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Enhancing the therapeutic effects of polyphenols with macromolecules

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Abstract

Well-known for their antioxidant properties, polyphenols are naturally occurring compounds containing one or more phenol groups. A high dietary intake of polyphenols has been linked to a reduced incidence of a number of diseases, including cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. Furthermore, *in vitro* and *in vivo* studies show the potential for polyphenols as therapeutic agents with cardioprotective, antimicrobial, anticancer, neuroprotective, and antidiabetic effects demonstrated. Despite their impressive therapeutic effects, polyphenols suffer from a number of drawbacks, including instability when exposed to light, heat and basic conditions; poor bioavailability; rapid metabolism; and poor membrane permeability. These drawbacks limit the clinical applications of polyphenols. Polymers and other macromolecules are well-known for their ability to stabilise and improve the bioavailability of therapeutic agents. A number of macromolecular systems have been developed that stabilise polyphenols whilst enhancing their therapeutic effects, including direct polymerisation of polyphenol monomers via step-growth, free radical and enzyme catalysed reactions; and conjugation with macromolecules via enzyme grafting, free-radical grafting, esterification and amidation. In this review, we will detail the key techniques employed to stabilise polyphenols with macromolecules and provide examples of each technique.

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1. Introduction

Ubiquitous in the plant kingdom, polyphenols are naturally occurring compounds containing one or more phenol groups, which are well-known for their antioxidant properties. Over 8000 phenolic structures have been identified[1] with polyphenols broadly classified into four main groups – flavonoids, stilbenes, lignans, and phenolic acids[2] (see **Figure 1**).

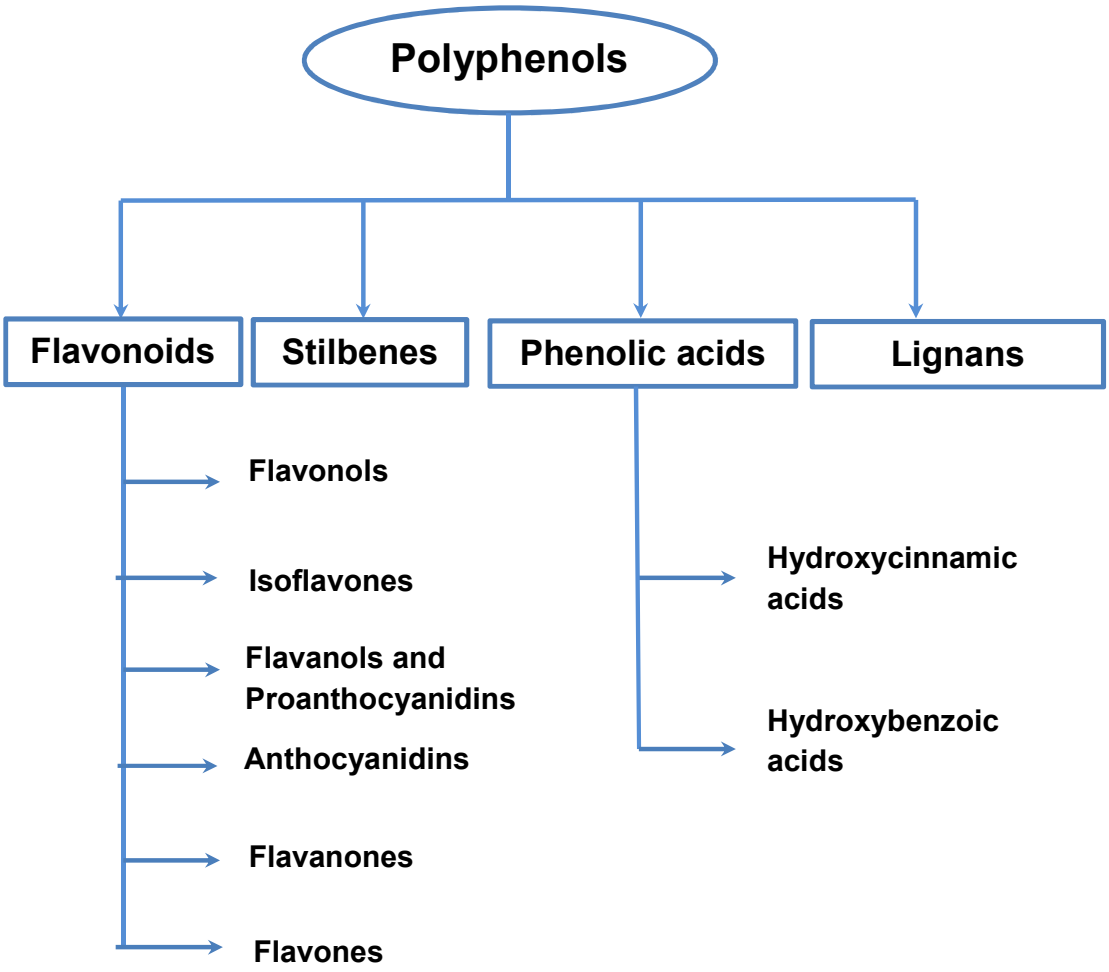

Figure 1: Classification of polyphenols

A large class of polyphenols are the flavonoids comprising flavonols, isoflavones, flavanols (also known as catechins), proanthocyanidins, anthocyanidins, flavanones and flavones. **Figure 2** shows the basic flavonoid structure and numbering system. Partly responsible for the colours displayed by flowers, fruits and leaves, over 4000 flavonoids have been identified in plants.[3] Rich sources of flavonoids include red wine, tea, citrus fruits, green leafy herbs and vegetables, soy beans, legumes and berries.[4] **Figure 3** shows the structure of some common flavonoids.

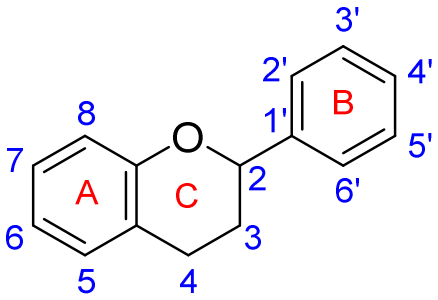

Figure 2: Flavonoid numbering system

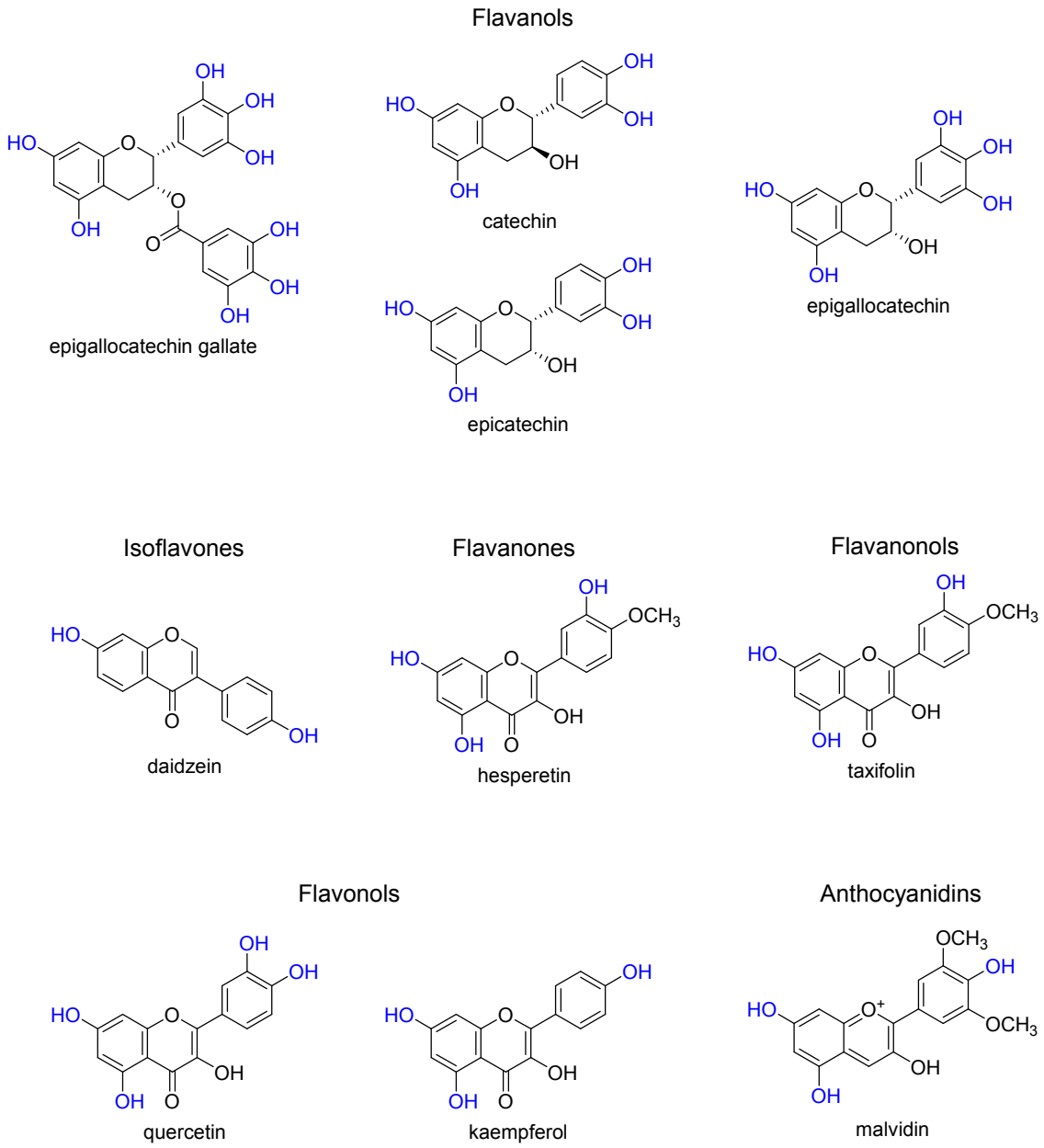

Figure 3: Structures of some common flavonoids

Phenolic acids are an important group among the non-flavonoid polyphenols and maybe divided into hydroxybenzoic acids and hydroxycinnamic acids. The hydroxycinnamic acids are the most common and are found in a number of fruits and vegetables as well as grains, cider and coffee.[5] Although only a small component

of the human diet, stilbenes are an important class of polyphenols with resveratrol having demonstrated anticarcinogenic effects.[5] **Figure 4** shows the structure of some common non-flavonoid polyphenols.

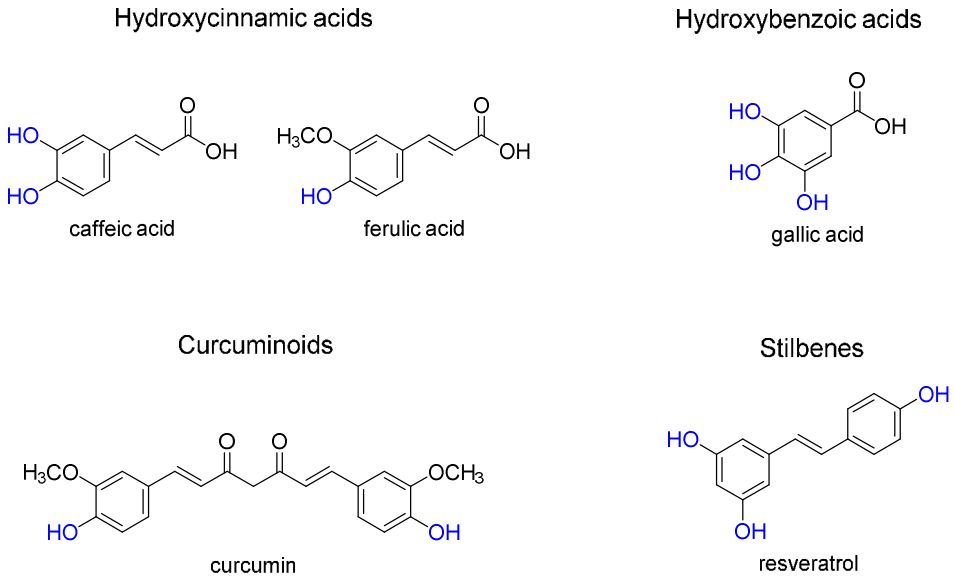

Figure 4: Structure of some common non-flavonoid polyphenols

Produced by plants for protection against other organisms and ultraviolet radiation,[3] polyphenols are associated with a wide range of health benefits. A high dietary intake of polyphenols has been linked to a reduced incidence of a number of diseases, including cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.[6-8] These population studies have been confirmed by subsequent *in vitro* and *in vivo* investigations showing the potential for polyphenols as therapeutic agents with cardioprotective,[9-11] antimicrobial,[12] anticancer,[13-15] neuroprotective[16,17] and antidiabetic[18,19] effects demonstrated.

The multitude of health benefits from polyphenols is largely attributed to their antioxidant activity. Reactive oxygen species (ROS), and reactive nitrogen species (RNS) are produced endogenously and play a vital role in a number of biological and biochemical processes. However, overproduction of these free radicals can lead to oxidative stress that can have deleterious effects, causing damage to lipids, proteins and DNA.[20,21] This damage can lead to a number of diseases, including cardiovascular disease, cancer, diabetes, rheumatoid arthritis, and septic shock as well as contribute to the aging process. The brain is particularly susceptible to oxidative stress as it is exposed throughout life to excitatory amino acids, such as glutamate, whose metabolism produces ROS, potentially leading to a number of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and cognitive dysfunction in the elderly.[22]

Polyphenols act as free radical scavengers by donating a hydrogen atom to form a phenoxy radical:[1]

$ROO^{\cdot} + PPH \rightarrow ROOH + PP^{\cdot}$

$RO^{\cdot} + PPH \rightarrow ROH + PP^{\cdot}$

As phenoxy radicals are resonance stabilised, they do not easily initiate new reactions but will further react with other free radicals thereby acting as chain breakers:

$ROO^{\cdot} + PP^{\cdot} \rightarrow ROOPP$

$RO^{\bullet} + PP^{\bullet} \rightarrow ROPP$

The antioxidant activity of polyphenols is largely dependent on their structure.[23] Antioxidant activity is greatly enhanced by: a catechol moiety on the B-ring; hydroxyl groups at the 3 (C-ring) and 5 (A-ring) positions; and a 2,3-double bond conjugated with a carbonyl group at position 4 on the C-ring (see **Figure 2**). Flavonoids are, therefore, particularly potent antioxidants.

The antioxidant activity of polyphenols is further enhanced by their metal chelating ability.[24,25] Hydroxyl radicals may be formed via the Fenton reaction whereby a coordinated ferrous ion (LFe^{2+}) is oxidised by H_2O_2 to form LFe^{3+}, a $HO^{\bullet}$ radical, and a HO^- ion:[26]

$LFe^{2+} + H_2O_2 \rightarrow LFe^{3+} + HO^{\bullet} + HO^-$

By chelating with transition metal ions such as Fe^{2+}, polyphenols can reduce the rate of Fenton reactions and thereby prevent oxidation from hydroxyl radicals.

Xanthine oxidase (XO) and other enzymes are involved in the generation of ROS:[27]

$$\text{xanthine} + H_2O + 2O_2 \xrightarrow{\text{XO}} \text{Uric acid} + 2O_2^{-\bullet} + 2H^+$$

$$\text{xanthine} + H_2O + O_2 \xrightarrow{\text{XO}} \text{Uric acid} + H_2O_2$$

Polyphenols are also able to inhibit the activity of xanthine oxidase (XO) and other enzymes involved in the generation of ROS thereby enhancing their antioxidant capacity.[24,25]

Although primarily antioxidants, under some conditions, such as high concentrations of transition metal ions, polyphenols can act as pro-oxidants.[28] Elevated copper levels have been reported in both the serum and tumours of cancer patients[29] and hence provide an environment with the requisite elevated transition metal ions. It is believed that this pro-oxidant action contributes to the anticancer effects of polyphenols.[28] Polyphenon E, a clinical grade mixture of green tea catechins, is under evaluation in multiple National Cancer Institute (Bethesda, MD) clinical trials and has been shown to have anticancer activity in a mouse model of neuroblastoma.[30]

Despite their impressive therapeutic effects, polyphenols suffer from a number of drawbacks, including instability when exposed to light, heat and basic conditions;[31] poor bioavailability; rapid metabolism; and poor membrane permeability.[5-7,32,33] These drawbacks limit the clinical applications of polyphenols.

A number of approaches have been utilised to stabilise polyphenols, including the synthesis of analogues/prodrugs, the development of novel drug delivery systems and stabilisation with macromolecules. Bansal et al.[34] have provided a comprehensive review of analogues/prodrugs and novel delivery systems that have been used to stabilise catechins. In this review we will explore the various approaches that have been used to stabilise polyphenols with macromolecules.

Polymers and other macromolecules are well-known for their ability to stabilise and improve the bioavailability of therapeutic agents. Accordingly, they can also be used

to improve the stability of polyphenols, either through polymerisation of polyphenols or via the conjugation of polyphenols to polymers. In the following sections, we will summarise the key techniques utilised for functionalising polyphenol compounds with natural and synthetic polymers, including direct polymerisation of polyphenol monomers via step-growth, free radical and enzyme catalysed reactions; and conjugation with macromolecules via enzyme grafting, free-radical grafting, esterification and amidation.

2. Polyphenol polymers

2.1. Polyphenol polymers prepared by step-growth polymerisation

The key step-growth polymerisation techniques employed with polyphenols are aldehyde condensations and glycerol diglycidyl ether condensations. **Table 1** details a number of polyphenols and comonomers that have been synthesised by step-growth polymerisations.

Table 1: Polyphenol polymers prepared by step-growth polymerisation

Polyphenol	Comonomer	catalyst	Molecular weight (dispersity)	Therapeutic application	Ref
catechin, epicatechin	acetaldehyde	acetic acid	-	-	[35,36]
epicatechin, malvidin 3-O-	acetaldehyde	tartaric acid	-	-	[37]

glucoside					
catechin	acetaldehyde, furfuraldehyde, 5-hydroxymethylfurfuraldehyde, 5-methylfurfuraldehyde, vanillin, syringaldehyde	tartaric acid	-	-	[38]
catechin	acetaldehyde	acetic acid	2760 (2.1), 890 (1.2)	antioxidant, XO inhibitor	[39]
catechin	acetaldehyde	acetic acid	3000 – 3700 (1.6-1.8)	antioxidant, XO inhibitor, proteinase inhibitor	[40-42]
catechin	acetaldehyde	HCl	2700 (1.6), 3400 (1.7)	antioxidant, XO inhibitor, proteinase inhibitor	[40-42]
catechin	glyoxylic acid	-	2300 (1.2)	antioxidant, XO inhibitor, proteinase inhibitor	[40-42]
catechin	pyruvic aldehyde	HCl	2300 (1.2)	antioxidant, XO inhibitor, proteinase	[40-42]

				inhibitor	
catechin	hydroxybenzaldehyde	HCl	1700 (1.1), 2000 (1.2)	antioxidant, XO inhibitor, proteinase inhibitor	[40-42]
EGCG	acetaldehyde	acetic acid	-	anticancer	[43]
quercetin	glycerol diglycidyl ether	-	-	antioxidant, antimicrobial	[44]
rutin	glycerol diglycidyl ether	-	-	antioxidant, antimicrobial, drug delivery	[45]
tannic acid	glycerol diglycidyl ether / trimethylolpropane triglycidyl ether	-	-	antioxidant, antimicrobial, anticancer, drug delivery	[46]

The initial studies of polyphenol polymers produced by aldehyde condensation polymerisation were undertaken in an effort to better understand the formation of oligomers and polymers in wine.[35-37,47,48] Fulcrand and co-workers[35] used liquid chromatography and ion spray mass spectrometry to elucidate the mechanism of the reactions of the flavanols, catechin and epicatechin, with acetaldehyde in the presence of acetic acid. The proposed mechanism is illustrated in **Figure 5**. The first step is the protonation of acetaldehyde to yield a carbocation, which subsequently undergoes nucleophilic attack by the flavanol (C-6 or C-8 of the A ring). A water

molecule is lost from the ethanol adduct formed to yield a new carbocation. A second flavanol molecule then attacks this carbocation to yield a dimer and the polymerisation continues in a similar manner. The reaction mechanism is quite similar to the well-known acid catalysed reaction between formaldehyde and phenols to form novolac resins[49] except, in the case of novolac resins, the phenol is in excess in the reaction mixture whereas acetaldehyde is in excess (50:1) in the case of the polymerisation of flavanols. It is believed that excess aldehyde favour the condensation reaction and, therefore, side reactions are avoided.[40]

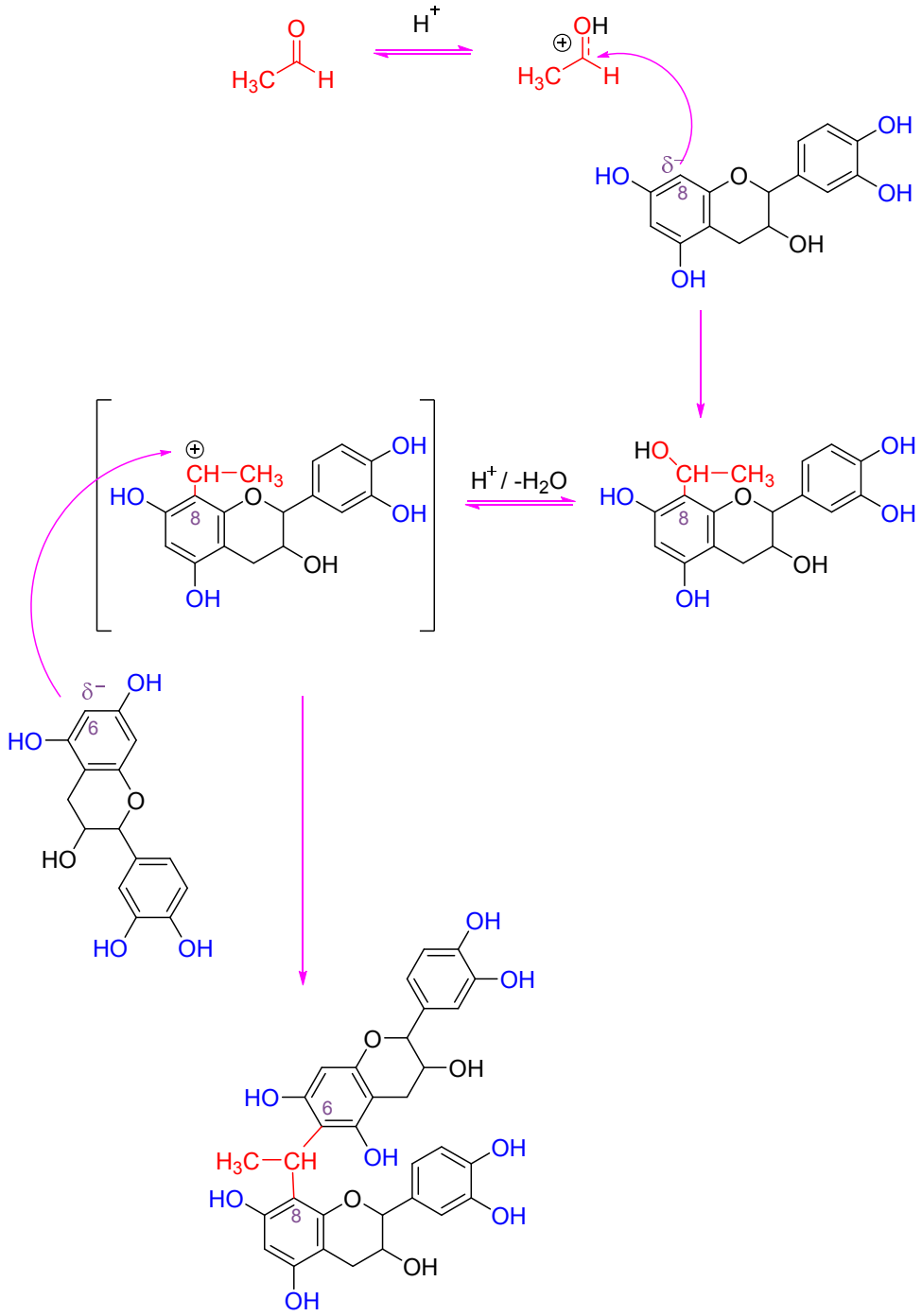

Figure 5: Mechanism of the acetaldehyde-induced polymerisation of flavan-3-ols. Adapted from reference [35]

A number of aldehydes were used to create polymers of catechin by Kim et al.[40] Catechin was reacted with acetaldehyde, glyoxylic acid, pyruvic aldehyde and *ortho*- and *para*-hydroxybenzaldehyde with either acetic acid or hydrochloric acid as the catalyst. All polymers were obtained in high yields ranging from 71% for the HCl catalysed reaction between catechin and *p*-hydroxybenzaldehyde to 94% for the HCl catalysed reaction between catechin and acetaldehyde. Prior to determining molecular weight by SEC, the polymers were acetylated to prevent the presence of multiple phenolic groups and improve the solubility. This modification affects the hydrodynamic volume resulting by some errors in the determination of molecular weight by SEC. The molecular weights achieved were not high (1700-3700 g/mol) with the *ortho*-and *para*-hydroxybenzaldehyde comonomers presenting the lowest molecular weights (1700- 2000 g/mol). The authors attributed this to likely steric hindrance. The polymers produced were soluble in acetone, DMF, DMSO, methanol, THF, and 1N NaOH, but only sparingly soluble (or insoluble) in water.

The above group undertook further studies to measure the superoxide scavenging activity,[42] xanthine oxidase (XO) inhibition[42] and proteinase inhibition[41] of the prepared polymers. All catechin polymers exhibited superior superoxide scavenging activity to catechin with the polymers prepared with glyoxylic acid and *p*-hydroxybenzaldehyde showing the strongest activity. In addition to being a biological source of reactive oxygen species (ROS), XO is the enzyme responsible for the production of uric acid. Excess uric acid leads to gout and is suspected of being a

risk factor in cardiovascular disease.[50] All polymers were effective inhibitors of XO with the *p*-hydroxybenzaldehyde showing the greatest effect. The catechin polymers also showed effective inhibition of the proteinases, collagenase and human neutrophil elastase (except catechin-*co*-glyoxylic acid), demonstrating potential as treatments for multiple sclerosis, Alzheimer's disease, and cancers.[41]

Self-assembled micellar nanocomplexes were prepared utilising an oligomeric EGCG/Herceptin core and a PEG-EGCG shell in a study by Chung and co-workers.[43] The OECGC was prepared by the acetic acid catalysed aldehyde polycondensation of ECGC and acetaldehyde whilst the PEG-EGCG was synthesised by reacting EGCG with an aldehyde terminated PEG in the presence of acetic acid. The Herceptin-loaded micellar nanocomplexes were tested *in vivo* in mice inoculated with BT-474 human breast cancer cells and demonstrated superior tumour selectivity and growth reduction compared with free Herceptin.

A different approach to preparing step-growth polymers from polyphenols was employed by Sahiner[44] who utilised glycerol diglycidyl ether as the comonomer with quercetin in a microemulsion polymerisation using lecithin as the surfactant and cyclohexane as the organic phase. In this polymerisation, the epoxy groups of glycerol diglycidyl ether react with the phenol groups in quercetin to link the monomers (see **Figure 6**). The synthesised quercetin polymers demonstrated antioxidant activity and showed antimicrobial effects against common bacteria - *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus.* Sahiner used the same

technique to polymerise rutin[45] and tannic acid[46] again establishing antioxidant and antimicrobial activity. Furthermore, Sahiner and co-workers established poly(tannic acid) was as effective as cisplatin against A549 cancerous cells and both poly(rutin) and poly(tannic acid) particles demonstrated effective drug release capabilities.

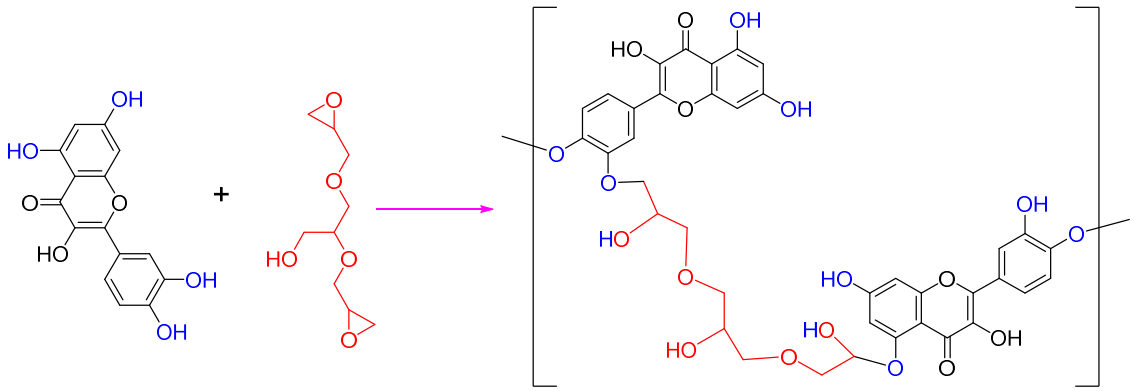

Figure 6: The schematic representation of the poly(quercetin) particle formation from quercetin. Adapted from reference [44]

2.2. Polyphenol polymers prepared by free radical polymerisation

Both traditional polymerisation initiators (AIBN)[51-53] and ascorbic acid/ H_2O_2 [54-56] have been used to copolymerise polyphenols with other monomers. Methacrylic acid (MAA) is typically used as the comonomer with additional comonomers, such as ethylene glycol dimethacrylate (EGDMA)[51,52] and N-isopropylacrylamide (NIPAAm),[51] sometimes included to add crosslinking and thermoresponsive

functionality respectively. **Table 2** details the polymers which have been synthesised by free radical polymerisation.

Table 2: Polyphenol polymers prepared by free radical polymerisation

Polyphenol	**Comonomer**	**Initiator**	**Molecular weight (dispersity)**	**Therapeutic application**	**Ref**
quercetin	MAA	AIBN	71 000 (1.7)	anticancer	[53]
ferulic acid	MAA/EGDMA	AIBN	-	antioxidant	[52]
catechin, quercetin	MAA/ NIPAAm/ EGDMA	AIBN	-	antioxidant, hydrogel	[51]
ferulic acid	MAA	ascorbic acid/H_2O_2	-	antioxidant, antifungal	[55,56]
quercetin	MAA	ascorbic acid/H_2O_2	-	anticancer	[54]

Early work on polyphenol free radical polymerisation was undertaken with ferulic acid which has a polymerisable styrenic bond[52,55,56] but later work has utilised polyphenols without double bonds, namely the flavonoids, catechin[51] and quercetin.[51,53,54] It is believed that these flavonoids are inserted into the growing polymer chains at positions 6, 8 (A ring) (refer **Figure 2** & **Figure 3**).

A trifunctional nanocomposite was prepared from the free radical copolymerisation of quercetin and MAA around carbon nanotubes by Cirillo and co-workers.[54] The

composite maintained the antioxidant properties of quercetin and displayed superior anticancer activity compared with free quercetin. Furthermore, the nanocomposite was non-toxic to healthy cells.

2.3. Enzymatic polyphenol polymerisation

Enzyme catalysed synthesis of macromolecules is common in nature and includes the synthesis of nucleic acids, proteins, polysaccharides and natural rubber.[57] A key class of enzymes are the oxidoreductases, which in cells play an important role in maintaining metabolism.[58] A number of oxidoreductases have been utilised to prepare polymers from polyphenols, including horseradish peroxidase (HRP), [59-62] soybean peroxidase, [62] bilirubin oxidase,[63] laccase,[64-67] and tyrosinase.[64] **Table 3** details some of the polyphenols that have been catalysed by oxidoreductase enzymes.

Table 3: Enzymatic polyphenol polymerisation

Polyphenol	**Enzyme**	**Molecular weight (dispersity)**	**Therapeutic application**	**Ref**
catechin	HRP	14000 (2.4)	antioxidant, XO inhibitor	59
taxifolin	bilirubin oxidase	2800 (8.6)	antioxidant	63
quercetin, kaempferol	laccase, tyrosinase	-	antioxidant	64
rutin	laccase	10000	antioxidant, LDL oxidation inhibitor	66

quercetin	HRP	14000 (3.64)		[60]
catechin	laccase	3000 (6.3)	antioxidant, XO inhibitor, LDL oxidation inhibitor	[65]
quercetin, rutin, catechin, daidzein, 5,6,4′-trihydroxyisoflavone	horseradish or soybean peroxidase	4000–12000	-	[62]
EGCG	laccase	4200 (1.8)	antioxidant, XO inhibitor	[67]
catechin, green tea extract	HRP	-	antimicrobial	[61]
quercetin, tannic acid*	laccase	-	antioxidant	[68]

*polymerisation was not successful

Oxidoreductases catalyse the polymerisation of polyphenols by facilitating the generation of phenoxy radicals. A metal in a low oxidative state forms the centre of most oxidoreductases. In horseradish peroxidase, the metal is Fe(III) and, in a typical enzyme catalysed polymerisation, it is oxidised by H_2O_2 to Fe(IV), forming a positively charged radical and water (see **Figure 7**). This radical, in turn, extracts a hydrogen from a phenol group thereby generating a phenoxy radical. The Fe(IV) is reduced back to Fe(III) by another phenol group, forming another phenoxy radical.[58]

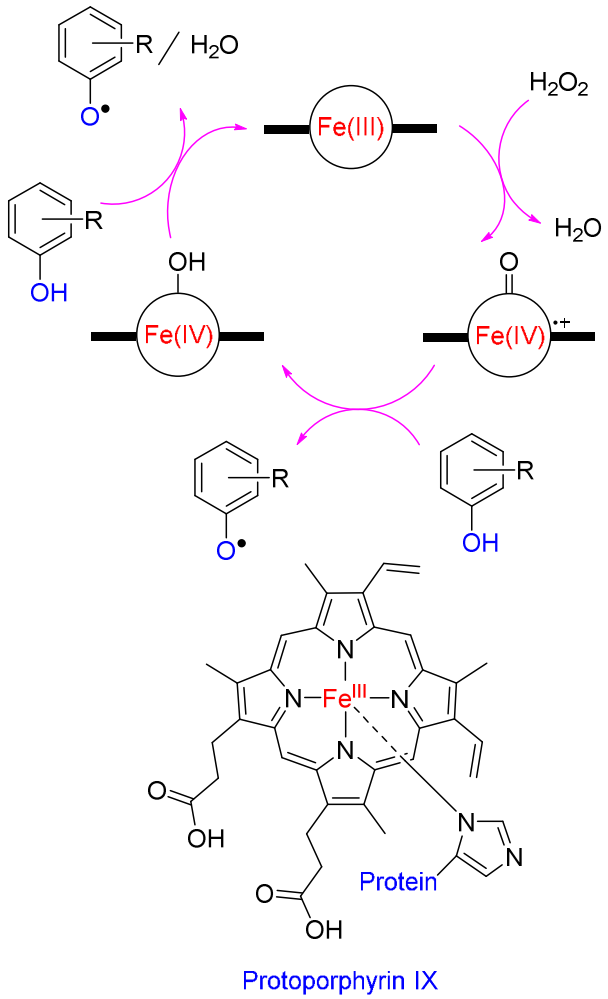

Figure 7: The catalytic cycle of HRP for a phenol substrate. Adapted from ref [58]

Kurisawa and co-workers have polymerised a number of polyphenols using oxidoreductases (HRP and laccase) as catalysts, including catechin (HRP and laccase),[59,65] rutin (laccase),[66] and EGCG (laccase).[67] The poly(catechin) from laccase was insoluble in water but soluble in DMF, DMSO, pyridine, and 1 N NaOH whereas poly(rutin) was soluble in water, DMF and DMSO. The solubility of the other polymers was not reported. In all cases, the polymer exhibited superior antioxidant and XO inhibition properties when compared with the monomer.

Furthermore, poly(catechin) and poly(rutin) from laccase showed improved inhibition effects on human low-density lipoprotein (LDL) oxidation initiated by 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) and protected endothelial cells from oxidative injury induced by AAPH.

Enhanced antioxidant effects have also been demonstrated for enzyme catalysed polymers of quercetin,[64,68] taxifolin,[63] and kaempferol[64] when compared with their respective monomers and oligomeric catechin from HRP has been shown to exhibit antimicrobial properties.[61] An attempt was made to polymerise tannic acid with laccase but the tannic acid, instead, was depolymerised forming gallic acid, gallic acid dimers, partially gallic acid esterified glucose and glucose.[68]

3. Polyphenol polymer conjugates

3.1. Polyphenol polymer conjugates prepared by enzyme grafting

Oxidoreductases have also been employed to catalyse the grafting of polyphenols onto synthetic and biopolymers, proceeding by a similar mechanism to enzymatic polyphenol polymerisation. A range of polymers that have been enzymatically grafted with polyphenols are detailed in **Table 4**.

Table 4: Polyphenol polymer conjugates prepared by enzyme grafting

Polyphenol	Polymer	Enzyme	Therapeutic application	Ref
catechin	poly(ε-lysine)	laccase	enzyme inhibitor	[69]
catechin, EGCG,	chitosan	tyrosinase	antioxidant, antimicrobial	[70]

epicatechin, epigallocatechin, quercetin, fisetin, rutin, hesperidin, daidzein				
catechin	porous acrylic polymer particles with amino group	laccase	antioxidant	[71]
chlorogenic acid	chitosan	tyrosinase	-	[72]
catechin	poly(allylamine)	laccase	antioxidant	[73-75]
catechin	gelatin	laccase	antioxidant, LDL oxidation inhibitor	[76]
catechin	polyhedral oligomeric silsesquioxanes (POSS)	horseradish peroxidase	antioxidant	[77]
catechin	chitosan	tyrosinase	-	[78]
quercetin, tannic acid	chitosan	laccase	antioxidant, antimicrobial	[68]
caffeic acid, gallic acid	chitosan	laccase	antioxidant, antimicrobial	[79,80]
ferulic acid, ethyl ferulate	chitosan	laccase	antioxidant, antimicrobial	[81,82]

Produced from the hydration of chitin (the main building block of crustacean shells), chitosan is a copolymer of N-acetyl-D-glucosamine and D-glucosamine. A natural antioxidant and antimicrobial, chitosan contains hydroxyl, amino and acetamido

functional groups and is the only cationic polysaccharide.[83] A number of flavonoids representing different classes – flavanols (catechin, EGCG, epicatechin, epigallocatechin), flavonols (quercetin, fisetin), flavones (rutin), flavanones (hesperidin), isoflavones (daidzein) – were conjugated to chitosan using tyrosinase by Sousa and co-workers.[70] All conjugates showed enhanced antioxidant efficacy compared with native chitosan and this enhancement was greatest for epigallocatechin. Furthermore, the antimicrobial activity of chitosan against *Bacillus subtillis* and *Pseudomonas aeruginosa* improved when conjugated with some flavonoids.

A number of phenolic acids have also been enzymatically grafted to chitosan. Aljawish and co-workers[81,82] used laccase to graft ferulic acid and ethyl ferulate to chitosan and demonstrated enhanced antioxidant activity – especially for the ferulic acid conjugate – and equivalent antimicrobial activity compared with native chitosan. Božič and co-workers[79,80] investigated the influence of pH on laccase mediated grafting of caffeic acid and gallic acid on chitosan. **Figure 8** shows the proposed functionalisation mechanisms for the enzymatic grafting of the two phenolic acids at pH 4.5 and pH 6.5. At pH 4.5, electrostatic interactions and ester bonds (gallic acid only) predominated whereas at pH 6.5, *o*-quinone-amino coupling reactions via Schiff-base and Michael addition mechanisms prevailed. The highest antioxidant activity was observed for the chitosan-phenolic acid conjugates

synthesised at pH 4.5 with these conjugates also demonstrating enhanced activity against *Escherichia coli* and *Listeria monocytogenes* compared with native chitosan.

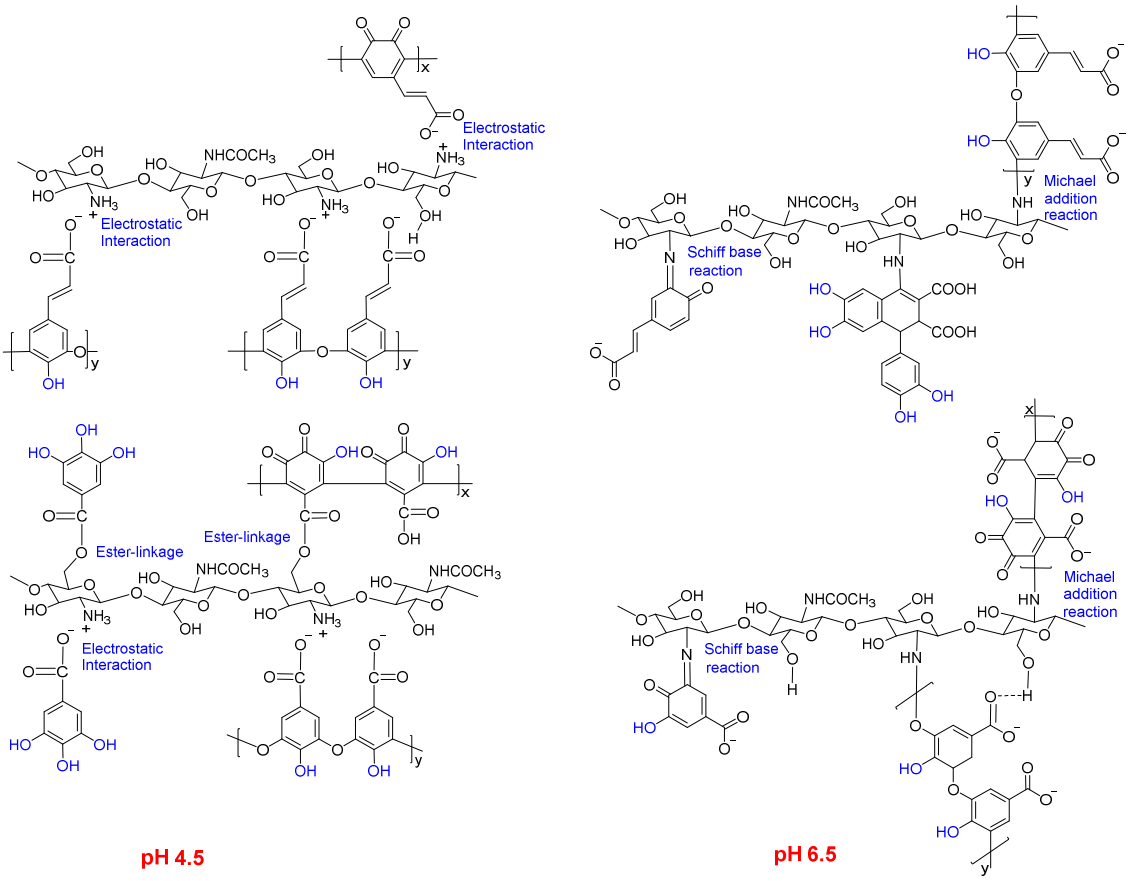

Figure 8: The proposed functionalisation mechanisms of chitosan with caffeic acid (top) and gallic acid (bottom) obtained by laccase catalysed reactions at different pHs. Adapted from ref [80]

Božič and co-workers[68] extended their work on pH dependence of enzymatic chitosan grafting to include quercetin and tannic acid. Quercetin was covalently bound to chitosan at pH 6.5 but covalent coupling was hindered at pH 4.5 due to protonation of the chitosan amine groups. In contrast, no covalent bonds were formed with the oxidative products of tannic acid at either pH but strong

crosslinking from hydrogen and electrostatic bonding did occur. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radicals (ABTS$^{\bullet+}$) are commonly used to determine the antioxidant properties of substances.[84] All conjugates prepared showed enhanced antioxidant effects against ABTS$^{\bullet+}$.

Other biopolymers used as substrates for enzymatic conjugation of polyphenols include poly(ε-lysine)[69] and gelatin.[76] The conjugation of catechin to poly(ε-lysine) resulted in improved inhibitory activity against a number of disease related enzymes and the conjugation of catechin to gelatin enhanced its antioxidant effects. Enhanced antioxidant activity has also been reported for polyphenols enzymatically conjugated to synthetic polymers, including poly(allylamine);[73] porous acrylic polymer particles with an amino group;[71] and polyhedral oligomeric silsesquioxanes (POSS).[77] The laccase catalysed conjugation of catechin with poly(allylamine) was further studied by Gogoi and co-workers.[74,75] They found a faster initial reaction rate with polar solvents[75] and that cross-linked enzyme crystals (CLEC) were preferable to free or immobilised laccase.[74]

3.2. Polyphenol polymer conjugates prepared by esterification and amidation

Carbodiimides (RN=C=NR′) have long been used to activate esterification[85] and amidation[86] reactions from carboxylic acids. A number of groups have employed carbodiimides to conjugate the carboxylic groups present on phenolic acids with

polymers containing hydroxyl and/or amine functional groups thereby enhancing the antioxidant capacity of the phenolic acid.[87-92] **Figure 9** shows a typical reaction pathway for the carbodiimide assisted conjugation of a phenolic acid to a polymer. Alternatively carboxylic groups on polymers have been utilised to form ester linkages with the phenol groups on polyphenols.[93,94] **Table 5** details the range of polyphenols and polymers which have been conjugated through esterification and amidation.

Table 5: Polyphenol polymer conjugates prepared by esterification and amidation

Polyphenol	**Polymer**	**Condensing agent**	**Therapeutic application**	**Ref**
gallic acid	chitosan	EDC/NHS	antioxidant	89,90,92
ferulic acid	dextran hydrogel	DCC/HBT	antioxidant, skin care	88
ferulic acid	poly(DMAA-*co*-AEMA)	DCC/HOBt	antioxidant	95
resveratrol	poly-ε-caprolactone-*g*-poly(acrylic acid)	EDC	bone regeneration	94
ferulic acid	cellulose-DMAA hydrogel	DCC/ HBT	antioxidant	91
caffeic acid	chitosan	EDC/Isourea	antioxidant	87
curcumin	cystamine core poly(amidoamine) dendrimer	DCC/NHS/TEA	anticancer	96
EGCG dimers	hyaluronic acid	EDC/NHS	hydrogel	93
caffeic acid, gallic acid	poly(GMA–TRIM), poly(NAT–GMA–BIS)	DCC/DMAP	antioxidant, food packaging	97

caffeic acid , gallic acid (acyl chlorides)	PP-g-HEMA, poly(GMA–TRIM), poly(NAT–GMA–BIS)	-	antioxidant, food packaging	97,98

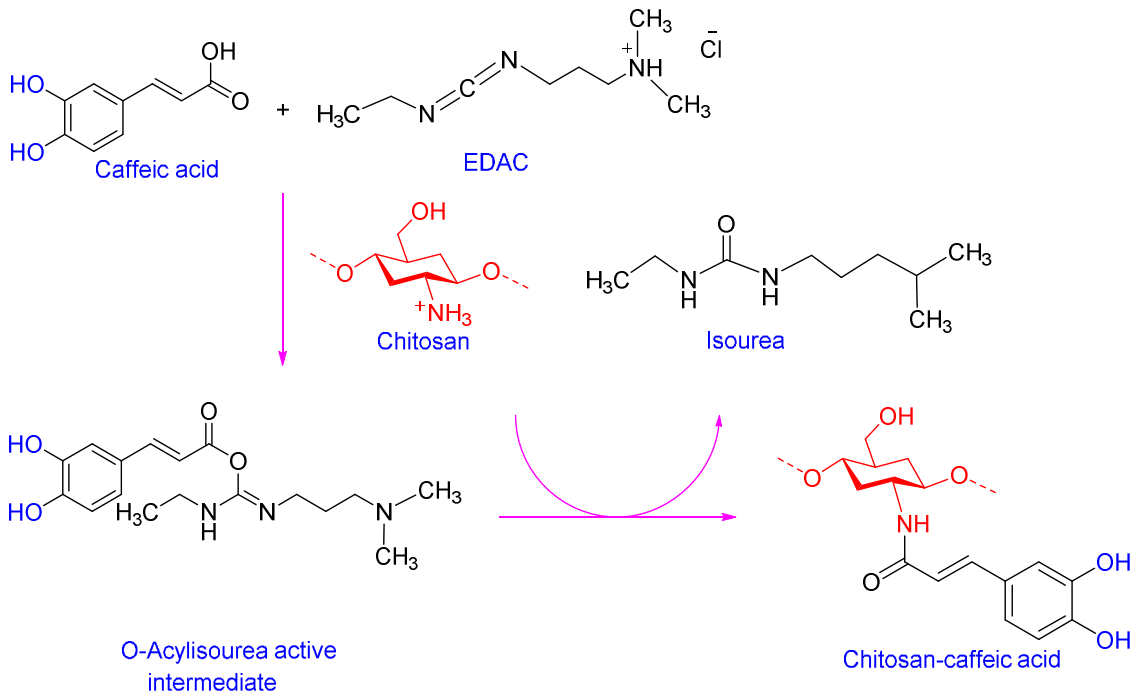

Figure 9: Grafting reaction of caffeic acid with chitosan. Adapted from ref [87]

Li and co-workers[94] used a vapour phase grafting method to covalently attach acrylic acid chains to porous poly-ε-caprolactone scaffolds. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was then used to activate the coupling of a resveratrol phenol group with a carboxylic group on the acrylic acid chain via a hydrolysable ester bond (see **Figure 10**). The degradable aliphatic ester linkage allowed the resveratrol moiety to be released by hydrolytic cleavage during *in vivo*

use, thereby providing a ready supply of the polyphenol to the host tissue. Osteogenic evaluation of the resveratrol functionalised scaffold formed demonstrated that it showed enhanced *in vitro* mineralisation and *in vivo* bone regeneration compared with the non-resveratrol functionalised scaffold.

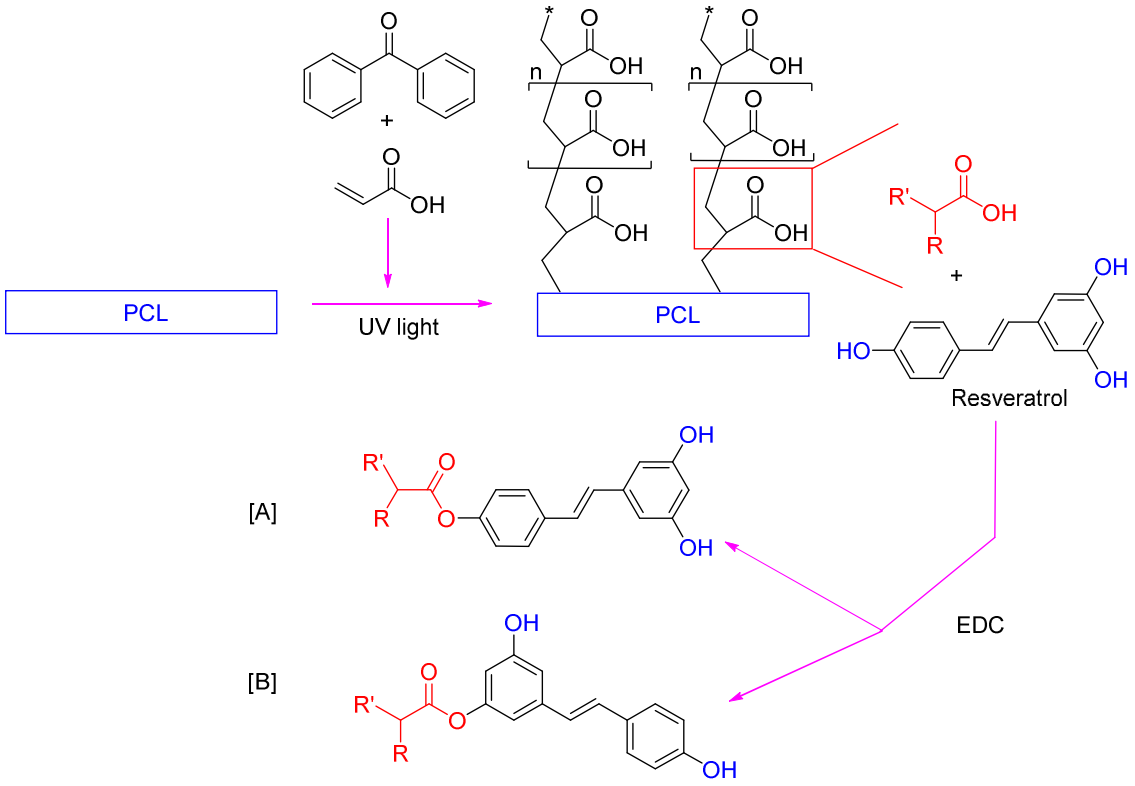

Figure 10: Strategy for coupling resveratrol through a hydrolysable linkage to poly(epsilon-caprolactone) surfaces. Reaction product B is less likely due to steric hindrance of the bisubstituted phenyl group. Adapted from ref [94]

An ethylamine bridged EGCG dimer was prepared by Lee and co-workers[93] and subsequently conjugated to hyaluronic acid via an amide bond employing EDC and NHS as condensing agents. The conjugate was found to autooxidise at pH 7.4

generating H_2O_2. This allowed the conjugate to be crosslinked using only horseradish peroxidase to form a hydrogel. When injected into subcutaneous tissue, the hydrogel maintained close to 100% of its weight after 42 days.

Shi and co-workers[96] synthesised a carboxylic acid derivative of curcumin via the reaction of glutaric anhydride in the presence of a base. The carboxylic acid group of this compound was subsequently bonded to a Generation 4 cystamine core poly(amidoamine) dendrimer using dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), and TEA in DMF. The dendrimer-curcumin conjugate is undergoing *in vitro* and *in vivo* evaluation.

An attempt was made to graft caffeic acid to hydroxyethyl methacrylate (HEMA) functionalised polypropylene film (PP-*g*-HEMA) by Arrua and co-workers[98] using DCC as the coupling agent but no covalent bonding occurred. Instead, the authors converted the caffeic acid to caffeoyl chloride and performed an esterification reaction in anhydrous THF. The conjugate produced exhibited good inhibition of ascorbic acid oxidation in orange juice. The same group compared the effectiveness of a one-step DCC/DMAP activated amidation with a two-step amidation via an acyl chloride to graft caffeic acid or gallic acid to two macroporous polymers, poly(glycidylmethacrylate-co-trimethylolpropane trimethacrylate) [poly(GMA–TRIM)] and poly(N-acryloyl-tris(hydroxymethyl)aminomethane-co-glycidylmethacrylate-co-N,N′-methylenebisacrylamide) [poly(NAT–GMA–BIS)].[97] (Amine groups were first introduced onto the surface of the polymers via reaction of

the epoxide groups with ethylenediamine.) For all conjugates tested, greater antioxidant activity was achieved with the two-step method whereby the phenolic acids were first converted to acyl chlorides.

3.3. Polyphenol polymer conjugates prepared by free radical grafting

The majority of studies of free radical grafting of polyphenols onto polymers have employed ascorbic acid and hydrogen peroxide to initiate the reaction. As a redox pair, ascorbic acid and hydrogen peroxide can be used to generate free radicals to initiate grafting or polymerisation reactions. The ascorbic acid is oxidised by H_2O_2 to form hydroxyl radicals and ascorbate radicals (see **Figure 11**). The advantage of the ascorbic acid/ H_2O_2 system is that reactions can be carried out at room temperature with water as the solvent thereby affording some protection against polyphenol degradation and reduced toxicity.

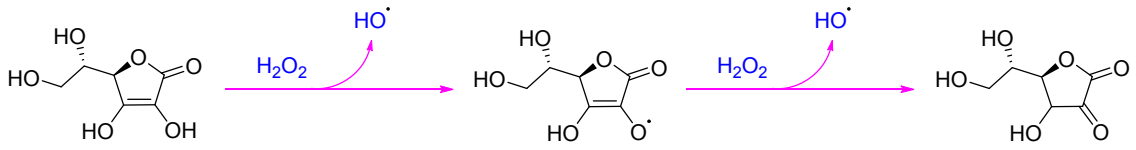

Figure 11: Interaction between ascorbic acid and hydrogen peroxide. Adapted from ref [99]

Ascorbic acid/ H_2O_2 grafting of polyphenols onto polysaccharides is typically a two-step process:[99] firstly, radicals are formed on the polymer chain via abstraction of a hydrogen from a hydroxyl group (or amine group if applicable); and secondly, the polyphenol reacts with the radical to form a covalent bond between the

polysaccharide and the polyphenol. **Figure 12** shows the proposed mechanism for the conjugation of catechin with inulin and alginate. It is hypothesised that the catechin is added at positions 2′, 5′ (B ring) and 6, 8 (A ring) (refer **Figure 2** & **Figure 3**). If the polyphenol being added is a phenolic acid, the bonding is believed to occur on the carboxyl group.[100,101]

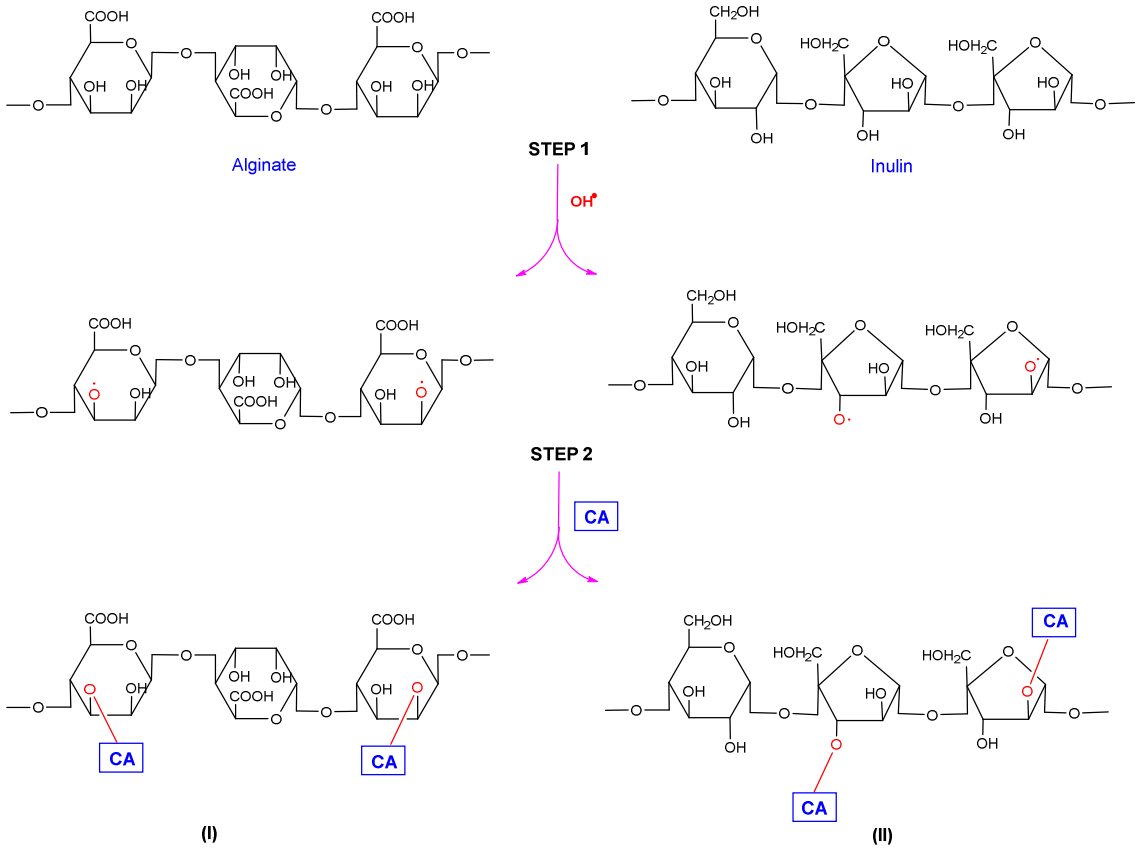

Figure 12: Insertion of catechin (CA) onto alginate (I) and inulin (II) backbones. Adapted from ref[99]

Polyphenols have been added to a number polysaccharides using ascorbic acid / H_2O_2 grafting, including dextran,[102,103] chitosan,[100,101,104-107] inulin,[99,108] alginate,[99] and starch.[109] All conjugates have demonstrated antioxidant effects and some have

shown potential therapeutic efficacy in diabetes,[109,110] Alzheimer's disease,[109] cancer,[102,103] bacterial infections[105] and skin care.[109] Proteins, including gelatin[111,112]and lactoferrin,[113] have also been conjugated to polyphenols, demonstrating antioxidant,[111-113] anti-inflammatory[112] and anticancer[112] effects. **Table 6** details the range of polyphenols and polymers which have been conjugated via free radical grafting.

Table 6: Polyphenol polymer conjugates prepared by free radical grafting*

Polyphenol	Polymer	Therapeutic application	Ref
catechin	inulin, alginate	antioxidant	99,108
catechin	chitosan	antioxidant, antidiabetic	104,110
catechin	dextran	antioxidant, anticancer	102,103
gallic acid	chitosan	antioxidant, antidiabetic	101,107
quercetin	starch	antioxidant, Alzheimer's disease, antidiabetic, skin care	109
gallic acid, caffeic acid, ferulic acid	carboxymethyl chitosan	antioxidant	100
caffeic acid, ferulic acid	chitosan	antioxidant	106
caffeic acid, ferulic acid, sinapic acid	chitosan	antioxidant, antimicrobial	105
EGCG, chlorogenic acid, gallic acid	lactoferrin	antioxidant	113
catechin, gallic acid	gelatin	antioxidant, anti-inflammatory, Alzheimer's disease, enzyme inhibition, anticancer	111,112

caffeic acid	chitosan /CPTMS hybrid scaffold	hard-tissue engineering	[114] *

*Ascorbic acid/H_2O_2 is used as the initiator with the exception of the grafting of caffeic acid onto a chitosan /CPTMS hybrid scaffold where potassium persulfate is used.

Additional functionality was added to a polyphenol/polysaccharide conjugate by Spizzirri and co-workers.[108] Ascorbic acid/H_2O_2 was used as an initiator to prepare a thermoresponsive antioxidant hydrogel by reacting NIPAAm (well-known for its thermoresponsive properties), N,N-ethylenebisacrylamide (a crosslinking agent) and catechin with inulin in a one-pot reaction. The hydrogels formed had an LCST between 31.3–33.1 ^{0}C and exhibited greater antioxidant effects below the LCST.

To enhance the anticancer efficacy of the conjugate and to exploit the targeting potential of a magnetic drug delivery system, Vittorio and co-workers[103] modified Endorem (iron oxide nanoparticles coated with dextran) with a dextran-catechin conjugate previously prepared via ascorbic acid/H_2O_2 grafting. A simple substitution procedure was utilised to replace the dextran coating of the iron oxide with the dextran-catechin conjugate. The resultant nanoparticles were tested on a pancreatic cancer cell line and increased anticancer efficacy was observed when the nanoparticles were placed under a magnetic field with 98% cell death within 24 hours.

4. Conclusion

This review has detailed a number of key methods used to enhance the therapeutic effects of polyphenols with macromolecules. Polyphenols can be stabilised through direct polymerisation of polyphenol monomers or via conjugation with macromolecules. Methods of direct polymerisation include step-growth, free radical and enzyme catalysed reactions. Polymers have been prepared from a number of polyphenols, including catechin, epicatechin, EGCG, quercetin, rutin and tannic acid with molecular weights ranging from 890 to 77 000. The antioxidant activity of polyphenols is maintained or enhanced by polymerisation and a number of potential therapeutic applications have been demonstrated, including XO, proteinase and LDL oxidation inhibition; drug delivery; and cancer, antimicrobial and antifungal treatments. Polyphenol polymer conjugates have been prepared by esterification, amidation, free radical grafting and enzyme assisted grafting. Polyphenols have been grafted onto both synthetic and natural polymers, including dextran, chitosan, hyaluronic acid, gelatin, inulin and alginate. A wide range of polyphenols have been conjugated to polymers including catechin, EGCG, epicatechin, quercetin, gallic acid, ferulic acid, caffeic acid, tannic acid and curcumin. As with direct polymerisation, antioxidant activity is maintained or enhanced when polyphenols are conjugated to polymers. Potential therapeutic applications of polyphenol polymer conjugates include hydrogels; bone regeneration and hard tissue engineering; skin care; LDL oxidation and enzyme inhibition; and diabetes, cancer, Alzheimer's disease,

antimicrobial and anti-inflammatory treatments. Although some *in vivo* work has been done with polyphenols stabilised by macromolecules, to date, the majority of studies have been *in vitro*. The challenge for the future will be to develop macromolecule stabilised polyphenols that translate *in vitro* results to *in vivo* studies and clinical trials.

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5. Abbreviations

AAPH	2,2′-azobis(2-amidinopropane)dihydrochloride
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AEMA	2-aminoethylmethacrylate
AIBN	2,2′-azoisobutyronitrile
BIS	N,N′-methylenebisacrylamide
CPTMS	3-chloropropyl trimethoxysilane
DCC	dicyclohexylcarbodiimide
DMAA	N,N-dimethylacrylamide
DMAP	dimethylaminepyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide

EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EGDMA	ethylene glycol dimethacrylate
EGCG	epigallocatechin gallate
GMA	glycidylmethacrylate
HBT	4-hydroxybenzotriazole
HOBt	1-hydroxybenzotriazole
HRP	horseradish peroxidase
MAA	methacrylic acid
NAT	N-acryloyl-tris(hydroxymethyl)aminomethane
NHS	N-hydroxysuccinimide
NIPAAm	N-isopropylacrylamide
PEG	poly(ethylene glycol)
RNS	reactive nitrogen species
ROS	reactive oxygen species
TEA	triethanolamine
THF	tetrahydrofuran
TRIM	trimethylolpropane trimethacrylate
XO	xanthine oxidase

6. References

(1) L. Bravo *Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance*, 1998; Vol. 56.

(2) V. Neveu; J. Perez-Jiménez; F. Vos; V. Crespy; L. du Chaffaut; L. Mennen; C. Knox; R. Eisner; J. Cruz; D. Wishart; A. Scalbert *Database* 2010, **2010**.

(3) J. B. Harborne; C. A. Williams *Phytochemistry* 2000, **55**, 481-504.

(4) G. R. Beecher *J. Nutr.* 2003, **133**, 3248S-3254S.

(5) C. Manach; A. Scalbert; C. Morand; C. Rémésy; L. Jiménez *Am. J. Clin. Nutr.* 2004, **79**, 727-747.

(6) G. Williamson; C. Manach *Am. J. Clin. Nutr.* 2005, **81**, 243S-255S.

(7) C. Manach; G. Williamson; C. Morand; A. Scalbert; C. Rémésy *Am. J. Clin. Nutr.* 2005, **81**, 230S-242S.

(8) A. Scalbert; C. Manach; C. Morand; C. Rémésy; L. Jiménez *Crit. Rev. Food Sci. Nutr.* 2005, **45**, 287-306.

(9) P. Gresele; C. Cerletti; G. Guglielmini; P. Pignatelli; G. de Gaetano; F. Violi *J. Nutr. Biochem.* 2011, **22**, 201-211.

(10) R. Kleemann; L. Verschuren; M. Morrison; S. Zadelaar; M. J. van Erk; P. Y. Wielinga; T. Kooistra *Atherosclerosis* 2011, **218**, 44-52.

(11) S. Khurana; M. Piche; A. Hollingsworth; K. Venkataraman; T. C. Tai *Can. J. Physiol. Pharmacol.* 2013, **91**, 198-212.

(12) T. P. T. Cushnie; A. J. Lamb *Int. J. Antimicrob. Agents* 2005, **26**, 343-356.

(13) M. Asensi; A. Ortega; S. Mena; F. Feddi; J. M. Estrela *Int. J. Antimicrob. Agents* 2011, **48**, 197-216.

(14) M. Kampa; A. P. Nifli; G. Notas; E. Castanas In *Reviews of Physiology, Biochemistry and Pharmacology*; Amara, S. G., Bamberg, E., Fleischmann, B., Gudermann, T., Hebert, S. C., Jahn, R., Lederer, W. J., Lill, R., Miyajima, A., Offermanns, S., Zechner, R., Eds.; Springer Berlin Heidelberg: 2007; Vol. 159, p 79-113.

(15) S. Ramos *Mol. Nutr. Food Res.* 2008, **52**, 507-526.

(16) D. H. Shin; Y. C. Bae; J. S. Kim-Han; J. H. Lee; I. Y. Choi; K. H. Son; S. S. Kang; W.-K. Kim; B. H. Han *J. Neurochem.* 2006, **96**, 561-572.

(17) O. Weinreb; S. Mandel; T. Amit; M. B. H. Youdim *J. Nutr. Biochem.* 2004, **15**, 506-516.

(18) Y. Shoji; H. Nakashima *Arch. Pharm. Res.* 2006, **29**, 786-794.

(19) H.-Y. Hung; K. Qian; S. L. Morris-Natschke; C.-S. Hsu; K.-H. Lee *Nat. Prod. Rep.* 2012, **29**, 580-606.

(20) B. Uttara; A. V. Singh; P. Zamboni; R. T. Mahajan *Curr. Neuropharmacol.* 2009, **7**, 65-74.

(21) M. Valko; D. Leibfritz; J. Moncol; M. T. D. Cronin; M. Mazur; J. Telser *The Int. J. Biochem. Cell Biol.* 2007, **39**, 44-84.

(22) Y. Gilgun-Sherki; E. Melamed; D. Offen *Neuropharmacology* 2001, **40**, 959-975.

(23) C. A. Rice-Evans; N. J. Miller; G. Paganga *Free Radical Biol. Med.* 1996, **20**, 933-956.

(24) R. Tsao *Nutrients* 2010, **2**, 1231.

(25) H. de Groot; U. Rauen *Fundam. Clin. Pharmacol.* 1998, **12**, 249-255.

(26) J. Prousek *Pure Appl. Chem.* 2007, **79**, 2325-2338.

(27) F. Lacy; D. A. Gough; G. W. Schmid-Schönbein *Free Radical Biol. Med.* 1998, **25**, 720-727.

(28) M. F. Ullah; A. Ahmad; H. Khan; H. Zubair; F. Sarkar; S. M. Hadi *Cell Biochem. Biophys.* 2013, **67**, 431-438.

(29) A. Gupte; R. J. Mumper *Cancer Treat. Rev.* 2009, **35**, 32-46.

(30) G. Santilli; I. Piotrowska; S. Cantilena; O. Chayka; M. D'Alicarnasso; D. A. Morgenstern; N. Himoudi; K. Pearson; J. Anderson; A. J. Thrasher; A. Sala *Clin. Cancer Res.* 2013, **19**, 1116-1125.

(31) M. A.-J. Rajae Saadeh, Ahmad Abdoh and Abeer Al-Bawab *Dirasat, Pure Sciences,* 2009, **36**, 62-75.

(32) D. Mereles; W. Hunstein *Int. J. Mol. Sci.* 2011, **12**, 5592-5603.

(33) J. Hong; H. Lu; X. Meng; J.-H. Ryu; Y. Hara; C. S. Yang *Cancer Res.* 2002, **62**, 7241-7246.

(34) S. Bansal; S. Vyas; S. Bhattacharya; M. Sharma *Nat. Prod. Rep.* 2013, **30**, 1438-1454.

(35) H. Fulcrand; T. Doco; N.-E. Es-Safi; V. Cheynier; M. Moutounet *J. Chromatogr. A* 1996, **752**, 85-91.

(36) N. E. Es-Safi; H. Fulcrand; V. Cheynier; M. Moutounet *J. Agric. Food Chem.* 1999, **47**, 2088-2095.

(37) N.-E. Es-Safi; H. Fulcrand; V. Cheynier; M. Moutounet *J. Agric. Food Chem.* 1999, **47**, 2096-2102.

(38) M.-F. Nonier; N. Vivas; N. Vivas de Gaulejac; I. Pianet; E. Fouquet *J. Agric. Food Chem.* 2007, **87**, 2081-2091.

(39) J. E. Chung; M. Kurisawa; Y.-J. Kim; H. Uyama; S. Kobayashi *Biomacromolecules* 2004, **5**, 113-118.

(40) Y.-J. Kim; J. E. Chung; M. Kurisawa; H. Uyama; S. Kobayashi *Macromol. Chem. Phys.* 2003, **204**, 1863-1868.

(41) Y.-J. Kim; H. Uyama; S. Kobayashi *Biochem. Biophys. Res. Commun. s* 2004, **320**, 256-261.

(42) Y.-J. Kim; J. E. Chung; M. Kurisawa; H. Uyama; S. Kobayashi *Biomacromolecules* 2004, **5**, 547-552.

(43) J. E. Chung; S. Tan; S. J. Gao; N. Yongvongsoontorn; S. H. Kim; J. H. Lee; H. S. Choi; H. Yano; L. Zhuo; M. Kurisawa; J. Y. Ying *Nat. Nano.* 2014, **9**, 907-912.

(44) N. Sahiner *Colloids Surf., A* 2014, **452**, 173-180.

(45) N. Sahiner *Materials Science and Engineering: C* 2014, **44**, 9-16.

(46) N. Sahiner; S. Sagbas; N. Aktas *Mater. Sci. Eng., C* 2015, **49**, 824-834.

(47) J. C. Rivas-Gonzalo; S. Bravo-Haro; C. Santos-Buelga *J. Agric. Food Chem.* 1995, **43**, 1444-1449.

(48) C. F. Timberlake; P. Bridle *Am. J. Enol. Vitic.* 1976, **27**, 97-105.

(49) M. Looney; D. Solomon *Aust. J. Chem.* 1995, **48**, 323-331.

(50) J. Dawson; M. Walters *Br. J. Clin. Pharmacol.* 2006, **62**, 633-644.

(51) M. Curcio; U. G. Spizzirri; G. Cirillo; T. Spataro; N. Picci; F. Iemma *Int. J. Polym. Mater. Polym. Biomater.* 2015, **64**, 587-596.

(52) O. I. Parisi; F. Puoci; F. Iemma; G. De Luca; M. Curcio; G. Cirillo; U. G. Spizzirri; N. Picci *Polym. Adv. Technol.* 2010, **21**, 774-779.

(53) F. Puoci; C. Morelli; G. Cirillo; M. Curcio; O. I. Parisi; P. Maris; D. Sisci; N. Picci *Anticancer Res.* 2012, **32**, 2843-2847.

(54) G. Cirillo; O. Vittorio; S. Hampel; F. Iemma; P. Parchi; M. Cecchini; F. Puoci; N. Picci *Eur. J. Pharm. Sci.* 2013, **49**, 359-365.

(55) F. Iemma; F. Puoci; M. Curcio; O. I. Parisi; G. Cirillo; U. G. Spizzirri; N. Picci *J. Appl. Polym. Sci.* 2010, **115**, 784-789.

(56) F. Puoci; F. Iemma; M. Curcio; O. I. Parisi; G. Cirillo; U. G. Spizzirri; N. Picci *J. Agric. Food Chem.* 2008, **56**, 10646-10650.

(57) S. Kobayashi; S.-i. Shoda; H. Uyama In *Polymer Synthesis/Polymer Engineering*; Springer Berlin Heidelberg: 1995; Vol. 121, p 1-30.

(58) S. Kobayashi; H. Uyama; S. Kimura *Chem. Rev.* 2001, **101**, 3793-3818.

(59) M. Kurisawa; J. E. Chung; Y. J. Kim; H. Uyama; S. Kobayashi *Biomacromolecules* 2003, **4**, 469-471.

(60) F. F. Bruno; A. Trotta; S. Fossey; S. Nagarajan; R. Nagarajan; L. A. Samuelson; J. Kumar *J. Macromol. Sci., Part A: Pure Appl.Chem.* 2010, **47**, 1191-1196.

(61) S. Hamada; M. Kontani; H. Hosono; H. Ono; T. Tanaka; T. Ooshima; T. Mitsunaga; I. Abe *FEMS Microbiol. Lett.* 1996, **143**, 35-40.

(62) L. Mejias; M. H. Reihmann; S. Sepulveda-Boza; H. Ritter *Macromol. Biosci.* 2002, **2**, 24-32.

(63) M. E. Khlupova; I. S. Vasil'eva; G. P. Shumakovich; O. V. Morozova; V. A. Chertkov; A. K. Shestakov; A. V. Kisin; A. I. Yaropolov *Biochem. Moscow* 2015, **80**, 233-241.

(64) R. M. Desentis-Mendoza; H. Hernández-Sánchez; A. Moreno; E. Rojas del C; L. Chel-Guerrero; J. Tamariz; M. E. Jaramillo-Flores *Biomacromolecules* 2006, **7**, 1845-1854.

(65) M. Kurisawa; J. E. Chung; H. Uyama; S. Kobayashi *Macromol. Biosci.* 2003, **3**, 758-764.

(66) M. Kurisawa; J. E. Chung; H. Uyama; S. Kobayashi *Biomacromolecules* 2003, **4**, 1394-1399.

(67) M. Kurisawa; J. E. Chung; H. Uyama; S. Kobayashi *Chem. Commun.* 2004, 294-295.

(68) M. Božič; S. Gorgieva; V. Kokol *Carbohydr. Polym.* 2012, **89**, 854-864.

(69) N. Ihara; S. Schmitz; M. Kurisawa; J. E. Chung; H. Uyama; S. Kobayashi *Biomacromolecules* 2004, **5**, 1633-1636.

(70) F. Sousa; G. M. Guebitz; V. Kokol *Process Biochem. Int.* 2009, **44**, 749-756.

(71) N. Ihara; Y. Tachibana; J. E. Chung; M. Kurisawa; H. Uyama; S. Kobayashi *Chem. Lett.* 2003, **32**, 816-817.

(72) G. Kumar; P. J. Smith; G. F. Payne *Biotechnol. Bioeng.* 1999, **63**, 154-165.

(73) J. E. Chung; M. Kurisawa; Y. Tachibana; H. Uyama; S. Kobayashi *Chem. Lett.* 2003, **32**, 620-621.

(74) P. Gogoi; S. Hazarika; N. N. Dutta; P. G. Rao *Chem. Eng. J.* 2010, **163**, 86-92.

(75) P. Gogoi; S. Hazarika; N. N. Dutta; P. G. Rao *Chem. Eng. J.* 2009, **155**, 810-815.

(76) J. Chung; M. Kurisawa; H. Uyama; S. Kobayashi *Biotechnol. Lett.* 2003, **25**, 1993-1997.

(77) N. Ihara; M. Kurisawa; J. E. Chung; H. Uyama; S. Kobayashi *Appl. Microbiol. Biotechnol*. 2005, **66**, 430-433.

(78) L.-Q. Wu; H. D. Embree; B. M. Balgley; P. J. Smith; G. F. Payne *Environ. Sci. Technol.* 2002, **36**, 3446-3454.

(79) M. Božič; J. Štrancar; V. Kokol *React. Funct. Polym.* 2013, **73**, 1377-1383.

(80) M. Božič; S. Gorgieva; V. Kokol *Carbohydr. Polym.* 2012, **87**, 2388-2398.

(81) A. Aljawish; I. Chevalot; B. Piffaut; C. Rondeau-Mouro; M. Girardin; J. Jasniewski; J. Scher; L. Muniglia *Carbohydr. Polym.* 2012, **87**, 537-544.

(82) A. Aljawish; I. Chevalot; J. Jasniewski; A. M. Revol-Junelles; J. Scher; L. Muniglia *Food Chem.* 2014, **161**, 279-287.

(83) J. L.-Z. Stefan, B.; Kaminski, K.; Szczubialka, K.; Nowakowska, M.; Korbut, R. *J. Physiol. Pharmacol.* 2014, **65**, 341– 347.

(84) D. Huang; B. Ou; R. L. Prior *J. Agric. Food Chem.* 2005, **53**, 1841-1856.

(85) B. Neises; W. Steglich *Angew. Chem. Int. Ed. i* 1978, **17**, 522-524.

(86) N. Nakajima; Y. Ikada *Bioconjugate Chem.* 1995, **6**, 123-130.

(87) A. O. Aytekin; S. Morimura; K. Kida *J. Biosci. Bioeng.* 2011, **111**, 212-216.

(88) R. Cassano; S. Trombino; R. Muzzalupo; L. Tavano; N. Picci *Eur. J. Pharm. Biopharm.* 2009, **72**, 232-238.

(89) W. Pasanphan; G. R. Buettner; S. Chirachanchai *Carbohydr. Polym.* 2010, **345**, 132-140.

(90) W. Pasanphan; S. Chirachanchai *Carbohydr. Polym.* 2008, **72**, 169-177.

(91) S. Trombino; R. Cassano; E. Bloise; R. Muzzalupo; L. Tavano; N. Picci *Carbohydr. Polym.* 2009, **75**, 184-188.

(92) M. Xie; B. Hu; Y. Wang; X. Zeng *J. Agric. Food Chem.* 2014, **62**, 9128-9136.

(93) F. Lee; J. E. Chung; K. Xu; M. Kurisawa *ACS Macro Lett.* 2015, 957-960.

(94) Y. Li; S. Dånmark; U. Edlund; A. Finne-Wistrand; X. He; M. Norgård; E. Blomén; K. Hultenby; G. Andersson; U. Lindgren *Acta Biomater.* 2011, **7**, 751-758.

(95) S. Trombino; R. Cassano; T. Ferrarelli; S. Leta; F. Puoci; N. Picci *Molecules* 2012, **17**, 12734.

(96) W. Shi; S. Dolai; S. Rizk; A. Hussain; H. Tariq; S. Averick; W. L'Amoreaux; A. El Idrissi; P. Banerjee; K. Raja *Organic Lett.* 2007, **9**, 5461-5464.

(97) R. D. Arrua; J. F. Basbus; M. C. Strumia; C. I. Alvarez Igarzabal; M. A. Nazareno *React. Funct. Polym.* 2012, **72**, 807-813.

(98) D. Arrua; M. C. Strumia; M. A. Nazareno *J. Agric. Food Chem.* 2010, **58**, 9228-9234.

(99) U. G. Spizzirri; O. I. Parisi; F. Iemma; G. Cirillo; F. Puoci; M. Curcio; N. Picci *Carbohydr. Polym.* 2010, **79**, 333-340.

(100) J. Liu; J. F. Lu; J. Kan; Y. Q. Tang; C. H. Jin *Int. J. Biol. Macromol.* 2013, **62**, 85-93.

(101) J. Liu; J. F. Lu; J. Kan; C. H. Jin *Int. J. Biol. Macromol.* 2013, **62**, 321-329.

(102) O. Vittorio; G. Cirillo; F. Iemma; G. Di Turi; E. Jacchetti; M. Curcio; S. Barbuti; N. Funel; O. I. Parisi; F. Puoci; N. Picci *Pharm. Res.* 2012, **29**, 2601-2614.

(103) O. Vittorio; V. Voliani; P. Faraci; B. Karmakar; F. Iemma; S. Hampel; M. Kavallaris; G. Cirillo *J. Drug Targeting* 2014, **22**, 408-415.

(104) M. Curcio; F. Puoci; F. Iemma; O. I. Parisi; G. Cirillo; U. G. Spizzirri; N. Picci *J. Agric. Food Chem.* 2009, **57**, 5933-5938.

(105) D.-S. Lee; J.-Y. Woo; C.-B. Ahn; J.-Y. Je *Food Chem.* 2014, **148**, 97-104.

(106) J. Liu; X. Y. Wen; J. F. Lu; J. Kan; C. H. Jin *Int. J. Biol. Macromol.* 2014, **65**, 97-106.

(107) Y.-S. Cho; S.-K. Kim; C.-B. Ahn; J.-Y. Je *Carbohydr. Polym.* 2011, **83**, 1617-1622.

(108) U. G. Spizzirri; I. Altimari; F. Puoci; O. I. Parisi; F. Iemma; N. Picci *Carbohydr. Polym.* 2011, **84**, 517-523.

(109) G. Cirillo; F. Puoci; F. Iemma; M. Curcio; O. I. Parisi; U. G. Spizzirri; I. Altimari; N. Picci *Pharm. Dev. Technol.* 2011, **17**, 466-476.

(110) W. Zhu; Z. Zhang *Int. J. Biol. Macromol.* 2014, **70**, 150-155.

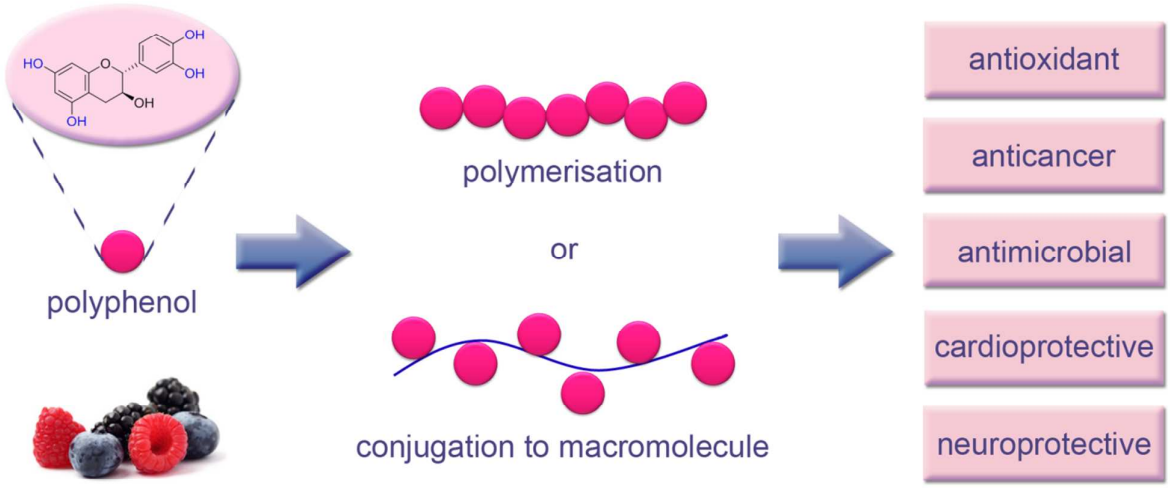
(111) U. G. Spizzirri; F. Iemma; F. Puoci; G. Cirillo; M. Curcio; O. I. Parisi; N. Picci *Biomacromolecules* 2009, **10**, 1923-1930.

(112) G. Cirillo; K. Kraemer; S. Fuessel; F. Puoci; M. Curcio; U. G. Spizzirri; I. Altimari; F. Iemma *Biomacromolecules* 2010, **11**, 3309-3315.

(113) F. Liu; C. Sun; W. Yang; F. Yuan; Y. Gao *RSC Adv.* 2015, **5**, 15641-15651.

(114) J. C. Shiu; M.-H. Ho; S.-H. Yu; A.-C. Chao; Y.-R. Su; W.-J. Chen; Z.-C. Chiang; W. P. Yang *Carbohydr. Polym.* 2010, **79**, 724-730.

Table of Contents Figure:



Short description: A review of key macromolecular systems employed to stabilise polyphenols, including direct polymerisation of polyphenol monomers and conjugation with macromolecules.