Solvent compatible polymer functionalization with adenine, a DNA base, for API degenotoxification: Preparation and characterization

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ABSTRACT

This work describes for the first time a novel functionalization of poly[2,2′-(m-phenylen)-5,5′-bisbenzimidazole] (PBI), a polymer stable in a wide range of solvents, with a DNA base, adenine (PBI-A$_{x\%}$). Functionalization was achieved through alkylation of N4 benzimidazole nitrogen with 9-(3-bromopropyl) adenine (9-BPA). The N9 adenine nitrogen that binds to deoxyribose in DNA molecule was inhere used for covalent binding to the PBI, leaving free the remaining adenine positions, as the ones available in a natural DNA molecule. Therefore, for the first time, the use of adenine based polymer is here suggested as a biomimicry strategy for degenotoxification of post-reaction synthetic active pharmaceutical ingredient (API) streams. The very same interactions responsible by in vivo genotoxicity, present in the novel adenine based polymer, PBI-A$_{x\%}$, were used for removal of a genotoxic impurity (GTI). Methyl p-toluenesulfonate (MPTS) at 100 ppm in dichloromethane was selected as a representative sulfonate GTI, which is present in a wide range of API post-reaction streams. The functionalization reaction efficiency was assessed using NMR at yields of 40–69% or 27–31%, for addition of 0.13–0.25 or 0.5–1.0 mol eq of 9-BPA to N4 PBI nitrogen, respectively. Synthesis and purification of 9-BPA was first optimized, increasing yields from previously reported values of 38–61%. The maximum removal of genotoxic impurity was obtained for PBI-A$_{12\%}$ polymer, obtained at 0.25 M equivalents of 9-BPA to N4 PBI nitrogen, at a value of $(96.6 \pm 0.2)\%$, against $(10.2 \pm 2.8)\%$ of the original non modified PBI.

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1. Introduction

The majority of the pharmaceutical products are synthetically obtained using a wide range of reactive reagents through catalyzed reactions. In those reactions, some of the reagents (added in molar excess), by-products, catalysts or solvents may be left as impurities in active pharmaceutical ingredients (APIs). When such impurities are prone to react with deoxyribonucleic acid, DNA, potentially preventing its normal replication and resulting in an associated carcinogenic risk, are categorized as genotoxic impurities (GTIs) [1,2]. Regulatory agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have high concerns relatively to the presence of the GTIs in the APIs. Therefore, there is a call to achieve simple and robust APIs synthetic processes with the lowest content of GTIs or to develop efficient purification methods to separate the API from the GTI impurity.

A considerable number of chemical compounds present in multistep synthesis and formulations of APIs, namely electrophilic reagents such as sulfonates, epoxides and primary alkyl monohalides have been identified as potential genotoxins [3,4]. Different chemical routes for API production, in which GTIs are prone to be present, have been recently presented in an extensive review [5]. In this paper we will focus on sulfonate agents, which are genotoxins that are considered as alkylating agents. They alkylate the DNA through a nucleophilic attack by the nitrogen or oxygen of the pyrimidine and purine bases present in the DNA to the electrophilic carbon of the sulfonate. Adenine and guanine are the DNA bases more prone to be attacked by sulfonates. Endocyclic nitrogens, N3 and N7, are the sites more nucleophilic of the DNA, while exocyclic oxygens are the less nucleophilic ones [6,7]. This particular class of genotoxic impurities has gained attention since the Viracept case, reviewed elsewhere [8]. Importantly, sulfonates can be formed in side reactions of sulfonic acids, or the corresponding sulfonyl chloride, with alcohols used as solvents, co-solvents in re-crystallization/precipitations, clean up procedures or washing equipment and, therefore, its prediction of their formation is not always straightforward.

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There are several methods to purify APIs, including conventional purification techniques [2], such as recrystallization, chromatography, and distillation, and emerging technologies, such as organic solvent nanofiltration (OSN) [9], supercritical extraction [10], resins and molecular imprinting [11,12]. In order to create a mimetic material for API degenotoxification, while still dissolved in organic solvent post-reaction streams, we select the polybenzimidazole (PBI) as starting polymer material. PBI is a versatile polymer, stable in a wide range of solvents, previously used for the manufacture of OSN membranes [13–17], that contains heterocyclic amine groups. PBI membranes were, for example, used in a two stage OSN membrane cascade to separate an API, roxithromycin, from two GTIs, 4-dimethylaminopyridine and ethyl tosylate, dissolved in methanol [18]. With this operating mode, a GTI concentration of less than 5 ppm was achieved in the final solution with 73% reduction in mass and solvent intensity when compared to a single-stage diafiltration mode. Moreover, PBI membranes have been manufactured in the presence of GTI templates with the aim to prepare PBI imprinted membranes for GTI removal [19]. However, the imprinted membrane specific performance was lost over filtration, with solute rejection being virtually the same as for the control non-imprinted membrane, probably due to compaction phenomenon. PBI has also been modified with different units, creating polymers with different properties, such as N-substituted [20], pyridine based [21], sulfonated [22], poly(2,5-benzimidazole) [23], crosslinked [24] and hyper branched [13,16,25].

In this study, we aim to functionalize PBI with an adenine, through covalent binding of the N9 adenine nitrogen (the position in which adenine in the DNA molecule is covalently bound to deoxyribose), conserving the remaining adenine positions free for further interactions with potential genotoxins. An alkyl chain is included between the adenine group and the PBI as an anchoring spacer to mitigate stereochemistry limitations. We aim to anchor a DNA base to this polymer as a side group to promote further interactions with sulfonate GTI molecules as an effective and novel system for GTI removal from the APIs solvent post-reaction streams. As PBI is stable in the solid form in many solvents, separation from the solid loaded with GTI, from the clean rich solution of API, will be facilitated. In this work the interaction of the adenine based polymer with methyl *p*-toluenesulfonate (MPTS) will be studied. MPTS is here used as representative of sulfonates, which are highly toxic alkylating agents present in pharmaceutical synthesis streams.

To the best of our knowledge, this is the first report regarding the functionalization of PBI with an adenine moiety in order to biomimicry the biologic interaction of GTIs with DNA and uses such interaction for removal of GTIs.

2. Materials and methods

2.1. Materials

Adenine, potassium carbonate (K_2CO_3), 1,3-dibromopropane, *p*-toluenesulfonic acid (PTSA), 1,3,5-trimethoxybenzene and triethylamine (NEt_3) were purchased from Sigma Aldrich. Methyl *p*-toluenesulfonate (MPTS) and sodium methoxide (NaOMe) solution 5.4 M in MeOH were purchased from Acros (Belgium). Polybenzimidazole (PBI) polymer 100 mesh powder was purchased from PBI Performance Products Inc. (USA). All these reagents were used as supplied without further purification. The ^1H and 2D NMR spectra were recorded using DMSO-d_6 (99.9%) purchased from Sigma Aldrich. Dichloromethane (DCM) and acetonitrile (MeCN) HPLC grade and hydrochloric acid (HCl) were purchased from Fisher Chemicals (USA). Formic acid was purchased from Panreac (Spain).

All solvents, dimethylformamide (DMF), methanol (MeOH), dimethylsulfoxide (DMSO) and dichloromethane (DCM) were used without further purification.

2.2. Apparatus and analysis

^1H and 2D NMR spectra were obtained on Bruker spectrometers MX300 and CXP400 operating at 300 and 400 MHz, respectively. The nitrogen adsorption isotherm was obtained at 77 K in adsorption apparatus (ASAP 2010 Micromeritics) and the samples were degasified at 80 °C, over 16 h. HPLC 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: aqueous 0.1% formic acid solution, B: MeCN 0.1% formic acid solution. For MPTS a flow rate of 2 mL min^{-1} and UV detection at 230 nm was used; method: 0–12 min., 70%A-30%B. For PTSA detection a flow rate of 1.5 mL min^{-1} and UV detection at 230 nm was used with the method: 0–10 min, 90%A–10%B. For both compounds the injection volume was 10 μL.

2.3. Procedure for the synthesis of 9-(3-bromopropyl) adenine (1)

To a round-bottom flask was added adenine (0.50 g; 3.8 mmol) and K_2CO_3 (1 g; 7.2 mmol) in DMF (3 mL) with stirring for approximately 15 min at room temperature and 1,3-dibromopropane (1.9 mL; 19 mmol) was slowly added and left stirring for, approximately, 3 h at room temperature. The solvent was evaporated and added a DCM/MeOH 4:1 solution to the solid and left stirring. The solution was filtered and evaporated. The product was purified by column chromatography (DCM/MeOH 10:1) and vacuum dried to obtain 0.55 g of a white solid (η = 61%). ^1H NMR (300 MHz, DMSO-d_6): δ 2.36 (quint., J = 6.7 Hz, 2H), 3.50 (t, J = 6.5 Hz, 2H), 4.26 (t, J = 6.8 Hz, 2H), 7.24 (br s, 2H), 8.14 3.50 (t, J = 1.2 Hz, 2H); ^{13}C (75 MHz, CDCl$_3$): 31.4, 32.2, 118.8, 140.9, 149.6, 152.5, 156.0.

2.4. Procedure for the synthesis of PBI-A$_{x\%}$

The following protocol was developed for functionalization of PBI with a purine base with different mol-degree of adenine integration percentages: A solution of 15% (wt/v) of PBI (2.00 g, 6.5 mmol) in DMSO was prepared by stirring this mixture for 3 h at 160 °C. Note, for molar equivalent calculations, that each PBI monomer has two N4 nitrogens. The solution was then cooled to 50 ^0C for addition of about 1 mol eq. of K_2CO_3 (1.74 g, 12.6 mmol) and 0.25 mol eq. of 9-(3-bromopropyl) adenine (0.82 g, 3.2 mmol) for each PBI N4 nitrogen, then the temperature was raised to 100 °C for 24 h. The reaction was stopped by adding 80 mL of water and left stirring at room temperature. The mixture was filtered and the solid was washed and filtered one time with MeOH and then with DCM. The final polymer was dried and obtained as a brown solid. ^1H NMR (300 MHz, DMSO-d_6): δ 2.03 (sl), 3.40 (sl), 4.01 (sl), 7.96–7.64 (m, 7H), 8.35 (d, J = 6 Hz, 2H), 9.16 e 8.68 (sl, 1H). The polymers with different insertion equivalents of 9-(3-bromopropyl) adenine, **1**, were synthesized with the previous protocol only by changing the 9-(3-bromopropyl) adenine moiety equivalents resulting into 0.13, 0.25, 0.5, 0.75 and 1 mol eq. of additions of 9-(3-bromopropyl) adenine to PBI N4 nitrogen.

2.5. Methyl p-toluenesulfonate binding experiments

Batch scavenging experiments were performed in duplicate, loading 2 mL Eppendorf tubes with 50 mg of each polymer and 1 mL of a 100 ppm solution of MPTS in DCM. The suspensions were stirred for 24 h at 200 rpm and at room temperature. After this time the samples were centrifuged and the supernatant filtered

and analyzed by HPLC for quantification of MPTS concentrations. The amount of GTI bound to the polymers was calculated from Eq. (1).

$$Q = V[C_0 - C_e]/M \qquad (1)$$

where Q (mg/g) is the amount of GTI bound to the polymer, C_0 (ppm) is the initial GTI concentration, C_e (ppm) is the equilibrium concentration of GTI in solution, V (L) is the volume of solution used and M (g) is the polymer mass.

2.6. Release and quantification of p-toluenesulfonic acid on PBI-A$_{12\%}$

Samples of 50 mg of PBI-A$_{12\%}$ were loaded into a 1000 ppm MPTS DCM solution. The suspensions were stirred for 24 h at 200 rpm and at room temperature. After this time the samples were centrifuged and the supernatant filtered and analyzed by HPLC for quantification of MPTS concentrations. The filtered solids, containing the polymer loaded with GTI, were then washed for 2 h at room temperature with 1 mL of a 0.05 M NaOMe solution prepared in MeOH or with 1 mL of a 5% NEt$_3$ solution prepared in MeOH. After this time the samples were centrifuged and the supernatants were filtered and analyzed by HPLC. The samples were further washed for 2 h at room temperature with 1 mL of MeOH and after this step the samples were again centrifuged and the supernatants were filtered and analyzed by HPLC for quantification of PTSA. The detection and quantification of PTSA in the polymer before and after the washes was also determined by ^{1}H NMR using an internal standard, 1,3,5-trimethoxybenzene.

3. Results and discussion

3.1. Synthesis of adenine derivative 1 and PBI-A$_{x\%}$

The functionalization of PBI polymer with a moiety of a purine base, adenine, consisted in the reaction of the halide alkylated adenine with the PBI in a DMSO solution in the presence of a base, K$_2$CO$_3$ (Scheme 1), to deprotonate the acidic proton from PBI N4

nitrogen, allowing such nitrogen to be alkylated by an organic halide with formation of potassium halide. To accomplish this goal, it was necessary to optimize first the synthesis and isolation of the halide alkylated adenine, in the particular case 9-(3-bromopropyl) adenine, followed by optimization of the alkylation of the polymer, PBI, modified with such purine base and of the isolation procedure for the polymer obtained.

3.1.1. Optimization of 9-(3-bromopropyl) adenine synthesis and purification

The first challenge of the synthesis of the PBI polymer incorporated with alkylated adenine was to obtain the 9-(3-bromopropyl) adenine, **1**, in higher yield than the ones reported of 8% [26] and 38% [27], which impairs effective purification of the product for its use for PBI functionalization. The main challenges in this synthesis are the formation of two side products, the dimer **A** and the tricyclic derivative **B** (Scheme 2). The optimization of such synthesis and purification procedure was achieved in iterative improvements over the information reported in the literature [28]. After initial studies, the optimization of the 9-(3-bromopropyl) adenine synthesis was studied assessing three different protocols resumed in Table 1. A detailed description and discussion of the results obtained are included as supplementary information. Overall, the synthesis was accomplished improving the yield to 61%, which is considerably higher than the reported data.

3.1.2. PBI functionalization with 9-(3-bromopropyl) adenine

The challenges to incorporation of 3-bromoadenine in the PBI are numerous and include hydrogen deprotonation of the PBI N4 nitrogen and the solubility of the polymer in a suitable solvent. PBI is reported to be a polymer stable in a wide range of solvents (i.e. this polymer is not soluble and does not swell in most of the solvents) However, PBI is soluble in DMSO and therefore the first attempt to synthesize a PBI-adenine polymer was using a solution of 1% (wt/v) of PBI in DMSO with 2.2 M eq. of K$_2$CO$_3$ and reaction of 1 mol eq. of the 9-(3-bromopropyl) adenine at room temperature
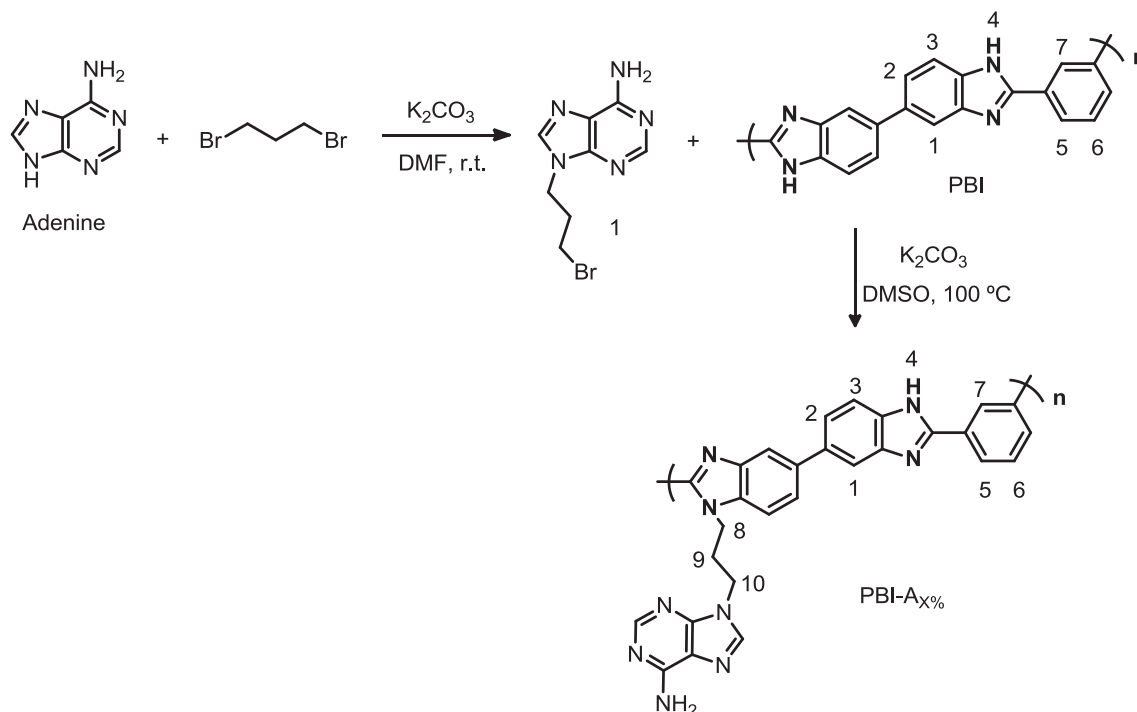
Scheme 1. Synthetic strategy of the alkylated adenine and the PBI-A$_{x\%}$ polymer.
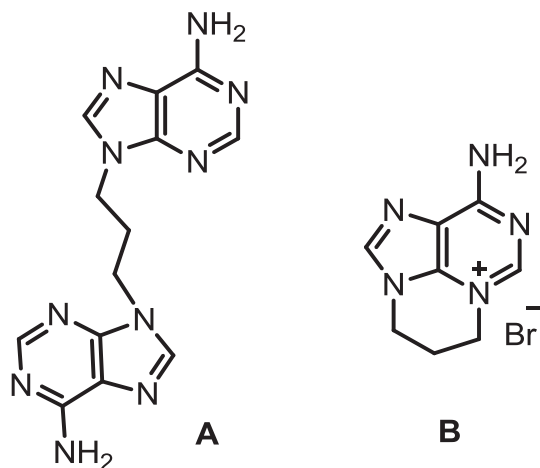
Scheme 2. Possible side products of the alkylation of the adenine, **A** and **B**.

Table 1
Reaction conditions assessed.

	Reaction I	Reaction II	Reaction III
Adenine	1 M	1 M	1.2 M
	0.50 g	0.50 g	0.50 g
	3.8 mmol	3.8 mmol	3.8 mmol
K_2CO_3	2 mol eq.	2 mol eq.	2 mol eq.
	1 g	1 g	1 g
	7.2 mmol	7.2 mmol	7.2 mmol
DMF	3.7 mL	3.7 mL	3.0 mL
1,3-dibromopropane	3 mol eq.	5 mol eq.	5 mol eq.
	1.14 mL	1.9 mL	1.9 mL
	11.4 mmol	19 mmol	19 mmol
Reaction time	16 h	16 h	3 h[a]
Yield	35%	50%	61%

[a] The reaction was followed by TLC.

for 24 h. Considering PBI aqueous insolubility, water was used as co-solvent in a first unsuccessful attempt to isolate the modified polymer. Then acetone was further added as second co-solvent and left at −20 °C overnight with successful isolation by filtration from such cold solution of a small amount of a thin solid powder.

To improve yields, the starting PBI concentration in DMSO was increased to 15% (wt/v) and, considering the solution viscosity, dissolution was achieved stirring the polymer in a round bottom flask in DMSO for approximately 3 h at 160 °C. The solution was then cooled down to room temperature for addition of the K_2CO_3 (2 eq.) and 9-(3-bromopropyl) adenine (1 eq.) and then, to allow stirring of this viscous solution, the reaction was carried out at a temperature of 50 °C for 24 h. Water was added to this solution to precipitate the resulting solid. Two additional temperatures, 80 °C and 100 °C were assessed for the reaction step, maintaining the procedure for the polymer reaction time of 24 h, reagents concentrations (PBI 15 wt/vol% in DMSO), K_2CO_3 and 9-(3-bromopropyl) adenine 2 and 1 mol eq. to PBI benzimidazole ring (i.e. N4 nitrogen), respectively, as well as polymer isolation procedure.

MPTS binding was preliminary assessed mixing the different polymers obtained (50 mg Polymer/mL) with 100 ppm MPTS in DCM solution for 24 h at room temperature, thus ensuring equilibrium was reached. The MPTS binding percentage obtained was similar at a value of 24% for both polymers prepared at lower reaction temperatures (50 °C or 80 °C), but a significantly higher MPTS binding was obtained, at a value of 59%, for polymers prepared at 100 °C. In the latter case similar MPTS affinities were obtained

regardless the K_2CO_3 content used. Therefore, for the following studies the polymers were prepared adding 1 mol eq. of K_2CO_3 and 9-(3-bromopropyl) adenine to a pre-prepared 15% (wt/v) PBI solution in DMSO at 50 °C and increasing the temperature to 100 °C for 24 h reaction under stirring.

In the preliminary binding assays, the HPLC chromatograms presented some peaks most probably related with products from reactions of MPTS with unreacted 3-bromoadenine species or impurities formed in the reaction for functionalization of the polymer. Therefore, the polymer isolation procedure was successfully upgraded to remove the species observed. After polymer precipitation, a first washing step with water was performed to remove K_2CO_3 and other salts formed and a final step with DCM, the solvent used in genotoxins binding, was also included to remove organic species. The success of such washing sequence depends on including an intermediate washing step, between water and DCM washing, with MeOH, a solvent mixable with both water and DCM. Note that both DCM and MeOH are able to solubilize alkylated adenine.

Considering the established protocols for PBI functionalization and isolation, the effect of different reaction molar equivalents (RME) of 9-(3-bromopropyl) adenine in the efficiency of GTI binding to the modified polymers was studied preparing polymers using 0.13, 0.25, 0.5, 0.75 and 1.0 RME of 9-(3-bromopropyl) adenine added per benzimidazole PBI ring. The polymers obtained will be named as PBI-Adenine, PBI-A$_{x\%}$, where X refers to the molar degree percentage of alkyl adenine incorporated in the N4 PBI nitrogen.

3.1.3. ^1H NMR quantification of incorporated adenine in PBI

To quantify the percentage of modified adenine that was inserted in the polymer, we obtained the ^1H NMR spectra of the several polymers at 80 °C dissolved in DMSO-d_6. The use of N4 nitrogen proton signal is not appropriate to assess the effectiveness of the reaction of PBI with 9-(3-bromopropyl) adenine, since it is a labile proton that exchanges with residual water present in the solvents. Therefore, other regions of the ^1H NMR spectra were used to assess the degree of PBI functionalization.

Scheme 3 illustrates the structure of the PBI-A$_{x\%}$ polymer with assignment of the protons used in the quantification of insertion of alkylated adenine. Fig. 2 presents the 5–1.5 ppm section of the ^1H NMR spectra of the several polymers modified with different equivalents of adenine moiety. Some new signals are visible in the modified polymer, comparatively to the initial PBI [29], but only the signals at 2.01, 3.42 and 4.03 ppm (Fig. 1) were confirmed by the COSY of the functionalized polymer, to be related with the
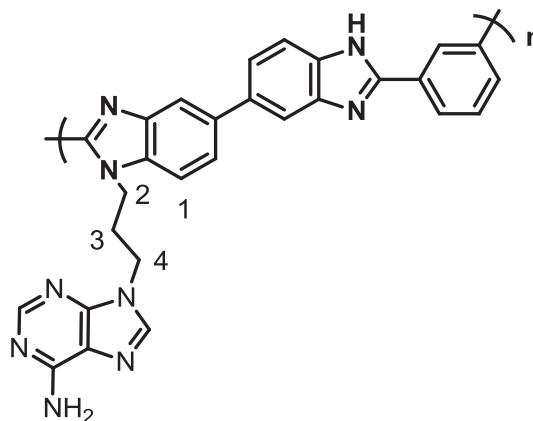
Scheme 3. Structure of the PBI-A$_{x\%}$ polymer with assignment of the protons used in the quantification of insertion of alkylated adenine.
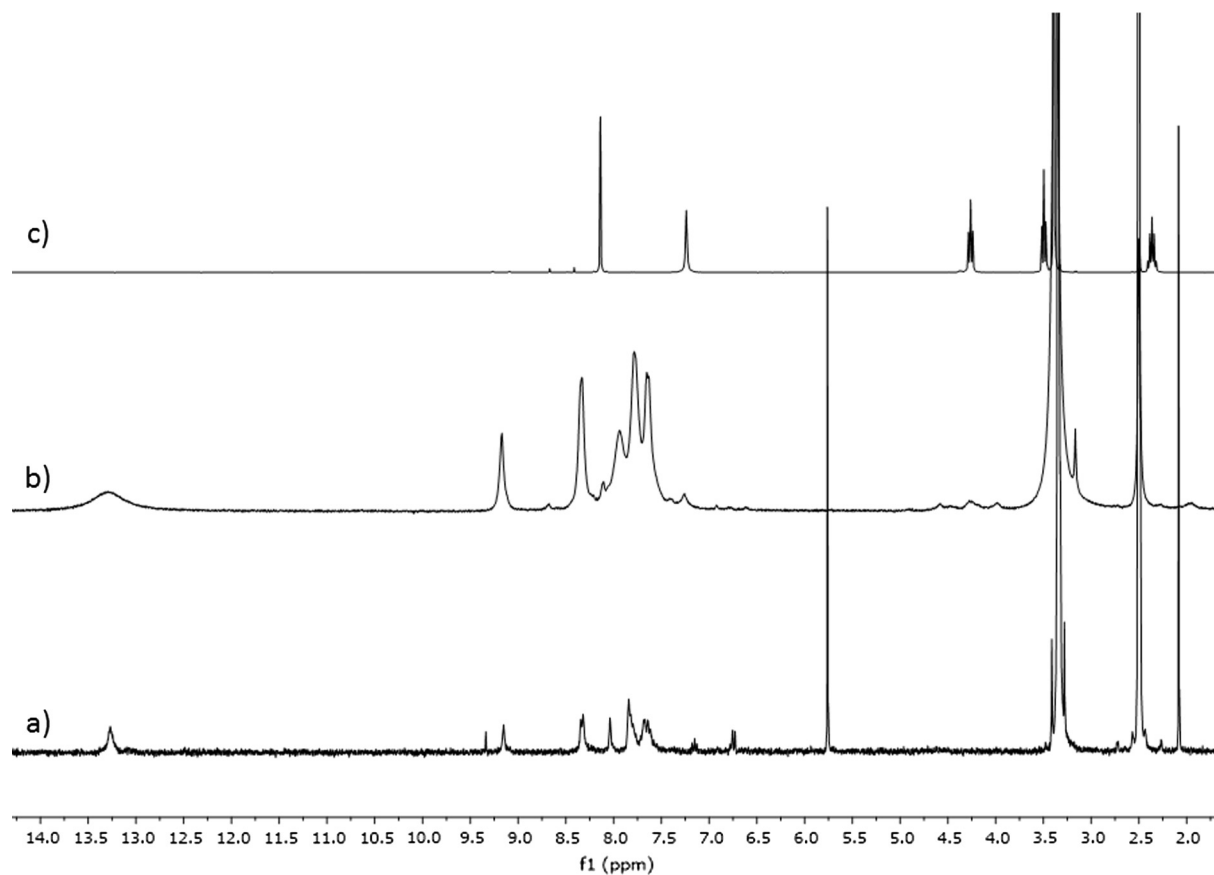
Fig. 1. Comparison of the several ^{1}H NMR spectra of the polymers (a) PBI as received, (b) PBI with adenine functionalization and (c) 9-(3-bromopropyl) adenine.
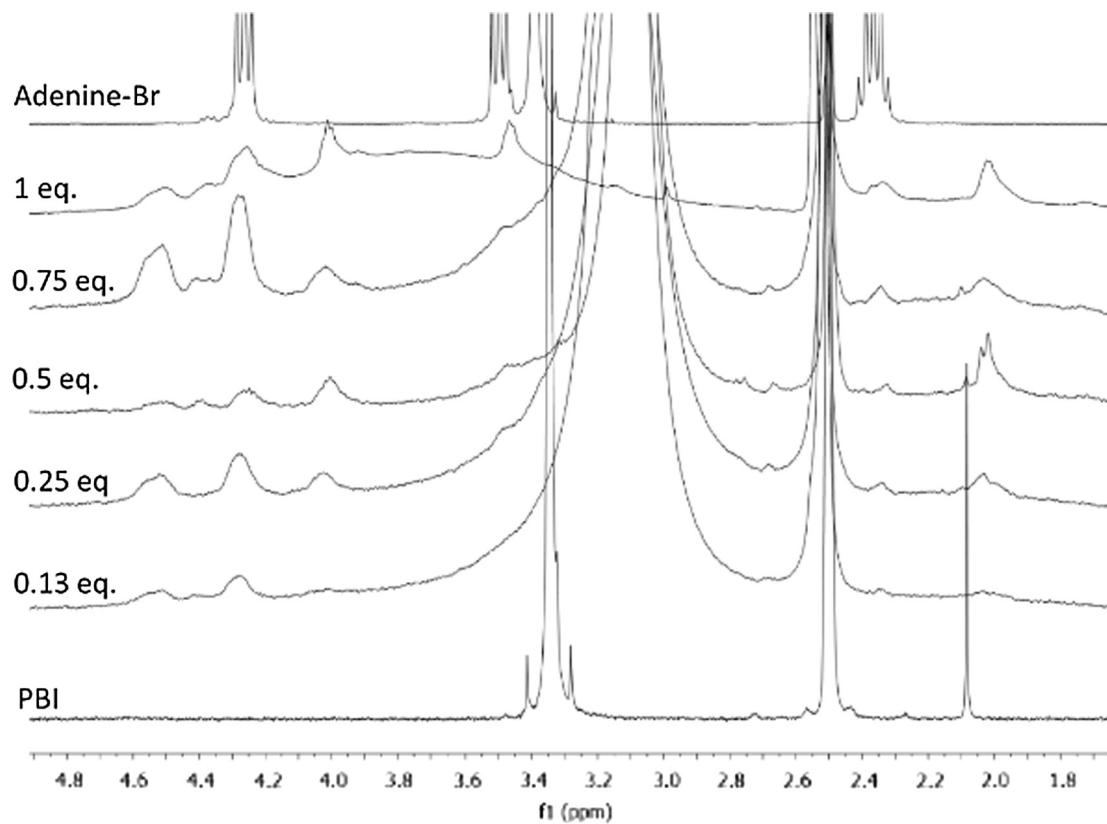

Fig. 2. ^{1}H NMR spectra of the several polymers modified with different equivalents of adenine moiety, 1, (5–1.5 ppm).

alkyl chain. In the spectrum resulting from the COSY experiment (Fig. 3), we observed correlation between the signal at 2.01 ppm with two others, at 3.42 and 4.03 ppm, which lead us to conclude that the 2.01 ppm signal relates to the H-3 of the propyl chain (Scheme 3). The peak at 3.42 ppm is overlapped with the water signal, so it will not be used to quantify the molar degree incorporation (MDI) of the alkyl adenine in the PBI polymer. The two other peaks of the alkyl chain of the adenine, at 2.01 and 4.03 ppm, are integrated, separately and used for MDI quantification. In the ^{1}H NMR section 9.5–6.7 ppm spectra for both untreated PBI and PBI-adenine polymers, is visible a signal at 9.16 ppm (Fig. 4), which is related to one of the aromatic protons of the PBI chain, H-1 (Scheme 3). Once N4 is alkylated, an additional signal is observable at 8.69 ppm, this peak represents a deviation of the original signal at 9.16 ppm (see 2D NMR spectrum in Fig. S3 in supplementary information), when the H-1 protons, due to different electronic environment resulting of the insertion of adenine moiety, appear at a higher field. So, for MDI quantification the sum of the integration area for signals at 9.16 and 8.69 ppm is considered as the representative of the total area of H-1.

Therefore, MDIs were estimated as the percentage ratios of the integrated area of the signal 2.01, 4.03 or 8.69 ppm by the sum of the integration area of signals at 9.16 and 8.69 ppm (Table 2). Incorporation efficiency (IE) is calculated as a yield from the ratio between MDI/RME (i.e. molar degree of incorporated alkyl adenine in the PBI polymer estimated from ^{1}H NMR by the reaction molar equivalents of 9-(3-bromopropyl) adenine added per PBI benzimidazole ring used in the respective reaction). We discarded the MDI calculated on the basis of 2.01 and 4.03, when a RME of 1 was used, since the values obtained were unreasonably high.

The values obtained using the different signals are coherent among each other (Table 2). Previous reports quantify the insertion of alkyl chains in the PBI chain by the ratio between the 10 protons of the aromatic backbone of PBI and the protons relatively to the moiety inserted. Applying this approach to our case, the obtained results are in agreement with the values assessed with our methods (Table 2) [30,31]. Overall, we observed that the reaction of PBI for higher RME, i.e. at higher equivalents of alkylated adenine, becomes less efficient, maybe due to the hindrance of the purine base that affects the structure of the polymer. It is reported that the degree of modification in the PBI is not dependent of the concentration of the base (K_2CO_3) or the electrophile (i.e. the 9-(3-bromopropyl) adenine), which is in accordance with our results [20 l].

3.2. Binding experiments

3.2.1. Binding experiments and nitrogen gas porosimetry

The several modified PBI-$A_{x\%}$ polymers prepared with different alkyl adenine incorporation moieties were assessed concerning their ability to bind methyl *p*-toluenesulfonate (MPTS) from a 100 ppm solution in DCM. This GTI was selected as a representative of the sulfonate ester family and easiness on development of a quantification method by HPLC-UV. Additionally, the commercial available PBI powder, i.e. the very same starting material used for PBI-$A_{x\%}$ preparation, was used to establish the MPTS binding in raw PBI. Results depicted in Fig. 5 show that almost all the alkyl adenine modified PBI polymers exhibit higher binding towards the sulfonate than the starting raw PBI material. An exception was the PBI-$A_{8\%}$, which presented the lowest GTI binding (15.7 ± 0.4)%, at a value not statistically different (p > 0.10) of the one obtained for raw PBI (10.2 ± 2.8)%, implying that a 8% adenine MDI was not sufficient to improve polymer binding towards MPTS. However, an increase in adenine MDI to 12% resulted in an extremely high binding (96.6 ± 0.2)% of PBI-$A_{12\%}$ towards the GTI assessed. Further improvements in adenine MDI resulted in higher MPTS binding percentage (>50% vs 10% for raw PBI), but at values lower than the ones obtained for PBI-$A_{12\%}$, which may result from
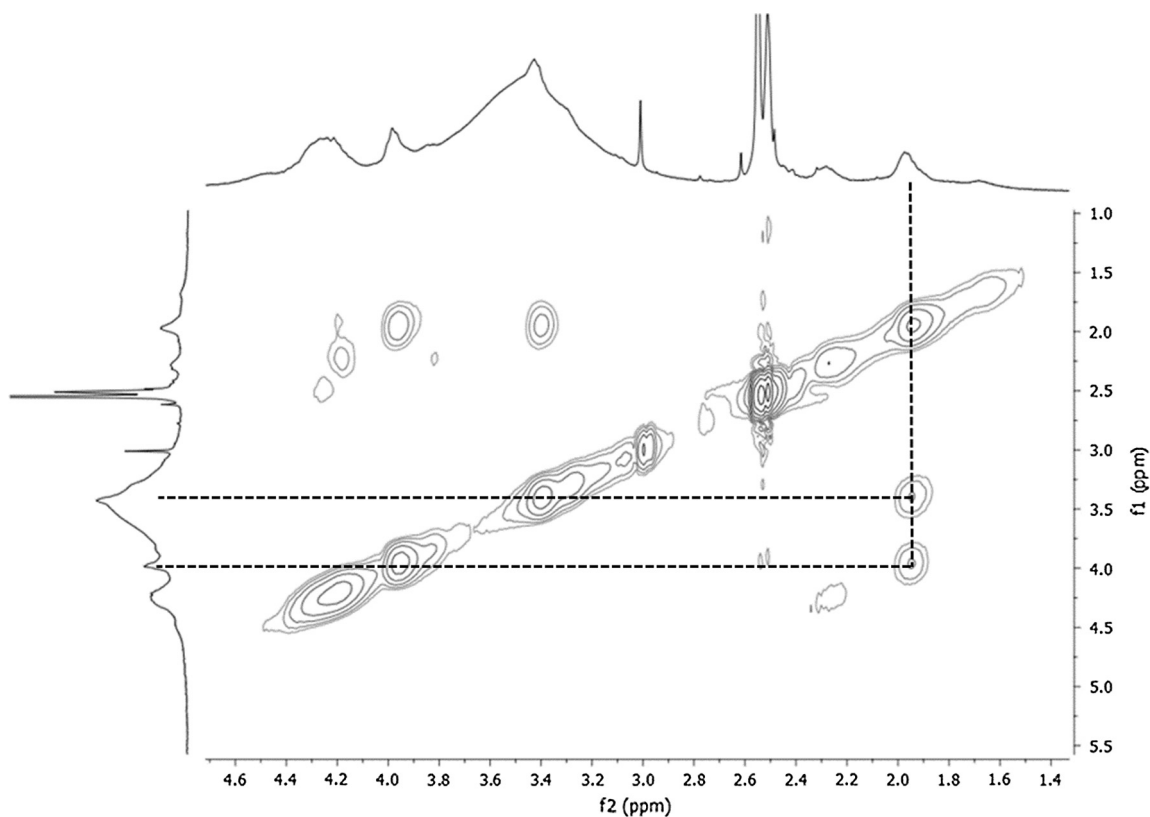

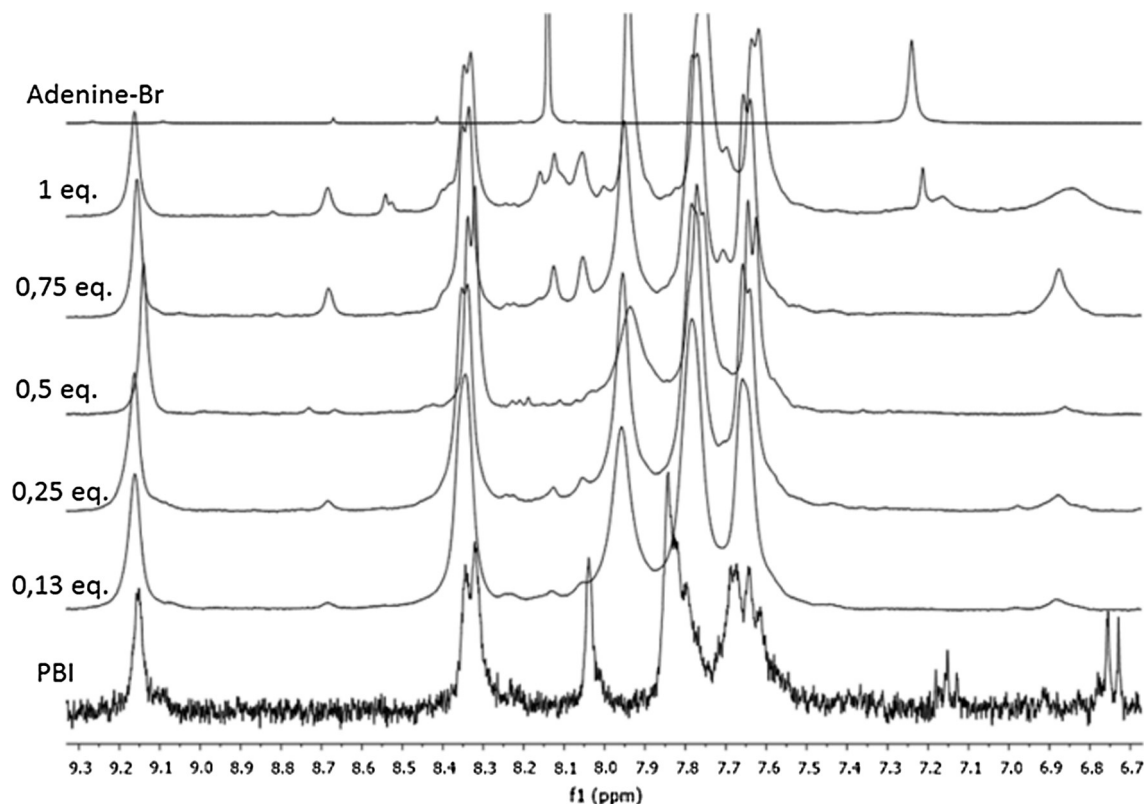**Fig. 3.** COSY NMR spectrum for the PBI that reacted with 1 equivalent of alkylated adenine.

Fig. 4. ^{1}H NMR spectra of the several polymers modified with different equivalents of adenine moiety, **1**, (9.5–6.7 ppm).

Table 2
Observed adenine moiety **1** incorporation efficiency and determined adenine incorporated in the PBI (mol%) for different amounts of **1** used (equivalents) based on three ^{1}H NMR signals.

Adenine (eq)[a]	Polymer	ppm (mol%)[b]			Incorporation efficiency of **1** (%)[c]
		4.03[d]	8.69	2.01[d]	
0.13	PBI-A$_{8\%}$	9	7	9	54–69
0.25	PBI-A$_{12\%}$	13	10	13	40–52
0.5	PBI-A$_{15\%}$	15	15	15	30
0.75	PBI-A$_{20\%}$	20	20	20	27
1	PBI-A$_{29\%}$	–	29	–	29

[a] Number of adenine moiety **1** equivalents used in the alkylation of PBI.
[b] Observed (mol%) of adenine incorporation in relation to the PBI monomer.
[c] Calculated (mol%) of adenine incorporation regarding to the amout (mol) of adenine moiety **1** used.
[d] Calculated by the ratio between the 10 protons of the backbone of PBI and the protons relatively to the moiety inserted.
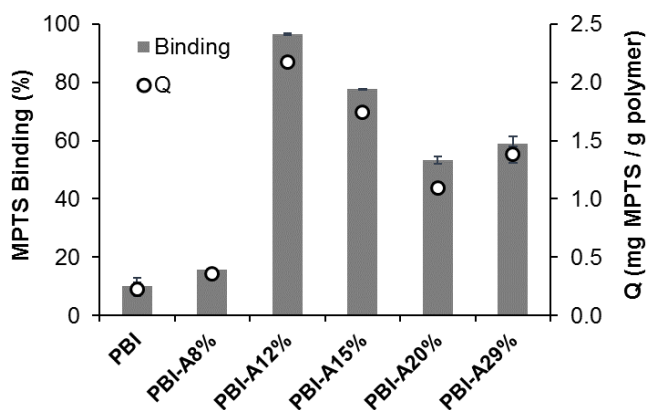

Fig. 5. Binding percentage and adsorption capacity of the several polymers towards MPTS. 1 mL of a 100 ppm solution of MPTS in DCM was loaded on 50 mg of raw and modified PBI polymers.

stereochemical hindrance on chain position to genotoxic molecule binding. The binding efficiencies were also obtained in specific experimental conditions from a 100 ppm solute concentration and a fixed amount of polymer, thus corresponding to a single point of adsorption isotherm. For further insights in MPTS binding to the modified PBI polymers is important to establish the surface area per g for each polymer tested and learn about the nature of the chemical bond established between the polymer and sulfonate. The MPTS binding values reported correspond to equilibrium values at room temperature, collected after 24 h assays; note that, for example for PBI-A$_{20\%}$, MPTS removal from a 100 ppm solution is limited to only 58%. Further improvement in kinetics may require operation temperature optimization.

In Fig. 5 is also reported the adsorption capacity achieved at the conditions assessed. The use of amine based functional groups to remove genotoxins, such as MPTS, has been presented as a promising strategy. Lee et al. [32] and Kecili et al. [33] report the use of nucleophilic ethylenediamine and trisamine (porous silica or

Table 3
Physical properties of PBI and PBI-adenine polymers, obtained by multipoint BET method.

	BET surface area ($m^2 g^{-1}$)	Total pore volume ($cm^3 g^{-1}$)	Average pore diameter (nm)
Raw PBI	n.d.	n.d.	n.d.
PBI-A$_{8\%}$	6.11	0.0225	16.1
PBI-A$_{12\%}$	20.18	0.139	24.9
PBI-A$_{15\%}$	32.13	0.181	22.6
PBI-A$_{20\%}$	36.30	0.213	23.5
PBI-A$_{29\%}$	36.06	0.212	22.6

n.d. = not determined.

macroporous polystyrene-divinylbenzene based) resins, respectively, to be able to remove 100% of MPTS from methanol or 2-propanol:THF (1:1) solutions. These studies were performed from a 1 ppm and 5 ppm solutions with 100 mg of diamine and 150 mg of trisamine based scavengers (corresponding to adsorption capacities of around 0.01 mg GTI/g diamine resin and 0.03 mg GTI/g trisamine scavenger). In the current work, 96.6% of GTI removal is achieved from a 100 ppm solution with 50 mg of polymer (adsorption capacity of 1.932 mg GTI/g polymer). Although, the diamine and trisamine functionalities may resemble the -NH$_2$ group interaction expected with the GTIs, it cannot replace the global intrinsic chemical structure of a DNA base. Therefore, the exploration of PBI-adenine is a more realistic approach to mimic interactions between potential GTI molecules and the DNA. Additionally, in a previous report, a specific molecular imprinted polymer was prepared targeting MPTS, achieving 15–45% MPTS removal from 1000 ppm solutions in DCM [34]. An additional binding efficiency of MPTS to PBI-A12% %, using the same from a 1000 ppm MPTS concentration, in DCM was established at a value of (82.9 ± 3.8)% after 24 h.

Nitrogen gas porosimetry and respective isotherms and Brunauer–Emmett–Teller (BET) theory was used to estimate BET surface area, the total pore volume and the pore size (Table 3). The isotherms for the raw PBI show an irregular behavior, not allowing to calculate BET parameters for these samples. In the isotherms of the modified polymers we observe that the BET surface area and the total pore volume per gram of polymer, as well as the pore size, increase for the polymers with higher amount of alkylated adenine incorporated. A significant increase in surface area from 6 to 20.15 $m^2 g^{-1}$ was observed from polymer PBI-A$_{8\%}$ to PBI-A$_{12\%}$, which can be correlated with the increase in MPTS binding efficiencies. However, further increases in surface area (observed with increased MDI values of 32.13, 36.30 and 36.06 $m^2 g^{-1}$ to PBI-A$_{15\%}$, PBI-A$_{20\%}$, PBI-A$_{29\%}$, respectively) result in decreases in MPTS binding, indicating that other factors than surface area affects binding efficiency.

Additionally, it was not found significant differences between the structure of the functionalized PBI particles, when examined by scanning electron microscopy (see supplementary information for images and details).

3.2.2. Assessment of binding by ^{1}H NMR

Methyl p-toluenesulfonate (MPTS) was selected as representative of sulfonate family, which are known GTIs. To understand the phenomena ruling the interaction between the adenine modified polymer and the GTI, we performed a ^{1}H NMR study in DMSO-d_6 at room temperature in which we obtained the spectra of PBI-A$_{12\%}$ before and after 2, 7 and 24 h of addition of the GTI (Figs. 6 and 7). The analysis of the spectra in Fig. 6 shows the disappearance of the aromatic signals (7.79 and 7.49 ppm) related with the sulfonate and the appearance of new signals (7.51 and 7.15 ppm). Fig. 7 shows the 4.2–2.2 ppm region of the spectra, where we observed the disappearance of the peaks relative to
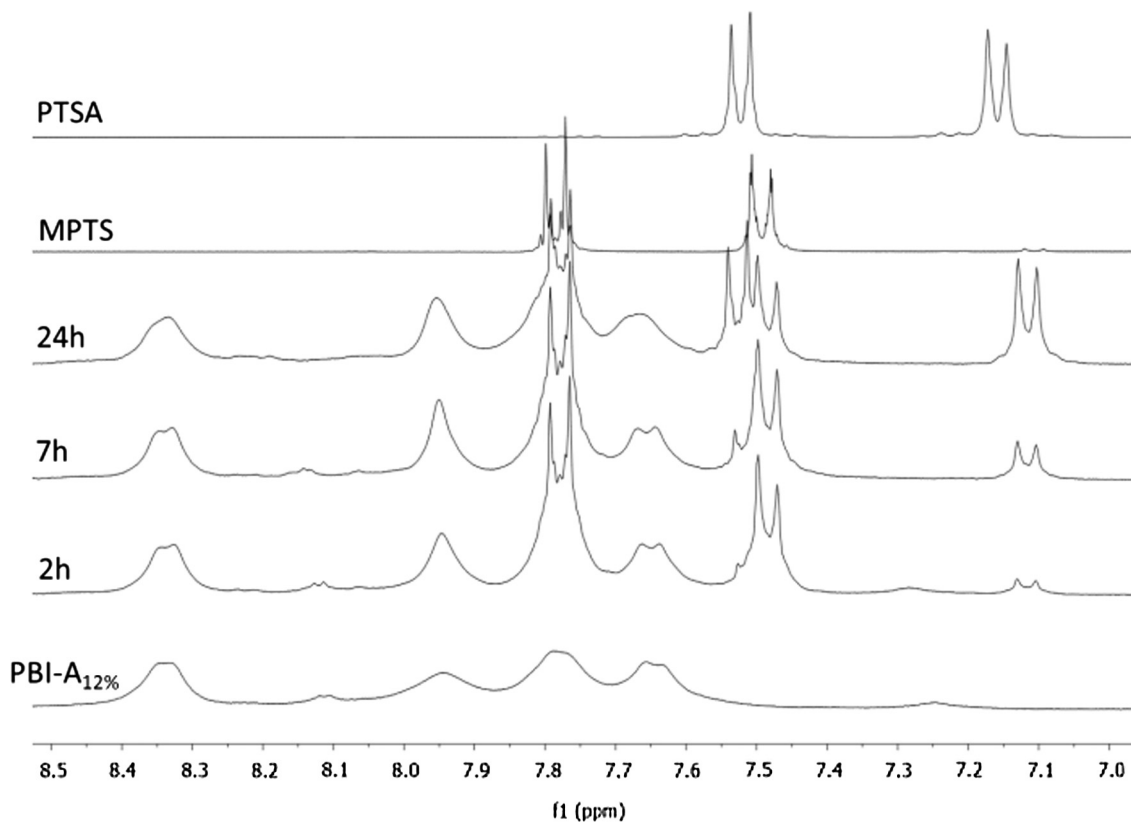

Fig. 6. ^{1}H NMR spectra of the PBI-A$_{12\%}$, the polymer in the presence of MPTS at different time, MPTS and PTSA in DMSO-d_6 (8.5–7.0 ppm).

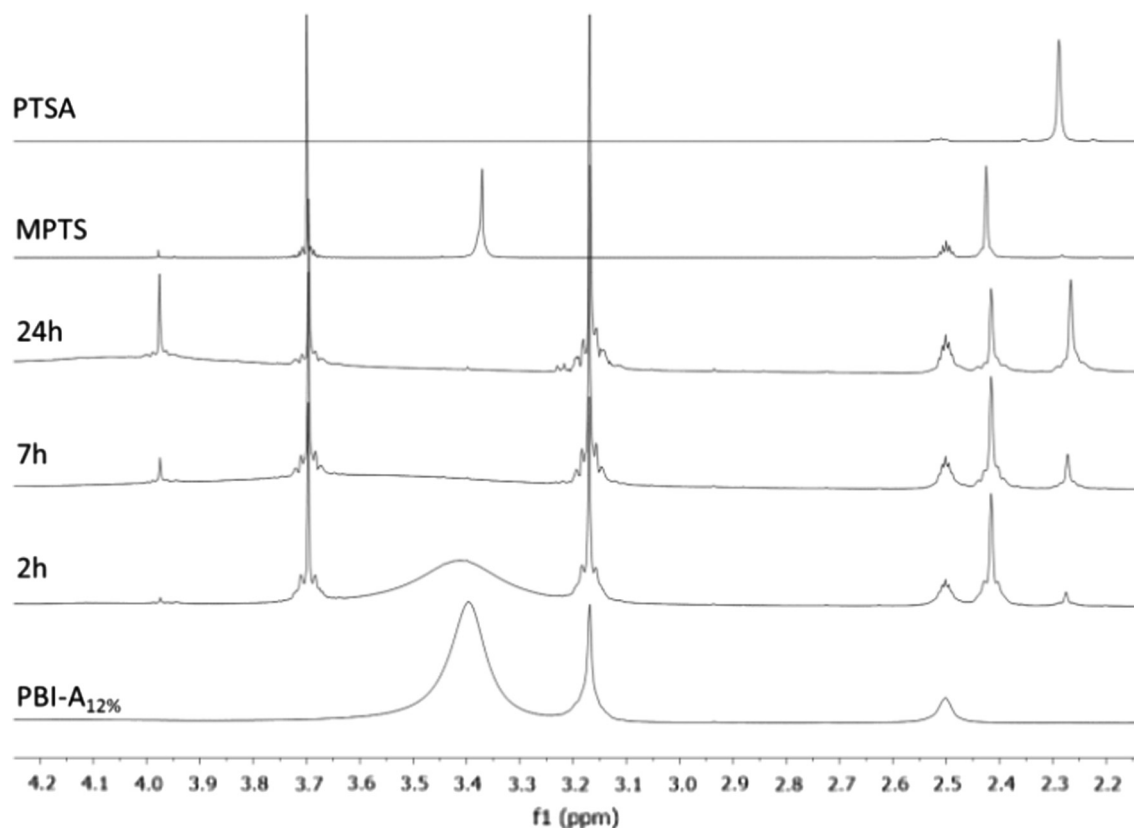**Fig. 7.** ^{1}H NMR spectra of the PBI-A$_{12\%}$, the polymer with MPTS at different time, MPTS and PTSA in DMSO-d_6 (4.2–2.2 ppm).

the methoxy protons, H-d (3.7 ppm) (Scheme 4) and the methyl protons, H-a (2.4 ppm), with simultaneous appearance of a new signal at 2.26 corresponding to the methyl group of PTSA. The behavior observed in the ^{1}H NMR spectra obtained along time supports the alkylation of the polymer adenine moiety by MPTS and consequent formation of the respective acid, PTSA (Scheme 4). In Figs. 6 and 7, in addition to the spectra of the modified polymer interacting with the GTI, it is also added the spectra of the PTSA and MPTS both alone, in DMSO-d_6. The appearance of the new peaks at 7.15 and 2.4 ppm are consistent with the formation of PTSA. However, the new signal at 3.96 ppm is not observed in

the spectrum of the PTSA, but could be explained by the methylation of the polymer (Fig. 7).

3.2.3. Release and quantification of PTSA of PBI-A$_{12\%}$ after binding with MPTS

Considering the additional binding efficiency of MPTS to PBI-A$_{12\%}$ from a higher, 1000 ppm MPTS concentration, still, no PTSA was detected in the analysis by HPLC of these DCM solutions in the end of the binding assays, suggesting that the PTSA generated remains strongly bound to the PBI-A$_{12\%}$ through ionic interactions.

To confirm that PTSA was indeed generated and attached to the polymer several attempts were made to assess the release of bound PTSA from the PBI-A$_{12\%}$ after binding with 1000 ppm of MPTS. Preliminary washes with MeOH or with a 0.1 M HCl solution in MeOH proved to be ineffective or only partially effective on removal of the PTSA from the polymer. However, washes with methanolic solutions of NaOMe or NEt$_3$ allowed the complete removal of the PTSA from the polymer. The analysis of the washing solutions by HPLC revealed only the presence of PTSA in quantitative recovery. These results were also supported by ^{1}H NMR. The washing solutions of the PBI-A$_{12\%}$ after binding, the washed polymer and PTSA were analyzed and compared. The efficiency of PTSA recovery through the washing procedures was confirmed by the observation of the ^{1}H NMR spectra of the polymer after washing and of the washing solution. For example, in the washing with NaOMe solution, no MPTS signals were observed and by superposition of the spectra (Fig. 8) we observed that PTSA was quantitatively removed from the polymer since the signals related to PTSA (7.51, 7.15 and 2.26 ppm) were absent from the spectrum of the washed polymer. Contrarily, these signals were only observed in the washing solution. This method revealed a quantitative removal of PTSA from the polymer which supports the previous results obtained by HPLC. This evidence is further supported
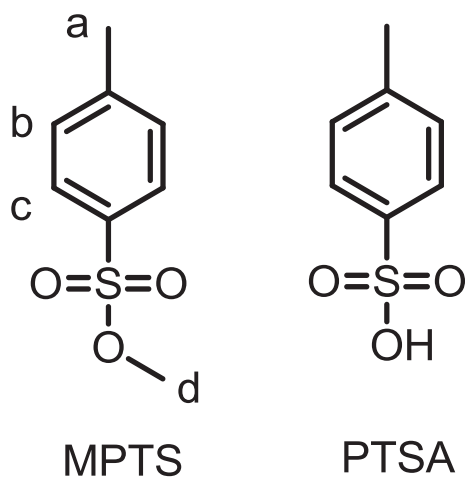
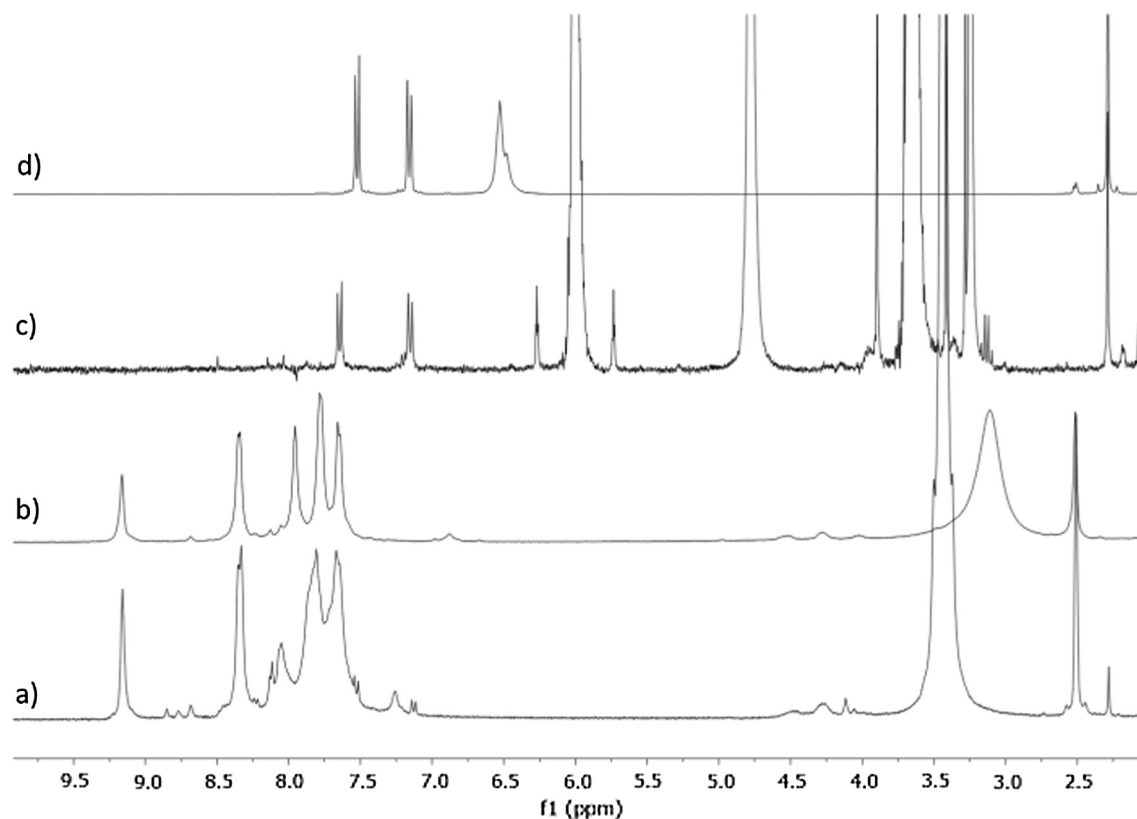**Scheme 4.** Structures of MPTS and the respective acid, PTSA.

Fig. 8. ^{1}HNMR spectra in DMSO-d_6 of: (a) the PBI-A$_{12\%}$ after binding with 1000 ppm of MPTS; (b) the PBI-A$_{12\%}$ after washing with methanolic solution of NaOMe; (c) washing solution; and (d) PTSA alone.

by the presence of the signals related to PTSA in the polymer spectrum after binding (Fig. 8a) and their absence in the spectrum of the polymer after washing with NaOMe solution (Fig. 8b).

4. Conclusions

The synthesis of an adenine-PBI polymer based was successfully accomplished with incorporations of adenine derivative between 8% and 29%. For the functionalized polymers we observed by nitrogen gas porosimetry, higher BET surface areas, pore and volume sizes for the polymers with higher incorporation of alkylated adenine. The highest MPTS binding percentage was obtained for the PBI-A$_{12\%}$ polymer at a value of 96.6%.

These results lead us to the conclusion that the binding with the GTI is not related with the BET surface areas, pore and volume sizes since there is no correlation between these values. The binding studies performed by ^{1}H NMR confirmed that the MPTS alkylates the PBI-A$_{x\%}$ polymer and PTSA is generated; with PTSA remaining strongly bound to PBI-A$_{x\%}$ and only efficiently removed using methanolic solutions of NaOMe or NEt$_3$.

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