Assessing separation processes for an aromatic and a chiral amine using Polybenzimidazole (PBI) membranes

Sara Alexandra da Silva Lopes
Instituto Superior Técnico, Universidade de Lisboa

Abstract: Amines are important chemical building block used in the organic synthesis and for production of compounds of pharmaceutical interest. In this context, it is important to develop separation processes and techniques to purify these types of compounds. Two types of separation are addressed in this thesis, resolution of chiral amines, i.e. separation of their enantiomers and removal of potential genotoxic impurities present in active pharmaceutical ingredients, which may cause DNA changes and subsequently cause cancer. Two amines were used as model for these studies in this thesis: Lupanine is a chiral amine and an interesting alkaloid to be used as a building block. 4-dimethylaminopyridine (DMAP) is regarded as a potentially genotoxic impurity because it contains alerting genotoxic structures. Several processes have been developed to solve the above-described problems, such as enzymatic resolution, chromatography, distillation, solvent extraction, nanofiltration, molecularly imprinted polymers. This thesis, explore the use of a material polybenzimidazole polymer (PBI) in various separation techniques. It was used successfully as scavenger, due to its ability of adsorb amines, either in beads or membranes adsorber or was used as a material to molecularly imprinted membranes used in nanofiltration and adsorption experiments. Additionally optimization of diastereomeric resolution was assessed, result on obtaining the fully pure D- (+)-Lupanine enantiomer using L-tartaric acid as resolving agent, however, using Di-*p*-toluoyl-tartaric acid (DTTA) as resolving agent resulted only in a maximum of 89.16% of L-(-)-Lupanine.

Key words: Amines, genotoxic impurity, active pharmaceutical ingredients, polybenzimidazole polymer, molecular imprinting membranes, diastereomeric resolution, Lupanine.

1. Introduction

Most pharmaceuticals are manufactured by applying an approach of total synthesis or modification of a natural product [1]. In both cases, a wide range of reactive molecules are used in synthetic reaction, many of these compounds are genotoxic or can form potential genotoxic compounds and thus may be present in the final active pharmaceutical ingredient (API) as impurities that can contaminate the final product and, ultimately reaching the patients. These type of impurities can induce genetic mutations which can result in cancer in humans [2]. A wide range of unrelated chemicals, with very different structures and from very different chemical families, have been categorized as genotoxic impurities (GTI) [3]. The development of simple and robust processes, using cost effective reagents to obtain high product yields through

selective reaction and purification steps, is extremely important for the industry. The presence of GTIs can be avoided or mitigated by developing new synthetic route or Quality by Design (QbD) strategies which includes adjusting parameters such as pH, temperature, reaction time or matrix. Moreover, synthetic routes already include several APIs and API precursors purification steps, which offer several purge options for PGTIS, still in spite of good practices in API synthesis development are enhanced purging of GTIS, there are cases in which addition separations steps may be necessary for GTI removal.

Others important separation in pharmaceuticals is resolution of chiral compounds. Louis Pasteur is known as the founder of stereochemistry because, in 1948, he was able to separate for the first time the two isomers of sodium ammonium tartrate [4]. However, only about a century later it was found that the phenomenon of chirality plays a key role not only in the life of plants and animals but also in pharmaceutical, agricultural and other chemical industries. In the pharmaceutical field, 56% of the drugs currently in use are chiral products and 88% of the last ones are marketed as racemic mixtures [5]. It is well established that the pharmacological activity is mostly restricted to one of the enantiomers (eutomer). In several cases, unwanted side effects or even toxic effects may occur with the inactive enantiomer (distomer). An enantiomer is one of two stereoisomers that are chiral, i.e., they are mirror images of each other are "non-superimposable" (not identical). Enantiomers nomenclature using (+) and (-) signs or d (dextro) and l (levo) or R and S. (+), d(dextro) or R means that the molecules rotate the plane of polarized light to the right (clockwise), whereas the and (-), l(levo) or S compounds make it rotate to the left or anticlockwise [4].

2- Methods

2.1- Binding’s tests

Binding assays were done by adding 50 mg of each PBI and 1 mL of lupanine or DMAP/Meta

2.2- Molecularly imprinted membrane

Polybenzimidazole (Fig.12) membranes with lupanine (Fig.4) imprinted were prepared by phase inversion. The commercial available 26 wt% PBI dope solution was diluted to 21% in DMAc and use as: (i) 21wt% PBI solution for preparation of non-imprinted membrane (NIM) and (ii) 21wt% PBI solution + 5 wt% (with regards to the polymer) of template for preparation of imprinted membrane (MIM). This solution was left under mechanical stirring at 50 rpm overnight in order to homogenize the solution. This solution was then cast on the polypropylene non-woven support using a casting knife of 250 µm. After the cast, the membranes were washed twice with distilled water (1h+1h) in the coagulation bath and then placed in a bath containing isopropanol for about 30 minutes (twice). The previous membranes were cut in 3 pieces: the first was used as control, the second was cross-linked with a solution of 3wt% of DBX in 100mL of MeCN and third was cross-linked with a solution of 3wt% of DBX in 100mL of MeCN + 1g of lupanine. The

cross-link reaction was carried out at 80ºC for 24h under constant stirring and reflux. After cross-linking, the membranes were first immersed in IPA to remove residual reagents.

2.3- Nanofiltration

The membranes were preconditioned by permeating pure acetonitrile solvent through the membrane until a constant solvent flux was obtained and for that, 200 mL feed solution was placed in the feed tank. 50 mL of a solution of lupanine in acetonitrile (1g/L) was passed through the membrane to quantify the rejection percentage of the membrane. As a final step we washed the membrane twice with 200mL of MeCN, in order to remove the lupanine trapped in the membrane.

2.4- Diastereomeric resolution

The general procedure for diastereomeric resolution consists in dissolving the racemic lupanine and the resolving agent (DTTA,TA), separately, in hot solvent and then mixing both solutions and leave to cool to room temperature. After that, the samples are left to recrystallize in the fridge.After 2-3 days, the mother liquor was separate from the crystals obtained and placed again in the fridge to allow the remaining compound to recrystallize (after the first crystallization, the other enantiomer is in excess and starts recrystallizing together with the chiral acid). The crystals formed are a salt of cationic lupanine (either its D or the L enantiomer) and the resolving agent used, in its anionic form.The crystals recovered after each crystallization step are washed with acetone, dried and then weighed to the yield of the recrystallization. After that, they are dissolved in approximately 20 mL of aqueous NaOH (1M) to neutralize the corresponding enantiomer. This leads to the deprotonation of the amine which has been protonated by the resolving agent, rendering it neutral and making possible its extraction from the solution with an organic solvent such as dichloromethane (DCM). The resolving agent is in the aqueous phase. The aqueous phase is extracted two times with 20ml of DCM. The organic phases are collected, dried over anhydrous sodium sulfate, filtered and evaporated to dryness in the rotavapor. The samples to be injected in the chiral HPLC (for quantification of the enantiomeric excess, e.e) were re-dissolved in DCM and passed through a Pasteur pipette with silica, to ensure the high purity of the samples injected in the chiral column. After evaporating the DCM to dryness, 2 mg of the lupanine were dissolved in 100 µL of IPA (HPLC grade), to which 900 µL of hexane (HPLC grade).

3- Results and discussion

3.1- PBI as scavenger

3.1.1- For Lupanine

In order to assess the PBI with higher lupanine binding in different solvents: H_2O, dichloromethane and acetonitrile with different PBIs were studied:

- PBI-Raw: PBI pristine

- PBI-T: PBI raw polymer with thermal treatment
- PBI-TA: PBI-T with acid treatment
- PBI-TB: PBI-T with basic treatment
- PBI-COOH (3C): PBI functionalized with carboxylic acid groups.

Table 1- Binding of lupanine

	% Binding Lupanine		
	1g/L		
	H_2O	DCM	MeCN
PBI-RAW	5.86 ± 1.50	2.3 ± 0.24	14.61 ± 2.95
PBI-T	20.76 ± 5.8	88.07 ± 3.7	54.86 ± 6.56
PBI-TA	31.55 ± 6.33	**93.62 ± 0.56**	**85.63 ± 1.96**
PBI-TB	**82.64 ± 2.65**	53.72 ± 10.20	66.39 ± 5.57
PBI-COOH (3C)	**>99.81**	49.21 ± 10.60	**92.66 ± 5.84**

At a concentration of 1 g/L in water, it can be observed that there is more binding with PBI-TB and PBI-COOH which may be due to the functional groups that these types of PBI contain. In the case of dichloromethane, the best result is observed with the PBI-TA that due to its acid composition ends up by better grasping the lupanine whereas in the case of acetonitrile the best binding is observed with the PBI-COOH where a hydrogen bond or even a covalent bond between the amine group (R-NH) of lupanine and the carboxylic group (R-COOH).

After the binding's, the supernatant was removed and PBI was allowed to dry and then 1mL of dichloromethane was added to the polymer leaving 24h under stirring at 200rpm.

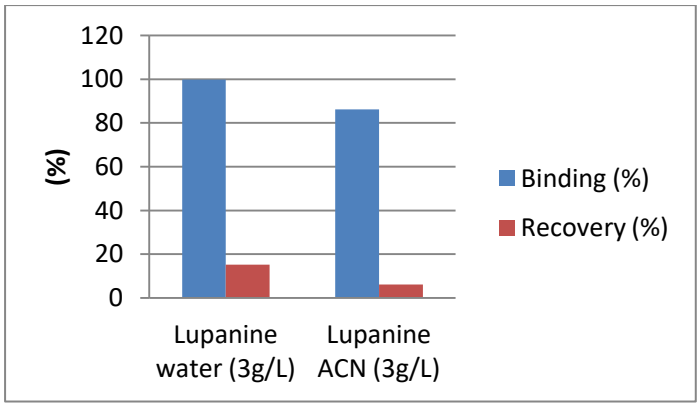

Fig. 1- Regeneration of PBI with DCM

As can be seen in figure 23, dichloromethane was not efficient in the regeneration of PBI. It is necessary, in future works, to strengthen and deepen the regeneration of PBI trying to find the best solvent to use. The use of alcohols such as methanol or ethanol may be a possibility as they break down hydrogen bonds that form during binding as well as exploit the use of NaOH or HCl to regenerate the PBI in order to break down previously formed ionic interactions.

3.1.2- For DMAP and Meta

Table 2- Binding of DMAP and Meta in DCM

Binding (%)		
	DMAP	Meta
Initial concentration	1000 ppm	10000 ppm
Raw PBI	2.88 ± 1.85	6.07 ± 0.34
PBI-T	78.90 ± 0.49	4.24 ± 3.47
PBI-TA	**> 99.95**	1.62 ± 0.32
PBI-TB	**93.55 ± 0.03**	8.90 ± 3.39
PBI-COOH (3C)	**95.77 ± 0.24**	3.92 ± 3.85

The best results are relative to the PBI-TA and PBI-COOH (3C) where an almost 100% DMAP adsorption and a low adsorption of Meta, which is the objective of our work, is verified.

Relative to the Meta molecule, low binding values can be caused by stereochemical impediment, thus making it difficult to bind the PBI to Meta due to the shape of its molecule. As can be seen in figure 41, the Meta molecule does not have many sites where it binds to the PBI, most likely it may be the formation of a hydrogen bond of the -OH group or a hydrogen bond of the oxygen double bond as shown in the figure. Access to the -OH group should be hampered by the geometry of the molecule.

In the case of PBI-COOH and PBI-TB there is an ionic interaction with the COO- of the PBI and the protonated nitrogen of the DMAP.

After the binding's, the supernatant was removed and PBI was allowed to dry and then 1mL of MeOH was added to the polymer leaving 24h under stirring at 200rpm. The same procedure was performed with DCM.

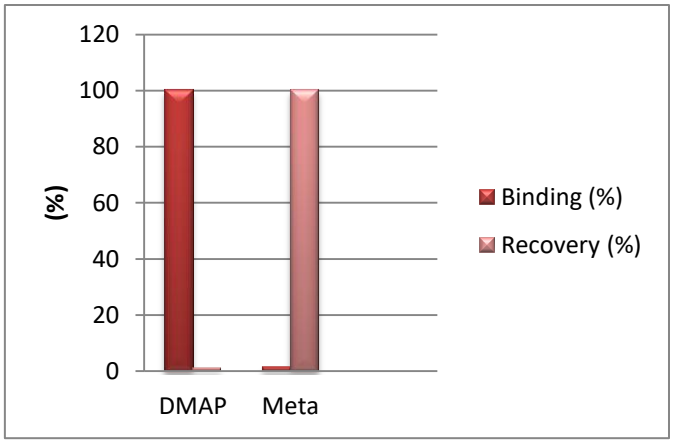

Fig. 2- Regeneration of PBI with DCM

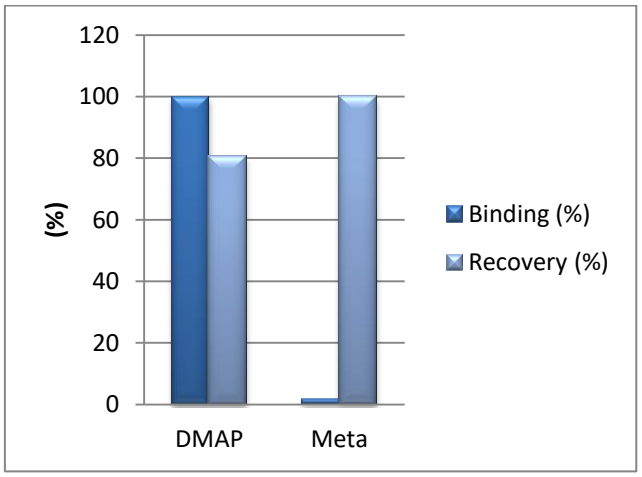

Fig. 3- Regeneration of PBI with MeOH

As can be seen in figure 27 and 28, dichloromethane was not efficient in the regeneration of PBI in the case of DMAP but with MeOH only about 20% of DMAP remained in PBI. In the case of Meta, both DCM and MeOH result for the total recovery of Meta and complete regeneration of PBI.

3.2- Nanofiltration with PBI membranes

In this section three different membranes were tested:

i. NIM: non-imprinted membrane
ii. MIM L-(-)- Lupanine: imprinted membrane with L-(-)-Lupanine as template
iii. MIM: imprinted membrane with racemic lupanine as template

Each membrane was placed in filtration cell and conditioned first with pure acetonitrile and after that a solution Lupanine in MeCN (1 g/L) was filtered to record rejection of each type of membrane.

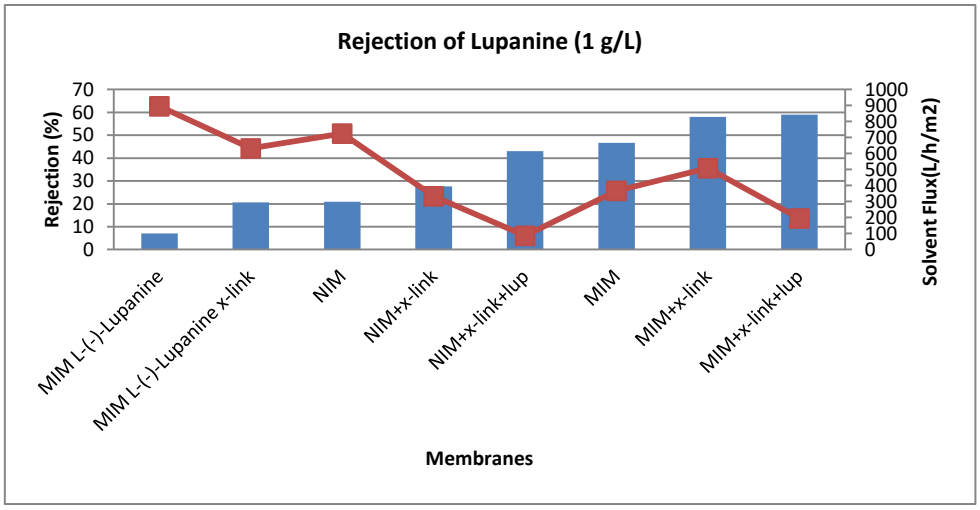

Fig. 4- Rejection and solvent performance of membranes

X-link: while the rejection increases, the solvent flow decreases because pores between polymer chains are smaller. With respect to the solvent flux In the non-imprinted membranes, the results show decrease flow with x-link as more closed/tight membrane with small pores is obtained. The same trend is observed for membranes prepared using as template the pure lupanine; however for membranes prepared using racemic lupanine as template this trend is no longer observed.

X-link+lupanine: In this case, the rejection is extremely low and the solvent flow extremely high compared to the other membranes. Since lupanine is a base it may be further closing the polymer matrix having greater interaction with PBI.

Rejection: in respect of imprinted membranes, the amount of template is the same but, the value of rejection it's quite different. The rejection is greater on the imprinted membranes than on the non-imprinted membranes and lower when imprinted with the enantiomer L-(-)-Lupanine. Higher rejection values means that more Lupanine is retained. The high rejection value may mean the molecular recognition of the Lupanine molecule and when the membrane undergoes the x-link process, it causes the molecule to be further retained; for membrane imprinted with the enantiomer as a template, this rejection value may mean facilitated transport of the molecule. For membranes imprinted with the pure enantiomer, the enantiomeric excess of permeate and retentate was analyzed and both was found to be racemic mixture, i.e. there was no selectivity for only one enantiomer of lupanine as had been thought.

3.4- Resolution by formation of diastereomeric salts

3.4.1- Optimization of diastereomeric resolution by recrystallization using as resolving agent TA and DTTA

i. **Diastereomeric resolution by recrystallization with tartaric acid: screening of the best solvents:** Initially, three samples with different solvents: EtOH, MeOH and MeOH+IPA were prepared. We started with 1g racemic lupanine and 1.1 mol equiv of L- tartaric acid.

Table 3- Sample of Lupanine with 1.1 mol equiv of L-tartaric acid

Sample	Concentration of lupanine (g/mL)	Solvent	Volume of solvent (mL)	Yield (%)	e.e (%)
A	0.334	Ethanol	7	9.47	20 % de D(+)
B	0.253	Methanol	6	21.47	Pure D-(+)-lupanine
C	0.342	Isopropanol +Methanol	6 (3+3)	42.36	Pure D-(+)-lupanine
D	0.286	Acetone	6 (3+3)	Formation of a viscous solution	

As shows in table 4, methanol and the mixture of isopropanol/methanol proved to be the best resolution solvent for the racemic lupanine with L-tartaric acid. In both cases, it was obtained the D-(+)-Lupanine enantiomer but, in the case of methanol the yield obtained was higher.

ii. **Diastereomeric resolution by recrystallization with Di-*p*-toluoyl-L-tartaric acid and Di-*p*-toluoyl-D-tartaric acid: screening of the best solvents:** Initially, four samples with different solvents: EtOH, MeOH, IPA and acetone were prepared. We started with 0.25g racemic lupanine and 1 mol equiv of L-DTTA or D-DTTA.

The only solvent which dissolved the two compounds well and recrystallized was acetone. With this, we have identified that acetone is the best solvent to be used in the recrystallization experiments of Lupanine with DTTA and therefore, all subsequent experiments were performed with this solvent.

iii. **Diastereomeric resolution by recrystallization with Di-*p*-toluoyl-L-tartaric acid with the best solvent (acetone):** We started with 0.25g racemic lupanine and 1 mol equiv of L-DTTA in acetone.

Table 4- Samples of Lupanine with 1 mol equiv of L-DTTA in 2mL of acetone

Sample	[ ] Lupanine (g/mL)	Days of Recrystallization	Yield (%)	e.e (%)
A	0.253	1	-	Racemic mixture
B	0.253	2	19.9	78.7 of L-Lupanine
C	0.251	3	15.7	79.5 of L-Lupanine
D	0.257	4	-	Racemic mixture
E	0.193	2	24.1	75.95 of L-Lupanine
F	0.130	3	18.3	83.16 of L-Lupanine

Conclusion

We can conclude that the process of diastereomeric resolution by recrystallization of lupanine works better with tartaric acid than with DTTA. Indeed, the use of tartaric acid as resolving agent allows to obtain enantiomerically pure L-Lupanine. PBI beads were successfully used as a scavenger for lupanine. A initial attempt to recover lupanine from PBI beads using DCM as eluent was not efficient enough. The use of conditioned PBI proved to be extremely efficient in the removal of DMAP in DMAP / Meta solution, and it was later possible to recover most of the compounds through DCM and MeOH elution. Molecularly imprinted membranes for both racemic lupanine and template as for pure enantiomers exhibited low selectivity to lupanine.The procedure of membrane recrystallization is still a recent concept and the results show that in this case proved to be inefficient on increasing diastereomeric resolution efficiency, since crystals obtained were racemic.

References

[1] G. Székely, “Degenotoxification of pharmaceuticals by molecular imprinting and organic solvent nanofiltration,” 2012.

[2] R. Viveiros, M. Inês, W. Heggie, and T. Casimiro, “Green approach on the development of lock-and-key polymers for API purification,” *Chem. Eng. J.*, vol. 308, pp. 229–239, 2017.

[3] G. Szekely, M. C. A. De Sousa, M. Gil, F. C. Ferreira, and W. Heggie, “Genotoxic Impurities in Pharmaceutical Manufacturing : Sources , Regulations , and Mitigation,” *Chem. Rev.*, 2012.

[4] L. N. Nchari, “Molecular Mechanisms during Amplification of Chirality in Organometallic Systems : In Situ Studies by X-ray Absorption Spectroscopy,” 2010.

[5] L. A. Nguyen, H. He, and C. Pham-huy, “Chiral Drugs : An Overview,” *Int. J. Biomed. Sci. Chiral*, pp. 85–100, 2006.