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Screening commercial available resins for simultaneous removal of two potential genotoxins from API methanolic streams

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ABSTRACT

This paper explores potential API loss mitigation during purification in recrystallization mother liquors, by including a resin adsorption step, to remove potential genotoxin impurities (PGTIs). Mometasone furoate (Meta) is used as model active pharmaceutical ingredient (API) in the presence of 4-dimethylaminopyridine (DMAP) and methyl *p*-toluenesulfonate (MPTS) as two model PGTIs. AG 50W-X2 and IRA68 resins efficiently removed DMAP and MPTS from methanol solutions, respectively, with adsorptions higher than 93% and Meta binding below 2%. Removal of GTIs using these resins sequentially, or combining them in a single step, was also assessed, with superior results for the later approach.

Abbreviations: API, active pharmaceutical ingredient; DMAP, 4-dimethylaminopyridine; DMAP-Me, methylated DMAP; GTI, potential genotoxin impurity; Meta, Mometasone furoate; MPTS, methyl *p*-toluenesulfonate; PTSA, *p*-toluenesulfonic acid; TTC, threshold of toxicological concern.

Introduction

The synthesis of active pharmaceutical ingredients (APIs) often requires the use of solvents and highly reactive molecules.[1,2] These species may have the potential to promote DNA aberrations, being globally referred as potential genotoxin impurities (PGTIs), representing a risk for patients' health.[3–5] To deal with this situation, regulatory entities have determined a threshold of toxicological concern (TTC) of 1.5 µg/day for GTIs, implying an acceptable limit for GTI content in APIs that consider the TTC value according to a maximum API daily dose (g/day).[6]

The best practices to decrease GTIs associated risk are to design API synthetic routes that eliminate their presence, with several existing routes for GTI purge in API synthetic processes clearly identified.[3] However, reaching acceptable GTI limits often requires additional purification stages including recrystallization, precipitation, solvent extraction, column chromatography, treatment with activated charcoal, the use of resins[7–10] or distillation[3] and, more recently, the use of molecularly imprinted polymers (MIPs)[11–15], DNA based polymers[16] and organic solvent nanofiltration (OSN) platforms.[17–20] The introduction of additional steps,

for removal of potential GTIs from API post reaction streams, is associated with API losses with high negative economic impact in API manufacture, in particular, when considering API generics production.

In this work, the removal of two PGTIs (4-dimethylaminopyridine and methyl *p*-toluenesulfonate), from Mometasone furoate (Meta) post reaction stream in dichloromethane (DCM), is used as a model study (Fig. 1). Meta, a glucocorticoid used in inflammatory diseases treatment[21], synthesis involves a sulfonylation reaction in DCM in the presence of a base. 4-Dimethylaminopyridine (DMAP) may be used as catalyst in this methodology[22] and has two genotoxic structural alerting functional groups: aromatic and alkyl amine.[23,24] Therefore, its control to TTC levels is suggested following ICH Guidelines.[4] Although primary and secondary aromatic amines are generally not inherently genotoxic, their metabolic activation *in vivo* generates electrophilic species, which are considered the proximate mutagen/carcinogen that binds to DNA.[25] On the other hand, alkyl and benzyl sulphate acids are widely used as counterions in API salt formation.[5] However, in the presence of alcohols, such as methanol (MeOH), they originate the corresponding
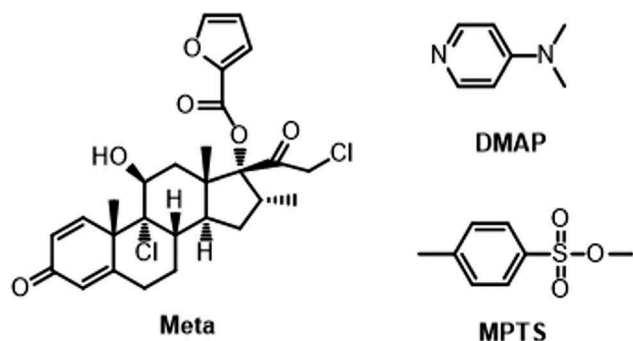
Figure 1. Structures of meta, DMAP and MPTS.

sulfonate esters[26], known to be DNA alkylating agents.[16] One of such cases is well documented in the literature for the API Viracept.[27]

A common strategy for API purification, including removal of GTIs from Meta, is recrystallization[28], with the advantage to isolate Meta as a solid ready to be put in storage or further processed into the final formulation. However, a large fraction of the API is often lost in recrystallization mother liquors.[29] This report starts by assessing the use of several readily available commercial resins for the removal of DMAP and MPTS from organic solvent solutions, discussing the effect of solvent matrix and pH on binding properties and establishing the kinetics and isotherms for the adsorption processes. The overall aim of the current paper is to discuss the possibility to reclaim the API lost in recrystallization mother liquor using inexpensive resins, which is not trivial considering the intricate relations between solute ionic states, resins and solvent matrix. Two routes are envisaged using such resins: (i) Direct API reclaiming approach: removal of GTIs from methanolic mother liquor to values at which GTI/API ratio in such solution complies with the TTC value; (ii) Recycle stream approach: to decrease GTI concentrations down to levels meeting the GTI/API ratio of the initial post-synthetic API crude stream, allowing recycling the API back into the next batch recrystallization cycle.

Materials and methods

Model compounds and solvents

4-Dimethylaminopyridine (DMAP), methyl *p*-toluenesulfonate (MPTS) and *p*-toluenesulfonic acid monohydrate (PTSA) were purchased from Acros (Belgium). Mometasone furoate (Meta) was kindly provided by Hovione PharmaScience Ltd (Portugal). Dichloromethane (DCM), methanol (MeOH) and acetonitrile (MeCN) HPLC grade were purchased from Fisher Chemicals (USA). Formic acid (FA) was purchased from Panreac

(Spain). All chemicals were of reagent grade or higher and were used as received.

Resins and adsorbents

Amberlite resins (CG400, IRA458, IRA68, IRC50, IRC86, XAD16 and XAD7) were purchased from Aldrich (Switzerland). Dowex resin AG 50W-X2 was purchased from BioRad (USA). Activated charcoal powder was purchase from Merck (Germany). The chemical nature of several resins used in this study is described in Table 1 and provides a variety of ionic resins with acidic or basic groups, as well as non-ionic resins with useful chemical functionalities to interact with DMAP and MPTS.

Purification processes using adsorbing or ionic exchange resins are well developed at the industrial scale and can be easily implemented for Meta purification that is lost in recrystallization mother liquor. However, the solvent in question is MeOH and most of the commercially available resins are designed to perform in aqueous solutions. Therefore, assessing DMAP and MPTS removal using different resins from MeOH is not without challenge. The strategy followed started by assessing removal of DMAP from water, then from a water:MeOH (1:1) mixture, to assess organic solvent versus water interference in binding process. Afterwards, only for the resins yielding higher DMAP binding in 1:1 mixture, its removal from pure MeOH was tested. Activated charcoal was also considered in these studies for performance comparison.

HPLC analyses

Measurements were performed on a Merck Hitachi pump coupled to a L-2400 tunable UV detector using an analytic Macherey-Nagel C18 reversed-phase column Nucleosil 100–10, 250 × 4.6 mm with eluents, A: 0.1% FA aqueous solution, B: 0.1% FA, MeCN solution. For Meta and DMAP a flow rate of 1 mL·min^{-1} was used with UV detection at 280 nm; method: 0–3 min, 60% to 20% A; 3–4 min, 20% A; 4–8 min, 20% to 60% A; 8–15 min 60% A. For MPTS a flow rate of 2 mL·min^{-1} was used with UV detection at 230 nm; method: 0–15 min, 70% A-30% B. For

Table 1. Chemical nature of the resins used in this study.

Resin	Functional group	Characteristic
AG 50W-X2	Sulfonic acid	Strong acid cation exchange
IRC50	Carboxylic acid	Weak acid cation exchange
IRC86	Carboxylic acid	Weak acid cation exchange
XAD16	Hydrophobic polyaromatic	Adsorption
XAD7	Acrylic ester	Adsorption
CG400	Quaternary amine	Strong base anion exchange
IRA458	Quaternary amine	Strong base anion exchange
IRA68	Tertiary amine	Weak base anion exchange

PTSA a flow rate of 1.5 mL·min^{-1} was used with UV detection at 230 nm; method: 0–10 min, 90% A-10% B.

Recrystallization process

To simulate a post reaction stream, a solution containing 1 g/L DMAP, 1 g/L MPTS and 10 g/L Meta was prepared in DCM, referred as crude solution. **Solvent exchange step protocol**: The initial DCM solution (50 mL) was concentrated to 10% of its initial crude volume (10%V_0, 5 mL) in a rotary evaporator (Buchi, Switzerland). Followed by addition of fresh MeOH (20% V_0, 10 mL), the solution was heated at about 50ºC until the volume was reduced (10%V_0, 5 mL) in the rotary evaporator. In this process crystals started to appear. This procedure was repeated twice. **Recrystallization step protocol**: The slurry obtained was allowed to cool to 20ºC for about 1 h, then to 10ºC for over 1 h, with a Haake D1 immersion circulator water bath, with stirring at 220 rpm and finally, left at 10ºC for 2 additional h. At this stage, Meta was filtered with a qualitative filter paper (Filter-Lab, Spain) with 2–4 μm pores and washed twice with 2 mL of cold MeOH (10ºC). The crystals were collected and dried in an oven for 24 h at 70ºC. The recrystallization mother liquor (about 10 mL) was analysed for DMAP, MPTS and Meta quantification.

Resin assessment for solute adsorption

Adsorption of DMAP and MPTS, unless otherwise stated, was assayed at a concentration of 1 g/L in 4 mL solution of water, MeOH or 1:1 of water:MeOH mixtures using 20 mg of resin and left at 220 rpm on a stirring plate (IKA, Germany) for 24 h at room temperature, after which the supernatant was filtered and analysed by HPLC for solute quantification and binding percentage determination. Experimental triplicates and controls without resin addition where carried out. pH was adjusted using 1M aqueous solutions of HCl and NaOH and measured using a 702 MS Titrino (Metrohm, Switzerland). The three different temperatures, 25ºC, 35ºC and 45ºC assessed, were controlled in an incubation chamber (J. P. Selecta, Spain). Adsorption isotherm studies where established by, at a fixed temperature and pH: (i) different amounts of resin (10, 20, 40, 80, 100, 200 or 400 mg) added to 4 mL solutions with an initial GTI concentration of 1 g/L; (ii) or 20 mg of resin added to 4 mL of GTI solutions with concentrations of 0.1, 0.25, 0.5, 0.75 or 1 g/L. For kinetic studies, identical solutions were prepared and the supernatant collected and filtered at 2, 4, 6, 10, 15, 30, 60, 120, 240, 480 and 1440 min.

The amount of GTI bound to the resins is calculated as follows:

$$q_{eq} = \frac{V\left[C_0 - C_{eq}\right]}{M} \quad (1)$$

where q_{eq} (mg/g) is the amount of GTI bound to the resin, C_0 (mg/L) is the initial GTI (DMAP or MPTS) concentration, C_{eq} (mg/L) is the equilibrium concentration of GTIs in solution, V (L) is the volume of solution used and M (g) is the resin mass. The assays were carried out in duplicates.

The adsorption models considered were as follows:

Langmuir: $\frac{q_{eq}}{q_m} = \frac{K_L\,C_{eq.}}{1 + K_L\,C_{eq.}}$

Freundlich: $q_{eq} = K_F\,C_{eq}^{\frac{1}{n}}$

where q_m (mg/g) is the maximum amount of GTI bound to the resin in a monolayer for the Langmuir model, whereas K_L and K_F are equilibrium constants (L/mg) for the Langmuir and Freundlich models, respectively, and are related with the energy taken for adsorption. n is a parameter related with the surface layer heterogeneity.[30–32]

Experimental data obtained from kinetic experiments were fitted to pseudo-first-order and pseudo-second-order kinetic models:[33]

$$\ln\left(q_{eq} - q_t\right) = \ln\left(q_{eq}\right) - k_1.t \quad (2)$$

$$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_{eq}^2} + \frac{t}{q_{eq}} \quad (3)$$

where q_{eq} and q_t (mg/g) are adsorption capacities at equilibrium and time t (min), respectively. k_1 (min^{-1}) and k_2 (g/(mg·min)) are pseudo-first-order and second-order rate constants for the models.

The same batch binding experiments described above, were performed for 4 mL of MeOH solutions with 10 g/L of Meta and 1 g/L of the GTI (DMAP or MPTS) with AG 50W-X2 or IRA68 resins. After 24 h in contact with 25 mg of each resin at 200 rpm and at room temperature, the mixtures were filtered and analysed by HPLC for solute quantification and binding percentage determination. Experimental triplicates and controls without resin addition where carried out.

Mother liquor purification

Adsorption experiments for the mother liquor solution in MeOH, with 6 g/L Meta, 5 g/L DMAP and 5 g/L MPTS, were assayed with AG 50W-X2 and IRA68 resins using 25 mg of resin for 1 mL of solution left

stirring at 220 rpm for 24 h at room temperature, for each adsorption stage. In the combined strategy, 25 mg of each resin (50 mg in total) were loaded on 1 mL of solution. After incubation time, the supernatant was filtered and analysed by HPLC for solute quantification and binding percentage determination. The assays were carried out in duplicates.

Results and discussion

Different types of studies are presented and discussed. The first sections report experimental studies for individual operations, recrystallization and screening of different resins for GTI adsorption. Then, selected resins following specific adsorption strategies are experimentally assessed, concerning GTIs and API removal from recrystallization synthetic mother liquors. Finally, the impact of the two suggested process approaches (direct API reclaiming or recycling stream) are theoretically assessed for different cases and a brief economic assessment is presented.

Recrystallization

The limit of GTI content allowed in an API formulation is determined by the TTC value and the API maximum daily dose (g/day).[6] For example, for a maximum daily dosage of 500 μg/day of Meta, the 1.5 μg/day TTC value corresponds to a GTI limit of 3 mgGTI/gAPI. In such case, considering a final Meta crude solution comprised by 10 g/L Meta, 1 g/L DMAP and 1 g/L MPTS, for an ideal purification system, where no Meta is lost, 97% of GTIs would need to be removed to comply with the TTC. Meta is a corticoid, which daily dosage administered varies with inflammatory conditions targeted: typical maximum Meta dosages of 200 μg/day or 2 mg/day are established for airways (e.g. allergic rhinitis and asthma) or skin (e.g. eczema and psoriasis) administration, corresponding to GTI limits of 7.5 and 0.75 mgGTI/gAPI, respectively. Considering case scenarios for GTI/API target limits, the previously mentioned Meta crude composition and no Meta losses during purification, GTI removals of 92.5% and 99.2% for airways or skin administration would be required. However, as a fraction of API is lost during purification steps, higher removal efficiencies are needed to reach the target GTI/API ratio.

For Meta, recrystallization is the purification process usually performed for removal of GTIs from the post reactional stream. DCM is the solvent usually used in Meta synthesis and a disclosed purification process comprises two recrystallizations from MeOH, an intermediate activated charcoal adsorption step from DCM

and the required solvent exchange steps.[28] This process was assessed in a previous study[29] in which, the overall purification allowed to decrease a GTI to API mass ratio from 200 mgGTI/gAPI (100 mgGTI/gAPI for each GTI) present in the initial solution, to a value of 3.1 mgGTI/gAPI in solid API, representing a total GTI removal of 98.7%, which fulfils the TTC for a case study of a maximum dosage of 200 μg/day for airways administration. The largest fraction of API loss was observed in the first recrystallization, accounting for about half of total API lost over the 3 steps, whereas GTI removal was not preferentially assigned to any stage. Nevertheless, DMAP removal tends to occur in the first recrystallization and activated charcoal adsorption steps, while the sulfonate ester (methyl methanesulfonate) removal was driven by recrystallization, with a higher efficiency in the second one, when DMAP was present at lower concentrations.

The specific allocation of API losses and GTI removals during a recrystallization process vary widely, mainly according to the scale used that impacts in losses through the washing and filtration operations. In this study, 10 g/L API and 1 g/L of each GTI in DCM were used, following the previous study with Meta[29], but focusing only in the first recrystallization step, which accounts for about half of the total API lost in the entire purification process. In this report, the recrystallization was performed at a scale 10 times lower than previously. Still, the results obtained are consistent concerning API yield [(91.1 ± 0.4)% vs. (91.4 ± 0.5)%[29]], API concentration in the mother liquor [(5.3 ± 0.7) g/L vs (4.8 ± 0.3) g/L[29]] as well as sulfonate ester removal [(26.2 ± 8.5)% for MPTS vs 36.8%[29] for methyl methanesulfonate] achieved in the first recrystallization. However, DMAP removal was higher in the current study than in the previous one [(81.9 ± 0.9)% vs 53.9%[29]] implying higher concentrations for DMAP than for MPTS in the mother liquor, (5.1 ± 0.7) g/l and (1.4 ± 0.4) g/L, respectively. An additional recrystallization of Meta from a Meta/MPTS solution (in the absence of DMAP), was performed, simulating the second recrystallization that targets sulfonate esters removal, after elimination of a larger DMAP fraction. In this recrystallization, API loss and concentration in the mother liquor were similar to values previously observed, but MPTS removal increased to 95.7%, corresponding to a 4.8 g/L MPTS concentration in the mother liquor.

The values described above allowed us to establish reference concentrations of API and GTIs in MeOH that should be loaded to a resin purification step, corresponding to about 6 g/L of API and 5 g/L for each GTI, i.e. a ratio of 1666.7 mgGTIs/gAPI (833.3 mgGTI/gAPI for

each GTI) that is about 8 times higher than the initial post-synthetic API crude stream (200 mgGTI/gAPI, considering both GTIs).

Screening scavengers for DMAP adsorption

The results obtained in water (Fig. 2a) showed that resins with acidic groups (AG 50W-X2, IRC50, IRC86), and also the activated charcoal were efficient for DMAP adsorption with removals above 80% for 1 g/L solutions. Intermediate DMAP bindings were obtained using non-ionic resins (XAD7 and XAD16), and a low performance was observed for amine based resins assessed. As MeOH was included in the solvent matrix, DMAP removal by non-ionic and amine based resins became negligible and activated charcoal ability also decreased, probably by possible MeOH adsorption, being a competitive factor in DMAP adsorption. The three resins with acidic groups (AG 50W-X2, IRC50, IRC86), showed a decrease in their performance in MeOH (Fig. 2a), but still reached acceptable values higher than 80% for AG 50W-X2 and about 60% for the IRC resins. This decrease in performance can possibly be attributed to resin swelling and competition of the solvent. These results prove that the use of this technology with such organic solvent is challenging.

DMAP binding is lower for the resins in all solvent matrices at pH 6–8 (Figure S1 in Supplementary Material), i.e. when DMAP is on its conjugated protonated acid form. However, the resins performance was improved for pH values around 10 (Fig. 2b), whereas for a value close to 12 the possible formation of competing ionic species (sodium ions towards sulfonic and carboxyl groups) can take place and the adsorption was low in all cases. The difference in response for the acidic groups (i.e. cationic resins) resins compared to

non-ionic or amine based (i.e. anionic) resins, suggested that the ionic interaction between DMAP and resin sulfonate and carboxyl groups is maintained in MeOH, whereas the solvent competes with the solute by non-ionic interactions with the adsorbent.

DMAP is a Brønsted base with a pKa of 9.7 with different inductions of charge distribution in the molecule according to solution pH. HCl or NaOH 1M solutions were used to adjust pH to values lower or higher than pKa, respectively. The activity of MeOH affects equilibrium constants of resins and solutes (i.e. pKa), as well as pH electrode readings scale. Moreover, the addition of HCl and NaOH may influence both DMAP and ionic exchange resins ionic states.[34] The ionic exchange resins with acidic groups are cationic resins, which are supplied in hydrogen form, i.e. protonated, and they are usually converted to their anionic form through a preconditioning step using NaOH for resin deprotonation and conjugation with sodium ions. These results are not of trivial reasoning, but it should be noted that, in this work, these cationic resins were used on the hydrogen form, which could sustain an explanation based on the action of DMAP as a base, and electrostatic interactions between the resins and DMAP, mediated by hydrogen bonding.

Considering the results previously discussed, a pH value around 10 was chosen to study the effect of temperature, adsorption kinetics, and establish binding isotherms with AG 50W-X2 resin in MeOH. Solvent effect in adsorption kinetics showed to be significant. The adsorption process was very fast in water and in water:MeOH mixture, with approximately 1–5 min being necessary for the resin to reach adsorption equilibrium (Figure S2 in Supplementary Material). On the other hand, in MeOH, the lower DMAP adsorption comprehends a slower adsorption process, and in this case about 15 minutes are needed for the system to
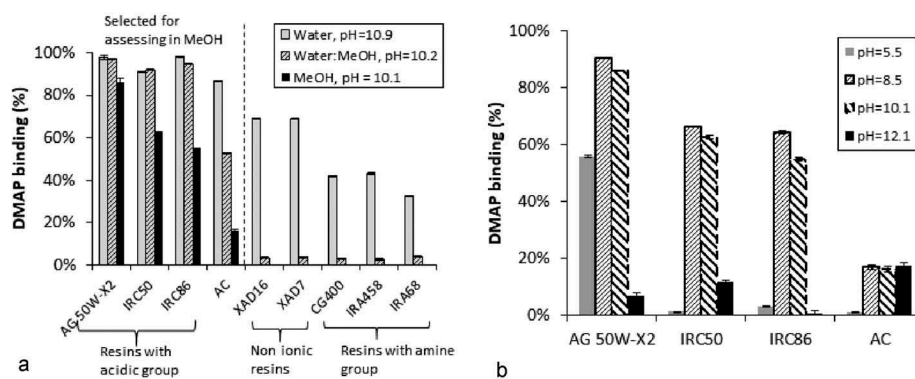

Figure 2. DMAP binding for different resins tested: (a) without pH adjustment for different solvent matrices. (b) Influence of pH on DMAP adsorption in MeOH. AC – activated charcoal.

reach the same equilibrium (Fig. 3). Furthermore, in MeOH, the temperature proved to have no effect in DMAP adsorption in the range (25–45) °C (Fig. 3).

The isotherm binding model behaviour for each scavenger in MeOH at 25°C contributes to provide additional information on equilibrium between DMAP and the scavenger, with the results presented in Fig. 4. The physical parameters determined for the theoretical models are included in Supporting Information (Table S1). AG 50W-X2 and IRC 50 resins follow the Freundlich isotherm model that assumes that the amount of DMAP adsorbed tends to infinity and that multylayers of adsorbed GTI molecules are formed. IRC 86 resin follows the Langmuir isotherm model suggesting the formation of a monolayer in a homogeneous surface.

From the studies for DMAP, and based on the results presented in Fig. 2a, AG 50W-X2 and IRC50 resins were selected to be assessed towards API binding in following sections, since these were the resins with higher DMAP adsorption in MeOH.

Screening scavengers for MPTS adsorption

MPTS removal from a MeOH solution was assessed at acidic and alkaline pH, following the same approach described in the previous section for DMAP (Fig. 5a). As expected, for the acidic resins, the absence of nucleophilic sites for sulfonate interaction, prevented any affinity towards the resins and almost no adsorption was observed. The activated charcoal also showed a low performance, with only 22% of binding, not affected by pH value. MPTS adsorption on IRA458 resin is pH dependent, being favoured at lower pH values. However, from the several scavengers assessed, the IRA68 resin, the only tertiary amine, showed a higher performance, regardless solution pH. The nucleophilic amine groups of this resin are prone to interact with the electrophilic MPTS groups with improved binding performance. Therefore, this resin ability for MPTS adsorption was further characterized concerning temperature effect, kinetics and equilibria isotherm models. Figure 5c shows a slow kinetic with only 67% and 90% of maximum resin capacity for MPTS reached after 4 and 8 h, respectively, which implies longer operation times. Moreover, MPTS removal over time is better described by a pseudo-first-order kinetic model ($r^2 = 0.993$) and the equilibrium isotherm is better described by the Langmuir adsorption model (Fig. 5d). Both kinetic and isotherm equilibrium data were obtained at 25°C, but the results
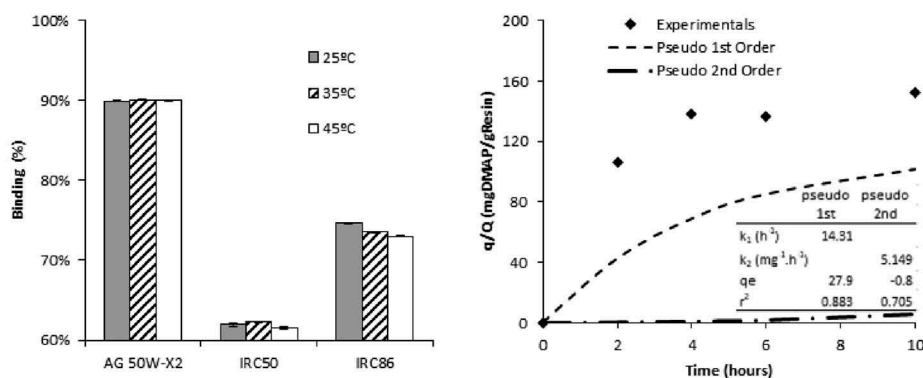

Figure 3. Left: Temperature influence in DMAP adsorption from MeOH. Right: DMAP binding capacity in AG 50W-X2 resin for a 1 g/L solution in MeOH along time at 25 °C.
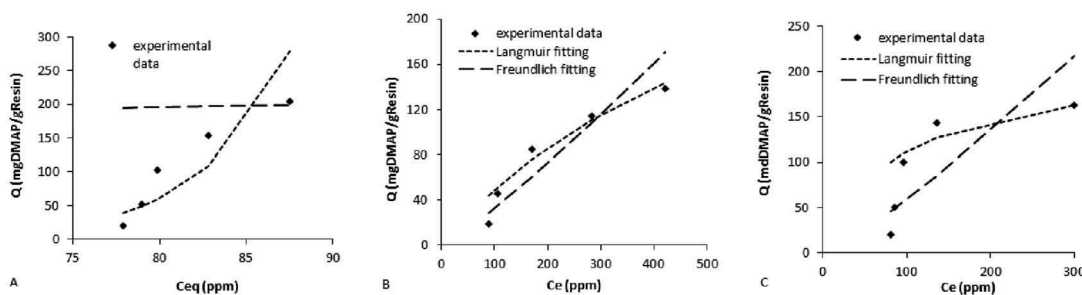

Figure 4. Adsorption isotherm models for DMAP in MeOH for several resins at 25 °C: (a) AG 50W-X2; (b) IRC50; and (c) IRC86. A good correlation of the Langmuir fitting with AG 50W-X2 resin could not be determined.
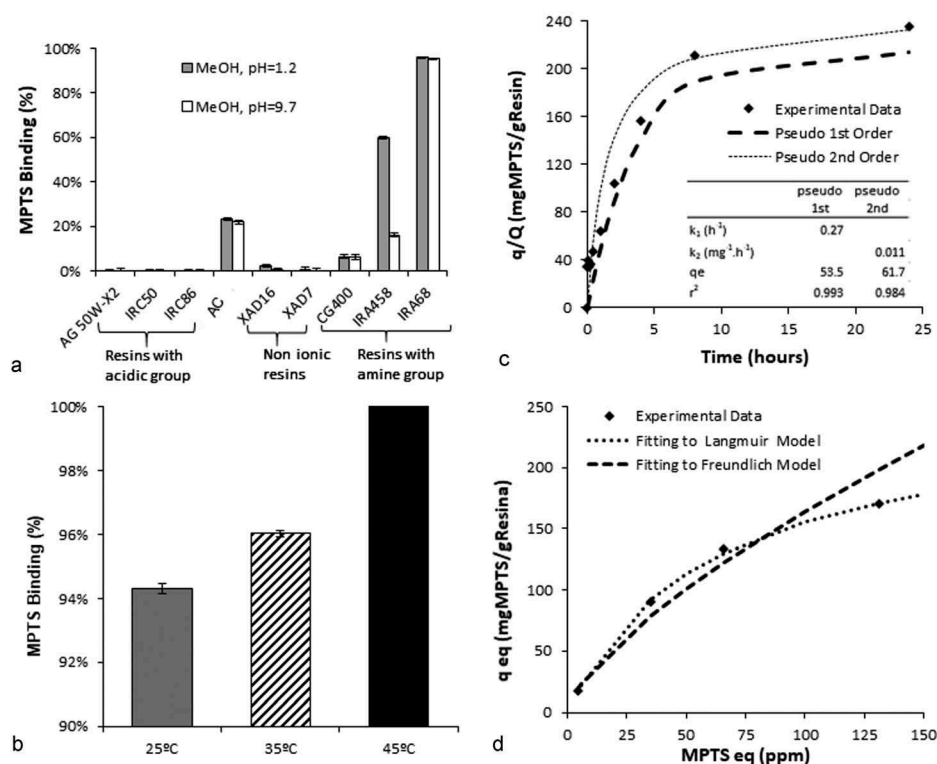
Figure 5. (a) MPTS equilibrium binding percentage for several scavengers from a 1 g/L solution in MeOH at different pH values at 25°C; (b) MPTS equilibrium binding percentage to IRA68 resin for a 1 g/L solution in MeOH at different temperatures; (c) MPTS binding capacity in IRA68 resin for a 1 g/L solution in MeOH along time; fitting trends to pseudo-first-order and second-order kinetic models and respective parameters; (d) MPTS equilibria isotherm in IRA68 resin and fitting trends to Langmuir and Freundlich models and respective parameters at 25°C. AC – Activated charcoal.

of assays at different temperatures (Fig. 5b) show that MPTS removal can be improved by increasing temperature.

Screening scavengers for low meta adsorption

From the studies in MeOH, the resins selected for potential use in removal of GTIs from recrystallization mother liquor were either AG 50W-X2 or IRC50 for DMAP, and IRA68 for MPTS. To establish at which extent Meta is adsorbed on these resins, a test solution with 10 g/L API and 1 g/L GTI was subjected to these resins: AG 50W-X2 and IRC50 resins were assessed with DMAP while IRA68 was assessed with MPTS. The results presented in Fig. 6 show that AG 50W-X2 resin was able to remove about 93% of DMAP with only about 2% of Meta loss reaching a final ratio of 7.14 mgDMAP/gMeta. For IRC50 resin, DMAP removal was lower (about 63%), without Meta loss, reaching a final ratio of 37 mgDMAP/gMeta. From these results, the AG 50W-X2 resin is the scavenger providing a lower mgDMAP/gMeta ratio, which may enable recycling the API lost in recrystallization mother liquor into the recrystallization process.
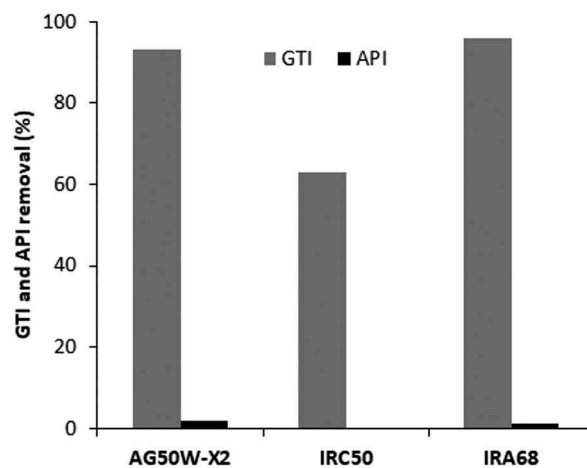

Figure 6. Amount of API and GTI removed in MeOH for Meta and DMAP mixture with AG 50W-X2 and IRC50 resins, and for meta and MPTS mixture with IRA68 resin.

As shown in Fig. 6, IRA68 resin was able to remove about 96% of MPTS with only about 1% of Meta loss reaching a final ratio of 4.04 mgMPTS/gMeta. This value is of the same order of magnitude to the one obtained for DMAP with the AG 50W-X2 resin

(7.14 mgDMAP/Meta) and accordingly, IRA68 resin may allow to recycle the API lost in recrystallization mother liquor.

Mother liquor purification using resins: assessment of different adsorption strategies

AG 50W-X2 and IRA68 resins were selected for the removal of DMAP and MPTS from Meta recrystallization mother liquor and evaluate possible API recovery. A synthetic mother liquor solution was prepared in MeOH with API and GTI concentrations based on recrystallization assays: 6 g/L for Meta and 5 g/L for each GTI (DMAP and MPTS). As a single resin able to remove both GTIs efficiently was not identified, different adsorption strategies were assessed. In strategies 1 and 2, two sequential adsorption steps were considered, i.e. after the mother liquor is treated in a first adsorption step (using IRA68 or AG 50W-X2, for strategy 1 or 2 respectively), the treated solution is then submitted to further treatment with the second resin (AG 50W-X2 or IRA68, for strategy 1 or 2, respectively). In strategy 3, the mother liquor is treated with both resins simultaneously in one single step.

Table 2 shows the results obtained, including the final ratios of GTI/API achieved. The presence of both GTIs (DMAP and MPTS) in solution, has effect in API adsorption, being as high as 13% (Table 2) compared with only (1–2)% for the same resins when only one of the GTIs and Meta were present in the solution (Fig. 6). Furthermore, in the case of two sequential adsorption steps, API loss is slightly higher (11–13%) than when GTI removal is carried out in one single step (9.6%), however the difference is not statistically significant (p > 0.10). With two sequential steps, the final ratios vary between (74.7–102.4) mgGTI/gMeta. These values are lower than the initial 200 mgGTI/gMeta present in Meta crude solution, allowing to use these strategies to recycle this API from the mother liquor to the next batch recrystallization.

The highest GTI removal and lowest API loss values were obtained for the one single adsorption step strategy 3, showing a synergistic effect of using both resins together. The final ratio of 1.2 mgGTI/gMeta, reached when using strategy 3, is considerably lower than the initial 200 mgGTI/gMeta present in the crude Meta solution. This promising pathway allows to recover 90% (5.4 g/L) of Meta present in the mother liquor. Note that, in the previous study, assessing Meta purification by recrystallization, from a total API loss of 15.6%, only 3.3% were lost through adsorption to activated charcoal, while the remaining 12.4% were lost through recrystallization mother liquor.[29]

MPTS resin adsorption studies confirmed the electrophilic interaction of sulfonate groups from MPTS with nucleophilic amine groups of IRA68 resin in MeOH, and that this reaction is favoured with temperature. During Meta recrystallization, temperature is used to promote solvent exchange from DCM to MeOH. Considering DMAP and MPTS in solution, this same interaction can take place between both GTIs. In fact, after the recrystallization, the appearance of two secondary products could be observed in HPLC spectra of the mother liquor. One of the products was identified as p-toluenesulfonic acid (PTSA) in its ionic form, by co-elution of the mother liquor test solution and PTSA commercially available. By ^{1}H NMR studies (Figures S4–S6 in Supplementary Material), it was postulated that the other species corresponded to methylated DMAP (DMAP-Me), which is formed based on the reaction depicted in Fig. 7. Accordingly, the mechanism previously proposed for binding between MPTS and amine-based nucleophilic scavengers involves the methylation of the resin amine group and formation of PTSA.[10]

The results suggest that recrystallization of the model system used in this study yields a mother liquor with the API and, instead of two, four species that should be removed to acceptable levels, which is an interesting challenge. The three strategies presented in Table 2 were explored to answer this new and interesting challenge (Table 3). In each situation, the species

Table 2. Strategies for ML purification with AG 50W-X2 and IRA68 resins in MeOH.

Strategy	GTI removal (%)			Meta loss (%)	[GTI] after resin		mgGTI/gMeta#
	DMAP	MPTS	Total		DMAP (ppm)	MPTS (ppm)	
1 (IRA/AG)	85.2 ± 0.2	> 99.8	92.5 ± 0.2	11.6 ± 1.9	636.8 ± 8.3	< 5.0	102.4*
2 (AG/IRA)	89.9 ± 0.1	> 99.8	94.9 ± 0.1	13.1 ± 1.2	455.6 ± 7.1	< 5.0	74.7**
3 (AG+IRA)	99.9 ± 0.1	> 99.8	99.8 ± 0.1	9.6 ± 2.7	2.5 ± 1.1	< 5.0	1.2

Note: #The mgGTI/gMeta ratio considers the sum of the 2 GTI species and Meta detected in solution after adsorption resin step(s); *101.6 mgDMAP/gAPI and < 0.8 mgMPTS/gAPI; **73.9 mgDMAP/gAPI and < 0.8 mgMPTS/gAPI.
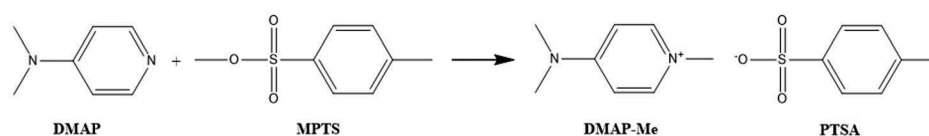
Figure 7. Proposed formation of DMAP-Me and PTSA in recrystallization mother liquor.

Table 3. Strategies for ML purification with AG 50W-X2 and IRA68 resins in MeOH in the presence of secondary species.

	GTI Removal (%)				[GTI] after resin		
Strategy	DMAP DMAP-Me	MPTS PTSA	Total	Meta loss (%)	MPTS (ppm)	PTSA (ppm)	mgGTI/gMeta[#]
1A (IRA/AG)	87.0 ± 0.7	55.4 ± 1.1	71.2 ± 1.3	28.3 ± 0.8	< 5.0	2232.32	670.0*
2A (AG/IRA)	92.5 ± 0.1	87.5 ± 1.3	90.0 ± 1.3	33.2 ± 0.3	< 5.0	626.26	249.9**
3A (AG+IRA)	99.8 ± 0.3	100	99.9 ± 0.3	19.0 ± 3.8	< 5.0	< 2.5	2.1

Note: [#]The mgGTI/gMeta ratio considers the sum of all the 4 GTI species and Meta detected in solution after adsorption resin step(s); *151.1 mgGTI/gAPI for DMAP/DMAP-Me and 518.9 mgGTI/gAPI for MPTS/PTSA; **93.6 mgGTI/gAPI for DMAP/DMAP-Me and 156.3 mgGTI/gAPI for MPTS/PTSA.

DMAP, DMAP-Me, MPTS and PTSA were considered as impurities and a stringent case of a ML with 6 g/L Meta, 5 g/L (DMAP+DMAP-Me) and 5 g/L (MPTS +PTSA) was explored.

The results presented in Table 3 confirm that, the simultaneous presence of the several GTIs in solution, leads to a higher API adsorption (19–33%). The lowest API losses (19%) are observed for strategy 3, when GTIs removal is carried out in one single step using both resins together. For strategy 1 and 2, when two sequential adsorption steps are used, the final ratios vary between 249.9–670.0 mgGTI/gMeta. These values, are higher than the 200 mgGTI/gMeta ratio of the post-reaction stream initially fed to the recrystallization, making challenging to explore these adsorption strategies to recycle the mother liquor back to the process.

A synergistic effect was again observed in strategy 3A (Table 3), with the highest GTI removal, when both resins are used together, reaching a final ratio of 2.1 mgGTI/gMeta and 81% (4.9 g/L) of Meta recovery from the recrystallization mother liquor. Therefore, strategy 3 is a promising pathway for API reclaiming from the mother liquor, allowing significant removal of GTIs and secondary species.

API reclaiming: assessment of different process approaches

This section presents theoretical calculations for generic GTI removal and API reclaiming considering, not only the results obtained, but several possible cases, following the two approaches illustrated in Fig. 8. Therefore, calculations include: (i) Direct API reclaiming from the mother liquor, by using the resins to remove GTIs to

ultra-low concentrations; or (ii) Recycle API rich stream obtained after resin treatment of the mother liquor to the recrystallization feed, where the resin separation reduces the GTI/API ratio from high values of the mother liquor to lower values present in the initial post-synthetic API crude stream. The higher the API loss, the higher the impact on the introduction of the resin reclaiming step. Therefore, the examples considered 25%, 20%, 15% and 10% API loss in the original recrystallization/activated charcoal adsorption (i.e. losses for the recrystallization mother liquor of 21.7%, 16.7%, 11.7% and 6.7%, considering a 3.3% API loss through adsorption to activated charcoal which, by using the resin API reclaiming approach, can be decreased to 7.6%, 6.6%, 5.6% and 4.6%). These cases are illustrated in Fig. 9a. Considering a 20% API binding to the resin and a stringent case where the methanolic mother liquor contains 6 g/L API and 10 g/L GTI (5 g/L of each GTI), a GTI removal higher than 99.6% (Table 3, strategy 3A) would ensure a value lower than 7.5 mgGTI/gMeta for the stream directly processed through the resins (e.g. for 99.9% GTI removal observed in strategy 3A of Table 3, a 2.1 mgGTI/gAPI is attained). This result complies with the TTC of 7.5 mgGTI/gAPI for the case study of maximum dosages of 200 μg/day for airways delivery.

For removal of potential GTIs from other API post reaction streams, the use of resins may not reach so high GTI removals and thus, the stream processed by the resins may not comply with TTC values. For example, considering the same 6 g/L API to 10 g/L GTI (5 g/L of each GTI), i.e. 1667 mgGTI/gAPI, GTI removals (about 71% or 90%) and API losses (28% or 33%), did result on GTI/API ratios of 670 or 250 mgGTI/API for strategies 1A and 2A (Table 3), respectively. These values are
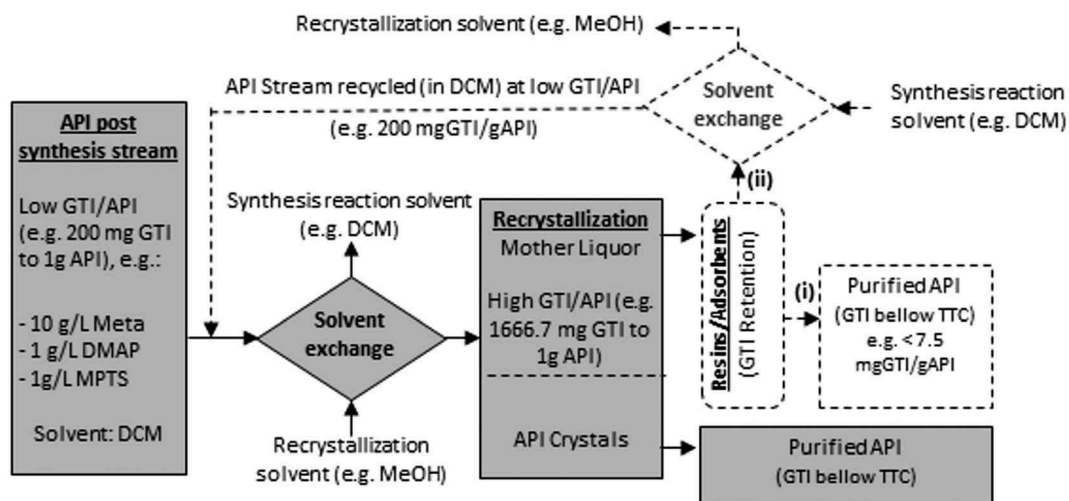
Figure 8. Schematic illustration of the role of a resin based step, in two different approaches, to (i) remove GTI from recrystallization mother liquor to an ultra-low level, allowing direct reclaim of API from mother liquor; or (ii) remove GTI from recrystallization mother liquor to an intermediate low level, decreasing the GTI/API ratio allowing mother liquor recycling to initial post-synthetic API crude stream fed into recrystallization. Grey filled boxes and solid black lines represent recrystallization process alone; black dotted lines and empty boxes represent steps for mother liquor subjected to resin treatment for API loss mitigation.

considerably higher than 7.5 mgGTI/gAPI. For such cases, the resin step can be used to decrease the high GTI to API ratio observed in mother liquor to the initial post-synthetic API crude stream level, allowing recycle back the mother liquor stream in the next batch recrystallization (after solvent exchange from MeOH to DCM). The 250 mgGTI/gAPI ratio obtained in strategy 2A (Table 3) is already quite close to the GTI/API ratio of the initial post-synthetic API crude stream fed into the recrystallization process at a value of 200 mgGTI/gAPI. Hypothetical cases able to meet such 200 mgGTI/gAPI ratio include resins performances for API bindings of 33%, 20% or 15% and GTI removals of 92%, 90.5% or 90%, respectively.

The calculation of the transition profile, in increase of API isolated in the crystals, as API in the mother liquor is recycled into the next batch recrystallization/activated charcoal cycle, is illustrated in Fig. 8b, showing the effect of three levels of API binding to resins for a recrystallization/activated charcoal process with an initial API loss of 15%. Calculations taken for Fig. 9b consider that the overall API fed to each new successive recrystallization is increasing over time since, the API in resin treated mother liquor of the previous recrystallization is added to the constant value of "fresh API" stream. Therefore, increase in percentage of "API in the crystals/fresh API fed" during the transition profile is not driven by an increase in recrystallization yield, but higher amount of API fed into each recrystallization, until convergence of this value, when resin treated mother liquor is recycled.

Economic assessment

The approaches suggested in this work to mitigate API losses are (i) to recover the API directly from the mother liquor using a resin step or, (ii) to recycle the API from recrystallization mother liquor, after the resin adsorption step that re-establishes the GTI to API mass ratio found in Meta crude solution, into a next API purification by batch recrystallization. The economic impact involved in these proposed API recovery strategies is here briefly considered. For example, in the central case scenario, in which 15% of API is lost to the mother liquor at a concentration of 6 g/L, the possibility to recover 80% of such API represents a gain in API yield of 12% (from 85% to 97%). The cost of introducing an additional resin adsorption step is preliminary assessed.

The major costs in adsorption processes is often related with infrastructure (Fig. 10), especially in the case of two sequential resin steps (70%) (Fig. 10, right). This cost analysis follows a previous work[29] considering batch operations of 1 m^3 featuring 10 kg of API, and an annual production of 10 batches. The current analysis considers only incremental capital and operation cost required to introduce the resin operation unit at total values of 66 k€ and 118 k€ per year, corresponding to 6.6 k€ and 11.8 k€ per batch, for direct API reclaiming from mother liquor or API recirculation, respectively.

For the central scenario, the capital cost was calculated assuming the use of non-depreciated equipment,
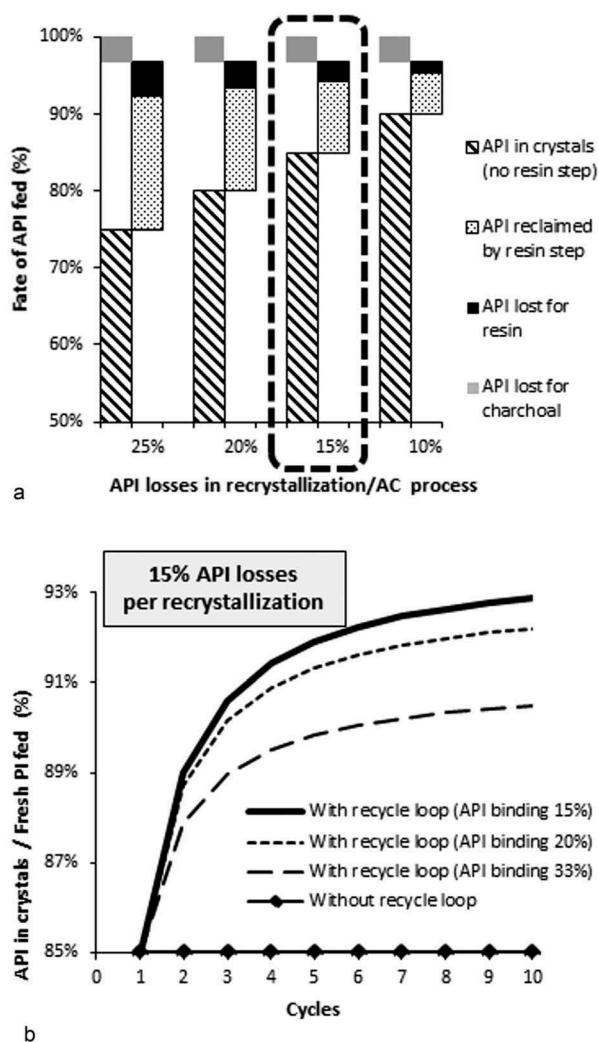
Figure 9. Potential to improve API yields in recrystallization/ activated charcoal process by API reclaiming directly from the mother liquor or recycling feedback loop of mother liquor after resin treatment: (a) calculations for potential direct API reclaim as function of API losses in recrystallization/activated charcoal process (assuming a 20% API biding to the resins), or after steady state is reached through successive recycling of resin treated mother liquor. (b) Transient profile of API isolated as crystals when resin treated mother liquor streams are recycled into next batch recrystallization/activated charcoal cycle (assuming a case of an initial recrystallization/activated charcoal process with 15% API losses and several percentages of API biding to the resins).

linear depreciation over 10 years and infrastructure and respective costs were calculated considering equipment allocation according with operation times. Facility maintenance was assumed to be 10 k€ and 19 k€, per year, which is significative.

Operation costs consider labour, energy requirements, resins and solid waste disposal. The analysis follows a conservative view in which resins are not recycled and the correspondent solid waste generated

is sent to disposal. In these case scenarios the resins correspond to about (5–10)% of total cost with solid waste disposal being evaluated in 3 k€ corresponding to (2–3)% of total adsorption cost. Labour and energy is estimated considering operations carried out. The mother liquor on the original recrystallization process requires to be distilled for solvent recycle or disposed, therefore we consider that the introduction of a resin step does not imply additional costs with solvent treatment or disposal.

Figure 10 represents central case scenarios in which an API cost of 6 k€/Kg was stipulated.[35] In the case of direct API reclaiming from the mother liquor, the API savings cover the costs associated with the additional adsorption step considering 80% recovery of the 6 g/L API (i.e. 15% API is initially present in the mother liquor and would be otherwise lost) (Fig. 11).

Figure 11 shows yearly API savings for different scenarios of API lost in the mother liquor, assuming a constant API reclaiming of 80%. The horizontal dashed line shows the yearly cost of introducing the resin step. For example, for the central case scenario, with an API price of 6 k€/Kg, the cost associated with the adsorption step is offset for an API loss of 14%. For an API that is 30% more expensive, the adsorption cost is offset for a 11% API loss. On the other hand, for an API that is 30% cheaper, the cost of adsorption is only offset at a higher API loss of 20%. Since APIs have an associated high production cost, these results show that the introduction of an additional adsorption step, after a recrystallization, is economically feasible for API recovery from mother liquor, that would be otherwise lost.

Conclusions

Several commercially available resins were screened for DMAP and MPTS, indicating that AG 50W-X2 and IRA68 resins were the ones able to efficiently remove these products, in MeOH in one single step, with adsorption of 99.8% of DMAP and its methylated conjugate (DMAP-Me) and the full removal of PTSA and MPTS from the mother liquor with an API loss of about 19%, reaching a final ratio of 2.1 mgGTI/gMeta, enabling the reintroduction of this enriched Meta solution to the process. The potential for improving the recrystallization economics, through mitigation of API losses is suggested based on: (i) direct reclaiming of API from a recrystallization mother liquor, when resin step is able to bring down GTI to ultra-low levels, and so GTI to API ratio in the mother liquor is able to comply with TTC or; (ii) through recycling recrystallization resin treated mother liquor into the next batch recrystallization/
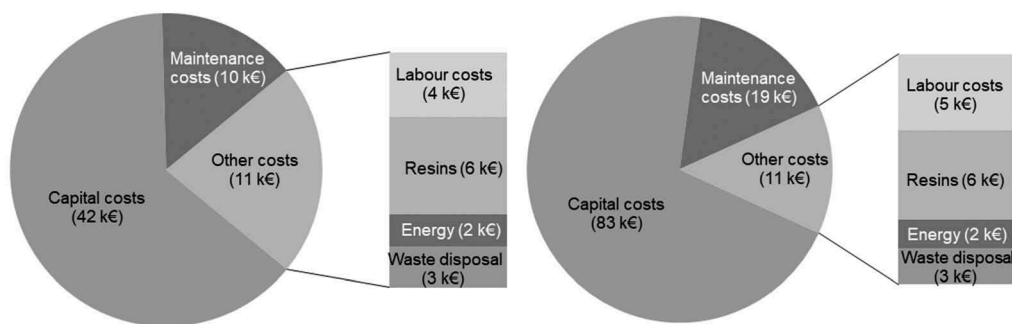
Figure 10. Costs associated with API resin adsorption step for: left – API reclaiming directly from mother liquor; right – recycling feedback loop of mother liquor after resin treatment. Assumptions: the resin is not recycled; no fresh solvent is added; solvent disposal is not considered; additional equipment concerns pumps, fluidized bed reactors for adsorption and extended period of hoven and dryer usage, with the later two already included in the recrystallization process.
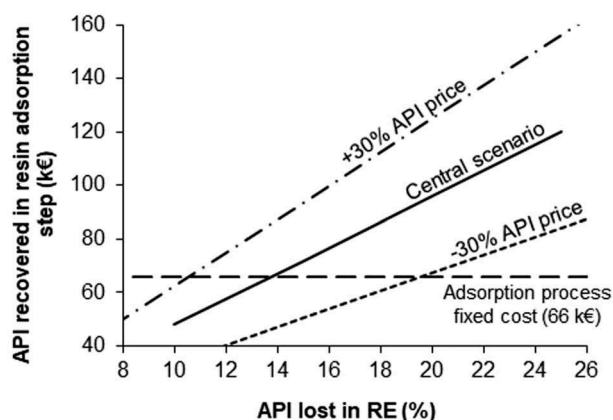

Figure 11. Potential gain in API reclaiming directly from the mother liquor with a resin adsorption step for several percentages (10–25)% of API loss in mother liquor (assuming a non-optimized case scenario considering the use of 50 kg of selective agent per 1 m^3 of mother liquor and a constant recovery of 80% of API) and considering several scenarios for API price in €/Kg.

activated charcoal cycle, where such mother liquor has GTI/API ratio that meets the value of the initial post-synthetic API crude stream. Recycling the mother liquor may be a more challenging strategy to implement, since the recrystallization step in many processes is also used to purge, through the mother liquor, additional impurities driven from the previous API synthetic steps and, recycling of this stream may require process requalification.

Supporting Information

Binding isotherm theoretical model parameters assessed in this report, pH influence on DMAP binding for several resins in water and in water:MeOH (1:1), kinetic profile of DMAP in water towards AG 50W-X2 resin and assessment of DMAP-ME and PTSA in Meta mother liquor by ^1H NMR in MeOH-d_4 can be found in Supporting Information.

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