POLITECNICO DI TORINO

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Crystal engineering: a novel strategy for the resolution of chiral compounds

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Abstract

The chemical industry often deals with chiral molecules, also called enantiomers. These are two molecules with the same chemical formula but that are mirror-like due to the different orientation of some functional groups. Enantiomers of the same molecule are often indicated with D and L or with R and S. Both enantiomers of the same molecule have the same physical-chemistry properties, but they might differ in pharmacological active and bioavailability.

In fact, one enantiomer might provide the desired effect while the other might be ineffective or, even worse, toxic. For this reason, chirality is an important issue in the pharmaceutical industry, where enantiomers of Active Pharmaceutical Ingredients (APIs) need to be carefully differentiated and separated.

To overcome this challenge, crystallization comes might be a suitable selective separation strategy. In fact, by creating a diastereomers salts of one specific enantiomer it is possible to separate two chiral components without the use of expensive techniques, such as chromatography.

This separation process, also called resolution of a racemic mixture, occurs by "Resolving agent" to the solution. The resolving agent can preferentially precipitate with a specific enantiomer, allowing the separation from the other.

In this work different amines with a chiral centre were tested as resolving agents for a model compound, Malic Acid. The natural enantiomer of the molecule is the L form; hence the use of resolving agents can allow the isolation of the D form. A screening of different compound was carried out through slurry crystallization.

Afterward, a complete characterization of the crystal is done extrapolating all the necessary information. Result show that (1S,2R)-2-Amino-1,2-diphenylethanol, (R)-1-Phenylethylamine, (S)-1-Phenylethylamine, (R)-1-(1-napthyl)ethylamine and (S)-1-(1-napthyl)ethylamine lead to diastereomeric salt formation.

Acknowledgments

First and foremost, I would like to show all my gratitude to Prof. Elena Simone, she made me discover a world foreign to me. Despite not physically close, her presence has been crucial, for any of my doubts, problems, or moments of discouragement. I would like to take this opportunity to publicly apologize for the drafts sent at unlikely times. Thank you especially for making me realize that it is not necessary to know everything as much as to want to learn.

This thesis was carried out at Université catholique de Louvain, in Louvain-la-Neuve in Belgium.

I was hosted by Prof. Tom Leyssens who I would like to thank both for the opportunity and also, his hospitality and kindness. As soon as you enter in his laboratory, it is impossible not to notice his helpfulness and care for all his students.

Lastly, I would like to thank all the people who are part of his group, starting with my supervisor Camila Caro Garido for having the patience to teach me and getting me to work on her project. I was fascinated by the environment of sharing among all of them, listening and seeing how they exchange ideas and advice, aroused in me lot of curiosity. I wish all of them to succeed. This experience and everything I have learned; I will always carry it with me.

Summary in Italian

Introduzione

La cristallizzazione viene utilizzata in vari ambiti dell'industria chimica, come metodo di produzione ma anche in operazioni di downstream come separazione e purificazione. Un composto chimico disciolto in soluzione può essere separato facilmente se questo cristallizza in fase solida. La cristallizzazione è un passo fondamentale nella produzione di Principi attivi farmaceutici (Active Pharmaceutical Ingredients) abbreviato APIs. Le caratteristiche dei cristalli come dimensione e forma saranno fondamentali per definire le performance della formulazione finale come solubilità, biodisponibilità, velocità di dissoluzione e stabilità.

La proprietà di una molecola organica di non essere sovrapponibile con la sua immagine speculare, né attraverso una rotazione né attraverso una traslazione viene definita chiralità. Due immagini speculari della stessa molecola vengono chiamate "Enantiomeri" ed entrambi presentano le stesse proprietà chimico-fisiche: densità, solubilità, temperatura di ebollizione, proprietà termodinamiche e spettrali ad eccezione dell'attività ottica. Per differenziare le due molecole, vengono spesso indicate con D ed L oppure con R ed S.
Una miscela contenente una stessa quantità dei due enantiomeri viene denominata "Racemica".
É molto importante, soprattutto in ambito farmaceutico, separare due enantiomeri. Questo perché è possibile che i due enantiomeri della stessa molecola abbiano differente attività biologica oppure un differente valore economico da cui deriva la necessità di separarli. Per separare un composto enantiopuro si possono seguire diverse strade; cristallizzazione preferenziale, cromatografia chirale oppure la formazione di sali diastereoisomerici.

La formazione di sali diastereoisomerici si ottiene aggiungendo un agente risolutivo chirale ad una miscela racemica. In questo modo i sali diastereoisomerici ottenuti non sono più composti chirali e presentano differenti proprietà fisico chimiche come la solubilità. Questo permette di poter separare l'enantiomero indesiderato attraverso un processo di cristallizzazione preferenziale. Infatti, mentre il diastereoisomero più solubile rimarrà nel liquido madre quello meno solubile cristallizza. Infine, il legame fra l'enantiomero e l'agente risolutivo potrà essere rotto sciogliendo il sale in un solvente puro per recuperare la molecola di interesse. L'agente risolutivo rappresenta un punto fondamentale. Questo deve avere i gruppi appropriati per formare un sale con l'enantiomero ed inoltre fornire un enantiomero puro e facilmente recuperabile.

In questo progetto di tesi sono state studiate le due forme enantiomeriche dell'Acido Malico (D-L). L'enantiomero presente in natura è la forma L, anche quella biologicamente attiva.

Il ruolo dell'agente risolutivo è affidato ad una lista di ammine chirali. L'obiettivo iniziale è quello di trovare un'ammina chirale in grado di creare sali diastereoisomerici. Successivamente sono state studiate diverse proprietà dei sali appena creati, incluse la morfologia, i valori di solubilità, le potenziali varie fasi solidi dei sali e le temperature di fusione e degradazione.

Materiali e metodi

Questo capitolo comprende un breve riassunto dei composti e dei solventi utilizzati. Successivamente, viene descritta in dettaglio la procedura per generare i cocristalli e viene fornita una panoramica completa delle apparecchiature e della metodologia utilizzata per l'analisi di ogni campione.

Materiali

In laboratorio è stata preparata una soluzione al 5% e 10 % in volume di etanolo con una soluzione buffer 1M di $KPO4^-$ e pH 7. Sono stati utilizzate le due forme enantiomeriche dell'acido malico D ed L con una serie di coformatori, ammine chirali, elencati nella tabella 1:

Tabella 1 *Elenco dei composti utilizzati*

Composti utilizzati	
(1S,2R)-(-)-1-Amino-2-indanol	(±)-trans-1,2-Diaminocyclohexane
(1S,2R)-2-Amino-1,2-diphenylethanol	(S)-(-)-1-(1-naphtyl)-ethylamine
(R)-(-)-2-Amino-1-butanol	Aspartame
(R)-(-)-2-Amino-3-methyl-1-butanol	(R)-1-phenylethylamine
(R)-(+)-1-(4-Bromophenyl)ethylamine	DL-2-Phenylglycinol
(R,S)-1-(4-bromophenyl)ethylamine	L-malic acid
(S)-(-)-2-Amino-3-phenyl-1-propanol	(S)-2-aminobutanol
(S)-(+)-2-Amino-1-propanol	(1R,2R)-(-)-2-Amino-1-phenyl-1,3-propanediol
(S)-(+)-2-Amino-3-methyl-1-butanol	Praziquantel
(S)-(+)-2-Phenylglycinol	(8s 9r)-(-)-n-benzylcinchonidinium chloride
(S)-(+)-2-Pyrrolidinemethanol	(1R,2R)-4-nitrophenyl-2-aminopropane-1,3-diol
(S)-(+)-Leucinol	(R)-(+)-1-(1-naphtyl)-ethylamine
D-2 Phenylglycine	Phenylglycinamide (D)
L-2 Phenylglycine	(S)-2-(phenylamino)caronyloxy propionic acid
L-Tyrosine Hydrazide	(1S,2S)-(+)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol
S-Methyl-L-cysteine	D-malic acid
Stanozolol	(S)-1-phenylethylamine

Metodi

Nella figura seguente vengono mostrati tutti i passaggi della metodologia utilizzata per ogni test svolto.

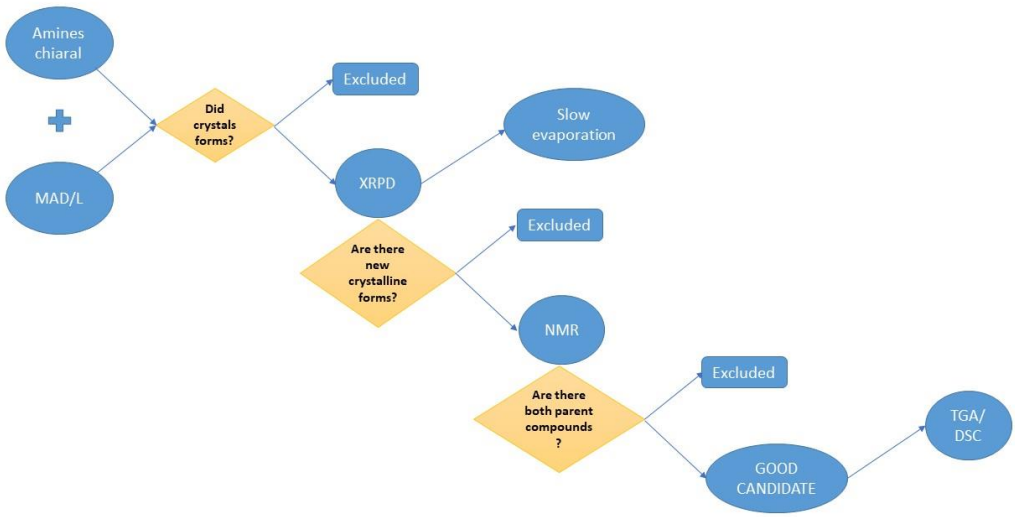

Figura 1 *Diagramma di flusso esplicativo della metodologia utilizzata*

Dopo aver preparato una soluzione supersatura (al 5% ed al 10% in volume in etanolo/soluzione di acqua) questa è stata posta in agitazione per 3-6 giorni a temperatura costante di 25°C fino ad un eventuale formazione del sale. Per ogni composto sono state effettuate un totale di 4 prove, l'ammina con la forma enantiomerica L in una soluzione al 5% ed al 10% e lo stesso con la forma D. I candidati che non hanno formato alcun cristallo sono stati scartati mentre per quanto riguarda gli altri campioni, si procede con la caratterizzazione.

Si inizia prelevando 1 ml di soluzione del sovranatante, che è stata inserita in dei flaconcini pesati con il tappo appositamente forato, poi destinati all'evaporazione lenta sotto una cappa controllata a temperatura costante di 25°C fino a completa asciugatura.

La rimanente parte è stata filtrata (la polvere) e posta all'interno dei tubi Eppendorf aperti per essiccare.

X-ray Powder Diffraction (XRPD)

Il primo step prevede di porre qualche mg di cristalli in un catodo di rame che inserito nell'apposita macchina verrà colpito da radiazioni elettromagnetiche. Le radiazioni interagiranno con il piano cristallino del solido ed il loro cambiamento di direzione è chiamato Diffrazione, fenomeno descritto dalla legge di Bragg. Dall'esperimento si ottiene un diffrattogramma, che registrata angoli 2θ da 5° a 35°, in cui sono visibili diversi picchi che forniscono informazioni sulla natura del solido, struttura, purezza e morfologia. Lo scopo di questa analisi è quello di verificare se il cristallo che si è formato presenta nel diffrattogramma nuovi picchi rispetto ai composti che l'hanno generato. Sono stati presi dal database CCDC le strutture 3D dell'acido malico L e dell'ammina chirale che poi sono state utilizzate nel programma Mercury per simulare il pattern 2D. Per l'acido malico sono stati usati due forme polimorfiche dell'enantiomero L ed una forma monoidrato come referenza.

Attraverso la sovrapposizione con i risultati dell'esperimento vengono fatti dei paragoni fra le posizioni dei picchi e la relativa intensità. I composti che non mostravano nuovi picchi sono stati esclusi mentre per i restanti si è proseguito con l'analisi.

Spettroscopia risonanza magnetica nucleare (NMR)

La risonanza magnetica nucleare è adatta alla risoluzione qualitative e quantitative di classi di composti organici in quanto consente di ottenere informazioni relative alla struttura del campione. Attraverso l'analisi spettrale dei protoni contenuti nei composti, si ottengono alcune informazioni relative al gruppo funzionale contenente i legami C-H. Il campione viene solubilizzato in DMSO (dimethyl sulfoxide), solvente deuterato per composti polari con un picco caratteristico a 2.5 ppm. Le informazioni che vengono estrapolate da questa prova sono

relative alla composizione chimica; quindi, possiamo stabilire se ci sono i relativi picchi dei composti di partenza e ipotizzare una stechiometria del cristallo.

In precedenza, sia l'AM che l'ammina chirale vengono esaminati separatamente ed i loro picchi registrati; quelli ampi derivano da ^{1}H labili che si scambiano con il solvente. Successivamente, dallo spettro risultante dall'esperimento si confrontano i picchi con quelli dei composti parentali, se ci sono nuovi picchi il composto viene escluso mentre se sono presenti si prosegue con la caratterizzazione termica.

Analisi Termogravimetrica (TGA)

L'analisi termogravimetrica è una tecnica molto utilizzata nello studio della stabilità termica e dei relativi meccanismi di degradazione. Una piccola quantità (5-7 mg) di campione viene riscaldata uniformemente in ambiente controllato e viene misurata la percentuale di massa persa in funzione di tempo e temperatura. Questa misura viene effettuata con una microbilancia, sensibile ai fenomeni fisici come l'adsorbimento superficiale, diverso dall'assorbimento in cui il solvente si trova nel reticolo cristallino, la decomposizione/degradazione e l'ossidazione attraverso la variazione di massa. Questo step serve per determinare dalla curva la temperatura di degradazione ma non si hanno informazioni né su come avviene né sulle transizioni di fase, quindi verrà accoppiata con un'altra tecnica, la calorimetria a scansione differenziale.

Calorimetria a scansione differenziale (DSC)

La calorimetria a scansione differenziale è una tecnica allo stato solido usata per la caratterizzazione termica di un solido cristallino che permette di rilevare tutti quei fenomeni che coinvolgono un cambiamento della capacità termica. La differenza di calore richiesta dai due porta-campioni, uno vuoto che servirà come riferimento e l'altro con il campione da analizzare, per mantenere la stessa temperatura, permette di identificare gli eventi termici.

Due diversi protocolli sono stati utilizzati perché i range di temperatura del primo erano troppo cautelativi per cui non permettevano di vedere alcun fenomeno ad eccezione per un composto. Come riferimento i fenomeni endotermici hanno un'area negativa mentre quelli esotermici un'area positiva.

Il primo viene mostrato in tabella 2, mentre il secondo in tabella 3:

Tabella 2 *Primo protocollo utilizzato per il DSC*

Step	Rate Temperature/ time
Hold	35° C during 5 min
Heating rate	5°C/min from 35°C to 150°C
Hold	150 during 5 min
Cooling rate	5°C/min from 150°C to 25°C
Hold	25°C during 5 min
Heating rate	10°C/min from 25°C to 150°C

Tabella 3 *Secondo protocollo usato per il DSC*

Step	Rate Temperature/ time
Hold	35° C during 15 min
Heating rate	5°C/min from 35°C to 200°C
Hold	250 during 5 min
Cooling rate	5°C/min from 200°C to 25°C
Hold	25°C during 5 min
Heating rate	5°C/min from 25°C to 200°C

Risultati :

Le ammine chirali che hanno portato alla formazione di sali sono elencate di sotto, ad ogni ammina è associato un numero.

- 4 is the (1S,2R)-2-Amino-1,2-diphenylethanol
- 63 is the (R)-1-Phenylethylamine
- 64 is the (S)-1-Phenylethylamine
- 71 is the (R)-1-(1-napthyl)ethylamine
- 72 is the (S)-1-(1-napthyl)ethylamine

(1S,2R)-2-Amino-1,2-diphenylethanol

Nella seguente figura sono raccolti e sovrapposti i pattern di riferimento e le prove MAD4 10% EtOH, MAD4 5% EtOH, MAL4 10% EtOH e MAL4 5%.

I picchi risultanti dell'esperimento sono diversi sia da quelli relativi all'acido malico che al coformer. Si può notare che c'è molto rumore e non si vede molto quindi si potrebbe ipotizzare che il sale non è puro ed è amorfo.

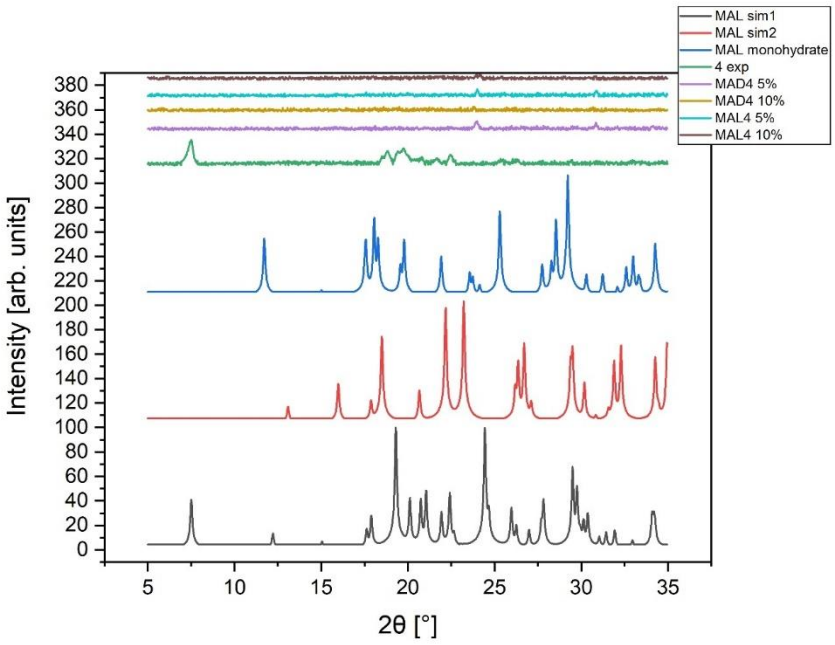

***Figura 2** XRPD del campione MA4*

Per quanto riguarda lo spettro NMR sono visibili il multipletto a 2.35 ppm ed il doppio doppietto a 3.8 ppm corrispondenti al -CH_2 e -CH dell'acido malico. Il multipletto a 7.19 ppm ed il doppietto a 4.76 e 4.09 ppm sono gli -^{1}H del (1S,2R)-2-Amino-1,2-diphenylethanol. Dall'integrazione dei picchi sembra che gli idrogeni dell'acido malico siano la metà per cui si potrebbe avanzare l'ipotesi di una stechiometria 2:1 del cristallo, che necessità però dell'analisi al cristallo singolo per conferma.

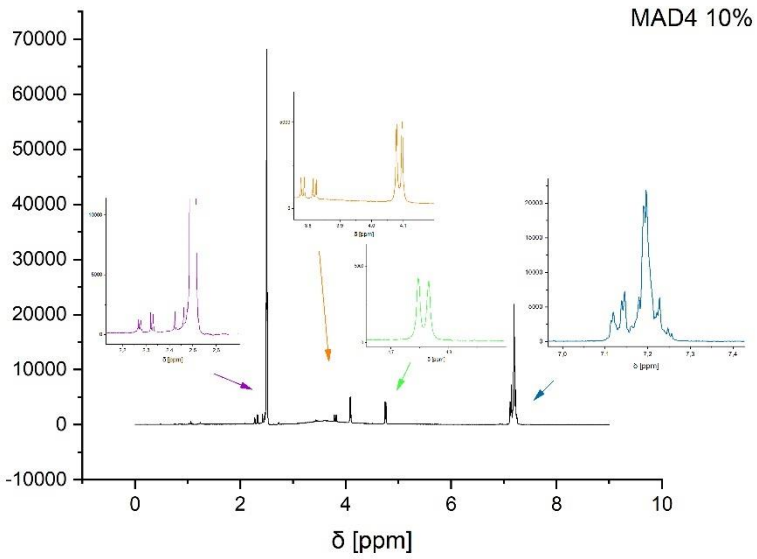

Figura 3 *Spettro NMR di MAD4 10%*

L'analisi termogravimetrica mostra una prima perdita di massa dovuta probabilmente a del solvente adsorbito in superficie, la temperatura si aggira sui 60°C, situazione molto comune nei sali ionici. Poi ci sono due step graduali che si osservano in caso di degradazione dai 120 °C ai 420°C.

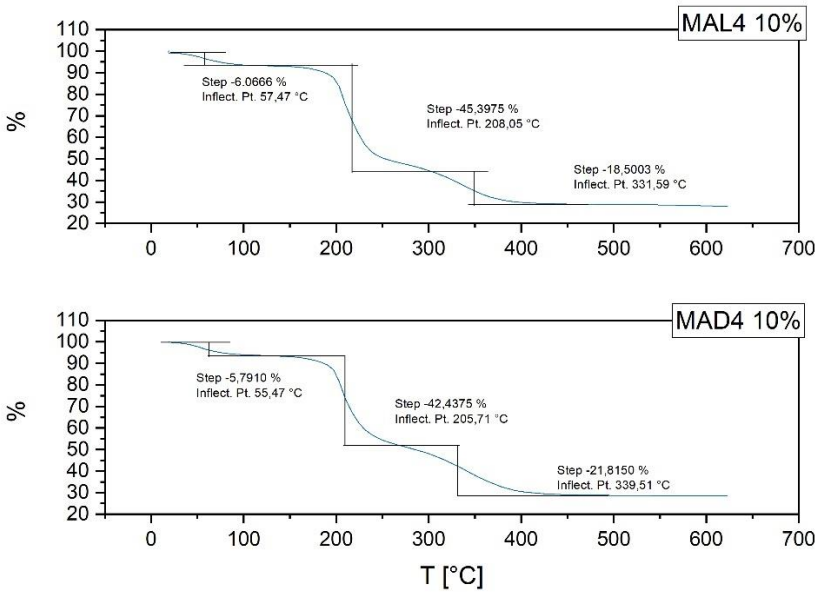

Figure 4 *TGA del MA4 10%*

Per la prova al DSC si è utilizzato il primo protocollo, in tabella 2. Per il campione MAD4 10% il primo picco ampio in nero si riferisce al solvente adsorbito in superficie, è molto largo perché il campione non è perfettamente asciutto mentre il secondo indica la transizione di fusione a 141.05 °C. Il fuso è poi raffreddato e cristallizzato a 133.38 °C (linea rossa) e questo viene confermato dal picco in blu alla stessa temperatura del secondo picco nero a 137.51 °C.

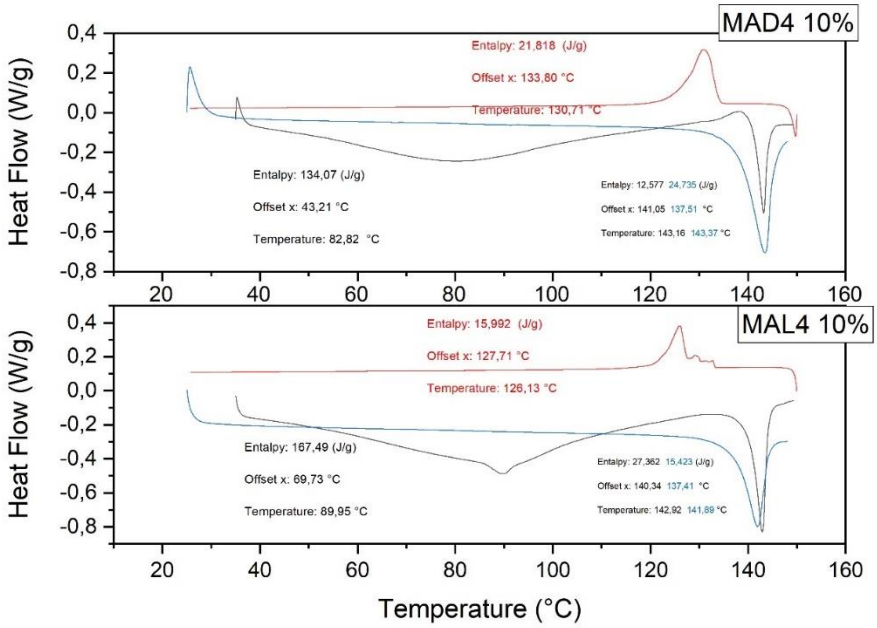

Figure 5 *DSC of MA4 10%*

(R)-1-Phenylethylamine and (S)-1-Phenylethylamine

I risultati dei due enantiomeri verranno presentati insieme perché presentano andamenti simili.

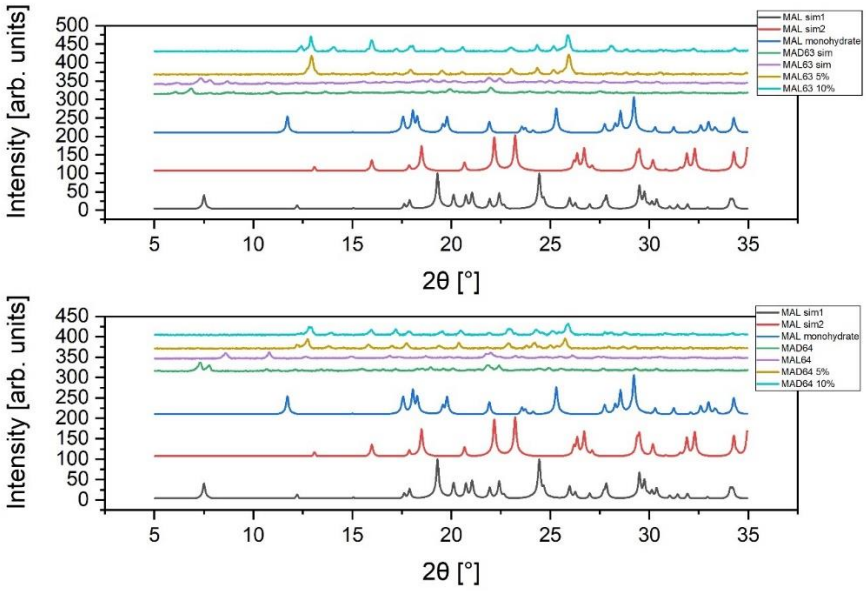

***Figure 6** XRPD MAL63 and MAD64*

Si vedono nuovi picchi rispetto all'acido malico e ad entrambi i coformers quindi si può assumere che è presente una nuova forma cristallina. Di notevole importanza il fatto che i sali si sono formati fra L-AM e (R)-1-Phenylethylamine e D-AM e (S)-1-Phenylethylamine, un risultato molto interessante.

Dopo aver analizzato tutti i campioni, si riportano a titolo esplicativo solo le figure relative a MAL63 perché uguali a quelle MAD64.

Dallo spettro NMR sono visibili il multipletto a 2.35 ppm e il doppio doppietto a 3.8 ppm riferiti all'acido malico. Ci sono poi un multipletto a 7.41 ppm corrispondente agli ^{1}H appartenenti all'anello aromatico della molecola; infatti, integrando l'area del picco sono cinque. Poi c'è un quadrupletto a 4.37 ppm ed il gruppo metilico a 1.46 ppm. Per l'altro enantiomero i picchi sono gli stessi, variano solo di qualche ppm.

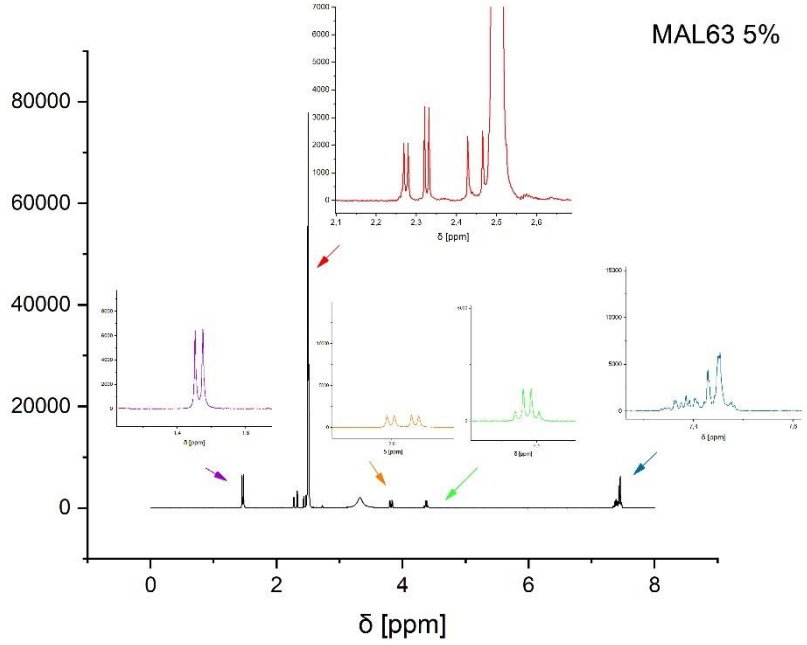

Figura 7 *Spettro di MAL63 5%*

Per MAD63 5% si ha una semplice degradazione del sale ionico, il campione perde ~ 95.8% della sua massa, quasi tutto degradato.

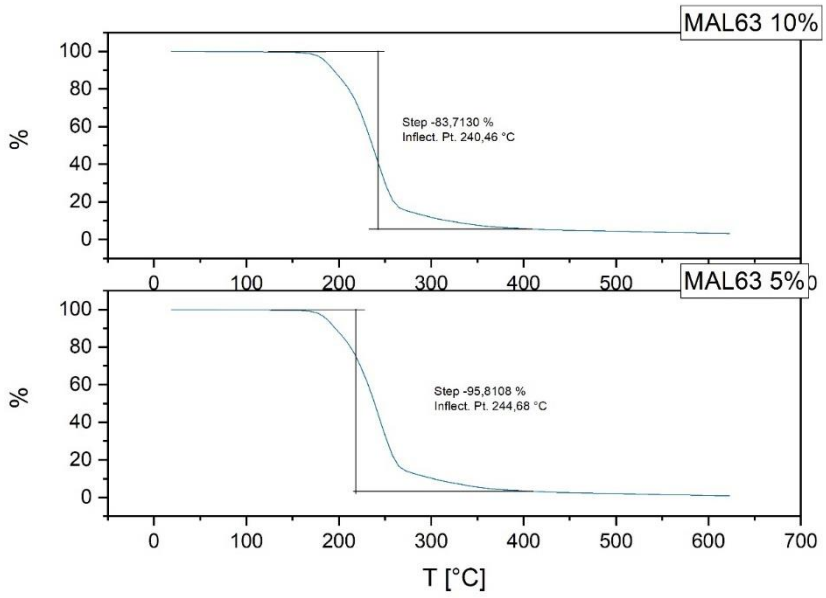

Figura 8 *TGA di MAL63*

Il grafico risultante dal DSC per la prova MAL63 è riportato in figura 9. Per MAL63 10% c'è un primo picco probabilmente relativo al solvente adsorbito, un secondo picco riferito forse ad un cambiamento di forma polimorfica ed infine il sale fonde e poi si degrada.

Per la prova MAL63 5% si vede solo un picco relativo alla fusione e la successiva degradazione come anche in MAD64 5% e MAD64 10%.

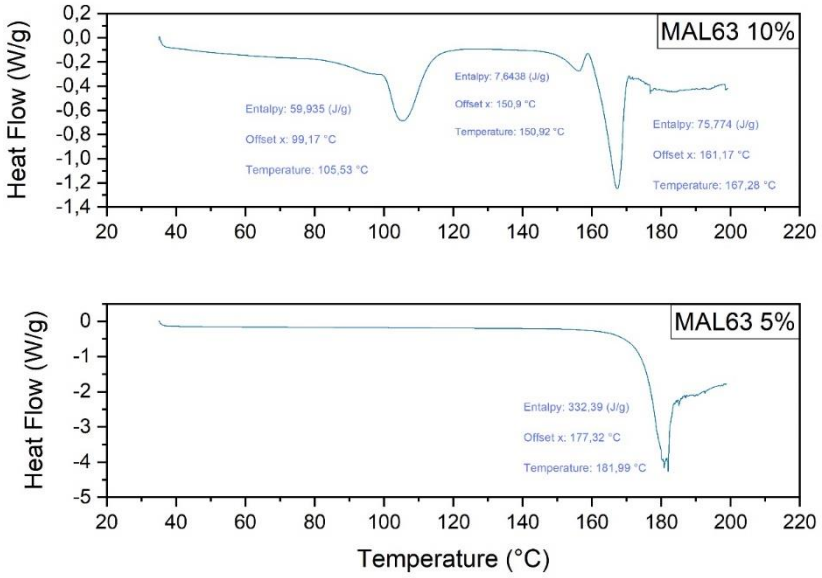

***Figura 9** DSC di MAL63*

(R)-1-(1-napthyl)ethylamine and (S)-1-(1-napthyl)ethylamine

Anche in questo caso i due enantiomeri verranno analizzati in concomitanza per le similarità che hanno mostrato. Nelle seguenti figure sono comparati tutte e quattro le prove di ogni enantiomero, quindi 71 e 72 con i pattern di riferimento in figura 10 e 11. La cosa interessante è che in una prima analisi dal primo grafico si vede che i pattern MAD71 al 5 ed al 10% sono uguali come dal secondo anche dal secondo per MAL72 al 5 e 10%. Ma da un'analisi globale di tutte le prove risulta che gli esperimenti MAL71 5% è uguale a MAD72 5% ed MAL71 10% è uguale a MAD72 10%.

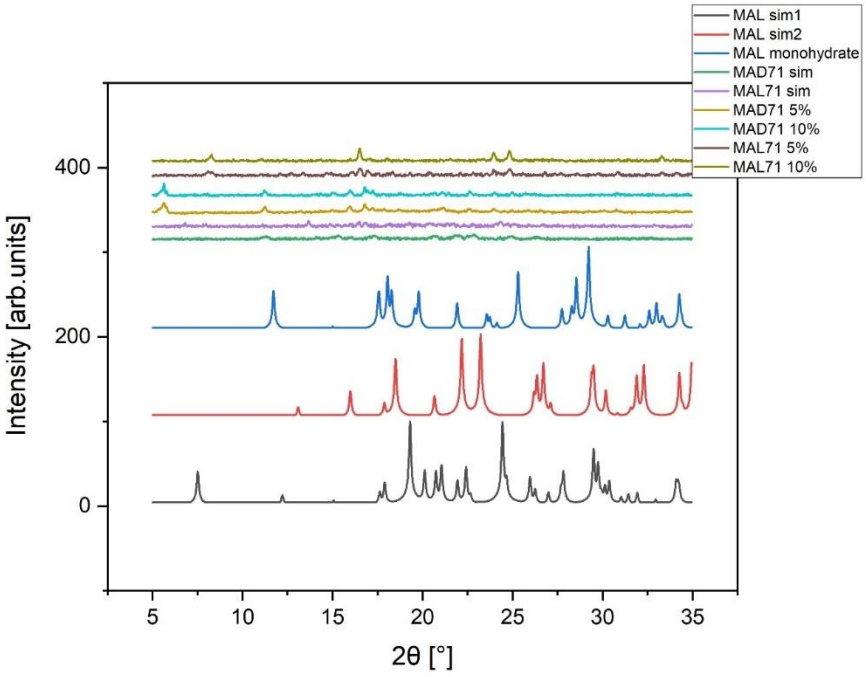

Figura 10 *XRPD di MA71*

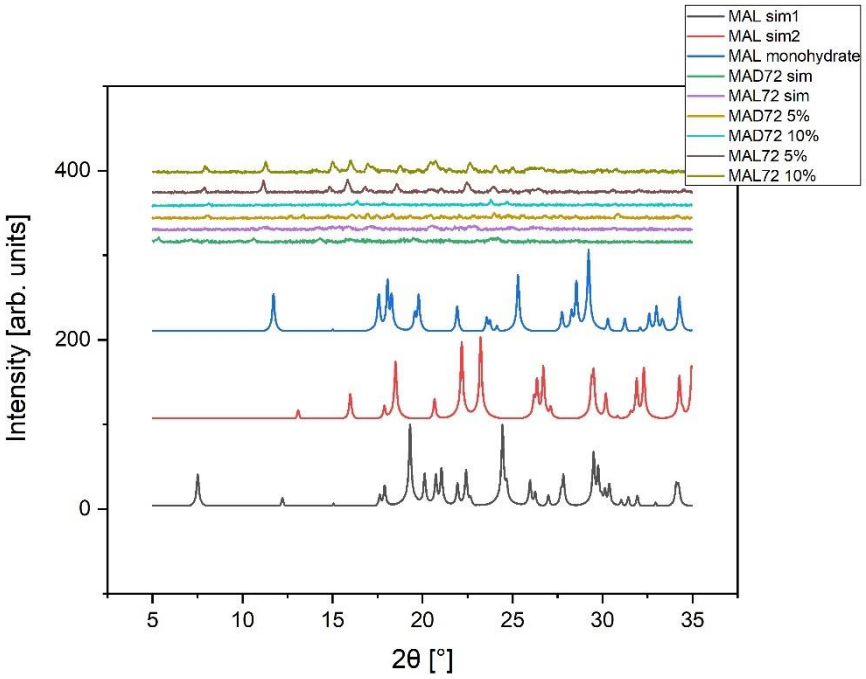

Figura 11 *XRPD di MA72*

Date queste similitudini verranno riportati solo i risultati di MAL71 5% e MAD72 5%.

Gli spettri mostrano i picchi relativi all'acido malico a 2.33 ppm e 3.79 ppm poi ci sono tre doppietti a 8.18 ppm, 7.86 ppm e 7.72 ppm con due multipletti a 7.95 e 7.57 ppm in blu che si riferiscono agli idrogeni dei due anelli aromatici; integrando l'area dei picchi infatti sono sette. Un doppietto a 4.99 ppm corrispondente al -CH ed un altro ad 1.46 ppm del $-CH_3$ del (R)-1-(1-napthyl)ethylamine

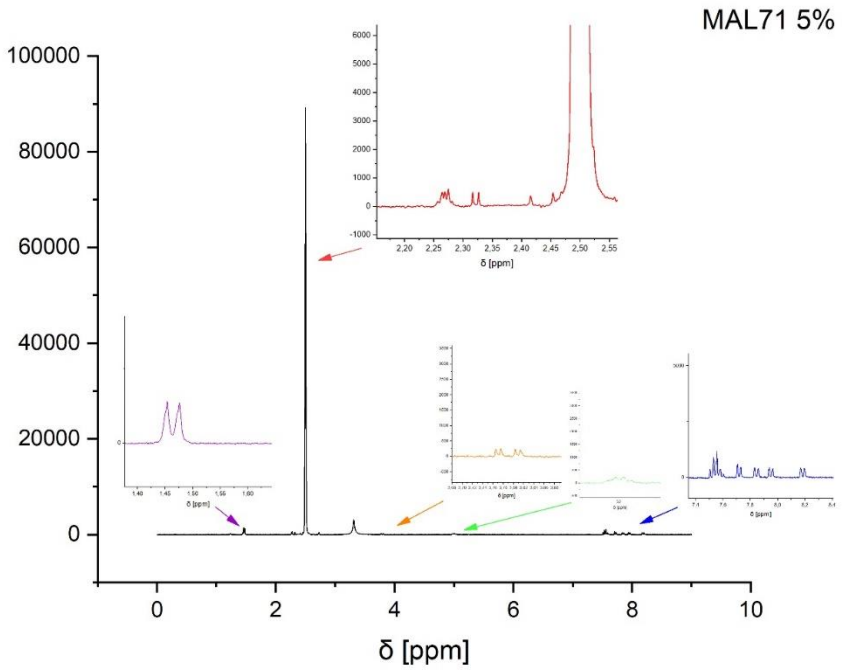

Figura 12 *Spettro NMR di MAL71 5%*

L'altro enantiomero, (S)-1-(1-napthyl)ethylamine come si può vedere in figura 13 è molto simile a quello appena descritto

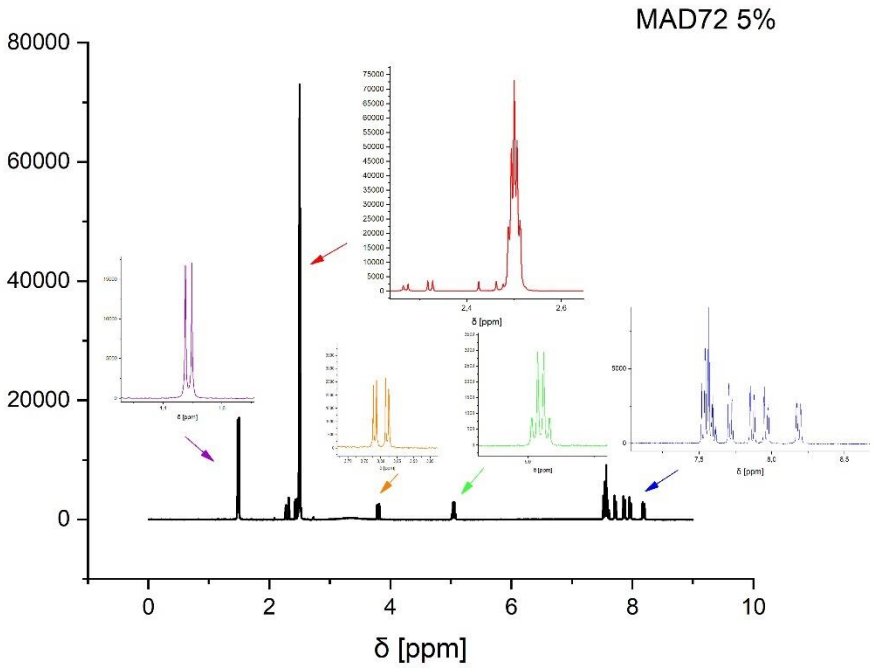

Figure 13 *Spettro NMR di MAD72 5%*

Come si vede nella figura successiva, il campione MAL71 5% perde all'incirca 1.30% di solvente adsorbito sulla superficie della polvere, questa assunzione i basa sul fatto che la temperatura è minore di 50°C. Poi segue la perdita del solvente absorbito all'interno del reticolo cristallino perché la temperatura è intorno ai 100°C. Infine si ha una lenta degradazione, non si vede un salto marcato, che parte da 120°C fino ad oltre i 600°C.

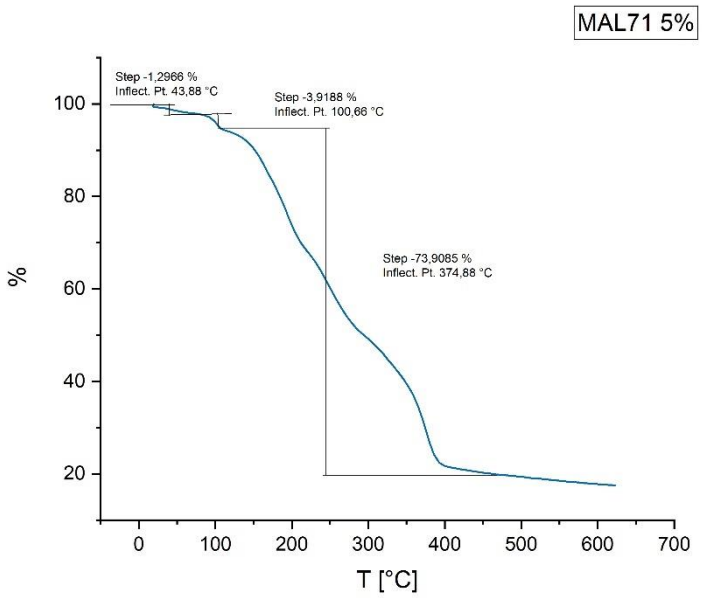

Figure 14 *TGA of MAL71 5%*

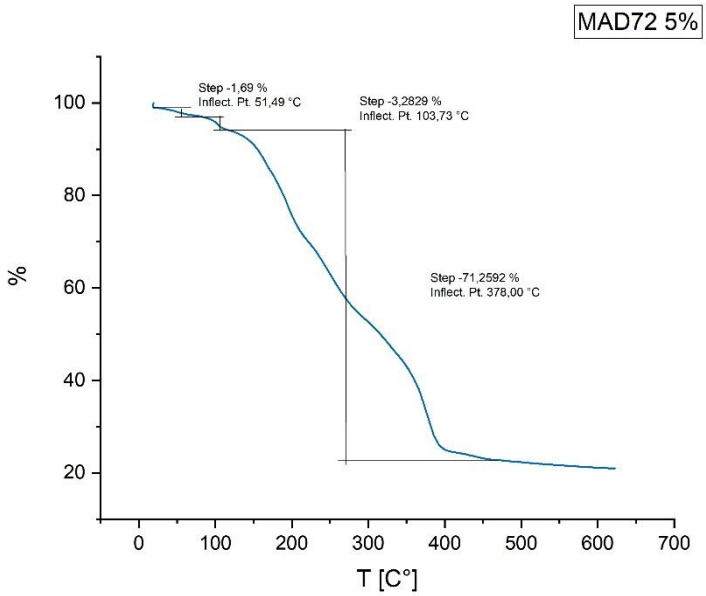

Figure 15 *TGA of MAD72 5%*

Dalle prove al DSC entrambi MAL71 5% e MAD72 5% mostrano un primo picco relativo al solvente absorbito, poi fondono ed infine vanno incontro a degradazione, in figura 16.

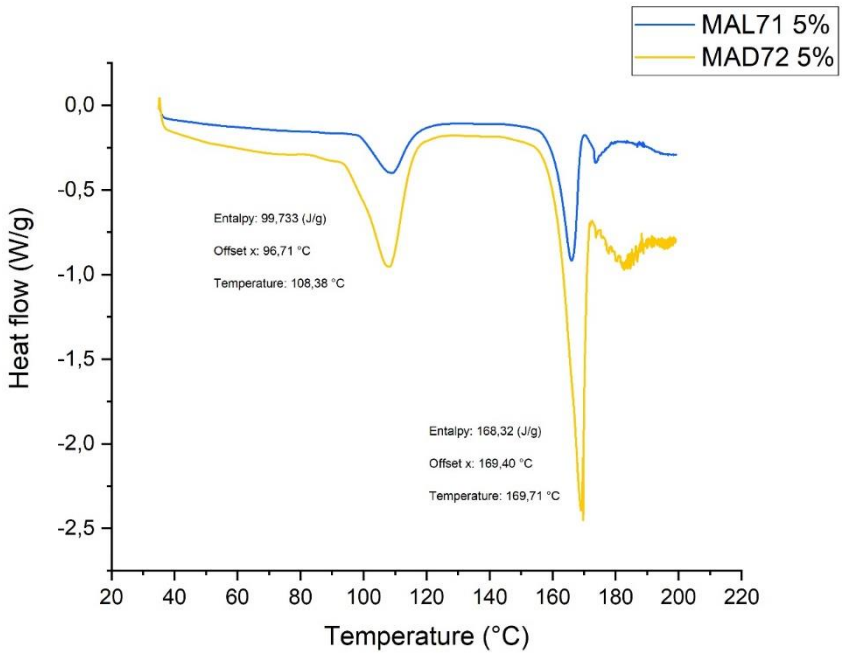

Figure 16 *DSC di MAL71 5% e MAD72 5%*

Solubilità

Nella prossima tabella sono stati raccolti tutti i valori di solubilità trovati sperimentalmente:

Tabella 4 *Valori si solubilità*

Compound		Solubility MAD [mg/ml]	Solubility MAL [mg/ml]
(1S,2R)-2-Amino-1,2-diphenylethanol	*10% EtOH*	140.38	141.03
	5% EtOH	91.47	161.71
(R)-1-phenylethylamine	*10% EtOH*		149.36
	5% EtOH		154.04
(S)-1-phenylethylamine	*10% EtOH*	166.86	
	5% EtOH	184.25	
(R)-(+)-1-(1-naphtyl)-ethylamine	*10% EtOH*	183.06	179.95
	5% EtOH	179.68	109.58
(S)-(-)-1-(1-naphtyl)-ethylamine	*10% EtOH*	197.65	142.80
	5% EtOH	162.94	117.86

Conclusioni

I diffrattogrammi sperimentali del solido proveniente dagli esperimenti fra l'acido malico e le quattro ammine chirali posti in agitazione in rapporto equimolare, esibiscono nuovi picchi paragonati al pattern dei composti parentali, suggerendo che una nuova fase cristallina si è formata. Per (1S,2R)-2-Amino-1,2-diphenylethanol sarebbe meglio ripetere la prova con l'XRPD dato che la misura è affetta da troppo rumore.

Le misure con l'NMR confermano che in tutti e quattro i casi, nello spettro, compaiono i picchi relativi all'acido malico e del coformer in questione. È necessaria un'ulteriore analisi, quella del cristallo singolo per validare la stechiometria ipotizzata e per ottenere informazioni addizionali.

Per quanto riguarda la caratterizzazione termica, alcuni test come MAL72 5% è necessario che siano ripetuti perché inconclusivi.

I risultati ottenuti permettono di affermare che si sono formati dei sali diastereoisomerici con (1S,2R)-2-Amino-1,2-diphenylethanol, (R)/(S)-1-Phenylethylamine, (R)/(S)-1-(1-napthyl) ethylamine e tutti loro condividono un elemento strutturale comune, il benzile. Per il futuro, si potrebbe continuare testando altre molecole con lo stesso gruppo funzionale.

Questo progetto è il punto di inizio di una tesi di dottorato che si prefissa come obiettivo quello di risolvere una miscela racemica attraverso la formazione di sali diastereoisomerici e l'aggiunta di un enzima che catalizza la reazione. Come è ben noto, gli enzimi sono estremamente sensibili all'ambiente che li circonda; quindi, il passo successivo sarà vedere come si comporta in un sistema con dei valori di solubilità molto alti.

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CHAPTER I

1 Introduction

1.1 Background

Crystallization is used in various fields such as the food industry, for pharmaceuticals, in the production of catalysts, and in generally for the manufacturing of fine chemicals.

The principle is the separation of a chemical compound dissolved in solution or in a melt by promoting the formation of a crystalline solid phase, then proceeding to the physical separation of the solid from the liquid medium.

Crystallization is a fundamental stage in the production of Active Pharmaceutical Ingredients, abbreviated API, that ensure their purity and important physical properties.

Crystallization is widely used both as a separation and purification technique that simplifiers the downstream operation(such as filtration, washing and granulation) and as a way to control the compound's physical properties. In particular, control of the crystalline form is important as it influences many properties which define the performance of the final formulation: solubility, bioavailability, dissolution rate and thermodynamic stability, (related to the product shelf life).

1.2 Chirality

In organic chemistry, the presence of an asymmetric carbon atom in a molecule (or sulfur, nitrogen and phosphorous) with different substituents leads to a molecule whose structure lacks any element of symmetry. The property of a molecule to be non-superimposable with its own mirror image through any translation or any rotation is termed chirality. The two-mirror images are defined as "Enantiomers"; both present the same physico-chemical properties such as density, solubility, boiling temperature, spectral and thermodynamic properties except for a different optical power (±).

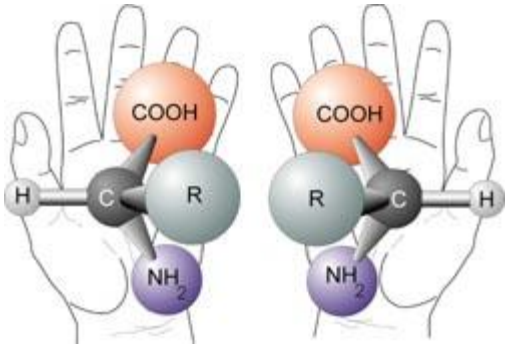


Figure 1.1 *Example of chiral enantiomers taken from https://www.wikidoc.org/index.php/Chirality_(chemistry)*

A mixture containing the same amounts (equimolar) of the two enantiomers is defined "Racemate". The crystal packing for racemates can be of three types:

- A racemic compound is the term used when a single crystalline phase contains both enantiomers in an even ratio. A given enantiomer has grater affinity for the opposite enantiomer. This occurs for most chiral drugs (90-95%).
- A conglomerate is a mixture of enantiomerically pure crystals. Conglomerates occur for 5-10% of cases.

– Solid solution or Pseudoracemate contain the same quantity of each enantiomer with a random distribution in the crystal lattice. This case seldom occurs for organic compounds (1%).

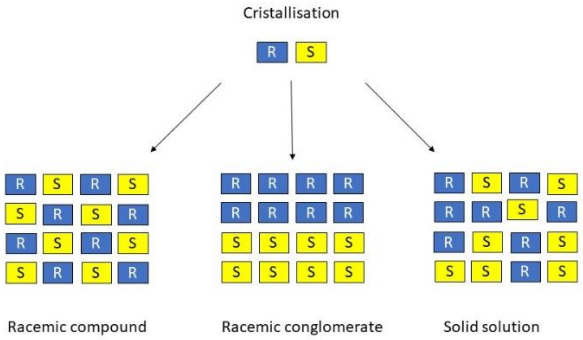


Figure 1.2 *Crystalline enantiomers packing as Racemic compound, Conglomerate and Solid solution. Imagine freerly adapted from Chem. Soc. Rev., 2015, 44, 6723.*

Separating two enantiomers in particularly important for the pharmaceutical industry .

A well-know example of pharmaceutical issue related to chirality is the case of Thalidomide, a drug marketed in the 1950s as an anti-nausea solution for pregnant woman. The R-enantiomer shows a sedative effects, but unfortunately, the S-enantiomers has teratogenic effects. Generally, two enantiomers may have different biological activity or just a different economic value; hence the need to find a method of separation.

In order to obtain an enantiopure compound from a racemic mixture three paths can be followed depending on the type of system:

- Preferential crystallization which occurs by adding a seed crystal of a given enantiomer to the racemic solution in the metastable region of crystallization. This technique can only be used for conglomerates.
- Chiral chromatography, which uses chiral selector to form different labile interactions with each enantiomer. By eluting the enantiomers through the column a separation is achieved.

- Diastereomeric Salt formation, which is a crystallization technique where a chiral resolution agent is added to a racemic mixture. In this way, diastereomeric salts can be obtained, which are no longer mirror images, and which hence have different physical properties such as solubility. A simple crystallization process can then be used to separate both enantiomers.

1.2.1 Salts and Cocrystals

APIs are very often administered in solid form as an oral dosage. Various crystalline solid forms of the same compound can exist, with an overview given below. Additionally a distinction must be made between amorphous and crystalline solids.

In an amorphous solid there is no long-range order of packing neither a well-defined conformation. These solids are isotropic. Amorphous solids are thermodynamically unstable compared to the crystalline form. They are nevertheless interesting, as they present higher and faster dissolution rate compared to the crystals. Their stabilization is, however, often a challenge.

Focusing on the crystals state, one can distinguish single- and multi-component systems. These latter, contain other pharmaceutically accepted compounds beside the main API.

The term polymorph is used to distinguish different crystalline forms of the same compound. Polymorphs present to differences in free energy that provokes different absorption rates, melting point and solubility properties which all affect the activity of a drug in the body. The thermodynamic stability of different polymorphs is crucial for their production and for their use in practical applications. Polymorphs exist both for multi-component as well as single component systems.

Multi-component systems can be divided in different categories according to the nature of the second component.

Solvates incorporate a solvent molecules into the crystalline lattice. Not all solvents are desired for pharmaceutical formulations. In fact, they must be safe for human consumption, limiting the pharmaceutically acceptable solvates to water, ethanol and ethyl acetate. When the solvent is water, they term hydrates is also encountered. Typically solvates and hydrates exist in precise stoichiometric ratio (mono, di, tri- hydrates) with main crystal component. Hydrates stability depends on temperature and relative humidity. By changing these parameter issues in production or stability of a drug formulation can occur.

Cocrystals are defined as: "Solids that are crystalline single-phase materials composed of two or more different molecular and/or ionic compounds generally in a stoichiometric ratio which are neither solvates nor simple salts". (Aitipamula et al., 2012)

In cocrystal intermolecular weak interactions such as hydrogen bonding, van der Waals interactions and π- π stacking occur.

In the cocrystals both components are neutral (no hydrogen bond transfer therefore occurs) and both components are solid considered on their own under ambient conditions (unlike solvates). When APIs are not ionizable, cocrystal formation is a good alternative to salt formation.

The majority of drugs are marketed as in the form of salts. This is because the ionization can improve solubility of the solid phase and increase thermodynamic stability. A salt often implies a proton transfer between two molecules (an acid and a base).

The boundary between the definition of a salt and a cocrystal is not sharp and well defined but often a $\Delta pKa > 3$, is considered sufficient to lead to salt formation.

1.2.2 Diastereomeric Salts

Among the various methods for separating two enantiomers in a racemate, diastereomeric salts formation is one of the most promising.

Both enantiomers interact with a chiral Resolving Agent, forming two diastereomers that reveal different properties such as the solubilities. The diastereomers can then be separated by the crystallization of the less soluble salt, while the more soluble one remains in the mother liquor. Ultimately, the bond between the enantiomer and the resolving agent needs to be broken to recover the target molecule.

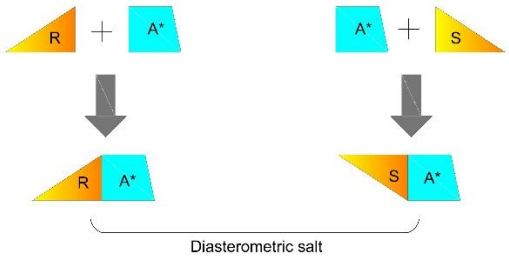


Figure 1.3 *Scheme of interaction between the enantiomers R S and the chiral resolving agent A**

The resolving agent plays a key role in the separation of enantiomers. This compound must have the appropriate groups to form a salt with the target molecule and the resulting diastereomeric salts need to show an important difference in solubility. Furthermore, the resolving agent must be affordable, the must provide a highly pure enantiomer and be easily retrievable.

1.3 Reactive crystallization

Crystallization is an operation highly sensitive to process conditions, so it is crucial to have control over it in order to obtain the desired chemical and physical properties in the crystals produced. Morphology, size distribution, shape and polymorphism are crystal properties that impact product performances as the taste for food ingredient, or the efficacy of pharmaceuticals.

Reactive crystallization is a process where a chemical reaction occurs in solution, and where the reaction product undergoes a crystallization process , which provides further driving force to the reaction itself (McDonald et al., 2021).

The combination of reaction and crystallization decreases the request for thermal energy, the number of unit operations need for synthesis and separation and hence production time.

If we are dealing with an equilibrium reaction, we can move to the products through their continuous removal according to the principle of Le Chatelier:

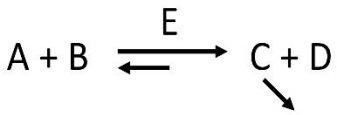


Figure 1.4 *Generic reaction with an enzyme that allows to shift the balance*

Enzymes often catalyze reactions with low yields; reactive crystallization can shift the equilibrium in the required direction and promote a faster reaction rate.

Biocatalytic reactions widely use enzymes. They manage to give stereo-, regio- and chemo selective conversion of the substrate, and are highly specific in enantiomeric recognition. Enzymes are also very sensitive, so by changing the conditions of the system they can be manipulated and push the reaction towards the desired product.
In the last few decades, reactive crystallizations involving enzymes have been developed (Hülsewede et al., 2019) ; they combine a biocatalytic reaction and a simultaneous crystallization of a product characterized by low solubility. Another advantage of crystallization is the use of low temperature which can be essential if we are dealing with thermally labile compounds such as enzymes.

1.3.1 State of the art

In 1998 a team of researchers (Michielsen, Meijer, et al., 1998) focused on the production of D-Malate from maleate through *Pseudomonas pseudoalcaligenes* . D-Maleate is rarely found in nature. Their purpose was to determine the best conditions of pH, temperature and initial concentration of substrate to maximize the yield of recovery of D-maleate.

In the same year another model reaction for the recovery of this enantiomer was studied: this involved the conversion of achiral solid Ca-maleate to solid Ca-D-Malate (Michielsen, Reijenga, et al., 1998). The authors started by dissolving achiral Ca-maleate and consequently converting from maleate^{2-} to D- malate^{2-} . They ended by crystallizing this latter , as in the figure 1.5.

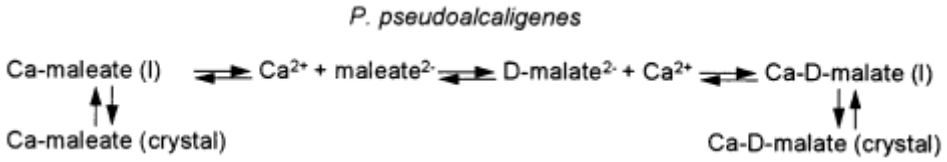


Figure 1.5 *Reaction Scheme for the conversion of solid Ca- maleate to solid Ca-D-maleate*

Regarding the process and equipment design in some cases the conventional bioreactor could be replaced by an immobilized biocatalyst reactor followed by a crystallizer. This configuration should avoid the mechanical destruction of the biocatalyst induced by the formation of crystals. (Tosa et al., n.d.).

1.4 Overview of this project

The aim of this thesis is to design a systematic procedure to create diastereomeric salts that can be separated by a simple crystallization process. As a model study system we focused on two

enantiomeric forms of Malic Acid (D-L). The role of resolving agent is played by a selection of chiral amine.

A supersaturated solution is created by adding the solvent (a 5% or 10% in volume of ethanol/water solution) to a mixture of the starting enantiomers in powder forms.

The suspension is left to slurry until the salts is eventually formed.

The time to reach equilibrium in these slurry experiments depends on the solubility of both components in the solvent mixture. Some tests did not lead to the formation of any crystal, this means that the candidate molecule was too soluble and was no longer considered. D-MA is the enantiomer not present in nature and therefore, of the highest economic value and interest. The initial goal wass to find the chiral amine capable of creating diastereomeric salts; subsequently provide a description of the salts just created, identifying the crystal morphology, the solubility values, potential various solid phases of this salt, and their melt/degradation temperature.

Chapter II

2 Literature review

In this chapter the theory of the crystallization and the main methods employed for the synthesis of crystals are reported . Thereafter, the experimental techniques used in this project for the characterization of the crystal produced are described.

2.1 Crystallization

There are many methods to produce crystals; some based on solid-state processing with no or little solvent used and others based on the use of solvent. The focus here is on crystallization form solution where the compounds is solubilized in a solvent.

A homogeneous mixture of two or more substances is defined as a solution where the solute is dispersed in the solvent. Choosing a suitable solvent for crystallization is a complex task. The solvent must not only withstand the process conditions, be cost effective and safe, but it must also reduce the solubility of the solute so that one can easily crystallize the solid and improve the separation yield. The term solubility is defined as the maximum amount of a solute that can be dissolved in a given amount of solvent, under specific conditions. The solubility curve can be function of temperature, but it is also affected by impurities or additives dissolved in solution. The solubility curve provides the information needed for the crystallization process. In the figure 2.1 illustrates the solubility curve and the different regions it delimits.

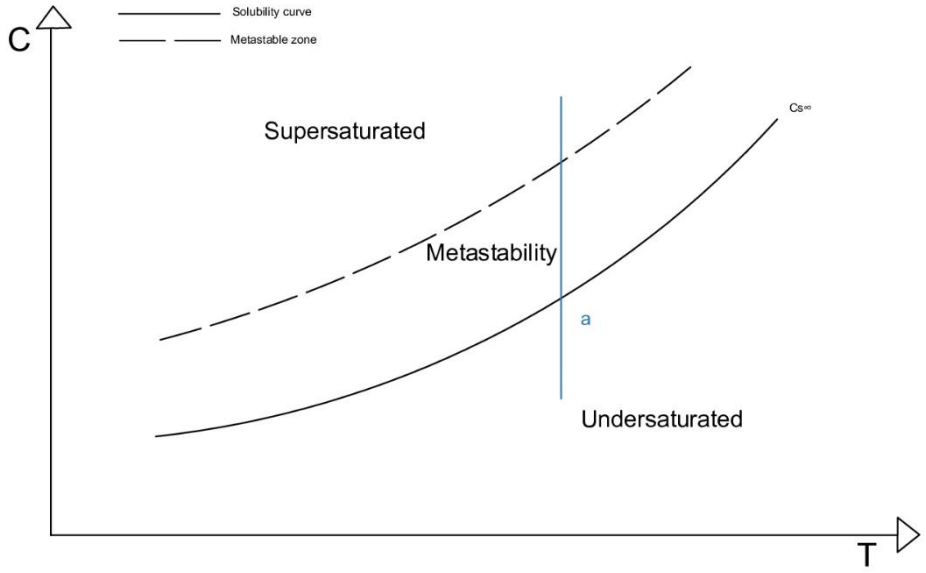


Figure 2.1 *Solubility diagram: solubility curve is represented by the continuous line while the supersolubility curve is represented by the dash line. The line a represent the "slow evaporation".*

Below the solubility curve the solution is stable and undersaturated. No crystal can exist because they will soon redissolve. Above there is the region of lability, where the solution is unstable and supersaturated so, after some time crystallization will occur; in this area crystals can nucleate and grow. In the case of metastable solution the concentration of the solute is above the solubility curve and the spontaneous crystallization should occour according to thermodynamics. Neverthless, the entire process is under the control of kinetic factors and in order to have spontaneous nucleation a critical level of supersaturation is required. In the metastable zone (MSZ) the formation of nuclei is kinetically hindered.

2.1.1 Nucleation

Two types of nucleation exist: primary and secondary nucleation. The term nucleation refers to the birth of a new crystal. Primary nucleation is the formation of nuclei from a supersaturated solution. When this nucleation occurs inside the solution, the term homogenous primary nucleation is used. When the nucleation occurs on a foreign surface, the term heterogeneous primary nucleation is used. Secondary nucleation, is the formation of nuclei from already existing crystals; however heterogenous nucleation occurs in which foreign particles or impurities acting like catalyst for nucleation.

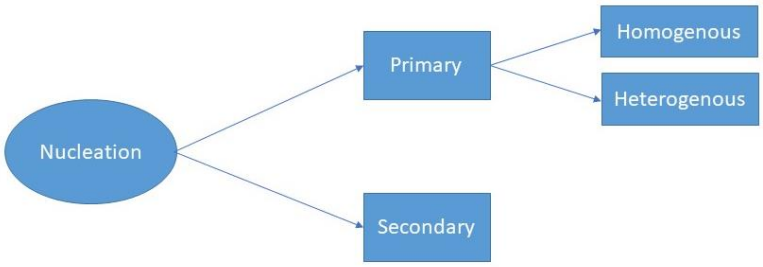


Figure 2.2 *Nucleation mechanism*

The classical nucleation theory (CNT) (Gibbs,1948) explains the mechanism of primary homogenous nucleation and claims that a cluster of molecules can either grow into a crystal nucleus or redissolve. Both the possibilities depend on the critical size radius r_c which correspond to the minimum size of a stable nucleus; for radius larger than r_c clusters continue to grow while cluster of radii smaller than that, re-dissolve.

2.1.2 Slow evaporation:

This is the most convenient method to grow single crystals or materials with high crystallinity. The path normally followed is illustrated in figure 2.1, line a.

The process starts by dissolving the starting material and supersaturation is reached by slowly evaporating the solvent.

2.1.3 Slurry crystallization for the preparation of co-crystals and salts

In slurry crystallization a suspension of starting materials is prepared and left to equilibrate for a certain time. The suspension is then seeded with cocrystal material to allow the nucleation and the growth of cocrystals. In practice the cocrystal/salt grows while the starting compounds dissolve. The equilibrium depends on the solubility of both coformers and in general, from the thermodynamic stability and the kinetics of the final composite system. When the solubility is too low, the system will need longer slurry times.

2.2 Solid state characterization technique

2.2.1 X- ray diffraction (XRD)

X-ray diffraction is a fundamental technique for the study of crystalline solids in which the arrangement in the space of atoms follows an ordered and regular pattern. X-rays are an electromagnetic radiation with a wavelength between 10^{-12} e 10^{-9}, and when a crystal reticle is hit by a radiation with a wavelength of the same order of the distance of the lattice planes, there are different interactions between photons and crystalline planes.

A crystalline plane is explain as a plane in the 3D space on which lie one or more atoms of the conventional cell; it is uniquely defined by a set of values called Miller indices (h,k,l) which specify the arrangement of the planes with respect to the three directions of the space. The change in the direction of propagation of the incident wave is called Diffraction.

The principle on which this phenomenon is based is described by Bragg's law:

$$n\lambda = 2d \sin\theta$$

where n is a positive integer number (0,1, 2...), λ is the wavelength of the radiation, d is the distance between two adjacent crystalline planes and θ "Bragg's angle" is the angle formed by the radiation beam and the crystalline plane. In the figure 2.3 are visible:

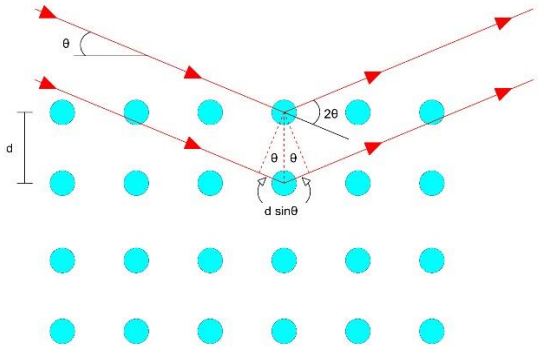


Figure 2.3 *Incident and diffracted X-rays following Bragg's Law*

The XRPD is carried out on a single crystal (SC-XRD) or on a powder (XRPD) where the various crystals are randomly oriented. A fixed source of X-rays generates a monochromatic beam directed towards the sample, both the sample and the detector can rotate to expose the greater number of crystalline planes within the sample. The detector is placed at 2θ angle with the direction of the beam emitted from the source, the double of the Bragg angle formed with the champion, to investigate the different directions of refraction.

The image obtained from an X-ray diffraction experiment is called Diffractogram or Diffraction pattern. The diffractogram has several peaks linked to the presence of parallel crystalline planes and from this we can get a lot of useful information on the crystal structure, the nature of the solid phase (if it is amorphous, semi-crystalline or crystalline), the purity, or the preferential orientation of the crystallographic planes (related to the morphology of the particles).

The SC-XRD provides more detailed information about the crystal including bond length, bond angle and crystallographic unit cell size.

The procedure for analyzing the diffractogram makes use of those present in a database "Powder Data File" PDF, by comparing the position and the intensities of the peaks, it is possible to identify the test compound and understand which crystalline phases are present in the sample analyzed.

2.2.2 Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy is suitable for the qualitative and quantitative resolution of classes of organic compounds, considering that it allows to obtain information related to the carbon structure of the sample. Through the spectral analysis of the proton contained in the compounds, some information regarding the functional group containing C-H bonds is obtained. The sample is solubilized in the appropriate deuterated solventand placed in an NMR tube.

When a compound is dissolved, the chemical shift values will depend on the interaction with the solvent molecules, usually $CDCl_3$ for apolar compounds, DMSO for polar compounds, or an anisotropic solvent like benzene.

The information than can be gained is:

- The peak integral: the area subtended by each of the peaks is defined as signal intensity and is represented with a stepped curve. In the ^1H spectroscopy, the areas associated with the various peaks are directly proportional to the number of protons that give rise to the signal itself.
- Chemical shift: The nuclei are shielded by the presence of electrons, sensing fields less than the one applied. The variation in magnetic field felt, allows to understand the chemical surrounding. Many factors contribute to the value of chemical shift, generally the more the groups are electronegative, the higher are the values.
- Multiplicity: Each proton gives rise to more than one signal; the spin states of a nucleus affect the resonance frequency of the surrounding nuclei.

2.2.3 Thermogravimetric Analysis (TGA)

The thermogravimetric analysis is a widespread technique used in the study of thermal stability and of related mechanisms of degradation.

A small amount of sample, it is heated at a uniform rate in an appropriate environment to measures the percent weight loss of a material as function of temperature and time.

This measurement in carried out with a microbalance, sensitive to physical phenomena such as solvent surface adsorption, different from absorption in which the solvent is in the crystal lattice, decomposition/degradation and oxidation through mass variation.

In the “pans” are pose 5-7 mg of sample, then it is placed in an oven above a weighting balance in a controlled atmosphere, under a nitrogen flow that is the gas for the purge.

The result of the experiment is a thermogram, a curve of thermal decomposition that reports in abscissa the temperature and the time and in ordinate the percentage variation in mass. The degradation temperature of the product can be determined from the curve but not the mechanism by which it occurs. State transitions cannot be detected by TGA because they do not involve mass changes. These limits may be overcome coupling the TGA with other techniques like differential scanning calorimetry (DSC).

2.2.4 Differential scanning calorimetry (DSC)

The differential scanning calorimetry (DSC) analysis is a solid-state technique used for thermal characterization of crystalline solids that allows to detect all those phenomena that involve a change in thermal capacity. This operation is based on the heat difference between the samples and a reference kept at the same temperature; it allows to identify the thermal events of the sample of interest. There are two sample-holders in the machine: one is empty and serve as the reference and the second, with the same morphology of the first, contains the sample to be analyzed.

If the sample undergoes a physical or chemical transformation, to maintain isothermal conditions the instrument will have to supply more or less heat, varying the heat flux. Consequently, the difference with the flux supplied to the reference sample also varies.

For explanatory purposes there is the figure 2.4:

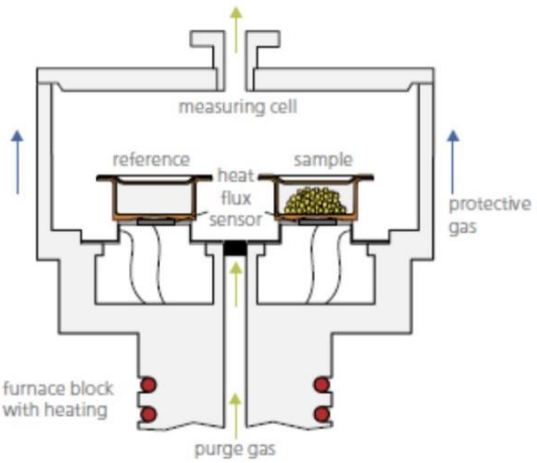


Figure 2.4 *The empty sample holder as reference and the one containing the powder are visible. Image taken from the material of the course of Principles of Pharmaceutical Biotechnology of Professor Pisano, 2020/2021.*

The thermogram resulting from this analysis shows the temperature against the heat flux and the differences in the heat flow positive or negative are referred respectively as endothermic or exothermic transformation. To calculate the enthalpy, change referred to chemical or physical phenomena, the area subtended by the peaks is integrated and then multiplied by a calorimetric constant.

Exothermic phenomena have positive area while the Endothermic ones have negative area as shown in Figure 2.5.

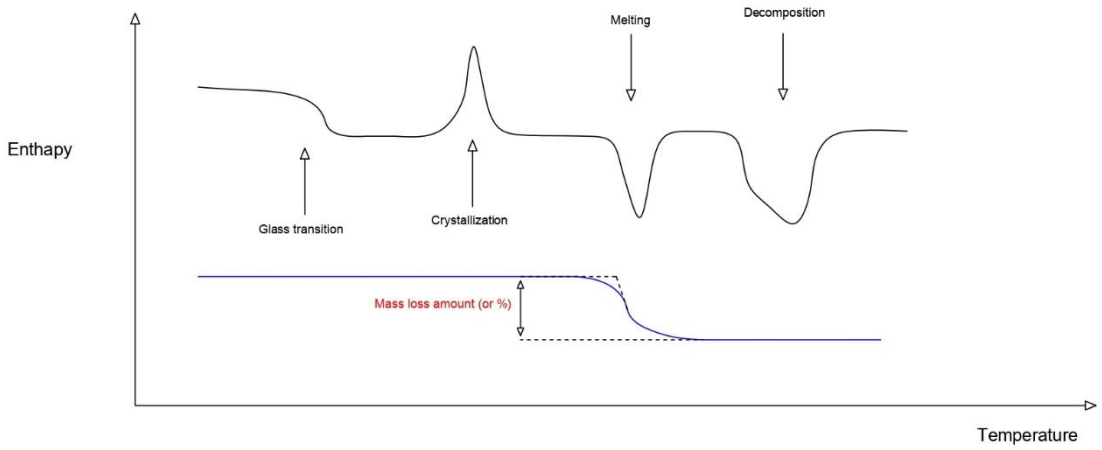


Figure 2.5 *Example of TGA (blue) and DSC (black) curve.*

Chapter III

3 Materials and methods

In this chapter, a brief summary of the compounds and solvent used is in this thesis formulated. The procedure to generate crystals are described and a complete documented overview of the equipment and the methodology used for each sample analysis is given.

3.1 Materials

The solutions at 5% and 10% by volume of ethanol have been prepared homemade with a buffer solution 1M KPO^{-}_{4} at pH 7. The tables below show where all the compounds used as conformers were purchased.

Table 3.1 *Compounds purchased from ABCR Gute Chemie*

ABCR GmbH
(1R,2R)-4-nitrophenyl-2-aminopropane-1,3-diol
(R)-(+)-1-(1-naphtyl)-ethylamine
(S)-2-aminobutanol

Table 3.2 Compounds purchsed from Acros Organics

Acros Organics
L-malic acid
(1S,2S)-(+)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol
(S)-1-phenylethylamine
Phenylglycinamide (D)
(S)-2-(phenylamino)caronyloxy propionic acid

Table 3.3 *Compounds purchased from Sigma-Aldrich*

Sigma-Aldrich
(8s 9r)-(-)-n-benzylcinchonidinium chloride

Table 3.4 *Compounds purchased from Alfa Aesar*

Alfa Aesar
Aspartame
(R)-1-phenylethylamine
Praziquantel

Table 3.5 *Compounds purchased from Apollo Scientific*

Apollo Scientific
D-malic acid
(S)-(-)-1-(1-naphtyl)-ethylamine
DL-2-Phenylglycinol
(1R,2R)-(-)-2-Amino-1-phenyl-1,3-propanediol

Table 3.6 *Compounds purchased from Fluorochem*

Fluorochem
S-Methyl-L-cysteine
(1S,2R)-2-Amino-1,2-diphenylethanol
(R)-(+)-1-(4-Bromophenyl)ethylamine
(S)-(+)-2-Amino-1-propanol
(R)-(-)-2-Amino-1-butanol
(R)-(-)-2-Amino-3-methyl-1-butanol
(S)-(+)-2-Amino-3-methyl-1-butanol
(S)-(+)-2-Pyrrolidinemethanol
(S)-(+)-Leucinol
(S)-(-)-2-Amino-3-phenyl-1-propanol
(S)-(+)-2-Phenylglycinol
(1S,2R)-(-)-1-Amino-2-indanol

Table 3.7 *Compounds purchased from TCI*

TCI
(R,S)-1-(4-bromophenyl)ethylamine
L-Tyrosine Hydrazide
Stanozolol
D-2 Phenylglycine
L-2 Phenylglycine

Table 3.8 Compounds purchased from Thermo Fisher Scientific

Thermo Fisher Scientific
(±)-trans-1,2-Diaminocyclohexane

3.2 Methods

All the equipment cited below are located in Louvain-la-Neuve. The flowchart in figure 3.1 shows the procedure designed to determine if salts of D-Malic Acid could be formed and for their characterization.

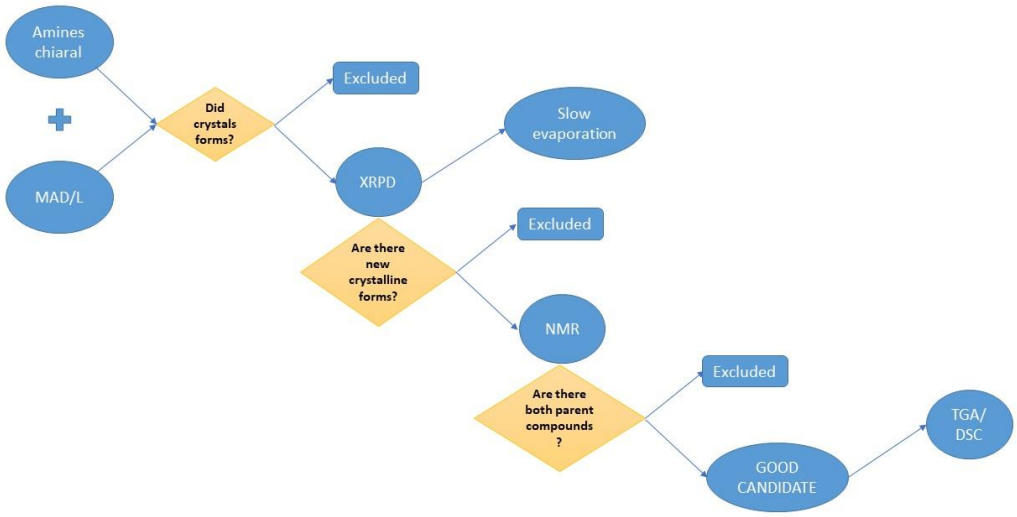


Figure 3.1 *Explanatory flowchart of the step followed*

First the vials are filled in a 1:1 ratio between the MA and the chiral ammine and are left to slurry from three to six days. If there are no visible crystals the compounds are excluded otherwise it proceeds with the characterization. 1ml of solution is taken from the supernatant and it is destined for slow evaporation, whereas the powder is filter and left to dry in the Eppendorf tubes. For the powder it is analysed through XRPD in order to confirm that a new crystal has been created and not just the undissolved parental compounds. If the diffractogram does not present new peaks the compound is excluded, on the contrary the characterization continue with the NMR analysis.

A total of four experiments are planned for one candidate (the chiral amine); two with D-Malic Acid (MAD) in 5% and 10% solution by volume in ethanol and the other two with L-Malic Acid (MAL) in 5% and 10% solution by volume in ethanol.

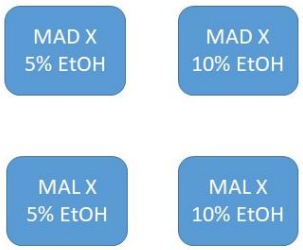


Figure 3.2 *Experiments done for each candidate (X)*

3.2.1 Slurry

The crystallization using slurrying was achieved by preparing an equimolar suspension of Malic acid (both R and S) and chiral ammines at 25°C in the previously mentioned solvent.

The quantity of the solvent used was from 1 to 3 ml, depending on the behavior of the vial. The suspension in sealed vials was left to stir at 600 rpm in the Cooling Thermomixer HLC at 25°C for 3 to 6 days. If in the first day's no thing or few is seeded in solid form, the quantity of the parent compound has been doubled.

All the experiment were ended when equilibrium was reached; the vials with the powders were filtered and let them dry before proceeding with XRPD, whereas in those in which nothing was formed the chiral ammines have been excluded.

3.2.2 Single crystal growth

After the eventual formation of salt cocrystals, 1ml of supernatant was taken and placed in previously weighed vials with a cap equipped with purposely created micropores. These vials with supersaturated solution were placed under a fumehood at a constant temperature of 25°C and were allowed to evaporate slowly, until the solids were completely dry. In this way the single crystal growth was conducted. From the weight difference between the empty vial and the vial after drying, solubility values were also collected. Instead, the powder was filtered and dried.

3.2.3 X-ray Powder Diffraction (XRPD)

The measurements were carried out on a Siemens D5000 machine equipped with a Cu cathode (I=1.5418 Å) by using a Bragg Brentano geometry, functioning at 40 kV e 40 mA.

The X-ray diagrams were recorded in 2θ angles from 5° to 35° by rising the step of 0.02°, with a speed of 0.6 °/min, under an integration time of 2s. The 3D structure of the compounds chosen as reference is taken from CCDC (Cambridge Crystallographic Data Centre) and then through the program mercury the 2D patter is simulated.

3.2.4 NMR

The ^{1}H-NMR spectra were recorded through the Bruker-300 MHz spectrometer. Firstly, a small amount of powder was placed in the sample holder tubes and then it is dissolved in deuterated solvent. The 1H chemical shifts are reported in parts per million ppm while the notation for the spectral multiplicities are singlet = s, doublet = d, triplet = t, quadruplet = q and multiplet = m.

The solvent chosen in the tests was dimethyl sulfoxide-d_6 (DMSO, $(CH_3)_2S=0$)) with a characteristic NMR peak at 2.5 ppm.

3.2.5 TGA

The thermogravimetric analysis (TGA) was executed putting (5-7 mg) of solid samples in aluminum oxide crucibles on a Mettler Toledo TGA-STDA 851e machine. The temperature was change from 25 to 400°C, at a scanning rate of 10°C/min. The nitrogen flows at 50 mL/min as a purge gas.

Subsequently, data were processed with the STARe 12.12 software.

3.2.6 DSC

The experiments were conducted on a TA DSC2500 equipment and the data were processed using the software TA instrument TRIOS v5.1.0.46403. Firstly, a small amount of solid phase was weighted (3-8 mg) in an aluminium pan that is closed with a lid which is perforated. The reference sample pan is in indium.

Two different protocols were used because the temperature ranges of the first showed no phenomenon except for one compound. For all other, the tests were repeated widening the temperature ranges. The first protocol used is described in the table 3.9 whereas the second in the table 3.10.

Table 3.9 *First protocol used for DSC*

Step	Rate Temperature/ time
Hold	35° C during 5 min
Heating rate	5°C/min from 35°C to 150°C
Hold	150 during 5 min
Cooling rate	5°C/min from 150°C to 25°C
Hold	25°C during 5 min
Heating rate	10°C/min from 25°C to 150°C

Table 3.10 *Second protocol used for DSC*

Step	Rate Temperature/ time
Hold	35° C during 15 min
Heating rate	5°C/min from 35°C to 200°C
Hold	250 during 5 min
Cooling rate	5°C/min from 200°C to 25°C
Hold	25°C during 5 min
Heating rate	5°C/min from 25°C to 200°C

Chapter IV

4 Result

In this chapter the main results of the experiments executed for this thesis will be presented. The chapter is divided in two sections: first a review of the compounds excluded at each step will be made. Then, the remaining compounds will be characterized because they are possible candidates for salt cocrystal formation and separation of the D-Malic acid from a racemic mixture.

4.1 Excluded compounds

The experiments were conducted with both pure enantiomers of malic acid in a ratio of 1:1 with the chiral amine in ethanol at 5% or 10% in volume.

A foremost skimming was done if the vials after 3-6 days stirred, even after doubling their concentration, were in the liquid phase. No crystal has formed, and this means that the components in solution were too soluble. They are collected in the following table:

Table 4.1 *Compounds that have not formed crystals because were too soluble or degraded*

Components	Solvent	V (mL)	Result for D-MA	Result for L-MA
S-Methyl-L-cysteine	10% EtOH	1,50	Too soluble, no chrystals observed	Too soluble, no chrystals observed
	5% EtOH	1,50	No crystals	No crystals

Aspartame	10% EtOH	3,00	No crystals	No crystals
	5% EtOH	3,00		No crystals
Phenylglycinamide (D)	10% EtOH			No crystals
	5% EtOH			No crystals
L-Tyrosine Hydrazide	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
(R)-1-phenylethylamine	10% EtOH	1,00	No crystals	
	5% EtOH	1,50	No crystals	
(S)-1-phenylethylamine	10% EtOH	1,00		No crystals
	5% EtOH	1,00		No crystals
(S)-2-aminobutanol	10% EtOH	1,50	No crystals	No crystals
	5% EtOH	1,50	No crystals	No crystals
Etiracetam	10% EtOH	1,00		
	5% EtOH	1,50	No crystals	No crystals
Levetiracetam	10% EtOH	1,00	No crystals	
	5% EtOH	1,50	No crystals	No crystals
(R)-(+)-1-(4-Bromophenyl)ethylamine	10% EtOH	1,50	No crystals	
	5% EtOH	1,50		
L-Alaninol	10% EtOH	1,00	No crystals	No crystals
(S)-(+)-2-Amino-1-propanol	5% EtOH	1,00	No crystals	No crystals
(R)-(-)-2-Amino-1-butanol	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
(R)-(-)-2-Amino-3-methyl-1-butanol	10% EtOH	1,00	No crystals	No crystals

	5% EtOH	1,00	No crystals	No crystals
(S)-(+)-2-Amino-3-methyl-1-butanol	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
(S)-(+)-2-Pyrrolidinemethanol	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
(S)-(+)-Leucinol	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
(±)-trans-1,2-Diaminocyclohexane	10% EtOH	1,00		
	5% EtOH	1,00	No crystals	No crystals
(S)-(-)-2-Amino-3-phenyl-1-propanol	10% EtOH	1,50	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
DL-2-Phenylglycinol	10% EtOH	1,00	Degradation	Degradation
	5% EtOH	1,00	Degradation	Degradation
(S)-(+)-2-Phenylglycinol	10% EtOH	1,50	No crystals	No crystals
	5% EtOH	1,50	No crystals	No crystals
(1S,2R)-(-)-1-Amino-2-indanol	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals
(1R,2R)-(-)-2-Amino-1-phenyl-1,3-propanediol	10% EtOH	1,00	No crystals	No crystals
	5% EtOH	1,00	No crystals	No crystals

Over the course of the salt cocrystal screening with the malic acid enantiomers and each compound screened in equimolar ratio (1:1) some crystal formed. The powder after being filtered and dried was analysed through X-Ray Powder Diffraction (XRPD) to determine if the sample was a cocrystal or a physical mixture of the two components. In fact, each crystalline solid has peculiar peaks; hence, the diffractograms of the two-parent compound might be used as a benchmark to establish if a new crystal structure has formed. The comparison was made using the simulated patterns of (L) malic acid and the chiral amine used in the experiment. The appearance of additional new peaks to sum of those of the single compounds, suggested that a new crystalline form has nucleated. The table 4.2 collects all the compounds that did not perform new peaks.

Table 4.2 *Compounds excluded because they did not show new peaks after XRPD analysis*

Components	Solvent	V (mL)	Result for D-MA	Result for L-MA
(R,S)-1-(4-bromophenyl) ethylamine	5% EtOH	3,00	XRPD shows only this compound and not MA	
Etiracetam	10% EtOH	1,00	XRPD shows only this compound and not MA	XRPD shows only this compound and not MA
D-2 Phenylglycine	10% EtOH	2	XRPD shows only this compound and not MA	XRPD shows only this compound and not MA
	5% EtOH	2	XRPD shows only this compound and not MA	XRPD shows only this compound and not MA
L-2 Phenylglycine	10% EtOH	1,5	XRPD shows only this compound and not MA	XRPD shows only this compound and not MA
	5% EtOH	1,5	XRPD shows only this compound and not MA	XRPD shows only this compound and not MA

Further screening is done for compounds with new peaks in the diffractogram through NMR analysis to confirm that a new structure has been created. Previously, both MA and the chiral amine are examinate by themselves and the trait peaks are recorder. The broad peaks result from labile ^{1}H exchanging with solvents. Then, these are compared with the spectrum of the experiment. As known, the chemical environment can affect intensity or shift values, for this reason it is necessary to evaluate if there are peaks for the non-labile ^{1}H of parental compounds. The following table lists all compounds that do not follow the previous condition:

Table 4.3 Compounds excluded after the NMR analysis

Compound	Solvents	V (mL)	Result for D-MA	Result for L-MA
Praziquantel	10% EtOH	3,00	NMR shows only this compound and not MA	NMR shows only this compound and not MA
	5% EtOH	3,00	NMR shows only this compound and not MA	NMR shows only this compound and not MA
(1S,2S)-(+)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol	10% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
	5% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
Phenylglycinamide (D)	10% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
	5% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
(1R,2R)-4-nitrophenyl-2-aminopropane-1,3-diol	10% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
	5% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
(S)-2-(phenylamino)caronyloxy propionic acid	10% EtOH	2,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
	5% EtOH	2,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
(8s 9r)-(-)-n-benzylcinchonidinium chloride	10% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA
	5% EtOH	1,50	NMR shows only this compound and not MA	NMR shows only this compound and not MA

(±)-trans-1,2-Diaminocyclohexane	10% EtOH	1,00	NMR shows only this compound and not MA	NMR shows only this compound and not MA

In conclusion, the remaining candidates are list in the table 4.2:

Table 4.2 *Compounds that have formed salts*

Compound	Solvent	V (mL)	Result for D-MA	Result for L-MA
(1S,2R)-2-Amino-1,2-diphenylethanol	10% EtOH	2,00	XRPD shows new peaks and NMR confirm,amorphous	XRPD shows new peaks and NMR confirm,amorphous
	5% EtOH	2,50	XRPD shows new peaks and NMR confirm,amorphous	XRPD shows new peaks and NMR confirm,amorphous
(R)-1-phenylethylamine	10% EtOH	1,00		XRPD shows new peaks and NMR confirm
	5% EtOH	1,50		XRPD shows new peaks and NMR confirm
(S)-1-phenylethylamine	10% EtOH	1,00	XRPD shows new peaks and NMR confirm	
	5% EtOH	1,00	XRPD shows new peaks and NMR confirm	
(R)-(+)-1-(1-naphtyl)-ethylamine	10% EtOH	2,00	XRPD shows new peaks and NMR confirm	XRPD shows new peaks and NMR confirm
	5% EtOH	2,00	XRPD shows new peaks and NMR confirm	XRPD shows new peaks and NMR confirm
(S)-(-)-1-(1-naphtyl)-ethylamine	10% EtOH	D=2,5 L=1	XRPD shows new peaks and NMR confirm	XRPD shows new peaks and NMR confirm
	5% EtOH	D=2,5 L=1	XRPD shows new peaks and NMR confirm	XRPD shows new peaks and NMR confirm

4.2 Characterization of possible D/L Malic Acid cocrystal Salts

Each chiral amines have been associated with a number.

- 4 is the (1S,2R)-2-Amino-1,2-diphenylethanol
- 63 is the (R)-1-Phenylethylamine
- 64 is the (S)-1-Phenylethylamine
- 71 is the (R)-1-(1-napthyl)ethylamine
- 72 is the (S)-1-(1-napthyl)ethylamine

4.2.1 Result of X-Ray powder diffraction of MA salts

Previously, the purpose for which X-ray analysis is used has been described. Diffractograms will be displayed and discussed below. For malic acid have been chosen as reference two simulated polymorphic and a monohydrated form of the L enantiomer were considered as references.

In figure 4.1 are collected and are overlapped the reference patterns to compare with MAD4 10% EtOH, MAD4 5% EtOH, MAL4 10% EtOH and MAL4 5% EtOH.

The peaks of sample nucleated during the experiments are different from both malic acid and the conformer (1S,2R)-2-Amino-1,2-diphenylethanol. In the result it is evident that there is a lot of noise, and it is not possible to see much, but it could be inferred that the salt is not pure it is perhaps amorphous.

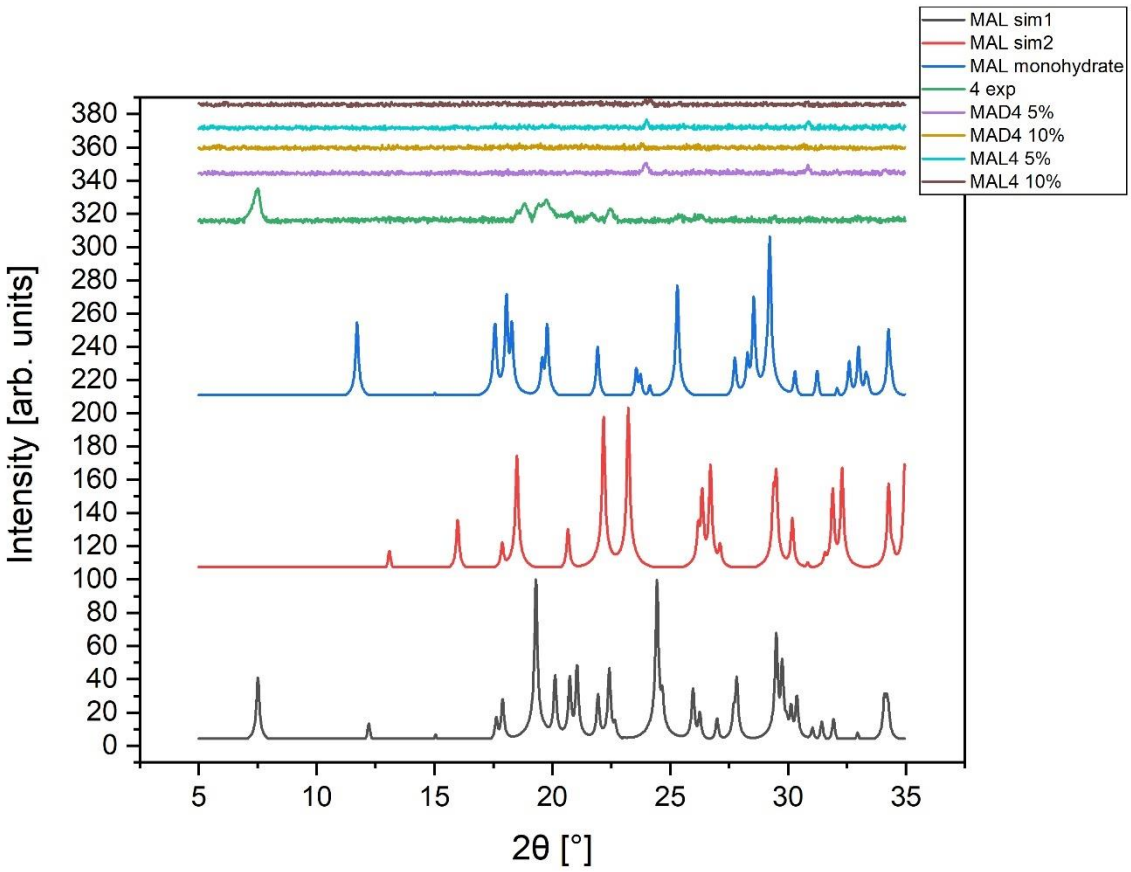


Figure 4.1 *XRPD diffractogram of (1S,2R)-2-Amino-1,2-diphenylethanol*

In figure 4.2 are collected and are overlapped the reference patterns to compare with MAL63 10% EtOH, MAL63 5% EtOH and MAD64 10% EtOH MAD64 5% EtOH.

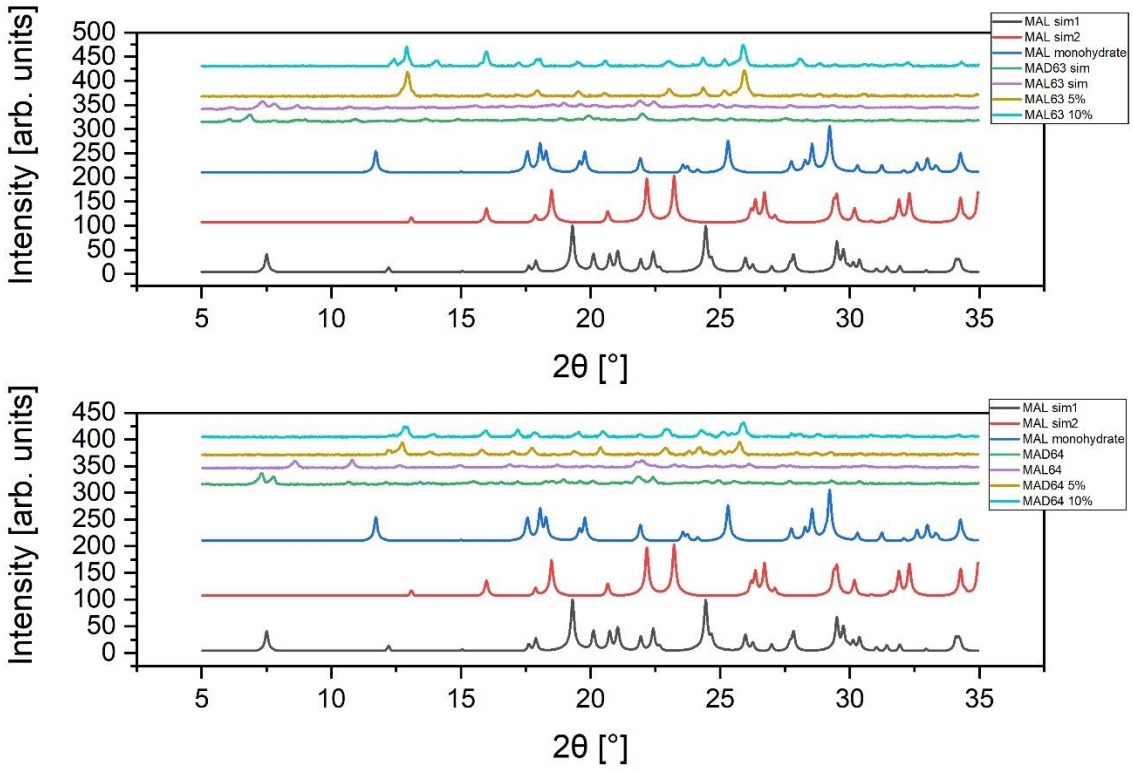


Figure 4.2 XRPD of (R)-1-Phenylethylamine (on the top) and (S)-1-Phenylethylamine (on the bottom).

New peaks are visible compared to malic acid and both coformers (R)-1-Phenylethylamine and (S)-1-Phenylethylamine, so it might be assumed that there is a new crystalline form.

Surprisingly, salts have formed between L-MA and (R)-1-Phenylethylamine and D-MA and (S)-1-Phenylethylamine and this is a very interesting result.

- In figure 4.3 are collected and are overlapped the reference patterns to compare with MAD71 10% EtOH, MAD71 5% EtOH , MAL71 10% EtOH, MAL71 5 % EtOH whereas in figure 4.4 MAD72 10% EtOH, MAD72 5% EtOH, MAL72 10% EtOH, MAL72 5% EtOH.

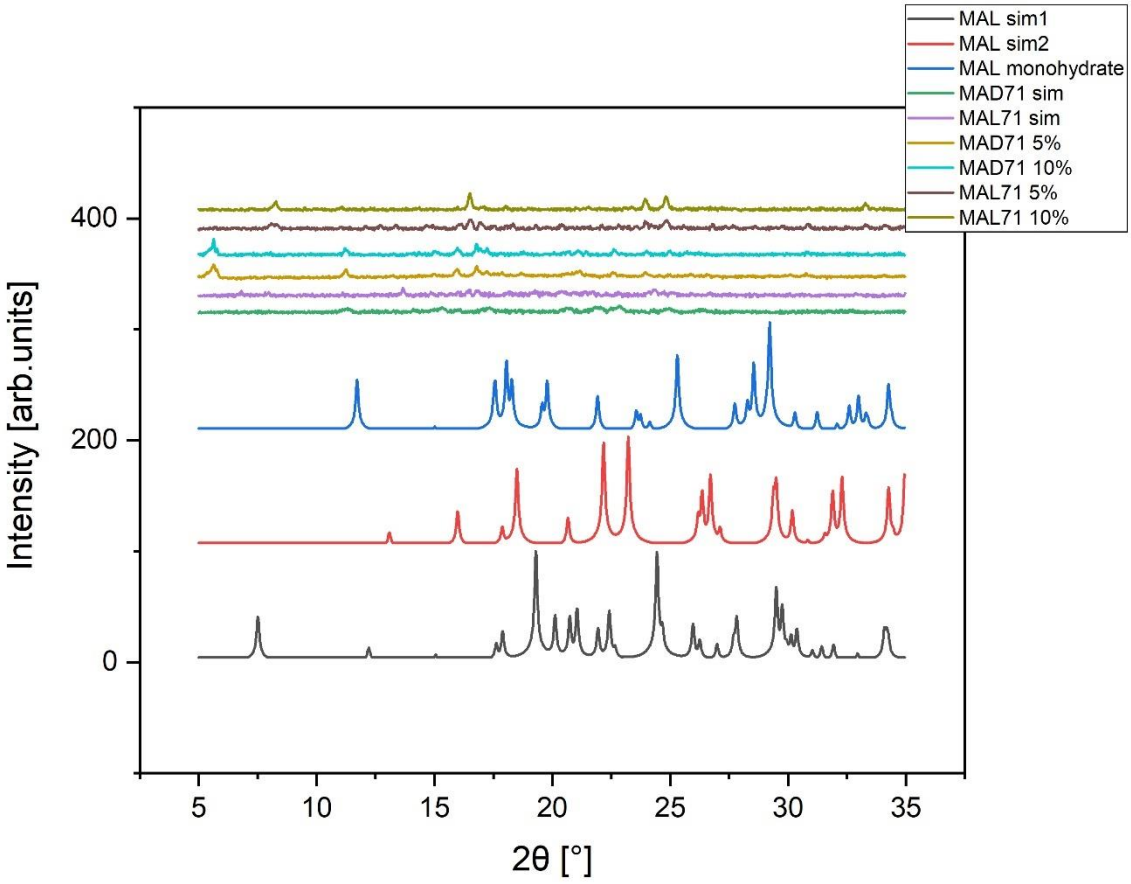


Figure 4.3 *XRPD of (R)-1-(1-napthyl)ethylamine*

The sample patterns of the experiments MAD71 5% and MAD71 10% are the same.

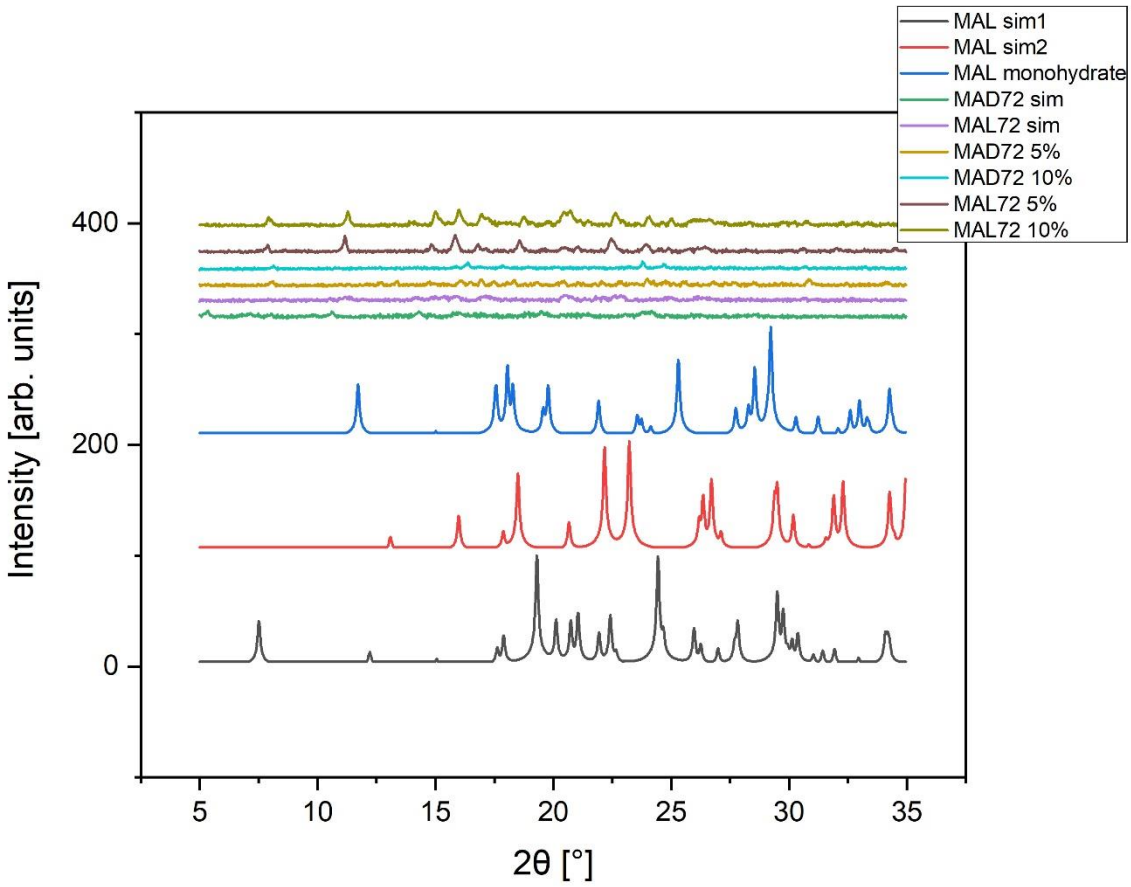


Figure 4.4 *XRPD of ()-1-(1-napthyl)ethylamine*

As in the previous figure, here MAL72 5% and MAL72 10% are the same.

It is possible to observe some similarities between the experiments because also MAL71 5% EtOH with MAD72 5% EtOH MAD72 10% EtOH with MAL71 10% EtOH have a comparable trend.

The latter two as before, are interesting results because it is as if there is a more favored crystallization between isomers.

4.2.2 Results of Nuclear magnetic resonance spectroscopy

For the NMR spectroscopy DMSO is often used as a solvent in its deuterated form (DMSO-d6) for its ability to dissolve a wide range of analytes and for the simplicity of its spectrum with a typical peak of 2.5 ppm. It was chosen as solvent to conduct the experiments in this work. The disadvantages of using DMSO-d6 are its viscosity, which broadens the NMR signals and its hygroscopicity. It is not unusual finding the H_2O resonance in the ^{1}H-NMR spectrum and sometimes a broad peak at 3.3 ppm could appear.

The characteristic spectrum of malic acid is shown as reference in figure

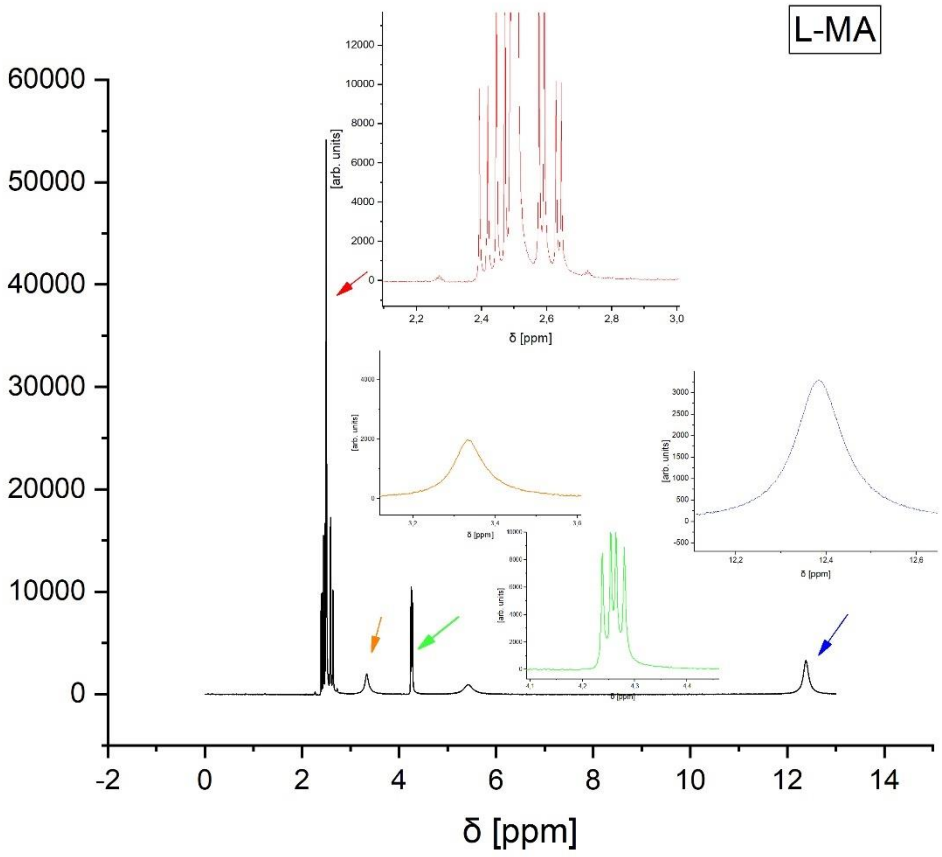


Figure 4.5 *NMR spectra of Malic Acid*

The properties of each characteristic NMR peak of this compound are instead reported in table 4.6 :

Table 4.3 *Characteristic peaks of malic acid*

shifts	multiplicity	∫	nH	
2.52	m	2.21	2H	$-CH_2$
3.34	bs	—	—	H2O
4.26	dd	0.96	1H	-CH
5.43	bs	0.80	1H	-OH
12.42	bs	1.77	2H	-COOH

In blue have been highlighted the peaks to be found in the spectrum of salt.

From the diffractogram evaluation of the salt formed with (1S,2R)-2-Amino-1,2-diphenylethanol, it was decided to continue with the analysis of MAD4 10% EtOH and MAL4 10% EtOH .

To make the NMR spectrum easier to understand, below is the molecule of (1S,2R)-2-Amino-1,2-diphenylethanol:

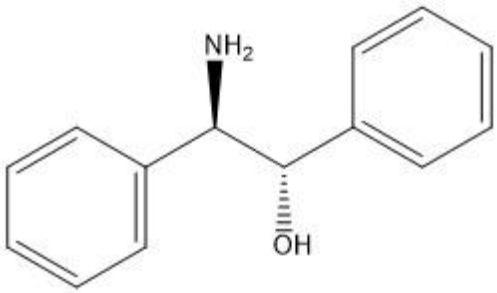


Figure 4.6 *Molecule of (1S,2R)-2-Amino-1,2-diphenylethanol*

What is expected from the spectrum is 10 -H absorbing approximately from 8.5 to 7 ppm, and the other two hydrogen between 4 and 5 pmm. The hydroxyl and amine group will not be visible because the hydrogens are labile. Hereinafter the spectra of the experiment of the MAD4 10% EtOH in the figure 4.7 which is really similar to that of MAL4 10% EtOH:

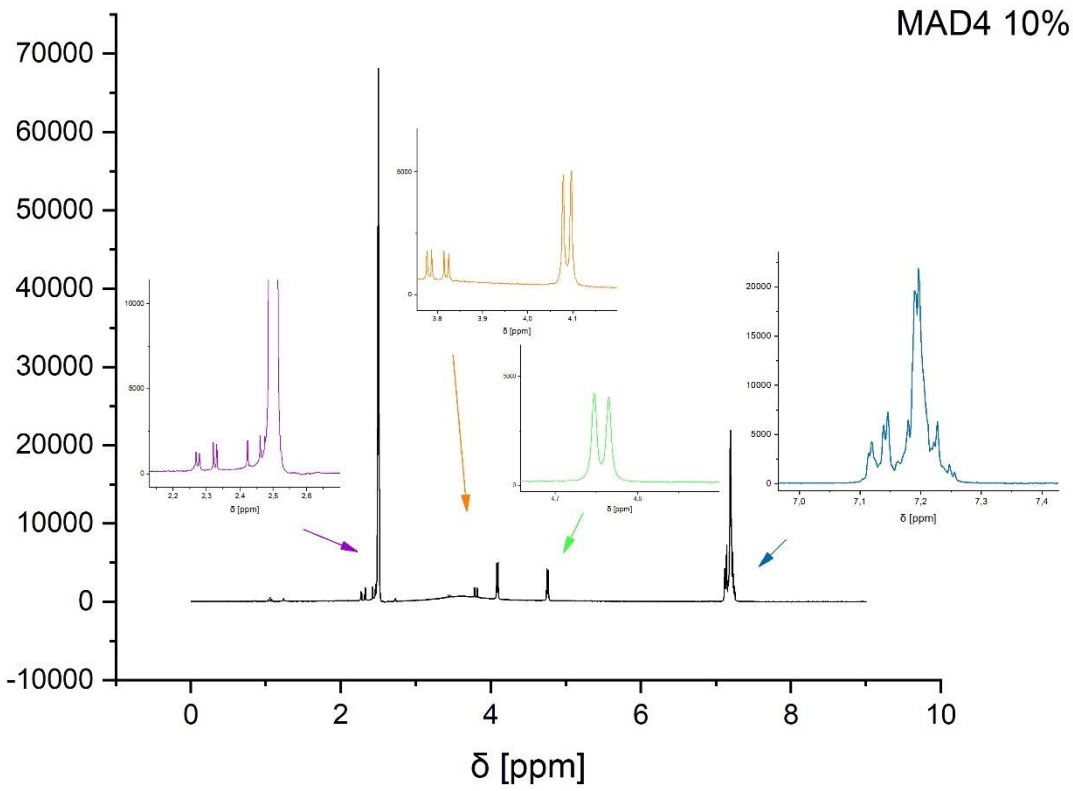


Figure 4.7 *NMR spectra of MAD4 10%*

The multiplet at 2.35 ppm and the doublet-doublet at 3.8 ppm are referred respectively to the -CH_2 and -CH of Malic Acid. The multiplet at 7.19 ppm represents the aromatics hydrogen, the doublets at 4.76 and 4.09 ppm are the -^{1}H from the (1S,2R)-2-Amino-1,2-diphenylethanol. For the sample MAL4 10 % the spectra is almost the same, only the values 4.75 and 4.07 were changed.

Table 4.4 *Characteristic peak of MAD4*

Shifts(ppm)	multiplicity	∫	nH	
2.35	m	0.94	1H	-CH_2
3.34	bs	—	—	H2O
3.8	dd	0.81	1H	-CH
4.07	d	1.29	1H	-H
4.75	d	1.07	1H	-H
7.19	m	10	10H	aromatics

Integrating the subtended area of these peaks gives us the number of hydrogens.

One inconsistency stands out from the table: from the integration of the peaks the number of hydrogens belonging to malic acid has decreased. The explanation could lie in the fact that

It could be assumed that the stoichiometry of the crystal is probably 2:1.

As mentioned above, all tests were performed in a 1:1 ratio, so the remaining malic acid will be non-converted and dissolved in solution.

Further analysis of the single crystal should be carried out to confirm whether the hypothesis just made is correct.

In the figure 4.8 is showed the molecule of the (R)-1-Phenylethylamine and the other enantiomer in the figure 4.9 the (S)-1-Phenylethylamine

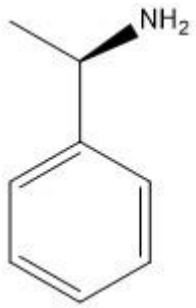


Figure 4.8 *Molecule of (R)-1-Phenylethylamine*

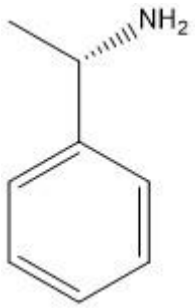


Figure 4.9 *Molecule of (S)-1-Phenylethylamine*

The molecule is comprises an aromatic ring so one would expect to find five hydrogens absorbing from 8.5 to 7 ppm , three hydrogen from the methyl group and the last one.

Two enantiomers analysed with NMR have the same spectra. As seen before for these enantiomers the D-MA had formed a salt with the (S)-1-Phenylethylamine and the L-MA with the (R)-1-Phenylethylamine. It is necessary to emphasise this point because all analysed samples had the same peaks, only the values differed slightly.

Only two spectra are therefore shown by way explanation: the MAL63 5% in the figure 4.10 and the MAD64 5% in the figure 4.11.

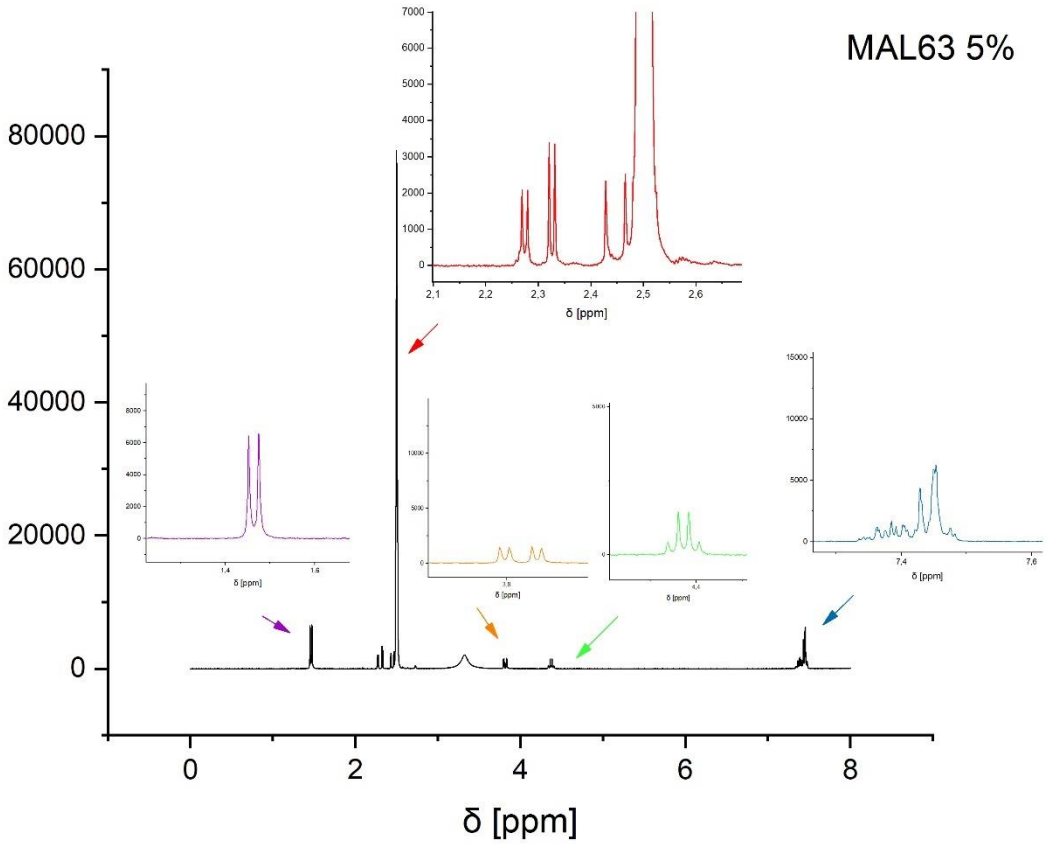


Figure 4.10 *NMR spectra of MAL63 5%*

The multiplet at 2.35 ppm and the doublet-doublet at 3.8 ppm are referred respectively to the -CH_2 and -CH of Malic Acid. There is a multiplet at 7.41 ppm corresponding to aromatics ^1H in fact, from the integration there are five. Then, a quadruplet at 4.37 ppm and a methyl group -CH_3 at 1.46 ppm, all belonging to the (R)-1-Phenylethylamine. For the other enantiomer, the (S)-1-Phenylethylamine the value of the quadruplet is 4.37 and the doublet doublet at 1.47 ppm.

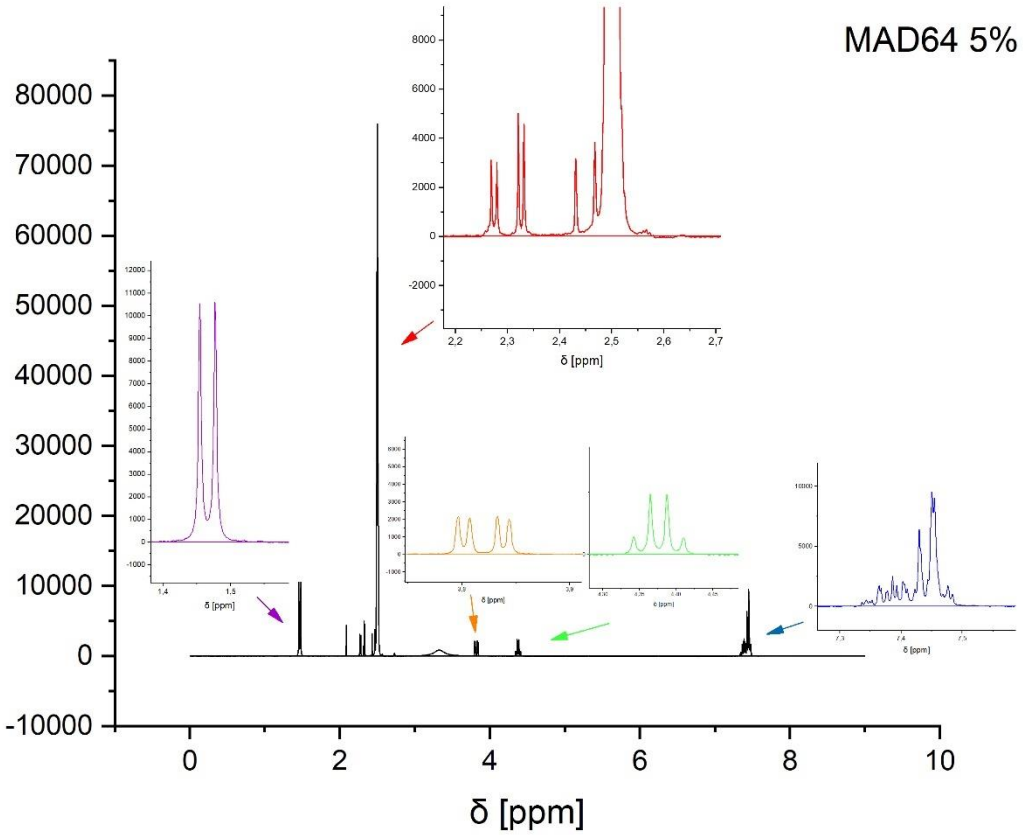
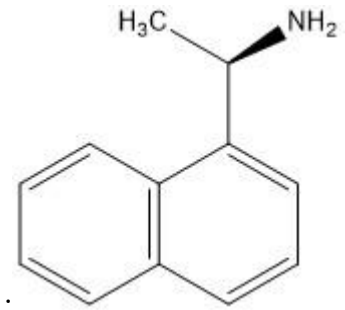


Figure 4.11 *NMR spectra MAD64 5%*

Such as the salts formed by compound 4, not all samples of compounds 71 and 72 were analysed; the diffraction patterns for MAD71 5% and MAD71 10% and for MAD72 5% and MAD71 10% are the same for which it was chosen to proceed with those at 5%. Initially are presented the results for MA71 and then results for MA72. The molecule of (R)-(+)-1-(1-naphtyl)-ethylamine is



:

Figure 4.12 *Molecule of (R)-(+)-1-(1-naphtyl)-ethylamine*

And the other enantiomer is the (S)-(+)-1-(1-naphtyl)-ethylamine, in the figure 4.13:

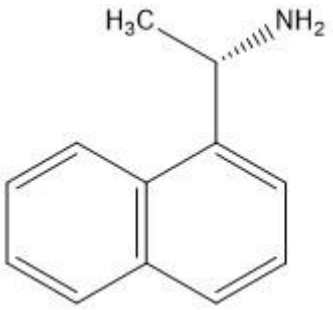


Figure 4.13 *Molecule of (S)-(+)-1-(1-naphtyl)-ethylamine*

From the structure of the molecule, it can be seen that there are two aromatic rings that involve seven hydrogen, a methyl group and another hydrogen from -CH, for a total of eleven hydrogen. The samples analysed all show the same traits, changing only for a few values but not the shape. In the previous step we saw that MAL71 5% EtOH and MAD72 5% EtOH 5% EtOH have the same pattern. For this reason, the two relevant spectra will be shown in the following figures:

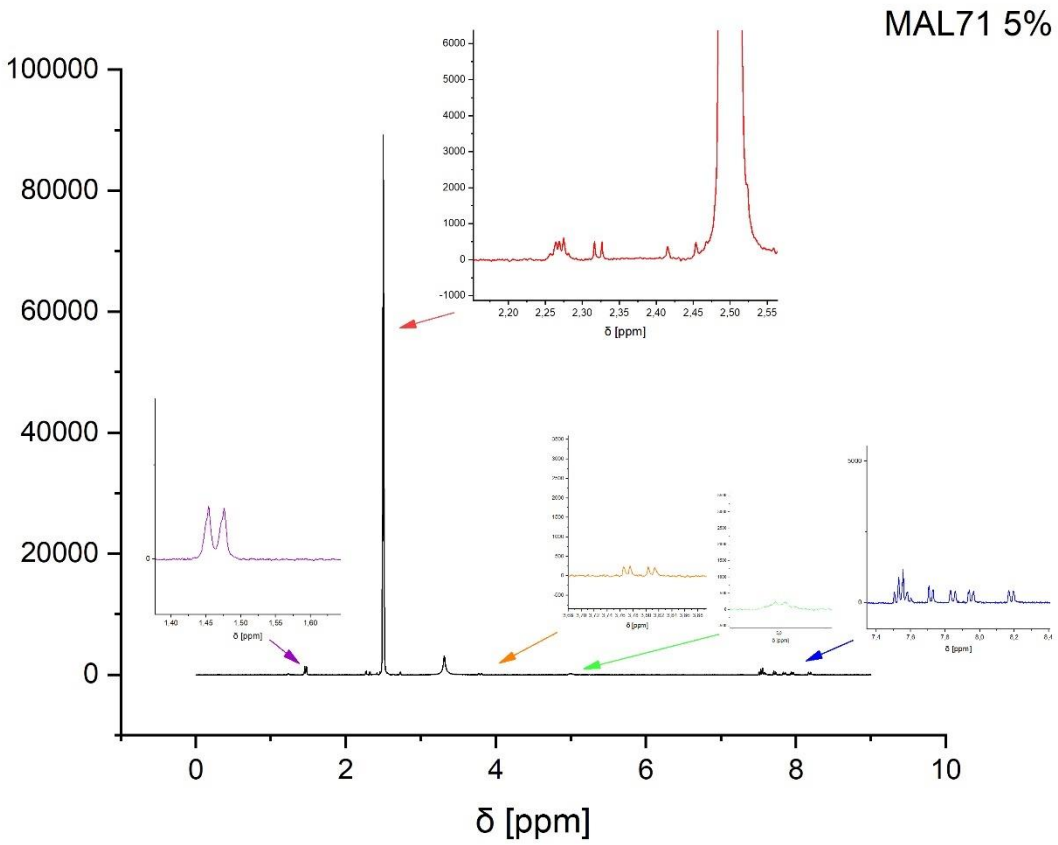


Figure 4.14 *NMR spectra of MAL71 5%*

The multiplet at 2.33 ppm and the doublet-doublet at 3.79 ppm are referred respectively to the $-CH_2$ and -CH of Malic Acid. There are three doublets at 8.18 ppm, 7.86 ppm and 7.72 ppm as well as two multiplet at 7.95 ppm and 7.57 ppm related to aromatics ^1H of (R)-(+)-1-(1-naphtyl)-ethylamine. From these integrated peaks result the seven hydrogens.

The "doublet" at 4.99 ppm corresponding to a -CH and the doublet at 1.46 ppm corresponding to a $-CH_3$ belong to the (R)-(+)-1-(1-naphtyl)-ethylamine.

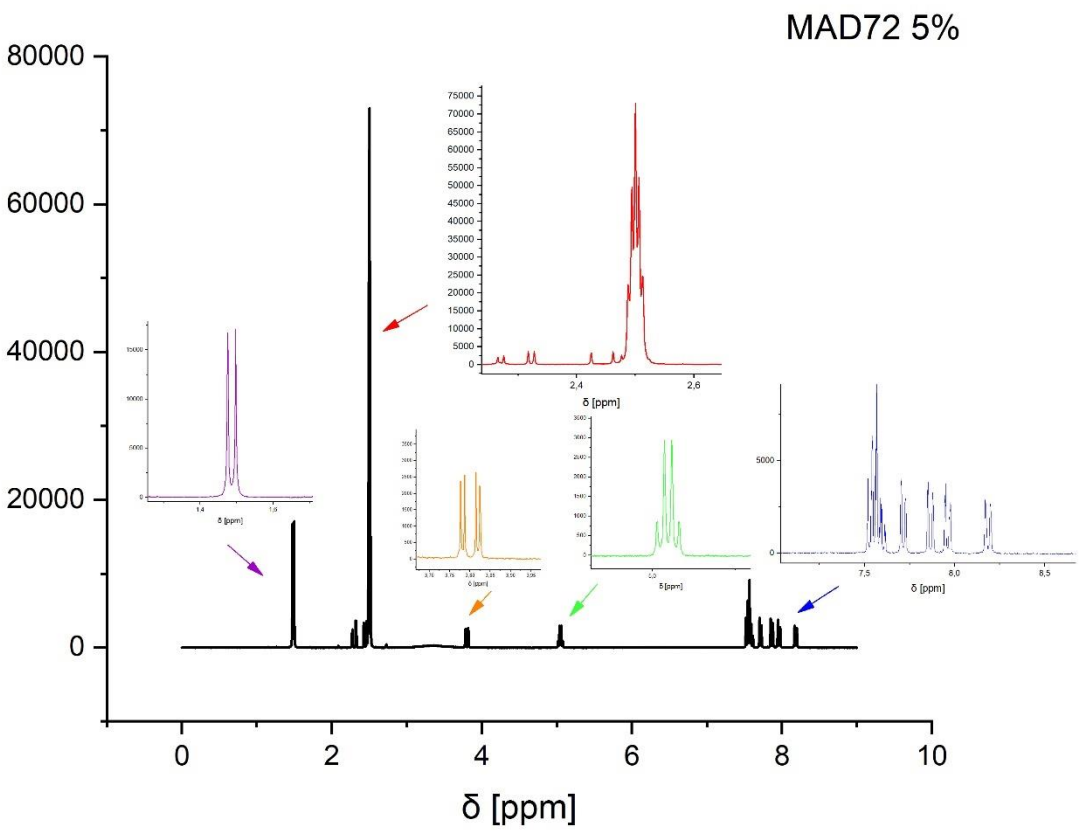


Figure 4.15 *NMR spectra of MAD72 5%*

4.2.3 Result for Thermogravimetric analysis:

Before discussing the results obtained from this analysis a foreword is necessary, as the salts have ionic bonds and charge-assisted hydrogen bond, the melting and boiling temperature of these salts are often intermediate or higher than those of the parent compound. In the following table physical properties will be listed:

- Malic acid:
  melting temperature = 130-133°C
  boiling/degradation temperature = 150°C

- MA4 (1S,2R)-2-Amino-1,2-diphenylethanol
  melting temperature = 142-143°C

- MA63-64 (R)/(S)-1-Phenylethylamine
  melting temperature = -10°C

  boiling temperature = 187 °C

- MA71-72 (R)/(S)-1-(1-napthyl)ethylamine
  boiling temperature = 154-153°C

The TGA analyses are carried out prior to the DSC tests to identify the temperature range within each sample degrades. The results of the tests with their description are grouped because the trends and the values are similar, and they will be presented below.

For the MAD4 10%, in the beginning the sample probably lose 5.7910% ~ 5.8% of solvent adsorbed at the surface of the powder. The adsorption hypothesis is deduced from the fact that the temperature is between 25-100°C. It is common to find solvent on the surface of the powder in ionic salt.

Then, two gradual "steps" of slow degradation are observed between 140-420°C that may derive from melting or degradation, which is very often encountered in the case of ionic salt with solvent adsorbed on the surface.

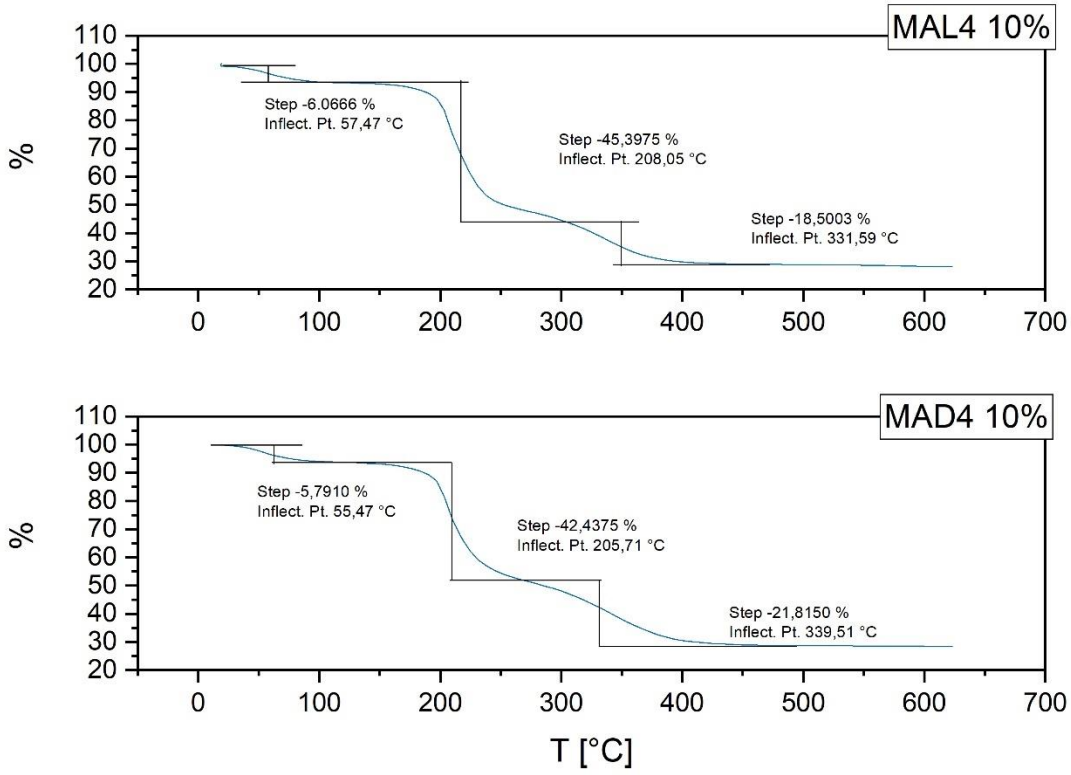


Figure 4.16 *TGA of MAD4 10%*

For the tests with (R)-1-Phenylethylamine the trend is slightly different. For the MAD63 5% this is probably a simple degradation of an ionic salt. The sample degrades from 120°C which could be melting or degradation. The sample lose ~ 95.8% of its mass (almost all of it, almost completely degraded).

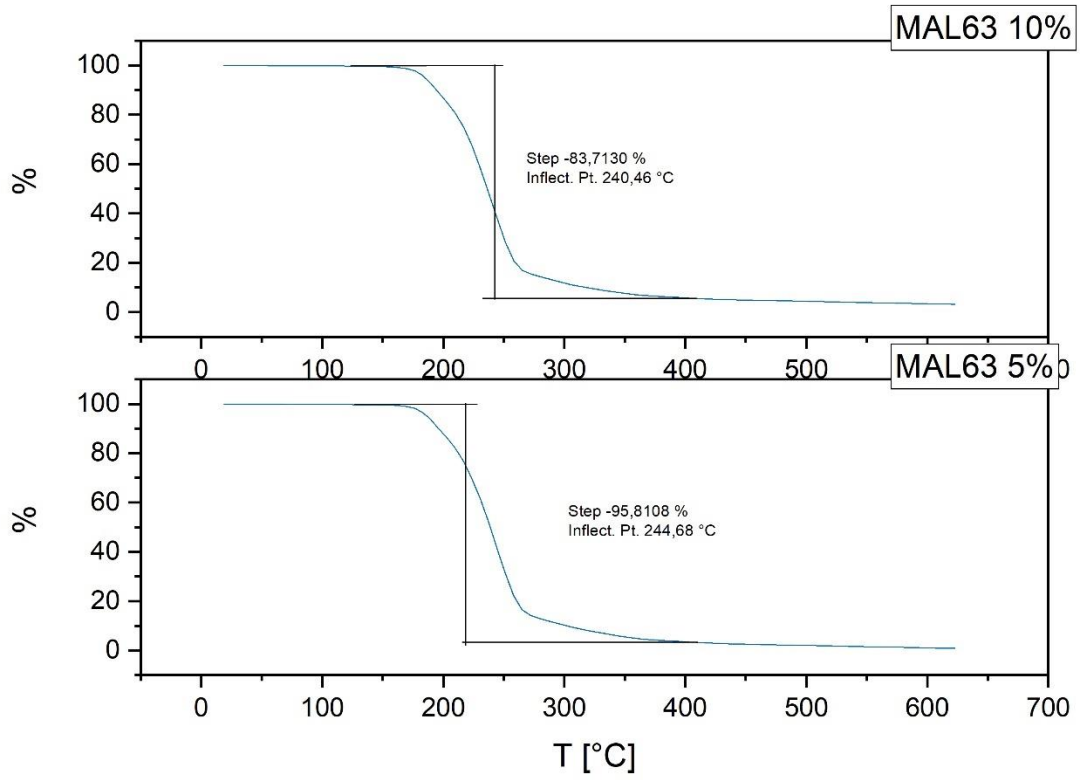


Figure 4.17 *TGA of MAL63 5% and 10%*

As before for MAL63 this is probably the degradation of the ionic salt. The sample degrades from 170°C. The sample lose 83.7130% ~ 83.7% of its mass.

The analysis for the other enantiomer, the (R)-1-Phenylethylamine the shape is similar.

The sample MAD64 10% degrades from 120°C and it loses ~ 94.7% of its mass (almost all of it, almost completely degraded).

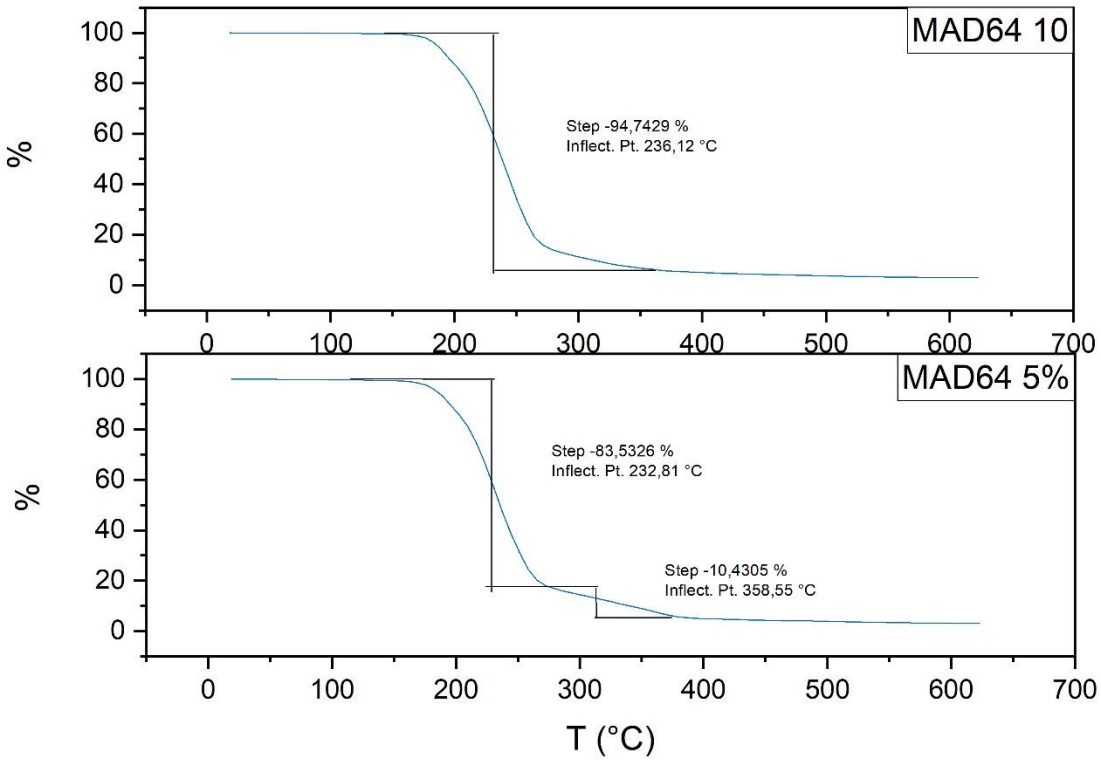


Figure 4.18 *TGA of MAD64 5% and MAD64 10%*

The sample MAD64 5% degrades from 150°C it lose in a more gradual way, as if there are two steps, almost all its mass (83.5326+10.4305=93.9631%).

In the previous step, only the graphs of MAL71 5% and MAD72 5% were exhibited. Hence, given that after the analysis of all samples, the graphs do not show any relevant discrepancies, only these will be commented.

As seen in the figure 4.19 the MAL71 5% sample probably lose ~ 1.30% of solvent adsorbed at the surface of the powder. This assumption is based on the fact that the temperature is less than 50 °C. Then, the sample probably lose solvent absorbed into the powder (-3.92%): the powder most likely went through dehydration, the temperature is around 100°C.

Finally, the continuation of the degradation starting from 120°C was analysed as a single step but it is actually there is no a clear jump but a consecutive series of steps of slow degradation which is very often encountered in the case of ionic salt with solvent adsorbed on the surface.

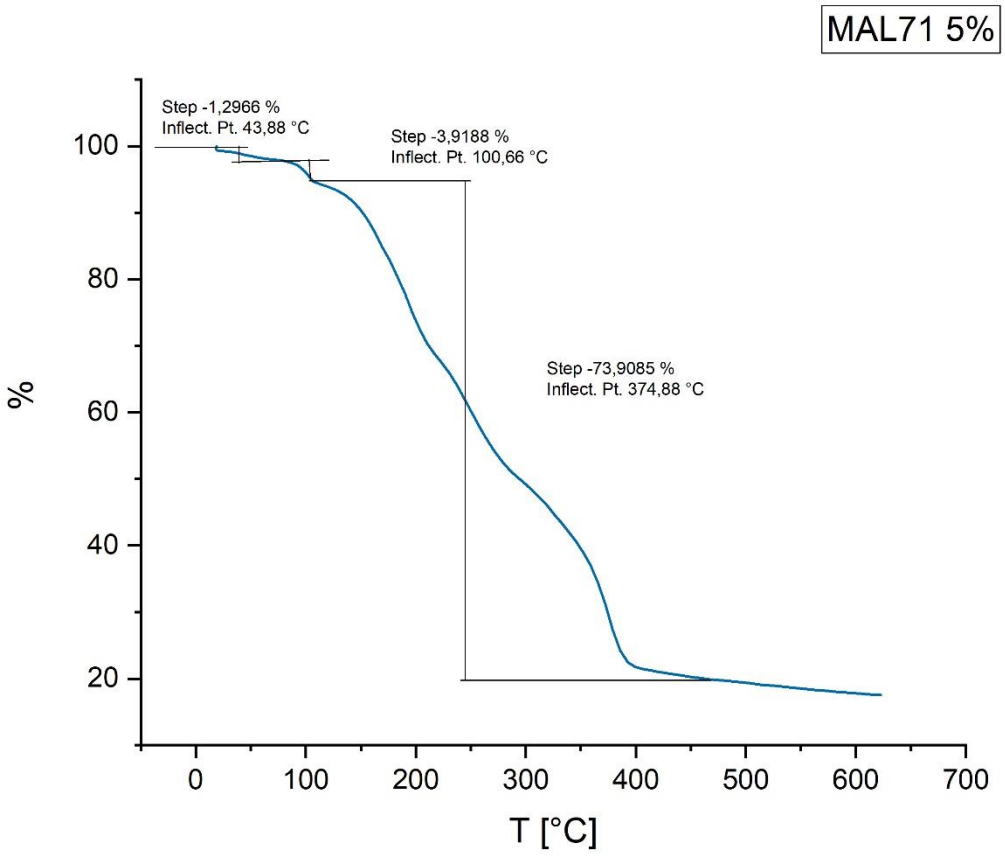


Figure 4.19 *TGA of MA71 5%*

Looking at the figure and comparing it with the previous one, trends are similar

The sample MAD72 5% probably lose 1.7% of solvent adsorbed at the surface of the powder.

Then, probably lose solvent absorbed into the powder (-3.3%) go through a slow degradation.

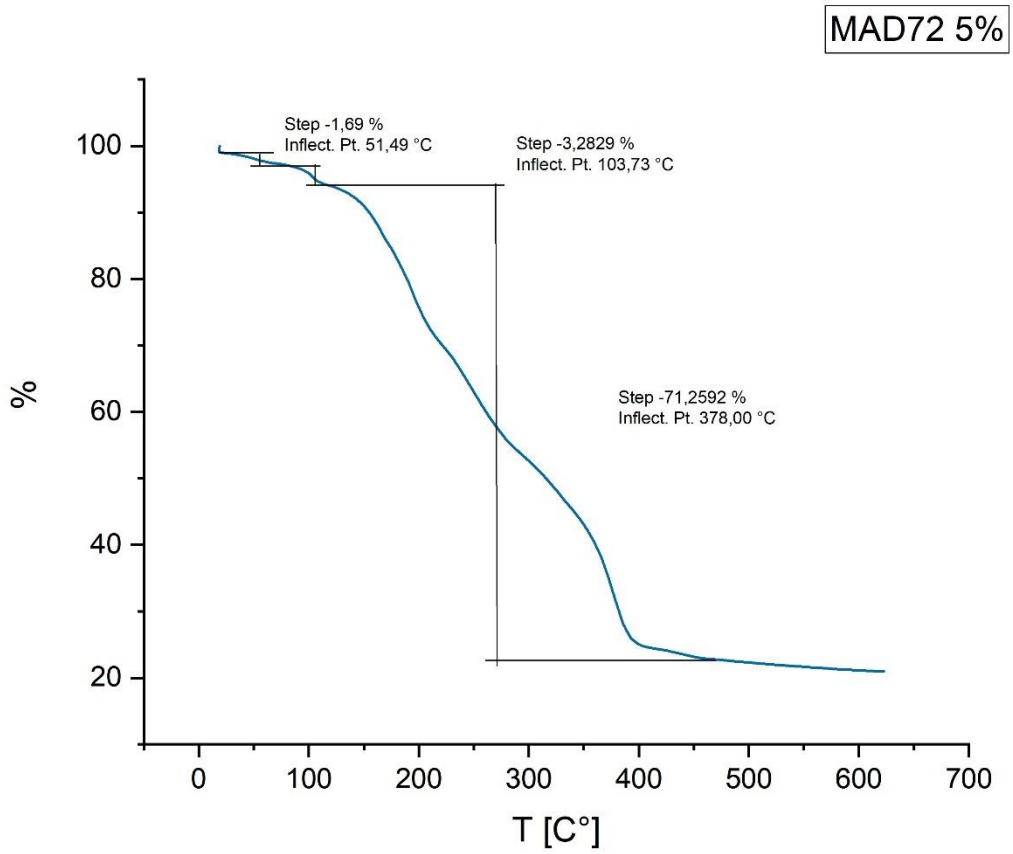


Figure 4.20 *TGA of MAD72 5%*

4.2.4 Results for Differential Scanning Calorimetry

This last analysis was made to characterize the phase changes of the salts, and to validate the hypotheses made on the graphs of the previous paragraph. (1S,2R)-2-Amino-1,2-diphenylethanol, which was then tested only once. For all other samples the tests were carried out twice, with a higher temperature range.

It was chosen to speed up the last heating cycle, so the melting value deviates slightly among repetitions.

The first black broad peak is referred to the solvent adsorbed at the surface, is very wide probably because the sample was not perfectly dry; whereas the second black thigh peaks indicate the melting transition at 141.,05 °C. The melt is then cooled and crystallized at 133.8 °C (red line) , this is confirmed by the red peak roughly at the same temperature as of the second green peak atone 137.51°C .

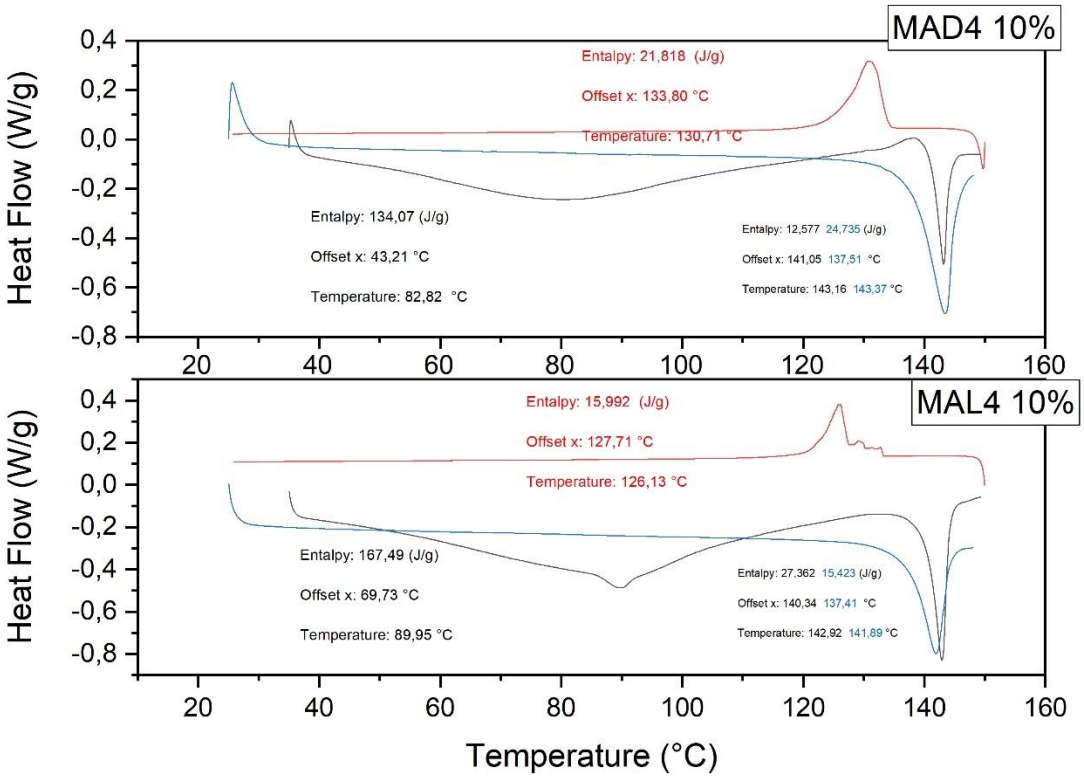


Figure 4.21 *DSC tests of MAD4 10% and MAL4 10%*

The trend is also similar for the sample MAL4 10% , the sample is probably wet and so the first black broad peak is referred to the solvent adsorbed at the surface , whereas the second black thigh peaks indicates the melting transition at 140.34 °C. The liquid is cooled and crystallized at 127.71 °C, this is confirmed by the blue peak roughly at the same temperature as the second green one 137.41°C .

The graphs resulting from MAL63 are shown in the figure 4.19.

In the case of MAL63 10 %there is a first peak which is probably related to adsorbed solvent and then a small peak perhaps referring to a transformation into a different polymorphic form. Lastly, there is the melting and then the complete degradation. Instead for the MAL63 5% the sample first melts and then degrades.

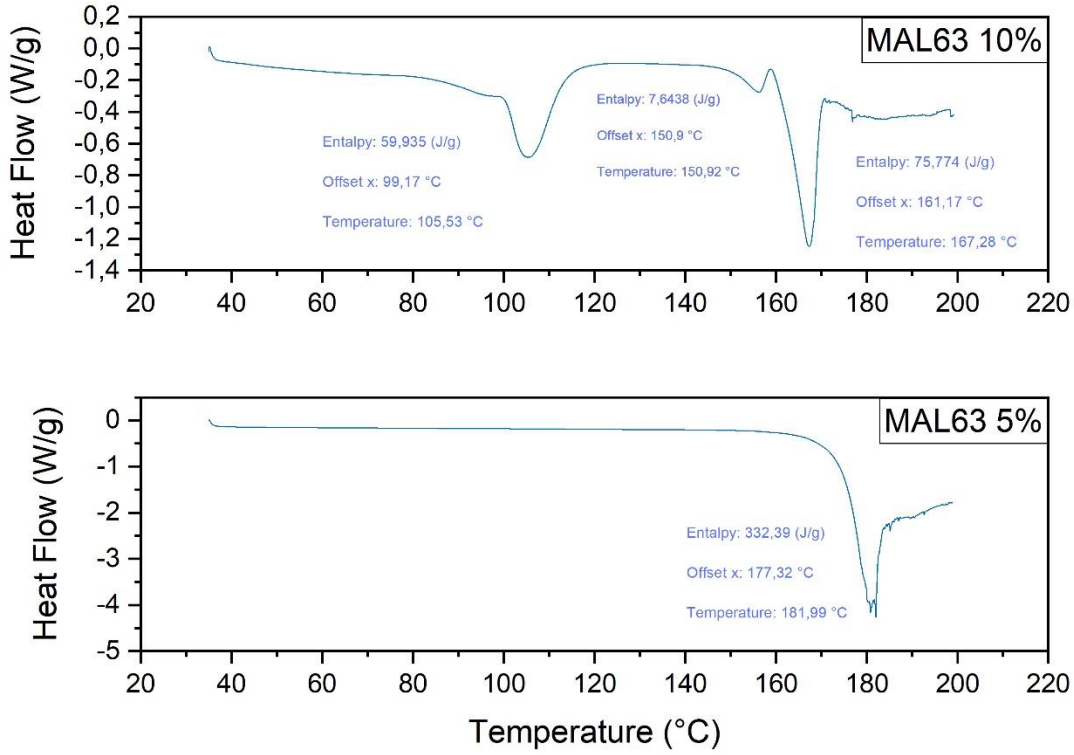


Figure 4.22 *DSC of the MAL63*

Both tests with (S)-1-Phenylethylamine (MAD64) are dried and so it simply melts and degrades.

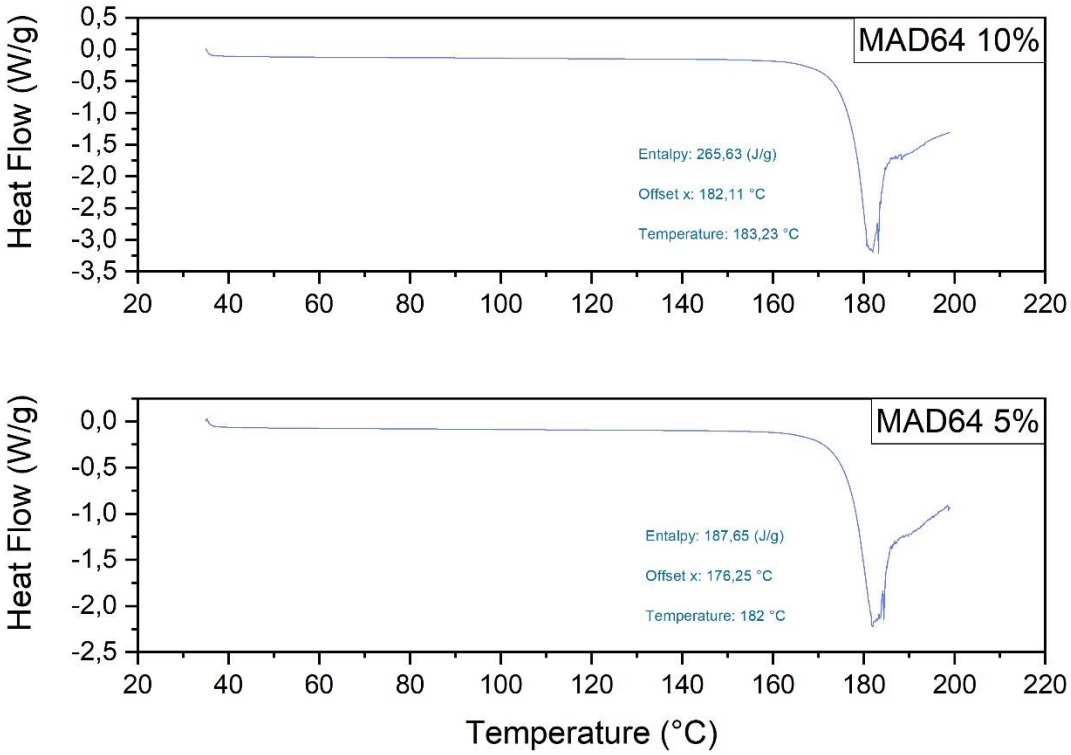


Figure 4.23 *DSC of the MAD64*

For the MAL71 5% and MAD72 5% both have a fist peak related to the solvent absorbed in different quantities and then go through melting and degradation.

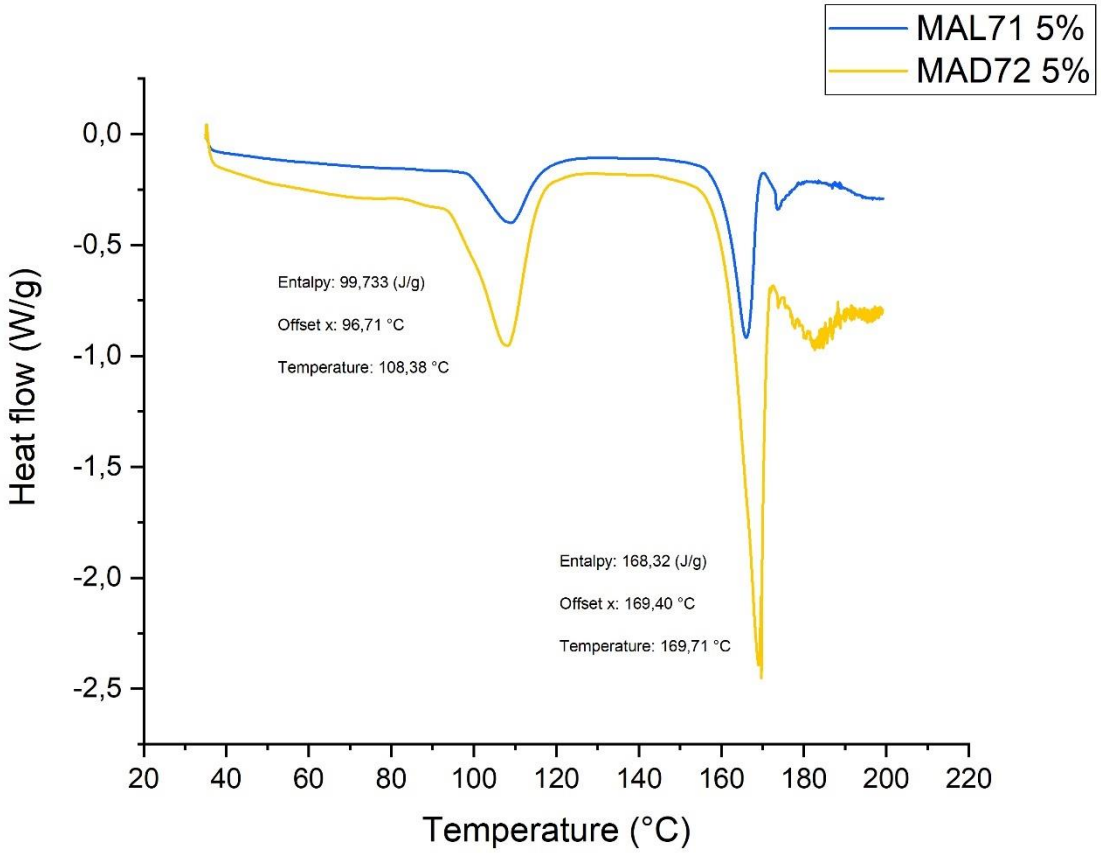


Figure 4.24 *DSC of MAL71 5% and MAD72 5%*

Further analysis will be done for all other samples of these two enantiomers because the graphs are not very comprehensive. By way of explanation in the figure 4.25 is presented the resultin graph of the salt MAL72 5%

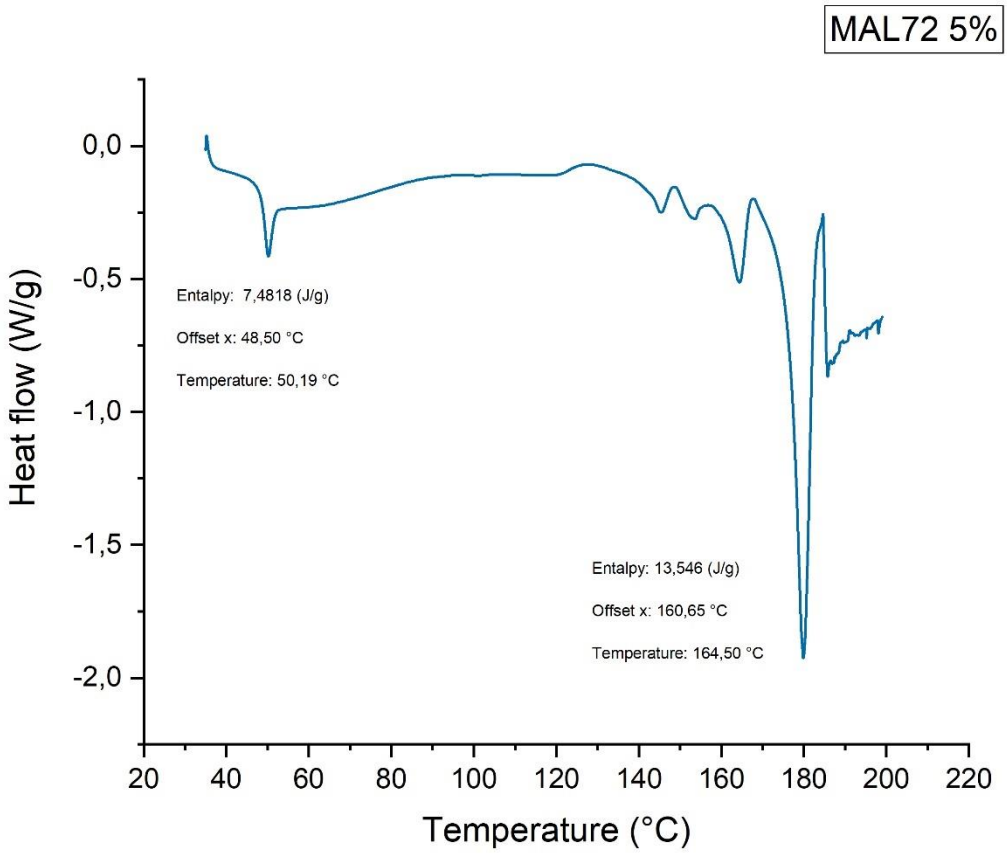


Figure 4.25 *DSC of MAL72 5 %*

4.2.5 Solubility determination

Before inserting 1 ml of solution into vials, they were weighed. Subsequently, after evaporating all the solvent, they were weighed again. From the weight difference, it is possible to derive the relative solubility values, which are tabulated:

Compound		Solubility MAD [mg/ml]	Solubility MAL [mg/ml]
(1S,2R)-2-Amino-1,2-diphenylethanol	*10% EtOH*	140.38	141.03
	5% EtOH	91.47	161.71
(R)-1-phenylethylamine	*10% EtOH*		149.36
	5% EtOH		154.04
(S)-1-phenylethylamine	*10% EtOH*	166.86	
	5% EtOH	184.25	
(R)-(+)-1-(1-naphtyl)-ethylamine	*10% EtOH*	183.06	179.95
	5% EtOH	179.68	109.58
(S)-(-)-1-(1-naphtyl)-ethylamine	*10% EtOH*	197.65	142.80
	5% EtOH	162.94	117.86

Table 4.5 *Solubility values*

Chapter V

5 Conclusion and Future Developments

The experimental diffractograms of the solid coming from slurry experiments between the malic acid and the four chiral ammine, in an equimolar ratio (1:1) , exhibits new peaks in comparison to the powder patterns of the two parent-components, suggesting the apparition of a new crystalline phase. Perhaps it would be better to repeat the XRPD analysis for the (1S,2R)-2-Amino-1,2-diphenylethanol because the measure is affected by too much noise.

The NMR measurements confirm that in all four cases the salts cocrystals have in their spectra the parental peaks. It is necessary to accompany these results with further analyses of the single crystal, validating stoichiometry hypotheses and to obtain additional information.

Regarding thermal characterisation, some DSC tests, as MAL72 5%, should be repeated because they are inconclusive.

The remarkable discovery is that for the tests 63/64 and 71/72 it is as if there is a tendency to form salts with only one of the enantiomers of malic acid.

The (1S,2R)-2-Amino-1,2-diphenylethanol, (R)/(S)-1-Phenylethylamine, (R)/(S)-1-(1-napthyl) ethylamine, we can state that have formed diastereomer salts and all of them shares a common structural element, the benzyl. For the future one could continue with this result testing other molecules with the same functional group.

5.1 Future developments

The results achieved in this thesis are a starting point for a larger project of the doctoral student Camila Caro Garrido who is a member of the research group of the professor Tom Leyssens at the Universitè catholique de Louvain.

The first step is the detection of the chiral amines that allow the formation of stable salt cocrystals with the malic acid and then the definition of their properties. The four compounds identified in this work will be then used in reactive crystallization systems.

The goal is to solve a racemate by preparing a supersaturated solution that led to two different diastereomeric salts thanks to a chiral coformer. Therefore, in the system is added a racemase enzyme whose task is to catalyze the equilibrium reaction (K_{eq}=1).

The racemace enzyme catalyzed the reaction which can be pushed towards the one og the two enantiomers. Enzymes have as a characteristic of being very sensitive, being influenced by the environment, hence by all variation of temperature, pressure, pH and the concentration of the compounds; they might change their activity.

The value of solubility is fundamental to recreate the condition under which diastereomeric salts are formed and understand how the enzyme works under this condition.

The behavior of the enzyme will be studied in the system found because the solubility values are very high and whether it is able to shift the equilibrium to the desired product.

Figure index

List of abbreviation

AM Acido Malico

APIs Active Pharmaceutical ingredients

CCDC Cambridge Crystallographic Data Centre

$CDCl_3$ Deuterated Chloroform

CNT Classical nucleation theory

D-MA or MAD D-Malic Acid

DMSO Dimethyl sulfoxide

DSC Differential scanning calorimetry

EtOH Ethanol

L-MA or MAL L-Malic Acid

MSZ Metastable zone

NMR Nuclear magnetic resonance spectroscopy

PDF Power Data File

SC-XRD Single crystal X-ray diffraction

SIM Simulated

TGA Thermogravimetric Analysis

XRPD X-ray powder diffraction

Table index

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