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Review article

# Inulin as a multifaceted (active) substance and its chemical functionalization: From plant extraction to applications in pharmacy, cosmetics and food

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ABSTRACT

This review is aimed at critically discussing a collection of research papers on Inulin (INU) in different scientific fields. The first part of this work gives an overview on the main characteristics of native INU, including production, applications in food or cosmetics industries, its benefits on human health as well as its main nutraceutical properties. A particular focus is dedicated to the extraction techniques and to the specific effects of INU on intestinal microbiota. Other than in food industry, the number of INU applications increases dramatically in the pharmaceutical field especially due to its simple chemical functionalization. Thus, aim of this review is also to give practical examples of chemical functionalization performed on INU also by including critical comments based on the direct experience of the Authors. With this aim, a full paragraph is dedicated to practical chemical experiences useful to reduce the efforts when establishing new experimental conditions. Moreover, the pharmaceutical technology is also taken in special consideration by underlining the aspects leading at the preparation of formulations based on INU. At the end of the review, a critical paragraph is intended to feed the scientists' curiosity on this versatile polysaccharide.

## 1. Introduction

The growing need in chemicals not deriving from petroleum-based sources, points on a marked direction of the scientific community: we have to explore natural products, which should not be a second choice, but effective alternatives. In this way, polysaccharides from renewable resources are milestones that must be taken into account. Cellulose derivatives, e.g., have been used for years in critical fields such as pharmaceutical, alimentary or cosmetic. Other important examples are chitosan, starch, dextran, pullulan, hyaluronic acid [1–4]. Some of these are extracted from plants or animals (crustaceous) or obtained by fermentative processes. Among the natural polysaccharides one of the

*Abbreviations:* 5-ASA, 5-aminosalicylic acid; AAc, acrylic acid (AAc); ACP, alternative complement pathway; AM, methacrylic acid; AOT, bis(2-ethylhexyl) sulfosuccinate; APS, using ammonium persulfate; BIO, biotin; BMAAB, bis(methacryloylamino)azobenzene; BNPC, bis-(4-nitrophenyl)carbonate; CAC, critical aggregation concentration; CDI, carbonyl diimidazole; CHPTMAC, 3-chloro-2-hydroxypropyl trimethyl ammonium chloride; CMI, carboxymethyl Cys, cysteine; DCC, dicyclohexylcarbodiimide; DDS, drug delivery system; DDSA, 2-dodecen-1-ylsuccinic anhydride; DLS, dynamic light scattering; DMA, dimethylacetamide; DMAP, 4-(dimethylamino)-pyridine; DMF, dimethylformamide; DMPC, dimyristoylphosphatidylcholine; DMSO, dimethyl sulfoxide; DS, degree of substitution; DTAB, dodecyltrimethylammonium bromide; DV, divinyl sulfone; EDA, ethylenediamine; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; FITC, fluorescein isothiocyanate; GMA, glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; IBD, inflammatory bowel diseases; IBU, ibuprofen; IgG, immunoglobulin G; INCN, cinnamoylated INU; INU, inulin, INU; MCA, monochloroacetic acid; INU-EDA, inulin-(2-aminoethyl)-carbamate; INU-MA, methacrylated INU; LA, lipoic acid; LUV, large unilamellar vesicles; MA, methacrylic anhydride; MAE, microwave assisted extraction; MWCO, molecular weight cut off; NHS, N-hydroxysuccinimide; NHSS, *N*-hydroxysulfosuccinimide; NIPAAm, *N*-isopropylacrylamide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; NIR, near infrared; NMP, N-methyl-2-pyrrolidone; OSA, 2-octen-1-yl-succinic anhydride; PAHy, α,β-polyaspartylhydrazide; PEG, polyethylene glycol; PEGBa, *O,O′*-bis(2-aminoethyl) polyethylene glycol; PEGDM, poly(ethylene glycol) dimethacrylate; PHEA, α,β-[*N*-(2-hydroxyethyl)-D,L-aspartamide], PLE, pressurized liquid extraction; PMDA, pyromellitic dianhydride; RA, rosmarinic acid; SA, succinic anhydride; SPIONs, superparamagnetic iron oxide nanoparticles; TEA, triethylamine; TEOS, tetraethyl orthosilicate; TMEDA, *N,N,N′,N′*-tetramethylethylenediamine; TT, trimethylolpropane tris(3-mercaptopropionate); VITE, vitamin E

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most emerging for its use in pharmaceutics, cosmetics or food, is Inulin (INU).

INU is a polymer from fructose, obtained from renewable resources such as plant extraction or even, more recently, from bio-based productions. In this way, INU is reported in literature as from renewable sources since the plant sources are currently cultivated in different countries and climatic conditions.

Historically, INU was indirectly known by ancient Greeks due to the use of chicory (*Cichorium intybus*) as food, which is rich in INU and one of the main source of extraction. On the other side, chicory was cultivated by Egyptians 5000 years ago. Both Greeks and Romans historical sources report on the beneficial effect of chicory when consumed by humans and, in particular, Galenus reported chicory as a "friend of liver". More recently (19th century), a chicory drink was used as coffee replacement due to the lack in coffee during the Napoleonic wars. Nowadays, chicory coffee is still used in several countries as either coffee replacement or as a drink itself. The spreading in the use of roasted chicory root extract, led to associate its beneficial effects on the intestinal tract to some of the components. The most evident sign for the presence of something belonging to the "sugars" family, was the darkening of the drink after the root roasting due to the INU caramelization. On the other side, even the extremely bitter taste of the original root was greatly reduced after roasting due to the "release" of fructose during the process.

Inulin was discovered in 1804 [V. Rose-Gehlen, Neues Allgem. J. Chem., 3, 217 (1804); and Nicholson, J. Nat. Phil. Chem., 12, 97 (1805)] and named "Inulin" by Thomson in 1818 [5,6]. Since then, numerous studies were carried on to isolate, identify and characterize this polysaccharide as well as to find new production methods by extraction or by the newest biotechnological techniques.

## 2. INU extraction from plants or production *via* biotechnological processes

INU production and industrial translation are processes that have been optimized for years. The main process consists in hot water extraction from the vegetable source (INU is almost insoluble in water at 25 °C while is soluble at 90 °C, about 35% w/v) plus numerous purification steps. It requires a considerable amount of energy and long processing time. The main sources of INU for industrial production are dahlia tubers (Genus: *Dahlia*), Jerusalem artichoke tubers (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) due to their high INU content of, respectively, 10–12%, 14–18% and 14.9–18.3% w/w, Fig. 1 [6,7]. Nowadays, according to Zhu et al., the most used source results the chicory root as this plant is the less sensible to seasonal changes in INU composition [6].

INU extracted from vegetable sources shows a broad molecular weight distribution due to the presence of oligo and polysaccharides in the mixture. These species are almost exclusively composed by fructose repeating units in the form of (2 → 1)-β-D-fructosyl-fructose with, possibly, a terminal glucose unit linked to a fructan structure by an α-D-glucopyranosyl bond, Fig. 2 [8].

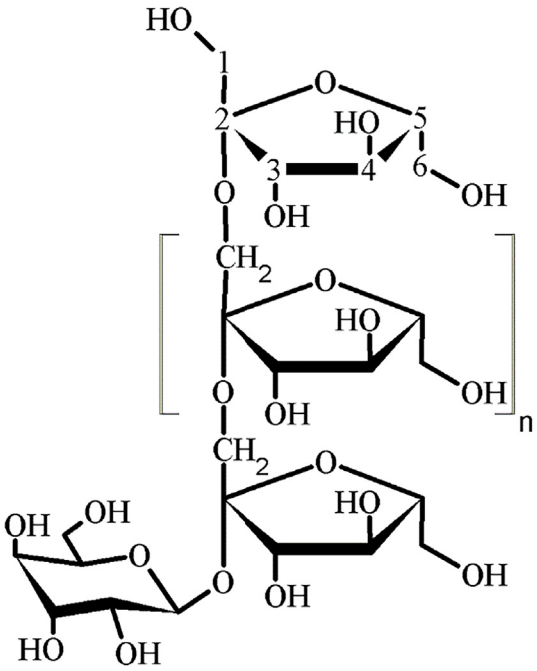



**Fig. 2.** Chemical structure of INU, in the upper fructose ring the carbons number is indicated.

The extraction mixture composition depends from both source and extraction method. The traditional hot water extraction method leads to different yields in extracted INU as depending from, e.g., pH and temperature. In particular, controlling the pH is fundamental since INU is sensitive to acidic hydrolysis when pH falls below about 6.0 [6]. Acidic degradation of INU should be taken into account also when INU is used for specific uses as in pharmacy, cosmetics and food that are the topic of this review.

To improve INU extraction, the enzyme-assisted extraction could be used, assuming that enzymatic degradation of INU could lead to the reduction of the higher molecular weight fractions of INU that results less soluble even at high temperatures. Moreover, enzyme-assisted extractions are even aimed at degrading the plant cell walls to facilitate the diffusion processes and speed up the overall process. An example of this approach is given by the use of protease and hemicellulose during the extraction of soluble fibres such as INU [9,10]. These enzymatic approaches are mostly propaedeutic to the main extraction methods.

Another applied technique for INU extraction is the ultrasound-assisted extraction. It is based on the cavitation phenomenon induced by ultrasounds that improves the plant cell walls disruption with consequent increasing in extraction yield. It would allow using lower temperatures as well as reducing the time of the process [11–13]. Furthermore, in a paper from Laura Ruiz-Aceituno et al., INU extraction


**Fig. 1.** Main vegetable sources of INU (a) Chicory root (*Cichorium intybus*), (b) Jerusalem artichoke (*Helianthus tuberosus*) root and (c) Dahlia tuber root (Genus: *Dahlia*) (Images tagged as free use from Google).

from artichoke (*Cynara scolymus* L.) was accomplished by microwave assisted extraction (MAE) and pressurized liquid extraction (PLE) [14].

Other than extraction methods, several new ways have been proposed for INU production including the biotechnological use of selected enzymes. In a recent (2018) paper from Dawei Ni et al., INU was synthesized from sucrose by using a inulosucrase generated in engineered *E. coli* [15]. The so produced inulosucrase, was used for INU production from sucrose by incubating the enzyme at 25 °C and pH 5.5 for 12 h with 4.5 U of inulosucrase per gram of sucrose. This procedure led to high molecular weight INU with polymer chains in the order of 1000–10,000 KDa. This molecular weight differs a lot from "natural" INU and also suggests a really low solubility of the polymer. Since the enzymatic breaking by inulinase is a well-known method for INU molecular weight reduction, it would not be considered as a drawback. An exhaustive list of commercially available products based on pure INU is reviewed [16].

## 3. From the '90 s to today: How INU beneficial effects on health were assessed and confirmed

In 1993, Roberfroid published one of the first review pointing out the effectiveness of INU as an active substance in improving human health. It was the starting point in exploring the multifaceted properties of this useful polysaccharide that plants use as energy storage [17]. In this first work, Roberfroid, defines INU and oligofructose as dietary fibers since it is not directly digested by human enzymatic pool, instead, it is "transformed" by the action of specific bacteria of the human colon. Thus, INU, into the colon, leads to the production of "by-products" that will be both substrate for the bacteria themselves and free actives for the human body. This concept was well reviewed by the same author with Gibson [18]. In this review, they introduced the concept of prebiotic that is a substance favouring the growth of a bacterial population, possibly selectively. The prebiotics are somehow in contrast with the concept of probiotic that points on adding, in number, from an external source, new bacteria to the gut microflora. Thus, while prebiotics increase the resident bacterial population, the probiotics add new bacteria that are exogenous. To use Gibson and Roberfroid's words: "… prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. Intake of prebiotics can significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota. Non-digestible oligosaccharides in general, and fructooligosaccharides in particular, are prebiotics.".

Starting from these studies a lot of scientific works were produced in multidisciplinary fields so that, while up to 1994 the great part of the literature on INU was on its use for the measurement of renal clearance, since 1995 up to today the great part of the literature is on its pharmacy and food applications.

Kaur and Gupta reviewed and summarized the main beneficial effect of INU when orally administered. In addition to the above-mentioned prebiotic effect, they also highlighted that the growing of *Bifidobacterium* promoted by INU fermentation in the colon, may lead to change the bacterial colonic population by inhibiting pathogenic bacteria also through the formation of bacteriocins. Thus, by competing for substrates or adhesion site on the epithelium, the *Bifidobacterium* growth "overcoming" pathogenic species and also stimulating the immune system [19,20]. In this way, INU is defined as "bifidogenic". Data from studies on humans with intestinal disorders or subject to serious illness, show that INU is capable of re-equilibrate the gut microflora when altered also by inhibiting the disease and by preventing a relapsing, Fig. 3 [21]. The antioxidant activity of INU was recently demonstrated both *in-vitro* and *in-vivo* [22].

One of the most evident effects after INU fermentation by *Bifidobacterium*, is the formation of short chain carboxylic acids (SCFAs) such as acetate, butyrate and propionate, lactate and gases. It has been reported that butyric acid could result in maintaining the health of colonic mucosa while propionic acid seems implied in carbohydrate and lipid metabolism also influencing the motility of the colon [24].

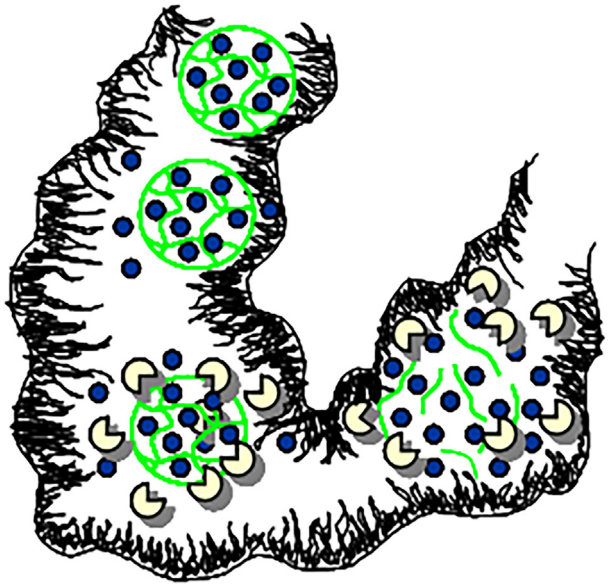


**Fig. 3.** Schematic representation of colonic degradation of a formulation (green lines) incorporating an active substance (blue circles) by enzymes/bacteria normally found into the colon (Readapted with permission from Elsevier, [23]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Different studies point on the positive effect of INU on diabetic patients, in particular for type 2 diabetes. Furthermore, INU has a glycaemic index lower than soluble carbohydrates as well as lower caloric value which helps in obesity treatment [25–28]. The caloric value of INU is only 1.5 Kcal/g instead of 3.5 Kcal/g of glucose [29]. It brings to the use of INU in managing obesity by fortifying foods with INU as a substitute of fats or sugars also thanks to an increased sense of satiety due to INU intake [16,30–32]. Differently from others dietary fibers, INU does not negatively influence the bioavailability of ions of nutritional importance like magnesium, calcium or iron. On the contrary, it has been demonstrated an increase in their adsorption in co-administration with INU, an increase measured in about 60% [33]. These findings brought to evaluate *in-vivo* on either animals or humans, the effect of co-administration of INU and calcium on bone remineralization in patients suffering from osteoporosis, some of the most recent research papers present in literature on this topic from different research groups are here indicated [34–38].

INU was shown effective also in the management of inflammatory bowel diseases (IBD) and colon cancer. In fact, INU intake would improve the general well-being of the gastrointestinal tract and may help in recovering after disturbances [21]. The growth of the beneficial bacteria would prompt the so-called "resistance to colonization" do the possibility to reduce the amount of available nutrients and to the reduction of the lumen pH which determines a lower survival rate for most of the pathogenic bacteria. All of these factors contribute to a proven reduction of infections and different pathologies of the gastrointestinal tract [21]. Treating the IBD seems one of the main outcome of INU consumption [39–41]. IBD are currently managed by the administration of mostly 5-aminosalicylic acid (5-ASA) or corticosteroids. Thus, the administration of INU may be useful in relieving IBD symptoms, in particular, due to the action of butyrate [42,43]. It has been demonstrated that butyrate can inhibit proinflammatory cytokines and reduce the inflammation also by inhibiting the nuclear factor kappa B (NF-κB) activation [44–46].

The role of INU intake in colon-cancer has been also discussed. In particular, it has been seen that INU can exert anticancerogenic properties due to the butyrate production and improvement in *Bifidobacterium* and *Lactobacillus* population [47–51].

Di Bartolomeo et al. published a review titled "Prebiotics in inflammatory bowel diseases: Reality or Fiction?". The question mark anticipates in some way the possibility that the beneficial effects attributed to INU would not be always demonstrated. They reviewed the literature available in that period and concluded, also supported by human *in-vivo* studies, that INU is effective in most of the attributed benefits. In particular, they assert that INU can have a real role in the prevention of mechanisms bringing to different diseases such as diabetes mellitus, colon cancer or even obesity [52]. Other important reviews on INU can be found here [53–55].

On the other side, the discovery of INU as an immune-active molecule was somehow casual. INU was, and is currently used, as a gold standard for the measurement of glomerular filtration and, thus, to asses kidney functionality. INU administered directly intravenously does not bind to plasma proteins and is fully filtered by kidney not being reabsorbed or secreted. It has been administered to thousands of patients with great safety and, only in a marginal number, it caused hypotension. This occasional symptom was further explored and it was found due to the complement activation *via* the so-called alternative complement pathway (ACP) [56–58]. This complement involvement was found caused by a particular form of INU not completely soluble but showing microcrystals from INU contaminants that, actually, activate the complement; this insoluble fraction was called gamma INU [59]. This finding led to the use of INU as a vaccine adjuvant due to the marked possibility of complement activation. INU, in different studies, showed positive effects on adaptive immune response. The first studies were performed *in-vivo* on mice, guinea pigs and rabbits reporting similar results [60]. The studies on INU as adjuvant for vaccines, comprised several microorganisms and animal species pointing on the clear direction of an important activity of this polyfructose with regards to vaccine formulation [61–65].

## 4. Chemical modification of INU as a useful tool for extending its application fields

### 4.1. Practical aspects on INU functionalization: Solubility, purification and recovery

One of the first paper reviewing INU chemical modifications shows that the first approaches to INU modification were mostly based on cellulose chemistry, they also do a comparative study on the chemical differences between INU, cellulose and starch [66].

INU chemistry is mostly related to carbohydrate characteristics plus the fact that the fructose repeating unit forms a polymeric chain that could be broken upon exposure to specific conditions. Keeping the pH of reaction as close as possible to neutrality is of crucial importance as INU is sensitive at both acidic and basic condition [66,67]. These assumptions are especially important for soluble derivatives of INU subjected to further functionalization in water. It should be clear that INU as such is soluble in water at around 60 °C while is almost insoluble at room temperature. This is due to a rather elongated helical structure in solution which results in low accessibility of the polymer to water molecules so reducing hydration of the fructose unites [68,69]. On the contrary, most of chemically functionalized INU derivatives may be soluble in water. Native INU, is soluble in different organic solvents, mostly polar solvents such as dimethyl sulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP), dimethylformamide (DMF) or dimethylacetamide (DMA). While, it is insoluble in solvents such as alcohols (even if, based on our experience, small amounts of methanol or ethanol could be well tolerated), tetrahydrofuran, diethyl ether, halogenated solvents or acetone. Obviously, the broad choice in non-solvents for INU gives a fundamental tool in the purification steps. The before mentioned non-solvents are all effective in precipitating INU but, very often, the solubility (or insolubility) of INU derivatives could change even upon minor modifications of the chemical structure. Thus, small variations in the degree of substitution (DS) of the same derivatizing agent could produce a final product more soluble in a solvent than in another. The trial and error approach, in this case, is always applicable and the starting point are the non-solvents above indicated or their mixtures. Using solvents with low boiling point in which most of the reaction components (by products or not reacted substances) are soluble and can be easily eliminated by supernatant discharge upon separation of the precipitate, e.g., by centrifugation or filtration, is always recommendable. However, collection by centrifugation is often suggested since filtration could prompt the hydration of the final product and subsequent premature solubilisation. Another technique applied to INU post-functionalization purification is the dialysis against water by using dialysis bags with a molecular weight cut off (MWCO) in the range 1–5 KDa. Finally, using an anionic or even cation exchange resin would result in a further purification bringing to products with high purity. Due to the widely available purification methods and to the efficiency of these techniques, INU derivatization is usually carried out with yields close to 100% w/w with respect to the starting polymer. Sometimes, some reagent could not be efficiently eliminated, e.g. dicyclohexylcarbodiimide (DCC), in those cases it is useful re-solubilizing the final product in, e.g., DMSO and to re-precipitate the polymer dispersion in acetone or diethyl ether or in a mixture of both. If the last purification step is performed in water, the best way to recover the final product is by lyophilization. On the other side, if the product is recovered from a volatile solvent, vacuum drying is often sufficient.

When establishing the reaction parameters for INU derivatization, one should always take into account that each fructose unit brings three hydroxyl groups, one (C6) is a primary OH and two (C3 and C4) are secondary OH. The difference in reactivity of these groups is such that, by fixing an adequate molar ratio between the reagent and INU repeating units, the primary OH will be derivatized preferentially.

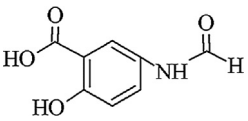
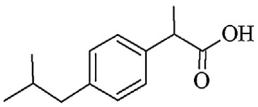
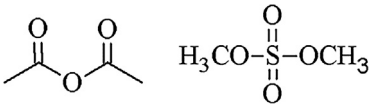
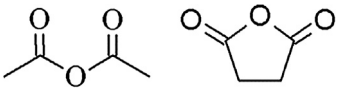
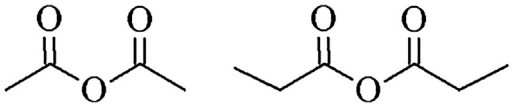
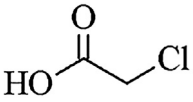
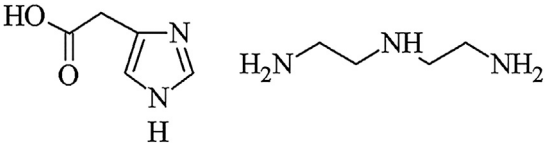
### 4.2. Chemical modification of INU by direct chemistry on fructose ring

Most of the chemical modifications belonging to this group of reactions, were firstly applied to cellulose or starch. An example is the cyanoethylation that was accomplished to apply INU in the textile industry as emulsifier [66]. The reaction was performed in a mixture water/NaOH at 45 °C for 30 min. As in many processes regarding INU, the solution concentration plays a fundamental role in the good course of the reaction, based on our experience, a concentration between 5 and 30 % w/v (g/100 ml) is optimal. The O-(cyanoethyl)inulin is usually used as a starting material for obtaining the corresponding poly-amine or amidoxime by reaction with hydroxylamine. One way to get the polyamine from O-(cyanoethyl)inulin is cyano group reduction by using sodium borohydride using a cobalt chloride hexahydrate as catalyst or Raney cobalt [70]. The –CN moiety can also be converted to carboxyl group in alkaline medium in the presence of sodium peroxide that results in the formation of an amide by the reaction with acrylamide and subsequent hydrolysis to carboxyl group [71]. A INU derivative that is obtained by directly modifying the fructose ring, is the formation of dialdehyde as a result of oxidation by periodate of the adjacent C3/C4 bringing the secondary OH groups. The dialdehyde derivative, can be further oxidized to di-carboxyl acid derivatives for the binding of cations, e.g., in detergent formulation. After the before mentioned method for dialdehyde formation, this product can be further oxidized by sodium chlorite/hydrogen peroxide or sodium hypochlorite or by a Pt catalyst. Also cationic derivatives of INU were prepared and are documented in literature [66].

### 4.3. Chemical functionalization of INU by small molecular weight molecules

Derivatization of INU with small molecular weight molecules such

**Table 1**
Chemical functionalization of INU by small molecular weight molecules.

| Substituent | Chemical formula | Nature of the new formed bond | Refs. |
|---|---|---|---|
| N-formyl-5-aminosalicylic acid |  | Ester | [72] |
| Ibuprofen |  | Ester | [73] |
| Acetic anhydride/Methyl sulphate |  | Ester/Ether | [74] |
| Acetic anhydride/Succinic anhydride |  | Ester | [76,77] |
| Acetic anhydride/Propionic anhydride |  | Ester | [78] |
| Chloroacetic acid |  | Ether | [79,80] |
| Spermine |  | Carbamate | [82] |
| 4-imidazoleacetic acid/diethylenetriamine |  | Ester/Carbamate | [83] |

as, e.g., acetyl groups, strongly influences the physicochemical behaviours of the final product depending from the kind and DS of the substituent, Table 1.

By controlling the reaction stoichiometry, most, if not all, of the INU functionalizations take place at the primary (C6) OH group of the fructose ring. Aimed at forming a prodrug-like structure, Hartzell et al. synthesized a 5-aminosalicylic acid (5-ASA) derivative of INU to be used in the therapy of IBD [72]. In particular, a formyl derivative of 5-ASA (to protect the amine group from 5-ASA) was reacted with INU using carbonyl diimidazole (CDI) as a coupling agent plus triethylamine (TEA) as organic base to deprotonate the primary OH group in C6 of fructose rings. The reaction product was directly dialyzed at the end of the reaction. The INU-5-ASA prodrug was characterized, in particular for its *in-vitro* fermentation in the presence of *Bifidobacterium*. As expected, the Authors found that the INU-5-ASA derivative was not digested but fermented by the gut microflora. It should be noted that the formyl-5-ASA released after fermentation, was not transformed in 5-ASA. In an attempt to introduce a lipophilic moiety to the INU backbone, ibuprofen (IBU) was conjugated to INU by direct esterification of IBU carboxyl group by the INU OH [73]. Also in this case, the reaction was accomplished by using as a coupling agent the CDI, in DMSO at 80 °C for 24 h. The Authors found that the corresponding INU-IBU conjugate self-assembled in micelle-like structures and exploited it to load the hydrophobic drug methylprednisolone. The INU-IBU derivative showed a critical aggregation concentration of $3.0 \cdot 10^{-4}$ g/L. Furthermore, it resulted in an excellent biocompatibility and the complete release of the incorporated drug was accomplished in almost 100 h.

Damian et al., aiming at synthesizing INU derivatives with low solubility in water, accomplished a full derivatization of INU at all or most of the three OH groups of the fructose ring [74]. This is one of the few examples in literature showing a functionalization, in this case, with acetyl or methyl groups not exclusively involving the primary (C6) OH of INU. The reaction was performed in pyridine by adding acetyl anhydride in large excess at 25 °C for 3 h to get the acetyl-INU. In another reaction, INU was solubilized in water plus NaOH 15% w/w and methylsulfate was added to get methyl-INU. The Authors obtained the acetyl-INU with a DS ranging 1.6–2.8, meaning that even at the lowest DS more than one OH for fructose ring was derivatized. Methyl-INU was obtained too but the DS was not calculated as the protons belonging from the methoxy groups and those from unmodified INU had similar chemical shifts. Macroscopically, acetyl-INU was insoluble in water while methyl-INU resulted soluble. Thus, one of the main aim of this paper, due to the modification of a great number OH groups, was to assess whether the final products were still recognized by *Bifidobacteria* even if the main structure of INU was strongly impaired by the modification. As first they tested the degradation of pure INU or the two derivatives in the presence of inulinase that is the enzyme degrading INU [75]. The not soluble acetyl-INU was not degraded by inulinase and also the soluble methyl-INU resulted slightly degradable. Even solubilizing the acetyl-INU in a mixture up to 50% v/v in DMSO, it was not degraded by inulinase. These results are extremely important and paved the way on a better understanding of the mechanisms by which a derivative from INU will degrade. In our opinion also the DS played an important role in strongly reducing the degradation of INU derivatives by inulinase since we found in other studies that not only the kind of the substituent is important but also the DS. The Authors also performed fermentation studies by *Bifidobacteria* on the methyl-INU assessing that even the selected bacteria are not capable of degrading the derivative. These results point in a direction by which each INU derivative,

intended for colon targeting, should be tested for its degradation at least by inulinase. Of the same year of the publication before reviewed, another paper points on the synthesis of INU esters of acetic acid and succinic anhydride; acetyl or succinic derivatives alone were further prepared [76]. In this paper, Wu et al. prepared acetyl-INU-succinate by using the respective anhydrides as starting reagents, differently from the previous work. The reactions were performed in dimethylformamide (DMF) at 40 °C for 24 h. Linking the result of this paper with those from the previous, it appears clear that the solubility in water of the INU derivatives strongly depends from the DS in acetyl groups while succinic group increases the water solubility. Another acetyl derivative of INU was published by Poulain et al. [77]. This paper was aimed at preparing microspheres for drug delivery applications by the coacervation method by exploiting the solubility of the gained acetylated conjugate in organic solvents such as acetone. More recently, Walz et al., published a paper in which acetyl and propionyl INU were synthesized [78]. The study was aimed at obtaining INU based derivatives to be used upon spray drying for the formation of microspheres for drug delivery applications. The hydrophobic model drug used for drug loading was the dexpanthenol that, being soluble in acetone, has been incorporated during the spray drying process. By a pharmaceutical point of view, this is an important feature of these INU esters that can be processed after dispersion in organic solvents where INU itself is insoluble. Unfortunately, except for the first study here reviewed relative to acetyl esters, no further data are available in terms of biodegradation but one can expect that the first shown results could only be confirmed and pointing on a low degradation/fermentation of these esters of INU.

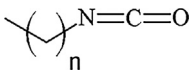
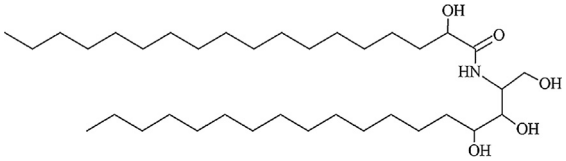
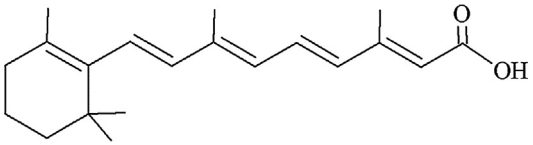
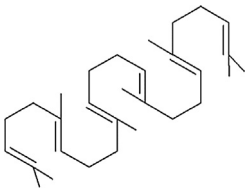
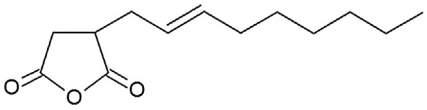
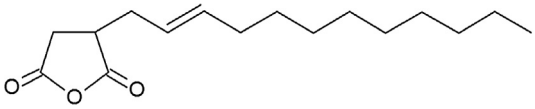
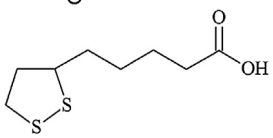
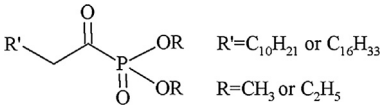
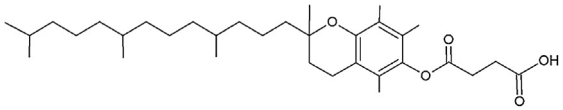
Another important reaction performed on INU, also in this case borrowed from cellulose chemistry, is the carboxymethylation to get the carboxymethyl INU (CMI) [79,80]. This reaction is mostly aimed at introducing pendant carboxyl groups to INU by etherification to obtain a polyanion to be used, e.g., as a dispersing agent or as metal ion carrier. The reaction is usually performed in a NaOH aqueous solution using monochloroacetic acid (MCA) as the derivatizing agent. For drug delivery application, CMI was linked to the surface of silver-graphene quantum dots by carboxymethyl INU by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/*N*-hydroxysuccinimide (NHS) coupling so obtaining a colloid showing on the external layer the biocompatible CMI. In fact, the Authors found that the coated particles had a lower cytotoxicity if compared to naked particles in *in-vitro* experiments. Of particular interest, Ren et al., synthesized a polyamine derivative of INU obtained by direct conversion of C6 primary OH in $–NH_2$ by following a complex scheme of reaction in 3 or 4 steps [81]. INU was further employed for gene delivery applications by providing the main backbone with cationic groups capable of complexing nucleic acids to be delivered intracellularly. In two different works, Sardo et al. functionalized INU with spermine or imidazole/diethylenetriamine to gain colloidal drug delivery systems for gene delivery applications [82,83].

### 4.4. Chemical functionalization of INU to obtain polymeric surfactants

INU is an ideal candidate to be hydrophobically functionalized to get amphiphilic derivatives, Table 2. First of all, its relatively low molecular weight allows the introduction of small molecular weight hydrophobic moieties that would marginally modify the original structure of the polymer. On the other side, functionalizing INU with medium to high molecular weight molecules would require a low number of them for polymer chain. It is particularly important if the bifidogenic properties of INU have to be maintained and also to exploit the favourable characteristics of INU (i.e., does not bind to plasma proteins) when administered parenterally. Obviously, the preservation of INU intrinsic properties should be evaluated case by case. Usually, after functionalization, INU becomes much more soluble even at room temperature reaching a solubility as high as 10–30% w/v in water. This fact not only allows using INU derivatives as surfactants even at high concentration but also prompt the micelle formation and results in low critical aggregation concentration (CAC) values for INU amphiphilic derivatives. As a demonstration of INU versatility in the construction of amphiphiles, several commercial and research products have been shown in the last years. Among the first papers introducing INU amphiphiles, the one belonging to Stevens and collaborators shows the chemical functionalization of INU by alkyl isocyanates known with the commercial name of INUTEC® [84,85]. The reaction was carried out in NMP as polar organic solvent at 80 °C up to 24 h. Interestingly, they also tested DMF which was found as a 3% impurity at the end of the purification process. Based on our experience, INU solubilization in NMP or DMF is a slow process that requires several hours to be accomplished. For this reason, when a reaction has to be performed in DMF or NMP, the dissolution time should be taken into account and the samples should be purged with argon or nitrogen to remove the atmospheric water that can affect the good course of the reaction reducing the overall reproducibility of the process. Even using dry solvents would help. Going back to Stevens's work, they formed alkyl carbamates which shown a stoichiometric DS depending from the amount of added alkyl isocyanates. The Authors underline that the presence of water should be avoided and even INU should be dried at 70 °C for 24 h before use. The purification of the reaction mixture was performed by repeated washing in a non-solvent such as acetone or diethyl ether while the final collection was performed by filtration on glass filters. CAC studies evidenced an aggregation of the octyl carbamates to form polymeric micelles at around 0.01 mg/100 ml. The same INU-based surfactant was also employed for the emulsion polymerization of styrene and methyl methacrylate comparing it with various commercially available surfactants [86]. The same Authors studied the interaction forces between a series of solid particles and an INU surfactant to evaluate its potential stabilization efficiency in aqueous suspension on the particles themselves [87,88]. They demonstrated that the INU surfactant covers the suspended particles by establishing specific interactions as demonstrated by adsorption isotherms studies. The coating is a result of multipoint attachments with loops around the particles that prevent the surfactant-particles system from strong instabilities. Atomic Force Microscopy (AFM) assessed the thickness of the polymeric layer around the particles, in water, that was found to be about 9 nm. Furthermore, the tested particles were found fully covered by INU surfactant at a concentration (in INU surfactant) of about $1 \times 10^{-4}$ mol/ml (0.4 M). Going deeper into the interaction between INU surfactant and hydrophobic surfaces, so considering the liquid/solid interface, Nedyalkov et al. calculated the contact angle and CAC of these surfactants. The contact angle measurements were performed on two different surfaces, both were quartz glasses but one was simply cleaned and used as hydrophilic substrate while the other was silanized so obtaining a hydrophobic substrate [89]. The INUTEC® surfactant was also used for the coating of inorganic particles to improve the stability of the aqueous particle dispersion [90]. The coated particles were obtained by simple dispersion in an INU surfactant solution and collected by centrifugation. The CAC of the surfactant that was found to be $2.2 \times 10^{-5}$ M. Most of the published works on INUTEC® pointed on a great stability of the emulsions formed by this surfactant even in the presence of high amount of salts, this feature allow to propose INU surfactants for their use in cosmetics [91,92].

Licciardi and Giammona developed different INU based surfactants by firstly synthesizing a polyamine derivative of INU that was exploited for further derivatization. Differently from the above mentioned work from Ren [81], Licciardi synthesized a INU polyamine by a one-step procedure by using as a coupling agent the bis-(4-nitrophenyl)carbonate (BNPC) which allowed the subsequent reaction of ethylenediamine (EDA) on the activated primary OH group of INU forming a carbamate and, in particular, the inulin-(2-aminoethyl)-carbamate (INU-EDA) [93]. The reaction was performed as a microwave-assisted synthesis in mild conditions for 1 h. The purification and collection of the reaction product was performed by solvent precipitation first (dichloromethane/

**Table 2**
Hydrophobic substituents used to obtain amphiphilic derivatives of INU.

| Hydrophobic substituent | Chemical formula | Nature of the new formed bond | Refs. |
|---|---|---|---|
| Alkyl isocyanate |  | Carbamate | [84,85] |
| Ceramide VI |  | Ethylene diamine linker Carbamate/amide | [93] |
| Retinoic acid |  | Ethylene diamine linker Carbamate/amide | [94,95] |
| Squalene |  | Ethylene diamine linker carbamate/amide | [96] |
| (2-Octen-1-yl)succinic anhydride (OSA) |  | Ester | [98,99,101,103] |
| (2-Dodecen-1-yl)succinic anhydride (DDSA) |  | Ester | [98,99,101,103] |
| Lipoic acid |  | Ester | [103,120] |
| Acyl phosphonates |  | Ester | [104] |
| Vitamin E |  | Ester | [105,106] |

diethyl ether), followed by SEC purification and collected by freeze drying, finally obtaining a yield close to 100% w/w. The high reactivity of the introduced amine group was exploited to insert as pendant groups the hydrophobic ceramide (via the succinyl ceramide) and a PEG aldehyde chains in a two-step reaction with isolation of the intermediates. The reactions were performed in DMF and collected after precipitation and washing in diethyl ether/THF or diethyl ether/dichloromethane non solvents. The amphiphilic co-polymer showed a CAC of $6 \times 10^{-2}$ and $5 \times 10^{-2}$ mg/mL for not pegylated or pegylated INU-ceramide derivatives, respectively.

A similar synthetic approach was further applied for obtaining self-assembling system based on INU hydrophobically derivatized with retinoic acid [94,95]. These derivatives showed a CAC of 0.135 mg/ml so slightly higher than the ceramide ones. Still belonging to the same class of INU derivatives, the same Authors introduced squalene moieties to form a new kind of INU self-assembling amphiphilic derivatives [96]. Interestingly, these derivatives were used to coat superparamagnetic iron oxide nanoparticles (SPIONs) with the aim of stabilizing these particles when suspended in aqueous environment. They found that INU-squalene derivative had a natural tendency to adhere on the surface of the SPION so exposing its hydrophilic groups belonging to INU to the water allowing a stabilization of the particles by reducing their sedimentation tendency due to their high density. Furthermore, when a magnetic field was externally applied, the coated particles were attracted in the direction of the magnetic field. They also found that the lipoic acid amphiphilic derivative of INU-ethylenediamine had similar results in terms of colloidal stability on SPIONs, magnetic activity and drug targeting [97].

Other examples of INU modifications to gain amphiphilic derivatives are those from alkyl derivatization to form alkyl-esters of INU. In a paper from Morros et al., INU was functionalized by 2-octen-1-yl-succinic anhydride (OSA) or 2-dodecen-1-ylsuccinic anhydride (DDSA) to obtain alkyl-esters with a DS ranged 0.02–2 [98]. The reaction of INU with OSA was accomplished in alkaline aqueous media in the pH range 8.3–9.5 depending from the applied reaction conditions. The mixture of reaction was then purified by dialysis against 50% EtOH/$H_2O$ and recovered by freeze drying. The reaction of INU with DDSA was carried out by dissolving INU in 1 M KOH and $NaBH_4$ (0.01 equivalents based on fructose units) at 25 °C plus 2.6 mmoles of dodecyltrimethylammonium bromide (DTAB) as surfactant to solubilize the

DDSA so allowing to perform the functionalization in emulsion. According to the Authors, DTAB being a cationic surfactant, would predictably be electrostatically bond to the anionic form of INU due a normal acid-base equilibrium, it would happen at the DTAB-micelle/water interface. The Authors hypothesized that this electrostatic interaction could increase the nucleophilic reactivity of INU hydroxyl groups together with the KOH-induced ionization. After equilibrating the reaction mixture, 0.15 equivalents of DDSA were added at 55 °C, the reaction time was about 1 h. The mixture was purified in two steps (1) dialysis against 50% EtOH/$H_2O$ (2) passage through a strong cationic exchange column and, finally, recovered by freeze drying.

Esterification of INU with OSA or DDSA was also performed by Kokubun et al. [99]. In this work, the reaction conditions were slightly modified with respect to Morro's method. In particular, while maintaining the basic aqueous conditions in terms of pH and with comparable time of reaction, both OSA and DDSA were added in ethanolic solution with a molar ratio of 6, 9 or 12%. The Authors found that OSA functionalized INU formed optically clear aqueous dispersions at room temperature while DDSA formed slightly cloudy solutions at room temperature that became clear on heating to 50 °C and remained clear on subsequent cooling. The CAC values of the various derivatives obtained in this study, were shown to be dependent from the kind of alkyl chain (OSA or DDSA) or the final DS of the final products. In particular, the Authors found lower CAC values for DDSA derivatives in the range 0.02–0.2 % w/v (2–20 mg/ml) than that from OSA that was found to be about 0.8% (80 mg/ml) independently from the DS. The hydrodynamic radius for INU-OSA was found in the range 7–13 nm while larger aggregates, 30 nm, were found for INU-DDSA aggregates [100]. The same Authors, further introduced OSA and DDSA by using the acyl chlorides of these molecules applying a synthesis before used for starch [101,102]. Lipoic acid (LA) was also linked to INU by Wang et al., In this work, differently from Scialabba et al. that linked LA through direct DCC coupling of LA carboxyl group on INU OH, they conjugated LA by using its anhydride form [97,103]. The micelles obtained from this INU-LA derivative showed a CAC value of 0.0669 mg/ml (for the sample with a DS of 32.05%) and shown a hydrodynamic size of 130–200 nm for empty micelles or 170–260 nm for transhinone loaded micelles, the zeta potential resulted negative (about −19 mV). Another method for introducing alkyl chains to INU backbone, was shown by Rogge et al. in 2007 [104]. They linked several alkyl chains by using as starting materials a series of acyl phosphonates, performing the coupling in NMP at 85 °C for 24 h. The resulting mixture was purified by dichloromethane or acetone washing and dried under vacuum to get the final products.

A different approach to obtain amphiphilic INU derivatives by coupling it with Vitamin E was shown for the first time by Mandracchia et al., Fig. 4 [105–108]. In this work the Authors coupled the succinylated vitamin E (VITE) to INU by using DCC and N-hydroxysulfosuccinimide (NHSS) in anhydrous DMF using TEA as organic base, the reaction was performed at 25 °C for 12 h. The reaction mixture was precipitated and purified in acetone and finally recovered by centrifugation and collected as a white powder after vacuum drying with yields close to 90% w/w. The effect of the reaction temperature on final DS by varying the molar ratio VITE/INU was evaluated and the Authors found that increasing the reaction temperature from 25 to 40 °C did not significantly changed the final DSs that remained within the range of the standard deviation. Interestingly, the whole reaction process, including the VITE activation by NHSS, was performed in bulk (one step) to reduce the environmental impact of the process. The CAC was calculated by applying two different methods, namely, the "classic" pyrene method and the curcumin method introduced in the literature by Mondal and Ghosh [109]. The values gained from CAC studies evidenced a clear dependence on the DS, as previously seen for others INU amphiphiles here reviewed, and in the range $10^{-2}$ to $10^{-3}$ mM (about 0.4 mg/ml) as from pyrene method. The obtained INVITE derivatives self-assembled in polymeric micelles showing, even in this case, a hydrodynamic size as determined by dynamic light scattering (DLS), dependent from the DS thus obtaining larger micelles 60 nm for lower DSs or 24 nm for higher DSs; the zeta potential resulted slightly negative for INVITE micelles (−20/−30 mV), it was attributed to a partial dissociation of hydroxyl groups from fructose that, as known, is the most acidic carbohydrate after lactose and maltose, this phenomenon would also be increased by the use of the TEA during the reaction; also the absorption of hydroxyl anions from water has been accounted as a reason for the negative zeta potential of polysaccharides [110].

Going deeper into the conformation of INVITE micelles, Catenacci et al., found that the hydrophobic core was mainly formed and stabilized by π-π stacking of the benzopyran-6-yl system belonging to VITE [111]. Starting from the assumption that micelle formation is thermodynamically driven by the hydrophobic association of the amphiphile in a core-shell structure, they hypothesized that, in water, the INVITE surfactant could form what they called a "dry zone" that is a water-exclusion zone formed by the association of the hydrophobic tails belonging to INU. The Authors found that registering the $^1$H NMR spectrum of INVITE in $D_2O$ the protons belonging to the alkyl chain of VITE were still detectable and quantifiable thus indicating that those tails were still exposed to the water even after the micelle formation. On the other side, the aromatic benzopyran-6-yl system from VITE resulted undetectable by $^1$H NMR in $D_2O$ because it excluded the water from the hydrophobic core of the micelle that, consequently, was mostly formed by the π-π stacking of these aromatic groups, Fig. 5.

Even at the dry state, e.g. after lyophilization, the INVITE micelles were well-detectable by SEM and with a good TEM resolution as discrete particles, Fig. 6 [112,113].

Further improvements of the previous amphiphilic INU derivatives were shown by Mandracchia et al. [114]. In this paper INVITE derivatives were derivatized to provide the resulting micelles with drug targeting molecules such as biotin (BIO). To characterize these systems either *in-vitro* or *in-vivo* they were additionally functionalized with fluorescein isothiocyanate (FITC) as a fluorescent probe or Cy5.5 as a probe for *in-vivo* biodistribution studies by near infrared (NIR) fluorescence imaging, Fig. 7.

This paper shows also one of the first *in-vivo* biodistribution study by using NIR imaging on INU derivatives, Fig. 8. The starting hypothesis was that both INU and BIO do not bind to plasma proteins, thus, when a system composed of both exposes these substances to plasma environment it may persist for time in the systemic circulation. For these reasons, the INVITE and INVITE-BIO systems were designed and tested as long-circulating/drug targeted (BIO) drug delivery system.

Finally, the INVITE system was further functionalized with succinic anhydride (SA) to introduce pH-sensitive moieties to the resulting nanomicelles [115,116]. This chemical modification was accomplished to obtain drug delivery systems for hydrophobic drugs for, e.g., colon delivery and targeting. The found CAC values for INVITESA derivative were in the order of $10^{-3}$ mM that resulted, so, very similar to those of INVITE, being of the same order of magnitude [117].

Lately, INU was also proposed for cosmetic applications as active ingredient or as surfactant with particular regard to stearoyl inulin [118,119].

### 4.5. Chemical functionalization of INU to obtain crosslinkable derivatives (hydrogels)

INU was chemically functionalized with specific substituents to obtain crosslinkable systems usable in a wide range of applications and, in particular, to design DDS, for the colon-specific release of bioactive molecules, Table 3, due to its specific fermentation by the colonic *Bifidobacteria* and *Lactobacilli* and the possibility to easily introduce functional groups in its side chain. Vervoot L. et al., prepared an INU hydrogel by free radical polymerization of aqueous solutions of methacrylated INU (INU-MA) using ammonium persulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) as radical initiators. INU-MA polymer was obtained by reaction of INU with glycidyl

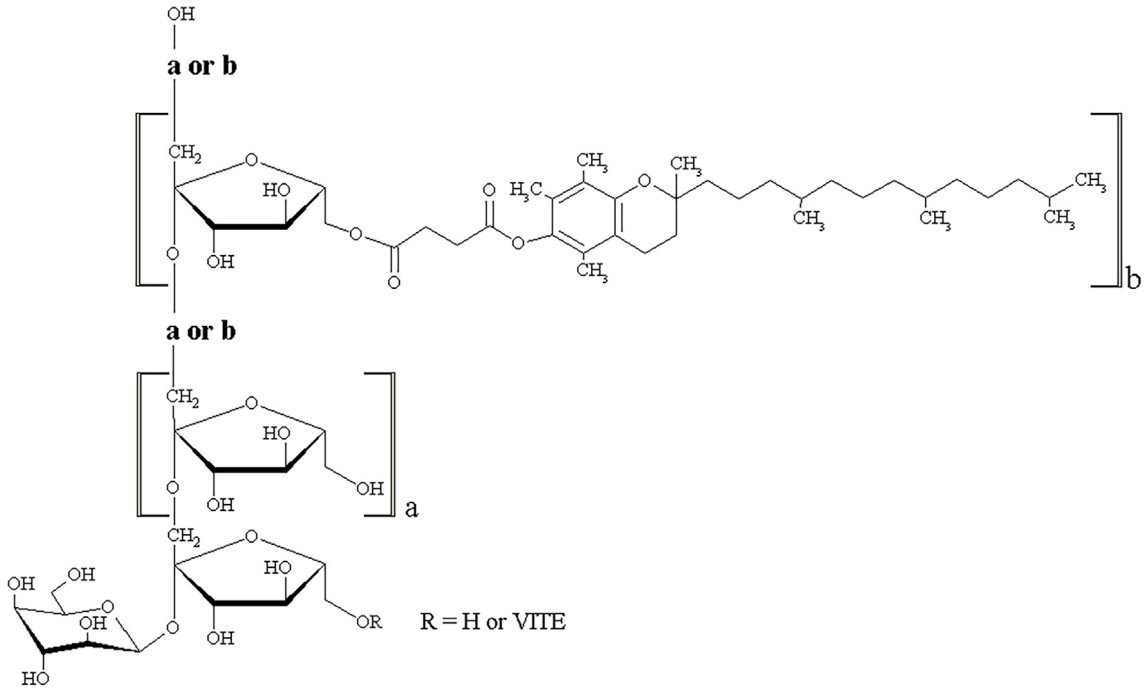



**Fig. 4.** Chemical structure of INU functionalized with vitamin E to gain the surfactant INVITE.

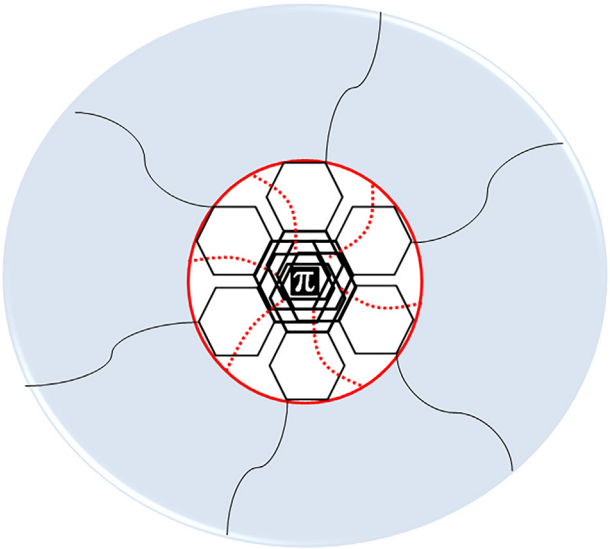


**Fig. 5.** Drawing of the hypothesized structure of INU-vitamin E (INVITE) micelles showing the hydrophobic core formed by π-π stacking of the benzopyran-6-yl system belonging to vitamin E while alkyl chains are found in the hydrophilic shell (Reproduced with permission from Wiley, [111]).

methacrylate (GMA) at room temperature using 4-(dimethylamino)-pyridine (DMAP) as a catalyst [121]. In a first research paper, the Authors analyzed the hydrogel formation process and its rheological properties [122].

The same Authors followed up the characterization of the prepared INU hydrogels by studying the dynamic and equilibrium swelling properties and the glass transition temperature (Tg) as a function of DS, polymer concentration (INU-MA) and concentrations of the initiators. Moreover, considering the potential application of INU hydrogel as colonic DDS, the Authors tested also the effect of pH, ionic strength and esterase degradation [123]. All tested hydrogels showed a high rate of swelling, within 1.12 h and the equilibrium swelling of the hydrogels exhibited a direct relationship with the DS, INU-MA concentration and ionic strength of the swelling media. But the equilibrium swelling ratio, evaluated in media at pH values mimicking those commonly encountered in the small intestine and in the colon with or without esterase, did not change, while at pH values of the stomach the hydrogels underwent an increase of swelling ratio or even completely dissolve the hydrogels, depending on the exposure time.

With the aim to investigate the enzymatic degradability of the prepared INU hydrogels Vervoort L. et al., evaluated the effect of enzyme concentration, incubation time, degree of substitution and concentration of polymer on INU hydrogel degradation. The obtained data revealed that INU hydrogels can be still enzymatically degraded by

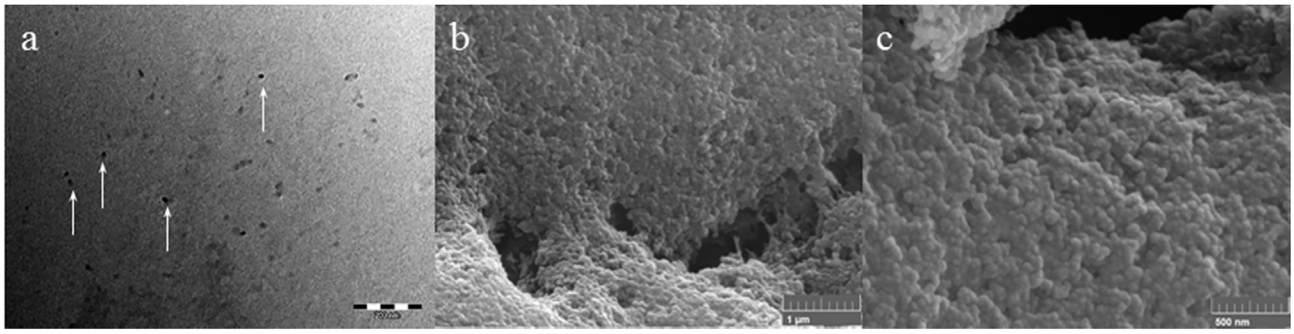



**Fig. 6.** TEM (a) and SEM (b and c) pictures of INU-vitamin E (INVITE) micelles (reproduced with permission from Elsevier, [112]).

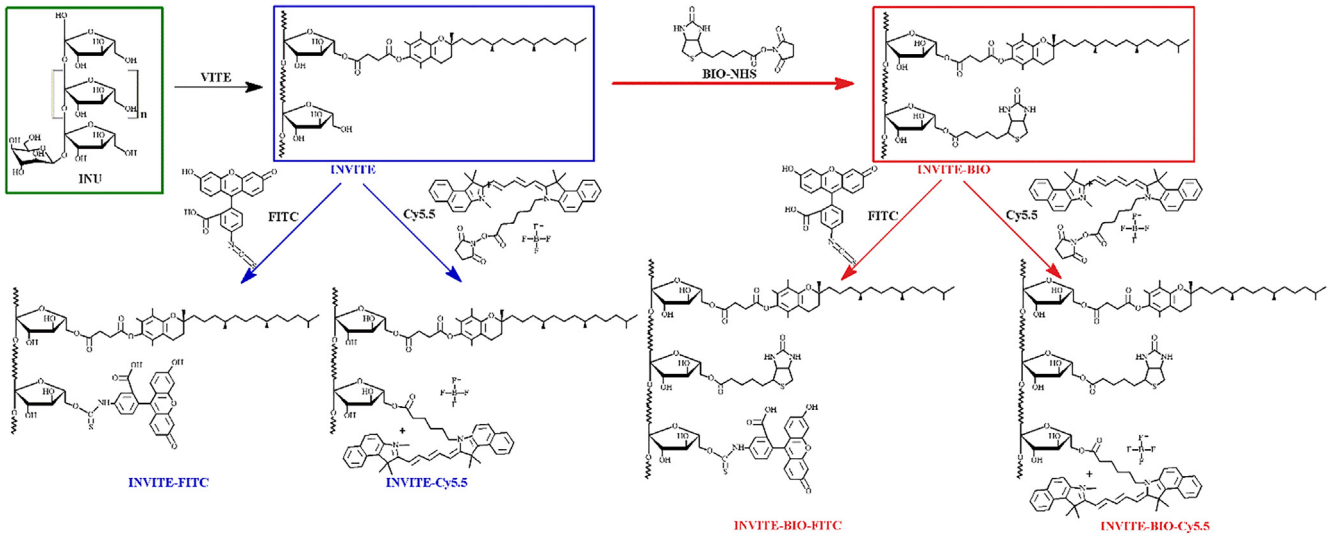



**Fig. 7.** Schematic representation of the reactions to gain amphiphilic INU derivatives (INVITE) further functionalized with biotin for tumor drug targeting (INVITE-BIO) and respective fluorescent (FITC) or NIR sensitive (Cy5.5) derivatives, (reproduced with permission from Elsevier, [114]).

inulinase, although the degradation was dependent on the incubation time, enzyme concentration and DS of functionalized INU. In 2001, the authors combined, within the same material, two different stimuli-sensitive functions in order to increase the susceptibility of the hydrogels toward bacterial degradation [124]. For example, Authors exploited the introduction of azo groups that are specifically reduced into the colon by azo-reductase normally present there. In particular, they crosslinked the methacrylated INU (INU-MA) with bis(methacryloylamino)azobenzene (BMAAB), bringing azo groups, and other monomers such as 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (AM), as hydrophilic and pH-sensitive moieties. The Authors evidenced some concern in obtaining a good compromise between the degree of swelling and drug release, often fast, due to a high degree of swelling.

In a similar study, Chiu H-C et al., prepared a pH-responsive INU hydrogels by radical copolymerization of methacrylated INU (INU-MA) with acrylic acid (AAc) in aqueous solution using APS and TMEDA as initiators and HEMA as hydrophilic comonomer [125]. Adding the AAc in the hydrogel caused an increase of the cross-linking density and a reduction of the swelling, but at the same time a higher pH sensitivity due to the augmented ionic osmotic pressure into the hydrogel. Moreover, the extent of swelling at pH 7.4 was reduced with increasing the DS of INU-MA (increases the effective network density of hydrogels). Overall, the swelling did not follow the expected pattern. The water uptake of hydrogels (DS 4.8) at pH 5.0 and 7.4 decreased first and then gradually increased with the increase in AAc content and the swelling at pH 2.2 decreased with increasing AAc to a more significant extent than expected. Although somewhat surprising, the results showed that the differences in swelling became significantly increased between high and low pH (for example, 7.4 and 2.0) with increasing the AAc content. Whereas, at low contents in AAc, the swelling of hydrogels at pH 7.4 was overcome by the increased crosslinking density, the swelling increased with increasing AAc owing to an enhanced ionic osmotic contribution and low polymerization efficiency.

In a subsequent study, Van den Mooter G. et al., investigated the release of bovine serum albumin (BSA) and lysozyme, chosen as model proteins, from the INU-MA hydrogels and the influence of several parameters on the release rate [126]. The study showed an influence of various parameters on the release of proteins derived both from hydrogel preparation and from protein specific characteristics.

Tripodo et al., prepared INU-based pH sensitive hydrogel by UV crossliking designed as colon drug delivery systems, by avoiding the use of radical initiators. In particular, they prepared INU-MA conjugates by reaction of INU and methacrylic anhydride (MA) or the INU-MA-SA derivative obtained by introducing carboxylic groups through the reaction with SA in the side chain of INU-MA, Fig. 9 [127].

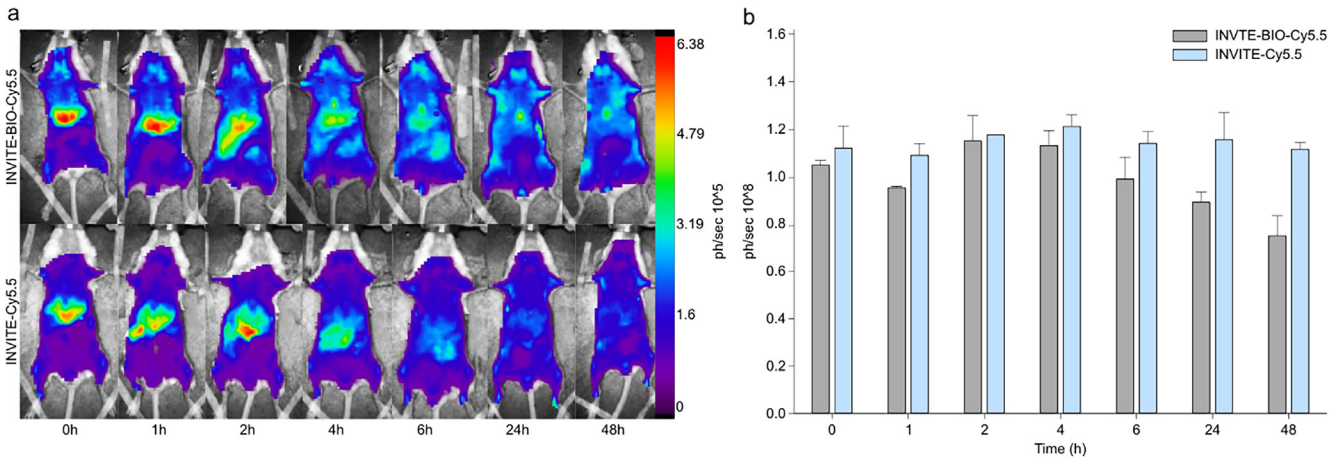
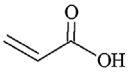
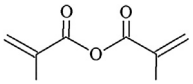
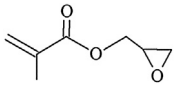
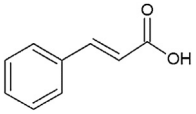
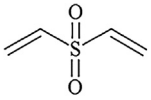
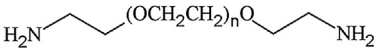
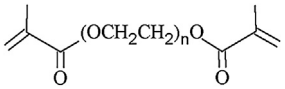
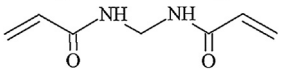
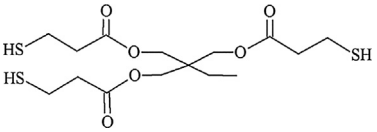
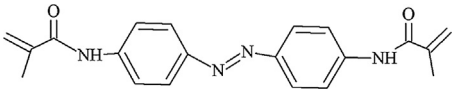
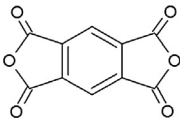



**Fig. 8.** The amphiphilic derivatives of INU, namely INVITE and INVITE-BIO, self-assembling in nanostructured micelles, were tested *in-vivo* by NIR imaging for their long-circulating behaviours, (reproduced with permission from Elsevier, [114]).

**Table 3**
Functionalizing agents used for INU to gain crosslinkable derivatives. Crosslinkers used for obtaining INU hydrogels are further indicated.

| Functionalizing agent | Chemical formula | Nature of crosslinking reaction | Refs. |
|---|---|---|---|
| Acrylic acid |  | Free-radical (initiator) | [125] |
| Methacrylic anhydride |  | Free-radical (UV) | [127,142] |
| Glycidyl methacrylate |  | Free-radical (initiator) | [121,122] |
| Cinnamic acid |  | Chemical/physical | [137] |
| Divinyl Sulfone |  | Chemical | [133,135] |
| *Crosslinker* | | | |
| O,O'-bis(2-aminoethyl)polyethyleneglycol |  | Chemical | [134] |
| Poly(ethylene glycol) dimethacrylate |  | Free-radical (UV) | [130] |
| N,N′-Methylenebis(acrylamide) |  | Free-radical (initiator) | |
| Trimethylolpropane tris(3-mercaptopropionate) |  | Chemical (Thiol-ene) | [133] |
| 4,4′-Di(methacryloylamino) azobenzene |  | Free-radical (initiator) | [124] |
| Pyromellitic dianhydride |  | Chemical | [141] |

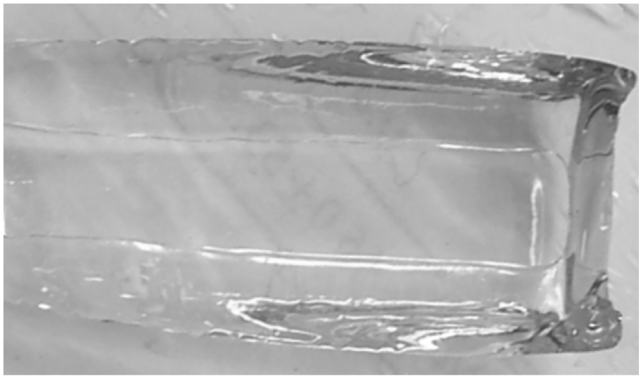


**Fig. 9.** An example of swollen hydrogel obtained by UV irradiation (Reprinted with permission from [128]. Copyright 2006 American Chemical Society).

The reactions were performed in mild conditions in aprotic polar solvent such as DMF, using TEA as organic base. Other than the introduction of methacrylic groups intended for UV crosslinking, the carboxyl groups were included to induce pH-sensitivity. The performed water-uptake studies on the resulting hydrogel evidenced a pH dependent swelling and a pronounced resistance to acidic degradation for INU-MA-SA derivatives, unlike starting INU or the original INU-MA hydrogels. The drug release from INU-MA-SA, unlike INU-MA, was lower in simulated gastric fluid and higher in simulated intestinal fluid. The ability to INU-MA-SA hydrogel to release an anti-inflammatory agent such as diflunisal has been further evaluated in another study by Castelli et al., where a DSC technique was used to evaluate drug release and diffusion from the hydrogels to the site mimicking the interaction with a biological membrane made of large unilamellar vesicles (LUV) of dimyristoylphosphatidylcholine (DMPC) [129].

From the same Authors, the INU-MA-SA derivative was employed for the crosslinking with a polyaminoacid polymer also containing vinyl photopolymerizable groups in its side chain, the methacrylated poly α,β-[*N*-(2-hydroxyethyl)-D,L-aspartamide] (PHEA), called PHM, to give the INUMASA/PHM hydrogels [130]. The network was again obtained by UV irradiation, always without the use of radical initiators and with or without the poly(ethylene glycol) dimethacrylate ($PEGDM_{550}$) used as a co-crosslinker in order to evaluate potential differences in terms of physicochemical properties and release profile. A proteic drug, namely the immunoglobulin G (IgG), was selected as a model of a monoclonal antibody used in the treatment of colon pathologies such as Crohn's disease and ulcerative colitis, and loaded into the hydrogels. The obtained hydrogels were degraded by inulinase and showed a high cell compatibility and modified released of loaded antibodies.

These papers bring to considerations about the new open panorama on the preparation of more sophisticated DDS by which only a tailored combination of polymers of different nature or a specific functionalization can allow to overcome the pharmacokinetic problems related to specific characteristics of active principles such as, stability or solubility as well as to design a real target-specific release and reduce or hopefully avoid toxic side effects.

In this context, Mandracchia *et al.* prepared polysaccharide/polyaminoacid hydrogels by cross-linking of succinic derivatives of INU (INU–SA) with an α,β-polyaspartylhydrazide (PAHy) obtaining the so called INUPAHy hydrogels [131]. The cross-linking reaction was performed by using N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) as a coupling agent and N-hydroxysulfosuccinimide (NHSS) to activate the INU-SA carboxyl group. The INU-SA derivative was further used to form hydrogels by iron coordination of the carboxyl anions from SA [132]. More specifically, INU was functionalized with succinic anhydride to gain the INU-SA derivative that was further functionalized with cysteine to gain the INU-SA-Cys derivative. Cysteine moieties were added to provide the polymer with mucoadhesive groups. Both INU-SA and INU-SA-Cys derivatives resulted degradable by inulinases and showed a high water solubility and resistance to acidic pH. These systems were crosslinked by coordination of carboxyl anions by ferric chloride which promptly oxidized to ferrous ions able to form the coordination network. Even the iron-crosslinked systems resulted degradable by inulinases and, for this reason, the system was proposed the oral therapy of iron deficiency anaemia.

Pitarresi et al., explored a different approach to obtain chemical hydrogels by using multifunctional crosslinkers bearing electron-rich terminal groups such as thiol or amine able to react with acceptor groups such as acrylic or vinyl-sulfone groups [133]. In particular, they reported the synthesis of new biocompatible, stimulus sensitive and biodegradable hydrogels, called INUDVSA-TT (DV = divinyl sulfone; TT = trimethylolpropane tris(3-mercaptopropionate)). The obtained hydrogels showed a good cell compatibility, pH-sensitivity, stability at acidic condition and biodegradability by inulinase and esterase. In another study, the INUDV derivative was employed to prepare hydrogels by crosslinking it with the crosslinker O,O′-bis(2-aminoethyl) polyethyleneglycol (PEGBa) tested at three different concentrations. The hydrogel was obtained in physiological conditions, i.e. in phosphate buffer solution pH 7.4, without use of initiators and at room temperature in 4 h. The obtained hydrogels were transparent, colourless, odourless and resistant to chemical hydrolysis, but degraded by inulinase [134].

In 2014, Sahiner et al., synthesized cross-linked INU microparticles in reverse micelles using a water-in-oil microemulsion polymerization technique [135]. In particular, they crosslinked INU with DV in a sodium bis(2-ethylhexyl) sulfosuccinate (AOT) inverse microemulsion under basic conditions. These particles were found to be excellent scaffolds for the *in situ* synthesis of CdS quantum dots (Q-dots). The INU-based particles were shown to be non-cytotoxic on fibroblast cell culture, and degradable under acidic and basic conditions. Furthermore, gallic acid and caffeine were used as model drugs for loading and release studies from these particles, illustrating their potential as drug carriers with controlled release.

Sahiner et al., in another work, prepared INU-silica and modified INU microgels in a single step via crosslinking within a microemulsion and used them as drug delivery devices [136]. INU-silica composite microparticles were also synthesized in the presence of tetraethyl orthosilicate (TEOS) via a water-in-oil microemulsion polymerization/crosslinking technique. To generate porous INU particles, INU-silica particles were treated with 0.5 M NaOH solution to dissolve silica particles. Furthermore, native INU INU and porous INU microgels P-INU were successfully quaternized by treatment with 3-chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTMAC) in aqueous solution, generating positive charges on the biopolymer as q-p(inulin). Rosmarinic acid (RA) was used as model drug for loading and release studies by synthesized INU-based microgels in phosphate buffer solution (PBS) at pH 7.4. It was shown that the absorption and release rate are influenced by zeta potential and porosity of the microgels. Another interesting work from Lopez-Molina et al., focused on the preparation of microspheres from a cinnamoylated derivative of INU (INCN) studied as a vehicle for the colonic controlled delivery of drugs [137].

Balan et al., studied the possibility to protect the natural antioxidants from the aqueous extract of carqueja by encapsulation in Ca–alginate microbeads and Ca–alginate microbeads containing 10% and 20% (w/v) of INU obtained by electrostatic extrusion process [138]. Results showed also the effect of the presence of INU in the microbeads and they indicated that the addition of INU did not have influence on antioxidant activity of the extract, INU reduced the stiffness of the hydrogel, and protected the bead structure from collapse upon freeze-drying, moreover, it delayed disintegration of the microbeads in simulated intestinal fluids, and reduced swelling rate. So, authors concluded that alginate–INU beads could be candidate for delivery of carqueja aqueous extract in functional food products.

The above-mentioned INUDV conjugate was further developed, in 2015 by Palumbo et al. for its ability to form an *in-situ* forming hydrogel by crosslinking with amino functionalized hyaluronic acid derivatives [139].

The group directed by Prof. Nevio Picci (†), proposed a INU derivative with an antioxidant molecule (catechin) prepared in the presence of the thermo-responsive co-monomer N-isopropylacrylamide (NIPAAm) to obtain hydrogels with controllable antioxidant properties depending on the external temperature [140]. The antioxidant activity of the conjugates was evaluated measuring the scavenger properties of the hydrogels against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and the total flavonoid content at different temperatures. These studies confirmed the controllable antioxidant properties of the hydrogels in response to the thermo-sensitive behaviour of the network.

The most recent paper considered in this part of the review is by Afinjuomo et al., who in 2019 proposed the preparation of a smart INU hydrogel with a pH sensitive release suitable for colon specific drug delivery [141]. The reported hydrogels were prepared by crosslinking INU with pyromellitic dianhydride (PMDA) at room temperature, introducing at the same time ionizable carboxylic acid groups.

When using hydrogel as DDSs, loading hydrophobic molecules could be a major problem. To overcome this issue, Mandracchia et al. in 2018, developed what they called a “nanogrid” where hydrophobic spots formed by INVITE micelles (see above in the INU surfactant part) were surrounded by a hydrophilic hydrogel composed by the methacrylated INU portions crosslinked by UV irradiation [142]. This system was specifically designed for the dual drug delivery of both hydrophilic and hydrophobic drugs for colon drug delivery. In this system INVITE amphiphiles were provided with methacrylic anhydride. The aqueous dispersion of the micelles was exposed to UV irradiation forming a hydrogel surrounding the hydrophobic core of the micelles. This “nanogrid” could load hydrophilic drugs into the hydrogel portion while loading hydrophobic drugs within the micelles core, Fig. 10.

## 5. Concluding critical remarks

Undoubtedly, producing chemicals from renewable resources has major advantages on petroleum-based ones. But not always natural is equal to ecologic since the production of pure materials from, e.g., plants and, in particular, the purification and isolation steps often require more energy and produce more wastes than other methods. Nonetheless, the agriculture processes should be mentioned. So, the process bringing to a full “natural” chemistry must involve the cooperation of a plethora of expertise ranging from engineers to chemists to biotechnologists. The latter contribute being of fundamental importance now and mostly in the next future for “natural” substances production. Obviously, also the fermentative processes have large drawbacks connected with the implementation of the methods and the

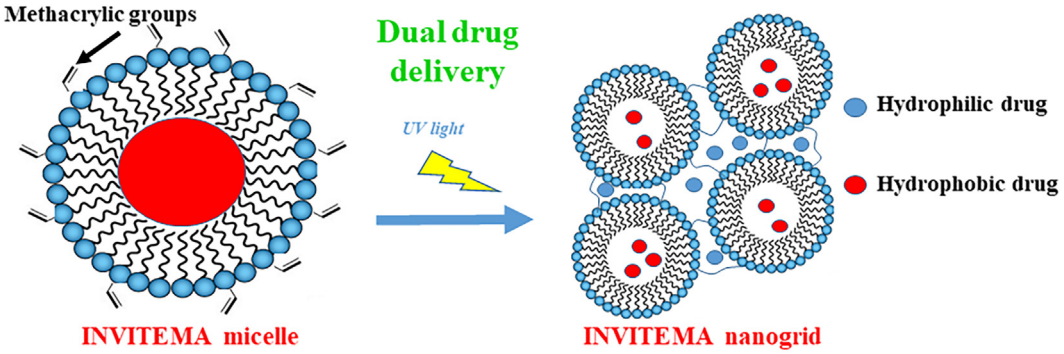



**Fig. 10.** Representation of the hydrogel "nanogrid" from INVITEMA crosslinked micelles for the dual delivery of hydrophilic and hydrophobic drugs (reproduced from MDPI open access, [142]).

management of wastes, but emerging technologies are coming up to improve the recycling operations. As shown in this review, INU has been largely and industrially produced by "classic" extraction methods but in the last years even biotechnological processes have been developed. The biotechnological way would further improve INU use and spreading in the market and in the uses. We have no doubts: Inulin is a versatile polysaccharide. When used as native molecule it finds different applications in particular in food industry and in the emerging application in cosmetics. In the pharmaceutical field it has been used for decades in the renal clearance evaluation and, lately, as adjuvant for vaccine formulation. But, and in this case our opinion is strongly supported by the last literature, INU gives its best when chemically functionalized. As shown here, it is an optimal candidate for the formation of surfactants and some of them are just on the market. INU is also an ideal choice when designed for colon drug targeting and delivery due to its biodegradation by *Bifidobacteria* and *Lactobaccilli* in human gut where, furthermore, it exerts beneficial effects on the mucosa and human health in general. In our opinion one of the most important findings on INU properties was establishing *in-vivo* that micelles intended for drug delivery have long-circulating behaviours up to 72 h. This is important because meaning that the INU micelles are neither eliminated by the immune system nor quickly cleared by the kidneys allowing the incorporated drug to be slowly released or taken up by specific cells, e.g. tumor cells, after providing them with targeting agents. Worth of mention is also the high thermodynamic stability of the colloidal systems formed by such amphiphiles. This stability could be also "transferred" to unstable colloids by coating them with an external layer of INU amphiphiles. This review is also demonstrating that the chemistry applied by different research groups is very often simple, fast, economic and reproducible and frequently performed in mild conditions for short times. It means low energy consumption, low amount of wastes and economy of the processes. If we would be asked to suggest a new product to develop in pharmaceutics, food or cosmetic fields, that would be based on Inulin.

## 6. Dedication

This work is dedicated to Michael Moeller, a very close friend, an extremely focussed and brilliant researcher, a lovely and dedicated husband and father, and a sincere and honest person.

Dear Michael, your premature death left such a big void in all the people that were lucky enough to know you. Thanks, Michael, for everything that you taught us and thanks for being such an inspiring man and researcher for us.

Your smile and the good times that we spent together will always be in our heart, where you will live forever.

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