

Phase Transitions of Ipidacrine Hydrochloride Polymorphs and Hydrates

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Abstract: Ipidacrine hydrochloride is pharmaceutically active compound that forms variety of crystalline modifications which differs by solvent type and content in crystalline structure. These crystalline forms analyzed by powder x-ray diffraction, differential thermal analysis, thermogravimetry and Karl Fisher titration.

Obtained crystalline forms include two monohydrates, two hemihydrates, two anhydrous forms, two non-stoichiometric hydrates and mixed solvates with octanol and water and methanol and water, solvate with chloroform and dimethylformamide.

Keywords: Ipidacrine, differential thermal analysis, powder X-ray diffraction, phase transitions.

INTRODUCTION

Ipidacrine hydrochloride (2,3,5,6,7,8-Hexahydro-1H-cyclopenta[b]quinolin-9-amine hydrochloride; amiridin) is pharmaceutically active compound which is a nootropic agent that acts as cholinesterase inhibitor and is used in treatment of Alzheimer disease. Usually it is prepared as hydrochloride monohydrate which is a white or slightly creamy powder, soluble in water and acid solutions, but practically insoluble in acetone, ether or chloroform [1].

Previous research [2] has stated that ipidacrine hydrochloride monohydrate exists in two polymorphic forms, abbreviated as monohydrate A and monohydrate B, where B is metastable form. Both monohydrates differ in dehydration temperatures: dehydration of monohydrate B starts at 80°C, while A – at 90°C.

The main objective of our research was chosen to further polymorph screening to discover formation of other possible solvates with water and organic solvents. These solvates could be taken into account in production of ipidacrine hydrochloride monohydrate as possible side products or may have potential for use in pharmacy. Results of this scientific research might be useful for pharmaceutical companies for ipidacrine hydrochloride quality control.

MATERIALS AND METHODS

Materials

Ipidacrine hydrochloride monohydrate was supplied by JSC Grindeks (Riga, Latvia). It was identified by gas-liquid chromatography and powder X-ray diffraction (PXRD; the observed pattern was compared with pattern in previous research [2]). PXRD pattern of ipidacrine used in this research completely matches the diffraction pattern of ipidacrine hydrochloride monohydrate A form from previous research [2]. It has characteristic diffraction peaks at 10.94°;

11.43°; 17.29°; 18.20°; 18.92°; 19.54°; 20.55°; 23.32°; 24.13°; 24.91°; 26.67° in 2 θ scale. Organic solvents were purchased from Acros Organics (basestock content above 98%). Water was deionised in laboratory (electrical conductivity below 0.01 $\mu\text{S} \cdot \text{cm}^{-1}$).

Exposure of Ipidacrine Samples to Different Moisture and Temperature Conditions

Ipidacrine samples were placed in plastic PXRD sample holders (sample thickness approx. 0.5 mm; surface area approx. 3 cm²) and placed in air thermostats at desired temperature. If it were necessary to maintain different moistures than that of laboratory air, then desiccators with different sulphuric acid concentration were used. For higher temperatures desiccators were placed in air thermostats.

Treatment of Ipidacrine Samples with Organic Solvents and Water

100-150 mg of ipidacrine monohydrate A or anhydrous form were placed in a pestle, then approx. 0.5 mL of solvent was added and the obtained mixture was pestled until the mixture reached consistency of a paste. This paste was dried at specified conditions (for example, hydrate formation at laboratory air (22°C, 55% relative humidity)) and PXRD analysis was performed.

Powder X-Ray Diffraction Studies

PXRD patterns were acquired on Bruker D8 Advance diffractometer, using copper radiation ($K_{\alpha 1}$ and $K_{\alpha 2}$; wavelength $\lambda = 0.15418$ nm). The tube voltage was set to 40 kV, and the current was 40 mA. The divergence, anti-scattering and receiving slits were 1.0 mm, 1.0 mm and 0.6 mm, respectively. The diffraction pattern was taken at a scanning speed of 0.02° step per 0.5 s from 3° to 30° on 2 θ scale. For calculation of lattice parameters the scanning speed was 0.02° per 5 s and 2 θ interval from 3° to 50°. If sample can absorb water from air humidity then it was covered with 10 μm polyethylene film (film forms additional diffraction signal at 2 $\theta = 21.5^\circ$).

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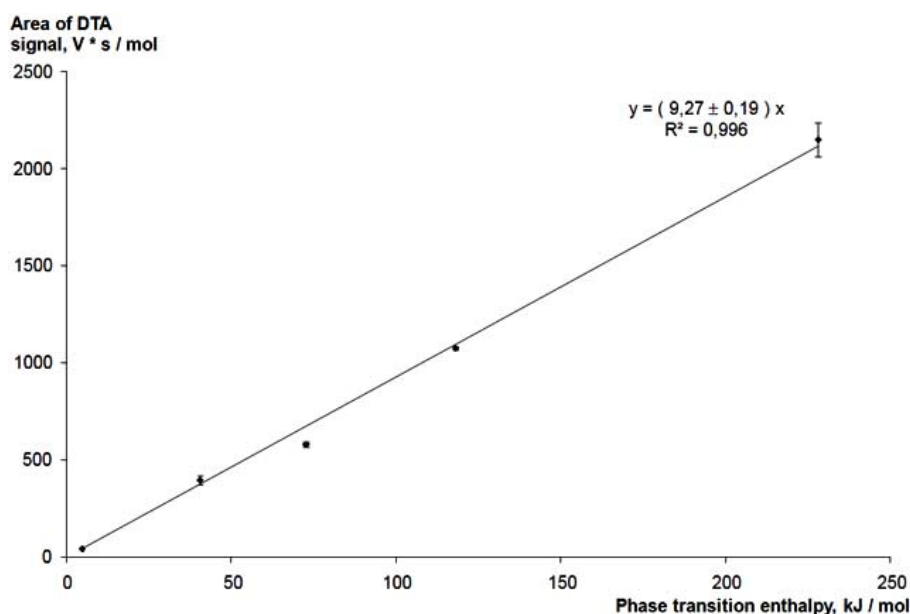


Fig. (1). Calibration curve of TG/DTA6300 machine for phase transition enthalpy determination.

DTA/TG Studies

DTA/TG experiments were performed on Exstar 6000 TG/DTA6300 machine. A sample of 10-15 mg was placed in the sample holder (aluminium pan P/N SS000E030) and heated from 30°C to 300°C. Scanning speed was 0.10°C or 5.0°C per minute. To determine dehydration enthalpies the instrument was calibrated with tin, lead, copper(II) sulphate pentahydrate, barium chloride dihydrate and distilled water, using their phase transition enthalpies from previous literature [3]. On the calibration curve DTA signal area (determined from five obtained DTA curves, scanspeed 5.0°C/min.) is plotted as a function from phase transition enthalpy. The calibration curve is shown in Fig. (1).

Karl Fischer (KF) Titration

Karl Fischer titration of ipidacrine samples was performed on Metrohm 836 Volumetric Karl Fischer Titrando, using the portions of samples which were analyzed by DTA/TG method.

X-Ray Fluorescence Analysis

X-ray fluorescence (XRF) analysis was made on Bruker S8 Tiger spectrometer. A sample of 150-200 mg was placed in sample holder where it was irradiated through 5 µm polypropylene film by Rh radiation using 8 mm mask.

RESULTS AND DISCUSSION

All tests performed with ipidacrine samples can be divided into two groups: 1) effects of temperature and air humidity on phase composition and 2) treatment of ipidacrine hydrochloride with organic solvents. An additional part of this research is lattice parameter calculation of obtained monohydrates.

Effects of Temperature and Air Humidity on Phase Composition of Ipidacrine Hydrochloride Samples

The starting point of this research was dehydration studies of ipidacrine monohydrate A form. It was analyzed using the DTA/TG method, and the obtained pattern is

shown in Fig. (2). Endothermic peak at 90-110°C corresponds to dehydration of ipidacrine monohydrate, weight loss of 7.30% ($\pm 0.05\%$, 3 measurements, 95% confidence interval) confirms the loss of one mole of water per 1 mole of ipidacrine hydrochloride.

The exothermic peak followed by an endothermic peak in temperature above 250°C corresponds to decomposition of ipidacrine hydrochloride to form ipidacrine base and melting of base. Total mass decrease of approximately 20% on heating till 290°C corresponds to loss of one mole of water and one mole of hydrochloric acid per one mole of ipidacrine base. Loss of HCl during heating at 290°C is confirmed by XRF analysis. It showed that chlorine content during 2 hour heating at 270°C decreased almost 20 times. Slow decomposition on heating is observed at temperatures above 160°C when ipidacrine hydrochloride turns to light brown or grey colour.

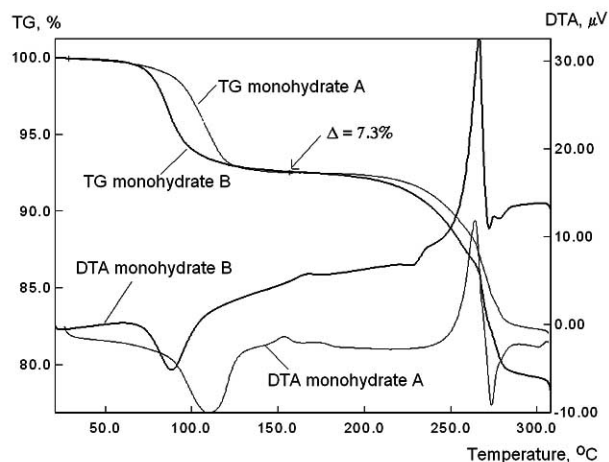


Fig. (2). DTA/TG curves of ipidacrine monohydrates A and B.

Monohydrates A and B were also examined by DTA/TG machine with purpose to determine phase transition

enthalpy. Using calibration graph shown in Fig. (1), it was determined that enthalpy of dehydration for ipidacrine hydrochloride is 53 ± 2 kJ/mol for monohydrate B and 70 ± 2 kJ/mol for monohydrate A. As seen from DTA curves in Fig. (2), monohydrate B has lower dehydration temperature than monohydrate A. These results confirm statement given in previous research [2] that monohydrate A is more stable than monohydrate B.

Phase transitions during heating were further investigated by PXRD method. While heating ipidacrine hydrochloride monohydrate (either A or B) at 90°C for 1-2 hours a new crystalline phase (abbreviated as C) was obtained. When using monohydrate B, the transformation to form C is more rapid. This phase is also formed in 20 hours in desiccator above phosphorous pentoxide at 70°C temperature or in 3 days in desiccator above concentrated sulphuric acid at laboratory temperature ($\sim 22^\circ\text{C}$). PXRD patterns of ipidacrine crystalline forms are given in Fig. (3). Further investigation of this form by DTA/TG analysis and KF titration leads to conclusion that ipidacrine hydrochloride form C is a non-stoichiometric hydrate where water content varies from 4.8% to 6.5%.

Further heating at $90 - 100^\circ\text{C}$ leads to changes in ipidacrine structure which are observed in PXRD pattern as new diffraction peak at $2\theta = 22.24^\circ$ and diffraction peak offset to 23.90° (Fig. 3, these peaks are marked with asterisk). This crystalline form (abbreviated as D) is obtained also in desiccator by holding ipidacrine hydrochloride monohydrate B or C form samples above phosphorous pentoxide for 30 hours. D form can also be obtained from monohydrate A, only in this case the time for complete conversion (measured as time after which characteristic diffraction peaks of other forms are not present) increase to 50 hours. Mentioned phase transition from A and B monohydrates proceeds through formation of non-stoichiometric hydrate form C. Investigation of form D by KF titration and DTA/TG analysis showed that water content in this form vary from 2.5 to 3.8%. It is also another non-stoichiometric hydrate.

Both forms C and D rapidly absorb water in ambient conditions and transform back to monohydrate B (water content 7.31 ± 0.02 %; 3 measurements; 95%). This transformation takes place approximately in half an hour (relative humidity 50%, 22°C). Analyzing C and D form samples by DTA/TG method there is mass increase that can be observed while samples till 70°C , then mass starts to decrease and at 120°C ipidacrine hydrochloride is completely dehydrated.

Complete dehydration of monohydrate B leads to formation of anhydrous form E which also in ambient conditions transforms back to monohydrate B. Dehydration of monohydrate A leads to formation of two crystalline phases abbreviated as F and G forms, where F is an ipidacrine hydrochloride hemihydrate and G is anhydrous form. Quantitative hydration – dehydration studies of G form showed that during hydration the mass increases by approximately 7%. Complete hydration of anhydrous form E takes approximately 2 hours to transform back to monohydrate B (relative humidity 50%, 22°C).

Anhydrous form E can be obtained also from monohydrate A by dehydration of this form at 120°C over phosphorous pentoxide.

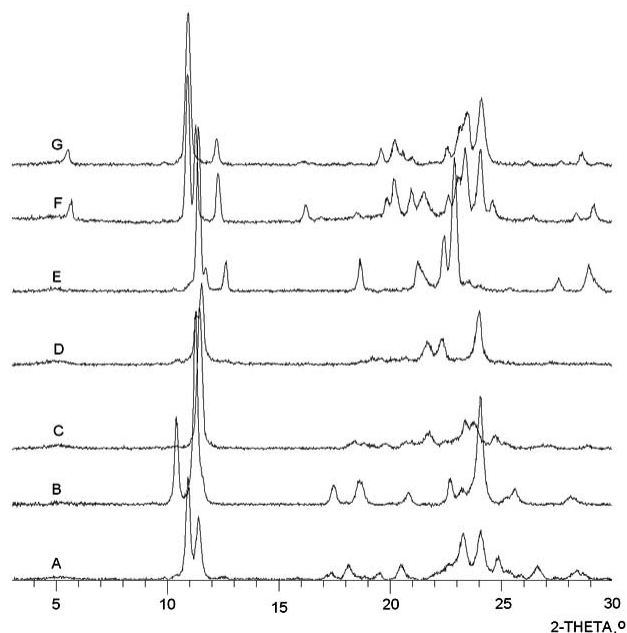


Fig. (3). PXRD patterns of ipidacrine hydrochloride crystalline forms.

Dehydration of monohydrate A at temperatures above 100°C leads to formation of hemihydrate F (weight loss during heating 3.51 ± 0.09 %; 3 measurements, 95%). This form is stable and does not undergo transformations during 1 hour heating at 150°C . At temperatures above 150°C , crystalline form F transforms to form G which, as confirmed by DTA/TG analysis and KF titration, is an anhydrous form. Both forms G and F transform to metastable forms at ambient conditions.

G form can be obtained from monohydrate A by heating it at temperature of 160°C for 3 days.

Hydration of form F (2 days for complete hydration) is faster than hydration of form G (5 days for complete hydration) and it is observed that G form is obtained from F and G mixtures by heating them in temperatures above 160°C . If we compare hydration rates of anhydrous forms G and E, then the rate of hydration for G form is lower than the rate of hydration for E form. This allows us to conclude that, at room temperature (22°C) and air humidity of 55%, G form is more stable than E form.

All ipidacrine hydrochloride phase transitions caused by change in temperature and humidity conditions are shown in Fig. (4).

Treatment of Ipidacrine Hydrochloride with Organic Solvents

The second part of this research focused on treatment of ipidacrine hydrochloride with wide range of organic solvents which were chosen according to solvent classification in literature [4] and evaluating possibilities of their use in industry. At first ipidacrine hydrochloride monohydrate A

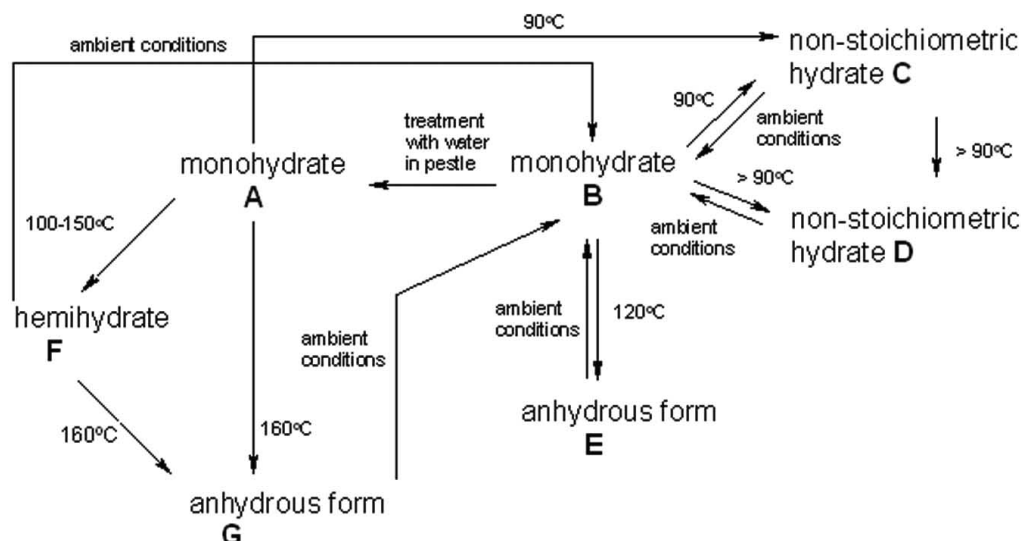


Fig. (4). Cross transitions of ipidacrine hydrochloride crystalline forms affected by change of relative humidity and temperature.

was treated with water but no changes were observed. After treatment of monohydrate B with water it transformed to monohydrate A, thus showing that monohydrate A is thermodynamically more stable than monohydrate B which forms during hydration of anhydrous forms due to kinetic factors. These results agree with previous research [2], where it is also stated that monohydrate B is a metastable form. To avoid water influence on solvate formation, treatment with organic solvents were repeated using anhydrous form E as starting material.

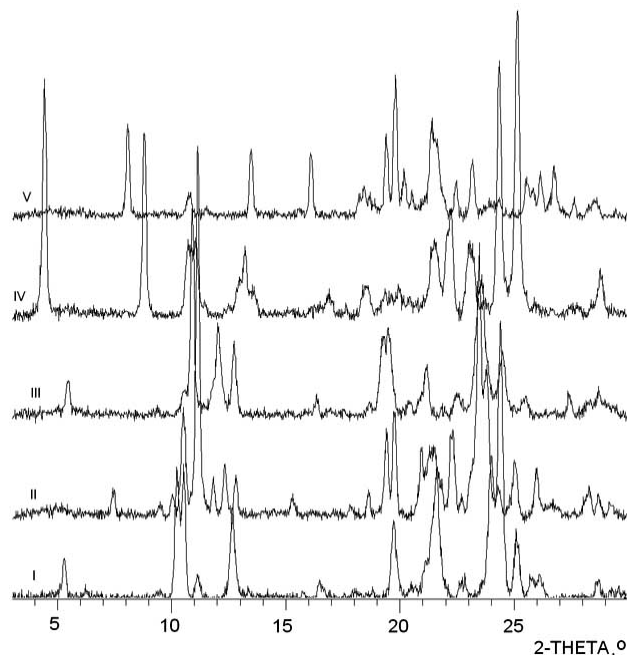


Fig. (5). PXRD patterns of ipidacrine hydrochloride solvates with organic solvents (I – methanol water solvate; II – hemihydrate H; III – octanol-water solvate; IV – DMF-water solvate; V – chloroform solvate).

Treatment of monohydrates A and B and anhydrous form E with methanol lead to formation of methanol – water solvate. Presence of water was also detected in case when

solvate was obtained from the anhydrous form E. It can be explained with small amounts of water in used methanol and with fact that ipidacrine hydrochloride has higher ability to bind with smaller water molecules than with bigger methanol molecules. Binding with methanol molecules is observed only in cases when there are no more water molecules available in reaction mixture. PXRD pattern of obtained solvate is shown in Fig. (5). Its composition was determined by DTA/TG and KF titration. DTA/TG analysis showed that it loses 1.4% of mass till 110°C and a total 6.2% of its mass on heating till 150°C. KF titration showed that the sample contains 1.40% of water. So the first step is dehydration of solvate while the second step is loss of methanol molecules. One possible explanation of this observation is also based on size difference between water molecules and methanol molecules, where methanol molecules are larger so it is harder for them to exclude themselves out of crystalline lattice. Such composition of solvate corresponds to one mole of water and two moles of methanol per five moles of ipidacrine hydrochloride.

By heating at temperatures above 90°C ipidacrine methanol-water solvate transforms to ipidacrine hydrochloride hemihydrate form F, but exposure to air humidity (~40%, 22°C) leads to formation of new ipidacrine hydrochloride hemihydrate form, abbreviated as H. Exposure of obtained solvates to room temperature and moisture (humidity 50%, 22°C) leads to formation of monohydrate B.

Hemihydrate H form has characteristic diffractions peaks at 7.40°; 9.47°; 10.01°; 10.48°; 11.80° on 2θ scale (Cu K_α radiation), KF titration determined that water content in this form is 3.0 ± 0.2%, which corresponds to one mole of water per two molecules of ipidacrine hydrochloride.

It was expected that other primary alcohols, such as ethanol and propanol, could also form solvates with ipidacrine hydrochloride, but treatment with these alcohols led to formation of ipidacrine hemihydrate form H (in both cases – using monohydrate A as starting material or using anhydrous form E as starting material). Same situation with formation of phase H was observed using all alcohols from ethanol to heptanol while treatment of ipidacrine

monohydrate A and the anhydrous form E with octanol led to a new crystalline phase with characteristic diffraction peaks at 5.43° ; 10.89° ; 12.02° ; 12.71° (see Fig. 5). DTA analysis of this solvate proves that it loses 3.3% of water till 140°C , but the total mass loss was 32.0%, which is higher than mass loss of pure ipidacrine monohydrate (22.0%). This fact allows concluding that it contains some octanol in its structure. Changes in PXRD pattern states that there is some interaction between octanol molecules and ipidacrine hydrochloride. Calculating solvate composition obtained that it contains 0.6 water moles and 0.5 octanol moles per one mole of ipidacrine hydrochloride.

Treatment of ipidacrine hydrochloride with chemically pure nonanol and decanol also did not lead to solvate formation. After this treatment monohydrate A stays as monohydrate A but the anhydrous form (E) transforms to hemihydrate H as nonanol and octanol contain small amount of water.

Another mixed solvate is formed by treating ipidacrine monohydrate A with N,N-dimethylformamide (DMF). Its PXRD pattern is shown in Fig. (5). Mass is lost in a two-stage process where during the first stage 1.76% is lost at 80°C but total mass loss of 18.9% takes place at 150°C . The first stage of dehydration corresponds to loss of water while the second stage – to loss of dimethylformamide. These results are confirmed with KF titration, where water content is determined to be $1.76 \pm 0.02\%$. Described ipidacrine hydrochloride DMF solvate composition is determined to be 5 moles of DMF and 2 moles of water per 7 moles of ipidacrine. Another experiment with preparation of ipidacrine hydrochloride DMF solvate leads to formation of solvate which loses 11.5% of its mass under heating till

180°C . This composition corresponds to 2 moles of DMF per 5 moles of ipidacrine hydrochloride. In both cases PXRD patterns of DMF solvates are corresponding, but determined solvate composition is different. These facts mean that obtained DMF solvate has non-stoichiometric solvate character.

Obtained DMF solvate is relatively unstable and on exposure to laboratory air it transforms back to monohydrate A in 2 hours, but heating at 60°C temperature leads to formation of non-stoichiometric hydrate form C.

There were several other organic solvents used such as 1,4-dioxane, dimethyl sulfoxide (DMSO), dichloromethane, 1,2-dichloroethane, ethylformiate, n-hexane, tetrahydrofurane (THF), butylacetate (BuOAc) and acetone. These solvents did not change the crystalline structure of the used substance. If it was anhydrous form E it remained as anhydrous form E, if it was monohydrate A it remained as monohydrate A.

Another solvent which led to structural changes is chloroform. Treatment of ipidacrine hydrochloride A and E forms leads to crystallization of chloroform solvate. By heating on DTA machine it loses 36.7% of its mass which corresponds to chloroform monosolvate. Exposition of chloroform solvate to air humidity leads to rapid formation of metastable monohydrate B (approximately 40 min., 22°C , 50% relative humidity). At dry air (relative humidity $<2\%$, 22°C) chloroform solvate is stable and does not change composition after one week storage at such conditions. Presence of water molecules in this solvate was not detected by Karl Fisher titration. Heating of obtained chloroform solvate at temperatures above 150°C leads to formation of form G which does not have mass loss till decomposition at

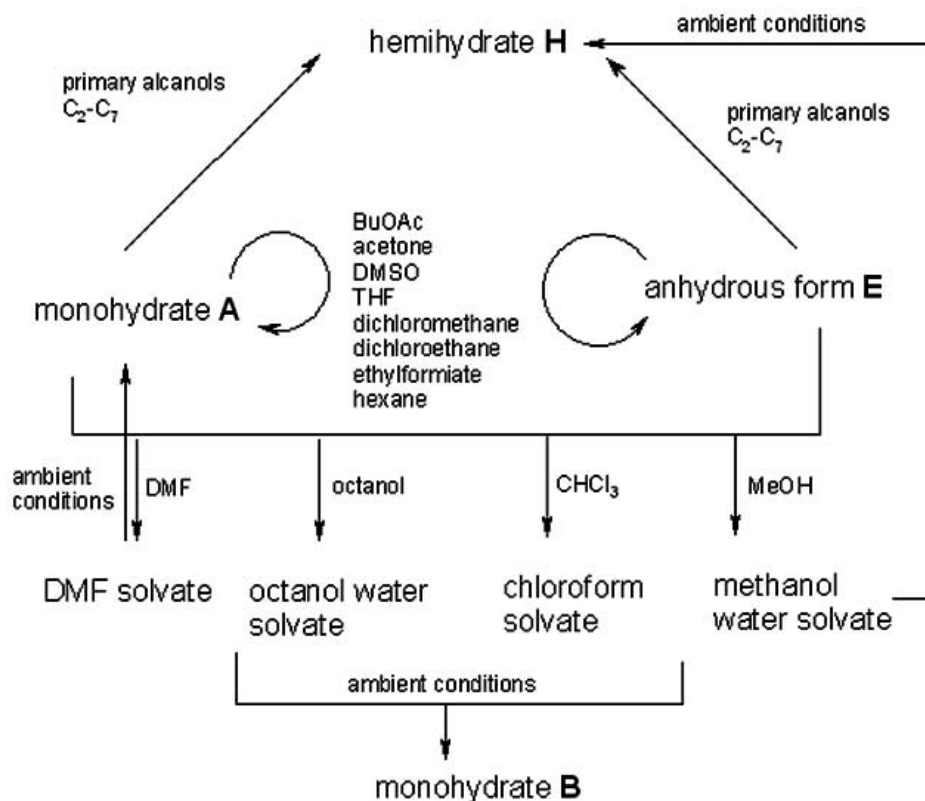


Fig. (6). Cross transitions of ipidacrine hydrochloride solvates with organic solvents.

Table 1. Comparison of the Obtained Ipidacrine Hydrochloride Crystalline Forms

Crystalline phase of ipidacrine hydrochloride	Water content, mass %	Organic solvent, included in structure	Organic solvent content, %	Stability in laboratory conditions (22°C, 55% rel. humidity)
A	7.4	-	-	Stable
B	7.3	-	-	Stable
C	~4.8	-	-	Transforms to monohydrate B
D	~3.8	-	-	Transforms to monohydrate B
E	~0	-	-	Transforms to monohydrate B
F	3.5	-	-	Transforms to monohydrate B
G	~0	-	-	Transforms to monohydrate B
H	3.0	-	-	Stable
Water/Methanol solvate	1.4	Methanol	4.8	Transforms to hemihydrate H
Monochloroform solvate	-	Chloroform	36.7	Transforms to monohydrate B
Water/Octanol solvate	3.3	Octanol	6.7	Transforms to monohydrate B
Water/DMF solvate	1.8	DMF	17	Transforms to monohydrate A

temperatures above 250°C. G form is determined to be nonsolvated form.

Phase transitions of ipidacrine by solvents are shown in Fig. (6). Summarising obtained results about solvate formation we can conclude that ipidacrine hydrochloride forms solvates with relatively polar solvents which can bind with ipidacrine molecules *via* hydrogen bonds or dipole-dipole interactions.

There are 12 crystalline phases described in this research paper, all these phases and their composition are given in Table 1.

CONCLUSION

The pharmaceutically active compound ipidacrine hydrochloride forms two monohydrates A and B which are mentioned in literature [2]. It is also shown by DTA/TG studies that monohydrate A is more thermodynamically stable than monohydrate B in all temperatures. Besides these two monohydrates two non-stoichiometric hydrates, two hemihydrates and two anhydrous forms were obtained. Some of these forms may have potential use in pharmacy due to different bioavailability, which is not further investigated in our studies.

Treating ipidacrine hydrochloride monohydrate A and the anhydrous form E with chloroform leads to formation of chloroform monosolvate while other solvents such as

methanol, octanol and DMF lead to formation of mixed solvents where the second solvent is water. All mentioned solvates absorb air humidity and convert to ipidacrine hydrochloride hemihydrate or monohydrate B (except DMF solvate) which, after processing with water, transforms to more thermodynamically stable A form.

By heating these forms in air they gradually lose weight, where at first at temperatures up to 160°C they lose water (if there is water in their structure) and afterwards, at temperatures above 250°C, they decompose to ipidacrine base.

ABBREVIATIONS

- PXRD = powder X-ray diffractometry
 DTA/TG = differential thermal analysis and thermogravimetry
 KF = Karl-Fisher titration
 XRF = X-ray fluorescence analysis
 DMF = dimethylformamide

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