Formulation Development of High Dose (-)-Epigallocatechin-3-gallate Immediate Release Tablets

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Pracę tą dedykuję
mojemu ukochanemu Dziadkowi

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INTRODUCTION

1.1. Green tea catechins

Green tea is derived from the leaves and leaf buds of Camellia sinensis (L.) and cultivated in more than 20 countries worldwide. Tea is one of the world's most consumed beverages, whereby green tea constitutes approximately 20 % of the tea consumed worldwide. High consumption is especially pronounced in Asian countries like Japan and China and epidemiological studies have been carried out to correlate longevity of the Japanese people to their traditional consumption of green tea (Kao Corporation, 2008). In traditional Chinese medicine, green tea is used for several discomforts like headaches, body aches and pains, depression and for detoxification (Cabrera et al., 2006).

Major components in green tea are polyphenols, accounting for 36 % of dry weight, whereby 30 % belong to the flavan-3-ol group, also known as catechins, followed by carbohydrates (25 %) and proteins (15 %) (Liu et al., 2009) (Graham, 1992). The four main representatives of green tea catechins are epicatechin (EC), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG) and their epimers respectively (Fig. 1). They are situated in the vacuole of cells; thus the highest amount of galloylated catechins can be found in apical bud and first leaves from the apex of the plant as these are the sites of active cell growth and therefore contain a large central vacuole. The further the plant matures, the less EGCG and ECG can be found but a high amount of the degalloylated form EGC (Forrest and Bendall, 1969) is present. The localization of catechins in vacuoles and chloroplasts (Liu et al., 2009) makes sense, as the pH of vacuole and thylakoid interior is acidic with pH 5 to 5.5 for the vacuole and pH 4 for the thylakoid, hence optimal in case of ECG and EGCG stability.

Despite the variation of galloylated catechins depending on plant maturation stage and part of plant, EGCG is the most abundant catechin in green tea and accounts for 55 up to 79 % of the total catechin content (Liu et al., 2009). The radical scavenging activities of tea catechins have been examined in numerous in vitro systems showing them to be efficient scavengers of singlet oxygen ($^1O_2$), superoxide radicals ($O^{\cdot-}_2$),

hydroxyl and peroxyl radicals (Higdon and Frei, 2003). In most of the studies, EGCG was more efficient than the other catechins. Especially on pathologies accompanied by oxidative stress like inflammation, cancer or infections it was noted that especially the galloylated catechins exhibit higher antioxidative potential than other antioxidants like ascorbic acid and tocopherol (Higdon and Frei, 2003). Consequently, it became the focus for several in vitro and in vivo studies to investigate its potential health benefits.

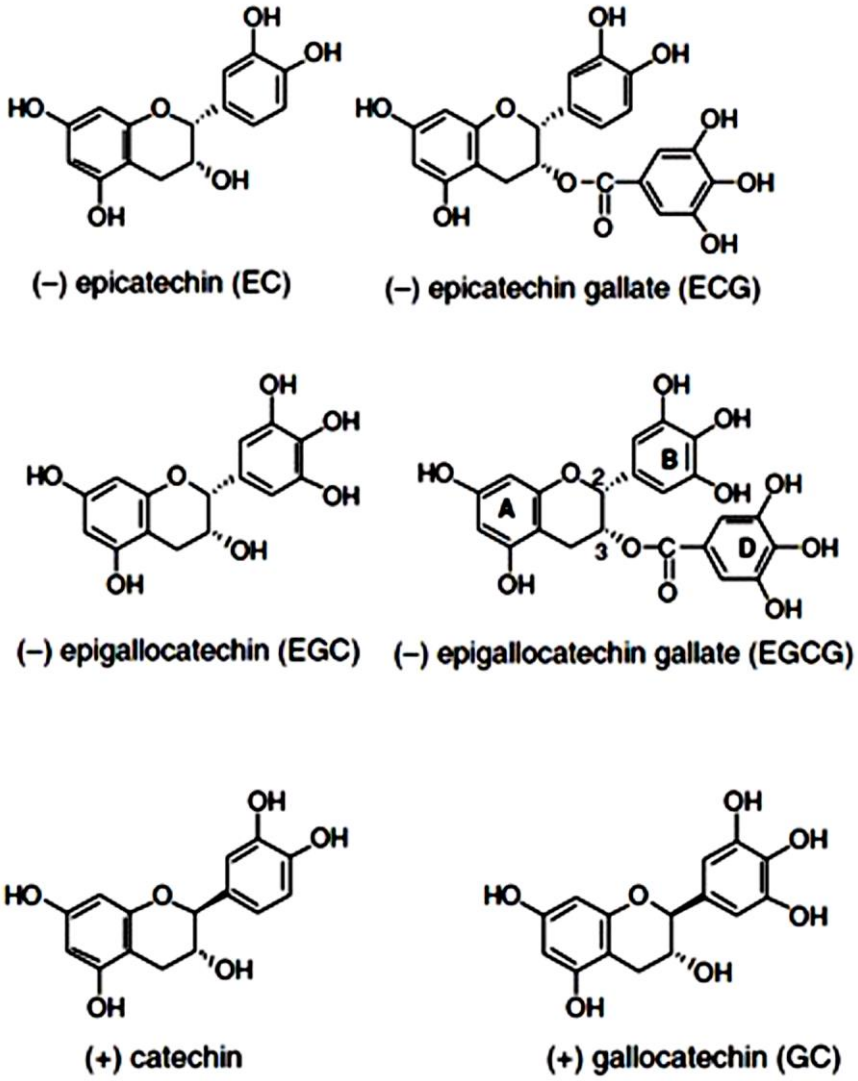

Figure 1: Structures of the major catechins in green tea (Zaveri, 2006) (Figure reprinted by permission)

1.2. Health benefits of (-)-Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG) is known for a series of beneficial effects in vitro and in vivo such as: protection against degenerative diseases; antiproliferative activity; hypolipidemic activity; prevention of hepatotoxicity; antitumorigenic effects; prevention of oxidative stress and thus anti-inflammatory actions; reducing blood glucose levels and body weight, thus reducing the risk of stroke and coronary heart disease (Chacko et al., 2010).

As shown by several scientific groups, the biological effects of EGCG do not just derive from its potential to scavenge radicals and by this to reduce the oxidative stress. EGCG is capable of interacting with proteins, enzymes and DNA/RNA, for example MAP kinases, phosphatases, DNA methyltransferase, topoisomerases and many others (Patra et al., 2008). As a consequence it induces apoptosis of cells, arrests cell cycle and modulates cell signaling (Khan et al., 2006) and in this way exhibits its pharmacological effects.

The list of investigated health benefits and/or pharmacological effects is long. Here are just few examples: EGCG shows antibacterial, antitoxin, antiviral, and antifungal activity by binding the toxins, binding to the cell wall components and blocking glycoprotein-mediated membrane fusion (Friedman, 2007). EGCG can improve endothelial function in the case of coronary artery disease by enhancing the production of nitric oxide by the endothelium (Widlansky et al., 2007).

It shows an effect on thermogenesis expressed as increased energy expenditure directly after meals as well as 24 h postprandial and as increased fat oxidation in humans by consumption of EGCG or green tea extract (Boschmann and Thielecke, 2007).

In cell lines and animal studies, EGCG showed chemoprevention and/or tumor inhibition for several cancer types such as lung, stomach, duodenal, breast and prostate cancer (Shim et al., 2010) (Yamane et al., 1995) (Fujita et al., 1989) (Gupta et al., 2000) (Liang et al., 1999). In case of human prostate cancer, it was shown that EGCG has no effect on normal human epithelial cells just on tumorigenic malignant prostate cells (Caporali et al., 2004). Hence it leads only in cancer cells to cell death, which therefore makes it an even more desirable therapeutic agent.

Consequently, it is nowadays an often investigated pharmaceutical active in clinical trials. Some of the ongoing studies are testing relevance even for severe diseases such as M. Huntington, M. Alzheimer and Duchenne Muscular Dystrophy (https://clinicaltrials.gov).

1.3. Physicochemical properties of (-)-Epigallocatechin-3-gallate

EGCG is, with a solubility in water (20°C) of 40 $^{g}/_{l}$, a soluble powder and sparingly good soluble powder in polar protic organic solvents like ethanol and polar aprotic solvents like dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). According to the BCS classification system, EGCG belongs to the BCS class 3 compounds, being in its dose of 50-300 mg a highly soluble but low permeable drug with an apparent permeability coefficient on Caco-2-layer around 0.4 • 10^{-6} $^{cm}/_{s}$ (Li and Gu, 2014), a log P_{ow} of 1.1 in pH 4.0 (Pharmachem Laboratories and DSM, 2008) (Cayman Chemical Company, 2012) and having calculated log D values in pH 5.5 of 2.08, in pH 7.4 of 1.92 (calculated by ACD/Labs' ACD/PhysChem Suite).

Furthermore, EGCG is a weak acid with a pKa value of 7.99 (calculated by ChemAxon on www.chemicalize.org). This is an important property as deprotonation is the first step towards autoxidation to dimeric and polymeric structures (Quideau et al., 2011) (Fig. 2).

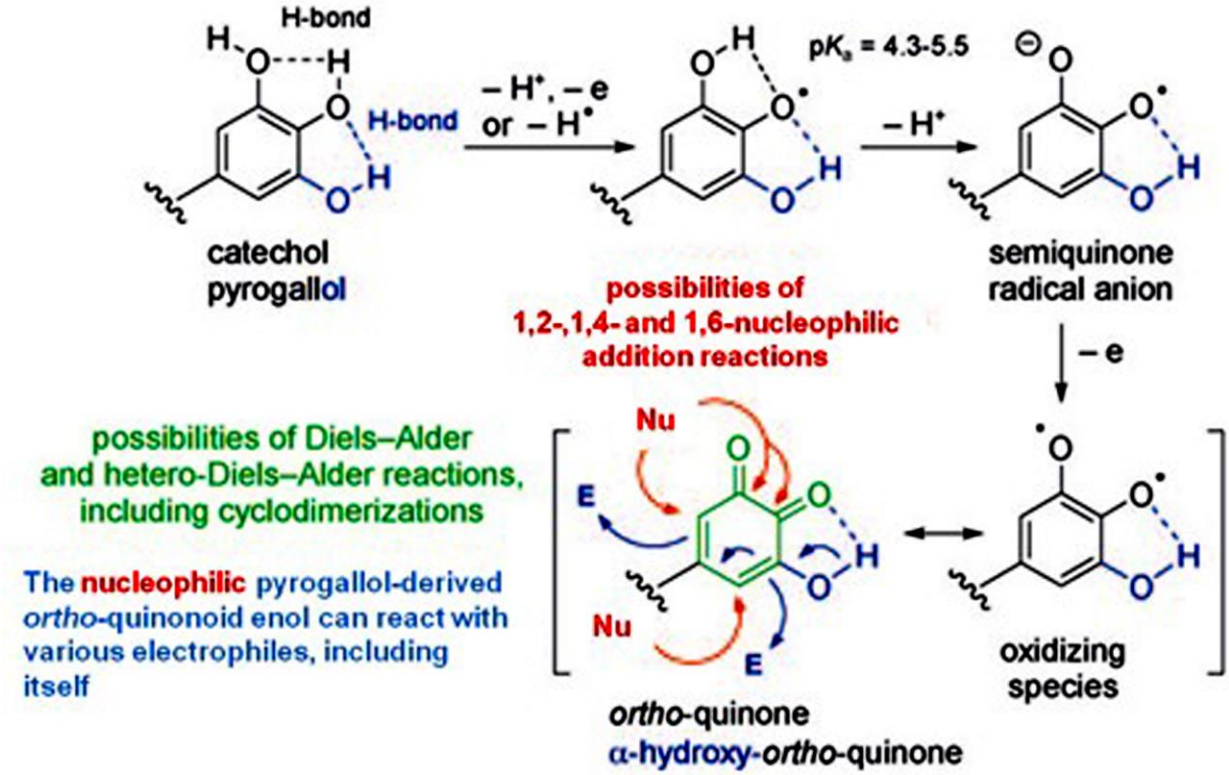

Figure 2: Oxidative dehydrogenation of catechol- and pyrogallol-type phenols into reactive quinonoid species (Quideau et al., 2011) (Figure reprinted by permission)

1.4. Autoxidative degradation of (-)-Epigallocatechin-3-gallate

EGCG is susceptible to degradation mainly through autoxidation which is a radical chain reaction. The degradation products are versatile in their structure due to the broad range of possible chemical interactions of two or more EGCG molecules (Fig. 2). The dimeric degradation products theaflavins and oligomeric thearubigins belong to one of them and are responsible for the browning of EGCG solutions and powder (Fig. 3).

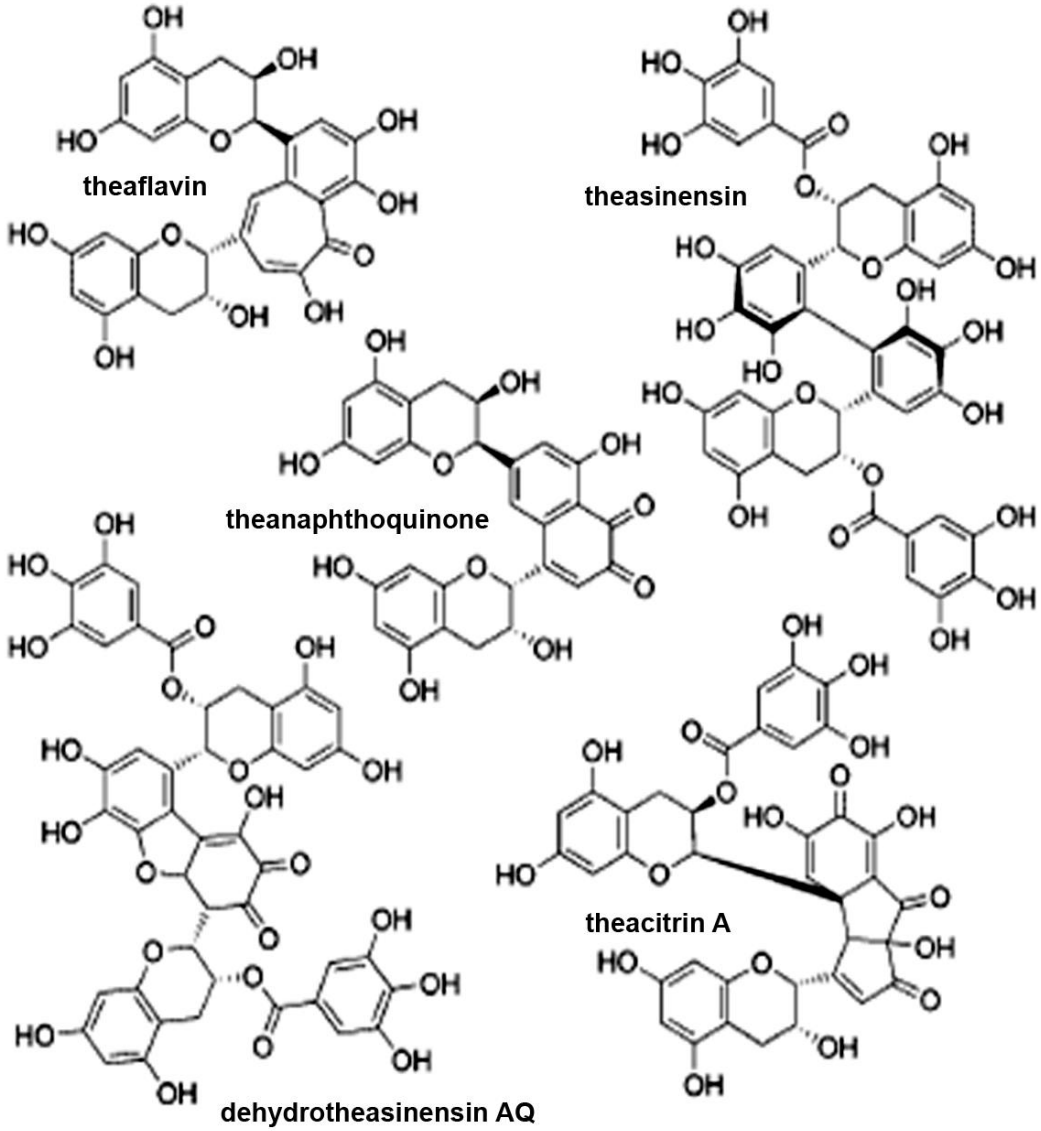

Figure 3: Theatannins resulting from autoxidative degradation of tea catechins (Quideau et al., 2011) (Figure reprinted by permission)

Many factors including pH trigger the autoxidative degradation (Mochizuki et al., 2002) (Hagerman et al., 2003). As a weak acid with a pKa of 7.99 EGCG deprotonation starts at pH > 3.6. Deprotonated EGCG is a stronger electron donor than its undissociated form. The higher the pH, the higher is the degradation rate as EGCG deprotonates to a higher degree. Furthermore, increased pH increases the stability of superoxide radicals, whereas protons counteract the radical chain reaction as the reactive superoxide radicals react with the protons and generate hydrogen peroxide, which cannot oxidize EGCG. Additionally, protons stabilize the EGCG semiquinone radicals.

The degradative reaction is described in detail by Mochizuki et al. (Fig. 4) by a first slow initiation reaction, which is slow due to the low redox potential of the educts oxygen and catechin. This initiation results in the formation of a semiquinone radical, a superoxide radical and two protons. The superoxide radical is a stronger oxidant than oxygen, hence the propagation proceeds much faster. Furthermore, the semiquinone contributes to the faster propagation as it represents a better electron donor than the catechin and the quinones can conproportionate with the catechins under the formation of two new semiquinones.

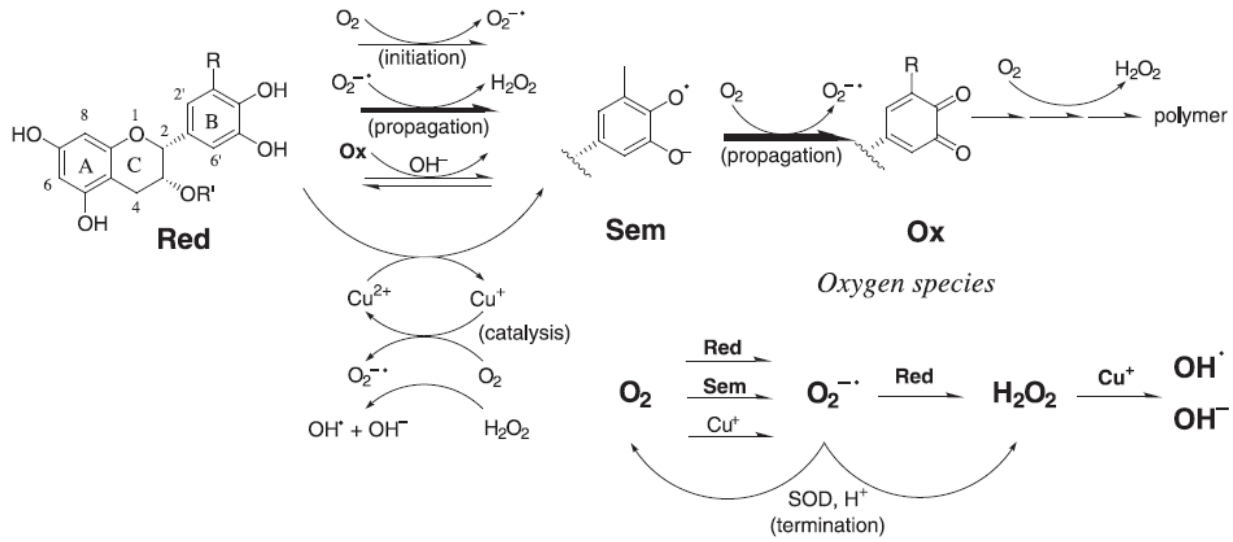

Figure 4: Mechanism of autoxidation proposed by Mochizuki et al. 2002 (Figure reprinted by permission)

But attention should not only be payed to the pKa value of EGCG but as well to the pKa of the semiquinone radicals. It is much lower than the pKa of EGCG. Hagerman et al. reported a pKa value of 4.8. Consequently, once the semiquinone radical is formed, pH would need to be adjusted much lower than pH 3.6 to prevent further degradation.
This difference in pKa values is as well the reason for the degradation of EGCG even at low pH over long term, as extracts might contain traces of these degradation intermediates of EGCG.
Furthermore, Li et al. investigated the pH optimum for EGCG in aqueous solution and found it around pH 3.7 at 25°C and pH 3.9 at 40°C with EGCG degradation occurring at this pH with the slowest degradation rate (Li et al., 2012). Above and below the pH optimum the degradation rate inclines. Thus simple acidification of products does not result necessarily in EGCG stabilization.

Understanding the autoxidative degradation, it becomes clear, that the presence of radicals and quinones within the EGCG extract, for example due to extraction or storage, can induce even at an acidic environment degradation. The presence of such byproducts is supported by the brownish colour of EGCG extract (Hagerman et al., 2003)(Severino et al., 2009). Severino et al. investigated the degradation of EGCG in non-aqueous systems with superoxide radicals and demonstrated that even in DMSO systems degradation might occur. This might be the reason for EGCG extract instability even in its solid form.

Another factor influencing the autoxidative degradation are metal ions. Especially trace metals like iron, magnesium, zinc and copper are capable of facilitating catechin deprotonation by complex formation. For example trivalent iron ions reduce the pKa value from 7.6 to 3.8, aluminium reduces it to 4.4 and by this facilitates the autoxidation (Kumamoto et al., 2001).
This complexity of degradation reactions and conditions leading to active loss emphasizes the need of proper stability studies in aqueous systems as well as in solid state and the care which needs to be taken when developing a delivery system for EGCG.

1.4.1. Effect of dissolution medium

Several working groups investigated the effect of dissolution medium on EGCG degradation. Zimeri and Tong investigated the effect of oxygen, temperature and pH of citrate buffer solutions from pH 4 to 7 and found a log-linear relationship for oxygen and pH (Zimeri and Tong, 1999). Interestingly, even the exclusion of oxygen does not prevent EGCG degradation, thus the formation of superoxide radicals from oxygen is not the only possibility to start the autoxidative degradation. But reducing the amount of dissolved oxygen clearly reduced the degradation rate.

Increasing temperature leads as well to stronger degradation of EGCG. This temperature-dependent degradation and epimerization occurs as well during infusion of green tea (Komatsu et al., 1993).

The osmolarity of medium is another factor contributing to the degradation of EGCG. Increasing the ionic strength of pH 5 solutions by addition of sodium chloride increased the degradation rate of EGCG (Proniuk et al., 2002).

Most of the dissolution media tested for EGCG were not digestive media but Neilson et al. investigated the fate of EGCG in a two-stage in vitro digestion model that took into account the proper pH adjustment due to gastric emptying into the intestine, digestive secretion of bile acids and their conjugates, reduced oxygen levels, absence of light and presence of digestive enzymes like pepsin, lipase and pancreatin. Under these conditions, around 5 % of EGCG is already lost during the gastric phase (simulated for 1 hour). After 2 hours under the digestive conditions of the small intestine, 65 up to 86 % EGCG is lost depending on its initial concentration (Neilson et al., 2007).
This demonstrates the high EGCG loss during the gastrointestinal passage and its contribution to the observed low oral bioavailability of EGCG.

Although the degree of degradation and its pattern is highly variable throughout the literature, all are consistent in the dissolution medium factors affecting EGCG stability:

- pH: autoxidation and epimerization rate increases with pH
- proteins: digestive enzymes and food proteins bind EGCG
- oxygen content: autoxidation rate increases with increasing oxygen content
- temperature: autoxidation rate increases with increasing temperature
- osmolarity: autoxidation rate increases with increasing osmolarity
- metal ions: type and amount of metal ions influence EGCG dissociation and consequently its degradation

1.4.2. Effect of (-)-Epigallocatechin-3-gallate concentration

EGCG is known to show concentration dependent degradation in aqueous solution at pH > 5 (Sang et al., 2005) (Li et al., 2012) with less EGCG being degraded percentage-wise when its concentration is increased.
The concentration-dependent degradation is found as well in in vitro digestive models (Neilson et al., 2007), thus might occur in vivo as well.
The mechanism underlying this phenomenon remained uninvestigated, and thus was investigated in this work.

1.4.3. Effect of additives on (-)-Epigallocatechin-3-gallate stability

Several attempts were undertaken to increase green tea catechin stability by the use of additives. Due to their oxidative degradation pattern, antioxidants or procedures to reduce oxidation were emphasized.
Superoxide dismutase, an enzyme present in plants, some bacteria and the human body, transforms superoxide radicals into oxygen and hydrogen peroxide. As EGCG cannot be radicalized by hydrogen peroxide but by superoxide radicals, capturing the superoxide radicals produced during EGCG degradation enhanced significantly its stability in solution (Sang et al., 2005).

Organic solvents like glycerin and diethylene glycol monoethyl ether are another option to increase EGCG stability. In organic solvents EGCG degradation is reduced due to the lack of dissociation and proton-transfer possibilities (Proniuk et al., 2002). On the other hand, propylene glycol and polyethylene glycol 200 were less effective according to Proniuk et al.. This is consistent with literature which describes formation of peroxide impurities upon storage by polyoxyethylene group containing excipients (Tamilvanan, 2008).

As some metals are capable of facilitating EGCG dissociation (Kumamoto et al., 2001), capturing them with the metal complexing agent ethylenediaminetetraacetic acid (EDTA) increases tea catechin stability as well (Sang et al., 2005).
Some proteins like human serum albumin and ß-casein were capable of stabilizing EGCG (Bae et al., 2009) (Arts et al., 2002). In the case of serum albumin, the sulfhydryl containing amino acids are responsible for the protective effect. Cysteine and glutathione have thiol groups which can undergo oxidation to form disulfide bonds with each other. This way they exhibit an antioxidant effect.
In the case of ß-casein, which contains a high amount of proline in its protein structure (35 of 224), the stabilization of EGCG is mainly mediated by the interaction of EGCG with the proline residues of the protein. Proline does not have any antioxidant property like cysteine or glutathione. Here the stabilization is derived by an interaction of ketone groups and amino groups through hydrogen bonds and hydrophobic interactions of EGCG ring system and the heterocycle of proline (Fig. 5).

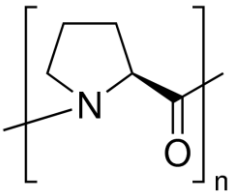

Figure 5: structure of poly-L-prolin

This interaction is comparable to the adsorption mechanism of polyphenols to polyvinylpolypyrrolidone (section 1.7.4) which is used to clear beverages like beer from polyphenols to reduce their haze formation (Markovic et al., 2003). Due to the interaction, EGCG vicinal hydroxyl groups become less reactive towards autoxidation.

But as postulated by Arts et al., these interactions reduce as well the antioxidant capacity and thus the pharmacological efficacy of EGCG. This was proven in a clinical study performed on healthy volunteers. The effect of drinking tea with and without milk was investigated. The outcome was that bioavailability of catechins in blood was reduced due to the interaction with milk proteins (Hof et al., 1998).
Complexation with proteins is a potential way to stabilize tea catechins within the gastrointestinal tract. This was not just shown for deliberately added proteins but as well for proteins present within the saliva. The interaction of EGCG with proline-rich proteins from saliva increased the stability of EGCG in simulated gastrointestinal fluids (Naz et al., 2011).

Accordingly, any other interaction with macromolecules which reduces the reactivity of the vicinal hydroxyl groups of EGCG will increase its stability.
But the problem arising from these interactions might be a reduced absorption due to the size of aggregates. 500 Da is set as the limit for passive diffusion according to “Lipinski’s rule of five”. As EGCG has already a molecular weight of 458 Da, an association with other molecules will increase the size above 500 Da and potentially counteract the passive diffusion process and make dissociation of the formed complex an additional necessary step before absorption. Hence, by this method of stabilization, neither an improvement of antioxidant capacity and probably nor of the bioavailability can be induced.
Nevertheless, it is noteworthy as this interaction is thought to be responsible for EGCG’s long half-life within blood.

1.4.3.1. Ascorbic acid as stabilizer for EGCG

Ascorbic acid is a well-known hydrophilic antioxidant which is often used as stabilizer in food and pharmaceutical products as it can act as reducing agent and free radical scavenger interacting with hydrogen peroxide, peroxyl radicals, superoxide radicals and many other oxygen radicals (Yen et al., 2002)(Niki, 1991) . This way it is capable of terminating radical chain reactions.

Additionally, it can regenerate other antioxidant radicals like α-tocopherol radicals by reducing them back into their native form (Niki, 1987). The reducing ability increases with concentration. And the concentration of ascorbic acid determines about its action either as pro- or antioxidant (Yen et al., 2002). Ascorbic acid first undergoes deprotonation to form dehydroascorbic acid, making the degradation kinetic pH-dependent (Fig. 6).

The further degradation occurs under the formation of several degradation products, whereby one volatile product is carbon dioxide (Shephard et al., 1999).

The reactions of ascorbic acid and its degradation products are not fully investigated yet. But it is known, that ascorbic acid can undergo anaerobic and aerobic oxidation (Yuan and Chen, 1998).

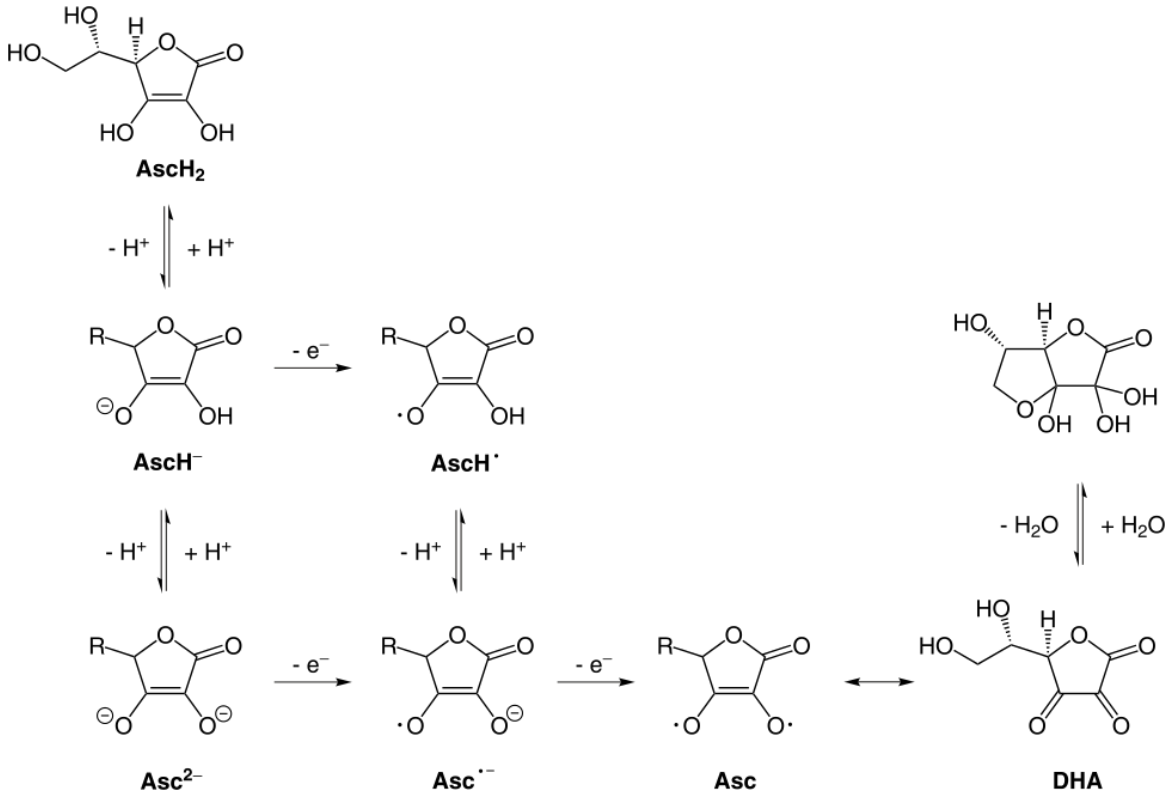

Figure 6: Radical formation by ascorbic acid (www.wikipedia.de)

Furthermore, it is also recognized that degradation products in solution differ from the ones in solid state. The degradation of ascorbic acid goes along with a discoloration up to a dark brown color. Interestingly, the color change appears before any

measurable degradation (Shephard et al., 1999). Nevertheless, it is an undesirable effect in beverages and pharmaceutics.

But ascorbic acid is not just an antioxidative stabilizer. It is an active ingredient itself that showed its beneficial effect in epidemiologic and in vitro studies, for example on cardiovascular diseases (Khaw et al., 2001) or lipid peroxidation in human low density proteins (Retsky and Frei, 1995).
Green tea catechins in aqueous solution of elevated pH can be stabilized by ascorbic acid (Chen et al., 1998). Chen et al. showed that addition of ascorbic acid to pH 7.4 buffer did not alter much the pH but showed significant stabilization of green tea catechins. But EGCG was just stabilized for a certain time. After 12 hours a steep decrease in EGCG content could be observed.

Beside in buffer systems, ascorbic acid also showed significant stabilization of EGCG in the two-stage in vitro digestive model of Green et al.. More than 10-fold higher EGCG amount could be recovered after the simulation of the gastro-intestinal passage of EGCG due to the addition of ascorbic acid. Other antioxidants like gallic acid, Trolox (water-soluble α-tocopherol derivative) or the synthetic antioxidant BHT (2,6-di-tert-butyl-4-methyl-phenol) were tested as well but ascorbic acid showed superiority over the other antioxidants in stabilizing EGCG (Green et al., 2007).
The stabilizing effect is dose dependent. Already at a 1:2 (w/w) ratio (ascorbic acid : EGCG) ascorbic acid is a potent stabilizer (Green et al., 2007). And a continuous increase of catechin recovery can be observed from 1:10 up to 1:2 (w/w) ratio (ascorbic acid : green tea extract) (Chen et al., 1998).
All authors concluded a more or less pronounced stabilization of EGCG in aqueous solutions due to the addition of ascorbic acid.
Additionally, the combination of EGCG and ascorbic acid enhances the pharmacological effects of EGCG. In a 1:1 (w/w) ratio the antibacterial effect of EGCG on MRSA was found to be prolonged and by this the MIC for the antibiotic oxacillin was reduced (Hatano et al., 2008). In summary, ascorbic acid is according to the literature a potent stabilizer for catechins like EGCG in buffer systems and digestive media and enhances their pharmacological effects in in-vitro studies.

But different observations were made in solid state. Ortiz et al. 2008 showed that the addition of ascorbic acid to green tea powder has up to a relative humidity of 70 % no effect on EGCG stability within the extract. But increasing the humidity results in a destabilization of EGCG. Furthermore, ascorbic acid/ green tea powder blends resulted in caking at 22°C/43 % RH, transformed into a plastic mass already at 69 % and at 75 % RH browning of ascorbic acid crystals could be observed. A transformation from solid to liquid occurs (deliquescence). The critical relative humidity of ascorbic acid is reduced by the green tea powder and ascorbic acid dissolves in the absorbed moisture (Ortiz et al., 2009). As a consequence of the higher moisture uptake degradation reactions are facilitated.

Hiatt et al. investigated the deliquescence behavior (lowering of critical relative humidity) of several ascorbic acid forms and found no deliquescence behavior for ascorbic acid up to 94 % relative humidity (RH) as single compound, but deliquescence for sodium ascorbate at relative humidity of 86 %. For a binary mixture of both, 82 % RH is already sufficient to induce deliquescence. Sodium ascorbate in a 1:1 (w/w) ratio with dehydroascorbic acid, which is a degradation product of ascorbic acid, lowered the deliquescence point even to 78 % RH. This points out that ascorbic acid in combination with other substances can result in deliquescence at lower relative humidity (Hiatt et al., 2011). The transition is clearly dependent on the compounds of mixture: thus it differs for individual ascorbic acid forms as well as for different combination partners. Reaching the deliquescence point goes along with high the moisture uptake and ascorbic acid degradation, thus radical formation. These radicals can act as pro-oxidants for other substances like EGCG.
This can occur as well in tablet formulations containing both substances and due to other excipients present within the tablet.
Ortiz et al. report as well the browning of ascorbic acid/citric acid blends without tea catechins. Two important points can be derived from this. First of all, ascorbic acid degradation in liquefied samples cannot be prevented by acidification. And secondly, it is not the presence of EGCG with its pro-oxidant property which starts the degradation reactions.

Furthermore, ascorbate can act as nucleophile and thus make substitution reaction and Michael-Additions with substances. Dehydroascorbate can act as electrophile (Kesinger and Stevens, 2009). All of these reactions can potentially occur with EGCG as it offers in its ring system positions for both kinds of reactions. Hashimoto et al. found indeed in oolong tea one of these products (Hashimoto et al., 1989). Although ascorbylated EGCG is not necessarily less active as shown by Oku et al. 2003, its formation should be avoided in dosage forms as it changes the pharmacological effects of EGCG (Kesinger and Stevens, 2009).

Focus on solid formulations containing EGCG and ascorbic acid should be taken under the aspect of their compatibility. Due to ascorbic acid potential to enhance EGCG stability within gastro-intestinal fluids (Green et al., 2007) and resulting enhancement of pharmacological effects of EGCG (Hatano et al., 2008), co-formulation of these two antioxidants is beneficial. Therefore stability studies of EGCG immediate release tablets containing different ascorbic acid forms at different loadings were investigated and deliquescence studies of ascorbic acid, sodium ascorbate and their binary 2:1 (w/w) mixture with EGCG and tableting excipients used for the formulation of EGCG immediate release tablets were performed in this work.

1.5. Epimerization

Beside autoxidation, EGCG can undergo epimerization to its stereoisomer gallocatechin-3-gallate (GCG). The epimerization occurs at a pH above 5 (Fig. 7). Analogical to the autoxidation, increasing the pH lead as well to a higher degree of epimerization and acidification reduces it (Komatsu et al., 1993).

Furthermore, increasing the temperature leads to a higher epimerization rate (Wang et al., 2008) and the epimerization is like the autoxidation dependent on the osmolarity of the medium: in tap water more conversion to GCG occurs than in purified water (Wang and Helliwell, 2000).

The epimer gallocatechin-3-gallate has at concentrations from 0.3 mM onwards the same radical scavenging effect as EGCG, and below this concentration it exhibits even a stronger radical scavenging effect (Guo et al., 1999).

Although conversion of EGCG is not a loss in pharmacological activity as was shown in a study by Hirai et al. where both catechins showed in guinea pigs with ischemia–reperfusion injury a pronounced effect on the recovery (Hirai et al., 2007), it still alters the pharmacological activity either in a positive or negative way as most of the molecular mechanism of EGCG arise from interactions with proteins. A change in its conformation can lead either to stronger interactions with target proteins or to weaker ones.

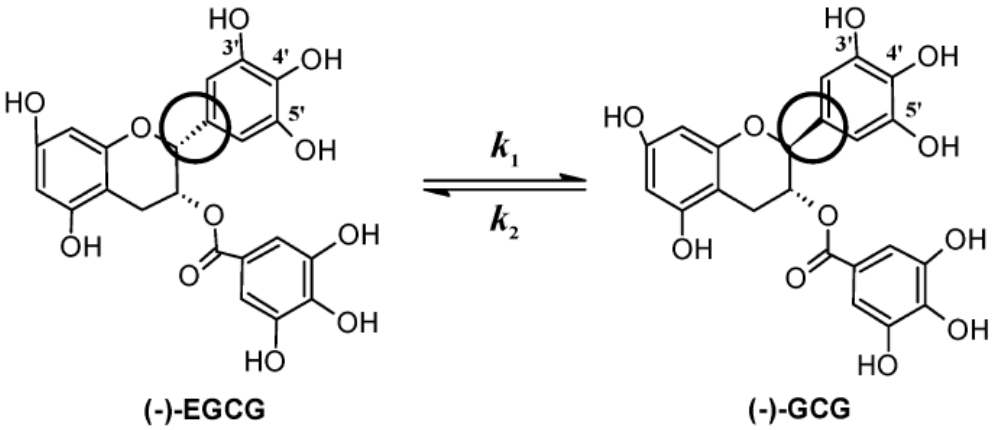

Figure 7: Epimerization of EGCG (Wang et al., 2008) (Figure reprinted by permission)

1.6. Self-association of (-)-Epigallocatechin-3-gallate

Self-association occurs when substances are amphiphilic in nature. The self-association of polyphenols is well known in the literature (Pianet et al., 2008). Wròblewski et al. investigated the self-association of EGCG in pH 3.0. They performed H-NMR-studies on EGCG solutions ranging from 0 to 110 mM (corresponding to 50.4 $^{g}/_{l}$). The strongest proton shift was observed for the protons from C-ring followed by the protons from B-ring (Fig. 8) indicating a hydrophobic interaction of the aromatic rings with each other.

The protons of the A-ring showed the smallest shift; thus the A-ring is less involved in the self-association of EGCG. And the galloylester group (D-ring) showed no shift at all, indicating that this ring is not involved in the self-association and thus represents the outer, hydrophilic side of the molecule. The reactive groups of the EGCG molecule are the vicinal hydroxyl groups from the B-ring that contribute to the associate formation. The interaction of the B- and C-ring system results in electron shifts that can change the reactivity. Hence, the formation of associates can alter the reactivity of EGCG and result in a decreased or enhanced degradation.

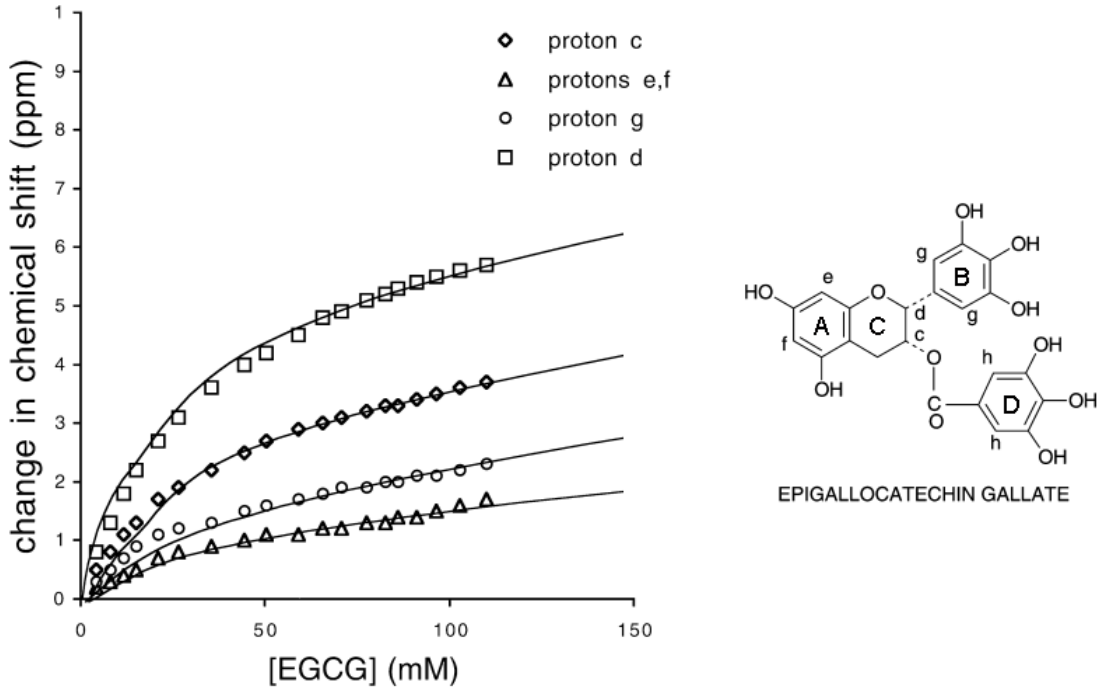

Figure 8: Proton chemical shifts measured by Wròblewski et al. 2001 upon EGCG self-assembly (Figures reprinted by permission)

The chemical structure of EGCG consists of a rigid hydrophobic ring structure (A- and C-ring) which is associated with rings containing hydrophilic hydroxyl groups (B- and D-ring) representing the hydrophilic side of the molecule. The hydrophobicity of a tea catechin molecule is mediated by the galloyl moiety increasing it, the hydrophilicity by the amount of hydroxyl groups (Hashimoto et al., 1999).

Wróblewski et al. assumed a stacking-type of association, i.e. the formation of dimers, trimers and oligomers and calculated from this a self-association constant for EGCG that lies around 20 M^{-1}.

In the literature, many papers can be found investigating surface active drugs (e.g. antibiotics, benzodiazepine, local anesthetics), their self-assembly and resulting modulation of physicochemical properties like chemical stability (Schreier et al., 2000).

But until now no one has correlated the concentration dependent degradation of tea catechins to their self-association. Therefore the phenomenon of concentration-dependent degradation of EGCG was investigated in this work under the scope of the formation of EGCG self-associates.

1.7. Complexation of (-)-Epigallocatechin-3-gallate

Several polyphenols are reported in the literature to interact with polymers, polysaccharides and proteins under the formation of complexes. The interaction of EGCG with macromolecules results either in coacervate formation or in the formation of unstable colloids that form precipitates. These interactions can lead to phase-separation and hence can slow down EGCG release and even reduce the dose of EGCG ready for absorption as reported ,for example, for naltrexone/Eudragit® L complexation (Alvarez-fuentes et al., 1997).

1.7.1. Complexation, coacervate formation and precipitation

When talking about the interactions of EGCG with other substances and the resulting physical instability, it is important to clarify all the different physical instabilities that can occur.

First of all, an interaction needs to take place by two different molecules due to hydrophobic and/or hydrophilic interactions. These interactions can be π-interactions, van-der-Waals interactions, Lewis-acid-base reactions and hydrogen bonds. This results in the formation of small complexes that can still be soluble in water. As further interactions of the small complexes can lead to bigger complexes, this results at a certain concentration or reactant ratio in the formation of insoluble complexes that separate from the solvent. This phase-separation can either result in a liquid-liquid system (coacervation) or in a solid-liquid system (flocculation/ precipitation). An important factor contributing to the phase-separation of EGCG with macromolecules is the multivalence of EGCG. EGCG with its eight hydroxyl groups offers several electron-donor and electron-acceptor sides and consequently enables the cross-linking after the first step of soluble complex formation. Furthermore, EGCG can interact by Lewis-acid-base reactions with some amino acids and act this way as a reversible or irreversible cross-linking agent. In a comparable way, polymer- or polysaccharide-EGCG complexes are formed. Depending on the strength of enthalpic interactions of the solvent and the complexes, a precipitate or a coacervate by complex coacervation can form (Gander et al., 2002).

The complex coacervation, takes place due to electrostatic or polar interactions in a Lewis-acid-base reaction, i.e. due to interaction between electron-donor and -acceptor sides of molecules. The resulting liquid phases contain both solutes. One phase is rich in both, the other poor in both. But the concentration ratio remains the same in both phases. This type of coacervation occurs at low concentrations of the reactants.
Coagulation of these coacervates can occur due to further electrostatic and/or interfacial attraction of the microdroplets (van Oss, 1988).

Flocculation or precipitation, on the other hand, is a solid-liquid phase-separation. It results when a colloidal unstable sol is formed upon the interaction of the solutes.
Van Oss published a table of the mechanism and conditions for coacervation and flocculation. First of all, he explains that the same mechanism is underlying both coacervation and flocculation and that coacervate can undergo flocculation when the complexes become insoluble in the liquid phase. Hence all three processes are connected with each other and the potential of EGCG to interact with several classes of macromolecules can go along with a phase-separation.

In the following sections, detailed examples of interactions with different classes of macromolecules will be emphasized as many of the macromolecules interacting with EGCG are common excipients used as binders, disintegrants or fillers in dosage forms.
Interactions occurring can lead to altered performance of the dosage forms such as a delayed release or a part of EGCG can be captured in insoluble complexes and result in an incomplete release. Furthermore, these interactions are accompanied by electron-shifts and they might induce chemical instability of EGCG upon storage.
And additionally, it is uncertain if the soluble complexes do not exhibit a lower partition coefficient across the intestinal epithelium because of high molecular weight and thus reduce the absorption of EGCG.

1.7.2. Complexation with proteins

Several working groups took a deeper look into the mechanism of EGCG/protein interactions and found them to be related to certain amino acids, with which EGCG can undergo hydrophobic and hydrophilic interactions.

The hydrophobic interactions of the catechin ring system are thought to be the driving force for this complexation, whereas the hydrophilic interactions of hydroxyl groups of polyphenols with the hydrogen acceptor sides of proteins reinforce the binding (Pascal et al., 2007) (Fig. 9).

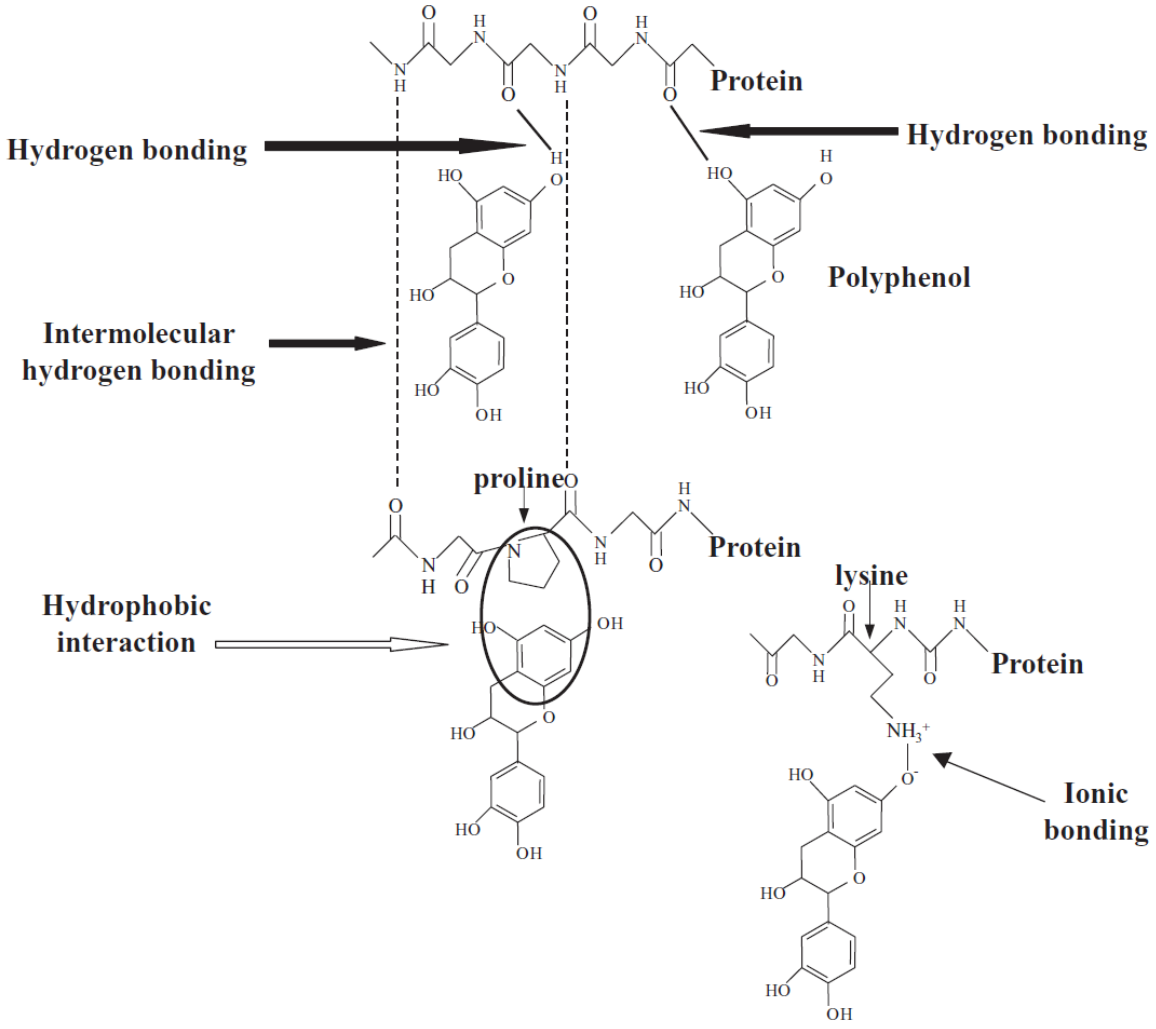

Figure 9: Mechanisms of interactions between proteins and polyphenols (Le Bourvellec and Renard, 2012) (Figure reprinted by permission)

The interaction is proposed to be a three-step mechanism (Fig. 10), starting with a first phase, where EGCG covers the protein and results in soluble complexes. The second step is a multivalent interaction resulting in protein cross-linking and thus the formation of insoluble complexes, the third step is the precipitation of aggregates which can be measured in form of turbidity (Le Bourvellec and Renard, 2012). The transition from one step to the other is mediated by the concentration of the reactants (Fig. 10).

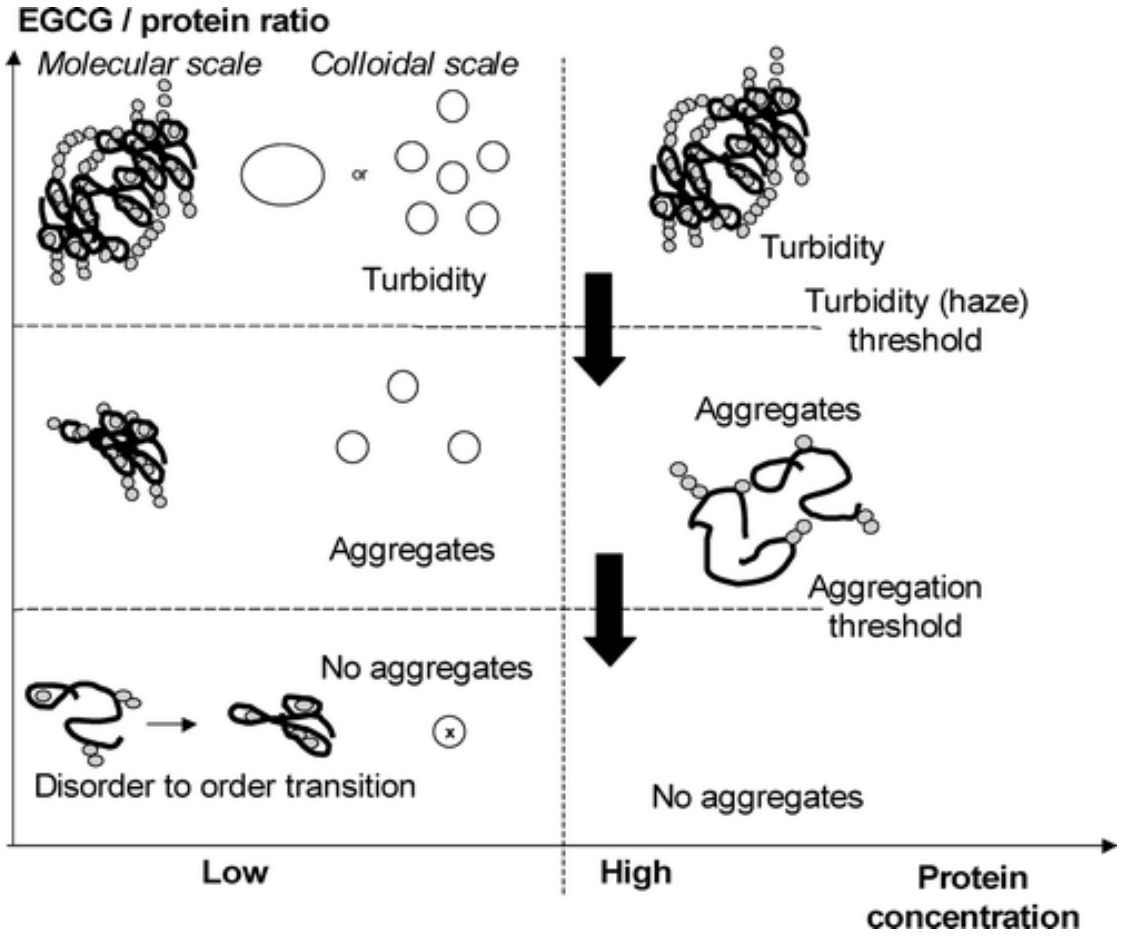

Figure 10: Proposed three-step mechanism of complex formation between proteins and polyphenols (Pascal et al., 2007) (Figure reprinted by permission)

A broad range of amino acids can be involved in EGCG complexation. In the context of human serum albumin interaction with EGCG, cysteine and the tripeptide glutathione are mainly responsible for the non-covalent bonds resulting in complex formation with serum albumin (Bae et al., 2009).

Other working groups demonstrated an affinity of EGCG to proline residues by investigating the interaction of EGCG with poly-L-proline (Poncetlegrand et al., 2006) and with human salivary proteins, which contain amongst others basic proline-rich proteins (Pascal et al., 2007). Gelatin that is hydrolysed collagen and consists of a mixture of proteins and peptides interacts as well with EGCG. The gelatin structure consists of a high proportion of tripeptide units out of glycine, proline/ hydroxyproline and a third amino acid which is variable (Graßmann et al., 1956). Consequently, gelatin contains a high amount of proline and hydroxyproline with which EGCG can undergo interactions.
Wróblewski et al. investigated the interaction of EGCG with another group of salivary proteins named histatin which contain nearly no proline. The interaction with histatins was shown to be even stronger than the interaction observed with the proline-rich proteins. They investigated the participation of the amino acids present in histatin in the complex formation by H-NMR measurements and found that phenylalanine, tyrosine, histidine, lysine and arginine interact with EGCG (Wróblewski et al., 2001).

The amino acids nowadays known to be involved in the complex formation of proteins with EGCG are arginine, cysteine, histidine, lysine, phenylalanine, proline and tyrosine. This elucidates the reason why EGCG can interact with such a broad range of proteins.

1.7.3. Complexation with polysaccharides

In the case of polysaccharides, as well several are capable of interacting with EGCG. For example EGCG forms 1:1 inclusion complexes with ß-cyclodextrin (ß-CD) and its derivatives dimethyl-ß-CD and 2-hydroxypropyl-ß-CD. The ring A and C of the EGCG molecule are localized within the hydrophobic cavity of ß-CD interacting through van-der-Waals and hydrophobic interactions. The ring B and B‘ form hydrogen bonds with the hydroxyl groups of the hydrophilic secondary rim of ß-CD (Ishizu et al., 2006) (Fig. 11+12). These interactions lead to an enhanced stability of EGCG that is even more pronounced in the case of ß-cyclodextrin derivatives as they contain side chains in the rim region for further interactions (Folch-Cano et al., 2010).

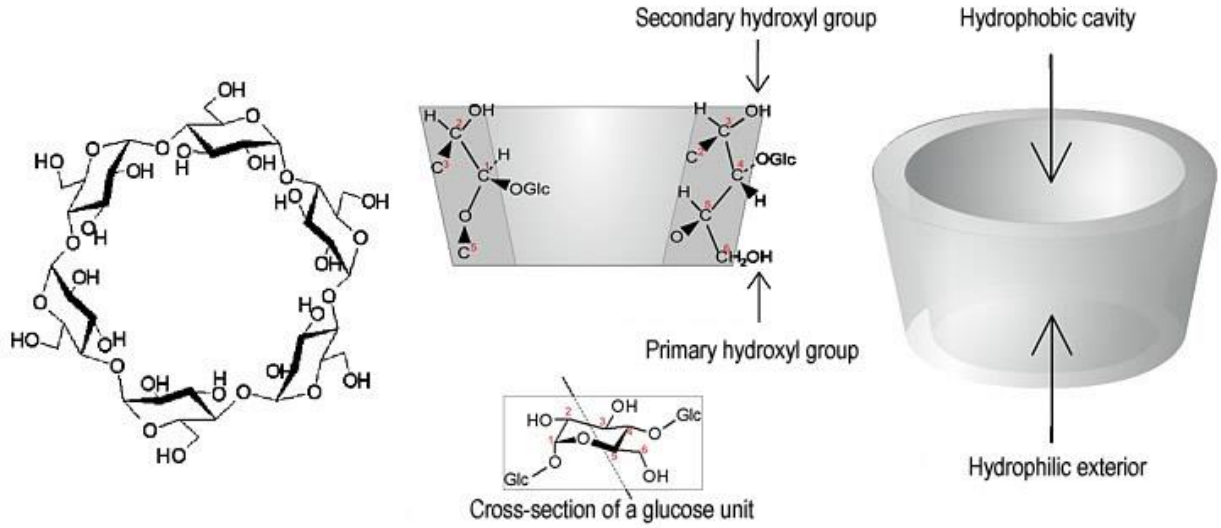

Figure 11: Structure of ß-cyclodextrins (www.chemiedidaktik.uni-wuppertal.de)

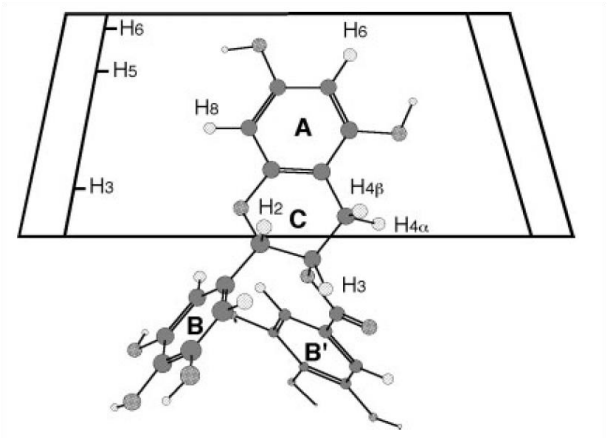

Figure 12: Proposed EGCG complexation by ß-cyclodextrin measured by Ishizu et al.2006 (Figure reprinted by permission)

Another polysaccharide interacting with EGCG is the heteropolysaccharide pectin (Fig. 13). Pectin is rich in galacturonic acid that can be esterified with methanol or amidated. Hayashi et al. found that citrus pectin forms soluble complexes with EGCG and thus reduces the astringency of tea catechin (Hayashi et al., 2005). The proton shifts indicate as well a hydrophobic interaction of the catechin ring system with the pectin whereby the galloylester group is not involved.

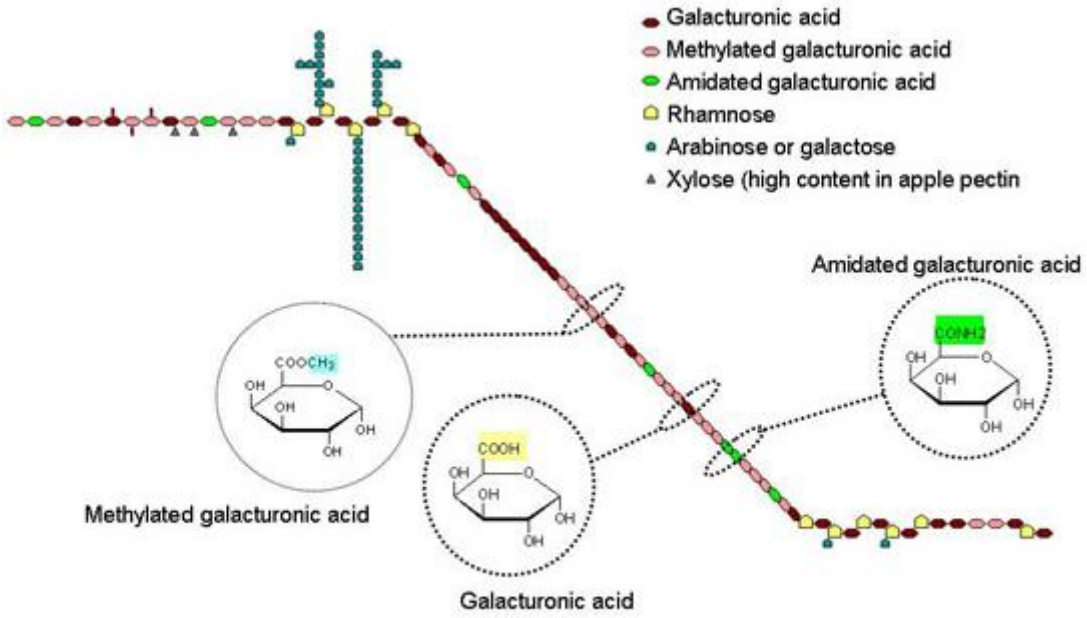

Figure 13: Pectin structure (http://cdavies.wordpress.com)

The polysaccharide chitosan (Fig. 14) interacts with EGCG in a comparable way to proteins by first forming soluble complexes. These complexes can aggregate and result in precipitates (Popa et al., 2000).

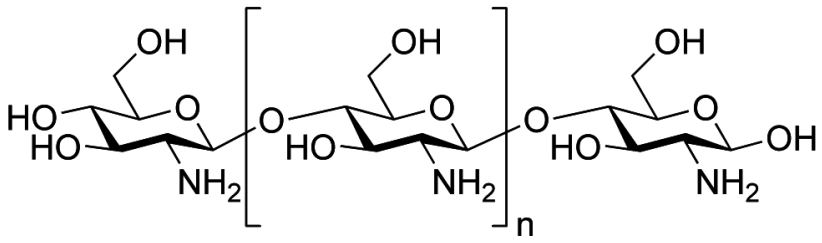

Figure 14: Structure of chitosan

In the case of chitosan, the aggregation of EGCG with chitosan was used for a new delivery system (section 1.13) from which EGCG was released in alkaline media (pH above 7.8). At alkaline pH the amino groups of chitosan were positively charged and so the interactions within the complex were weakened.
Furthermore, in in vivo studies performed with mice an enhanced intestinal stability of EGCG was observed due to its complexation with chitosan (Dube et al., 2011).

Starch is another commonly used polysaccharide in beverage and pharmaceutical products which also interacts with polyphenols. Wu et al. found that tea polyphenols reduce the viscosity of gelatinized starch. By the use of FT-IR they found hydrogen bonds being responsible for the reduced viscosity. Thus tea polyphenols and starch can interact with each other, the properties of the polysaccharide can be changed and hence the performance of a dosage form (Wu et al., 2011)**.**

1.7.4. Complexation with synthetic and semisynthetic polymers

Patel et al. investigated the interaction of EGCG with the water-soluble cellulose ethers methylcellulose and hydroxypropyl methylcellulose. They found the formation of particles from 95 up to 300 nm dependent on the chosen weight ratio of EGCG to methylcellulose (A. R. Patel et al., 2011). They prepared even firm beads of EGCG with a diameter of 0.6 up to 2.5 mm by dropping cellulose ether solutions into an EGCG solution (Ashok R. Patel et al., 2011). Although presented by Patel et al. as an opportunity for improved EGCG delivery, the increased stability which was postulated by Patel et al. in the case of EGCG-methylcellulose colloids exhibited just half of the antioxidant activity of free EGCG. Furthermore, the EGCG release from these particles in simulated gastric fluid and simulated intestinal fluid was just 40 % and 60 %, respectively, indicating that EGCG was captured in insoluble complexes within the beads.

Likewise, the interaction of EGCG with PVP is well known. Polyvinylpolypyrrolidone (Fig. 15) is used to filter beverages like beer to get rid of polyphenols and thus clear and stabilize the beverage (Siebert, 1999). The interaction is reported to be through

hydrogen bonds between the hydroxyl groups from the polyphenol and the carbonyl group from PVPP, hydrophobic interactions and an additional delocalization of electrons due to π-electron interactions (Rehmanji et al., 2005).

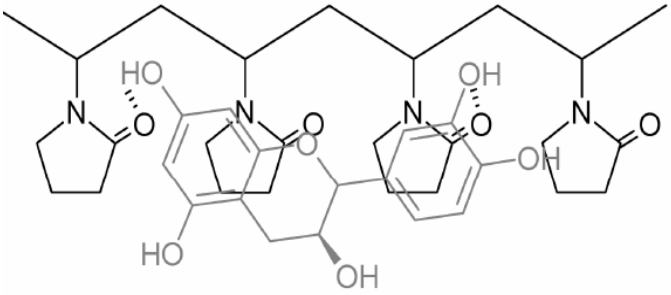

Figure 15: Proposed interaction of polyvinylpolypyrrolidone (PVPP) with polyphenols (Rehmanji et al., 2005) (Figure reprinted by permission)

1.8. Oral bioavailability of (-)-Epigallocatechin-3-gallate

Bioavailability is, according to the FDA, “the rate and extent that the active drug is absorbed from a dosage form and becomes available in the systemic circulation”. There are several factors which can affect the oral bioavailability of a drug. Some derive from the dosage form itself and its manufacturing process such as poor disintegration/ dissolution and physical and chemical incompatibilities of the dosage form ingredients. Others arise from the physiological circumstances of the administration route. In the case of peroral dosage forms, important physiological factors are: pH in the gastrointestinal tract which can result in chemical instability of the active pharmaceutical ingredient (API); the absorption of the API within the intestine which might be influenced by transporters; phase I and phase II biotransformation within the gut, cells and liver; and the enterohepatic circulation of the drug.
In particular the factors arising from the physiological circumstances can show high inter- and intraindividual variation. But by an appropriate dosage form development, the weight of all these factor can be reduced and bioavailability increased.

As intravenous administration of EGCG or green tea extract cannot be performed in humans due to safety reasons, absolute bioavailability data of EGCG can only be derived from animal studies. It has to be considered that metabolism and elimination varies among the species as well as the doses in the in vivo studies. Consequently, a broad range of values for the oral bioavailability of EGCG can be found ranging from 0.1 % up to 12.4 % (Feng, 2006) (Chen et al., 1997) (Swezey et al., 2003). Nevertheless, all the results reflect a poor bioavailability of EGCG.
It is certain, that EGCG can be absorbed in the intestine. Tea catechins given orally to rats were identified in the portal vein, i.e. were absorbed within the intestine (Higdon and Frei, 2003). Furthermore, Yang et al. found that EGCG is absorbed through the oral cavity mucosa. Human volunteers who held tea catechin solutions in their mouth without swallowing it exhibited plasma peaks of tea catechins. The possibility of small amounts swallowed was excluded as the t_{max} after peroral dosing of tea catechins was between 1 and 3 hours, but in their study, c_{max} was reached

after 15 min (Yang et al., 1999). This indicates that EGCG can be absorbed through passive diffusion across the epithelium. Thus the observed low oral bioavailability of EGCG has to be attributed to other factors than just poor permeability.

Ullman et al. investigated the pharmacokinetics of EGCG in the case of single and/or repeated dosing of EGCG. EGCG was administered pre-prandial to healthy volunteers as hard gelatin capsule formulations. The first food intake was 2 hours after the swallowing of the capsules. Hence no food effect can be expected. This is important to mention, as other working groups investigated the uptake of tea catechins from capsules administered after a meal or with a meal. The consequence was a significant reduction in the bioavailability of the tea catechins.

The kinetic profile of EGCG followed a fast absorption with a one-peak-plasma concentration versus time profile and the maximum plasma concentration was reached within 1 to 3 hours independent of the administration regime. Hence, EGCG must exhibit a high absorption rate with an absorption window in the upper intestinal tract.

The maximum plasma concentration was dependent on the administered dose. According to Ullmann et al., dose linearity from 200 to 1600 mg could be observed for a single dose as well as a constant elimination half-life of approximately 3 hours (Ullmann et al., 2003) (Ullmann et al., 2004). For low doses represented by 200 mg, repeated dosing resulted in a decline in systemic availability and the elimination half-life became faster. Ullmann et al. attributes this to an induced elimination by the alteration of metabolism and/or elimination. But due to the high inter- and intravariability of the data significant results could hardly be generated in this study.

This high variability in pharmacokinetic parameters was described as well by Lee et al. 2002 who repeated the same dosing on individuals three times with a wash-out period of at least one week.

The areas under the curve (AUC) showed in the case of one subject a 4-fold decrease of AUC on repetition. In other subjects, 2-fold higher or lower AUCs were reached.

In other subjects no difference was observed. The AUC differences were accompanied by a difference in the elimination half-lives and in the maximum

plasma-concentrations. This high variability was more pronounced for EGCG than for the ungalloylated tea catechins after ingestion of green tea formulations (Lee et al., 2002).

Chow et al. investigated as well the pharmacokinetics of tea catechin following a single dose administration. Although their volunteers took the green tea formulation with food, and thus food interactions influenced the pharmacokinetic parameters, their analysis of the metabolic fate of the single catechins is interesting in the context of variability.

They found that no glucuronides or sulfates of EGCG can be found in the plasma samples, although they were clearly present in the case of the ungalloylated catechins EGC and EC (Chow et al., 2001). Thus when EGCG reaches the blood stream, it is present in its free unconjugated form. Vice versa this means that metabolites cannot enter the blood stream. Thus the conjugation must play a crucial role in the high inter- and intravariability.

In the following, the physiological circumstances EGCG encounters after peroral administration will be reviewed. From this, an appropriate dosage form design will be deduced to potentially reduce the EGCG loss and thus to increase its oral bioavailability.

1.8.1. Effect of metabolism

EGCG and the other green tea catechins undergo extensive metabolism within the intestine by catechol-O-methyltransferase (COMT), UDP-glucuronosyltransferases (UGT) and sulfotransferases (SULT).

Major metabolites are monomethylated, monosulfated, monoglucuronated and monoglucuronated and -methylated EGCG. Some of the metabolites show activity similar to EGCG. Examples of such metabolites are EGCG-3'-, EGCG-3"- and EGCG-4"-glucuronide (Sang et al., 2007). This arises from the fact, that the activity of EGCG is correlated to the presence of vicinal hydroxyl groups (Higdon and Frei, 2003). When these groups are not affected by the biotransformation, EGCG still can undergo autoxidation and hence is still active. In case of glucuronidation at the 3'-C

position, two vicinal hydroxyl groups are still present. The same holds when the conjugation occurs at the galloylester ring (D-ring) (Fig. 16). On the other hand, if the methylation occurs at the 4'-C position, no vicinal hydroxyl groups are present in the B-ring to undergo autoxidation.

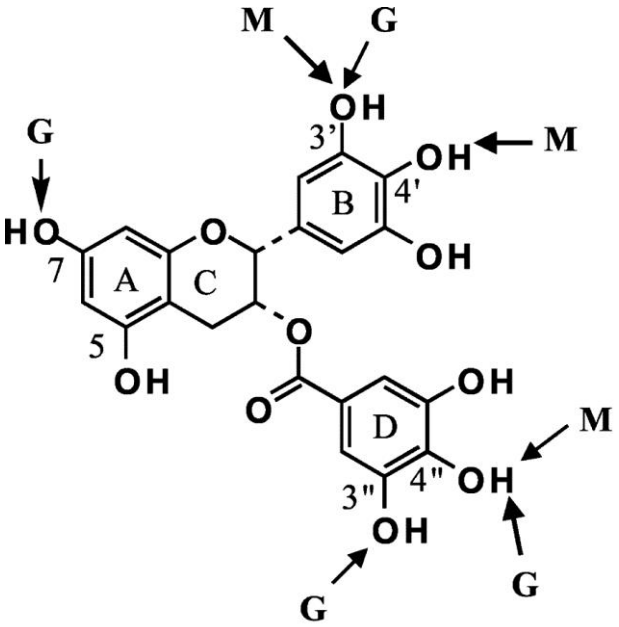

Figure 16: Sides of methylation and glucuronidation of EGCG (Lu et al., 2003) (Figure reprinted by permission)

SULT and COMT are present in the cytosol as well as membrane-bound, and UGTs are membrane-bound enzymes located in the membrane of the endoplasmatic reticulum. UGTs are besides SULTs the main conjugating enzyme in humans (Jancova et al., 2010). But the amount of EGCG-sulfate conjugates recovered after ingestion is lower than the one of EGCG-glucuronides.

Lambert et al. showed that inhibition of the intestinal UGT in mice by piperine resulted in a significant increase of the amount of EGCG available in the small intestine as well as a significantly reduced amount of glucuronated EGCG within the enterocytes (Lambert et al., 2004). But as shown by Lambert et al., the inhibition of glucuronidase by piperine just significantly increased the amount of methylated EGCG.

The enzymes responsible for EGCG glucuronidation, sulfation and methylation are inducible enzymes by external factors like smoking, consumption of drugs, alcohol consumption and nutrition and additionally can differ in their activity due to genetic

disposition (polymorphism), internal factors like age, sex, and disease. For example, a genetic polymorphism in catechol-O-methyltransferase (COMT) results in a 3- to 4-fold change in its activity (Scalbert and Williamson, 2000). Smoking and alcohol consume can result in an alteration of enzymatic activity such as a reduced glucuronidation by UDP-glucuronosyltransferase (UGT) up to 35 % of its normal activity.

But the expression of the metabolizing enzymes varies along the whole intestine. The expression of UGT and SULT is lower in the ileum than in the upper parts of the small intestine; thus less conjugation occurs there (Pang, 2003).
The consequence for a dosage form is the release of EGCG in the ileum when the conjugation wants to be avoided as here the expression of glucuronidase and sulfotransferase is lower than in the other segments of small intestine. This way the amount of EGCG being conjugated into sulfates and glucuronides could be decreased.

1.8.2. Effect of efflux transporters

A large amount of EGCG is transported via passive diffusion through the intestinal membrane (Lambert et al., 2006). EGCG uptake in human colon adenocarcinoma cells was found to be taken up fast into the cells within 5 minutes and then slows then down due to the decreased gradient with further absorption over 3 hours and to be unsaturable up to 640 µM (293 $^{mg}/_{l}$). This indicates that EGCG uptake is mediated by passive diffusion (Hong et al., 2002). But especially at higher concentrations and for the methylated and glucuronated metabolites, multi-drug-resistance-associated proteins (MRP) play a crucial role in absorption and excretion of EGCG. Hong et al. showed in an in vitro model of Madin–Darby canine-kidney type II cells and HT-29 human colon adenocarcinoma cells a significant increase in EGCG uptake by co-administration of MRP-inhibitors. In the case of HT-29 cells, a 2-fold higher uptake of EGCG was observed when an MRP inhibitor (indomethacin and/or probenecid) was co-administered. But not just EGCG uptake was affected by MRP-inhibitors. The

uptake of 4”-EGCG glucuronide, 4”- methyl-EGCG and 4’4’’-dimethyl-EGCG was increased as well (Hong et al., 2002) (Hong et al., 2003).

The multi-drug-resistance-associated proteins belong to the family of ATP-binding cassette transporters (Dietrich et al., 2003). They transport organic anions such as glutathiones, glucuronides and sulfates (Hipfner et al., 1999) and neutral/ unconjugated organic drugs associated with glutathione (Borst et al., 2000).
Seven members are nowadays known, with different distribution in humans. Borst et al. listed their main locations. They are found in high proportions in the liver, kidney and gut as these are the main organs responsible for drug absorption and excretion. Furthermore, they can be found in other organs like lung, pancreas, testis, ovaries, bladder and gallbladder. Even in muscles they are present. Some of them are ubiquitous like MRP1 and 5; others are restricted to certain organs.
The MRP-isoforms also differ in their localization within the cells. They can be located at the basolateral side, within the cell or at the apical side of the membrane. Consequently, they induce flux of substrates out of the cell back into the intestinal lumen or from the cell into the blood (Prime-Chapman et al., 2004).
MRP2 is an isoform localized at the apical side within the intestine and thus transports EGCG and its conjugates back into the lumen. MRP1 is located at the basolateral side and transports EGCG from the cell into the interstitial space (Lambert et al., 2007).
Thus MRP1-expression is desirable in the release window and areas of high MRP2 expression should be avoided. The expression throughout the human intestine was investigated by PCR analysis of human tissue and revealed MRP-isoforms in general being less expressed in the duodenum than in the jejunum and ileum. MRP2 is more pronounced in jejunum and ileum, but lower expression comparable to the one in duodenum was found in the ileocecal region. MRP1 is most pronounced in the jejunum, followed by the ileum and the ileocecal region and is least pronounced in the duodenum (Prime-Chapman et al., 2004). Consequently, the duodenum or ileocecal region should be targeted as release window for substrates of MRP like EGCG.

For the consistence of data it needs to be mentioned that although P-glycoprotein (Pgp) belongs to the same family of transporters as MRP, Pgp-inhibitors had no effect on EGCG uptake, demonstrating that EGCG is not a substrate of Pgp (Hong et al., 2002). But EGCG can modulate Pgp by binding to its substrate side, thus inhibiting and modulating the transport of other substrates (Jodoin et al., 2002). This process is rapid and reversible.

1.8.3. Effect of membrane interaction

In several studies it has been shown that EGCG interacts with lipid bilayers and membranes. Hong et al. tested the recovery of EGCG in HT-29 human colon adenocarcinoma cells and found that approximately 24 % of absorbed EGCG is placed within the membrane and 76 % in cytosol (Hong et al., 2002).Looking in depth at the cell membrane association, EGCG binds 10 times more to the raft fraction than to the lipid bilayer, whereby 80 % is adsorbed by the cholesterol fraction of the lipid rafts (Tachibana et al., 2004) (Fig. 17).

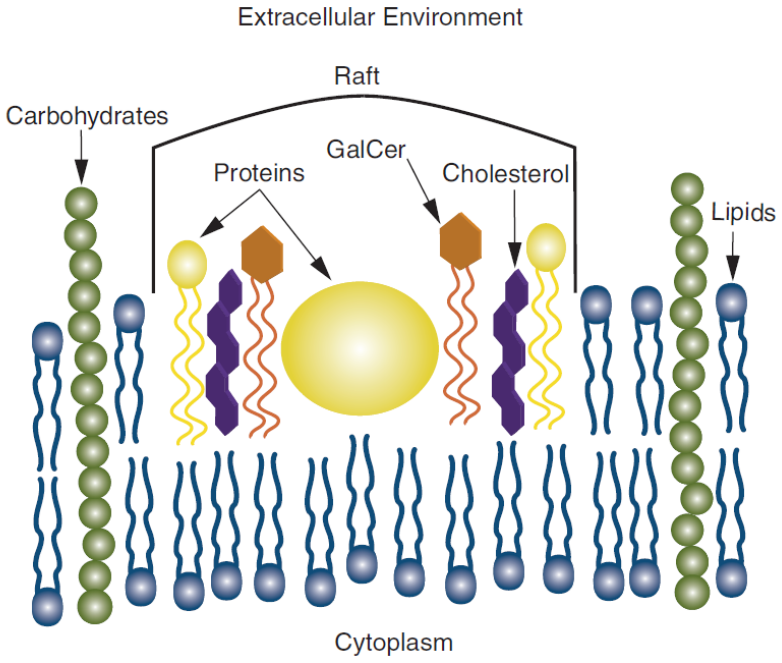

Figure 17: Membrane lipid rafts enriched with cholesterol and galactosyl ceramide (GalCer) and studded with proteins (Alchorn, 2007) (Figure reprinted by permission)

This interaction is thought as well to alter cell signalizing by influencing the membrane fluidity and proteins located in these lipid rafts (Pajak et al., 2011). Thus around one fourth of an orally administered dose of EGCG is lost for systemic absorption due to its accumulation in the cell membrane of epithelial cells.
And it is not surprising, that as a consequence, the highest amount of EGCG after intragastric administration can be recovered in the intestine and colon.

As this fact cannot be altered without the risk of altering membrane fluidity and thus cell viability by additives, a 25 % reduction in oral bioavailability has to be taken into account when administering EGCG over the peroral route.

1.9. Consequences for a peroral dosage form containing (-)-Epigallocatechin-3-gallate

From the factors deriving from the dosage form, complexation, coacervation, precipitation and chemical instability might result due to the presence of some excipients and reduce the liberated dose of EGCG (section 1.7.).
Furthermore, the complexes might not be dissociated within the gastrointestinal tract and hence have a reduced absorption due to their large molecular weight. Thus these excipients should be avoided or their amount within the dosage form should be adjusted.

In the context of the physiological factors, EGCG undergoes autoxidative degradation in the intestinal fluids due to the elevated pH of around 6 in the duodenum up to 7.4 in the terminal ileum (Fallingborg, 1999). Thus EGCG should be released either in the stomach and the absorption should take place in the upper intestinal tract or, if the absorption of EGCG is targeted to the ileocecal region, appropriate acidification is necessary.
Furthermore, EGCG is transported back into the lumen by MRP2 which is expressed most in the jejunum and ileum, followed by the ileocecal region and least in the duodenum; thus EGCG should be ready for absorption either in the duodenal or in the ileocecal region.
And EGCG is extensively metabolized into sulfates, glucuronides and methyl conjugates. To decrease the amount of EGCG being conjugated, it needs to be released in the ileum as here the expression of glucuronidase and sulfotransferase is lower than in the other segments of the small intestine.

The only factor that cannot be influenced by the peroral dosage form and its release window is the interaction of EGCG with lipid rafts present in the cell membrane and its concomitant EGCG loss of 25 % as it increases the risk of damaging cells and inducing cell death.

1.10. Green tea infusions

One question arising when talking about a peroral dosage form for EGCG is the question why preparing green tea infusions and drinking them is not sufficient.
The biggest issue in the administration of EGCG through green tea consumption is the high variability of catechins within the extracts as well as in the brewed tea.
In the case of green tea, the standardization of EGCG content is problematic, as it strongly depends on the origin of the green tea plant since soil and climate have an influence on the polyphenol content. Additionally, the manufacturing process influences the content starting from the way the tea is gathered and then further processed either by steaming or pan-frying. In a study of 19 green tea samples, the total phenolic compounds varied in water extracts from 21.38 to 228.20 mg/g of dry plant material (Unachukwu et al., 2010).
A final factor is the storage by the customer which might result in variation of polyphenol content due to exposure to light, humidity and temperature. Friedman et al. stored 8 different green tea brands for 6 months at 20°C and found an EGCG loss upon storage of 28 % (Friedman et al., 2009).

These were just the content variations before the beverage preparation. The leaf size, tea bag or loose tea, kind of tea bag, the brewing time and temperature exhibit as well an effect on the EGCG content within the tea. The following results were obtained by investigating these factors (Astill et al., 2001) (Price and Spitzer, 1994):

- leaf size: the bigger the leaves, the higher is the extracted catechin content (small particles are more susceptible to oxidation)
- brewing time: the longer the higher was the catechin content
- brewing temperature: the higher the more catechin was extracted due to an enhanced diffusion
- form of dry tea: loose tea provided a better extraction than tea in bags
- kind of tea bag: with higher porous bags better extraction was obtained

Consequently, it is impossible for the patient to take in a defined dose of EGCG by tea consumption. Furthermore, in some clinical trials high EGCG doses are applied

up to 1200 mg. Just to visualize the necessary tea intake for such a dose: assuming an EGCG content of 50-100 mg per cup of tea (200 ml), it would mean drinking 2.4 to 4.8 liters of green tea for a dose of 1200 mg. And even assuming a smaller dose like 300 mg, the patient would need to drink 0.6 to 1.2 liters and this all at once. These are unrealistic quantities for a patient compliant therapy and it emphasizes the need for high dose EGCG dosage forms.

1.11. Commercial products of (-)-Epigallocatechin-3-gallate

Green tea and its catechins belong to dietary supplements; thus no governmental approval is necessary and the control of the marketed product is in the hand of the producer alone. The consequence was shown by Seeram et al., who tested several marketed products with the result that EGCG content varied in 19 products from 12 up to 143 % of the labeled content (Seeram et al., 2006).

As a dietary supplement, green tea extract and EGCG products are wide spread all over the world. The main peroral dosage forms for EGCG are capsule formulations. But green tea extract and EGCG are not just marketed as single compounds but as well in combination with several other dietary ingredients such as vitamin C (Grüner Tee + C – Kapseln®; Canea Pharma GmbH, Germany), melon extract and red grape extract (Ladival® SONNEN KAPSELN; STADA GmbH, Germany) and many more.
The capsule material used is in most cases hydroxypropyl methylcellulose due to its vegetarian character, for example in Teavigo® Vcaps (BCT Nutraceuticals, Germany. Just a few products consist of a hard gelatin capsule shell, for example Grüner Tee Kapseln® (Allpharm Vertriebs GmbH, Germany).

In contrast, tablets are rare probably due to the complexity of formulation development and the need of other excipients but they can be found on the dietary supplement market too. Two examples are Source Naturals® EGCG tablets (Source Naturals, USA) containing 350 mg EGCG and GRÜNTEE Tabletten® (Hannes Nutripharm GmbH, Germany) which contain 2.4 g green tea concentrate.
Concerning other application routes, topical formulations are sold too, Veregen® (Medigene AG, Germany), for example, an ointment containing 10 % green tea extract which is prescribed in case of anogenital warts.
Furthermore, food and beverages are enriched with green tea extract or EGCG.

1.12. Disadvantages of capsule formulations of (-)-Epigallocatechin-3-gallate

In clinical trials, capsule formulations are unavoidable. But if a dosage form should be introduced to the market and thus to the patients, focus needs to be taken on patient compliance. Capsules are restricted by volume. Doses of powder above 600 mg cannot be filled in an acceptably-sized capsule (Stegemann, 2002). EGCG extract is a fine powder with low bulk density. In the case of a high dose application, either one capsule of very large size or several smaller capsules need to be swallowed by the patient.

As already recommended by the FDA in the “Guidance for size, shape, and other physical attributes of generic tablets and capsules”, small tablets are easier to swallow by patients. In fact, patients admit skipping a dose due to swallowing difficulties (U.S. Department of Health and Human Services Food and Drug Administration (CDER), 2013). Tablets consisting of compacted powder can lead to a reduced dosage form size and/or reduce the unit doses that need to be taken. This can increase patient compliance.

Another disadvantage of EGCG capsule formulations is the tendency of EGCG to interact with polymers, proteins and polysaccharides (section 1.7). Most of the marketed EGCG capsule formulations consist of a hydroxypropyl methylcellulose shell as it originates from natural non-animal sources. But traditional hard gelatin capsules are sold as well despite the fact that the literature reports that EGCG interacts with gelatin (Chen et al., 2010) as well as with water-soluble cellulose derivatives (Ashok R. Patel et al., 2011)(Ashok R. Patel et al., 2011).

The interactions result in concentration-dependent coacervate and precipitate formation and can lead to chemical instability due to their amorphous nature, to altered release patterns as already shown for green tea extract (Glube et al., 2013) (A. Solik and R. Bodmeier, 2013) and consequently to altered pharmacokinetics. According to the “FDA Guidance for Dissolution Testing of Immediate Release Solid Oral Dosage Forms”, 85 % of a BCS class 3 drug should be dissolved in 0.1 M HCl after 15 minutes to ensure its full dissolution and its exiting the stomach as drug

solution (U.S. Department of Health and Human Services Food and Drug Administration, 1997).

Another interesting aspect found by Chen et al. is, that beside the interaction of gelatin with EGCG a decreased enzymatic degradation of gelatin can be observed. This is attributed to the enzyme inhibitory effect of tea catechins on trypsin, as well as the interaction of specific gelatin sides with EGCG which make cleavage difficult by trypsin (Chen et al., 2010). Thus the use of gelatin capsules as dosage form for EGCG should be rethought for several reasons: first, as the diffusion through the intestinal epithelium might be reduced due to the formation of complexes that are larger in size than the EGCG molecule; secondly, due to the reduced activity of trypsin and potentially other digestive enzyme necessary to cleave the EGCG/gelatin complexes; and finally due to potential instabilities during storage which might arise from the interactions.

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1.13. State of art in new delivery systems for (-)-Epigallocatechin-3-gallate

Several new delivery systems for EGCG have been proposed in the literature. Most of them are derived from EGCG interactions with excipients such as layer-by-layer-coated gelatin nanoparticles, chitosan nanoparticles and ß-lactoglobulin-based nanocomplexes (Shutava et al., 2009) (Dube et al., 2011) (Shpigelman et al., 2010).
These nanoparticles are reported to increase EGCG stability. The authors attribute this to the interactions of EGCG with the matrix and/or macromolecules in solution. But the drawback of these interactions is that not all EGCG is released from the matrix. Furthermore, the loading of EGCG within the particles is low with just 20 % (wt.) or less.
Besides the nanoparticles derived from interactions of EGCG with macromolecules, PLA-PEG nanoparticles with EGCG and the stabilizer polyvinylalcohol were prepared and lyophilized to form a powder. These nanoparticles showed a 10-fold dose advantage on prostata cancer cells and an increased half-life in mice (Siddiqui et al., 2009).
One liposomal delivery system is even marketed under the name Greenselect® Phytosome® (Indena, Italy) (www.phytosomes.info). It contains besides other tea catechins atleast 13 % EGCG and is produced by complexation of the polyphenol with soy phospholipids that form a liposome-like structure in aqueous media. Phytosome® is reported by the manufacturer to increase the bioavailability of EGCG in human (www.indena.com).

Not taking the expensive manufacturing of these systems into account, the main disadvantage of all these systems is the low EGCG loading. For the intake of 300 mg, EGCG administration of at least 1.5 g of nanoparticles would be necessary, based on a 20 % loading. Chitosan nanoparticles, for example just contain around 4 % EGCG, increasing the necessary intake even up to 7.5 g.
Furthermore, no one has investigated the long-term stability of EGCG within these particles. It is unclear if a chemical destabilization of EGCG would result in solid state due to the interactions of EGCG with the matrix excipients.

1.14. Research objective

The main aim of this work was to formulate a solid peroral dosage form containing EGCG with potentially improved oral bioavailability.

As chemical degradation within the dosage forms and during the gastrointestinal passage is one of the first factors leading to reduced bioavailability, focus was made on the chemical stability of EGCG within the dosage form as well as on finding the appropriate release window and pattern for EGCG to enhance its chemical stability in the dissolved state during the gastrointestinal passage.

The first target was to investigate the mechanism of EGCG degradation in solution and the effect of pH and concentration to adjust a proper release window and pattern for EGCG. Due to the pharmacokinetic data found in the literature, fast absorption within the duodenum was the goal.

Secondly, the existing immediate release formulations for EGCG were investigated to analyze their drawbacks and to develop a better immediate release formulation under the aspect of its performance and EGCG stability within it. As commercial EGCG products are mainly capsule formulations and few tablet formulations exist, HPMC capsule formulations, hard gelatin capsule formulations and one tablet formulation were chosen as reference products.

2. MATERIALS AND METHODS

2.1. Materials

Purified (-)-Epigallocatechin-3-gallate (EGCG) extract > 90 % (Teavigo®, DSM Nutritional Products Europe Ltd., Basel, Switzerland), acetaminophen with 3 % (w/w) polyvinylpyrrolidon (Rhodapap® DCP3, Rhodia (Solvay Group), Courbevoie, France). Gelatin powdered pure Ph. Eur. grade (Applichem GmbH, Darmstadt, Germany), hydroxypropyl methylcellulose (HPMC) (Methocel® E5 LV, Colorcon, Orpington, UK), HPMC capsules size 2 (Vcaps® plus, Capsugel, Bornem, Belgium), HPMC capsules with gellan gum as gelling aid size 2 (Vcaps®, Capsugel, Bornem, Belgium) and hard gelatin capsules size 1 (Coni-Snap ®, Capsugel, Bornem, Belgium).

Pregelatinized starch (Lycatab® C, Roquette, Tunbridge Wells, Lestrem, France), sodium carboxymethyl cellulose (Cellogen ® HP-SB, Daiichi Kogyo Seiyaku Co. LTD., Kyoto, Japan), microcrystalline cellulose (Avicel® PH 102, FMC BioPolymers, Cork, Ireland), dibasic calcium phosphate dihydrate (Emcompress® premium, JRS Pharma Gmbh & Co, Rosenberg, Germany), monobasic calcium phosphate anhydrate (Chemische Fabrik Budenheim KG, Budenheim, Germany), sodium starch glycolate (Explotab® low pH, JRS Pharma Gmbh & Co, Rosenberg, Germany), cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol®, FMC BioPolymers, Cork, Ireland), cross-linked polyvinylpyrrolidone (PVP) (Kollidon® CL, BASF AG, Ludwigshafen, Germany), magnesium stearate (Baerlocher GmbH, Unterschleissheim, Germany), colloidal silicium dioxide (Aerosil® 200, Evonik Industries AG, Darmstadt, Germany), polyvinylpyrrolidone (Kollidon® 30LP, BASF AG, Ludwigshafen, Germany). Potassium dihydrogen phosphate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), talc (Luzenac ® Pharma, Luzenac Europe SAS, Toulouse, France), ascorbic acid Ph.Eur. grade (gesund leben, GEHE Pharma Handel GmbH, Dresden, Germany), sodium ascorbate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), basic butylated methacrylate copolymer (Eudragit® E 100, Evonik Industries AG, Darmstadt, Germany).

Disodium hydrogen phosphate dodecahydrate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), polysorbate 20 and polysorbate 80 (Tween® 20 and Tween® 80, Sigma-Aldrich, USA), sorbitan esters (Span® 40 and 65, Sigma Aldrich Chemie GmbH, Munich, Germany), polyethoxylated castor oil (Cremophor® EL, BASF AG, Ludwigshafen, Germany), poloxamer 188 (Pluronic® F68, BASF AG, Ludwigshafen, Germany), sodium dodecyl sulfate (SDS) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and sodium docusate (DSS) (Sigma Aldrich Chemie GmbH, Munich, Germany).

Chemicals for HPLC analysis were acetonitrile HPLC-grade (HiPerSolv® CHROMANORM, VWR International GmbH, Darmstadt, Germany), ultrapure water, trichloroacetic acid ACS reagent Ph.Eur. (Merck KGaA, Darmstadt, Germany), 0.1 M hydrochloric acid solution (HCl).

All excipients and chemicals were used as received.

2.2. Quantification of (-)-Epigallocatechin-3-gallate

The EGCG content was analyzed with HPLC-UV (Lee and Ong, 2000) on a Waters® HPLC system consisting of two Waters® 515 HPLC pumps in gradient mode, a Waters® 717plus autosampler, column oven set at 32°C and a dual wavelength absorbance detector Waters® 2487 set at 275 nm. The injection volume was 20 µl. Empower® software was used for peak integration.
Another HPLC-system used was from Shimadzu® consisting of two LC-10ADvp pumps, SIL-10ADvp autosampler, a CTO-10ACvp column oven set at 32°C and SPD-10AV UV-Vis spectrophotometric detector set at 275 nm. The injection volume was 10 µl and 5 µl. The software used for peak integration was LabSolution® (Shimadzu, Japan).
The column was an Inertsil® ODS-4 C18 reversed-phase column (4.0 x 125 mm I.D., 5µm) from GL Sciences Inc. (Tokyo, Japan).

Standard solutions of EGCG were prepared with 0.1 M HCl and isopropanol/0.1 M HCl 1:1 (v/v) for the quantification of EGCG in precipitate forming sample solutions.

The gradient was run according the literature method with a mobile phase A consisting of 5 % (v/v) acetonitrile, 95% (v/v) ultrapure water, 0.07 % (w/v) trichloroacetic acid and a mobile phase B of 50 % (v/v) acetonitrile, 50 % (v/v) ultrapure water and 0.05 % (w/v) trichloroacetic acid. Standard solutions of EGCG for calibration were prepared in 0.1 M HCl or in 0.1 M HCl/ isopropanol (1:1 v/v), respectively.

2.3. Stability studies of (-)-Epigallocatechin-3-gallate in aqueous solution

2.3.1. Stability study of EGCG at different pH and concentration

The buffer solutions were hydrochloric acid/ sodium chloride pH 1.2, citrate/ hydrogen phosphate buffer pH 4.0, 5.0, 6.0 and USP phosphate buffers of pH 6.5, 6.8, 7.4. The buffer solutions were tempered at 37°C in a GFL® incubation shaker 3033 (GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany) set at 80 rpm. Teavigo® powder was dissolved at a concentration of 0.3 $^{g}/_{l}$ in the tempered buffer solutions and kept at 37°C shaking for 6 hours (n=3). Additionally, Teavigo® powder was dissolved at concentrations of 1.0 and 2.5 $^{g}/_{l}$ in tempered phosphate buffer solutions of pH 6.5, 6.8 and 7.4 and kept at 37°C shaking for 180 minutes (n=3). Samples were taken at predetermined time points and EGCG was quantified with HPLC-UV (section 2.2).

2.3.2. Stabilization of EGCG in solution by sodium ascorbate

100 ml phosphate buffer solutions of pH 6.5, 6.8 and pH 7.4 were tempered at 37°C in a GFL® incubation shaker 3033 (GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany) set at 80 rpm. 100 mg Teavigo® and 44 mg sodium ascorbate, resulting in a 1:1 molar ratio of EGCG to sodium ascorbate, were dissolved in the tempered buffer solutions and kept at 37°C shaking for 180 minutes (n=3). Furthermore, 100 mg Teavigo® with 88 mg, 176 mg and 2220 mg sodium ascorbate were dissolved in tempered phosphate buffer pH 6.8 to investigate the effect of sodium ascorbate on EGCG at higher molar ratios of 1:2, 1:4 and 1:50 (EGCG to sodium ascorbate) over 6 days at 37°C (n=3).

The sequence of powder dissolution was kept constant, with first dissolving sodium ascorbate and afterwards Teavigo® powder. Samples were taken at predetermined time points and EGCG was quantified with HPLC-UV (section 2.2).

2.3.3. Contact angle measurement of EGCG solutions

As the Du Noüy ring tensiometer cannot be applied in case of surface active substances which reduce the surface tension only slightly, contact angle measurement was used to assess the surface tension decrease of 0.1 M HCl by EGCG.

For this purpose, EGCG solutions were prepared in 0.1 M HCl at varying concentrations from 0.1 $^{g}/_{l}$ up to 6.0 $^{g}/_{l}$. 7.5 µl of the solutions were pipetted on a glass slide with teflon layer (n=3). Pictures were immediately taken of the droplets with a camera. The picture processing was performed with the IC Capture® 2.1 software (The Imaging Source Europe GmbH, Bremen, Germany) and contact angle was measured between droplet and teflon layer (Fig. 18). 0.1 M HCl was used as the blank.

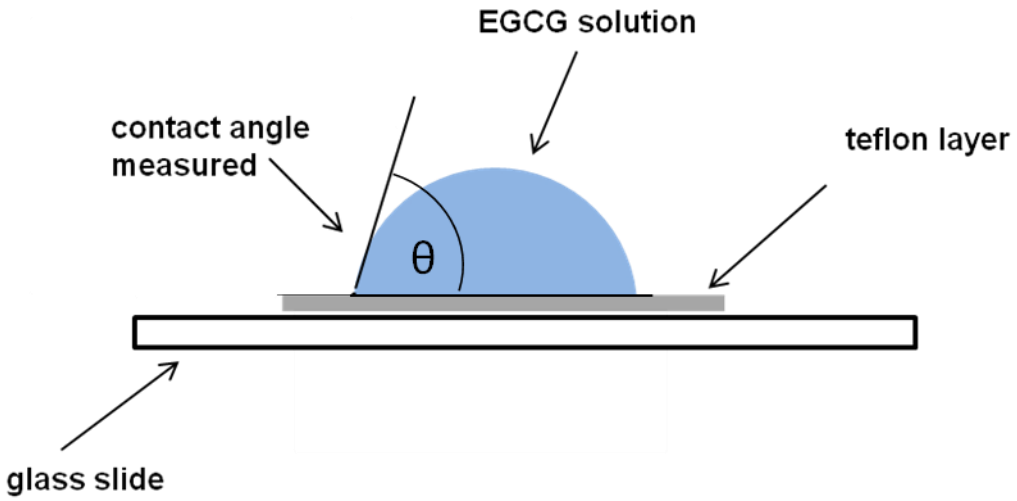


Figure 18: Scheme of contact angle measurement

2.3.4. Photon correlation spectroscopy of EGCG solutions

0.1 M HCl and USP phosphate buffer pH 7.4 were prepared with ultrapure water (18.4 MΩ) and filtered through a polyamide membrane filter with a pore size of 0.2 µm (Whatman®, GE Healthcare Europe GmbH, Freiburg, Germany). Teavigo® powder was dissolved in the filtered solvents at varying concentrations from 0.3 $^{g}/_{l}$ up

to 4.0 $^{g}/_{l}$. As EGCG undergoes oxidative degradation with concomitant formation of hydrogen peroxide (section 1.4) and hydrogen peroxide decomposes into oxygen that volatilizes, the particle size measurements in pH 7.4 were performed after 45 minutes. This way, the first fast degradation phase of EGCG was completed and the oxygen amount was kept as low as possible. The Teavigo® solutions were filtered through 0.2 µm syringe filters (CHROMAFIL® PET 20/25) directly into plastic cuvettes just before starting the measurement.

The solutions were analyzed for the mean particle size, polydispersity index and mean light scattering (mean count rate) with a Zetasizer® Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany) equipped with a He-Ne-Laser (633 nm) at a backscattering angle of 173° and cell temperature of 20°C. 10 runs were performed with a measuring time of 25 seconds each.

2.3.5. Effect of EGCG pre-aggregation on its stability in solution

0.016 M HCl was tempered at 37°C in a GFL® incubation shaker (GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany) set at 80 rpm. 30 and 100 mg Teavigo® powder were dissolved in 100 ml of the tempered 0.016 M HCl, resulting in a concentration of 0.3 $^{g}/_{l}$ and 1.0 $^{g}/_{l}$, respectively. The solutions were shaken for 30 minutes at 37°C to ensure that the aggregate formation reached its equilibrium. After 30 minutes 0.3 g disodium hydrogen phosphate dodecahydrate was added as powder to the EGCG solutions to buffer the pH to 7.4. The solutions were further kept at 37°C and shaken. After the adjusting the solutions to pH 7.4, samples were taken over 180 minutes at predetermined time points and analyzed for their EGCG content with HPLC-UV (section 2.2).

Finally, the pH of each solution was measured with a Sartorius® pH-meter PB11 (Sartorius AG, Goettingen, Germany) to test, if the pH of the samples remained at pH 7.4.

2.4. Coacervation study

2.4.1. Polarized light microscopy

Polarized light microscopy allows differentiation between crystalline and amorphous particles. Furthermore, the pictures taken and processed with EasyMeasure® software (Inteq Informationstechnik GmbH, Berlin, Germany) show the morphology of the particles and allow distinguishing between coacervates and precipitates.
0.2 % (w/v) stock solutions of Teavigo®, Kollidon® LP30, Methocel® E5 LV, powdered gelatin and dissolved/ disintegrated HPMC-gellan gum capsules (Vcaps®) were prepared in 0.1 M HCl representing simulated gastric fluid. The stock solutions were mixed at a 1:1 (v/v) ratio, resulting in a final concentration of 0.1 % (w/v) for Teavigo® and for the macromolecules. The turbid solutions of EGCG with HPMC, HPMC-gellan gum, gelatin and PVP were pipetted on a glass slide and observed under a polarized light microscope (Zeiss® Axioscope; Carl Zeiss AG, Oberkochen, Germany).
Additionally, the process of coacervate/precipitate formation of EGCG (Teavigo®) with hydroxypropyl methylcellulose (HPMC) (Methocel® E5 LV), polyvinylpyrrolidone (PVP) (Kollidon® LP30) and gelatin was observed in-situ by placing the powders on a glass slide beside each other. A small gap was left between them. The powders were covered with a glass plate and 0.1 M HCl was pipetted at the edge of the glass plate to be aspired by capillary forced into the gap between the two solids. Upon contact with the fluid, the powders dissolved under the formation of saturated solutions. Once these saturated solutions get in contact, the interaction of both reactants can be observed in-situ and reflects the situation within a dosage form such as capsule which is penetrated by the dissolution medium.

2.4.2. Turbidimetric studies

Stock solutions of Teavigo®, Methocel® E5 LV and powdered gelatin were prepared in 0.1 M HCl and mixed at defined ratios at room temperature. The investigated reactant concentrations ranged from 0.0005 % (w/w) up to 0.2 % (w/w). The sample volume was 10 ml. The samples were analyzed spectrophotometrically at 400 nm to

quantify the turbidity of samples with an Agilent 8453 UV-Vis spectrophotometer series 8453 equipped with a photodiode array detector (Agilent Technologies®, Santa Clara, CA, USA). Absorbance values of ≥ 0.050 were postulated as turbidity. The results were processed with Chemstation® software (Agilent Technologies®, Santa Clara, CA, USA) and plotted with the OriginPro® 8.5 software (OriginLab Corporation, Northampton, MA, USA) in 3-dimensional ternary plot (contour coacervation triangles) to assess qualitatively the extent of coacervation and the coacervation boundary.
Additionally, samples of EGCG and gelatin were prepared at 37°C and the absorbance at 400 nm was measured in a heatable cell holder for the cuvette at 37°C to assess the temperature effect on EGCG/gelatin coacervates since gelatin solubility is strongly enhanced above 30°C. For HPMC, the temperature dependence was not investigated as HPMC solubility does not differ from 10°C up to 55°C (Chiwele et al., 2000).

2.4.3. Quantification of EGCG within coacervates and precipitates

Samples were prepared at biorelevant EGCG concentrations in the stomach of 0.375 mg/ml up to 2.25 mg/ml corresponding to an EGCG dose of 75 mg to 450 mg EGCG. HPMC (Methocel® E5 Premium LV), PVP (Kollidon® 30LP) and gelatin stock solutions were added to give a final ratio of 1:1 (w/w) (macromolecule : EGCG) in order to assess the concentration dependent phase-separation and concomitant EGCG loss. Additionally, 1:50, 1:10, 1:5, 1:2, 1:1 and 2:1 (w/w) ratios (macromolecule : EGCG) were prepared with a constant EGCG concentration of 1.5 mg/ml to investigate the EGCG loss upon phase-separation dependent on the macromolecule amount in combination with 300 mg EGCG in a dosage form.
The turbid samples were centrifuged at 13000 rpm for 5 minutes (Heraeus™ Biofuge™, Thermo Fisher Scientific Inc., Waltham, MA, USA) and filtered through syringe filters with a pore size of 0.20 µm (CHROMAFIL® PET 20/25) to remove the particles before HPLC analysis. The amount of EGCG in the supernatant was quantified with HPLC-UV (section 2.2). This amount corresponded to the free EGCG in solution. The amount lost was taken as EGCG loss upon coacervation/precipitation with the tested excipients.

2.4.4. Extraction of EGCG from precipitates

The extraction out of EGCG/gelatin particles can be performed by simple dilution as the occurring phase-separation is coacervation. But in the case of EGCG precipitates with PVP or HPMC, simple dilution is not sufficient to dissociate the particles. Addition of co-solvents was investigated by the addition of the polar protic organic solvent isopropanol to the turbid samples. The ratio of isopropanol to 0.1 M HCl was 1:1 (v/v) to ensure that the macromolecules do not precipitate out of the solutions. The efficacy of isopropanol to recover EGCG out of the precipitates was investigated at different reactant ratios (polymer : EGCG) of 1:10, 1:5, 1:2, 1:1, 2:1, 3:1. Therefore, stock solutions of EGCG and polymers were prepared in 0.1 M HCl and mixed at defined ratios to lead to a final EGCG concentration of 1.5 mg/ml. Furthermore, it was investigated whether the extraction of EGCG out of the precipitate is completed immediately or if overnight extraction is necessary.
The amount of EGCG recovered was analyzed with HPLC-UV (section 2.2.). The running standard and standards of EGCG for the calibration curves were prepared as well with 1:1 (v/v) isopropanol and 0.1 M HCl.

2.4.5. Effect of surfactants on HPMC-EGCG coacervates and precipitates

0.2 % (w/v) solutions of EGCG and HPMC were prepared in 0.1 M HCl separately and then mixed at a 1:1 (v/v) ratio leading to a final concentration of 0.1 % (w/v) each. Surfactants were added above their critical micelle concentration (CMC) from 0.5 % up to 2.0 % (w/v) to the turbid solutions and stirred for 5 minutes at 500 rpm with a magnetic stirrer. Pictures were taken with a camera prior to surfactant addition and after stirring to visualize the clearance, i.e. effectiveness of surfactants on dissociation of the coacervates/ precipitates.
The surfactants were ionic or non-ionic in nature to investigate the effect of electric and steric stabilizers and differed according to their HLB value, i.e. solubility in the aqueous phase.

Additionally, an HPMC-gellan gum capsule (Vcaps®) was disintegrated/ dissolved in 0.1 M HCl at 37°C and mixed with an EGCG stock solution to give a final concentration corresponding to 1 capsule with 150 mg EGCG in 250 ml gastric fluid. The surfactants that were effective in dissociating the EGCG/HPMC precipitate were investigated as to their required concentrations to clear the turbid solutions resulting from coacervation of EGCG with disintegrated/ dissolved HPMC-gellan gum capsules.

2.5. Tablet preparation

Excipients and the active pharmaceutical ingredient (API) were weighed and blended manually according to the formulations in Table 1 without stabilizer and Table 2 with ascorbic acid, sodium ascorbate or a binary mixture of both as stabilizer. 2 % (w/w) magnesium stearate as lubricant and 0.5 % (w/w) Aerosil® 200 to reduce electrostatic charging of Teavigo® powder were added to all formulations at the end.

Powder blends were mixed thoroughly and sieved through a 500 µm sieve to destroy agglomerates formed during blending. Compaction into tablets was performed with an instrumented single-punch tableting machine (EK-0 Korsch, Erweka Apparatebau, Heusenstamm, Germany). Powder blends were filled manually into the tableting die and directly compressed into 7 mm flat-faced bevel-edged tablets of 100 mg weight.

The compression force was monitored with Catman® Easy software (HBM, Darmstadt, Germany).

For the dissolution study, tablets were up-scaled to 13 mm flat-faced bevel-edged tablets with an EGCG content of 300 mg.

Table 1: Tablet formulations containing excipients that are according the storage stability study compatible or *incompatible with EGCG

Excipients	Amount (%) wt.					
Teavigo®	70	70	70	75	70	70
Avicel® PH102	20	-	20	-	-	-
Emcompress® premium	-	20	-	-	20	-
Lycatab® C*	-	-	-	-	-	20
Kollidon® CL*	-	-	-	-	10	-
Ac-Di-Sol®*	-	-	10	25	-	-
Explotab® low pH	10	10	-	-	-	10

Table 2: Tablet formulations containing one *incompatible excipients and sodium ascorbate, ascorbic acid or its mixture as stabilizer

Excipients	Amount (%) wt.									
Teavigo®	75	65	75	65	55	60	50	60	50	40
Avicel® PH102	-	-	-	-	-	-	-	-	-	-
Emcompress® prem.	-	-	-	-	-	-	-	-	-	-
Lycatab® C*	-	-	-	-	-	20	20	20	20	20
Ac-Di-Sol®*	25	25	25	25	25	-	-	-	-	-
Explotab® low pH	-	-	-	-	-	10	10	10	10	10
Sodium ascorbate	10	20	-	-	10	10	20	-	-	10
Ascorbic acid	-	-	10	20	20	-	-	10	20	20

2.6. Characterization of tablets

2.6.1. Tablet hardness and porosity

Tablet hardness (radial fracture strength), diameter, height and weight were measured with an Erweka® Multicheck tablet tester (Erweka Apparatebau, Germany). The tablet apparent density (ϱ_{app}) was calculated from the tablet weight and the calculated volume according to equation 1. No volume correction for the bevel-edged shape was performed.

The tablet porosity (ε) in percent was derived according to equation 2, whereby the true density of the tablets (ϱ_{true}) was calculated by the use of the true density of the individual excipients found in the literature and their weight fraction within the tablet formulation (Table 3).

$$\rho_{app} = \frac{m_{tablet}}{V_{tablet}} \quad \text{(Eq.1)}$$

$$\varepsilon = 100 \cdot (1 - \frac{\rho_{app}}{\rho_{true}}) \quad \text{(Eq.2)}$$

Table 3: True density of excipients

Excipient	ϱ_{true} ($^{g}/_{ml}$)
Teavigo®	1.590[a]
Avicel® PH102	1.56[c]
Emcompress® premium	2.389[b]
Lycatab® C	1.516[b]
Kollidon® CL	1.26[c]
Ac-Di-Sol®	1.543[b]
Explotab® low pH	1.51[e]
Sodium ascorbate	1.826[b]
Ascorbic acid	1.688[b]
Magnesium stearate	1.092[b]
Talc	2.75[b]
Potassium dihydrogen phosphate	2.338[a]

[a] MSDS [b] Handbook of Pharm. Excipients 6th Ed. 2009 [c](Kuentz and Leuenberger, 1999) [d](Schmidt and Kleinebudde, 1999) [e] (Shah and Augsburger, 2002)

2.6.2. Compactibility

Tablets out of pure Teavigo® powder with 0 %, 1 % and 2 % (w/w) magnesium stearate as lubricant were prepared according to section 2.5 at varying compression forces ranging from 0.9 to 7.8 kN. As reference for the compactibility of Teavigo®, blends of Rhodapap® DCP, a special acetaminophen grade containing 3 % PVP with excellent compactibility, with 1 % (w/w) magnesium stearate were compressed at different compression forces. The measured hardness of the tablets was plotted against the compaction force to evaluate the compactibility of Teavigo® powder in comparison to the Rhodapap® DCP 3 reference tablets.

The effect of sodium starch glycolate (Explotab® low pH) and cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol®) as superdisintegrant on the compactibility was assessed as superdisintegrants are elastic polymers, and thus can potentially influence the tablet strength at high loadings (Edge et al., 2002). Their effect was investigated by preparing powder blends of Teavigo® with a high superdisintegrant loading of 25 % (w/w). The blends were prepared and compressed according to section 2.5 at varying compaction forces and their hardness was plotted against the compaction force. The compaction profile was compared to the one of pure Teavigo® powder.

2.6.3. Disintegration studies

The disintegration studies were performed in 800 ml 0.1 M HCl at 37°C in a triple basket disintegration testing instrument compliant with USP (PharmaTest® DIST 3, Pharma Test Apparatebau AG, Hainburg, Germany). The stroke speed was 30 per minute. Disks were used to restrict tablet floating. The disintegration time was evaluated visually with an endpoint set when no tablet residues remained in the basket.

The effect of superdisintegrants with and without additives on the tablet disintegration behavior of pure Teavigo® tablets was investigated. The superdisintegrants Explotab® low pH and Ac-Di-Sol® were investigated at 5 %, 10 % and 25 % (w/w) loading. The effect of tablet hardness, and thus tablet porosity, on the disintegration

efficacy of Ac-Di-Sol® and Explotab® low pH was investigated for tablets containing 25 % (w/w) superdisintegrant. The additives tested to improve the tablet disintegration efficacy of Explotab® low pH within Teavigo® tablets were potassium dihydrogen phosphate and talc at 10 % (w/w) loading. The tablets were prepared as mentioned in section 2.5.

2.6.4. Dissolution study

The dissolution studies were performed with the USP apparatus 2 (Erweka® Dissolution Tester DT 700, Erweka Apparatebau, Heusenstamm, Germany) with a paddle rotating speed of 50 rpm as recommended for immediate release formulations by the FDA (U.S. Department of Health and Human Services Food and Drug Administration, 1997).

The dissolution medium was 500 ml 0.01M HCl tempered at 37°C ± 0.1°C to better simulate to the concentrations occurring in the stomach in vivo.

5 ml samples were taken every 15 minutes, filtered through 0.20 µm syringe filters (CHROMAFIL® PET 20/25) and analyzed for the released EGCG. The dissolution medium was not replaced.

2.6.4.1. Immediate release tablets

Tablet formulations with maximum 5 % EGCG loss upon storage at 40°C/75 % RH were scaled up to 300 mg flat-faced bevel-edged tablets with a diameter of 13 mm and release tests were performed over 2 hours.

Source Naturals® tablets with a declared content of 350 mg EGCG and hard gelatin (Coni-Snap® size 1) capsules filled with 150 mg Teavigo® were released as reference formulations.

The amount of EGCG in the release medium was quantified by HPLC-UV (section 2.2.). The 100 % value corresponded to the EGCG content in the medium after destruction of the remaining tablet by 5 minutes fast agitation of the paddles (250 rpm).

2.6.4.2. Hard capsule formulations

Vcaps® plus size 2 (HPMC), Vcaps® size 2 (HPMC-gellan gum) and Coni-Snap® (hard gelatin capsules) size 1 were filled manually with 150 mg EGCG.
Additionally, HPMC-gellan gum (Vcaps® size 2) capsules were filled with EGCG triturated with Tween® 80 (15 % (w/w) of dosage form), EGCG blended with Pluronic® F68 (10 % (w/w) of dosage form) and an oily suspension of EGCG in peanut oil (60% (w/w)) to investigate the potential of these additives to prevent the capsule shell solidification and precipitation of EGCG with HPMC as capsule material.

The capsules were placed in sinkers to avoid their floatation. Two capsules corresponding to 300 mg EGCG were released per vessel to keep the concentration within the dissolution medium equal to the release tests performed with immediate release tablets and as this concentration represents the intake of 1 commercial EGCG capsule with a glass of water. Investigating the release at this concentration is important as the concentration of the capsule material as well as of EGCG is the main factor inducing coacervate formation and concomitant release modification of EGCG from capsule formulations.

The dose-effect on EGCG release from hard gelatin capsules was assessed over 1 hour as this time was sufficient to reach 100 % release.
All other capsule formulations were released over 2 hours as 100 % release could not be achieved within 1 hour. In the case of capsule formulations, the released amount of EGCG was quantified spectrophotometrically at 275 nm with single wavelength background correction at 400 nm with an Agilent 8453 UV-Vis spectrophotometer series 8453 equipped with a photodiode array detector and Chemstation® software (Agilent Technologies®, USA).

2.7. Stability studies of (-)-Epigallocatechin-3-gallate in solid state

2.7.1. Storage of EGCG powder blends and tablets

Binary mixtures of EGCG with common tableting excipients and hard capsule materials were prepared with a mortar and pestle in a 1:1 (w/w) ratio. In the case of magnesium stearate and polyvinylpyrrolidone, 1:5 (w/w) ratios were prepared as these excipients are commonly used at low doses in tablets. The EGCG content was quantified with HPLC-UV (section 2.2.). The amount of EGCG lost due to the sample preparation was assessed directly after blending and was presented as 0 weeks value. The blends were put in petri dishes and stored in an incubation chamber at isothermal stress conditions of 40°C and 75 % relative humidity (RH) for 26 weeks (6 months). Samples were taken after 6, 12, 26 weeks (n=3) according to the ICH guideline Q1A (R2) “Stability Testing of New Drug Substances and Products” and analyzed for their EGCG content with HPLC-UV. As recommended by the International Conference on Harmonization, the 6 weeks value was included as significant changes over the testing period were expected. A significant change is according to the above mentioned guideline, a 5 % change from initial (0 weeks) value.

Due to the non-linear degradation pattern of EGCG, the statistical analysis of the results was performed with a one-tailed dependent two-sample t-test by analyzing the EGCG content for a significant change between two time points. The highest average content, mostly the initial content at t = 0 weeks, was compared to the average content at t = 26 weeks with an alpha-level of 0.05 and p-value above 0.25 as rejection criterion for significance. The statistical analysis was performed with Microsoft® Excel Pro 2010. Furthermore, the results which showed a significant difference in their content were analyzed with the above mentioned t-test if the occurring content difference is significant 5 % or more.

Accordingly, the stress tests of immediate release tablets were performed and analyzed statistically. The tablets were prepared according the tablet formulations

described in section 2.5. Each tablet was weighed prior to storage and put into the incubation chamber in separate open roll rim glasses. The testing period was 26 weeks (6 months) with samples taken after 6, 12 and 26 weeks. Three tablets were analyzed per time point. The initial EGCG content within the tablets was measured right after tableting and is presented as 0 weeks value. The tablets were extracted in 0.1 M HCl and analyzed for their EGCG content with HPLC-UV (section 2.2).

2.7.2. Moisture uptake upon storage

Powder blends and tablets kept for storage at 40°C/ 75 % RH were immediately weighed with a Mettler Toledo® analytical balance model AT261 Delta Range before storage. Powder blends were weighed after 3, 6 and 12 weeks and tablets after 6, 12 and 26 weeks of storage (n=9).

The weight gain was taken as moisture uptake in percent on dry weight basis of the sample prior to the storage at equilibrium at standard ambient temperature and pressure (SATP) because the compendial method “Loss on Drying” cannot be applied with EGCG as heating induced strong degradation.

Additionally, the single excipients were stored as powders at 40°C/75 % RH as well as the superdisintegrants Ac-Di-Sol®, Explotab® low pH and Kollidon® CL in binary 1:1 (w/w) blends with EGCG. The weight gain was measured after 1 and 2 weeks of storage (n=6).

To investigate the effect of EGCG on the moisture uptake of the superdisintegrants, the calculated moisture uptake of the superdisintegrant within the blends was compared to the moisture uptake of the as single compounds, and the reduction in moisture uptake due to the presence of EGCG was calculated according to equation 3.

$$reduction\ in\ moisture\ uptake\ (\%) = 100 \cdot \left(1 - \frac{\%moisture\ uptake_{in\ binary\ blend}}{\%moisture\ uptake_{single\ compound}}\right) \quad \text{(Eq.3)}$$

2.7.3. Microenvironmental pH

The microenvironmental pH within the stored powder blends as well as within the tablets was assessed with the pH-slurry method. For this purpose, the pH of excipient slurries with and without EGCG was measured in demineralized water (pH 6.08) with a Sartorius® pH-meter PB11 (Sartorius AG, Germany) after 15 minutes equilibration time necessary to result in a final pH.

Following amounts of excipients were dispersed in 5 ml demineralized water to be in excess of their solubilities: Teavigo®, Avicel® PH102, Lycatab® C, Explotab® low pH, Ac-Di-Sol®, Kollidon® CL and magnesium stearate at 10 % (w/v). Emcompress® premium, sodium carboxymethyl cellulose, Methocel® E5 LV and gelatin at 20 % (w/v), ascorbic acid and Kollidon® 30LP at 40 % (w/v) and sodium ascorbate at 80 % (w/v).First, the pH of slurries without EGCG was measured and then again once 10 % (w/v) Teavigo® was added to the slurries.
The pH-change resulting from the addition was calculated as Δ pH (Eq.4). A negative value represents a pH-reduction due to EGCG and a positive value an increase in pH.

$$\Delta pH = pH_{blend} - pH_{excipient} \qquad \text{(Eq.4)}$$

The pH-change to lower pH-values was attributed to the deprotonation of EGCG and the necessary concentration ($^{mol}/_{l}$) of hydroxonium ions was calculated according to equation 5 and converted into $^{mg}/_{l}$.

$$c_{H_3O^+} = 10^{-pH\ blend} - 10^{-pH\ excipient} \qquad \text{(Eq.5)}$$

The microenvironmental pH of immediate release tablets was investigated accordingly. Slurries of all tablet excipients were prepared in 5 ml demineralized water, whereby colloidal silicium dioxide was omitted as it accounts for just 0.5 % of the tablet formulations and hence does not markedly influence the microenvironmental pH. Following amounts of excipients were dispersed in 5 ml demineralized water: Kollidon® CL and magnesium stearate at 5 % (w/v). Teavigo®,

Avicel® PH102, Lycatab® C, Ac-Di-Sol® and Explotab® low pH at 10 % (w/v). Emcompress® premium at 20% (w/v), ascorbic acid at 40 % (w/v) and sodium ascorbate at 70 % (w/v).

In the same procedure as for the binary mixtures, the microenvironmental pH was measured first without and then with the addition of EGCG, and the pH-change (ΔpH) as well as the necessary concentration ($^{mol}/_{l}$) of hydroxonium ions to reduce the pH was calculated.

From literature data, the corresponding degradation velocity (k) of EGCG in solution as well as ascorbic acid was taken and correlated to the measured microenvironmental pH (Li et al., 2012) (Golubitskii et al., 2007) to explain the difference in EGCG degradation within these formulations.

2.7.4. Deliquescence study of ascorbic acid and its sodium salt

To assess the deliquescence behavior of ascorbic acid, sodium ascorbate and their binary 1:2 (w/w) mixture, the substances were blended with the used tableting excipients within the stabilized tablet formulations (Ac-Di-Sol®, Explotab® low pH, Lycatab® C, Teavigo®) at 1:1 (w/w) ratios and stored for 2 weeks in open roll rim glasses at 40°C/ 75 % RH.

After 1 and 2 weeks, the samples were weighed and the mass gain calculated as moisture uptake (section 2.7.2.). Samples which showed the same weight on 2 consecutive weeks were taken as average moisture uptake values (n=6). When the weight gain increased upon storage, the 2 weeks' value was taken as average moisture uptake (n=3).

To compare the excipient effect on deliquescence, antioxidants were stored as well as single compounds and assessed upon their moisture uptake.

Additionally, pictures were taken with a camera to illustrate the deliquescence and the extent of discoloration upon storage as a color change correlates with the degradation of ascorbic acid and the concomitant radical formation.

3. RESULTS AND DISCUSSION

3.1. Stability of (-)-Epigallocatechin-3-gallate in aqueous solution

3.1.1. Surface activity of EGCG

The addition of EGCG to 0.1 M HCl resulted in a reduced contact angle between the droplet and Teflon surface (Fig. 19). As the contact angle is, according to Young's equation, directly proportional to surface tension, EGCG reduced the surface tension of 0.1 M HCl and thus can be considered as surface active.

Although the reduction was less pronounced than for typical detergents, a drop of contact angle from 0 to 0.25 $^{g}/_{l}$ could be observed with a further decline of the contact angle up to 6 $^{g}/_{l}$. This indicates a critical aggregation concentration (CAC) of 0.25 $^{g}/_{l}$.

The further reduction in surface tension above the CAC is likely due to further occupation of the air-water interface by EGCG molecules forming a denser package of molecules at the interfaces and thus exhibiting a further reduction in the surface tension (Menger and Rizvi, 2011).

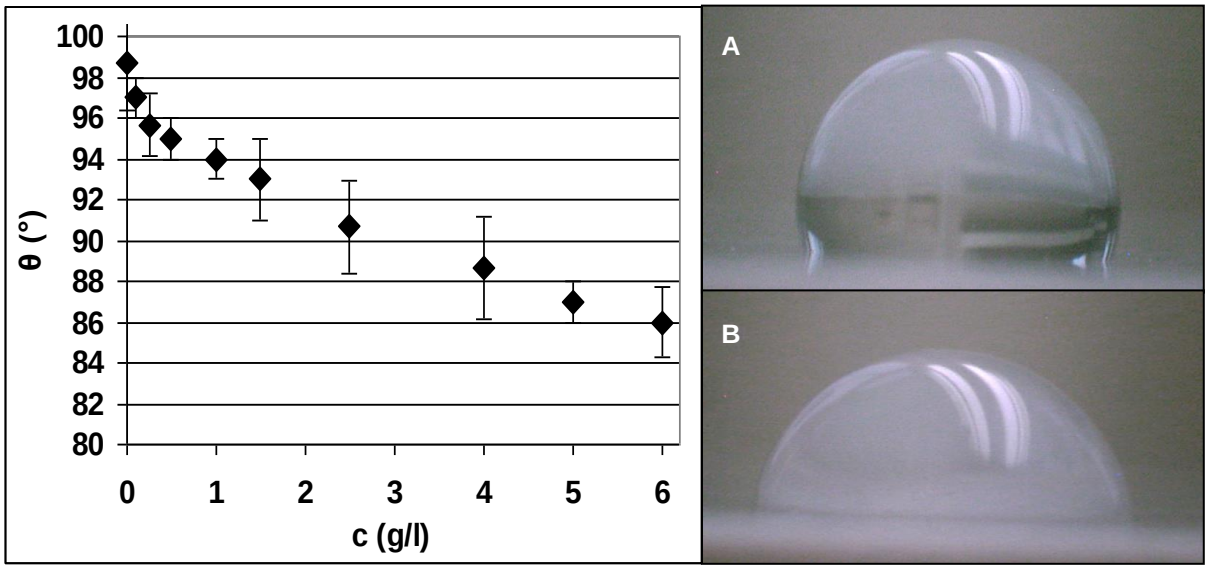


Figure 19: Contact angle measured in dependence of EGCG concentration in 0.1 M HCl at room temperature (n=3); A) droplet picture of 0.1 M HCl and B) droplet picture of 5 g/l EGCG solution in 0.1M HCl

3.1.2. Particle size of EGCG self-associates

The mean light scattering, expressed as mean count rate, increases when particles increase in size or the amount of particles increases. For EGCG solutions in 0.1 M HCl, an increase of light scattering could be observed up to a concentration of 0.5 $^{g}/_{l}$ where a plateau is reached (Fig. 20). According to these results, the EGCG molecules form aggregates in 0.1 M HCl which increase in size or amount with increasing concentration up to a critical concentration of 0.5 $^{g}/_{l}$.

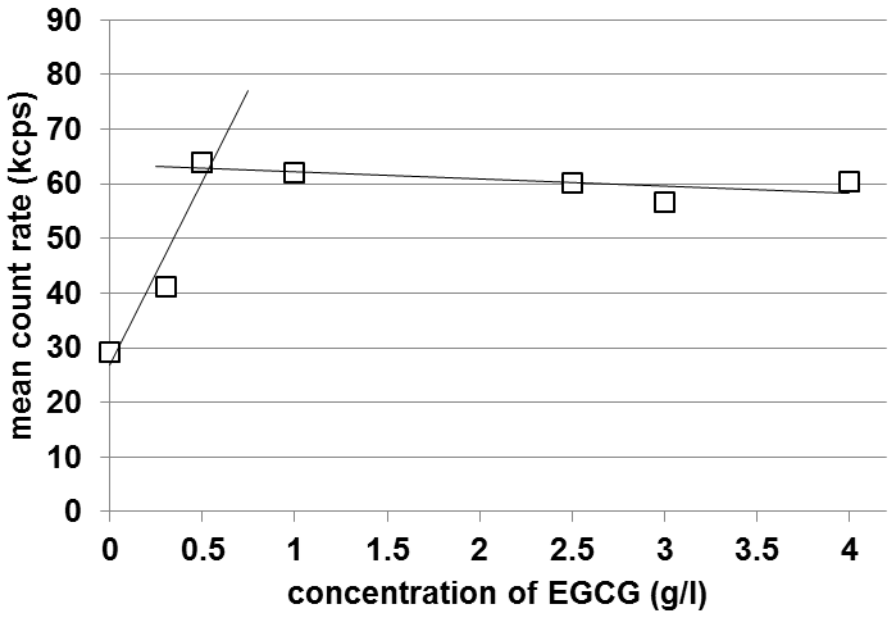


Figure 20: Mean light scattering (10 runs) of EGCG solutions in 0.1 M HCl at varying EGCG concentrations

The particle size derived from the dynamic light scattering reveals three different particle size populations. One population consisted of particles below 5 nm, that are attributed to impurities and/or EGCG di- and trimers as the molecule size of EGCG is around 1.5 nm (www.chemspider.com). The second population consisted of particles above 4000 nm, which are impurities such as dust particles from air and adhered to the cuvettes. They are recorded as well due to the overall small amount of particles within the solutions. The third and main population corresponded to the EGCG self-

associates and was examined instead of the Z-average values as the Z-average values include all particle size populations (Fig. 21).

The particle size increased up to an EGCG concentration of 1.0 $^{g}/_{l}$. Hence the observed increase in mean light scattering is due to the formation of larger particles. Above 1.0 $^{g}/_{l}$, the mean particle size remained constant ranging between 439 nm and 515 nm. Above the critical concentration of 1.0 $^{g}/_{l}$, neither the light scattering, nor the particle size changed. But the polydispersity index (PDI) values are relatively high with up to 0.492 due to the presence of several particle size populations, to the non-spherical nature of the self-aggregates and the low particle concentration in solution. Nevertheless, the existence of particle as well as their tendency to increase in size could be deduced from these results.

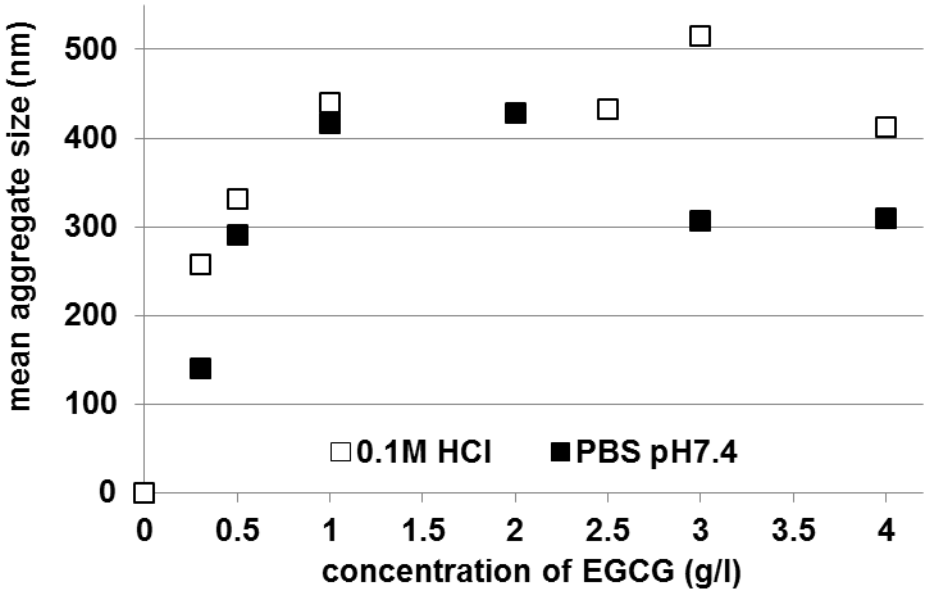


Figure 21: Mean aggregate size at different EGCG concentrations in 0.1 M HCl (pH 1) and phosphate buffer pH 7.4 at 20°C (average of 10 runs)

In pH 7.4 EGCG undergoes oxidative degradation which goes along with the formation of hydrogen peroxide (section 1.4). Hydrogen peroxide in turn decomposes into oxygen that volatilizes. The presence of the volatile oxygen during the PCS measurement lead to higher mean count rates (not presented here) and an increased PDI lying between 0.444 and 1.000 in comparison to the EGCG solutions in 0.1 M HCl. But EGCG formed self-associates in phosphate buffer solutions of pH 7.4 too.

The mean particle size of the main population was comparable with the one measured in 0.1 M HCl with a size increase up to a concentration of 1.0 $^{g}/_{l}$ at which the mean particle size remained constant ranging between 307 nm and 428 nm.
These results show that EGCG self-aggregation even occurs when EGCG is partially deprotonated and/or degraded.

3.1.3. pH-dependence of EGCG degradation

EGCG degrades dependent on pH and its concentration (section 1.4.1.): the higher the pH of the aqueous medium, the higher the degradation of EGCG (Fig. 22).

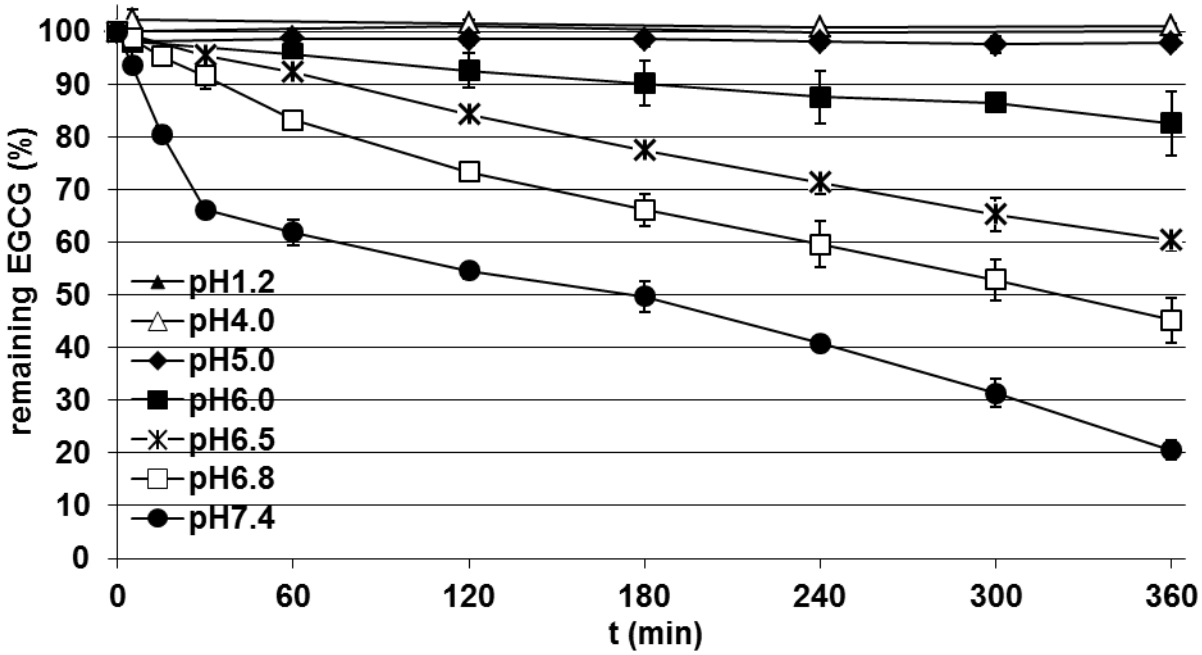


Figure 22: pH-dependent degradation of 0.3 g/l EGCG solutions at different pH at 37°C (values presented as an average of n=3 ±SD)

Furthermore, EGCG degradation in pH > 6.5 follows a biphasic pattern. The first degradation phase of 30 to 60 minutes (initial degradation) is followed by a second slower degradation phase. Both phases fit best a zero order kinetic with regression coefficients of 0.9918 and 0.9885 for first and second phase in pH 7.4 and 0.9986 and 0.9957 in pH 6.8, respectively. In the case of pH 6.5 and lower, the two phases of degradation cannot be distinguished anymore, but they follow as well zero order degradation with regression coefficients of 0.991.

Interestingly, the biphasic degradation can be divided into a first pH-dependent phase and a second pH-independent phase (Table 4). Overall, the first phase results in faster degradation velocity than the second phase. After 30 minutes in the case of pH 7.4 and after 60 minutes in the case of pH 6.8, the second degradation phase starts with approximately the same degradation rate of 18.6 $^{mg}/_{l*h}$ ± 2.4 $^{mg}/_{l*h}$ to 25.8 $^{mg}/_{l*h}$ ± 3.0 $^{mg}/_{l*h}$ independent of the pH.

Table 4: Amount of EGCG degraded within 60 minutes (initial degradation phase) and degradation rate (k) after 60 minutes (second degradation phase) at 37°C and varying pH (values presented as an average of n=3 (SD))

Concentration$_{degraded}$ ($^{mg}/_{l}$) at t = 60 min			**Degradation rate k ($^{mg}/_{l*h}$) at t > 60 min**		
pH 6.5	**pH 6.8**	**pH 7.4**	**pH 6.5**	**pH 6.8**	**pH 7.4**
26.7 (3.5)	50.6 (4.0)	115.8 (5.9)	22.8 (4.7)	25.8 (3.0)	18.6 (2.4)

3.1.4. Effect of EGCG concentration on degradation

The concentration-dependence of the degradation of EGCG is an important aspect for the dosage form formulation as the release of EGCG from the dosage form can be modulated and consequently, the stability of EGCG within the intestinal fluid could be enhanced without the need of stabilizing additives like antioxidants.

For this purpose three different EGCG concentrations were chosen: a low concentration (0.3 $^{g}/_{l}$) representing the release of 60 mg EGCG in the stomach, an intermediate concentration after taking 1 capsule with 200 mg EGCG (1.0 $^{g}/_{l}$); and high concentration representing the administration of a 500 mg dose (2.5 $^{g}/_{l}$) or the release in areas with less fluid such as the ileal region.

As expected, increasing the EGCG concentration reduced percentage-wise the degradation of EGCG in pH 6.5 up to 7.4. In the case of 0.3 $^{g}/_{l}$ EGCG in pH 7.4, 50 % degraded over 3 hours, whereas at 1.0 $^{g}/_{l}$ just 21 % and at 2.5 $^{g}/_{l}$ just 13 % EGCG degraded (Fig. 23).

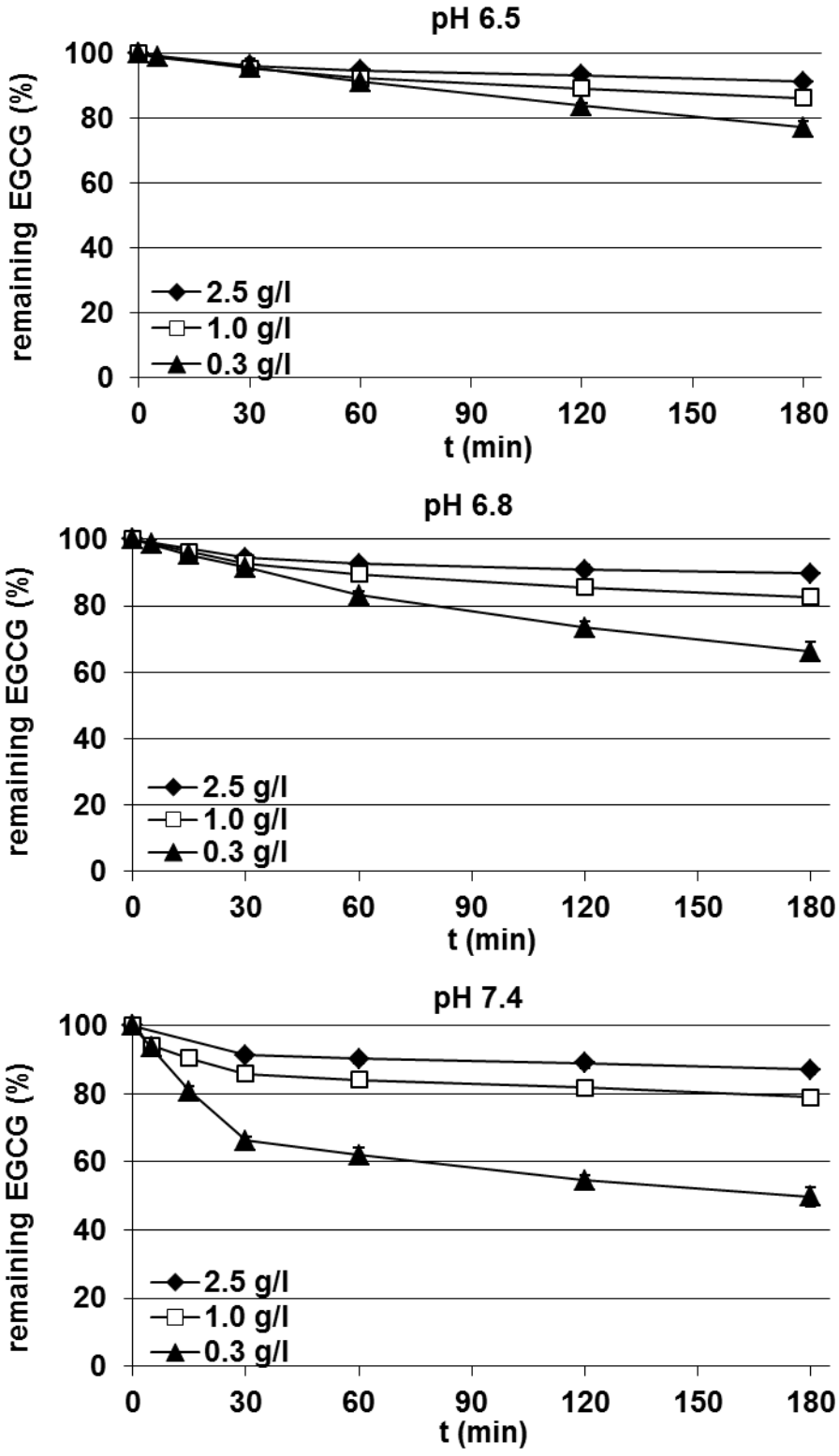


Figure 23: Concentration- and pH-dependent degradation of EGCG in phosphate buffer solution at 37°C (values presented as an average of n=3 ±SD)

The observed degradation was not proportional to the EGCG concentration either percentage-wise or absolutely. Increasing the EGCG concentration 2.5-fold from 1.0 $^{g}/_{l}$ to 2.5 $^{g}/_{l}$ increases the amount degraded within 60 minutes not even 2-fold (Table 5).

The biphasic degradation pattern occurred as well at the different concentrations. Besides, the susceptibility of the first phase to pH, a concentration-dependence could be observed, whereas the second phase showed no significant difference in its reaction velocity at the different concentrations. Thus, the first phase is pH- and concentration-dependent and the second phase is independent of both factors.

Table 5: Amount of EGCG degraded within 60 minutes (initial degradation phase) and degradation rate (k) after 60 minutes (second degradation phase) at 37°C, varying pH and concentrations of EGCG (values presented as an average of n=3 (SD))

Concentration ($^{g}/_{l}$)	Concentration $_{degraded}$ ($^{mg}/_{l}$) at t = 60 min			Degradation rate k ($^{mg}/_{l*h}$) at t > 60 min		
	pH 6.5	pH 6.8	pH 7.4	pH 6.5	pH 6.8	pH 7.4
0.3	26.7 (3.5)	50.6 (4.0)	115.8 (5.9)	22.8 (4.7)	25.8 (3.0)	18.6 (2.4)
1.0	72.6 (5.0)	106.7 (7.4)	159.1 (14.7)	29.0 (3.8)	31.6 (4.5)	25.8 (4.9)
2.5	129.9 (5.2)	185.1 (7.4)	233.7 (27.6)	35.4 (4.2)	29.8 (9.6)	40.4 (0.7)

A reasonable explanation for this phenomenon is the self-aggregation of EGCG and the resulting binary system which consists of EGCG monomers in equilibrium with EGCG self-associates.

With increasing concentrations a further decline in surface tension could be observed (section 3.1.1.) and thus more EGCG must have been present in its monomeric form at the interfaces. The occupation of the interfaces is concentration dependent. The more EGCG is dissolved, the more monomers gather at the interfaces and form a denser package. These monomers are responsible for the first degradation phase. But the occupation of the interfaces does not increase proportional to the dissolved EGCG amount. Thus more EGCG degrades absolutely at higher concentrations, but not with the same factor as the total concentration of EGCG in solution is increased.

On the other hand, the self-aggregates are responsible for the second degradation phase. EGCG forms even at elevated pH thus in the presence of degradation products and deprotonated EGCG aggregates (section 3.1.2.). Hence degradation

products and deprotonated EGCG can be incorporated within the aggregates and induce the autoxidative degradation of EGCG inside the aggregates. The reduced degradation velocity originates from van-der-Waals and hydrophobic interactions of the B- and C-ring of EGCG, hydrogen bonds and the subsequent reduced reactivity of vicinal hydroxyl groups. So once the monomers are degraded, the degradation velocity slows down but the autoxidative degradation is not terminated.
In terms of pH-dependence, EGCG in its monomeric form is surrounded by hydration water, whereas aggregate formation is accompanied by the expulsion of hydration water. The hydration water is necessary to exhibit a pH effect; thus the second phase arising from aggregates follows a pH-independent degradation pattern.

3.1.5. Effect of pre-aggregation on degradation

To investigate whether the self-aggregation of EGCG is responsible for the biphasic degradation pattern and for the concentration-dependent increase in stability, the effect of first dissolving EGCG under stable pH conditions (0.016 M HCl) prior to buffering to pH 7.4 was investigated. The pH of the solutions remained unaltered over the time of the experiments, excluding the hypothesis that EGCG as weak acid reduces at high concentration the pH of the solution and this way enhances its stability.
The results reveal that in fact, the pre-aggregation in acidic pH has a positive effect on EGCG stability (Fig. 24). The second phase of degradation (t > 60 min) levels off to a degradation rate of nearly 0 $^{mg}/_{l*h}$, whereas in the first degradation phase (t < 60 min) the same EGCG loss can be observed with and without pre-aggregation step (Table 6). This arises from the fact, that even after aggregate formation, EGCG monomers are still present in equilibrium to EGCG aggregates. Since the equilibrium is concentration-dependent, the first degradation phase remains concentration-dependent and since the monomers have a hydration shell, the phase remains pH-dependent.

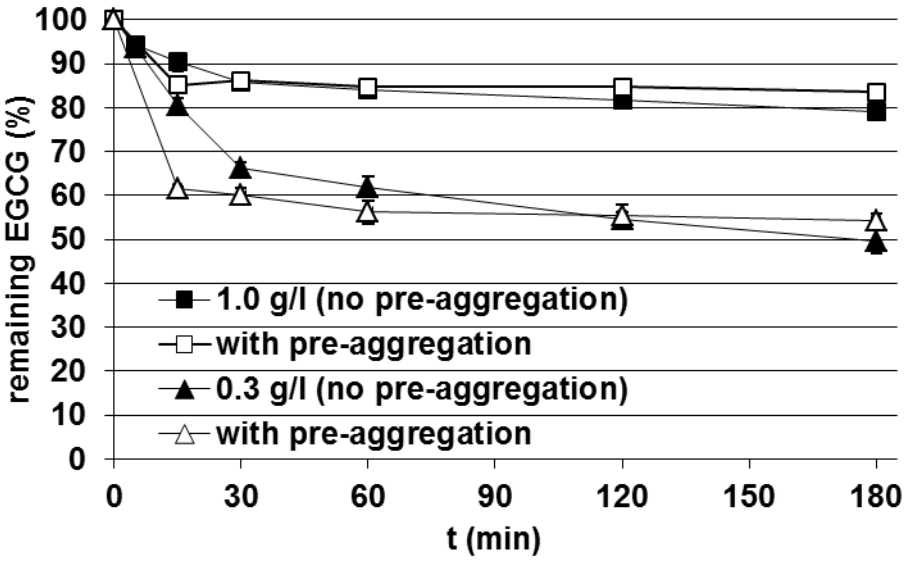


Figure 24: Effect of EGCG pre-aggregation in acidic pH on EGCG degradation at 37°C in pH 7.4 (values presented as an average of n=3)

Furthermore, a faster change from first to second degradation phase can be observed in the case of pre-aggregation. The monomers degrade within 15 minutes, whereas without pre-aggregation the first phase takes 30 to 60 minutes. It seems that under acidic pH the aggregates form faster than at elevated pH because no other thermodynamic processes take place which are coupled to the autoxidative degradation of EGCG.

The second phase of degradation (t > 60 min) levels off to a degradation rate of 2.3 $^{mg}/_{l*h}$ ± 2.0 $^{mg}/_{l*h}$ and 5.8 $^{mg}/_{l*h}$ ± 8.8 $^{mg}/_{l*h}$ with no significant degradation occurring for 2 hours (Table 6) because the aggregates consist of undissociated, undegraded EGCG (beside degradation products present in the Teavigo® powder itself) and consequently, EGCG does not or just little degrade inside the self-associates.

Table 6: Effect of 30 minutes pre-aggregation in acidic pH on the initial degradation phase and degradation rate (k) after 60 minutes (second degradation) of EGCG at 37°C in pH 7.4 at different concentrations (values are presented as average of n=3 (SD))

Concentration ($^{g}/_{l}$)	Concentration $_{degraded}$ ($^{mg}/_{l}$) at t = 60 min		Degradation rate k ($^{mg}/_{l*h}$) at t > 60 min	
	No pre-aggregation	With pre-aggregation	No pre-aggregation	With pre-aggregation
0.3	115.8 (5.9)	133.2 (8.0)	18.5 (5.7)	2.3 (2.0)
1.0	159.1 (14.7)	151.6 (9.6)	25.9 (4.8)	5.8 (8.8)

As a result, 5 % more EGCG can be recovered after 3 hours exposure to higher pH at low concentrations of 0.3 $^{g}/_{l}$ as well as at higher concentrations of 1.0 $^{g}/_{l}$.
These results can be transferred to the in-vivo situation where the dissolution of EGCG in the stomach would lead to aggregate formation under acidic conditions. These aggregates would leave the stomach into the intestine and could exhibit higher stability.
Consequently, immediate release formulations are an appropriate peroral dosage form for EGCG. Alternatively, the release could occur as well in a desired part of the intestine like the ileocecal region, where fewer phase 2 metabolizing enzymes are present, but would need to be combined with an acidifier to ensure that EGCG aggregates can form under stable pH-conditions.

Referring to the study of EGCG stability in the two-stage digestive model of Neilson et al. mentioned in the introduction, where EGCG degraded to a high extent despite the pre-incubation in acidic environment, a deeper look into the model needs to be taken.
First of all they investigated concentrations below the determined CAC; thus any aggregate present would be just of minor size (dimers, trimers). Additionally, they conducted the stage change from gastric phase to intestinal phase by strong dilution of 3 ml samples up to a volume of 50 ml. But dilution processes have an effect on the thermodynamics of systems. Dilution results in the reduction of system enthalpy and entropy with the consequence of aggregate dissociation and monomer hydration. Hence the thermodynamic changes by dilution processes can result in an increased EGCG loss. Such a negative effect of dilution processes was proven as well during the design of the pre-aggregation study. Adjusting the pH by the addition of sodium hydrogen phosphate solution instead of sodium hydrogen phosphate powder resulted in an increase in EGCG loss of 20 %.

Furthermore, this more than 10-fold dilution in the above-mentioned digestive model does not occur in-vivo. Studies performed on human digestion found just a 3- to 5-fold dilution of administered meals in the duodenum (Borgström et al., 1957). Studies performed on rats that had drug solutions administered showed in the stomach and

the duodenum the same concentration of drug with concentration even increasing in the jejunum due to fluid absorption (Masaoka et al., 2006).
This indicates that increasing the drug concentration in a digestive model would be more comparable to the in-vivo situation to assess drug stability within the gastrointestinal tract than the dilution of drug solutions. In the case of EGCG, this would mean that stable aggregates could exist even in the lower parts of the intestine if they are formed under acidic pH-conditions.

In accordance with these results, immediate release tablets with a fast disintegration and dissolution of EGCG in the stomach were chosen as a peroral dosage form for EGCG and were investigated in detail.

3.2. Coacervate/ precipitate formation of (-)-Epigallocatechin-3-gallate with common excipients

EGCG forms complexes with different classes of macromolecules. Many commonly used excipients are macromolecules and some of them are already reported to interact with EGCG (section 1.7). The phase-separation in aqueous systems containing EGCG is mainly driven by hydrophobic interactions of the solutes. The EGCG ring system interacts with the polymer backbone chain and as second step hydrophilic interactions of the side chains and functional groups reinforce the formed complex. At first, soluble complexes are formed. When more reactants are present, insoluble complexes are formed which can further associate through van-der-Waals interactions into precipitates. This phase-separation can occur as well in the gastrointestinal fluids with EGCG and dosage form excipients. And this can affect EGCG release from the dosage form as well as its absorption and hence can influence the oral bioavailability of EGCG. For this reason, the phase-separation in aqueous systems containing EGCG and gelatin, HPMC and PVP was investigated.

3.2.1. Polarized light microscopy

First of all, the capsule materials HPMC, gelatin and HPMC with gellan gum as gelling aid were tested as capsule formulations are the main dosage form for EGCG. The combination of EGCG with these capsule materials resulted in a phase-separation in 0.1 M HCl (Fig. 25). Round coacervate droplets were formed with EGCG and disintegrated HPMC-gellan gum capsules as well as with gelatin (Fig. 25A+C). In the case of pure HPMC, a network structure was formed consisting of small spherical particles (Fig. 25B).

Polyvinylpyrrolidone was tested as well as it is a commonly used binder in peroral dosage forms; similar to HPMC it formed a network structure but no round particles could be distinguished (Fig. 25D). Consequently, HPMC-gellan gum and gelatin capsules formed coacervate droplets with EGCG whereas PVP and pure HPMC precipitated to form a solid phase.

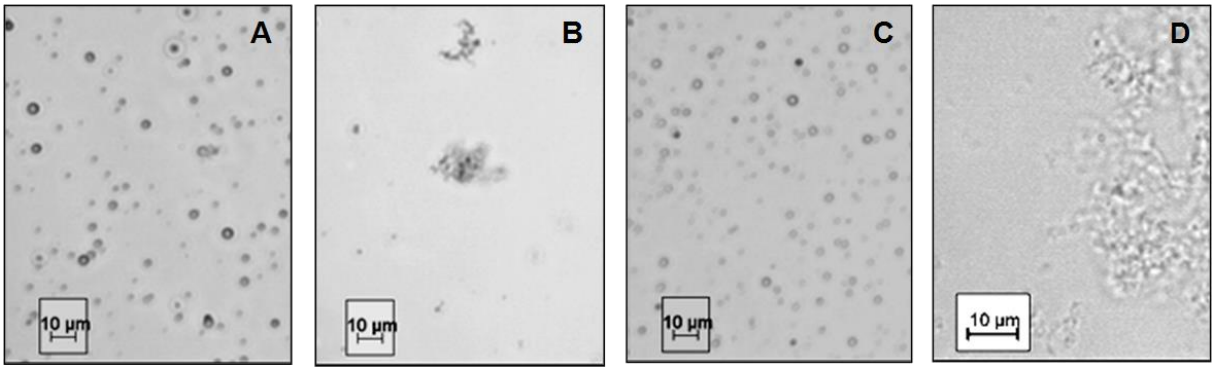


Figure 25: Microscope pictures of particles formed by mixing solutions of EGCG with A) disintegrated HPMC-gellan gum capsule, B) HPMC, C) gelatin and D) PVP in 0.1M HCl

The process of coacervation / precipitation was observed under the polarized microscope. The addition of 0.1 M HCl between both solids (Fig. 26A+D) resulted in a fast dissolution of the two reactants and where the saturated reactant solutions came in contact, a boundary layer was formed immediately.

The strength of the layer is proportional to its darkness in the picture. The denser the aggregates, the darker the layer appears. In the case of HPMC a strong layer is formed which penetrates in both solutions forming a network structure (Fig. 26E+F). This process reflects the formation of solid capsule shell observed during dissolution studies (section 3.2.5) when the capsule material and content is wetted and saturated solutions of each substance get in contact.

The interaction of EGCG with PVP on the other hand led to a transparent film (Fig. 26B+C).

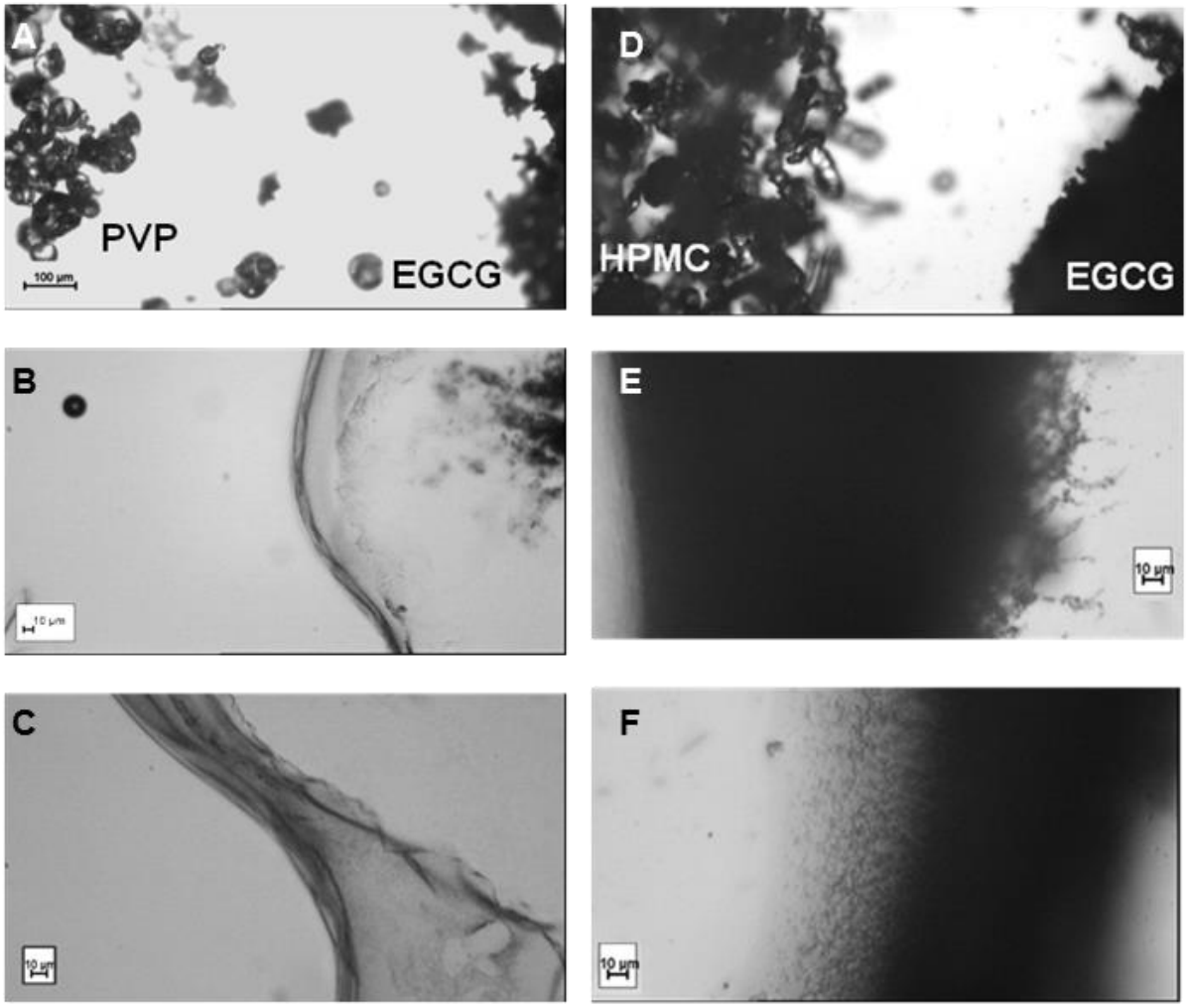


Figure 26: Phase-separation occurring with PVP or HPMC and EGCG in 0.1 M HCl observed under the polarized light microscope: A) and D) represents the solids without 0.1 M HCl, B) and C) the boundary layer of PVP and EGCG, E) and F) the boundary layer of HPMC and EGCG

In the case of gelatin, round coacervates were observed (Fig 27B+C). The boundary layer differed from the one observed with PVP and HPMC. It resembles more a line consisting of densely packed coacervates which in fact coalesced into one layer. This might be the reason for the better disintegration behavior of hard gelatin capsules and faster EGCG release observed in the dissolution studies (section 3.2.5).

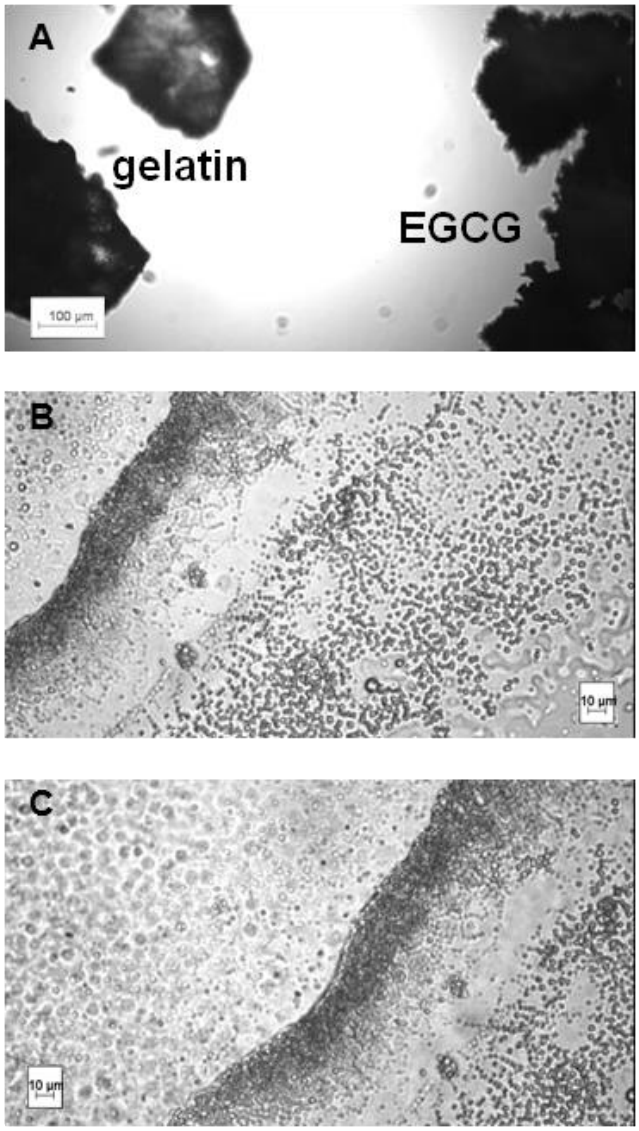


Figure 27: Phase-separation occurring with gelatin and EGCG in 0.1 M HCl observed under the polarized light microscope with boundary layer formed out of coalesced coacervates

In the case of HPMC-gellan gum capsules, gellan gum has to form the observed coacervates in Figure 25A as pure HPMC formed a precipitate with EGCG (Fig. 25B). The inability of this capsule material to disintegrate arises consequently from a combination of liquid phase-separation (coacervation) with gellan gum and solid phase-separation (flocculation) with HPMC.

3.2.2. Turbidimetric studies of EGCG with the hard capsule materials HPMC and gelatin

Turbidimetric studies are considered as an indirect method to qualitatively investigate the phase-separation. The particles result in a reduction of the intensity of the transmitted light which is expressed as turbidity. With this method, the phase-separation boundary and the extent of phase-separation depending on different concentrations and ratios of reactants can be analyzed. Due to the different phase-separations observed in EGCG-HPMC and EGCG-gelatin systems and the different particle sizes, the absorbance values could not be compared with each other. Furthermore, at high concentrations of the reactants in solution large particles are formed which can result in sedimentation; thus the turbidity will be reduced in the incident beam.

Nevertheless, the turbidimetric studies of EGCG in 0.1 M HCl with hydroxypropyl methylcellulose and gelatin allowed estimating the boundary at which precipitation or coacervation started. Ternary phase diagrams are useful to represent the coacervation/precipitation boundaries and extent of the phase-separation in one single graph. The boundary lies for both hard capsule materials at low concentrations of the reactants (Fig. 28). At room temperature, the precipitation with HPMC started at 0.005 % (w/w) of EGCG in combination with 0.09 % (w/w) HPMC or with 0.0005 % (w/w) HPMC in the presence of 0.01% (w/w) EGCG (Fig. 28A). In the case of gelatin, higher concentrations are necessary for the coacervation to start: 0.07 % (w/w) of EGCG and 0.01 % (w/w) gelatin or 0.2 % (w/w) EGCG and 0.001 % (w/w) gelatin (Fig. 28B).

Furthermore, it could be observed that increasing EGCG concentration resulted in an increased turbidity (Fig. 28). On the other hand, increasing the gelatin or HPMC concentration at constant EGCG concentrations resulted only in a slight increase of turbidity. The concentration of EGCG is thus more critical than the concentration of the macromolecules. Nevertheless, the coacervation is dependent on the concentration of both reactants.

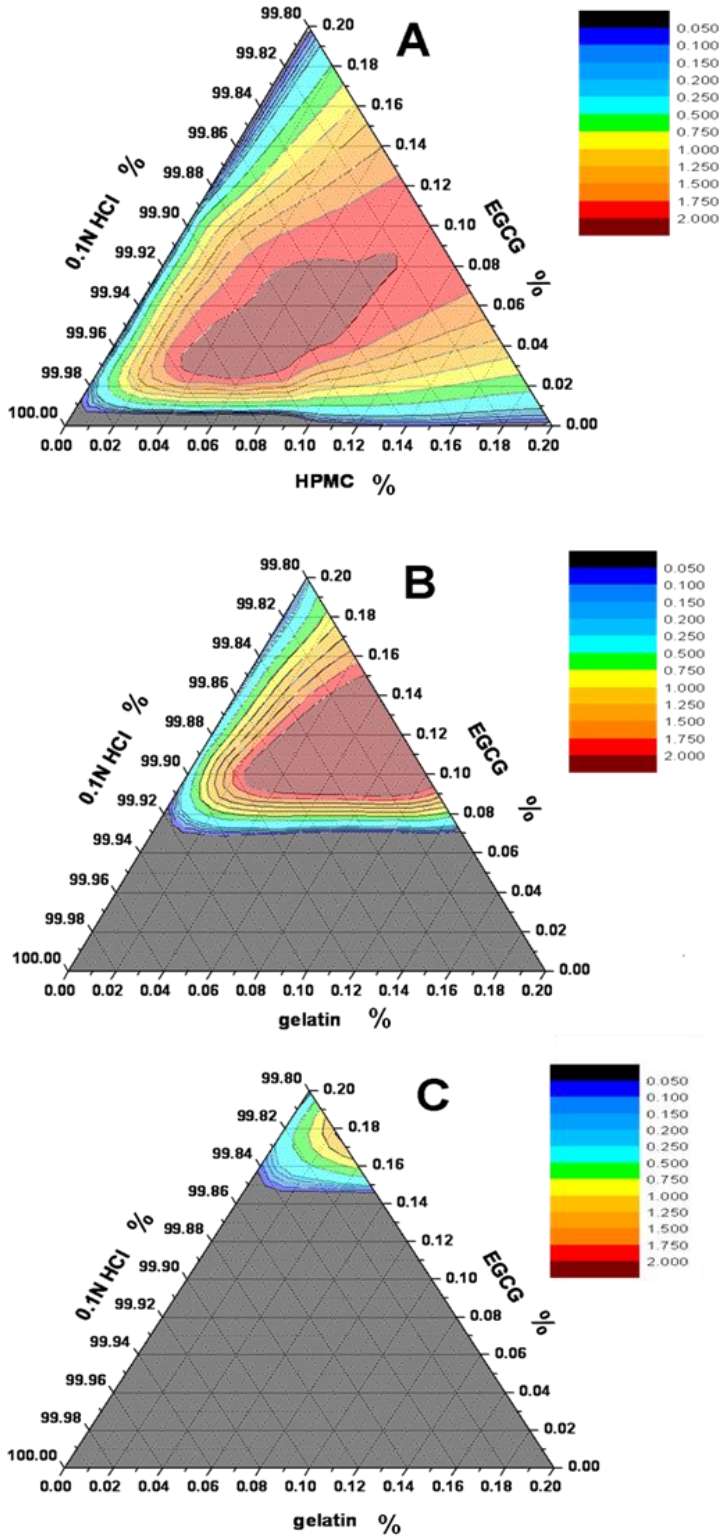


Figure 28: Coacervation triangles of EGCG in 0.1 M HCl at room temperature with A) HPMC B) gelatin and C) at 37°C with gelatin

As coacervation occurs due to solubility effects, temperature can affect the coacervates. With EGCG-gelatin coacervates, increasing the temperature from room temperature to 37°C resulted in a shift in coacervation boundary to higher EGCG concentrations (0.15 % (w/w)) (Fig. 28C) due to the increased solubility of gelatin.

3.2.3. Extraction of EGCG from coacervates/precipitates

Two approaches were followed up to prevent the phase-separation observed in section 3.2.. One was inducing steric and electrostatic hindrance of the colloids to each other by addition of stabilizers such as surfactants analogue to nanoparticle stabilization. When the complexes are covered by the surfactant and this way have a certain distance to each other, the attractive forces are not strong enough to result in agglomeration and precipitation. Furthermore, surfactants can be ionic and thus result in an electrostatic repulsion of the particles.
Another possibility is to dissociate the complexes by enhancing their dissolution, for example by the addition of organic co-solvents.
Both strategies were investigated in this work as dissociation of complexes and precipitates was necessary for precise quantification of EGCG loss due to degradation upon storage at 40°C/75 % RH. Furthermore, the findings of these studies could be used to tackle the shell solidification of EGCG capsules.

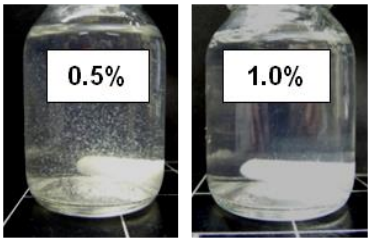
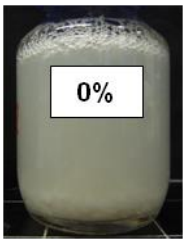
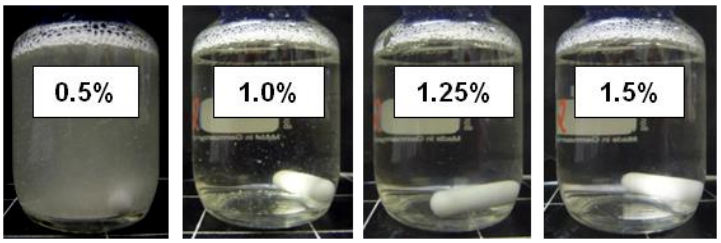
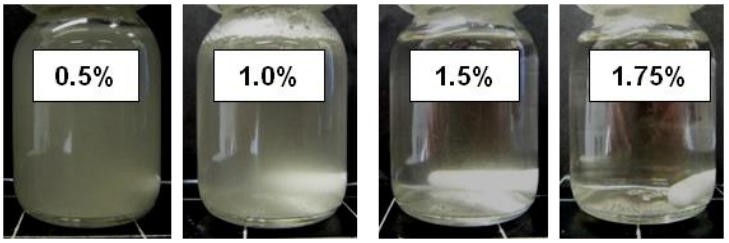
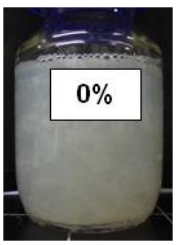
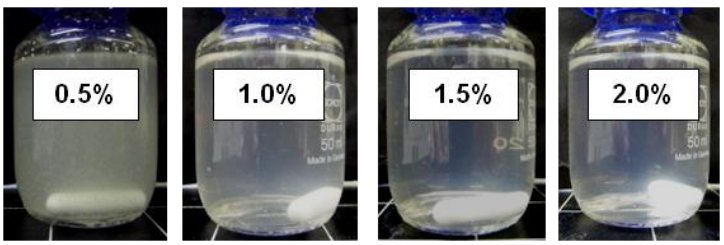
3.2.3.1. Effect of surfactants on HPMC-EGCG precipitates and HPMC-gellan gum-EGCG coacervates

According to Patel et al., the interaction of EGCG with water-soluble cellulose ethers results in colloidal complexes forming nanoparticles with the size of 30 nm up to 700 nm which further agglomerate to form precipitates. The agglomeration was mainly due to van-der-Waals interactions. If the precipitate consists of agglomerated nanoparticles, it can be classified as lyophobic colloids. In lyophobic colloidal systems the reduction of free energy is the driving force for agglomeration (Burgess, 2002).

Beside surface coverage and steric hindrance, surfactants reduce the interfacial tension and by this way reduce the free energy of the systems. Hence the addition of surfactants should inhibit their agglomeration and result in a clear solution.
Additionally, surfactants can increase the solvation power of solvents and thus increase the solubility of EGCG-macromolecule complexes in aqueous media. For this purpose, ionic and non-ionic surfactants of different HLB values were added to solutions containing HPMC-EGCG precipitates.

In fact, some surfactants were capable of disrupting the particles when they were added after the phase-separation and they were able to prevent the precipitation when they were added prior to the mixing of the macromolecule and EGCG solutions (Table 7). The addition of 1 % (w/v) polysorbate to turbid solutions of EGCG and HPMC resulted in a clear solution. At a concentration of 0.5 % (w/v) polysorbate 80 results in less turbidity than the addition of 0.5 % (w/v) polysorbate 20 (Table 7). Polysorbate 20 is an ester of lauric acid and polysorbate 80 an ester of oleic acid. A longer hydrophobic side chain results in more efficient particle dissociation. The bulkier spatial structure of polysorbate 80 creates more steric hindrance between the nanoparticles and thus reduces the interactions already at a lower concentration than polysorbate 20.
Poloxamer 188 (Pluronic® F68) is a commonly used stabilizer for nanosuspensions. The effect of Pluronic® F68 originates from particle surface coverage. This way it increases the spatial distance between the colloids. Consequently, it was also effective but a higher amount (1.5 % (w/v)) was necessary for the stabilization.
Likewise, the addition of 1.5 % (w/v) Cremophor® EL was effective, though the addition resulted in an opalescent solution. But this opalescence comes from Cremophor® EL itself, not from the colloids (Technical information on Kolliphor® EL former Cremophor® EL) and hence can be considered as an effective stabilizer, too.

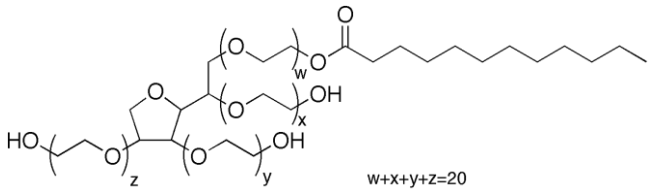
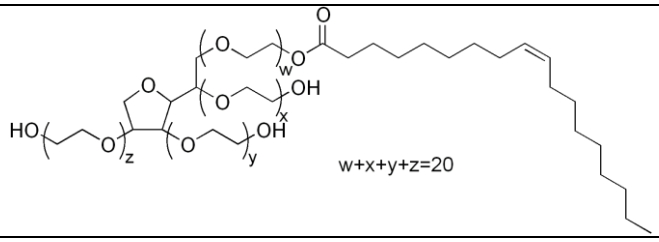
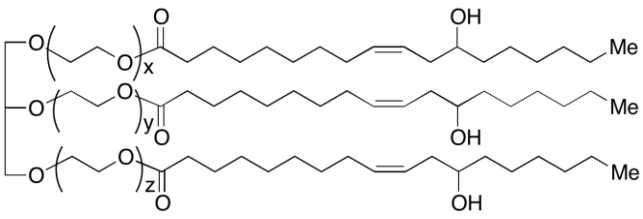
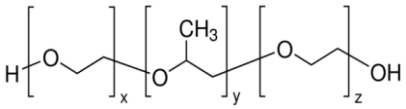
Table 7: Turbid solution resulting from precipitate formation between HPMC and EGCG in 0.1 M HCl and resulting clearance by the addition of surfactants (amount of added surfactant presented as (w/v))

Tween® 80	
	
Tween® 20	
	
Pluronic® F68	
	
Cremophor® EL	
	

In summary, EGCG-HPMC precipitates can be disrupted by the addition of 1 % (w/v) nonionic surfactant and result in clear solutions (Table 7). The concentrations of surfactants were well above their critical micelle concentration (CMC); thus the efficacy of stabilization is not only mediated by the improved solvation power of the medium toward the complexes and the reduced interfacial tension (Table 8). The most important factor is the surface coverage of colloidal particles and the subsequent resulting steric hindrance preventing their agglomeration.

Two important attributes of effective surfactants can be deduced from the results: first a HLB value above 12, i.e., they are hydrophilic in nature and belong to the oil-in-water emulsifiers. Second, they all comprise polyoxyethylene groups and due to their molecular structure offer steric hindrance (Table 8).

Table 8: Name, structure, HLB value and critical micelle concentration of effective surfactants

Name	Structure	HLB	CMC (%)
Polysorbate 20 (Tween® 20)	 w+x+y+z=20	16.7*	0.007*
Polysorbate 80 (Tween® 80)	 w+x+y+z=20	15.0*	0.002*
Polyethoxylated castor oil (Cremophor® EL =Kolliphor® EL)		12-14**	0.02**
Poloxamer 188 (Pluronic® F68)	 y=31; x=z=75	n.d.	0.03*

* Sigma-Aldrich

** BASF

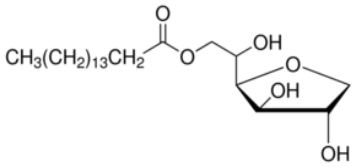
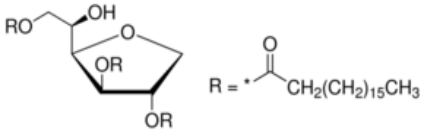
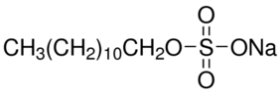
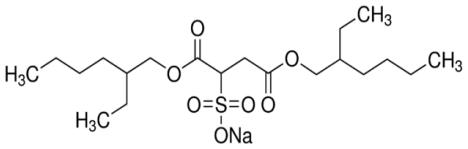
In the case of other surfactants, even additions of up to 2 % (w/v) resulted in no clearing of the suspensions and thus they were classified as ineffective (Table 9).
Sorbitan fatty esters belong to the water-in-oil emulsifiers and thus are poorly soluble in water. This might have caused their failure in disrupting the precipitates.
A rational explanation for the failure of the ionic surfactants sodium lauryl sulfate (SDS) and sodium docusate (DSS) is that colloids always have a charged surface, whereby most particles carry a negative charge. Thus if the surfactant carries the same charge as the colloid, the surfactant molecules will be repulsed, making a surface coverage impossible. Furthermore, their lack of stabilization might arise from the fact that they consist of a linear hydrophobic chain which offers less steric hindrance. The effective surfactants have a bulkier structure resulting from polyoxyethylene chains.

Table 9: Remaining turbid solutions after addition of 2 % (w/v) ineffective surfactants and PEG

Span® 40	Span® 65	SDS	DSS	PEG 4000
				

All ineffective surfactants (Table 10) have in common that they have no polyoxyethylene groups in their structure. To ensure that the polyoxyethylene chains were not the key factor for surfactant effectiveness, the addition of PEG 4000 to the suspensions was investigated, but was ineffective too (Table 9). Thus the polyoxyethylene chains were not the only critical attribute for effective dissociation of agglomerates.

Table 10: Name, structure, HLB value and critical micelle concentration of ineffective surfactants

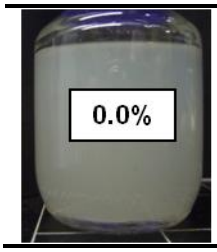
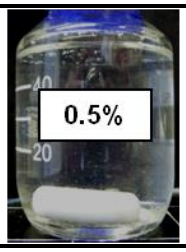
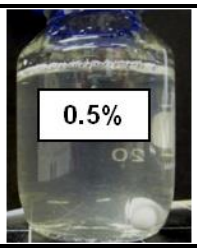
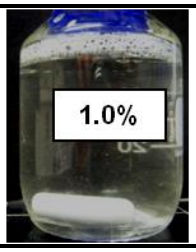
Name	Structure	HLB	CMC (%)
Sorbitan monopalmitate (Span® 40)		6*	n.d.
Sorbitan tristearate (Span® 65)		2.1±1.0*	n.d.
Sodium lauryl sulfate (SDS)		n.d.	0.2-0.3*
Sodium docusate (DSS)		n.d.	0.02-0.03**

* Sigma-Aldrich

** W. G. Chambliss et al. 1981

In contrast to pure HPMC which resulted in solid-liquid phase-separation in form of solid nanoparticles, disintegrated HPMC-gellan gum capsules formed additionally coacervates with EGCG (section 3.2.1); thus liquid-liquid and solid-liquid phase-separation occurred. Therefore it was tested, whether the addition of surfactants would be effective in this system as well. The test was performed at physiological concentrations, i.e. upon the intake of one capsule filled with 150 mg EGCG. In this case 0.5 % (w/v) of most of the effective surfactants was sufficient for the dissociation of EGCG-HPMC-gellan gum coacervates. Just in the case of Pluronic® F68 a higher amount (1 % (w/v)) was needed (Table 11).

Table 11: Turbid solution resulting from coacervate formation between HPMC-gellan gum capsule and EGCG in 0.1 M HCl and resulting clearance by addition of surfactants (amount of added surfactant presented as (w/v))

None	Tween® 80	Tween® 20	Cremophor® EL	Pluronic® F68
				

But despite a visual clearance of the systems, the complexes were not fully dissociated. The extraction of EGCG out of precipitates with the help of surfactants did not lead to full EGCG recovery. In EGCG-HPMC systems consisting of 1 $^{g}/_{l}$ of each reactant in 0.1 M HCl, at the most 85.1 % ± 0.1 % and 91.7 % ± 0.5 % of EGCG could be recovered by addition of 1.5 % (w/v) of Tween® 20 and Cremophor® EL, respectively. This loss in recovery was not mediated by micellization of EGCG, as the validated calibration curves of EGCG with Tween® 20 and pure EGCG solutions yielded the same calibration function with only 3.5 % difference in the slop.

3.2.3.2. Extraction of EGCG from precipitates with isopropanol

As the addition of surfactant resulted in suspension clearance but did not recover EGCG fully out of complexes, the addition of the co-solvent isopropanol was investigated. Isopropanol was used as an extraction solvent in a 1:1 (v/v) ratio to the aqueous phase.

Addition of an organic co-solvents results in an exchange of water molecules by organic solvent molecules in the solvation shell. The kind of organic solvent determines the size increase of these cavities as it changes the surface tension of the medium. Thus it can hinder the fusion into a bigger cavity and as a consequence hinder the formation of complexes (Connors and Khossravi, 1993).

Furthermore, the added organic solvents need to have a solvation effect on the single compounds and increase the hydrophobicity of medium as the complex

formation of EGCG and macromolecules results in expulsion of hydration water and creation of hydrophobic areas to minimize the energy of the system.

Thus, polar organic solvents like ethanol, isopropanol or acetone can be chosen to dissociate the lyophobic colloids and complexes. They reduce the surface tension of aqueous solutions (solvent-solvent interaction), they can dissolve EGCG, PVP and HPMC (solvent-solute interaction) and they increase the hydrophobicity of medium and reduce the free energy of the system. Consequently, the complexes have no driving force to agglomerate.

EGCG was successfully recovered from the precipitates with HPMC and PVP by the addition of 50 % (v/v) isopropanol to 0.1 M HCl (Table 12). In the case of lower polymer to EGCG ratios, 96.7% to 101.3% of EGCG was recovered when the EGCG content was quantified immediately within 1 hour after solvent addition with HPLC-UV. But when the polymer to EGCG ratio was above 2:1 (w/w) in the case of HPMC and above 1:2 (w/w) in the case of PVP, overnight extraction (14 hours) became necessary prior HPLC-analysis.

Table 12: Recovery of EGCG out of precipitates with HPMC and PVP by the addition of 50 % (v/v) isopropanol and EGCG quantification immediately after solvent addition of after 14 hours (overnight extraction) (values presented as an average of n=3 ±SD)

Weight ratio (polymer:EGCG)	HPMC		PVP	
	Extraction time in isopropanol/ 0.1 M HCl (h)			
	< 1	14	< 1	14
1:10	100.5 ± 0.6	-	99.8 ± 2.4	-
1:5	101.3 ± 0.5	-	100.8 ± 2.9	-
1:2	99.0 ± 2.1	-	96.7 ± 2.8	98.1 ± 0.5
1:1	97.0 ± 3.1	-	90.1 ± 3.9	94.6 ± 1.2
2:1	91.3 ± 4.1	98.1 ± 0.9	83.4 ± 1.7	88.4 ± 2.6
3:1	64.9 ± 12.3	99.1 ± 0.7	82.9 ± 5.3	95.3 ± 4.9

With increasing polymer content, bigger particles were formed and hence the dissolution process of EGCG and polymer took longer. The EGCG recovery after overnight extraction from EGCG-HPMC precipitates was approximately 100 %, whereas from EGCG-PVP precipitates just 88.4 % up to 98.1 % could be recovered.

Due to the good EGCG recovery results for the precipitates resulting from a polymer to EGCG ratio of 1:1 (w/w) and lower, isopropanol was chosen as the extraction medium for all further experiments with precipitate forming excipients.

3.2.4. Quantification of EGCG loss due to coacervation/ precipitation

The quantification of EGCG loss due to phase-separation was investigated upon mixing of stock solutions of reactants at room temperature in 0.1 M HCl. The quantitative results were consistent with the qualitative results from the turbidimetric studies. A higher EGCG loss was observed by the addition of HPMC than by the addition of gelatin (Fig. 29A). Just in the case of low concentrations of the capsule materials (below 20 % (w/w) on EGCG), gelatin showed a higher EGCG loss than HPMC.

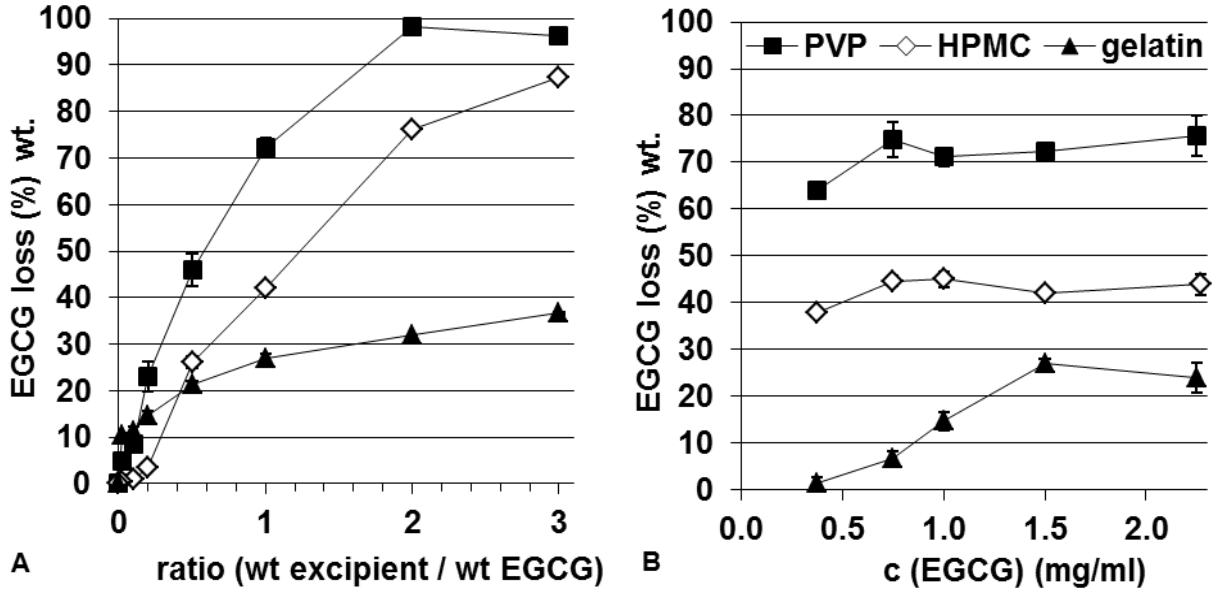


Figure 29: EGCG captured by coacervate and precipitate forming excipients in 0.1 M HCl A) at a constant EGCG concentration of 1.5 mg/ml (300 mg dose) and varying amount of excipient B) at a constant EGCG to excipient ratio of 1:1 (w/w) and varying EGCG concentration

Comparing the capsule materials, EGCG loss increased less steeply with increasing gelatin concentration (Fig. 29A) than with HPMC.

Overall, the highest EGCG loss resulted by the addition of PVP, followed by HPMC and gelatin. PVP addition (2:1 (w/w) ratio) resulted in almost 100 % EGCG loss, explaining its use as filtration agent in beverages to get rid of polyphenols. At the same ratio, HPMC addition resulted in 75 % and gelatin in 30 % loss of EGCG.

In all three systems, the EGCG loss did not increase linearly with increasing macromolecule concentrations. Hence the interaction did not occur at a constant molar ratio of the reactants. This can be explained by the three-step mechanism of polyphenol interaction with proteins described by Le Bourvellec and Renard (section 1.7.2.) that was postulated for polysaccharides as well. First of all, EGCG covers the macromolecule chain. For the coverage many EGCG molecules start interacting with one macromolecule; thus a steep increase in EGCG loss can be observed. Secondly, a cross-linking of covered macromolecules by multivalent interactions follows. This step consumes less EGCG than the first step of macromolecule coverage. Consequently, the slope of the curve declines. This can be observed for all three systems, however in the case of gelatin the cross-linking occurs already at lower macromolecule to EGCG ratio of 1:1 (w/w) whereas with HPMC or PVP 2:1 (w/w) ratio is necessary. This can be attributed to the special structure of proteins in contrast to synthetic polymers. Proteins exhibit several levels of structure so does gelatin. The 3-dimensional structure, e.g. a triple helical structure in the case of gelatin, is mediated by hydrogen bonds and hydrophobic interactions in which amino acid residues of the protein interact with each other. As a consequence, these sides are not or hardly available for EGCG. In contrast, the synthetic polymers do not offer such conformations and thus the H-donor and -acceptor sides are easily accessible for EGCG and more EGCG can interact with the synthetic polymers PVP and HPMC in contrast to gelatin.

Furthermore, keeping the reactant ratio constant but changing the EGCG concentration revealed, that in the case of PVP and HPMC always the same percentage of EGCG would be captured in precipitates, whereas with gelatin a constant incline in loss was observed (Fig. 29B). This can be related as well to the conformational changes of biopolymers such as gelatin in dependence of their

concentration in solution and the therefrom resulting ease or unease for EGCG to approach sufficiently the amino acid residues for interactions.

These results can be transferred to drug delivery systems containing 300 mg of EGCG and HPMC, PVP or gelatin as excipients.
In the case of 300 mg hard gelatin capsules, 21 % of EGCG would get captured in particles assuming the intake of two size 1 capsules (150 mg gelatin), respectively. In the case of 300 mg HPMC capsules, 19 % of EGCG would get captured using two size 2 capsules (120 mg HPMC).
In a delivery system consisting of 300 mg EGCG and 6 mg PVP, HPMC or gelatin as binder in wet granulation processes, 5 %, 0.3 % and 10 % of EGCG would be lost, respectively.
HPMC matrix systems with EGCG would result in a large EGCG loss. If the formulation would consist of 300 mg EGCG and 150 mg or 300 mg HPMC between 26 to 42 % of the total EGCG dose would get captured. Thus EGCG matrix systems with water-soluble cellulose derivatives such as HPMC should not be used at all.
But consideration should be given to the experimental set-up. The phase-separation investigated here was induced by mixing stock solutions of the substances. This does not reflect the microenvironmental conditions when a dosage form containing the reactants is wetted and saturated concentrations of reactants get in contact with each other. Consequently, the loss represented here is underestimated in the case of insoluble complexes such as for PVP and HPMC. It has to be considered as best case scenario of EGCG loss upon co-formulation of these substances under the terms that EGCG and the excipient have not contact until they separately dissolved in the gastrointestinal tract. But if coacervates are formed upon dosage form wetting such as in the case of gelatin, their dilution within the gastric or intestinal fluid could result in the EGCG loss presented here.

3.2.5. Effect of coacervation/ precipitation on EGCG release from different hard capsule materials

The interaction of EGCG with HPMC and gelatin resulted in phase-separation and high EGCG loss upon capturing it within precipitates. Hence the release of EGCG from different hard capsule materials was tested, setting the conditions to concentrations and ratios representing the intake of one capsule containing 150 mg EGCG with 200 ml fluid. In the case of Teavigo® Vcaps (HPMC-gellan gum as capsule material) and Vcaps® plus (HPMC as capsule material) the release medium turned turbid and the capsule shells formed a strong gum-like material in both cases (Fig. 30).

This shell solidification resulted in restricted disintegration and both capsule materials showed incomplete EGCG release. After 2 hours, just 40 % EGCG was released from the HPMC-gellan gum capsules (Teavigo® Vcaps) and 80 % from pure HPMC-capsules (Vcaps® plus) (Fig. 31). The hard gelatin capsules (Coni-Snap®) exhibited complete release and the best release profile with approximately 80 % release after 15 minutes. Also no turbidity of the release medium was observed in the case of hard gelatin capsules.

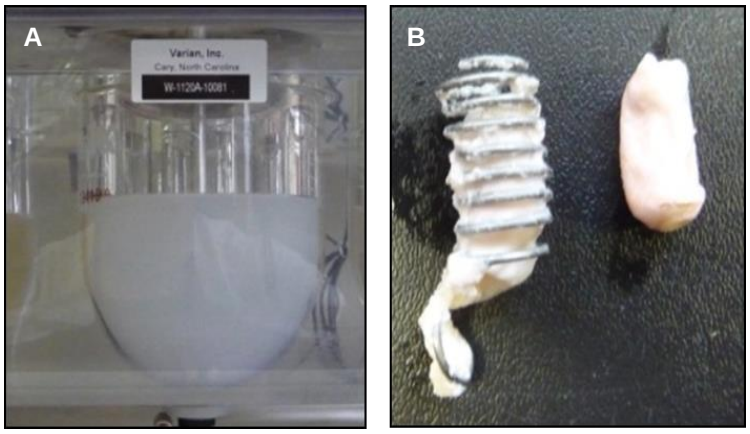


Figure 30: A) turbid dissolution medium (0.01 M HCl) during the dissolution study of Teavigo® Vcaps B) capsule shell solidification

But none of the capsule formulations met the requirements of the guideline of immediate release formulations containing BCS class 3 substances set by the FDA. According to these guidelines, 85 % of drug release within 15 minutes is required as this correlates with gastric emptying of fluids and ensures drug absorption independent of its dissolution.

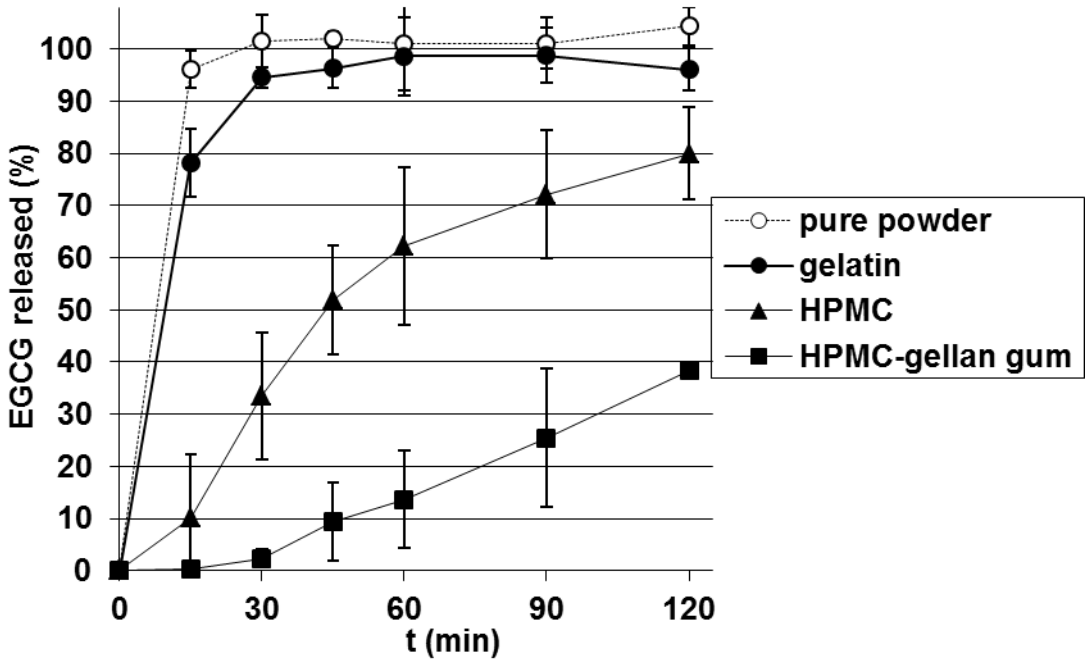


Figure 31: Dissolution study of different EGCG capsules in comparison to pure Teavigo® powder in 0.01 M HCl at 37°C performed with USP apparatus 2 set at 50 rpm (n=3)

As shown with the turbidimetric studies, gelatin can form coacervates depending on EGCG to gelatin ratio, EGCG amount and medium temperature. Consequently, increasing the dose of EGCG within the capsule, decreasing drinking fluid volume or taking the capsule with cold water carries the risk of coacervate formation and thus decreased release with failure of requirements. Research done concerning the amount of liquid patients use to swallow their tablets or capsules showed how the drug concentrations within the stomach can in fact differ interindividually (Fuchs, 2009). Out of 136 participants, 15.4 % took below 60 ml with their dose of medicine, 20.6 % took it with 61 ml to 100 ml and 64.0 % with 101 ml to 150 ml whereas just 6 individuals took it with the suggested volume of 150 ml.

Moreover, the temperature of fluid can be a true risk for lag phase development and undesired release profiles in the case of capsule formulations. Chiwele *et. al.* performed release tests from capsules assuming the intake of a 10°C cold water. In their preliminary experiments, it took 7 minutes until 150 ml 10°C cold water equilibrated to 37°C. This can result in coacervate formation of EGCG with gelatin already at low concentration of the reactants (section 3.2.2.).

Thus the fate of EGCG by increasing the dose was investigated (Fig. 32). In the case of 400 mg or 600 mg EGCG doses administered in the form of two 200 mg or two 300 mg hard gelatin capsules, the release was 65 % within 15 minutes but still complete. Upon the results from section 3.2.4., hard gelatin capsules are supposed to retain the same amount of EGCG as HPMC capsules. This could not be proven by the dissolution studies.

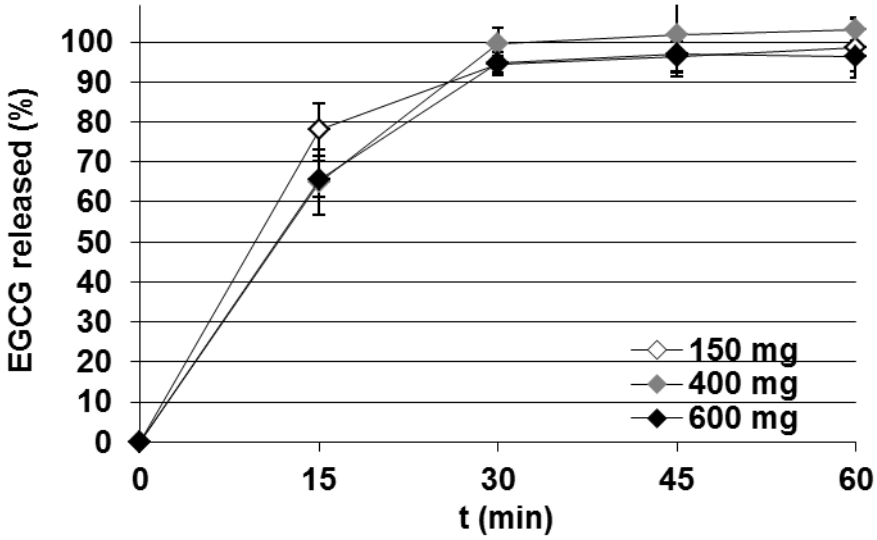


Figure 32: Dose-dependent EGCG release from hard gelatin capsules representing the intake of 1 capsule with 150 mg, 2 capsules with 200 mg and 2 capsules with 300 mg EGCG in 0.01 M HCl at 37°C performed with USP apparatus 2 set at 50 rpm (n=3)

In summary, there is a risk of coacervate and precipitate formation, i.e., release modification if EGCG is administered in capsule formulations especially due to their lack of release robustness in the case of changes of the release volume and temperature of release medium.

3.2.6. Effect of additives on EGCG release from HPMC-gellan gum capsules

Surfactants like polysorbates and poloxamer were capable of disrupting formed EGCG-HPMC-gellan gum coacervates (section 3.2.3.1). For this purpose HPMC-gellan gum capsules containing EGCG in combination with Tween® 80 and Pluronic® F68 were tested on their release behavior. The effective concentration for Tween® 80 was 0.5 % (w/v) and 1.0 % (w/v) for Pluronic® F68. This amount would correspond to the intake of around 1 g Tween® 80 and 2 g Pluronic® F68. As these concentrations are too high to be used with patients due to the mucolytic effect of surfactants, lower amounts of 15 % (w/w) Tween® 80 and 10 % (w/w) Pluronic® F68 on the total dosage form were investigated as these are concentrations typically used in dosage forms (Rowe et al., 2009) corresponding to a concentration of 0.14 % (w/v) and 0.08 % (w/v) within the gastric fluid, respectively.

Although these concentrations are lower than the effective concentrations identified in section 3.2.3.1, consideration has to be given to the small volume within the capsule which is commonly below 1 ml. Hence, the used amounts of surfactants within the capsule would be higher than the necessary concentration. Consequently, the solidification of the capsule shell could be tackled from inside and by this the release of EGCG could be increased.

But neither surfactant improved the release of EGCG from HPMC-gellan gum capsules. In the case of Tween® 80, a further decrease in release was observed, and the incorporation of Pluronic® F68 showed a release pattern comparable to HPMC-gellan gum capsules containing only EGCG (Fig. 33).

Furthermore, as EGCG does not dissolve in oils, an oily suspension of EGCG was investigated as capsule filling. This way the interaction of EGCG with capsule material was expected to be reduced.

The release of EGCG out of the oily suspension did not succeed in improving the release rate either. It was even reduced in comparison to all other EGCG capsules formulations.

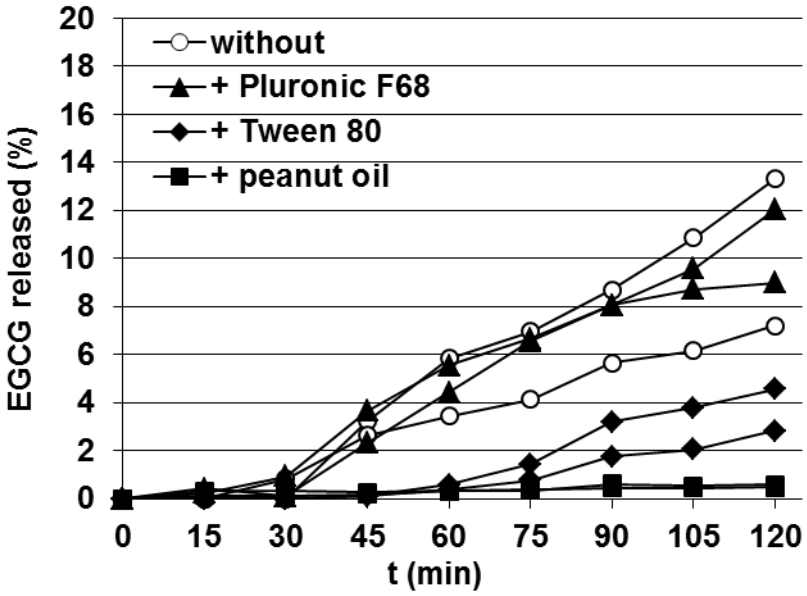


Figure 33: EGCG release from Vcaps® (HPMC-gellan gum) with and without additives in 0.01 M HCl at 37°C performed with USP apparatus 2 at a stirring rate of 50 rpm (n=2; presented as single curves)

The oily suspension was released from the capsules through shell ruptures and the released droplets could be observed floating in the release medium (Fig. 34).Polarized light microscopy revealed a shell formation around the released oil droplets formed out of crystalline EGCG (Fig. 35).

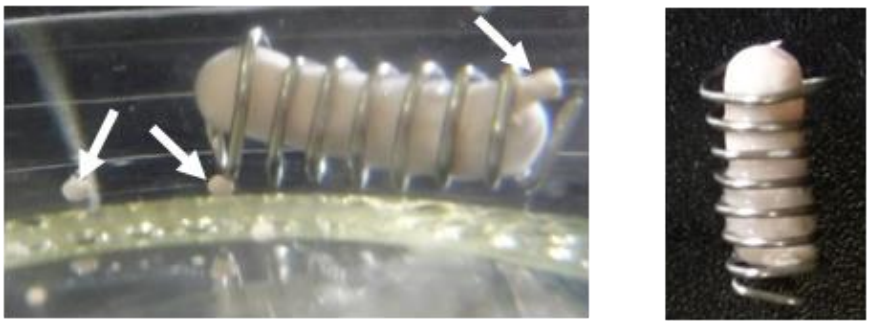


Figure 34: Oily droplets released from capsule shell rupture (left side); HPMC-gellan gum capsule filled with EGCG oil suspension after the dissolution test (right side)

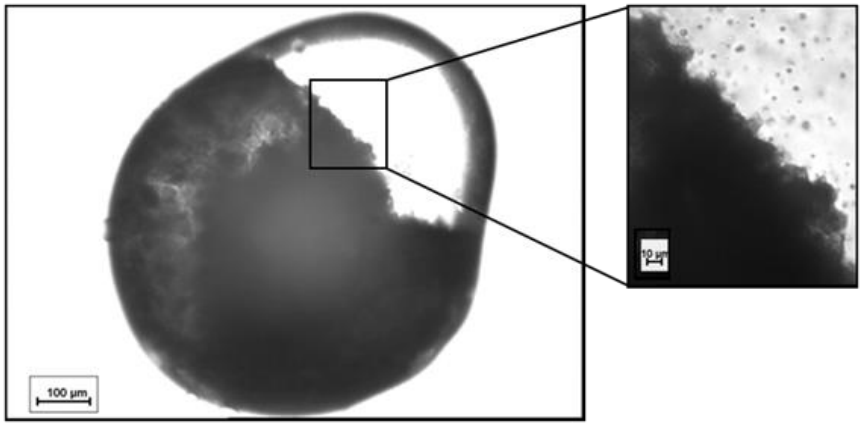


Figure 35: Microscope picture of oily droplet with solid EGCG shell

As EGCG is surface active, it gathered at the oil/water interface and being insoluble in oil but soluble in water, EGCG recrystallized at the oil/water-interface. This recrystallization process slowed down the release rate of EGCG. Hence, oily suspensions are also not an effective way to circumvent the interaction of EGCG with the capsule materials.

As no investigated additive was capable of avoiding coacervate/ precipitate formation with the capsule shell materials, the further focus of this work was aimed at tablets as alternative immediate release formulations for EGCG. Tablets offer a broader range of applicable excipients and consequently by appropriate excipient choice a dosage form for EGCG with higher robustness could be attained. Furthermore, higher unit doses can be achieved with tablets due to the compaction of loose powder. Therefore, EGCG stability in solid state with common tableting excipient was investigated to formulate stable high dose EGCG immediate release tablets.

3.3. Stability of (-)-Epigallocatechin-3-gallate in solid state

3.3.1. Storage stability with common tableting excipients and hard capsule materials in binary blends

Stability tests at accelerated stress conditions of 40°C/75 % relative humidity (RH) showed that EGCG remains stable with 2.2 % ± 1.5 % loss over 26 weeks. But several excipients are not compatible with EGCG leading to its degradation. According to the ICH guidelines for stability testing, a 5 % change from initial value (0 weeks value) or within the test period of 26 weeks represents a significant change in content and is considered as instability. Therefore, all excipients showing a significant change in EGCG content of more than 5 % over the test period of 26 weeks (Fig. 36) are considered as incompatible and thus should be avoided in tablet formulations, or their amount within the dosage form should at least be chosen carefully. The excipients were divided into three groups: coacervate/precipitate forming excipients, no coacervate forming incompatible excipients and compatible excipients.

The differentiation between coacervate-forming excipients and non-coacervate forming excipients is necessary as EGCG undergoes a transition from crystalline to amorphous state within the precipitates. Amorphous forms are known to suffer from low chemical stability as also reported for EGCG (Li et al., 2013). The consequence could be observed for HPMC and gelatin as a constant decline of EGCG within the 1:1 (w/w) binary blends over the test period. Statistically both capsule materials induced a significant change of more than 5 % in the EGCG content upon storage.

For HPMC /EGCG blends, the EGCG loss was 13.6 % ± 5.0 % (Fig. 36A). After 6 weeks Gelatin/EGCG blends resulted in an increased content of EGCG up to 124.4 % ± 0.6% which then started declining and resulted in an overall EGCG loss of 33.1 % ± 9.0 %. The increase in content after 6 weeks can be attributed to the incompatibility of gelatin with oxidizing agents, the thereof resulting decomposition of gelatin, and its cross-linking by EGCG. The decomposition lead to a mass loss of gelatin in the binary blend and changed the weight ratio of EGCG to gelatin from 1:1 to a higher EGCG content. This decomposition was proven as well by the results

from the microenvironmental pH of these blends (section 3.3.1.2). Upon the addition of EGCG to the gelatin slurry, the pH increased from pH 5.4 to 5.8. This increase can be attributed to the oxidative deamination of lysine residues and concomitant dehydration reaction (Digenis et al., 1994).

Polyvinylpyrrolidone (PVP) as well forms precipitates with EGCG. In 1:1 (w/w) binary mixtures this resulted in an EGCG loss of 6.7 % ± 1.2 %. According to the statistical test, the result was significantly higher than the threshold loss of 5 %. But at a lower concentration represented by 1:5 (w/w) binary mixtures, it was an acceptable combination partner for EGCG with 2.7 % ± 2.0 % EGCG loss over the test period (Fig. 36A). This was consistent with the results of the microenvironmental pH study (section 3.3.1.2), as PVP did not induce EGCG deprotonation. However, attention should be paid to the grade of PVP used. In this study a special low peroxide grade of PVP was chosen to assess EGCG instability due to PVP itself and not the peroxide byproducts. With other grades having a higher amount of peroxide impurities, most likely higher degradation could occur.

EGCG/pregelatinized starch (Lycatab® C) blends resulted in a decline of EGCG content within the first 6 weeks up to 15.6 % ± 5.7 % and then leveled off with a significant EGCG loss of more than 5 % (Fig. 36B). Blends containing sodium carboxymethyl cellulose (Na CMC) and EGCG resulted as well in a significant reduction of content over 26 weeks of 10.7 % ± 1.8 %.
Magnesium stearate compatibility with EGCG was tested at a lower ratio of 1:5 (w/w) but even at this low proportion it resulted in a significant EGCG loss over 26 weeks of 9.7 % ± 1.5 %. These excipients were considered incompatible as well and when possible should be avoided in tablet formulations.

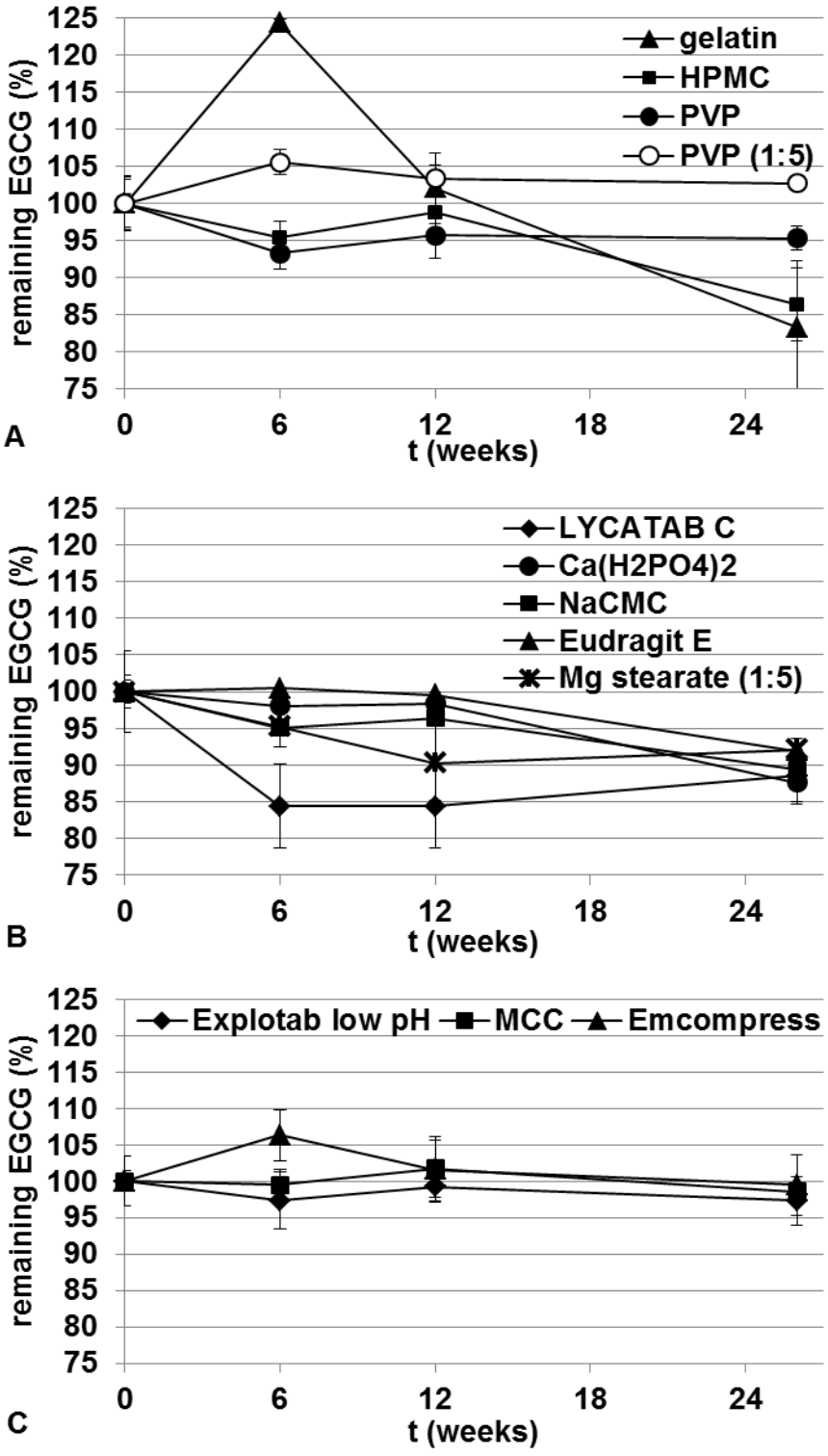


Figure 36: Storage stability of EGCG in 1:1 (w/w) binary powder blends at 40°C/75 % RH: A) coacervate/ precipitate forming excipients B) incompatible no coacervate forming excipients and C) compatible excipients

Blends of EGCG with the methacrylate copolymer Eudragit® E showed no significant difference in content over 12 weeks. But between week 12 and 26 a steep decline in the EGCG content occurred with a final loss of 8.1 % ± 2.9 % resulting in a significant change of more than 5 % ($p < 0.04$) (Fig. 36B). Likewise, EGCG blends with monobasic calcium phosphate were stable over 12 weeks but a steep decline in EGCG content could be observed between week 12 and 26 of storage with an EGCG loss of 12.4 % ± 0.6 % ($p < 0.01$). Consequently, both excipients were considered incompatible.

Dibasic calcium phosphate dihydrate (Emcompress® premium) showed a borderline value like PVP with an EGCG loss of 6.4 % ± 5.7 % over the test period (Fig. 36C). But statistically there was no significant difference in EGCG content with $p < 0.18$; therefore dibasic calcium phosphate was considered compatible with EGCG. The evaluation of the EGCG loss was assessed from week 6 up to week 26 as the dibasic calcium phosphate was present as dihydrate and it is known to lose its hydrate water upon storage at 40°C/75 % RH (section 3.3.1.1). Hence after 6 weeks of storage, the concentration of EGCG within the blend was elevated up to 106.3 % ± 3 %.
It is noteworthy, that although statistically Emcompress® premium can be considered compatible with EGCG, the results indicate that at higher ratios than 1:1 (w/w) problems with instability of EGCG might occur. Therefore dibasic calcium phosphate like PVP should be used at a lower ratio within dosage forms.

Of all tested excipients, only blends with microcrystalline cellulose (MCC) and sodium starch glycolate (Explotab® low pH) were clearly stable over the whole test period with no significant change in EGCG content and EGCG losses within the samples of 1.4 % ± 1.0 % and 2.7 % ± 3.3 %, respectively (Fig. 36C).

The results in the following two sections will show that it is a combined effect of moisture uptake, microenvironmental pH and EGCG dissociation reinforced by drug-excipient interactions that determines the stability of EGCG in blends with excipients.

Moisture uptake studies were conducted and the microenvironmental pH with and without EGCG was measured. These simple tests are effective methods to exclude excipients in the early stage of preformulation studies without the necessity of long-term stability studies.

3.3.1.1. Moisture uptake studies of binary blends and single compounds

Excipients that take up water have the risk of inducing EGCG degradation as in the presence of water, EGCG can dissolve and undergo chemical degradation.
Therefore, moisture uptake upon storage at 40°C/75 % RH was investigated and calculated as percent moisture content upon dry weight of powders at SATP conditions (standard ambient temperature and pressure). Due to the susceptibility of EGCG to degrade upon heating, “loss on drying” as regulated by the compendia was not applied.
Teavigo® powder did not take up moisture. In blends with EGCG only few excipients caused a measurable moisture uptake (Table 13). The highest moisture uptake in binary blends could be observed for the incompatible excipients sodium carboxymethyl cellulose and PVP with 6.3 % and 5.7 %, respectively. Gelatin and pregelatinized starch, which are as well incompatible with EGCG, showed the lowest moisture uptake with 2.1 % and 1.6 %, respectively. Of the compatible excipients, only the superdisintegrant sodium starch glycolate exhibited a moisture uptake in blends with EGCG of 3.0 %.
In the case of all the other compatible excipients, no moisture uptake could be detected for the EGCG blends.
Emcompress® premium (dibasic calcium phosphate dihydrate) lost its hydration water upon storage at 40 °C/75 % RH as reported in the literature (de Haan et al., 1990). In combination with EGCG, the mass loss after 6 weeks was 3.3 %. As single compound Emcompress® premium showed a mass loss of 1.2 % after 6 weeks of storage with no further change. Thus the expulsion of hydrate water is finished after 6 weeks and the EGCG loss in blends observed after 6 weeks can be attributed to pure EGCG degradation without concentration changes due to the loss of hydrate water.

Table 13: Moisture uptake of single excipients and 1:1 (w/w) binary powder blends with EGCG upon storage at 40 °C/75% RH (values presented as average of n=9 (SD) and *n=6 (SD))

Incompatible excipient	Moisture uptake (%)	
	Single compound	Blend with EGCG
Na carboxymethyl cellulose	18.4 (0.3)	6.3 (1.5)
PVP	31.3 (0.5)	5.7 (1.5)
Gelatin	7.3 (0.2)	2.1 (1.1)
Pregelatinized starch	5.8 (0.2)	1.6*(0.9)
HPMC	7.0 (0.4)	-
$Ca(H_2PO_4)_2$	4.0 (0.1)	-
Compatible excipient	**Single compound**	**Blend with EGCG**
Na starch glycolate	17.9 (0.3)	3.0 (1.7)
MCC	3.5 (0.2)	-
$Ca(HPO_4) \bullet 2\ H_2O$	-1.2 (0.2)	-3.3 (1.2)

Overall, the combination with EGCG resulted in a reduced moisture uptake of the excipients. This effect was more pronounced for some excipients such as PVP, HPMC and sodium starch glycolate and less pronounced for others such as sodium carboxymethyl cellulose and pregelatinized starch.

Some excipients such as HPMC, microcrystalline cellulose (MCC) and monobasic calcium phosphate showed no moisture uptake in EGCG blends, although as single compounds moisture uptake was observed (Table 13). Teavigo® powder altered the moisture uptake by the interaction of EGCG with macromolecules such as HPMC and PVP resulting in complex formation and amorphous precipitates with altered chemical and physical properties. Complexation goes along with hydrophilic interactions of EGCG with macromolecules (section 1.7.) and concomitant increase of hydrophobicity. This will consequently reduce the moisture uptake.

Another reason accounting for substances which do not interact with EGCG by any complex formation such as microcrystalline cellulose and monobasic calcium phosphate is the fact that Teavigo® powder as single compound does not attract

water. Its particle size is around 3 µm and the particles readily form agglomerates due to electrostatic forces. Due to these attributes, the Teavigo® powder particles can adhere to the bigger sized excipient particles of around 100 µm, and this way impede the moisture to come in close contact with the excipient particles comparable to the effect of magnesium stearate. Consequently, a lower moisture uptake of the binary blends can be observed.

3.3.1.2. Microenvironmental pH of binary blends and single compounds

As explained in the introduction, the autoxidative degradation as well as the epimerization of EGCG is pH-dependent. Thus microenvironmental pH is an important factor governing EGCG stability. With increasing pH the degradation rate increases. Furthermore, there is a pH-optimum at pH 3.9 (measured at 40 °C) where the degradation rate of EGCG is lowest. This correlates with the pKa of EGCG which lies at 7.99. For this reason, the microenvironmental pH generated by excipients and of binary blends with EGCG was investigated (Fig. 37).

The extent to which EGCG degrades is a combination of moisture uptake, as it results in the dissolution of EGCG and allows acid-base reactions between the excipients and EGCG; the microenvironmental pH of the excipient and its change upon EGCG addition, as it decides about the amount of EGCG being deprotonated and thus in its activated form; and the final microenvironmental pH with EGCG as it influences the degradation rate of EGCG. The consideration of just the microenvironmental pH of the blends is not sufficient in the investigation of potential incompatibilities as will be explained below. Only few excipients exhibited a microenvironmental pH close to the pH-optimum of EGCG. The compatible excipient sodium starch glycolate (Explotab® low pH) is one of them, inducing a microenvironmental pH of 4.0. As a consequence, the addition of EGCG to the sodium starch glycolate slurry did not result in a pH-alteration and Explotab® low pH consequently did not induce any significant EGCG loss in the storage stability study despite the moisture uptake of these blends.

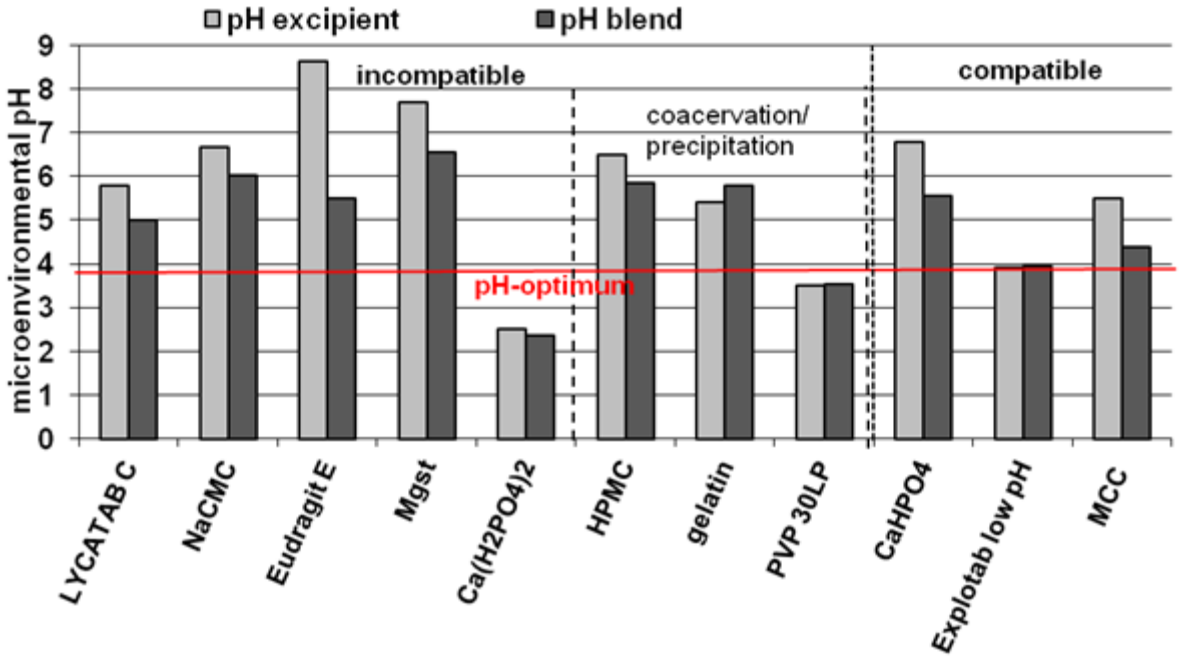


Figure 37: Microenvironmental pH measured by the pH-slurry method of single excipients and binary blends with EGCG, red line represents the pH-optimum of EGCG

The binary blends of Kollidon® 30LP with EGCG exhibited with pH 3.5 as well an microenvironmental pH close to the pH-optimum. Nevertheless, Kollidon® 30LP induced more than 5 % EGCG degradation upon storage. The reason can be found in their physical incompatibility: gelatin, HPMC and Kollidon® 30LP belong to the group of coacervate/precipitate forming excipients. In blends with coacervate/ precipitate forming excipients, EGCG is partially present in an amorphous form. It is known that especially in the amorphous state EGCG, like other substances, is more prone to chemical instability due to the molecular mobility within this state (Li et al., 2013). Therefore, these excipients induced stronger degradation than the non-coacervate/precipitate forming ones at the same microenvironmental conditions.

MCC and dibasic calcium phosphate, on the other hand, belong to the compatible excipients. EGCG addition to the MCC slurry lead to a pH-reduction of 1.1 caused by EGCG deprotonation (Table 14) and the final microenvironmental pH results in a low EGCG degradation rate of $1.39 \cdot 10^{-6}$ min^{-1} (Table 15). But the blends do not take up moisture, hence the pH-alteration does not play an important role and accordingly, the degradation of EGCG is within an acceptable range. Dibasic calcium phosphate does neither take up moisture, and in combination with the low EGCG deprotonation

(Table 14) and the induced moderate degradation rate of 3.12 • 10^{-6} min^{-1} (Table 15) it results as well in an acceptable EGCG degradation although already at the borderline (section 3.3.1).

Table 14: Microenvironmental pH of excipients, pH-change resulting from the addition of EGCG to the excipient slurries and thereof calculated concentration of EGCG necessary to deprotonate

Incompatible excipients	$pH_{excipient}$	*pH-change upon EGCG addition*	$Concentration_{EGCG}$ ($^{mg}/_{l}$) *deprotonated to alter pH*
Lycatab® C	5.8	-0.8	3.95
Eudragit® E	8.6	-3.1	1.48
Na carboxymethyl cellulose	6.7	-0.6	0.34
Magnesium stearate	7.7	-1.1	0.12
Monobasic calcium phosphate	2.5	-0.2	-
HPMC	6.5	-0.7	0.53
Gelatin	5.4	0.4	-
Kollidon® 30LP	3.5	-	-
Compatible excipients	$pH_{excipient}$	*pH-change upon EGCG addition*	$Concentration_{EGCG}$ ($^{mg}/_{l}$) *deprotonated to alter pH*
MCC	5.5	-1.1	17.69
Dibasic calcium phosphate	6.8	-1.2	1.19
Explotab® low pH	4.0	-	-

The incompatible excipients exhibited a microenvironmental pH far from the optimum. The highest value could be observed for Eudragit® E slurries with pH 8.6, followed by magnesium stearate slurries with pH 7.7 and sodium carboxymethyl cellulose slurries with pH 6.7 (Fig. 37). The consequence is that upon the addition of EGCG the polyphenol deprotonates (Table 14) and thus becomes activated for autoxidative degradation. In combination with the high microenvironmental pH of the blends with EGCG ranging from pH 5.5 up to 6.6, this leads to an enhanced EGCG degradation rate of 2.90 • 10^{-6} min^{-1} up to 5.39 • 10^{-6} min^{-1}(Table 15).

Table 15: Resulting degradation rate (k) of EGCG due to microenvironmental pH of binary blends based on the published results of Li et al. 2012

Incompatible excipients	***Microenvironmental pH of blend***	***Degradation rate k • 10 $^{-6}$ (min^{-1})***
Magnesium stearate	6.6	5.39
Na carboxymethyl cellulose	6.0	4.03
HPMC	5.8	3.58
Gelatin	5.8	3.58
Eudragit® E	5.5	2.90
Lycatab® C	5.0	2.01
Monobasic calcium phosphate	2.4	1.97
Kollidon® 30LP	3.5	1.15
Compatible excipients	***Microenvironmental pH of blend***	***Degradation rate k • 10 $^{-6}$ (min^{-1})***
Dibasic calcium phosphate	5.6	3.12
MCC	4.4	1.39
Explotab® low pH	4.0	0.98

It is noteworthy, that Eudragit® E and magnesium stearate belong to the excipients, which do not take up moisture in blends with EGCG. Nevertheless, they induced an EGCG degradation of more than 5 % upon storage. The assumption that excipients which do not take up moisture result in an acceptable EGCG stability does not hold true in all cases. When the excipients exhibit as single compounds a microenvironmental pH above 7, the presence of low proportions of surface water is already sufficient to induce significant degradation of EGCG. A combination of Eudragit® E and EGCG with an excipient that takes up moisture would induce a far higher degradation. But according to the results from section 3.3.1., Eudragit® E at a lower weight ratio could be used in formulations as it does not take up moisture.

Furthermore, the strongest pH-alteration by EGCG was not necessarily coupled to the highest microenvironmental pH induced by the excipient (Fig. 37). Examples are Lycatab® C and sodium carboxymethyl cellulose: although sodium carboxymethyl

cellulose induced a higher microenvironmental pH than Lycatab® C (Fig. 37), Lycatab® C induced a stronger pH-alteration upon EGCG addition and as consequence a stronger deprotonation of EGCG (Table 14). Hence, although sodium carboxymethyl cellulose induced a 2-fold higher degradation rate of EGCG than Lycatac® C (Table 15), Lycatab® C with the highest EGCG deprotonation among the tested excipients (Table 14) activated more EGCG for autoxidation and results in combination with its 4-fold lower moisture uptake in a slightly higher EGCG loss of 15.6 % ± 5.7 % than sodium carboxymethyl cellulose with 10.7 % ± 1.8 %. This points out the necessity of investigating not just the final microenvironmental pH, but as well considering the pH-change upon the addition of EGCG to the slurries.

The incompatible excipient monobasic calcium phosphate exhibited an acidic pH of 2.5. In combination with the resulting low EGCG degradation rate of $1.97 \bullet 10^{-6}$ min^{-1} and as these blends do not take up moisture, acceptable EGCG stability would be expected. But the addition of EGCG to the slurry resulted in a further reduction in pH that cannot arise from a deprotonation of EGCG.

More probably the reduction is induced by the decomposition of monobasic calcium phosphate in the presence of water into phosphoric acid and dibasic calcium phosphate (Hinman et al., 1962) with hydroxylapatite as final product (Schmidt and Herzog, 1993) (Chen et al., 2013). The phosphoric acid lowers the pH and can be reduced into phosphorous acid by oxidation of EGCG and EGCG degrades despite the acidic microenvironmental pH and the corresponding low degradation rate.

In general, all excipients which take up moisture, do not generate a microenvironmental pH around 3.9 and undergo alteration of microenvironmental pH upon addition of EGCG induce EGCG degradation (pregelatinized starch, sodium carboxymethyl cellulose, gelatin).

Excipients which do not take up moisture but do have a microenvironmental pH above 7 are incompatible as well, since the low amount of surface water is already sufficient to induce enough EGCG deprotonation to result in a significant degradation (magnesium stearate, Eudragit® E).

All excipients inducing a microenvironmental pH close to pH 3.9 are compatible no matter if they take up moisture or not (Explotab® low pH) as long as they do not show coacervate/precipitate formation with EGCG.
Furthermore, excipients which do not take up moisture in combination with EGCG and have a microenvironmental pH below 7 are compatible (microcrystalline cellulose, dibasic calcium phosphate).

In summary, investigating the moisture uptake of EGCG blends with excipients as well as the microenvironmental pH with and without EGCG and the pH-change due to EGCG addition allows a prediction of the potential of excipients to induce EGCG degradation within a dosage form.

3.3.2. Storage stability of EGCG in tablets containing compatible and incompatible excipients

EGCG showed stability problems in binary 1:1 (w/w) mixtures under accelerated stress conditions with several common tableting excipients. In compacts, the contact of excipient and API is more intimate than in powder blends and the contact area with moisture/ sorbed water is smaller. For this reason, EGCG tablets containing compatible and incompatible tableting excipients were formulated and were exposed again to accelerated stress conditions (40 °C/75 % RH) for 6 months to assess EGCG stability within tablets with these excipients.
Only the tablet formulation containing the compatible excipients Emcompress® premium as filler and Explotab® low pH as superdisintegrant and the tablet formulation containing the compatible binder/filler Avicel® PH102 and incompatible superdisintegrant Ac-Di-Sol® showed sufficient stability with 99.6 % ± 3.8 % and 99.1 % ± 0.5 % EGCG recovery after 26 weeks respectively (Table 16) with no significant difference of 5 % or more with $p < 0.12$ and $p < 0.10$, respectively.
All other incompatible formulations exhibited significant differences from their initial EGCG contents and the differences were 5 % or more with $p < 0.05$.
As the high dose EGCG tablets just consisting of API and Ac-Di-Sol® at 25 % (w/w) loading result in 9.5 % ± 2.8 % loss, i.e. exhibited insufficient stability, but the

formulation containing 10 % (w/w) Ac-Di-Sol® in combination with Avicel® PH102 was stable, it can be assumed that tablets with acceptable stability could be formulated by adjusting the ratio of EGCG to Ac-Di-Sol®.

Table 16: Recovery of EGCG after storage of 100 mg tablets at 40°C/75% RH over 6 months (values presented as average of n=3 (SD));*significant difference with p < 0.05; **no significant difference with p < 0.10; ***no significant difference with p > 0.25 but difference below 5% with p < 0.12

	Unstable formulations				***Stable formulations***	
Filler/ Binder	**Avicel**	**Emcompress**	**-**	**Lycatab**	**Avicel**	**Emcompress**
Disint.	**Explotab**	**Kollidon CL**	**Ac-Di-Sol**	**Explotab**	**Ac-Di-Sol**	**Explotab**
t (w)						
0	100.0 (1.5)	100.0 (1.8)	100.0 (1.0)	100.0 (0.5)	100.0 (2.6)	100.0 (2.4)
6	93.5 (2.7)	98.5 (1.6)	93.0 (1.2)	95.2 (2.9)	104.5 (2.3)	101.2 (3.7)
12	94.4 (2.1)	91.7 (1.8)*	90.5 (2.8)	90.6 (1.5)	91.6 (9.3)	101.1 (5.0)
26	92.1 (0.6)*	92.1 (3.2)	94.3 (2.4)*	89.6 (1.1)*	99.1 (0.5)**	99.6 (3.8)***

Using the incompatible binder Lycatab® C instead of the compatible Emcompress® premium at a loading of 20 % (w/w) within the tablets resulted in a significant EGCG loss of 10.4 % ± 1.1 %. Incorporation of Kollidon® CL which is no special low peroxide grade at a loading of 10 % (w/w) resulted in 8.3 % ± 1.8 % EGCG loss.

3.3.2.1. Moisture uptake study of immediate release tablets

Moisture uptake of immediate release tablets was assessed after 6, 12 and 26 weeks of storage. The weight gain was constant over the test period of 26 weeks and the maximum was already reached after 6 weeks. The highest weight gain upon storage was observed for formulations containing only EGCG and Ac-Di-Sol® with 2.0 %, followed by the combination of EGCG, Avicel® PH102 and Explotab® low pH with 1.1 % and EGCG, Lycatab® C and Explotab® low pH with 0.9 % (Table 17). This corresponds to the observed EGCG degradation within these tablets. The other formulations exhibit a moisture uptake of less than 1.0 % and result in acceptable EGCG stability.

Table 17: Moisture uptake of EGCG immediate release tablets upon storage at 40 °C/75% RH over 6 months (values presented as average of n=9 (SD))

Tablet formulation	Moisture uptake (%)
Ac-Di-Sol®	2.0 (0.2)
Avicel® PH102 + Explotab® low pH	1.1 (0.2)
Lycatab® C + Explotab® low pH	0.9 (0.2)
Emcompress® premium + Explotab® low pH	0.6 (0.1)
Avicel® PH102 + Ac-Di-Sol®	0.4 (0.1)

Only tablets containing EGCG, Emcompress® premium and Kollidon® CL showed a constant decrease of mass up to week 12 and then remained constant in their weight (Fig. 38A). This loss is not attributed to the hydrate water loss of dibasic calcium phosphate as the hydrate water expelled by Emcompress® premium should be directly absorbed by Kollidon® CL. Furthermore, the expulsion of hydrate water is finished after 6 weeks (section 3.3.1.1).

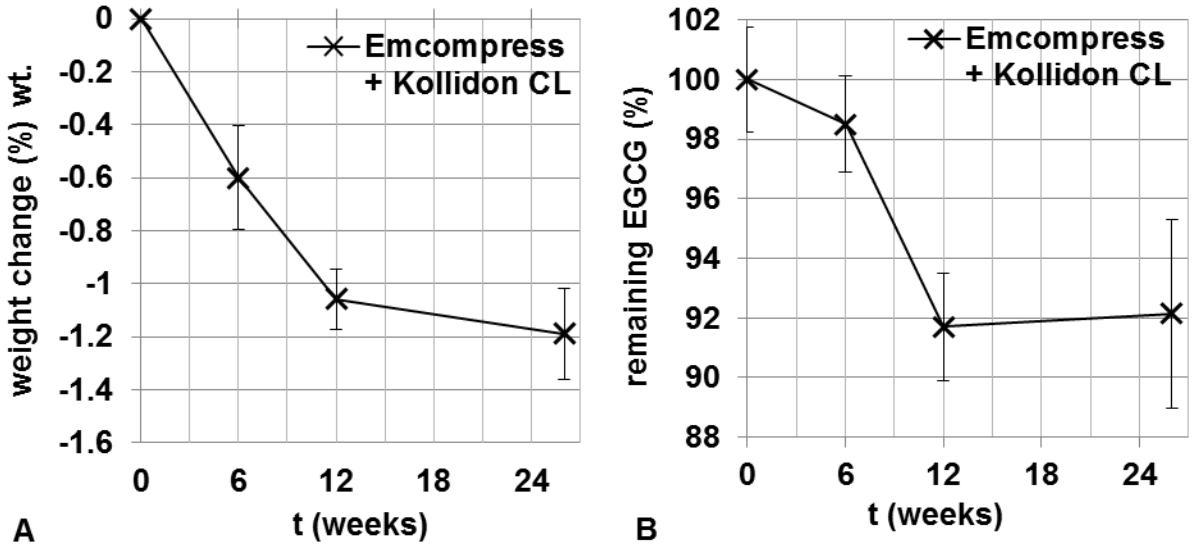


Figure 38: Weight loss of EGCG immediate release tablets containing Emcompress® premium and Kollidon® CL upon storage at 40 °C/ 75% RH and concomitant EGCG loss (values presented as average of n=3 ± SD)

A more reasonable explanation for this weight loss upon storage is the degradation of peroxide impurities present within Kollidon® CL. These impurities can interact with

EGCG under the formation of volatile products such as hydrogen peroxide. This explanation is also consistent with the degradation pattern of these tablets over 26 weeks (Fig. 38B). The EGCG content within the tablets is constantly decreased up to week 12 with an EGCG recovery of 91.7 % ± 1.8 % and levels off with 92.1 % ± 3.2 % recovered at 26 weeks.

3.3.2.2. Microenvironmental pH of immediate release tablets

Three factors induce EGCG instability in formulations: the moisture uptake, the microenvironmental pH of the tablet formulation and the pH alteration due to the addition of EGCG (section 3.3.1.2). Investigating these three factors predicts the stability of EGCG in formulations. This can be applied as well to the immediate release tablet formulations investigated here.

Just two formulations have a low moisture uptake of around 0.5 % (w/w). They consist of Teavigo®, Emcompress® premium with Explotab® low pH and Teavigo®, Avicel® PH102 with Ac-Di-Sol®. They both exhibited acceptable stability. The tablet formulation containing Emcompress® premium as filler and Explotab® low pH as superdisintegrant showed additionally the lowest and thus best microenvironmental pH with 5.0 (Fig. 39) and the lowest degradation rate of $2.12 \cdot 10^{-6}$ min^{-1} (Table 18). Furthermore, no marked pH-alteration occurred by the addition of EGCG, and thus less EGCG is activated for autoxidation and the tablets exhibit acceptable stability in the storage stability study.

The formulation containing Avicel® PH102 in combination with Ac-Di-Sol® exhibits a microenvironmental pH of 6.7 (Fig. 39) and results in a pH-change of 0.4 (Table 19) by the addition of EGCG. The induced degradation rate of EGCG is as well high with $4.71 \cdot 10^{-6}$ min^{-1}, but due to the low moisture uptake and as the microenvironmental pH is still below pH 7.0, this formulation showed as well acceptable EGCG stability.

The formulation containing Teavigo® and Ac-Di-Sol® takes up the most moisture of all tablet formulations (2.0 % (w/w)). The microenvironmental pH is far above the pH-optimum of EGCG resulting in a high degradation rate of $5.61 \cdot 10^{-6}$ min^{-1}. Together

with the deprotonation of EGCG to change the pH, the EGCG content upon storage is significantly reduced to 90.5 % ± 2.8 %.

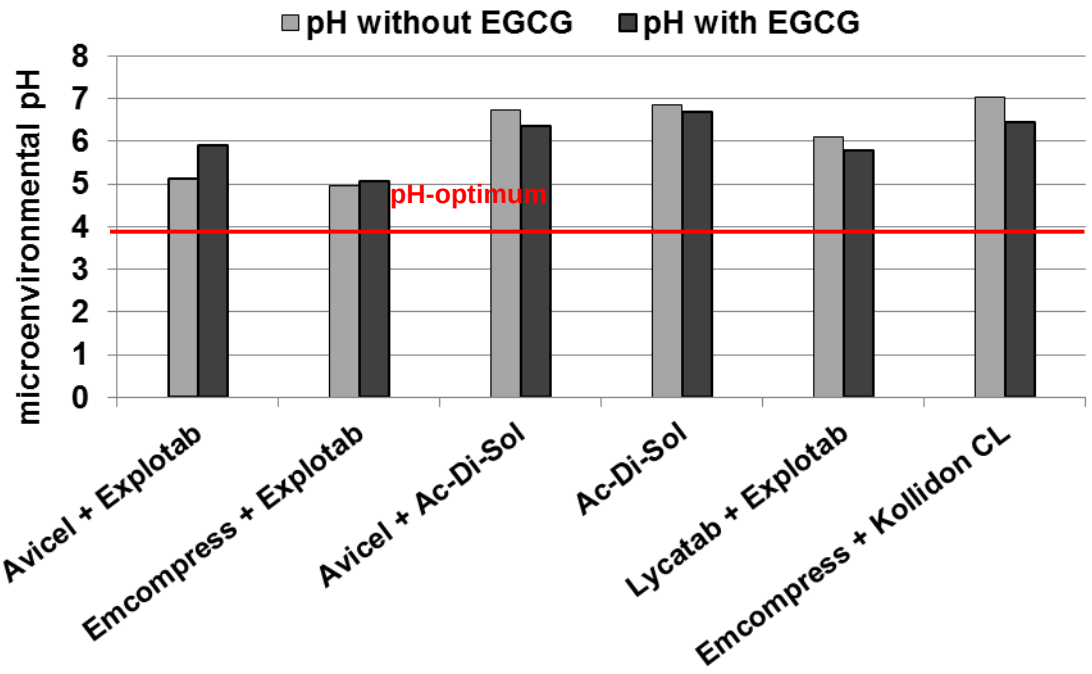


Figure 39: Microenvironmental pH measured by the pH-slurry method of the tablet formulations with and without EGCG; red line represents pH-optimum of EGCG

Formulations containing Teavigo®, Explotab® low pH as superdisintegrant and either Lycatab® C or Avicel® PH102 as binder also take up moisture (around 1.0 % (w/w)) and have a microenvironmental pH above the pH-optimum of EGCG leading to a degradation rate of EGCG of $3.58 \cdot 10^{-6}$ min^{-1} and $3.80 \cdot 10^{-6}$ min^{-1}, respectively. They result as well in EGCG degradation of 7.9 % ± 0.6 % and 10.4 % ± 1.1 %, respectively. Furthermore, the addition of Teavigo® to the formulation containing Lycatab® C resulted in a pH-reduction, i.e. deprotonation of EGCG.

Table 18: Resulting degradation rate (k) of EGCG due to the microenvironmental pH of the tablet formulation containing EGCG based on the published results of Li et al. 2012

Tablet formulation	*Microenvironmental pH of tablets*	*Degradation rate $k \cdot 10^{-6}$ (min^{-1})*
Emcompress® premium + Explotab® low pH	5.1	2.12
Lycatab® C + Explotab® low pH	5.8	3.58
Avicel® PH102 + Explotab® low pH	5.9	3.80
Avicel® PH102 + Ac-Di-Sol®	6.3	4.71
Emcompress® premium + Kollidon® CL	6.4	4.93
Ac-Di-Sol®	6.7	5.61

Table 19: Microenvironmental pH of tablet excipients of tablet formulations without EGCG, pH-change resulting from the addition of EGCG to the slurries of tablet excipients and thereof calculated concentration of EGCG necessary to deprotonate

Tablet formulation	$pH_{tablet\ excipients}$	*pH-change upon EGCG addition*	*$Concentration_{EGCG}$ ($^{mg}/_{l}$) deprotonated to alter pH*
Avicel® PH102 + Explotab® low pH	5.1	0.80	-
Emcompress® premium + Explotab® low pH	5.0	0.10	-
Ac-Di-Sol®	6.8	-0.17	0.03
Lycatab® C + Explotab® low pH	6.1	-0.34	0.42
Avicel® PH102 + Ac-Di-Sol®	6.7	-0.39	0.12
Emcompress® premium + Kollidon® CL	7.0	-0.60	0.12

The question arises why this incompatibility and concomitant EGCG loss could be observed for the formulation containing Avicel® PH102 and Explotab® low pH but not in the case of the formulation where Avicel® PH102 is combined with Ac-Di-Sol®. Avicel® PH102 did not induce EGCG degradation in binary blends (section 3.3.1), because the blends did not take up moisture. But the tablet formulations contain superdisintegrants that take up moisture. And the moisture uptake is around 2-fold higher when Avicel® PH102 is combined with Explotab® low pH in contrast to Ac-Di-Sol®. Furthermore, the formulation of Teavigo® with Avicel® PH102 and Explotab® low pH, both as single compounds compatible with EGCG, resulted as only formulation in a positive pH-alteration which corresponds to proton acceptance (Fig. 39). But a proton acceptance by EGCG is unlikely. More reasonable is that some other reactions are occurring when these two excipients are combined with Teavigo® which results in the degradation of EGCG.

The formulation containing Teavigo®, Emcompress® premium with Kollidon® CL showed no measurable moisture uptake. Instead, the tablets constantly lose weight. Furthermore, the microenvironmental pH of the excipient slurry is above 7 and a pH-change of 0.6 could be observed by the addition of EGCG (Table 19). Consequently, the low amount of surface water is already sufficient to induce enough EGCG deprotonation to result in a significant degradation EGCG. Together with the final pH that results in a degradation rate of $4.93 \cdot 10^{-6}$ min^{-1} (Table 18) a reduction of 8.3 % ± 1.8 % in the EGCG content upon storage could be observed over 26 weeks at 40°C/75 % RH.

These results show that even for blends containing more than two ingredients the investigation of the microenvironmental pH, the pH-alteration due to EGCG addition and the moisture uptake are sufficient to predict EGCG instability within formulations and reduces the effort in preformulation studies with EGCG.

3.3.3. Stabilization of EGCG in aqueous solution by the addition of sodium ascorbate

Short term stabilization against autoxidative degradation of EGCG by sodium ascorbate over 3 hours at 37 °C was investigated in pH 6.5, 6.8 and 7.4 phosphate buffer solutions representing the passage of EGCG through the intestine (Fig. 40). For this purpose, an equimolar concentration of 1 $^{g}/_{l}$ EGCG to 0.44 $^{g}/_{l}$ sodium ascorbate was chosen. Up to 15 % more of EGCG remained in solution after 3 hours, showing its efficiency in stabilizing EGCG during the intestinal passage. Thus co-administration of EGCG with ascorbic acid or its salt could increase the dose of EGCG present for absorption within the intestine and thus increase the concentration gradient across the intestinal epithelium and consequently its uptake.

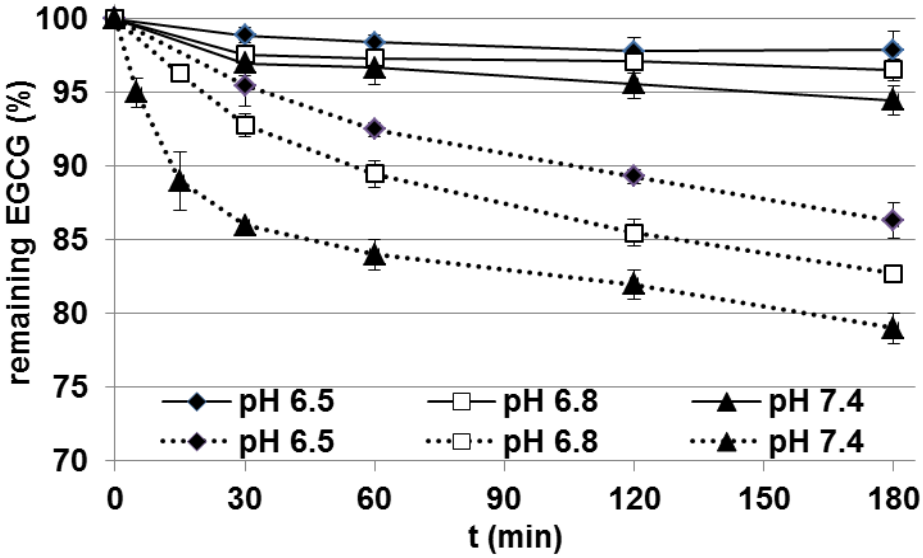


Figure 40: pH-dependent degradation of EGCG in phosphate buffer solutions at 37°C without addition of sodium ascorbate (dashed lines) and with equimolar addition of sodium ascorbate (continuous line) (values presented as average of n=3 ± SD)

Additionally, stability studies over 6 days in pH 6.8 at 37°C were performed to assess its potential to stabilize EGCG within dosage forms. For this purpose, molar ratios from 2:1 up to 50:1 (ascorbate : EGCG) were chosen, as a molar ratio of 35:1 would represent the ratio present at saturated state of both substances and thus the microenvironmental concentrations within tablets (Fig. 41).

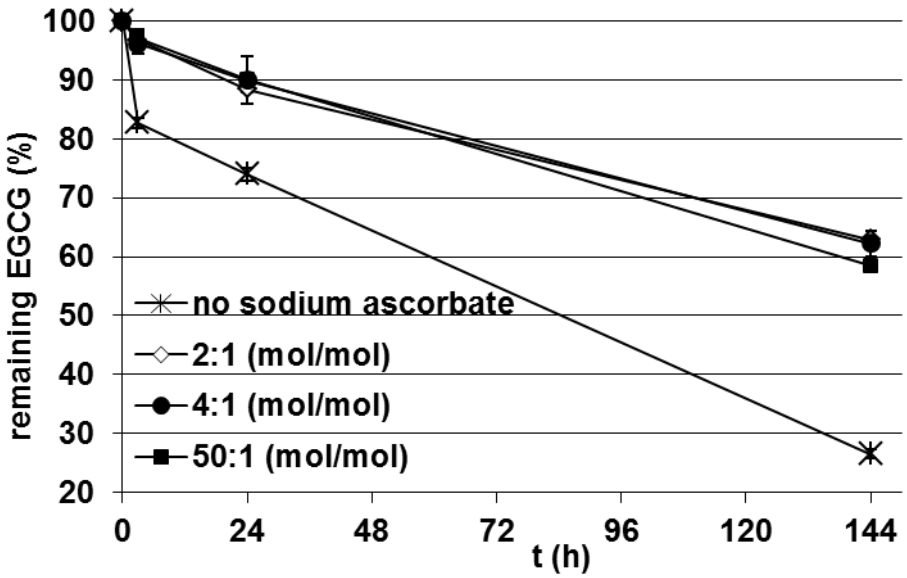


Figure 41: Degradation of EGCG in phosphate buffer pH 6.8 at 37°C without sodium ascorbate and at different molar ratios of ascorbate to EGCG (values presented as average of n=3 ± SD)

Contrary to the report of Chen et al. 1998 that ascorbic acid can stabilize EGCG in solution for only 12 hours and results then in a sudden concentration drop, addition of sodium ascorbate in this study resulted in improved stability of EGCG over 6 days. Without sodium ascorbate 26.5 % ± 0.7% EGCG could be recovered, whereas the addition of sodium ascorbate at molar ratios of 2:1, 4:1 and 50:1 resulted in 62.8 % ± 2.1 %, 62.2 % ± 1.3 % and 58.4 % ± 0.4 % EGCG recovery, respectively. Hence, 32 to 36 % more EGCG remained stable over the test period of 144 hours.
According to these results, ascorbic acid or its salts could not only stabilize EGCG during the gastrointestinal passage but also stabilize EGCG within tablet formulations.

3.3.4. Stabilization of EGCG within tablets by incorporation of different ascorbic acid forms

As shown by the stabilization of EGCG solutions with sodium ascorbate (section 3.3.3.), the addition of ascorbate as antioxidant might as well enhance the stability of

EGCG within tablet formulations containing incompatible binders, fillers or superdisintegrants.

Therefore, ascorbic acid, sodium ascorbate and a mixture of both were investigated as to their stabilization efficacy, since they all result in different microenvironmental pH. Furthermore, the dose-dependent effect of antioxidants was analyzed by incorporation of 10 and 20 % (w/w) of single antioxidants within the tablets.

The results showed, quite contrary to the expectations generated from the stabilization of EGCG in solution, that the addition of ascorbic acid alone, in mixture with its sodium salt or as sodium salt induced EGCG degradation in all tablets besides the tablets containing Teavigo®, Lycatab® C and Explotab® low pH in combination with 10 % (w/w) sodium ascorbate. The stability of EGCG was improved from 89.6 % ± 1.1 % to 93.6 % ± 3.3 %. The EGCG content after storage does not show a significant difference from its initial content with $p < 0.45$ (Table 20).

Table 20: Recovery of EGCG after storage of 100 mg tablets consisting of incompatible Lycatab® C, compatible Explotab® low pH with different ascorbic acid forms as stabilizer and different stabilizer amount at 40°C/75 % RH over 6 months (values presented as average of n=3 (SD)) ; *significant difference with p < 0.03; **no significant difference with p > 0.25

Stabilizer	Na ascorbate		Ascorbic acid		Ascorbic acid + Na ascorbate
Loading (%)	10	20	10	20	20 + 10
t (weeks)					
0	100.0 (6.0)	100.0 (2.7)	100.0 (1.3)	100.0 (1.4)	100.0 (1.5)
6	97.4 (5.9)	66.6 (4.5)	92.1 (6.1)	93.3 (3.0)	60.7 (1.9)
12	98.2 (1.9)	55.5 (6.4)	77.2 (6.2)	75.5 (11.3)	51.5 (2.0)
26	93.6 (3.3)**	48.0 (4.7)*	84.4 (5.1)*	77.6 (3.7)*	15.6 (1.8)*

Although the probability of error is above 0.25 and thus the rejection criterion is fulfilled, taking a look at the whole degradation curve points out that the degradation rate of EGCG was reduced (Fig. 42).

In the other tablet formulations, ascorbic acid, sodium ascorbate and a mixture of both exhibited a destabilizing effect on EGCG with a decrease in EGCG content varying from 15.6 % ± 5.1 % up to 84.4 % ± 1.8 % (Table 20).

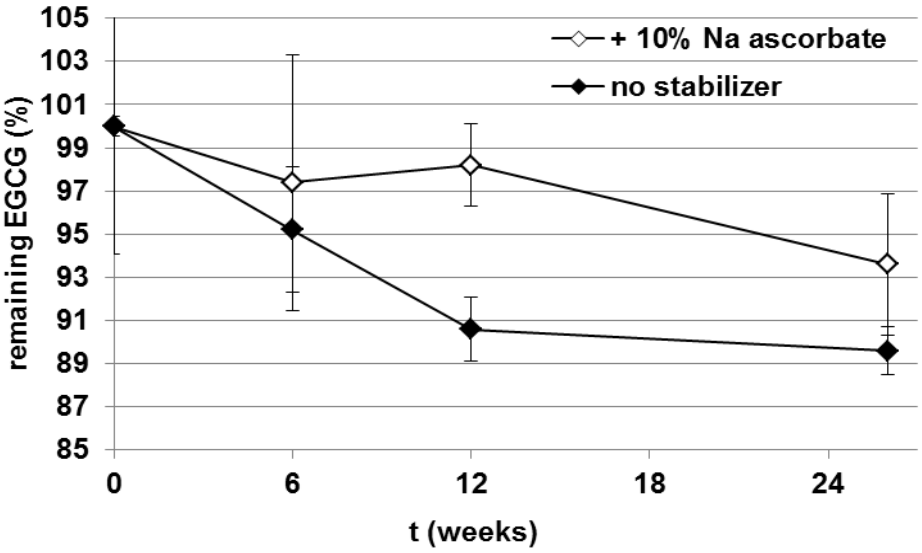


Figure 42: Degradation pattern of EGCG in tablets containing Lycatab® C and Explotab® low pH with 10 % sodium ascorbate and without it upon storage at 40°C/ 75 % RH over 6 months (values presented as average of n=3 ± SD)

The immediate release tablets consisting of just Teavigo® powder and the incompatible superdisintegrant Ac-Di-Sol® could not be improved at all with regard to EGCG stability. They showed an EGCG loss of 17.1 % ± 2.7 % in the case of incorporation of 10 % (w/w) ascorbic acid up to 72.3 % ± 5.3 % EGCG loss when ascorbic acid and sodium ascorbate as a mixture were incorporated into the tablets (Table 21).

Overall, the highest degradation of EGCG with 84.4 % ± 1.8 % and 72.3 % ± 5.3 % loss occurred due to the incorporation of ascorbic acid and sodium ascorbate together in tablets, although they bring the microenvironmental pH of the tablet formulations close to the pH-optimum of EGCG. Furthermore, the incorporation of sodium ascorbate at a high loading of 20 % (w/w) resulted in a significant stronger destabilization of EGCG than the incorporation of the same amount ascorbic acid.

Table 21: Recovery of EGCG after storage of 100 mg tablets consisting of incompatible Ac-Di-Sol® with different ascorbic acid forms as stabilizer and different stabilizer amount at 40°C/75 % RH over 6 months (values presented as average of n=3 (SD)); all values show significant difference with p < 0.03

Stabilizer	Na ascorbate		Ascorbic acid		Ascorbic acid + Na ascorbate
Loading (%)	10	20	10	20	20 + 10
t (weeks)					
0	100.0 (2.1)	100.0 (0.3)	100.0 (2.3)	100.0 (3.5)	100.0 (1.3)
6	89.6 (4.9)	61.6 (5.8)	84.4 (1.0)	83.7 (5.8)	73.5 (2.6)
12	79.6 (11.4)	70.1 (5.3)	86.0 (3.0)	74.7 (3.9)	32.8 (5.5)
26	79.4 (4.7)	45.8 (3.1)	82.9 (2.7)	63.7 (1.2)	27.7 (5.3)

Beside the induced EGCG degradation upon antioxidant addition, browning of tablets could be observed during the storage at 40°C/75 % RH (Table 22). This browning was accompanied by a caking of the tablet matrix. The tablets did not disintegrate properly anymore and brown sponge-like eroded particles could be observed in the extraction medium. The browning is dependent on the amount of stabilizer incorporated with more brown spots occurring at a higher loading (Table 22).

The strongest browning of tablets occurred upon the addition of ascorbic acid and sodium ascorbate as a binary mixture. This is consistent with the observed degradation of EGCG that was highest in these tablets followed by the addition of sodium ascorbate as single stabilizer.

Table 22: Color change of immediate release tablets after 26 weeks of storage at 40°C/ 75% RH

Stabilizer	None	Na ascorbate		Ascorbic acid		Ascorbic acid + Na ascorbate
Loading (%) (w/w)	0	10	20	10	20	20 + 10
Lycatab® C + Explotab® low pH						
Ac-Di-Sol®						

The immediate release tablets containing the incompatible binder Lycatab® C and the compatible superdisintegrant Explotab® low pH in combination with 10 % (w/w) sodium ascorbate exhibited brown spots as well, although they showed slightly improved stability of EGCG. The browning was even more pronounced than in the case of the immediate release tablets containing Ac-Di-Sol® with 10 % (w/w) sodium ascorbate. Nevertheless, the EGCG loss in Ac-Di-Sol® immediate release tablets containing 10 % (w/w) sodium ascorbate is 20.6 % ± 4.7 %, whereas tablets containing Lycatab® C, Explotab® low pH and 10 % (w/w) sodium ascorbate showed just 6.4 % ± 3.3 % EGCG loss.

Through this study the potential of ascorbate to improve EGCG stability within tablet formulations was shown by the example of the incompatible binder Lycatab® C, but the downside is a discoloration of the product. Furthermore, an anti- as well as pro-oxidant effect of ascorbic acid forms on EGCG was observed within tablet formulations and the reasons and influencing factors were investigated in the following sections.

3.3.4.1. Weight change upon storage of EGCG immediate release tablets containing ascorbic acid and its sodium salt

The addition of ascorbic acid and its sodium salt into tablets resulted in an increased weight of the tablets after 6 weeks at 40°C / 75 % RH, which declined upon further storage (Fig. 43). Such a behavior was not observed in the case of stabilizer-free EGCG immediate release tablets.

The highest weight gain was observed for tablets containing sodium ascorbate. Incorporation of 20 % (w/w) sodium ascorbate into tablets with Lycatab® C as binder and into tablets containing just Teavigo® and Ac-Di-Sol® resulted after 6 weeks in a weight gain of tablets of 6.4 % and 5.6 %, respectively (Fig. 43A). The weight gain was proportional to the amount of sodium ascorbate within the tablets with approximately half the weight gain upon the incorporation of just 10 % (w/w) sodium ascorbate.

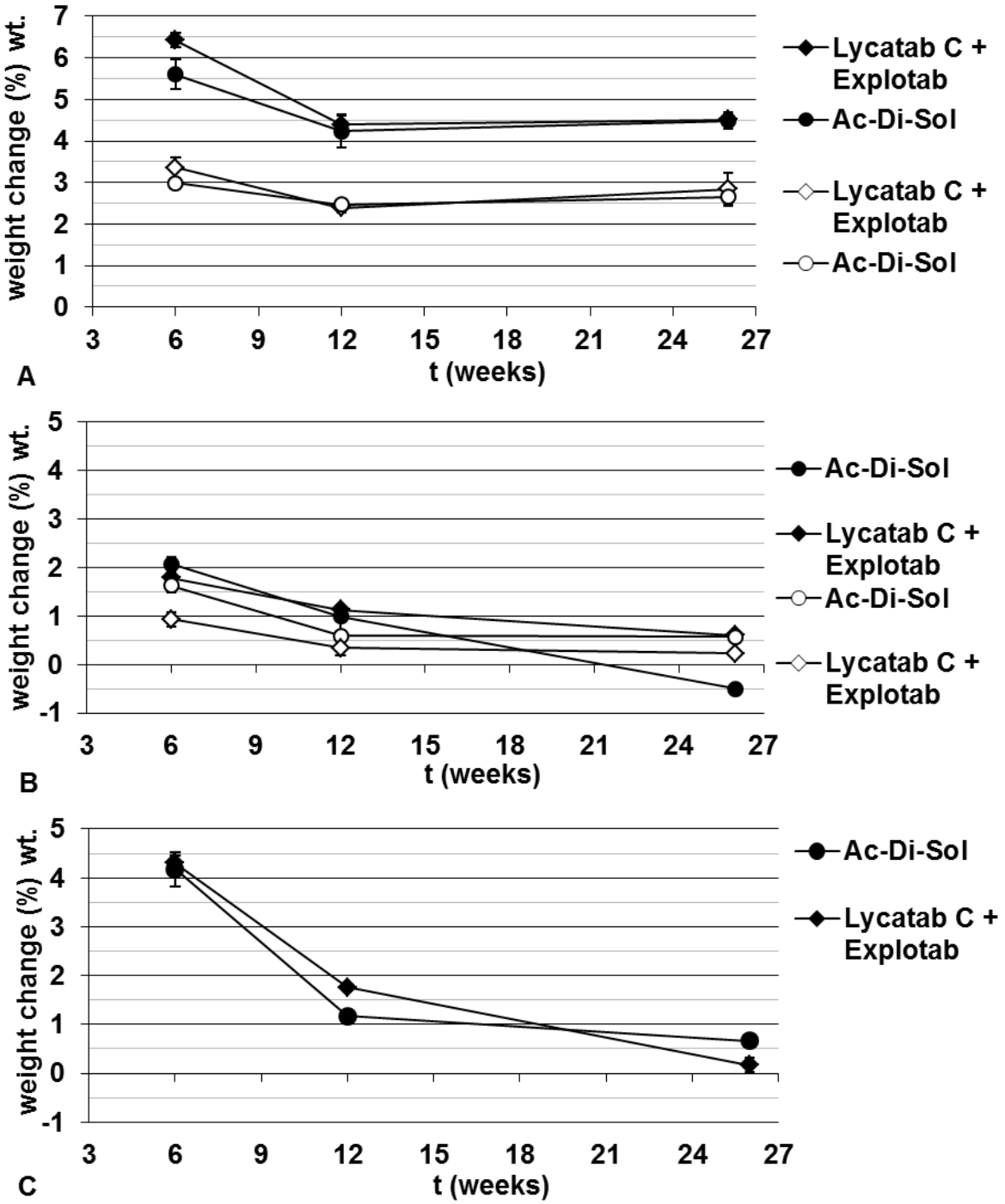


Figure 43: Weight change upon storage at 40°C/ 75% RH of immediate release tablets containing A) sodium ascorbate B) ascorbic acid + sodium ascorbate (20% + 10%) and C) ascorbic acid as stabilizer. Open symbol represent a stabilizer loading of 10 %, closed symbols represent a stabilizer loading of 20 % (all values presented as average of n=3 ± SD)

The addition of a binary mixture of ascorbic acid and sodium ascorbate resulted in a lower weight gain after 6 weeks of 4.2 % and 4.3 % than the addition of just sodium ascorbate (Fig. 43B). And the lowest weight gain was observed for tablets containing ascorbic acid with just 2.1 % and 1.8 % at a loading of 20 % (w/w) and 1.6 % and 0.9 % at a loading of 10 % (w/w) (Fig. 43C).

Another important result is the weight loss of tablets. In particular, the tablets containing ascorbic acid and sodium ascorbate in a mixture exhibited a high weight loss (Fig. 43B). The tablets reduce their mass from week 6 up to 26 about 3.5 % to 4.1 %. In the case of tablets containing either sodium ascorbate or ascorbic acid, the reduction is below 2.5 %. A reduction of weight must be associated with some reactions: either the transformation of the ingredients to substances which attract less water with the consequence that they expulse the absorbed water or the degradation to volatile products.

Ascorbic acid decomposes under the formation of 2,3-diketo-L-gulonic acid and oxalic acid. Oxalic acid is a reducing agent and upon reduction it transforms into the volatile product carbon dioxide. Furthermore, 2,3-diketo-L-gulonic acid transforms upon decarboxylation into L-threosone.

This can lead to the observed mass loss of the tablets. Consequently, the observed reduction in weight is an indicator for degradative reactions. As such a weight change was not observed for the tablets containing no stabilizer (section 3.3.2.1) it cannot be the result from EGCG degradation and must originate from ascorbic acid/ ascorbate degradation. And as ascorbic acid and ascorbate degrade under radical formation, these radicals can either induce EGCG degradation (pro-oxidant effect) or react with EGCG degradation products and reduce the autoxidative degradation (antioxidant effect).

3.3.4.2. Deliquescence study of ascorbic acid and its sodium salt

The deliquescence (lowering of the critical RH (RH_0) for dissolution) of ascorbic acid and its forms in combination with other substances is known (section 1.4.3.1.), as is the fact that this deliquescence is accompanied by a discoloration and might result in a degradation of ascorbic acid. Moisture uptake is one of the three factors enhancing

the autoxidation of EGCG as EGCG can dissolve and react (section 3.3.1.1.). Furthermore, the moisture uptake and deliquescence of ascorbic acid and ascorbate results in their degradation with concomitant radical formation and can be seen by their discoloration.

To investigate which excipients within the formulations are responsible for this deliquescence, and if Teavigo® induces it as reported in the literature, ascorbic acid, sodium ascorbate and a mixture of both were analyzed for their moisture uptake at 40°C/ 75 % RH over 2 weeks as single compounds and in combination with the individual excipients from the tablet formulations as well as with Teavigo® powder. Additionally, the discoloration was monitored and the extent of discoloration was compared to rate which excipients induce the strongest solid-liquid transformation.

As single compounds, the binary mixture of ascorbic acid and sodium ascorbate took up the most moisture with 19.2 % followed by sodium ascorbate with 8.1 % and ascorbic acid showed no measurable moisture uptake (Table 23). The discoloration after 2 weeks of storage at 40°C/ 75 % RH correlated with these results. Ascorbic acid remained a crystalline white powder (Fig. 44E), i.e. the RH_0 was above the storage conditions, whereas sodium ascorbate and the mixture of sodium ascorbate and ascorbic acid changed their color. The crystalline sodium ascorbate became a pasty, dark brown substance (Fig. 45E) and the mixture of both became a sticky orange semisolid (Fig. 46E).

Table 23: Moisture uptake of ascorbic acid, its sodium salt, the mixture of both and their binary 1:1 (w/w) blends with excipients and EGCG upon storage at 40°C/75% RH over 2 weeks (all values are understood as average of n=6 (SD) or * n=3 (SD))

	Moisture uptake (%)		
Excipient	**Ascorbic acid**	**Na ascorbate***	**Na ascorbate + Ascorbic acid***
None	-0.9 (0.8)	8.1 (1.6)	19.2 (0.6)
Teavigo®	-0.4 (0.1)	1.3 (0.1)	14.1 (0.1)
Lycatab® C	2.8 (0.1)	5.0 (0.1)	11.5 (0.2)
Ac-Di-Sol®	8.0 (0.5)	18.2 (1.8)	17.6 (1.0)
Explotab® low pH	11.7 (0.1)	24.8 (0.5)	22.4 (0.5)

The browning observed within the immediate release tablets thus does originate from degradation products of ascorbate. Consequently, the higher the amount of sodium ascorbate within the tablet, the more brown degradation products were present and could be observed as brown spots at the surface of the stored tablets.

Although EGCG is reported in the literature to induce a deliquescence lowering of ascorbic acid, this could not be observed in our study with Teavigo® powder. The combination with Teavigo® powder even reduced the moisture uptake of ascorbic acid and sodium ascorbate as single compounds (Table 23). Consequently, Teavigo® can be combined with sodium ascorbate or ascorbic acid in one dosage form. Only in combination with its binary mixture Teavigo® powder induced a deliquescence lowering and a sticky paste was formed (Fig. 46D).

Therefore, the other excipients must be responsible for the lowering of the critical relative humidity (RH_0) and the concomitant moisture uptake observed for the tablets containing sodium ascorbate and ascorbic acid (section 3.3.4.1).

The superdisintegrants Ac-Di-Sol® and Explotab® low pH in particular lead to an increased moisture uptake of ascorbic acid and sodium ascorbate of up to 24.8 %, whereas upon Lycatab® C as well as upon Teavigo® addition the moisture uptake of sodium ascorbate was reduced (Table 23). Consequently, the amount of ascorbyl radicals is lower in Lycatab® C tablets with 10 % (w/w) Explotab® low pH than in the tablets containing 25 % (w/w) Ac-Di-Sol®. These results explain why Lycatab® C formulations containing EGCG could be stabilized by the addition of 10 % (w/w) sodium ascorbate whereas tablets containing 25 % (w/w) Ac-Di-Sol® could not be stabilized.

In the case of the binary blends with ascorbic acid, only the superdisintegrants induced a browning (Fig. 44). On the other hand, all binary mixtures of sodium ascorbate with the formulation excipients showed a color change (Fig. 45). This was most pronounced for the combination with Explotab® low pH, followed by Ac-Di-Sol® and Lycatab® C. This is in accordance with the moisture uptake results of these binary blends that declined in the same order (Table 23). And for the binary mixture of ascorbic acid and sodium ascorbate, discoloration could be observed with all combined excipients as well as with Teavigo® powder (Fig. 46).

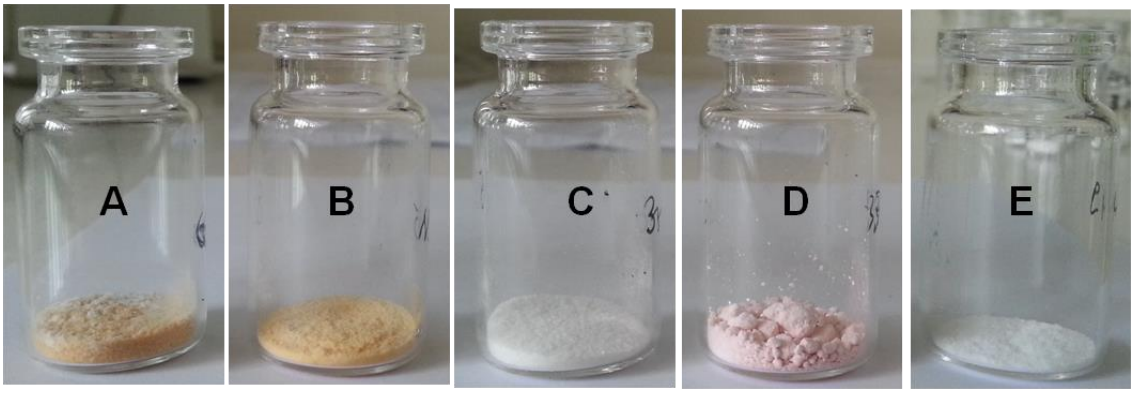


Figure 44: Binary 1:1 (w/w) mixtures of ascorbic acid with excipients from tablet formulations after 2 weeks of storage at 40°C/75 % RH: A) with Ac-Di-Sol®, B) with Explotab® low pH, C) with Lycatab® C, D) with Teavigo®, E) ascorbic acid

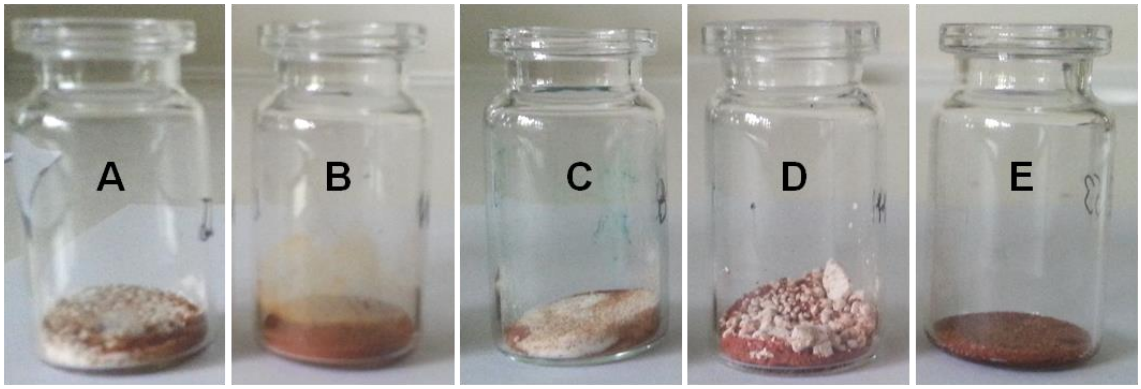


Figure 45: Binary 1:1 (w/w) mixtures of sodium ascorbate with excipients from tablet formulations after 2 weeks of storage at 40°C/75 % RH: A) with Ac-Di-Sol®, B) with Explotab® low pH, C) with Lycatab® C, D) with Teavigo®, E) sodium ascorbate

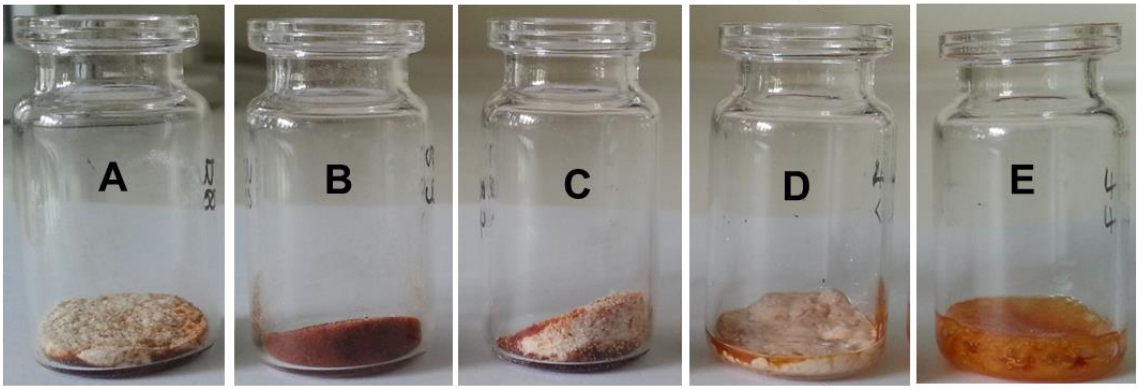


Figure 46: Binary 1:1 (w/w) mixtures of sodium ascorbate/ ascorbic acid with excipients from tablet formulations after 2 weeks of storage at 40°C/75 % RH: A) with Ac-Di-Sol®, B) with Explotab® low pH, C) with Lycatab® C, D) with Teavigo®, E) sodium ascorbate/ ascorbic acid

An important finding of this study was that EGCG in form of Teavigo® powder induced neither a discoloration of ascorbic acid nor of sodium ascorbate (Fig. 44D+45D) but the combination of sodium ascorbate and ascorbic acid should be avoided. And if a stabilization of EGCG by ascorbic acid or sodium ascorbate is emphasized, the other excipients within the dosage form need to be chosen carefully.

Furthermore, Lycatab® C showed less moisture uptake and less browning with ascorbic acid than with sodium ascorbate, but it was sodium ascorbate at a loading of 10 % (w/w) which stabilized EGCG in the tablet formulations containing Lycatab® C. This fact demonstrates that the degradation of ascorbate needs to occur at a certain ratio to the degradation of EGCG to act as an antioxidant for EGCG. Ascorbic acid at the same loading had a destabilizing effect.
Hence if the degradation products of ascorbate are present at a too high amount in relation to the EGCG degradation products, ascorbate acts as a pro-oxidant and leads to an elevated EGCG degradation. Both antioxidants show a pH-dependent radicalization. Thus the amount of degradation products is not only mediated by the moisture uptake and deliquescence, but also by the microenvironmental pH.

3.3.4.3. Microenvironmental pH of immediate release tablets containing ascorbic acid and its sodium salt

The incorporation of ascorbic acid, sodium ascorbate or a mixture of both into the Lycatab® C tablets (Fig. 47) and Ac-Di-Sol® tablets (Table 24) generated different microenvironmental pH. Ascorbic acid turned the microenvironmental pH acidic to pH 2.4 and 3.0, sodium ascorbate incorporation resulted in pH 6.4 and 6.5, and the incorporation of a mixture of both resulted in pH 4.3 and 4.4. Although the incorporation of ascorbic acid together with ascorbate into the tablets brought the pH the closest to the pH-optimum of EGCG, an excessive degradation of EGCG with 72.3 % up to 84.4 % loss could be observed upon storage (section 3.3.4).

Table 24: pH-change resulting from the addition of EGCG to the tablet excipient slurries and resulting degradation rate (k) of EGCG and ascorbic acid due to microenvironmental pH of tablets based on the published results of Li et al. 2012 and Golubitskii et al. 2007

Tablet formulation	Microenvironmental pH		Degradation rate	
	Tablets without EGCG	Tablets with EGCG	$k_{EGCG} \cdot 10^{-6}$ (min^{-1})	$k_{ascorbic\ acid} \cdot 10^{-3}$ (min^{-1})
Lycatab® C + Explotab® low pH + Na ascorbate + ascorbic acid	4.3	4.3	1.29	7.64
Lycatab® C + Explotab® low pH + ascorbic acid	2.8	2.4	1.97	1.55
Lycatab® C + Explotab®low pH + Na ascorbate	6.7	6.4	4.93	4.08
Ac-Di-Sol® + ascorbic acid	3.1	3.0	1.32	2.40
Ac-Di-Sol® + Na ascorbate + ascorbic acid	4.3	4.4	1.39	8.31
Ac-Di-Sol® + Na ascorbate	6.5	6.5	5.16	3.54

The reasons are first of all the enhanced deliquescence of ascorbic acid/ascorbate blends and secondly the pH-dependent degradation behavior of ascorbic acid. The pKa of ascorbic acid is 4.37; thus at pH 1.4 all ascorbic acid is present in its protonated stable form. This pH corresponds to the first pH-optimum. The second pH-optimum can be found, according to the literature, at pH 7.2. Both pH-optima deviate from the pH-optimum of EGCG that lies around pH 3.9. Consequently, at pH 3.9 ascorbic acid exhibits a high degradation rate whereas at the pH-optimum of ascorbic acid, the degradation rate of EGCG is high (Fig. 47).

A high degradation rate of ascorbate and a low degradation of EGCG can lead to a pro-oxidant effect of ascorbic acid on EGCG with the consequence of an enhanced EGCG degradation as observed in section 3.3.4. In combination with an enhanced deliquescence (section 3.3.4.2.) for ascorbic acid/sodium ascorbate blends, more EGCG as well as ascorbate is in solution and can degrade.

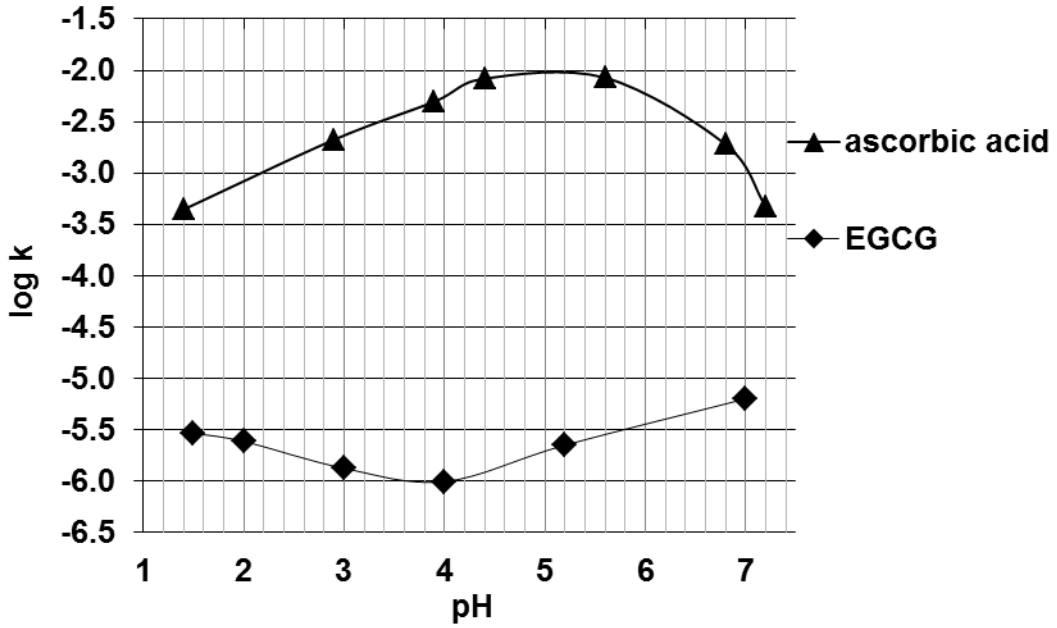


Figure 47: Log-linear relationship between the degradation rates (k) of ascorbic acid at room temperature and of EGCG at 40°C in solution and the pH (Li et al., 2012) (Golubitskii et al., 2007)

Additionally, the microenvironmental pH induced by the other tablet ingredients can cause a protonation of ascorbate or deprotonation of ascorbic acid resulting in a binary mixture of both forms. When in addition the moisture uptake is enhanced, this can lead to deliquescence and degradation of ascorbate as could be observed in the case of Lycatab® C tablets as well as Ac-Di-Sol® tablets containing ascorbic acid.
The incorporation of ascorbic acid within the tablets generated an acidic microenvironmental pH of 2.4 in the case of Lycatab® C tablets and pH 3 in the case of Ac-Di-Sol® tablets (Table 24). This decrease in microenvironmental pH from 5.8 to 2.4 and 6.7 to 3 resulted from ascorbic acid deprotonation. The resulting ascorbate induced a deliquescence lowering, degraded, and resulted in a browning of the tablets. EGCG, on the other hand, does not deprotonate at these pH-values.

Furthermore, the microenvironmental pH affected the degradation rate of ascorbic acid as well as of EGCG. The Ac-Di-Sol® tablets resulted in a higher microenvironmental pH of 3 which induced a lower EGCG degradation rate of $1.32 \cdot 10^{-6}$ min^{-1}, but on the other hand, a higher degradation rate for ascorbic acid of $2.40 \cdot 10^{-3}$ min^{-1} (Table 24). Thus a pro-oxidant effect on EGCG can be observed with 36.3 % EGCG loss.

The pH of 2.4 within the Lycatab® C tablets induced a higher EGCG degradation rate of $1.97 \cdot 10^{-6}$ min^{-1} and a lower ascorbic acid degradation rate of $1.55 \cdot 10^{-3}$ min^{-1}. Together with the lower moisture uptake, an unsufficient antioxidant effect is obtained and 24.5 % is lost. But overall, the degradation for both formulations was lower than in the case of tablets containing sodium ascorbate because ascorbic acid in blends at 75 % RH exhibits a lower moisture uptake than sodium ascorbate in blends and just a small amount of ascorbate is present that originates from ascorbic acid deprotonation.

The incorporation of sodium ascorbate resulted in a microenvironmental pH of 6.4 and 6.5, thus in an ascorbate degradation rate of $4.08 \cdot 10^{-3}$ min^{-1} and $3.54 \cdot 10^{-3}$ min^{-1}, respectively. At this pH, EGCG shows a high degradation rate of $4.93 \cdot 10^{-6}$ min^{-1} and $5.16 \cdot 10^{-6}$ min^{-1}, higher than at the acidic pH when it is combined with ascorbic acid (Table 24). Furthermore, blends containing sodium ascorbate exhibited after 2 weeks the highest moisture uptake (section 3.3.4.2.). Nevertheless, despite the elevated pH, an improvement in EGCG stability could be observed for the Lycatab® C tablets containing 10 % (w/w) sodium ascorbate. The degradation reactions of ascorbate and EGCG seem to occur at a suitable ratio resulting in an antioxidant instead of pro-oxidant effect on EGCG. The reason for the failed stabilization of EGCG in Ac-Di-Sol® tablets containing the same amount of sodium ascorbate can be found in the enhanced moisture uptake by Ac-Di-Sol® (section 3.3.4.2.). This resulted in a higher amount of dissolved ascorbate within the tablets that could degrade and act as pro-oxidant. Additionally, the ratio of the degradation rates of ascorbic acid and EGCG differed stronger than within the Lycatab® C tablets.

These results lead to the conclusion that the combination of Teavigo® with ascorbic acid or ascorbate is challenging but possible. It can result in a stability-enhancement of EGCG even in solid state, but attention should be paid to several factors: appropriate microenvironmental pH for both antioxidants, appropriate dose of both antioxidants within the dosage form as well as a suitable excipient choice under the scope of moisture uptake, and deliquescence of ascorbate. The major challenge is to find the appropriate microenvironmental pH, as their pH-dependent degradation behavior is antagonistic and a balance must exist between EGCG and ascorbate

degradation to avoid a pro-oxidant effect as shown for most of the combinations investigated here. But as these two dietary supplements are often combined in dosage forms, food products and beverages, it could offer a stabilization of EGCG not just within a dosage form but additionally during the gastrointestinal passage as well. The investigation of their appropriate loading within a dosage form and their combined pH-optimum should be the task of future studies.

3.4. (-)-Epigallocatechin-3-gallate immediate release tablets

3.4.1. Compactibility

Restrictions in drug loading and the need of additional tableting excipients are caused by the compactibility of active pharmaceutical ingredients, making compactibility testing an important preformulation step in the development of new tablet formulations. Hence, the compactibility of Teavigo® powder was investigated and compared to Rhodapap® DCP 3, a special grade of paracetamol with improved compactibility.

Teavigo® exhibited a superior compaction profiles (Fig. 48), but the tablets tend to stick to punches and to cap with increasing compression force, resulting in a poor regression coefficient for the linearity of the compaction profile, and made the addition of lubricant necessary. With increasing amounts of magnesium stearate as lubricant, the regression coefficient improved from 0.9807 in the case of no magnesium stearate up to 0.9991 in the case of 2 % (w/w) (Fig. 48) and the sticking and capping was reduced. Furthermore, the force necessary to compress Teavigo® into tablets decreased with increasing magnesium stearate amount.

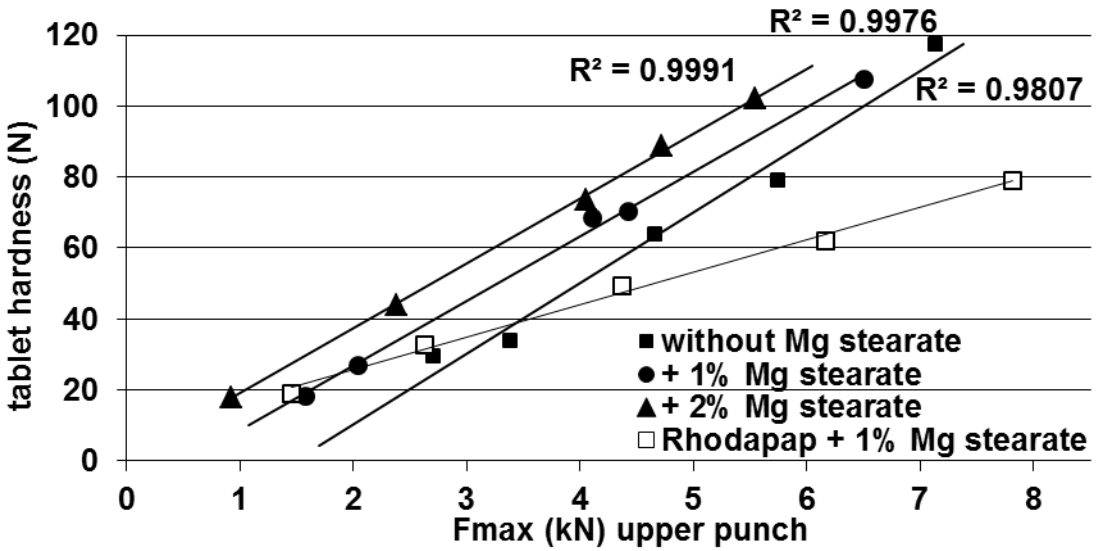


Figure 48: Improvement of the compaction profile of Teavigo® powder by the addition of different amounts magnesium stearate (n=1)

Consequently, 2 % (w/w) magnesium stearate was chosen as lubricant level for all tablet formulations.

As already mentioned, 85 % EGCG needs to be released within 15 minutes to ensure that the drug is dissolved when it enters the intestine. As the disintegration of tablets and drug dissolution correlate, fast disintegration of the tablets is necessary. Ac-Di-Sol® (cross-linked carboxymethyl cellulose) and Explotab® low pH (sodium starch glycolate) belong to the group of superdisintegrants and are often used in immediate release tablets to enhance their disintegration. Therefore, their effect on compactibility was investigated by compacting pure Teavigo® powder with 25 % (w/w) of the superdisintegrants.

The compactibility of Teavigo® was reduced but it still resulted in a better compaction profile than the one of Rhodapap® DCP 3. The effect of superdisintegrant on the compactibility was more pronounced for the incorporation of Explotab® low pH than of Ac-Di-Sol® (Fig. 49).

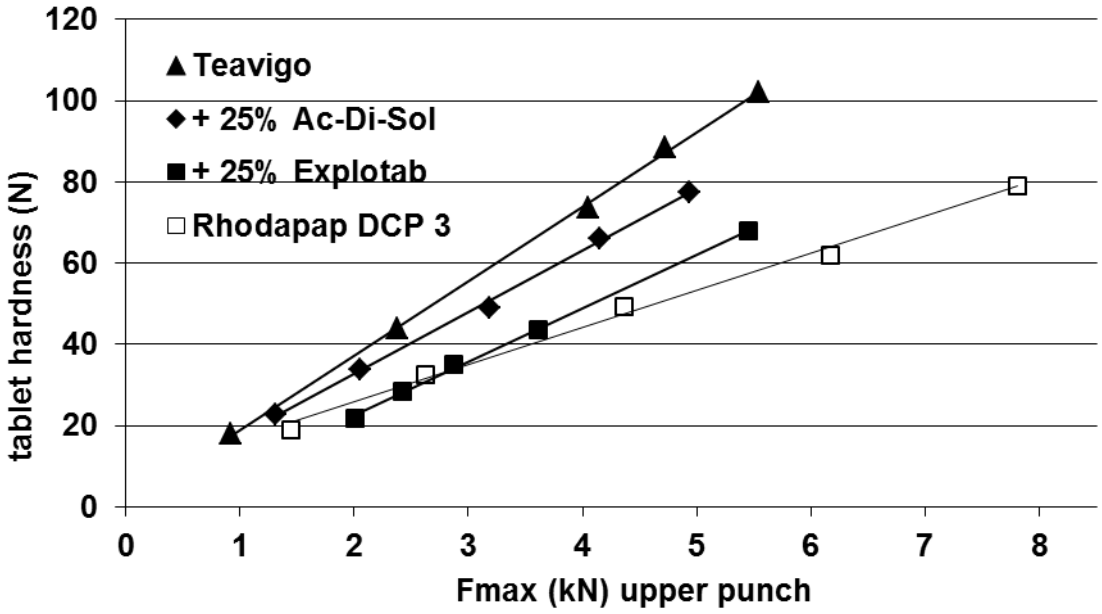


Figure 49: Compaction profile of Teavigo® powder with and without superdisintegrants compared to pure Rhodapap® DCP 3 tablets (n=1)

3.4.2. Disintegration study

3.4.2.1. Pure EGCG tablets with superdisintegrants

Due to the broad range of incompatible excipients, it would be preferably to avoid other excipients within EGCG immediate release tablets or at least keep their amount as low as possible. Pure Teavigo® tablets show a large disintegration time of around 13 minutes and additives to enhance their disintegration time become necessary.
Ac-Di-Sol® and Explotab® low pH are capable of disintegrating tablets already at loadings of 0.5-5 % and 2-8 % , respectively (Rowe et al., 2009). But in the case of EGCG tablets, the disintegrant efficacy of both superdisintegrants is decreased. Incorporation of up to 10 % (w/w) Explotab® low pH showed no significant disintegration enhancement (Fig. 50). Even at 25 % (w/w) loading, the disintegration efficacy was still insufficient to ensure that all drug would be released within 15 minutes.

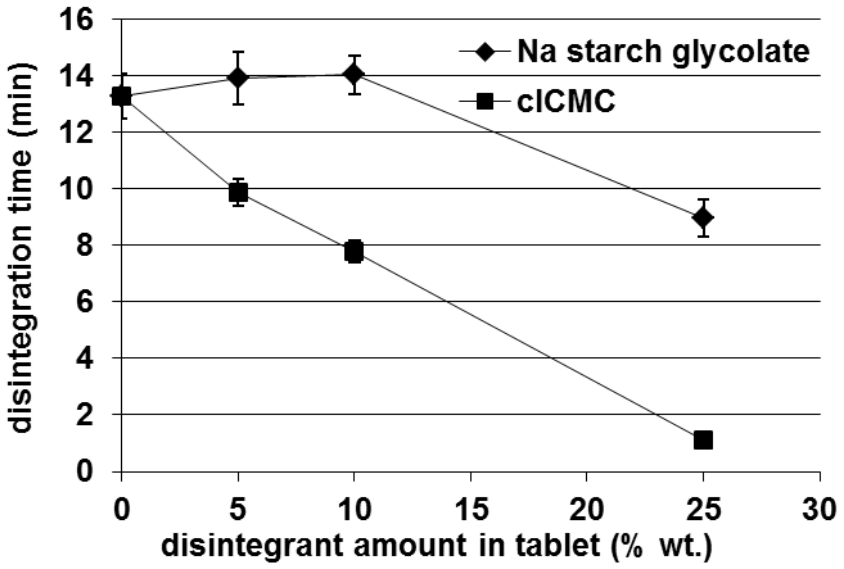


Figure 50: Disintegration time of EGCG tablets in 0.1 M HCl at 37°C in dependence of incorporated superdisintegrant amount (values presented as an average of n=3 ±SD)

Ac-Di-Sol® on the other hand, improved the disintegration time linearly with increasing amount, and at a loading of 25 % (w/w) exhibited a disintegration time of around 1 minute. It is noteworthy, that this loading is far above the recommended and normally used amount within immediate release tablets and thus was investigated.

3.4.2.2. Effect of EGCG on superdisintegrant efficacy

The main mechanism of disintegration by superdisintegrants is their fast water uptake, which results in swelling and concomitant swelling pressure bursting the tablets. Several factors can result in decreased disintegration efficacy. One important factor is the tablet porosity. The higher the porosity, the more fluid can penetrate the tablet and thus increase the wetting and swelling of the disintegrant and this way improve the disintegration. But not only too high but also too low porosity needs to be avoided as it may counteract the swelling pressure. Too low porosity or too strong sintering of the matrix can either result in swelling of the superdisintegrant particles which is too low, or the swelling pressure of the superdisintegrant becomes insufficient to disintegrate the strong bonds in the tablet. Too high porosity, however, can result in strong swelling but as there is a lot of void space, the swelling pressure remains too small to disintegrate the tablet matrix.
And finally, interactions of tablet ingredients can result in decreased hydration and thus swelling of the superdisintegrant.

The effect of porosity was investigated by reducing the tablet hardness and so increasing the tablet porosity. This resulted in further improvement of disintegration (Fig. 51). In the case of EGCG tablets containing either 25 % (w/w) Ac-Di-Sol® or Explotab® low pH, a constant decline in the disintegration time could be noticed with decreasing tablet hardness. In particular, the formulations containing Explotab® low pH exhibited strong dependence of hardness versus disintegration time although sodium starch glycolate and cross-linked carboxymethyl cellulose are reported to exhibit the same dependence of disintegration time on compression pressure; furthermore they have above 7 % (w/w) loading a disintegration behavior independent of compression force (Augsburger and Hahm, 2002). In the case of Ac-

Di-Sol®, increasing the hardness from 30 up to 70 N still resulted in fast disintegration within 1 to 2 minutes. Ac-Di-Sol® exhibited the behavior described in the literature whereas Explotab® low pH did not.

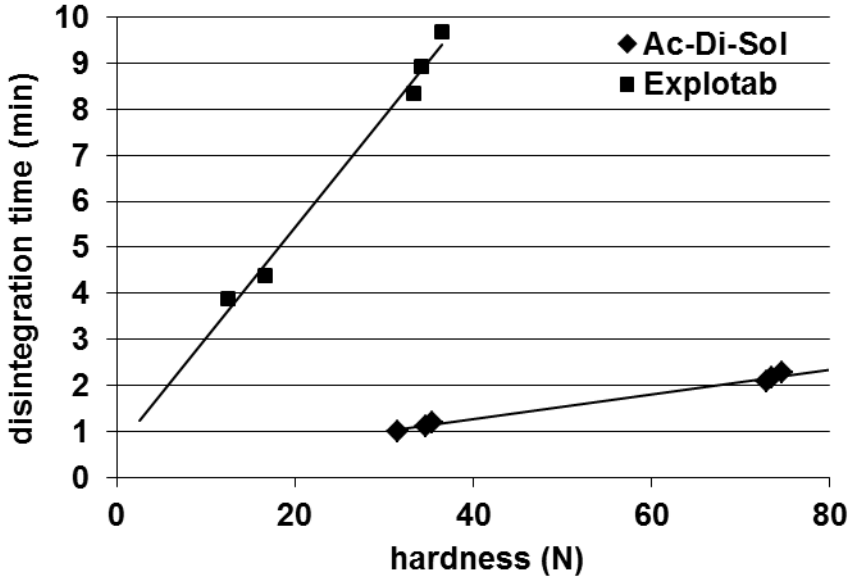


Figure 51: Disintegration time of EGCG tablets containing 25 % (w/w) superdisintegrant in 0.1 M HCl at 37°C in dependence of tablet hardness (n=1)

As porosity is not responsible for the slow disintegration of EGCG tablets containing Explotab® low pH, the most reasonable explanation for this phenomenon is the interaction of sodium starch glycolate with EGCG which reduces its swelling rate and pressure.

This explanation would also account for the steep slope of hardness versus disintegration time profile exhibited by tablets containing Explotab® (Fig. 51). Increasing the compression force and thus the hardness of tablets leads to a denser matrix and consequently closer contact of superdisintegrant particles with EGCG, which facilitates its interaction. As polyphenols are prone to interact with polysaccharides such as starch, and storage at 40°C/75 % RH conducted with 1:1 (w/w) powder blends of pregelatinized starch with EGCG results in its degradation, the reduced disintegration efficacy of sodium starch glycolate is likely due to hydrophilic interactions and thus reduced hydration capacity of the superdisintegrant molecules.

To prove that the reduced swelling is caused by EGCG, moisture uptake of 1:1 (w/w) binary mixtures of the superdisintegrants with EGCG was investigated and compared to their moisture uptake as single compounds (Table 25).

A slight insignificant reduction in moisture uptake can be observed for Ac-Di-Sol® in combination with Teavigo® but a significant reduction for Explotab® low pH with $p < 0.001$ / $\alpha = 0.05$. Explotab® low pH takes up 15.3 % less moisture when it is combined with Teavigo®. As a consequence of the altered water uptake, the disintegration efficacy of this superdisintegrant is reduced.

Table 25: Moisture uptake of superdisintegrants as single compounds and compounds in binary 1:1 (w/w) blends with Teavigo® (EGCG) over 2 weeks of storage at 40°C/75% RH and resulting reduction in moisture uptake of superdisintegrants (*values presented as average of n=6 (SD))

Superdisintegrant	Moisture uptake (%)		Reduction in moisture uptake (%)
	Single compound	Compound in EGCG blend	
Ac-Di-Sol®	13.6 (0.3)	13.3 (0.2)	2.1
Explotab® low pH	17.9 (0.3)	15.1 (0.3)	15.3

Unfortunately, sodium carboxymethyl cellulose belongs to the incompatible excipients for EGCG; hence the cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol®) is also expected to be incompatible.

And though Explotab® low pH is a compatible superdisintegrant, in order to formulate immediate release tablets with the same disintegration efficacy as the tablets containing Ac-Di-Sol®, the tablet porosity would need to be increased and thus hardness reduced. A reduction in hardness down to 10 N and below would be necessary and tablets would have the drawback of low mechanical stability. Hence different additives were tested to improve Explotab® low pH disintegration efficacy. The addition of potassium dihydrogen phosphate as a soluble salt with the aim to weaken the matrix upon its dissolution resulted in a significantly poorer disintegration time with $p < 0.01$ / $\alpha = 0.05$ (Table 26). The increased ionic strength within the tablets further reduced the swelling capacity of Explotab® low pH.

The addition of talc reduced the disintegration time significantly with $p < 0.01$ / $\alpha = 0.05$ despite the fact that hydrophobic lubricants normally increase the disintegration time of tablets. The explanation for the efficacy enhancement by talc is its unique lamellar structure which weakens the cohesive forces within the tablet and thus makes lower swelling pressure sufficient to disrupt the compact. But the disintegration still was not as fast as that of immediate release tablets containing Ac-Di-Sol® as superdisintegrant.

Table 26: Porosity, hardness and disintegration time of pure Teavigo® tablets and Teavigo® tablets containing 25 % (w/w) Explotab® low pH with and without additives in 0.1 M HCl at 37°C (values presented as an average of n=3 ±SD)

Tablet formulation	Porosity ε (%)	Hardness (N)	Disintegration time t_{dis} (min)
2 % Mg stearate	24 ± 0	47 ± 1	13.27 ± 0.80
+ 25 % Explotab	19 ± 1	37 ± 2	8.97 ± 0.67
+ 10 % KH_2PO_4 + 25 % Explotab	11 ± 2	40 ± 5	12.43 ± 1.13
+ 10 % Talc + 25 % Explotab	20 ± 2	24 ± 4	4.54 ± 2.07

The addition of the plastic, swellable binder microcrystalline cellulose (Avicel® PH102) to support the swelling or the brittle filler dibasic calcium hydrogen phosphate (Emcompress® premium) to facilitate matrix disintegration improved the disintegration time significantly with $p < 0.05$ / $\alpha = 0.05$ (Table 27) but still resulted in a too large disintegration time of 12 minutes and 13 minutes, respectively. Just the tablets containing Explotab® low pH and pregelatinized starch (Lycatab® C) as binder showed an acceptable disintegration time of 8 minutes. The interaction of EGCG with Explotab® low pH, that is a starch derivative, seemed to be shifted in favor of an interaction with Lycatab® C, enabling a sufficient disintegration effect of Explotab® low pH.

3.4.2.3. EGCG immediate release tablets

All immediate release tablet formulations tested upon their storage stability were tested as to their disintegration behavior (Table 27). Cross-linked polyvinylpyrrolidone (Kollidon® CL) is an unsuitable superdisintegrant as it interacts with EGCG by formation of complexes and precipitates. Consequently, 12 minutes disintegration time was obtained for the immediate release tablets containing Kollidon® CL, although these tablets exhibit the highest porosity and thus ease of water penetration within the tablet.

Both formulations containing Ac-Di-Sol® showed the fastest disintegration with t_{dis} below 2 minutes.

Table 27: Porosity, hardness and disintegration time of different EGCG immediate release tablets in 0.1 M HCl at 37°C (values presented as an average of n=3 ±SD)

Tablet formulation	Porosity ε (%)	Hardness (N)	Disintegration time t_{dis} (min)
Emcompress® premium+ Explotab® low pH	26 ± 0	34 ± 5	13.13 ± 0.86
Avicel® PH102 + Explotab® low pH	26 ± 2	38 ± 1	11.79 ± 0.79
Emcompress® premium + Kollidon® CL	37 ± 3	37 ± 3	12.10 ± 2.04
Avicel® PH102 + Ac-Di-Sol	28 ± 1	36 ± 2	1.28 ± 0.09
Ac-Di-Sol®	26 ± 0	59 ± 7	1.62 ± 0.07
Lycatab®C + Explotab® low pH	26 ± 1	48 ± 7	7.63 ± 1.75
Lycatab®C + Explotab® low pH + 10 % Na ascorbate	21 ± 1	45 ± 3	5.82 ± 0.51

The tablets containing the incompatible binder Lycatab ® C with Explotab® low pH were tested regarding their disintegration time with and without 10 % (w/w) sodium ascorbate as antioxidants to ensure that the disintegration behavior was not altered due to the stabilizer addition. But no significant difference could be observed in the disintegration time. The tablets disintegrated within 8 minutes.

3.4.3. Dissolution studies of stable EGCG immediate release tablets

The immediate release tablets that showed sufficient EGCG stability over 6 months at 40°C/75 % RH were up-scaled to immediate release tablets containing 300 mg EGCG and dissolution studies were performed. The release patterns and disintegration times of these formulations were compared to a commercial tablet formulation (SOURCE NATURALS® EGCG tablet) and self-prepared hard gelatin capsules as these showed the best release pattern among the capsule formulations (Table 28 + Fig. 54). The one-tailed independent two-sample t-test with unequal variance was used to analyze the released amount after 15 minutes upon significant difference in release with an alpha error set at 5 %.

Table 28: Porosity, disintegration time and amount of EGCG released after 15 minutes in 0.01 M HCl at 37°C of up-scaled EGCG immediate release tablets in comparison to one commercial EGCG tablet and self-prepared hard gelatin capsules as reference products (*values presented as an average of n=6 ±SD and average of 3 ±SD)

Tablet formulations	Porosity ε (%)	Hardness (N)	Disintegration time t_{dis} (min)*	EGCG released at t=15 min (%)
SOURCE NATURALS® tablet	n.d.	195 ± 27	22.55 ± 4.35	35.7 ± 6.0
Hard gelatin capsule	n.d.	n.d.	n.d.	78.2 ± 6.6
Emcompress® premium + Explotab® low pH	46 ± 0	17 ± 5	15.49 ± 3.57	61.1 ± 5.9
Avicel® PH102 + Ac-Di-Sol®	36 ± 0	40 ± 4	5.91 ± 1.29	89.5 ± 2.6
Lycatab® C + Explotab® low pH + 10 % Na ascorbate	34 ± 2	38 ± 2	15.47 ± 5.86	49.2 ± 4.7

As the formulations containing Ac-Di-Sol® exhibited the fastest disintegration behavior, they released EGCG the fastest as well. The immediate release tablets with Avicel® PH102 as binder and Ac-Di-Sol® as superdisintegrant were superior to both reference formulations and was the only tablet formulation releasing more than 85 % within 15 minutes, thus significantly faster than both reference formulations with

p < 0.05. The up-scaled immediate release tablets containing Emcompress® premium or Lycatab® C and Explotab® low pH as superdisintegrant showed a longer disintegration time of around 15 minutes and consequently as well did not fulfill the FDA requirements for BCS class 3 substances of 85 % drug release in 15 minutes (Table 28). Even the reduction of hardness to 17 N in case of Emcompress® premium tablets containing Explotab® low pH as superdisintegrant did not enhance the dissolution. The release is too slow to ensure full API dissolution prior to stomach exiting.

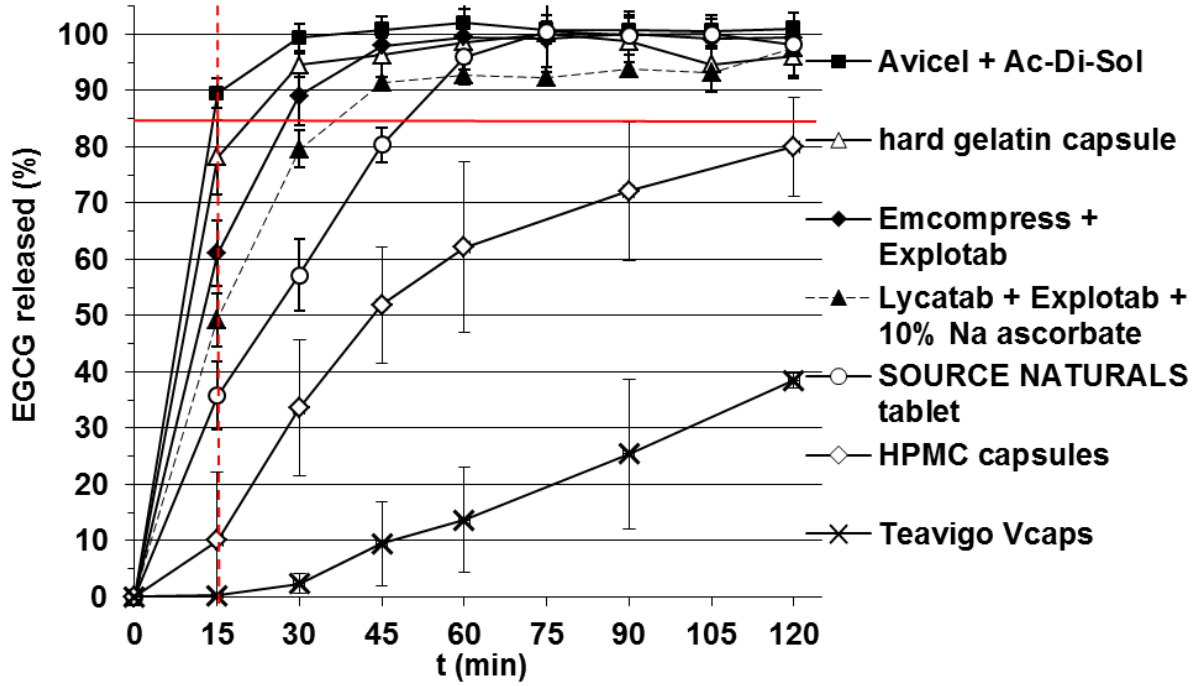


Figure 52: Dissolution study of stable EGCG immediate release tablets in comparison to a commercial tablet and self-prepared hard gelatin capsules as reference products performed in the USP 2 apparatus with a stirring rate of 50 rpm in 0.01 M HCl at 37°C (values presented as an average of n=3 ±SD)

Overall, the immediate release tablet formulations released EGCG significantly faster than the commercial tablet formulation with p < 0.05 but just the tablets containing Avicel® PH102 and Ac-Di-Sol® showed better release than hard gelatin capsules and thus offer an alternative peroral delivery system for EGCG to capsule formulations.

4. SUMMARY

(-)-Epigallocatechin-3-gallate (EGCG) exhibits a low oral bioavailability which is attributed to several factors. The aim of this work was to develop a solid oral dosage form containing EGCG with potentially improved oral bioavailability. The chemical degradation during the gastro-intestinal passage and within the dosage form is one of the factors leading to the poor bioavailability. As a consequence, finding the appropriate release window and pattern as well as investigating the physical and chemical compatibility of EGCG with excipients is crucial for the formulation development.

First of all, the autoxidative degradation of EGCG in aqueous solution was investigated. The pH-stability study showed that the degradation occurs at pH ≥ 5 and follows a biphasic degradation pattern with a first fast degradation phase of 30 minutes which is pH- and concentration-dependent. The second phase of degradation after 30 minutes is pH- and concentration-independent and slower with a constant degradation rate over 6 hours of approximately 20 to 40 $^{mg}/_{l*h}$. This degradation pattern could be attributed to the formation of EGCG aggregates. The self-aggregation occurred from a critical aggregation concentration (CAC) of 0.25 $^{g}/_{l}$ onwards as was determined by contact angle measurements. The aggregate formation could be proven by dynamic light scattering studies in pH 1 as well as in pH 7.4. The particle size increased with increasing EGCG concentration up to a mean particle size of 439 nm at 1 $^{g}/_{l}$ in pH 1 and 416 nm in pH 7.4 with no further size increase up to a concentration of 4 $^{g}/_{l}$. The first degradation phase represents the degradation of EGCG monomers, which are present in equilibrium with the aggregates. The amount of monomers present in solution and at the interfaces is dependent on the EGCG concentration. From this originates the further decline in contact angle observed above the CAC. The second degradation phase occurs within the aggregates. When the aggregate formation takes place at pH > 5, reactive degradation products and dissociated EGCG are incorporated in the aggregates. But the reactivity of the vicinal hydroxyl groups within the aggregates is reduced due to interactions of the EGCG molecules. Hence a slower degradation can be observed. Furthermore, the formation of aggregates is accompanied by the expulsion of

hydration water, by which the pH of the solution no longer exhibits an effect on the degradation. In contrast, the monomers in solution still possess their hydration shell; hence an effect of the pH of solution on the degradation can be observed.
If EGCG was now first dissolved under acidic and thus stable pH-conditions and aggregates have been already formed when the pH was increased to pH 7.4, the degradation rate after 30 minutes was reduced to approximately 0 $^{mg}/_{l*h}$ as no reactive species were incorporated within the aggregates and thus no degradation within the aggregates occurred. According to these studies, the pre-aggregation in acidic conditions might increase the amount of EGCG ready for absorption up to 5 %. This shows the benefit of first dissolving EGCG in the stomach prior to its entering the intestine where pH is above 5.

The most common commercial dosage forms for EGCG are capsule formulations. Most of them consist of a hydroxypropyl methylcellulose capsule shell. Capsule formulations belong to immediate release formulations and according to the FDA for EGCG as BCS class 3 substance, a release of 85 % within 15 minutes is required. Therefore hard capsule formulations were investigated in this work. The tested hard capsule materials were gelatin (Coni-Snap®), hydroxypropyl methylcellulose (HPMC) (Vcaps® plus) and hydroxypropyl methylcellulose with gellan gum as gelling aid (Vcaps®). All these capsule materials interacted with EGCG and resulted in a phase-separation. In the case of gelatin and HPMC with gellan gum, a liquid-liquid phase-separation was observed in form of coacervates. With pure HPMC, a solid-liquid phase-separation occurred in form of precipitates out of colloidal particles. The phase-separation already takes place at low reactant concentrations and is more sensitive to changes in the EGCG concentration than in the macromolecule concentration as was investigated by turbidimetric studies. At an elevated temperature of 37°C, the coacervation boundary of gelatin and EGCG was shifted to higher concentrations due to an improved solubility of the formed complexes.
As shown by polarized light microscopy, the phase-separation occurs immediately when concentrated solutions of both reactants come in contact. A firm layer was formed with HPMC in the contact area of the concentrated solutions and a layer out of agglomerated partially coalesced coacervates in the case of gelatin. This is the

reason for the observed capsule shell solidification during the release studies of EGCG from HPMC capsules. The capsule shell containing HPMC turned into a strong gum-like material with insufficient disintegration. The consequence was a retarded incomplete release of EGCG from HPMC capsules with just 80 down to 40 % EGCG release in 2 hours in 0.01 M hydrochloric acid solution (HCl). In the case of hard gelatin capsules, EGCG was released immediately and completely. But like the other capsule formulations, they failed to meet the requirements of 85 % release within 15 minutes.

Consequently, phase-separation can occur in the stomach when EGCG is administered in capsule formulations. Especially when the dose of EGCG or unit doses of capsules are increased, the patient reduces the recommended drinking volume of 150 ml or the hard gelatin capsule is taken with a cold drink. The consequence would be a release alteration as was proven by the dose-dependent dissolution study of EGCG dose from hard gelatin capsules. The increase in EGCG dose from one 150 mg capsule to two 300 mg capsules resulted in just 65 % EGCG release within 15 minutes instead of 80 %. Furthermore, even if soluble complexes are formed, the extent of absorption might be reduced when the complexes do not dissociate within the intestine. Particularly as EGCG inhibits trypsin and other protein digesting enzymes as well, the gelatin-EGCG complexes might remain in the intestine and be too large in size for passive diffusion processes.

The incorporation of additives into the capsules was an attempt to prevent the lack in disintegration by phase-separation occurring with HPMC gellan gum capsules. Nonionic surfactants with an HLB value above 12, a bulky structure and with polyoxyethylene chains such as polysorbate 20 and 80 and poloxamer 188 were capable of disrupting the coacervates and precipitates and even to prevent their formation when they were added first to the medium. The necessary amounts under physiological concentrations of the reactants were 0.5 % (w/v) for polysorbate 20 or 80 and 1.0 % (w/v) for poloxamer 188. Thus poloxamer 188 and polysorbate 80 were combined with EGCG and filled into HPMC capsules. But both additives failed to enhance EGCG release from the capsules. In the case of polysorbate 80, the release was even further decreased and poloxamer 188 showed no effect on the release.

Another approach investigated in this work to circumvent the coacervate/ precipitate formation was the preparation of an oily suspension of EGCG which was filled into the capsules. But as EGCG is surface active, it gathered at the oil/water interface once the oily suspension came in contact with the dissolution medium.
EGCG crystallized out at the surface of the released oily droplet and formed a crystalline shell. This crystallization process reduced the dissolution of EGCG further.

Consequently, preformulation studies to develop an immediate release tablet formulation were performed to replace the existing capsule formulations on the market. As EGCG readily interacts with polysaccharides, proteins and polymers, 6 months storage stability studies at 40°C/75 % relative humidity (accelerated stress conditions) were performed with capsule materials and common tableting excipients. The storage studies over 6 months showed that EGCG is not just physically incompatible with capsule materials but results as well in high EGCG degradation during storage. In combination with gelatin 33.1 % and with HPMC 13.6 % EGCG degraded upon 6 months of storage.
Furthermore, a series of tableting excipients showed chemical incompatibility in binary 1:1 (w/w) powder blends with a significant EGCG loss upon storage of more than 5 %. Of the group of binders and fillers, pregelatinized starch, sodium carboxymethyl cellulose and monobasic calcium phosphate are incompatible. Additionally, the combination with methacrylate copolymer (Eudragit® E) and magnesium stearate showed insufficient stability. Only powder blends with dibasic calcium hydrogen phosphate, microcrystalline cellulose (MCC) as filler and sodium starch glycolate of a special low pH grade (Explotab® low pH) as superdisintegrant showed acceptable EGCG loss of less than 5 % after 6 months.
The chemical instability was triggered by three factors generated by the excipients: the moisture uptake, microenvironmental pH generated by the excipient as it leads to a deprotonation of EGCG, and the final microenvironmental pH upon the addition of EGCG that differed among the excipient blends and influenced the degradation velocity of EGCG. It was shown, that the investigation of these three factors is sufficient to predict EGCG stability with excipients. When excipients were taking up moisture and had a microenvironmental pH above 3.9 (pH-optimum of EGCG),

degradation occurred immediately upon storage. When excipients did not take up moisture but resulted in a microenvironmental pH above 7, thus far removed from the pH-optimum of EGCG, the blends showed sufficient stability over a certain time, but in the end, the permanent contact with humid air and the presence of surface water were sufficient to result in EGCG degradation.

When moisture was taken up but the microenvironmental pH was close to the pH-optimum, sufficient stability was obtained, and no moisture uptake in combination with a microenvironmental pH below pH 7 lead to stable formulations.

Coacervate and precipitate formation is a factor enhancing the degradation as well. The coacervates as well as precipitates are amorphous in nature, as could be assessed by polarized light microscopy. Due to this solid state, they are more susceptible to degradation hence a lower pH is already sufficient to induce high EGCG degradation. Polyvinylpyrrolidone (PVP) forms precipitates with EGCG as well. And although it exhibited a microenvironmental pH close to the pH-optimum of EGCG, significant degradation of EGCG in 1:1 (w/w) binary powder blends occurred.

These three parameters could be applied as well to the formulated and stored immediate release tablets containing incompatible and compatible tableting excipients. Just two of the six tablet formulations showed sufficient EGCG stability: the combination of EGCG with the compatible excipients dibasic calcium phosphate and sodium starch glycolate; and the combination of the compatible filler MCC with 10 % of the incompatible superdisintegrant cross-linked carboxymethyl cellulose (Ac-Di-Sol®).

Due to the series of incompatibilities with excipients and since Teavigo® powder showed excellent compactibility, pure ,i.e., high dose immediate release tablets of EGCG without disintegrant were first investigated. Although easily processible, the formulations lacked fast disintegration with a disintegration time in 0.1 M HCl of 13 minutes. As disintegration time and dissolution rate correlates, the attempt was undertaken to improve the disintegration behavior by the addition of superdisintegrants. Although they are known for their high disintegration efficacy at low loadings below 8 % (w/w) within tablets, in the case of EGCG immediate release tablets higher amounts of disintegrants were necessary to result in a fast

disintegration. Above 15 % (w/w) loading of the incompatible superdisintegrant cross-linked carboxymethyl cellulose (Ac-Di-Sol®) was necessary to result in a disintegration time of 5 minutes. The only compatible superdisintegrant sodium starch glycolate (Explotab® low pH) failed even at a loading of 25 % (w/w) to disintegrate the tablets within 5 minutes. The only way to improve the tablet disintegration of immediate release formulations containing Explotab® low pH was the additional incorporation of 10 % (w/w) talc or to reduce the tablet hardness up to 10 N. But in both cases, insufficient mechanical stability of tablets resulted. Even the combination with the brittle filler dibasic calcium hydrogen phosphate or the swellable binder microcrystalline cellulose did not improve the disintegration time sufficiently. Just the combination with pregelatinized starch (Lycatab® C) as binder showed an acceptable disintegration time of 8 minutes. The interaction of EGCG with Explotab® low pH, that is a starch derivative, seemed to be shifted in favor of an interaction with Lycatab® C, enabling a sufficient disintegration effect of Explotab® low pH.

The poor efficacy of the superdisintegrants could be traced back to a reduction in water uptake in combination with Teavigo® powder. In the case of Explotab® low pH, the moisture uptake was reduced about 15.3 %, whereas cross-linked carboxymethyl cellulose took up just 2.1 % less moisture. As their main effect derives from swelling and the concomitant buildup of pressure within the tablets, the reduction in water uptake resulted in a considerably lowered disintegration enhancement of the EGCG tablets.

Due to the poor disintegration, the stable immediate release tablets with sodium starch glycolate did not meet the requirement of 85 % active release after 15 minutes in 0.01 M HCl. Just the fast disintegrating formulations containing 10 % (w/w) cross-linked carboxymethyl cellulose in combination with MCC were capable of meeting these requirements and even showed 89.5 % release after 15 minutes. Hence they were superior in their release not only over commercially available EGCG immediate release tablets but also over hard gelatin capsules as reference formulation as well.

As the chemical incompatibilities reduce the range of useful excipients, immediate release tablets were prepared which contained either an incompatible disintegrant (Ac-Di-Sol®) or an incompatible filler (Lycatab® C) and stabilization approaches were

investigated with ascorbic acid and its sodium salt. Sodium ascorbate was capable of stabilizing EGCG at an equimolar concentration in buffer solutions of pH 6.5, 6.8 and 7.4. And even at an excess molar ratio (50:1) in pH 6.8, such as would be present in the water sorption layer within the tablets, it showed a stabilizing effect on EGCG over 6 days with 58.4 % EGCG recovery instead of 26.5 %. Consequently, the effect of ascorbic acid, sodium ascorbate and a mixture of both on EGCG stability within immediate release tablets was investigated. Depending on the chosen stabilizer, the tablets exhibited different microenvironmental pH of 2.4 or 3, 6.4 or 6.5, and 4.3 or 4.4, respectively. The pH-optimum for EGCG lies around pH 3.9. At this pH the degradation rate of EGCG is the lowest. Thus it was expected that the combination of EGCG with a mixture of ascorbic acid and sodium ascorbate would be most favorable for EGCG.

But the stabilizing effect of sodium ascorbate on EGCG in solution could not be observed in solid state during the 6 months storage at 40°C/ 75 % RH. A deliquescence of ascorbic acid and its salt occurred. Upon the deliquescence, radicals of ascorbate were generated and a pro-oxidant effect on EGCG could be observed. The degradation of EGCG was highest for the tablets containing ascorbic acid and sodium ascorbate in a mixture with up to 84.4 % EGCG loss, followed by sodium ascorbate with up to 54.2 % and ascorbic acid with 36.3 %. This was in accordance with the results of the deliquescence study as the moisture uptake with concomitant discoloration of them as single compounds declined in the same order. The amount of stabilizer within the tablets had an effect on EGCG degradation as well, with more EGCG being lost at 20 % (w/w) loading than at 10 % (w/w) loading. Furthermore the deliquescence study showed that the moisture uptake was not enhanced by EGCG in form of Teavigo® powder but by the excipients present in the tablets. In particular, the superdisintegrants Ac-Di-Sol® and Explotab® low pH increased the moisture uptake of the ascorbic acid forms and resulted in a sponge-like matrix due to their interaction.

Only the tablet formulation containing the incompatible binder Lycatab® C (pregelatinized starch) in combination with EGCG showed an improved stability of EGCG due to the incorporation of 10 % (w/w) sodium ascorbate from 10.4 % EGCG loss to 6.4 %. It was found, that several factors contributed to this stabilization.

First of all, Lycatab® C reduced the moisture uptake of sodium ascorbate. Additionally, the microenvironmental pH of 6.4 induced sufficiently low ascorbate degradation in combination with a sufficiently high degradation of EGCG and this way a pro-oxidant effect of ascorbate on EGCG could be avoided. This is an important finding as EGCG and ascorbic acid are a common combination throughout the supplementary market and claim that it increases EGCG stability within the intestine and enhances its therapeutic effectivity. The results show, that EGCG and ascorbic acid and its sodium salt are compatible as long as care is taken with the microenvironmental pH and moisture uptake. Furthermore, sodium ascorbate is capable of improving EGCG stability in dosage forms as long as the other excipients do not induce too high ascorbate degradation leading to a pro-oxidant effect on EGCG.
Tablet formulations containing the incompatible superdisintegrant Ac-Di-Sol® at 25 % loading could neither be stabilized by ascorbic acid nor its sodium salt. But when adjusting the amount of an incompatible excipient like Ac-Di-Sol® to 10 % (w/w), as well an acceptable active loss below 5 % can be achieved.

Overall, this work broadens the fundamentals of solid dosage form formulations with EGCG. The deficiency of existing formulations was elucidated. And the investigation of the chemical and physical stability of EGCG with common excipients offers the possibility to facilitate the development of new EGCG formulations. This work gives an overview of factors influencing the instability of EGCG and allows pre-estimation of its compatibility with excipients. Additionally, this work contributes to a better understanding of the degradation pattern of EGCG in regard to its concentration- and pH-dependence as well as its biphasic pattern. This knowledge helps to adjust the release window and release pattern of EGCG according to its chemical stability in solution and thus to increase its oral bioavailability.

5. ZUSAMMENFASSUNG

(-)-Epigallocatechin-3-gallate (EGCG) weist eine niedrige orale Bioverfügbarkeit auf, die mehreren Faktoren zugeschrieben werden kann. Ziel der Arbeit war es eine feste orale Darreichungsform für EGCG zu entwickeln mit einer möglicherweise verbesserten oralen Bioverfügbarkeit. Die chemische Zersetzung während der Magen-Darm-Passage und innerhalb der Arzneiform ist ein Faktor, der zur geringen Bioverfügbarkeit führt. Daher ist es für die Formulierungsentwicklung äußerst wichtig, ein geeignetes Freisetzungsfenster und Freisetzungsprofil zu finden, sowie die physikalische und chemische Kompatibilität von EGCG mit Hilfsstoffen zu untersuchen.

Zunächst einmal wurde der autoxidative Abbau von EGCG in wäßrigen Lösungen untersucht. Die pH-Stabilitätsstudie zeigte, dass die Zersetzung bei pH ≥ 5 erfolgt und einen zweiphasigen Abbauverlauf aufweist mit einer ersten schnellen Abbauphase von 30 Minuten, die pH- und konzentrationsabhängig verläuft. Die zweite Phase des Abbaus nach 30 Minuten war pH- und konzentrationsunabhängig und langsamer mit einer konstanten Abbaurate über 6 Stunden von 20 bis 40 $^{mg}/_{l*h}$. Dieser Abbauverlauf konnte zurückgeführt werden auf die Ausbildung von Aggregaten bestehend aus EGCG Molekülen. Die Ausbildung der Aggregate erfolgte ab einer kritischen Aggregationskonzentration (CAC) von 0.25 $^{g}/_{l}$ wie mithilfe von Kontaktwinkelmessungen ermittelt wurde. Die Aggregatbildung konnte mittels dynamischer Lichtstreuungsmessung sowohl in pH 1 als auch in pH 7,4 nachgewiesen werden. Die Partikelgröße wuchs mit steigender EGCG-Konzentration bis zu einer mittleren Partikelgröße von 439 nm in pH 1 und 416 nm in pH 7,4, wobei von 1 $^{g}/_{l}$ bis hin zu 4 $^{g}/_{l}$ kein Partikelwachstum mehr erkennbar war. Die erste Abbauphase ist bedingt durch den Abbau der EGCG Monomere, die sich im Gleichgewicht mit den Aggregaten befinden. Je nach EGCG-Konzentration befinden sich unterschiedliche Mengen an Monomeren in Lösung sowie an den Grenzflächen. Daher auch der weitere Abfall des Kontaktwinkels selbst nach dem Erreichen der CAC. Die zweite Abbauphase findet innerhalb der Aggregate statt. Wenn die Aggregatbildung in pH über 5 vonstattengeht, werden auch reaktive Abbauprodukte von EGCG und dissoziiertes EGCG in die Aggregate eingebaut. Jedoch ist die

Reaktivität der benachbarten Hydroxylgruppen durch die Wechselwirkungen der EGCG-Moleküle innerhalb der Aggregate herabgesetzt. Deshalb kann ein langsamerer Abbau beobachtet werden. Des Weiteren geht die Entstehung von Aggregaten mit dem Verdrängen von Hydratationswasser einher, wodurch der pH keinen Einfluss mehr auf den Abbau hat. Im Vergleich dazu besitzen die Monomere in Lösung noch ihre Hydrathülle und es kann ein pH-Effekt auf die erste Abbauphase beobachtet werden.
Wenn nun EGCG zunächst unter sauren demzufolge stabilen pH-Bedingungen gelöst wurde, somit Aggregate bereits vorhanden waren, wenn der pH auf pH 7,4 angehoben wurde, kam es nach 30 Minuten zu einer Verringerung der Abbaurate auf etwa 0 $^{mg}/_{l*h}$ da keine reaktiven Abbauprodukte in die Aggregate eingebaut werden und somit kein Abbau innerhalb der Aggregate stattfindet. Das zeigt den Vorteil auf, wenn EGCG zuerst im Magen aufgelöst wird und dann in den Dünndarm gelangt, wo Bedingungen über pH 5 herrschen. Diese Voraggregation im sauren Milieu könnte entsprechend der hier durchgeführten Studien die Menge an EGCG, die zur Resorption zur Verfügung steht, erhöhen und zwar um bis zu 5 %.

Die gängigsten Arzneiformen für EGCG auf dem Markt sind Kapselformulierungen. Die meisten bestehen aus einer Hydroxypropylmethylcellulose-Kapsel. Kapselformulierungen gehören zu schnell freisetzenden Arzneiformen und entsprechend der FDA wird für EGCG, da es zur BCS-Klasse 3 gehört, eine Freisetzung von mindestens 85 % innerhalb von 15 Minuten gefordert. Daher wurden in dieser Arbeit Hartkaspel-formulierungen untersucht. Die getesteten Hartkaspelmaterialien waren Gelatine (Coni-Snap®), Hydroxypropylmethylcellulose (HPMC) (Vcaps® plus) und Hydroxypropyl-methyl-cellulose-Kapseln mit Gellan als Gelierungshilfsstoff (Vcaps®). Alle getesteten Hartkapselmaterialien interagierten mit EGCG, was eine Phasentrennung zur Folge hatte. Im Fall von Gelatine und HPMC in Kombination mit Gellan konnte eine flüssig-flüssig Phasentrennung in Form von Koazervaten beobachtet werden. Mit purem HPMC kam es zur fest-flüssig Phasentrennung in Form von Präzipitaten bestehend aus kolloidalen Partikeln. Wie die turbidimetrischen Untersuchungen zeigten erfolgte die Phasentrennung bereits bei niedrigen Konzentrationen der Reaktanden und ist sensitiver hinsichtlich

Veränderungen in der EGCG-Konzentration als von der der Makromoleküle. Bei einer erhöhten Temperatur von 37°C wurde die Koazervationsgrenze von Gelatine und EGCG zu höheren Konzentrationen verschoben, da sich die Löslichkeit der geformten Komplexe verbessert.

Wie mit Hilfe von Polarisationslichtmikroskopie gezeigt werden konnte, läuft die Phasentrennung sofort ab, wenn konzentrierte Lösungen der beiden Reaktanden aufeinandertreffen. Mit HPMC wird eine dichte Schicht ausgeformt im Bereich, in dem die Lösungen aufeinandertreffen und eine Schicht aus agglomerierten, partiell koaleszierten Koazervaten im Falle von Gelatine. Das ist auch der Grund für die während der Freisetzungsstudien von EGCG aus HPMC-Kapseln beobachtete Kapselhüllenverfestigung. Die Kapselhüllen bestehend aus HPMC verwandeln sich in ein widerstandsfähiges, gummiartiges Material, welches mangelnden Zerfall aufweist. Die Konsequenz war eine verzögerte unvollständige Freisetzung von EGCG aus HPMC-Kapseln mit nur 80 % bis hinunter zu 40 % freigesetztem EGCG in 2 Stunden in 0.01 M verdünnter Salzsäure. Im Fall von Hartgelatinekapseln erfolgt die Freisetzung schnell und vollständig. Aber wie die anderen Kapselmaterialien, erfüllen sie nicht die Anforderungen von 85 % Freisetzung binnen 15 Minuten.

Demzufolge kann auch eine Phasentrennung im Magen erfolgen, wenn EGCG in Form von Kapselformulierungen verabreicht wird. Vor allem wenn die Dosis von EGCG oder die Anzahl der einzunehmenden Dosiseinheiten erhöht wird, der Patient die Kapseln mit weniger Flüssigkeit als vorgeschrieben oder die Hartgelatinekapseln mit einem kalten Getränk zu sich nimmt. Die Konsequenz wäre eine Veränderung des Freisetzungsprofils, wie bei der dosis-abhängigen Freisetzung von EGCG aus Hartgelatinekapseln gezeigt wurde. Die Erhöhung der EGCG Dosis von einer 150 mg Kapsel zu zwei 300 mg Kapseln führte zu nur 65 % freigesetztem EGCG innerhalb von 15 Minuten statt 80 %. Außerdem selbst wenn lösliche Komplexe gebildet werden, könnte es zu einer verringerten Resorption kommen, wenn die Komplexe im Dünndarm nicht dissoziieren. Vor allem da EGCG Trypsin sowie auch andere Verdauungsenzyme für Proteine hemmt, könnten Gelatine-EGCG-Komplexe im Dünndarm bestehen bleiben und zu groß sein um über passive Diffusion aufgenommen zu werden.

Es wurde versucht den mangelnden Zerfall von HPMC-Gellan-Kapseln aufgrund der Phasentrennung durch Zusätze zur Kapselfüllung zu verhindern. Nichtionische Emulgatoren mit einem HLB-Wert von über 12, einer sperrigen Struktur und mit Polyoxyethylen-Seitenketten wie Polysorbat 20 und 80 sowie Poloxamer 188 waren in der Lage geformte Koazervate und Präzipitate zu trennen und sogar deren Bildung zu verhindern, wenn sie vor der Koazervatbildung zum Medium hinzugefügt wurden. Die hierfür nötigen Mengen unter physiologischen Konzentrationsbedingungen der Reaktanden waren 0,5 % (m/V) Polysorbat 20 oder 80 und 1 % (m/V) Poloxamer 188. Daher wurden Poloxamer 188 und Polysorbat 80 mit EGCG kombiniert und in HPMC-Kapseln gefüllt. Beide Zusätze waren nicht in der Lage, die Freisetzung von EGCG aus den Kapseln zu erhöhen. Polysorbat 80 führte sogar zu einer Freisetzungs-verschlechterung. Der Zusatz von Poloxamer 188 hatte keinen Einfluss auf die Freisetzung.
Ein anderer Ansatz der in dieser Arbeit bearbeitet wurde, war die Koazervation zu umgehen, indem man eine ölige Suspension von EGCG herstellt und diese in die Kapseln füllt. Da aber EGCG oberflächenaktiv ist, sammelte es sich an der Öl/Wasser-Grenzfläche an sobald die ölige Suspension mit dem Freisetzungsmedium in Kontakt kam. EGCG kristallisierte an der Oberfläche der freigesetzten öligen Tröpfchen aus und bildete eine Kristallhülle aus. Dieser Kristallisationsvorgang reduziert die Freisetzung von EGCG nur noch weiter.

Daher wurden Präformulierungsstudien durchgeführt um schnell freisetzende Tabletten als Ersatz für die bestehenden Kapselformulierungen zu entwickeln. Da EGCG bereitwillig mit Polysacchariden, Proteinen und Polymeren wechselwirkt, wurden 6 monatige Lagerstabilitätsstudien unter 40°C/ 75 % relativen Luftfeuchte (forcierten Stressbedingungen) mit Kapselmaterialien und gängigen Tablettierungshilfsstoffen sowie daraus hergestellten EGCG Tabletten durchgeführt. Die Lagerstudien über 6 Monate zeigten, dass EGCG mit den Kapselmaterialien nicht nur physikalisch inkompatibel ist, sondern deren Kombination während der Lagerung auch zu einem hohen Abbau von EGCG führt. Im Fall von Gelatine wurden über die 6 monatige Lagerung 33,1 % und im Falle von HPMC 13,6 % EGCG abgebaut.

Zudem wies eine Reihe von Tablettierungshilfsstoffen chemische Inkompatibilitäten in binären 1:1 (m/m) Pulvermischungen unter den Lagerungsbedingungen auf mit einem signifikanten EGCG Verlust von mehr als 5 %. Von den Trockenbindemitteln und Füllstoffen sind vorgelatinierte Stärke, Natriumcarboxymethylcellulose sowie Calciumdihydrogenphosphat inkompatible. Außerdem wiesen die Kombinationen mit Dimethylaminoethyl-methacrylatcopolymeren (Eudragit® E) und Magnesiumstearat unzureichende Stabilität auf.

Nur Pulvermischungen mit den Füllstoffen mikrokristalline Cellulose (MCC), Calciumhydrogenphosphat und Natriumstärkeglykolat als Sprengmittel mit einem speziell niedrig gehaltenen pH (Explotab® low pH) wiesen einen akzeptablen EGCG Verlust von weniger als 5 % nach 6 Monaten auf.

Die chemische Instabilität von EGCG wurde durch drei Faktoren beeinflusst, die von den Hilfsstoffen herrührten: die Feuchtigkeitsaufnahme, den UmgebungspH erzeugt durch den Hilfsstoff, der zu einer Deprotonierung von EGCG führt und dem End-pH nach der Zugabe von EGCG, der sich bei den verschiedenen Hilfsstoffmischungen unterschied und die Abbaugeschwindigkeit von EGCG bestimmte. Es konnte gezeigt werden, dass die Untersuchung dieser 3 Parameter ausreicht um die Stabilität von EGCG mit Hilfsstoffen vorherzusagen. Wenn die Hilfsstoffe Feuchtigkeit aufnahmen und einen UmgebungspH hervorriefen, der über 3,9 lag (pH-Optimum für EGCG), kam es während der Lagerung sofort zum Abbau. Sofern die Hilfsstoffe keine Feuchtigkeit aufnahmen, aber einen UmgebungspH über 7 erzeugten, also fernab vom pH-Optimum von EGCG, wiesen sie zunächst über eine gewisse Zeit Stabilität auf, aber zum Ende hin resultiert der permanente Kontakt mit feuchter Luft und die Anwesenheit von Oberflächenwasser dennoch im Abbau von EGCG.

Wenn Feuchtigkeit aufgenommen wurde, aber der UmgebungspH nahe am pH-Optimum war, erhielt man ausreichende Stabilität. Und sofern keine Feuchtigkeit aufgenommen wurde und der UmgebungspH unter pH 7 lag, führte dies zu stabile Formulierungen.

Auch die Entstehung von Koazervaten oder Präzipitaten ist ein Faktor, der den Abbau begünstigt. Sowohl die Koazervate als auch die Präzipitate sind amorpher Natur wie unter dem Polarisationslichtmikroskop beobachtet werden konnte. Aufgrund dieses Feststoffzustandes ist EGCG anfälliger für den Abbau und daher ist

auch ein niedriger pH ausreichend um einen starken EGCG-Abbau zu bewirken. Polyvinylpyrrolidon (PVP) bildet ebenfalls Präzipitate mit EGCG. Und obwohl es einen UmgebungspH erzeugt, der nahe am pH-Optimum von EGCG liegt, kam es zu einem signifikanten Abbau von EGCG in binären 1:1 (m/m) Pulvermischungen.

Diese drei Parameter konnten auch für die formulierten und gelagerten schnell freisetzenden Tabletten mit kompatiblen und inkompatiblen Hilfsstoffen herangezogen werden. Nur zwei der sechs getesteten Tablettenformulierungen wiesen eine ausreichende EGCG-Stabilität auf: die Kombination von EGCG mit den kompatiblen Hilfsstoffen Calciumhydrogenphosphat und Natriumstärkeglykolat, sowie die Kombination mit dem kompatiblen Füllstoff MCC und 10 % des inkompatiblen Sprengmittels quervernetzte Carboxymethylcellulose (Ac-Di-Sol®).

Aufgrund der Reihe von Inkompatibilitäten mit Hilfsstoffen und da Teavigo® Pulver in den durchgeführten Untersuchungen zur Tablettierbarkeit eine exzellente Kompaktierung aufwies, wurde zunächst untersucht pure und somit hochdosierte, schnell freisetzende Tabletten von Teavigo® ohne Sprengmittelzusatz herzustellen. Obwohl die Formulierung problemlos zu tablettieren war, wiesen die Tabletten eine mangelhafte Zerfallszeit in 0.1 M HCl von 13 Minuten auf. Da die Zerfallszeit mit der Freisetzung korreliert, wurde versucht das Zerfallsverhalten durch den Zusatz von Sprengmitteln zu verbessern. Obwohl sie für ihre hohe Effizienz in der Zerfallsbeschleunigung bereits bei Beladungen von unter 8 % (m/m) in Tabletten bekannt sind, waren im Fall von schnell freisetzenden EGCG-Tabletten höhere Mengen der Sprengmittel nötig um einen schnellen Zerfall zu erzeugen. Über 15 % (m/m) des inkompatiblen Sprengmittels quervernetzte Carboxymethylcellulose (Ac-Di-Sol®) waren nötig um einen Zerfall binnen 5 Minuten zu erhalten. Das einzige kompatible Sprengmittel Natriumstärkeglykolat (Explotab® low pH) scheiterte sogar bei einer Beladung von 25 % (m/m) daran die Tabletten binnen 5 Minuten zum Zerfallen zu bringen. Der einzige Weg den Tablettenzerfall der schnell freisetzenden Tabletten mit Explotab® low pH zu verbessern, war der Zusatz von 10 % (m/m) Talkum oder die Tablettenhärte auf bis zu 10 N zu reduzieren. In beiden Fällen wurde jedoch eine mangelhafte mechanische Stabilität der Tabletten erhalten. Auch die Kombination mit dem spröden Füllstoff Calciumhydrogenphosphat oder dem

quellenden Trockenbindemittel mikrokristalline Cellulose (MCC) verbesserte die Zerfallszeit nicht. Nur die Kombination mit vorgelatinierter Stärke (Lycatab® C) als Bindemittel wies eine akzeptable Zerfallszeit von 8 Minuten auf. Die Wechselwirkungen zwischen EGCG und Explotab® low pH, welches ein Stärkederivat ist, scheinen verschoben worden zu sein zugunsten einer Wechselwirkung mit Lycatab® C, und ermöglichten somit eine ausreichende Zerfallsbeschleunigung durch Explotab® low pH.
Die schlechte Effizienz der Sprengmittel konnte auf eine Verringerung der Wasseraufnahme dieser in Kombination mit Teavigo® zurückgeführt werden.
Im Fall von Natriumstärkeglykolat kommt es zu einer Reduktion der Feuchtigkeitsaufnahme um 15,3 %, wohingegen bei quervernetzter Carboxymethylcellulose nur 2,1 % weniger Feuchtigkeit aufgenommen wird. Da deren Wirkung hauptsächlich durch Quellung und den damit verbundenen Druckaufbau innerhalb der Tablette zustande kommt, führt die verringerte Wasseraufnahme zu einer deutlich geringeren Zerfallsbeschleunigung im Fall von EGCG Tabletten.
Aufgrund des schwachen Zerfallsverhaltens erfüllten deshalb auch die stabile schnell freisetzenden Tabletten mit Natriumstärkeglykolat nicht die Anforderung von 85 % freigesetztem Wirkstoff nach 15 Minuten in 0,01 M HCl. Nur die schnell zerfallenden Formulierungen, die 10 % (m/m) quervernetzte Carboxymethylcellulose in Kombination mit MCC enthielten, waren in der Lage diese Anforderungen zu erfüllen und zeigten sogar 89,5 % Freisetzung nach 15 Minuten. Und waren daher in ihrer Freisetzung nicht nur handelsüblichen EGCG Tabletten sondern auch den Hartgelatinekapseln als Referenzformulierung überlegen.

Da die chemischen Instabilitäten die Auswahl an verwendbaren Hilfsstoffen reduzieren, wurden schnell freisetzende Tabletten hergestellt die entweder ein inkompatibles Sprengmittel (Ac-Di-Sol®) oder einen inkompatiblen Füllstoff (Lycatab® C) enthielten und die Stabilisierungsmöglichkeit von EGCG mit Ascorbinsäure und dessen Natriumsalz untersucht. Natriumascorbat war in der Lage EGCG in Lösungen von pH 6,5, 6,8 und pH 7,4 bei äquimolaren Zusatz zu stabilisieren. Und sogar im molaren Überschuss von (50:1) in pH 6,8, so wie es auch der Fall wäre in der

adsorbierten Wasserschicht innerhalb der Tabletten, wies es eine Stabilisierung von EGCG in pH 6,8 über 6 Tage auf mit einer verbleibenden Menge von 58,4 % EGCG anstatt 26,5 %. Daher wurde der Effekt von Ascorbinsäure, Natriumascorbat und einer Mischung aus beiden auf die Stabilität von EGCG in schnell freisetzenden Tabletten untersucht. In Abhängigkeit des gewählten Stabilisators, wiesen die Tabletten unterschiedliche UmgebungspH-Werte auf von pH 2,8 oder 3, 6,4 oder 6,5 beziehungsweise 4,3 oder 4,4. Das pH-Optimum von EGCG liegt laut Literatur bei etwa pH 3.9. Bei diesem pH ist die Abbaurate von EGCG am kleinsten. Daher wurde erwartet, dass die Kombination von EGCG mit der Mischung aus Ascorbinsäure und Natriumascorbat die vorteilhafteste für EGCG wäre.

Aber der stabilisierende Effekt von Natriumascorbat auf EGCG in Lösung konnte nicht in Feststoffsystemen während der 6 monatigen Lagerung bei 40°C/ 75 % relativer Luftfeuchte beobachtet werden. Eine Verflüssigung von Ascorbinsäure und dessen Salz trat auf. Aufgrund der Verflüssigung wurden Radikale von Ascorbat gebildet und ein pro-oxidativer Effekt auf EGCG konnte beobachtet werden. Der EGCG-Abbau war am höchsten in Tabletten, die Ascorbinsäure und Natriumascorbate als ein Gemisch enthielten mit bis zu 84,4 % EGCG Verlust, gefolgt von Natriumascorbat mit bis zu 54,2 % EGCG Verlust und Ascorbinsäure mit 36,3 %. Das entsprach auch den Ergebnissen der durchgeführten Verflüssigungsstudien mit Ascorbinsäure, Natriumascorbat und der Mischung beider. Die Feuchtigkeitsaufnahme mit begleitender Verfärbung von ihnen als Einzelkomponenten fiel in der gleichen Reihenfolge. Auch die Menge an Stabilisator hatte einen Einfluss auf den Abbau von EGCG mit stärkerem Verlust bei 20 % (m/m) Stabilisatorbeladung als bei 10 % (m/m). Des Weiteren zeigte die Verflüssigungsstudie, dass die Feuchtigkeitsaufnahme nicht durch EGCG in Form von Teavigo® Pulver hervorgerufen wurde, aber durch die in den Tabletten vorkommenden Hilfsstoffe.

Vor allem die Sprengmittel Ac-Di-Sol® und Explotab® low pH erhöhten die Feuchtigkeitsaufnahme der Ascorbinsäureformen und resultierten in einer schwammartigen Matrix aufgrund ihrer Wechselwirkung. Allein die Tablettenformulierung, die das inkompatible Trockenbindemittel Lycatab® C (vorgelatinierte Stärke) in Kombination mit EGCG enthielt zeigte eine verbesserte

Stabilität von EGCG durch die Einarbeitung von 10 % (m/m) Natriumascorbat von 10,4 % EGCG Verlust auf nur 6,4 %. Es konnte ermittelt werden, dass verschiedene Faktoren zur Stabilisierung beitrugen. Zunächst einmal reduzierte Lycatab® C die Feuchtigkeitsaufnahme von Natriumascorbat. Zusätzlich führte der UmgebungspH-Wert von 6.4 zu einem ausreichend niedrigen Abbau von Ascorbat in Kombination mit einem ausreichend hohen Abbau von EGCG und verhinderte so einen pro-oxidativen Effekt auf EGCG. Dies ist eine wichtige Erkenntnis, da EGCG und Ascorbinsäure eine oft anzutreffende Kombination über den Nahrungsergänzungsmittelmarkt hinweg sind und diese Kombination hat ihre Berechtigung, da sie in der Lage ist EGCG im Dünndarm zu stabilisieren und dessen therapeutische Effizienz zu erhöhen. Die Ergebnisse zeigen, dass EGCG und Ascorbinsäure sowie dessen Natriumsalz kompatibel sind, wenn auf den UmgebungspH, sowie die Feuchtigkeitsaufnahme geachtet wird. Zudem haben die Ergebnisse gezeigt, dass Natriumascorbat in der Lage ist die Stabilität von EGCG in Darreichungsformen zu stabilisieren, solange andere Hilfsstoffe nicht einen allzu hohen Abbau von Ascorbat hervorrufen, der zu einem pro-oxidativen Effekt auf EGCG führt.
Tablettenformulierungen die das inkompatible Sprengmittel Ac-Di-Sol® mit einer Beladung von 25 % (m/m) enthielten konnten weder durch Ascorbinsäure noch durch dessen Natriumsalz stabilisiert werden. Wenn jedoch die Menge Ac-Di-Sol® angepasst wurde zu 10 % (m/m) wurde ein akzeptabler Wirkstoffverlust von unter 5 % (m/m) erhalten.

Im Großen und Ganzen vertieft diese Arbeit das Grundwissen von festen Darreichungsformulierungen von EGCG. Die Defizite existierender Formulierungen wurden ausgewertet. Die hier durchgeführten Untersuchungen der chemischen und physikalischen Stabilität von EGCG mit gängigen Hilfsstoffen eröffnen die Möglichkeit die Entwicklung neuer Formulierungen von EGCG zu vereinfachen. Die Arbeit gibt einen Überblick über Faktoren, die die Instabilitäten von EGCG beeinflussen und erlaubt die Vorwegeinschätzung von dessen Auftreten. Zusätzlich trägt diese Arbeit zu einem besseren Verständnis des Abbauverlaufes von EGCG bei im Hinblick auf dessen konzentrations- und pH-Abhängigkeit sowie des

zweiphasigen Verlaufes. Dieses Wissen hilft das Freisetzungsfenster und den Freisetzungsverlauf von EGCG hinsichtlich seiner chemischen Stabilität in Lösung anzupassen und auf diesem Wege dessen orale Bioverfügbarkeit zu erhöhen.

6. LIST OF REFERENCES

Alchorn, A.L., 2007. Lipid Rafts Observed in Cell Membranes. Sci. Technol. Rev. 24–25.

Alvarez-fuentes, J., Caraballo, I., Boza, A., Llera, J.M., Holgado, M.A., 1997. Study of a complexation process between naltrexone and Eudragit ® L as an oral controlled release system. Int. J. Pharm. 148, 219–230.

Arts, M.J.T.J., Haenen, G.R.M.M., Wilms, L.C., Beetstra, S. a J.N., Heijnen, C.G.M., Voss, H.-P., Bast, A., 2002. Interactions between flavonoids and proteins: effect on the total antioxidant capacity. J. Agric. Food Chem. 50, 1184–7.

Astill, C., Birch, M.R., Dacombe, C., Humphrey, P.G., Martin, P.T., 2001. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J. Agric. Food Chem. 49, 5340–7.

Augsburger, L.L., Hahm, H.A., 2002. Super disintegrants: characterization and function, in: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, Inc., New York, Basel, pp. 2623–2638.

Bae, M.-J., Ishii, T., Minoda, K., Kawada, Y., Ichikawa, T., Mori, T., Kamihira, M., Nakayama, T., 2009. Albumin stabilizes (-)-epigallocatechin gallate in human serum: binding capacity and antioxidant property. Mol. Nutr. Food Res. 53, 709–15. doi:10.1002/mnfr.200800274

Borgström, B., Dahlqvist, A., Lundh, G., Sjövall, J., 1957. Studies of intestinal digestion and absorption in the human. J. Clin. Invest. 36, 1521–1536.

Borst, P., Evers, R., Kool, M., Wijnholds, J., 2000. A family of drug transporters: the multidrug resistance-associated proteins. J. Natl. Cancer Inst. 92, 1295–1302.

Boschmann, M., Thielecke, F., 2007. The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: a pilot study. J. Am. Coll. Nutr. 26, 389S–395S. doi:10.1080/07315724.2007.10719627

Burgess, D.J., 2002. Colloids and colloid drug-delivery systems, in: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, Inc., New York, Basel, pp. 497–508.

Cabrera, C., Artacho, R., Giménez, R., 2006. Beneficial effects of green tea—a review. J. Am. Coll. Nutr. 25, 79–99. doi:10.1080/07315724.2006.10719518

Caporali, A., Davalli, P., Astancolle, S., D'Arca, D., Brausi, M., Bettuzzi, S., Corti, A., 2004. The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression. Carcinogenesis 25, 2217–24. doi:10.1093/carcin/bgh235

Cayman Chemical Company, 2012. safety data sheet epigallocatechin gallate.

Chacko, S.M., Thambi, P.T., Kuttan, R., Nishigaki, I., 2010. Beneficial effects of green tea: a literature review. Chin. Med. 5, 1–9. doi:10.1186/1749-8546-5-13

Chen, L., Lee, M., Li, H.E., Yang, C.S., Al, C.E.T., 1997. Absorption , distribution , and elimination of tea polyphenols in rats. Drug Metab. Dispos. 25, 1045–1050.

Chen, S.-Y., Ou, S.-F., Teng, N.-C., Kung, C.-M., Tsai, H.-L., Chu, K.-T., Ou, K.-L., 2013. Phase transformation on bone cement:mono calcium phosphate monohydrate into calcium-deficient hydroxyapatite during setting. Ceram. Int. 39, 2451-2455. doi:10.1016/j.ceramint.2012.08.097

Chen, Y.-C., Yu, S.-H., Tsai, G.-J., Tang, D.-W., Mi, F.-L., Peng, Y.-P., 2010. Novel technology for the preparation of self-assembled catechin/gelatin nanoparticles and their characterization. J. Agric. Food Chem. 58, 6728–34. doi:10.1021/jf1005116

Chen, Z., Zhu, Q.Y., Wong, Y.F., Zhang, Z., Chung, H.Y., 1998. Stabilizing effect of ascorbic acid on green tea catechins. J. Agric. Food Chem. 46, 2512–2516.

Chiwele, I., Jones, B.E., Podczeck, F., 2000. The shell dissolution of various empty hard capsules. Chem. Pharm. Bull. (Tokyo). 48, 951–6.

Chow, H.H., Cai, Y., Alberts, D.S., Hakim, I., Dorr, R., Shahi, F., Crowell, J. a, Yang, C.S., Hara, Y., 2001. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol. Biomarkers Prev. 10, 53–8.

Connors, K.A., Khossravi, D., 1993. Solvent effects on chemical processes . 4 . complex formation between naphthalene and theophylline in binary aqueous-organic solvents. J. Solution Chem. 22, 677–694.

De Haan, P., Kroon, C., Sam, A.P., 1990. Decomposition and stabilization of the tablet excipient calcium hydrogenphosphate dihydrate. Drug Dev. Ind. Pharm. 16, 2031–2055.

Dietrich, C.G., Geier, A., Elferink, R.P.J.O., 2003. ABC of oral bioavailability: transporters as gatekeepers in the gut. Gut 52, 1788–1795. doi:10.1136/gut.52.12.1788

Digenis, G.A., Gold, T.B., Shah, V.P., 1994. Cross-linking of gelatin capsules and its relevance to their in vitro-in vivo performance. J. Pharm. Sci. 83, 915–921.

Dube, A., Nicolazzo, J. a, Larson, I., 2011. Chitosan nanoparticles enhance the plasma exposure of (-)-epigallocatechin gallate in mice through an enhancement in intestinal stability. Eur. J. Pharm. Sci. 44, 422–6. doi:10.1016/j.ejps.2011.09.004

Edge, S., Steele, D.F., Staniforth, J.N., Chen, A., Woodcock, P.M., 2002. Powder compaction properties of sodium starch glycolate disintegrants. Drug Dev. Ind. Pharm. 28, 989–999. doi:10.1081/DDC-120006430

Fallingborg, J., 1999. Intraluminal ph of the human gastrointestinal tract. Dan. Med. Bull. 46, 183–96.

Feng, W.Y., 2006. Metabolism of green tea catechins: an overview. Curr. Drug MeTable 7, 755–809.

Folch-Cano, C., Jullian, C., Speisky, H., Olea-Azar, C., 2010. Antioxidant activity of inclusion complexes of tea catechins with β-cyclodextrins by ORAC assays. Food Res. Int. 43, 2039–2044. doi:10.1016/j.foodres.2010.06.006

Forrest, G.I., Bendall, D.S., 1969. The distribution of polyphenols in the tea plant (Camellia sinensis L.). Biochem. J. 113, 741–55.

Friedman, M., 2007. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol. Nutr. Food Res. 51, 116–34. doi:10.1002/mnfr.200600173

Friedman, M., Levin, C.E., Lee, S.-U., Kozukue, N., 2009. Stability of green tea catechins in commercial tea leaves during storage for 6 months. J. Food Sci. 74, H47–51. doi:10.1111/j.1750-3841.2008.01033.x

Fuchs, J., 2009. The amount of liquid patients use to take tablets or capsules. Pharm. Pract. 7, 170–174.

Fujita, Y., Yamane, T., Tanaka, M., Kuwata, K., Okuzumi, J., Takahashi, T., Fujiki, H., Okuda, T., 1989. Inhibitory effect of (-)-epigallocatechin gallate on carcinogenesis with N-ethyl-N'-nitro-N-nitrosoguanidine in mouse duodenum. Japanese J. cancer Res. 80, 503–505.

Gander, B., Blanco-Príeto, M.J., Thomasin, C., Wandrey, C., Hunkeler, D., 2002. Coacervation/phaseseparation, in: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, Inc., New York, Basel, pp. 481–496.

Glube, N., Moos, L. Von, Duchateau, G., 2013. Capsule shell material impacts the in vitro disintegration and dissolution behaviour of a green tea extract. Results Pharma Sci. 3, 1–6. doi:10.1016/j.rinphs.2013.08.002

Golubitskii, G.B., Budko, E. V., Basova, E.M., Kostarnoi, a. V., Ivanov, V.M., 2007. Stability of ascorbic acid in aqueous and aqueous-organic solutions for quantitative determination. J. Anal. Chem. 62, 742–747. doi:10.1134/S1061934807080096

Graham, H.N., 1992. Green tea composition, consumption, and polyphenol chemistry. Prev. Med. (Baltim). 21, 334–50.

Graßmann, W., Hannig, K., Endres, H., 1956. Aminosäuresequenzen des Kollagens. I. Zur Bindungsweise des Prolins und Hydroxyprolins. Hoppe. Seylers. Z. Physiol. Chem. 306, 123–131.

Green, R.J., Murphy, A.S., Schulz, B., Watkins, B. a, Ferruzzi, M.G., 2007. Common tea formulations modulate in vitro digestive recovery of green tea catechins. Mol. Nutr. Food Res. 51, 1152–62. doi:10.1002/mnfr.200700086

Guo, Q., Zhao, B., Shen, S., Hou, J., Hu, J., Xin, W., 1999. ESR study on the structure-antioxidant activity relationship of tea catechins and their epimers. Biochim. Biophys. Acta 1427, 13–23.

Gupta, S., Ahmad, N., Nieminen, a L., Mukhtar, H., 2000. Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (-)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol. Appl. Pharmacol. 164, 82–90. doi:10.1006/taap.1999.8885

Hagerman, A.E., Dean, R.T., Davies, M.J., 2003. Radical chemistry of epigallocatechin gallate and its relevance to protein damage. Arch. Biochem. Biophys. 414, 115–120. doi:10.1016/S0003-9861(03)00158-9

Hashimoto, F., Nonaka, G., Nishioka, I., 1989. Tannins and related compounds. XC. 8-C-ascorbyl (-)-epigallocatechin 3-o-gallate and novel dimeric flavan-3-ols, oolonghomobisflavans A and B, from oolong tea. (3). Chem. Pharm. Bull. (Tokyo). 37, 3255–3263.

Hashimoto, T., Kumazawa, S., Nanjo, F., Hara, Y., Nakayama, T., 1999. Interaction of tea catechins with lipid bilayers investigated with liposome systems. Biosci. Biotechnol. Biochem. 63, 2252–5.

Hatano, T., Tsugawa, M., Kusuda, M., Taniguchi, S., Yoshida, T., Shiota, S., Tsuchiya, T., 2008. Enhancement of antibacterial effects of epigallocatechin gallate, using ascorbic acid. Phytochemistry 69, 3111–6. doi:10.1016/j.phytochem.2007.08.013

Hayashi, N., Ujihara, T., Kohata, K., 2005. Reduction of catechin astringency by the complexation of gallate-type catechins with pectin. Biosci. Biotechnol. Biochem. 69, 1306–10.

Hiatt, A.N., Ferruzzi, M.G., Taylor, L.S., Mauer, L.J., 2011. Deliquescence behavior and chemical stability of vitamin C forms (ascorbic acid, sodium ascorbate, and calcium ascorbate) and blends. Int. J. Food Prop. 14, 1330–1348. doi:10.1080/10942911003650338

Higdon, J. V, Frei, B., 2003. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit. Rev. Food Sci. Nutr. 43, 89–143. doi:10.1080/10408690390826464

Hinman, W.C., Beaton, J.D., Read, D.W.L., 1962. Some effects of moisture and temperature on transformation of monocalcium phosphate in soil. Can. J. Soil. Sci. 42 , 229-39

Hipfner, D.R., Deeley, R.G., Cole, S.P., 1999. Structural, mechanistic and clinical aspects of MRP1. Biochim. Biophys. Acta 1461, 359–76.

Hirai, M., Hotta, Y., Ishikawa, N., Wakida, Y., Fukuzawa, Y., Isobe, F., Nakano, A., Chiba, T., Kawamura, N., 2007. Protective effects of EGCG or GCG, a green tea catechin epimer, against postischemic myocardial dysfunction in guinea-pig hearts. Life Sci. 80, 1020–32. doi:10.1016/j.lfs.2006.11.032

Hof, K.H. Van, Kivits, G.A.A., Weststrate, J.A., Tijburg, L.B.M., 1998. Bioavailability of catechins from tea : the effect of milk. Eur. J. Clin. Nutr. 52, 356–359.

Hong, J., Lambert, J.D., Lee, S.-H., Sinko, P.J., Yang, C.S., 2003. Involvement of multidrug resistance-associated proteins in regulating cellular levels of (−)-epigallocatechin-3-gallate and its methyl metabolites. Biochem. Biophys. Res. Commun. 310, 222–227. doi:10.1016/j.bbrc.2003.09.007

Hong, J., Lu, H., Meng, X., Ryu, J., Hara, Y., Yang, C.S., 2002. Stability , cellular uptake , biotransformation , and efflux of tea polyphenol (-)-epigallocatechin-3-Gallate in HT-29 human colon adenocarcinoma cells. Cancer Res. 62, 7241–7246.

Ishizu, T., Hirata, C., Yamamoto, H., Harano, K., 2006. Structure and intramolecular flexibility of beta-cyclodextrin complex with (-)-epigallocatechin gallate in aqueous solvent. Magn. Reson. Chem. 44, 776–83. doi:10.1002/mrc.1848

Jancova, P., Anzenbacher, P., Anzenbacherova, E., 2010. Phase II drug metabolizing enzymes. Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc. Czech. Repub. 154, 103–16.

Jodoin, J., Demeule, M., Beliveau, R., 2002. Inhibition of the multidrug resistance P-glycoprotein activity by green tea polyphenols. Biochim. Biophys. Acta 1542, 149–59.

Kao Corporation, 2008. GRAS Notice 000259: Green tea catechins. Tokyo.

Kesinger, N.G., Stevens, J.F., 2009. Covalent interaction of ascorbic acid with natural products. Phytochemistry 70, 1930–9. doi:10.1016/j.phytochem.2009.09.028

Khan, N., Afaq, F., Saleem, M., Ahmad, N., Mukhtar, H., 2006. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer Res. 66, 2500–5. doi:10.1158/0008-5472.CAN-05-3636

Khaw, K.-T., Bingham, S., Welch, A., Luben, R., Wareham, N., Oakes, S., Day, N., 2001. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. Lancet 357, 657–663. doi:10.1016/S0140-6736(00)04128-3

Komatsu, Y., Suematsu, S., Hisanobu, Y., Saigo, H., Matsuda, R., Hara, K., 1993. Effects of pH and temperature on reaction kinetics of catechins in green tea infusion. Biosci. Biotechnol. Biochem. 57, 907–910.

Kuentz, M., Leuenberger, H., 1999. Pressure susceptibility of polymer tablets as a critical property: a modified Heckel equation. J. Pharm. Sci. 88, 174–9. doi:10.1021/js980369a

Kumamoto, M., Sonda, T., Nagayama, K., Tabata, M., 2001. Effects of pH and metal ions on antioxidative activities of catechins. Biosci. Biotechnol. Biochem.

Lambert, J.D., Hong, J., Kim, D.H., Mishin, V.M., Yang, C.S., 2004. Piperine enhances the bioavailability of the tea polyphenol (-)-epigallocatechin-3-gallate in mice. J. Nutr. 1948–1952.

Lambert, J.D., Lee, M.-J., Diamond, L., Ju, J., Hong, J., Bose, M., Newmark, H.L., Yang, C.S., 2006. Dose-dependent levels of epigallocatechin-3-gallate in human colon cancer cells and mouse plasma and tissues. Drug MeTable Dispos. 34, 8–11. doi:10.1124/dmd.104.003434.(Mrp)

Lambert, J.D., Sang, S., Yang, C.S., 2007. Biotransformation of green tea polyphenols and the biological activities of those metabolites. Mol. Pharm. 4, 819–825.

Le Bourvellec, C., Renard, C.M.G.C., 2012. Interactions between polyphenols and macromolecules: quantification methods and mechanisms. Crit. Rev. Food Sci. Nutr. 52, 213–48. doi:10.1080/10408398.2010.499808

Lee, B.L., Ong, C.N., 2000. Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis. J. Chromatogr. A 881, 439–47.

Lee, M.-J., Maliakal, P., Chen, L., Meng, X., Bondoc, F.Y., Prabhu, S., Lambert, G., Mohr, S., Yang, C.S., 2002. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol. biomarkers Prev. 11, 1025–32.

Li, N., Taylor, L.S., Ferruzzi, M.G., Mauer, L.J., 2012. Kinetic Study of Catechin Stability: Effects of pH, concentration, and temperature. J. Agric. Food Chem. 60, 12531–12539.

Li, N., Taylor, L.S., Ferruzzi, M.G., Mauer, L.J., 2013. Color and chemical stability of tea polyphenol (−)-epigallocatechin-3-gallate in solution and solid states. Food Res. Int. 53, 909–921. doi:10.1016/j.foodres.2012.11.019

Li, Z., Gu, L., 2014. Fabrication of Self-Assembled (−)-epigallocatechin gallate (EGCG) ovalbumin − dextran conjugate nanoparticles and their transport across monolayers of human intestinal epithelial Caco - 2 cells. J. Agric. Food Chem. 62, 1301–1309.

Liang, Y.C., Lin-Shiau, S.Y., Chen, C.F., Lin, J.K., 1999. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (-)-epigallocatechin-3-gallate. J. Cell. Biochem. 75, 1–12.

Liu, Y., Gao, L., Xia, T., Zhao, L., 2009. Investigation of the site-specific accumulation of catechins in the tea plant (Camellia sinensis (L.) O. Kuntze) via vanillin-HCl staining. J. Agric. Food Chem. 57, 10371–6. doi:10.1021/jf902614n

Lu, H., Meng, X., Yang, C.S., 2003. Enzymology of methylation of tea catechins and inhibition of catechol- O -methyltransferase by (-) -epigallocatechin gallate. Drug MeTable Dispos. 31, 572–579.

Markovic, R.S., Grujic, O.S., Pejin, J.D., 2003. Conventional and alternative principles for stabilization of protein and polyphenol fractions in beer. Acta Period. Technol. 34, 3–12.

Menger, F.M., Rizvi, S. a a, 2011. Relationship between surface tension and surface coverage. Langmuir 27, 13975–7. doi:10.1021/la203009m

Mochizuki, M., Yamazaki, S., Kano, K., Ikeda, T., 2002. Kinetic analysis and mechanistic aspects of autoxidation of catechins. Biochim. Biophys. Acta 1569, 35–44.

Naz, S., Siddiqi, R., Dew, T.P., Williamson, G., 2011. Epigallocatechin-3-gallate inhibits lactase but is alleviated by salivary proline-rich proteins. J. Agric. Food Chem. 59, 2734–8. doi:10.1021/jf103072z

Neilson, A.P., Hopf, A.S., Cooper, B.R., Pereira, M. a, Bomser, J. a, Ferruzzi, M.G., 2007. Catechin degradation with concurrent formation of homo- and heterocatechin dimers during in vitro digestion. J. Agric. Food Chem. 55, 8941–9. doi:10.1021/jf071645m

Niki, E., 1987. Interaction of Ascorbate and alpha-Tocopherol. Ann. N. Y. Acad. Sci. 498, 186–99.

Niki, E., 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. Am. J. Clin. Nutr. 54, 1119S–1124S.

Ortiz, J., Kestur, U.S., Taylor, L.S., Mauer, L.J., 2009. Interaction of environmental moisture with powdered green tea formulations: relationship between catechin stability and moisture-induced phase transformations. J. Agric. Food Chem. 57, 4691–7. doi:10.1021/jf8038583

Pajak, B., Kania, E., Gajkowska, B., Orzechowski, A., 2011. Lipid rafts mediate epigallocatechin-3-gallate- and green tea extract-dependent viability of human colon adenocarcinoma COLO 205 cells; clusterin affects lipid rafts-associated signaling pathways. J. Physiol. Pharmacol. 62, 449–59.

Pang, K.S., 2003. Modeling of intestinal drug absorption: roles of transporters and metabolic enzymes (for the Gillette Review Series). Drug MeTable Dispos. 31, 1507–19. doi:10.1124/dmd.31.12.1507

Pascal, C., Poncet-Legrand, C., Imberty, A., Gautier, C., Sarni-Manchado, P., Cheynier, V., Vernhet, A., 2007. Interactions between a non glycosylated human proline-rich protein and flavan-3-ols are affected by protein concentration and polyphenol/protein ratio. J. Agric. Food Chem. 55, 4895–901. doi:10.1021/jf0704108

Patel, A.R., Nijsse, J., Velikov, K.P., 2011. Novel polymer–polyphenol beads for encapsulation and microreactor applications. Soft Matter 7, 4294–4301. doi:10.1039/c1sm05135k

Patel, A.R., Seijen-ten-Hoorn, J., Velikov, K.P., 2011. Colloidal complexes from associated water soluble cellulose derivative (methylcellulose) and green tea polyphenol (epigallocatechin gallate). J. Colloid Interface Sci. 364, 317–23. doi:10.1016/j.jcis.2011.08.054

Patra, S.K., Rizzi, F., Silva, A., Rugina, D.O., Bettuzzi, S., 2008. molecular targets of (–)-epigallocatechin-3-gallate (EGCG): specificity and interaction with membrane lipid rafts. J. Physiol. Pharmacol. 59, 217–235.

Pharmachem Laboratories, DSM, 2008. Safety Data Sheet Teavigo (TM).

Pianet, I., André, Y., Ducasse, M.-A., Tarascou, I., Lartigue, J.-C., Pinaud, N., Fouquet, E., Dufourc, E.J., Laguerre, M., 2008. Modeling procyanidin self-association processes and understanding their micellar organization: a study by diffusion NMR and molecular mechanics. Langmuir 24, 11027–35. doi:10.1021/la8015904

Poncetlegrand, C., Edelmann, a, Putaux, J., Cartalade, D., Sarnimanchado, P., Vernhet, a, 2006. Poly(l-proline) interactions with flavan-3-ols units: Influence of the molecular structure and the polyphenol/protein ratio. Food Hydrocoll. 20, 687–697. doi:10.1016/j.foodhyd.2005.06.009

Popa, M.-I., Aelenei, N., Popa, V.I., Andrei, D., 2000. Study of the interactions between polyphenolic compounds and chitosan. React. Funct. Polym. 45, 35–43. doi:10.1016/S1381-5148(00)00009-2

Price, W.E., Spitzer, J.C., 1994. The kinetics of extraction of individual flavanols and caffeine from a Japanese green tea (Sen Cha Uji Tsuyu) as a function of temperature. Food Chem. 50, 19–23. doi:10.1016/0308-8146(94)90086-8

Prime-Chapman, H.M., Fearn, R.A., Cooper, A.E., Moore, V., Hirst, B.H., 2004. Differential multidrug resistance-associated protein 1 through 6 Isoform expression and function in human intestinal epithelial Caco-2 cells. J. Pharmacol. Exp. Ther. 311, 476–484. doi:10.1124/jpet.104.068775.important

Proniuk, S., Liederer, B.M., Blanchard, J., 2002. Preformulation study of epigallocatechin gallate, a promising antioxidant for topical skin cancer prevention. J. Pharm. Sci. 91, 111–6.

Quideau, S., Deffieux, D., Douat-Casassus, C., Pouységu, L., 2011. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew. Chem. Int. Ed. Engl. 50, 586–621. doi:10.1002/anie.201000044

Rehmanji, M., Gopal, C., Mola, A., 2005. Beer stabilization technology — clearly a matter of choice. Master Brew. Assoc. Am. 42, 332–338.

Retsky, K.L., Frei, B., 1995. Vitamin C prevents metal ion-dependent initiation and propagation of lipid peroxidation in human low-density lipoprotein. Biochim. Biophys. Acta 1257, 279–87.

Rowe, R.C., Sheskey, P.J., Quinn, M.E., 2009. Handbook of Pharmaceutical Excipients, 6th ed. Pharmaceutical Press and American Pharmacists Association, London, Chicago.

Sang, S., Lee, M.-J., Hou, Z., Ho, C.-T., Yang, C.S., 2005. Stability of tea polyphenol (-)-epigallocatechin-3-gallate and formation of dimers and epimers under common experimental conditions. J. Agric. Food Chem. 53, 9478–84. doi:10.1021/jf0519055

Sang, S., Yang, I., Buckley, B., Ho, C.-T., Yang, C.S., 2007. Autoxidative quinone formation in vitro and metabolite formation in vivo from tea polyphenol (-)-epigallocatechin-3-gallate: studied by real-time mass spectrometry combined with tandem mass ion mapping. Free Radic. Biol. Med. 43, 362–71. doi:10.1016/j.freeradbiomed.2007.04.008

Scalbert, A., Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. J. Nutr. 2073–2085.

Schmidt, C., Kleinebudde, P., 1999. Influence of the granulation step on pellets prepared by extrusion / spheronization. Chem. Pharm. Bull. (Tokyo). 47, 405–412.

Schmidt, P. C., Herzog, R., 1993. Calcium phosphates in pharmaceutical tableting. 1. physico-pharmaceutical properties. Pharm World Sci. 15(3):105-15.

Schreier, S., Malheiros, S. V, de Paula, E., 2000. Surface active drugs: self-association and interaction with membranes and surfactants. Physicochemical and biological aspects. Biochim. Biophys. Acta 1508, 210–34.

Seeram, N.P., Henning, S.M., Niu, Y., Lee, R., Scheuller, H.S., Heber, D., 2006. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. J. Agric. Food Chem. 54, 1599–603. doi:10.1021/jf052857r

Severino, J.F., Goodman, B. a, Kay, C.W.M., Stolze, K., Tunega, D., Reichenauer, T.G., Pirker, K.F., 2009. Free radicals generated during oxidation of green tea polyphenols: electron paramagnetic resonance spectroscopy combined with density functional theory calculations. Free Radic. Biol. Med. 46, 1076–88. doi:10.1016/j.freeradbiomed.2009.01.004

Shah, U., Augsburger, L., 2002. Multiple sources of sodium starch glycolate, NF: evaluation of functional equivalence and development of standard performance tests. Pharm. Dev. Technol. 7, 345–59. doi:10.1081/PDT-120005731

Shephard, A.B., Nichols, S.C., Braithwaite, A., 1999. Moisture induced solid phase degradation of l-ascorbic acid part 2, separation and characterization of the major degradation products. Talanta 48, 595–606.

Shim, J.-H., Su, Z.-Y., Chae, J.-I., Kim, D.J., Zhu, F., Ma, W.-Y., Bode, A.M., Yang, C.S., Dong, Z., 2010. Epigallocatechin gallate suppresses lung cancer cell growth through Ras-GTPase-activating protein SH3 domain-binding protein 1. Cancer Prev. Res. 3, 670–9. doi:10.1158/1940-6207.CAPR-09-0185

Shpigelman, A., Israeli, G., Livney, Y.D., 2010. Thermally-induced protein-polyphenol co – assemblies : beta lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG. Food Hydrocoll. 24, 735–743. doi:10.1016/j.foodhyd.2010.03.015

Shutava, T.G., Balkundi, S.S., Vangala, P., Steffan, J.J., Bigelow, R.L., Cardelli, J. a, O'Neal, D.P., Lvov, Y.M., 2009. Layer-by-layer-coated gelatin nanoparticles as a vehicle for delivery of natural polyphenols. ACS Nano 3, 1877–85. doi:10.1021/nn900451a

Siddiqui, I. a, Adhami, V.M., Bharali, D.J., Hafeez, B.B., Asim, M., Khwaja, S.I., Ahmad, N., Cui, H., Mousa, S. a, Mukhtar, H., 2009. Introducing nanochemoprevention as a novel approach for cancer control: proof of principle

with green tea polyphenol epigallocatechin-3-gallate. Cancer Res. 69, 1712–6. doi:10.1158/0008-5472.CAN-08-3978

Siebert, K.J., 1999. Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. J. Agric. Food Chem. 47, 353–62.

Solik, A., Bodmeier, R., 2013. Coacervation study of (-)-epigallocatechin-3-gallate with common hard capsule materials and its effect on drug release. Annual Meeting of the American Association of Pharmaceutical Scientists, AAPS, San Antonio, USA

Stegemann, S., 2002. Hard gelatin capsules today – and tomorrow. Capsugel Libr. 2, 1–23.

Swezey, R.R., Aldridge, D.E., LeValley, S.E., Crowell, J. a., Hara, Y., Green, C.E., 2003. Absorption, tissue distribution and elimination of 4-[3 H]-epigallocatechin gallate in beagle dogs. Int. J. Toxicol. 22, 187–193. doi:10.1080/10915810305101

Tachibana, H., Fujimura, Y., Yamada, K., 2004. Tea polyphenol epigallocatechin-3-gallate associates with plasma membrane lipid rafts: lipid rafts mediate anti-allergic action of the catechin. Biofactors 21, 383–5.

Tamilvanan, S., 2008. New dosage forms: progress in the design of biodegradable polymer-based microspheres for parenteral controlled delivery of therapeutics, in: Cox Gad, S. (Ed.), Pharmaceutical Manufacturing Handbook: Production and Processes. John Wiley & Sons, Inc., New Jersey, pp. 393–442.

U.S. Department of Health and Human Services Food and Drug Administration, 1997. Guidance for industry dissolution testing of immediate release solid oral dosage forms.

U.S. Department of Health and Human Services Food and Drug Administration (CDER), 2013. Guidance for industry size , shape , and other physical attributes of generic tablets and capsules.

Ullmann, U., Haller, J., Decourt, J.D., Girault, J., Spitzer, V., Weber, P., 2004. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. Int. J. Vitam. Nutr. Res. 74, 269–278. doi:10.1024/0300-9831.74.4.269

Ullmann, U., Haller, J., Decourt, J.P., Girault, N., Girault, J., Richard-Caudron, a S., Pineau, B., Weber, P., 2003. A single ascending dose study of epigallocatechin gallate in healthy volunteers. J. Int. Med. Res. 31, 88–101.

Unachukwu, U.J., Ahmed, S., Kavalier, A., Lyles, J.T., Kennelly, E.J., 2010. White and green teas (Camellia sinensis var. sinensis): variation in phenolic,

methylxanthine, and antioxidant profiles. J. Food Sci. 75, C541–8. doi:10.1111/j.1750-3841.2010.01705.x

Van Oss, C.J., 1988. Coacervation, complex-coacervation and flocculation. J. Dispers. Sci. Technol. 9, 561–573. doi:10.1080/01932698808944011

Wang, H., Helliwell, K., 2000. Epimerisation of catechins in green tea infusions. Food Chem. 70, 337–344.

Wang, R., Zhou, W., Jiang, X., 2008. Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range. J. Agric. Food Chem. 56, 2694–701. doi:10.1021/jf0730338

Widlansky, M.E., Hamburg, N.M., Anter, E., Holbrook, M., 2007. Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. J. Am. Coll. Nutr. 26, 95–102.

Wróblewski, K., Muhandiram, R., Chakrabartty, A., Bennick, A., 2001. The molecular interaction of human salivary histatins with polyphenolic compounds. Eur. J. Biochem. 268, 4384–97.

Wu, Y., Lin, Q., Chen, Z., Xiao, H., 2011. The interaction between tea polyphenols and rice starch during gelatinization. Food Sci. Technol. Int. 17, 569–77. doi:10.1177/1082013211430294

Yamane, T., Takahashi, T., Kuwata, K., Oya, K., Inagake, M., Kitao, Y., Suganuma, M., Fujiki, H., 1995. Inhibition of N-methyl-N'-nitro-N-nitrosoguanidine-induced carcinogenesis by (-)-epigallocatechin gallate in the rat glandular stomach. Cancer Res. 55, 2081–4.

Yang, C.S., Lee, M., Chen, L., 1999. Human salivary tea catechin levels and catechin esterase activities : implication in human cancer prevention studies. Cancer Epidemiol. biomarkers Prev. 8, 83–89.

Yen, G.-C., Duh, P.-D., Tsai, H.-L., 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem. 79, 307–313. doi:10.1016/S0308-8146(02)00145-0

Yuan, J.-P., Chen, F., 1998. Degradation of ascorbic acid in aqueous solution. J. Agric. Food Chem. 46, 5078–5082. doi:10.1021/jf9805404

Zaveri, N.T., 2006. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci. 78, 2073–80. doi:10.1016/j.lfs.2005.12.006

Zimeri, J., Tong, C.H., 1999. Degradation kinetics of (-) -epigallocatechin gallate as a function of pH and dissolved oxygen in a liquid model system. J. Food Sci. 64, 753–758.

7. LIST OF PUBLICATIONS

1. Solik, A., Bodmeier, R., 2011. PH-dependent degradation of (-)-epigallo-catechin-3-gallate under physiological conditions of the gastro-intestinal tract and possible stabilization approaches. 4th World Congress on Tea and Health, Berlin, Germany.

2. Solik, A., Bodmeier, R., 2013. Coacervation study of (-)-epigallocatechin-3-gallate with common hard capsule materials and its effect on drug release. Annual Meeting of the American Association of Pharmaceutical Scientists, AAPS, San Antonio, USA.

8. CURRICULUM VITAE

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