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Mimicking DNA alkylation: Removing genotoxin impurities from API streams with a solvent stable polybenzimidazole-adenine polymer

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ABSTRACT

This study reports the application of a novel polybenzimidazole (PBI) polymer modified with an alkylated DNA base - adenine - as an effective scavenger for several families of DNA alkylating agents. This new material addresses an important issue in active pharmaceutical ingredients (APIs) manufacture, the removal of genotoxic impurities (GTIs) to strictly low regulated limits. Instead of targeting individual GTIs removal, **PBI-adenine** scavenger mimics the concept of DNA-GTI adduct formation that takes place in vivo, but in this case, in an organic solvent matrix where APIs are chemically synthesized. Removal of eleven GTIs from five different chemical families is assessed with > 80% removal. Slow binding kinetics for some GTIs at room temperature was identified as one of the limitations of the **PBI-adenine** polymer. API purification is addressed and an efficient process is presented for two APIs studied, mometasone furoate and betamethasone acetate, affording high impurity removals (> 96%) and high API recovery with low API loss (3.5%) for these case studies. The possible application of this straightforward strategy in API post-reaction stream purification, is able to attain GTI imposed limits as low as 0.6 mg GTI/g API respecting the Threshold of Toxicological Concern (TTC) value.

1. Introduction

Pharmaceutical regulatory authorities have shown increased concern about impurities - especially genotoxic impurities (GTIs) – in active pharmaceutical ingredients (APIs) due to their adverse effects on human health [1, 2]. Sources for organic impurities in APIs include unreacted starting materials and reagents, intermediary products, catalysts, by-products formed, and degradation and storage products [3, 4]. The best route to prevent GTI presence in the final formulations is their elimination from synthetic pathways. However, when the formation of GTIs in APIs production cannot be prevented, purification of the API must be performed until the GTI is removed to satisfying levels: a Threshold of Toxicological Concern (TTC) value of 1.5 μg/day imposed by strict regulatory guidelines [1, 2].

Conventional separation techniques used in API purification include crystallization, filtration, distillation, the use of adsorbents, resins and column chromatography [4–6]. However, since these operation units are not GTI selective, significant amounts of API can be lost with great economic impact for pharmaceutical companies [5]. More recently, the use of organic solvent nanofiltration (OSN) [5–9], molecular imprinting techniques [10–12] and combinations thereof [13–15] have been suggested to address this challenge, based on size discrimination and specific interactions to target molecules.

Reactive resins as adsorbents, bearing specific functional groups, are versatile and robust materials with vast application in aqueous systems [16–21]. Nevertheless, API manufacturing synthetic processes often take place in organic solvent media, rendering their application challenging. For this reason, the development of a versatile organic solvent compatible material, for DNA alkylating agents scavenging, is a huge achievement with promising successful applications in pharmaceutical industry, ultimately contributing for API patients' wellbeing.

Several authors have been pursuing the aim of finding good performing organic solvent compatible adsorbers useful in the context of API purification [10–13, 22, 23]. For sulfonate GTIs, scavenging

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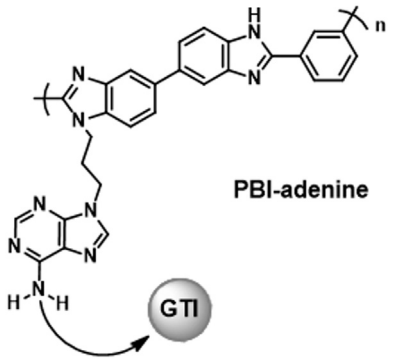


Fig. 1. Example of **PBI-adenine**-GTI adduct formation.

nucleophilic resins [22, 23] or molecular imprinted polymers (MIPs) [10] have been explored, taking advantage of specific interactions established between the polymers functional groups and the target sulfonate molecules. The amount of adsorber varies between 50 and 200 mg per 1 mL of solution to be treated [10, 22, 23] and generally, when GTI removal is around 100% there is still a considerable API loss in some cases [23]. Therefore, the challenge remains to find a platform suitable to perform in organic solvents, able to remove the highest amount of GTI with the lowest API loss possible.

GTIs cover a wide range of compounds from different chemical families including electrophilic reagents such as sulfonates, alkyl halides or epoxides, which are genotoxins that act as DNA alkylating agents. These species alkylate DNA through a nucleophilic attack by the nitrogen or oxygen of the pyrimidine and purine bases present in DNA to the electrophilic carbon of the GTIs [4, 24–27]. In order to mimic the process that takes place in vivo, herein we explore the potential of a recent material developed within our group, based on polybenzimidazole (PBI) polymer with an appending adenine moiety (**PBI-adenine**, Fig. 1) for API purification [28]. PBI is a versatile organic solvent compatible polymer that contains heterocyclic amine groups that can be modified with adequate chemical functionalities. In this case, PBI was modified to present as side group a DNA base, namely adenine, originating a new powder porous material suitable to interact with a wide range of DNA alkylating agents. The modification of PBI with adenine had never been attempted in order to mimic what happens in biologic systems, where alkylating GTIs interact with DNA originating DNA-GTI adducts [24], as exemplified in Fig. 1.

The synthesis of modified polymer (**PBI-adenine**) is presented elsewhere [28] and the current study is focused on exploring the capability of this innovative material to remove a broad range of DNA alkylating agents from API organic solvent solutions, identify limitations for the use of **PBI-adenine** for API degenotoxification and, to define strategies and operation conditions at which **PBI-adenine** can successfully remove GTIs down to TTC values, with minimal API losses.

2. Experimental

2.1. Materials

All chemicals were of reagent grade or higher and used as received. Methyl *p*-toluenesulfonate (MPTS), methyl methanesulfonate (MMS), ethyl methanesulfonate (EtMS), 1,3-dibromopropane (DBP), dimethyl sulfate (DMS) and dodecane (DDC) were purchased from Acros (Belgium). Ethyl *p*-toluenesulfonate (EPTS), 1,4-dibromobutane (DBB) and epichlorohydrin (EPI) were purchased from Alfa Aesar (United Kingdom). Butyl *p*-toluenesulfonate (BPTS) was purchased from TCI (Japan). 1,2-Dibromoethane (DBE) and glycidol (GCD) were purchased from Aldrich (USA). Mometasone furoate (Meta) and betamethasone acetate (Beta) were kindly provided by Hovione PharmaScience Ltd. (Portugal). Dichloromethane (DCM) and acetonitrile (MeCN) HPLC grade solvents were purchased from Fisher Chemicals (USA). Formic acid (FA) was purchased from Panreac (Spain). The synthesis and full characterization of **PBI-adenine** was performed previously and the results are published elsewhere [28].

2.2. Apparatus and analysis

The experiments at 55 °C were controlled in an incubation chamber from J. P. Selecta (Spain). HPLC measurements for MPTS, EPTS, BPTS, Meta and Beta 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 10 µL injection volume and eluents, A: aqueous 0.1% FA solution, B: MeCN 0.1% FA solution. For MPTS, EPTS and BPTS a flow rate of 2 mL min^{-1} and UV detection at 230 nm was used with the following methods: MPTS: 12 min, 70% A, t_R = 7.80 min; EPTS: 10 min, 60% A, t_R = 5.38 min; BPTS: 6 min, 40% A, t_R = 3.12 min. For Meta and Beta a flow rate of 1 mL·min^{-1} and UV detection at 280 nm was used; method: 0–3 min, (60–20) % A; 3–4 min, 20% A; 4–8 min, (20–60) % A; 8–15 min 60% A, t_R (Meta) = 7.25 min, t_R (Beta) = 6.12 min. GC measurements for MMS, EtMS, DBE, DBP, DBB, GCD, EPI and DMS were performed on a GC-2010 Plus by Shimadzu (Japan) equipped with a TRB-5 column (30.0 m × 0.25 mm, 0.12 µm film thickness) from Teknokroma (Spain) using an injection volume of 1.0 µL, a 1:2 split ratio and DDC as internal standard. Ultra-high purity helium was used as carrier gas and column flow was kept constant throughout the runs at 1 mL min^{-1}. Both injector and detector were set at 250 °C. MMS: oven at 50 °C for 9 min, ramp 30 °C/min to 120 °C, 4 min at 120 °C and ramp 25 °C/min to 180 °C, t_R (MMS) = 6.62 min, t_R (DDC) = 15.53 min. EtMS: oven at 60 °C for 9 min, ramp 25 °C/min to 120 °C, 4 min at 120 °C and ramp 25 °C/min to 150 °C, t_R (EtMS) = 7.47 min, t_R (DDC) = 15.75 min. DBE: oven at 40 °C for 8 min, ramp 40 °C/min to 120 °C, 4 min at 120 °C and ramp 30 °C/min to 150 °C, t_R (DBE) = 6.04 min, t_R (DDC) = 14.63 min. DBP: oven at 60 °C for 9 min, ramp 30 °C/min to 120 °C, 4 min at 120 °C and ramp 30 °C/min to 150 °C, t_R (DBP) = 7.77 min, t_R (DDC) = 14.75 min. DBB: oven at 80 °C for 9 min, ramp 40 °C/min to 120 °C, 4 min at 120 °C and ramp 30 °C/min to 150 °C, t_R (DBB) = 8.58 min, t_R (DDC) = 12.30 min. GCD and EPI: oven at 40 °C for 5 min, ramp 50 °C/min to 120 °C, 2 min at 120 °C, ramp 30 °C/min to 180 °C, and 180 °C for 2 min, t_R (GCD) = 4.00 min, t_R (EPI) = 3.89 min, t_R (DDC) = 10.69 min. DMS: oven at 40 °C for 9 min, ramp 40 °C/min to 120 °C, 4 min at 120 °C and ramp 30 °C/min to 180 °C, t_R (DMS) = 9.03 min, t_R (DDC) = 15.61 min.

2.3. Binding experiments

For each GTI, 50 mg of polymer (**PBI-adenine**) were placed in 2 mL round bottom tubes and 1 mL of a 100 ppm solution of GTI, prepared in DCM, was added. The suspension mixtures were magnetically stirred at 200 rpm for 24 h or 2 weeks at room temperature. After this time the suspensions were centrifuged at 13,000 rpm for 20 min and the supernatants were filtered and analysed by HPLC or GC. All experiments were carried out in duplicate. The percentage of GTI bound to the polymer was calculated from eq. (1) where C_0 (mg/L) is the initial GTI concentration and C_f (mg/L) is the final GTI concentration in solution.

$$\% = \frac{[C_0 - C_f]}{C_0} \times 100 \quad (1)$$

The amount of GTI bound to the polymer was calculated from eq. (2) where q (mg/g) is the amount of GTI bound to the polymer, C_0 (mg/L) is the initial GTI concentration, C_f (mg/L) is the final GTI concentration in solution, V (L) is the volume of solution used and M (g) is the polymer mass.

$$\mathbf{q} = \frac{\mathbf{V} \times [\boldsymbol{C_0} - \boldsymbol{C_f}]}{\mathbf{M}} \quad (2)$$

For experiments at 55 °C, the suspension mixtures were stirred at 200 rpm for 2–8 h at 55 °C in glass test tubes with screw caps. Afterwards, the suspensions were centrifuged and processed as described above. These experiments were carried out in duplicate. The percentage of GTI bound to the polymer was calculated from Eq. (1).

The binding experiments performed for the APIs and GTI/API mixtures followed the procedures described above at room temperature for 24 h and at 55 °C for 2–8 h. In these experiments, the GTIs were present at a concentration of 100 ppm and the APIs were present at a concentration of 10 g/L. The percentage of API bound to the polymer was calculated from Eq. (1) where, in this case, C_0 (g/L) is the initial API concentration and C_f (g/L) is the final API concentration in solution. All experiments were carried out in duplicate and compared to blank samples.

2.4. API recovery experiments

1 mL of DCM was added to 50 mg of polymer used in API batch binding experiments. The suspension mixtures were stirred at 200 rpm for 24 h at room temperature. After this time, the suspensions were centrifuged and the supernatants were filtered and analysed by HPLC for API quantification. All experiments were carried out in duplicate.

2.5. Kinetic studies

Several solutions were prepared with 50 mg of polymer and 1 mL of a 100 ppm solution of MPTS or MMS prepared in DCM. The suspension mixtures were stirred at 200 rpm at room temperature or 55 °C. At certain time intervals of 5, 15 and 30 min and 1, 2, 4, 6, 8, 24 and 27 h, the suspensions were centrifuged and the supernatants were filtered and analysed by HPLC or GC. All experiments were carried out in duplicate. The percentage and amount of GTI bound to the polymer was calculated from Eqs. (1) and (2). Experimental data were fitted to pseudo-first and pseudo-second order kinetic models [29] according to Eqs. (3) and (4) respectively, where q_f and q_t (mg/g) are the adsorption capacities at the final and time t (min) respectively, and k_1 (min^{-1}) and k_2 (g/(mg·min)) are the pseudo- first and second order rate constants for the models.

$$\ln(q_f - q_t) = \ln(q_f) - k_1 . t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 . q_f^2} + \frac{t}{q_f} \quad (4)$$

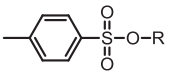
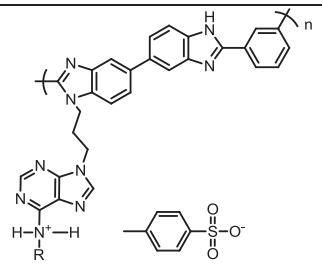
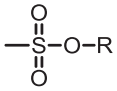
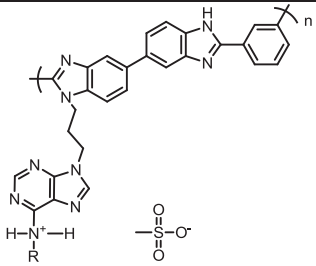
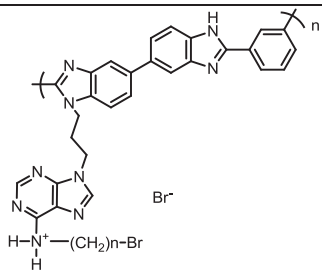
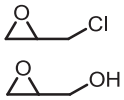
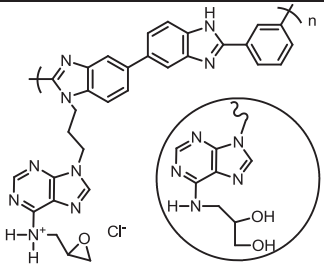
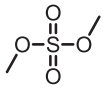
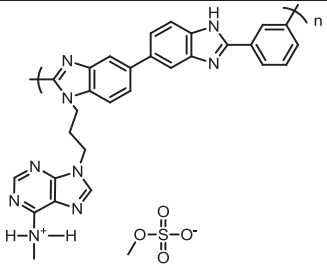
2.6. Adsorption isotherm studies

For adsorption isotherm experiments, 1 mL of MPTS or MMS solutions prepared in DCM, with different initial concentrations (5–1000 ppm), were added to 50 mg of **PBI-adenine**. The mixtures were stirred at 200 rpm for 24 h at room temperature, or for 3–8 h at 55 °C. After that, the suspensions were centrifuged and the supernatants were filtered and analysed by HPLC or GC. All experiments were carried out in duplicate. The percentage and amount of GTI bound to the polymer was calculated from Eqs. (1) and (2). Experimental data were fitted to the Langmuir and Freundlich isotherm models [30] according to Eqs. (5) and (6) respectively, where q_m (mg/g) is the maximum amount of GTI bound to the polymer 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.

$$\frac{q_f}{q_m} = \frac{K_L C_f}{1 + K_L C_f} \quad (5)$$

$$q_f = K_F C_f^{\frac{1}{n}} \quad (6)$$

Table 1
Proposed **PBI-adenine** polymer adduct formation with several DNA alkylating agents. The interaction between **PBI-adenine** polymer and MPTS was studied in detail using ^{1}H NMR in a previous study. [28]

GTI family	Polymer-GTI adduct
Alkyl tosylate (MPTS, EPTS, BPTS) 	
Alkyl mesylate (MMS, EtMS) 	
Dihalo alkane (DBE, DBP, DBB) Br—$(CH_2)n$—Br	
Epoxide (GCD, EPI) 	
Dimethyl sulfate (DMS) 	

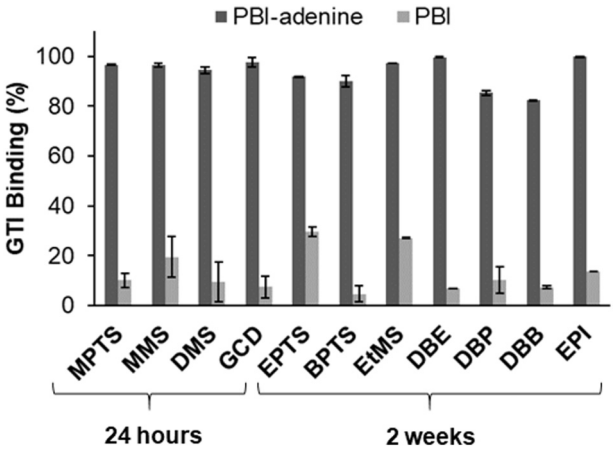


Fig. 2. GTI binding to PBI and **PBI-adenine** scavengers, for 50 mg of polymer in 1 mL of a 100 ppm solution in DCM of each GTI after 24 h or 2 weeks at room temperature.

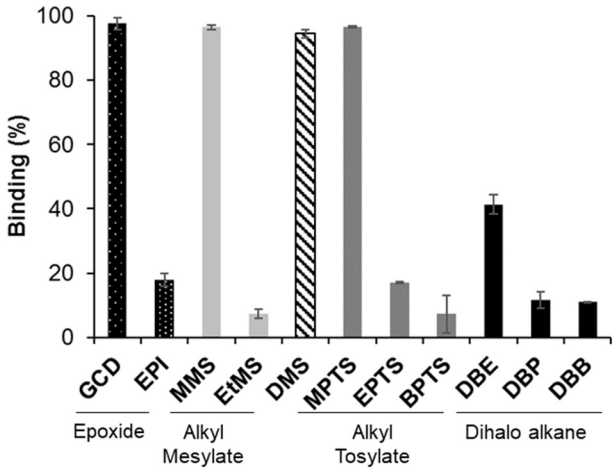


Fig. 3. GTI binding to **PBI-adenine** scavenger, for 50 mg of polymer in 1 mL of a 100 ppm solution in DCM of each GTI after 24 h at room temperature. GTIs within the same family are ordered by increasing molecular weight from left to right.

3. Results and discussion

3.1. GTI binding experiments

The first objective of this study was to develop a versatile material, compatible with organic solvents, able to scavenge a broad range of DNA alkylating molecules presenting different chemical functionalities. In order to assess the versatility of **PBI-adenine** polymer, batch binding experiments in dichloromethane (DCM) were performed for several GTIs belonging to the following different five chemical families: (i) alkyl tosylates (MPTS, EPTS, BPTS); (ii) alkyl mesylates (MMS, EtMS), (iii) di-halo alkanes (DBE, DBP, DBB), (iv) epoxides (GCD, EPI), and (v) dimethyl sulfate (DMS). For all GTIs assessed in this report, the expected alkylation interactions with the scavenger are represented in Table 1. After alkylation, is expected that ionic interaction may also occur, as well as some pi-pi interaction between the aromatic heterocycle and the tosyl group. In case of the other tested genotoxic impurities the molecule is covalently bonded to the adenine.

For all cases, we obtained a GTI removal higher than 80% for the same initial concentration of 100 ppm, at room temperature (Fig. 2). For performance comparison, in blank experiments, performed with, PBI raw polymer we obtained GTI removals lower than 40%, under the same operation conditions. These results show the efficiency obtained after chemical modification of PBI with adenine side chains that should derive mainly from nucleophilic substitution by adenine unit on the electrophilic carbon present in the tested genotoxic impurities (Table 1). DCM was selected as solvent for the different experiments as it is a solvent with high solvability properties and low boiling point. Therefore, in spite of the environmental issues raised, it is still a solvent broadly used in synthesis in the pharmaceutical industry, allowing reagents ready dissolution, product isolation and low energy intensive solvent recycling. Specifically, for the synthesis of steroids, the model APIs selected for this study, DCM is typically used as solvent on the final synthetic reaction steps.

However, MPTS, MMS, DMS and GCD needed 24 h to achieve removals higher than 94%, while the remaining GTIs required an extended period of about 2 weeks to reach higher removals. The slower kinetics observed at room temperature represent the first identified limitation for the novel **PBI-adenine** polymer and can probably be attributed to structural constraints presented by the GTIs. For example, the alkyl side chains present in the structures of EPTS, BPTS and EtMS, may cause some steric hindrance, not allowing a good proximity or interaction between adenine side chains of the polymer and GTI molecules. A similar observation was reported by Lee et al. [23] in which several nucleophilic resins were screened for sulfonate esters removal from solutions prepared in methanol (MeOH). The authors assigned this behaviour to the increased steric bulkiness of EPTS and EtMS, for example, compared to MPTS or MMS. This tendency can be easily observed in Fig. 3 where, within the same GTI family, the binding percentage is represented as function of increasing molecular weight of GTIs. On the other hand, the presence of electron withdrawing elements such as −Cl or −Br in EPI, DBE, DBP and DBB, seems to also have some negative influence in the interaction between these GTIs and the polymer, leading to an extended incubation time to achieve GTI removals comparable to GCD, for example.

We also assessed solvent compatibility of **PBI-adenine** polymer in MeOH and performed binding studies in this solvent. We observed a good solvent resistance of the material but the results in Fig. 4 show that in MeOH, the binding is less favoured than in DCM. This may be explained by a possible competition between the solvent and GTIs towards recognition sites, since –OH groups of MeOH may interact with adenine -NH_2 groups by hydrogen bonding. Additionally, the different swelling of the polymer in these solvents can also have different impacts on GTI binding (see supplementary data).

3.1.1. Temperature effect on GTI binding

Decreasing operation times is crucial to make the use of **PBI-**

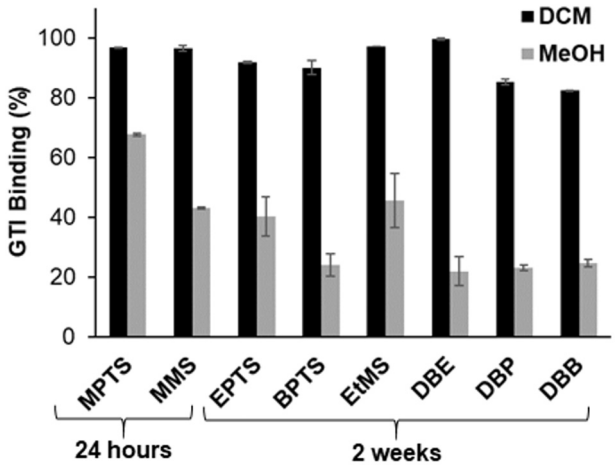


Fig. 4. GTI binding to **PBI-adenine** scavenger, for 50 mg of polymer in 1 mL of a 100 ppm solution in DCM or MeOH of each GTI after 24 h or 2 weeks at room temperature.

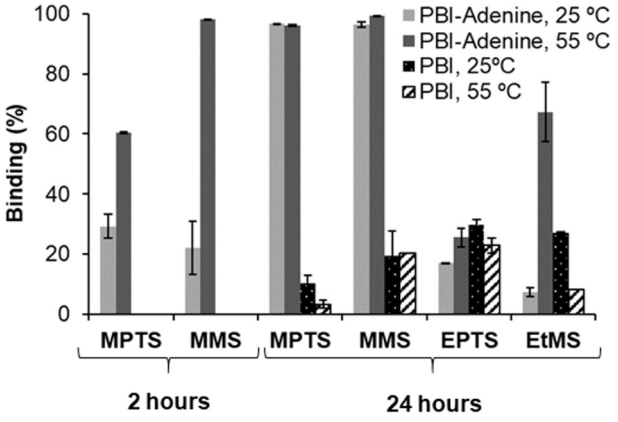


Fig. 5. MPTS, MMS, EPTS and EtMS binding to PBI and **PBI-adenine** at 25 °C and 55 °C in DCM, for 50 mg of polymer in 1 mL of a 100 ppm solution of each GTI for 2 h or 24 h in contact with the polymer.

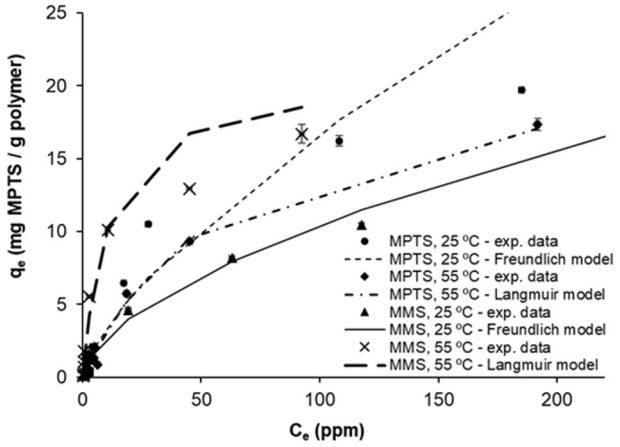


Fig. 7. Isotherm adsorption models for MPTS and MMS at 25 °C and 55 °C in DCM.

adenine a viable alternative for removal of GTIs. Therefore, we explored the influence of temperature in GTI binding in order to increase kinetics and improve GTI removal by assessing binding experiments performed at 55 °C. For these experiments, the same temperature and contact time with the polymer were used to assess the binding of two GTIs with low binding rate, EPTS and EtMS, and compared against two other GTIs with faster binding rates, MPTS and MMS (which reached > 95% GTI removal at room temperature within 24 h).

From Fig. 5 we observe a positive effect of temperature in MPTS, MMS and EtMS binding. However, EPTS gain in binding from 17% at 25 °C to 25% at 55 °C is not statistically significant (p value > .05) and the EtMS nine-fold increase improvement in binding from 7% at 25 °C to 67% at 55 °C does not reach the high desirable binding values superior to 95% obtained for MPTS or MMS at the GTI/scavenger ratios used and in a single binding step. For PBI raw polymer, we did not observe a positive effect in binding with temperature, indicating that the PBI itself is not contributing to binding to the GTIs and the adenine functionalization is promoting the interaction with the solutes.

In the case of MPTS and MMS at 55 °C, after only 2 h, MPTS registered a two-fold increase in binding to the polymer (from 29% to 60%), whereas for MMS there is a four-fold increase (from 22% to 98%). We also observed that, at 25 °C for both GTIs, a pseudo first order kinetic model is followed, while at 55 °C a pseudo second order kinetic model is followed instead (Fig. 6). Moreover, for MMS at 55 °C around 60% of the GTI is removed after only 30 min, while MPTS requires a longer time period, since 60% of this GTI is removed only after 2 h. These data were obtained from the binding kinetic studies at 25 °C and 55 °C in DCM for both GTIs which mathematical parameters are presented in Supporting Information. The results support the hypothesis that bulkier side chains may pose some steric hindrance in the interaction between the polymer and the GTIs as discussed above. In this case, the aromatic moiety of MPTS may impair the close proximity to adenine moieties, requiring a longer time period to interact, compared to MMS. Moreover, at a higher temperature, the polymer side chains may move more freely in solution, favouring accessibility between GTIs and -NH_2 adenine groups, making the binding process to reach equilibrium faster.

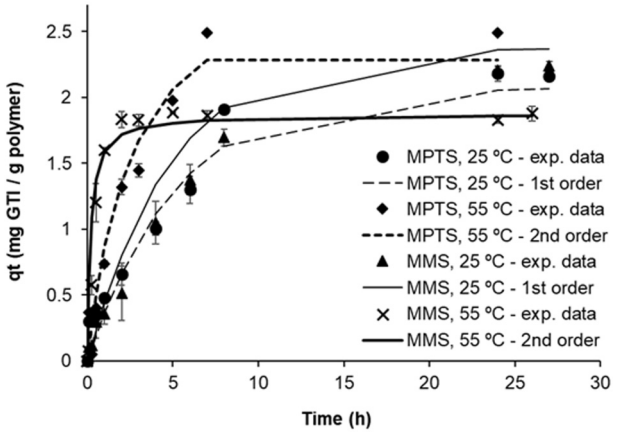


Fig. 6. Kinetic models for MPTS and MMS at 25 °C and 55 °C in DCM.

3.1.2. Adsorption isotherm characterization

Adsorption isotherm studies were performed for MPTS and MMS in DCM at 25 °C and 55 °C. The experimental data were fitted to different mathematical models and the parameters determined are presented in Supporting Information. Both GTIs presented the same behaviour. At 25 °C, the binding of MPTS and MMS follows the Freundlich model (Fig. 7). This model assumes that the adsorber presents a heterogeneous binding site distribution and that as the GTI concentration increases, its concentration on the polymer will also increase with the amount bound being the sum on all sites [30]; the 1/n values of 0.55 and 0.69 estimated for the Freundlich model suggest that the binding sites available are saturated resulting in relatively lower binding. However, at 55 °C for both GTIs the Langmuir model is followed, assuming a monolayer adsorption taking place at definite localized sites with no interaction or steric hindrance between the GTI bound molecules [30]. This behaviour may be explained by the polymer chains being less constrained at a higher temperature and the proximity and interaction with the GTIs being favoured that way.

While **PBI-adenine** proved to be already extremely efficient for removal of smaller GTIs, the results obtained suggest a slow performance for larger GTIs, which has still room for binding rates improvement by increasing temperature or surface area.

3.2. API binding experiments

Considering the GTI removal efficiencies, the next step was to assess **PBI-adenine** binding ability towards the APIs, to quantify possible losses and recoveries. This was performed for two glucocorticoid steroids readily soluble in DCM: mometasone furoate (Meta) and betamethasone acetate (Beta), both represented in Fig. 8. Meta is used topically to reduce inflammation on skin (eczema, psoriasis) or airways (allergic rhinitis, some asthma patients) pathologies [5, 31], while Beta is used as an oral suspension to treat arthritis, allergic or inflammatory conditions or reactive airways diseases [32].

A solution of each API was prepared in DCM at a concentration of 10 g/L and assessed alone or in the presence of 100 ppm of MPTS. After 24 h in contact with the polymer at room temperature, or 8 h at 55 °C,

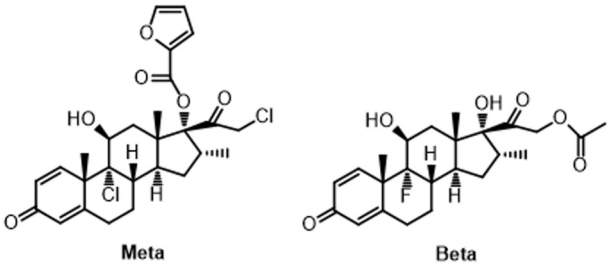


Fig. 8. Chemical structures of the APIs studied in this work: mometasone furoate (Meta) and betamethasone acetate (Beta).

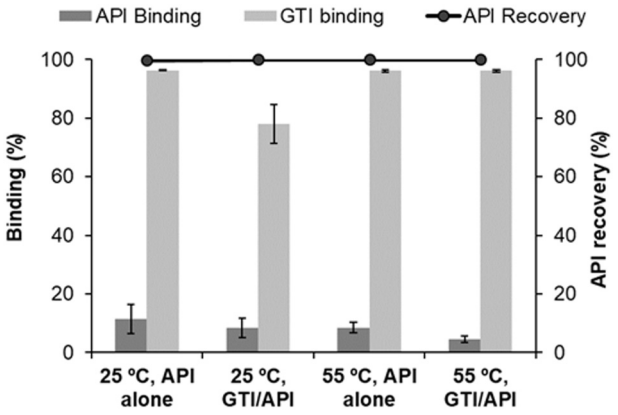


Fig. 9. MPTS and Meta binding to **PBI-adenine** when present alone or together in solutions at 25 °C and 55 °C in DCM. Meta recovery in washing steps after binding.

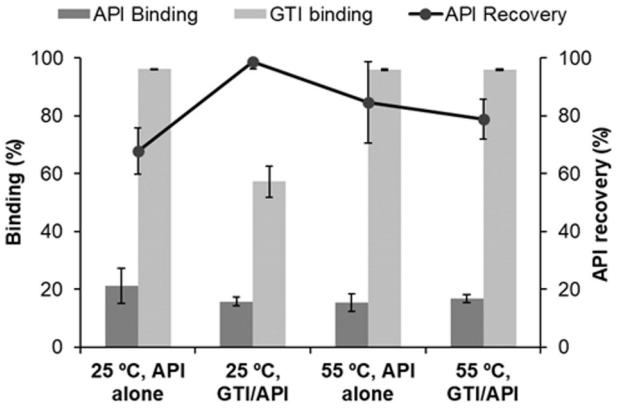


Fig. 10. MPTS and Beta binding to **PBI-adenine** when present alone or together in solutions at 25 °C and 55 °C in DCM. Beta recovery in washing steps after binding.

the amount of API present in solution was quantified. For both APIs there was no influence in binding towards the polymer caused by temperature change or the presence of the GTI (p value > .05) with an average binding value around 8% for Meta (Fig. 9) and 17% for Beta (Fig. 10). The relatively high percentage of API binding to **PBI-adenine** may be identified as the second limitation of this new material.

GTI removals were similar at a value around 96% when the GTI is alone or in the API mixtures at 55 °C with no significant differences (p value > .05) found. However, at 25 °C there is a considerable impairment in GTI binding to the polymer with Meta and Beta presence leading to GTI removals decrease to values as low as 78% and 57%, respectively. Therefore, the use of a higher temperature (e.g. 55 °C) seems to be beneficial not only to increase binding rate, but also to prevent API inhibition of GTI binding.

In a previous study [28] it was shown that **PBI-adenine** washing with DCM or MeOH alone was inefficient to remove MPTS bound to this polymer, since the adsorption takes place through a covalent interaction between this GTI and adenine moieties of the polymer. Release and recovery of *p*-toluenesulfonic acid (PTSA) formed was only possible using methanolic solutions of triethylamine or sodium methoxide (see Table 1 for alkyl tosylates), as **PBI-adenine** polymer scavenges MPTS by adenine alkylation and then, behaves as an ionic exchanger, in the presence of an organic base, for PTSA formed during this reaction. Therefore, considering that the GTI is not easily removed from the polymer, we envisaged the possibility to decrease API losses (8–17%) by straightforward recovery of the API, eventually trapped in **PBI-adenine**, by a simple solvent washing procedure, potentially without any GTI back extraction. The implementation of such washing step using DCM allowed full Meta recovery, with virtually no API loss, whereas for Beta an average recovery of about 83% was achieved leading to a loss around 3% of this API. No PTSA, formed during binding, was detected in API recovered solutions, proving that APIs are recovered without any GTI back contamination.

Beta recovery from **PBI-adenine**, using DCM at room temperature, is significantly higher when the polymer is used in the previous binding step, with Beta/GTI mixtures (around 98%) than when Beta individual solutions were used. The impossibility to have a full recovery of this API suggests that some covalent interaction may be taking place with the polymer. The ester functional group present in Beta may be forming amide bonds with -NH_2 groups of the polymer. In this way, some API remains bound to the polymer and is not fully recovered. Nevertheless, for both APIs, HPLC chromatograms did not reveal the presence of other impurities that could be originated from polymer treatment.

Due to sulfonates intrinsic genotoxicity, several authors explored different adsorbers for the removal of MPTS from API solutions, employing different procedures (Table 2). For example, Székely et al. reported a low GTI removal of (15–45) % with a considerable API loss of (10–15) % using a MIP specifically designed for this GTI [10]. Lee et al. reported 100% removal of MPTS but also a high API loss, around 10% [23]. Furthermore, Kecili et al. also reported a 100% removal of this GTI with full recovery of the API. However, the authors needed 150 mg of adsorber per 1 mL of solution to achieve this result [22]. With **PBI-adenine** polymer we are able to remove > 96% of the same GTI with full API recovery with only one third of that amount of adsorber (50 mg/mL). These results illustrate the high efficiency and improved performance of the developed PBI modified polymer comparing with other adsorbing systems.

3.3. Process design for API purification

Considering the results presented in Figs. 9 and 10, we envisaged a binding step process at 55 °C with the potential to eliminate a GTI from an API solution. In these conditions, the **PBI-adenine** polymer is able of 96% GTI removal with a total recovery of Meta and only 3.5% loss of Beta.

Table 2
MPTS vs API binding data in batch experiments, for previously reported cases and current work.

Reference	[10]	[22]	[23]	Current work
Adsorber	MIP	Nucleophilic resin		PBI-adenine
Solvent	DCM	2-propanol	MeCN, MeOH	DCM
[GTI] (ppm)	1000	5	100	100
[API] (ppm)	10,000	500	100	10,000
GTI:API	1:10	1:100	1:1	1:100
Time/Temperature	24 h / RT	2 h / RT	1 h / 40 °C	8 h / 55 °C
Adsorber amount	50 mg /mL	150 mg/mL	200 mg /mL	50 mg/mL
API loss	(10–15)%	100%recovery	< 10%	3.5%
GTI removal	(15–45)%	100%	100%	96%

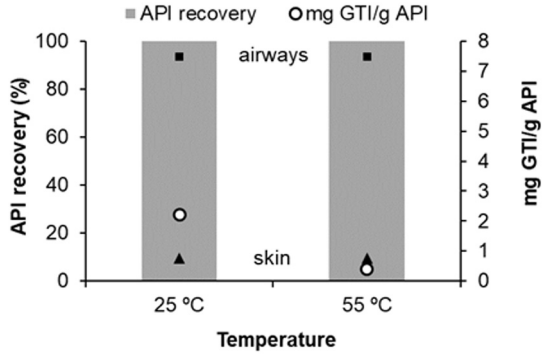


Fig. 11. Performance of a single step process concerning API recovery and level of degenotoxification of MPTS to Meta as a function of temperature (white dots). Squares represent the limit for airways treatment of 7.5 mg GTI/g API. Triangles represent the limit for skin treatment of 0.75 mg GTI/g API.

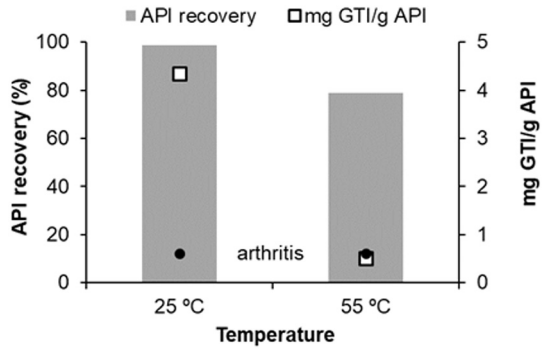


Fig. 12. Performance of a single step process concerning API recovery and level of degenotoxification of MPTS to Beta as a function of temperature (white squares). Dots represent the limit for arthritis treatment of 0.6 mg GTI/g API.

For Meta degenotoxification, we consider two case scenarios for the therapeutic use of this corticosteroid: nebulization for the treatment of airways diseases or topical application in the treatment of eczema, with administered daily dose of 200 µg or 2 mg of API, respectively. The amount of GTI allowed is determined considering a TTC value of 1.5 µg GTI/day and the maximum daily dose in g API/day at the values of 7.5 or 0.75 mg GTI/g API for the airways or the skin treatment, respectively.

For Beta, we consider a case scenario in which the patient is administered an initial high dose of 2.5 mg/day for the treatment of rheumatoid arthritis. For the imposed TTC value of 1.5 µg GTI/day, this case implies the need to reach a limit of 0.6 mg GTI/g API.

For both cases, we considered a post-reaction stream to be treated, with an API load of 10 g/L and a GTI contamination at a concentration of 100 ppm, simulating an industrial batch production in which the API is found in a higher concentration compared to the GTI with a ratio of 10 mg GTI/g API. We performed bindings at 25 °C and 55 °C and compared results for both strategies.

For Meta, the API is totally recovered for both operational temperatures (Fig. 11). However, at 25 °C about 22% of the GTI remains in solution, reaching a final ratio of 2.2 mg GTI/g Meta, which is an acceptable value for the airways treatment case (target limit set at 7.5 mgGTI/gAPI), but not for the skin treatment (target limit of 0.75 mgGTI/gAPI). Nevertheless, when the process takes place at 55 °C only about 4% of GTI remains in solution, reaching a final ratio of 0.39 mg GTI/g Meta, which is suited for both case scenarios.

For the more challenging case of Beta, (Fig. 12) when the process takes place at 25 °C there is a good recovery of the API around 98%, but 43% of the GTI remains in solution reaching a final ratio of 4.33 mg GTI/g Beta, which is a value far from the 0.6 mg GTI/g API limit required. However, when the process takes place at 55 °C, it is possible, using the solvent washing step to recover about 79% of the initial 13% of API bound to **PBI-adenine**, with 96% removal of the GTI, leading to an overall loss of about 3.5% of API. Since only 4% of the GTI remains in solution, a final ratio of 0.50 mg GTI/g Beta is reached, which is a value within the limit imposed by legislation of 0.6 mg GTI/g API.

Overall, for both APIs the best GTI to API ratio is always achieved when the binding process takes place at 55 °C, which contributes to a faster GTI removal and prevented API inhibition of GTI binding, leading to a final GTI to API ratio that is within the limits imposed by strict regulatory authorities, with virtually no losses of API for the case of Meta and about 3.5% loss in the case of Beta.

4. Conclusions

The potential development of a versatile material able to scavenge a broad range of DNA alkylating agents from organic solvent based solutions was investigated. Adsorption of GTIs from different chemical families, on an adenine modified PBI polymer, was found to be effective (> 80%) at room temperature. Our results show that in a typical industrial scenario, where the GTI is present in low concentration compared to the API, the efficiency and GTI removal rate can be improved with temperature increase. Furthermore, a simple solvent washing step was implemented to recover the API trapped in **PBI-adenine** polymer without GTI back contamination, exploring the fact that, the GTI is not easily retrieved from the adsorbing platform. Based on these achievements, a strategy is proposed for the efficient removal of a DNA alkylating GTI from an API solution in an organic solvent, leading to GTI to API ratios within the limits imposed by legislation, as low as 0.6 mg GTI/g API with only a 3.5% loss of API for the worst-case scenario considered. From the point of view of an industrial application this is a major advantage, since with one simple washing step it could be possible to recover the API, minimizing its loss, addressing the economic impact for the pharmaceutical companies associated with API losses in time consuming and material demanding elaborated purification strategies.

Data availability statement

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reactfunctpolym.2018.07.019.

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