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Elimination of Chromium (Vl) from Industrial Effluent through the Utilization of Water Hyacinth weed

Anuradha Tiwari[1], Nand Lal[2], Ashish Kr Singh[3], Tamanna Begam[4], Neelam Pal[5]

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Abstract

Biosorption is a physico-chemical binding of a substance to biological material. Water hyacinth has been successfully utilized for the removal of chromium (VI) from waste water samples. The plants were divided in to two part (i) root (ii) foliage. Each part was dried at 75⁰C for 48 hours and then its weighed was taken. The tissue samples weight then ash in the muffle-furnace at 350⁰C for 5 hours. Ash samples were digested with acids (HNO_3, HCl) and diluted with 100 ml of distilled water. The detection of chromium (VI) carried out by Atomic Adsorption Spectrophotometer (AAS). This method of removing chromium from water is cost-effective, eco-friendly and has been shown to be effective in laboratory studies.

Keywords: Chromium; Biosorption; Water Hyacinth; Effluent.

INTRODUCTION

India is a major contributor to the industrial[1] effluent problem[2] in the region, with many of these facilities releasing large amounts of waste water containing Cr (VI) into the surrounding environment. Two tanning methods are used in leather tannery. Vegetable tanning or chrome tanning. Approx 90% of tanneries around the world today use salts of trivalent chromium (chromium (III) hydroxide sulphate) for tanning. The main hazards related to chromium (III) can oxidized to chromium (VI) at very low pH values when oxygen is present. Chromium (VI) is a toxic metal[3] that can be harmful to both humans and the environment if released into the water sources in excessive amounts.

In Uttar Pradesh particularly in and around Kanpur upto 70-80 percent tanneries are located on the bank of river Ganga.[4] In Kanpur, tanneries generate large amount of waste water as a result of the leather processing operations[5], which can contain high levels of chromium (VI). Chromium (VI) is widely used in various industries, such as leather tanning, wood preservation and electroplating, which can lead to its release into the environment in the form of effluent. Tanneries must reduce chromium level in their effuents.[6] The ability of biological materials[7] to concentrate pollutants, often too many thousands of time the level in the

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surrounding environment has long been known.[8] The use of water weeds, such as water hyacinth, to remove chromium from tannery effluent is a highly effective and sustainable solution.

To mitigate the negative impacts of chromium (VI)[9] on the environment, various methods have been developed for removing it from effluent. One such method is the use of water weeds, such as water hyacinth[10], to absorb chromium (VI) from the water. Water hyacinth is a highly efficient and low-cost method for removing chromium (VI) from effluent. This plant has the ability to absorb and accumulate heavy metals, including chromium (VI), from the water due to its high biomass[11] and extensive root system. The absorption process is a result of the interaction between the heavy metal ions and the organic compounds in the water hyacinth. Studies have shown that water hyacinth is highly effective in removing chromium (VI) from water, with removal efficiencies of upto 95%, observed in some cases. Further more, water hyacinth has a relatively fast removal rate, making it an ideal method for removing chromium (VI) from effluent in real-time. In addition to its removal efficiency, water hyacinth is also a sustainable method for removing chromium (VI) from effluent. The plant is non-toxic, renewable[12] and can be grown easily in a variety of water sources. More over, water hyacinth is a versatile plant that can be used for other purposes, such as for bio-fuel production[13] and as a source of food for livestock. So, water hyacinth is an effective, low-cost and sustainable method for removing chromium (VI) from effluent. By utilizing the absorbent properties of this water weed, industries[14] can reduce the release of chromium (VI) into the environment and help to protect both human health[15] and the environment.[16] The suitability of this method will be tested for industrial use. Biosorption of heavy metals will studies on water weed (water hyacinth) for removal of Chromium. The effect of pH and uptake time will be investigated to achieve maximum absorption.

METHODOLOGY

The tannery effluent obtained from the tanneries at Jajmau in Kanpur bank of Ganga River. Plant weighed approx 5 to 7 Kg allow growing in 5 plastic containers, which have 20 litres capacity.

AR grade chemicals use in acids digestion (HNO3, HCl). The ratio of water and effluents are given in the table 1. pH of tannery effluents was in between 7 to 8 approx.

Table 1: Preparation of Sample

Sample	Water (%)	Effluent (%)
1	0	100
2	50	50
3	80	20
4	90	10
5	100	0

After 15 days observation the plant were divided in root and foliage (bunch of leaves). Root and foliage both are dried for 48 hours at 75ºC. Dried plant tissue were grounded with a mill tissue sample weighed approx. 0.3 gm were ash in a muffle furnace at 350 ºC for 5 hours.

These ash samples were digested with acids (HNO_3, HCl) and diluted with 100ml of distilled water digested plant samples and water were analyzed for chromium by atomic absorption spectrophotometer. The pH of the effluents which was taken from tanneries is near to neutral or basic in nature.

RESULT AND DISCUSSION

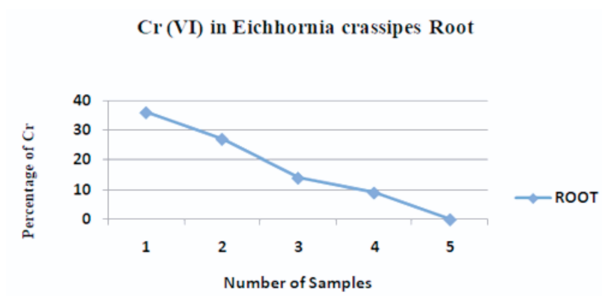

Fig. 1: Presence of Chromium in different samples of EC plant root

Fig. 1. Shows that in sample no. 1 (100% effluent) absorb 36 %, sample no. 2 (50% effluent) absorb 27%, sample no. 3 (20% effluent) absorb 14% and sample no. 4 (10% effluent) absorb 7% Cr (VI).

It means that the samples which have higher

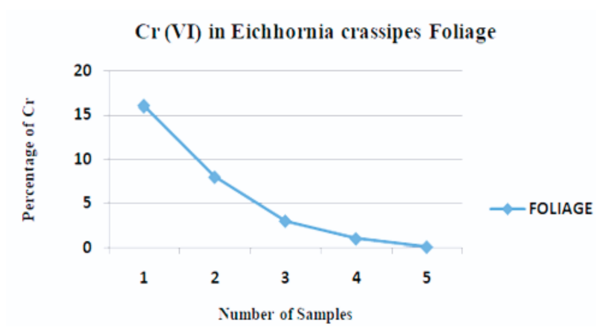

Fig. 2: Presence of Chromium in different samples of EC plant foliage

concentration show higher percentage of Cr (VI) absorption. This indicates that the Cr (VI) is absorbed by root of waterweed Eichhornia crassipes (EC).

Similarly, in *Fig. 2* absorption of Cr (VI) also observed by foliage of water weed Eichhornia crassipes (EC). Presence of Cr (VI) in sample no. 1 (100% effluent) absorb 16%, sample no. 2 (50% effluent) absorb 8%, sample no. 3 (20% effluent) absorb 3%, sample no. 4 (10% effluent) absorb 1% Cr (VI).

It reveals that maximum Cr (VI) absorbed by roots than the foliage. The mechanism of biosorption in hydrosphere plant are same as lithosphere. The oxygen released by photosynthesis of water hycinth gets dissolved into water. This dissolved oxygen is used by water hycinth plants for respiration. This water hyacinth can tolerate heavy metals in the substrate up to a threshold concentration. In addition to the ability to tolerate high concentrations of Chromium these plants can actively take them up and accumulate them in their parts. The removal of Cr (VI) metal by water hyacinth (EC) plant root are maximum absorption then the foliage. Thus with the help of this scientific work we can use the water weed EC in removing of Cr (VI) from tannery effluent.

CONCLUSION

The toxic metals pollution is global issues. The toxic metals are most frequently identifying problems in the aquatic ecosystem. Excellent removal capabilities of the water hycinth (EC) for several metals of environmental concern were known. These result indicated the possibility of using water weed to removal of chromium in tannery effluents which pose an environmental problem. Water hyacinth has been used successfully to remove heavy metal. This proves removal of chromium for industrial effluent is very effective, rapid and cheap.

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Interesting Nuclear Magnetic Resonance studies of some N, N-bis(2-methoxyethyl) substituted Benzamides

Mamta Sharma[1], Sujeet Kumar Mewar[2]

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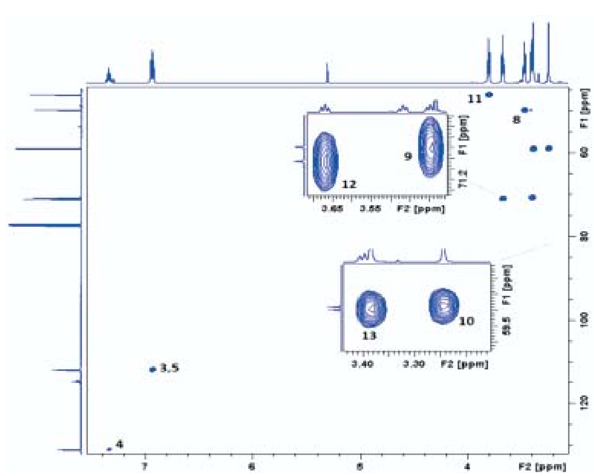
Abstract

DEET (*N,N* Diethyl m-toluamide) and DEPA (Diethyl phenyl acetamide) are synthetic compounds and proven potent insecticide and repellent respectively. In the search of effective mosquito repellent, different derivatives of DEPA or substituted benzamides were synthesized and their NMR analysis was carried out at low temperature. Sterically Crowded Bis (2-methoxyethyl) substituted Benzamides possess low rotational barrier and are floppy at room temperature. Alkyl arms attached to nitrogen become magnetically nonequivalent even at low temperature. Di ortho Substitution in benzamides enhanced hindered internal rotation and resulted splitting in methylene proton signals. The effect of substitution in benzene ring, on the splitting of methylene proton NMR is very well explained and the complete NMR data of substituted benzamides is described in this study for reference purpose.

Keywords: Proton NMR; Carbon NMR; Heteronuclear Single Quantum Coherence Spectroscopy (HSQC); Rotamers.

Synopsis

Herein, Synthesis, NMR characterization of nineteen substituted benzamides at low temperature and study of formation of rotamers are reported. The results suggest that molecules are floppy in nature and form number of rotamers at room temperature. The ortho substitution on benzene ring possess restriction in rotation and reduce the formation of rotamers and causes clear splitting of methylene signals on NMR Chemical shift scale.



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INTRODUCTION

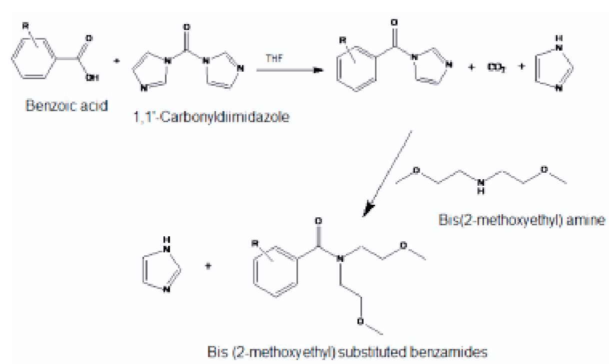
Mosquitoes are the major source of vector borne diseases like Malaria, Dengue and Chikungunya etc. Globalization, frequent movement of travelers are one of the causes of these fast spreading diseases. In-spite of lot of development in the field of insect or mosquito repellent, a mosquito free environment still a challenge for developing countries. Its complete eradication seems merely impossible and it has become a horrifying dream for scientific community.[1] Therefore continuous research towards the effective control of population of mosquitoes is a prime requirement of present world. Currently much emphasis is being given on the insect repellent research and taken it as an alternative of the insecticides. This is all because of the toxic effects of the latter on the human being and environment, and being a less toxic, former has potential to apply on skin and to prevent the stored products by repelling insects. In this way insect repellents effectively help to prevent and control the insect borne diseases. In recent past out breaks of chikv fever[2], yellow fever[3] and dengue[4] once again strongly fixed the requirement of mosquito repellents. So far, this field has not been explored fully, hence there is a need to develop new effective insects repellent which should be nontoxic and more environmental friendly. Various insect repellents have been discovered so far like Dimethyl phthalate (1929), Indalone (1937), military formulation 6-2-2, DEET (1953), DEPA (1990), n-methylneo decanamide (1998), 1-piperidine carboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester (Yap *et al.*, 2000). Among these DEET (N, N Diethyl m-toluamide) is a well known effective and versatile mosquito repellent. In this connection, DEPA (Diethyl phenyl acetamide)[5, 6] was reported as cockroach/multi insect repellent long back. Some naturally occurring sources are also reported as repellent to certain insects while they may act as insecticide for others. The natural compound 'Repel Lemon Eucalyptus' has been reported as more active in comparison to synthetic DEET, when the activity of different synthetic, chemical and herbal repellents were compared.[7-9] Most of the reported insect repellents are having benzamide linkages, so keeping this as target, number of benzamide derivatives were synthesized with different substitution in the benzene ring and their efficacy as mosquito repellent is under evaluation.

This paper describes the complete NMR structure elucidation of substituted benzamides, synthesized by simple and easy method. The ^1H and ^{13}C NMR assignment of 19 benzamides, having single and double substitution in their aromatic ring presented very interesting changes in splitting pattern of methylene proton signals with substitution in benzene ring. To the best of our knowledge and literature surveyed, no unambiguous NMR assignment for both the nuclei ^1H and ^{13}C is available in the literature. These data obtained by a combination of 1D ^1H, ^{13}C and 2D ^1H-^1H Correlation Spectroscopy (COSY), Heteronuclear single quantum coherence spectroscopy (HSQC), Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC), Two Dimensional Nuclear Overhauser Effect Spectroscopy (2D-NOESY) NMR experiments.

EXPERIMENTAL METHODS

2.1 Synthesis

All the substituted benzamides (1-19) were synthesized as shown in scheme 1. Substituted Benzoic acid was dissolved in THF solvent. After this 1, 1′ -Carbonyldiimidazole reagent[10] was added to make the inter mediate and kept for 10 to 15 minutes at RT. The evolved gas carbon dioxide during the reaction was removed by applying vacuum using rotavac. Bis(2-methoxyethyl) amine was added to the reaction mixture and kept for stirring for 10 to 15 minutes. Reaction was monitored by TLC and GC up to the completion. Reaction mixture was neutralized with 10% Sodium bicarbonate solution. The organic layer was separated out and THF was



Scheme 1: Synthesis of *N,N*-bis(2-methoxyethyl) substituted benzamides entries (1-19)

removed by vacuum to obtain the desired amide.

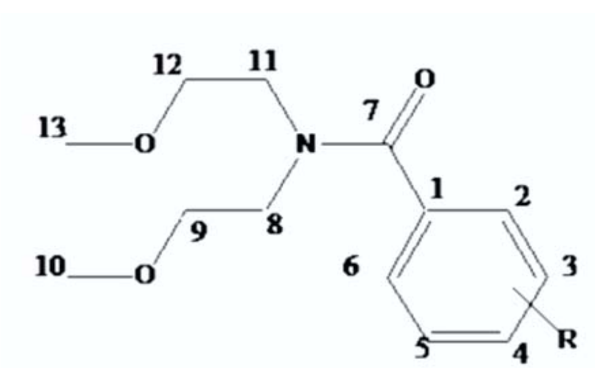
2.2 Materials and Physical measurements

The chemical shifts of ^1H and ^{13}C nuclei were referenced keeping spectrometer reference frequency at 0 ppm (SR=0). The synthetic Bis(2-methoxyethyl) substituted benzamide derivatives

1–19 were dissolved in CDCl3 for NMR analysis. Spectra were scanned by taking 80 µl concentration of compounds in the 500 µl of solvent in 5 mm wilmad quartz NMR tube. All the NMR data were recorded on a Bruker Avance III 600 MHz spectrometer system (14 T; Ultra shield plus, Bruker Germany) at topspin 3.1 version and temperature range 288 to 293K using QNP cryoprobe. The ^{1}H NMR experiments were carried out by keeping relaxation delay 1 sec., spectral width 20ppm, and 90° pulse 5.25 µsec., pulse programme zg30, and fid resolution 0.366 Hz. For the ^{13}C NMR experiments the relaxation delay, spectral width and 90° pulse were kept 2s, 239 ppm, and 9.47 µsec. respectively. 2-dimensional experiments including COSY, HMBC were performed by acquiring 2048 data points for t2 and 128 data points for t_1 while in HSQC t_2 and t_1 were adjusted at 1024 and 256 respectively. The long range coupling time for HMBC was 70 ms prior to Fourier transformation, zero filling of 2k and a sine squared bell window function were applied using topspin model 3.1. *Noesy* experiments were performed with 16 acquisitions for 256 increments in F1 and 1024 data points in F2. The spectral width in both dimensions was 10 ppm. Gradient based Phase sensitive noesys pulse program was used with mixing time 80 msec to 300 ms, 2s relaxation delay at temp 290° K.

RESULTS AND DISCUSSION

Amides have been studied considerably, more effectively, by the use of Nuclear Magnetic Resonance Spectroscopy. It was realized that unambiguous ^{1}H and ^{13}C NMR assignment should be available in the literature for substituted benzamides, synthesized in laboratory. The structure of all the compounds and numbering used in proton and carbon nuclei assignment are shown in scheme 2. Resonance assignment of ^{1}H and ^{13}C is done based on the multiplicity pattern, δ and J, which is further confirmed by 2D NMR experiments like COSY, HSQC, HMBC and NOESY.



Entry	R*	Entry	R*
1	0	11	2,6-OMe
2	4-Cl	12	3,4-OMe
3	2-OMe	13	2,6-F
4	3-OMe	14	2,3-F
5	4-OMe	15	2,4-F
6	2-F	16	2,5-F
7	4-F	17	3,5-F
8	2,6-Cl	18	2-NO2
9	3,5-Cl	19	4-NO2
10	3,4-Cl		

R* = Substitution on Benzene Ring

Scheme 2. Structure of *N,N*-bis(2-methoxyethyl) Substituted Benzamides (1-19).

Proton NMR

The full characterization of benzamides containing chemical shift (δ) of ^{1}H nucleus is shown in the Table 1 and coupling constant (J) of ^{1}H nucleus is given in Supplementary information. The ^{1}H NMR spectra of entries 1-19 showed clearly resolved two distinct regions, temperature independent aromatic region and temperature dependent aliphatic region with $A_2B_2C_2D_2$ spin system for methylene protons. Entries 3/18 presented $A_2BB'CC'D_2$ / $A_2B_2CC'DD'$ systems for alkyl protons respectively. As far as the proton NMR spectrum of precursor Bis 2-methoxyethyl amine is concerned, this exhibited single peak for two methoxy groups at δ 3.36 ppm, while this signal further segregated into two for protons of both the methoxy groups after the formation of amide, resulting shifting of one signal towards the high field and other shifts at low field. In the similar manner, both the methylene protons attached to nitrogen and oxygen exhibited same δ value at 2.80 ppm and 3.50 ppm for protons of N-$(CH_2)_2$ and (O-$CH_2)_2$ groups respectively in amine, but these appeared as four well resolved signals, representing four separate methylene groups of aliphatic chain, in benzamides (Table 1, Fig. 2). This confers that protons of all four methylene groups of both the aliphatic chains attached to nitrogen and oxygen become magnetically different after formation of benzamides. In some cases this separation was observed at low temperature. This is well documented that internal rotation around single bond of amide (CO-N) is some what restricted due to partial double bond character of bond and this rotation is affected by various substitutions of amide nitrogen and carbon. The internal rotation around the bond normally occur via two transition states; anti TS_1 or syn TS_2 and causes rotamers formation in amides.[11-23]

Table 1A: [1]H NMR Chemical shifts of *N,N*-bis (2-methoxyethyl) Substituted Benzamides for entries 1 to 19 in ppm. (for 2H to 6H Protons)

Entry	R	2H	3H	4H	5H	6H
1	-	7.41 m	7.38 m	7.39 m	7.38 m	7.41 m
2	-	7.40 d	7.39 d	-	7.39	7.40
3	3.83 s	-	6.91 dd	7.33dt	6.98 dt	7.22 dd
4	3.84 s	6.98 t	-	6.93 ddd	7.31 t	6.99 td
5	3.85	7.42 d	6.92 d	-	6.92 d	7.42 d
6	-		7.06 ddd	7.32 dt	7.15 dt	7.34
7	-	7.42 dd	7.04 t	-	7.04 t	7.42 dd
8	-		7.33 d	7.26 dd	7.33 d	-
9	-	7.34 d	-	7.37 t	-	7.34 d
10	-	7.58 d	-	-	7.48 d	7.31 dd
11	3.82	-	6.58 d	7.28 t	6.58 d	-
12	3.87, 3.88	7.06 d	-	-	6.84 d	7.03 dd
13	-	-	6.93 dd	7.34 tt	6.93 dd	-
14	-	-	-	7.18 dtd	7.12 dt	7.11m
15	-	-	6.83 dt	-	6.90 dt	7.33 dt
16	-	-	7.05 m	7.06 m	-	7.07 m
17	-	7.00 m	-	6.85 tt	-	7.01 m
18	-	-	8.22 dd	7.57 dt	7.72 dt	7.47 dd
19	-	7.58 d	8.21 d	-	8.21 d	7.58 d

S: singlet, m: multiplet, t: triplet, td: triplet of doublet, d: doublet, dd: doublet of doublet, ddd: doublet of doublet of doublet, tt: triplet of triplet, dt: doublet of triplet, dtd: doublet of triplet of doublet.

Table 1B: [1]H NMR Chemical shifts of *N,N*-bis(2-methoxyethyl) Substituted Benzamides for entries 1 to 19 in ppm. (for 8H to 13H Protons)

Entry	8H	9H	10H	11H	12H	13H
1	3.54,bs	3.44,bs	3.28,s	3.78,bs	3.67,bs	3.41,s
2	3.53,bs	3.45,bs	3.31,s	3.76,bs	3.68,bs	3.40,s
3	3.40,bs	3.39,3.35,bs	3.23,s	3.74,3.82,bs	3.68,t	3.41,s
4	3.55,bt	3.46,bt	3.30,s	3.77,bt	3.69,bt	3.41,s
5^	3.59,bs	3.47,bs	3.31,bs	3.76,bs	3.68,bs	3.40,bs
6	3.44,t	3.36,t	3.20,s	3.78,bs	3.64,t	3.35,s
7	3.49,bs	3.41,bs	3.24,s	3.71,bs	3.63,bs	3.34,s
8	3.43,t	3.47,t	3.29,s	3.85,t	3.73,t	3.40,s
9	3.51,bt	3.45,bt	3.30,s	3.74,bt	3.66,bt	3.39,s
10	3.52,s	3.45,s	3.30,s	3.74,s	3.66,s	3.39,s
11	3.39,t	3.38,t	3.25,s	3.80,t	3.70,t	3.42,s
12^	3.58,bs	3.47,bs	3.29,bs	3.71,bs	3.65,bs	3.36,bs
13	3.47,t	3.40,t	3.24,s	3.78,t	3.67,t	3.38,s
14	3.46,t	3.40,t	3.23,s	3.77,bs	3.66,t	3.38,s
15	3.44,t	3.37,t	3.22,s	3.75,bs	3.62,t	3.36,s
16	3.49,t	3.42,t	3.27,s	3.78,bs	3.67,t	3.39,s
17	3.52bs	3.46,bs	3.31,s	3.75,bs	3.67,bs	3.39,s
18	3.41,bs	3.42,bs	3.26,s	3.96,3.63,bs	3.74,3.82,bs	3.44,s
19	3.44,bt	3.39,bt	3.24,s	3.73,bt	3.64,bt	3.35,s

bs=broad signal, bt=broad triplet, ^=broad merged six single peaks with joined base at 10°C t=triplet.

Plethora of papers witnessed that the barrier of rotation is governed by various factors like solvent polarity,[24-30] temp,[20,31-33] molecular phases,[20,26] substitution on carbonyl Carbon,[20,34-39] amide nitrogen and carbine of benzene ring attached to carbonyl.[24,33] Reports deduce that to attain the optimum possible stable ground state geometry, amides possess twist around the C_{ar}-C(O),[40] C(O)-N[41] and N-CR[40,41] bonds. The steric repulsion between alkyl substitutes of amide nitrogen further destabilizes moleculeand lead to frequent changes in alkyl chain geometry with respect to the benzene ring plane. In our case N, N-dialkyl benzamides are sterically crowded, having long alkyl chain and bulky substitutions on benzene ring, hence these molecules always try to adopt the twisted confirmation (Fig. 1a) by twisting C_{ar}-CO and N-CR bond axis.[42] This is clearly visible by changing splitting pattern of proton signals of methylene groups with placement of substitution in benzene ring at low temperature.

In all benzamides, entries 1 to 19, proton signals of two methylene groups attached to nitrogen (N-$(CH_2)_2$) (H-8 and H-11, δ range is 3.38-3.59 ppm and 3.63-3.96 ppm respectively) are more deshielded in comparison to the proton signals of their respective adjacent partners, two methylene groups attached to oxygen (O-$CH_2)_2$ (H-9, and H-12, δ range is 3.35-3.47 ppm and 3.62-3.82 ppm respectively) and appeared at low field always (Table 1). This low field shifting of N-$(CH_2)_2$ group may be attributed to the carbonyl group adjacent to Nitrogen.

Cis-trans

Moreover within the two N-CH_2 groups, H-8 protons are always shielded by 0.13ppm to 0.42 ppm than the H-11 protons in all the entries (Scheme 2, Table 1). The available literature reveals that protons anti to carbonyl oxygen are always shielded in benzamides.[34-38],[43,44] The literature values of protons of different substituted group of benzamides are given in supplementary information. Our observation is congruent with the aforementioned findingsof literature. H-8, trans to carbonyl group is shielded than H-11 [Cis to C(O)] for all the entries. The magnitude of shielding of H-8 protons is high for mono and di ortho substituted benzamides than other entries, which is 0.42 ppm (entries 8 & 11), 0.39 ppm (entries 18 & 3), 0.34ppm (entry 6) and 0.30 ppm (entries 13 to 16). (Table 1). The preponderance of the conformer in solution is further suggested by 2D NOESY experiment at 20ºC in $CDCl_3$ solvent. Which established that H-8 and H-9 methylene protons remain away from carbonyl group and hence near to protons of benzene ring in space. The 3D spatial hydrogen interaction provided by 2D NOESY is shown in Fig. 1b, exhibiting correlation between H-8/H-9 and H-6/ H-3 protons respectively.

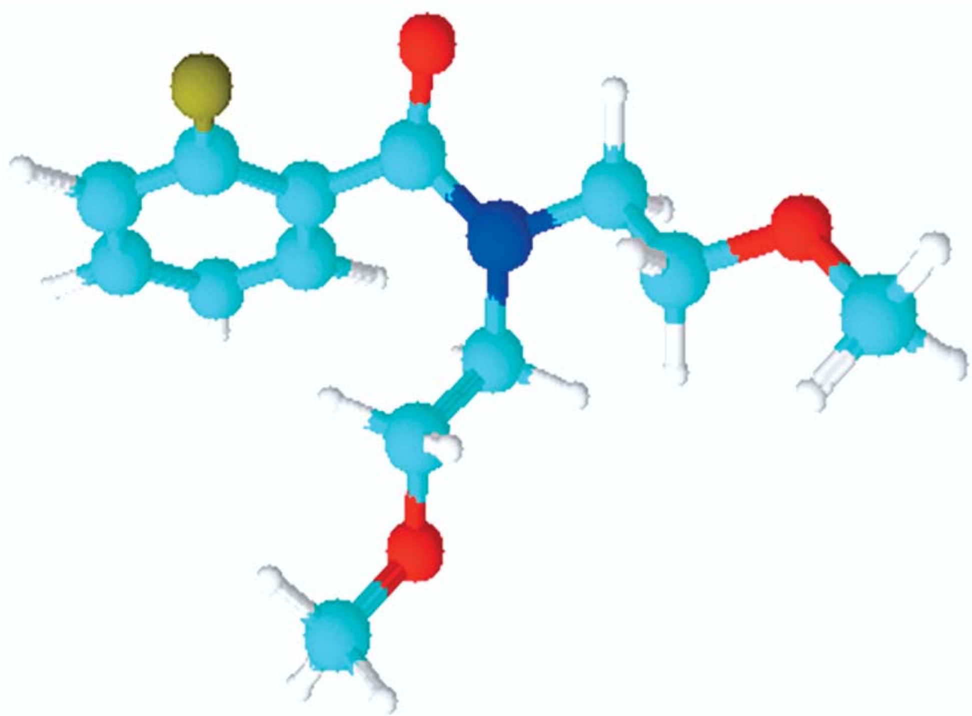


Fig. 1a: 3D Structure of 2-fluoro-*N,N*-bis (2-methoxyethyl)benzamide (entry-6) showing connectivity of protons in Space

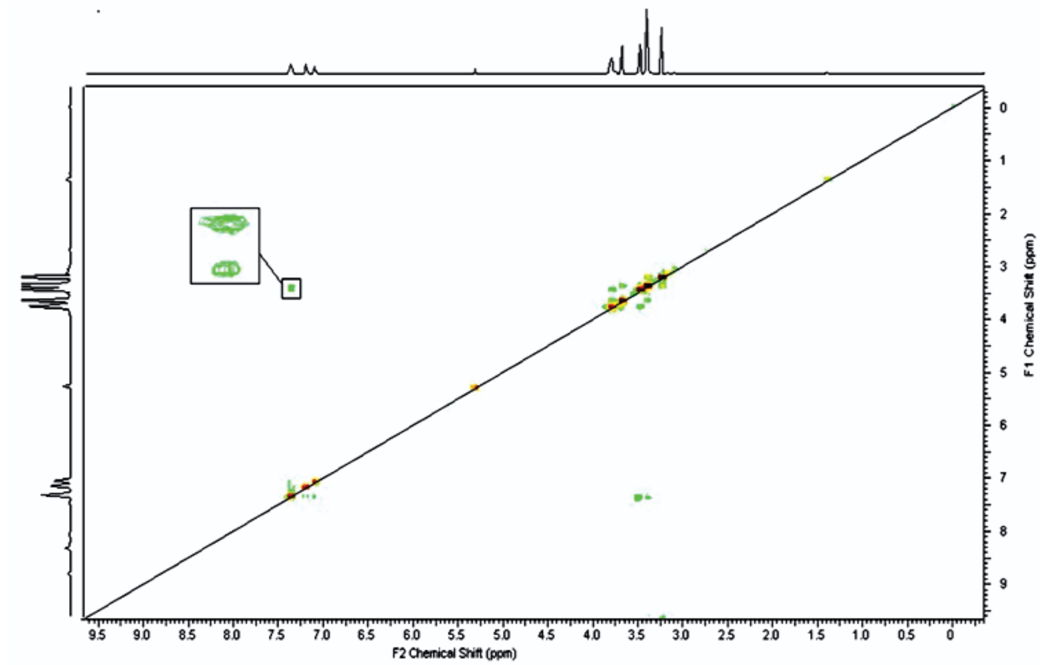


Fig. 1b: H-[1]H, 2D-NOESY Spectrum of Benzamide entry 6 Showing Connectivity of H8/H9 with H-6.

Chemical shift sequence of aliphatic chain

Now discussion is made on the δ sequence pattern of methylene and methyl signals on the chemical shift scale, the sequence in which these are appeared at NMR Chemical shift scale. In general proton δ of most of the benzamides (entries 1, 2, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16, 17, and 19) methylene and methyl protons appeared in the order NCH_2 (H-11), OCH_2 (H-12), NCH_2 (H-8), OCH_2 (H-9), OCH_3 (H-13) and OCH_3 (H-10) starting from low field to high field. Under lined fonts are shown to differentiate one aliphatic chain from other in benzamides. The chemical shift sequence of protons is clearly demonstrated in the graph (Fig. 2), where δ values of methylene and methyl protons are plotted against their respective entries. This clearly demonstrated the δ trend, in which H-8, H-9 and H-10 of one alkyl chain are more shielded than H-11, H-12 and H-13 of another alkyl chain, when compared vis a vis with in molecule (Graph, Fig. 2). This distinctly shows that one alkyl arm, cis to the carbonyl oxygen is completely deshielded than the other arm, trans to carbonyl. Di ortho substituted chloro benzamide entry 8, follows slight variation in chemical shift sequence, which is H-11, H-12, H-9, H-8, H-13and H-10 towards high field, where H-8 (δ 3.43 ppm) is slightly shielded than H-9 (δ 3.47 ppm) (Table 1). Entries 3, 11, 18 showed different patterns and exhibited complete true shielding of one arm (H-8, H-9, H-10) than other (H-11, H-12, H-13) irrespective of methyl and methylene groups. H-8 (3.40 ppm), H-9 (3.39 ppm), H-10 (3.23ppm) protons of benzamide entry 3 are shielded than their respective H-11, (δ 3.77ppm) H-12, (δ 3.68 ppm) and H-13 (δ 3.41 ppm) protons. Similarly H-8 (3.39ppm), H-9 (3.38 ppm), H-10 (3.25ppm) of benzamide entry 11 are more shielded than their respective H-11, (δ 3.80ppm) H-12, (δ 3.70ppm) and H-13 (3.42 ppm) protons. Entry 18 follows δ sequence pattern H-11, H-12, H-13, H-9, H-8, and H-10 towards high field, where H-8 (δ 3.41ppm) protons are slightly shielded than H-9 (δ 3.42 ppm) by 0.01 ppm. These all molecules are having bulky substitution at ortho position that is methoxy (entries 3, 11), chloro (entry 8) and nitro (entry 18) as mono and di substitution. Hence presence of the bulky substitution at ortho position brings variation in chemical shift sequence of methylene and methyl protons of aliphatic chain.

Splitting of Proton Signals

The broadening and splitting of the proton signals of methylene group is dependent on the rotation around the C_{ar}-C(O), C(O)-N, and N-C_{alkyl} bonds and substitutions in amide benzenering. All the benzamides showed broadening/hump for the proton signals at room temperature therefore NMR spectra were recorded at low temperature ranging from 20°C to 10°C for all the compounds and resulted splitting and separation of signals for methylene protons of benzamides. Ortho substituted benzamides exhibited clear splitting/ distorted triplet for methylene proton signals which is generated due to hindered rotation, while

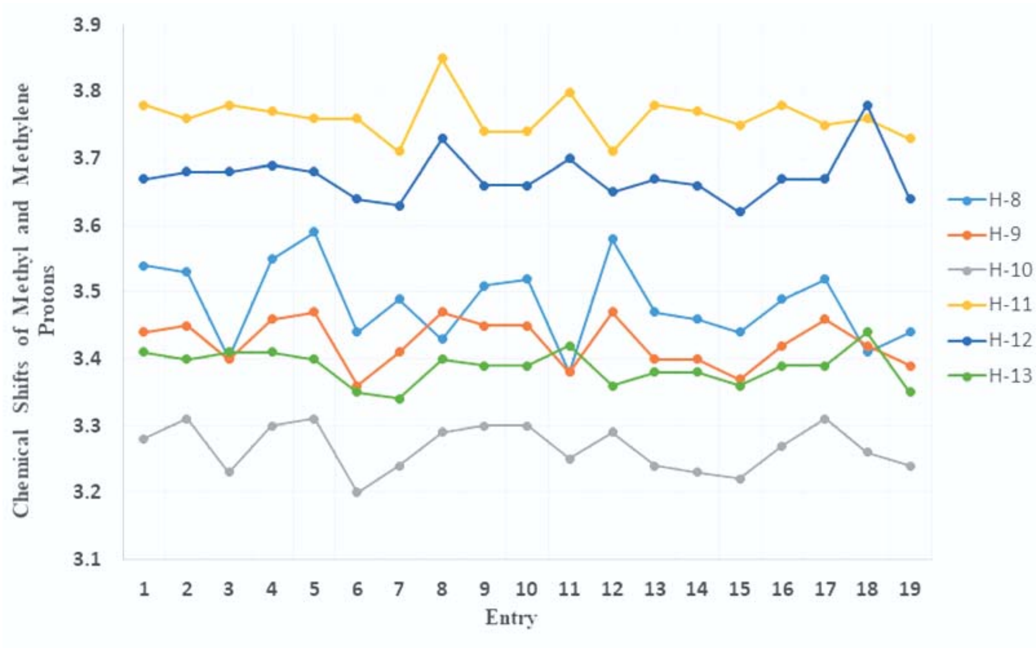


Fig. 2: Graph depicting the chemical shift trend in methyl and methylene protons in entries 1 to 19

for other pera/meta substituted molecules, unsplit singlets were obtained even at 10°C for methylene protons. This shows that barrier of rotation for ortho substituted benzamides is higher than the pera/meta substituted benzamides in the temperature range 15°C to 20°C, which leads to the more rotamers formation for pera/meta substituted benzamides and resulted broad humps/singlets for methylene proton signals on NMR Scale. On the basis of available literature data (given as supplementary information) on free energies of activation of the amide bond rotation around (CO)-N bond for the amides/benzamides,[20,34-48] it can be concluded that at RT the barrier of rotation decreases with the increasing size of alkyl groups on nitrogen and carbonyl carbon of amide functionality[20,49] which leads to destabilization at ground state, intern facilitate the rotamers formation fast, and causes broadening of signals also. But on increasing the bulkiness at the ortho position of phenyl ring the barrier of rotation is increased around the amide bond and rotation becomes restricted which leads to the stabilization and facilitate the clear splitting. Therefore benzamides with di ortho, mono ortho, and no ortho substitution showed clear triplets (due to restricted rotation), incomplete splitting pattern (due to intermediate rotation), and unsplit signals (due to fast rotamer formation) for methylene proton signals respectively (Fig. 3). The electron with drawing/donating nature of substituents and their position on benzene ring influence the splitting of methylene proton signals also. The ^1H spectra showing variation in NMR signals with temperature is given in supplemenatry information. After examining the proton spectra of all compounds meticulously it was concluded that Di ortho substituted benzamides entries 8, 11 and 13 presented very clear splitting pattern with little roofing, for protons of all four CH_2 groups. The proton spectra of mono ortho fluorine substituted benzamides entries 6, 14, 15 and 16 appeared as 1+3 pattern (one unsplit signal + 3 split signals) i.e. one CH_2 (H-11) as broad signal while other three CH_2 (H-8, H-9, H-12) appeared as distorted triplet as shown in the fig. 3. Molecule 3 with strong electron donating methoxy group at ortho position exhibited 3+1 (unsplit/ split signals) pattern. Splitting was observed only for H-12 (3.68 ppm), while both the geminal protons of H-9 (3.393, 3.353 ppm) and H-11 (3.74, 3.82 ppm) exhibited two separate δ with broad signals. Mono ortho nitro substituted benzamide 18 presented broad resonances for all the methylene protons and separation in resonances of geminal protons of H-11 and H-12 appeared at δ 3.968, 3.637 ppm and 3.74, 3.82 ppm respectively. Rest of the benzamides 1, 2, 4, 5, 7, 9, 10, 12, 17 and 19 showed unsplit broad singlets for all protons of methylene groups. Hence when rotamers formation fast and slow on NMR chemical shift scale the broadening and splitting of signals occur respectively. The three bond ^1H-^1H coupling for methylene protons was found 5.5 Hz in distorted triplets of benzamides. Similar type coupling magnitude in X-CH_2-CH_2-Y type systems is reported.[50]

After overlapping the aromatic regions of pera substituted benzamides 2 and 5 (given as supplementary information) it was concluded that chlorine exerts more deshielding on ring protons H-3/5, than fluorine. (Table 1).

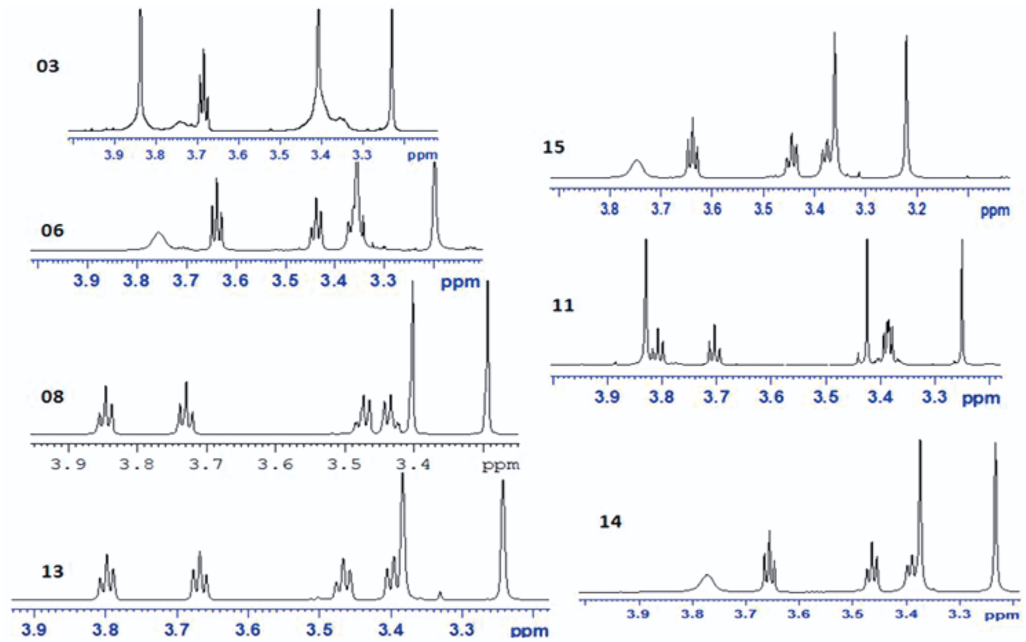


Fig. 3: Expanded ^{1}H NMR Spectra of *N,N*-bis(2-methoxyethyl) substitued benzamides for entries 3,6,8,11,13,14,15, in $CDCl_3$ showing spitting patterns of methylene protons in the chemical shift range 3 to 4 ppm.

42D-COSY

The three bond proton connectivity for protons of N-CH_2 and O-CH_2 is confirmed by 2D-^{1}H-^{1}H COSY (Homonuclear Correlation Spectroscopy) NMR experiment (Fig. 4), which deduced that adjacent proton signals are of the adjacent methylene groups and coupling with each other. Protons H-11/H-8 are coupling with H-12/H-9 protons respectively and showing correlation contours.

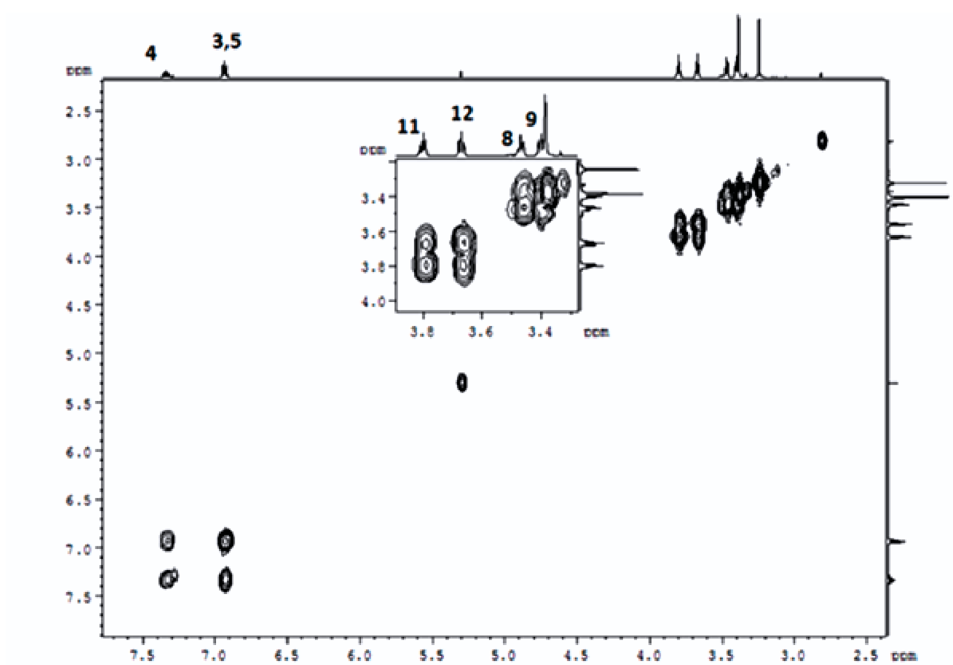


Fig. 4: ^{1}H-^{1}H COSY Spectrum of entry 13 showing three bond Connectivity for adjacent protons of methylene groups in $CDCl_3$

Carbon NMR

The full characterization of compounds containing chemical shift (δ) of $^{13}C\{^1H\}$ nucleus is shown in the Table 2 and coupling constant (J) is given in 2b table. The interpretation of ^{13}C NMR signals of *N,N*-Bis-(2-methoxyethyl) substituted benzamide entries 1 to 19 was done with the aid of 2D heteronuclear correlation (HSQC) experiment, which allowed the unambiguous assignments of all protonated carbon resonances. For instance the carbon resonances of all the methoxy groups can easily be assigned in the range 55.34–55.90 ppm, as well as the aromatic and aliphatic carbon resonances at 103.95-130.86 ppm and 44.98-71.30 ppm respectively (Table 2). Chemical shift of carbonyl carbon of all the compounds shifted high field in comparison to carbonyl carbon of unsubstituted benzamide entry 1 (Table 2). This chemical shift varies from 0.05 to 10.13 ppm, in all benzamides, while this variation is 3 to 10 ppm in mono and di ortho substituted compounds. Further high range 5 to 10 ppm shift belongs to only halo substitution. The maximum 10 ppm and minimum 0.05 ppm shift was obtained for entries 13 (ortho di fluoro) and 4 (pera-methoxy) respectively (Table 2). Hence it can be concluded that Carbonyl carbon is more shielded in weakly ring deactivating halo ortho substituted benzamides in comparison to the strong electron donating/ with drawing substituents, irrespective to their position in benzene ring (o/p/m). Proton and carbon single bond correlation is elaborated with the help of HSQC (Heteronuclear single quantum correlation experiment) experiment. Aliphatic carbon resonances C-8 to C-13 (δ range 44.98 ppm–71.30 ppm) presented correlations with H-8 to H-13 Proton resonances (δ range 3.968 ppm–3.20 ppm). This helped in sequencing the resonances of protons and carbons on chemical shift scale. To understand the single bond correlation of proton with carbon, HSQC spectrum of benzamide 13 is shown in Figure 5 as example, which emphasizes the correlation of C-10, δ 58.84 ppm / C-13, δ 58.94 ppm with H-10, δ 3.24 ppm / H-13, δ 3.38 ppm respectively. Simultaneously this established the other correlations also like C-11, δ 45.97 ppm /C-12, δ 70.85 ppm with H-11 δ 3.78ppm/H-12, δ 3.67 ppm and C-8, δ 49.58 ppm /C-9, δ 70.58 ppm with H-8, δ 3.47 ppm /H-9, δ 3.40 ppm. Aromatic protons H-3/5 δ 6.93 ppm, H-4, δ 7.34 ppm correlated with C-3/5, δ 111.70 ppm, and C-4, δ 130.86 ppm respectively. (Figure 5) The NMR spectra of various ortho, meta, pera, diortho, and dimeta, placed methoxy in benzamides entries 3, 4, 5, 11, and 12 showed singlet in the δ range 3.82–3.88 ppm and 55.34–55.90 ppm for proton and carbon resonances respectively. This assignment is done through single bond correlation of carbon and protons in HSQC NMR experiment.

Table 2: $^{13}C\{^1H\}$ NMR Chemical Shifts of *N,N*-bis(2-methoxyethyl) Substituted Benzamides for Entries 1 to 19 (1C to 7C).

Entry	1C	2C	3C	4C	5C	6C	7C
1.	136.79	126.86	128.37	129.22	128.37	126.86	172.31
2.	135.17	128.59	128.57	135.25	128.57	128.59	171.29
3.	126.35	155.04	110.95	130.18	120.80	127.92	169.82
4.	137.93	112.23	159.43	115.23	129.53	119.03	172.05
5.	132.29	128.84	113.61	160.32	113.61	128.85	172.27
6.	124.90d	157.94d	115.76d	128.87d	124.42d	130.86d	167.21
7.	132.76d	129.24d	115.32d	163.02d	115.32d	129.24d	171.40
8.	135.27	131.88	128.14	130.35	128.19	131.88	165.55
9.	139.26	125.74	135.06	129.31	135.06	125.74	169.53
10.	136.50	129.43	132.63	133.49	130.43	126.59	169.94
11.	115.03	156.45	103.95	130.24	103.95	156.45	167.27
12.	128.91	110.86	148.61	149.81	110.45	120.03	172.09
13.	114.57t	158.79dd	111.70dd	130.86t	111.70dd	158.79dd	162.18
14.	127.08dd	150.31dd	146.37dd	117.93dd	124.78dd	123.61dd	165.92dd
15.	121.22dd	158.40dd	104.17t	163.20dd	111.80dd	130.20dd	166.46
16.	126.11dd	158.49dd	117.08dd	117.39dd	153.99dd	115.72dd	165.87
17.	139.69t	110.52dd	162.70dd	104.71t	162.70dd	110.52dd	169.64t
18.	133.05	145.16	124.66	129.64	134.20	128.95	168.28
19.	143.03	128.27	123.59	147.92	123.59	128.27	170.14

d=doublet, dd=doublet of doublet, t=triplet, Other Carbon signals, where it is not mentioned, appeared as single peak.

Table 2: Continued $^{13}C\{^1H\}$ NMR Chemical Shifts of *N,N*-bis(2-methoxyethyl) for Entries 1 to 19. (8C to 13C)

Entry	8C	9C	10C	11C	12C	13C	OCH3
1.	49.68	70.63	58.90	45.41	70.97	58.90	-
2.	49.81	70.31	58.90	45.37	70.90	58.90	-
3.	49.13	71.05	58.76	45.60	70.97	58.94	55.52
4.	49.68	70.63	58.91	45.37	70.93	58.92	55.34
5.	49.89	70.64	58.94	45.53	70.90	58.96	55.34
6.	49.32	70.52	58.75	45.47	70.81	58.90	-
7.	49.77	70.22	58.84	45.29	70.80	58.86	-
8.	49.32	70.75	58.92	45.92	70.69	58.89	-
9.	49.90	69.87	58.93	45.32	70.82	59.07	-
10.	49.85	69.90	58.94	45.22	70.81	58.94	-
11.	49.13	71.07	58.77	45.97	71.30	58.89	55.79
12.	49.91	70.66	58.88	45.42	70.94	58.88	55.90
13.	49.58	70.58	58.84	45.97	70.85	58.94	-
14.	49.46	70.29	58.80	45.48	70.77	58.92	-
15.	49.56	70.28	58.88	45.45	70.80	58.83	-
16.	49.44	70.26	58.84	45.46	70.81	58.96	-
17.	49.70	69.89	58.94	45.20	70.77	58.95	-
18.	49.56	69.92	58.87	45.26	70.48	58.98	-
19.	49.70	69.62	58.86	44.98	70.69	58.89	-

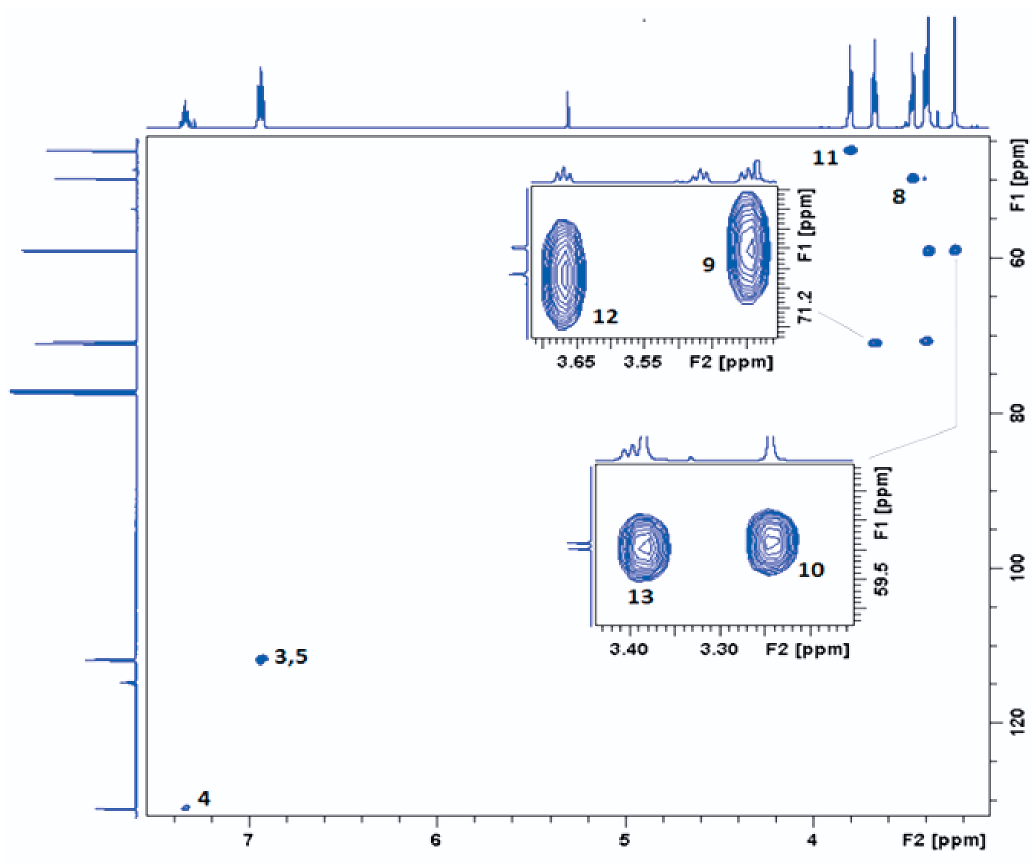


Fig. 5: 2D ^1H-$^{13}C\{^1H\}$, HSQC NMR spectrum of entry 13 in $CDCl_3$ showing single bond connectivity of Carbons and Protons

Heteronuclear Multiple Bond Correlation (HMBC)

To confirm the assignments made by the use of HSQC experiment and to assign the signals corresponding to quaternary carbons, HMBC (Heteronuclear Multiple Bond Correlation) NMR spectra of benzamides were recorded (Fig. 6). This experiment established the connectivity of carbon with neighboring protons via two/three bond. In the HMBC spectra, of all benzamides H-8 protons were long range coupled to carbon signals at δ 69.62–71.07 ppm, 44.98-45.97 ppm and 162.18-172.31 ppm (Fig. 6, Scheme 2). The first resonances were assigned as C-9, second as C-11 and third were attributed to C-7 resonance. In the similar manner H-11, δ 3.63-3.82 ppm exhibited long range connectivity with C-12, δ 70.48-71.30 ppm C-8, δ 49.13-49.91 ppm and C-7. Methyl carbons of alkyl chains C-10, δ 58.75-58.94 ppm, C-13, δ 58.83-59.07 ppm, were long range coupled to the H-9, δ 3.36-3.47 ppm and H-12, δ 3.62-3.82 ppm respectively, and these in tern coupled to the respective carbons C-8 and C-11. C-1 (114.57-143.03ppm) was assigned through the long range couplings with H-5 δ 6.57-8.21 ppm and H-3 δ 6.57-8.76 ppm in benzamides where both positions C-5 and C-3 are not occupied by substitution on benzene ring. Here to demonstrate the two and three bonds important connectivity between carbon and protons, HMBC spectrum of entry 13 is shown as example in the figure 6. The over lapped signals of two Quaternary carbons C-2 and C-6 of entry 13 were assigned separately, at δ 159.46 ppm and 157.06 ppm, respectively with the help of 2D HMBC, experiment and shown their long range coupling with H-4,3 and H-4,5 of ring protons respectively. In all mono and di substituted methoxy benzamides entries 3, 4, 5, 11 and 12 the methoxy protons at δ 3.83, 3.84, 3.85, 3.82 and 3.87/3.88 ppm respectively were long range coupled to their respective quaternary ^{13}C signals at δ 155.04(C-2), 159.43(C-3), 160.31(C-4), 156.45(C-2/6) and 149.81(C-3)/148.61(C-5) ppm, respectively (Scheme 2). Beside that carbon resonances showed splitting due to the coupling with fluorine in benzamides 6, 7, and 13-17. Fluorine coupling with carbon was observed in the range 244 -251 Hz, 12-26 Hz, 4-13 Hz and 2-6 Hz for one, two, three and four bond respectively.

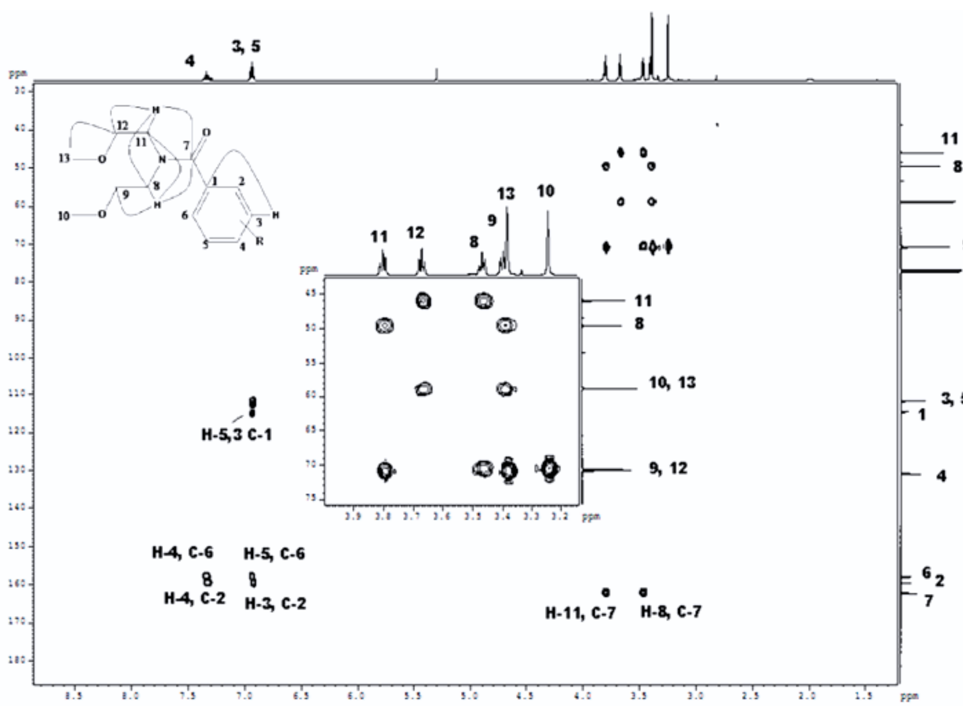


Fig. 6: 2D ^{1}H-$^{13}C\{^{1}H\}$, HMBC NMR Spectrum of Entry 13 in $CDCl_3$ showing long connectivity of Carbon with Proton

CONCLUSION

In summary, here we report the complete ^{1}H and ^{13}C NMR data of benzamide derivatives. The results obtained from the present investigation revealed that the *N,N* Dialkyl substituted benzamides are sterically crowded and floppy at room temperature, possess low barrier of rotation, cause formation of multiple rotamers. This rotation further creates broadening in the methylene proton signals. Low temperature lowers the tumbling especially in ortho substituted benzamides and exhibited clear splitting for alkyl chain methylene proton signals. The Splitting and Sequencing of chemical shift of methylene/methyl protons at NMR Scale is affected by Substitution in benzene ring. Molecules adopted twisted geometry because of bulky alkyl chain and bulky substitution in benzene ring.

Supplementary Information (SI)

Coupling constant values of Proton and Carbon NMR, literature values of ^{1}H NMR chemical shifts of cis-trans alkyl groups, Activation energy literature values of rotamers and over lapped ^{1}H spectra of aromatic region and ^{1}H NMR showing variable temperature analysis for this article can be accessed from supplementary information.

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Case Report

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Postmortem Computed Tomography: A Supplant Technique to Autopsy for Firearm Injuries in the Head

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Abstract

Background: Autopsy is more time consuming if the bullet/bullets are lodged at the difficult to access sites of the head especially maxillofacial regions. The procedure of suturing the deceased becomes even more difficult after the retrieval of bullets post dissection of facial tissues. The altered aesthetics psychologically and emotionally disturbs the already bereaved next of kin. The authors explored the utility of PMCT and propose a methodology of targeted dissection/minimally invasive approach to retrieve the bullet. The authors discussed the feasibility to conclude the cause of death in cases of single/multiple firearm injuries tothe head using PMCT alone.

Methods: The authors evaluated three cases of firearm deaths at a distance lesser than close range to the head. The deceased was subjected to PMCT scanning using a 16 slice Multi-Slice CT spiral scanner and findings were analyzed using the Vitrea software v.6.9.1 with the slice thickness ranging from 0.5mm to 5mm. A routine conventional autopsy was conducted post-scanning. Two of the three cases were suicide and succumbed to a single firearm injury while the third case was a homicide due to multiple firearm injuries.

Conclusion: PMCT alone can beutilized and relied upon in deaths due to a single shot to the head and suggest a combined methodology of PMCT evaluation and minimally invasive approach in cases of multiple firearm injuries for better correlation of wound track. Lastly, PMCT helped in a targeted approach to reach the in situ bullets more precisely than any other radiological technique which upholds the humanitarian forensic aspect.

Keywords: Firearm; Bullets; Wound Ballistics; PMCT; Autopsy; Minimal Invasive Autopsy; Head Injury.

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INTRODUCTION

Ballistics is a word derived from Greek, which means “the study of objects that are thrown and of their trajectories”. The “fir earm” is a common term that means all processes related to the motion of a bullet. The field of forensic medicine is mainly concerned with a part of terminal ballistics referred to as wound ballistics which is concerned primarily with the effects of a bullet after penetration into a person’s body.[1] Autopsy is a conventional procedure for understanding the wound ballistics of the deceased who succumbed to gun shot wounds.[2,3] It is time consuming if the bullet/bullets are lodged at the difficult to access sites of the head especially maxillofacial regions. The procedure of suturing the deceased becomes even more difficult after the retrieval of bullets post-dissection of facial tissues. The altered aesthetics psychologically and emotionally disturbs the already bereaved next of kin, even though the autopsy surgeons try their best to restore the original look of the deceased.

Postmortem Computed Tomography (PMCT) has evolved as a promising tool in the field of forensic medicine in recent years.[4,5] The scanned images being highly objective has the ability to be as evidence post-cremation and answer most of the medicolegal queries by up holding the dignity of the dead. Several studies have proven that the diagnostic yield using PMCT increases, especially to detect fractures, hemorrhage, and gas collections, such as pneumothorax, pneumoperitoneum, and pneumocephalus which could alone be the cause of death in most man-made and accidental scenarios, when PMCT is considered as a screening technique prior to the conduction of an autopsy.[6-10] Considering the humanitarian forensic aspects, the authors explored the utility of PMCT and propose a methodology of targeted dissection/minimally invasive approach to retrieve the bullet. The authors discussed the feasibility to conclude the cause of death in cases of single/multiple firearm injuries to the head using PMCT alone and a combination of techniques as well.

Methods

The authors evaluated three cases of firearm deaths at a distance lesser than close range to the head. Two of the three cases were suicide and succumbed to a single firearm injury while the third case was a homicide due to multiple firearm injuries. The authors analyzed the efficacy of PMCT alone with the combination of both techniques to conclude the cause of death in the following cases.

PMCT Examination: All the deceased were subjected to PMCT scanning using a 16-slice Multi-Slice CT spiral scanner, Toshiba America Medical Systems, Inc Aquilion Lightning TSX-035A CT. Scanning parameters were 120kV and 70 mAs. 16 x 1 mm collimation was used for all the cases for data acquisition. The findings were analyzed using the Vitrea software v.6.9.1 with the slice thickness ranging from 0.5mm to 5mm. The reconstructions were performed in the soft tissue, bone, and lung window for the thorax (FC18).

The dissection procedure was carried out by three forensic specialists and included a detailed external examination followed by a complete internal examination. Virchow's & Ghon's Technique of dissection was performed in suicide cases where injuries were only to the head. However, all cavities were dissected even in cases where firearm injury was limited to head alone. The Letulle method (En-masse) of dissection was performed in the homicide case so as to interpret the wound track. A routine conventional autopsy was conducted post-scanning and wherever the bullets were insitu, PMCT scans were referred for the exact location of the bullets there by minimizing unwanted dissection of tissues.

CASE HISTORY

Case 1

Alleged history of self-inflicted firearm injury by a 30-year-old male using arifle.

Autopsy findings: An irregular bullet entry wound with an elliptical abrasion collar was present over the left temple region 6 cm below the left frontal eminence and 3.5 cm from the lateral end of the left eyebrow. Singeing of hair, blackening, and tattooing of skin were present (Fig. 1A, Yellow colored arrow shows the direction of bullet entry). Bevelling of the inner table of the skull is present underlying the entry wound (Fig. 1B). The track of the wound runs upwards, backward, and to the right and passing through the left temporal, parietal lobes, and right parietal lobe and exiting through the right parietal region of the scalp. A comminuted fracture is present involving the left tempo-parietal, frontal, right parietal, and occipital bones (Fig. 1C, yellow arrows mark the direction of the wound track). The comminuted base of skull fracture is present involving the bilateral roof of the orbit and the body of sphenoid bones. The entire wound track was hemorrhagic. Anir regularly shaped exit wound of size was present over the right parietal region of the scalp 3 cm from the parietal eminence (Fig. 1D). The exit wound was at a higher level than the entry wound. A Bevelling outer table of the skull was present underlying the exit wound.

PMCT Findings: The 3D volume rendered technique (3D VRT) of the skull showsa comminuted fracture surrounding the entry wound without artefactual displacement of fragments (Fig. 2A). The presence of Pneumocranium is seen and displaced bone fragments are noted in the parenchyma between the entry wound at a lower level on the left temporal region and the exit wound higher level on the right parietal region. The direction of the track is depicted by the displaced fractured fragments from left to right (Fig. 2B). Yellow colored arrow denotes the direction of the pathway of the bullet. However, the wound track is not clearly seen due to the loss of parenchyma. The sagittal section of the Head and neck in the soft tissue window shows the presence of air in the spinal column from the head continuously at the cervical level (Pneumorrhachis) (Fig. 2C). The 3D VRT skull in skeletal filter shows the comminuted fracture of the right parietal region surrounding the exit wound without artefactual displacement of fragments (Fig. 2D).

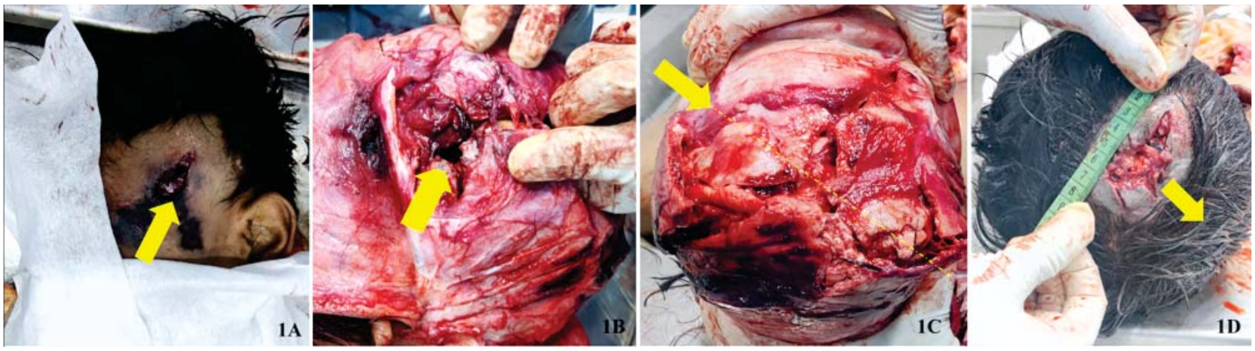

Fig. 1: *Case 1* Conventional Autopsy Findings

1A: Bullet entry wound over the left lateral forehead. **1B:** Communited fracture surrounding the stellate shaped defect. **1C:** Communited fracture seen from head end. **1D:** Bullet exit wound on right parietal region.

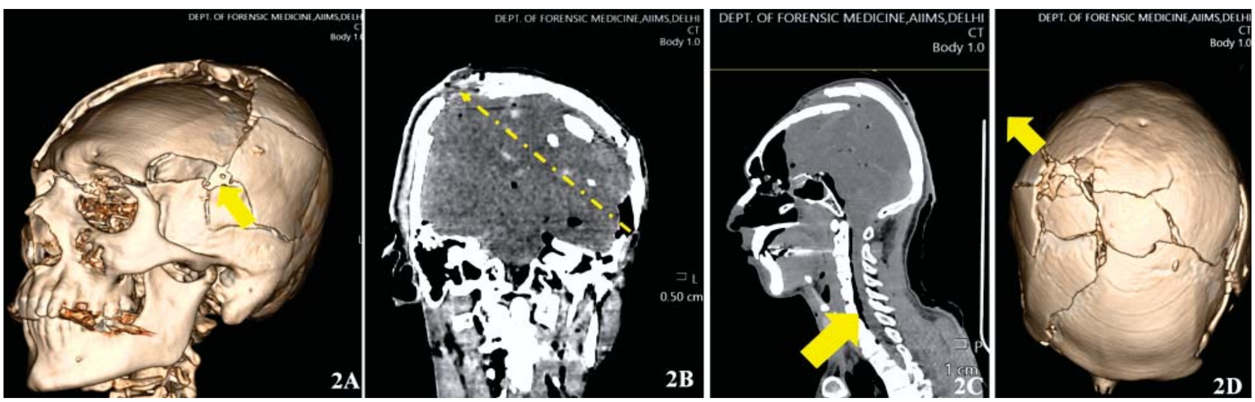

Fig. 2: *Case 1* Virtual Autopsy findings.

2A: Communited fracture surrounding the entry. **2B:** PMCT (coronal) head - Pneumocranium and displaced bone fragments. **2C:** Pneumorrhachis. **2D:** Communited fracture of skull vault along the direction of bullet.

Case 2

Alleged history of self-inflicted firearm injury by a 30-year-old male using a pistol. The room in which the incident happened was locked from the inside and he was last seen alive a few minutes back by his colleagues sitting in the living hall. He was declared brought dead and an autopsy was requested.

Autopsy Findings: An irregularly shaped firearm entry wound was present on the left temporal region, with an oval abrasion at the base suggesting muzzle impression with associated tattooing and singeing of the hairs (Fig. 3A, yellow colored arrow showing the direction of bullet entry). A bony defect with bevelling in wards was present on the left temporal bone surrounded by a depressed comminuted fracture involving the left temporoparietal region (Fig. 3B). Two linear fractures of lengths 15 cm and 9 cm are radiating from the bony defect and extend on the skull vault towards the right frontal and right occipital bones respectively. The above-mentioned comminuted fracture on the left temporoparietal region extends into the middle cranial fossa, as a fissure fracture involving the bilateral greater wing of the sphenoid through the pituitary fossa (Type I Base of skull fracture). The wound track extends from the above mentioned entry wound in an upward and backward direction through a contused laceration of the left temporal lobe, left parietal lobe, bilateral lateral ventricles, and right parietal lobe of the brain (Fig. 3C). A stellate shaped firearm exit wound is present on the right parietal region (Fig. 3D). A bony defect is present on the right parietal bone measuring with everted edges surrounded by a comminuted fracture of the right temporoparietal region overlying the laceration on the right parietal lobe (Fig. 3E, Yellow colored circle denotes a mismatch between the autopsy image and PMCT image).

PMCT Findings: 3D-VRT shows the comminuted fracture surrounding the entry wound without artefactual displacement of fragments (Fig 4A). The presence of Pneumocranium is seen and the hyperdensity of brain parenchyma is due to hemorrhagic contusion along the wound track. The entry wound (left) and exit wound (right) both lie at the same level; the direction is depicted by the inward displacement of bone fragments near the entry wound and outward displacement near the exit wound. Yellow colored arrow denotes the direction of the pathway of the bullet (Fig 4B). The sagittal section of the Head and neck in the soft tissue window shows the presence of air in the spinal column from the head continuously at the cervical level (Fig. 4C). The 3D-VRT of the skull in the skeletal filter shows the comminuted fracture of the right parietal region surrounding the exit wound without artefactual displacement of fragments. (Fig. 4D)

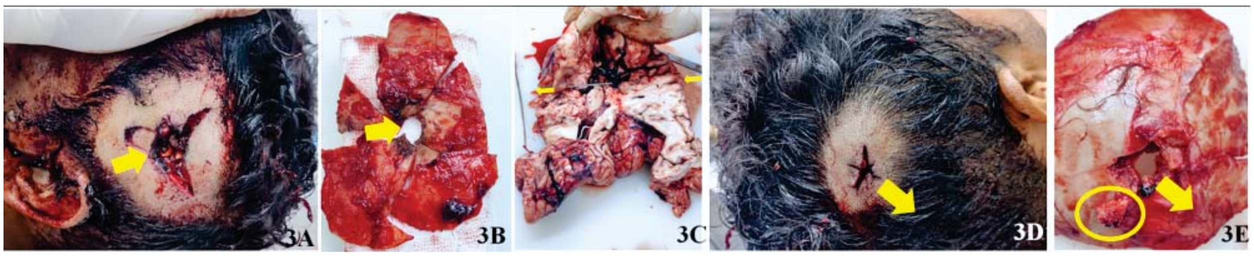

Fig 3: *Case 2* Conventional autopsy findings.

3A: Entry wound on left temporal region. **3B:** Communited fracture surrounding the entry wound. **3C:** Wound track in brain parenchyma. **3D:** Exit wound on right parietal region. **3E:** Bevelled-out margins of exit wound.

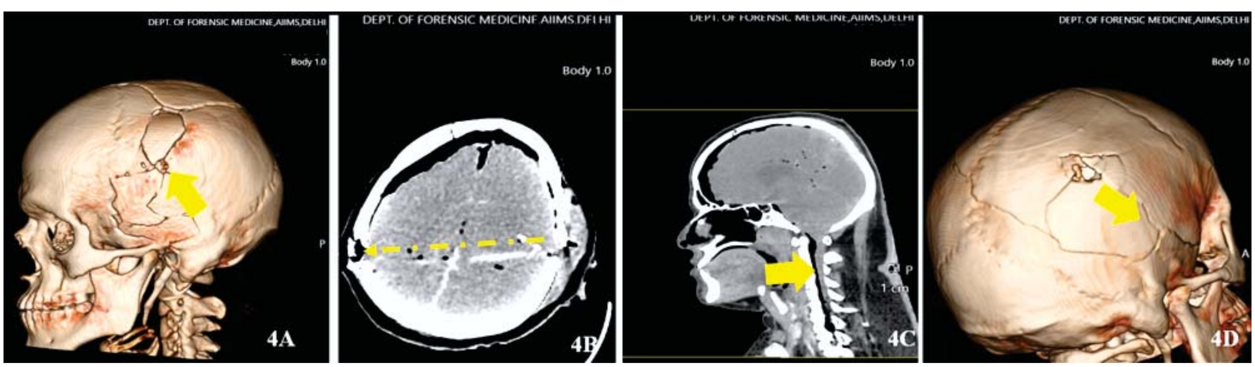

Fig 4: *Case 2* Virtual autopsy findings.

4A: Communited fracture surrounding entry wound. **4B:** Pneumocranium-anterior part, hyperdensity of brain parenchyma along wound track. **4C:** Pneumorrhachis. **4D:** Exit wound showing bevelled margins.

Case 3

Alleged history of the deceased being shot by multiple persons from multiple directions using country made guns.

Autopsy Findings: There were multiple injuries all over the body, only the injuries sustained to the head are discussed below.

- A bullet entry wound with irregular margins surrounded by an abrasion collar was present over the left occipital region, directed forwards medially and downwards (Fig. 5A, 1). The track passes through the left occipital lobe, and right temporal lobe and exits through the skull through an exit wound on the lesser wing of the sphenoid (Fig. 5B, yellow circle depicts laceration and yellow arrow depicts the bullet direction). The track ends below the scalp, a bullet was retrieved (Fig. 5C, 1).

- A bullet entry wound with irregular margins surrounded by an abrasion collar was present over the left side of the lower lateral face with associated tattooing on the skin surrounding the left ear (Fig. 5A, 2). The wound was directed medially upwards from left to right. The track passes through the ramus of the left side of the mandible making a comminuted fracture and then through the left maxilla further progressing through the right side of the maxilla. The track ends subcutaneously in the right zygoma area, a bullet was retrieved from the subcutaneous tissue just below the temporal process of zygomatic bone (Fig. 5D, 2).
- A bullet entry wound with irregular margins surrounded by an abrasion collar was present over the left side of the lower lateral face (Fig. 5A, 3). The wound is directed medially upwards from left to right. The track passes through the ramus of the left side of the mandible making a comminuted fracture, further progressing through the left maxilla, the right side of the maxilla with associated maxillary sinus fractureto exit through an exit wound in the maxillary areaof the right side of the face (Fig. 5 E, 3). The entire base of the brain had subarachnoid hemorrhage due to the effects of the bullet (Fig. 5F).

PMCT Findings: 3D-VRT of the skull in skeletal filter showed irregularly defined defects on the left occipital region and the left ramus of the mandible associated with the fracture due to the entry of a bullet (Fig. 6A) The coronal section of the PMCT head shows the presence of hyperdense objects confirmed as bullets due to high Hounsfield units (HU) at the subcutaneous plane on the right temporal region due to entry wound '1' and the second bullet underlying to right zygomatic process from the entry wound '2'. Yellow arrows show the tracks of the wounds '1, 2 & 3' (Fig. 6B). The sagittal section of the PMCT in the soft tissue window shows the non-continuous presence of air in the spinal column at the thoracic level (Fig. 6C).

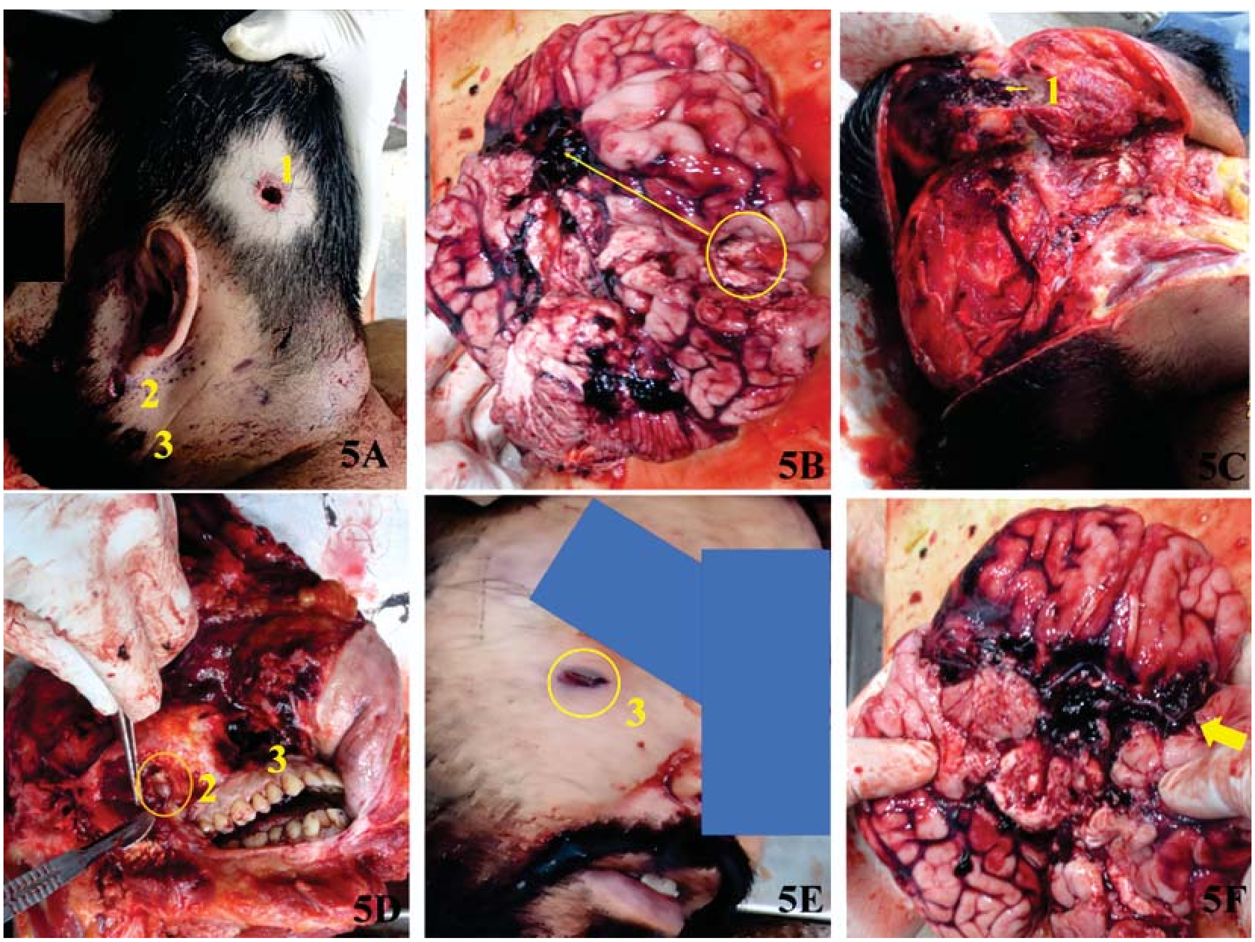

Fig 5: *Case 3* Conventional Autopsy findings

5A: Entry wound (1,2,3). **5B:** Laceration on left occipital region. **5C:** Insitu Bullet temporal region. **5D:** Fracture of right maxillary sinus. **5E:** Exit wound. **5F:** Intracranial hemorrhages on the base of skull.

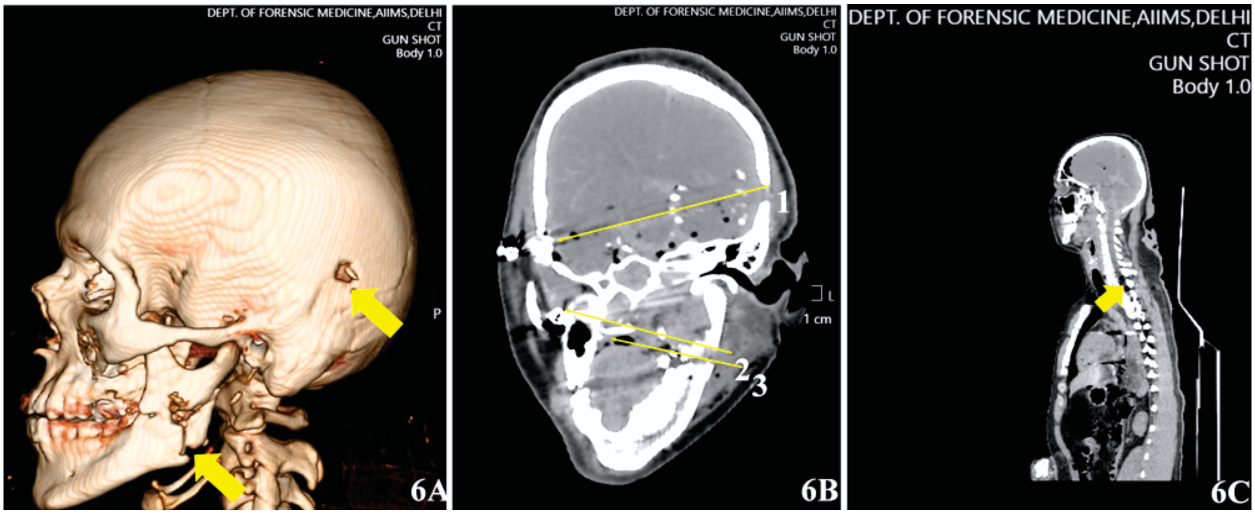

Fig 6: *Case 3* Virtual Autopsy findings.

6A: Entry wound on occipital region and mandible. **6B:** Foreign bodies at right temporal region due to entry wound (1) and under right zygomatic process from the entry wound (2). **6C:** Pneumorrhachis.

DISCUSSION

The current discussion related to firearms is not the first. Researchers had explored several aspects of PMCT and concluded that PMCT could be a good complementary tool for the determination of the cause of death and for localization and diagnosis of different types of lesions in gunshot injuries.[11-14] The authors with the help of these cases would like to compare the utility of PMCT in a single gunshot and multiple gunshots to the head region. The entry wound was present on the left side of the head in both suicide cases. In the first case, the room in which the incident happened was locked from the inside and he was last seen alive half an hour back by his colleagues during dinner. Upon breaking open the room, the deceased was lying on his back with his left upper limb crossing his body at the chest level holding the barrel of the rifle pointing towards his shoulder. The right hand was in an extended position with the presence of a cadaveric spasm in the triggering position on his right hand. It was concluded from the crime scene that he was sitting on the cot near by while performing the act of triggering, following which he fell down from the cot. There was the presence of shattered cement from the roof at places over his body and floor. An empty cartridge and a fired bullet were found inside the room. The cadaveric spasm in addition to the crime scene images helped in concluding the case as a suicide. In the second case, the muzzle imprint was at the entry wound on the left side. It was confirmedin the investigation that the deceased was ambidextrous in handling the pistol. There were no other external wounds over the body other than the entry and exit. It was correlated from the history and confirmed by external examination at autopsy that a single firearm was fired. The role and advantage of PMCT in these types of cases is to document the internal findings in a detailed manner. In the authors' experience, using PMCT.

- The entry and exit wounds can be confirmed by the direction of displaced fragments and can be precisely measured.
- The comminuted fractures of the vault were exactly seen devoid of iatrogenic artifacts.
- The level of entry and exit was accurately seen which helps in correlating the wound track externally.
- Internally the wound track was interpreted by the presence of hyperdensity suggestive of hemorrhage along the lacerated parenchyma in the brain.
- Lastly, PMCT had the benefit of witnessing air inside the cranial cavity (Pneumocranium) and air inside the spinal canal (Pneumorrhachis) which is not possible by the conventional autopsy.

The third case was a case of homicide due to multiple firearm injuries from multiple directions. It was noted from the CCTV videos, the range was a close range shot to the face fired by the same individual multiple times. The challenge faced here was to identify the track and retrieve the insitu bullet from the face. The X-ray is highly efficient in demonstrating the presence of a bullet inside the body but is not as equally efficient as PMCT. The reason being the difference of planes the exact location is difficult to understand and approach in dissection.The authors felt that even though PMCT alone is considered highly efficient in single firearm cases to head, it was difficult alone in PMCT aloneto handle multiple firearms to head. The reason was the abnormal positioning of the hefty deceased and the development of rigor mortis. In this scenario, there would be multiple fractures and mispositioning would create additional queries while correlating the wound track. Therefore, we suggest a combined approach of minimal dissection together with the correlation of the findings seen to interpret the track.

Hence, the authors propose the following methodology in cases of single firearm wounds which would answer all the medicolegal queries.

- A proper history needs to be elicited from the investigation officer.
- A thorough external examination must be conducted to rule out any other injuries.
- Collection of required evidentiary material collections like Nail clippings, Gun Shot Residue (GSR) collection in the form of swabs from hands and wounds, peripheral blood, vitreous humour, or cerebrospinal fluid as per need and protocols.
- A complete screening of PMCT images for the presence of foreign bodies like a bullet.
- A minimal targeted dissection if the bullet is present inside the body and if the bullet is not present the dissection could be curtailed.

CONCLUSION

The authors briefly conclude that the PMCT alone can be utilized and relied upon in deaths due to a single shot to the head. PMCT identified the exact location of the bullet in the subcutaneous tissues of the head and underlying the zygomatic process. It has the ability to preserve the altered anatomy of the disrupted skull post-bullet entry and exit there by avoiding artefactual fracture interpretation. The wound tracks in the head could be interpreted at PMCT by the presence of the displaced bone fragments, hemorrhage along the disrupted parenchyma together with the presence of air along the track in cases of single firearm shot. We suggest a combined methodology of PMCT evaluation and a minimally invasive approach in cases of multiple firearm injuries to the head for better correlation of wound track as it would be difficult on PMCT alone due to postmortem changes. Lastly, PMCT helps in a targeted approach to reach the insitu bullets more precisely curtailing unwanted dissection than any other radiological technique which up holds the humanitarian forensic aspect.

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