Alma Mater Studiorum - Università di Bologna

SCUOLA DI SCIENZE

Dipartimento di Chimica Industriale "Toso Montanari"

Corso di Laurea Magistrale in

Chimica Industriale

Classe LM-71 - Scienze e Tecnologie della Chimica Industriale

"*Synthesis in millireactor system and stability of intermediates for the functionalization of imidazole*"

Tesi di laurea sperimentale

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Sessione III

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1. Abstract

This Master thesis presents the results obtained in the curricular traineeship, carried out within the laboratories of the Department of Chemistry of the University of Bergen, during the Erasmus period, and within the Department of Industrial Chemistry of the University of Bologna. The project followed in Bergen concerned the synthesis of key intermediates used for the functionalization of the backbone of imidazole, using N,N'-diiodo-5,5-dimethylhydantoin ("DIH") as an iodinating agent, and employing an innovative kind of chemical reactor: the "Multijet Oscillating Disc Millireactor" (MJOD Reactor). Afterwards, the work performed in Bologna consisted in verifying the stability in solution of the above mentioned N,N'-diiodo-5,5-dimethylhydantoin utilising spectrophotometric techniques and High Performance Liquid Chromatography analyses (HPLC).

In questa tesi di laurea magistrale si presentano i risultati ottenuti nel tirocinio curricolare, effettuato nei laboratori del Dipartimento di Chimica dell'Università di Bergen, durante il periodo Erasmus, e all'interno del Dipartimento di Chimica Industriale dell'Università di Bologna. Il progetto seguito a Bergen ha riguardato la sintesi di intermedi chiave utilizzati per la funzionalizzazione del "backbone" dell'imidazolo (i carboni C4 e C5), utilizzando N,N'-diiodo-5,5-dimetilidantoina ("DIH") come agente iodinante, e impiegando un innovativo tipo di reattore chimico: il "Multijet Oscillating Disc MilliReactor" (Reattore MJOD). Successivamente, il lavoro svolto a Bologna è consistito nel verificare la stabilità in soluzione della suddetta N,N'-diiodo-5,5-dimetilidantoina utilizzando tecniche spettrofotometriche ed analisi con cromatografia liquida ad alte prestazioni (HPLC).

2. Introduction

2.1 The role of functionalized imidazoles in medicinal chemistry

During the last years, the research of chemical synthesis explored in different ways the functionalization of imidazoles, due to the high importance of functionalized imidazoles in the field of medicinal chemistry. Imidazoles are a well known class of heterocycles: they include several substances that were studied because of their relevant chemical and biological interest. It is also known that they are part of a great number of very significant biomolecules, for instance: the important amino acid “histidine”, some other related compounds as biotin, and the imidazole alkaloids too.

Since imidazole drugs have several applications in different areas of clinical medicine, it is clear that the insertion of the imidazole nucleus is a very important synthetic strategy in the discovery of new effective drugs. More specifically, the imidazoles are also known for being antifungal azole derivatives that have a large range of significant activities, both in vivo and in vitro. Therefore, these molecules are currently used as strategic tools in many pharmacological studies. Because of their remarkable therapeutic properties, imidazole related drugs gave reasons to the medicinal chemists to develop and test a considerable number of new molecules, and some of these have shown interesting chemotherapeutic properties[1].

Furthermore, functionalized imidazoles are useful precursors for N-heterocyclic carbenes ligands not only in organocatalysis, but also in homogeneous transition metal catalysis[2]. It was reported in previous studies that N-heterocyclic carbenes can be compared with phosphines and cyclopentadienyls because of their capability of bonding and reactivity[3]. For all these reasons, achieving improvements in the synthesis of this kind of molecules results particularly interesting. It is of primary importance to research and develop novel procedures for the functionalization of the imidazole that can be reproduceble, selective and possibly “green”, both for future industrial applications and for further academic research. It is particolarly important to discover efficient synthetic strategies that permit unsymmetrical substitution on the C-backbone of the imidazole skeleton.

2.2 Scope of the present research

Specifically, this project investigated the possibilities to trasfer some previously know

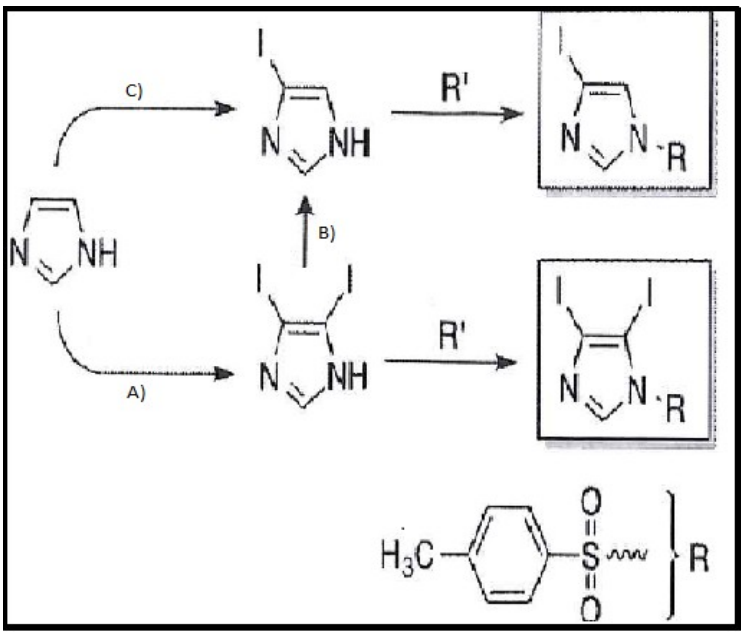
reaction from bench scale to a bigger scale (procedure of "*number-up*", in a scale-up perspective), utilising a particular kind of reactor: the "Multijet Oscillating Disc Millireactor (MJOD)"[4]. Both this specific type of reactor and the considered reaction were previously developed in the laboratories of the Department of Chemistry of the University of Bergen from the research team directed from professor Hans-René Bjørsvik, through an extensive period of dedicated studies and researches[5]. The reactions that were investigated are represented in the following image, which shows synthetically the pathways that were taken in consideration in order to get to the molecules of interest: imidazoles substituted with iodine on the C-backbone (C4 and C5). In the picture:

A): di-iodination reaction[5];

B): selective de-iodination reaction[6];

C): mono-iodination reaction[5].

This reactions lead either to 4,5-Diiodo-1H-imidazole or to 4(5)-Diiodo-1H-imidazole. Then, it is possible to perform another reaction with these molecules and p-toluenesulfonyl chloride in order to obtain, respectively, N-tosyl-4,5-diiodoimidazole or N-tosyl-4(5)-iodoimidazole, which would be the products of major interest, in this case.

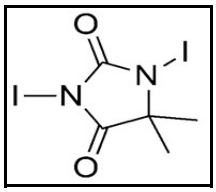


- Picture 1: Different synthetic pathways leading to iodinated imidazoles

The reason why these two chemical species would be of so great importance is because there is currently a collaboration between the mentioned Department of Chemistry and the Hospital of Bergen, which is interested in these molecules for their potential applications as “tumour marking compounds”. Nowadays there is a significative attention for further development in this area of medicinal chemistry, and this project takes its part in it.

2.3 The iodinating agent: N,N'-diiodo-5,5-dimethylhydantoin (DIH)

The team of Bjørsvik firstly developed a process for being able to produce mono- and di-iodinated imidazoles using N,N'-Diiodo-5,5-dimethylhydantoin (DIH, C5H6I2N2O2, CAS: 2232-12-4) as iodinating agent[5]. DIH at room temperature is a light brown powder, which is stable when kept dry at relatively low temperature (ambient temperature or below) and, since it possesses a high content of iodine, it has a light iodine odor. DIH was prepared for the first time by Orazi and his collegues in 1965 by reacting iodine monochloride (ICl) under basic conditions with 5,5-dimethylhydantoin (DMH, $C_5H_8N_2O_2$)[7]. In their work, Orazi reported that the DIH showed "general applicability for nuclear iodination of homo- and heteroaromatic compounds activated by electrondonating substituents". His research team was also able to prove that the iodination of amino and acetylamino aromatic substrates happens via the intermediate formation of N-iodo derivatives, and this was demonstrade by isolation of 2-(iodoamin0)-4,6dimethylpyrimidine and 2,4-dimethyl-6-(N-iodo-N-acetylamino)-pyrimidine.



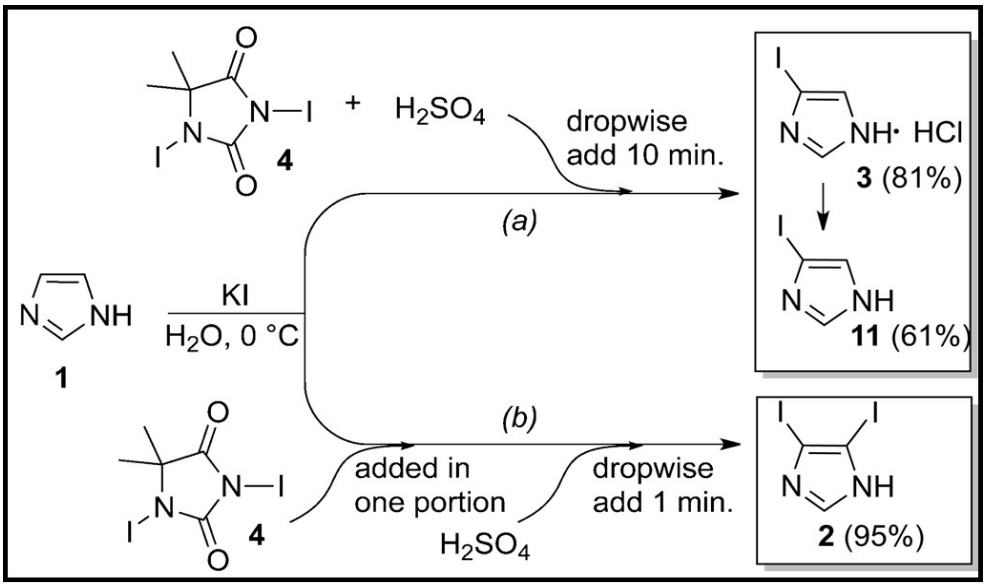
- Picture 2: Molecular structure of N,N'-diiodo-5,5-dimethylhydantoin (DIH)

DIH manifests a reactivity comparable to that of molecular iodine, but it is far more simple to handle because it is a solid reagent, as said, and it does not sublimate as iodine does. It is worth of metioned that DIH possesses the same selectivity as *N*-iodosuccinimide (NIS) and equal, or even better, halogenating capability. Orazi observed that DIH can react with enol acetates derived from both saturated and unsaturated

ketones, like N-iodosuccinimide does, giving α-iodo ketones in satisfactory yields in the process[7]. DIH can be more economical in comparison to NIS, thanks to its two N–I bonds, and it has been used as an oxidizing agent or as an iodizing agent in several production processes in different fields: (agricultural, pharmaceutical, food industries, and so on). It is quite easy to find examples in the scientific literature that can testify this[8][9][10]. For all these reasons, DIH was taken in consideration as potential iodinating reagent in the previous project of Bjørsvik's group of research. Thanks to the Multijet Oscillating Disc Reactor developed in their laboratories, team Bjørsvik managed to produce a large quantity of DIH, which was used in the experiments and tests that were performed in this project.

2.4 The reaction mechanism of the iodinating process

Bjørsvik and his colleagues reported in their paper[5] that the iodination reaction can be performed following two different synthetic routes that give, respectively, the mono-iodinated or the di-iodinated imidazole (the specific procedures to do these reactions are reported at paragraph 3.2 and 3.4)[5].



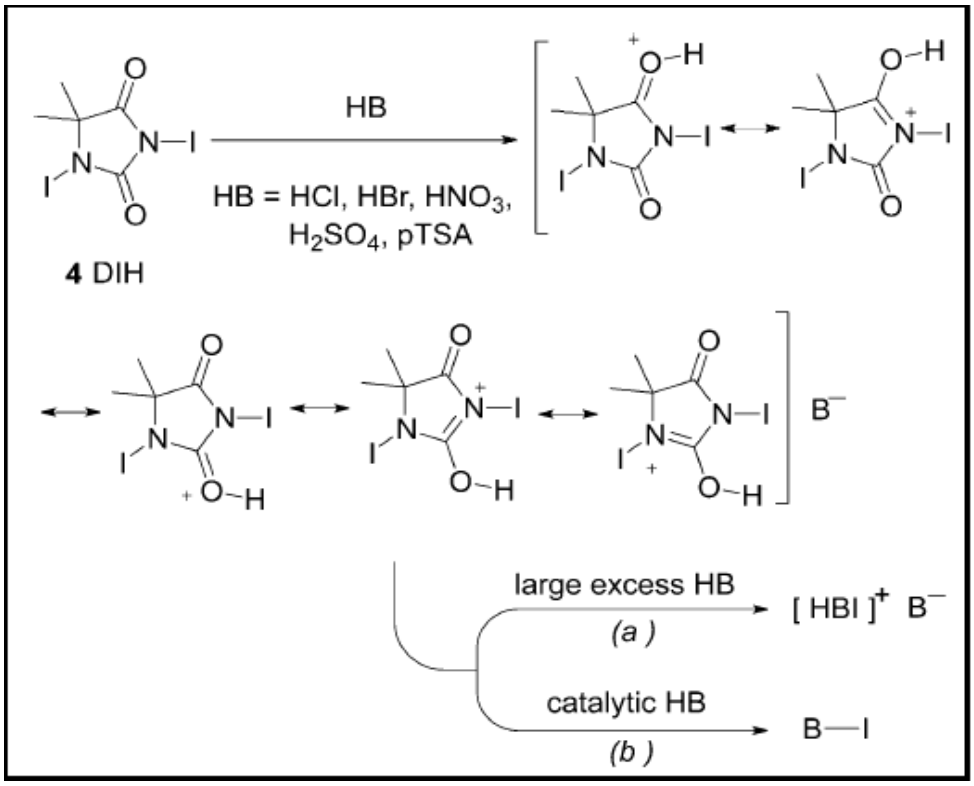
- Picture 3: the mono-iodination and the di-iodination reactions

Orazi and his collegues[7] discussed for the first time a possible radical mechanism for iodination using DIH as iodinating agent but, since they observed that the DIH did not have a role in the iodination of toluene (utilising benzoyl peroxide as radical initiator),

this appeared in turn to be improbable. Then, they made deeper researches, after which they ended up proposing a mechanism that involved the production of an electrophilic I^+. This specie was thought to be the iodinating agent, at that time. After some years, Chaikovskii and his collegues[11] found out that the DIH is able to succesfully iodinate, aromatic amines, phenyl ethers and alkylbenzenes in organic solvents. They also discovered that the reactivity of electrophilic iodine is controlled by the acidity of the medium where the reaction takes place, since a superelectrophilic iodine is generated via dissolution of DIH in sulfuric acid. Then, this iodine reacts with electron-deficient arenes leading to formation of the respective iodo derivatives, at 0 to 20°C, giving interesting yields in the process. Basically, when DIH is treated with concentrated H_2SO_4, both of its two N-I bonds are broken and the iodine atoms are removed from DIH. This experimental observation induced Chaikovskii to propose the formation of an iodine hydrogen sulphate ($HOSO_2OI$) as the molecula to conduct the pre-iodination in the reaction. Some DFT calculations were also executed, and the results gave reasons to suppose that the homolytic disassociation of iodine hydrogen sulphate was more probable, if compared to a heterolytic bond dissociation. Afterwards, Bjørsvik and Sandtorv[5] decided to explore more in depth some mechanistic aspects, taking in consideration the di-iodination of imidazole as a model reaction in their experiments. From their experiments, it emerged that the first step of the reaction mechanism involves the protonation of DIH, performed by a strong acid (HB). The following step depends on the quantity of the mentioned strong acid.

Fundamentally, there are two main possibilities:

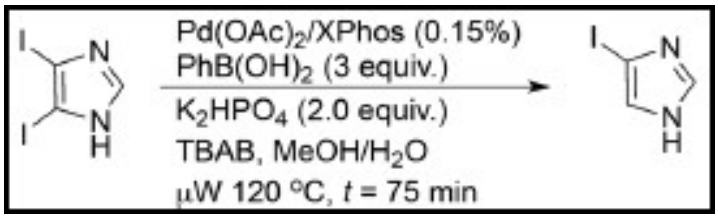
- If we are under conditions of a relevant excess of the strong acid HB, then the DIH becomes protonated and it reacts further to give the active iodinating specie [HBI+] B. This specie is very reactive and it has a strong electrophilic character, and it can iodinate even highly deactivated aromatic rings.
- Instead, In the other case, if we are working just with catalytic quantities of the acid HB, then the protonated DIH undergoes N-I cleavage and produces the iodonium species “BI”, which is significantly less reactive and less electrophilic than [HBI+] B.



- Picture 4: Reaction mechanism of the iodination reaction

2.5 The selective deiodination reaction

In another previous study, Bjørsvik and Sandtorv projected, developed and optimized a three-way switchable Pd-catalysed process that can effect transformations on the carbon atoms of the imidazole backbone (C4 and C5)[6]. This process is microwave assisted and it provides a hydrodehalogenation or a selective arylation of the imidazole backbone. The hydrodehalogenation and a cross-coupling reactions were conducted one after another with good results in "the third switch position" that realizes an assisted tandem reaction sequence, giving 4(5)-aryl-1H-imidazole as a final product. The "arylation switch position", instead, was optimized to synthetise the 4,5-diaryl-1H-imidazole. Finally, the "hydrodehalogenation switch position" was utilised to produce the 4(5)-iodo-1H-imidazole. This last reaction, in particular, is the one that interests us the most in this case: it was tried in order to look for alternative ways to produce 4(5)-iodo-1H-imidazole by deiodination of the 4,5-iodo-1H-imidazole previously synthetised via di-iodination reaction with DIH, as discussed in the former paragraph.



- Picture 5: The Pd-catalysed selective deiodination reaction

2.6 The microreactor technology

During the last years, there has been a substantial demand for synthetic processes to be as "environment-friendly" as possible, especially in the fields of pharmaceutical chemistry and fine chemistry. This implies finding synthetic routes that are able to provide the target molecules with the highest possible yield and selectivity, while at the same time producing the least wastes and minimizing the use of hazardous or toxing reagents and solvents. The significative progress in the synthetic processes, with and without catalysis, is also testified by the development of a new type of technology: the so called "flow chemistry" in the form of "microreactors", which were designed and presented in the scientific community as optimal and advantageous tools to perform various kinds of sythesis, for the production of pharmaceutical ingredient and fine chemicals[12]. Microreactors give the possibility to conduct organic synthesis in a continuous flow, which is a valuable alternative to the "classical" batch system. Naturally, when we talk about "batch", we usually refer to the laboratory flask on a laboratory scale and to the stirred tank reactor (STR) on an industrial scale (both for pilot reactors and for full scale plants). The microreactor technology is caracterised by excellent mass- and heat-transfer properties, if compared to the common batch, and therefore it is easy to understand why, nowadays, several kinds of microreactors are commercially sold from various companies[13]. The miniaturisation of the equipments is revolutionising chemical synthesis, generally producing with higher yield and purity and in a shorter time. The progressive expansion of this kind of technology is also leading to a new way of conceive the implementation of synthetic processes from the laboratory scale to a pilot plant and, at the end, to an industrial production on a larger scale. Generally, a process of organic synthesis that is completely developed can be transferred from the laboratory directly to the production scale thanks to the concept of "number-up" (instead of "sizing-up") during a scale-up procedure. Currently, two different types of microreactors have been designed

and developed: the "micro-structured reactors" and the "chip-based microreactors". The formers are commonly employed in organic process chemistry, instead the latters are tipically utilized for academic research purposes[14]. Concerning the structure of microreactors, they are generally made of miniaturized channels incorporated in a flat surface, which can be made of different materials such as: glass[15], silicon[16], stainless steel[17], or also some kind of polymers (like polydimethylsiloxane, for instance)[18]. One of the most common material used to construct various equipment for chemistry purposes is glass, because of its high resistance against different acids, bases, solvents and numerous reagents. Silicon is another option: it is more used in those reactions that must be performed at high (or low) temperatures[16], because of its good heat-transfer capacity and thermal conductivity; and it manifests similar properties to those of glass when it is in its oxidized form, which may also be exploitable to our advantage. The most commonly used material for building microreactors though is still Stainless steel, especially in the area of process chemistry where it is employed in pilot plant. It is also utilised in fine chemical industry, where usually a battery of microreactors work in parallel at the same time. Microreactors made of polymers also exist and they have been used to conduct reactions at atmospheric pressure in aqueous medium[19], but they generally tend to have lesser performance because of the lower tolerance against the majority of the solvents and reagents. Microreactors, and flow systems in general, have proven their uselfulness in many different areas of the organic process research, as it is attested by several examples in the scientific literature[20-22]. However, it is also true that microreactor systems possess some important limitations when it comes about performing synthetic reactions, namely:

- it may happen that solid particles can obstruct the network of the thin channels of the microreactor during the course of liquid-solid reactions;
- when a solid catalyst is necessary, then it must be present as cartridges inserted inside the reactor, included in the microreactor and immobilized by grafting on the channel walls, or located in poles of very small dimentions in the reactor channels which may be tricky to do[23-25];
- if there are any solubility problems, then the undissolved solid particles may cause clogging of the reactor channels;
- it is required a consistent number-up proceduure in order to compensate the

reduced capacity of production;

- it is complicated to perform telescoped processes, where two or more reaction are done in sequence into the same reactor body;
- it is challenging to conduct multi-phasic reactions (gas-liquid, liquid-solid, or gas-liquid-solid);
- some processes need long reaction times (and long residence time in the reactor, as consequence).

In the scientific literature there are examples of gas-liquid two phase reactions that were conducted using microreactors, such as halogenations by elemental fluorine and chlorine gas[26,27], and also some cases of liquid-gas-solid multiphase reactions[28,29], but to do them utilising microreactor systems has been proved to be a complicated operation. Moreover, concerning multistep syntheses using microreactors, some examples have been reported , but they are still few[30,31]. Nowadays, some other systems to conduct continuous flow organic syntheses exist, but they are still of limited number. Both the standard tubular (plug) flow reactors and the oscillatory flow mixing reactor[32] currently have heavy disadvantages, when compared to a microreactor. This is due to the fact that a tubular reactor usually requires a high length-to-diameter ratio in order to perform reactions that are caracterized by a long residence time, and they are rather difficult to control properly. On the other hand, this is not a problem for an oscillatory flow mixing reactor, but the "contact area versus reactor volume" ratio is not optimal enough if compared with a microreactor. It must be pointed out that this is a parameter of critical importance when it comes to keep a precise control on the reaction temperature and on the heat that is generated in an exothermic reaction (since the selectivity of the reaction itself is related to it).

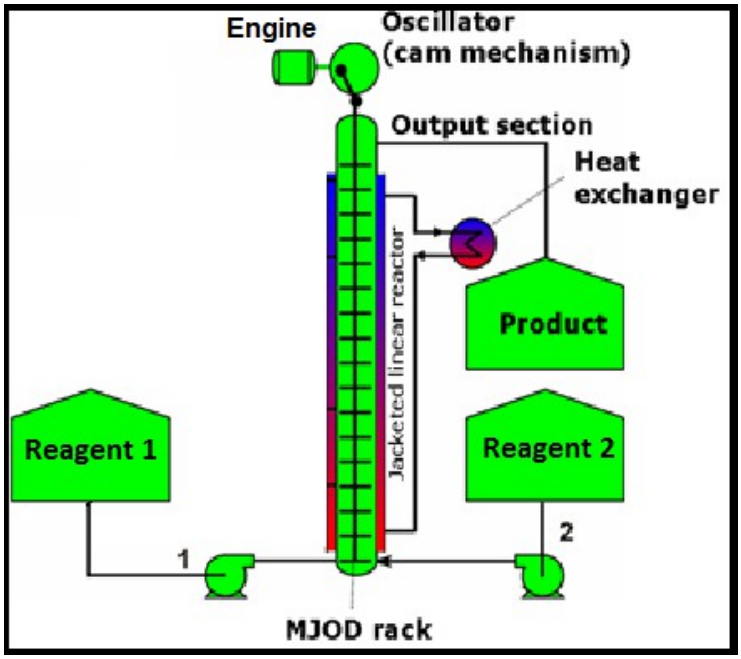
2.7 The Multijet Oscillating Disc Millireactor (MJOD Reactor)

In the last years, Bjørsvik's research team projected and developed several different approaches for performing organic reactions in a continuous flow reactor system. This paragraph presents an outlook about this specific kind of approach, as studied and described by Bjørsvik himself and his colleagues[4], which keeps the advantages of microreactor systems (the good mass-transfer and heat-transfer properties) while at the same time not suffering for their most common disadvantages. Nowadays, the

microreactor technology is already well established in the scientific community. This technology is usually characterised by mixing the reagents in channels of micrometric dimensions. Instead, the approach for continuous flow processing is executed in multimillimeter-sized channels, hence using a "millireactor system". In this particular system, the reagents and the catalysts (if any) undergo a mechanical mixing that generates an optimal heat and mass transfer. The flow reactor system was designed and built up in order to do continuous flow organic synthesis on a milli-scale, utilising the so called "Multi-jet Oscillating Disc Reactor" (MJOD reactor).

This reactor possesses four different parts:

1. the section(s) to feed the reagents;
2. the section(s) of the reactor and of the heat exchanger;
3. the outlet and pressure regulator section;
4. the oscillator.



- Picture 6: Synthetic scheme of the "Multijet Oscillating Disc (MJOD) Reactor"

It is possible to connect these segments in multiple ways in order to customize the reagent inlet patterns and the reactor length as necessary, thanks to the fact that each reactor section has a set of male and female joints. In particular, the male joint of each unit is provided with four reagent inlet channels (the input channel is made to connect standard flangeless nut and ferrule to the reactor body). Furthermore, each section is also

connected to a standard size flange (o.d. 40 mm). The different reactor elements are connected to each other by several flange swing clamps. These are standard swing clamps (o.d. 50 mm) for flange fittings, as used for vacuum lines and fittings for oil vacuum pumps. Moreover, the reactor includes some external supporting units too, which are:

- pumps to feed the reagent with reservoirs;
- heating and cooling machine(s), which are provided with a circulator pump to allow the flow of any heating (or cooling) fluid. This fluid circulates through the heat exchanger cap that enfolds the whole reactor tube;
- a direct current power supply (U = 0 – 24 V), so that it is possible to controll the frequency of the oscillator (by monitoring the number of revolutions and the rotation of the electrical motor,).

The MJOD unit is located inside the reactor tube, which consists in the perforated discs (the multijet discs) that are integrated on the oscillator piston shaft with some space between each other, always providing a standardized volume of 0.6 mL in this way. Each section of the reactor is also provided with a male and female joint pair. These joints are what make the MJOD system assemblable in various ways, in order to be customized for the particular necessities of the specific reaction. This configuration consents to set up the residence time inside the reactor both with the pump rate and by varying the length the reactor body. It is also possible to keep a precise control on the temperature of the reaction mixture and to adjust the mass-throughput when collecting the product. Both the reactor and the heat-transfer chamber are made of stainless steel tubes. The reactor tube (o.d. 12 mm, i.d. 10 mm) is surrounded by a second larger tube (o.d. 37 mm, i.d. 33 mm), made of stainless steel, and the space that is created in between is used to let the cooling fluid (water) circulate in order to keep the temperature under control. The inlets and outlets can be disposed at different levels of the length of the reactor, as required for the specific experiment, thus allowing the heat-transfer fluid to flow in and out at different points of the reactor itself. More specifically, the tube of the MJOD reactor is surrounded by an insulating coat, and the cooling, or heating, fluid (water, in this case) is let to flow inside it, by means of a pump. This cooling/heating coat can be divided different sections. Therefore, it is practically possible to divide the body of the reactor in more different "temperature zones". For instance, this is a necessary feature if one desires to

perform a "telescoped multistep synthesis" (i.e. synthetic processes that are composed of several reactions), since different reaction temperatures are required in the process. Thanks to the DC power supply, the reactor tube has an adjustable frequency (0.5 Hz) and amplitude (0.25 mm), which helps to achieve an optimal mixing, and an oscillator that moves the multijet disk unit back and forth longitudinally. The MJOD unit is basically a piston engine with multiple piston heads (the equally spacied four-jet discs) disposed on one piston shaft. The reagents are feeded to the reactor tube by the pumps, as previously mentioned. By means of the pressure generated by the pumps, the reagents will flow through the holes (the jets) of the discs that are located on the piston of the MJOD unit, with a high rate. There is a technical problem that must be avoided, though: the alternating pressure produced by the oscillating piston shaft could potentially provoke a “back kick” of the reaction mixture into the reagent feeding tubes (and into the pumps, as a conseguence). This issue is easily bypassed by supplying the reagent inlet lines with "one-way valves". When the reaction mixture passes through the disc holes and arrives into an area of a larger cross section (like the reactor cavity), then the flow rate decreases, which produces vortexes and very turbulent movements that make the mixing of the reagents so optimal. Furthermore, the mixing inside the reactor is improved even more by the movement of the discs fixed on the oscillating piston. The oscillator is made of two parts: the engine unit and the agitation. The agitation unit consists of a piston shaft with numerous perforated piston heads equally spaced in the length of the piston itself. The piston length is egual to the reactor length plus the distance between the end of the reactor tube and the joining point on the cam wheel of the electrical motor. The piston heads are perforated ring-formed discs (o.d. = 10 mm, L = 4 mm) made of Teflon (PTFE), chosen for its well know high chemical resistance. Each one of these discs has four jets with a diameter in the range d = 1.00-1.30 mm. The piston shaft with its discs fits very closely to the reactor tube walls. With this configuration, there is a thin annular cavity between the inner surface of the rector tube, two discs, and the piston. This cavity is what generates the internal surface of the reaction cavity. In general, the number of reaction cavities can be modified as necessary, and specifically in this project the piston shaft supported 60 equally spaced discs (10 mm o.d. with 4 x 1.25 mm diameter jets). The 59 cavities that are located between the discs, along with the reaction cavities, provided a total volume of ≈38 mL. Also the length of the reactor tube can be varied as

desired, but it was observed that one meter of length from the first feeding point to the product outlet point is usually the best option for several kinds of reactions and processes. Therefore, this was the tube length that has been used for all of the performed experiments. Since many different set-ups can be arranged with the MJOD reactor, it has been tested by performing various reactions that are currently in use both on research and on industrial level (using both batch and microreactor protocols), in particular: the Nef reaction, sodiumborohydride reduction , the Haloform reaction, the Paal-Knorr pyrrole synthesis, nucleophilic aromatic substitution, O-allylation, N-acylation, the Suzuki cross-coupling reaction and the Hofmann rearrangement. For instance, using the MJOD reactor while performing the Paal-Knorr reaction resulted in a 2837 mmol/h production of 2-(2,5-dimethylpyrrol-1-yl)ethanol, starting from 51,4 mL of a β-aminoethanol solution (851 mmol) and 100 mL of a acetonylacetone solution (852 mmol)[4]. In following projects, the MJOD reactor was also succesfully used to conduct other reactions: organocatalyzed epoxidation of alkenes[33], synthesis of phenylboronic acids at cryogenic temperatures[34], and lithiation/borylation reactions[35], giving satisfactory results in all the cases. Currently, moreover, there are more studies and project designs that are under progress concerning the MJOD system. These researches are founding evidences that this kind of reactor may be suitable to conduct gas- liquid reactions with molecular oxygen as oxidant agent, different metallorganic reactions that need low reaction temperature metallorganic reactions that need low reaction temperature, telescoped reactions, olefin metathesis reactions, and also other different reactions that require a protecting atmosphere with an inert gas, such as nitrogen or argon.

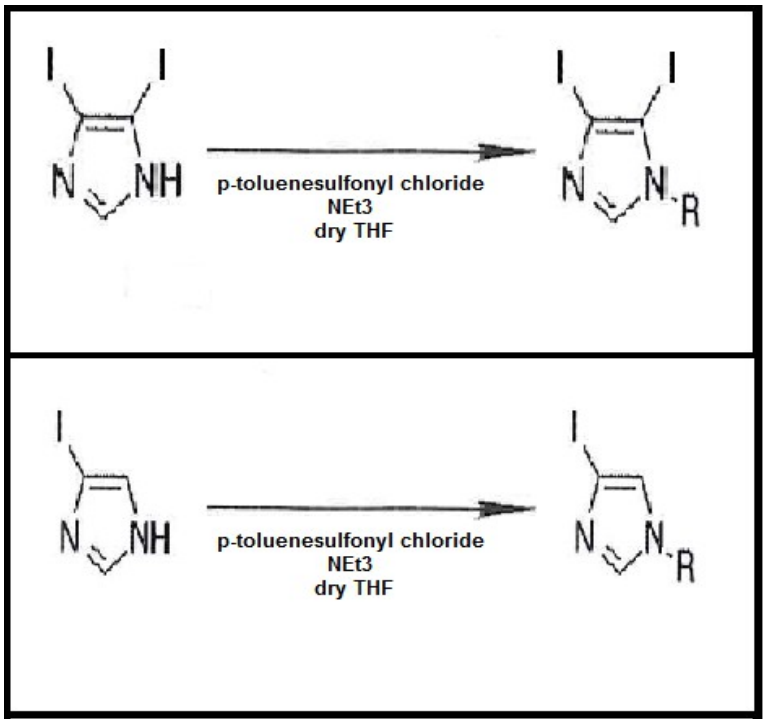
2.8 The stability of N,N'-Diiodo-5,5-dimethylhydantoin in solution

Since it was previously known that DIH could present some form of instability in a solution, it would be necessary to develop and implement a quantitative analytical method, based on HPLC analysis, to observe the stability of DIH in different solvents. At the best of our knowledge, although some former reserches were know to employ HPLC techniques to analize and separate different kinds of hydantoins, no specific study concerning the DIH was done until now[36-38]. Therefore, in order to draft an analytical protocol as previously mentioned, some preliminary analyses are necessary. More specifically, since the HPLC instrument uses a spectrophotometric detector which works

in the UV-Visible range of wavelength, it is first needed to verify where does the DIH absorbs in that range. Since the DIH solutions are coloured, it is easy to suppose that it should give some absorption in the visible range. In the scientific literature there are no data whatsoever concerning UV-Vis spectra for DIH, and this is the reason why some preliminary tests are required. At the same time, monitoring the variations of absorbance with time, it is possible to perform a qualitative observation of the stability of DIH in solution, using water, acetone and methanol as solvents.

2.9 The stability of p-toluenesulfonyl chloride in solution

As seen in the previous *Picture 1*, p-toluenesulfonyl chloride (tosyl chloride) is the reagent that has to be employed in the final steps of the synthetic procedure, in order to obtain N-tosyl-4,5-diiodoimidazole and N-tosyl-4(5)-iodoimidazole. Because of its importance in this synthetic route, it was decided to test its stability in solution in an analogous way as done for the DIH. For tosyl chloride, however, no HPLC resulted necessary: its stability was verified just using spectrophotometric methods. The details concerning the spectrophotometric analyses are reported in the following paragraph 3.6

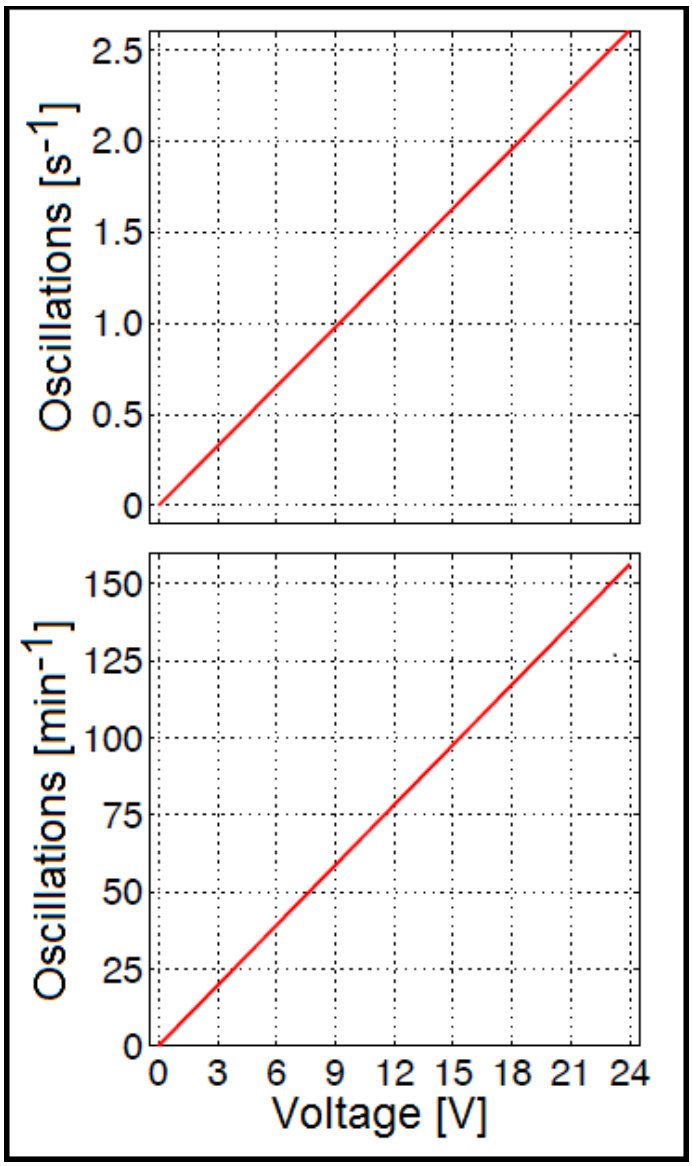


- Picture 7: Synthesis of N-tosyl-4,5-diiodoimidazole and N-tosyl-4-iodoimidazole. "R" represents a tosyl group ($CH_3C_4H_6SO_2$).

3. Experimental section

3.1 Methods

The MJOD reactor has a total volume of 65 mL and it was provided with two "QG low speed – low flows" pumps by Fluid Metric Incorporated (60 Hz, shaded 2 pole, enclosed ventilated, thermally protected, pump drive "QG20", piston code "Q1"). The pumps were set in order to have the reservoirs with reagents completely depleted at the same time. The engine was set up so that the oscillations of the piston shaft increased proportionally to the applied voltage, as shown in the following picture.



- Picture 8: Correlation between the piston shaft oscillations and the applied voltage in the MJOD reactor.

Water was used as cooling fluid, which circulated through a set of tubes. In order to keep the water at 0°C its main reservoir has been filled with ice.

Control ^{1}H NMR spectra were obtained on a NMR spectrometer, which operated at 400 Mhz/52 MM (Bruker). Chemical shifts were referenced to the deuterated solvent (D_2O or $CDCl_3$).

Control GC analyses were performed on a capillary gas chromatograph "GC 800 Series" (Fisons Instruments), equipped with a fused silica column (*l* 25 m, 0.20 mm i.d., 0.33 mm film thickness) at a helium pressure of 200 kPa, split less/split injector and flame ionization detector. Mass spectra were obtained on a GC-MS instrument, using a gas chromatograph equipped with fused silica column (*l* 30 m, 0.25 mm i.d., 0.25 mm film thickness) and helium as carrier gas.

Control FT-IR spectra were recorded on a "Nicolet 380 FT-IR" instrument (Thermo Electron Corporation).

The routine pH tests were performed using a B27 pH Lab instrument ("Metrohm"), equipped with a 3 M KCl glass electrode, which has been calibrated with two buffer solutions (pH 4 and 7).

The UV-Vis spectra were obtained using the following instruments:

- Double beam spectrophotometer: "Cary 1E UV/Vis Spectrophotometer" (Varian), which can record spectra in the wavelength region λ = 190 – 900 nm, equipped with a "Cary WinUV" program;
- Single beam spectrophotometer: "Jenway 6405 UV-Vis Spectrophotometer" (Barloworld Scientific), which can record spectra in the wavelength region λ = 320 – 1100 nm;

The microwave reactor was a "Initiator" equipment (Biotage®).

The instrument utilised for the ultrasonic bath was a "Transsonic 700/H" (Tamro) machine.

The rotavapor machine was a "Rotavapor R-3" (Buchi Switzerland) with a vacuum controller V-850 and a vacuum pump V-700.

Operations of purification were performed using an Autoflash "Reveleris® X2 Flash Chromatography System", equipped with two UV detectors (set up at λ=254 nm and

λ=280 nm) and one ELSD detectors (threshold detection: 6mV), using a "Grace Resolv™" column (silica, 4 g/5 mL), isopropanol as carrier and hexane/ethyl acetate as eluents, with a flow rate of 36 mL/min.

All the chemicals were used as received, except N,N'-diiodo-5,5-dimethylhydantoin (DIH) that was previously produced with the MJOD reactor during a foregoing project, as previously mentioned.

All of the data analysis were performed using the software "OpenOffice Calc".

3.2 Synthesis of 4(5)-Iodoimidazolium chloride by iodination reaction

A round-bottom flask was filled with water (50 mL) and immersed in an ice-bath. Imidazole (0.388 g, 5.70 mmol) and KI (12 g, 72 mmol) were transferred to the flask and the mixture was stirred until the solids were dissolved. NaOH (3.7M, 50 mL) was then added. Sulphuric acid (5 mL) was added to N,N'-diiodo-5,5-dimethylhydantoin (0.519 g, 1.4 mmol) in a separate flask and manually stirred in a vigorous way. The resulting dark and viscous mixture was slowly added drop-wise with a Pasteur pipette to the imidazole solution over 10 min of time.

After the addition, the reaction mixture was neutralized with acetic acid (pH~6). A saturated solution of K_2SO_3 (1 mL) was added and finally the solution was saturated with NaCl. During all these steps, the mixture needs to be kept in the ice bathThe clear solution was extracted with ether (3x40 mL) and the combined, organic phases were again extracted with 10% HCl (3x10 mL). The aqueous solution was evaporated with a rotavapor to about half the volume and the white precipitation filtered off. Yellow crystals of the title compound were crystallized from the aqueous hydrochloric acid solution.

3.3 Synthesis of 4-Iodoimidazole by selective deiodination reaction

4,5-diiodo-1H-imidazole (0.34 mmol), XPhos (0.15 mol%), phenylboronic acid (3.0 equiv.), K_2HPO_4 (2.0 equiv.) and TBAB (0.025 mmol) were transferred to a micro-wave reactor tube equipped with a magnetic stirring bar. The vial was sealed and carefully flushed with argon. Methanol/water (4:1) (5mL) was added to the tube and a fresh solution of $Pd(OAc)_2$ in methanol was prepared. To the vial, $Pd(OAc)_2$ solution was

added (0.15 mol%) by means of syringe. The vial was then submerged into the micro-wave cavity and heated to 120°C for 75 minutes.

3.4 Synthesis of 4,5-Diiodo-1H-imidazole by diiodination reaction

Imidazole (0.076 g, 1.1 mmol) and N,N'-diiodo-5,5-dimethylhydantoin (0.345 g, 0.91 mmol) were transferred to a round-bottom flask immersed in an icebath. Water (2 mL) was added and the heterogeneous mixture was stirred during the drop-wise addition of sulphuric acid (1 mL) over 1 min. After the addition, NaOH (3.9M, 15 mL) was added. The resulting milky solution was then neutralized (pH~6) with acetic acid which resulted in precipitation of the product. The crystals were filtered, washed multiple times with cold water and saturated K_2SO_3 solution (3x3 mL) and allowed to air-dry to constant weight to give the product as creamy crystals. Traces of 4-(5)-iodo-1H-imidazole were also observed in the isolated product.

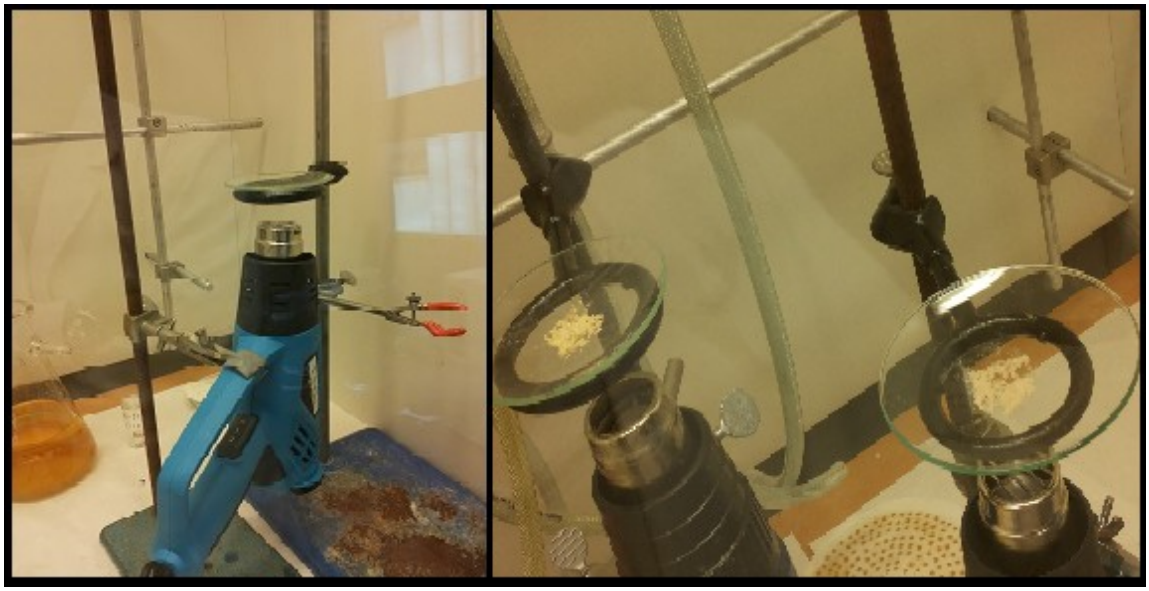
3.5 Synthesis of N-tosyl-4-Iodoimidazole

4(5)-diiodo-1H-imidazole (5.46 g, 28.2 mmol) and *p*-toluenesulfonyl chloride (5,38 g, 28,2 mmol) were transferred to a Schlenk tube under argon atmosphere. Then dry THF (40 mL) was added to dissolve the solids. After that, NEt_3 (4.0 mL, 28.2 mmol) was added drop-wise, using a syringe. It is important that the reaction is conducted under completely dry conditions, therefore it is necessary to preventively dry all the equipment (Schlenk tubes, magnetic stirring bar, etcetera) with a heat gun before proceeding. The reaction mixture was stirred for 24h at ambient temperature while keeping the argon atmosphere. This procedure has been employed to try to synthesize N-tosyl-4,5-diiodoimidazole starting from 4,5-diiodo-1H-imidazole.

3.6 Analyses to determine the quality of the DIH

To estimate the quality and the purity of the DIH two different kind of analyses were performed: FT-IR analyses and thermogravimetric analyses. Regarding the recording of the FT-IR spectra, the only required precaution is to preventively dry the DIH sample, since otherwise the water band could cover part of the spectrum. Therefore, the DIH samples were left to dry under vacuum for 2 days before recording the FT-IR spectra. Concerning the thermogravimetric tests, during these experiments a know amount of

DIH was deposited on an appropriate support which was slowly heated from the bottom with a heat gun (see the following *Picture 9*), until fumes of iodine were released. This phenomenon is usually accompanied also by the solid turning of a darker colour.



- Picture 9: Disposition of the equipment during the thermogravimetric analyses.

Weighting the support before and after the heating allows to find out the quantity of lost iodine, and thus the DIH quantity. In this way, it is easy to obtain an estimation of the DIH purity.

3.7 Solubility tests for the DIH in different solvents

It is useful to know the DIH solubility in the solvents that are supposed to be used in the spectrophotometric analyses and HPLC tests, before actually performing them. Since at the best of our knowledge no specific data about can be found in the scientific literature, we had to try to obtain at least an estimation of its solubility by ourselves.

These preliminary experiments consisted in weighting a known amount of DIH in a flask and progressively adding the chosen solvent, one millilitre at time using a calibrated pipette, until the solid was completely dissolved at room temperature. In order to generate an optimal mixing, a vigorous magnetic stirring was also applied to the solutions. Experiments were made using distilled water, acetone and methanol as solvents, and the obtained results are reported in the following paragraph 4.3.

3.8 Spectrophotometric analysis of DIH solutions

Every DIH solution was sonicated for 30 minutes in an ultrasonic bath, in order to provide a better dissolution of the solid in the three different solvents (distilled water, acetone and methanol). The complete spectra of DIH were further obtained using a double beam spectrophotometer. This operation also allowed us to find out the wavelength where the DIH has its maximum peak of absorption (λ_{max}), in every solvent. The solution stability tests has been performed utilising both a single- and a double-beam UV-Vis spectrophotometer.

All the spectrophotometric analysis were conducted using sealed quartz cuvettes, in order to prevent any evaporation of the solvent. During the analysis with the single beam spectrophotometer, the cuvette containing the blank (the pure solvent) was put inside the spectrophotometer first, in order to register the baseline of the blank itself, and only after this registration the cuvette containing the DIH solution was put in the spectrophotometer to read the absorbance value at the λ_{max}. Instead, when operating with the double beam spectrophotometer, the two cuvettes could be placed inside the instrument at the same time while registering the continuous spectrum of the solution.

This procedure has been repeated at specific time intervals and, when the two cuvettes were not been analysed, they were placed closed in a dark place, far from any source of heat or light. This precaution is necessary in order to prevent some kind of undesired degradation of the DIH between one analysis and the following. This same operative methodology was also applied to the spectrophotometric analysis of p-toluenesulfonyl chloride. For the tosyl chloride, acetone and dry THF were employed as solvents.

3.9 HPLC analysis of DIH solutions

As for the spectrophotometric analysis, also for these experiments every DIH solution that was used has been sonicated for 30 minutes, for the same reasons mentioned above. The HPLC (High Performance Liquid Chromatography) system that has been used, connected with the thermostatation equipment, is represented in the following image.

In the picture, from the left to the right, we can see:

1. The thermostating bath, which uses distilled water as heat exchange fluid and an alcohol thermometer to monitor the temperature;
2. The HPLC system (Varian, v. 2.1, HPLC pump 2510), equipped with a

spectrophotometric detector (with an adjustable spectrophotometric range), a 50 µL calibrated loop, and the column;

3. the recorder (Amersham Pharmacia Biotech, REC 1111).

- Picture 10: The thermostated HPLC system.

The column that has been employed was a Discovery® C18 (504971), 25cm x 4,6 mm, 5 µm of diameter for the silica particles (Supelco). The eluent flow in the HPLC system was always kept at 1,0 mL/min, and the eluent was let to run in the column for at least 15 minutes before doing any kind of test, in order to appropriately condition the column itself. We employed UPP H_2O and "Hipersolv Chromanorm" CH_3OH (HPLC gradient grade, CAS 67-56-1, VWR International) as eluents. Morever, as previously mentioned for the spectrophotometric analyses, also for these experiments every DIH solution has been preventively sonicated for 30 minutes in an ultrasonic bath. This is particularly important to prevent any clogging during the injection of the solution inside the system, due to particulate that could be present in the solution.

The injections were executed with a plastic syringe (5 mL "Fortuna®" syringes) provided with a “Hamilton” needle. The syringe has to be connected to a filter (“Econofilter”, Agilent Technologies) too, which is located between the syringe and the needle. This filter helps to further prevent any remaining particulate (or suspension) to clog the system. It is also necessary to sonicate the eluent as well to degas it, for at least 5 minutes, and this is necessary to avoid that air bubbles may get stuck inside the loop, thus provoking a sudden obstruction of the calibrated loop. In fact, when we degas the eluent, usually some gas bubble are released, and after that this phenomenon is observed the

eluent is generally ready to be employed.

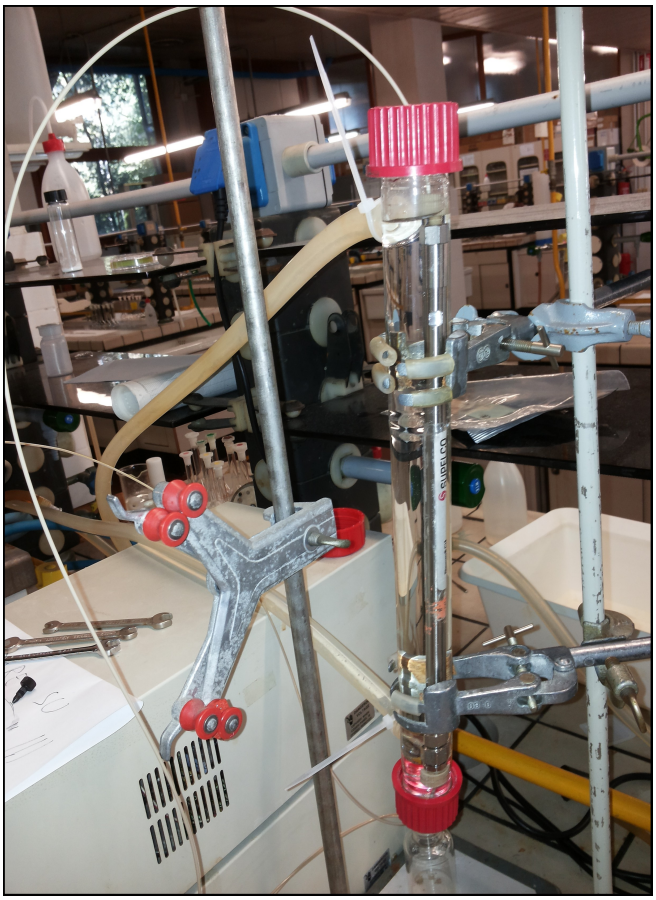
Concerning the analyses: the volume of DIH solution that was injected in the HPLC system at every analysis was 0,1 mL, and these injection were executed at specific time intervals (one every 15 minutes). It is worthy to mention, though, that any injection should be avoided before that the pressure of the HPLC has not been completely stabilized, otherwise this could result in unattainable data. Another important detail is the fact that no air bubble should be present inside the syringe before the first injection, for all the above mentioned reasons. This is avoided simply by filling the syringe with the solution and then “spilling” some of the liquid outside, and in this way the air bubbles that may be present will be easily ejected.

One must also be sure to reset the baseline of the recorder before every injection, to prevent any drift of the baseline itself. Moreover, in order to reduce the interferences as much as possible, two operations can be done. First of all, one has to appropriately clean the syringe with the eluent (methanol, UPP water, or else), before and after every injection. Secondly, after the first injection, the syringe will be left inside the HPLC injector, to avoid air to get in.

Having said that, the HPLC pump is able to handle a pressure between 0 and 200 atm, and it is constantly monitored by the internal software of the system. With this equipment, the analyses were made at increasing temperatures (25°, 30°, 35°C), in order to study the effect of the temperature on the degradation process of the DIH (if any).

The following picture shows the employed C18 column when put under thermostatation. The heat exchanging fluid is water that continuously circulates in the glass cylinder that contains the column itself, through two plastic tubes. The whole system is sustained by metallic arm connected to a support.

The next picture shows the C18 HPLC column that has been utilised for the vast majority of the experiments. During the tests performed in water, where the analyses were more complicated to perform, we also try to use a different C18 column, which was a “Kinetex 5u C18 100A", 250 x 4,60 mm, 643153-12, purchased by "Phenomenex". The results did not improve while using this column though, and therefore after some trials it was discarded, since probably it was not what created the encountered issues (see the following paragraph 4.15).



- Picture 11: The C18 HPLC column (Discovery®) under thermostatation.

The experimental results obtained both with the spectrophotometric and HPLC analyses are reported in the following paragraph, with some considerations regarding their interpretation.

4. Results e Discussion

4.1 Synthesis of 4(5)-Iodoimidazolium chloride

Table 1 reports the results, in terms of crude yield, of some experiments performed to produce 4(5)-iodoimidazolium chloride, via the procedure reported at the previous paragraph 3.2. In fact, the iodination reaction was performed several times in order to test its reproducibility and to see if it has passages that may be difficult to reproduce on a bigger scale, using the MJOD reactor.

Table 1: Reproduction of the monoiodination reaction.

Test n°	g DIH	g 4(5)-Iodoimidazolium chloride	Crude yield (%)
1	0,523	0,235	37,0
2	0,520	0,210	33,3
3	0,521	0,228	36,1

The nature of the product was verified recording the FT-IR and GC-MS spectra. In particular, while doing the infrared spectra, it is important that the solid is as dry as possible, otherwise the large band of the water will cover a considerable part of the spectrum. Hence, before doing the FT-IR spectra is usually useful to dry the product with a flow of N_2. Having said that, the obtained yields were not satisfactory, considering that the article that contained the procedure[5] reported a yield equal to 81%. While performing the reactions, it became clear that the two most critical steps of this synthetic path were: the mixing of the DIH with concentrated sulphuric acid; and the separation of the product (which involves some steps of extraction, filtration and crystallisation).

Indeed, if the manual mixing is not appropriate, then the DIH does not become activated and the already mentioned super-elecrophilic iodinating agent $[HBI^+]\ B^-$ is not formed in a sufficient quantity, thus resulting in a lower yield (or, even worse, the reaction does not start at all). In addiction, the removal of the aqueous fractions (most of the times performed with a rotavapor), may be very slow, due to the large amount of water, which in turn slows the entire process. To remove the last portions of water, moreover, it can be also helpful to use a high vacuum pump (and this is always necessary if one wants to prepare a sample to run on a GC-MS machine, since it can not allow any water inside). Concerning the filtrations, both the conventional vacuum pump and the ”water vacuum

pump" are suitable for this separation. In any case, though, this could be a stage where part of the product is lost, and handle the instruments with extreme care becomes critical. In particular, it is better to avoid to filtrate utilising a funnel with glass filter, since the product tends to remain stuck on its surface and it gets very difficult to remove it. The classic paper filter is more appropriate for this operation.

Trying to remove all the solvent by evaporation gave a dense and viscous oil, of a yellow/orange colour, as result. The reason is easy to understand, considering that the mono-iodoimidazole is not volatile because it forms hydrogen bonds between its own molecules. Regarding the crystallisation, some trials were made using an ice bath with salt (NH_4Cl). Naturally, if it is used a salt which is not a powder, like $CaCl_2$, one must grind the salt in advance in order to make it a powder and thus providing a better contact between the ice and the salt itself. However, this kind of approach did not give good results. Therefore, several re-crystallization trials were conducted in order to look for a suitable solvent to further purify the 4(5)-iodoimidazole from the remaining salt (CH_3COONa, which is present as a consequence of the neutralisation of NaOH with acetic acid). This new set of experiments gave the results reported in the following ***Table 2***:

Table 2: Results of the crystallization of 4(5)-Iodoimidazolium chloride, after three days.

Sample n°	Solvent	Colour of the solution	Results
1	DCM	Light pink	-
2	1,2-Dichloroethane	Light yellow	Long transparent needles
3	$CHCl_3$	Light yellow	Few little needles
4	Ethyl acetate	Light orange	-
5	Hexane[a]	Pink	-
6	Toluene	Light pink	-
7	Petroleum ether	Pink	-
8	Penthane	Transparent	-
9	Acetone	Light orange	Few brown grains
10	THF	Yellow	Brown nails
11	Diethyl ether	Light yellow	Little ochre grains
12	Methanol	Transparent	-

[a] The hexane is a mixture of isomers, boiling point: 68-70°C.

The most interesting observations were obtained employing 1,2-dichloroethane, THF and chloroform. In general, it seems like chlorinated solvents can provide better results. Performing GC-MS analyses confirmed the presence of the desired product. Anyway,

after considering different possibilities regarding the separation stage of this synthetic route, it was decided to switch to the di-iodination reaction, basically because it required less tricky passages to obtain the final product (4,5-diiodoimidazole).

4.2 Synthesis of 4,5-Diiodo-1H-imidazole in batch

The di-iodination reaction was performed several times, in different batches, in order to see how reproducible it was and to find out eventual difficulties that could be detrimental for the process while using the MJOD reactor. In the following ***Table 1***, the crude yields were calculated considering the DIH as being the limiting reagent in the adopted conditions. The presence of the desired product in the solid that was produced was verified with GC-MS tests.

Table 3: Yields of different batches performed to produce 4,5-Diiodoimidazole.

Batch n°	g DIH	g 4,5-Diiodoimidazole obtained	Crude Yield (%)
1	0,345	0,907	312,3
2	0,353	0,472	158,8
3	0,345	0,514	177,0
4	0,345	0,322	110,9
5	0,348	0,231	78,8

The reason why the yields, apparently, are higher than 100% is because one fact has to be considered: the followed procedure[5] required a separation phase in order to get the product, which consisted in washing multiple times the obtained solid with water and a saturated solution of K_2SO_3 (to remove the remaining iodine). This stage often caused the deposition of K_2SO_3 itself on the solid containing the product, thus making higher the yields. Therefore, more washing with distilled water were necessary. Hence, after a new series of washes with distilled water, the masses of recovered product were as reported in the following ***Table 2***:

Table 4: Yields of different batches performed to produce 4,5-Diiodoimidazole (after a new series of washes).

Batch n°	g DIH	g 4,5-Diiodoimidazole obtained	Crude Yield (%)
1	0,345	0,082	28,23
2	0,353	0,079	26,58
3	0,345	0,091	31,33
4	0,345	0,037	12,74
5	0,348	0,062	21,16

The low yields achieved this time are mainly due to the fact that there were problems in the stage of recovering the product. The actual masses of 4,5-diiodoimidazole were probably higher, but since, as mentioned, repeated washings with H_2O were required, this usually produced a loss of product. In fact, in the following experiments, the washing with K_2SO_3 were generally removed from the procedure for this reason. This was one of the first issues that we had to deal with in the first troubleshooting phase.

We also performed a scale-up attempt on batch scale, multiplying the quantities of all reagents by ten times. Being aware of the technical issues encountered in the foregoing experiments, this time the washing of the final product was done mainly with water and eventually just with smaller quantities of K_2SO_3 solution. This resulted in an increase of the yield, as reported in the next ***Table 3***. Naturally, due to the bigger quantity of solid involved in this experiment, also a greater volume of water has been necessary in order wash the product in the best way possible. Therefore, 5x10 mL of water were employed, instead of 3x3 mL.

Table 5: Diiodination reaction, x10 scale-up.

g imidazole	g DIH	g KI	g 4,5-Diiodoimidazole obtained	Crude Yield (%)
0,761	3,454	22,431	2,432	83,6

The purity of the obtained product was checked through ^{1}H-NMR analyses. These spectra showed that the purity of the 4,5-diiodoimidazole was on average ≈80%, and that the remaining 20% was actually 4(5)-iodoimidazole. This is expected, since it was previously reported in the article that contained the procedure that this phenomenon could happen. Hence, in this experiment, the actual yield would be 68.7%, which is still a result that is closer to the ones reported in the scientific literature[5]

It was also done an attempt to separate the 4,5-diiodoimidazole from the 4(5)-iodoimidazole and thus improving its purity. It was used an automatic chromatographic system (“autoflash”), with the equipment previously mentioned at paragraph 3.1, imposing a solvent gradient that went from 20%-80% ethyl acetate/hexane eluent to a 90%-10% mixture of the mentioned solvents. Unluckily, though, it emerged that the retention times of the two compounds were too close to allow a proper separation and a purification with this system.

More experiments were made with these reaction in order to evidence more eventual

issues that could potentially complicate transferring this synthetic path on the MJOD reactor. In fact, it is also necessary to consider other factors, to avoid conditions that may negatively affect the course of the reaction, and the yield as consequence. It emerged that the pH is another key factor. After adding the NaOH 3,9 M the pH of the solution is naturally very high and it is necessary to lower it by adding an aliquot of acetic acid. Otherwise, if the pH is above 10, it is not possible to have the precipitation of 4,5-diiodoimidazole. It is worth to underline that, due to the viscosity of the reaction mixture, it is complicated (if not impossible) to check the pH using common pH paper ("Panpeha" paper), and a pH-meter is usually required. Usually, a dark, viscous and slurrish reaction mixture is observed at the equivalent point between H_2SO_4 and NaOH. To try to overcome this issue, it becomes fundamental to maintain the best possible mixing. Otherwise, the high viscosity could create some pH gradients in the volume of the solution, thus complicating the reach of a suitable equivalent point. To improve the mixing, it is also helpful to operate using an "egg-shaped" magnetic stirring bar rather than a cylindric one. At higher pH (9-10), the solution becomes yellowish and almost transparent and it is possible to see crystals of product. Moreover, if the pH increases too fast, the 4,5-diiodoimidazole does not precipitate. This explains the necessity of monitoring the acidity level during the reaction.

The last major parameter to keep under observation is the temperature: theoretically, the di-iodination should take place at 0°C, and the reaction mixture would need to be kept in an ice bath for all the steps before the filtration stage. However, it was observed that if the temperature drops too quickly or if the baloon containing the reaction mixture is kept at low temperatures for too long, a considerable gelification of the mixture itself occours, which makes impossible to proceed any further due to his high viscosity (it is not possible even to titrate). It is in this kind of situation that measuring the pH become almost impracticable, since the mixture is so "gellish" and viscous that it remains attached to the surface of the pH-meter glass electrode, which as consequence senses pH always lower and lower as the seconds pass.

This tendency to give gelification apparently increases when smaller volumes of solvent are used, probably due to the increase of viscosity. This phenomenon is probably due to the presence of CH_3COONa in solution, after the neutralization of NaOH with acetic acid. After this preliminary tests, we proceeded at the following phase: to transfer the

reaction on the MJOD reactor.

4.3 Synthesis of N-tosyl-4,5-diiodoimidazole

The procedures reported at paragraph 3.5 was utilised to try to produce N-tosyl-4,5-diiodoimidazole, starting from 4,5-diiodoimidazole (previously made) as substrate instead of 4(5)-iodoimidazole. Because, this reaction has never been performed in this way, two attempts were done at the same time, but the results were different to the ones expected. In fact, after the recrystallisation step with DCM, some GC-MS analyses were executed in order to verify the nature of the product. In the recorded GC-MS spectra, though, it was not possible to find any peak corresponding to the expected molecular mass of the final product (MM=470,017 g/mol), and probably the reaction did not even started.

In subsequent TLC analysis, using a 20:80 ethyl acetate/hexane solution as eluent (λ=254 nm), showed that the two reaction mixtures did not contain traces of N-tosyl-4,5-diiodoimidazole. Therefore, assuming that all the different phases of the procedure were reproduced correctly, the experimental observation suggests to make changes to the operative conditions while using 4,5-diiodoimidazole, rather than 4(5)-iodoimidazole. To find them out, however, would require a separated study, and at this stage of the project it was decided rather to focus all the effort on the production of 4,5-diiodoimidazole with the MJOD reactor.

4.4 Synthesis of 4-Iodoimidazole by selective deiodination

The procedure reported at paragraph 3.3 was used to try to reproduce the selective deiodination reaction, thus producing 4-iodoimidazole. This reaction was performed three times, but some difficulties were encountered to reproduce the expected results reaction in an fast and efficient way. More specifically:

- in the first experiment, all the reagents were employed as prescribed by the procedure, with no modifications;
- in the second one, all the mentioned reagents were used except the phenylboronic

acid (Ph-$B(OH)_2$), no other changes were made;

- in the third one, all the reagents were utilised except phenylboronic acid and potassium hydrogen phosphate (K_2HPO_4), with no other changes.

Besides this, the rest of the procedure was conducted as prescribed. Concerning the execution of the reaction, it is worth to mention that, in order to provide a better dissolution of the catalyst ($Pd(OAc)_2$), it is helpful to use an ultrasonic bath and sonicate the palladium solution in methanol for few minutes.

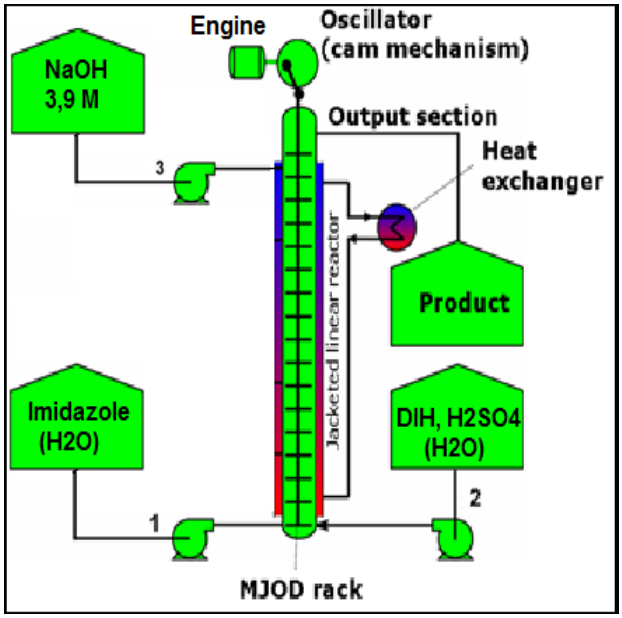
After performing the reactions with the microwave reactor, GC-MS analyses were made to look for the presence of the product, but it wasn't find any. It was firstly supposed that the product needed to be purified better, so that it could have been possible to see its presence via GC-MS. Hence, a new work-up procedure was elaborated to reach this goal. This work-up involved:

1. extraction with 10% HCl (20 mL);
2. extraction with diethyl ether (20 mL);
3. neutralization with NaOH (3M);
4. filtration of the solid, washing it with distilled water, and let it air dry.

After performing this procedure, though, new GC-MS analyses were performed, but again no trace of the expected product was found. In any case, after this last series of experiments, it was decided to fully concentrate all the efforts on the reaction of di-iodination, since it is the one with the procedure that involves less passages and is therefore simpler to transfer on the MJOD reactor.

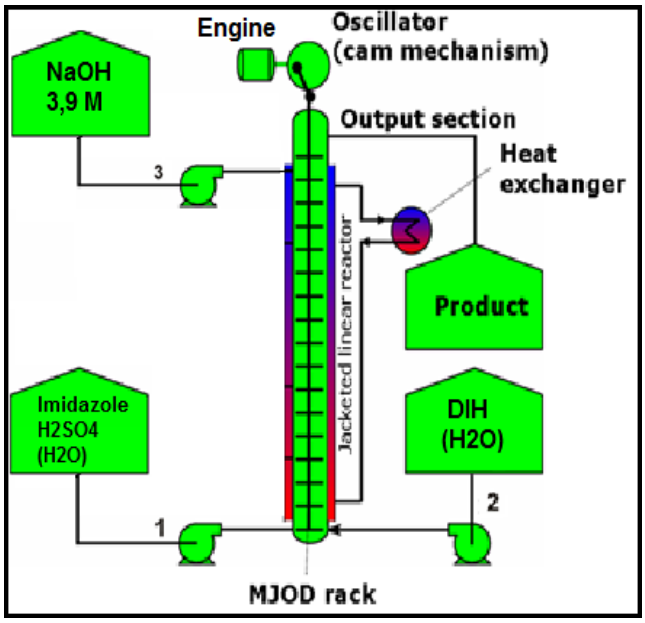
4.5 Synthesis of 4,5-Diiodo-1H-imidazole using the MJOD reactor

When we tried to transfer the reaction of di-iodination from batch scale to the flow reactor, different configurations of the instruments were possible. Different trials were made in order to find out the one which was more appropriate for our experiments. The first configuration that was employed consists of putting imidazole with water in the first reservoir, while having the DIH with water and sulphuric acid in the second one, as is presented in the following *Picture 11*:



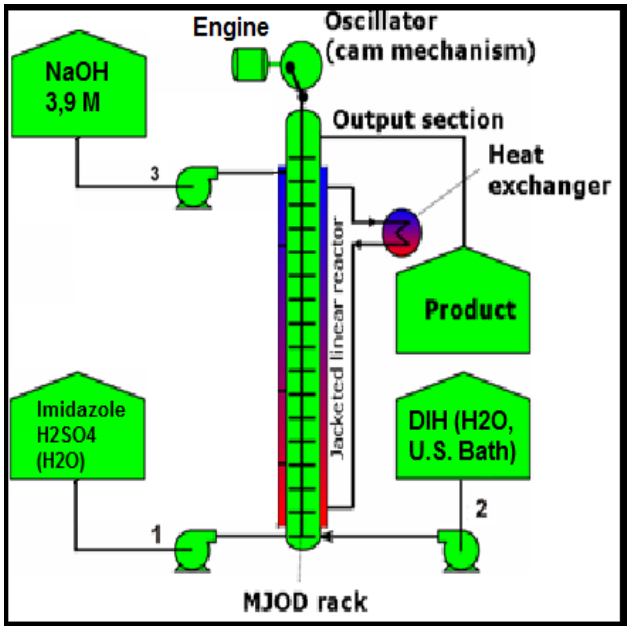
- Picture 11: First configuration of the MJOD reactor.

This configuration turned out not to be optimal, though. In fact, when the DIH is put in contact with the sulphuric acid, it quickly starts to release the iodine, which is easy to spot since the solution manifests a characteristic purple colour. Since the sulphuric acid is the catalyst that activated the DIH, it is important to not let it have any contact with the DIH itself before that the reagent solutions are pumped inside the reactor. If DIH is mixed with H_2SO_4 before the feeding, then it releases all the iodine too early, giving a quite viscous slurry which is difficult to pump inside the reactor. It is important that the contact between the two reagents and H_2SO_4 takes place only inside the body of the MJOD and not before. Otherwise, the yield decreases significantly and the results are lower than usual (see the following ***Table 6*** for further details). Therefore, it was decided to switch the configuration of the reactor. The imidazole was put with water and H_2SO_4 in the first reservoir, and instead the DIH with only water in the second one, as *Picture 12* shows. This new configuration has been maintained for several experiments, in order to prevent an undesired decomposition of the DIH. In particular, the water was added to the DIH reservoir only when needed, thus immediately before the feeding. Even if this prevented the degradation phenomenon, another technical problem had to be solved. In fact, some experiments had to be aborted because of considerable clogging issues with the feeding pumps that made impossible to proceed further.



- Picture 12: Second configuration of the MJOD reactor.

Basically, the DIH is not just a powder, but it can be also a granular substance. Hence, if it is not properly dissolved in the solution, the bigger grains can get stuck in the feeding pumps and in the feeding tubes. When this happens, the pumps have to be thoroughly washed to make them operative again. After observing this, a new modification to the process was made (see *Picture 13*).



- Picture 13: Third configuration of the MJOD reactor.

Where “U.S. Bath” means “ultrasonic bath”, which was used in order to improve the dissolution of the DIH. This was necessary because DIH solutions are usually quite viscous and “slurrish", which may cause clogging of the inlet valves of the MJOD reactor. This issue leads to very low yields or no product at all several times at the end of the run. The introduction of the ultrasonic bath for the DIH solution helped to overcome this problem, as it was also employing wider tubes for the feeding. Another significant modification was a change in the solvent of the DIH solution in some experiments: from water to methanol, since the DIH is better soluble in the last one. Also, since methanol is less viscous than water, the reduced viscosity of the DIH leads to an easier flow inside the pumps and tubes. As consequence, thanks to the lesser pressure drops, the clogging problems of the MJOD reactor were reduced. Anyhow, more factors need to be taken in consideration in order to run the process in a smoother way. Also the temperature is an important variable: since the reaction has to take place at 0°C, it is necessary that there are no flaws in the heat exchanging system (which, in this case, used cold water as refrigerant fluid). Lastly, there is one more technical issue that has to be handled in order to give significantly good yields using the MJOD. The last experiments showed that the NaOH could get stuck inside the reactor, like “coating” the walls, and this was continuously quenching the reaction, giving yield no higher than 70%. After we discovered this, we had to change the cleaning procedure of the reactor, and after this it was possible to achieved a very pure product: 97% 4,5-diiodoimidazole and 3% 4(5)-iodoimidazole (checked with ^{1}H-NMR), which was the best result obtained so far. The former washing procedure involved fluxing the inside of the reactor with distilled water, then with acetone, and finally fluxing with water again. Also washing pure ethyl acetate was tried, but it resulted ineffective. Instead, the new improved procedure required fluxing thoroughly the reactor with hot water and soap (“Renax Ultra”, without phosphate), and then slowly removing the liquid; no acetone was employed. With the former washing method the results were, on average, quite fluctuating; meanwhile ,with the new one, more stable yield values were observed. The next ***Table 6*** reports a summary of the main experiments that were performed, showing the results and the eventual modifications of the different variables of the diiodination reaction that were investigated (reagents quantity, solvents, volumes, and so on). The quantities of the reagents are mainly a x10 scale-up respect the original quantities planned in the original

procedure (see paragraph 3.4), and they are calculated so that the ratio between imidazole and DIH is 1:1. All the experiments had a reaction time of 4 minutes.

Table 6: Experiments with the diiodination reaction, using the MJOD reactor.

Experiment n°	g imidazole	g DIH	Solvent for imidazole	Solvent for DIH	Volume H_2SO_4 96% (mL)	Volume NaOH 3.9M (mL)	g product	Crude Yield (%)
1[e]	0,618	3,450	H_2O, 55mL	H_2O, 45mL	10	95	0,546	18,8
2[f]	0,618	3,450	H_2O, 45mL	H_2O, 55mL	10	95	1,054	36,3
3[f]	0,618	3,451	H_2O, 45mL	H_2O, 55mL	10	95	0,835	28,7
4[e]	0,620	3,452	H_2O, 45mL	H_2O, 55mL	10	95	0,041	1,4
5[g]	0,761	2,122	H_2O, 45mL	H_2O, 55mL	10	95	0,436	24,4
6	0,650	3,492	H_2O, 20mL	CH_3OH, 5mL	5	95	1,631	55,5
7	0,618	3,450	H_2O, 5mL	H_2O, 15mL	10	95	1,089	37,5
8	0,618	3,450	H_2O, 5mL	H_2O, 15mL	10	95	0,722	24,9
9	0,618	3,450	0 mL[a]	H_2O, 15mL	15	95	0,548	18,9
10	0,618	3,450	H_2O, 5mL	H_2O, 15mL	10	95	0,811	27,9
11	0,618	3,450	0 mL[a]	CH_3OH, 4mL	10	95	0,766	26,4
12	0,618	3,450	0 mL[a]	CH_3OH, 5mL	10	95	1,228	42,3
13	0,618	4,140	H_2O, 5mL	CH_3OH, 5mL	10	95	1,340	46,2
14[c]	3,070	17,250	H_2O, 25mL	CH_3OH, 20mL	50	475	3,967	27,5
15	0,618	3,450	H_2O, 5mL	CH_3OH, 5mL	5	95	1,213	41,8
16	0,618	3,450	H_2O, 5mL	CH_3OH, 5mL	1	45	2,010	69,2
17	0,618	3,450	H_2O, 5mL	CH_3OH, 5mL	15	144	-[b]	-[b]
18	0,618	1,725	0 mL[a]	CH_3OH, 3mL	5	46	-[b]	-[b]
19	0,618	6,929	H_2O, 10mL	CH_3OH, 7mL	1	95	-[b]	-[b]
20	0,618	4,485	H_2O, 10mL	CH_3OH, 7mL	1	95	1,282	34,0
21[d]	0,618	4,485	H_2O, 5mL	CH_3OH, 5mL	10	95	4,332	114,7

[a] "0 mL" of solvent for the imidazole means that it was dissolved only in 10 mL of H_2SO_4.

[b] These experiments gave no product, mainly due to technical problems with the MJOD reactor.

[c] This experiment was performed as a x50 scale-up from the original procedure.

[d] The yield of this experiment is apparently higher than 100% because, even if the product was left to air dry overnight, it still retained a considerable amount of water.

[e] This experiment was conducted using the configuration presented in *Picture 11*.

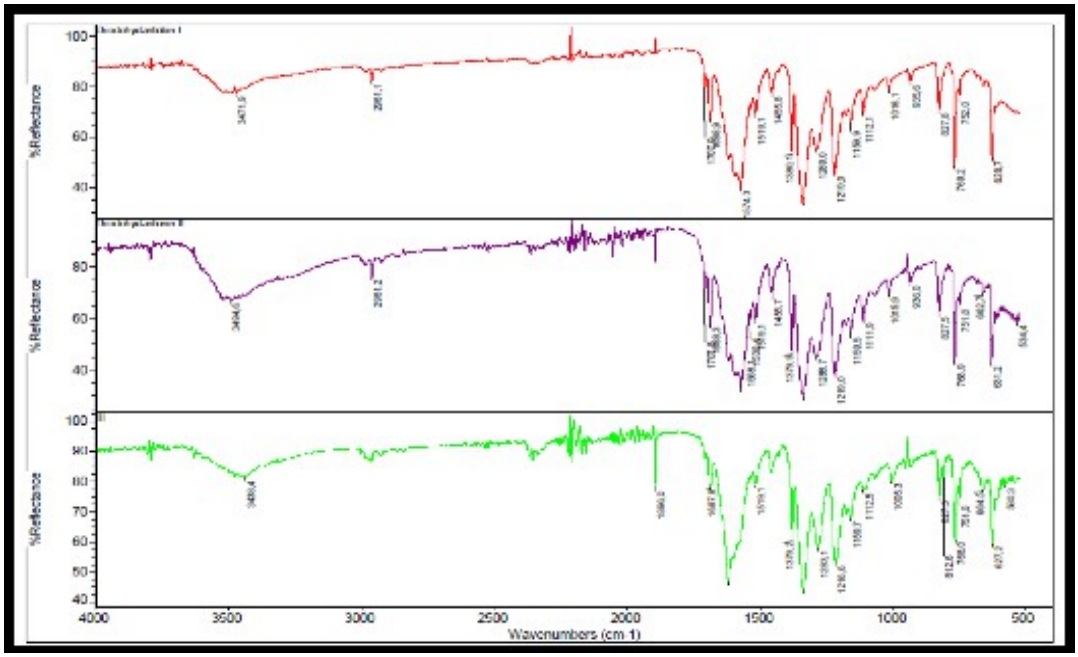
[f] This experiment was conducted using the configuration presented in *Picture 12*.

[g] This experiment and all the following were performed using the configuration presented in *Picture 13*.

4.6 Quality control of the DIH

Since some of the reactions performed using DIH as iodinating agent did not give the

expected results in terms of yield, some investigations were made to discover the cause of these observation. Some analyses were conducted on the DIH itself, in order to verify its purity and quality. The DIH, as previously mentioned, were product in large quantities in a foregoing project that involved the MJOD reactor as well. During this thesis, several different batches of DIH were made and kept in the laboratory. These samples of DIH were the ones utilised to perform all the iodination reactions involved in our project. The point is, though, that during the previous project the operators made some analyses to verify the DIH purity mainly on the last batches product, when the production process was already consolidated and the DIH was obtained in satisfactory yields. In particular, TGA analyses were made to check the DIH quality, which gave promising results. This kind of tests were not performed on the first product samples of DIH, when the procedure was still "work in progress". However, all the produced DIH was conserved anyway, both the batches of know purity and the ones with uncertain purity. FT-IR analyses were executed on some different samples of DIH and the obtained spectra are reported in the following *Picture 14*:



- Picture 14: FT-IR spectra of different samples of N,N'-diiodo-5,5dimehtylhydantoin.

Repeated tests provided analogous results. The spectra are consistent with the ones that were previously registered in the foregoing project, therefore it is possible to confirm that the actual nature of the substance is assured. Afterwards, as described in the

paragraph 3.6, some thermogravimetric tests were performed on the same DIH samples, in order to estimate of its purity. Two different types of support were utilised for his kind of tests, and the results of these tests are reported in the following ***Table 7***:

Table 7: Thermogravimetric analyses on DIH samples.

Sample of DIH n°	Type of support	g DIH before heating	g DIH after heating	g I_2 lost	Effective g DIH	Purity of DIH (%)
1	MW vial	0,065	0,065	0,024	0,036	**55,3**
1	MW vial	0,065	0,065	0,021	0,031	**48,4**
1	hourglass	0,051	0,023	0,028	0,042	**82,2**
1	hourglass	0,050	0,023	0,027	0,040	**80,8**
2	hourglass	0,051	0,022	0,029	0,043	**85,1**
2	hourglass	0,051	0,025	0,026	0,039	**76,3**
2	hourglass	0,050	0,022	0,028	0,042	**83,8**

The experiments made using the microwave vials as supports for the DIH are clearly not consistent with the ones performed utilising the hourglass, since the resulted estimation of the purity are significantly distant from the other ones. It must be considered that probably the test that involved the use of hourglasses are the one that provided the most reliable data, since with this kind of support the heat-transfer area is larger and more optimal than with the microwave vial (which is much narrower). Hence, one can conclude that the analysed samples are the ones with the better quality of DIH. In fact these were the ones employed when conducting the last series of experiments with the MJOD reactor, which gave the most promising results.

4.7 Results of the solubility tests for the DIH in different solvents

The results obtained from the experiments that lead to an estimation of the DIH solubility in water, acetone and methanol, employing the procedure in the earlier paragraph 3.7, are reported in the following ***Table 8)***.

Table 8): Solubiliy tests for DIH in different solvents.

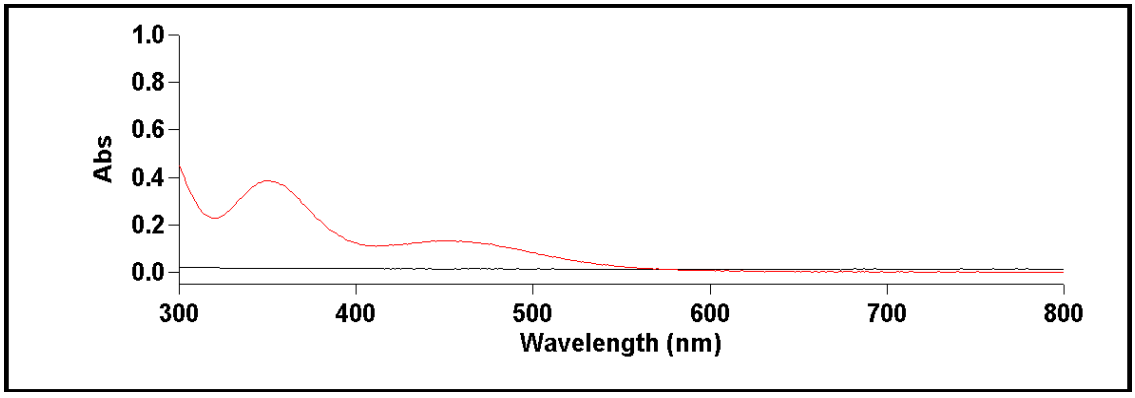
Test n°	g DIH	Solvent	Volume (mL)	Solubility (mol/L)	Solubility (g/L)
1)	0,0102	H_2O	1	0,0269±0,0003	10,2±0,1
2)	0,0108	CH_3COCH_3	1	0,0284±0,0005	10,8±0,2
3)	0,0103	CH_3OH	1	0,0271±0,0005	10,3±0,2

All the tests were conducted at room temperature (~25°C). Repeated proves showed

analogous results. The solubility of the DIH appears to be more than sufficient in all of the three solvents, at least for our purposes. There is one aspect than needs to be pointed out, though: during the tests performed in distilled water, no kind of remaining solids were observed; instead, during the experiments in acetone and methanol there was always some insoluble solid on the bottom of the flask that was impossible to dissolve (at least a room temperature), even applying an energetic magnetic stirring. It is easy to supposed that this solid is due to some kind of reaction impurity that was present in the DIH sample from the beginning. We can expect this problem, since it is know that the DIH was of course not reagent pure grade, but it was produced in the laboratories of the Department of Chemistry of Bergen with the MJOD reactor in a previous project, as mentioned. This has to be taken into account during the execution of the following analysis because, theoretically, these impurities may somehow interfere with the spectrophotometric and HPLC tests. This needs to be verified, though.

4.8 Spectrophotometric analysis of a DIH solution in water

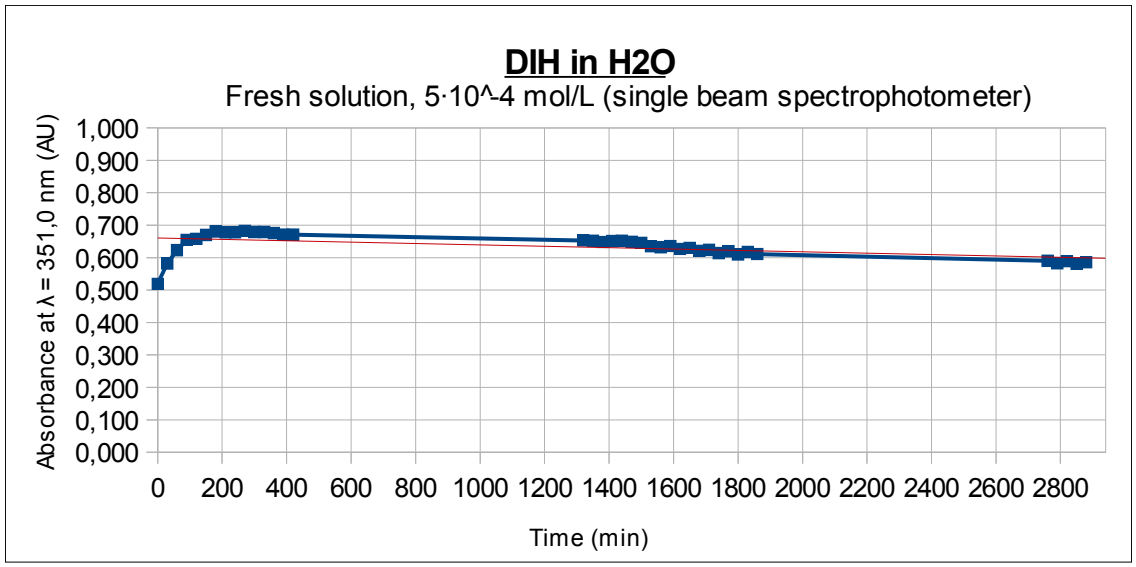
For every DIH solution that was prepared, the absorption spectrum was registered using a double beam spectrophotometer in order to find any absorption peak for that solution in the considered range of wavelength. Then, in a second moment, we monitored how did the absorbance of the peak change with time using a single beam spectrophotometer. The experimental observations are presented below, and the discussion of the results is summarized in the paragraph 4.11. In this first experiment, a $5 \cdot 10^{-4}$ mol/L DIH solution in H_2O was prepared and analysed with a double beam spectrophotometer, which resulted in the following absorption spectrum:



- Graphic 1: UV-Vis spectrum of DIH in distilled water in a range of λ = 300-800 nm; λ_{max} = 351.0 nm.

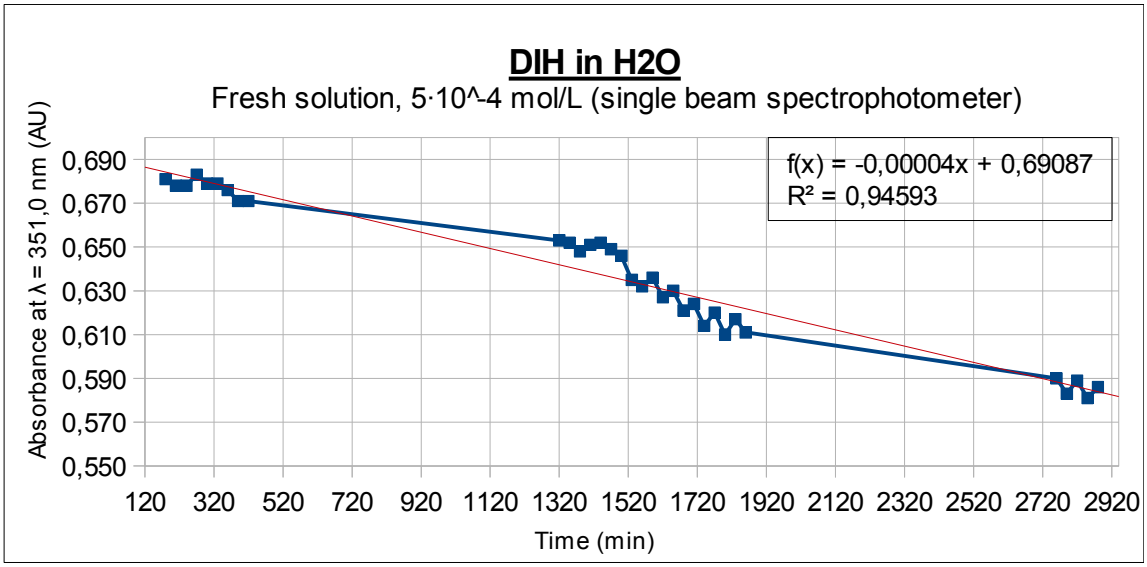
Concerning the recording of the spectra: the double beam UV-Vis spectrophotometer does an automatic subtraction of the signal of blank; hence the peak intensity is the difference between the peak maximum and the bottom of the scale. The same thing applies also for all the other following spectra recorded with the double beam spectrophotometer.

The considered wavelength range for this experiment was from 800 nm to 300 nm. The absorption peak was located at 351,0 nm. The absorbance of the sample was monitored for 48 hours, checking its value every 30 minutes with a single beam spectrophotometer, and observing the trend showed below. Since the photometric accuracy of the spectrophotometer is ±0,005 Absorbance Units (AU), this is the error that should be considered related to every single measure that is made.



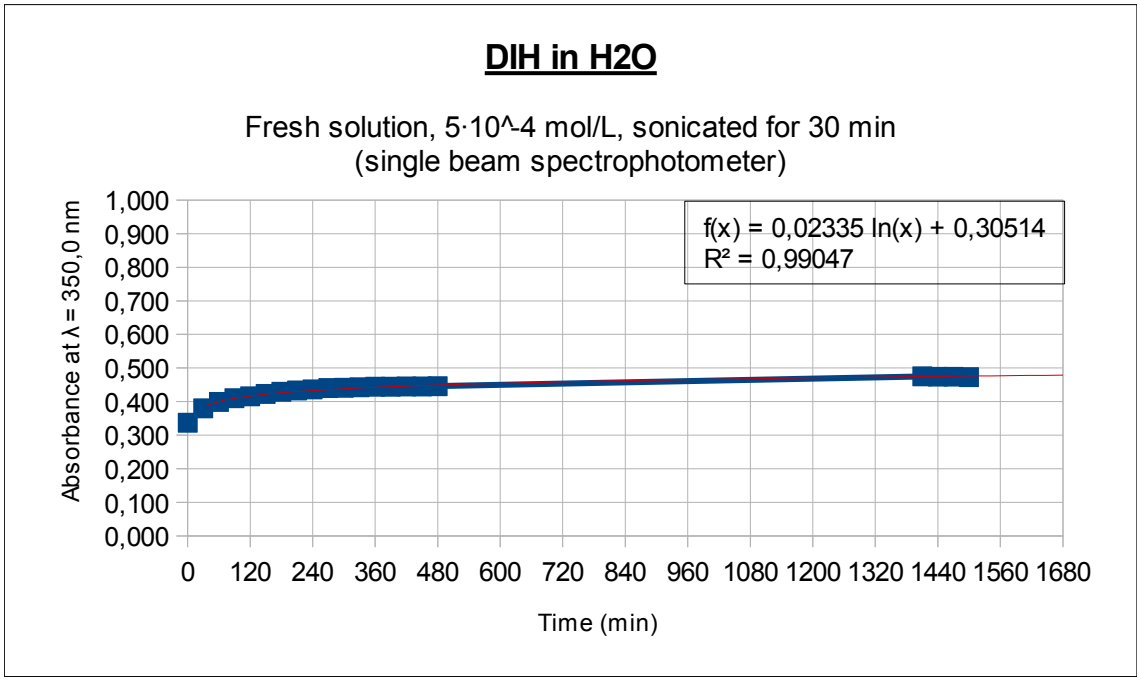
- Graphic 2: Absorbance trend of DIH in distilled water at λ = 351.0 nm in a 48 hours timespan.

The increasing of absorbance during the first three hours is probably due to the fact that the DIH wasn't still completely dissolved, even after the magnetic stirring of the solution, and hence its dissolution with time causes this increasing of absorbance. After three hours, the absorbance decreases very slowly with a linear trend (with some normal statistical fluctuations in the signal), as shown in the next *Graphic 3* which presents a magnification of the fluctuations from t = 120 minutes to t = 2880 minutes.



- Graphic 3: Absorbance trend of DIH at λ = 351.0 nm in a 48 hours timespan (from hour 3 to 48).

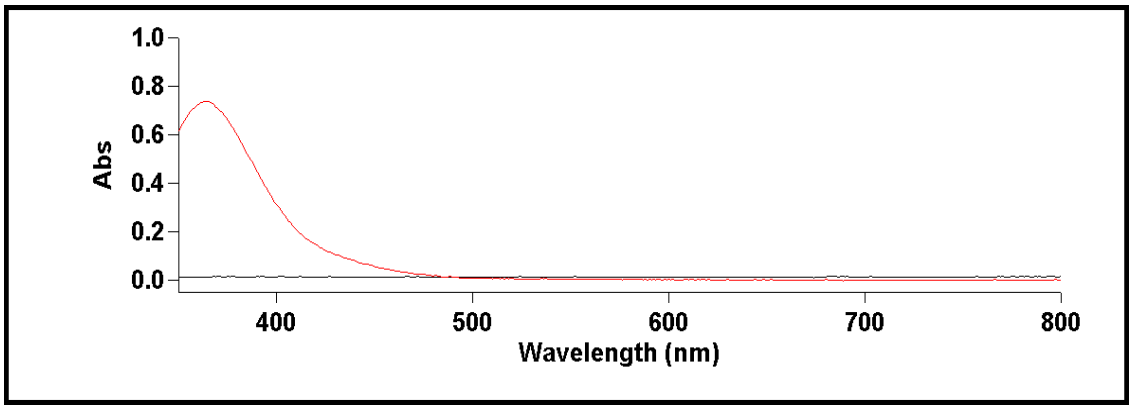
Repeated analyses gave compatible results with what presented above. It was also tried to sonicate the DIH solution for 30 minutes before doing the analyses, to check if it was possible to have an improved dissolution of the solute in the water, but as we can see from the next *Graphic 4*, the results were practically the same as before.



- Graphic 4: Absorbance trend of DIH at λ = 351.0 nm in a 6 hours timespan.

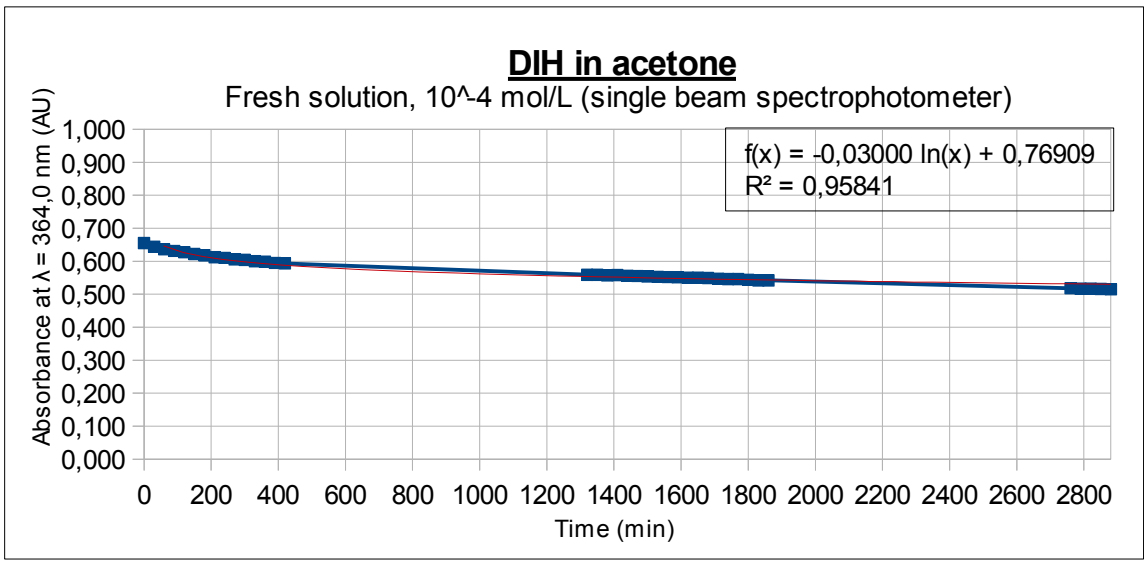
4.9 Spectrophotometric analysis of a DIH solution in acetone

A 10^{-4} mol/L DIH solution in acetone was prepared and analysed with a double beam spectrophotometer. Here is the registered absorption spectrum:



- Graphic 5: UV-Vis spectrum of DIH in acetone in a range of λ = 350-800 nm; λ_{max} = 364.0 nm.

The considered wavelength range was from 800 nm to 350 nm. The absorption peak was situated at 364,0 nm. Similarly as the previous solution, the absorbance of this sample was monitored for 48 hours, checking it every 30 minutes. The registered trend is presented below.

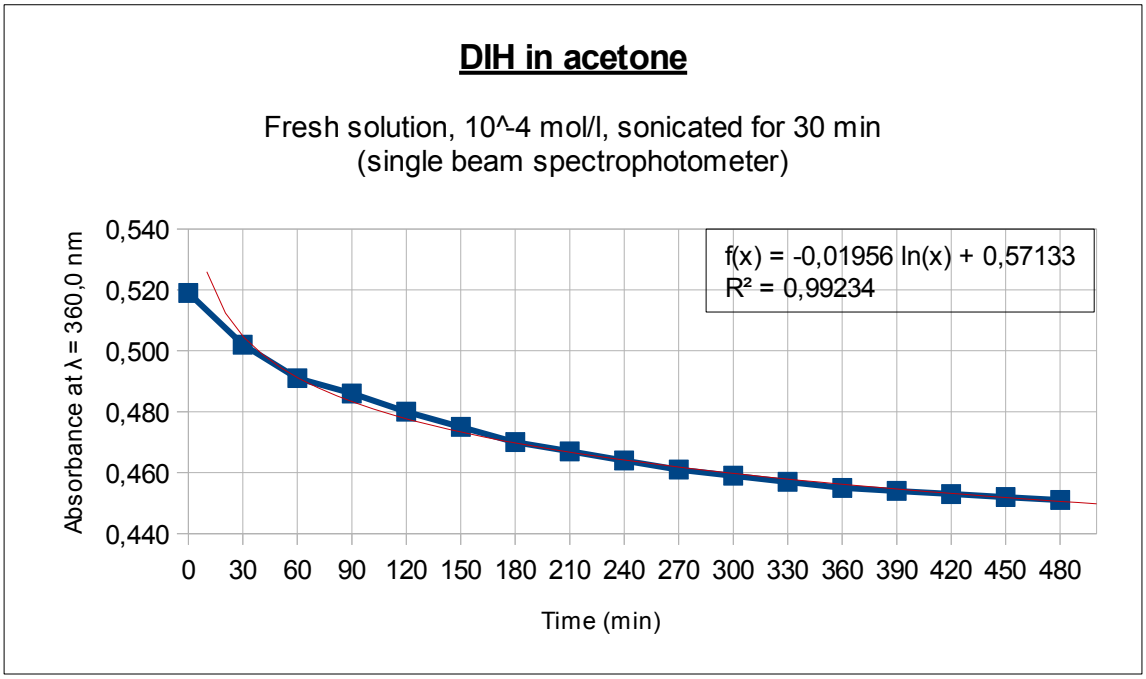


- Graphic 6: Absorbance trend of DIH in acetone at λ = 364.0 nm in a 48 hours timespan.

Again, repeated analyses gave data that were consistent with this trend, with a very good correlation coefficient of Pearson (R^2), as shown in the previous chart.

Furthermore, similarly to the previous tests executed for the DIH in water, also in this set of experiments the N,N'-diiodo-5,5-dimethylhydantoin solution was sonicated in an ultrasonic bath for 30 minutes before performing the analyses, in order to have a better dissolution of the DIH itself in acetone. This time, the correlation between the absorbance value is even stronger than before, following a significant decrease in a timespan of eight hours.

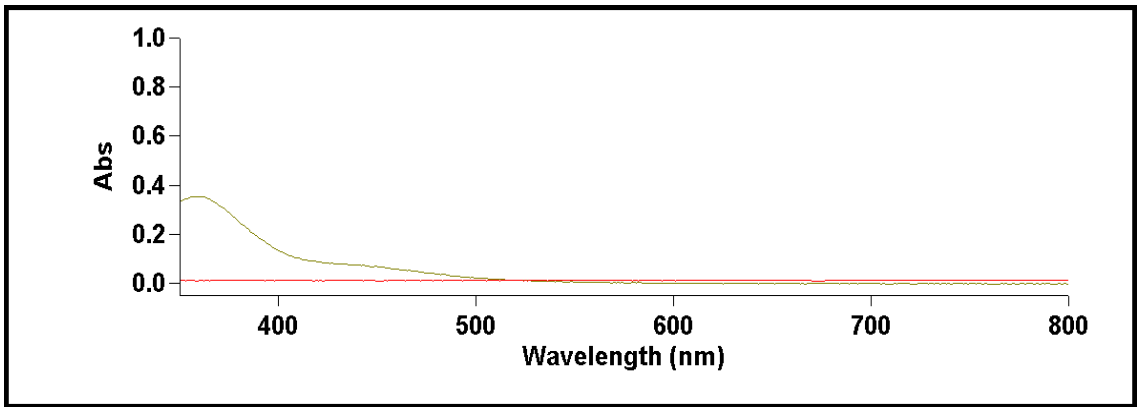
The successive graphic illustrates the trend of the values. Looking at this charts, that is something that appears rapidly clear: somehow, the DIH seems to be unstable and to undergo some kind of degradation when put in acetone. Therefore, it would not be possible to perform any kind of calibration curve with this solvent, since the concentration of DIH decreases too quickly and this would obviously falsify the results of the analysis. This is the reason why performing HPLC test with DIH in acetone was not even tried during the work, since it would be impracticable.



- Graphic 7: Absorbance trend of DIH in acetone at λ = 360.0 nm in a 8 hours timespan.

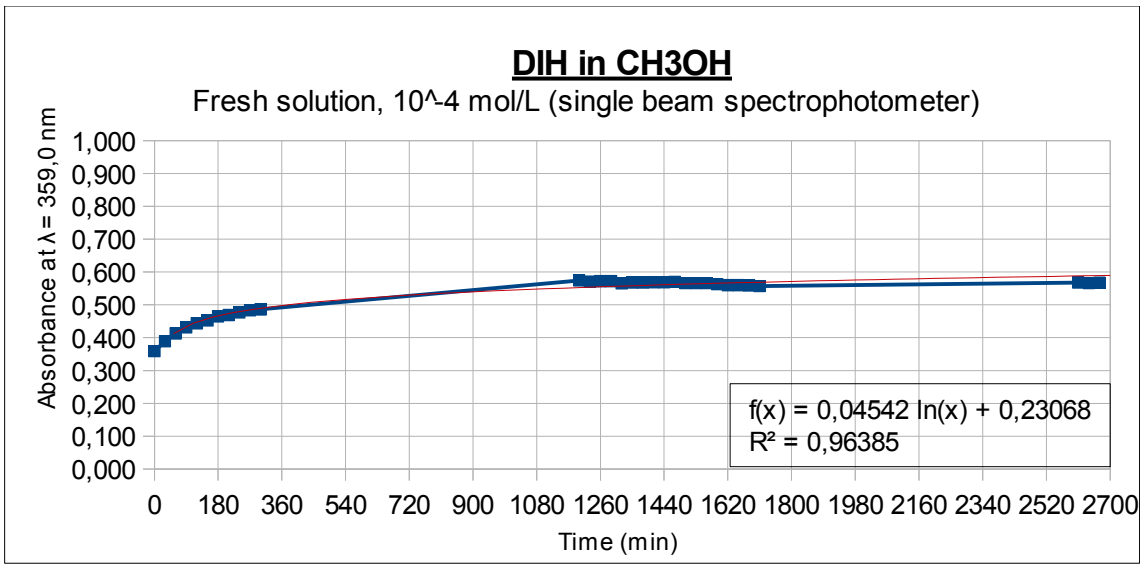
4.10 Spectrophotometric analysis of a DIH solution in methanol

A 10^{-4} mol/L DIH solution in CH3OH was prepared and analysed with a double beam spectrophotometer. The observed absorption spectrum is the following:



- Graphic 8: UV-Vis spectrum of DIH in methanol in a range of λ = 350-800 nm; λ_{max} = 359.0 nm.

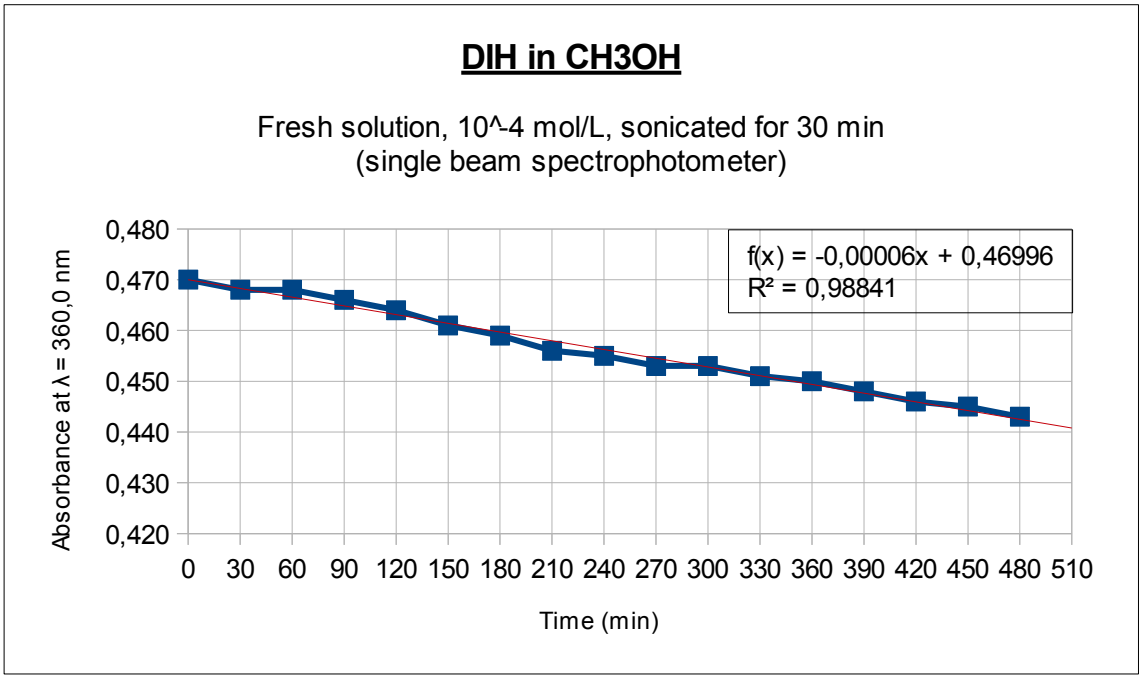
The wavelength range was from 800 nm to 350 nm. The absorption peak was at 359,0 nm. The absorbance of this solution was monitored for more than 44 hours, checking its value every 30 minutes as usual. The following graph reports the results.



- Graphic 9: Absorbance trend of DIH in methanol at λ = 359.0 nm in a 44 hours timespan.

In a similar way to the DIH solution in H_2O, also here the increasing of absorbance is likely to be explained with an incomplete dissolution of the DIH in the CH_3OH, even

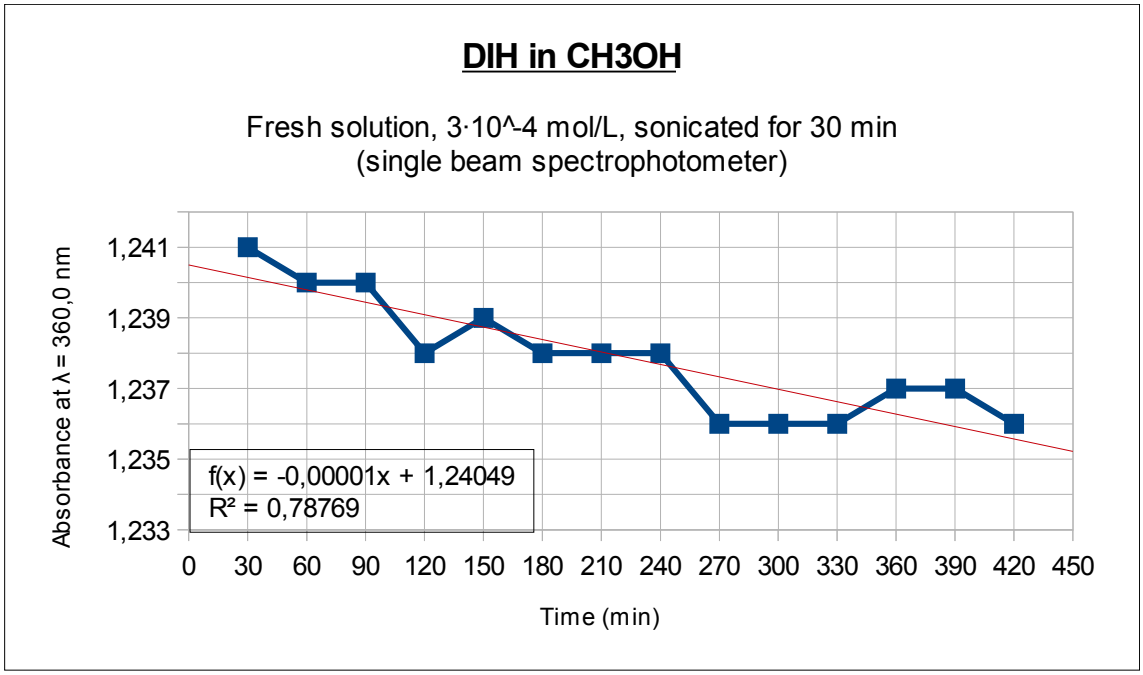
after an extended and vigorous magnetic stirring. In this case, the absorbance seems to reach a sort of plateau with time. While doing the experiments with sonication of the solution, we also looked for any sort of variation in results due to the concentration. On a practical level, three solutions of DIH in methanol of three different concentrations were prepared ($1 \cdot 10^{-4}$ M, $3 \cdot 10^{-4}$ M, and $5 \cdot 10^{-4}$ M), and they were all analysed with the same procedure that was applied in the previous experiments. In particular, the methanol that was utilised as solvent was "Chromanorm" CH_3OH, which is methanol that is usually employed in HPLC systems. This was done foreseeing that the first HPLC tests would have been performed in methanol, and so having a better consistency between the two kind of analyses. For the $1 \cdot 10^{-4}$ M solution, the following trend was observed:



- Graphic 10: Absorbance trend of $1 \cdot 10^{-4}$ DIH solution in methanol at λ = 360.0 nm in a 8 hours timespan.

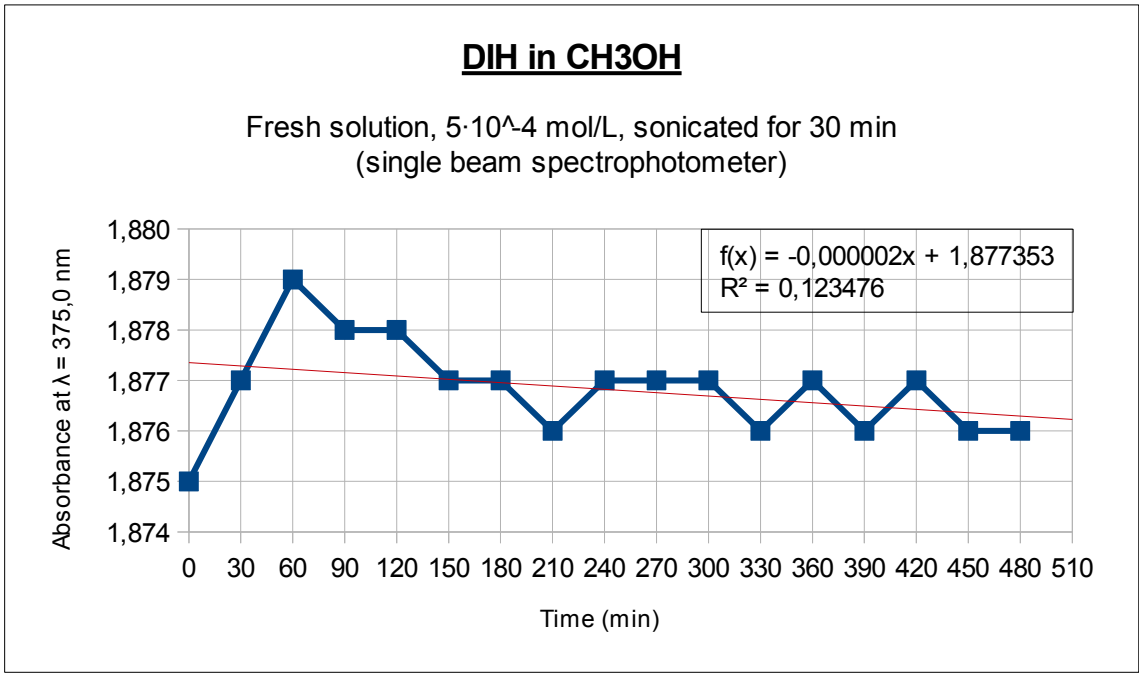
The absorbance decreases with time, but very slowly as we can see by the value of the slope in the regression line. Moreover, the correlation between the data is again very strong, which may lead to think at a good consistency between the data themselves. The same very slow absorbance decrease also occurs with a $3 \cdot 10^{-4}$ M solution, as seen in the next *Graphic 11*. There are a bit more instrumental fluctuations of absorbance in this case, but this is not so strange considering that the absorbance values are slightly higher

than 1, so this is predictable and still acceptable.



- Graphic 11: Absorbance trend of $3 \cdot 10^{-4}$ DIH solution in methanol at λ = 360.0 nm in a 7 hours timespan.

Finally, a test with $5 \cdot 10^{-4}$ M DIH solution was also performed, even if this is at the limit of acceptability concerning the absorbance values. But even though it is so, the trend that resulted is surprisingly regular, as we see from the following chart:



- Graphic 12: Absorbance trend of $5 \cdot 10^{-4}$ M DIH solution in methanol at λ=375.0 nm in a 8 hours timespan

Considering that, as previously mentioned, the spectrophotometer has a photometric accuracy of ±0,005, this basically means that the absorbance values are, in reality, the exact same value from a statistically point of view and the fluctuations are just due to the instrument, which is normal. This also explains why the slope in the regression line is so low and therefore again, as in the previous experiments, DIH shows a good stability in solution in the timespan of some hours. A part from this, anyway, it would not be possible to perform this type of test on a more concentrated solution, because in that case the absorbance would rapidly go above 2 and at that level there would be no consistency between the data at all. This also implies that the concentration range that could be useful to make a calibration curve is, on a practical level, very narrow. Naturally, this has to be taken into proper account when operating with the HPLC equipment. The following ***Table 9*** summarizes the operative conditions employed for all the spectrophotometric analyses.

Table 9: Summary of the operative conditions for spectrophotometric analyses.

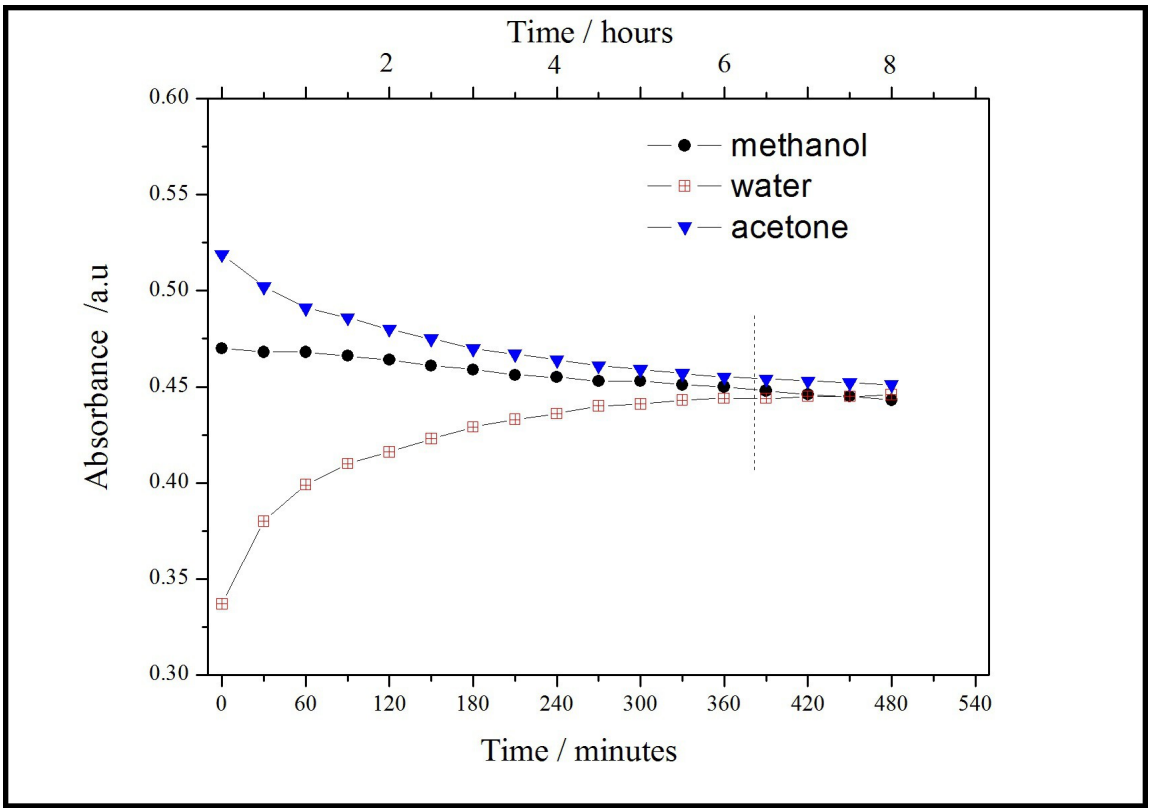
Solvent	DIH Concentration (mol/L)	λ (nm)	Temperature (°C)	Monitoring time (h)
H_2O[a]	$5 \cdot 10^{-4}$	351,0	25	48
H_2O	$5 \cdot 10^{-4}$	351,0	25	26
Acetone[a]	$1 \cdot 10^{-4}$	364,0	25	48
Acetone	$1 \cdot 10^{-4}$	360,0	25	8
CH_3OH[a]	$1 \cdot 10^{-4}$	359,0	25	44
CH_3OH	$1 \cdot 10^{-4}$	360,0	25	8
CH_3OH	$3 \cdot 10^{-4}$	360,0	25	7
CH_3OH	$5 \cdot 10^{-4}$	375,0	25	8

[a] These tests were performed without sonication of the DIH solution.

4.11 Considerations about the spectrophotometric analysis of DIH

Looking at the trends displayed by the absorbance of DIH in water, acetone and methanol, it seems like it is quite stable in all three solvents, at least on the short run (below the 24 hours). In fact, the slope of the above mentioned trends is actually quite little, which means that the DIH absorbance (and, in the same way, its concentration) decreases only at a very slow rate. It is necessary to wait at least 48 hours or more to notice any statistically relevant decrease of the absorbance values. Considering the usual fluctuations of values (due to normal instrumental noise) and the error associated at every measure (due to the photometric accuracy of the spectrophotometer itself), it's possible to say that in a short period of time (3-6 hours) the absorbance is almost in a

situation of plateau, and only after more hours it start to diminish. These tests would lead us to be optimistic concerning the stability of DIH in the three solvents, at least at room temperature. The following *Graphic 13* shows a comparison between the trends of the DIH solutions in the different solvents with time. It is interesting to notice how all the three trends come together towards the same average values, but only the methanol shows an almost linear progress. Therefore, it makes sense to use CH_3OH as solvent for HPLC analyses, if one wants to try to build a calibration curve for DIH.

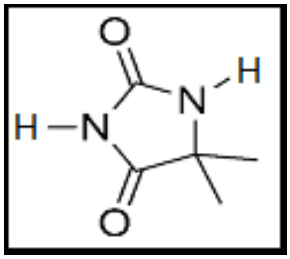


- Graphic 13: Comparison between the absorbance trends of DIH solutions in H_2O, acetone, and CH_3OH.

4.12 Spectrophotometric and HPLC analysis of DMH solutions in methanol

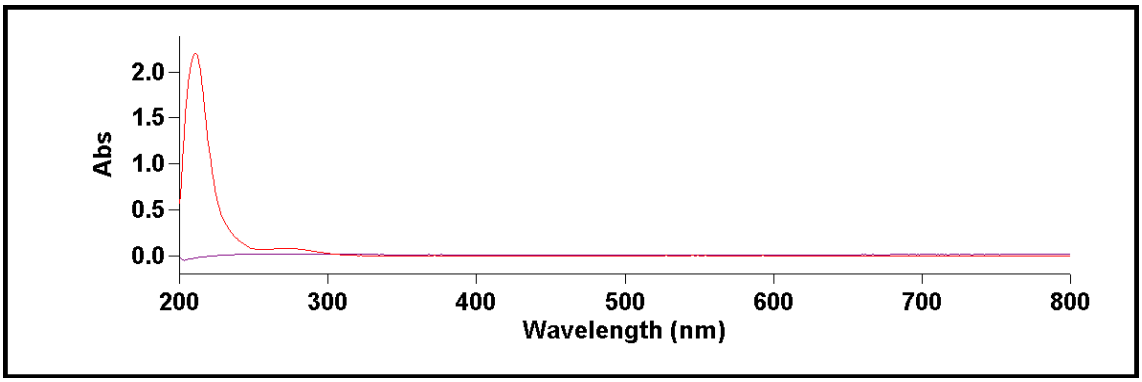
As a customary for any HPLC analyses in their first applications, it is necessary to have a reference material for retention time (t_{REF}). In this case, we used a sample of 5,5-dimethylhydantoin ("DMH") as a reference, which is the equivalent of DIH without the two iodine atoms. It is also a by-product of the iodination reaction, since this is the form that DIH assumes after having iodinated the imidazole (both via a di-iodination or a

mono-iodination).



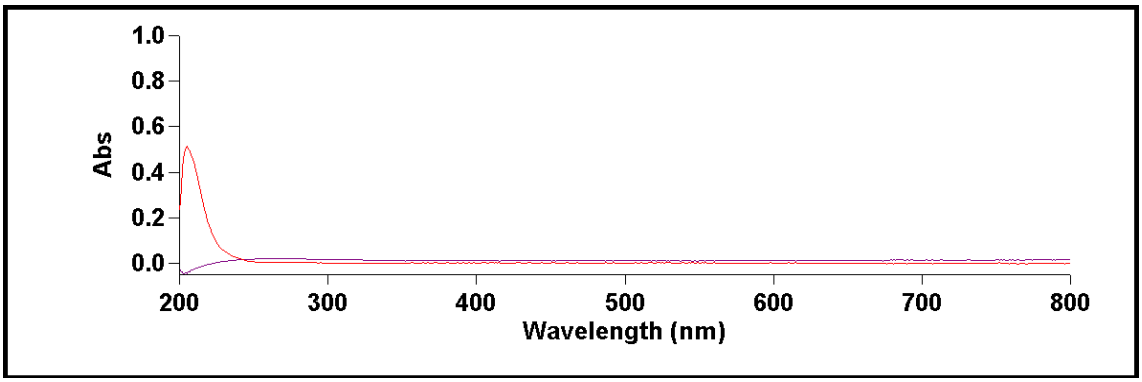
- Picture 15: Molecular structure of 5,5-dimethylhydantoin (DMH)

Firstly, a 10^{-3} M DMH solution in Chromanorm CH_3OH was analysed with a double beam spectrometer to obtain its UV-Vis spectrum, which is reported in the next graphic. The reason why "Chromanorm" methanol was also used as solvent in the following HPLC tests.



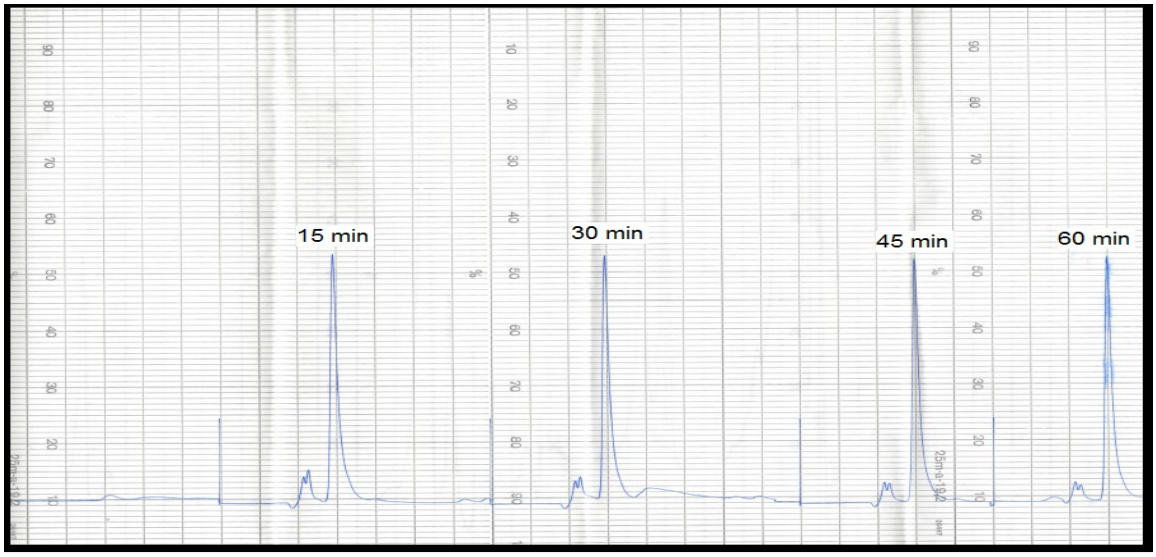
- Graphic 14: UV-Vis spectrum of a 10^{-3} M DMH in methanol in a range of λ=200-800 nm; λ_{max}=211.0 nm.

The wavelength range was from 800 nm to 200 nm. The absorption peak was at 211.0 nm, with a secondary peak at 270.0 nm. The same test was performed also on a 10^{-4} M solution, and the results were the following:

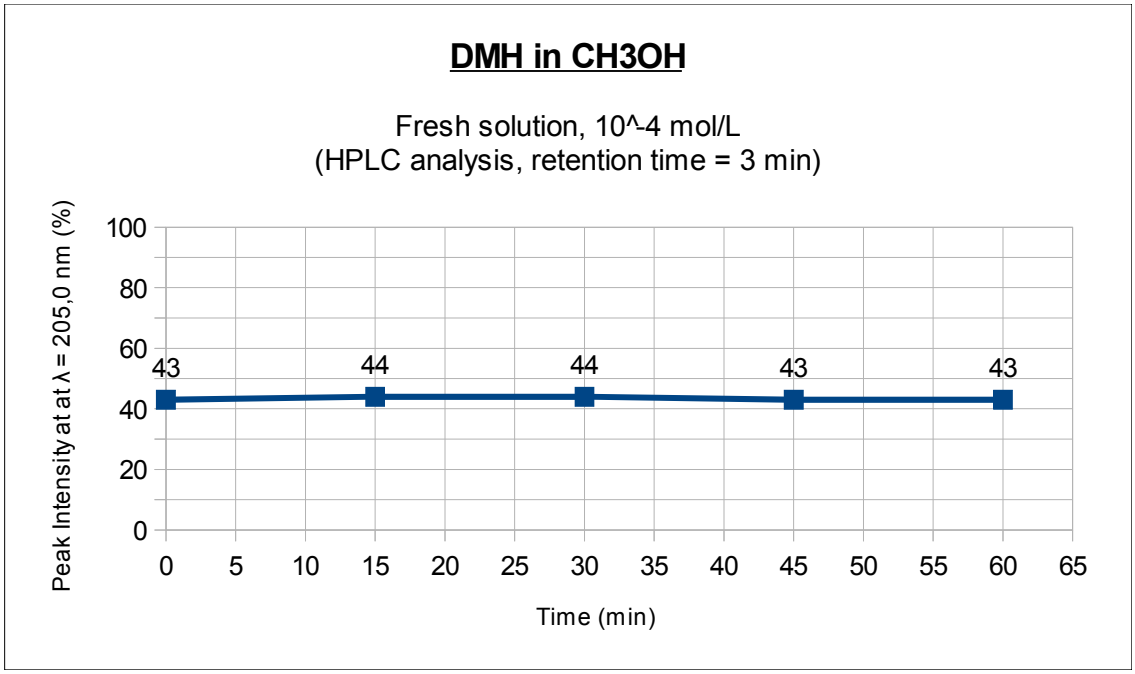


- Graphic 15: UV-Vis spectrum of a 10^{-34} M DMH in methanol in a range of λ=200-800 nm; λ_{max}=205.0 nm

The wavelength range was the same as before, with an absorption peak at 205.0 nm. This is the wavelength that has been set up in also in the HPLC system. The reason is that this last experiment was probably more consistent than the previous one, since in the previous the absorbance reached a value higher than 2, so we are already outside the linearity range of the Lambert-Beer Law. After these preliminary experiments, an HPLC test was performed with a 10^{-4} M DMH solution, in order to find out its retention time, as reported in the following *Picture 16* and *Graphic 16*.



- Picture 16: Sample of HPLC peaks of 10^{-4} M DMH solution in CH_3OH at λ = 205.0 nm.



- Graphic 16: Peak intensity trend of a 10^{-4} M DMH solution at λ = 205.0 nm in a one hour timespan.

The DMH signal is, as foreseen, very homogeneous and linear since it is 99% pure. The retention time that was observed was 3 minutes, and this has been the "reference t_{REF}" for the next DIH HPLC analyses. In the next *Picture 16* is reported the image of the recorded HPLC peaks of DMH.

4.13 HPLC analysis of a DIH solution in methanol

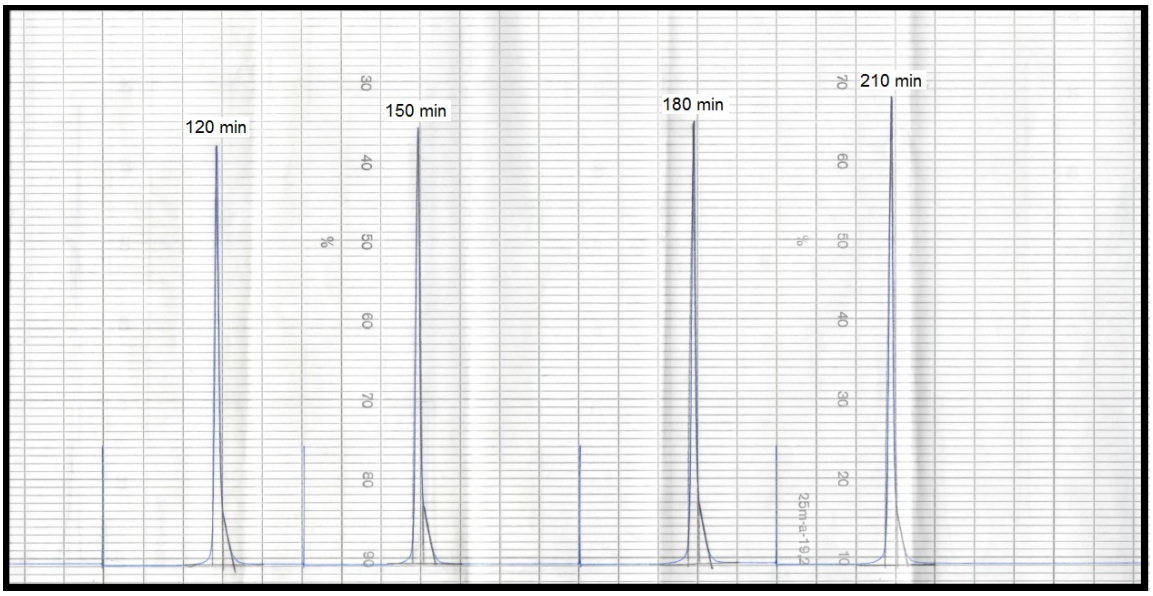
First of all, some preliminary tests with DIH solutions in methanol were required, in order to find which was the appropriate range both for the spectrophotometric detector and the recorded of the HPLC. This as proved to be a quite delicate operation, since setting the above mentioned ranges to lower levels (thus increasing the sensitivity) also implied to increase the background noise and, eventually, even provoking a significant drift in the baseline toward higher values. This last technical issue can seriously interfere with the interpretation of the signals and it has to be controlled as much as possible at hardware level. Furthermore, also in this series of analyses, performing a sonication of t he solutions in an ultrasonic bath resulted very important: often the simple magnetic stirring, though vigorous, is not sufficient to properly dissolve the solute, and this could cause the results to be not completely consistent between each other, thus nullifying the value of the analysis itself. Therefore, each solution has been preventively sonicated for 30 minute before injecting it in the HPLC.

In this series of experiments, both the effect of concentration and temperature were considered. The analyses were conducted using solutions at different concentrations of DIH, namely: $1 \cdot 10^{-4}$ M, $2{,}5 \cdot 10^{-4}$ M, $3 \cdot 10^{-4}$ M, and $5 \cdot 10^{-4}$ M. All this HPLC experiments were executed at room temperature (25°C). Afterwards, new tests were done on a $5 \cdot 10^{-4}$ M solution at different temperatures: 30°, 35° and 45°C. Subsequently, we moved to performing the analyses using UPP (ultra-purified pure) water. In this paragraph we present the results obtained with the DIH methanol solutions, as long with the encountered issues and some considerations concerning the empirical observations. Correlations were outlined between the intensity of the HPLC peaks and time, as well as between the peak area and time, in order to look for evidences regarding the DIH stability in methanol. It has to be underlined that the sensitivity of the spectrophotometric detector of the HPLC has needed to be changed and adapted to every different concentration, so that it is possible to properly appreciate the signals. This is the reason

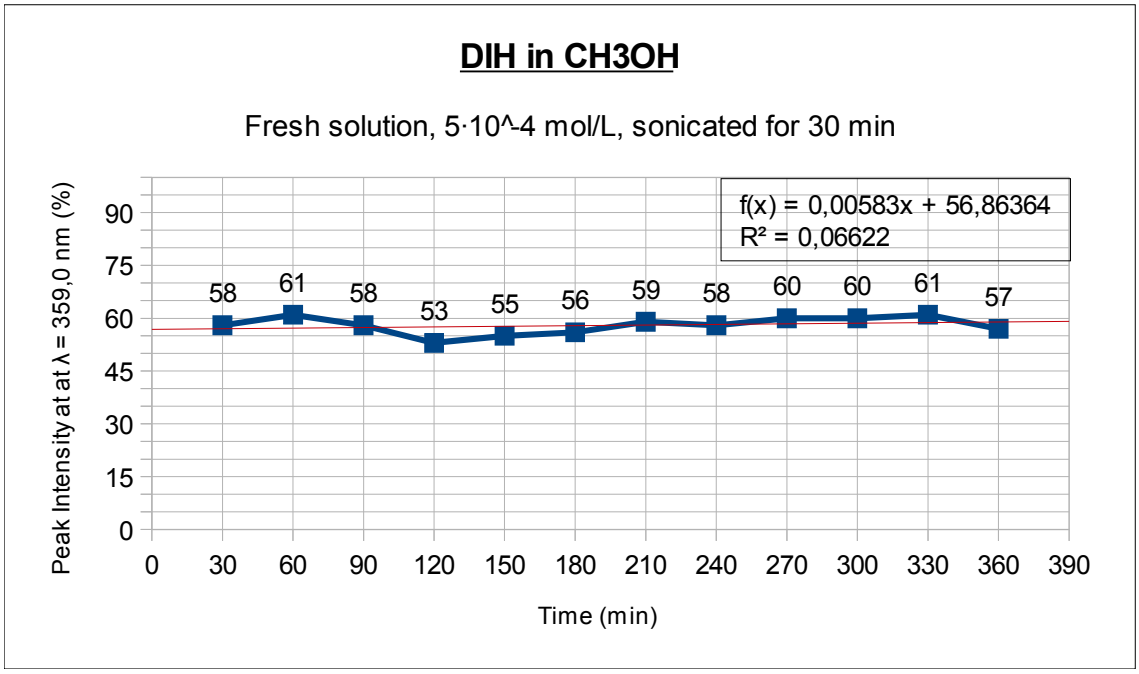
why, in the following graphics, the intensity of the signals do not grow proportionally while, instead, the concentration does: the sensitivity simply has been modified to every specific case.

The first test was performed using a $5 \cdot 10^{-4}$ M DIH solution, prepared with the usual methodology, which has been monitored for six hours. Every injection gave a very sharp single peak (see *Picture 17*), whose intensity (and area) remained mostly uniform during the considered timespan. Some fluctuations are present, but they are still in an acceptable range. *Graphic 17* shows how varies the peak intensity of the solution with time, the slope of the regression line is very close to zero. The retention time was 2 minutes and 54 seconds. A similar trend is also observed when we use the peak area in the y-axis (see the following *Graphic 18*).

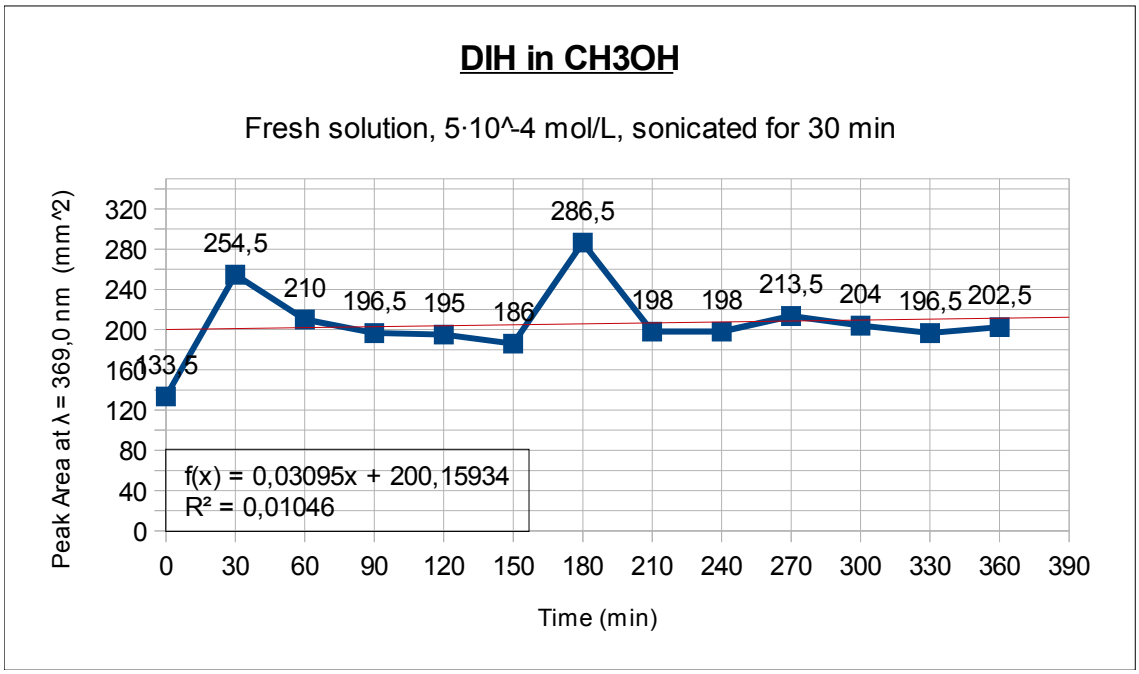
In such case there are some probable outlayers that can not be ignored. Nevertheless, even with these minor fluctuations, the peak area is regular and the slope is very close to zero. As usual, repeated tests conducted in the same way gave analogous results. Therefore, apparently this DIH methanol solution showed a good degree of stability at least at room temperature and in a period of six hours. *Picture 17* represents a sample of the recorded peaks for this solution.



- Picture 17: Sample of HPLC peaks of a $5 \cdot 10^{-4}$ M DIH solution in CH_3OH at λ = 359.0 nm.

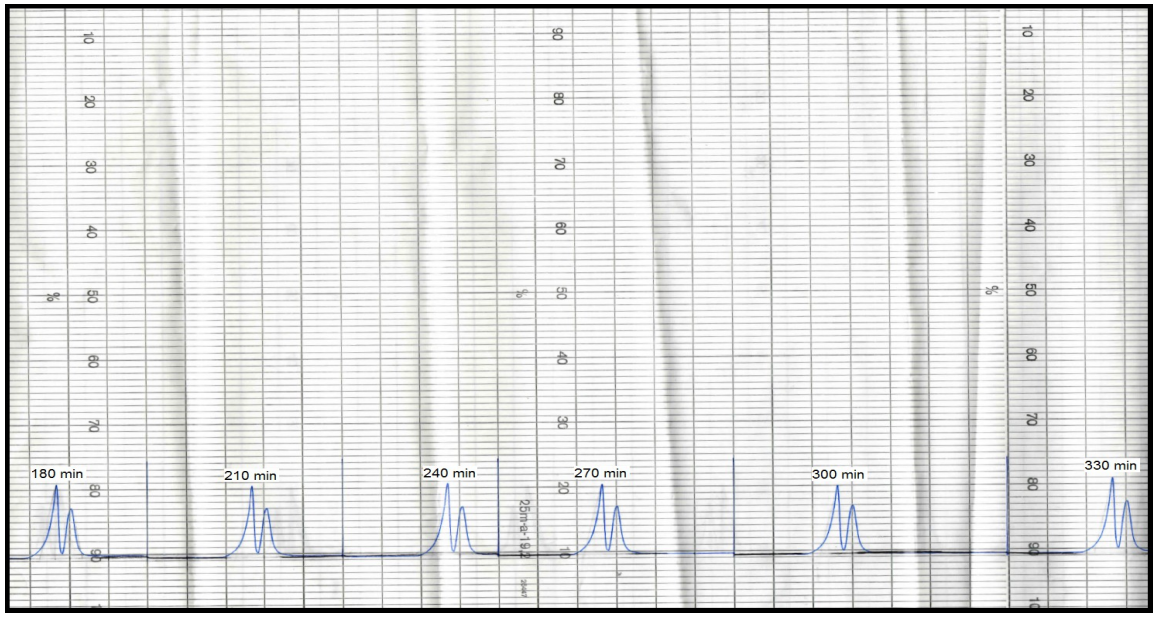


- Graphic 17: Peak intensity trend of a 5·10^{-4} M DIH solution at λ = 359.0 nm in a six hours timespan.

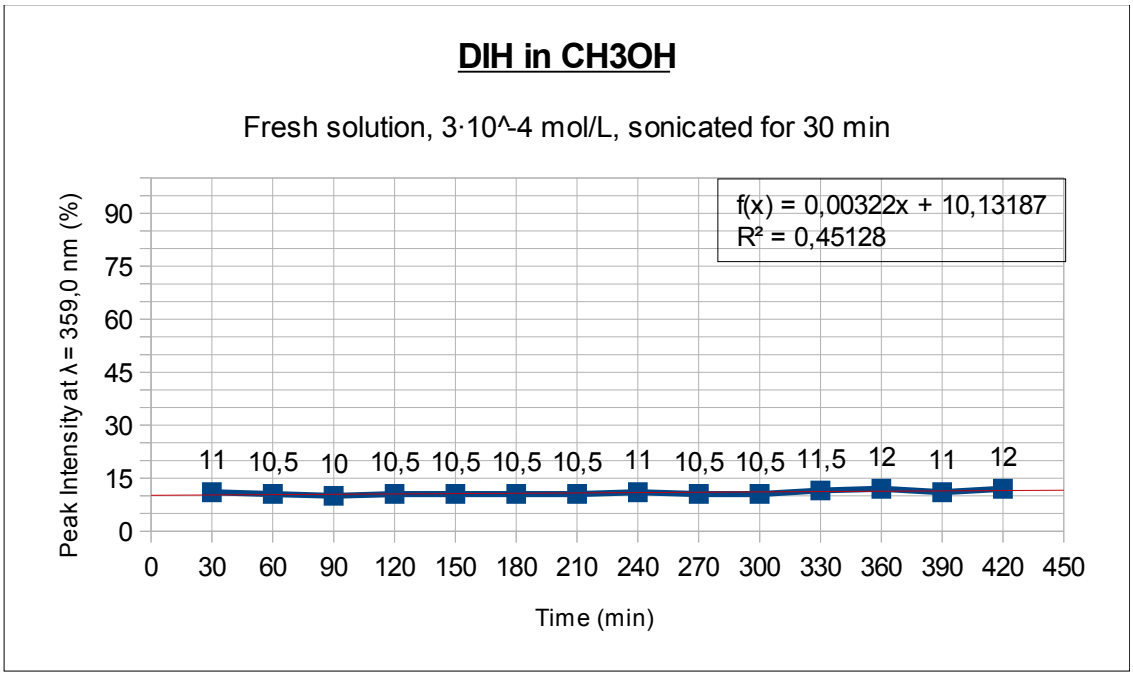


- Graphic 18: Peak area trend of a 5·10^{-4} M DIH solution at λ = 359.0 nm in a six hours timespan.

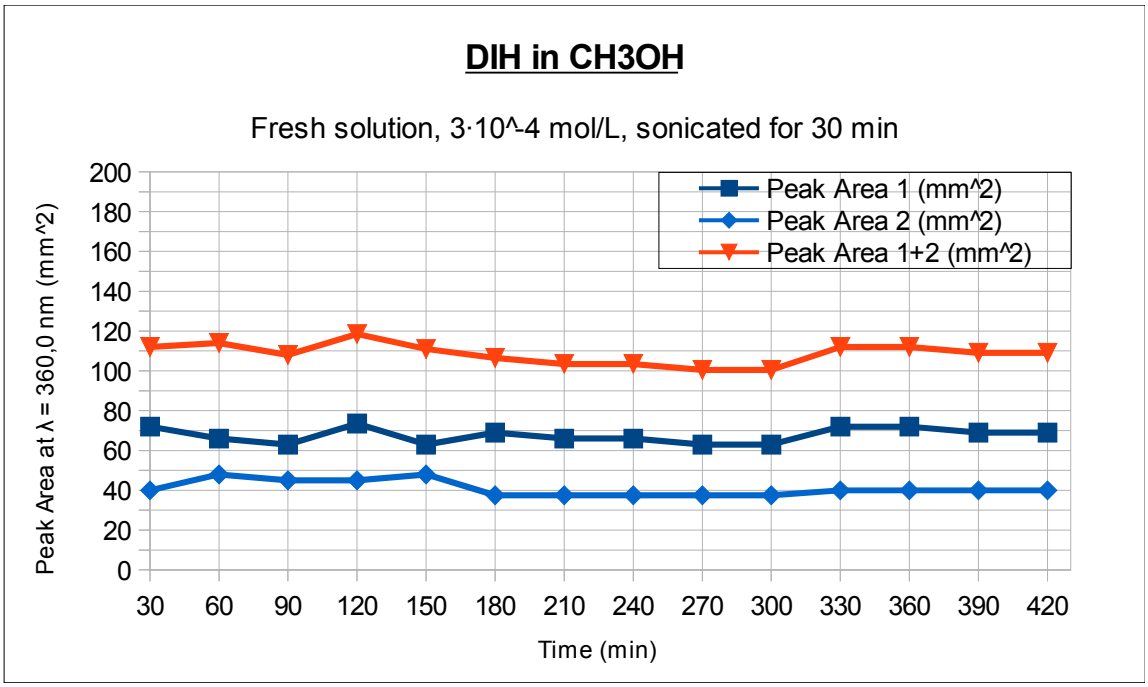
Successively, a new set of analyses were performed on a $3 \cdot 10^{-4}$ M solution. Surprisingly, this time we observed two peaks: a first, higher, peak with the same retention time as before (2'54"); and a second, shorter, peak with a longer t_{RET} of 3 minutes and 6 seconds, which was not expected (see the following *Picture 18*). From now, the first peak is called "peak 1", and the second peak "peak 2". *Graphic 19* reports the variation of the intensity of peak 1 with time, which resulted once again in a trend with no particular fluctuations, except the normal instrumental ones. In *Graphic 20* are reported the correlation between Peak 1 area with time, Peak 2 area with time, and finally the sum of the two areas "Peak 1 + Peak 2" with time. The nature of the second peak is not clear, but some considerations can be presented. The fractions of the HPLC runs were collected and analysed wit a GC-MS instrument but, even applying a "soft" programming with the lowest temperature increase possible, the species contained in the solutions were too fragile and heat-sensitive to be detected by the machine. As result, only the background noise was observed in the GC-MS spectra. It is known, though, that N,N'-diiodo-5,5-dimethylhydantoin can quickly undergo some sort of degradation due to heat, so this is not a surprise and it could be expected that it decomposes during the injection in the GC-MS system that occurs at 200°C minimum. Anyhow, the monitoring was performed for seven hours with no interruption according to the standard procedure, at the same wavelength as in the previous experiments.



- Picture 18: Sample of HPLC peaks of a $3 \cdot 10^{-4}$ M DIH solution in CH_3OH at λ = 359.0 nm.

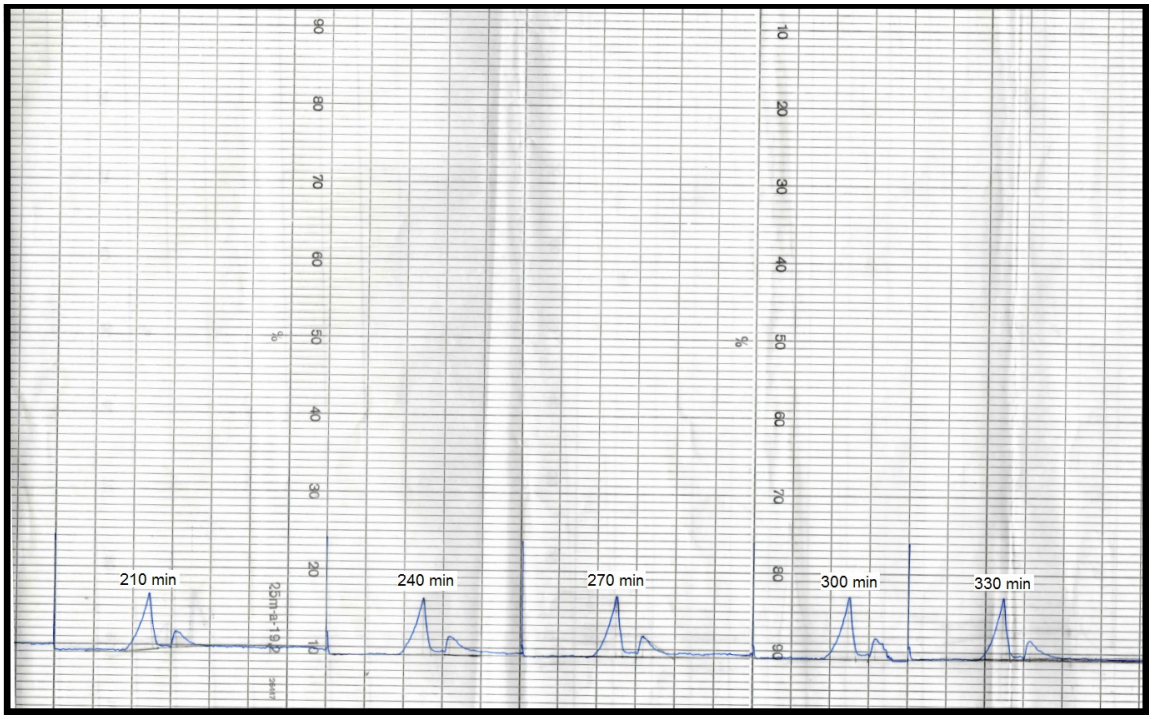


- Graphic 19: Peak 1 intensity trend of a $3 \cdot 10^{-4}$ M DIH solution at λ = 359.0 nm in a seven hours timespan.

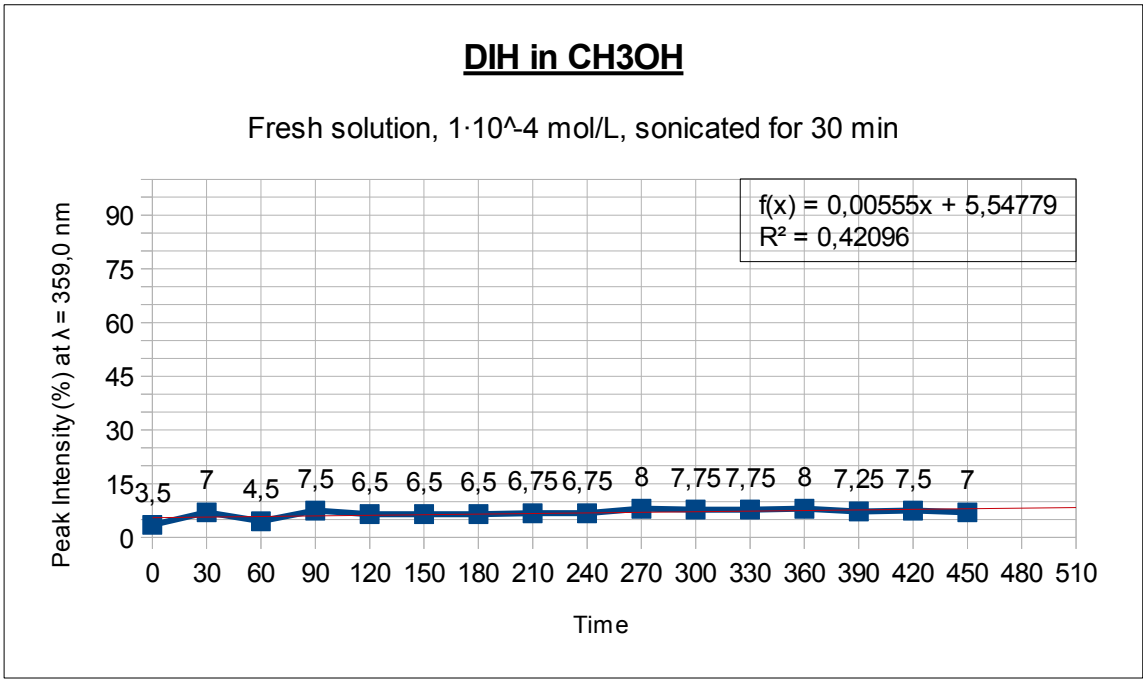


- Graphic 20: Peak area trends of a $3 \cdot 10^{-4}$ M DIH solution at λ = 359.0 nm in a seven hours timespan.

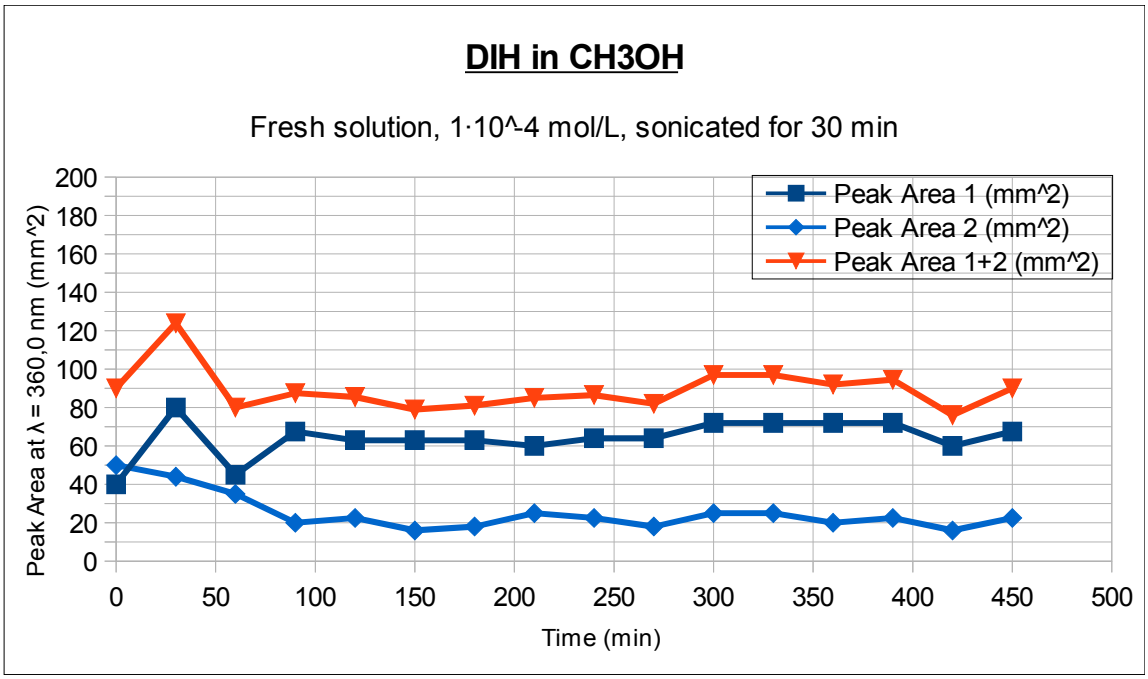
There is an hypothesis that can be made regarding this phenomenon. During the synthesis experiments conducted in Bergen, GC-MS spectra were usually performed to verify the nature of the product of the reactions, and very often a problem emerged: it happened multiple times that the background noise of these spectra was increased due to the presence of iodine, which got stuck to the column of the GC-MS machine. This iodine was present because of the unreacted DIH that remained in the reaction mixture. Basically, the problem is that this remaining iodine progressively clogged the GC-MS column, thus causing a slower passage of the chemical species through the column itself. Thus, it is possible that this time the DIH remained partially stuck inside the HPLC column and that it came out in two different portions, which could explain the two separated peaks. Something similar happened also with the next $1 \cdot 10^{-4}$ M DIH solution (see *Picture 19*) that was employed in another series of analyses, since we observed two close peaks again during an eight hours monitoring. In *Graphic 21* we show the intensity variation of the major peak (Peak 1, retention time of 2 minutes and 40 seconds). The second smaller peak had a t_{RET} = 3' 15". Similarly as before, in the following *Graphic 22* the correlation between Peak 1 area with time, Peak 2 area with time, and the total area "Peak 1 + Peak 2" with time are presented.



- Picture 19: Sample of HPLC peaks of a $1 \cdot 10^{-4}$ M DIH solution in CH_3OH at λ = 359.0 nm.



- Graphic 21: Peak intensity trend of a 1·10^{-4} M DIH solution at λ = 359.0 nm in a eight hours timespan.



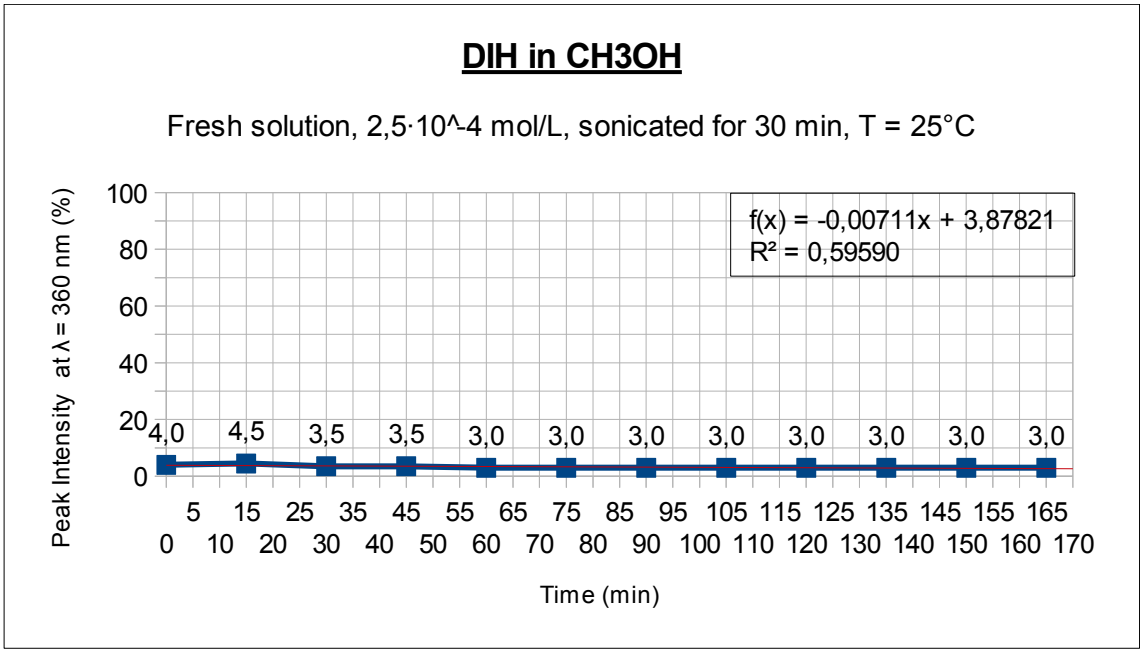
- Graphic 22: Peak area trends of a 1·10^{-4} M DIH solution at λ = 359.0 nm in a eight hours timespan.

In all the cases we observe that, even with some fluctuations and some possible outlayers the slope of the regression lines (thus the values of both the intensity peaks and the peak areas) is generally quite low and apparently there is no particular tendency from the DIH to decompose quickly by any means. The only unexpected thing is the fact that two close peaks are observed. It could be supposed that the second smaller peak belongs to some sort of degradation product that, for pure coincidence, absorbs at the same wavelength (359.0 nm) as the N,N'-diiodo-5,5-dimethylhydantoin.

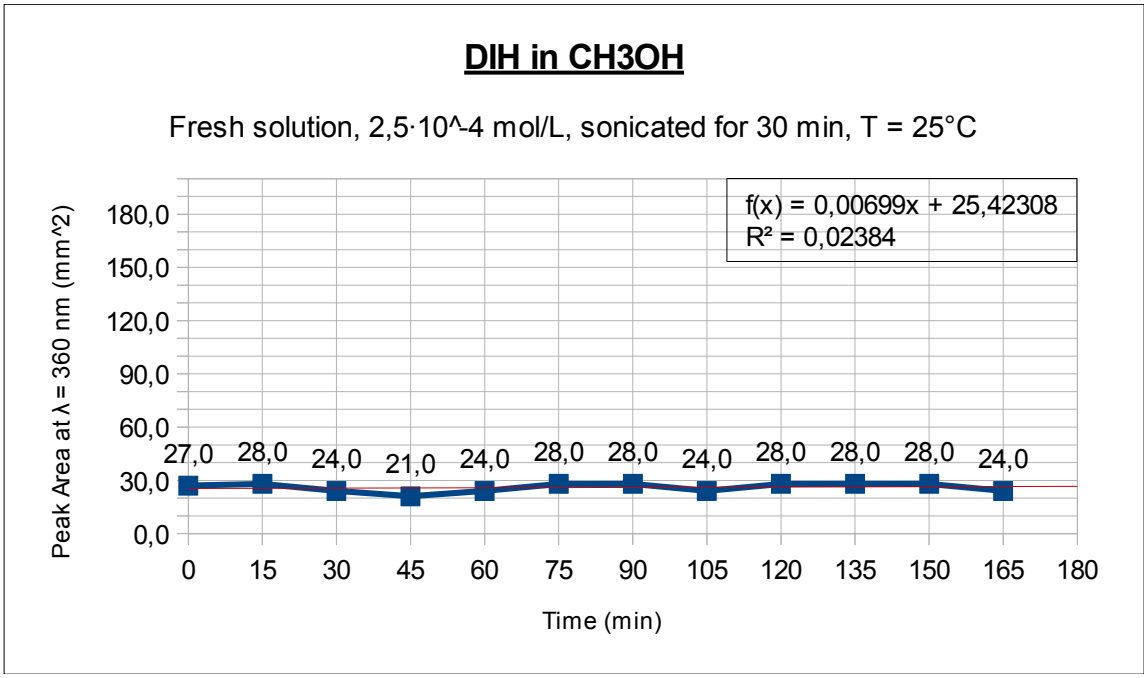
This degradation could be the "normal" DIH decomposition in methanol, or hypothetically it could also be an undesired side effect of the sonication process. Though this is not theoretically impossible, it is still improbable that any degradation product absorbs at the exact same wavelength of DIH, and we have to assume something else to explain this technical issue. Usually, the eluent is left to run inside the HPLC column both before and after the analysis period, as well as overnight, although with a very small flow of 0.1 mL/min. It is possible that, even with these "washing periods", some DIH remains stuck inside the column, and it is more slowly released during the following injections, in a not dissimilar way as what was observed in the GC-MS routine analyses performed during the synthetic stage of the project.

At this point it could be helpful to change the HPLC column with something different for a C18 column. To remove impurities, we also wash the column with the maximum flow. Eventually, there is also another hypothesis that can not be excluded: as the DIH sample is not pure, it may be that contains some minor impurities that absorb at 359.0 nm in methanol as well, thus causing the second peak. The last experiment conducted at room temperature required a $2.5 \cdot 10^{-4}$ M DIH solution. This test has been shorter than the previous ones, as it lasted for less than three hours. In this case, the HPLC analyses showed one single peak: it was a slightly larger than usual, but it was still possible to calculate its area with sufficient accuracy.

Below we report two *Graphics 23* and *24* showing the correlations between the peak intensity and the peak area with time. There is not much of specific to signal, since as usual the presented trend resembles almost a straight line, with a very small slope of the regression line. In particular, the observed fluctuations were especially restrained (both concerning the peak intensity trend and the peak area trend).



- Graphic 23: Peak intensity trend of a 2,5·10^{-4} M DIH solution at λ = 359.0 nm, in a 165 min timespan.

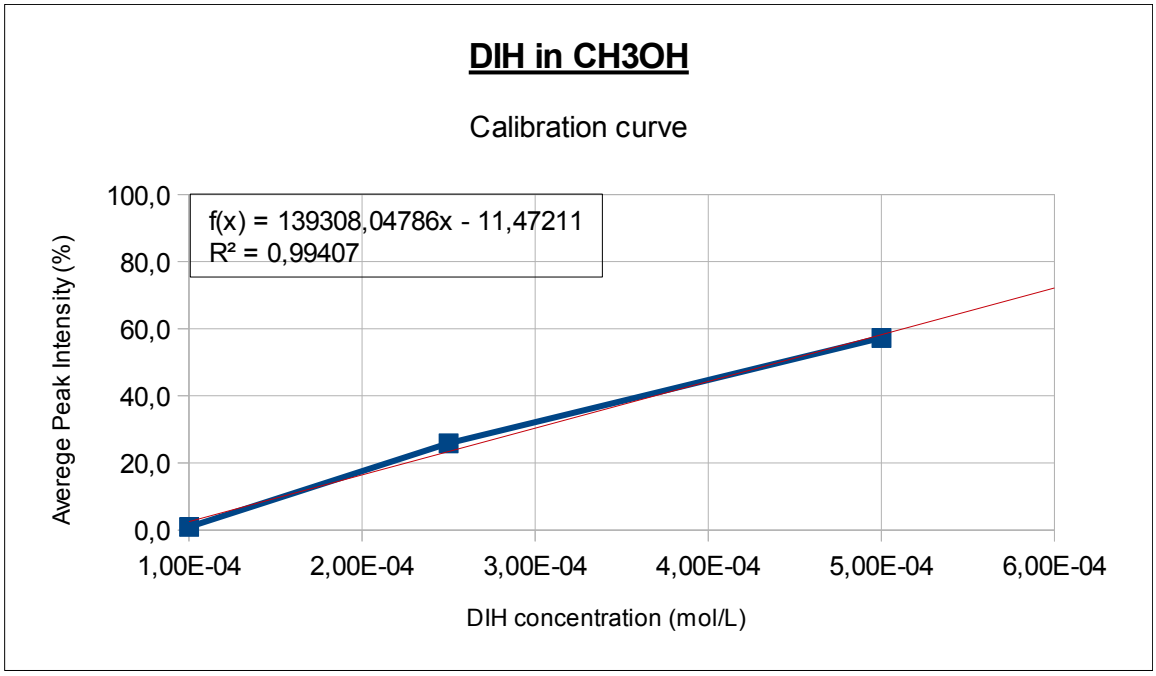


- Graphic 24: Peak area trend of a 2,5·10^{-4} M DIH solution at λ = 359.0 nm in a 165 min timespan.

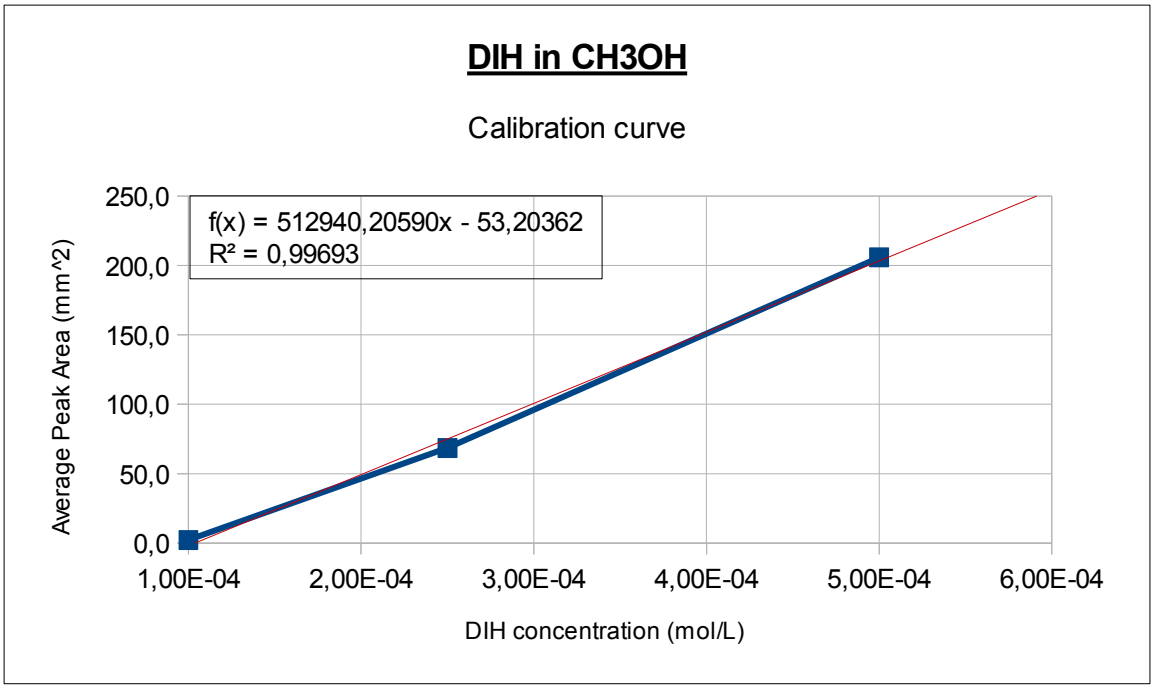
This last experiment were done to try to build a calibration curve for DIH in methanol, in a narrow concentration range, to be able to perform quantitative analyses of the degradation process of the DIH. The DIH absorbance, at the different concentration level, changed consistently, as expected.

This implies that it was necessary to adapt the range of the spectrophotometric detector of the HPLC system at different values every time. Basically every specific concentration required its own particular spectrophotometric range, since it is not always possible to use the same spectrophotometric range and to observe the signals of different concentrations.

Reporting the obtained average values of peak intensity and peak area to the same spectrophotometric range, it is possible to appropriately compare them and thus to build a calibration curve, which is presented in *Graphic 25* and *Graphic 26*. As we can see, the correlation between the data is very strong. With a Pearson correlation coefficient (R^2) higher than 99 in both cases. Hence, it could be possible to perform quantitative analyses of DIH solutions in methanol, since this molecule shows the best stability in this solvent. Nevertheless, the procedure still needs to be further improved (from an instrumental point of view) in order to increase its reproducibility and repeatability.



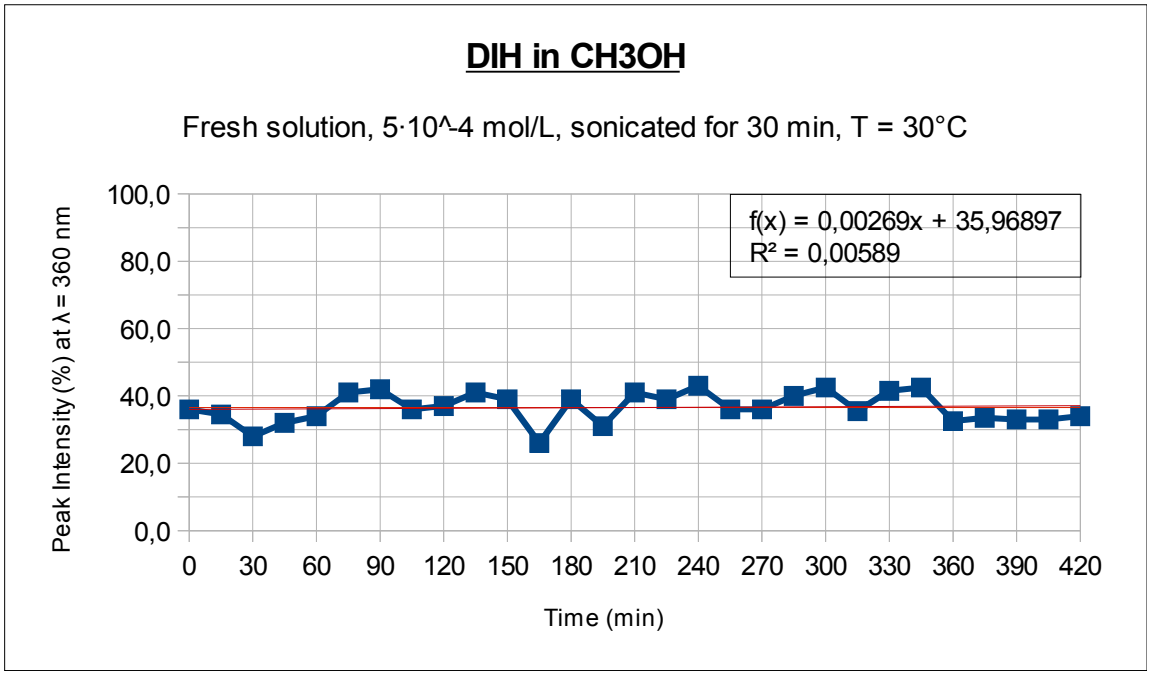
- Graphic 25: Calibration curve with three DIH solutions (average peak intensity correlation).



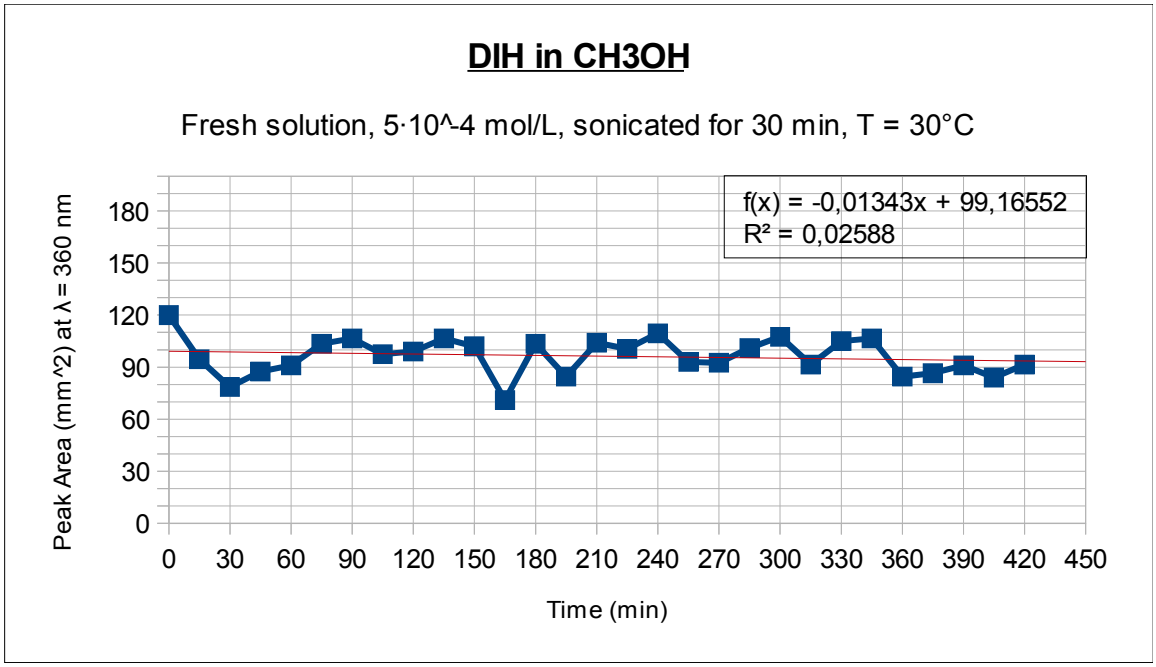
- Graphic 26: Calibration curve with three DIH solutions (average peak area correlation).

4.14 HPLC analysis in methanol – effect of the temperature

After the analyses performed at room temperature, a new series of test at different temperature were executed, utilising always $5 \cdot 10^{-4}$ M solutions. The experiments were done at 30°, 35°, and 45° C in order to look for any influence of the temperature in the degradation process of DIH. The following two graphics show the observed trends for a $5 \cdot 10^{-4}$ M solution, with the HPLC column thermostated at 30°C, and monitored for seven hours, which gave a set of pretty sharp peaks with time. It is possible to notice that, on average, there are some more fluctuations if compared with all the previous experiments, and there are some values that could be considered as possible outlayers, from a statistical standpoint. Nevertheless, in both charts the slope is always near zero, so apparently no particular degradation is observed in the considered timespan. This could mean that the temperature is not high enough to trigger the degradation of DIH, or simply that no decomposition occurs whatsoever (unless at considerably higher temperature than room temperature). On the other hand, one could also argue that the average values both of the peak intensity and of the peak area are lower at 30°C than the ones measured at 25°C, so this could be a sign that some sort of degradation happens quickly just in the beginning of the process and then almost "stalls", proceeding at a very slow rate with time. At this point, though, the results were still uncertain.

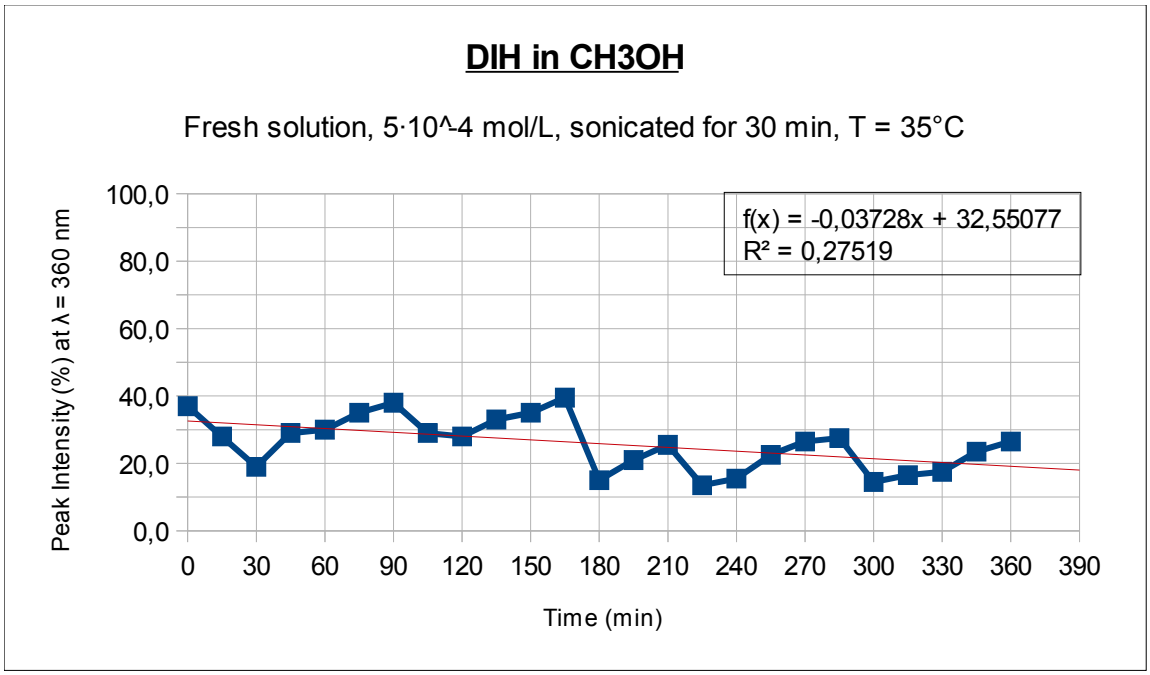


- Graphic 27: Peak intensity trend of a 5·10^{-4}M DIH solution, λ=359.0nm, 30°C, in a seven hours timespan

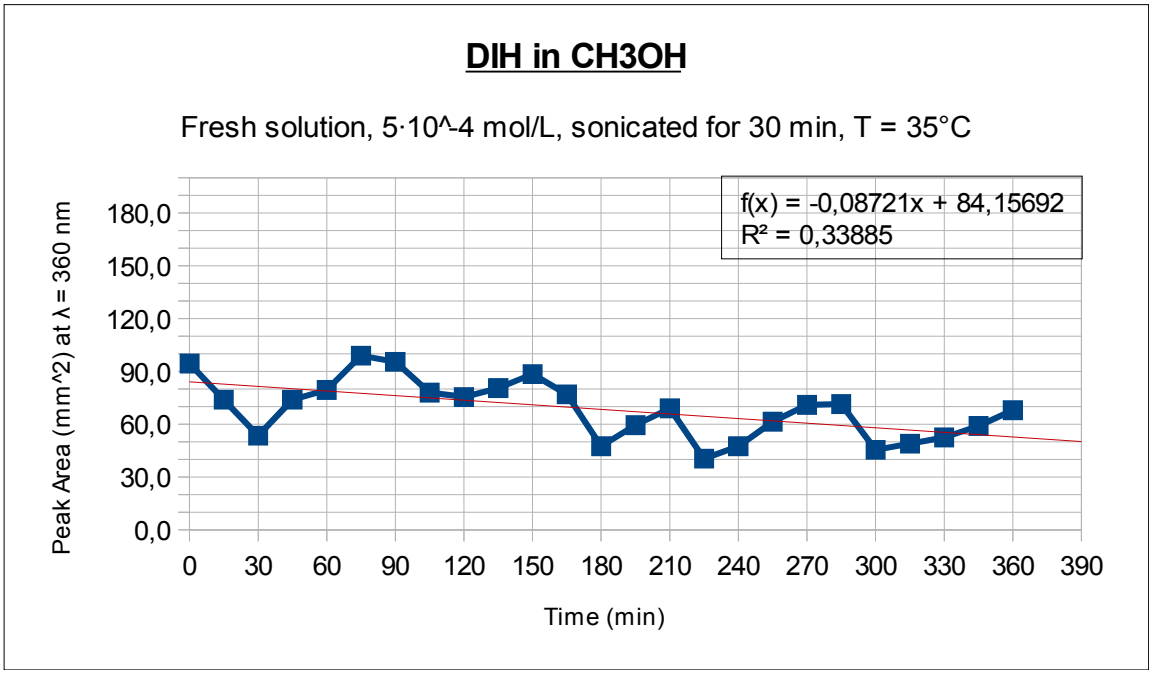


- Graphic 28: Peak area trend of a 5·10^{-4} M DIH solution, λ=359.0 nm, 30°C, in a seven hours timespan.

The measured retention time for the DIH was consistent with the ones detected in the previous analyses. To further investigate, more test were performed rising the temperature of thermostatation at 35°C. Monitoring the $5 \cdot 10^{-4}$ M solution for six hours, the obtained results have been the following:



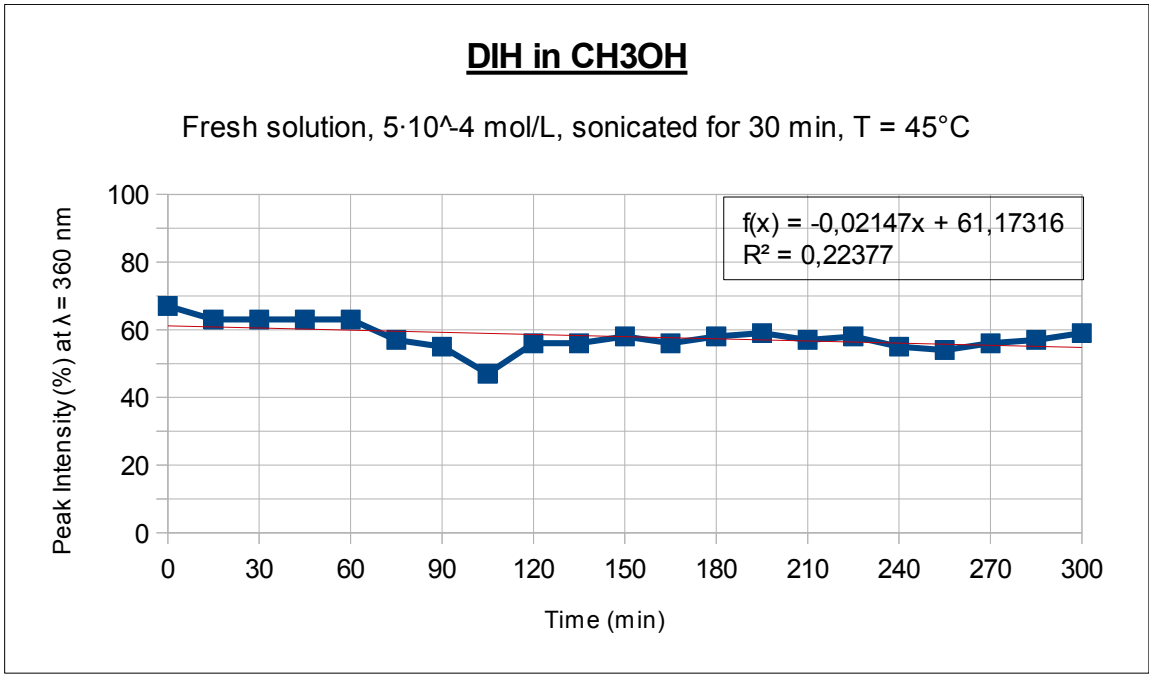
- Graphic 29: Peak intensity trend of a $5 \cdot 10^{-4}$ M DIH solution, λ=360.0 nm, 35°C, in a six hours timespan.



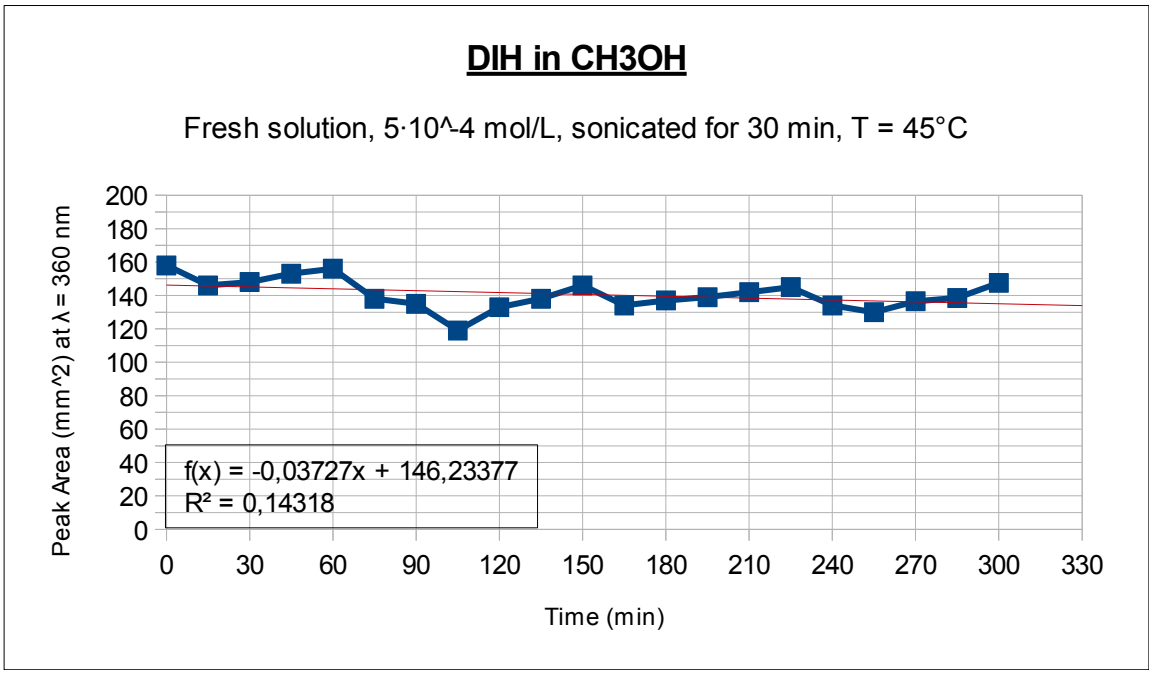
- Graphic 30: Peak area trend of a $5 \cdot 10^{-4}$ M DIH solution, λ=360.0 nm, 35°C, in a six hours timespan.

It can be noticed that in this case, even if the slope of the regression line is again quite small, it is slightly higher than in the previous case, so apparently the degradation process becomes a bit faster if the temperature is increased of 5°C. It is worth noticing also the there are some significant fluctuations over time, and the trends both of the peak intensity and of peak area almost have a "wavy" progression. This is a bit unexpected, but it is probably a side effect of the increase of temperature. In any case, although the slope has a negative value, the decreasing of the signals is still very slow with time. Therefore, it would probably require a considerable amount of hours to produce a significant degradation of the N,N'-diiodo-5,5-dimethylhydantoin.

Because of this, it was decided to try to rise the temperature even further, thus finally reaching 45°C. This is considerably above any normal storage temperature, that in the vast majority of cases would not exceed 30°C anyway. This has been the last test conducted in using methanol as solvent and also the last one that allowed to maintain a satisfactory control over the thermostatation. In fact, managing the temperature with a sufficient precision while going above 50°C would have been rather difficult, from an instrumental point of view, and not worthy at all to our purpose. The peak intensity and the peak area trends are reported in the next two graphics.

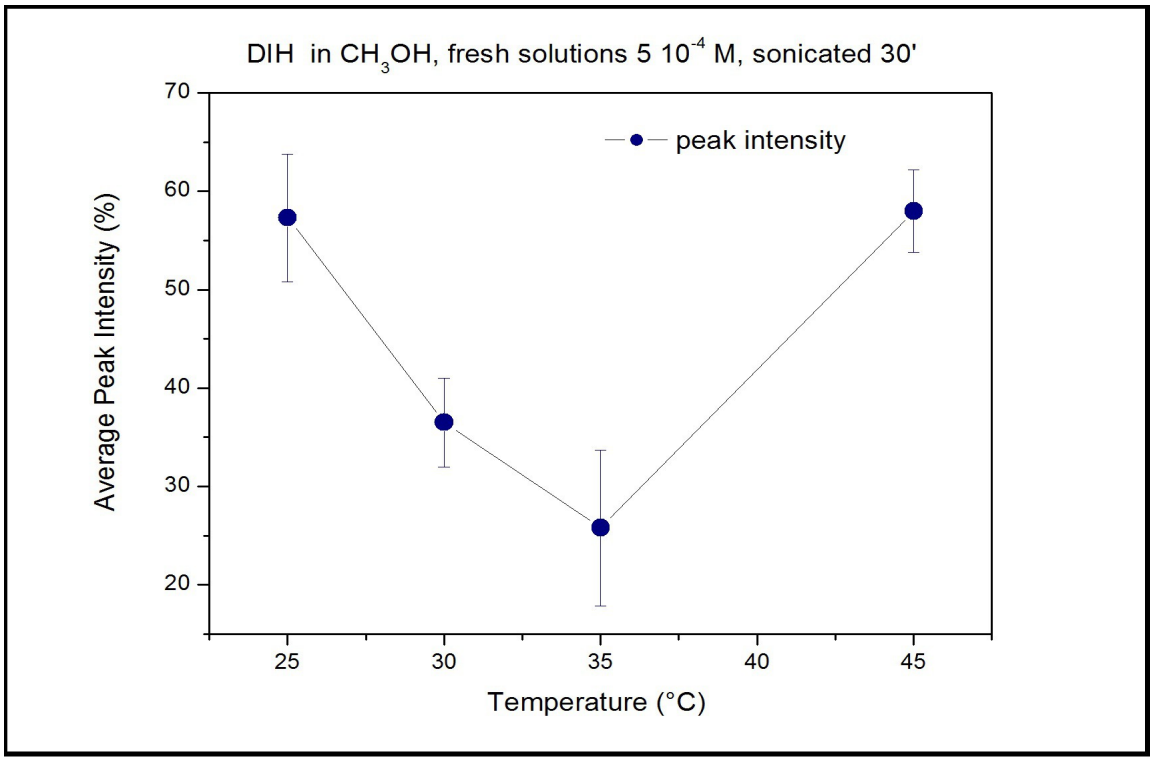


- Graphic 31: Peak intensity trend of a 5·10^{-4} M DIH solution, λ=360.0 nm, 45°C, in a five hours timespan.

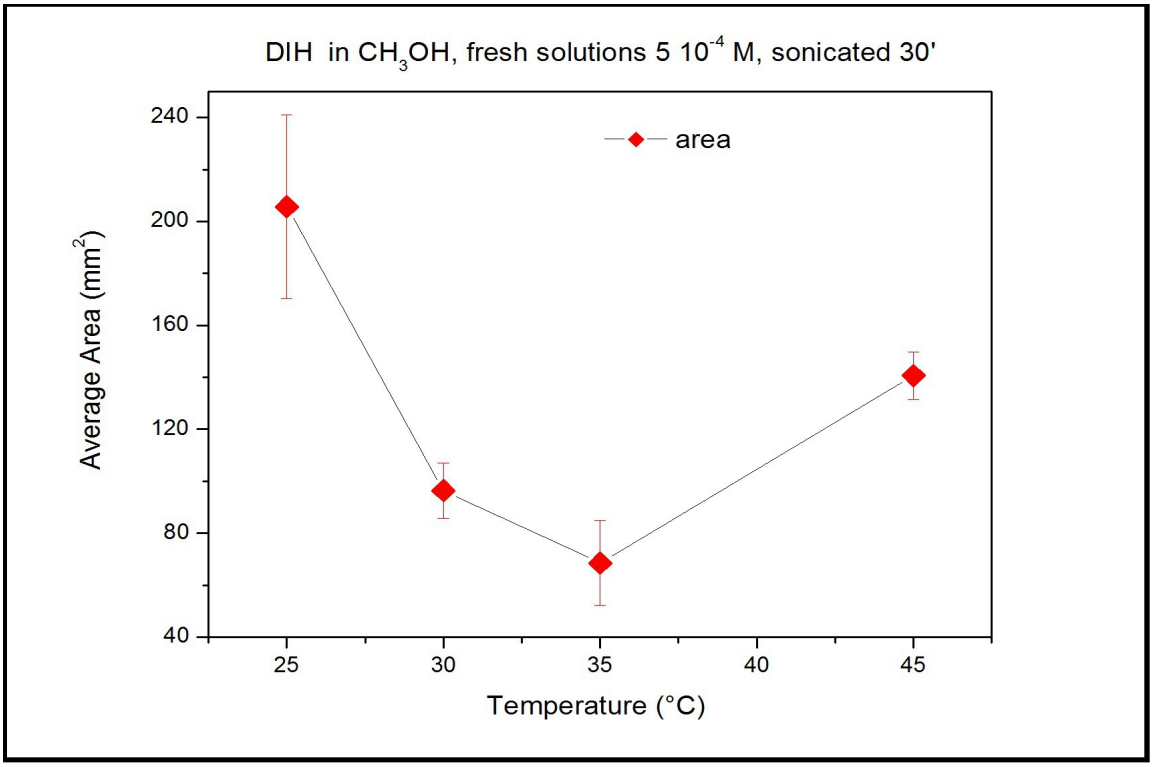


- Graphic 32: Peak area trend of a 5·10^{-4} M DIH solution, λ=360.0 nm, 45°C, in a five hours timespan.

As in the previous set of analyses, also here the slopes of the regression lines have negative values and 10^{-2} as order of magnitude, so it seems like there is no substantial increase in the degradation speed for the DIH once the temperature is set. Consequently, there seem to be no risk regarding performing a reaction with the DIH in the MJOD reactor, since usually the synthetic processes do not require such a long time that DIH would need to eventually degrade. In the following *Graphic 33* and *34* the trend of average peak intensity and average peak area with temperature are reported. It is interesting to notice how both the peak intensity and peak area first decrease progressively as the temperature increases, but then suddenly they increase again when temperature reaches 45°C. It is expected to see the peaks decrease in this way, but the sudden increase at 45°C is actually surprising. Two hypothesis can be made to explain this: an instrumental one and chemical-physical one. The first explanation is simply that some DIH remained stuck inside the column during the previous experiments and, with the higher temperature, it has been released, which caused an increase of the signal. The second explanation is that the higher temperature provoked a rise of the energy levels of the molecule, thus causing an alteration of its response to the UV-Vis wavelength. It is know, in fact, that the molar absorptivity (ε_λ) may vary with temperature, and it is not impossible that putting the DIH solution at 45°C may have caused this phenomenon.



- Graphic 33: Average peak intensity trend of 5·10^{-4}M DIH solutions at different temperatures, λ=360.0 nm



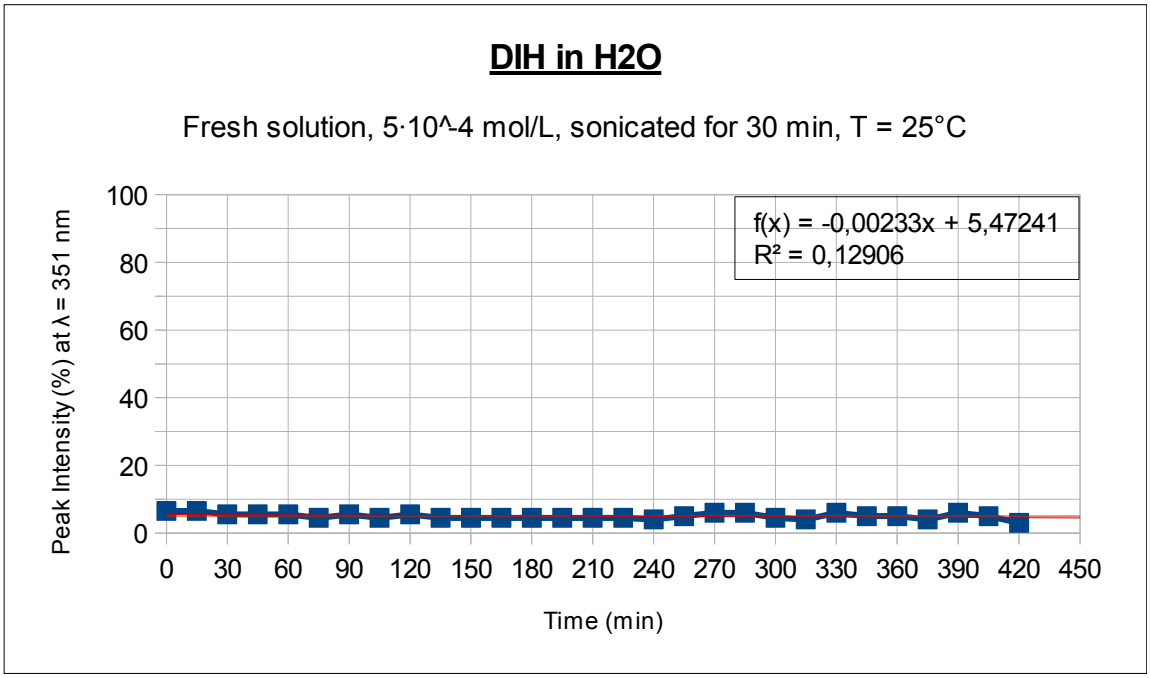
- Graphic 34: Average peak area trend of 5·10^{-4}M DIH solutions at different temperatures, λ=360.0 nm

Hence, after looking at the trends displayed in the previous graphics, apparently some sort of degradation with temperature for DIH actually exists, which becomes severe after that 30°C are overcome. This does not affect the production process with MJOD reactor since it works at room temperature, as soon as the heat-transfer system works properly. It effects how the DIH has to be stored though: at a temperature not higher than 25°C.

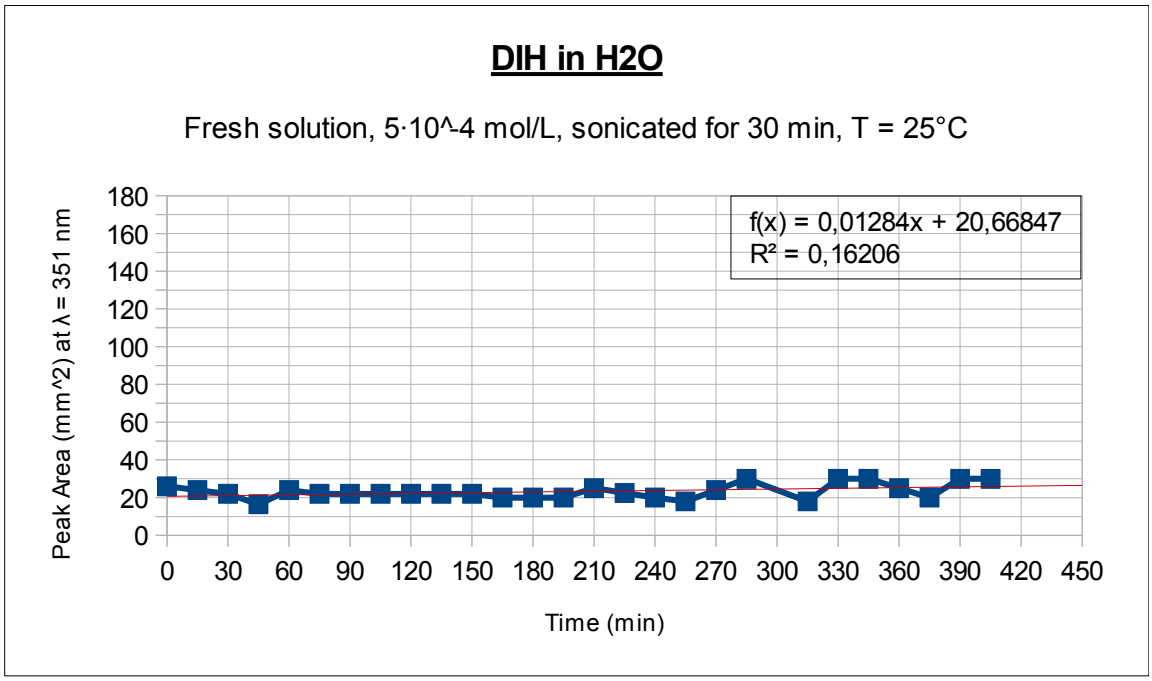
4.15 HPLC analysis of a DIH solution in water

Before doing HPLC analyses using UPP water as solvent for the DIH solution, the HPLC column was previously conditioned by flowing UPP H_2O through it for 6 hours with a reduced flow (0,1 mL/min), in order to clean it from potential impurities as thoroughly as possible.The solutions were prepared using an analogous procedure as the previous ones, and these experiments were conducted at room temperature (25°C). The water used as eluent was preventively sonicated in an ultrasonic in order to degas it. Firstly, tests were performed utilising $5 \cdot 10^{-4}$ M DIH solution. *Graphic 33* and *34* the variations of the peak intensity and of the peak area with time.

The fluctuations are not particularly substantial, and the slope of the regression line is very close to zero in the time interval that was considered. Overall, the peak trend is quite regular.

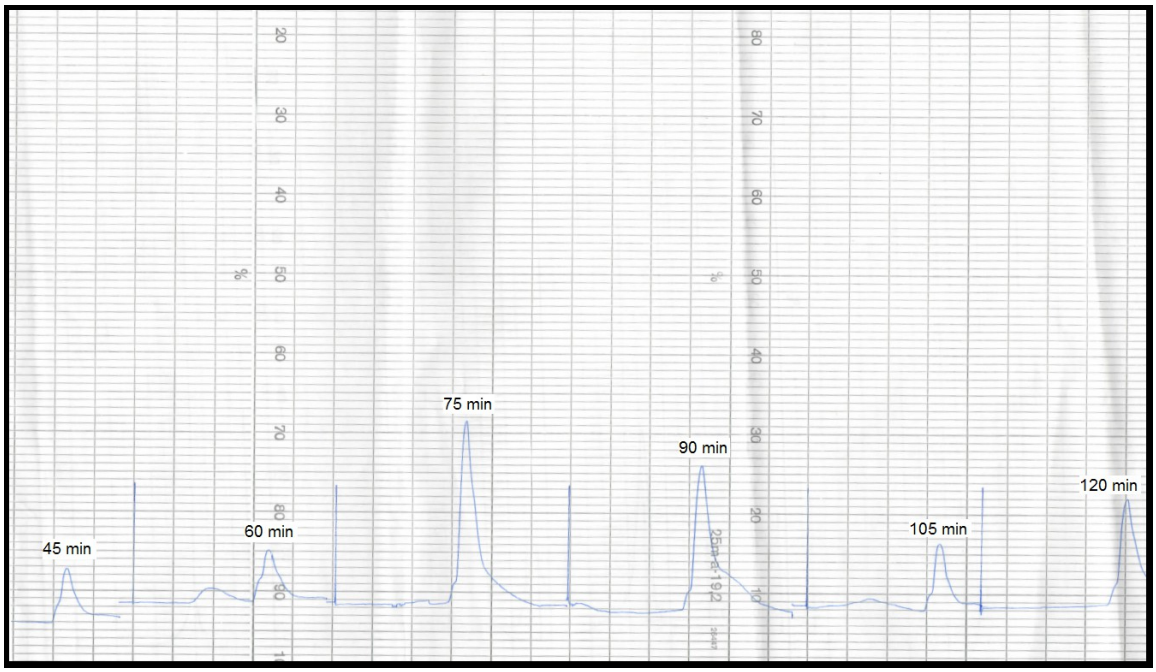


- Graphic 35: Peak intensity trend of a $5 \cdot 10^{-4}$M DIH solution, λ=351.0nm, 25°C, in a seven hours timespan

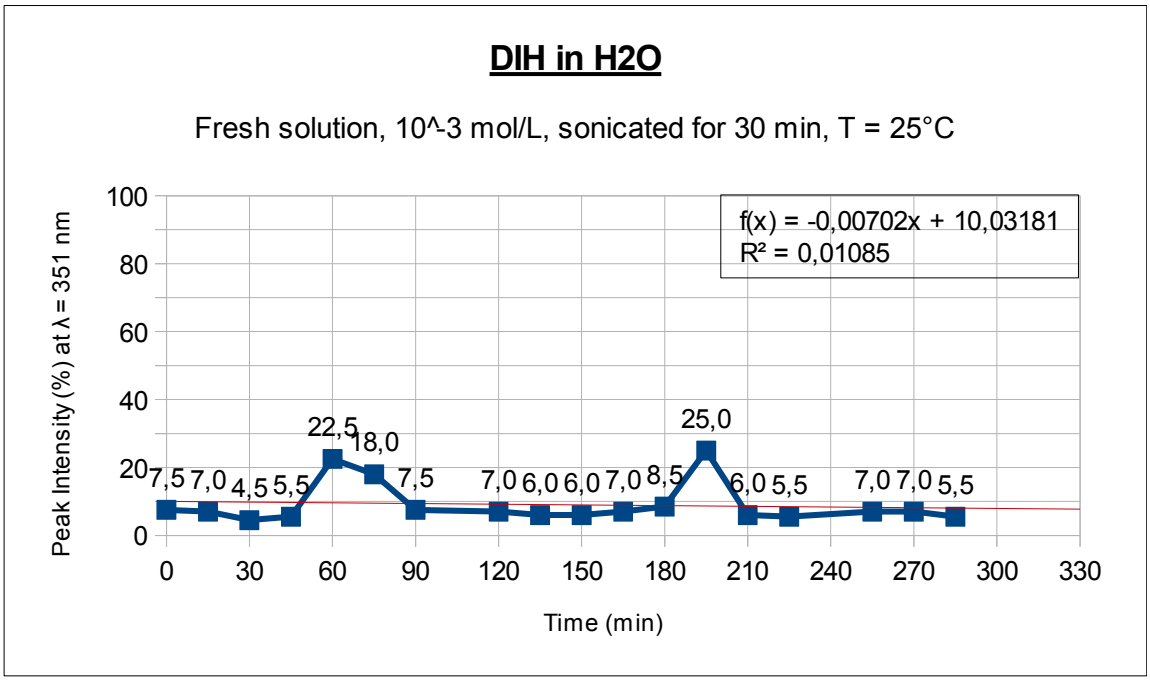


- Graphic 36: Peak area trend of a 5·10^{-4} M DIH solution, λ=351.0 nm, 25°C, in a seven hours timespan.

Secondly, experiments were made utilising a 1·10^{-3} M DIH solution. This time though, we encountered some serious difficoluties while performing the analyses. The peaks were significantly broadened and it is not possible to calculate their area (see *Picture 20*). *Graphic 37* shows the peak intensity trend during a seven hours time interval.



- Picture 20: Sample of HPLC peaks of a 1·10^{-3} M DIH solution in UPP water at λ = 351.0 nm.



- Graphic 37: Peak intensity trend of a 1·10^{-3}M DIH solution, λ=351.0nm, 25°C, in a seven hours timespan

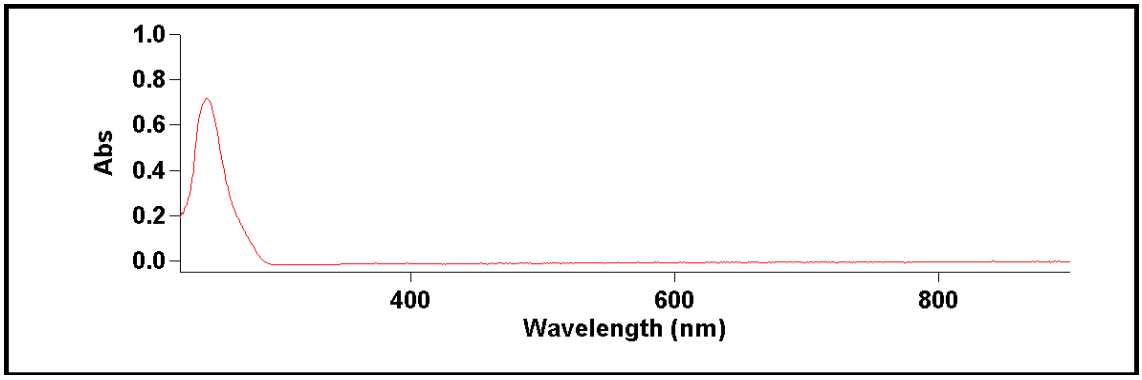
The outlayers are clearly visible. Repeated experiments showed the same trend, with very widened peaks that sometimes almost "collided" into each ther forming an uncomprehensible ensamble of peaks, which nearly resembled a "band". Changing the eluent and going back to methanol did not improve the results, nor did changig the HPLC column with a new one ("Kinetex"). It is possibile that impurities in the DIH sample somehow interfere with the proces even if it is difficult to identifiy them more specifically. The following ***Table 10*** summarizes the operative conditions employed for all the HPLC analyses.

Table 10: Summary of the operative conditions for the HPLC analyses.

Solvent	DIH Concentration (mol/L)	λ (nm)	Temperature (°C)	Monitoring time (h)
CH_3OH	$5\cdot10^{-4}$	359,0	25	6,00
CH_3OH	$3\cdot10^{-4}$	359,0	25	7,00
CH_3OH	$1\cdot10^{-4}$	359,0	25	8,00
CH_3OH	$2,5\cdot10^{-4}$	359,0	25	2,75
CH_3OH	$5\cdot10^{-4}$	360,0	30	7,00
CH_3OH	$5\cdot10^{-4}$	360,0	35	6,00
CH_3OH	$5\cdot10^{-4}$	360,0	45	5,00
H_2O	$5\cdot10^{-4}$	351,0	25	7,00
H_2O	$1\cdot10^{-3}$	351,0	25	7,00

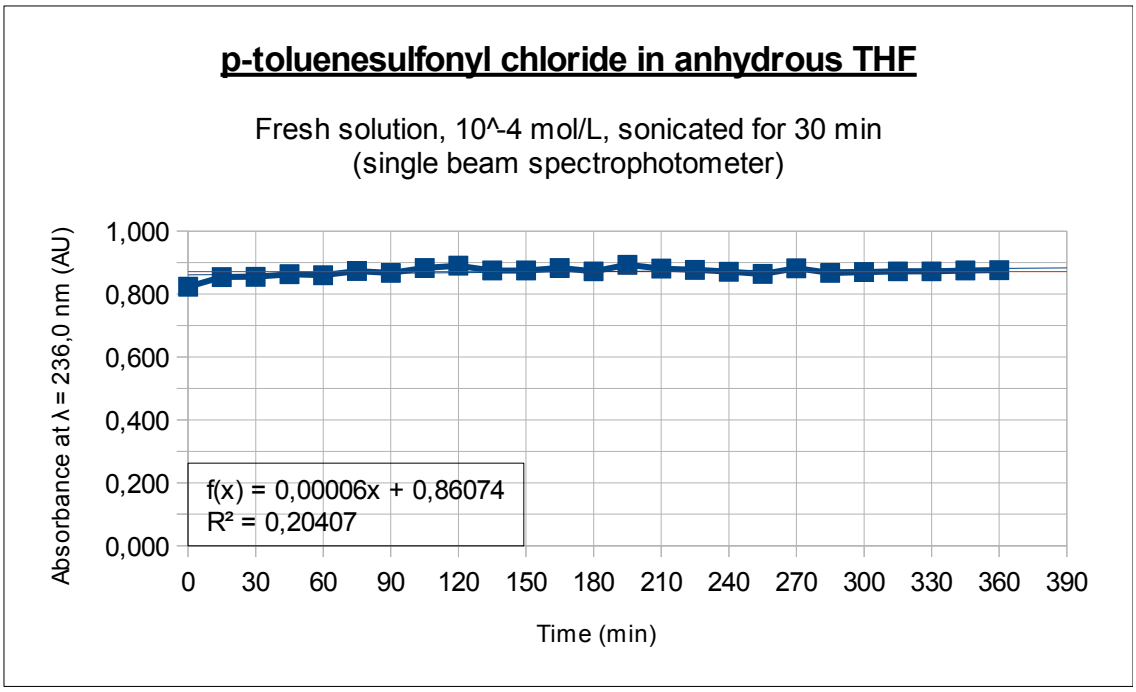
4.16 Spectrophotometric analysis of p-toluenesulfonyl chloride solutions

The last stages of the synthetic route involves the reaction between 4,5-Diiodo-1H-imidazole (or to 4(5)-Diiodo-1H-imidazole) with tosyl chloride to give N-tosyl-4,5-iodoimidazole (or N-tosyl-4(5)-iodoimidazole), as previously described. The reaction could take place in dry THF or, potentially, in acetone but, since it is planned to perform the entire synthetic path in the same MJOD Reactor from the first reaction to the last one, this implies that the p-toluenesulfonyl chloride would get in contact with water (because H_2O is the solvent for the iodination reaction). This means that tosyl chloride could potentially undergo an hydrolysis reaction, at least partially, and this could affect the last stage of the synthetic process. Hence, it is necessary to verify how fast this potential hydrolysis can happen. To test this, it was chosen to perform a spectrophotometric analysis. Using a double beam spectrophotometer, we obtained the tosyl chloride spectrum in anhydrous THF from 221 nm to 1000 nm (since the cut-off limit of the THF is 220 nm), which showed that the maximum peak of absorption of the analyte is at 237,0 nm. See *Graphic 38*.



- Graphic 38: UV-Vis spectrum of tosyl chloride in dry THF in a range of λ=221-1000 nm; λ_{max}=237.0 nm.

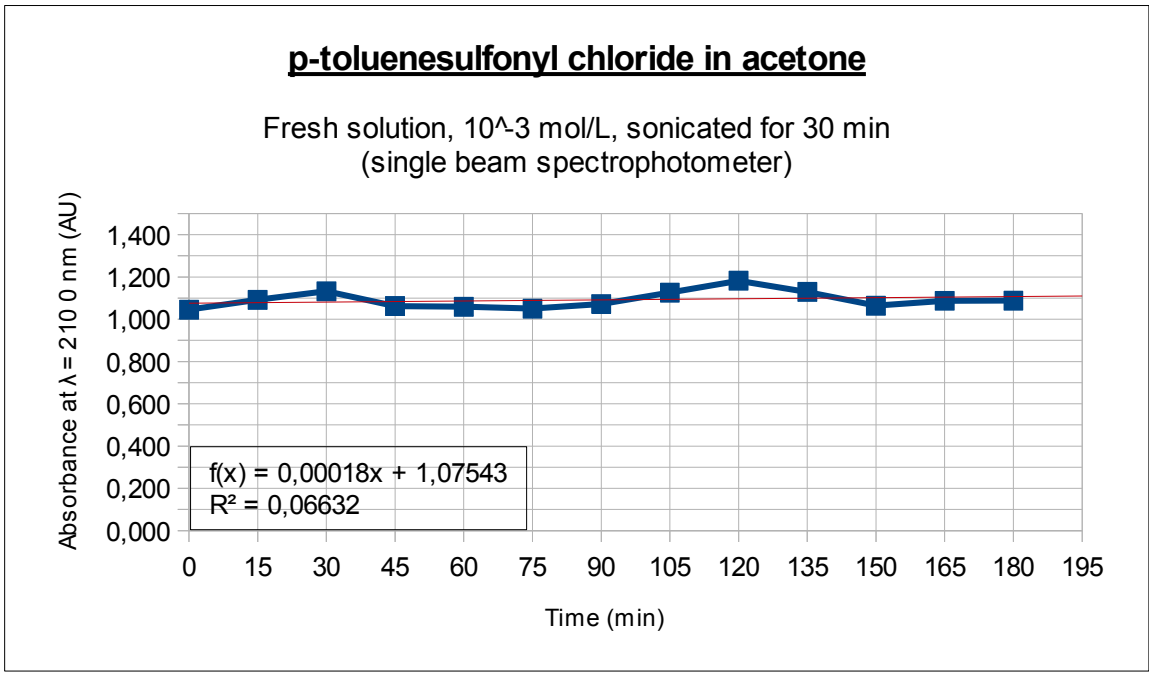
Afterwards, we observed the absorbance trend of tosyl chloride in THF when it is put in contact with water. In particular, an large excess of H_2O was added to the solution containing p-toluenesulfonyl chloride which was equal to ten times the amount of tosyl chloride. After some preliminary tests, needed to find the appropriate range of tosyl chloride concentration with whom conduct the analysis, the monitoring of the absorbance lasted for 6 hours, as it is possible to see from *Graphic 39*.



- Graphic 39: Absorbance trend of a $1 \cdot 10^{-4}$ tosyl chloride solution at. λ = 237.0 nm in a 6 hours timespan.

Repeated analyses gave analogous results. We can therefore easily assume that tosyl chloride does not undergo any kind of fast hydrolysis, at least in THF and at room temperature, even with a large amount of water. The same kind of experiment was also tried using acetone as a solvent, but in this case it is necessary to underline that the absorbance data may not be completely consistent.

In fact, we observed that the maximum peak of absorption of tosyl chloride in acetone lies around 210,0 nm, but the cut-off limit off acetone is 330,0 nm, which implies that the absorbance value would be partly affected by the absorption of acetone itself. Nevertheless, a test was carried out anyway with the same excess of water as in the previous one, which resulted in the trend reported in *Graphic 40*.



- Graphic 40: Absorbance trend of a $1 \cdot 10^{-3}$ tosyl chloride solution at λ = 210.0 nm in a 3 hours timespan.

The absorbance values fluctuate a bit more than in the previous test. In any case, though, even with these fluctuations the absorbance lies around a small set of values. So, apparently, no quick hydrolysis takes place in the considered timespan and the tosyl chloride is stable with time even when it is put in contact with a considerable amount of water.

5. Conclusions

Concluding, progress were made in transferring iodination reaction from batch scale to the Multijet Oscillating Disc Millireactor. The latest results may indicate that it is actually possible to product larger quantities of 4,5-diiodoimidazole with this kind of technology. The following stages of the study will probably investigate the possibility to implement also the synthesis of N-tosyl-4,5-Iodoimidazole on the same reactor. Basically, the ideal scenario would be to be able to go from imidazole, to 4,5-diiodoimidazole and finally to N-tosyl-4,5-Iodoimidazole in the same run with the MJOD reactor. Otherwise, following the other synthetic route, going from imidazole, to 4(5)-iodoimidazole, to N-tosyl-4(5)-Iodoimidazole. Anyhow, some aspects concerning both the chemical and the engineering aspects of these routes still have to be properly explored in order to actually perform a “tandem reaction” with the MJOD reactor in a smooth and reproducible way. The informations that were obtained during this project will be taken into consideration for further developments of the process, looking for synthesizing imidazole with functionalized backbone on bigger quantities. Moreover, the spectrophotometric and HPLC analyses made possible to assess the stability in solution for both the N,N'-diiodo-5,5-dimethylhydantoin and the p-toluenesulfonyl chloride: these two compounds are stable enough in the timespan that is usually required to perform the reactions with the MJOD reactor (few hours), therefore the process should not suffer problems due to degradation or decomposition of these chemical species.

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Acknowledgements

I want to sincerely thank my supervisor, professor Marco Giorgetti, and I have more than one reason to do it. Firstly, because he managed to put me in contact with my co-supervisor, professor Hans-René Bjørsvik, and with this he made this journey possible for me: my Erasmus, my experience in Norway, with everything that it this includes. Secondly, for his support throughout all the phases of this work until its end. He has been an helpful, reliable and respectful collaborator during the project that I worked in during my bachelor degree, and he behaved in the same way also this time. I am honestly glad that I have had the opportunity to work with him.

Thank you to my co-supervisor, professor Hans-René Bjørsvik, for having accepted my in his research team, giving me the opportunity to observe and work in a new academic reality and in a scientific area that I had never experience before. This project, overall, put to test my strengths on an intellectual, psychological and sometimes even physical level. Completing it until the end left me undoubtedly a stronger person than I was before, and this is valuable beyond everything else.

I want to thank professor Paolo Righi, Dr. Silvia Orlandi and ms. Guro Kristin Øvsthus for their fundamental help with the bureaucreacy of the Erasmus, the acceptance in the University of Bergen, and everything related. There is a serious probability that I could have benn lost in all this complications without their support.

Thank you to all the members of the research team of professor Bjørsvik, because each one of them, in a moment or another, was able to give me a useful suggestion, informations, help, support, collaboration or even friendship: Sergii Tretiakov, Marta Ferreri, Audun Drageset, Alexander H. Sandtorv, Vijayaragavan Elumalai, Ignacio Chamorro Burgos, Erlend Nesje Brevik.

Finally, I want to thank the technicians of the Industrial Chemistry Laboratory of the University of Bologna for their practical support with the instrumentations during the last phases of my project: Dr. Alberto Mucchi, Dr. Stefano Cerini and Dr. Sandra Stipa.