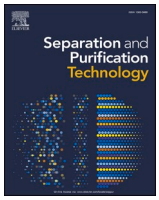
Separation and Purification Technology 353 (2025) 128462

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur



Enantioseparation of ofloxacin using a liquid–liquid extraction system based on hydrophobic eutectic solvents

Mahtab Moradi [a,b], Ana M. Ferreira [a,*], Catarina M.S.S. Neves [a], Filipe H.B. Sosa [a], Gholamreza Pazuki [b,*], João A.P. Coutinho [a]

[a] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro (UA), 3810-193 Aveiro, Portugal
[b] Department of Chemical Engineering, Amirkabir University of Technology (Tehran Polytechnic), 1591634311 Tehran, Iran

ARTICLE INFO

Editor: B. Van der Bruggen

Keywords:
Enantiomers
Chirality
Liquid–liquid extraction
Deep eutectic solvents
Cyclodextrin
Response surface methodology
Fluoroquinolones

ABSTRACT

The enantioseparation of chiral drugs is a major challenge for the pharmaceutical industry as the pharmacological activities of the enantiomers may differ, which can lead to severe secondary effects in the treatment of diseases. The aim of this study is to develop an enantioseparation platform for the resolution of ofloxacin (OFX) using enantioselective liquid–liquid extraction (ELLE) based on a hydrophobic eutectic solvent (HES) in conjunction with one chiral selector. β-Cyclodextrin (β-CD) derivatives, including β-CD, hydroxypropyl-β-cyclodextrin (HP-β-CD), carboxymethyl-β-cyclodextrin sodium salt (CM-β-CD), and sulfated-β-cyclodextrin sodium salt (S-β-CD), were investigated as potential chiral selector for the separation of (R/S-OFX), with CM-β-CD proving to be the most promising. The efficiency of the HES-based ELLE system was then studied, and a total of fourteen systems were evaluated using HES from four groups: L-menthol: fatty acid, L-menthol: fatty alcohol, fatty acid: fatty acid, and fatty acid: fatty alcohols. Decanoic acid: dodecanoic acid (C10 acid: C12 acid) in a molar ratio of 2:1 was identified as the optimal HES-based ELLE system for the enantioseparation of R/S-OFX. This selected system was further used to optimize enantioseparation conditions, including pH, HES-water ratio (v/v), and excess of chiral selector (CM-β-CD), using response surface methodology (RSM). Under optimal conditions, i.e., pH 3.6, HES-water ratio (v/v) of 1:2 and a 77-fold molar excess of chiral selector, an OFX selectivity (α) of 3.8 ± 0.3 was achieved in a single-step.

1. Introduction

Chirality is a significant factor in drug development, with approximately 50 % of marketed drugs being chiral [1]. While the approval of racemates (mixtures of enantiomers) is declining, many chiral drugs are still used as racemates [1,2]. Regulatory concerns, such as those from the Food and Drug Administration of the United States (FDA) and the European Medicines Agency (EMA), arise due to the differing biological activities of enantiomers [3,4]. Typically, only one enantiomer (the eutomer) exhibits desired effects, while the other (the distomer) may cause adverse effects. Although certain racemic drugs are considered safe, regulatory authorities impose stringent requirements on pharmaceutical companies for the provision of comprehensive pharmacological, pharmacokinetic, and toxicological data on individual enantiomers and their racemates [5]. Therefore, the need for enantiopure compounds over racemic mixtures is growing, aiming to improve drug safety and efficacy.

Ofloxacin (OFX), a widely used quinolone antibiotic, is marketed both as levofloxacin (S-OFX) and in racemic form (S-OFX/R-OFX) for treating bacterial infections. Its antibacterial activity is highly dependent on its stereostructure, with S-OFX exhibiting significantly greater biological activity than R-OFX [6]. Due to R-OFX's lower activity and S-OFX's reduced toxicity, OFX has been exclusively marketed in its S-isomer form since 1998 [7]. Given the pivotal role of S-OFX in therapeutic effect, the enantioseparation of OFX holds significant importance.

The resolution of racemates to isolate individual enantiomers remains the primary approach in chiral separation. Various techniques, such as capillary electrophoresis (CE) [8], cocrystallization [9], high performance liquid chromatography (HPLC) [10], thin-layer chromatography (TLC) [11], and ligand exchange chromatography/capillary electrophoresis (LEC/LECE) [12,13], have been used to separate enantiomers, like the OFX. While these methods are effective, they have some drawbacks, including the need for expensive equipment, high operational costs, and environmental hazards [14,15], which complicate the

* Corresponding authors.
E-mail addresses: ana.conceicao@ua.pt (A.M. Ferreira), ghpazuki@aut.ac.ir (G. Pazuki).

https://doi.org/10.1016/j.seppur.2024.128462
Received 21 March 2024; Received in revised form 12 May 2024; Accepted 15 June 2024
Available online 15 June 2024

scaling-up of enantioseparation processes. This highlights the need for more cost-effective and environmentally friendly techniques.

Enantioselective liquid–liquid extraction has recently emerged as a versatile technology for recovering single enantiomers from racemic mixtures, suitable for continuous operation [16]. ELLE is known for its scalability, tunability, and integration of enantiomer recognition and solvent extraction in a single-step [17]. In ELLE, there is a chiral selector that preferentially interacts with one of the enantiomers and forming a complex through various intermolecular interactions (e.g. hydrogen bonding, π-π interactions, dipole and van der Waals interactions) [18]. This complex shows a different partitioning behavior from the other enantiomer and ensures effective enantioseparation [19]. The composition of ELLE also strongly influences enantiomer separation. Therefore, thoughtful selection of phase composition along with the chiral selector makes ELLE adaptable and versatile for enantiomer separation.

Jiao et al. [20] investigated the enantioseparation of OFX using a traditional ELLE based on n-octanol–water. Using a combination of hydrophilic (β-cyclodextrin) and hydrophobic (complex formed by O'O-dibenzoyl-(2R,3R)-tartaric acid (L-DBTA) and di(2-ethylhexyl)-phosphoric acid (D_2EHPA)) chiral selectors, they optimized conditions to enhance enantioselectivity, achieving a selectivity of 2.48. In another study in which different organic solvents for ELLE were investigated, octanol–water was found to be the most effective ELLE for OFX enantioseparation [21]. Tartaric acid derivatives and β-cyclodextrin (β-CD) were used as chiral selectors, achieving an enantioselectivity of 2.4 with di-p-toluoyl-l-tartaric acid (L-DTTA) and β-CD. Molecular simulations clarified the selectivity, highlighting the significant roles of interaction sites and steric effects in chiral recognition. In addition, Jiao et al. [22] utilized an aqueous biphasic system (ABS) – a specific type of ELLE – composed of polyethylene glycol (PEG) and ammonium sulfate for OFX enantioseparation. By adjusting the concentrations of L-tartaric acid (L-TA) and hydroxypropyl-β-cyclodextrin (HP-β-CD) and maintaining a pH of 4, they achieved a selectivity of 1.32.

However, conventional ELLE systems face issues like the volatility, flammability, and toxicity of some organic solvents, while polymer-based ELLE (ABS) struggles with high viscosity and limited polarity range, affecting extraction efficiency and selectivity, which hinders the achievement of high extraction efficiency [23]. Therefore, ELLE based on emerging alternative solvents, such as eutectic solvents (ES), has gained attention.

Typically, ES consist of a mixture of two (or more) compounds known as the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) [24]. These solvents are highly customizable and allow fine-tuning of physicochemical properties, such as hydrophobicity and hydrophilicity, by selecting the appropriate components [25]. Given the hydrophobic nature of OFX and the advantages offered by hydrophobic eutectic solvents (HES), such as higher water stability [25], the development of a HES-based ELLE emerges as a viable alternative.

The aim of this study was to investigate the potential of HES as an alternative solvent for the enantioseparation of OFX by ELLE. First, four different β-CD derivatives were investigated as chiral selectors for OFX enantioseparation. Then, fourteen HES-based ELLE systems were investigated, with HES based on compounds such as L-menthol, fatty acids and fatty alcohols. Finally, optimization of the most effective system and the chiral selector was performed using a response surface method (RSM). The operating parameters, including pH, excess of chiral selector and HES-water ratio (v/v), were optimized to achieve high selectivity for R/S-OFX.

2. Material and methods

2.1. Materials

All the chemical reagents used in the present work are reported in Table 1. Moreover, in all experiments ultrapure water was used, which underwent a rigorous double distillation procedure and meticulously treated through a reverse osmosis system and further refined using a Milli-Q plus 185 water purifier.

2.2. HES preparation

This work focused on the use of HES for ELLE (Table 2), from which four groups were evaluated: (i) L-menthol: fatty acid; (ii) L-menthol: fatty alcohol; (iii) fatty acid: fatty acid and (iv) fatty acid: fatty alcohol. The selection of these HES was based on our previous study [26]. In particular, the COSMO-RS predictions showed that the most promising HES for OFX extraction are fatty acids with a higher fatty acid molar ratio and longer alkyl chains. The selected HES were prepared with the indicated molar ratio of HBA and HBD (Table 2) by heating the mixture at 80 °C for 4–5 h with constant stirring until a clear and homogeneous liquid was obtained. These HES remained stable (clear and homogeneous liquid), with no detectable changes when cooled to 25 °C. In

Table 1
List of compounds used in this work, including the abbreviation, CAS number, supplier and purity.

Compound	Abbreviation	CAS number	Supplier	Purity (wt%)
HES compounds				
1-Decanol	C10 alcohol	112-30-1	TCI	98.0
1-Dodecanol	C12 alcohol	112-53-8	Alfa Aesar	98.0
Decanoic acid	C10 acid	334-48-5	Thermo Scientific	99.0
Dodecanoic acid	C12 alcohol	143-07-7	Acros Organics	99.0
L-menthol	––	1490-04-6	Acros Organics	99.5
Octanoic acid	C8 acid	124-07-2	Thermo Scientific	98.0
Chiral selectors				
Carboxymethyl-β-cyclodextrin sodium salt, DS* ~3	CM-β-CD	2828447-14-7	BLD Pharmatech	99
Hydroxypropyl-β-cyclodextrin, DS* ~ 5–6	HP-β-CD	128446-35-5	Sigma	––
Sulfated-β-Cyclodextrin sodium salt, DS* ~12–15	S-β-CD	37191-69-8	Sigma	––
β-Cyclodextrin	β-CD	7585-39-9	Sigma	≥ 97
Standards and compounds for NMR quantification				
1-Butyl-3-methylimidazolium trifluoromethanesulfonate	$[C_4mim][CF_3SO_3]$	174899-66-2	Iolitec	99
Deuterium oxide-D	D_2O	7789-20-0	Aldrich	99.9
Dimethyl sulfoxide-D6	DMSO	2206-27-1	Euriso-top	99.80
Levofloxacin	S-OFX	100986-85-4	TCI	98
Ofloxacin	R/S-OFX	82419-36-1	TCI	98
Sodium fluoride	NaF	7681-49-4	Thermo scientific	99

* DS – degree of substitution.

Table 2
List of HES studied in this work.

HBA	HBD	Abbreviation	Molar ratio
L-menthol	Octanoic acid	L-menthol: C8 acid	1:2
	Decanoic acid	L-menthol: C10 acid	1:2; 1:3
	1-Decanol	L-menthol: C10 alcohol	2:1
	1-Dodecanol	L-menthol: C12 alcohol	2:1
Octanoic acid	Decanoic acid	C8 acid: C10 acid	2:1
	Dodecanoic acid	C8 acid: C12 acid	2:1
	1-Decanol	C8 acid: C10 alcohol	2:1
	1-Dodecanol	C8 acid: C12 alcohol	2:1
Decanoic acid	Dodecanoic acid	C10 acid: C12 acid	2:1; 3:1
	1-Decanol	C10 acid: C10 alcohol	2:1
	1-Dodecanol	C10 acid: C12 alcohol	2:1; 3:1

addition, their stability in water was previously studied confirming it [25].

2.3. Selection of the best chiral selector

First, the efficacy of β-cyclodextrin derivatives were investigated – namely β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), carboxymethyl-β-cyclodextrin sodium salt (CM-β-CD) and sulfated-β-cyclodextrin sodium salt (S-β-CD) – as chiral selectors for the enantioseparation of OFX. To evaluate the suitability of these selectors and determine the initial appropriate molar excess for evolute HES-based ELLE, a solution containing racemic OFX (1 mg/ml) was mixed with different molar excesses of the chiral selector (20x, 40x, 80x, 120x, and 160x) in relation to OFX. For β-CD, the evaluation was limited to a 20-fold excess of the chiral selector, whereas HP-β-CD was evaluated in a range of 20 to 80-fold due to solubility issues. The mixtures were then stirred at 50 rpm and room temperature (25 ± 1 °C) for at least 12 h to ensure optimal contact between the chiral selector and R/S-OFX. Afterwards, ^{19}F NMR spectroscopy was used to determine whether the R/S-OFX enantiomers were separated or not, as explained below.

2.4. HES-based enantioselective Liquid-Liquid extraction (ELLE)

The HES-based ELLE was chosen as the platform for the enantioseparation of R/S-OFX. A total of fourteen systems (see Table 2) with an equal volume ratio of HES and water (HES-water ratio, v/v) were analyzed for enantioseparation. The water phase contained the racemic mixture of OFX (2 mg/ml) and the previously identified and optimized chiral selector. The mixture was stirred at 50 rpm for at least 12 h at room temperature (25 ± 1 °C) to ensure the contact between the phases. Then, the system was centrifuged at 10,000 rpm for 10 min. Subsequent separation of the HES phase (top) from the aqueous phase (bottom) was performed and analyzed by ^{19}F NMR spectroscopy to quantify the OFX enantiomers in each phase as described below. This entire procedure was repeated three times in order to determine the average of the partition coefficients and selectivity, as well as the respective standard deviations. Fig. 1 gives an overview of the experimental process of OFX enantioseparation using HES-based ELLE.

2.5. Identification and quantification of OFX enantiomers by ^{19}F NMR

The NMR technique can be used to identify and quantify enantiomers, especially for fluorine-containing compounds such as OFX, as shown in Fig. 2. The identification and quantification of OFX enantiomers were performed using the ^{19}F NMR spectroscopic method, following the established approach by Rofouei *et al.* [7]. This method is characterized by the observation of a doublet signal in the ^{1}H-decoupled ^{19}F NMR spectrum of OFX, which is crucial for accurate enantiomeric analysis.

All samples were analyzed by ^{1}H-decoupled ^{19}F recorded on a Bruker Avance 300 at 282 MHz to obtain their spectrum, and the depending on the chiral selector and its concentration different signals of R/S-OFX (ppm) were observed.

To quantify OFX enantiomers using ^{19}F NMR, sodium fluoride (NaF) was employed as an internal standard, as it presents a distinct signal (−118.8 ppm) compared to OFX (−115.7 ppm) in the ^{1}H-decoupled ^{19}F NMR spectrum (Fig. S1 in Supplementary Material). A solution of NaF at a concentration of 2 mg/ml in deuterated water (D_2O) was prepared and distributed into separate capillaries to avoid interactions with the OFX enantiomers and to ensure accurate quantification. Each capillary was standardized using a reference solution of 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([C_4mim][CF_3SO_3]). Then, these calibrated capillaries were used as an internal standard for the quantification of OFX enantiomers in each phase. For OFX quantification, 400 µl of each phase was weighed individually (±10^{-4} g) in a NMR tube with a NaF standardized capillary. Then, the samples were analyzed by ^{1}H-

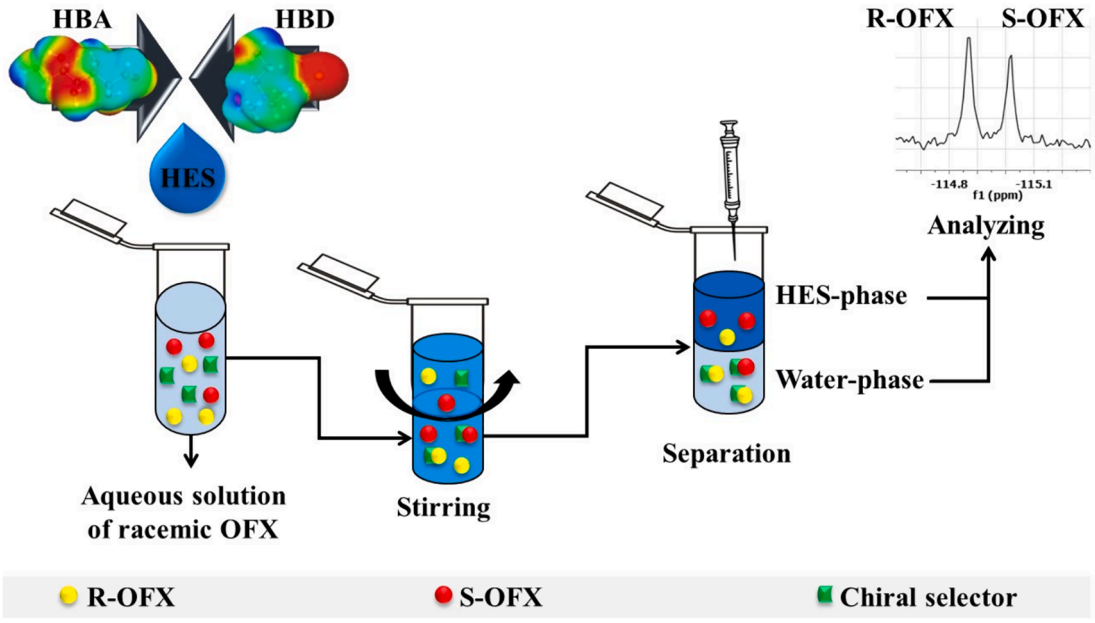


Fig. 1. Schematic diagram of the enantioseparation of OFX using HES-based ELLE.

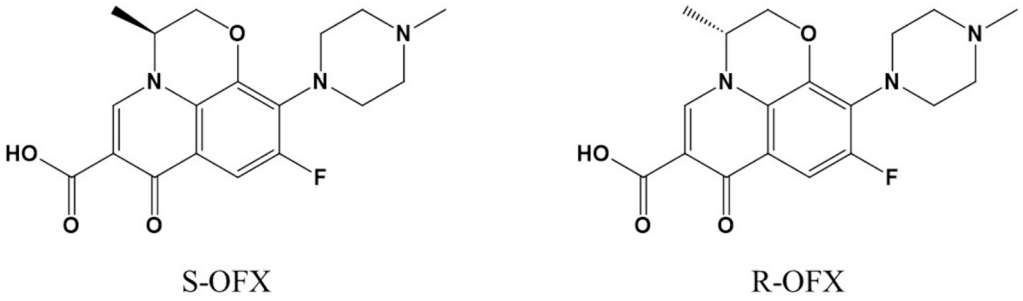


Fig. 2. The structure of the OFX enantiomers.

decoupled ^{19}F recorded and their spectrum recorded. The gathered spectra were analyzed in order to calculate the amount of R/S-OFX enantiomers present in the phases, aiming to determine the partition coefficient and the selectivity.

The partition coefficient (K) for each enantiomer were calculated separately through Eq. (1):

$$K_{S/R-OFX} = \frac{C_{botS/R-OFX}}{C_{topS/R-OFX}} \quad (1)$$

where $C_{botS/R-OFX}$ and $C_{topS/R-OFX}$ refer to the concentration of R-OFX or S-OFX in the bottom phase (aqueous phase) and in the top phase (HES phase), respectively. Moreover, the selectivity (α) of each system was calculated as described in Eq. (2):

$$\alpha_{R-OFX/S-OXF} = \frac{K_{R-OFX}}{K_{S-OFX}} \quad (2)$$

where K_{R-OFX} and K_{S-OFX} represent the partition coefficient of R-OFX and S-OFX, respectively.

2.6. Computational details

Initially, the geometry optimization of the β-CD trimer, CM-β-CD trimer, S-β-CD trimer, and OFX (in both zwitterionic and positive forms) was conducted using Turbomole v4.5.2 (TmoleX19 software package) at the BP86/TZVP level. In this step, a separate *.cosmo file was generated for each molecule, which was then used in the COSMOthermX software. To confirm the stability of the most stable conformer, a final single-point calculation was conducted to eliminate any oscillation frequencies [27].

The intermolecular interactions energies – specifically, hydrogen bonding (H-bond), electrostatic misfit (misfit) and van der Waals forces (vdW) – between the chiral selectors and OFX were then calculated. These calculations used the COSMO-RS (COnductor-like Screening MOdel for Real Solvents) approach [28], a thermodynamic model that combines quantum chemistry and statistical thermodynamics. All COSMO-RS calculations were performed utilizing the COSMOthermX software package (Version 21.0, COSMOlogic GmbH) at the BP86/TZVP level with the BP_TZVP_21.ctd parameterization [28,29].

2.7. Optimization of the operating extractions conditions

In order to investigate synergies or antagonisms in the extraction process under different conditions, we employed the response surface methodology (RSM). RSM uses mathematical and statistical methods to determine correlations and optimize response(s) [30]. In a 2^{k} RSM, k variables contribute to different response (y), and the data are treated according to the following second-order polynomial equation:

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j \quad (3)$$

where, β_0, β_i, β_{ii}, and β_{ij} refer to the adjusted coefficients for the intercept, linear, quadratic, and interaction terms, respectively, while X_i and X_j represent the operational variables.

A 2^3 RSM was used to optimize the selectivity of OFX by evaluating three operating variables, namely pH (between 3 and 5), excess of chiral selector (ranging from 20 to 121 times the amount of OFX) and HES-water ratio (between 1:2 and 2:1 v/v). A total of twenty experiments were performed. Detailed information on the RSM can be found in Tables S1 and S2 in the Supplementary Material.

Statistical analysis of the results was performed using Statistic 10.0 software (StatSoft, Tulsa, OK, USA) with a 95 % confidence level. This analysis included various methods, including analysis of variance (ANOVA), regression analysis and contour plots. These analysis techniques were used to determine and define the optimal operating conditions in this study.

3. Results and discussion

3.1. Selection of the best chiral selector

In enantioseparation, the interactions of the chiral selectors with the enantiomers are crucial for effective separation of the enantiomers [31]. Cyclodextrins (CD) are preferred as chiral selectors due to their cost efficiency, stability and water solubility [32]. Recently, β-CD derivatives with neutral or charged groups have proven to increase the solubility and enantioseparation efficiency. In this context, we investigated the efficiency of four β-CD derivatives (neutral β-CD: β-CD and HP-β-CD and anionic β-CD: S-β-CD, CM-β-CD) at different molar excesses of chiral selector (20x, 40x, 80x, 120x and 160x), at 25 °C for the enantiomeric separation of OFX. The ^{19}F NMR results obtained are shown in Fig. 3. Please note that the results for the excess of β-CD from 40x to 160x and HP-β-CD from 120x to 160x are not shown in the figure, as it was not possible to dissolve these chiral selectors in water at these concentrations.

As shown in Fig. 3, among the studied chiral selectors, the neutral β-CDs, namely β-CD and HP-β-CD, exhibit a negligible effect on the enantioseparation of OFX. This is evident from their ^{19}F NMR spectra, which lack two distinct peaks even with a high excess of the chiral selector. These findings align with previous studies [7,33,34]. In contrast, anionic β-CD namely, CM-β-CD and S-β-CD showed considerable potential for the enantioseparation of OFX, since two peaks were observed in the NMR spectra, confirming the separation of OFX enantiomers. These results show that, while in neutral β-CD hydrophobic interactions are usually responsible for the formation of host–guest complexes by entrapping the aromatic part of the analytes into the CD cavity, in anionic β-CD electrostatic interactions and hydrogen bonding are the main driving forces for chiral recognition, in addition to the well-known hydrophobic interactions [35]. This allows the formation of a shallow external complex rather than an inclusion complex between anionic β-CD and analytes, which may explain the good enantiomeric results observed for anionic β-CD. However, note that the structure of the analytes also significantly influences the enantioseparation. Furthermore, two distinct peaks were observed with increasing excess of the anionic chiral selector, especially at 120-fold excess of the chiral

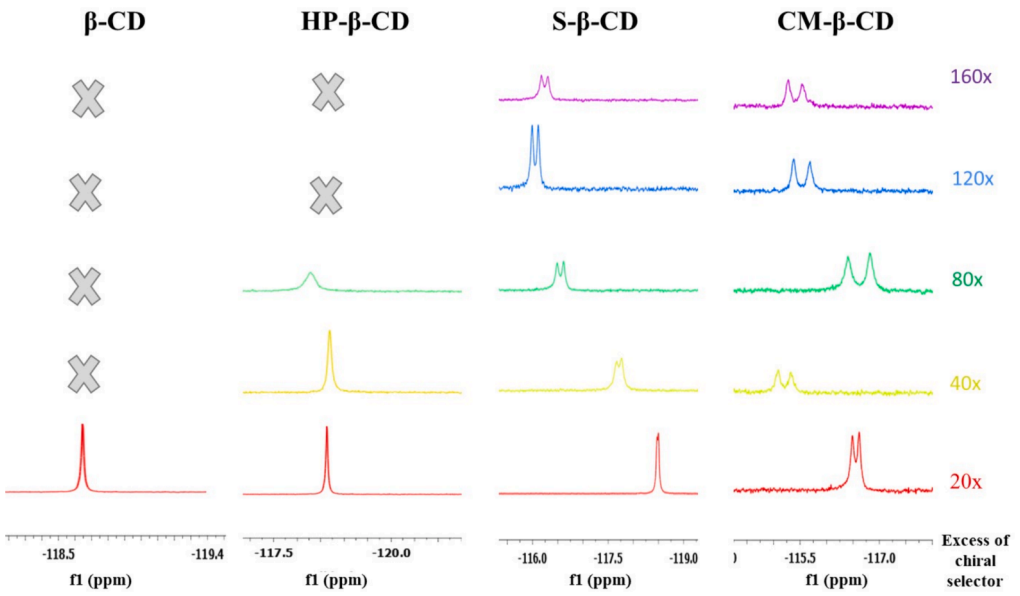


Fig. 3. The ^{1}H-decoupled ^{19}F NMR spectrum of R/S-OFX in the presence of different chiral selectors and molar excess, at 25 °C.

selector. This improved separation can be associated with the formation of additional complexes when the concentration of the chiral selector was increased [36]. However, a further increase in the excess of the anionic chiral selector to 160-fold leads to a decrease in the separation efficiency for the R/S-OFX peaks.

It is worth noting that despite some studies indicating successful OFX enantiomer separation with HP-β-CD [33,34,37], our observations are not consistent with this, suggesting that its efficacy may be limited to certain experimental conditions. On the other hand, only when CM-β-CD was used as a chiral selector, especially at 120-fold excess, two clearly separated peaks were observed, highlighting its superiority in the enantioseparation of OFX. These results are consistent with previous studies showing that anionic derivatives of β-CD, particularly CM-β-CD, produce the most stable complexes and the highest resolution in enantioseparation [33,38].

To gain insights into why the chiral selector CM-β-CD performs better than S-β-CD in the enantiomer separation of OFX, we calculated the intermolecular interaction energies, including hydrogen bonding (H-

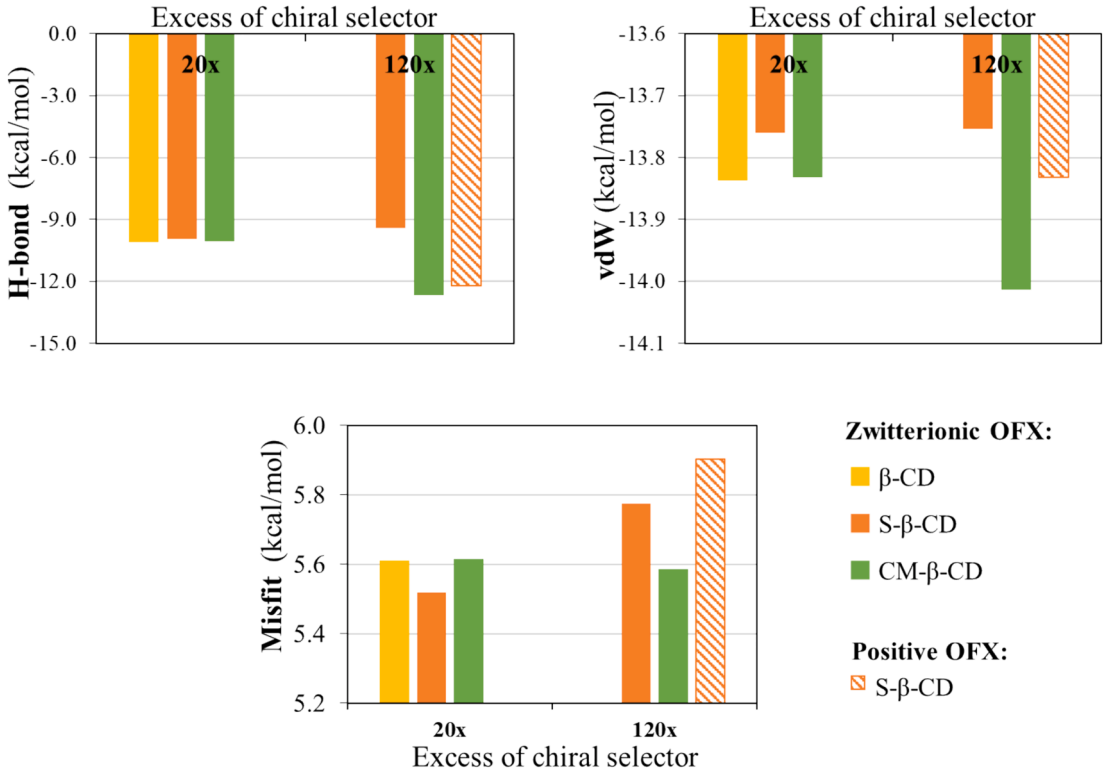


Fig. 4. Interaction energies, including hydrogen bonding (H-bond), electrostatic misfit (misfit), and van der Waals forces (vdW), calculated using COSMO-RS for aqueous mixtures of β-CD (yellow), S-β-CD (orange), or CM-β-CD (green) with OFX in its zwitterionic (solid line) or positive (dashed line) form, at 20-fold and 120-fold excess of the chiral selector. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bond), electrostatic misfit (misfit), and van der Waals forces (vdW). These calculations were performed using COSMO-RS for water mixtures with β-CD, S-β-CD or CM-β-CD and OFX at 20- or 120-fold excess of the chiral selector. The results are shown in Fig. 4 and Table S3 in the Supplementary Material. β-CD was not included at 120-fold excess because it is not water soluble at this concentration. In addition, the mixture of S-β-CD and OFX at 120-fold excess had a pH of 5.7, meaning that OFX was present in both its zwitterionic and positive forms (OFX $pK_{a1} = 5.2$ and $pK_{a2} = 8.4$ [39]), so calculations were performed for both OFX forms. For the other mixtures, the interaction energies were only calculated for this form, as they had a pH of about 6.1 and OFX was predominantly present in its zwitterionic form.

Fig. 4 shows that both H-bond and vdW interactions are slightly more negative (with greater intensity) for the chiral selector CM-β-CD at an excess of 120x. Conversely, under these same conditions, the misfit energy interactions have less intensity. Negative values for H-bond and vdW interactions indicate stronger interactions between the chiral selector and OFX, while positive misfit interactions are generally disadvantageous – the lower the better [40]. These results show that CM-β-CD at 120x has the strongest interactions with OFX, as observed experimentally (Fig. 3). When comparing different chiral selectors at 20x excess, especially in terms of H-bond energy, they show similar values, indicating a comparable affinity for OFX; consequently, no significant differences were expected under these conditions, as observed experimentally (Fig. 3). In addition, for S-β-CD at 120x, it should be noted that half of the OFX is in its positive form and the other half in the zwitterionic form, which could explain why S-β-CD was less effective than CM-β-CD in enantiomer separation at this concentration (Fig. 3). In this context, it is understandable that CM-β-CD at 120x shows the highest efficiency in enantiomer separation, as it has the most negative values for both H-bond and vdW energies and the lowest misfit energy among the chiral selectors studied.

Since CM-β-CD with a 120-fold excess showed the most promising results in OFX enantiomer separation, this chiral selector and molar excess were chosen for subsequent studies.

3.2. Selection of the optimal enantioselective Liquid-Liquid extraction (ELLE)

To identify the optimal HES-based ELLE for OFX enantioseparation, fourteen systems were explored, assessing four different HES families (L-menthol: fatty acid, L-menthol: fatty alcohol, fatty acid: fatty acid, and fatty acid: fatty alcohol) with various molar ratios of HES (Fig. 5). The experiments were conducted under the following conditions: 1:1 (v/v) HES-water ratio, 120-fold excess of CM-β-CD, stirring at 50 rpm for at least 12 h at (25 ± 1 °C). Fig. 5 shows the results obtained regarding the selectivity of the systems for the enantioseparation of OFX and initial pH of each system (for more details see Table S4 Supplementary Material). The pH was not adjusted during these experiments. Furthermore, CM-β-CD prefers the aqueous phase due to its high hydrophilicity, and since the partition coefficient of R-OFX exceeds the partition coefficient of S-OFX in the aqueous phase, this indicates that R-OFX is predominantly in this phase (water). As a result, the chiral cavities of CM-β-CD exhibit a specific affinity for the R-enantiomer of OFX due to its high hydrophilicity [38]. This selective recognition is consistent with the three-point interaction model, in which optimal chiral discrimination occurs through at least three simultaneous interaction points (hydrogen bonds, hydrophobic effects and van der Waals forces) [38]. These results support the complex formation with the preferred enantiomer and promote its separation from the racemic mixture.

As shown in Fig. 5, HES-based ELLE are in general promising for OFX enantioseparation, which is consistent with the hypothesis that highly hydrophobic solvents are advantageous to achieve robust enantiorecognition when β-CD derivatives are used as chiral selectors [18]. The only exception is the HES group based on L-menthol: fatty alcohol, which has a significantly lower selectivity, suggesting that this group may not be suitable for enantioseparations. These systems are characterized by being those with higher pH values (around 6.3), which could explain these results as the OFX molecule is in its zwitterionic form at this pH (OFX $pK_{a1} = 5.2$ and $pK_{a2} = 8.4$) [39], which could lead to weaker interactions with the chiral selector [41]. The impact of pH on enantioseparation will be further discussed in more detail ahead. On the other hand, fatty acid-based HES proves to be a good option for OFX enantioseparation and shows the most promising results. It is noteworthy that these systems have pH values between 4.5 and 5.3, where the molecules are positively monocharged, and therefore have a higher affinity to interact with the anionic chiral selector. It is also observed that increasing the alkyl chain length of the fatty acids (e.g., L-menthol: C8 acid (1:2) with α of 1.19 ± 0.03 *vs.* L-menthol: C10 acid (1:2) with α of 1.42 ± 0.04) and their molar ratio (e.g., L-menthol: C10 acid (1:2) with an α of 1.42 ± 0.04 *vs.* L-menthol: C10 acid (1:3) with an α of 1.85 ± 0.05) contribute to a better separation of the OFX enantiomers. An exception was observed with HES from fatty acid:fatty acid, where a higher molar ratio of fatty acids led to a decrease in the selectivity of OFX enantiomer separation (i.e., C10 acid:C12 acid (2:1) with an α-value of 2.01 ± 0.06 versus C10 acid:C12 acid (3:1) with an α-value of

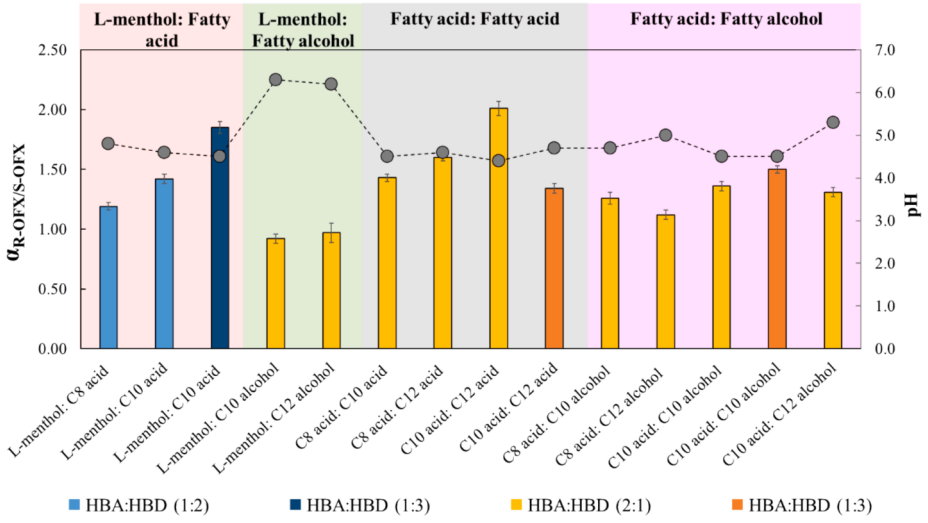


Fig. 5. Selectivity of OFX ($\alpha_{R\text{-}OFX/S\text{-}OFX}$) for each system (bars) and pH (circles), using the chiral selector CM-β-CD at a 120-fold excess. Experiments were conducted at 25 °C.

1.34 ± 0.04).

In summary, among the HES-based ELLE systems, the one utilizing a C10 acid:C12 acid (in a 2:1 ratio) demonstrated the highest effectiveness, boasting a selectivity of up to 2.01 ± 0.06. Consequently, this system was chosen for further evaluation in subsequent steps.

3.3. Optimization of the operating extractions conditions

A RSM was used to optimize the operating conditions for OFX enantiomer separation in order to analyze different variables simultaneously and to identify the most significant parameters and their interaction. The independent variables considered were pH, excess of chiral selector and HES-water ratio (v/v), and the dependent variable was the selectivity of OFX ($\alpha_{R\text{-}OFX/S\text{-}OFX}$). Details about the results, the statistical analyses and fitted model can be found in the Supplementary Material (Tables S5–S7, Figs. S2 and S3). The fitted model was obtained using the analysis of variance (ANOVA) to estimate the statistical significance of the variables and their interactions. It shows satisfying predictability at a confidence level of 95 % with R^2 = 0.92 and F-calculated > F-tabulated, demonstrating the model reliability in describing experimental findings. The impact of these three variables on the selectivity of OFX is illustrated in Fig. 6. In addition, the Pareto chart (Fig. S2 in Supplementary Material) highlights the significant effects of operating variables, including pH, excess of chiral selector, and HES-water ratio, along with some of their interactions. The order of variables with statistical significance is HES-water ratio ≥ pH > excess of chiral selector^2 > pH^2 > excess of chiral selector. The effects of each variable will be discussed in more detail below.

3.3.1. Effect of HES-water ratio (v/v) on the enantioseparation of OFX

The volume ratio of the phases typically plays an important role in the enantioseparation in ELLE [20,31]. In our case, the HES-water ratio (v/v) was the most important parameter, together with pH, as mentioned above. The results show a higher selectivity at a lower HES-water ratio (v/v), highlighting the significant influence of a higher ratio of the aqueous phase on the enantiomer distribution [20]. This can be explained by the lower volume of HES, which allows more OFX to enter the hydrophilic phase. Consequently, CM-β-CD can interact more effectively with the enantiomers enhancing enantioseparation [31].

3.3.2. Effect of pH on enantioseparation of OFX

The significance of pH in enantioseparation has been extensively investigated in previous studies [20,22], and the OFX enantiomers can usually be separated in a wide pH range, except when the molecule is in its neutral zwitterionic form (OFX has two pK_a values: pK_{a1} = 5.2 and pK_{a2} = 8.4, with the neutral zwitterionic form prevailing between these pK_a values) [39]. Therefore, a pH range of 3.0 to 5.0 was chosen for enantioseparation in this study.

As expected, the pH plays a crucial role in the separation of OFX enantiomers emerging as the second most significant variable. The results show that the quadratic effect of pH has a negative influence on the enantioseparation of OFX (Table S6 in the Supplementary Material). Thus, maximum selectivity is observed at intermediate pH values, where an increase from 3.0 to 3.5 enhances selectivity, but further increases above 3.5 lead to decreased selectivity.

This behavior can be attributed to the chemical structure of OFX, characterized by the presence of a carboxyl group (pK_{a1} = 5.2) and a piperazine group (pK_{a2} = 8.4) [39]. The charge of these functional groups varies with pH, becoming positively charged in an acidic medium [39]. Additionally, CM-β-CD is negatively charged at acid medium (pK_a = 4) [41]. As a result, there is a strong ionic interaction between R-OFX and CM-β-CD, enhancing the selectivity. These results are consistent with previous research [33,38] showing that the complexes formed between R-OFX and CM-β-CD remain stable at acidic pH. Jiao et al. [22] also demonstrated the favorable influence of acidic conditions, specifically at pH 4, on the enantioseparation of OFX using an ABS based on PEG and ammonium sulfate, with two chiral selectors, L-TA and HP-β-CD.

On the other hand, a reduction in selectivity can be observed when the pH value increases. This is attributed to the increased deprotonation

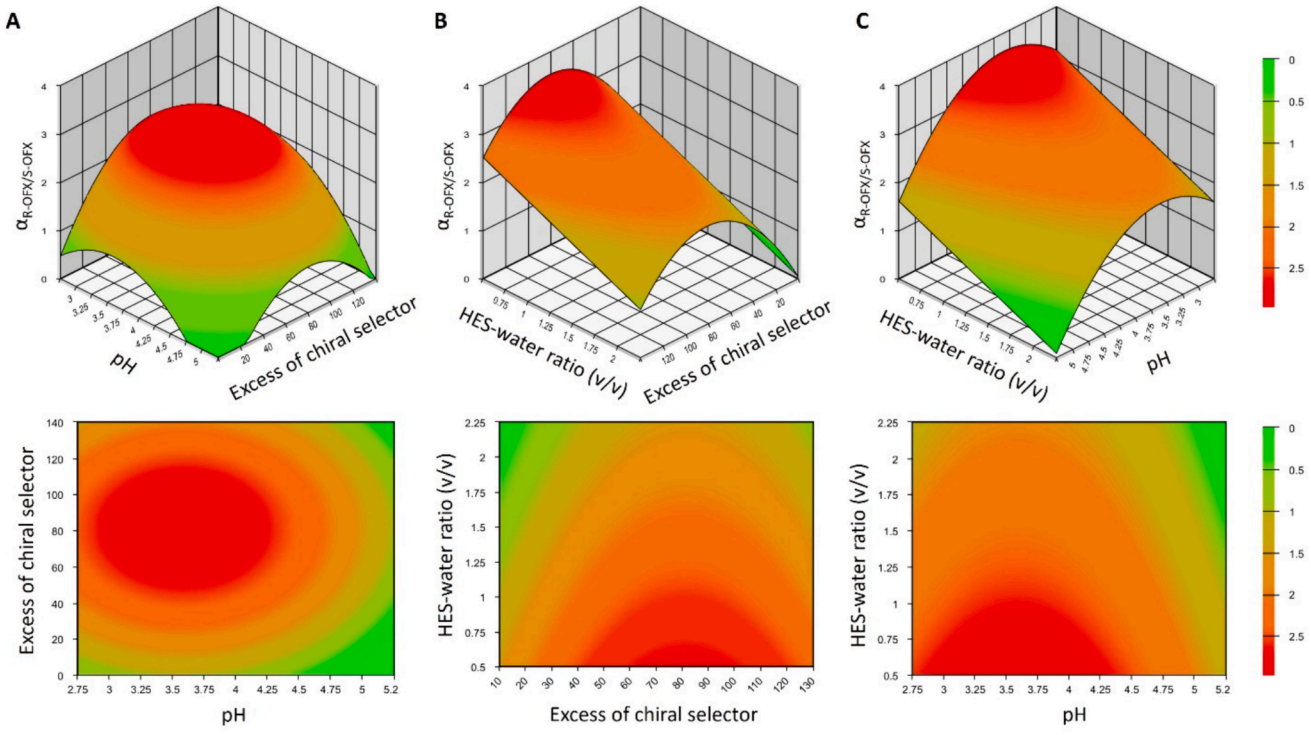


Fig. 6. Responsive surface and contour plots of the selectivity of OFX ($\alpha_{R\text{-}OFX/S\text{-}OFX}$) with the combined effects: (**A**) pH and excess of chiral selector, (**B**) HES-water ratio (v/v) and excess of chiral selector and (**C**) HES-water ratio (v/v) and pH.

of the carboxyl groups of OFX, which leads to an increase in the zwitterionic form. Simultaneously, the electrostatic attraction between OFX and CM-β-CD decreases, contributing to a less efficient enantioseparation. In summary, the most efficient enantioseparation is achieved at a pH around 3.5, where CM-β-CD is negatively charged while OFX is positively charged.

3.3.3. Effect of excess of chiral selector on enantioseparation of OFX

The excess of the chiral selector is considered a critical factor in enantioseparation. Chiral selectors not only improve the selectivity of enantiomers in ELLE, but also influence the partitioning of enantiomers between phases [22]. The data from Fig. 6 and Table S6 in the Supplementary Material show a positive correlation between CM-β-CD excess and the selectivity of R/S-OFX. This can be explained by the fact that an increase in CM-β-CD excess enhances interactions such as hydrogen bonding and dipole–dipole interactions between the enantiomers and the chiral selector, which facilitates the formation of more complexes and improves chiral recognition [20,33]. However, Fig. 6 shows that exceeding a 100-fold excess of CM-β-CD leads to a decrease in the selectivity of OFX enantioseparation. This decrease is consistent with the negative effects of the quadratic effect of excess CM-β-CD. The excess concentration of CM-β-CD likely leads to saturation of the binding sites for the OFX enantiomers, disrupting the chiral recognition process. This saturation could affect the separation efficiency and even lead to non-selective interactions between the enantiomers and the chiral selector. These findings are consistent with the results of previous studies [20,42]. Jiao et al. [20] demonstrated that in an ELLE system composed of n-octanol and water, increasing the concentration of the hydrophobic chiral selector (a complex of D_2EHPA and L-DBTA) improved the distribution, enantiomeric excess, and separation factor of OFX enantiomers up to a maximum. Beyond this optimal concentration, however, further increases led to a decrease in enantioseparation efficiency.

3.3.4. Discussion

After analyzing the surface plots shown in Fig. 6, the optimal operating conditions for the enantioseparation of OFX with ELLE based on HES-C10 acid:C12 acid (2:1) and CM-β-CD as chiral selector are an excess of chiral selector of 77, an HES-water ratio of 1:2, and a pH of 3.5. Under these optimized conditions, a selectivity of R/S-OFX of 3.81 ± 0.33 was achieved. Remarkably, this experimental result aligns with the value predicted by multiple regressions using RSM analysis, which estimated a selectivity of R/S-OFX at 3.75, resulting in a relative error of only 1.6 %. This validated the model with a confidence interval of 95 %, underlining the robustness of the fitted model.

Table 3 shows a comparison of the developed method for the enantioseparation of OFX with previous ELLE approaches. Jiao et al. [22] used an ABS consisting of PEG 2000 and ammonium sulfate using L-TA in the PEG-rich phase and HP-β-CD in the salt-rich phase as chiral selectors and achieved a maximum selectivity of 1.32 for OFX under optimal conditions. In contrast, in another study [20], a higher separation factor of 2.48 was obtained for R/S-OFX using conventional ELLE with n-octanol, using both hydrophilic and hydrophobic chiral selectors; however, the hydrophobic component was a complex of L-DBTA and D_2EHPA. In addition, Li et al. [21] investigated different organic solvents and chiral selectors in ELLE for OFX extraction. Their most effective system, which used n-octanol and water with L-DTTA as the hydrophobic chiral selector and β-CD as the hydrophilic selector, achieved an enantioselectivity of 2.4 under optimized conditions. This demonstrates that complex chiral selectors are not always necessary, depending on the specific conditions of enantioseparation. Overall, research on OFX enantioseparation in two-phase systems, as described both in the literature and in this study, highlights the importance of careful solvent and chiral selector selection, as well as optimized operating factors such as pH control. These elements are crucial for increasing the efficiency of OFX enantioseparation. Notably, the HES-based ELLE system using a C10 acid:C12 acid (2:1) HES with CM-B-CD as chiral selector at pH 3.5 proves to be the most promising approach.

Table 3
A summary of extraction conditions for some enantioselective liquid–liquid systems in OFX enantioseparation from the literature.

Enantioseparation method	Chiral selector	Extraction conditions	Selectivity (α)	Ref.
Enantioselective liquid–liquid extraction (ELLE), including aqueous biphasic system (ABS)	L-TA; HP-β-CD	[OFX] = 0.04 mg/ml, pH = 4, [HP-β-CD] = 0.02 mol/l, [L-TA] = 0.05 mol/l, 40 wt% of PEG 2000, 25 wt% of ammonium sulfate. (ABS − a specific type of ELLE)	1.32	[22]
	β–CD;	[OFX] = 0.002 g/ml, pH = 6.5, [β–CD] = 0.011 g/ml, [L-DBTA] = 0.055 g/ml and [D_2EHPA] = 0.3 g/ml, organic phase volume (n-octanol) = 14 ml, aqueous phase volume = 20 ml, temperature = 25 °C.	2.48	[20]
	L-DTTA;β-CD	[OFX] = 1 mg/ml, [L-DTTA] = 0.012 g/ml and [β-CD] = 0.006 g/mL, organic phase volume (n-octanol) = 3 ml, aqueous phase volume = 3 ml, temperature = 25 °C.	2.4	[21]
	CM-β-CD	[OFX] = 2 mg/ml, pH = 3.5, molar excess of chiral selector = 77-fold, HES-water volume ratio = 1:2 (V/V), temperature = 25 °C.	3.8 ± 0.3	This work

Expanding the discussion to include process development, it is crucial to develop effective method for recovering the active pharmaceutical ingredient and to understand the toxicity (both eco- and cytotoxicity) of HES.

Regarding the recovery of the target enantiomer, we did not directly study the extraction of S-OFX from the HES phase in this study. However, Li et al. [21] investigated this aspect using an acetic acid solution to recover S-OFX from octanol, a highly hydrophobic solvent, and showed effective recovery. This suggests that this approach could be effective for the extraction of S-OFX from the HES phase, as it is also a highly hydrophobic solvent. Moreover, this method not only facilitates the recovery of the enantiomer, but also enables the reuse of HES in subsequent extractions, which is crucial for the development of a sustainable process. Nevertheless, further studies are needed to evaluate the stability of HES after the recovery process.

Regarding the ecotoxicity of our HES, although no specific tests have yet been carried out, the analysis of only the C10 and C12 acids can provide information on their hazard potential. Their slow degradation in water poses a risk to aquatic ecosystems and requires proper disposal to minimize the impact on the environment [43,44]. On the other hand, the low solubility of HES (2 wt%) helps to reduce water pollution and improve environmental sustainability [45]. The challenge of complete separation of HES from compounds also requires an evaluation of the

cytotoxicity of HES. Studies suggest different cytotoxic effects of fatty acid-based HES on yeasts (*C. albicans*) [46], bacteria and HaCaT [47], so further targeted research is needed. Although the Food and Drug Administration (FDA) classifies the HES components themselves as safe [48], and they are therefore widely used in the industry, high doses can cause irritation, so further research is needed.

Taking into account all the aforementioned points, the development of advanced recycling methods and the provision of comprehensive toxicity data are essential for a more effective integration of HES into industrial applications.

4. Conclusions

Aiming at the enantioseparation of OFX, this study developed an ELLE method based on HES, utilizing both neutral and anionic derivatives of β-CD as chiral selectors. An initial screening was conducted to identify the most promising chiral selector for the enantioseparation of OFX. The results highlighted the potential efficacy of anionic β-CD derivatives, particularly CM-β-CD, in the effective separation of R/S-OFX. After that, a screening of fourteen HES-based ELLE systems revealed that an increased molar ratio of fatty acids, coupled with an extension of their alkyl chain length, significantly improved the efficiency of enantioseparation of OFX. Among these combinations, C10 acid: C12 acid at a ratio of 2:1 was found to be the most favorable extraction solvent. Finally, an experimental design was carried out to investigate the most important factors in enantioseparation with C10 acid: C12 acid (2:1) HES and CM-β-CD as a chiral selector. The results showed that the three variables evaluated, i.e. pH, excess of chiral selector and HES-water ratio (v/v), had an effect on improving the selectivity of R/S-OFX separation. Under the optimal conditions, i.e. 77-fold excess of chiral selector, pH of 3.5 and HES-water ratio (v/v) of 0.54, the highest selectivity (α) of 3.8 ± 0.3 was achieved in a single-step.

These results emphasize the critical significance of carefully selecting appropriate solvents and chiral selectors while evaluating key factors for the separation. This approach has the potential to significantly improve the efficiency of enantioseparation of chiral compounds. This research not only addresses the separation of OFX enantiomers, one of the most common quinolone antibiotics, but may also be of interest to the pharmaceutical industry at large, which needs to commercialize chiral drugs as single enantiomers.

CRediT authorship contribution statement

Mahtab Moradi: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Ana M. Ferreira:** Writing – review & editing, Supervision, Formal analysis, Data curation. **Catarina M.S.S. Neves:** Writing – review & editing, Formal analysis, Data curation. **Filipe H.B. Sosa:** Data curation, Software, Writing – review & editing. **Gholamreza Pazuki:** Writing – review & editing, Supervision, Conceptualization. **João A.P. Coutinho:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was developed partly within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 (DOI 10.54499/UIDB/50011/2020), UIDP/50011/2020 (DOI 10.54499/UIDP/50011/2020) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). AMF, CSSN and FHBS acknowledge FCT for the research contracts CEECIND/00361/2022 (DOI 10.54499/2022.00361.CEECIND/CP1720/CT0020), CEECIND/01975/2017 (DOI 10.54499/CEECIND/01975/2017/CP1459/CT0037) and CEECIND/07209/2022 (DOI 10.54499/2022.07209.CEECIND/CP1720/CT0019) under the Scientific Stimulus – Individual Call, respectively.

Appendix A. Supplementary data

Experimental details of optimization of separation conditions by RSM (Table S1 and S2). The ^{19}F NMR spectrum of OFX and NaF (Figure S1). COSMO-RS results (Table S3). Screening results for choosing the best system and chiral selector ratio (Table S4). Experimental results on RSM using the best HES and chiral selector (Table S5 to S7 and Figs. S2 and S3). Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2024.128462.

References

[1] S. Orlandini, G. Hancu, Z.I. Szabó, A. Modroiu, L.A. Papp, R. Gotti, S. Furlanetto, New trends in the quality control of enantiomeric drugs: quality by design-compliant development of chiral capillary electrophoresis methods, Molecules 27 (2022) 7058, https://doi.org/10.3390/MOLECULES27207058.
[2] R.U. McVicker, N.M. O'Boyle, Chirality of new drug approvals (2013–2022): trends and perspectives, J. Med. Chem. 67 (2024) 2305–2320, https://doi.org/10.1021/ACS.JMEDCHEM.3C02239.
[3] A. Calcaterra, I. D'Acquarica, The market of chiral drugs: chiral switches versus de novo enantiomerically pure compounds, J. Pharm. Biomed. Anal. 147 (2018) 323–340, https://doi.org/10.1016/j.jpba.2017.07.008.
[4] S.W. Smith, Chiral toxicology: it's the same thing…only different, Toxicological Sci 110 (2009) 4–30, https://doi.org/10.1093/toxsci/kfp097.
[5] FDA'S policy statement for the development of new stereoisomeric drugs, Chirality 4 (1992) 338–340. https://doi.org/10.1002/CHIR.530040513.
[6] C. Zhao, W. Lu, X. Meng, Using chiral magnetic surface molecularly imprinted polymers for chiral separation of Ofloxacin, J. Polym. Res. 28 (2021) 1–8, https://doi.org/10.1007/S10965-021-02581-3.
[7] M.K. Rofouei, N. Sohrabi, S. Haghgoo, Application of 19 F NMR spectroscopic method to the enantioresolution and quantitative determination of ofloxacin in pharmaceutical formulations, Recent Res. Modern Med. (2011) 385–394.
[8] S. Deng, J. Pan, M. Wang, Y. Huang, Z. Xia, Study on improvement of chiral separation of capillary electrophoresis based on cyclodextrin by deep eutectic solvents, Talanta 220 (2020) 121419, https://doi.org/10.1016/j.talanta.2020.121419.
[9] L. He, X. Chen, X. Li, Z. Zhou, Z. Ren, Chiral co-selector induced chirality switching in the enantioseparation of ofloxacin by forming a co-crystal, New J. Chem. 43 (2019) 15048–15051, https://doi.org/10.1039/C9NJ02921D.
[10] I. Ali, K. Sekkoum, N. Belboukhari, M.N. Rebizi, M.E.A. Zaid, K. Yusuf, A. A. Alothman, B.A. AlJumah, M. Ouladsmane, Determination of enantio-separation, absolute configuration and chiral recognition mechanism of ofloxacin and flumequine by HPLC and modeling studies, J. Chem. Technol. Biotechnol. 96 (2021) 2901–2908, https://doi.org/10.1002/JCTB.6843.
[11] N. Salama, H. Zaazaa, L. Abd El Halim, M. Salem, L. Abd El Fattah, Thin-layer chromatographic enantioseparation of ofloxacin and zopiclone using hydroxypropyl-beta-cyclodextrin as chiral selector and thermodynamic studies of complexation, J. Planar Chromatogr.–Mod. TLC 27 (2014) 166–173, https://doi.org/10.1556/JPC.27.2014.3.4/METRICS.
[12] C. Aydoğan, V. Karakoc, F. Yılmaz, H. Shaikh, A. Denizli, Enantioseparation of ofloxacin by ligand exchange capillary electrophoresis using L-histidine modified nanoparticles as chiral ligand, J Biol & Chem 41 (2013) 29–36.
[13] N. Dat, N.Q. Vượng, T.H. Hanh, Enantioselective separation of ofloxacin by ligand exchange chromatography, Vietnam J. Chem. 53 (2015) 65–68, https://doi.org/10.15625/0866-7144.2015-2e-01.
[14] M. Gumustas, S.A. Ozkan, B. Chankvetadze, Analytical and preparative scale separation of enantiomers of chiral drugs by chromatography and related methods, Curr. Med. Chem. 25 (2018) 4152–4188, https://doi.org/10.2174/0929867325666180129094955.
[15] A.R.F. Carreira, A.M. Ferreira, M.R. Almeida, J.A.P. Coutinho, T.E. Sintra, Propranolol resolution using enantioselective biphasic systems, Sep. Purif. Technol. 254 (2021) 117682, https://doi.org/10.1016/J.SEPPUR.2020.117682.

[16] B. Schuur, B.J.V. Verkuijl, A.J. Minnaard, J.G. De Vries, H.J. Heeres, B.L. Feringa, Chiral separation by enantioselective liquid–liquid extraction, Org. Biomol. Chem. 9 (2010) 36–51, https://doi.org/10.1039/C0OB00610F.
[17] T.G. Susanti, E.B. Meinds, B. Pinxterhuis, J.G.D. Schuur, B.L. Vries, J.G.M. Feringa, J. Winkelman, H.J. Yue, Heeres, Proof of concept for continuous enantioselective liquid–liquid extraction in capillary microreactors using 1-octanol as a sustainable solvent, Green Chem. 19 (2017) 4334–4343, https://doi.org/10.1039/C7GC01700F.
[18] B. Zhu, H. Qiu, C. Ma, S. Chen, J. Zhu, S. Tong, Recent progress on chiral extractants for enantioselective liquid-liquid extraction, J. Chromatogr. A 1709 (2023) 464389, https://doi.org/10.1016/J.CHROMA.2023.464389.
[19] M. Steensma, N.J.M. Kuipers, A.B. de Haan, G. Kwant, Modelling and experimental evaluation of reaction kinetics in reactive extraction for chiral separation of amines, amino acids and amino-alcohols, Chem. Eng. Sci. 62 (2007) 1395–1407, https://doi.org/10.1016/J.CES.2006.11.043.
[20] F. Jiao, W. Yang, D. Huang, J. Yu, X. Jiang, X. Chen, Enantioseparation of ofloxacin enantiomers by mixed extractants in biphasic system, Sep. Sci. Technol. 47 (2012) 1971–1976, https://doi.org/10.1080/01496395.2012.659784.
[21] J. Li, H. Cheng, Z. Qi, Enhancement of separation performance for ofloxacin enantiomers by a biphasic recognition chiral extraction system, AIChE J. (2024) e18367.
[22] F. Jiao, J. Wang, X. Jiang, H. Yang, S. Shi, X. Chen, J. Yu, Biphasic recognition enantioseparation of ofloxacin enantiomers by an aqueous two-phase system, J. Chem. Technol. Biotechnol. 90 (2015) 2234–2239, https://doi.org/10.1002/JCTB.4538.
[23] D. Sun, R. Wang, F. Li, L. Liu, Z. Tan, Enantioselective extraction of phenylalanine enantiomers using environmentally friendly aqueous two-phase systems, Processes 6 (2018) 212, https://doi.org/10.3390/PR6110212.
[24] M. Moradi, M. Mohammadian, G. Pazuki, S. Shahriari, Partitioning of crocin in a novel aqueous two-phase system composed of a deep eutectic solvent and acetonitrile, J. Chem. Eng. Data 67 (2022) 1205–1213, https://doi.org/10.1021/acs.jced.2c00027.
[25] M. Moradi, G. Pazuki, S. Zarei Mahmoudabadi, A comprehensive study of the water stability of eutectic solvents using COSMO-SAC, J. Mol. Liq. 387 (2023) 122605, https://doi.org/10.1016/J.MOLLIQ.2023.122605.
[26] M. Moradi, A.M. Ferreira, C.M.S.S. Neves, S.Z. Mahmoudabadi, G. Pazuki, J.A. P. Coutinho, Extraction of ofloxacin from water using hydrophobic eutectic solvents, J. Environ. Chem. Eng. 12 (3) (2024) 113075.
[27] R. Santiago, F.H.B. Sosa, I. Díaz, M. González-Miquel, J.A.P. Coutinho, Predicting partition coefficients in organic biphasic systems using COSMO-RS, Ind. Eng. Chem. Res. 62 (2023) 17905–17913, https://doi.org/10.1021/ACS.IECR.3C02303.
[28] F. Eckert, A. Klamt, Fast solvent screening via quantum chemistry: COSMO-RS approach, AIChE J. 48 (2002) 369–385, https://doi.org/10.1002/AIC.690480220.
[29] BIOVIACOSMOtherm, DassaultSystèmes, (2021). https://www.3ds.com/products/biovia/cosmo-rs/cosmotherm (accessed June 12, 2023).
[30] M. Moradi, S.N. Ashrafizadeh, Nitrate removal from tapwater by means of electrocoagulation-flotation process, Sep. Sci. Technol. 55 (2020) 1577–1587, https://doi.org/10.1080/01496395.2019.1602143.
[31] S. Ma, F. Li, L. Liu, L. Liao, L. Chang, Z. Tan, Deep-eutectic solvents simultaneously used as the phase-forming components and chiral selectors for enantioselective liquid-liquid extraction of tryptophan enantiomers, J. Mol. Liq. 319 (2020) 114106, https://doi.org/10.1016/J.MOLLIQ.2020.114106.
[32] J.M. Saz, M.L. Marina, Recent advances on the use of cyclodextrins in the chiral analysis of drugs by capillary electrophoresis, J. Chromatogr. A 1467 (2016) 79–94, https://doi.org/10.1016/J.CHROMA.2016.08.029.
[33] B. Zhu, S. Xu, X. Guo, L. Wei, J. Yu, T. Wang, Use of various β-cyclodextrin derivatives as chiral selectors for the enantiomeric separation of ofloxacin and its five related substances by capillary electrophoresis, J. Sep. Sci. 40 (2017) 1784–1795, https://doi.org/10.1002/JSSC.201601403.
[34] T. De Boer, R. Mol, R.A. De Zeeuw, G.J. De Jong, K. Ensing, Enantioseparation of ofloxacin in urine by capillary electrokinetic chromatography using charged cyclodextrins as chiral selectors and assessment of enantioconversion, Electrophoresis (2001), https://doi.org/10.1002/1522-2683.
[35] D. Boczar, K. Michalska, Cyclodextrin inclusion complexes with antibiotics and antibacterial agents as drug-delivery systems—A pharmaceutical perspective, Pharmaceutics 14 (2022), https://doi.org/10.3390/PHARMACEUTICS14071389.
[36] Q. Ma, W. Cong, Y. Liu, Z. Geng, Y. Lin, Z. Wang, Experimental and computational study on the enantioseparation of four chiral fluoroquinolones by capillary electrophoresis with sulfated-β-cyclodextrin as chiral selector, Chirality 33 (2021) 549–557, https://doi.org/10.1002/CHIR.23340.
[37] F.E.O. Suliman, A.A. Elbashir, O.J. Schmitz, Study on the separation of ofloxacin enantiomers by hydroxyl-propyl-β-cyclodextrin as a chiral selector in capillary electrophoresis: a computational approach, J. Incl. Phenom. Macrocycl. Chem. 83 (2015) 119–129, https://doi.org/10.1007/S10847-015-0547-2.
[38] G. Tóth, R. Mohácsi, Á. Rácz, A. Rusu, P. Horváth, L. Szente, S. Béni, B. Noszál, Equilibrium and structural characterization of ofloxacin-cyclodextrin complexation, J. Incl. Phenom. Macrocycl. Chem. 77 (2013) 291–300, https://doi.org/10.1007/S10847-012-0245-2.
[39] X. Liang, L. Zhao, M. Deng, L. Liu, Y. Ma, X. Guo, Separation of ofloxacin and its six related substances enantiomers by chiral ligand-exchange chromatography, Chirality 27 (2015) 843–849, https://doi.org/10.1002/CHIR.22527.
[40] I. Khan, K.A. Kurnia, T.E. Sintra, J.A. Saraiva, S.P. Pinho, J.A.P. Coutinho, Assessing the activity coefficients of water in cholinium-based ionic liquids: experimental measurements and COSMO-RS modeling, Fluid Phase Equilib. 361 (2014) 16–22, https://doi.org/10.1016/J.FLUID.2013.10.032.
[41] M. Fillet, I. Bechet, P. Hubert, J. Crommen, Resolution improvement by use of carboxymethyl-β-cyclodextrin as chiral additive for the enantiomeric separation of basic drugs by capillary electrophoresis, J. Pharm. Biomed. Anal. 14 (1996) 1107–1114, https://doi.org/10.1016/S0731-7085(95)01756-9.
[42] B. Chankvetadze, W. Linder, G.K.E. Schriba, Enantiomer separations in capillary electrophoresis in the case of equal binding constants of the enantiomers with a chiral selector: commentary on the feasibility of the concept, Anal. Chem. 76 (2004) 4256–4260, https://doi.org/10.1021/AC0355202.
[43] European Food Safety Authority, Conclusion on the peer review of the pesticide risk assessment of the active substance Fatty acids C7 to C18 (approved under Regulation (EC) No 1107/2009 as Fatty acids C7 to C20), EFSA J. 11 (2013) 3023. https://doi.org/10.2903/J.EFSA.2013.3023.
[44] K. Boyle, K. Leifer, IIFG Decision documents on reassessment of excemptions from the requirement of a tolerance for fatty acids, Washington, 2002. https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-079029_31-Jul-02_a.pdf (accessed March 20, 2024).
[45] C. Florindo, F. Lima, L.C. Branco, I.M. Marrucho, Hydrophobic deep eutectic solvents: a circular approach to purify water contaminated with ciprofloxacin, ACS Sustain. Chem. Eng. 7 (2019) 14739–14746, https://doi.org/10.1021/acssuschemeng.9b02658.
[46] J.M. Silva, E. Silva, R.L. Reis, A.R.C. Duarte, A closer look in the antimicrobial properties of deep eutectic solvents based on fatty acids, Sustain. Chem. Pharm. 14 (2019) 100192, https://doi.org/10.1016/j.scp.2019.100192.
[47] J.M. Silva, C.V. Pereira, F. Mano, E. Silva, V.I.B. Castro, I. Sá-Nogueira, R.L. Reis, A. Paiva, A.A. Matias, A.R.C. Duarte, Therapeutic role of deep eutectic solvents based on menthol and saturated fatty acids on wound healing, ACS Appl. Bio Mater. 2 (2019) 4346–4355, https://doi.org/10.1021/acsabm.9b00598.
[48] U.S. Food and Drug Administration, Food Additives Permitted for Direct Addition to Food for Human Consumption, Subpart I — Multipurpose Additives, (2023). https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-172/subpart-I/section-172.860 (accessed March 20, 2024).