

**UNIVERSITY OF MEDICINE AND PHARMACY  
OF CRAIOVA  
DOCTORAL SCHOOL**



# **PhD THESIS SUMMARY**

**CONTRIBUTIONS TO THE PREPARATION OF  
CAPSAICIN-CYCLODEXTRIN INCLUSION  
COMPLEXES AND THEIR INCLUSION INTO  
SEMISOLID PHARMACEUTICAL FORMS**

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**Keywords:** inclusion complexes, capsaicin,  $\alpha$ -cyclodextrins, ointments

### ABBREVIATIONS

DPPH•	1,1-diPhenyl-2-picrylhydrazyl radical
SRO	Reactive oxygen species
IC50	Concentration causing 50% inhibition of a given parameter
GC-MS	Gas chromatography coupled with mass spectrometry
FT-IR	Fourier Transform Infrared Spectroscopy
HPLC	High performance liquid chromatography
DAD	Diode array detector
XRD	X-ray diffraction
FDA	Food and Drug Administration
UFC	Colony forming units
CLSI	Clinical and Laboratory Standards Institute
CMI	MIC
OMS	World Health Organization
F.R.X	Romanian Pharmacopoeia X edition
$\alpha, \beta, \gamma$ - CD	$\alpha, \beta, \gamma$ - Cyclodextrins
C	Capsaicin
UV-VIS	Ultraviolet-visible spectrophotometry
PEG	Polyethyleneglycols
H,L	Hydrophilic, lipophilic
CMC	Carboxymethylcellulose
MC	Methylcellulose
APV	Polyvinyl alcohol
DHC	Dihydrocapsaicin
NDHD	Nordihydrocapsaicin
HDHC	Homodihydrocapsaicin
TRPV	Transient receptor potential cation channel
DL50	The medium lethal dose

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Rpm	Revolutions per minute
2-HP $\beta$ CD	2-hydroxypropil-beta-cyclodextrin
MS	Mass Spectrometry
GAE	Gallic acid equivalents
QE	Quercetin equivalents
TEAC	Trolox equivalents

## INTRODUCTION

### GENERAL CONSIDERATIONS

Capsaicin is a natural active ingredient, the major component of pungent *Capsicum* genus species (Fam. Solanaceae).

Capsaicin has been shown to have a variety of pharmacological effects, and it is used in recent studies published in the literature as anti-cancer<sup>1</sup> agent in several types of cell lines, initiated in different kinds of tumors such as breast cancer<sup>2</sup>, colon carcinoma<sup>3</sup>, nasopharyngeal cancer<sup>4</sup>, gastric cancer and pancreatic cancer<sup>5</sup>.

A drawback of the use of this substance is represented by the poor solubility which reduces its bioavailability in the human body.

On the other hand, it is known that cyclodextrins are chemical substances with high molecular weight that form inclusion complexes with various drugs, thereby helping to easier release the drug. Those CD are transportation agents with a hydrophilic exterior cavity providing an internal hydrophobic matrix.

In literature there are many studies of the synthesis of complex type drug /  $\beta$ -cyclodextrin. Studies with  $\alpha$ -CD are quite limited, however, as those with  $\gamma$ -CD that are affordable and cost less.

### PROPOSED OBJECTIVES

For this thesis, capsaicin was chosen as an active ingredient and  $\alpha$ -CD of the aforementioned considerations in order to synthesize a complex liquid phase capsaicin /  $\alpha$ -CD which was subsequently included in semisolid pharmaceutical formulations.

Another argument for the choice of this product was the spread *Capsicum annum* species, species that underlies natural capsaicin obtaining in our country and its accessibility.

In order to obtain the best results a multidisciplinary study was realised (instrumental analysis, pharmaceutical technology, biochemistry, pharmacognosy, microbiology, cell biology) and it is applied in several methodologies that have led to the development of a pharmaceutical formulation with the active ingredient capsaicin.

Research methodology included:

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1. Qualitative and quantitative analysis of the composition of capsaicin *Capsicum annuum*.
  2. Establish anatomical parts with maximum capsaicin pepper.
  3. The development of methods for extraction and concentration of capsaicin from biological material with maximum efficiency.
  4. Spectral analysis of the main components involved in the research aspect: capsaicin, alpha-cyclodextrin complex thereof.
  5. Determining the physicochemical methods of analysis of the combination and the easiest conditions for obtaining a bigger quantity of complex.
  6. Determination of the main auxiliary components used for the application of an ointment.
  7. Follow opportunities to interact with complex combination ointment base and creating optimal conditions for the use of this ointment for purpose.

## **CURRENT STATE OF KNOWLEDGE**

### **CHAPTER 1. Literature review**

**Chapter 1** contains a bibliographic study of literature describing semisolid pharmaceutical preparation. According to FR. X ointments are semisolid pharmaceutical preparations applied on the skin or mucous membranes, protective or therapeutic purposes. They are made up of excipients (ointment base) which may incorporate the active ingredients<sup>6</sup>. Here, in subsection 1.2 described as an active ingredient, capsaicin has numerous pharmacological properties. The last chapter (1.3) is dedicated to studies regarding the use of  $\alpha$ -cyclodextrin drug formulations.

### **CHAPTER 2. Preliminary studies of the species *Capsicum annuum***

In this chapter there were conducted preliminary studies on the species *Capsicum annuum*. Thus, there were determined the percent of water, dry matter and ash powder plant. FT-IR analysis performed to confirm the presence of important classes of secondary metabolites which have subsequently been identified by GC-MS. Among the identified compounds, an important number was shown to have pharmaceutical properties, including capsaicin.

Subchapter 2.2. It is dedicated exclusively to determining polyphenolic extracts profile of *Capsicum annuum* ultrasonicated by HPLC. Using a simple method for quantification, we succeeded eleven dosing acid derived from hydroxycinnamic acids and five flavonoids. It has been found to be present in relatively high concentration compared to other species of ellagic acid in all samples. Framed same chapter was aefectuat and a parallel study which determined the total polyphenol (Folin-Ciocalteu method), total flavonoids and antioxidant activity. The results of this

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study revealed that alcoholic extracts of *Capsicum annuum* exhibit significant antioxidant, the highest antioxidant activity being obtained extract of green peppers (723,795 mg / L GAE, 3.315 mg / L equivalents trolox and 327,394 mg / L QE). In addition, the antibacterial activity of the tested extracts was significantly higher compared to the standard capsaicin used as a positive control against *Enterococcus faecalis*, *E. coli* and *Bacillus subtilis*, those having similar capsaicin strain with *Staphylococcus aureus*. The results of the *in vitro* cytotoxicity tests revealed that the extracts tested showed a good biocompatibility and did not alter the morphology of HaCaT cells. Taken together, these biological properties indicate that these extracts can be used in various biomedical applications, such as anti-rheumatic agents, anti-inflammatory and anti-microbial.

### **CHAPTER 3. DETERMINATION OF CAPSAICIN ROM CAPSICUM ANNUUM BY HPLC**

**Chapter 3** is a brief survey of a study where two strains of *Capsicum annuum* L. were analyzed for their content in capsaicin using a simple RP-HPLC. Capsaicin extraction was accelerated by ultrasonication. The highest content was found in dried green pepper (85,262 mg / L), followed by dried red peppers with the seeds and ribs removed (75,457 mg / L).

### **CHAPTER 4. SYNTHESIS AND CHARACTERIZATION OF A-CYCLODEXTRIN-COMPLEX CAPSAICIN**

**Chapter 4** covers the synthesis of alpha-cyclodextrin inclusion complex of capsaicin for which both fitting equation obtained by UV-Vis and fluorescence complexing showed a 1: 1 ratio. HPLC assay confirmed qualitatively and quantitatively the presence of capsaicin in the complex, to give a good yield of incorporation thereof.

### **CHAPTER 5. PHARMACEUTICAL APPLICATIONS OF THE COMPLEX A-CD / CAPSAICIN**

In **chapter 5** it was found that on a 6 hours period there is a 59% more release of the active principle contained in conveyor, the inclusion complex formulation helping to increase the amount of capsaicin which diffuses through the membrane<sup>7</sup>. The percentage can be adjusted by changing the type of the used membrane, we intend such an approach in the following studies.

In section 5.3 rheological tests were performed on the simple ointment base, then we included the capsaicin and the capsaicin- $\alpha$ -CD complex in the simple ointment base, and measured for each compound its viscosity. The values of the shear rate of 240 s<sup>-1</sup> over the capsaicin H/L ointment produced a thixotrope removal properties of the ointment base. The same can be observed at shear rates below 240 s<sup>-1</sup> but this time in reverse. Based on these findings we can say that the H/L ointment has greater stability.

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