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**Topical Salicylic acid and Lactic acid
Microemulsion and Co-crystal**

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Microemulsion and Co-crystal**

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Thesis approval

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Dedication:

This thesis is dedicated to my beloved mother who raised me from child hood to man hood and who devoted her entire life for me, my education and my well being. Also my thesis is dedicated to my children's and my beloved wife, who encouraged me to continue my education in this stage of my life, and gave me her entire efforts and love, in which my achievements have never been accomplished without her unlimited love and devotion.

Finally, chemists have to decide their life road, either to be or not to be, by means to succeed or not ...! And my decision is always within and after BA degree is to be (to succeed) even with hard practice work.

Maher Abdul Kareem Mohammad Aljamal

Date 9/1/2016

Declaration:

I certify that this thesis is submitted for the degree of Master graduation in applied industrial technology is my own research.

Maher Abdul Kareem Mohammad Aljamal

Signed:

Date 9/1/2016.

Acknowledgment:

First and foremost I give thank and praise to God, through him all things are possible and could be effective

My deep gratitude is expressed to my beloved pace maker **Dr. Ibrahim Kayali**, who encourages me to love chemistry by his personality, humanitarian and by giving me the concepts easily, and for his special tremendous efforts to complete my project. As well as to my new beloved known **Dr. Mohammad Abul-Haj**, who encourage me to co-crystal topic and to succeed my new paradigm?

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Lastly, my special thank to all members of doctors in Al-Quds University for their support and help.

Abstract:

This study aims to prepare topical salicylic acid and lactic acid microemulsion with a different formulations applied using the minimum amount of Tweens. The sugar surfactant used in this study is sorbitan monooleate (Tween 80). Short chain alcohol used as a co-surfactant which is propylene glycol. The oil phase used is castor oil. The aqueous phase is purified water. Also this study aims to prepare a co-crystal paradigm between salicylic acid and lactic acid as the active pharmaceutical ingredients, in presence of using different co-solvents.

In this research, we studied the effect of different percentage of surfactants on the phase behavior of the systems suggested at different temperatures 25, 37 and 45°C. We also explored the effect of adding co-surfactant (propylene glycol) on the phase behavior. The addition of propylene glycol as a co-surfactant contributes clearly in forming much smallest and stable micro emulsion droplet size besides giving pliability to the infected skin. Lastly the phase behavior of sorbitan monooleate studied as a function of temperature and surfactant concentration; that is presented in the form of the well known phase diagram that shows an isotropy microemulsion solution (using visual inspection, cross polarizer and dynamic light scattering) as low as 4% water addition at all temperatures (25°C, 37°C & 45°C).

Also in this research, we studied the ability of different co-solvents used in the formation of co-crystal paradigms, such as ethanol (96%), methanol (99%), diethyl ether and acetonitrile either in reflux or grinding techniques. The co-crystal paradigm was obtained in all reflux techniques applied successfully; more than 80% from the grinding technique of the samples obtained creates a merged compound successfully. All paradigms are tested using Fourier Transform Infra Red spectroscopy (FTIR) and the melting point range is tested for part of them. The co-crystals obtained were tested for solubility modifications and the results show a clear change in their solubility to be sparingly soluble to soluble in water. Also the co-crystal solids were tested for their melting point and the variation change observed is dependent on the target active pharmaceutical concentration and depending on the molar ratio for each co-crystal tested. But even so the melting point was changed to be lower than pure salicylic acid melting point and higher than lactic acid.

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Abbreviations, Symbols and Terminology:

HLB	H idrophile L ipophile B alance
L1	Spherical normal micelle
L2	Reversed micelle
CMC	C ritical M icelle C oncentration
o/w	o il in w ater
w/o	w ater in o il
NMR	N uclear M agnetic R esonance
API	A ctive P harmaceutical I ngredient
FTIR	F ourier T ransform I nfra R ed
PG	P ropylene G lycol

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Chapter one

Introduction

1. Introduction:

To make a review about topical microemulsion therapeutic meaning let us consider these two therapeutic words individually, what do we mean by topical?, And what is microemulsion?, What are the main microemulsion component?

1.1 Surfactants:

Surfactants (or ‘surface active agents’) are organic compounds with at least one lyophilic (‘solvent-loving’) group and one lyophobic (‘grease-loving’) group in the molecule. If the solvent in which the surfactant is to be used is water or an aqueous solution, then the respective terms ‘hydrophilic, water loving,’ and ‘hydrophobic, grease loving,’ are used. In the simplest terms, a surfactant contains at least one non-polar group and one polar (or ionic) group and is represented in a somewhat stylized form shown in Figure 1. Adsorption of a surfactant at the water/oil interface produces a surface with a significant very low interfacial energies, the formation of this type of low interfacial energy surface is the basis of the stability of most oil and water emulsions and all microemulsions as shown in Figure 2 [1].

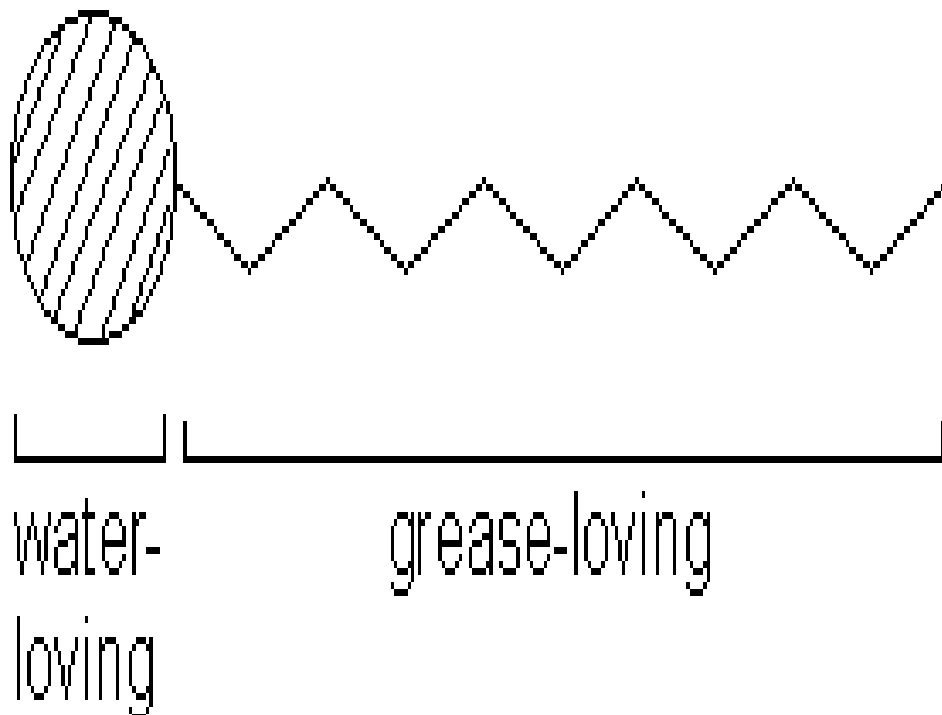


Figure 1 Schematic illustration of surfactant molecule .[1]

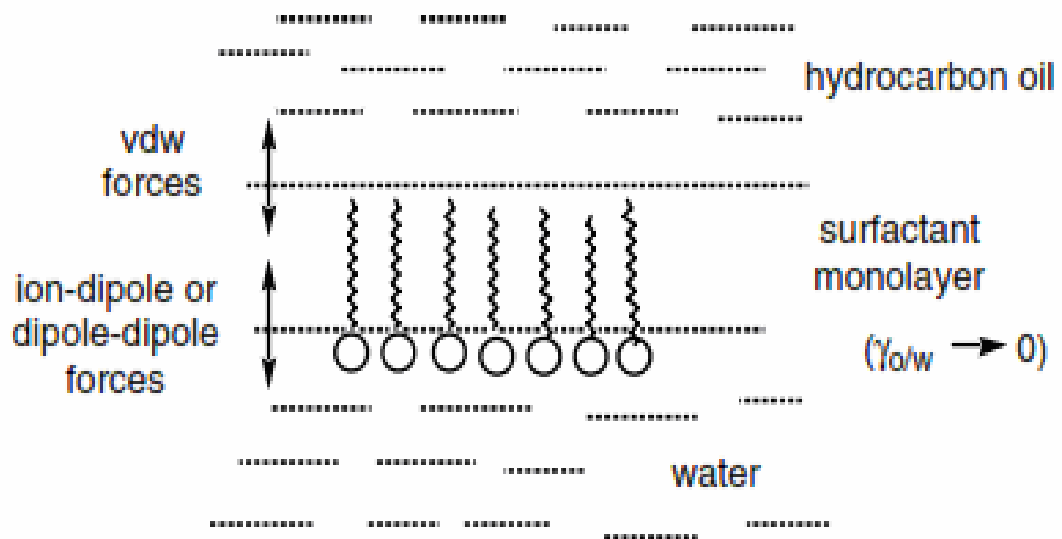


Figure 2 Illustration of the effect of an adsorbed surfactant layer on the interfacial energy between oil and water .[1]

The most useful chemical classification of surfactant is based on the nature of the hydrophilic part (head), with subgroups based on the nature of the hydrophobic part (tail). The four basic classes of surfactants are [2]: Anionic in which the hydrophilic part is a negatively charged group, cationic in which the hydrophilic part is a positive charge, nonionic the hydrophilic has no charge, but derives its water solubility from highly polar groups such as sugars and amphoteric groups (zwitterionic). It is not surprising that the selection of a suitable surfactant for a given application can become a significant problem. Thus an empirical numbering system has been developed to enable the correct type of surfactant to be chosen. The system is called the 'hydrophile-lipophile balance' (HLB). Therefore polysorbate 80 (Tween 80) is selected in this project preparation to achieve the drug product microemulsion formation since it had an HLB=15.0

Self-Assembled Surfactant Structures:

In aqueous solution dilute concentrations of surfactant act much as normal electrolytes, but at higher concentrations very different behavior results will be obtained. This behavior is explained in terms of the formation of organized aggregates of large numbers of surfactant molecules called micelles as shown in Fig. 3. In which the lipophilic parts of the surfactants will be associated in the interior of the aggregate leaving hydrophilic parts to face the aqueous medium. Meanwhile, that happens at a specific, higher, surfactant concentration, known as the critical micelle concentration (CMC). Besides, the physico-chemical properties of surfactants vary markedly above and below the CMC value [2,3].

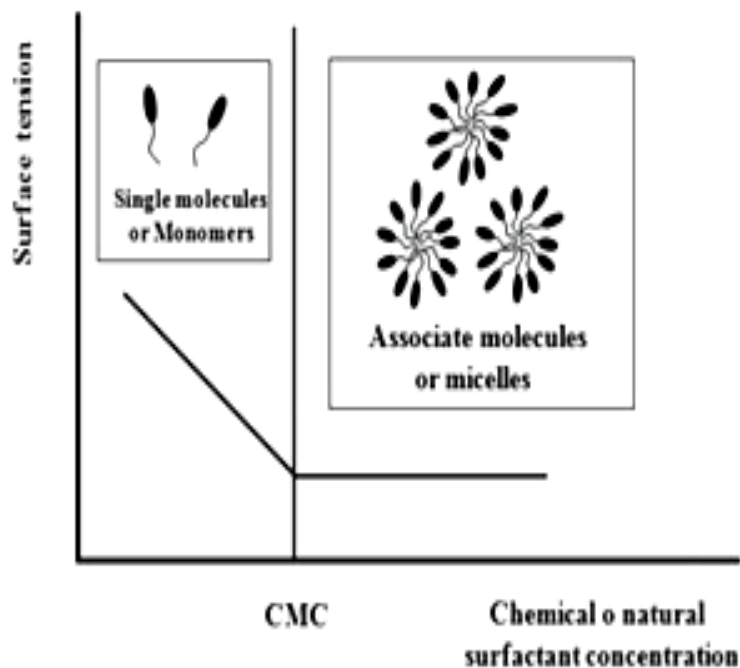


Figure 3 Schematic diagram of a surfactant micelle [3].

In addition, acting surfactants at oil/water interface formed a large number of self-assembled structures depending on the type of surfactants [4]. Spherical normal micelles (L1) are formed at high water content (oil/water), while reversed micelle (L2) are formed at low water content (water/oil), between these two extremes different isotropic and anisotropic liquid crystalline phases with decreased water content or increased temperature or electrolyte concentration may be formed. The following sequence of anisotropic liquid crystalline phase may take place for the surfactant systems as shown in Fig.4:

The variation and complexity of these structures has led to much research on potential industrial applications. However, microemulsion is the most important structures in which single low viscous isotropic phase formed in L1 and L2 regions of the phase diagram.

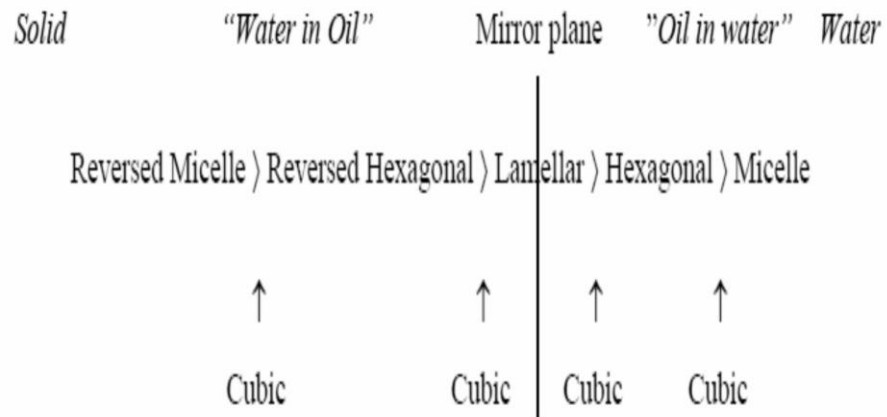


Figure 4 The fontell scheme, showing the idealized sequence of association structures of surfactants [4].

1.1.2 Sugar-Based Surfactants:

Sugar-based surfactants are characterized by having the hydrophilic sugar head group and the hydrophobic alkyl chain. This structural feature provides unique physicochemical properties to these surfactants. Among the characteristic properties of Sugar-based surfactants, a frequently remarked fact is that they can be produced from renewable resources and exhibit excellent environmental behavior. Certainly, there is currently a clear tendency to replace conventional surfactants with more environmentally good naturally compounds. Although interest in Sugar-based surfactant was traditionally, they have recently become the object of increasing attention for many researchers. The behavior of Sugar-based surfactants is also critically influenced by the nature of the substituent groups that are bounded to the individual monosaccharides. This substituent's can be of natural origin or semi natural quality. Whether they are natural or semi natural, the classification of Sugar-based surfactants falls into one of five categories, based primarily on the polysaccharide charge. These include: anionic, cationic, nonionic, amphoteric, and hydrophobically modified Sugar based surfactants [4]. It is clear from an industrial perspective that only a few carbohydrates fulfill the criteria of price, quality, and availability. Today, one of the most important sugar-based surfactants is sorbitan esters, which is used in pharmaceutical drugs such as polysorbate 80 (Tween 80), see figure 5.

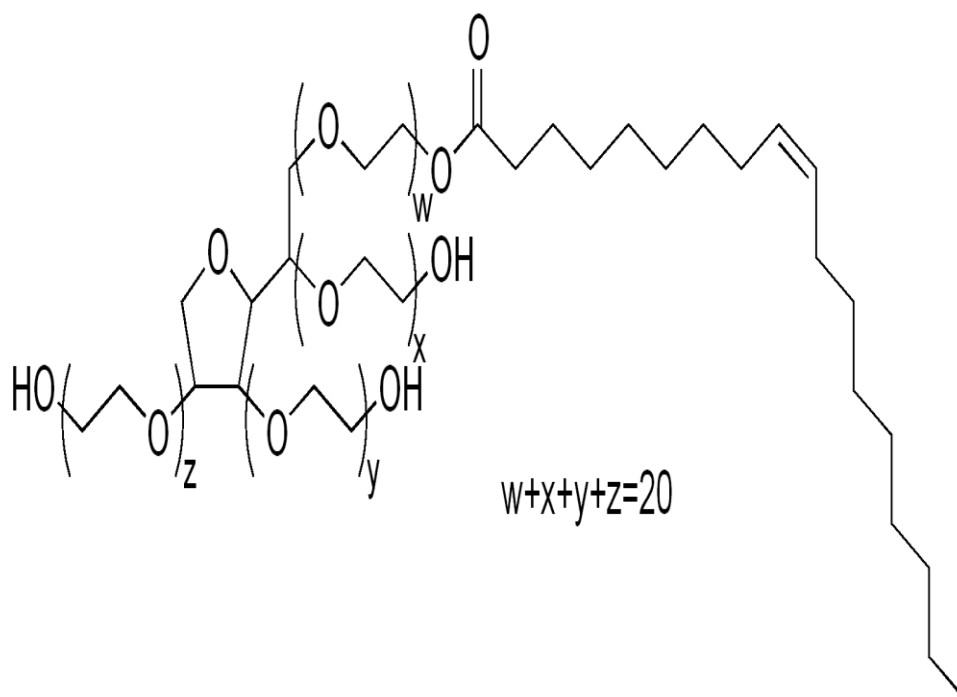


Figure 5 Polyoxyethylene (20) sorbitan monooleate (Tween 80), [4]

1.2 Alcohol Conventional Effects:

Three effects of an alcohol additive in the formulation of microemulsion have been mentioned so far [5]. **First**, it contributes to the general formulation as a co-surfactant, slightly hydrophilic contribution for methanol and ethanol; lipophilic contribution for n-butanol and longer linear alcohols, **Secondly**, as a co-solvent. The alcohol will be adsorbed with the surfactant at the interface and changes the overall interaction of the amphiphilic film with the adjacent solvents. The longer alcohol chain will give a lower tendency to act as co-surfactant, because it is partly soluble in the oil phase. Consequently, the co-surfactant effect may be said to fade away. As the alcohol mostly partitions into the water or oil phase it behaves either as a co-solvent. When such alcohol co-solvents are present in small proportion, they might not mix uniformly in the bulk of the oil or water phase and they could exhibit a **thirdly** effect called lipophilic linker Fig. 6.

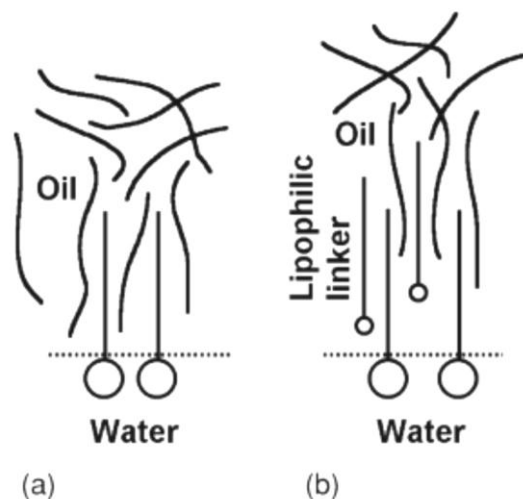


Figure 6 Solubilisation improvement of a conventional surfactant (a) by alipophilic linker (b) [5].

The high surfactant concentration required to formulate a microemulsion, usually remains a major concern for the user. In addition, much of the work on microemulsions has employed alcohols as co-surfactants and as co-solvent in order to decrease surfactant film rigidity, thus promoting microemulsion formation and delay the occurrence of liquid crystalline phases. Alcohol can also reduce the time needed for equilibration to be reached in multi phase systems [6].

1.3 Microemulsion:

Microemulsions are clear, thermodynamically stable, optically isotropic systems of at least a hydrophilic, a hydrophobic and an amphiphilic component (surfactants/co-surfactants). It is composed of submicron sized droplets that plays an important role to distinguish and characterize them from ordinary emulsions which are thermodynamically unstable [7]. Also microemulsion can be considered as small scale versions of emulsions with droplet dispersions either of oil-in-water (o/w) or of water-in-oil (w/o), with a size range in the order of 5–50 nm in drop radius. Microemulsions advantages include improvement in poorly drug solubility, enhancement of bioavailability, protection of the unstable drugs against environmental conditions and a long shelf life [8].

It is known that oil and water are not miscible at ambient temperature; a small amount of surfactant is able to co-solubilize them. Generally **non-ionic** surfactants are chosen because of their good co-tolerance, lower irritation potential and toxicity, such as polysorbate 80 (Tween 80). The co-surfactants even though being indispensable in the formulation of microemulsions, have exhibited toxicity such as ethanol [9]. Microemulsion formulation has to be controlled accurately, which is very critical because of the high number of degrees of freedom in any practical case. This is why formulation is sometimes considered as earnestly magic formulation. Investigations the phase behavior of salicylic acid and lactic acid with a specified Castor oil using surfactants/co-surfactant introduced to use these two drugs in different formulations, for other routes of drug administration [10].

Microemulsion stabilized by sugar surfactants usually contains a co-surfactant/ co-solvent. Since without a co-surfactant/ co-solvent only o/w microemulsions are formed, which is mainly due to the fact that the high hydrophilicity of sugar surfactants cannot be changed significantly by temperature variation [11]. The proposed co-surfactant in this project is propylene glycol.

Microemulsion applications:

Microemulsions had been used in a variety of chemical and industrial processes, such as in enhanced oil recovery, fuel, coatings and textile finishing, lubricants, cutting tools oils and corrosion inhibitors, detergency, cosmetics, agrochemicals, food, biotechnology, environmental remediation and detoxification, analytical applications, microemulsion gel technique, liquid membranes and in pharmaceuticals [12]. Microemulsion applications as drug delivery would be in different routes of administrations, such as in Oral, Ophthalmic, Parenteral, Nasal, Periodontal, Drug Targeting, Antiacne, Antioxidants (in dermatological and cosmetic products), Antifungal, Antiviral, Ocular, Spermicidal, oral, mucosal, transdermal and dermal drug delivery.

Drug delivery to or through the skin offers various advantages over oral and parenteral routes of drugs for local and systemic effects. However, the natural protective barrier nature of the skin poses a challenge to deliver drugs in therapeutic topical quantities [7]. Topical preparations mean in pharmacists that it will pertain to increase the absorption of the medicament (active drug agents) applied to a specific area of the body. So, topical microemulsion is applied directly to or through the skin [13].

The skin is the largest human organ of the integumentary system and also an excellent biological barrier against chemical and biological insult. It is about 2 mm in thickness and accounts for nearly 4% of the total body weight. It is composed of three major components: the epidermis, dermis, and subcutaneous fat layer (hypodermis). The epidermis layer, which is the outermost skin layer, it is typically 50-150 μm in thickness and it contains the stratum corneum in the outer layer, which is the main barrier for the permeation of drugs across the skin). Stratum corneum is 10-20 μm thick and made up of dead keratinized cells called corneocytes, which are embedded in a lipid matrix that renders the membrane practically impermeable to large and hydrophilic molecules [14, 15,16]. The goal in topical microemulsion is to maximize drug absorption in the systemic circulation. The rate and extent of drug penetration into different layers of skin and into systemic circulation are governed by the drug properties and formulation characteristics.

One of the advantages of microemulsions lies in the 'phase inversion' that takes place at a given water to oil concentration ratio. This means, at lower water content the microemulsion consists of very small water droplets dispersed in oil (W/O), while at higher water content the situation is reversed and the system consists of oil

droplets dispersed in water (O/W). Between these two phases exists an intermediate situation in which the system consists of layers of surfactant separating alternate layers of water and oil? By increasing the water content the droplets of water initially increase their radius slightly, and then the system passes directly to the o/w droplets situation. The existence of this kind of structural change has been predicted on the basis of many different measurements, such as x-ray, optical properties, dielectric properties, viscosity and NMR. From the thermodynamic point of view, microemulsions are rather complicated systems, mainly because of the existence of at least four components, and because of the electric double layer surrounding the droplets, or the rods, or the layers that contribute clearly to the free energy of the system.

The microemulsion region is usually characterized by constructing ternary phase diagrams. The basic three components required to form a microemulsion are: an oil phase, an aqueous phase and a surfactant. If a co-surfactant is used, it may sometimes be represented at a fixed ratio to surfactant or to any component of the phase diagram as a single component, and treated as a single “**pseudo-component**”. The relative amounts of these three components can be represented in a ternary phase diagram. The three components composing the system are each found at the head of the triangle, where their corresponding volume fraction is 100%. The relative amounts of A, B and C are expressed in percentage of the selected parameter, such as: $A\% + B\% + C\% = 100\%$ [17].

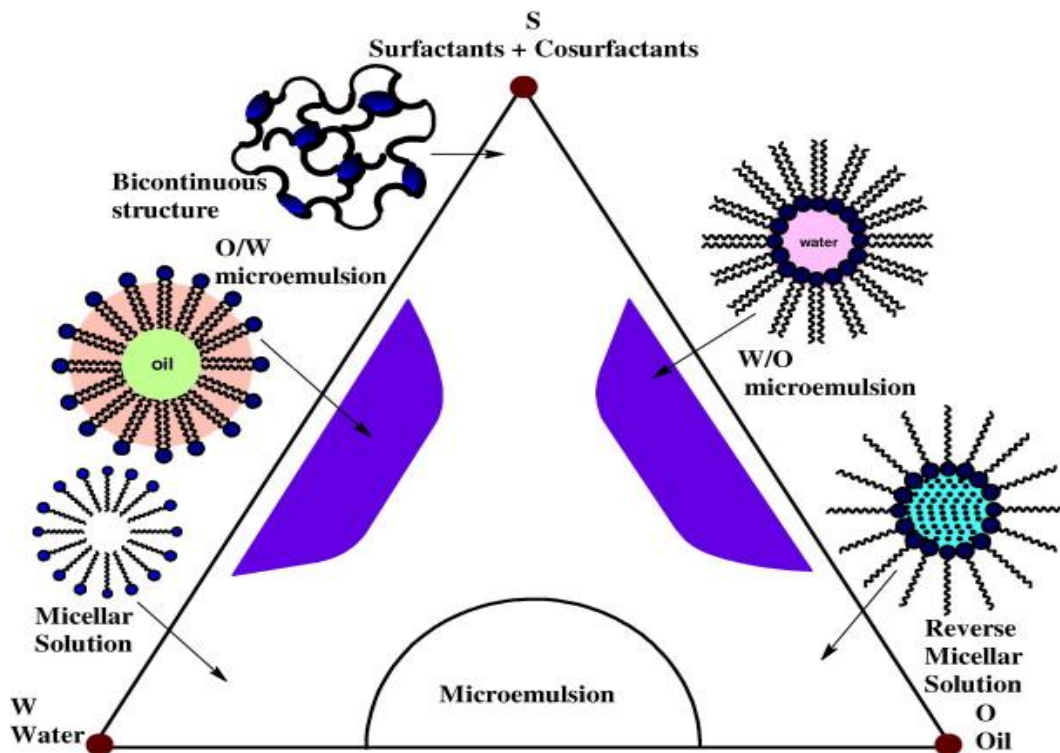


Figure 7 Hypothetical phase regions of microemulsion systems [17]

1.4 Factors affecting drug release:

- **Amount of drug**

The large amount of drug incorporated in the internal phase leads to development of a concentration gradient between the internal and external phase, increasing the thermodynamics of the drug and providing for the diffusion of drug from the internal phase into the skin layers [16].

- **Type and amount of surfactant**

Various types of surfactants have been reported in the use of topical microemulsions, such as oil in water or water in oil. The non-ionic surfactants are preferred over the anionic or cationic surfactants, since the latter are reported to be harsh on the skin. An inverse relationship exists between the amount of surfactant and drug permeation since a large amount of surfactant reduces the thermodynamic activity of the system hampering drug diffusion [18, 19].

- **Amount of alcohols**

Alcohols are used as skin penetration enhancers, and they are able to lower the microemulsion melting point and have been known to disorganize the stratum corneum lipid structure, enhancing the partitioning and permeation of drugs [20,21].

- **Amount of water**

At low water concentrations, all water molecules are used in hydrating the polar heads of the surfactant molecule leaving no free water available for hydrating the stratum corneum, thus affecting the permeation of both lipophilic as well as hydrophilic drug that provides a strong force to push the drug molecules across skin layers [22, 23].

1.5 Co-crystallization:

Poorly water soluble drugs pose significant hurdles for drug bioavailability that in turn affect in vivo efficacy and safety in all stages of formulation. Among the biopharmaceutical properties, solubility remains a key issue with drugs often discarded during commercial production due to their low solubility. Improving the solubility of drugs is currently one of the main challenges for the pharmaceutical industry. Many approaches have been adopted for improving the aqueous solubility of drugs including micronisation, salt formation, emulsification, solubilisations using co-solvents. Over the last decade, there has been growing interests in the design of pharmaceutical co-crystals, which emerges as a potential method for enhancing the bioavailability of drugs with low aqueous solubility [24]. The ability to deliver the drug to the patient in a safe, efficient and cost effective manner depends largely on the physicochemical properties of the active pharmaceutical ingredient (API) in the solid state being studied.

Co-crystals can be defined as crystalline complexes of two or more neutral molecular constituents bound together in the crystal lattice through non covalent interactions (primarily hydrogen bonding). The formation of pharmaceutical co-crystals involves incorporation of a given active pharmaceutical ingredient with another pharmaceutically acceptable molecule in the crystal lattice. A pharmaceutical co-crystal can be designed by crystal engineering with the intention to improve the pure solid-state properties of an active pharmaceutical ingredient without affecting its intrinsic structure. Crystal engineering affords a paradigm for rapid development of pharmaceutical co-crystals. It can be defined as an application of the concepts of supramolecular chemistry to the solid state with particular emphasis upon the idea that crystalline solids are actual manifestations of self-assembly [25, 26].

Co-crystals are constructed from intermolecular interactions such as hydrogen bonding contact forces, and hydrogen bonding [27]. The term supramolecular synthon is frequently used in the research field of co-crystals. It is defined as structural units within supramolecules which can be formed and/or assembled by known conceivable synthetic operations involving intermolecular interactions. Supramolecular synthons are spatial arrangements of intermolecular interactions and the overall goal of crystal engineering is therefore to recognize and design synthons that are robust enough to be interchanged between network structures.

A pharmaceutical co-crystal is simply a co-crystal in which at least one of the molecular components is an active pharmaceutical ingredient (API) in conjunction

with another type of molecule termed a co-crystal former. More strictly, in order to be useful, the non-API component should be non-toxic with no adverse side effects. Interest in pharmaceutical co-crystals arises from the fact that, as different crystal forms to the pure API, they dramatically expand the range of solid forms available for formulation. Co-crystals have different physical properties such as habit, bulk density, and solubility; compressibility, friability, melting point, hygroscopy and dissolution rate. Formation of a co-crystal often offers scope to transform an amorphous or hard to crystallize active pharmaceutical ingredient into a readily handled, stable crystalline solid. Indeed, it is far more likely to be poor biopharmaceutical characteristics rather than toxicity or lack of efficacy that prevent a candidate active compound progressing in clinical trials [28,29]. Discovery or design of a new, useful co-crystal solid form also offers new opportunities for the exploitation of intellectual property, the most common supra molecular synthons utilized in pharmaceutical co-crystals are shown in Fig.8 , the circled co-crystal design is the expected model in my co-crystal paradigm

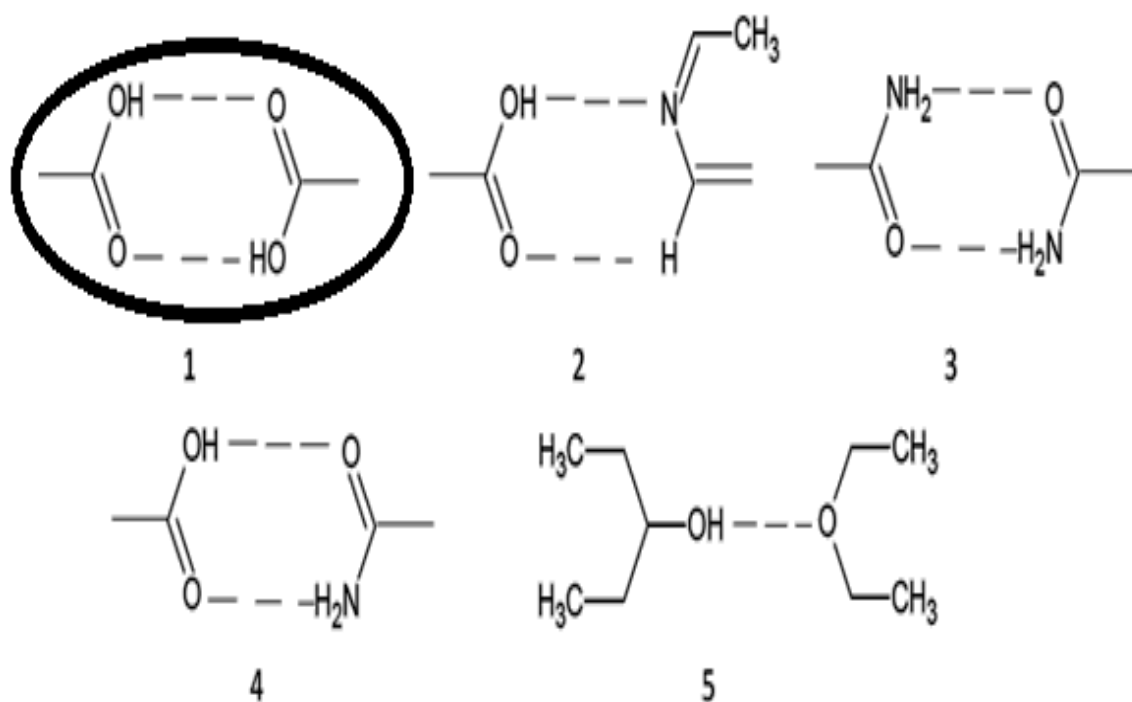


Figure 8 Typical hydrogen bonds utilized in crystal engineering [28]

The salicylic acid structure is composed of dimeric units through hydrogen bonding as expected with more hydrogen bonding from the hydroxyl group with the carboxylate of the same molecule, as shown in figure 9, [30]. While in lactic acid, the hydrogen bonding between the different molecules is between the hydroxyl groups of one molecule with the carbonyl of the adjacent molecule forming cyclic arrangement composed of six lactic acid molecules as shown in figure 10, [31]

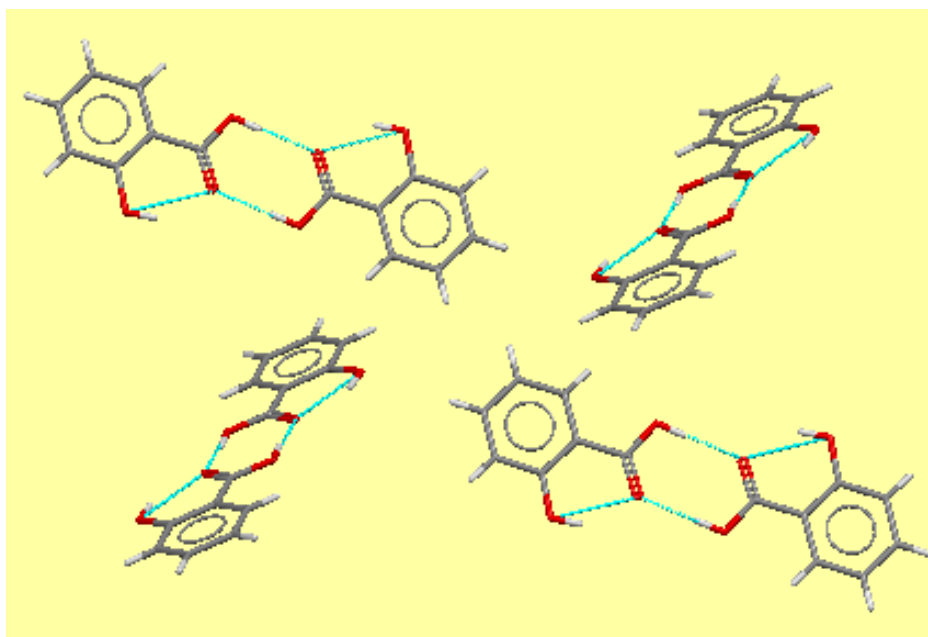


Figure 9 Salicylic acid crystal structures in solution [30]

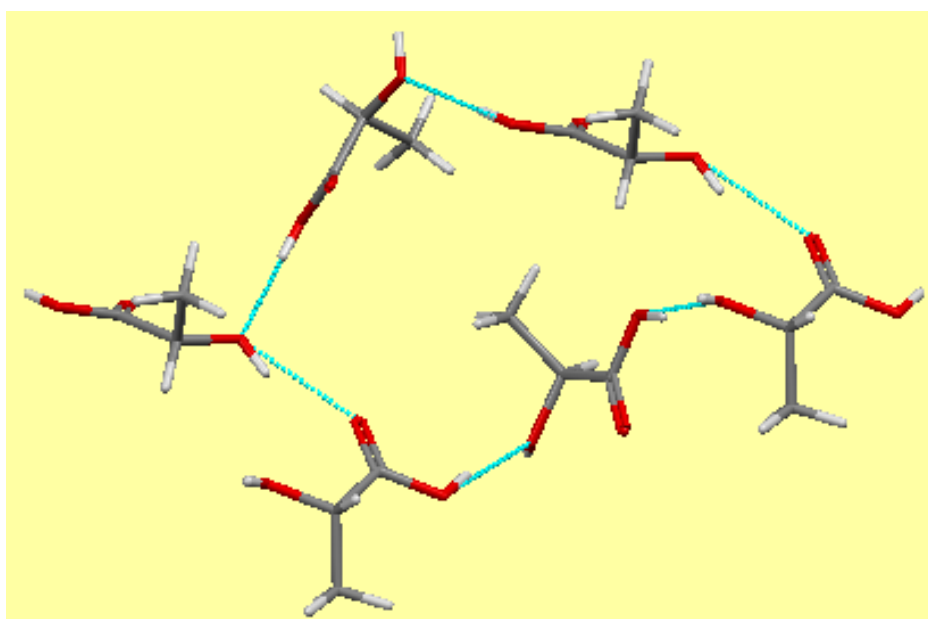


Figure 10 Lactic acid crystal structures in solution [31]

1.6 Salicylic acid and Lactic acid chemical structures:

Salicylic acid is the main therapeutic compound in the proposed drug which is used for a number of different skin conditions caused by thickened, hard skin, such as warts, verrucas, psoriasis, scaly skin conditions and some nail infections. It is a keratolytic, which means that it works by softening the outer layer of your skin allowing it to loosen and shed [32]. While Lactic acid used to severe forms of dry scaly skin since it is a humectants and supporter. Exactly how it works is unknown but it increases the amount of water in the skin, making it softer and more pliable [33]. Salicylic acid is generally the most prominent ketrolytic compound in the medication product and lactic acid is the booster ketrolytic (humectants) in the medication product [34].

The following are chemical structures for salicylic acid and lactic acid [32, 33] respectively.

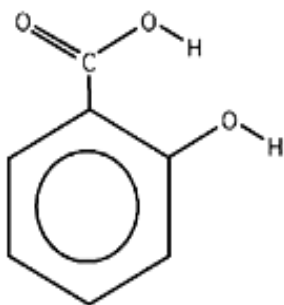


Figure 11 Salicylic acid chemical structure
(2-hydroxy benzoic acid)

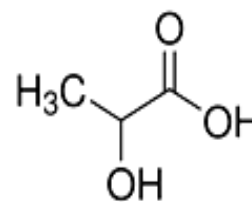


Figure 12 Lactic acid chemical structure
(2-hydroxypropanoic acid)

Chapter Two

- **Literature review**
- **Problem**
- **Research objectives**
- **Hypothesis**

2. Literature Review:

The topical microemulsion therapeutic products are of an important drug delivery systems and their use in therapy is becoming more widespread. Topical formulations to treat ailments have existed since ancient times. The purpose of topical dosage forms is to conveniently deliver drugs to a localized area of the skin. Although microemulsions can be used to deliver drugs via several routes, these multi use microemulsions have been extensively studied as vehicles for topical administration. Their composition and structure enables them to incorporate greater amount of drug than other topical formulations such as ointments, creams, gels and lotions. Delivery of drugs using these microemulsions through skin increases the local/systemic delivery of the drug by different mechanisms that make them suitable vehicles for the delivery of Antifungal [35].

Salicylic acid is a gentle acid derived from the bark of the willow tree, which has long been used as a traditional treatment for pain, inflammation and fever. Salicylic acid in ancient times was used by the ancient Greeks in about 500 BC [36]. Because pharmacists, chemists, and doctors were already familiar with salicylic acid's anti-inflammatory and pain-relieving properties, they sought to create a widely usable version of the drug. Salicylic acid has been described as a β -hydroxy acid and classified as a phenolic aromatic acid. The hydroxyl group of true β -hydroxy acids is neutral, and not acidic. Salicylic acid has a pKa of 2.98. To obtain a significant exfoliative effect, salicylic acid must be formulated at a proper pH close to the pka to allow enough free acid to be present, to give significantly more exfoliation than formulations at any pH significantly greater than the pKa [13].

While Lactic acid was discovered in 1780 by Swedish chemist, Carl Wilhelm Scheele, who isolated the lactic acid from sour milk as impure brown syrup and gave it a name based on its origins. Lactic acid is produced by the fermentation of sugar and water or by chemical process and is commercially usually sold as a liquid. Pure and anhydrous racemic lactic acid is a white crystalline solid with a low melting point. Lactic acid is the biological isomer as it is naturally present in the human body. The greatest benefit of having a professional lactic acid peel is that it is gentler on your skin than most other types of peels [37].

Microemulsion emulsifying, Oxidative stability and the properties of prepared topical therapeutic drug based on salicylic acid and lactic acid with chemical emulsifiers (surfactants) and with a co-surfactant addition have been studied [9]. The primary use of surfactant is to lower the interfacial tension to a very small value which will facilitates dispersion process during the preparation of the

microemulsion and provide a flexible film that can readily deform around the droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region. The tested chemical nonionic surfactants (Tween 20, 40, 80 and Span 20, 60) and results have shown that stable topical microemulsions can be obtained with the aid of co-surfactant addition. The short to medium chain alcohols are generally considered as co-surfactants in the microemulsion system. The presence of surfactant and co-surfactant in the system makes the interfacial tension very low. Therefore the microemulsions form spontaneously, with a small droplet diameter. The nonionic surfactants appeared to be more suitable surfactants as it was predicted by the HLB theory, these components can be used both for their antioxidant and their emulsifying properties [9].

As known, one of the major properties of salicylic acid is its ability to remove skin cells of the most upper layer of the skin, the stratum corneum. This keratolytic effect is dependent on the concentration of salicylic acid used in. For example, at a concentration of 10 - 15% salicylic acid shows a keratolytic effect already after 2 or 3 days. At 5% and 1% desquamation of skin cells is seen after 7 and 10 days, respectively. Hence, salicylic acid is widely used as major ingredient for peelings or other exfoliant or abrasive skin treatments. Such peeling products contain salicylic acid usually at concentrations between 0.5 - 3%. Whereas the upper limit of 20% of is applied only in dermatological (prescription) products, the use of salicylic acid at this level has also been recommended for face masks. For anti-warts products salicylic acid is usually used between 12 - 40%. Besides removing old cells and horny debris from the skin, keratolysis has also the advantage that it facilitates the penetration of other active ingredients into the skin. Salicylic acid is therefore often added to skin care products that contain active ingredients in order to increase their absorption and efficacy [36].

Many alpha-hydroxy acid (lactic acid) products, containing low- or high-acid concentrations, are being used. It is not known whether different products perform differently or whether they modulate changes in both the epidermis and dermis. The purpose was to examine whether treatment with 5% and 12% lactic acid produces different cosmetic results and produces changes in the epidermis and dermis [38]. Test participants applied either 5% or 12% lactic acid twice a day for 3 months. Changes in skin smoothness and texture, the depth and number of lines and wrinkles, and epidermal and dermal firmness and thickness were determined. Treatment with 12% lactic acid resulted in increased epidermal and dermal firmness and thickness and clinical improvement in skin smoothness and in the appearance of lines and wrinkles. No dermal changes were observed after treatment

with 5% lactic acid; however, similar clinical and epidermal changes were noted. The results demonstrate that cosmetic benefits from the use of alpha-hydroxy acids are caused by modification of the skin surface, the epidermis and the dermis. Although 5% lactic acid modulates surface and epidermal changes, 12% lactic acid influences both the epidermis and the dermis [39].

Microemulsions are clear, thermodynamically stable systems. They were used to solubilize drugs and to improve topical drug availability. Salicylic acid is a keratolytic agent used in topical products with antimicrobial actions. The objective of their work was to prepare and evaluate salicylic acid microemulsion systems. Different concentrations of salicylic acid were prepared: S_{2%}, S_{5%}, and S_{10%} which contain 2%, 5%, and 10% of salicylic acid, respectively. Stability study for 6 months under ambient conditions was carried out for S_{10%}. No remarkable changes were recorded except a decrease in the viscosity value after 1 month. The results suggested that microemulsion could be a suitable vehicle for topical application of different concentrations of salicylic acid [40].

Salicylic acid has bacteriostatic and fungicidal actions as well as keratolytic properties. Its effectiveness for topical treatment of hyperkeratotic skin lesions is based on mild keratolytic action which produces slow and painless destruction of the epithelium. In the treatment of warts, a mild irritant reaction, which may render the virus more prone to immunologic stimulation or response, may add to the mechanical removal of infected cells [41]. Salactol contains 16.7% salicylic acid and 16.7% lactic acid in flexible collodion. The bioavailability of salicylic acid is reduced as the collodion film dries on the skin due to entrapment of the drug which inhibits release. The addition of lactic acid to salicylic acid collodion provides more efficient release of the salicylic acid, since the non volatile lactic acid remains in the film, thus permitting continued release of the keratolytic which may otherwise be entrapped within the dried collodion film. Systemic absorption of salicylic acid or lactic acid after application to small circumscribed areas is exceedingly unlikely [42].

Salatac Gel presents 12% salicylic acid and 4% lactic acid in an evaporative collodion-like gel which forms a cohesive and adhesive film on the skin. This minimizes the spread of the preparation onto the surrounding healthy skin. The gel quickly forms a surface film, well before it dries completely, thereby prolonging the period during which the keratolytic solution can properly infiltrate and achieve intimate contact with the surface layers of the thickened stratum corneum. It is used for the topical treatment of warts, verrucas, corns and calluses [20, 43].

Pharmaceutical **co-crystals** are emerging as a new class of solid drugs with improved physicochemical properties, which has attracted increased interests from both industrial and academic researchers. They are attractive to pharmaceutical scientists because they can significantly diversify the number of crystal forms that exist for a particular active pharmaceutical ingredient (API), and they can lead to improvements in physical properties of clinical relevance. Co-crystals not only provide a technique for improvement of physicochemical property! but also provide opportunity to the researchers of pharmaceutical companies regarding intellectual property. Co-crystal approach especially used to enhance the specific properties of pharmaceutical solids such as dissolution rate of poorly water soluble API and the physical stability of moisture liable APIs [28].

Solubility is essential for the therapeutic effectiveness of the drug, independent of the route of administration. Poorly soluble drugs are often a challenging task for formulators in the industry. Conventional approaches for enhancement of solubility have limited applicability, especially when the drugs are poorly soluble simultaneously in aqueous and in non-aqueous media. Solubilization may be affected by co-solvent water interaction, micellar solubilization, reduction in particle size, inclusion complexes, solid dispersion, and change in polymorph. Some new technologies are also available to increase the solubility like microemulsion, self emulsifying drug delivery system and supercritical fluid technology. This review focuses on the recent techniques of solubilization for the attainment of effective absorption and improved bioavailability [44].

A review on pharmaceutical co-crystals, nutraceutical co-crystals and pharmaceutical co-crystal polymorphs depicting their relevance both in academia and pharmaceutical industry because of their potential as new solid forms of the active pharmaceutical ingredient. The over view of crystal engineering to design co-crystals for altered and improved physicochemical properties such as solubility, dissolution rate, bioavailability, hygroscopicity etc. [45].

3. Problem:

Microemulsions were used in several fields as routes of administration; this sample application, which delivered the drug in sustained or controlled manner and prolonged delivery as compared to conventional dosage form for topical applications which exhibit minimal systemic absorption. Microemulsion can be prepared using a low-energy emulsification method, depending on the phase behavior and properties of the constituents, to promote the formation of high small droplets using co-surfactants that include self emulsification. **So, why not using?** the low energy technique in our medicine factories?, and introduce the new nanotechnology in Palestinian medicine factories as seen in all over the world in general using the phase behavior and studying its available applications for the topical treatment of warts, verrucas, corns and calluses. **Why not enhancing** the solubility of poor water soluble pharmaceutical drugs? The crystal engineering and super molecular chemistry were used in co-crystal formation, to improve the active pharmaceutical ingredients solubility as well as their bioavailability to and through the skin.

4. Research objectives:

The objective of this research is to study the stability and phase behavior of Salicylic acid and lactic acid in a therapeutic product, in order to formulate a stable, biocompatible microemulsion between Salicylic acid, Lactic acid and a sugar/nonionic surfactant like tween 80 as well as a co-solvent such as ethanol, propylene glycol and water. This microemulsion could be used in the formulation of topical or in other routes of pharmaceutical administrations or products, depending on the results that will be obtained from the pseudo ternary phase diagram. The secondary objective is to prepare salicylic acid and lactic acid co-crystal using co solvents in crystal engineering techniques. In which the crystal engineering is the design of molecular solid state structures with desired properties, based on intermolecular interactions. The two main intermolecular interactions are based on hydrogen bonding and coordination complexation.

To obtain this formula there are specific objectives needed to be achieved:

- To estimate phase diagram of the best components concentration for microemulsion preparation.
- To assess the patient's satisfaction with a topical microemulsion treatment, by addressing the affected area of the skin very effectively.
- To determine the surfactant ratios which have highly solubilization capacity as well as highly surfactant efficiency?
- To determine the water and ethanol ratios which have high solubilization capacity as well as high efficiency?
- To identify, approve and justify the output results: by dynamic light scattering, crossed polarizers and polarizing microscope.
- To prepare and characterize co-crystals of salicylic acid and lactic acid (API) in order to improve bioavailability and efficacy as an antifungal drug.
- To use the following instruments in identifying the co-crystal output: FTIR, melting point apparatus, X-ray diffraction,

5. Hypothesis:

There have been several topical microemulsion therapeutic products and their affectivity on the skin diseases as a route of drug administration without affecting the interior human body by any side effect even they are. Depending on these studies, it is expected that Palestinian Pharmaceutical Company to be entered this production field by a colloidal microemulsion product using the low energy technique, since they have a significant therapeutic activity. Additionally, have shown that salicylic acid and lactic acid pharmaceutical ingredients are used in the formulation of microemulsions to help clear and prevent pimples and skin blemishes in people who have acne, also used to treat skin conditions that involve scaling or overgrowth of skin cells such as psoriasis, scaly patches, ichthyoses, dandruff, corns, calluses, and warts on the hands or feet [46].

A pharmaceutical co-crystal can be designed by crystal engineering with the intention to improve the solid state properties of an active pharmaceutical ingredient without affecting its intrinsic structure. Crystal engineering affords a paradigm for rapid development of pharmaceutical co-crystals. Therefore crystal engineering was applied to increase the Bioavailability of salicylic acid and lactic acid by merging them in one co-crystal solid product. [47].

Chapter Three

- **Instrumentation and Methodology**

6. Instrumentation & Methodology:

6.1 Instrumentation:

Analytical balance (Precisa 125-A) , Vortex (VELP), culture tubes sealed with Viton lined screw caps, Incubators (WTB Binder), crossed polarizers, polarizer microscope and Dynamic light scattering, reflux unit apparatus, glass wares, FTIR (AVATAR 320), Melting point apparatus (BUCHI B-545), X-ray diffraction,

6.2 Materials:

Salicylic acid 100% (Rhodia), Lactic acid 90% (Merck), ethanol 96% (shitzer), methanol (J.T.Baker), diethyl ether (Merck), acetonitrile (J.T.Baker), polysorbate 80 or Tween 80 (Eigenmann & Veronelli), purified water, castor oil (gustavheess) and propylene glycol (Dow). All components were used as supplied and tested in Beit Jala Pharmaceutical Company for further purification.

6.3 Phase diagram Methodology:

6.3.1 Dissolution of Salicylic acid and Lactic acid

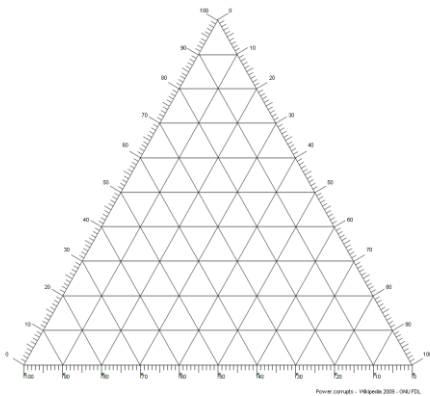
Salicylic acid is an organic soluble (ethanol) while lactic acid is an aqueous (water) and organic (ethanol) miscible, therefore it was suggested to use the minimum amount of ethanol that is able to dissolve them in a solution (to enhance the drugs release). → Salicylic acid and lactic acid solution (Ethanolic solution)

6.3.2 Constructing of Phase Diagram:

The phase behavior of the systems consisting of water (with or without Co-solvent (propylene glycol)), castor oil (with or without Ethanolic solution), surfactant (Tween 80) may be described on a phase tetrahedron whose apexes respectively represent the pure components. 1g mixture consisting of Ethanolic solution (with or without castor oil) and surfactant (Tween 80) at different weight ratios were prepared in culture tubes sealed with Viton lined screw caps and stirred by vortex until clear solution was obtained. See table below for each phase diagram tube components. These samples were titrated with water (with or without Co-solvent (propylene glycol)) which was added drop wise until its solubilization limit was reached. See table below for each titration percentage required amount. Vigorous stirring followed after additions on a vortex mixer. The time for equilibration between each addition was typically, from a few minutes up to 24 hours (therefore

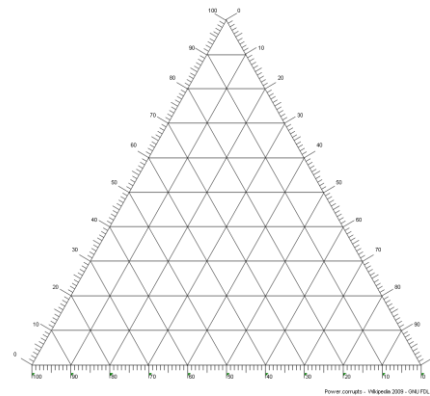
the reading will be taken after 24 hours). Each phase diagram was investigated at three temperatures 25, 37 and 45°C and detecting the number of phase by bare eye, the anisotropy by cross polarizer. The single isotropic sample which will be dark under cross polarizer will be regarded to either cubic or micelle; which can be distinguished by its viscosity. The anisotropic lamellar liquid crystal and hexagonal liquid crystal are determined by the cross polarizer and polarizing microscope. Detect the boundary of single phase; four phase diagrams were installed and each phase was tested at three temperatures 25, 37 and 45°C. Finally draw the phase diagram using specified computer software.

Oil+ Ethanolic solution



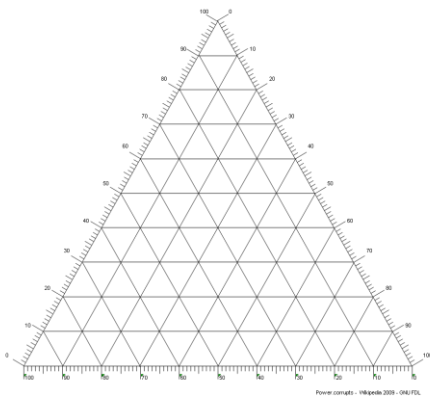
Water Tween 80
Figure 13 Phase Diagram 1 Proposed constructions

Ethanolic solution



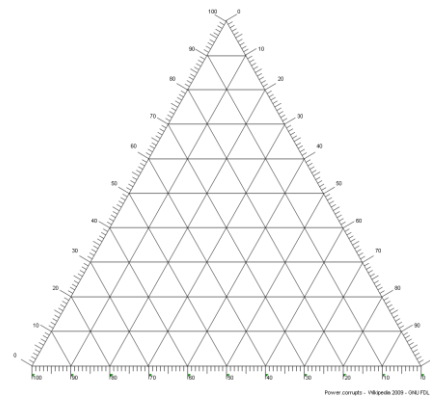
Water Tween 80
Figure 14 Phase Diagram 2 Proposed constructions

Ethanolic solution



Water+PG Tween 80
Figure 15 Phase Diagram 3 Proposed constructions

Oil+ Ethanolic solution



Water+PG Tween 80
Figure 16 Phase Diagram 4 Proposed constructions

Tube components amount for each phase diagram:

Table 1 Phase diagram 1 components

Tube code#	Surfactant (g) ---(B)	Oil+EtOH (g) --- (C)
100% (C)	-----	0.9998
0.5,9.5	0.0499	0.9501
19	0.1004	0.9005
28	0.2002	0.8003
37	0.2999	0.7002
46	0.4002	0.5995
55	0.4992	0.5004
64	0.6003	0.3998
73	0.7002	0.3002
82	0.7997	0.2004
91	0.8998	0.1010
9.5,0.5	0.9499	0.0495
100 % (B)	0.9996	-----

Table 2 Phase diagram 2 components

Tube code#	Surfactant (g) ---(B)	EtOH (g) --- (C)
100% (C)	-----	1.0003
0.5,9.5	0.0505	0.9501
19	0.1000	0.9003
28	0.2008	0.8007
37	0.3006	0.6996
46	0.3995	0.6003
55	0.5004	0.4996
64	0.5997	0.4004
73	0.7000	0.2997
82	0.7996	0.2005
91	0.9002	0.1005
9.5,0.5	0.9498	0.0498
100 % (B)	1.0003	-----

Table 3 Phase diagram 3 components

Tube code#	Surfactant (g) ---(B)	Oil+EtOH (g) --- (C)
100% (C)	-----	1.0003
0.5,9.5	0.0503	0.9499
19	0.1003	0.9005
28	0.1997	0.7999
37	0.3002	0.7003
46	0.4000	0.5998
55	0.5004	0.5004
64	0.5997	0.4002
73	0.7002	0.2997
82	0.8005	0.2002
91	0.8998	0.0997
9.5,0.5	0.9498	0.0501
100 % (B)	1.0002	-----

Table 4 Phase diagram 4 components

Tube code#	Surfactant (g) ---(B)	Oil+EtOH (g) --- (C)
100% (C)	-----	0.9997
0.5,9.5	0.0496	0.9498
19	0.0997	0.9000
28	0.2001	0.8000
37	0.3005	0.7004
46	0.4000	0.5998
55	0.4997	0.4998
64	0.6002	0.3999
73	0.6996	0.3005
82	0.8002	0.1999
91	0.9000	0.0997
9.5,0.5	0.9497	0.0502
100 % (B)	0.9999	-----

Note: The tube code refers to the amount of surfactant and oil components in each tube. For example tube code # 19 contains 0.1g of surfactant and 0.9g of oil component phase.

Titration amount required in grams for each percentage addition:

Table 5 Titration percentage versus weight

%	Total added weight (g)	Net weight Each interval (g)
4	0.0417	0.0417
8	0.0870	0.0453
12	0.1364	0.0494
16	0.1905	0.0541
20	0.2500	0.0595
24	0.3158	0.0658
28	0.3889	0.0732
32	0.4706	0.0817
36	0.5625	0.0919
40	0.6667	0.1042
44	0.7857	0.1191
48	0.9231	0.1374
52	1.0833	0.1603
56	1.2727	0.1894
60	1.5000	0.2273
64	1.7778	0.2278
68	2.125	0.3472
72	2.5714	0.4464
76	3.1667	0.5953
80	4.0000	0.8333
84	5.2500	1.2070
88	7.3330	2.0833
92	11.500	4.1667
96	24.000	12.580

The total added Weight (g) can be calculated as the term x from the following equation: $\# \% * (1+x) = x * 100$

6.4 Co-crystallization methodology:

Salicylic acid and Lactic acid that are proposed as active pharmaceutical ingredients (API's) in this project, they are used as ketolytic compounds. The proposed solvents were Ethanol, Methanol, Diethyl ether and Acetonitrile to be used for the co-crystal formation each one in both co-crystal techniques (solution and Grinding). Currently the most established methods for co-crystal formation are reflux solution and mechanical techniques. In reflux co-crystal synthesis, stoichiometric ratios of active pharmaceutical ingredients (API's) are dissolved in a solvent of choice and super saturation is achieved either through a temperature difference or through evaporation of the solvent.

6.4.1 Reflux Co-crystallization technique:

API's target concentration expected to be formulated were mixed with 10 ml of the selected solvent in a round bottom flask and refluxed for 2.5 hours. Collecting the reflux solution in a capped glass ware and loosen the cap screw slightly or making holes in the top part of the cap, allowed to stand for several days (14-21 day). Co-crystals were obtained in each solvent, in which the physicochemical properties are notified and tested.

Table 6 salicylic acid and lactic acid target concentrations

Target Concentration %	Salicylic acid and Lactic acid (2:1) molar ratio		
	Solvent	Salicylic acid (g)	Lactic acid (g)
100%	Ethanol	1.1998	0.4468
100%	Methanol	1.2001	0.4428
100%	Diethyl ether	1.2004	0.4440
100%	Acetonitrile	1.2004	0.4440

A new reflux drug concentration is done according to the reflux technique for more co-crystal clarifications, by means of duplication the lactic acid molar ratio concentration and fixing the salicylic acid molar ratio. API's new target concentration expected to be formulated were mixed with 10 ml of the selected solvent in a round bottom flask and refluxed for 2.5 hours. Collecting the reflux solution in a capped glass ware and loosen the cap screw slightly or making holes in the top part of the cap, allowed to stand for several days (30-40 day). Co-crystals were obtained in each solvent, in which the physicochemical properties are notified and tested.

Table 7 salicylic acid and lactic acid concentrations

Salicylic acid and Lactic acid (2:2) molar ratio			
Target Concentration %	Solvent	Salicylic acid (g)	Lactic acid (g)
100%	Ethanol	2.3997	1.7796
100%	Methanol	2.4005	1.7786
100%	Acetonitrile	2.4011	1.7767

6.4.2 Grinding (mechanical) Co-crystallization technique:

There have been great progresses in co-crystal formation via grinding method over the past few years. There are different techniques for co-crystal formation via grinding. Stoichiometric ratios of active pharmaceutical ingredients (API's) are mechanically agitated (e.g. by grinding in a mill) to induce phase transformations from a physical mixture into co-crystal. Drops of solvent, which are considered as plasticizers, have been shown to impact the crystallization outcome.

Mechanical methods are often favored due to their speed, procedural simplicity, and potential for green chemistry. These two methods were largely successful in the discovery of co-crystals.

The grinding method was applied on different molar ratios for each solvent chosen, depending on the accuracy and linearity tests of active pharmaceutical ingredients (API's) in the pharmaceutical validation procedure 50%, 75%, 100%, 125% and 150 % of the target concentration dose. By fixing one active pharmaceutical ingredient (API) concentration and making the variations with the other, as listed in the following table:

Table 8 Salicylic acid and Lactic acid variable molar ratios / grinding co-crystallization technique

Concentration %	Variable Salicylic acid and constant Lactic acid			Constant Salicylic acid and Variable Lactic acid		
	Tube #	Salicylic acid (g)	Lactic acid (g)	Tube #	Salicylic acid (g)	Lactic acid (g)
50%	1	0.06	0.0444	1	0.12	0.0222
75%	2	0.09	0.0444	2	0.12	0.0333
100%	3	0.12	0.0444	3	0.12	0.0444
125%	4	0.15	0.0444	4	0.12	0.0555
150%	5	0.18	0.0444	5	0.12	0.0666

This procedure was applied for the four solvents chosen and each solvent for ten different molar ratios.

Chapter Four

- Results And Discussion

7. Results and Discussions:

7.1 Microemulsion phase diagram results:

7.1.1 Pseudo ternary phase diagram 1 Tween 80 in the corner B and Castor oil with Ethanolic solution (1:1) in the corner C demonstrates that the microemulsion was obtained and started as a single clear, isotropic and not shiny solution upon the addition of the first 4% water to the system in the tube number 46 which contains 0.4g B and 0.6g C at 25, 37 and 45°C, up to tube 100%B. The microemulsion region remains the same with increasing the temperature, which refers to the fact that the non-ionic surfactant used is soluble in water, while it is soluble in castor oil with the aid of ethanol in a specific ratio. Since castor oil is insoluble in water, therefore ethanol is used as a tuning parameter for all ingredients and contributes clearly in forming the microemulsion for tube 46 up to 100% B. Thus, an increase in the temperature will not have an effect on the non-ionic surfactant micelle structure (W/O). As shown in the bellow pseudo ternary phase diagram at all studied temperature conditions.

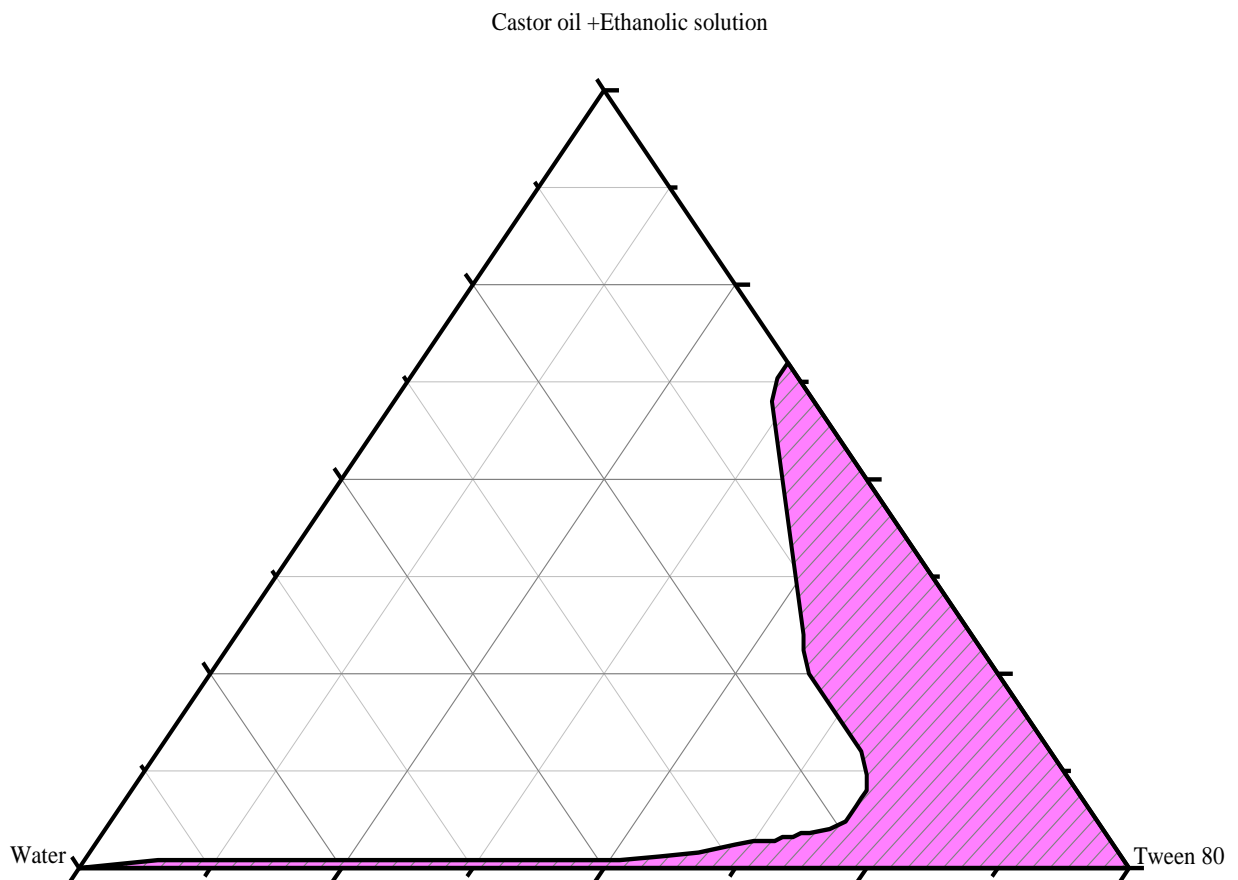


Figure 17 Pseudo ternary phase diagram 1

Where: Ethanolic solution is 12.0g of Salicylic acid and 4.44g of Lactic acid in 50ml of ethanol (96%)

7.1.2 Pseudo ternary phase diagram 2, Tween 80 in the corner B and Ethanolic solution in the corner C demonstrates that the microemulsion was obtained and started as a single clear isotropic and not shiny solution upon the addition of 4% water to the system in a wide region from 100%C up to 100%B tubes at 25, 37 and 45°C with a different viscosity that is increased with increasing the surfactant concentration. The microemulsion region remains the same with increasing the temperature, which refers to the fact that the non-ionic surfactant used is soluble in water and in ethanol in a wide ratio, in which ethanol is used as a tuning parameter for all ingredients and ethanol is soluble and miscible in water by nature knowledge. Thus, an increase in the temperature will not have an effect on the non-ionic surfactant micelle structure (W/O), and the absence of castor oil increases the non-ionic surfactant solubility region as shown in the bellow pseudo ternary phase diagram at all studied temperature conditions.

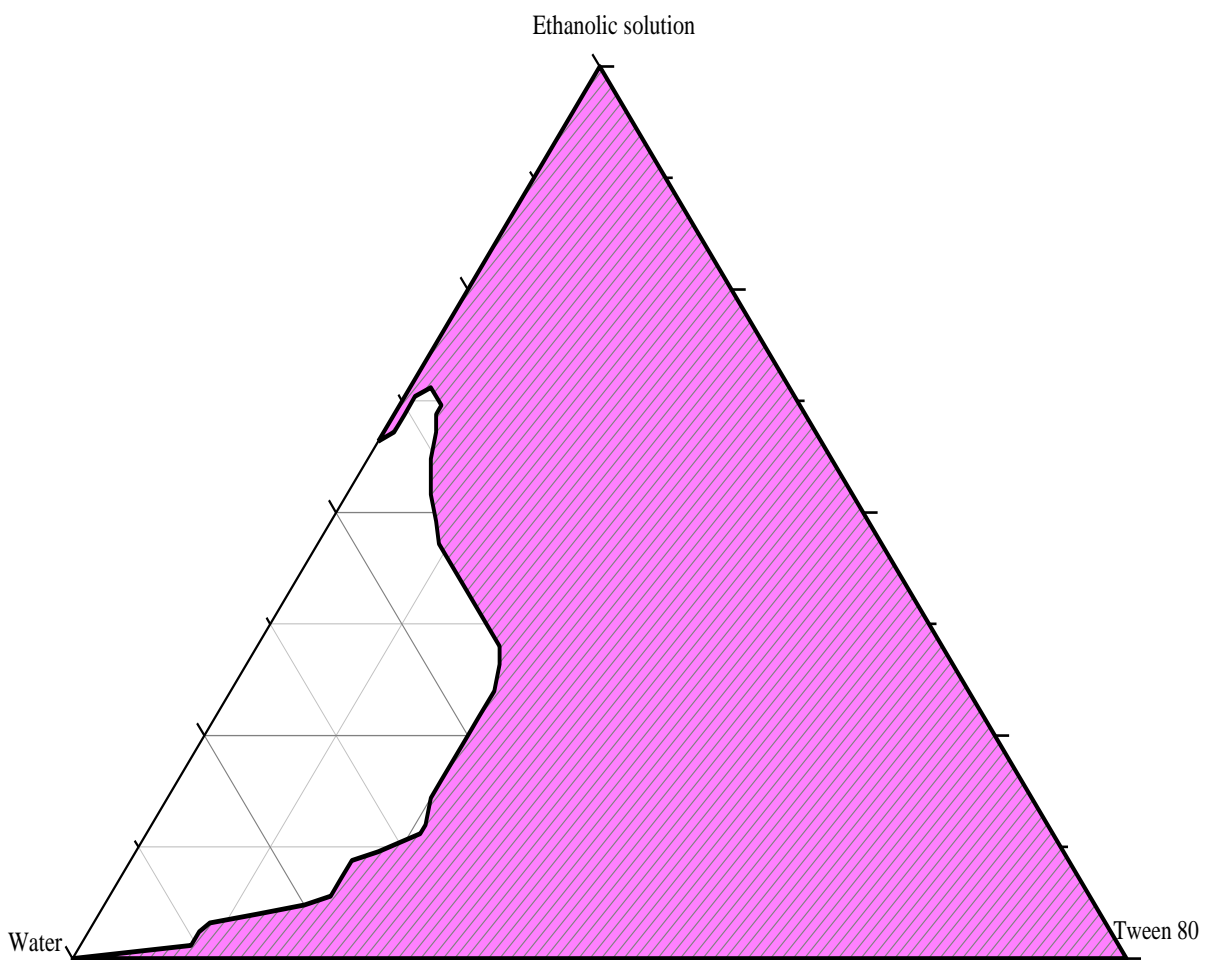


Figure 18 Pseudo ternary phase diagram 2

Where: Ethanolic solution is 12.0g of Salicylic acid and 4.44g of Lactic acid in 50ml of ethanol (96%)

7.1.3 Pseudo ternary phase diagram 3, Tween 80 in the corner B and Ethanolic solution in the corner C demonstrates that the microemulsion was obtained and started as a single clear isotropic and not shiny solution upon the addition of 4% water and propylene glycol (1:1) to the system in a wide region from 100%C up to 100%B tubes at 25, 37 and 45°C with a different viscosity which is increased with increasing the surfactant concentration. The microemulsion region remains the same with increasing the temperature, which refers to the fact that the non-ionic surfactant used is soluble in water and also in the propylene glycol used as co-surfactant, which shares the non-ionic surfactant in making a smallest droplet size to form this wide microemulsion region. Also ethanol is used as a tuning parameter and co-solvent for all ingredients. The non-ionic surfactant is water and ethanol soluble, therefore, an increase in the temperature will not have an effect on the non-ionic surfactant micelle structure (W/O) or (O/W) . As shown in the bellow pseudo ternary phase diagram at all studied temperature conditions.

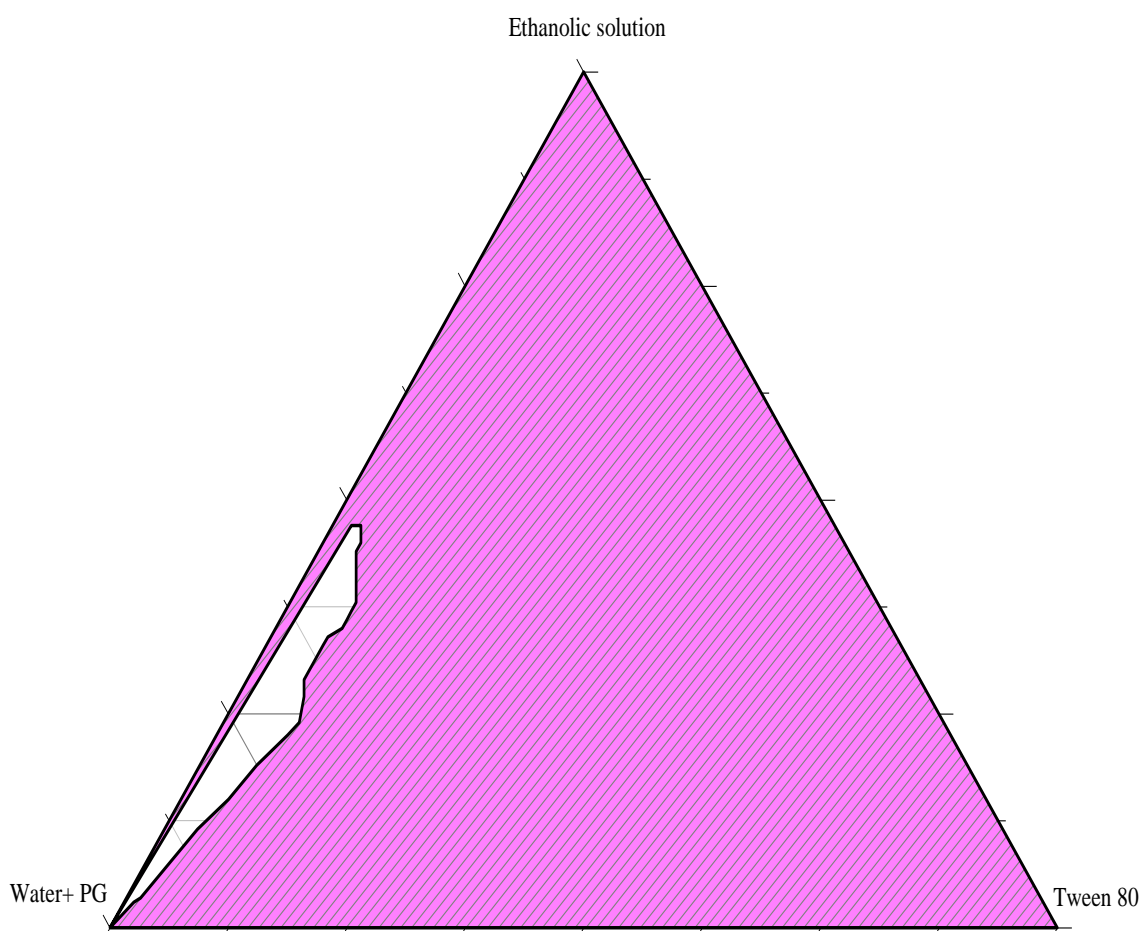


Figure 19 Pseudo ternary phase diagram 3

Where: Ethanolic solution is 12.0g of Salicylic acid and 4.44 g of Lactic acid in 50ml of ethanol (96%)

7.1.4 Pseudo phase diagram 4, Tween 80 in the corner B and Castor oil with Ethanolic solution (1:1) in the corner C demonstrates that the microemulsion was obtained and started as a single clear isotropic and not shiny solution upon the addition of 4% water and propylene glycol (1:1) to the system in the tube 100% C up to 100% B tubes at 25, 37 and 45°C with a different microemulsion viscosity that is increased with increasing the surfactant concentration. The microemulsion region remains the same with increasing the temperature, which refers to the fact that the non-ionic surfactant used is soluble in water, and also in the propylene glycol used as co-surfactant, which shares the non-ionic surfactant in making a smallest droplet size to form this microemulsion region. Castor oil is insoluble in water but the presence of propylene glycol shares with 4% solubility region near the corner C, and with increasing the surfactant concentration the microemulsion region becomes larger up to 100% B concentration with the aid of ethanol in a specific ratio, in which ethanol is used as a tuning parameter for all ingredients. Thus, an increase in the temperature will not have an effect on the non-ionic surfactant micelle structure (W/O). As shown in the bellow pseudo ternary phase diagram at all studied temperature conditions.

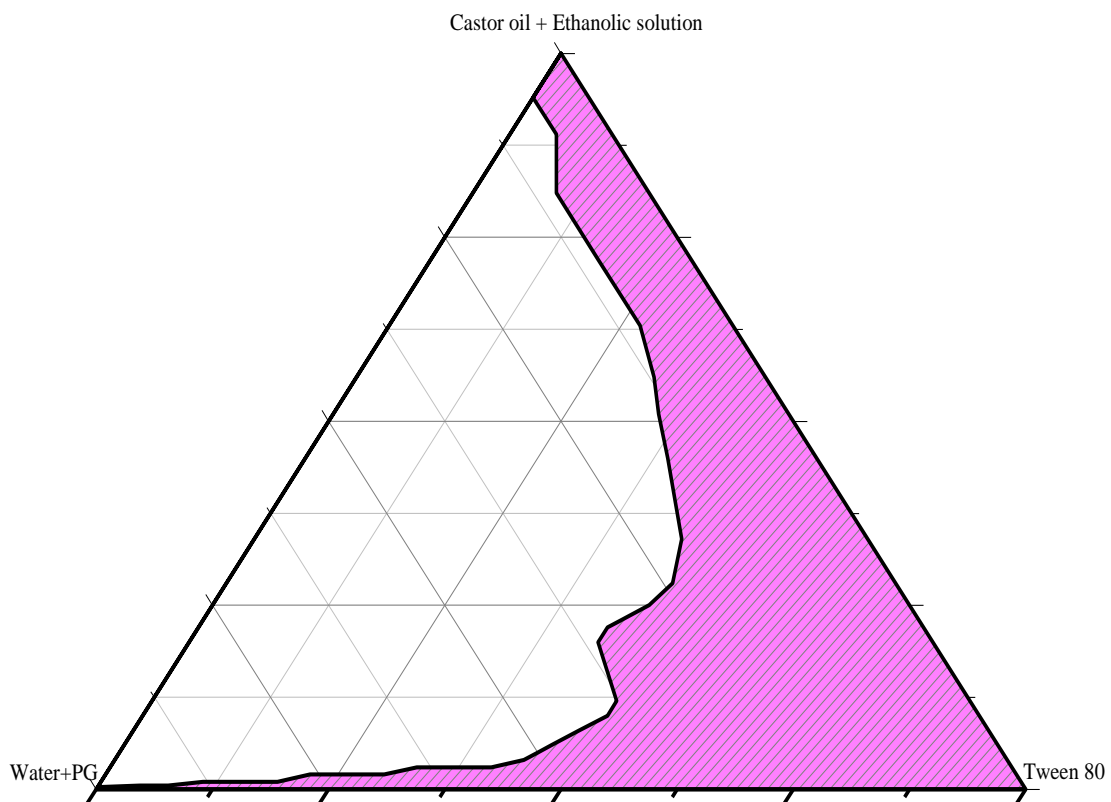


Figure 20 Pseudo ternary phase diagram 4

Where: Ethanolic solution is 12.0g of Salicylic acid and 4.44 g of Lactic acid in 50ml of ethanol (96)

7.2 Co-crystal results:

7.2.1 Reflux Co-crystallization technique:

The co-crystals obtained from each solvent used in the reflux co-crystal technique were amazing and impressive in their uniformity, physicochemical properties, shape and their order of formation. See the photo pictures bellow for the pure active pharmaceutical ingredient and the co-crystal paradigm obtained in each selected solvent.

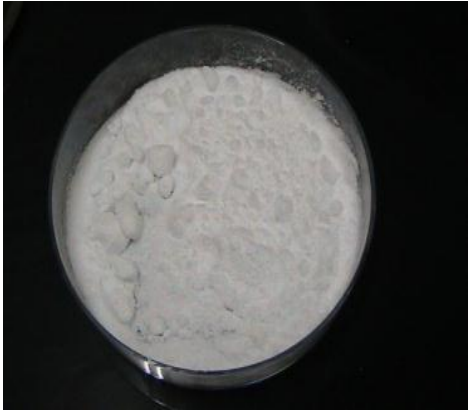





Salicylic acid (pure)		Lactic acid (pure)	
			
Ethanol Co-crystal	Methanol Co-crystal	Diethyl ether Co-crystal	Acetonitrile Co-crystal
			

Figure 21 Co-crystals obtained from each co-solvent / Reflux technique

Note: Diethyl ether Co-crystals seems white in its color due to the co solvent effect of drying, while when they are wet they behave like other solvents in the physical appearance.

The obtained co-crystals were tested individually for their melting point and FTIR spectrum test. The co-crystal formation is readily apparent from the resulting physical properties of the new material. Formation of a co-crystal from Salicylic acid (white powder) and Lactic acid (viscous clear liquid) is immediately apparent from the transparent color of the co-crystal, despite the fact that salicylic acid is white solids and lactic acid is clear liquid. The color arises from the fact that the co-crystal as part of the overall hydrogen bonded crystal packing arrangement, with concomitant reduction of the π - π^* energy gap [48].

The data showed the initial appearance of 1.8:1 ratio for salicylic acid and lactic acid respectively, that the melting point is about 120°C. In addition to the melting point, FTIR spectroscopy of solid samples (measured for example using KBr salt) can also give a characteristic fingerprint of a particular solid form. Because vibrational spectroscopy depends on bond vibrational modes which are only moderately worried by the molecule's solid state environment, the differences in FTIR spectra between pure active pharmaceutical ingredients forms, or between co-crystal and pure forms, can be relatively major appeared. However, if particular bands are sensitive to solid form (*e.g.* when there is a significant change in hydrogen bonding mode in different forms) then vibrational spectra represent a useful and facile method of distinguishing different polymorphs and co-crystals. Among many recent patents relating to potential commercial co-crystal products, the possibility of combining two active ingredients in a single co-crystal is an interesting one and has been claimed in the co-crystallization. The combination drug has been suggested to have physical properties and biological activity that are distinct from the individual properties of the two components [49].

The original known and tested FTIR spectrum was as followed for each pure active pharmaceutical ingredient:

API Major peak Pure Salicylic acid API FTIR spectrum

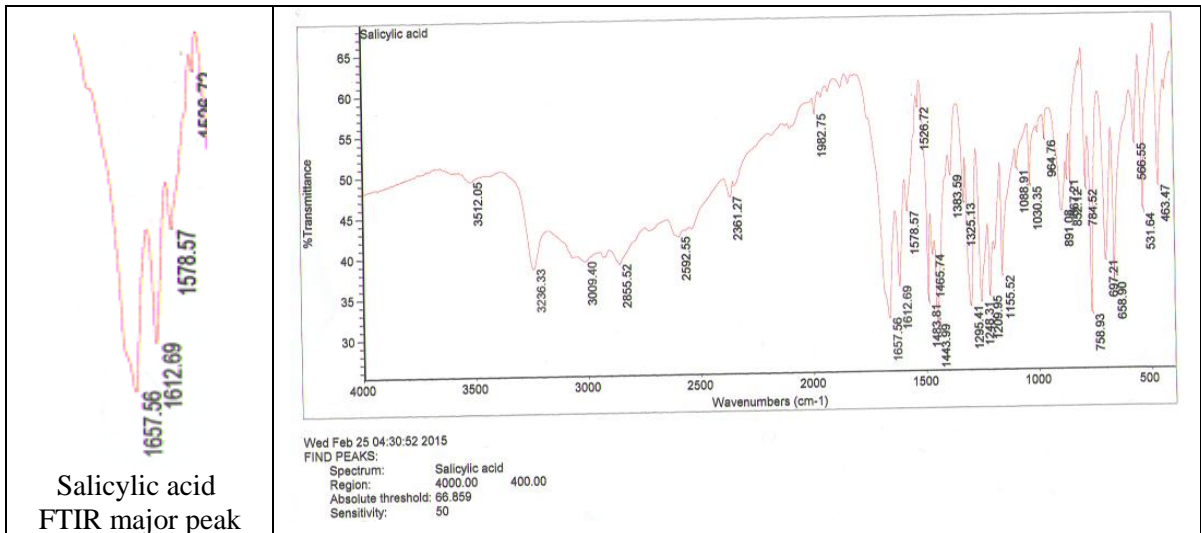


Figure 22 pure Salicylic acid FTIR spectrum

API Major peak Pure Lactic acid API FTIR spectrum

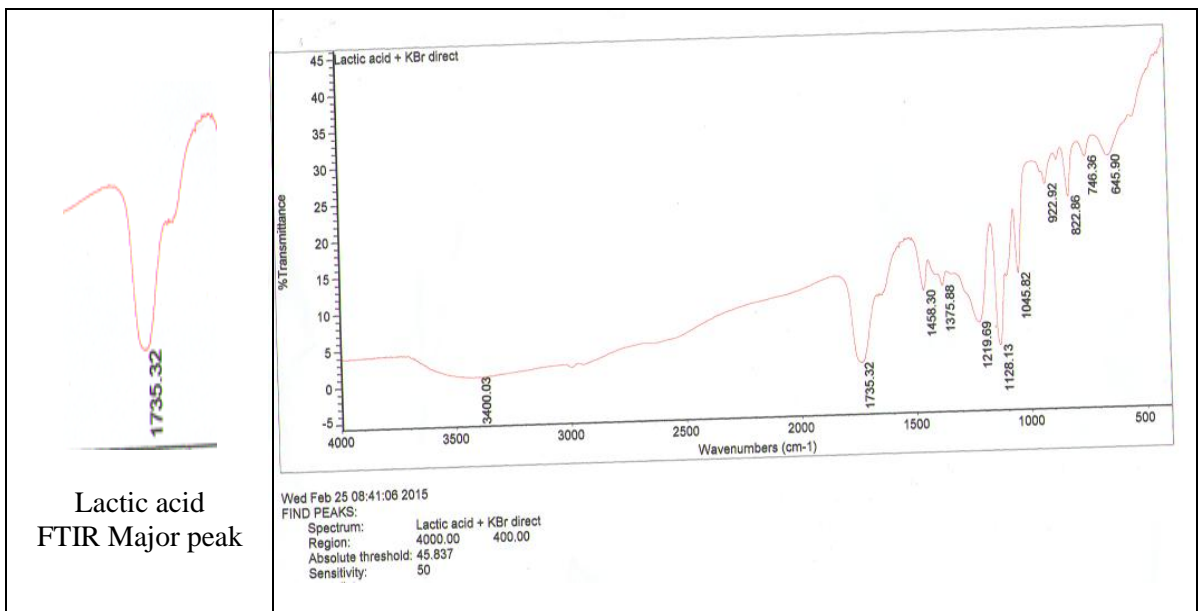


Figure 23 Pure Lactic acid FTIR spectrum

7.2.2 Grinding (mechanical) Co-crystal technique

The co-crystals obtained from each co-solvent used in the grinding mechanical co-crystal technique were amazing in their uniformity, shape and their order of formation. See the photo pictures bellow for each

Ethanol co-solvent



Methanol co-solvent



Diethyl ether co-solvent

Acetonitrile co-solvent



Figure 24 Co-crystal obtained from each co-solvent / grinding technique

The obtained co crystals from Grinding (mechanical) Co-crystal technique were tested individually for FTIR spectroscopy of solid samples using KBr salt and compared with the pure samples spectrum that was tested before at the beginning.

Table 9 Grinding co-crystallization technique FTIR results

Solvent	Constant Salicylic acid and Variable Lactic acid		Variable Salicylic acid and Constant Lactic acid	
Ethanol co-solvent	Tube #	Result	Tube #	Result
	1	Positive	1	Positive
	2	Positive	2	Positive
	3	Positive	3	Positive
	4	Positive	4	Positive
	5	Positive	5	Positive
Methanol co-solvent	Tube #	Result	Tube #	Result
	1	Negative	1	Positive
	2	Negative	2	Positive
	3	Partial	3	Positive
	4	Positive	4	Positive
	5	Positive	5	Positive
Diethyl ether co-solvent	Tube #	Result	Tube #	Result
	1	Positive	1	Positive
	2	Positive	2	Positive
	3	Positive	3	Positive
	4	Positive	4	Positive
	5	Positive	5	Positive
Acetonitrile co-solvent	Tube #	Result	Tube #	Result
	1	Negative	1	Positive
	2	Negative	2	Positive
	3	Positive	3	Positive
	4	Positive	4	Negative
	5	Positive	5	Negative

Note: - Negative means no co-crystal obtained
 - Partial means weak co-crystal obtained, expected to negative results
 - Positive means a clear repeatable co-crystal obtained

The obtained co-crystal paradigm FTIR spectrum in diethyl ether as co-solvent:

FTIR spectrum for the obtained co-crystals paradigm in Diethyl ether as a co-solvent according to the grinding technique

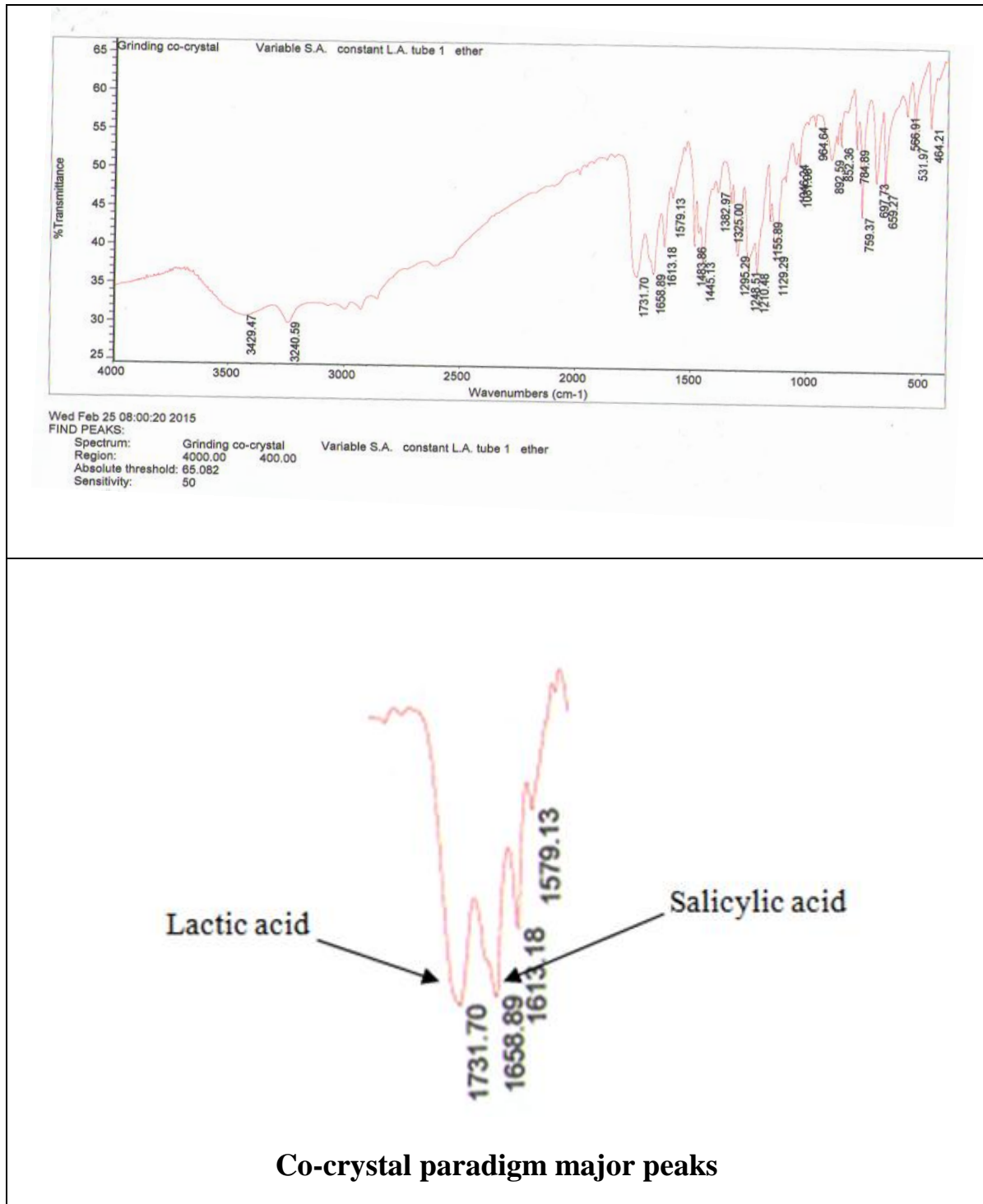


Figure 25 Co-crystal obtained from diethyl ether FTIR spectrum / grinding technique

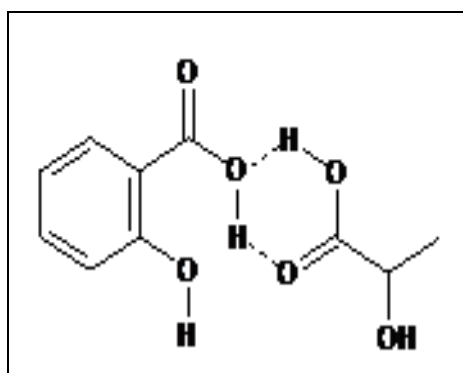
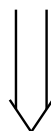
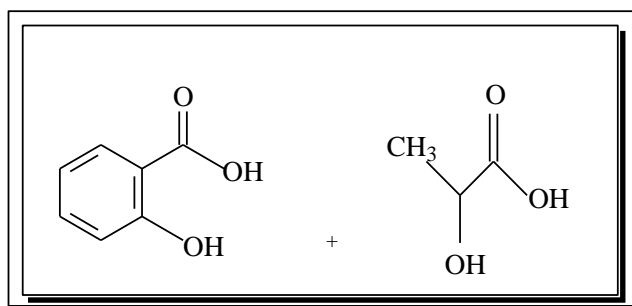
The co-crystals obtained either from reflux or grinding techniques were tested using FTIR showing an impressive merged active pharmaceutical ingredient. By comparing the major peaks obtained with those in the pure API's spectrums, the following table will show how many wave lengths shifted in the co-crystal spectrums:

Table 10 Wave length degree shift in cm^{-1} for the major peaks

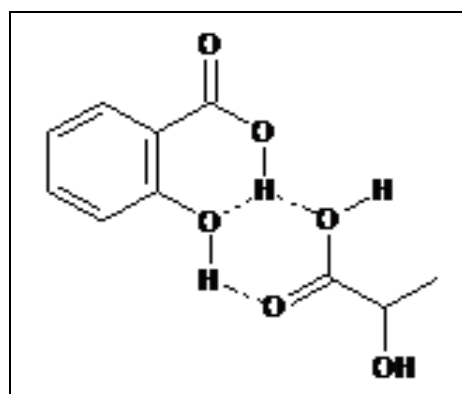
Solvent	Constant Salicylic acid and Variable Lactic acid							Variable Salicylic acid and Constant Lactic acid						
	Wave number degree shift in cm^{-1} for major peaks							Wave number degree shift in cm^{-1} for major peaks						
Ethanol co-solvent	Tube #	3235	1735	1657	1444	1128	1046	Tube #	3235	1735	1657	1444	1128	1046
	1	+1	-3	+3	+1	+2	+2	1	+5	-3	+2	+1	+2	+1
	2	-1	-3	+4	+1	+2	+1	2	+6	-3	+2	+1	+2	+1
	3	+4	-3	+1	+1	+2	---	3	+4	+1	-1	+1	+2	+1
	4	+3	-3	+1	+1	+2	+1	4	+5	-3	+2	+1	+2	+1
	5	+1	-6	-2	---	---	+3	5	+2	-6	+2	---	+3	+3
Reflux	+5	-3	+2	+2	+2	+1								
Methanol co-solvent	Tube #	3235	1735	1657	1444	1128	1046	Tube #	3235	1735	1657	1444	1128	1046
	1	+2	---	-1	---	---	---	1	+8	+0.5	+3	+1	+2	+1
	2	+2	---	+2	---	---	---	2	+4	-3	+2	+1	+3	---
	3	+7	-3	+2	---	---	+4	3	+2	-5	+2	+1	+3	+3
	4	+7	+3	+2	+1	+3	+2	4	+5	+5	+5	+0.5	+4	+4
	5	+1	+3	+2	+1	+3	+2	5	+5	-4	+3	+0.5	+3	+3
Reflux	+2	-3	+2	+0.5	---	+5								
Diethyl ether co-solvent	Tube #	3235	1735	1657	1444	1128	1046	Tube #	3235	1735	1657	1444	1128	1046
	1	+5	-4	+2	+1	+2	+1	1	+5	-3	+2	+1	+1	---
	2	+4	-3	+3	+1	+1	---	2	---	-3	+3	+1	+1	+1
	3	+4	+1	+1	+1	+1	+1	3	+4	-4	+1	+1	+2	+1
	4	+2	-6	+2	+1	---	+2	4	+6	-4	+2	+1	+1	+1
	5	+3	-5	+1	+1	+1	+1	5	+2	-7	+2	+1	+2	+1
Reflux	+5	-4	+2	+1	+1	+1								
Acetonitrile co-solvent	Tube #	3235	1735	1657	1444	1128	1046	Tube #	3235	1735	1657	1444	1128	1046
	1	+3	---	-1	---	---	---	1	---	+0.5	-1	+3	+1	+1
	2	+4	---	+1	---	---	---	2	+5	-3	+3	+1	+1.5	+1
	3	+8	-3	+3	+1.5	+1	+0.5	3	+5	+0.5	-2	+1	+1	---
	4	+2	-3	+3	+1	+1	+1	4	+3	---	-1	---	---	---
	5	+3	+8	+2	+0.5	+2.5	+2	5	+2	---	+1	---	---	---
Reflux	+6	-3	+3	+1	+2	+1								

7.2.3 The proposed compounds from co-crystallization paradigms expected:

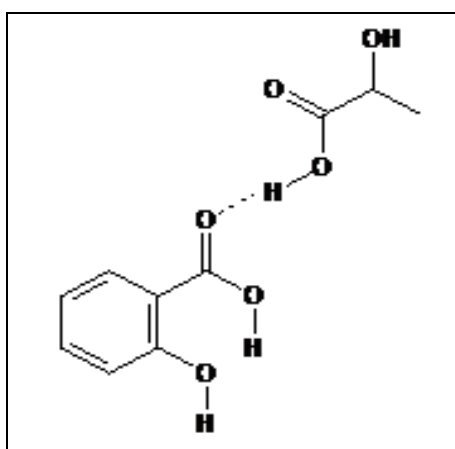
The figure bellow represents the expected products paradigms formed by hydrogen bonding due to co-solvents utilized between lactic acid and salicylic acid.



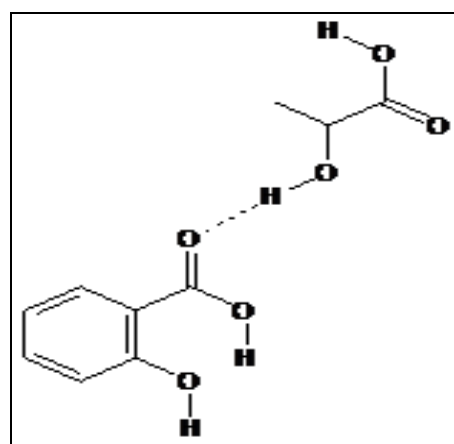
Paradigm 1



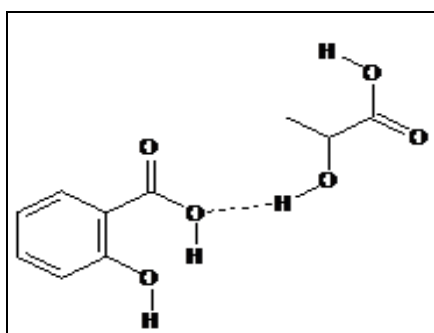
Paradigm 2



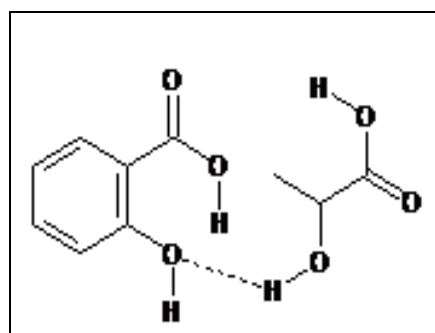
Paradigm 3



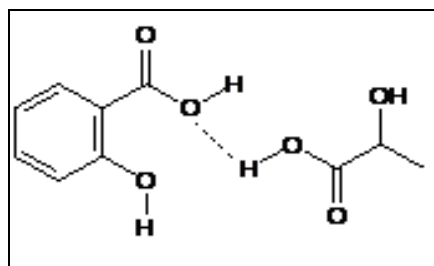
Paradigm 4



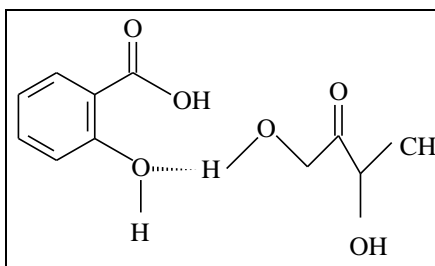
Paradigm 5



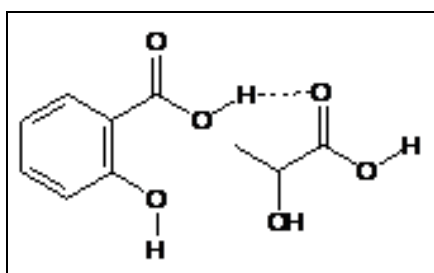
Paradigm 6



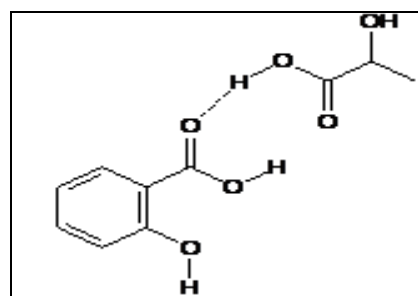
Paradigm 7



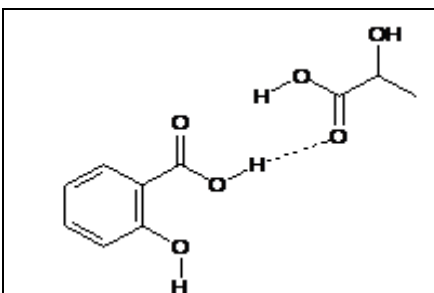
Paradigm 8



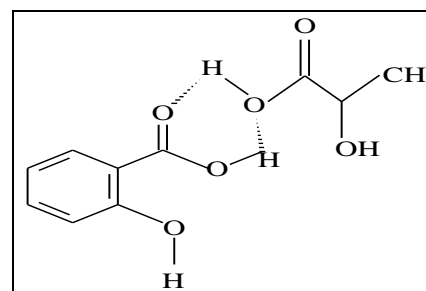
Paradigm 9



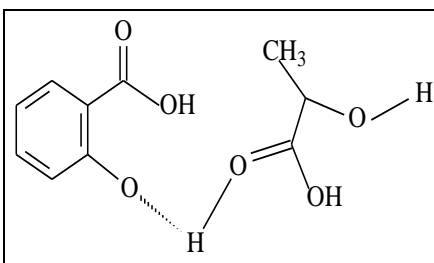
Paradigm 10



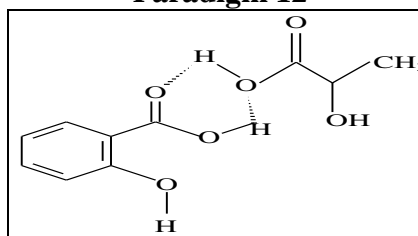
Paradigm 11



Paradigm 12



Paradigm 13



Paradigm 14

Figure 26 Proposed co-crystal paradigms (1 – 14) structures

7.2.4 Co-crystal solubility in water:

The general United State Pharmacopeia solubility requirements are listed in the following table:

Table 11 United State Pharmacopeia solubility criteria

Classification	Parts of solvent required for one part of solute (between 15°C and 25°C)
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	More than 10 to 30
Sparingly soluble	More than 30 to 100
Slightly soluble	More than 100 to 1000
Very slightly soluble	More than 1000 to 10000
Practically insoluble	More than 10000

It was known that salicylic acid solubility is slightly soluble in water and freely soluble in ethanol (96 per cent), while lactic acid solubility is miscible with water and with ethanol (96 per cent).

From previous co-crystal aims mentioned before in the text, it was supposed to change and modify the solubility of the active pharmaceutical ingredients in the new paradigms obtained. The surprise is obtained after testing the co-crystal paradigm, in each solvent prepared previously, in water as a solubility solvent used and the results are:

Sample taken from each co-crystal paradigm is about 0.5 g, an attempt to dissolve it in 10 ml purified water, using volumetric flask, by sonication for 15 minutes, and the result was not totally soluble. Then the same solution from each paradigm was transferred totally to 25 ml volumetric flask and diluted up to volume with purified water and sonicated for 15 minutes, and the result was also not totally soluble. Then the same solution from each paradigm was transferred totally to 50 ml volumetric flask and diluted up to volume with purified water and sonicated for 15 minutes, and the result was totally soluble.

As a conclusion for the solubility test, since (1) salicylic acid is sparingly soluble in water (which means each 1.0 g powder is sparingly soluble in 30-100 ml of solvent), and (2) lactic acid is miscible in water (which means no problem with lactic acid solubility in water). Then it was found that each 0.5 g co-crystal paradigm is soluble in 50 ml of purified water. Therefore the solubility is modified in each paradigm co-crystal, which means and assures that new compound is formed.



Figure 27 Photos for sample representing co-crystal obtained from each co-solvent used in water, at the beginning

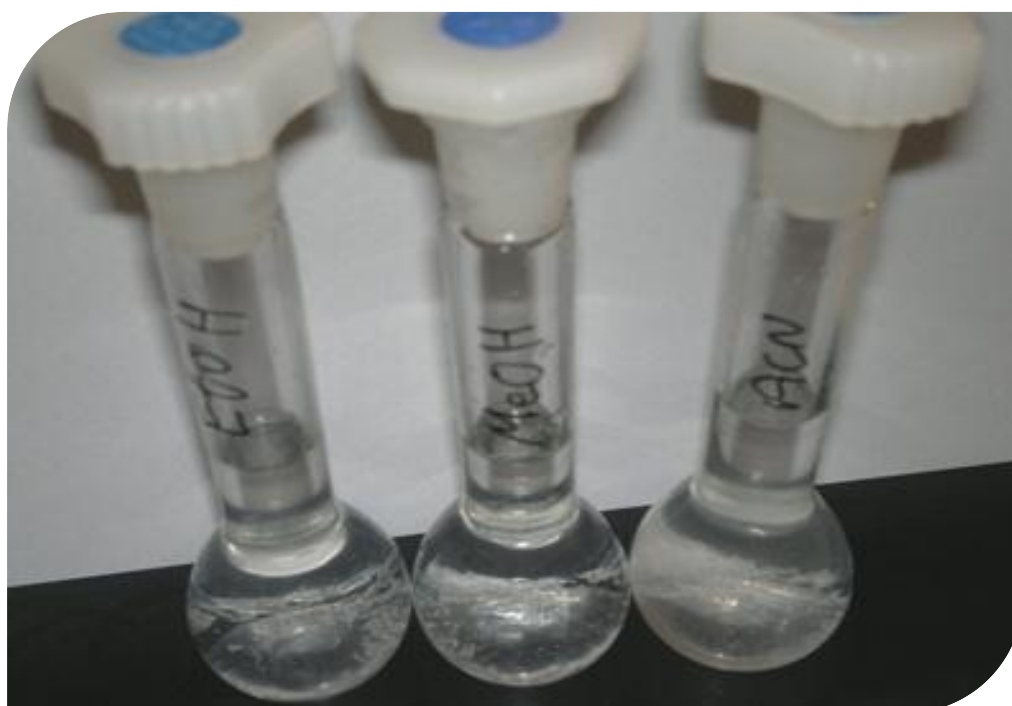


Figure 28 Photos for sample representing co-crystal obtained from each co-solvent used in water, after sonication for 15 minutes, few is dissolved



Figure 29 Photos for sample representing co-crystal obtained from each co-solvent used in water, after the transference in 50ml volumetric flask



Figure 30 Photos for sample representing co-crystal obtained from each co-solvent used in water, after sonication for 15 minutes. **It is completely dissolved**

7.2.5 The co-crystal melting point:

Pure salicylic acid pharmaceutical ingredient melting point is about 159°C, while lactic acid pure pharmaceutical ingredient melting point is about 53°C. The expected new co-crystal paradigm melting point is to be lower than 159°C and higher than 53°C, if the paradigm obtained is succeeded as expected **and yes it is**, the following data result are for part of the co-crystals tested:

- Co-crystal sample melting Range is 127.6-127.7°C
- Co-crystal sample melting Range is 138.3-139.4°C
- Co-crystal sample melting Range is 141.5-147.8°C
- Co-crystal sample melting Range is 145.2-149.1°C

The variation change between the co-crystal melting point ranges is dependent on the target active pharmaceutical concentration and depending on the molar ratio for each co-crystal tested. But even so, there is a clear change in the melting point range which will assure that a new paradigm (composed of merging the two active pharmaceutical ingredients) is obtained by using any of the solvents suggested ethanol, methanol, diethyl ether or acetonitrile.

Note: for data results print out refer to the figures 31& 32 in the list of appendices.

8. Conclusion

This work shows that the addition of short chain alcohol as a co-surfactant at different mass fraction in the dissolution of active pharmaceutical ingredients mixture is necessary to initiate the formation of drug product microemulsion in the formula of water, propylene glycol and sugar ester nonionic surfactant such as polysorbate 80 (Tween 80) due to temperature insensitivity of sugar ester; In addition, short chain alcohol is used as "tuning parameter "to increase the flexibility of the surfactant film. The increase of the amount of ethanol concentration supports w/o microemulsion to form o/w microemulsion in a wide concentration range within the phase diagram regions.

The microemulsion drug product was obtained in the four phase diagrams upon the addition of 4% water (with or without propylene glycol), in which it is isotropic, clear and non shiny microemulsion at three temperatures 25, 37 and 45°C in the same phase behavior at all temperature conditions and continued up to 100% aqueous addition, successfully.

The co-crystal paradigm of salicylic acid and lactic acid was obtained clearly in the reflux technique for each co-solvent used, which was tested firstly using FTIR, as well as in the grinding technique at different molar ratios, secondly using melting point range.

9. Future work

The co-crystal paradigms obtained will be tested externally for determining the lattice structure of the new compound structure obtained, using x-ray structure analysis.

10. References:

- [1] Richard J. Farn, (2006), "Chemistry and Technology of Surfactants" by Blackwell Publishing Ltd
- [2] Drew Myers, (2006), "Surfactant Science and Technology". Third Edition, John Wiley & Sons, Inc., Hoboken, New Jersey.
- [3] Schramm et al. (2003), "Surfactants and their applications". Annu.Rep. Prog. Chem., Sect. C, Vol99, PP. 3–48.
- [4] Johanna Borne, (2002), "lipid Self-Assembly and lipase Action". Thesis presented to the graduate faculty of Lund University, Sweden.
- [5] Cosima Stubenrauch.,(2009) , "Microemulsions Background, New Concepts, Applications, Perspectives", 1 st Ed., A John Wiley and Sons Ltd, Inc., New Delhi, India.
- [6] Ibrahim Kayali, et al., (2012):"Water-Diesel Microemulsions Stabilized by an Anionic Extended Surfactant and a Cationic Hydrotrope ". Journal of Dispersion Science and Technology, Vol 33, PP. 516-520.
- [7] Jadhav C. M.; Shinde S. M.; Kate U. K.; Payghan S. A; Disouza J. I., (2014), "Investigating Application of Non Aqueous Microemulsion for Drug Delivery". Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (29); 2014; 1-9.
- [8] Jorg Kreuter, (1994), "Colloidal Drug Delivery Systems". Volume 66, 31–71
- [9] Dr. Bhanuben Nanavati, (2011), Ind J Pharm Edu Res, Jan-Mar, "Microemulsions for Topical Use– A Review" / Vol 45/ Issue 1
- [10] Diane L. Buhr et al., (2012), "Use of micro-emulsion technology for the directed evolution of antibodies" Published by Elsevier Inc.10.1016/j.ymeth.07.007
- [11] Cristobal Carnero Ruiz, (2009), "Sugar-Based Surfactant: Fundamentals and Applications", 1 st Ed., Taylor & Francis Group.London
- [12] Bidyut KP, Satya PM. (2001), "Uses and applications of Microemulsions". Current Science; 80(8): 990-1001
- [13] Kalpana S Paudel et al., (2010), "Challenges and opportunities in dermal / transdermal delivery"; 1(1):109-131
- [14] Barry BW., (2004), "Breaching the skin's barrier to drugs.Nat Biotechnol" 22(2):165-167
- [15] Morrow DIJ, McCarron PA, Woolfson AD, Donnelly RF., (2007), "Innovative Strategies for Enhancing Topical and Transdermal Drug Delivery". The Open Drug Delivery Journal 136-59
- [16] Kemken J, Ziegler A, Muller BW., (1992), "Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using Microemulsions as vehicle". Pharm Res 9(4):554-558.
- [17] Maqsood Ahmad Malik et al., (2012), Arabian Journal of Chemistry, Volume 5, Issue 4, Pages 397–417
- [18] Lawrence MJ., (1994), "Surfactant systems: their use in drug delivery". Chemical Society Reviews 23(6):417-424

- [20] Babu RJ, Singh M, Kanikkannan N., (2005), "Fatty alcohols and fatty acids". In Smith E, Maibach HI, editors. *Percutaneous Penetration Enhancers*. New York: CRC Press; pp: 137-158.
- [21] Mittal A, Sara UV, Ali A, Aqil M., (2009), "Status of fatty acids as skin penetration enhancers-a review". *Curr Drug Deliv* 6(3):274-279.132
- [22] Zhang J, Michniak-Kohn B., (2011), "Investigation of microemulsion microstructures and their relationship to transdermal permeation of model drugs": ketoprofen, lidocaine, and caffeine. *Int J Pharm* 421(1):34-44.
- [23] Changez M, Varshney M, Chander J, Dinda AK.,(2006), "Effect of the composition of lecithin/n-propanol/isopropyl myristate/water Microemulsions on barrier properties of mice skin for transdermal permeation of tetracaine hydrochloride": in vitro. *Colloids Surf B Biointerfaces* 50(1):18-25.
- [24] Ning Qiao, et, (2011), "Pharmaceutical cocrystals": An over view, *International Journal of Pharmaceutics*
- [25] Almarsson O and Zaworotko MJ., (2004), "Crystal engineering of the composition of pharmaceutical cocrystals", Do pharmaceutical co-crystals represents a new path to improved medicines? *Chem Comm* , 1889–1896
- [26] Khan M, Enkelmann V and Brunklaus G., (2010), "Crystal engineering of pharmaceutical co-crystals": application of methyl paraben as molecular hook. *J Am Chem Soc.*132, 5254–5263.
- [27] Aakeröy CB et al., (2007). "Structural competition between hydrogen bonds and halogen bonds". *J Am Chem Soc.*;129:13772–13773.
- [28] R. Vir Prasad* et al., (2012), "Pharmaceutical Cocrystallization", *International journal of pharmaceutical and chemical sciences*, ISSN: 22775005, Vol.1 (3)
- [29] Desiraju GR., (1995), "Supramolecular synthons in crystal engineering a new organic synthesis", *Angew Chem Int Ed Engl*,34:2311–2327
- [30] B. Rajnicova, D. Valigura, Z. Padelkova, J. Moncol, (2011), salicylic acid reference from Cambridge crystallographic database, private communication
- [31] A. Schouten, J.A. Kanters, J. Van Krieken, (1994), lactic acid reference from *Journal of molecular structure* volume323, page 165
- [32] Rhodia, (2011), "salicylic acid": Rev 1, EC 1907/2006 (reach)
- [33] Michael Boivin BSc.Pharm., (2009), "Managing dry skin", 1.0 CEU
- [34] Hellen Allen, (2012), *British National Formulary; 63rd Edition*, British Medical Association and Royal Pharmaceutical Society of Great Britain, London
- [35] K. Rajagopal et al., (2010), "Diversity of Fungal Endophytes in Few Medicinal Herbs of South India" *ASIAN J. EXP. BIOL. SCI. VOL 1 (2): 415- 418*
- [36] DiSalvo R.,(2002), "Salicylic acid. The chemistry and manufacture of cosmetics". Volume III. Edited by M.L. Schlossman
- [37] JohnWiley & Sons, Ltd., (2014), "Topical treatments for cutaneous warts (Review)", *The Cochrane Collaboration*

- [38] Tommaso Casalini et al., (2011), “ Structural Characterization of Poly-L-lactic Acid (PLLA) and Poly(glycolic acid)(PGA) Oligomers”, *Int. J. Mol. Sci.* 2011, 12, 3857-3870;doi:10.3390/ijms12063857
- [39] Jones Am.,(1996), “Epidermal and dermal effects of topical lactic acid” ;35(3Pt1):388-91
- [40] Alia A. Badawi, et al., (2009), “Preparation and evaluation of microemulsion systems containing salicylic acid”, 10(4):1081-4. doi: 10.1208/s12249-009-9301-7
- [41] Gosmore,Hitchin, (2013), Irish Medicines Board, “Salactol colloidon”, PA 278/6/1
- [42] Hitchin, Herts, (2011), SPC Dermal Laboratories limited, “salactol collidon”, SG4 7QR, UK. 00173/5006R.
- [43] Gosmore,Hitchin, (2009),Irish Medicines Board, “Salatac gel”, PA 278/15/1
- [44] Arun et al., (2012), “TECHNIQUES TO IMPROVE THE ABSORPTION OF POORLY SOLUBLE DRUGS”, INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY 2781–ISSN: 2231.
- [45] Altaf Ahmad Najar and Yasser Azim*, (2014), “Pharmaceutical Co-Crystals: A New Paradigm of Crystal Engineering”, *Journal of the Indian Institute of Science* VOL 94:1 Jan.–Mar. 2014
- [46] Liliana L Jorge, Caroline C Feres, and Vitor EP Teles, (2011), “Topical preparations for pain relief: efficacy and patient adherence”, 4:11-24
- [47] R. Vir Prasad et al., (2012),International journal of pharmaceutical and chemical science ISSN: 2277-5005, Vol. 1 (3) Jul-Sep 2012
- [48] Sander, J. R. G., et al., (2010), “A red zwitterionic co-crystal of acetaminophen and 2,4-pyridinedicarboxylic acid”. *J.Pharm. Sci.* 99, 3676-3683
- [49] Brader, M. L., et al., (2002), “Hybrid insulin cocrystals for controlled release delivery’. *Nature Biotech.* 20, 800-804

مستحلب بجزئيات صغيرة جداً يحتوي على حمض الساليسيليك وحمض اللاكتيك، واستخدام طريقة البنية البلورية في دمج الاحماض المستخدمة

إعداد الطالب: ماهر عبد الكريم محمد الجمل.

إشراف: البرفسور إبراهيم كيالي.
مشرف ثاني: الدكتور محمد ابو الحاج.

الملخص:

تهدف هذه الدراسة الى تحضير منتج دوائي لعلاج الامراض الجلدية الموضعية (Topical) على شكل مستحلب بجزئيات صغيرة ويحوي حمض الساليسيليك salicylic acid وحمض اللاكتيك lactic acid لعلاج مناطق معينة من الجلد، بتركيبات مختلفة وباستخدام أقل كمية ممكنة من مركب سوربتان مونواولييات المعروف تجارياً باسم توين 80 (surfactant). استخدام المركبات الكحولية قصيرة السلسلة مثل بروبيلين جلايكول propylene glycol كموا د مساعدة للذوبان. ان طور الزيت المستخدم هو زيت الخروع (castor oil). والطور المائي المستخدم هو الماء النقي. بالاضافة الى أن هذه الدراسة تهدف لتحضير نموذج الكريستال المشترك بين حمض الساليسيليك salicylic acid وحمض اللاكتيك lactic acid كموا دوائية فعالة باستخدام محاليل شبيكة مختلفة.

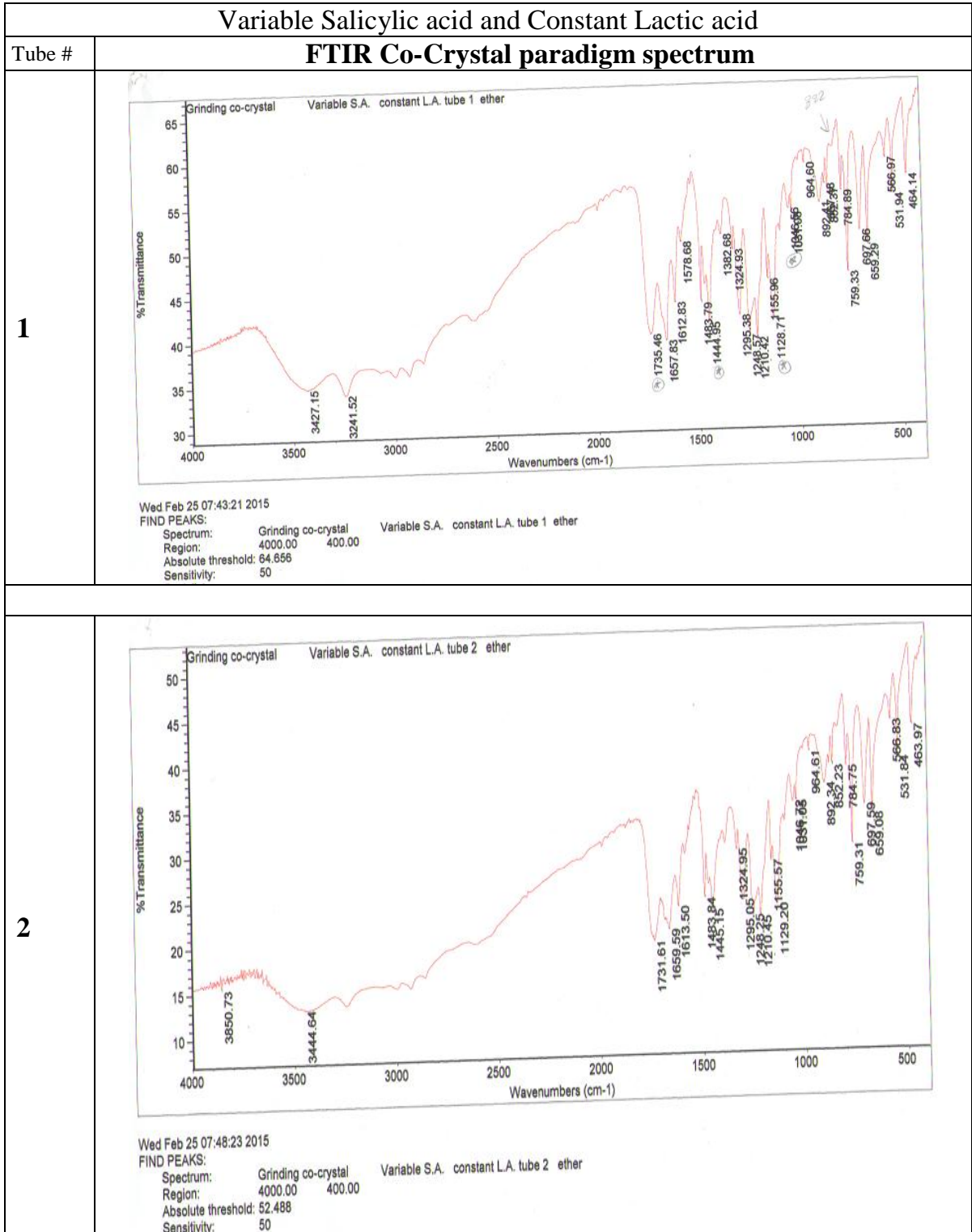
في هذا البحث، قمنا بدراسة أثر نسب مختلفة من مركب سوربتان مونواولييات المعروف تجارياً باسم توين 80 (surfactant) على المرحلة المقترحة على درجات حرارة مختلفة (25, 37 and 45°C). قمنا باكتشاف أثر اضافة المذيب الشريك (propylene glycol) على سلوك المرحلة المقترح. أخيراً، دراسة فعالية سلوك المرحلة المقترح ل سوربتان مونواولييات (sorbitan monooleate) وبتأثير درجة الحرارة، أعطى محلول مستحلب موحد الخواص isotropy (Microemulsionsolution) باستخدام العين المجردة، (using visual inspection, cross polarizers and dynamic light scattering) بأقل كمية اضافة وهي 4% من الطور المائي على كل درجات الحرارة (25°C, 37°C & 45°C).

بالاضافة الى انه في هذا البحث، قمنا بدراسة امكانية استعمال محاليل شريكة مختلفة في تكوين نموذج الكريستال الشريك، مثل الايثانول، الميثانول، داي ايتل ايثر و اسيتونايتريل. باستخدام تقنيات مختلفة مطبقة، سواء كانت تقنية Reflux او تقنية الطحن للتراكيز المقترحة ولتراكيز أخرى بنسب مولية مختلفة. نموذج الكريستال الشريك تم الحصول عليه في جميع تقنية الريفلكس بنجاح وبحوالي 80% في تقنية الطحن بنجاح مؤكداً. تم فحص كل النماذج باستخدام الفحص الطيفي بالاشعة تحت الحمراء (Fourier transform infrared spectroscopy) وفحص مدى درجة الانصهار (melting point range) لبعض العينات كمرحلة أولى للتحقق، بالاضافة لفحص الذاتية (solubility) ومدى تحسنها وتطورها.

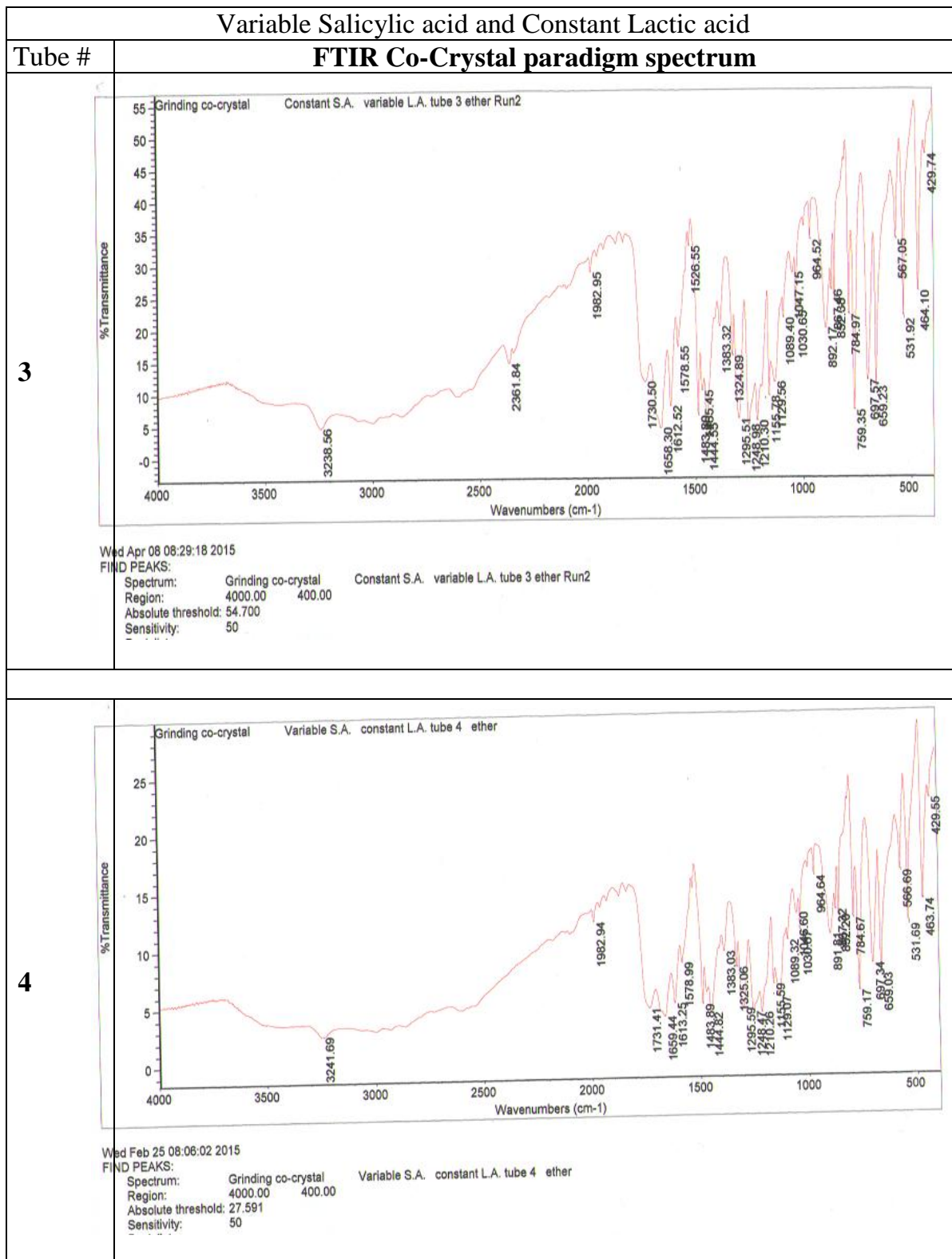
The following FTIR spectrums refers to all tested samples in this research either for reflux or grinding techniques each are ordered related to the co-solvent used;

Appendix 1 :

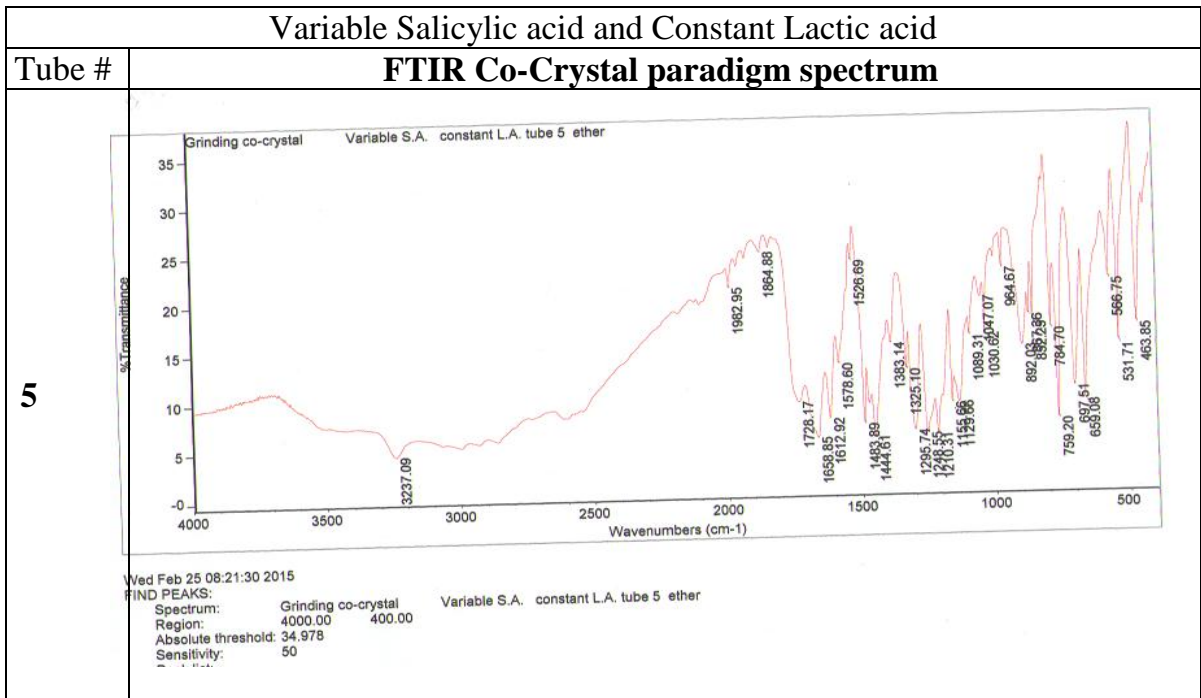
FTIR spectrums for grinding technique in diethyl ether co-solvent



FTIR spectra for grinding technique in diethyl ether co-solvent

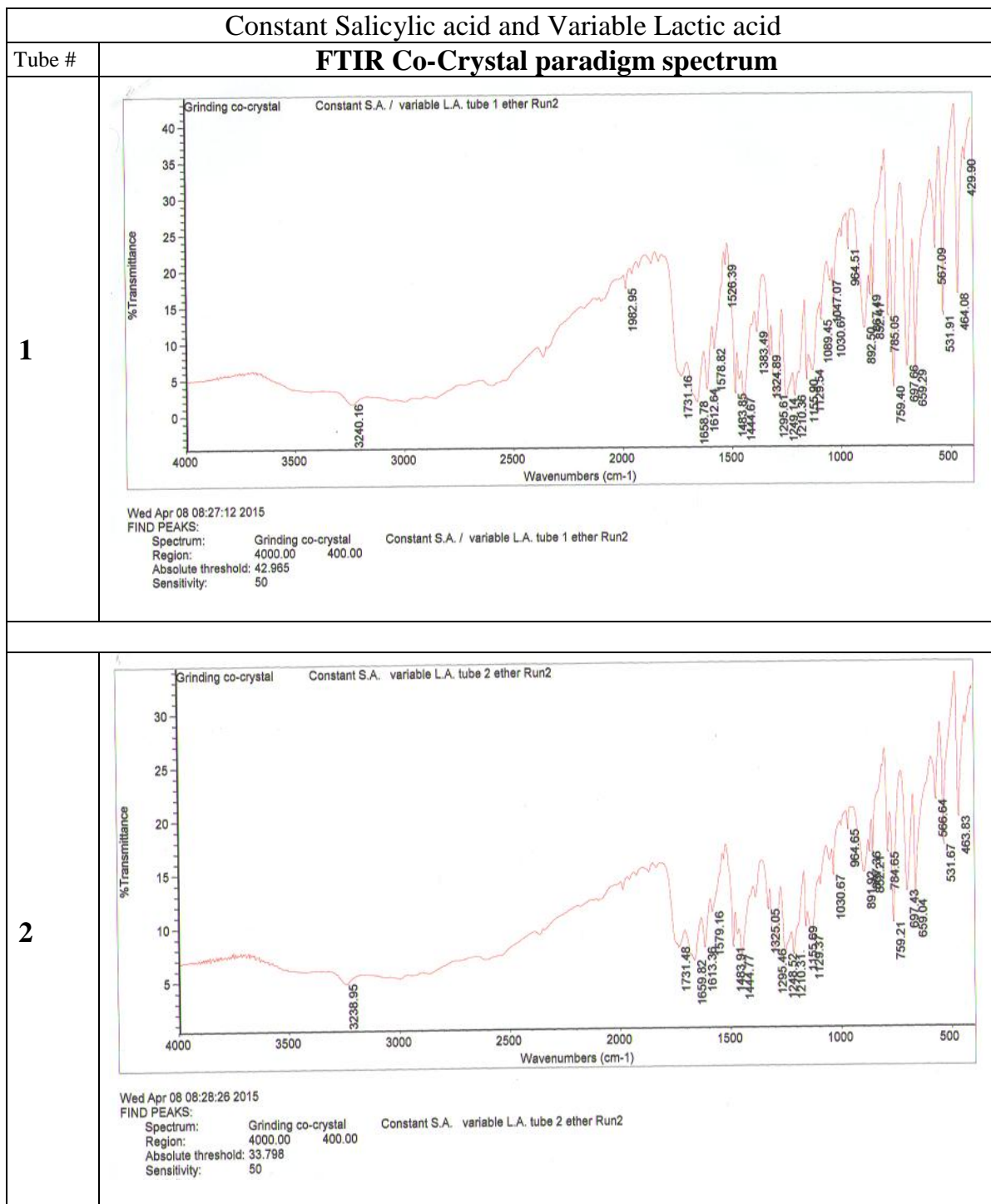


FTIR spectrums for grinding technique in diethyl ether co-solvent



Appendix 2 :

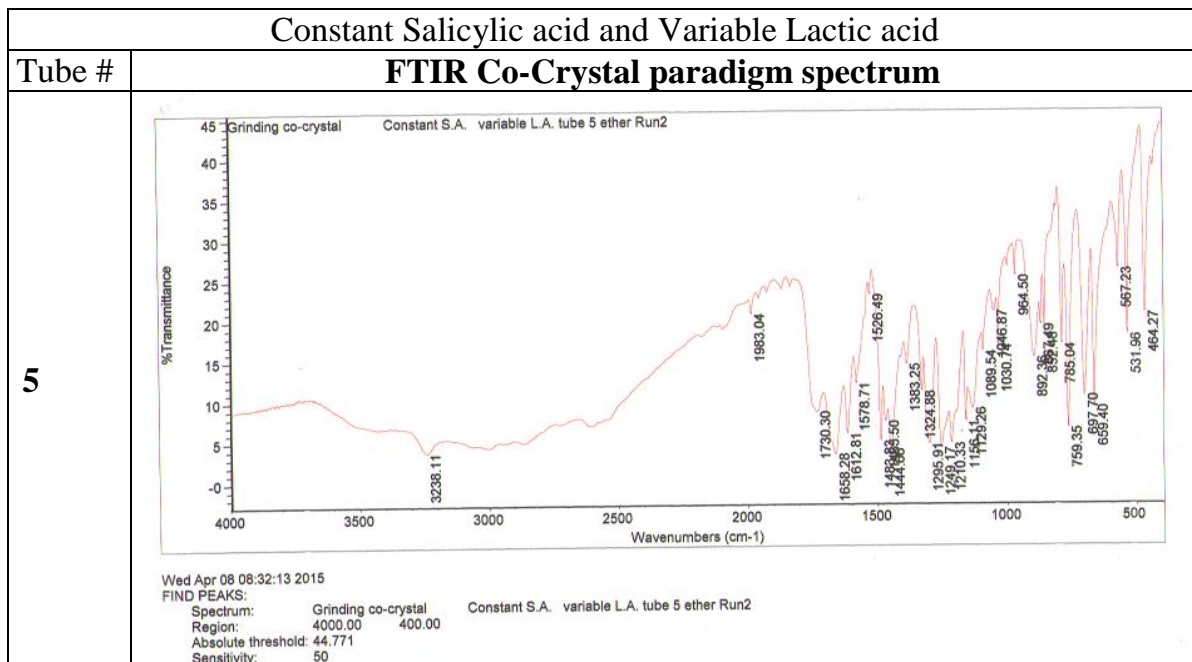
FTIR spectrums for grinding technique in diethyl ether co-solvent



FTIR spectrums for grinding technique in diethyl ether co-solvent

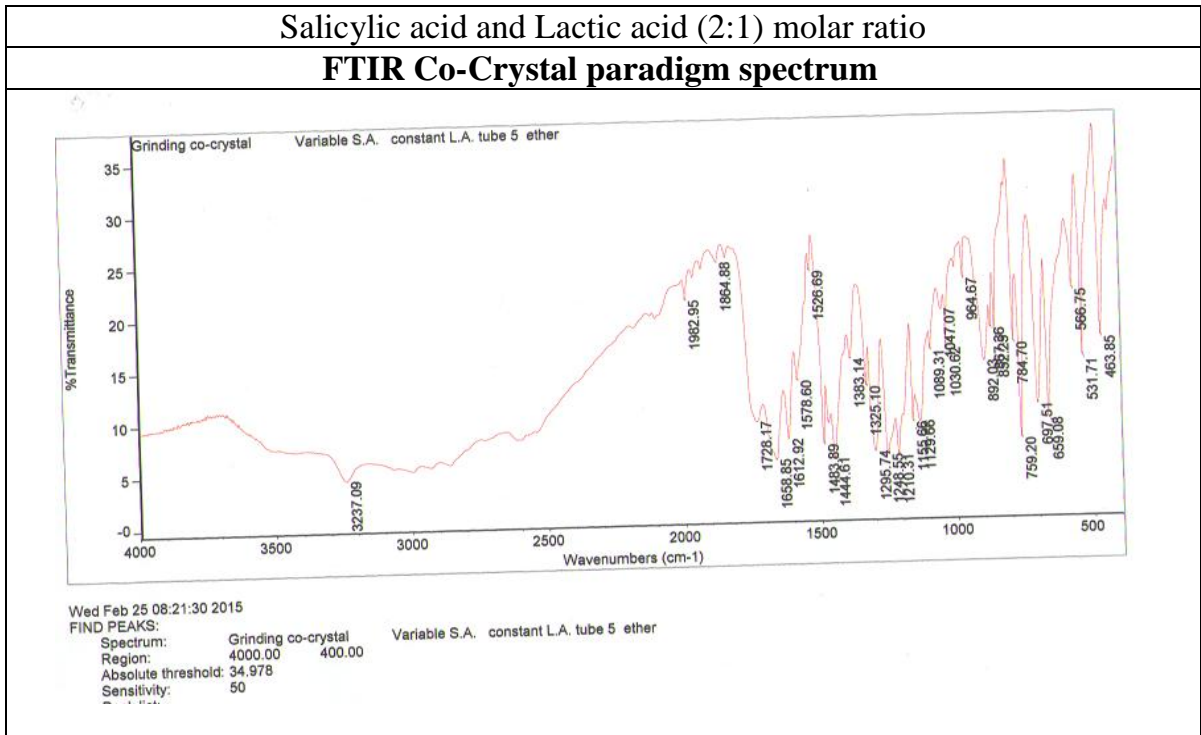
Constant Salicylic acid and Variable Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
3	<p>Grinding co-crystal Variable S.A. constant L.A. tube 3 ether</p> <p>Wed Feb 25 07:57:22 2015 FIND PEAKS: Spectrum: Grinding co-crystal Variable S.A. constant L.A. tube 3 ether Region: 4000.00 400.00 Absolute threshold: 62.493 Sensitivity: 50</p>
4	<p>Grinding co-crystal Constant S.A. variable L.A. tube 4 ether Run2</p> <p>Wed Apr 08 08:31:08 2015 FIND PEAKS: Spectrum: Grinding co-crystal Constant S.A. variable L.A. tube 4 ether Run2 Region: 4000.00 400.00 Absolute threshold: 56.857 Sensitivity: 54</p>

FTIR spectrums for grinding technique in diethyl ether co-solvent



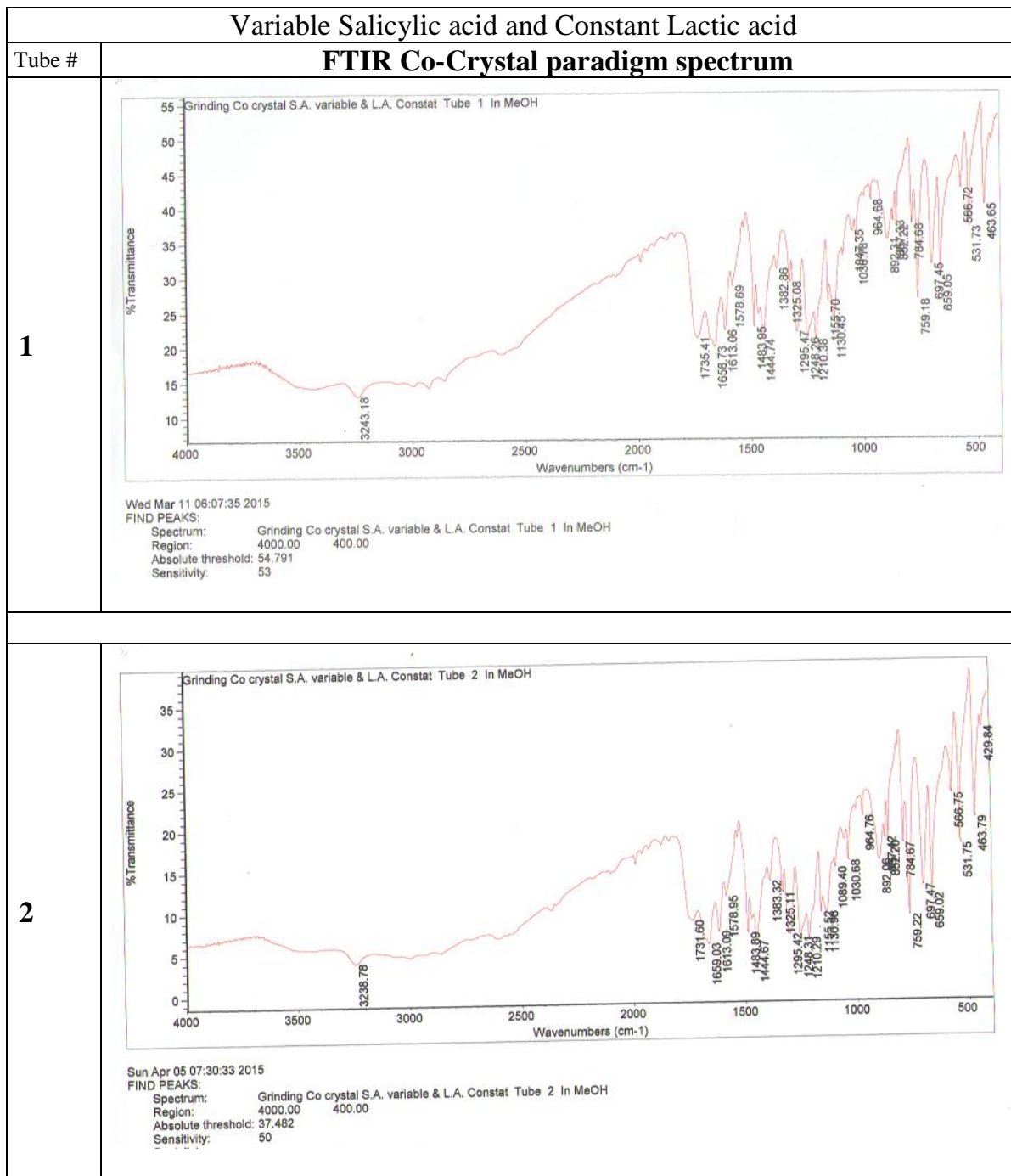
Appendix 3 :

FTIR spectrums for Reflux technique in diethyl ether co-solvent

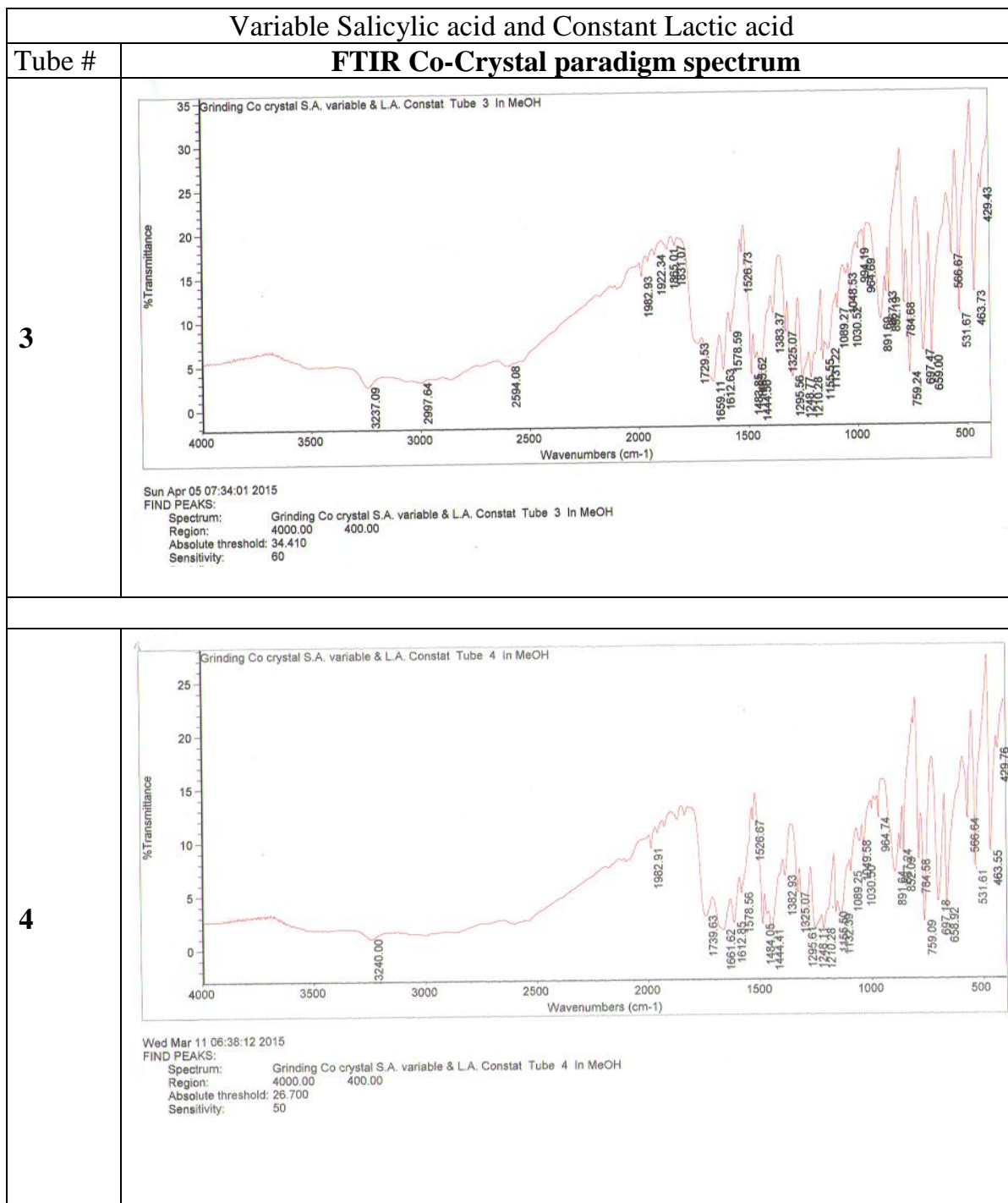


Appendix 4:

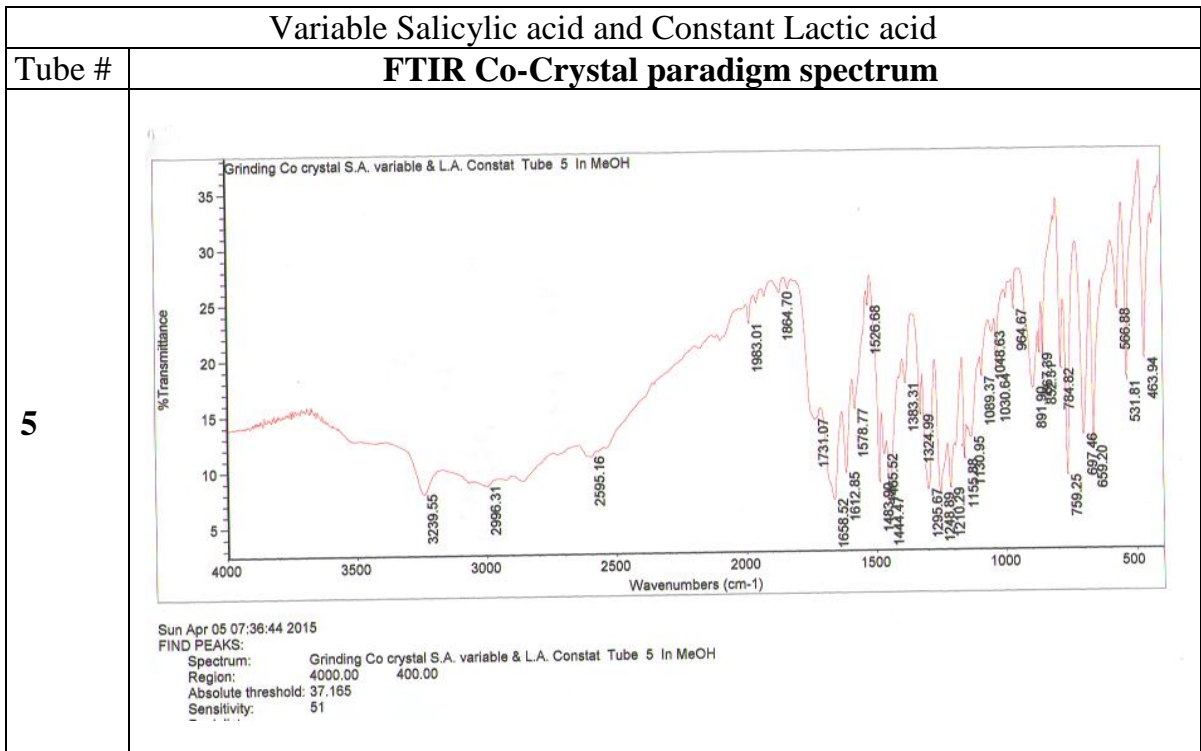
FTIR spectrums for grinding technique in Methanol co-solvent



FTIR spectrums for grinding technique in Methanol co-solvent

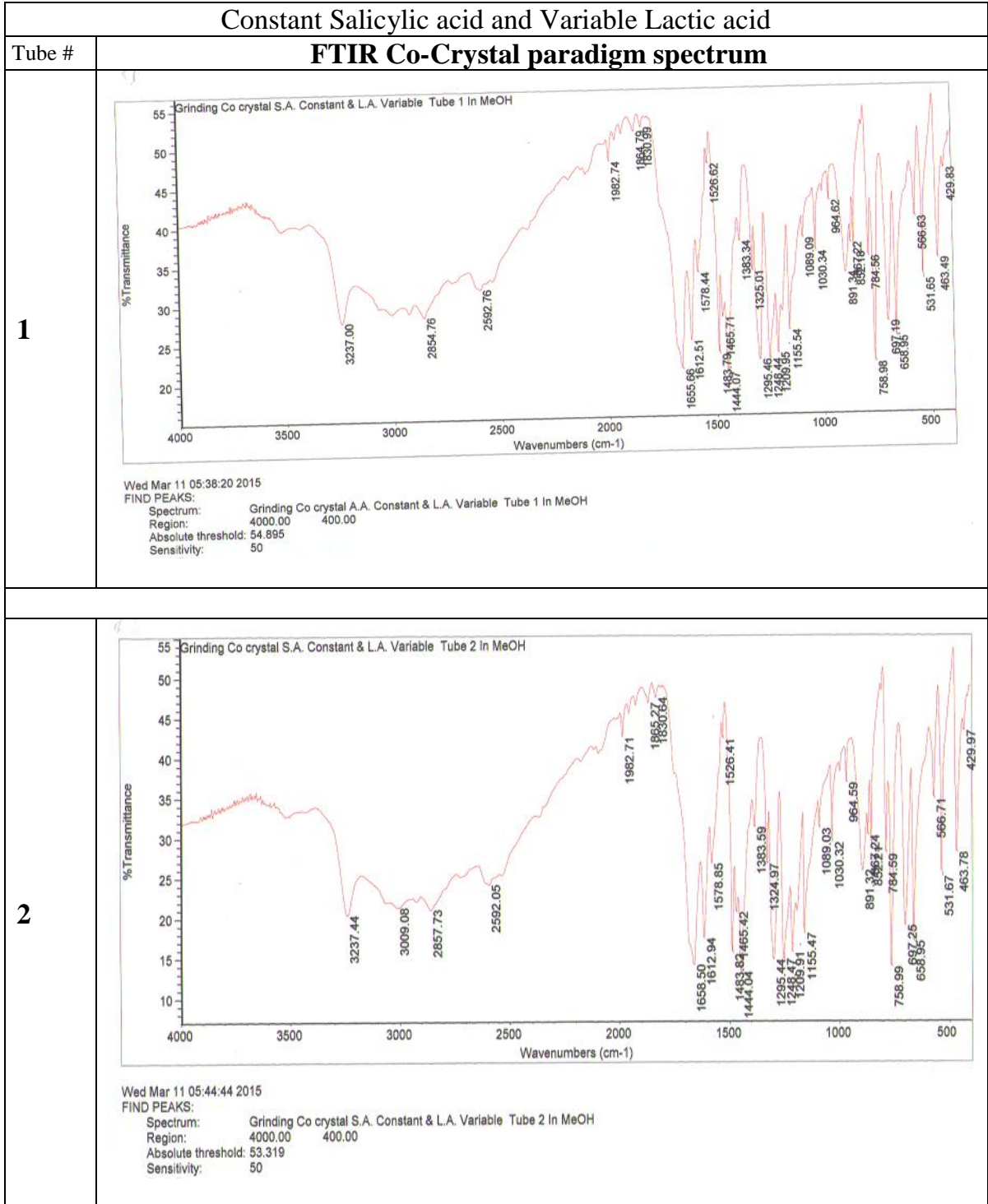


FTIR spectrums for grinding technique in Methanol co-solvent



Appendix 5:

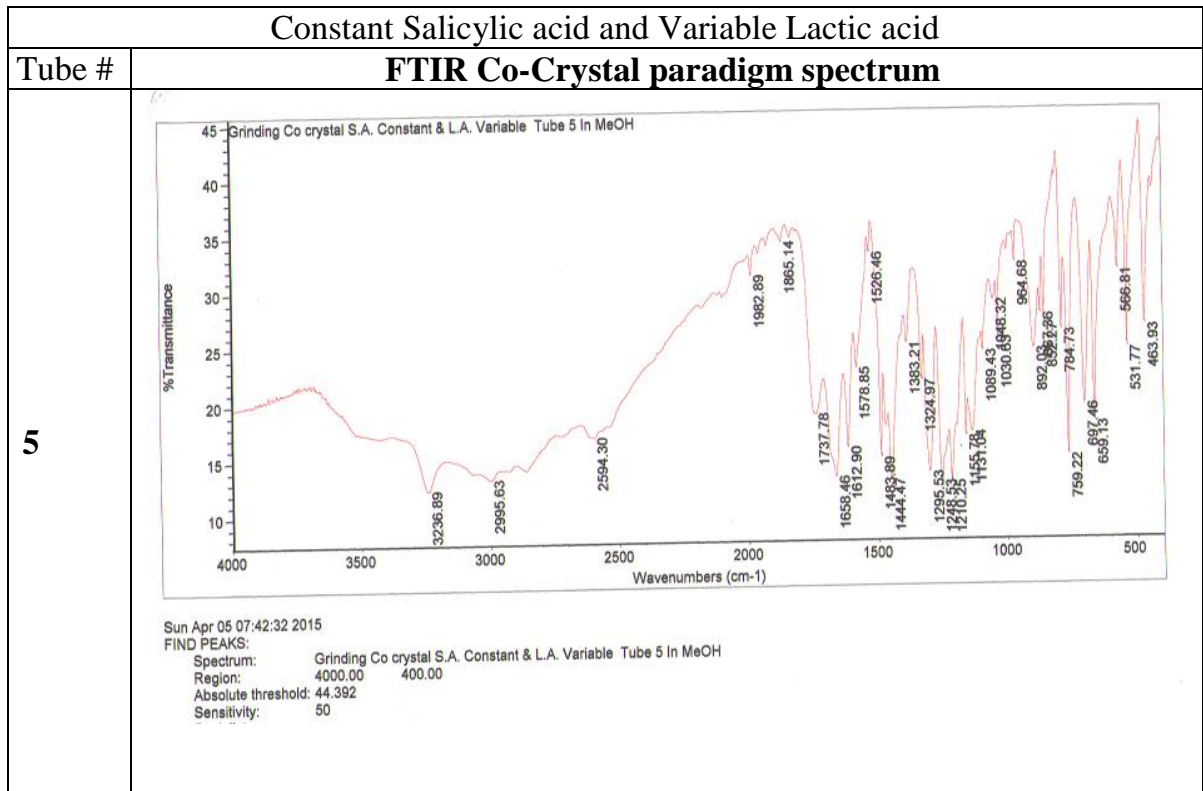
FTIR spectrums for grinding technique in Methanol co-solvent



FTIR spectrums for grinding technique in Methanol co-solvent

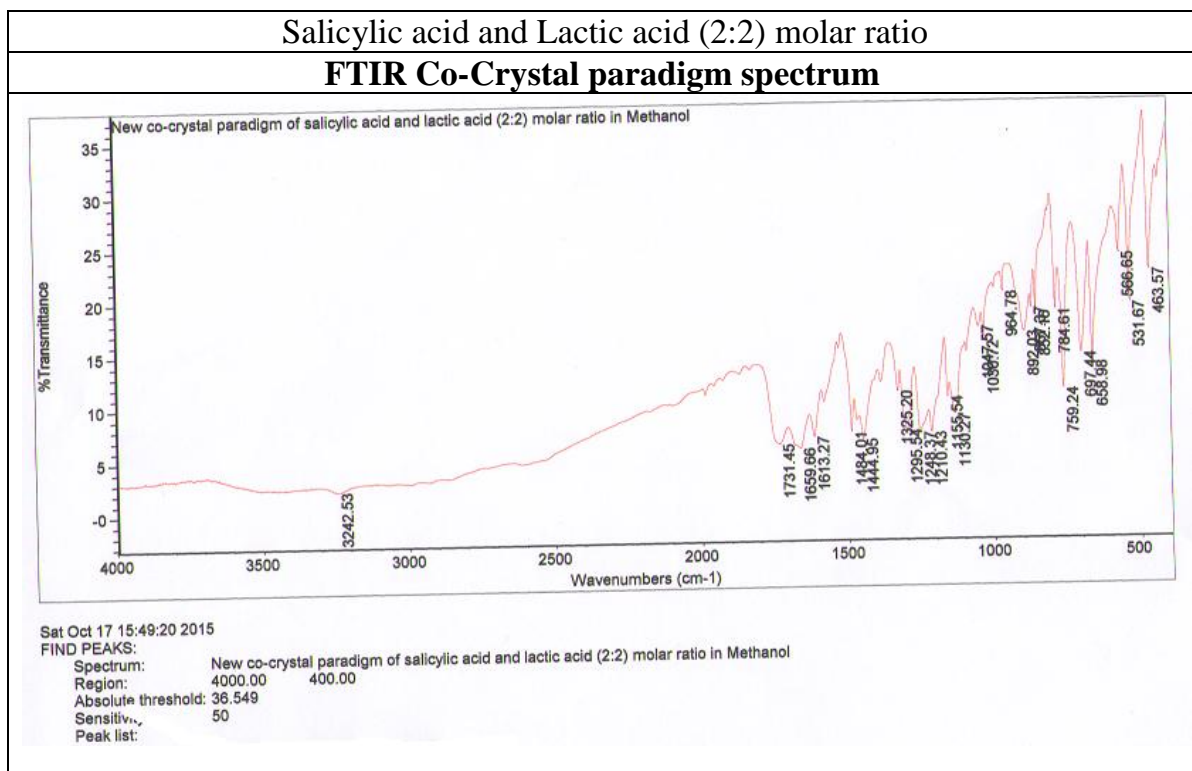
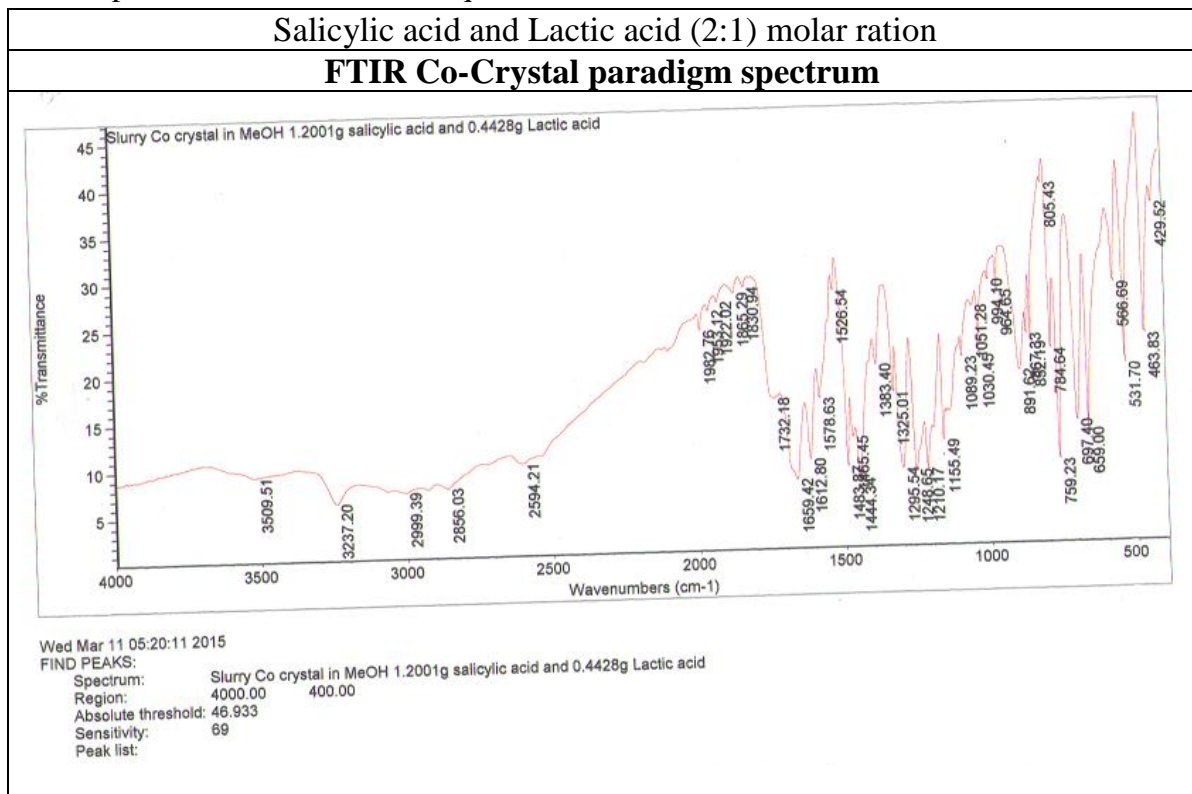
Constant Salicylic acid and Variable Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
3	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 3 In MeOH Run2 68% sensitivity</p> <p>Sun Apr 05 07:41:12 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Constant & L.A. Variable Tube 3 In MeOH Run2 68% sensitivity Region: 4000.00 400.00 Absolute threshold: 31.840 Sensitivity: 50</p>
4	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 4 In MeOH</p> <p>Wed Mar 11 05:56:22 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Constant & L.A. Variable Tube 4 In MeOH Region: 4000.00 400.00 Absolute threshold: 52.727 Sensitivity: 53</p>

FTIR spectrums for grinding technique in Methanol co-solvent



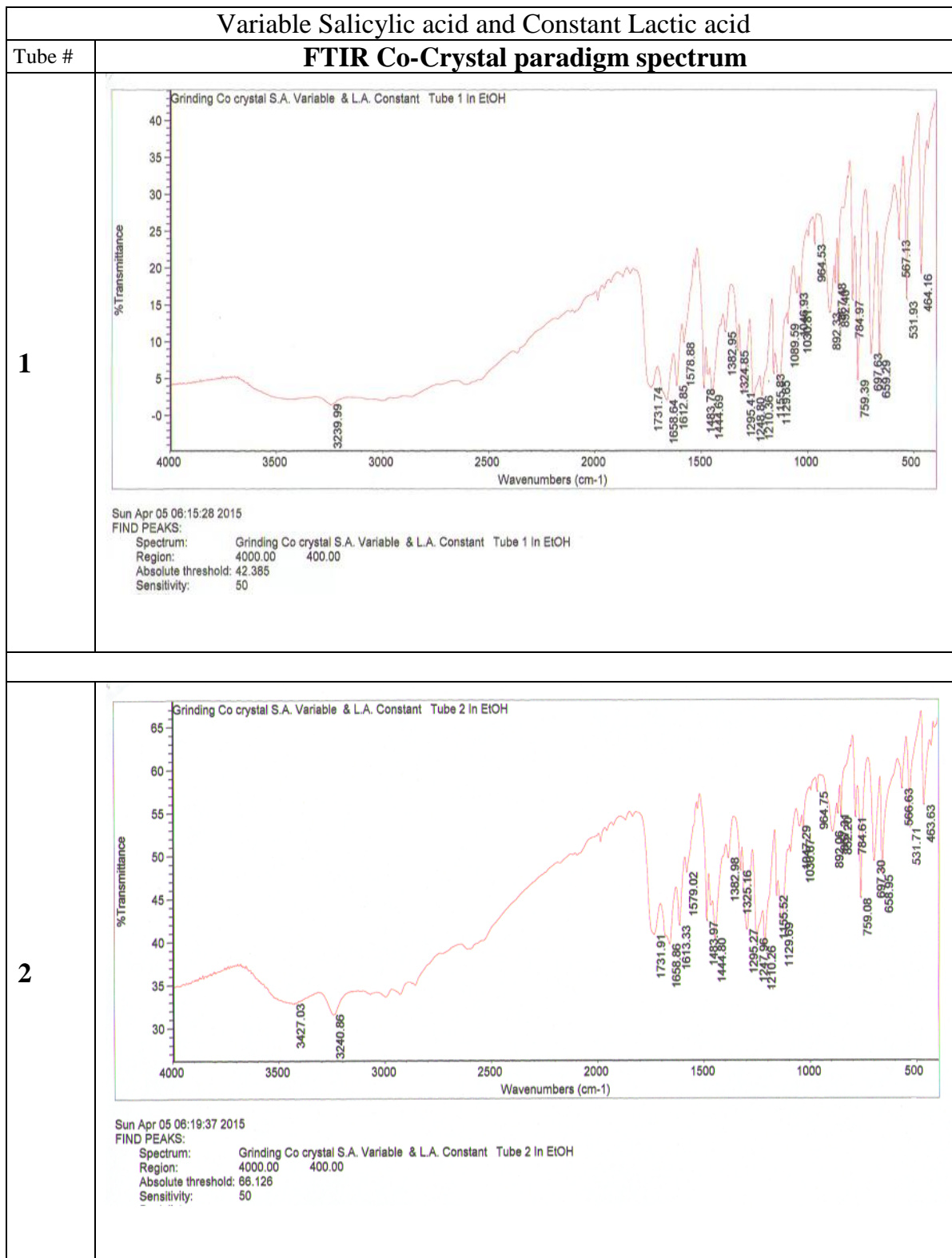
Appendix 6:

FTIR spectrums for Reflux technique in Methanol co-solvent



Appendix 7:

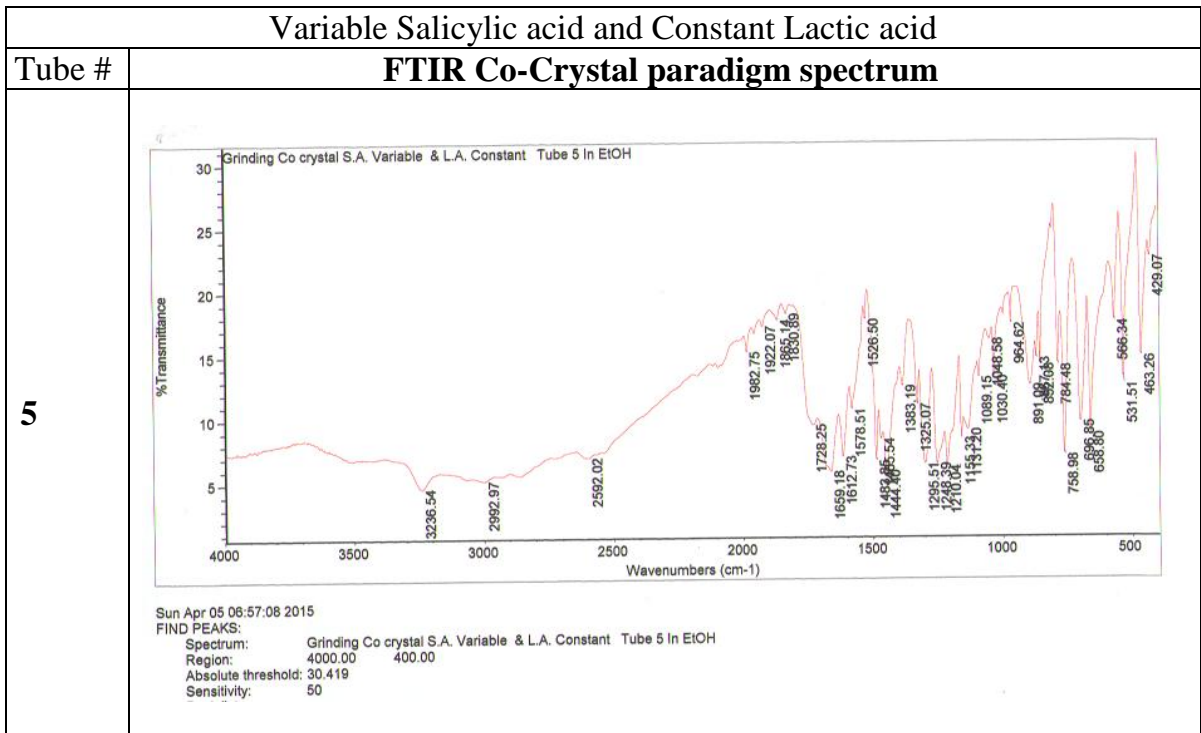
FTIR spectrums for grinding technique in Ethanol co-solvent



FTIR spectrums for grinding technique in Ethanol co-solvent

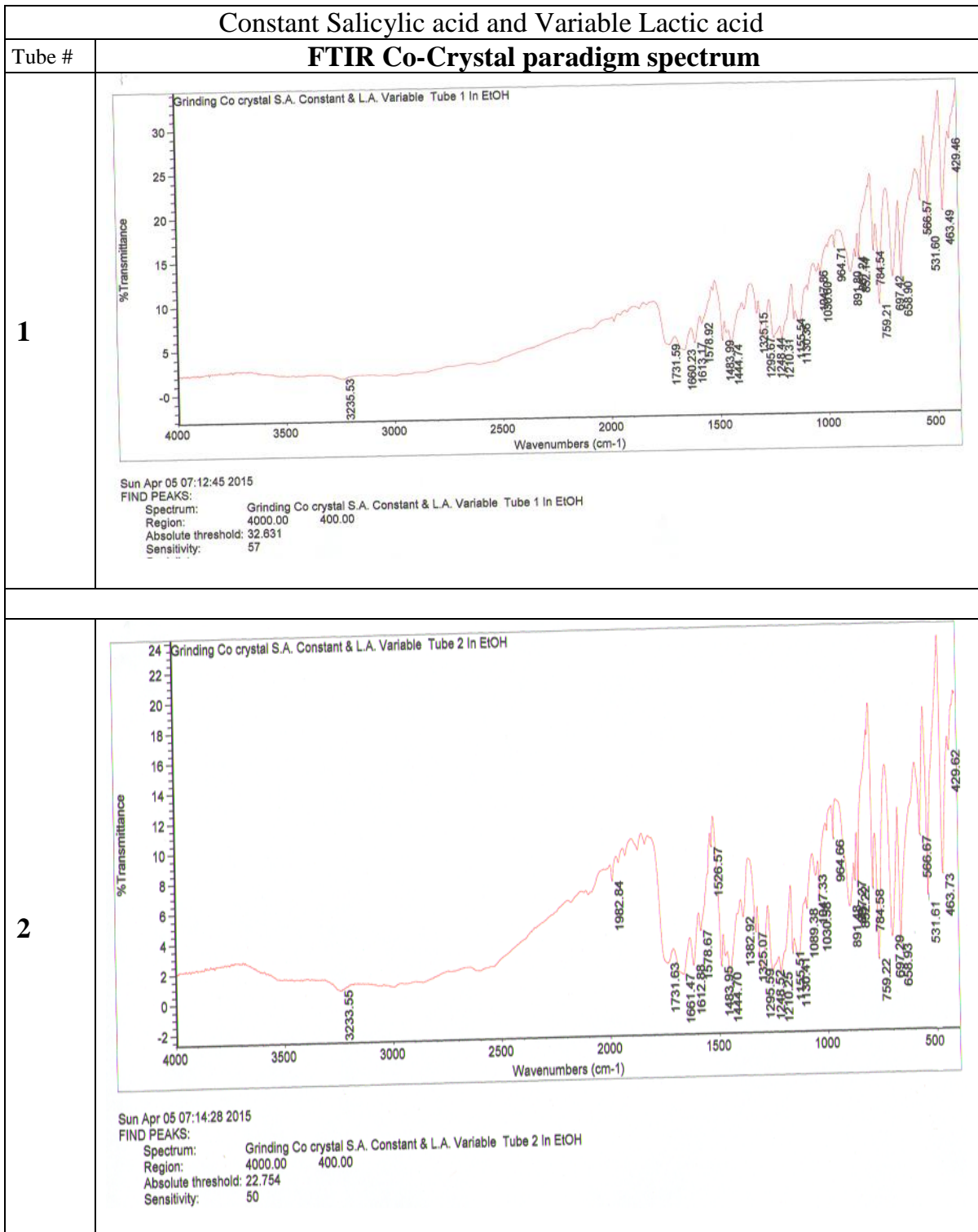
Variable Salicylic acid and Constant Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
3	<p>Grinding Co crystal S.A. Variable & L.A. Constant Tube 3 In EtOH</p> <p>Sun Apr 05 06:30:48 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Variable & L.A. Constant Tube 3 In EtOH Region: 4000.00 400.00 Absolute threshold: 63.795 Sensitivity: 50</p>
4	<p>Grinding Co crystal S.A. Variable & L.A. Constant Tube 4 In EtOH</p> <p>Sun Apr 05 07:00:03 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Variable & L.A. Constant Tube 4 In EtOH Region: 4000.00 400.00 Absolute threshold: 52.988 Sensitivity: 50</p>

FTIR spectrums for grinding technique in Ethanol co-solvent



Appendix 8:

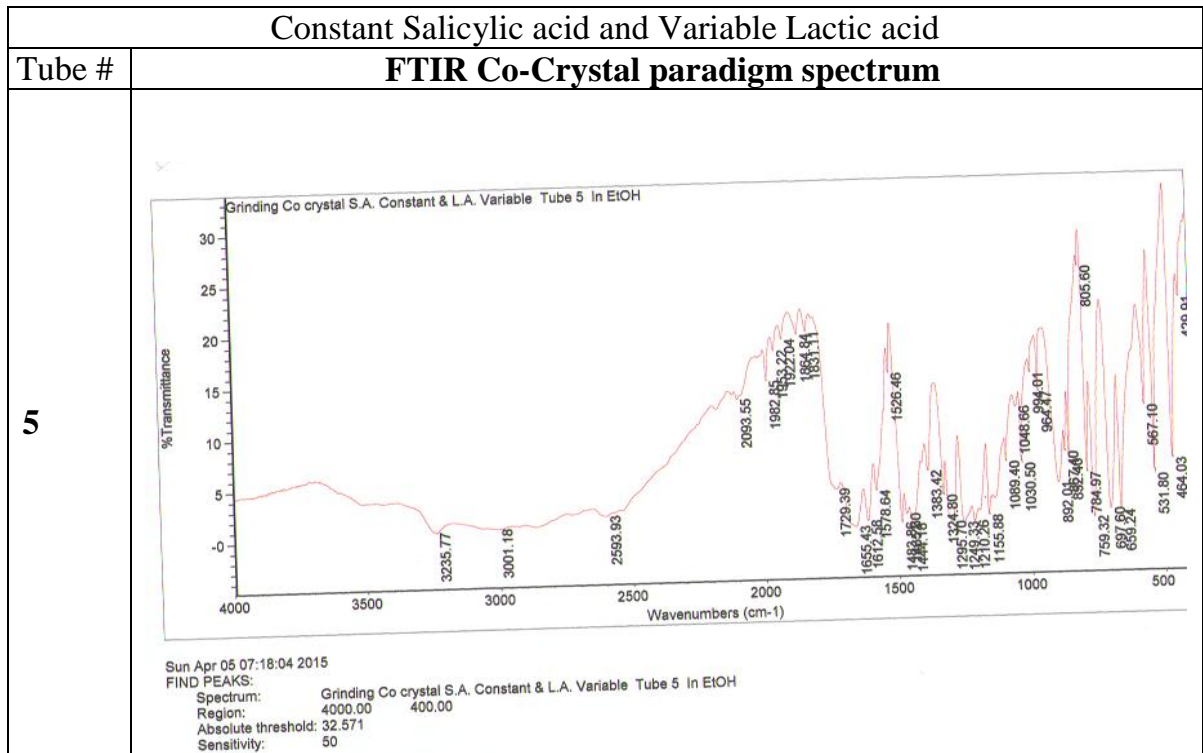
FTIR spectrums for grinding technique in Ethanol co-solvent



FTIR spectrums for grinding technique in Ethanol co-solvent

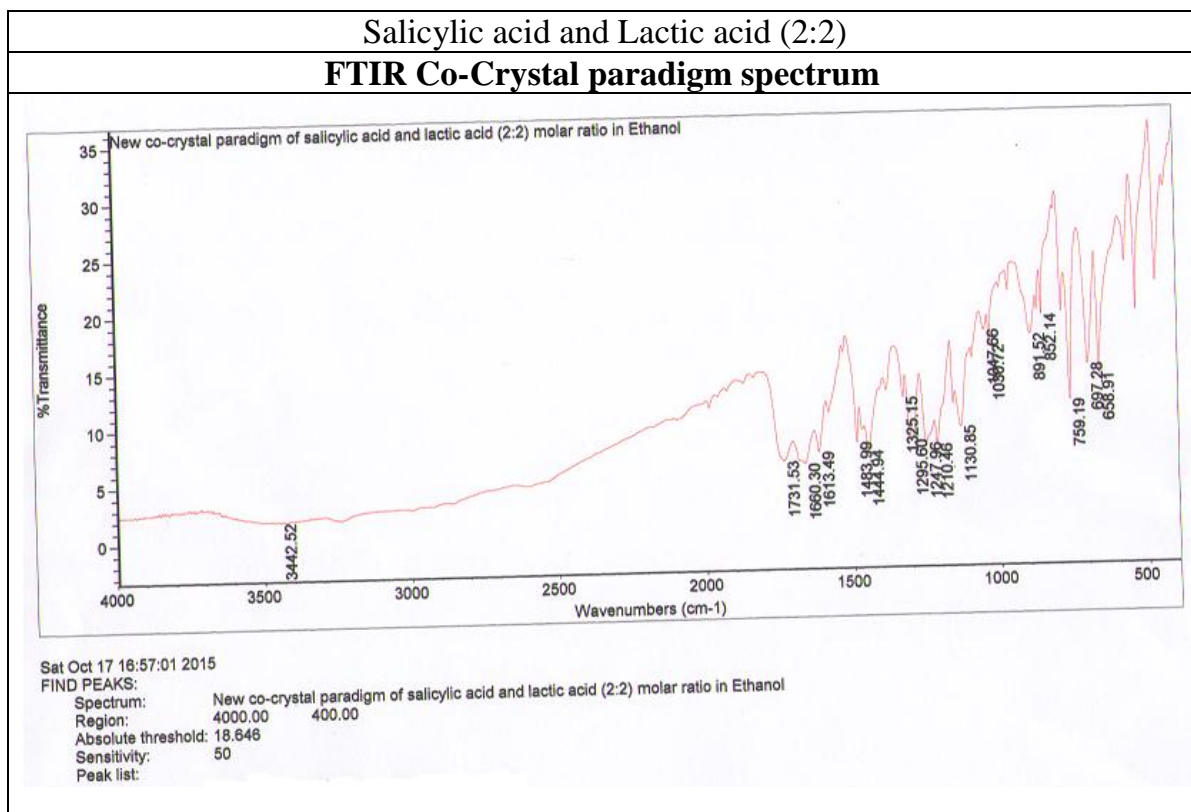
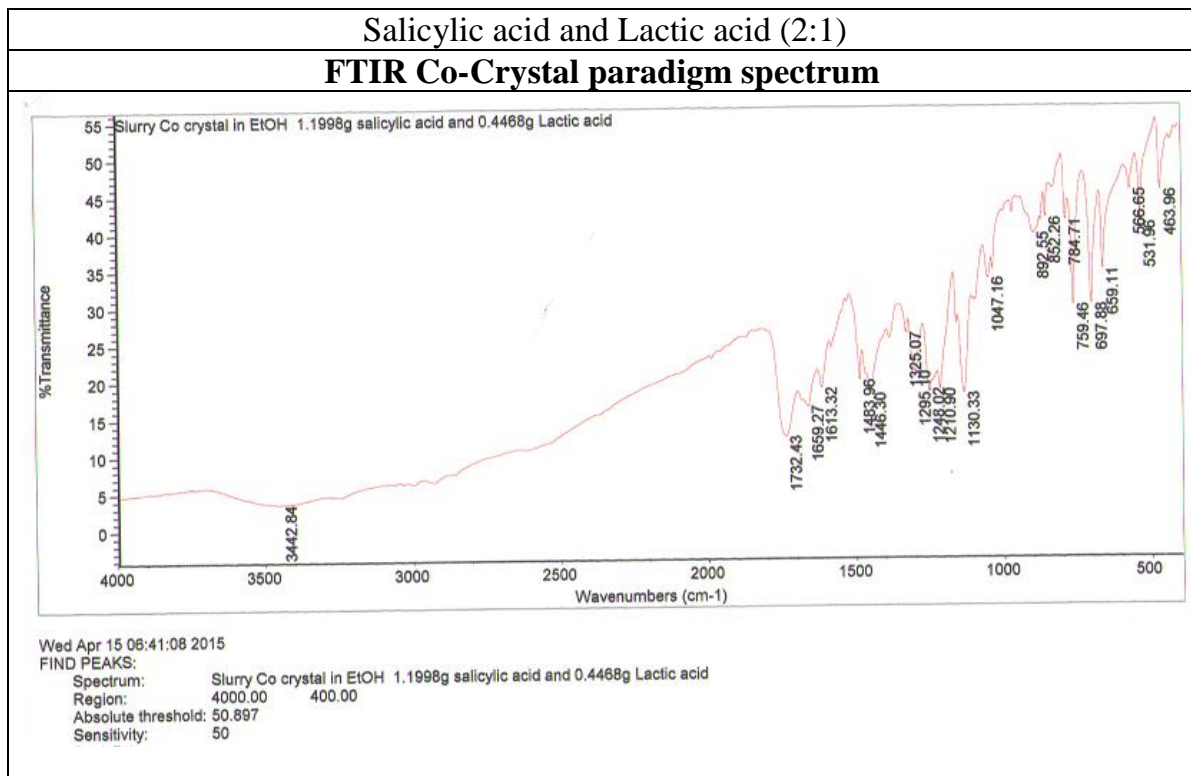
Constant Salicylic acid and Variable Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
3	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 3 In EtOH</p> <p>Sun Apr 05 07:16:08 2015 FIND PEAKS: Grinding Co crystal S.A. Constant & L.A. Variable Tube 3 In EtOH Spectrum: 4000.00 400.00 Region: 4000.00 400.00 Absolute threshold: 48.569 Sensitivity: 50</p>
4	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 4 In EtOH</p> <p>Sun Apr 05 07:16:58 2015 FIND PEAKS: Grinding Co crystal S.A. Constant & L.A. Variable Tube 4 In EtOH Spectrum: 4000.00 400.00 Region: 4000.00 400.00 Absolute threshold: 33.693 Sensitivity: 50</p>

FTIR spectrums for grinding technique in Ethanol co-solvent



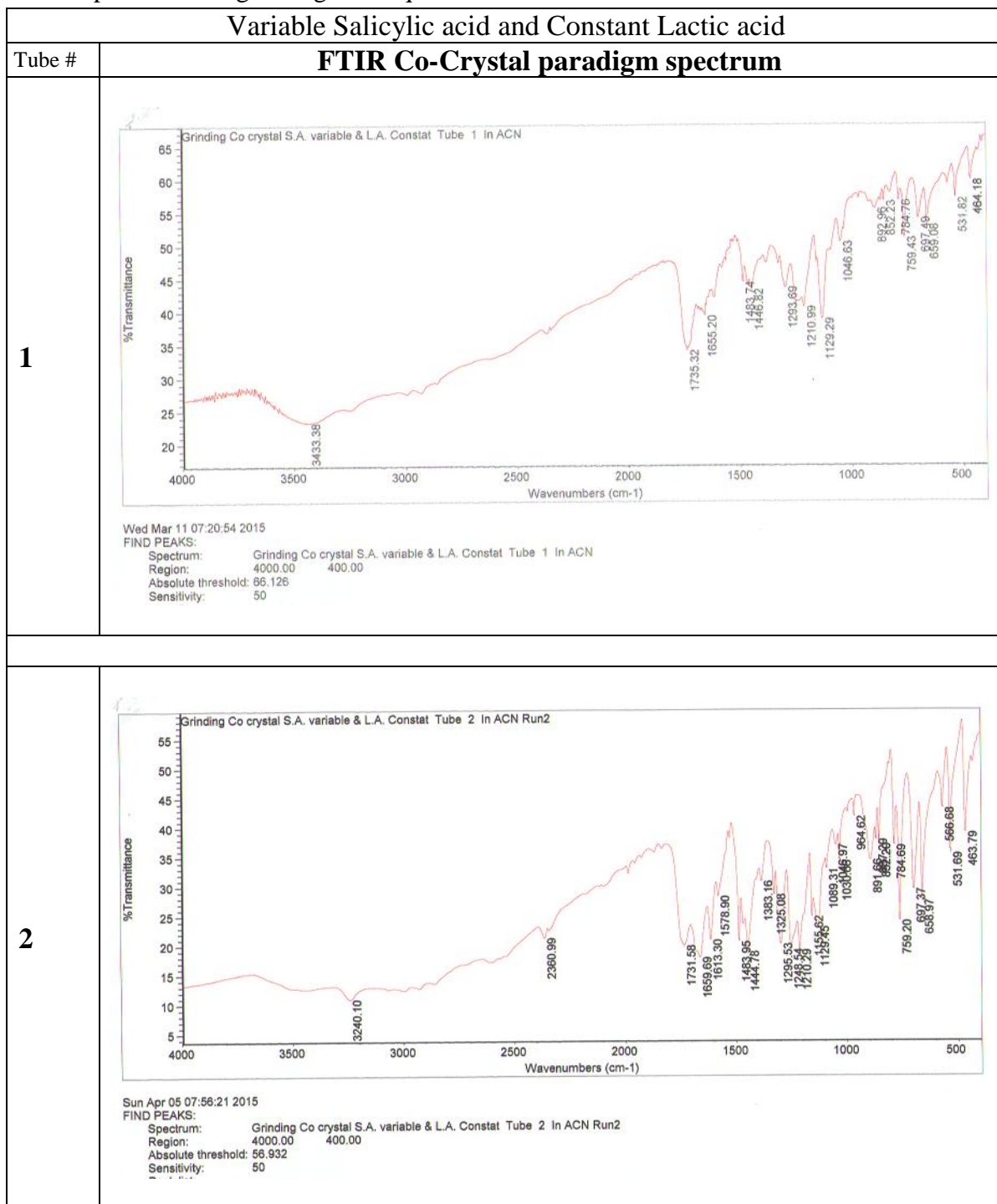
Appendix 9:

FTIR spectrums for Reflux technique in Ethanol co-solvent



Appendix 10:

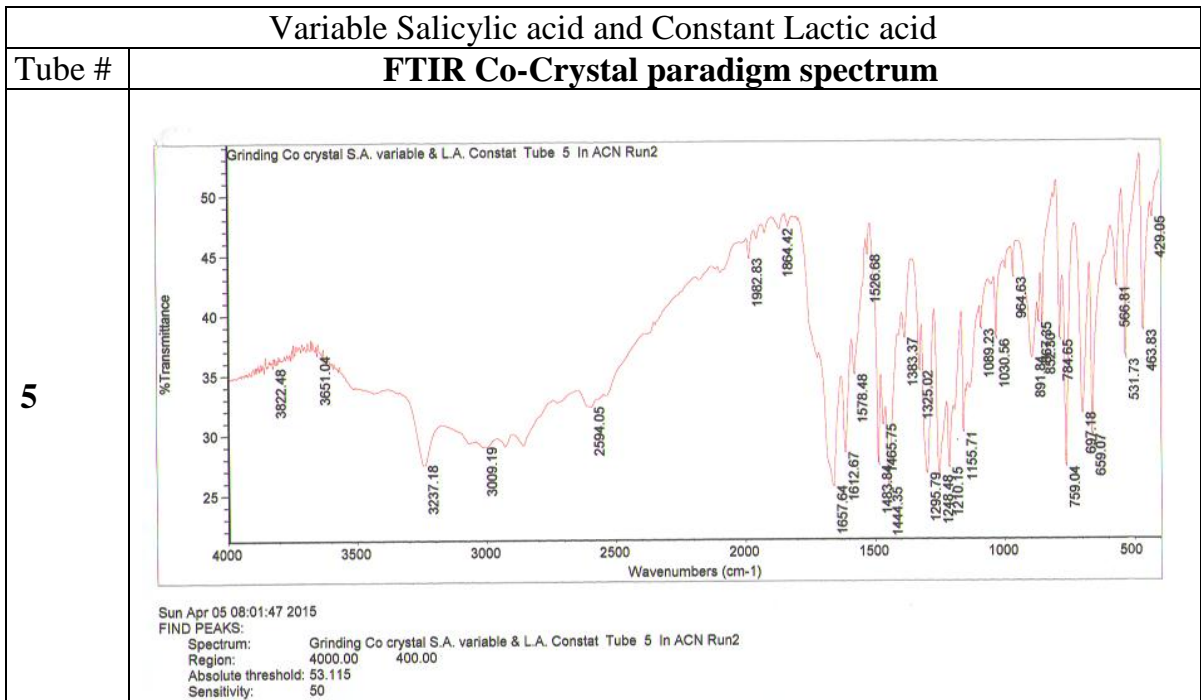
FTIR spectrums for grinding technique in Acetonitrile co-solvent



FTIR spectrums for grinding technique in Acetonitrile co-solvent

Variable Salicylic acid and Constant Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
3	<p>Grinding Co crystal S.A. variable & L.A. Constat Tube 3 In ACN Run2</p> <p>Sun Apr 05 07:57:14 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. variable & L.A. Constat Tube 3 In ACN Run2 Region: 4000.00 400.00 Absolute threshold: 72.982 Sensitivity: 50</p>
4	<p>Grinding Co crystal S.A. variable & L.A. Constat Tube 4 In ACN Run2</p> <p>Sun Apr 05 08:00:27 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. variable & L.A. Constat Tube 4 In ACN Run2 Region: 4000.00 400.00 Absolute threshold: 73.091 Sensitivity: 50</p>

FTIR spectrums for grinding technique in Acetonitrile co-solvent



Appendix 11:

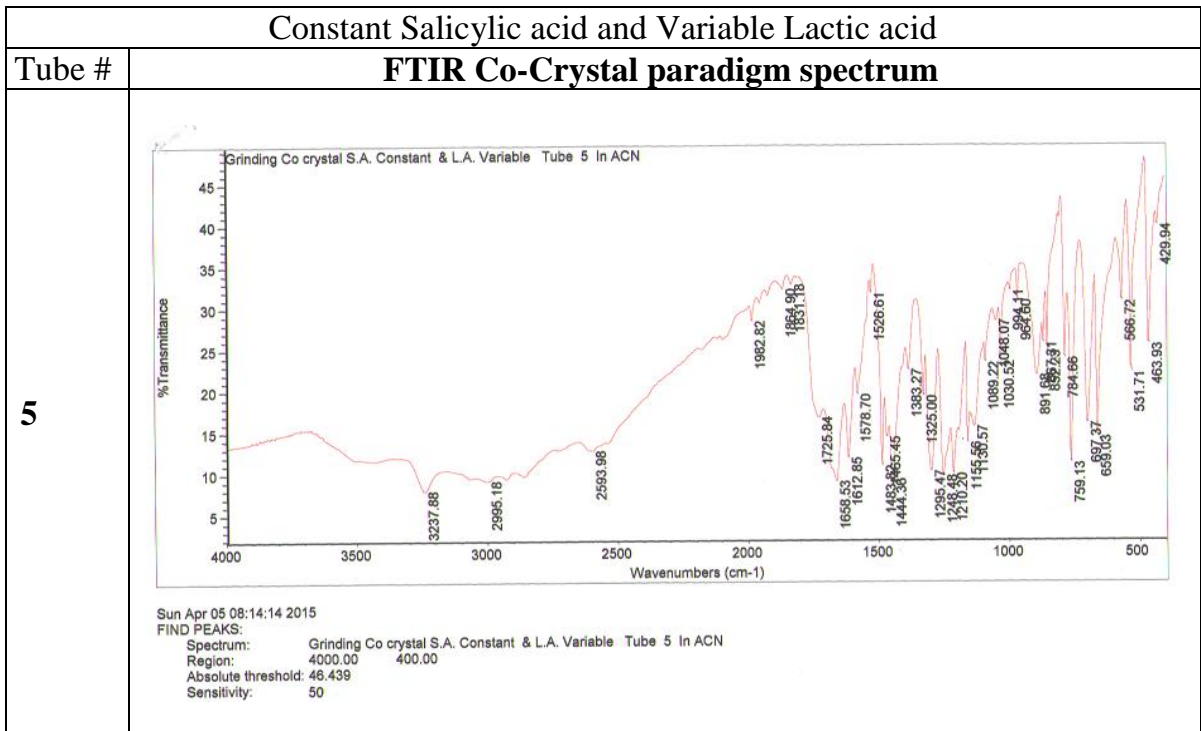
FTIR spectrums for grinding technique in Acetonitrile co-solvent

Constant Salicylic acid and Variable Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
1	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 1 In ACN Run2</p> <p>Wavenumbers (cm-1): 4000, 3500, 3000, 2500, 2000, 1500, 1000, 500</p> <p>Peak List (cm-1): 3237.84, 2857.38, 2593.33, 1962.84, 1921.62, 1850.96, 1656.27, 1612.76, 1578.72, 1526.42, 1483.86, 1444.06, 1383.57, 1295.62, 1324.93, 1248.74, 1210.01, 1155.70, 1089.18, 1030.37, 964.58, 891.88, 853.23, 784.73, 759.05, 697.40, 658.11, 531.75, 566.95, 429.74</p> <p>Sun Apr 05 08:10:18 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Constant & L.A. Variable Tube 1 In ACN Run2 Region: 4000.00 400.00 Absolute threshold: 44.228 Sensitivity: 50</p>
2	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 2 In ACN Run2</p> <p>Wavenumbers (cm-1): 4000, 3500, 3000, 2500, 2000, 1500, 1000, 500</p> <p>Peak List (cm-1): 3238.87, 2998.57, 2592.79, 1982.84, 1851.06, 1657.76, 1612.73, 1578.85, 1526.46, 1483.78, 1444.2, 1383.60, 1295.50, 1324.86, 1248.75, 1210.01, 1155.81, 1089.30, 1030.49, 964.57, 891.88, 853.27, 784.79, 759.11, 697.44, 658.19, 531.81, 566.88, 430.10</p> <p>Sun Apr 05 08:11:24 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Constant & L.A. Variable Tube 2 In ACN Run2 Region: 4000.00 400.00 Absolute threshold: 46.390 Sensitivity: 50</p>

FTIR spectrums for grinding technique in Acetonitrile co-solvent

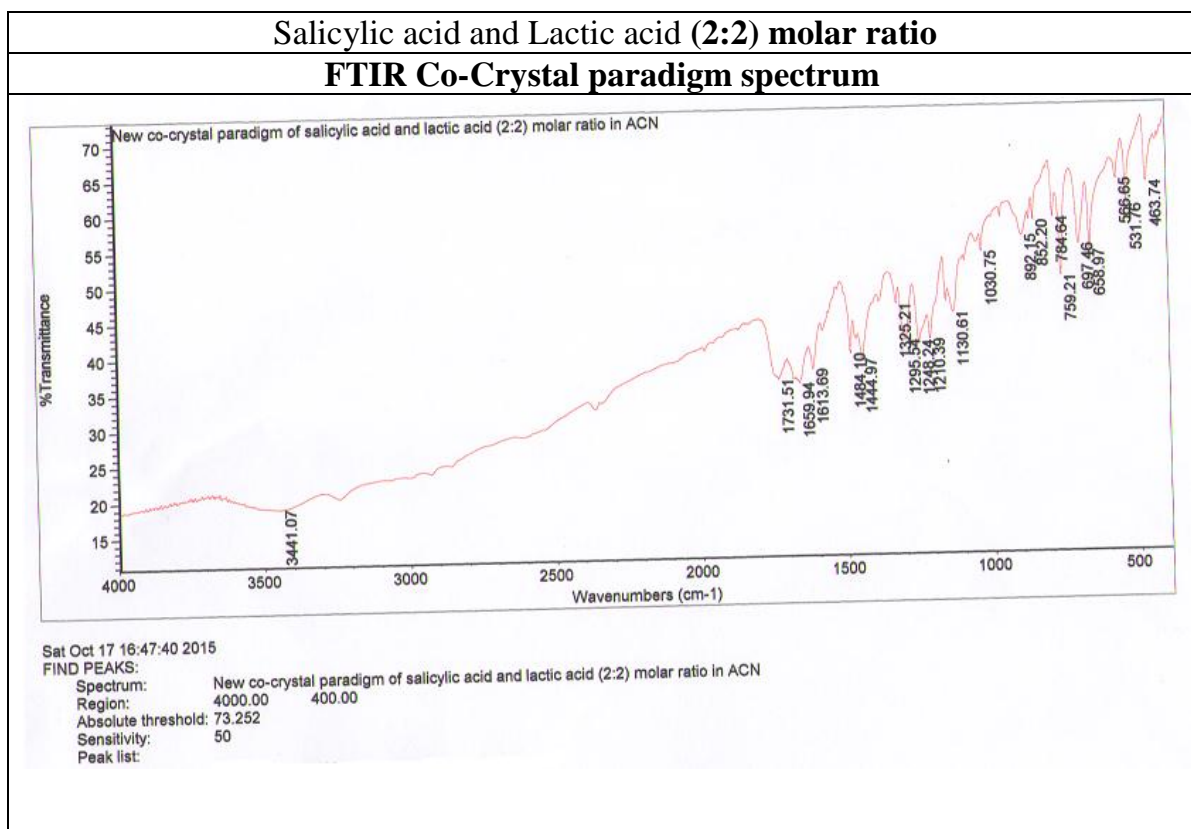
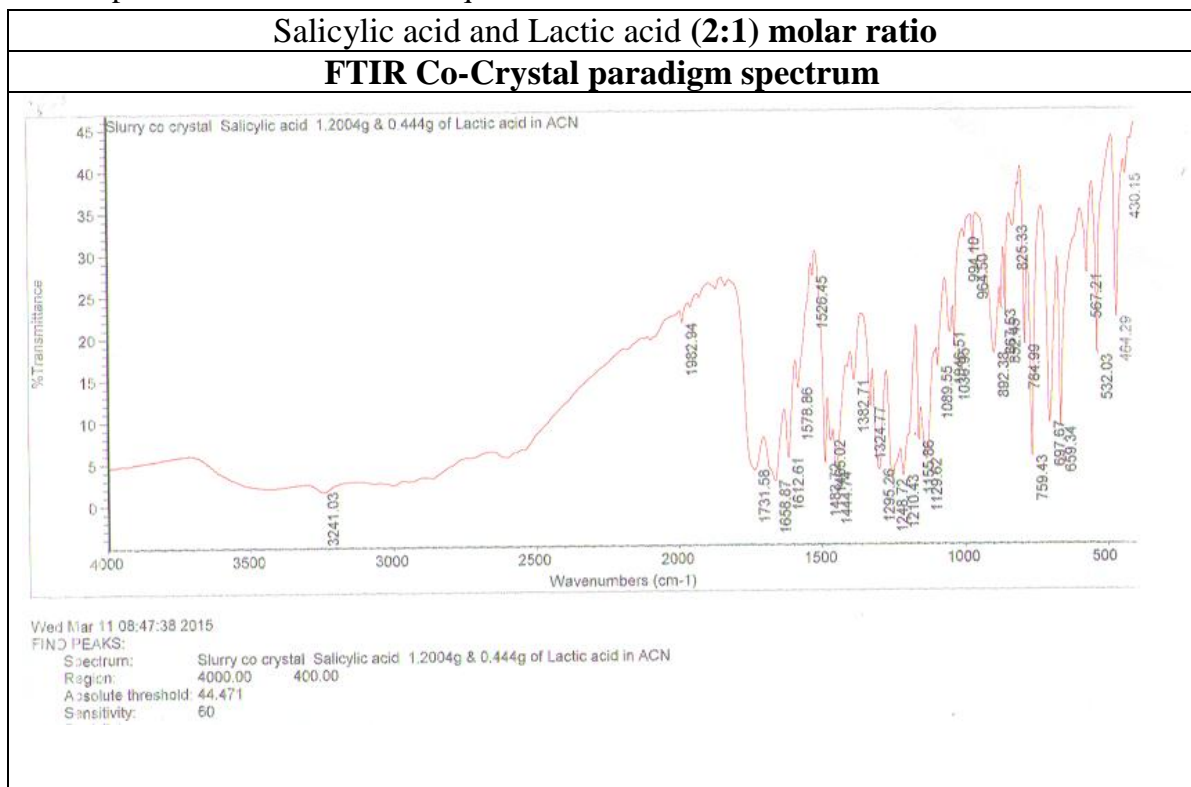
Constant Salicylic acid and Variable Lactic acid	
Tube #	FTIR Co-Crystal paradigm spectrum
3	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 3 In ACN Run2</p> <p>Peak list for Tube 3:</p> <ul style="list-style-type: none"> 3850.56 3741.78 3686.06 3243.34 1731.54 1659.97 1613.67 1484.05 1445.45 1295.37 1275.10 1217.91 1210.38 1128.66 1086.96 964.64 892.21 852.18 784.90 758.96 696.93 658.94 531.57 566.70 463.45 <p>Sun Apr 05 08:12:00 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Constant & L.A. Variable Tube 3 In ACN Run2 Region: 4000.00 400.00 Absolute threshold: 48.980 Sensitivity: 50</p>
4	<p>Grinding Co crystal S.A. Constant & L.A. Variable Tube 4 In ACN Run2</p> <p>Peak list for Tube 4:</p> <ul style="list-style-type: none"> 3237.32 1731.62 1659.97 1613.31 1483.94 1445.44 1295.23 1225.12 1210.43 1128.41 1086.84 964.65 892.17 852.19 784.64 759.21 697.40 658.99 531.63 566.67 463.67 <p>Sun Apr 05 08:12:42 2015 FIND PEAKS: Spectrum: Grinding Co crystal S.A. Constant & L.A. Variable Tube 4 In ACN Run2 Region: 4000.00 400.00 Absolute threshold: 37.310 Sensitivity: 50</p>

FTIR spectrums for grinding technique in Acetonitrile co-solvent



Appendix 12:

FTIR spectrums for Reflux technique in Acetonitrile co-solvent





Co-crystal sample melting Range is 127.6-127.7°C



Co-crystal sample melting Range is 138.3-139.4°C

Figure 31 Co-crystal sample melting point ranges



Co-crystal sample melting Range is 141.5-147.8°C

Co-crystal sample melting Range is 145.2-149.1°C

Figure 32 Co-crystal sample melting point ranges