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Oxidative Degradation of Fentanyl in Aqueous Solutions of Peroxides and Hypochlorites

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ABSTRACT

Fentanyl widely used in clinics as practices is potentially utilised as an incapacitant in countering terrorism and its analogues would be used as an addictive drug, so the degradation of these compounds need to be investigated for protecting environment and human health. In this work, the degradation of fentanyl was examined in a series of oxidant aqueous solutions. Meanwhile, the degradation pathways of fentanyl in the oxidant solution were discussed according to all products identified by GC/MS and LC/MS.

Keywords: Fentanyl, degradation pathway, countering terrorism, addictive drugs, incapacitants

1. INTRODUCTION

Fentanyl, N-[1-(2-phenethyl)-4-piperidyl] propionanilide, a narcotic analgesic of the 4-anilidopiperidine series, which was first introduced into clinical practice in the early 1960s[1], has been reported to be much more efficient than meperidine or morphine. However, these opioid compounds have a large number of side effects including respiratory depression, nausea, miosis, and alterations in patterns of behaviour and activities in case of overdose. Since the late 1970s, ten kinds of homologues and six kinds of analogues of fentanyl have appeared in the illicit market and have even led to death because of overdose[2,3]. Nevertheless, these kinds of compounds can also be utilised as incapacitants in countering terrorism. In October 2002, the analogues of fentanyl were reported to be successfully used in the accident of rescuing hostages in Russia[4]. In recent years, the analgesic and anesthetic medicines have gained attention in the worldover. The dealing methods of these compounds are of great importance to criminalistics and countering terrorism.

Highly sensitive methods of analysis for fentanyl have been established with gas chromatography (GC) and high-performance liquid chromatography (HPLC), especially in human plasma, hair or urine[5-19]. Nevertheless, very few methods of the degradation of fentanyl are reported in literatures. Lambropoulos[20], *et al.* had investigated the fate of fentanyl in acidic, basic, and hydrogen peroxide solutions. They found that fentanyl could be converted into N-phenyl-N-(4-piperidinyl) propionamide (PPA) via deacylation in 3 M hydrochloric acid at 90 °C, while 3 per cent H_2O_2 or basic solution could hardly destroy fentanyl at room temperature.

As a common and highly effective approach, oxidative degradation is preferable to destroy organic compounds, typically using hypochlorites and peroxides[21]. To evaluate the oxidative degradation efficiency of fentanyl, a series of oxidants such as $KHSO_5$, $K_2S_2O_8$, H_2O_2, peracetic acid (CH_3CO_3H), sodium percarbonate (SPC), sodium percarbonate/ N,N,N,N-tetraacetylethylene diamine (SPC/TAED), magnesium monoperoxyphtalate (MMPP), trichloroisocyanuric acid (TCCA, $C_3O_3N_3Cl_3$) and $Ca(ClO)_2$ have been examined. GC/MS and LC/MS approaches were employed to identify the degradation products.

2. EXPERIMENTAL STUDY

2.1 Materials

Fentanyl hydrochlorate has a purity >99 per cent, which was synthesised according to the procedure described by Janssen[22]. Methanol (HPLC grade) was obtained from Fisher Chemicals (Leicester, UK). The oxidants used included MMPP, $KHSO_5$ which were procured from Aldrich. The other oxidants were purchased from Beijing Chemical Reagent Company.

2.2 Degradation of Fentanyl in Peroxide and Hypochlorite Solutions

The active oxygen concentration of the prepared oxidant solutions were 0.2 mol/l. It should be noted that SPC/ TAED dissolved in water can produce peracetic acid. Phosphate salts as buffer reagent were used to adjust the p*H* value of oxidant solution. The hypochlorite solution was obtained by dissolving $Ca(ClO)_2$ or TCCA into water with available chlorine of 0.2 mol/l. Stock standard aqueous solution of fentanyl hydrochlorate (10 mg/ml) was prepared with the purified chemicals. The degradation reaction was conducted in a flask with stirring at a rate of 1320 r/min, where

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5.0 ml of stock standard fentanyl hydrochlorate solution and 50 ml of oxidant solution were added.

2.3 Analysis of Fentanyl and its Degradation Products

Fentanyl residual in the oxidant solution was extracted by organic solvent and analysed using GC-FID approach. To achieve an optimised extraction ratio, the pH value of the solution was adjusted to around 12 with 2 M sodium hydroxide solution[4,23]. Equivalent volume of dichloromethane was added to extract the residue of fentanyl. The extractant was analysed by GC-FID (Agilent 6890N) equipped with a HP-5 capillary column (30 m × 0.32 mm × 0.25 ìm), employing the temperature ramp 100-280 °C at 15 °C /min.

For identification of the degradation products, dichloromethane were used to extract the residue of fentanyl and less polar degradation products. The extractant was analysed by an Agilent 7890/5975 GC/MSD equipped with a HP-5 capillary column (30 m × 0.25 mm × 0.25 ìm), employing the temperature range 40-280 °C at 10 °C/min. The energy of EI was 70eV and scan range was 10-500 m/z. The polar degradation products were qualitatively analysed by the following method:

Dry the oxidant solution by N_2 stream, then extract the solid residue with methanol, and analyse the extractant with Agilent 1100 LC/MS equipped with a non-polar column (Zorbax SBC18 column, 30 mm × 2.1 mm × 3 ìm). The used mobile phase was 0.1 per cent acetic acid at a rate of 0.2 ml/min, the column temperature was 40 °C, and scan range was 50-2000 m/z.

3. RESULTS AND DISCUSSION

3.1 Oxidative Degradation of Fentanyl in the Oxidant Solutions

When fentanyl solution was added to the oxidant solution, solid fentanyl segregated from the aqueous solution of $Ca(ClO)_2$ due to the light solubility of fentanyl in strong basic solution. This may lead to the uncertainty of the concentration analysis of fentanyl. The degradation ratios of fentanyl in various oxidant solutions has been listed in Table 1. It was seen that fentanyl could be degraded in all the oxidant solutions. Among all the oxidants, TCCA solution achieved the best degradation ratio of fentanyl, followed by SPC/TAED and peracetic acid. Furthermore, it was found that the degradation efficiency of fentanyl in acidic solution was higher than that in basic solution.

Table 1. The degradation ratio of fentanyl in several oxidant solutions

Type	Oxidants	pH value	Degradation ratio (per cent)				
			2 min	5 min	10 min	30 min	60 min
Peroxide	$K_2S_2O_8$	6	-	4.0	6.4	33.0	45.6
	H_2O_2	5	-	10.4	14.5	34.6	53.0
	$KHSO_5$	5	-	24.3	28.0	44.6	43.5
	MMPP	5	-	47.0	52.1	63.0	76.5
	SPC	4	-	18.7	36.0	24.4	45.3
	CH_3CO_3H	8	-	90.7	92.1	93.0	95.1
	SPC+TAED	8	81.6	91.9	92.4	95.7	98.6
	SPC+TAED	10	76.3	81.2	91.0	91.4	93.0
hypochlorite	$C_3O_3N_3Cl_3$	5	96.5	98.6	99.0	99.5	N.D.
	$Ca(ClO)_2$	12	85.9	59.2	41.7	38.9	36.9

N.D. not detected, >99.9 per cent;
– the sample was not prepared at the certain reaction time

3.2 The Degradation Products of Fentanyl

The GC/MS spectrum with total ion chromatography for the degradation products of fentanyl by various peroxides over 1 h are presented in Fig. 1. The possible structural formulae were determined according to the retention time and mass spectra of detected species and are presented in Table 2. It indicates that the identified products were mainly derived from fentanyl via the cleavage of *C-N* bond and *C-C* bond in the oxidant solution.

Table 2. Possible degradation products of fentanyl with peroxide aqueous solutions over 1 h based on GC/MS

Retention time (min)	Name	Main mass fragments
5.0	Phenethylene	104(100),78,77,63,51.39
6.1	Benzaldehyde	106(100),105,77,51,39
7.3	Benzenemethanol	108,107,91,79(100), 77,65,51,39
7.4	Benzeneacetaldehyde	120,92,91(100),77, 65,51,39
13.3	N-Phenylpropanamide	149,120,93(100),77,66, 65,57,39,29
17.9	UC	223,149(100),121, 104,76,57
19.1	N-Phenyl-N-(4-piperidinyl) propanamide	232,175,159,120,93, 83(100),82,68,57
22.5	N-(1-formylpiperidine-4-yl)-N-phenylpropionamide	260,231,187,150,132, 111,93(100),77,57
25.7	Fentanyl	245(100),189,146, 132,105,91,57,42

UC unknown compound

Figure 2 illustrates the GC/MS spectrum with total ion chromatography for the reaction products of fentanyl in TCCA aqueous solution. Except for the cleavage of *C-N* bond and *C-C* bond, chlorination species derived from fentanyl were also detected. It is mentioned that the detected ring-chlorinated aromatic products might be similar with the reaction products of chlorine gas on benzaldehyde-di-*n*-alkyl acetals[24]. Nevertheless, the product phenethylene detected in the peroxide solution wasn't found in TCCA aqueous solution.

The polar compounds involved in the products were analysed by LC/MS. The obtained liquid chromatograms with extracted ion chromatograms ($M \pm H^+$) are presented as Fig. 3. One could deduce the possible structural formulae

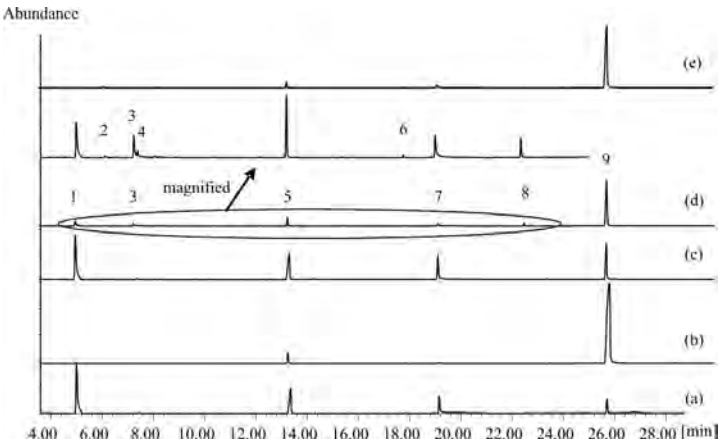


Figure 1. GC/MS total ion chromatogram for degradation products of fentanyl in five peroxide aqueous solution over 1 h, (a) CH_3CO_3H, (b) H_2O_2, (c) $2NaCO_3{\cdot}3H_2O_2$, (d) $KHSO_5$, (e) $K_2S_2O_8$.

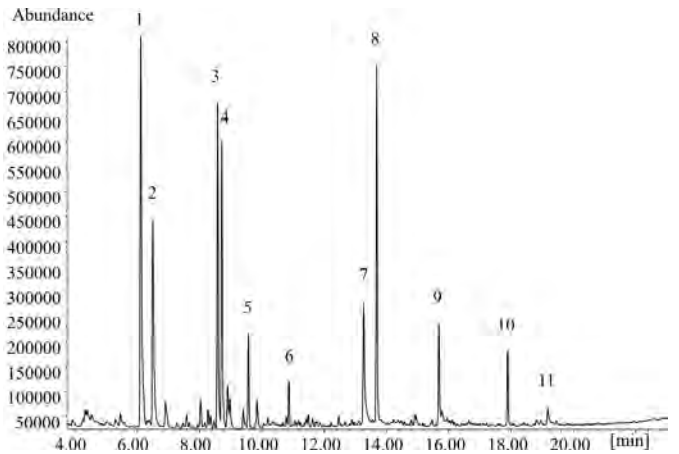


Figure 2. GC/MS total ion chromatogram for degradation products of fentanyl in trichloroisocyanuric acid aqueous solution over 1 h.

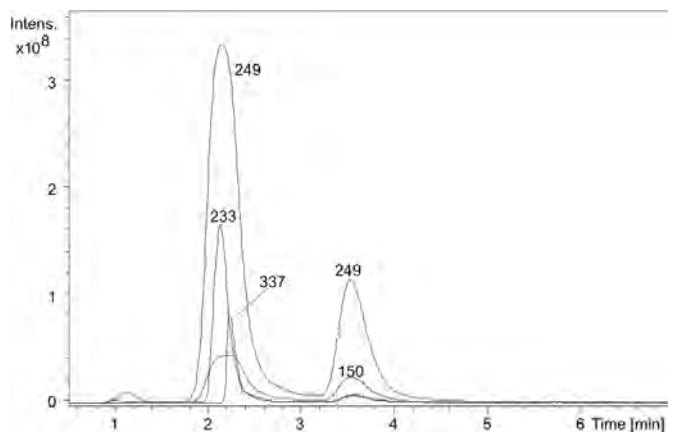


Figure 3. Liquid chromatogram with extracted ion chromatograms for degradation products of fentanyl in peracetic acid solution over 1 h, showing the $M+H^+$ peaks.

for the detected species based on the retention time and the $M \pm H^+$ peaks, as presented in Table 4. It was observed that the compounds via direct cleavage of *C-N* bond and oxidation of carbon atom next to nitrogen at the piperidine ring, and several oximes from the oxidation of the formed secondary amine were also found by LC/MS.

For TCCA aqueous solution, the LC/MS with extracted ion chromatograms ($M \pm H^+$) of the degradation products are presented in Fig. 4, and their possible structural formulae are presented in Table 5. It could be seen that several chlorination products and small amounts of dehydrogenation products were detected, except for the oxidation products.

3.3 Possible Degradation Pathways of Fentanyl

In term of clinical practices, the potency of the fentanyl series mainly depends upon the substituent at tertiary amine (Site 1), and their duration of action rely on the substituent at Site-4, as shown in Fig. 5[19,25]. In human body, fentanyl is extensively metabolised and only several per cent of the original dose is excreted through urine. The main metabolic pathway is the oxidative *N*-dealkylation at the piperidine, resulting in the formation of nor-metabolitie[3].

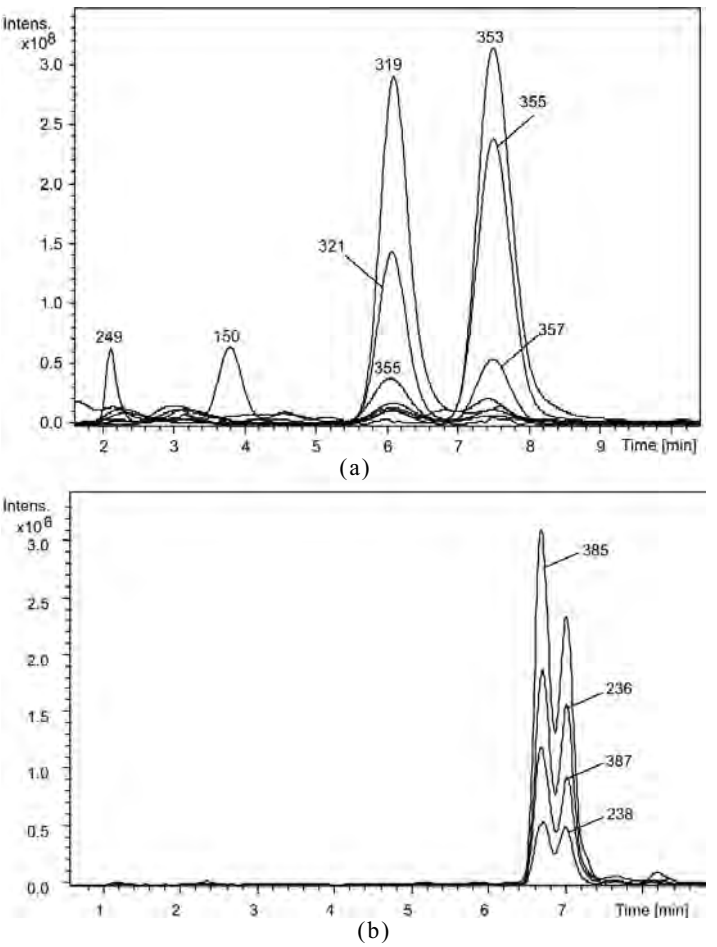


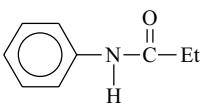
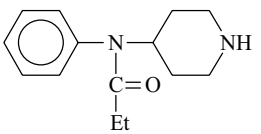
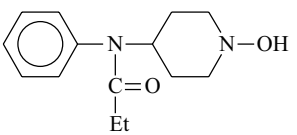
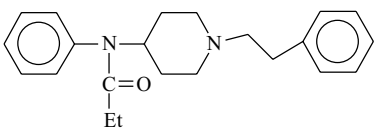
Figure 4. Liquid chromatogram with extracted ion chromatograms for degradation products of fentanyl in trichloroisocyanuric acid aqueous solution over 1 h, showing the (a) $M+H^+$ and (b) $M-H^+$ peaks.

Table 3. Possible degradation products of fentanyl with trichlorocyanuric acid aqueous solution over 1 h based on GC/MS.

Retention Time (min)	Name	Mass Ion
6.1	Benzaldehyde	106(100),105,77,51,39
6.5	Benzonitrile	103(100),76,50,39
8.5	2-Chlorobenzaldehyde	140(140:142=3:1),139(139:141=3:1)(100), 111(111:113=3:1),77,76,75,50
8.7	4-Chlorobenzaldehyde	140(140:142=3:1),139(139:141=3:1)(100), 111(111:113=3:1),77,,75,50
9.5	2-Chlorobenzyl chloride	160(160:162:164=9:6:1),125(125:127=3:1)(100), 89,63,44
10.8	2,6-Dichlorobenzyl chloride	194(194:196:198:200=27:27:9:1),174, 159(159:161:163=9:6:1)(100),125(125:127=3:1), 89,71,63,57,43
13.3	N-Phenylpropanamide	149,120,93(100),77,66,65,57,39,29
13.6	4-Chloropropionanilide	183(183:185=3:1),148, 127(127:129=3:1) (100),99, 57,29
15.7	Propanil	217(217:219:223=9:6:1),182, 161(161:163:165=9:6:1)(100),133,57,29
17.9	UC	223,149(100),121,104,76,57

UC unknown compound

Table 4. Possible degradation products of fentanyl with peracetic acid based on LC/MS profile for $M+H^+$

Structural formula	Mass
	$M+H^+$=150
	$M+H^+$=233
	$M+H^+$=249
	$M+H^+$=337

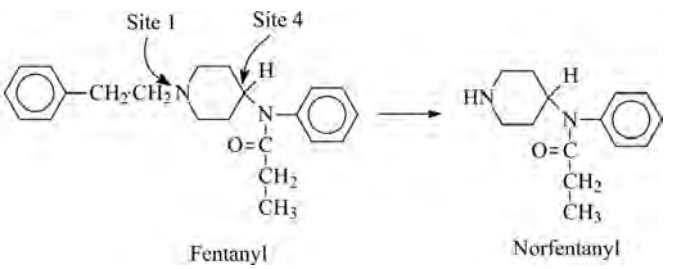
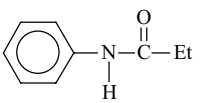
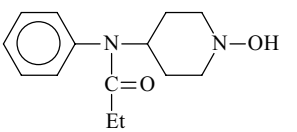
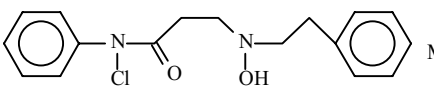
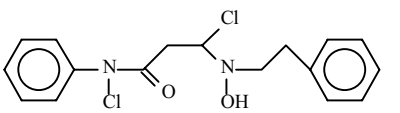
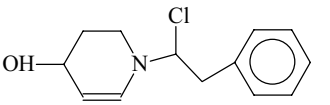
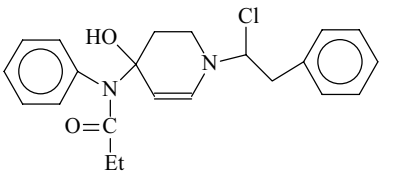
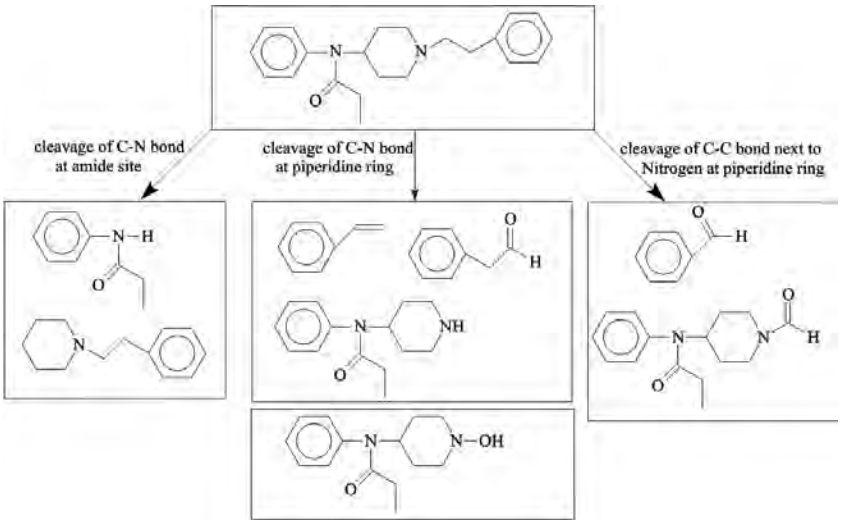


Figure 5. The structure of fentanyl and nor-metabolitie.

Table 5. Possible degradation products of fentanyl with trichlorocyanuric acid aqueous solution based on LC/MS profile for $M\pm H^+$

Structural formula	Mass
	$M+H^+$=150
	$M+H^+$=249
	$M+H^+$=319:321=3:1
	$M+H^+$=353:355:357=9:6:
	$M-H^+$=236:238=3:1
	$M-H^+$=385:387=3:1



Scheme 1. The degradation pathways of fentanyl in peracetic acid solution

It has been experimentally demonstrated that the molecule of fentanyl could be destroyed in the oxidant solutions. On the basis of the detected degradation products in the peroxide solution, three principal pathways should be considered for degradation of fentanyl, as summarised in Scheme 1.

- Firstly, oxidative *N*-dealkylation at the piperidine ring is the main pathway, and resulting in the formation of the secondary amine and styrene, which is quite similar to the metabolic pathway in human body. Consequently, the secondary amine may be further oxidized into oxime in the degradation process.
- Secondly, the *N*-dealkylation can also occur at the site of amide in fentanyl molecule.
- Thirdly, the carbon atom next to nitrogen at the piperidine ring can be oxidized and may lead to the cleavage of *C-C* bond.

However, the degradation of fentanyl in hypochlorite solution seemed to be more complicated according to the identified products. The chlorination reaction of fentanyl molecular and its oxidation products with active chlorine was taking place, except for the above-mentioned oxidation routes.

4. CONCLUSIONS

The oxidative degradation of fentanyl was examined in aqueous solution of various oxidants. It has been experimentally shown that trichloroisocyanuric acid solution achieved the best degradation efficiency to fentanyl, followed by SPC/TAED and peracetic acid. Based on the degradation products identified by GC/MS and LC/MS, it was suggested that fentanyl in the peroxide solution might undergo an oxidative N-dealkylation reaction at the piperidine ring and amide sites, as well as oxidation of carbon next to nitrogen at the piperidine ring might take place. The degradation pathway of fentanyl in hypochlorite solution might include, not only the above-mentioned oxidation reaction patterns, but also chlorination reaction of fentanyl molecule and its degradation products.

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Contributors

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