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

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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.

1. Introduction

Looking at the pharmaceutical products, their manufacturing might follow 2 different pathways, either through a total synthesis approach or through modification of a naturally occurring product. In both situations, indispensable reactive reagents can be involved, and the final drug product may present these or side products as impurities, which could be related with genotoxicity, mutagenicity, and carcinogenicity.[1] These terms, despite being associated, it is important to not confound them.[1,2] A certain compound presenting a carcinogenic or mutagenic effect will surely react with DNA. According to James and Elizabeth Miller theory, genotoxins attack DNA molecules due to presence of nitrogen and oxygen atoms on pyrimidine and purine bases, as well as on phosphodiester backbone, which constitute the nucleophilic sites. In some cases, this mechanism of action could lead to strand breaks.[1,3] Beyond the chemical nature and structure of the GTI, 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 are endocyclic nitrogens (N3 and N7 of guanine and adenine) and the less nucleophilic are the exocyclic oxygens.[1,2] However, it is necessary to attend to situations where there is an overprediction of mutagenicity since some structural alerts do not consider factors such as steric hindrance and hydrophilicity.[1,4,5]

Since GTIs have been at the center of increasing regulatory and industry attention, the main key actions through the years toward regulations must be presented. Regarding the timeline, this can be traced back to late 1990s, where the ICH Q3 guidelines used the general term "unusual toxicity". Looking at the ICH Q3, this guideline presented several topics, namely Q3A (control of impurities in API), Q3B (degradants in pharmaceutical products) and Q3C (address residual solvents).[1,6] However, these guidelines did not effectively address the requirements for controlling GTIs trace levels.[7] Still within this late 1990s, two ICH safety guidelines (S2A – 1995 and 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."* [8] In 2002, a position paper was published by Committee for Proprietary Medicinal Products (CPMP) focusing on finding GTI-free routes for API production or providing a justification for GTIs unavoidable presence on the final product..[9,10] 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.[1] 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.[1] In this way, the implementation of this concept and its limit of 1.5 $\mu g.day^{-1}$ for known and potential carcinogens was made.[1] 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.[1,9] Still looking at EMA guideline[9,11], finalized in 2006, there was an update on the meaning of GTI term, which refers to "positive findings in established *in vitro* or *in vivo* genotoxicity tests with the focus on DNA reactive substances".[9] Still in 2006, a staged TTC approach was proposed considering acceptable limits for GTIs in final APIs related with exposure duration, suggested by the Pharmaceutical Research and Manufacturers of America (PhRMA).[1,8] The same document also defined 5 separate classes for the impurities attending to the structure−activity relationship (SAR). 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 from EMA. One year later, the Food and Drug Administration (FDA) released their draft guidance.[9,12]. This guideline provided recommendations on acceptable exposure limits for GTIs during either marketing applications or clinical testing. Beyond this, FDA draft guidance suggested changing API synthetic route for minimizing the formation of GTIs or maximize its removal and introduced lower limits for different patient populations.[1,9] In 2010, the Safety Working Party (SWP) published a Question and Answers (Q&A) document to complement the guideline from EMA (2006).[9,10] For the staged TTC approach, SWP introduced a new dose rate correction factor to consider deviations from the original linear extrapolation model. 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. Finally, SWP stated 2 different situations regarding the moment of introduction/formation of the GTI in the synthesis and its inclusion in the API specification, which depended on GTI exceeding the acceptable TTC in 30%.[9] In 2011, ICH S2A and S2B guidelines were replaced by the ICH S2(R1). Here, a reorganization and a restructuration were 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 positive findings, its interpretation and management should be enhanced through risk assessment improvement.[13] In 2014, the ICH M7 guideline: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk[14] reached Step 4 of the ICH process, which means that the final draft became recommended for adoption by the ICH regulatory entities. The purpose of this guideline was to provide a practical framework for identification, categorization, qualification, and controlling of mutagenic impurities (MIs) to limit carcinogenic risks associated, with a wide-ranging application.[9] However, for ICH M7, exceeding the TTC does not automatically mean an expanded cancer risk, being stated that this concept is highly hypothetical.[9] ICH M7 stated that a (Q)SAR assessment would be required when carcinogenicity and bacterial mutagenicity data were not available. For this, 2 (Q)SAR computational methodologies (one rule-based and other statistical-based expert) were indispensable and, depending on the outcome, an analytical test could be performed to properly classify an impurity. In relation to the less than lifetime (LFL) treatments, the cumulative effect was considered for determining the acceptable lifetime dose attending to treatment duration. Beyond this, exceptions regarding the TTC limits could be done for severe disease or limited therapeutic alternatives cases.[9] Moving to control strategy, ICH M7 introduced 4 different approaches to develop it. So, API specification (option 1) or raw, starting, and intermediate material specifications (option 2) included a test for MI (and in-process controls for option 2), having been used an analytical method for establishing the acceptance criterion. Option 3, presenting same specifications from option 2, set the acceptance criterion above the acceptable limit of MI by combining an analytical method with a purge factor analysis. Option 4, through purge factors analysis, did not involve any specification and test for MI if its level was below the TTC limit, being the scientific risk assessment used to justify

this approach. When this analysis alone was not satisfactory, analytical data to validate would be expected.[9] Due to the difficulty to apply this ICH M7, an Addendum was suggested and released for public consultation (Step 2) in 2015: Application of the principles of the ICH M7 guideline to calculation of compound specific AIs.[9,15] In 2017, adoption of this Addendum was recommended by the ICH regulatory bodies.[9,16] In 2018, a version of ICH M7 (R2) was released by the Expert Working Group (EWG), in which acceptable limits for new MIs and a revision of these for the MIs already listed on the Addendum were included. As a result, a Q&A document was presented. This document was able to clarify the justification of control strategy in marketing authorization applications, the (Q)SAR models predictions validation, the risk assessment, and the meaning of "mutagenic and genotoxic potential" and "significant increase in clinical dose of marketed products", as well as recommending the elements to be considered when using predictive purge calculations.[18] Thus, M7 Q&A was signed off as a Step 2 document in 2020, to be issued for public consultation. The Step 4 Q&A document was signed off in April 2021 and the Step 4 M7(R2) sign off occurred in July 2021.[17,18]

The development of synthetic processes to mitigate GTIs should be considered since their presence in pharmaceutical streams during API synthesis is normally challenging to avoid.[1] Chemical synthetic approaches are related with synthesis alteration and reaction conditions adjustment. Regarding the first one, it relies on avoiding generating or using GTIs by applying different production steps.[1] However, in some cases, the reactivity of a reagent is an indispensable feature for assuring a proper API or intermediate synthesis without significative loss of yield, despite this reactivity being also related with GTIs formation. In relation to reaction conditions adjustment, this strategy relies on eliminating or reducing the presence of GTIs by changing reaction conditions. However, all this must be performed without significative yield reduction.[1,19] Both previous strategies are linked with the use of another strategy - Quality by Design (QbD) approach, which aims also to control GTI levels below acceptable limits, decreasing the need of using routine testing.[1,20] However, 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 and intermediates.[1,19] Beyond all this, several stages and routes of API isolation and purification could be included in the production process as a last resort. In this way, the purge factor analysis could be introduced. Teasdale, A., *et al.*[7] presented a semiquantitative "assessment purge tool". 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 the use of analytical tests (Table 1).[1,9]

Table 1. Presentation of an example of key parameters in purge factors in the tool proposed by Teasdale, A., *et al.* Purge factor is defined as the ratio of [GTI] before and after purging.[1,7]

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

Several other publications and authors applied the theoretical purge factor assessment tool, although presenting some minor alterations on its conception. However, regardless the publication or case-study, by comparing theoretical with experimental purge factors, an underprediction of the purge capacity was always seen so that the proposed tool could gain acceptance.[9] Due to the continuous adherence to this tool, a new semi-automated system for assessing the purge of MIs was created – Mirabilis (2014).[21] The original paper-based scoring approach was applied within this new *in silico* system to ensure the maintenance of the conservative positioning, while improving the efficiency and transparency of purge predictions. Thus, resorting to a complete dataset, systematic models were provided to facilitate the prediction of purges, including always regulatory inputs. Mirabilis will continually be supported and developed to meet present and future user needs by becoming a regular practice to help the pharmaceutical industry, while not raising risks for patients' health.[21]

During API synthesis, there are some purification steps that contribute to GTIs removal. For GTI removal, the higher the selectivity of a purification step, the lower will be API loss and the higher will be removal efficiency. However, this efficiency is compromised when large quantities of GTI are removed to reach demanded ultralow levels, leading to high API losses.[1] Regarding the different pathways to 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. Some of the conventional purification steps include crystallization, precipitation, distillation, solvent extraction, and treatment with activated carbon and resins. The efficiency of the separation is based on the differences in the physicochemical properties of the compounds to be separated and their relative affinities for a selective agent. During last years, some advanced techniques, such as membrane separations or molecularly imprinted polymers, have been developed.[1]

Adsorption, a conventional purification process, is widely used for removing GTIs.[22] Here, the adsorbate is attracted to the adsorber surface and, hence, the surface free energy is reduced. The transference proceeds until equilibrium is achieved between the amount of adsorbate in solid phase and the amount of adsorbate still present in liquid phase. These quantities will vary according to affinity degree of the adsorbate for the adsorber. From a pharmaceutical point of view, high affinity of GTI, to adsorber, combined with lower binding of API is intended.[23] Looking at the advantages of this process, it is of highlighting its low-cost due to possible adsorber recycle. 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 may be necessary (e.g., filtration).[23] Then, to have a proper adsorption system, it is primarily necessary to choose the most adequate adsorber. Thus, features such as low cost, efficiency, high surface area, stability at mechanic, chemical and thermal level, and ease of desorption make generally a good adsorber.[24] After choosing a proper adsorber, it is necessary to proceed to adsorption isotherms. After obtaining isotherm curves, their shape provides information regarding physical nature of both adsorbate and adsorber.[25,26] Then, rising a classification system for liquid-solid adsorption isotherms was indispensable (Figure 1).

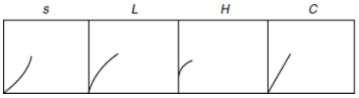


Figure 1. Presentation of the 4 main classes of curves classified according to their initial slope: S curve (vertical orientation isotherm); L curve (normal or "Langmuir" isotherm); H curve (high affinity isotherms); C curve (constant partition isotherm).

Regarding the models to adjust experimental data of isotherm studies, the commonly used are Langmuir and Freundlich. The physical simplicity of Langmuir model is based on 4 assumptions: adsorption cannot take place in multilayers; each site only binds to one adsorbate molecule; adsorber surface is uniform and all binding sites are energetically equivalent; capacity of an adsorbate molecule to bind in a site is independent of the occupation at adjacent site.[23] This model is represented by equation 1:

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

Where q_e is the adsorption capacity at equilibrium, q_m is the maximum adsorption capacity, 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 is possible to obtain the parameters. The linearized form of this isotherm is presented in equation 2:

$$\frac{C_e}{q_e} = \frac{1}{K_L \times q_m} + \frac{1}{q_m} C_e \quad (2)$$

When an initial adsorbed layer becomes a surface capable of being involved in adsorption, multilayers formation can be expected. Freundlich model assumes that adsorber surface is heterogeneous due to possibility of interaction between adsorber particles.[23] This model is defined by equation 3:

$$q_e = K_F \times C_e^{\frac{1}{n}} \quad (3)$$

Where K_F is the Freundlich constant, related with the energy taken for adsorption, and 1/n is the heterogeneity factor. Regarding parameters determination, linearization is crucial. So, these 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 to $\ln(K_F)$, as seen in equation 4:

$$ln\ q_e = ln\ K_F + \frac{1}{n} ln\ C_e \quad (4)$$

PBI, an organic solvent stable polymer, has been explored for API purification resorting to adsorption. To find optimal properties that could improve impurity removal with lowest API losses, new adsorbers derived from pristine PBI were obtained through physicochemical alterations.[27] According to Ferreira, F. A., *et al.*[27], the PBI with thermal and acidic treatment (PBI-TA) presented high efficiency on 4-dimethylaminopyridine (DMAP) removal in DCM, even at high concentrations, with an API loss nearly null after recovery. 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 methyl *p*-toluenesulfonate (MPTS) and an API loss virtually null after recovery. In this way, the previous GTIs could be efficiently removed depending on the pH conditioning selected for the PBI, making the previous adsorbers good platforms for purification. Beyond this, these PBI adsorbers revealed to be versatile since both PBI-TA and -TB could be produced as

beads or electrospun fibers without compromising their performance, being used in applications like adsorption column (beads) and membrane (fibers).[27] PBI-Adenine, another new adsorber from a different study, obtained by chemical functionalization, presented an efficient removal for 5 different families of DNA alkylating agents with an API loss virtually null after recovery. So, this polymer could simulate the double helix of DNA and be effective on removal of intercalating agents of DNA.[28] However, there is always room for improvement, with the purpose of finding economic and environmentally attractive new adsorbers for industry.

Thus, in the present study, the main objective is to implement an API purification process by exploring, for the first time, 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 evaluation of their ability for specific removal of GTIs and, the second, concerns developing a post-binding step, that is, using a recovery step for mitigating API losses and a regeneration step to try to recycle the adsorber.

2. Research Strategy

The model APIs and GTIs selected, as well as the rationale behind their choice are presented. Starting by model APIs, the selected ones were Halobetasol Propionate (Halo), Betamethasone Acetate (Beta), and Roxithromycin (Roxi). Halo is a glucocorticoid steroid that reduces skin inflammation or infection in airways by topical administration. It is prescribed for treatment of allergic rhinitis and asthma.[29,30] Regarding its genetic toxicology profile, this API gave positive findings in 2 genotoxicity studies, but being administrated as lotion, the systemic exposure will be much lower than the initial quantity applied on skin. This API is well study and widely used in purification processes studies.[31] Beta is a glucocorticoid used for treating various disorders like arthritis or allergic conditions related with airways diseases by several routes of administration.[32,33,34] This API does not present any relevant genotoxic data. However, its study has been earning some relevance in purification processes studies. Roxi is a semi-synthetic macrolide that acts as antibiotic for treatment of urinary and respiratory tract infections[35], not being associated with genotoxicity effects. Roxi has been studied to increase its oral bioavailability[36] and to discover possible degradation pathways to avoid or reduce its incidence in environment.[37] However, its study in purification processes, especially adsorption, is not widely reported. Thus, for the first time, the purification of these APIs by using PBI membrane adsorbers is going to be reported. Despite all 3 APIs being selected as model, the one presenting more relevance will be Roxi due to lack of prior studies about its purification processes based on adsorption. Thus, while Halo and Beta are only included in binding experiments, Roxi will be addressed in both binding and post-binding experiments with the purpose of finding a proper purification strategy for this API. In Figure 2, molecular structure, and molecular weight of all APIs are displayed.

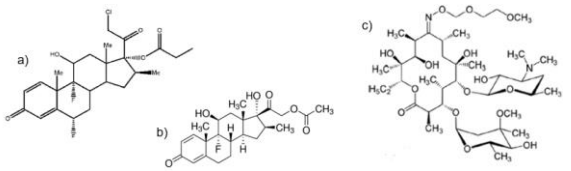


Figure 2. Molecular structure of model APIs considered in this study: a) Halo (MW: 484.96 g/mol); b) Beta (MW: 434.50 g/mol); c) Roxi (MW: 837.04 g/mol).

Regarding GTIs, DMAP and MPTS were selected. Despite not being involved or produced on synthesis of previous APIs, these GTIs were used since they are well-studied and -characterized compounds. For the first time, these GTIs were being submitted to adsorption using PBI membranes. Regarding DMAP, this aromatic amine is a highly efficient catalyst used for acylation reactions[38] and presents a structural alert. Despite not being innately genotoxic, DMAP can originate electrophilic species through its metabolic activation *in vivo*. Thus, for this compound, which may be involved in reactions like the formation of Meta[39], its presence in this API must be avoided. With respects to MPTS, this sulfonate ester is seen as a potential GTI, being part of a widely studied family of GTIs (alkylating agents).[40] These GTIs and their precursors may be used for groups protection or act 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.[1] In Figure 3, molecular structure, and molecular weight of both GTIs are presented.

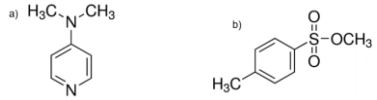


Figure 3. Molecular structure of model GTIs considered in this study: a) DMAP (MW: 122.17 g/mol); b) MPTS (MW: 186.23 g/mol).

The organic solvents are commonly used in synthesis of APIs. Thus, for the binding experiments in this study, Acetonitrile (MeCN) was the solvent selected, presenting a role as polar aprotic solvent, and having not been classified as to human carcinogenicity.[41] So, from several organic solvents tested, MeCN was chosen since all APIs and GTIs selected were soluble at all experimental concentration range used. Beyond this, the preference for this solvent is also related with the fact of being a volatile compound that presents a lower boiling point than water, being both solvents studied in the post-binding step. The water was used to introduce a green solvent in the purification strategy. The use of these type of solvents in pharmaceutical industry has been encouraged, but their implementation is still difficult. Attending to the need for developing robust and suitable adsorbers, PBI polymer has gained some relevance in API purification strategy due to its stability at thermal, chemical, and mechanical level, not being soluble on most organic solvents used on pharmaceutical industry. Thus, 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 TTC value and the maximum daily dose of API (mg/day).[42]

$$GTI\ limit\ (mgGTI/gAPI) = \frac{TTC\ value\ (\mu g.day^{-1})}{maximum\ daily\ dose\ of\ API\ (mg.day^{-1})} \quad (5)$$

Attending to the model APIs selected, depending on their route of administration, their respective maximum daily dosage may change. For Halo, it is reported that administration of 50 g per week of the lotion containing 0.5 mg of Halo per gram of lotion should not be exceeded.[43] Thus, 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 1 mL per week of an injection presenting 3 mg of API should not be exceeded.[44] Using the same reasoning applied for Halo, a value of approximately 3.5 mgGTI/gAPI is obtained. For Roxi, due to its instability in gastric acid media, a high maximum daily dosage of 300 mg must be administered orally.[45] Thus, for this case, a GTI limit of 0.005 mgGTI/gAPI is obtained. Therefore, assuming a situation without API losses during purification process, a GTI removal of 99.58%, 96.5%, and 99.995% would be required to comply with the TTC for case-studies involving Halo, Beta, and Roxi, respectively. However, as it is going to be seen, there is API loss during purification steps which means that higher removal efficiencies will be needed to reach desirable GTI/API ratio.

3. Materials and Methods

3.1. Materials

Fluticasone Propionate (FP), Beta, Mometasone Furoate (Meta) and Halo were kindly provided by *Hovione PharmaScience Ltd*. Roxi was acquired from *Alfa Aesar*. The GTIs selected for this study, DMAP and MPTS, were purchased from *ACROS Organic* and *Alfa Aesar*, respectively. Both APIs and GTIs mentioned were used as supplied, so no further purification was needed. 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*.

3.2. Experimental methods description

3.2.1. Membranes manufacturing

The *Casting* of 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 membrane 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 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. 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), being the selected areas 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. 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 membranes having been 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 and 800 ppm, respectively. The membranes were submitted to continuous agitation at 200 rpm for 24 h at room temperature. After 24 h, the solution was 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 membrane was calculated using equation 6, 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.[23]

$$Binding\ (\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \qquad (6)$$

The amount of GTI or API bound to the membrane was calculated from equation 7, where q_e (mg/g) is 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, V (L) is volume of solution and M (g) is quantity of PBI used.[23]

$$q_e = \frac{V \times (C_0 - C_e)}{M} \qquad (7)$$

3.2.5. Binding adsorption isotherm experiments

Isotherms were determined by varying the quantity of PBI (20, 9.4, 4.5, 3, 1.5, 0.84 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 testing solutions, the procedure was the same as the one presented in section 3.2.4 for binding experiments. The percentage and amount of API or GTI bound to the PBI membrane was obtained by using the previous equations 6 and 7. Regarding the isotherm models, Langmuir and Freundlich were the ones used for data treatment. For Langmuir model, equations 1 and 2 were used while for Freundlich model, equations 3 and 4. The suitability between experimental and predicted values from isotherm studies was described by χ^2 (equation 8).[46]

$$\chi^2 = \sum \frac{(q_e - q_{e,m})^2}{q_{e,m}} \qquad (8)$$

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. Thus, the lower the Chi-square, the better the fit.[46]

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 model API while both DMAP and MTPS as model GTIs. After 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.

API case:

$$\%Recovery = \frac{C_{f\ after\ recovery}}{C_0} \times 100 \qquad (9)$$

$$\%API\ lost = \%Binding - \%Recovey \qquad (10)$$

GTI case:

$$\%Genotoxic\ leaching = \frac{C_{f\ after\ recuperation}}{C_0} \times 100 \qquad (11)$$

$$\%GTI\ removed = \%Binding - \%Genotoxic\ leaching \qquad (12)$$

For membrane regeneration, the following equations were used.

GTI case:

$$\%GTI\ eluted\ (from\ membrane) = \frac{C_{f\ after\ regeneration}}{C_0} \times 100 \qquad (13)$$

$$\%GTI\ in\ membrane = \%Binding - \%GTI\ eluted \qquad (14)$$

For 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. After *Conditioning* phase, a MPTS solution of 80 ppm in MeCN was added, letting the binding occur for 24 h at 200 rpm at room temperature. Then, the solution was analysed by UV-Vis spectroscopy for solute quantification.

4. Results and Discussion

4.1. Solubility Experiments

4.1.1. MeCN - solubility test

For decision making of which API or GTI would be used as a case-study in this work, 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 experimental work is according with the literature, as presented in Table 2.

Table 2. Comparison between experimental and prior data results regarding solubility in MeCN of 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 [49]
	Meta	Insoluble	Insoluble	Negligible [32]
	Roxi	Insoluble	Soluble	Soluble (at 800 ppm) [32]
	Halo	Soluble	Soluble	Highly Soluble [32]
	Beta	Soluble	Soluble	Highly Soluble [48]
GTIs	DMAP	Soluble	Soluble	Soluble [38,47]
	MPTS	Miscible	Miscible	Miscible/Soluble [47]

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.[27,47] 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[32] and Beta[48], the experimental results agree whit what is stated in the literature. Attending to the API concentrations previously reported when using MeCN[35], 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[32], and FP was slightly soluble[49]. Thus, both APIs were discarded for further studies. Regarding Roxi, this was soluble at 800 ppm, which was according to previous studies[32]. Thus, 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.

4.1.2. 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 3.

Table 3. Comparison between experimental and prior data results regarding 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 [50]	**NF**	**NF**
7	This work	Soluble	Soluble	Miscible
	Literature	Poorly Soluble [50]	Highly Soluble [52]	Insoluble [53]
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.[50] 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.[50] In previous studies, despite their difference regarding the saturation value of 187 ppm[51] and 283 ppm[36], 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.[52] For MPTS, although it is reported to be insoluble at pH 7[53], 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 H2O 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.

4.2. Binding Adsorption Experiments

Due to the possibility of inputting severe chemical conditions on API synthesis, such as high temperatures and acidic or basic conditions, it is necessary to promote the development of robust and adequate adsorbers. Beyond this, organic solvents are commonly used in API synthesis (including its purification process(es)). Thus, an organic solvent compatible polymer is needed, being PBI an example of this type of polymer. In the present study, all binding experiments were performed considering a PBI membrane at 26 wt% as adsorbing material. Thus, the phenomenon that underlies the experiments performed in this study is adsorption. The solid phase is known as adsorbent or adsorber and the liquid phase, in these experiments, contains just one compound to be adsorbed.[23] 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 3 independently produced PBI membranes at 26 wt% for solutions around 800 ppm for the APIs and 80 ppm for the GTIs.

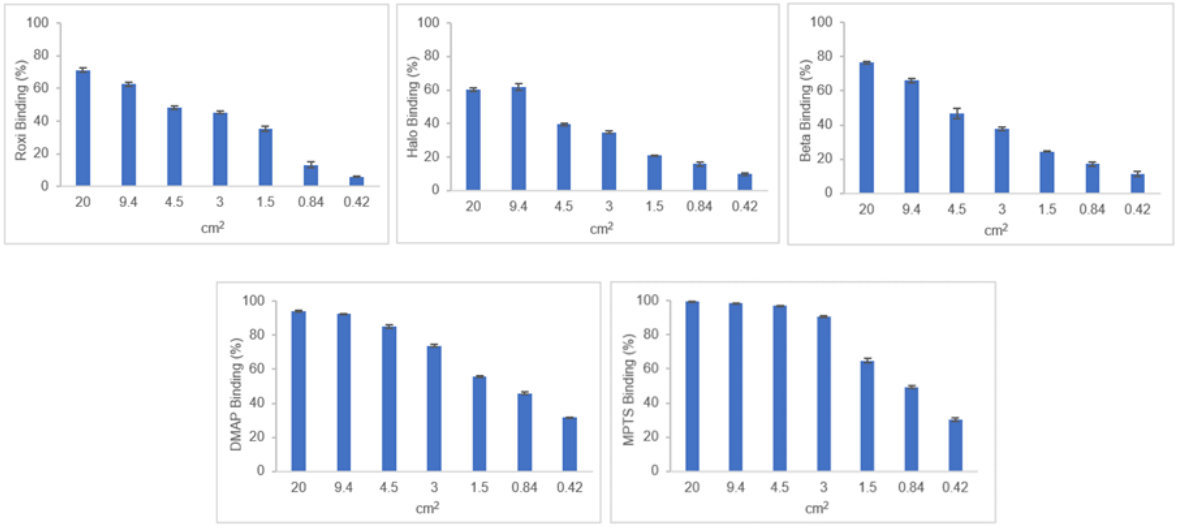


Figure 4. Binding adsorption experiments in MeCN for different membrane areas (A_m) of PBI.

Regardless the API or GTI, the binding increases with the A_m. 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 A_m of 9.4 cm^2 and below 20% for the two smallest A_m. However, as intended, these binding results were lower when comparing with the ones obtained for the GTIs. DMAP presented a maximum binding above 80% for A_m of 4.5, 9.4 and 20 cm^2 and MPTS above 90% for the same A_m and 3 cm^2. Since the membranes were placed in 2 mL Eppendorf vials, for A_m higher than 1.5 cm^2, it was necessary to roll them to fit properly and assure that they were totally covered by the solutions. Despite the shape similarity with spiral wound membranes (normally used in OSN studies), the ones used in this study 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 adsorber surface area for the binding. In this way, the binding capacity for higher A_m (>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.
Despite binding results for GTIs were higher than the ones for APIs, it is crucial to refer that 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.*[27] However, in this former study, a recuperation and regeneration step were also performed. As earlier mentioned in the present study, 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 API adsorption, leading to a proper API purification. According to Ferreira, F. A., *et al.*[27], 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, physical adsorption was expected. Here, the process can be reversible and multilayer adsorption, as well as desorption, were possible. Regarding the type of interactions, these can be electrostatic, hydrogen bonds, Van der Waals, or dipole–dipole.[23,56] So, taking a look at the molecular structure of the APIs, it is possible to see that all present both hydrogen bond donor and acceptor sites.[51,57,58] 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 5. 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.[27,54] 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. In this way, a multilayer adsorption could be possible.

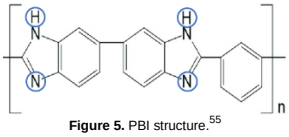


Figure 5. PBI structure.[55]

Regarding DMAP, this molecule could also interact with PBI through hydrogen bonding.[52] However, this 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.[27] 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.[23] 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, chemical adsorption was expected, being the process irreversible, and desorption difficult. Regarding type of interactions, these can occur by ionic or covalent bonds.[23] So, the interaction with PBI was expected to occur through methylation reaction of the amine groups of imidazole rings of adsorber.[27] Beyond this, PBI behaves additionally as ion exchanger, interacting ionically with GTI anion that is formed.[27,59] Hence, 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.

4.3. Binding Isotherm studies

After choosing the adsorbent for binding experiments, it is necessary to obtain the adsorption isotherms. Isotherms are diagrams presenting the variation of concentration at equilibrium in the adsorbent solid as a function of the concentration of 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.

For Halo and Beta, Langmuir and Freundlich models were only used to adjust the experimental data from the binding adsorption studies.

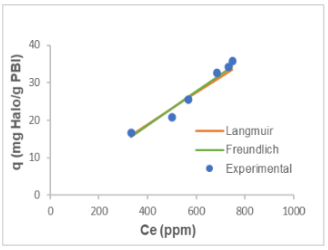


Figure 6. Binding isotherm fitting models for Halo. It is presented the experimental values and the ones predicted by isotherm models (Langmuir and Freundlich).

Table 4. Binding isotherm physical parameters for Halo.

	Parameters	Halo
Langmuir	K_L (L/mg)	1.66x10^{-4}
	q_m (mg/g)	303.48
	R^2	0.977
	X^2	0.583
Freundlich	K_F (L/mg)	5.33 x10^{-2}
	n	1.02
	R^2	0.980
	X^2	0.430

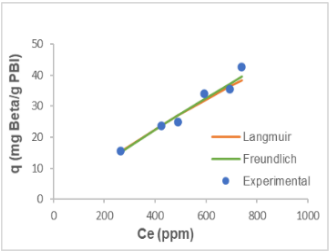


Figure 7. Binding isotherm fitting models for Beta. It is presented the experimental values and the ones predicted by isotherm models (Langmuir and Freundlich).

Table 5. Binding isotherm physical parameters for Beta.

	Parameters	Beta
Langmuir	K_L (L/mg)	3.02x10^{-4}
	q_m (mg/g)	209.20
	R^2	0.991
	X^2	0.822
Freundlich	K_F (L/mg)	9.12 x10^{-2}
	n	1.09
	R^2	0.987
	X^2	0.599

From Figures 6 and 7 and Tables 4 and 5, either Halo or Beta seems to follow Freundlich isotherm model on PBI membrane since $\chi 2$ 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.[23] Regarding the shape of equilibrium curve, 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.[23] Looking at both Halo and Beta cases, the n parameter is close to 1. So, probably, the number of adsorption sites is greater than the number of molecules to be adsorbed. Here, the Freundlich model could be simplified to Henry model since K_F values are associated with the initial slope of the isotherm curve.[23] The Henry model suggests that adsorption capacity is proportional to the solute concentration, up until the maximum possible adsorption, where an abrupt change to a horizontal plateau would occur. For this model, the isotherm with partition constant is characterized by a linear behaviour of the equilibrium data at low concentrations.[23] So, from the prior results, K_F here is equivalent to K_H. In this way, the Freundlich model suitable for both APIs ended up evolving to a Henry model. Hence, for a proper isotherm study, a broader range of concentrations should be used for both APIs to be possible to obtain more experimental points useful for describing the suitable isotherm model. With both Halo and Beta presenting a physical adsorption, there is no change in molecular state of adsorption.[23,60,61] So, a multilayer adsorption would probably not occur.

For Roxi, DMAP and MPTS, Langmuir and Freundlich models were also used to adjust experimental data from binding studies. Moreover, by changing concentration of Roxi, DMAP and MPTS solutions for the same quantity of PBI, it was also possible to determine isotherms. The reason for choosing Roxi as only API in this additional isotherm study was due to its higher binding value at 4.5 cm^2, when comparing with the values for other APIs. Thus, for Roxi, saturation of membrane at given concentration was expected, whose value would be lower than the one for Halo or Beta.

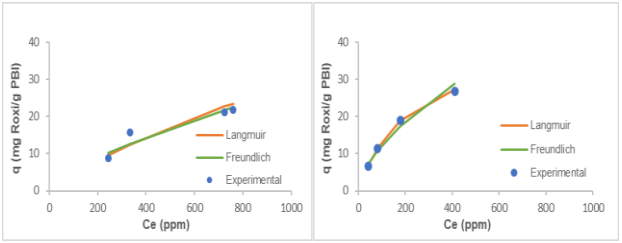


Figure 8. Langmuir and Freundlich binding isotherm fitting models for Roxi. Left: Plot obtained by varying the quantity of PBI; Right: Plot obtained by varying the solution concentration.

Table 6. Binding isotherm physical parameters for Roxi varying the quantity of PBI.

	Parameters	Roxi
Langmuir	K_L (L/mg)	$5.87x10^{-4}$
	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 7. Binding isotherm physical parameters for Roxi varying the concentration of API solution.

	Parameters	Roxi
Langmuir	K_L (L/mg)	$4.60x10^{-3}$
	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

From Figure 8 and Tables 6 and 7, there is a divergency on the results obtained. By varying the quantity of PBI used (Figure 8 left), Roxi seems to follow Freundlich model on PBI membrane. However, by varying API solution concentration (Figure 8 right), the model that fits the data is 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 8 right) and other at higher concentrations (Figure 8 left). So, for 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 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 $\chi 2$ obtained (Table 7). 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.[23] 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 8 right.

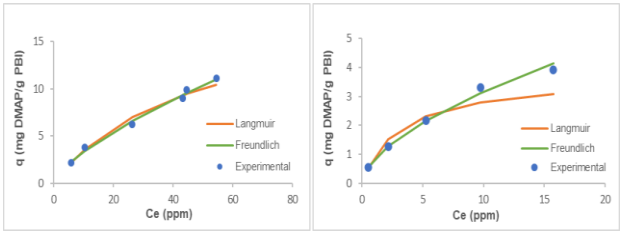


Figure 9. Binding isotherm fitting models for DMAP (Langmuir and Freundlich). Left: Plot obtained by varying the quantity of PBI; Right: Plot obtained by varying the solution concentration.

Table 8. Binding isotherm physical parameters for DMAP varying the quantity of PBI.

	Parameters	DMAP
Langmuir	K_L (L/mg)	$2.23x10^{-2}$
	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 9. 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

From Figure 9 and Tables 8 and 9, it is concluded that DMAP follows Freundlich model on PBI membrane since χ^2 presented a significative lower value for this model regardless the approach used for determining isotherms. This means that when initial adsorbed layer becomes a surface for more adsorption, the formation of multilayers is expected. The type of curve, described by Freundlich model, representative of DMAP case is a class L since n > 1.[23] This one indicates that adsorption occurs due to relatively weak forces (e.g., van der Waals)[23], which agrees with what was previously supposed on binding studies (section 4.2) for DMAP case.

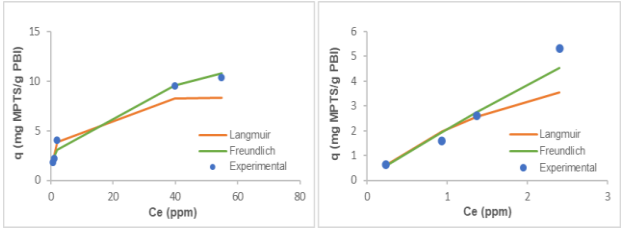


Figure 10. Binding isotherm fitting models for MPTS (Langmuir and Freundlich). Left: Plot obtained by varying the quantity of PBI; Right: Plot obtained by varying the solution concentration.

Table 10. 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

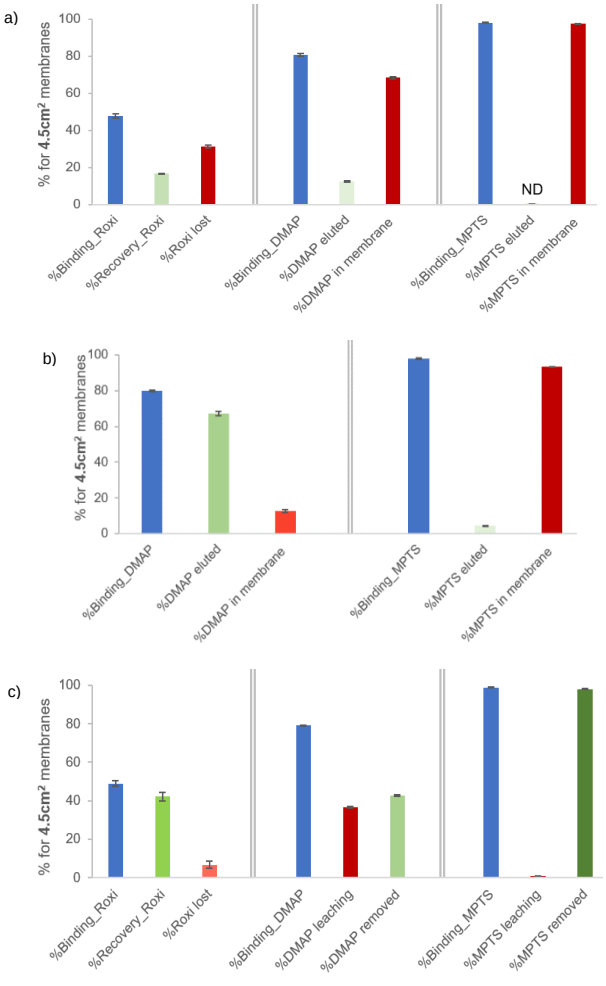
Table 11. Binding isotherm physical parameters for MPTS varying the concentration of GTI solution.

	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 10 and Tables 10 and 11, MPTS seems to follow Freundlich model since χ^2 was lower for this model regardless the approach used. So, MPTS seems to follow an adsorption on multilayers, being n higher than 1. Regarding the isotherm shape, the H type is an isotherm curve that occur when adsorption sites were not fully occupied, or there was not a complete vertical orientation of the solvent molecules.[23] 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. This agrees with what was said about the type of adsorption since these H curves are indicative of chemisorption.[23] 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 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.

4.4. API Recuperation and Membrane Regeneration and Reusability

At section 4.2, the non-differentiated selectivity that PBI membrane presented for APIs and GTIs was noticed. Thus, due to significative binding for APIs, which would lead to huge negative economic impact to the pharmaceutical industry, the recovery of API that remained bound to the adsorber is crucial. Here, the results presented and discussed are correlated with experiments performed using A_m of 4.5 cm^2. This is due to the significative binding adsorption values obtained for GTIs (≥80%). In this section, only Roxi would be subjected to recovery experiments due to lack of prior studies about its purification processes based on adsorption.



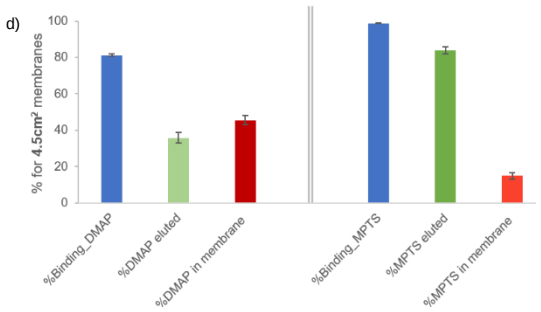


Figure 11. Representation of Binding and Recovery experiments for Roxi, and Binding, Regeneration and Leaching experiments for DMAP and MPTS, using **a)** MeCN for Binding and Regeneration **b)** MeCN for Binding and H_2O at pH 1.2 for Regeneration **c)** MeCN for Binding and H_2O at pH 7 for Recovery and Leaching **d)** MeCN for Binding and H_2O at pH 13 for Regeneration. ND means Non-Detectable.

From **Figure 11 a)**, binding for Roxi of around 48% was obtained. After washing with MeCN, it was possible to recover approximately 17% of Roxi in relation to feed solution. Knowing the value of both %Binding and %Recovery, the %Roxi lost was obtained, corresponding to 31% of API still adsorbed on the membrane after binding and recovery steps in relation to its initial amount on feed solution. Thus, using MeCN for Roxi recovery as API purification strategy is not suitable, since the %Roxi lost was significative. So, despite the results not having been promising, there is still possible to observe some recovery. This happens because the adsorption of Roxi to membrane is a physical process, where desorption is possible.[23] 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. Regarding membrane regeneration, a GTI elution step was performed by assessing DMAP and MPTS desorption using MeCN. Being MeCN infeasible for API recovery, it was then investigated if MeCN could be efficient for regeneration. For DMAP, after the 80% binding to the adsorber, a value of around 12% of DMAP eluted was obtained. Thus, it was determined the %DMAP in membrane corresponding to 68% in relation to its initial amount on feed solution. So, using MeCN for regeneration is not feasible since a significative amount of DMAP continued adsorbed after washing, that is, MeCN was inefficient in surpassing the extent of interaction between DMAP and PBI. Here, a physical adsorption has been reported and, consequently, desorption would be possible since MeCN would be capable of solubilizing DMAP adsorbed, being this partitioned for both phases. Looking at %DMAP eluted (~12%) and %Recovery of API (~17%), the higher value for API could be due to higher concentration and molecular weight of Roxi. For MPTS, after 98% binding to adsorber, there is practically no elution of GTI (0.41%) with MeCN washing. This means that regeneration would be unsuitable. As previously mentioned, this is due to interaction with PBI through a methylation reaction, being this adsorber capable of acting additionally as an ion exchanger.[27,59] Having in mind other objective, the results obtained for MPTS would be interesting if MeCN could be efficient at API recovery since there would be minimum MPTS back contamination (0.41%). According to Ferreira, F. A., *et al.*, the resulting salt of MPTS presented poor solubility in DCM solvent and, thus, practically all this salt had remained precipitated with PBI.[27] Looking at MPTS-MeCN case, the results obtained here were probably due to the same reason.

After using MeCN, the recuperation and regeneration studies proceeded resorting to H_2O at pH 1.2, 7 and 13, with the aim of studying pH influence on desorption process. From **Figure 11 b)**, API recuperation was not performed since Roxi presents acid instability at pH 1.2.[62] So, H_2O at pH 1.2 could never be seen as a washing solvent for API recovery. It is now important to interpret the results for GTIs to verify if regeneration would be possible using this solvent. To regenerate the membrane, a GTI elution step was performed by assessing DMAP and MPTS desorption. For both GTIs, after binding (~80% for DMAP and ~98% for MPTS), a %GTI eluted of around 67% for DMAP and 4% for MPTS was obtained. Then, 13% and 94% represent the quantity of DMAP and MPTS, respectively, still adsorbed after binding and regeneration steps in relation to their respective initial amount on feed solution. Thus, H_2O at pH 1.2 for regenerating the membrane when DMAP was the GTI was satisfactory. However, this regeneration would be impractical for MPTS case. For DMAP, comparing with the results from MeCN, the use of H_2O at pH 1.2 allowed to discover a new approach to be used for regeneration. This could be explained attending to pKa values of DMAP (9.7)[63] and PBI (5.23)[54]. So, at pH 1.2, PBI would be in its protonated form and, hence, interaction adsorber-adsorbate would end. The same protonation would happen to DMAP and, thus, re-interaction between adsorber and adsorbate would not be favoured due to the possible electrostatic repulsion of both protonated species. For MPTS, as mentioned, a chemical interaction is established with PBI. So, the resulting salt of MPTS presented poor solubility in this H_2O and practically all this salt had remained precipitated with PBI.[27] The 4% from %GTI eluted could possibly be due to the use of same Eppendorf where binding test took place and some residual MPTS could have been left.

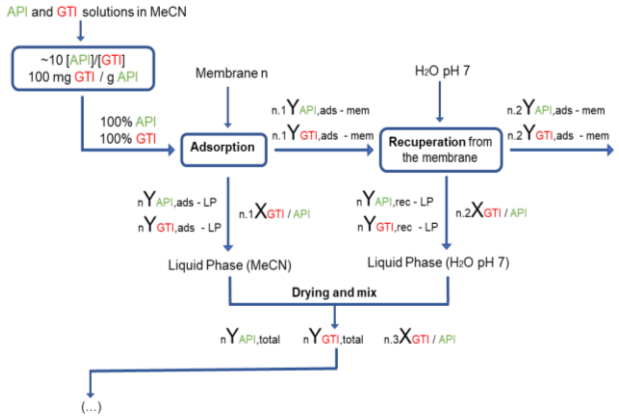
From **Figure 11 c)**, an API recuperation scenario will be viable. For Roxi, after binding (49%), approximately 42% was recovered from the feed solution, corresponding to about 7% of %Roxi lost. So, using H_2O at neutral pH for Roxi recovery as a part of API purification strategy would be feasible. Comparing with results from Ferreira, F. A., *et al.*, where all 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 is below 10%. Also, resorting to H_2O instead of organic solvent could be seen as a positive aspect from the environmental impact of the process. So, assuming API recuperation in H_2O at neutral pH viable, the results obtained for GTIs would allow to verify if a possible GTI back contamination (GTI leaching) would occur. Thus, a GTI elution step was performed. 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. So, 43% and 98.05% represents the quantity of DMAP and MPTS, respectively, still adsorbed after binding and GTI leaching steps in relation to their respective initial amount on feed solution. Therefore, using H_2O at pH 7 for a Roxi-DMAP case would not be acceptable since there would be a relevant DMAP back contamination. For Roxi-MPTS case, using this solvent as a step for API purification strategy would be viable as there is nearly no MPTS back contamination. For DMAP, comparing with the results from MeCN studies, using H_2O at pH 7 allowed to elute a more significative amount of GTI. So, DMAP would be more partitioned for liquid phase when this was H_2O at pH 7 than when it was MeCN due to possibility of DMAP establishing hydrogen bonds with H_2O molecules, contributing to its high solubility.[52] Comparing with the studies for H_2O at pH 1.2, GTI elution was more significative for acidic conditions due to possible protonation and, thus, potential electrostatic repulsion, as mentioned. For MPTS, comparing with results from MeCN and H_2O at pH 1.2 studies, there was almost no GTI eluted since the resulting salt of MPTS presented, probably, poor solubility in H_2O at pH 7 and practically all this salt had remained precipitated with the PBI.[27,59]

Now, it would be interesting if MPTS or its salt could be eluted. For membrane regeneration, a GTI elution step was performed by assessing MPTS desorption using H_2O at pH 13. For H_2O at pH 1.2, a good result was obtained when DMAP was the GTI, despite degradation of Roxi in acidic conditions constituting a disadvantage since, after regeneration, the residual Roxi still present on the membrane is possibly its degradation products. By reusing this membrane, there is a possibility of these products being eluted, as impurities, with the API in the recovery step. So, regeneration using H_2O at pH 13 was also performed for DMAP since its previous results for 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, using H_2O at pH 1.2 for regeneration should be considered. For DMAP (**Figure 11 d)**), comparing with results from MeCN and H_2O at pH 1.2 and 7, a %DMAP eluted (~36%) similar to the one for H_2O at pH 7 (named as %DMAP leaching) was obtained. At pH 13, there are excess OH^- species in solution, promoting PBI deprotonation. This could lead to competition since proton donor hydrogen bonding site of PBI is the one through which DMAP establishes hydrogen bonding and the one being involved in deprotonation. So, DMAP would be partitioned for both solid and liquid phase since there was no total solubilization of the adsorbed DMAP. For MPTS (**Figure 11 d)**), comparing with results from MeCN and H_2O at pH 1.2 and 7, it was obtained a significative value for %MPTS eluted (~84%), possibly due to solvent capability for solubilizing MPTS salt. According to Ferreira, F. A., *et al.*[27], it was possible to detect the GTI anion (*p*-toluenesulfonate) on a MeOH washing solution since this solvent could solubilize MPTS salt. Thus, for H_2O at pH 13, there are both Na^+ and OH^- species in solution, being Na^+ capable of stabilizing the anion of MPTS, leading to its solubilization.

Attending to all previous results, for Roxi-MPTS case, using H_2O at pH 7 would be viable as a step for API purification strategy as there is nearly no MPTS back contamination (0.85%). Beyond this, using H_2O at pH 13, a significative amount of MPTS could be eluted (~84%) and, hence, the membrane reusability could be possible. Thus, a study to assess this reusability was performed by submitting the membrane to a new binding step after having been regenerated, obtaining around 44% of MPTS that could still bind to the membrane. This could be due to the fact that after 1st binding, membrane was possibly 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 restoration of PBI is impaired by the nature of the reaction between MPTS and this adsorber.

4.5. API purification strategy

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



Scheme 1. API purification strategy for Roxi-MPTS case-study. **n**, which could be 1, 2 and 3, corresponds to the cycle number.

Table 12. Results found by applying API purification strategy outlined in Scheme 1.

	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

To comply with TTC, GTI limit of 0.005 mgGTI/gAPI needs to be achieved since maximum daily dosage for Roxi is 300 mg/day. Thus, from **Scheme 1**, strategy starts with a solution comprising Roxi at 800 ppm and MPTS at 80 ppm, being the ratio 100 mgGTI/gAPI. Firstly, an adsorption process is conducted by putting in contact the previous solution with PBI membrane (4.5 cm^2). Thus, it is obtained a liquid phase (LP) with a specific GTI/API ratio. The membrane, after adsorption, is placed in contact with fresh H_2O at pH 7 for API recovery and a LP with specific GTI/API ratio is obtained. Then, the 2 previous LPs need to be submitted to drying step to obtain resultant API powder. This powder needs later to be solubilized in MeCN, being this solution subjected to all preceding steps again. At the end of 3 cycles, a GTI/API ratio below 0.005 mgGTI/gAPI is achieved, being %Roxi lost around ~19% ($_{3.2}Y_{API,ads-mem}$).

For Roxi-DMAP case-study, a proper purification strategy would not be achieved since using H_2O at pH 7, a significative DMAP back contamination (37%) would be observed, and using H_2O at pH 1.2 for regeneration, the degradation of Roxi in acidic conditions constitutes a disadvantage. However, for comparison, API purification strategy was developed for this case-study, using H_2O at pH 7 for API recovery. So, the strategy follows same rational as the one presented on **Scheme 1** for Roxi-MPTS case-study. Since there is no step on purification process capable of either removing DMAP or recovering 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 TTC (1.5 µg/day), obtaining a value around 69% for %Roxi lost.

4.5.1. Scale-up simulation for API purification strategy

At laboratory scale, for both previous case-studies, after adsorption, 3 mL of MeCN were used. Moving to recuperation step, 1.5 mL of H_2O at pH 7 was used. After this, membrane was not recycled. So, for each cycle, 3 mL of MeCN, 1.5 mL of H_2O at pH 7, and 1 PBI membrane (4.5 cm^2, i.e., 23.6 mg) were used, while 1.2 mg was the amount of API inputted. Thus, a prediction for the quantity of material used in API purification strategy was performed by considering 1 Kg of API as reference.

For Roxi-MPTS case-study, at the end of 3 cycles, a value below GTI limit would be reached, despite having lost around 19% of Roxi. Thus, for 1 Kg of API, 59 Kg of membrane, 3750 L of H_2O and 7500 L of MeCN would be used. If, hypothetically, the maximum daily dosage was about 12 mg, this GTI limit would be of 0.125 mgGTI/gAPI (25 times higher). Thus, at the end of 2nd cycle, a value below this limit would be achieved. This would lead to a reduction of around 33% of each material used 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. However, maximum daily dosage would have to be 800 times lower (0.375 mg), leading to a GTI limit of 4 mgGTI/gAPI. For this hypothetical situation, %Roxi lost would be around 7%. Table 13 summarises results when considering these 3 different daily doses for Roxi-MPTS case.

Table 13. 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 is by far higher than the one determined for Roxi-MPTS case. In Roxi-DMAP case-study, only at the end of 16 cycles, a value below 0.005 mgGTI/gAPI would be reached. Thus, for 1 Kg of API, 315 Kg of membrane, 20000 L of H_2O and 40000 L of MeCN would be used. A situation involving a daily dose of 0.375 mg 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. In this hypothetical situation, %Roxi lost would be around 35%. For 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 limit at the expense of a loss of 55% for API. This would lead to the use of 217 Kg of membrane, 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. Table 14 summarises results obtained when considering these 3 different daily doses for Roxi-DMAP case.

Table 14. 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

Regarding the solvents from previous purification processes, it would be possible to reuse them since 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 due to the lower boiling point of MeCN (82 ºC) in relation to the one of H_2O (100 ºC) and, simultaneously, the obtention of dried API powder.

5. Conclusion

Attending to the main goal of this study - assess 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 - an efficient GTI removal without significative API loss. This is mainly due to non-differentiated selectivity that PBI membrane presented for APIs and GTIs. Thus, despite being developed a purification strategy, divided into 2 main moments (binding and post-binding), 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 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 future work. Looking at the purification strategy, especially to post-binding step, this would constitute an opportunity to increase the performance of API purification process through recuperation of the API still bound to the adsorber. 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 extremely low GTI limit of 0.005 mgGTI/gAPI that Roxi presents. Thus, none of the results obtained all the way through this work were illustrative of good outcome that would lead to consider the study of this purification process at industrial scale. Beyond this recuperation procedure, regeneration of 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, 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 GTIs were rapidly adsorbed while APIs presented a slow adsorption. Then, by adjusting the time of adsorption process, it could be possible to obtain a good result. However, in this work, all 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.

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