

Yenepoya University

GUIDELINES FOR THE PREPARATION OF Ph.D. Thesis

THESIS

The arrangement of parts of Ph.D. Thesis

The sequence in which the thesis material should be arranged and bound should be as follows:

1. Cover page
2. Inside cover page
3. Dedication page (Optional)
4. Certificates from Research Guide and co-guide on letter head
5. Declaration by the candidate
6. Acknowledgements
7. Table of Contents
8. Abstract
9. List of Tables
10. List of Figures
11. Annexures
12. List of Symbols, Abbreviations or Nomenclature (Optional)
13. Chapters – (1) Introduction, (2) Literature review, (3) Methodology, (4) Results, (5) Discussion, (6) Summary and Conclusion
14. References
15. List of papers based on Thesis (Published /acceptance letter for publication) hard copies to be Submitted.
16. Doctoral Committee

The formats in various headings are given below

Cover page See Appendix I

Inside cover page	Same as Cover page
Dedication page (if Any)	Should not exceed one page
Bonafide Certificate Guide	See Appendix II
Bonafide Certificate CoGuide	See Appendix III
Declaration of the Candidate	See Appendix IV
Abstract	Should not exceed two pages (about 600 words and should contain a maximum of 6 key words)
Acknowledgements	Should not exceed two pages
Table of Contents	See Appendix V
List of Tables	
List of Figures	
List of Symbols, Abbreviations or Nomenclature (Optional)	
Contents (Sample)	See Appendix VI

GUIDELINES FOR PREPARATION OF THESIS

RECOMMENDATION : TOTAL NUMBER OF PAGES IN THE THESIS NOT TO EXCEED 200- 250

THESIS FORMAT:

The thesis manuscript has three sections basically :

- the preliminary pages
- the Chapters
- the reference list.

PRELIMINARY PAGES :

The preliminary pages consist of the :

- I. Title Page
- II. Certificate from Research Guide
- III. Certificate from Research Coguide
- IV. Declaration Certificate
- V. Abstract
- VI. Acknowledgements
- VII. Dedication (optional)
- VIII. Table of contents
- IX. Abstract (start numbering pages in small roman numerals)
- X. List of tables
- XI. List of figures
- XII. Other lists if any.

Preliminary pages are numbered separately from the rest of the text.

Numbering of Pages :

- The title page has no number but is counted for the total number of pages.
- Page numbers shall be placed in lowercase, Roman numerals centered at the bottom of the preliminary pages. This numbering is continued in all the preliminary pages (up to the first page of the text).

ORDER OF PRELIMINARY PAGES:

1. Title Page

- The title of the thesis should be as concise and congruent in every respect including - punctuation, capitalization, hyphenation, on the abstract, approval forms and other documents submitted along with the thesis.
- On the title page, the identical title must appear in all capital letters with every line centered on the page.
- The month in which the thesis is submitted, e.g., May / August / January with the year of submission is to be printed at the bottom of the page (as shown in the annexure).

2. Abstract

- The abstract should provide a brief, descriptive account of the thesis not exceeding 600 words, 1.5 spaced lines and should adhere to the same style as the thesis manuscript. The abstract may include specific names of places and other proper nouns.

3. Dedication (optional)

- The dedication is brief, single-spaced, and centered on the page (horizontally and vertically). Dedication customarily begins with the word "To".

4. Acknowledgement

- Title of this page ACKNOWLEDGEMENTS is centered in all capital letters. The following may be thanked in this section :
- Persons who have been rendered help to the student in completing the degree requirements.
- Acknowledgement of grants and special funding from funding agencies, received in support of the research.

5. Table of Contents

- The words TABLE OF CONTENTS must be centered, page two inches from the top of the first page only.
- The Table of Contents must include all chapter headings, the bibliography and appendices.
- Line spacing : in an entry 1.15; between two different Entries - 1.5.
- The headings of major sections (i.e., chapters, bibliography, references, appendices) are written in all capital letters.
- Headings must be identical to both in the Table of Contents and in the text.

6. List of Tables (if tables appear in the thesis)

- The heading, LIST OF TABLES, appears centered on the page two inches from the top of the first page only.
- All table numbers and captions are listed exactly as they appear in the text.

7. List of Figures (if figures appear in the thesis)

- The heading, LIST OF FIGURES, appears centered on the page two inches from the top of the first page only.
- All figure numbers and captions are listed exactly as they appear in the text.

8. List of Abbreviations (nomenclature) used:

- The heading, ABBREVIATIONS, appears centered on the page two inches from the top of the first page only.
- Symbols used, their meaning, and units are given in this section
- The expansions of all the abbreviations, symbols used should be listed in alphabetical order.

9 Other Lists (Definitions, Glossary of terms, etc.)

- The appropriate title in all capital letters is centered two inches from the top of the first page only for each of these sections.

10. References:

- The references should be in **VANCOUVER** style
- For eg: Miller DJ, Shugars DA. The Health of the Dental Professional: American Dental Association Councils. J Am Dent Assoc. 1987; 114(4):515-8.
- line spacing in the references should be 1.15 and the spacing between the references 1.5

PAGE DIMENSIONS AND MARGIN

QUALITY OF THE PAPER : Good quality white paper preferably not lower than 80 gsm.
SIZE OF THE PAPER : **Standard A4 size** (210 mm X 297 mm) paper should be used for preparing the copies.

DIMENSIONS OF THE PAGE MARGINS:

Top edge : 1 inch (25 mm)
Left side : 1 ½ inch (38 mm) [For binding]
Bottom edge : 1 inch (25 mm)
Right side : 1 inch (25 mm)

TYPE-SETTING, TEXT PROCESSING AND PRINTING

PRINTING : Laserjet /Inkjet printer, the text having been processed using a standard text processor.

STANDARD FONT TYPE : Times New Roman

FONT SIZE : 12 pts

LINE SPACING : 1.5

The text must be 1.5 lines spaced and printed on only one side of each page.

TEXT

- The text must be divided on a style that is followed consistently throughout the document.
- Chapter organization as practiced by the discipline should be followed.
- The larger divisions and more important minor divisions are indicated by suitable, consistent headings / sub-headings.
- The student, the Guide, the co-guide and the academic department are responsible for the quality and content of the text.

Some specific requirements for text presentation :

1. Headings and Subheadings

- Headings (Font Size 14, bold, Times New Roman) and sub-headings (Font Size 13, bold, Times New Roman) maybe used to subdivide chapters or sections, but a consistent sequence of headings as identified in the style selected by the Research Guide must be followed.
- The student shall not change the sequence and style of headings from chapter to chapter.
- Once the sequence is chosen, it must be followed consistently throughout the thesis.

2. Pagination

- No number appears on that title page.
- In the subsequent pages, lower-case, Roman numerals are used sequentially to number all the preliminary pages preceding the text.
- Beginning with the first page of the text, all pages are to be numbered with Arabic numerals, positioned at the bottom of the page, centered between the margins, consecutively, throughout the thesis document, including the appendix, the bibliography, list of references.
- Page headers or running heads may not be used in the thesis.

3. Tables and Figures (Floaters):

- "Table" refers to a columnar arrangement of information, often data sets, organized to save space and convey relationships and comparisons at a glance.
- The tables should be numbered in Arabic numerals in sequential order as they appear in the text as shown in example cited below.
- Each table should have a title (header) comprising of a brief description, highlighting the relationships of the data contents in the table.
- For eg : Table 1.3 : A comparison of the enzyme activity at 30° C and 37° C. (table number 1.3, 1 stands for chapter number and .3 for the table number in the chapter).
- The term "figure" refers to graphs, drawings, diagrams, charts, maps, illustrations or photographs.
- The figures should be numbered in Arabic numerals in sequential order as they appear in the text.
- Each figure should have a legend below the figure / picture / graph, comprising of a brief description, of the figure / picture / Illustrations / photographs / graph with experiment.
- For eg : Fig. 1.6 : Effect of temperature on enzyme activity at 30° C and 37° C. (table number 1.6, 1 stands for chapter number and .6 for the figure in the chapter).
- The header (**bold**) and the legend should be in font size 10, italics, Times New Roman and 1.15 line spacing.
- Tables and Figures should be inserted in the text near where they are first mentioned.
- A table or figure may appear on the same page as the text that refers to it or on a separate page. However, each figure or table must be numbered and have a legend or title respectively.

4. Illustrations

- Every illustration material
- must be referred to in the text
- must be placed after and as near as possible to the first reference to it in the text.
- must be prepared on paper that is the same weight (or stronger).
- use the same font type as elsewhere in the manuscript.
- If illustrations are mounted
- dry mounting must be used.
- may not be mounted with rubber cement, staples, mucilage, or photomounting corners.
- Illustrations must be
- drawn or computer-generated in black ink.
- may be laser-printed or drawn in waterproof, permanent ink.
- Color should be used only if it is essential to the thesis.

5. Photographs

- High quality, high contrast copying machine should be used to reproduce photographic material for submission in lieu of photographs.
- Original photographs should be printed on single-weight, fiber-based paper with a matte finish.
- Photographic prints must be processed for established standards for chemical permanence.
- Black-and-white prints are preferable.
- Photograph page number placement follows the standard pagination style.

6. Appendix / Appendices

- The appendix or a series of appendices maybe included immediately following the main

text.

- The appendix includes material that is helpful to the reader of the thesis but is too lengthy for inclusion in the text or footnotes.
- The title, APPENDIX, appears only on the first page of the section, in capital letters centered two inches from the top.
- Examples of such material include questionnaires, letters, original data, sample forms and vitae.
- Appendix is numbered with roman numerals, uppercase, as follows Appendix I.
- Each appendix is a separate subdivision of the text and must begin on a separate page
- The materials in the Appendix should be referred to in appropriate places in the text as follows (Appendix I) .
- Each appendix must be listed in the List of Appendices.

ORGANISATION OF THE THESIS

- The thesis shall be presented in a number of *chapters*, starting with **Introduction** and ending with **Summary and Conclusions**.
- Each of the chapters shall have an accurate title indicating the contents of the chapter.
- A chapter may be subdivided into *sections*, *sub-sections* and *subsub-section* so as to present the content systematically and with due emphasis.
- **Each chapter shall begin on a fresh page**
- However the sections / subsections in the chapter will be numbered as follows :

1. Chapter and Section format : Introduction

- The title of **CHAPTER 1** shall be **Introduction**. Introduction justifies and highlights the problem poser and defines the research problem / topic, the aim, objectives and scope of the work presented in the thesis. It may also highlight the significant contributions from the investigation.
- Chapter numbering should be centered on the top of the page using large bold print using only Arabic numerals.

Constitution of a chapter :

- A chapter can be divided into **Sections, Sub-sections and Sub-sub-sections** so as to present different concepts separately and in a sequential manner.
- Sections and sub-sections can be numbered using decimal points, e.g., 3.2 for the second Section in Chapter 3 and 3.2.4 for the fourth Subsection in second Section of Chapter 3.
- Only Arabic Numerals with decimals may be used.
- Section numbering should be left justified using large bold print.

2. Review of Literature

- This is normally the **CHAPTER 2** and presents an critical overview of the previous work published in the literature pertaining to the topic of the investigation. The extent and emphasis of the chapter shall depend on the nature of the investigation to be done in the project.
- Each literature discussed shall have a backup with the appropriate reference inserted in Vancouver style.
- **For Example,**
Several researchers attempted to develop mathematical models to simulate the activated sludge process. Some of these models simulate the organic removal mechanisms in wastewater treatment field (4 -7) [the appropriate references in the reference list will be numbered from 4 to 7].
or

The oxygen transfer mechanism has an important place in the activated sludge process. An estimation technique for the oxygen transfer capacity has already been established (8).

3. Methodology:

- The chemicals, reagents used in the study shall be listed in alphabetical order with the names of the manufacturing company
- The source from which the data was collected for the study with names of Institutions involved. For example, in clinical settings, it may be patients in hospitals, or in community settings, or it may be households or it may be laboratory based study.
- The method of collection of data. - interview / study of records / by animal experiments / by performing tests or laboratory Investigations / even through some intervention.
- In case of human / animal study, mention the inclusion and exclusion criteria. If there were any issues involved, mention them and state how you overcame.
- The sampling procedure and sample size.
- The parameters studied and the procedures used with appropriate references. The procedures shall be in a format such that the experiment could be repeated preferably with similar outcome with the given protocol.
- Give the operational definitions.

4. Results:

- This chapter shall comprise of results of the investigations carried out in the form of figures (either graphs, tables, histograms, piecharts, etc) in sequential order of the experiments outlined in the materials and methods section.
- Every figure will have a number followed by a legend (printed in the specification already mentioned) below it.
- Figure should be in the vicinity of the referral text.
- The Figure number shall be quoted in the appropriate text.
- The inference of every figure has to be mentioned in the referred text of the figure

Table / Figure Format

- As far as possible tables and figures should be presented in portrait style. Small size table and figures (less than half of writing area of a page) should be incorporated within the text. However, larger figures / tables may be presented on separate pages.
- Table and figures shall be numbered chapter –wise.
- For example, the fifth figure in Chapter 4 will bear the number - *Fig. 4.5*
- Table number and title will be placed above the table while the figure number and caption will be located below the figure.
- Reference for Table and Figures reproduced from elsewhere shall be cited in the last, for e.g. [12].

Equations

- All the equations should be typed in equation editor and should be properly numbered
For Example,
$$\Delta X \propto X \Delta t \quad (2.1)$$

5. Discussion :

- This shall form the penultimate chapter of the thesis and shall include a thorough evaluation of the results of the investigation carried out and highlight the contributions from the study.

- The discussion should logically lead to inferences of the results of the study leading to the conclusions.
- The conclusions should also lead way to the scope for possible further future work.

6. Summary and Conclusions

- This is the final chapter of the thesis.
- 6.1 - A concise report of the work done.
- 6.2 - Findings of the study from results.
- 6.3 - logical analysis presented in the discussion
- 6.4 – Conclusions & limitations of the study.
- 6.5 – Scope of the future work should be clearly stated.

Appendices:

Should include scanned copies of the original ethical clearances obtained, permission letters to collect data from all the centers (if data has been collected from one or more centers), Request letter for validation of the tools, Copy of the tools used, Request letter for conducting the pre-testing, reliability and pilot study, copy of the informed consent, letter requesting the conducting of the study proper.

Binding

The thesis shall have a black laminated cover with golden letters.

Front Covers

The front cover shall contain the following details:

- Full title of thesis – Font size :20; Style : Times New Roman; Position : center justified and at the top, one and a half inches from the top edge.
- Full name of the candidate in 14 point's size font properly centered at the middle of the page. 1" below the title.
- A 1.25 " × 1.25 " size of the University emblem.
 - Name of the Department
 - Name of the Institute
 - The year of submission

each in a separate line, Times, New Roman, Bold, Capital, Font size 14 and properly centered and located at the bottom of the page.

TITLE OF THE THESIS

A thesis submitted for the award of the degree
of

**DOCTOR OF PHILOSOPHY
(Ph.D)
in**

Name of the Department

by

**Name of the Ph.D Scholar
workplace address of the Ph.D Scholar**

UNIVERSITY EMBLEM

**NAME OF THE DEPARTMENT
YENEPOYA MEDICAL COLLEGE
YENEPOYA UNIVERSITY
MANGALORE 575018
KARANTAKA, INDIA.**

AUGUST 2011

CERTIFICATE

This is to certify that the thesis entitled **TITLE OF THE THESIS** submitted by **NAME OF THE CANDIDATE** to the Yenepoya University, Mangalore 575 018, for the award of the degree of **Doctor of Philosophy (PhD)** is a bonafide record of research work carried out by him under my / our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Place: Mangalore

Date:

(Name of the Guide)

Research Guide

Department of
Name of Institute,
Yenepoya University
Mangalore 575 018.

CERTIFICATE

This is to certify that the thesis entitled **TITLE OF THE THESIS submitted by NAME OF THE CANDIDATE** to the Yenepoya University, Mangalore 575 018, for the award of the degree of **Doctor of Philosophy (PhD)** is a bonafide record of research work carried out by him under my / our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Place: Mangalore

Date:

(Name of the Coguide)

Coguide

Department of
Name of Institute,
Yenepoya University
Mangalore 575 018.

DECLARATION

I hereby declare that the thesis titled -----
submitted to the Yenepoya University, Mangalore 575018 for the award of the degree of **Doctor of
Philosophy (PhD)** in the faculty of _____ is the result of the
study carried out by me under the guidance of **Name & address of the Research Guide** during the
period 20__ to 20__ .

I further declare that I have not submitted any of the results of the study for the award of any other
degree or diploma.

Place: Mangalore

Date:

**Name & Signature
of the Ph.D Scholar**

Title	Page
Abstract	i
List of tables	ii
List of figures	iii
List of Appendices	iv
List of Abbreviations	v
Definition of terms	vi

CONTENTS (Sample)

Chapter	Topic	Page
1.	Introduction	1
1.1.	5' Nucleotidase	1
1.1.1	Distribution of 5' nucleotidase	2
1.1.2	Plant nucleotidase	2
1.1.3	Bacterial 5' nucleotidase	3
1.1.4	Animal 5' nucleotidase	4
	Membrane bound 5' nucleotidase	4
	Structure of 5' nucleotidase	6
	GPI- anchor of the enzyme	7
	Cytosolic forms of 85' nucleotidase	8
	Mitochondrial 5' nucleotidase	10
1.2	Other nucleotidase	11
1.2.1	Pyrimidine 5' nucleotidase	11
1.2.2	3' nucleotidases	11
1.2.3	Serum nucleotidase	12
1.3	5' nucleotidase inhibitors	13
1.3.1	Purification of the 5' nucleotidase inhibitor	14
1.3.2	Non-protein inhibitors	15
1.4	Functions of 5' nucleotidase	16
1.4.1	Adenosine receptors	20
1.4.1.1	Structure of adenosine receptor -AR	21
1.4.1.2	Regulation of transmembrane signalling system	27
1.4.1.3	Receptor effector coupling	31
1.4.2	Role of adenosine in cardiac tissues	31
1.4.3	Role of adenosine in inflammation	37
1.5	Background of the work	40
1.6	Purpose of the present study	41
1.7	Objective of the present study	41
2.	Review of Literature	
3.	Methodology	43
3.1	Materials	43
3.2	Methods	45
3.3	5' nucleotidase enzyme assay	45
3.3.1	Assay of inorganic phosphate by malachite green method	45
3.3.1.1	Effect of various ions on malachite green method of	46
3.3.1.2	phosphorus estimation	
3.4	Effect of heat treatment on 5' nucleotidase activity of serum	46
3.5	Effect of heat serum sample on normal serum	46

3.6 Partial purification of 5' nucleotidase from placental homogenate for inhibitor assay	47
3.6.1 Preparation of placental homogenate (source of 5' nucleotidase for inhibitor assay)	47
3.6.2 Preparation of AMP Sepharose column	47
3.6.3 Separation of 5' nucleotidase from placental homogenate on AMP Sepharose column	47
3.7 Characterization of placental 5' nucleotidase	48
3.7.1 Stability of the placental 5' nucleotidase	48
3.7.2 Effect of pH on placental 5' nucleotidase	48
3.7.8 Effect of various heavy metal ions on placental 5' nucleotidase activity	48
3.8 Inhibitor assay	49
3.8.1 5' nucleotidase inhibitor assay	49
3.8.2 Inhibitor assay with heat treated serum samples	49
3.9 Isolation of the inhibitor of 5' nucleotidase from serum	50
3.9.1 Isolation of inhibitor by ion-exchange chromatography – DEAE-Sephadex column	50
3.9.2 Isolation of inhibitor by gel filtration- Sephadex G-100 column	50
3.9.2.1 Comparison of elution pattern of 4 different serum samples through the same Sephadex G-100 gel filtration column	51
3.9.3 Isolation of inhibitor from normal urine using Sephadex G-100 column	52
3.10 Kinetic studies with the inhibitor	52
3.10.1 Determination of K_m and V_{max}	52
3.10.2 Determination of IC_{50}	53
3.10.3 Calculation of K_i from IC_{50}	53
3.11 Characterization of the inhibitor	53
3.11.1 SDS polycrylamide gel electrophoresis (SDS-PAGE)	53
3.11.2 Dialysis of inhibitor	53
3.11.3 Lyophilization of inhibitor fraction	53
3.11.4 Stability of the inhibitor	54
3.12 Qualitative tests done to identify the inhibitor from normal constituents of serum	54
3.12.1 Molisch test	54
3.12.2 Benedict's test	54
3.12.3 Colour reaction for sialic acid	55
3.12.4 Colour reaction for fructose	55
3.12.5 Biuret test for protein	56
3.12.6 Ninhydrin test for amino acids	56
3.13 Qualitative tests done to differentiate the inhibitor from the normal constituents in urine	56
3.13.1 Phosphotungstic acid reduction test for uric acid	57
3.13.2 Schiff's test for uric acid	57
3.13.3 Jaffe's test for urobilinogen	57
3.13.4 Erlich's test for urobilinogen	58
3.13.5 Enzymatic method (uricase enzyme) for uric acid	58
3.14 Quantitative Analysis	58
3.14.1 Total carbohydrate content	58
3.14.1.1 Estimation of glucose	59
3.14.1.2 Hydrolysis of inhibitor before glucose estimation	59
3.14.2 Protein content	60

3.15 PAPER CHROMATOGRAPHY OF INHIBITOR	60
3.15.1 Hydrolysis of inhibitor for chromatography	60
3.15.2 Carbohydrate paper chromatography	61
3.16 TREATMENT STUDIES	61
3.16.1 Precipitation studies with inhibitor	61
3.16.2 Hydrolytic studies	61
3.16.2.1 Acid hydrolysis	61
3.16.2.2 Alkali hydrolysis	62
3.16.2.3 Enzymatic hydrolysis- trypsin	62
3.17 Dilution studies of the inhibitor	62
3.17.1 Acid medium	62
3.17.2 Neutral medium	62
3.17.3 Alkaline medium	62
3.18 Glucuronic acid as inhibitor	63
3.19 Ninhydrin test with glucosamine	63
3.20 Test of the inhibitor for direct bilirubin (bilirubin glucuronide)	63
3.21 Creatinine as inhibitor	64
3.22 Heparin as inhibitor	64
3.23 Reviparin as inhibitor	64
3.24 Clinical studies	64
3.24.1 Enzyme 5' nucleotidase and its inhibitor activity in hyperuricemic samples	64
3.25 Test for rheumatoid factor (RF) with the inhibitor – Humatex RF Test	64
3.26 Studies with cultured cells	65
3.26.1 Preparation of polymorphonuclear leukocytes	65
3.26.2 Total leukocyte count	66
3.26.3 Differential count of WBC	66
3.26.4 Labelling of PMNLs with ¹¹¹ indium (¹¹¹ in)	67
3.26.5 HUVEC cultures	67
3.26.5.1 Isolation of HUVECs from umbilical cords	67
3.26.5.2 Passages of HUVEC cultures	69
3.26.6 Standardization of PMNL adhesion assay	69
3.26.6.1 Stimulation of HUVECs	69
3.26.6.2 Standardization of time of adhesion	70
Standardization of number of PMNL used in the adhesion assay	70
3.27 Treatment of HUVECs with inhibitor	71
3.27.1 PMNL adhesion	71
3.27.2 Measurement of calcium transients	71
3.27.3 Inhibition of 5' nucleotidase on the HUVECs	72
3.27.4 Effect of 5' nucleotidase inhibitor on adhesion molecules:	72
3.27.5 Inhibition of action of Staphylococcus Aureus sphingomyelinase	73
3.27.6 Action of adenosine on LPS mediated cell adhesion:	73
3.28 IL-6 ELISA	
4. Results	75
4.1 Malachite green method	75
4.2 Effect of ions on malachite green method	76
4.3 5' nucleotidase enzyme assay	77
4.4 Effect of heat on 5' nucleotides activity of serum	77

4.5 Effect of mixing heated serum with untreated serum	78
4.6 Assay of inhibitor activity	78
4.7 Separation of 5' nucleotidase from placental homogenate on AMP- Sepharose column	79
4.8 Characterization of the placement enzyme	80
4.8.1 Stability of the placement enzyme	80
4.8.1.1 Effect of ph on placental 5' nucleotidase	81
4.8.1.2 Effect of various ions on the activity of placental 5' nucleotidase	82
4.9 Inhibitor assay	83
4.9.1 5' nucleotidase inhibitor assay	83
4.10 Effect of heat treated serum on 5' nucleotidase activity	84
4.11 Isolation of the inhibitor of 5' nucleotidase from serum	85
4.11.1 Isolation of inhibitor by gel filtration on DEAE-Sephadex	85
4.11.2 Purification of inhibitor from serum in a Sephadex G-100 column	86
4.11.2.1 Comparison of elution profiles of four different serum samples through Sephadex G-100 column	87
4.11.3 Isolation of the inhibitor of 5' nucleotidase from urine using a Sephadex G-100 column	88
4.12 Kinetic studies with the inhibitor	89
4.12.1 Determination of K_m and V_{max}	89
4.13 Characterization of inhibitor	92
4.13.1 SDS- Polyacrylamide gel electrophoresis: (SDS- PAGE)	92
4.13.2 Dialysis of the inhibitor	92
4.13.3 Lyophilization of the inhibitor fractions	92
4.13.4 Stability of the inhibitor	92
4.13.5 Qualitative tests done to identify the inhibitor from normal constituents of serum	93
4.13.6 Qualitative tests done to identify the inhibitor from normal constituents of urine	94
4.13.7 Quantitative analysis	95
4.13.7.1 Total carbohydrate content	95
4.13.7.1.1 Estimation of glucose in the inhibitor before and after hydrolysis	95
4.13.7.2 Protein content content	95
4.13.8 Paper chromatography of inhibitor	95
4.13.8.1 Carbohydrate paper chromatography	95
4.13.9 Treatment studies	96
4.13.9.1 Precipitation studies with the inhibitor	96
4.14 Dilution studies with the inhibitor	98
4.15 Glucuronic acid as inhibitor	99
4.16 Ninhydrin test with glucosamine	100
4.17 Test for direct bilirubin (bilirubin diglucuronide) with the inhibitor	100
4.18 Creatinine as inhibitor	100
4.19 Heparin as inhibitor	100
4.20 Reviparin as inhibitor	100
4.21 Clinical studies	101
4.21.1 5' nucleotidase enzyme and its inhibitor activity in hyperuricemia sample	101
4.21.2 Test of inhibitor for RA factor	102
4.22 Effect of inhibitor on the 5' nucleotidase activity in HUVECs at seven passages	102

4.22.1	Inhibition of 5' nucleotidase of HUVECs	104
4.22.2	Standardization of PMN- Leukocyte adhesion assay	105
4.22.2.1	Effect of number of PMN- Leukocytes added on percent adhesion	106
4.22.2.2	Effect of the number of PMN- Leukocytes added on the DPM adherence	106
4.22.2.3	Day to day variation in the percentage adhesion	107
4.22.2.4	Effect of time of adhesion	108
4.22.2.5	Effect of total radio active counts in the PMN- Leukocytes on the percentage adhesion	109
4.22.2.6	Effect of HUVEC cell density on percentage adhesion	110
4.22.3	Effect of adenosine on LPS induced PMN adhesion	112
4.22.4	Effect of 5' nucleotidase inhibitor on adhesion molecules	114
4.23	Estimation of IL-6	115
4.24	Inhibition of action of <i>Staphylococcus aureus</i> sphingomyelinase	117
4.25	Effect of adenosine and 5' nucleotidase inhibitor on calcium modilization in PMN Leukocytes	118
4.25.1	Effect of adenosine and 5' nucleotidase inhibitor on calcium modilization in HUVECs	119

5. Discussion 120

5.1	Purification of the 5' nucleotidase inhibitor from human serum	124
5.2	Characterization of the inhibitor	128
5.2.1	Molecular weight	144
5.2.2	Treatment studies	144
5.2.3	Hydrolytic studies	145
5.2.4	Trypsin Hydrolytic studies	146
5.3	Dilution studies	146
5.4	Kinetic characterization	147
5.5	Effect on endothelial cells and WBCs	148
5.6	Action of the inhibitor through adenosine	150
5.3	Inflammation	155
5.3.1	Vascular endothelial cells	155
5.3.2	Polymorphonuclear leukocytes (PMNL)	156
5.3.3	Role of adenosine	157
5.3.4	Role of the 5' nucleotidase inhibitor	158

6. Summary and conclusions 160

References	164
-------------------	------------

Appendices