



(51) International Patent Classification:

A61K 31/6615 (2006.01) A61K 36/82 (2006.01)
A61K 31/715 (2006.01) A61P 1/00 (2006.01)
A61K 31/734 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/GB2012/000380

(22) International Filing Date:

25 April 2012 (25.04.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1107068.7 26 April 2011 (26.04.2011) GB

(71) Applicant (for all designated States except US): **THE UNIVERSITY OF BIRMINGHAM** [GB/GB]; Edgbaston, Birmingham B15 2TT (GB).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **TSELEPIS, Chris** [GB/GB]; School of Cancer Sciences, University of Birmingham, Vincent Drive, Edgbaston, Birmingham, West Midlands B15 2TT (GB).

(74) Agent: **WARD, David**; Marks & Clerk LLP, Alpha Tower, Suffolk Street Queensway, Birmingham, B1 1TT (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

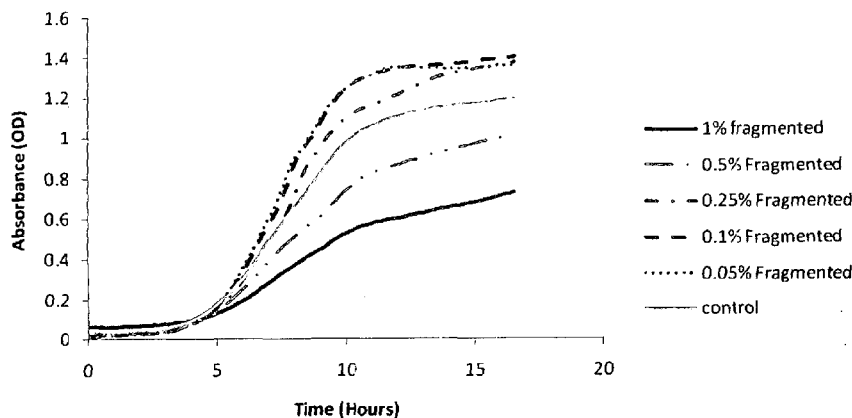
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: PREBIOTIC AGENT

Figure 1



(57) Abstract: A composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal bacterial population is provided. The composition comprises a prebiotic, is adapted for selective targeting of the prebiotic to the colon and is substantially free from probiotic.

Prebiotic Agent

5 This invention relates to methods and medicaments for the enhancement of gastrointestinal health, and/or for the improvement of the gastrointestinal bacterial population.

10 It is well known that the mammalian gastrointestinal (GI) tract, and particularly the human colon, are inhabited by a large number of bacterial species. These microbiota are believed to be important to the health of the host individual, not least in reducing or preventing colonisation by pathogenic microorganisms.

15 It is known to supply the GI tract, and particularly the colon, with 'probiotics', i.e. live microorganisms which confer a health benefit to the host. Microbes utilised for this purpose include lactic acid bacteria (LAB), bifidobacteria, and certain yeasts and bacilli. It is thought that the main mechanism of action is by adjustment of the microbiota population in favour of non-pathogenic microorganisms and away from pathogenic organisms. Such treatment, including the encapsulation of probiotics to ensure delivery to the colon are discussed in Possemiers et al., *International Journal of Food Microbiology*, 141 (2010), 97-103; Graff et al., *Pharmaceutical Research*, 25:6 (2008), 1290-1296; Chávarri et al., *International Journal of Food Microbiology*, 142 (2010), 185-189; Islam et al., *Journal of Microbiology and Biotechnology*, 20:10 (2010), 1367-1377; Rose et al., *Molecular Nutrition and Food Research*, 53 (2009), S121-S130; and Tuohy et al., *Drug Discovery Today*, 8:15 (2003), 692-700.

25 It is also known to supply the GI tract with 'prebiotics', i.e. indigestible food ingredients which stimulate the growth and/or activity of bacteria in the colon and thus improve health.

30 However, there remains a need for alternative medicaments and methods for the improvement of gastrointestinal health and/or for the improvement of the gastrointestinal bacterial population.

According to a first aspect of the invention, there is provided a composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal

bacterial population, comprising an iron chelator, wherein the composition is adapted for the selective targeting of the iron chelator to the colon.

5 According to a second aspect of the invention, there is provided a composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal bacterial population, comprising an iron chelator, wherein the iron chelator is unable to bind iron until the composition reaches the colon.

10 It will be understood that a chelator binds free metal ions and removes them from solution. Metal ions bound by a chelator molecule are effectively inactivated because they are no longer available to react with other chemical species, or for certain biological actions. Chelators are usually specific for particular metal ions. An iron chelator is a chelator that binds Fe ions. In certain embodiments said chelator may be iron specific.

15 The iron chelator may be naturally occurring, or it may be man-made. In a particular embodiment, the chelator is naturally-occurring.

20 In an embodiment, the iron chelator comprises phytic acid, tea flavonoids (catechins), melanoidins, curcumin, capsaicin, polyphenols, alginates, iron-binding proteins (such as for example lactoferrin), bacterial siderophores (such as for example enterobactin) or a combination of any of these. Advantageously, since these potential iron chelators are found in foods, it is known that they are safe for human consumption, and it is likely that they will impact on the levels of luminal iron when ingested.

25 In some embodiments, the iron chelator is non-digestible, non-absorbable and non-fermentable in the gastrointestinal tract. A chelator which is not digested or degraded by the body will be more effective at binding excess iron. If the chelator cannot be absorbed, any iron bound by the chelator will also be prevented from being absorbed
30 by the body. The iron will therefore be removed from the body as an iron-chelator complex.

According to a third aspect of the invention, there is provided a composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal

bacterial population, comprising a prebiotic, wherein the composition is adapted for selective targeting of the prebiotic to the colon and is substantially free from probiotic.

5 For the avoidance of doubt, it will be understood that, in some embodiments, the prebiotic may also be an iron chelator.

The following comments apply to the first, second and third aspects of the invention.

10 By "selective targeting to the colon" and "selective administration", it will be understood that the composition has no iron-chelating effect or other biological (such as prebiotic) activity (as appropriate) until it reaches the colon. This may also be referred to as "colonic delivery".

15 Colonic delivery of the iron chelator or prebiotic may be achieved by oral or rectal administration of the composition.

20 For rectal administration, the composition may be in the form of a suppository incorporating the iron chelator or prebiotic and a carrier such as cocoa butter, or in the form of an enema.

25 For oral administration, the composition may be in the form of discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the iron chelator or prebiotic; in the form of a powder or granules; in the form of an emulsion, suspension in an aqueous liquid or non-aqueous liquid. The composition may also be in the form of a bolus, electuary or paste. In some embodiments, the composition may be formulated for incorporation into a foodstuff.

30 For oral administration, colonic delivery of the iron chelator or prebiotic may be achieved by ingestion of the iron chelator or prebiotic in an inactivated form, or in a form which otherwise prevents the iron chelator from binding iron, or the prebiotic from having any biological activity, until it reaches the colon. Alternatively, the iron chelator may be ingested in the form of a precursor or prodrug, which is activated or modified *in vivo* such that the active iron chelator or prebiotic is only released in the colon.

In an embodiment, the composition comprising the iron chelator or prebiotic is encapsulated by a coating which remains intact while the composition passes through the stomach and small intestine, but which degrades in the colon. In a series of embodiments, colonic delivery is achieved using a coating which is pH sensitive, time-dependent, pressure-dependent or degradable by colonic bacteria. In a particular embodiment, the composition is encapsulated by a biodegradable coating. The coating may be a polymer. In some embodiments, the coating comprises colon-targeted microsponges, or a microbially-triggered osmotic pump. The composition may be targeted to the colon by a combination of the above strategies.

In some embodiments, the coating comprises a biodegradable polysaccharide. The coating may comprise one or more biodegradable polysaccharides selected from the group consisting of albizia gum, alginates, amylose, arabinogalactan, cellulose, chitosan, chondroitin sulphate, curdlan, cyclodextrin, dextran, furcelleran, galactomannan, gellan gum, guar gum, hyaluronic acid, inulin, kara gum, karaya gum, locust bean gum, scleroglucan, starch, pectin, pullulan or xylan.

The use of colonic targeting may reduce the incidence of side effects caused by action of the iron chelator or prebiotic in other parts of the GI tract. Alternatively or additionally, the use of colonic targeting may prevent degradation of the iron chelator or prebiotic in other parts of the GI tract, thereby providing a relative increase in bioavailability of the iron chelator or prebiotic in the colon.

According to a fourth aspect of the invention, there is provided a method for the improvement of gastrointestinal health and/or for the improvement of the gastrointestinal bacterial population of a human or animal patient, comprising administering to the patient a composition according to any of the first, second or third aspects of the invention.

The following comments apply to all aspects of the invention.

As used herein, the improvement of a gastrointestinal bacterial population is intended to refer to an increase in the levels of health promoting bacteria and/or a reduction in the levels of pathogenic bacteria. Health promoting bacteria comprise the genera of bifidobacterium (e.g. *B. longum*, *B. reve*) and lactobacillus (e.g. *L. acidophilus*, *L.*

rhamnosus, *L. gasserii*). Pathogenic bacteria comprise the genera clostridia, bacteriodes, enterobacteriaceae and desulfovibrio.

Bacterial levels may be assessed by any suitable method, including for example by high throughput pyrosequencing, such as high throughput Roche-454 pyrosequencing. In brief, this involves extraction of faecal DNA followed by amplification with conserved MID-tagged primers (such as for example those of the V1-V3 region of bacterial 16S rDNA), followed by sequencing with Roche-454 Titanium amplicon protocols. Typically, thousands of sequence reads per sample can then be obtained routinely to assess the proportions of different bacterial taxa. In some further embodiments, increasing or reducing the levels of bacteria comprises increasing or reducing the relevant bacterial populations by at least 5%, at least 10%, at least 15%, at least 20% or at least 50%. Alternatively or additionally, increasing the levels of bacteria may comprise increasing the bacterial levels above zero, and/or reducing the levels of bacteria may comprise reducing the bacterial levels to zero.

In some embodiments, the improvement of a gastrointestinal bacterial population is intended to refer to an increase in the ratio of the level of health promoting bacteria to the level of pathogenic bacteria. In some further embodiments, increasing the ratio of the level of health promoting bacteria to the level of pathogenic bacteria comprises increasing the ratio by at least 5%, at least 10%, at least 15%, at least 20% or at least 50%.

In some embodiments, the improvement of gastrointestinal health comprises the treatment of irritable bowel syndrome (IBS). Measurement of treatment of irritable bowel syndrome may be made by any suitable method, such as for example by assessing patient symptom scores based on questionnaires designed to assess quality of life (QoL). Different QoL assessments may be used in parallel, such as disease specific tools such as the IBS-QOL and generic instruments such as the well-validated 36 item short form general health survey (SF-346) (described in Ware et al., *Med Care*, 30(6), 1992, 473-83) or the sickness impact profile (SIP) (described in Bergner et al, *Med Care*, 19(8), 1981, 787-805).

In some embodiments, the improvement of gastrointestinal health comprises the treatment of inflammatory bowel disease (IBD). Measurement of treatment of

inflammatory bowel disease may be made by any suitable method, such as for example well validated symptom scores for patients with either ulcerative colitis (UC) (such as the simple clinical colitis index – SCCI) or Crohn's disease (CD) (such as the Crohn's disease activity index – CDAI). Additionally or alternatively, well validated QoL tools, either specific for IBD (IBDQ) or generic (SF36) allow comparison with studies in other patient groups (such as IBS). These clinical scores may be complemented by endoscopic and biopsy data to measure inflammation and gene/protein expression in the epithelium, and evaluation of the effect of treatment on markers of systemic (such as CRP) and colonic (such as faecal calprotectin) inflammation.

In some embodiments, the improvement of gastrointestinal health comprises the treatment of colorectal neoplasia. Measurement of treatment of colorectal neoplasia may be made by any suitable method, such as for example by visualisation of adenoma numbers and cancers from colonoscopies and pathological examination of collected biopsy material.

In some embodiments, the improvement of gastrointestinal health comprises the treatment of antibiotic-associated colitis. Measurement of treatment of antibiotic-associated colitis may be made by any suitable method, such as for example by measurement of production of *C. difficile* toxin, microbiota populations, and diarrhoea (using the Bristol stool chart).

In some embodiments, the composition comprises alginate. In some further embodiments, the composition comprises sodium alginate. It will be understood that the alginate (such as sodium alginate) may function as an iron chelator and/or prebiotic. Thus, in an embodiment of the first and second aspects of the invention, the iron chelator comprises alginate. In an embodiment of the third aspect of the invention, the prebiotic comprises alginate.

In some further embodiments, the alginate is covalently complexed to a further iron chelator molecule. In particular, the alginate may be covalently complexed to an iron-chelator molecule having a greater affinity for iron than the alginate. In some still further embodiments, the iron chelator molecule may have a greater specificity for iron than the alginate.

In addition to an iron chelator or prebiotic, the composition of the present invention may include one or more additional pharmaceutically acceptable ingredients such as excipients, bulking agents, diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants and preservatives.

5

In some embodiments, the amount of alginate administered to the patient is between 0.1g and 4g per day, between 0.2g and 3g per day, between 0.4g and 2g per day or between 0.5g and 1.5g per day, which may be administered as a single dose or multiple doses (e.g. 2, 3 or 4 doses at intervals of e.g. 3, 6 or 8 hours).

10

To reduce iron levels in the colon, the composition according to the first and second aspects of the invention comprises a chelator – a chemical which binds iron. Alginates are an example of a naturally-occurring chelator of iron and are used by the UK food industry as gelling, emulsifying and stabilising agents and thus are extensively found in products such as ice creams, jams, sauces and desserts. Alginates are also found in medicines, particularly in anti-reflux medicines. Alginates are therefore known to be safe for consumption by humans and animals. A further advantage of alginates is that they cannot be digested or absorbed by the human body. Both *in vitro* and *in vivo* studies have shown that alginates can stably bind iron and may prevent iron absorption. In particular, studies carried out by the inventors have shown that iron-mediated cellular proliferation is inhibited in the presence of alginate (unpublished data).

15

20

25

30

A number of studies have been carried out which attempt to show the effect of diet on bacterial populations, particularly in relation to iron. Werner et al., *Gut*, 60 (2011), 325-333 showed that certain bacterial genera are depleted in animal models after feeding with an iron-deficient diet. Tako et al., *British Journal of Nutrition*, 99 (2008), 472-480 showed that supplementing the diet of piglets with inulin resulted in increases in the populations of *Lactobacillus* and *Bifidobacterium*. Tomkins et al., *Current Microbiology*, 43 (2001), 38-42 showed that, with the exception of coliforms, all examined bacterial groups were significantly elevated in the colons of iron-deprived mice.

35

Griffiths et al., *Digestive Diseases and Sciences*, 48:7 (2003), 1324-1332, described the effects of lactoferrin, an iron-binding glycoprotein found in human breast milk, on the microecology of the human GI tract.

Akiyama et al., *Biosci. Biotech. Biochem.*, 56:2 (1992), 355-356 and Terada et al., *Microbial Ecology in Health and Disease*, 8 (1995), 259-266 described the effect of depolymerised alginates on GI microbiota.

5

However, none of these documents describes the targeting of an iron chelator and/or a prebiotic to the colon for the improvement of gastrointestinal health and/or the improvement of the gastrointestinal bacterial population.

10 Figure 1 shows the growth kinetics of *L. acidophilus* in the presence of different concentrations of fragmented alginate; Figure 2 shows the growth kinetics of *L. acidophilus* in the presence of 0.1% whole and fragmented alginate; Figure 3 shows the growth kinetics of *L. acidophilus* in the presence of 0.1% whole and fragmented alginate, and 0.1% whole alginate pre-loaded with iron.

15

Example Oral Formulations

A. Iron Chelators	B. Delivery Component
1. Phytic acid	a. pH-sensitive (e.g. Eudragit FS30D)
2. Polyphenols	b. Time-dependent (e.g. the Pulsincap TM and Chronotopic systems)
3. Alginates	c. Pressure-dependent (e.g. pressure-controlled colon-delivery capsules have been formulated utilising ethylcellulose)
	d. Biodegradable (e.g. pectin, guar gum and chitosan)
	f. Microsponges (e.g. microsphere®5640)
	g. Osmotic pump (e.g. the microbially-triggered colon-targeted osmotic pump)

20 A composition comprising an iron chelator selected from list A is coated with a colonic-delivery component selected from list B to give compositions 1a to 1g, 2a to 2g and 3a to 3g.

Example 1

A composition comprising microcrystalline cellulose, sugar, propyl paraben and 0.5g sodium alginate is formulated into a 1g tablet and coated with a biodegradable polysaccharide. Two tablets are orally administered to a patient with inflammatory bowel disease three times a day at 4 hourly intervals.

Example 2

10 Materials and methods

Materials and bacterial strains

The following strains were used in this study: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Lactobacillus acidophilus*. The anaerobic bacteria (*L. Acidophilus*) were cultured in anaerobic conditions at 37°C in anaerobic jars (BD anaerobic systems with GasPak) using Wilkins-Chalgren agar (Oxoid) supplemented with 5% defibrinated horse blood or Wilkins-Chalgren broth (Oxoid). Aerobic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*) were cultured using Luria Bertani (LB) broth and agar (Sigma). Broth and agar were prepared according to the manufacturers instructions.

Preparation of alginate solutions

To prepare 'whole' alginate solution, 2g of alginate as purchased was dissolved in 100ml of appropriate broth. To prepare 'fragmented' alginate solution, the 2% 'whole' alginate solution was incubated with 10U of alginate lyase (Sigma) in a shaking incubator at 37°C for 12 hours. The 'iron loaded' alginate solutions were prepared by dialysing the 'whole' or 'fragmented' alginate solutions across a 12 KDa membrane (Sigma) against 0.2 mMol iron sulphate dissolved in broth for four hours. The resultant solutions were autoclaved at 121°C for 15 minutes to sterilise.

30

Minimum Inhibitory Concentration Assay

The Minimum Inhibitory Concentration (MIC) was performed using a microdilution technique. Alginate solutions prepared as described above (initial concentration 2% w/v) were diluted in broth in serial twofold dilutions in a 96 well microtitre plate. An overnight broth of the required bacteria was diluted to 1:100. 50µl of diluted bacterial

35

solution was added to 50µl of the diluted alginate solution, the plate covered and incubated at 37°C in the appropriate atmosphere for 18 hours. The MIC was determined as the lowest concentration of the alginate showing no visible bacterial growth at 18 hours, i.e. the minimum concentration of alginate required to inhibit bacterial growth. The assay was repeated with all bacterial strains and alginate solutions.

Growth kinetics

A 96 well microtitre plate was set up as described for MIC. This was incubated at 37°C on a microplate reader (FLUOstar optima) for 12-18 hours. Absorbance (OD) was measured at 10 minute intervals at 600nm. This was repeated for all species and all alginate solutions. Anaerobes were incubated under a 50µl layer of sterile mineral oil to replicate anaerobic conditions. This was carried out on 4 independent occasions.

Results

Sodium alginate was tested against a variety of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Lactobacillus acidophilus*) using a minimum inhibitory concentration (MIC) assay. The whole alginate (i.e. whole molecules of alginate which have not been broken down) exhibited a bacteriostatic effect against all of the bacterial strains tested, although the effectiveness of the alginate varied between different bacteria (see Table 1). The susceptibility of each species to alginate was variable with *E. coli* demonstrating the most resistance to the effect of alginate, as evidenced by a much higher MIC (1% vs <0.5% for other strains).

The bacteriostatic effect of alginate was also observed when using fragmented alginate (i.e. alginate molecules which have been cleaved/broken down enzymatically into smaller subunits) in the MIC assay in all species. However, the fragmented alginate was less efficacious, giving a higher MIC. The MIC assay gives a result from a single time point and so in order to investigate the consequences of alginate on bacterial growth curves in more detail, growth kinetics were studied using a microplate absorbance reader.

Table 1. Minimum percent concentration of alginate required to inhibit growth

Bacteria	Alginate form	Minimum percent concentration (w/v)
<i>S. aureus</i>	whole	0.12
	fragmented	0.35
<i>S. typhimurium</i>	whole	0.12
	fragmented	0.25
<i>E. coli</i>	whole	1
	fragmented	1
<i>L. acidophilus</i>	whole	0.12
	fragmented	0.25

- The growth kinetics studies supported the finding of a bacteriostatic effect of whole alginate against all bacterial strains tested, as previously demonstrated by the MIC assay. When using fragmented alginate, the growth suppression effect against *S. aureus*, *E. coli* and *S. typhimurium* was diminished as compared to whole alginate, and the growth curves of these species in the presence of fragmented alginate approached the control curve (data not shown).
- L. acidophilus* similarly demonstrated diminished growth in the presence of the whole alginate. However, in the presence fragmented alginate at concentrations below 0.5%, the growth of *L. acidophilus* was augmented, as shown in Figure 1. In the presence of 0.5% and 1% fragmented alginate, growth was diminished compared to the control.
- Exposure of *L. acidophilus* to 0.1% fragmented alginate resulted in a significant increase ($p < 0.05$) in the final OD of the kinetics curves compared to control, as shown in Figure 2. In contrast, the generation time of lactobacillus was decreased by 18.5% at 7 hours when growing in the presence of 0.1% fragmented alginate.
- Pre-loading the whole alginate with iron partially reversed the inhibitory effect on *L. acidophilus* growth, as shown in Figure 3, suggesting that the iron binding action of alginate may in part be responsible for its bacteriostatic effect.

CLAIMS:

1. A composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal bacterial population, comprising a prebiotic, wherein
5 the composition is adapted for selective targeting of the prebiotic to the colon and is substantially free from probiotic.
2. The composition of claim 1, wherein the prebiotic is an iron chelator.
- 10 3. A composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal bacterial population, comprising an iron chelator, wherein the composition is adapted for the selective targeting of the iron chelator to the colon.
- 15 4. A composition for the improvement of gastrointestinal health and/or for the improvement of a gastrointestinal bacterial population, comprising an iron chelator, wherein the iron chelator is unable to bind iron until the composition reaches the colon.
5. The composition of any one of claim 2 to 4, wherein the iron chelator is iron specific.
- 20 6. The composition of any one of claims 2 to 5, wherein the iron chelator is man-made.
7. The composition of any one of claims 2 to 5, wherein the iron chelator is naturally-occurring.
- 25 8. The composition of any one of claims 2 to 7, wherein the iron chelator comprises phytic acid, tea flavonoids, melanoidins, curcumin, capsaicin, polyphenols, alginates, iron-binding proteins, bacterial siderophores or a combination of any of these.
- 30 9. The composition of any one of claims 2 to 8, wherein the iron chelator is non-digestible, non-absorbable and non-fermentable in the gastrointestinal tract.
10. The composition of any one of the preceding claims, wherein colonic delivery of the iron chelator or prebiotic may be achieved by rectal administration of the composition.

11. The composition of claim 10, wherein rectal administration is in the form of a suppository or in the form of an enema.
12. The composition of any one of claims 1 to 9, wherein colonic delivery of the iron chelator or prebiotic may be achieved by oral administration of the composition.
13. The composition of claim 12, wherein the composition is in the form of capsules, cachets, tablets, lozenges, powder, granules, an emulsion, a suspension in an aqueous liquid or non-aqueous liquid, a bolus, an electuary or a paste.
14. The composition of claim 12 or 13, which is encapsulated by a coating which remains intact while the composition passes through the stomach and small intestine, but which degrades in the colon.
15. The composition of claim 14, wherein the coating comprises one or more biodegradable polysaccharides selected from the group consisting of albizia gum, alginates, amylose, arabinogalactan, cellulose, chitosan, chondroitin sulphate, curdlan, cyclodextrin, dextran, furcelleran, galactomannan, gellan gum, guar gum, hyaluronic acid, inulin, kara gum, karaya gum, locust bean gum, scleroglucan, starch, pectin, pullulan or xylan.
16. A method for the improvement of gastrointestinal health and/or for the improvement of the gastrointestinal bacterial population of a human or animal patient, comprising administering to the patient a composition according to any one of the preceding claims.
17. The composition or method of any one of the preceding claims, wherein the improvement of a gastrointestinal bacterial population is an increase in the levels of health promoting bacteria and/or a reduction in the levels of pathogenic bacteria.
18. The composition or method of claim 17, wherein the health promoting bacteria comprise the genera of bifidobacterium (e.g. *B. longum*, *B. reve*) and lactobacillus (e.g. *L. acidophilus*, *L. rhamnosus*, *L. gasseri*).

19. The composition or method of claim 17, wherein the pathogenic bacteria comprise the genera clostridia, bacteriodes, enterobacteriaceae and desulfovibrio.

5 20. The composition or method of any one of claims 17 to 19, wherein increasing or reducing the levels of bacteria comprises increasing or reducing the relevant bacterial populations by at least 5%.

10 21. The composition or method of any one of claims 17 to 20, wherein the improvement of a gastrointestinal bacterial population is intended to refer to an increase in the ratio of the level of health promoting bacteria to the level of pathogenic bacteria.

22. The composition of any one of claims 1 to 15 for use in the treatment of irritable bowel syndrome (IBS).

15 23. The composition of any one of claims 1 to 15 for use in the treatment of inflammatory bowel disease (IBD).

20 24. The composition of any one of claims 1 to 15 for use in the treatment of colorectal neoplasia.

25 25. The composition of any one of claims 1 to 15 for use in the treatment of antibiotic-associated colitis.

26. The composition of any one of claims 1 to 15 comprising alginate.

27. The composition of claim 26, wherein the alginate is covalently complexed to a further iron chelator molecule.

30

35

Figure 1

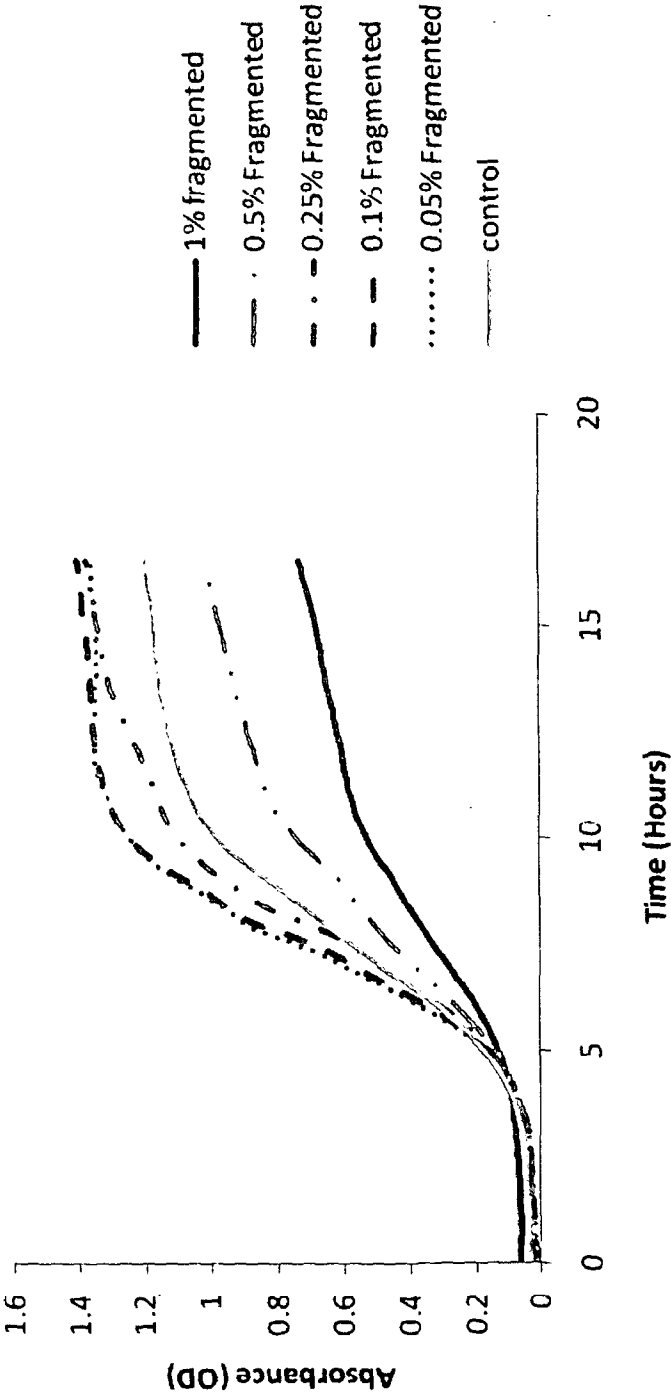


Figure 2

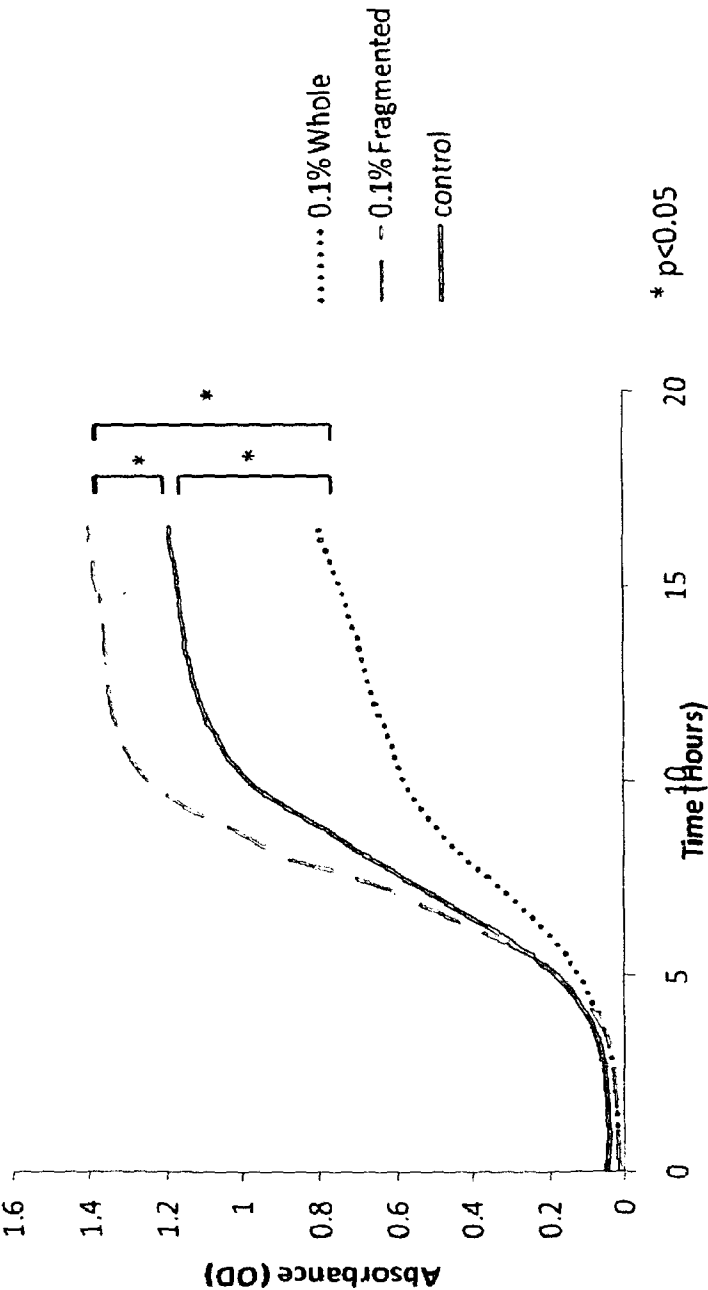
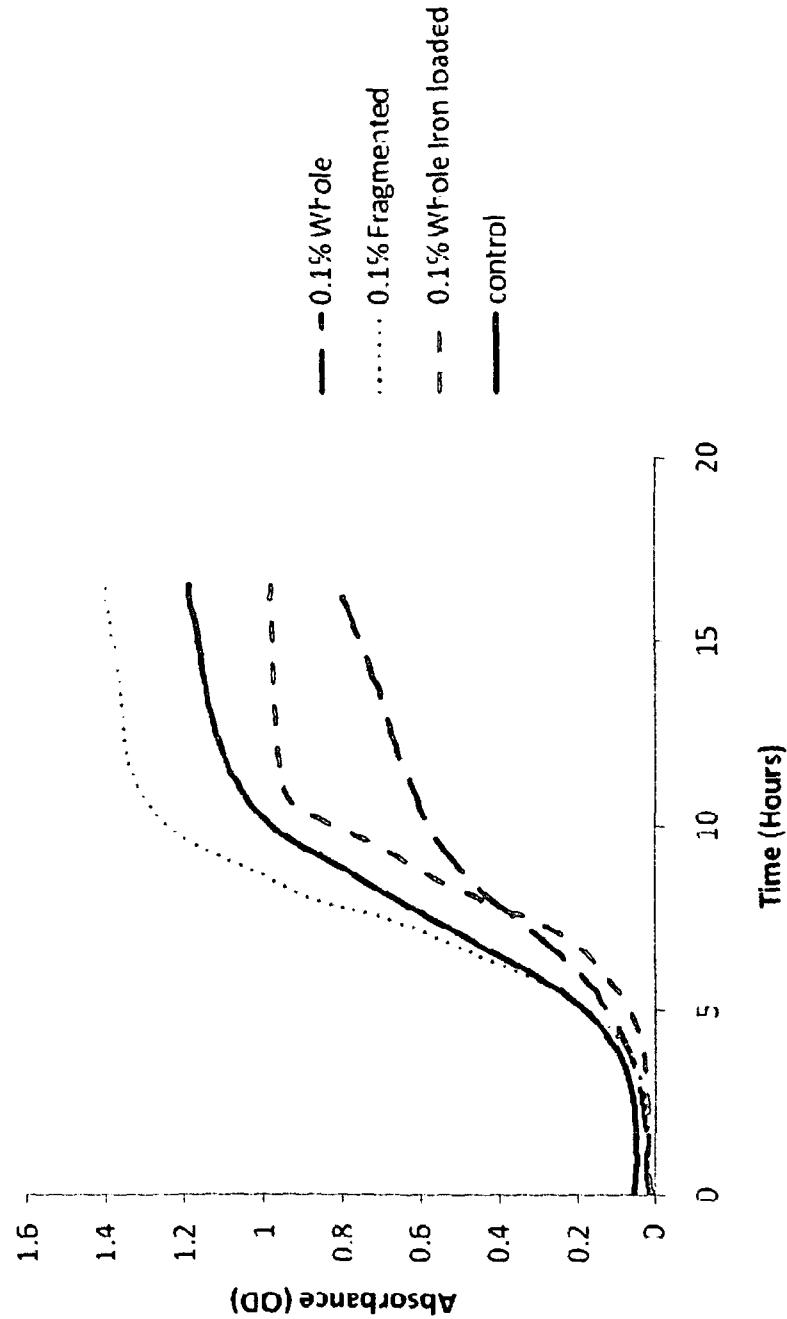


Figure 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/000380

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/6615 A61K31/715 A61K31/734 A61K36/82 A61P1/00 A61P35/00 ADD. According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, SCISEARCH, WPI Data														
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category*</th> <th style="width: 70%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width: 20%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="vertical-align: top;">X,P</td> <td style="vertical-align: top;"> WO 2011/051665 A1 (UNIV BIRMINGHAM [GB]; TSELEPIS CHRIS [GB]; IQBAL TARIQ [GB]) 5 May 2011 (2011-05-05) the whole document, particularly page 7 lines 4-7; claims; examples ----- </td> <td style="vertical-align: top;"> 1-16,24, 26 </td> </tr> <tr> <td style="vertical-align: top;">X</td> <td style="vertical-align: top;"> US 2007/248649 A1 (SAWATZKI GUNTHER [DE] ET AL) 25 October 2007 (2007-10-25) the whole document ----- </td> <td style="vertical-align: top;"> 1,12,13, 16-18,21 </td> </tr> <tr> <td style="vertical-align: top;">X</td> <td style="vertical-align: top;"> US 2009/022852 A1 (SIMMONS DONALD L [CA] ET AL) 22 January 2009 (2009-01-22) paragraph [0007] - paragraph [0016]; examples ----- -/-- </td> <td style="vertical-align: top;"> 1,12,13, 16-18, 21,24 </td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X,P	WO 2011/051665 A1 (UNIV BIRMINGHAM [GB]; TSELEPIS CHRIS [GB]; IQBAL TARIQ [GB]) 5 May 2011 (2011-05-05) the whole document, particularly page 7 lines 4-7; claims; examples -----	1-16,24, 26	X	US 2007/248649 A1 (SAWATZKI GUNTHER [DE] ET AL) 25 October 2007 (2007-10-25) the whole document -----	1,12,13, 16-18,21	X	US 2009/022852 A1 (SIMMONS DONALD L [CA] ET AL) 22 January 2009 (2009-01-22) paragraph [0007] - paragraph [0016]; examples ----- -/--	1,12,13, 16-18, 21,24
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X,P	WO 2011/051665 A1 (UNIV BIRMINGHAM [GB]; TSELEPIS CHRIS [GB]; IQBAL TARIQ [GB]) 5 May 2011 (2011-05-05) the whole document, particularly page 7 lines 4-7; claims; examples -----	1-16,24, 26												
X	US 2007/248649 A1 (SAWATZKI GUNTHER [DE] ET AL) 25 October 2007 (2007-10-25) the whole document -----	1,12,13, 16-18,21												
X	US 2009/022852 A1 (SIMMONS DONALD L [CA] ET AL) 22 January 2009 (2009-01-22) paragraph [0007] - paragraph [0016]; examples ----- -/--	1,12,13, 16-18, 21,24												
<div style="display: flex; justify-content: space-between;"> <div> ☒ Further documents are listed in the continuation of Box C. </div> <div> ☒ See patent family annex. </div> </div>														
<div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </div> </div>														
Date of the actual completion of the international search <div style="text-align: center; font-size: 1.2em;">28 September 2012</div>		Date of mailing of the international search report <div style="text-align: center; font-size: 1.2em;">09/10/2012</div>												
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-size: 1.2em;">Venturini, Francesca</div>												

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2012/000380

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/000380

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/297058 A1 (BANERJEE GAUTAM [IN] ET AL) 25 November 2010 (2010-11-25) paragraph [0019] - paragraph [0026]; claims -----	1,12,13, 16-18, 21-23
X	WO 2009/027955 A2 (SHANNON MINERALS PATENTS LTD [IE]; O'MARA ANN MARIE [IE]; O'MARA BREND) 5 March 2009 (2009-03-05) page 2, lines 4-7, paragraph 28; claims; examples -----	1,12,13, 16-18, 21,26
X	EP 1 600 061 A1 (COGNIS IP MAN GMBH [DE]) 30 November 2005 (2005-11-30) paragraph [0029] - paragraph [0030]; claims; examples -----	1,12-18, 21
X	US 2009/035371 A1 (KATZ DANIEL [IL]) 5 February 2009 (2009-02-05) claims; examples -----	3-5,7-9, 12,14, 16,23,24
X	US 2010/303904 A1 (KATZ DANIEL [IL]) 2 December 2010 (2010-12-02) claims; examples -----	3-5,7-9, 12,14, 16,23,24
X	WO 00/72874 A1 (NAIDU A SATYANARAYAN [US]) 7 December 2000 (2000-12-07) page 7, lines 8-15 page 15, line 28 - page 16, line 10 page 24, lines 23-27; claims -----	3-5, 7-14,16, 17,19

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2(completely); 10-27(partially)

Composition comprising a prebiotic adapted for selective targeting to the colon and substantially free from probiotic

2. claims: 3-9(completely); 10-27(partially)

Composition comprising an iron chelator adapted for selective targeting to the colon

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2012/000380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011051665 A1	05-05-2011	EP 2493481 A1	05-09-2012
		WO 2011051665 A1	05-05-2011

US 2007248649 A1	25-10-2007	AT 220511 T	15-08-2002
		AU 766429 B2	16-10-2003
		AU 5514699 A	06-03-2000
		BR 9912959 A	08-05-2001
		CA 2340103 A1	24-02-2000
		CA 2740912 A1	24-02-2000
		CN 1312685 A	12-09-2001
		DE 19836339 A1	24-02-2000
		DK 1105002 T3	11-11-2002
		EP 1105002 A2	13-06-2001
		ES 2180321 T3	01-02-2003
		ID 29945 A	25-10-2001
		JP 4972606 B2	11-07-2012
		JP 2002522461 A	23-07-2002
		JP 2008222720 A	25-09-2008
		JP 2009203236 A	10-09-2009
		NZ 510347 A	25-07-2003
		PL 346642 A1	25-02-2002
		PT 1105002 E	31-12-2002
		US 2007248649 A1	25-10-2007
		US 2008207559 A1	28-08-2008
		US 2010016214 A1	21-01-2010
		WO 0008948 A2	24-02-2000

US 2009022852 A1	22-01-2009	NONE	

US 2010297058 A1	25-11-2010	CA 2762665 A1	25-11-2010
		CN 102427824 A	25-04-2012
		EA 201171421 A1	29-06-2012
		EP 2432485 A1	28-03-2012
		US 2010297058 A1	25-11-2010
		WO 2010133404 A1	25-11-2010

WO 2009027955 A2	05-03-2009	US 2009060942 A1	05-03-2009
		WO 2009027955 A2	05-03-2009

EP 1600061 A1	30-11-2005	EP 1600061 A1	30-11-2005
		JP 2008500032 A	10-01-2008
		US 2008268024 A1	30-10-2008
		WO 2005115170 A1	08-12-2005

US 2009035371 A1	05-02-2009	NONE	

US 2010303904 A1	02-12-2010	NONE	

WO 0072874 A1	07-12-2000	AT 505200 T	15-04-2011
		AU 776657 B2	16-09-2004
		AU 5449100 A	18-12-2000
		AU 2003204900 A1	24-07-2003
		BR 0011021 A	29-04-2003
		BR 0011558 A	26-02-2002
		EP 1181044 A2	27-02-2002
		JP 2003500425 A	07-01-2003
		US 6172040 B1	09-01-2001
		US 7375080 B1	20-05-2008

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2012/000380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		W0 0072690 A2	07-12-2000
		W0 0072874 A1	07-12-2000
