CALCIUM LIGNOSULFONATE (40-65)

Chemical and Technical Assessment

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1. Summary

Calcium lignosulfonate (40-65) is an amorphous light-yellow-brown powder obtained from the sulfite pulping of softwood. The organic framework of the additive is a sulfonated random polymer of three aromatic alcohols: coniferyl alcohol, *p*-coumaryl alcohol, and sinapyl alcohol, of which coniferyl alcohol is the principle unit. The commercial product has a weight-average molecular weight range of 40,000 to 65,000 and the name of the additive is intended to reflect this range and to distinguish it from other calcium lignosulfonates in the market place. The intended use of calcium lignosulfonate (40-65) is as a carrier (encapsulating agent) for fat-soluble vitamins, carotenoids, and other functional ingredients in, e.g., fruit-based beverages,vitamin drinks, dairy products, and hard candies. Preparations of such substances will contain calcium lignosulfonate (40-65) at ratios of active principle to lignosulfonate between 1:5 to 1:200. Stability studies of the additive 1) stored in polyethylene containers (36 months) and in aluminium-foil bags (24 months); 2) as a component of a formulation of the additive with ß-carotene (48 weeks); and 3) in a ß-carotene-containing product form in a non-pasteurised, non-carbonated soft drink (3 months) all support the intended uses.

2. Introduction

Lignosulfonates are commercially available as sodium and calcium salts and have been used by industry in a wide variety of applications. The usefulness of commercial lignosulfonate products comes from their dispersing, binding, complexing, and emulsifying properties. The additive calcium lignosulfonate as described in the *Food Chemicals Codex* (FCC, 6th ed, US Pharmacopeia, Rockville, MD 20852 USA (2008)) has been used for a number of years in the food industry, serving, for example, as an emulsifier in animal feed, as raw material in the production of vanillin, and as a boiler water additive. The additive calcium lignosulfonate (40-65), evaluated by the 69th JECFA, is of higher purity than the calcium lignosulfonate described in the FCC, with a higher degree of polymerization and a lower content of sugars. The intended use of calcium lignosulfonate (40-65) is as a carrier for fat-soluble vitamins, carotenoids, and other functional ingredients. Calcium lignosulfonate (40-65) corresponds to the material on which the toxicological studies have been performed and evaluated by JECFA.

3. Description

Calcium lignosulfonate (40-65) is an amorphous material derived from lignin. It is a light-yellow-brown powder that is soluble in water, but practically insoluble in organic solvents. The product exhibits a weight-average molecular weight in the range of 40,000 to 65,000 with greater than 90% ranging from 1,000 to 250,000 .

4. Manufacturing process

Calcium lignosulfonate (40-65) is produced from softwood in the sulfite pulping method for manufacturing paper. The wood chips are digested with acidic calcium bisulfite solution in large reaction vessels where they are processed through cooking cycles of 6 - 10 hours. The highest temperature during a cooking cycle is

[1] This document is based primarily on a draft CTA provided by DSM Nutritional Products, Basel Switzerland, for which the Committee is greatly appreciative.

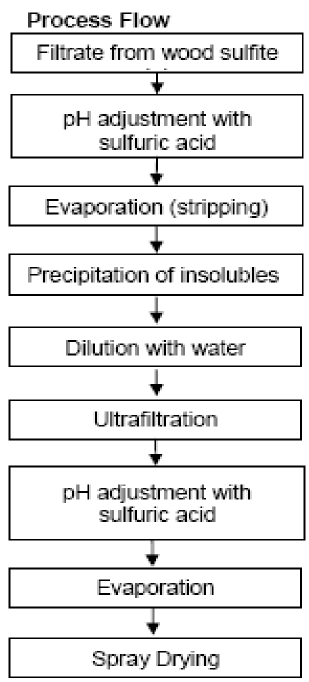
approximately 130°. In this process, bisulfite ions react with the native lignin polymer of the wood to form sulfonated lignin (i.e., lignosulfonate). This reaction increases the water-solubility of the hydrophobic lignin polymer. The calcium bisulfite provides the calcium ions that stabilise the anionic sulfonate groups in the lignosulfonates. After the completion of pulping, water-insoluble cellulose and soluble calcium lignosulfonate are separated by filtration.

The brownish filtrate, containing the lignosulfonates, will also contain residual amounts of sulfite salts and reducing-sugar monomers formed from wood cellulose during pulping. The pH of the filtrate is adjusted by addition of concentrated sulfuric acid. The water and sulfite (as sulfur dioxide) content are reduced by subsequent evaporation.

After the first evaporation step, the filtrate may be diluted with water before it is subjected to further purification by ultrafiltration at moderately elevated temperatures. Ultrafiltration is a liquid/liquid separation method whereby the filtrate is separated by molecular size through a semi-permeable membrane. The ultrafiltration step separates the high-molecular weight lignosulfonate fraction from depolymerisation products, such as low-molecular weight lignosulfonates, and reducing-sugar monomers. According to the sponsor, the use of other than softwood as a source material would not yield a product with the desireable high-molecular weight distribution.

The purified calcium lignosulfonate (40-65) from ultrafiltration may be pH-adjusted by addition of dilute sulfuric acid. This solution is then evaporated at a temperature of 95-105° to a dry-matter content that is appropriate for spray-drying. The final product is spray-dried to a moisture content in accordance with the specification for Loss on drying and filled into containers suitable for holding food.

A process flow diagram is shown below:



5. Chemical characterization

5.1 Composition

Lignin is the second largest component of wood. It is a highly polymerized material that makes up the middle lamella of woody fibers and holds the fibers together. The basic units of the polymeric structure consist of three aromatic propenyl alcohols (monolignols): coniferyl alcohol (4-(3-hydroxy-1-propenyl)-2-methoxyphenol), *p*-coumaryl alcohol (4-[(*E*)-3-hydroxyprop-1-enyl]phenol), and sinapyl alcohol (4-hydroxy-3,5-dimethoxycinnamyl alcohol), of which coniferyl alcohol represents the principle unit in lignin. Methoxy groups are abundant on the polymeric structure providing many reactive sites and promoting its low water solubility. In calcium lignosulfonate (40-65), however, sulfonate groups are attached to the alkane backbone units to confer water solubility and calcium anions, introduced with the calcium sulfite during the process of manufacture, stabilize them. The degree of sulfonation on the alkane backbone has also been established as a criterion for the identification of the additive. An upper limit of 5.0 % for calcium content was also set.

Ultraviolet spectroscopy confirms the presence of phenyl groups in the additive and infrared spectroscopy reveals bands characteristic of sulfonate at 1210-1220 cm^{-1}, 1037 cm^{-1}, and 655 cm^{-1}. A proposed structure of the polymeric fraction of calcium lignosulfonate (40-65) is presented in Figure 5.1. Representative IR and UV spectra are presented in Figures 5.2 and 5.3, respectively.

5.2 Impurities (including degradation products)

Impurities in calcium lignosulfonate (40-65) include monosaccharides from wood cellulose, sulfite residues, and potentially arsenic and various metallic elements. Filtration, evaporation and ultrafiltration aim at reducing the content in the final product to not more than 5 % reducing sugars and not more than 0.5 % sulfite, on the dried basis, which are the bases for the establishment of corresponding limits in the specifications monograph. A typical analysis provided by the sponsor of a single sample of the additive showed the presence of the following reducing sugars: mannose (2.2 %), xylose (1.1 %), galactose (0.6 %), glucose (0.4 %), arabinose (0.3 %), and rhamnose (0.1%). The specifications monograph also contains maximum limits on Loss on drying (8.0 %), Total ash (14.0%), arsenic (1 mg/kg), and lead (2 mg/kg).

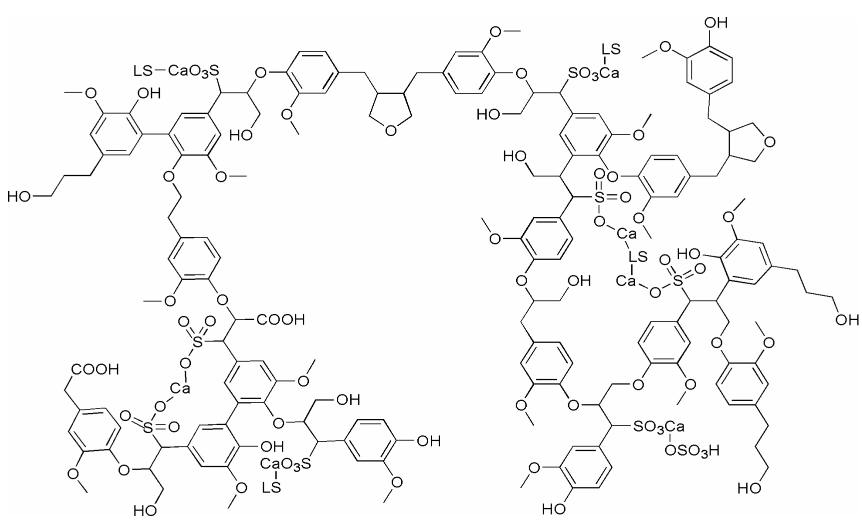


Figure 5.1- Proposed structure of the polymeric fraction of calcium lignosulfonate (40-65)

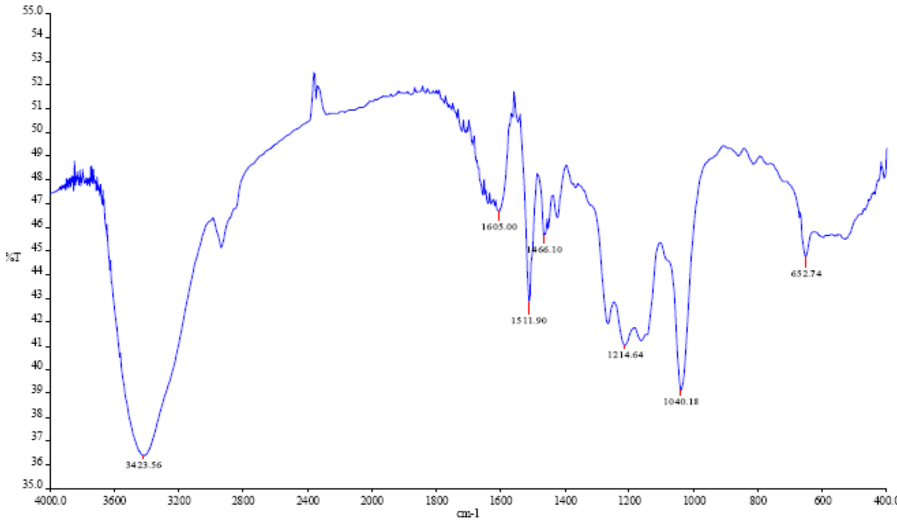


Figure 5.2 - Infrared spectrum of calcium lignosulfonate (40-65)

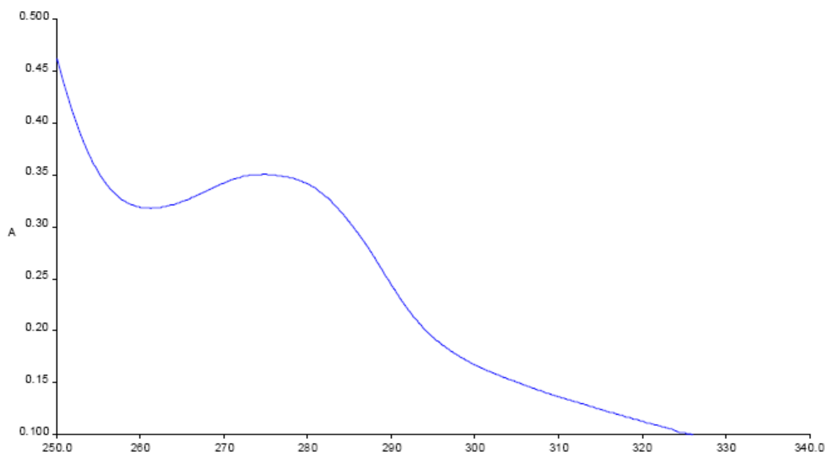


Figure 5.3 - UV Spectrum of calcium lignosulfonate (40-65)

5.3 Analytical methods

Analytical methods used to support the specifications for calcium lignosulfonate (40-65) are based on general tests for identy and purity (solubility, IR spectrum, UV spectrum, pH, calcium, loss on drying, arsenic and lead) published in the *FAO Combined Compendium of Specifications* (FAO JECFA Monographs 1, vol. 4, 2006). Other methods (for weight-average molecular weight, degree of sulfonation, total ash, reducing sugars, and sulfite) are described in the new specifications monograph.

5.4 Rationale for proposed specifications

The specifications for calcium lignosulfonate (40-65) were developed based on the *FCC* specifications for food-grade calcium lignosulfonate (FCC, 6th ed, 2008) with modifications added as appropriate. The major differences between the two products are that calcium lignosulfonate (40-65) contains substantially lower amounts of low-molecular weight polymeric material and reducing sugars than does the *FCC*-specified material. Because of these differences and the end uses for the material reviewed by the 69^{th} JECFA, the expert committee decided that the *FCC* material and the material it evaluated were sufficiently different as to require different names, hence calcium lignosulfonate (40-65). The committee adopted this nomenclature to reflect the high-molecular weight distribution (40,000-65,000) of the evaluated material.

6. *Functional use*

6.1 Technological function

Calcium lignosulfonate (40-65) is intended for use as a carrier for the production of encapsulated fat-soluble vitamins (A, D, E, and K), carotenoids (e.g. β-carotene, β-apo-8'-carotenal, zeaxanthin, canthaxanthin, lutein, and lycopene), and other functional ingredients to facilitate their introduction into water-based foods. It has an adequate emulsifying and film-forming effect and a viscosity that ensure the formation of droplets of appropriate size in the final step of the encapsulation process.

According to the sponsor, gelatins, gum arabic, soy protein hydrolysates, and starches (including chemically modified starches, such as octenylsuccinate starches) are the most commonly used water-soluble matrix materials for the production of encapsulated nutrients; in general, gelatins are preferred if a barrier against oxygen is required. The sponsor notes, however, that gelatins of animal origin have shortcomings, for example, in terms of kosher/halal, BSE, and allergen labeling issues, compared to calcium lignosulfonate (40-65), which is of plant origin.

6.2 Food categories and use levels

The sponsor intends that vitamin and carotenoid preparations containing calcium lignosulfonate (40-65) will be used as nutrient sources in various food applications, such as fruit-based beverages and vitamin drinks. Carotenoid preparations may also be used as food colours. The levels of calcium lignosulfonate (40-65) in foods will depend on the application and permitted levels of use of food colours and nutrients. The carrier would normally provide about 50% of the mass of vitamin and nutrient products with the active ingredient comprising about 10%, the rest being made up of other food ingredients/additives. However, according to the sponsor, for technological reasons the ratio of active principle to calcium lignosulfonate (40-65) in powdered products will vary between 1:5 to 1:200 resulting in levels of lignosulfonate typically in the range of 2 to 100 mg/kg in fortified or coloured foods and beverages. The intended food uses are the same as for the commonly used water-soluble matrix materials gelatins, gum arabic, soy protein hydrolysates, and modified starches.

The sponsor provided the following table of applications:

Product	**Declared vitamins where preparations with lignosulfonate exist**	**Declared amount of vitamins/ carotenoids** **per 1000 ml**	**Resulting calcium lignosulfonate content** **per 1000 ml**
Drink	β-Carotene	5 mg	**25 mg**
	E	2 mg	**2 mg**
High enriched drink	β-Carotene	10 mg	~ **50 mg**
Ice cream	β-Carotene	~10 mg	~ **50 mg**
Dairy products	β -Carotene	Up to 30 mg	**Up to 150 mg**
Jelly babies	β -Carotene	10 mg	**50 mg**
Hard candies	β-Carotene	10 mg	**50 mg**

Additionally, the sponsor provided a typical composition of a carotenoid/vitamin product:
Carotenoid/Vitamin, 5-20 %; DL-α-Tocopherol, 1-3 %; Corn oil, 2.5-10 %; Calcium-lignosulfonate (40-65), 30- 60 %; Glucose syrup, 5- 40 %; and Corn starch, 10- 20 %.

7. Reactions and fate in foods

As there is no analytical method available that permits the quantification of calcium lignosulfonate (40-65) at the low levels that are expected to be present in food resulting from its use with nutrient and food-colour preparations, no studies are availble on its reaction and fate in food. Data available from studies submitted by the sponsor on the stability of the additive itself and from the stability of the additive in carotenoid preparations provide information that allows extrapolation to the possible reactions and fate in food.

7.1 Stability of calcium lignosulfonate (40-65)

The sponsor provided stability data (pH of a 10% solution, molecular weight, % reducing sugars) for one lot of powdered calcium lignosulfonate (40-65) stored for 36 months in polyethylene containers and for one lot stored in closed aluminium-foil bags for 24 months. See Appendix 1, below. The results show that the powdered product was stable over the time of the tests. Weight-average molecular weight showed a variation of ± 6 %, within the expected variation of the method of analysis, and reducing sugar levels remained stable and were within the limit given in the specifications (<5.0 %).

7.2 Stability of calcium lignosulfonate (40-65) in carotenoid preparations

The sponsor tested three batches of a calcium lignosulfonate (40-65)-containing formulation with ß-carotene and a blank formulation, all prepared from a single lot of the additive, for stability over 48 weeks. Powdered samples were stored in closed aluminium-foil bags under normal (25°/60% relative humidity) and accelerated (40°/75% relative humidity) conditions. The formulations and blank also contained corn oil, corn starch, and glucose syrup. The content of calcium lignosulfonate (40-65) was determined by nitrosating the phenolic groups of the polymeric fraction of the additive and spectrophotometrically observing the absorbance at 440 nm of the nitrosated products; ß-carotene was determined by HPLC. See Appendix 2, below. Stability was satisfactory for the length of the tests.

7.3 Stability of calcium lignosulfonate (40-65) in beverages

The sponsor also tested a ß-carotene/calcium lignosulfonate (40-65)-containing product form for stability in a non-pasteurised, non-carbonated soft drink. The calcium lignosulfonate (40-65)-based product form showed good physical stability over 3 months and performed equally to that of a product form based on fish gelatin.

7.4 Possible effects on nutrition

The sponsor has noted that calcium lignosulfonate (40-65) is water-soluble and concluded that no interaction with fat-soluble compounds in the diet is expected, especially because levels of the additive in foods as consumed are low. The sponsor also provided a report on intake of the additive. A conservative estimate from its use as a carrier for carotenoids indicates that consumption is unlikely to exceed about 100 mg/day (above the 90^{th} percentile). The report also states that the intakes resulting from vitamin E use in multi-vitamin products could result in lignosulfonate intakes up to 300 mg/day. Adding vitamins A, D and K at highest supplement levels would result in a maximum total intake of lignosulfonate of less than 400 mg/day, when the proportion of vitamin preparations ingested in powdered form is taken into account.

The sponsor also speculates that, as a non-digestible dietary fiber, calcium lignosulfonate (40-65), when consumed at high levels, might produce the same side effects (e.g., laxation) as other dietary fibers, such as lignin, that are consumed at much higher levels in normal human diets. It appears, however, that the intended applications of calcium lignosulfonate (40-65) will not likely result in a significant contribution to the over-all intake of non-digestible dietary fiber.

APPENDIX 1: Stability of powdered calcium lignosulfonate

Packaging: Polyethylene sample containers

Storage (months):	**0**	**3**	**6**	**9**	**12**	**18**	**24**	**32**	**36**
pH 10 % solution:	2.8	2.8	2.8	2.9	2.9	2.9	2.8	2.9	2.9
Mol. Wt. (wt. avg.):	51100	48400	51200	51700	51700	54100	52600	53300	54000
Reducing sugars, %:	4.7	4.7	4.6	4.8	4.8	3.7	4.6	4.5	4.5

Packaging: Aluminium-foil bags

Storage (months):	**0 Liquid***	**14 Powder**	**18 Powder**	**24 Powder**
pH 10 % solution:	3.5	3.5	3.4	3.5
Mol. Wt. (wt. avg.):	40000	42500	42600	39900
Reducing sugars, %:	3.5	4.1	3.8	2.9

* Initial measurements were carried out on liquid material before spray-drying. Stability over time carried out on powder.

APPENDIX 2: Stability of powdered calcium lignosulfonate (40-65) containing ß-Carotene (ca. 10 %)
(All samples were stored in closed aluminium-foil bags. ß-Carotene was determined using HPLC.)

	Storage Conditions (Trial no. 1)				**Storage Conditions** (Trial no. 2)			
	25°/60% relative humidity		**40°/75% relative humidity**		**25°/60% relative humidity**		**40°/75% relative humidity**	
Storage Time	ß-Carotene content	Lignos. content	ß-Carotene content	Lignos. content	ß-Carotene content	Lignos. content	ß-Carotene content	Lignos. content
0 weeks Start	10.7%	38.2%	10.7%	38.2%	11.2%	39.8%	11.2%	39.8%
4 weeks	10.9%	39.3%	10.8%	38.9%	11.3%	40.1%	11.2%	41.2%
12 weeks	11.1%	38.6%	11.1%	38.7%	11.6%	38.2%	11.6%	40.0%
24 weeks	10.5%	36.8%	10.8%	37.0%	11.3%	37.3%	11.3%	38.1%
48 weeks	11.0%	38.6%	10.9%	38.1%	11.6%	38.5%	11.4%	38.1%

	Storage Conditions (Trial no. 3)				**Storage Conditions** (Blank)	
	25°/60% relative humidity		**40°/75% relative humidity**		**25°/60% relative humidity**	**40°/75% relative humidity**
Storage Time	ß-Carotene content	Lignos. content	ß-Carotene content	Lignos. content	Lignos.content	Lignos.content
0 weeks Start	10.8%	39.1%	10.8%	39.1%	39.5%	39.5%
4 weeks	10.9%	40.2%	11.1%	40.1%	40.2%	38.3%
12 weeks	11.1%	39.7%	11.0%	40.9%	39.9%	38.3%
24 weeks	10.7%	39.7%	10.5%	38.1%	37.5%	37.0%
48 weeks	10.9%	41.4%	10.8%	40.6%	39.5%	38.3%