

Ain Shams University Faculty of Science Chemistry Department

EFFECT OF NATURAL PRODUCT EXTRACTS IN CALCIUM OXALATE MONOHYDRATE CRYSTALLIZATION

A Thesis
Submitted to
Chemistry Department
Faculty of Science – Ain Shams University

For
The Degree of Doctor of Philosophy in Science (Ph.D.)
(Inorganic Chemistry)

By Amany Mohamed Kamal Yassin

Supervised by

Prof. Dr. M. Fathy. El-Shahat
Prof. of Analytical and Inorganic
Chemistry, Faculty of Science
Ain Shams University

Prof. Dr. El-Sayed A. Abdel-Aal
Prof. of Minerals Technology,
Central Metallurgical Research
and Development Institute



Faculty of Science Chemistry Department

Approval Sheet for Submission

A Thesis Title

EFFECT OF NATURAL PRODUCT EXTRACTS IN CALCIUM OXALATE MONOHYDRATE CRYSTALLIZATION

A Thesis Submitted by

Amany Mohamed Kamal Yassin M. Sc. (Analytical & Inorganic Chemistry)

Submit For

The Degree of Doctor of Philosophy in Science (Ph.D.)

(Inorganic Chemistry)

This thesis has been approved for submission by supervisors

Thesis Advisors:

- 1. Prof. Dr. Mohamed Fathy El-Shahat

 Professor of Analytical and Inorganic Chemistry,
 Faculty of Science -Ain Shams University
- 2. Prof. Dr. El-Sayed Ali Abdel-Aal
 Professor of Minerals Technology, Central Metallurgical
 Research and Development Institute (CMRDI)

Head of Chemistry Department Faculty of Science-Ain Shams University

Prof. Dr. Ibrahim H. A. Badr

ACKNOWLEDGEMENTS

All gratitude is due to Allah Almighty, who guided and aided me to bring-forth to right this thesis.

This thesis presented to the spirit of my father's mercy be upon him.

I would like to express my sincere appreciation and deep thanks to **Prof. Dr. Mohamed F. El-Shahat**, Prof. of Analytical and Inorganic Chemistry, Faculty of Science, Ain Shams University for kind support, fruitful discussions and concern in this work.

My warm and sincere appreciations and gratitude are directed to **Prof. Dr. El-Sayed A. Abdel-Aal**, Prof. of Minerals Technology, Central Metallurgical Research and Development Institute (CMRDI), for suggesting the point of research, suggesting the topics of this thesis, continues encouragement, supervision and valuable discussion and fruitful advice and kind helping during this work.

I am very grateful to my Mather, brothers and sisters for helping me and for their continuous encouragement.

My thanks to all members in minerals technology Lab., Central Metallurgical Research and Development Institute for their facilities provided throughout this work.

Amany M. K. Yassin

ABSTRACT

Kidney stone formation is a major health problem all over the world. Calcium oxalate monohydrate (COM) kidney stones are the most common type of kidney stone (renal calculi); about 70% to 80% of kidney stones are formed from COM crystals. The mechanism underlying calcium oxalate stone formation is complex and not completely understood. Therefore, better understanding of calcium oxalate monohydrate crystallization is in urgent need to find out much efficient drug for inhibition kidney stone formation.

This study was carried out using herbal extracts as crystallization inhibitors of kidney stone. Crystallization kinetics of calcium oxalate monohydrate (COM) was studied with and without an aqueous *Ammi Visnaga* (Khella) and *Nigella Sativa*(Habbet El-Barakah) extracts individually.

To our knowledge, it is the first time to study the effect of an aqueous Nigella Sativa extract on COM crystallization as well as it is the first time to compare Nigella Sativa extract effect with the effect of an aqueous Khella extract on the COM crystallization. The induction period was determined under different supersaturation ratios at 37 °C using the conductivity method. The induction time decreased exponentially with increasing super saturation ratio whereas the induction time was increased with addition of an aqueous Egyptian khella and

Nigella Sativa extracts individually compared to without additives. By using theory of classical homogenous nucleation, the calculated surface energy was increased from 7.97 mJ/m² without additive to 9.15 mJ/m² and 10.31 mJ/m² with Khella and Nigella Sativa extracts, respectively. However, the nucleation rate at a supersaturation ratio of 3.26 corresponding to 3.27 and 5.44 with Khella and Nigella Sativa extracts, respectively was decreased from 3.9 x 10²⁹ nuclei/cm³.s (without additive) to 2.4×10^{29} nuclei/cm³.s and 1.3×10^{29} nuclei/cm³.s with Khella and Nigella Sativa extracts, respectively. The results indicate that increasing surface energy leads to decreasing nucleation rate. The number of molecules required for the formation of stable nucleus was calculated with and without addition of extracts at different supersaturation ratios. COM crystals were investigated by X Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X Ray Spectroscopy (EDX). SEM photomicrographs show formation of small crystals, less aggregated with extracts inhibitor compared with the baseline. This study can help us to find out much efficient medicine from herbs extract for removal/inhibition kidney stone formation.

Keywords: Calcium oxalate monohydrate; Crystallization; Crystal morphology; Induction time; Crystal growth inhibitor.

ABBREVIATIONS

COM Calcium oxalate monohydrate

CaOx Calcium oxalate

CaP Calcium Phosphate

RS Relative supersaturation

S Supersaturation

W/O Without

K.E. Khella Extract

N.S. Nigella Sativa

UTI Urinary tract infection

GAG Glycols amino glycan

GAGS Glycols amino glycans

M Molarity

mM Milli molar

mL Milliliter

XRD X-ray powder diffraction

SEM Scanning electron microscopy

EDX Energy-dispersive X-ray spectroscopy

CONTENTS

CHAPTER I

I. INTRODUCTION& LITERATURE SURVAY1
I.1. Kidney Stones 3
I.1.1. Composition and Types of Kidney Stones4
I.1.1.1. Calcium Stones 6
A. Calcium Oxalate Stone6
B. Calcium Phosphate Stone7
I.1.1.2. Uric Acid Stones 8
I.1.1.3. Struvite Stones 9
I.1.1.4. Cystine Stones 9
I.2. Risk Factors for the Formation of Kidney Stone 10
I.2.1. Urinary Calcium11
I.2.2. Urinary Oxalate
I.2.3. Urinary Magnesium 13
I.2.4. Urinary Citrate
I.2.5. Urinary Uric Acid 14
I.2.6. Urinary Phosphate 14

I.2.7. Urinary Pyrophosphate	15
I.2.8. Urinary pH	15
I.2.9. Urinary Volume	16
I.3. Physicochemical Mechanisms of C	Calcium Oxalate
Monohydrate Formation	18
I.3.1. Nucleation and Crystallization	18
I.3.1.1. Supersaturation	18
I.3.1.2. Nucleation	21
A. Homogeneous Nucleation	26
B. Heterogeneous Nucleation	29
I.3.1.3. Crystal Growth	31
I.3.1.4. Aggregation	34
I.3.1.5. Induction Time	35
I.3.2. Inhibitors of Calcium Oxalate Monohydrate C	rystal Growth37
I.3.2.1. Urinary Inhibitors	37
7	
I.3.2.2. Citrate Inhibitor	39
I.3.2.3. Magnesium salt Inhibitor	39
I.3.2.4. Medicinal Plants Inhibitor	41
A. Ammi Visnaga (Khella) Plant	42

B. Nigella Sativa (Black Cumin) Seeds		4:	5
---------------------------------------	--	----	---

CHAPTER II

II. EXPERIMENTAL	. 49
II.1. MATERIALS	49
II.2. APPARATUS	- 51
II.3. PROCEDURE	- 52
II.3.1. Experimental Technique	
II.3.1.1. Extract Preparation	- 52
II.3.1.2. Calcium Oxalate Monohydrate Preparation	- 54
II.3.1.3. Conductivity Measurements	· 58
II.3.1.4. Crystallization Kinetics	. 59
i. Calculation of Induction Time	- 59
ii. Calculation of Supersaturation	60
iii. Calculation of Surface Energy	60
iv. Calculation of Nucleation Rate	61
v. Calculation of Free Energy Change (ΔGcr)	62
vi. Calculation of Critical Nucleus Radius (r) and N	umber of
Molecules in the Critical Nucleus (i)	63
II.3.2. Characterization	64
II.3.2.1. Chemical Analysis	- 64

a. EDX. Elemental Analysis	64
b. C, H Elemental Analysis	64
II.3.2.2. Physical Analysis	64
A. Morphology Investigation	64
B. Particle Size Distribution	64
II.3.2.3. Mineralogical Analysis	65
II.3.3. The Flow Sheet of the Whole Experimental Procedure	66
CHAPTER III	
III. RESULTS AND DISCUSSION	67
III.1. CRYSTALLIZATION OF CALCIUM OF	XALATE
MONOHYDRATE	67
III.1.1 Crystallization of Calcium Oxalate Monohydrate	without
Additives (Base Line)	67
III.1.1.1 Calculation of Induction Time	68
III.1.1.2. Relation between Induction Time and Supersa	aturation
Ratio	70
III.1.1.3. Results of Surface Energy	70
III.1.1.4. Results of Nucleation Rate	72

of
H
n

III.1.2.1.7. Characterization of Formed Crystals 93
A. X-Ray Diffraction Analysis 93
B. Particle Size Distribution 95
C. SEM Photomicrographs of COM Crystals with and
without Khella Extract96
D. Chemical Analysis 98
III.1.2.2. Effect of an Aqueous Extract of Nigella Sativa (Habbet El-
Barakah)100
III.1.2.2.1. Effect of Nigella Sativa Extract Concentration on
Induction Time of COM 100
III.1.2.2.2. Calculation of Induction Time101
III.1.2.2.3. Surface Energy104
III.1.2.2.4. Nucleation Rate 105
III.1.2.2.5. Free Energy Change (ΔGcr)107
III.1.2.2.6.Critical Nucleus Radius (r) and Number of Molecules in
the Critical Nucleus (i)108
III.1.2.2.7. Characterization of Formed Crystals111
A. X-Ray Diffraction Analysis111
B. Particle Size Distribution 113
C. SEM Photomicrographs of COM Crystals with and
without Habbet El-Barakah Extract114
D. Chemical Analysis 116

III.2. COMPARISON FOR AMMI VISNAGA [KHELLA] AND
NIGELLA SATIVA [HABBET EL-BARAKAH] EXTRACTS ON COM
CRYSTALS
118
III.2.1. Effect of Additives Concentration on the COM Crystals
Induction Time118
III.2.2. Effect of Supersaturation Ratios on Induction Time of COM
with and w/o Khella and Nigella Sativa Extracts119
III.2.3. Results of Surface Energy120
III.2.4. Results of Nucleation Rate122
III.2.5. Results of Free Energy Change (ΔGcr)123
III.2.6. Results of Critical Nucleus Radius (r) and Number of Molecules
in the Critical Nucleus (i)125
III.2.7. Characterization of Formed Crystals129
A. X-Ray Diffraction Analysis129
B. Particle Size Distribution131
C. Scanning Electron Microscopy132
D. Chemical Analysis 134

CHAPTER IV

IV. SAMARRY & CONCLUSION	135
REFERENCES	144
ARABIC SUMMARY	164

LIST OF TABLES

Serial		Page
No.		No.
1 1	Table I.1. Different Types of Kidney Stones, Their Formulas and Percent Occurrences.	5
2	Table I.2. Classification and Characterization of Different Types of Kidney Stones and its Urinary Risk Factor	17
3	Table I.3 . Surface Tension Factor for Heterogeneous Nucleation	31
4	Table II.1. Chemical Analysis for Aqueous Extract of Ammi Visnaga (Khella)	53
5	Table.II.2. Chemical Composition of the Nigella Sativa Seeds	53
6	Table II.3. Experimental Procedures of Different COM Supersaturation Ratios with Ammi Visnaga Extract	55
7	Table II.4. New Calculated Supersaturation Ratios of COM System with an Aqueous Extract of Ammi Visnaga	56
8	Table II.5. Experimental Procedure of Different COM Supersaturation Ratios with and without Nigella Sativa Extract	57
9	Table III.1. Effect of S on the Induction Time w/o Additives	69
10	Table III.2. Nucleation Rate, Free Energy Change for Formation of Critical Nucleus Size of COM Crystals at Different Supersaturation Ratios (S)	74
11	Table III.3. Radius of Critical Nucleus and Number of Molecules in the Critical Nucleus at Different Supersaturation Ratios (S)	77
12	Table III.4. XRD Data of Calcium Oxalate Monohydrate w/o Additives	78
13	Table III.5. Particle Size Distribution and Mean Diameter of COM Crystals w/o Additives at 4.51 S	79
14	Table III.6. Theoretical and Experimental Chemical Analyses of COM w/o Additives	82
15	Table III.7. Induction Time at Different Supersaturation Ratios with and without Khella Extract	86
16	Table III.8. Effect of KE on Nucleation Rate, Free Energy Change for Formation of Critical Nucleus Size of COM Crystals at Different Supersaturation Ratios (S)	90
17	Table III.9. Effect of Khella Extract on Radius of Critical Nucleus and Number of Molecules in the Critical Nucleus at Different Supersaturation Ratios	93