy papers readily available in Australia agrees with the observations compiled by Maunu *et al*. [24-25] and suggests that, while chromatography grade cellulose is a suitable "clean" allegory for paper substrates, research also needs to be performed on operational substrates. Unfortunately, we were not able to repeat the *in situ* NMR studies on the copy papers using the same NMR spectrometer; however, replication and expansion of this study is a focus of our research.

Figure 6: *A comparison of fibre distribution, morphology and Ind-Zn development on Reflex Ultrawhite, EXP 800 and Evolve Everyday by incident light and fluorescence microscopy (8x objective; Leica FSM macroscope). Top: female donor; bottom: male donor.*

4. Conclusions

Cross polarisation ^{13}C-MAS NMR proved to be a suitable method for studying both fingermark enhancement reagents *in situ* and the dried reaction products extracted from cellulose substrates. Using this method, it was possible to compare the relative reaction rates of each of the fingermark enhancement reagents, with the slow reaction rate of DFO compared to ninhydrin and 1,2-indanedione agreeing with observations recorded during latent fingermark enhancement procedures. The combined use of ^{13}C-MAS-NMR and liquid NMR was also able to provide information regarding the stoichiometry of each reaction, which is fundamental information for understanding the limits of chemical fingermark enhancement reagents.

These results demonstrate that cross-polarisation ^{13}C-MAS NMR is a viable technique for not only studying the reactions of latent fingermark enhancement reagents with latent fingermark

residues *in situ*, but also for investigating the interactions between paper-based substrates, latent fingermark residues and enhancement reagents. This widens the scope of fingermark enhancement research to characterise and explain the rudimentary factors that are responsible for the reported variability in the performance of latent fingermark reagents across jurisdictions. Further research into the physico-chemical origins of the divergent success rates observed between virgin and reclaimed fibre (recycled) copy papers, as well as archival versus non-archival papers, could lead to the targeted application of specific fingermark enhancement reagents for defined classes of paper. This would ultimately ensure that fingermark development schemes are applied in the most efficient manner to give the best possible results in operational casework.

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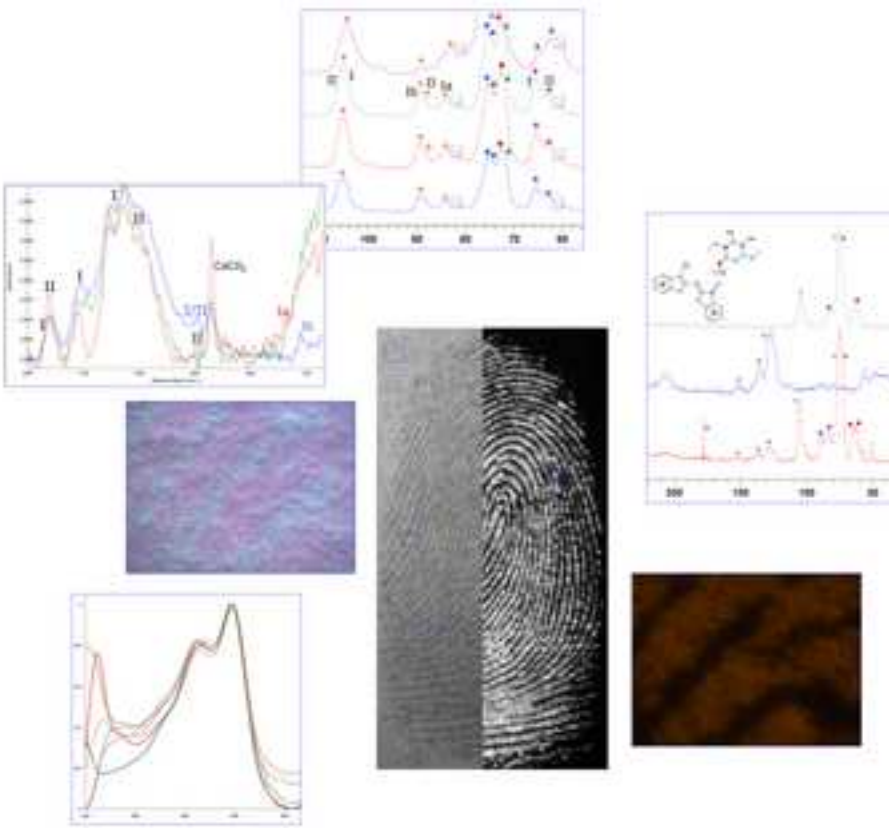
Highlights:

- Cross-polarisation ^{13}C -MAS-NMR was used to study fingermark enhancement reagents
- Ind-Zn, ninhydrin, DFO and isatin interact differently with cellulose
- Three copy papers could be differentiated by NMR, FTIR and morphology
- Chemical and physical attributes of cellulose in paper affect fingermark quality
- ^{13}C-MAS-NMR is a promising technique for studying fingermark enhancement reagents



Paper
Additives
Composition
Interactions
Structure
Donor
Targets
Quality
Reagent
Optimisation
Development

Figure 1

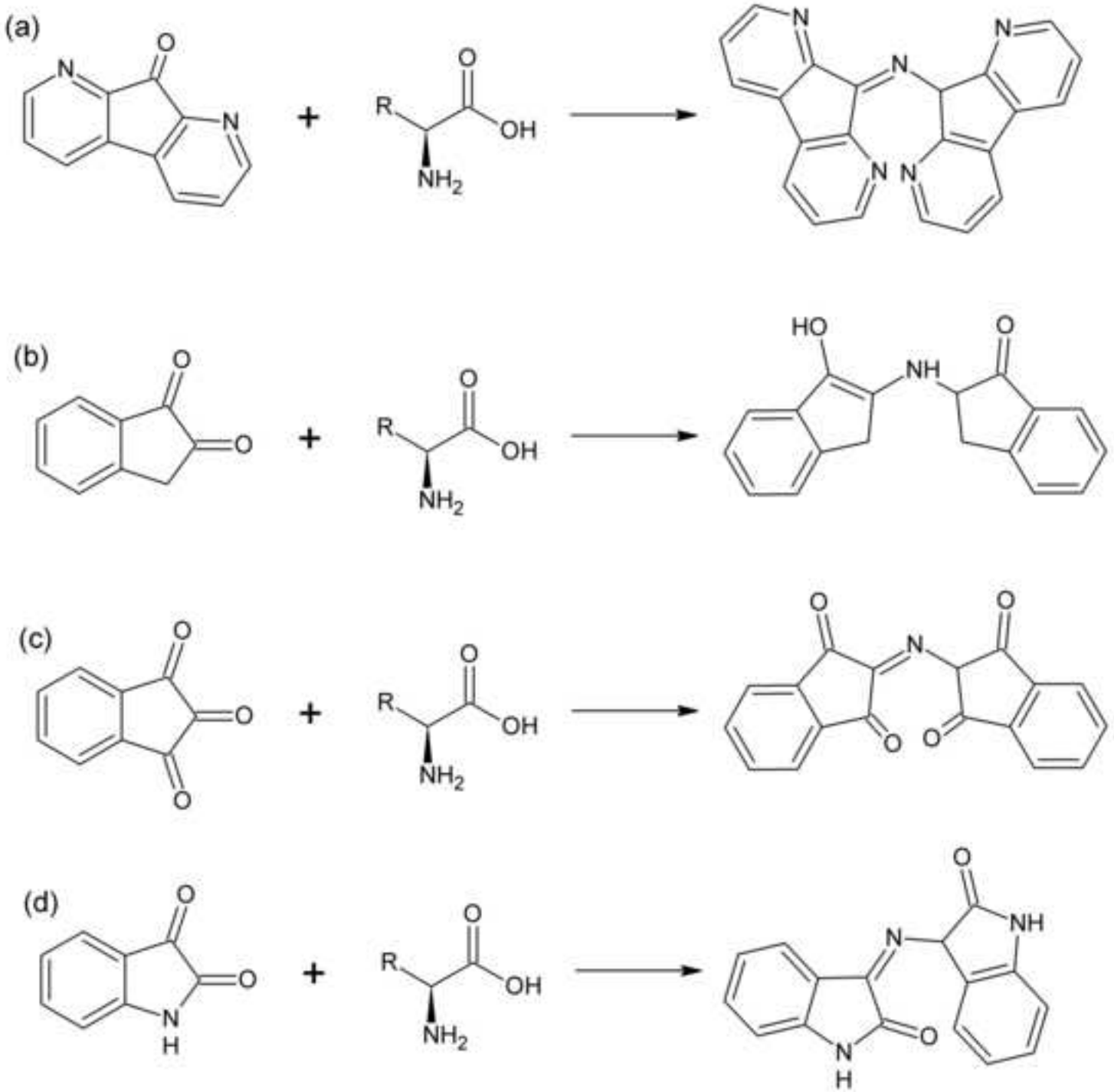
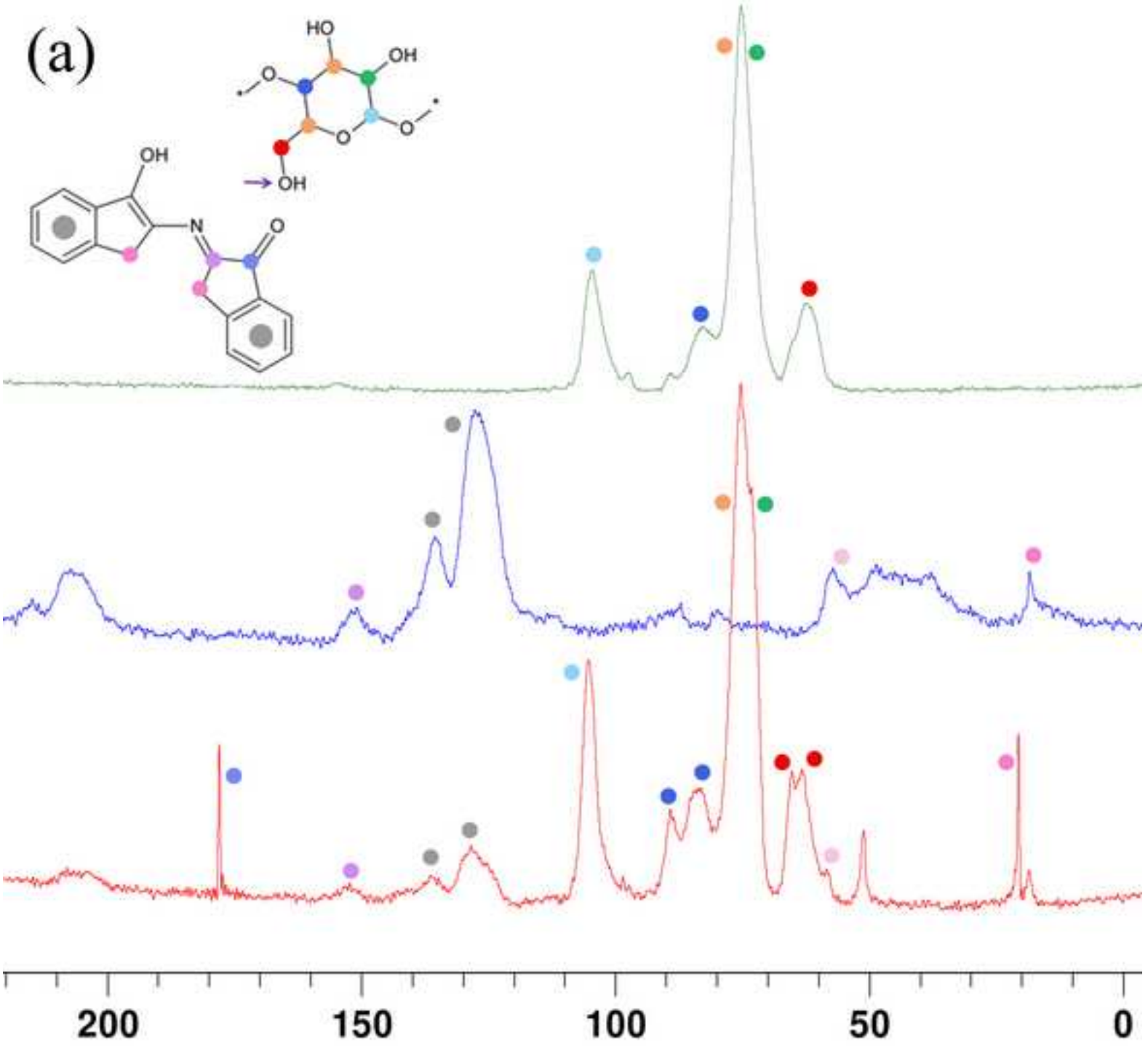

(a)
O
N
N
+
R
O
OH
NH2
N
N
N
N
N
(b)
O
O
+
R
O
OH
NH2
HO
NH
O
(c)
O
O
O
+
R
O
OH
NH2
O
N
O
O
O
(d)
O
O
N
H
+
R
O
OH
NH2
O
NH
N
O
N
H

Figure 2a



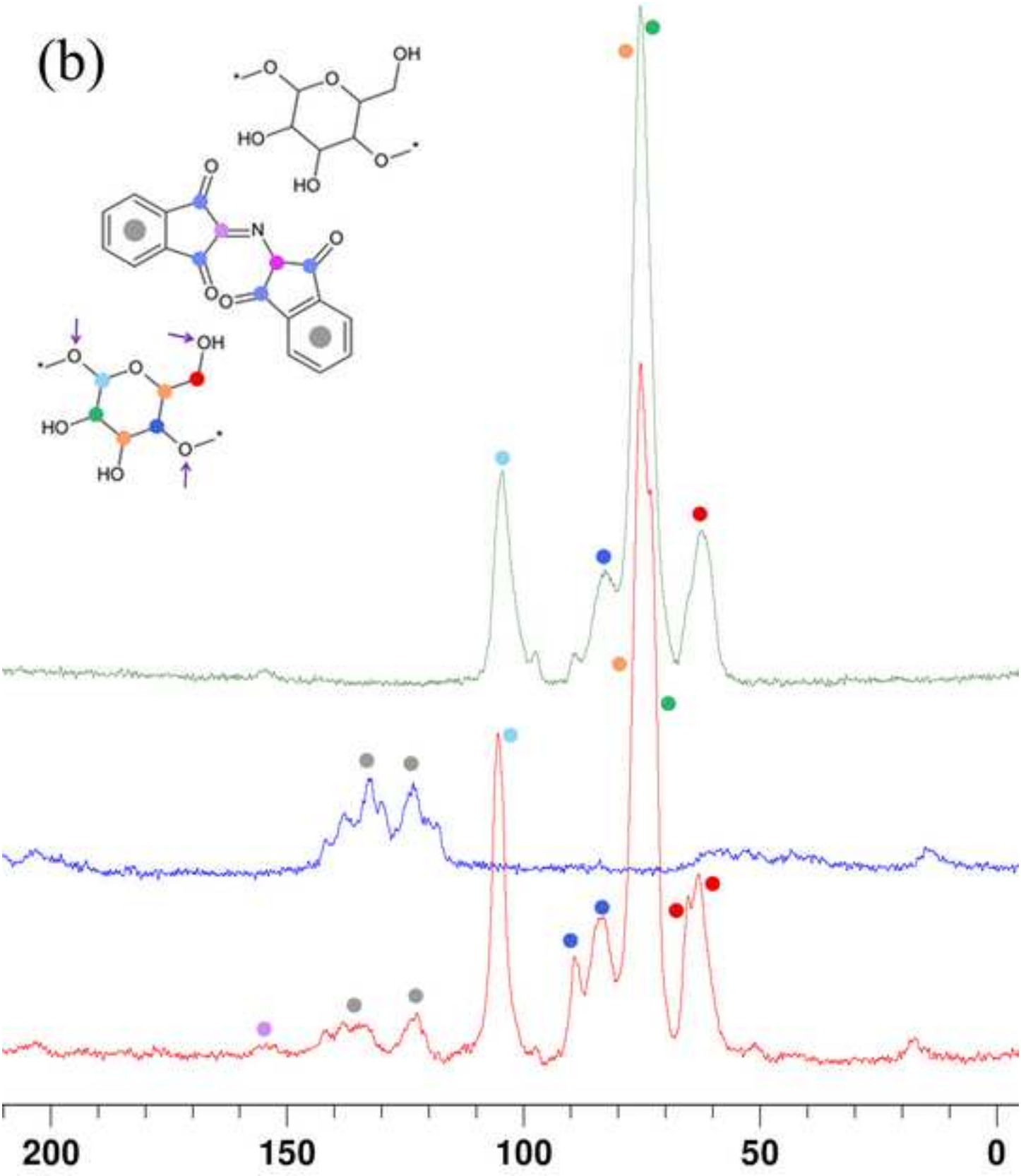


Figure 2c

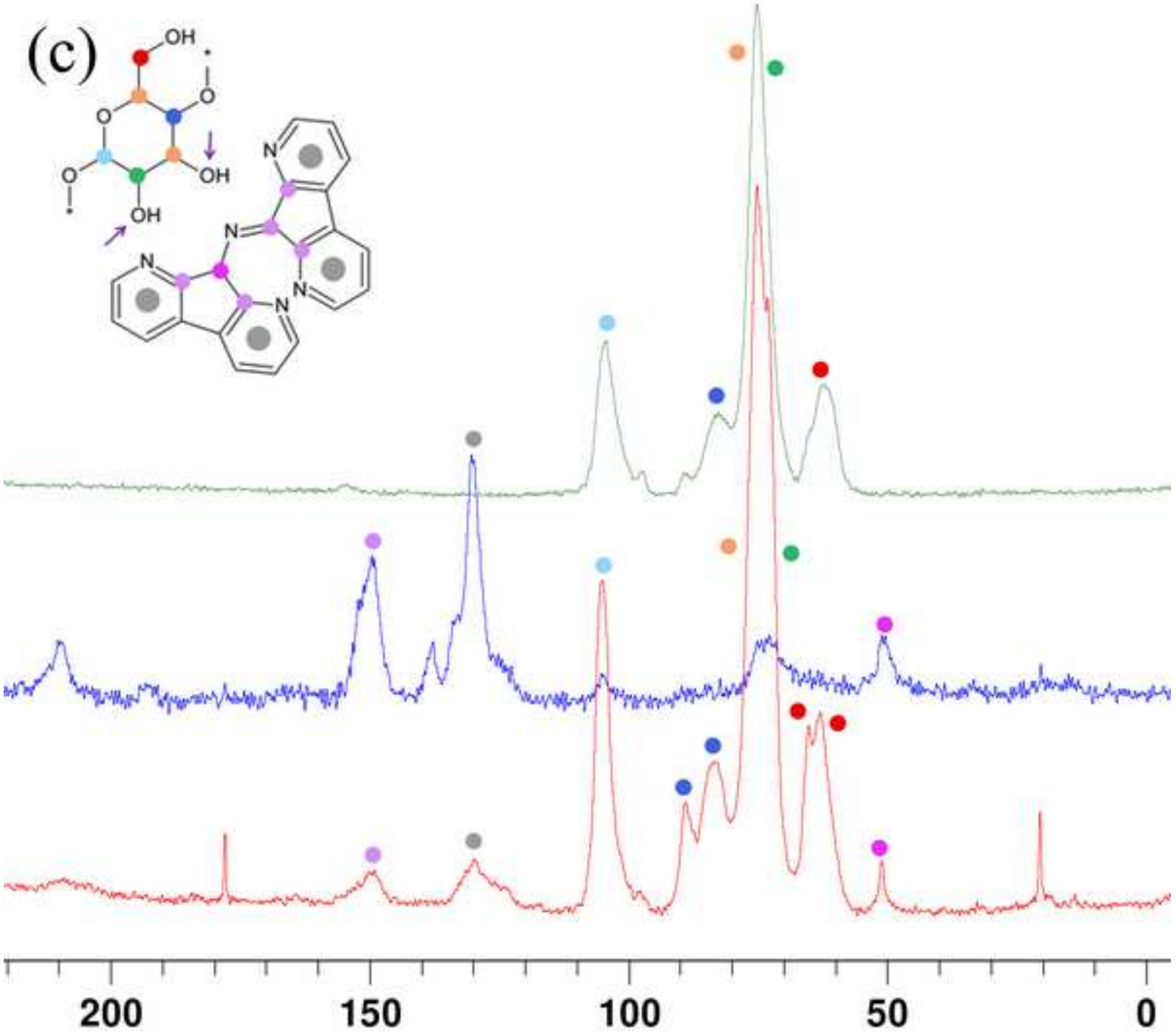
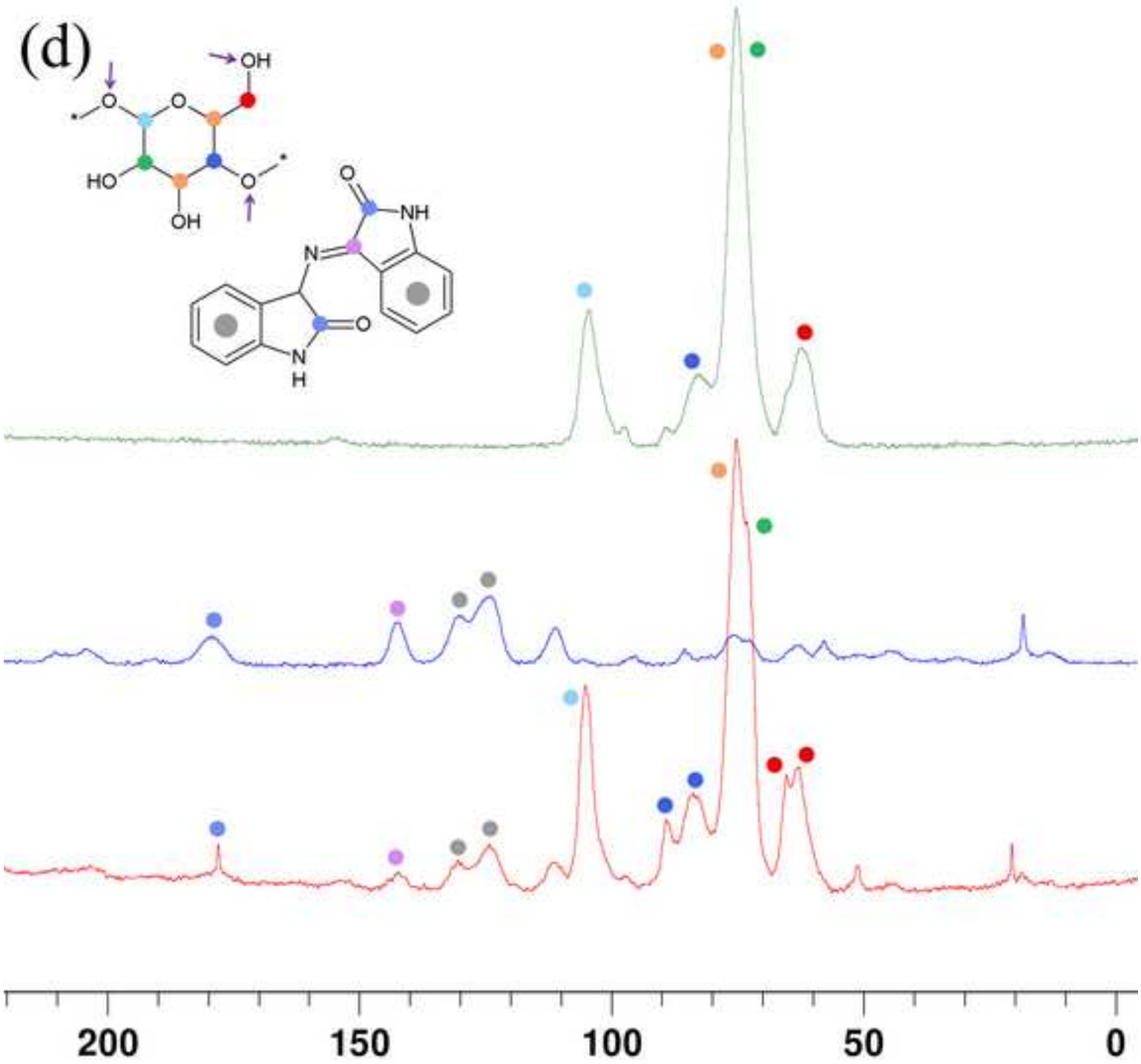


Figure 2d



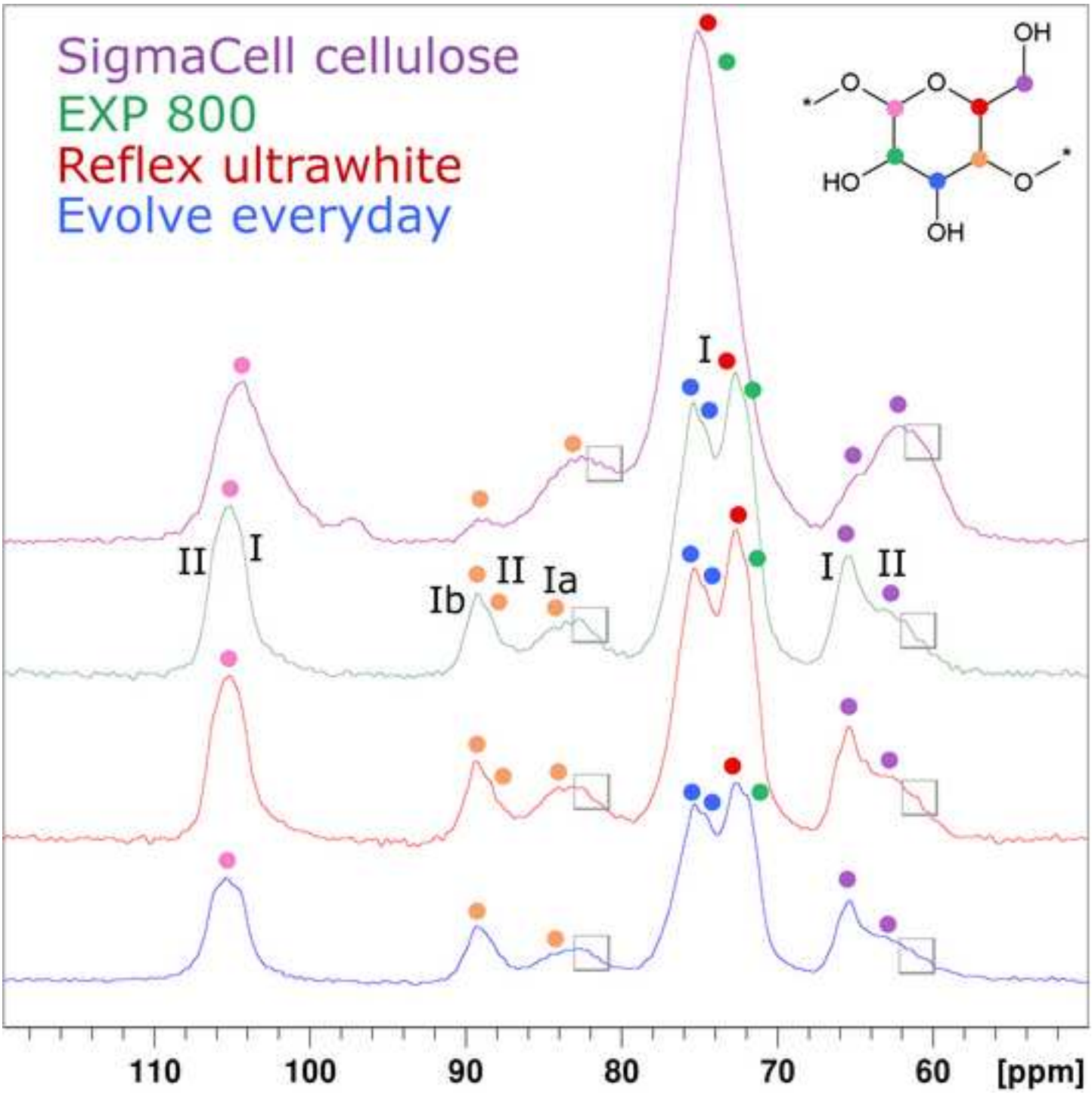
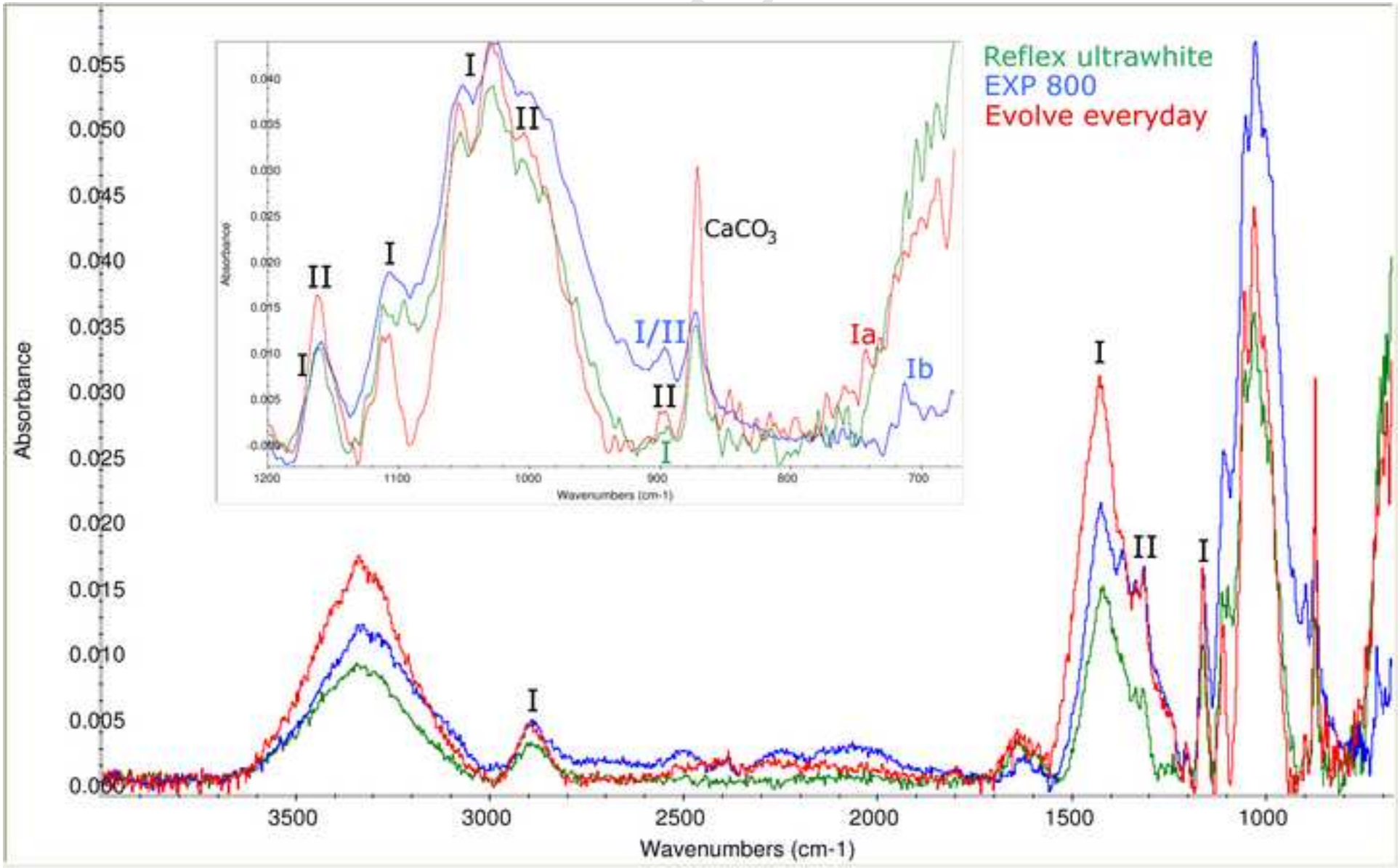


Figure 4


Reflex ultrawhite
EXP 800
Evolve everyday
Absorbance
Wavenumbers (cm-1)
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
3500
3000
2500
2000
1500
1000
I
II
CaCO3
I/II
Ia
Ib
1200
1100
1000
900
800
700