Studies on removal of genotoxic impurities from pharmaceuticals streams using polybenzimidazole membrane adsorbers

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Preface

The work presented in this thesis was performed at BERG/IBB Department of Bioengineering - Instituto Superior Técnico – Alameda (Lisbon, Portugal), during the period March-July 2021, under the supervision of Doctor Frederico Castelo Alves Ferreira and Doctor Teresa Sofia Araújo Esteves.

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Declaration

I declare that this document is an original work of my own authorship and that it fulfils all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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Resumo

Indústria farmacêutica e agências reguladoras têm demonstrado avanços no campo de estudo da genotoxicidade ao longo dos anos. Esta evolução pretende reduzir os níveis genotóxicos nos produtos farmacêuticos para não comprometer a saúde dos pacientes, podendo recorrer-se a processos de purificação. Contudo, apesar de haver a redução dos níveis de impureza genotóxica (GTI) até aos impostos, estes processos podem levar a perdas significativas de Ingrediente Farmacêutico Ativo (IFA). Assim, ao produzir um IFA seguro, o rendimento dessa produção poderia ficar comprometido, resultando em perdas económicas.

Portanto, o desenvolvimento de um processo de purificação adequado é obrigatório, devendo este ser capaz de promover uma remoção eficiente do GTI, obedecendo ao valor do limiar toxicológico geral (TTC), e garantir uma perda de IFA mínima, preferencialmente abaixo dos 10%.

Assim, esta tese foca-se na implementação de um processo de purificação por adsorção, usando membranas de polibenzimidazolo (PBI) como adsorventes. É reportada a avaliação da sua capacidade para remover seletivamente um GTI e uma possível estratégia de purificação de IFA é sugerida. Com o objetivo de mitigar as perdas de IFA e regenerar a membrana, um passo de pós-ligação foi aplicado dentro desta estratégia e avaliado. Os resultados obtidos sugerem que as membranas e a estratégia adotada não permitiram obter um processo de purificação eficaz devido ao baixo limite de GTI imposto para Roxitromicina (IFA selecionado). Contudo, H_2O a pH 1.2 para regeneração quando 4-dimetilaminopiridina é o GTI em estudo e H_2O a pH 13 para eluir metilo *p*-toluenossulfonato da membrana apresentaram bons resultados.

Palavras-chave: impurezas genotóxicas, ingrediente farmacêutico ativo, adsorção, membranas de polibenzimidazolo adsorventes, estratégia de purificação.

Abstract

Pharmaceutical industry and regulatory agencies have been demonstrated advances through the years at the field of genotoxicity study. This evolution aims to reduce genotoxic levels on final pharmaceutical products to not compromise patients' health, being purification processes one of the pathways to follow. However, despite assuring reduction of genotoxic impurity (GTI) levels to the ones imposed by regulatory authorities, these processes may lead to significative active pharmaceutical ingredient (API) losses. Thus, by guaranteeing the manufacturing of safe API, its production yield could get compromised, resulting in economic losses.

Therefore, development of suitable purification process is mandatory. This must be capable of promoting an efficient GTI removal, complying to the threshold of toxicological concern (TTC), and ensure a minimal API loss, preferably below 10%.

Thus, this thesis focuses on implementation of an adsorption-based purification process by using polybenzimidazole (PBI) membrane. It is assessed the ability of these adsorbers for performing a specific GTI removal and a possible API purification strategy is suggested. With the objective of mitigating API losses and regenerating the membrane, a post-binding step was applied within this strategy and evaluated. The obtained results suggest that PBI membranes and the experimental strategy followed did not lead to an efficient purification process due to low GTI limit imposed for Roxithromycin (API selected). However, the use of H_2O at pH 1.2 for membrane regeneration when 4-dimethylaminopyridine is the concerned GTI and of H_2O at pH 13 for eluting methyl *p*-toluenesulfonate from the membrane presented good results to be further explored.

Keywords: genotoxic impurities, active pharmaceutical ingredient, adsorption, polybenzimidazole membrane adsorbers, purification strategy.

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General abbreviations and mathematical symbols

ADI – Allowable Daily Intake

AI – Allowable/Acceptable Intake

ALARP – As Low as Reasonably Practical

A_m – Membrane Area

API – Active Pharmaceutical Ingredient

Beta – Betamethasone Acetate

C_e – Equilibrium concentration

C_f – Final concentration

C_0 – Initial concentration

χ^2 – Chi-square

CPMP – Committee for Proprietary Medicinal Products

CHMP – Committee on Human Medicinal Products

DMAc – Dimethylacetamide

DMAP – 4-dimethylaminopyridine

EMA - European Medicines Agency

EWG – Expert Working Group

FDA - Food and Drugs Administration

FP – Fluticasone Propionate

GAC – Granular Activated Carbon

GTI – Genotoxic Impurity

H^+ - Free hydrogen ion

Halo – Halobetasol Propionate

HCl – Hydrochloric Acid

H_2O – Water (Milli-Q)

ICH – International Conference for Harmonization

IPA – 2-Propanol

IPC – In-Process Control

K_F – Freundlich's adsorption constant

K_H – Henry´s adsorption constant

K_L – Langmuir's adsorption constant

LP – Liquid Phase

LFL – Less than Lifetime

λ_{max} – Wavelength of maximum absorbance

M – weight or mass

MeCN – Acetonitrile

Meta – Mometasone Furoate

MI – Mutagenic Impurity

MPTS – Methyl *p*-toluenesulfonate

MW – Molecular Weight

NaOH – Sodium Hydroxide

n – Parameter from Freundlich model related with the surface layer heterogeneity

OSN – Organic Solvent Nanofiltration

PBI – Polybenzimidazole

PBI-TA – PBI with thermal and acidic treatment

PBI-TB – PBI with thermal and basic treatment

PDE – Permitted Daily Exposure

pH – Negative decadic logarithm of the free hydrogen ion concentration

PhRMA - Pharmaceutical Research and Manufacturers of America

QA – Quality Attribute

Q&A – Question and Answers

QbD – Quality by Design

QbT – Quality by Testing

QSAR – Quantitative Structure-Activity Relationship

q – Binding Capacity

q_e – Binding Capacity at equilibrium

$q_{e,m}$ – Binding Capacity at equilibrium obtained from the model

q_m – Maximum adsorption capacity

R – Correlation coefficient

R^2 – Coefficient of determination

Roxi – Roxithromycin

SAR – Structure-Activity Relationship

SWP – Safety Working Party

TTC – Threshold of Toxicological Concern

V - Volume

wt% - weight percent

1. Objective, Research Strategy, and Thesis Outline

1.1. Objective

Genotoxicity, mutagenicity, and carcinogenicity are terms that have been earning some relevance in pharmaceutical and health sectors in the last two decades, despite its origins could be traced back to the late 90s.

All this raising concern has been reflected through implementation of several guidelines and their continuous updates with the purpose of helping the pharmaceutical industry manufacturing drug products without presenting genotoxic risks associated. These are normally promoted by using highly reactive materials, which sometimes cannot be avoided or substituted since its reactivity is essential for a reaction to happen. Thus, this same reactivity from synthesis reagents associated or not with the use of catalysts in organic solvents could originate, beyond the intended active pharmaceutical ingredient (API), some impurities which may be directly or indirectly, as precursors, involved in mutagenicity or carcinogenicity effects. These effects constitute a situation to be prevented because, otherwise, a cancer condition could potentially take place.

In this way, the development of purification processes and strategies to assure the total removal of certain genotoxic compounds or its reduction until a level imposed by the regulatory agencies is indispensable.

To not represent a risk for patients' health, this extremely low limit of impurities is crucial but, at the same time, its achievement leads to a significative loss of API, which denotes an unfavourable situation. Therefore, it is important to develop a purification process that ensures both efficient genotoxic impurity (GTI) removal and insignificant API loss with the purpose of avoiding production yield reduction and, hence, a raise on the market price of the pharmaceutical product.

In this thesis, the main objective is to implement an API purification process by exploring polybenzimidazole (PBI) membrane adsorbers and assess their capability for efficient GTI removal without significative API losses. In this way, a purification strategy will be developed, consisting in two different moments. The first is related with the use of these membrane adsorbers and the evaluation of their ability for specific removal of GTIs and, the second, concerns developing a post-binding step, that is, resorting to a recovery step for mitigating the API losses and a regeneration step to try adsorber reusability.

1.2. Research strategy

After presenting the main objective, the model APIs and GTIs selected after solubility experiments, as well as the rationale behind their choice to fulfil the purpose(s) of this thesis, are presented. In this way, starting by the model APIs, the selected ones were Halobetasol Propionate (Halo), Betamethasone Acetate (Beta), and Roxithromycin (Roxi).

Halo is a glucocorticoid steroid capable of reducing skin inflammation or infection in the airways by topical administration. It is prescribed for the treatment of allergic rhinitis, asthma, and inflammatory skin disorders (e.g., eczema) [1,2]. Regarding its genetic toxicology profile, this API gave positive findings in two genotoxicity studies, despite presenting negative results in others. However, being administrated as a lotion, the systemic exposure to this API will be much lower than its initial quantity applied on the skin due to the low level of dermal absorption after topical administration. This drug substance is well characterized and widely used as model API in purification processes studies, being a medical alternative to Mometasone Furoate (Meta), an API also well-studied in purification processes [3].

Beta is a glucocorticoid used for treating various disorders like arthritis or allergic/inflammatory conditions related with airways diseases by several routes of administration (oral, topical, parental) [4,5,6]. Its lack of mineralocorticoid properties makes it suitable for cerebral edema treatment [4,5]. This API does not present any relevant genotoxic data result and, hence, it is not related with genotoxicity. However, the study of this API has been earning some relevance in purification processes/strategies studies.

Roxi is a semi-synthetic macrolide that acts as an antibiotic for the treatment of urinary, soft tissue, and respiratory tract infections [7], not being associated with genotoxicity effects. Roxi has been studied with the purpose of increasing its oral bioavailability through hydrophilicity improvement and complete or partial transformation to an amorphous form. With this, the goal was to increase its saturation solubility in aqueous systems [8]. Beyond this, its significative presence in surface waters and even in drinking water, due to its widespread use, led to perform studies about degradation pathways for Roxi with the purpose of avoiding or reducing its incidence in the environment [9]. However, its study in purification processes, especially resorting to adsorption, is not widely reported.

In this way, for the first time, the purification of these APIs by using PBI membrane adsorbers is going to be reported here. Despite all three drug substances being selected as model APIs, the one presenting more relevance in this thesis will be Roxi due to lack of prior studies about its purification processes based on adsorption phenomena. Thus, while Halo and Beta are only included in binding experiments, Roxi will be addressed in both binding and post-binding (recuperation and regeneration) experiments with the purpose of finding a proper purification strategy for this API.

In figure 1.1, the molecular structure and respective molecular weight of the three APIs are displayed.

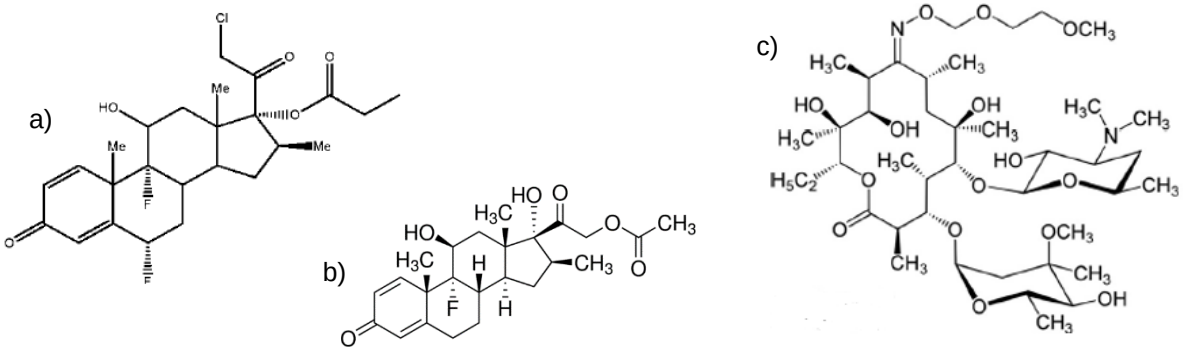


Figure 1.1. Molecular structure of model APIs considered in this thesis: a) Halo (MW: 484.96 g/mol); b) Beta (MW: 434.50 g/mol); c) Roxi (MW: 837.04 g/mol).

Now, moving to the GTIs, 4-dimethylaminopyridine (DMAP) and methyl *p*-toluenesulfonate (MPTS) were the ones selected to be part of this thesis. Despite not being involved or produced on the synthesis of the previous APIs or even being formed through metabolic pathways of these APIs after ingestion, the concerned GTIs were used due to their significant number of prior studies where they have been reported, which make them well-studied and -characterized compounds. However, submitting these GTIs to adsorption phenomena using PBI membranes is being described here for the first time.

Regarding DMAP, this aromatic amine is a highly efficient catalyst used for acylation reactions [10] and presents a structural alert. So, despite not being innately genotoxic, primary and secondary amines (like DMAP) can originate electrophilic species through their metabolic activation *in vivo*. These species are associated with mutagenicity and carcinogenicity effects [11]. Therefore, in fact, DMAP is not classified as a GTI itself but may be involved in reactions like the formation of Meta [12] and, consequently, the presence of DMAP in this final API must be avoided. Otherwise, after Meta being administered, the metabolization of DMAP *in vivo* can originate compounds involved in mutagenicity and carcinogenicity effects. With respects to MPTS, this sulfonate ester is seen as a potentially genotoxic impurity, being part of a widely studied family of GTIs (alkylating agents) [13], as it is going to be seen on the second chapter.

In figure 1.2, the molecular structure and respective molecular weight of both GTIs are presented.

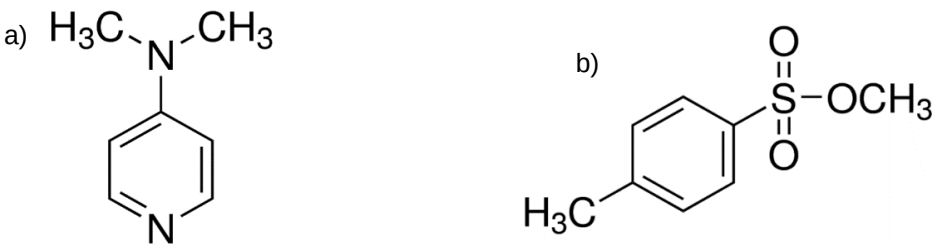


Figure 1.2. Molecular structure of model GTIs considered in this thesis: a) DMAP (MW: 122.17 g/mol); b) MPTS (MW: 186.23 g/mol).

Organic solvents are commonly used in synthesis and purification processes of APIs. Thus, for the binding experiments in this thesis, Acetonitrile (MeCN) was the solvent selected, presenting a role as a polar aprotic solvent [14]. Being aprotic, it lacks an acidic proton and so a hydroxyl and/or amine groups. This means that it is not capable to donate protons in hydrogen bonding, despite being able to accept them. Beyond this, this solvent has not been classified as to human carcinogenicity [14]. Therefore, from several organic solvents tested, MeCN was chosen since all APIs and GTIs selected for this thesis were soluble in this solvent at all experimental concentration range used.

Beyond this reason for its choice, the preference for this organic solvent is also related with the fact of being a volatile compound and having a lower boiling point than water, which is another solvent used in the purification process presented in this thesis, being both solvents studied in the post-binding step. The water was used with the purpose of introducing a green solvent in the purification strategy. The use of these type of solvents in pharmaceutical and chemical industry has been encouraged, but their implementation is still difficult as viable alternatives to some organic solvents.

Bearing in mind the input of severe chemical conditions on certain API synthesis, the development of robust and suitable adsorbers, used for drug substance purification processes, is indispensable. PBI is an organic solvent compatible polymer that has gained some relevance in API purification strategy due to its stability at thermal, chemical, and mechanical level. Beyond this, not being soluble on most organic solvents used on pharmaceutical industry, makes it very desirable as a feasible choice. Thus, in this thesis, as PBI was not dissolved by MeCN or water, the physical integrity of its membranes would not be compromised.

Regarding the maximum quantity of GTI allowed in an API, this is determined by resorting to the Threshold of Toxicological Concern (TTC) value and the maximum daily dose of API (mg/day) [15], as presented on the following equation.

$$GTI\ limit\ (mgGTI/gAPI) = \frac{TTC\ value\ (\mu g.day^{-1})}{maximum\ daily\ dose\ of\ API\ (mg.day^{-1})} \qquad (1.1)$$

Attending to the model APIs selected, depending on their route of administration, their respective maximum daily dosage may change. For Halo, usually administered topically, it is reported that administration of 50 g per week of the lotion containing this API, in the amount of 0.5 mg of Halo per gram of lotion, should not be exceeded [16]. Then, through this data, it is possible to determine the maximum daily dose for this API. After this and knowing the well-established TTC (1.5 µg/day), a value of approximately 0.42 mgGTI/gAPI is obtained. For Beta, which could be administered through several routes, it is reported that an administration of 1mL per week of an injection presenting 3 mg of API should not be exceeded [17]. Using the same reasoning previously applied for Halo, a value of approximately 3.5 mgGTI/gAPI is obtained for Beta. For Roxi, due to its instability in gastric acid media, a high maximum daily dosage of 300 mg must be administered orally [18]. In this way, for this case, a GTI limit of 0.005 mgGTI/gAPI is obtained.

Therefore, assuming a situation without API losses during the purification process, a GTI removal of 99.58%, 96.5%, and 99.995% would be necessary to comply with the TTC for case-studies involving Halo, Beta, and Roxi, respectively. However, as it is going to be seen, there are API losses during purification steps, which means that higher removal efficiencies will be needed to reach the desirable GTI/API ratio.

1.3. Thesis Outline

This thesis is structured in 5 chapters which are intercorrelated. Regarding the chapters, these are:

1st Chapter

Objective, Research Strategy and Thesis Outline are presented in this 1st chapter.

2nd Chapter

Theoretical Introduction make up this chapter, contextualizing and addressing the main topics to be presented and discussed in this thesis. Starts with a general introduction of concepts associated with GTIs, as well as their sources and related reactions. A detailed review on the legislation context regarding GTI presence in drug products will be provided. Beyond this, different strategies to mitigate GTIs are presented. So, chemical synthetic approaches, purge factors and separation or purification processes (either conventional or advanced ones) will be addressed, giving emphasis to the adsorption process.

3rd Chapter

Materials and methods will be part of this chapter, where all the experimental work carried out is described, since the production of the PBI membranes until the post-binding step procedure, comprising both recuperation and regeneration steps.

4th Chapter

This corresponds to the results and discussion chapter, where all the outcomes of the experimental work performed are presented and properly discussed. It involves solubility results, from where, the model APIs and GTIs for the upcoming experiments are selected. Then, binding adsorption results are presented and the performance of the PBI membrane (for API purification) is evaluated, being as well the outcomes from the respective isotherm studies presented and discussed. After this, the post-binding step and its results and discussion are addressed with the purpose of finding a proper API purification strategy to mitigate API losses.

5th Chapter

This chapter closes the thesis by presenting a general conclusion and the topics that were left unaddressed on this thesis (as a future work).

2. Introduction

2.1. GTIs – General Overview

Looking at the pharmaceutical products, their manufacturing might follow two different pathways. One of them resorts to a total synthesis approach and the other to the modification of a naturally occurring product. However, in both situations, reactive reagents can be involved. Then, the final drug product may present these reagents or side products as impurities, at low levels. Being impurities, it is inevitable that they do not present toxicities, which could be related with genotoxicity and carcinogenicity[19].

Both terms, genotoxicity, and carcinogenicity, as well as a mutagenicity, despite being associated, it is important to not confound them and, hence, leading to its misuse. Regarding genotoxicity, this term covers a broader range of genetic damage, regardless if such damage is or not corrected through a cell DNA-repairing mechanism. In relation to mutagenicity, this is directly correlated with processes involving genetic change, that is, mutation. This includes a perpetual change in the genome and, consequently, may be reflected on the phenotype, being all this promoted by a substance (mutagen) that increases the frequency of these changes. Carcinogenicity involves processes leading to tumour development because of mutagenic processes. Therefore, carcinogen can induce unregulated growth processes at cellular level and, in its turn, leading probably to cancer situation due to cell metabolic effects or genome damaging [19,20,21].

Having been properly clarified the difference between those previous terms, it is now important to understand how the genotoxic impurities (GTIs) could get incorporated in the final active pharmaceutical ingredient (API). According to Pounikar, A., *et al.*, there are several sources through which GTIs could end up being present in the drug product [22]. Synthesis components such as solvents, reagents or even catalysts, which are involved in drug production, could be genotoxic impurities. Starting material and its impurities as genotoxic intermediates or process related by-products involved in API synthesis could be another source. Beyond all this, some specific situations at storage conditions such as exposure to light and air oxidation could lead to drug degradation or its hydrolysis, which might result in formation of genotoxic impurities. At last, but not least, it is also mentioned a possible generation of chiral impurities, which may be genotoxic, in APIs due to the synthesis of stereoselective drugs, which might present stereoisomers of raw material or intermediates [22,23].

In this way, attending to what has been referred, the presence of these genotoxic impurities in the final drug products has been leading to an increasing concern in health sector, including not only pharmaceutical companies and regulatory agencies, but also patients and doctors. Therefore, attending to the risks associated for patient's health, a significative rise on the number of publications involving "genotoxicity" and other related terms has been noticed in the last years [19].

2.2. Mechanism of action

Attending to what has been referred, a certain compound presenting a carcinogenic or mutagenic effect will surely react with DNA. According to James and Elizabeth Miller theory, the actuation of genotoxins upon DNA molecules occurs due to the presence of nitrogen and oxygen atoms on pyrimidine (Cytosine and Thymine) and purine bases (Adenine and Guanine), as well as on phosphodiester backbone, which constitute the nucleophilic sites where the electrophilic attack by the GTIs takes place (Figure 2.1). In some cases, this mechanism of action could lead to strand breaks[19,21,24].

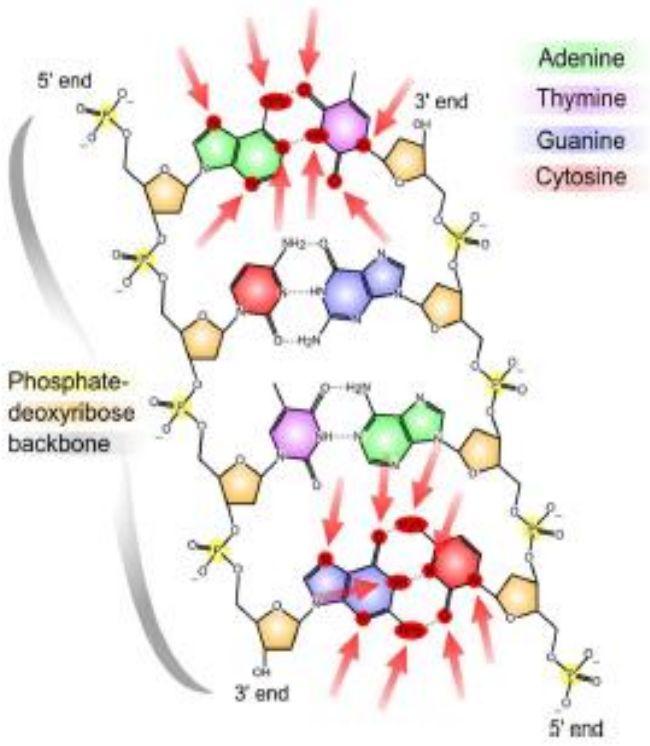


Figure 2.1. Attack on the DNA by genotoxins, where the arrows indicate the targeted nucleophilic sites of DNA bases (based on Madeleine Price Ball's figure, GNU Free Documentation License) [19].

Beyond chemical nature of the GTI, and so its reactivity, there are other factors influencing the reaction site, namely steric factors and nucleophilicity. In this way, due to the stereospecificity of the reactions, the most nucleophilic sites within DNA bases and, hence, most probable to suffer an electrophilic attack are endocyclic nitrogens (N3 and N7 of guanine and adenine) and the less nucleophilic are the exocyclic oxygens [19,20,25].

2.3. Classes of Genotoxic Impurities

After understanding how the GTIs can act upon DNA molecules, it is now important to take a deeper look at the chemical nature of the GTI and the reason behind its reactivity. There is a variety of genotoxic molecules presenting different chemical structures that will lead to a reaction between these molecules and the DNA. Some of these GTIs show known genotoxic effects while others are labelled as dangerous due to their connection with reactive groups known as structural alerts [25]. However, it is necessary to attend to situations where there is an overprediction of mutagenicity since some structural alerts do not take into account factors such as steric hindrance, hydrophilicity, among others [19,26,27].

As previously reported, there are several routes through which the GTI could get incorporated in the final API. This would happen because a certain GTI could play an important role in the manufacture of an API. Thus, genotoxic chemicals could be used as reactants or as organic solvents, as well as being formed in side reactions [19].

The reactants are part of a chemical API synthesis due to their reactivity, which despite being crucial for the reaction to happen, could be associated with genotoxicity. In the pharmaceutical industry, there are several reactants, from different families, presenting genotoxicity. Thus, as alkylating agents, there are alkyl halide and dialkyl sulfate; aromatic amines are also commonly used, usually as building blocks; epoxides are used in several addition reactions; there are also hydrazines and TEMPO (cyclic amine oxide radical). All these reactants, when they are not totally consumed, their genotoxicity end up being boosted [19].

Regarding the GTIs formed in side reactions, like alkyl halides or acetamides, one of the main classes studied is the sulfonate esters. These GTIs and their precursors may be used either in catalytic amounts for cyclizations and specific groups protection or in stoichiometric amounts, acting as API salt forming agents and good leaving groups. Being alkylating agents, they act upon DNA bases through electrophilic attacks by adding alkyl residues in nucleophilic sites. Being formed in secondary reactions (with alcohols or on cleanup processes), their detection on the API synthesis would not be immediate. In this way, the detection and identification of their precursors is indispensable since if a specific precursor is present in an API synthesis, there is a possibility for genotoxic sulfonates formation (in certain conditions) [19]. However, its formation could be reverted through its decomposition into a sulfonic acid and an ether, leading to a reduction on the formation of these genotoxic sulfonate esters [28,29]. In this way, for the APIs commercialized as sulfonic acid salt, the removal of all sulfonate ester through purification processes needs to be proven, according to the European Pharmacopoeia [30].

In relation to organic solvents, it is important to highlight its wide range of uses in several pharmaceutical production processes. However, these solvents presenting various roles could be related with genotoxicity and carcinogenicity. Then, a specific guideline addressing organic solvents is crucial, as it is going to be seen on the next section [19,31].

2.4. Regulation

Since genotoxic impurities have been at the center of increasing regulatory and industry attention, a presentation of the main key actions through the years toward regulations must be included here with the purpose of demonstrating the evolution of this raising concern [19].

In this way, the main regulatory authorities from Europe, United States and Japan got together to create the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. This would be involved in the analysis of scientific and technical aspects of pharmaceutical product registration.

Regarding the timeline, this can be traced back to late 1990s, where the ICH Q3 guidelines used the term “unusual toxicity” instead of genotoxicity. However, unusual toxicity, being a general term, it ended up including many of the genotoxic impurities. Looking at the ICH Q3, this guideline presented several topics, namely Q3A (control of impurities in drug substance); Q3B (degradants in pharmaceutical products) and Q3C (address residual solvents) [19,32]. However, these existing regulatory guidelines did not effectively address the requirements for controlling GTIs trace levels [33]. Still within this late 1990s, two ICH safety guidelines (ICH S2A – 1995; ICH S2B – 1997) presented a general framework for genotoxicity testing of pharmaceuticals. According to Muller, L., *et al.*, both guidelines stated: *“For compounds giving negative results, completion of the standard battery of tests, performed and evaluated in accordance with current recommendations, will usually provide a sufficient level of safety to demonstrate the absence of genotoxic activity.”* [34]. So, this standard set of genotoxicity assays used for testing the API provided crucial information regarding the diversity of genotoxicity mechanisms, directly and indirectly associated with effects on DNA [34]. In this way, for a specific compound, a positive result produced in one or more of those genotoxicity assays led to label this compound as genotoxic, being further testing for risk assessment advised. However, despite all this, in this context, genotoxicity is still a term involving mutagenicity and its effects through DNA damage and reactivity [34].

In 2000, an article published by PharmEuropa about the formation of sulfonate esters in API salt production was disclosed, being the first example addressing a specific regulatory concern with GTIs[19,35]. Two years later, a position paper led the scientific and industrial community trying to find GTI-free routes for API production or to provide a justification for GTIs unavoidable presence on the final drug product when the first approach was not possible. So, this first draft paper, published by the Committee for Proprietary Medicinal Products (CPMP) was about GTIs presenting sufficient evidence for the existence of a respective threshold mechanism [36,37]. In this way, a model of virtual safe dose concept was suggested as an alternative to *in vivo* studies and the terminology “as low as technically feasible” was established [19]. With this model, the conditions were created to soon introduce the threshold of toxicological concern (TTC) concept that would be finally able to identify an acceptable risk exposure level for genotoxic carcinogens, without being necessary to assure a complete elimination of GTIs from the API [36].

Thus, a draft guideline on the limits of GTIs was released in 2004 by the Committee on Human Medicinal Products (CHMP) from European Medicines Agency (EMA) and the TTC concept was

introduced [19,38]. With this, it was possible to establish a safe level of exposure for all chemicals, regardless existing or not toxicity data, and below which there would not be considerable risk to human health. In this way, the implementation of the TTC concept and, hence, of its limit of 1.5 μg.day^{-1} for known and potential carcinogens was made, unless experimental evidence justifies higher limits. These higher limits constitute an exception, being only applied in short-term studies for clinical testing of APIs. The reasoning for the limit presenting so small value is to assure that a specific substance, regardless exhibiting or not a negligible carcinogenic risk, would not constitute a risk for patients' health [19,39].

So, this TTC approach, presenting a conservative behaviour, would lead the pharmaceutical industry to address it in all control and purification strategies, remaining the need of justifying a selected route. Still within this draft guideline "as low as technically feasible" terminology was replaced with the "as low as reasonably practical" (ALARP) principle, and the need for introducing alternative routes was omitted. However, in this draft, guidance on permissible doses during short-term studies was missing [36,19].

Still looking at EMA guideline [36,40], which was finalized in 2006, there was an update on the meaning of genotoxic impurity term, which refers to "positive findings in established *in vitro* or *in vivo* genotoxicity tests with the focus on DNA reactive substances" [36]. When such information was missing, *in vitro* genotoxics were typically considered *in vivo* mutagens and carcinogens [32]. According to EMA guideline [40], attending to the significance of mechanism and dose-response relationship in the assessment of GTIs, there was presented two classes of genotoxic compounds:

"1. Genotoxic compounds with sufficient (experimental) evidence for a threshold-related mechanism, which were regulated according to the procedure as outlined for class 2 solvents in the "Q3C Note for Guidance on Impurities: Residual Solvents"." [32].

"2. Genotoxic compounds without sufficient (experimental) evidence for a threshold-related mechanism. In this case, the guideline proposed a policy of controlling levels to "as low as reasonably practicable" (ALARP) principle, where avoiding is not possible" [32].

Still in 2006, a staged TTC approach was proposed (presented in Table 2.1), considering acceptable limits for GTIs in final drug products related with exposure duration, suggested by the Pharmaceutical Research and Manufacturers of America (PhRMA) [19,34]. Therefore, for a quantitative risk assessment, the concerned approach could be used for GTIs presenting genotoxicity data [32,34]. The same document also defined five separate classes for the impurities attending to the structure−activity relationship (SAR).

Table 2.1. Presentation of the Proposed Allowable Daily Intake (µg/day) for GTIs during clinical development using the staged TTC approach. This table constituted the PhRMA task force proposal for the GTIs [32].

	Duration of clinical trial exposure				
	≤1 month	>1-3 month	>3-6 month	>6-12 month	>12 month
Allowable Daily Intake (µg/day) – for all development phases	120	60	20	10	1.5
Alternative maximum level of allowable impurity attending to the percentage of impurity in API	0.5%	0.5%	0.5%	0.5%	0.5%

In 2007, since the excipients were excluded from the 2006's finalized EMA guideline, a specific position paper addressing excipients was disclosed by the CHMP of the European Medicines Agency.

One year later, the Food and Drug Administration (FDA) released their draft guidance addressing the issue of GTIs [36,41]. This guideline has never been finalized and contained nonbinding recommendations to the pharmaceutical industry. In this way, the FDA draft guideline provided recommendations on acceptable exposure limits for genotoxic and carcinogenic impurities during either marketing applications or clinical testing, while EMA guideline only provided these recommendations to products for marketing applications. Beyond this, FDA draft guidance suggested changing API synthetic route for minimizing the formation of GTIs or maximize its removal with the purpose of reducing the potential lifetime cancer risk associated with genotoxic and carcinogenic impurities [36]. Despite FDA having considered acceptable the EMA guideline approach related with the exposure limit for genotoxic or carcinogenic impurities, there is a main difference between these two guidelines regarding the requirements for the degree of lower GTI limits. In FDA draft, the introduction of lower limits for different patient populations is recommended and, hence, different staged TTC values for short term studies are proposed. Beyond this, FDA demands specific genotoxicity tests for GTIs above the ICH qualification thresholds [19,36].

In September 2010, the Safety Working Party (SWP) published a Question and Answers (Q&A) document to complement the "Guideline on the limits of genotoxic impurities" (2006) from EMA. This document ended up being useful since it allows to clarify certain key topics that were left unaddressed on the EMA guideline, leading the industry to some issues regarding the interpretation and understanding of certain matters [36,37]. In this way, the main points clarified were:

- If a potential GTI, not belonging to a class of very potent genotoxic carcinogens, is controlled at the TTC level, no genotoxicity test or ALARP principle is necessary.
- Negative Ames test (bacterial mutagenicity test) overrules a structural alert and, consequently, no further studies are needed.
- If the impurity has no genotoxicity concern by resorting to the quantitative structure-activity relationship (QSAR) assessment, then no further qualification studies will be necessary.

It has also been clarified that for investigational studies, minor durational adjustments to the TTC limit are tolerable. Beyond all this, for the staged TTC approach, SWP introduced a new dose rate correction factor to consider deviations from the linear extrapolation model, giving slightly different values in relation to the ones from the original TTC approach (from PhRMA).

Also, for controlling multiple GTIs, SWP stated that a TTC value of 1.5 µg/day can be applied to each individual and structurally unrelated impurity present in the API. Therefore, the sum of the GTIs at 1.5 µg/day is recommended for the cases where the impurities present the same mode of action and have the same molecular target, exerting the effect in an additive manner [36]. It is also important to mention the introduction of the "cause of concern" terminology, being this related with a material presenting either a pre-existing or new genetic toxicology indications.

Finally, SWP document presented two different situations regarding the moment of introduction/formation of the GTI in the synthesis. In the case that this moment corresponds to the step before the final synthetic step, the inclusion of the impurity in the drug substance specification was not necessary. However, if resorting to analysis, it was proved the presence of this impurity exceeding 30% of the acceptable TTC, then the impurity must be included in the drug substance specification. Regarding the situation when a GTI is formed/introduced in the final synthesis step, it should always be included in the specifications. However, if the presence of this impurity does not exceed 30% of the acceptable TTC, it is possible to skip testing. For this to be feasible, data from at least 6 consecutive pilot scale or 3 consecutive production scale batches should be submitted [36].

Looking back to the first ICH S2 guidelines, it was concluded that a revision of the principles of both S2A and S2B guideline was indispensable. In this way, at the end of 2011, these previous guidance documents were replaced by the ICH S2(R1). In this revised guideline, a battery of different genotoxicity tests to address several genotoxic mechanisms related with carcinogenesis was presented with the purpose of assuring the removal of potentially genotoxic carcinogens in the initial phase of the API development [42]. In this way, reorganization and restructuring should be done, by reducing the number of animals involved in routine testing through current procedures improvement and by clarifying the specific tests performed in the case of positive findings. Regarding irrelevant findings, its management and interpretation should be enhanced with the purpose of reducing barriers in early stages of API production through risk assessment improvement for carcinogenic effects. Lastly, the standards established for testing and interpretation of positive results from *in vitro* assays must be internationally agreed [42].

Regarding the principles ruling the genetic toxicology testing and its use, it was possible to see that for Ames test, despite being an indispensable part of regulatory testing, there was no more need for repeating fully adequate negative tests. It was also presented the *in vitro* micronucleus test as an alternative and valid option to some of the previous tests involving animals. Beyond this, reducing 10 times the concentration for non-toxic compounds in mammalian cells *in vitro* assays was feasible. In relation to irrelevant positive findings in mammalian cell tests *in vitro*, their growing number was counterbalanced by limiting the levels of cytotoxicity for *in vitro* chromosomal aberration and

micronucleus tests. An optimised management for animal usage could also be achieved by integrating the assessment of genotoxicity into the rodent repeat-dose toxicity study when it is feasible. Finally, it was stated that an *in vivo* test studying the genotoxic damage in two tissues instead of conducting mammalian cells tests *in vitro* followed by an *in vivo* test would be more rationale [42].

Thus, the ICH S2(R1) enables a better risk assessment for genotoxicity of pharmaceuticals.

Moving to the year of 2014, in June, the ICH M7 guideline: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk [43] reached Step 4 of the ICH process, which means that the final draft became recommended for adoption by the three regulatory entities (EU, Japan and USA) ruling the ICH. According to Kragelj Lapanja, N., *et al.*, the purpose of this new guideline was to provide *"a practical framework that is applicable to the identification, categorization, qualification, and control of mutagenic impurities (MIs) to limit potential carcinogenic risk"*[36]. Its application is quite wide-ranging since it is valid for new drug products during either marketing applications or clinical testing, post-approval submissions of marketed products and even for products with an API already present in an approved product but associated with a new marketing application [36]. Now, regarding the use of the TTC approach in the evaluation of acceptable limits for a new chemical, the ICH M7 also considered it to be crucial. In a similar way as presented in previous EMA and FDA guidelines, higher Allowable Intakes (AI) of impurities in short time exposures would be permitted. However, in this new guideline, the fact of exceeding the TTC does not automatically mean an expanded cancer risk, being stated that this concept is a highly hypothetical one and, hence, not always indicating an actual risk [36].

According to ICH M7, all actual and potential impurities with a high probability of arising either during API synthesis and storage or during manufacturing and storage of the drug product should be included in the impurity assessment analysis. Therefore, resorting to previous data regarding carcinogenicity and bacterial mutagenicity, the impurities may be classified into five separate classes (as previously reported by Muller *et al*., 2006 [34]) [32] (Table 2.2).

Table 2.2. Genotoxic impurities classification. [32,36]

Class	Description
1	Impurities that are known mutagenic carcinogens
2	Impurities that are known mutagens with unknown carcinogenic potential
3	Impurities with alerting structure, unrelated to the structure of the drug substance and no mutagenicity data
4	Impurities with alerting structure, same alert in drug substance or compounds related to the drug substance which have been tested and are non-mutagenic
5	Impurities with no structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity

ICH M7 stated that a (Q)SAR assessment would be required for the cases when carcinogenicity and bacterial mutagenicity data were not available. For this, two (Q)SAR computational methodologies (one rule-based and the other statistical-based expert) were indispensable. When from both methods, structural alerts did not outcome, then it could be assumed that the concerned impurity belonged to class 5. On the other hand, being identified a structural alert, an Ames test (or an equivalent) should be performed. From here, two outcomes were possible. Being the result negative, then no further genotoxicity assessment would be necessary and, hence, the impurity was from class 5. In its turn, a positive result would mean the need for more assessment and control strategy (class 2) [36].

Regarding class 1 impurities, a specific AI obtained resorting to carcinogenic potency and linear extrapolation could be used. For the cases when an impurity is chemically similar to another one, which was classified as a known carcinogen compound class, the AIs typical from this class could be applied to the first [36].

In relation to the less than lifetime (LFL) treatments, the cumulative effect was considered for determining the acceptable lifetime dose attending to the total number of exposure treatment days. Once again, the TTC-based AIs should be employed to each individual and unrelated impurity. For the cases involving three or more class 2 or 3 impurities in the APIs, limits should also be imposed to the total mutagenic impurities (Table 2.3).

Table 2.3. Presentation of AI for LFL to lifetime exposures for a) an individual impurity and b) multiple impurities [36,43].

Treatment Duration	Maximum Daily Dose (µg/day)	
	a)	b)
≤1month	120	120
>1-12 months	20	60
> 1-10 years	10	30
>10 years to lifetime	1.5 (TTC limit)	5

Regarding a class 1 impurity presenting class-related AI limits, the values from the previous table were not applicable. Beyond this, impurities created by degradation of drug products also required to be controlled individually and exceptions regarding the TTC limits could be done in specific cases like a severe disease, reduced life expectancy, or limited therapeutic alternatives [36].

Moving to the control strategy mentioned at ICH M7 for impurities classified as class 1, 2 or 3, this strategy would be crucial to make sure that a specific impurity would be in an API or drug product presenting a level below the acceptable limit. Therefore, this guideline introduced 4 different approaches for developing a control strategy [36]:

- **Option 1** – Drug substance specification included a test for the mutagenic impurity (MI), having been used a suitable analytical method for establishing the acceptance criterion. A periodic

verification testing would be possible in specific conditions involving levels of the impurity less than 30% of the acceptable limit.

- **Option 2** – Raw and starting material, as well as intermediate specifications included a test for MI, being also possible the use of in-process controls (IPCs). Like in option 1, the acceptance criterion was set at or below the TTC limit using an analytical method.

- **Option 3** – Test for MI was included in the same previous specifications from option 2. However, the acceptance criterion was set above the acceptable limit of the impurity in API, resorting to a combination of an analytical method with a suitable purge factor analysis. In this way, it could be ensured that the level in API was below the acceptable limit without needing further testing.

- **Option 4** – There was no specification where a test for MI should be included if the level of this impurity in API was proven to be below the TTC limit without resorting to analytical method. So, the purge factor analysis, relying on process parameters understanding and scientific principles, would be sufficient. When this analysis alone was not satisfactory, analytical data to validate this control strategy would be expected [36]. Regarding the scientific risk assessment used to justify this approach, it could be seen as a projected purge factor for removal of the impurity [36].

With respect to the ALARP principle in ICH M7, its application was not needed every time the level of MI was below the limits. Beyond this, the demonstration of exploring alternative routes of API synthesis would not be necessary, which in turn, was needed at EMA guideline [36].

As a conclusion, ICH M7 was mostly capable of addressing many points that were left unclear in EMA and FDA guidelines. However, the difficulty from the pharmaceutical industry to properly apply it was still visible. In this way, an Addendum to ICH M7 was suggested and released for public consultation (Step 2) in June 2015: Application of the principles of the ICH M7 guideline to calculation of compound-specific AIs [36,44]. Through this document, useful AIs information of 14 well-known mutagenic/carcinogenic impurities was summarised. In May 2017, this Addendum reached Step 4 of the ICH process, that is, its adoption was recommended by the ICH regulatory bodies [36,45,46].

Later, a version of ICH M7 (R2) was released by the Expert Working Group (EWG), in which acceptable limits (AIs or Permitted Daily Exposures (PDEs)) for new mutagenic impurities as well as a revision of these limits for the MIs already listed on the ICH M7 (R1) (Addendum) were included. Thus, in November 2018, the new area of work of the M7 (R2) Maintenance EWG was approved as it has been outlined in the revised Concept Paper (July 2018) granted by the ICH Management Committee. As a result, a Q&A document was presented to address some quality and safety issues that had been identified from experience since M7 implementation in 2014 [46,47]. So, this Q&A document was able to clarify some specific aspects from the previous guideline, namely the justification of control strategy in marketing authorization applications, the (Q)SAR systems, and so on. Beyond this, this document also aims to promote further harmonization in M7 guidance utilization for MI regulation by facilitating the implementation and interpretation of ICH M7 recommendations [48].

Taking a deeper look at M7 (R2) document, it is possible to see its structuration into several sections and their addressed points [48]:

- **Section 1** – Clarification of the meaning of mutagenic and genotoxic potential; presentation of recommendations for impurities evaluation (at/below/above 1 mg).
- **Section 2 and 3** – Explanation of non-mutagenic carcinogens being out of scope; MIs being non-carcinogens were considered similar to class 5 impurities; clarification of semi-synthetic drugs being in scope.
- **Section 4 and 5** – Clarification of the meaning of "significant increase in clinical dose of marketed products", corresponding to any increase in dose that would raise any MI above acceptable limits.
- **Section 6** - Recommendations for validation and documentation of (Q)SAR models; Ames negative impurities identified as class 5 without addressing positive clastogenicity results.
- **Section 7** – Clarification of LTL approach not being considered acceptable for PDEs; *in vivo* mutation assays were not considered sufficiently validated to derive compound specific limits; explanations for the HIV disease case, which had been moved from a "treatment duration < 10 years" to "lifetime treatment".
- **Section 8** – Focusing on option 4 control strategy; clarifying and recommending the elements that should be considered when using predictive purge calculations to claim no analytical testing; explanation of the timing for periodic verification testing; recommendation of batch scales for providing supporting data for options 3 and 4 control strategy.
- **Section 9** – Clarification of the validity of (Q)SAR predictions made earlier in development for market authorization; recommendations for clarifying the ICH M7 risk assessment and control strategy are presented.

Regarding the M7 Q&A, this guideline was signed off as a Step 2 document by the Assembly (Step 2a) and their Regulatory Members (Step 2b) in June 2020, to be issued by the ICH Regulatory Members for public consultation. The Step 4 Q&A document was signed off in April 2021 and concerning the Step 4 M7(R2) sign off, this occurred in July 2021[47,48].

2.5. GTI Mitigation

From the previous section about Regulation, it is possible to retain the importance of identifying GTIs on initial phases of APIs process development (when possible) through analytical methods implementation. If possible, developing synthetic processes to control or limit GTIs should be considered since their presence in pharmaceutical streams during API synthesis is normally challenging to avoid.

Bearing this in mind, original routes of API synthesis end up being improved through optimization of reaction conditions or substituting specific reaction steps with the purpose of achieving GTI levels below the acceptable limits imposed by regulatory entities. Resorting to these changes, there would be expected higher yields and efficiency on using reactants, leading to lower quantities of unreacted compounds and side products formed [19]. Then, according to Szekely, G., *et al.*, *"the ideal solution consists of the simplest possible, robust process, using cost-effective reagents to obtain high product yields through selective reactions and purification steps"* [19].

2.5.1. Chemical synthetic approaches

Chemical synthetic approaches are related with two main first strategies for mitigating GTIs: synthesis alteration and reaction conditions adjustment.

Regarding synthesis alteration, the preferred strategy, it relies on avoiding generating or using GTIs by applying different production steps or sequences to obtain the same API or a specific intermediate [19,49]. However, in some cases, the reactivity of a specific starting material or reagent is an indispensable feature for assuring a proper API or intermediate synthesis without significative loss of yield, despite this reactivity being also responsible for generating GTIs reacting with DNA. Then, altering the synthesis during process development could be challenging and impractical [19,50].

In relation to reaction conditions adjustment, this strategy relies on eliminating or reducing the presence of GTIs by changing reaction conditions through switching the order of addition of the reactants or altering the proportions of the components to be added on the synthesis or even attending to other factors like reaction time and quantity of reactants used. However, as always, all this must be performed without significative yield reduction [19,50]. As a brief example of this strategy, it is going to be possible to see the positive effects that adjusting reaction parameters could have on mitigating the GTIs formation. In this way, the effect of pH, temperature, and water content on the formation of sulfonate esters (obtained through the reaction between acids and an alcohol (solvent)) will be presented [19]:

- **pH:** Using a molar excess (even being minor) of a base prevents sulfonate esters formation. Then, it is possible to mitigate this GTI formation through either avoiding acidic conditions or adding a base [29].
- **Temperature:** Using lower temperatures significantly decreases the rate of formation of this GTI even without adding a base. So, lower temperatures are recommended for both reaction and workup [29].
- **Water:** The use of water has a significant effect on reducing this GTI formation, even without using base, since it can promote impurity hydrolysis and compete with the solvent (alcohol) for protonation [28].

Beyond all this, an extended storage time for solutions presenting both sulfonic acid and alcohol mixed should be avoided [51].

Looking at both previous strategies for mitigating GTI formation, they are linked with the use of another strategy that, somehow, is closely related with the two previous ones. The concerned strategy is the Quality by Design (QbD) approach, which aims also to control GTI levels below acceptable limits by designing and producing APIs for which the final quality should be ensured *a priori*. In this way, the need of using routine testing started decreasing [19,52]. Regarding its structuration, this strategy includes four stages [19]:

- **1st stage** – Definition of the targeted quality profile.
- **2nd stage** – Designing manufacture process and product to reach certain quality.
- **3rd stage** – Identification and selection of quality attributes (QAs), process parameters and sources of variability.
- **4th stage** – Developing control mechanisms to assure quality over time.

Despite all this, the main approach supported by regulatory entities is the Quality by Testing (QbT), which consists in developing analytical tools, methods, as well as intensive screening for GTIs in APIs, starting materials or even in intermediates [19,50].

Before getting into purification processes, there is another strategy that could be applied, despite not being mentioned in all guidelines. This strategy relies on conducting toxicity studies for proving that a suspect impurity does not represent a risk to patient health at low levels. However, carrying out this experimental investigation constitutes a very expensive and time-consuming situation, whereby it is only used in extreme circumstances. Regarding the guidelines mentioning this strategy, EMEA insists on its application for the most potent classes of carcinogen (e.g. N-nitroso compounds and azoxy compounds) while PhRMA, usually, for its Class 1 impurities (known to be carcinogenic) [50].

2.5.2. API purification

2.5.2.1. Purge Factors

Beyond what was previously presented, several stages and routes of API isolation and purification could be included in the production process as a last resort. Then, most of the GTIs could be eliminated (together with other impurities) during synthesis.

In this way, the purge factor analysis could be introduced. Its first mention could be traced back to 2009, where Pierson, D. A., *et al.* [30] considered satisfactory the number of steps between the appearance of GTI and the final production step to evaluate the risk of a GTI to be present in the final API. Here, a chemical rational should be used to decide if a specific impurity removal was required or not when the GTI was present more than four steps away in relation to the final step. However, due to the subjectivity implicated on the previous approach, Teasdale, A., *et al.* [33] presented a semiquantitative "assessment purge tool", which would constitute the ICH M7 option 4 control strategy. This tool would

be able to evaluate the risk assessment by resorting to physicochemical properties and process factors that influenced the fate and purge of a GTI, being unnecessary to resort to analytical tests [19,36].

Still within this proposed tool, the key parameters to which were attributed purge factors are:

- **Reactivity** – contributes to how impurities (as reactants) are purged as they are consumed in the reaction, being eliminated either by reaction with acids/bases or through work up.
- **Solubility** – involves crystallization or extraction; GTIs can be dissolved either in mother liquors or discarded phase.
- **Volatility** – a feasible route for low boiling point compounds removal, being the impurity removed with the solvent through separation techniques like distillation or solvent exchange.
- **Ionizability** – pH adjustments are crucial for assuring a separate partition of GTI and API between two phases (aqueous and organic) due to the different ionization state of the compounds.
- **Physical processes** – could be chromatography (for API purification).

It is also important to mention that this tool used a score scale for each purge factor, as it can be seen on Table 2.4.

Table 2.4. Presentation of an example of key parameters in purge factors in the tool proposed by Teasdale, A., *et al*. The purge factor is defined as the ratio of [GTI] before and after purging [19].

Physicochemical parameters	Purge Factor
Reactivity	High reactivity = 100
	Moderately reactivity = 10
	Low/no reactivity = 1
Solubility	Freely soluble = 10
	Moderately soluble = 3
	Sparingly soluble = 1
Volatility	Boiling Point > 20 ºC below that of the reaction solvent = 10
	Boiling Point ±10 ºC that of the reaction solvent = 3
	Boiling Point > 20 ºC above that of the reaction solvent = 1
Ionizability	Ionization potential of GTI significantly different
Physical processes (e.g. chromatography)	GTI elutes prior to the desired product = 10
	GTI elutes after to the desired product = 10

After scores being attributed, these are multiplied to give a purge factor for each stage of the process. Then, multiplying the purge factors from each individual stage yields an overall purge factor [36].

After this publication of Teasdale, A., *et al.*, several other publications and authors applied the theoretical purge factor assessment tool, although presenting some minor alterations on its conception. For example, in 2013, Elder, D. P., *et al.* [36] described a specific case-study where, comparing with the original approach, isolation steps within the physical process parameter were included, being a factor 3 used if the isolation step was present and 1 if not. Regarding the results, it was concluded that the tool was generally capable of correctly predicting the purging capacity for the concerned MIs studied [36]. Later, in 2015, two more practical applications of the proposed tool were published by McLaughlin, M., *et al.* [53] and Lapanja, N., *et al.* [36], where, once again, a minor modification at the physical process parameter was suggested, which corresponded to the inclusion of recrystallization step, instead of its inclusion within solubility parameter, as previously described by Teasdale, A., *et al*. [36].

However, it is important to refer that regardless the publication or case-study, from the comparison between theoretical and experimental purge factors, it was always seen an underprediction of the purge capacity. Then, the proposed tool (with or without minor modifications) tends to assume a more conservative side, being reliable. In this way, despite differing too much from the measured experimental values, the theoretical purge factors present an intentional underprediction with the purpose of the approach gaining acceptance [36].

Beyond all this, since expert systems have been widely used in different applications inside pharmaceutical industry, namely for predicting synthetic reactions [54,55] or designing routes via retrosynthetic analysis [56,57], a new semi-automated system for assessing the purge of MIs was created (Mirabilis) [58].

The initial prototype version of Mirabilis (version 1.0) was announced in late 2014. Its development was possible due to a consortium of seven pharmaceutical firms, established in December 2013, being now part of this consortium 21 companies. The scoring approach mentioned on the previous paragraphs was applied within this new *in silico* system to ensure the maintenance of the conservative positioning, which underestimates the true purge factor. Therefore, while improving the efficiency and transparency of purge predictions, it is crucial to preserve the simplicity associated with the original paper-based approach [58].

The objective of this system creation was to apply the best practices related to purge prediction, including always regulatory inputs through interaction with regulatory entities. Thus, resorting to a complete dataset, to which all the companies contributed, systematic models were provided with the purpose of facilitating the prediction of purges [58].

Mirabilis will continually be supported, developed, and redefined through collaborative efforts within the consortium to meet present and future user needs. Therefore, it is to be hoped this approach to become a regular practice to help the pharmaceutical industry, while not raising any risk for patients' health [58].

2.5.2.2. Separation techniques

During the synthesis of an API, there are some purification steps that contribute to GTIs removal, despite not being specific for these impurities and their elimination. However, when a GTI removal is intended, the selection of the purification process depends on the physicochemical properties of the GTI, which will decide the relative "purge" factors [19].

Beyond this, it is also important to bear in mind the selectivity of a purification step for a specific impurity. The higher this aspect, the lower will be the API loss and the higher will be the efficiency of GTI removal. Therefore, from a process chemistry point of view, delivering a safe API will require a proper application of a purification strategy capable of reducing a GTI level to values below the acceptable limit. However, being these levels ultralow, the efficiency of the purification could be compromised since large quantities of GTI should be removed. As a first thought, increasing the number of cycles of a purification step for a significative GTI removal would be considered. However, this strategy would lead to high API losses, which constitutes a situation to avoid [19].

In this way, instead of increasing the number of cycles of a purification process, two different pathways could be followed. On one hand, a final purification step could be included on the process where intercalated purification procedures are already present. On the other hand, identifying and mapping the reactions where GTIs are found constitutes another strategy, especially for cases where the GTI concentration is low [19].

Regardless the separation technique, according to Szekely, G., *et al.*, *"the efficiency of the separation depends on the differences in chemical and physical properties of the two entities to be separated and/or their relative affinities for a selective agent"* [19].

On the following paragraphs, some conventional purification steps will be presented.

Regarding **Crystallization**, which promotes crystal formation in solution, the isolation of the API in relation to the impurities is achieved by letting the GTIs to remain dissolved in the mother liquors (liquid phase) while the separated API remains as a solid phase. Regarding the process itself, its parameters like robustness, temperature, kinetics, or pH are important to bear in mind [59,60] since crystallization is responsible for establishing the crystalline properties (related with the drug bioavailability), crystal habit, size distribution or even bulk density, affecting all these the downstream processing [61,62]. Beyond all this, in some cases, a fraction up to 30% of the API can continue in the mother liquors [63] or be lost through washes of the crystalline solids, resorting to filtration, which constitutes an inefficient purification process. Also contributing to this inefficiency, is the situation where the GTIs remain as part of the crystal lattice due to washing procedure ineffectiveness [19].

Regarding **Precipitation**, promoted by adding a nonsolvent to a solution of API or vice-versa, the separation of the API in relation to the impurities is similar to what happens in Crystallization. Then, once again, the solvent system used should present higher solubility for the GTIs than for API, presenting or not a crystalline form. This solubility parameter usually depends on the polarity of both GTI and solvent.

For the case of the impurity being precipitated, its removal is performed by using filtration and the solvents, with low boiling points, can be evaporated by distillation [19].

In relation to **Distillation** process, it is necessary to highlight its application for several purposes like purifying volatile APIs [64] and removing or exchanging solvents, attending to the boiling points of the solvents. Beyond this, volatile organic GTIs, mostly rising from residual solvents, might be removed through a distillation process [65,66].

Resorting to **solvent liquid-liquid extraction**, this process depends on the relative partition coefficients of API and GTI in different solvents. So, the API can be retained in an aqueous phase as a salt and the impurities, present in the organic phase, are removed. The opposite could also happen. Then, the organic salt may be converted to neutral species, considering the pKa of the API, and re-extracted with a second organic solvent [19].

With respect to advanced purification techniques in API synthesis, there are the supercritical extraction process; organic solvent nanofiltration (OSN) and the molecular imprinting technology.

The **supercritical extraction** is seen as a highly rated purification technique capable of providing an effective and clean path for GTI removal. The supercritical fluid (CO_2, which is an ideal supercritical solvent) used in this process presents a high solvation power and an improved diffusivity, whereby changing from a supercritical state to a gaseous one, it is possible to isolate the solute efficiently.

Regarding **organic solvent nanofiltration**, this process relies mostly on the differences in molecule size, despite polarity and molecular shape contributions. The efficiency of this process for API purification is highly depending on the membrane selected and respective rejection curve. This separation technique might be also used for switching a solvent with high boiling point by another with a lower boiling point [19,63].

In relation to **molecular imprinting technology**, the MIPs are prepared through incorporation of a target molecule, acting as a template, into a polymeric matrix. Then, this template is removed by washing, leaving a potential binding site within the final structure of the polymer. This will lead to an improved affinity for removing molecules similar to the template [19].

2.5.2.3. Adsorption – Taking a deeper look

Adsorption, being a conventional purification process, is widely used for removing GTIs [67], resorting to some specific adsorbents like granular activated carbon (GAC) [68] and resins [19,69]. However, recently, PBI, a solvent stable polymer has been explored for API purification through adsorption process [70].

In this purification process, the adsorbate is attracted to the adsorber surface and, hence, the surface free energy is reduced. The transference proceeds until the equilibrium is achieved between the amount of adsorbate in the solid phase and the amount of adsorbate still present in the liquid phase. These quantities will vary according to the affinity degree of the adsorbate for the adsorber. From a

pharmaceutical point of view, high affinity of the GTI, to the adsorber, combined with lower binding of the API is intended [71].

Regarding the type of interaction that could occur between the adsorber and the adsorbate, there are two possibilities. For the case when an electron transfer occurs, this interaction is the chemical type, known as chemical adsorption or chemisorption. This process is of high energy (40 to 800 kJ/mol) and, hence, desorption is difficult. Beyond this, the process itself is irreversible and only a monolayer could be observed. Then, the interactions occur by ionic or covalent bonds [72]. On the other hand, if there is no electron transfer, a physical adsorption (or physisorption) occurs, being desorption possible. Then, the process may be reversible, and a multilayer adsorption is possible. Regarding the type of interactions, electrostatic, van der Waals, dipole–dipole, or hydrogen bonds are the typical ones [71,73].

Looking at the advantages of adsorption processes, it is interesting to mention their low-cost since there are situations where it is possible to recycle and reuse the adsorber several times. On the other hand, the selectivity is a parameter extremely important to make this unit operation even more efficient at removing or recovering all the specific adsorbate from the liquid phase. Beyond this, comparing with other processes, the adsorption in liquid medium presents low energetic requirement and its implementation and operation are simple. However, it is not always possible to achieve a proper separation, due to lack of selectivity, and an additional operation might be necessary to provide a good solid–liquid separation (e.g., filtration or centrifugation) [71].

Then, to be possible to have a proper adsorption system, it is primarily necessary to choose the most adequate adsorber. In this way, features like low cost, efficiency, high pore volume, high surface area, availability, stability at mechanic, chemical and thermal level, ease of desorption and capability for providing fast kinetics and high adsorption capacity make generally a good adsorber [74].

After this first and fundamental step, addressing the adsorber choice, it is necessary to proceed towards the second step for developing an adsorption operation. In this last step it is aimed to obtain the adsorption isotherms [71], intended to relate the amount of adsorbate in the solid phase (q_e) with the amount of adsorbate still present in the liquid phase (C_e) when the two phases are in equilibrium at a certain temperature. For the proper examination of the equilibrium isotherms, it is important to include several experimental points. After obtaining the isotherm curves, it is crucial to proceed to their classification and then to the fit of suitable models with the purpose of finding adequate parameters. For this fitting, a proper statistic treatment is required. After all this, the isotherms are then capable of providing information regarding the adsorption process [71].

As above mentioned, q_e and C_e are critical and indispensable parameters for the adsorption system design. Beyond this, the isotherm shape could also present an important role to explain certain phenomena related with the adsorption process, not only providing information about the affinity but also indicating the mode of interaction between adsorber and adsorbate [75]. In this way, being the isotherm shape capable of analysing adsorption mechanism and, hence, providing information regarding physical nature of both adsorbate and adsorber, the rising of a classification system for liquid-solid adsorption

isotherms was indispensable [76]. In this classification, the curves are categorized into four main classes, according to their initial slope. Thus, in Figure 2.2, these four classes are presented.

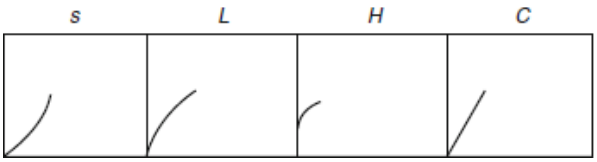


Figure 2.2. Presentation of the four main classes of curves classified according to their initial slope: S curve (vertical orientation isotherm); L curve (normal or "Langmuir" isotherm); H curve (high affinity isotherms); C curve (constant partition isotherm).

Looking at each main class, the S type presents an inclined slope followed by a vertical orientation. In this curve, instead of observing a shape that would induce a possible future plateau (occupation of all binding sites), there is an increase of the slope. This is due to the vertical orientation tendency of the solute molecules in higher concentrations and, hence, more sites will be available for binding. Regarding the L curves, these are typically found in adsorption processes in aqueous solutions. Their initial shape corresponds to the situation where the higher the concentration, the bigger the adsorption capacity until the number of adsorption sites available is limited, leading to the competition between adsorbate molecules. In relation to H curves, they are similar to L curves, differing on the beginning. H type presents an initial portion with a vertical orientation (q_e values higher than zero), even for solute concentrations close to zero, while L type presents its beginning in the origin. For C curves, it is possible to observe a linear behaviour of the equilibrium data. This suggests that the adsorption capacity is proportional to the solute concentration, at low levels, until the maximum adsorption, where a horizontal plateau will be expected to occur [71].

Regarding the models to adjust the experimental data of the isotherm studies, the commonly used are Langmuir and Freundlich.

The physical simplicity of Langmuir model is based on four assumptions [71,77]:

- Adsorption cannot take place in multilayers.
- Each site only binds to one adsorbate molecule.
- The adsorber surface is uniform, and all binding sites are equivalent (in energetic terms).
- The capacity of an adsorbate molecule to bind in a site is independent of the occupation at adjacent site.

The Langmuir model is represented by equation 2.1:

$$q_e = \frac{q_m \times K_L \times C_e}{1 + K_L \times C_e} \qquad (2.1)$$

Where q_e is the adsorption capacity at equilibrium, q_m is the maximum adsorption capacity (which occur when all sites of the monolayer are occupied), C_e is the concentration of the adsorbate in liquid

phase at equilibrium, and K_L is the ratio of adsorption and desorption constants and is related with the energy taken for adsorption.

The linearization is important, since through its application it is possible to obtain the parameters. The linearized form of this isotherm is presented in equation 2.2:

$$\frac{C_e}{q_e} = \frac{1}{K_L \times q_m} + \frac{1}{q_m} C_e \qquad (2.2)$$

For the case when an initial adsorbed layer becomes a surface capable of being involved in adsorption processes, the multilayers formation can be expected. Then, the Freundlich model assumes that the adsorber surface is heterogeneous due to the possibility of interaction between adsorber particles. Beyond this, the amount that is adsorbed increases infinitely with an increase in concentration and this isotherm tries to include the role of the interactions between adsorbate molecules on the surface[71]. The Freundlich model is represented by equation 2.3:

$$q_e = K_F \times C_e^{\frac{1}{n}} \qquad (2.3)$$

Where K_F is the Freundlich constant, which is related with the energy taken for adsorption, and 1/n is the heterogeneity factor. Regarding parameters determination, once again, linearization is important. Here, the parameters are obtained through graphical representation of $\ln(q_e)$ as a function of $\ln(C_e)$ with the slope being equal to 1/n, and the intercept corresponding to $\ln(K_F)$, as seen in equation 2.4:

$$ln\ q_e = ln\ K_F + \frac{1}{n} ln\ C_e \qquad (2.4)$$

2.5.2.4. PBI – Taking a deeper look

PBI, a solvent stable polymer, has been explored for API purification resorting to either conventional separation technique, namely adsorption, or advanced purification technique, OSN.

Regarding OSN, PBI polymer has been experienced as a membrane for purifying APIs, attending always to the compliance with the TTC. During production of this membrane, the thermodynamic properties of the system and the kinetics of the exchange of solvent and non-solvent during Phase Inversion have a strong impact on the membrane morphology, affecting its performance [78].

Thus, sometimes, membrane processes might present limitations such as low product yield, compromising their application in the pharmaceutical industry. To overcome this, a two-stage cascade configuration could be employed, leading to product yield enhancement, and making OSN a highly competitive unit operation when compared to other traditional processes. Therefore, this configuration allows membranes with insufficient separation performance to achieve high yields, improving their versatility. By removing two classes of GTIs in a single unit operation, this size exclusion membrane process has revealed an advantage over affinity-based separations [79].

Regarding adsorption processes, new adsorbers derived from PBI polymer were obtained through physic-chemical alterations, that is, thermal treatment or acidic/basic treatment (alone or combined) were imposed to PBI. Here, the pristine PBI was subjected to those treatments with the purpose of finding optimal properties that could improve impurity removal with lowest API losses, allowing to develop a cost efficient and sustainable strategy for API purification [70].

According to Ferreira, F. A., *et al.* [70], the PBI with thermal and acidic treatment (PBI-TA) presented high efficiency on DMAP removal in DCM, even at high concentrations, with an API loss nearly null after recovery step. Here, there was the possibility of recycling the PBI after DMAP elution. Regarding PBI with thermal and basic treatment (PBI-TB), there was also an efficient removal of MPTS and an API loss virtually null after applying a recovery step. In this way, the previous GTIs from different families could be efficiently removed depending on the pH conditioning selected for the PBI, making the previous adsorbers good platforms for API purification in organic solvent media [70].

Beyond this, these PBI adsorbers abovementioned revealed to be versatile since both PBI-TA and PBI-TB could be produced as beads or electrospun fibers without compromising their adsorption performance. In this way, due to the different morphologies in which these polymers could be obtained, their use in applications like adsorption column (beads) and membrane (fibers) was possible [70].

PBI-Adenine, another new adsorber, obtained by chemical functionalization, presented an efficient removal for 5 different families of DNA alkylating agents with minimal API loss, complying with the TTC. Here, a recuperation step allowed to practically recover all the API adsorbed, being API loss nearly null. Therefore, this polymer could simulate the double helix of DNA and, hence, be effective on removal of intercalating agents of DNA [80].

For PBI-TB and PBI-Adenine, it was observed that an increase of the temperature was important to decrease operation time of GTI binding [70,80].

The scale-up of these new efficient PBI adsorbers and, consequently, their application at industrial level requires their previous well-established API purification strategy, not only focusing in their performance for removing GTIs with minimal API loss (that could be recovered), but also giving relevance to the ability of these platforms to be regenerated and reused. However, there is always room for their improvement, making these polymers even more economic and environmental attractive for the industry.

3. Materials and methods

3.1. Materials

Fluticasone Propionate (FP), Betamethasone Acetate (Beta), Mometasone Furoate (Meta) and Halobetasol Propionate (Halo) were kindly provided by *Hovione PharmaScience Ltd*. Roxithromycin (Roxi) was acquired from *Alfa Aesar*.

The GTIs selected for this present study, 4-dimethylaminopyridine (DMAP) and methyl *p*-toluenesulfonate (MPTS), were purchased from *ACROS Organic* and *Alfa Aesar*, respectively.

Both APIs and GTIs mentioned above were used as supplied, which means that no further purification was needed.

Polybenzimidazole (PBI) S26 dope solution in dimethylacetamide (DMAc) at 26 wt% was purchased from *PBI Performance Products Inc*.

2-Propanol (IPA) was provided by *Scharlau*. MeCN (HPLC grade solvent) and sodium hydroxide (NaOH) pellets were purchased from *Fisher Chemicals*. Hydrochloric acid (HCl) 37% solution was purchased from *Honeywell Fluka*. Milli-Q water was provided by using a water purification system from *Merck* company.

3.2. Experimental methods description

3.2.1. Membranes manufacturing

The *Casting* of the membrane adsorber took place at room temperature. Here, the PBI S26 dope solution was spread on a glass plate directly and manually, resorting to a casting knife set at 250 µm, which was slowly filled to avoid creating bubbles with dope solution and a parallel movement to the glass plate disposition was performed with this knife. This movement had to be executed continuously and applying the same strength all the way through with the purpose of guaranteeing homogeneity of the membrane. The glass plate was fixed on a bench top laboratory casting machine from *RK PrintCoat Instruments Ltd*. During this phase, the humidity value between 40-50% was recommended. Afterwards, the dope solution was immersed in a water Milli-Q coagulation bath and a film rapidly precipitated from the top surface down, due to water absorption and loss of solvent - phase inversion. Then, after 1 h in this coagulation bath, the glass plate containing the membrane was once again immersed in a new coagulation bath. After 1 h in this bath, the membrane was immersed in IPA for membrane storage. This step was repeated twice more with 1 h intervals.

3.2.2. Solubility experiments

3.2.2.1. Using MeCN

10 mL solutions of APIs (FP, Beta, Meta, Halo and Roxi) at 10000 ppm and GTIs (DMAP and MPTS) at 1000 ppm, in volumetric flasks, were prepared in MeCN using a Sartorius CPA64 digital scale to

weight the required amount of the different compounds. For MPTS, instead of weighting, a volumetric measurement was performed resorting to a proper micropipette from *VWR*. The volumetric flasks were gentle stirred, left to rest and analysed to check if there were particles in suspension. Since MPTS is in liquid form, its miscibility was investigated instead.

Beyond these previous concentrations, lower concentrations were also tested. Thus, 20 mL solutions of all 5 APIs at 800 ppm and both GTIs at 80 ppm were prepared. Once again, the volumetric flasks were gentle stirred, left to rest and analysed to check if there were particles in suspension, while for MPTS, its miscibility was investigated instead.

3.2.2.2. Using H_2O at different pHs (1.2, 7 and 13)

Regarding the solubility tested in aqueous system, Roxi was the only model API while both DMAP and MPTS were the model GTIs selected. These experiments were performed after both API and GTI concentrations being well-defined, being 800 ppm for the first and 80 ppm for the second. Thus, it was necessary to check the solubility of these compounds at these respective concentrations for H_2O at different pHs. These different pHs were obtained using HCl 0.25M solution (for pH 1.2) or NaOH 1M solution (for pH 13). For each API and GTI solution prepared, its respective preparation and analytical procedure was similar to the one presented on the previous section.

3.2.3. λ_{max} determination and calibration curves assessment

After solubility experiments using MeCN, the solutions of the APIs (Roxi, HP, Beta) and GTIs (DMAP, MPTS) selected for further studies were analysed by UV-Vis spectroscopy in a Hitachi UH5300 spectrophotometer to determine λ_{max} in the range 200-800 nm, for further quantification and calibration curve assessment.

These UV-Vis spectroscopy analyses were also performed for the aqueous solutions with the purpose of obtaining the λ_{max} for Roxi, DMAP and MPTS cases. However, for Roxi aqueous solutions at pH 7 and 13, syringe tip filters (0.22 µm) were used and then the solutions filtered were analysed for determination of λ_{max}, and later, were diluted for the calibration curves.

3.2.4. Binding adsorption experiments

Binding experiments were performed by placing different quantities of PBI membrane in 2 mL Eppendorf vials. These different quantities were represented as A_m (Area of membrane). In these experiments, the selected areas were 20 cm^2 (maximum area possible), 9.4 cm^2, 4.5 cm^2, 3 cm^2, 1.5 cm^2, 0.84 cm^2 and 0.42 cm^2. Since the manufactured membranes were stored in IPA, it was necessary to wash them with MeCN (2–3 times) before putting them into Eppendorfs. After this, it was rapidly added to the vials (with the membranes already) 1.5 mL of MeCN, corresponding this step to the

Conditioning phase. Here, the membranes were subjected to continuous agitation at 200 rpm for 24 h (at room temperature) resorting to small magnetic agitators and an agitation plate. After this phase, where the membranes were conditioned, MeCN present in the vials was exchanged for 1.5 mL of a solution of each GTI (DMAP, MPTS) or API (Roxi, Halo, and Beta) alone prepared in MeCN at concentrations of 80 ppm and 800 ppm, respectively. The membranes were submitted to continuous agitation at 200 rpm for 24 h (at room temperature). After this time, the solution was transferred to another Eppendorf to be analysed by UV-Vis spectroscopy for API and GTI quantification. These assays were performed with triplicate samples and the absorbance values obtained were corrected by measuring the absorbance of an Eppendorf containing MeCN and PBI membrane as the control.

The percentage of GTI or API bound to the membranes was calculated resorting to equation 3.1, where C_0 (g/L) is the initial API or GTI concentration and C_e (g/L) is the final API or GTI concentration in solution (at equilibrium).

$$Binding\ (\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \qquad (3.1)$$

The amount of GTI or API bound to the membrane was calculated from equation 3.2, where q_e (mg/g) is the amount of GTI or API bound to the membrane at equilibrium, C_0 (mg/L) is the initial API or GTI concentration, C_e (mg/L) is the final API or GTI concentration in solution (at equilibrium), V (L) is the volume of solution and M (g) is the quantity of PBI used.

$$q_e = \frac{V \times (C_0 - C_e)}{M} \qquad (3.2)$$

3.2.5. Binding adsorption isotherm experiments

Isotherms were determined by varying the quantity of PBI (20 cm^2, 9.4 cm^2, 4.5 cm^2, 3 cm^2, 1.5 cm^2, 0.84 cm^2 and 0.42 cm^2) placed in contact with the APIs at 800 ppm and GTIs at 80 ppm solutions prepared in MeCN. After 24 h under agitation at 200 rpm at room temperature, the solutions were analysed by UV-Vis spectroscopy for quantification of the solutes. For Roxi, DMAP and MPTS the isotherms were also determined for a constant amount of PBI (4.5 cm^2) and varying the solutes concentrations in MeCN. Roxi solutions presented concentrations varying from 50 to 800 ppm; DMAP solutions presented concentrations varying from 10 to 80 ppm while for MPTS the concentrations varied from 2.5 to 80 ppm.

After preparation of the testing solutions, the procedure was the same as the one presented in previous section (**3.2.4**) for batch binding experiments comprising a conditioning step of the membranes, followed by the binding step after 24 h at 200 rpm at room temperature and solutes quantification. As described on section **3.2.4**, also in these isotherm experiments, the absorbance values measured had to be corrected.

The percentage and amount of API or GTI bound to the PBI membrane was obtained by using the previous equations 3.1 and 3.2. Regarding the isotherm models, Langmuir and Freundlich were the ones used for data treatment. For the Langmuir model, equations 2.1 and 2.2 from the section **2.5.2.3** were used while for Freundlich model, equations 2.3 and 2.4 from the same section were applied.

The suitability between experimental and predicted values from isotherm studies was described by Chi-square, which was obtained using the equation 3.3 [81]:

$$\chi^2 = \sum \frac{(q_e - q_{e,m})^2}{q_{e,m}} \qquad (3.3)$$

Where $q_{e,m}$ is the equilibrium capacity obtained from the model (mg/g) and q_e is the equilibrium capacity (mg/g) obtained from the experimental data. In this way, the lower the Chi-square, the better the fit [81].

3.2.6. API Recuperation and Membrane Regeneration and Reutilization experiments

MeCN and H_2O at different pHs (1.2, 7 and 13) were tested as washing solvents. Roxi was selected as the model API while both DMAP and MTPS as model GTIs. After the binding step, the membranes were washed at room temperature with 1.5 mL of the washing solvents for 24 h at 200 rpm. Then, the solutions were analysed by UV-Vis spectroscopy for solutes quantification. These experiments were performed for a A_m of 4.5 cm^2.

Both API recovery and GTI removal were calculated by using simple percentage.

For API recuperation from the membrane, the following equations were used.

<u>Recuperation scenario:</u>

- API

$$\%Recovery = \frac{C_{f\ after\ recovery}}{C_0} \times 100 \qquad (3.4)$$

$$\%API\ lost\ = \%Binding - \%Recovey \qquad (3.5)$$

- GTI

$$\%Genotoxic\ leaching = \frac{C_{f\ after\ recuperation}}{C_0} \times 100 \qquad (3.6)$$

$$\%GTI\ removed\ = \%Binding - \%Genotoxic\ leaching \qquad (3.7)$$

For membrane regeneration, the following equations were used.

Regeneration scenario:

- GTI

$$\%GTI\ eluted\ (from\ membrane) = \frac{C_{f\ after\ regeneration}}{C_0} \times 100 \qquad (3.8)$$

$$\%GTI\ in\ membrane = \%Binding - \%GTI\ eluted \qquad (3.9)$$

With the purpose of assessing the reusability of the membrane, after a first MPTS binding step in MeCN followed by membrane regeneration using H_2O at pH 13, it was necessary to conditionate the membrane with fresh MeCN before performing a second binding experiment. After 24 h of the *Conditioning* phase, a MPTS solution of 80 ppm in MeCN was added, letting the binding experiment occur for 24 h at 200 rpm at room temperature. Then, the solution was analysed by UV-Vis spectroscopy for solute quantification.

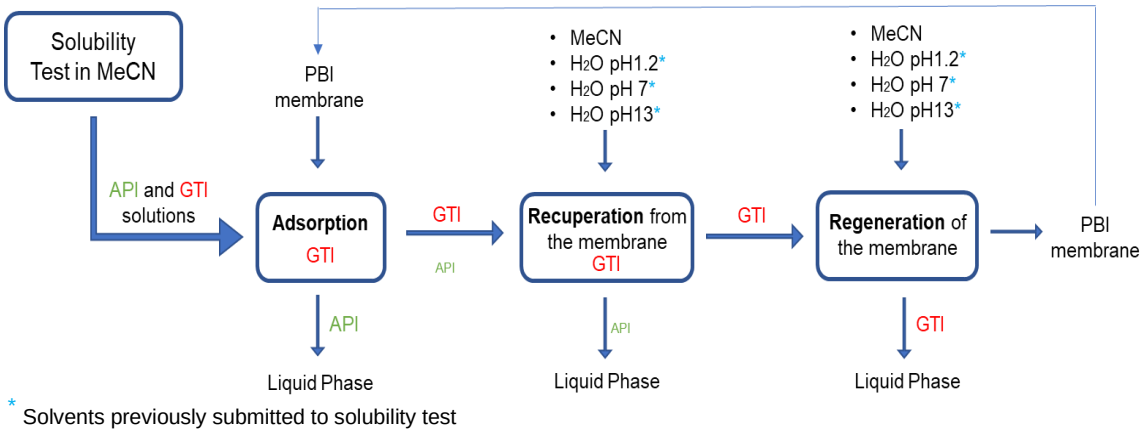
4. Results & Discussion

4.1. Outline

This thesis reports a new approach to attempt API purification through GTI removal using polybenzimidazole (PBI) membrane adsorbers. This chapter starts with a solubility study in MeCN (section **4.2**) of the following APIs: Fluticasone Propionate (FP), Betamethasone Acetate (Beta), Mometasone Furoate (Meta), Halobetasol Propionate (Halo) and Roxithromycin (Roxi). Still within this solubility study, two GTIs from different families, 4-dimethylaminopyridine (DMAP) and methyl *p*-toluenesulfonate (MPTS), were also tested. The solubility experiments were also performed using H_2O at different pHs (1.2, 7 and 13) for Roxi, DMAP and MPTS. The solubility experiments, with this solvent, were indispensable for the API recuperation and membrane regeneration studies (section **4.5**). Then, from here, it was possible to proceed to binding studies for Roxi, Halo, Beta, DMAP and MPTS.

Moving through this chapter, binding adsorption studies are the next section (**4.3**). Here, it was studied the effect of different quantities of PBI membrane adsorber used for each API (Roxi, Halo and Beta) and GTI (DMAP and MPTS) in single solute solutions. After that, another dedicated study of the adsorption of APIs and GTIs reporting their isotherms is presented. In this section (**4.4**), the isotherms were determined either varying the quantity of adsorber placed in contact with the APIs at 800 ppm and GTIs at 80 ppm solutions or changing the concentration of these solutes for the same quantity of PBI, considering a membrane area of 4.5 cm^2. Regarding the first situation, the isotherms were obtained resorting to the binding adsorption results from previous section (**4.3**). In section **4.5**, a post binding step is developed, which includes recuperation of non-specifically bound API (Roxi) and regeneration of the membrane.

Attending to what has been presented, from the solubility tests (section **4.2**) until the post binding step (section **4.5**), it is possible to represent in scheme 4.1 the experimental work outlined, step by step. Here, starting with the solubility tests in MeCN, it is defined the APIs and GTIs solutions selected as case studies. These solutions, as single solute, are subjected to adsorption studies. Here, the goal is to obtain a significative amount of API in liquid phase and GTI adsorbed. Consequently, in the case of some API being adsorbed, this compound needs to be recovered for the liquid phase without contamination, that is, without GTI desorption. Thus, fresh solvent is added (MeCN and H_2O at different pHs). Finally, with the purpose of reusing the membrane for further purifications, it is necessary to elute or desorb the remaining GTI from the membrane.



Scheme 4.1. Diagram representing the experimental thesis work outlined.

At section **4.6**, an API purification strategy is developed attending to the experimental results from the previous sections. The purpose of this section is to verify if it is or not possible to achieve a value of GTI per API that does not exceed the limit of GTI content allowed in an API formulation imposed by applying the TTC. In these studies, Roxi is the model API selected.

4.2. Solubility Experiments

4.2.1. MeCN - solubility test

For decision making of which API or GTI would be used as a case-study in this thesis, it was necessary to attend not only to experimental outcomes but also to the data previously reported in other studies. The obtained data in this work is according with the literature, as presented in Table 4.1.

Table 4.1. Comparison between the experimental and prior data results regarding the solubility in MeCN of the several APIs at 800 and 10000 ppm, and GTIs at 80 and 1000 ppm considered in this work.

		Solubility		
		This work [API] = 10000 ppm [GTI] = 1000 ppm	**This work** [API] = 800 ppm [GTI] = 80 ppm	**Literature**
APIs	**FP**	Insoluble	Insoluble	Slightly Soluble [83]
	Meta	Insoluble	Insoluble	Negligible [4]
	Roxi	Insoluble	Soluble	Soluble (at 800 ppm) [4]
	Halo	Soluble	Soluble	Highly Soluble [4]
	Beta	Soluble	Soluble	Highly Soluble [84]
GTIs	**DMAP**	Soluble	Soluble	Soluble [10,82]
	MPTS	Miscible	Miscible	Miscible/Soluble [82]

Firstly, these experiments started by testing the solubility of API solutions at 10000 ppm and GTI solutions at 1000 ppm, being the ratio between these of 100 mgGTI/gAPI. The reasoning behind the choice for these concentrations was related with the purpose of minimizing the error associated to the weight of the solutes and reducing the quantity of solvent used. Regarding the GTIs at 1000 ppm, both ended up being selected for the study since they showed to be soluble, in case of DMAP, and miscible, in the case of MPTS. This observation agrees with the literature where DMAP and MPTS at 1000 ppm were tested in MeCN for binding studies [70,82]. For the APIs, the results showed that Roxi, Meta and FP were insoluble at 10000 ppm. In its turn, Halo and Beta were soluble at 10000 ppm according to experimental results. For Halo [4] and Beta [84], the experimental results agree whit what is stated in the literature.

In this way, attending to the API concentrations previously reported when using MeCN [7], a value of 800 ppm for the APIs that were not soluble at 10000 ppm was set. The results showed that both Meta and FP were insoluble at 800 ppm, which was according to previous studies, where Meta presented a negligible solubility [4], and FP was slightly soluble [83]. Thus, both APIs were discarded for further studies. Regarding Roxi, this was soluble at 800 ppm, which was according to previous studies [4].

Bearing this in mind, a concentration of 800 ppm was established for Halo, Beta and Roxi, which were the APIs selected for further studies due to their good solubility in MeCN at this concentration. For both GTIs, a concentration of 80 ppm was set with the purpose of maintaining the ratio of 100 mgGTI/gAPI. At this concentration, both GTIs were soluble as expected.

4.2.2. H_2O at different pHs - solubility test

Solubility tests were performed using H_2O at different pHs as model solvent, whose capacity for Roxi recuperation or membrane regeneration, by removing DMAP and MPTS adsorbed, would be later evaluate. This capacity depends on the affinity, and so solubility, of these solutes for the H_2O at different pHs. Once again, the experimental outcomes were compared to data reported in the literature and presented in Table 4.2.

Table 4.2. Comparison between the experimental and prior data results regarding the solubility in H_2O at different pHs for 800 ppm solutions of Roxi and 80 ppm solutions of DMAP and MPTS.

		API at 800 ppm	GTI at 80 ppm	
pH	**Solubility**	**Roxi**	**DMAP**	**MPTS**
1.2	This work	Soluble	Soluble	Miscible
	Literature	Less Poorly Soluble [85]	**NF**	**NF**
7	This work	Soluble	Soluble	Miscible
	Literature	Poorly Soluble [85]	Highly Soluble [87]	Immiscible [88]
13	This work	Insoluble	Soluble	Miscible
	Literature	**NF**	**NF**	**NF**

NF – Not Found

In these solubility experiments performed in water, the API solutions were prepared for a concentration of 800 ppm while the GTI solutions presented a concentration of 80 ppm since these values of concentration were the same used for the binding step. For pH 1.2, Roxi was experimentally soluble at 800 ppm, with prior data results appointing to its low solubility in dilute hydrochloric acid, despite not clarifying its pH value [85]. Although for both GTIs, prior data, regarding their solubility at pH 1.2, were not found, it was observed that DMAP and MPTS were soluble or miscible at 80 ppm.

For pH 7, Roxi seemed to be soluble at 800 ppm, which was not in accordance with prior data [85]. In previous studies, despite their difference regarding the saturation value of 187 ppm [86] and 283 ppm [8], it was possible to notice that these were significantly lower than 800 ppm considered in the present study. Since this study was not performed in duplicate, the validity of experimental results here obtained may be compromised. Regarding DMAP, this compound was observed to be soluble at 80 ppm, which was according with the literature, where the high solubility of DMAP in neutral water was reported [87]. For MPTS, although it is reported to be insoluble at pH 7 [88], it was not observed any droplets in the solution prepared.

For pH 13, the Roxi solution prepared at 800 ppm seemed to not present any particles suspended but ended up displaying values of absorbance close to zero after UV-Vis spectroscopy analysis. Then, further studies for Roxi in this solvent were not performed. This could be due to the use of syringe tip filters (0.22 µm) to obtain a filtered solution. These filters were used with the purpose of confirming the solubility of this API. So, even seeming that Roxi was soluble in H_2O at pH 13, by filtering the solution and analysing it through UV-Vis spectroscopy, it was possible to conclude that this API was probably insoluble at 800 ppm. Regarding DMAP, this compound was observed to be soluble at 80 ppm while for MPTS, there were no experimental indications of its immiscibility at 80 ppm. For both GTIs, prior data regarding their solubility at pH 13 were not found.

4.3. Binding Adsorption Experiments

Due to some severe chemical conditions, such as high temperatures and acidic or basic conditions, that could be employed on API synthesis, it is necessary to promote the development of robust and adequate adsorbers. Beyond this, as previously mentioned, organic solvents are commonly used in API synthesis, also including the purification of this compound. Thus, an organic solvent compatible polymer is needed. PBI is an example of this type of polymer that has gained some relevance for API purification strategies.

In this present thesis, all binding experiments were performed considering a PBI membrane at 26 wt% as the adsorbing material.

The phenomenon that underlies the experiments performed in the present thesis is **adsorption**, which involves the contact of a solid phase with a fluid phase (liquid or gas) [89]. The solid phase is known as adsorbent or adsorber and the liquid phase, in these experiments, contains just one compound to be adsorbed. [71].

As it follows, a deeper analysis will be taken to the binding results obtained to all compounds selected as case-studies. So, the performance of different areas or quantities of adsorber for the selected APIs and GTIs was assessed resorting to three independently produced PBI membranes at 26 wt% for solutions around 800 ppm for the APIs and 80 ppm for the GTIs.

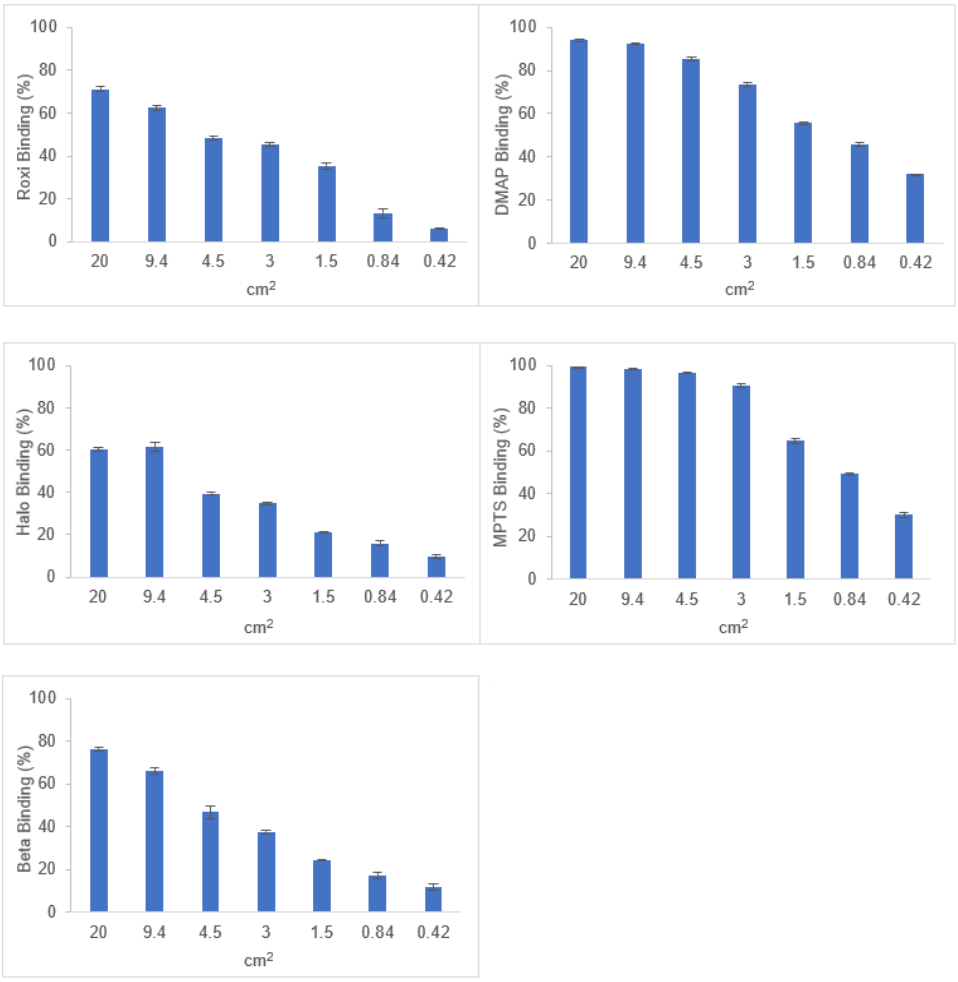


Figure 4.1. Binding adsorption experiments in MeCN for different membrane areas (A_m) of PBI.

Table 4.3. Different A_m and respective quantities of PBI.

Area (cm²)	PBI (mg)
20	104.25 ± 2.10
9.4	49.00 ± 2.42
4.5	23.63 ± 2.57
3	17.77 ± 1.80
1.5	8.47 ± 0.12
0.84	6.10 ± 0.17
0.42	3.43 ± 0.42

From Figure 4.1 it is observed that, regardless the API or GTI, the binding increases with the membrane area. In relation to the APIs, despite some differences, they presented, in a general way, similar behaviour and results throughout all different PBI quantities used with a maximum binding around

60% for the membrane area of 9.4 cm^2 and below 20% for the two smallest membrane areas. However, as intended, these binding results were lower when comparing with the ones obtained for the GTIs, for the same membrane area. DMAP presented a maximum binding above 80% for membrane areas of 4.5, 9.4 and 20 cm^2 and MPTS above 90% for the same membrane areas and 3 cm^2.

As previously reported, the membranes were placed in 2 mL Eppendorf vials. However, for membrane areas higher than 1.5 cm^2, it was necessary to roll them to fit properly and assure that they were totally covered by the API or GTI solutions, as presented in Figure 4.2. Despite the shape similarity with spiral wound membranes (normally used in OSN studies), as seen in Figure 4.3, the ones used in this thesis did not present a spacer. This last one would be capable of avoiding the proximity and contact between parts of the membrane, which would lead to a total availability of all this adsorber surface area for the binding.

Figure 4.2. PBI membrane spirally placed into the eppendorf.

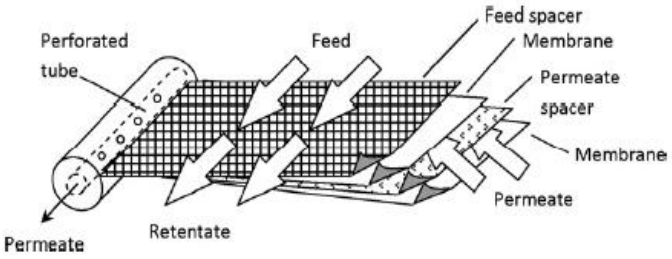


Figure 4.3. Schematic of spiral-wound module, function, and design [90].

In this way, the binding capacity for the higher membrane areas (>1.5 cm^2) would probably be underestimated since not all the membrane surface would be available for adsorption to take place. Looking at the results, an example of this could be the binding values obtained for Halo at 3 cm^2 (~35%) and 4.5 cm^2 (~40%), or for Roxi at 9.4 cm^2 (~63%) and 20 cm^2 (~71%). However, these previous results could be related with another situation. Since both APIs present a significative molecular weight, then a steric hindrance phenomenon could occur and, hence, the capacity for some random molecule of Roxi or Halo adsorbing in a specific site could be limited by the occupation of neighbouring binding sites.

Now, attending to all binding results depicted on Figure 4.1 and despite the ones for GTIs were higher than the ones for APIs, it is crucial to refer that, in fact, the results obtained will not probably lead to a proper API purification due to the non-differentiated selectivity that PBI membrane presented for APIs and GTIs studied. In this way, recuperation of the API and regeneration of the membrane are the next processes to be considered.

Hypothetically, an indicative of an effective API purification would be if the binding was 10-20% (at maximum) for APIs and above 90% for the GTIs at the same quantity of PBI used, like the results described by Ferreira, F. A., *et al.* [70]. However, in this former study, a recuperation and regeneration step were also performed. As previously mentioned, in this thesis, only single solute solutions were used for the experiments. Thus, solutions presenting both API and GTI, where a possible competition between

these species for available binding sites of the adsorber could take place, were not used for the binding studies. So, despite of not knowing the outcome of applying this condition, one of the hypotheses could involve an efficient GTI removal and a low adsorption for the API, which could lead to a proper API purification. According to Ferreira, F. A., *et al.* [70], for one of the cases, the adsorber performance was not affected by the presence of both species in solution, while for another, a reduction of GTI removal and an increase in API adsorption was observed. However, optimizing the experimental conditions for this last case, GTI removal was re-established to previous values from single solute solutions and a lower API adsorption value was obtained comparing with the value achieved from single solute solutions.

For Roxi, Halo, Beta and DMAP, it was expected that a physical adsorption occurred. Here, the process could be reversible and multilayer adsorption, as well as desorption, were possible. Regarding the type of interactions, as mentioned before, these could be electrostatic, hydrogen bonds, Van der Waals, or dipole–dipole [71,73]. So, taking a deeper look at the molecular structure of the APIs, it is possible to see that all present both hydrogen bond donor and acceptor sites [86,94,95]. Then, it is possible to infer that these APIs could interact with PBI through hydrogen bonding, which could be established with the amine groups of imidazole rings of PBI, being these binding sites presented with blue circles in Figure 4.4. Also, attending to PBI structure and its pKa of 5.23, the imidazole ring can act either as an electron acceptor or donor and be present in different protonation states depending on the pH [70,91,92]. Beyond this, attending to the different atoms present on these API molecules, and so different electronegativity, partial charges could be formed within one molecule. These are then attracted to an opposite partial charge in a nearby molecule. So, dipole-dipole interactions could be also involved in adsorption between API molecules [96]. In this way, a multilayer adsorption could be possible.

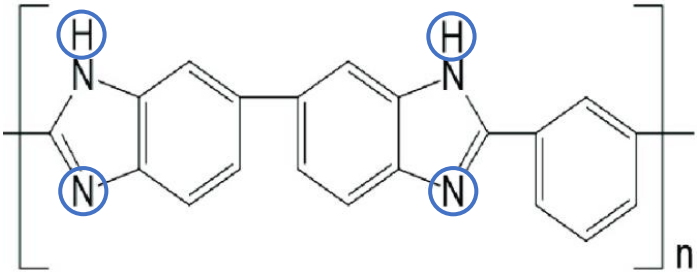


Figure 4.4. PBI structure presenting with blue circles the sites involved in hydrogen bonding [93].

Regarding DMAP, this molecule could also interact with PBI through hydrogen bonding [87]. However, this bonding interaction is only possible between the nitrogen (hydrogen bond acceptor site) of aromatic ring of DMAP and the hydrogen bond donor site of the amine groups present in imidazole ring of PBI [70]. Once again, comparing the binding values obtained for DMAP with the ones obtained for the APIs, it is possible to see that these values were more significative for the GTI regardless the A_m. This could be related with the fact that DMAP presents a much lower molecular weight than APIs and, in this way, the steric hindrance phenomenon would be more common on the API cases, mostly when A_m was low. Beyond this, the API solutions presented a concentration (800 ppm) 10 times higher than the one for GTI solutions (80 ppm). It is also important to refer that the bonding forces between the

solute (API or GTI) and the solvent are weak and depend on the liquid phase concentration [71]. Both DMAP and MeCN (aprotic solvent) do not present hydrogen bond donor site and, hence, between these two, hydrogen bonding would difficulty happen. On the other hand, for all the APIs, this hydrogen bonding with the solvent could be possible. In this way, this situation could constitute another reason for DMAP presenting more affinity to PBI adsorber.

For MPTS, it was expected that a chemical adsorption occurred. Here, the process is irreversible, and desorption is difficult. Regarding the type of interactions, these can occur by ionic or covalent bonds[71,72].

In this case, the interaction with PBI was expected to occur through a methylation reaction of the amine groups of the imidazole rings of the adsorber [70], in a similar way as presented on Figure 4.5, where it is possible to observe that the adsorber behaves additionally as an ion exchanger, interacting ionically with the GTI anion that is formed [97]. Therefore, the high affinity established through this ionic bond is representative of the binding results obtained since, regardless the A_m, these values were generally higher than the ones obtained for the other 4 compounds.

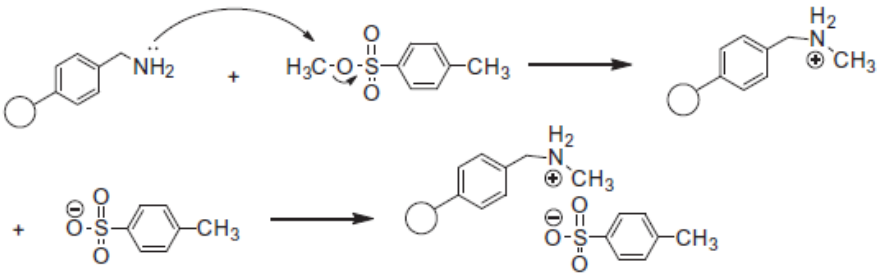


Figure 4.5. Adsorption mechanism of the removal of MPTS using amine-based nucleophilic adsorber[97].

4.4. Binding Isotherm studies

For a specific adsorption operation, after choosing the adsorbent for binding experiments, it is then necessary to obtain the adsorption isotherms. Isotherms are diagrams presenting the variation of C_e (concentration at equilibrium) in the adsorbent solid as a function of the concentration of the liquid phase at a given temperature.

In this section, the isotherm models explored were Langmuir and Freundlich, being both generally applied for adjusting the data from experimental binding isotherms.

As it was previously mentioned, the isotherms were determined by choosing two different pathways. On one hand, from the previous binding adsorption results obtained at section **4.3**, it was possible to determine the isotherms. Here, a variation of the quantity of PBI used for APIs at 800 ppm and GTIs at 80 ppm solutions was inputted. On the other hand, by changing the concentration of APIs and GTIs solutions for the same quantity of PBI (4.5 cm^2), it was possible to determine the isotherms. However, for this last approach, only Roxi (API), DMAP (GTI) and MPTS (GTI) were used as models. Here, the reason for choosing Roxi as the only API in study was due to its higher binding value obtained at 4.5 cm^2, when comparing with the values obtained for the other APIs. Then, for Roxi case, it was expected to achieve the saturation of membrane at a given concentration, whose value would be lower than the one obtained for Halo and Beta. Regarding the reasoning for choosing this specific area of 4.5 cm^2, it is related with the fact that both GTIs presented a high binding result, that is, above 80% for this A_m.

In this way, for Halo and Beta, Langmuir and Freundlich models were only used to adjust the experimental data from the binding adsorption studies (section **4.3**).

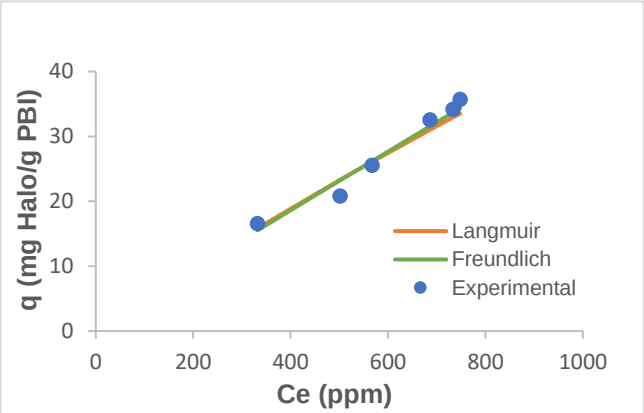


Figure 4.6. Binding isotherm fitting models for Halo. It is presented the experimental values and the ones predicted by isotherm models (Langmuir and Freundlich).

Table 4.4. Binding isotherm physical parameters for Halo.

	Parameters	Halo
Langmuir	K_L (L/mg)	1.66×10^{-4}
	q_m (mg/g)	303.48
	R^2	0.977
	X^2	0.583
Freundlich	K_F (L/mg)	5.33×10^{-2}
	n	1.02
	R^2	0.980
	X^2	0.430

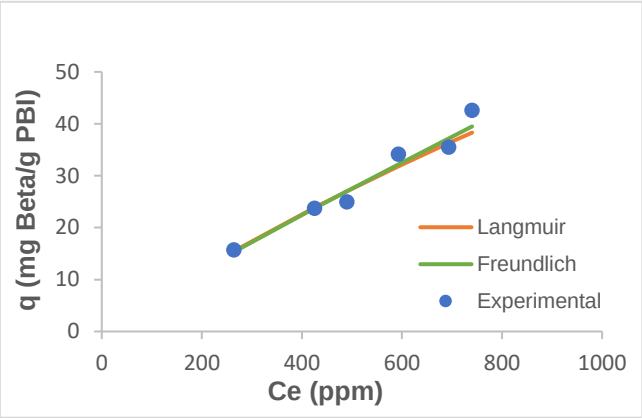


Figure 4.7. Binding isotherm fitting models for Beta. It is presented the experimental values and the ones predicted by isotherm models (Langmuir and Freundlich).

Table 4.5. Binding isotherm physical parameters for Beta.

	Parameters	Beta
Langmuir	K_L (L/mg)	3.02×10^{-4}
	q_m (mg/g)	209.20
	R^2	0.991
	X^2	0.822
Freundlich	K_F (L/mg)	9.12×10^{-2}
	n	1.09
	R^2	0.987
	X^2	0.599

From Figures 4.6 and 4.7 and Tables 4.4 and 4.5, either Halo or Beta seems to follow Freundlich isotherm model on PBI membrane since χ^2 presented a lower value for this model for both APIs. So, it is expected that adsorption occurs on a heterogeneous surface, and the amount adsorbed increased infinitely with an increase of solute concentration [71].

Knowing that the shape of an equilibrium curve could be useful for explaining certain phenomena associated with the interaction between the adsorbate and adsorbent, it is important to mention that Freundlich model can describe the adsorption isotherm data of types S, L, and C (subclass 1) curves. Thus, when $0 < n < 1$, the isotherm is of class S (unfavourable); when $n > 1$, the isotherm is of class L (favourable), and for $n = 1$, the isotherm is of class C [71]. For Halo and Beta, n parameter is close to 1, which means that the number of adsorption sites is greater than the number of molecules to be adsorbed. Thus, Freundlich model could be simplified to Henry model being K_F values associated with the initial slope of isotherm curve [71]. The Henry model suggests that adsorption capacity is proportional to solute concentration, up until the maximum possible adsorption, where an abrupt change to a horizontal plateau would occur. Here, the isotherm with partition constant is characterized by a linear behaviour of the equilibrium data at low solute concentrations. Thus, the adsorption equilibrium constant is referred to as Henry constant (K_H) and may be expressed in terms of concentration ($q_e = K_H \times C_e$) [71]. So, from the experimental results, it is possible to admit that K_F here is equivalent to K_H.

In this way, attending to what has been reported, the Freundlich model described for both Halo and Beta ended up evolving to a Henry model since a linear behaviour of the equilibrium data at low concentrations of solute was visible. Hence, for a proper isotherm study, a broader range of concentrations should be used either for Halo or Beta to be possible to obtain more experimental points useful for describing the suitable isotherm model. Here, with the two APIs presenting a physical adsorption, there is no change in molecular state of adsorption, that is, for adsorption on a uniform surface at sufficiently low concentration, all molecules are isolated from their nearest neighbours [71,89,98]. So, at these conditions, a multilayer adsorption would probably not occur.

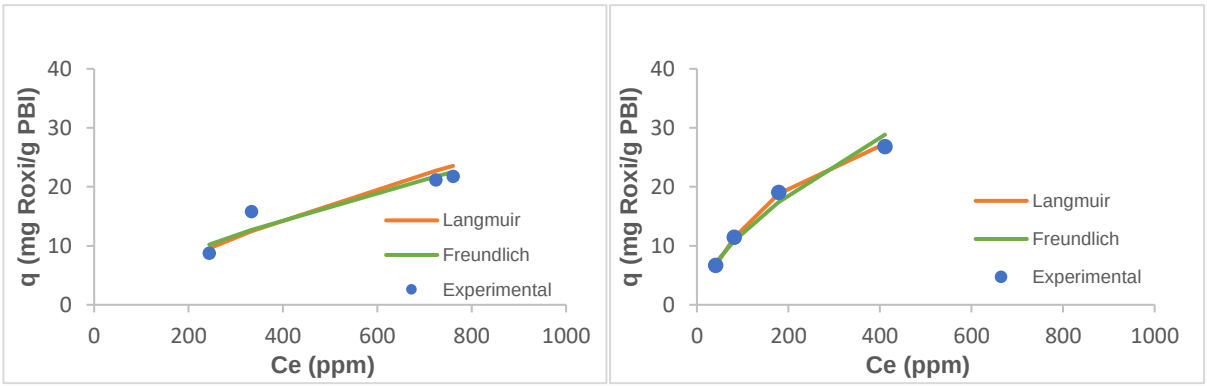


Figure 4.8. Langmuir and Freundlich binding isotherm fitting models for Roxi. Left: Plot obtained by varying the quantity of PBI; Right: Plot obtained by varying the solution concentration.

Table 4.6.1. Binding isotherm physical parameters for Roxi varying the quantity of PBI.

	Parameters	Roxi
Langmuir	**K_L (L/mg)**	5.87×10^{-4}
	q_m (mg/g)	76.28
	R^2	0.932
	X^2	1.202
Freundlich	**K_F (L/mg)**	0.22
	n	1.44
	R^2	0.928
	X^2	1.029

Table 4.6.2. Binding isotherm physical parameters for Roxi varying the concentration of API solution.

	Parameters	Roxi
Langmuir	**K_L (L/mg)**	4.60×10^{-3}
	q_m (mg/g)	41.75
	R^2	0.999
	X^2	0.013
Freundlich	**K_F (L/mg)**	0.75
	n	1.65
	R^2	0.990
	X^2	0.374

Regarding Roxi, from Figure 4.8 and Tables 4.6.1 and 4.6.2, it is possible to observe a divergency on the results obtained. By varying the quantity of PBI used (Figure 4.8 left), Roxi seems to follow the Freundlich model on PBI membrane. On the other hand, by varying the API solution concentration (Figure 4.8 right), the model that fits the data is the Langmuir. A possible reason for this could be related with the small range of concentrations used to obtain both isotherm curves, not being possible to compare them since these curves represent different parts of the isotherm, that is, one was obtained at low concentrations (Figure 4.8 right) and the other at higher concentrations (Figure 4.8 left). So, once again, for a proper isotherm study, a broader range of concentrations should be used for both cases to be possible to obtain more experimental points useful for describing the suitable isotherm model. For example, looking at Figure 4.8 right, more experimental points at higher concentrations would be valuable for obtaining a proper isotherm model, what could lead to discard the linear profile.

Then, with some caution, it is possible to admit that Roxi seems to follow the Langmuir isotherm due to the lowest χ^2 obtained (Table 4.6.2). So, the formation of a monolayer presenting a maximum adsorption (q_m) of 41.75 mg of Roxi per gram of PBI would be expected. In this isotherm model, the ability of a molecule to adsorb in each site is independent of the occupation of neighbouring sites [71]. However, attending to Roxi structure and molecular weight, this macrolide in fact could be associated

with steric hindrance phenomenon and, hence, the capacity for some random molecule of Roxi adsorbing in a specific site could be limited by occupation of neighbouring sites. However, that limitation would not happen possibly due to the low concentration of Roxi solutions and an A_m of 4.5 cm^2, instead of a lower one like 0.42 cm^2 or 0.84 cm^2, used for the binding isotherm study depicted in Figure 4.8 right.

The q_m can vary due to many factors, such as chemical structure of the adsorbate and adsorbent, molecular size, and nature of the adsorbent [71]. Therefore, attending to the objective of API purification, the lower the q_m for an API, the better the purification. Looking at q_m, related with the complete saturation of the monolayer adsorbate [71], the value obtained in the present experiments (41.75 mg/g) is higher than the one obtained by Ferreira, F. A., *et al.* for Meta (8.22 mg/g) [70], where the adsorbers (PBI beads) were subjected to thermal and acidic/basic treatment. Bearing this in mind, it is possible to refer that Roxi would probably not be efficiently purified resorting to adsorption phenomena using PBI membranes since its loss would probably be significative attending to the high q_m obtained. Thus, attending to this, the only way to provide a proper purification for Roxi, without a significative loss of it, that is, below 10%, would be through implementation of a selective API recuperation step.

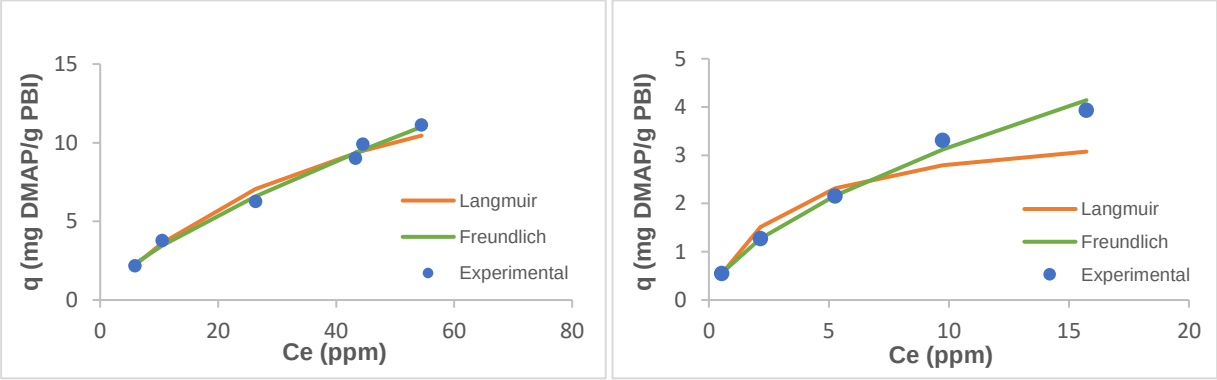


Figure 4.9. Binding isotherm fitting models for DMAP (Langmuir and Freundlich). Left: Plot obtained by varying the quantity of PBI; Right: Plot obtained by varying the solution concentration.

Table 4.7.1. Binding isotherm physical parameters for DMAP varying the quantity of PBI.

	Parameters	DMAP
Langmuir	K_L (L/mg)	2.23×10^{-2}
	q_m (mg/g)	19.06
	R^2	0.996
	X^2	0.172
Freundlich	K_F (L/mg)	0.66
	n	1.42
	R^2	0.995
	X^2	0.081

Table 4.7.2. Binding isotherm physical parameters for DMAP varying the concentration of GTI solution.

	Parameters	DMAP
Langmuir	K_L (L/mg)	0.32
	q_m (mg/g)	3.68
	R^2	0.992
	X^2	0.383
Freundlich	K_F (L/mg)	0.81
	n	1.68
	R^2	0.999
	X^2	0.023

Moving to the GTIs, two different approaches for their binding isotherm studies were adopted, in a similar way to what happened for Roxi. For DMAP, from Figure 4.9 and Tables 4.7.1 and 4.7.2, it is possible to conclude that DMAP follows the Freundlich model on PBI membrane since χ^2 presented a significative lower value for this model regardless the approach used for determining the isotherms. Then, this means that when the initial adsorbed layer becomes a surface for more adsorption, the formation of multilayers can be expected. Despite Freundlich model being able to describe the adsorption isotherm data of several types of curves, the one representative of DMAP case is a class L since n > 1 (favourable) [71]. This isotherm type indicates that the adsorption occurs due to relatively weak forces (e.g., van der Waals) [71], which is in accordance with what was previously supposed on the binding adsorption section (**4.3**) for DMAP case-study.

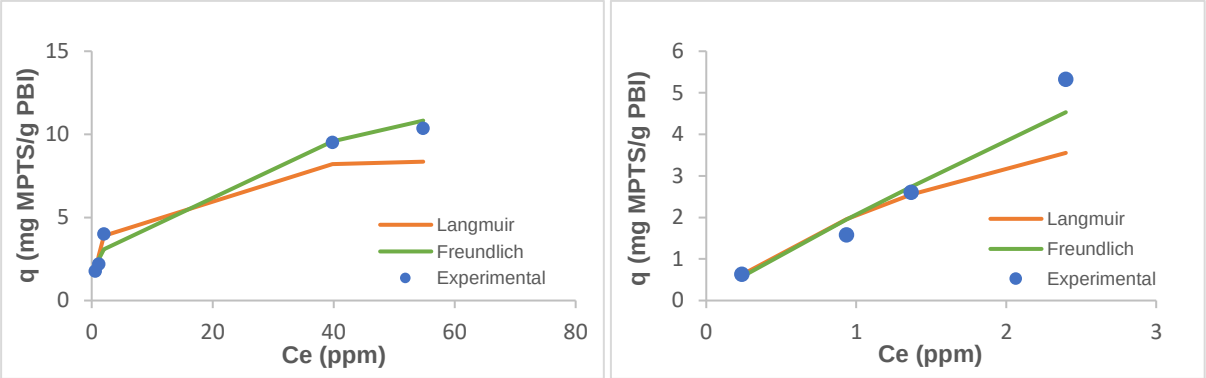


Figure 4.10. Binding isotherm fitting models for MPTS (Langmuir and Freundlich). Left: Plot obtained by varying the quantity of PBI; Right: Plot obtained by varying the solution concentration.

Table 4.8.1. Binding isotherm physical parameters for MPTS varying the quantity of PBI.

	Parameters	MPTS
Langmuir	K_L (L/mg)	0.39
	q_m (mg/g)	8.74
	R^2	0.969
	X^2	0.799
Freundlich	K_F (L/mg)	2.35
	n	2.62
	R^2	0.983
	X^2	0.341

Table 4.8.2. Binding isotherm physical parameters for MPTS varying the concentration of GTI solution.

	Parameters	MPTS
Langmuir	K_L (L/mg)	0.39
	q_m (mg/g)	7.39
	R^2	0.990
	X^2	0.957
Freundlich	K_F (L/mg)	2.07
	n	1.12
	R^2	0.983
	X^2	0.220

From Figure 4.10 and Tables 4.8.1 and 4.8.2, MPTS adsorption seems to follow the Freundlich model since χ^2 presented a lower value for this model regardless the approach used for determining the isotherms. So, facing these results, MPTS seems to follow an adsorption on multilayers, being n higher than 1.

With respects to the isotherm shape, in a similar way with what happen in the case of classes C and L, the H type is as well an isotherm curve that occurs when adsorption sites were not fully occupied, or there was not a complete vertical orientation of the molecules of the solvent [71]. This class H can also be described by Freundlich model whereby it is possible to say that MPTS case-study presents an isotherm of class H, being n >1 (favourable). This is according to what was said about the type of adsorption since these H curves are indicative of chemisorption [71].

Once again, it is of highlighting the small range of concentrations used to obtain both isotherm curves. So, for a proper isotherm study, a broader range of concentrations should be used for both cases to be possible to obtain more experimental points useful for describing the suitable isotherm model. For example, looking at Figure 4.10 right, more experimental points at higher concentrations would be useful for obtaining a proper isotherm model, what could lead to discard the linear profile that the respective curve seems to present.

4.5. API Recuperation and Membrane Regeneration and Reusability

Attending to the results discussed on the section **4.3**, it was possible to notice the non-differentiated selectivity that PBI membrane presented for APIs and GTIs studied. Thus, due to the significative binding obtained for APIs, which would lead to a huge negative economic impact to the pharmaceutical industry, the **recovery or recuperation** of API that remained bound to the adsorber is crucial.

In this section, all the results presented and discussed are correlated with experiments performed using a A_m of 4.5 cm^2. The reasoning behind this choice is due to the significative binding adsorption values obtained for both GTIs (≥80%). Beyond this, from the three APIs studied on the previous sections, only Roxi would be subjected to the recovery experiments due to lack of prior studies about its purification processes based on adsorption phenomena, as previously stated at section **1.2**. In these recuperation and regeneration studies, MeCN and H_2O at different pHs were considered.

Regarding the graphics to be presented in this section **4.5**, depending on the compound (API or GTI), they are going to present a different nomenclature. For API, it is possible to see in the graphics, bars named as **%Recovery** and **%API lost**. For GTIs, there are bars named as **%Genotoxic leaching** and **%GTI removed** (from API stream). This last case is for API recuperation scenarios. However, not being possible or viable this recuperation, **regeneration** scenario comes up to be discussed. In this last one, **%GTI eluted** (from membrane) and **%GTI in Membrane** are the terms used. Lastly, for all the graphics, the error bars represent the standard deviation of the experimental results obtained (triplicates).

4.5.1. API Recuperation and Membrane Regeneration in MeCN

In order to reduce API loss, a recovery step was performed by assessing Roxi desorption from the adsorber using MeCN.

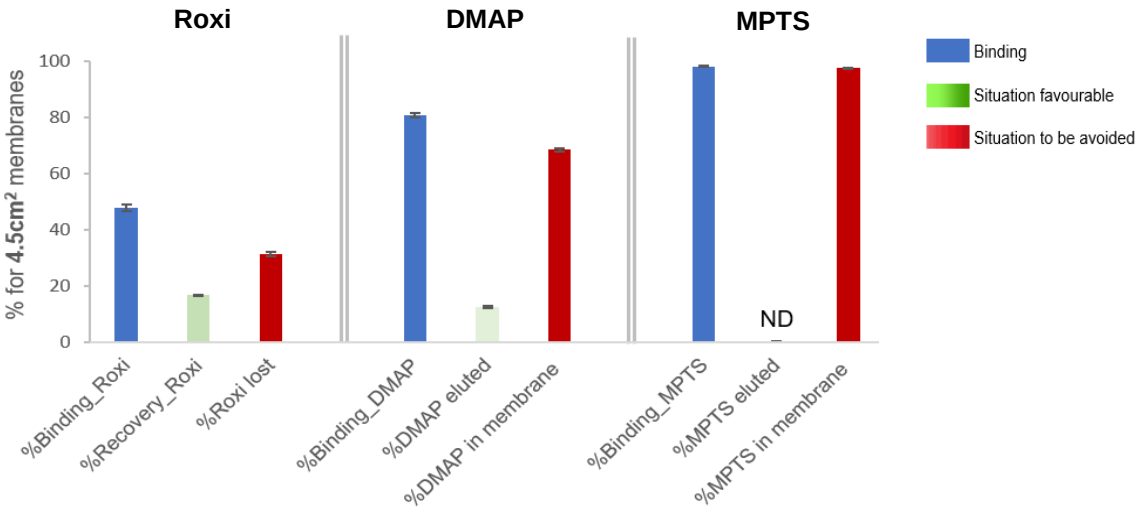


Figure 4.11. Representation of Binding and Recovery experiments for Roxi, and Binding and Regeneration experiments for DMAP and MPTS. ND means Non-Detectable.

For Roxi**,** in Figure 4.11, we obtained around 48% of binding to the adsorber. After a washing step with MeCN, it was possible to recover approximately 17% of Roxi in relation to the feed solution. Then, knowing the value of both %Binding and %Recovery for Roxi, it was possible to determine the %Roxi lost corresponding to 31% still adsorbed on the membrane after binding and recovery steps in relation to its initial amount on feed solution.

In this way, it is not advisable to use MeCN for Roxi recovery as an API purification strategy, since the percentage of Roxi lost was significative.

Despite the results obtained resorting to a single recovery step, experiments for four recovery steps were also performed (Figure 19, *Appendix 3*). However, the results were far from promising due to the fact that a significative amount of Roxi was still lost (~19%). Thus, resorting to four recovery steps instead of one, did not allow to obtain a relevant percentage of Roxi recovered, that is, an increase of 12% in API recovery was not significative if time and resources consumed are considered as well as the %Roxi lost.

Therefore, for Roxi, despite the results not having been promising, there is still possible to observe some recovery of the API. This happens because the adsorption of Roxi to the membrane is a physical process, where energies are low, and, consequently, desorption is possible [73]. Thus, using fresh MeCN, which is a polar solvent capable of solubilizing Roxi, there will be affinity and, consequently, Roxi will be partitioned for both adsorber and liquid phase since there was no total recovery. However, the fresh MeCN was not the solvent able of guaranteeing a total solubilization of Roxi adsorbed in PBI membrane.

Besides API recovery it is also important to consider the adsorber regeneration and reuse. Then, to regenerate the membrane, a GTI elution step was performed by assessing DMAP and MPTS desorption from the adsorber using MeCN. Being MeCN infeasible for API recovery and, consequently, necessary to find another solvent for this recuperation, it was then investigated if MeCN could be efficient for regeneration of the membrane.

For DMAP, from figure 4.11, after the 80% binding to the adsorber, a value of approximately 12% of DMAP eluted with MeCN was obtained, which means that from all the initial quantity of DMAP, only this small percentage was removed from the membrane. Then, knowing the value of both %Binding and %DMAP eluted, it was possible to determine the %DMAP in membrane corresponding to 68% in relation to its initial amount on feed solution.

Therefore, the use of MeCN for regenerating the membrane when DMAP was the concerned GTI is not feasible because a significative amount of DMAP continued adsorbed after washing.

For DMAP, it was also tested if four regeneration steps instead of only one would have an impact on the results. However, as presented in Figure 20 (*Appendix 3*), about 48% of DMAP is still adsorbed to the membrane after four GTI elution/regeneration steps. Thus, comparing with only one regeneration step, four of these would not still allow to look at MeCN washing as an important strategy for membrane regeneration.

Therefore, for DMAP, it was concluded that membrane regeneration would not be possible using MeCN, although a small quantity of this GTI would be eluted when washing the membrane with this solvent. Thus, similarly to Roxi, a physical adsorption has been reported here and, consequently, a desorption phenomenon would be possible. Therefore, MeCN, being polar like the GTI itself, would be capable of solubilizing the DMAP adsorbed. The outcome led to suggest that, despite this solubilization, DMAP was partitioned for both phases (solid and liquid) at the equilibrium. Now, looking at the results obtained by Ferreira, F. A., *et al.*, the use of MeOH for the regeneration step presented a good result. In this case, 80-90% of DMAP was removed from the adsorber since MeOH was effective in ending the ionic interaction and hydrogen bonding between DMAP and the adsorber (PBI-TA) [70]. So, for DMAP-MeCN case, this solvent was inefficient in surpassing the extent of interaction between DMAP and PBI membrane, so that just a small amount of GTI was eluted. Looking at %DMAP eluted (~12%) and %Recovery of API (~17%), the last one presented a higher value. This could be due to the higher concentration and molecular weight of the API.

For MPTS, after 98% binding to the adsorber, from Figure 4.11 it is possible to observe that there is practically no elution of GTI (0.41%) from the adsorber with MeCN washing. This means that, the regeneration would be unsuitable, as it can be seen in the bar related to %MPTS in membrane. As previously mentioned, this is due to the interaction with PBI through a methylation reaction, being this adsorber capable of acting additionally as an ion exchanger, interacting ionically with the GTI anion that is formed [70,97].

Therefore, for MPTS, it was observed that regeneration would be unsuitable since practically none of the GTI was eluted using MeCN to wash the membrane. Looking from other perspective and having in mind other objective, the results obtained for MPTS would be interesting if the MeCN could be efficient at API recovery since there would be a minimum MPTS back contamination (0.41%). So, for this situation, 97.51% represents the percentage of MPTS removed after binding and genotoxic leaching steps, that is, the percentage of MPTS still adsorbed on the membrane after these steps in relation to its initial amount on feed solution. According to Ferreira, F. A., *et al.*, it was referred that the resulting salt of MPTS presented a poor solubility in DCM solvent and, thus, practically all this salt had remained precipitated with the PBI [70]. Then, looking at MPTS-MeCN case, the results obtained here were probably due to the same reason presented in Ferreira, F. A., *et al.*, where it was suggested that chemical adsorption is the type of interaction established between MPTS and PBI, occurring a methylation reaction.

4.5.2. API Recuperation and Membrane Regeneration in H_2O at different pHs

After the results obtained in MeCN, the recuperation and regeneration studies proceeded resorting to another solvent. Here, H_2O was used at pH 1.2, 7 and 13 with the purpose of studying the pH influence on the desorption process.

4.5.2.1. H_2O at pH 1.2

API recuperation step was not performed since Roxi molecule presents acid instability, being rapidly degraded at pH 1.2 [99]. In this way, being the API degraded in these conditions, the H_2O at pH 1.2 could never be seen as a washing solvent for API recovery.

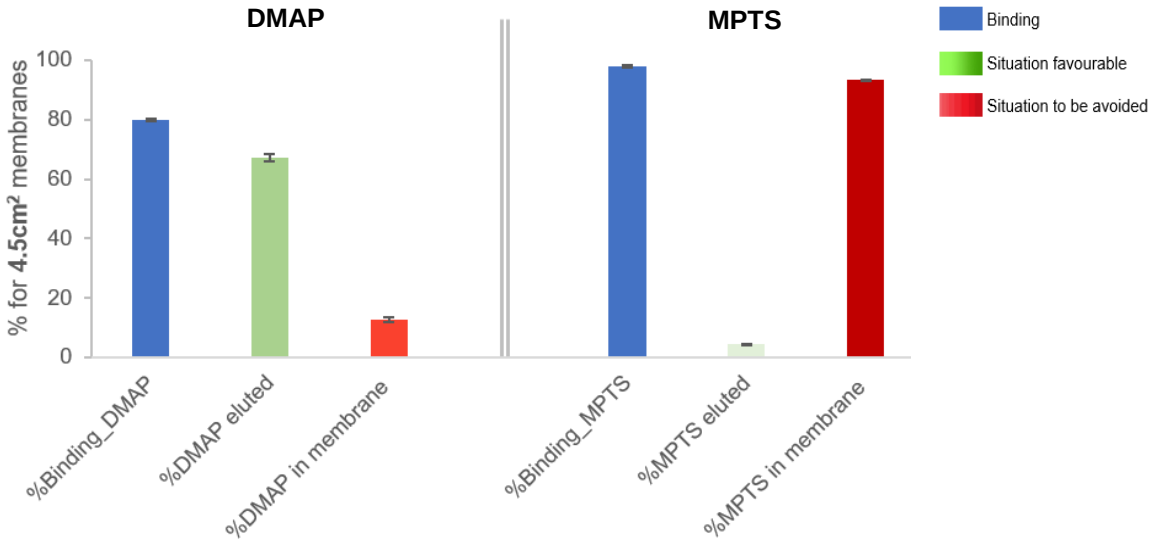


Figure 4.12. Representation of Binding and Regeneration experiments for DMAP and MPTS. The Bindings were performed in MeCN while the Regeneration in H_2O at pH 1.2.

Being the recuperation of API in H_2O (pH 1.2) inviable, it is now important to interpret the results obtained for the GTIs with the purpose of verifying if the regeneration of the membrane would be possible using this solvent. Then, to regenerate the membrane, a GTI elution step was performed by assessing DMAP and MPTS desorption from the adsorber using H_2O at pH 1.2.

For both GTIs, attending to the results from Figure 4.12, after the binding (~80% for DMAP and ~98% for MPTS), a value for %GTI eluted of around 67% for DMAP was obtained while for MPTS, about 4% was achieved. So, this means that from all the initial quantity of GTI, a significative percentage of DMAP and a small percentage of MPTS were removed from the membrane. Then, 13% and 94% represent the quantity of DMAP and MPTS, respectively, still adsorbed on the membrane after binding and regeneration/GTI elution steps in relation to their respective initial amount on feed solution.

Therefore, analysing the previous results, the use of H_2O at pH 1.2 for regenerating the membrane when DMAP was the concerned GTI was satisfactory. On the other hand, the use of this solvent for regeneration would be impractical when MPTS was the GTI considered.

For DMAP, comparing with the results from MeCN studies, the use of H_2O at pH 1.2 allowed to discover a new approach to be used for regeneration of the membrane. This could be explained attending to the pKa values of DMAP (9.7) [100] and PBI (5.23) [91,92]. So, knowing that pH of solvent is 1.2, this means that probably PBI would be in its protonated form and, consequently, the interaction adsorber-adsorbate would end. The same protonation would happen to DMAP (Figure 4.13) and, thus,

the re-interaction between adsorber and adsorbate would not be favoured due to the possible electrostatic repulsion of both protonated species in solution.

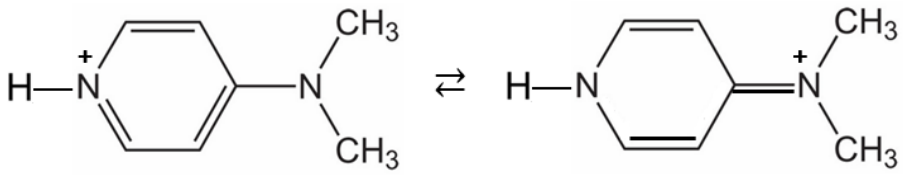


Figure 4.13. Protonated species of DMAP.

For MPTS, comparing with the results from MeCN studies, once again the regeneration was infeasible. As previously mentioned, the type of interaction established between MPTS and PBI is a chemical one (irreversible). Then, perhaps, the resulting salt of MPTS presented a poor solubility in this H_2O and, thus, practically all this salt had remained precipitated with the PBI [70]. So, the 4% obtained for %GTI eluted could possibly be due to the use of the same Eppendorf vial where the binding experiment took place and, hence, some residual MPTS could have been left.

4.5.2.2. H_2O at pH 7

To reduce API loss, a recovery step was performed by assessing Roxi desorption from the adsorber using H_2O at neutral pH.

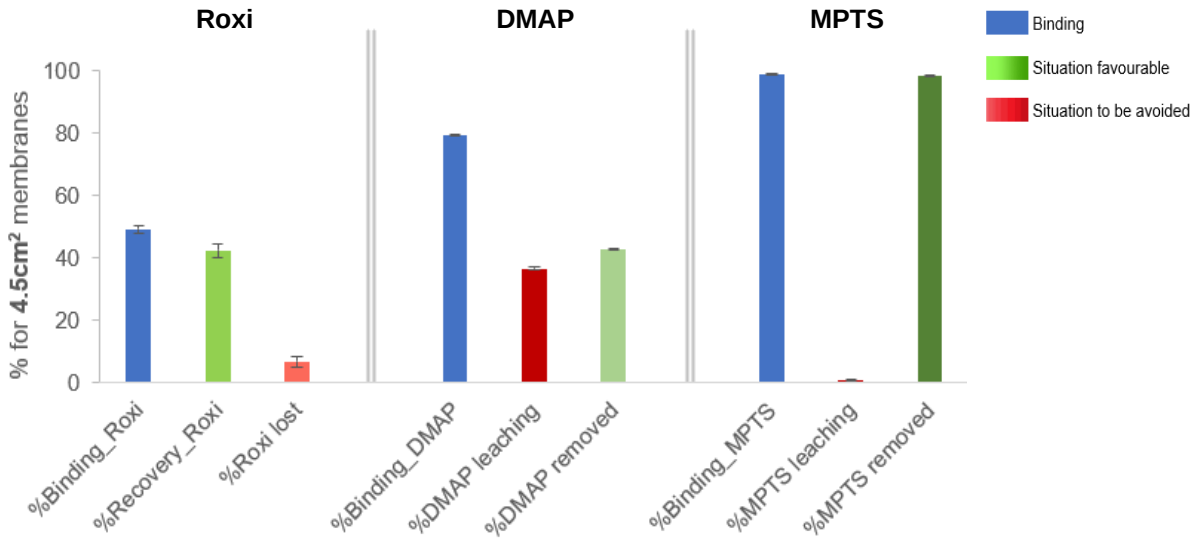


Figure 4.14. Representation of Binding and Recovery experiments for Roxi, and Binding and Leaching experiments for DMAP and MPTS. The Bindings were performed in MeCN while the Recovery and Leaching in H_2O at pH 7.

From Figure 4.14, for Roxi, after the binding (49%), it is possible to see that from the feed solution, approximately 42% of Roxi was recovered, corresponding to %Roxi lost of about 7%.

Thus, from here, it is possible to refer that the use of H_2O at neutral pH for Roxi recovery as a part of an API purification strategy would be feasible.

Comparing with the previous results obtained using MeCN, it is important to highlight that after resorting to four recovery steps at MeCN, the %Recovery ended up being significative lower than the one obtained resorting to only one recovery step using H_2O at neutral pH. On the other hand, comparing with the results from Ferreira, F. A., *et al.*, where all the API (Meta) was recovered with DCM washing, it is important to infer that a loss of 7% for Roxi using H_2O at pH 7 is a good result since API loss should be preferentially below 10%. Thus, despite this result, the fact of resorting to water instead of an organic solvent could be seen as a positive aspect from the environmental impact of the process. This could end up being more relevant since DCM has been categorized as an undesirable solvent in the pharmaceutical industry both by GSK [101,102] and Pfizer[103,104]. This categorization is due to its negative effects on both health and environment.

Assuming the recuperation of API in H_2O at neutral pH is viable, it is now important to interpret the results obtained for the GTIs with the purpose of verifying if a possible GTI back contamination (GTI leaching) would occur. Thus, a GTI elution step was performed by assessing DMAP and MPTS desorption from the adsorber using H_2O at neutral pH.

Looking at Figure 4.14, after GTI binding (~80% for DMAP and ~99% for MPTS), a value of approximately 37% of DMAP and 0.85% of MPTS leaching was obtained. Then, knowing the value of both %Binding and %Genotoxic leaching, it was possible to determine the %GTI removed from API stream. So, 43% and 98.05% represents the quantity of DMAP and MPTS, respectively, still adsorbed on the membrane after binding and GTI leaching steps in relation to their respective initial amount on feed solution.

Therefore, the use of H_2O at pH 7 for a case involving Roxi and DMAP would not be acceptable as a step for API purification strategy since, despite a significative recuperation of API, there would be a relevant DMAP back contamination. On the other hand, for the case Roxi – MPTS, the use of this solvent would be viable as a step for API purification strategy as there is nearly no MPTS back contamination.

For DMAP, comparing with the results from MeCN studies, the use of H_2O at pH 7 allowed to elute from the membrane a more significative amount of GTI. Despite in both cases the polar solvents having been able to partially solubilize DMAP adsorbed, it would be possible to infer that this GTI would be more partitioned for the liquid phase when this was H_2O at pH 7 than when it was MeCN. So, DMAP presented more affinity for H_2O at pH 7 than for MeCN, which could be due to the possibility of DMAP establishing hydrogen bonds with H_2O molecules, contributing to its high solubility in this solvent [87]. On the other hand, comparing with the studies for H_2O at pH 1.2, GTI elution was more significative for acidic conditions due to the possible protonation phenomenon and, hence, the potential electrostatic repulsion between PBI and DMAP, as mentioned before.

For MPTS, comparing with the results from MeCN and H_2O at pH 1.2, once again, there was almost no GTI eluted. As it has been already said, perhaps, after methylation reaction, the resulting salt of

MPTS presented a poor solubility in H_2O at pH 7 and, thus, practically all this salt had remained precipitated with the PBI. This is due to the capability of PBI adsorber acting additionally as ion exchanger, stabilizing the GTI anion formed [70,97].

4.5.2.3. H_2O at pH 13

From the previous results at pH 7, the recovery of Roxi presented a good result. However, it would be interesting if MPTS or its salt could be eluted and, consequently, stop being precipitated with PBI. Then, as an attempt to regenerate the membrane, a GTI elution step was performed by assessing MPTS desorption from the adsorber using H_2O at pH 13.

Despite the use of H_2O at pH 1.2, as washing solvent, having presented a good result for the regeneration of the membrane when DMAP was the GTI, the degradation of Roxi in acidic conditions constitutes a disadvantage. Thus, after regeneration step, the residual Roxi still present on the membrane is, in fact, possibly some degradation products of the API. In this way, by reusing this membrane, there is a possibility of these products being eluted, as impurities, with the API in the recovery step, being a situation to be avoided. Therefore, the regeneration experiment using H_2O at pH 13 was also performed for DMAP since the previous promising results obtained for this GTI using H_2O at pH 1.2 would only be relevant if Roxi was not degraded in these conditions. However, for other APIs (stable at pH 1.2) and their purification processes using PBI membranes, if DMAP is the GTI to be removed, the use of H_2O at pH 1.2 should be considered for the regeneration experiments.

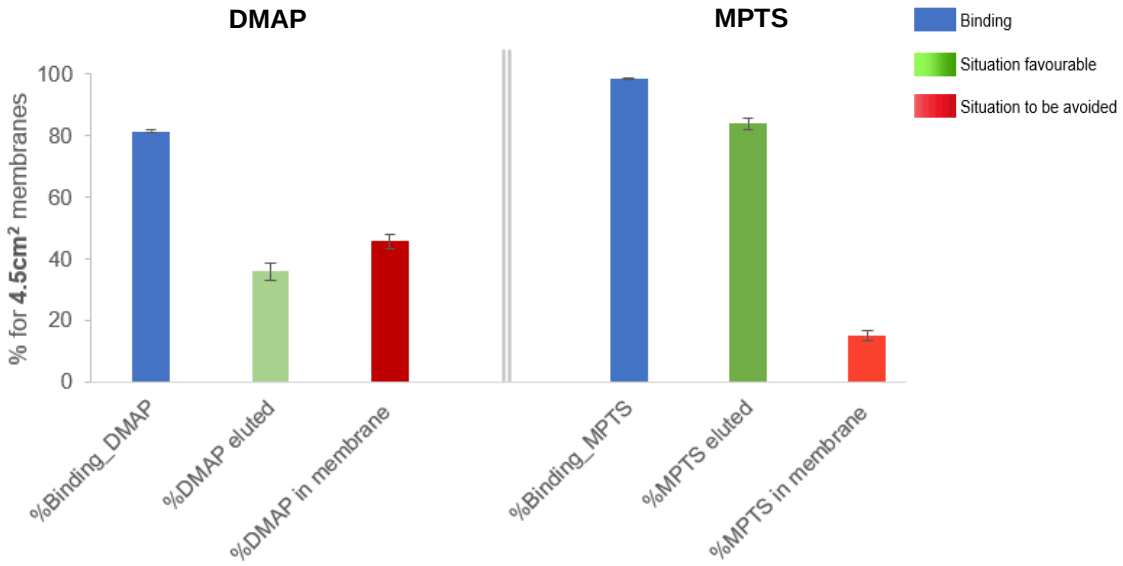


Figure 4.15. Representation of Binding and Regeneration experiments for DMAP and MPTS. The Bindings were performed in MeCN while the Regeneration in H_2O at pH 13.

For DMAP, comparing with previous results from MeCN and H_2O at pH 1.2 and 7, it was obtained a %DMAP eluted value (~36%) similar to the one for H_2O at pH 7 (named as %DMAP leaching, as seen

in Figure 4.14). At pH 13, there are excess OH^- species in solution, which could promote PBI deprotonation. So, this could lead to a competition since the proton donor hydrogen bonding site from PBI is the one through which DMAP establishes hydrogen bonding and the one being involved in deprotonation. Thus, DMAP would be partitioned for both adsorber and liquid phase since there was no total solubilization of the adsorbed DMAP in H_2O at pH 13.

For MPTS, comparing with previous results from MeCN and H_2O at pH 1.2 and 7, it was obtained a significative value for %MPTS eluted (~84%). This was possibly due to the solvent capability for solubilizing the MPTS salt. According to Ferreira, F. A., *et al*., it was possible to detect the GTI anion (*p*-toluenesulfonate) on a MeOH washing solution because this solvent could solubilize the MPTS salt. Then, for H_2O at pH 13, there are both Na^+ and OH^- species in solution, being this sodium ion capable of stabilizing the anion of MPTS, leading to its solubilization.

4.5.3. Membrane Reusability after MPTS adsorption

As previously mentioned, the use of H_2O at pH 7 for a case involving Roxi and DMAP would not be acceptable as a step for API purification strategy since there would be a relevant DMAP back contamination (37%). For case Roxi – MPTS, the use of this solvent would be viable as a step for API purification strategy as there is nearly no MPTS back contamination (0.85%). Beyond this, bearing in mind the impossibility of using H_2O at pH 1.2 for Roxi purification strategy as a regeneration step, then the membrane reusability when DMAP was the concerned GTI would not be possible. In its turn, using H_2O at pH 13, a significative amount of MPTS could be eluted from the membrane (~84%) and, hence, the membrane reusability could be possible. In this way, a study to assess this reusability was performed by submitting the membrane to a new binding step after having been regenerated.

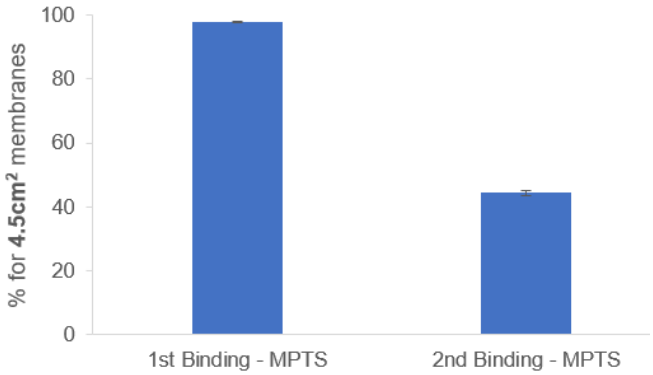


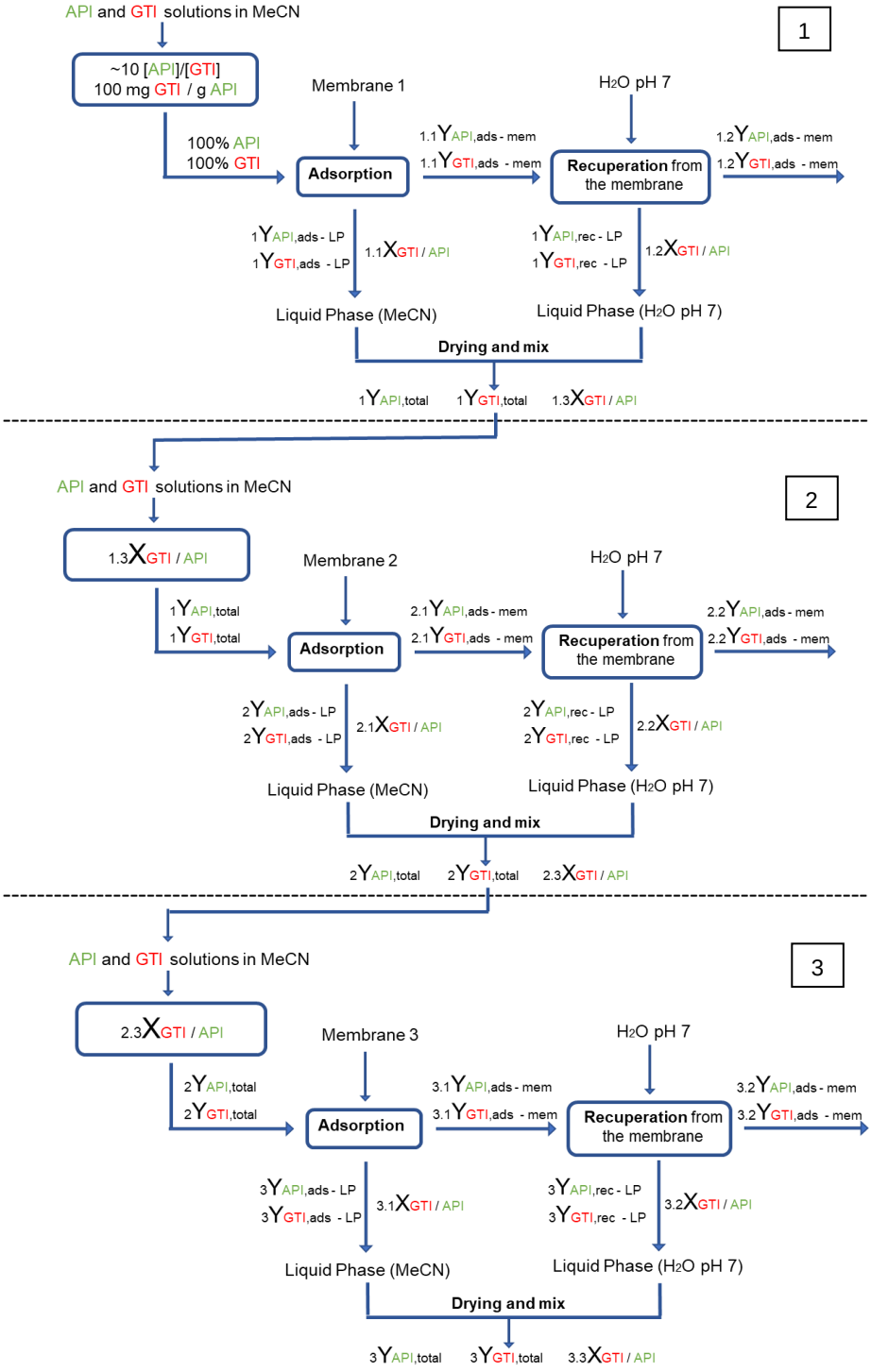
Figure 4.16. MPTS binding in MeCN before (left bar) and after (right bar) membrane regeneration step in H_2O at pH 13.

From Figure 4.16, around 44% of MPTS could still bind to the membrane. So, probably, after 1st binding, it is possible that the membrane was not saturated, and some binding sites were available for 2nd binding. Thus, this could be the reason for obtaining that binding result after regeneration since the restoration of PBI is impaired by the nature of the reaction between MPTS and this adsorber.

4.6. API purification strategy

4.6.1. Roxi – MPTS case-study

From the results obtained, an API purification strategy could be developed attending to API recovered and insignificant MPTS back contamination when using H_2O at pH 7. However, the reusability results were not good so, this step would not be used in API purification strategy developed for Roxi-MPTS case-study, presented on Scheme 4.2.



Scheme 4.2. API purification strategy for Roxi-MPTS case-study.

Table 4.9. Results obtained by applying the API purification strategy outlined in Scheme 4.2.

	1st Cycle		2nd Cycle		3rd Cycle	
Adsorption Step	$_1Y_{API,ads-LP}$	51.83%	$_2Y_{API,ads-LP}$	46.06%	$_3Y_{API,ads-LP}$	42.84%
	$_1Y_{GTI,ads-LP}$	3.18%	$_2Y_{GTI,ads-LP}$	0.09%	$_3Y_{GTI,ads-LP}$	$2.99x10^{-3}$ %
	$_{1.1}X_{GTI/API}$	4.77 mg GTI/ g API	$_{2.1}X_{GTI/API}$	0.16 mg GTI/ g API	$_{3.1}X_{GTI/API}$	$5.40x10^{-3}$ mg GTI/ g API
	$_{1.1}Y_{API,ads-mem}$	48.17%	$_{2.1}Y_{API,ads-mem}$	47.29%	$_{3.1}Y_{API,ads-mem}$	43.99%
	$_{1.1}Y_{GTI,ads-mem}$	96.82%	$_{2.1}Y_{GTI,ads-mem}$	3.92%	$_{3.1}Y_{GTI,ads-mem}$	0.12%
Recuperation Step	$_1Y_{API,rec-LP}$	41.52%	$_2Y_{API,rec-LP}$	40.77%	$_3Y_{API,rec-LP}$	37.92%
	$_1Y_{GTI,rec-LP}$	0.83%	$_2Y_{GTI,rec-LP}$	0.04%	$_3Y_{GTI,rec-LP}$	$1.01x10^{-3}$ %
	$_{1.2}X_{GTI/API}$	1.57 mg GTI/ g API	$_{2.2}X_{GTI/API}$	0.06 mg GTI/ g API	$_{3.2}X_{GTI/API}$	$2.20x10^{-3}$ mg GTI/ g API
	$_{1.2}Y_{API,ads-mem}$	6.65%	$_{2.2}Y_{API,ads-mem}$	13.17%	$_{3.2}Y_{API,ads-mem}$	19.24%
	$_{1.2}Y_{GTI,ads-mem}$	95.99%	$_{2.2}Y_{GTI,ads-mem}$	99.87%	$_{3.2}Y_{GTI,ads-mem}$	99.996%
Drying and mixing Step	$_1Y_{API,total}$	93.35%	$_2Y_{API,total}$	86.83%	$_3Y_{API,total}$	80.76%
	$_1Y_{GTI,total}$	4.01%	$_2Y_{GTI,total}$	0.13%	$_3Y_{GTI,total}$	$4.00x10^{-3}$%
	$_{1.3}X_{GTI/API}$	3.34 mg GTI/ g API	$_{2.3}X_{GTI/API}$	0.11 mg GTI/ g API	$_{3.3}X_{GTI/API}$	$3.9x10^{-3}$ mg GTI/ g API

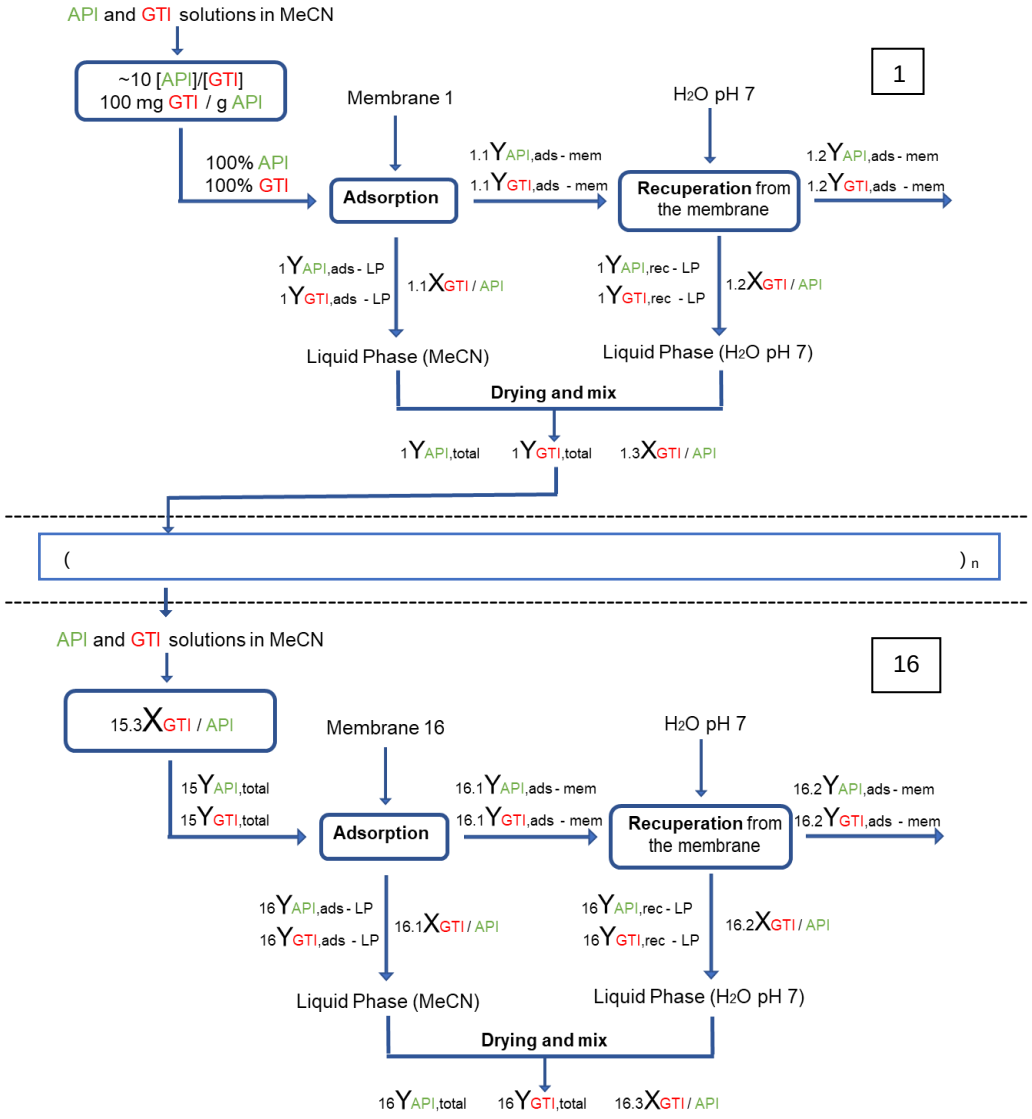
As previously mentioned, the maximum daily dosage for Roxi is 300 mg/day. In this way, to comply with the TTC (1.5 µg/day), a GTI limit of 0.005 mgGTI/gAPI needs to be achieve.

From Scheme 4.2, the strategy starts with a solution comprising Roxi at 800 ppm and MPTS at 80 ppm, being the ratio between these compounds of 100 mgGTI/gAPI. Firstly, an adsorption process is conducted by putting in contact the previous solution with a PBI membrane (4.5 cm^2), which has already been conditioned with fresh MeCN. From here, it is obtained a liquid phase (LP) with a specific GTI/API ratio associated. The membrane, after the adsorption, is placed in contact with fresh solvent (H_2O at pH 7) for API recuperation and, hence, a liquid phase with a specific GTI/API ratio is obtained in the end of this recovery step. Then, the two previous liquid phases (MeCN and H_2O at pH 7) need to be submitted to a drying step with the purpose of obtaining the resultant API powder, although contaminated with GTI. This powder needs later to be solubilized in MeCN, being the respective solution subjected to all the preceding steps again. At the end of 3 cycles, it is possible to achieve a GTI/API ratio below the GTI limit of 0.005 mgGTI/gAPI, being the %Roxi lost around **~19%** ($_{3.2}Y_{API,ads-mem}$). Thus, the API purification strategy outlined ended up leading to a significative loss of API. All the results obtained in each step and cycle are presented in Table 4.9, being their respective calculations/formulas in *Appendix 4*. Beyond this, a glossary is also presented in *Appendix 4* to clarify the meaning of each term used in the scheme.

4.6.2. Roxi – DMAP case-study

Regarding Roxi-DMAP case-study, attending to the results obtained, it is possible to infer that a proper purification strategy involving both Roxi and DMAP would not be achieved. As previously referred, the use of H_2O at pH 7 for a case involving these two compounds would not be acceptable for API purification strategy since a significative DMAP back contamination (37%) would be obtained.

However, for a comparison study, an API purification strategy was developed for this Roxi-DMAP case-study, using H_2O at pH 7 for the API recuperation step. Regarding the use of H_2O at pH 1.2, as washing solvent, despite this solvent having presented a good result for the regeneration of the membrane when DMAP was the GTI, the degradation of Roxi in acidic conditions constitutes a disadvantage. In this way, by reusing the membrane, there is a possibility of the degradation products being eluted, as impurities, with the API in the recovery step, being a situation to be avoided. On Scheme 4.3, an API purification strategy adopted for Roxi-DMAP case-study is observed.



Scheme 4.3. API purification strategy for Roxi-DMAP case-study. Only the first and the last cycles (16th) are presented, being n equal to 14.

Table 4.10. Results obtained by applying the API purification strategy outlined in Scheme 4.3.

		1st Cycle		16th Cycle	
Adsorption Step	$_1Y_{API,ads-LP}$	51.83%	$_{16}Y_{API,ads-LP}$	16.70%
	$_1Y_{GTI,ads-LP}$	14.78%	$_{16}Y_{GTI,ads-LP}$	$1.70x10^{-4}$%
	$_{1.1}X_{GTI/API}$	24.08 mg GTI/ g API	$_{16.1}X_{GTI/API}$	$8.00x10^{-4}$ mg GTI/ g API
	$_{1.1}Y_{API,ads-mem}$	48.17%	$_{16.1}Y_{API,ads-mem}$	17.15%
	$_{1.1}Y_{GTI,ads-mem}$	85.22%	$_{16.1}Y_{GTI,ads-mem}$	$2.73x10^{-3}$%
Recuperation Step	$_1Y_{API,rec-LP}$	41.52%	$_{16}Y_{API,rec-LP}$	14.78%
	$_1Y_{GTI,rec-LP}$	39.30%	$_{16}Y_{GTI,rec-LP}$	$1.23x10^{-3}$%
	$_{1.2}X_{GTI/API}$	79.49 mg GTI/ g API	$_{16.2}X_{GTI/API}$	$7.00x10^{-3}$ mg GTI/ g API
	$_{1.2}Y_{API,ads-mem}$	6.65%	$_{16.2}Y_{API,ads-mem}$	68.52%
	$_{1.2}Y_{GTI,ads-mem}$	45.92%	$_{16.2}Y_{GTI,ads-mem}$	99.9986%
Drying and mixing Step	$_1Y_{API,total}$	93.35%	$_{16}Y_{API,total}$	31.48%
	$_1Y_{GTI,total}$	54.08%	$_{16}Y_{GTI,total}$	$1.4x10^{-3}$%
	$_{1.3}X_{GTI/API}$	48.63 mg GTI/ g API	$_{16.3}X_{GTI/API}$	$3.8x10^{-3}$ mg GTI/ g API

Regarding Scheme 4.3, the strategy proposed follows the same rational as the one presented on Scheme 4.2 for Roxi-MPTS case-study. However, since there is no step on the purification process capable of either removing DMAP or recovering the API efficiently, the outcome was unacceptable. Thus, for Roxi-DMAP case-study, only at the end of 16th cycle it was possible to comply with the TTC (1.5 µg/day) by achieving a GTI limit below of 0.005 mgGTI/gAPI. Looking at the %Roxi lost, a value around **69%** ($_{16.2}Y_{API,ads-mem}$) was obtained. All the results obtained in each step from the 1st and 16th cycle are presented in Table 4.10, being their respective calculations/formulas in *Appendix 4*.

4.6.3. Scale-up simulation for API purification strategy

For both Roxi-MPTS and Roxi-DMAP case-studies, the strategy started by using an API/GTI solution prepared for a final volume of 1.5 mL, being MeCN the selected solvent. So, at laboratory scale, after adsorption step, 3 mL of MeCN were used. 1.5 mL were from the *Conditioning* phase and the remaining volume was from API at 800 ppm and GTI at 80 ppm solution submitted to adsorption. Moving to the recuperation step, 1.5 mL of washing solvent (H_2O at pH 7) was used. After this, the membrane was not recycled for either Roxi-MPTS or Roxi-DMAP case-studies. In this way, for each cycle, 3 mL of MeCN, 1.5 mL of H_2O at pH 7, and 1 PBI membrane (4.5 cm^2, equivalent to 23.6 mg) were used, while the amount of API inputted was 1.2 mg. Bearing this in mind, a prediction for the quantity of material used in an API purification strategy for both cases was performed by considering 1 Kg of API as reference.

Looking at what has been referred, Roxi presents a high value for the maximum daily dosage and, consequently, this leads to a low GTI limit of 0.005 mgGTI/gAPI. For **Roxi-MPTS** case-study, at the end of 3 cycles, a value below this limit would be reached, as previously stated, despite having lost around

19% of Roxi. Thus, for 1 Kg of API, 59 Kg of membrane (PBI), 3750 L of H_2O and 7500 L of MeCN would be used. If, hypothetically, the maximum daily dosage was about 12 mg (instead of 300), this GTI limit would be of 0.125 mgGTI/gAPI (25 times higher). In this way, at the end of the 2nd cycle, a value below this limit would be achieved. This would lead to a reduction of around 33% of each material used (membrane and solvents) in all purification process and to a %Roxi lost around **13%**. If after 1 cycle, a value below GTI limit could be obtained, this would mean a reduction of around 66% for the materials used in the purification process. However, for this last case, the maximum daily dosage would have to be 800 times lower (0.375 mg), what would lead to a GTI limit of 4 mgGTI/gAPI. Within this hypothetical situation, the %Roxi lost would be around **7%**. Table 4.11 summarises the results obtained when considering these three different daily doses for Roxi-MPTS case-study.

Table 4.11. Quantity of each material used (per Kg of API) for **Roxi-MPTS** case-study, attending to the daily dose applied and, consequently, to the GTI limit established.

Daily dose (mg)	GTI limit (mgGTI/gAPI)	no. of Cycles	Membrane (Kg)	H_2O (L)	MeCN (L)	%API lost
300	0.005	3	59.08	3750	7500	19
12	0.125	2	39.38	2500	5000	13
0.375	4	1	19.69	1250	2500	7

For **Roxi-DMAP** case-study, since a proper purification strategy was not found, the quantity of materials used in the process is by far higher than the one determined for Roxi-MPTS case-study. In Roxi-DMAP case-study, only at the end of 16 cycles involving a %Roxi lost of around **69%**, a GTI limit value below 0.005 mgGTI/gAPI would be reached, as previously stated. Thus, for 1 Kg of API, 315 Kg of membrane (PBI), 20000 L of H_2O and 40000 L of MeCN would be used. Here, since there is no efficient step for separating the API from the GTI, a situation involving a daily dose of 0.375 mg of API would lead to a value below the GTI limit of 4 mgGTI/gAPI only at the end of 6 cycles, instead of 1 as presented for Roxi-MPTS. So, a quantity of material 6 times higher would be necessary to accomplish the TTC. In this hypothetical situation, the %Roxi lost would be around **35%**. In relation to the use of a daily dose of 12 mg, which would impose a GTI limit of 0.125 mgGTI/gAPI, only at the end of 11 cycles it would be possible to achieve a value below this GTI limit, although at the expense of a loss of **55%** for the API. This would lead to the use of 217 Kg of membrane (PBI), 13750 L of H_2O and 27500 L of MeCN, which corresponds to a quantity of material more than 5 times higher than the one needed for Roxi-MPTS case-study when considering the same daily dose of 12 mg. Table 4.12 summarises the results obtained when considering these three different daily doses for Roxi-DMAP case-study.

Table 4.12. Quantity of each material used (per Kg of API) for **Roxi-DMAP** case-study, attending to the daily dose applied and, consequently, to the GTI limit established.

Daily dose (mg)	GTI limit (mgGTI/gAPI)	no. of Cycles	Membrane (Kg)	H_2O (L)	MeCN (L)	%API lost
300	0.005	16	315.07	20 000	40 000	69
12	0.125	11	216.59	13 750	27 500	55
0.375	4	6	118.14	7500	15000	35

However, regarding the solvents used on both purification processes, it would be possible to reuse them. Thus, knowing the boiling point of MPTS (292 ºC) and the melting points of Roxi (120 ºC) and DMAP (113 ºC), which are all higher than the boiling point of the solvents (MeCN and H_2O), it is possible through distillation, followed by condensation, to guarantee the separated recuperation of the solvents and, simultaneously, the obtention of dried API powder. This separated recuperation would be possible due to the lower boiling point of MeCN (82 ºC) in relation to the one of H_2O (100 ºC).

Regarding the quantity of PBI membrane used when performing this scale-up simulation, for Roxi-DMAP case study, the values obtained are impractical. For Roxi-MPTS case-study, despite being also obtained high values, these are significative lower than the ones obtained for Roxi-DMAP case. So, the use of 59 Kg, at maximum, obtained for Roxi-MPTS case could be scaled-up by resorting to several modules of spiral wound membranes to reach this demanded quantity of material necessary for the API purification process. However, for this scenario, the scale-up would not be suitable due to the significative %Roxi lost (~19%).

5. Conclusion

Attending to the main goal of this thesis, which was about assessing the viability of using PBI membrane adsorbers to perform a successful API purification process, it is possible to conclude that the results obtained do not reflect what would constitute a desirable situation, that is, an efficient GTI removal without significative API loss, preferentially below 10%. This is mainly due to a non-differentiated selectivity that PBI membrane presented for APIs and GTIs.

Thus, despite being developed a purification strategy, divided into two main moments (binding and post-binding step), it is possible to infer that this adsorption/desorption unit operation was not effective. Therefore, it would be interesting if other unit operation, namely OSN, was performed with the purpose of testing its feasibility for API purification, since the APIs, especially Roxi, present a well-differentiated and higher molecular weight in comparison with the GTIs in study. Beyond this, by combining these two unit operations and, hence, evaluate the capability of this approach against each one of the unit operations (alone) could be another pathway to be studied as a future work.

Now, looking detailly at the purification strategy, especially to the post-binding step, this would constitute an opportunity to increase the performance of the API purification process through recuperation of the API still bound to the adsorber as seen at the first moment of the API purification strategy – binding experiments. However, even attending to the best result, it is possible to notice a loss of around 19% of API and the need to perform 3 cycles to comply with the TTC. This is mainly due to the fact that Roxi presents an extremely low GTI limit of 0.005 mgGTI/gAPI. Thus, none of the results obtained all the way through the thesis were representative of a good outcome that would lead to consider the study of this purification process at industrial scale. Beyond this recuperation procedure, the regeneration of the PBI membrane was also studied within the post-binding step since recycling polymers applied in API purification processes makes their use more economically and environmentally attractive for the industry. Looking at the results obtained for the case-studies, this regeneration was inviable either due to the kind of adsorption where MPTS is involved or due to the degradation of Roxi in acidic conditions. However, it is of highlighting that for other APIs, stable at pH 1.2, and their purification processes using PBI membranes, if DMAP is the GTI to be removed, the use of H_2O at pH 1.2 should be considered for regeneration experiments.

Another study that could be performed is about the kinetics of adsorption. Resorting to this, it could be possible to know if the compounds selected to be API and GTI models would present a slow or a fast adsorption. The ideal situation would be if the GTIs were rapidly adsorbed while the APIs presented a slow adsorption. Then, by adjusting the time of the adsorption process, it could be possible to obtain a good result. However, in this thesis work, all the experiments were performed during 24 h, not being possible to add a potential selectivity, that could be conferred by the kinetic aspects, to the purification process.

6. References

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Appendix

Appendix 1 – Calibration Curves

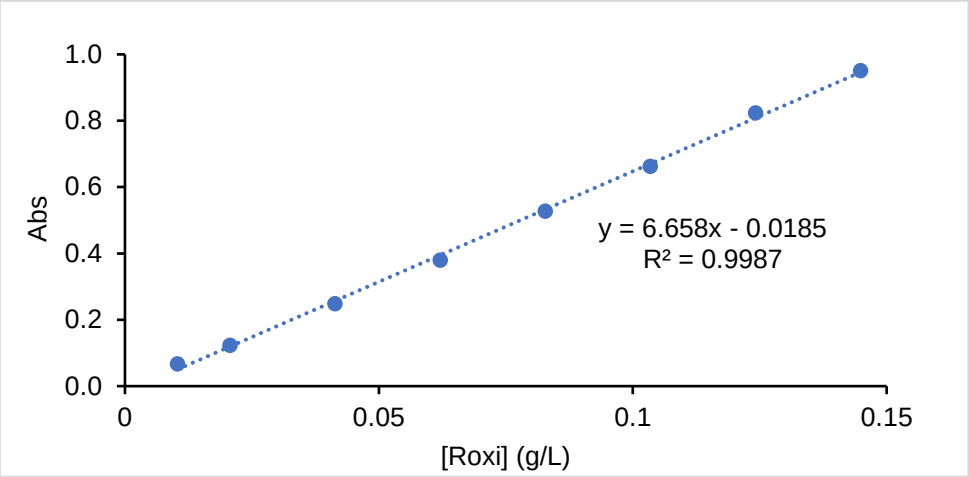

Figure 1. Calibration Curve for Roxi in MeCN (at 205 nm).

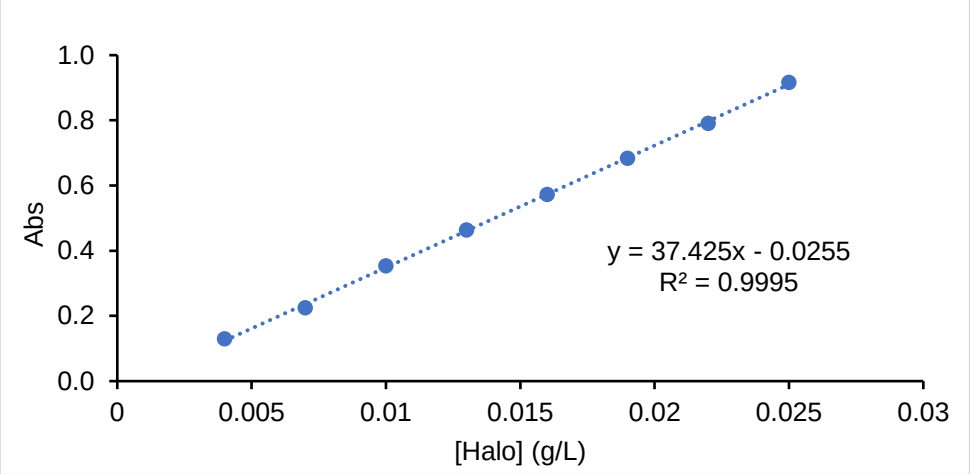

Figure 2. Calibration Curve for Halo in MeCN (at 234 nm).

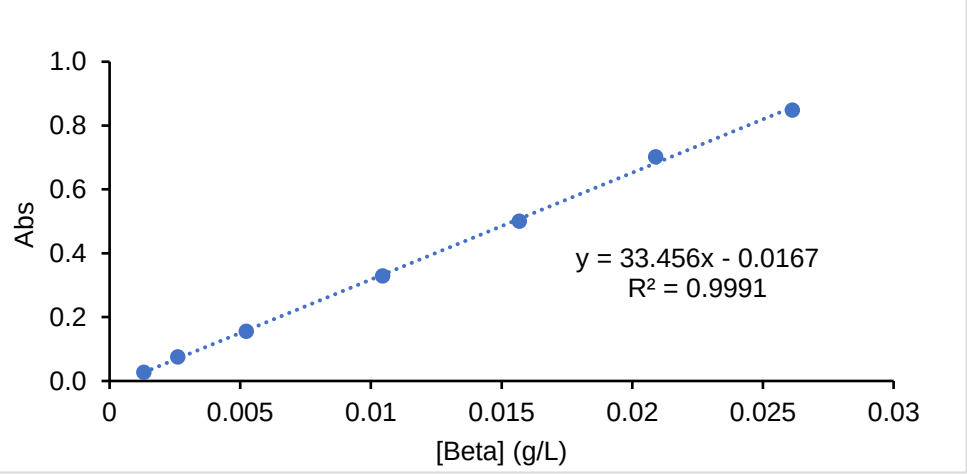

Figure 3. Calibration Curve for Beta in MeCN (at 236 nm).

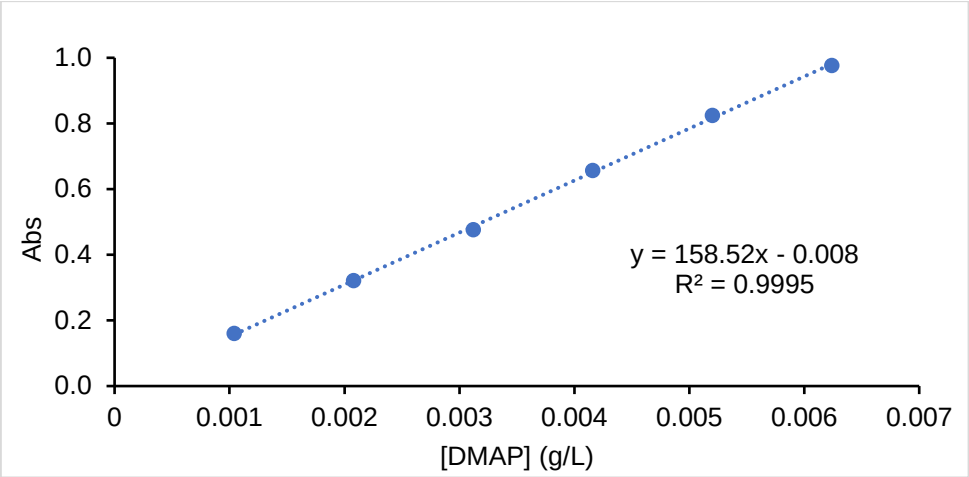

Figure 4. Calibration Curve for DMAP in MeCN (at 256 nm).

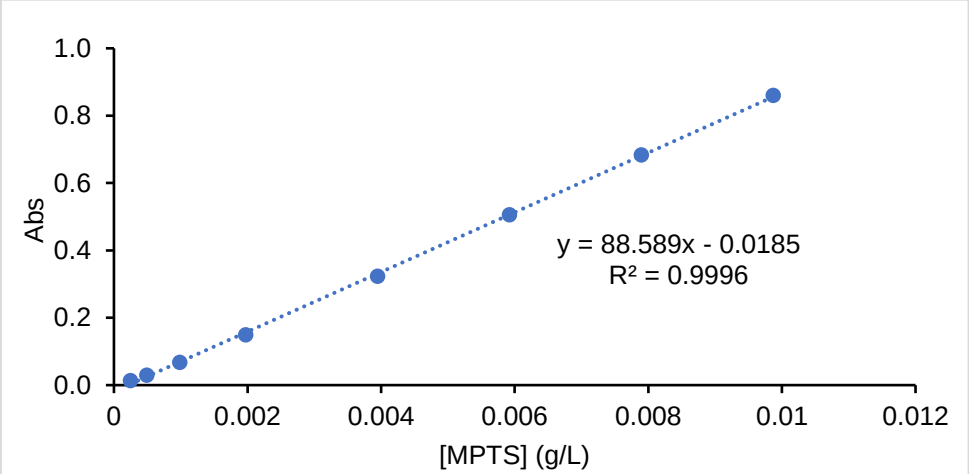

Figure 5. Calibration Curve for MPTS in MeCN (at 224 nm).

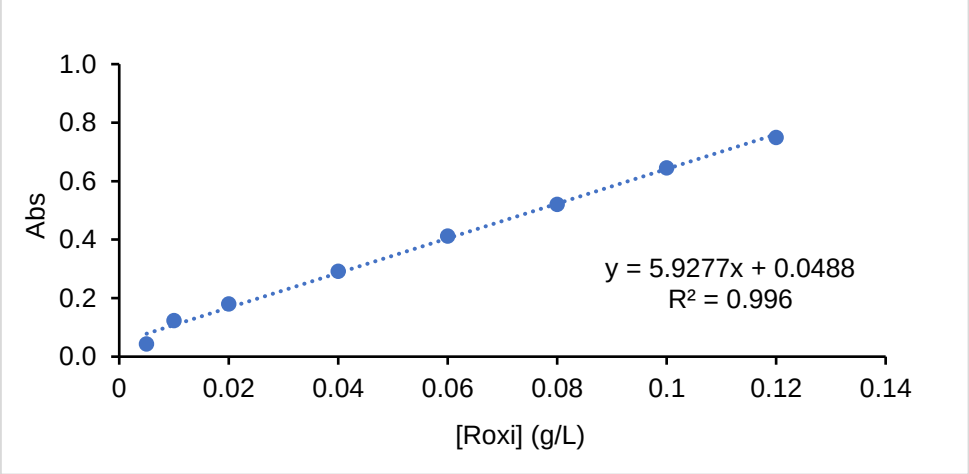

Figure 6. Calibration Curve for Roxi in H_2O pH 1.2 (at 202 nm).

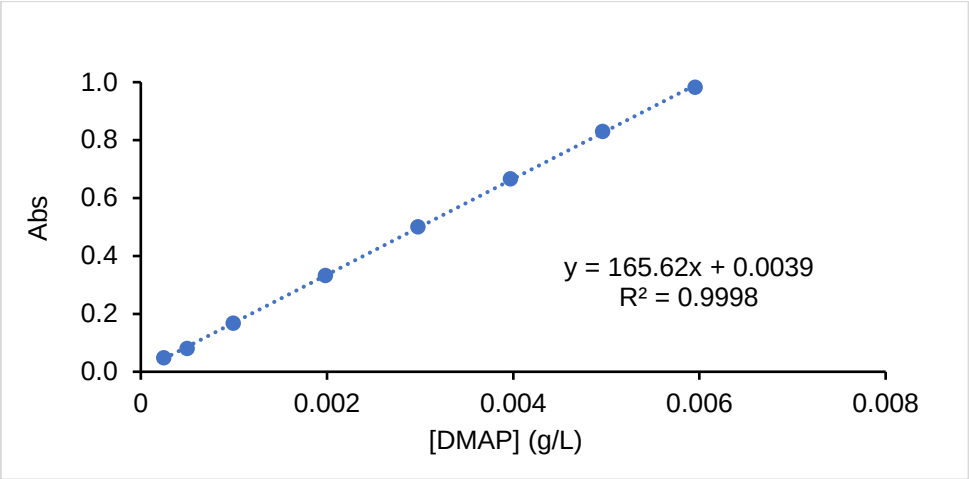

Figure 7. Calibration Curve for DMAP in H_2O pH 1.2 (at 280 nm).

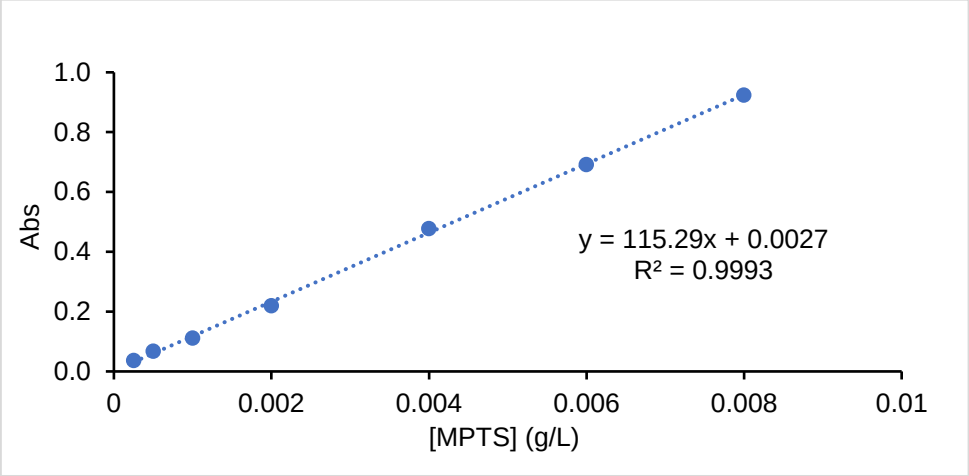

Figure 8. Calibration Curve for MPTS in H_2O pH 1.2 (at 226 nm).

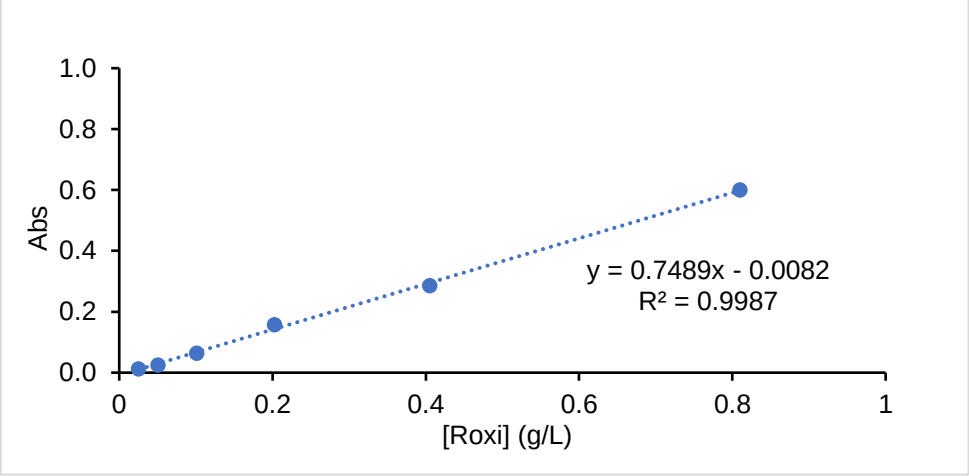

Figure 9. Calibration Curve for Roxi in H_2O pH 7 (at 205 nm).

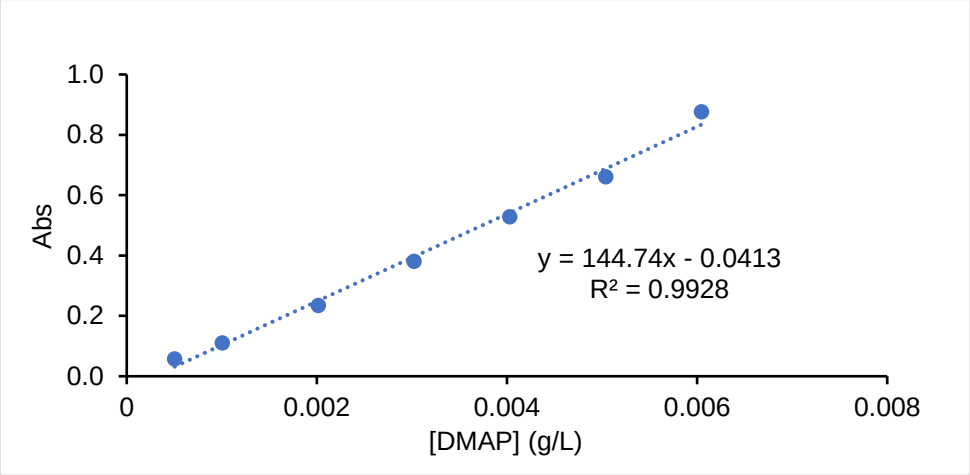

Figure 10. Calibration Curve for DMAP in H_2O pH 7 (at 261 nm).

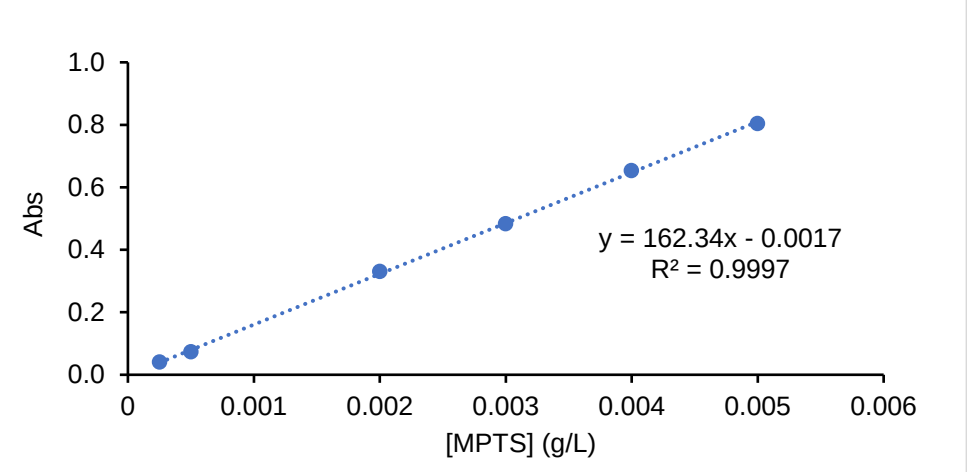

Figure 11. Calibration Curve for MPTS in H_2O pH 7 (at 226 nm).

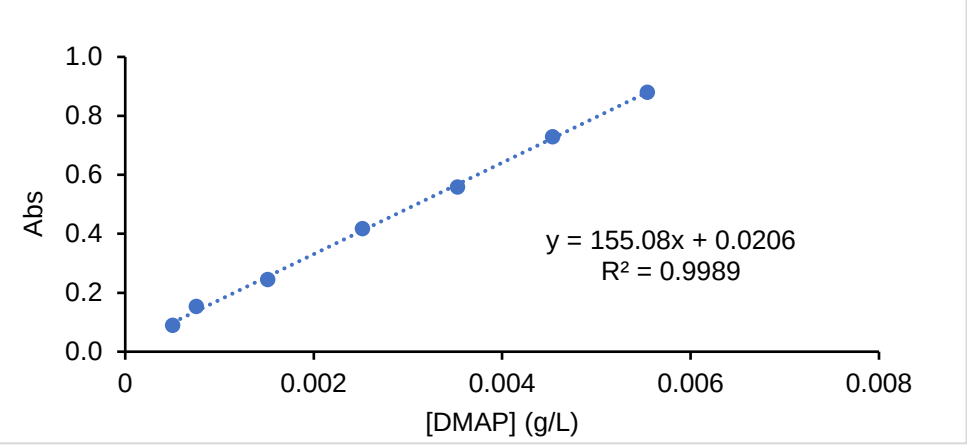

Figure 12. Calibration Curve for DMAP in H_2O pH 13 (at 261 nm).

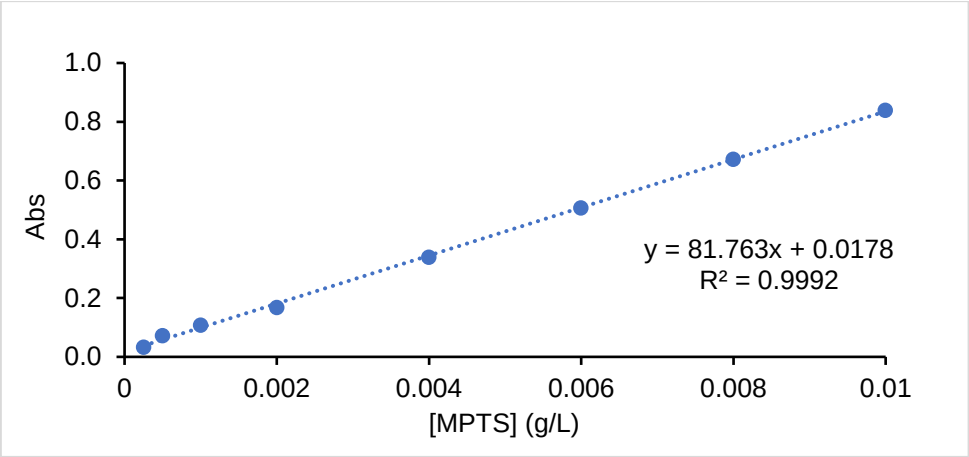

Figure 13. Calibration Curve for MPTS in H_2O pH 13 (at 224 nm).

Appendix 2 – Binding Adsorption Results

Despite the results here presented having been obtained resorting to three independently produced PBI membranes, all three were manufactured by using a dope solution at 26 wt%.

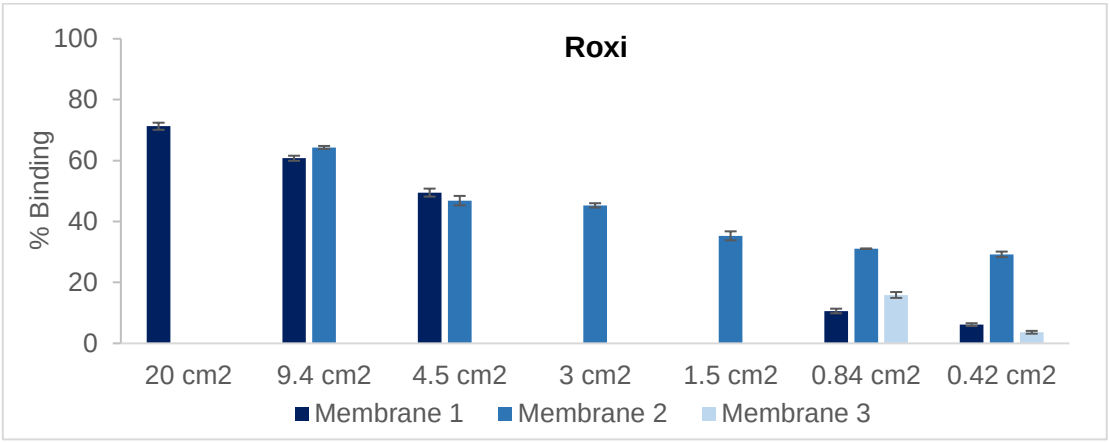

Figure 14. Binding adsorption experiments in MeCN at different PBI quantities (depicted as A_m) for **Roxi case**.

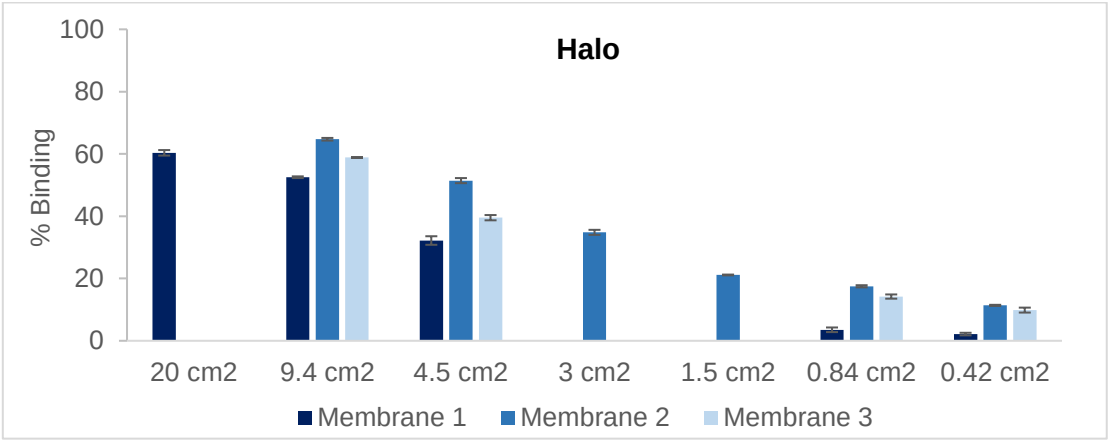

Figure 15. Binding adsorption experiments in MeCN at different PBI quantities (depicted as A_m) for **Halo case**.

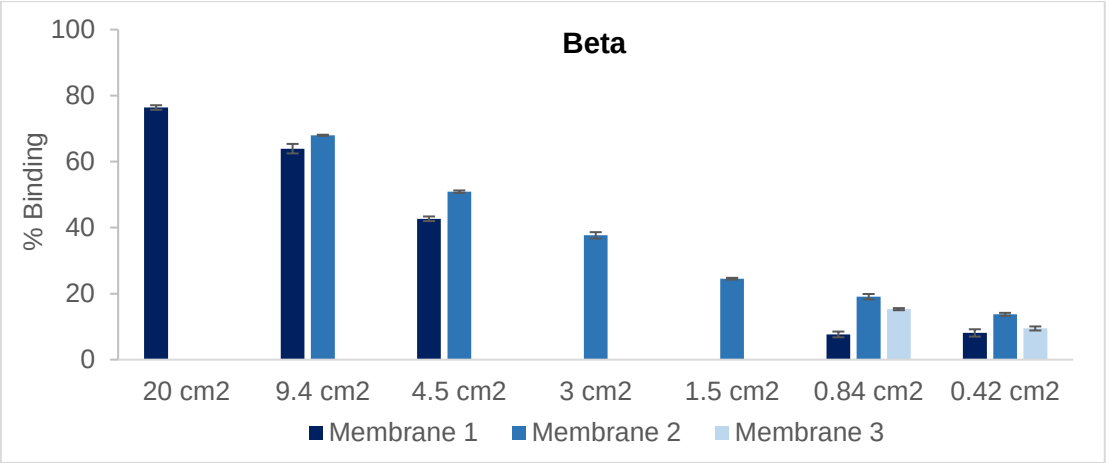

Figure 16. Binding adsorption experiments in MeCN at different PBI quantities (depicted as A_m) for **Beta case**.

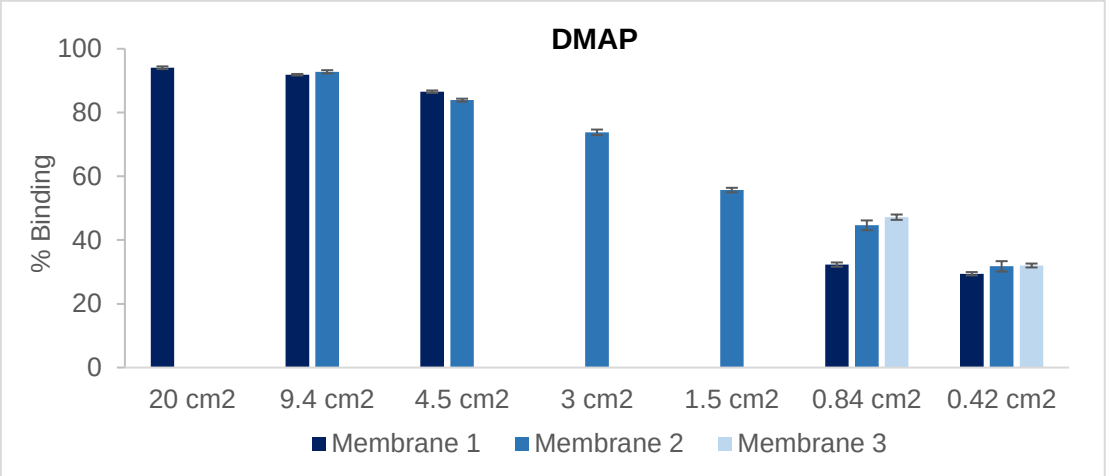

Figure 17. Binding adsorption experiments in MeCN at different PBI quantities (depicted as A_m) for **DMAP case**.

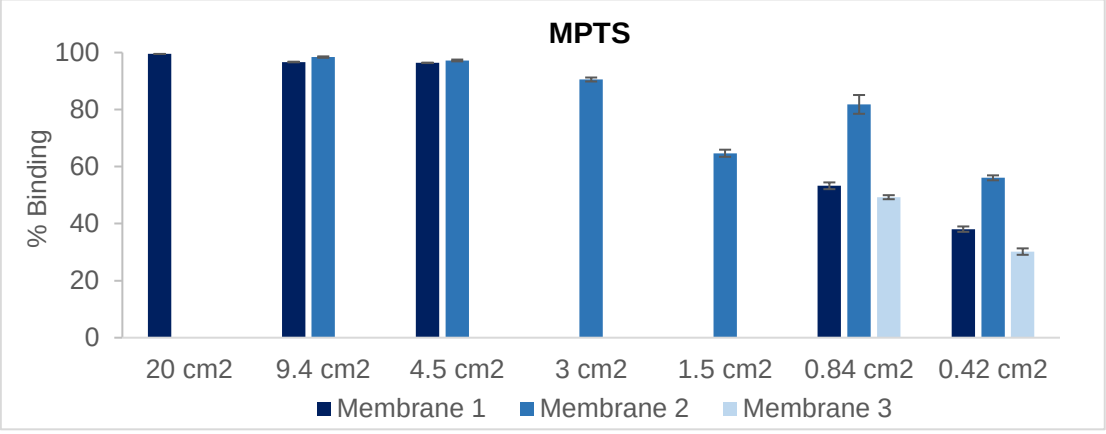

Figure 18. Binding adsorption experiments in MeCN at different PBI quantities (depicted as A_m) for **MPTS case**.

Appendix 3 – Post-binding step results

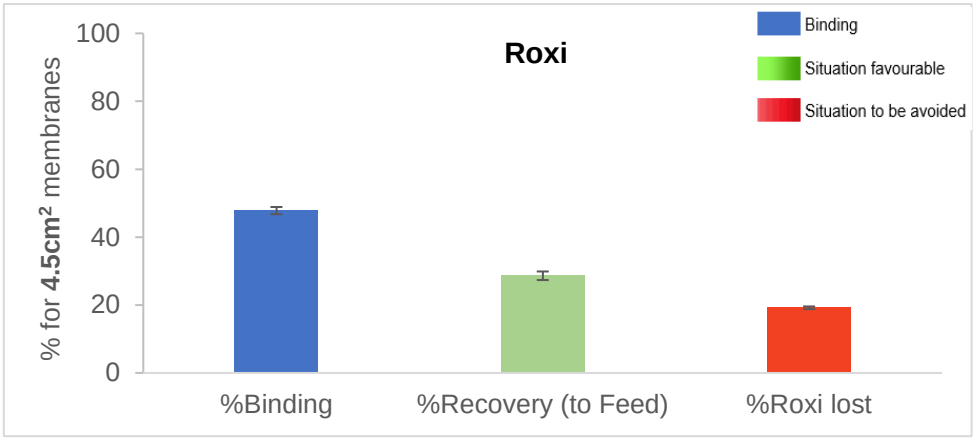

Figure 19. Binding and Recovery (4 steps) experiments for **Roxi**. For all the experiments, the same PBI quantity was used.

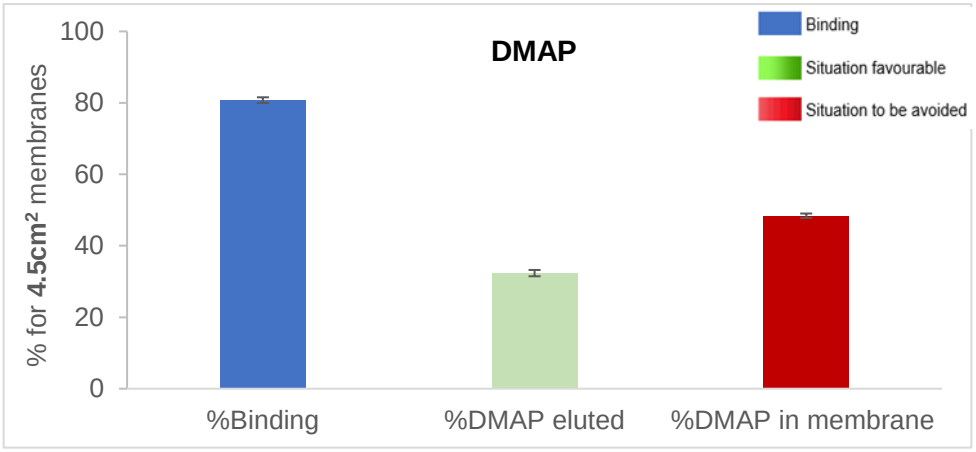

Figure 20. Binding and Recovery (4 steps) experiments for **DMAP**. For all the experiments, the same PBI quantity was used.

Appendix 4 – API purification strategy

Before getting into this section, it is important to refer that all **percentage values** obtained are always in relation to the total amount of API/GTI used at the beginning of API purification strategy, that is, quantity of API/GTI in the feed solution from the first cycle.

Glossary

Table 1. Meaning of each term used in the API purification strategy.

$_{n}Y_{API,ads - LP}$	Quantity of API in liquid phase (MeCN), after adsorption, in relation to its initial amount (feed solution) – presented as percentage
$_{n}Y_{GTI,ads - LP}$	Quantity of GTI in liquid phase (MeCN), after adsorption, in relation to its initial amount (feed solution) – presented as percentage
$_{n.1}X_{GTI / API}$	GTI/API ratio in mgGTI/gAPI (being MeCN the liquid phase)
$_{n.1}Y_{API,ads - mem}$	Quantity of API in the membrane n, after adsorption, in relation to its initial amount (feed solution) – presented as percentage
$_{n.1}Y_{GTI,ads - mem}$	Quantity of GTI in the membrane n, after adsorption, in relation to its initial amount (feed solution) – presented as percentage
$_{n}Y_{API,rec - LP}$	Quantity of API in liquid phase (H_2O pH 7), after recovery, in relation to its initial amount (feed solution) – presented as percentage
$_{n}Y_{GTI,rec - LP}$	Quantity of GTI in liquid phase (H_2O pH 7), after recovery, in relation to its initial amount (feed solution) – presented as percentage
$_{n.2}X_{GTI / API}$	GTI/API ratio in mgGTI/gAPI (being H_2O pH 7 the liquid phase)
$_{n.2}Y_{API,ads - mem}$	Quantity of API lost in the membrane n, after recovery, in relation to its initial amount (feed solution) – presented as percentage
$_{n.2}Y_{GTI,ads - mem}$	Quantity of GTI in the membrane n, after recovery, in relation to its initial amount (feed solution) – presented as percentage
$_{n}Y_{API,total}$	Quantity of API total (after drying and mixing), in relation to its initial amount (feed solution) – presented as percentage
$_{n}Y_{GTI,total}$	Quantity of GTI total (after drying and mixing), in relation to its initial amount (feed solution) – presented as percentage
$_{n.3}X_{GTI / API}$	Final GTI/API ratio (at each cycle n) in mgGTI/gAPI

Blue – Adsorption step; ***Grey*** – Recuperation step; ***White*** – Drying and mixing step;
n – number of cycles.

Equations

As an example, the equations applied for the cycles n=1 and n=2 are presented. For the remaining cycles, the same rational is followed.

For n=1

- Adsorption step

$$_{1}Y_{API,ads-LP} = \frac{quantity\ of\ API\ in\ LP}{quantity\ of\ API\ in\ Feed_{n=1}} \times 100 \qquad (1.1)$$

$$_{1}Y_{GTI,ads-LP} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ GTI\ in\ Feed_{n=1}} \times 100 \qquad (1.2)$$

$$_{1.1}X_{GTI/API} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ API\ in\ LP} \times 1000 \qquad (1.3)$$

$$_{1.1}Y_{API,ads-mem} = 100 - {}_1Y_{API,ads-LP} \quad (1.4)$$

$$_{1.1}Y_{GTI,ads-mem} = 100 - {}_1Y_{GTI,ads-LP} \quad (1.5)$$

- Recuperation step

$$_1Y_{API,rec-LP} = \frac{quantity\ of\ API\ in\ LP}{quantity\ of\ API\ in\ Feed_{n=1}} \times 100 \quad (1.6)$$

$$_1Y_{GTI,rec-LP} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ GTI\ in\ Feed_{n=1}} \times 100 \quad (1.7)$$

$$_{1.2}X_{GTI/API} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ API\ in\ LP} \times 1000 \quad (1.8)$$

$$_{1.2}Y_{API,ads-mem} = 100 - {}_1Y_{API,ads-LP} - {}_1Y_{API,rec-LP} \quad (1.9)$$

$$_{1.2}Y_{GTI,ads-mem} = 100 - {}_1Y_{GTI,ads-LP} - {}_1Y_{GTI,rec-LP} \quad (1.10)$$

- Drying and mixing step

$$_1Y_{API,total} = {}_1Y_{API,ads-LP} + {}_1Y_{API,rec-LP} \quad (1.11)$$

$$_1Y_{GTI,total} = {}_1Y_{GTI,ads-LP} + {}_1Y_{GTI,rec-LP} \quad (1.12)$$

$$_{1.3}X_{GTI/API} = \frac{final\ quantity\ of\ GTI\ (at\ n=1)}{final\ quantity\ of\ API\ (at\ n=1)} \times 1000 \quad (1.13)$$

For n=2

- Adsorption step

$$_2Y_{API,ads-LP} = \frac{quantity\ of\ API\ in\ LP}{quantity\ of\ API\ in\ Feed_{n=1}} \times 100 \quad (2.1)$$

$$_2Y_{GTI,ads-LP} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ GTI\ in\ Feed_{n=1}} \times 100 \quad (2.2)$$

$$_{2.1}X_{GTI/API} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ API\ in\ LP} \times 1000 \quad (2.3)$$

$$_{2.1}Y_{API,ads-mem} = {}_1Y_{API,total} - {}_2Y_{API,ads-LP} \quad (2.4)$$

$$_{2.1}Y_{GTI,ads-mem} = {}_1Y_{GTI,total} - {}_2Y_{GTI,ads-LP} \quad (2.5)$$

- Recuperation step

$$_2Y_{API,rec-LP} = \frac{quantity\ of\ API\ in\ LP}{quantity\ of\ API\ in\ Feed_{n=1}} \times 100 \qquad (2.6)$$

$$_2Y_{GTI,rec-LP} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ GTI\ in\ Feed_{n=1}} \times 100 \qquad (2.7)$$

$$_{2.2}X_{GTI/API} = \frac{quantity\ of\ GTI\ in\ LP}{quantity\ of\ API\ in\ LP} \times 1000 \qquad (2.8)$$

$$_{2.2}Y_{API,ads-mem} = 100 - {}_2Y_{API,ads-LP} - {}_2Y_{API,rec-LP} \qquad (2.9)$$

$$_{2.2}Y_{GTI,ads-mem} = 100 - {}_2Y_{GTI,ads-LP} - {}_2Y_{GTI,rec-LP} \qquad (2.10)$$

- Drying and mixing step

$$_2Y_{API,total} = {}_2Y_{API,ads-LP} + {}_2Y_{API,rec-LP} \qquad (2.11)$$

$$_2Y_{GTI,total} = {}_2Y_{GTI,ads-LP} + {}_2Y_{GTI,rec-LP} \qquad (2.12)$$

$$_{2.3}X_{GTI/API} = \frac{final\ quantity\ of\ GTI\ (at\ n{=}2)}{final\ quantity\ of\ API\ (at\ n{=}2)} \times 1000 \qquad (2.13)$$