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Stability Assessment Of Donepezil Hydrochloride Using Validated RP-HPLC Method

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ABSTRACT

Stability of donepezil hydrochloride was investigated using stability indicating high performance liquid chromatography (HPLC) utilizing C-18 column and mobile phase containing methanol, acetate buffer (pH 4.25) and triethylamine in ratio of 50:50:0.5 at flow rate of 1 ml min^{-1}. Peaks of donepezil and degradation products were well resolved at retention times < 7 min. Stability was performed in 1N hydrochloric acid, 2N sodium hydroxide, 6 % hydrogen peroxide, neutral, photolytic and dry heat conditions. Fast hydrolysis was seen in alkaline condition as compared to oxidative and neutral conditions. Study of degradation kinetics was carried out in 2N sodium hydroxide, 6 % hydrogen peroxide and neutral solutions at 40^0 C, 80^0 C and at boiling under reflux. The decomposition followed first order kinetics and rate constants were 0.13, 0.379 and 0.541 hr^{-1} in 2N NaOH, 0.0032, 0.0124 and 0.0232 min^{-1} in 6% H_2O_2 and 0.026, 0.219 and 0.345 hr^{-1} in neutral condition.

Keywords: Donepezil hydrochloride; HPLC; acidic, alkaline, neutral, oxidative, degradation kinetics

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INTRODUCTION

Donepezil hydrochloride, (±) – 2 –[(1 – benzylpiperidin – 4 – yl) methyl] – 5, 6 – dimethoxyindan – 1 – one monohydrochloride [1], is a reversible cholinesterase inhibitor that exhibits high specificity for centrally active cholinesterase. Cholinergic deficit is one of the major pathological features of Alzheimer's disease. This deficit has been associated with the loss of cognition and memory, the primary symptoms of this disorder. Donepezil HCl (also known as E2020 or Aricept) is the first of the "new generation" acetylcholinesterase inhibitors, and is currently used for the symptomatic treatment of Alzheimer's disease in many countries [2]. There exists a report on stability indicating HPLC method [3] but it has not studied the degradation products and degradation kinetics. Other methods include HPLC-UV analysis of donepezil in human plasma [4], analysis and enantioresolution of donepezil by capillary electrophoresis [5] and simultaneous determination of donepezil enantiomers in human plasma by liquid chromatography-electrospray-tandem mass spectrometry [6]. The present work describes the utility of HPLC in forced degradation-stability study to determine the degradation kinetics of donepezil under different chemical conditions at different temperatures.

EXPERIMENTAL

Chemicals

Donepezil hydrochloride was supplied by the Hetero Drugs Pvt. Ltd. India and was used without further purification. Methanol, triethylamine and water (HPLC grade) were purchased from Apchem, Ashonuj Chem. Pvt. Ltd., Navi Mumbai. MS, India. All other chemicals were of analytical reagent grade.

Instrumentation

The HPLC system consisted of a pump (model Jasco 2000 series) with rheodyne injector at 20µl capacity per injection was used. The detector consisted of a UV/VIS (Jasco UV 2075) model operated at a wavelength of 268 nm. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Hypersil C-18 (250 mm × 4.6 mm with particle size of 5.0 µ).

Degradation studies

All degradation studies were done at a drug concentration of 1mg ml^{-1}. For acid decomposition studies, drug was dissolved in 1N HCl and solution was boiled under reflux for 8 h. The studies in alkaline conditions were done in 2N NaOH boiling under reflux for 8 h. For study in neutral conditions, drug in water was boiled under reflux for 8 h. For oxidative conditions, initial studies were done in 3% H_2O_2 solution. The solution was kept at room temperature for 6 h. Subsequently, the drug was exposed to 6% H_2O_2 at boiling under reflux for 4 h. Photolytic studies were done by exposing solid drug directly to sunlight for 48 h. Control

samples were kept in dark for the same period. Thermal decomposition studies were performed by exposing solid sample of drug to dry heat at 80 ^{0}C for 48 h.

HPLC analysis

HPLC studies were first performed on stressed samples individually (after appropriate dilution), and later on a mixture of those samples in which sufficient degradation products were formed. Studies on individual reaction solutions were carried out using methanol-acetate buffer pH 4.25-triethylamine (50:50:0.6) as a mobile phase. In all HPLC runs, the mobile phase was filtered before analysis, through 0.45 µm nylon membrane and degassed before use. The injection volume was 20 µl and the flow rate was 1 ml min^{-1} for initial studies. The detection wavelength was 268 nm.

Validation of the developed method

Linearity of the method was established by injecting solution containing 10-100 µg ml^{-1} of the drug in triplicate. Repeatability studies were performed by hexaplicate injections of the drug at three concentrations (40, 80, 100 µg ml^{-1}) on the same day. The studies were also repeated on different days to determine inter-day precision. Accuracy of the method was evaluated by spiking the drug at three different concentrations in a mixture of stressed solutions and determining recovery of the added drug. The specificity of the method was determined by the complete separation of donepezil hydrochloride in presence of its degradation products and determining the resolution of drug peak from closest eluting degradant. Robustness was verified by studying the resolution of the drug in a mixture of degraded samples on a different chromatographic system on different day.

Calculation of the degradation kinetics

The logarithmic values of percentages of the remaining concentrations of donepezil (R_t ~ 7.4 min) at zero and at different time-intervals were used to establish the degradation plots of donepezil in 2N NaOH solution, 6% hydrogen peroxide solution and neutral solution respectively. Degradation kinetic parameters such as the degradation rate constant (K_{deg}) and degradation half life ($t_{1/2}$) at 40, 80^{0}C temperature and at boiling under reflux were derived from the plots. The predicted kinetics parameters for the degradation of donepezil at 25 ^{0}C were extrapolated from Arrhenius plots.

RESULTS

Degradation behavior

HPLC studies on donepezil under different stress conditions using a mobile phase composition of methanol: acetate buffer pH 4.25: triethylamine (50:50:0.6) as the solvent system suggested the following degradation behavior.

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Acidic conditions

On heating the drug in 1N HCl at boiling under reflux for 8 h, negligible degradation was seen with corresponding rise in degradation peaks but after heating in more severe condition like 2N HCl for 2, 4 and 8 h, peaks for secondary degradation products were observed but not at significant level. (Data not shown).

Alkaline conditions

Donepezil was found to be labile in alkali. Measurable degradation of the drug was observed in 2N NaOH at boiling under reflux for 8 h. As shown in HPLC chromatogram (Fig. 1A), degradation of the drug resulted in the rise of the five major degradation products at 3.6, 4.3, 4.5, 5.2 and 5.5 min.

Oxidative conditions

Donepezil was found to degrade in 6% H_2O_2 after boiling for 4 h under reflux. Peak area of the drug was reduced to a measurable extent but there was no corresponding increase in the intensity of degradation peaks (Fig. 1B). It indicates that there might be formation of nonchromophoric products besides three major degradation products at 5.01, 5.43 and 5.95 min.

Neutral condition

Degradation of the drug was seen in neutral (water) condition after boiling under reflux for 8 h with the generation of seven degradation peaks at retention times 2.7, 3.0, 3.7, 4.1, 4.6, 5.3 and 5.6 min respectively (Fig. 1C).

Photolytic conditions

Donepezil HCl was exposed to UV light (sunlight) for 48 h. Donepezil sample was then analyzed by HPLC. It showed no additional peaks in the chromatogram indicating that donepezil HCl is stable in photolytic condition to which it was exposed. (Data not shown).

Thermal condition (Dry heat)

Degradation of the drug was not seen under dry heat for 48 h at 80 ^{0}C. HPLC analysis of donepezil sample showed generation of not a single additional peak in the chromatogram. So it can be concluded that donepezil HCl is stable under dry heat condition. (Data not shown).

Development and validation of HPLC method

HPLC separation of donepezil and its degradation products was achieved using Hypersil C-18 (250 mm × 4.6 mm, 0.5 µm) and a mobile phase containing methanol, acetate buffer pH

4.25 and triethylamine (50:50:0.6 v/v/v) at the flow rate of 1 ml min $^{-1}$. The chromatograms exhibited well resolved peaks at retention time < 7 min for donepezil HCl and the degradation products. These peaks were monitored at 268 nm using UV detection. Linear correlation between the peak area and various concentrations of donepezil HCl in the range 10-100 µg ml^{-1} was obtained. Using least square regression, the linear equation was, y=35183x-7360.6 (r^2 = 0.999), using three determinations. Accuracy of the method was assessed through recovery studies. The percentage recovery was found to be in the range of 99.15-101.33 (Table 1). Data obtained from precision experiments are given in Table 2 for repeatability and intermediate precision studies. The RSD values, ranging from 0.315 to 1.12 % for repeatability study, from 0.689 to 1.733 for intra-day precision and from 0.632 to 1.587 % for inter-day precision study respectively, confirm that the method was sufficiently precise.

Study of degradation kinetics

The kinetics of degradation of donepezil was investigated in 2N NaOH, 6% w/v H_2O_2 and neutral solution. The regular decrease in the concentration of donepezil with increasing time intervals was observed. The degradation kinetics for alkaline, oxidative and neutral condition was studied at selected temperatures such as 40, 80^{0}C and at boiling under reflux. Plotting of the logarithmic values of the remaining concentrations of donepezil hydrochloride, expressed as percentages versus time indicated that alkaline (Fig. 2A-C), water hydrolysis (Fig. 3A-C) and oxidation (Fig. 4A-C) followed first-order kinetics which was distinctly catalyzed by the presence of alkali and hydrogen peroxide in respective condition and was significantly enhanced by elevation of temperature.

From the slopes of the straight lines it was possible to calculate apparent first order degradation constants, half life ($t_{1/2}$) and t_{90} (time where 90% of original concentration of the drug is left) at each temperature for alkaline, oxidative and neutral degradation processes. Table 3 displays the calculated kinetic parameters of donepezil in 2N NaOH, 6% w/v H_2O_2 and neutral solutions respectively. The data showed higher values of the degradation rate constants with shorter half-lives in 2N NaOH solution compared with 6% H_2O_2 and neutral solutions. Furthermore, the degradation was progressively enhanced by increase of temperature.

To determine the degradation kinetics of donepezil HCl at 25^{0}C, Arrhenius plots (data not shown) were constructed by plotting the logarithmic values of the observed K_{deg} values, computed from the degradation plots at different temperatures, versus 1/T. Using least square regression, linear relationships with correlation coefficients (r = 0.9763-0.9977) were obtained (Table 4). As derived from Arrhenius plots, the degradation rate constant (K_{deg}), half life ($t_{1/2}$) and shelf life (t_{90}) for base catalyzed hydrolysis of donepezil were 0.083 hr^{-1}, 8.34 and 1.265 hr respectively, 0.00516 hr^{-1}, 134.3 and 20.41 hr for hydrogen peroxide catalyzed oxidation respectively, whereas in neutral solution, the values of K_{deg}, $t_{1/2}$ and t_{90} were 0.011 hr^{-1} , 63.0 and 9.576 hr, respectively (Table 5). The calculated kinetic parameters proved that donepezil was quite unstable in strongly alkaline medium while slightly labile in hydrogen peroxide and neutral solutions.

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Table 1: Recovery studies

Actual concentration (µg/ml)	Calculated concentration (µg/ml) ± SD; RSD %	Recovery (%)
20	19.83 ± 0.145, 0.731	99.15
50	49.96 ± 0.161, 0.322	99.92
80	81.06 ± 0.677, 0.835	101.33

Table 2: Intra-day and inter-day Reproducibility and precision.

Actual concentration (µg/ml)	Calculated concentration ± SD (µg/ml); RSD%.		
	Injection Repeatability (n=6)	Intra-day precision (n=3)	Inter-day precision (n=3)
40	39.89±0.126, 0.315	40.1± 0.689, 1.722	40.71 ± 0.646, 1.587
80	79.92 ± 0.896, 1.12	80.19 ± 0.39, 1.733	80.56 ± 0.755, 0.937
100	99.7 ± 0.499, 0.5	99.49 ±0.722, 0.726	100.78 ± 0.637, 0.63

Table 3: Kinetics parameters for the degradation of donepezil hydrochloride

Temperature (^{0}C)	K_{deg} (hr^{-1})	$T_{1/2}$ (hr)	t_{90} (hr)
	2N NaOH		
40	0.13	5.33	0.81
80	0.379	1.82	0.268
Boiling under reflux	0.541	1.28	0.194
6% H_2O_2			
40	0.0032	216.56	32.91
80	0.0124	55.88	8.43
Boiling under reflux	0.0232	29.87	4.5
Neutral condition			
40	0.0032	216.56	32.91
80	0.0124	55.88	8.43
Boiling under reflux	0.0232	29.87	4.5

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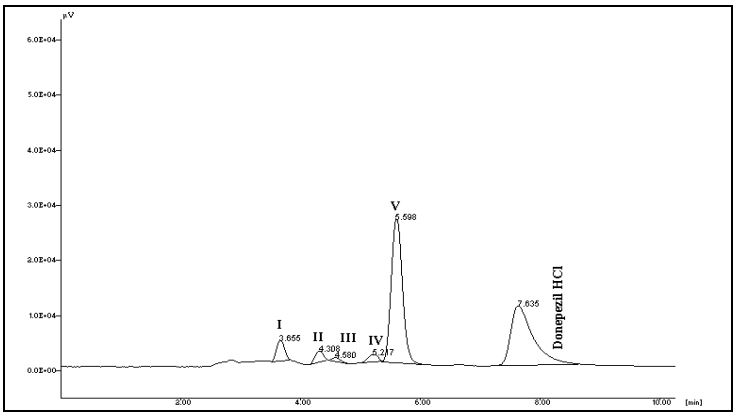
Table 4: Arrhenius plots at different chemical conditions

Chemical condition	Arrhenius plot
2N NaOH	Log K_{deg} = 3.0416 – 1229.6/T (r = 0.9977)
6% H_2O_2	Log K_{deg} = 8.6382 – 3787.2/T (r = 0.9763)
Neutral	Log K_{deg} = 5.6806 – 2269.9/T (r = 0.9844)

Table 5: Summary of the predicted K_{deg}, $t_{1/2}$, t_{90} of donepezil hydrochloride in various chemical conditions as derived from Arrhenius plots at 25 ^{0}C.

Chemical condition	K_{deg} [a] (hr)	$t_{1/2}$ [b] (hr)	t_{90} [c] (hr)	E_a [d] (Kcal/deg mol)	A [e] Frequency factor
2N NaOH	0.083	8.34	1.265	2.443	0.9950
6% H_2O_2	0.00516	134.30	20.41	7.525	0.9716
Neutral	0.011	63.0	9.576	4.51	0.9852

[a]= rate of degradation at 25 ^{0}C, [b=] Half life, [c=] Time left for 90% potency, [d=] Activation energy, [e=] Arrhenius frequency factor



(A)

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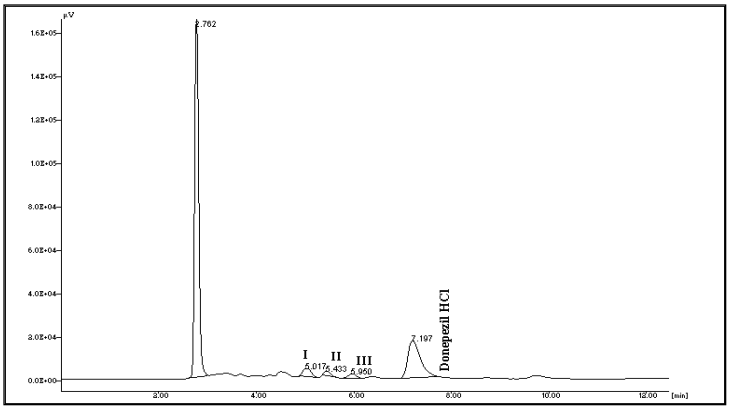

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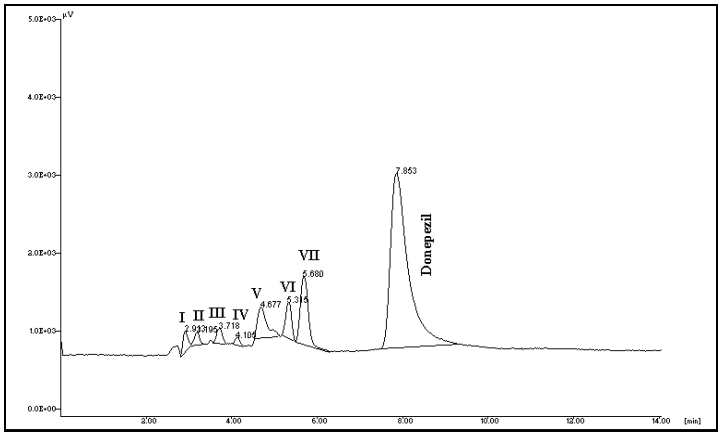
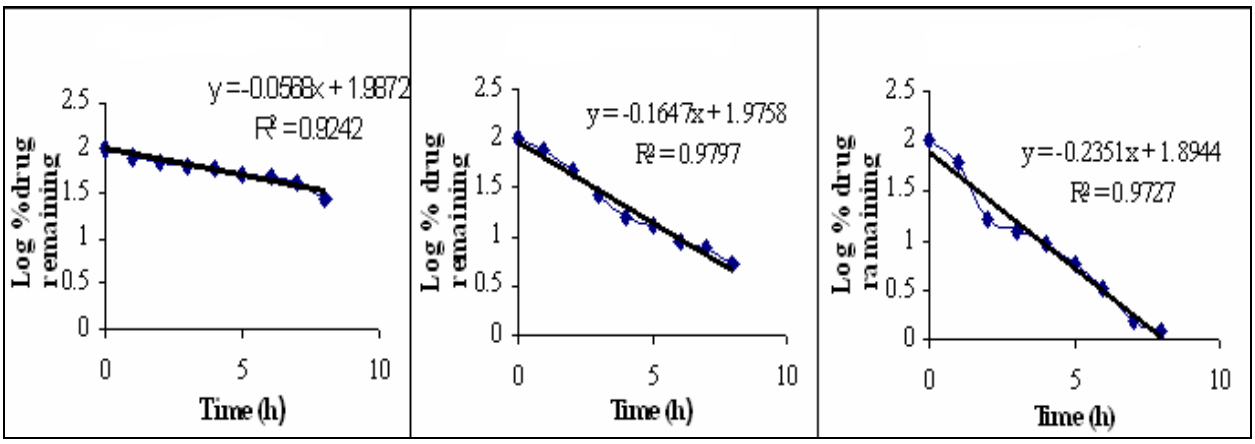


(C)

Figure 1: Representative HPLC chromatogram of donepezil hydrochloride (40µg/ml) degraded in (A) 2N NaOH, (B) 6% H_2O_2 solution, and in (C) neutral solution.



(A) **(B)** **(C)**

Figure 2: First order plot of donepezil hydrochloride at (A) 40 ^{0}C, (B) 80 ^{0}C and (C) boiling under reflux in 2N NaOH solution.

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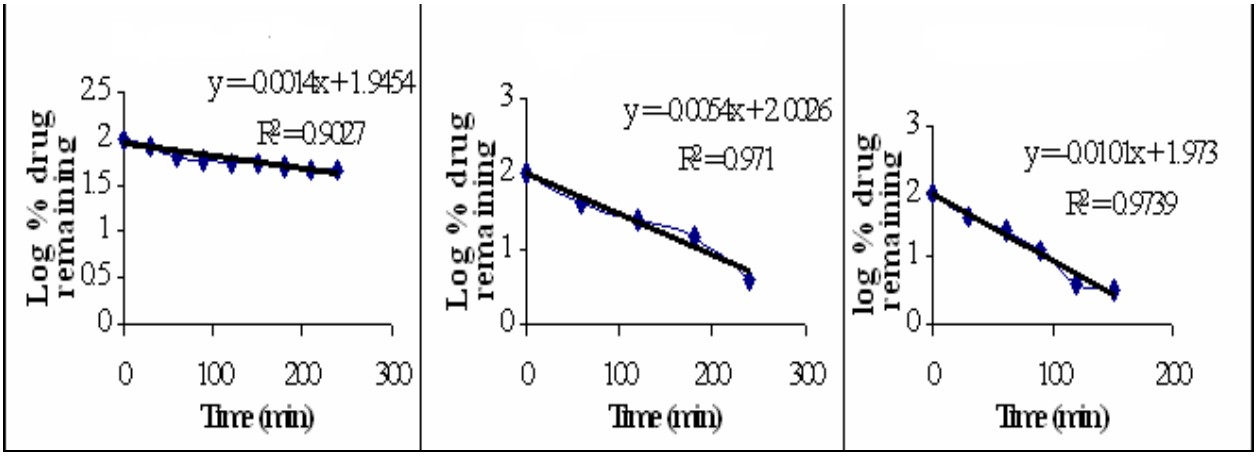
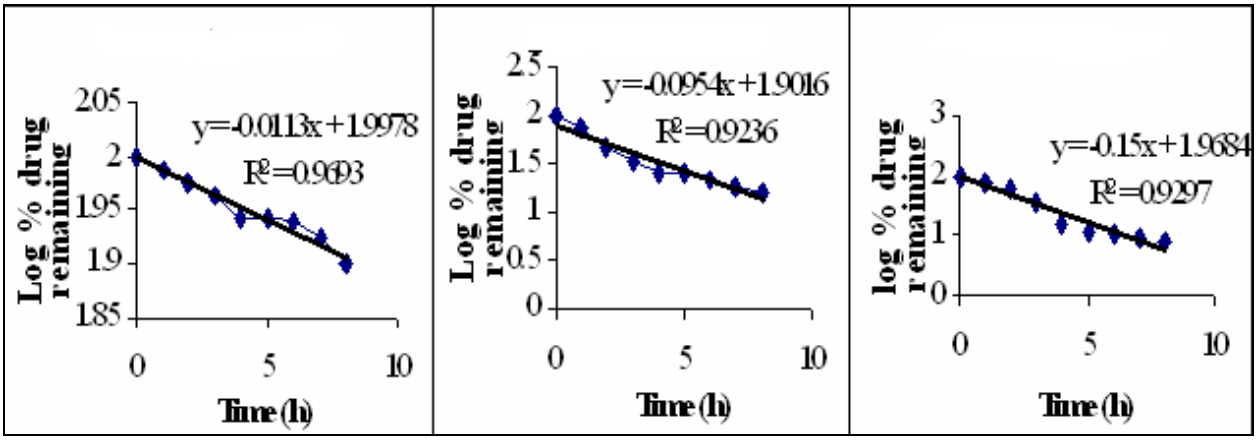


(A) (B) (C)

Figure 3: First order plot of donepezil hydrochloride at (A) 40^{0}C, (B) 80^{0}C and (C) at boiling under reflux in 6% w/v H_2O_2 solution.



(A) (B) (C)

Figure 4: First order plot of donepezil hydrochloride at (A) 40^{0}C, (B) 80 ^{0}C and (C) at boiling under reflux in neutral condition.

DISCUSSIONS

The stability of the new donepezil hydrochloride is investigated using stability indicating HPLC procedure. The method permits detection and quantitation of donepezil hydrochloride in the presence of its degradation products. It was subjected to stress conditions as per ICH guidelines. The drug was found to degrade in alkaline, oxidative and neutral conditions while it was found to be stable under photolytic and dry heat conditions. The drug can be analyzed specifically in the presence of different chromophoric degradation products by using isocratic

conditions and mobile phase containing methanol-acetate buffer pH 4.25- triethylamine in the ratio of 50:50:0.6. The method was validated for parameters like linearity, precision, accuracy, specificity and ruggedness.

The degradation rate constant, half-life and t_{90} were predicted for donepezil hydrochloride for each condition and data obtained from degradation kinetics was further treated to get Arrhenius plot. From this study rate of degradation (K_{deg}), $t_{1/2}$, t_{90} activation energy (Ea) and Arrhenius frequency factor (A) at 25^0C were calculated for donepezil hydrochloride. Degradation kinetics study showed higher degradation rate and shorter half-life in 2N NaOH compared with 6% H_2O_2 and neutral solution.

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