DEVELOPMENT OF FLUORESCENT AND COLORIMETRIC RHODAMINE CHEMOSENSORS FOR SELECTED METAL IONS

CHEAH POH WEI

FACULTY OF SCIENCE UNIVERSITI MALAYA KUALA LUMPUR

2021

DEVELOPMENT OF FLUORESCENT AND COLORIMETRIC RHODAMINE CHEMOSENSORS FOR SELECTED METAL IONS

CHEAH POH WEI

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY FACULTY OF SCIENCE UNIVERSITI MALAYA KUALA LUMPUR

2021

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: CHEAH POH WEI

Matric No: SVA180023

Name of Degree: DOCTOR OF PHILOSOPHY

Title of Thesis ("this Work"):

DEVELOPMENT OF FLUORESCENT AND COLORIMETRIC RHODAMINE CHEMOSENSORS FOR SELECTED METAL IONS

Field of Study: INORGANIC CHEMISTRY

I do solemnly and sincerely declare that:

- (1) I am the sole author/writer of this Work;
- (2) This Work is original;
- (3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
- (4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
- (5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any mean whatsoever is prohibited without the written consent of UM having been first had and obtained;
- (6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature

Date: 30 September 2021

Subscribed and solemnly declared before,

Witness's Signature

Date: 5 October 2021

Name:

Designation:

DEVELOPMENT OF FLUORESCENT AND COLORIMETRIC RHODAMINE CHEMOSENSORS FOR SELECTED METAL IONS

ABSTRACT

Metal pollution as a result of industrialisation has been a recurring environmental issue for humans and aquatic ecosystem. However, conventional methods used to detect these metals are deemed inconvenient and impractical for on-site sensing, hence an alternative method is highly sought after. Thus far, rhodamine based probes have shown encouraging results in this area but limitations such as low sensitivity and interference from other metal ions still persist. Therefore, in an effort to improve the efficacy of metal detection, four rhodamine Schiff base chemosensors were synthesized from the condensation of rhodamine B hydrazide with different ketone and aldehydes, namely 2,4,6trihydroxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, 6-bromoveratraldehyde, and methyl ethyl ketone to form sensors THB, HMB, BVT, and MEK respectively. Characterization of the chemosensors were carried out with FTIR, ¹H-NMR, ¹³C-NMR, and X-ray crystallography. The selectivity, sensitivity, and specificity of each sensor towards various metal ions were studied with UV-vis and fluorescence spectroscopy. Results obtained have revealed different metal preferences of each sensor through colour changes, enhanced absorbance, and fluorescence, whereby BVT, HMB, and MEK were selective towards Al³⁺ and Sn²⁺ ions, while **THB** was shown to be selective towards Cu²⁺ ions. The detection limits of these sensors were also evaluated and compared with WHO acceptable limit of Al³⁺, Cu²⁺, and Sn²⁺ in drinking water. Among the sensors synthesized, THB displayed the lowest limit of detection for Cu^{2+} (0.48 μ M) followed by MEK, HMB, and lastly BVT towards their respective target metal ion. Additionally, the formation of the sensor-metal complex was confirmed to be reversible through the

utilization of EDTA, with MEK having the highest reproducibility (up to 6 cycles) amongst the four. While the binding stoichiometry determined through Job's plot was different for each sensor, the most probable binding site involves the amide carbonyl of rhodamine spirolactam ring, as revealed by the shifting of this characteristic peak to a lower frequency in the sensor-metal complex FTIR spectra. Then, the concept of these four Schiff base ligands as an on-site sensor that provides 'naked-eye' recognition of Al^{3+} , Cu^{2+} , and Sn^{2+} in 100% aqueous condition was also effectively developed with paper test strips. Furthermore, the potential of these Schiff base ligands as cancer inhibitory agents were studied using colorectal adenocarcinoma cell line (HT-29) and non-cancerous colon cell line (CCD-18Co) via MTT assay. Both BVT and HMB were not cytotoxic towards cancerous HT-29 cells, but MEK exhibited comparable cytotoxicity with cisplatin and THB was weakly cytotoxic. Nonetheless, we have successfully synthesized four new rhodamine Schiff base ligands that provides rapid "naked-eye" detection through off-on colour changes in this study. The convenience, portability, and facile synthesis of these ligands further suggest its potential application as an on-site testing kit for the detection of Al^{3+} , Cu^{2+} and Sn^{2+} .

Keywords: Rhodamine, chemosensor, "naked-eye" detection, selective, "off-on" probe.

PEMBANGUNAN KEMOSENSOR FLUORESEN DAN KOLORIMETRIK BERASASKAN RHODAMINE UNTUK ION LOGAM SELEKTIF

ABSTRAK

Pencemaran logam akibat industrialisasi merupakan isu alam sekitar yang berterusan bagi manusia dan hidupan aquatik. Walaubagaimanapun, kaedah konvensional yang digunakan untuk mengesan logam ini dianggap tidak sesuai dan tidak praktikal untuk pengesanan, oleh itu kaedah alternatif sangat diperlukan. Setakat kini, sensor berasaskan rhodamine telah menunjukkan hasil yang menggalakkan tetapi batasannya merangkumi kepekaan yang rendah dan gangguan dari ion logam lain. Oleh itu, dalam usaha untuk meningkatkan keberkesanan pengesanan logam, kajian ini akan melibatkan empat kemosensor bes Schiff berasaskan rhodamine yang disintesis dari tindak balas kondensasi rhodamine B hidrazida dengan keton dan aldehid yang berbeza, iaitu 2,4,6trihidroksibenzaldehid, 3-hidroksi-4-metoksibenzaldehid, 6-bromoveratraldehid, dan metil etil keton untuk membentuk sensor THB, HMB, BVT, dan MEK. Bes Schiff yang dihasilkan telah dicirikan dengan kaedah FTIR, CHN, ¹H-NMR, ¹³C-NMR dan kristalografi sinar-X. Selektiviti, kepekaan, dan speksifisiti setiap sensor terhadap pelbagai ion logam dikaji dengan UV-vis dan spektroskopi fluoresen. Keputusan yang diperoleh telah menunjukkan selektivit logam yang berbeza bagi setiap sensor melalui perubahan warna, penyerapan, dan fluoresen, dimana BVT, HMB, dan MEK menunjukkan sifat selektif terhadap ion Al³⁺ dan Sn²⁺, sementara **THB** menunjukkan sifat selektif terhadap ion Cu²⁺ sahaja. Had pengesanan sensor ini juga dinilai dan dibandingkan dengan had yang boleh diterima oleh WHO untuk Al³⁺, Cu²⁺, dan Sn²⁺ dalam air minuman. Di antara sensor yang disintesis, **THB** menunjukkan had pengesanan terendah untuk Cu²⁺, diikuti oleh MEK, HMB, dan akhirnya BVT dengan ion logam

sasaran masing-masing. Selain itu, pembentukan kompleks sensor-logam dalam tindak balas berbalik disahkan melalui penggunaan EDTA, seperti yang ditunjukkan oleh MEK yang mempunyai kebolehulangan tertinggi (hingga 6 kitaran) di antara keempat-empat sensor. Walaupun stoikiometri pengikatan yang ditentukan melalui plot Job adalah berbeza untuk setiap sensor, molekul yang paling mungkin terlibat dalam pengesanan logam adalah karbon amida dari spirolaktam rhodamine, seperti yang ditunjukkan oleh peralihan puncak ini ke frekuensi yang lebih rendah dalam spektrum FTIR kompleks sensor-logam. Selain itu, ligan bes Schiff sebagai sensor in-situ yang membolehkan pengesanan 'mata-kasar' terhadap ion Al³⁺, Cu²⁺, dan Sn²⁺ dalam keadaan 100% berair juga berjaya dikembangkan dengan ujian helaian kertas. Di samping itu, potensi ligan bes Schiff ini sebagai agen sitotosik dikaji dengan sel kanser kolon (HT-29) dan sel normal (CCD-18Co) melalui ujian MTT. Kedua-dua BVT dan HMB tidak sitotoksik terhadap sel HT-29, tetapi MEK menunjukkan aktiviti sitotoksik yang setanding dengan cisplatin manakala **THB** lebih lemah dalam aktiviti sitotoksik. Walaubagaimanapun, kami telah berjaya menghasilkan empat rhodamine bes Schiff ligan yang memberikan pengesanan "mata-kasar" yang cepat melalui perubahan warna dalam kajian ini. Kaedah penggunaan yang mudah, kemudahalihan, dan sintesis ligan yang ringkas ini juga mencadangkan potensi penggunaannya sebagai kit ujian untuk mengesan $A1^{3+}$, Cu^{2+} , dan Sn^{2+} di tapak.

Kata kunci: Rhodamine, kemosensor, "mata-kasar" pengesanan, selektif, "tutup-buka" sensor.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisor, Dr. Tan Kong Wai for his never-ending support, patience, encouragement, and guidance throughout this project. His expertise and continuous support made it possible for me to work on this topic that is of great interest to me. Besides that, I am most grateful to Dr. Sim Kae Shin for her help and advice in some of the biological works.

I am hugely indebted to my lab seniors, Heng Mok Piew and Lok Jun Wei for being ever so helpful and for sharing valuable advice regarding problems I have faced during my research. I am also truly grateful for my labmates, Chan Wei Chuen, Nur Amira Solehah bt Pungut, and Savina Savir for being a constant source of motivation.

Furthermore, I would like to take this opportunity to express my sincere thanks to all staff of the Department of Chemistry for their help and providing me with all the necessary facilities for this research. I also thank my friends and family for their unceasing love and support throughout this period. Last but not least, I would like to acknowledge the scholarship and financial support from MyBrainSc throughout my study.

TABLE OF CONTENTS

ORIGINAL LITERARY WORK DECLARATION	ii
ABSTRACT	iii
ABSTRAK	V
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES	xii
LIST OF TABLES	xviii
LIST OF SYMBOLS AND ABBREVIATIONS	xix
LIST OF APPENDICES	xxi

CHAPTER 1: INTRODUCTION	1
1.1 Research background	1
1.2 Problem statements	3
1.3 Research objectives	4
1.4 Scope of studies	4

CHAPTER 2: LITERATURE REVIEW	5
2.1 Metal pollution & common metal pollutants	5
2.1.1 Aluminium	7
2.1.2 Copper	9
2.1.3 Tin	10
2.2 Chemosensors	12
2.3 Schiff base chemosensor	15
2.4 Rhodamine B chemosensor	20

2.5 Rhodamine B hydrazide chemosensor	.24
2.6 Rhodamine B hydrazide substituted chemosensor	.31

CHAPTER 3: METHODOLOGY4	0
3.1 Materials	0
3.2 Physical measurements	1
3.3 Syntheses	1
3.3.1 Synthesis of rhodamine B hydrazide (RBH)4	1
3.3.2 Synthesis of 6-bromoveratraldehyde rhodamine B hydrazone (BVT)	2
3.3.3 Synthesis of 3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone	
(HMB)	3
3.3.4 Synthesis of methyl ethyl ketone rhodamine B hydrazone (MEK)4	5
3.3.5 Synthesis of 2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone (THB).4	6
3.4 X-ray crystallography	7
3.5 Spectral characteristics of chemosensors	7
3.5.1 Metals selectivity	8
3.5.2 Varying pH	8
3.5.3 Varying concentration of metal ions	9
3.5.4 Competing metals	9
3.5.5 Reversibility	0
3.6 Formation of sensor-metal complex	1
3.6.1 Job's plot	1
3.6.2 FTIR, ¹ H- and ¹³ C-NMR spectra of sensor-metal complex	1
3.7 Practical applications with chemosensors	2
3.7.1 On-site assay	2
3.7.2 Cell culture and MTT cytotoxicity assay	2

CHAPTER 4: RESULTS AND DICUSSION	54
4.1 Syntheses	54
4.1.1 Synthesis of rhodamine B hydrazide (RBH)	55
4.1.2 Synthesis of 6-bromoveratraldehyde rhodamine B hydrazone (BVT)	55
4.1.3 Synthesis of 3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone	
(HMB)	56
4.1.4 Synthesis of methyl ethyl ketone rhodamine B hydrazone (MEK)	56
4.1.5 Synthesis of 2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone (THB).56
4.2 Fourier transform infrared spectra (FTIR)	57
4.3 Nuclear magnetic resonance spectra (NMR)	58
4.4 Crystal structures of Schiff-base ligands	58
4.4.1 Crystal structure of BVT	60
4.4.2 Crystal structure of HMB	62
4.4.3 Crystal structure of MEK	64
4.4.4 Crystal structure of THB	66
4.5 Spectral characteristics of chemosensors	68
4.5.1 Colour and spectral changes upon addition of metal ions	68
4.5.2 Effects of pH	74
4.5.3 Sensitivity of the sensors as probe	89
4.5.4 Limit of detection (LOD) of the sensors	94
4.5.5 Binding affinity (K _a) of the sensors	. 102
4.5.6 Selectivity of sensors in the presence of other metals	. 105
4.5.7 Reversibility of the metal bound sensors	. 109
4.6 Possible sensing mechanism of sensors	. 112
4.6.1 Binding stoichiometry of the sensors with respective metal ions	. 112

4.6.2 Evaluation of sensor-metal complex interactions through FTIR, ¹	H- and ^{13}C -
NMR spectra	114
4.7 Feasible applications of rhodamine Schiff-base chemosensors	
4.7.1 Application in 'naked-eye' identification of metal ions	
4.7.2 Colorectal adenocarcinoma cells inhibitory properties of sensors.	

.1 Conclusions	
.2 Recommendations for future work	
REFERENCES	
IST OF PUBLICATIONS AND PAPERS PRESENTED	164
APPENDIX	

LIST OF FIGURES

Figure 2.1	:	Visual representation of a chemosensor	13
Figure 2.2	:	Mechanism for the condensation between amines and carbonyl compounds	16
Figure 2.3	:	The three forms of Schiff base reactions: (a) condensation (b) exchange, and (c) metathesis	17
Figure 2.4	:	Cyclization between open and closed form of rhodamine B.	21
Figure 2.5	:	Spirolactam ring-opening process and colour changes of rhodamine derivative	23
Figure 2.6	:	Synthesis of rhodamine B hydrazide from rhodamine B	24
Figure 2.7	:	Cu ²⁺ -assisted spirolactam ring opening of rhodamine B hydrazide (RBH)	25
Figure 2.8	:	HPLC chromatograms of (a) chemosensor (b) metal- chemosensor complex	27
Figure 2.9	:	Infrared spectra of (a) sensor and (b) sensor in presence of metal	28
Figure 2.10	:	¹ H NMR spectra of (a) sensor, (b) sensor + 0.5 equivalent of metal, and (c) sensor + 1 equivalent of metal	29
Figure 2.11	:	Proposed binding mechanism of sensor with target metal ion (Al^{3+})	29
Figure 2.12	:	One-pot synthesis of (a) rhodamine B hydrazide and (b) rhodamine derivative chemosensor	32
Figure 2.13	:	Reversible Schiff base condensation mechanism between a carbonyl group with an aldehyde	33
Figure 2.14	:	X-ray structure of rhodamine derivative bearing the spirolactam form	34
Figure 2.15	:	Effects of solvent on fluorescence emission of rhodamine derivative chemosensor	36
Figure 2.16	:	Colour change of gel balls in pure water, from brown to light green upon adsorption of Cu^{2+} ions. Left: Aqueous copper chloride solution, Middle: Gel balls in pure water, Right: Gel balls added to aqueous copper chloride solution	38

:	Synthetic pathway of RBH and chemosensors BVT , HMB , MEK , and THB	53
:	X-ray crystal structure of chemosensor BVT . Hydrogen atoms are removed for clarity and structure drawn with 50% ellipsoid	60
:	Honeycomb packing formed from Van der Waals interactions of complex BVT	60
:	X-ray crystal structure of chemosensor HMB . Hydrogen atoms are removed for clarity and structure drawn with 10% ellipsoid	62
:	Zigzag chain formed from Van der Waals interactions of complex HMB	62
:	X-ray crystal structure of chemosensor MEK . Hydrogen atoms are removed for clarity and structure drawn with 30% ellipsoid	64
:	Zig-zag chain formed from Van der Waals interactions of complex MEK	64
:	X-ray crystal structure of chemosensor THB . Hydrogen atoms are removed for clarity and structure drawn with 10% ellipsoid	66
:	Zig-zag chain formed from Van der Waals interactions of complex THB	66
	Colour changes of chemosensors upon addition of various metal ions. (a) BVT (100 μ M) turned pink in presence of Al ³⁺ and Sn ²⁺ ions (300 μ M) in 50% acetonitrile solution, (b) Addition of Al ³⁺ and Sn ²⁺ (100 μ M) to HMB (50 μ M) in 30% acetonitrile solution changed from colourless to pink, (c) MEK (50 μ M) solution turned pink upon introduction of Al ³⁺ and Sn ²⁺ (50 μ M) in 30% acetonitrile solution, and (d) Only Cu ²⁺ turned pink in the presence of THB in 30% acetonitrile solution.	70
:	UV-vis spectra of chemosensors in the presence of various cations at room temperature. (a) BVT in 50% acetonitrile solution, $\lambda_{max} = 562$ nm, (b) HMB in 30% acetonitrile solution, $\lambda_{max} = 563$ nm, (c) MEK in 30% acetonitrile solution, $\lambda_{max} = 563$ nm, and (d) THB in 30% acetonitrile solution, $\lambda_{max} = 559$ nm.	71
		 Synthetic pathway of RBH and chemosensors BVT, HMB, MEK, and THB

Figure 4.12	:	Colour changes of chemosensors upon addition of various metal ions under UV lamp (short wavelength). (a) BVT (100 μ M) turned brighter orange fluorescence in presence of Sn ²⁺ compared to Al ³⁺ (300 μ M) in 50% acetonitrile solution, (b) Addition of Al ³⁺ and Sn ²⁺ (100 μ M) to HMB (50 μ M) in 30% acetonitrile led to slight orange fluorescence solution, (c) Introduction of Sn ²⁺ (50 μ M) to MEK (50 μ M) resulted in brighter orange fluorescence compared to Al ³⁺ (50 μ M) in 30% acetonitrile solution, and (d) Detection of Cu ²⁺ by THB is not fluorescent.	72
Figure 4.13	:	Fluorescence spectra of chemosensors in the presence of various cations at room temperature. (a) BVT in 50% acetonitrile solution, $\lambda_{ex} = 562 \text{ nm}$, $\lambda_{em} = 588 \text{ nm}$ (b) HMB in 30% acetonitrile solution, $\lambda_{ex} = 563 \text{ nm}$, $\lambda_{em} = 588 \text{ nm}$, and (c) MEK in 30% acetonitrile solution, $\lambda_{ex} = 563 \text{ nm}$, $\lambda_{em} = 588 \text{ nm}$.	73
Figure 4.14	:	Colour changes of BVT upon addition of various metal ions at different pH values in 50% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 6.0, (d) pH 7.2, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	80
Figure 4.15	:	Colour changes of HMB upon addition of various metal ions at different pH values in 30% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	81
Figure 4.16	:	Colour changes of MEK upon addition of various metal ions at different pH values in 30% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	82
Figure 4.17		Colour changes of THB upon addition of various metal ions at different pH values in 30% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	83
Figure 4.18	:	Effect of pH on the absorbance at (a) 562 nm of BVT (100 μ M), BVT + Al ³⁺ (300 μ M), and BVT + Sn ²⁺ (300 μ M), (b) 563 nm of HMB (50 μ M), HMB + Al ³⁺ (100 μ M), and HMB + Sn ²⁺ (100 μ M), (c) 563 nm of MEK (50 μ M), MEK + Al ³⁺ (50 μ M), and MEK + Sn ²⁺ (50 μ M), and MEK + Sn ²⁺ (50 μ M), and (d) 559 nm of THB (10 μ M) and THB + Cu ²⁺ (10 μ M).	84
Figure 4.19	:	Colour changes of BVT upon addition of various metal ions at different pH values in 50% acetonitrile solution under a UV lamp. (a) pH 2.0, (b) pH 4.0, (c) pH 6.0, (d) pH 7.2, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	85

Figure 4.20	Colour changes of HMB upon addition of various metal ions at different pH values in 30% acetonitrile solution under a UV lamp. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	86
Figure 4.21	Colour changes of MEK upon addition of various metal ions at different pH values in 30% acetonitrile solution under a UV lamp. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0	87
Figure 4.22	Effect of pH on the fluorescence intensity at 588 nm of (a) BVT (100 μ M), BVT + Al ³⁺ (300 μ M), and BVT + Sn ²⁺ (300 μ M), (b) HMB (50 μ M), HMB + Al ³⁺ (100 μ M), and HMB + Sn ²⁺ (100 μ M), and (c) MEK (50 μ M), MEK + Al ³⁺ (50 μ M), and MEK + Sn ²⁺ (50 μ M)	88
Figure 4.23	: Absorption spectra of (a) BVT $(100 \ \mu\text{M}) + \text{Al}^{3+} (0-600 \ \mu\text{M})$ and (b) BVT $(100 \ \mu\text{M}) + \text{Sn}^{2+} (0-360 \ \mu\text{M})$ in 50% acetonitrile solution, (c) HMB $(50 \ \mu\text{M}) + \text{Al}^{3+} (0-175 \ \mu\text{M})$ and (d) HMB $(50 \ \mu\text{M}) + \text{Sn}^{2+} (0-150 \ \mu\text{M})$ in 30% acetonitrile solution, (e) MEK $(50 \ \mu\text{M}) + \text{Al}^{3+} (0-130 \ \mu\text{M})$ and (f) MEK $(50 \ \mu\text{M}) + \text{Sn}^{2+} (0-60 \ \mu\text{M})$ in 30% acetonitrile solution, and (g) THB $(10 \ \mu\text{M}) + \text{Cu}^{2+} (0-30 \ \mu\text{M})$ in 30% acetonitrile solution.	91
Figure 4.24	Fluorescence emission spectra of (a) BVT $(100 \mu\text{M}) + \text{Al}^{3+}$ (0–600 μ M) and (b) BVT $(100 \mu\text{M}) + \text{Sn}^{2+}$ (0–360 μ M) in 50% acetonitrile solution, (c) HMB $(50 \mu\text{M}) + \text{Al}^{3+}$ (0– 175 μ M) and (d) HMB $(50 \mu\text{M}) + \text{Sn}^{2+}$ (0–150 μ M) in 30% acetonitrile solution, (e) MEK $(50 \mu\text{M}) + \text{Al}^{3+}$ (0–130 μ M) and (f) MEK $(50 \mu\text{M}) + \text{Sn}^{2+}$ (0–60 μ M) in 30% acetonitrile solution, and (g) THB $(10 \mu\text{M}) + \text{Cu}^{2+}$ (0–30 μ M) in 30% acetonitrile solution.	92
Figure 4.25	Linearized absorbance changes of (a) $\mathbf{BVT} + Al^{3+}$, (b) $\mathbf{BVT} + Sn^{2+}$, (c) $\mathbf{HMB} + Al^{3+}$, (d) $\mathbf{HMB} + Sn^{2+}$, (e) $\mathbf{MEK} + Al^{3+}$, (f) $\mathbf{MEK} + Sn^{2+}$, and (g) $\mathbf{THB} + Cu^{2+}$	96
Figure 4.26	Linearized fluorescence intensity changes of (a) $\mathbf{BVT} + Al^{3+}$, (b) $\mathbf{BVT} + Sn^{2+}$, (c) $\mathbf{HMB} + Al^{3+}$, (d) $\mathbf{HMB} + Sn^{2+}$, (e) $\mathbf{MEK} + Al^{3+}$, and (f) $\mathbf{MEK} + Sn^{2+}$	97
Figure 4.27	Benesi-Hildebrand plot on the absorption of (a) $\mathbf{BVT} + Al^{3+}$, (b) $\mathbf{BVT} + Sn^{2+}$, (c) $\mathbf{HMB} + Al^{3+}$, (d) $\mathbf{HMB} + Sn^{2+}$, (e) \mathbf{MEK} $+ Al^{3+}$, (f) $\mathbf{MEK} + Sn^{2+}$, and (g) $\mathbf{THB} + Cu^{2+}$	102
Figure 4.28	Benesi-Hildebrand plot on the fluorescence emission ($\lambda_{em} = 588 \text{ nm}$) of (a) BVT + Al ³⁺ , (b) BVT + Sn ²⁺ , (c) HMB + Al ³⁺ , (d) HMB + Sn ²⁺ , (e) MEK + Al ³⁺ , and (f) MEK + Sn ²⁺	103

Figu	ure 4.29	:	UV–vis spectra changes for (a) BVT (100 μ M) containing 3 equivalent of various cations in presence of 3 equivalent of Al ³⁺ plotted at $\lambda_{max} = 562$ nm, (b) BVT (100 μ M) containing 3 equivalent of various cations in presence of 3 equivalent of Sn ²⁺ plotted at $\lambda_{max} = 562$ nm, (c) HMB (50 μ M) containing 2 equivalent of various cations in presence of 2 equivalent of Al ³⁺ plotted at $\lambda_{max} = 563$ nm, (d) HMB (50 μ M) containing 2 equivalent of various cations in presence of 2 equivalent of Sn ²⁺ plotted at $\lambda_{max} = 563$ nm, (e) MEK (50 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Al ³⁺ plotted at $\lambda_{max} = 563$ nm, (f) MEK (50 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Sn ²⁺ plotted at $\lambda_{max} = 563$ nm, and (g) THB (10 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Cu ²⁺ plotted at $\lambda_{max} = 563$ nm, and (g) THB (10 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Cu ²⁺ plotted at $\lambda_{max} = 563$ nm,	106
Fig	ure 4.30	:	Fluorescence spectra changes plotted at $\lambda_{em} = 588$ nm for (a) BVT (100 µM) containing 3 equivalent of various cations in presence of 3 equivalent of Al ³⁺ , (b) BVT (100 µM) containing 3 equivalent of various cations in presence of 3 equivalent of Sn ²⁺ , (c) HMB (50 µM) containing 2 equivalent of various cations in presence of 2 equivalent of Al ³⁺ , (d) HMB (50 µM) containing 2 equivalent of various cations in presence of 2 equivalent of Sn ²⁺ , (e) MEK (50 µM) containing 1 equivalent of various cations in presence of 1 equivalent of Al ³⁺ , and (f) MEK (50 µM) containing 1 equivalent of various cations in presence of 1 equivalent of Sn ²⁺	107
Fig	ure 4.31	:	Reversible cycle of (a) $\mathbf{BVT} + Al^{3+}$, (b) $\mathbf{BVT} + Sn^{2+}$, (c) $\mathbf{HMB} + Al^{3+}$, (d) $\mathbf{HMB} + Sn^{2+}$, (e) $\mathbf{MEK} + Al^{3+}$, (f) $\mathbf{MEK} + Sn^{2+}$, and (g) $\mathbf{THB} + Cu^{2+}$, with quencher EDTA by absorbance changes	109
Figu	ure 4.32	·	Reversible cycle of (a) $BVT + Al^{3+}$, (b) $BVT + Sn^{2+}$, (c) $HMB + Al^{3+}$, (d) $HMB + Sn^{2+}$, (e) $MEK + Al^{3+}$, and (f) $MEK + Sn^{2+}$, with quencher EDTA by fluorescence intensity changes	110
Figu	are 4.33	:	Job's plot of (a) $BVT + Al^{3+}$, (b) $BVT + Sn^{2+}$, (c) $HMB + Al^{3+}$, (d) $HMB + Sn^{2+}$, (e) $MEK + Al^{3+}$, (f) $MEK + Sn^{2+}$, and (g) $THB + Cu^{2+}$	112
Fig	ure 4.34	:	Infrared spectra of (a) BVT , (b) BVT with Al^{3+} , (c) BVT with Sn^{2+}	115
Figu	ure 4.35	:	Infrared spectra of (a) HMB , (b) HMB with Al^{3+} , (c) HMB with Sn^{2+}	115
Fig	ure 4.36	:	Infrared spectra of (a) MEK, (b) MEK with Al^{3+} , (c) MEK with Sn^{2+}	116

:	Infrared spectra of (a) THB , (b) THB with Cu ²⁺	116
:	¹ H NMR spectra (400 MHz, CDCl ₃) of (a) BVT , (b) BVT + Al ³⁺ , and (c) BVT + Sn ²⁺	119
:	¹ H NMR spectra (400 MHz, CDCl ₃) of (a) HMB, (b) HMB + Al^{3+} , and (c) HMB + Sn^{2+}	119
:	¹ H NMR spectra (400 MHz, CDCl ₃) of (a) MEK, (b) MEK + Al^{3+} , and (c) MEK + Sn^{2+}	120
:	¹³ C NMR spectra (100 MHz, CDCl ₃) of (a) BVT , (b) BVT + Al^{3+} , and (c) BVT + Sn^{2+}	120
:	¹³ C NMR spectra (100 MHz, CDCl ₃) of (a) HMB , (b) HMB + Al ³⁺ , and (c) HMB + Sn ²⁺	121
:	^{13}C NMR spectra (100 MHz, CDCl ₃) of (a) MEK, (b) MEK + Al ³⁺ , and (c) MEK + Sn ²⁺	121
:	Test strips for the 'naked-eye' detection of different metal ions in 100% aqueous medium with fabricated chemosensors. (a) BVT (10 mM) + metals (100 mM), (b) HMB (10 mM) + metals (100 mM), (c) MEK (10 mM) + metals (10 mM), and (d) THB (0.1 mM) + metals (0.1mM)	124
:	Test strips for the 'naked-eye' detection of different metal ions in 100% aqueous medium with fabricated chemosensors under UV lamp (a) BVT (10 mM) + metals (100 mM), (b) HMB (10 mM) + metals (100 mM), (c) MEK (10 mM) + metals (10 mM), and (d) THB (0.1 mM) + metals (0.1mM)	124
		 Infrared spectra of (a) THB, (b) THB with Cu²⁺

LIST OF TABLES

Table 4.1	:	Selected infrared spectroscopic peak assignments of RBH , BVT , HMB , MEK , and THB	56
Table 4.2	:	Crystal data and structure refinement parameters of BVT , HMB , MEK , and THB	58
Table 4.3	:	Selected bond lengths and bond angles for ligand BVT	59
Table 4.4	:	Selected bond lengths and bond angles for complex HMB	61
Table 4.5	:	Selected bond lengths and bond angles for complex MEK	63
Table 4.6	:	Selected bond lengths and bond angles for complex THB	65
Table 4.7	:	Summarized total metal ion concentration added into chemosensors and their respective absorbance and fluorescence intensities	90
Table 4.8	:	Summarized limit of detection (LOD) of chemosensors BVT , HMB , MEK , and THB with their respective absorption and fluorescence spectrum	95
Table 4.9	:	LOD comparison among a selection of reported chemosensors for detection of Al^{3+} , Cu^{2+} , and Sn^{2+}	98
Table 4.10	:	Summarized binding constants (K _a) of chemosensors BVT , HMB , MEK , and THB with their respective absorption and fluorescence spectrum	101
Table 4.11	:	Summarized number of reversible cycles of chemosensors with its target metal ions through absorption and fluorescence changes	109
Table 4.12	÷	Summarized binding stoichiometry of chemosensors BVT , HMB , MEK , and THB with their respective target metal ions	111
Table 4.13	:	Selected infrared spectroscopic peak assignments of the chemosensors BVT , HMB , MEK , and THB with its respective metal complexes.	114
Table 4.14	:	Proposed binding mechanism of the chemosensors with their respective target metal ions	117
Table 4.15	:	Cytotoxic activity (IC ₅₀ values) of BVT , HMB , MEK , and THB against HT-29 and CCD-18Co cell lines	126

LIST OF SYMBOLS AND ABBREVIATIONS

Å	:	Angstrom
°C	:	Degree celsius
μ	:	Micro
%	:	Percent
λ	:	Wavelength
B.C.	:	Before Christ
BVT	:	6-bromoveratraldehyde rhodamine B hydrazone
CDCl ₃	:	Chloroform-d
cm	:	Centimeter
cm ⁻¹	:	Reciprocal centimeter
DMF	:	N,N-dimethylformamide
DMSO	:	Dimethyl sulfoxide
DNA	:	Deoxyribonucleic acid
EDTA	:	Ethylenediamine tetraacetic acid
Eq.	:	Equivalent
Etc.	÷	Et cetera
FL	÷	Fluorescence
g	:	Gram
h	:	Hour
HMB	:	3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone
HSAB	:	Hard and soft acids and bases
IC50	:	Median inhibition concentration
IR	:	Infrared
IUPAC	:	International union of pure and applied chemistry

:	Binding constant
:	Kilogram
:	Liter
:	Limit of detection
:	Molar
:	Methyl ethyl ketone rhodamine B hydrazone
:	Milligram
:	Megahertz
:	Milliliter
:	Millimolar
:	Millimole
:	Melting point
:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
:	Nanometer
:	Nuclear magnetic resonance
:	Parts per million
:	Rhodamine B Hydrazide
:	2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone
;	Tetrahydrofuran
:	Tetramethylsilane
:	Ultraviolet-visible
:	World health organization

LIST OF APPENDICES

Appendix A	:	IR spectrum of rhodamine B hydrazide (RBH)	164
Appendix B	:	¹ H NMR of RBH	165
Appendix C	:	¹³ C NMR of RBH	166
Appendix D	:	IR spectrum of 6-bromoveratraldehyde rhodamine B hydrazone (BVT)	167
Appendix E	:	¹ H NMR of BVT	168
Appendix F	:	¹³ C NMR of BVT	169
Appendix G	:	IR spectrum of 3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone (HMB)	170
Appendix H	:	¹ H NMR of HMB	171
Appendix I	:	¹³ C NMR of HMB	172
Appendix J	:	IR spectrum of methyl ethyl ketone rhodamine B hydrazone (MEK)	173
Appendix K	:	¹ H NMR of MEK	174
Appendix L	:	¹³ C NMR of MEK	175
Appendix M	:	IR spectrum of 2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone (THB)	176
Appendix N	:	¹ H NMR of THB	177
Appendix O	:	¹³ C NMR of THB	178

CHAPTER 1: INTRODUCTION

1.1 Research background

In recent years, globalization, industrialization, and urbanization have undoubtedly left a profound effect on the environment worldwide as increasing amounts of toxic waste are being released into the atmosphere (Fashola et al., 2016). Even though this occurrence benefits the socioeconomic of each nation, improper metal waste disposal from such activities have led to various environmental and health related issues (Baldi et al., 1989; Park et al., 2020). On the other hand, it is undeniable that metal ions play important roles in most biological processes, but excessive quantities can result in biological and physiological disorders such as Alzheimer's, Parkinsons', Wilson's disease, and many more health complications (Bag & Pal, 2011). Therefore, proactive measures should be taken to identify and handle these metal waste accordingly to prevent unceasing adverse effects to the environment and human health (Nath, 2008).

Generally, several conventional methods that have been used to detect these metal ions include atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICPMS), ion chromatography (IC), high performance liquid chromatography (HPLC), and anodic stripping voltammetry (ASV), but these analytical methods are restricted by its immobility, tedious sample preparation, lengthy data acquisition time and requires sophisticated instruments along with skilled operators (Gao et al., 2012; Nayab et al., 2015; Rasheed et al., 2019; Wang et al., 2007; Wolle et al., 2014). To overcome these issues, chemically synthesized probes with optical, colorimetric, and fluorometric properties were widely explored and studied with UV-vis and fluorescence spectroscopy methods (Soares-Paulino et al., 2020; Yang et al., 2014).

These techniques are favored due to its simplicity, portability, faster response time, higher selectivity and sensitivity in contrast to conventional methods (Cai et al., 2013; Gao et al., 2014; Hazra et al., 2019; Kaur et al., 2014). Production of the aforementioned colorimetric and fluorometric methods for detection of metal ions at low concentrations have been crucial for researchers as metal ions play an important role in various environmental, medicinal, and biological processes (Cotruvo Jr et al., 2015; Valeur & Leray, 2000). Hence, the design of a fast, simple, and convenient device for monitoring these metal ions are of utmost importance (Phapale et al., 2017).

For the past twenty years, much attention have been garnered on rhodamine based derivatives as chemosensors for various metal ions detection (Bae & Tae, 2007; Ko et al., 2006). This approach was initially experimented in the pioneering work done by Dujols et al. in 1997, whereby the ring-opening mechanism of rhodamine was established (Dujols et al., 1997). The underlying principle behind this mechanism is that the closed-spirocyclic structure of rhodamine based derivatives are colourless, while the opened-spirocyclic structure prompted by the addition of target metal ion changes colour together with significant absorbance and fluorescence enhancements (Xiang & Tong, 2006). Thus, this ideal 'off-on' framework which undergoes equilibrium between the closed and open spirocyclic forms that results in optically active, colorimetric, and fluorometric properties allow extensive studies to be carried out for the detection of metal ions (Roy et al., 2019). For example, these derivatives are extensively used as industrial colouring, biomarkers, and sensing probes owing to their good photostability, high absorption coefficient, and excellent spectroscopic characteristics (Leite et al., 2013; Xu et al., 2017).

However, previously reported chemosensors have displayed limited solubility, low sensitivity, and restricted reproducibility. Hence, in this study, we have employed the same approach of the 'off-on' spirolactam ring opening mechanism and synthesized four new rhodamine based chemosensors, namely **BVT**, **HMB**, **MEK**, and **THB**. The substitution of each aldehyde or ketone moiety (2,4,6-trihydroxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, 6-bromoveratraldehyde, and methyl ethyl ketone) was anticipated with the production of a chemosensor for metal ion detection with better solubility, lower detection limit, higher selectivity, and sensitivity. Subsequent changes in colour or fluorescence upon addition of different metal ions were compared and its sensing behaviour was studied with UV-vis and fluorescence spectroscopy.

1.2 Problem statements

Metal pollution is a growing problem in the country due to the prevalent industrial activities and unregulated disposal of toxic wastes. Generally, Malaysia lacks literature study on potential rapid and convenient method of detection of these metal pollutants. Although metals are recognized as essential elements for the human body, prolonged exposure will lead to detrimental effects on bodily functions. For this reason, the study of a different approach for recognition of metal pollutants without the need of immobile and expensive instrument is important for a better environmental health.

1.3 Research objectives

This research aims to investigate the chemosensors' selectivity and binding behaviour through UV-vis and fluorescence spectroscopy techniques. Besides that, this research attempts to explore an alternative pathway that provides simple and rapid response for on-site studies. The four main objectives of this research are as follows:

- To synthesize rhodamine based chemosensors for rapid and naked eye detection of metal ions.
- 2. To investigate the preferences and sensitivity of chemosensors towards different metal ions.
- 3. To study the interactions or potential binding mode of chemosensors with different metal ions.
- 4. To evaluate the potential applications of the chemosensors.

1.4 Scope of studies

The research scope for this thesis is to study the characteristics of newly synthesized rhodamine Schiff-base compounds and its selectivity towards various metal ions. Overall, the scope is divided into four main sections based on the stated objectives. This includes the development of colorimetric and fluorometric chemosensors to be employed as real-time monitoring with "off-on" colorimetric changes upon recognition of metal ions. Its coordination mechanism with the target metal will be investigate with FTIR and NMR approaches. Lastly, the modelling of an on-site testing kit for metal detection will also be examined in fully aqueous medium for practical applications.

CHAPTER 2: LITERATURE REVIEW

2.1 Metal pollution & common metal pollutants

The rise of global population and various products developed from the start of this century came with the consequence of increased metal production (De Bartolomeo et al., 2004). As living conditions have improved over the years, the production of sophisticated products containing metals with specified functions are in demand due to its unique chemical and physical properties individually (Nriagu, 1990). However, with every innovation and development of gadgets that eases our daily lives, comes with a growing problem of metal wastes, and the improper disposal of these metal wastes into the environment will eventually disrupt the ecosystem and jeopardize human health (Babel & Kurniawan, 2003).

The main industrial activities that contribute to the growing environmental metal pollution are smelting, mining, burning of fossil fuels, and manufacturing of commercial metallic products (Miró et al., 2004). Natural occurrences (for example, volcanic eruptions and soil weathering) was also reported to contribute a certain extent to metal pollution (He et al., 2005). Unfortunately, metal pollutants display contrasting behaviour compared to organic pollutants, since the latter may possibly decompose to less harmful substituents through chemical or biological processes, but metals cannot be degraded naturally. Metal waste disposal from these situations eventually seep into the soils, water, and air surrounding it (Amiard et al., 1985).

Commonly found metal pollutants include aluminium (Al), cadmium (Cd), copper (Cu), lead (Pb), tin (Sn), and zinc (Zn), all of which affect the environment as well as human health (Jaishankar et al., 2014). Although these metals are reported to be essential nutrients which play an important role in physiological and biochemical functions, excessive exposure at high concentrations can become toxic and result in various health diseases (Brown & Kozlowski, 2004). A surplus of metals can displace essential metals from its usual binding site due to ionic interactions, such occurrence would result in obstructed enzymatic function and altered protein and DNA conformation, subsequently causing metal induced toxicity (Beyersmann & Hartwig, 2008). For example, enzymes become inactive when cadmium supersedes catalytic zinc, and thus causing a singlestrand break for binding nonspecifically to DNA (Neumann & Leimkühler, 2008). Meanwhile, presence of lead even in picomolar amount is capable of replacing calcium during biological processes, altering the regulation of memory storage and neural excitation of the cell (Flora et al., 2008).

As rapid urbanization continues, the importance of proper regulatory and management of industrial chemical waste has often been overlooked (Afroz et al., 2014). The increasing number of metal pollution cases reported has become a major distress around the world, and such incident is apparent in our country, Malaysia, as well (Abdullah, 1995; Dahbi et al., 2002). In 2019, nearly fifty factories in Nilai were involved in the unlawful discharge of toxic waste in the Indah Water consortium sewage, this unpleasant event has led to the closure of the relevant water treatment plants and more than 300 areas (including Hulu Langat, Petaling, and Sepang) were affected (Goi, 2020). Another infamous case of chemical waste pollution occurred in Kim Kim River, which harmed two thousand over residents as they had to be hospitalized. This crisis was unique because it began with water pollution and then it progressed into air pollution because numerous toxic gases (methane, toluene, and xylene) were emitted, breathing difficulties, nausea, and dizziness were reported amongst students and schools were ordered to close (Yap et al., 2019). Furthermore, abrupt water disruption as a consequence of these issues have evidently caused inconvenience to every household and surrounding community (Afroz & Rahman, 2017). Hence, a better comprehension of potential environmental hazards as a result of extensive industrial activities should be well established along with strict policy and legislation to prevent further damage to our earth (Parikh, 1994). Together with a practical and convenient method to recognize these metal pollutants before it goes out of hand, maintaining a balanced ecosystem while safeguarding human health is achievable (Kabir et al., 2012).

2.1.1 Aluminium

Aluminium is one of the major metal elements on earth and it constitutes 8% of the total mineral deposits in the earth's lithosphere (Maity & Govindaraju, 2010). Generally, aluminium exists in its ionic form, Al³⁺, and its toxicity is influenced by surrounding pH as well as organic content (Cannata Andia, 1996). External factors, like acid rain, causes a change in pH of surrounding water and soil that leads to leeching of this metal into the ecosystem (Barabasz et al., 2002). This produces a spike in aluminium ions which is harmful to humans and surrounding environment (Delhaize & Ryan, 1995). Approximately 40% of the earth acidic soils was found to be contaminated due to aluminium toxicity, which subsequently impedes crop yield (Álvarez et al., 2005; Poléo et al., 1997).

Apart from that, the accumulation of Al³⁺ also originates from its extensive use in food additives, cooking utensils, packaging, and construction materials (Soni et al., 2001; Stahl

et al., 2017). Due to its frequent usage in our daily lives, Al^{3+} accumulates in our body through the food chain since intakes of aluminium could go as high as 5 g/day (Edition, 2011). Although aluminium is not necessary for biological processes, imbalance of Al^{3+} could displace Mg^{2+} and Fe^{3+} in the human body, disturbing cellular growth and intercellular communication (Jaishankar et al., 2014). Over the century, aluminium is a known neurotoxin for humans, it was discovered that health issues such as osteomalacia and risk of breast cancer can be linked back to aluminium poisoning (Woodson, 1998). Besides that, this metal is also toxic to plants, fishes, and multiple biological activities through acid rain as a result of human activities (Gensemer & Playle, 1999).

The World Health Organization (WHO) stated that the acceptable amount of weekly human dietary uptake of aluminium is approximated to be 7 mg/kg body weight, excessive exposure to Al³⁺ ions will cause neurodegenerative, Alzheimer's, and Parkinson's disease (Barcelo & Poschenrieder, 2002; Fasman, 1996; Krejpcio & Wojciak, 2002; Nayak, 2002). Additionally, overconsumption of aluminium would affect iron and calcium absorption, resulting in bone softening, anemia, and atrophy (Berthon, 2002). For these reasons, detection of Al³⁺ ions are crucial in regulating its influence on human health and the environment (Kan et al., 2020b). However, its strong hydration ability, weak coordination capacity, and poor spectroscopic behavior have caused its detection problematic compared to other metal ions (Darbre, 2005). Considering Al³⁺ is a hard-acid, chelation with a receptor unit containing nitrogen and oxygen as the hard-base donor sites can be expected (Kim et al., 2016). Therefore, the design and production of a selective aluminium sensor that can overcome its' drawback is still in great demand (Sahana et al., 2013).

2.1.2 Copper

Copper is one of the widely occurring metals on Earth and in fact, the discovery of copper can be traced back to prehistoric eras, where it is used for weapons and tools (Cutler, 2018). Its malleability, ductility, coupled with excellent heat and electrical conductivity, have provided better living standards for us over the past few decades (Al-Fartusie & Mohssan, 2017). The versatility of copper has allowed it to be used in numerous applications such as manufacturing of mobile phones, batteries, semiconductors, fertilizer industry, and catalysts (Labidi et al., 2016).

Aside from the applications with copper that ease our daily lives, copper is also an essential trace element and ranks third in abundance in the human body since the brain makes up the largest proportion of copper content, followed by the liver (Bulcke et al., 2017; Meng et al., 2015). Such plentitude of copper ions is attributable to its vital roles in various biochemical and physiological processes (Scheiber et al., 2014). Copper naturally exists as Cu⁺ and Cu²⁺ ions, the ability of copper to interchange between the reduced (Cu⁺) and oxidized (Cu²⁺) state is crucial in redox reaction (Tchounwou et al., 2008). For instance, copper functions as a cofactor that is involved in various oxidative stress-related enzymes like catalase, peroxidase, and ferroxidases (Stern, 2010). Other biological processes such as hemoglobin development, carbohydrate metabolism, and hair keratin cross linking have deemed its essentiality in bodily functions as well (Harvey & McArdle, 2008).

Normally, ingestion of copper through daily diet is approximately 0.6 - 1.6 mg (Linder et al., 1998). Although deficiency of copper amongst healthy individual is rare, it may occur among infants due to malnutrition, where the side effects include anemia, fatigue, and damaged nerves (Kodama & Fujisawa, 2009; Tsugutoshi, 2004). Whilst consumption of essential trace elements is key for maintaining better health and wellbeing, the WHO advisable upper limit of copper in drinking water is 31.5 μ M, any amount higher than that is considered poisonous for the human body (Edition, 2011). Excess amount of copper was reported to result in many health complications, such as Wilson's disease, type 2 diabetes, cell hypopigmentation anemia, and systemic lupus erythematosus (Wang et al., 2016; Ye et al., 2015). Copper can also impede iron-sulfur protein clusters and displace zinc from metalloproteins, thereby hindering its activity (Festa & Thiele, 2011).

Nonetheless, it is noteworthy that copper is a significant common environmental pollutant due to its extensive usage in agriculture, industry, and water pipe systems (Fanna et al., 2018). Immoderate exposure to copper has evidently caused harmful side effects to the ecosystem, environment, and human health (Bandmann et al., 2015; Elmorsi et al., 2017). Hence, the development of a quick and effective method for recognition of copper ions is of utmost importance to prevent further detrimental effects of this metal (Yoon et al., 2017).

2.1.3 Tin

Tin is the most prevalent heavy metal present in air, soil, and water (Arakawa, 1997). Due to its abundant occurrence, the discovery of the hardening effect of alloying tin with other metals in 3000 B.C. have since contributed to its widespread usage for domestic and technological development (Smith, 2012). Tin alloys have been extensively used to produce bronze, wine capsules, tableware, and battery. Meanwhile, almost 50% of tin is involved in plumbing and electronics because this field has mostly been substituted by tin-based solders owing to their flexibility in melting ranges compared with lead-tin solders (Cutler, 2018). Furthermore, canning of foods makes up of 15% of tin usage, where a thin metal plating is laced along the interior of steel cans as means of food preservation and corrosion prevention (Blunden & Wallace, 2003).

Regrettably, industrialisation and globalization without the practice of appropriate treatment and handling of metal wastes have caused increasing amounts of tin being leeched into the environment through dentifrices, pesticides, and tin plates (Adhikari et al., 2016; Florea & Büsselberg, 2006; Wang et al., 2015a). Typically, tin exist in two forms, organic and inorganic tin, where both display contrasting characteristics in terms of biochemical, chemical, and physical properties (Cigala et al., 2012). Even though inorganic tin is relatively less toxic due to its poor absorption, it has caused symptoms of tin poisoning in mammals, including nausea, diarrhea, nose irritation, paralysis, and growth retardation (Boogaard et al., 2003).

Considering tin is not a major pollutant in drinking water, there is no threshold concentration set by WHO, but its contribution is 4 μ g based on a maximum value of 2 μ g/litre (Edition, 2011). Despite being mildly toxic to humans, accumulated ingestion of tin at 0.1 – 1.0 g/L through contaminated water was found to affect the brain, digestive, and respiratory systems (Mahapatra et al., 2013; Patil et al., 2017; Sherman et al., 1986). On the other hand, dietary uptake of tin is mostly from packaged foods in unrefined tin cans caused by slow dissipation and formation of insoluble tin complexes (Berković et al., 1995). The adults' average inorganic tin consumption was recorded to be less than 10% of recommended provisional tolerable weekly intake (14 mg/kg body weight)

(Blunden & Wallace, 2003). However, a few scientific reports suggest that prolonged oral administration of inorganic tin (0.5 - 1 mg/kg body weight per day) could trigger carcinogenicity and mutagenicity, as well as influence homeostasis (Benoy et al., 1971; Dehghan & Khoshkam, 2012; Hamasaki et al., 1993; Sherlock & Smart, 1984).

Malaysia have remained one of the main tin-producing country apart from China, Indonesia, and Thailand (Smith, 2012). As seen by its prevailing usage in an extensive range, from food packaging to construction materials, the probability of tin accumulating and infiltrating the ecosystem and ultimately into the human food chain should not be taken lightly (Weber, 1985). Otherwise, the oversight of handling these metals may cause adverse effects to the environment and human health (Cima, 2011). Hence, alternative ways to identify these metals and proactive measures to curb further damages should be executed (Ravichandiran et al., 2020).

2.2 Chemosensors

As the growing problem of environmental issues due to metal pollution will eventually eradicate mankind, the need for selective, sensitive, and specific sensors that are able to provide fast, cheap, and real-time monitoring of analyte of interest (biological, chemical, and environmental field) are of greatest importance (Prodi et al., 2000). One of the appealing solutions to this issue involves the formation of chemosensors (Gale & Quesada, 2006). By IUPAC definition, a chemical sensor, or a chemosensor, is a device that is able to convert chemical data into a noticeable signal, where the information ranges from the amount of target analyte (measured variable) to the total composition analysis (Hulanicki et al., 1991). A lot of research has gone into designing unique chemosensors that can alter one or more macroscopic properties in response to the introduction of the target species (Suksai & Tuntulani, 2003). Chemosensors were reported to be developed as early as the 1980s, where various types of luminescent probes for ions and molecules in mixture of solvent and intracellular cells were successfully executed for real-time applications (De Silva & Rupasinghe, 1985; Huston et al., 1989; Minta & Tsien, 1989). The output signals involved in the production of these optically active chemosensors include differences in colour, absorbance and/or fluorescence, and it has been proved useful for identifying various analytes (Beer & Smith, 1998; Gunnlaugsson et al., 2006).

Typically, a chemosensor contains a receptor moiety (recognition site), that is linked to a signal reporter (active unit) by a spacer which transform the recognition event into a detectable signal (Figure 2.1) (De Silva et al., 1997). The function of these three components are as follows: a receptor which is in charge of specific analyte binding, a spacer that changes the geometry of the framework and alter the electronic interaction between the two other moieties, lastly the signal reporter whose properties ought to change upon complexation. Signal transduction of the analyte binding is a crucial component of chemosensors as it offers the likelihood to track its concentration progressively (Bargossi et al., 2000). Therefore, by linking a recognition event with a quantifiable signal event, detection of target analyte through chemosensors become easily accessible (Krämer, 1998).



Figure 2.1: Visual representation of a chemosensor (Bargossi et al., 2000).

Conversion of molecular recognition into highly sensitive and readily obtained result by chemosensors have been constantly studied in recent years (Lavigne & Anslyn, 2001). The most conventional approach used in chemosensors are optical retention, absorption, luminescence, redox potential and so on, but sensors in view of different spectroscopies and optical parameters, for example, refractive index and reflectivity, have likewise been produced (McDonagh et al., 2008). Despite the many choices, an extensive variety of books and review articles has been published by specialists in the field who have highlighted the benefits of optical detecting over other transduction techniques (Stock et al., 2019). By observing the absorbance/fluorescence change as well as the colour intensity upon selective binding of target analyte, this method can be used as a straightforward "naked-eye" detection for instantaneous monitoring (Li et al., 2015b). Such phenomenon is also facilitated by chemosensors that have structures with extensive delocalization because the wavelength absorbed will eventually be high enough to be in the visible region of the electromagnetic spectrum, resulting in a coloured complex solution (Santos-Figueroa et al., 2013). Hence, the quick, selective, and sensitive colour change of chemosensors have been widely employed as it does not require the use of complicated equipments while providing sufficient information needed to carry out further analysis as well (Valeur, 2003).

Chemosensors can be classified into a few categories: optical sensors, electrochemical sensors, electric sensors, mass sensitive sensors, magnetic sensors, and thermometric sensors. They are then further grouped by its recognized analyte form such as ionic species (Ca^{2+} , H^+ , K^+ , Na^+), small molecules (O_2 , NH_3 , H_2O_2), and larger molecules (glucose, citrate, glutathione) (Cammann et al., 1991). Among the diverse forms of analytes, development of chemosensors for the detection of heavy metal ions (ionic species) has been of particular interest in recent years (Kaur et al., 2015). Although
commonly used analytical techniques like atomic absorption spectroscopy (AAS), inductively coupled atomic emission spectrometry (ICPAES), X-ray fluorescence spectroscopy (XFS), and capillary electrophoresis (CE) can identify and quantify metal ions at low concentrations, they are limited by its immobility, high maintenance cost, requires well-trained personnel, and involves careful sample preparations (Sahoo et al., 2012). Therefore, the ability of chemosensors to perform real-time on-site measurements on metal ions have provided a better alternative for a wide range of applications in the field of clinical, medicinal, analytical and environmental sciences (Bargossi et al., 2000).

As mentioned previously, various researchers have found that optical chemosensors (chromogenic and fluorogenic) are able to associate metal ions concentration with changes in colour and spectroscopic characteristics (De Silva et al., 2001). In view of its "off-on" interchangeability, ease-of-use, combined with its ability to provide qualitative and quantitative analysis, we have chosen to focus on optical sensors due to the need of a convenient on-site testing of metal ions without the requirement of expensive instruments in this study. Along with proper methodological plans, variation in photophysical procedure and evidence *via* output signal, these guidelines are able to assist in producing a highly selective and sensitive chemosensor (Jiang et al., 2012).

2.3 Schiff base chemosensors

The term "Schiff base" was named after Hugo Schiff, where he discovered Schiff bases are formed when a condensation reaction occurs between an amine and a carbonyl compound, resulting in the formation of an imine bond with the elimination of a water molecule, its general structure is $R_1R_2C=NR'$ ($R' \neq H$) (Schiff, 1864). The "Schiff base" designation applies when they act as ligands to coordinate with metal ions and form metal complexes (McNaught & Wilkinson, 1997). This classic reaction has proven to be a versatile method in organic synthesis, particularly when creating complex molecular structures (Xavier & Srividhya, 2014). Based on the mechanism illustrated in Figure 2.2, the formation of imine linkage is reversible, and thus the capacity for "error-rectification" is an appealing feature of this chemical reaction (Borisova et al., 2007). Furthermore, expensive catalysts are not required in this reaction and the yield is mostly high (Jin et al., 2013).



Figure 2.2: Mechanism for the condensation between amines and carbonyl compounds (Borisova et al., 2007).

Generally, Schiff bases engage in three forms of equilibrium-regulated reactions (Figure 2.3) (Belowich & Stoddart, 2012):

- a) Hydrolysis Formation of starting materials (amine and carbonyl compound) upon addition of water.
- b) Exchange The original Schiff base can undergo transimination when a second amine is introduced, resulting in the exchange of R (alkyl or aryl) groups.
- Methathesis Both imines will undergo a reaction in which the two R (alkyl or aryl) groups are interchanged upon addition of a second Schiff base.

Besides that, Schiff bases can be reduced to yield a secondary amine, thereby "fixing" the products formed under thermodynamic equilibrium through kinetic manners (Brady & Sanders, 1997). While this process (reductive amination) is practical and widely used for the synthesis of substituted amines, it lacks the reversibility trait that distinguishes imines

from other compounds (Greig & Philp, 2001). Hence, these factors should be taken into account when handling Schiff bases in order to achieve the desired outcome.



Figure 2.3: The three forms of Schiff base reactions: (a) condensation (b) exchange, and (c) metathesis (Belowich & Stoddart, 2012).

In light of the unique features of Schiff bases stated above, it has allowed studies in a broad range of contexts, such as antiviral, antimicrobial, anticancer agents, catalysts, and chemosensors (Bajema et al., 2019; Udhayakumari & Inbaraj, 2020). They are found to be selective metal chelators whose biological and chemical properties changes upon coordination with transition metals (Abu-Dief & Mohamed, 2015). Owing to their flexibility through synthesis that result in different structures, starting materials can be planned beforehand so that the final product can be anticipated, for example, introducing a particular steric or electronic moiety for better selectivity and reactivity towards the target reactant (Corbett et al., 2006). From the aspect of chemosensors, the synthesized Schiff base ligand unit binds selectively with the metal ion is accompanied with transformation in terms of colour (chromogenic sensors) or fluorescence (fluorogenic sensors) as a result of chelation, substitution, or redox reaction (Cheng et al., 2014). Incorporation of an extended π -conjugation system within the chemosensor was reported to further enhance the colour upon complexation with metal ions, subsequently aiding

naked-eye detection (Kundu et al., 2015). The following π - π interaction allows modification of the electronic properties over the structure, absorption, and fluorescence by conjugation of suitable ligands (Gondia & Sharma, 2019).

Over the past few years, multiple reports have revealed the usage of Schiff base ligands as an alternative to conventional methods for metal ion analysis (Liu et al., 2013; Vashisht et al., 2020; Wang et al., 2012c). A number of Schiff base entities was successfully synthesized specifically for Cu²⁺ detection because of its importance ascommon essential trace metal in the human body with prescribed consumption limits (Kim et al., 2015b; Manna et al., 2020; Venkatesan et al., 2019). Meanwhile, the probability of tin leeching into the food content through food packaging and tinned beverages is relatively high due to its widespread use, but reported Schiff base sensors for Sn²⁺ identification are limited (Kolcu et al., 2021). In contrast to transition metals, Al³⁺ has always been difficult to detect due to its lack of spectroscopic characteristics and weak coordination performance (Azadbakht et al., 2013). However, optical sensing ability of newly synthesized Schiff base chemosensor was proven effective by the significant colour, absorption, and fluorescence changes upon addition of Al³⁺ but not other metal ions, subsequently enabling the observation of a "off-on" colorimetric conversion with the naked eye (Gupta et al., 2014). Moreover, utilization of different solvent environment and substituent affects the metal ion detected, for instance, changes in the substituent and solvent in the coumarin based chemosensor resulted in tunable Mg²⁺ fluorescence sensing (Dong et al., 2011). Imidazole derivative sensor demonstrated preference towards different metals in different solvents, reflected by its sensitivity towards Zn^{2+} in acetonitrile solution but Al^{3+} in DMF (Choi et al., 2014). Varying selectivity for a range of metal ions was observed with porphyrin based fluorophore by modifying the electron donating groups (Güngör et al., 2016). Although there are Schiff base chemosensors for detection of Co^{2+} (Alamgir

et al., 2020; Liu et al., 2015), Mn²⁺ (He et al., 2015; Roy et al., 2015), Ni²⁺ (Chowdhury et al., 2018; Wang et al., 2012b), and Pb²⁺ (Ghorai et al., 2016; Karachi et al., 2018), but reports on simultaneous detection of multiple metal ions are sparse thus far (Hariharan & Anthony, 2014; Xu et al., 2014).

Apart from metal ions sensing abilities, multiple reports of Schiff bases derivatives have found to be linked with anticancer characteristics (Zhang et al., 2012). Cancer, also known as malignant neoplasm, is a category of diseases characterised by uncontrolled cell proliferation, invasion, and, in some cases, metastasis (Vita et al., 1986). It is also the most dreaded diagnosis in the world, posing a major public health challenge given that it is the second leading cause of death in both developing and developed countries, after cardiovascular diseases (Bandgar et al., 2010). Over the last 50 years, much effort has been made to create improved drugs for treating cancer patients. Currently, cancer is treated mainly with surgery and chemotherapy, but the curative benefits of present chemotherapeutic medications are lacking, and they come with a slew of side effects (Bernardi et al., 2006). On the contrary, antiproliferative effects studied on a copper (II) Schiff base complex against HT-29 colon cancer cells exhibited potent side effects as the cells treated underwent immediate apoptosis (Hajrezaie et al., 2014). Furthermore, another study has shown that substitution of electron withdrawing groups improved the antiproliferative effects compared with electron donating groups, thereby corroborating the modifiability of Schiff base ligands for desired treatment (Sun et al., 2020).

Nonetheless, Schiff bases are a type of organic compound that is essential considering their ability to form complexes with metals and provide pharmacological properties. Other than its robust photophysical properties, excellent biological activities, and selective chelation ability, Schiff bases' resounding capability to be tailored through synthesis to be utilized as a metal tagging agent and provide various therapeutic options should be explored to maximize its potential (Xavier & Srividhya, 2014).

2.4 Rhodamine B chemosensor

Rhodamines are chromophore derivatives of xanthene and a family of dyes. Key members of the rhodamine family including rhodamine 123, rhodamine 6G, and rhodamine B, are commonly used in dyes, printing, textiles, and leather industry owing to its vibrant colour and pigmentation (Maeda, 1984). Between these three, rhodamine B is preferred as it is less corrosive and harmful compared with rhodamine 123 and rhodamine 6G (Gessner & Mayer, 2000). Aside from being used as dyes, colorimetric and fluorometric probes based on rhodamine B have been widely created for various analyte as early as 1997 because of their excellent fluorescent properties, low detection limit, and photostability (Dujols et al., 1997). Even though there is a plethora of fluorophores to choose from (coumarin, oxazines, cyanines, pyrromethenes, etc.), rhodamine based chemosensors display diverse advantages in comparison to smaller organic compounds, these include substantial signal amplification, high sensitivity, and option to incorporate multiple recognition elements that can increase both the binding efficiency and recognition selectivity for specific analytes (Kim et al., 2011).

Rhodamine B can exist in two states as illustrated in Figure 2.4, the open (coloured/fluorescent) form and closed (colourless/nonfluorescent) form in equilibrium. In acidic conditions, the opened ring form dominates, while in basic conditions, the closed ring form persists (Birtalan et al., 2011). Knowing that pH values are crucial in homeostasis, biochemical processes, and environmental system, this equilibrium allows rhodamine compounds to act as pH probes on top of being metal ions sensors (Zhang et al., 2015). Additionally, earlier findings suggested that the optimum concentration for rhodamine B investigation is in the micromolar range of dilute solution, because most of it is dissolved in ethanol in the concentration range of $6 - 8 \ge 10^{-6}$ M (Hinckley et al., 1986). However, owing to the rotational movement of the n-diethyl groups in the xanthene moiety, the photophysical behaviour of rhodamine derivatives are dependent on solvent polarity, environment, and also viscosity (Chang & Cheung, 1992).



Figure 2.4: Cyclization between open and closed form of rhodamine B (Birtalan et al., 2011).

Among different chemosensors available, rhodamine chemosensors are the most userfriendly since they allow recognition of a particular target analyte directly through inducing colour and fluorescence changes and do not involve complicated sample pretreatment (Kataria et al., 2019; Tang et al., 2017). It is noteworthy that rhodamine derivatives exhibit longer emission wavelengths (over 550 nm), which is favored to serve as a reporting unit for the target analyte as it reduces errors contributed by background fluorescence that usually occurs below 500 nm (Lee et al., 2007). Considering rhodamine dyes fluoresce, its employment for metal detection can be carried out inexpensively and easily with spectrophotometers (Yang et al., 2002). Its remarkable absorbance and fluorescence intensity coupled with intense colour formation towards specific metal ions is a feature that strongly facilitates "naked-eye" detection (Yang et al., 2005). Based on the "off-on" signaling feature, rhodamine derivatives have since been successfully designed as metal ion probes, pH probes, and biological imaging agent (Best et al., 2010; Kan et al., 2020a). Analytes can trigger a ring opening process that results in colour changes, absorption and fluorescence emission, which aids in identification (Yin, 2017).

To bring rhodamine compounds to greater heights, another variation of this compound is modified at the carboxylic acid moiety, where it can be converted to an amide group to form rhodamine spirolactams. Likewise, this molecule exists in two forms as well, its native form (closed spirolactam ring) is colourless, whereas ring opening process give rise to a reddish pink colour solution (Figure 2.5) (Kim et al., 2008a). In 2006, when Xu and co-workers replaced the carbonyl group's oxygen atom in rhodamine B to a sulphur atom using Lawesson's reagent, the recognition preference changed from Cu²⁺ to Hg²⁺ (Zheng et al., 2006). A few years later, Ma and co-workers took advantage of the strong nucleophilicity and affinity of sulfur towards Hg2+ and synthesized rhodamine B thiolactone that could selectively detect Hg²⁺ (Shi & Ma, 2008). From this event, it can be inferred that functional groups based on sulfur (soft base) can be considered and incorporated for selective binding of soft metal ions (Hg²⁺), with nitrogen and sulfur atoms participating as potential selective receptor (Nolan & Lippard, 2003). Such phenomenon can be explained with reference to the hard and soft acids and bases (HSAB) theory, whereby soft acids have an affinity and form stronger bonds with soft bases, and vice versa. Subsequent complex formed between a hard base and hard acid or soft base with soft acid is stable (Minkin, 1999). In accordance with the hard or soft acids and bases' strengths, this theory lays out the possibility to formulate ligands with improved selectivity towards a certain metal ion (Chereddy et al., 2012). While the HSAB theory applies, rhodamine B derivatives was also reported to recognize simple molecules like hypochlorous acid (Kenmoku et al., 2007), phosgene gas (Wu et al., 2012), and carbon monoxide (Zhang et al., 2019).



Figure 2.5: Spirolactam ring-opening process and colour changes of rhodamine derivative (Kim et al., 2008a).

For typical absorption or fluorescence spectroscopy experiments, the possibility of cyclization poses a disadvantage for rhodamine molecules. Grote's study discovered that fluorescein derivates reacted quickly in N,N-dimethylformamide (DMF) with primary amines at room temperature, the starting material that was a reddish orange solution turned almost colourless (Adamczyk & Grote, 2001). The amide bond formed can be cyclized to form a spirocyclic compound, which is colourless and deemed unsuitable for absorption or fluorescence studies (Fölling et al., 2008). To overcome this issue, a new procedure suggested to prevent cyclization is to methylate the amide nitrogen. Nguyen's group successfully functionalized rhodamine B with piperazine by refluxing to avoid generation of spirolactam ring (Nguyen & Francis, 2003). Boyarskiy and co-workers designed a sulfonated rhodamine with N-alkyl group which exhibited remarkable

fluorescence properties and photostability (Boyarskiy et al., 2008). Whilst rhodamine B compounds are excellent dyes, they are mostly hydrophobic. Its performance in purely aqueous solutions can be hindered by aggregation and formation of dimers (Valdes-Aguilera & Neckers, 1989). Hence, a suitable ratio of solvent solution and better modifications applied to rhodamine B will be discussed in the next section.

2.5 Rhodamine B hydrazide chemosensor

Rhodamine B hydrazide (**RBH**) are analogues of rhodamine B and it is expected that they operate in the same way spectroscopically. The synthesis of this compound is shown in Figure 2.6, where rhodamine B is refluxed with hydrazine hydrate in methanol to obtain another variant of rhodamine based chemosensor (Jiang et al., 2010).



Figure 2.6: Synthesis of rhodamine B hydrazide from rhodamine B (Jiang et al., 2010).

Rhodamine based chemosensors were already known for their exceptional spectroscopic properties, intense fluorescence, and visible excitation wavelengths, these characteristics undoubtedly eased the synthesis of an "off-on" type probe (Yang et al., 2007). It was understood that by altering certain atoms of rhodamine compounds, an obvious change in colour would develop in response to selected ions, thus allowing "naked-eye" detection together with enhancement in absorption and fluorescent intensity that can easily be measured with spectrophotometers (Zheng et al., 2006). Without metal

ions, these sensors remain in the spirocyclic form that is colourless, significant change in colour (from colourless to pink) upon the addition of ions in the presence of chemosensor can be attributed to the spirolactam ring-opening process (Saleem & Lee, 2014). This unique process was employed in one of the few pioneering work where rhodamine B hydrazide was used as a fluorescent chemodosimeter for Cu²⁺, the mechanism of the spirolactam ring opening is illustrated in Figure 2.7 (Dujols et al., 1997). However, one prominent disadvantage of chemodosimeters is that the ring-opening process induced by metal ions are irreversible. The irreversibility of a probe will restrict its application because then the compound can only be utilized in a stoichiometric manner and will act as a "reagent" instead (Wang & Anslyn, 2011). On the contrary, reversibility of a chemosensor plays a fundamental role in analyzing its interaction with the target analyte, reason being that a reversible reaction allows real-time monitoring of an analyte's concentration which would aid on-site decision making (McQuade et al., 2000). Hence, the attention is being drawn towards the production of reusable chemosensors for practical detection of various metal ions without omitting the rationale behind Czarnik's work (Zhang et al., 2007).



Figure 2.7: Cu²⁺-assisted spirolactam ring opening of rhodamine B hydrazide (RBH) (Dujols et al., 1997).

In consideration of synthesizing a reusable chemosensor besides understanding that the closed spirocyclic and ring-open forms show distinguishable absorption and fluorescent properties, introduction of a stronger ligand was found to reverse the coordination between sensor and metal complex (Sun et al., 2011). Commonly, addition of EDTA caused fading of the pink colour solution to almost colourless followed by the loss of distinguished peaks in the ensuing absorption and fluorescence spectrum. No doubt, the colour, absorption, and fluorescence was restored upon steady addition of the target metal ions. This interchangeability coupled with indistinct signal decline proves its practical application in various analytical fields (Li et al., 2015a). Furthermore, testing on the reversibility is not only limited to EDTA, multiple reports have shown to use diverse ligands and solvents, to name a few, tetrasodium pyrophosphate (Song et al., 2019), THF (Li et al., 2014a), and cyanide ions, to promote reusability of the respective probes (Pei et al., 2017). Hence, these outcomes suggests that the coordination of sensor with target metal is reversible, ergo crucial for inspecting dynamic shifts in metal ion concentration (Koide et al., 2012).

Several techniques have been applied to study the interaction between chemosensor and metal ions that would allow us to have a better understanding towards their binding mechanism. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) curves have shown that the electrochemical properties of a chemosensor is altered upon the addition of metal ions, together with the increased peak current that can be attributed to the formation of metal-chemosensor complex (Rana et al., 2017). This formation can also be further confirmed by high-performance liquid chromatography (HPLC) via comparing the peak intensity of chemosensor and metal-chemosensor chromatograms along with the emergence of a new peak for metal-chemosensor complex as seen in Figure 2.8 (Dong et al., 2010). Unfortunately, this technique is not favored as the peak formed is minimal and inconspicuous, the appearance of this peak due to presence of impurities is also very likely. One study found no obvious peak in the HPLC chromatogram of rhodamine solution, the corresponding complex had to be further investigated by liquid chromatography–mass spectrometry (LC-MS) instead, which defeats the purpose of a simple, fast, and effective sensing system (Guo et al., 2020).



Figure 2.8: HPLC chromatograms of (a) chemosensor (b) metal-chemosensor complex (Dong et al., 2010).

Interestingly, some studies have verified the binding mechanism through fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. It was found that the characteristic amide carbonyl absorption peak of rhodamine based compounds at ~1690 cm⁻¹ shifted to a lower frequency in presence of metal ions (Figure 2.9), suggesting that the carbonyl O of the rhodamine B unit is directly involved in the recognition of target metal ions (Kwon et al., 2005). Besides that, the ¹H NMR peaks of the benzene moiety broadened along with the protons of the xanthene moiety shifting downfield due to the decrease in electron density of the rhodamine moiety upon binding with the selected metal ion (Figure 2.10) (Jeong et al., 2015). Another

resounding proof of the lactam ring opening process is reflected by the tertiary carbon peak at ~66 ppm which diminished remarkably upon presence of metal ions in the ¹³C NMR spectrum (Rasheed et al., 2018). Thereafter, the proposed binding mechanism supported by these data obtained is explained in Figure 2.11, where the chemosensor likely coordinates via the spirolactam carbonyl O and amide N atoms (Jeong et al., 2015). Hence, this approach provides a better understanding and strongly endorse the claim that the amide carbonyl of rhodamine participates in the coordination with metal ions, whereby this encounter enables the spirocyclic ring-opening process and result in changes of the absorption and emission spectra (Liu et al., 2014).



Figure 2.9: Infrared spectra of (a) sensor and (b) sensor in presence of metal (Kwon et al., 2005).



Figure 2.10: ¹H NMR spectra of (a) sensor, (b) sensor + 0.5 equivalent of metal, and (c) sensor + 1 equivalent of metal (Jeong et al., 2015).



Figure 2.11: Proposed binding mechanism of sensor with target metal ion (Al³⁺) (Jeong et al., 2015).

Recently, the idea of immobilizing rhodamine molecules on supporting solid phase to extend their range of functions have been explored (Trigo-López et al., 2015). The process of combining diverse materials with different characteristics leads to the possibility of obtaining additional benefits, such as the ability to be used sustainably and recycled (Shakeela et al., 2020). The immobilized sensing molecules should be designed in a way that it is non-leaching on the supporting material as well as concomitantly ensuring its accessibility for contact with the metal ions (Favaretto et al., 2019). Much research efforts have been committed to produce befitting solid phase materials together

with optimization of such techniques, whereby efficient, facile, and low-cost detection is made possible. Ventura's group reported the effective immobilization of rhodamine B hydrazide onto polymeric materials for Cu²⁺ recognition through colour and fluorescent changes of the dye (Morsi et al., 2020a). A year later, they pushed the limits and managed to construct a biocompatible polymer fabric by using cellulose acetate, a natural polymer derivative, further upgrading the sensory feedback of the dye attached (Morsi et al., 2020b). Undeniably, this approach has open new doors for the application of rhodamine dyes, not just as chemosensors, but also the ability to attach onto a solid phase enhances its portability, convenience, and diverse usage.

As much as designing new chromogenic and fluorogenic chemosensors for targeting biologically essential metal cations are of great importance, some of these compounds are limited by certain circumstances, such as low solubility in aqueous solution, interference from other analytes, and optical signals disrupting the visible wavelength region (Descalzo et al., 2003). For example, Guo's group required as much as 90% of organic solvent for absorption and fluorescence measurements (Liu et al., 2011) and Tang's group showed inconsistent absorption values in presence of competing analytes (Tang et al., 2011a). In the next section, the broad possibilities of various substituents for specific functions with rhodamine B hydrazide will be discussed.

2.6 Rhodamine B hydrazide substituted chemosensor

Clearly, the family of rhodamine dyes are ideal for fabrication of colorimetric and fluorometric chemosensors by virtue of their distinctive structure (Li & Meng, 2014). Rhodamine B hydrazide derivatives definitely do not fall far behind from their parent compounds as they are praised for their properties: tremendous photostability, operational simplicity, rapid response, non-invasiveness, and high signal-to-noise ratio (Bao et al., 2019). They are a class of Schiff base chemosensors that is used for monitoring and signaling ionic species in aqueous solution as well (Zhao et al., 2009). Depending on the size of the cavity and the target ionic species, they have the ability to chelate to a specific analyte through carbonyl oxygen and imine nitrogen to stabilise the target species (Erdemir et al., 2015). Most rhodamine derivatives conformation interchange between spirocyclic and ring-open forms, and both forms usually have its own absorbance and fluorescence properties. This distinctive feature is suitable to produce "off-on" switch sensors since the commonly colourless or nonfluorescent closed-ring form ("off" signal) results in the development of strongly coloured or fluorescent open-ring states ("on" signal) when the target metal ions are detected by these chemosensors (Jiao et al., 2018). In a nutshell, rhodamine spirolactams have been poised and hallmarked to form colorimetric and fluorometric chemosensors through appropriate requirements via ring opening process (Li et al., 2011).

Without limiting to rhodamine B hydrazide chemosensors only, the amine terminal allows substitution of various carbonyl compounds which can alter selectivity, sensitivity, and reversibility (Long et al., 2019). Hu's group demonstrated the formation of desired chemosensor starting from rhodamine B to rhodamine B hydrazide, and the one-pot synthesis of the rhodamine derivative sensor, where rhodamine B hydrazide is refluxed

with 2-hydroxy-5-methoxybenzaldehyde in ethanol (Figure 2.12) (Hu et al., 2019). Although the imine bond is strong, it is susceptible to hydrolysis according to the mechanism of reaction as depicted in Figure 2.13, and the formation of rhodamine Schiff base chemosensors are therefore thermodynamically controlled. The formation and dissolution of covalent bonds in organic substances is normally slow. It is thus irreversible and inert, and its formation is kinetically controlled. Contrarily, a number of reversible binding interactions occur in nature, such as the hydrogen bond which are weak, reversible, fast to form or break, and function under thermodynamic regulation. Just like the hydrogen bond, the imine formation is labile, at the same time it is also strong like a covalent bond, which makes this chemistry unique. Hence, this dual feature enables the one-pot synthesis of rhodamine molecular recognition systems, where the reactants mixed in the same vessel are subjected to an infinite number of quick and reversible attempts before the desired product is obtained, eventually forming a thermodynamically stable complex (Fabbrizzi, 2020).



Figure 2.12: One-pot synthesis of (a) rhodamine B hydrazide and (b) rhodamine derivative chemosensor (Hu et al., 2019).

$$\underset{R_{1}}{\overset{H}{\longrightarrow}} O + \underset{H}{\overset{R_{2}}{\longrightarrow}} \underset{H}{\overset{(i)}{\longleftarrow}} \left[\underset{H}{\overset{(i)}{\longrightarrow}} \left[\underset{H}{\overset{R_{2}}{\longrightarrow}} \right] \underset{H}{\overset{(ii)}{\longleftarrow}} \left[\underset{H}{\overset{HO}{\longrightarrow}} \right] \underset{H}{\overset{R_{2}}{\longleftarrow}} \right] \underset{H}{\overset{(ii)}{\longleftarrow}} \left[\underset{H}{\overset{R_{2}}{\longrightarrow}} \right] \underset{H}{\overset{(ii)}{\longleftarrow}} \underset{H}{\overset{R_{1}}{\longrightarrow}} \underset{H}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{H}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\longrightarrow}} \underset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\overset}} \underset{R_{2}}{\overset{R_{2}}{\overset{R_{2}}{\overset}} \underset{R_{2}}{\overset{R_{2}}{\overset}} \underset{R_{2}}{\overset{R_{2}}{\overset}} \underset{R_{2}}{\overset{R_{2}}{\overset}} \underset{R_{2}}{\overset{R_{2}}{\overset}} \underset{$$

Figure 2.13: Reversible Schiff base condensation mechanism between a carbonyl group with an aldehyde (Fabbrizzi, 2020).

In connection with the ease of rhodamine derivatives' synthesis, this allows an extensive range of substitution and modification for metal ions detection (Wang et al., 2012a). As seen previously, the preference of the chemosensor changed from detecting Cu²⁺ to Hg²⁺ just by altering one atom, so there are endless possibilities for further designs of rhodamine B hydrazide derivatives for multi analyte analysis (Zheng et al., 2006). One of the first few rhodamine derivative sensor was reported by Yoon and co-workers, where rhodamine B was acylated to react with an amine compound and the formation of this fluorescent chemosensor was supported by the X-ray structure showcasing the spirolactam moiety (Figure 2.14). When tested against various metal cations, it showed significant spectral enhancements with Pb²⁺ in acetonitrile with small fluorescence interference from Zn²⁺ and Cu²⁺ (Kwon et al., 2005). Apart from that, rhodamine based derivatives as chemosensors for trivalent metal ions (Al³⁺, Fe³⁺, Cr³⁺) was successfully synthesized by incorporating triazatruxene, a fluorophore with three reactive sites (Sadak & Karakus, 2020). Ever since Czarnik's rhodamine probe for Cu²⁺ was reported, more rhodamine derivatives for Cu²⁺ have been researched on as well. Intriguingly, most polyhydroxy substituents with rhodamine B hydrazide displayed preference towards Cu2+ over other metal ions. For example, Xu's group reacted rhodamine B hydrazide with 2,4dihydroxybenzaldehyde that resulted in a colorimetric and fluorometric chemosensor selective for Cu²⁺ in 10% DMSO (Xu et al., 2011). Pisitsak and co-worker condensed rhodamine B hydrazide with 2,5-dihydroxybenzaldehyde which exhibited "off-on"

sensing of Cu^{2+} ions in 50% ethanol (Milindanuth & Pisitsak, 2018). Meanwhile, the coupling with 4-bromo-2-hydroxybenzaldehyde displayed simultaneous recognition of multi analyte (Co^{2+} and Cu^{2+}) (Zhang et al., 2020). Although it can be predicted that polyhydroxy compounds will have an affinity for Cu^{2+} ions, Bag *et al.* studied the effect of increasing alkyl chain lengths on Cu^{2+} sensing by rhodamine B derivatives and found that their chelating ability towards Cu^{2+} is proportional to the length of the alkyl chain. This research also revealed that the nature of the substituent affected the photophysical properties, affinity for sensor-metal complex formation, and detection limit (Mishra et al., 2020). Evidently, the amount and nature of external coordinating moieties integrated with rhodamine are consequential (Mao et al., 2007). Owing to its less labour intensive and straightforward production, rhodamine derivative chemosensors can be tailored accordingly for various chemical and biological applications where better photostability, increased selectivity, and contrasting spectral characteristics are apparent (Liu et al., 2020). These attributes can be applied to our advantage for the development of new chemosensors that exhibit selectivity over a single metal ion in a competing environment.



Figure 2.14: X-ray structure of rhodamine derivative bearing the spirolactam form (Kwon et al., 2005).

Despite multiple approaches taken to optimize the selectivity and sensitivity of rhodamine based chemosensors, another factor affecting the performance includes the medium in which the chemosensor is employed. One of the many studies have proven that the pH environment is indeed critical in determining the rhodamine spirolactam structure and the behavior of target analyte. It was found that in weak acidic medium (pH 4.8), the increased concentration of target analyte in the reaction system caused fluorescence quenching, presumably due to the strong oxidation of target analyte in acidic media that led to the destruction of the rhodamine structure. However, in neutral medium (pH 7.4), continuous addition of target analyte did not cause any disruption in fluorescence enhancement. Therefore, improper selection of pH system would also vastly affect the accuracy of the experimental methods and alter the fluorescence stability between the chemosensor and target analyte (Zhang et al., 2011b). Likewise, the established rhodamine B hydrazide is selective towards Cu²⁺ ions in water, but in acetate buffer at pH 5.0, it can now selectively detect Hg⁺ ions (Kim et al., 2008b). Additionally, Wang's study discovered that rhodamine based chemosensors demonstrated stronger absorption and fluorescence in protic solvents (methanol, ethanol, water) compared to aprotic solvents (acetone, THF, DMF) (Figure 2.15) (Li et al., 2013). Yang and coworkers also studied the effect of solvent media on the reaction system of rhodamine derivative, it was found that the compound exhibited spectral response upon addition of Cu²⁺ ions in DMSO-water, acetonitrile, and acetonitrile-water solution but insensitive to DMF, acetone, and pure DMSO solvent. Further testing on the selected solvents revealed that different ratio of solvent solution gave rise to different absorbance values, hence these combinations can be carefully chosen to produce the most suitable spectrum for additional investigation (Xu et al., 2011). Xu's group applied this to their advantage and developed a system where the rhodamine probe selectively detects only Cu2+ in DMSO-water solution, but simultaneous coordination with A1³⁺ and Ca²⁺ in EtOH-water solution (Zhao

et al., 2020a). Earlier findings revealed that some groups require as much as 90% of organic solvent for spectroscopic measurements. However, this is not encouraged because organic solvents are volatile, the concentration will be subjected to changes if the liquid detection system is left unattended for a period of time, and thus the resulting spectral data would be affected and inconsistent (Yang & Wu, 2013). As it is generally known that rhodamines in a spirocyclic form have neither absorbance nor fluorescence in the visible region, suggesting that the spirocyclic form exists predominantly, regulation of the equilibrium between the spirocyclic form and the open-ring form through different solvent ratio provides a strategy for controlling the absorption and fluorescence data (Sivaraman et al., 2012).



Figure 2.15: Effects of solvent on fluorescence emission of rhodamine derivative chemosensor (Li et al., 2013).

Previously, we have seen immobilization of rhodamine on silica gel (Praikaew et al., 2015), polymeric materials (Kaewtong et al., 2011), and electrospun nanofibers (Cho et al., 2016). Whilst it fulfills the need of a solid support for chemosensors, the cons of these methods include lengthy sample preparation, multiple starting material, and progressive degradation may occur (Ju et al., 2011; Wang et al., 2020). For instance, Wang et al. immobilized rhodamine on silica gel to observe the adsorption affinity and selective sensing of Hg²⁺, but they required scanning electron microscope (SEM) to verify the rhodamine-gel functionalized beads and subsequent investigation of sensor-metal interaction entails isolation from the aqueous suspension (Huang et al., 2012). Yuan's research involved polyacrylic acid-based gel balls as the solid phase indicator of Cu²⁺ due to its high adsorption efficiency. However, the steps to prepare the gel balls are tedious and the final colour change is not obvious (Figure 2.16) (Yang et al., 2021). For these reasons, the process of conducting on-site research should be convenient, fast, and simple. Since the detection system was done in liquid form previously, alternative methods are proposed along the way to translate a liquid system to a solid system while retaining its "off-on" properties (Yang & Wu, 2013). There have been many innovative ways reported, for example, TLC strips (Rasheed et al., 2018), ultrathin platinum films (Kim et al., 2008c), sol-gel technique (Pipattanawarothai & Trakulsujaritchok, 2020), and etc., but the most eye-catching approach is by using filter papers, which is much less expensive and easily available (Sheng et al., 2008). In Sheng's and Wu's study, they effectively demonstrated the use of filter paper for adsorption of rhodamine chemosensors, where the colourless test strips turned pinkish-purple in presence of the target metals in a mixture of solvents (Xiong et al., 2016; Yang et al., 2011). Wang's work successfully demonstrated the detection of metals in 100% aqueous medium as reflected by the colour change and fluorescence under a UV lamp, which is useful in monitoring real-time samples (Li et al., 2018). Apart from its low-cost starting materials, they are simple to prepare and provides "naked-eye" recognition upon contact with metal ions as it changes from colourless to pink (Wang et al., 2015b). Hence, this alternative method to immobilize rhodamine based chemosensors on filter papers can be considered since it is non-damaging to the reacting components and proved its affordable diagnostic testing for on-site study in the future (Ahmed et al., 2016).



Figure 2.16: Colour change of gel balls in pure water, from brown to light green upon adsorption of Cu²⁺ ions. Left: Aqueous copper chloride solution, Middle: Gel balls in pure water, Right: Gel balls added to aqueous copper chloride solution (Yang et al., 2021).

As the growing environmental pollution from metal wastes disposal has become a major concern of the world, it is imperative to develop a fast, simple, and accessible device to identify these pollutants before the concentration levels become hazardous (Amde et al., 2016; Sullivan & Krieger, 2001). The disadvantages of conventional methods for metal ion detection outweigh the advantages as they are immobile, expensive, and requires elaborate sample preparation (Forzani et al., 2005). Fortunately, the field of chemosensors have positively mitigated this issue through rapid "off-on" colour changes based on rhodamine spirolactam ring opening mechanism (Fan et al., 2014). There has been many chemosensors synthesized for the recognition of various

metal ions but none for simultaneous detection of Al^{3+} and Sn^{2+} hitherto (Bao et al., 2014; Brulíkova et al., 2020; Han et al., 2012; Tian et al., 2012). However, one of the first few reported rhodamine based derivatives that can function as a dual recognition chemosensor for metal ions was by Yin's group. They managed to differentiate these two metals (copper and vanadium) by using different spectroscopy techniques as both metals exhibited contrasting spectral properties (Huo et al., 2010). Annaraj's study discovered the differing selectivity of the fluorescent rhodamine based chemosensor towards iron and copper ions by varying pH values (Senthil Murugan et al., 2018). Moreover, the new dual colorimetric and fluorometric sensor by Xu et al. displayed merits not only in its excellent selectivity and sensitivity, but also in the obvious colour change against the colourless blank during the sensing process of copper and cobalt ions (Wang et al., 2019). Such chemosensor with dual sensing ability clearly proves its advantage over conventional methods for heavy and transition metals detection (Kim et al., 2015a). Whilst there is no one-for-all solution, more efforts should be put into finding suitable substituting moiety and solvent system since rhodamine based chemosensors can be further fine-tuned to selectively detect different metal ions (Singh et al., 2009). Herein, we employ the same approach of spirolactam ring opening mechanism in hopes to develop a chromogenic and/or fluorogenic rhodamine based chemosensor that would behave in an analogous manner in the presence of metal cations to ease detection in future analytical applications.

CHAPTER 3: METHODOLOGY

3.1 Materials

All chemical reactants for synthesis were purchased from SIGMA while the solvents and methyl ethyl ketone were purchased from MERCK. rhodamine B, 6bromoveratraldehyde, 3-hydroxy-4-methoxybenzaldehyde and 2,4,6trihydroxybenzaldehyde were purchased from Alfa Aesar.

The solutions of Al^{3+} , Ca^{2+} , Co^{2+} , Cu^{2+} , Na^+ , Sn^{2+} , and Zn^{2+} were prepared from their chloride salts; the solutions of Ag^+ , Cd^{2+} , La^{3+} , Pb^{2+} and Ni^{2+} were prepared from their nitrate salts; the solution of Mn^{2+} was prepared from its sulphate salt.

Stock solution of the chemosensors methyl ethyl ketone rhodamine B hydrazone (**MEK**) (10 mM) and 6-bromoveratraldehyde rhodamine B hydrazone (**BVT**) (10 mM) were prepared in acetone, while stock solutions of the chemosensors 3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone (**HMB**) (10 mM) and 2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone (**THB**) (10 mM) were prepared in DMSO. The metal ions stock solutions (100 mM) were prepared in a similar way dissolved in distilled water.

3.2 Physical measurements

Melting points were determined on Laboratory Devices Mel-Temp II. IR spectra were recorded as KBR pellets by using a Perkin-Elmer Spectrum RX-1 spectrometer. NMR spectra were recorded in deuterated CDCl₃ and DMSO-d₆ on a JEOL ECX 400 MHz instrument. UV-Vis spectroscopic measurements were performed on a Shimadzu UV-2600 spectrophotometer. All absorption spectra were performed in a quartz optical cell of 1 cm optical path length at room temperature in the range of 200-700 nm. Fluorescence spectroscopic measurements were performed on a Cary Eclipse fluorescence spectrophotometer in a quartz optical cell of 1 cm optical path length. Both excitation and emission slit widths were 5.0 nm.

3.3 Syntheses

3.3.1 Synthesis of rhodamine B hydrazide (RBH)

Rhodamine B hydrazide was synthesized according to literature method (Gupta et al., 2016). Rhodamine B (2.0 g, 5mmol) was dissolved in ethanol (50 mL). Hydrazine hydrate (2.5 mL) was then added into the solution in a drop wise manner with vigorous stirring at room temperature. The solution was refluxed for 5 hours until the colour changed from purple to orange. Once the mixture was cooled, hydrochloric acid (50 mL, 1 M) was added drop wise followed by addition of sodium hydroxide (55 mL, 1 M). The resulting precipitate was filtered and washed 4 times with distilled water (15 mL, room temperature) and left to dry at room temperature. The solid obtained was then characterized with FTIR, ¹H NMR and ¹³C NMR.

Yield: 89%; M.P.: 180-182 °C. Anal. calcd. for C₂₈H₃₂N₄O₂: C, 73.66; H, 7.06; N, 12.27. Found: C, 73.53; H, 7.15; N, 12.19.

FTIR (KBr), ν, cm⁻¹: 3334m (C-H), 3250m (N-H), 2965m (C-H), 2926m (C-H), 2868m (C-H), 1687s (C=O), 1329m (C-N), 1216s (C-O-C), 1116s (C-O) (br, broad; w, weak; m, medium; s, strong).

¹H NMR (400 MHz, CDCl₃, TMS, s = singlet; d = duplet; t = triplet; q = quadruplet; m = multiplet), δ (ppm): 1.16 (t, J = 6.8 Hz, 12H, NCH₂CH₃); 3.33 (q, J = 6.8 Hz, 8H, NCH₂CH₃); 3.61 (s, 2H, NH₂); 6.28 (d, J = 7.8 Hz, 2H, Xanthene-H); 6.42 (d, J = 6.0 Hz, 2H, Xanthene-H); 6.47 (d, J = 6.0 Hz, 2H, Xanthene-H); 7.09 (m, 1H, Aromatic-H); 7.43 (m, 2H, Aromatic-H); 7.93 (m, 1H, Aromatic-H).

¹³C NMR (100 MHz, CDCl₃, TMS), δ (ppm): 12.70 (NCH₂CH₃); 44.45 (NCH₂CH₃); 66.00 (C-N); 98.02, 104.60, 108.10, 123.07, 123.91, 128.18, 130.10, 132.60, 148.96, 151.63, 153.93 (Aromatic-C); 166.23 (C=O).

3.3.2 Synthesis of 6-bromoveratraldehyde rhodamine B hydrazone (BVT)

6-Bromoveratraldehyde (0.13 g, 0.5 mmol) was dissolved in ethanol (10 mL) and then added into a round bottom flask containing **RBH** (0.24 g, 0.5 mmol). The solution was refluxed at 60°C for 5 hours until the colour changed from pink to purple. The solution was then poured into a beaker and left to cool at room temperature. The crystals formed were rinsed with methanol (room temperature) and further characterized with FTIR, CHN, ¹H NMR, ¹³C NMR and X-ray crystallography.

Yield: 85%; M. P.: 206-208 °C. Anal. calcd. for C₃₇H₃₉BrN₄O₄: C, 65.01; H, 5.75; N, 8.20. Found: C, 64.90; H, 5.83; N, 7.98.

FTIR (KBr), ν, cm⁻¹: 2970m, 2925m (C-H), 1691s (C=O), 1612s (C=N), 1308s (C-N), 1215s (C-O-C), 1118s (C-O) (br, broad; w, weak; m, medium; s, strong).

¹H NMR (400 MHz, CDCl₃, TMS, s = singlet; d = duplet; t = triplet; q = quadruplet; m = multiplet), δ (ppm): 1.12 (t, *J* = 6.8 Hz, 12H, NCH₂CH₃); 3.32 (q, *J* = 7.6 Hz, 8H, NCH₂CH₃); 3.80 (s, 6H, O-CH₃); 6.24 (s, 2H, Xanthene-H); 6.42 (d, *J* = 2.8 Hz, 2H, Xanthene-H); 6.52 (d, *J* = 8.8 Hz, 2H, Xanthene-H); 6.83 (s, 1H, Aromatic-H); 7.15 (d, *J* = 2.0 Hz, 1H, Aromatic-H); 7.50 (m, 3H, Aromatic-H); 7.99 (d, *J* = 2.0 Hz, 1H, Aromatic-H).

¹³C NMR (100 MHz, CDCl₃, TMS), δ (ppm): 12.69 (NCH₂CH₃); 44.45 (NCH₂CH₃); 56.07 (O-CH₃); 66.06 (C-N); 98.06, 105.712, 108.19, 108.94, 114.76, 116.16, 123.45, 124.00, 127.00, 128.07, 128.46, 133.54, 148.46, 148.99, 150.86, 151.79, 153.32 (Aromatic-C); 145.03 (C=N); 164.93 (C=O).

3.3.3 Synthesis of 3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone (HMB)

Powder of 3-hydroxy-4-methoxybenzaldehyde (0.08 g, 0.5 mmol) and **RBH** (0.24 g, 0.5 mmol) were dissolved separately in ethanol (10 mL each). Both solutions were then added together in a round bottom flask and the mixture was refluxed for 6 hours with continuous stirring until the colour changed from light pink to dark purple. The solution was then left to cool, and crystals were formed at room temperature. The crystals were

then collected and rinsed with ethanol (room temperature). The crystal was harvested as product and it was studied with FTIR, CHN, ¹H NMR, ¹³C NMR and X-ray crystallography.

Yield: 80%; M. P.: 140-142 °C. Anal. calcd. for C₃₆H₃₈N₄O₄: C, 73.20; H, 6.48; N, 9.48. Found: C, 73.02; H, 6.40; N, 9.50.

FTIR (KBr), ν, cm⁻¹: 3281s (OH), 2967s (C-H), 1694s (C=O), 1609s (C=N), 1307s (C-N), 1215s (C-O-C), 1112s (C-O) (br, broad; w, weak; m, medium; s, strong).

¹H NMR (400 MHz, CDCl₃, TMS, s = singlet; d = duplet; t = triplet; q = quadruplet; m = multiplet), δ (ppm): 1.14 (t, *J* = 6.8 Hz, 12H, NCH₂CH₃); 3.32 (q, *J* = 7.6 Hz, 8H, NCH₂CH₃); 3.83 (s, 3H, O-CH₃); 5.65 (s, 1H, OH); 6.24 (s, 2H, Xanthene-H); 6.43 (d, *J* = 2.4 Hz, 2H, Xanthene-H); 6.52 (d, *J* = 8.4 Hz, 2H, Xanthene-H); 6.74 (d, *J* = 2.4 Hz, 1H, Aromatic-H); 7.06 (m, 3H, Aromatic-H); 7.46 (m, 2H, Aromatic-H); 7.98 (d, *J* = 6.4 Hz, 1H, Aromatic-H); 8.63 (s, 1H, N=CH).

¹³C NMR (100 MHz, CDCl₃, TMS), δ (ppm): 12.72 (NCH₂CH₃); 44.38 (NCH₂CH₃); 56.02 (O-CH₃); 66.09 (C-N); 98.00, 106.19, 108.00, 110.20, 113.00, 120.71, 123.33, 123.85, 128.08, 128.26, 129.15, 129.51, 133.22, 147.71, 148.15, 148.94, 151.97, 153.17 (Aromatic-C); 145.50 (C=N); 164.96 (C=O).

3.3.4 Synthesis of methyl ethyl ketone rhodamine B hydrazone (MEK)

RBH (0.24 g, 0.5 mmol) was dissolved and refluxed in methyl ethyl ketone (10 mL, 0.12 mol) for 5 hours until the colour changed from pink to purple. The solution was then left to cool at room temperature. Crystals formed from slow evaporation of the solvent at room temperature were collected and rinsed with ethanol (cold). The crystal collected was then evaluated with FTIR, CHN, ¹H NMR, ¹³C NMR and X-ray crystallography.

Yield: 86%; M. P.: 170-172 °C. Anal. calcd. for C₃₂H₃₈N₄O₂: C, 75.26; H, 7.50; N, 10.97. Found: C, 75.13; H, 7.54; N, 10.71.

FTIR (KBr), v, cm⁻¹: 2971m (C-H), 2926m (C-H), 1689s (C=O), 1611s (C=N), 1326s (C-N), 1219s (C-O-C), 1118s (C-O) (br, broad; w, weak; m, medium; s, strong).

¹H NMR (400 MHz, CDCl₃, TMS, s = singlet; d = duplet; t = triplet; q = quadruplet; m = multiplet), δ (ppm): 0.94 (t, J = 4.4 Hz, 3H, CH₂CH₃); 1.16 (t, J = 4.8 Hz, 12H, NCH₂CH₃); 1.73 (s, 3H, N=CCH₃); 2.20 (q, J = 7.6 Hz, 2H, CH₂CH₃); 3.34 (q, J = 3.6 Hz, 8H, NCH₂CH₃); 6.26 (d, J = 2.8 Hz, 2H, Xanthene-H); 6.37 (d, J = 2.4 Hz, 2H, Xanthene-H); 6.52 (d, J = 4.4 Hz, 2H, Xanthene-H); 7.16 (m, 1H, Xanthene-H); 7.45 (m, 2H, Aromatic-H); 7.89 (m, 1H, Aromatic-H).

¹³C NMR (100 MHz, CDCl₃, TMS), δ (ppm): 11.18 (CH₂CH₃); 12.70 (NCH₂CH₃); 19.56 (N=CCH₃); 44.43 (NCH₂CH₃); 66.40 (C-N); 97.92, 107.79, 122.92, 123.97, 128.23, 128.62, 131.56, 148.70, 151.20, 153.76, 153.90 (Aromatic-C); 160.17 (C=N); 178.69 (C=O).

3.3.5 Synthesis of 2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone (THB)

Powder of **RBH** (0.24 g, 0.5 mmol) and 2,4,6-trihydroxybenzaldehyde (0.08 g, 0.5 mmol) were weighed and dissolved in ethanol (10 mL) before the pinkish solution was refluxed in boiling ethanol with continuous stirring for 5 hours until the solution turned to purple colour. The solution was then left to cool and pink crystals that are suitable for X-ray crystallography were formed from slow evaporation of the solvent. The crystals were collected and rinsed with excess ethanol (room temperature). The crystal obtained was then characterized with methods including FTIR, CHN, ¹H NMR, ¹³C NMR and X-ray crystallography.

Yield: 88%; M. P.: 284-286 °C. Anal. calcd. for C₃₅H₃₆N₄O₅: C, 70.93; H, 6.12; N, 9.45. Found: C, 69.97; H, 6.38; N, 9.63.

FTIR (KBr), ν, cm⁻¹: 3210 (OH), 2972s (C-H), 2930s (C-H), (C-H), 1616s (C=O), 1605s (C=N), 1309s (C-N), 1220s (C-O-C), 1119s (C-O) (br, broad; w, weak; m, medium; s, strong).

¹H NMR (400 MHz, DMSO-d₆, δ (ppm): 1.05 (t, *J* = 4.0 Hz, 12H, NCH₂CH₃); 3.25 (q, *J* = 6.8 Hz, 8H, NCH₂CH₃); 5.75 (s, 2H, OH); 6.30 (m, 6H, Xanthene-H); 6.39 (m, 2H, Aromatic-H); 6.98 (d, *J* = 4.8 Hz, 1H, Aromatic-H); 7.47 (d, *J* = 5.2 Hz, 2H, Aromatic-H); 7.89 (m, 1H, Aromatic-H); 9.12 (s, 1H, N=CH); 10.82 (s, 1H, OH).

¹³C NMR (100 MHz, DMSO-d₆), δ (ppm): 12.71 (NCH₂CH₃); 44.14 (NCH₂CH₃); 65.84 (C-N); 94.79, 97.62, 99.70, 104.69, 108.60, 123.40, 123.95, 128.01, 129.04, 129.37, 134.22, 150.20, 151.45, 153.11, 160.20, 162.39, 163.74 (Aromatic-C); 149.01 (C=N); 166.46 (C=O).

3.4 X-ray crystallography

The unit cell parameters and the intensity data of the crystals obtained (MEK, BVT, HMB and THB) were analyzed on a Oxford Rigaku SuperNova Dual diffractometer, equipped with a Mo-K α X-ray source ($\lambda = 0.71073$ Å) with Atlas detector used to analyze the intensity data of the crystals and the unit cell parameters (Rigaku, 2015). Data acquisition, cell refinement and reduction were carried out with CrysAlis Pro software (CrysAlis, 2009). Absorption corrections on the data were done using SADABS. The structure were solved and refined by SHELXL97 (Sheldrick, 2008). Molecular graphics were drawn by using Mercury v3.9 (Macrae et al., 2008).

3.5 Spectral characteristics of chemosensors

All experiments were carried out in triplicates and the best results were used. The concentration and solvent at which the experiments were carried out is the combination that produced the most suitable absorbance. No other metals could be detected even at different concentrations.

3.5.1 Metals selectivity

The sensing behavior of the chemosensors with 13 different metals (Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, La³⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Sn²⁺, and Zn²⁺) in an aqueous solution were evaluated with UV-vis and fluorescence spectroscopy. For chemosensors **MEK**, **HMB**, and **THB**, all experiments were carried out in 30% acetonitrile solution and the spectral data (200 nm – 700 nm) were recorded immediately after the addition of the metal ions. The test samples were prepared by placing 1, 2, and 1 equivalent of the cation stock solution respectively in 2 mL of the **MEK** (50 μ M), **HMB** (50 μ M), and **THB** (10 μ M) solution. On the other hand, all experiments for chemosensor **BVT** (100 μ M) were carried out in 30% acetonitrile solution with 3 equivalents of cation stock solution. Meanwhile, the fluorescence measurements were carried out at excitation and emission slit width of 5.0 nm, excitation was provided at 563 nm, and the emission was collected from 565 nm to 750 nm (Wan et al., 2017).

3.5.2 Varying pH

The effects of pH on the response of chemosensors in the presence of different metal ions were investigated. Briefly, acetonitrile solutions of two different concentrations 50% (**BVT**) and 30% (**HMB**, **MEK**, and **THB**) were prepared and adjusted to six different pH values (2, 4, 6, 8, 10, and 12) via 4 M HCl and 4 M NaOH. Chemosensor (**BVT**, 100 μ M; **HMB**, 50 μ M; **MEK**, 50 μ M; **THB**, 10 μ M) was then introduced into each solution prior to the addition of metal ion (**BVT**, 300 μ M; **HMB**, 100 μ M; **MEK**, 50 μ M; **THB**, 10 μ M), and the UV-vis spectra were recorded immediately (Chen et al., 2017).

3.5.3 Varying concentration of metal ions

The sensitivity of the chemosensors was better apprehended via titration experiments for the respective metal ions of interest. Briefly, the sensitivity of **BVT** (100 μ M) towards Al³⁺ and Sn²⁺ was elucidated by gradual addition of 0-600 μ M of Al³⁺ and 0-360 μ M of Sn²⁺, respectively. Then, the respective absorption and fluorescent spectra were recorded from 200 nm – 700 nm and 565 nm – 800 nm respectively. Similar procedures were repeated for **HMB**, **MEK**, and **THB** (concentrations of Al³⁺ tested are: **HMB**, 0-175 μ M; **MEK**, 0-130 μ M; concentrations of Sn²⁺ tested are: **HMB**, 0-150 μ M; **MEK**, 0-60 μ M; concentrations of Cu²⁺ tested are: **THB**, 0-30 μ M) (Liao et al., 2013).

3.5.4 Competing metals

The selectivity of chemosensors towards the metal ion of interest, as well as the potential interference from other metal ions were evaluated through the introduction of other metal ions into the solution containing chemosensor and the metal ion of interest. Firstly, the complex solution of Al³⁺/competing metal ion/**BVT** was prepared by adding Al³⁺ (300 μ M) and 300 μ M of Ag⁺ (as competing metal ion) into a solution containing 50% acetonitrile. After the solution was well mixed, the chemosensor **BVT** (100 μ M) was then added into it. The absorption (200 nm – 700 nm) and fluorescent (565 nm to 800 nm) spectra were then recorded. Complex solutions of other competing metal ions were prepared by replacing Ag⁺ with other metal ions including: Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, La³⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Sn²⁺, and Zn²⁺, whilst Al³⁺ ion always present. The same procedure was repeated by replacing Al³⁺ with Sn²⁺ ion for chemsosensor **BVT**. Unlike **BVT**, experiments involving the other chemosensors **HMB** (100 μ M), **MEK** (50 μ M) and **THB** (10 μ M) were carried out in 30% acetonitrile solution. Moreover, the

experiments involving **HMB** was performed with concentration of both Al^{3+} and Sn^{2+} remained at 100 µM respectively. Meanwhile, the procedures involving **MEK** was done with both Al^{3+} and Sn^{2+} at 50 µM respectively. Nevertheless, concentration of Cu^{2+} was kept constant at 10 µM in the studies involving **THB** (Li et al., 2016).

3.5.5 Reversibility

The reversibility of the chemosensors were evaluated by introducing a commonly used quencher, EDTA (ethylenediaminetetraacetic acid disodium salt), into the mixture of chemosensor and metal cation to determine if the coordination of the sensor with target metal ion can be reversed by chemical process. For instance, 100 μ M of **BVT** in 2 mL of 50% acetonitrile was mixed with 550 μ M of Al³⁺, resulting in a pinkish solution and the absorbance and fluorescence at the respective λ_{max} were recorded. EDTA was then added into the mixture until it has become colourless, followed by measurement of absorbance and fluorescence. Subsequently, appropriate amount of Al³⁺ was added until the solution turned back to pink and the procedures were repeated several times until the absorbance and fluorescence drop below half its original intensity. The same procedure was repeated by replacing Al³⁺ with (360 μ M, 0.72 μ L) amount and volume of Sn²⁺ ion. On the other hand, reversibility for **HMB** in 30% acetonitrile with Al³⁺ (130 μ M, 2.6 μ L) and Sn²⁺ (60 μ M, 1.2 μ L), and **THB** in 30% acetonitrile with Cu²⁺ (26 μ M, 5.2 μ L) (Rai et al., 2018).
3.6 Formation of sensor-metal complex

3.6.1 Job's plot

The binding stoichiometry of the chemosensors toward the metal ions detected was determined via Job's plot. In brief, the binding stoichiometry of **BVT** towards Al^{3+} was evaluated by preparing a series of solutions containing **BVT** and Al^{3+} ions where the mole fraction ([sensor]/[sensor]+[metal ion]) was varied from 0 to 1 while the sum of the total **BVT** and Al^{3+} concentration remained constant at 500 µM. The absorbance at 563 nm was then recorded and plotted against the mole fraction of the Al^{3+} solution. Similar procedure was repeated for Sn^{2+} (sum of total concentration = 500 µM; $\lambda_{max} = 562$ nm). On the other hand, the sum of the total concentration for **HMB** with both Al^{3+} ($\lambda_{max} = 563$ nm) and Sn^{2+} ($\lambda_{max} = 563$ nm) were 250 µM, whilst **MEK** with both Al^{3+} ($\lambda_{max} = 563$ nm) and Sn^{2+} ($\lambda_{max} = 563$ nm) were carried out at 100 µM, and **THB** with Cu^{2+} ($\lambda_{max} = 559$ nm) was performed at 20 µM (Lee et al., 2017).

3.6.2 FTIR, ¹H- and ¹³C-NMR spectra of sensor-metal complex

In order to achieve better understanding on the sensing mechanisms of the chemosensors, the sensor-metal complexes formed were investigated via different methods. In general, appropriate amount of sensor was mixed with metal of interest and the solid formed was then harvested and subsequently studied with different methods. For instance, chemosensor **BVT** (10 mM) was added to a solution containing Al³⁺ ion (10 mM) and left to dry at room temperature. The solids formed were dried at room temperature, collected and further elucidated with FTIR, ¹H- and ¹³C-NMR. Similar procedure was repeated by replacing Al³⁺ with Sn²⁺ (10 mM). As for other chemosensors, **HMB** (10 mM) was reacted with Al³⁺ (10 mM) and Sn²⁺ (10 mM) respectively, while

MEK (10 mM) with Al³⁺ (10 mM) and Sn²⁺ (10 mM) respectively and **THB** (10 mM) was added to a solution containing Cu²⁺ ion (10 mM). The respective sensor and sensormetal complex's FTIR, ¹H and ¹³C NMR spectra obtained was compared and studied (Li et al., 2014b).

3.7 Practical applications with chemosensors

3.7.1 On-site assay

The potential application of the chemosensor as an on-site assay test kit for 'nakedeye' detection, along with the selectivity of these chemosensors were examined by using filter paper test strips cut into a standard size of 1 cm x 1 cm (width x height). Firstly, the chemosensors were dissolved in acetone before the test strips were submerged in it and then left to dry. Afterwards, the dried test strips containing the chemosensor were immersed in aqueous solutions containing one of the 13 different metal ions to be tested (Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, La³⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Sn²⁺, and Zn²⁺) and allowed to dry. Any recognizable or observable colour change was recorded and photographed under visible and UV light. In particular, concentrations of the chemosensor prepared are: **BVT**, 10 mM; **HMB**, 10 mM; **MEK**, 10 mM; **THB**, 0.1 mM. Meanwhile, concentrations of the metal ions for the respective chemosensors tested are: **BVT**, 100 mM; **HMB**, 100 mM; **MEK**, 10 mM; **THB**, 0.1 mM (Zhang et al., 2018).

3.7.2 Cell culture and MTT cytotoxicity assay

All cell lines, including the human colorectal adenocarcinoma HT-29 and human fibroblast CCD-18Co were purchased from American Type Culture Collection (ATCC, USA). These cells were cultured in McCoy's 5A medium (HT-29) and DMEM (CCD-

18Co) supplemented with 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere containing 5% CO₂.

The MTT assay was performed according to the methods described by Mossmann et al. with some modifications (Mosmann, 1983; Navanesan et al., 2015). Briefly, the cells were seeded into a 96-well microplate, incubated overnight (24 h) prior to treatment with various concentrations of **THB** for 72 h whilst cisplatin was included as positive reference standard. Then, MTT was added into each well and further incubated for another 3 h before the addition of DMSO. The absorbance of each well was measured using a Tecan M200 Infinite Pro Microplate Reader at 570 and 650 nm as reference wavelength. The cytotoxicity was determined as IC₅₀ values by plotting percentage of viability against the concentration of treatment on a logarithmic scale using GraphPad Prism 7 software (Graphpad software Inc., CA, USA).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Syntheses

Rhodamine B hydrazide (**RBH**) and the respective chemosensors (**BVT**, **HMB**, **MEK**, and **THB**) were synthesized according to the scheme as shown in Figure 4.1. The selectivity, sensitivity, and binding mechanism of these chemosensors were studied through visual assessment, absorption, and emission experiments.



Figure 4.1: Synthetic pathway of RBH and chemosensors BVT, HMB, MEK, and THB.

4.1.1 Synthesis of rhodamine B hydrazide (RBH)

The reaction of rhodamine B with hydrazine hydrate in excess ethanol yielded rhodamine B hydrazide (**RBH**) with the formula, C₂₈H₃₂N₄O₂. The reaction mixture was refluxed for 5 hours as the colour of the solution changed from dark purple to orange. Once the reaction completed, hydrochloric acid was added to the solution and turned the orange solution into a light pink mixture. Then, the pH of the solution was adjusted to 9-10 with the addition of sodium hydroxide solution. The resulting orange precipitate was filtered, washed with distilled water, and left to dry. The crude product recrystallized with ethanol yielded purple crystals. The structure of **RBH** was characterized by FTIR, CHN, ¹H NMR, and ¹³C NMR.

4.1.2 Synthesis of 6-bromoveratraldehyde rhodamine B hydrazone (BVT)

The one-step synthesis of **BVT** from the condensation reaction between rhodamine B hydrazide (**RBH**) with 6-bromoveratraldehyde in ethanol yielded purple crystals. The complex **BVT** was then characterized by FTIR, CHN, ¹H NMR, ¹³C NMR, and X-ray crystallography. It is soluble in organic solvents such as acetone, methanol, ethanol, chloroform, DMF, and DMSO.

4.1.3 Synthesis of 3-hydroxy-4-methoxybenzaldehyde rhodamine B hydrazone (HMB)

The sensor **HMB** was synthesized in a way similar to that of **BVT**, the condensation reaction between **RBH** with 6-bromoveratraldehyde was replaced with 3-hydroxy-4-methoxybenzaldehyde. This reaction yielded light pink crystals and is soluble in various organic solvents like acetone, methanol, ethanol, chloroform, DMF, and DMSO. The synthesized **HMB** was also confirmed with FTIR, CHN, ¹H NMR, ¹³C NMR, and X-ray crystallography.

4.1.4 Synthesis of methyl ethyl ketone rhodamine B hydrazone (MEK)

The sensor **MEK** was prepared from the condensation of **RBH** with excess methyl ethyl ketone. This reaction yielded pink crystals and they are soluble in acetone, acetonitrile, chloroform, ethanol, methanol, DMF, and DMSO. The formation of this ligand **MEK** was confirmed with FTIR, CHN, ¹H NMR, ¹³C NMR, and X-ray crystallography.

4.1.5 Synthesis of 2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone (THB)

Sensor **THB** was synthesized by refluxing **RBH** with 2,4,6-trihydroxybenzaldehyde in excess ethanol solution. The resulting product formed from condensation resulted in dark purple crystals. **THB** was found to be soluble in most organic solvents such as acetone, acetonitrile, ethanol, methanol, DMF, and DMSO. Meanwhile, FTIR, CHN, ¹H NMR, ¹³C NMR, and X-ray crystallography confirmed the formation of this ligand **THB**.

4.2 Infrared spectra

The main IR vibrational bands of **RBH**, **BVT**, **HMB**, **MEK**, and **THB** were found in their expected regions. Their characteristic IR bands were listed in the experimental section, important infrared bands were selected and tabulated in Table 4.1. Formation of **BVT**, **HMB**, **MEK**, and **THB** Schiff-base ligands by condensation was indicated by the disappearance of characteristic peak of amine group ν (NH₂), which is present in the IR spectra of **RBH** at 3334 cm⁻¹ and 3250 cm⁻¹. The Schiff-base formation of **BVT**, **HMB**, **MEK**, and **THB** was also further confirmed by the strong band attributed to imine linkage, ν (R₂C=NR) at 1612 cm⁻¹, 1611 cm⁻¹, 1611 cm⁻¹, and 1607 cm⁻¹ respectively, which is similar to reported value (Rai et al., 2016).

Table 4.1: Selected infrare	d spectroscopic p	peak assignments	of RBH, BVT	, HMB,
MEK, and THB.				

Complex	ν (NH ₂), cm ⁻¹	ν (C=O), cm ⁻¹	ν (C=N), cm ⁻¹
RBH	3334, 3250	1687	-
BVT	<u> </u>	1691	1612
НМВ	-	1695	1611
МЕК	-	1689	1611
тнв	-	1619	1607

4.3 ¹H-NMR and ¹³C-NMR

Structural information of the synthesized Schiff-base sensor can be deduced from ¹H NMR and ¹³C NMR spectra. The chemical shift for the proton of terminal amine of rhodamine B hydrazide that was observed at 4.21 ppm is similar to reported value in literature (Kai et al., 2014). However, in the ¹H NMR spectra of the ligands **BVT**, **HMB**, **MEK**, and **THB**, this characteristic NH peak was not found, indicating the formation of Schiff-base linkage upon condensation. Meanwhile, the characteristic imine (C=N) peak of the respective ligands found at 145.03 ppm, 145.50 ppm, 160.15 ppm, and 149.01 ppm in the ¹³C NMR spectra (Ozdemir, 2016). Hence, this further supports that an imine linkage was formed via condensation between the protons of terminal amine of rhodamine B hydrazide with their respective carbonyl group.

4.4 Crystal structures of Schiff-base ligands

The single crystal X-ray analysis revealed that **BVT**, **HMB**, **MEK**, and **THB** crystallized in the monoclinic crystal system with the space group of P2₁/c. Details of crystallographic data and structure refinement parameters are summarized in Table 4.2.

Compound	BVT	HMB	MEK	ТНВ
Empirical formula	C37H39BrN4O4	$C_{36}H_{38}N_4O_4$	$C_{32}H_{38}N_4O_2$	$C_{35}H_{36}N_4O_5$
Formula weight	684.63	590.70	510.66	592.68
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$	$P2_1/c$
Unit cell dimensions				
a (Å)	10.6244(2)	10.847(7)	12.5912(4)	9.3416(10)
b (Å)	14.8219(2)	21.18(3)	12.4388(3)	26.994(3)
c (Å)	22.0012(4)	13.942(12)	18.9631(6)	12.3493(12)
α (°)	90	90	90	90
β (°)	98.947(2)	101.10(7)	107.628(4)	104.725(10)
γ(°)	90	90	90	90
$V(Å^3)$	3422.46(10)	3143(6)	2830.53(16)	3011.9(6)
Z	4	4	4	4
F (000)	1428	1256.0	1096	1256
$D_x (Mg m^{-3})$	1.329	1.248	1.198	1.310
T (K)	293	293	293	293
Absorption coefficient, μ (mm ⁻¹)	1.994	0.658	0.569	0.72
θ ranges (°)	3.6-75.5	3.8–64.6°	4.3-74.4	4.9-73.9
Reflections collected	13677	3350	11647	10097
Independent reflections	6878	1816	5621	5789
Data/Restraints/Parameters	6878/0/425	1816/0/403	5621/0/326	5789/0/404
Range of <i>h</i> , <i>k</i> , <i>l</i>	-9/13, -12/18, -27/25	-7/9, -13/17, -11/11	-15/11, -14/16, -23/23	-11/9, -31/32, -10/14
Goodness-of-fit on F ²	1.02	1.013	1.025	1.038
$R[F^2 > 2\sigma(F^2)]$	0.038	0.094	0.048	0.077
$wR(F^2)$	0.105	0.290	0.149	0.235

 Table 4.2: Crystal data and structure refinement parameters of BVT, HMB, MEK, and THB.

4.4.1 Crystal structure of BVT

Single crystal of **BVT** complex was obtained by slow evaporation from ethanol solution and the crystallographic data confirmed the presence of the spirolactam form of the structure, with the lactam and xanthene moiety coordinated in a mutually perpendicular position as shown in Figure 4.2. Its bond lengths and angles are within normal ranges. Selected bond lengths and angles are shown in Table 4.3. The bond length of the imine linkage (N2=C29) is 1.281 (2) Å, it is similar to reported value in literature, which further supports that an imine linkage was formed via condensation (Wu et al., 2009). **BVT** forms a honeycomb packing that runs along the *b*-axis of the monoclinic unit cell as illustrated in Figure 4.3.

Bond lengths (Å)		Bond an	gles (°)
N1-N2	1.373 (3)	C1-N1-N2	129.89 (15)
N2-C29	1.281 (2)	C29-N2-N1	120.01 (16)
N4-C12	1.383 (3)	C12-N4-C26	120.40 (2)
C1-N1	1.371 (2)	C12-N4-C28	121.10 (2)
C1-O1	1.226 (2)	C10-O2-C20	117.73 (15)
C8-N1	1.506 (2)	N1-C8-C9	109.41 (14)
C10-O2	1.381 (2)	C30-C31-Br1	121.80 (14)
Br1-C31	1.905 (18)	C32-C31-Br1	116.04 (14)

Table 4.3: Selected bond lengths and bond angles for ligand BVT.



Figure 4.2: X-ray crystal structure of chemosensor BVT. Hydrogen atoms are removed for clarity and structure drawn with 50% ellipsoid.



Figure 4.3: Honeycomb packing formed from Van der Waals interactions of complex BVT.

4.4.2 Crystal structure of HMB

Single crystal of **HMB** collected at room temperature was dried and studied with Xray crystallography. Selected bond lengths and bond angles are shown in Table 4.4. Its bond lengths and bond angles are within reported values. The presence of imine bond was demonstrated by the shorter bond length of N4-C29 (1.294 Å) compared with N1-C2 (1.500 Å) (Munro et al., 2006). As illustrated in Figure 4.4, the formation of the **HMB** spirolactam structure was confirmed by the orthogonal position of the lactam moiety to the xanthene moiety. Meanwhile, **HMB** forms a zig-zag chain *via* hydrogen bonds of the type O3–H3···O2 that runs along the *c*-axis of the monoclinic unit cell as illustrated in Figure 4.5.

Bond lengths (Å)		Bond angles (°)	
N1—C2	1.500 (2)	C33—O4—C36	118.1 (13)
N4—C29	1.294 (17)	N1—C3—C4	114.0 (15)
N2—C16	1.400 (2)	N4—N3—C11	131.1 (12)
N2—C18	1.490 (19)	N4—N3—C28	113.8 (14)
N2—C20	1.460 (2)	C28—N3—C11	114.3 (14)
C16—C15	1.406 (19)	C5—N1—C2	115.0 (12)
C16—C17	1.39 (2)	C3—N1—C5	119.1 (14)
O3—C34	1.399 (18)	C3—N1—C2	112.7 (15)

Table 4.4: Selected bond lengths and bond angles for complex HMB.



Figure 4.4: X-ray crystal structure of chemosensor HMB. Hydrogen atoms are removed for clarity and structure drawn with 10% ellipsoid.



Figure 4.5: Zigzag chain formed from Van der Waals interactions of complex HMB.

4.4.3 Crystal structure of MEK

Single crystal of **MEK** grown in acetonitrile solution crystallized in a monoclinic crystal system. The crystallographic data obtained confirms the spirolactam form of the rhodamine ring-closed structure, where the xanthene and lactam moiety are positioned in a perpendicular manner as depicted in Figure 4.5. Its bond lengths and angles were found within normal ranges while selected bond angles and bond lengths are tabulated in Table 4.6. The bond length of C30-N4 was found to be 1.280 (2) Å, which is reflective towards the formation of imine linkage (Akerman & Chiazzari, 2014). **MEK** forms a zig-zag chain that runs along the *c*-axis of the monoclinic unit cell as shown in Figure 4.7.

Bond lengths (Å)		Bond an	gles (°)
N2-C15	1.378 (2)	C15-N2-C20	122.55 (16)
N3-N4	1.407 (17)	C15-N2-C19	121.24 (16)
N4-C30	1.280 (2)	C28-N3-N4	125.45 (12)
C13-O1	1.376 (15)	C30-N4-N3	115.86 (14)
C11-N3	1.491 (17)	C7-O1-C13	118.65 (9)
C28-N3	1.365 (2)	N3-C11-C12	111.45 (11)
C28-O2	1.223 (19)		

Table 4.5: Selected bond lengths and bond angles for complex MEK.



Figure 4.6: X-ray crystal structure of chemosensor MEK. Hydrogen atoms are removed for clarity and structure drawn with 30% ellipsoid.



Figure 4.7: Zig-zag chain formed from Van der Waals interactions of complex MEK.

4.4.4 Crystal structure of THB

Condensation reaction between **RBH** with 2,4,6-trihydroxybenzaldehyde yielded **THB** upon the formation of imine bond. X-ray crystallography shown that **THB** crystallizes in the space group of P2₁/c with the monoclinic crystal system. The bond lengths and angles are in typical ranges. Selected bond lengths and angles in Table 4.6 exhibited that the imine bond length of C29-N4 (1.247 Å) is shorter compared to that of C2-N1 (1.463 Å), thereby suggesting the establishment of imine linkage (Munro et al., 2004). Furthermore, the perpendicular orientation of the lactam moiety with the xanthene moiety of the rhodamine structure was illustrated in Figure 4.8, where **THB** was also shown to have adopted an E configuration. Apart from that, hydrogen bonds of the type O3–H3···O5, O4–H4···O2, and O5–H5···N4 links **THB** by forming a zig-zag chain that runs along the *c*-axis of the monoclinic unit cell as illustrated in Figure 4.9.

Bond lengths (Å)		Bond angles (°)		
N1-C2	1.463 (6)	C7-C10-C22	111.7 (2)	
N3-N4	1.387 (3)	C26-C22-C10	128.5 (3)	
N4-C29	1.247 (4)	C29-N4-N3	121.4 (3)	
C11-C12	1.381 (4)	C3-C2-N1	111.1 (5)	
O1-C12	1.380 (3)	O1-C8-C9	114.1 (3)	
N1-C4	1.385 (4)	O2-C2-N3	125.9 (3)	
C28-O2	1.223 (19)			

Table 4.6: Selected bond lengths and bond angles for complex THB.



Figure 4.8: X-ray crystal structure of chemosensor THB. Hydrogen atoms are removed for clarity and structure drawn with 10% ellipsoid.



Figure 4.9: Zig-zag chain formed from Van der Waals interactions of complex THB.

4.5 Spectral characteristics of chemosensors

4.5.1 Colour and spectral changes upon addition of metal ions

The selectivity of all the chemosensors synthesized (**BVT**, **HMB**, **MEK**, and **THB**) towards different metal ions was evaluated by observing colour changes, as well as UV-vis absorption and fluorescence spectroscopy in the absence and presence of several metal ions (Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, La³⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Sn²⁺, and Zn²⁺). The experiments were performed in 30% acetonitrile solution for chemosensors **HMB**, **MEK**, and **THB** while chemosensor **BVT** was carried out in 50% acetonitrile solution due to poorer solubility compared to the other 3 chemosensors.

The results illustrated in Figure 4.10a shows the colour of **BVT** chemosensor solutions in absence and presence of different metals. All solutions did not show any observable difference except in the presence of AI^{3+} and Sn^{2+} ions, which led to a remarkable change in colour (from colourless to pink), presumably due to the transformation of the closed spirolactam ring into the open spirolactam ring upon coordination with the metal ions (Kilic & Bozkurt, 2018). Similarly, **HMB** and **MEK** displayed similar preference of metal ions detected, reflected by the solutions that turned pink upon addition of AI^{3+} and Sn^{2+} ions as shown in Figure 4.10b and Figure 4.10c respectively. In contrast to these findings, Figure 4.10d revealed that these metal ions were unable to induce any observable colour changes in the case of **THB**. However, the introduction of Cu^{2+} ions was able to turn the colourless solution of **THB** into pink, thereby suggested that the substitution of methyl ethyl ketone with 2,4,6-trihydroxybenzaldehde has altered the preferences of rhodamine-based chemosensor towards different metal ions. The reason behind **THB**'s preference towards Cu^{2+} ions is that the presence of polyhydroxy substituents have been reported to display varied sensitivity towards copper.

In the absence of metal ions, two defined peaks at ~260 and ~320 nm observed in the UV-vis spectrum of the free **BVT** corresponds to $\pi \to \pi^*$ transitions. Meanwhile, in accordance with the change in colour (from colourless to pink) upon introduction of Al³⁺ and Sn^{2+} ions into solutions of **BVT**, a new band was observed at 562 nm in their respective UV-vis absorption spectra (Figure 4.11a), which is ascribed to the $n \rightarrow \pi^*$ transitions (Mandal et al., 2019). Similarly, the UV-vis spectrum of HMB alone is weak, but the addition of Al^{3+} and Sn^{2+} ions into the solutions of **HMB** led to a significant increase in the UV-vis absorption spectra at 563 nm respectively, where the absorption of Sn^{2+} is superior to Al^{3+} ions (Figure 4.11b). Likewise, in the case of **MEK**, the UV-vis spectrum showed no observable difference in the absence of metal ions. However, the presence Al³⁺ and Sn²⁺ ions resulted in remarkable absorption enhancement at 563 nm (Figure 4.11c), where the absorption of Sn^{2+} supersedes Al^{3+} signifying higher affinity of MEK towards Sn^{2+} ions. Besides, addition of Cu^{2+} has led to the emergence of a new absorption band at 559 nm in the spectrum of **THB** (Figure 4.11d), owing to the $n \rightarrow \pi^*$ transitions, whilst the presence of other metal ions did not induce any significant changes in their respective UV-vis spectrum, and thus chemosensor THB displayed excellent selectivity towards Cu²⁺ ions only.

Apart from colour changes, emission of fluorescence in the presence of metal ions under a UV light source (short wavelength) allows the ease of 'naked-eye' detection of these metals. Results in Figure 4.12a demonstrated that addition of Sn^{2+} into **BVT** was capable of inducing bright orange fluorescence under the UV lamp, while the lower emission intensity of Al^{3+} was reflected by the weak orange fluorescence. Similarly, the higher absorption of **HMB** towards Sn^{2+} was reflected by the brighter orange fluorescence under UV lamp as shown in Figure 4.12b, while the lower absorption of **HMB** towards Al^{3+} results in a weaker fluorescence. In the case of **MEK**, Figure 4.12c illustrated that introduction of Sn^{2+} into **MEK** was capable of inducing bright orange fluorescence under the UV lamp. Despite the emission is of lower intensity, presence of Al³⁺ in the solution of **MEK** was able to cause comparably bright orange fluorescence under UV light source too. The differences in the fluorescence intensity between these three chemosensors (**BVT**, **HMB**, and **MEK**) might be utilized as a pivotal parameter in differentiating Al³⁺ and Sn²⁺ ions respectively, considering the latter induced a brighter orange fluorescence despite both metals turned colourless solutions of the respective chemosensors into pink coloured solutions. Unlike these three chemosensors mentioned previously, **THB** did not display any fluorescence amid presence of Cu²⁺ (Figure 4.12d), potentially due to the paramagnetic nature of Cu²⁺ ion that quenched the fluorescence of the rhodamine compound (Liu & Lu, 2007).

Similar to that of UV-vis spectroscopy, the fluorescence properties of the chemosensors in the absence and presence of multiple cations were also studied by investigating the respective prospects in fluorescence emission with fluorescence spectroscopy. For example, the fluorescence emission band of **BVT** alone is weak as shown in Figure 4.13a, but the addition of Al^{3+} and Sn^{2+} resulted in a strong emission band at 588 nm, with Sn^{2+} ions causing a greater increase in fluorescence intensity (~ 237 a.u.) compared with Al^{3+} ions (~ 173 a.u.). The change in spirolactam ring (from closed to opened) was observed in other chemosensors too. For instance, Figure 4.13b exhibited that **HMB** showed no observable fluorescence intensity at 588 nm. However, the introduction of Al^{3+} and Sn^{2+} induced significant fluorescence enhancement at 588 nm, both displayed comparable fluorescence intensity which is ~ 123 a.u. and ~ 138 a.u. respectively. Likewise, the weak fluorescence emission band of **MEK** at 588 nm (Figure 4.13c) was amplified by Al^{3+} and Sn^{2+} , the differences in the amplitude correlated well with the fluorescence intensity observed in Figure 4.13c, where the presence of Sn^{2+} ions

have induced brighter fluorescence and higher fluorescence intensity (~ 259 a.u.), while presence of Al^{3+} resulted in a lower fluorescence intensity (~ 164 a.u.). Ultimately, these results together with changes in colour and UV-vis absorption spectra cumulatively suggested the transformation of the dominant form (closed spirolactam ring) of the chemosensor into its amide form (open spirolactam ring) upon coordinating with the metal ions, potentially due to metal-induced delocalization of rhodamine (Yu et al., 2019). On the other hand, no emission spectra were recorded for **THB** since the coordination of **THB** with all the thirteen metal cations do not possess any fluorescence property.



Figure 4.10: Colour changes of chemosensors upon addition of various metal ions. (a) BVT (100 μ M) turned pink in presence of Al³⁺ and Sn²⁺ ions (300 μ M) in 50% acetonitrile solution, (b) Addition of Al³⁺ and Sn²⁺ (100 μ M) to HMB (50 μ M) in 30% acetonitrile solution changed from colourless to pink, (c) MEK (50 μ M) solution turned pink upon introduction of Al³⁺ and Sn²⁺ (50 μ M) in 30% acetonitrile solution, and (d) Only Cu²⁺ turned pink in the presence of THB in 30% acetonitrile solution.



Figure 4.11: UV–vis spectra of chemosensors in the presence of various cations at room temperature. (a) BVT in 50% acetonitrile solution, $\lambda_{max} = 562$ nm, (b) HMB in 30% acetonitrile solution, $\lambda_{max} = 563$ nm, (c) MEK in 30% acetonitrile solution, λ_{max} = 563 nm, and (d) THB in 30% acetonitrile solution, $\lambda_{max} = 559$ nm.



Figure 4.12: Colour changes of chemosensors upon addition of various metal ions under UV lamp (short wavelength). (a) BVT (100 μ M) turned brighter orange fluorescence in presence of Sn²⁺ compared to Al³⁺ (300 μ M) in 50% acetonitrile solution, (b) Addition of Al³⁺ and Sn²⁺ (100 μ M) to HMB (50 μ M) in 30% acetonitrile led to slight orange fluorescence solution, (c) Introduction of Sn²⁺ (50 μ M) to MEK (50 μ M) resulted in brighter orange fluorescence compared to Al³⁺ (50 μ M) in 30% acetonitrile solution, and (d) Detection of Cu²⁺ by THB is not fluorescent.



Figure 4.13: Fluorescence spectra of chemosensors in the presence of various cations at room temperature. (a) BVT in 50% acetonitrile solution, $\lambda_{ex} = 562 \text{ nm}$, $\lambda_{em} = 588 \text{ nm}$ (b) HMB in 30% acetonitrile solution, $\lambda_{ex} = 563 \text{ nm}$, $\lambda_{em} = 588 \text{ nm}$, and (c) MEK in 30% acetonitrile solution, $\lambda_{ex} = 563 \text{ nm}$, $\lambda_{em} = 588 \text{ nm}$.

4.5.2 Effects of pH

The stability of the chemosensors under various acidic and alkaline conditions were investigated over a range of pH values from 2 to 12, where pH 5.3 is the native pH of 30% acetonitrile solution (used for chemosensors **HMB**, **MEK**, and **THB**) and pH 7.2 is the native pH of 50% acetonitrile solution (used for chemosensor **BVT**). Changes in colour, absorption, fluorescence emission, and fluorescence intensity were recorded in the absence and presence of the respective target metal ion.

Figure 4.14a illustrated that all solutions (50% acetonitrile) containing **BVT** turned pink at pH 2.0, presumably due to the opened spirolactam ring under extreme acidic conditions (Mironenko et al., 2017). As the pH increases, **BVT** solution turns colourless while the solutions containing Al³⁺ and Sn²⁺ remained pink up to pH 8 (Figure 4.14b-e). However, only the solution of **BVT** containing Sn²⁺ remained pink colour at pH 10 (Figure 4.14f), but the dominant form of the closed spirolactam ring persisted at pH 12, as shown by the colourless solutions of the respective metals containing **BVT** (Figure

4.14g). Likewise, all the 30% acetonitrile solutions with **HMB** turned pink as well at pH 2.0 (Figure 4.15a). When the pH increased to pH 4.0, all the solutions were light pink except for Al^{3+} and Sn^{2+} , which displayed darker pink as illustrated in Figure 4.15b. From pH 5.3 – pH 6.0, both solutions comprising of **HMB** with Al^{3+} and Sn^{2+} retained its pink colour respectively (Figure 4.15c-d). Although the detection of Al^{3+} was not possible at pH 8.0, reflected by its colourless solution, recognition of Sn²⁺ with HMB was still achievable as demonstrated by its pink coloured solution (Figure 4.15e). Meanwhile, the identification of these metal ions were not viable at pH 10.0 and 12.0 as depicted by the solutions that remained colourless in Figure 4.15f and Figure 4.15g respectively. In the case of MEK, Figure 4.16a-b revealed that in presence of all thirteen metal cations, the solutions containing MEK remained pink from pH 2.0 - pH 4.0. As the pH increases, the solutions remained colourless except for Al^{3+} and Sn^{2+} which turned pink at the native pH of 30% acetonitrile (pH 5.3), as shown in Figure 4.16c. Once it reaches pH 6.0, only MEK with Sn²⁺ was still detectable as illustrated by the only pink colour solution in Figure 4.16d. Eventually, no observable changes in colour was recorded from pH 8.0 pH 10.0 as shown in Figure 4.16e-g, such occurrence can be ascribed to the closed spirolactam ring of MEK in alkaline conditions (Chen et al., 2012). On the other hand, Figure 4.17a shows that solutions comprising of **THB** with the respective metal ions were light pink under extremely acidic conditions (pH 2.0). Although these lightly pink coloured solutions were still observable at pH 4.0 (Figure 4.17b), the introduction of Cu²⁺ ions were able to induce an intense pink coloured solution, discerning itself from the other metal cations. As the pH increases from pH 5.3 - pH 12, Figure 4.17c-g have desmonstrated the excellent selectivity of **THB** for Cu²⁺ ions as the solution retained its pink colour amongst other metal ions even at extremely alkaline conditions.

The absorption profile of **BVT** with Al^{3+} and Sn^{2+} respectively were recorded over a range of pH from 2.0 - 12.0 and the results are illustrated in Figure 4.18a. The ringopened spirolactam form of BVT recorded at pH 2.0 was supported by the strong absorption obtained, but BVT was found to be impervious with increasing pH as reflected by the minimal absorbance, indicating that the spirolactam ring is in the closed form (Shen et al., 2015). The introduction of Al³⁺ and Sn²⁺ resulted in significant difference in absorption from pH 4.0 - 10.0, signifying the ring opening process of **BVT** upon binding with these metals (Lee et al., 2007). However, the optimal pH detection range of Al³⁺ and Sn^{2+} ions is from pH 6.0 – 10.0 since interference from **BVT** was low in this region. Similarly, HMB displayed spirolactam ring opening behavior under acidic conditions (from pH 2.0 - 4.0) as revealed by the increase in absorption in Figure 4.18b, whereas the closed spirolacatam ring of HMB was dominant from pH 5.3 - 12.0 as shown by the unaffected absorption intensity. Subsequently, addition of Al³⁺ and Sn²⁺ into the solution containing **HMB** led to substantial absorption enhancement throughout pH 4.0 - 8.0, but the ideal pH range for the recognition of these metals is from pH 5.3 - 8.0 since absorption intensity from HMB alone is weak in this region. Likewise, the effect of pH on the absorbance of MEK was investigated as well. Results illustrated in Figure 4.18c shows that the absorption of MEK enhanced with decreasing pH from pH 5.3 to 2.0, such observation can be ascribed to the spirolactam ring opening process under acidic conditions. On the other hand, the absorbance of MEK was steady as it is not affected throughout pH 5.3 - 12.0, owing to the predominant closed spirolactam ring form. Meanwhile, MEK in presence of Al³⁺ and Sn²⁺ was capable of inducing strong absorption between pH 4.0 - 6.0, but the optimum pH region for the sensing of these metals is from pH 5.0 - 6.0, because absorption from MEK is weak in this range. Besides that, THB alone exhibited slight absorption at pH 2.0 - 4.0 but was relatively stable throughout pH 5.3 - 12.0 (Figure 4.18d). The solution of **THB** containing Cu²⁺ did not display any

noticeable absorption enhancements at pH 2.0, but remarkable enhancement in the absorption intensity was recorded from pH 4.0 onwards. Although, a small drop in absorbance was recorded as the basicity of the solutions increases (above pH 8.0), the retention of strong absorption intensity up to pH 12.0 reflects the high affinity of **THB** towards Cu^{2+} . Nonetheless, these findings are in line with the persistence of colour (colourless to pink) upon addition of Cu^{2+} ions from pH 4.0 – 12.0.

Fluorescence emission of the respective chemosensors with metal ions at various pH were observed under a UV light source to facilitate 'naked-eye' recognition of the target metal ions. Figure 4.19a showed that BVT in presence of all thirteen metal ions at pH 2.0 emitted bright orange fluorescence under UV lamp, indicating the spirolactam ring-open state in acidic conditions (Tan et al., 2015). Even though slight orange fluorescence was still observed at pH 4.0 (Figure 4.19b), the presence of Al^{3+} and Sn^{2+} managed to induce a much brighter orange fluorescence compared to the other cations. As the pH of the solution increases from pH 6.0 - 8.0, only Al³⁺ and Sn²⁺ maintained bright orange fluorescence while no noticeable fluorescence was observed from the other metal cations (Figure 4.19c-e). Interestingly, the solution of **BVT** with Sn²⁺ remained fluorescent up to pH 10.0 whereas **BVT** with Al³⁺ turned colourless as illustrated by Figure 4.19f, but when the pH of the solution reaches 12.0, all fluorescence emission were quenched (Figure 4.19g), presumably due to the closed spirolactam ring at extremely basic conditions (Tang et al., 2011b). Similarly, Figure 4.20a illustrated that all solutions containing HMB induced bright orange fluorescence at pH 2.0, owing to the opened spirolactam form. These solutions maintained relatively fluorescent at pH 4.0, but the solutions with Al³⁺ and Sn²⁺ were lightly distinguishable from the other metal cation solutions as shown in Figure 4.20b. When the pH increases from 5.3 - 6.0, the respective solutions of HMB with Al^{3+} and Sn^{2+} displayed weaker fluorescence in accordance with the lighter pink

colour solution obtained, while the latter exhibited significant difference in colour (bright orange fluorescence) when compared with the colourless solutions (Figure 4.20c-d). As the basicity of the solution increases, only Sn²⁺ was lightly fluorescent at pH 8.0 (Figure 4.20e), and the solution eventually becomes colourless from pH 10.0-12.0 (Figure 4.20fg), indicating the inactive spirolactam ring at basic conditions. In the case of MEK, the solutions were brightly fluorescent from pH 2.0 onwards (Figure 4.21a) and the presence of Al^{3+} and Sn^{2+} were hardly discernible from one another even at pH 4.0 (Figure 4.21b). Subsequently, Figure 4.21c reveals the significant difference in fluorescence emission of **MEK** upon binding with Al^{3+} and Sn^{2+} when the pH reaches 5.3 (native value of 30%) acetonitrile). Meanwhile, the recognition of these metals can be distinguished at pH 6.0 since the only solution capable of inducing bright orange fluorescence is MEK with Sn²⁺ among other metals as demonstrated in Figure 4.21d. However, all fluorescence emission were quenched from pH 8.0 – 12.0 as reflected by the colourless solution in Figure 4.21eg, ascribed to the predominant spirolactam ring. On the other hand, fluorescence emission of **THB** in presence of Cu^{2+} that was quenched throughout pH 2.0 – 12.0 is attributed to the paramagnetic properties of the Cu^{2+} ions' d₉ system (Wang et al., 2014b).

Corresponding to UV-vis spectroscopy, the fluorescence characteristics of the chemosensors at various pH conditions were studied with fluorescence spectroscopy as well. For instance, Figure 4.22a illustrated that the fluorescence emitted at pH 2.0 (λ_{em} = 588 nm) by **BVT** with and without Al³⁺ and Sn²⁺ were equally strong, owing to the opened rhodamine spirolactam ring in acidic environment (Zhang et al., 2011a). As the pH increases, the fluorescence intensity of **BVT** alone decreases but the presence of Al³⁺ and Sn²⁺ induced remarkable fluorescence enhancement from pH 4.0 – 8.0. Although, the recognition of Sn²⁺ ions with **BVT** were detectable up to pH 10.0, as proven by the significant fluorescence intensity, all fluorescence intensities were quenched once it

reaches pH 12.0. Nevertheless, such results have revealed the ideal pH range for the detection of $A1^{3+}$ and Sn^{2+} ions, which is from pH 6.0 – 10.0 since interference from **BVT** was minimal in this region. Likewise, the fluorescence intensity of HMB increases with decreasing pH from 4.0 - 2.0, but the fluorescence emission of HMB becomes stable as the pH increases from 5.3 - 12.0 as shown in Figure 4.22b. The addition of Al^{3+} and Sn^{2+} into solutions containing HMB resulted in a spike in fluorescence intensity from pH 4.0 - 6.0, while only the solution with Sn²⁺ was capable of inducing fluorescence enhancement at pH 8.0. Even though no significant fluorescence changes were observed from pH 10.0 – 12.0, the optimum pH for the recognition of Al^{3+} and Sn^{2+} is from pH 5.3 - 8.0 since fluorescence emission from HMB is weak in this range. Similarly, Figure 4.22c demonstrated the proton induced spirolactam ring opening of MEK in acidic conditions, as reflected by the enhanced fluorescence intensity from pH 2.0 - 4.0. On the other hand, the predominant closed spirolactam ring was present from pH 5.3 - 12.0, indicated by the low fluorescence intensity recorded. Subsequently, the introduction of Al^{3+} and Sn^{2+} amplified the fluorescence emission from pH 4.0 – 6.0, but the optimal pH range for the detection of these metals is from pH 5.3 - 6.0, since fluorescence emission from MEK alone is weak in this region. Conversely, no fluorescence emission spectra were recorded for **THB**, attributed to the fluorescence quenching properties of Cu^{2+} upon binding with THB.

To summarize, the differentiation between the detection of Al^{3+} and Sn^{2+} could possibly be done so at pH 10.0, 8.0, and 6.0 with chemosensors **BVT**, **HMB**, and **MEK**, respectively. Mainly due to the retention of the pink colour and bright orange fluorescence solution under UV lamp by these sensors with Sn^{2+} ions, which allows naked eye detection at the aforementioned pH values. Although **THB** was not fluorescent, **THB** demonstrated excellent stability and selectivity towards Cu^{2+} ions over a wide pH range as reflected by the pink-coloured solution and strong absorption intensity from pH 4.0 – 12.0. Such observations are promising since numerous sensors have been reported to exist in the closed spirolactam ring form at basic conditions (Yan et al., 2012). Nevertheless, the effect of pH did not influence the fluorescence measurements and these chemosensors (**BVT**, **HMB**, and **MEK**) could be used concurrently to detect Al^{3+} and Sn^{2+} ions in both acidic and basic conditions, while the versatility of **THB** in a broad pH range could be used for the recognition of Cu²⁺ ions.

(a)

$$Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$$

(b)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$
(c)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$
(d)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$
(e)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$
(f)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$
(g)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$

Figure 4.14: Colour changes of BVT upon addition of various metal ions at different pH values in 50% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 6.0, (d) pH 7.2, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.

(a)

$$Ag^{+} AI^{3+} Ca^{2+}Cd^{2+}Co^{2+} Cu^{2+}La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$$

(b)
 $Ag^{+} AI^{3+} Ca^{2+}Cd^{2+}Co^{2+} Cu^{2+}La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$
(c)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+}La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$
(d)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$
(e)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$
(f)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$
(g)
 $Ag^{+} AI^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$

Figure 4.15: Colour changes of HMB upon addition of various metal ions at different pH values in 30% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.

Figure 4.16: Colour changes of MEK upon addition of various metal ions at different pH values in 30% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.

Figure 4.17: Colour changes of THB upon addition of various metal ions at different pH values in 30% acetonitrile solution. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.



Figure 4.18: Effect of pH on the absorbance at (a) 562 nm of BVT (100 μ M), BVT + Al³⁺ (300 μ M), and BVT + Sn²⁺ (300 μ M), (b) 563 nm of HMB (50 μ M), HMB + Al³⁺ (100 μ M), and HMB + Sn²⁺ (100 μ M), (c) 563 nm of MEK (50 μ M), MEK + Al³⁺ (50 μ M), and MEK + Sn²⁺ (50 μ M), and (d) 559 nm of THB (10 μ M) and THB + Cu²⁺ (10 μ M).



Figure 4.19: Colour changes of BVT upon addition of various metal ions at different pH values in 50% acetonitrile solution under a UV lamp. (a) pH 2.0, (b) pH 4.0, (c) pH 6.0, (d) pH 7.2, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.


Figure 4.20: Colour changes of HMB upon addition of various metal ions at different pH values in 30% acetonitrile solution under a UV lamp. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.



Figure 4.21: Colour changes of MEK upon addition of various metal ions at different pH values in 30% acetonitrile solution under a UV lamp. (a) pH 2.0, (b) pH 4.0, (c) pH 5.3, (d) pH 6.0, (e) pH 8.0, (f) pH 10.0, and (g) pH 12.0.



Figure 4.22: Effect of pH on the fluorescence intensity at 588 nm of (a) BVT (100 μ M), BVT + Al³⁺ (300 μ M), and BVT + Sn²⁺ (300 μ M), (b) HMB (50 μ M), HMB + Al³⁺ (100 μ M), and HMB + Sn²⁺ (100 μ M), and (c) MEK (50 μ M), MEK + Al³⁺ (50 μ M), and MEK + Sn²⁺ (50 μ M).

4.5.3 Sensitivity of the sensors as probe

The sensitivity of chemosensors **BVT**, **HMB**, and **MEK** towards Al^{3+} and Sn^{2+} , as well as **THB** towards Cu^{2+} were evaluated with increasing concentration of the target metal ions to the respective chemosensor solution. Any changes in successive addition of metal ions were recorded with UV-vis and fluorescence spectroscopy.

The free sensor **BVT** exhibited no distinguishable absorption band at 562 nm as illustrated in Figure 4.23a-b. However, the absorption spectra of **BVT** upon gradual addition of Al^{3+} increased significantly and the absorbance achieved a maximum at 600 μ M of Al^{3+} . Meanwhile, the addition of Sn^{2+} raised the absorbance significantly as the absorption intensified regularly and reached a maximum at 360 μ M of Sn^{2+} (Figure 4.23b). Likewise, in the case of **HMB**, Figure 4.23c-d illustrated no observable absorption band by the free **HMB** at 563 nm, whilst subsequent addition of Al^{3+} and Sn^{2+} led to a consistent absorption enhancement and eventually achieved maximum absorption at 175 μ M of Al^{3+} (Figure 4.23c) and 150 μ M of Sn^{2+} (Figure 4.23d). Similarly, prior to the

addition of Al^{3+} and Sn^{2+} , the free **MEK** displayed no significant absorption band at 563 nm. However, the absorption spectra of **MEK** with Al^{3+} and Sn^{2+} progressively increased and attained maximum intensity at 130 µM of Al^{3+} (Figure 4.23e) and 60 µM of Sn^{2+} (Figure 4.23f) respectively. Besides that, similar observations were obtained for **THB** as reflected in Figure 4.23g, where the free **THB** exhibited no obvious absorption band at 559 nm but the gradual addition of Cu^{2+} into the solution of **THB** have resulted in significant increase in absorption as the cumulative concentration of Cu^{2+} reaches 30 µM, further addition of Cu^{2+} did not lead to any noticeable absorption enhancement as the sensor has reached its capacity for accommodating more copper ions.

Furthermore, results obtained through fluorescence spectroscopy were comparable to that of UV-vis spectroscopy. For instance, **BVT** showed no observable emission band at 588 nm as depicted in Figure 4.24a-b, but the presence of Al^{3+} and Sn^{2+} led to progressive fluorescence intensity enhancement in their respective emission spectra and eventually reached a maximum as the concentration increases from $0 - 600 \mu M$ for A1³⁺ (Figure 4.24a) and $0 - 480 \mu$ M for Sn²⁺ (Figure 4.24b). Similarly, Figure 4.24c-d demonstrated that the free sensor HMB did not exhibit any fluorescence emission band at 588 nm. However, the gradual addition of $Al^{3+}(0 - 250 \mu M)$ and $Sn^{2+}(0 - 125 \mu M)$ resulted in consistent increase in the fluorescence intensity as reflected in the emission spectra in Figure 4.24c and Figure 4.24d respectively, further addition of these metals did not cause any significant changes. Likewise, MEK shown no noticeable emission band at 588 nm as illustrated in Figure 4.24e-f, successive introduction of Al³⁺ and Sn²⁺ led to a consistent emission enhancement and achieved maximum fluorescence intensity at 120 µM of Al³⁺ (Figure 4.24e) and 60 μ M of Sn²⁺ (Figure 4.24f) respectively. On the other hand, no fluorescence emission spectra were recorded upon progressive addition of Cu²⁺ to THB since no fluorescence properties were observed to begin with.

The absorption and fluorescence intensity of the chemosensors upon addition of the respective target metal ion that is required to reach saturation are tabulated in Table 4.7. The results obtained through UV-vis and fluorescence spectroscopy have demonstrated that the continuous introduction of the target metal ion is accompanied with simultaneous colour changes from colourless (closed-ring) to pink (open-ring), thereby indicating the spirolactam ring opening process (Mahato et al., 2014). Interestingly, chemosensors **BVT**, **HMB**, and **MEK** shown greater sensitivity towards Sn²⁺ ions compared to that of Al³⁺ ions, evidenced by the lesser amount of Sn²⁺ needed to obtain similar absorbance and fluorescence intensity value despite its lower saturation concentration.

Compound	[metal]	Absorbance	[metal]	Fluorescence
Compound	added (µM)	Absolutice	added (µM)	intensity (a.u.)
BVT	0	0.003	0	2.464
$BVT + Al^{3+}$	600	0.431	600	254.927
$BVT + Sn^{2+}$	360	0.482	480	255.268
HMB	0	0.004	0	0.616
$\mathbf{HMB} + \mathrm{A1}^{3+}$	175	0.673	250	142.677
HMB + Sn^{2+}	150	0.806	125	138.103
MEK	0	0.002	0	3.126
$MEK + Al^{3+}$	130	0.738	120	219.620
$MEK + Sn^{2+}$	60	0.613	60	233.710
ТНВ	0	0.014	-	-
THB + Cu^{2+}	30	0.935	-	-

 Table 4.7: Summarized total metal ion concentration added into chemosensors and

 their respective absorbance and fluorescence intensities.



Figure 4.23: Absorption spectra of (a) BVT (100 μ M) + Al³⁺ (0–600 μ M) and (b) BVT (100 μ M) + Sn²⁺ (0–360 μ M) in 50% acetonitrile solution, (c) HMB (50 μ M) + Al³⁺ (0–175 μ M) and (d) HMB (50 μ M) + Sn²⁺ (0–150 μ M) in 30% acetonitrile solution, (e) MEK (50 μ M) + Al³⁺ (0–130 μ M) and (f) MEK (50 μ M) + Sn²⁺ (0–60 μ M) in 30% acetonitrile solution, and (g) THB (10 μ M) + Cu²⁺ (0–30 μ M) in 30% acetonitrile solution.



Figure 4.24: Fluorescence emission spectra of (a) BVT (100 μ M) + Al³⁺ (0–600 μ M) and (b) BVT (100 μ M) + Sn²⁺ (0–360 μ M) in 50% acetonitrile solution, (c) HMB (50 μ M) + Al³⁺ (0–175 μ M) and (d) HMB (50 μ M) + Sn²⁺ (0–150 μ M) in 30% acetonitrile solution, (e) MEK (50 μ M) + Al³⁺ (0–130 μ M) and (f) MEK (50 μ M) + Sn²⁺ (0–60 μ M) in 30% acetonitrile solution, and (g) THB (10 μ M) + Cu²⁺ (0–30 μ M) in 30% acetonitrile solution.

4.5.4 Limit of detection (LOD) of the sensors

The sensitivity of the chemosensors towards the target metal ions was further gauged with its respective detection limits, where this value is inversely proportional to its responsiveness and practicality (Wang et al., 2014a; Zhao et al., 2020b). Thus, this value is calculated based on the relationship defined by IUPAC, where $LOD = 3S_b/m$ ($S_b =$ standard deviation of the blank solution measured 10 times; m = slope of the calibration curve) (Mahapatra et al., 2013).

The limit of detection calculated for chemosensors BVT, HMB, MEK, and THB through absorption and fluorescence spectroscopy are tabulated in Table 4.8. According to the results obtained, chemosensor **MEK** displayed the lowest detection limit for Al^{3+} and Sn^{2+} , followed by HMB and BVT. Additionally, the detection limits of BVT towards Al^{3+} and Sn^{2+} through UV-vis spectroscopy was two times lower in contrast to fluorescence spectroscopy, indicating that BVT works better as a colorimetric chemosensor compared to a fluorometric chemosensor. Conversely, HMB and MEK both exhibited relatively better detection limits through fluorescence spectroscopy compared to UV-vis spectroscopy. The superior colorimetric properties of **BVT** in contrast to its fluorometric response is likely due to its weak fluorescent properties resulting from the substituent and solvent environment. Although BVT, HMB, and MEK revealed that their respective detection limits are higher than the WHO acceptable limit for Al³⁺ (7.41 μ M) and Sn²⁺ (0.02 μ M; based on a maximum value of 2 μ g/litre because no maximum concentration is set by WHO) in drinking water (Edition, 2011), with only **MEK** towards Al^{3+} that exhibited slightly lower detection limit (6.23 µM) through fluorescence spectroscopy, these chemosensors can still be used as an indicator when drinking water is above or approaching hazardous levels for consumption. In the case of **THB**, the limit of detection calculated for Cu^{2+} was 0.48 μ M, not only this value is much lower compared with earlier findings of polyhydroxy compounds with rhodamine B conjugates, for instance, $LOD = 3.42 \ \mu$ M with 2,4-dihydroxybenzaldehyde rhodamine B derivative (Xu et al., 2011), it is also well below the allowable WHO limit for Cu^{2+} in drinking water (31.5 μ M) (Edition, 2011), thus demonstrating its efficient identification of Cu^{2+} in low concentrations at various practical applications.

Furthermore, the limit of detection for several other literature findings are summarized in Table 4.9. For sensors that detect Al³⁺, there are sensors with comparable detection limit for Al^{3+} , ~50 µM (Kumar et al., 2015), while there are also detection limit as low as 4.28 nM (Jeong et al., 2015). In the case of Sn^{2+} detection, literature methods were found to be much lower than this research (chemosensors BVT, HMB, and MEK). Additionally, very few sensors were reported for simultaneous detection of Al^{3+} and Sn^{2+} , hence the slightly higher detection limit of BVT, HMB, and MEK does not entirely strip away its potential to discern between these two metals. It is also noteworthy that while the literature sensors demonstrated comparably low detection limit for Al³⁺ and Sn²⁺, their solvent system consist of at least 90% solvent, which is relatively volatile and would subsequently affect the absorption and/or fluorescence characteristics (Arbeloa & Rohatgi-Mukherjee, 1986; Nag & Goswami, 2009). Meanwhile, the chemosensors for Cu²⁺ detection tabulated below was found to have a higher detection limit than THB, thereby favoring **THB** to be selected for copper detection in low concentrations. Nonetheless, the sensors synthesized in this study (BVT, HMB, MEK, and THB) have its own distinctive properties that can be utilized simultaneously for detection of Al³⁺, Cu^{2+} , and Sn^{2+} .

Table 4.8: Summarized limit of detection (LOD) of chemosensors BVT, HMB, MEK, and THB with their respective absorption and fluorescence spectrum.

Compound	Absorption spectrum		Fluorescence spectrum	
Compound	LOD (µM)	Figure	LOD (µM)	Figure
$\mathbf{BVT} + \mathrm{Al}^{3+}$	48.05	4.25a	64.14	4.26a
$\mathbf{BVT} + \mathbf{Sn}^{2+}$	28.57	4.25b	43.47	4.26b
$\mathbf{HMB} + \mathrm{Al}^{3+}$	24.36	4.25c	17.81	4.26c
HMB + Sn ²⁺	15.63	4.25d	7.17	4.26d
$MEK + Al^{3+}$	8.96	4.25e	6.23	4.26e
$MEK + Sn^{2+}$	5.10	4.25f	5.05	4.26f
$THB + Cu^{2+}$	0.48	4.25g	-	-



Figure 4.25: Linearized absorbance changes of (a) BVT + Al³⁺, (b) BVT + Sn²⁺, (c) HMB + Al³⁺, (d) HMB + Sn²⁺, (e) MEK + Al³⁺, (f) MEK + Sn²⁺, and (g) THB + Cu²⁺.



Figure 4.26: Linearized fluorescence intensity changes of (a) BVT + Al³⁺, (b) BVT + Sn²⁺, (c) HMB + Al³⁺, (d) HMB + Sn²⁺, (e) MEK + Al³⁺, and (f) MEK + Sn²

Structure of chemosensor	Solvent system	Detection method	Metals detected	LOD	Reference
N HE	CH ₃ CN/H ₂ O (1:1, v/v)	UV-vis	Al ³⁺	50 µM	(Kumar et al., 2015)
OH HO NO ₂	MeOH/H ₂ O (9:1, v/v, pH 7.4)	Fluorescence	Al ³⁺	4.39 µM	(Roy et al., 2017)
OH N	MeOH/H ₂ O (9:1, v/v)	Fluorescence	Al ³⁺	4.32 μΜ	(Kumar et al., 2018)
	CH ₃ CN	Fluorescence	Al ³⁺	4.28 nM	(Jeong et al., 2015)

Table 4.9: LOD comparison among a selection of reported chemosensors for detection of Al³⁺, Cu²⁺, and Sn²⁺.

	17	idle 4.9, continueu.			
Structure of chemosensor	Solvent system	Detection method	Metals detected	LOD	Reference
	EtOH	Fluorescence	Sn ²⁺	2.27 μM	(Patil et al., 2017)
	CH ₃ CN/H ₂ O	UV-vis	Sn ²⁺	0.12 μM	(Yan et al., 2018b)
	(99:1, v/v, pH 7.0)				
HO OH	Acetone/H ₂ O	Fluorescence	Al^{3+}	63.1 nM	(Gul et al., 2020)
$ \begin{array}{c} \begin{pmatrix} n \\ l \\$	(1:1, v/v)		Sn ²⁺	64.8 nM	

Table 4.9, continued.

Table 4.9, continueu.					
Structure of chemosensor	Solvent system	Detection method	Metals detected	LOD	Reference
CI N OH	DMSO	UV-vis	Cu ²⁺	5.8 μM	(Anbu Durai & Ramu, 2020)
N HO	DMF/bis-tris buffer (7:3, v/v, pH 7.0)	UV-vis	Cu ²⁺	3.89 µM	(You et al., 2016)
	DMSO/Tris-HCl buffer (1:9, v/v, pH 7.0)	UV-vis	Cu ²⁺	3.42 µM	(Xu et al., 2011)

Table 4.9, continued.

4.5.5 Binding affinity (Ka) of the sensors

The complexation kinetics of the chemosensors with the respective metal ions through UV-vis and fluorescence spectroscopy were recorded as binding constant (K_a) via Benesi-Hildebrand plot (Benesi & Hildebrand, 1949):

$$\frac{1}{A-A_0} = \frac{1}{K_a \left(A_{max} - A_0\right)[M^+]} + \frac{1}{A_{max} - A_0}$$
(4.1)

$$\frac{1}{F - F_0} = \frac{1}{K_a (F_{max} - F_0)[M^+]} + \frac{1}{F_{max} - F_0}$$
(4.2)

Where $[M^+]$ is the concentration of target metal ion, A_0/F_0 is the absorbance/emission without the metal ion, A/F is the absorbance/emission at any given metal ion concentration, and A_{max}/F_{max} is the saturated absorbance/emission in presence of metal ion. By substituting the values obtained from the absorption and fluorescence titration assay, the binding constants calculated for chemosensors **BVT**, **HMB**, **MEK**, and **THB** through absorption and emission are tabulated in Table 4.10. The variations in binding constants could be due to different substituents and solvent environment, which in return affects the effective binding of the respective chemosensors towards the target metal ion (Fang et al., 2018).

Table 4.10: Summarized binding constants (K_a) of chemosensors BVT, HMB, MEK, and THB with their respective absorption and fluorescence spectrum.

Compound	Absorption spectrum		Fluorescence spectrum	
Compound	$K_{a}(M^{-1})$	Figure	$K_{a}(M^{-1})$	Figure
$BVT + Al^{3+}$	$1.40 \ge 10^4$	4.27a	6.13×10^2	4.28a
$BVT + Sn^{2+}$	1.36 x 10 ⁴	4.27b	1.97 x 10 ³	4.28b
$HMB + Al^{3+}$	1.43 x 10 ⁶	4.27c	$2.36 \ge 10^8$	4.28c
HMB + Sn ²⁺	$1.11 \ge 10^{11}$	4.27d	4.91 x 10 ⁹	4.28d
$MEK + Al^{3+}$	$3.58 \ge 10^{10}$	4.27e	1.82 x 10 ⁹	4.28e
$MEK + Sn^{2+}$	$2.72 \ge 10^6$	4.27f	2.08 x 10 ⁹	4.28f
THB + Cu^{2+}	$5.95 \ge 10^3$	4.27g	-	-



Figure 4.27: Benesi-Hildebrand plot on the absorption of (a) BVT + Al³⁺, (b) BVT + Sn²⁺, (c) HMB + Al³⁺, (d) HMB + Sn²⁺, (e) MEK + Al³⁺, (f) MEK + Sn²⁺, and (g) THB + Cu²⁺.



Figure 4.28: Benesi-Hildebrand plot on the fluorescence emission ($\lambda_{em} = 588$ nm) of (a) BVT + Al³⁺, (b) BVT + Sn²⁺, (c) HMB + Al³⁺, (d) HMB + Sn²⁺, (e) MEK + Al³⁺, and (f) MEK + Sn²⁺.

4.5.6 Selectivity of sensors in the presence of other metals

The ability of the synthesized chemosensors (**BVT**, **HMB**, **MEK**, and **THB**) to establish anti-interference properties among other interfering metal cations were investigated. The recognition of the chemosensors with the respective target metals in presence of other metal ions were studied with UV-vis and fluorescence spectroscopy by ensuring signal amplification was detected.

As depicted in Figure 4.29a-b, the absorption of **BVT** plotted at its λ_{max} (562 nm) in the presence of single metal ions showed no obvious absorption enhancement apart from Al^{3+} and Sn^{2+} ions (blue bars), while the addition of Al^{3+} (Figure 4.29a) and Sn^{2+} (Figure 4.29b) to the solutions containing other metal ions resulted in significant absorption enhancement (orange bars). Likewise, all interfering cations did not affect the detection of Al^{3+} and Sn^{2+} ions in the case of **HMB** as well as **MEK**, reflected by the significant absorption amplification upon the introduction of these metals in Figure 4.29c-d (HMB $\lambda_{\text{max}} = 563 \text{ nm}$) and Figure 4.29e-f (MEK $\lambda_{\text{max}} = 563 \text{ nm}$) respectively. Furthermore, the recognition of Cu²⁺ with **THB** was not interfered by the presence of competing metals as well (orange bars), proven by the enhanced absorption intensity in comparison to the absorption of single metals (blue bars) plotted at its λ_{max} (559 nm) as illustrated in Figure 4.29g. Although the presence of some interfering ions caused slightly reduced absorption and fluorescence, the resulting outcome is still much more significant than the absorption of the single metal ion only. Moreover, the presence of both Al^{3+} and Sn^{2+} in the same solution was successfully detected by the sensors BVT, HMB, and MEK as reflected by the multiplied absorption.

Similar observations were also recorded from the fluorescence emission data plotted at $\lambda_{max} = 588$ nm for **BVT** (Figure 4.30a-b), **HMB** (Figure 4.30c-d), and **MEK** (Figure 4.30e-f) respectively, where the blue bars represent the absence of competing metal ions and the orange bars represent the presence of competing metal ions upon addition of target metal ions (Al³⁺ and Sn²⁺). The fluorescence emission results obtained have also demonstrated the selective preference of these three chemosensors (**BVT**, **HMB**, and **MEK**) towards Al³⁺ and Sn²⁺ through the retainment of enhanced emission intensity despite presence of interfering metals.

Such outcomes are encouraging since earlier findings have reported that the maximum absorption/fluorescence intensity achieved was affected by the presence of various metal ions. This occurrence is attributable to the similar ionic radius of interfering ion with the target metal ion, which subsequently hinders the effective binding of the chemosensor when both species are present (Tang et al., 2020; Wechakorn et al., 2020). Hence, the results obtained for chemosensors **BVT**, **HMB**, **MEK**, and **THB** have cumulatively suggested that the metal ion induced spirolactam ring opening process is in preference of $Al^{3+}/Sn^{2+}/Cu^{2+}$ amidst the presence of other interfering metal cations.



Figure 4.29: UV–vis spectra changes for (a) BVT (100 μ M) containing 3 equivalent of various cations in presence of 3 equivalent of Al³⁺ plotted at $\lambda_{max} = 562$ nm, (b) BVT (100 μ M) containing 3 equivalent of various cations in presence of 3 equivalent of Sn²⁺ plotted at $\lambda_{max} = 562$ nm, (c) HMB (50 μ M) containing 2 equivalent of various cations in presence of 2 equivalent of Al³⁺ plotted at $\lambda_{max} = 563$ nm, (d) HMB (50 μ M) containing 2 equivalent of various cations in presence of 2 equivalent of Sn²⁺ plotted at $\lambda_{max} = 563$ nm, (e) MEK (50 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Al³⁺ plotted at $\lambda_{max} = 563$ nm, (f) MEK (50 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Sn²⁺ plotted at $\lambda_{max} =$ 563 nm, and (g) THB (10 μ M) containing 1 equivalent of various cations in presence of 1 equivalent of Cu²⁺ plotted at $\lambda_{max} = 559$ nm.



Figure 4.30: Fluorescence spectra changes plotted at $\lambda_{em} = 588$ nm for (a) BVT (100 µM) containing 3 equivalent of various cations in presence of 3 equivalent of Al³⁺, (b) BVT (100 µM) containing 3 equivalent of various cations in presence of 3 equivalent of Sn²⁺, (c) HMB (50 µM) containing 2 equivalent of various cations in presence of 2 equivalent of Al³⁺, (d) HMB (50 µM) containing 2 equivalent of various cations in presence of 2 equivalent of Sn²⁺, (e) MEK (50 µM) containing 1 equivalent of various cations in presence of 1 equivalent of Al³⁺, and (f) MEK (50 µM) containing 1 equivalent of Sn²⁺.

4.5.7 Reversibility of the metal bound sensors

The reproducibility of the chemosensors (**BVT**, **HMB**, **MEK**, and **THB**) from its coordination with the respective target metal ($Al^{3+}/Sn^{2+}/Cu^{2+}$) was investigated with UV-vis and fluorescence spectroscopy. Alternate addition of EDTA as the competing ligand and target metal to the respective solutions of chemosensors were recorded until the absorbance/fluorescence intensity decreased to half its original value. The respective spectrum and number of cycles that the chemosensors could be reproduced was tabulated in Table 4.11.

Upon introduction of EDTA to the sensor-metal complex, the pink/orange solution turns colourless with the absorption/fluorescence intensity quenched, which clearly indicates the regeneration of the free sensor (Ju et al., 2011). Subsequent addition of the respective target metals successfully recovered most of its absorption/fluorescent signals and turned the colourless solutions pink/orange again. This suggests the reversibility of rhodamine's spirolactam ring opening and closing mechanism in the coordination of sensor-metal complex and thus ruling out the likelihood of an irreversible chemical reaction (Nayab & Shkir, 2017; Yang et al., 2016). As the number of cycles increases, the absorption/fluorescence intensity decreases, possibly due to excess EDTA remained in the solution that obstructs the effective binding of the chemosensors. Although each chemosensor exhibited disparities in the reversibility cycles with their respective target metals, such differences in the number of reversible cycles are helpful in differentiating between the detection of these metal ions. Moreover, the data obtained for these four chemosensors shown to have achieve a higher number of reversible cycles compared with previously reported sensors, which further supports its application as a reusable portable device in various on-site application (Park et al., 2014; Yilmaz et al., 2020).

 Table 4.11: Summarized number of reversible cycles of chemosensors with its target

 metal ions through absorption and fluorescence changes.

Compound	Absorption	Absorption spectrum		e spectrum
Compound	Cycles	Figure	Cycles	Figure
$BVT + Al^{3+}$	4	4.31a	5	4.32a
$BVT + Sn^{2+}$	3	4.31b	3	4.32b
$HMB + A1^{3+}$	2	4.31c	4	4.32c
$\mathbf{HMB} + \mathbf{Sn}^{2+}$	2	4.31d	3	4.32d
$MEK + Al^{3+}$	4	4.31e	6	4.32e
$MEK + Sn^{2+}$	5	4.31f	6	4.32f
$THB + Cu^{2+}$	3	4.31g	-	-



Figure 4.31: Reversible cycle of (a) BVT + Al³⁺, (b) BVT + Sn²⁺, (c) HMB + Al³⁺, (d) HMB + Sn²⁺, (e) MEK + Al³⁺, (f) MEK + Sn²⁺, and (g) THB + Cu²⁺, with quencher EDTA by absorbance changes.



Figure 4.32: Reversible cycle of (a) $BVT + Al^{3+}$, (b) $BVT + Sn^{2+}$, (c) $HMB + Al^{3+}$, (d) $HMB + Sn^{2+}$, (e) $MEK + Al^{3+}$, and (f) $MEK + Sn^{2+}$, with quencher EDTA by fluorescence intensity changes.

4.6 Possible sensing mechanism of sensors

4.6.1 Binding stoichiometry of the sensors with respective metal ions

The stoichiometric interaction of the chemosensors with the respective target metal ions were established with Job's plot method, where the total concentration of chemosensor and metal were kept constant as the mole fraction varied from 0 to 1. Table 4.12 records the figures and mole fraction at which peak absorption occurs, subsequently indicating the binding stoichiometry. Results obtained have revealed that different substituent in the chemosensors resulted in different binding stoichiometry. For example, **BVT**, **HMB**, and **MEK** exhibited same preference towards the target metals (Al³⁺ and Sn²⁺), but its binding coordination is different for each metal. Meanwhile, **THB** demonstrated a 1:1 binding ratio with Cu²⁺, which is similar with previously reported rhodamine B conjugate with polyhydroxy compounds, possibly due to the nature of the polyhydroxy substituent and the placement of hydroxy (-OH) groups (Milindanuth & Pisitsak, 2018; Xu et al., 2011). Hence, such findings further support that different substituents with rhodamine based chemosensors will lead to different coordination with the target metal ion, mainly ascribable to the altered stereo-electronic environment derived from the substitued component (Mishra et al., 2020).

and THB with their respective target metal lons.						
Compound	Figure	Mole fraction [Sensor]/[Sensor]+[metal]	Binding stoichiometry (sensor:metal)			
$\mathbf{BVT} + \mathrm{Al}^{3+}$	4.33a	0.5	1:1			
$\mathbf{BVT} + \mathbf{Sn}^{2+}$	4.33b	0.5	1:1			
$\mathbf{HMB} + \mathrm{Al}^{3+}$	4.33c	0.3	1:2			

0.6

0.3

0.5

0.5

HMB + Sn²⁺

MEK + $A1^{3+}$

 $MEK + Sn^{2+}$

THB + Cu²⁺

4.33d

4.33e

4.33f

4.33g

Table 4.12: Summarized binding stoichiometry of chemosensors BVT, HMB, MEK, and THB with their respective target metal ions.

2:1

1:2

1:1

1:1



Figure 4.33: Job's plot of (a) BVT + Al³⁺, (b) BVT + Sn²⁺, (c) HMB + Al³⁺, (d) HMB + Sn²⁺, (e) MEK + Al³⁺, (f) MEK + Sn²⁺, and (g) THB + Cu²⁺.

4.6.2 Evaluation of sensor-metal complex interactions through FTIR, ¹H- and ¹³C-NMR spectra

In order to study the binding interaction between the chemosensors with the respective target metal ion, the role of **BVT**, **HMB**, **MEK**, and **THB** acting as a ligand that chelates and forms a coloured coordination compound with Al³⁺/Sn²⁺/Cu²⁺ was investigated. Apart from the Job's plot analysis that suggested their respective binding stoichiometry, the mechanism of these rhodamine-based sensor was further studied with FTIR, ¹H, and ¹³C NMR spectroscopy.

Selected FTIR peaks and the corresponding figures are tabulated in Table 4.13. The participation of the amide carbonyl group (C=O) of the respective chemosensors in the coordination with target metals can be suggested by the shifting of this characteristic FTIR peak from ~1690 cm⁻¹ (BVT, HMB, MEK) to ~1615 cm⁻¹ upon addition of Al³⁺ and to $\sim 1587 \text{ cm}^{-1}$ for Sn^{2+} . Although the characteristic amide carbonyl peak of **THB** is slightly lower (~1619 cm⁻¹), the introduction of Cu²⁺ have also shifted this peak to a lower frequency (~1586 cm⁻¹) (Kwon et al., 2005). Additionally, the chemosensors in absence and presence of the target metal showed noticeable difference in the respective ¹H NMR spectra. In particular, downfield shifting and broadening of the peaks upon the formation of sensor-metal complex was observed in the xanthene and benzene peaks region. The 1 H NMR spectra for BVT, HMB, and MEK with their metal complexes are illustrated in Figure 4.38, Figure 4.39, and Figure 4.40 respectively. Furthermore, the most resounding proof of the BVT, HMB, and MEK chemosensors' (Figure 4.41, Figure 4.42, and Figure 4.43 respectively) spirolactam ring opening upon binding with target metal ion was obtained from the corresponding ¹³C NMR spectra, where the distinctive tertiary carbon peak at ~ 66.3 ppm diminished drastically in the presence of Al^{3+} and Sn^{2+} (Patil & Das,

2019). However, no signals were recorded in the ¹H and ¹³C NMR spectra of THB, owing to the formation of paramagnetic Cu^{2+} complex (Song et al., 2020), but it can be inferred that the binding of **THB** with Cu^{2+} is through the carbonyl and hydroxy group's oxygen. subsequently triggering the spirolactam ring opening mechanism. Thus, these observations are in line with previously reported outcomes of similar chemosensors, where the chemosensor's most probable binding site is through carbonyl O, imine N, and bromide Br (BVT)/phenol O (HMB and THB). Additionally, the ESI-MS data further endorses this binding mechanism, as the peak due to the molecular-ion [MEK+H]⁺ was found at m/z 511.25 ($m/z_{calcd} = 511.29$), while the base peak of the metal complexes corresponding to $[MEK+2A1+H]^+$ ($m/z_{calcd} = 739.10$) and $[MEK+Sn-H]^+$ ($m/z_{calcd} = 739.10$) 630.20) was found at m/z 739.42 and 630.72 respectively, which correlates well with the results from Job's plot and collectively reflects the formation of 1:2 complex of MEK-Al³⁺ and 1:1 complex of **MEK**-Sn²⁺. In conjunction with the Job's plot analysis in Section 4.6.1, proposed structure of the respective sensor-metal coordinating mechanism summarized in Table 4.14 further corroborates the participation of rhodamine's amide carbonyl and the respective substituents in the recognition of $Al^{3+}/Sn^{2+}/Cu^{2+}$ (Gul et al., 2020; Yu et al., 2018).

Compound	C=O	C-O	Figure
BVT	1694	-	4.34a
$BVT + Al^{3+}$	-	1615	4.34b
$BVT + Sn^{2+}$	-	1587	4.34c
HMB	1695	-	4.35a
$HMB + A1^{3+}$	-	1612	4.35b
$HMB + Sn^{2+}$	-	1586	4.35c
MEK	1693	-	4.36a
$MEK + A1^{3+}$	-	1618	4.36b
$MEK + Sn^{2+}$	-	1588	4.36c
THB	1619	-	4.37a
THB + Cu^{2+}	-	1585	4.37b

Table 4.13: Selected infrared spectroscopic peak assignments of the chemosensorsBVT, HMB, MEK, and THB with its respective metal complexes.



Figure 4.34: Infrared spectra of (a) BVT, (b) BVT with Al³⁺, (c) BVT with Sn²⁺.



Figure 4.35: Infrared spectra of (a) HMB, (b) HMB with Al³⁺, (c) HMB with Sn²⁺.



Figure 4.36: Infrared spectra of (a) MEK, (b) MEK with Al³⁺, (c) MEK with Sn²⁺.



Figure 4.37: Infrared spectra of (a) THB, (b) THB with Cu²⁺.

Table 4.14: Proposed binding mechanism of the chemosensors with their respective

target metal ions.

Compound	Binding stoichiometry (sensor:metal)	Proposed binding structure
BVT + A1 ³⁺	1:1	N O A^{13+} O O Br Br O
BVT + Sn ²⁺	1:1	n n n n n n n n n n
HMB + Al ³⁺	1:2	CI CI CI CI CI CI CI OH
HMB + Sn ²⁺	2:1	$ \begin{array}{c} & & \\ & & $

Compound	Binding stoichiometry (sensor:metal)	Proposed binding structure
MEK + Al ³⁺	1:2	CI- CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNN CI- NNNNN CI- NNNNNNNNNN
MEK + Sn ²⁺	1:1	
THB + Cu ²⁺	1:1	HO N-N OCu ²⁺ H O H
<u> </u>		



Figure 4.38: ¹H NMR spectra (400 MHz, CDCl₃) of (a) BVT, (b) BVT + Al³⁺, and (c) BVT + Sn²⁺.



Figure 4.39: ¹H NMR spectra (400 MHz, CDCl₃) of (a) HMB, (b) HMB + Al³⁺, and (c) HMB + Sn²⁺.



Figure 4.40: ¹H NMR spectra (400 MHz, CDCl₃) of (a) MEK, (b) MEK + Al³⁺, and (c) MEK + Sn²⁺.



Figure 4.41: ¹³C NMR spectra (100 MHz, CDCl₃) of (a) BVT, (b) BVT + Al³⁺, and (c) BVT + Sn²⁺.



Figure 4.42: ¹³C NMR spectra (100 MHz, CDCl₃) of (a) HMB, (b) HMB + Al³⁺, and (c) HMB + Sn²⁺.



Figure 4.43: ¹³C NMR spectra (100 MHz, CDCl₃) of (a) MEK, (b) MEK + Al³⁺, and (c) MEK + Sn²⁺.
4.7 Feasible applications of rhodamine Schiff-base chemosensors

4.7.1 Application in 'naked-eye' identification of metal ions

The feasibility of the chemosensors (**BVT**, **HMB**, **MEK**, and **THB**) as an on-site testing kit that allows 'naked-eye' recognition of metal ions were investigated with filter papers. Sensing strips prepared from the respective chemosensor were dried prior to soaking them into different metal cations solutions (Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, La³⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Sn²⁺, and Zn²⁺) in distilled water. Any changes in colour observed by the 'naked-eye' were recorded while fluorescence properties were detected with the help of a UV lamp.

Results obtained have revealed that **BVT** (Figure 4.44a) and **HMB** (Figure 4.44b) turned pink in presence of Al^{3+} and Sn^{2+} , where the addition of Al^{3+} resulted in a darker pink colour and Sn^{2+} turned light pink among thirteen metals tested. On the contrary, the colourless test strips containing **MEK** (Figure 4.44c) selectively turned pink in the presence of Sn^{2+} , whereas test strips stained with **THB** (Figure 4.44d) became pink upon addition of Cu^{2+} . Apart from that, fluorescence emission under a UV light source was observed for chemosensors **BVT**, **HMB**, and **MEK**, thus revealing its consistency with the test strips that turned pink previously. Results illustrated in Figure 4.45a and Figure 4.45b have respectively demonstrated that **BVT** and **HMB** emits bright orange fluorescence for Sn^{2+} . Meanwhile, the test strip containing Sn^{2+} exhibited bright orange fluorescence along with Al^{3+} slightly fluorescence in the case of **MEK** (Figure 4.45c). However, no fluorescence response was recorded for **THB** (Figure 4.45d) in the presence of Cu^{2+} due to its paramagnetic effect (Yang et al., 2015).

Although multiple reported literatures have produced immobilised hydrogel (Ozay et al., 2014; Ozay & Ozay, 2013) and rhodamine based polymeric films (Kaewtong et al., 2011; Ma et al., 2010) for on-site recognition of metal ions, these approaches require the utilization of various chemical reactants and tedious sample preparation, which is deemed to be cost ineffective and inconvenient as the pursuit of a simple and rapid on-site testing kit is highly sought after. Conversely, the findings in this study are promising as an alternative method for on-site sensing without the requirement of expensive equipment and complex synthesis was successfully established. As shown by the results above, the test strips containing **BVT**, **HMB**, and **MEK** (along with a simple UV light source) could be used to differentiate between Al³⁺ and Sn²⁺ at different concentrations, whereas **THB** could be used to selectively detect Cu²⁺, and thus demonstrating its capability to be used as a compact and convenient on-site sensor that provides straightforward and immediate colour changes which allows 'naked-eye' detection.

(a)	
	$Ag^{+} Al^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} BVT$
(b)	
	$Ag^+ Al^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^+ Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} HMB$
(c)	
	$Ag^{+} Ai^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} MEK$
(d)	$Ag^{+} Al^{3+} Ca^{2+} Cd^{2+} Co^{2+} Cu^{2+} La^{3+} Mn^{2+} Na^{+} Ni^{2+} Pb^{2+} Sn^{2+} Zn^{2+} THB$

Figure 4.44: Test strips for the 'naked-eye' detection of different metal ions in 100% aqueous medium with fabricated chemosensors. (a) BVT (10 mM) + metals (100 mM), (b) HMB (10 mM) + metals (100 mM), (c) MEK (10 mM) + metals (10 mM), and (d) THB (0.1 mM) + metals (0.1 mM).



Figure 4.45: Test strips for the 'naked-eye' detection of different metal ions in 100% aqueous medium with fabricated chemosensors under UV lamp. (a) BVT (10 mM) + metals (100 mM), (b) HMB (10 mM) + metals (100 mM), (c) MEK (10 mM) + metals (10 mM), and (d) THB (0.1 mM) + metals (0.1mM).

4.7.2 Colorectal adenocarcinoma cells inhibitory properties of sensors

Aside from the 'off-on' sensing characteristics, rhodamine B Schiff base compounds as cancer inhibitory agents were also widely explored, mainly attributable to its mitochondria targeting properties combined with its potential as a theranostic agent (Liu et al., 2018; Sommerwerk et al., 2017; Wolfram et al., 2018). While nanomaterials-based theranostic agents are prevalent, rhodamine based theranostic agents provide more benefits in terms of ease of reproducibility and modification by synthesis (Yan et al., 2018a; Zhen et al., 2018; Zhu et al., 2014). Therefore, a preliminary study of the cancer cell growth inhibitory behavior of the chemosensors **BVT**, **HMB**, **MEK**, and **THB** was carried out and the results are summarized in Table 4.15.

As shown in Table 4.15, **BVT** and **HMB** displayed no cytotoxic effect towards both the cancerous (HT-29) and non-cancerous (CCD-18Co) colon cell line, thus rendering it futile as a theranostic agent. Conversely, **MEK** exhibited relatively good cytotoxicity against HT-29 cells as its IC₅₀ value is comparable with cisplatin, but **MEK** is two times more toxic towards the non-cancerous CCD-18Co cell line. Meanwhile, weak inhibition towards the colorectal adenocarcinoma cell line (HT-29) was demonstrated by **THB**, as evidenced by its IC₅₀ value that is roughly five times weaker than that of cisplatin. Moreover, **THB** is fairly non-cytotoxic towards the non-cancerous human colon cell line (CCD-18Co). Results have concluded that **BVT**, **HMB**, and **THB** may not be suitable candidates to serve as cancer inhibitory agents for colorectal cancer cells whereas **THB** could be further explored as a cell imaging agent since it is weakly cytotoxic and highly sensitive in the recognition of Cu^{2+} ions. Nevertheless, only **MEK** demonstrated the possibility to be used as a cytotoxic agent for colorectal cancer cells, but it needs to be used with caution since its potency against non-cancerous CCD-18Co cells are higher than cisplatin.

Compound	IC ₅₀ (μM)		
Compound	HT-29	CCD-18Co	
BVT	> 43.82	> 43.82	
HMB	> 50.79	> 50.79	
MEK	10.20 ± 0.63	22.58 ± 0.86	
THB	39.24 ± 1.59	> 50.62	
Cisplatin ^a	7.90 ± 0.80	55.60 ± 3.19	

Table 4.15: Cytotoxic activity (IC₅₀ values) of BVT, HMB, MEK, and THB against HT-29 and CCD-18Co cell lines.

^a Positive control. Values were expressed as mean \pm standard deviation (n = 3).

CHAPTER 5: CONCLUSION

5.1 Conclusions

In conclusion, four new rhodamine based chemosensors (**BVT**, **HMB**, **MEK**, and **THB**) were successfully synthesized for the detection of Al^{3+} , Sn^{2+} , and Cu^{2+} ions. Out of these chemosensors, **BVT**, **HMB**, and **MEK** were fluorescent and could recognize Al^{3+} and Sn^{2+} ions simultaneously, while chemosensor **THB** was capable of selectively detecting only Cu^{2+} ions amongst thirteen other metal cations through colour changes in lieu of fluorescence, owing to the paramagnetic nature of copper ions. The extensive spectroscopic studies carried out have provided an understanding on the mechanism of the rhodamine spirocyclic framework, where it changes from the predominant closed ring form (colourless), to the opened ring form upon coordination with metal ion (pink colour).

As the pursuit of a selective, specific, and sensitive chemosensor is highly sought after, the simultaneous detection of AI^{3+} and Sn^{2+} by the three chemosensors **BVT**, **HMB**, and **MEK** could be differentiated at pH 10.0, 8.0, and 6.0 respectively. Such method is supported by the retention of pink colour upon addition of Sn^{2+} at these pH values, which allows rapid and "naked-eye" recognition of Sn^{2+} ions. Nonetheless, the outcomes at different pH environment cumulatively suggest that the identification of AI^{3+} and Sn^{2+} ions in both acidic and basic environments can be carried out with these chemosensors (**BVT**, **HMB**, and **MEK**) in tandem. Meanwhile, the tremendous stability and selectivity of **THB** towards Cu^{2+} throughout pH 4.0 – 12.0 was demonstrated by the persistence of enhanced absorbance and pink-coloured solution, thus proving its versatility in a wide pH range and harsh conditions. Besides that, the respective chemosensors' superiority in terms of detection limits towards Al^{3+} and Sn^{2+} are in the following order: **BVT**, **HMB**, and **MEK**, with **MEK** being the most superior among the three for exhibiting the lowest detection limit for these metals. Even though their corresponding detection limits were found to be higher than the WHO acceptable limit for Al^{3+} and Sn^{2+} in drinking water, these chemosensors can still function as an indicator and provide warning when drinking water is above harmful levels for consumption. On the contrary, the detection limit of **THB** for Cu²⁺ is much below the WHO permissible limit, therefore supporting its effective recognition of Cu²⁺ ions in low concentrations.

The substitution of different aldehyde/ketone moiety have shown to affect the binding modes upon the introduction of different target metals. From the Job's plot analysis, it was found that **BVT**, **HMB**, **MEK**, and **THB** each have its own characteristic coordination stoichiometry with the respective target metal ion (Al³⁺, Sn²⁺, and Cu²⁺), such findings are also useful in differentiating the metal ions detected when field study is carried out. Regardless of the variations in binding stoichiometry, the coordination of the sensor-metal complex is reversible through the introduction of common quencher EDTA. Hence, the reproducibility of the chemosensors synthesized have ruled out the possibility of an irreversible reaction of the rhodamine spirolactam ring opening mechanism and proves its feasible application as a reusable portable device. Whilst these chemosensors show distinctive binding modes and reversible properties, the most probable binding site ultimately involves the amide carbonyl of rhodamine's spirolactam ring, evidenced by the shifting of the characteristic amide peak to a lower frequency upon the formation of sensor-metal complex in the FTIR and ¹H-NMR spectra. Moreover, the disappearance of the tertiary carbon peak in the ¹³C-NMR of the sensor-metal complex further corroborates

the ring-opened amide form, which results in colour change, enhanced absorbance, and fluorescence that enables "naked-eye" detection.

Additionally, the ease and prospect of these chemosensors for on-site application was effectively demonstrated *via* filter paper. The results obtained have displayed the chemosensors' excellent selectivity towards Al^{3+} (**BVT** and **HMB**), Cu^{2+} (**THB**), and Sn^{2+} (**MEK**) in 100% aqueous solution, as reflected by the test strips colour change from colourless to pink. Such observations conveniently allow rapid optical recognition of the target metal ion without the use of expensive equipment, and thus rendering the employment of these four chemosensors beneficial, economical, and practical in environmental analysis studies.

Although **BVT**, **HMB**, and **THB** displayed poor cancer inhibitory properties on colorectal adenocarcinoma cell line (HT-29), **MEK** showed comparable cytotoxicity with cisplatin against the growth of the cancerous colorectal adenocarcinoma HT-29 cells, but **MEK** needs to be used with caution since its potency against non-cancerous CCD-18Co cells are higher than that of cisplatin. Despite **BVT**, **HMB**, and **THB** not showing any inhibitory effects on HT-29 cell line, the fluorescence properties of these sensors (**BVT** and **HMB**) and weakly cytotoxic behavior (**THB**) could be useful in future studies to explore cancer inhibitory strength on other cell lines as well as its potential as cell imaging agent.

Nevertheless, these chemosensors successfully demonstrated real-time monitoring and "off-on" colorimetric changes that allows ease of "naked-eye" recognition of Al^{3+} , Sn^{2+} , and Cu^{2+} ions with fast response time. For instance, **BVT**, **HMB**, **MEK** showed pH-dependent dual-channel recognition of Al^{3+} and Sn^{2+} , while the detection of Cu^{2+} by **THB** was pH-independent. The outcomes in this study have essentially provided an inexpensive, simple, and facile alternative to detect these metals, such approach established have rendered these chemosensor promising for further research and development.

5.2 Recommendations for future work

The recommendations are as follows:

- 1. Multiple solvent combinations and introducing more metals to be tested could be explored to increase its selectivity, sensitivity, and specificity in order to further improve its application in metal detection.
- 2. While on-site testing kit was successfully established, its detected metal concentration could be lowered through adhering the sensors on different types of solid phases which are also cheap and simple to increase its practicality.
- 3. Cell imaging could be carried out to study the sensors' ability to detect metal contamination in living cells.
- 4. Computational studies can be applied to study the probability of formation and energy levels of the sensors with the target analyte.

REFERENCES

- Abdullah, A. R. (1995). Environmental pollution in Malaysia: trends and prospects. *TRAC Trends in Analytical Chemistry*, 14(5), 191-198.
- Abu-Dief, A. M., & Mohamed, I. M. (2015). A review on versatile applications of transition metal complexes incorporating Schiff bases. *Beni-suef university Journal of Basic and Applied Sciences*, 4(2), 119-133.
- Adamczyk, M., & Grote, J. (2001). Efficient fluorescein spirolactam and bis-spirolactam synthesis. *Synthetic Communications*, *31*(17), 2681-2690.
- Adhikari, S., Ghosh, A., Guria, S., & Sahana, A. (2016). A through bond energy transfer based ratiometric probe for fluorescent imaging of Sn 2+ ions in living cells. *RSC Advances*, 6(46), 39657-39662.
- Afroz, R., Masud, M. M., Akhtar, R., & Duasa, J. B. (2014). Water pollution: Challenges and future direction for water resource management policies in Malaysia. *Environment and Urbanization ASIA*, 5(1), 63-81.
- Afroz, R., & Rahman, A. (2017). Health impact of river water pollution in Malaysia. International Journal of Advanced and Applied Sciences, 4(5), 78-85.
- Ahmed, S., Bui, M.-P. N., & Abbas, A. (2016). based chemical and biological sensors: Engineering aspects. *Biosensors and Bioelectronics*, 77, 249-263.
- Akerman, M. P., & Chiazzari, V. A. (2014). An X-ray crystallographic and DFT study of the complementary hydrogen bonding of bidentate pyrrolide-imine Schiff base ligands. *Journal of Molecular Structure*, 1058, 22-30.
- Al-Fartusie, F. S., & Mohssan, S. N. (2017). Essential trace elements and their vital roles in human body. *Indian J Adv Chem Sci*, 5(3), 127-136.
- Alamgir, S., Rhaman, M. M., Basaran, I., Powell, D. R., & Hossain, M. A. (2020). Colorimetric and spectroscopic cobalt (II) sensing by a simple Schiff base. *Polyhedron*, 187, 114681.
- Álvarez, E., Fernández-Marcos, M., Monterroso, C., & Fernández-Sanjurjo, M. (2005). Application of aluminium toxicity indices to soils under various forest species. *Forest Ecology and Management, 211*(3), 227-239.

- Amde, M., Yin, Y., Zhang, D., & Liu, J. (2016). Methods and recent advances in speciation analysis of mercury chemical species in environmental samples: a review. *Chemical Speciation & Bioavailability*, 28(1-4), 51-65.
- Amiard, J., Amiard-Triquet, C., & Metayer, C. (1985). Experimental study of bioaccumulation, toxicity and regulation of some trace metals in various estuarine and coastal organisms. Paper presented at the Symposia Biologica Hungarica.
- Anbu Durai, W., & Ramu, A. (2020). Hydrazone Based Dual Responsive Colorimetric and Ratiometric Chemosensor for the Detection of Cu2+/F– Ions: DNA Tracking, Practical Performance in Environmental Samples and Tooth Paste. *Journal of fluorescence*, 30(2), 275-289.
- Arakawa, Y. (1997). Biological activity of tin and immunity. Sangyo eiseigaku zasshi= Journal of Occupational Health, 39(1), 1-20.
- Arbeloa, I. L., & Rohatgi-Mukherjee, K. K. (1986). Solvent effects on the photophysics of the molecular forms of rhodamine B. Internal conversion mechanism. *Chemical Physics Letters*, 129(6), 607-614.
- Azadbakht, R., Almasi, T., Keypour, H., & Rezaeivala, M. (2013). A new asymmetric Schiff base system as fluorescent chemosensor for Al3+ ion. *Inorganic Chemistry Communications*, 33, 63-67.
- Babel, S., & Kurniawan, T. A. (2003). Low-cost adsorbents for heavy metals uptake from contaminated water: a review. *Journal of Hazardous Materials*, 97(1-3), 219-243.
- Bae, S., & Tae, J. (2007). Rhodamine-hydroxamate-based fluorescent chemosensor for FeIII. *Tetrahedron Letters*, 48(31), 5389-5392.
- Bag, B., & Pal, A. (2011). Rhodamine-based probes for metal ion-induced chromo-/fluorogenic dual signaling and their selectivity towards Hg (II) ion. *Organic & Biomolecular Chemistry*, 9(12), 4467-4480.
- Bajema, E. A., Roberts, K. F., & Meade, T. J. (2019). Cobalt-Schiff base complexes: preclinical research and potential therapeutic uses. *Essential metals in medicine:* therapeutic use and toxicity of metal ions in the clinic, vol. 19 of metal ions in life sciences, 267-295.
- Baldi, F., Filippelli, M., & Olson, G. J. (1989). Biotransformation of mercury by bacteria isolated from a river collecting cinnabar mine waters. *Microbial Ecology*, 17(3), 263-274.

- Bandgar, B. P., Gawande, S. S., Bodade, R. G., Totre, J. V., & Khobragade, C. N. (2010). Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. *Bioorganic & Medicinal Chemistry*, 18(3), 1364-1370.
- Bandmann, O., Weiss, K. H., & Kaler, S. G. (2015). Wilson's disease and other neurological copper disorders. *The Lancet Neurology*, 14(1), 103-113.
- Bao, G., Wong, K. L., & Tanner, P. A. (2019). A reversible rhodamine B based pH probe with large pseudo-Stokes shift. *ChemPlusChem*, 84(7), 816-820.
- Bao, X., Cao, X., Nie, X., Jin, Y., & Zhou, B. (2014). RBAP, a rhodamine B-based derivative: Synthesis, crystal structure analysis, molecular simulation, and its application as a selective fluorescent chemical sensor for Sn2+. *Molecules*, 19(6), 7817-7831.
- Barabasz, W., Albinska, D., Jaskowska, M., & Lipiec, J. (2002). Ecotoxicology of aluminium. *Polish Journal of Environmental Studies*, 11(3), 199-204.
- Barcelo, J., & Poschenrieder, C. (2002). Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environmental and Experimental Botany*, 48(1), 75-92.
- Bargossi, C., Fiorini, M. C., Montalti, M., Prodi, L., & Zaccheroni, N. (2000). Recent developments in transition metal ion detection by luminescent chemosensors. *Coordination Chemistry Reviews*, 208(1), 17-32.
- Beer, P. D., & Smith, D. K. (1998). Tunable bis (ferrocenyl) receptors for the solutionphase electrochemical sensing of transition-metal cations. *Journal of the Chemical Society, Dalton Transactions*(3), 417-424.
- Belowich, M. E., & Stoddart, J. F. (2012). Dynamic imine chemistry. *Chemical Society Reviews*, *41*(6), 2003-2024.
- Benesi, H. A., & Hildebrand, J. (1949). A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. *Journal of the American Chemical Society*, 71(8), 2703-2707.
- Benoy, C. J., Hooper, P. A., & Schneider, R. (1971). The toxicity of tin in canned fruit juices and solid foods. *Food and Cosmetics Toxicology*, 9(5), 645-656.
- Berković, K., Pavić, M., Ciković, N., & Gačić, M. (1995). Corrosion of iron, tin and aluminium in fruit juices. *Acta Alimentaria*, 24(1), 31-38.

- Bernardi, D., Errante, D., Tirelli, U., Salvagno, L., Bianco, A., & Fentiman, I. S. (2006). Insight into the treatment of cancer in older patients: developments in the last decade. *Cancer Treatment Reviews*, 32(4), 277-288.
- Berthon, G. (2002). Aluminium speciation in relation to aluminium bioavailability, metabolism and toxicity. *Coordination Chemistry Reviews*, 228(2), 319-341.
- Best, Q. A., Xu, R., McCarroll, M. E., Wang, L., & Dyer, D. J. (2010). Design and investigation of a series of rhodamine-based fluorescent probes for optical measurements of pH. Organic Letters, 12(14), 3219-3221.
- Beyersmann, D., & Hartwig, A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Archives of Toxicology*, 82(8), 493-512.
- Birtalan, E., Rudat, B., Kölmel, D. K., Fritz, D., Vollrath, S. B., Schepers, U., & Bräse, S. (2011). Investigating rhodamine B-labeled peptoids: Scopes and limitations of its applications. *Peptide Science*, 96(5), 694-701.
- Blunden, S., & Wallace, T. (2003). Tin in canned food: a review and understanding of occurrence and effect. *Food and Chemical Toxicology*, *41*(12), 1651-1662.
- Boogaard, P. J., Boisset, M., Blunden, S., Davies, S., Ong, T. J., & Taverne, J.-P. (2003). Comparative assessment of gastrointestinal irritant potency in man of tin (II) chloride and tin migrated from packaging. *Food and Chemical Toxicology*, 41(12), 1663-1670.
- Borisova, N. E., Reshetova, M. D., & Ustynyuk, Y. A. (2007). Metal-free methods in the synthesis of macrocyclic Schiff bases. *Chemical Reviews*, 107(1), 46-79.
- Boyarskiy, V. P., Belov, V. N., Medda, R., Hein, B., Bossi, M., & Hell, S. W. (2008). Photostable, amino reactive and water-soluble fluorescent labels based on sulfonated rhodamine with a rigidized xanthene fragment. *Chemistry–A European Journal*, 14(6), 1784-1792.
- Brady, P. A., & Sanders, J. K. (1997). Thermodynamically-controlled cyclisation and interconversion of oligocholates: metal ion templated 'living'macrolactonisation. *Journal of the Chemical Society, Perkin Transactions 1*(21), 3237-3254.
- Brown, D. R., & Kozlowski, H. (2004). Biological inorganic and bioinorganic chemistry of neurodegeneration based on prion and Alzheimer diseases. *Dalton Transactions*(13), 1907-1917.
- Brulíkova, L., Voln, T., & Hlavac, J. (2020). Bis-Rhodamine B System as a Tin Detector or Molecular Electronics Device. *ACS Omega*, 5(16), 9324-9333.

- Bulcke, F., Dringen, R., & Scheiber, I. F. (2017). Neurotoxicity of copper. In *Neurotoxicity of Metals* (pp. 313-343): Springer.
- Cai, S., Lu, Y., He, S., Wei, F., Zhao, L., & Zeng, X. (2013). A highly sensitive and selective turn-on fluorescent chemosensor for palladium based on a phosphine– rhodamine conjugate. *Chemical Communications*, 49(8), 822-824.
- Cammann, K., Lemke, U., Rohen, A., Sander, J., Wilken, H., & Winter, B. (1991). Chemical sensors and biosensors—principles and applications. *Angewandte Chemie International Edition in English*, 30(5), 516-539.
- Cannata Andia, J. (1996). Aluminium toxicity: its relationship with bone and iron metabolism. *Nephrology Dialysis Transplantation*, 11(supp3), 69-73.
- Chang, T. L., & Cheung, H. C. (1992). Solvent effects on the photoisomerization rates of the zwitterionic and the cationic forms of rhodamine B in protic solvents. *The Journal of Physical Chemistry*, *96*(12), 4874-4878.
- Chen, H., Bao, X., Shu, H., Zhou, B., Ye, R., & Zhu, J. (2017). Synthesis and evaluation of a novel rhodamine B-based 'off-on'fluorescent chemosensor for the selective determination of Fe3+ ions. *Sensors and Actuators B: Chemical, 242*, 921-931.
- Chen, X., Pradhan, T., Wang, F., Kim, J. S., & Yoon, J. (2012). Fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives. *Chemical Reviews*, 112(3), 1910-1956.
- Cheng, J., Ma, X., Zhang, Y., Liu, J., Zhou, X., & Xiang, H. (2014). Optical chemosensors based on transmetalation of salen-based Schiff base complexes. *Inorganic Chemistry*, 53(6), 3210-3219.
- Chereddy, N. R., Suman, K., Korrapati, P. S., Thennarasu, S., & Mandal, A. B. (2012). Design and synthesis of rhodamine based chemosensors for the detection of Fe3+ ions. *Dyes and Pigments*, *95*(3), 606-613.
- Cho, C.-J., Lu, S.-T., Kuo, C.-C., Liang, F.-C., Chen, B.-Y., & Chu, C.-C. (2016). Pyrene or rhodamine derivative-modified surfaces of electrospun nanofibrous chemosensors for colorimetric and fluorescent determination of Cu2+, Hg2+, and pH. *Reactive and Functional Polymers*, *108*, 137-147.
- Choi, Y. W., Park, G. J., Na, Y. J., Jo, H. Y., Lee, S. A., You, G. R., & Kim, C. (2014). A single schiff base molecule for recognizing multiple metal ions: a fluorescence sensor for Zn (II) and Al (III) and colorimetric sensor for Fe (II) and Fe (III). *Sensors and Actuators B: Chemical, 194*, 343-352.

- Chowdhury, B., Karar, M., Paul, S., Joshi, M., Choudhury, A. R., & Biswas, B. (2018). Salen type ligand as a selective and sensitive nickel (II) ion chemosensor: a combined investigation with experimental and theoretical modelling. *Sensors and Actuators B: Chemical*, 276, 560-566.
- Cigala, R. M., Crea, F., De Stefano, C., Lando, G., Milea, D., & Sammartano, S. (2012). The inorganic speciation of tin (II) in aqueous solution. *Geochimica et Cosmochimica Acta*, 87, 1-20.
- Cima, F. (2011). Tin: environmental pollution and health effects. *Encyclopedia of Environmental Health*, 10, 351-359.
- Corbett, P. T., Leclaire, J., Vial, L., West, K. R., Wietor, J.-L., Sanders, J. K., & Otto, S. (2006). Dynamic combinatorial chemistry. *Chemical Reviews*, 106(9), 3652-3711.
- Cotruvo Jr, J. A., Aron, A. T., Ramos-Torres, K. M., & Chang, C. J. (2015). Synthetic fluorescent probes for studying copper in biological systems. *Chemical Society Reviews*, 44(13), 4400-4414.

CrysAlis, P. (2009). Oxford Diffraction Ltd. Yarnton, England.

- Cutler, C. P. (2018). Use of metals in our society. In Metal Allergy (pp. 3-16): Springer.
- Dahbi, S., Azzi, M., Saib, N., De la Guardia, M., Faure, R., & Durand, R. (2002). Removal of trivalent chromium from tannery waste waters using bone charcoal. *Analytical and Bioanalytical Chemistry*, 374(3), 540-546.
- Darbre, P. D. (2005). Aluminium, antiperspirants and breast cancer. *Journal of Inorganic Biochemistry*, 99(9), 1912-1919.
- De Bartolomeo, A., Poletti, L., Sanchini, G., Sebastiani, B., & Morozzi, G. (2004). Relationship among parameters of lake polluted sediments evaluated by multivariate statistical analysis. *Chemosphere*, 55(10), 1323-1329.
- De Silva, A. P., Fox, D. B., Moody, T. S., & Weir, S. M. (2001). The development of molecular fluorescent switches. *TRENDS in Biotechnology*, 19(1), 29-34.
- De Silva, A. P., Gunaratne, H. N., Gunnlaugsson, T., Huxley, A. J., McCoy, C. P., Rademacher, J. T., & Rice, T. E. (1997). Signaling recognition events with fluorescent sensors and switches. *Chemical Reviews*, 97(5), 1515-1566.

- De Silva, A. P., & Rupasinghe, R. D. (1985). A new class of fluorescent pH indicators based on photo-induced electron transfer. *Journal of the Chemical Society, Chemical Communications*(23), 1669-1670.
- Dehghan, G., & Khoshkam, Z. (2012). Tin (II)-quercetin complex: Synthesis, spectral characterisation and antioxidant activity. *Food Chemistry*, 131(2), 422-426.
- Delhaize, E., & Ryan, P. R. (1995). Aluminum toxicity and tolerance in plants. *Plant Physiology*, 107(2), 315.
- Descalzo, A. B., Martínez-Máñez, R., Radeglia, R., Rurack, K., & Soto, J. (2003). Coupling selectivity with sensitivity in an integrated chemosensor framework: design of a Hg2+-responsive probe, operating above 500 nm. *Journal of the American Chemical Society*, 125(12), 3418-3419.
- Dong, L., Zeng, X., Mu, L., Xue, S.-F., Tao, Z., & Zhang, J.-X. (2010). The synthesis of a rhodamine B schiff-base chemosensor and recognition properties for Fe3+ in neutral ethanol aqueous solution. *Sensors and Actuators B: Chemical*, 145(1), 433-437.
- Dong, Y., Li, J., Jiang, X., Song, F., Cheng, Y., & Zhu, C. (2011). Na+ triggered fluorescence sensors for Mg2+ detection based on a coumarin salen moiety. *Organic Letters*, 13(9), 2252-2255.
- Dujols, V., Ford, F., & Czarnik, A. W. (1997). A long-wavelength fluorescent chemodosimeter selective for Cu (II) ion in water. *Journal of the American Chemical Society*, 119(31), 7386-7387.
- Edition, F. (2011). Guidelines for drinking-water quality. WHO chronicle, 38(4), 104-108.
- Elmorsi, T. M., Aysha, T. S., Machalický, O., Mohamed, M. B. I., & Bedair, A. H. (2017). A dual functional colorimetric and fluorescence chemosensor based on benzo [f] fluorescein dye derivatives for copper ions and pH; kinetics and thermodynamic study. *Sensors and Actuators B: Chemical, 253*, 437-450.
- Erdemir, S., Kocyigit, O., & Malkondu, S. (2015). Detection of Hg2+ ion in aqueous media by new fluorometric and colorimetric sensor based on triazole–rhodamine. *Journal of Photochemistry and Photobiology A: Chemistry*, 309, 15-21.
- Fabbrizzi, L. (2020). Beauty in Chemistry: Making Artistic Molecules with Schiff Bases. *The Journal of Organic Chemistry*, 85(19), 12212-12226.

- Fan, L., Qin, J.-c., Li, T.-r., Wang, B.-d., & Yang, Z.-y. (2014). A novel rhodamine chromone-based "Off–On" chemosensor for the differential detection of Al (III) and Zn (II) in aqueous solutions. *Sensors and Actuators B: Chemical*, 203, 550-556.
- Fang, Y., Li, X., Li, J.-Y., Wang, G.-Y., Zhou, Y., Xu, N.-Z., ... Yao, C. (2018). Thiooxo-Rhodamine B hydrazone derivatives bearing bithiophene group as fluorescent chemosensors for detecting mercury (II) in aqueous media and living HeLa cells. *Sensors and Actuators B: Chemical*, 255, 1182-1190.
- Fanna, D. J., Lima, L. M., Craze, A. R., Trinchi, A., Wuhrer, R., Lindoy, L. F., ... Li, F. (2018). Ultrasensitive colorimetric and ratiometric detection of Cu2+: acid–base properties, complexation, and binding studies. ACS Omega, 3(9), 10471-10480.
- Fashola, M. O., Ngole-Jeme, V. M., & Babalola, O. O. (2016). Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. *International Journal of Environmental Research and Public Health*, 13(11), 1047.
- Fasman, G. D. (1996). Aluminum and Alzheimer's disease: model studies. *Coordination Chemistry Reviews*, 149, 125-165.
- Favaretto, L., Manoli, F., Zambianchi, M., Bocchi, L., Ventura, B., Manet, I., & Melucci, M. (2019). Immobilization of Perylene-3, 4, 9, 10-Tetracarboxylic Dianhydride on Hollow Polysulfone Fibers: Primary Amine Coupling and Fluorescence Reporting. *ChemPlusChem*, 84(9), 1299-1304.
- Festa, R. A., & Thiele, D. J. (2011). Copper: an essential metal in biology. *Current Biology*, 21(21), R877-R883.
- Flora, S., Mittal, M., & Mehta, A. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian Journal of Medical Research*, *128*(4), 501.
- Florea, A.-M., & Büsselberg, D. (2006). Occurrence, use and potential toxic effects of metals and metal compounds. *Biometals*, 19(4), 419-427.
- Fölling, J., Polyakova, S., Belov, V., Van Blaaderen, A., Bossi, M. L., & Hell, S. W. (2008). Synthesis and characterization of photoswitchable fluorescent silica nanoparticles. *Small*, 4(1), 134-142.
- Forzani, E. S., Zhang, H., Chen, W., & Tao, N. (2005). Detection of heavy metal ions in drinking water using a high-resolution differential surface plasmon resonance sensor. *Environmental Science & Technology*, 39(5), 1257-1262.

- Gale, P. A., & Quesada, R. (2006). Anion coordination and anion-templated assembly: Highlights from 2002 to 2004. *Coordination Chemistry Reviews*, 250(23-24), 3219-3244.
- Gao, L., Lu, H., Lin, H., Sun, X., Xu, J., Liu, D., & Li, Y. (2014). KOH direct activation for preparing activated carbon fiber from polyacrylonitrile-based pre-oxidized fiber. *Chemical Research in Chinese Universities*, 30(3), 441-446.
- Gao, Y., Shi, Z., Long, Z., Wu, P., Zheng, C., & Hou, X. (2012). Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry. *Microchemical Journal*, 103, 1-14.
- Gensemer, R. W., & Playle, R. C. (1999). The bioavailability and toxicity of aluminum in aquatic environments. *Critical Reviews in Environmental Science and Technology*, 29(4), 315-450.
- Gessner, T., & Mayer, U. (2000). Triarylmethane and diarylmethane dyes. Ullmann's Encyclopedia of Industrial Chemistry.
- Ghorai, A., Mondal, J., Saha, R., Bhattacharya, S., & Patra, G. K. (2016). A highly sensitive reversible fluorescent-colorimetric azino bis-Schiff base sensor for rapid detection of Pb 2+ in aqueous media. *Analytical Methods*, 8(9), 2032-2040.
- Goi, C. L. (2020). The river water quality before and during the Movement Control Order (MCO) in Malaysia. Case Studies in Chemical and Environmental Engineering, 2, 100027.
- Gondia, N., & Sharma, S. (2019). Comparative optical studies of naphthalene based Schiff base complexes for colour tunable application. *Materials Chemistry and Physics*, 224, 314-319.
- Greig, L. M., & Philp, D. (2001). Applying biological principles to the assembly and selection of synthetic superstructures. *Chemical Society Reviews*, 30(5), 287-302.
- Gul, A., Oguz, M., Kursunlu, A. N., & Yilmaz, M. (2020). A novel colorimetric/fluorometric dual-channel sensor based on phenolphthalein and Bodipy for Sn (II) and Al (III) ions in half-aqueous medium and its applications in bioimaging. *Dyes and Pigments, 176*, 108221.
- Güngör, S. A., Köse, M., Tümer, F., & Tümer, M. (2016). Photoluminescence, electrochemical, SOD activity and selective chemosensor properties of novel asymmetric porphyrin-Schiff base compounds. *Dyes and Pigments, 130*, 37-53.

- Gunnlaugsson, T., Glynn, M., Tocci, G. M., Kruger, P. E., & Pfeffer, F. M. (2006). Anion recognition and sensing in organic and aqueous media using luminescent and colorimetric sensors. *Coordination Chemistry Reviews*, 250(23-24), 3094-3117.
- Guo, N., Liu, H., Fu, Y., & Hu, J. (2020). Preparation of Fe2O3 nanoparticles doped with In2O3 and photocatalytic degradation property for rhodamine B. Optik, 201, 163537.
- Gupta, V. K., Mergu, N., & Kumawat, L. K. (2016). A new multifunctional rhodaminederived probe for colorimetric sensing of Cu (II) and Al (III) and fluorometric sensing of Fe (III) in aqueous media. Sensors and Actuators B: Chemical, 223, 101-113.
- Gupta, V. K., Singh, A. K., & Kumawat, L. K. (2014). Thiazole Schiff base turn-on fluorescent chemosensor for Al3+ ion. Sensors and Actuators B: Chemical, 195, 98-108.
- Hajrezaie, M., Paydar, M., Zorofchian Moghadamtousi, S., Hassandarvish, P., Gwaram, N. S., Zahedifard, M., . . . Ali, H. M. (2014). A Schiff Base-derived copper (II) complex is a potent inducer of apoptosis in colon cancer cells by activating the intrinsic pathway. *The Scientific World Journal*, 2014.
- Hamasaki, T., Sato, T., Nagase, H., & Kito, H. (1993). The mutagenicity of organotin compounds as environmental pollutants. *Mutation Research/Genetic Toxicology*, 300(3-4), 265-271.
- Han, T., Feng, X., Tong, B., Shi, J., Chen, L., Zhi, J., & Dong, Y. (2012). A novel "turnon" fluorescent chemosensor for the selective detection of Al3+ based on aggregation-induced emission. *Chemical Communications*, 48(3), 416-418.
- Hariharan, P., & Anthony, S. P. (2014). Selective turn-on fluorescence for Zn2+ and Zn2++ Cd2+ metal ions by single Schiff base chemosensor. *Analytica Chimica Acta*, 848, 74-79.
- Harvey, L. J., & McArdle, H. J. (2008). Biomarkers of copper status: a brief update. *British Journal of Nutrition, 99*(S3), S10-S13.
- Hazra, S., Bodhak, C., Chowdhury, S., Sanyal, D., Mandal, S., Chattopadhyay, K., & Pramanik, A. (2019). A novel tryptamine-appended rhodamine-based chemosensor for selective detection of Hg 2+ present in aqueous medium and its biological applications. *Analytical and Bioanalytical Chemistry*, 411(6), 1143-1157.

- He, Y., Liao, L., Xu, C., Li, S., Wu, R., & Yang, Y. (2015). Resonance light scattering study on the formation of a manganese (II) coordination supramolecular polymer and its analytical application. *Spectroscopy Letters*, 48(8), 616-621.
- He, Z. L., Yang, X. E., & Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. *Journal of Trace elements in Medicine and Biology*, 19(2-3), 125-140.
- Hinckley, D. A., Seybold, P. G., & Borris, D. P. (1986). Solvatochromism and thermochromism of rhodamine solutions. *Spectrochimica Acta Part A: Molecular Spectroscopy*, 42(6), 747-754.
- Hu, J.-H., Long, C., Fu, Q.-Q., Ni, P.-W., & Yin, Z.-Y. (2019). A novel highly selective colorimetric and "turn-on" fluorimetric chemosensor for detecting Hg2+ based on Rhodamine B hydrazide derivatives in aqueous media. *Journal of Photochemistry* and Photobiology A: Chemistry, 379, 105-111.
- Huang, W., Wu, D., Wu, G., & Wang, Z. (2012). Dual functional rhodamine-immobilized silica toward sensing and extracting mercury ions in natural water samples. *Dalton Transactions*, 41(9), 2620-2625.
- Hulanicki, A., Glab, S., & Ingman, F. (1991). Chemical sensors: definitions and classification. *Pure and Applied Chemistry*, 63(9), 1247-1250.
- Huo, F.-J., Su, J., Sun, Y.-Q., Yin, C.-X., Tong, H.-B., & Nie, Z.-X. (2010). A rhodaminebased dual chemosensor for the visual detection of copper and the ratiometric fluorescent detection of vanadium. *Dyes and Pigments*, 86(1), 50-55.
- Huston, M. E., Akkaya, E. U., & Czarnik, A. W. (1989). Chelation enhanced fluorescence detection of non-metal ions. *Journal of the American Chemical Society*, 111(23), 8735-8737.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2), 60-72.
- Jeong, J. W., Rao, B. A., & Son, Y.-A. (2015). Rhodamine-chloronicotinaldehyde-based "OFF–ON" chemosensor for the colorimetric and fluorescent determination of Al3+ ions. *Sensors and Actuators B: Chemical, 208*, 75-84.
- Jiang, J., Gou, C., Luo, J., Yi, C., & Liu, X. (2012). A novel highly selective colorimetric sensor for Ni (II) ion using coumarin derivatives. *Inorganic Chemistry Communications*, 15, 12-15.

- Jiang, L., Wang, L., Zhang, B., Yin, G., & Wang, R. Y. (2010). Cell compatible fluorescent chemosensor for Hg2+ with high sensitivity and selectivity based on the Rhodamine fluorophore. In: Wiley Online Library.
- Jiao, Y., Zhou, L., He, H., Yin, J., Gao, Q., Wei, J., . . . Peng, X. (2018). A novel rhodamine B-based "off-on" fluorescent sensor for selective recognition of copper (II) ions. *Talanta*, 184, 143-148.
- Jin, Y., Zhu, Y., & Zhang, W. (2013). Development of organic porous materials through Schiff-base chemistry. *CrystEngComm*, 15(8), 1484-1499.
- Ju, H., Lee, M. H., Kim, J., Kim, J. S., & Kim, J. (2011). Rhodamine-based chemosensing monolayers on glass as a facile fluorescent "turn-on" sensing film for selective detection of Pb2+. *Talanta*, 83(5), 1359-1363.
- Kabir, E., Ray, S., Kim, K.-H., Yoon, H.-O., Jeon, E.-C., Kim, Y. S., . . . Brown, R. J. (2012). Current status of trace metal pollution in soils affected by industrial activities. *The Scientific World Journal*, 2012.
- Kaewtong, C., Wanno, B., Uppa, Y., Morakot, N., Pulpoka, B., & Tuntulani, T. (2011). Facile synthesis of rhodamine-based highly sensitive and fast responsive colorimetric and off-on fluorescent reversible chemosensors for Hg 2+: Preparation of a fluorescent thin film sensor. *Dalton Transactions*, 40(46), 12578-12583.
- Kai, Y., Yang, S., Gao, X., & Hu, Y. (2014). Colorimtric and "turn-on" fluorescent for Hg2+ based on rhodamine-3, 4-ethylenedioxythiophene derivative. Sensors and Actuators B: Chemical, 202, 252-256.
- Kan, C., Song, F., Shao, X., Wu, L., & Zhu, J. (2020a). Fe (III) induced fluorescent probe based on triamine and rhodamine derivatives and its applications in biological imaging. *Journal of Photochemistry and Photobiology A: Chemistry*, 390, 112306.
- Kan, C., Wu, L., Shao, X., Wang, X., Zhang, Y., Zhu, J., & Qiu, S. (2020b). A new reversible fluorescent chemosensor based on rhodamine for rapid detection of Al (III) in natural environmental water samples and living organisms. *Tetrahedron Letters*, 61(42), 152407.
- Karachi, N., Azadi, O., Razavi, R., Tahvili, A., & Parsaee, Z. (2018). Combinatorial experimental and DFT theoretical evaluation of a nano novel thiodicarboxaldehyde based Schiff base supported on a thin polymer film as a chemosensor for Pb2+ detection. *Journal of Photochemistry and Photobiology A: Chemistry, 360*, 152-165.

- Kataria, R., Sethuraman, K., Vashisht, D., Vashisht, A., Mehta, S. K., & Gupta, A. (2019). Colorimetric detection of mercury ions based on anti-aggregation of gold nanoparticles using 3, 5-dimethyl-1-thiocarboxamidepyrazole. *Microchemical Journal*, 148, 299-305.
- Kaur, B., Srivastava, R., & Satpati, B. (2015). Ultratrace detection of toxic heavy metal ions found in water bodies using hydroxyapatite supported nanocrystalline ZSM-5 modified electrodes. *New Journal of Chemistry*, 39(7), 5137-5149.
- Kaur, P., Kaur, N., Kaur, M., Dhuna, V., Singh, J., & Singh, K. (2014). 'Turnon'coordination based detection of Pd 2+ and bioimaging applications. RSC Advances, 4(31), 16104-16108.
- Kenmoku, S., Urano, Y., Kojima, H., & Nagano, T. (2007). Development of a highly specific rhodamine-based fluorescence probe for hypochlorous acid and its application to real-time imaging of phagocytosis. *Journal of the American Chemical Society*, 129(23), 7313-7318.
- Kilic, H., & Bozkurt, E. (2018). A rhodamine-based novel turn on trivalent ions sensor. Journal of Photochemistry and Photobiology A: Chemistry, 363, 23-30.
- Kim, H.-S., Angupillai, S., & Son, Y.-A. (2016). A dual chemosensor for both Cu2+ and Al3+: a potential Cu2+ and Al3+ switched YES logic function with an INHIBIT logic gate and a novel solid sensor for detection and extraction of Al3+ ions from aqueous solution. *Sensors and Actuators B: Chemical*, 222, 447-458.
- Kim, H., Rao, B. A., Jeong, J. W., Mallick, S., Kang, S.-M., Choi, J. S., . . . Son, Y.-A. (2015a). A highly selective dual-channel Cu2+ and Al3+ chemodosimeter in aqueous systems: sensing in living cells and microfluidic flows. *Sensors and Actuators B: Chemical, 210*, 173-182.
- Kim, H. N., Guo, Z., Zhu, W., Yoon, J., & Tian, H. (2011). Recent progress on polymerbased fluorescent and colorimetric chemosensors. *Chemical Society Reviews*, 40(1), 79-93.
- Kim, H. N., Lee, M. H., Kim, H. J., Kim, J. S., & Yoon, J. (2008a). A new trend in rhodamine-based chemosensors: application of spirolactam ring-opening to sensing ions. *Chemical Society Reviews*, 37(8), 1465-1472.
- Kim, H. Y., Lee, H. J., & Chang, S.-K. (2015b). Reaction-based colorimetric signaling of Cu2+ ions by oxidative coupling of phenols with 4-aminoantipyrine. *Talanta*, 132, 625-629.

- Kim, K.-N., Choi, M.-G., Noh, J.-H., Ahn, S.-D., & Chang, S.-K. (2008b). Rhodamine B hydrazide revisited: chemodosimetric Hg 2+-selective signaling behavior in aqueous environments. *Bulletin of the Korean Chemical Society*, 29(3), 571-574.
- Kim, Y. R., Kim, H. J., Kim, J. S., & Kim, H. (2008c). Rhodamine-Based "Turn-On" Fluorescent Chemodosimeter for Cu (II) on Ultrathin Platinum Films as Molecular Switches. *Advanced Materials*, 20(23), 4428-4432.
- Ko, S.-K., Yang, Y.-K., Tae, J., & Shin, I. (2006). In vivo monitoring of mercury ions using a rhodamine-based molecular probe. *Journal of the American Chemical Society*, 128(43), 14150-14155.
- Kodama, H., & Fujisawa, C. (2009). Copper metabolism and inherited coppertransport disorders: molecular mechanisms, screening, and treatment. *Metallomics*, 1(1), 42-52.
- Koide, Y., Kawaguchi, M., Urano, Y., Hanaoka, K., Komatsu, T., Abo, M., . . . Nagano, T. (2012). A reversible near-infrared fluorescence probe for reactive oxygen species based on Te-rhodamine. *Chemical Communications*, 48(25), 3091-3093.
- Kolcu, F., Erdener, D., & Kaya, İ. (2021). Synthesis and characterization of a highly selective turn-on fluorescent chemosensor for Sn2+ derived from diimine Schiff base. *Synthetic Metals*, *272*, 116668.
- Krämer, R. (1998). Fluorescent chemosensors for Cu2+ ions: fast, selective, and highly sensitive. *Angewandte Chemie International Edition*, *37*(6), 772-773.
- Krejpcio, Z., & Wojciak, R. (2002). The influence of Al[^] 3⁺ ions on pepsin and trypsin activity in vitro. *Polish Journal of Environmental Studies*, 11(3), 251-254.
- Kumar, J., Sarma, M. J., Phukan, P., & Das, D. K. (2015). A new simple Schiff base fluorescence "on" sensor for Al3+ and its living cell imaging. *Dalton Transactions*, 44(10), 4576-4581.
- Kumar, M., Kumar, A., Faizi, M. S. H., Kumar, S., Singh, M. K., Sahu, S. K., . . . John, R. P. (2018). A selective 'turn-on' fluorescent chemosensor for detection of Al3+ in aqueous medium: Experimental and theoretical studies. *Sensors and Actuators* B: Chemical, 260, 888-899.
- Kundu, A., Hariharan, P., Prabakaran, K., & Anthony, S. P. (2015). Developing new Schiff base molecules for selective colorimetric sensing of Fe3+ and Cu2+ metal ions: Substituent dependent selectivity and colour change. *Sensors and Actuators B: Chemical, 206*, 524-530.

- Kwon, J. Y., Jang, Y. J., Lee, Y. J., Kim, K. M., Seo, M. S., Nam, W., & Yoon, J. (2005). A highly selective fluorescent chemosensor for Pb2+. *Journal of the American Chemical Society*, 127(28), 10107-10111.
- Labidi, A., Salaberria, A. M., Fernandes, S. C., Labidi, J., & Abderrabba, M. (2016). Adsorption of copper on chitin-based materials: Kinetic and thermodynamic studies. *Journal of the Taiwan Institute of Chemical Engineers*, 65, 140-148.
- Lavigne, J. J., & Anslyn, E. V. (2001). Sensing a paradigm shift in the field of molecular recognition: from selective to differential receptors. *Angewandte Chemie International Edition*, 40(17), 3118-3130.
- Lee, M. H., Wu, J.-S., Lee, J. W., Jung, J. H., & Kim, J. S. (2007). Highly sensitive and selective chemosensor for Hg2+ based on the rhodamine fluorophore. *Organic Letters*, 9(13), 2501-2504.
- Lee, S., Jang, H., Lee, J., Jeon, S. H., Sohn, Y., & Ra, C. S. (2017). Selective and sensitive morpholine-type rhodamine B-based colorimetric and fluorescent chemosensor for Fe (III) and Fe (II). Sensors and Actuators B: Chemical, 248, 646-656.
- Leite, A., Silva, A. M., Cunha-Silva, L., de Castro, B., Gameiro, P., & Rangel, M. (2013). Discrimination of fluorescence light-up effects induced by pH and metal ion chelation on a spirocyclic derivative of rhodamine B. *Dalton Transactions*, 42(17), 6110-6118.
- Li, D., Li, C.-Y., Qi, H.-R., Tan, K.-Y., & Li, Y.-F. (2016). Rhodamine-based chemosensor for fluorescence determination of trivalent chromium ion in living cells. *Sensors and Actuators B: Chemical, 223*, 705-712.
- Li, G., Bai, L., Tao, F., Deng, A., & Wang, L. (2018). A dual chemosensor for Cu 2+ and Hg 2+ based on a rhodamine-terminated water-soluble polymer in 100% aqueous solution. *Analyst*, 143(22), 5395-5403.
- Li, G., Tao, F., Wang, H., Li, Y., & Wang, L. (2015a). A novel reversible colorimetric chemosensor for rapid naked-eye detection of Cu2+ in pure aqueous solution. *Sensors and Actuators B: Chemical, 211*, 325-331.
- Li, H., Guan, H., Duan, X., Hu, J., Wang, G., & Wang, Q. (2013). An acid catalyzed reversible ring-opening/ring-closure reaction involving a cyano-rhodamine spirolactam. *Organic & Biomolecular Chemistry*, 11(11), 1805-1809.
- Li, K., Xiang, Y., Wang, X., Li, J., Hu, R., Tong, A., & Tang, B. Z. (2014a). Reversible photochromic system based on rhodamine B salicylaldehyde hydrazone metal complex. *Journal of the American Chemical Society*, *136*(4), 1643-1649.

- Li, L.-Q., & Meng, L.-P. (2014). Novel rhodamine derivate as high selective detection lead sensor. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 122, 772-775.
- Li, N., Dai, J.-K., Du, H.-T., Yuan, M.-S., Zhang, J.-W., & Wang, J.-R. (2015b). β-Carboline-functionalized dithioacetal as Hg2+-selective fluorescence probe in water. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 136, 900-905.
- Li, Z., Hu, Q., Li, C., Dou, J., Cao, J., Chen, W., & Zhu, Q. (2014b). A 'turnon'fluorescent chemosensor based on rhodamine-N-(3-aminopropyl)-imidazole for detection of Al3+ ions. *Tetrahedron Letters*, 55(6), 1258-1262.
- Li, Z., Wu, S., Han, J., & Han, S. (2011). Imaging of intracellular acidic compartments with a sensitive rhodamine based fluorogenic pH sensor. *Analyst*, *136*(18), 3698-3706.
- Liao, Z.-C., Yang, Z.-Y., Li, Y., Wang, B.-D., & Zhou, Q.-X. (2013). A simple structure fluorescent chemosensor for high selectivity and sensitivity of aluminum ions. *Dyes and Pigments*, 97(1), 124-128.
- Linder, M. C., Wooten, L., Cerveza, P., Cotton, S., Shulze, R., & Lomeli, N. (1998). Copper transport. *The American Journal of Clinical Nutrition*, 67(5), 965S-971S.
- Liu, H., Wan, X., Liu, T., Li, Y., & Yao, Y. (2014). Cascade sensitive and selective fluorescence OFF–ON–OFF sensor for Cr3+ cation and F– anion. *Sensors and Actuators B: Chemical, 200*, 191-197.
- Liu, J., & Lu, Y. (2007). A DNAzyme catalytic beacon sensor for paramagnetic Cu2+ ions in aqueous solution with high sensitivity and selectivity. *Journal of the American Chemical Society, 129*(32), 9838-9839.
- Liu, R. Q., Ding, G. H., Li, J. L., Feng, H. J., He, W. Y., & Wu, L. Y. (2020). A triazolebased fluorescence probe for detecting Hg2+ ions and its biological application. *Luminescence*, 35(1), 129-137.
- Liu, Y., Lee, D., Wu, D., Swamy, K., & Yoon, J. (2018). A new kind of rhodamine-based fluorescence turn-on probe for monitoring ATP in mitochondria. *Sensors and Actuators B: Chemical*, 265, 429-434.
- Liu, Y., Sun, Y., Du, J., Lv, X., Zhao, Y., Chen, M., . . . Guo, W. (2011). Highly sensitive and selective turn-on fluorescent and chromogenic probe for Cu 2+ and ClO– based on a N-picolinyl rhodamine B-hydrazide derivative. Organic & Biomolecular Chemistry, 9(2), 432-437.

- Liu, Z., Jia, X., Bian, P., & Ma, Z. (2013). A simple and novel system for colorimetric detection of cobalt ions. *Analyst*, 139(3), 585-588.
- Liu, Z., Wang, W., Xu, H., Sheng, L., Chen, S., Huang, D., & Sun, F. (2015). A "naked eye" and ratiometric chemosensor for cobalt (II) based on coumarin platform in aqueous solution. *Inorganic Chemistry Communications*, 62, 19-23.
- Long, C., Hu, J.-H., Fu, Q.-Q., & Ni, P.-W. (2019). A new colorimetric and fluorescent probe based on Rhodamine B hydrazone derivatives for cyanide and Cu2+ in aqueous media and its application in real life. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 219, 297-306.
- Ma, B., Wu, S., & Zeng, F. (2010). Reusable polymer film chemosensor for ratiometric fluorescence sensing in aqueous media. *Sensors and Actuators B: Chemical*, 145(1), 451-456.
- Macrae, C. F., Bruno, I. J., Chisholm, J. A., Edgington, P. R., McCabe, P., Pidcock, E., . . Wood, P. A. (2008). Mercury CSD 2.0–new features for the visualization and investigation of crystal structures. *Journal of Applied Crystallography*, 41(2), 466-470.
- Maeda, M. (1984). Laser dyes: properties of organic compounds for dye lasers: Academic Press.
- Mahapatra, A. K., Manna, S. K., Mandal, D., & Mukhopadhyay, C. D. (2013). Highly sensitive and selective rhodamine-based "Off-On" reversible chemosensor for Tin (Sn4+) and imaging in living cells. *Inorganic chemistry*, 52(19), 10825-10834.
- Mahato, P., Saha, S., Das, P., Agarwalla, H., & Das, A. (2014). An overview of the recent developments on Hg 2+ recognition. *RSC advances*, 4(68), 36140-36174.
- Maity, D., & Govindaraju, T. (2010). Conformationally constrained (coumarintriazolyl- bipyridyl) click fluoroionophore as a selective Al3+ sensor. *Inorganic Chemistry*, 49(16), 7229-7231.
- Mandal, J., Ghorai, P., Pal, K., Bhaumik, T., Karmakar, P., & Saha, A. (2019). Development of Rhodamine 6G-Based Fluorescent Chemosensors for Al3+-Ion Detection: Effect of Ring Strain and Substituent in Enhancing Its Sensing Performance. ACS Omega.
- Manna, A. K., Sahu, M., Rout, K., Das, U. K., & Patra, G. K. (2020). A highly selective novel multiple amide based Schiff base optical chemosensor for rapid detection of Cu2+ and its applications in real sample analysis, molecular logic gate and smart phone. *Microchemical Journal*, 157, 104860.

- Mao, J., Wang, L., Dou, W., Tang, X., Yan, Y., & Liu, W. (2007). Tuning the selectivity of two chemosensors to Fe (III) and Cr (III). *Organic Letters*, 9(22), 4567-4570.
- McDonagh, C., Burke, C. S., & MacCraith, B. D. (2008). Optical chemical sensors. *Chemical Reviews*, 108(2), 400-422.
- McNaught, A. D., & Wilkinson, A. (1997). *Compendium of chemical terminology* (Vol. 1669): Blackwell Science Oxford.
- McQuade, D. T., Pullen, A. E., & Swager, T. M. (2000). Conjugated polymer-based chemical sensors. *Chemical Reviews*, 100(7), 2537-2574.
- Meng, Q., Zhang, R., Jia, H., Gao, X., Wang, C., Shi, Y., . . . Zhang, Z. (2015). A reversible fluorescence chemosensor for sequentially quantitative monitoring copper and sulfide in living cells. *Talanta*, 143, 294-301.
- Milindanuth, P., & Pisitsak, P. (2018). A novel colorimetric sensor based on rhodamine-B derivative and bacterial cellulose for the detection of Cu (II) ions in water. *Materials Chemistry and Physics, 216*, 325-331.
- Minkin, V. I. (1999). Glossary of terms used in theoretical organic chemistry. *Pure and Applied Chemistry*, 71(10), 1919-1981.
- Minta, A., & Tsien, R. Y. (1989). Fluorescent indicators for cytosolic sodium. Journal of Biological Chemistry, 264(32), 19449-19457.
- Miró, M., Estela, J. M., & Cerdà, V. c. (2004). Application of flowing stream techniques to water analysis: part III. Metal ions: alkaline and alkaline-earth metals, elemental and harmful transition metals, and multielemental analysis. *Talanta*, 63(2), 201-223.
- Mironenko, A. Y., Tutov, M., Sergeev, A., & Bratskaya, S. Y. (2017). On/off rhodamine based fluorescent probe for detection of Au and Pd in aqueous solutions. *Sensors and Actuators B: Chemical, 246*, 389-394.
- Mishra, S. K., Dehuri, S., & Bag, B. (2020). Effect of n-alkyl substitution on Cu (ii)selective chemosensing of rhodamine B derivatives. *Organic & Biomolecular Chemistry*, 18(2), 316-332.
- Morsi, R. E., Elsawy, M., Ali, M. M., Gentili, D., Cavallini, M., Manet, I., . . . Ventura, B. (2020a). Rhodamine B hydrazide loaded polysulfone fabrics for Cu (II) detection: Morphological and optical properties. *Journal of Applied Polymer Science*, 137(9), 48408.

- Morsi, R. E., Elsawy, M., Manet, I., & Ventura, B. (2020b). Cellulose Acetate Fabrics Loaded with Rhodamine B Hydrazide for Optical Detection of Cu (II). *Molecules*, 25(16), 3751.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63.
- Munro, O. Q., Joubert, S. D., & Grimmer, C. D. (2006). Molecular recognition: Preorganization of a bis (pyrrole) Schiff base derivative for tight dimerization by hydrogen bonding. *Chemistry–A European Journal*, 12(31), 7987-7999.
- Munro, O. Q., Strydom, S. D., & Grimmer, C. D. (2004). Complementary hydrogen bonding in a new tridentate Schiff base ligand: X-ray, DFT and solution NMR studies. *New Journal of Chemistry*, 28(1), 34-42.
- Nag, A., & Goswami, D. (2009). Solvent effect on two-photon absorption and fluorescence of rhodamine dyes. *Journal of Photochemistry and Photobiology A: Chemistry, 206*(2), 188-197.
- Nath, M. (2008). Toxicity and the cardiovascular activity of organotin compounds: a review. *Applied Organometallic Chemistry*, 22(10), 598-612.
- Navanesan, S., Wahab, N. A., Manickam, S., & Sim, K. S. (2015). Leptospermum flavescens constituent-LF1 causes cell death through the induction of cell cycle arrest and apoptosis in human lung carcinoma cells. *PloS One*, *10*(8), e0135995.
- Nayab, P. S., Pulaganti, M., & Chitta, S. K. (2015). A new isoindoline based schiff base derivative as Cu (II) chemosensor: synthesis, photophysical, DNA binding and molecular docking studies. *Journal of Fluorescence*, 25(6), 1763-1773.
- Nayab, P. S., & Shkir, M. (2017). A dual responsive colorimetric and fluorescent reversible turn-on chemosensor for iron (Fe3+): Computational and spectroscopic investigations. *Sensors and Actuators B: Chemical*, 245, 395-405.
- Nayak, P. (2002). Aluminum: impacts and disease. *Environmental Research*, 89(2), 101-115.
- Neumann, M., & Leimkühler, S. (2008). Heavy metal ions inhibit molybdoenzyme activity by binding to the dithiolene moiety of molybdopterin in Escherichia coli. *The FEBS Journal*, 275(22), 5678-5689.
- Nguyen, T., & Francis, M. B. (2003). Practical synthetic route to functionalized rhodamine dyes. *Organic Letters*, 5(18), 3245-3248.

- Nolan, E. M., & Lippard, S. J. (2003). A "turn-on" fluorescent sensor for the selective detection of mercuric ion in aqueous media. *Journal of the American Chemical Society*, 125(47), 14270-14271.
- Nriagu, J. O. (1990). Global metal pollution: poisoning the biosphere? *Environment:* Science and Policy for Sustainable Development, 32(7), 7-33.
- Ozay, H., Kagit, R., Yildirim, M., Yesilot, S., & Ozay, O. (2014). Novel hexapodal triazole linked to a cyclophosphazene core rhodamine-based chemosensor for selective determination of Hg 2+ ions. *Journal of Fluorescence*, 24(6), 1593-1601.
- Ozay, H., & Ozay, O. (2013). Rhodamine based reusable and colorimetric naked-eye hydrogel sensors for Fe3+ ion. *Chemical Engineering Journal*, 232, 364-371.
- Ozdemir, M. (2016). A rhodamine-based colorimetric and fluorescent probe for dual sensing of Cu2+ and Hg2+ ions. *Journal of Photochemistry and Photobiology A: Chemistry, 318*, 7-13.
- Parikh, J. K. (1994). *Trade and Environment Linkages: a case study of India*: Indira Gandhi Institute of Development Research.
- Park, G. J., Lee, M. M., You, G. R., Choi, Y. W., & Kim, C. (2014). A turn-on and reversible fluorescence sensor with high affinity to Zn2+ in aqueous solution. *Tetrahedron Letters*, 55(15), 2517-2522.
- Park, S.-H., Kwon, N., Lee, J.-H., Yoon, J., & Shin, I. (2020). Synthetic ratiometric fluorescent probes for detection of ions. *Chemical Society Reviews*, 49(1), 143-179.
- Patil, K. S., Mahajan, P. G., & Patil, S. R. (2017). Fluorimetric detection of Sn2+ ion in aqueous medium using Salicylaldehyde based nanoparticles and application to natural samples analysis. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 170, 131-137.
- Patil, S. K., & Das, D. (2019). A nanomolar detection of mercury (II) ion by a chemodosimetric rhodamine-based sensor in an aqueous medium: Potential applications in real water samples and as paper strips. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 210, 44-51.
- Pei, P.-X., Hu, J.-H., Ni, P.-W., Long, C., Su, J.-X., & Sun, Y. (2017). A novel dualchannel chemosensor for CN– based on rhodamine B hydrazide derivatives and its application in bitter almond. *RSC Advances*, 7(74), 46832-46838.

- Phapale, D., Gaikwad, A., & Das, D. (2017). Selective recognition of Cu (II) and Fe (III) using a pyrene based chemosensor. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 178, 160-165.
- Pipattanawarothai, A., & Trakulsujaritchok, T. (2020). Hybrid polymeric chemosensor bearing rhodamine derivative prepared by sol-gel technique for selective detection of Fe3+ ion. *Dyes and Pigments, 173*, 107946.
- Poléo, A. B., Østbye, K., Øxnevad, S. A., Andersen, R. A., Heibo, E., & Vøllestad, L. A. (1997). Toxicity of acid aluminium-rich water to seven freshwater fish species: a comparative laboratory study. *Environmental Pollution*, 96(2), 129-139.
- Praikaew, P., Duangdeetip, T., Chimpalee, N., Wainiphithapong, C., Swanglap, P., & Wanichacheva, N. (2015). Colorimetric sensor for detection of Hg2+ in aqueous samples utilizing rhodamine B hydrazide-modified silica. *Materials Express*, 5(4), 300-308.
- Prodi, L., Bolletta, F., Montalti, M., & Zaccheroni, N. (2000). Luminescent chemosensors for transition metal ions. *Coordination Chemistry Reviews*, 205(1), 59-83.
- Rai, A., Kumari, N., Srivastava, A. K., Singh, S. K., Srikrishna, S., & Mishra, L. (2016). Rhodamine hydrazone as OFF–ON–OFF type selective sequential sensor of Al3+ and N3– ions. *Journal of Photochemistry and Photobiology A: Chemistry*, 319, 78-86.
- Rai, A., Singh, A. K., Tripathi, K., Sonkar, A. K., Chauhan, B. S., Srikrishna, S., . . . Mishra, L. (2018). A quick and selective rhodamine based "smart probe" for "signal-on" optical detection of Cu2+ and Al3+ in water, cell imaging, computational studies and solid state analysis. *Sensors and Actuators B: Chemical*, 266, 95-105.
- Rana, S., Mittal, S. K., Singh, N., Singh, J., & Banks, C. E. (2017). Schiff base modified screen printed electrode for selective determination of aluminium (III) at trace level. Sensors and Actuators B: Chemical, 239, 17-27.
- Rasheed, T., Li, C., Nabeel, F., Huang, W., & Zhou, Y. (2019). Self-assembly of alternating copolymer vesicles for the highly selective, sensitive and visual detection and quantification of aqueous Hg2+. *Chemical Engineering Journal*, 358, 101-109.
- Rasheed, T., Li, C., Zhang, Y., Nabeel, F., Peng, J., Qi, J., ... Yu, C. (2018). Rhodaminebased multianalyte colorimetric probe with potentialities as on-site assay kit and in biological systems. *Sensors and Actuators B: Chemical, 258*, 115-124.

- Ravichandiran, P., Prabakaran, D., Bella, A. P., Boguszewska-Czubara, A., Masłyk, M., Dineshkumar, K., . . . Kim, H. G. (2020). Naphthoquinone-Dopamine Linked Colorimetric and Fluorescence Chemosensor for Selective Detection of Sn2+ Ion in Aqueous Medium and Its Bio-Imaging Applications. ACS Sustainable Chemistry & Engineering, 8(29), 10947-10958.
- Rigaku, O. (2015). Crysalis pro. Rigaku Oxford Diffraction Ltd, Yarnton, Oxfordshire, England.
- Roy, A., Das, S., Sacher, S., Mandal, S. K., & Roy, P. (2019). A rhodamine based biocompatible chemosensor for Al 3+, Cr 3+ and Fe 3+ ions: extraordinary fluorescence enhancement and a precursor for future chemosensors. *Dalton Transactions*, 48(47), 17594-17604.
- Roy, A., Dey, S., Halder, S., & Roy, P. (2017). Development of a new chemosensor for Al3+ ion: Tuning of properties. *Journal of Luminescence*, 192, 504-512.
- Roy, N., Dutta, A., Mondal, P., Paul, P. C., & Singh, T. S. (2015). A new turn-on fluorescent chemosensor based on sensitive Schiff base for Mn2+ ion. *Journal of Luminescence*, 165, 167-173.
- Sadak, A. E., & Karakuş, E. (2020). Triazatruxene–rhodamine-based ratiometric fluorescent chemosensor for the sensitive, rapid detection of trivalent metal ions: Aluminium (III), iron (III) and chromium (III). Journal of Fluorescence, 30(1), 213-220.
- Sahana, A., Banerjee, A., Lohar, S., Sarkar, B., Mukhopadhyay, S. K., & Das, D. (2013). Rhodamine-based fluorescent probe for Al3+ through time-dependent PET– CHEF–FRET processes and its cell staining application. *Inorganic chemistry*, 52(7), 3627-3633.
- Sahoo, S. K., Sharma, D., Bera, R. K., Crisponi, G., & Callan, J. F. (2012). Iron (III) selective molecular and supramolecular fluorescent probes. *Chemical Society Reviews*, *41*(21), 7195-7227.
- Saleem, M., & Lee, K.-H. (2014). Selective fluorescence detection of Cu2+ in aqueous solution and living cells. *Journal of Luminescence*, 145, 843-848.
- Santos-Figueroa, L. E., Moragues, M. E., Climent, E., Agostini, A., Martínez-Máñez, R., & Sancenón, F. (2013). Chromogenic and fluorogenic chemosensors and reagents for anions. A comprehensive review of the years 2010–2011. *Chemical Society Reviews*, 42(8), 3489-3613.
- Scheiber, I. F., Mercer, J. F., & Dringen, R. (2014). Metabolism and functions of copper in brain. *Progress in Neurobiology*, 116, 33-57.

- Schiff, H. (1864). Mittheilungen aus dem Universitätslaboratorium in Pisa: eine neue Reihe organischer Basen. *Justus Liebigs Annalen der Chemie*, 131(1), 118-119.
- Senthil Murugan, A., Vidhyalakshmi, N., Ramesh, U., & Annaraj, J. (2018). In vivo bioimaging studies of highly selective, sensitive rhodamine based fluorescent chemosensor for the detection of Cu2+/Fe3+ ions. Sensors and Actuators B: Chemical, 274, 22-29.
- Shakeela, K., Guru, S., & Rao, G. R. (2020). Cationic dye adsorption by phosphomolybdate nanoclusters immobilised on polyelectrolyte matrix. *Journal of Chemical Sciences*, 132(1), 1-10.
- Sheldrick, G. M. (2008). A short history of SHELX. Acta Crystallographica Section A: Foundations of Crystallography, 64(1), 112-122.
- Shen, S.-L., Zhang, X.-F., Bai, S.-Y., Miao, J.-Y., & Zhao, B.-X. (2015). A novel ratiometric pH probe for extreme acidity based on FRET and PET. *RSC Advances*, 5(18), 13341-13346.
- Sheng, R., Wang, P., Gao, Y., Wu, Y., Liu, W., Ma, J., ... Wu, S. (2008). Colorimetric test kit for Cu2+ detection. *Organic Letters*, 10(21), 5015-5018.
- Sherlock, J., & Smart, G. (1984). Tin in foods and the diet. Food Additives & Contaminants, 1(3), 277-282.
- Sherman, L. R., Masters, J., Peterson, R., & Levine, S. (1986). Tin concentration in the thymus glands of rats and mice and its relation to the involution of the gland. *Journal of Analytical Toxicology*, 10(1), 6-9.
- Shi, W., & Ma, H. (2008). Rhodamine B thiolactone: a simple chemosensor for Hg 2+ in aqueous media. *Chemical Communications*(16), 1856-1858.
- Singh, N., Kaur, N., Choitir, C. N., & Callan, J. F. (2009). A dual detecting polymeric sensor: chromogenic naked eye detection of silver and ratiometric fluorescent detection of manganese. *Tetrahedron Letters*, 50(29), 4201-4204.
- Sivaraman, G., Anand, T., & Chellappa, D. (2012). Turn-on fluorescent chemosensor for Zn (II) via ring opening of rhodamine spirolactam and their live cell imaging. *Analyst*, 137(24), 5881-5884.

Smith, P. J. (2012). Chemistry of tin: Springer Science & Business Media.

- Soares-Paulino, A. A., Giroldo, L., Pradie, N. A., Reis, J. S., Back, D. F., Braga, A. A., . . Dos Santos, A. A. (2020). Nanomolar Detection of Palladium (II) through a Novel Seleno-Rhodamine-based fluorescent and colorimetric chemosensor. *Dyes* and Pigments, 108355.
- Sommerwerk, S., Heller, L., Kerzig, C., Kramell, A. E., & Csuk, R. (2017). Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations. *European Journal of Medicinal Chemistry*, 127, 1-9.
- Song, F., Shao, X., Zhu, J., Bao, X., Du, L., & Kan, C. (2019). Reversible "turn-off-on" fluorescence response of Fe (III) towards Rhodamine B based probe in vivo and plant tissues. *Tetrahedron Letters*, 60(20), 1363-1369.
- Song, Y., Tao, J., Wang, Y., Cai, Z., Fang, X., Wang, S., & Xu, H. (2020). A novel dualresponsive fluorescent probe for the detection of copper (II) and nickel (II) based on BODIPY derivatives. *Inorganica Chimica Acta*, 120099.
- Soni, M. G., White, S. M., Flamm, W. G., & Burdock, G. A. (2001). Safety evaluation of dietary aluminum. *Regulatory Toxicology and Pharmacology*, 33(1), 66-79.
- Stahl, T., Falk, S., Rohrbeck, A., Georgii, S., Herzog, C., Wiegand, A., . . . Brunn, H. (2017). Migration of aluminum from food contact materials to food—a health risk for consumers? Part I of III: exposure to aluminum, release of aluminum, tolerable weekly intake (TWI), toxicological effects of aluminum, study design, and methods. *Environmental Sciences Europe*, 29(1), 19.
- Stern, B. R. (2010). Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. *Journal of Toxicology and Environmental Health, Part A*, 73(2-3), 114-127.
- Stock, R. I., Dreyer, J. P., Nunes, G. E., Bechtold, I. H., & Machado, V. G. (2019). Optical Chemosensors and Chemodosimeters for Anion Detection Based on Merrifield Resin Functionalized with Brooker's Merocyanine Derivatives. ACS Applied Polymer Materials, 1(7), 1757-1768.
- Suksai, C., & Tuntulani, T. (2003). Chromogenic anion sensors. *Chemical Society Reviews*, 32(4), 192-202.
- Sullivan, J. B., & Krieger, G. R. (2001). *Clinical environmental health and toxic exposures*: Lippincott Williams & Wilkins.
- Sun, C., Chen, J., Ma, H., Liu, Y., Zhang, J., & Liu, Q. (2011). A new Cu 2+-induced color reaction of a rhodamine derivative N-(3-carboxy) acryloyl rhodamine B hydrazide. *Science China Chemistry*, 54(7), 1101-1108.

- Sun, Y., Lu, Y., Bian, M., Yang, Z., Ma, X., & Liu, W. (2020). Pt (II) and Au (III) complexes containing Schiff-base ligands: A promising source for antitumor treatment. *European Journal of Medicinal Chemistry*, 113098.
- Tan, J.-L., Zhang, M.-X., Zhang, F., Yang, T.-T., Liu, Y., Li, Z.-B., & Zuo, H. (2015). A novel "off-on" colorimetric and fluorescent rhodamine-based pH chemosensor for extreme acidity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 140, 489-494.
- Tang, F.-K., Chan, S.-M., Wang, T., Kwan, C.-S., Huang, R., Cai, Z., & Leung, K. C.-F. (2020). Highly selective detection of Pd2+ ion in aqueous solutions with rhodamine-based colorimetric and fluorescent chemosensors. *Talanta*, 210, 120634.
- Tang, L., Li, F., Liu, M., & Nandhakumar, R. (2011a). Single sensor for two metal ions: Colorimetric recognition of Cu2+ and fluorescent recognition of Hg2+. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 78(3), 1168-1172.
- Tang, R., Lei, K., Chen, K., Zhao, H., & Chen, J. (2011b). A rhodamine-based off-on fluorescent chemosensor for selectively sensing Cu (II) in aqueous solution. *Journal of Fluorescence*, 21(1), 141-148.
- Tang, X., Han, J., Wang, Y., Ni, L., Bao, X., Wang, L., & Zhang, W. (2017). A multifunctional Schiff base as a fluorescence sensor for Fe3+ and Zn2+ ions, and a colorimetric sensor for Cu2+ and applications. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 173, 721-726.
- Tchounwou, P. B., Newsome, C., Williams, J., & Glass, K. (2008). Copper-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma (HepG2) cells. Paper presented at the Metal ions in biology and medicine: proceedings of the... International Symposium on Metal Ions in Biology and Medicine held...= Les ions metalliques en biologie et en medecine:... Symposium international sur les ions metalliques.
- Tian, M., Peng, X., Fan, J., Wang, J., & Sun, S. (2012). A fluorescent sensor for pH based on rhodamine fluorophore. *Dyes and Pigments*, 95(1), 112-115.
- Trigo-López, M., Pablos, J. L., Muñoz, A., Ibeas, S., Serna, F., García, F. C., & García, J. M. (2015). Aromatic polyamides and acrylic polymers as solid sensory materials and smart coated fibres for high acidity colorimetric sensing. *Polymer Chemistry*, 6(16), 3110-3120.

Tsugutoshi, A. (2004). Copper deficiency and the clinical practice. Trace Elements, 365.

- Udhayakumari, D., & Inbaraj, V. (2020). A review on Schiff Base fluorescent Chemosensors for cell imaging applications. *Journal of fluorescence*, 1-21.
- Valdes-Aguilera, O., & Neckers, D. (1989). Aggregation phenomena in xanthene dyes. Accounts of Chemical Research, 22(5), 171-177.
- Valeur, B. (2003). Molecular fluorescence. *Digital Encyclopedia of Applied Physics*, 477-531.
- Valeur, B., & Leray, I. (2000). Design principles of fluorescent molecular sensors for cation recognition. *Coordination Chemistry Reviews*, 205(1), 3-40.
- Vashisht, D., Sharma, S., Kumar, R., Saini, V., Saini, V., Ibhadon, A., . . . Kataria, R. (2020). Dehydroacetic acid derived Schiff base as selective and sensitive colorimetric chemosensor for the detection of Cu (II) ions in aqueous medium. *Microchemical Journal*, 155, 104705.
- Venkatesan, V., Kumar, R. S., Kumar, S. A., & Sahoo, S. K. (2019). Dual optical properties of new schiff base based on bisthiophene for sensing of Cu2+ in protic media. *Journal of Molecular Structure*, 1198, 126906.
- Vita, V., Hellman, S., Rosenberg, S., & Markoe, A. (1986). Cancer: principles and practice of oncology. *American Journal of Clinical Oncology*, 9(1).
- Wan, J., Zhang, K., Li, C., Li, Y., & Niu, S. (2017). A novel fluorescent chemosensor based on a rhodamine 6G derivative for the detection of Pb2+ ion. Sensors and Actuators B: Chemical, 246, 696-702.
- Wang, B., & Anslyn, E. V. (2011). *Chemosensors: principles, strategies, and applications* (Vol. 15): John Wiley & Sons.
- Wang, J., Lv, M., Wang, Z., Zhou, M., Gu, C., & Guo, C. (2015a). Highly sensitive and selective fluorescent detection of rare earth metal Sn (II) ion by organic fluorine Schiff base functionalized periodic mesoporous material in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*, 309, 37-46.
- Wang, J., Xie, Y., Wang, Z., & Song, Q. (2014a). A highly sensitive and selective nakedeye probe for detecting copper ion based on 2, 3-modified Bodipy derivatives. *Sensors and Actuators B: Chemical*, 194, 149-155.
- Wang, L., Yan, J., Qin, W., Liu, W., & Wang, R. (2012a). A new rhodamine-based single molecule multianalyte (Cu2+, Hg2+) sensor and its application in the biological system. *Dyes and Pigments*, 92(3), 1083-1090.

- Wang, L., Ye, D., & Cao, D. (2012b). A novel coumarin Schiff-base as a Ni (II) ion colorimetric sensor. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 90, 40-44.
- Wang, M., Feng, W., Shi, J., Zhang, F., Wang, B., Zhu, M., . . . Chai, Z. (2007). Development of a mild mercaptoethanol extraction method for determination of mercury species in biological samples by HPLC–ICP-MS. *Talanta*, 71(5), 2034-2039.
- Wang, M., Liu, X., Lu, H., Wang, H., & Qin, Z. (2015b). Highly selective and reversible chemosensor for Pd2+ detected by fluorescence, colorimetry, and test paper. ACS Applied Materials & Interfaces, 7(2), 1284-1289.
- Wang, M., Yan, F., Zou, Y., Chen, L., Yang, N., & Zhou, X. (2014b). Recognition of Cu2+ and Hg2+ in physiological conditions by a new rhodamine based dual channel fluorescent probe. *Sensors and Actuators B: Chemical*, 192, 512-521.
- Wang, P., Wu, J., Su, P., Xu, C., Ge, Y., Liu, D., . . . Tang, Y. (2016). Fluorescence "onoff-on" peptide-based chemosensor for the selective detection of Cu 2+ and S 2and its application in living cell bioimaging. *Dalton Transactions*, 45(41), 16246-16254.
- Wang, Y., Ding, H., Zhu, Z., Fan, C., Tu, Y., Liu, G., & Pu, S. (2020). Selective rhodamine-based probe for detecting Hg2+ and its application as test strips and cell staining. *Journal of Photochemistry and Photobiology A: Chemistry*, 390, 112302.
- Wang, Y., Wang, L., Shi, L. L., Shang, Z. B., Zhang, Z., & Jin, W. J. (2012c). Colorimetric and fluorescence sensing of Cu2+ in water using 1, 8dihydroxyanthraquinone-β-cyclodextrin complex with the assistance of ammonia. *Talanta*, 94, 172-177.
- Wang, Y., Wu, H., Wu, W.-N., Mao, X.-J., Zhao, X.-L., Xu, Z.-Q., ... Fan, Y.-C. (2019). Novel rhodamine-based colorimetric and fluorescent sensor for the dual-channel detection of Cu2+ and Co2+/trivalent metal ions and its AIRE activities. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 212, 1-9.
- Weber, G. (1985). The importance of tin in the environment and its determination at trace levels. *Fresenius' Zeitschrift für Analytische Chemie*, *321*(3), 217-224.
- Wechakorn, K., Chomngam, S., Eiamprasert, U., & Kongsaeree, P. (2020). A rhodamine– bistriazole based fluorescent and colorimetric sensor containing a phenyl linker for Fe (III) detection. *Chemical Papers*, 1-10.
- Wolfram, R. K., Heller, L., & Csuk, R. (2018). Targeting mitochondria: Esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis. *European Journal of Medicinal Chemistry*, 152, 21-30.
- Wolle, M. M., Fahrenholz, T., Rahman, G. M., Pamuku, M., & Browne, D. (2014). Method development for the redox speciation analysis of iron by ion chromatography-inductively coupled plasma mass spectrometry and carryover assessment using isotopically labeled analyte analogues. *Journal of Chromatography A*, 1347, 96-103.
- Woodson, G. (1998). An interesting case of osteomalacia due to antacid use associated with stainable bone aluminum in a patient with normal renal function. *Bone*, 22(6), 695-698.
- Wu, G. H., Wang, D. X., Gao, Y., & Wang, Z. Q. (2009). Highly sensitive optical chemosensor for the detection of Cu 2+ using a rhodamine B spirolatam. *Journal* of Chemical Sciences, 121(4), 543-548.
- Wu, X., Wu, Z., Yang, Y., & Han, S. (2012). A highly sensitive fluorogenic chemodosimeter for rapid visual detection of phosgene. *Chemical Communications*, 48(13), 1895-1897.
- Xavier, A., & Srividhya, N. (2014). Synthesis and study of Schiff base ligands. IOSR Journal of Applied Chemistry, 7(11), 06-15.
- Xiang, Y., & Tong, A. (2006). A new rhodamine-based chemosensor exhibiting selective FeIII-amplified fluorescence. *Organic Letters*, 8(8), 1549-1552.
- Xiong, J.-J., Huang, P.-C., Zhang, C.-Y., & Wu, F.-Y. (2016). Colorimetric detection of Cu2+ in aqueous solution and on the test kit by 4-aminoantipyrine derivatives. Sensors and Actuators B: Chemical, 226, 30-36.
- Xu, Q.-C., Zhu, X.-H., Jin, C., Xing, G.-W., & Zhang, Y. (2014). Dual off–on and on– off fluorescent detection of Zn 2+/Cd 2+ ions based on carbazolone substituted 2aminobenzamides. *RSC Advances*, 4(7), 3591-3596.
- Xu, Z.-Q., Mao, X.-J., Wang, Y., Wu, W.-N., Mao, P.-D., Zhao, X.-L., . . . Li, H.-J. (2017). Rhodamine 6G hydrazone with coumarin unit: a novel single-molecule multianalyte (Cu 2+ and Hg 2+) sensor at different pH value. *RSC Advances*, 7(67), 42312-42319.
- Xu, Z., Zhang, L., Guo, R., Xiang, T., Wu, C., Zheng, Z., & Yang, F. (2011). A highly sensitive and selective colorimetric and off-on fluorescent chemosensor for Cu2+ based on rhodamine B derivative. *Sensors and Actuators B: Chemical*, 156(2), 546-552.

- Yan, C., Guo, Z., Shen, Y., Chen, Y., Tian, H., & Zhu, W.-H. (2018a). Molecularly precise self-assembly of theranostic nanoprobes within a single-molecular framework for in vivo tracking of tumor-specific chemotherapy. *Chemical science*, 9(22), 4959-4969.
- Yan, F., Cao, D., Yang, N., Yu, Q., Wang, M., & Chen, L. (2012). A selective turn-on fluorescent chemosensor based on rhodamine for Hg2+ and its application in live cell imaging. *Sensors and Actuators B: Chemical*, 162(1), 313-320.
- Yan, Z., Wei, G., Guang, S., Xu, M., Ren, X., Wu, R., ... Xu, H. (2018b). A multidentate ligand chromophore with rhodamine-triazole-pyridine units and its acting mechanism for dual-mode visual sensing trace Sn2+. *Dyes and Pigments*, 159, 542-550.
- Yang, B., & Wu, W. (2013). Fabrication of a novel natural cellulose-based paper chemodosimeter via grafting-to of Rhodamine B moieties for detection of Hg2+. *Reactive and Functional Polymers*, 73(11), 1553-1558.
- Yang, H., Zhou, Z., Huang, K., Yu, M., Li, F., Yi, T., & Huang, C. (2007). Multisignaling optical-electrochemical sensor for Hg2+ based on a rhodamine derivative with a ferrocene unit. Organic Letters, 9(23), 4729-4732.
- Yang, L., Zhang, X., Yang, J., Yuan, M.-S., & Wang, J. (2021). A rhodamine-based chemosensor and functionalized gel ball for detecting and adsorbing copper ions. *Tetrahedron*, 80, 131893.
- Yang, S., Yin, B., Xu, L., Gao, B., Sun, H., Du, L., ... Cao, F. (2015). A natural quercetinbased fluorescent sensor for highly sensitive and selective detection of copper ions. *Analytical Methods*, 7(11), 4546-4551.
- Yang, X.-F., Guo, X.-Q., & Zhao, Y.-B. (2002). Development of a novel rhodamine-type fluorescent probe to determine peroxynitrite. *Talanta*, *57*(5), 883-890.
- Yang, Y.-K., Yook, K.-J., & Tae, J. (2005). A rhodamine-based fluorescent and colorimetric chemodosimeter for the rapid detection of Hg2+ ions in aqueous media. *Journal of the American Chemical Society*, *127*(48), 16760-16761.
- Yang, Y., Gao, C.-Y., Zhang, N., & Dong, D. (2016). Tetraphenylethene functionalized rhodamine chemosensor for Fe3+ and Cu2+ ions in aqueous media. *Sensors and Actuators B: Chemical*, 222, 741-746.
- Yang, Y., Gao, W., Sheng, R., Wang, W., Liu, H., Yang, W., . . . Zhang, X. (2011). Rhodamine-based derivatives for Cu2+ sensing: Spectroscopic studies, structurerecognition relationships and its test strips. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 81(1), 14-20.

- Yang, Z., Cao, J., He, Y., Yang, J. H., Kim, T., Peng, X., & Kim, J. S. (2014). Macro-/micro-environment-sensitive chemosensing and biological imaging. *Chemical Society Reviews*, 43(13), 4563-4601.
- Yap, C. K., Peng, S. H. T., & Leow, C. S. (2019). Contamination in Pasir Gudang Area, Peninsular Malaysia: What can we learn from Kim Kim River chemical waste contamination? *Journal of Humanities and Education Development (JHED)*, 1(2), 84-87.
- Ye, Y., Lv, M., Zhang, X., & Zhang, Y. (2015). Colorimetric determination of copper (II) ions using gold nanoparticles as a probe. *RSC advances*, 5(124), 102311-102317.
- Yilmaz, B., Keskinates, M., & Bayrakci, M. (2020). Novel integrated sensing system of calixarene and rhodamine molecules for selective colorimetric and fluorometric detection of Hg2+ ions in living cells. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 245, 118904.
- Yin, C. X. (2017). 8.18 Fluorescent Probes for Reactive Oxygen Species. In J. L. Atwood (Ed.), Comprehensive Supramolecular Chemistry II (pp. 401-421). Oxford: Elsevier.
- Yoon, J. W., Chang, M. J., Hong, S., & Lee, M. H. (2017). A fluorescent probe for copper and hypochlorite based on rhodamine hydrazide framework. *Tetrahedron Letters*, 58(40), 3887-3893.
- You, G. R., Jang, H. J., Jo, T. G., & Kim, C. (2016). A novel displacement-type colorimetric chemosensor for the detection of Cu2+ and GSH in aqueous solution. *RSC advances*, 6(78), 74400-74408.
- Yu, C., Jian, L., Ji, Y., & Zhang, J. (2018). Al (iii)-responsive "off-on" chemosensor based on rhodamine derivative and its application in cell imaging. *RSC Advances*, 8(54), 31106-31112.
- Yu, H.-X., Zhi, J., Chang, Z.-F., Shen, T., Ding, W.-L., Zhang, X., & Wang, J.-L. (2019). Rational design of aggregation-induced emission sensor based on Rhodamine B for turn-on sensing of trivalent metal cations, reversible data protection, and bioimaging. *Materials Chemistry Frontiers*, 3(1), 151-160.
- Zhang, C., Xie, H., Zhan, T., Zhang, J., Chen, B., Qian, Z., . . . Zhou, J. (2019). A new mitochondrion targetable fluorescent probe for carbon monoxide-specific detection and live cell imaging. *Chemical Communications*, 55(64), 9444-9447.
- Zhang, E., Ju, P., Li, Q., Hou, X., Yang, H., Yang, X., . . . Zhang, Y. (2018). A novel rhodamine 6G-based fluorescent and colorimetric probe for Bi3+: synthesis,

selectivity, sensitivity and potential applications. Sensors and Actuators B: Chemical, 260, 204-212.

- Zhang, L.-F., Zhao, J.-L., Zeng, X., Mu, L., Jiang, X.-K., Deng, M., . . . Wei, G. (2011a). Tuning with pH: the selectivity of a new rhodamine B derivative chemosensor for Fe3+ and Cu2+. Sensors and Actuators B: Chemical, 160(1), 662-669.
- Zhang, N., Fan, Y.-h., Zhang, Z., Zuo, J., Zhang, P.-f., Wang, Q., . . . Bi, C.-f. (2012). Syntheses, crystal structures and anticancer activities of three novel transition metal complexes with Schiff base derived from 2-acetylpyridine and l-tryptophan. *Inorganic Chemistry Communications, 22*, 68-72.
- Zhang, X., Jing, S.-Y., Huang, S.-Y., Zhou, X.-W., Bai, J.-M., & Zhao, B.-X. (2015). New fluorescent pH probes for acid conditions. *Sensors and Actuators B: Chemical*, 206, 663-670.
- Zhang, X., Shiraishi, Y., & Hirai, T. (2007). A new rhodamine-based fluorescent chemosensor for transition metal cations synthesized by one-step facile condensation. *Tetrahedron Letters*, 48(31), 5455-5459.
- Zhang, Y., Wang, W., Li, R., Zhang, E., Li, Z., Tang, L., . . . Wang, J.-J. (2020). A novel rhodamine-based colorimetric and fluorometric probe for simultaneous detection of multi-metal ions. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 230, 118050.
- Zhang, Z., Zheng, Y., Hang, W., Yan, X., & Zhao, Y. (2011b). Sensitive and selective off-on rhodamine hydrazide fluorescent chemosensor for hypochlorous acid detection and bioimaging. *Talanta*, 85(1), 779-786.
- Zhao, G., Yi, C., Wei, G., Wu, R., Gu, Z., Guang, S., & Xu, H. (2020a). Molecular design strategies of multifunctional probe for simultaneous monitoring of Cu2+, Al3+, Ca2+ and endogenous L-phenylalanine (LPA) recognition in living cells and zebrafishes. *Journal of Hazardous Materials*, 389, 121831.
- Zhao, M., Zhang, Y., Zheng, X., Li, Z., & Xu, S. (2020b). High selective and sensitive optical probe with effective recognition for Cu2+ based on a novel aniline squarylium dye. *Inorganic Chemistry Communications*, 121, 108198.
- Zhao, Y., Zhang, X.-B., Han, Z.-X., Qiao, L., Li, C.-Y., Jian, L.-X., ... Yu, R.-Q. (2009). Highly sensitive and selective colorimetric and off– on fluorescent chemosensor for Cu2+ in aqueous solution and living cells. *Analytical Chemistry*, 81(16), 7022-7030.
- Zhen, X., Zhang, J., Huang, J., Xie, C., Miao, Q., & Pu, K. (2018). Macrotheranostic probe with disease-activated near-infrared fluorescence, photoacoustic, and

photothermal signals for imaging-guided therapy. Angewandte Chemie, 130(26), 7930-7934.

- Zheng, H., Qian, Z.-H., Xu, L., Yuan, F.-F., Lan, L.-D., & Xu, J.-G. (2006). Switching the recognition preference of rhodamine B spirolactam by replacing one atom: design of rhodamine B thiohydrazide for recognition of Hg (II) in aqueous solution. Organic Letters, 8(5), 859-861.
- Zhu, Z., Wang, X., Li, T., Aime, S., Sadler, P. J., & Guo, Z. (2014). Platinum (II)– Gadolinium (III) Complexes as Potential Single-Molecular Theranostic Agents for Cancer Treatment. *Angewandte Chemie*, 126(48), 13441-13444.

163