Stability of para-aminobenzoic acid in cell culture media

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ABSTRACT

Para-aminobenzoic acid (PABA) is a compound commonly added to chemically defined (CD) cell culture media and is utilized by cells in the pathway of folate synthesis (Figure 1 and 2) [1]. PABA is expected to be stable under media manufacturing and storage conditions. However, PABA in some CD formulations showed unexpected low recoveries. CD media stored at 4°C for 30 months showed PABA dropped to <20% of the theoretical levels. In an aqueous mixture of D-glucose and PABA heated at 100°C for 4 hours, 15.7% of PABA was recovered. Similar observation was made when PABA and D-glucose were milled and stored at 35°C, 55% relative humidity (RH) for four weeks. PABA reacted with D-glucose to give 4-carboxyphenyl-D-glucosamine during storage and under study conditions. The reaction product was confirmed by HPLC-MS analysis via comparison with the authentic sample synthesized in the lab.

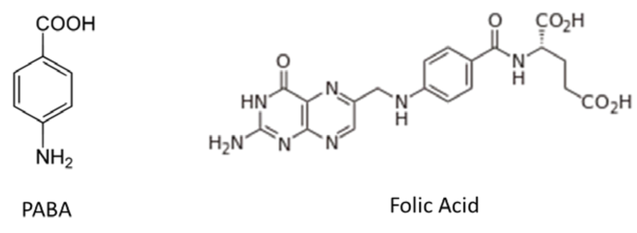

Figure 1: PABA and folic acid.

INTRODUCTION

Para-aminobenzoic acid (PABA) is an aromatic compound that is sometimes referred to as vitamin H1, Bx, or B10. Most water-soluble vitamins used in cell culture media undergo chemical degradation when exposed to excessive heat, moisture, light, and oxidizing/reducing conditions. PABA, however, is a very stable molecule (resonance-stabilized, weak base, and nucleophile). PABA is used as a UV filter in personal care products, such as sunscreen, and is difficult to degrade in surface water or wastewaters using conventional treatment methods [2]. It can only be degraded through advanced oxidation processes involving UV/Fe2+/persulfate.

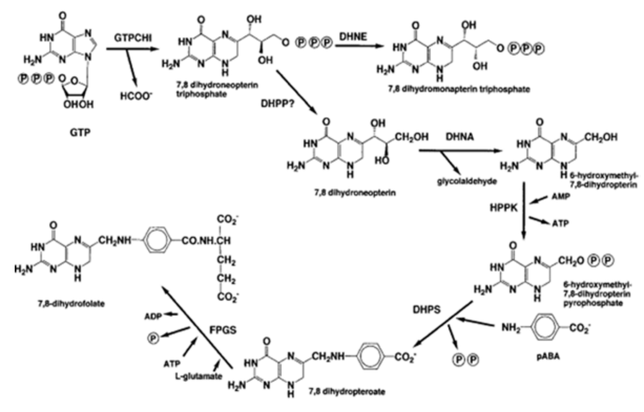

Figure 2: Biosynthesis of 7,8-dihydrofolate.

MATERIALS AND METHODS

PABA and vitamin analysis

- Vitamins, including PABA and any reaction product(s), were separated by an Agilent™ 1100 reversed-phase HPLC and detected by UV (absorbance wavelength for PABA = 270 nm) or mass spectrometry.

Accelerated studies

- Aqueous solutions of PABA, as an individual or mixture of components, were refluxed at 10X the concentration found in CD media for 4 hours.
- A powder mixture of PABA and D-glucose (milled or unmilled) was incubated in a stability chamber at 35.5 °C and 55% RH for 7-30 days.

Synthesis of carboxyphenyl-D-glucosylamine

- Carboxyphenyl-D-glucosylamine was synthesized by condensation of molar equivalents of para-aminobenzoic acid with D-glucose in the presence of glacial acetic acid as a catalyst (Figure 3) [3].

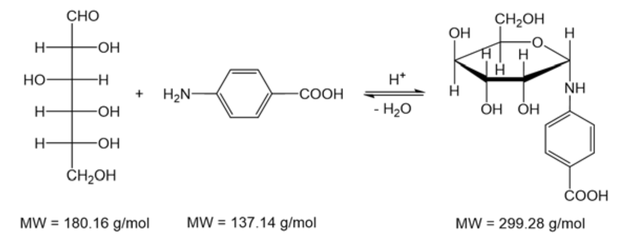

Figure 3: Synthesis of carboxyphenyl-D-glucosylamine.

RESULTS AND DISCUSSION

Investigation of lower PABA recoveries chemically defined media

- For chemically defined media stored for approximately 30 months under refrigerated conditions, HPLC analysis revealed that all vitamins were in the 80-120% recovery range, except PABA, which had a low recovery of <20%

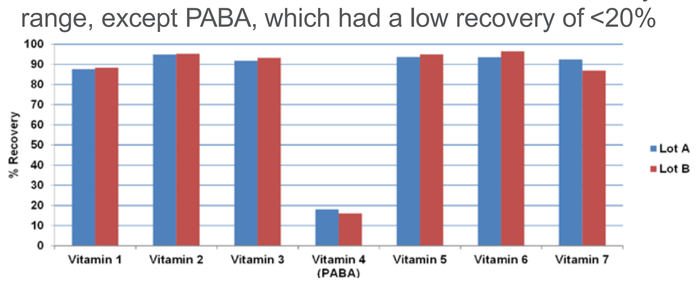

Figure 4: % Recovery of vitamins for 2 lots of the same chemically defined medium stored for 30 months at 2-8°C.

Defined components

- Except D-glucose, selected cell culture media components did not react with or degrade PABA. PABA reacted with D-glucose under forced conditions (4 hrs at 100°C in aqueous solution) resulting in 15.7% recovery of PABA compared to control (Table 1).

Reactant	Control	Ferrous Sulfate heptahydrate	Glutathione reduced	Glutathione reduced and sodium selenite	Riboflavin and tryptophan	D-glucose
% PABA recovery	100	98.4	103.6	98.2	98.2	15.7

Table 1: % Recovery of PABA when refluxed with media components.

- PABA reacted with D-glucose during ball milling (40 min) and during storage at 35°C, 55% RH for four weeks, resulting in approximately 77% and 6.8% recovery of PABA, respectively (Table 2).

Sample	% PABA recovery
PABA+D-glucose – Time 0	77.1
PABA+D-glucose – Week 1	22.2
PABA+D-glucose – Week 4	6.8

Table 2: % Recovery of PABA in milled mixture of PABA and D-glucose.

HPLC and LC-MS: monitoring formation of unknown peak (compound)

- HPLC-MS analysis of PABA showed that the level of PABA, reduced with storage time and unknown peak, increased during the same time (Figure 5A). The unknown peak has a mass of 300 m/z.

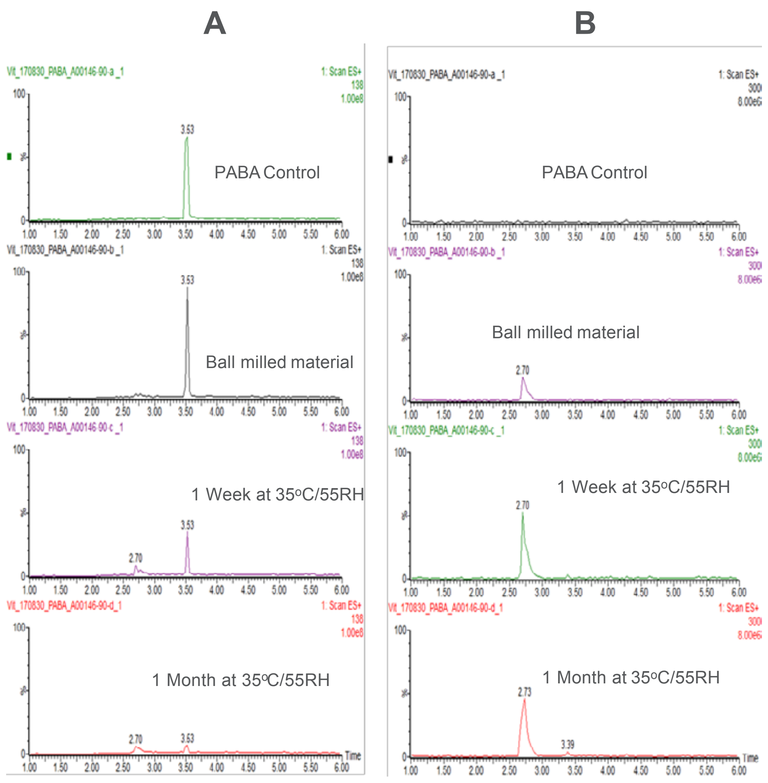

Figure 5: Decrease in the intensity of PABA (A) and increase in the intensity of an unknown peak (B) with a retention time of 2.7 min.

LC-MS: identification and confirmation of the unknown peak in CD media

- Based on the reaction mechanism, the unknown peak was proposed to be carboxyphenyl-D-glucosylamine.
- The proposed structure of carboxyphenyl-D-glucosylamine was confirmed by LC-MS analysis via parent and fragment ion match with the authentic samples (Figure 6).

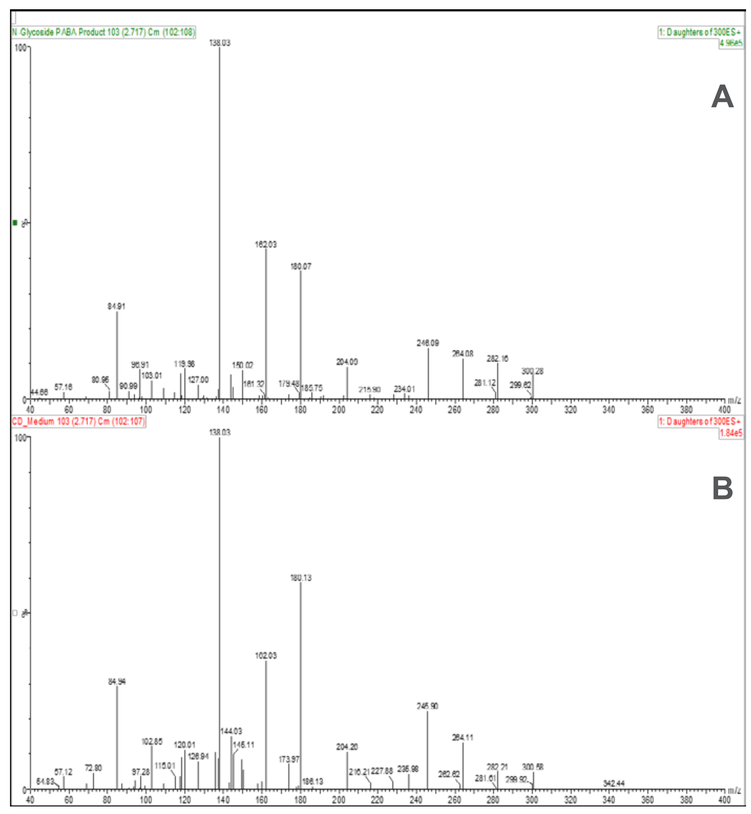

Figure 6: Mass spectrum of (A) synthesized carboxyphenyl-D-glucosylamine and (B) the unknown peak in CD medium at RT 2.7 min.

CONCLUSIONS

- In chemically defined cell culture media that contain PABA, the presence of D-glucose will lead to reduced PABA stability.
- PABA reacts with D-glucose thermodynamically to give carboxyphenyl-D-glucosylamine.
- Reaction of PABA with D-glucose was confirmed using an authentic sample synthesized in the lab and HPLC/LC-MS analysis.
- It is recommended that D-glucose and PABA should not be in the same CD cell culture media.

REFERENCES

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