Degradation Studies on Bronopol in Oral Hygiene Formulations

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ABSTRACT
This study investigated the decomposition of Bronopol and its degradation products how they produce Nitrosamines in oral hygiene products. Based on this study Bronopol was degradable in oral hygiene products which are containing aqueous medium, higher pH values (4 to10) and which are presented in higher temperatures and then gives formaldehyde, and nitro compounds. These nitro compounds are acting as a nitrosating agent and react with secondary amines then give Nitrosamines in the products. The degradation study was done by using determination of mass by LCMS and determination of formaldehyde content by Head-Space GC. These studies strongly demonstrate the Bronopol was degradable in aqueous medium and give formaldehyde and nitro compounds. Bronopol was not acting itself as a nitrosating agent and its degradation products act as a nitrosating agent. The products which are containing the secondary amines and the nitrosating agent then the Nitrosamines are formed in that product.

Keywords: Bronopol, Degradation, Nitrosamines, Formaldehyde, Oral hygiene products.

INTRODUCTION
Bronopol is an organic compound also called as a 2-bromo-2-nitropropane-1, 3-diol (Molecular formula: C(Br)(NO₂)(CH₂OH)₂). In cosmetics Bronopol is used as an antibacterial preservative to prevent spoilage due to microbial contamination [1]. Bronopol structure was shown in fig1. Bronopol is an active against a broad spectrum of bacteria, including gram-negative species that are resistant to many antibacterial agents ([2], [3], [4]). Many countries are restricted the usage of Bronopol in cosmetic products [5], because under conditions where it decomposes (alkaline solution and/or elevated temperatures) and liberate nitrite.
and low levels of formaldehyde, these decomposition products can react with any contaminant secondary amines or amides in a personal care formulation to produce significant levels of nitrosamines may therefore cause consumer exposure to a recognized carcinogen ([6], [7]).

![Fig1: The structure of Bronopol](image1)

Bronopol was degraded in to formaldehyde (Molecular formula: HCHO), 2-Bromo, 2-Nitroethanol (Molecular formula: CH(Br)(NO$_2$)-CH$_2$-OH) and tris-(hydroxyl methyl)-nitro methane (Molecular formula: C(NO$_2$)(CH$_2$-OH)$_3$).

![Fig2: The structure of 2-Bromo, 2-Nitroethanol](image2)

![Fig3: The structure of tris-(hydroxyl methyl)-nitro methane](image3)

C(Br)(NO$_2$)(CH$_2$OH)$_2$ → HCHO + CH(Br)(NO$_2$)-CH$_2$-OH
CH(Br)(NO$_2$)-CH$_2$-OH+HCHO+H$_2$O → HCOOH+C(NO$_2$)(CH$_2$-OH)$_3$+HBr

Compare with primary and tertiary amines, secondary amines are more reactive in Nitrosamines formation process. Primary alkyl amines react with nitrosating agents to give short-lived, highly reactive diazonium ions (the species also formed by metabolic activation of nitrosamines). These reactive intermediates decompose to give molecular nitrogen by substitution, elimination, and molecular rearrangement pathways. Nitrosamines may also be formed from tertiary amines. The transformation requires the cleavage of the carbon-nitrogen bond of one of the alkyl groups attached to the nitrogen atom. The rates and the nature of these processes depend significantly on the structure of the tertiary amine. Any compound containing the secondary amine functional group is expected to react with nitrosating agents to produce a nitrosamine. The extent of nitrosamine
formation will depend upon the concentrations of the amine and the nitrosating agent as well as structural features of the amine influencing reaction rates [8].

In these degradation products tris-(hydroxyl methyl)-nitro methane act as a nitrosating agent and formaldehyde act as a catalyst in the formation of Nitrosamines. If the product containing secondary amines these nitrosating agent reacts with secondary amines and give corresponding Nitrosamines [8]. For example if the product containing diethanolamine in presence of Bronopol degradation products it forms N-Nitroso - diethanolamine (NDELA), product containing Bis (2-hydroxypropylamine) in presence of Bronopol degradation products it forms N-Nitroso – bis (2-hydroxypropylamine)(NBHPA). Similarly N-Nitroso-dimethyamine (NDMA), N-Nitroso-diethylamine (NDEA), N-Nitroso-N-methyl-N-dodecylamine, N-Nitroso- morpholine (NMOR), N-Nitroso-pyrollidine (NPYR), N-Nitroso-bis (2-hydroxypropylamine)(NBHPA) Nitrosamines are major Nitrosamines formed in the cosmetic products.

\[
\text{NO}_2^- + 2\text{H}^+ \rightarrow \text{NO}^+ + \text{H}_2\text{O} \\
(\text{Nitrosating Agent}) \quad (\text{Nitrosonium electrophile})
\]

\[
\text{R}_2\text{NH} + \text{NO}^+ \rightarrow \text{R}_2\text{N}-\text{NO} + \text{H}^+ \\
(\text{Secondary amine}) \quad (\text{Nitrosamine})
\]

In the presence of acid or heat nitrosamines are converted to diazonium ions. That diazonium ion reacts with biological nucleophiles (such as DNA or an enzyme) in the cell. If this nucleophilic substitution reaction occurs at a crucial site in a biomolecule, it can disrupt normal cell functions, leading to cancer or cell death [9].

\[
\text{R}_2\text{N-N}=\text{O} + \text{(acid or heat)} \rightarrow \text{R-N}_2^+ \\
(\text{Nitrosamine}) \quad (\text{Diazonium ion})
\]

\[
\text{R-N}_2^+ \rightarrow \text{R}^+ + \text{N}_2 + :\text{Nu} \rightarrow \text{R-Nu} \\
(\text{Diazonium ion}) \quad (\text{Carbocation}) \quad (\text{Biological nucleophiles})
\]

The presence of Nitrosamines in the cosmetic products was hazard to the humans. If we are using Bronopol as a preservative then the possibility is there for formation of nitrosamines. In this study for the determination of formaldehyde content, we used Head-space GC with FID method [10]. We did this study for the degradation of Bronopol and the formation of nitrosamines in oral hygiene products, when we are using Bronopol as a preservative.

**MATERIALS AND METHODS**

**Apparatus**

UFLC-Sil 20AC HT prominence Auto sampler, LC-20AD pump, SPD-M20A Diod Array Detector, Make: Shimadzu, LCMS (API-2000, Turbo spray source, triple quadruple analyzer), ODS Hypersil column( 250 x 4.6mm, 5µ, Make: Thermo), Gas Chromatography with FID and AOC-20i Auto injector (model: GC-2010 Plus, make: Shimadzu), Column-ZB-624 (Length-30m, Diam-0.32mm, Film-1.80µm, Make: phenomenon), Head-Space Gas Chromatography (Model: Versa, Make: Teledyne Tekmar), 20 mL crimp cap Head-Space GC vials, Crimper, Sonicator (model: Soltec, Make: Sonica), Electronic Weighing Balance (Model: ML204 1a01, Make: Mettler Toledo), Volumetric flasks (100± 0.2mL, Make: Borosil).
Chemicals and Reagents
Acetonitrile (HPLC grade) (Make: Rankem), Formaldehyde solution (37%) (ARgrade) (Make: Sigma-Aldrich), Tetrahydrofuran (ARgrade) (Make: sd-fine-chem-limited), O-(2,3,4,5,6-Pentafluoro benzyl) hydroxyl amine hydrochloride (Analytical grade) (Alfa-Aesar), Sodium Chloride (AR grade) (Make: Rankem) and water (HPLC Grade).

LCMS Analysis
We prepared the 1% Bronopol solution in water, in methanol and in acetonitrile for standard. We prepared the 1% oral hygiene products solution (in which product Bronopol used as a preservative) in water, in methanol and in acetonitrile. Analysed the Bronopol standard solution and sample solution (oral hygiene products solution) in LCMS using ODS Hypersil column, 80% water and 20% acetonitrile as a mobile phase with 1.0mL/minute flow rate. In negative mode analysis, we got m/z 167.59 with bromo pattern. Based on LCMS data Bronopol was degraded and then gave 2-Bromo, 2-Nitroethanol. The mass spectrum for Bronopol standard was shown in fig4 and the mass spectrum for cosmetic product was shown in fig5. The oral hygiene products which are having Bronopol as preservative and amine free ingredients that samples were analyzed by LCMS for nitrosamines determination, but we found the results bellow detection limit for nitrosamines like N-Nitroso-Diethanol Amine, N-Nitroso-Dimethyl Amine, N-Nitoso-Diethyl Amine (the method detection limit for the corresponding Nitrosamines were 0.05ppm). So, based on that, amines required for react with nitrosating agent and formation of Nitrosamines.

Fig 4: Mass spectrum of Bronopol standard
Fig 5: Mass spectrum of oral hygiene products

**Formaldehyde content Determination by Head-space GC**

For the determination of formaldehyde content we used derivatization process by O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride. Formaldehyde content analyzed by using Head-space GC with ZB-624 capillary column and nitrogen carrier gas. In this method internal standard (tetrahydrofuran) was used. In gas chromatography injector temperature 180 °C, detector temperature 240°C, column program initial 80 °C and hold 5 minutes, increase 12 °C up to 150 °C and hold 4 minutes then increase 30 °C up to 220 °C and hold 12 minutes, column flow rate 1.3mL/minute and split ratio 10 were used. In Head-space GC cycle time 40 minutes, volume oven temperature 110 °C, transfer line temperature 140 °C, Platen/Sample temperature 80 °C, sample mixing time 20 minutes were used. For this study we used 0.1% Bronopol standard solution in water and 0.1% oral hygiene product (Its containing Bronopol as a preservative 0.05%, water 40% and PH 5.5) solution in water. We performed this study after solution preparation of Bronopol standard and oral hygiene products immediately, 2nd day of the preparation and 8th day of the preparation for formaldehyde determination in room temperature and 5th day of the preparation in 60°C. We found formaldehyde content in the aqueous solution of Bronopol standard and Bronopol containing oral hygiene products. Based on this study Bronopol was degrading in aqueous medium and release formaldehyde. The results are shown in table 1.

<table>
<thead>
<tr>
<th>Name of the sample solution (0.1% in water)</th>
<th>1st Day formaldehyde content (RT)</th>
<th>2nd Day formaldehyde content (RT)</th>
<th>8th Day formaldehyde content (RT)</th>
<th>5th Day formaldehyde content (60°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral hygiene product</td>
<td>0.0001%</td>
<td>0.0026%</td>
<td>0.0022%</td>
<td>0.0075%</td>
</tr>
<tr>
<td>Bronopol Standard</td>
<td>0.3870%</td>
<td>2.00%</td>
<td>2.42%</td>
<td>5.79%</td>
</tr>
</tbody>
</table>

As per theoretical one molecule of Bronopol degraded and give one molecule of formaldehyde. Based on that Bronopol degradation was given in table 2.
Table 2 Degradation of Bronopol in percentages

<table>
<thead>
<tr>
<th>Name</th>
<th>RT(8th Day)</th>
<th>60°C(5th Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral hygiene products</td>
<td>29.33%</td>
<td>100%</td>
</tr>
<tr>
<td>Bronopol standard</td>
<td>16.13%</td>
<td>38.60%</td>
</tr>
</tbody>
</table>

Based on this study Bronopol was degradable with temperature, pH and aqueous medium.

CONCLUSION

This study strongly says the Bronopol was degrading in aqueous solution in to formaldehyde and nitro compounds. The degradation was increased with increase of temperature and aqueous medium. If the Bronopol used as a preservative in oral hygiene products which are having aqueous medium, that Bronopol should be degradable. If these products are having amino compounds (secondary amines), these amino compounds are react with Bronopol degardent (Nitrosating agent) and form nitrosamines. As per best of my knowledge this study gave more information regarding degradation of Bronopol and formation of nitrosamines in oral hygiene products. So, this study is more useful for usage of Bronopol in oral hygiene products.

REFERENCES