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STRUCTURES AND PROPERTIES OF STABILIZED VITAMIN AND CAROTENOID DRY POWDERS

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Abstract

The development of special formulated forms of vitamin and carotenoid dry powders solved most of the technical problems encountered in handling the original pure crystalline or oily compounds. Various procedures to obtain stabilized products have been introduced on an industrial scale for feed, food and pharmaceutical purposes. Proven modes of manufacture of formulated dry powders includes the numerous spraying processes for emulsions, dispersions or solutions, and the production of adsorbates of fat-based products.

The objective of this paper is to provide an overview of stabilized vitamin and carotenoid dry powders. This contribution is intended to demonstrate the usefulness of microscopy in characterizing pure and formulated vitamins and carotenoids. Scanning electron microscopy is considered to be a reliable and fast method for analyzing various parameters of these compounds, such as, morphology, particle size distribution, and formation system of emulsion droplets.

Introduction

Vitamins are essential for life in both men and animals. They are considered to fulfill catalytic functions. Without vitamins vital processes do not work properly. Vitamins form a mixed group of chemical compounds and are important constituents of food. By virtue of their organic nature vitamins can be differentiated from the trace elements. Vitamins are distinguished from other organic compounds in the diet being likewise fundamental for health, as, for example, the essential fatty acids. This distinction is largely based on the low quantities of the material which must be present in an adequate diet. The vitamins may be classified as water-soluble or fat-soluble.

The biological and biochemical significance of the 13 vitamins is basically known [9]. The addition of vitamins and carotenoids to food and feed is also generally accepted [2].

Before production of vitamins and carotenoids in an economic way by chemical synthesis they were extracted from natural sources. Vitamins A and D for example were obtained from fish liver, vitamin E from wheat germ and vitamin K from alfalfa meal. The carotenoids were principally gained from carrots, grass meal, from marigold petals or yellow corn products. Today, the worldwide industrial production of many forms of all vitamins and of many carotenoids takes place in various industrial companies on a large scale.

The development of special formulations of fat and water soluble vitamins and of carotenoids eliminated the solubility and stability problems encountered in handling the crystalline forms. The dry forms of vitamins and of carotenoids are manufactured by a special process which provides free-flowing powders. The dependable stability has been demonstrated in extensive laboratory tests, as well as by many years of practical experience. The particular physical properties of these powders allow ready mixing with other materials and uniform distribution in tablet granulation, capsules mixtures and other dry pharmaceutical formulations. Also, the production of high-quality, economical feed mixtures were only made possible through carefully formulated vitamin supplements.
Table 1. Possible forms of stabilized fat-soluble vitamin dry powders.

<table>
<thead>
<tr>
<th>Group of Dry Powders</th>
<th>Form of Dry Powders</th>
<th>Production Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>adsorbates [7] and mixtures</td>
<td></td>
<td>mechanical mixing</td>
</tr>
<tr>
<td></td>
<td>pulverized particles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>granulates</td>
<td>air- and spray-mixing, granulating, crushing</td>
</tr>
<tr>
<td>fat based dry powders [5, 11]</td>
<td>microspheres</td>
<td>spray-congealing</td>
</tr>
<tr>
<td></td>
<td>flakes</td>
<td>drum-cooling</td>
</tr>
<tr>
<td></td>
<td>powdered round particles</td>
<td>spraying/dropping into liquids, comminuting dispersion in aqueous liquids, mixing</td>
</tr>
<tr>
<td>dried emulsion and suspension</td>
<td>spherical beadlets</td>
<td>spraying into hot gas, spraying into liquids, spraying into powders, double dispersion process (double emulsion), air suspension coating,</td>
</tr>
<tr>
<td></td>
<td>flakes</td>
<td>spraying onto a moving or a stationary surface, comminuting sheets of dried emulsion</td>
</tr>
<tr>
<td>microcapsules [6]</td>
<td>spherical or spheroidal beadlets, irregular or spherical agglomerates</td>
<td>coacervation, air suspension coating, multi-orifice, vacuum encapsulation</td>
</tr>
<tr>
<td>inclusion compounds and various</td>
<td>crystals</td>
<td>crystallization</td>
</tr>
<tr>
<td>products [7, 14]</td>
<td>pulverized particles</td>
<td>precipitation and coagulation</td>
</tr>
<tr>
<td></td>
<td>granulates</td>
<td>chemical reaction</td>
</tr>
</tbody>
</table>

The technical procedures and aspects for the manufacture of dry powders were reviewed some time ago [7]. Manufacturers of such equipment have presented spraying [16] and drying apparatus [10, 11]. Problems with galenic formulations of vitamins have been recognized [15]. Recently, an application of the scanning electron microscopy (SEM) and the energy-dispersive spectrometry (EDX) in the pharmaceutical industry has been described [17].

The present work concentrates on the appearance and microstructures of marketable and formulated vitamin and carotenoid dry powders. It gives a pragmatic description of the application SEM and EDX in formulation work. Examples include the characterization of pure vitamin forms and of raw materials.

**Materials and Methods**

The vitamin and carotenoid products which were either of crystalline nature or fine powders were mounted on SEM sample holders either with double sided adhesive tape or with a conductive cement. Alternatively, very fine compounds were attached to the sample holder with an electrically conducting polymer (TEMPPFIX, Polaron Equipment Ltd.). The mounted samples were made electrically conducting by applying a layer of gold, or gold/palladium (25 nm). Since most products under investigations had a rough surface the coating technique by diode sputtering gave better results than the high vacuum evaporation coating technique.

X-ray microanalysis was performed using a Tracor Analytical 5502 spectrometer with a ultrathin window detector fitted to the specimen chamber of the JEOL JSM 840 SEM. Specimen for EDX-analysis were always mounted on carbon stubs using a conductive carbon cement. EDX-analysis has been performed with samples which had been covered with a thin (10 nm) layer of carbon (high vacuum coating technique). The elemental maps were printed by means of a color video copy processor (Mitsubishi CP 200B).

When observing dry powders in the SEM and the EDX the use of rather low acceleration voltages (10 to 15 kV) is advisable, i.e., an acceleration voltage giving good X-ray yield without loss of spatial resolution caused by excessive penetration of the electron beam into the specimen [3]. However, Figure 11 which has been taken with an older microscope an acceleration voltage of 20 kV, has been used because it shows good resolution and contrast. In order to detect all possible elements in a specimen by EDX a first run with high acceleration voltages (> 25 kV) was usually performed.

Occasionally (though not shown here), uncoated dry powders were investigated in a LaB₆ cathode equipped SEM. However, since very low acceleration
voltage (< 0.8 kV) had to be employed, the usable magnification, in such instances, was below 3,000.

Results and Discussion

Pure Vitamin Forms

Water-soluble vitamin forms: Pure water-soluble vitamins are crystalline (Figure 1). Depending on the chemical and physical characteristics, their morphology differs markedly. Water-soluble vitamins may form needles, as e.g., thiamine, riboflavin, biotin, pyridoxine hydrochloride, nicotinamide. Ascorbic acid forms cube-like crystals, whereas folic acid is composed of sheet-like components. Pyridoxine hydrochloride crystals may also form aggregated plates. Pure cyanocobalamin crystals are very small crystalline components and, finally, the salts of pantothenic acid form either rectangular or rhombic plates (sodium-D-pantothenate), or very fine long needles (calcium-D-pantothenate).

Fat-soluble vitamin forms: Two of the four oil-soluble vitamins are crystalline (vitamin A and D), whereas the other two are viscous oils at room temperature (vitamin E and K). As an example, the pure finely crystallized vitamin D₃-form is shown in Figure 2. The shape and size of vitamin D₃ crystals differ markedly from most forms of water soluble vitamins. Pure beta-carotene crystals consist of agglomerated crystalline platelets which typically show rounded edges and corners (Figure 3). The oil-soluble vitamins are highly sensitive to oxidation and are stable only under special conditions, e.g., within a limited pH range, or under inert gas. In order to improve miscibility, dispersion in aqueous media, and particularly stability, the fat-soluble vitamins are transformed into dry powders. Therefore, in most instances, the modern marketed forms of fat-soluble vitamins are formulated products that are produced by special processes which provide a stable and free-flowing dry powder. Such forms may be easily mixed with food or feed constituents.

Development of Stabilized Dry Forms of Fat-soluble Vitamins

As a general assumption, the separation of active compounds from all the factors which are responsible for a loss in activity should lead to an optimally stabilized product. The formulation has three main objectives:

1. Isolation of the vitamins from all factors having a detrimental effect, realized by covering with a solid matrix;
2. Aiming at most favorable conditions for bioavailability, achieved by fine distribution of active vitamin in the matrix,
3. Homogeneous distribution in food/feed, or pharmaceuticals is possible since the produced vitamin dry powders contain a very large number of particles in which the vitamin product is evenly distributed.

For fat-soluble vitamins and carotenoids, perfect separation from all detrimental effects, such as oxygen, humidity, ultraviolet rays, light, metals, acidity, is of paramount importance. The coating materials should be fully digestible, should not be toxic, nor interact with the embedded vitamin. They should be easily available, inexpensive, as impermeable to oxygen as possible and should not hamper the intestinal uptake of the enclosed or admixed vitamin.

Gelatin is an optimal coating material and most widely used in formulating fat-soluble vitamins and carotenoids [7]. It is a superb gel-forming agent, has excellent emulsifying properties, and is a good hydrophilic protective colloid since the films produced with gelatin are particularly impervious to oxygen. In combination with antioxidants, gelatin has a stabilizing effect on the fat-soluble vitamin. Examination of patent literature reveals a great number of special matrix compositions that have been claimed to be useful or to be particularly adaptable to special requirements, such as starches, lactose, gum arabic, gum acacia, dextrin, hydrolyzed collagen and others.

Table I (based mainly on a survey of patent literature [7]), summarizes the common forms of stabilizing fat-soluble vitamin dry powders and their production processes. Several of these forms of stabilization have also been applied to carotenoids.

Adsorbates

Adsorption on powders is a very simple process for the transformation of oils into dry powders due to an enormously increased surface. However, this technique is usually not suitable for increasing the stability of a product and is mostly restricted to the rather stable vitamin E-acetate. The adsorbate form does not sufficiently protect vitamin A especially when mineral salt mixtures, i.e., compositions often present in feed mixtures, are added. The protection of adsorbate forms is also insufficient when the product has to be stored under humid conditions [7] and is exposed to air.

It is possible to produce powders which contain 50% of its weight as pure vitamin E (Figure 4). This vitamin E adsorbate consists of 10 to 100 μm wide, free-flowing aggregates.

Fat-based Dry Powder

Fat-based powders are principally superior in stability when compared with adsorbates, although the fat part limits the heat resistance of such products. Furthermore, the fat coating may be affected by oxygen. Therefore, the addition of emulsifying agents has been suggested [7]. Fat-based dry powders are mostly prepared by a spray-congealing, or also called spray-chill process. Spray-congealed products show smooth, round and mostly uniform particles. The particle size varies depending on the spray conditions and is usually 50 to several 100 μm (Figure 5). It is obvious that the behavior of the resulting formulated products depends mainly on the properties of the particular matrix chosen. Significant contributions to the characteristics of the congealed products, as, e.g., bulk density, grittiness, size distribution and coverage of the active component in the matrix, come from their particle size which is mainly
Structure of formulated vitamin dry powders

**Figure 4.** Vitamin E adsorbate, consisting of 50% oily vitamin E which has been absorbed on highly dispersed, porous silicic acid. The final product is a free flowing dry powder; note the wide range of size. **Figure 5.** Formulated Vitamin E dry powder, manufactured according to the spray-chill process. Survey showing particle size distribution. **Figure 6.** Spray-dried vitamin A dry powder. a. Size distribution pattern with dimension of particles. b. Occurrence of grooves, caused by drying parameter. c. Occurrence of holes as a consequence of evaporated liquid phase. **Figure 7.** Various surface structures of formulated vitamin A dry powders produced according to powder catch processes. Each product contains 500,000 IU/g of vitamin A, but the four products (a - d) have been produced by different manufacturers (scale bars represent 75 μm).

**Figure 1.** Forms of pure crystalline water-soluble vitamins, ranging from fine crystalline needles, aggregated, plate-formed and sheet-like constituents, to rectangular crystals. a. Thiamine (Vitamin B₁). b. Riboflavin (Vitamin B₂). c. Pyridoxine (Vitamin B₆). d. Cyanocobalamin (Vitamin B₁₂). e. Nicotinamide. f. Folic acid. g. Ascorbic acid (Vitamin C). h. Ca-Pantothenate. j. d-Biotin (Vitamin H). **Figure 2.** Pure Vitamin D₃ crystals, showing very fine needles. **Figure 3.** Pure beta-Carotene crystals, showing round edges.
given by the atomization condition \([11]\).

**Powders Based on Dried Emulsions and Suspensions**

A great part of marketable stabilized fat-soluble vitamin and carotenoid bulk products consists of powders based on dried emulsions. The principle of such a production includes the following main steps \([7]\):

1. Dispersing the vitamin in an aqueous solution of an organic, film-forming colloid (see above; besides gelatin a number of special matrix compositions have been claimed to be useful or to be particularly adapted to special requirements).

2. Disintegration of the emulsion and formation of small particles (formation of droplets to be achieved by spraying, by dispersing the emulsion in a non-miscible liquid, by air suspension technique, or by comminuting small fractions of the dried emulsions).

3. Keeping the particles separated from each other until they can be dried without formation of sticky agglomerates (use of a suitable pulvulent).

4. Drying (e.g., by spraying the droplets in hot gas, into moving warm air stream, or, also by spraying the droplets into a dehydrating atmosphere).

In a first step vitamin containing finely dispersed fat or oil droplets, preferably below \(1 \mu m\) in size, are produced by means of homogenizing devices, such as colloid mills. In the aqueous phase one or several matrix constituents and antioxidants are present. The choice of the particular parameters of the spraying device (second step), such as speed of the (rotating) unit, diameter of the wall openings of the atomizing nozzle, and the properties of the emulsion, e.g., surface tension, primarily affects the size and the morphology of the produced particles \([4]\). In other words, the morphology, particle size distribution and ultrastructure of a particular dry powder product can be associated with a specific spraying method. Furthermore, each manufacturer’s spraying method very often leaves typical traces and foot-prints on a resulting particular dry powder. After the consecutive collecting (third step) and drying process (fourth step) a free flowing dry powder originates in which the vitamin is homogeneously distributed.

Electron micrographs in Figures 6-9 demonstrate the unequivocal divergences in morphology and ultrastructure of the various vitamin dry powders.

The contact of the spray with air is a characteristic feature of spray drying. The optimum evaporation conditions are determined by the choice and handling of the atomizer and the air dispenser \([8]\). The powder recovery is usually collected in cyclones. Spray-drying procedure can be operated both for small quantities (few grams per hour) and for large amounts of dry powder (several hundred kg per day).

Spray-drying of emulsions and dispersions produces typically round particles which mostly show a smooth surface. Occasionally, grooves are found on the particle’s surface. Gaps are formed when the internal vapor pressure overcomes the particle’s wall strength (Figure 6). The atomizing conditions and the initial concentrations directly influence the powder bulk properties. Most importantly size, density and flowability are affected by the process conditions. Spray-dried vitamin beadlets include particles in the size range of a few to 100 \(\mu m\).

In a so-called powder catch procedure a continuous large scale production of bulk products takes place by spraying the finely divided particles of the emulsion into a pulvulent product, such as corn starch. This brings about a protection of the particles which, immediately after spraying, are still wet and sticky. Furthermore, the applied layer of starch dehydrates the product by absorbing the moisture. The starch granules stick, more or less uniformly, to the surface of the particles and give them a characteristic grainy aspect (Figure 7).

Drum drying is a very simple drying method (pouring or spraying the emulsion onto a roller). It produces, after grinding, a fine dry powder which consists of rather flat components (Figure 8) which are obviously markedly different to the round forms of dry powders presented in Figures 6 and 7. The bubble-like dark structures in the inside of a beadlet (Figure 9) are vitamin droplets surrounded by the protective colloid. The size of these droplets is a very important parameter for the biological uptake by governing the completeness of the uptake \([7]\).

A drum-dried vitamin powder would be always less stable than a product produced by spray-drying, by double dispersion or by a so-called catch procedure, since the dried emulsion does not seem to be entirely protected by the colloidal matrix (arrow, Figure 8).

**Application of Vitamins and Carotenoids**

The application of all fat soluble and water soluble vitamins in food and feed is always connected with the particular properties of these ingredients, such as the high sensitivity towards oxidation, pH, moisture and light. The use of vitamin A and E in their pure forms is limited to standardized oily solutions for enriching of various types of fats, as, e.g., margarine and some other fat based foods \([2]\). The oily products are not appropriate for direct applications to water based nutrients. Therefore, they have to be transformed into dry powders in order to improve also their miscibility and dispersion in aqueous media. The application of vitamin A and of vitamin D_3_ (cholecalciferol) as stabilized powders eliminated the risk of isomerization and of degradation which is high when the active compound is not protected against oxygen and acid agents.

Solubility and stability problems encountered in handling crystalline carotenoids have been overcome in the same way by the use of special formulated forms. In a first approach an oily suspension of micronized crystals was produced, and later, emulsion or beadlet forms containing the carotenoid in supersaturated solution of the solvent or in colloid form were developed. Recently, the treatment of micronized beta-carotene by a very short heat application to 180\(^\circ\)C was proposed \([5]\). The range of the amount of a vitamin recommended \([13]\) runs from a few micrograms for cyanocobalamin, to about 100 mg in the case of ascorbic acid. Vitamin supple-
mentation levels adopted by the food industry are mainly based on such requirements. The addition of vitamins to food products is generally described as vitaminization. According to the type of food these supplements have different aims which can be defined as follows:

-- Revitaminization is the compensation for losses in processing, i.e., the restoration of the naturally present vitamin content. With the help of SEM and EDX the flour particles and the admixed B-complex vitamins, iron and calcium phosphate are easily differentiated (Figure 10);

-- Standardization is the compensation of natural variation, e.g., in fruit juices, when the customer wants to be sure that a certain amount of vitamin C is in the drink regardless of the origin of the fruits and without regard to the season;

-- Enrichment is the addition over and above the initial natural level. The principle of vitamin enrichment is demonstrated by the example of margarine;

-- Vitaminization is the addition of vitamins to foods which represent ideal vehicles for a particular vitamin, but which do not necessarily contain that vitamin. Hypovitaminosis A may be largely responsible for the occurrence of eye lesions and even blindness that has been documented in certain poor regions of the world [12]. The vitaminization of sugar with vitamin A practiced in Central America is an outstanding example of a successfully realized nutrification concept [1]. The principle of adhering vitamin particles to a sugar crystal by means of a natural oily compound can be studied by SEM (Figure 11).

Conclusions

This article describes the use of SEM and of EDX in combination with SEM in millimeter, micrometer and submicrometer particle sizing. SEM and EDX applied on crystalline and formulated vitamin forms provide valuable information on the marketable vitamin and carotenoid products. The techniques described in this study permit microstructural knowledge of vitamin dry powders. For example, the information of shape and particle size distribution pattern may enable a prediction for the term of solubility. The morphological and ultrastructural description of all types of crystals is especially important when aggregated forms are present. The microstructures of formulated products, as seen with SEM, may also enable a statement for the quality of a product. The use of EM appears necessary when fine emulsion droplets of fat-soluble vitamin dry powders (sizing range below 0.2 μm) have to be visualized. During formulation work the application of SEM enables, at least in special cases, a prediction of product stability. The EDX seems to be a very useful method for describing the homogeneity of dry powder mixtures; sulfur-containing biotin (vitamin H) and thiamine (vitamin B₁) are readily traceable by EDX even in multicomponent mixtures.

References


Figure 8. Drum-dried formulated vitamin A dry powder. The inner phase is not entirely protected by the colloidal matrix (arrows).

Figure 9. Inner phase (longitudinal section) of formulated vitamin A dry powder. The round dark openings with a dimension of 1 to 2 μm are the emuligated vitamin A droplets surrounded by the protective colloid.

The products have been prepared by the double dispersion process (a), or the powder catch procedure (b), note the presence of corn starch on the outside of this vitamin beadlet.

Figure 10 (color plate on facing page). Mixture of vitamins of the B-complex, containing for the flour enrichment also iron, calcium phosphate and corn starch (scale bars represent 10 μm). a. SEM micrograph (SE detector); image resolution 512 x 512 pixels. b-e. EDX elemental distribution map (same area); image resolution 256 x 256 pixels. b. Iron. c. Sulfur, indicating presence of thiamine (Vitamin B₁). d. Calcium. e. Phosphorus.

Figure 11. Vitaminization of sugar. Concept applied in the Central-American region. The vitamin A beadlets adhere to the sugar crystals due to the use of a natural oil, as e.g., compounds containing arachic acid.
Structure of formulated vitamin dry powders

Discussion with Reviewers

J.G. Szekely: You demonstrate the usefulness of SEM and EDX for showing the size and morphology of dry vitamin powders. At the present time, what methods are being used to study these powders?

Author: Formulation work needs equipment for measuring the various physico-chemical parameters. Other needed equipment include: viscometers (viscosity), particle size measuring devices (e.g. photon correlation spectroscopy; Fraunhofer scattering device), stirring equipment, pH-measuring units. According to the type of vitamin the activity of the vitamin is measured by high pressure liquid chromatography (HPLC), gas chromatography (GC), or UV-spectrometry.

M.S. Kerley: Can SEM analysis of dried vitamin powder products be used to determine their availability in the digestive tract?

Author: Formulating of vitamin products has two main goals: Firstly, the vitamin product has to be readily available in the digestive tract, and, secondly, the formulated product should exert great stability and withstand effects which would otherwise deteriorate the unprotected vitamin. In the case of vitamin A, a most favorable intestinal uptake was observed with emulsion droplets which were smaller than 0.2 μm. This order of magnitude can be easily detected with SEM.

S-G.G. Cheng: Does the information from the micrograph actually co-relate to the information from the stability study?

Author: In some cases, e.g., as stated in the chapter of drum-dried products, a prediction of the stability of a vitamin product is possible. Otherwise, much experience is needed in order to correlate information from the micrograph with information from stability studies. However, SEM is often used during the formulation work for investigating the size of emulsion droplets, for estimating coating processes, or, for determining the surface structure of formulate products.

D.F. Lewis: To what extent does the crystal form of the vitamins depend on the conditions under which they are formed?

Author: One must differentiate between crystallization and spraying processes. During crystallization crystals are formed in a process which is dependent upon the type and amount of the solvent, the saturation conditions of the crystallizing compound, the temperature of the solution and the temperature gradient of the solvent. In spray-drying either a solution or a dispersion may be sprayed into hot air. In the case of fat-soluble vitamins there are no crystals but round particles which contain solidified emulsion droplets generating during spray-drying (see Figure 9). Spray-drying of a dispersion which contains a vitamin compound dispersed in a matrix produces small round beadlets in which the crystalline vitamin is coated by the matrix; these crystals would have essentially the same shape as the crystals which were produced by a crystallization process.