separations

MDPI

Article

A Cyanoalkyl Silicone GC Stationary-Phase Polymer as an Extractant for Dispersive Liquid–Liquid Microextraction

Mohamed A. Abdelaziz [1,2] and Neil D. Danielson [1,*]

[1] Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA; alima@miamioh.edu
[2] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh 33511, Egypt
* Correspondence: danielnd@miamioh.edu; Tel.: +1-513-529-2872

Abstract: In this work, three cyanoalkyl silicone GC stationary-phase polymers, namely OV-105, OV-225, and OV-275, were investigated as potential extractants for dispersive liquid–liquid microextraction (DLLME). The OV-225 polymer (cyanopropylmethyl-phenylmethylsilicone) exhibited the cleanest chromatographic background and was extensively studied. The proposed polymer was tested through the DLLME of four non-steroidal anti-inflammatory drugs from aqueous samples, followed by HPLC separation with UV detection at 230 nm. To achieve the maximum enrichment, the experimental conditions that influence the DLLME process were optimized using one-factor-at-a-time and design-of-experiment (DoE) approaches. The extraction variables (polymer mass, dispersive solvent volume, buffer pH, and mixing time) were screened by implementing a two-level full factorial design (FFD). Significant variables were fine-tuned using response surface methodology based on a face-centered central composite design (CCD). The optimum conditions were 10 mg of polymer (extraction medium); 50 µL of tetrahydrofuran (dispersive solvent); 100 µL of phosphate buffer pH 2.75 ($[PO_4^{3-}]$ = 100 mM); and 3 min of vortex mixing. The addition of salt had a minimal effect on the enrichment factors. In the optimum conditions, enrichment factors up to 46 were achieved using 1.5 mL samples. Calibration curves exhibited correlation coefficients > 0.999 using 4-pentylbenzoic acid as an internal standard. The limits of quantitation were 5 ng/mL for naproxen, 10 ng/mL for diflunisal, 25 ng/mL for indomethacin, and 75 ng/mL for ibuprofen. The analysis of spiked tap water samples showed adequate relative recoveries and precision. In conclusion, the proposed polymer (OV-225) is a potential greener alternative to traditional organic extractants used in DLLME.

Keywords: dispersive liquid–liquid microextraction; silicone GC polymer; non-sterodaanti-inflammatory drugs

Citation: Abdelaziz, M.A.; Danielson, N.D. A Cyanoalkyl Silicone GC Stationary-Phase Polymer as an Extractant for Dispersive Liquid–Liquid Microextraction. *Separations* **2024**, *11*, 18. https://doi.org/10.3390/separations11010018

Academic Editors: Dimosthenis Giokas and Manolis Manos

Received: 27 November 2023
Revised: 1 January 2024
Accepted: 3 January 2024
Published: 5 January 2024

1. Introduction

Since its development in 2006 [1], dispersive liquid–liquid microextraction (DLLME) has become one of the most widely used sample preparation techniques, as evidenced by a large number of publications present in the literature (Figure 1a). The popularity of this technique can be attributed to its apparent simplicity, low solvent consumption, short extraction time, and achievement of high enrichment factors (EFs). Notwithstanding these merits, the basic DLLME technique relies on the use of chlorinated organic solvents (such as dichloromethane, chloroform, carbon tetrachloride, and chlorobenzene) for extraction. According to our bibliometric analysis of DLLME publications (Figure 1b), a chlorinated hydrocarbon solvent was used for the extraction in 40% of the cases. The wide use of these solvents for DLLME stems from their very low water solubility, which is favorable for efficient extraction. Nevertheless, the use of these solvents violates the current principles of green chemistry, as they are volatile and toxic to the environment and biological systems [2]. Moreover, they are highly hydrophobic and have limited interaction mechanisms with solutes, which may render them inappropriate for the preconcentration of polar analytes

or when analytes vary considerably in polarity. Over the past decade, efforts have been focused on finding safer and more efficient extraction solvents [3]. The use of ionic liquids as extraction solvents for DLLME has proven successful in many applications [4–6]. Although they are considered greener and more versatile alternatives, many of the reported ionic liquids are lighter than water [7,8] and therefore require special apparatus to facilitate extract collection. This complicates the DLLME procedure and could also affect the measurement precision and accuracy.

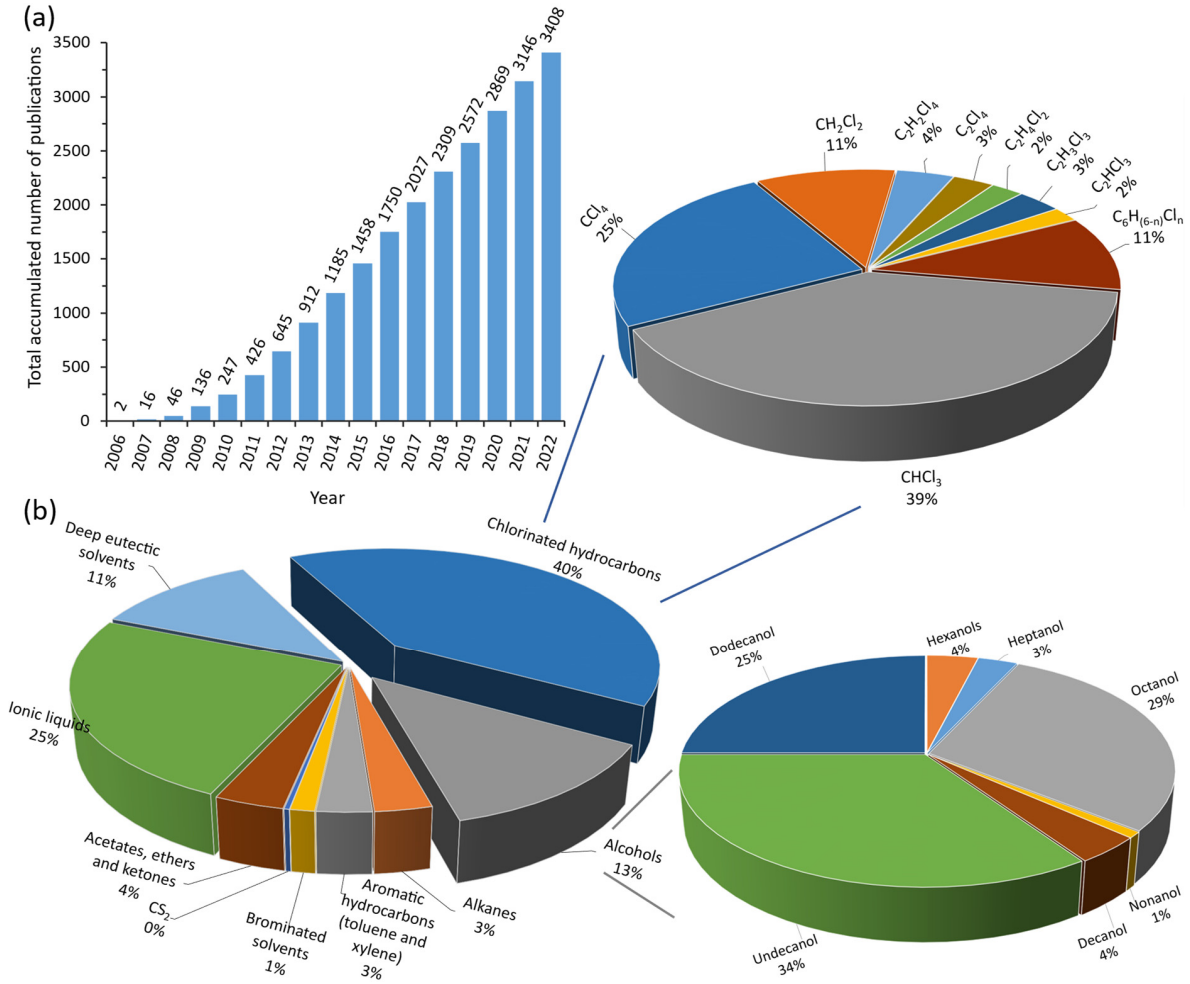


Figure 1. Bibliometric analysis of dispersive liquid–liquid microextraction (DLLME) publications: (**a**) year-wise publications between 2006 and 2022; (**b**) extraction solvents used for DLLME from aqueous samples and their distributions. Data were retrieved from the Scopus database. The detailed methodology of the bibliometric study is discussed in the Supplementary Material.

To the best of the authors' knowledge, the use of polymers as extraction solvents for DLLME has not been sufficiently explored. Out of the 3216 original articles on Scopus related to DLLME (from 2006–July 2023), only about 3% (~100 reports) have the word "polymer" or "poly" mentioned in the abstract.

In most of these reports, the polymer is either the analyte of interest or not a part of the extraction process at all. Only a few of these reports have a polymer used for extraction [9,10]. Liu and coworkers [9] prepared a thermally controlled copolymer of methacryl-polyhedral oligomeric silsesquioxane (MA-POSS) and 2-(dimethylamino) ethyl methacrylate (DMAEMA). The prepared polymer p(POSS-co-DMAEMA) is water-miscible at room temperature, but hydrophobic at high temperatures. The polymer was successfully applied to the DLLME of five phthalic acid esters from water samples. After extraction, the solution temperature was raised to 60 °C, and the floating extract layer was collected,

diluted with 500 µL methanol, and injected into the HPLC. However, the EFs obtained with 25 mL samples ranged only from 15.9 to 48.4. In another article [10], Xu et al. prepared a copolymer of *N*-isopropylacrylamide and maleic anhydride-modified β-cyclodextrin (p(MAH-β-CD-*co*-NIPAM)), which undergoes a phase transition at room temperature. The polymer was used for the extraction of bisphenols from milk. Nevertheless, in both reports, the polymer was not commercially available, and its preparation was tedious and time-consuming. After preparation and purification of the modified monomer, a 24 h reaction under N_2 was required for polymerization, followed by purification by dialysis and freeze-drying. Furthermore, the extract formed after phase separation floats on the solution surface, meaning great caution has to be taken during collection. Notably, the use of polymers as extractants for DLLME could address all the limitations resulting from the use of traditional solvents. In particular, the physicochemical characteristics of polymers can be readily tuned (by proper selection of monomers and molar ratio during copolymerization) to modify selectivity to suit different analytes of interest, and to facilitate phase separation. Moreover, the polymers selected could favorably be bioderived, non-hazardous, and biodegradable.

Polysiloxanes, also known as silicones, are a large class of polymers characterized by a silicon-oxygen backbone with organic substituents attached to the silicon atoms [11]. In this study, several polysiloxanes (commonly used as GC stationary phases) were examined for use as extraction solvents for DLLME. Initial experiments found that OV-225 (cyanopropylmethyl-phenylmethylsilicone, see chemical structure in Table 1) was the most promising extraction medium because it exhibited the cleanest chromatographic background. OV-225 has negligible vapor pressure, a high flash point, high thermal stability, and is not classified as hazardous by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Therefore, it represents a potential greener alternative to toxic organic solvents commonly used in DLLME. Unlike extractants lighter than water (such as decanol, dodecanol [12], and many ionic liquids), OV-225 has a relative density greater than one, which simplifies its collection from the aqueous samples after phase separation and promotes the precision of the extraction step. Furthermore, no solvent removal is required after extraction. Additionally, the polymer is commercially available, eliminating the need for time-consuming synthesis and purification procedures. A Scopus search for DLLME of non-steroidal anti-inflammatory drugs (NSAIDs) yields 25 reports employing various extractants and phase separation procedures. Herein, the potential use of OV-225 polymer as an extraction medium for DLLME was investigated using four NSAIDs (see chemical structures and physicochemical properties in Table 1) as test analytes, and employing HPLC/UV for separation and detection. The log P and log D values in Table 1 are measures of the lipophilicity of the molecule. The full factorial and face-centered central composite designs were used for screening and optimization of the DLLME conditions.

Table 1. Chemical structures and properties of the studied analytes.

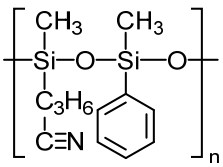
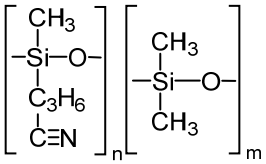
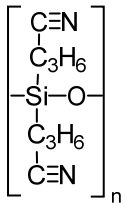
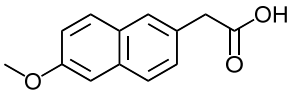
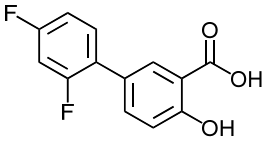
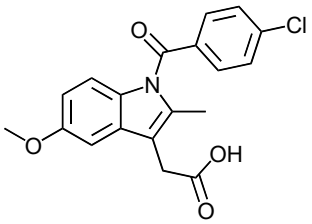
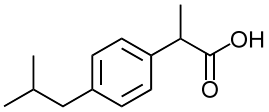
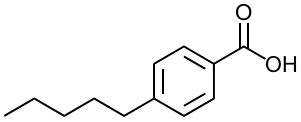
Name	Chemical Structure	Classification	Log P *	Log D * (pH = 2)	pK_a *
Silicone OV-225		Extraction medium	**	**	N/A
Silicone OV-105		Extraction medium	**	**	N/A

Table 1. *Cont.*

Name	Chemical Structure	Classification	Log P *	Log D * (pH = 2)	pK_a *
Silicone OV-275		Extraction medium	**	**	N/A
Naproxen (NAP)		Analyte	2.876 ± 0.239	2.88	4.84 ± 0.30
Diflunisal (DIF)		Analyte	3.652 ± 0.530	3.37	2.94 ± 0.10
Indomethacin (IND)		Analyte	4.251 ± 0.796	4.25	3.96 ± 0.30
Ibuprofen (IBU)		Analyte	3.502 ± 0.227	3.50	4.41 ± 0.10
4-pentylbenzoic acid (internal standard		Internal standard	4.034 ± 0.210	4.03	4.35 ± 0.10

* Log P is the partition coefficient of a molecule between octanol and water; log D considers the same partition coefficient measure but at pH 2, ensuring the molecule is neutral. Values were obtained from SciFinder and calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02. ** Not available. N/A: Not applicable.

2. Experimental Procedure

2.1. Chemicals and Stock Solutions

Silicone OV-225 (cyanopropylmethyl-phenylmethylsilicone), silicone OV-105 (cyanopro pylmethyl-dimethylsilicone), and silicone OV-275 (dicyanopropylsilicone) were purchased from Ohio Valley Specialty (Marietta, OH, USA). Diflunisal (≥99.0%), ibuprofen (≥99.0%), indomethacin (99.0%), naproxen sodium (≥98.0%), and phosphoric acid (≥99.999% trace metal basis) were obtained from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade), tetrahydrofuran (HPLC grade, ≥99.9%), sodium phosphate dibasic heptahydrate (≥99.2%), sodium phosphate monobasic dihydrate (≥99.9%), sodium chloride (≥99.0%), and sodium hydroxide (ACS, ≥99.5%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Both 4-hexylbenzoic acid (≥99.0%) and 4-pentylbenzoic acid (≥99.0%) were obtained from Thermo Scientific Chemicals (Oxford, UK). Acetone (ACS, ≥99.6%) was purchased from Acros Organics™ (Geel, Belgium). Stock standard solutions of diflunisal, ibuprofen, indomethacin, naproxen sodium, and 4-pentylbenzoic acid (internal standard), were individually prepared at 1 mg/mL in methanol and kept refrigerated at 4 °C when not in use.

2.2. Instrumentation and Chromatographic Conditions

Throughout this study, an UltiMate 3000 UHPLC system (Thermo Fisher Scientific™, Sunnyvale, CA, USA) was used for the analysis of all samples. The instrument consisted of a DGP-3600RS ternary pump, WPS-3000RS autosampler, TCC-3100RS column thermostat, and MWD-3000RS UV-VIS multiwavelength detector. Chromeleon 7.2.1 software was used for instrument operation and data processing. Separation was performed using a Hypersil Gold™ C_{18} column (150 mm × 4.6 mm, 5 µm) obtained from Thermo Fisher Scientific™ (Sunnyvale, CA, USA). The mobile phase consisted of two solvents: acetonitrile and phosphate buffer ($[PO_4^{3-}]$ = 20 mM, pH 2.4), which were pumped at a ratio of 53:47% (*v*/*v*), respectively. The flow rate was 0.9 mL/min. The column thermostat was set at 45 °C. The injection volume was 20 µL, and detection was carried out at 230 nm. Total recovery autosampler vials (SKU: 186000385C, Waters™, Milford, MA, USA) were used throughout this study to enable the analysis of samples as small as a few microliters in size. A 10K variable-speed minicentrifuge (BT Lab Systems, Saint Louis, MO, USA) was employed for centrifugation, and an Accumet® Research AR 15 pH meter (Fisher Scientific, Waltham, MA, USA) was used for pH measurements. Minitab 17.1.0 software was used to build and analyze the designs during the screening and optimization phases of the DLLME conditions.

2.3. Screening and Optimization of the DLLME Conditions

For the screening and optimization studies of the DLLME conditions, an aqueous standard mixture of the four NSAIDs was prepared daily by appropriate dilution of the stock solutions in ultrapure water so that the concentrations of NAP, DIF, IND, and IBU were 0.2, 0.5, 1.0, and 2.0 µg/mL, respectively. The general DLLME procedure (Figure S1, Supplementary Material) was performed by transferring 1.5 mL of the aqueous NSAIDs mixture into a 2 mL Eppendorf tube. A 100-microliter volume of phosphate buffer ($[PO_4^{3-}]$ = 100 mM) was added for pH adjustment. The premixed solution of the extractant polymer and the dispersive solvent was added to the sample solution, and the mixture was then vortex mixed. The resultant cloudy dispersion was centrifuged at 5000 rpm for 3 min, allowing for phase separation. Afterward, the aqueous phase was discarded, and the sedimented extract was diluted with acetonitrile and transferred into a total recovery HPLC vial for analysis.

To achieve maximum EFs with all the studied analytes (EF = C_e/C_i, where C_e is the concentration of the analyte in the extract and C_i is the initial concentration of the analyte in the original sample solution before extraction), the experimental conditions that influence the DLLME process were studied using both one-factor-at-a-time and design-of-experiment (DoE) approaches. Firstly, the effect of the dispersive solvent type on the EFs was studied as a single variable, employing acetone, acetonitrile, and tetrahydrofuran (measurements were made in duplicate for error bar calculations). Secondly, a two-level full factorial experimental design (2^k-FFD where k is the number of factors) was conducted to screen the other parameters for significance, considering the polymer mass (mg), dispersive solvent volume (µL), sample pH, and mixing time (s). The 2^4-FFD was developed using 16 combinations in the base design with a replicate per combination, thus generating a total of 32 runs. Experiments were performed at random to minimize the effect of uncontrolled variables. The levels of the factors were (A) extractant polymer mass 10, 30 mg; (B) disperser solvent volume 50, 150 µL; (C) sample pH 2.2, 8.0; and (D) mixing time 10, 120 s. The Pareto chart was used to evaluate the significance of the main and interaction effects of the studied factors, allowing the inclusion of only the significant factors in the final optimization model. Third, a face-centered central composite design (CCD) was applied to fine-tune the three factors found significant during the factorial analysis. The CCD consisted of 16 cube points, 12 axial points, and 8 center points. The design consisted of three base blocks, with each block corresponding to experiments carried out on the same day. In both FFD and CCD, the responses considered were the EFs for all analytes. The factors, their levels, and all combinations are presented in Tables S1 and S2, Supplementary Material. The results were analyzed to build a mathematical model for each response, considering the linear

variation as well as the two-way and quadratic interactions. The validity of the proposed model was assessed using an analysis of variance (ANOVA), where fitting was deemed adequate when the *p*-value for lack-of-fit was greater than 0.05. The adjusted coefficient of determination (R^2_{adj}) was also used to evaluate the percentage of variance explained by the given model. After obtaining the best-fitting mathematical models, the response optimizer tool, which utilizes the desirability function, was applied to predict the experimental DLLME conditions that would achieve the criterion of maximizing the EF with all analytes. Afterwards, the salting-out effect on the EFs was studied using sample mixtures containing NaCl at concentrations of 0, 5, and 10% *w*/*v* (measurements were made in duplicate for error bar calculations). The addition of an internal standard was deemed important in the subsequent calibration and recovery studies in order to significantly improve the precision.

2.4. Analytical Performance and Recovery from Tap Water Samples

In order to evaluate the performance of the whole analytical method, calibration standard mixtures of the four NSAIDs were prepared in ultrapure water so that the concentrations (ng/mL) were in the range of 5–250 for NAP, 10–500 for DIF, 25–1250 for IND, and 75–3000 for IBU. In all calibration mixtures, the concentration of the IS (4-pentylbenzoic acid) was held constant at 250 ng/mL. A standard curve was constructed for each analyte by plotting the peak area ratio of the analyte to the internal standard ($AUC_{analyte}/AUC_{IS}$) against the analyte concentration in ng/mL. The method was evaluated in terms of linearity range, trueness, precision, and concentration limit for quantitation (LOQ). The LOQ was considered as the lowest concentration on the calibration curve that achieves a correlation coefficient greater than 0.995, a signal-to-noise ratio greater than 10, and a % RSD less than 15.

The method was applied to the analysis of dissolved NSAIDs In tap water. Tap water samples were collected in glass amber bottles and filtered through a 0.45 μm nylon membrane to remove particulate matter. Samples were analyzed immediately after collection, and before and after spiking with NSAIDs at low and high concentration levels, and the percentage relative recoveries were determined.

3. Results and Discussion

3.1. Selection of Cyanopropyl Silicone Polymer (OV 225) as an Extraction Medium for DLLME

In the first set of experiments, the miscibility of several polysiloxanes with the LC mobile phase was analyzed to identify those that can be directly injected into the HPLC system. It was found that the 100% dimethyl polysiloxane and most phenylmethyl polysiloxanes were not suitable for direct injection, as they were immiscible with the mobile phase. However, the tested cyanopropyl polysiloxanes (OV-105, OV-225, and OV-275) were found to be adequately miscible with the mobile phase, presumably due to the greater polarity imparted by the cyanoalkyl substituent group (see chemical structures in Table 1). Initial DLLME experiments were carried out on blank ultrapure water samples using the three studied cyanopropyl polysiloxanes. The results indicated that OV-225 provided a much cleaner chromatographic background compared to OV-105 and OV-275 (Figure S2). This is likely due to the presence of UV-absorbing impurities in OV-105 and OV-275. As a result, OV-225 was chosen for further testing and investigation.

3.2. Screening and Optimization of the DLLME Conditions

The potential use of OV-225 silicone as an extraction medium in DLLME was investigated via the extraction of four NSAIDs from aqueous samples (Figure S1) followed by HPLC/UV for separation and detection, according to the procedure described in the Section 2. The DLLME conditions were optimized to achieve the highest EF with each of the studied analytes. Notably, the rate and yield of extraction are greatly influenced by the efficiency of extractant dispersion through the sample solution. Hence, efficient extractant dispersion into an enormous number of fine droplets increases the sample/extractant interfacial area, and leads to more effective extraction. This effect could also be perceived

from the well-known mathematical perspective of extraction: $R = 1 - [1/(1 + k\beta)]^n$, where R is the analyte's recovered fraction, β is the extractant–sample volumetric ratio, K is the distribution constant, and n is the number of extraction steps. Here, in DLLME n is related to the number of localized extraction processes that simultaneously take place within the sample, which is largely determined by the dispersion efficiency. Based on the importance of the dispersion step in DLLME, three dispersive solvents were tested: acetonitrile, acetone, and tetrahydrofuran. Results with acetonitrile roughly showed an enrichment of 3 with each of the studied analytes. Although better enrichment was obtained with acetone, the enrichment obtained with tetrahydrofuran was the highest with all analytes (Figure 2). Therefore, tetrahydrofuran was used as the disperser solvent in subsequent factor screening and optimization experiments.

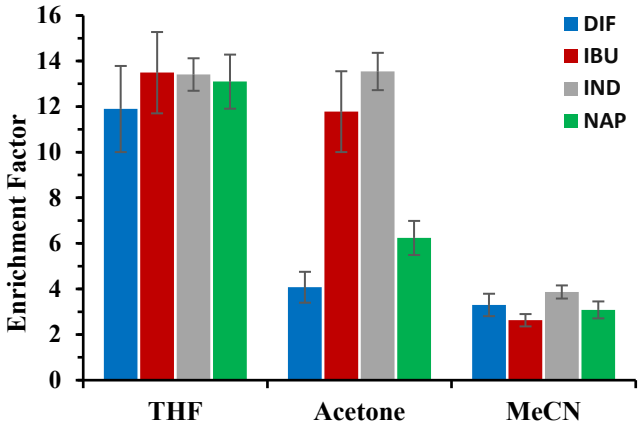


Figure 2. Effect of dispersive solvent on the enrichment factor of the studied non-steroidal anti-inflammatory drugs. Extraction conditions: sample volume, 1.5 mL; extraction solvent mass, 20 mg; dispersive solvent volume, 80 µL; extraction time, 120 s; pH, unadjusted. DIF: diflunisal, IBU: ibuprofen, IND: indomethacin, and NAP: naproxen. Error bars based on n = 2.

Among the factors that can affect DLLME efficiency are extractant mass, disperser solvent volume, vortex time, and pH of the buffered sample solution. These variables were tested for significance using a 2^4-FFD consisting of 16 combinations in the base design, with 32 runs in all, as experiments were carried out in two replicates. The EFs of the studied analytes were the measured responses (Table S1). The Pareto charts (Figure S3) show that most of the effect values for the extractant polymer mass (mg), pH of the buffered sample solution, and vortex time fall above Bonferroni's statistical limit of significance. In addition, varying the tetrahydrofuran volume over a range of 50–150 µL did not significantly affect the obtained EFs, even though the addition of tetrahydrofuran is still important for efficient dispersion and extraction, as shown by the preceding experiment for dispersive solvent optimization. Consequently, the polymer mass, sample pH, and mixing time were considered in subsequent fine-tuning optimization experiments, whereas the tetrahydrofuran volume was kept constant at 50 µL.

According to Mousavi et al. [13], the CCD is the most widely used optimization design in combination with any of the factor screening designs. Therefore, the CCD was used for the optimization of the three parameters found to be important by the factorial analysis. Notably, data from the FFD revealed higher EFs with longer extraction times and smaller amounts of the extractant polymer mass (Figure S4). It appears that decreasing the extractant mass (within the studied range of masses) resulted in a significant proportional decrease in the extract volume, with a small effect on the amount of analyte recovered, thereby forming a more concentrated extract and giving higher enrichment factors. Also, it was noticed that higher EFs were obtained with all analytes as the sample pH was lowered (Figure S5). This stems from the predictable lower ionization of the acidic NSAIDs at pH values below their pK_a (see pK_a values in Table 1). Accordingly, as the sample pH decreases, the proportion of the unionized species, which partition more readily to the

polymer extractant phase, increases, leading to higher EFs. Based on these findings, the three effective factors mentioned above were included in the CCD so that the extractant polymer mass was fine-tuned over the range from 10 mg to 30 mg, the buffered sample pH was studied from 2.2 to 3.6, and the extraction time was optimized from 60 s to 180 s. All the experiments of the CCD as well as the corresponding responses (EFs) are presented in Table S2. The experimental data showed adequate fitting to a second-order polynomial function for each response, as demonstrated by p values of the lack-of-fit greater than 0.05 (Table S3). The adjusted coefficients of determination (R^2_{adj}) were in the range of 0.72–0.84, indicating that an adequate percentage of data variability is explained by the developed mathematical model. Afterward, the desirability function was applied (using the response optimizer tool in Minitab) to predict the best combination of conditions to achieve the goal of maximizing the EF of each analyte. In calculating the global desirability, all responses were given the same importance. The highest value of global desirability (D = 0.95) was obtained under the following conditions: 10 mg extractant polymer mass, 2.7 added buffer pH, and 3 min of vortex mixing (Figure 3).

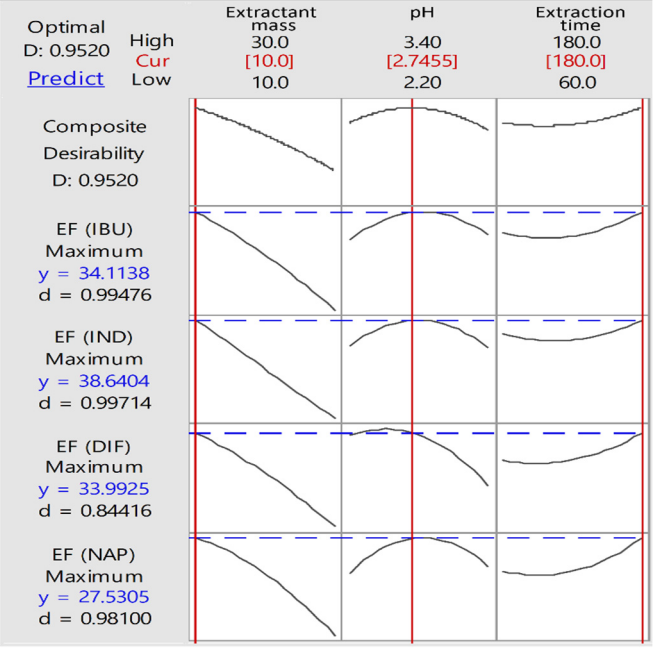


Figure 3. Composite desirability (D) plot from the central composite design for the enrichment factors of the studied non-steroidal anti-inflammatory drugs. DIF: diflunisal, IBU: ibuprofen, IND: indomethacin, and NAP: naproxen. The denoted current settings were employed to establish the calibration curve. The horizontal dashed blue lines represent the current response values. The vertical red lines on the graph represent the current settings.

Thereafter, the salting-out effect on the DLLME extraction efficiency was examined using sample mixtures containing NaCl at concentrations 0, 5, and 10% (w/v). As shown in Figure S6, only slight changes in the EFs of the four NSAIDs were observed. Therefore, NaCl was not added in subsequent experiments.

In all previous screening and optimization experiments, after phase separation and discard of the aqueous layer, a volume of 20 µL acetonitrile was added to the extract to reduce its viscosity and facilitate transfer into HPLC vials. However, the optimization study concluded that the highest EFs were obtained with an extractant amount of 10 mg, which in turn yields a small extract volume after phase separation (about 10 µL). Therefore, it was found that a 15-µL volume of acetonitrile diluent would be more appropriate, since it roughly led to a 20% increase in EFs, while maintaining an adequate viscosity and volume to facilitate extract transfer and injection into the HPLC instrument.

In conclusion of the optimization study, the following conditions were adopted: extractant polymer mass, 10 mg; disperser solvent, 50 µL tetrahydrofuran; buffer, 100 µL phosphate buffer pH 2.75 ($[PO_4^{3-}]$ = 100 mM); vortex mixing time, 3 min; and extract diluent, 15 µL acetonitrile. Under these conditions, the average EFs obtained were 33, 40, 41, and 46 for NAP, DIF, IND, and IBU, respectively. The absolute recoveries obtained were 55, 67, 68, and 77%, respectively, which are adequate, considering the low extractant/sample volumetric ratio. Noticeably, the increase in EF and absolute recovery is consistent with the increase in analyte retention time on the reversed-phase stationary phase. This indicates that OV-225 may be more suitable for the preconcentration of compounds of moderate to high hydrophobicity.

3.3. Analytical Performance and Recovery from Tap Water Samples

To evaluate the analytical performance of the developed method, two compounds were first tested to select an internal standard: 4-pentylbenzoic and 4-hexylbenzoic acids. These compounds have comparable structural and physicochemical properties to the studied analytes (Table 1) and, therefore, they should similarly respond to small extraction process and instrumental variations. Under the employed chromatographic conditions, both compounds came out after the last eluting analyte. The compound, 4-pentylbenzoic acid, was selected because it showed reasonable retention time, enabling faster analysis. All chromatographic peaks were symmetric and well resolved in an 8 min run time (Figure 4). The peak just after 2.50 min was attributed to the GC stationary phase extractant. Analyte signals were barely detectable before the extraction and became strong and evident after the DLLME. All standard curves (peak area ratio analyte/IS vs. analyte concentration in ng/mL) showed excellent linearity over the studied concentration ranges, with correlation coefficients above 0.999. The linearity ranges and concentration limits of quantitation are summarized in Table 2. The method has shown good precision and adequate trueness, as indicated by the RSD and error levels presented in Table 2. For instance, the analysis of NAP at 100 ng/mL showed a relative error of −0.52% and an RSD of 0.7%.

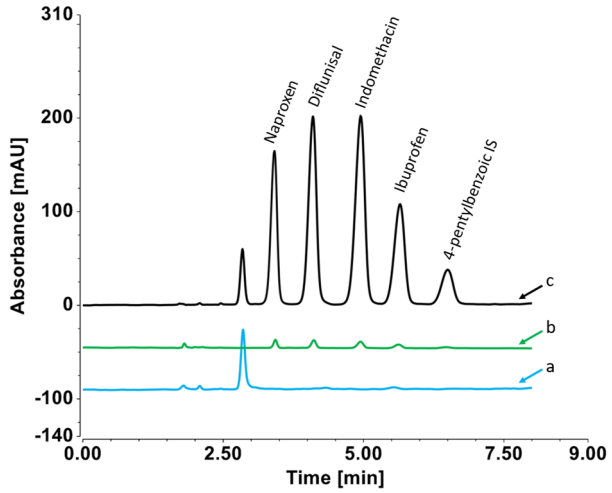


Figure 4. Chromatograms of (a) the blank tap water sample after DLLME; (b) the spiked tap water sample without extraction; and (c) the spiked tap water sample after DLLME. Extraction conditions: sample was a mixture of NAP, DIF, IND, IBU and IS at 200, 500, 1000, 2000 and 250 ng/mL, respectively; sample volume, 1.5 mL; extractant mass, 10 mg; dispersive solvent volume, 50 µL THF; buffer volume, 100 µL phosphate buffer (0.1 mol/L, pH 2.7). NAP: naproxen, DIF: diflunisal, IND: indomethacin, and IBU: ibuprofen.

Table 2. Figures of merit of the optimized DLLME HPLC/UV method.

Analyte	Linear Range (ng/mL)	*r*	Slope ± Error (10^{-3})	LOQ (ng/mL)	Error (%) (Indicated Level)	RSD (%) (Same Level as Error)	RSD (%) (at LOQ)
Naproxen	5–250	0.9995	11.0 ± 0.1	5.0	−0.5 (100 ng/mL)	0.7	4.5
Diflunisal	10–500	0.9997	6.22 ± 0.05	10.0	−0.4 (400 ng/mL)	6.7	9.6
Indomethacin	25–1250	0.9998	3.23 ± 0.02	25.0	−0.8 (500 ng/mL)	0.4	3.1
Ibuprofen	75–3000	0.9999	0.977 ± 0.004	75	−0.06 (3000 ng/mL)	1.9	10.8

LOQ, Limit of quantitation; RSD, Relative standard deviation; *r*, Correlation coefficient.

The developed OV-225 polymer-based DLLME HPLC/UV method was applied under optimized experimental conditions for the determination of dissolved NSAIDs in tap water. Chromatograms of non-spiked tap water samples did not show any measurable peaks at the retention times of the studied analytes after DLLME (Figure 4). After analysis of spiked tap water samples, the relative recovery and RSD (%) were calculated. The results shown in Table 3 indicate an adequate level of precision and accuracy without appreciable matrix interference.

Table 3. Relative recoveries of NSAIDs from spiked tap water samples obtained using the developed OV-225 polymer-based DLLME-HPLC/UV method.

Analyte	Added (ng/mL)	Mean Found ± Standard Deviation (ng/mL)	RSD (%)	Recovery (%)
Naproxen	15	15.6 ± 0.3	1.9	103.9
	200	195 ± 6	3.1	97.3
Diflunisal	30	34 ± 3	8.8	112.5
	200	200 ± 9	4.5	100.1
Indomethacin	75	85 ± 3	3.5	97.5
	1000	975 ± 23	2.4	113.6
Ibuprofen	225	208 ± 3	1.5	92.3
	1500	1529 ± 2	0.2	101.9

Finally, the developed method was compared with other methods reported for the extraction of NSAIDs from aqueous samples in terms of the extraction technique, extraction time, sample volume, instrument used for analysis, LOQ, and RSD (Table S4). Many of these methods rely on solid-phase extraction (SPE) [14], molecularly imprinted solid-phase extraction (MISPE) [15,16], and magnetic solid-phase extraction (MSPE) [17,18]. However, these techniques require a large sample volume and a long time for analyte extraction (10–160 min) and desorption. On the other hand, DLLME [19,20] is much faster requiring only a few minutes (Table S4). Notably, the LOQ for naproxen obtained with this developed DLLME method is fairly close to that obtained with other extraction methods when HPLC/UV is used for separation and detection. Determination of NASIDs using this method would be viable for most African environmental water matrices in which mean concentrations are in the µg/L range [21]. The authors believe that the full potential of the developed method in trace-level analysis could be achieved using larger sample volumes and utilizing MS or MS/MS for detection. LOQ values for LC-MS of naproxen and ibuprofen are about 1000 times better than those found using LC with UV detection [22,23]. Unfortunately, LC-MS was not explored in this study due to the lack of the necessary instrumentation. Polysiloxanes and co-polymers of siloxanes have been characterized by LC-MS [24], indicating that the compatibility of our GC stationary phase extraction method with LC-MS should be viable.

4. Conclusions

In this work, the OV-225 silicone polymer was successfully applied as an extraction medium for DLLME. Up to 46-fold enrichment of NSAIDs from aqueous samples was

achieved using a 1.5 mL sample size. The polymer has a relative density greater than one, which facilitated its collection after phase separation. It also has favorable physicochemical properties that make it a potential greener alternative to commonly used toxic organic extractants. The current research may represent a promising step towards exploration of the full potential of using polymers for DLLME. Our future research will focus on the rationale design and synthesis of multimode extraction polymers with a pH-controllable ion exchange as well as hydrophobic interaction.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations11010018/s1, Figure S1: A general schematic overview of the dispersive liquid-liquid micro-extraction procedure; Figure S2: Dispersive liquid-liquid micro-extraction of blank ultrapure water samples using the investigated cyanopropyl polysiloxanes: OV-275, OV-105, and OV-225; Figure S3: Pareto charts from the 24-FFD showing the main and interaction effects of the investigated experimental variables on the preconcentration of the studied NSAIDs. The dotted red line represents Bonferroni's statistical limit of significance; Figure S4: Main effects plots from the 24-FFD showing the main effects of the investigated experimental variables on the pre-concentration of the studied NSAIDs; Figure S5: Interaction plots from the 24-FFD showing the interaction effects of the investigated experimental variables on the pre-concentration of the studied NSAIDs; Figure S6: Salting-out effect on enrichment factors of the studied analytes. Note: the x-axis is the NaCl concentration in the sample solution before extraction. n = 2. Table S1: The 24-FFD experiment of independent DLLME variables and their corresponding responses. Table S2: The FCCD experiment of independent DLLME variables and their corresponding responses. Table S3: Mathematical model fitting for each response. Table S4: Comparison with other reported liquid chromatographic methods. Bibliometric Analysis Search Key also present.

Author Contributions: Conceptualization, N.D.D.; methodology, M.A.A.; software, M.A.A.; validation, M.A.A.; formal analysis, M.A.A. and N.D.D.; investigation, M.A.A.; resources, N.D.D.; data curation, M.A.A.; writing—original draft preparation, M.A.A.; writing—review and editing, N.D.D.; visualization, M.A.A.; supervision, N.D.D.; project administration, N.D.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Rezaee, M.; Assadi, Y.; Milani Hosseini, M.R.; Aghaee, E.; Ahmadi, F.; Berijani, S. Determination of Organic Compounds in Water Using Dispersive Liquid–Liquid Microextraction. *J. Chromatogr. A* **2006**, *1116*, 1–9. [CrossRef]
2. Leong, M.I.; Chang, C.C.; Fuh, M.R.; Huang, S.D. Low Toxic Dispersive Liquid–Liquid Microextraction Using Halosolvents for Extraction of Polycyclic Aromatic Hydrocarbons in Water Samples. *J. Chromatogr. A* **2010**, *1217*, 5455–5461. [CrossRef]
3. Grau, J.; Azorín, C.; Benedé, J.L.; Chisvert, A.; Salvador, A. Use of Green Alternative Solvents in Dispersive Liquid-Liquid Microextraction: A Review. *J. Sep. Sci.* **2022**, *45*, 210–222. [CrossRef]
4. Abdelaziz, M.A.; Saleh, A.M.; Mansour, F.R.; Danielson, N.D. A Gadolinium-Based Magnetic Ionic Liquid for Dispersive Liquid–Liquid Microextraction of Ivermectin from Environmental Water. *J. Chromatogr. Sci.* **2022**, *61*, 988–994. [CrossRef]
5. Abdallah, I.A.; Hammad, S.F.; Bedair, A.; Abdelaziz, M.A.; Danielson, N.D.; Elshafeey, A.H.; Mansour, F.R. A Gadolinium-Based Magnetic Ionic Liquid for Supramolecular Dispersive Liquid–Liquid Microextraction Followed by HPLC/UV for the Determination of Favipiravir in Human Plasma. *Biomed. Chromatogr.* **2022**, *36*, e5365. [CrossRef]
6. Rykowska, I.; Ziemblińska, J.; Nowak, I. Modern Approaches in Dispersive Liquid-Liquid Microextraction (DLLME) Based on Ionic Liquids: A Review. *J. Mol. Liq.* **2018**, *259*, 319–339. [CrossRef]
7. Yu, H.; Merib, J.; Anderson, J.L. Faster Dispersive Liquid-Liquid Microextraction Methods Using Magnetic Ionic Liquids as Solvents. *J. Chromatogr. A* **2016**, *1463*, 11–19. [CrossRef]
8. Abdelaziz, M.A.; Mansour, F.R.; Danielson, N.D. A Gadolinium-Based Magnetic Ionic Liquid for Dispersive Liquid–Liquid Microextraction. *Anal. Bioanal. Chem.* **2021**, *413*, 205–214. [CrossRef]
9. Liu, Q.; Wei, L.; Chen, X.; Xu, Y.; Gao, X.; Zhao, J. Three-Dimensional (3D) Thermal Controlled Polymer for Simplified Dispersive Liquid-Liquid Microextraction in Phthalic Acid Easters Detection of Straw. *Microchem. J.* **2022**, *181*, 107668. [CrossRef]

10. Xu, Y.; Wei, L.; Chen, X.; Zhao, J.; Wang, Y. Application of the Liquid–Liquid Dispersed Microextraction Based on Phase Transition Behavior of Temperature Sensitive Polymer to Rapidly Detect 5 BPs in Food Packaging. *Food Chem.* **2021**, *347*, 128960. [CrossRef]
11. Colas, A.; Curtis, J. Silicone Biomaterials: History and Chemistry & Medical Applications of Silicones. In *Biomaterials Science. An Introduction to Materials in Medicine*; Ratner, B.D., Hoffman, A.S., Schoen, F.J., Lemons, J.E., Eds.; Academic Press: San Diego, CA, USA, 2004; pp. 80–86.
12. Kocúrová, L.; Balogh, I.S.; Šandrejová, J.; Andruch, V. Recent Advances in Dispersive Liquid–Liquid Microextraction Using Organic Solvents Lighter than Water. A Review. *Microchem. J.* **2012**, *102*, 11–17. [CrossRef]
13. Mousavi, L.; Tamiji, Z.; Khoshayand, M.R. Applications and Opportunities of Experimental Design for the Dispersive Liquid–Liquid Microextraction Method—A Review. *Talanta* **2018**, *190*, 335–356. [CrossRef]
14. Shafiei-Navid, S.; Hosseinzadeh, R.; Ghani, M. Solid-Phase Extraction of Nonsteroidal Anti-Inflammatory Drugs in Urine and Water Samples Using Acidic Calix[4]Arene Intercalated in LDH Followed by Quantification via HPLC-UV. *Microchem. J.* **2022**, *183*, 107985. [CrossRef]
15. Olcer, Y.A.; Demirkurt, M.; Demir, M.M.; Eroglu, A.E. Development of Molecularly Imprinted Polymers (MIPs) as a Solid Phase Extraction (SPE) Sorbent for the Determination of Ibuprofen in Water. *RSC Adv.* **2017**, *7*, 31441–31447. [CrossRef]
16. Madikizela, L.M.; Chimuka, L. Determination of Ibuprofen, Naproxen and Diclofenac in Aqueous Samples Using a Multi-Template Molecularly Imprinted Polymer as Selective Adsorbent for Solid-Phase Extraction. *J. Pharm. Biomed. Anal.* **2016**, *128*, 210–215. [CrossRef]
17. Mohd Marsin, F.; Wan Ibrahim, W.A.; Abdul Keyon, A.S.; Sanagi, M.M. Box–Behnken Experimental Design for the Synthesis of Magnetite–Polypyrrole Composite for the Magnetic Solid Phase Extraction of Non-Steroidal Anti-Inflammatory Drug Residues. *Anal. Lett.* **2018**, *51*, 2221–2239. [CrossRef]
18. Liu, S.; Li, S.; Yang, W.; Gu, F.; Xu, H.; Wang, T.; Sun, D.; Hou, X. Magnetic Nanoparticle of Metal-Organic Framework with Core-Shell Structure as an Adsorbent for Magnetic Solid Phase Extraction of Non-Steroidal Anti-Inflammatory Drugs. *Talanta* **2019**, *194*, 514–521. [CrossRef]
19. Parrilla Vázquez, M.M.; Parrilla Vázquez, P.; Martínez Galera, M.; Gil García, M.D.; Uclés, A. Ultrasound-Assisted Ionic Liquid Dispersive Liquid–Liquid Microextraction Coupled with Liquid Chromatography-Quadrupole-Linear Ion Trap-Mass Spectrometry for Simultaneous Analysis of Pharmaceuticals in Wastewaters. *J. Chromatogr. A* **2013**, *1291*, 19–26. [CrossRef]
20. Toledo-Neira, C.; Álvarez-Lueje, A. Ionic Liquids for Improving the Extraction of NSAIDs in Water Samples Using Dispersive Liquid–Liquid Microextraction by High Performance Liquid Chromatography-Diode Array–Fluorescence Detection. *Talanta* **2015**, *134*, 619–626. [CrossRef]
21. Mlunguza, N.Y.; Ncube, S.; Mahlambi, P.N.; Chimuka, L.; Madikizela, L.M. Adsorbents and Removal Strategies of Non-Steroidal Anti-Inflammatory Drugs from Contaminated Water Bodies. *J. Environ. Chem. Eng.* **2019**, *7*, 103142. [CrossRef]
22. Patel, P.N.; Samanthula, G.; Shrigod, V.; Modh, S.C.; Chaudhari, J.R. RP-HPLC Method for Determination of Several NSAIDs and Their Combination Drugs. *Chrom. Res. Int.* **2013**, *2013*, 242868. [CrossRef]
23. Wabaidur, S.M.; AlOthman, Z.A.; Siddiqui, M.R.; Mohsin, K.; Bousiakou, L.G.; Karikas, G.A. UPLC–MS Method for the Simultaneous Determination of Naproxen, Fluvastatin and Ibuprofen in Waste Water Samples. *J. Ind. Eng. Chem.* **2015**, *24*, 302–307. [CrossRef]
24. Desmazieres, B.; Buchmann, W.; Terrier, P.; Tortajada, J. APCI Interface for LC- and SEC-MS Analysis of Synthetic Polymers: Advantages and Limits. *Anal. Chem.* **2008**, *80*, 783–792. [CrossRef]