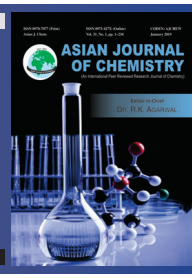
Asian Journal of Chemistry; Vol. 31, No. 2 (2019), 417-421

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.21669



Effect of Sonication on Certain Antiepileptic Drugs: Approach to Drug Delivery Mechanism Through Perfluorocarbon

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Received: 22 August 2018; *Accepted*: 20 October 2018; *Published online*: 31 December 2018; AJC-19219

Lennox-Gastaut type of seizures that affects the central nervous system (CNS) are facing drug administration problems. One such problem is that the delivery mechanism of antiepileptic drugs (AEDs) to the affected seizures is haunting the complete treatment of epilepsy. One of the better methods to overcome this problem is to attach the drug with a carrier agent and making it deliver at the proper site. In this paper, a novel attempt has been made to identify all possible attachment portions in the antiepileptic drugs like topiramate, carbamazepine and lamotrigine by sonicating (ultrasonic) the drugs at different timings and amplitudes. The disintegration of the drugs and the possible attachment points, for every different timing and amplitude, were analyzed through high performance liquid chromatography. An extensive study has been made on topiramate by reacting it with the chosen drug delivering agent perfluorodecalin (PFD) and subjected to sonication. The choice of perfluorodecalin is a novel intuition since they are traditionally used as oxygen carrier and blood substitute. The topiramate + perfluorodecalin emulsion is grown into a crystal. Complete structure has been elucidated through X-ray crystallographic studies. Results show that the sonication is one of the much easier and handy technique to break chemical bonds and to create cavitations thus allowing other molecules to occupy that space. Investigation further reveals that, more the number of nitrogen atoms, less they are interactive and less it responds to sonication effect. Hence we prefer to safely conclude that topiramate is more adaptive in the study of drug delivering mechanism though it possesses a bulk structure than lamotrigine and carbamazepine.

Keywords: Antiepileptic drugs, HPLC, Sonication, Ultrasonic, Perfluorodecalin, Drug delivery mechanism.

INTRODUCTION

Epilepsy is a nervous condition affecting the central nervous system. In humans it creates individual familial and social problems and cries for effective treatment, which is hampered by drug administration problems. It is well know fact that the modern drugs creates much side effects rather than effectiveness [1-5]. Topiramate (TPM) (2,3:4,5-*bis*-*o*-methylethylidene-β-D-fructopyranose sulfamate), carbamazepine (CBZ) (5*H*-dibenzo-azepine-5-carboxamide) and lamotrigine (LMG) (3,5-diamino-6-2-3-dichlorophenyl-1,2,4-triazine) are certain new generation anticonvulsant drugs which are suitable for treating Lennox-Gastaut epilepsy syndrome and central nervous system disorders. The anticonvulsant drugs are typically tri cyclic in structure. Blocking the voltage-dependant sodium channels, stabilizing neuronal membrane, reducing the release of excitatory neuro transmitters, particularly glutamate and γ-aminobutyric acid (GABA) are some of the mode of action by these drugs on the affected seizures [6-8]. Typically these actions are effectively achieved by methylation of any of these drugs. Successful attempts have been made on methylation of anticonvulsant sulfamate derivative by so many researchers and have even filed patents too [9,10].

The drawback of methylation is that the mentholated structures do not remain stable for a longer period. Methylation is possible by a straight forward chemical reaction [11]. Further the attachment of methyl group is expected to be in the amino group portion, which is relatively weakly bonded than any other core portion of the structure. Removal of hydrogen, NH or even NH_2 through a prescribed chemical reaction does not remain stable for a long period.

The aim of this study is to break other weak interaction portions (including the H, H_2 in the NH_2) of these drugs through the process of sonication (ultrasonic) and to identify the potential portion for not only methylation and its stability analysis but also to attach drug delivering agents like perfluorodecalin (PFD, $C_{10}F_{18}$) with prescribed conditions. This analytical method is used to develop a novel drug-delivering mechanism. The choice of using perfluorodecalin is novel intuition since they are high oxygen carrying agents which are widely used as blood substitute, oxygen carriers and so on [12-14]. It is evident that, both the drug and the delivering agents are bulk in structure needs much energy to break a prescribed bond. Sonication is one of the much easier and handy techniques used to break the chemical bond and to create cavitations thus allowing other molecules to occupy that space [15,16].

Hence, in this work, topiramate, carbamazepine and lamotrigine are subjected to sonication for different specifications. The effects of dissolution of drugs due to sonication were identified using RP-HPLC analysis. The valuable information produced by the RP-HPLC analysis helps us to validate the application of sonication and an attempt to attach any preferred drug delivering agents (perfluorodecalin). Though all the solutions were subjected to crystal growth, topiramate alone is considered for extensive study. Whereas, the HPLC analysis reports of all the solutions are reported. The possibilities of attachment of perfluorodecalin are explored for topiramate by developing the reaction products into a crystal. X-ray crystallography studies were carried out and the structure is determined using SHELX software.

EXPERIMENTAL

Topiramate, carbamazepine and lamotrigine provided by Sigma-Aldrich are HPLC grade and were used without further purification. They were certified to contain 99.8 % of drugs. HPLC grade acetonitrile, ether, pyridine obtained from Merck scientific Inc was used for HPLC analysis. Analytical grade perfluorodecalin purchased from Sigma-Aldrich were directly used for chemical reactions. HPLC grade water prepared from reverse osmosis and filtered through Millipore Mille-Q plus system is used as the only solvent.

Sonicator, HPLC and X-ray specifications: Probe sonicator instrument supplied by PCI model KS-250F module has been used for sonication. It has a sweep frequency range of 0-20 KHz. The probe is made up of Titanium. It possesses a temperature sensor and R-F frequency generator. The amplitude can be varied from 10 to 90 %. To negotiate the heat generated during sonication, an ice-cage filled with dry ice is setup. The temperature could be reduced up to even 0 °C.

The instrument used for HPLC studies is a Shimadzu make LC-2010 RP-HPLC–LC-2010 HT module instrument. It contains UV-visible detector. RP-HPLC is just opposite of normal-phase chromatography, with a non-polar stationary phase C-18 and a polar largely aqueous mobile phase. Data were recorded, monitored and evaluated using a Lab solution LC-solution. It has a UV wavelength of relatively good range of nm.

X-ray data for the compounds were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromatic MoK_α radiation (λ = 0.71073 Å) with ω-scan method. Integration and scaling of intensity data was accomplished using SAINT program. The structure was solved by direct methods using SHELXS97 and refinement was carried out by full-matrix least-squares technique using SHELXL97.

Preparation of standard solutions: A stock standard solution of topiramate (1 mg/mL) was prepared by dissolving 10 mg of topiramate into a 25 mL A-grade volumetric flask and diluted to 10 mL with HPLC grade water. The powder was completely dissolved using a magnetic stirrer at 10 °C. The standard solution was stable during this process and stored at 4 °C. The standard topiramate was prepared in their solubility limit in water which is 9.8 mg/mL [17].

Similarly a stock standard solution of carbamazepine (2 mg/mL) was prepared by dissolving 60 mg of carbamazepine into a 50 mL A-grade volumetric flask and diluted to 30 mL with HPLC grade water. The powder was completely dissolved using a magnetic stirrer at 10 °C. The standard solution was stable during this process and stored at 4 °C. The standard carbamazepine was prepared in their solubility limit in water [18].

A stock standard solution of lamotrigine (0.2 mg/mL) was prepared by dissolving 10 mg of lamotrigine into a 100 mL A-grade volumetric flask and diluted to 50 mL with HPLC grade water. The powder was completely dissolved using a magnetic stirrer at 10 °C. The standard solution was stable during this process and stored at 4 °C. The standard lamotrigine was prepared in their solubility limit in water which is 0.17 mg/mL [19].

Sonication procedures: 5 mL aliquots of standard topiramate solution has been accurately measured and transferred into 25 mL A-graded volumetric flask, 10 mL aliquots of standard carbamazepine solution has been accurately measured and transferred to 25 mL A-graded volumetric flask, 15 mL aliquots of standard lamotrigine has been accurately measured and transferred into 25 mL A-graded volumetric flask. Each aliquot was divided into three sets ($3 \times 3 = 9$). One to perform HPLC studies without sonication, the second to perform sonication for 5 min and the third to perform sonication for 15 min respectively and then sent for HPLC analysis.

For all the six set of solutions (3×2) sonication is performed using the probe sonicatior with the specifications mentioned previously. The amplitude is varied between the ranges of 10-60 % in order to avoid over heating of the sample. The sweep frequency is reduced to 10 KHz for 15 min sonication and raised to 18 KHz for 5 min sonication to maintain the dissociation rate of the solution. The temperature inside the sonicator unit has been well maintained at the range of 0-15 °C with the help of dry-ice cage. Utmost care has been taken to maintain the temperature because the dissolution is much dependant on temperature.

Chromatographic procedures: All the nine sets, 3-non sonicated solutions, 3-sonicated for 5 min and 3-sonicated for 15 min were all subjected for HPLC for dissolution analysis. Chromatographic separation was performed using Xterra C18G, 250 mm × 4.6 mm i.d., 5 µm column. The mobile phase consisting of acetonitrile: 1.0 g of ammonium acetate in 1000 mL of HPLC grade water buffer (pH-7) in ratio of 35:65 %.

In order to make the dissolution more sensitive and accurate, the mobile phase filtered through 0.22 µm membrane filter paper and degassed by sonication for 15 min. Samples were injected at a constant flow rate of 1 mL/min for 15 min. The column was maintained at 40 °C and 20 µL of samples were injected onto the column. These conditions were maintained for all the nine samples to maintain consistency. The UV wavelengths set for topiramate at 264 nm, carbamazepine at 280 nm and for lamotrigine at 225 nm.

Growth of topiramate crystal: 10 mg of Topiramate powder was reacted with 2.5 mL of perfluorodecalin ($C_{10}F_{18}$, PFD) in the presence of pyridine as base and ether as solvent. The mixture is maintained at 5 °C. The reaction is left undisturbed for 1 day. The prepared sample is subjected for sonication for 15 min. The resultant mixture is collected and made up with NaOH base and then maintained at 5 °C for nearly a week. Then it is left for slow evaporation process. It took nearly a month time for the crystal was grown. The crystal thus grown is subjected to X-ray diffraction. The possibility of perfluorodecalin getting attached with the basic structure of topiramate is explored and discussed.

RESULTS AND DISCUSSION

Effect of sonication: The influence of time of sonication on these drugs were analyzed with the nature of peak resolution, peak height, peak area and the retention time of the peaks produced through chromatography. The peak formed for the standard solution *i.e.* without sonication is compared with the peaks formed after sonication of the solution for all the three drugs. The dissolution of the drugs is evident from the peaks formed by the HPLC analysis.

Lamotrigine: The retention time (Table-1) of pure lamotrigine (without sonication) is approximately 5.075 min [20] and that of lamotrigine solution with sonication (5 and 15 min) are also the same *viz*., 5.076 min (Figs. 1-3). Whereas it is interesting to note that the peak area and tailing factor is very low for 5 min sonication but both peak area and tailing factor of both pure lamotrigine (without sonication) and 15 min sonicated solutions are identical. This indicates the possibility of reunion of the molecules when the sonication time is increased, since the molecular interaction increases with sonication time. Probably drugs with two benzene rings and too much of nitrogen-amino groups may not allow any other molecules to attach with it even if their bonds are broken through sonication.

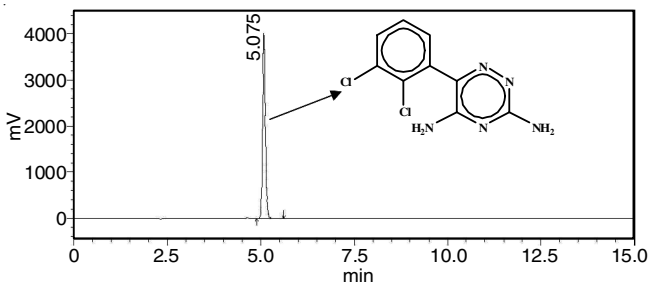

Fig. 1. Typical chromatograph of lamotrigine without sonication

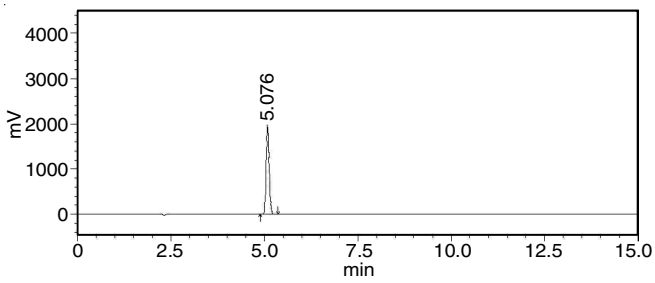

Fig. 2. Chromatograph of lamotrigine with 5 min sonication

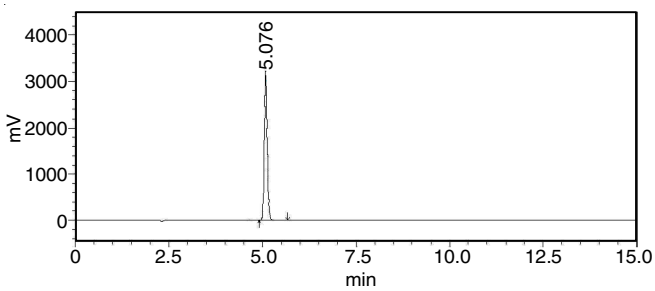

Fig. 3. Chromatograph of lamotrigine with 15 min sonication

Even for 5 min sonication, the breaking up of bonds is feeble which reflects the same retention time for all the solutions.

Carbamazepine: Carbamazepine was well resolved on stationary phase and the retention time (Table-2) was approximately 10.176 for pure carbamazepine (without sonication [21,22]). Whereas, there is a decent shift in the retention time for both 5 min sonication and 15 min sonication with the peak appearing at the same value of 10.319-10.320 (Fig. 4-6). But it is interesting to note that the peak area and tailing factor steeply increases with sonication effect. This is a clear indication of the dissolution of the bonds in the solution thus

TABLE-1
CHROMATOGRAPHIC DETAILS OF LAMOTRIGINE

Sample name	Retention time	Area	Tailing factor	Theoretical plane
Lamotrigine without sonication	5.075	20695042	1.249	21262.269
Lamotrigine with 5 min sonication	5.076	11111778	1.225	15750.030
Lamotrigine with 15 min sonication	5.076	17317494	1.241	16953.003

TABLE-2
CHROMATOGRAPHIC DETAILS OF CARBAMAZEPINE

Sample name	Retention time	Area	Tailing factor	Theoretical plane
Carbamazepine without sonication	10.176	15103572	1.166	22329.180
Carbamazepine with 5 min sonication	10.319	9652050	1.188	21463.983
Carbamazepine with 15 min sonication	10.320	10467965	1.185	21578.808
	12.295	20876	1.472	21626.029

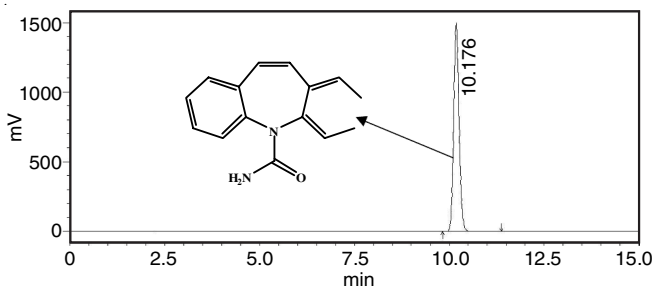

Fig. 4. Typical chromatography of carbamazepine without sonication

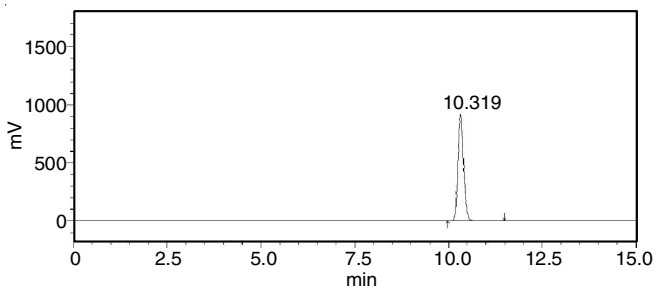

Fig. 5. Chromatography of carbamazepine with 5 min sonication

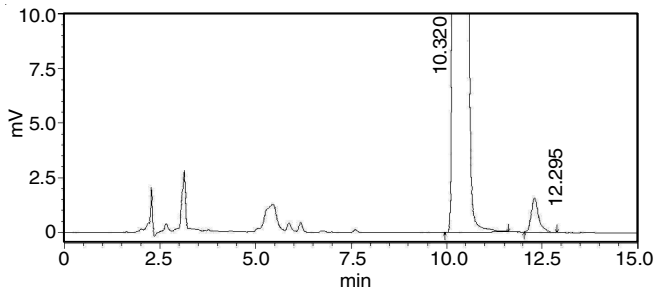

Fig. 6. Chromatography of carbamazepine with 15 min sonication

reducing the molecular interaction. This permits the drug the possibility of getting attached to any other molecule during chemical reactions. The increase in peak area with sonication time indicates that the drug once dissociated does not tend to reunite. Hence, retaining carbamazepine in its intermediate state and then attaching it with any of the drug delivering agent could only be feasible. In contrast to lamotrigine, carbamazepine possess only one nitrogen and amino groups. Further it has three benzene rings. One additional peak will be eluted in carbamazepine with 15 sonication sample and retention time is 12.295 min in Fig. 6.

Topiramate and its crystal structure: Topiramate is structurally distinct from both lamotrigine and carbamazepine. It possesses three benzene rings with a bulk sulfamate substituted monosaccharide, no exclusive nitrogen and only one amino group. This may be the reason for the previous workers [9-11,23,24] to choose topiramate for methylation. The retention time, fluctuate slight around 2.1 (Table-3). The peak area and tailing factor slightly increases with sonication time (15 min) (Figs. 7 and 8). This clearly indicates the possibility of allowing the weakly interacted bond to be easily mutilated. This helps to attach the desired drug delivering agent to desired position. Unlike lamotrigine and carbamazepine, topiramate possess only one NH_2 and in fact no nitrogen at all.

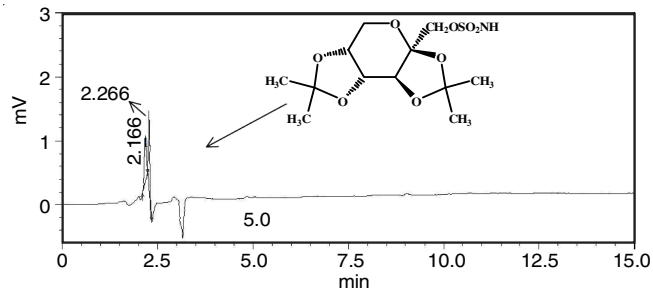

Fig. 7. Typical chromatography of topiramate without sonication

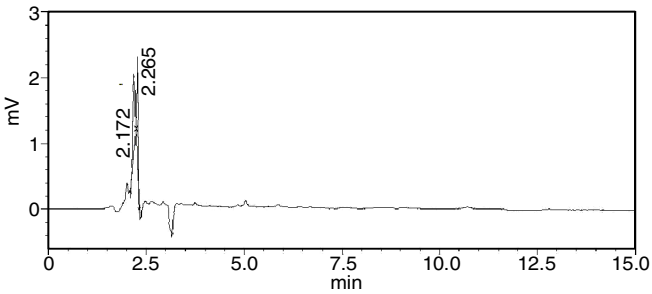

Fig. 8. Chromatography of topiramate with 15 min sonication

The crystal structure elucidated through X-ray crystallography-SHELX software is presented in Fig. 9. The formation of cavitations is clearly seen. The presence of complete structure of perfluorodecalin is not completely visible and they are supposed to be non-bonded in C2-C3 positions. Though the attachment is not complete, the possibility is very much evident. There is no bond formation between sulphur and carbon atom indicating the possible position of attaching the bulk structured perfluorodecalin and to act as a delivering agent.

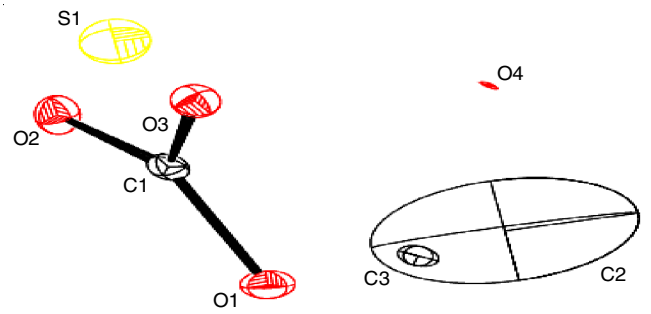

Fig. 9. Typical crystal structure of topiramate + perfluorodecalin crystal evolved through X-ray crystallography

TABLE-3
CHROMATOGRAPHIC DETAILS OF TOPIRAMATE

Sample name	Retention time	Area	Tailing factor	Theoretical plane
Topiramate without sonication	2.166	2817	1.115	5868.472
	2.266	2688	1.592	13636.102
Topiramate with 15mints. sonication	2.172	4600	1.095	5709.986
	2.265	3679	1.433	14559.802

Conclusion

The dissolution nature of these drugs with respect to time of sonification effect has been dealt. The result shows that it is easy to break the chemical bond and change the inter-molecular interaction of these drugs. Investigation reveals that the more the number of nitrogen atoms, less they are interactive and less it responds to sonication effect. Hence we prefer to safely conclude that topiramate is more effective in the study of drug delivering mechanism by attaching a delivering agent to it through sonication.

ACKNOWLEDGEMENTS

The authors acknowledge the financial assistance from Defence Research & Development Organization (DRDO), New Delhi, India (ERIP/ER/0804422/M/01/1447/Dt. 28th Sep 2012).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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