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Critical Review

Going Natural: Using polymers from nature for gastroresistant applications

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ABSTRACT

Nutraceuticals provide an additional health or medicinal benefit besides their nutritional value and are therefore marketed for the prevention and treatment of certain conditions. Nutraceuticals contain natural ingredients, usually presented in the form of functional foods or as dietary supplements. Many of the ingredients are susceptible to degradation by gastric acid or can provoke nauseatic feelings or induce vomiting on oral administration. Gastroresistant coatings, widely researched and used in pharmaceuticals, employ enteric polymers which are not regarded as natural ingredients or do not possess GRAS (generally regarded as safe) status by the regulatory bodies, thus cannot be used for nutraceutical products. Consequently, most nutraceuticals are not formulated as gastroresistant and can therefore lack efficacy or are well tolerated. This manuscript provides a critical review of natural substances employed in producing gastroresistant products, their shortcomings, and potential industrial applications. It also identifies current gaps in our knowledge to encourage further research in this area.

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INTRODUCTION

In recent years, nutraceuticals have been widely adopted in the western world with a market value of over US\$50bn in the US and US\$30bn in Europe in 2010 (Frost and Sullivan, 2010). Innovation and new product development have been the focus of development activities with reported increases in research and development expenditure over recent years increasing the global nutraceuticals market value to US\$165.62bn in 2014, with a predicted value of US\$278.96bn by the end of 2021 (TMP 2015).

Products such as dietary supplements, functional foods or herbal products were given the term, nutraceuticals (from the combination of “nutrition” and “pharmaceuticals”), in 1989 (Kalra, 2003). They are reported to provide numerous medical or health

benefits and their therapeutic value has been documented as anti-fatigue, natural antioxidants and anti-inflammatory agents (Kuppusamy et al., 2014; Zaki, 2014). More significantly, it is claimed they have roles in the prevention or delay of several diseases such as arthritis (Akhtar and Haqqi, 2012), cancer (Kuppusamy et al., 2014; Salami et al., 2013; Wargovich et al., 2010), metabolic (Davi et al., 2010) and cardiovascular diseases (Garcia-Rios et al., 2013; Ramaa et al., 2006; Zuchi et al., 2010), neurodegenerative diseases (Mecocci et al., 2014; Rigacci and Stefani, 2015) and even osteoporosis (Nieves, 2013). However, despite these claims, no clinical evidence has been published so far regarding these products. The desired effects of these substances can only be achieved when the substance is released intact at the correct place in the

gastrointestinal (GI) tract. For example, some of these substances are susceptible to degradation by gastric acid or can provoke nauseatic feelings or induce vomiting on oral administration. Particularly, some probiotic-containing formulations may even need to pass through the small intestine to the colon in order to exert their effects. Therefore, in order to achieve its goal, the formulation has to be stable during residence within the challenging environment in the stomach.

The design of these formulations can be very diverse and a plethora of materials, both natural and synthetic, are already described (Agyilira and Banker, 1991; Hussan et al., 2012; Kendall and Basit, 2006; Rajpurohit et al., 2010). The first attempt to create an enteric formulation, exploiting the insolubility of materials in the stomach, is attributed to a German physician who reported clinical case studies using keratin-coated carbolic acid pills, in 1884 (Unna, 1884). A range of different approaches has been designed ever since in order to provide gastric protection to acid-labile substances, from tablets with enteric coatings, to emulsions intended to be added to food or beverages (Chen et al., 2014; Ron et al., 2010; Sansone et al., 2011; Semo et al., 2007; Shpigelman et al., 2014). The mechanisms behind many of these approaches exploit the significant pH changes across the GI tract, from very acidic in stomach (pH 1-2) to less acidic in the duodenum (pH 6-7) (Ibekwe et al., 2008).

Thus, formulations can be designed to respond to changes in the pH and disintegrate at the desired location. Polymeric coatings with carboxylic moieties are usually used to achieve this purpose. When in contact with gastrointestinal milieu at a pH higher than the polymer's pKa, the ionised form of the polymer is predominant, thus increasing the solubility of the polymer, leading to the disintegration of the coating system and drug release.

Simpler formulations have also been designed to delay the release of the active substance in a pH independent manner so that it is contained within the delivery system until gastric emptying, or even until almost complete passage through the small intestine (Tubic-Grozdanis et al., 2008). Although the pH in the stomach may vary depending on prandial state,

intra-subject and inter-subject variability thus leading to inconsistency in delivery (Fallingborg et al., 1989; McConnell et al., 2008), the delayed-release approach is similarly subject to variation. This is due to the fact that stomach residence time also fluctuates greatly, (from 30 min to 5 h) (McConnell et al., 2008), whereas water empties from the fasted stomach in times as short as 10-15 min (Ziessman et al., 2009). In some conditions, gastric time can be even more delayed (up to 10h post-dosing (Davis et al., 1984)), particularly in the case of single-unit dosage forms in the event of the dosage form having not emptied the stomach prior to the subsequent ingestion of a meal. In the fasted state, gastric motility is under the control of the migrating myoelectric complex (MMC), which helps to empty larger objects from stomach. However, the MMC is disrupted in the presence of food, contributing to longer gastric retention of modified release single-unit dosage forms in particular (Varum et al., 2010). Moreover, and even though the small intestine transit times are regularly reported as constant at 3-4 h (Davis et al., 1986), some studies have reported significant variability amongst small intestine transit times (Fallingborg et al., 1989; McConnell et al., 2008). These variations in transit times may cause the formulation to pass through the GI tract without completely releasing the active substance or, by staying in the stomach for a prolonged time, even prematurely releasing it in the stomach.

A range of polymeric materials has been successfully used to prepare gastroresistant pharmaceutical dosage forms, with poly(meth)acrylates, (copolymer of methacrylic acid and either methyl-methacrylate or ethyl acrylate), cellulose-based materials and polyvinyl derivatives being most common (Cole et al., 2002). However, these polymers are of synthetic or semi-synthetic origin and cannot be used routinely in delivering nutritional substances needing to bypass the stomach. Hence, there is a need for natural polymers offering sufficient enteric protection for the nutraceutical industry. Just like other natural substances, these polymers are also subject to batch-to-batch variability and to the difficulty of eliminating or reducing contaminants, which may have consequences in the performance of dosage forms. Despite these issues, natural polymers are needed to address this gap in nutraceutical market

until a desired synthetic coating is approved for nutraceutical use globally.

The list of natural materials that are already listed as “generally regarded as safe” (GRAS) is long, and can be readily exploited for this application. There is a significant amount of research in formulating novel solutions for gastroresistant nutraceutical products, so-called *traditional* formulations, using natural polymers. This manuscript provides a critical review of the published literature in this area, highlighting shortcomings, and potential industrial applications. It also identifies current gaps in our knowledge to encourage further research in this area.

CELLULOSE-BASED MATERIALS

Cellulose is a polysaccharide consisting of a linear chain of glucose (Fig. 1) and is a constituent of most plant cell walls, some algae and is excreted by some bacteria, making it the most abundant biopolymer in nature (Klemm et al., 2005).

Cellulose has been used since the 19th century in industrial applications such as leather, paints, plastics, fibres and films (Kamide, 2005). Its biocompatibility, biodegradability and low toxicity ensured its generalised use. However, due to its insolubility in water, several cellulose derivatives were produced, mainly ester and ether derivatives. Most of these modifications afford cellulose a greater solubility in water, along with different physicochemical and mechanical properties (Shokri and Adibki, 2013).

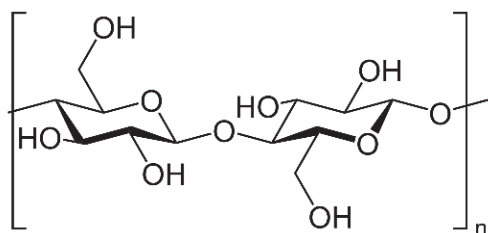


Fig. 1. Chemical structure of cellulose

In pharmaceutical industries, cellulose derivatives have been applied in very diverse fields such as: bioadhesive and mucoadhesive drug delivery systems, general coating processes, extended release dosage forms, osmotic drug delivery systems, enteric coated solid dosage forms, etc (Shokri and Adibki, 2013). Cellulose derivatives are also used as enteric coatings in pharmaceutical applications, being

resistant to acidic pH while becoming soluble in less acidic conditions. These derivatives include cellulose acetate phthalate (CAP), hydroxypropylmethyl cellulose phthalate (HPMCP) or hydroxypropyl methyl cellulose acetate succinate (HPMC-AS) (Shokri and Adibki, 2013). These polymers are however not considered ‘generally recognized as safe’ (GRAS) substances, due to their chemical modifications and to the lack of studies regarding possible side effects. For this reason their use for nutraceuticals is not allowed, as they may not be considered as food grade products.

A commercial HPMC (hydroxypropyl methyl cellulose) capsule, co-formulated with gellan gum, has been recently marketed under the brand name DRCaps™, which claims to provide protection to acid-sensitive ingredients by a delaying-release mechanism (Marzorati et al., 2015). The combination of HPMC and the gum forms a gel on contact with gastric fluids, which delays the capsule disintegration in anticipation of a delayed release until capsule is emptied in small intestine. This approach is however, prone to inter- and intra-subject variability in gastric retention time, as mentioned earlier, hence significantly affecting its gastroresistant functionality.

Due to its good film-forming properties and polymer-to-polymer adhesion, HPMC is sometimes used with other enteric materials in order to improve the plasticity of the main polymer or as a pre-coating (Cole et al., 2002). Additionally, a cellulose-based enteric coating, Nutrateric®, was developed by Colorcon® to be used for nutraceutical products. Nutrateric® is an ethylcellulose coating, containing sodium alginate as a pH-dependent pore former (Colorcon®, 2015). When using coating levels equal to, or higher than, 6.7 mg/cm², tablets containing aspirin or caffeine remained intact for 2 h in 0.1M HCl, while disintegrating quickly (2-9 min) in 0.05M pH 6.8 phosphate buffer (Young et al., 2006). Sodium alginate dissolves at intestinal pH, hence forming pores in the ethylcellulose films, enabling pH dependent release. However, in another study (Merchant et al., 2009) the robustness of Nutrateric®-coated tablets was investigated. The Nutrateric® -coated tablets were found intact in pH 1.2 HCl after two hours, however swelling was noticed at pH 2.0. Complete disintegration of the coated tablets was

observed at pH 2.5 and 80% drug release was observed within 75 min in this medium. A further study by Czarnocka and Alhnan (2015) also confirmed the inability of Nutrateric[®] to withstand gastric conditions at pH 2.0 or higher. The basal gastric pH, measured in 252 healthy men and 113 healthy women under fasted conditions, has been reported as pH 2.16 ± 0.09 (males) and 2.79 ± 0.18 (females) (Feldman and Barnett, 1991). This indicates that Nutrateric[®] may result in premature drug release in the stomach. Although the coating was intact at pH ≤ 2.0 , when tablets were transferred to pH 6.8 USP phosphate buffer, the drug release was very slow, with a lag time of 70 min (6% coating weight gain). This is in contrast to the <10 minutes disintegration observed by Young et al. (2006) in 0.05M pH 6.8 phosphate buffer.

The use of compendial phosphate buffer in dissolution testing as surrogate for the GI fluids is a dramatic oversimplification. The ionic composition and the buffer capacity of phosphate-based media are not close to the components in the intestinal milieu (Liu et al., 2011). For example, jejunal fluid contains mainly bicarbonate, chloride and calcium ions. However, none of these ions are present in phosphate buffers. Some phosphate buffered saline solutions contain additional ions, however their concentration is not comparable to those *in vivo*. Due to the similarity in ionic strength and the buffer capacity, *m*Hanks buffer (predominantly buffered by bicarbonate ions) provides a much closer surrogate to jejunal fluids. Therefore, bicarbonate buffers offer a more suitable option to simulate intestinal conditions for *in-vitro* release assays and dissolution tests. In particular, a study by Liu et al. (2011) highlighted differences in the release behaviour from these coated formulations when bicarbonate buffer was used to simulate the intestinal phase, instead of conventional phosphate buffer, suggesting the importance of physiological resemblance of the test medium to the GI fluids. This is further supported by evidence from other studies (Goyanes et al., 2015; Merchant et al., 2014; Varum et al., 2014).

Apart from ionic strength, buffering species and strength, other components of the gastrointestinal milieu have also proven to be important, leading to the development and constant improvement of fasted/fed state simulated gastric and intestinal

fluids (Dressman et al., 1998; Jantratid et al., 2008; Khoshakhlagh et al., 2015; Klein, 2010; Soderlind et al., 2010; Vertzoni et al., 2005; Vertzoni et al., 2004). Soderlind et al. (2010) studied the solubility of a range of neutral compounds using fasted state simulated intestinal fluid (FaSSIF), FaSSIF-V2, pH 6.5 phosphate buffer and human intestinal fluids and highlighted interesting differences in the solubility profiles, further emphasising the importance of physiological relevance of the media in dissolution testing.

Table 1 provides a summary of natural-based polymeric formulations already commercialised or with potential use for gastroresistant applications.

STARCH

Starch is a carbohydrate composed of glucose units bonded by glycosidic bonds to form amylose and amylopectin (Fig. 2.) and is present in most plants as a source of energy. It is abundant in the human diet and it can be found in potatoes, wheat, corn and rice. Due to this, the investigation of starch and its role as an excipient has been popular, considering it is degradable and GRAS.

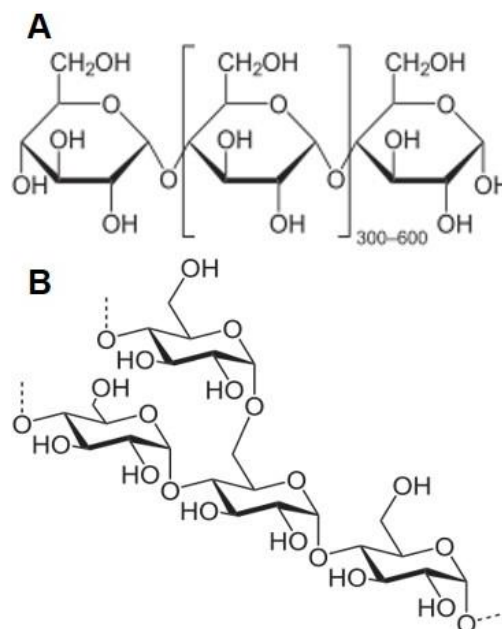


Fig. 2. Chemical structure of amylose (A) with $\alpha(1,4)$ glycosidic bonds and amylopectin (B) with $\alpha(1,4)$ and $\alpha(1,6)$ glycosidic bonds, both constituents of starch.

Similar to other natural polymers, starch was initially explored as a film-forming agent for food packaging (Arvanitoyannis et al., 1998). Later, starch capsules

Table 1. Summary of gastroresistant formulations for nutraceutical applications.

Material	Product and formulation	Description	Reference
1. CELLULOSE			
Hydroxypropyl methylcellulose (HPMC)+ gellan gum	DRCaps™* (Capsules)	Claimed to provide protection for acid sensitive ingredients using a delayed-release mechanism. Prone to significant inter- and intra-subject variability in gastric emptying affecting its effectiveness.	Marzorati et al. (2015)
Ethylcellulose (EC) + sodium alginate (SA)	Nutrateric®* coated tablets	Comprises EC coating containing SA, which dissolves at intestinal pH and forms pores in the EC coatings, enabling drug release. Although coated tablets remain intact in 0.1M HCl (pH≤1.2) for 2 h, coatings are not robust to resist gastric conditions pH >2.0, possibly leading to premature drug release in the stomach.	Czarnocka and Alhnan (2015), Merchant et al. (2009), Young et al. (2006)
2. STARCH			
Maize starch	EUDRAGUARD®* Natural coated tablets or pellets	Based on maize starch and claimed to provide taste-masking properties and acid-resistance. However, no further information is available on coating composition and on mechanisms of gastroresistance achieved using maize starch. It is not clear if this is also a delayed release approach, as in DRCaps™, or is a pH sensitive coating.	Evonik (2015), Kuntz (2016)
High amylose corn starch (HACS)	Coated glass beads	HACS is highly resilient to both gastric (0.1M, pH1.6, 2h) and neutral (pH 7.0. 0.1M phosphate buffer, 3h) conditions. However, it is shown to dissolve in a medium containing pancreatic amylases.	Dimantov et al. (2004)
3. SHELLAC			
Shellac succinate	Cast films	Chemically modified shellac, where esterification with succinic anhydride and manipulation of annealing time allows the tailoring of the polymer's dissolution pH. However, due to chemical modification, it potentially loses its GRAS status.	Limmatvapirat et al. (2008)
Shellac + inulin	Coated tablets	Coating resisted 0.1M HCl for 2 h, yet drug release was initiated when in phosphate buffer pH 7.4. Shellac provides the enteric resistance, while inulin purports to retard the drug release until the formulation reaches the colon.	Ravi et al. (2008)
Shellac + sodium alginate	Protect™ Enteric* coated tablets	Claimed to remain intact in 0.1M HCl for 2h, while disintegrating at pH 6.8 (phosphate buffer). However, in a recent study by Czarnocka and Alhnan (2015), a slower release rate was observed after the acid stage when transferred to pH 6.8 phosphate buffer (<50% release in 4h) than in pH 7.4 phosphate buffer (80% release in 2h).	Fraser and Young (2010), Czarnocka and Alhnan (2015)
4. ZEIN			
Zein + PEG-400 or glycerol	Coated tablets	While both organic and aqueous solutions resisted 2h at pH=1.2 for 2h, different lag times for drug release were observed when tested in pH 6.8 phosphate buffer. PEG-400 and glycerol were shown to influence the drug release, with PEG 400 formulation having a lower water uptake than glycerol, thus causing a more delayed onset of release.	Li et al. (2010)
Carboxymethyl zein	Tablet matrix	Authors claim that with carboxymethyl modification, zein becomes soluble at pH 4.5, thus, dissolving at the pH of the small intestine, yet being resistant to fasted gastric pH. However, due to chemical modification it potentially loses its GRAS status.	Yin et al. (2015)

Table 1 (continued). Summary of natural-based polymeric formulations for gastroresistant applications.

5. ALGINATE			
Sodium alginate + pectin	Probiotic pearls™* (coated pellets)	Triple-layered beads containing a core of probiotics in an oil suspension. It is claimed that the outer layer of sodium alginate and pectin offers the gastric protection, creating a gel layer under acidic conditions for 2 h, which is then expected to dissolve in the distal gut, hence releasing the probiotics. Due to the delayed release in intestine, it is not suitable for conventional gastroresistant applications however, it may be suitable for colon targeting.	Nature's Way Products (2011 a,b)
Sodium alginate + chitosan	Microparticles	Probiotic containing microcapsules claimed to resist acidic conditions from pH 1.0 to 4.0 (HCl with 0.85% NaCl) for up to 8 h and subsequently released probiotic when tested in intestinal buffer containing 0.3% bile salts. It was reported that the probiotic viability was maintained throughout the test conditions.	Wu et al. (2016)
6. OTHERS			
Soy hydrogel	Tablet matrix	Tablets composed of lyophilised soy hydrogel, containing riboflavin, were shown to resist 0.1M HCl (pH 1.2) for 30 min. When in pH 7.5 phosphate buffer, 50% of the riboflavin was released in 2h with complete release in 5h. Shorter testing duration in acidic conditions and slower and delayed release in the small intestine could be a concern.	Maltais et al. (2010)
Whey protein + gum arabic	Coated microcapsules	Microcapsules containing hydrolase enzyme as a nutraceutical agent, resisted simulated gastric conditions (both in fed and fasted state, pH 4.5 and 2.0, respectively) for 15 min. The enzyme was then shown to be fully active when at pH 7.0 (phosphate buffer). Nevertheless, the reduced testing duration (only 15 min) in the gastric conditions is a serious limitation of this study, and it is not known if the encapsulated enzyme will remain stable under acidic conditions for longer than 15 min.	Lambert et al. (2008)
Marine sponge collagen	Spongicol®* (coated tablets)	Tablets coated with 12.9mg/cm ² complied with the Ph. Eur. specifications for delayed-release tablets with no drug release for 2h in 0.1M HCl, yet disintegrating within 10min in pH 6.8 phosphate buffer. This system is currently used in a nutraceutical formulation for ulcerative colitis.	KliniPharm (2015)

* commercialised products for nutraceutical use.

were developed and studied for their physical characteristics and their enteric targeting ability (Vilivalam et al., 2000). These capsules had similar characteristics to traditional gelatine capsules, with notable advantages in some cases. In terms of gastrointestinal drug delivery, starch has been mainly explored for colonic targeting (Kotla et al., 2014; Norman, 2011). High-amylose starch is highly resilient to gastric conditions (pH 1.6, 0.1M HCl, 2 h) and also at neutral pH (pH 7.0, phosphate buffer 0.1 M, 3 h), however when pancreatic enzymes are present, amylose is enzymatically digested, causing the disintegration of the dosage form (Dimantov et al., 2004). Carboxymethyl high amylose starch (CM-HAS) has been used to produce oral vaccines for the delivery of F4 *fimbriae* to the colon and also as a complex with lecithin for a mesalazine-based colonic delivery system (Mihaela Friciu et al., 2013).

Recently, a starch-based aqueous coating has been commercialized by Evonik Industries under the brand name of Eudraguard® Natural (Evonik, 2015). This coating is a GRAS substance and has been licensed to be used for nutraceutical formulations in the United States and in Europe (Hauschildt, 2016). It is claimed that this coating provides taste-masking and acid-resistant properties (Kuntz, 2016), however some information such as the type of starch and other composition of the coating system is not disclosed.

SHELLAC

Shellac is a resin secreted by the lac bug (*Kerria lacca*) and has been used as a protective layer for wood, leather or paper, insulating material (Azouka et al., 1993), as a colorant, food glaze or acting as a sanding sealant, odour-blocker or as a high-gloss varnish (Baldwin et al., 1994).

Besides these, shellac was also used in the pharmaceutical industry as a sealant, glaze and in particular, in sugar coating. Due to its acidic properties, shellac also offers applications in developing gastroresistant products. There have been several reports describing various preparations of shellac-based films for enteric coatings however, due to several reported disadvantages, shellac has decreased in popularity as a material for acid protection. Problems such as its tackiness (Goorley and Lee, 1938) or changes in the dissolution profile of shellac-coated formulations caused by ageing (crosslinking and esterification) caused the decline of this material for enteric coating purposes (Agyilirah and Banker, 1991). Moreover, despite reports suggesting shellac's resistance to the stomach's acidic conditions, problems were reported when shellac was subject to the weakly-acidic conditions found during the ingestion of food. These caused the coating to dissolve prematurely, hence the loss of gastroresistance. Interest in shellac has resurfaced recently, with research being carried out regarding modifications of shellac to improve its quality as a gastroresistant material. Limmatv-apatir et al. (2008) developed a modified form of shellac (shellac succinate) in an attempt to improve its solubility in the small intestine. The unmodified shellac presented good gastric resistance in the fasted state, however it is not readily soluble at the small intestinal pH (Limmatvapirat et al., 2008; Ravi et al., 2008). The authors suggest that esterification with succinic anhydride, and the change in annealing time, made it possible to control the pH at which the polymer dissolves. Different annealing times influence the acid value of shellac (measure of free acid content), thus modifying the number of available carboxylic acids and consequently altering its solubility to occur at lower pH values. Moreover, Signorino and colleagues (Signorino, 2003; Signorino et al., 2010) found that formulations containing up to 60% of shellac would allow the tailoring of the pH at which the coating dissolves (ranging from 6.8 to above 7.4). This was achieved using different grades and types of shellac, bearing different acid numbers, and therefore controlling the release of the active ingredient. Also, Ravi et al. (2008) developed an enteric coating using shellac, and showed that the coating integrity was maintained when using 0.1M HCl for 2 h, yet drug release was initiated when the

medium was changed to phosphate buffer pH 7.4. This particular formulation was designed with two different coatings, a shellac outer coating and an inulin-based inner coating. The shellac coating provides the ability to withstand the acidity of the stomach, whereas the inulin coating is present to retard drug release until it reaches the colon. These results may, however, not reflect the biological pH changes across the gastrointestinal tract. The authors reported that phosphate buffer (pH 7.4) was used to mimic conditions in the small intestine. Firstly, the USP test to mimic small intestinal conditions refers to pH 6.8, not 7.4. Moreover, as shown by Liu et al. (2011), the use of phosphate buffer as a release medium is not the most representative procedure to assess the release of enteric coated solid dosage forms, with bicarbonate buffer yielding more realistic results.

Recently, Encap Drug Delivery, a division of Capsugel®, has developed a commercial shellac-based coating formulation, described as containing GRAS materials and suitable for the delivery of pharmaceuticals and nutraceuticals (Fraser and Young, 2010; Young, 2006). This formulation has been shown to be resistant to the acidic pH of the stomach (0.1M HCl, 2h), using different coating thicknesses, yet disintegrating when in more neutral pH values (pH 6.8, phosphate buffer).

Moreover, a combination of shellac and alginate was recently commercialised by Sensient Pharmaceuticals under the name of Protect™ Enteric (Sensient®, 2014). Czarnocka and Alhnan (2015) compared its performance with Nutratric® (ethylcellulose films with alginate as pore-former) using theophylline tablets. Nutratric® (6.5% and 7% weight gain) and Protect™ Enteric (2.75% and 3% weight gain) resisted the acid stage (0.1 M HCl for 2 h). However, the shellac-containing product had a slower release rate in pH 6.8 phosphate buffer (less than 50% in 4 h) when using 2.75 and 3% coating level, whereas formulations coated with Nutratric® released 80% of the drug in 65 or 90 min for 6.5% and 7% coating weight gains respectively. Moreover, when tested in phosphate buffer pH 7.4, Protect™ Enteric formulations had a faster drug release, reaching 80% of the release in 2 h. This may indicate a possible use of this coating system for colonic delivery. The authors also studied the resistance of

the enteric coating at elevated stomach pH (pH 2.0, 3.0 and 4.0), and where Nutratric® failed, with dissolution times of 70 min (pH 2.0), 30 min (pH 3.0) and 55 min (pH 4.0), Protect™ Enteric managed to fully retard drug release at all the pH values tested. Additionally, pH 7.4 Krebs bicarbonate buffer was used as a release medium. Nutratric® formulations demonstrated similar release profiles in bicarbonate buffer (at pH 7.4) to those obtained in phosphate buffer (at pH 6.8), however, Protect™ Enteric formulations experienced a significant decrease in the release rate, exhibiting only < 20% drug release after 4 h in bicarbonate buffer. Although the authors do not present an explanation for these differences, this can be attributed to the polymers used in both drug delivery systems. Even though both systems have embedded alginates to act as pore-formers in response to pH changes, drug release from Protect films is mediated by the ionisation of both shellac and alginate, whereas in Nutratric there is reliance on an alginate-only based pore-forming mechanism. It has previously been reported that drug release from ionisable polymeric films in phosphate buffer is faster than physiological bicarbonate buffers mainly due to the differences in ionic strength and buffer capacity (Fadda et al., 2009; Liu et al., 2011). Therefore, it is reasonable to expect a higher rate of release from Protect formulations when tested in phosphate buffer than in bicarbonate, presuming most of the drug release in phosphate buffer is attributed to the ionisation of shellac whereas drug release in bicarbonate may be attributed to its pore-forming effect. This study also reiterates the importance of the physiological relevance of the release medium.

As discussed previously, and as shown by Liu et al. (2011) and Czarnocka and Alhnan (2015), phosphate buffer may not indeed be the most suitable medium to mimic intestinal conditions, often generating misleading results. Although the results by Limmatvapirat et al. (2008), Fraser and Young (2010), Ravi et al. (2008) and Young (2006) using shellac as a possible enteric coating are promising, these may be different when a different media simulating intestinal conditions is used. Studies using bicarbonate buffer or other simulated intestinal fluids would thus provide a more comprehensive drug release profile.

ZEIN

Zein belongs to the family of alcohol-soluble proteins extracted from corn. Based on solubility and amino acid sequence, zein can be divided into four classes: α -zein (19 and 22 kDa), β -zein (14 kDa), γ -zein (16 and 27 kDa) and δ -zein (10 kDa) (Esen, 1987; Thompson and Larkins, 1989). Fig. 3 shows the amino acid sequence of α -zein (22kDa), with the nine repeat sequences aligned to show the greatest number of identities between sequences. Each repeat sequence contains a glutamine (Glu - Q) rich end, represented by the black line connecting each α -helix. This structural arrangement creates rectangular "tri-blocks", with non-polar amino acids in the hydrophobic core and Glu-rich loops forming two hydrophilic edges (Auke de et al., 2014). Owing to its unique amino-acid composition, zein has a distinctive solubility profile, with its primary solvents being glycols, glycol-ethers, amino-alcohols, nitro-alcohols acids, amides, and amines (Lawton, 2002). In terms of binary solutions, zein is soluble in water combined with a lower aliphatic alcohol (methanol, ethanol, isopropanol or butanol), acetone or dioxane. Moreover, a study by Li and colleagues described the mechanism of dissolution of α -zein in aqueous ethanol and acetic acid (Li et al., 2012) and revealed that acetic acid is a better solvent for zein than aqueous ethanol. Zein is widely used in the food industry as a coating for candies, nuts and fruits. Being labelled in the US as "confectioner's glaze", it is found as an ingredient of various food products, such as the caramel sweets, "sugar babies". Zein has also been used to form edible films exhibiting antioxidant, antimicrobial or simple barrier properties for food packaging (Cheng et al., 2015; Escamilla-García et al., 2013; Gezer et al., 2015; Güçbilmez et al., 2007; Liang et al., 2015; Ünalán et al., 2013).

The first reported use of zein as a coating material for tablets dates back to 1956 by Winters and Deardorff. They demonstrated that zein could be used as a coating to improve resistance against abrasion, humidity and heat compared to sugar-coated tablets. Moreover, zein has also been used as a coating to produce modified release formulations. For example, using a dry-coating technique, Guo and Shi (2009) prepared sustained-release tablets coated with zein

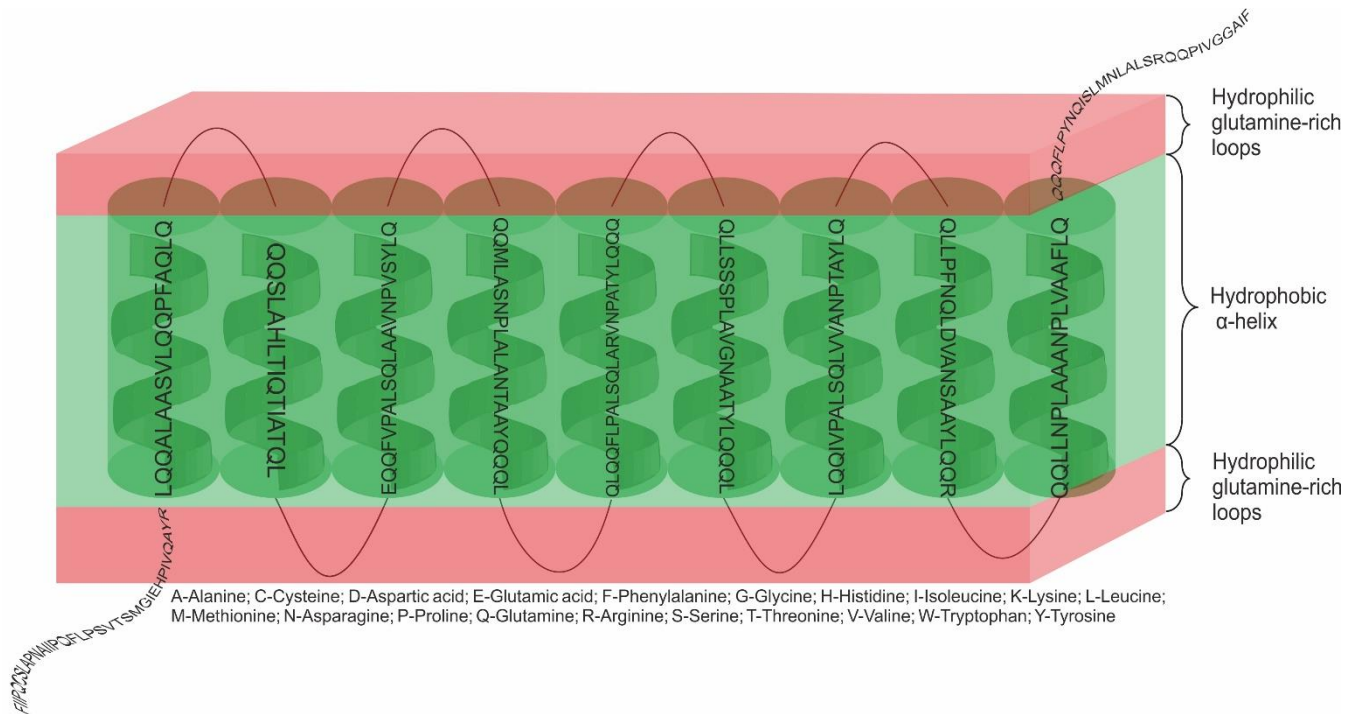


Fig. 3. Structural representation of α -zein (22kDa). Amino acid sequence, with the nine repeat sequences forming α -helixes linked by Glutamine (Q) rich loops, creating rectangular "tri-blocks", with non-polar amino acids in the hydrophobic core and Q-rich loops forming two hydrophilic edges. Adapted from (Argos et al., 1982; Auke de et al., 2014).

and microcrystalline cellulose (MCC) or starch. Further work on zein films by the same group evaluated the effects of plasticisers, pH and electrolytes on film formation and physical stability (Guo et al., 2008). More recently, zein was used to coat selenite-loaded chitosan nanoparticles (Luo et al., 2010).

Zein has also been a candidate of interest for enteric coatings, due to its poor solubility at low pH, and good solubility in intestinal conditions. Reports concerning the application of zein in enteric coatings are somewhat contradictory (Li et al., 2010; O'Donnell et al., 1997).

An early report of zein for enteric applications from Kanig and Goodman (1962) reported that zein films, containing oleic acid as a plasticiser, were insoluble in USP simulated gastric media (pH 1.2) for 2 hours, but completely dissolved in intestinal conditions (pH 6.8 phosphate buffer USP) within a few seconds. A different formulation containing zein was developed by O'Donnell et al. (1997). The paracetamol tablets were coated with zein dispersions containing propylene glycol as a plasticiser and methyl or propyl parabens as preservatives. Compared to the zein films reported by Kanig and Goodman (1962), formulations prepared by O'Donnell et al., had a

higher (~2x) water content. A faster release rate of paracetamol was observed from these coated formulations in acidic medium than in the intestinal buffer. It is not clear as to why the drug release in acidic medium was faster, however the authors concluded that this was due to the zein coating applied to the tablets. Li et al. (2010) further studied organic solutions and aqueous dispersions of zein for coating purposes and also investigated the influence of plasticisers (PEG400 and glycerol). The formulations were tested in acidic conditions (0.1M HCl for 2 hours) followed by pH 6.8 phosphate buffer. All formulations have shown to resist the acidic conditions for the tested time. However, when these were transferred to pH 6.8 phosphate buffer, the lag time for aqueous formulations was significantly higher than the organic formulations. 80% drug release was noted at 12h and 30h hours for aqueous formulation with glycerol and PEG400 respectively. Apart from the difference between organic and aqueous formulations, this study also showed that the choice of plasticiser greatly influences drug release from these dosage forms. Even though the preparation method for the aqueous formulations was similar to that of O'Donnell et al. (1997), the results are contradictory. A reason for these may be pH-dependant solubility of the drug

but also to the fact that zein can be supplied with different grades, with the possibility of significant variability existing between suppliers, causing variations in the obtained results. Nevertheless, the results by Li et al. (2010) are in accordance with the results by Kanig and Goodman (1962).

Stability of aqueous dispersions of zein at different pH values was studied for 30 days by Guo et al. (2008). Dispersions in which pH was adjusted to between 2.5 and 4.5 were shown to be more stable. Interestingly, phase separation was observed when the dispersion pH was adjusted above 4.5, which was attributed to the reduction in absolute zeta potential of the dispersion, causing particles to not repel each other, leading to agglomeration. This may also suggest that at pH 2.5-4.5, zein was ionised to some extent, which kept the dispersion stable under those conditions. Recently, Yin et al. (2015) proposed a modification of zein by carboxymethylation using sodium monochloro-acetate. With this modification, the authors suggest that the pH at which the polymer becomes soluble is changed from pH ~9 to around 4.5, hence being resistant to fasted gastric pH, yet soluble at the pH of small intestine, and therefore producing a promising candidate for gastroresistant applications.

CASEIN

Milk proteins are a heterogeneous mixture of two main groups, casein and whey proteins (El-Zahar et al., 2004). This section focuses on the properties of casein, with whey proteins being explored in the following section in detail. Mainly four casein phosphoproteins exist in cow milk, α (S1 and S2)-, β - and κ -casein, in an approximate proportion of 4:1:4:1 respectively, with a molecular weight of 19 to 25 kDa and an isoelectric point between 4.6 and 4.8 (Elzoghby et al., 2011). Casein has been widely explored for a range of pharmaceutical applications, for example in producing hydrogels for drug delivery (Li et al., 2014; Song et al., 2010; Song et al., 2009; Zhang et al., 2015), floating devices for gastroretentive formulations (Bulgarelli et al., 2000; Elzoghby et al., 2015; Mishra et al., 2008) and drug-casein compacts to improve drug solubility (Gubbins et al., 2003; Millar and Corrigan, 1991, 1993). Moreover, several recent studies have reported casein-based nano-vehicles for oral delivery of folic

acid (Penalva et al., 2015), anticancer agents (Shapira et al., 2010a; Shapira et al., 2012; Shapira et al., 2010b) and other compounds (Bachar et al., 2012; Esmaili et al., 2011; Luo et al., 2015).

Additionally, this material was proposed as a potential coating agent for solid dosage forms. Abu Diak et al. (2007) studied the influence of different plasticisers on the formation of casein films as well as the influence of different coating levels (5-15% weight gain) and different heat treatment temperatures (60-100°C) for 24 h. The coated tablets that had greater resistance to gastric conditions (15% coating level, 100°C heat treatment and 20% oleic acid as plasticiser), released only 2.6% of the drug (diltiazem HCl) after 2 h in 0.1 N HCl (pH 1.2), however, after 2 h in phosphate buffer pH 6.8, only approximately 20% of the drug was released. Alternatively, when decreasing the heat treatment temperature to 60°C, the release in acidic conditions slightly increased to 9.4% however after 2 h in nearly neutral conditions, the release increased to around 80%. A high percentage of coating (15% weight gain) and plasticiser (20% oleic acid) provided more effective gastric protection. The curing temperature clearly influences the resistance of the coating to both acidic and alkaline conditions. The temperature range tested and the long curing time (24 h) may however pose as a challenge when trying to employ this material with particularly heat labile drugs.

More recently, Huang et al. (2015) designed a casein-coated drug delivery system composed of doxorubicin-loaded iron oxide nanoparticles, pre-coated with poly(maleic acid), which were then over-coated with casein. The coating with casein provided an additional barrier, decreasing the amount of drug released in simulated gastric fluids (pH 2.0 containing 1.0 mg/mL pepsin, 2h) from 70% to 40%. Also, when the system was tested at different pH values, the drug release decreased with increasing pH. However, when trypsin was added to the release medium, the released rate increased. This confirms the importance of using release media, not only with the right pH, but also containing relevant enzymes in the gastric and intestinal phases for particular applications.

WHEY PROTEIN

As mentioned in previous section, whey proteins are one of the main groups of proteins present in milk, along with casein. Whey proteins in milk are mostly β -lactoglobulin, α -lactalbumin and serum albumin (El-Zahar et al., 2004). Bovine β -lactoglobulin is highly stable at acidic pH, resisting denaturation at pH values as low as 1, but is easily denatured at alkaline pHs (above pH 9) (Taulier and Chalikian, 2001). Studies by Fu et al. (2002) and Takagi et al. (2003) demonstrated that native β -lactoglobulin is almost entirely resistant to pepsin degradation at low pH and also that the protein remained intact in simulated gastric fluids (3.2 mg/mL pepsin in 0.03 M NaCl, pH adjusted to 1.2 with HCl). β -lactoglobulin represents around 60% of the whey proteins in bovine milk (Nicolai et al., 2011), and consequently is also present in high amounts in whey protein isolate (WPI). Thus, WPI could be an interesting material to develop gastroresistant formulations. Lambert et al. (2008) used whey protein together with gum arabic to produce microcapsules containing the model enzyme bile salt hydrolase for targeted delivery to the small intestine. Their study found that these microencapsulates resist simulated gastric conditions (fed state: pH 4.5, 5 mM acetate buffer, ionic strength 70 mM, 15 min; fasted state: pH 2, 5 mM phosphate buffer, ionic strength 70 mM, 15 min) and that the enzyme remains fully active in intestinal conditions (50 mM phosphate buffer, pH 7; ionic strength, 111 mM). These microencapsulates were formulated using 350-500 μ m microcrystalline cellulose spheres, which were sprayed sequentially with the model enzyme, whey protein and then gum Arabic. It appears that the gelling of the gum Arabic in acidic environments and the acid resistance of the whey protein protected the enzyme from acid (at least during the duration of the study). However, at higher intestinal pH, the coating porosity increases due to degradation of whey protein layer, enabling the enzyme release. Even though these results appear encouraging, again, the use of appropriate simulated conditions is of great importance. Despite the fact that the authors did test their sample in the fasted and fed state, the time in the simulated gastric fluid was much reduced (15 min), compared to the USP recommended test (2 h). Literature on the use of WPI for enteric coatings is still scarce and is a possible avenue for further development.

SOY PROTEIN

Soy protein is extracted from soy beans and contains high amounts of aspartic acid (ASP) and glutamic acid (GLU) (Asif and Acharya, 2013). Since these two amino acids contain an acidic side chain (Fig. 4.) with pKas of 3.71 (ASP) and 4.15 (GLU), they are potential candidates for enteric coatings. However, soy protein, or soy protein isolate (SPI), as is commercially available, has not yet been explored for this application.

Incorporating soy in a tablet matrix, Maltais et al. (2010) developed a riboflavin-loaded lyophilised soy hydrogel. The lyophilised powder was then compressed into tablets and then tested for 30 min in simulated gastric fluid (pH1.2, with and without 3.2 g/L of pepsin) followed by 6 h in simulated intestinal conditions (phosphate buffer pH 7.5, with and without 10.0g/L of pancreatin).

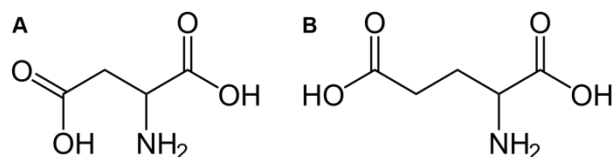


Fig. 4. Structure of aspartic acid (A) and glutamic acid (B).

The tablet was shown to withstand acidic conditions, with negligible release of the active substance (less than 3%), with 50% of the drug released after 2h in intestinal conditions (with pancreatin), and nearly 100% after 5 h in these conditions. The acid stage in this work only lasted 30 min, as opposed to the standard 2 hours compendial acid challenge test. However, riboflavin release was also assessed separately in simulated gastric fluids (same composition as before) for 24 h, exhibiting about 5% drug release after 2 h, suggesting soy may be a promising candidate for gastro-resistant applications.

Nonetheless, there are not many studies employing soy protein in enteric formulations, hence the evidence to support such an application is weak and warrants further research.

ALGINATE

Alginate is an anionic polysaccharide composed of β -D-mannuronate and α -L-guluronate (Fig. 5) present in cell walls of brown algae (Lee and Mooney, 2012).

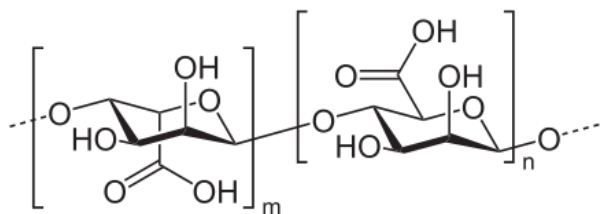


Fig. 5 - Representation of alginate structure, with m repetitions of α -L-guluronate and n repetitions of β -D-mannuronate, linked by $\beta(1-4)$ bonds.

Alginate has been used to provide enteric protection using triple-layered granules containing probiotics. Probiotic Pearls™ are an example of commercially available, triple-layered beads containing different probiotics (such as *Lactobacillus* and *Bifidus spp*), which purport to use this technology, where the outer layer contains a blend of alginate and pectin to withstand the acidic conditions of the stomach (Nature's Way Products, 2011a,b), however, several patents have been submitted using similar technology (Penhasi, 2012; Zorea, 2011). The outer layer forms an acidic gel under gastric conditions, which is expected to subsequently dissolve in the distal gut, thus releasing the encapsulated probiotics. In another study, alginate/chitosan microparticles containing probiotic cells (*Bacillus licheniformis*) were developed by Wu et al. (2016); these were able to maintain cell viability under acidic conditions (pH 1.0 to pH 4.0) for up to 8 h. However, such systems (Penhasi, 2012; Wu et al., 2016; Zorea, 2011) may not work for conventional gastroresistant applications which require the active ingredient to be released higher in the proximal small intestine. These systems, in contrast, are rather more suitable for further delayed release applications, for instance colonic targeting.

OTHERS

In addition to the above systems, a few other natural polymers have also been studied for gastroresistant applications, such as marine sponge collagen (Nicklas et al., 2009). This material resisted the compendial acid challenge (2h in 0.1M HCl), contrasting with type I collagen, which displays quick degradation into tropocollagen in acidic media (Heinemann et al., 2007). Tablets coated with 12.9mg/cm² complied with the Ph. Eur. specifications for delayed-release tablets, showing no disintegration or degradation for 2h in 0.1M HCl, yet disintegrating within 10min when in pH6.8

phosphate buffer. This system is currently being used in a nutraceutical formulation for ulcerative colitis under the name Spongicol® (KliniPharm, 2015).

CONCLUSIONS

Studies on naturally occurring materials with potential gastroresistant properties that can be employed for nutraceutical products are quite scarce. Even though some materials have been developed using polymers from nature, more accurate and better designed dissolution studies are needed to understand the dissolution profile of these substances.

Recently several natural materials like pectin/alginate (Nature's Way Products 2011a), shellac (Fraser and Young, 2010) and alginate (Colorcon®, 2015) have been employed. However their gastroresistant properties are not comparable to those employed in pharmaceutical products and lack robustness over a wider spectrum of gastric pH (Czarnocka and Alhnan, 2015; Merchant et al., 2009), and residence time accounting for inter- and intra-subject variability (time of the day, type and size of meal, demographics etc.). Therefore, there is still a need for a better solutions for the fast-growing nutraceutical market, and further research in this area is needed.

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