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Experimental Determination of Drug Diffusion Coefficients in Unstirred Aqueous Environments by Temporally Resolved Concentration Measurements

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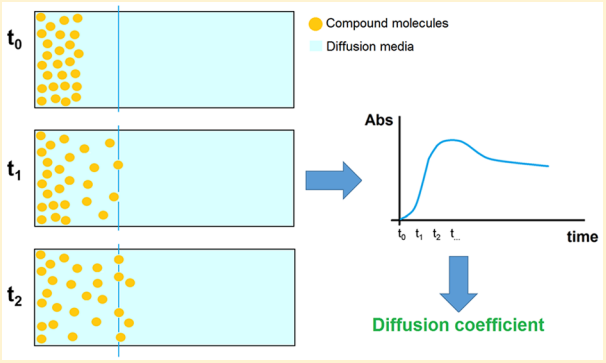
Supporting Information

ABSTRACT: The diffusion coefficient (also known as diffusivity) of an active pharmaceutical ingredient (API) is a fundamental physicochemical parameter that affects passive diffusion through biological barriers and, as a consequence, bioavailability and biodistribution. However, this parameter is often neglected, and it is quite difficult to find diffusion coefficients of small molecules of pharmaceutical relevance in the literature. The available methods to measure diffusion coefficients of drugs all suffer from limitations that range from poor sensitivity to high selectivity of the measurements or the need for dedicated instrumentation. In this work, a simple but reliable method based on time-resolved concentration measurements by UV−visible spectroscopy in an unstirred aqueous environment was developed. This method is based on spectroscopic measurement of the variation of the local concentration of a substance during spontaneous migration of molecules, followed by standard mathematical treatment of the data in order to solve Fick's law of diffusion. This method is extremely sensitive and results in highly reproducible data. The technique was also employed to verify the influence of the environmental characteristics (i.e., ionic strength and presence of complexing agents) on the diffusivity. The method can be employed in any research laboratory equipped with a standard UV−visible spectrophotometer and could become a useful and straightforward tool in order to characterize diffusion coefficients in physiological conditions and help to better understand the drug permeability process.



KEYWORDS: *diffusion coefficients, UV−visible spectroscopy, Fick's laws of diffusion, data fitting, molecular modeling, ion strength*

1. INTRODUCTION

Diffusion is the spontaneous migration of molecules of a substance from regions of higher chemical activity (high concentration) to regions of lower chemical activity (low concentration) through one or several isotropic environments. This process is of capital importance in pharmaceutics since passive transport of active pharmaceutical ingredients (APIs) remains a significant (and often primary) mechanism of biological membrane permeation,[1] and therefore, the diffusion process is crucial for reaching an appropriate bioavailability. According to the Stokes−Einstein equation (eq 1), the diffusivity D (cm^2/s) is directly proportional to the absolute temperature T (K) and Boltzmann constant K_b ($cm^2 \cdot g/s^2 \cdot K$) but inversely proportional to the viscosity of the media η (g/cm·s) and the hydrodynamic radius r (cm) of the molecules.

$$D = \frac{k_B T}{6\pi\eta r} \quad (1)$$

The relation between diffusion and flux of a drug substance in aqueous environment can be described by Fick's first law as

$$j = -D\frac{dc}{dx} \quad (2)$$

where j represents the net flux ($mol/cm^2 \cdot s$) of drug substance through the media, dc/dx represents the concentration gradient

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Table 1. General Characteristics and Composition of the Aqueous Solutions Used as Donor Environments[a]

diffusional medium	compound name	molecular weight (g/mol)	nominal conc. (mM)	buffer conc. (mM)	density[b] (g/mL)	λ_{max} (nm)	ε (cm^2/μmol)
DW	caffeine	194.2	1.23	72.8	1.0086	269	11.3
DW	calcein	622.6	1.23	72.8	1.0083	294	11.3
DW	chloramphenicol	323.2	1.20	72.8	1.0095	277	10.0
DW	ketoprofen	254.3	1.17	72.8	1.0064	257	18.4
DW	nitrofurantoin	238.2	0.77	72.8	1.0113	274	13.7
DW	paracetamol	151.2	1.42	72.8	1.0079	236	13.2
DW	penicillin G	334.4	0.60	72.8	1.0071	207	19.9
DW	tetracycline	444.4	0.83	72.8	1.0197	361	14.8
DW	trimethoprim	290.3	1.19	72.8	1.0085	278	5.6
DW	vancomycin	1449.3	1.20	72.8	1.0011	280	6.7

[a]The diffusional medium was composed of plain distilled water (DW, density of 1.0015 g/mL). Each of the compounds was detected at its maximum wavelength of absorption (λ_{max}) and the local concentration was calculated using its specific absorptivity (ε). [b]Data are reported as mean of three measurements. SD below 0.5%.

and D is the diffusion coefficient. D is an essential parameter to understand pharmaceutically relevant processes such as mass transport through barriers (i.e., absorption) and bioavailability of APIs. However, pharmaceutical research has for years overlooked the importance of this parameter in favor of the measurement of the apparent permeability coefficient (P_{app},[2−4]). Since water is the primary component of the human body, a proper quantification of D values for APIs should be of crucial importance for a proper evaluation of the permeability process. Reliable D values, together with a proper evaluation of partition coefficients (LogP), could help to elucidate the role of unstirred water layers (UWL)[5] and interface transitions in passive transport through barriers.[6] The scientific literature is significantly lacking in diffusivity values for APIs in aqueous environments and methods to measure them in a simple but reliable manner. Currently, several techniques for measuring diffusion coefficients are available, including holographic laser interferometry (HLI[7]), electron speckle pattern interferometry (ESPI[8]), mass transfer through barrier studies,[9] Taylor dispersion analysis (TDA[10,11]), nuclear magnetic resonance (NMR[12]), dual-focus fluorescence correlation spectroscopy (FCA[13]), and fluorescence recovery after photobleaching (FRAP[14]) just to mention the most employed ones. Just a few of these techniques (TDA, NMR, and mass transfer studies) have been applied to estimate diffusivity of APIs, the reason being that all the techniques listed above show limitations when facing pharmaceutically relevant problems such as poor sensitivity (e.g., NMR), high specificity (e.g., FRAP), and the need for dedicated instrumentation (e.g., HLI, ESPI, FCA, TDA, and NMR, which are not standard instrumentations in most pharmaceutical research laboratories).

Another approach to estimate diffusivities of small molecules in aqueous environment is to predict them from molecular properties such as molecular volume and molar volume or by molecular simulation.[15] However, all these *in silico* approaches are prone to uncertainty and do not provide a complete interpretation of diffusion in the case of environmental changes such as ion strength or presence of complexing agents. In this work, a simple, versatile, and easily implemented spectroscopic method[16,17] for diffusion coefficient measurement is introduced. This versatile method is based on the measurement of variation of local concentration of a compound in aqueous media and subsequent temporal resolution of diffusion equation by means of analytical as well as numerical mathematical methods. By comparing different approaches to the solution of Fick's diffusion equation, we show that simple nonlinear least-squares fitting is sufficient to extract the diffusion coefficient. The experiments are performed employing a regular quartz cuvette and a standard UV−visible spectrometer. This method is simple, reliable, reproducible, and extremely sensitive. Moreover, it has the significant advantage that it could be employed in any research laboratory equipped with a standard UV−visible spectrophotometer, and very small changes related to environmental alterations can be measured.

2. EXPERIMENTAL SECTION

2.1. Materials. Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl, ≥ 37%), caffeine, calcein, chloramphenicol, ketoprofen, nitrofurantoin, tetracycline hydrochloride, trimethoprim, vancomycin, and β-cyclodextrin (βCD) were all purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Paracetamol was purchased from Norsk Medisinaldepot AS (Oslo, Norway). Penicillin G employed was USP Reference Standard (Timbrook, USA). All chemicals were of analytical grade.

2.2. UV−Visible Localized Spectroscopy. *2.2.1. Preparation of the Solutions.* Phosphate buffer saline (PBS) 73 mM was prepared mixing a solution of $NaH_2PO_4{\cdot}H_2O$ (2.2% W/V) with a solution of $Na_2HPO_4{\cdot}12H_2O$ (1.8% W/V) in a ratio of 1 to 5. The pH of the PBS was adjusted to 7.3−7.4 (pH meter Lab 744, Metrohm AG, Herisau, Switzerland) by the addition of NaOH solid pellets. Tonicity of the PBS solution was adjusted to 280−290 mOsm (Semi-Micro Osmometer K-7400, Knauer, Berlin, Germany) by the addition of NaCl. Each of the compounds was then adequately weighted and dissolved in the PBS in order to achieve a concentration of approximately 0.5−1.5 mM. The molecular weights of the compounds and the characteristics of the donor solutions (compound concentrations, buffer concentrations, solution densities, wavelengths of maximal absorption (λ_{max}), and specific absorptivities (ε)) are reported in Table 1.

Solutions of βCD (used in the diffusion experiments to investigate complexation effects) were prepared by adding solid powder of the dextrin derivative (3.4−4 mg) directly into 2 mL of ketoprofen neutral and acidic solution (pH 1.0, obtained by mixing 1.9 mL of neutral ketoprofen solution plus 0.1 mL of HCl) in order to achieve a molar ratio drug/βCD of approximately 1:1.

2.2.2. Experimental Procedure. For the spectrophotometric measurements, a double array VWR (VWR International, Radnor, USA) UV−visible spectrophotometer (model UV-6300 PC) equipped with a Hellma Suprasil (Sigma-Aldrich) quartz absorption cuvette (chamber volume of 700 μL and path length of 10 mm) was employed. The experimental setup is schematized in Figure 1.

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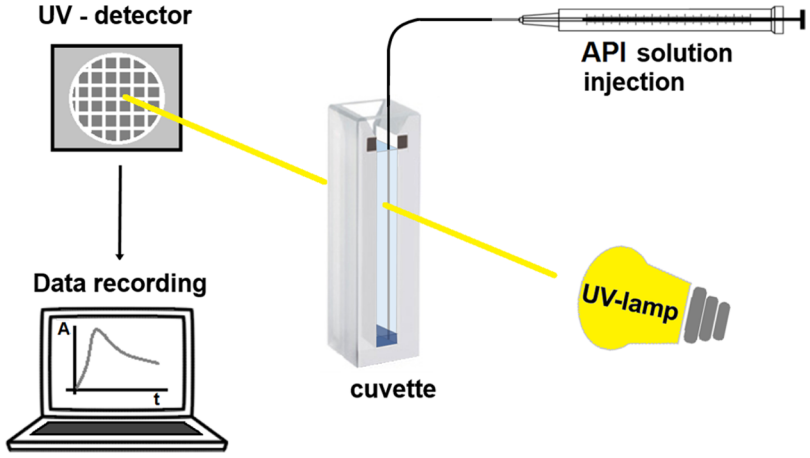

Figure 1. Schematic representation of localized UV−visible spectroscopy experimental setup.

Both reference and sample cuvette were filled with 675 μL of distilled water and placed in the respective compartment of the spectrophotometer. At time (t) = 0 s (start of the experiment), 25 μL of drug solution was gently injected in the bottom of the sample cuvette by a needle syringe. The reference and sample cuvette were both sealed with parafilm in order to avoid evaporation of the solvent. Absorbance readings were recorded at fixed wavelength (Table 1) at regular time intervals (120 s) for 24 h at room temperature (23−24 °C). Absorbance was recorded at a specific position (x_1) corresponding to 0.51 cm from the bottom of the cuvette (Figure 2). This was obtained by keeping the sample cuvette lifted 0.60 cm (Hard Head calliper, Jula Norge AS, Lørenskog, Norway) from the bottom of the cuvette holder.

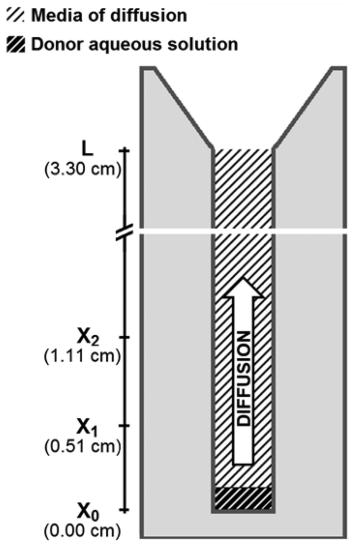

Figure 2. Adaptation of the monodimensional diffusional model used the cuvette geometry and related relevant spatial points (initial point x_0, measurement points x_1 and x_2, and total length of the diffusion chamber L).

For each of the API studied, two independent experiments were conducted (n = 2), and the final diffusional profile (employed for the mathematical data treatment and diffusivity calculations) was obtained by averaging the respective absorbances at each time point. The average variance (in percentage, %) between the absorbance registered at each time point between the two parallel experiments was used to estimate the experimental error (≤5%).

2.2.3. Mathematical Model and UV−Visible Spectroscopy Data Interpretation. The mathematical approach used in this work to fit the experimental data obtained by UV−visible spectroscopy to calculate the diffusion coefficients was based on the standard diffusion equation (eq 3) and well-known classical physical models.[9,18] Due to the geometry of the experimental setup (Figure 2), the diffusion process was approximated with a one-dimensional model. The diffusion equation (eq 3) describes the concentration of a solute over time and space in a homogeneous medium (assuming constant diffusivity) as

$$\frac{\partial c(x,\,t)}{\partial t} = D\frac{\partial^2 c(x,\,t)}{\partial x^2} \tag{3}$$

where c represents the concentration of the substance, t the time, x the position, and D the diffusivity. In theory, any x between 0.51 and 1.11 cm can be selected (Figure 2) by lifting the cuvette from its standard recording position. In this work, the recording point was primarily set to 0.51 cm (see experimental procedure section).

The first mathematical approach used to find numerical solution of D was analytical. This approach is based on the assumption that for times t, $t \ll L^2/D$, and $x \ll L$ (where L is 3.30 cm, Figure 2), the container can be considered to be infinite in length, and the differential equation can be solved via its Fourier transform.

Based on the governing equation (eq 3) and the given boundary conditions (see Supporting Information A), the following analytical solution was derived (eq 4, see Supporting Information A for a detailed description of this equation):

$$c(x,\,t) = \frac{A}{\sqrt{\pi}}\frac{e^{-x^2/2\sigma^2+4Dt}}{\sqrt{2\sigma^2+4Dt}} \tag{4}$$

In this equation, σ represents the width of the initial distribution, and it is used as a fitting parameter. Equation 4 was fitted to the experimental data (concentration over time profiles) using a nonlinear fitting program in order to find diffusivity values for each API investigated.

An alternative approach was to use a numerical approach to find diffusivity values. In this case, the initial concentration distribution was approximated by a standard Gaussian distribution centered in the origin as (Figure 3[18]):

$$c(x,\,0) = \alpha\, e^{-x^2/2^2} \tag{5}$$

where σ is the full width at half-maximum and α the maximum of the initial Gaussian distribution obtained by the equilibrium

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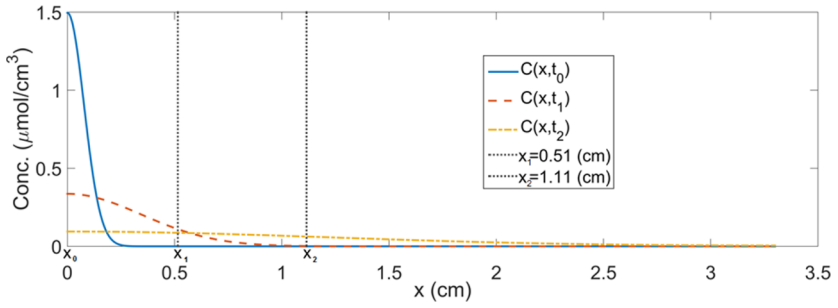

Figure 3. Example of concentration vs position (assuming diffusion over one-dimension). The concentration distribution is initially (t_0) represented by a Gaussian curve (blue line) centered on the origin. The red and the yellow lines respectively represent the concentration distribution after a short time (t_1) and a longer time (t_2). The black-dotted lines represent the two different observation points (x_1 and x_2).

concentration (c_f, i.e., the final equilibrium concentration of the compound injected into the cuvette homogeneously diffused) and the length of the diffusional pathway (L) (see Supporting Information B for a detailed description of the calculation of these parameters). Since there was no flux into or out of the cuvette (conditions of mass preservation), the boundary conditions at x_0 (0.00 cm) and L (3.30 cm) (Figure 2) were (eq 6a,b)

$$\frac{\partial c(0,t)}{\partial x} = 0 (a) \quad \frac{\partial c(L,t)}{\partial x} = 0 (b) \tag{6}$$

The diffusion equation (eq 3) with the initial condition (eq 5) and the boundary conditions (eq 6) has been solved numerically with a finite differences scheme[19] (for additional information, see Supporting Information B). To estimate the parameters D and σ (variable parameters), a global optimization method based on simulated annealing was used (Matlab program from Mathworks Inc., Natick, USA).

Diffusion experiments of caffeine were used in order to calibrate x in the experimental setup. Specifically, two diffusion experiments were conducted at recording at two different measurement points: x_1 (0.51 cm) and x_2 (1.11 cm, measured by caliper). In each of the calibration experiments, recording position (x_1 or, alternatively, x_2) was let to optimization. The best fitting was obtained with x values of 0.51 and 1.11 cm, respectively, in good agreement with direct caliper measurement.

2.3. Estimation of Diffusion Coefficients from Molecular Modeling. Diffusion coefficients of the APIs were estimated from molecular properties using two different approaches. The first was to calculate the molecular radii from the solvent-accessible molecular volumes (SAMV) of the APIs, which were determined using the QikProp program (QikProp, Schrödinger, LLC, New York, NY, 2017).

The second approach was to use the molar volume, which was calculated employing the ChemSketch software (Advanced Chemistry Development Inc., Toronto, Canada). In both cases, the API molecules were approximated to be spherical and the calculated radii (Table 2) were used to estimate the diffusion coefficients employing Stokes–Einstein equation (eq 1).

3. RESULTS AND DISCUSSION

3.1. Validation Experiment with Caffeine. One of the biggest challenges to overcome in the proposed diffusional model (mimicking the UWL) was to avoid turbulence during injection of the donor solution to the diffusion media (Figure 2). To solve this problem, a very simple but effective expedient based on density gradient was used. In Table 1, the general properties of all solutions used as donor media and the diffusion media are reported. The presence of buffering salts and compounds in solution (Table 1) resulted in a slightly higher density for the donor aqueous environment compared to the diffusion media (just plain distilled water). This density difference allowed injection/deposition of the donor solution in the bottom of the cuvette without any turbulence or movement of liquids that could hamper proper measurement of diffusion (as represented in Figure 2). It should be underlined that both solutions (donor and diffusing media) were primarily composed of water so there was no interphase between them (isotropic media, one-phase compartment). In Figure 4, the concentration vs time curve obtained for caffeine at x_1 is reported as an example, but this could be generalized to all investigated compounds. As can be seen in Figure 4, the concentration of the drug increased locally to a maximum value (0.14 μmol/cm^3) and then the concentration slowly decreased until reaching equilibrium (not reached in 24 h, period of the experiments). The best fit obtained with simulated annealing (Figure 4, blue-dashed line) was x = 0.51 cm, confirming the caliper measurement.

To verify that the diffusion process measured by UV–visible spectroscopy was reliable and that the diffusional model designed was appropriate, a second data set was recorded for caffeine at x_2 (1.11 cm) using the previously detected diffusion coefficient (at x_1 = 0.51 cm) as a constant and setting x as variable. In Figure 5, the variation of the concentration of caffeine recorded at x_2 over time is reported. As expected, the increase of the concentration over time was slower (highest concentration approximately 0.06 μmol/cm^3) due to the observation point being further from x_0.

Table 2. Molecular Volumes and Solvent-Accessible Molecular Volumes (SAMV) Estimated by Molecular Modelling and the Respective Calculated Molecular Radii

compound name	molar volume[a] (cm^3/mol)	molecular radii estimated from molar volume (Å)	SAMV[b] (Å^3)	molecular radii estimated from SAMV (Å)
caffeine	133.3	3.7	646.9	5.4
calcein	378.6	5.3	1611.0	7.3
chloramphenicol	208.8	4.4	881.4	5.9
ketoprofen	212.2	4.4	865.7	5.9
nitrofurantoin	163.5	4.0	766.0	5.7
paracetamol	120.9	3.6	567.8	5.1
penicillin G	235.1	4.5	1044.2	6.3
tetracycline	273.1	4.8	1203.9	6.6
trimethoprim	231.8	4.5	928.7	6.1
vancomycin	912.3	7.1	3687.7	10.0

[a]Calculated with ChemSketch software (Advanced Chemistry Development Inc.). [b]Calculated with QikProp program (QikProp, Schrödinger, LLC).

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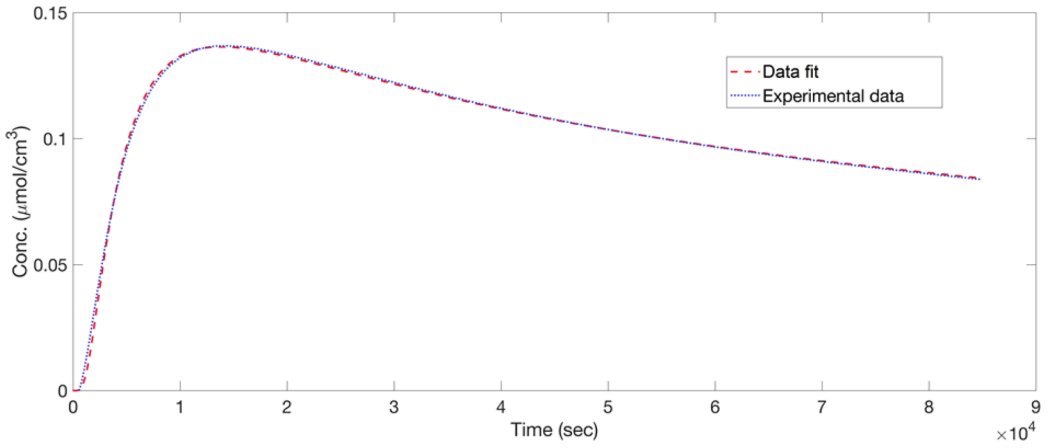

Figure 4. Variation of local concentration of caffeine over time measured at x_1 (0.51 cm). The blue-dotted line represents the experimental data, whereas the red-dashed line represents the best fit (numerical approach).

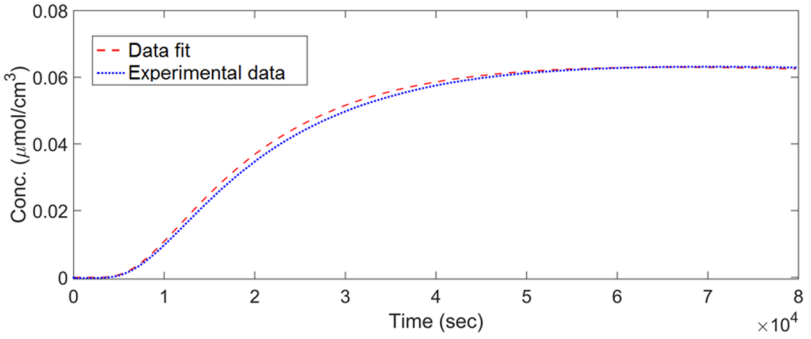

Figure 5. Variation of local concentration of caffeine over time measured at x_2 (1.11 cm). The blue-dotted line represents the experimental data, whereas the red-dashed line represents the best fit obtained with the simulated annealing ($D = 9.4 \times 10^{-6}$ cm^2/s).

Interestingly, the data fitting of the curve obtained by simulated annealing (red-dashed line) mimicked closely the behavior observed experimentally (blue-dotted line) also in this case. It should be noted that, even though in this experiment D was used as constant and x let to optimization, the x found was in very good agreement with the caliper measured (1.11 cm) certifying the validity of the experimental setup for diffusion studies developed. We also investigated an analytical-based method employing the value of 0.51 cm as fixed parameter for the measurement position (x_1).

3.2. Experimental Diffusion Measurements. In Table 3, the diffusion coefficients calculated with the numerical and analytical approaches are reported together with the data obtained from the literature (when applicable) for the compounds investigated.

As shown in Table 3, the diffusivities obtained with the two different mathematical approaches are quite similar, with a variation between 0% (calcein, trimethoprim) and 6% (for ketoprofen). This justifies the thought that both mathematical approaches are equally good for solving local concentration profiles in such geometrical diffusional parameters (i.e., size of a cuvette). From these results, it appears that the analytical approach works well with the geometry of a standard quartz cuvette and small to medium-sized molecules. However, it cannot be excluded that this assumption might not be valid anymore for smaller molecules (with higher D); however, such scenarios were not investigated in this work. In cases where diffusivities might be affected by specific parameters (e.g., high viscosity, strong electrostatic field) also the numerical approach should be adjusted, with the application of more complex equation. However, for the experimental conditions employed in this work, the standard diffusion equation resulted adequate (fitting error below 1%). Moreover, the experimental data results are quite comparable with the available literature data (refs 20−25, linear fitting R^2 of 0.8). Due to the limited data available in the literature, it is difficult to clearly state if our method is more accurate than others. It should be noted that the D values obtained in this work are generally higher than the ones reported in the literature. Interestingly,

Table 3. Diffusion Coefficients Obtained Experimentally by the UV−Visible Spectroscopic Method and Subsequent Analytical and Numerical Data Treatment (Fitting Error below 1%)[a]

	D (10^{-6} cm^2/s)		
compound name	analytical approach	numerical approach	literature values
caffeine	9.0	9.4	7.8,[20] 6.2[21]
calcein	3.8	3.8	3.3[22]
chloramphenicol	6.6	6.3	5.7[23]
ketoprofen	6.4	6.8	
nitrofurantoin	7.1	7.0	
paracetamol	7.8	7.7	6.6[24]
penicillin G	6.5	6.4	4.0[25]
tetracycline	5.8	5.9	
trimethoprim	5.6	5.6	
vancomycin	2.9	3.0	

[a]When applicable, results are compared with available literature data. Data recorded at x_1 position (0.51 cm).

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using the caffeine literature value (7.8×10^{-6} cm^2/s) to rescale our data, our values became identical to the literature values for calcein, chloramphenicol, and paracetamol. A discrepancy still exists for penicillin G (rescaled value obtained 5.7×10^{-6} cm^2/s), but the difference is much smaller. However, the literature data[25] refers to an experiment performed with potassium penicillin in plain water; therefore, the discrepancy is most probably due to the different experimental conditions (i.e., pH, tonicity[26]) used. These data suggest that the D values we identified should therefore be considered "relative", but, using a standard, the spectroscopic method described in this work results suitable in order to measure absolute diffusivities.

3.3. Comparison with Model Predictions. In Figure 6, the experimentally determined diffusion coefficients (analytical approach) are compared with the diffusivities calculated by two different *in silico* parameters (solvent-accessible molecular volume (SAMV) and molar volume).

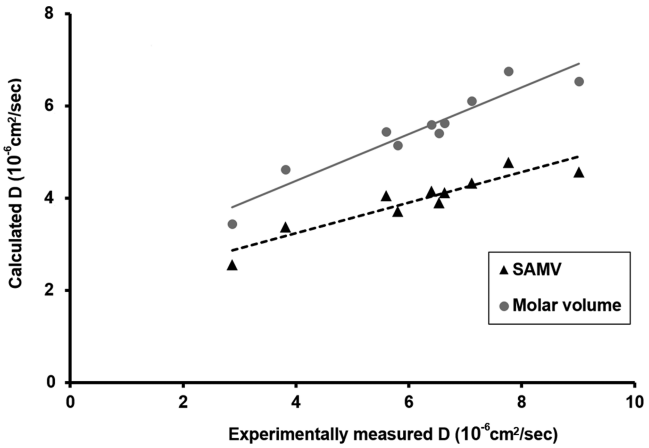

Figure 6. Correlation between the diffusivity constants measured experimentally (and subsequent analytical data treatment) and estimated by molecular properties (solvent-accessible molecular volume (SAMV) and molar volume). Calculated D error was below 1%.

As it can be seen, the correlation between experimentally determined and calculated diffusivities is quite linear ($R^2 = 0.9$), independent of the molecular descriptor used to estimate diffusivity. D values measured in this work result generally slightly higher than the one obtained by *in silico* prediction (Figure 6). One of the reasons might be that we measure the diffusivity of the chromophore, which can be higher than the diffusivity of the center of mass. The diffusivities measured via the UV-technique should thus be consider "relative" diffusivities and not absolute values. However, the method disclosed in this work can be considered extremely reliable and can monitor even very small changes in diffusivity.

3.4. Effect of Ion Strength and Complexation on Diffusivity. In order to explore the potentials of this technique to solve relevant pharmaceutical issues, we applied this technique to investigate whether it was possible to measure the influence of ion strength and the presence of βCD on the diffusion process. First, we tried to verify if the alteration of the environment of diffusion could affect the diffusion constants. In the first experiments we wanted to verify if the ion strength (i.e., tonicity) could influence the diffusion constant of caffeine. As it can be seen from Table 4, reducing the tonicity of donor solution did not significantly alter the diffusivity of caffeine. On the contrary, the diffusivity of caffeine was highly increased (25% increase) by the increased ionic strength (i.e., tonicity).

Table 4. Influence of Environmental Ion Strength on the Diffusion Coefficients of Caffeine[a]

	caffeine conc. (μmol/cm^3)	pH	ionic strength (mOsm)	diffusivity (10^{-6} cm^2/s)
isotonic media	1.23	7.4	290	9.0
hypotonic media	1.42	7.6	35	9.1[b]
hypertonic media	1.34	7.2	521	11.3[b]

[a]Hypertonic solution was obtained by the addition of NaCl. [b]Single experiment.

This phenomenon can be explained by the fact that at higher concentration of ions, electrical fields and osmotic gradient may start to play a stronger role in the passive diffusion of APIs,[26] synergistically promoting passive diffusion of the API molecules.

In the second experiment, we wanted to verify the effect of complexing agents on passive diffusion. For this purpose, a solution of ketoprofen and βCD was prepared at pH 1 and used as donor solution. At acidic pH, ketoprofen in solution is primarily in its unionized form (pK_a 4.7), and therefore, it expresses the highest affinity for the dextrin rings.[27] In fact, the equilibrium constant of the ketoprofen-βCD complex is approximately 20% higher in acidic pH (unionic ketoprofen) than in neutral/basic pH (ionic ketoprofen).[27] Using data fitting and simulated annealing, it was possible to calculate a new diffusion coefficient of 5.04×10^{-6} cm^2/s for ketoprofen in the presence of βCD that corresponded to a 25% reduction in comparison to ketoprofen alone. This discrepancy was also confirmed by applying analytical mathematical interpretation of the data. In order to verify if the reduction of D could be related to other factors rather than complexation (e.g., viscosity increment given by the presence of βCD), a diffusion experiment of ketoprofen in the presence of βCD was also conducted as reference at pH 7.4. At this pH, ketoprofen molecules are expected to be primarily ionized, therefore having reduced affinity for βCD (i.e., ketoprofen molecules should be primarily uncomplexed). In this case, the reduction in D value of ketoprofen was just 7% (analytical solution). This result clearly shows that the presence of complexing agents has a significant effect on the diffusivity of ketoprofen and that the magnitude of this effect is directly related to the drug-βCD equilibrium constant (pH dependent).

4. CONCLUSION

In this work, a simple and versatile technique based on temporal resolution of concentration measurement by UV−visible spectroscopy for the determination of API diffusion coefficients in unstirred aqueous environment has been successfully developed. This technique is simple and of high sensitivity, and it could be potentially applied to almost all drugs as well as to new chemical entities. In this work, the technique has been successfully employed to verify the influence of environmental characteristics on diffusion. This technique might become an important tool to gain a better understanding of relevant biopharmaceutical problems such as permeability of drugs through biological barriers and comprehension of the effect of the diffusional environment on diffusion.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.7b01053.

Detailed descriptions of the analytical solution of the diffusion equation and the numerical approach employed (PDF)

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Notes

The authors declare no competing financial interest.

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