Membrane/adsorption hybrid processes for water purification and lupanine isolation from lupin beans debittering wastewater

Ana Teresa Afonso Parente Mota

Instituto Superior Técnico, Universidade de Lisboa

December 2017

Abstract

Lupanine is a quinolizidine alkaloid (QA) that can be found in white lupin beans. This compound can be utilized to synthetize an essential chiral ligand for asymmetric synthesis. Lupanine is considered to be toxic and confer a bitter taste to the beans. The food industry developed a “debittering” process for removing this alkaloid from these beans so that their consumption is safe. This process of lupin beans “debittering” uses large amounts of fresh water and yields wastewater that is rich in lupanine. In this thesis, a multidisciplinary approach was explored to isolate lupanine and fresh purified water from the lupin beans debittering wastewater. A viable process starts with a centrifugation to remove suspense solids followed by two filtration steps, ultrafiltration to remove the (macro)molecules, and a nanofiltration for wastewater volume reduction and lupanine concentration in the retentate and purified water in the permeate. Then, a weak acid cation exchanger resin (Dowex MAC-3) was selected to isolate lupanine from the nanofiltration retentate. Resin regeneration and lupanine recovery is done using a solution of NaOH in water, followed by a liquid-liquid extraction with dichloromethane. Molecularly Imprinted Polymers (MIPs) were synthesized using lupanine as a template, to be included as the last unit operation of the process to isolate the alkaloid. The most efficient MIP for lupanine was obtained using itaconic acid as the functional monomer.

Keywords

Lupin beans debittering wastewater, lupanine, ultrafiltration, nanofiltration, resin, liquid-liquid extraction, molecularly imprinted polymers.

1. Introduction

Quinolizidine alkaloids (QAs) are natural compounds with a ring structure and a nitrogen atom, derived from lysine. Lupanine (Figure 1.) is one of the most common QAs [1]. They are metabolites that give the plants some resistance to pathogens and also to herbivores as they confer a bitter taste in addition to their toxicity [2,3,4]. QAs are neurotoxins that affect nicotinic and muscarinic acetylcholine receptors and ion channels [5].

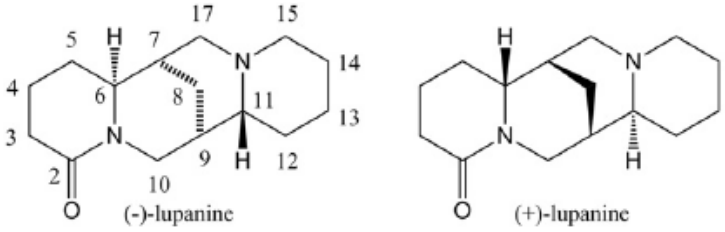
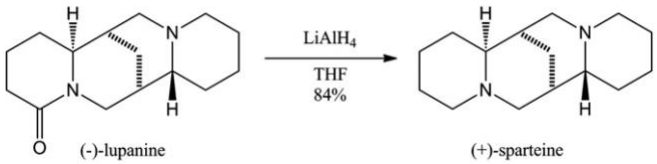


Figure 1. (-)- and (+)-enantiomer of lupanine [10].

Traditional debittering process involves boiling the lupin beans which allows the QAs to be released into the water [8]. The alkaloid content of the beans is reduced from 1-2% (10-20 g/kg) to 0.05% (0.5 g/kg) [1]. Lupanine content in *L. albus* beans corresponds to 1.4% (14 g/kg plant material) [5]. Therefore, since hundreds of tons of white beans are debittered per year, a high amount of water rich in QAs, especially lupanine, is disposed as wastewater.

There are evidences showing that lupanine may be a compound of pharmaceutical interest, especially due to the fact that it may have a hypoglycemic effect as it can enhance insulin secretion [6].

Lupanine is chemically related to sparteine, and it can be converted into the latter through a reduction reaction (Figure 2).



(-)-lupanine (+)-sparteine

Figure 2. Reduction of (-)-lupanine to (+)-sparteine using $LiAlH_4$ in THF [10].

Sparteine is a very important chiral ligand for asymmetric synthesis. It is the most investigated chiral diamine that is used with organolithium reagents in stereoselective deprotonation, oxidation, reduction, substitution and addition reactions to obtain chiral compounds. The great majority of synthesis reactions involves one or more steps that are based on these lithium reagents [7].

For a certain period, (-)-sparteine was unavailable in the market and it was suggested that lupanine could be a useful compound to obtain sparteine [8].

A previous academic study performed also in our team at iBB-IST provided the preliminary information for the development of a membrane-based process to recover lupanine, that included an ultrafiltration followed by a nanofiltration step. Resins and liquid-liquid extractions were then also studied as viable options to be part of the process. Several ultrafiltration and nanofiltration membranes were assessed and characterized. This study allowed to select an appropriate nanofiltration membrane (NF270) but none of the ultrafiltration membranes was adequate for this process. [9].

The present work aims to validate and improve some of these unit operations towards optimal performance. A screening of several resins (some of them already tested) was performed, including binding and regeneration assays. Liquid-liquid extractions were also done for either already tested solvents or new ones to decide which one could be more adequate to extract lupanine. A nanofiltration membrane performance was analyzed and an ultrafiltration membrane performance was also assessed. A new unit operation was also suggested: MIPs for lupanine and the enzymatic transformation. In the end, a process comprising some of the unit operations above mentioned was suggested and discussed to obtain pure lupanine from lupin beans debittering wastewater.

2. Methods

2.1. Lupanine quantification

Lupanine was quantified by HPLC. The mobile phase was constituted by a mixture of acetonitrile (15 %) and Na_2HPO_4 buffer (85 %), and the analysis was performed at a flow rate of 1 mL min^{-1} for 24 minutes, at room temperature. The volume of each injection was equal to 20 µL and the detection was done at 220 nm.

Na_2HPO_4 buffer was prepared by dissolving 1.8 g of this reagent in 1 L of mili-Q water. The pH was then adjusted to 10.5 with some drops of a NaOH solution (50 g/L).

The samples were previously basified with around 1 KOH pellet (pH between 13-13.5), centrifuged at 14000 rpm for 4 minutes and filtered into vials using nylon syringe filters.

2.2. Liquid-liquid extractions

Nine different organic solvents were selected to be assessed according the criteria defined in the introduction and to provide examples of different chemical classes: dichloromethane, MTBE, MIBK, heptane, hexane, toluene, diethyl ether, 1-octanol and 1-butanol. 4 mL of phase 3 (previously basified to pH between 12-13 with approximately 0.20 g of NaOH) and 2 mL of each solvent were added to a test tube. The tubes were vortexed for 2-3 minutes, the mixture was transferred into a graduated cylinder and allowed to stand for 8-10 minutes. Then, the volumes of both organic and aqueous phase were annotated, the aqueous phase was collected with a Pasteur pipette and put in a new test tube. A second extraction was performed by adding 2 mL of the same organic solvent. The test tube was again vortexed, and the same procedure described above was done. After the two extractions, 1.5 mL of the aqueous phase was recovered, basified with KOH pellets (13-13.5) and centrifuged for 4 minutes at 14000 rpm. The samples were then filtered into vials and analyzed by HPLC for lupanine quantification.

2.3 Resins

Binding assays

Firstly, 18 different resins were tested: IRC 50, IRC 86, IRC 7481, AG-50W-X2, AG-50W-X8, Amberlyst 16, Amberlyst 36, Purolite PD206 Amberlite IRA 68, Amberlite IRA 458-Cl, Amberlite IRA 400-Cl, Amberlite IRA 410, Amberlite CG-400, Amberlite XAD-1, Amberlite XAD-7, Amberlite XAD-16, Dowex 1X8-50 and Dowex MAC-3.

Binding assays were done by adding 0.15 g of each resin and 1.5 mL of phase 3 to 2 mL Eppendorf tubes. The tubes were allowed to stand for approximately 15 hours at room temperature, under agitation (260 rpm) with magnetic stirrers. Each resin was tested in duplicate. After 15 hours, the tubes were centrifuged at 14000 rpm for 4 minutes. The supernatant was recovered and basified with KOH pellets (pH between 13 - 13.5). After this, the samples were analyzed by HPLC for lupanine quantification.

Regeneration assays

After the binding assays, the best resins (IRC 50, IRC 86, AG-50W-X2, AG-50W-X8, Amberlyst 16, Amberlyst 36, Purolite PD206 and Amberlite XAD-16) were regenerated using five different solutions: HCl 10% (w/w) in water, NaOH 10% (w/w) in water, HCl 10% (w/w) in ethanol/water (70:30 v/v), NaOH 10% (w/w) in ethanol/water (70:30 v/v) and ethanol absolute. 1.5 mL of a regeneration solution were added to the eppendorfs containing the resins. After this, the procedure was the above-described for the binding assays, except for the regeneration solutions with ethanol. In these cases, the supernatant was recovered after the first centrifugation, filtered to new eppendorfs which were left in the fume hood for two days to promote the evaporation of the organic solvent, and then a stream of nitrogen was used. The volume was refilled with water (final volume approximately 1.5 mL), the samples were basified with KOH, centrifuged and filtered to vials for lupanine quantification.

2.4. Molecularly Imprinted Polymers

Synthesis of MIPs

MIPs were synthesized by bulk polymerization. 5 different monomers were tested: Methacrylic acid, Itaconinc acid, Methyl methacrylate, Styrene and N-isopropylacrylamide. A monomer:cross-linker:template ratio of 0.4:2.0:0.1 was used. 750 µL of DCM were used per 0.1 mmol template, and the quantity of AIBN corresponds to 1% w/w of monomer+cross-linker weight.

Firstly, the functional monomer and the template (lupanine) were dissolved in dichloromethane, inside a glass tube, for 5 minutes under agitation with a magnetic stirrer, at room temperature. After this, the initiator (AIBN) and the cross-linker (EGDMA) were added to the polymerization solution, that was purged with a stream of nitrogen for 10 minutes at room temperature. The tube was closed and placed at 40 ^{o}C overnight (15 hours), under agitation. Then, the temperature was increased with 5 ^{o}C/20 min. increments up to 65 ^{o}C. At this temperature, the tube was left for 4 hours. In the end of the polymerization reaction, a rigid bulk polymer was obtained. The tube was opened and the polymer was crushed in a mortar. The non-imprinted polymers (NIPs) were synthesized using the same experimental conditions, except that no template was added.

After crushing, the polymers obtained with IA, MAA and MMA were transferred into a thimble to be washed using a Soxhlet-apparatus. In the case of the MIPs obtained with MAA and IA, the template molecule was removed using 70 mL of a solution of 0.1 M HCl in MeOH for 48 hours. The traces of HCl were then removed from the polymers with 70 mL of MeOH for 24 hours. NIPs obtained with methacrylic acid and itaconic acid were washed with 70 mL of MeOH for 24 hours. In the case of the MIPs obtained with MMA, the template molecule was removed using 70 mL of dichloromethane for 48 hours. NIPs obtained with MMA were washed with 70 mL of dichloromethane for 24 hours.

The polymers obtained with styrene and N-isopropylacrylamide were transferred into a glass beaker after crushing. The template was removed from MIPs with four sequential washings, using 25 mL of 0.1 M HCl in MeOH at a time, under agitation with a magnetic stirrer. Each washing step consisted of adding 25 mL of the HCl solution that was left in contact with the polymer for 3 minutes. Then, the solution was decanted and another 25 mL of the washing solution were added. This procedure was repeated four times. The traces of HCl were then removed from the polymers with three sequential washings using 25 mL of MeOH at a time, under agitation as described above for the template removal. NIPs obtained with styrene and N-isopropylacrylamide were washed with three sequential additions of 25 mL of MeOH at a time, under agitation as described above.

Two samples of all washing solutions of MIPs were and analyzed by HPLC for lupanine quantification, to confirm that the template was removed.

After washing, the polymers were placed in a Petri dish, left in the fume hood for at least 15 hours and dried under vacuum at 40 ^{o}C.

In the end, the polymers were grounded in a mechanical mortar and sieved through sieves of 38 µm and 63 µm pore size. The fraction between 38 µm and 63 µm was used for the binding experiments.

Binding assays

Binding assays with MIPs were done by adding 0.075 g of each MIP and 1.5 mL of a solution of pure lupanine in dichloromethane (1 or 0.5 g/L) to 2 mL Eppendorf tubes. The tubes were allowed to stand for approximately 24 hours at room temperature, under magnetic agitation (60 rpm). Each MIP was tested in duplicate. After 24 hours, the tubes were centrifuged at 14000 rpm for 20 minutes. The supernatant was filtered using PTFE syringe filters to new Eppendorf tubes, and left in the fume hood

for 1 day to promote the evaporation of the organic solvent. Then, the volume recovered was refilled with distilled water, the samples were basified with KOH pellets (pH between 13-13.5), centrifuged for 4 minutes at 14000 rpm, filtered into vials and analyzed by HPLC.

2.5. Nanofiltration and Ultrafiltration

Around 400 mL (or 1500 mL) of lupin beans debittering wastewater were centrifuged at 6000 rpm and 20 ^{o}C for 30 minutes. The supernatant was recovered to be used as feed of the filtrations.

The conditioning of the membranes was done by introducing around 400 mL of distilled water into the filtration cell, applying pressure (20 bar for the nanofiltration and 5 bar for the ultrafiltration) and setting the gear pump flow rate for 420 mL/min. (or 600 mL/min.). Then, the permeate was recovered and the membranes were ready when the flux was constant.

After conditioning, the supernatant of the wastewater was introduced in the filtration cell (maximum 400 mL), the gear pump flow rate was set for 420 mL/min. (or 600 mL/min.) and the feed was left to recirculate (with no pressure) for approximately 5 minutes. After this time, two samples of 1.5 mL were recovered (initial samples) and the pressure was applied to the system (20 bar for the nanofiltration and 5 bar for the ultrafiltration).

The permeate was recovered into graduated cylinders, the flux was registered over time and some permeate and retentate samples were taken during the experiments for lupanine quantification, COD measurement, pH and conductivity control. Depressurizations were done every time a retentate sample was taken.

3. Results and discussion

3.1. Ultrafiltration and Nanofilitration membranes assessment

Ultrafiltration is a process that can be used before the nanofiltration to retain most of the proteins, for example, while generating a permeate that is rich in lupanine. The ultrafiltration was done using a thin film membrane with a molecular weight cut-off of 3000 Da. Around 400 mL of wastewater were filtered (after centrifugation) under 5 bar, and 420 mL/min. In the end, approximately 250 mL of permeate and 150 mL of retentate were obtained.

Table 1. COD, lupanine concentration, pH and conductivity values obtained for each fraction recovered after ultrafiltration of 400 mL of lupin beans wastewater.

Fraction	Lupanine (g/L)	COD (g O_2/L)	% COD rejection	pH	Conductivity (µS)
Feed	4.804 ± 0.549	23.09 ± 0.41	12.46 ± 1.93	4.01	5700
Permeate	5.622 ± 0.410	20.21 ± 0.41		3.75	5460
Retentate	4.988 ± 0.505	31.17 ± 0.41		3.91	6860

The % COD rejection obtained for the ultrafiltration membrane that was utilized is very low (Table 1.)., which means that the great majority of the macromolecules that are present in the wastewater can pass through it. This result shows the ultrafiltration membrane that was tested is inadequate to retain COD, which means that a membrane with cut-off much lower than 3000 Da is required.

The nanofiltration experiments were performed comparing the use of wastewater (after centrifugation) directly or using around 250 mL of the ultrafiltration permeate, to mitigate fouling of the nanofiltration membrane. Figure 3. shows that the flux is higher for the nanofiltration that was performed after the ultrafiltration. Although the COD rejection for the ultrafiltration membrane that was tested was low, it seems to retain some macromolecules that will have an impact in the nanofiltration membrane performance.

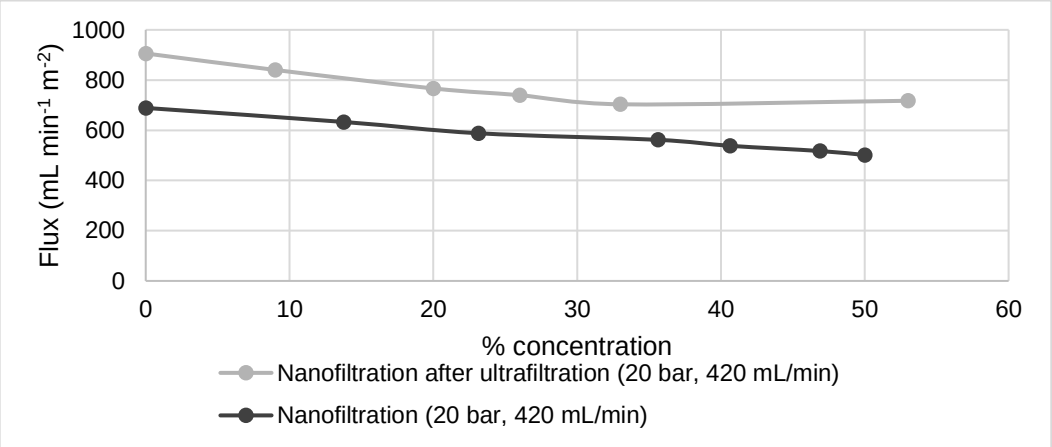


Figure 3. Variation of permeate flux with % concentration, during the nanofiltration of 400 mL of lupin beans debittering water, and the nanofiltration of 250 mL of permeate from the ultrafiltration experiment. The % concentration is the ratio between the volume of permeate over volume of feed.

3.2. Extraction of lupanine with organic solvents

The solvents for liquid-liquid extractions must be selected essentially according to five parameters: the percentage of extraction (% extraction), COD retained, water contamination with extraction solvent, solvent inherent environmental impact (GSK guide) and the boiling point for easy recover of lupanine and extraction solvent recycling.

The % extraction was obtained for each solvent was determined using wastewater (previously basified) and using a solution of lupanine in water (3.2 g/L) to study matrix effects (Figure 4.).

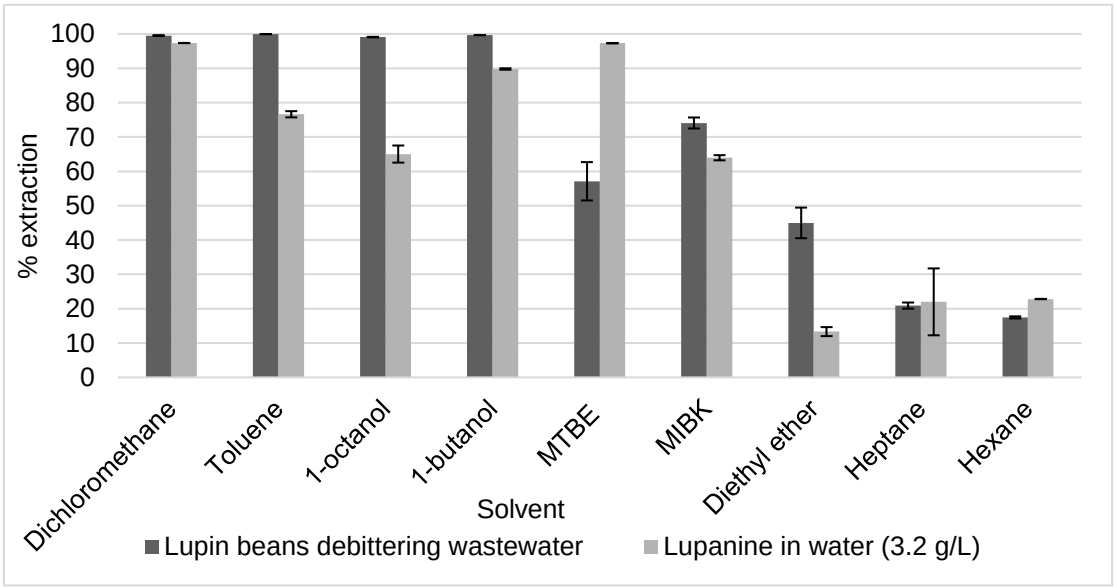


Figure 4. % extraction calculated according to equation 14 for lupin beans debittering wastewater and a solution of pure lupanine.

The solvents to extract lupanine showing higher efficiency on lupanine extraction were dichloromethane, toluene, 1-octanol, 1-butanol and MTBE. Extraction of lupanine from aqueous wastewater using toluene, 1-octanol, diethyl ether and MTBE seems to be influenced by the matrix. There might be some ions in the wastewater which presence will interfere with the passage of lupanine from de aqueous to the organic phase during the extractions.

1-octanol has a high boiling point. Although 1-octanol seems to be efficient to extract lupanine, recovery of this compound will then be challenging due to difficulties on evaporating this extracting solvent.

In the context of the experiments that were performed, COD retained is defined as the capability of a given organic solvent to separate COD from lupanine. When doing liquid-liquid extractions, COD is supposed to remain in the aqueous phase. All five solvents showed similar results regarding COD retained (data not shown). 1-butanol and 1-octanol should be excluded because the first one causes substantial water contamination and octanol cannot be easily evaporated. Toluene has a significant environmental impact, and DCM is a very hazardous solvent which leaves MTBE as the most suitable solvent for lupanine extraction. Both MTBE and DCM have low boiling points (40 and 55 ^{o}C, respectively), so they can be easily evaporated. DCM would be more appropriate because lupanine dissolved in this solvent could be directly used for MIPs assays.

3.3. Extraction of lupanine with resins

From the resins assessed, the ones with higher binding efficient for lupanine were the strong and weak acid cation exchangers. Lupin beans wastewater contains lupanine in the acidic form, which means that this molecule is protonated (because the pH of the wastewater is between 3 and 4 and the pKa of lupanine is 9.1). Thus, lupanine will have a positive charge and the strong sulfonic acid or weak carboxylic groups cation exchange resins will exchange the hydrogen ions for the lupanine molecules. The polymeric resin that promotes higher adsorption of lupanine is the one with a hydrophobic polymeric chain, XAD-16. It interacts with organic compounds (like lupanine) through hydrophobic and polar effects, due to the presence of the aromatic rings. The most efficient resins to extract lupanine from phase 3 were selected for lupanine recovery (Figure 5.).

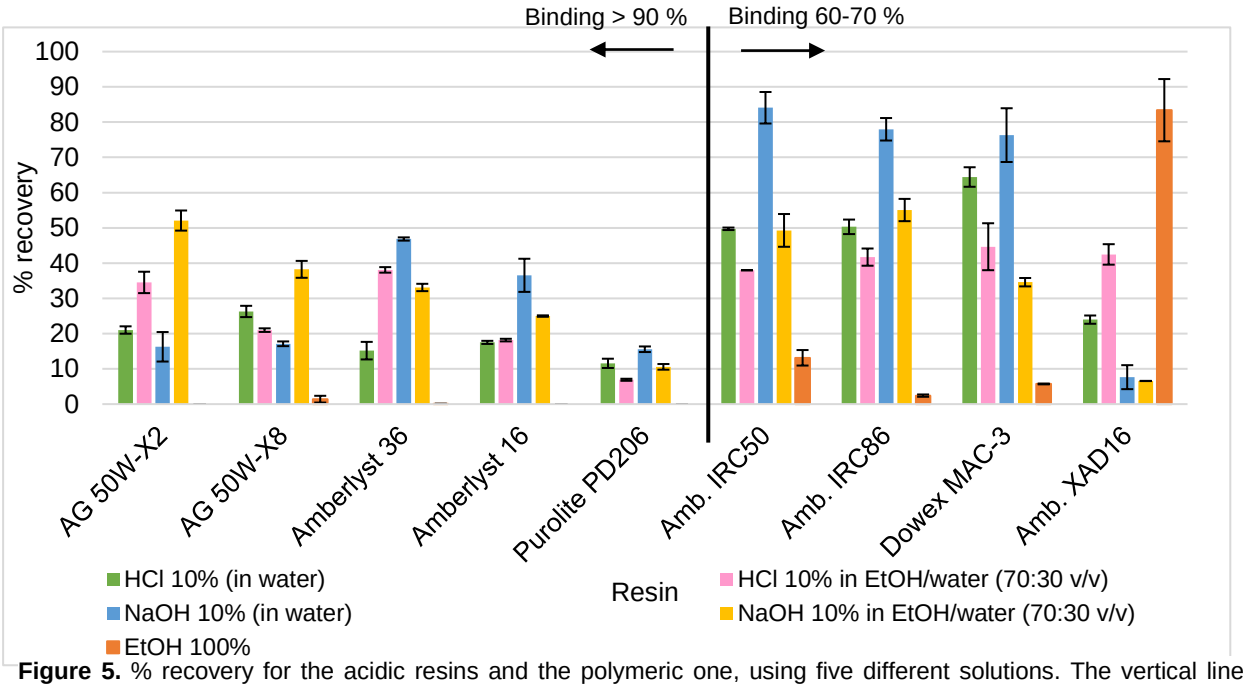


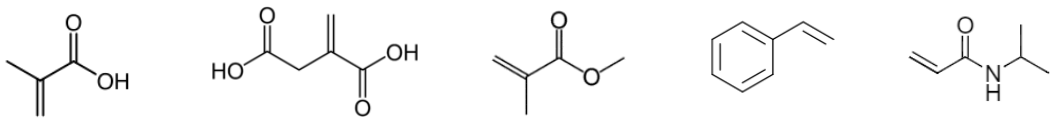
Figure 5. % recovery for the acidic resins and the polymeric one, using five different solutions. The vertical line signalizes the resins with % binding higher than 90% (on the left) and % binding between 60 and 70% (on the right).

Regeneration with NaOH and HCl is mainly based on ionic interactions, while the regeneration with ethanol is based on weaker interactions (dipole-dipole or hydrophobic interactions). Ethanol seems to be useful to recover lupanine that was adsorbed on XAD-16. In general, regeneration of the acidic resins with NaOH in water seems to be more efficient than with HCl. Na^+ ions will compete with protonated lupanine to bind the resin, lupanine will dissociate from the resin and it will move to the solution. In the case of HCl, it will dissociate in the water, and the H^+ will protonate the sulfonic or carboxylic groups, allowing lupanine to be recovered

Considering the results of binding and regeneration, the best option seems to be choosing the weak acid cation exchanger resin (Dowex MAC-3) or the polymeric adsorbent resin (XAD 16) to recover lupanine.

3.4. Molecularly Imprinted Polymers for lupanine

Besides membrane-based processes, liquid-liquid extractions and resins, an additional unit operation consisting in the isolation of lupanine using molecularly imprinted polymers (MIPs) was tested. Five monomers with different chemical properties were used to obtain MIPs, using racemic lupanine as template (Figure 6.).



(A) (B) (C) (D) (E)

Figure 6. Structure of each monomer utilized to synthetize MIPs using lupanine as template. (A) Methacrylic acid (MAA), (B) Itaconic acid (IA), (C) Methyl methacrylate (MMA), (D) styrene, (E) N-isopropylacrylamide (NIPAM).

Considering the chemical structure of lupanine, it is possible to see that this molecule is able to form hydrogen bonds because it has two electronegative atoms that work as hydrogen acceptors. The carboxylic groups of MAA and IA can act as hydrogen donors since they contain a hydroxyl group. It was expected that the polymers obtained with itaconic acid could be more efficient to extract lupanine because each molecule of IA can form two hydrogen bonds.

Ionic interactions are possible between IA and MAA. These monomers contain carboxylic groups that are able to lose the acidic protons to the tertiary amine of lupanine. Thus, lupanine acquires a positive charge that will be electrostatically attracted by the carboxylic acid groups.

NIPAM contains an amide group that is capable of forming hydrogen bonds with lupanine. Considering that nitrogen is less electronegative than oxygen, then it is expected that the interaction between lupanine and NIPAM through hydrogen bonding is weaker than between MAA and IA.

Styrene is a derivative of benzene, which means that the establishment of hydrogens bonds or dipole-dipole interactions is not possible, since there are only carbon and hydrogen atoms. Dipole-induced dipole forces are the only possible intermolecular forces between styrene and lupanine.

MMA contains an ester group, which means that it cannot establish hydrogen bonds or ionic interactions with lupanine, only weaker forces (dipole-dipole and dipole-induced dipole).

The preliminary results were obtained using a 0.1 g/L solution of pure lupanine in dichloromethane. After the preliminary assays, new binding experiments were performed with all polymers using a more concentrated solution of lupanine in DCM (1 g/L) (Figure 7.).

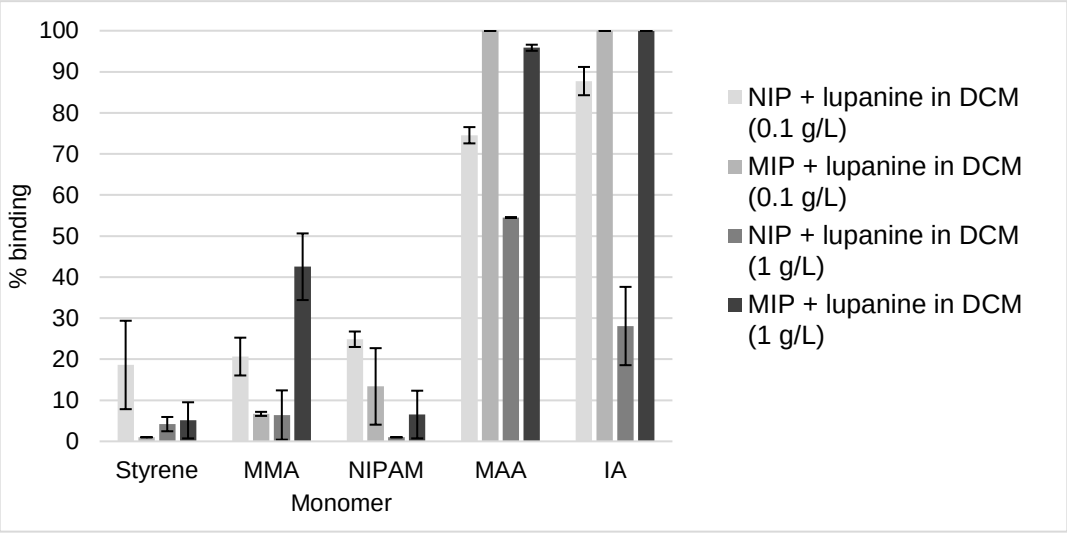


Figure 7. Binding results for NIPs and MIPs obtained with the monomers indicated and two solutions of racemic lupanine in dichloromethane (0.1 and 1 g/L).

Regarding styrene, MMA and NIPAM, the NIPs showed higher % binding than the correspondent MIPs, using the solution of lower concentration (0.1 g/L). This unexpected situation may have happened eventually due to the arrangement of the monomers in the NIP, that favored the interaction with lupanine more than in MIPs, since a low concentration was used.

Although there is no significative difference between the % binding for the MIPs and NIPs obtained with MAA and IA, these two seem to be the most promising monomers to produce MIPs for lupanine, according to the results obtained with the solution of 0.1 g/L. When a more concentrated solution of lupanine was utilized (1 g/L), it was possible to confirm that styrene and NIPAM are not adequate to produce MIPs for lupanine as the % binding is very low.

The polymers obtained with MMA show an improvement when the concentration of lupanine is increased, but the MIP has a % binding that is lower than MAA and IA MIPs. The more efficient MIPs to bind lupanine seem to be the ones synthesized with MAA and IA. These results are in agreement with the predicted interactions that were previously analyzed.

IA was selected to synthesize a chiral MIP, using a lupanine enantiomer as template, but the polymer did not exhibit enantioselectivity.

3.5. Lupanine Recovery Process

An efficient process to extract lupanine was assessed using lupin beans wastewater, and it should start with a centrifugation to eliminate as much solid particles as possible, followed by an ultrafiltration to remove the macromolecules and the resultant permeate will then be subjected to a nanofiltration. This sequence of steps allows to reduce the amount of water from which lupanine will be extracted, while concentrating this alkaloid. Then, ion exchanger resin (MAC-3) is introduced, which regeneration is made with NaOH 10% in water, followed by a liquid-liquid extraction with DCM. For the organic extractions, lupanine must be deprotonated, and the use of a strong base for regeneration of the resin makes this sequence of steps very suitable. After the extraction, the organic phase (that contains lupanine) will be used for binding assays with MIPs to obtain racemic lupanine or chiral MIPs for the enantiomeric resolution of this alkaloid.

4. Conclusions

It was possible to suggest a membrane-based processes to isolate lupanine from lupin beans debittering wastewater, that include also resins, liquid-liquid extractions and MIPs. The process is composed by a centrifugation, ultrafiltration, nanofiltration, and the resultant retentate can be applied to either an ion exchange resin (Dowex MAC-3), followed by a liquid-liquid extraction. MIPs constitute the last unit operation of the process.

A different ultrafiltration membrane, with MWCO lower than 3000 Da should be tested for organic matter retention and fouling mitigation.

MIPs obtained with IA and MAA, showed promising results for racemic lupanine. An alternative to be explored is the synthesis of a chiral using a chiral monomer, to be included in the process as an enantioselective unit.

References

[1] I. I. Koleva, T. A. van Beek, A. E. M. F. Soffers, B. Dusemund, and I. M. C. M. Rietjens, "Alkaloids in the human food chain - natural occurrence and possible adverse effects," *Mol. Nutr. Food Res.*, vol. 56, no. 1, pp. 30–52, 2012.

[2] F. M. C. Santana, A. M. Fialho, I. Sá-Correia, and J. M. A. Empis, "Isolation of bacterial strains capable of using lupanine, the predominant quinolizidine alkaloid in white lupin, as sole carbon and energy source," *J. Ind. Microbiol. Biotechnol.*, vol. 17, no. 2, pp. 110–115, 1996.

[3] H. Reinhard, H. Rupp, F. Sager, M. Streule, and O. Zoller, "Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing food," *J. Chromatogr. A*, vol. 1112, no. 1–2, pp. 353–360, 2006.

[4] D. J. Hopper and M. A. Kaderbhai, "The quinohaemoprotein lupanine hydroxylase from Pseudomonas putida," *Biochim. Biophys. Acta - Proteins Proteomics*, vol. 1647, no. 1–2, pp. 110–115, 2003.

[5] M. Wiedemann, C. M. Gurrola-D'\iaz, B. Vargas-Guerrero, M. Wink, P. M. Garc'\ia-López, and M. Düfer, "Lupanine Improves Glucose Homeostasis by Influencing KATP Channels and Insulin Gene Expression," *Molecules*, vol. 20, no. 10, pp. 19085–19100, 2015.

[6] T. Bobkiewicz-Kozłowska, M. Dworacka, S. Kuczyński, M. Abramczyk, R. Kolanoś, W. Wysocka, P. M. Garcia Lopez, and H. Winiarska, "Hypoglycaemic effect of quinolizidine alkaloids - lupanine and 2-thionosparteine on non-diabetic and streptozotocin-induced diabetic rats," *Eur. J. Pharmacol.*, vol. 565, no. 1–3, pp. 240–244, 2007.

[7] N. M. Clark, P. García-Álvarez, A. R. Kennedy, C. T. O'Hara, and G. M. Robertson, "Reactions of (−)-sparteine with alkali metal HMDS complexes: conventional meets the unconventional," *Chem. Commun.*, no. 39, p. 5835, 2009.

[8] S. K. Ritter, "Where has all the sparteine gone?," *Chem. Eng. News*, vol. 95, no. 17, pp. 18–20, 2017.

[9] C. Barbeitos, "Towards the development of a process for lupin beans detoxification wastewater with lupanine recovery," University of Lisbon, 2016.

[10] A. K. Przybył and M. Kubicki, "Simple and highly efficient preparation and characterization of (-)-lupanine and (+)-sparteine," *Tetrahedron*, vol. 67, no. 40, pp. 7787–7793, 2011.