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SPECIFICATIONS FOR THE IDENTITY AND PURITY OF FOOD ADDITIVES AND THEIR TOXICOLOGICAL EVALUATION: EMULSIFIERS, STABILIZERS, BLEACHING AND MATURING AGENTS

Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives

WORLD HEALTH ORGANIZATION

GENEVA

1964

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 18-25 February 1963

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* Professor Truhaut also acted as observer on behalf of the International Union of Pure and Applied Chemistry (IUPAC).

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SPECIFICATIONS FOR THE IDENTITY AND PURITY OF FOOD ADDITIVES AND THEIR TOXICOLOGICAL EVALUATION: EMULSIFIERS, STABILIZERS, BLEACHING AND MATURING AGENTS

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INTRODUCTION

The Joint FAO/WHO Expert Committee on Food Additives met in Rome from 18-25 February 1963. The meeting was opened by Dr O. E. Fischnich, Assistant Director-General, Technical Department, FAO, on behalf of the Director-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization. Professor A. C. Frazer and Professor J. F. Reith were unanimously elected Chairman and Vice-Chairman respectively. Professor Sv. Dalgaard-Mikkelsen and Mr. L. L. Ramsey agreed to act as Rapporteurs.

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955 [1] six Joint FAO/WHO Expert Committees on Food Additives have met and issued the following reports: " General Principles Governing the Use of Food Additives: First Report ",[2] " Procedures for the Testing of Intentional Food Additives to Establish their Safety for Use: Second Report ",[3] " Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants): Third Report ",[4] " Specifications for Identity and Purity of Food Additives (Food Colours): Fourth Report ",[5] " Evaluation of the Carcinogenic

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[1] *FAO Nutrition Meetings Report Series*, 1956, No. 11; *Wld Hlth Org. techn. Rep. Ser.*, 1956, **107**.

[2] *FAO Nutrition Meetings Report Series*, 1957, No. 15; *Wld Hlth Org. techn. Rep. Ser.*, 1957, **129**.

[3] *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, **144**.

[4] These specifications were subsequently revised and published as *Specifications for identity and purity of food additives. Vol. I. Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962.

[5] These specifications were subsequently revised and published as *Specifications for identity and purity of food additives. Vol. II. Food Colors*, Rome, Food and Agriculture Organization of the United Nations, 1963.

Hazards of Food Additives : Fifth Report ",[1] " Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants : Sixth Report ".[2]

This meeting was convened on recommendations made in the previous reports of the Joint FAO/WHO Expert Committee on Food Additives, its terms of reference being to draw up specifications for emulsifiers, stabilizers, maturing and bleaching agents and related substances used as food additives, and to evaluate the toxic hazards involved in their use.

GENERAL CONSIDERATIONS

General principles

Attention is again drawn to certain important principles set out in earlier reports [3] that should be taken into consideration when making decisions on the use and control of food additives.

1. Food additives should not be used to disguise faulty processing or handling techniques, nor to deceive the consumer with regard to the nature or quality of food.

2. Special care should be exercised in the use of additives in foods that may form a major part of the diet of some sections of a community, or that may be consumed in especially large quantities at certain seasons.

3. The choice of food additives should be related to the prevailing dietary patterns within a community. The availability of essential nutrients and their distribution in the various foods consumed should be taken into account before the true significance of making a further addition of a particular nutrient (e.g., calcium or phosphorus), or of using an additive that may change the pattern of nutrients in a food (e.g., an oxidizing agent), can be assessed.

4. The specifications needed for each food additive have been compiled with three main objectives in mind :

(*a*) To identify the substance that has been subjected to biological testing.

(*b*) To ensure that the substance is of the quality required for safe use in food.

(*c*) To reflect and encourage good manufacturing practice.

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[1] *FAO Nutrition Meetings Report Series*, 1961, No. 29 ; *Wld Hlth Org. techn. Rep. Ser.*, 1961, **220**.

[2] *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**.

[3] For references, see page 3, footnotes 1-5, and this page, footnotes 1 and 2.

Substances studied

The Committee had before it a provisional list of emulsifiers, stabilizers and selected substances for which as much information as possible had been collected. It was agreed that it would be advisable to delete from this list any substance that was not known to be in use as a food additive, or on which the information available to the Committee was insufficient for compilation of specifications or for toxicological evaluation. On this basis, nitrosyl chloride was deleted as it was thought to be no longer in use, while acetone peroxide, calcium peroxide, ammonium persulfate, the propylene glycol ester of alginic acid, the sulfoacetate and the acetyl tartaric esters of mono- and diglycerides, and the monosodium phosphate derivatives of mono- and diglycerides were deleted because adequate information on them was not available to the Committee and could not be found in the literature.

Consideration was then given to the advisability of adding further substances to the list. It was noted that:

(*a*) Wide circulation had been given to the provisional list when information about the substances had been requested but, at that time, no indication was given that additions might be made to the list.

(*b*) The number of substances that can be dealt with by the Committee in one meeting is necessarily limited.

It was therefore agreed that any addition to the list would be undesirable at this stage.

The Committee wishes to emphasize that *the fact that a substance does not appear in this report is not intended to imply any doubt about its technical usefulness or safety in use*. All available information about any compounds that are not included among the monographs in this report but that are already in use, or may soon be introduced, as emulsifiers, stabilizers, bleaching or maturing agents should be sent to FAO or WHO [1] so that specifications may be compiled and any toxic hazard evaluated by a future Committee.

Specifications

With regard to specifications, the Committee agreed to follow the principles already laid down in the third and fourth reports of the Expert Committee on Food Additives and to adopt the same presentation. Certain points of detail were discussed, some of which had been raised since the circulation of the third report. It had been suggested that the limits for

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[1] Information relating to specifications should be sent to: Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy; toxicological data should be sent to: Food Additives, World Health Organization, Geneva, Switzerland.

heavy metal contaminants might become too rigorous if the proposals contained in the third report were followed. It was recalled that the limits were introduced to encourage good manufacturing practice and not, in most cases, because of any great toxicological concern, since food additives are commonly used in relatively low concentrations. However, the Committee agreed that it is always desirable to keep the level of lead in the food as low as possible. It was also considered important that food additives that might be used in ice cream or soft drinks, which were often consumed in large quantities by children as well as adults, should conform to heavy metal limits approximating to those required for foods. It was felt that limits should not be made unnecessarily rigorous, but that the general principles laid down in the third report were reasonable and that each case should be decided on its merits. The limits for lead, arsenic and total heavy metals contained in the specifications in this report are therefore those that are considered to be capable of achievement by manufacturers without undue difficulty and to be necessary in the interests of the consumer.

The Committee also discussed the need for the inclusion of some requirements for microbiological control. It was agreed that some general guidance on this matter might be given in the specifications where this was required in the interest of public health. It was also agreed that a short section should be included in each specification indicating the main uses of each substance in food at the present time and the main foods to which it is at present added.

NOTES ON THE USE OF THE MONOGRAPHS

The most effective way of using the information contained in the monographs has been described in detail in the Committee's sixth report.[1] The following supplementary information is given on the conventions adopted in the specifications and on the evaluation of toxicological data.

Specifications

Nomenclature

The title of each monograph contains the name by which the substance is most commonly known in food manufacture. Where other recognized names exist, they are listed as synonyms. For the chemical names, the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) and the usages of the various national chemical societies have been taken into consideration. Where the structure or composition of a food

[1] *FAO Nutrition Meetings Report Series*, 1962, No. 31, p. 13; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**, 13.

additive has not been clearly elucidated, a description of its chemical nature is given instead of the chemical name.

Formulas

A chemical formula is given for each inorganic substance and empirical and structural formulas are shown, where known, for each organic substance. All formulas represent the pure compounds and serve only a descriptive purpose.

Molecular weight

For purposes of information and description, the molecular weights of the compounds are given where known. These have been calculated from the table of atomic weights approved by the Commission on Atomic Weights of the International Union of Pure and Applied Chemistry in 1961.

Temperature

All temperatures are given in degrees centigrade.

Solubility

The solubilities of the substances contained in the monographs are given without reference to possible chemical changes. Unless otherwise stated, the standards adopted are room temperature and normal atmospheric pressure.

Solvents

Unless otherwise stated, reference to water in the monographs presumes distilled water. The term "ethanol" is used as referring to 95% v/v ethyl alcohol, and the term "absolute ethanol" for solvent containing not less than 98.8% v/v ethyl alcohol. Other v/v concentrations of ethanol may be specified in individual cases.

Assays

The assay methods described for the compounds are those that are considered to be the most suitable. A second method is included if the preferred procedure requires instrumentation not widely available.

Methods

Procedures for the determination of loss on drying, sulfated ash and physical constants, and tests for the presence of certain metals, are presented in Annex 1.

Test solutions

All reagents referred to in the identification and purity tests and in the assays are assumed to be analytical grade, unless otherwise specified.

Test solutions (TS) are given in alphabetical order in Annex 2; the indication (PbT) means that the test solution used must be free of lead.

In general, solutions are brought to approximate normality or to a simple multiple or fraction of normality. When the normality or percentage composition of acids is not indicated, the concentrated form should be used.

Where the use of a test solution as indicator is specified in a test or assay, 3 drops of the solution should be added, generally as the end-point is approached, unless otherwise directed.

Evaluation of Toxicological Data

For assessment of the carcinogenic and toxicological risks, the Committee agreed to abide by the principles and procedures set out in the second, fifth and sixth reports and to use the same general form of presentation as previously developed. However, it was agreed to place the section on biochemical aspects at the beginning of each monograph and to adopt such modifications as might be necessary to deal with substances that did not fit conveniently into the usual pattern. The Committee considered that the use of acceptance zones as given in the sixth report was satisfactory, but it was felt that some further description of their significance and use should be included in the present report.

Relationship between specifications and toxicological evaluation

As already mentioned, one of the main objectives of the specifications is to identify the substance studied biologically. In the case of a single, well-defined chemical compound this presents no great problem, but the situation is very different when mixtures, polymers or some natural products come under consideration. Many of these are required to conform to physicochemical specifications. If these specifications are too narrow, many substances that are equally useful and acceptable may be excluded and, if they are too wide, substances might be introduced as food additives that are not satisfactorily covered by the biological tests. The Committee has endeavoured to give specifications that ensure safety without being unduly restrictive. Certain substances in the same general class as those considered in this report may be found not to conform to the specifications suggested, although apparently having similar biological properties. In such cases, full details should be sent to FAO or WHO [1] so that the matter can be

[1] See the footnote on page 5.

considered by a future Committee. The groups of substances that may present problems of this nature are :

1. *Agar :* Agar must be prepared from specified natural raw materials. Its safety in use is based largely on extensive human experience.

2. *Alginic acid and alginates ; methyl celluloses, sodium carboxymethyl-celluloses :* It is not considered that there are any differences of toxicological significance between alginic acid and sodium, ammonium, calcium or potassium alginates, or between the methyl celluloses and sodium carboxymethyl celluloses conforming to the specifications laid down in this report.

3. *Polyphosphates :* Although there are many different substances in this group, the toxicological situation is relatively simple, since that part of these phosphates that is hydrolyzed and absorbed is metabolized in the body as monophosphate. They can therefore be considered together.

4. *Polyoxyethylene esters :* In this group the polyoxyethylene moiety includes polymers covering a wide range of average molecular weights. Considerable biological differences have been demonstrated between polyoxyethylene glycols with about 8 oxyethylene units and those with 20 units or more. It seems desirable, therefore, to consider polyoxyethylene glycols containing approximately 8 units separately from those with 20 or more units as the predominating polymer.

5. *Fatty acid moieties :* Many of the substances considered, especially the polyoxyethylene esters, sorbitan esters, glycerides and lecithins contain a number of fatty acids, of which one may predominate. Extensive biological studies have often been carried out on only one or a few of the esters and the question arises whether the results of such studies could be accepted as applicable also to other esters of the same class. The Committee concluded that no general ruling could be given and that each group of substances should be considered separately. In the case of the substances included in this report, the Committee considered that for the purposes of toxicological evaluation there is no indication that any differentiation need be made within the following groups of esters :

(*a*) polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene (20) sorbitan monopalmitate, polyoxyethylene (20) sorbitan monostearate, and polyoxyethylene (20) sorbitan tristearate.

(*b*) Sorbitan monopalmitate, sorbitan monostearate and sorbitan tristearate.

(*c*) Monoglycerides and diglycerides containing the long-chain fatty acids commonly found in food.

(*d*) Lecithins of natural origin.

The significance of unconditional and conditional zones of acceptability

The concept of using zones of acceptability was put forward in the Committee's sixth report [1] for several reasons. First, the primary intention of the Committee is to give guidance to national bodies to assist them in their choice of food additives which are suitable for their particular needs, safe in use, and can be regarded as acceptable for purposes of international trade. Secondly, it is one of the principles underlying food additive control that the quantity of a food additive used should not be greater than is necessary to ensure the technological effect required. Thirdly, the dietary pattern differs greatly from one country to another and consequently the possible benefits and risks may also differ ; the final decision on the use of any particular food additive can only be usefully taken on a national or regional rather than an international basis.

The zone of acceptability represents the limits of intake that can be regarded as presenting no significant hazard to health on the basis of the evidence available. However, the problems that may arise from the introduction of a food additive into the diet may be complex and may sometimes require further study by experts in nutrition or other related fields. This is more likely to occur when high levels of dosage are used or if the food additive is to be used in foods mainly consumed by some special group in the community, such as children. It is for this reason that many countries tie proposed levels of a permitted substance to a specified use or uses. The Committee felt, however, that any attempt to tie zones of acceptability to specified uses would be too restrictive at an international level, and some other solution of this problem was therefore sought. To ensure that expert opinion would be consulted whenever higher dosage levels of certain food additives were used or when special circumstances might arise, the Committee decided to split the zone of acceptability into two parts in selected cases. The first part has been termed the unconditional zone of acceptability and this represents levels of use that are effective technologically, at least for some purposes, and can be safely employed without further expert advice. The second part consists of a conditional zone of acceptability and represents levels of use that can be employed safely, but at which it is thought desirable that some degree of expert supervision and advice should be readily available. The unconditional zones of acceptability are therefore intended mainly to help developing countries that may not be able to call upon appropriate experts to guide them in the handling of particular problems in the field. The conditional zones of acceptability, on the other hand, are more likely to be of interest to those countries that have a more elaborate organization for dealing with food policy and the health hazards

[1] *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**.

to the consumer. It must be emphasized that the whole zone of acceptability may be safely employed, that it provides for an adequate margin of safety, and that it is based on a careful consideration of the evidence available. The added caution imposed by restricting levels of use to the unconditional zone of acceptability is only necessary in the special circumstances already described.

ACCEPTABLE DAILY INTAKE ZONES FOR MAN
(mg/kg body weight)

Compounds	*Unconditional*	*Conditional*	*Page*
Monosodium monophosphate Disodium monophosphate Trisodium monophosphate Monopotassium monophosphate Dipotassium monophosphate Tripotassium monophosphate Disodium diphosphate Tetrasodium diphosphate Pentasodium triphosphate Sodium polyphosphate	up to 30 [1]	30-70 [1]	31, 37, 47
Calcium acetate Calcium chloride		not limited	53
Sodium citrate Potassium citrate Calcium citrate		not limited	59
Sodium tartrate Potassium sodium tartrate	0-6 [2]	6-20 [2]	64
Agar Sodium alginate Potassium alginate Ammonium alginate Calcium alginate Alginic acid	0-50		67, 81
Methyl cellulose Sodium carboxymethylcellulose	0-30		87, 95
Sorbitol	0-150	not limited	101
Sorbitan monopalmitate Sorbitan monostearate Sorbitan tristearate	0-25 [3]	25-50 [3]	113
Propylene glycol	0-20	20-40	118
Polyoxyethylene (8) stearate	0-25 [4]	25-50 [4]	126

[1] As total dietary intake of phosphorus from both foods and food additives.

[2] As tartaric acid.

[3] Calculated as sorbitan monostearate.

[4] As total polyoxyethylene ester intake; Professor R. Truhaut wished to record a reservation in regard to these figures.

Compounds	*Unconditional*	*Conditional*	*Page*
Polyoxyethylene (40) stearate Polyoxyethylene (20) sorbitan monolaurate Polyoxyethylene (20) sorbitan monooleate Polyoxyethylene (20) sorbitan monopalmitate Polyoxyethylene (20) sorbitan monostearate Polyoxyethylene (20) sorbitan tristearate	0-25 [1]	25-50 [1]	130, 144
Mono- and diglycerides	0-125	not limited	151
Lecithin	0-50	50-100	155

ACCEPTABLE TREATMENT LEVELS OF FLOUR IN PARTS PER MILLION

Compound	*Average level*	*Level for special purposes* [2]	*Page*
Benzoyl peroxide	0-40	40-75	158
Chlorine dioxide	0-30	30-75	163
Potassium bromate	0-20	20-75	167

[1] As total polyoxyethylene intake.

[2] e.g., for certain biscuit flours.

GENERAL RECOMMENDATIONS TO FAO AND WHO

In conclusion, the Committee recommends the following further action to FAO and WHO.

1. FAO and WHO should continue to use all means available to encourage more complete publication of the results of investigations necessary for the toxicological evaluation of food additives.

2. FAO and WHO should explore the possibility of expanding their current activities on collecting and disseminating information on the toxicological and legislative aspects of food additives, and of developing further co-operation with IUPAC and other international organizations on the compilation of specifications and the establishment of standard methods of analysis.

3. A Joint FAO/WHO Expert Committee on Food Additives should be convened as soon as practicable to evaluate the toxic hazards involved in the use of food colours.

4. A further Joint FAO/WHO Expert Committee on Food Additives should be convened as soon as practicable to draw up specifications for additional emulsifiers, stabilizers, bleaching and maturing agents, as well as other food additives, and to evaluate the toxic hazards involved in their use. This Committee might also review the specifications and toxicological evaluations of previous Committees in the light of subsequent published work.

ACKNOWLEDGEMENTS

In compiling the specifications in the monographs that follow, the Committee made extensive use of material from the following publications:

The British Pharmacopoeia

The British Pharmaceutical Codex

The International Pharmacopoeia

The National Formulary of the American Pharmaceutical Association

Official Methods of Analysis of the Association of Official Agricultural Chemists

Official and Tentative Methods of the American Oil Chemists' Society

Pharmacopeia of the United States of America.

The Committee wishes to acknowledge its indebtedness to the respective publishers and other bodies concerned for permission to quote from these publications.

MONOGRAPHS

MONOSODIUM MONOPHOSPHATE *

Chemical names Monosodium monophosphate; monosodium dihydrogen monophosphate; sodium biphosphate; sodium dihydrogen phosphate; acid sodium phosphate; monosodium orthophosphate; primary sodium phosphate; sodium phosphate monobasic.

Chemical formula
Anhydrous : NaH_2PO_4
Monohydrate : $NaH_2PO_4.H_2O$
Dihydrate : $NaH_2PO_4.2H_2O$

Molecular weight
Anhydrous : 119.98
Monohydrate : 138.00
Dihydrate : 156.01

Definition Monosodium monophosphate, after drying at 60° for one hour and then at 105° for 4 hours, contains not less than 97% of NaH_2PO_4 and conforms to the following specifications.

Description Monosodium monophosphate occurs as a white, odourless, slightly deliquescent powder, crystals, or granules.

Uses Buffer, neutralizing agent, and sequestrant in cheese, milk, fish and meat products.

Identification Tests

A. *Solubility :*

Water : freely soluble.
Ethanol, ether, chloroform : insoluble.

B. To a 1% solution of monosodium monophosphate add silver nitrate TS ; the yellow precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of monosodium monophosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of monosodium monophosphate add a few drops of barium nitrate TS. The solution remains limpid. Add 2 N sodium acetate. The white precipitate formed is soluble in acetic acid TS.

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* For biological data and toxicological evaluation see pp. 29-32.

E. To 5 ml of a 1% solution of monosodium monophosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few minutes.

Purity Tests

Loss on drying : The anhydrous salt loses not more than 2%, the monohydrate not more than 15%, and the dihydrate not more than 25% when dried first at 60° for 1 hour, then at 105° for 4 hours.

pH of a 1% solution : 4.2-4.6.

Free acid and disodium phosphate : 2.00 g of monosodium monophosphate dissolved in 40 ml of water require for neutralization not more than 0.3 ml of either N sodium hydroxide or N sulfuric acid, using methyl orange TS as indicator.

Arsenic : Not more than 2 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Potentiometric method. Transfer 0.7000 g of monosodium monophosphate, previously dried at 60° for 1 hour and then dried at 105° for 4 hours, to a 250-ml beaker, add 50 ml of 0.1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and add slowly from a burette, with constant stirring, 0.1 N sodium hydroxide until a pH of 3.3 is attained. Continue to add sodium hydroxide solution until the next ml or 0.5-ml graduation mark on the burette is reached. Record the burette reading under column 1 of a suitable data sheet and record the pH under column 2. Continue the addition of 0.1 N sodium hydroxide in 0.5-ml increments until a pH of 6.0 is attained, recording the burette reading and the pH after the addition of each increment. Then proceed with the titration in the usual manner until a pH of 8.0 is attained, and again continue to add the solution until the next ml or 0.5-ml graduation mark is reached before recording the burette reading and the pH. Then continue adding 0.1 N sodium hydroxide in 0.5-ml increments until the pH is 10.0, again recording the burette reading and the pH after the addition of each increment. In column 3 of the data sheet, note the values for ΔpH obtained by subtracting each pH value recorded from the next higher value. In column 4, note the values for Δ_2pH, i.e., the differences between successive ΔpH values, recording them

as plus or minus depending upon whether the value of ΔpH is higher or lower than the preceding one. The end-point lies in the 0.5-ml increment of sodium hydroxide that gives the highest value for ΔpH, its exact position being calculated by adding 0.5 b/B to the next lower burette reading, where b is the last Δ_2pH value having a plus sign and B is the sum, without regard to sign, of the last Δ_2pH value having a plus sign and the first Δ_2pH value having a minus sign. Two end-points are calculated, that occurring between pH 3.3 and pH 6.0 being designated F and that between pH 8.5 and pH 10.0 being designated T. The volume of sodium hydroxide used in the titration is obtained by subtracting F from T. Each ml of 0.1 N sodium hydroxide is equivalent to 0.01200 g of NaH_2PO_4.

DISODIUM MONOPHOSPHATE *

Chemical names Disodium monophosphate; disodium monohydrogen monophosphate; disodium orthophosphate; disodium hydrogen phosphate; secondary sodium phosphate; sodium phosphate dibasic.

Chemical formula
Anhydrous : Na_2HPO_4
Dihydrate : $Na_2HPO_4.2H_2O$
Heptahydrate : $Na_2HPO_4.7H_2O$
Dodecahydrate : $Na_2HPO_4.12H_2O$

Molecular weight
Anhydrous : 141.96
Dihydrate : 177.99
Heptahydrate : 268.06
Dodecahydrate : 358.14

Definition Disodium monophosphate, after drying at 60° for one hour and then at 105° for 4 hours, contains not less than 98% of Na_2HPO_4. All forms conform to the following specifications.

Description Disodium monophosphate anhydrous occurs as a white, hygroscopic, odourless powder.

Disodium monophosphate dihydrate occurs as a white, crystalline, odourless solid.

Disodium monophosphate heptahydrate occurs as white, odourless, efflorescent crystals or granular powder.

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* For biological data and toxicological evaluation see pp. 29-32.

Disodium monophosphate dodecahydrate occurs as a white, efflorescent, and odourless powder or crystals.

Uses Buffer, neutralizing agent, and sequestrant in cheese and certain milk, fish, and meat products.

Identification Tests

A. *Solubility*

Water : freely soluble.
Ethanol, ether, chloroform : insoluble.

B. To a 1% solution of disodium monophosphate add silver nitrate TS ; the yellow precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of disodium monophosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of disodium monophosphate add a few drops of barium nitrate TS. The white precipitate formed is soluble in acetic acid TS.

E. To 5 ml of a 1% solution of disodium monophosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few minutes.

Purity Tests

Loss on drying : When dried at 60° for one hour and then at 105° for 4 hours, the losses in weight of the salts are as follows :

Anhydrous : not more than 5.0% ; *dihydrate :* not more than 21.0% ; *heptahydrate :* not more than 50.0% ; *dodecahydrate :* not more than 61.0%.

pH of a 1% solution : 8.7-9.2.

Arsenic : Not more than 2 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Potentiometric method. Transfer 0.6000 g of disodium monophosphate, previously dried at 105° to constant weight, to a 250-ml beaker, add 50 ml of 0.1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and add

slowly from a burette, with constant stirring, 0.1 N sodium hydroxide until a pH of 3.3 is attained. Continue to add sodium hydroxide solution until the next ml or 0.5-ml graduation mark on the burette is reached. Record the burette reading under column 1 of a suitable data sheet and record the pH under column 2. Continue the addition of 0.1 N sodium hydroxide in 0.5-ml increments until a pH of 6.0 is attained, recording the burette reading and the pH after the addition of each increment. Then proceed with the titration in the usual manner until a pH of 8.0 is attained, and again continue to add the solution until the next ml or 0.5-ml graduation mark is reached before recording the burette reading and the pH. Then continue adding 0.1 N sodium hydroxide in 0.5-ml increments until the pH is 10.0, again recording the burette reading and the pH after the addition of each increment. In column 3 of the data sheet, note the values for ΔpH obtained by subtracting each pH value recorded from the next higher value. In column 4, note the values for Δ_2pH, i.e., the differences between successive ΔpH values, recording them as plus or minus depending upon whether the value of ΔpH is higher or lower than the preceding one. The end-point lies in the 0.5-ml increment of sodium hydroxide that gives the highest value for ΔpH, its exact position being calculated by adding 0.5 b/B to the next lower burette reading, where b is the last Δ_2pH value having a plus sign and B is the sum, without regard to sign, of the last Δ_2pH value having a plus sign and the first Δ_2pH value having a minus sign. Two end-points are calculated, that occurring between pH 3.3 and pH 6.0 being designated F and that between pH 8.5 and pH 10.0 being designated T. The volume of sodium hydroxide used in the titration is obtained by subtracting F from T. Each ml of 0.1 N sodium hydroxide is equivalent to 0.01420 g of Na_2HPO_4.

TRISODIUM MONOPHOSPHATE *

Chemical names Trisodium monophosphate; trisodium orthophosphate; trisodium phosphate; sodium phosphate tribasic.

Chemical formula
Anhydrous : Na_3PO_4
Monohydrate : $Na_3PO_4.H_2O$
Dodecahydrate : $Na_3PO_4.12H_2O$

Molecular weight
Anhydrous : 163.94
Monohydrate : 181.96
Dodecahydrate : 380.12

* For biological data and toxicological evaluation see pp. 29-32.

Definition Trisodium monophosphate, after ignition at 800° for 30 minutes, contains not less than 97.0% of Na_3PO_4. All forms conform to the following specifications.

Description Trisodium monophosphate occurs as a white, odourless powder, crystals, or granules.

Uses Neutralizing agent and sequestrant in drinking water and cheese.

Identification Tests

A. *Solubility*

Water : freely soluble.
Ethanol, ether, chloroform : insoluble.

B. To a 1% solution of trisodium monophosphate add silver nitrate TS ; the yellow precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of trisodium monophosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of trisodium monophosphate add a few drops of barium nitrate TS. The white precipitate formed is soluble in acetic acid TS.

Purity Tests

Loss on heating : Trisodium monophosphate anhydrous ignited at 800° for 30 minutes loses not more than 2.0% of its weight. Trisodium monophosphate monohydrate ignited at 800° for 30 minutes loses not more than 9.0% of its weight. Trisodium monophosphate dodecahydrate ignited at 800° for 30 minutes loses not more than 55.0% of its weight.

pH of a 1% solution : 11.5-12.0.

Arsenic : Not more than 2 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Trisodium monophosphate and excess alkali. Dissolve 5.000 g of trisodium monophosphate, previously ignited at 800° for 30 minutes, in 40 ml of water in a 400-ml beaker. Add 100 ml of N hydrochloric acid and pass a stream of carbon-dioxide-free air in fine bubbles through the solution for 30 minutes to expel carbon dioxide. The beaker must be loosely covered

to prevent any loss by spraying. Wash down the cover and sides of the beaker. Titrate the solution with N sodium hydroxide to pH 4.0 using a standard pH meter and glass electrodes. Record the number of ml (A) of hydrochloric acid used in this titration.

Protect the solution from absorbing CO_2 from the atmosphere and continue the titration with N sodium hydroxide to pH 9.0. Record the number of ml (B) of N sodium hydroxide required for this titration.

Calculation :

If A is equal to 2B the sample contains no Na_2HPO_4 or excess NaOH.

$$\% \ Na_3PO_4 = \frac{B \times 0.1639}{\text{weight of sample}} \times 100$$

If A is less than 2B the sample contains some Na_2HPO_4.

$$\% \ Na_3PO_4 = \frac{(A - B) \times 0.1639}{\text{weight of sample}} \times 100$$

If A is greater than 2B the sample contains excess NaOH.

$$\% \ NaOH = \frac{(A - 2B) \times 0.040}{\text{weight of sample}} \times 100$$

MONOPOTASSIUM MONOPHOSPHATE *

Chemical names	Monopotassium monophosphate ; monopotassium dihydrogen monophosphate ; potassium biphosphate ; potassium acid phosphate ; potassium dihydrogen phosphate ; monopotassium phosphate ; potassium phosphate monobasic.
Chemical formula	KH_2PO_4
Molecular weight	136.09
Definition	Monopotassium monophosphate, after drying at 105° for 4 hours, contains not less than 98% of KH_2PO_4 and conforms to the following specifications.
Description	Monopotassium monophosphate occurs as a white odourless powder or crystals.
Uses	Buffer, neutralizing agent, and sequestrant in milk products and meat products.

* For biological data and toxicological evaluation see pp. 29-32.

Identification Tests

A. *Solubility*

Water : freely soluble.
Ethanol, ether, chloroform : insoluble.

B. To a 1% solution of monopotassium monophosphate add silver nitrate TS ; the yellow precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of monopotassium monophosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of monopotassium monophosphate add a few drops of barium nitrate TS. The solution remains limpid. Add 2 N sodium acetate. The white precipitate formed is soluble in acetic acid TS.

E. Add to a 1% solution of monopotassium monophosphate 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

Purity Tests

Loss on drying : Not more than 2.0% after drying at 105° for 4 hours.

pH of a 1% solution : 4.2-4.6.

Sodium : To 5 ml of a 1% solution of monopotassium monophosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. No precipitate is formed.

Arsenic : Not more than 2 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Potentiometric method. Transfer 0.7000 g of monopotassium monophosphate, previously dried at 105° for 4 hours, to a 250-ml beaker, add 50 ml of 0.1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and add slowly from a burette, with constant stirring, 0.1 N sodium hydroxide until a pH of 3.3 is attained. Continue to add sodium hydroxide solution until the next ml or 0.5-ml graduation mark on the burette is reached. Record the burette reading under column 1 of a suitable data sheet and

record the pH under column 2. Continue the addition of 0.1 N sodium hydroxide in 0.5-ml increments until a pH of 6.0 is attained, recording the burette reading and the pH after the addition of each increment. Then proceed with the titration in the usual manner until a pH of 8.0 is attained, and again continue to add the solution until the next ml or 0.5-ml graduation mark is reached before recording the burette reading and the pH. Then continue adding 0.1 N sodium hydroxide in 0.5-ml increments until the pH is 10.0, again recording the burette reading and the pH after the addition of each increment. In column 3 of the data sheet, note the values for ΔpH obtained by subtracting each pH value recorded from the next higher value. In column 4, note the values for Δ_2pH, i.e., the differences between successive ΔpH values, recording them as plus or minus depending upon whether the value of ΔpH is higher or lower than the preceding one. The end-point lies in the 0.5-ml increment of sodium hydroxide that gives the highest value for ΔpH, its exact position being calculated by adding 0.5 b/B to the next lower burette reading, where b is the last Δ_2pH value having a plus sign and B is the sum, without regard to sign, of the last Δ_2pH value having a plus sign and the first Δ_2pH value having a minus sign. Two end-points are calculated, that occurring between pH 3.3 and pH 6.0 being designated F and that between pH 8.5 and pH 10.0 being designated T. The volume of sodium hydroxide used in the titration is obtained by subtracting F from T. Each ml of 0.1 N sodium hydroxide is equivalent to 0.01361 g of KH_2PO_4.

DIPOTASSIUM MONOPHOSPHATE *

Chemical names	Dipotassium monophosphate; dipotassium monohydrogen monophosphate; dipotassium phosphate; dipotassium hydrogen phosphate; potassium phosphate dibasic.
Chemical formula	K_2HPO_4
Molecular weight	174.18
Definition	Dipotassium monophosphate, after drying at 105° for 4 hours, contains not less than 98% of K_2HPO_4 on a dry-weight basis and conforms to the following specifications.
Description	Dipotassium monophosphate occurs as a deliquescent, colourless or white, granular salt.

* For biological data and toxicological evaluation see pp. 29-32.

Uses Buffer, neutralizing agent, and sequestrant in cheese, milk products, and ice-cream.

Identification Tests

A. *Solubility*

Water : freely soluble.
Ethanol : very slightly soluble.

B. To a 1% solution of dipotassium monophosphate add silver nitrate TS ; the yellow precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of dipotassium monophosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of dipotassium monophosphate add a few drops of barium nitrate TS. The white precipitate formed is soluble in acetic acid TS.

E. Add to a 1% solution of dipotassium monophosphate 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

Purity Tests

Loss on drying : Not more than 2.0% after drying at 105° for 4 hours.

pH of a 1% solution : 8.7-9.2.

Sodium : To 5 ml of a 1% solution of dipotassium monophosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. No precipitate is formed.

Arsenic : Not more than 2 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Potentiometric method. Transfer 0.7000 g of dipotassium monophosphate, previously dried at 105° for 4 hours, to a 250-ml beaker, add 50 ml of 0.1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and add slowly from a burette, with constant stirring, 0.1 N sodium hydroxide until a pH of 3.3 is attained. Continue to add sodium hydroxide solution until the next ml or 0.5-ml graduation mark on the burette is reached. Record the burette reading under column 1 of a suitable data sheet and record the

pH under column 2. Continue the addition of 0.1 N sodium hydroxide in 0.5-ml increments until a pH of 6.0 is attained, recording the burette reading and the pH after the addition of each increment. Then proceed with the titration in the usual manner until a pH of 8.0 is attained, and again continue to add the solution until the next ml or 0.5-ml graduation mark is reached before recording the burette reading and the pH. Then continue adding 0.1 N sodium hydroxide in 0.5-ml increments until the pH is 10.0, again recording the burette reading and the pH after the addition of each increment. In column 3 of the data sheet, note the values for ΔpH obtained by subtracting each pH value recorded from the next higher value. In column 4, note the values for Δ_2pH, i.e., the differences between successive ΔpH values, recording them as plus or minus depending upon whether the value of ΔpH is higher or lower than the preceding one. The end-point lies in the 0.5-ml increment of sodium hydroxide that gives the highest value for ΔpH, its exact position being calculated by adding 0.5 b/B to the next lower burette reading, where b is the last Δ_2pH value having a plus sign and B is the sum, without regard to sign, of the last Δ_2pH value having a plus sign and the first Δ_2pH value having a minus sign. Two end-points are calculated, that occurring between pH 3.3 and pH 6.0 being designated F and that between pH 8.5 and pH 10.0 being designated T. The volume of sodium hydroxide used in the titration is obtained by subtracting F from T. Each ml of 0.1 N sodium hydroxide is equivalent to 0.01742 g of K_2HPO_4.

TRIPOTASSIUM MONOPHOSPHATE

Chemical names Tripotassium monophosphate; tripotassium phosphate; tripotassium orthophosphate; potassium phosphate tribasic.

Chemical formula $K_3PO_4 \cdot XH_2O$

Molecular weight 212.28 (anhydrous)

Definition Tripotassium monophosphate, after ignition at 800° for 30 minutes, contains not less than 80% K_3PO_4 and conforms to the following specifications.

Description Tripotassium monophosphate occurs as an odourless, coarse granular powder.

Identification Tests

A. *Solubility*

Water: freely soluble.
Ethanol: insoluble.

B. To a 1% solution of tripotassium monophosphate add silver nitrate TS. The yellow precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of tripotassium monophosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of tripotassium monophosphate add a few drops of barium nitrate TS. The white precipitate formed is soluble in dilute acetic acid TS.

E. Add to a 1% solution of tripotassium monophosphate 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

Purity Tests

Loss on heating : Not more than 20% of its weight after ignition at 800° for 30 minutes.

pH of a 1% solution : 11.5-12.0.

Arsenic : Not more than 2 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Dissolve 8.000 g of tripotassium monophosphate, previously ignited at 800° for 30 minutes, in 40 ml of water in a 400-ml beaker. Add 100 ml of N hydrochloric acid and pass a stream of carbon-dioxide-free air in fine bubbles through the solution for 30 minutes to expel carbon dioxide. The beaker must be covered with a perforated cover to prevent loss of any of the solution by spraying. Wash down the cover and sides of the beaker, and place the electrodes of a standardized pH meter in the solution. Titrate the solution with N sodium hydroxide to the inflection point occurring about pH 4. Record the number of ml (A) of N hydrochloric acid consumed. Protect the solution from absorbing carbon dioxide from the atmosphere, and continue the titration with N sodium hydroxide to the inflection point occurring about pH 8.8. Record the number of ml (B) of N sodium hydroxide consumed in this titration. If A is equal to or greater than 2B, then

$$\% \ K_3PO_4 = \frac{B \times 0.2123}{\text{weight of sample}} \times 100$$

If A is less than 2B, then

$$\% \ K_3PO_4 = \frac{(A - B) \times 0.2123}{\text{weight of sample}} \times 100$$

Biological Data

Biochemical aspects

The level of inorganic phosphate in the blood is stabilized by exchange with the mineral depot in the skeleton through the action of the hormone of the parathyroid glands. This hormone inhibits tubular reabsorption of phosphates by the kidney and brings about demineralization of bone tissue through the action of osteoclasts. The amount of parathyroid hormone that enters the circulation is probably regulated by the calcium level of the blood. As a result of these mechanisms, man and animals can tolerate large variations in phosphate intake without the balance being upset.

Some investigators have considered that the formation in the intestinal tract of insoluble salts of phosphate with calcium, iron and other metal ions might result in decreased absorption of such minerals. From studies dealing with this aspect,[8, 11, 15, 18] it is concluded that moderate dose levels of phosphates do not impair absorption as shown by results from carcass analyses or haemoglobin determinations.

Little specific toxicological information on potassium monophosphates is available. There is no reason to consider that the potassium salts, in the amounts that could be used as food additives, behave differently from the sodium salts. They will be dealt with together as monophosphates.

Acute toxicity

Compound	Animal	Route	Minimum lethal dose (g/kg body weight)	Reference
NaH_2PO_4	Guinea-pig	oral	> 2	5
Na_2HPO_4	Rabbit	i.v. infusion	> 0.985, ≤ 1.075	5

Short-term studies

Rat

There are many reports of short-term studies to determine the effects of the addition of monophosphates to the diet of rats.[6, 7, 8, 9] Those do not provide for a differentiation between the action of the mono-, di- and trisodium or potassium salts; several authors have used "neutral" mixtures, e.g., of mono- and disodium monophosphates. There is no reason to expect a specific action on the part of one of these three monophosphates, the relevant factors being the phosphate content and the acidity of the food mixture as a whole. On high-dose levels, hypertrophy of the parathyroid glands has been observed. A more important and more sensitive criterion for the deleterious action of phosphate overdosage is the appearance of metastatic

calcification in soft tissues, especially in the kidney, stomach and aorta. Kidney calcification may be observed in a few weeks or months, depending on the dose level. The pathology of calcification and necrosis of the tubular epithelium in the kidneys (nephrocalcinosis) has been studied in detail.[6, 7, 9, 10]

It is difficult to indicate a border line between dose levels that do not produce nephrocalcinosis and those that produce early signs of such changes, because :

(1) even on diets to which no phosphate has been added, rats in apparently healthy condition may have a few isolated areas of renal calcification ;

(2) the composition of the diet (amount of calcium, acid-base balance, vitamin D), has an important influence on the appearance of renal calcification.

There are numerous reports of experimental phosphate-containing diets that do not produce kidney damage by excessive calcification, e.g., the Sherman diet, which contains 0.47-0.51% P,[1, 8, 11] the diet used by McKay & Oliver[6] containing 0.62% P, and the commercial " Purina A " diet, containing 0.90% P.[11]

Early calcification has been observed in rats on a Sherman diet to which 1% of a 2 : 3 mixture of NaH_2PO_4 and Na_2HPO_4 was added, bringing the P-content to 0.71%.[8] Similar effects were observed with the addition of a phosphate mixture resulting in a P-content of 0.89%,[1] and with levels of phosphate in the diet corresponding to a P-content varying from 1.25% to 2.85%.[5, 6, 7, 8, 11, 12]

In recent experiments, however,[2] a diet to which K_2HPO_4 had been added and containing 1.3% P and 0.5% Ca did not produce nephrocalcinosis in a group of 12 mice within a period of 150 days, although the weight of the kidneys was increased. Also, food and protein efficiency was diminished as compared with the animals on the control diet. These effects may have resulted from the large amount of salts added to the diet in these experiments.

Guinea-pig

Diets containing 0.9% P and 0.8% Ca or higher levels of phosphate produced calcification in the soft tissues.[13, 14]

Man

The long-continued daily intake of 5-7 g of NaH_2PO_4 (corresponding to 1.0-1.5 g of P) did not produce adverse effects.[11] Similarly a daily intake of 6 g of $NaH_2PO_4 \cdot 2H_2O$ was tolerated without difficulty.[15]

Long-term studies

No long-term studies have been found in the literature.

Comment on experimental studies reported

The highest levels of P in food for rats that did not cause significant kidney damage were 0.90%[11] and 1.30%.[2]

The lowest levels of phosphate in the diet of rats that produced the first signs of calcification in the kidneys corresponded to 0.71% P and 0.89% P ; these were found in experiments using diets with excess base (the Sherman diet, which contains $^1/_3$ whole-milk powder).[1, 8] This may have increased the tendency toward calcification.

From consideration of the complete experimental evidence it is estimated that diets containing 1% P or more may be nephrocalcinogenic in rats.[11, 16]

Since nearly every food normally contains phosphates, it is impossible to indicate acceptable intakes of these compounds as food additives without regard to the phosphate intake from the food itself. For this reason, acceptable intakes are given as total daily intakes both from food and from food additives. These should also include phosphoric acid, for which an estimate of acceptable daily intakes for man has been given in the Committee's sixth report,[3] and phosphorus from polyphosphates (see monograph, p. 43) and diphosphates (see monograph, p. 36).

Evaluation

The usual evaluation procedure of taking the highest dose level in animals that does not produce any toxicological effects and applying a safety factor to arrive at an estimate of acceptable daily intakes for man is not appropriate in this case. The lowest dose levels that produce nephrocalcinosis overlap the highest dose levels failing to do so. The calculation based on body weight is probably not suitable for food additives that are also nutrients.

The lowest level that produced nephrocalcinosis in the rat (1% P in the diet) is used as the basis for the evaluation and, by extrapolation based on a daily food intake of 2800 calories, this gives a dose level of 6.6 g of P per day as the best estimate of the lowest level that might cause nephrocalcinosis in man.

Since acceptable dose levels of phosphate intake depend on the amount of calcium in the diet, it is difficult to indicate zones that apply uniformly to countries having widely differing levels of dietary calcium. Therefore, the unconditional acceptance zone for phosphate intake may be regarded as suitable for communities with a low calcium intake and the conditional acceptance zone for those with a high calcium intake in the normal diet.

Estimate of acceptable daily intakes for man

	mg/kg body weight [a]
Unconditional acceptance	up to 30
Conditional acceptance	30-70

[a] As total dietary phosphorus intakes from both food and food additives.

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DISODIUM DIPHOSPHATE *

Chemical names Disodium diphosphate; disodium dihydrogen diphosphate; disodium dihydrogen pyrophosphate; disodium pyrophosphate; sodium acid pyrophosphate.

Chemical formula $Na_2H_2P_2O_7$

Molecular weight 221.94

Definition Disodium diphosphate, after drying at 105° for 4 hours, contains not less than 95.0% of $Na_2H_2P_2O_7$ and conforms to the following specifications.

* For biological data and toxicological evaluation see pp. 36-38.

Description Disodium diphosphate occurs as white, fused masses or as a white powder.

Uses Buffer, stabilizer, sequestrant, and peptizing agent in cheese and meat products, and leavening agent in baking powder.

Identification Tests

A. *Solubility*

Water : freely soluble.

B. To a 1% solution of disodium diphosphate add silver nitrate TS ; the white precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of disodium diphosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of disodium diphosphate add a few drops of barium nitrate TS. The white precipitate formed is insoluble in acetic acid TS.

E. To 5 ml of a 1% solution of disodium diphosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few minutes.

F. Paper chromatography for distinction from triphosphate and higher polyphosphates :

Chromatographic solvent : Mix 75 ml of isopropanol, 10 ml of water, 20 ml of 20% trichloroacetic acid and 0.3 ml of 20% ammonia. Make fresh every week.

Chromatographic spray : Dissolve 1.0 g of ammonium molybdate in 85 ml of water, 10 ml of N hydrochloric acid and 5 ml of 60% perchloric acid.

Sample solution : Dissolve 1.0 g of disodium diphosphate in 50 ml of water.

Reference solution : Dissolve 1.0 g of a standard sample of disodium diphosphate in 50 ml of water.

Procedure : Place 0.01 ml of the sample solution and 0.01 ml of the reference solution on the starting line of the chromatographic paper and allow to dry in a stream of warm air. Use ascending chromatography at 18°-20° until the solvent has ascended about 25 cm from the starting line (12-15 hours). Dry at 60° in an oven and spray with the chromatographic spray. Place the paper under an ultraviolet lamp and irradiate until the phosphates are visible as blue spots (about 2 minutes). Disodium diphosphate shows only two spots : one from the technically

unavoidable monophosphate (Rf = 0.69), and one from the diphosphate (Rf = 0.44).

Purity Tests

Loss on drying : Not more than 0.5% after drying at 105° for 4 hours.
pH of a 1% solution : 3.8-4.2.
Arsenic : Not more than 5 mg/kg.
Lead : Not more than 10 mg/kg.
Heavy metal : Not more than 40 mg/kg.
Fluorine : Not more than 10 mg/kg.

Assay

Weigh 0.5000 g of disodium diphosphate into a 400-ml beaker and dissolve in 100 ml of water. Adjust pH of the solution to exactly 3.8 with hydrochloric acid (pH meter). Add 50 ml of a 12.5% solution of zinc sulfate (125 g of $ZnSO_4.7H_2O$ dissolved in water, diluted to 1 litre, filtered, and adjusted to pH 3.8) and allow to stand for two minutes. Titrate the liberated acid with 0.1 N sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the end-point, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 N sodium hydroxide is equivalent to 0.01110 g of $Na_2H_2P_2O_7$.

TETRASODIUM DIPHOSPHATE

Chemical names — Tetrasodium diphosphate ; sodium pyrophosphate ; tetrasodium pyrophosphate.

Chemical formula — *Anhydrous :* $Na_4P_2O_7$
Decahydrate : $Na_4P_2O_7 \cdot 10H_2O$

Molecular weight — *Anhydrous :* 265.90
Decahydrate : 446.05

Definition — Tetrasodium diphosphate, after ignition at 800° for 30 minutes, contains not less than 98% of $Na_4P_2O_7$ on a dry-weight basis and conforms to the following specifications.

Description — Tetrasodium diphosphate occurs as a white, crystalline powder.

Uses — Buffer, stabilizer, sequestrant, and peptizing agent in cheese and meat products.

Identification Tests

A. *Solubility*

Water : 1 g of the anhydrous salt is soluble in about 20 ml ; 1 g of the decahydrate is soluble in about 12 ml.

B. To a 1% solution of tetrasodium diphosphate add silver nitrate TS ; the white precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of tetrasodium diphosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

D. To 5 ml of a 1% solution of tetrasodium diphosphate add a few drops of barium nitrate TS. The white precipitate formed is insoluble in acetic acid TS.

E. To 5 ml of a 1% solution of tetrasodium diphosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few minutes.

F. Paper chromatography for distinction from triphosphate and higher polyphosphates :

Chromatographic solvent : Mix 75 ml of isopropanol, 10 ml of water, 20 ml of 20% trichloroacetic acid and 0.3 ml of 20% ammonia. Make fresh every week.

Chromatographic spray : Dissolve 1.0 g of ammonium molybdate in 85 ml of water, 10 ml of N hydrochloric acid and 5 ml of 60% perchloric acid.

Sample solution : Dissolve 1.0 g of tetrasodium diphosphate anhydrous or 1.0 g of the dried decahydrate in 50 ml of water.

Reference solution : Dissolve 1.0 g of a standard sample of tetrasodium diphosphate anhydrous in 50 ml of water.

Procedure : Place 0.01 ml of the sample solution and 0.01 ml of the reference solution on the starting line of the chromatographic paper and allow to dry in a stream of warm air. Use ascending chromatography at 18°-20° until the solvent has ascended about 25 cm from the starting line (12-15 hours). Dry at 60° in an oven and spray with the chromatographic spray. Place the paper under an ultraviolet lamp and irradiate until the phosphates are visible as blue spots (about 2 minutes). Tetrasodium diphosphate shows only one spot, from the diphosphate ($R_f = 0.44$).

Purity Tests

Loss on ignition : Not more than 0.5% for the anhydrous salt and not more than 42% for the decahydrate after ignition at 800° for 30 minutes.

pH of a 1% solution : 9.9-10.3.
Arsenic : Not more than 5 mg/kg.
Lead : Not more than 10 mg/kg.
Heavy metals : Not more than 40 mg/kg.
Fluorine : Not more than 10 mg/kg.

Assay

Weigh 0.5000 g of tetrasodium diphosphate into a 400-ml beaker and dissolve in 100 ml of water. Adjust pH of the solution to exactly 3.8 with hydrochloric acid (pH meter). Add 50 ml of a 12.5% solution of zinc sulfate (125 g of $ZnSO_4.7H_2O$ dissolved in water, diluted to 1 litre, filtered and adjusted to pH 3.8) and allow to stand for two minutes. Titrate the liberated acid with 0.1 N sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the end-point, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 N sodium hydroxide is equivalent to 0.01330 g of $Na_4P_2O_7$.

Biological Data

Biochemical aspects

In the animal body diphosphate is formed from adenosyl triphosphate (ATP) in many enzymatic reactions. It is either utilized by entering phosphorolytic reactions, or it is hydrolysed by an inorganic diphosphatase to monophosphate.[1] Ingested diphosphate is readily converted to monophosphate ;[2, 3] no diphosphate was found in faeces or urine of rats treated with diets containing up to 5% tetrasodium diphosphate. In these experiments diphosphate was almost completely absorbed by the gut and excreted as monophosphate in the urine.

Acute toxicity

Animal	Route	Minimum lethal dose (mg/kg body weight)	Reference
Rabbit	i.v.	About 50	4
Rat	oral	LD_{50} ($Na_4P_2O_7$) : > 4000	5

Short-term studies

Rat

In a series of successive experiments,[6, 7] tetrasodium diphosphate ($Na_4P_2O_7$) was added in concentrations of 1.8%, 3% and 5% to a modified Sherman diet and fed to groups of 34-36 young rats for 6 months. The

studies also included control groups and groups receiving the same levels of sodium monophosphate. With 3% and 5% diphosphate diets growth was significantly decreased and at both these concentrations nephrocalcinosis appeared as the main toxic effect. The degree of damage to the kidneys was about the same as that observed in the corresponding monophosphate groups.

With the 1.8% diphosphate and monophosphate diets, normal growth occurred, but a slight yet statistically significant increase in kidney weight was noted. Microscopic examination revealed kidney calcification in some of the animals, both in the diphosphate and monophosphate groups. This was more extensive than the calcification occasionally found in the control animals. In an additional experiment, 1.1% of diphosphate and of monophosphate were used.[8] There was a slight growth retardation in the first part of the experiment. After 39 weeks, a slight degree of kidney calcification was noted and this was the same for both phosphates.[8]

In a recent series of experiments [5] Sherman diets containing 1%, 2.5% and 5% tetrasodium diphosphate ($Na_4P_2O_7$) were fed for 16 weeks to groups of 20 male and 20 female rats weighing between 90 and 115 g; a similar group received a diet containing 5% monophosphate. In the sodium diphosphate groups, growth was normal up to the 2.5% level; kidney weight was increased at the 2.5% level (females) and above; kidney function, as determined by a concentration test, decreased at the 2.5% level (males) and above. Kidney damage (calcification, degeneration and necrosis) was observed in a greater percentage of the rats in the 1% group than in the controls. At the higher concentrations of sodium diphosphate more severe kidney damage occurred and, in addition, some of the animals had hypertrophy and haemorrhages of the stomach. The latter abnormality was not found in rats in the 5% monophosphate group.

Long-term studies

Rat

No specific long-term studies with diphosphates have been made, but in one series of long-term experiments a mixed preparation was used which consisted of $^2/_3$ disodium and tetrasodium diphosphate ($Na_2H_2P_2O_7$ and $Na_4P_2O_7$) and $^1/_3$ Kurrol's salt.[a] Concentrations of 0.5%, 1%, 2.5% and 5% were added to a Sherman diet and given to groups of 10 male and 10 female rats. From these animals a second and third generation were produced, during which the treatment with phosphates was continued. Growth and fertility and average life-span were normal and the life-span was not significantly reduced up to the 2.5% level. Nephrocalcinosis

[a] Kurrol's salt is a polymer of high molecular weight obtained by fusion of monopotassium monophosphate. The formula is $(KPO_3)_n \cdot H_2O$, where n = 400-5000.

occurred at the 1% level and above. At 0.5% no abnormalities were observed that were not also present in control animals. At none of the concentrations did tumours appear with a higher frequency than in the controls.[9]

Comments on experimental studies reported

The main conclusion from the data on diphosphate is that kidney damage is the most sensitive criterion of its toxic action. This damage is of the general type of nephrocalcinosis and there is no evidence that in this respect the action of diphosphate differs from that of monophosphate.

At higher concentrations in the diet and in acute experiments, an additional toxic action of diphosphate may appear which is probably mainly due to the hydrolysis and resulting acidification occurring in the intestinal tract. This effect, however, is not relevant to the evaluation of daily intake levels where the more sensitive criterion of kidney damage is used. Undesirable acute effects from the hydrolysis of diphosphate in the stomach are not likely to occur in the concentrations that are at present used in food products, since the resulting concentrations in the final products as consumed are much below the concentration that produced such effects in the animal experiments. Since the nephrotoxic action of diphosphate is no greater than that of monophosphate, there is no basis for an estimate of acceptable daily intakes different from that for monophosphate. It is the total load of phosphate in the food which determines whether nephrotoxic effects will appear. Therefore, for the purpose of estimating acceptable intake zones diphosphates should be treated as if they were monophosphates on the basis of their phosphorus content.

Evaluation

The acceptable daily intakes for man of monophosphate (polyphosphates, diphosphate and phosphoric acid inclusive) are given in the monograph on monophosphate (see page 31).

Further work considered desirable

Toxicological studies in non-rodent species.

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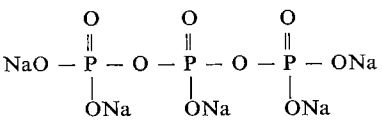
PENTASODIUM TRIPHOSPHATE *

Synonyms Tripolyphosphate; sodium tripolyphosphate.

Chemical names Pentasodium triphosphate; sodium triphosphate.

Chemical formula $Na_5P_3O_{10}$

Structural formula



Molecular weight 367.86

Definition Pentasodium triphosphate, after ignition at 550° for 30 minutes, contains not less than 85% of $Na_5P_3O_{10}$ (the remainder being other phosphates) and conforms to the following specifications.

Description Pentasodium triphosphate occurs as white, slightly hygroscopic granules or powder.

Uses Sequestrant, peptizing agent and stabilizer in fruit juice, canned fish, cheese, meat products and shortening.

Identification Tests

A. *Solubility*

Water: freely soluble.

B. To a 1% solution of pentasodium triphosphate add silver nitrate TS; the white precipitate formed is soluble in dilute nitric acid TS.

C. To 5 ml of a 1% solution of pentasodium triphosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

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* For biological data and toxicological evaluation see pp. 43-48.

D. To 5 ml of a 1% solution of pentasodium triphosphate add a few drops of barium nitrate TS. The white precipitate is insoluble in acetic acid TS.

E. To 5 ml of a 1% solution of pentasodium triphosphate add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few minutes.

F. Paper chromatography for distinction from diphosphate and higher polyphosphates :

Chromatographic solvent : Mix 75 ml of isopropanol, 10 ml of water, 20 ml of 20% trichloroacetic acid and 0.3 ml of 20% ammonia. Make fresh every week.

Chromatographic spray : Dissolve 1.0 g of ammonium molybdate in 85 ml of water, 10 ml of N hydrochloric acid and 5 ml of 60% perchloric acid.

Sample solution : Dissolve 1.0 g of pentasodium triphosphate in 50 ml of water.

Reference solution : Dissolve 1.0 g of a standard sample of pentasodium triphosphate in 50 ml of water.

Procedure : Place 0.01 ml of sample solution and 0.01 ml of reference solution on the starting line of the chromatographic paper and allow to dry in a stream of warm air. Use ascending chromatography at 18°-20° until the solvent has ascended about 25 cm from the starting line (12-15 hours). Dry at 60° in an oven and spray with the chromatographic spray. Place the paper under an ultraviolet lamp and irradiate until the phosphates are visible as blue spots (about 2 minutes). Pentasodium triphosphate shows three spots : one from monophosphate (Rf = 0.69), a second from the diphosphate (Rf = 0.44) and the third from the triphosphate (Rf = 0.29).

Purity Tests

Loss on ignition : Not more than 0.5% after ignition at 550° for 30 minutes.

pH of a 1% solution : 9.5-9.9.

Arsenic : Not more than 5 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Paper chromatography. After developing the spots corresponding to the various phosphates, cut them out following their shapes, roll them and

put them into 50-ml flasks ; put spots which are less intensively coloured into 25-ml flasks. Add 2 ml of a 60% perchloric acid ($HClO_4$) solution and heat the flasks on a sand-bath until the paper is carbonized. Add 3-5 drops of nitric acid (HNO_3) and continue to heat the flasks strongly. As soon as the water and the nitric acid have boiled off and the white perchloric acid fumes are visible, shake the flask well to determine whether the content is colourless. Heat till the content of the flask is clear. Then add 20 ml of water, or 10 ml for the 25-ml flasks, and hydrolyse on the sand-bath at low temperature for about 30 minutes. After cooling, neutralize with 2 N NaOH using phenolphthalein TS and take back the small excess of NaOH with a few drops of 0.1 N H_2SO_4. Add the following solutions to each of the flasks while shaking :

(1) 0.2 ml of a citric acid solution (20 g of anhydrous citric acid to 100 ml of water).

(2) 0.8 ml of a sodium pyrosulfite solution (35 g of $Na_2S_2O_5$ to 100 ml of water).

(3) 1.6 ml of an ammonium molybdate solution (50 ml of concentrated H_2SO_4 in 900 ml of water, mixed with 50 g of ammonium molybdate, and made up to 1 litre).

(4) 1.6 ml of a solution of 2 g of *p*-methylaminophenol sulfate $[(HO \cdot C_6H_4 \cdot NHCH_3)_2 \cdot H_2SO_4]$ and 0.1 ml of concentrated H_2SO_4 in 1 litre of water. This solution must be renewed about every 4 days.

Heat the flasks for 10 minutes in a water bath up to 60°, cool and fill to the mark. Run blanks with the same reagents and water in 50-ml and 25-ml flasks. The flasks should be protected from solar radiation. The blue colour resulting from the formation of the phosphormolybdenum blue is measured after about 30 minutes in 5-cm trays in the Elko II colorimeter with filter S 72 E against the blank. The respective P_2O_5-values can be read off by using a standard extinction curve which has been prepared both for the 25-ml and the 50-ml flasks. It is recommended that a piece of chromatographic paper of the same size should be developed and treated at the same time and the determined value subtracted from the obtained P_2O_5-content.

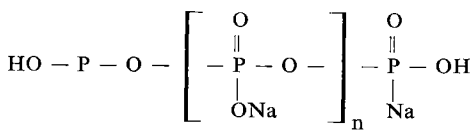
Multiply the obtained P_2O_5-content by the factor 1.728 for pentasodium triphosphate.

SODIUM POLYPHOSPHATE (Graham's sodium polyphosphate)

Chemical description A polymer of high molecular weight obtained by fusion of monosodium monophosphate.

Chemical formula $(NaPO_3)_n \cdot H_2O$ (n = 10-300)

Structural formula



Definition Graham's sodium polyphosphate, after ignition to dull red, contains not less than 66.5% P_2O_5 and conforms to the following specifications.

Description Colourless, glassy, transparent, hygroscopic platelets, granules or powder.

Uses Sequestrant and stabilizer in fruit juice, canned fish, cheese and meat products and shortening.

Identification Tests

A. *Solubility*

Water : freely soluble.

B. To 5 ml of a 1% solution of Graham's sodium polyphosphate add 1 ml of nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

C. Paper chromatography for distinction from diphosphate and triphosphate :

Chromatographic solvent : Mix 75 ml of isopropanol, 10 ml of water, 20 ml of 20% trichloroacetic acid and 0.3 ml of 20% ammonia. Make fresh every week.

Chromatographic spray : Dissolve 1.0 g of ammonium molybdate in 85 ml of water, 10 ml of N hydrochloric acid and 5 ml of 60% perchloric acid.

Sample solution : Dissolve 1.0 g of sodium polyphosphate in 50 ml of water.

Reference solution : Dissolve 1.0 g of a standard sample of sodium polyphosphate in 50 ml of water.

Procedure : Place 0.01 ml of the sample solution and of the reference solution on the starting line of the chromatographic paper and allow to dry in a stream of warm air. Use ascending chromatography at 18°-20° until the solvent has ascended about 25 cm from the starting line (12-15 hours). Dry at 60° in an oven and spray with the chromatographic spray. Place the paper under an ultraviolet lamp and irradiate until the phosphates are visible as blue spots (about 2 minutes). Sodium polyphosphate shows a main spot on the starting point and different spots of oligophosphates.

Purity Tests

Loss on ignition : Not more than 0.5% after ignition at a dull red heat.

pH of 1% solution : About 6.6.

Arsenic : Not more than 5 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Fluorine : Not more than 10 mg/kg.

Assay

Dissolve 0.2500 g of Graham's sodium polyphosphate in 25 ml of water, add 5 ml of nitric acid and heat on a steam bath for 10 minutes. Then dilute to 100 ml, heat to 60°, add an excess of ammonium molybdate TS, and heat at 50° for 30 minutes. Filter, wash the precipitate with dilute nitric acid (1 in 36), followed by potassium nitrate solution (1 in 100) until the filtrate is no longer acid to litmus. Dissolve the precipitate in 50 ml of N sodium hydroxide, add phenolphthalein TS, and titrate the excess sodium hydroxide with N sulfuric acid. Each ml of N sodium hydroxide is equivalent to 0.003086 g of P_2O_5.

Biological Data

Biochemical aspects

Several studies indicate that polyphosphates can be hydrolysed *in vivo* by enzymes with the formation of monophosphate. The localization of different polyphosphatases in the nuclei of animal cells has been demonstrated.[1] Injected hexametaphosphate is more slowly degraded than tripolyphosphate,[2] and the highly polymerized Tammann's salt [a] is even more slowly eliminated from the blood after intravenous injection than is Graham's salt.[3] When administered parenterally, a small part of these products may escape in the urine as oligophosphates.[2, 3] The higher polyphosphates are probably not absorbed as such in the intestinal tract. After hydrolysis into smaller units absorption takes place. The larger the molecule, the less the speed of hydrolysis and absorption.

After giving hexametaphosphate to rats and rabbits by stomach tube, no more than trace amounts of labile phosphate were found in the urine.[2] The oral administration of radioactively-labelled Tammann's salt did not give rise to radioactivity in the blood.[3] With Graham's salt and Kurrol's

[a] Tammann's salt is potassium sodium polyphosphate.

salt,[a] 10-30% was absorbed as monophosphate and small amounts of oligophosphates were found in the urine.[4, 5] In experiments in rats with labelled tripolyphosphate and Graham's salt these polymers were not absorbed as such, but were taken up after hydrolysis into monophosphate and diphosphate. In a period of 18 hours, only 40% of the dose of Graham's salt was hydrolysed and absorbed. The bacterial flora of the intestinal tract may contribute to the hydrolysis of the polyphosphates.[6] In other experiments, radioactively-labelled Kurrol's salt was given orally to rats. About half the radioactivity was recovered from the faeces, mainly as polymeric phosphate, and only a small percentage of the dose was found in the urine, in this case in the form of monophosphate.

It is to be noted that, for practical reasons, in the studies cited high dosages were given to the animals. The efficiency of hydrolysis and absorption may be greater at lower dose levels, such as were used in the short-term and long-term feeding experiments quoted. In some of these [7] the " monophosphate action ", as demonstrated by the production of nephrocalcinosis, was not much smaller than when the same dose level was administered by the addition of monophosphate to the food. In another study, this applied only to tripolyphosphate, while Graham salt had definitely less effect on the kidney.[8]

The possibility of the intermediate formation of small amounts of trimetaphosphate in the hydrolysis of polyphosphates has been considered.[9] Today, the only possible method of production of sodium polyphosphates is by the fusion process. In this process metaphosphates are also formed in amounts up to 8% and their presence is technically unavoidable. It is of interest to note that these metaphosphates (sodium trimetaphosphate and sodium tetrametaphosphate) have been tested in short-term experiments in rats and dogs in conjunction with polyphosphates.[10] The metaphosphates are also hydrolysed to monophosphates. No specific action of these metaphosphates different from that of the other phosphates has been observed and it is concluded that the presence of these impurities does not present a hazard. It is also noted that the preparations of sodium polyphosphates used in the toxicological studies mentioned always contained metaphosphates in amounts up to 8%.

It has been considered by many authors that the ingestion of polyphosphate in the food may result in a loss of minerals (calcium, iron, copper, magnesium), which are bound to the polyphosphate and are lost in the faeces with unhydrolysed polyphosphate. For this reason, in most of the toxicological studies cited, particular attention has been paid to the mineral composition of the carcass and to the possible development of anaemia.

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[a] See footnote on page 37.

The experimental results available indicate that such an action, if it occurs at all, is not significant. Anaemia is not a characteristic feature of treatment with high dose levels of polyphosphate, and hexametaphosphate had no effect on iron utilization by rats.[11]

Acute toxicity

Animal	Substance	Route	LD_{50} (mg/kg body weight)	Approx. lethal dose (mg/kg body weight)	Reference
Mouse	Hexametaphosphate (neutralized sodium salt)	oral		> 100	12
Rabbit	,,	i.v.		about 140	12
Rat	Mixed water-soluble and neutral preparation of $^1/_3$ Kurrol's salt and $^2/_3$ tetra- and disodium diphosphates	oral	4000		7
Rat	,,	i.v.	18		7

Short-term studies

Rat

Groups of 5 male rats were fed for a period of one month on diets containing 0.2%, 2% and 10% sodium hexametaphosphate or 0.2%, 2% and 10% sodium tripolyphosphate. Control groups were given the standard diet, or diets with the addition of 10% sodium chloride or 5% disodium phosphate.[10]

With 10% of either of the polyphosphate preparations and also with 10% sodium chloride in the diet, growth retardation occurred, but none of the rats died. Increased kidney weights and tubular necrosis were, however, observed. With 2% of polyphosphate in the diet, growth was normal, but in the kidneys inflammatory changes were found which were different from the tubular necrosis observed in the 10% groups. With 0.2% of polyphosphate in the diet, normal kidneys were seen. In another series of experiments,[8, 13, 14] 3% and 5% of sodium tripolyphosphate (pH = 9.5 in 1% solution), and 1.8%, 3% and 5% of Graham's salt (pH = 5) were added to a modified Sherman diet, which was then fed during 24 weeks to groups of 36 male and 36 female rats. Growth retardation was exhibited by the rats in the 5% polyphosphate groups. With 3% of either preparation, a temporary growth inhibition was observed, and with 1.8% of Graham's salt (male animals), growth was normal. Nephrocalcinosis was observed in the 3% and 5% groups. It was noted that the degree of damage with Graham's salt was less than that in control groups treated with the same

concentrations of orthophosphate; with tripolyphosphate, however, kidney damage was practically identical with that exhibited by the animals in the orthophosphate control group. In the animals on a diet containing 1.8% Graham's salt, calcification in the kidneys was slight or absent and the kidney weights were normal.[14]

In a further group of experiments [7, 15] Kurrol's salt was used in a commercial preparation consisting of $^1/_3$ Kurrol's salt and $^2/_3$ of a mixture of disodium and tetrasodium diphosphate ($Na_2H_2P_2O_7$ and $Na_4P_2O_7$). Kurrol's salt is practically insoluble in water, but the mixture with diphosphate can be dissolved and a 1% solution had a pH of 7.6. Groups of 10 male and 10 female rats were fed for a period of 12 weeks on a Sherman diet to which 0.5%, 1%, 2.5% and 5% of the mixed preparation had been added. Normal growth was observed in the groups treated with the 0.5%, 1% and 2.5% concentrations of the polyphosphate mixture, but in those receiving the 5% concentration growth retardation was exhibited. Kidney weights were normal in the 0.5% group, slightly increased (males significantly) in the 1% group, and further increased in the 2.5% and 5% groups. The histopathological examination revealed that in the kidneys of the animals of the 5% group definite nephrocalcinosis had occurred, with extensive damage to the tubular tissue. Calcification was also observed in other tissues. In the 2.5% group a less extensive nephrocalcinosis was exhibited, and in the 1% group isolated areas of calcification with lymphocyte infiltrations were found. In the 0.5% group kidney structure was normal. The results obtained with this polyphosphate preparation were practically identical, qualitatively and quantitatively, with the results of a similar experiment made with a neutral mixture of NaH_2PO_4 and Na_2HPO_4 carried out at a later date in the same laboratory.[3, 8]

In other experiments, groups of 12 male rats were treated with diets to which 0.9% and 3.5% of sodium hexametaphosphate had been added (corresponding to 0.46% and 1.20% P). Other groups received the control diet alone (0.4% P and 0.5% Ca), or with addition of potassium monophosphate. To the experimental diets, different amounts of salts were added to replace cornstarch in order to equalize the levels of major minerals; this resulted in a rather high salt concentration. The duration of treatment was up to 150 days. With 3.5% added hexametaphosphate growth and food and protein efficiency were poorest. The kidneys of the animals fed the high level of hexametaphosphate were significantly heavier than those of the control rats. This was perhaps a manifestation of the high salt load on the kidneys. No histological abnormalities were observed in kidney sections from animals taken from any of the groups.[16]

Dog

Sodium tripolyphosphate (pentasodium triphosphate, $Na_5P_3O_{10}$) and sodium hexametaphosphate were fed to one dog each in a dose of 0.1 g/kg

per day for one month ; two other dogs received daily doses which increased from 1.0 g/kg at the beginning to 4.0 g/kg at the end of a 5-month period.

The dog treated with a starting dosage of 1.0 g/kg/day of hexametaphosphate began to lose weight when the daily dose reached 2.5 g/kg, while the one receiving gradually increasing doses of tripolyphosphate lost weight only when its diet contained 4.0 g/kg/day. In other respects (urine analysis, haematology, organ weights), the animals were normal, with the exception of an enlarged heart, due to hypertrophy of the left ventricle, in the dog receiving gradually increasing doses of sodium tripolyphosphate. In addition, tubular damage to the kidneys was observed in both dogs on the higher dose regime. In the tissues of the dogs fed 0.1 g/kg/day, no changes were found that could be attributed to the treatment.[10]

Long-term studies

Rat

To a Sherman diet containing 0.47% P, a mixture of $^1/_3$ Kurrol's salt and $^2/_3$ diphosphate was added in concentrations of 0.5%, 1%, 2.5% and 5% and fed to groups of 30 male and 10 female rats from weaning to the end of their life-span (see short-term studies).[7] Two successive generations of offspring were produced on these diets. Significant growth inhibition was observed in the 5% groups of both first and second generations. In the other groups growth was normal. Fertility was normal in the 0.5%, 1% and 2.5% groups, but much decreased in the 5% group. Haematological tests were carried out only in the 0.5% and 2.5% groups ; they showed a decreased number of erythrocytes in the 2.5% group, second generation only. The life-span of the first generation was decreased in the 5% groups only, probably as a result of the extensive damage to the kidneys. In the 0.5% group no kidney damage attributable to the polyphosphate treatment was observed, but in the groups having higher intakes renal calcification occurred in a degree increasing with the dose level.

In another series of feeding tests,[17] diets containing 0.05%, 0.5% and 5% sodium tripolyphosphate were given for two years to groups of 50 male and 50 female weanling rats. Only when 5% of polyphosphate was added to the diet was growth reduced ; the reduction was significant in males but slight and delayed in females. A smaller number of rats survived in the 5% groups than in the other groups. A low grade of anaemia was sometimes observed in the 5% groups only. Increased kidney weights were noted in the 5% group ; pathological changes which could be ascribed to treatment were not observed in the 0.5% and 0.05% groups. In the control group and the 0.5% tripolyphosphate group reproduction studies were carried out over three generations involving the production of two litters

in each generation. Reproduction was normal and no changes in the offspring were observed.

A long-term study[18] of the same design was made with sodium hexametaphosphate, also at concentrations of 0.05, 0.5 and 5% in the diet. Growth retardation occurred only in the 5% groups. Mortality was high in all groups but had no relation to the amount of hexametaphosphate in the diet. Periodic blood examination gave normal haematological values. Kidney weights were increased in the 5% group, and calcification was present. Rats given the 0.5% diet did not have significant changes in the kidneys. Reproduction studies for three generations in the 0.5% group revealed normal performance in every respect.

Comment on experimental studies reported

The most important observation is that polyphosphates are not absorbed as such to any significant extent, but only in the form of monophosphates to which they are broken down in the intestine. The biological effects of ingested polyphosphate are, therefore, determined by the amount of monophosphate formed and absorbed. Since the extent of hydrolysis of polyphosphates in the intestine is difficult to predict, the safest course is to assume that conversion to monophosphate is complete. Thus, for purposes of toxicological evaluation, polyphosphates may be considered equivalent to monophosphates.

Evaluation

The acceptable daily intake of polyphosphate is related to the total dietary phosphate (see under toxicological evaluation of monophosphates, page 31).

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CALCIUM ACETATE *

Chemical name Calcium acetate.

Empirical formula *Anhydrous:* $C_4H_6O_4Ca$
Monohydrate: $C_4H_6O_4Ca.H_2O$
$C_4H_6O_4Ca \cdot XH_2O (X<1)$

Structural formula

Molecular weight *Anhydrous:* 158.17
Monohydrate: 176.18

Definition Calcium acetate, after drying at 200°, contains not less than 98% of $C_4H_6O_4Ca$ and conforms to the following specifications.

Description Calcium acetate is a white, hygroscopic, bulky, crystalline solid with a slightly bitter taste. A slight odour of acetic acid may be present.

Uses Stabilizer in sausage casings. Antimould and anti-rope agent in bakery products.

Identification Tests

A. *Solubility*

Water: 1 g is soluble in 3.5 ml.

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* For biological data and toxicological evaluation see pp. 53-54.

B. Cover the open end of a hard glass tube containing solid calcium acetate with a piece of filter paper, slightly larger than the mouth of the tube and moistened with freshly prepared alkaline *o*-nitrobenzaldehyde TS. Suspend the tube through an asbestos plate and heat slowly with a gas flame. The filter paper turns blue to blue-green, indicating the presence of acetate.

C. When warmed with sulfuric acid, acetic acid is evolved, recognizable by its odour.

D. React a solution of calcium acetate with ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

Purity Tests

Loss on drying : Not more than 11% after drying at 200°.

Water-insolubles : Dissolve 10.00 g of calcium acetate in 100 ml of hot water, filter through a Gooch crucible, tared to an accuracy of ± 0.0002 g, and wash any residue with hot water. Dry the crucible for 2 hours at 105°. The weight of the dried residue should not exceed 0.0300 g.

pH of a 10% solution : 6-9.

Formic acid and oxidizable impurities : Not more than traces. Dissolve 1 g of calcium acetate in 5 ml of water. Add 2.5 ml of 0.1 N potassium dichromate and 6 ml of sulfuric acid and allow to stand for 1 minute ; add 20 ml of water, cool to 15° and add 1 ml of potassium iodide TS. A faint yellow or brown colour is produced immediately.

Aldehydes : Dissolve 2 g of calcium acetate in 10 ml of water and distil. To the first 5 ml of the distillate, add 10 ml of mercuric chloride TS and make alkaline with N sodium hydroxide ; allow to stand for 5 minutes, and acidify with dilute sulfuric acid TS. The solution shows only a faint turbidity.

Arsenic : Not more than 5 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 30 mg/kg.

Assay

Calcium content. Dissolve in a beaker 2.500 g of calcium acetate in 5 ml of hot dilute hydrochloric acid TS. Cool, transfer the solution to a 250-ml volumetric flask, dilute to volume with water, and mix. Transfer 50 ml of the solution to a 400-ml beaker, add 100 ml of water, 25 ml of sodium

hydroxide TS, 40 mg of murexide indicator preparation,[a] and 3 ml of naphthol green TS and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 0.007909 g of $C_4H_6O_4Ca$.

Acid content. Dissolve 0.5000 g of calcium acetate in 25 ml of water and pour this solution slowly on to a hydrogen-ion-exchange column of about 50-60 ml exchange volume. Wash with water until the eluate is free from acid and titrate with 0.1 N sodium hydroxide using phenolphthalein TS. Each ml of 0.1 N sodium hydroxide is equivalent to 0.007909 g of $C_4H_6O_4Ca$.

CALCIUM CHLORIDE

Chemical name Calcium chloride.

Chemical formula
Anhydrous : $CaCl_2$
Dihydrate : $CaCl_2 \cdot 2H_2O$
Hexahydrate : $CaCl_2 \cdot 6H_2O$

Molecular weight
Anhydrous : 110.99
Dihydrate : 147.02
Hexahydrate : 219.08

Definition
Calcium chloride anhydrous contains not less than 95% $CaCl_2$.

Calcium chloride dihydrate contains not less than 99% $CaCl_2 \cdot 2H_2O$.

Calcium chloride hexahydrate contains not less than 98% $CaCl_2 \cdot 6H_2O$.

All three forms conform to the following specifications.

Description
Calcium chloride anhydrous occurs as white, deliquescent, odourless lumps or porous masses.

Calcium chloride dihydrate occurs as white, hard, deliquescent, odourless fragments or granules.

Calcium chloride hexahydrate occurs as colourless, very deliquescent, odourless crystals, with a slightly bitter taste.

Uses Stabilizer in milk for cheese production and firming agent in canned tomatoes.

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[a] An alternative indicator is hydroxynaphthol blue, of which 0.25 g is used. In this case the naphthol green TS is omitted.

Identification Tests

A. *Solubility*

Calcium chloride anhydrous :

Water : 1 g is soluble in 1.5 ml.
Ethanol : 1 g is soluble in 8 ml.

Calcium chloride dihydrate :

Water : 1 g is soluble in 1.2 ml.
Ethanol : 1 g is soluble in 10 ml.

Calcium chloride hexahydrate :

Water : 1 g is soluble in 0.2 ml.
Ethanol : 1 g is soluble in 0.5 ml.

B. React a solution of calcium chloride with silver nitrate TS. The white, curdy precipitate formed is insoluble in nitric acid, but is soluble in a slight excess of dilute ammonia TS.

C. React a solution of calcium chloride with ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

Purity Tests

Water insolubles and ammonium hydroxide precipitate : Dissolve 5.0 g of anhydrous calcium chloride or the corresponding weight of a hydrate in 100 ml of water and heat to boiling. Add 0.10 ml of methyl red TS, make slightly alkaline with carbonate-free ammonium hydroxide, and boil for 5 minutes. Filter through a small paper, and wash with a little hot water. Dissolve the precipitate in hot dilute hydrochloric acid (1 : 3), wash the paper free from acid, boil the solution (about 30 ml) for 1-2 minutes, add 1 drop of methyl red TS, make slightly alkaline with dilute ammonia TS, and boil gently to coagulate the precipitate. Filter through the same paper, wash thoroughly, and ignite at 800° ± 25°. The weight of ignited residue should not exceed 10 mg (0.1%).

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

Weigh 1.000 g of anhydrous calcium chloride or the corresponding weight of a hydrate, transfer to a 250-ml beaker, and dissolve in a mixture of 100 ml of water and 5 ml of dilute hydrochloric acid TS. Transfer the

solution to a 250-ml volumetric flask, dilute with water to volume and mix. Pipette 50 ml of the solution into a suitable container, add 100 ml of water, 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation,[a] and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 0.005550 g of $CaCl_2$; 0.007351 g of $CaCl_2 \cdot 2H_2O$; or 0.01095 g of $CaCl_2 \cdot 6H_2O$.

Biological Data

Biochemical aspects

Calcium is an essential nutrient. It is present in large quantities in the body. Its absorption and metabolism are related to vitamin D and phosphorus intake and to the functional activity of the parathyroid glands. It is unlikely that a relatively small addition to the daily calcium intake would have any effects in the body and even larger doses would be likely to cause effects only if vitamin D intake were also increased.

The acetate and chloride moieties of these two compounds can be disregarded from a toxicological point of view. They enter naturally into the metabolism of the body.

No definitive estimates of the LD_{50} for calcium acetate or calcium chloride have been found in the literature.

Short-term studies

Nothing was found in the literature concerning the toxicology of calcium acetate or calcium chloride specifically. Acetic acid given to rats in the drinking-water at a level of 0.25% caused no toxic symptoms, while 0.5% produced a slight retardation of growth.[1]

Long-term studies

No animal data have been found in the literature. About 1 g a day of acetic acid, present in vinegar and other items of food and drink, has been consumed by man for centuries, apparently without giving rise to any ill effects.

Evaluation

Judging from the report of the Joint FAO/WHO Expert Group on Calcium Requirements,[2] the average daily intake of this element for man

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[a] An alternative indicator found to be useful is hydroxynaphthol blue. In this case 0.25 g of indicator is used and the naphthol green TS is omitted.

may safely extend from about 400 mg up to 2 or even 3 g. The latitude for dietary variation without ill-effects due to calcium is therefore wide. The contribution of calcium derived from compounds used as food additives according to present practice is unlikely to alter substantially the total intake. For this reason, no specific figure has been proposed as an acceptable daily intake for calcium acetate or calcium chloride.

References

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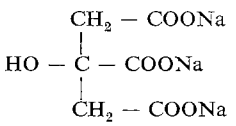
2. *FAO Nutrition Meetings Report Series*, 1962, No. 30; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **230**

SODIUM CITRATE *

Chemical names Trisodium citrate; trisodium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid; trisodium salt of β-hydroxytricarballylic acid.

Empirical formula
Anhydrous : $C_6H_5O_7Na_3$
Dihydrate : $C_6H_5O_7Na_3 \cdot 2H_2O$
Pentahemihydrate : $C_6H_5O_7Na_3 \cdot 5\frac{1}{2}H_2O$

Structural formula



Molecular weight
Anhydrous : 258.07
Dihydrate : 294.10
Pentahemihydrate : 357.16

Definition Sodium citrate, after drying at 180° to constant weight, contains not less than 99.0% of $C_6H_5O_7Na_3$ and conforms to the following specifications.

Description Sodium citrate occurs as colourless crystals, or as a white, crystalline powder; it is odourless and has a cooling, saline taste.

Uses Sequestrant, stabilizer and buffer in certain milk and meat products.

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* For biological data and toxicological evaluation see p. 59.

Identification Tests

A. *Solubility*

Water : 1 g of $C_6H_5O_7Na_3 \cdot 2H_2O$ is soluble in 2 ml.
Ethanol : insoluble.

B. To 5 ml of a 10% solution of sodium citrate add 1 ml of calcium chloride TS and 3 drops of bromothymol blue TS, and slightly acidify with dilute hydrochloric acid TS. Add sodium hydroxide TS until the colour changes to a clear blue, then boil the solution for 3 minutes, agitating it gently during the heating period : a white, crystalline precipitate appears which is insoluble in sodium hydroxide TS but is soluble in acetic acid TS.

C. To 10 ml of a 10% solution of sodium citrate add 1 ml of mercuric sulfate TS. Heat the mixture to boiling, and add a few drops of potassium permanganate TS : a white precipitate of the acetone dicarboxylic acid salt of mercury is formed.

D. To a solution of sodium citrate add uranyl zinc acetate TS ; a yellow, crystalline precipitate appears within a few minutes.

Purity Tests

Loss on drying : After drying at 180° to constant weight : not more than 1% for the anhydrous form ; not less than 10% and not more than 13% for the dihydrate ; not less than 25.0% and not more than 30.0% for the pentahemihydrate. Alternatively, the water content may be determined by the Karl Fischer method.

pH of a 10% aqueous solution : 7.0-8.5.

Oxalate : Add 5 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of sodium citrate. No turbidity should be produced after one hour.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 5 mg/kg.

Heavy metals : Not more than 20 mg/kg.

Assay

Titrimetric method. Heat until carbonized 1.500 g of sodium citrate, previously dried at 180° to constant weight; cool, and boil the residue with 50 ml of 0.5 N sulfuric acid. Filter, wash the filter with water, and titrate the excess of acid in the filtrate and washings with 0.5 N sodium hydroxide, using methyl orange TS. Each ml of 0.5 N sulfuric acid is equivalent to 0.04301 g of $C_6H_5O_7Na_3$.

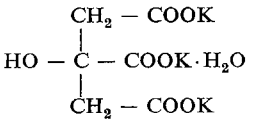
Alternative method. Weigh 0.3500 g of sodium citrate, previously dried at 180° to constant weight, and transfer to a 250-ml beaker. Add 100 ml of glacial acetic acid, and stir the solution (e.g., with a magnetic stirrer) until the sample is dissolved. Titrate the solution with 0.1 N perchloric acid in glacial acetic acid, adding the titrant in 0.2-ml increments as the end-point is approached, and determine the end-point by the potentiometric method as described in the assay for monosodium monophosphate (page 17). Each ml of 0.1 N perchloric acid used is equivalent to 0.008602 g of $C_6H_5O_7Na_3$.

POTASSIUM CITRATE *

Chemical names Tripotassium citrate ; tripotassium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid ; tripotassium salt of β-hydroxytricarballylic acid.

Empirical formula $C_6H_5O_7K_3 \cdot H_2O$

Structural formula



Molecular weight 324.42

Definition Potassium citrate, after drying at 180° for 4 hours, contains not less than 99% of $C_6H_5O_7K_3$ and conforms to the following specifications.

Description Potassium citrate occurs as transparent crystals, or as a white, granular powder. It is odourless, has a cooling, saline taste, and is deliquescent.

Uses Sequestrant, stabilizer, and buffer in certain milk and meat products.

Identification Tests

A. *Solubility*

Water : 1 g is soluble in 1 ml.
Ethanol : insoluble.

B. To 5 ml of a 10% solution of potassium citrate add 1 ml of calcium chloride TS and 3 drops of bromothymol blue TS, and slightly acidify

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* For biological data and toxicological evaluation see p. 59.

with dilute hydrochloric acid TS. Add sodium hydroxide TS until the colour changes to a clear blue, then boil the solution for 3 minutes, agitating it gently during the heating period : a white, crystalline precipitate appears which is insoluble in sodium hydroxide TS, but is soluble in acetic acid TS.

C. To 10 ml of a 10% solution of potassium citrate add 1 ml of mercuric sulfate TS. Heat the mixture to boiling, and add a few drops of potassium permanganate TS : a white precipitate of the acetone dicarboxylic acid salt of mercury is formed.

D. When hydrochloric acid is present, a solution of potassium citrate gives with platinum chloride TS a yellow, crystalline precipitate which on ignition leaves a residue of potassium chloride and platinum.

Purity Tests

Loss on drying : When dried for 4 hours at 180°, not more than 6.0%.

pH of a 10% aqueous solution : 7-8.

Oxalate : Add 5 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of potassium citrate. No turbidity is produced within one hour.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 5 mg/kg.

Heavy metals : Not more than 20 mg/kg.

Assay

Titrimetric method. Heat until carbonized 1.500 g of potassium citrate previously dried at 180° to constant weight ; cool, and boil the residue with 50 ml of 0.5 N sulfuric acid. Filter, wash the filter with water and titrate the excess of acid in the filtrate and washings with 0.5 N sodium hydroxide, using methyl orange TS as indicator. Each ml of 0.5 N sulfuric acid is equivalent to 0.05107 g of $C_6H_5O_7K_3$.

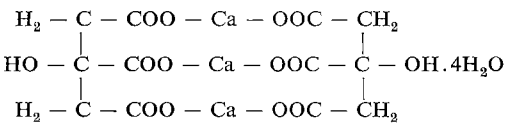
Alternative method. Weigh 0.4500 g of potassium citrate previously dried at 180° to constant weight, and transfer to a 250-ml beaker. Add 100 ml of glacial acetic acid, and stir the solution (e.g., with a magnetic stirrer) until the sample is dissolved. Titrate the solution with 0.1 N perchloric acid in glacial acetic acid, adding the titrant in 0.2-ml increments as the end-point is approached, and determine the end-point by the potentiometric method as described in the assay for monosodium monophosphate (page 17). Each ml of 0.1 N perchloric acid is equivalent to 0.01021 g of $C_6H_5O_7K_3$.

CALCIUM CITRATE

Chemical names Tricalcium citrate; tricalcium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid, tricalcium salt of β-hydroxytricarballylic acid.

Chemical formula $C_{12}H_{10}O_{14}Ca_3 \cdot 4H_2O$

Structural formula



Molecular weight 570.51

Definition Calcium citrate, after drying at 180° to constant weight, contains not less than 97.5% $C_{12}H_{10}O_{14}Ca_3$ and conforms to the following specifications.

Description Calcium citrate occurs as an odourless, fine, white powder.

Uses Sequestrant, stabilizer and buffer in meat and cheese products.

Identification Tests

A. *Solubility*

Water: 1 g is soluble in 1050 ml.
Ethanol: insoluble.

B. Dissolve 1.0 g of calcium citrate in 20 ml of water and 5 ml of dilute nitric acid TS. Dilute 5 ml of this solution to 10 ml. Add 1 ml of mercuric sulfate TS, heat to boiling and add 0.1 N potassium permanganate. A white precipitate is formed.

C. Ignite 0.5 g of sample completely at as low a temperature as possible, cool, and dissolve the residue in 10 ml of water and 1 ml of glacial acetic acid. Filter and add 10 ml of ammonium oxalate TS. A voluminous white precipitate appears which is soluble in hydrochloric acid.

Purity Tests

Loss on drying: Not more than 13.0% after drying to constant weight at 180°.

Carbonate: Dissolve 1 g of calcium citrate in 10 ml of dilute hydrochloric acid TS. Only a few isolated bubbles escape.

Oxalate : Dissolve 1 g of calcium citrate in 5 ml of dilute hydrochloric acid TS, filter if necessary, add 2 g of sodium acetate, and water to 10 ml. No turbidity is produced within one hour.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 5 mg/kg.

Heavy metals : Not more than 20 mg/kg.

Assay

Weigh 0.4000 g of calcium citrate, previously dried to constant weight at 180°, and transfer to a 250-ml beaker. Dissolve the sample in 100 ml of water containing 2 ml of dilute (10%) hydrochloric acid. Add 15 ml of sodium hydroxide TS, 50 ml of water, 40 mg of murexide indicator preparation,[a] and 3 ml of naphthol green TS ; titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 0.008307 g of $Ca_3(C_6H_5O_7)_2$.

Biological Data

Citric acid and citrates occur in many foods and are normal metabolites in the body.[1]

Potassium and sodium citrate in doses of up to 4 g have been extensively used in medical practice for many years without giving rise to ill-effects. The calcium salt probably behaves similarly in the body. As food additives, therefore, all the citrates can be considered together.

There is no reason to believe that the use of these citrates as food additives constitutes a significant toxicological hazard to man.

Evaluation

Estimate of acceptable daily intakes for man

Not limited.[b]

Reference

1. *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**

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[a] An alternative indicator is hydroxynaphthol blue, of which 0.25 g is used. In this case the naphthol green TS is omitted.

[b] In the sixth report of the Joint FAO/WHO Expert Committee on Food Additives,[1] limits were given for citric acid intake. These limits may be too strict and will need reconsideration by a future Committee.

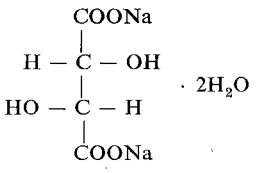
SODIUM TARTRATE *

Synonym Sodium *dextro*-tartrate.

Chemical names Disodium tartrate; sodium *d*-tartrate.

Empirical formula $C_4H_4O_6Na_2 \cdot 2H_2O$

Structural formula



Molecular weight 230.08

Definition Sodium tartrate is the disodium salt of L-(+)-tartaric acid. After drying at 150° for 3 hours, it contains not less than 99% of $C_4H_4O_6Na_2$ and conforms to the following specifications.

Description Sodium tartrate occurs as transparent, colourless and odourless crystals.

Uses Sequestrant and stabilizer in meat products and sausage casings.

Identification Tests

A. *Solubility*

Water: 1 g is soluble in 3 ml.
Ethanol: insoluble.

B. On ignition, sodium tartrate emits the odour of burning sugar and leaves a residue which is alkaline to litmus and effervesces with acids.

C. Heat a few milligrams of sodium tartrate on a steam bath with 2 ml of sulfuric acid containing 0.5% pyrogallol. An intense violet colour is produced.

D. A solution of sodium tartrate acidified with dilute acetic acid TS, filtered if necessary, and treated with uranyl zinc acetate TS, yields a yellow crystalline precipitate within a few minutes.

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* For biological data and toxicological evaluation see pp. 63-64.

Purity Tests

Loss on drying : Not more than 17% and not less than 14% after drying at 150° for 3 hours.

pH of a 10% solution : 7.0-7.5.

Oxalate : Add 5 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of sodium tartrate. No turbidity is produced within one hour.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 5 mg/kg.

Heavy metals : Not more than 20 mg/kg.

Assay

Sodium analysis on ash. Weigh 1.500 g of sodium tartrate, previously dried at 150° for 3 hours, into a tared porcelain crucible, ignite gently at first, until the salt is thoroughly carbonized, protecting the carbonized salt from contact with the flame at all times. Cool the crucible, place in a glass beaker and break up the carbonized mass with a glass rod. Without removing the glass rod or the crucible, add 50 ml of water, 50 ml of 0.5 N sulfuric acid, cover the beaker, and boil the solution for 30 minutes. Filter, and wash with hot water until the last washing is neutral to litmus. Cool the combined filtrate and washings, add methyl orange TS, and titrate the excess acid with 0.5 N sodium hydroxide. Each ml of 0.5 N sulfuric acid is equivalent to 0.04851 g of $C_4H_4O_6Na_2$.

Potentiometric titration : Weigh 0.4500 g of sodium tartrate, previously dried at 150° for 3 hours, and transfer to a 250-ml beaker. Add 100 ml of glacial acetic acid, and stir the solution (e.g., with a magnetic stirrer) until the sample is dissolved. Titrate the solution with 0.1 N perchloric acid in glacial acetic acid, adding the titrant in 0.2-ml increments as the end-point is neared, and determine the end-point by the potentiometric method as described in the assay for monosodium monophosphate (page 17). Each ml of 0.1 N perchloric acid is equivalent to 0.009703 g of $C_4H_4O_6Na_2$.

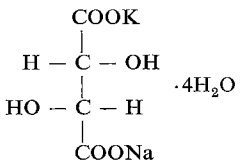
POTASSIUM SODIUM TARTRATE

Synonyms Rochelle salt; Seignette salt; potassium sodium *dextro*-tartrate.

Chemical names Potassium sodium tartrate; potassium sodium *d*-tartrate.

Empirical formula $C_4H_4O_6KNa \cdot 4H_2O$

Structural formula



Molecular weight 282.23

Definition Potassium sodium tartrate is the salt of L-(+)-tartaric acid. After drying at 150° for 3 hours, it contains not less than 99% of $C_4H_4O_6KNa$ and conforms to the following specifications.

Description Potassium sodium tartrate occurs as colourless crystals, or as a white, crystalline powder, having a cooling saline taste.

Uses Sequestrant, stabilizer in cheese products, minced meat, and sausage casings.

Identification Tests

A. *Solubility*

Water : 1 g is soluble in 1 ml.
Ethanol : insoluble.

B. On ignition, potassium sodium tartrate emits the odour of burning sugar and leaves a residue which is alkaline to litmus and effervesces with acids.

C. Heat a few mg of potassium sodium tartrate on a steam bath with 2 ml of sulfuric acid containing 0.5% pyrogallol. An intense violet colour is produced.

D. A solution of potassium sodium tartrate, acidified with dilute acetic acid TS, filtered if necessary, and treated with uranyl zinc acetate TS, yields a yellow crystalline precipitate within a few minutes.

E. A solution of potassium sodium tartrate acidified with dilute hydrochloric acid TS gives with platinic chloride TS a yellow crystalline precipitate which on ignition leaves a residue of potassium chloride and platinum.

Purity Tests

Loss on drying : Not more than 26.0% and not less than 21.0% after drying at 150° for 3 hours.

pH of a 10% solution : 6.5-7.5.

Oxalate : Add 3 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of potassium sodium tartrate. No turbidity is produced within one hour.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 5 mg/kg.

Heavy metals : Not more than 20 mg/kg.

Assay

Potassium and sodium analysis on ash. Weigh 1.500 g of potassium sodium tartrate, previously dried at 150° for 3 hours, into a tared porcelain crucible and ignite. Heat gently at first, until the salt is thoroughly carbonized, protecting the carbonized salt from contact with the flame at all times. The final temperature must not be above that of a dull read heat. Cool the crucible, place in a glass beaker, and break up the carbonized mass with a glass rod. Without removing the glass rod or the crucible, add 50 ml of water, 50 ml of 0.5 N sulfuric acid, cover the beaker, and boil the solution for 30 minutes. Filter, and wash with hot water until the last washing is neutral to litmus. Cool the combined filtrate and washings, add methyl orange TS, and titrate the excess acid with 0.5 N sodium hydroxide. Each ml of 0.5 N sulfuric acid is equivalent to 0.05254 g of $C_4H_4O_6KNa$.

Biological Data

Sodium tartrate in daily doses of up to 10 or even 20 g has been used in medical practice as a laxative. It has been tested for this action in a clinical study involving the application of daily doses of 10 g of sodium tartrate to 26 patients for an average of 11.8 doses, giving laxative responses in 66% of the subjects. The only side-effects noticed were nausea or vomiting (1.6%) and abdominal cramps (2.1%).

Renal damage has been observed only after the intravenous administration of tartaric acid in doses of 0.2-0.3 g in rabbits and rats.[1, 2]

Comment on experimental studies reported

In the sixth report of the Joint FAO/WHO Expert Committee on Food Additives,[3] the acceptable daily intake for tartaric acid was calculated from the dietary level of 1.2% found to produce no ill-effects in rats over a 2-year feeding study ; a safety factor of 200 was applied to arrive at the unconditional acceptance zone. Diets containing more than 1.2% of tartaric acid were not tested, so that there is no evidence that the feeding of higher levels would be harmful. The acceptance zone for tartaric acid

given in the sixth report may therefore have been unduly restrictive and this question should be re-examined by a future Committee.

Moreover, the acceptance zone for the salts may not necessarily be similar to that for the acid.

Evaluation

Estimate of acceptable daily intakes for man

	mg/kg body weight [a]
Unconditional acceptance	0-6
Conditional acceptance	6-20

[a] Calculated as tartaric acid.

References

1. Gold, H. & Zahm, W. (1943) *J. Amer. pharm. Ass., sci. Ed.*, **32**, 173
2. Bodansky, O., Gold, H. & Zahm, W. (1942) *J. Amer. pharm. Ass., sci. Ed.*, **31**, 1
3. *FAO Nutrition Meetings Report Series*, 1962, No. 31; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**

AGAR

Synonyms Agar-agar; gelose; Japan agar; Bengal, Ceylon, Chinese or Japanese isinglass; Layor Carang.

Chemical description Agar is a polygalactoside, about 90% of the galactose-molecules being of the D-form and 10% of the L-form. On about every tenth D-galactopyranose unit one of the CH_2OH- or CHOH-groups is esterified with one of the two hydrogen atoms of sulfuric acid; the other hydrogen atom of the sulfuric acid molecule is replaced by calcium, magnesium, potassium, or sodium.

Definition Agar is the dried hydrophilic, colloidal substance extracted from certain marine algae of the class Rhodophyceae. It conforms to the following specifications.

Description Agar is odourless or has a slight characteristic odour.

Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips, or in cut, flaked or granulated forms. It may be light yellowish

orange, yellowish grey to pale yellow, or colourless. It is tough when damp, brittle when dry.

Powdered agar is white to yellowish white or pale yellow.

Microscopic appearance : Place a few fragments or some powder on a slide and add some drops of water : the agar appears granular and somewhat filamentous ; a few fragments of the spicules of sponges and a few frustules of diatoms may be present. In chloral hydrate TS the fragments are more transparent than in water, more or less granular, striated, angular and occasionally contain frustules of diatoms.

Uses Thickening agent and stabilizer in whipped cream, ice-cream, jams, marmalades, and confectionery.

Identification Tests

A. Agar is slowly soluble in cold water and soluble in boiling water.

B. Prepare a 1.0% solution in boiling water in a flask ; place the flask in water at 30° for 15 minutes : a firm, resistant gel is formed. Place the flask in water at 70° for 1 hour ; the gel is not molten.

C. A warm (40°) 0.5% solution of agar gives a precipitate with half its volume of warm (40°) 40% ammonium sulfate solution. This test distinguishes agar from alginate, ghatti gum, gum arabic (acacia), karaya gum, pectin and tragacanth.

D. A warm 0.5% solution of agar gives a precipitate with one-fifth its volume of basic lead acetate. This test distinguishes agar from methyl cellulose.

Purity Tests

Loss on drying : Cut unground agar into pieces from 2 to 5 mm square before drying. Dry at 105° until the difference between two weighings is less than 1 mg (about 5 hours). The loss on drying is not more than 22%.

Total ash : Not more than 6.5% on a dry-weight basis.

Acid-insoluble ash : Not more than 0.5% on a dry-weight basis.

Foreign insoluble matter : Boil 5 g of agar with 500 ml of water and 12 ml of sulfuric acid under a reflux condenser for 2 hours. Allow to cool and filter through a tared, fine, sintered glass crucible ; wash flask and filter

with 50 ml of water, dry at 105° and weigh. The insoluble matter weighs not more than 0.05 g (1%).

Starch and dextrines : Add 2 drops of iodine TS to the 0.5% warm aqueous solution (40°). Where the drops fall a red-violet colour appears. After mixing, the solution is golden brown and not blue or reddish.

Gelatin and other proteins : A warm (40°) 0.5% solution gives no turbidity with 1 volume of warm (40°) picric acid TS within 10 minutes.

Water absorption : Place 5 g of agar in a 100-ml graduated cylinder, fill to the mark with water, mix, and allow to stand at 25° for 24 hours. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into a second 100-ml graduated cylinder : not more than 75 ml of water is obtained.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

Threshold gel concentrations. Prepare serial dilutions of known solids content (0.15%, 0.20%, 0.25%, etc.) and place in tubes 150 mm long by 16 mm internal diameter, stoppered at both ends. Cool for 1 hour at 20°-25° and then hold for 1 hour at 2°-4° and for 1 hour at 20°-25°. Allow cylinders of gel to slide from the tubes to a level surface. The lowest concentration of gel that resists gravity without rupture for 5-30 seconds is the threshold concentration of the sample. The threshold concentration is not higher than 0.25%.

Biological Data

Acute toxicity

LD_{50} values have not been found in the literature.

Subcutaneous implantation or injection into rats produced a foreign-body giant-cell reaction.[1, 2]

Short-term studies

Rat

Four groups of 6 rats received diets containing 5%, 10%, 20% and 30% of agar respectively for 10 weeks. The group receiving the 10% diet gained weight about 20% faster than the controls ; the other groups gained weight at the same rate as the controls. The rats fed diets containing 20% and 30% of agar required significantly more feed and water per gram of weight gain than the control group.[3]

Six weanling male rats fed a diet containing 25% of agar for 4 weeks showed growth retardation during the third week. The dry weight of the cleaned stomach and caecum was found moderately increased. Colon and rectum were more than twice as heavy as in the controls.[4]

Man

For the last 50 years agar has been used as a mild laxative for human subjects in daily doses of 4-15 g.[5]

Comment on experimental studies reported

Although fewer formal toxicological studies have been carried out than would normally be required for a food additive, it is considered that the extensive consumption of seaweeds by man, their wide use in animal diets, and the clinical experience with agar as a laxative compensate for the incompleteness of the animal data.

The effect on weight gain in rats was probably due to the lack of utilization of the agar and/or its laxative effects.[3] It is therefore considered that the 10% level did not cause any toxic effects.

Evaluation

Level causing no significant toxicological effect in the rat

10% (= 100 000 p.p.m.) in the diet, equivalent to 5000 mg/kg body weight per day.

Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance	0-50

References

1. Zbinden, G. & Studer, A. (1957) *Schweiz. Z. allg. Path.*, **20**, 469
2. Tedeschi, G. G. & Mangiantini, M. T. (1956) *Arch. Sci. biol.*, **40**, 504
3. Nilson, H. W. & Schaller, J. W. (1941) *Food Res.*, **6**, 461
4. Fischer, J. E. (1957) *Amer. J. Physiol.*, **188**, 550
5. Sollmann, T. (1957) *A Manual of Pharmacology*, Philadelphia & London, Saunders, p. 207

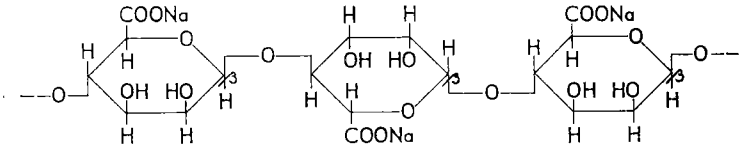
SODIUM ALGINATE *

Synonym Alginate.

Chemical names Sodium salt of alginic acid; sodium polymannuronate.

Molecular formula $(C_6H_7O_6Na)_n$

Structural formula



Molecular weight *Structural unit :* 198.11.
Macromolecule : 32 000-250 000.

Definition Sodium alginate is a hydrophilic, colloid substance. It contains not less than 98% of $(C_6H_7O_6Na)_n$ on a dry-weight basis and conforms to the following specifications.

Description Sodium alginate occurs in filamentous, grainy, granular, and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

Uses Thickening agent and stabilizer in ice-cream, cheese products, canned fruits and sausage casings.

Identification Tests

A. *Solubility*

Water : slowly soluble forming a viscous solution.
Ethanol : insoluble.
Ether : insoluble.
Chloroform : insoluble.

B. To a 0.5% aqueous solution of sodium alginate add one-fifth of its volume of a 2.5% aqueous solution of calcium chloride. A voluminous gelatinous precipitate is formed. This test distinguishes sodium alginate from gum arabic, carboxymethylcellulose, carboxymethyl starch, gelatine, ghatti gum, Irish moss, karaya gum, locust bean gum, methylcellulose, pectin and tragacanth.

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* For biological data and toxicological evaluation see pp. 80-81.

C. To a 0.5% aqueous solution of sodium alginate add one-half its volume of saturated ammonium sulfate solution. No precipitate is formed. This test distinguishes sodium alginate from agar, carboxymethylcellulose, de-esterified pectin, gelatine, Irish moss, locust bean gum, methylcellulose and starch.

D. Clarify a 0.5% aqueous solution of sodium alginate with kieselguhr and determine the rotation in a 2-dm tube. The specific rotation is not less than —0.8° at 20°.

E. Moisten 1-5 mg of sodium alginate with water and add 1 ml of acid ferric sulfate TS. Within five minutes a cherry-red colour develops, finally becoming deep purple.

F. Dissolve the sulfated ash of sodium alginate in dilute acetic acid TS and filter. Add to the filtrate uranyl zinc acetate TS. A yellow, crystalline precipitate is formed within a few minutes.

Purity Tests

Colour, odour, pH : A 1% aqueous solution of sodium alginate is almost limpid and colourless or has at most a very slight brownish-yellow colour. At 50° the solution is odourless or possesses only a very faint odour. The pH is between 6.0 and 8.0.

Loss on drying : Dry 1.000 g of sodium alginate at 105° until the difference between two successive weighings is less than 1 mg (about 8 hours). The loss on drying is not more than 20.0%.

Sulfated ash : 30.0%-35.9% calculated on a dry-weight basis.

Water insolubles : Dissolve 1 g of sodium alginate in 100 ml of water, centrifuge and decant; wash the residue five times with water by mixing, centrifuging and decanting. Transfer the residue by means of water to a tared fine glass filter, dry for 1 hour at 105°, cool and weigh. The increase of weight is not more than 10 mg (1.0% calculated on a dry-weight basis).

Phosphate : To a 0.5% aqueous solution of sodium alginate add one-fifth of its volume of 4 N nitric acid and 1 volume of ammonium molybdate TS and warm. No yellow precipitate is formed.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 15 mg/kg.

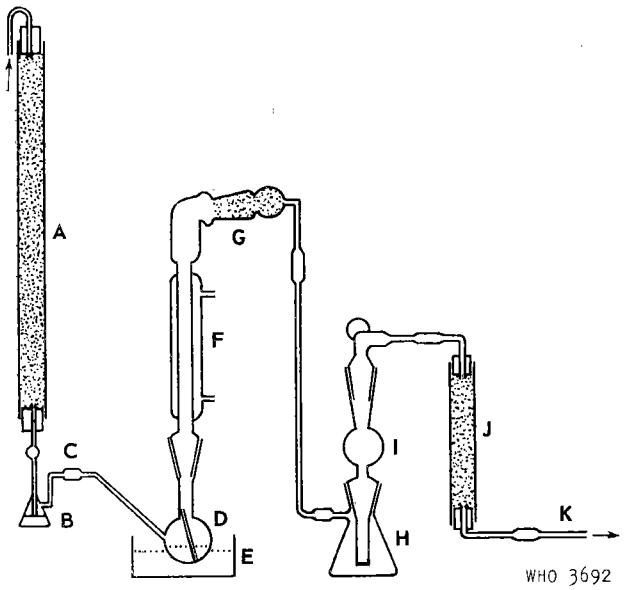
Heavy metals : Not more than 40 mg/kg.

Assay

Decarboxylation method. This depends on the determination of the carbon dioxide evolved when sodium alginate is heated with dilute hydrochloric acid. The apparatus used is shown in Fig. 1.

Carbon-dioxide-free air is produced by passing air through the soda lime column A. The air then passes through a mercury valve B and a connecting tube C into the reaction flask D. The latter is a 100-ml round-bottomed, long-necked boiling flask with a standard ground-glass joint. It is immersed in an oil bath E which is maintained at 145° by a thermo-regulator and immersion heater. The air and evolved gases pass upwards through the condenser F and the trap G, which contains 25 g of 20-mesh granulated zinc or tin. Finally, the carbon dioxide is absorbed in the absorption flask H and the absorption tower I. A medium-porosity Pyrex glass disc is sealed to the lower end of the absorption tower, which is placed 1-2 mm above the bottom of the flask. The absorption tower is protected by the soda lime tube J, which is connected via the capillary tube regulator K to a water aspirator capable of drawing carbon-dioxide-free air through the apparatus at the rate of 6 litres per hour.

FIG. 1. APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE EVOLVED FROM ALGINIC ACID BY HEATING WITH HYDROCHLORIC ACID



A. Soda lime column
B. Mercury valve
C. Connecting tube
D. Reaction flask
E. Oil bath
F. Condenser
G. Trap containing granulated zinc or tin
H. Absorption flask
I. Absorption tower
J. Soda lime tube
K. Capillary tube regulator

Reproduced from *Industrial and Engineering Chemistry, Analytical Edition*, 1946, Vol. 18, page 290, by permission of the American Chemical Society.

Procedure. Weigh 0.2500 g of sodium alginate and transfer it to the reaction flask D. Add 30 ml of 19% hydrochloric acid and an ebullition tube. Connect the reaction flask to the condenser F after lubricating the connexions with syrupy phosphoric acid. Attach the soda lime tube A and trap B after lubricating the connexions with stopcock grease. Place the oil bath close to the reaction flask so as to warm it to 45°-50° and draw carbon-dioxide-free air through the apparatus at the rate of 6 litres per hour for 10 minutes. Stop the current of air, disconnect the top of the absorption tower I, rapidly pipette in 25.0 ml of 0.25 N sodium hydroxide, add 5 drops of 1-butanol, and replace the top of the tower. Raise the oil bath until the oil level is several millimetres above the level of the liquid in the flask. Draw carbon-dioxide-free air through the apparatus at the rate of 2 litres per hour for 2 hours. Stop the water aspirator, lower the oil bath and disconnect the reaction flask D. Force the sodium hydroxide solution in the absorption tower back into the flask H by means of gentle pressure from an aspirator bottle. Rinse down the absorption tower I with approximately four 8-ml portions of distilled water, forcing each washing through the sintered glass disc into the absorption flask H. Avoid as far as possible the entrance of any CO_2-laden air. Disconnect the absorption flask H and add to the contents 10.0 ml of a 10% aqueous solution of $BaCl_2 \cdot H_2O$. Stopper the flask H and shake gently for 1 minute. Add 2 drops of phenolphthalein TS and titrate at a moderate rate with 0.1 N hydrochloric acid, shaking all the time, until a colourless end-point is reached. Determine in equivalents the gross quantity of alkali consumed. Perform a blank run, which should consume approximately 14×10^{-5} equivalent of alkali. Subtract this amount from the gross quantity consumed in order to obtain the net equivalents of alkali consumed. Divide this figure by 2 to give the net gram moles of carbon dioxide evolved.

Calculation :

$$\% \ CO_2 = \frac{(\text{Net gram moles of } CO_2 \text{ evolved}) \times 44}{\text{weight of sample}} \times 100$$

$$\% \text{ sodium alginate} = \frac{\% \ CO_2 \times 1.125}{23.8} \times 100$$

Gravimetric method. Dissolve 0.500 g of sodium alginate in 100 ml of water and filter if necessary. Add 15 ml of 4 N hydrochloric acid and 100 ml of 90% v/v ethanol. Allow this mixture to stand for 2 hours, decant the supernatant liquid as far as possible, and centrifuge. Decant the liquid and replace it by 90% v/v ethanol. Mix well, centrifuge and decant again. This washing is repeated until the hydrochloric acid is removed. Then transfer the precipitate by means of 90% v/v ethanol to a fine glass filter, wash with dry acetone, place the filter in a vacuum desiccator and dry to constant weight at 100°.

Calculation :

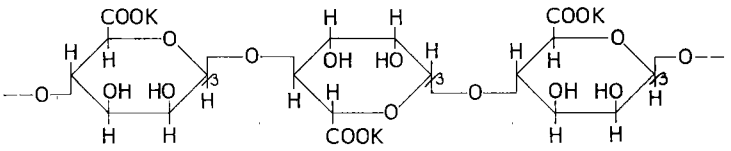
$$\% \text{ sodium alginate} = \frac{200 \times 1.125 \times \text{weight of precipitate}}{\% \text{ dry substance in the sample}} \times 100$$

POTASSIUM ALGINATE *

Chemical name Potassium salt of alginic acid ; potassium polymannuronate

Molecular formula $(C_6H_7O_6K)_n$

Structural formula



Molecular weight *Structural unit :* 214.22
Macromolecule : 32 000-250 000

Definition Potassium alginate is a hydrophilic, colloid substance. It contains not less than 98% of $(C_6H_7O_6K)_n$ on a dry-weight basis and conforms to the following specifications.

Description Potassium alginate occurs in filamentous, grainy, granular, and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

Uses Thickening agent and stabilizer in ice-cream, cheese products, canned fruits and sausage casings.

Identification Tests

A. *Solubility*

Water : slowly soluble forming a viscous solution.
Ethanol : insoluble.
Ether : insoluble.
Chloroform : insoluble.

B. To a 0.5% aqueous solution of potassium alginate add one-fifth of its volume of a 2.5% aqueous solution of calcium chloride. A voluminous gelatinous precipitate is formed. This test distinguishes potassium

* For biological data and toxicological evaluation see pp. 80-81.

alginate from gum arabic, carboxymethylcellulose, carboxymethyl starch, gelatine, ghatti gum, Irish moss, karaya gum, locust bean gum, methylcellulose, pectin and tragacanth.

C. To a 0.5% aqueous solution of potassium alginate add one-half its volume of saturated ammonium sulfate solution. No precipitate is formed. This test distinguishes potassium alginate from agar, carboxymethylcellulose, de-esterified pectin, gelatine, Irish moss, locust bean gum, methylcellulose and starch.

D. Clarify a 0.5% aqueous solution of potassium alginate with kieselguhr and determine the rotation in a 2-dm tube. The specific rotation is not less than —0.8° at 20°.

E. Moisten 1-5 mg of potassium alginate with water and add 1 ml of acid ferric sulfate TS. Within five minutes a cherry-red colour develops, finally becoming deep purple.

F. Dissolve the sulfated ash of potassium alginate in dilute acetic acid TS and filter. Add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

Purity Tests

Colour, odour, pH : A 1% aqueous solution of potassium alginate is almost limpid and colourless or has at most a very slight brownish-yellow colour. At 50° the solution is odourless or possesses only a very faint odour. The pH is between 6.0 and 8.0.

Loss on drying : Dry 1.000 g of potassium alginate at 105° until the difference between two successive weighings is less than 1 mg (about 8 hours). The loss on drying is not more than 20.0%.

Water insolubles : Dissolve 1 g of potassium alginate in 100 ml of water, centrifuge and decant; wash the residue five times with water by mixing, centrifuging, and decanting. Transfer the residue by means of water to a tared fine glass filter, dry for 1 hour at 105°, cool, and weigh. The increase of weight is not more than 10 mg (1.0% calculated on a dry-weight basis).

Sodium : Dissolve the ash of potassium alginate in dilute acetic acid TS and filter. Add to the filtrate uranyl zinc acetate TS. No precipitate is formed.

Phosphate : To a 0.5% aqueous solution of potassium alginate add one-fifth of its volume of 4 N nitric acid and 1 volume of ammonium molybdate TS and warm. No yellow precipitate is formed.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 15 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

Decarboxylation method. As for sodium alginate, but using a conversion factor of 1.215, instead of 1.125, in the calculation, thus :

$$\% \ CO_2 = \frac{(\text{Net g moles } CO_2 \text{ evolved}) \times 44}{\text{weight of sample}} \times 100$$

$$\% \text{ potassium alginate} = \frac{\% \ CO_2 \times 1.215}{23.8} \times 100$$

Gravimetric method. As for sodium alginate, but using the conversion factor 1.215 in the calculation, thus :

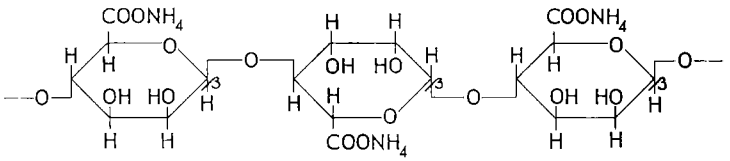
$$\% \text{ potassium alginate} = \frac{200 \times 1.215 \times \text{weight of precipitate}}{\% \text{ dry substance in the sample}} \times 100$$

AMMONIUM ALGINATE *

Chemical name Ammonium salt of alginic acid; ammonium polymannuronate.

Molecular formula $(C_6H_{11}O_6N)_n$

Structural formula



Molecular weight *Structural unit :* 193.16
Macromolecule : 32 000-250 000

Definition Ammonium alginate is a hydrophilic, colloid substance. It contains not less than 98% of $(C_6H_{11}O_6N)_n$ on a dry-weight basis and conforms to the following specifications.

Description Ammonium alginate occurs in filamentous, grainy, granular, and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

Uses Thickening agent and stabilizer in ice-cream, cheese products, canned fruits and sausage casings.

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* For biological data and toxicological evaluation see pp. 80-81.

Identification Tests

A. *Solubility*

Water : slowly soluble forming a viscous solution.
Ethanol : insoluble.
Ether : insoluble.
Chloroform : insoluble.

B. To a 0.5% aqueous solution of ammonium alginate add one-fifth of its volume of a 2.5% aqueous solution of calcium chloride. A voluminous gelatinous precipitate is formed. This test distinguishes ammonium alginate from gum arabic, carboxymethylcellulose, carboxymethyl starch, gelatine, ghatti gum, Irish moss, karaya gum, locust bean gum, methylcellulose, pectin and tragacanth.

C. To a 0.5% aqueous solution of ammonium alginate add one-half its volume of saturated ammonium sulfate solution. No precipitate is formed. This test distinguishes ammonium alginate from agar, carboxymethylcellulose, de-esterified pectin, gelatine, Irish moss, locust bean gum, methylcellulose and starch.

D. Clarify a 0.5% aqueous solution of ammonium alginate with kieselguhr and determine the rotation in a 2-dm tube. The specific rotation is not less than —0.8° at 20°.

E. Moisten 1-5 mg of ammonium alginate with water and add 1 ml of acid ferric sulfate TS. Within five minutes a cherry-red colour develops, finally becoming deep purple.

F. Heat a solution of ammonium alginate in sodium hydroxide TS. Ammonia is evolved, recognizable by its odour, and by its reaction on moist, red litmus paper.

Purity Tests

Loss on drying : Dry 1.000 g of ammonium alginate at 105° until the difference between two successive weighings is less than 1 mg (about 8 hours). The loss on drying is not more than 20.0%.

Sulfated ash : Not more than 5% calculated on a dry-weight basis.

Water insolubles : Dissolve 1 g of ammonium alginate in 100 ml of water, centrifuge and decant ; wash the residue five times with water by mixing, centrifuging, and decanting. Transfer the residue by means of water to a tared fine glass filter, dry for 1 hour at 105°, cool and weigh. The increase of weight is not more than 10 mg (1.0% calculated on a dry-weight basis).

Phosphate : To a 0.5% aqueous solution of ammonium alginate add one-fifth of its volume of 4 N nitric acid and 1 volume of ammonium molybdate TS and warm. No yellow precipitate is formed.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 15 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

Decarboxylation method. As for sodium alginate, but using a conversion factor of 1.096, instead of 1.125, in the calculation, thus :

$$\% \ CO_2 = \frac{(\text{Net g moles } CO_2 \text{ evolved}) \times 44}{\text{weight of sample}} \times 100$$

$$\% \text{ ammonium alginate} = \frac{\% \ CO_2 \times 1.096}{23.8} \times 100$$

Gravimetric method. As for sodium alginate, but using the conversion factor 1.096 in the calculation, thus :

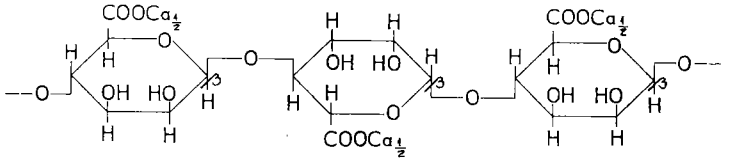
$$\% \text{ ammonium alginate} = \frac{200 \times 1.096 \times \text{weight of precipitate}}{\% \text{ dry substance in the sample}} \times 100$$

CALCIUM ALGINATE *

Chemical name Calcium salt of alginic acid

Molecular formula $(C_6H_7O_6Ca_{\frac{1}{2}})_n$

Structural formula



Molecular weight *Structural unit :* 195.16

Macromolecule : 32 000-250 000

Definition Calcium alginate is a colloid substance. It contains not less than 98% of $(C_6H_7O_6Ca_{\frac{1}{2}})_n$ on a dry-weight basis and conforms to the following specifications.

Description Calcium alginate occurs in filamentous, grainy, granular, and powdered forms. It is colourless or

* For biological data and toxicological evaluation see pp. 80-81.

slightly yellow and may have a slight characteristic smell and taste.

Uses Thickening agent and stabilizer in ice-cream, cheese products, canned fruits and sausage casings.

Identification Tests

A. *Solubility*

Insoluble in water. Dissolves slowly in solutions of sodium polyphosphate, sodium carbonate, and substances that combine with the calcium.

B. Moisten 1-5 mg of calcium alginate with water and add 1 ml of acid ferric sulfate TS. Within five minutes a cherry-red colour develops, finally becoming deep purple.

C. Dissolve the sulfated ash of calcium alginate in dilute acetic acid TS and filter. Treat the filtrate with ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid.

Purity Tests

Loss on drying : Dry 1.000 g of calcium alginate at 105° until the difference between two successive weighings is less than 1 mg (about 8 hours). The loss on drying is not more than 20.0%.

Phosphate : To a 0.5% aqueous solution of calcium alginate add one-fifth of its volume of 4 N nitric acid and 1 volume of ammonium molybdate TS and warm. No yellow precipitate is formed.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 15 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

Decarboxylation method. As for sodium alginate, but using a conversion factor of 1.108, instead of 1.125, in the calculation, thus :

$$\% \, CO_2 = \frac{(\text{Net g moles } CO_2 \text{ evolved}) \times 44}{\text{weight of sample}} \times 100$$

$$\% \text{ calcium alginate} = \frac{\% \, CO_2 \times 1.108}{23.8} \times 100$$

Gravimetric method. As for sodium alginate, but using the conversion factor 1.108 in the calculation, thus :

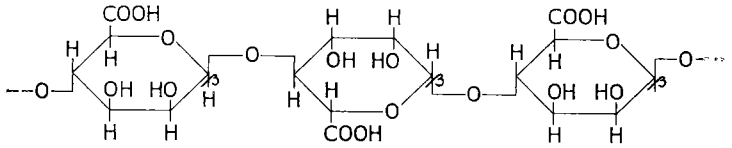
$$\% \text{ calcium alginate} = \frac{200 :: 1.108 \times \text{weight of precipitate}}{\% \text{ dry substance in the sample}} \times 100$$

ALGINIC ACID

Chemical name High-polymer polymannuronic acid. Linear polymer of β-1,4-linked D-mannuronic acid in the pyranose ring form.

Molecular formula $(C_6H_8O_6)_n$

Structural formula



Molecular weight *Structural unit :* 176.13
Macromolecule : 32 000-250 000

Definition Alginic acid is a colloid substance obtained from various species of brown seaweed (Phaeophyceae). It contains not less than 98% of $(C_6H_8O_6)_n$ on a dry-weight basis and conforms to the following specifications.

Description Alginic acid occurs in filamentous, grainy, granular, and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

Uses Thickening agent and stabilizer in ice-cream and sausage casings.

Identification Tests

A. *Solubility*

Insoluble in water. Dissolves slowly in solutions of sodium carbonate, sodium hydroxide, and trisodium monophosphate.

B. To a 0.5% solution of alginic acid in sodium hydroxide TS add one-fifth of its volume of a 2.5% aqueous solution of calcium chloride. A voluminous gelatinous precipitate is formed. This test distinguishes alginic acid from gum arabic, carboxymethylcellulose, carboxymethyl starch, gelatine, ghatti gum, Irish moss, karaya gum, locust bean gum, methylcellulose, pectin and tragacanth.

C. To a 0.5% solution of alginic acid in sodium hydroxide TS add half its volume of saturated ammonium sulfate solution. No precipitate is formed. This test distinguishes alginic acid from agar, carboxymethylcellulose, de-esterified pectin, gelatine, Irish moss, locust bean gum, methylcellulose and starch.

D. Clarify a 0.5% solution of alginic acid in sodium hydroxide TS with kieselguhr and determine the rotation in a 2-dm tube. The specific rotation is not less than –0.8° at 20°.

E. Dissolve, as completely as possible, 0.01 g of alginic acid by shaking with 0.15 ml of 0.1 N sodium hydroxide, and add 1 ml of acid ferric sulfate TS. Within 5 minutes a cherry-red colour develops, finally becoming deep purple.

Purity Tests

Loss on drying : Not more than 15%.

Sulfated ash : Not more than 5% calculated on a dry-weight basis.

Sodium hydroxide insolubles : Dissolve 1 g of alginic acid in 100 ml of sodium hydroxide TS, centrifuge and decant; wash the residue five times with water by mixing, centrifuging and decanting. Transfer the residue by means of water to a tared fine glass filter, dry for 1 hour at 105°, cool and weigh. The increase of weight is not more than 10 mg (1.0% calculated on a dry-weight basis).

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

Decarboxylation method. As for sodium alginate, but using a conversion factor of 1.000 instead of 1.125 in the calculation, thus :

$$\% \, CO_2 = \frac{(\text{Net gram moles } CO_2 \text{ evolved}) \times 44}{\text{weight of sample}} \times 100$$

$$\% \text{ alginic acid} = \frac{\% \, CO_2}{23.8} \times 100$$

Gravimetric method. Dissolve 0.500 g of alginic acid in 10 ml of sodium hydroxide TS. Add 90 ml of water and filter if necessary. Add 15 ml of 4 N hydrochloric acid and 100 ml of 90% v/v ethanol. Allow this mixture to stand for 2 hours, decant the supernatant liquid as far as possible, and centrifuge. Decant the liquid and replace it by 90% v/v ethanol. Mix well,

centrifuge and decant again. This washing is repeated until the hydrochloric acid is removed. Then transfer the precipitate by means of 90% v/v ethanol to a fine glass filter, wash with dry acetone, place the filter in a vacuum desiccator and dry to constant weight at 100°.

Calculation :

$$\% \text{ alginic acid} = \frac{200 \times 1.000 \times \text{weight of precipitate}}{\% \text{ dry substance in the sample}} \times 100$$

Biological Data

Biochemical aspects

Calcium balance experiments on 6 healthy adults taking 8 g of sodium alginate daily for 7 days failed to show any interference with the absorption of calcium from a normal mixed diet.[1]

From the clinical experiments reported,[2, 3] it appears that alginic acid does not bind sodium in man to any great extent.

Acute toxicity

Compound	Animal	Route	LD_{50} (mg/kg body weight)	Reference
Sodium alginate	Mouse	i.v.	Less than 200	4
" "	Rabbit	i.v.	Approx. 100	4
" "	Cat	i.p.	Approx. 250	5
Alginic acid	Rat	i.p.	1 600	6

Subcutaneous and intramuscular injections of 0.1 ml of a 1% dispersion of alginic acid were not followed by any injurious reactions in mice or rats.[5]

Short-term studies

Rat

Groups of 6 rats were fed sodium alginate for 10 weeks at levels of 5%, 10%, 20% and 30% in the diet. The mortality rate was high in the 20% and 30% groups during the first two weeks, apparently due to inanition. The weight gains of the 5% and 10% groups were slightly decreased.[7]

Potassium alginate at a level of 5% in the feed acted as a laxative; calcium alginate 5% was without this effect.[6]

Groups of 5 rats were fed 5%, 10% and 20% of alginic acid in the diet for two months. Rats on the 20% diet showed a decreased rate of weight gain. Those on the lower levels were unaffected.[6]

Man

Six healthy adults were given 8 g of sodium alginate daily for 7 days without untoward effects.[1]

Three patients whose clinical condition warranted sodium restriction were given oral doses of 15 g of alginic acid 3 times daily for 7 days. A slightly increased faecal sodium and potassium excretion was noted, but no changes in plasma electrolyte concentration.[2]

Six patients with essential hypertension were given daily doses of 45 g of alginic acid containing 10% of potassium alginate for 5-9 weeks and 3 patients in an oedematous state were given the same dosage for about a week. It was well tolerated and produced no gastro-intestinal disturbance.[3]

Long-term studies

Rat

Two groups of 10 male albino rats were fed two different commercial preparations of sodium alginate at the 5% level over their life-span (maximum 128 weeks). Data on longevity, maximum weight and food and water consumption indicate no adverse effect. Gross necropsy studies revealed no abnormalities. Histopathological examination was not carried out.[7]

Comment on experimental studies reported

Although fewer experimental data are available than would normally be required, reliance can be placed on practical experience; not only alginic acid and the alginates but also the algae from which these substances are derived have been used in man for many years. It seems reasonable to consider alginic acid and the four alginates together.

Evaluation

Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance	0-50

Further work considered desirable

Observations on the effect of alginic acid and the alginates on the absorption of nutrients from the diet, especially in man.

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METHYLCELLULOSE

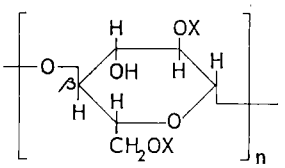
Synonym MC

Chemical names Methyl ether of cellulose; cellulose methyl ether.

Molecular formula $[C_6H_7O_2(OH)_x(OCH_3)_y]_n$

Where $x = 0.80$ to 1.40
$y = 2.20$ to 1.60
$x + y = 3.00$

Structural formula



Where $X = H$ or CH_3

Molecular weight

Unsubstituted structural unit : 162.14
Monosubstituted structural unit : 176.17
Disubstituted structural unit : 190.20
Trisubstituted structural unit : 204.23
Low polymers (n about 200) : about 40 000
High polymers (n about 900) : up to 180 000

Definition Methylcellulose is prepared from wood pulp or cotton by treatment with alkali and methylation of the alkali cellulose with methyl chloride. The final product contains not less than 25% and not more than 33% of methoxygroups. It conforms to the following specifications.

Description Methylcellulose occurs as white or slightly yellowish or grayish, odourless and tasteless fine granules, filaments or powder.

Uses Thickener, binder, emulsifier, stabilizer and colloidal suspending agent in salad dressing, fruit pie fillings, baked goods, dietetic foods and many others.

Identification Tests

A. *Solubility*

Methylcellulose swells in water and, in the proportion of 1 g to 100 ml of water, produces a clear to opalescent, viscous, colloidal solution which is stable in the presence of most electrolytes and of ethanol in concentrations up to 40%. It is insoluble in ethanol, in ether and in chloroform. It is soluble in glacial acetic acid. Methylcellulose containing 28% or more methoxygroups is soluble in a mixture of 30-35 volumes of ethanol and 70-65 volumes of chloroform. Under the microscope, formations like strings of pearls are observed during the swelling and before the final dissolution.

B. Heat a 1% solution of methylcellulose. It becomes cloudy and a flaky precipitate appears which dissolves as the solution cools.

C. Pour a few ml of a 1% solution of methylcellulose on to a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

D. Add 5 drops of 10% acetic acid solution and 5 ml of 1% tannic acid solution to 5 ml of 1% methylcellulose solution. A yellowish-white flocculent precipitate is formed which is soluble in dilute ammonia TS. This test distinguishes methylcellulose from sodium carboxymethylcellulose.

E. Add 1 ml of 5% copper sulfate solution or aluminium sulfate solution to 5 ml of 1% methylcellulose solution. The solution remains clear. This test distinguishes methylcellulose from sodium carboxymethylcellulose.

Purity Tests

Loss on drying : Not more than 7% after drying at 105° for 1 hour.

Sulfated ash : Not more than 1.5%.

Arsenic : Not more than 3 mg/kg.

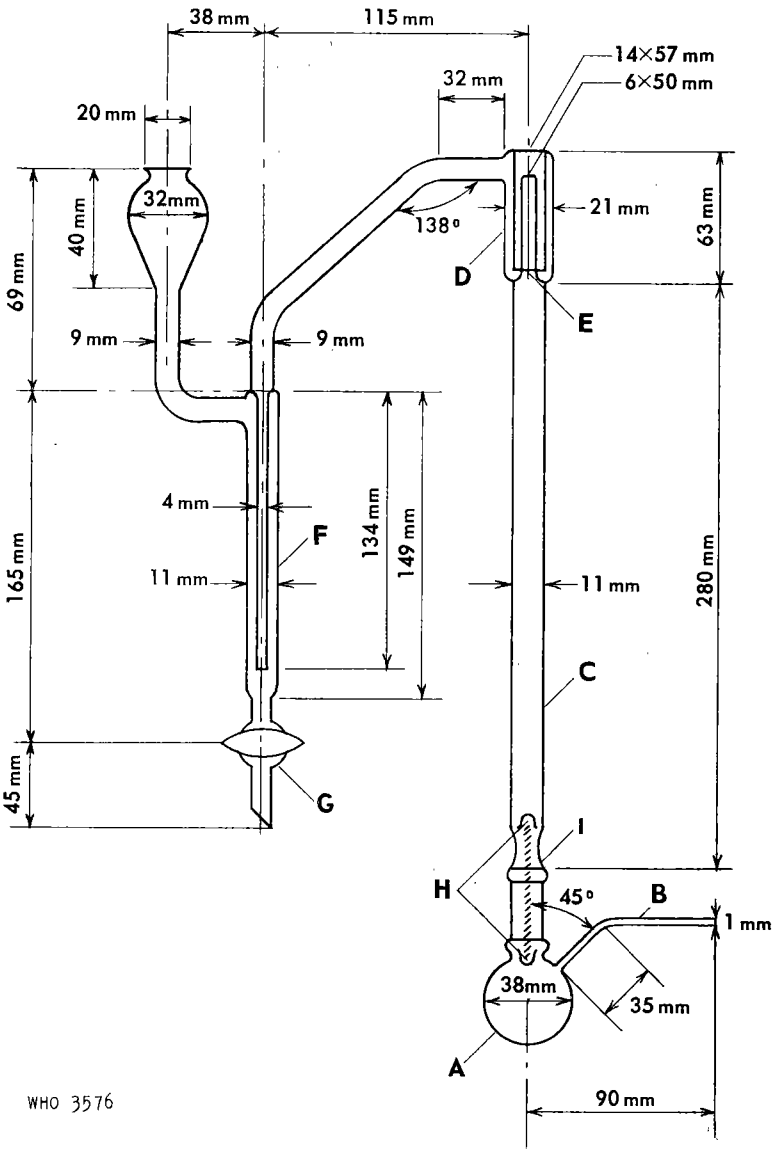
Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

Assay

The apparatus used for the methoxy determination is shown diagrammatically in Fig. 2. The boiling flask, A, is fitted with a capillary side-arm, B, for the introduction of carbon dioxide and is connected to a column, C, which serves to separate aqueous hydriodic acid from the more volatile methyl iodide. The methyl iodide passes through an aqueous red phosphorus suspension in a scrubber trap, D, and is finally absorbed in the bromine-

FIG. 2



A. Boiling flask
B. Side-arm capillary, 1 mm internal diameter
C. Condenser
D. Trap
E. 4 slots, each 1 × 3 mm
F. Absorption tube
G. 2-mm stopcock
H. Hooks and tension springs each side
I. 14/20 $ joint

acetic-acid solution in absorption tube F. The carbon dioxide is introduced from a device arranged to minimize pressure fluctuations and connected to the apparatus by a small capillary containing a small cotton plug.

Prepare the apparatus by placing in trap D, through the funnel on tube F and the connecting side-arm, a volume sufficient to make trap D half-full of a suspension of about 60 mg of red phosphorus in 100 ml of water. Rinse the tube F and the side-arm with water into trap D. Dry carefully the absorption tube F and pour down the funnel 7 ml of bromine-acetic acid TS. Weigh 0.050 g of the methylcellulose in a tared gelatin capsule, and place it in the boiling flask along with a few glass beads or pieces of porous plate. Finally, add 6 ml of hydriodic acid TS and attach the flask to the condenser, using a few drops of the acid to seal the junction. Bubble the carbon dioxide through the apparatus at the rate of about 2 bubbles per second, place the boiling flask in an oil bath heated to 150°, and continue the reaction for 40 minutes. Drain the contents of the absorption tube into a 500-ml Erlenmeyer flask containing 10 ml of sodium acetate solution (1 in 4). Rinse the tube with water, adding the rinsings to the flask, and finally dilute with water to about 125 ml. Add formic acid, dropwise, with swirling, until the reddish-brown colour of the bromine is discharged, then add 3 additional drops. A total of 12 to 15 drops are usually required. Let stand for 3 minutes, and add 15 ml of dilute sulfuric acid TS and 3 g of potassium iodide, and titrate immediately with 0.1 N sodium thiosulfate, using starch TS. Perform a blank determination, including also a gelatin capsule, and make any necessary correction. Each ml of 0.1 N sodium thiosulfate is equivalent to 0.517 mg of $-OCH_3$.

Biological Data

Biochemical aspects

Methylcellulose is usually resistant to microbial attack.[1] In rats it was not hydrolyzed to cellulose and methanol in the intestinal tract and it did not appear to be absorbed.[2]

Investigations on two male adults and one 10-year old girl showed that methylcellulose passed through the digestive tract practically unaltered. When 5-10 g of methylcellulose were ingested the recovery of methoxyl groups from the faeces was almost quantitative. Methanol formation after the taking of methylcellulose was not significantly different from that under normal conditions.[3]

It has been stated that methylcellulose could be partly hydrolyzed in the digestive tract to units of lower molecular weight, as indicated by a decrease in viscosity. Such intermediate products obtained after acid hydrolysis were fed to mice at a dose of 1 g daily for 28 days without any demonstrable effect on growth rate.[4]

Acute toxicity

LD_{50} values have not been found in the literature. In dogs, single intravenous injections of 40 ml of 0.7-2.8% solutions of methylcellulose in saline resulted, within 24 hours, in a moderate anaemia and leucopenia and an increased sedimentation rate.[5] In rabbits, intravenous injections of 10 mg/kg were followed by leucopenia. Injections of 10-100 mg/kg in a 1% solution had no effect on the blood pressure or respiration.[6] In man, single oral doses of 5 g and 10 g were well tolerated.[3]

Short-term studies

Rat

A group of 10 rats (5 male and 5 female) was fed a diet containing 10% methylcellulose for 95 days. The male rats gained weight at the same rate as the controls. The females showed lower food intake and slight growth depression. No abnormalities were found at autopsy or on microscopic examination. Weights of heart, liver, spleen and kidney were normal. The stomachs were 15% heavier in the experimental group than in the controls.[7]

Eighty rats received methylcellulose at the level of 0.8% in the diet and 1% in the drinking-water for 8 months. This was equivalent to an average total daily intake of 436 mg of methylcellulose per animal. No effect on growth rate was observed in any of the animals. Water and food intake were normal. No gross or microscopic pathological changes were found post mortem.[8]

Groups of 5 female rats were fed diets containing 1.66% and 5% methylcellulose for 6 months without any adverse effects.[9]

Three groups of 10 rats (5 male and 5 female) were given diets containing 0.17% (changed after 6 weeks to 0.5%) and 5% methylcellulose for 8 months. No deleterious effect on growth was recorded, and macroscopic and microscopic examination of representative animals revealed essentially normal tissues. Deposition of abnormal material in the tissues was not observed. Reproduction was unimpaired through three generations. Second and third generation rats were fed a diet containing 5% methylcellulose for 4 months and responded normally.[2]

A modified paired feeding experiment was conducted on 3 groups of rats for 90 days : one group received a diet containing 50% methylcellulose, one a diet containing 50% cellulose powder, and one the basal diet. Growth depression was seen in the first two groups. Subsequent replacement of the methylcellulose or cellulose diet by the basal diet resulted in marked weight gain.[2]

In 28-day experiments with groups of 10 rats, some normal and others vitamin-depleted, the oral administration of 50 mg of methylcellulose did not affect the absorption of either 6 µg of thiamine or 3 units of vitamin A per day, as determined by weight gain.[10]

Four intraperitoneal injections over 10 days of a maximal total dose of 160 mg of methylcellulose produced arterial hypertension and glomerulonephritis in rats given a 1% NaCl solution to drink. Typical foam cells were observed in the glomeruli on microscopic examination.[11]

Dog

Two dogs were fed doses of methylcellulose increasing from 2 g to 100 g daily for one month without any noticeable effect.[12]

Solutions containing 0.7-2.8% of methylcellulose of different molecular weights in 1% NaCl were administered by intravenous injection to 18 dogs in doses of 40-130 ml for 5 days a week. The maximum total dose of 5720 ml was injected within 6 months. Most of the animals died. Haematological reactions and the formation of foam cells were observed.[5]

Man

In 3 healthy adults 5 g of methylcellulose given twice a day for 8 days approximately doubled the volume of the stools and increased their frequency slightly.[7]

There was no evidences of toxicity when 1-6 g were taken daily as a laxative for 4-240 days (maximum 6 g for 240 days) by 37 patients,[13] but doses of 2.5-5.25 g of methylcellulose taken orally as a gel in 250 ml of water were mildly constipating.[12]

Patients have been given 2 g of methylcellulose before meals without toxic reactions.[14]

Carcinogenicity

Subcutaneous implantation of 500 mg of methylcellulose as a powder in 25 rats failed to demonstrate carcinogenic properties.[15]

Comment on experimental studies reported

The results of the short-term studies in rats, two of them for periods of 8 months, are supported by evidence from the treatment of human patients without any adverse effects. As metabolic studies show no absorption from the intestinal tract, toxic reactions resulting from parenteral administration can be left out of consideration.

Evaluation

Estimate of acceptable daily intake for man

	mg/kg body weight
Unconditional acceptance	0-30

Further work considered desirable

Long-term studies in animals. Influence on the absorption of nutrients from the digestive tract.

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SODIUM CARBOXYMETHYLCELLULOSE

Synonyms Sodium cellulose glycolate; Na-CMC; CMC.

Chemical name Sodium salt of a carboxymethyl ether of cellulose.

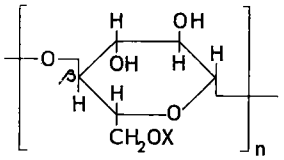
Chemical formula $[C_6H_7O_2(OH)_x(OCH_2COONa)_y]_n$

where $x = 2.00$ to 2.40

$y = 1.00$ to 0.60

$x + y = 3.00$

Structural formula



where X = H or CH_2COONa

Molecular weight *Unsubstituted structural unit :* 162.14

Monosubstituted structural unit : 242.16

Macromolecules : from about 21 000 (n about 100) up to 500 000 (n about 2000)

Definition Sodium carboxymethylcellulose is the sodium salt of a carboxymethyl ether of cellulose, where the substitution degree ranges from 0.60 to 1.00. After drying at 110° to constant weight it contains not less than 99.5% of sodium carboxymethylcellulose. It conforms to the following specifications.

Description Sodium carboxymethylcellulose is a white or a slightly yellowish, almost odourless and tasteless hygroscopic powder, consisting of very fine particles, fine granules, or fine fibres.

Uses Thickener, binder, emulsifier, stabilizer and colloidal suspending agent in salad dressing, fruit pie fillings, baked goods, dietetic foods and many others.

Identification Tests

A. *Solubility*

Sodium carboxymethylcellulose yields viscous colloidal solutions with water. It is insoluble in ethanol and in ether.

B. Add about 1 g of powdered sample to 100 ml of warm (60°-70°) water while stirring to produce a uniform dispersion. Continue the stirring until a colloidal solution is produced, cool to room temperature, and use the solution for the following tests.

(*a*) To part of the solution add 1 volume of uranyl zinc acetate TS and shake. A yellow precipitate is formed within a few minutes.

(*b*) Boil part of the solution during 5 minutes ; the solution remains limpid. This test distinguishes sodium carboxymethylcellulose from methylcellulose.

(*c*) Dilute 1 ml of the solution with water to 100 ml. To 1 ml of the dilution add 2 ml of naphthalenediol TS and place in a boiling water bath for 20 minutes. A deep red colour develops.

(*d*) Add iodine TS to part of the solution ; no blue colour appears. This test distinguishes sodium carboxymethylcellulose from carboxymethyl starch.

(*e*) Add a solution of copper sulfate to the sample ; a blue precipitate is formed. This test distinguishes sodium carboxymethylcellulose from gelatin, locust bean gum, methylcellulose, and tragacanth.

Purity Tests

Loss on drying : Dry sodium carboxymethylcellulose at 110° until constant in weight ; it loses not more than 6% of its weight.

pH of a 1% solution : 6-8.

Sodium chloride : Char carefully 5.000 g of sodium carboxymethylcellulose in a platinum or porcelain crucible. After cooling, pulverize the ashes thus obtained and extract several times with warm water. Filter the extracts into a 500-ml volumetric flask, acidify with nitric acid and dilute to the mark. Determine the NaCl content of 100 ml of this extract by the method of Volhard, using 0.02 N silver nitrate and 0.02 N ammonium rhodanide. Each ml of 0.02 N silver nitrate is equivalent to 0.001169 g NaCl. The sodium chloride content can be calculated according to the formula :

$$\% \text{ NaCl} = \frac{a \times 0.001169 \times 5}{b} \times 100$$

where a = ml of 0.02 N silver nitrate used and
b = dry weight of 5.000 g of the sample.

Sodium carboxymethylcellulose should not contain more than 0.5% NaCl on a dry-weight basis.

Free glycolate : Carboxymethylcellulose should not contain free glycolate. This impurity is absent if the following test is met. Extract 5 g of the dried sample in a Soxhlet apparatus with absolute ethanol (less than 2% water) for 15 hours. Dilute the ethanolic extract with water to 100 ml. Transfer 1 ml of this dilute ethanolic solution to a 50-ml volumetric flask, and add 20 ml of naphthalenediol TS. At the same time, prepare a standard sample containing 0.5 ml of standard glycolic acid solution [a] and enough water to make 1 ml. Stopper the flasks and mix. Loosen the stoppers and place in a boiling water bath for 30 minutes. Cool to room temperature and dilute slowly to 50 ml with water, cooling during the dilution in water at 0°. The colour of the sample solution is not more intense than the colour of the standard solution.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 10 mg/kg.

Heavy metals : Not more than 40 mg/kg.

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[a] *Preparation of standard glycolic acid solution.* Dry glycolic acid in a vacuum desiccator for at least 16 hours, weigh 0.1000 g of the dry substance, dissolve in water and make up to 1 litre. Do not keep the solution longer than 30 days.

Assay

Sodium potentiometric method : Weigh 0.500 g of sodium carboxymethylcellulose in a beaker, add 80 ml of glacial acetic acid, heat the mixture on a boiling water bath for 20 minutes, and cool to room temperature. Dry the exterior of a glass-calomel electrode assembly on a pH meter and place the electrodes in the solution. Set the pH meter on the +mV circuit and add 0.1 N perchloric acid in large increments until the deflection of the needle becomes noticeable ; then reduce the added fraction to 0.1 ml until the variation in the readings passes through a maximum. Plot the number of millilitres added against the mV readings and determine the quantity of the titrant corresponding to half-way up the steepest gradient. Each ml of 0.1 N perchloric acid is equivalent to 0.00230 g of sodium.

Substitution degree and content of sodium carboxymethylcellulose : uranyl method. Weigh accurately about 2 g of the substance (p_1 g), transfer into a 1000-ml volumetric flask with the aid of a little methanol and dissolve in about 500 ml of water under constant shaking until complete dissolution. Fill up to the mark and mix thoroughly. Pipette 100 ml of the solution into an 800-ml beaker, add 400 ml of water, 3 drops of a 0.05% bromothymol blue solution and dilute nitric acid until a yellowish-green colour is obtained (pH = 6.3-6.5). Heat the solution to 70°, and add 30 ml of a 2% uranyl nitrate solution drop by drop by means of a pipette or burette under vigorous stirring. Continue stirring for 5-10 minutes after adding the uranyl nitrate solution. At the end of the precipitation the pH is approximately 4.0. The precipitate should settle leaving a clear supernatant solution within a few minutes. Should this not be the case, heat the solution again to 60°-70° under constant stirring.

Allow the precipitate to settle and decant the supernatant liquor through a tared fine glass filtering crucible under vacuum. Add some water to the fine-grained precipitate in the beaker, whirl, allow to settle, and decant into the filter. Repeat this washing subsequently with water, 75% methanol, and absolute methanol. Let each washing liquid be 200 ml. Transfer the precipitate by means of methanol to the filter. Test the last portion of the filtrate for nitrate ions with a 0.1% solution of diphenylamine in sulfuric acid. The reaction should be negative ; if not, continue washing with absolute methanol. Dry the precipitate for 1 hour at 110°, allow to cool in a desiccator and weigh (p_2 g). Transfer the larger part (q_1 g) of the precipitate into a glowed and weighed porcelain crucible, char at 750°-800° (red-hot) for 20-30 minutes and weigh the urane octoxide formed (q_2 g).

$$\text{Degree of substitution (DS)} = \frac{162\,f}{135\,000 - 192\,f}$$

$$\text{where :} \qquad f = \frac{q_2 \times 0.962}{q_1}$$

$$\%\ \text{sodium carboxymethylcellulose} = \frac{p_2 \times (162 + 80\ DS) \times 10}{p_1 \times (162 + 192\ DS)} \times 100$$

Biological Data

Biochemical aspects

Sodium carboxymethylcellulose (CMC) is readily hydrolysed by micro-organisms.[1] Diastase and cellulases are stated to bring about the breakdown of this compound.[2] Pepsin and pancreatin, separately or in combination, do not attack the substance.[3]

In 5 rats fed 5 g of CMC collectively, approximately 90% of the dose was recovered in the faeces.[4] Experiments on 6 rats during 4 periods of 10 days each showed that CMC given in the diet at levels of 5%, 10% and 14% was reclaimed quantitatively in the faeces.[5]

^{14}C-tagged CMC, containing up to 0.34% radioactive sodium glycolate, was given orally to 2 groups of 5 male rats each in a dose of 400 mg. No detectable activity (less than 0.02% of the dose) was found in the livers and kidneys and about 0.14% of the administered radioactivity was found in the 48-hour urine samples. This amount, however, could be accounted for by the free radioactive glycolate present in the test compound.[6]

Only about 50% of the intake of CMC could be recovered from the faeces of 2 rabbits on diets containing 4.76% and 9% CMC.[5]

Two dogs received 10 g of CMC for one day and 20 g daily for the following 5 days. The total doses were recovered quantitatively in the faeces.[3]

Two human adults were given 30 g of CMC by mouth daily for 4 days and a third was given 20 g/day. About 90% of the compound was recovered from the faeces.[5]

Acute toxicity

Animal	Route	LD_{50} (mg/kg body weight)	Reference
Rat	oral	27 000	4
Guinea-pig	oral	16 000	4

Rats, guinea-pigs and rabbits showed no symptoms after administration by stomach tube of 3000 mg/kg in 3 divided doses.[7]

Six rats given an intravenous injection of 1 ml of a 1.6% solution of CMC showed 48 hours later the presence of particles localized in cells of the reticulo-endothelial system.[8]

Four dogs given an intravenous injection of 40 ml of 0.25% CMC in 1% sodium chloride solution reacted with a transitory leucopenia.[9]

Short-term studies

Rat

Ten rats received 300-500 mg of CMC daily for 2 months without any adverse effect.[10]

One group of rats (10 male and 15 female) was fed on a diet containing 5% CMC for 201-250 days. Judged by growth rate, mortality, organ weight and the results of histopathological examination of the liver, kidney, spleen, pancreas, adrenal gland, testis and gastro-intestinal tract, there was no significant difference between the treated and the control group.[7]

Another group of 10 rats received a diet containing 20% of CMC for 63 days. Slight growth retardation and a laxative effect were observed. Organ weights and both gross and microscopic pathological examination revealed no abnormalities.[7]

Two groups of 100 rats received daily 500 and 1000 mg/kg body weight of CMC mixed in their diets for 6 months. No adverse effects were observed in any of the experimental animals as determined by growth rate, fertility and examination of the blood, urine, and main tissues.[4]

Six rats were fed 14% of CMC in the diet for 5 weeks without demonstrable deleterious effect.[5]

Ten rats given subcutaneous injections of CMC showed mast-cell-like elements within the adrenal medulla. Changes in the adrenocortical cells and the presence of granules in the adrenal-vein were noted occasionally.[11]

Guinea-pig

Two groups of 100 animals were fed CMC for 6 months at the levels of 500 and 1000 mg/kg body weight daily mixed in the diet. No signs of toxicity were observed.

Two groups of 20 guinea-pigs received CMC in their diet at rates of 500 and 1000 mg/kg body weight daily for one year. As judged by weight gain, gross and histopathological examination, no adverse effects were noted.[4]

Rabbit

Three rabbits were fed CMC at levels of 4.8% and 9% in their diet for two periods of 15 days without any detectable toxic effects.[5]

Dog

Two dogs were given daily doses of CMC (0.3-0.4 g/kg body weight) in water by mouth for two months without adverse effects.[10]

Groups of 10 dogs were fed CMC daily in the diet at levels of 500 and 1000 mg/kg body weight for 6 months. Growth rate was the same in all groups. Six animals from each group were examined post mortem. Histologically the stomach, intestines, spleen, kidney, heart, lung and pancreas in the treated animals were no different from those of the controls.[4]

Five dogs received intravenous injections of 0.25% CMC in 1% sodium chloride solution in doses increasing from 40 ml to 150 ml for a maximum

of 3 months. There were no gross pathological changes. Histopathological studies revealed uptake in the reticulo-endothelial cells in the aorta.[9]

Man

Daily oral doses of 20-30 g for 7 days were well tolerated by 3 human subjects.[5]

Eleven patients received 10 g daily for 6 months without complaint, but in 7 further cases the dose had to be reduced owing to abdominal discomfort. No haematological changes were observed.[12]

Adult patients have been treated for more than a year with daily oral doses of 2-6 g of CMC as a laxative and there were no ill-effects.[13, 14, 15]

Skin tests on 100 men and 100 women demonstrated that sodium carboxymethylcellulose is not a primary irritant, nor a sensitizer.[4]

Long-term studies

Rat

Thirty rats were given weekly injections of 1 ml of a 2% aqueous solution of CMC subcutaneously. After 73 weeks, 43% of the animals showed tumours at the site of injection, characterized as fibrosarcomas of moderate malignancy by histological standards.[16]

Twenty rats were given subcutaneous injections once a week of 2% aqueous solution of CMC. In 4 animals tumours developed at the site of injection within 13-16 months. Two of the neoplasms were fibromas and two fibrosarcomas.[17]

Groups of 25 rats, divided about equally for sex, were placed for 2 years on diets containing CMC in concentrations providing 100, 500 and 1000 mg/kg body weight daily. Three generations of litters were produced and kept on the same diet as their parents. According to growth rates, monthly urine and blood examinations, fertility, and histopathological examination of the main organs, there were no differences between the test rats and the controls. No neoplasms were found in any of the experimental animals.[4]

Comments on experimental studies reported

Significant amounts of CMC do not appear to be absorbed from the digestive tract in man. The injurious properties that have been demonstrated in animals following parenteral administration can therefore be left out of consideration. (For more detailed discussion see the fifth report of the Joint FAO/WHO Expert Committee on Food Additives.)[18] The same reasoning therefore applies to CMC as to methylcellulose and the acceptable intake can similarly be based on human findings.

Evaluation

Estimate of acceptable daily intake for man

	mg/kg body weight
Unconditional acceptance	0-30

Further work considered desirable

1. Further long-term studies, especially in other species than the rat.

2. Further investigations concerning the influence of the substance on the digestibility and absorption of nutrients from the digestive tract.

3. Study of the possible relationships between the chemical and physical properties of sodium carboxymethylcellulose and its biological effects.

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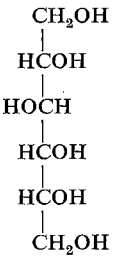
SORBITOL

Synonyms D-Glucitol; D-sorbitol; D-sorbit.

Chemical name 1,2,3,4,5,6-Hexanehexol.

Empirical formula $C_6H_{14}O_6$

Structural formula



Molecular weight 182.17

Definition Sorbitol consists essentially of D-glucitol with a small quantity of D-mannitol and traces of other polyhydric alcohols. The material is available both as an almost anhydrous crystalline solid, and as a 70% by weight aqueous solution.

Crystalline Sorbitol contains not less than 91% of D-glucitol.

Sorbitol Solution contains not less than 64 g of D-glucitol per 100 g of solution.

Description Crystalline Sorbitol is a white hygroscopic powder with a sweet taste.

Sorbitol Solution is a clear, colourless, syrupy liquid with a pleasantly sweet taste.

Both conform to the following specifications.

Uses Sweetening agent, sequestrant, humectant, e.g., in diabetic foods and special dietary products and in soft drinks.

Identification Tests

A. *Solubility*

Crystalline Sorbitol is very soluble in water. Sorbitol Solution is miscible with water, glycerol and propylene glycol. Crystalline Sorbitol

is sparingly soluble in cold methanol and ethanol, but insoluble in most other common organic solvents.

B. The melting point of the metastable form is 93° and of the stable form 97.5°.

C. To 5 g of Crystalline Sorbitol or 6 ml of Sorbitol Solution, add 7 ml of methanol, 1 ml of benzaldehyde, and 1 ml of hydrochloric acid. Mix and shake in a mechanical shaker until crystals appear. Filter with the aid of suction, dissolve the crystals in 20 ml of boiling water containing 1 g of sodium bicarbonate, filter while hot, cool the filtrate, filter with suction, wash with 5 ml of methanol-water mixture (1 in 2) and dry in air : the sorbitol monobenzylidene derivative so obtained melts between 173° and 177°.

D. Dissolve 1 g of sorbitol solids by boiling in a mixture of 1 ml of pyridine and 10 ml of acetic anhydride for 10 minutes, cool and add 25 ml of water. Cool again and a white, crystalline precipitate of sorbitol hexa-acetate is formed. Wash the crystals with water, recrystallize from 10 ml of ethanol and dry ; the crystals melt at 98°-101°.

Purity Tests

Loss on drying of Crystalline Sorbitol : Not more than 1%. Weigh 1.000 g of Crystalline Sorbitol in a suitable, tared weighing bottle, dry at 80° and at a pressure not exceeding 5 mm of mercury for 6 hours, transfer to a desiccator, cool and weigh.

Water content of Sorbitol Solution : 29-31% as determined by the Karl Fischer method.

Reducing sugars as dextrose : Transfer 10.00 g of Sorbitol Solution or 7.00 g of Crystalline Sorbitol to a 400-ml beaker with the aid of 40 ml of water and mix. Add 25 ml of copper sulfate solution TS and 25 ml of alkaline tartrate solution TS. Cover the beaker with glass, heat the mixture at such a rate that it comes to a boil in approximately 4 minutes and boil for exactly 2 minutes. Filter the precipitated cuprous oxide through a tared Gooch crucible previously washed with hot water, ethanol, and ether, and dried at 100° for 30 minutes. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 ml of ethanol and finally with 10 ml of ether, and dry at 100° for 30 minutes. The weight of the cuprous oxide does not exceed 50 mg, corresponding to not more than 0.21% of reducing sugars in the case of Sorbitol Solution, or 0.30% of reducing sugars in the case of Crystalline Sorbitol.

Total sugars : Transfer 3.00 g of Sorbitol Solution or 2.10 g of Crystalline Sorbitol to a 250-ml flask fitted with a ground glass joint, add 40 ml of approximately 0.1 N hydrochloric acid. Attach to a reflux condenser, and

heat at reflux temperature for 4 hours. Transfer to a 400-ml beaker, rinsing the flask with 10 ml of water. Add 25 ml of copper sulfate solution TS and 25 ml of alkaline tartrate solution TS and continue as described above for reducing sugars. The weight of the cuprous oxide does not exceed 50 mg, corresponding to not more than 0.7% of total sugars in the case of Sorbitol Solution or 1.0 % of total sugars in the case of Crystalline Sorbitol.

Sulfated ash : Not more than 0.1%.

Arsenic : Not more than 3 mg/kg.

Lead : Not more than 5 mg/kg.

Heavy metals : Not more than 20 mg/kg.

Assay

Insert a cotton plug on the removable porous plate of a slightly tapered chromatographic tube (38×230 mm) which is inserted in a 500-ml suction flask. Add, with full vacuum, 80 g of a mixture of 5 parts of very fine chromatographic fuller's earth and 1 part of chromatographic siliceous earth, in portions of 2 to 3 g, tapping the tube gently after each addition. Apply suction gently to compress the absorbant to the column to a height of about 200 mm, level the top of the column with a spatula, and cover it with a disk of filter paper.

To 0.3000 g of Crystalline Sorbitol or 0.4500 g of Sorbitol Solution add 1 ml of water and 5 ml of isopropanol solvent (85% v/v isopropanol in water), mix, and pour the solution on the prepared column. Complete the transfer with the aid of three 10-ml portions of the solvent, waiting after each addition until the solvent has practically disappeared into the column. Attach a separator, containing 325 ml of the solvent, to the tube by means of a rubber stopper inserted to a point 16 mm above the top of the column. Admit enough solvent to make a 1-cm layer, and adjust the separator so that its tip is just below the solvent surface. As soon as an even front of the liquid has advanced about 1.3 cm to 2.0 cm down the tube, apply full suction and open the separator stopcock to admit solvent so that the effluent collects initially at a rate of approximately 3 ml per minute and continues until all the solvent has been added, taking care that the column does not run dry.

When all the solvent has been added from the separator and the liquid layer has just disappeared into the column, continue full vacuum for 1 to 3 minutes, then disconnect the system promptly, and extrude the column on to glazed paper. Paint several streaks the length of the column with a solution consisting of 1 g of potassium permanganate and 10 g of sodium hydroxide in sufficient water to make 100 ml. Note and mark the zones where the streaks are decolourized. Cut out with a sharp blade the second

zone, which usually starts about 45 mm to 50 mm from the top of the column and extends 125 mm to 150 mm ; return this portion of the column to the tube in which a fresh plug of cotton has been placed on the porous plate, replace the separator and, with the aid of gentle suction, draw the column down the chromatographic tube until it rests snugly on the cotton plug. Elute the absorbed sorbitol with 200 ml of water, applying full vacuum when the water has advanced about 13 mm down the column. Transfer the eluate to a 500-ml volumetric flask, dilute to the mark with water, and mix.

Pipette 5.00 ml of the diluted eluate into a 250-ml conical flask, and add 50 ml of potassium periodate reagent prepared by mixing 40 ml of dilute sulfuric acid (1 in 20) and 60 ml of potassium periodate solution (1 in 1000) that has been acidified with 5 drops of sulfuric acid. Heat the solution on a steam bath for 15 minutes, cool to room temperature, and add 1 g of potassium iodide. Allow to stand for 5 minutes, and then titrate the solution with 0.02 N sodium thiosulfate, using starch TS as indicator. Perform a blank determination, using water in place of sorbitol, and subtract the volume used for the sorbitol solution from that used in the blank determination. Each ml of 0.02 N sodium thiosulfate is equivalent to 0.3643 mg of sorbitol.

Biological Data

Biochemical aspects

The absorption of sorbitol is much slower than that of glucose or fructose. Both normal and diabetic human subjects excreted in the urine less than 3% of an oral dose of 35 g. No sorbitol was detected in the faeces. In experiments with uniformly labelled ^{14}C-sorbitol (sorbitol-U-^{14}C), at least 75% of the dose given orally was metabolized to CO_2. In normal subjects, there was no significant increase in the blood sugar levels ; in diabetic subjects the blood sugar increased slightly. The concentration of sorbitol in the blood was immeasurably small.[1] In experiments on rats given sorbitol-U-^{14}C by intraperitoneal injection, 57.4% of the activity was excreted as CO_2, 17.3% in the urine, 4.2% was found as liver glycogen and 0.6% as liver fatty acids. In diabetic rats, a smaller proportion was oxidized and the major portion was excreted in the urine.[2] Sorbitol has a strongly glycogenic effect in the fasted diabetic rat.[2, 3] The polyol has a more efficient antiketogenic effect in liver slices of fasted rats than glucose or fructose,[4] and behaved similarly in the intact rat.[5] Sorbitol was oxidized to fructose by a DPN-linked polyol dehydrogenase.[6, 7] Experiments with sorbitol-U-^{14}C in rats indicated further that sorbitol is first oxidized to fructose.[8] The intravenous infusion of sorbitol in rabbits caused a prompt fructosaemia and a variable secondary glucosaemia occurred later.[9] Experiments on rats [10] given sorbitol-U-^{14}C support the view that at least two

pathways exist for the oxidation of sorbitol in the body : (*a*) oxidation after conversion to glucose, and (*b*) the direct oxidation of the primarily formed fructose. Sorbitol was not metabolized by hepatectomized animals.[11]

Sorbitol has a sparing effect for some B-vitamins (thiamine, pyridoxine, biotin).[12, 13] It has been shown that the slowly absorbed polyol promotes the proliferation of intestinal bacteria which synthesize B-vitamins.[14] When administered in amounts of 20-40 g daily, sorbitol increased the excretion of thiamine, riboflavin and N^1-methylnicotinamide in man.[15] Sorbitol is an inhibitor of the intestinal absorption of vitamin B_{12} in man, and in the rat, guinea-pig and pig, as shown by administering physiological doses of the ^{60}Co-labelled vitamin.[16] In rats, sorbitol enhances the absorption of iron,[17] and also that of calcium and strontium. In feeding experiments on rats, sorbitol showed the same caloric value as glucose.[18]

Acute toxicity

Animal	Route	LD_{50} (mg/kg body weight)	Reference
Mouse — male	oral	23 200	19
Mouse — female	oral	25 700	19
Rat — male	oral	17 500	19
Rat — female	oral	15 900	19
Rat — male	i.v.	7 100	19
Rat — female	i.v.	7 300	19

Short-term studies

Rabbit

The intravenous infusion of sorbitol together with amino-acids for 10 days was associated with a positive nitrogen balance. The histopathological examination of the organs showed no abnormalities.[20]

Dog

Sorbitol was excreted by glomerular filtration ; the renal clearance in the dog was found to be 74-77 ml/min.[21] Injection of 2.5 ml/kg body weight of a 50% solution had a marked diuretic effect for about one hour.[22]

Man

In amounts of 40 g daily, spread throughout the day's intake of food, sorbitol was well tolerated for a long period.[23, 24] A total of 25 g daily in two doses caused no laxative effect in 86 subjects. In about 5% of these subjects a somewhat increased amount of gas appeared in the bowel.[25] Quantities greater than 50 g daily were laxative. This effect was presumably due to the relatively slow rate at which sorbitol was absorbed from the small bowel.[26]