ENCAPSULATED DRUGS

FIELD OF THE INVENTION

This invention relates to orally administrable formulations for the controlled release of a drug, and to consumable products and food supplements comprising them.

BACKGROUND OF THE INVENTION

Turmeric (*curcuma longa* L.) a member of the ginger family is extensively used in Ayurveda, Unani and Siddha medicine in India as home remedy for various diseases. Curcumin is the principal curcuminoid in turmeric. Curcuminoids are polyphenolic compounds that give turmeric its yellow color.

Clinically, curcumin has already been used to reduce post-operative inflammation. Safety evaluation studies indicate that both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases.

Orally administered Curcumin exhibits low bioavailability due to a poor absorption and a rapid metabolism *in vivo (*Anand et al., 2007). Bioavailability studies in humans reported variable but always low plasma levels of curcumin ranging from 0.016 to 11 μmol/L after acute or chronic supplementation of the pure compound at dosage ranging from 2 to 12 g. All the pharmacokinetic studies concord that once absorbed, curcumin undergoes extensive reduction, most likely through alcohol dehydrogenase, followed by conjugation at various tissue sites mainly in the liver, kidney and intestinal mucosa. Thus curcumin glucuronides, sulphated and hexahydrocurcumin, are the major curcumin metabolites; glucuronides representing in many cases up to 99% of total conjugates in the plasma. Anyway less than 1% curcumin ingested is retrieved in the blood, trace amount are generally found principally metabolized in urines and from 40 up to 75% of curcumin ingested is excreted in unchanged form in faeces.

Kurien and Scofield, 2009 disclose encapsulation of curcumin in PEG. Curcumin is heated in order to increase its solubility and facilitate encapsulation. Shaikh et al, 2009 describe nanoencapsulation of curcumin. In both cases curcumin is encapsulated as a single agent.

A clinical study by Cheng and colleagues (Cheng et al (2001) Anticancer Res. 21, pp: 2895-2900) shows that after oral intake of 8 grams of curcumin, serum concentrations of the drug peaked 1-2 hours after intake to a level of about 1.75μM and declined gradually within 12 hours.

Shoba and colleagues administered 2 grams of pure curcumin powder to fasting volunteers resulting in low curcumin concentrations detected in plasma (less than 10ng/ml) one hour post dosing. Co-ingestion of curcumin with 20 mg of the pepper constituent 1-piperoylpiperidine appeared to increase curcumin's bioavailability (Shoba et al. (1998) Planta Med 64, pp: 353-356).

It has been suggested that the bioavailability of curcumin can be enhanced by inhibition of the gut enzymes responsible for the inactivation of curcumin. Specifically, it was suggested that such enzyme inhibitors should be consumed 15 to 30 minutes prior to consumption of curcumin (Alvin Hashimoto http://drzarkov.com/delanoblog/?p=56).

SUMMARY OF THE INVENTION

The present invention provides a platform for enhanced bioavailability and delivery of biologically active compounds.

Accordingly, by a first of its aspects, the present invention provides an orally administrable formulation for the controlled release of a drug, said formulation comprising:

(a) A mixture of microcapsules each consisting of a coating material comprising an encapsulation agent and an active agent, wherein said mixture comprises microcapsules comprising as an active agent at least one inhibitor of gut enzymatic and/or transport activity and microcapsules comprising as an active agent at least one drug; and

(b) at least one carrier, adjuvant or excipient therefore.

In certain embodiments the microcapsules are being formulated so as to allow differential release rates of said at least one drug and said at least one inhibitor of gut enzymatic and/or transport activity.

In one embodiment the at least one inhibitor of gut enzymatic and/or transport activity is released in the gut prior to the drug.

The at least one inhibitor of gut enzymatic and/or transport activity and the drug may be released gradually or in a pulse mode.

In certain embodiments the coating material comprises a multilayered coating.

In certain embodiments said encapsulation agent is selected from the group consisting of resistant starch, fat coatings, mono and di glycerides, wax, shellac, cellulose based coatings, and any combination thereof.

Specifically said cellulose based coatings are selected from the group consisting of Methyl cellulose (MC), Hydroxypropyl cellulose (HPC), Ethyl Cellulose (EC), Hydroxy ethyl cellulose (HEC), hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), Hydroxypropyl methyl cellulose acetate, cellulose acetate phthalate, hydroxymethyl cellulose phthalate, cellulose trimellitate, hydroxymethyl cellulose acetate succinate and any combination thereof.

In certain embodiments said coating material further comprises Hydrogenated Vegetable oils.

The microcapsules may comprise between about 5% and about 20% w/w coating material.

In certain embodiments said drug is selected from the group consisting of curcumin, digoxin, antibiotics (e.g. cyclosporine A), anti neoplastic agent, and viral inhibitors (e.g. HIV protease inhibitors).

In certain embodiments said inhibitor inhibits the activity of an enzyme selected from the group consisting of UGT (UDP-glucuronosyltransferase) enzyme family, sulfotransferase enzymes, alcohol dehydrogenase, and p450 enzymes.

In specific embodiments said inhibitor is selected from the group consisting of Piperine, Quercetin, Genistein, Glabridin, and any combination thereof.

In a specific embodiment the orally administrable formulation of the invention comprises the inhibitors Piperine, Quercetin, Genistein, and the drug curcumin.

In another aspect, the present invention provides an orally administrable formulation for the controlled release of curcumin wherein said formulation comprises:

(a) microcapsules consisting of a coating material comprising an encapsulation agent;

(b) Curcumin; and

(c) at least one carrier, adjuvant or excipient therefore.

In certain embodiments said microcapsules comprise between about 60% and about 90% curcumin and between about 10% and 30% coating material.

In another aspect, the present invention provides a consumable product comprising the orally administrable formulation of the invention.

In certain embodiments said product is selected from the group consisting of dry pasta, bread and bread crisps.

In another aspect, the present invention provides a pharmaceutical or nutraceutical composition comprising the orally administrable formulation of the invention.

In certain embodiments said pharmaceutical composition is for the treatment of inflammatory diseases, infectious diseases, or cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a graph demonstrating the content (as a percent of total weight) of bisdesmethoxycurcumin, desmethoxycurcumin, curcumin and total curcuminoids in dry (A) and cooked pasta (B).

Fig. 2 is a graph demonstrating the content (as a percent of total weight) of bisdesmethoxycurcumin (A), desmethoxycurcumin (B), curcumin (C) and total curcuminoids (D) levels in bread crisps baked at 180 ° C for 15 and 30 minutes.

Fig. 3 is a graph demonstrating the mean concentration of curcumin and metabolized curcumin (total curcumins) in subjects' sera collected over the experiment day. The subject groups consumed bread supplemented with free curcumine (Free); bread supplemented with ecapsulated-curcumin (Enc Curc); and bread supplemented with encapsulated curcumin + enhancers, namely gut enzyme inhibitors (Enc Curc + Enhanc). (n = 10).

Fig. 4 is a graph demonstrating the mean concentration of curcumin and metabolized curcumin (total curcumin metabolites) in subjects' urine collected 24 hours after the commencement of the experiment. The subject groups consumed bread supplemented with free curcumin (FC); bread supplemented with ecapsulated-curcumin (EC); and bread supplemented with encapsulated curcumin + enhancers, namely gut enzyme inhibitors (EC + EN). (n = 10).

Fig. 5 is a graph demonstrating the mean concentration of total curcumin phenolic acids in subjects' urine collected 24 hours after the commencement of the experiment. The subject groups consumed bread supplemented with free curcumin (FC); bread supplemented with ecapsulated-curcumin (EC); and bread supplemented with encapsulated curcumin + enhancers, namely gut enzyme inhibitors (EC + EN). (n = 10).

DETAILED DESCRIPTION OF EMBODIMENTS

The cells lining the intestine are equipped with metabolizing enzymes that convert biologically active compounds into non active metabolites, and molecular transporters that pump these compounds or their metabolite byproducts out of the intestinal lining back into the intestine. These enzymatic mechanisms result in a reduction in drug availability and thus compromise their therapeutic effect.

The present invention provides a method for increasing the bioavailability of pharmaceutically active compounds (drugs) which are orally ingested, using microencapsulation as a vector for compound delivery.

The microencapsulation of a drug in a coating allows an attenuated time release mode of absorption through the gut.

Without wishing to be bound by theory, the encapsulation attenuates the release of the drug within the digestive system. Specifically it allows "safe passage" through areas of the digestive system which are rich in digestive enzymes (e.g. the stomach). The capsule is degraded with time and the drug is finally released into the intestine in an area which contains a less degrading enzymatic activity.

The invention further provides a composition comprising a mixture of microcapsules said composition containing at least two separate components, a first component being microcapsules comprising the active agent (i.e. drug) and the second component being microcapsules comprising an inhibitor of gut enzymatic and/or

transport activity. In one embodiment of the invention, the microcapsules in the composition are constructed so as to allow differential release rates of the at least two components.

As used herein the term ***"encapsulation"*** and in particular ***"microencapsulation"*** refers to a process wherein a core composed of the compound of interest in solid, liquid or gaseous form is covered by a thin film of a coating agent. The encapsulation generates a physical barrier having different morphological and resistance characteristics depending on the coating material. Most microcapsules have diameters between a few micrometers and a few millimeters.

Techniques for microencapsulation of compounds intended for human or animal consumption are well known in the art (see for example in Handbook of Food Preservation, Second Edition, Chapter 22 "Encapsulation, Stabilization, and Controlled Release of Food Ingredients and Bioactives" by Pegg and Shahidi, pages 510-568) and depend on the physical and chemical properties of the material to be encapsulated.

Such techniques include, but are not limited to spray drying, spray cooling and spray chilling, fluidized bed coating, extrusion, centrifugal extrusion, lyophilization, coacervation, centrifugal suspension separation, cocrystalization, liposome entrapment, interfacial polymerization, inclusion complexation (molecular inclusion).

Encapsulation further encompasses nanoparticulate delivery systems (nanoencapsulation), which relate to techniques for generating nano scale capsules, i.e. in the range of 1-100 nm.

Encapsulation is largely used in the pharmaceutical industry as well as in the food industry. In one application, encapsulation serves to allow a controlled release of bioactive ingredients in various parts of the gastro intestinal tract.

In accordance with the invention the encapsulation is performed using an encapsulation agent (also termed herein "coating material"). Non-limiting examples of an encapsulation agent are resistant starch, Hydrogenated Vegetable oils (HVO), fat coatings, mono and di glycerides, wax, shellac, Methyl cellulose (MC), Hydroxypropyl cellulose (HPC), Ethyl Cellulose (EC), Hydroxy ethyl cellulose (HEC), hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), Hydroxypropyl methyl cellulose acetate and combinations thereof. These coatings are gradually dissolved in the gut in a time-dependent manner.

Additional examples of cellulose-based coatings are cellulose acetate phthalate, hydroxymethyl cellulose phthalate, cellulose trimellitate, and hydroxymethyl cellulose acetate succinate. These coatings are pH sensitive and may dictate differential dissolution in the gut based on the local pH.

The core may be of 50 – 3000 microns, the coating may be between 5% - 80%, and may contain additional components e.g. oil or a combination of the above substances, for example a cellulose derivative and wax or hydorgenated vegetable oil.

In certain embodiments the microcapsules comprise between about 70% and about 90% Curcumin Particle (CP) and between about 5% and about 20% Coating material (CM). In certain embodiments the microcapsules further comprise between about 10% and 20% Hydrogenated vegetable oil (HVO).

In specific embodiments the microcapsules comprise 90% CP and 10% CM; 85% CP and 15% CM; 76.5% CP, 8.5% CM and 15%HVO; or 72.25% CP, 12.75% CM and 15% HVO.

In one embodiment the composition comprises an encapsulated drug as a single agent.

In another embodiment, the composition comprises an encapsulated drug in combination with at least one encapsulated gut enzyme inhibitor.

In accordance with the present invention, the drug may be any pharmaceutically active ingredient which is taken orally and may be compromised by the activity of gut enzymes. Non-limiting examples of such drugs include: curcumin, digoxin, antibiotics (e.g. cyclosporine A), anti neoplastic agents and anti viral agents (e.g. HIV protease inhibitors).

Curcumin is a polyphenolic compound ($C_{21}H_{20}O_6$) having a molecular weight of 368.37 and a melting point of 183^{o}C.

Curcumin is relatively insoluble in water, but dissolves in acetone, dimethylsulphoxide and ethanol. It is unstable at basic pH, and degrades within 30 minutes to trans-6-(40-hydroxy-30-methoxyphenyl)-2, 4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin.

Curcumin has been largely investigated in the last few years showing a broad array of biological activities, such as those described in Aggarwal et al (Phytopharmaceuticals in Cancer Chemoprevention (2005) 349-387). A growing body of literature has demonstrated the antioxidant, anti inflammatory, anti-carcinogenic, and

anti-infectious activity of curcumin based on the ability of this compound to regulate a number of cellular signal transduction pathways (Calabrese et al., 2008).

Curcumin is readily conjugated in the intestine and liver to form curcumin glucuronides and curcumin sulfates or reduced to hexahydrocurcumin (Ireson et al. (2002) Cancer Epidemiol. Biomarkers Prev. 11 (1), pp: 105-111).

Examples of converting enzymes which may act in the gut on an orally taken drug include, but are not limited to: The UGT (UDP-glucuronosyltransferase) enzyme family; sulfotransferase enzymes; alcohol dehydrogenase; and p450 enzymes.

Non limiting examples of gut enzyme inhibitors include but are not limited to Piperine (which inhibits UGT (UDP-glucuronosyltransferase), p450 enzymes, P-glycoprotein and CYP3A4 (Rajinder et al The J. of Pharmacology and Experimental Therapeutics Vol. 302, No.2 pages 645-650)), Quercetin (which inhibits sulfotransferase enzymes), Genistein (which inhibits alcohol dehydrogenase), and Glabridin (which inhibits p450 enzymes).

In certain embodiments wherein the composition comprises an encapsulated drug and an encapsulated enzyme inhibitor the release mode would be two fold, namely the enzyme inhibitor is released first while the drug is released at a later stage. The sequential release of the compounds may be gradual and continuous or in a pulse mode. In the pulse mode the enzyme inhibitor is release in a first pulse and the drug is released in a second belated pulse.

In one embodiment, the encapsulation of the drug and the enzyme inhibitor is performed using a multilayered coating. The multilayered coating will allow release of the active compounds at different times, different areas of the intestine and at specific release rates. In one specific embodiment said drug is encapsulated in a multilayered coating comprising for example one layer of Ethocel 100 and a second layer being HVO (Hydrogenated Vegetable Oil) ; while the enzyme inhibitor is coated with a single layer coating allowing an enhanced disintegration of the coating in the gut and an enhanced release of the enzyme inhibitor prior to the release of the drug. Furthermore, the drug and/or the enzyme inhibitor may each be coated with different coating material allowing gradual release of the drug and/or the enzyme inhibitor within the gut.

The present invention thus provides microcapsules encapsulating bioactive compounds, which in the absence of the coating are normally absorbed at the upper

portion of the gut. The encapsulation allows these compounds to reach the lower gut and to exert their beneficial action, without being inactivated prematurely.

The examples provided herein refer to curcumin but are applicable to any orally administered drug. Examples of drugs known to be metabolized by gut enzymes, e.g. P450 are well known in the art and may be found for example in "Text book of personalized Medicine" - Kewal K. Jain; Springer Science business madia; 2009; pp 73-74.

Accordingly, in one specific embodiment the present invention provides encapsulated curcumin. Encapsulation may be performed by any method known in the art, for example, a fluidized bed coater – using a Wurster process. In accordance with the invention curcumin may be obtained from various sources including, but not limited to, Oleoresin Turmeric, or curcuma root.

In one embodiment, curcumin content in the microcapsule is in the range of about 60% to about 90%. In one embodiment, curcumin content in the microcapsule is at least 80%. In another embodiment, curcumin content in the microcapsule is at least 85%. The remaining constituents of the microcapsule include the coating material and optionally additional active ingredients e.g. gut enzyme inhibitors which enhance curcumin activity.

In one embodiment, gut enzyme inhibitor content in the microcapsule is between about 5% and 10%. In one specific embodiment gut enzyme inhibitor content in the microcapsule is about 8.5%. In another specific embodiment said gut enzyme inhibitors include at least one of Piperine, Quercitin or Genistein or any combination thereof. In one specific embodiment said gut enzyme inhibitors are provided in equal proportions. In another embodiment said gut enzyme inhibitors are provided in unequal proportions.

In a non-limiting example Curcumin is encapsulated with a double coating, the first being Ethocel 100 and the second being HVO while the gut enzyme inhibitors are divided into aliquots and coated with 5% Ethocel 100, 10% Ethocel 100, 15% Ethocel 100 and 20% Ethocel 100. In one aspect the microcapsules of the invention are incorporated into a consumable product, i.e. food or beverage for human or animal consumption.

In one embodiment, the microcapsules of the invention are incorporated into starch-based material.

In one embodiment, the microcapsules of the invention are incorporated into dry pasta.

In another embodiment, the microcapsules of the invention are incorporated into bread or bread crisps.

The present invention therefore pertains to consumable products containing the microcapsules of the invention.

In one embodiment, said consumable products have a curcumin content of between about 0.5% to 5% w/w. In one specific embodiment, said consumable products have a curcumin content of about 1% or 1.2% w/w.

In one embodiment the composition of the invention is for use as a food supplement. In one specific embodiment curcumin content in said composition for use as food supplement is between about 85% and 95%. In certain embodiments said composition is provided as a tablet, powder or capsule.

In another aspect, the present invention provides a pharmaceutical composition comprising encapsulated gut enzyme inhibitors and encapsulated curcumin. Said pharmaceutical composition may be used for the treatment of any disease known to be affected by curcumin e.g. inflammatory diseases, infectious diseases, or cancer.

In one specific embodiment the present invention provides a pharmaceutical composition comprising encapsulated gut enzyme inhibitors and encapsulated curcumin for the treatment of cancer.

In said pharmaceutical composition the gut enzyme inhibitor content in the microcapsule is between about 5% and 10%. In one specific embodiment said gut enzyme inhibitors include at least one of Piperine, Quercitin or Genistein or any combination thereof. In one specific embodiment said gut enzyme inhibitors are provided in equal proportions. In another embodiment said gut enzyme inhibitors are provided in unequal proportions.

In a non-limiting example Curcumin is encapsulated with a double coating, the first being Ethocel 100 and the second being HVO while the gut enzyme inhibitors are divided into aliquots and coated with 5% Ethocel 100, 10% Ethocel 100, 15% Ethocel 100 and 20% Ethocel 100.

Examples

Production of encapsulated curcumin

Different types of curcumin-containing capsules were produced, having varying characteristics including different compositions (e.g. resistant starch or cellulose), size, thickness of the coating, and the presence or absence of oil in the coating, as follows:

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 85% ± 2%

Coating material: Cellulose derivatives (total of 15% ± 2%) including: Ethocel 10 (13.2%) + Castor oil (1.8%)

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Coating material: Cellulose derivatives (total of 15% ± 2%) including: Ethocel 100 (13.2%) + Castor oil (1.8%).

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 90% ± 2%

Coating material: Cellulose derivatives (total of 10% ± 2%) including: Ethocel 100 (8.8%) + Castor oil (1.2%).

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 80% ± 2%

Coating material: Cellulose derivatives (total of 20% ± 2%) including: Ethocel 10 (17.6%) + Castor oil (2.4%).

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 76.5% ± 2%

Coating material: Cellulose derivatives (total of 8.5% ± 2%) including: Ethocel 100 (7.48%) + Castor oil (1.02%); + Hydrogenated Vegetable Oil 15% ± 2%.

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 72.25% ± 2%

Coating material: Cellulose derivatives (total of 12.75% ± 2%) including: Ethocel 100 (11.22%) + Castor oil (1.53%); + Hydrogenated Vegetable Oil 15% ± 2%.

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 72.25% ± 2%

Coating material: Cellulose derivatives (total of 12.75% ± 2%) including: Ethocel 10 (11.22%) + Castor oil (1.53%); + Hydrogenated Vegetable Oil (15% ± 2%).

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 68.0% ± 2%

Coating material: Cellulose derivatives (total of 17% ± 2%) including: Ethocel 10 (14.96%) + Castor oil (2.04%); + Hydrogenated Vegetable Oil (15% ± 2%).

Production of encapsulated curcumin + gut enzyme inhibitors

Active material: Oleoresin Turmeric (as Curcumin > 95%) – 70.0% ± 2%
Encapsulated gut enzyme inhibitors: (Piperine, Quercitin and Genistein in equal proportions) – 8.5%

Coating material: Cellulose derivatives (total of 7.77% ± 2%) including: Ethocel 100 (6.84%) + Castor oil (0.93%); + Hydrogenated Vegetable Oil (HVO) (13.73% ± 2%).

Curcumin was encapsulated with a double coating, the first being Ethocel 100 and the second being HVO. The mixture of gut enzyme inhibitors was divided to four equal portions: one coated with 5% Ethocel 100, the second coated with 10% Ethocel 100, the third coated with 15% Ethocel 100 and the fourth coated with 20% Ethocel 100.

The curcumin capsules and the gut enzyme inhibitors capsules were mixed and incorporated into the consumable products as described below.

The source of curcumin was an Oleoresin Turmeric (having a content of bioactive compounds higher than 95%). The encapsulation was performed by the use of a fluidized bed Wurster coater using the various formulations.

Analytical procedure to quantify curcuminoids in starch based material

Curcuminoids content in dry pasta and bread crisps was determined after extraction with ethanol and following analysis by HPLC equipped with UV-VIS detector. A novel extraction procedure was developed in order to obtain a recovery of near 100%. Each sample was extracted after freeze drying and grinding. Briefly, 50 mg of each sample were weighted and a pre-digestion step with 30 µl of Viscozyme™ mix (purchased from Fluka) and 1 ml of acidified water (pH = 3.0) was performed in a shaking bath for 16 hours at 40 °C. This pre-digestion step was necessary for 2 main reasons 1) during the pasta and bread crisps preparation process curcumin forms non covalent complexes with starch; 2) in food products enriched with encapsulated curcumin, coating material can be either intact or partially destroyed by food processing. Both factors may affect extraction efficiency, making curcuminoids partially inaccessible during solvent extraction. After enzymatic digestion, extraction was carried out for three times with 10 ml of ethanol and one more time with 5 ml

ethanol. After solvent addition, samples were vortexed for 2 minutes and then centrifuged at 4000 rpm for 10 minutes at 4 °C. All upper phases were collected together and total volume was adjusted to 40 ml. After ultracentrifugation at 12000 rpm for 5 minutes at 4 °C, 20 µl of extract was injected. Chromatographic separation was performed with Shimadzu 10A VP HPLC system, using a Prodigy 5u ODS3 100 A column, size 250 x 4.60 mm purchased by Phenomenex. Elution was carried out with isocratic method with 50% A phase (0.1 M formic acid in water) and 50% B phase (acetonitrile) and carrier flow was 0.800 ml/min. Curcuminoids were detected by UV-vis detector at a wavelength of 262 nm.

Analytical procedure to quantify curcuminoids in human plasma, urine and feces

Extraction of curcumin and its metabolites curcumin glucuronide, curcumin sulfate and hexahydrocurcumin from serum and urine was performed by method described by Shaikh et al. (Eu J Pharm Sci (2009) 37, pp: 223-30) with some modifications. For serum, 500 µl of sample were extracted twice with 2 ml of ethyl acetate, vortexed 30 sec and centrifuged at 4000 rpm for 10 minutes at 4 °C. Upper phases were collected together and total extract was dried under nitrogen flow. Dried extract was re-suspended with 50 µl of methanol. For urine samples the same procedure was employed but with 3 ml of sample and 4 extraction steps. Curcumin and curcumin sulfate extraction from feces was carried out by a method reported by Sharma et al. (Clin Cancer Res (2001) 7, pp: 1894-1900) with some modifications. 2 ml of acetonitrile:water 70:30 mix were added to 1 ml of fecal suspension (10% w/v in PBS containing 2mM BHT). After shaking by vortex 30 sec and centrifugation at 4000 rpm for 10 minutes at 4 °C, curcuminoids in the upper phase were separated from other fecal constituents by C18 solid phase extraction and eluted from the cartridge with acetonitrile (2 ml). Finally, 30 µl of curcuminoids extract from feces, and re-suspended extracts from serum and urine, were injected into LC/MS/MS system, that consisted in PerkinElmer Series 200 LC system, coupled with API3000 LC/MS/MS system. Curcumin and metabolites separation was accomplished using the same column, liquid phases (with A:B phases ratio of 30:70), and carrier flow used for curcuminoids analysis in food products.

RESULTS

Production of dry pasta with curcumin in free and encapsulated form

Dough was prepared with the addition of the functional ingredient. A longer pre hydration step of semolina and the functional ingredient was necessary. Laminated pasta was produced as well as an extruded product (Rigatoni).

Sensory acceptance in curcumin-enriched pasta

Two kinds of curcumin-enriched pasta shaped like Italian “tagliatelle” were made: one with curcumin in encapsulated form (curcumin content about 85%), and the other with a free curcumin powder (curcumin content 95%).

These two products were made by partially replacing flour with the curcumin-based ingredient, to obtain a curcumin content corresponding to 1% in the final product. The drying process, to reach a moisture content of 12 % was made at low temperature (25-32°C) and for a long time (24 hours) to reduce, as much as possible, the degradation of bioactive ingredient.

Data indicate that colour intensity and pungent smell were more pronounced in pasta containing free curcumin powder. No significant differences were found for sweet taste whereas the encapsulation process masked the unpleasant curcumin bitter taste perception.

This bitter taste, which is a typical feature of curcumin based products, was one of the main difficulties for the consumer acceptance of this kind of products. In fact, the pasta enriched with encapsulated curcumin was preferred by the large majority of the subjects.

Curcumin stability to the preparation process

To evaluate curcuminoids stability, chemical analysis of dry pasta and cooked pasta was performed.

The pasta was subjected to extraction as described above and its content was analyzed using HPLC.

Data reported in Figure 1 indicated that there is no significant difference in curcuminoids stability between pasta enriched with encapsulated curcumin and free curcumine, both in dry or cooked pasta.

To gain further information on thermal stability bread crisps containing curcumin were also produced. In this product much higher temperature (up to 180°C) is reached during the preparation process and the differences in the degree of thermal degradation between free and encapsulated curcumine can be better appreciated. Bread crisps toasted at 180° C for 15 and 30 minutes, with free curcumin and four kinds of encapsulated curcumin were made (1.2% final content).

The types of encapsulated curcumin are as follows:

- 76.5% ± 2% curcumin + Coating material: Cellulose derivatives (8.5% ± 2%) including Ethocel 100 (7.48%) + Castor oil (1.02%); + Hydrogenated Vegetable Oil 15% ± 2%.
- 90% ± 2% curcumin + Coating material: Cellulose derivatives (10% ± 2%) including Ethocel 100 (8.8%) + Castor oil (1. 2%).
- 85% ± 2% curcumin + Coating material: Cellulose derivatives (15% ± 2%) including Ethocel 100 (13.2%) + Castor oil (1.8%).
- 72.25% ± 2% curcumin + Coating material: Cellulose derivatives (12.75% ± 2%) including Ethocel 100 (11.22%) + Castor oil (1.53%); + Hydrogenated Vegetable Oil 15% ± 2%.

Table 1 shows the ingredients for preparing bread crisps with free and encapsulated curcumin.

Table 1

Sample	**Ingredients amount for 100g of dough**				
	Flour	**Curcumin powder**	**Water**	**Salt**	**Yeast**
Free curcumin	60.08	1.00	36.65	0.73	1.53
CP 76,5% + CM 8,5% + HVO 15%	59.79	1.30	36.65	0.73	1.53
CP 90% + CM 10%	59.98	1.11	36.65	0.73	1.53
CP 85% + CM 15%	59.90	1.18	36.65	0.73	1.53
CP 72,25% + CM 12,75% + HVO15%	59.70	1.38	36.65	0.73	1.53

As shown in Figure 2, encapsulation with a higher percentage coating material (12.75% and 15%) is associated with higher curcuminoids stability in the enriched product, on dry basis, compared to the product enriched with free curcumin. Particularly, this protective effect is more appreciable when toasting is carried out for 30 minutes. With regards to HVO addition, for capsules with high percentage of coating material, it seems to be an additional protective agent for both toasting times. This additional protection is not kept during 30 minutes toasting in capsules containing a lower percentage of coating material.

Bio availability studies in human subjects

The bioavailability of the microencapsulated compounds compared to free curcumin was examined in human subjects.

Three types of bread containing curcuminoids in free form, in microencapsulated form and in microencapsulated form + microencapsulated enzyme inhibitors (2g per 100g serving) were produced.

Ten healthy subjects with no type of gastrointestinal disorders, no recent body weight changes, or any drug therapy, were selected and enrolled to participate in the study.

In the study, volunteers were randomized to receive bread with free curcumin, with encapsulated curcumin or with encapsulated curcumin and encapsulated gut enzyme inhibitors.

Briefly, the volunteers had a polyphenol-free diet 2 days before the experiment and throughout. In particular the volunteers had to avoid certain foods such as fruits and

fruit juices, vegetables, chocolate, coffee, tea, whole grains and legumes while bread, pasta, rice, fish, milk and dairy products, meat and meat products were allowed.

Blood samples were collected from the volunteers before and at 0.5, 1, 2, 4, 6 and 24 hours after the consumption of 100 g of the curcumin–enriched bread.

Urine and feces samples were also collected. Two supplements, 100g each, of the curcumin-enriched bread were consumed by the subjects at the experiment day. Thus in the experiment days subjects ingested totally 6 g curcumin.

All biological samples were analyzed to evaluate the concentration of curcuminoids and metabolites using the methods previously described.

Analysis of the level of curcumin and its various metabolites (curcumin glucuronide, esahydroxy curcumin glucuronide and desmethoxy curcumin) in the subjects' sera revealed that consumption of bread containing encapsulate curcumin + the gut enzyme inhibitors (Piperine, Quercitin and Genistein) resulted in an increased and prolonged level of curcumin and its metabolites in the sera as compared with encapsulated curcumin alone and free curcumin (see Fig. 3).

As shown in Figure 4, analysis of total curcumin metabolite content in urine samples 24 hours after curcumin consumption revealed that the highest level was found in subjects who consumed encapsulated curcumin + the gut enzyme inhibitors (Piperine, Quercitin and Genistein, also termed the "enhancer").

Interestingly, consumption of encapsulated curcumin and to an even higher extent, consumption of encapsulated curcumin + enzyme inhibitors significantly increased the concentration of phenolic acids (chlorogenic acid, Ferulic acid, Vanillic acid, diHPA, HPA and HPP) in urine samples obtained 24 hours after curcumin consumption (Figure 5).

Urine and blood data indicated that curcumin encapsulation determined a slowed but continuous absorption of the compound over a supplementation day.

A lower 24h fecal excretion was found after consumption of encapsulated curcumin + enzyme inhibitors (0.52 ng) as compared with free curcumin (31.32 ng). Interestingly, the level of total phenolic acids in faeces samples obtained 24 hours after consumption of encapsulated curcumin + enzyme inhibitors were significantly lower as compared with the phenolic acid content in faeces samples obtained 24 hours after consumption of free curcumin (51.46ng compared with 0.13ng, respectively)

The overall results obtained in the bioavailability tests suggest that encapsulated curcumin + enzyme inhibitors may elicit its bioactivity over a longer time-period compared to the free-compound, or the encapsulate curcumin without an enzyme inhibitor.

References

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CLAIMS:

1. An orally administrable formulation for the controlled release of a drug, said formulation comprising:

(a) A mixture of microcapsules each consisting of a coating material comprising an encapsulation agent and an active agent, wherein said mixture comprises microcapsules comprising as an active agent at least one inhibitor of gut enzymatic and/or transport activity and microcapsules comprising as an active agent at least one drug; and

(b) at least one carrier, adjuvant or excipient therefore.

2. An orally administrable formulation in accordance with claim 1 wherein said microcapsules are being formulated so as to allow differential release rates of said at least one drug and said at least one inhibitor of gut enzymatic and/or transport activity.

3. An orally administrable formulation in accordance with any of the preceding claims wherein said at least one inhibitor of gut enzymatic and/or transport activity is released in the gut prior to the drug.

4. An orally administrable formulation in accordance with any of the preceding claims wherein the at least one inhibitor of gut enzymatic and/or transport activity and the drug are released gradually or in a pulse mode.

5. An orally administrable formulation in accordance with claim 1 wherein said coating material comprises a multilayered coating.

6. An orally administrable formulation in accordance with claim 1 wherein said encapsulation agent is selected from the group consisting of resistant starch, fat coatings, mono and di glycerides, wax, shellac, cellulose based coatings, and any combination thereof.

7. An orally administrable formulation in accordance with claim 6 wherein said cellulose based coatings are selected from the group consisting of Methyl cellulose (MC), Hydroxypropyl cellulose (HPC), Ethyl Cellulose (EC), Hydroxy ethyl cellulose (HEC), hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), Hydroxypropyl methyl cellulose acetate, cellulose acetate phthalate, hydroxymethyl cellulose phthalate, cellulose trimellitate, hydroxymethyl cellulose acetate succinate and any combination thereof.

8. An orally administrable formulation in accordance with claim 6 wherein said coating material further comprises Hydrogenated Vegetable oils.

9. An orally administrable formulation in accordance with claim 1 wherein said microcapsules comprise between about 5% and about 20% w/w coating material.

10. An orally administrable formulation in accordance with claim 1 wherein said drug is selected from the group consisting of curcumin, digoxin, antibiotics (e.g. cyclosporine A), anti neoplastic agent, and viral inhibitors (e.g. HIV protease inhibitors).

11. An orally administrable formulation in accordance with claim 1 wherein said inhibitor inhibits the activity of an enzyme selected from the group consisting of UGT (UDP-glucuronosyltransferase) enzyme family, sulfotransferase enzymes, alcohol dehydrogenase, and p450 enzymes.

12. An orally administrable formulation in accordance with claim 1 wherein said inhibitor is selected from the group consisting of Piperine, Quercetin, Genistein, Glabridin, and any combination thereof.

13. An orally administrable formulation in accordance with claim 1 wherein said inhibitor is a combination of Piperine, Quercetin, Genistein, and said drug is curcumin.

14. An orally administrable formulation for the controlled release of curcumin wherein said formulation comprises:

(a) microcapsules consisting of a coating material comprising an encapsulation agent;

(b) Curcumin; and

(c) at least one carrier, adjuvant or excipient therefore.

15. An orally administrable formulation in accordance with claim 14 wherein said microcapsules comprise between about 60% and about 90% curcumin and between about 10% and 30% coating material.

16. A consumable product comprising the orally administrable formulation in accordance with any of the preceding claims.

17. A consumable product in accordance with claim 16 wherein said product is selected from the group consisting of dry pasta, bread and bread crisps.

18. A pharmaceutical or nutraceutical composition comprising the orally administrable formulation in accordance with any of the preceding claims.

19. A pharmaceutical composition according to claim 18 for the treatment of inflammatory diseases, infectious diseases, or cancer.

Figure 1

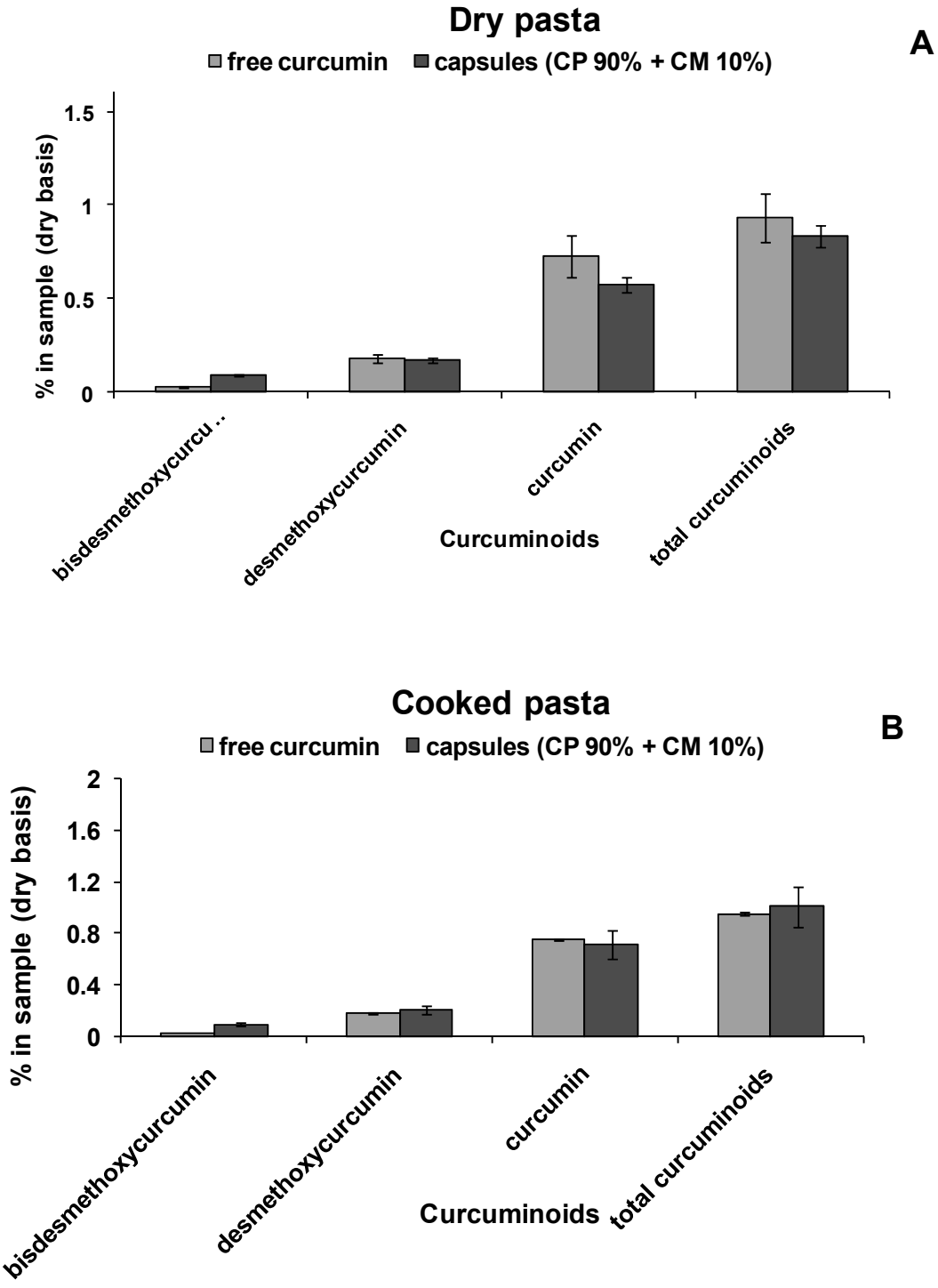

Dry pasta
A
free curcumin
capsules (CP 90% + CM 10%)
% in sample (dry basis)
1.5
1
0.5
0
bisdesmethoxycurcu ..
desmethoxycurcumin
curcumin
total curcuminoids
Curcuminoids
Cooked pasta
B
free curcumin
capsules (CP 90% + CM 10%)
% in sample (dry basis)
2
1.6
1.2
0.8
0.4
0
bisdesmethoxycurcumin
desmethoxycurcumin
curcumin
total curcuminoids
Curcuminoids

Figure 2

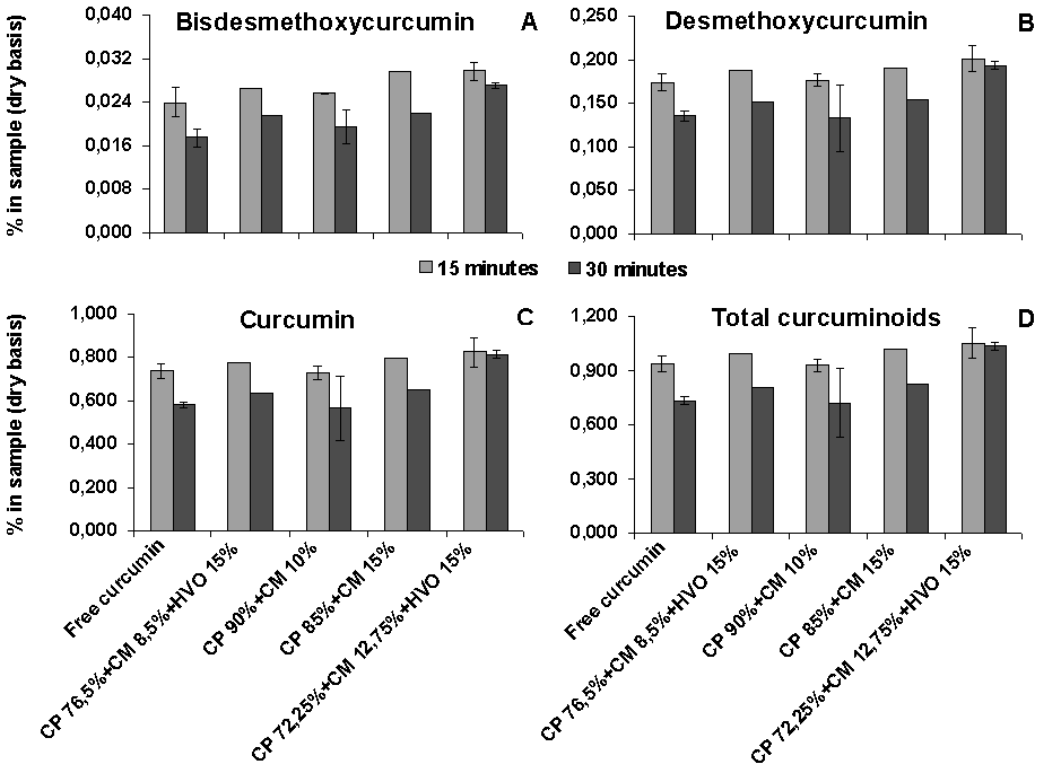

Bisdesmethoxycurcumin
A
Desmethoxycurcumin
B
Curcumin
C
Total curcuminoids
D
% in sample (dry basis)
15 minutes
30 minutes
Free curcumin
CP 76,5%+CM 8,5%+HVO 15%
CP 90%+CM 10%
CP 85%+CM 15%
CP 72,25%+CM 12,75%+HVO 15%

Figure 3

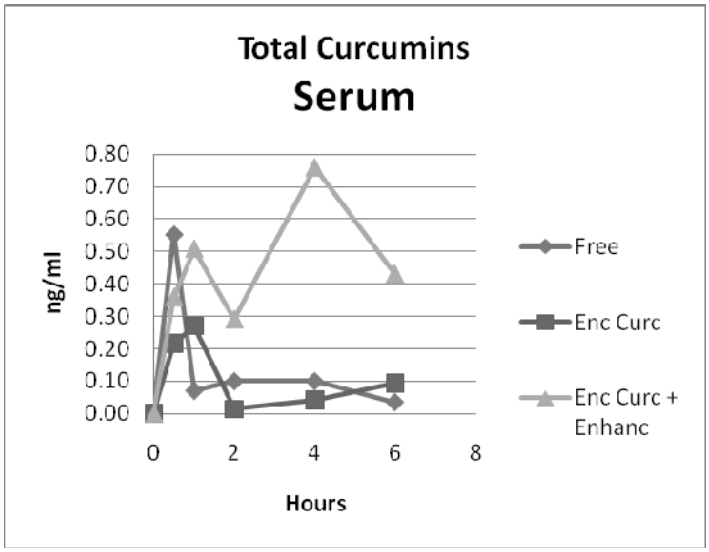

Total Curcumins
Serum
ng/ml
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
2
4
6
8
Hours
Free
Enc Curc
Enc Curc + Enhanc

Figure 4

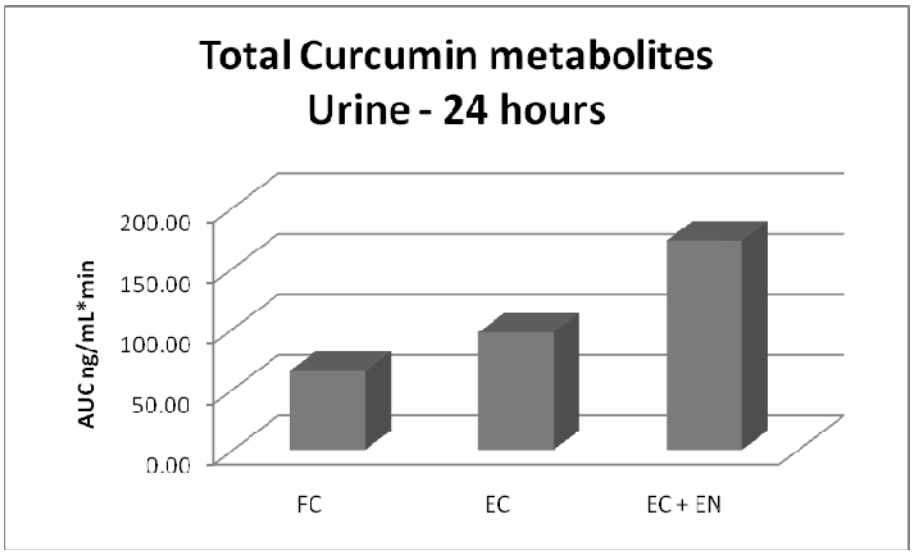
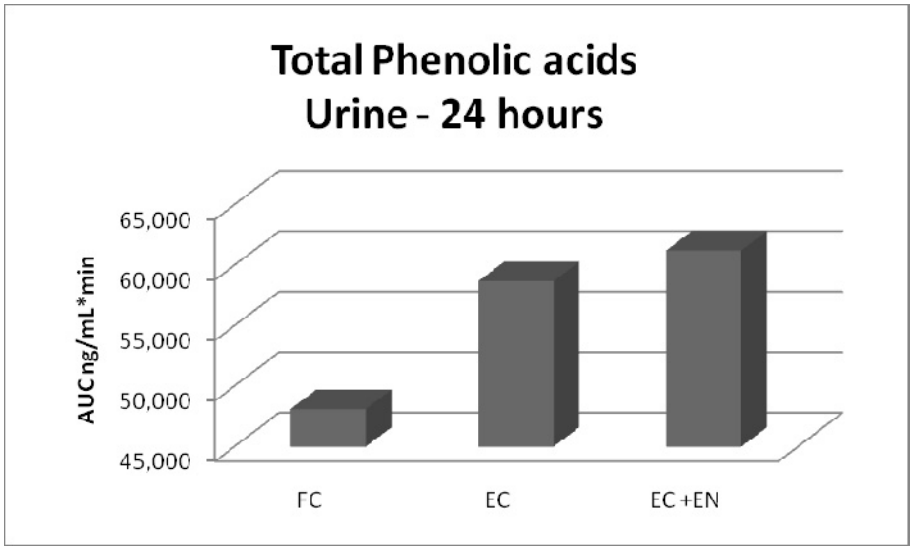

Total Curcumin metabolites
Urine - 24 hours
AUC ng/mL*min
200.00
150.00
100.00
50.00
0.00
FC
EC
EC + EN

Figure 5


Total Phenolic acids
Urine - 24 hours
AUC ng/mL*min
65,000
60,000
55,000
50,000
45,000
FC
EC
EC+EN