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A NOVEL SYNTHESIS OF MAGNESIUM ENRICHED ARTIFICIAL SWEETENERS

Ainy Ilyas[1], Dr. Arjumand Iqbal Durrani [2]
Department of chemistry, University of Engineering and Technology Lahore, Pakistan.
Email: Princessgul.pg@gmail.com[1] arjumand@uet.edu.pk[2]

ABSTRACT: *Mg deficiency is the leading cause of hypertension and obesity. The goal of this project was to formulate magnesium in sweeteners to suppress Mg deficiency in humans. The sweeteners selected for this purpose were Saccharin, Sorbitol & Xylitol, approved as GRAS by the FDA. This formulation was done by synthesizing Mg- saccharin, Mg-sorbitol & Mg-xylitol complexes. Mg $(NO_3)_2.6H_2O$ was allowed to react with selected sweeteners under the pH = 9 with different molar solutions of NaOH. The percentage yield of Mg-saccharin was 76%, Mg- xylitol was 75% and Mg-sorbitol was 84%. The Proximate analysis such as ash content, moisture content, crude protein content, crude fiber content, and energy value was also calculated. Mg-saccharin complex gave a sharp peak at 1411cm^{-1}, for Mg-xylitol the peak value was 1625.214cm^{-1} while Mg- sorbitol showed a broad peak at 3271cm^{-1} during IR analysis. The resulting compounds were further characterized by AAS and HPLC analysis.*

Keywords: Artificial sweeteners, Magnesium, magnesium nitrate, sugar complexes.

INTRODUCTION

Sugar alternatives are substances that are utilized instead of sugars with sugar (sucrose) or sugar alcohols. They may likewise be called counterfeit sugars, non-nutritive sugars (NNS), and non-caloric sugars. Sugars are delivered to be utilized in a few items running from treats to sodas, so as to fulfill the shoppers. Sweet substances are mixes with assorted concoction structures and sizes, for instance, sulfonyl amides (saccharin), sugars (sucrose), sugar alcohols (xylitol and sorbitol), proteins (thaumatin), D-amino acids (D-tryptophan) and peptides (aspartame) (Das, A., &, Chakraborty, R. 2018). Remarkably, Brew Economics Handbook (2017) revealed that the world utilization of artificial sugars was evaluated to be in excess of 159,000 metric tons with a market estimation of USD $2 billion. China was distinguished as the nation which expended the most counterfeit sugars (32%), trailed by Asia (23%), United States (23%), Europe (12%) and Africa (7%). What's more, refreshment items showed aspartame has the most elevated use with 18.5 thousand metric tons pursued by saccharin (9.7 thousand metric tons), acesulfame (6.8 thousand metric tons) and sucralose (3.3 thousand metric tons). Magnesium is the eighth-most basic component in the outside layer of the Earth and its common minerals are magnesite and dolomite. It is an essential alkaline earth metal that is required by the body for more than 300 enzymatic biochemical metabolisms. Magnesium salts break down effectively in water and are a lot more solvent than the particular calcium salts. Therefore, magnesium is promptly accessible to living beings.

Classification of artificial sweeteners

Artificial sweeteners are a vast branch of sweeteners. They are classified as nutritive and non-nutritive as showed in the figure.

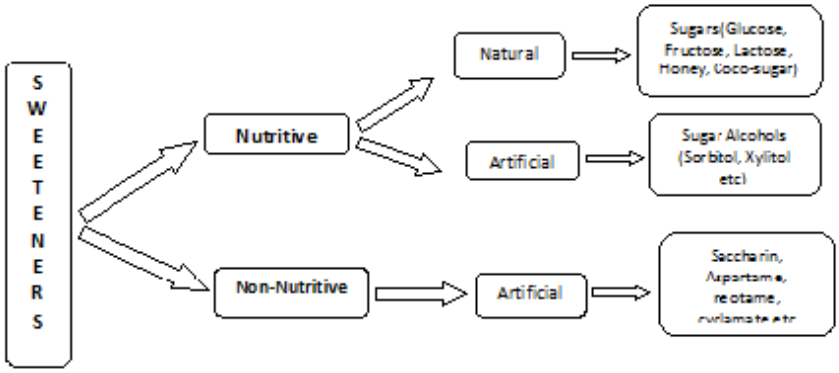

Figure 1 shows the types and classifications of the sweeteners

LITERATURE REVIEW

Artificial sweeteners are low- or no-calorie sugar substitutes that provide little nutritional value, but then modify the flavor of foods or beverages so that they become more appealing to the consumer. These sweet alternatives can help to cut calories on anything from cookies to iced tea. While magnesium helps to maintain normal nerve and muscle function, supports a healthy immune system, keeps the heartbeat steady, and helps bones remain strong. It also helps adjust blood glucose levels. However, studies showed that a major part of the population is unaware of their Mg deficiencies. As no work was done in the past in this regard, therefore, this research not only focuses on the importance of magnesium but also it's used with artificial sweeteners for wellbeing of the world. Fake sugars were found fortunately in research center mishaps, man's mission for sweetness in nourishment and beverages, combined with a craving for low-caloric sustenance's, has kept on starting the quest for new counterfeit sugars. The vast majority of the sugars have encountered stormy and questionable accounts as different specialists have investigated conceivable unfavorable impacts from their utilization (Sardarodiyan, M., & Hakimzadeh, V., 2016). U.S sustenance and Drug organization have demonstrated some counterfeit sweeteners for use according to satisfactory day by day allow esteem (ADI). These sweeteners incorporate acesulfame-K, neotame, cyclamate aspartame, and alitame. On the opposite side, monosaccharides are seemed, by all accounts, to be uncommon sugars that do not utilize in our body (Sanchari, C., Utpal, R & Runu C., 2014). The improvement of new artificial sugars has expanded the potential for supplanting sugar in prepared merchandise. Individual types and measures of fake sugar will cause modifications in appearance, surface and, taste when contrasted with sucrose. The goal is to show the different outcomes when substituting 25%, 33%, half, and 100% parts of, maltitol, mannitol sucralose, xylitol, isomalt and stevia for sucrose. The outcomes yielded rise, appearance, surface, delicacy, and flavor contrasts among the fake sugars when contrasted with the 100%

sucrose cupcake by a trial board (Edelstein, et, al, 2008). A to a great extent utilized fixing in the sustenance business is sorbitol. Truth be told, sorbitol is low-calorie sugar liquor which is utilized on the modern scale because of its sweetness and high solvency. Amid the union of sorbitol, it is discovered that a lactic corrosive bacterium Lactobacillus Plantarum delivers sorbitol by returning the catabolic pathway of sorbitol in a distorted strain lacking for both L-and D-lactate dehydrogenase exercises. Around 25% of sugar is changed over into sorbitol after streamlining under precise PH (Victor, et, al, 2017). Sugar-free sustenances are particularly outstanding as a direct result of their less calorie content. Thusly, instead of greasy sugar distinctive fake sugars that are low in calorie content are used by the differing adventures. As a matter of fact, the articulation "fake sugars" or low-calorie sugars implies the sugars that are built and can in like manner be gotten from the customary substances, for instance, herbs or sugar itself. As they are normally better than the standard sugar so they furthermore called genuine sugars. As such, in the taking care of and limit of sustenance, it is essential to grasp their science in association with their quality in these sustenance things (Kapadiya, D and Aparnathi, K, D, 2017).

Magnesium (Mg^{+2}) is a fundamental particle to the human body, assuming an instrumental job in supporting and continuing wellbeing and life. As the second most plentiful intracellular cation after potassium, it is associated with more than 600 enzymatic responses including vitality digestion and protein blend (Jeroen, et, al, 2015). The negative effects of magnesium deficiency suggests the possible role of hypomagnesaemia as cardiovascular risk factor and the use of serum magnesium level for the screening and prevention of cardiovascular diseases. Moreover, it might help with the identification of new therapeutical strategies for the management of cardiovascular disease through magnesium supplementation (Paolo et, al, 2018). As a cofactor in various enzymatic responses, magnesium satisfies different intracellular physiological capacities.44 Vitamin D and magnesium (Mg) is the absolute most contemplated themes in medication with gigantic ramifications for human wellbeing and infection. Mg and vitamin D are utilized by every one of the organs in the body, and their insufficiency states may prompt a few ceaseless ailments. In light of the epidemiologic examinations, ∼75% of all grown-ups worldwide have serum 25(OH) D degrees of <30 ng/mL.45. Magnesium is a basic component required as a cofactor for more than 300 enzymatic responses and is along these lines vital for the biochemical working of various metabolic pathways. Lacking magnesium status may impede biochemical forms subject to the adequacy of this component. Developing proof affirms that about 66% of the populace in the western world isn't accomplishing the prescribed day by day stipend for magnesium. (Schwalfenberg, G. K and Genuis, S. J, 2017)

MATERIAL AND METHODS

Prior to use, all the glassware was carefully clean by boiling in a 1:1 solution of nitric acid and sulfuric acid followed by boiling in ultrapure water. Then rinsed gently with ultrapure water and acetone and dried in an oven at 80^{o}C for about 1 hour. All solutions were made in distilled water.

Reagent	Chemical formula	Manufacturer	Grade/Purity
Magnesium nitrate	$Mg(NO_3)_2$	Merck	Analytical Grade
Sodium saccharine	$C_7H_5NO_3S$	Merck	Analytical Grade
Sorbitol	$C_6H_{14}O_6$	Merck	70% solution
Xylitol	$C_5H_{12}O_5$	Merck	Analytical Grade
Hydrochloric Acid	HCl	Merck	99.99%
Nitric acid	HNO_3	Merck	99.9%
Ethanol	C_2H_5OH	Merck	99.9%
Sodium Hydroxide	NaOH	Merck	Analytical grade
methanol	CH_3OH	Merck	99.9%

Table 1 shows the reagent with chemical formula and manufacturer details

Apparatus:

- Beakers
- Funnel
- magnetic stirrer
- Hotplate
- Glass rod
- measuring cylinders
- Iron Stand
- Measuring flask
- Suction pump

Solutions Required

- 1M methanolic solution of xylitol = 15.21g of xylitol dissolved in 100ml methanol
- 1M methanolic solution of NaOH = 3.99 g of NaOH dissolved on 100ml methanol
- 0.1M ethanolic solution of NaOH = 0.399 g of NaOH dissolved on 100ml ethanol
- 2M $Mg(NO_3)_2$ solution = 29.66g of Mg(NO3)2 dissolved in 100ml distilled water
- 1M ethanolic solution of 70% sorbitol = 19.9 ml sorbitol dissolved in 50 ml ethanol

Characterization technique

The final product was characterized by following quantitative and qualitative techniques and methods

1. AAS
2. FTIR
3. HPLC
4. Proximate analysis
5. Qualitative analysis
6. Energy calculation

Instrumentation

Hot plate:

EHP-001 portable single hot plate was used for concentrating the solution of lactose zinc to ¼ in the experimentation.

Figure 2 shows the hot plate used for solution

PH Meter:

Deluxe pH meter model #151-R was used for pH measurements. The electrode was standardized and calibrated against a standard buffer solution of pH #7 prior to use.

Fourier-transform Infra-red spectrophotometer (IR):

IR ranges were gathered utilizing an Agilent Cary Infrared spectrophotometer. Ranges are gathered applying the recurrence go from 600 to 4000 cm^{1-}. There are three principal areas of infrared range i.e. close to the infrared area, which is likewise called overtone area, second is center infrared area which is vidration-turn area, and third is far-infrared area which is revolution area. 2.5-25 micomerters is the primary area of concern for diagnostic purposes,

Figure 3 shows the Fourier transform infra-red spectrophotometer machine

Atomic Absorption Spectroscopy (AAS):

It is a system where gaseous molecules retain radiations to produce signals. The quality of the sign is legitimately relative to the quantity of retaining molecules present in the sample. Test is changed over in vaporous state by atomizer.

Figure 4 shows the atomic absorption spectroscopy system

Determination of crude protein:

Crude protein of the samples was determined by Kjeldahl method. This method consists of three steps;

- **Digestion** was done in digestion flask. 2g of sample was taken in sample flask. Then added 15ml of H_2SO_4 & 8g of digestion mixture (K_2SO_4, $CuSO_4$; 8:1). Place the flask on heater till the solution becomes clear.solution was cooled and transfeered to the 100ml volumetric flask and diluted it upto the mark with distilled water.
- **Distillation** was done in distillation apparatus. 10ml of disgest was added in the digestion flask. Then 10ml of 0.5N NaOH was added gradually in the flask. Distillation was continued for 10 minutes. NH_3 gas was produced which was collected in a flask containing 20ml of 4% boric acid solution and few drops of methyl red indicator.
- **Titration** was done using distillation solution and titrated against 0.1N HCl solution till pink color appeared.
- A blank was laso run using all these steps and crude protein content was calculated by following formula;

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt. of sample} \times V}$$

Where

S = samples tirating reading B= blanlk reading

N = normality of HCl D= dilution of sample after digestion

V= vol. taken for distillation 0.014= milli eq. wt of nitrogen

Figure 5 shows the Kjeldahl method

Nitrogen free extract
Nitrogen free extract (NFE) was calculated after all other analysis was done. Following formula was used to calculate NFE.
NFE= (100- % moisture+ % crude protein+ % crude fat+ % crude fibre + % ash)

Energy calculation
The percent calories was calculated by multiplying % crude protein and carbohydrate with 4 and crude fat with 9. The values was then converted to calories per 100g of sample.

Qualitative analysis for magnesium

Ash test
The color of the ash was observed by dipping a filter paper strip in a solution of sample and Co $(NO_3)_2$ and burnt it.

Wet test
NH4Cl solution was added to the sample solution. Then the solution was boiled and cooled. Then solution of NH_4OH and $(NH_4)_2HPO_4$ was added into the solution. Color of the precipitates was then observed.

Confirmatory tests
Confirmatory tests for magnesium was done by following procedure:

- NaOH solution was added into the sample solution
- Na_2CO_3 solution was added into sample solution.
- $(NH_4)_2CO_3$ solution was added into the sample solution

RESULTS AND FINDINGS

1. Mg-saccharinato complex:

- **Percentage yield**

Theoretical yield of product = 52.87g/mol
Actual yield of product = 40.23g/mol

$$\% \text{ yield} = \frac{\text{actual yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{40.23}{52.87} \times 100$$

$$= 76\%$$

- **IR analysis**

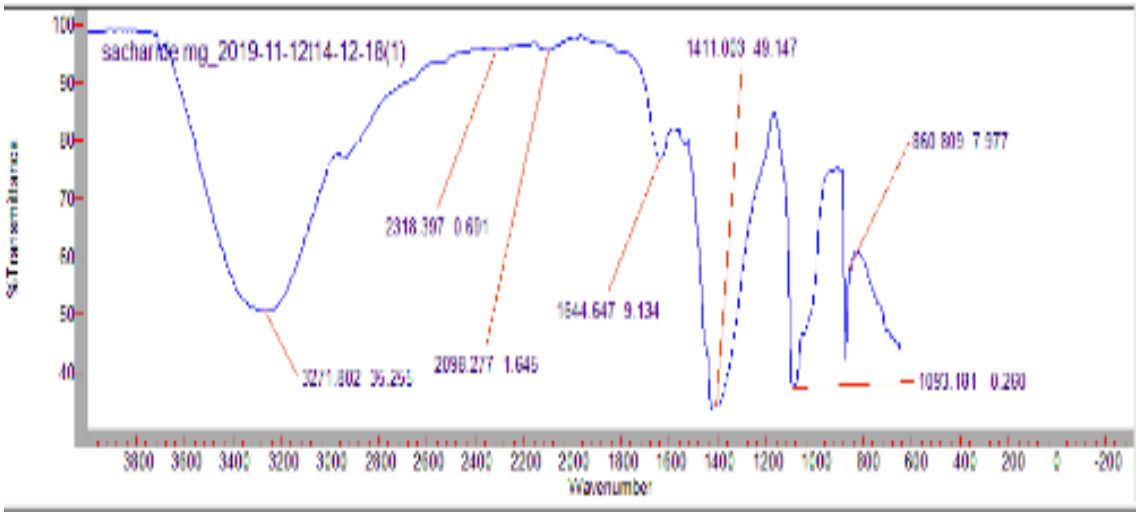

Figure 6 shows the Infra-red reading and analysis bar

HPLC analysis

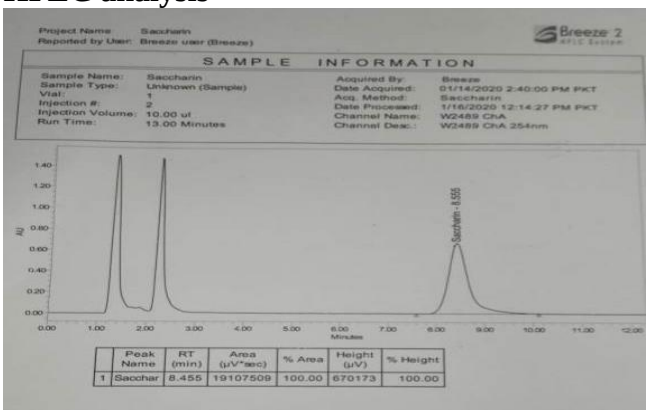

Figure 7 shows the sample information report

Xylitol- Mg complex

- **Percentage yield**

Theoretical yield of product = 40.15g/mol
Actual yield of product = 30.05g/mol

$$\% \text{ yield} = \frac{\text{actual yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{30.05}{40.15} \times 100$$

$$= 75\%$$

- **IR analysis**

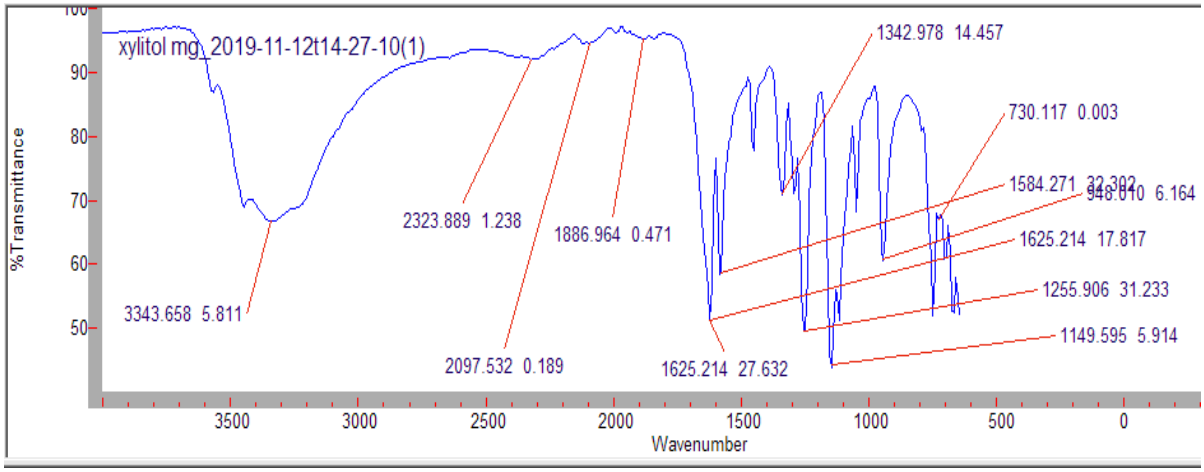

Figure 8 shows the infra-red reading and analysis of xylitol mg-complex

HPLC analysis

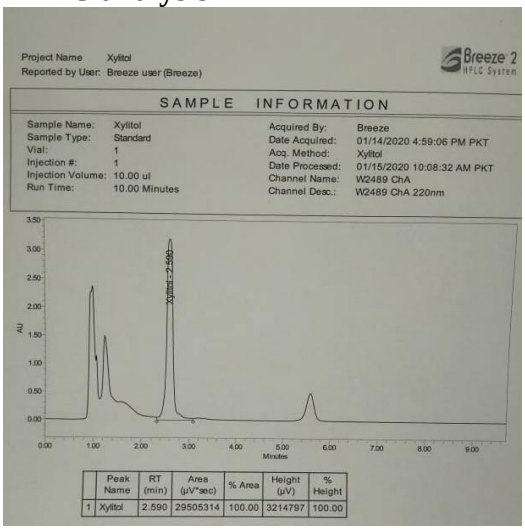

Figure 9 shows the sample analysis report

Sorbitol Mg- complex:

- **Percentage yield**

Theoretical yield of product = 40.75g/mol
Actual yield of product = 35.62g/mol

$$\% \text{ yield} = \frac{\text{actual yield}}{\text{Theoretical yield}} \times 100$$

$$= \frac{34.52}{40.75} \times 100$$

$$= 84\%$$

- **IR analysis**

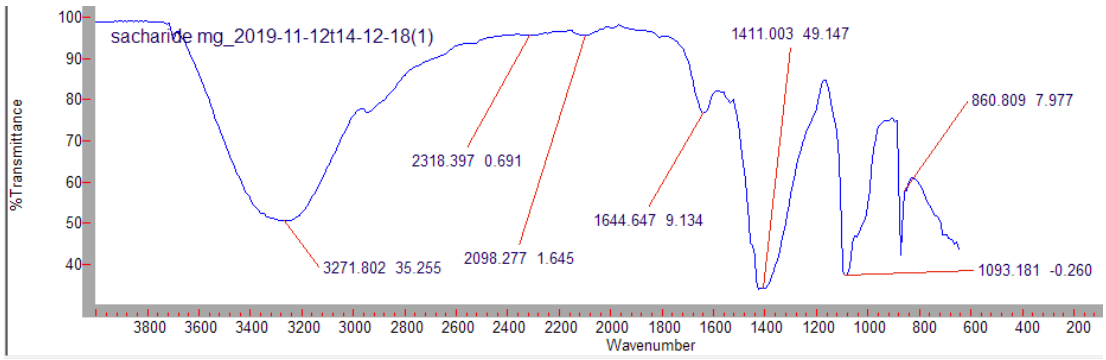

Figure 10 shows the infra-red analysis of sorbitol mg-complex

HPLC analysis

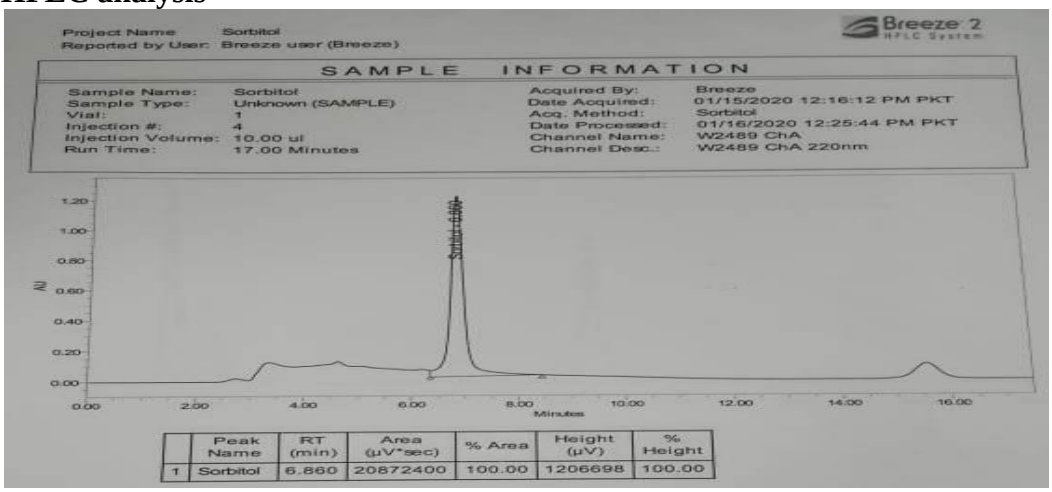

Figure 11 shows the sample information report of sorbitol mg-complex.

AAS

Four Mg-sugar samples are prepared. All the samples are prepared under similar conditions. Atomic absorption spectroscopy technique is employed to quantitatively determine the amount of magnesium in all the samples. The active concentration of magnesium captured in all Mg-sugar complexes such as Mg- saccharine, Mg- xylitol and Mg- sorbitol is demonstrated in the following table.

Table. AAS results presenting magnesium amount in all Mg- sugar complexes discussed in this study

AAS Readings of Concentration (mg/g) of magnesium	
Saccharin- Mg	0.104
Xylitol - Mg	0.13
Sorbitol- Mg	0.118

Table 2 shows the readings of concentration magnesium

Qualitative analysis:

TEST	SACCHARIN-Mg	XYLITOL-Mg	SORBITOL-Mg
Flame test	Pink ash is formed		
Confirmatory tests	White precipitates		
Wet tests	White precipitates		

Table 3 shoes the qualitative analysis of tests

Proximate analysis:

TEST	SACCHARIN-Mg	XYLITOL-Mg	SORBITOL-Mg
Ash Content	5%	0.5%	1%
Moiature content	2.39%	0.75%	3.27%
Crude fat content	0.79%	0.27%	0.75%
Crude protein content	0%	0%	0%
N- free extract	98.18%	98.48%	94.98%
Energy value	1%	8.25%	1.76%

Table 4 shows the proximate analysis of magnesium levels

DISCUSSION

The aim of this research work was to synthesize Mg enriched artificial sweeteners in which Mg was fully fortified with the help of different chemical reagents to give the certain products such as Saccharin complex, xylitol- Mg complex and sorbitol- Mg complex. Saccharin- Mg complex was synthesized by the reaction of Magnesium nitrate hexahydrate with sodium saccharin. The needle like white crystals of Mg-Saccharin complex was appeared within one week. The yield of the observed product was 76%. The analysis was done with methanol:water mobile phase having ratio 85%: 15% with buffer solution of PH=3 having retention time 6.45 minutes with the peak value of 1910750uVwhile the reported retention time for the HPLC analysis of saccahrin was 4-6 minutes with concentration ranges from 10mg/L-100mg/L with mobile phase of phosphate buffer solution: MeCN, 86%: 14%. (Trandafir, I., Nour, V and Ionica, M. E, 2009). It was observed that Xylitol- Mg complex can be conveniently

prepared by the reaction of magnesium nitrate with xylitol. In this reaction methanolic solution was used for precipitates formation. The yield of the product is 74%. Then the compound was subjected to HPLC analysis which shows that the retention time of xylitol is 2.53 minutes with a peak value of 2739341uV while the reported retention time for xylitol 4minutes with concentration ranges from 0.12mg-0.44mg on amino column (Shah, R., Jager, D and Lowri, S, 2017). It was observed that sorbitol- Mg complex can be prepared by the reaction of magnesium nitrate with 70% solution of sorbitol. The reaction was done in the ethanolic solution for the convenient formation of precipitates. The resulting product was gel like material which was then centrifuged and the precipitates were formed after 24 hours. The retention time for Mg-sorbitol was 6.66minutes with the peak value of 20672400uv while the reported retention time for sorbitol was given as 12-14 minutes with ELSD having concentration from 30ug-70ug/mL (Simonzadeh, N and Ronsen, B, 2012). All the samples were subjected to AAS analysis for the determination of Magnesium in the desired compounds. AAS analysis results showed that Mg was fortified in the desired sweeteners. The reported values for the concentration of magnesium for medicated herbs was 2.03ppm- 1.85ppm while in blood the normal value of magnesium was reported as 17.0-28.0mg/L (Akram, et, al, 2015).

CONCLUSION

Magnesium deficiency is now becoming a point to concern of the era due to its adverse effect on physical and mental growth of the people. Here, we have disclosed a novel and simplest method to develop bio-friendly and cost-effective Mg-sweetener complexes by following a modified chemical synthesis. Mg is a metal of importance as it is involved in complex reactions from behaving as a cofactor leading to the maintenance of its content in the body. While artificial sweeteners for last few years emerged as “dietary sugars” and now has become a modern trend. Upon complex formation with Mg these sweeteners showed different coordination chemistry and bonding properties. Here, the various concentration of magnesium nitrate is incorporated into the target sweeteners .i.e. saccharine, sorbitol and xylitol to achieve a maximum yield of the product with significant amount of Magnesium. The percentage yield of Mg-saccharin was 76%, Mg- xylitol was 75% and Mg-sorbitol was 84%. The three compositions i.e. Mg- saccharine complex, Mg- xylitol and Mg- sorbitol complex were tested via different analytical techniques to confirm the formulation of magnesium in the desired products. The peaks in IR analysis were at 1411cm^{-1} for Mg-saccharin complex, 1625.214cm^{-1} for Mg- xylitol while Mg- sorbitol gave a broad peak at 3271cm^{-1}. AAS results showed the amount of magnesium in three complexes as 0.1044mg/g, 0.13mg/g and 0.1184 mg/g for Mg-saccharin, Mg-sorbitol and Mg-xylitol complex while HPLC analysis further confirm the production of complexes. Different qualitative tests were also performed to confirm the presence of magnesium in the sewwteners. These complexes may work as a replacements of the medicines in the future to overcome the magnesium deficiencies without effecting the sugar level of diabetic and hypertension patients.

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