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Research Article

EFFECT OF SOME PRESERVATIVES ON THE AQUEOUS STABILITY OF AN ORAL REHYDRATION SALT SOLUTION INTENDED FOR USE IN DEVELOPING COUNTRIES.

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ABSTRACT

The investigation was concerned with formulating and evaluating the effect of some preservatives on the aqueous stability of an oral rehydration salt (ORS) solution intended for use in developing countries. Nine batches of ORS solutions were prepared. Eight of the batches coded A, A1, B, B1, C, C1, D and D1 were prepared with four different preservatives (Sodium benzoate, Methyl paraben, Propyl paraben and Methyl/propyl paraben) in addition to a flavour and colourant. One batch coded E containing no preservative served as a control. Stability studies were carried out on the prepared solutions after 0, 3, 6, 9, 12, 18 and 24 months. The parameters evaluated include: Physical characteristics (clarity, palatability, odour, colour and mould status/purity) and physico-chemical properties (pH, viscosity, specific gravity and determination of sodium chloride, potassium citrate and glucose contents). Results indicated that all the ORS solutions had good organoleptic and physico-chemical properties at preparation. After 24 months of storage, there were no significant ($P > 0.05$) changes in the potassium citrate and sodium chloride content of all the batches. The percentage glucose content in the batches ranged from 100.05 ± 0.00 to 99.28 ± 0.01 with significant changes ($P < 0.05$) in the quantity of glucose occurring only in B, B1, C, C1 and the control batch (E) after 24 months. The overall results, indicated that only A and A1 which contained sodium benzoate as preservative maintained 99% of all the physical characteristics and stability parameters assessed after 24 months.

Keywords: Oral rehydration salt solution, aqueous stability, sodium benzoate, methyl paraben, propyl paraben.

INTRODUCTION

Diarrhoeal diseases caused by bacteria or viruses are very common among young children and is a leading child killer in developing countries. About 2.2 million children die yearly from dehydration due to diarrhoeal diseases, 80% of them within the first two years of life.[1], [2] Previously, it could only be treated by qualified nurses or doctors using expensive intravenous feeding in an often inaccessible hospital. With the discovery of oral rehydration therapy (ORT), it can easily be treated by a mother or any adult using either a pre-packed formula called oral rehydration salts (ORS) available in a sachet or a simple home solution of sugar, salt (sodium chloride) and water. [3], [4] The major drawbacks to the use of ORS during diarrhoeal disease are cultural practices,[5] lack of parental knowledge,[6] lack of training of medical professionals, and cost of commercially available ORS.[7] Among physicians, preference for IV hydration (even where evidence indicates improved results from oral rehydration) [8], [9] remains a major barrier. The overriding need to make essential drugs available through the simplest and most appropriate technology at an affordable price, has made UNICEF/WHO to primarily concentrate their efforts during the last decade on ORT in powder form. Other presentations of ORT are also available.[10] Of all the presentations available, liquid ORS remain the best choice for fluid and electrolyte replacements. It has a quick onset of action, increased bioavalability, easy usability and is relatively cheap. It is considered the most efficacious oral rehydration therapy especially in locations where good water supply is a major problem and a source of public health concern. Good water supply is a source of public health concern in third world countries such as Nigeria and especially in the northern part of Nigeria where only about 30% of the population has access to safe drinking water.[11] Because of the use of unsafe water for drinking, cooking and washing, there is a relatively high prevalence of preventable water-borne diseases such as cholera, diarrhoea, typhoid and malaria among others with a high impact on infant mortality rates.[11] Formulation of a liquid ORS preparation with a shelf-life of at least 2 years will reduce the need for reconstitution of OR salts with water from a questionable source. This would reduce the prevalence of cholera, typhoid, diarrhoea and other water-borne diseases, as well as add variety to the already existing forms of the therapy in the market. Furthermore, solution dosage forms are prone to a number of stability problems which include hydrolysis, oxidation and microbial attack. In liquid formulations where hydrolysis and oxidation may not constitute problems, contamination and subsequent degradation by micro-organisms (bacteria, fungi etc) may contitute a major stability problem. It is against this background that we formulated and evaluated the effect of some preservatives on the aqueous stability of an ORS solutions meant for use in developing countries.

EXPERIMENTAL

Materials

Sodium chloride, Potassium citrate, Glucose, Potassium chromate (Fluka, USA), Sodium citrate, 1- Napthol benzein, Bromothymol blue, Phenolphthalein, Sodium hydroxide (Sigma-aldrich, USA), Sodium benzoate, Methyl paraben, Propyl paraben, Sodium metabisulphite (Merck, Germany), Refractometer (RFM 470, Bellingham and Stanley), digital pH meter (Somtex T5-2, Taiwan).

Methods

Nine batches of ORS solutions were prepared according to the formula shown in Table I. Eight of the batches coded A, A1, B, B1, C, C1, D and D1 were prepared with four different preservatives (Sodium benzoate, Methyl paraben, Propyl paraben and Methyl/propyl paraben) in addition to a flavour and colourant. One batch coded E containing no preservative and colorant served as the control. A 1000 mL of distilled water was heated to 100°C in a beaker and 400 mL portion was allowed to cool to 80°C. Starting with the preservative, the solutes were added individually to the solvent in a mixing vessel and stirred continously until dissolution was complete. The mixture was filtered using a filter cloth with 0.22 mm aperture and the filterate allowed to cool to ambient temperature (30°C). The colourant and the flavour were added to the solution. The solution was made up to 1000 mL volume with distilled water and distributed in sterile 500 mL colourless bottles.

Product Evaluation

Stability testings of the ORS solutions was carried out by monitoring changes in the organoleptic properties, physico-chemical properties and content of the active substances over a 24 month time interval. The content of sodium chloride, potassium citrate and glucose were assayed using non-aqueous titrations (method A).[12, 13]The stability of the ORS preparations was monitored under ambient

temperatures of 30°C. The test for microbial contamination was performed using the direct inoculation method as described in the 4th edition of the International Pharmacopoeia (3.2 Test for sterility).[13] Stability studies were carried out on the prepared solutions after 0, 3, 6, 9, 12, 18 and 24 months. Parameters evaluated include; Physical characteristics (clarity, palatability, odour, colour and mould status) and physico-chemical properties (pH, viscosity and specific gravity and assays of sodium chloride, potassium citrate and glucose). The mean, standard deviation and standard error of the mean of three replicate determinations were calculated.

Solutions were considered to be stable over the time period studied (24 months) if there was no statistically significant difference at the 95% confidence level in the mean percentage assays between the initial time point (0 month) and that under consideration or the mean difference between the sets of results was less than 2.0%.

Quantitative Assay

Assay of Sodium Chloride

A 20 mL volume of each of the prepared ORS solutions was pipetted into a conical flask. Two drops of already standardized Potassium chromate indicator was added. The solution was titrated using 0.1M Silver nitrate. The end point (i.e the first excess of titrant in the formation of a red Silver chromate precipitate) was noted. Appropriate calculations were done to ascertain the amount of Sodium chloride contained in 1000 mL of the solution (where each ml of 0.1M Silver nitrate is equivalent to 0.005845g of Sodium chloride).

Assay of Potassium Citrate

Preparation of 0.1N Perchloric acid

A 8.5 mL volume of Perchloric acid (72%) was gradually mixed with 900 mL of glacial Acetic acid with vigorous and continuous stirring. Thirty millilitres (30 mL) of Acetic anhydride was then added with stirring. The volume of the mixture was made up to 1000 mL with glacial Acetic acid and allowed to stand for 24 hours before use.

Standardization of 0.1N Perchloric acid

A 0.5g quantity of Potassium hydrogen phthalate was weighed in a 100 mL conical flask. A 25 mL volume of glacial Acetic acid was added. The set up was attached to a reflux condenser with a silica-gel drying tube and warmed until the salt dissolved completely. The mixture was allowed to cool and then titrated with 0.1N Perchloric acid using α-Naphtol benzein as indicator.

Assay of Potassium citrate

A 20 mL volume of each of the prepared ORS solutions was pipetted into a conical flask. Two drops (2 drops) of α- Naphtol benzein indicator was added and the solution was titrated using the standardized 0.1N Perchloric acid. The end point (i.e the first excess of titrant in the formation of an orange precipitate) was noted. Appropriate calculations were done to ascertain the amount of potassium citrate contained in 1000 ml of the solution (where each ml of 0.1N Perchloric acid is equivalent to 0.01021g of potassium citrate).

Assay of Glucose

The assay of glucose was carried out using a refractometer (RFM470, Bellingham and Stanley). The equipment was properly caliberated using distilled water before taking the readings for each ORS solution. A drop from each solution was placed on the refractometer and the cover was closed. Readings were quickly taken off the scale (the line at the top of the dark area). Readings were taken to the nearest 0.1 percent . All the components were assayed at 0, 6, 12, 18 and 24 months post preparation.

RESULTS AND DISCUSSIONS

According to WHO's working document QAS/06.179/Rev.1 on guidelines for stability testing of active pharmaceutical ingredients and products, 2007; a significant change in stability of a product is defined as a 5% change in assay from it's initial value or if it fails to meet the acceptance criteria for potency as stated in the WHO document .[14] If no significant change occurs in the physical and chemical properties during six months of either accelerated or real time stability testing, the product is taken as stable and can be placed in the market with a provisional shelf life of 24 months. [14]

The freshly prepared solution batches were crystal clear and without particulate matter or precipitation upon preparation. After 24 months of storage, the five (5) panel of test subjects indicated that there seemed to be no change in the colour and odour of any of the batches. This indicated a likely Compactibility between the colourant and other ingredients in the solution. Twenty-four hours post preparation, batches D and D1 containing a combination of methyl and propyl paraben as preservative, were observed to have developed particulate matter that were not microbial in nature. This could be as a result of precipitation, incompactibility between some of the ingredients or method of incorporation of the preservatives.[15], [16], [17] Also, batches B and B1 (containing methyl paraben as preservative) and batches C and C1 (containing propyl paraben as preservative) was observed to contain particulate matter after nine and six months of storage respectively. The particulate matter observed in batches B, B1, C and C1 could be attributed to microbial contamination, particularly mould. This could be an indication that the preservatives, methyl and propyl paraben were not totally effective in assuring the integrity of the product after more than 9 months of storage. The results after 24 months indicates that, only batches A and A1 containing sodium benzoate as preservative was effective in maintaining 99% of the physical characteristics evaluated. This indicates a likely Compactibility between the ingredients of the solution of batches A and A1. The effectiveness of sodium benzoate as anti-mould growth is attributed to its undissociated, acidic form.[17], [18] Sodium benzoate has been known to have activity against mould, yeast and bacteria. The effectiveness of sodium benzoate as a preservative increases with decreasing pH because the ratio of undissociated (i.e. free) benzoic acid to ionized benzoic acid increases as the pH decreases. It is generally accepted that the undissociated benzoic acid is the active antimicrobial agent. Although no definite theory has been yet proposed to explain this antimicrobial effect, it is believed to be related to the high lipoid solubility of the undissociated benzoic acid which allows it to accumulate on the cell membranes or on various structures and surfaces of the bacterial cell, effectively inhibiting its cellular activity.[17], [18], [19], [20], [21]

Physico-chemical Properties

Batches A and A1 that contained sodium benzoate, had a higher pH value of 5.18 than the rest of the batches (Table 2). This high value in pH, is due to the acidic undissociated benzoic acid, which is the active antimicrobial agent of sodium benzoate. According to Howard (1972), "only the undissociated fraction or molecular form of preservatives possess preservative capabilities, since the ionized portion is incapable of penetrating the microrganism". Therefore, the preservative to be selected for any formulated product must be largely undissociated in the pH of the formulation being prepared[10]. There was no significant change ($P > 0.05$) of pH of all the batches after storage for 24 months except the batch that served as control.

The viscosity values of the batches ranged from 1.00 to 1.10 poise (Table 3). The slight variation in the viscosity of some of the batches from distilled water (1.00 poise at 25°C), is as a result of the presence of other excipients in the solution. The overall results of the viscosity test indicated that there was no significant ($P > 0.05$) change in the viscosity of the batches after storage for 24 months. The batches would therefore not present pourability problems during use.

The specific gravity values of the batches ranged from 1.00 to 1.19 (Table 4). Specific gravity is commonly used in industries as a simple means of obtaining information about the concentration of solutions of various materials [11]. A high specific gravity value (relative to that of water) indicates a likely contamination with particulate matter. Such contaminations could be as a result of improper clarification during production or contamination from other sources such as dust which carry microorganisms, that can contaminate a product, or contain toxic waste or chemicals, which can react with the drug

product and cause degradation.[15], [16], [17] Results in Table 4 indicate that the solutions were free from particulate matter at preparation. Results after 24 months relative to that of water, indicated significantly ($P < 0.05$) high specific gravity values in batches B, B1, C, C1, D, D1 and the control. This is likely an indication of contamination with particulate matter that resulted from precipitation or products of degradation by microorganisms. The results were consistent with the results obtained from the clarity test. Only Batch A and A1 remained significantly unchanged ($P > 0.05$) after storage for 24 months.

Sodium Chloride, Potassium citrate and Glucose content of the ORS solutions

At preparation, the percentage of Sodium chloride obtained from the assay, was lowest in the batches B1, C, C1, D and D1 and highest in batches A, A1, B and E (Table 5). The assay was stopped at 6, 9, 12 and 18 months for batches C, C1, D, D1, B and B1 respectively in accordance with WHO's working document QAS/06.179/Rev.1 on guidelines for stability testing because these batches failed to meet the acceptance criteria for clarity and appearance. After 24 months, there was a slight decrease in the content of sodium chloride in all the batches with batches A and A1 showing the least decrease. The changes in the sodium chloride content were however, not statistically significant ($P > 0.05$). This could be an indication that sodium benzoate could be the best choice of preservative for the formulation.

The percentage of glucose obtained from the assay at preparation was highest in batches A, A1, D, D1 and E and lowest in batches B, B1, C and C1 (Table 6). The glucose content in the batches gradually decreased over the 24 month period studied with significant changes ($P < 0.05$) in the quantity of glucose occuring only in batches B, B1, C, C1 and E. This could be an indication ingress of microbes (mould) in these batches that were feeding on the glucose. [22] This is consistent with the results obtained from the clarity test. The glucose content of batches A, A1, D and D1 remained significantly unchanged indicating that sodium benzoate and methylpropyl paraben are the best preservatives for these formulations in terms of maintainance of the stability of the glucose content. The overall results indicate that the preservatives methyl paraben, propyl paraben and methylpropyl paraben were not totally effective in assuring the integrity of the products. Sodium benzoate is considered the best preservative for the formulations.

The percentage of potassium citrate obtained from the assay for all the batches were within 100.05 except for the control batch, E which had a potassium citrate content of 99.26 (Table 7). After 24 months of storage, there was a decrease in the content of potassium citrate in all the batches. The control showed the highest decrease in content while batches A and A1 showed the least decrease. Overall the changes in the potassium citrate content of the preparations were not statistically significant ($P > 0.05$).

Table 1: Formula for preparing 1000 ml Oral rehydration solutions

INGREDIENTS	A	AI*	B	BI*	C	CI*	D	DI*	E
Sodium chloride (g)	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Sodium citrate (g)	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Potassium citrate (g)	2.16	2.16	2.16	2.16	2.16	2.16	2.16	2.16	2.16
Glucose (g)	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6
Lemon Flavouring (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sunset Yellow	-	2.0	-	2.0	-	2.0	-	2.0	-
Sodium benzoate (g)	2.0	2.0	-	-	-	-	-	-	-
Methyl Paraben	-	2.0	2.0	2.0	-	-	-	-	-
Propyl paraben	-	-	-	-	2.0	2.0	-	-	-
Methylpropyl paraben (1:10)	-	-	-	-	-	-	2.0	2.0	-
Sodium metabisulphite (g)	1	1	1	1	1	1	1	1	1
Distilled water (ml) q.s	1000	1000	1000	1000	1000	1000	1000	1000	1000

Table 2: Average pH Values of the Samples

Sample Code	At preparation	6months	12months	18months	24months
A	5.20 ± 0.01	5.20 ± 0.00	5.20 ± 0.17	5.19 ± 0.03	5.19 ± 0.01
A1	5.19 ± 0.01	5.19 ± 0.02	5.19 ± 0.01	5.18 ± 0.02	5.18 ± 0.00
B	7.42 ± 0.01	7.41 ± 0.01	7.41 ± 0.01	7.40 ± 0.01	7.40 ± 0.17
B1	7.45 ± 0.01	7.45 ± 0.15	7.43 ± 0.01	7.42 ± 0.06	7.42 ± 0.02
C	7.64 ± 0.00	7.65 ± 0.04	7.66 ± 0.01	7.67 ± 0.31	7.68 ± 0.02
C1	7.86 ± 0.05	7.87 ± 0.12	7.88 ± 0.00	7.89 ± 0.00	7.90 ± 0.56
D	7.74 ± 0.01	7.73 ± 0.06	7.73 ± 0.06	7.71 ± 0.00	7.70 ± 0.00
D1	7.74 ± 0.01	7.73 ± 0.00	7.73 ± 0.06	7.71 ± 0.00	7.70 ± 0.00
E	7.38 ± 0.01	7.56 ± 0.03	7.75 ± 0.09	7.93 ± 0.03	8.11 ± 0.01

Table 3: Average Viscosity values (poise) of the Samples

Sample Code	At preparation	6 months	12 months	18 months	24 months
A	1.00 ± 0.02	1.01 ± 0.10	1.02 ± 0.06	1.02 ± 0.06	1.02 ± 0.00
A1	1.01 ± 0.07	1.01 ± 0.04	1.01 ± 0.00	1.01 ± 0.67	1.01 ± 0.29
B	1.01 ± 0.48	1.01 ± 0.00	1.02 ± 0.05	1.02 ± 0.03	1.02 ± 0.57
B1	1.01 ± 0.03	1.01 ± 0.01	1.02 ± 0.06	1.02 ± 0.00	1.02 ± 0.05
C	1.01 ± 0.03	1.03 ± 0.00	1.04 ± 0.00	1.04 ± 0.00	1.04 ± 0.00
C1	1.01 ± 0.01	1.02 ± 0.01	1.02 ± 0.00	1.02 ± 0.01	1.02 ± 0.01
D	1.01 ± 0.05	1.02 ± 0.02	1.03 ± 0.01	1.03 ± 0.03	1.03 ± 0.54
D1	1.02 ± 0.00	1.04 ± 0.44	1.07 ± 0.00	1.07 ± 0.01	1.07 ± 0.01
E	1.02 ± 0.04	1.08 ± 0.00	1.10 ± 0.36	1.10 ± 0.66	1.10 ± 0.10

Table 4: Average specific gravity values in A - E

Sample Code	At preparation	6 months	12months	18 months	24months
A	1.00 ± 0.01	1.00 ± 0.00	1.08 ± 0.04	1.08 ± 0.04	1.09 ± 0.00
A1	1.01 ± 0.01	1.01 ± 0.01	1.09 ± 0.02	1.09 ± 0.02	1.09 ± 0.01
B	1.01 ± 0.00	1.01 ± 0.00	1.01 ± 0.01	1.01 ± 0.01	1.11 ± 0.01
B1	1.01 ± 0.01	1.03 ± 0.01	1.06 ± 0.06	1.09 ± 0.01	1.09 ± 0.00
C	1.02 ± 0.00	1.03 ± 0.00	1.06 ± 0.00	1.08 ± 0.00	1.08 ± 0.02
C1	1.04 ± 0.01	1.07 ± 0.02	1.09 ± 0.00	1.10 ± 0.00	1.11 ± 0.02
D	1.03 ± 0.00	1.04 ± 0.01	1.06 ± 0.03	1.07 ± 0.06	1.08 ± 0.00
D1	1.07 ± 0.02	1.07 ± 0.03	1.06 ± 0.00	1.13 ± 0.01	1.11 ± 0.01
E	1.10 ± 0.01	1.14 ± 0.01	1.17 ± 0.04	1.18 ± 0.04	1.19 ± 0.06

Table 5: Percentage Sodium Chloride content in A -E

Sample Code	0 Month	3 Months	6 Months	9 Months	12 Months	18 Months	24 Months
A	100.81±0.06	100.86±0.25	100.59±0.00	100.29±0.15	100.01±0.10	99.44±0.06	99.44±0.06
A1	100.75±0.26	100.56±0.15	100.36±0.06	100.17±0.17	99.98 ±0.17	99.60±0.06	99.44±0.06
B	100.66±0.10	99.06 ± 0.06	98.83 ± 0.06	98.60 ± 0.10	-	-	98.21±0.23
B1	99.34 ± 0.10	98.93 ± 0.12	98.67 ± 0.06	98.60 ± 0.10	-	-	98.21±0.11
C	99.53 ± 0.10	98.21 ± 0.15	98.06 ± 0.23	-	-	-	97.67±0.00
C1	99.76 ± 0.10	98.42 ± 0.06	98.29 ± 0.21	-	-	-	97.90±0.11
D	99.99 ± 0.00	98.44 ± 0.10	-	-	-	-	98.15±0.11
D1	99.76 ± 0.06	98.37 ± 0.06	-	-	-	-	97.44±0.11
E	100.81±0.06	100.38±0.35	99.06 ± 0.06	98.88 ± 0.12	98.83 ± 0.15	98.83±0.10	98.60±0.11

- = the test was stopped at 6, 9, 12 and 18 months for batches C, C1, D, D1, B and B1 respectively because they failed to meet the acceptance criteria for clarity and appearance.

Table 6: Percentage Glucose content in A - E

Sample Code	At Preparation	6 Months	12 Months	18 Months	24 Months
A	2.76 ± 0.00	2.76 ± 0.01	2.75 ± 0.01	2.75 ± 0.00	2.75 ± 0.00
A1	2.76 ± 0.01	2.76 ± 0.01	2.75 ± 0.01	2.75 ± 0.01	2.75 ± 0.01
B	2.75 ± 0.01	2.74 ± 0.01	2.72 ± 0.01	2.72 ± 0.01	2.71 ± 0.01
B1	2.75 ± 0.00	2.74 ± 0.01	2.72 ± 0.01	2.72 ± 0.00	2.70 ± 0.01
C	2.74 ± 0.00	2.73 ± 0.01	2.72 ± 0.00	2.70 ± 0.01	2.69 ± 0.01
C1	2.74 ± 0.01	2.72 ± 0.01	2.69 ± 0.01	2.69 ± 0.00	2.68 ± 0.01
D	2.76 ± 0.01	2.76 ± 0.01	2.75 ± 0.01	2.75 ± 0.01	2.74 ± 0.01
D1	2.76 ± 0.00	2.76 ± 0.01	2.75 ± 0.00	2.74 ± 0.00	2.74 ± 0.00
E	2.76 ± 0.01	2.73 ± 0.01	2.70 ± 0.01	2.67 ± 0.01	2.67 ± 0.01

Table 7: Percentage Potassium Citrate content in A - E

Sample Code	At preparation	24 months
A	100.05 ± 0.15	99.26 ± 0.10
A1	100.05 ± 0.06	99.26 ± 0.10
B	100.05 ± 0.06	98.48 ± 0.12
B1	100.05 ± 0.15	99.26 ± 0.17
C	100.05 ± 0.06	97.69 ± 0.06
C1	100.84 ± 0.15	98.48 ± 0.06
D	100.05 ± 0.06	99.26 ± 0.10
D1	100.05 ± 0.06	99.26 ± 0.17
E	99.26 ± 0.10	96.11 ± 0.15

CONCLUSION

The overall results obtained, show that all the ORS solutions had good physical and physico-chemical characteristics at preparation. However, after 3, 6 and 9 months, batches D and D1, C and C1 and B and B failed to meet the acceptance criteria for appearance and physical attributes. They also failed to meet the acceptance criteria for quantitative characteristics assessed. This indicates that the preservatives methyl paraben, propyl paraben and a combination of methyl/propyl paraben may not be suitable in maintaining the integrity of the preparation for a provisional shelf life of 24 months. Only A and A1 which contained sodium benzoate as preservative maintained 99% of all the stability parameters assessed and this makes it the best choice of preservative for the formulation of oral rehydration salt solution. The two preparations (A and A1) were therefore coded Sabilyte Oral Rehydration solutions**.**

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