

# **Ultrafast Spectroscopy and Drug Delivery of the Medicinal Pigment Curcumin in Molecular Assemblies**



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This dissertation is submitted in total fulfilment of the requirements  
for the degree of Doctor of Philosophy

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## Abstract

Curcumin is a natural pigment extracted from turmeric. It is well known as a spice and herbal medicine in east Asia. The medicinal effects of curcumin have been demonstrated for cancer, inflammation, Alzheimer's disease, and cystic fibrosis. Recent studies have explored a number of delivery systems to suppress rapid aqueous degradation of curcumin and improve its bioavailability. Previously, we have demonstrated that diamide linked  $\gamma$ -cyclodextrin dimers, namely  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$ , suppress the degradation of curcumin by forming strong 1:1 cooperative binding complexes under physiological conditions. This result indicates the potential for  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$  as curcumin delivery systems.

As a part of the thesis work, both  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$  are used as molecular-scale delivery agents for curcumin in potential treatment of cancer. Cellular viability assays and gene regulation in human prostate cancer (PC-3) cells show an anti-proliferative effect of curcumin complexed with  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$ , which is comparable with that of curcumin alone. Both  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$  carriers show a lack of toxicity to the cells. Fluorescence studies show the intracellular delivery of curcumin by  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$ . Our results strongly suggest the potential of these carriers for future studies involving animal models.

To further understand the properties of curcumin, particularly its photo-therapeutic effect, ultrafast dynamics of curcumin complexed with  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$  are investigated using femtosecond transient absorption spectroscopy. Both curcumin complexes show only an excited state absorption (ESA) band without any stimulated emission signals. The ESA decay kinetics reveals the non-radiative relaxation of curcumin through solvent reorganization, excited state intramolecular hydrogen atom transfer, and other slow dynamics of inclusion molecules and flexibility of the  $\gamma$ -CD moieties of  $66\gamma\text{CD}_2\text{su}$  and  $66\gamma\text{CD}_2\text{ur}$ . In addition, transient absorption anisotropy studies reveal slow rotational motions of the curcumin complexes due to their large hydrodynamic volumes.

Hydrophobically modified polyacrylates are also potential delivery systems for curcumin because they suppress its degradation under physiological conditions. The

3 % octadecyl randomly substituted polyacrylate, PAAC18, shows a remarkable ability to suppress the degradation of curcumin, which is attributed to strong hydrophobic interactions between curcumin and the octadecyl substituents of PAAC18 within the micelle-like aggregates and the hydrogel. In contrast, the 3 % dodecyl randomly substituted polyacrylate, PAAC12, shows a negligible effect on slowing the degradation of curcumin, which is consistent with the dodecyl substituents being insufficiently long to capture curcumin in an adequately hydrophobic environment.

The ultrafast dynamics of water molecules and curcumin in the PAAC18 hydrogel are also studied using ultrafast spectroscopic techniques. The solvation dynamics (reorganization) of water molecules in the PAAC18 hydrogel exhibit a triexponential characteristic, as shown using femtosecond fluorescence upconversion spectroscopy. We attribute the slow solvation dynamics to the confinement of water molecules in the three-dimensional cross-linking network of the octadecyl substituents of PAAC18. Moreover, non-radiative relaxation processes of curcumin were investigated using femtosecond transient absorption spectroscopy.

## List of Publications

The following publications are presented as a part of the thesis work.

- Leung, M. H. M.<sup>†</sup>; Harada, T.<sup>†</sup>; Kee, T. W., Delivery of Curcumin and Medicinal Effects of the Copper(II)-Curcumin Complexes. *Curr. Pharm. Des.* **2013**, 19, 2070-2083 (Published in April 2013). <sup>†</sup>These authors contribute equally to this review article. Reprinted by permission of Eureka Science Ltd. Copyright (2013) Eureka Science Ltd.
- Harada, T.; Giorgio, L.; Harris, T. J.; Pham, D.-T.; Ngo, H. T.; Need, E. F.; Coventry, B. J.; Lincoln, S. F.; Easton, C. J.; Buchanan, G.; Kee, T. W., Diamide Linked  $\gamma$ -Cyclodextrin Dimers as Molecular-scale Delivery Systems for the Medicinal Pigment Curcumin to Prostate Cancer Cells. *Mol. Pharmaceutics* **2013**, 10, 4481-4490 (Published in December 2013). Adapted with permission from this journal article. Copyright (2013) American Chemical Society.
- Harada, T.; Pham, D.-T.; Lincoln, S. F.; Kee, T. W., The Capture and Stabilization of Curcumin Using Hydrophobically Modified Polyacrylate Aggregates and Hydrogels. *J. Phys. Chem. B* **2014**, 118, 9515–9523 (Published in July 2014). Adapted with permission from this journal article. Copyright (2014) American Chemical Society.
- Harada, T.; McTernan, H. L.; Pham, D.-T.; Lincoln, S. F.; Kee, T. W., Femtosecond Transient Absorption Spectroscopy of the Medicinal Agent Curcumin in Diamide Linked  $\gamma$ -Cyclodextrin Dimers *J. Phys. Chem. B* **2015**, 119, 2425-2433 (Published in September 2014). Adapted with permission from this journal article. Copyright (2015) American Chemical Society.

The following publication is presented as a part of the thesis work and will be submitted to a journal article.

- Harada, T.; Pham, D.-T.; Lincoln, S. F.; Kee, T. W., Ultrafast Dynamics of the Medicinal Pigment Curcumin and Solvation Dynamics of Water in Octadecyl Substituted Polyacrylate Hydrogel (In preparation).

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# List of Abbreviations

BBO	$\beta$ -Barium borate
10%-PAAC12	10 % Dodecyl randomly substituted polyacrylate
10%-PAAC18	10 % Octadecyl randomly substituted polyacrylate
PAAC12	3 % Dodecyl randomly substituted polyacrylate
PAAC18	3 % Octadecyl randomly substituted polyacrylate
66 $\gamma$ CD <sub>2</sub> su	<i>N,N'</i> -Bis(6 <sup>A</sup> -deoxy- $\gamma$ -cyclodextrin-6 <sup>A</sup> -yl)succinamide
66 $\gamma$ CD <sub>2</sub> ur	<i>N,N'</i> -Bis(6 <sup>A</sup> -deoxy- $\gamma$ -cyclodextrin-6 <sup>A</sup> -yl)urea
DMPC	1,2-Dimyristoyl- <i>sn</i> -glycero-3-phosphocholine
DHAQ	1,8-Dihydroxy-9,10-anthraquinone
NMP	1-Methyl-2-pyrrolidone
6 $\gamma$ CDN <sub>3</sub>	6 <sup>A</sup> -Azido-6 <sup>A</sup> -deoxy- $\gamma$ -cyclodextrin
6 $\gamma$ CDTs	6 <sup>A</sup> - <i>O</i> -(4-methylbenzenesulfonyl)- $\gamma$ -cyclodextrin
AOT	Aerosol OT
AR	Analytical Reagent
BSA	Bovine Serum Albumin
<i>BRCA2</i>	Breast Cancer 2 gene
CTAB	Cetyltrimethylammonium bromide
CMOS	Complementary Metal–Oxide–Semiconductor
CW	Continuous Wave

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CD	Cyclodextrin
COX-II	Cyclooxygenase-II
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
DNA	Deoxyribonucleic acid
DCC-FBS	Dextran-Coated Charcoal Stripped Fetal Bovine Serum
DCC	Dicyclohexylcarbodiimide
DMSO	Dimethyl sulphoxide
DPSS	Diode-Pumped Solid State
DTAB	Dodecyltrimethylammonium bromide
eq	Equation
ESA	Excited State Absorption
ESIHT	Excited-State Intramolecular Hydrogen atom Transfer
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FWHM	Full Width at Half Maximum
GVD	Group Velocity Dispersion
<i>GADD45A</i>	Growth Arrest and DNA-Damage-inducible protein alpha gene
IC50	Half maximal inhibitory concentration
<i>HMOX1</i>	Heme Oxygenase 1 gene
HPLC	High Pressure Liquid Chromatography
PC-3	Human Prostate Cancer
HSA	Human Serum Albumin
H/D exchange	Hydrogen/Deuterium exchange
IgG	Immunoglobulin G

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LBO	Lithium triborate
mp	melting point
mRNA	messenger Ribonucleic acid
Mw	Molecular weight
NIR	Near Infra-Red
Nd:YLF	Neodymium-doped yttrium lithium fluoride
Nd:YVO <sub>4</sub>	Neodymium-doped yttrium vanadate
<i>n.s.</i>	not significant
<i>NFκBIA</i>	Nuclear Factor κ light polypeptide gene enhancer in B-cells Inhibitor, Alpha gene
NRF2	Nuclear Factor like 2
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
OD	Optical Density
OC	Output Coupler
PPAR	Peroxisome Proliferator-Activated Receptor
PRF	Phenol Red-Free
PBS	Phosphate Buffer Saline
PD	Photodiode Detector
PC	Pockels Cell
PLGA	Poly(lactic- <i>co</i> -glycolic acid)
QPCR	Quantitative Real Time Polymerase Chain Reaction
RI	Refractive Index
SPM	Self Phase Modulation

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SDS	Sodium dodecyl sulphate
SD	Standard Deviation
SEM	Standard Error of the Mean
SE	Stimulated Emission
Mw/Mn	The Weight Average Molecular Weigh over The Number Average Molecular Weight
Ti:sapphire	Titanium-doped sapphire
TX-100	Triton-X 100
<i>TNFRSF10B</i>	Tumour Necrosis Factor Receptor Superfamily, member 10B gene
UV-Vis	Ultraviolet-Visible
VRR	Vertical Retro-Reflectors