Sys Rev Pharm 2020; 11(6): 96 – 100
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Determination of Chemical Potential for Stavudine (D4T) Diffusion through SDS Micelle Solution

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Article History: *Submitted: 02.03.2020* *Revised: 10.04.2020* *Accepted: 31.05.2020*

ABSTRACT

This study include a spectroscopic measurements of Stavudine diffusion in cell membrane alternative model in two different polar solutions; buffer phosphate solution (Polar solution) and N-Hexane (Non-polar solution). Consistent with the standard values, a clear maximum absorption peaks at 266 nm was noted for Stavudine in buffer phosphate solution. The data also showed that the value of the extension coefficient and λ_{max} reduced in the non-polar medium compare to polar medium which was noted a s a part of the spectroscopic properties of Stavudine in polar and non-polar medium. Stavudine express a high stability with time in pH 7.4 .

SDS was used as a cell membranes substitute model, and the diffusion rate of Stavudine through SDS micelles solution (with a concentration of 0.2 $\times 10^{-2}$ M)was examined. The chemical potential was calculated which was equal to -2489.4 J mol^{-1}which indicate the impulsiveness of the diffusion process for the compound. The results suggested that Stavudine can diffuse (in a rate constant of 0.0183 min^{-1}) to inside micelle from the aqueous medium. Of other detected factors; the equilibrium constant for diffusion rate was detected and was equal to 2.7313.

Keywords: Stavudine, diffusion, SDS, micelle solution

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DOI: 10.31838/srp.2020.6.17

INTRODUCTION

Stavudine (D4T) is a synthetic nucleoside (thymidine) analogue reverse transcriptase inhibitor (NARTI) with activity against HIV-1 and HBV [1][2]. The chemical name of Stavudine is 21, 31-didehydro-31-deoxythymidine, it has a molecular formula of C10H12N2O4 (Figure 1) and a molecular weight of 224.22 g/mol [3].

D4T is converted to triphosphate inside the cell and cease the DNA synthesis of retroviruses via competing with reverse transcriptase enzyme inhibitory effect and incorporation into viral DNA. (Basavaiah, et al., 2008).Stavudine is a white to off-white crystalline powder. It is freely soluble in ethanol (95%) with a solubility of about 83 mg/ml in water at 23°C [3][4].

It was suggested that Stavudine can distributes into body fluid and used the non- facilitated diffusion to enter body cells [5]

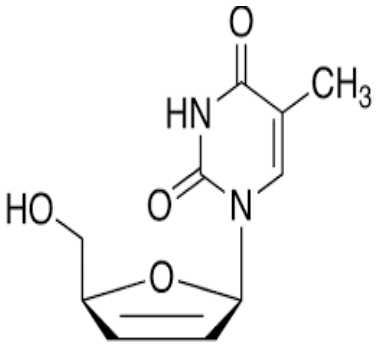

Figure 1: Chemical structure of Stavudine

Several methods of analysis been used to determine Stavudine in biological fluids or pharmaceutical preparations. Nevertheless, for simple bio-analytical assays, UV- visible spectrophotometry is continued to be used [2].

Sodium dodecyl sulfate (SDS), otherwise known as lauryl sulfate, with the chemical formula C12H25NaO4S or CH3=(CH2)11- O-SO3-Na+is an alcohol detergent derivative of Alcohol Sulfates, it also considered as an ionic detergent which play a role in the rapid disruption of biological membranes[6]. SDS is consists of a 12-carbon tail attached to a sulfate group. This sulfate group represent the ester of sulfuric acid and dodecyl alcohol and the sodium salt of *dodecyl hydrogen sulfate*, . The hydrocarbon tail of SDS together with the polar "head group" provide the amphiphilic properties of the compound and make it of use as a detergent[7] [8].

Micelles are a set of amphiphilic surfactant molecules which aggregate together impulsively, once contact an aqueous medium, as a spherical vesicles [9].

Synthetic polymers and Surfactant molecules interactions in aqueous medium remain significant to many detergents applications, chemical, pharmaceutical, health products, petroleum industries. Generally, the existence of surfactant molecules and polymer mutually modify the solutions rheological characteristics, such as the colloidal dispersions stability, the adsorption characteristics at solid–liquid interfaces, liquid–liquid interfacial tensions and the solubilization capacities in water for sparingly soluble molecules. The capability of the polymer molecules in addition to the surfactant to affect the solution and interfacial features is rolled via their state of occurrence in the aqueous medium and the nature of their microstructures if they made a mixture of aggregates in solution[10].

Micellar Solubilisation is considered as one of the most important characteristic of surfactant solution that has been widely used in pharmaceutical formulations, particularly, to increase drugs bioavailability [11].

EXPERIMENTAL.

Aqueous solutions of buffer phosphate was prepared by mixing a particular volume of KH_2PO_4 with a concentration of 0.0667 M ,then the volume completed with $Na_2HPO_4 \bullet 2H_2O$ with a concentration of 0.0667M to 100 mL, followed by PH adjusting . Aqueous solutions of Stavudine (D4T) with a concentration of 1 $x10^{-4}$ M was prepared as a stock solution.

Spectroscopic measurements was implemented at 37^0C for drug (1$x10^{-5}$ M) solution , n-Hexane and drug solution in Sodium dodecyl sulfate micelle(with respect to critical micelle concentration in the preparation process) using Shimadzu 1800 UV-spectrometer in the range of 200-400 nm.

RESULTS AND DISCUSSION

Spectroscopic study of Stavudine

Spectroscopic properties of Stavudine (D4T)(1 ×10^{-5} M)were tack place in a different polarity media at 37 C° (Figure -2). Blue shift and hypo chromic effect was observed when solution changed from polar to nonpolar ,where in buffer phosphate solution λmax = 266 nm which was compatible with previous studies [12][13] while λmax was equal to 259 nm in cyclohexane (Table 1) .

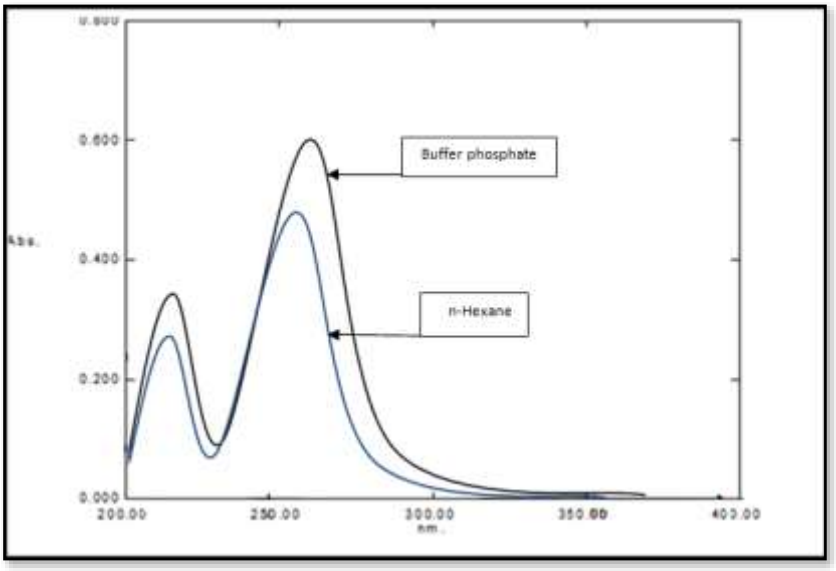

Figure 2: Ultra- Violet spectrum of Stavudine (D4T) with a concentration of 1×10^{-5} M in buffer phosphate pH=7.4 and n-Hexane

Table 1: Molar attenuation coefficient of Stavudine (D4T) in different solutions

Wavelength /nm	attenuation coefficient/ $mol^{-1}.L.cm^{-1}$	
	Buffer	n-Hexane
241	17500	17500
243	19600	19600
245	28000	28000
247	32400	32400
249	38100	38100
251	40000	40000
253	42500	42500
255	46000	43500
257	48400	45500
259	51000	46500
261	54400	43500
263	57500	38500
266	60000	35000
268	57000	32500
270	54500	29000
272	51500	25500
274	46500	23500
276	42000	20000
278	38000	18500

The solvatochromism phenomenon was clearly observed in the behavior of drug solutions , the term solvatochromism is used to depict the proclaimed change in position and intensity of a UV-visible absorption band tack place according to a change in the polarity of the medium. When absorption spectra were measured in different polarity solvents , it was noted that not only the position of the absorption band can vary but also the intensity and sometimes the shape , depending on the nature of the solvent.[14]

The blue shift of the band in drug solution in n-Hexane may be interpret according the change in geometry of molecule as resulted of decreasing in the energy level of the molecular orbital π caused by the solvent . A remarkable note which give additional explanation is a difference in dielectric constant of solvents (it is 1.882 $F{\cdot}m^{-1}$for n-Hexane compare with 80 $F{\cdot}m^{-1}$ for water) in addition to the difference in the solvent –solute interaction in the ground and excited state.

Intensity of absorption band also increased with polarity of the media, where the polar solvent increases the polarity of excited state π* which leads to increasing in the value of attenuation coefficient [15][16]

The specific solute-solute and solute-solvent interaction and bulk solvent properties were the main reasons of the spectral shifts. For systems without intermolecular hydrogen bond, the spectral shifts might be interpreted by the sensitivity to the solvent polarity. Accordingly, in many molecules a bathochromical shifting of the bands (π-π*) noticed with increasing the polarity of the solvent [14][17]

Diffusion study of Stavudine (D4T) through SDS micelle solutions

An investigation of the diffusion rate through SDS micelles for pharmaceutical compound with a concentration of 0.2 $\times 10^{-2}$ M,was doneas and the chemical potential was calculated.

A remarkable change in the absorbance of Stavudine with time was recorded (Figures 3,4) and (Tables 2,3). The calculations was at a λ_{max} = 266 nm:

$$\mathbf{A_{tot} = A_{aq} + A_{org}}$$

$$\mathbf{A_{tot} = \varepsilon_{aq} A_{aq} + \varepsilon_{org}[C_{initial} - C_{aq}]}$$

$$C_{aq} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} C_{org}$$

out side micelle — in side micell

where :

Atot: is the total absorbance of stavudiene

Aaq: is the absorbance of stavudiene in aquaouse solution

Aorg: is the absorbance of stavudiene in organic solution

ε_{aq} : is the attenuation coefficient of stavudiene in aquaouse solution

ε_{org} : is the attenuation coefficient of stavudiene in organic solution

$C_{initial}$: is the initial concentration of stavudiene

C_{aq} : is the concentration of stavudiene in aqueouse solusion

From table (1) :

$A_{tot} = 60000C_{aq} + 35000[C_{initial} - C_{aq}]$

Use initial concentration and rearrangement the equation:

$A_{tot} - 0.35 = 25000\ Caq$

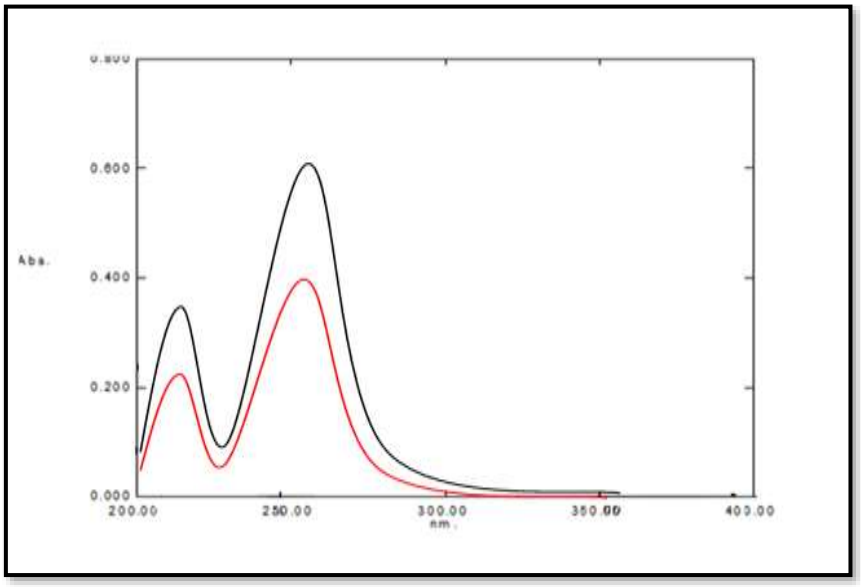

Figure 3: Initial and final absorption spectrum of stavudiene diffusion through SDS .

Table 2: stavudiene diffusion behavior with time

λ / nm	Time/min										
	1	10	20	30	40	60	80	100	120	160	1440
241	0.171	0.163	0.152	0.147	0.133	0.114	0.094	0.082	0.066	0.062	0.062

243	0.192	0.169	0.158	0.150	0.144	0.128	0.111	0.093	0.086	0.081	0.081
245	0.272	0.265	0.258	0.236	0.214	0.181	0.174	0.164	0.161	0.158	0.158
247	0.314	0.319	0.281	0.269	0.243	0.225	0.212	0.194	0.186	0.181	0.180
249	0.372	0.364	0.332	0.288	0.256	0.244	0.239	0.219	0.192	0.189	0.189
251	0.380	0.374	0.358	0.332	0.275	0.261	0.248	0.222	0.214	0.212	0.212
253	0.415	0.408	0.385	0.363	0.337	0.312	0.293	0.267	0.257	0.254	0.254
255	0.453	0.446	0.433	0.395	0.374	0.332	0.324	0.312	0.305	0.303	0.303
257	0.478	0.469	0.442	0.426	0.402	0.381	0.368	0.356	0.339	0.337	0.337
259	0.500	0.491	0.475	0.467	0.442	0.429	0.397	0.383	0.371	0.366	0.366
261	0.532	0.525	0.489	0.473	0.452	0.427	0.408	0.391	0.388	0.379	0.379
263	0.565	0.554	0.536	0.514	0.487	0.465	0.449	0.424	0.394	0.391	0.391
266	0.582	0.569	0.544	0.528	0.486	0.454	0.427	0.419	0.417	0.417	0.417
268	0.560	0.558	0.538	0.521	0.489	0.461	0.434	0.406	0.359	0.351	0.351
270	0.538	0.523	0.497	0.472	0.454	0.438	0.409	0.372	0.269	0.261	0.261
272	0.501	0.493	0.462	0.442	0.418	0.397	0.365	0.354	0.231	0.225	0.225
274	0.454	0.433	0.392	0.366	0.327	0.295	0.254	0.221	0.189	0.185	0.185
276	0.372	0.356	0.372	0.346	0.311	0.273	0.242	0.189	0.171	0.168	0.168

Table 3: Stavudiene concentration in buffer phsphate and n-Hexane at each time at λ_{max}

Time/min	M $C_{.aq}/10^{-5}$	$C_{.org}/10^{-5}$ M	Xe/(Xe-X)	Ln [Xe/(Xe-X)]
1	1	0	1	0
10	9.28	0.72	1.109091	0.103541
20	8.76	1.24	1.203947	0.185606
30	7.76	2.24	1.440945	0.365299
40	7.12	2.88	1.648649	0.499956
60	5.44	4.56	2.652174	0.97538
80	4.16	5.44	3.893617	1.359339
100	3.08	6.92	18.3	2.906901
120	2.86	7.14	40.66667	3.705409
160	2.68	7.32	x	x

Where :
X : concentration of Stavudiene in n-Hexane at time t
Xe: : concentration of Stavudiene in n-Hexane at equilibrium
C_{eq}: concentration of Stavudiene in buffer phosphate
C_{org}: concentration of Stavudiene in n-Hexane
From the equation of reversible reaction which is first order in both direction :

$$t = \frac{1}{k_1 + k_{-1}} \ln \frac{Xe}{Xe - X}$$

$$t_{0.5} = \frac{0.693}{k_1 + k_{-1}}$$

$$\mathbf{k_{eq}} = \frac{\mathbf{k_1}}{\mathbf{k_{-1}}}$$

$$\therefore k_{eq} = \frac{Xe}{a - Xe}$$

plutting $\ln \frac{Xe}{Xe-X}$ aganist time give a stright line with a slop equal to($k_1 + k_{-1}$) figure(4) :

Slop = 0.025
So: $k_1 + k_{-1} = 0.025\ \mathbf{min^{-1}}$

$$\mathbf{k_{eq}} = \frac{\mathbf{k_1}}{\mathbf{k_{-1}}} = 2.7313$$

K_1 =0.0183 min^{-1}
K_{-1} = 0.0067 min^{-1}
$t_{0.5}$= 27.72 min
$\Delta G° = -RTLn\ K_{eq}$
ΔG^o = -2489.4 J mol^{-1}

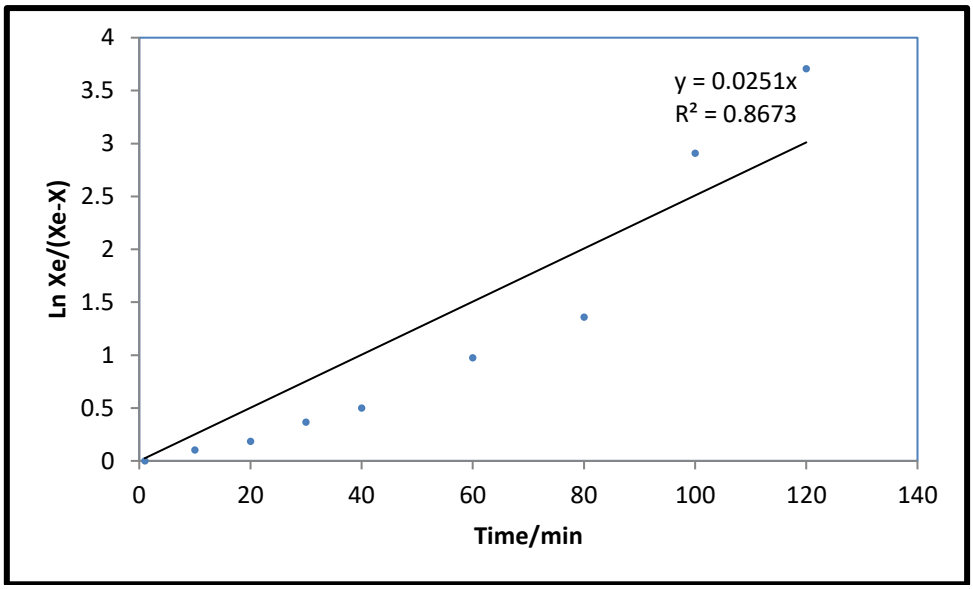

Figure 4: Ln [Xe/(Xe-X)] against time for the diffusion of Stavudine through SDS .

According to the negative value of Gibbs free energy (chemical potential) it could be clearly observed that the diffusion of Stavudine through SDS micelle is a spontaneous process.

Decreasing the absorbance value of Stavudine in SDS is an indicate to entering of Stavudine from the aqueous solution outside the micelle into the organic media inside the micelle, it is important to notied that there was no reaction between Stavudine with buffer phosphate which used as a solvent in preparation of SDS solution.

The absence of any reaction between buffer phosphate component and Stavudine, and the low value of attenuation coefficient of Stavudine in organic non polar solvent comparing to the high value in aqueous media, also the dropped of Stavudine absorbance in SDS solution, can all suggest that Stavudine was enter the organic media inside the micelle from the aqueous solution outer of micelle.

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