Report on the PhD thesis:

'Study of the mechanism of radiation- and photoinduced oxidation of methionine containing peptides'

by Marta Teresa Ignasiak

Peter Wardman, (formerly) University of Oxford, Gray Cancer Institute

This thesis is of the conventional 'monograph' form. The candidate has already published three full papers based on the work described:

> M Ignasiak, D Scuderi, P de Oliveira, T Pedzinski, Y Rayah and C Houée-Levin, Characterization by mass spectrometry and IRMD spectroscopy of the sulfoxide group in oxidized methionine and related compounds. *Chemical Physics Letters,* 2011, **502**, 29–36.
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> M Ignasiak, P de Oliveira, C Houée-Levin and D Scuderi, oxidation of methionine-containing peptides by $^{\bullet}$OH radicals: is sulfoxide the only product? *Chemical Physics Letters,* 2013, **590**, 35–40.
>
> M Ignasiak, B Marciniak and C Houée-Levin. A long story of sensitized one-electron oxidation of methionine. *Israel Journal of Chemistry,* 2014, **54**, 248–253.

and a further paper has been submitted to the *Journal of Photochemistry and Photobiology, A: Chemistry.*

The thesis describes a substantial body of work; it is written in English, with Polish and French summaries. The English is of a good standard: ignoring the occasional missing 'the' or 'a(n)', doubtless reflecting differing structures of English and Polish, the grammatical and typographical errors encountered were of the same order as those typically found in comparable theses written by native English speakers.

The standard of presentation is good and the layout conventional, in five chapters, with the usual *Introduction, Methodology* (photochemistry/radiation chemistry/general), *Results and Discussion,* and *Conclusions.* Each chapter has its own bibliography. Minor criticisms of presentational style are: the section numbering mixes Roman and Arabic numbers (the use of the former seems quite unnecessary); the list of abbreviations is at the end rather than the beginning, as more usual; and the limitation of author names in the bibliographies, truncating to the first author when there are more than three authors (as is the case with most papers) – I would have preferred a full author list. Any space penalty would be easily outweighed by having a single bibliography rather than added to each chapter, which introduces some duplication.

The topic is well set out in the *Introduction.* Methionine is an amino acid with special characteristics resulting from the sulfur (thioether) centre, oxidation of which has major repercussions in ageing and oxidative stress generally. While the topic has been studied to varying degrees over many years, this thesis applies advanced methodology, particularly mass spectrometric techniques, to advance knowledge

significantly in this important area. A principal aim was to extend earlier studies to include amino acid sequences, investigating the effects of neighbouring groups on methionine oxidation. This is a complex and ambitious project. The *Introduction* explains in detail why the topic is of importance and provides good background information, with plenty of detail without being too long. The candidate demonstrated a good knowledge of previous work in this area. The existing literature is surveyed adequately.

The work described generated significant new knowledge, as attested to by the publication of three papers (to date) in good journals. Particularly noteworthy was the identification of methionine sulfoxide residues after oxidation by photosensitizer excited states and hydroxyl free radicals in oxygen-free solutions. A clearer understanding of the roles of different transient species (three-electron bonded atoms) in peptide oxidation was achieved. Photochemical studies extended work with established photosensitizers to similar molecules which are constituents of sunscreens.

The methodology used well-established time-resolved radiation- and photo-chemical techniques but also included extensive studies of stable product analyses utilizing state-of-the-art mass spectrometry and infrared spectroscopy. It is fairly unusual for the two different approaches to aid mechanistic understanding (ultra-fast phenomena and stable product measurement) to be combined in a single study, and the applicant (and her supervisors) can be commended for their ambition in this regard. The description of the work is generally sufficient, although there seemed to be no mention of the sources of chemicals (usually a matter of routine detail), and in one or two places there was a lapse where some minor experimental detail was lacking. However, it could easily be inferred from similar studies described.

The thesis described the results clearly, with plenty of diagrams, tables of data and mechanistic schemes. Occasionally jargon crept in, e.g. the description of one reaction pathway as the 'k_{NH}' pathway, rather than something more descriptive (after all, *k* here is a rate constant rather than a reaction path). It might have been better to have a slightly shorter description of some of the competing reaction pathways in the *Introduction*, and have some of the reaction schemes there moved to the *Results and Discussion* section, avoiding the need to turn back to the *Introduction*. However, this is a trivial criticism. The interpretation of the data is reasonable and well-argued.

In one or two instances the results occasionally contradict or question related work in the literature. This is handled well and the advanced methodology applied in this study gives confidence in the newer work. The implications of the study beyond the narrow focus of particular problems are adequately described.

In conclusion, this thesis is of a standard, both in content and presentation, which is certainly comparable to international acceptance for the requirements for the degree of PhD.

P. Wardman

Peter Wardman DSc PhD Chem FRSC
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