Enhanced extraction of levodopa from *Mucuna pruriens* seeds using aqueous solutions of eutectic solvents

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ABSTRACT: Levodopa (L-dopa) is an amino acid precursor of the catecholamines dopamine, norepinephrine and epinephrine, which can be used in the treatment of Parkinson's disease. Levodopa is present in several vegetable sources, such as *Mucuna pruriens* seeds. However, the extraction of levodopa from vegetable matrices is usually carried out with volatile organic solvents (methanol, hexane and chloroform). In this work we demonstrate that aqueous solutions of eutectic solvents (ES) can be used as alternative solvents for the extraction of levodopa. Eutectic solvents based on carboxylic acids or polyols combined with cholinium chloride ([Ch]Cl) were studied. Experimental conditions such as temperature, solid-liquid (solvent-biomass) ratio and ES concentration in aqueous solution were optimized by a response surface methodology, with the aim of maximizing the levodopa extraction yield. Extraction yields up to 9.9 ± 1.0 wt.% (levodopa per dry weight of *Mucuna pruriens* seeds) were obtained at a temperature of 56 °C, solid-liquid ratio of 1:7 and ES concentration close to 35 wt.%. The recovery of levodopa from the ES aqueous solutions was achieved by a subsequent solid-phase extraction step, allowing to recover 87% of the extracted levodopa with high purity. This step further allowed the solvent recovery and reuse, demonstrating that the solvent can be reused at least three times without compromising the extraction yield for levodopa. This work shows the remarkable capacity of ES aqueous solutions to extract the value-added compound levodopa from biomass and the possibility of applying reusable solvents, paving the way for their use as alternative solvents to extract bioactive compounds from natural sources.

KEYWORDS: Levodopa, eutectic solvents, *Mucuna pruriens*, extraction yield, purity.

Introduction

According to the Parkinson's Foundation more than 1 million people worldwide live with Parkinson's disease.[1] This is a neurodegenerative disorder,[2] partially defined by a decrease in dopamine production, characterized by fear and bradykinesia.[3] However, the direct administration of dopamine neurotransmitter for Parkinson's treatment is ineffective due to its inability to cross the blood-brain barrier.[4] Levodopa (*L*-dopa) (Figure 1) is a neutral amino acid precursor of the catecholamines dopamine, norepinephrine and epinephrine.[5] Unlike dopamine, this amino acid can cross the blood brain barrier, reaching the central nervous system where it is converted into dopamine.[6] Today, the main treatment for Parkinson's disease involves the administration of synthetic levodopa. Its administration provides a rapid and effective control of motor symptoms for virtually all patients. However, one of the common side effects of synthetic levodopa is dyskinesia (involuntary muscle-induced muscle movement),[5,7] reinforcing the need of investigating and use levodopa extracted from natural sources.

Mucuna pruriens is one of the largest natural sources of *L*-dopa, from which it was first isolated in 1937.[7] Studies have shown that treatment with natural levodopa can control the symptoms of Parkinson's disease,[8] reinforcing the interest in levodopa-rich plants.[9] Misra and Wagner studied levodopa extraction from *Mucuna* seeds using different mixtures of ethanol:water in a 1:1 weight ratio under ascorbic acid protection, and chloroform in basic medium, obtaining extraction yields close to 1.78 wt.% and 4 wt.%, respectively.[10] Anosike et al. [11] evaluated the antioxidant properties of extract from *Mucuna pruriens* seeds obtained using 99.8% methanol, and found extraction yields of approximately 12.16 wt.% of *L*-dopa. Cassani et al.[12] used an acidic aqueous solution composed of 0.01 mol.L^{-1} of hydrochloric acid with the aid of ultrasounds to extract *L*-Dopa from *Mucuna pruriens* and obtained average concentrations of levodopa of 5.29 wt.%. Pulikkalpura et

al.[13] evaluated the extraction of levodopa from the same biomass using a mixture of formic acid-alcohol (1:1, w:w) with extraction yields of 6.42 wt.%. Overall, several solvents have been used to extract levodopa from biomass, with extraction yields ranging between 1 wt.% and 12 wt.% from *Mucuna pruriens* seeds. The best results were obtained with methanol, which is however a hazardous solvents; therefore, alternative and effective solvents should be studied to extract levodopa from biomass. Amongst neoteric alternative solvents and co-solvents it is important to find solvents that lead to high extraction yields, while being of low toxicity, and with high thermal stability and biodegradability.[14,15] Amongst these, deep eutectic solvents (DES) have been considered as promising alternatives in this field.[16] DES are mixtures of two or more compounds with a much lower melting temperature than the melting temperatures of the pure constituents, and also present negative deviations from the ideal behaviour.[17] This lowering of the melting temperature of the mixture makes possible to prepare new solvents which may be liquid at temperatures close to or below room temperature.[18] To prepare a mixture of two solids that can be liquid at room temperature does not strictly requires negative deviations to ideality, and many of the mixtures reported as DES are actually near ideal mixtures.[19] In this work, we will name the studied eutectic mixtures as eutectic solvents (ES). These mixtures, if properly designed, may offer important benefits compared to conventional volatile organic solvents, such as simple preparation, low cost, low toxicity and high biodegradability.[20,21]

Studies report the use of eutectic solvents to extract compounds of interest from biomass.[16,22–24] However, there are no reported results in the literature for the extraction of levodopa using eutectic solvents. Even though high amounts of *L*-dopa have been extracted from *Mucuna pruriens* seeds (4–12 wt.%) with conventional solvents,[7,10,11] its extraction is not selective. This work reports, for the first time, the use of aqueous solutions of eutectic solvents (ES) as effective alternatives for

the extraction of levodopa from biomass, additionally showing that they act as highly selective solvents. An initial screening on the structural characteristics of the eutectic mixtures was performed, followed by a response surface methodology (RSM) used to optimize operating conditions, such as extraction temperature (°C), solid-liquid ratio (S:L, biomass:solvent) and ES concentration (wt.%). The recovery of levodopa from the ES aqueous solutions was additionally investigated, as well as the recyclability and reuse of eutectic solvents.

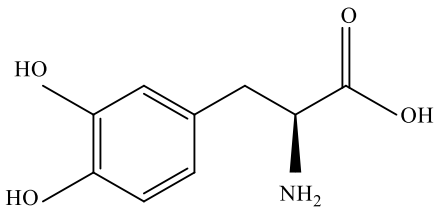


Figure 1. Chemical structure of levodopa.

Materials and methods

Biomass

Mucuna pruriens (Figure 2) seeds were purchased from BRSeeds, São Paulo, Brazil. According to the distributor, the seeds were collected and naturally dried with controlled humidity. The samples were reduced to a fine powder (40 to 20 mesh) and stored in sealed and protected plastic vacuum bags until subsequent assays. In all experiments in this work, biomass pre-treatment was not applied.

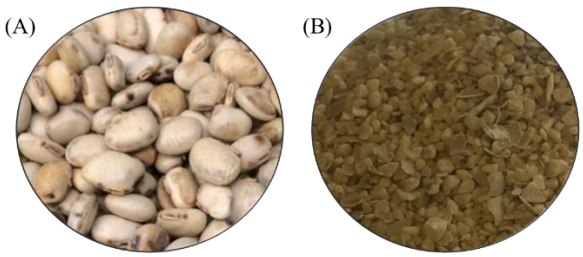


Figure 2. (a) *Mucuna pruriens* seeds *in natura* (b) *Mucuna pruriens* after grinding (40 to 20 mesh).

Standards and Reagents

HPLC grade acetonitrile was from Fisher Chemical (Lisbon, Portugal). Water used was treated with a Milli-Q water drilling system (Millipore, model A10, Billerica, MA, USA). The amino acid standard (3,4-Dihydroxy-L-phenylalanine), >99.9%, was acquired from Sigma-Aldrich (Germany). The detailed purity of the chemicals Acetic Acid (AA), Citric Acid (CA), Glycolic Acid (GA), Lactic Acid (LA), Propionic Acid (PA), Ethylene glycol (EG), Glycerol (G) and Cholinium Chloride ([Ch]Cl) used in the DES preparation is given in Table S1 in the Supporting Information. The water content in these compounds was measured by a Metrohm 831 Karl Fisher coulometer to ensure a correct molar ratio ES preparation and their aqueous solutions at known concentrations. The combinations used to prepare ES, i.e. the hydrogen-bond donor (HBD) and hydrogen-bond acceptor (HBA) species, are summarized in Table 1.

Operating conditions screening for the extraction

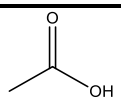
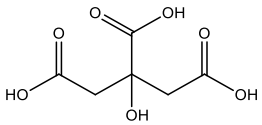
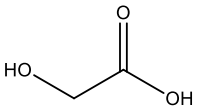
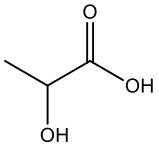
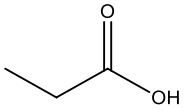
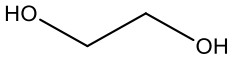
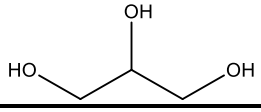
The ES investigated in this study (Table 1) were prepared by mixing in the desired molar proportions under constant agitation at a temperature (maximum 80° C).[25] Eutectic mixtures were prepared gravimetrically (± 10^{-4} g) in closed glass vials. Extractions were performed using a Carousel Radleys Tech commercial equipment, capable of stirring and maintaining the

temperature within ± 0.5 ° C. In preliminary extraction tests, 40 to 28 mesh dried *Mucuna pruriens* seeds were mixed with aqueous ES (at 50 wt.%) solutions under the following conditions: constant stirring at 300 rpm, for 90 min, at 1:10 S:L ratio, at 50 °C. This set of assays allowed to appraise the effect of the molecular structure of carboxylic acids and polyols on the levodopa extraction yield. After extraction, samples were subjected to filtration to separate the solid particles present in the extract. An aliquot of the 20 μL extract was diluted in 4980 μL Milli-Q water and subjected to filtration (0.45 μm sterile polypropylene syringe filters). After filtration, levodopa was quantified by HPLC-DAD (Shimadzu, model PROMINENCE). These analyses were performed with a Phenomenex Kinetex 5 μm C18 100 Å reverse phase analytical column (250 × 4.60 mm). The mobile phase consisted of 90% acetonitrile and 10% ultrapure water at a flow rate of 0.7 $mL.min^{-1}$ and using a 20 μL injection volume. DAD was adjusted to 280 nm. Each sample was analyzed in triplicate. Calibration curves were prepared using pure levodopa aqueous solutions. The DAD-HPLC results show that aromatic compounds are not being co-extracted from the seeds of *Mucuna pruriens*, at least at concentrations higher than the detection limit of the equipment at the given wavelength. The extraction yield (EY%) of levodopa was determined using equation 1:

$$EY\% = \frac{\text{weight of levodopa}}{\text{weight of biomass}} \cdot 100 \quad (1)$$

At least three individual samples were prepared, and three samples from each aqueous phase were quantified, allowing the determination of the average extraction yield and corresponding standard deviation.

Table 1. List of HBA, HBD, molar ratio, structural formula and abbreviations of ES used in this work.

Hydrogen bond acceptor (HBA)	Hydrogen bond donor (HBD)	Structural Formula	HBA:HBD Molar ratio	Abbreviation
Cholinium chloride	Acetic Acid		1:2	[Ch]Cl:AA (1:2)
			1:1	[Ch]Cl:AA (1:1)
	Citric Acid		1:2	[Ch]Cl:CA (1:2)
			1:1	[Ch]Cl:CA (1:1)
	Glycolic Acid		1:2	[Ch]Cl:GA (1:2)
			1:1	[Ch]Cl:GA (1:1)
	Lactic Acid		1:2	[Ch]Cl:LA (1:2)
			1:1	[Ch]Cl:LA (1:1)
			2:1	[Ch]Cl:LA (2:1)
	Propionic Acid		1:2	[Ch]Cl:PA (1:2)
			1:1	[Ch]Cl:PA (1:1)
	Ethylene Glycol		1:2	[Ch]Cl:EG (1:2)
			1:1	[Ch]Cl:EG (1:1)
	Glycerol		1:2	[Ch]Cl:G (1:2)
			1:1	[Ch]Cl:G (1:1)

Surface response methodology

After an initial screening on the best ES and operating conditions to extract levodopa, an experimental design was then used to optimize the levodopa extraction. Initially, single factor studies were performed to identify which variables should be optimized and the range of application (in SI Figure S1). The monitored factors in the levodopa extraction were temperature, S:L ratio and ES concentration. The 19 experimental points studied are reported in the Supporting Information (Tables S2-S3). The obtained results were statistically analysed with a confidence

level of 95%. The adequacy of the model was determined by evaluating the lack of fit, the regression coefficient (R^2) and the F-value obtained from the analysis of variance (ANOVA). Three-dimensional surface response plots were generated by varying two parameters within the experimental range and holding the other factors constant at the central point. In each factorial planning the central point was experimentally measured at least in triplicate. The quantification of levodopa was carried out as described before.

Levodopa extraction kinetics

After obtaining the optimized conditions for the factorial design, a kinetic study of extraction was investigated to obtain the ideal condition of time in extracting higher yields of *L*-dopa. The experiments were carried out at intervals of 1, 5, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 minutes.

Recovery of levodopa from the ES aqueous solutions

Once the ideal conditions for the extraction of levodopa were identified, the separation of the compound of interest was investigated. The levodopa recovery was carried using a Dowex 500W X8 cation exchange column. The solutions containing Levodopa were adjusted to pH 1 when necessary with HCl. Then the column was pre-treated with 2 mL of HPLC grade methanol, followed by 2 mL of a concentrated HCl solution (pH=1). 2 mL of the extracted levodopa solutions were then passed through the column, followed by the elution of 2 mL of deionized water through the column to recover levodopa. Finally, the column was regenerated with 5 mL of methanol. All the fractions were recovered and analysed through DAD-HPLC. The recovery efficiency (RE%)

of levodopa was determined according to the weight of recovered levodopa after elution in respect to that present in the ES aqueous solution.

Scanning electron microscopy (SEM) and Nuclear magnetic resonance (NMR)

The surface seeds of *Mucuna pruriens* were analysed by SEM by cutting an appropriately sized sample, while for cross-sectional images the *Mucuna pruriens* were broken after immersion in liquid nitrogen. The samples were then covered with carbon and analysed using a Hitachi SU-70 (Japan) microscope at 4 and 15 kV. Original and recycled solvents, and pure and recovered levodopa, were analysed by ^{1}H and ^{13}C NMR, using a Bruker Avance 300 (France) at 300.13 MHz and 75.47 MHz, respectively, and deuterium oxide (D_2O) as a solvent and trimethylsilylpropanoic acid (TSP) as the internal reference.

Results and Discussion

ES screening

Different combinations of eutectic solvents were studied in the attempt to selectively extract levodopa from *Mucuna pruriens* seeds. This set of assays was carried out to investigate the effect of the molecular structure of carboxylic acids and polyols on the levodopa extraction yield. Different types of hydrogen bond donors (HBD) were chosen, namely linear monocarboxylic acids (AA and PA), hydroxy monocarboxylic acids (GA and LA) and a hydroxy tricarboxylic acid (CA), as well as polyols (G and EG). These HBD species were always combined [Ch]Cl as the HBA. In the first screening test, only aqueous ES solutions were investigated. In preliminary studies, we identified that the pretreatment of the seeds of *Mucuna pruriens*, for instance by previous washing

with hexane to remove lipids, resulted in lower levodopa yields. Therefore, in all experiments in this work, biomass pre-treatment was not applied, which can be seen as an additional advantage when considering the process sustainability character.

The same operating conditions were maintained in all experiments, namely a biomass-solvent weight ratio of 1:10, a 50 wt.% ES concentration, an extraction time of 90 min and a temperature of 50 °C. The performance of the different aqueous solutions of eutectic solvents to extract levodopa is shown in Figure 3. The purity of levodopa in each extraction solvent, at least considered to other species that could absorb in a similar wavelength and be eluted at retention times up to 2.5 min, is also given in Figure 3. The purity of levodopa was determined by dividing the HPLC peak area of the target compound by the total area of all peaks present under the studied conditions.

Regarding the solvents structural differences given in Figure 3, some trends can be drawn. The addition of a hydroxyl group (-OH) to the acid used in the ES leads to higher extraction yields, as shown for the pairs glycolic/acetic acids or lactic/propionic acids. Therefore, the [Ch]Cl combined with LA in a (1:2) ratio provides a yield (8.2 ± 0.8% wt.%) of levodopa, with a purity of 98% at the conditions described before. Significant yields were also obtained with [Ch]Cl:CA and [Ch]Cl:GA, with yields of 7.2 ± 0.3 wt.% and 7.1 ± 0.6 wt.% and purities of 98% and 95%, respectively. Regarding the stoichiometric ratio, significant changes in the extraction yield were obtained when the ratio 1:2 is used, except for [Ch]Cl:GA and [Ch]Cl:G. Therefore, the extraction mechanism may be mainly related to the increased solubility of *L*-dopa in the mixture and the effect of the solvent on the biomass structure, as discussed below.

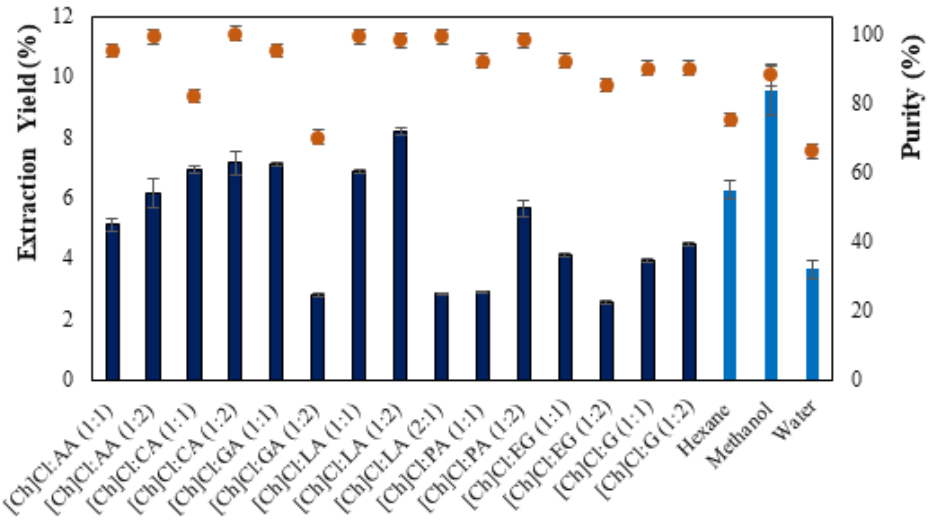


Figure 3. Extraction Yield (■) and Purity (●) of levodopa using aqueous solution of ES at 50 wt.% and Extraction Yield (■) and Purity (●) using hexane, methanol and water (T = 50°C, S:L = 1:10, time = 90 min).

Due to the good performance of the ES [Ch]Cl:LA in the extraction of levodopa, this ES was then chosen for further studies with the aim of tailoring the molar ratio and also understanding the effect of the ES individual components. The results obtained by Ruegas-Rámon et.al [26] confirm the efficiency of [Ch]Cl:LA in extracting biomolecules from a plant matrix. The evaluation of the performance of the individual compounds in the extraction yields was deemed important to better understand the possible synergistic effects between the two compounds in the ES, and to analyse the performance of the mixtures with respect to the individual compounds, whose results are presented in Figure 4.

The eutectic solvent [Ch]Cl:LA leads to a higher extraction yield than the pure LA and LA aqueous solutions (at 50 wt.%). A better performance of [Ch]Cl:LA (1:2) when compared to [Ch]Cl:LA (1:1), [Ch]Cl:LA (2:1) and [Ch]Cl at 50 wt.% in aqueous solution is also noticed. The process mechanism allows the selective extraction of L-dopa from other aromatic compounds due to the

acidity of the eutectic solvent, since lactic acid is in greater quantity in the mixture. As levodopa is an amino acid, it will be protonated in the ES solvent, exhibiting an alkaline behavior, thus resulting in an acid-base extraction. The results were compared with the solvents: methanol, as they present the highest yield of levodopa extraction from *Mucuna pruriens* seeds, hexane, as it could help remove lipids present in the seeds and water, since the solvents used are water-based (acting as a control). Furthermore, it can be observed that water alone does not show a high extraction performance (3.64 wt.%); however, when added to ES it increases the extraction capacity of the solvents, probably by increasing their polarity and reducing their viscosity.

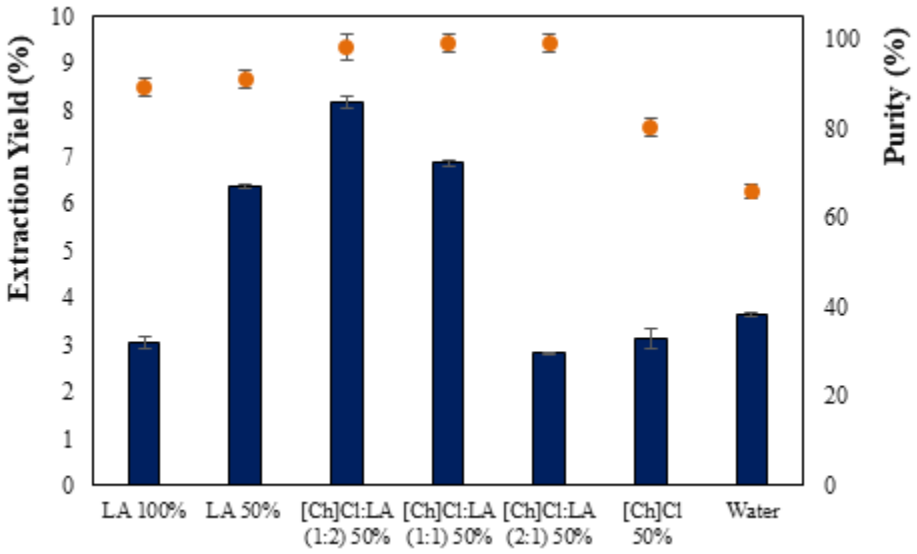


Figure 4. Extraction Yield (■) and Purity (●) of levodopa using lactic acid, aqueous solutions of lactic acid and aqueous solutions of ES based on LA (T = 50°C, S:L = 1:10, time = 90 min).

Optimization of the operating conditions: response surface methodology

To maximize the extraction yield of levodopa from *Mucuna pruriens* seeds, it is important to evaluate the effect of variables such as temperature, solid:liquid ratio and solvent concentration to

obtain the most effective and economic extraction system. The response surface methodology (RSM) allows the optimization of all variables simultaneously, considering the interactions between the effect while predicting the most efficient conditions. In this work, an experimental design 2^3 (3 factors and 2 levels) was performed with 5 central points and axial axes. In the proposed design the following variables were considered: temperature (X_1), solid:liquid ratio (X_2) and solvent concentration (X_3). The experiments were performed in random order, aiming to avoid systematic errors in the design. The coded and decoded levels and factors, as well as the data obtained for the response variable extraction yield are presented in Table S2 in the Supporting Information.

The influence of the studied variables on the levodopa extraction yield is illustrated in Figure 5. From the factorial planning, it was possible to evaluate the correlation coefficients, the variance analysis and the optimal conditions to maximize the levodopa extraction yield. From the analysis of Figure 4, it can be seen that extraction yields increase with decreasing the [Ch]Cl:LA concentration, down to 30-50 wt.%. Increasing water content in ES can decrease the viscosity of the extraction system, which is beneficial for the extraction.[27,28] However, for higher water contents there is the increase of the solvent polarity, which may affect the interaction between the ES and the levodopa, and decrease the acidity required for biomass attack. Other authors also reported that adding 25-30 wt.% of water to a ES may improve the solvent extraction efficiency.[29,30]

Extraction temperatures in the range of 25-75 °C were investigated, being the results presented in Figure 5 (i) and (ii). The temperature rise leads to an increased extraction yield due to an increase on the solubility of the levodopa on the solvent and the decrease of the solvent viscosity.[31] The swelling of the biomass particles also increases with the temperature, loosening the bonds between

the molecules, promoting the dissolution of the biomass in the solvent. However, high temperatures increase the energy consumption of the process.[32] By investigating the effect of the S:L ratio on the extraction yield, given in Figure 5 (i) and (iii), it is shown that yields increase with increasing the solid:liquid ratio and then decreased with a further increase of this parameter.

The ANOVA table, as well as all statistical analysis, are shown in the Supporting Information (Tables S4-S5 and Figures S2-S4). These results reveal that the significant parameters ($P<0.05$) are temperature (X_1), solid-liquid ratio (X_2), concentration (X_3), quadratic concentration $(X_3)^2$ and the combined effects of temperature and solid-liquid ratio (X_{12}), temperature and concentration (X_{13}), solid-liquid ratio and concentration (X_{23}). Therefore, the resulting model for the evaluated extraction technique is presented by equation 2:

$$Y = 5.53 + 0.53_{X_1} - 0.32_{X_2} - 1.46_{X_3} - 1.13_{{X_3}^2} - 0.50_{X_{12}} - 0.64_{X_{13}} - 0.91_{X_{23}} \quad (2)$$

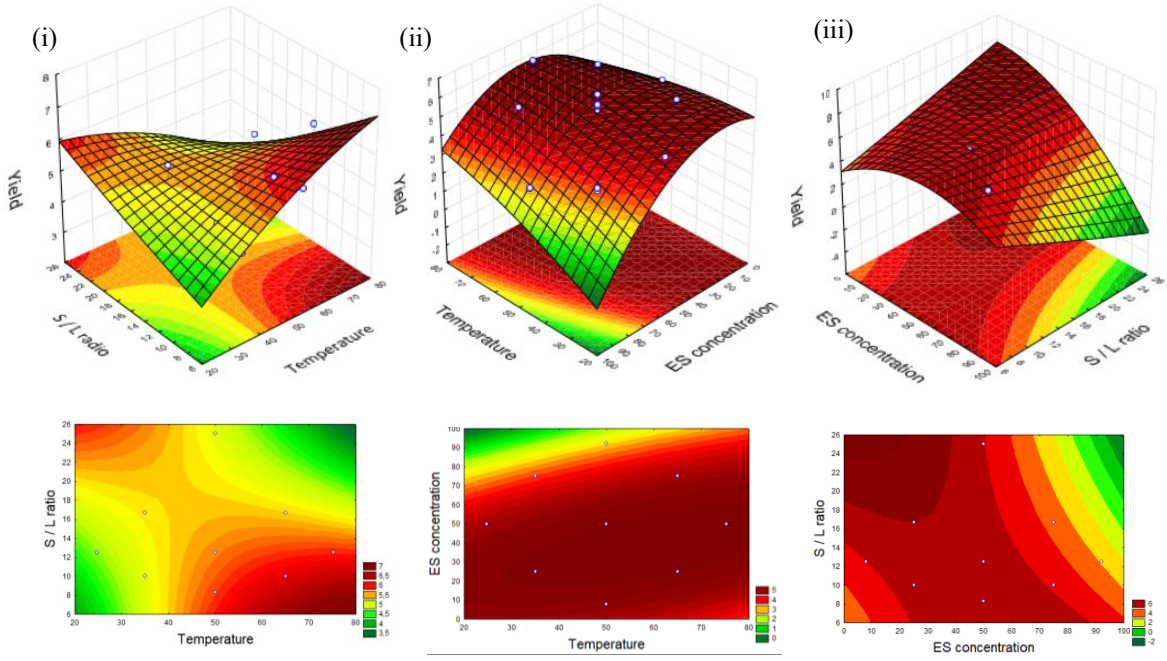


Figure 5. Response surface graphs on the levodopa extraction yield with the combined effects of (i) Temperature and S:L ratio, (ii) ES concentration and S:L ratio, (iii) Temperature and ES concentration using aqueous [Ch]Cl:LA (1:2) solutions for 90 minutes of extraction time.

The average relative deviation between the observed and predicted values is -0.17%, demonstrating a good description of the experimental results by the statistical model. The optimized conditions are 56 °C for low solid-liquid ratios and concentration of ES of 35.5 wt.%. At the optimized conditions of the factorial design, levodopa extraction yields up to 9.9 ± 1.0 wt.% were obtained. The extraction achieved with this ES in aqueous solution is equivalent to that obtained with pure methanol, suggesting that all *L*-dopa is being extracted, and that the aqueous solution of the ES can replace the volatile organic solvent methanol.

The extraction kinetic curve in Supporting Information (Figure S6) shows that up to 90 minutes there was an increase in the extraction yield of *L*-dopa, but after 100 minutes there is the formation of a plateau.

Solvent reuse and levodopa recovery

Once the extraction conditions were optimized, the separation of levodopa from the aqueous medium and the reuse of the solvent were investigated. Each extraction step was carried out according to the optimized conditions (temperature = 56 °C, S:L ratio = 1:7 and ES at 35.5 wt.%). After each extraction, levodopa was separated from the aqueous medium and the solvent was directly subjected to a new extraction cycle with new dry biomass particles. The results obtained (Figure 6) show that the aqueous ES solution presents high extraction capacity without reaching saturation. Figure 6 also shows that the purity of levodopa is not compromised using the recycled

solvent. About 9-10 wt.% of levodopa is extracted with the 35 wt.% [Ch]Cl:LA (1: 2) aqueous solution.

Although the extraction yield and purity of levodopa are not compromised using the recycled solvent, some losses of levodopa occur. Even though, in the first cycle, about 87% of levodopa was recovered, decreasing to 83% in the third cycle. The recovered ES integrity was confirmed by ^{1}H and ^{13}C liquid NMR, performed on the recovered ES. Figure 7 illustrates an optimized strategy for extracting levodopa from *Mucuna pruriens* seeds, highlighting the levodopa recovery from the solvent and reuse of the aqueous ES solutions.

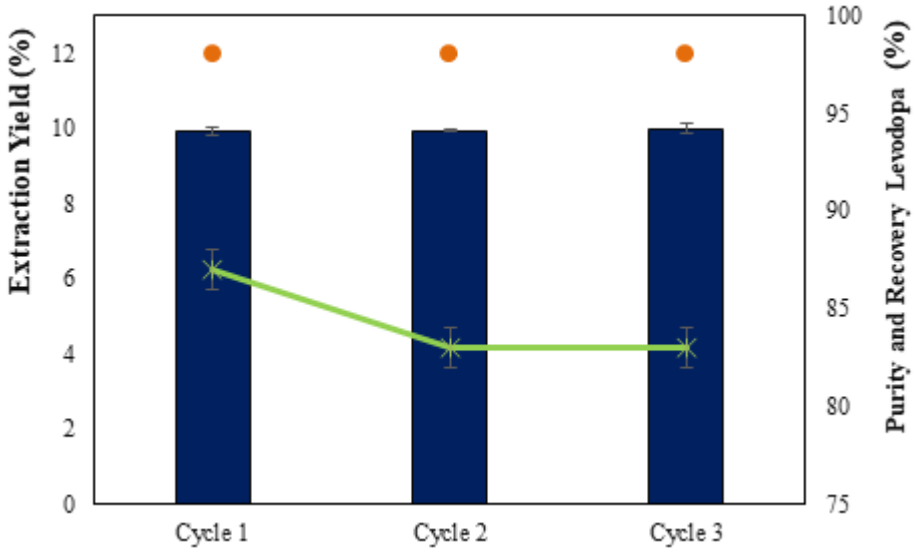


Figure 6. Results of Extraction Yield (■), Recovery (✳) and Purity (●) of Levodopa using the ES [Ch]Cl:LA (1:2) aqueous solutions at the optimized conditions and a cation exchange column for levodopa recovery.

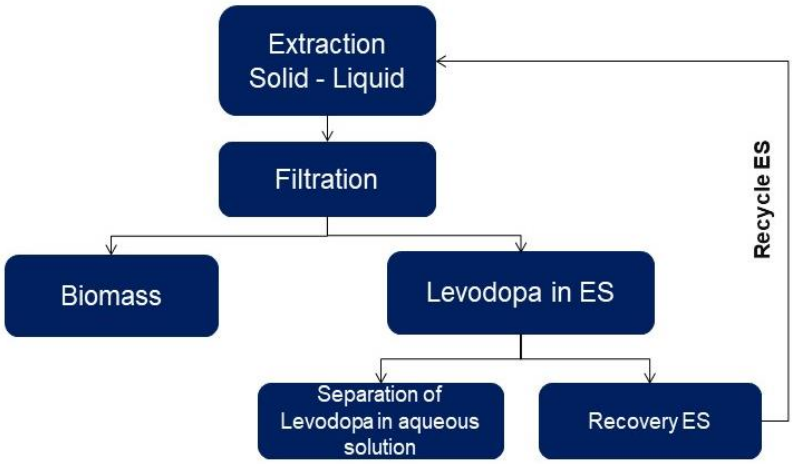


Figure 7. Flowchart for the extraction and separation of levodopa from *Mucuna pruriens* seeds.

The main challenge that the biorefinery faces today is to develop sustainable processes and apply the use of "green" solvents to guarantee maximum efficiency and yield of the extraction, together with the minimum generation of waste. The recovery of levodopa, the recycling of the solvent and the low environmental impact of the process allow validating the technique when compared to conventional separation strategies in which methanol is used as a solvent.

After optimizing the whole process, SEM analyses were performed on the dried *Mucuna pruriens* seeds after the extraction step. SEM images after treatment with water and an aqueous [Ch]Cl:LA (1:2) solution under the optimal conditions described above are shown in Figure 8.

The structure of the biomass sample after extraction with water and ES aqueous solutions shows significant disruptions. Although broken cells are seen in both samples, there is an increase in the ratio of broken cells to intact cells in the presence of ES. This change in structure may be responsible for the increased extraction of L-dopa with ES aqueous solutions. Thus, the amino acid extraction yield is improved in the presence of acids since, in addition to increasing the solubility

of the amino acid in aqueous medium, they also allow better access to the biocompounds present in the biomass matrix.

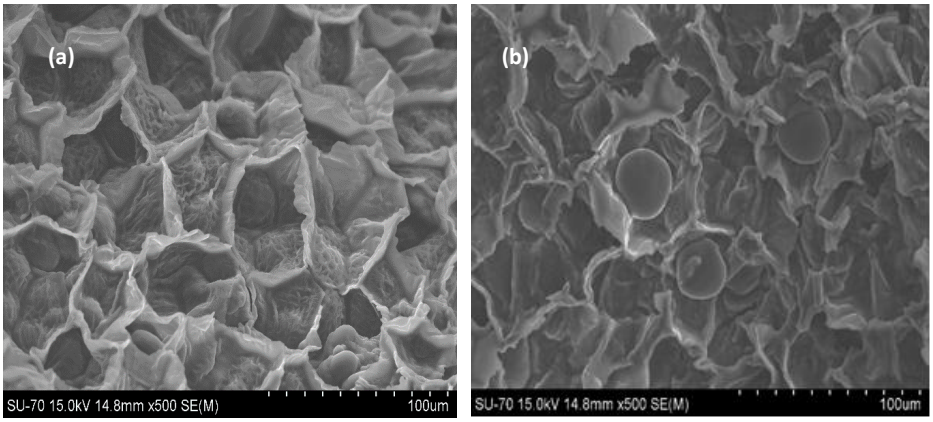


Figure 8. SEM images of the *Mucuna pruriens* samples after extraction with (a) water and (b) an aqueous solution of [Ch]Cl:LA (1:2).

Finally, ^{1}H NMR was used to confirm the integrity and purity of the recovered levodopa after the extractions. As shown in Figure 9, all the proton peaks for levodopa can be identified as follows: ^{1}H NMR (D_2O, 300 MHz, [ppm]): δ 6.89 (d, 1H, (COC<u>H</u>=CH)), 6.83 (d, 1H, (COC<u>H</u>=CH)), 6.74 (dd, 1H, ((COCH=C<u>H</u>)), 3.93 (dd, 1H, (CH=C<u>H</u>CH_2CHNH_3)), 3.14 (dd, 2H, (CH=CHC<u>H</u>$_2$$CHNH_2$), 3.02 (dd, 2H, (CH=CHC<u>H</u>$_2$$CHNH_3$). These results reveal that not only levodopa was extracted in high yields with ES aqueous solutions, but also that it has a very high purity, which is essential for further applications.

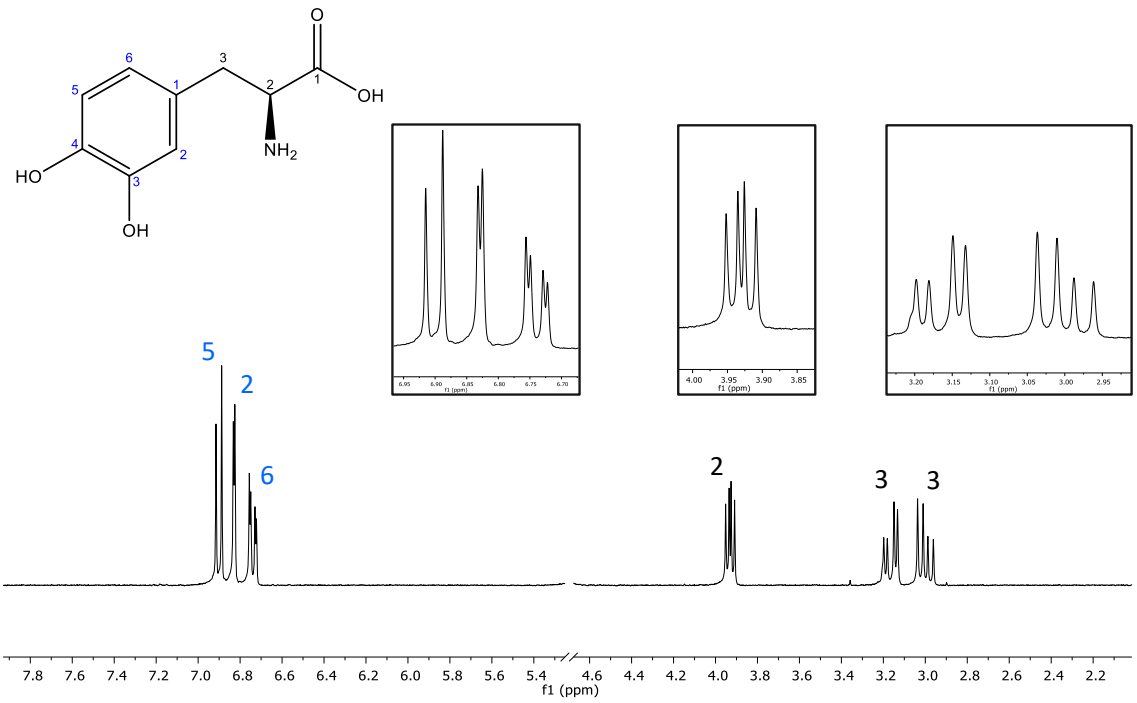


Figure 9. ^{1}H NMR spectra of the recovered levodopa from [Ch]Cl:LA (1:2) aqueous solutions.

Conclusions

This work reports the use of ES and their aqueous solutions as alternative solvents in the solid-liquid extraction of levodopa from *Mucuna pruriens* seeds. The use of ES made possible to efficiently and selectively extract this compound with a high purity. At the optimized extraction conditions (56 °C, 1:7 S:L ratio and [Ch]Cl:LA (1:2) concentration of 35.5 wt.%) for 90 minutes, a maximum extraction yield of 9.92 wt.% of levodopa was obtained. In addition, it was possible to recover the compound of interest by 87% from the ES aqueous solution, as well as to recover the solvent and apply it in further extractions without compromising its extraction efficiency. In summary, these results show the ability of ES aqueous solutions to act in solid-liquid extraction processes, selectively increasing the interaction between solvent and solute, leading to high extraction yields of high-value compounds.

Supporting Information

The Supporting Information is available free of charge. SI includes compound descriptions, molecular formula, CAS number, purity and supplier of the ES components investigated; Levels of process factors in the experimental design; Experimental single factor analysis ; Response surface methodology; Estimated coefficients obtained from the polynomial model and statistical analyses; Chromatogram; Kinetic curve of the extraction of levodopa.

Acknowledgment

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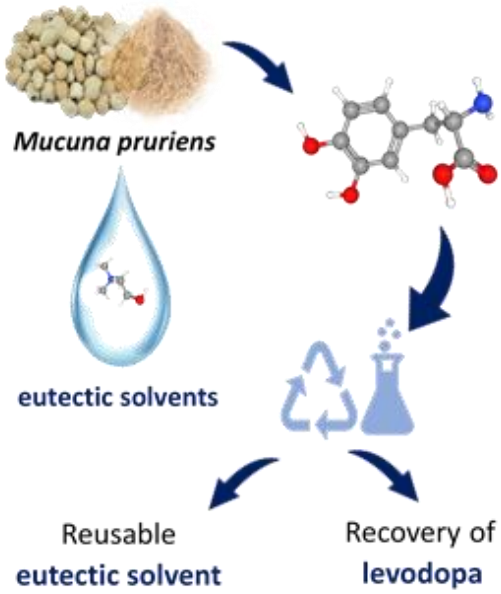

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Synopsis sentence

Sustainable extraction using eutectic solvents to obtain levodopa from *Mucuna pruriens* seeds: levodopa recovery and solvent recycling.