Bioactively filled gelatin gels; challenges and opportunities

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Abstract

Soft, chewable gelatin matrices represents an excellent alternative to traditional oral administration forms (tablets, soft and hard capsules) for pharma- and nutraceuticals; especially for the pediatric and geriatric segments as well as for those suffering from dysphagia. As of today, chewable delivery units, most commonly produced using gelatin, are a very popular formulation design for vitamin and dietary supplements. Bioactive components can be present in such formulations as lipids in O/W emulsions, as dispersed particulate matter or dissolved in the aqueous phase or in the lipid phase in the case of O/W emulsions. Challenges do however exist: many of the bioactive ingredients give a distinct taste, are vulnerable to degradation or may influence the gelling properties of gelatin. This is highlighted by many of the current chewable multivitamins only containing a small fraction of the whole array of vitamins/minerals included in traditional tablets and soft gels. Pharmaceuticals have many of the same issues, coupled with stricter regulatory demands in regards to quality, stability and bioavailability compared to nutraceuticals, making formulation of pharmaceutical chewables potentially even more challenging. However, many of these challenges may be solved through innovative formulation design. By adjusting pH or using buffer systems, by adding taste masking or stability enhancing excipients or by using encapsulation techniques, even the more challenging active ingredients may potentially be incorporated into gelatin-based chewables.

Keywords: chewable tablets, gelatin, nutraceuticals, pharmaceuticals, filled gels, emulsions

1. Introduction

Oral delivery of active ingredients is generally considered to be the safest and most convenient route of administration.(York, 2007) Different types of oral administration forms exist, including tablets, soft and hard gel capsules, elixirs, suspensions, chewables, gummies among other.(York, 2007) For pharmaceutical products, traditional administration forms such as tablets are most common, while nutraceuticals come in a wider range of delivery forms. This can to a large degree be attributed to very strict regulatory demands for pharmaceutical delivery forms (stability, bioavailability, clinical studies), while the regulatory demands for nutraceuticals are more lenient. However, nutraceutical regulations are gradually getting more comprehensive, strict and standardized.(Witt & Kotwal, 2011) A common delivery form, especially for nutraceuticals is chewable delivery units, often made with gelatin. Soft chewable delivery units are easier to administrate/swallow compared to tablets and capsules, making it a very good administration form in the pediatric and geriatric segments as well as for those suffering from dysphagia. In one questionnaire 26 % reported having problems with swallowing conventional tablets, potentially leading to significant issues with patient compliance and showing a demand for dosage forms that are easier to ingest.(Andersen, Zweidorff, Hjelde, & Rodland, 1995) In addition, taste profile (sweetness, sourness and aroma) can be adjusted according to customer preferences, which may be important attributes to improve user acceptance. These advantages have made soft chewables a very popular administration form and it is now a billion dollar industry, with a broad portfolio of ingredients.

However, there are several challenges linked to chewable delivery units. A wide range of nutraceuticals and pharmaceuticals give a profound off-taste when released in the mouth. This is not a problem for traditional tablets and capsules that are swallowed whole, but for chewable delivery units it can be difficult to avoid some release of active ingredients during chewing. Solutions to this problem can be using strong sweeteners and flavor compounds to mask off-taste, or using techniques such as buffer systems, complexation, emulsification or encapsulation to keep the active ingredients isolated and/or undissolved through the chewing process. Another potential limitation is stability. As many chewable supplements, such as those based on gelatin are water based, active ingredients may be partially or fully solubilized in the formulation, leading to potentially reduced stability or undesired ingredient interactions. These issues can lead to deactivation of active ingredients, changes in taste or colour, or formation of degradation products with negative health effects. The stability of the gelling agent itself during manufacture and storage might also be a problem, possibly leading to sub-optimal gel texture or even total loss of gelling capacity of the chewable dose.

A large range of gelling agents can be used for the preparation of soft chewable delivery units, although gelatin is the most popular. This can be attributed to gelatin's good availability and ease of use, but also the sol-gel transition temperature of (mammalian) gelatin gels close to physiological conditions giving a very unique melt in the mouth texture.(Haug & Draget, 2009;

80   Schrieber & Gareis, 2007) As gelatin-based candies are already a popular and well liked type of
81   confectionary, most people are familiar with the texture, mouth-feel and appearance of gelatin
82   gels, perhaps especially an advantage with regards to the pediatric segment of the population.
83   Besides the nice palatability, gelatin also provides very fast and consistent dissolution kinetics of
84   the delivery unit in the gastrointestinal tract.(Hattrem, Molnes, & Draget, 2014b) This is usually
85   considered beneficial, as a fast dissolution/disintegration of the delivery unit may promote rapid
86   dissolution and/or absorption of the active ingredient in the gastrointestinal tract.
87
88   Despite all the challenges, chewable delivery units based on gelatin can offer a great alternative
89   to traditional tablets, perhaps especially for the benefit of the pediatric and geriatric populations
90   as well as people suffering from dysphagia. To allow for incorporation of a wider range of active
91   ingredients and ingredient combinations into these chewable gelatin-based delivery units, smart
92   formulation design has to be applied. In this review, different ways to incorporate active
93   ingredients into chewable formulations are highlighted. This is followed by an examination of
94   gelatin and its properties in regards to forming chewable delivery units. Then some issues with
95   certain problematic active ingredients and chewables in general are described, with some
96   potential solutions listed. Finally a case-study is presented where stability and taste-masking are
97   optimized for an API-containing chewable by using a buffer system to keep the active ingredient
98   in an undissolved particulate form.
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103   2. Incorporation of bioactive compounds into a gel matrix

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105   2.1 Types of chewable tablets and gelling agents

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107   The main focus of this review is water-based chewables made with gelatin. However, chewable
108   tablets containing bioactive compounds can also be made using other gelling agents, or even
109   without water. Through wet granulation or dry blending, followed by compression, chewable
110   tablets looking similar to traditional tablets can be formed. By adjusting tablet forming pressure,
111   and using fillers like sorbitol or mannitol, a tablet can be made that can be chewed and
112   disintegrates in the mouth.(Alderborn, 2007) By including disintegrating agents that absorb
113   saliva and swell, and/or effervescent agents, you can also make oro-dispersible tablets that
114   disintegrate so quickly that chewing is nearly or even completely unnecessary.(Hahm &
115   Augsburger, 2008) These chewable tablets are fairly easy/cheap to make using common
116   granulation and pressing techniques, and with a low/non-existent water content active ingredients
117   will stay in an undissolved form potentially minimising or avoiding several stability issues.
118   However, they can be sensitive to moisture during storage, and can also give a drying/powdery
119   feeling in the mouth upon chewing/disintegrating, perhaps especially a problem for those with
120   reduced saliva production such as a large fraction of the geriatric population.(Hahm, et al., 2008)

121

122   Gel-based chewable tablets generally offer a texture and mouth-feel more similar to traditional
123   jelly or gummy candy. Gelatin, which is further discussed in chapter 3 is the most common
124   gelling agent for such products, due to good availability, ease of use and a pleasant mouthfeel,
125   but other alternatives exist. Some examples are described below.

126

127   Pectin is a structural polysaccharide found in the cell walls of terrestrial plants. Commercial
128   pectin is most commonly produced from citrus peel and apple pomace, byproducts from juice
129   production. Generally pectin forms brittle gels with a short texture, and forms stronger gels in the
130   presence of acids, calcium and/or sugars depending on pectin structure.(Endress & Christensen,
131   2009) Some pectins (e.g. highly esterified pectin) may also function as emulsifier in the case of
132   gelled emulsions.(Ngouémazong, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015)

133

134   Starch is a mixture of the polysaccharides amylose and amylopectin, and is used by plants as an
135   energy reserve. Starches can be chemically or structurally modified, giving them the ability to
136   form gels and/or stabilize emulsions.(Taggart & Mitchell, 2009)

137

138   Carrageenans are linear polysaccharides extracted from red seaweed. The most common types of
139   carrageenans are lambda-, kappa- and iota-carrageenan, differing in the amount of sulfated
140   monomers. While kappa-carrageenan forms firm gels with potassium ions, iota-carrageenan
141   forms soft gels in combination with calcium ions. By using different combinations of

142 carrageenans, or combining with e.g. galactomannans, a wide range of gel textures can be
143 obtained.(Imeson, 2009)
144
145
146 2.2 Incorporation of active ingredients into water-based chewables
147
148 There are three main ways to include bioactive compunds in a water-based chewable gel matrix:
149
150 1. As solid aggregates/particles suspended throughout the gel matrix.
151 2. Dissolved in the water phase of the gel matrix.
152 3. Dissolved in or dispersed as lipid based emulsion droplets, which are then suspended
153 throughout the gel matrix.
154
155 The aggregate/particle based and emulsion droplet based strategies are shown schematically in
156 figure 1.
157
158
159 2.2.1 Solid particle suspensions
160
161 In a chewable aggregate/particle formulation, the active ingredient is suspended as solid particles
162 throughout the gelled matrix. As the gelled product is chewed and digested the gel matrix
163 dissolved/disintegrates, and the active ingredient particles are released. Using a gelling agent
164 such as gelatin, which quickly melts at body temperature, ensures rapid release of the active
165 ingredient.
166
167 Including the bioactive compound as aggregates/particles is obviously dependent on the
168 compound not dissolving in the water phase. Some compounds have an overall low water
169 solubility, which makes them easy to incorporate in this way. Other compounds have a water
170 solubility highly dependent on e.g. pH of the solution. The pH of a chewable formulation can be
171 adjusted, however certain gelling agents have suboptimal stability or reduced gelling power at
172 acidic or alkaline conditions. As an example, gelatin stability and gelling capacity decreases
173 rapidly outside the pH range of 4 - 10(Haug, et al., 2009). Many active ingredients have been
174 synthesized or extracted in alternative forms with molecular differences, such as different
175 salts/counter-ions, conjugated/esterified with fatty acids/methyl-groups, etc. These changes to the
176 molecular structure may significantly influence the solubility of the active ingredient. For
177 instance vitamin C is freely soluble in water in its sodium ascorbate form. Meanwhile calcium
178 ascorbate has a lower solubility and ascorbyl palmitate is barely water-soluble at all. A different
179 example is various mineral salts: while e.g. carbonate, oxide and phosphate salts often have low
180 water solubilites (dependent on pH), chloride salts are generally highly soluble. If aggregate
181 formulation is the preferred administration form (due to issues with stability, taste etc.), selecting
182 the optimal molecular form of the active ingredient is of crucial importance. It is, however,

183   important to note that molecular form may also affect rate and extent of gastrointestinal
184   absorption of the active ingredient, an important consideration, especially in the case of
185   pharmaceutical products.(Wells & Aulton, 2007)
186

187   For active ingredient forms with a high water solubility at all pH values, aggregate formulations
188   may still be possible. By using micro-encapsulation techniques solid particles may be formed
189   where the active ingredient is protected from the water phase, potentially ensuring improved
190   stability and efficient taste masking. This micro-encapsulation can also function as an enteric
191   coating, dissolving and releasing the active ingredient only upon reaching e.g. the small
192   intestine.(Augustin & Sanguansri, 2012) As the chewable unit is water based, the encapsulation
193   material has to be uniform, impermeable and stable in water solution over long storage periods
194   (months or even years). Even a tiny rift/imperfection in the surface of an encapsulated aggregate
195   can lead to gradual leakage of the water-soluble active ingredient, or migration inward of water
196   leading to swelling and the potential of the encapsulation layer bursting. The encapsulation
197   material also has to be completely stable during processing, for gelatin based delivery units this
198   entails heat and shear stability. Considering these limitations, the use of micro-encapsulated
199   ingredients in water-based chewables is challenging.
200

201   An important consideration for particle suspension formulations is to avoid excipients or
202   ingredients that may promote solubilisation of the active ingredient. As an example, some active
203   ingredients that are stable as aggregates in a pure water solution, may if there are lipid droplets
204   present dissolve in this lipid phase instead, potentially decreasing the stability and increasing the
205   off-taste of the compound. Depending on the active ingredient, even just traces of lipid
206   compounds (e.g. from flavorings) might be enough to get this effect and result in an unpalatable
207   product.(Unpublished data)
208
209

210   2.2.2 Dissolved
211

212   For ingredients with good stability and no off-taste issues in a solubilized state, it is easy and
213   convenient to include the active ingredient simply dissolved in the aqueous phase of the
214   chewable product. However, while the active ingredient might be stable in solution on its own, it
215   might still be unstable when combined with certain other active ingredients or excipients. These
216   issues are further examined in chapter 4.
217

218   In addition, having active ingredients dissolved may increase the ionic strength of the solution,
219   which will affect the gelling capacity of e.g. gelatin. As an example, Haug et al. (2004) tested 10
220   (w/w)% fish gelatin gels with varying concentrations of added NaCl. Up to about 0.1 M NaCl,
221   the gel formed had (after 2h at 4 °C) a higher gel modulus compared to a gel without salt added,
222   with a maximum modulus increase of approximately 50 % at 12 mM NaCl, possibly due to
223   screening of long range electrostatic interactions, allowing tighter association of gelatin chains.

However, at higher salt concentrations, the gel modulus decreased: at 0.25 M and 0.5 M NaCl the modulus after 2h at 4 °C was 75 % and 10 % of the modulus of the salt free gel, respectively, which might be due to screening of the important short range electrostatic interactions at higher salt concentrations. However, it is difficult to estimate theoretically exactly how the addition of an amount of a specific salt will affect a formulation, as other excipients present such as sugars, as well as the type of salt and type and amount of gelatin will affect the final gel texture in various ways.(Choi & Regenstein, 2000; Sarabia, Gómez-Guillén, & Montero, 2000)

To increase solubility of certain ingredients, and/or reduce off-taste of compounds with poor palatability in the dissolved state, complexation may be a solution. Cyclodextrins are cylindrical polysaccharides consisting of 6 - 8 glucose monomers in a ring, giving a hydrophilic exterior with a hydrophobic inner cavity. Many active ingredient molecules with hydrophobic residues have an affinity for this cavity and may form complexes with cyclodextrin.(Challa, Ahuja, Ali, & Khar, 2005; Szejtli & Szente, 2005) These complexes will often be more soluble, and possibly also more stable than the active ingredient alone, and through mastication the complex may keep the active ingredient from interacting with taste receptors, significantly reducing off-taste.(Hattrem, Kristiansen, Aachmann, Dille, & Draget, 2015a; Loftsson & Brewster, 1996)

2.2.3 Emulsion

Lipophilic active ingredients may be solubilized in a lipid carrier, which can further be dispersed throughout a aqueous phase (containing a gelling agent), giving a chewable gelled emulsion. Bioactive lipids, such as fish oil (high in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)), flaxseed oil (high in alpha-linolenic acid (ALA)), borage oil (high in gamma-linolenic acid (GLA)) among others, may be dispersed in the same way. In this case using gelatin or other surface active gelling agents for the chewable tablet is highly advantageous, as it will improve the emulsion stability. This is further explained in chapter 2.3.

The approach of solubilising the active ingredient in a lipid carrier is used in the pharmaceutical industry to improve the bioavailability of certain drugs.(Hauss, 2007; Pouton, 2000, 2006) Many drugs suffer from low aqueous solubility, which gives a slow drug dissolution in the gastrointestinal tract further giving a slow or incomplete absorption of the active pharmaceutical ingredient (API). By pre-dissolving the drug in a lipid carrier the rate limiting step of drug dissolution can be removed resulting in improved bioavailability of the administered API.(Hauss, 2007) Similarly several lipid-soluble nutraceutical compounds may also achieve improved bioavailability when dissolved in a lipid carrier or when taken at the same time as fat rich foods, some examples include fat-soluble vitamins such as vitamin E (Leonard, Good, Gugger, & Traber, 2004) and vitamin K (Gijsbers, Jie, & Vermeer, 1996), and carotenoids such as lycopene (Gartner, Stahl, & Sies, 1997), astaxanthin (Odeberg, Lignell, Pettersson, & Höglund, 2003) and beta-carotene(Dimitrov, et al., 1988; Jayarajan, Reddy, & Mohanram, 1980).

265

Including loaded carrier lipid, or bioactive lipid compounds as an emulsion may further improve bioavailability compared to delivery as bulk lipids in e.g. hard-/soft-gel capsules. Studies have shown that the increased interfacial area/low droplet size of these emulsions may result in a higher bioavailability of the bioactive lipid or lipid-soluble ingredient(s). Some examples include algae oil/DHA(Lane, Li, Smith, & Derbyshire, 2014), fish oil/EPA(Raatz, Johnson, & Bukowski, 2016), curcumin(Zou, et al., 2015) and co-enzyme Q10(Hatanaka, Kimura, Lai-Fu, Onoue, & Yamada, 2008).

More complex emulsion structures are also possible, such as double emulsions. A W/O/W-double emulsion consists of water droplets inside oil droplets that are then dispered throughout an outer aqueous phase. By keeping the active ingredient inside the inner water droplets, the compound can be protected from interactions with taste receptors upon chewing and possibly also protected from gastric conditions.(Dickinson, 2011; Garti & Aserin, 1996) When the oil droplets are digested in the small intestine, the inner water phase, and thus the active ingredient, is finally released. As an example, Hattrem et al. (2015a) successfully made stable double emulsions with ibuprofen confined to the inner water droplets. By addition of cyclodextrins, the ibuprofen formed highly water-soluble complexes which reduced any migration of ibuprofen from the inner to the outer water phase. Such double emulsions can also be made solid, using e.g. gelatin in the outer water phase, which gives it a potential for use in chewable formulations for both taste-masking, stability and gastric protection purposes.(Hattrem, Dille, Seternes, & Draget, 2014a)

2.3 The advantages of surface active gelling agents in stabilizing filled gels

Some gelling agents, such as gelatin and other proteins, modified starches and some pectins have significant surface active properties, due to their amphiphilic structure. In relation to gelled emulsions, this means that the gelling agent may interact with and adhere to the emulsion droplets in addition to forming a gel, functioning as an emulsifier and providing connectivity between the gelled continuous and the lipid discontinuous phases.(Ward & Courts, 1977) This will lead to emulsion droplets that are structurally arrested in a gelled matrix, improving the stability of the emulsion towards e.g. coalescence or flocculation, which is a large advantage for chewable supplements containing lipids.(Dickinson, 2012; Dille, Draget, & Hattrem, 2015)

2.3.1 Active and inactive filler

Droplets or particles that interact strongly with the gel network are generally referred to as *active fillers*. The opposite, *inactive filler*, describes droplets or particles that are distributed through a gel with little or no interaction between gel network and particles. While inactive filler particles always give a composite modulus of the filled gel lower than the modulus of the gel alone, active

306 filler particles may increase or decrease this composite modulus depending on the modulus of the
307 filler particles/droplets.(Dickinson & Chen, 1999) This is shown schematically in figure 2.
308
309 For gelled emulsions using a surface active gelling agent, such as gelatin, the emulsion droplets
310 will usually behave as active filler particles. The modulus of the droplets can be approximated to
311 be the same as the Laplace pressure of the droplets, which is proportional to the surface tension
312 divided by droplet radius.(van Vliet, 1988) This means that the modulus of the composite gelled
313 emulsion will increase as the droplet size decreases, and thus the texture of the finalized
314 chewable product can be optimized to some degree by adjusting the oil droplet size through e.g.
315 changing homogenisation parameters.(Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken,
316 2009) The amount of oil in the product is also important, as the active filler contribution from the
317 oil droplets will be more dominating the more droplets are present.(Dickinson, Stainsby, &
318 Wilson, 1985; Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009)
319
320 The presence of other surface active compounds, such as small molecular surfactants (e.g.
321 lecithins or monoglycerids), may strongly influence the parameters of a gelled emulsion.(Dille,
322 et al., 2015) These compounds may displace the surface active gelling agent at the oil-water-
323 interface leading to oil droplets that are no longer bound to the gel network, and thus act more
324 like inactive fillers. When the oil droplets of a chewable supplement gelled emulsion are present
325 as inactive fillers, the emulsion may not be fully stable over time, potentially leading to emulsion
326 destabilisation and sweating of oil. In addition oil may be released when shear is applied to the
327 chewable product, such as during mastication, which may lead to a strongly negative sensoric
328 experience for the consumer.(Sala, van de Velde, Stuart, & van Aken, 2007) Some examples and
329 issues with active/inactive filler for gelatin-based chewable supplements (containing e.g. omega-
330 3 oils) are described in chapter 3.5.
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332
333

3. Physicochemical properties of gelatins

Gelatin is a versatile biopolymer with a wide range of uses, for example it is used as a gelling/thickening agent in foods, confectionaries and cosmetics, as a fining agent for various beverages and as a material used in making hard and soft capsules for pharma- and nutraceuticals.(Haug, et al., 2009)

Gelatin is produced from collagen by the process of partial hydrolysis. Collagen is a structural fibrous protein mostly found in the skin, bones and connective tissues of animals. The raw material used for gelatin production is most commonly from pork, bovine and fish sources. Native collagen molecules usually form a triple-helical structure, consisting of three polypeptide chains (called alpha-chains), each with a molecular weight of approximately 100 kDa. The collagen molecule has a unique amino acid sequence, comprising of repeating units of glycine-X-Y, where X is usually proline and Y is usually hydroxyproline. This sequence favours the formation and stability of the triple helical structure through the formation of intramolecular hydrogen bonds. Gelatin's ability to form gels can be attributed to its ability to partially reform this triple-helical structure in an aqueous solution. This reformed triple-helical structure is however thermoreversible, upon heating a gelatin gel will melt again with a low hysteresis compared to many other gelling agents.(Veis, 1964; Ward, et al., 1977)

In comparison to other protein sources, gelatin is a heterogenous product, with a broad molecular weight distribution and differences in isoelectric point depending on the preparation procedure and source of raw material. In the preparation of gelatin-based soft chewables, it is of importance to consider type of gelatin (molecular weight, bloom, type A and B gelatin, raw material source) as this can significantly influence the properties of the final product.(Hattrem, Molnes, Haug, & Draget, 2015b) In addition, after production, long term stability of the gelatin matrix is of large importance. Many parameters can influence this, among other pH, water activity, ionic strength, excipients and type of active ingredients.(Haug, et al., 2009)

3.1 Effect of gelatin raw material

Gelatin is produced from connective tissues, bones and skin, with the most common raw material being pig skin.(Schrieber, et al., 2007) The raw material has a large influence on the resulting gelatin. The transition temperature of the gelatin is dependent on the amount of proline and hydroxyproline in the collagen amino acid sequence. Animals with a high body temperature, such as mammals, birds and warm water fish generally have high proline and hydroxyproline content, giving a gelatin with a higher transition temperature. Meanwhile, animals with lower body temperatures, such as cold water fish, have less of these amino acids and thus resulting gelatin with a lower transition temperature. Generally, the transition temperature of a gelatin

375 corresponds roughly to the body temperature of the animal the gelatin is extracted from.(Haug, et
376 al., 2009; Veis, 1964)
377
378 Gelatin from mammalian sources have transition temperatures fairly similar to human body
379 temperature, giving gels that are solid at room temperature, but melt in the mouth and/or the
380 gastrointestinal system upon ingestion providing good mouthfeel and rapid release of active
381 ingredients.(Haug, et al., 2009; Nussinovitch & Hirashima, 2013; Veis, 1964)
382
383
384 3.2 Type A vs B
385
386 In the production of gelatin, the collagen raw material is pre-treated with either acid (most
387 commonly for fish and pork raw material) or alkali (most commonly for bovine raw material),
388 and the resulting gelatin is referred to as type A and type B, respectively.(Schrieber, et al., 2007)
389
390 The acid pre-treated gelatin (type A) has a isoelectric point (IEP) of 7 – 9 and an amino acid
391 sequence similar to the original collagen molecule. The alkali pre-treated gelatin (type B) on the
392 other hand, has a lower IEP of ~5, due to conversion of glutamine and asparagine to their acid
393 precursors during the processing.(Veis, 1964; Ward, et al., 1977)
394
395
396 3.3 Molecular weight
397
398 During processing of the collagen the covalent bonds between the alpha-chains are broken, but
399 some breakage also occurs in the primary structure. In addition, some covalent bonds between
400 alpha-chains might survive the hydrolyzation process, giving rise to a gelatin fraction of very
401 high molecular weight. Thus the resulting gelatin usually has a fairly wide molecular weight
402 distribution, as shown in figure 3. There is also a significant difference between type A and type
403 B gelatins. Type B gelatins usually have a molecular size distribution peak around 100kDa
404 (corresponding to the collagen alpha-chain average molecular weight), and also a more narrow
405 distribution compared to type A gelatins.(Hattrem, et al., 2014b)
406
407 A higher molecular weight average for a gelatin generally corresponds to an increase in bloom
408 value, which is essentially a way to denote the gel strength of the resulting gelatin gel. It is
409 measured using a standardized method, where a 6.67% gelatin gel in a predefined shape is
410 matured at 10 °C for 18 hours, and then compressed 4 mm using a flat bottomed cylinder probe.
411 The force (in grams) necessary for this compression is defined as the bloom value for the
412 gelatin.(Eysturskarð, Haug, Ulset, & Draget, 2009; Haug, et al., 2004; Schrieber, et al., 2007) It
413 should be noted that the Bloom value is a single point measurement that does not fully describe
414 the non-equilibrium gelling behavior of a gelatin gel. By performing small strain oscillatory

415 measurements a more comprehensive characterization of the rheological properties can be
416 obtained.(Eysturskarð, Haug, Elharfaoui, Djabourov, & Draget, 2009)
417
418
419 3.4 Gel dissolution by bloom strength
420
421 Commercial gelatins come in a wide range of bloom strengths, with higher bloom gelatins
422 generally having a larger average molecular weight, as described in chapter 3.3. The dissolution
423 profile of a gelatin-based delivery unit is of crucial importance as it may influence the
424 dissolution kinetics of the embedded active ingredient further influencing absorption in the
425 gastrointestinal tract. Studies have shown that gelatin with larger molecular weight can give a gel
426 with higher transition temperature potentially giving slower dissolution kinetics of the final
427 gelatin gel at body temperature, especially for type A gelatins.(Hattrem, et al., 2014b) This can
428 be seen in figure 4, where the dissolution of gels made with 160 and 260 bloom gelatins of type
429 A and type B in simplified gastric conditions are shown.
430
431 As already described gelatin-based chewables may contain different types of filler particles. This
432 may influence both the mechanical properties (as described in previous sections), but also the
433 gelling kinetics(Hattrem, et al., 2015b). Thus, filled gels may have different dissolution profiles
434 compared to a pure gelatin gel, also affecting the dissolution and absorption of the active
435 ingredient. Therefore it is of importance to consider both the rheological properties as well as
436 performing simulated dissolution studies to evaluate the properties of the delivery system.
437
438
439 3.5 Gelatin-based gelled emulsions and gel strength
440
441 As previously mentioned, gelatin has surface active properties, allowing it to act as emulsifier in
442 addition to gelling agent, making it a good choice as gelling agent for producing chewable gelled
443 emulsions. A gelatin-based gelled emulsion will most likely experience the active filler effect,
444 giving an increase in total gel modulus for the gelled emulsion compared to an oil-free gel,
445 dependent on droplet size, as described in chapter 2.3.1.
446
447 In addition to droplet size, the amount of oil has a large effect on the system. As long as the
448 droplet size is mostly constant, more oil present will result in a higher modulus of the gelled
449 emulsion, and thus changes in texture and possibly mouthfeel.(Dickinson, et al., 1985) In
450 addition it will also result in a higher viscosity of the melted gel, which can be important in
451 regards to production parameters. The connection between gel strength (storage modulus) and oil
452 content for gelatin-based gelled emulsions using 200 bloom type A and type B gelatin is shown
453 in figure 5.
454

455   As can be seen in the figure above, the modulus increase with increasing oil concentration is
456   larger for the type A gelatin compared to the type B gelatin. This has previously been attributed
457   to the formation of hydrogen bonds between asparagine residues and the gelatin backbone,
458   promoting flocculation of oil droplets providing a structural reinforcement of the gelled
459   emulsion.(Hattrem, et al., 2015b; Vijayakumar, Qian, & Zhou, 1999) This effect is not seen for
460   type B gelatin due to the degradation of asparagine (and glutamine) amino acids during
461   extraction.
462
463   As previously mentioned in chapter 2.3, the presence of other surface active compounds in the
464   gelled emulsion might have undesirable effects on gel texture or emulsion stability. Gelatin-
465   based chewable supplements might contain a wide range of compounds with varying surface
466   activities, and it is important to be aware of the potential effects of such compounds if a
467   chewable gelled emulsion is the desired product. An example of this might be in the production
468   of gelatin-based chewable emulsified omega-3 supplements. Some omega-3 oils (e.g. algae oil)
469   may contain small surface active molecules such as lecithins or free fatty acids, depending on
470   purification and refining methods.(Winwood, 2013) After the emulsion is made, these small
471   surfactants might adhere to the oil droplet surfaces and displace the gelatin, giving an
472   unexpectedly weak gel due to inactive filler effects, and possibly also an oily unpalatable final
473   product.
474
475
476   4.General challenges with water-based chewables and active ingredients
477
478   A wide range of active ingredients are already being formulated into chewable delivery units:
479   vitamins, minerals, lipids, pharmaceuticals, etc. However, several of these compounds have
480   potential issues when formulated into chewables, such as poor taste upon chewing, poor stability
481   in a water-based system or negative interactions with other ingredients or with the gelling agent
482   itself. Relevant issues related to some common nutraceutical active ingredients are summarized
483   in table 1, and are further explained in the following sub-chapters, along with potential solutions.
484   General challenges with pharmaceutical ingredients in chewable tablets are also discussed.
485
486
487   4.1 Vitamins
488
489   Vitamins are a diverse group of molecular compounds, and the potential issues with
490   incorporation into water-based chewables are different from vitamin to vitamin. Some of these
491   issues are summarized below, along with potential solutions.
492
493   Thiamine, also known as vitamin B1, has a bitter taste and a distinct pungent aroma, and is
494   usually added to supplements as either thiamine hydrochloride or thiamine mononitrate, most
495   commonly the nitrate form as it is known to be the most stable.(Bettendorff, 2013; Macek,

496   Feller, & Hanus, 1950) Both these salts are fairly water-soluble, and will most likely be
497   solubilized in a water-based chewable supplement increasing the potential for taste receptor
498   interactions. Riboflavin (vitamin B2) is another vitamin with a very strong bitter flavor, and a
499   moderate solubility. These two vitamins can be very challenging to include in chewable
500   formulations as these products often are intended for children, who are more sensitive to bitter
501   off-taste compared to adults.(Walsh, et al., 2014) However, many compounds are available in
502   several different molecular forms, potentially with differing bitterness profiles. As an example
503   riboflavin sodium phosphate is known to be less bitter than riboflavin, but special care has to be
504   taken using this salt as it is considered less stable in the presence of light. The light degradation
505   products of riboflavin are very strong oxidizing agents, which may catalyze degradation of e.g.
506   vitamin C and PUFAs present in the formulation.(Coultate, 2009; DSM Nutritional Products
507   Ltd., 2012; Smith, 1991)
508
509   A common general approach for reducing bitterness is adding very potent sweeteners, such as
510   artificial sweeteners (sucralose, acesulfame k, aspartame, etc.) or natural stevia.(Ley, 2008;
511   Schiffman, et al., 1994) In addition, adding strong or complementary flavors (e.g. grapefruit,
512   coffee, cocoa/chocolate, etc.) might mask/reduce the bitter off-taste further.(Ley, 2008)
513   However, to satisfy the palate of especially the pediatric segment, this might not be sufficient.
514
515   Another potential approach for reducing vitamin aroma and off-taste is the addition of beta-
516   cyclodextrin. This type of cyclodextrin will form complexes with both thiamine and riboflavin,
517   ideally giving a more palatable, and also more stable supplement.(Szejtli, et al., 2005; Terekhova
518   & Obukhova, 2005) Another method to reduce off-taste, and also potentially increase stability of
519   thiamine, riboflavin or other compounds is through encapsulation, previously mentioned in
520   chapter 2.2.1. Some possible approaches include encapsulation within solid microcapsules,
521   encapsulation within liposomes(Ahmad, et al., 2015; Liu, Ye, & Singh, 2015) and double
522   emulsions (described in chapter 2.3).
523
524   Vitamin C, in the form of ascorbic acid is a very common ingredient in multivitamin
525   formulations, including gummies produced with gelatin. This compound is prone to degradation
526   through a number of different routes, including both oxidative and non-oxidative routes as well
527   as through the Maillard reaction, and is therefore a challenging molecule to include in products
528   requiring long-term stability(Coultate, 2009). Degradation of ascorbic acid is affected by
529   different parameters, including time, temperature, pH(Wilson, Beezer, & Mitchell, 1995), water
530   activity(Lee & Labuza, 1975) and the presence of metallic catalyzers, oxygen, amino compounds
531   and enzymes (Gallarate, Carlotti, Trotta, & Bovo, 1999; Santos & Silva, 2008; Stešková,
532   Morochovičová, & Lešková, 2006; Yu, Tan, & Wang, 2013). Besides loss of vitamin C activity
533   decomposition of ascorbic acid may also lead to formation of undesirable flavor compounds and
534   discoloration, both highly disadvantageous in chewable products(Coultate, 2009; Shinoda,
535   Murata, Homma, & Komura, 2004). By keeping a low pH in the solution as well as removing
536   excess oxygen, degradation can be minimized. To remove dissolved oxygen, an oxygen

537   scavenger such as cysteine can be included in the formulation. Cysteine is very easily oxidized
538   by oxygen, thereby protecting other compounds such as ascorbic acid from oxidative
539   degradation.(Elias, McClements, & Decker, 2005) However, cysteine has a distinct off-taste
540   (sulfur-like), which may influence palatability. It is also of importance to avoid certain metallic
541   ions (e.g. copper and iron) in the product as these may act as catalyzer of ascorbic acid
542   degradation.(Santos, et al., 2008) Iron and copper are often considered important ingredients in
543   multivitamin/mineral formulation and related issues and potential solutions are further explored
544   in chapter 4.2.
545
546   4.2 Minerals
547
548   Supplement minerals are available in a wide range of different salt forms. As previously
549   mentioned in chapter 2.2.1, these salts have differing solubilites and choosing the optimal salt
550   might be very important to obtain optimal taste, texture and stability in a chewable tablet. One
551   example is calcium, which is a popular mineral supplement with a daily recommended dose of
552   1.0 - 1.3 g for adults(US Institute of Medicine, 2011). Due to the high recommended daily dose,
553   using a soluble salt form of calcium such as calcium chloride in a gelatin-based chewable
554   supplement will lead to an unpalatable salty flavor and very high ionic strength, influencing gel
555   strength significantly. Instead tricalcium phosphate is commonly used in chewables, as it has a
556   low solubility, affecting taste and gel texture to a much lesser degree, as well as a high payload
557   of calcium. The particle size of the insoluble salt is also important, as too large particles may
558   impart a "sandy" mouthfeel to the chewable product. Some other common supplement minerals
559   such as zinc and magnesium have similar issues to calcium that also can be solved by using a
560   less soluble salt form, but with a lower RDI the problems for these are usually of a lesser
561   magnitude. In addition, it is important to keep in mind that salt form and solubility may affect
562   bioavailability of the active compound.
563
564   Iron, as well as other transition metals such as copper can be difficult minerals to include in
565   water-based chewable supplements. Due to their variable oxidation states they can take part in
566   catalyzing degradation of various other active ingredients, such as vitamin C and omega-3 fatty
567   acids. Ferrous iron (Fe^{2+}) in aqueous solution reacts readily with e.g. oxygen, forming ferric iron
568   (Fe^{3+}) and reactive free radicals. Meanwhile, reducing agents such as vitamin C may react with
569   ferric iron, converting it back to ferrous iron while at the same time oxidizing the
570   vitamin.(Buettner & Jurkiewicz, 1996; Murray-Kolb & Beard, 2010) Highly soluble iron salts,
571   such as ferrous sulfate, have a high bioavailability, but a strong metallic off-taste and large
572   potential for reactions, while less soluble iron compounds have more variable
573   bioavailabilities.(Murray-Kolb, et al., 2010) Ferrous fumarate may be a good salt form for
574   inclusion in chewables, as it has fairly low solubility and little off-taste, yet good
575   bioavailability.(Degerud, Manger, Strand, & Dierkes, 2015) However, this compound will still
576   be slightly in solution, giving the potential for reactions with other ingredients.
577

578 Microencapsulation, where the iron and other transition metal salts are fully compartmentalized
579 away from all other ingredients might thus be the optimal solution for water-based chewable
580 multivitamin supplements. However, as mentioned in chapter 2.2.1, there are also a wide range
581 of potential issues with encapsulation in water-based chewables. In addition, trace amounts of
582 transition metal salts are also often present in other excipients or may be introduced during
583 processing.(Buettner, et al., 1996) Thus using deionized water and high quality ingredients, as
584 well as adding metal chelators, can be beneficial to minimize free transition metal ions in the
585 aqueous phase of the chewable formulation and maximize stability of sensitive active
586 ingredients.
587
588
589 4.3 Polyunsaturated fatty acids
590
591 PUFAs, such as omega-3s and omega-6, are highly susceptible to oxidation, due to their
592 molecular structure with multiple cis-double bonds. During production and storage of
593 supplements containing PUFAs it is nearly impossible to avoid *some* oxidation of the oil, and
594 especially the volatile secondary lipid oxidation products give a fishy/rancid aroma and off-taste,
595 even at very low concentrations.(Jacobsen, 1999) As these degradation products may have
596 negative health effects in addition to bad off-taste, minimising PUFA oxidation in such
597 supplements is very important. Maintaining anoxic conditions during production, using
598 packaging impermeable to oxygen and light, addition of antioxidants such as ascorbyl palmitate
599 or vitamin E and total avoidance of metal ions through using deionized water, refined ingredients
600 and possibly addition of metal ion binding agents (chelators) are all common strategies for
601 optimising PUFA stability in supplements.(Winwood, 2015) There are currently a wide range of
602 soft and hard gel capsule PUFA supplements on the market. As these are swallowed whole, any
603 oil off-taste upon ingestion is negated, even though poor off-taste may present itself later through
604 reflux. For chewable supplements on the other hand, chewing may release the volatile oil off-
605 taste compounds in the oral cavity, promoting a nauseating taste sensation. In chewable
606 supplements containing lipid active ingredients, the oil is generally included as an emulsion,
607 which may reduce this off-taste. Including the oil as an emulsion may also minimize problems
608 related to oil reflux.(Haug, et al., 2011) For further information on emulsions, see chapter 2.2.3
609 or chapter 3.5 (gelatin based gelled emulsions).
610
611 Addition of volatile and aromatic flavor compounds, such as lemon or peppermint flavors may
612 overpower and mask the aroma of oxidation products released during chewing. There are also
613 many compounds with the ability to bind to the secondary oxidation products and potentially
614 reduce their release during mastication. A couple of examples include cyclodextrins(Astray,
615 Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009) and amylose rich pea
616 dextrin(Böttcher, Steinhäuser, & Drusch, 2015). It is, however, important to note that some of
617 these compounds may affect the properties of the chewable gelled product in various ways, such

618 as amylose which can increase the viscosity during production significantly.(Böttcher, et al.,
619 2015)
620
621 4.4 Pharmaceutical compounds
622
623 Pharmaceutical compounds are often unpalatable due to a bitter/metallic off-taste, even at very
624 low concentrations.(York, 2007) This is especially apparent for easily soluble APIs, as a high
625 solubility maximizes the potential API-taste receptor interactions when the tablet is chewed. As
626 previously mentioned, strong sweeteners or complementary flavors may reduce bitterness. In
627 addition, sodium salts have been shown to reduce bitterness of some APIs. In a study by Keast et
628 al. (2002) on the bitter APIs pseudoephedrine, ranitidine and acetaminophen, the largest
629 bitterness inhibition effect was seen with sodium glutamate and sodium adenosine
630 monophosphate followed by sodium chloride. Salts with other cations, such as ammonium,
631 magnesium or potassium also had some effect, but less than sodium.
632
633 Many APIs, such as ibuprofen have a solubility strongly dependent on pH due to the presence of
634 e.g. carboxyl groups in the API molecule.(Shaw, Irwin, Grattan, & Conway, 2005) By adjusting
635 the pH in the chewable dosage form, the API can be kept mainly as solid aggregates/particles.
636 This may significantly reduce off-taste when chewing, as the API aggregates have to dissolve in
637 the saliva before their taste can be detected. By including high strength buffer systems in the
638 dosage form, the pH in the oral cavity upon chewing can be kept at an advantageous low level
639 sufficient to keep the aggregates from dissolving before they are swallowed. It is, however,
640 important to note that both low or high pH values or high ionic strength buffer systems may
641 affect other excipients present, such as the gelling agent, as mentioned in chapter 2.2.2. Gelatin-
642 based chewables containing ibuprofen aggregates using a buffer system are further explored in
643 chapter 5.
644
645 Having an excellent stability of the API in the formulation during processing and storage is of
646 absolute importance, as it is essential for the regulatory approval of the pharmaceutical product.
647 Many APIs are sensitive to heat, oxygen, moisture or light, thus taking care to minimising these
648 influences are important.(Billany, 2007; York, 2007) Interactions between API and excipients
649 present in the dose are also a possibility that must be anticipated, both in regards to direct
650 chemical/physical interactions as well as indirect interactions that may affect bioavailability of
651 the API. Some examples include aspirin which may interact with the common tablet excipient
652 magnesium stearate(York, 2007), trace amounts of metal ions (from excipients) that might
653 catalyse oxidation of API and grapefruit flavorings which might interact with the bioavailability
654 of several drugs(Seden, Dickinson, Khoo, & Back, 2010). To detect chemical interactions,
655 calorimetric methods may be used(Abrantes, Duarte, & Reis, 2016).
656
657
658 4.5 Water activity and microbial stability

659

660   Many chewables, including ones made with gelatin, are generally based around an aqueous
661   solution, as opposed to traditional dry tablets. As water is present, it opens for the possibility of
662   microbial growth. However, by reducing the amount of free water, through e.g. a high content of
663   water-binding sugars, microbial growth is inhibited and addition of additional preservatives is
664   often unnecessary. The amount of free water in a sample is usually described through the *water*
665   *activity* (a_w), with a value between 0.0 (no free water) and 1.0 (pure water). Generally, bacteria
666   and fungi require a certain minimum water activity to be able to grow. At neutral pH values, an
667   a_w of 0.9 or below is generally considered enough to inhibit most bacterial growth, while many
668   fungi/molds can grow all the way down to an a_w of around 0.7.(Fontana & Campbell, 2004)
669   However, by decreasing the pH of the water phase, the necessity of low a_w is reduced, down to a
670   pH of 4 - 4.5 where all common bacterial growth is halted at any a_w.(FDA, 2015) This minimum
671   pH for bacterial growth is also dependent on which acidulant is used. Chung and Goepfert (1970)
672   showed that growth of Salmonella spp. in laboratory media was inhibited at a pH of 5.5 when
673   using propionic or acetic acid, but when using citric or hydrochloric acid the bacteria could grow
674   down to a pH value of 4.05. Those experiments were however performed in a simplified system,
675   real food/supplement products are very complex and determining the exact minimum pH for
676   optimal microbial stability is difficult. In addition, some fungi can grow all the way down to a
677   pH of 2.(Wheeler, Hurdman, & Pitt, 1991) Thus, it is recommended to keep both a low a_w and
678   low pH, if possible, and if not, addition of preservative agents can be used.
679

680   Another potential problem with water-based dosage forms is the fact that active ingredients can
681   become solubilized in the formulation, potentially leading to a large reduction in stability.
682   However, a reduced water activity may decrease solubility and reduce freedom of movement for
683   solubilized molecules, possibly increasing active ingredient stability.(Fontana, et al., 2004; Lee,
684   et al., 1975) Traditional gummy and jelly candies generally have a_w values between 0.50 -
685   0.75(Ergun, Lietha, & Hartel, 2010), which might be a good aw target to strive for in the
686   formulation of chewable nutraceutical and pharmaceutical products to obtain microbial stability
687   and improve chemical stability.
688

689

690   5. Chewable API formulations; filled particle-gels case study
691

692   Delivery formulations containing APIs have in general much higher regulatory demands
693   compared to their nutraceutical counterparts. Briefly, these demands are linked to:
694

695   ●   excipients (requires pharmaceutical quality)
696   ●   product stability (mainly API)
697   ●   disintegration/dissolution
698   ●   bioavailability
699

700 For chewable API formulations there is another challenge; as a direct consequence of chewing
701 the API will inevitably get into contact with both the tongue taste buds as well as the mucosa in
702 the oral cavity. Given the variation of gelatins and gelatin gels that have been outlined in
703 previous chapters, it is fair to ask how APIs may be incorporated into gelatin based chewables in
704 a regulatory acceptable way.
705
706 To anticipate events somewhat, partly because it is also intuitively clear, all these challenges are
707 most easily met by applying the aggregate/suspension approach. Dispersing solid API-particles
708 into a gelatin gel will limit off-taste by limiting contact with taste buds and mucosal surfaces as
709 well as improve stability. Crystalline forms of a component are generally much less susceptible
710 to hydrolysis compared to the dissolved state. The main challenge therefore becomes how to
711 control API solubility.
712
713 Ibuprofen is a most challenging API in a chewable formulation; it causes a specific throat
714 irritation when present in a solubilized form(Breslin, Gingrich, & Green, 2001). Fortunately,
715 ibuprofen has a carboxylic group with a pKa value of approximately 4.9 and at pH values below
716 this its solubility is greatly reduced(Lindqvist, Tuhkanen, & Kronberg, 2005; Shaw, et al., 2005).
717 It is therefore possible to keep ibuprofen mainly in its crystalline form, and at the same time
718 maintain gelatin stability, by adjusting the pH to 4.5(Dille, Hattrem, & Draget, paper in prep.).
719 Keeping pH at 4.5 within the chew results in a 2 year stability of the ibuprofen as well as
720 comparable dissolution with a marketed ibuprofen solid tablet(Dille, et al., paper in prep.).
721
722 The main challenge with an ibuprofen chew, because of its profound throat irritation, is if
723 keeping the formulation pH at 4.5 is sufficient with respect to reduction of off-taste. A non-
724 trained taste panel consisting of 6 persons was asked to chew and spit ibuprofen containing
725 gelatin chews (100 mg/g) at different buffered pH values ranging from below to above the
726 ibuprofen pKa value in a randomized order, and rate the off-taste on a scale from 0 (no off-taste)
727 to 9 (significant off-taste). As control a formulation containing the same amount of inert SiO_2
728 particles was used. The panel's taste impression is summarized in figure 6. The overall result
729 points towards that keeping the product pH below the pKa value of the ibuprofen molecule (pH
730 4.5) has a profound effect on off-taste sensation due to a greatly reduced API solubility.
731
732 Perhaps the most difficult challenge with respect to regulatory requirements of new drug
733 formulations is related to bioavailability. Deviations in bioavailability relative to market
734 approved formulations will inevitably lead to a much longer and costly regulatory process. A
735 clinical investigation on bioavailability of GMP manufactured ibuprofen chews was initiated and
736 compared to a standard marketed dry tablet(Hattrem, Dille, & Draget, paper in prep.). The
737 ibuprofen blood concentration was recorded over a period of 24 hours and some of the results
738 from this study is summarized in Table 2.
739

740 This small clinical trial also included the influence of the degree of chewing. As can be seen
741 from Table 2 there are no significant differences between the bioavailability of the standard dry
742 tablet ('Advil') and the ibuprofen containing chewable formulations. There is an indication (not
743 statistically significant) that chewing 8 times may cause a slight reduction in bioavailability. This
744 may be due to a low number of participants in this study and/or increased buccal loss with
745 increased chewing.
746
747 Overall, this study suggests that bioequivalence of API containing chewable formulations
748 compared to standard dry tablets is within reach.
749
750
751
752 6. Conclusions
753
754 As highlighted in this article, chewable delivery units offer a good alternative to traditional
755 tablets, especially for children, the elderly and for patients suffering from dysphagia. By offering
756 an easier and more comfortable administration of active ingredients, chewables have the
757 potential to increase compliance and reduce tablet-related discomfort.
758
759 Unfortunately, there are also many challenges, as highlighted in this article. Many active
760 ingredients are potentially unstable when dissolved, they give unacceptable off-taste in chewable
761 products, or they interact with each other in products with multiple active ingredients. However,
762 some of these issues may be solved through innovative formulation design: by adjusting
763 parameters such as pH or water activity, by using other molecular forms of the active
764 ingredients, by using emulsification, solid encapsulation or complexation or by addition of strong
765 sweeteners or flavor compounds.
766
767 Overall, gelatin-based chewable formulations offer a good vehicle for delivery of a wide range of
768 active ingredients, even if significant development work might be needed for formulations
769 including certain ingredients and ingredient combinations.
770
771
772
773
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775

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